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SID 5 Research Project Final Report

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Companies making submissions to PSD for registration of herbicides are often required to propose a resistance management strategy. Research studies are required to help PSD validate these proposed strategies. This project was conducted by Rothamsted Research and ADAS Boxworth to provide relevant information in support of PSD. Black-grass (*Alopecurus myosuroides*), the main resistance problem, was the subject of most studies, but work was also conducted on wild-oats (*Avena* spp.) and Italian rye-grass (*Lolium multiflorum*). The overall scientific objective was to model herbicide resistance in order to determine risk profiles for different resistance strategies. A series of field, glasshouse and laboratory studies was undertaken on a range of relevant topics. The main findings were:

Black-grass

1. There was a correlation between the use of ACCase inhibitors and the level of ACCase target site resistance as measured by response to sethoxydim. The greater the use of ACCase inhibiting herbicides, the greater the level of ACCase target site resistance.
2. The most widely promoted mitigation strategy, the use of mixtures and sequences of ACCase inhibitors in combination with herbicides with other modes of action, did not prevent an increase in ACCase target site resistance, but did tend to slow down the rate of increase.
3. The reductions in the increase in resistance achieved by mixtures and sequences were relatively modest, and certainly could not be considered as a resistance prevention strategy. Consequently, a main implication is that, while mixtures and sequences have a useful role in mitigation strategies, this is likely to have relatively short term benefits and they should not be considered a sustainable solution in the longer term.
4. Resistance to both clodinafop and flupyrsulfuron increased over a three year period. Resistance to clodinafop increased more rapidly than resistance to flupyrsulfuron, probably reflecting rapid selection for ACCase target site resistance by clodinafop and slower selection for enhanced metabolism by flupyrsulfuron.
5. An important finding was that the value of mixtures was dependent on the herbicide under

evaluation. Mixtures of clodinafop+flupyr-sulfuron had not reduced the otherwise low level of selection for resistance to flupyr-sulfuron. In contrast, mixtures had substantially reduced the otherwise high rate of selection to clodinafop. The results with mixtures were a reflection of the interaction between the herbicide and resistance mechanisms.

6. There was no consistent effect of low v high rates of herbicides on resistance development.
7. Rotational use of herbicides sometimes resulted in marginally lower levels of resistance after three years, but overall showed only a modest benefit at reducing resistance development.
8. In the absence of herbicides there was little decline in ACCase target site resistance. The implication is that management strategies need to be effective enough to minimise increases in ACCase target site resistance because the process cannot easily be reversed.
9. In contrast, there was evidence of a loss of resistance to urea herbicides, in the absence of selection. This was probably due to changes in degree of enhanced metabolism. However, the degree of deselection was relatively modest and probably of limited significance.
10. Mesosulfuron+iodosulfuron, (a mixture of two sulfonylureas, ALS inhibitors), was very effective and gave good control of populations resistant to both ACCase inhibiting herbicides and another sulfonylurea, flupyr-sulfuron. However, mesosulfuron+iodosulfuron was not immune from resistance.
11. Three years of use of one sulfonylurea herbicide (flupyr-sulfuron) selected for probable enhanced metabolism resistance to another sulfonylurea (mesosulfuron+iodosulfuron) to a greater extent than non-sulfonylureas such as isoproturon, cycloxydim and clodinafop. The differences in efficacy were relatively small and may not impact significantly on efficacy in the field in the short term.
12. Laboratory studies with flupyr-sulfuron confirmed that enhanced metabolism is a mechanism of resistance to this sulfonylurea herbicide in black-grass in the UK.
13. Farm monitoring showed that resistance to flupyr-sulfuron had increased significantly since the 1990's. This was probably due to selection by many other herbicides, (not just flupyr-sulfuron), for increasing levels of enhanced metabolism.
14. Monitoring showed that a high degree of resistance to fenoxaprop was very widespread, probably due to enhanced metabolism and/or ACCase target site resistance.
15. Resistance to chlorotoluron appeared to increase quite slowly with time. Varying degrees of resistance occurred, but these were maintained at a fairly constant level in the medium term. Such herbicides appear to be 'lower resistance risk' from a development perspective. They are not 'no risk', as testified by the small reductions in efficacy recorded at several sites.
16. Ploughing had benefits, not only at reducing weed populations by roughly half, but also by reducing the increase in ACCase target site resistance. This was probably due to the 'diluting' effect of bringing older, less selected, buried seeds back to the soil surface. However, as with mixtures and sequences, the additional use of ploughing, did not prevent an increase in ACCase target site resistance, so again is not in itself a long term solution.
17. An outline decision model framework for the production of herbicide resistance risk profiles was devised for incorporation into Weed Manager (WMSS). See www.wmss.net.

Wild-oats, Rye-grass & Broad-leaved weeds

1. Very rapid selection for ACCase target site resistance was demonstrated in wild-oats. This was in contrast to the lack of selection recorded with a population with enhanced metabolism in previous experiments. The implications of this is that the mechanism of resistance in wild-oats can have a huge influence on the risk of selection for greater resistance in the longer term.
2. No fitness differences were found between resistant and susceptible wild-oats, regardless of whether resistance was due to enhanced metabolism or ACCase target site insensitivity.
3. Studies showed that early herbicide timing is of critical importance in achieving maximum control of both resistant wild-oats and rye-grass.
4. Studies on the biochemical basis of resistance in Italian rye-grass showed that enhanced metabolism and ACCase target site resistance occurred. It was concluded that enhanced metabolism is currently the major mechanism of resistance in Italian rye-grass

5. Resistance in broad-leaved weeds to ALS inhibiting herbicides was demonstrated for the first time in the UK. The relationship between resistance to different ALS inhibitors and insensitivity to herbicides with different modes of action (such as mecoprop and fluroxypyr) needs further investigation as some unexpected relationships were identified in this study.

Implications of the research results

Mitigation strategies for ACCase target site resistance based on herbicide mixtures and/or sequences are unlikely to be effective in the long-term. Mixtures and sequences help in the short term, but their contribution in these studies at reducing the threat from resistance was relatively modest. It is probable, but needs to be confirmed, that this will also be true for target site resistance to other herbicide classes sharing similar characteristics (e.g. high resistance risk). With enhanced metabolism resistance, the situation is more complex. This mechanism appears to develop more slowly, confer partial resistance but affect a wider range of modes of action. The use of a wide a range of different herbicide modes of action appears a valid means of reducing overall selection for enhanced metabolism resistance, although this requires verification. The studies highlight the importance of monitoring the status of resistance on individual fields or farms. The highly variable nature of weeds, means that accurate predictions of the rate of development of resistance is always going to be difficult. Hence the need to monitor individual fields so that success of any resistance management strategy can be measured. This will be particularly important in relation to ALS target site resistance in grass and broad-leaved weeds, and the possibility of ‘double target site resistance’ - ACCase + ALS target site resistance in the same population. It is also vital that cultural control measures are used in order to reduce reliance on herbicides.

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
- the scientific objectives as set out in the contract;
 - the extent to which the objectives set out in the contract have been met;
 - details of methods used and the results obtained, including statistical analysis (if appropriate);
 - a discussion of the results and their reliability;
 - the main implications of the findings;
 - possible future work; and
 - any action resulting from the research (e.g. IP, Knowledge Transfer).

PT0225: Herbicide Resistance Management: Evaluation of Strategies (HeRMES)

Background

Companies making submissions to PSD for registration, or re-registration, of herbicides are required to consider the risk that resistance poses to their active ingredients, and propose a resistance management strategy. For PSD to properly evaluate such strategies, data are required to design, test and validate the resistance management strategies proposed by registrants, and to provide a comparative assessment of different assumptions or models, so that resistance risk profiles can be formulated. Black-grass (*Alopecurus myosuroides*) is the major resistant weed of arable crops in the UK, so this was the focus for most studies in this project.

This research aims to support regulatory decision-making by helping PSD in its assessment of applicant's herbicide resistance management strategies, and in its targeting of future data requirements. In addition, the research should contribute to the development of policy on registration requirements and labelling. PSD supports research that addresses regulatory needs rather than control or farmer issues. Thus the primary user of the outputs should be PSD, although the production and publication of information which is relevant to assist farmers combat resistance is compatible with PSD's and national policy for more sustainable use of herbicides.

Scientific objectives

The overall scientific objective was to model herbicide resistance in order to determine risk profiles for different resistance strategies. There were four more specific objectives:

1. To determine how quickly resistance evolves to different herbicides, and how this is affected by mechanisms, population genetics, herbicide timing and rate.
2. To quantify resistance status, biochemical mechanisms, characteristics and occurrence.
3. To determine the influence of cultural factors on selection pressure for resistance.
4. To integrate the new information generated into a decision model framework, to allow resistance risks to be better evaluated.

The report deals with each of these objectives, and their sub-objectives, in turn including an outline of the methods used, key results and conclusions. A final section discusses the results in terms of their wider significance, implications and limitations, and also details publications, contributions to regulatory affairs and technology transfer initiatives.

Objective 1. To determine how quickly resistance evolves in black-

grass to different herbicides, and how this is affected by mechanisms, population genetics, herbicide timing and rate.

1.1 Field experiments

Three field experiments (Woburn, Boxworth, Drayton) were conducted, each running for 4 years. The aim during the first three years was to study the rate of development of ACCase target site resistance in black-grass in response to the use of a range of herbicides with different modes of action. Crops were grown as normal commercial crops in terms of inputs (e.g. fertilizer, fungicides, broad-leaved weed herbicides) with the exception of the grass-weed herbicide treatments. Weed populations were assessed by counting plants or heads in 10 x 0.1 m² random quadrats per plot.

1.1.1 Woburn experiment.

This was set up in Warren field at Rothamsted Research's Woburn farm in Bedfordshire. Plots were 6 x 6 m with a 12 m uncropped area between plots to minimise black-grass cross-pollination. The baseline frequency of ACCase target site resistance (ACCcase TSR) was determined by collecting seeds, and surface soil (0-5cm) samples, in July/August 2000 from the site of each of 32 plots in the wheat crop preceding the start of the experiment. Seed samples were subsequently collected annually to monitor changes in resistance. Seed samples were evaluated for resistance to sethoxydim (10 ppm) in a Petri-dish assay (Moss, 2000) and seedlings emerging from the soil samples sprayed with sethoxydim (145 g a.i./ha) in a glasshouse assay. Resistance to sethoxydim was taken as an indicator of the form of ACCcase target site resistance conferred by an Isl-418-Leu substitution (Brown *et al.*, 2002) that appears to be the commonest type of ACCcase target site resistance in several species worldwide. The mean initial frequency of ACCcase target site resistance was 29% based on seeds collected and 26% based on soil samples – an excellent agreement. The initial frequency between individual plots varied considerably (4 – 69% seeds; 7 - 60% soil samples) but ACCcase target site resistance was present on each of the 32 plots of the experiment and there was a good correlation (coefficient = 0.70) between the two sampling methods. Winter oil-seed rape was sown in the first year (2000/01) and separate plots were treated with cycloxydim (200 g a.i./ha) + oil, tepraloxymid (50 g a.i./ha), fluazifop-P-butyl (187.5 g a.i./ha) + 'Partna' (two plots per rep), propyzamide (700 g a.i./ha) (two plots per rep), propyzamide+cycloxydim + oil and untreated (Table 1). There were four replicates and cultivations in all years were by shallow tine/discs to 10 cm maximum depth.

Years 1 – 3.

The % reductions in black-grass plants achieved in the first year with the different herbicides were: cycloxydim - 76%; tepraloxymid - 49%; fluazifop - 39%; propyzamide - 91%; propyzamide +cycloxydim - 98%. The control by cycloxydim was consistent with the efficacy of this herbicide being only affected by ACCcase target site resistance, and not enhanced metabolism. Tepraloxymid was expected to give better control, being less affected than cycloxydim by ACCcase TSR but this did not happen for unknown reasons. Fluazifop being vulnerable to both ACCcase TSR and enhanced metabolism (EM) gave poorer control while propyzamide, being unaffected by any known mechanisms of resistance, gave good control alone, and excellent control in mixture with cycloxydim. The very high rainfall in autumn 2000 would probably have assisted the movement of immobile propyzamide down the soil profile, thus enhancing its efficacy. These weed control values are largely consistent with expectations in relation to the resistance profile at this site.

In the second and third years (2001/02 & 2002/03) the cycloxydim, tepraloxymid, propyzamide+cycloxydim and the untreated plots continued to be sown with oil-seed rape and treated in the same way as in the first year. The double plots treated with fluazifop and

propyzamide in the first year were sown with winter wheat in the second and third years, with the pairs of plots treated with clodinafop (30 g a.i./ha) + mineral oil alone or in mixture with flupyr-sulfuron (10 g a.i./ha) (Table 1). Control of black-grass in the oil-seed rape crops in the second (70 – 79%) and third years (0-23%) tended to be poor, due to increasing levels of ACC TSR, but also due to poor competition from the oil-seed rape which suffered due to repeated sowing. In the wheat crops, on the plots that had been treated with propyzamide in year 1, black-grass control in yrs 2 & 3 was respectively 65% and 43% with clodinafop alone, and 90% and 65% with clodinafop+flupyr-sulfuron. On the plots that had been treated with fluazifop in year 1, black-grass control in the wheat crops in yrs 2 & 3 was respectively 55% and 33% with clodinafop alone, and 83% and 72% with clodinafop+flupyr-sulfuron. Consequently the addition of flupyr-sulfuron consistently increased control in both years, but overall control appeared to decline between yrs 2 & 3.

The degree of control in the field, although critical from a practical viewpoint, is a crude means of identifying subtle changes in resistance as so many other factors determine herbicide efficacy (e.g. environmental conditions). Consequently changes in the resistance status of seeds collected from survivors, and the soil seed-bank, are much better indicators. Table 1 shows the changes in ACCase TSR detected over a three year cropping cycle by comparing % change in incidence in samples collected in 2003 relative to the baseline in 2000. As the initial frequency of ACCase TSR varied between plots, % changes were used for analysis. The % ACCase inhibitors used represent the proportionate usage over the 3 experimental years. The treatments are listed in order of decreasing use.

Table 1. Changes in ACCase target site resistance at the Woburn field experiment based on seed sampling and testing in Petri-dish assays with sethoxydim

Crop and herbicide treatments			ACCase TSR % change over 3 years	% ACCase inhibitors used
Year 1 OSR	Year 2 Wheat	Year 3 Wheat		
Fluazifop*	Clodinafop*	Clodinafop*	+52	100
Propyz.	Clod*	Clod*	+32	67
Fluazifop*	Clod*+Flupyr.	Clod*+Flupyr.	+26	60
Propyz.	Clod*+Flupyr.	Clod*+Flupyr.	+18	40
NIL	NIL	NIL	-3	0
Year 1 OSR	Year 2 OSR	Year 3 OSR		
Tepraloxym*	Tepraloxym*	Tepraloxym*	+61	100
Cycloxydim*	Cycloxydim*	Cycloxydim*	+54	100
Propyz+Cyclox*	Propyz+Cyclox*	Propyz.+Cyclox*	+56	50
		S.E. ±	7.0	

* = ACCase inhibiting herbicides

These results show:

- In the absence of herbicide there was only a very small decline in frequency of ACCase TSR, indicating little or no fitness penalty to this form of resistance. Without any selection pressure, the frequency of resistance did not decline.
- In the OSR/wheat rotation, there was a direct correlation between increasing use of ACCase inhibitors and increase in ACCase TSR. The greater the use of ACCase inhibiting herbicides, the greater the increase in ACCase TSR.
- The addition of flupyr-sulfuron to clodinafop reduced the increase in ACCase TSR, both where propanil was used in yr 1 (+18% v +32%) and where fluazifop was used in yr 1 (26% v 52%).
- The use of propanil instead of fluazifop in the oil-seed rape crop, reduced the increase in ACCase TSR, both where clodinafop alone was used in yrs 2 & 3 (+32% v +52%) and to a lesser extent where clodinafop+flupyr-sulfuron was used in yrs 2 & 3 (18% v 26%).
- In the continuous oil-seed rape, there was a large increase in frequency of ALS TSR with all three treatments. The actual frequency of ACCase TSR in seed samples by the end of the third year was 77- 82% for these three treatments.
- The lack of a difference between the ACCase inhibitors used alone or in mixture was probably a result of a 'plateau' effect – the rapid selection for ACCase resistance meant that there was little scope for further increases after the first two years.
- The use of mixtures and sequences of ACCase inhibitors in combination with herbicides with other modes of action did not prevent an increase in ACCase TSR, but did tend to reduce the increase relative to use of ACCase inhibitors alone.

Year 4

All plots were uncropped, and uncultivated, during 2003/04. Soil samples (0-5 cm and 10-20 cm depth) were taken in September 2004 from all plots treated with cycloxydim during the first three years of the experiment. Soil samples were placed in germination trays in the glasshouse and emerging plants sprayed with sethoxydim as an indicator of ACCase TSR as previously described. 89% of seedlings from the surface soil samples survived sethoxydim, compared with 25% in the baseline soil samples collected from the same plots in 2000. Over the 4 year period, there had been a 64% increase – similar to the 54% increase recorded for the seed samples in Table 1. In contrast, only 25% of seedlings from the 10-20 cm soil samples survived sethoxydim – the same as the baseline figure. As cultivations had been to a 5-10 cm, seeds in the 10-20 cm depth samples should represent old seeds, not subjected to any selection during the four year period of the experiment, unless new seeds fell down cracks or got buried by soil fauna. The number of seeds recovered in the depth samples was small, but the results indicate that there had been no change in the frequency of ACCase TSR in seeds in the deeper seedbank, in marked contrast to the increase recorded in the surface seedbank.

- The selection imposed by cycloxydim was reflected in a considerable increase in the frequency of ACCase TSR in the *surface* soil seedbank, and was similar to that recorded for seed samples.
- In the absence of selection by cycloxydim, there was no change in the frequency of ACCase TSR in the *deeper* soil seedbank below cultivation depth, indicating no fitness penalty to this form of resistance.

These results indicate that effective ploughing can 'turn the clock back' by returning to the surface, older, less selected seeds. In this experiment, this could potentially restore the level of ACCase TSR in the surface soil (0-5 cm, from where most black-grass seedlings emerge), to the same level as 4 years previously.

1.1.2 Boxworth and Drayton experiments.

These two field experiments were established in autumn 2000, at ADAS Boxworth, Cambridgeshire and ADAS Drayton, Warwickshire. The experimental design was identical for both sites consisting of a randomised block design with six treatments (Table 2) and four replicates, with plots 24 x 18m. In 2000/01 (yr 1) and 2002/03 (yr 3) winter wheat and in 2001/02 (yr 2) winter oilseed rape crops were established in the autumn at both sites. Herbicide treatments were applied at approximately the 2 leaf growth stage of black-grass. In autumn 2003/04 (yr 4) winter wheat was sown following the same cultivation treatments and mesosulfuron+iodosulfuron (12 + 2.4 g a.i./ha) + 'Biopower' adjuvant, an ALS inhibitor, applied to *all* plots. Black-grass seeds were collected in summer from the centre of each plot, to minimise any effects of cross-pollination, and tested in the same manner as described for the Woburn experiment.

Years 1 - 3

The pre-spray black-grass plant populations at Boxworth averaged 111/m² (range 91-133) in yr 1 but increased on all treatments in the subsequent two years. The least increase occurred on the ploughed plots, with 200-206 plants/m² surviving spraying in yr 3, and the greatest increase on disc/continuous 'fop' plots where 751 plants/m² survived spraying in yr 3. Ploughing resulted in consistently lower populations in comparisons with disced plots receiving identical herbicide treatments (1 v 2 and 3 v 4 in Table 1). Average over yrs 1-3, the reduction was 47% based on pre-spray and 42% based on post-spray survivor counts. The advantage to ploughing tended to increase with time, with reductions relative to disc cultivations, based on post-spray survivor counts averaging 23% in yr 1, 35% in yr 2 and 66% in yr 3.

The pre-spray black-grass plant populations at Drayton in yr 1 was extremely high and averaged 3196/m² (range 1547 - 4345). However, the lesser resistance status of the Drayton population resulted in more effective control than at Boxworth. Compared with this pre-spraying baseline in yr 1, there was an overall reduction in the number of black-grass plants/m² surviving herbicide in yr 3 on all disced plots, although very high populations were still present (mean 2322, range 1226 – 3618 plants/m²). Ploughing was very effective at reducing black-grass populations, with 284 plants/m² (treatment 2) and 433 plants/m² (treatment 4) surviving herbicide in yr 3. As at Boxworth, ploughing resulted in consistently lower populations in comparisons with disced plots receiving identical herbicide treatments (1 v 2 and 3 v 4 in Table 1). Averaged over yrs 1-3, the reduction was 59% based on pre-spray and 61% based on post-spray survivor counts. In contrast to Boxworth, there was no clear tendency for the advantage to ploughing to increase with time.

The results of the seed assays used for determining changes in ACCase TSR are given in Table 2. As the sites for the Boxworth and Drayton experiments could not be fixed precisely prior to the start of the experiments, baseline seed sample were not collected for individual plots. Previous testing had indicated that ACCase TSR was present at Boxworth but not at Drayton. Samples collected from a small unsprayed area in 2003, indicated that the baseline incidence of ACCase TSR were: Boxworth – 53%; Drayton – 0%. Due to the absence of individual plot baselines, the actual frequencies measured at the end of yr 3 are presented in the table, rather than changes relative to a baseline.

The results show:

- At Boxworth the level of ACCase TSR remained high with all treatments (56-85%).
- At Drayton, despite no previous record of ACCase TSR, there was clear evidence that this was present at the end of yr 3 (3-33%). This indicates that this resistance mechanism was present at a level below the limits of detection at the start of the experiment, as also appeared to be the case with the Faringdon population used in containers (see next section).

Table 2. ACCase target site resistance in the Drayton and Boxworth field experiment based on seed samples collected in summer 2003, at end of yr 3, and tested in Petri-dish assays with sethoxydim

Cults	Crop and herbicide treatments			% ACCase TSR after 3 years at Boxworth	% ACCase TSR after 3 years at Drayton	% ACCase inhibitors used
	Year 1 Wheat	Year 2 OSR	Year 3 Wheat			
1. Disc	Clod*	Fluaz*	Clod*	85%	33%	100%
2. Plough	Clod*	Fluaz*	Clod*	73%	13%	100%
3. Disc	Clod*	Propyz	Clod*	64%	15%	67%
4. Plough	Clod*	Propyz	Clod*	65%	7%	67%
5. Disc	Clod* +Flup	Fluaz*	Clod* +Flup	64%	8%	60%
6. Disc	Clod* +Flup	Propyz	Clod* +Flup	56%	3%	40%
			S.E. ±	5.7	4.4	

* = ACCase inhibiting herbicides

The % ACCase inhibitors used represent the proportionate usage over the 3 experimental years.

Clod = clodinafop (30 g a.i./ha) + oil; Flup = flupyr-sulfuron (10 g a.i./ha); Fluaz = fluazifop-P-butyl 188 g a.i./ha + 'Partna'; Propyz = propyzamide (700g a.i./ha).

- As at the Woburn site, there was a direct correlation between increasing use of ACCase inhibitors and level of ACCase TSR at both Boxworth and Drayton. The greater the use of ACCase inhibiting herbicides, the greater the level of ACCase TSR.
- The actual levels were much higher at Boxworth than at Drayton for each treatment almost certainly due to the different baseline levels.
- For identical herbicide histories, ploughing tended to result in lower levels of ACCase TSR (Treatment 1 v 2 Boxworth 85% v 73%, Drayton 33% v 13%; Treatment 3 v 4 Boxworth 64% v 65%, Drayton 15% v 7%). However, only one of these differences was statistically significant.
- The addition of flupyr-sulfuron to clodinafop reduced the level of ACCase TSR, both where propyzamide was used in yr 2 (Boxworth 56% v 64%; Drayton 3% v 15%) and where fluazifop was used in yr 2 (Boxworth 64% v 85%; Drayton 8% v 33%).
- The use of propyzamide instead of fluazifop in the oil-seed rape crop, reduced the level of ACCase TSR, both where clodinafop alone was used in yrs 1 & 3 (Boxworth 64% v 85%; Drayton 15% v 33%) and to a lesser extent where clodinafop+flupyr-sulfuron was used in yrs 1 & 3 (Boxworth 56% v 64%; Drayton 3% v 8%).
- The results from Boxworth and Drayton support those from Woburn. The use of mixtures and sequences of ACCase inhibitors in combination with herbicides with other modes of action, or the additional use of ploughing, did not prevent an increase in ACCase TSR, but did tend to reduce the increase relative to use of ACCase inhibitors alone.
- Overall, the result support the view that with high resistance risk herbicides, such as

ACCCase inhibitors, their use alone can result in rapid selection for increased resistance. Mixtures or sequences with other modes of action reduce the rate of selection, but do not prevent it, at least not at the proportions used in these studies ($\geq 40\%$ ACCCase use).

Year 4

At Boxworth in yr 4, despite very high pre-spray populations averaging 2780 plants/m² (range 446 – 4982) control by mesosulfuron+iodosulfuron was outstanding with an average of only 12 plants/m² surviving, equivalent to a 99.6% reduction. Control by mesosulfuron+iodosulfuron was not obviously affected by differences in herbicide treatments in yrs 1-3, giving very similar levels of control (99.4% – 99.7% reductions based on pre v post spray plant assessment comparisons) with all six previous treatments. At Drayton in yr 4, there were very high pre-spray populations averaging 2202 plants/m² (range 830 – 3154) but, in contrast to Boxworth, control by mesosulfuron+iodosulfuron was poorer with an average of 466 (mean 17 on ploughed and 691 on disced treatments) plants/m² surviving, equivalent to a 79% reduction. Control by mesosulfuron+iodosulfuron was much better on ploughed (98-99%, leaving a mean of 17 plants/m²) than on disced plots (72-79%, leaving a mean of 691 plants/m²), based on pre v post spray plant assessment comparisons. The similar levels of control on all the disced plots indicate that, as at Boxworth, degree of control was not obviously affected by differences in herbicide treatments in yrs 1-3. However it was observed that black-grass germinated over a protracted period in yr 4 at Drayton, resulting in many plants emerging after the application of mesosulfuron+iodosulfuron, especially on disc cultivated plots.

Seed samples collected from surviving plants in summer 2004 from each plot of both the Boxworth and Drayton experiments were used in a glasshouse pot assay to determine whether there was any evidence of resistance to mesosulfuron+iodosulfuron, which had been applied to all plots in yr 4. Plants grown from seed were treated with field rate (12+2.4 g a.i./ha), and half field rate (6+1.2 g a.i./ha) mesosulfuron+iodosulfuron in a randomised block experiment with 5 replicates. A susceptible standard (Rothamsted) and baseline samples collected in 2000 before each field experiment started were also included. At the field rate, % control (as % reduction in foliage fresh weight relative to untreated controls) of the susceptible standard was 96%, and 91% for the Boxworth and 95% for the Drayton baseline populations. The mean control of the 2004 samples from the six treatments at Boxworth and Drayton was 84% (range 68-91%) and 90% (range 85-95%) respectively. For the same herbicide treatments (in yrs 1-3), control at Drayton was always slightly better than at Boxworth, probably reflecting the generally higher level of resistance at Boxworth and the fact that many plants at Drayton emerged after herbicide application in yr 4.

There was no evidence that samples from plots treated with an ALS inhibiting herbicide (flupyr-sulfuron) in mixture with clodinafop, during yrs 1-3 showed greater resistance than other treatments (mean % control values for these ALS treatments 5 & 6 v four other treatments = Boxworth 86% v 83%; Drayton 92% v 87%). At half rate, % control of the susceptible standard was 95%, and 92% for the Boxworth and 94% for the Drayton baseline populations, little different to results at the field rate. At half rate, mean control of the 2004 samples from the six treatments at Boxworth and Drayton was lower than at the field rate, at 64% (range 32-82%) and 87% (range 82-91%) respectively. As at the field rate, there was no evidence that samples from plots treated with an ALS inhibiting herbicide (flupyr-sulfuron) in mixture with clodinafop, during yrs 1-3 showed greater resistance than other treatments (mean % control values for these ALS treatments 5 & 6 v four other treatments = Boxworth 72% v 60%; Drayton 87% v 86%). There was no clear cultivation effect at either rate. Overall there was some evidence that control by mesosulfuron+iodosulfuron was slightly poorer in the 2004 samples, from plots treated with various herbicides for 4 years, compared with the baseline populations collected in 2000. The mean % control values for 2004 samples v 2000 baseline samples were: Boxworth 84% v 91% at field rate, 64% v 92% at half rate; Drayton 90% v 95% at field rate; 87% v 94% at half rate. However most of these differences were not statistically significant ($P \leq 0.05$).

These results show that:

- Mesosulfuron+iodosulfuron, a mixture of two ALS inhibitors, is a very effective herbicide capable of giving good control of populations highly resistant to ACCase inhibiting herbicides.
- Mesosulfuron+iodosulfuron is not immune from resistance, and there were indications that its efficacy could be reduced following use of a range of herbicides.
- It is not clear to what degree its efficacy is affected by use of herbicides of the *same* mode of action (i.e. ALS inhibitors) as compared with herbicides with *different* modes of action (e.g. ACCase inhibitors). A new LINK project will address this issue and aim to determine whether ALS TSR or enhanced metabolism poses the greater threat.

1.2 Outdoor large container experiments

Containers successfully mimic field conditions but allow more controlled studies to be made on rate of development of resistance and were complementary to the field experiments. The aim was to grow resistant populations with known mechanisms and apply herbicides which are considered to impose a 'higher' (e.g. ACCase and ALS inhibitors) and 'lower' resistance risk (e.g. ureas). Rotations and mixtures of herbicides were also included. Seed samples were collected each summer and re-sown each year for the duration of the project with the emphasis on establishing changes in *proportion* of resistant individuals rather than population density. Changes in level of resistance were assessed in glasshouse and Petri-dish assays. Experiments were conducted on black-grass (for 4 years starting in autumn 2000) and wild-oats (for 3 years) at two sites.

1.2.1 Black-grass experiment

The same treatments and experimental design (randomised block with four replicates) were used at both sites. Three black-grass populations were used: Rothamsted 2000 (=Roth), a susceptible standard; Faringdon 2000 (=Far), a population with a low/moderate level of enhanced metabolism and a low incidence of ACCase TSR (1.5%); Islip Ptch 2000 (=Isl), a population with a low/moderate level of enhanced metabolism and a higher incidence of ACCase TSR (17%). In each container (40 x 33 x 16 cm deep), 650 black-grass and 15 wheat seeds were sown into the surface 2.5 cm of a Kettering loam soil. Seeds were sown in early October each year and herbicides applied at the 3 leaf stage in November/December. Plants per container were assessed prior to spraying and survivors recorded in the spring (Feb-April). Containers for each treatment were isolated in early May to prevent cross-pollination. Seeds were collected as they matured from each individual container in July and August. Containers were re-sown each autumn with seeds collected from the same treatment that summer, except for the treated susceptible standard which was sown each year with seed from the same original sample to act as reference.

The following herbicide treatments were applied to the Faringdon and Islip populations in each of yrs 1 - 3: the ACCase inhibitor clodinafop at 15 and 30 g a.i./ha + oil; the ALS inhibitor flupyr-sulfuron at 5 and 10 g a.i./ha; a mixture of half rate clodinafop+flupyr-sulfuron (15 + 5 g a.i./ha) + oil; a rotation of clodinafop (yr 1 & 3) at 30 g a.i./ha and flupyr-sulfuron (yr 2) at 10 g a.i./ha; the ACCase inhibitor cycloxydim at 200 g a.i./ha; the urea isoproturon at 1.5 kg a.i./ha. In each case the higher rate is the field recommended rate. The Rothamsted susceptible standard was treated with the higher rates of clodinafop, flupyr-sulfuron and isoproturon only. There were also untreated controls for all three populations.

In yr 4 (2003/04), seed samples treated in yrs 1-3 with the higher rate of clodinafop and flupyr-sulfuron, the herbicide mixture, cycloxydim, isoproturon and untreated were sown into containers and *all* (except untreated) subsequently treated with mesosulfuron+iodosulfuron (12 + 2.4 g a.i./ha) to determine whether its activity was affected by the different herbicide treatments in yrs 1 - 3. The baseline populations, Faringdon 2000 and Islip 2000, and a susceptible standard (Rothamsted) were also included.

Years 1 - 3 plant counts

- Control of the susceptible standard, Rothamsted, was very good at both locations in all three years demonstrating that application method and environmental conditions were conducive to good control by all herbicides. Clodinafop gave 96 – 100% control, flupyr-sulfuron 90 – 100% control and isoproturon 93 – 100% control of plants.
- Summaries of plants counts for yrs 1 - 3, in terms of % reduction relative to untreated controls for the same population, are given in the following tables with comments below.

Table 3.

	% reduction in plant numbers (meaned over 16 herbicide treatments applied to Far and Isl)	
Site...	Rothamsted	Boxworth
Year 1	67%	63%
Year 2	44%	24%
Year 3	20%	17%
	S.E. ± 1.59	S.E. ± 2.14

- Meaned over all 16 treatments (both populations and 8 herbicide treatments), % control of plants declined over the three year period at both locations. Results for both sites were similar in yr 1 and 3, but poorer at Boxworth in year 2. There was a clear indication that resistance increased substantially during the three year period.

Table 4.

	% reduction in plant numbers (meaned over 8 herbicide treatments applied to Far and Isl)			
Site...	Rothamsted		Boxworth	
Population...	Faringdon	Islip	Faringdon	Islip
Year 1	66	69	68	59
Year 2	41	47	33	16
Year 3	15	25	23	10
	S.E. ± 3.59		S.E. ± 3.81	

- The two populations behaved in a broadly similar way at both locations. Control of each population declined over the three year period at both locations, but control of Islip was slightly better than Faringdon at Rothamsted, whereas at Boxworth the reverse occurred.

Table 5.

	% reduction in plant numbers (meaned over 2 populations and 2 dose rates)			
Site...	Rothamsted		Boxworth	
Herbicide...	Clodinafop	Flupyr-sulfuron	Clodinafop	Flupyr-sulfuron
Year 1	82	39	79	37
Year 2	44	34	23	12
Year 3	28	8	26	19
	S.E. ± 4.21		S.E. ± 4.71	

- Clodinafop gave better control than flupyr-sulfuron at both locations, although control with both herbicides declined over the three years. At Rothamsted the decline was progressive whereas at Boxworth the greatest decline tended to occur between years 1 & 2 with both herbicides, with little subsequent change between years 2 & 3.

Table 6.

	% reduction in plant numbers (meaned over 2 populations)			
Site...	Rothamsted			
Herbicide...	Clodin.+Flupyr. in mixture	Clodin./Flupyr. in rotation	Clodinafop higher rate	Flupyr-sulfuron higher rate
Year 1	72	88	89	42
Year 2	48	29	43	35
Year 3	25	29	29	15
	S.E. \pm 5.26			

	% reduction in plant numbers (meaned over 2 populations)			
Site...	Boxworth			
Herbicide...	Clodin.+Flupyr. in mixture	Clodin. (yr 1&3) Flupyr (yr 2)	Clodinafop higher rate	Flupyr-sulfuron higher rate
Year 1	53	82	82	38
Year 2	27	27	11	20
Year 3	11	16	24	23
	S.E. \pm 6.24			

- Overall, use of mixtures of clodinafop+flupyr-sulfuron or rotation of the same herbicides (clodinafop in years 1 & 3, flupyr-sulfuron in year 2) did not reduce the decline in herbicide performance recorded over the three years at either location. Meaned over both populations, the mixture (of half rates) gave poorer control (Roth/Boxw = 72/53) than full rate clodinafop (89/82), but better control than full rate flupyr-sulfuron (42/38), at both locations in the first year. After 3 years, control by both herbicides used alone or in mixture or rotation was poor: Rothamsted 15 – 29%; Boxworth 11 – 24%. At neither location was the mixture or rotation giving better control than full rate clodinafop alone. At Rothamsted the mixture and rotation gave slightly better (but n.s.) control than full rate flupyr-sulfuron whereas as Boxworth full rate flupyr-sulfuron gave slightly better (but n.s) control than the mixture or rotation. However at both sites control by all these treatments was clearly inadequate. Control by clodinafop declined relatively more than control by flupyr-sulfuron over the three years at both locations.
- Cycloxydim gave good control of the Faringdon population in the first year at both locations, but control declined substantially over the 3 years. For the 3 years respectively, % control of plants at Rothamsted/Boxworth sites were: 97%/91%, 58%/48%, 19%/18%. Control of the Islip population was poorer than Faringdon at both locations in every year, with % control of plants at Rothamsted/Boxworth sites being: 88%/80%, 46%/1%, 8%/-2%. The Islip population was known to possess a small % of ACCase target site resistant (TSR) plants (17%) whereas the Faringdon population was initially thought have no ACCase TSR. Subsequent more detailed tests indicated that about 1.5% of the original Faringdon 2000 population did possess ACCase TSR. Thus these results are supportive of an initial difference in level of ACCase TSR and subsequent rapid selection for ACCase TSR in both populations. This conclusion was supported by a Petri-dish test with 10ppm sethoxydim conducted on seed samples from both sites, which confirmed that very rapid increases in resistance had occurred as a consequence of annual use of cycloxydim.
- Isoproturon gave poor to mediocre control of plants of both populations at both locations. Control of Islip tended to be poorer than for Faringdon, the only exception being in year 3 at Rothamsted. Meaned over both populations and sites, % control was: yr1 50%; yr2 58%; yr 3 15%. At both sites control in year 3 was poorer than in year 1.

The degree of control of plants in the containers gives an indication of the likely impact of the different herbicide regimes in the field, but is a relatively crude means of identifying subtle

changes in the resistance status of the populations as other factors will influence herbicide efficacy in outdoor conditions (e.g. environmental conditions). Consequently changes in the resistance status of seeds collected from survivors should be much better indicators.

Years 1 - 3 glasshouse dose assay

At the end of yr 3, seed samples collected in summer 2003 were evaluated in a glasshouse dose response assay to determine the degree of change in resistance to clodinafop and flupyr-sulfuron compared with the original populations. A susceptible standard was also included. Seven doses in the range 0.625 – 160 g flupyr-sulfuron/ha and 0.9375 – 480 g clodinafop/ha were applied to plants at the 3 leaf stage growing in individual 5 cm pots with 12 replicate pots per dose. Foliage fresh weight was recorded 21-27 days post spraying as a measure of herbicide efficacy.

Table 7. Response to **flupyr-sulfuron** based on glasshouse dose response assay conducted on plants grown from seeds collected from containers in summer 2003 after three years of annual selection (mean of two sites)

Population...	Faringdon		Islip	
	Log ₁₀ ED ₅₀	ED ₅₀ g/ha (<i>Ratio to Baseline</i>)*	Log ₁₀ ED ₅₀	ED ₅₀ g/ha (<i>Ratio to Baseline</i>)*
<i>Roth 00 (S standard)</i>	0.766	5.8 (-)	0.766	5.8 (-)
Baseline population (2000)	1.627	42.4 (1.0)	1.463	29.0 (1.0)
Flupyr-sulfuron Low (5 g/ha)	1.828	67.4 (1.6)	2.090	123.1 (4.2)
Flupyr-sulfuron High (10 g/ha)	1.927	84.5 (2.0)	1.951	89.3 (3.1)
Clod + Flup (15+5 g/ha)	2.069	117.3 (2.8)	2.002	100.4 (3.5)
Clod rotated with Flup (30/10 g/ha)	1.795	62.4 (1.5)	1.665	46.3 (1.6)
Combined S.E. ±	0.099	-	0.082	-

* = Resistance index (RI) in brackets, which is the ratio of ED₅₀ values relative to the baseline population, *not* the susceptible standard.

Flupyr-sulfuron results

- There were no consistent differences in the response of samples from the two sites where the container experiments were conducted (Rothamsted and Boxworth) so a combined analysis was done and results presented in Table 7.
- Both baseline populations, collected from fields at Faringdon and Islip in 2000, showed partial resistance to flupyr-sulfuron (RI relative to the susceptible standard, Rothamsted 2000: Faringdon = 7.3; Islip = 5.0).
- All herbicide treatments had selected for slightly greater resistance to flupyr-sulfuron relative to the baseline, and this was statistically significant with Flup High and Clod+Flup on both populations and Flup Low on Islip.
- Dose rate had no consistent, or significant effect on the degree of resistance. With Faringdon, the higher rate, and with Islip the lower rate of flupyr-sulfuron resulted in slightly higher levels of resistance. However none of these differences were significant.
- Mixtures with clodinafop had not decreased selection, but actually gave slightly higher levels of resistance than full rate flupyr-sulfuron alone with both populations, although these effects were not significant.
- Rotational use of clodinafop and flupyr-sulfuron (clodinafop in years 1 & 3; flupyr-sulfuron in year 2), resulted in the lowest degree of resistance selection over the three years with both populations. Compared with annual applications of full rate flupyr-sulfuron alone, this difference was significant with the Islip, but not the Faringdon population. One interpretation of this is that clodinafop was not selecting for enhanced metabolism (EM) resistance in the same way as flupyr-sulfuron. This could be due to selection for resistance conferred by different enzyme systems that have differential abilities to metabolise flupyr-sulfuron. Alternatively, as clodinafop would be expected to select very strongly for

ACCCase TSR, the degree of EM in TSR plants would be irrelevant. Thus clodinafop would select for EM resistance to a lesser degree than flupyr-sulfuron – hence lower degrees of resistance following rotational use.

- The mean ED₅₀ value for the four herbicide treatments was 82.9 for Faringdon and 89.8 for Islip – very similar. The generally greater increases in resistance over the three year period for Islip compared with Faringdon, appeared to be due to the lower baseline resistance in the latter. This supports the idea of a ‘plateauing’ effect with enhanced metabolism based resistance.

Table 8. Response to **clodinafop** based on glasshouse dose response assay conducted on plants grown from seeds collected from containers in summer 2003 after three years of annual selection (mean of two sites)

Population...	Faringdon		Islip	
	Log ₁₀ ED ₅₀	ED ₅₀ g/ha (<i>Ratio to Baseline</i>)*	Log ₁₀ ED ₅₀	ED ₅₀ g/ha (<i>Ratio to Baseline</i>)*
<i>Roth 00 (S standard)</i>	0.801	6.3 (-)	0.801	6.3 (-)
Baseline population (2000)	1.414	25.9 (1.0)	1.374	23.7 (1.0)
Clodinafop Low (5 g/ha)	2.390	245.6 (9.5)	2.400	250.9 (10.6)
Clodinafop High (10 g/ha)	2.286	193.3 (7.5)	2.650	446.3 (18.8)
Clod + Flup (15+5 g/ha)	1.766	58.3 (2.3)	1.826	67.0 (2.8)
Clod rotated with Flup (30/10 g/ha)	2.167	146.8 (5.7)	2.624	420.7 (17.8)
Combined S.E. ±	0.089	-	0.093	-

* = Resistance index (RI) in brackets, which is the ratio of ED₅₀ values relative to the baseline population, *not* the susceptible standard.

Clodinafop results

- There were no consistent differences in the response of samples from the two sites where the container experiments were conducted (Rothamsted and Boxworth) so a combined analysis was done and results presented in Table 8.
- Both baseline populations, collected from fields at Faringdon and Islip in 2000, showed partial resistance to clodinafop (RI relative to the susceptible standard, Rothamsted 2000: Faringdon = 4.1; Islip = 3.8). A similar overall level of resistance, but frequency of ACCCase TSR is known to be higher in Islip (17%) than in Faringdon (1.5%).
- All herbicide treatments had selected for significantly greater resistance to clodinafop relative to the baseline.
- Increases for clodinafop applied alone at both rates were higher than those recorded with flupyr-sulfuron (Faringdon clod 7.5-9.5 v flup 1.6-2.0; Islip clod 10.6-18.8 v flup 3.1-4.2). This probably reflected stronger selection for ACCCase target site resistance by clodinafop than selection for enhanced metabolism by flupyr-sulfuron.
- Dose rate had no consistent, or significant, effect on the degree of resistance. With Faringdon the lower rate, and with Islip the higher rate of clodinafop resulted in slightly higher levels of resistance. The result with Islip was almost statistically significant.
- Mixtures with flupyr-sulfuron had substantially reduced the rate of selection, giving only a 2.3 (Faringdon) or 2.8 (Islip) fold increase in resistance index, whereas the other three treatments gave 5.7-9.5 (Faringdon) or 10.6-18.8 (Islip) fold increases. However, there was no evidence to support this in terms of the % control of plants in the third year. It would appear that despite roughly equally poor control of plants of both populations in year 3, the seed produced by surviving plants showed big differences in degree of resistance in glasshouse assays. One explanation for this apparent contradiction could be that, despite the apparent big differences recorded in the glasshouse assays, the degree of

resistance was high enough in all populations to result in substantial reductions in efficacy. This appears a valid explanation as the clodinafop resistance indices in the glasshouse dose response assay were very high – even those for the clodinafop+flupyrulfuron mixture population were 9.3 for Faringdon and 10.6 for Islip *relative to the Rothamsted susceptible standard*.

- Rotational use of clodinafop and flupyrulfuron (clodinafop in years 1 & 3; flupyrulfuron in year 2) gave RI values of 5.7 (Faringdon) and 17.8 (Islip) which were marginally lower or similar to values for clodinafop alone at low or high rate (Faringdon, 7.5 - 9.5; Islip, 10.6-18.8). None of these differences were significant.
- The mean ED₅₀ value for the four herbicide treatments was 161.0 for Faringdon and 296.2 for Islip. This difference with clodinafop, despite similar baselines (25.9 v 23.7), contrasts with the lack of difference after 3 years recorded with flupyrulfuron in selected populations, despite larger differences in baselines (Faringdon slightly more resistant to flupyrulfuron than Islip). The generally greater increases in resistance with clodinafop with Islip treatments compared with Faringdon, was probably associated with a greater increase in ACCase TSR, which was present at a higher initial frequency in Islip.
- A Petri-dish test using sethoxydim at 10 ppm was used to confirm that the increases in resistance to clodinafop at both sites were linked to ACCase TSR. Poor control (<32%) of both populations treated in the containers with the higher rate of clodinafop or the clodinafop/flupyrulfuron rotational treatment was achieved, and reduced control (49-80%) with the mixture of the two herbicides. This was consistent with the glasshouse dose response conclusion that clodinafop alone or in rotation had selected for greater ACCase TSR in both populations over 3 years.

The overall conclusions from the container studies for years 1 – 3 were:

- Resistance to both clodinafop and flupyrulfuron increased over the three years
- Resistance to clodinafop increased more rapidly than resistance to flupyrulfuron, probably reflecting rapid selection for ACCase target site resistance by clodinafop and slower selection for enhanced metabolism by flupyrulfuron.
- There was no consistent effect of low v high rates on resistance development with either flupyrulfuron or clodinafop.
- Rotational use of clodinafop and flupyrulfuron sometimes resulted in marginally lower levels of resistance after three years, but overall showed only a modest benefit at reducing resistance development
- The value of the clodinafop+flupyrulfuron mixture was dependent on the herbicide under evaluation. Mixtures of clodinafop+flupyrulfuron had not reduced the otherwise low level of selection for resistance to flupyrulfuron. In contrast, mixtures of clodinafop+flupyrulfuron had substantially reduced the otherwise high rate of selection to clodinafop. Mixture had not prevented an increase in resistance, but in some cases had slowed the process, although not always to a degree that was reflected in adequate herbicide efficacy.
- The results with mixtures were probably a reflection of the interaction between herbicide and resistance mechanisms. Each herbicide in the mixtures was likely to be selecting for enhanced metabolism resistance to flupyrulfuron in both populations, and hence the mixture showed little advantage over flupyrulfuron alone. In contrast, clodinafop was probably selecting very strongly for ACCase TSR, and the degree of enhanced metabolism in ACCase target site resistant plants would have been irrelevant. Thus clodinafop may have selected for enhanced metabolism resistance *in plants with ACCase*

target site resistance to a lesser degree than flupyr sulfuron. Thus at least some of the clodinafop selected plants would have a lower level of enhanced metabolism and thus would have been well controlled by flupyr sulfuron. Consequently the mixture would have selected less strongly for resistance to clodinafop than clodinafop alone. ACCase target site resistance occurred in *both* baseline populations, although at much higher level in Islip than Faringdon. This may explain the greater benefit with the mixture in Islip compared with Faringdon.

Yr 4 (2003/04) results

Mesosulfuron+iodosulfuron gave very good control of all the populations in the containers in yr 4. Control of plants was always over 87% at both sites, and mostly over 95%. There was no clear evidence that previous treatment in yrs 1-3 with the ALS inhibitor flupyr sulfuron predisposed plants to be more resistant to mesosulfuron+iodosulfuron (also an ALS inhibitor) than pre-treatment with other modes of action. Efficacy of mesosulfuron+iodosulfuron on Islip samples collected in 2004 tended to be slightly less than on the 2000 baseline sample, but this effect was marginal. Control of samples derived from containers of both populations treated annually with the mixture of clodinafop+flupyr sulfuron during yrs 1-3 tended to be slightly poorer (87% – 92%) than control of all other samples (Rothamsted mean 96%; Boxworth mean 97%). This effect was observed at both sites but is difficult to explain.

To determine whether there were more subtle differential degrees of selection, not detectable in terms of plant counts in containers, seed samples were collected from surviving plants in summer 2004. Plants grown from these seeds were tested in a glasshouse pot assay in which plants (6 per pot) were treated with mesosulfuron+iodosulfuron at field (12 + 2.4 g a.i./ha) and half rate (6 + 1.2 g a.i./ha), and flupyr sulfuron at the field rate (10 g a.i./ha). Plants were sprayed at the 3 leaf stage and foliage fresh weight was recorded 28 days after spraying. There were four replicates.

The Rothamsted 2000 susceptible standard, and the Faringdon 2000 and Islip 2000 baseline populations were well controlled (84% – 94%) by all three herbicide treatments. The lower rate of mesosulfuron+iodosulfuron gave generally moderate to good control of all populations but there were differences between original (Yrs 1-3; 2000-2003) treatments. Eight (out of 20; 10 treatments x 2 sites) original treatments gave values that were statistically significantly less than the 2000 baseline population. Five of these involved flupyr sulfuron, either alone or in mixture with clodinafop. The higher rate of mesosulfuron+iodosulfuron gave generally good control of all populations, over 90% in most cases. Four original treatments gave values that were statistically significantly less than the baseline population. Three of these involved flupyr sulfuron, either alone or in mixture with clodinafop. Thus for both mesosulfuron+iodosulfuron treatments, a total of 12 original treatments (out of 40) gave values that were statistically significantly less than the baseline populations, and 8 (67%) involved flupyr sulfuron, 3 clodinafop alone and 1 isoproturon. None involved cycloxydim, even though this had been shown in other experiments to have considerably increased the level of ACCase target site resistance in both populations. Flupyr sulfuron alone or in mixture was used in 16 (40%) out of the 40 original treatment comparisons. These 40 comprised the two sites x two populations x five herbicide treatments (clodinafop and flupyr sulfuron at full rate, mixture of these two herbicides, isoproturon, cycloxydim) x the two rates of mesosulfuron+iodosulfuron used.

In the glasshouse pot assay, flupyr sulfuron gave generally moderate control but with more variability. Three original treatments gave values that were statistically significantly less than the baseline populations and all of these involved flupyr sulfuron, either alone or in mixture with clodinafop.

Table 9. Glasshouse pot assay: results pooled over both populations and sites.

	Herbicide used in <i>initial</i> 3 years of container experiments (pooled % reduction value in glasshouse assay)					S.E. ±
Assay herbicide	Most resistant				Least resistant	
Meso+iodo. Half rate	Flup (73)	Clod+Flup (74)	Clod (78)	IPU (80)	Cyc (89)	3.01
Meso+iodo. Field rate	Flup (77)	Clod (85)	Clod+Flup (88)	Cyc (91)	IPU (93)	2.47
Flupyr sulfuron	Flup (60)	Clod+Flup (63)	IPU (78)	Clod (80)	Cyc (81)	4.80
Overall mean	Flup (70)	Clod+Flup (75)	Clod (81)	IPU (84)	Cyc (87)	(pooled) 3.59

The lowest level of control (most resistant) for all three assay herbicides was with seeds from containers that had been treated with flupyr sulfuron in each of the first three years (2000-2003). The mixture of clodinafop+flupyr sulfuron and clodinafop alone were next in terms of association with resistance whereas isoproturon (IPU) and especially cycloxydim were associated with the least increase in resistance. Indeed, none of the cycloxydim results were significantly different to the baseline populations. The differences between the most and least resistant treatments in the table above were always statistically significant. The differences between flupyr sulfuron and isoproturon were significant for field rate mesosulfuron+iodosulfuron and flupyr sulfuron, but not quite significant for half rate mesosulfuron+iodosulfuron.

The predictions made in the HeRMES extension proposal (Annex to CS7 for project PT0225, October 2003) were that the treatments would have selected for resistance in the following order: flupyr sulfuron (most resistance), clodinafop+flupyr sulfuron, clodinafop alone, isoproturon, cycloxydim (least resistance). Although the differences in efficacy are relatively small and may not impact significantly on efficacy in the field in the short term, the overall mean results from this experiment are in *precise* agreement with this prediction. Amazing!

An important implication is that the use of one sulfonylurea herbicide (e.g. flupyr sulfuron) selects for resistance to another sulfonylurea (e.g. mesosulfuron+iodosulfuron) to a greater extent than non-sulfonylureas such as isoproturon and cycloxydim, and to a lesser extent clodinafop. Mixtures help – but only to a limited degree. This result would be expected with ALS target site resistance, but in this experiment enhanced metabolism is much more likely due to the relatively low initial population sizes in the containers and the fact that resistance was partial, not absolute.

1.2.2 Wild-oats experiment

Previous container studies in wild-oats had shown, rather surprisingly, that five annual applications of herbicides to which partial resistance already existed (fenoxaprop, tralkoxydim, imazamethabenz), did not result in any increase in level of resistance in a population (T/11) in which resistance was conferred by enhanced metabolism. However it was predicted that resistance conferred by target site insensitivity could increase more rapidly. Hence container experiments were conducted at Rothamsted and Boxworth within the HeRMES project to investigate this aspect. A wild-oat (*Avena sterilis* ssp. *ludoviciana*) population from Essex (T/41 1994) with confirmed ACCase target site resistance (Cocker *et al.*, 2000) was used. As a very high proportion of this population is resistant, it was used in mixture with a susceptible standard (LLUD95) to simulate a population with 10% TSR. 120 wild-oat seeds (12 T/41 + 108 LLUD) and 30 wheat seeds were sown in early October each year in a Kettering loam soil in containers using the identical method used in previous wild-oat experiments (Moss *et al.*, 2001). A susceptible standard (LLUD95) was also used. The following herbicides were applied at the 1 - 3 tiller stage in February/March each year: fenoxaprop-P-ethyl 27.5 and 55 g a.i./ha;

imazamethabenz 300 and 600 g a.i./ha + ‘Agral’. These are half and full recommended field rates. Herbicide efficacy was determined as % reduction in seed production relative to untreated controls. Seeds were collected as they matured from each individual container in July and August and containers re-sown each autumn with seeds collected from the same treatment that summer. The experiments ran for three successive years (2000-2003) at both sites.

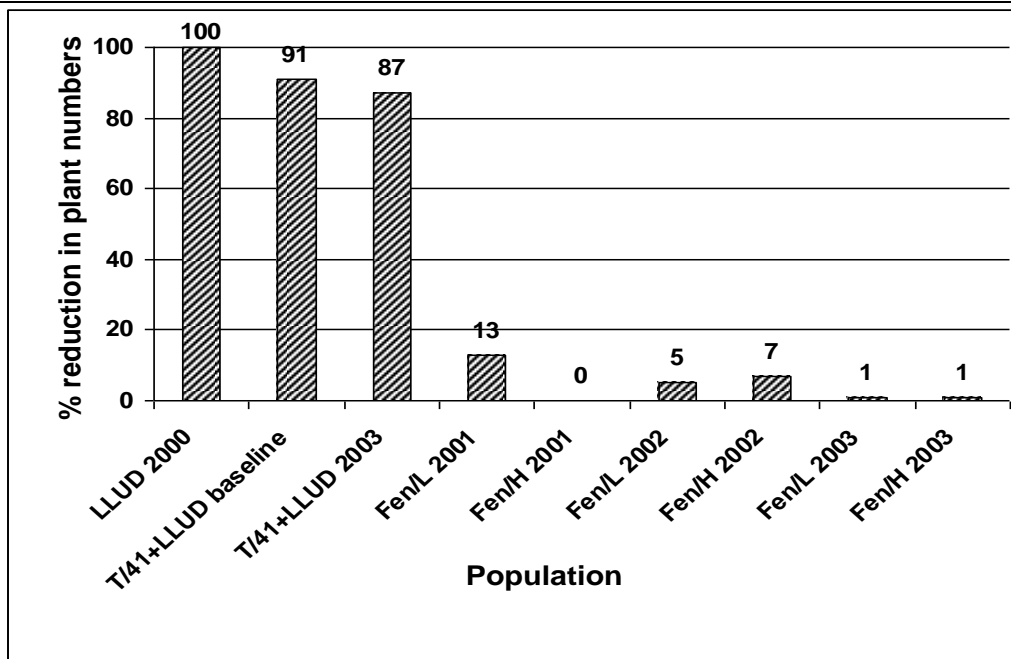
Table 10. Wild-oat container experiment: % reduction in seed return of the resistant population (initially 10% ACCase TSR)

Site...	Year 1 (2000/01)		Year 2 (2001/02)		Year 3 (2002/03)	
	Roth.	Boxw.	Roth.	Boxw.	Roth.	Boxw.
Fenox. 55 g/ha	63%	81%	52%	9%	17%	0%
Fenox. 27.5 g/ha	77%	81%	52%	24%	13%	3%
Imaz. 600 g/ha	96%	78%	95%	89%	91%	41%
Imaz. 300 g/ha	77%	60%	98%	76%	74%	33%
S.E.±	9.95	7.01	2.31	11.75	3.75	15.65

- Fenoxaprop gave excellent (99 – 100%) control of the susceptible standard, LLUD, every year, showing that the application and environmental conditions were conducive to good herbicide activity. Control by imazamethabenz was slightly more variable (83 – 93%), with a mean of 89% control over the three years.
- The control of plants of the resistant population in the first years by fenoxaprop, an ACCase inhibitor, averaged 88% over both rates which was consistent with this initial mixed population comprising 10% resistant seeds.
- Control of the resistant population with fenoxaprop, in terms of seed return (Table 10), declined over the three year period at both sites, and very poor control was achieved after three years (0 – 17%).
- Dose rate of fenoxaprop had no significant impact on the decline in control at either site.
- At Rothamsted, the higher rate of imazamethabenz, an ALS inhibitor, gave good and consistent levels of control in all three years averaging 94%, which was comparable to the control of the susceptible standard. At Boxworth, there was more variation between years. Overall, there was no clear evidence for resistance to imazamethabenz, which was consistent with the T/41 component of the population being only resistant to ACCase inhibitors.
- The lower rate of imazamethabenz gave more variable control.

To quantify changes in degree of resistance, seed samples collected each year from plants surviving both rates of fenoxaprop, the baseline original mixed population, seeds collected in 2003 from the original population grown on for three years without any herbicide application, together with a susceptible standard (LLUD00) were evaluated for resistance to fenoxaprop in a glasshouse assay. For each sample, there were four treated and one untreated germination tray, each sown with 40 pre-germinated seeds. Plants were counted, sprayed at the 3 leaf stage with fenoxaprop-P-ethyl (110 g a.i./ha), and the number of surviving plants recorded 3 weeks after spraying. The results for the Rothamsted and Boxworth sites showed very similar trends, so mean results are presented in Figure 1.

Figure 1. % reduction in wild-oat plant numbers by fenoxaprop (110 g/ha) in glasshouse assay of seeds from wild-oat container experiment (mean of Rothamsted and Boxworth sites).



- Despite the use of twice the field rate of fenoxaprop, plants were either killed, or survived showing few herbicidal symptoms, which was consistent with the presence of ACCase target site resistance, rather than the ‘shades of grey’ response more typical of enhanced metabolism.
- The susceptible standard (LLUD 2000) was completely controlled, as would be expected.
- The T/41+LLUD baseline population consisting of 10% resistant seeds, as used for sowing the containers at the start of the experiment in autumn 2000, was controlled by almost exactly the level that would be predicted (90% reduction).
- The T/41+LLUD 2003 population, grown on for three years without herbicide, was controlled to a similar degree (87%) to the baseline population from which it originated. Consequently there was no evidence of any fitness penalty to ACCase target site resistance in wild-oats. If there had been, an increase in degree of control would have been expected.
- After only one year’s selection, control was very poor at both dose rates. Control continued to be very poor for all three years. There was no clear effect of dose rate.
- In comparison with the values for reductions in seed return in Table 10, which showed a more progressive decline in activity, there appeared to be a much more dramatic selection for resistance in the seed samples, especially at Rothamsted. However, the seeds tested in each year were collected from surviving plants, so the degree of control of seed return, and resistance status of those seeds, need not be correlated. However, the degree of control of seed return in the second year (2001/02) at Rothamsted in Table 10 is higher than would be predicted from the tests on seeds collected at the end of the first year (2001) in Figure 1.
- The results were consistent with a very rapid selection for ACCase target site resistance. This was in marked contrast to the lack of selection recorded with a population with enhanced metabolism in previous experiments.
- The implications of this, and previous studies, are that the mechanism of resistance in wild-oats can have a huge influence on the risk of selection for greater resistance in the longer term. This effect appears to be greater than that recorded in black-grass, and may be a consequence of wild-oats being a self pollinating species.

1.3 Outdoor small container experiments

These were used to study the interaction of resistance and herbicide application timing and rate. Populations of black-grass, wild-oats and rye-grass with known resistance mechanisms were sown in outdoor containers (27 x 18 x 10 cm deep) and treated with herbicides at different timings (autumn v spring). Susceptible populations were included for comparison. Methodology was otherwise similar to that used for the large containers, in section 1.2 above. The principal aim was to study the influence of any interaction between resistance and growth stage of plants on overall herbicide efficacy.

1.3.1 Black-grass experiment

An experiment conducted in 2001/02 to study the interaction of resistance and herbicide timing produced unexpected results. Later applications (January) of field rate flupyr-sulfuron (10 g a.i./ha) and clodinafop (30 g a.i./ha) at 2-3 tillers gave better control than earlier applications at the 3 leaf stage (November). The mean % reductions (late v early) in foliage weight for the two resistant populations used (Faringdon and Islip) were: flupyr-sulfuron 86% v 56%; clodinafop 86% v 53%. Spring (March) applications to plants at the 3 leaf stage of the same populations gave even poorer control (flupyr-sulfuron 46%; clodinafop 18%). This indicates that herbicide efficacy against resistant plants is not solely a consequence of growth stage of the weed, but that environmental conditions are also important. The susceptible standard, Rothamsted, was well controlled (88 – 99%) at all timings with both herbicides. Past experiments have consistently indicated that control of resistant populations is best achieved by application to smaller plants. This experiment indicated that while early applications can normally be expected to give superior control, there are exceptions.

1.3.2 Wild-oat experiment

In a 2002/03 experiment, a susceptible (LLUD) and partially (enhanced metabolism) resistant (T/11) population were equally well controlled (98%) by autumn applications (3 leaf, December) of clodinafop (30 g a.i./ha). In contrast, with spring applications (2-3 tillers, April), control of the partially resistant population was much poorer (48% reduction in foliage fresh weight; 20% of panicle number) than the susceptible standard (99%). This shows that early herbicide timing is of critical importance in achieving maximum control of partially resistant wild-oats.

1.3.3 Italian rye-grass (*Lolium multiflorum*) experiment

In a 2002/03 experiment, a susceptible (Trajan) and partially (enhanced metabolism) resistant (Wilts B1) population were treated pre-emergence (October), at 2 leaf (November) and 1 tiller (January) stage with chlorotoluron at 2.75 kg a.i./ha. The susceptible standard was well controlled (% reduction in foliage fresh weight) at all three timings, 100%, 100% and 91% respectively. In contrast, control of the partially resistant population declined from 74% pre-emergence, to 71% at the 2 leaf and to only 41% at 1 the tiller timing. This shows that, as with wild-oats, early herbicide timing is of critical importance in achieving maximum control of partially resistant rye-grass.

Objective 2. To quantify resistance status, biochemical mechanisms,

characteristics and frequency of occurrence.

2.1 & 2.2 Refine and validate assays for evaluating resistance in wild-oats, rye-grass and black-grass

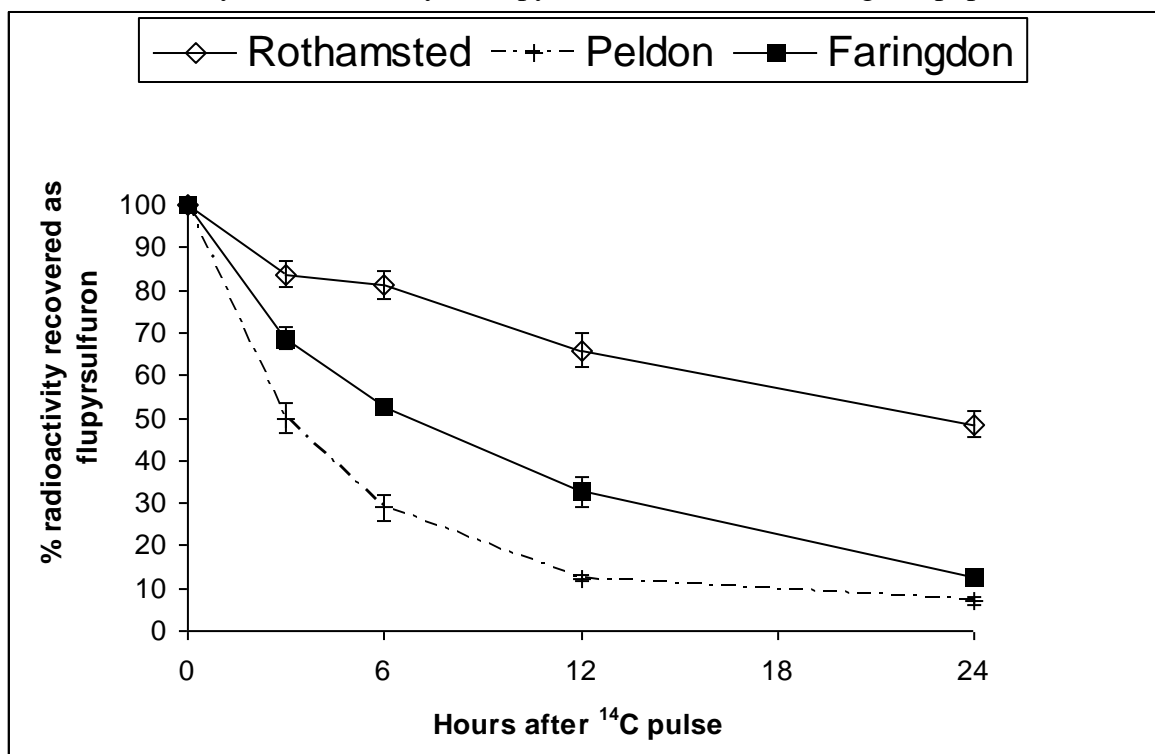
Glasshouse pot assays proved the most robust test system and the use of fenoxaprop (55 g a.i./ha), tralkoxydim (43.75 g a.i./ha) imazamethabenz (300g a.i./ha) for wild-oats and diclofop-methyl (1.134 kg a.i./ha), tralkoxydim (87.5 g a.i./ha), cycloxydim (75 g a.i./ha) and isoproturon (1 kg a.i./ha) for Italian rye-grass provided excellent discrimination between differing degrees of resistance. Glasshouse pot tests were the most reliable method for identifying resistance to ALS inhibitors in black-grass, although a low organic matter soil must be used to avoid potentially highly misleading results. The Petri-dish method for detecting resistance in rye-grass was revised and a new protocol published (Moss, 2003). Generally results in Petri-dishes and pots were well correlated, but a small number of populations of rye-grass showed strikingly different results in the two test systems, possibly due to the presence of novel mechanisms. This is currently being investigated further in associated BBSRC funded biochemical/molecular studies.

2.3 Investigation into the biochemical basis of resistance to ALS inhibitors

Laboratory studies were conducted in black-grass using radio-labelled flupyrsulfuron donated by DuPont. Three populations were compared: Rothamsted, a susceptible standard; Faringdon (partially resistant); Peldon highly resistant. The % reductions in foliage fresh weight obtained for these three populations with flupyrsulfuron at 10 g a.i./ha in glasshouse tests have been 99%, 67% and 39% respectively, showing the quantitative differences between them.

In the laboratory studies, the % recovery of radioactivity as flupyrsulfuron over a 24 hour period declined much more rapidly in the Peldon, than in the susceptible Rothamsted population, with Faringdon intermediate (Figure 2).

Figure 2. % recovery of radioactivity as flupyrsulfuron in three black-grass populations



The proportion of radioactivity recovered in metabolites was the reverse, being highest in Peldon

and lowest in Rothamsted (data not presented). These results correlate perfectly with the responses seen at the whole plant level and demonstrate convincingly that enhanced metabolism is a mechanism of resistance to ALS inhibitors in black-grass in the UK. More recent studies, conducted by a BBSRC CASE (DuPont) PhD student at Rothamsted indicate that ALS target site resistance is also present in at least one UK population of black-grass. Current molecular studies are in progress and if confirmed, this will be the first demonstration of ALS target site resistance in black-grass. This has serious implications for herbicide resistance to ALS inhibitors in the UK.

2.4 Determine the cross-resistance patterns and biochemical basis of resistance in Italian rye-grass

Detailed studies on the biochemical basis of resistance in populations of Italian rye-grass were undertaken. These showed that enhanced metabolism of diclofop-methyl existed in three populations from Essex, Lincolnshire and Wiltshire but ACCase target site resistance occurred in a population in Yorkshire. These studies were written up and published by Cocker *et al.*, (2001). It was concluded that enhanced metabolism is the major mechanism of resistance in Italian rye-grass, but ACCase target site resistance also occurs.

2.5 Watching brief on potential new types of resistance in weeds

During the past five years, new forms of resistance have been investigated. ALS resistance in poppies (*Papaver rhoeas*) and chickweed (*Stellaria media*) was identified for the first time (see below). Several samples of canary grass (*Phalaris paradoxa*) and annual meadow grass (*Poa annua*) were assayed against fenoxaprop and isoproturon respectively, but none were found to be resistant. Seeds and plants from three suspected cases of glyphosate-resistant black-grass were collected and tested, but no resistance was demonstrated.

Chickweed and poppy

Sample of suspected sulfonyl-urea resistant chickweed (from Scotland and England) and poppy (from England) were tested for resistance to metsulfuron in glasshouse tests initially. ALS-resistance was confirmed in both species. A subsequent glasshouse pot experiment was conducted to look at cross-resistance patterns in chickweed. Metsulfuron (6 g a.i./ha), florasulam (5 g a.i./ha), mecoprop 1200 g a.i./ha) and fluroxypyr (200 g a.i./ha) were applied to plants grown from seed of nine populations, including a susceptible standard. Herbicides were applied when plants were up to 15 cm across and assessed for foliage fresh weight 21 days after spraying. There were 12 replicates and untreated controls for all populations.

Results – see Table 11.

- All 8 test populations, including the two from England, showed evidence of resistance to metsulfuron in comparison with the susceptible standard, which was well controlled. The FORFAR and CORNWALL samples showed lower levels of resistance than the other populations and this appeared to be due to *partial* effects on most plants rather than due to a higher proportion of susceptible individuals in the population sample.
- Two test populations (ABERDEEN and IRELAND) showed clear evidence of resistance to florasulam, whereas all other populations were controlled. (The high negative control values for these two populations are due to the later spraying and assessment of the florasulam treatments which necessitated calculation of % reduction values relative to the earlier harvested untreated pots).
- All populations were well controlled by mecoprop, although there was some evidence of a low level of insensitivity in the ABERDEEN, IRELAND, ARBROATH and KENT populations (the last two almost rating R?).

Table 11. Results of single dose glasshouse screen of nine chickweed populations

Population	% reduction in foliage weight (and R ratings - see note)							
	Metsulfuron 6 g a.i./ha		Florasulam 5 g a.i./ha		Mecoprop-P 1200 g a.i./ha		Fluroxypyr 200 g a.i./ha	
WE 97 (susceptible)	96	S	94	S	96	S	99	S
SCOT 2001 g/h	-19	RRR	96	S	99	S	97	S
SCOT 2001 field	-18	RRR	86	S	97	S	87	R?
ABERDEEN 2001	2	RRR	-55	RRR	81	R?	71	RR
ARBROATH 2001	-3	RRR	83	R?	88	S	80	R?
FORFAR 2001	61	RR	89	S	92	S	90	S
KENT 2001	8	RRR	86	S	88	S	83	R?
CORNWALL 2001	50	RR	92	S	96	S	90	S
IRELAND 1996?	12	RRR	-34	RRR	84	R?	72	RR
S.E. ±	7.9		3.8		1.5		2.1	

Note: R ratings (RRR, RR, R? and S) have been assigned in the same manner as for grass weeds to highlight differing degrees of resistance.

- All populations were well controlled by fluroxypyr, although there was some evidence of a low level of insensitivity in the ABERDEEN, IRELAND, ARBROATH, KENT and SCOT 2001 field populations (all rated R? or RR). Interestingly four of these also showed a low level of insensitivity to mecoprop.
- The R rating tend to exaggerate perceived resistance as all plants of all populations were severely affected by fluroxypyr and, as with mecoprop, virtually all plant weights were less than 25% of the untreated means for the same population.
- It is especially noteworthy that the only two populations which showed resistance to florasulam (ABERDEEN and IRELAND), were the same two populations which showed the greatest (albeit marginal) insensitivity to fluroxypyr *and* to mecoprop. This was an unexpected result - but unlikely to be a chance occurrence.
- The relationship between resistance to different ALS inhibitors and insensitivity to herbicides with different modes of action (such as mecoprop and fluroxypyr) needs further investigation as some unexpected relationships were identified in this study.
- These results confirmed the presence of ALS resistant chickweed in England, and confirm that it not confined to the single resistant site identified in Scotland in 2000. Resistance in the SCOT site has been shown to be due to target site resistance (insensitive ALS) and it is probable that this is also the main mechanism of resistance in the other resistant populations.
- This study also showed for the first time that some, but not all, metsulfuron (sulfonylurea) resistant populations in both Scotland and Ireland (ABERDEEN and IRELAND), are also resistant to florasulam (a triazolopyrimidine). This is probably due to a different target site mutation in these populations.

Objective 3. To determine the influence of cultural factors on selection pressure for resistance.

3.1 Fitness experiments

Any fitness penalty associated with resistance will potentially reduce the rate of increase of resistance. How significant this factor is, will depend largely on the degree of any fitness penalty. It is important that studies include a range of populations, as simply comparing a single susceptible with a single resistant population may simply highlight chance differences not specifically associated with resistance. Consequently, “fitness” studies were conducted on a range of resistant black-grass and wild-oat populations.

3.1.1 Black-grass experiments

Glasshouse studies were undertaken using four black-grass populations: Rothamsted and LARS which are both susceptible, and the Peldon (enhanced metabolism) and Notts A1 (ACCase target site) resistant populations. Replacement series competition studies were conducted between all six combinations of pairs of populations. Populations were also grown individually.

All plants when grown in competition were significantly smaller and produced fewer tillers than when grown without competition. With one exception, there were no significant differences in number of tillers produced or foliage weight by any population when grown in competition with one another, in any combination. The exception was Notts A1 which was significantly less competitive when grown in combination with the LARS susceptible population. However, there was no significant difference when Notts A1 was grown in combination with the other susceptible standard, Rothamsted. Consequently there was no consistent fitness penalty associated with the herbicide resistant populations studied, that could be detected in these experiments.

3.1.2 Wild-oat experiments

Glasshouse studies were undertaken using three wild-oat populations: LLUD (susceptible), T/11 (enhanced metabolism) and T/41 (ACCase target site) resistant populations. Replacement series competition studies were conducted between all three combinations of pairs of populations. Populations were also grown individually.

There were no significant differences in number of tillers produced or foliage weight by any population when grown in competition with one another, in any combination. Consequently there was no consistent fitness penalty associated with the herbicide resistant populations studied, that could be detected in these experiments.

3.2 Deselection experiments with black-grass

An important aspect of long-term resistance management is whether any deselection, or loss of resistance occurs, in the absence of selection by herbicide. This could potentially happen in situations where herbicides ceased to be used, or more practically, where herbicides were used which were selection neutral, being equally active on resistant and susceptible plants. Consequently studies were undertaken to investigate this aspect using populations with known resistance mechanisms.

3.2.1 Container experiments.

Two populations of black-grass were used. Peldon, is known to exhibit enhanced metabolism, but not ACCase target site resistance. In contrast, Notts A1, possesses a high degree of ACCase target site resistance, but no enhanced metabolism. Seeds were sown with wheat in separate outdoor containers in autumn 2000, using the methodology as described in section 1.2 above. No herbicides were applied and seeds collected each year were resown into containers the following autumn for three successive years. About 150 black-grass and 15 wheat plants were present in each of two containers per population each year. Seeds collected in summer 2003 were evaluated in dose response assays to determine the degree of change in resistance.

3.2.1.1 Peldon deselection. The response of the Peldon baseline population (actually collected from the field in 1996), Peldon 2001 (1 yr deselection), Peldon 2003 (3 yr deselection) and a susceptible standard (Roth 2000) were compared in a glasshouse pot assay. Eight doses within the range 0.1094 – 56 kg chlorotoluron/ha and 3.438 – 880 g fenoxaprop-P-ethyl/ha were applied to plants at the 3 leaf stage growing in individual 5 cm pots with 14 replicate pots per dose. Foliage fresh weight was recorded 22-26 days post spraying as a measure of herbicide efficacy. The dose response curves are shown in Figures 3 & 4.

Figure 3. Deselection for resistance to chlorotoluron in the Peldon population: glasshouse dose response assay

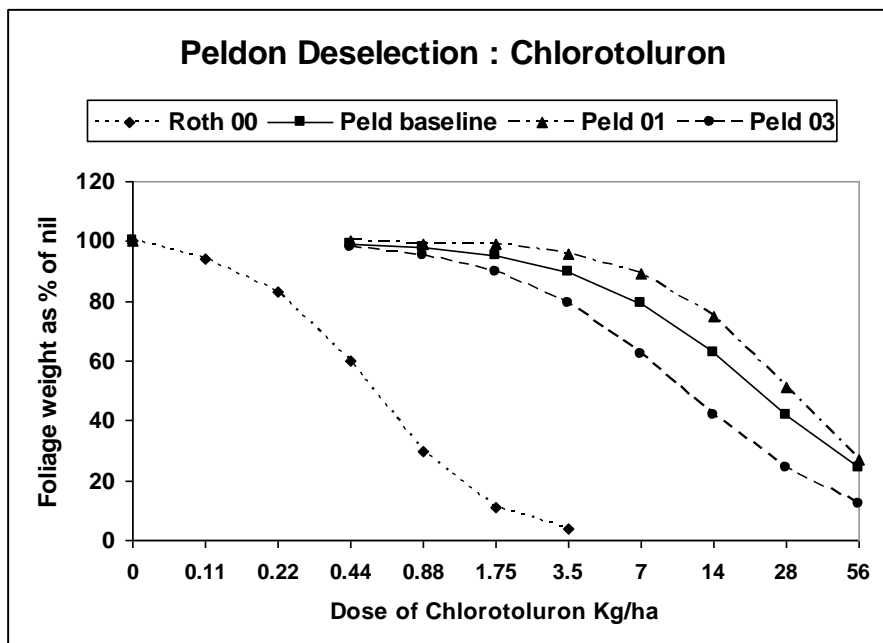
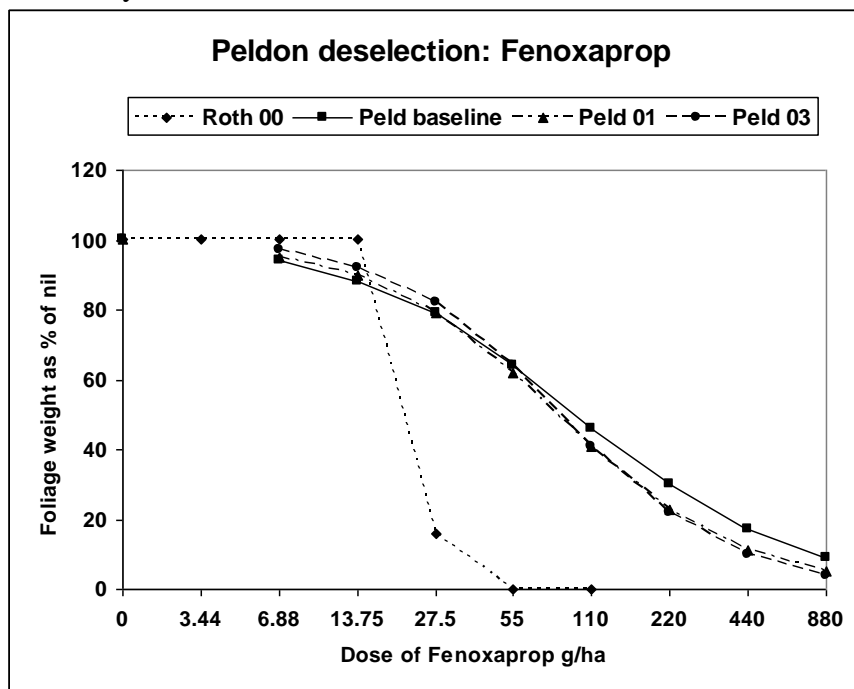


Figure 4. Deselection for resistance to fenoxaprop in the Peldon population: glasshouse dose response assay



Chlorotoluron – Figure 3

- The ED₅₀ value for the Rothamsted susceptible standard was 0.54 kg chlorotoluron/ha, and 21.11 kg/ha for the Peldon baseline population, which showed very high resistance (Resistance index (RI) = 39).
- Three years deselection in outdoor containers resulted in a significant ($P \leq 0.05$) reduction (about 50%) in the degree of resistance (Peld03 ED₅₀ value = 10.57 kg a.i./ha, giving a RI of 19). However the population was still highly resistant bearing in mind that maximum field rate of chlorotoluron is 3.5 kg a.i./ha. The apparent increase after 1 year of

deselection (Peld01 ED₅₀ value = 28.72 kg a.i./ha giving a RI of 53) was not statistically significant.

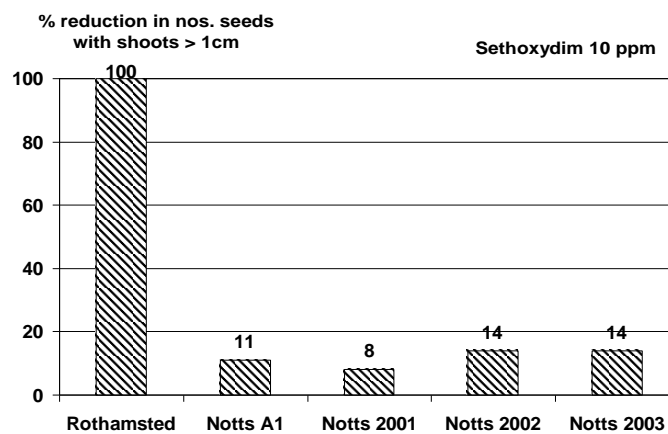
- Consequently this experiment indicates that there can be some degree of deselection for resistance to chlorotoluron in the absence of herbicide use, but the degree of deselection is relatively modest and of limited significance on an agronomically relevant timescale.

Fenoxaprop – Figure 4

- The ED₅₀ value for the Rothamsted susceptible standard was 26.1 g fenoxaprop/ha, and 95.8 g/ha for the Peldon baseline population, which showed resistance to fenoxaprop (RI = 3.7), but to a much lower degree than chlorotoluron. This supports previous findings with this population.
- In contrast to the chlorotoluron results, three years deselection in outdoor containers resulted in no significant reductions in the degree of resistance to fenoxaprop (Peld 01/03 ED₅₀ values = 81.7/84.5 g a.i./ha, giving RIs of 3.1/3.2) There was a trend for reducing resistance with RI declining from 3.7 to 3.2 over 3 years, but this was very slight.
- Consequently this experiment indicates no change in the degree of resistance to fenoxaprop over a 3 year period, with very similar dose response curves. The population was as resistant to fenoxaprop after three years of no herbicide use as at the outset.
- These differences between herbicides may reflect the involvement of different forms (P450 v GST) of enhanced metabolism.

3.2.1.2 Notts deselection. The response of the Notts A1 baseline population (actually collected from the field in 1993), Notts 01 (1 yr deselection), Notts 02 (2 yrs deselection), Notts 03 (3 yrs deselection) and a susceptible standard (Roth 2000) were compared in a Petri-dish assay. A single concentration of sethoxydim (10 ppm) was used and there were 6 replicate treated dishes and 2 untreated dishes per population. The number of shoots > 1 cm was assessed after two weeks as a measure of resistance and % reductions relative to untreated dishes calculated.

Figure 5. Deselection for resistance to sethoxydim (indicator for ACCase target site resistance) in the Notts. population: Petri-dish assay



- The Rothamsted susceptible standard was well controlled (100%)
- All the Notts populations were poorly controlled (8 – 14%), indicating a high level of ACCase target site resistance in all populations.
- There was a very slight indication of an increase in % reduction between the baseline figure (11%) and the 2002 and 2003 values (both 14%). However this trend was very slight and certainly not statistically significant (S.E.± 3.18).
- Consequently this experiment indicates no change in the degree of ACCase target site

resistance to over a 3 year period. If there had been any deselection, there should have been increases in the % reductions, but none were evident. The population was as resistant to sethoxydim after three years of no herbicide use as at the outset, indicating no change in ACCase target site resistance.

3.2.2 Deselection (and selection) for resistance to isoproturon in Faringdon and Islip populations.

Black-grass samples from the Rothamsted container experiments in Section 1.2 above were used to investigate whether deselection occurs in the absence of use of isoproturon. For comparative purposes, resistance selection following the annual use of isoproturon was also studied. The samples used were: Faringdon and Islip 2000 baseline samples; Faringdon and Islip 2004 Nil samples which had been grown on for 4 years without any herbicide application; Faringdon and Islip 2003 samples which had been treated annually with isoproturon (1.5 kg a.i./ha) for 3 successive years; Rothamsted 2000 susceptible standard. A glasshouse dose response experiment was conducted in which nine doses within the range 0.0393 – 5 kg isoproturon/ha were applied to plants at the 3 leaf stage growing in individual 5 cm pots with 16 replicate pots per dose. Foliage fresh weight was recorded 19-22 days post spraying as a measure of herbicide efficacy.

Table 12. Deselection, and selection, for resistance to isoproturon in the Faringdon and Islip populations in dose response assay using seeds from container experiments

Isoproturon g a.i./ha (but based on kg data)

Population	Log ₁₀ ED ₅₀ values	ED ₅₀ values (g/ha) (RI in brackets – relative to baseline)
Rothamsted 2000 (susceptible standard)	2.090	123
Faringdon 2000 (baseline population)	2.135	137 (1.0) (1.1 x Roth)
Faringdon 2003 IPU (3 yrs selection)	2.539	346 (2.5) (2.8 x Roth)
Faringdon 2004 Nil (4 years deselection)	2.053	113 (0.8) (0.9 x Roth)
Combined S.E. ±	0.0583	
Rothamsted 2000 (susceptible standard)	2.090	123
Islip 2000 (baseline population)	2.482	303 (1.0) (2.5 x Roth)
Islip 2003 IPU (3 yrs selection)	2.770	589 (1.9) (4.8 x Roth)
Islip 2004 Nil (4 years deselection)	2.168	147 (0.5) (1.2 x Roth)
Combined S.E. ±	0.0568	

- Deselection for 4 years (no herbicide applied) resulted in a loss of resistance in both populations. (Resistance Indices (RIs) relative to the *original* populations: Faringdon = 0.8; Islip = 0.5). This change was significant with the Islip population but not with Faringdon.
- Selection with isoproturon applied for 3 successive years significantly ($P \leq 0.05$) increased resistance in both populations by a modest amount (RIs relative to the *original* populations: Faringdon = 2.5; Islip = 1.9).
- The actual levels of resistance recorded were relatively low in terms of RIs. This was expected as resistance to isoproturon has been partial in all populations so far tested, because enhanced metabolism appears to be the only mechanism responsible. Some farmers have commented that isoproturon “works better” after a period of years when it has not been used. These results support this comment, although it must be stressed that

in this experiment, no herbicides at all were used during the deselection phase. In most field situations, other herbicides would be used (e.g. flupyr-sulfuron) and it has been assumed that virtually all herbicides select for enhanced metabolism to some degree. What is not clear is to what extent herbicides select for enhanced metabolism that is specific to the applied herbicide's mode of action.

- These results show that selection *and* deselection for resistance to isoproturon can occur within a 3-4 year period. The impact of these findings on the field efficacy of isoproturon is harder to predict, and needs to be addressed.

3.2.3 Deselection in the Faringdon and Islip populations to flupyr-sulfuron and clodinafop.

Black-grass samples from containers from both sites (see previous section and Section 1.2) which had been grown for four successive years without any herbicide application were used in a dose response assay, along with the baseline populations from 2000 and a susceptible standard (Rothamsted 2000). Glasshouse dose response experiments were conducted in which eight doses within the range 1.25 – 160 g flupyr-sulfuron/ha (Faringdon) and 1.88 – 240 g clodinafop/ha (Islip) were applied to plants at the 3 leaf stage growing in individual 5 cm pots with 12 replicate pots per dose. Foliage fresh weight was recorded 21-23 days post spraying as a measure of herbicide efficacy.

- Pooled over both sites, there was a small reduction in ED₅₀ value with the Faringdon population untreated for 4 successive years, relative to the baseline population (ED₅₀ 1.40 v 1.87 g flupyr-sulfuron/ha, RI = 0.7). This difference was not statistically significant. Consequently there was no clear evidence for deselection to flupyr-sulfuron over the 4-year period. The precision of this assay was poorer than that for clodinafop as the dose range used was not ideal – lower dose should have been included.
- Pooled over both sites, there was no reduction in ED₅₀ value with the Islip population untreated for 4 successive years, relative to the baseline population (ED₅₀ 14.5 v 12.7 g clodinafop/ha, RI = 1.1). This difference was not statistically significant. Consequently there was no evidence for deselection to clodinafop over the 4-year period.

3.3 Effect of cultivation system on rate of development of resistance in black-grass

Cultural measures are an essential part of any resistance management strategy as they can reduce the overall weed population, and hence reduce the requirement for herbicide. This may reduce the rate of development of resistance by reducing selection pressure. However, unless cultural measures act differentially on resistant and susceptible individuals, they will not in themselves influence the selection process (they may affect the total population size, but not the proportion of resistant:susceptible plants). To investigate the effect of cultivations on this process, an experiment in outdoor containers was conducted.

3.3.1 Container experiment.

This was conducted over three years (2000-2003) with two populations of black-grass, Faringdon and Islip (see Section 1.2 above for details of these populations). Cultivations (ploughing v non-inversion tillage) were simulated each autumn by: either resowing seed comprising 90% collected from the same treatment that summer plus 10% original baseline seeds (simulating non-inversion tillage); or with 10% seed collected from the same treatment that summer plus 90% original baseline seeds (simulating ploughing). Herbicides were applied each year: clodinafop (30 g a.i./ha + oil) to the Islip population, and flupyr-sulfuron (10 g a.i./ha) to the Faringdon population.

Methodology otherwise was same as in Section 1.2. At the end of yr 3, seed samples collected in summer 2003 were evaluated in a glasshouse dose response assays to determine the degree of change in resistance to clodinafop and flupyr sulfuron compared with the original populations. A susceptible standard (Rothamsted 2000) was also included. Eight doses in the range 0.3125 - 160 g flupyr sulfuron/ha and 0.9375 – 960 g clodinafop/ha + oil and were applied to plants growing in individual 5 cm pots with 16 replicate pots per dose. Foliage fresh weight was recorded 22-26 days post spraying as a measure of herbicide efficacy. There were also untreated controls.

Table 13. Response to flupyr sulfuron in the Faringdon and clodinafop in the Islip populations in relation to different simulated cultivation systems conducted for 3 years. Results from glasshouse dose response assay conducted on plants grown from seeds collected from containers in summer 2003 after three years of annual selection.

Population...	Faringdon		Islip	
Herbicide...	Flupyr sulfuron		Clodinafop	
	Log ₁₀ ED ₅₀	ED ₅₀ g/ha (Ratio to Baseline)*	Log ₁₀ ED ₅₀	ED ₅₀ g/ha (Ratio to Baseline)*
<i>Roth 2000 (S standard)</i>	- 0505	0.312 (-)	0.742	5.5 (-)
Baseline population (2000)	- 0.223	0.598 (1.0)	1.494	31.2 (1.0)
Plough (end of yr 3 - 2003)	0.253	1.791 (3.0)	2.717	521.5 (16.7)
Shallow cults. (end of yr 3 - 2003)	0.252	1.788 (3.0)	2.760	575.3 (18.4)
Combined S.E. ±	0.1213	-	0.143	-

* = Resistance index (RI) in brackets, which is the ratio of ED₅₀ values relative to the baseline population, *not* the susceptible standard.

Faringdon result.

- Faringdon Plough and Faringdon Shallow cults. were both significantly more resistant to flupyr sulfuron than the Faringdon baseline population (Table 13). However, there was no difference between the two cultivations. The RI values (both 3.0) showed that the change in degree of resistance over the 3 years was far lower than for the equivalent changes with clodinafop in the Islip population (see next section) where RI values = 16.7 – 18.4.
- In terms of the annual plant counts done on the outdoor containers, there was not a consistent difference between cultivations in level of control of plants. For Faringdon Plough and Faringdon Shallow cults. respectively, % reduction in plant numbers for the 3 years were: yr 1 40%/40%; yr 2 52%/42%; yr 3 14%/-6%. The glasshouse studies support the view that there was little difference between cultivations, although there was evidence that resistance reduced the activity of flupyr sulfuron to a greater extent in the third year.
- Overall the results indicate that selection with flupyr sulfuron does increase resistance in the Faringdon population, but by a relatively modest amount. The increase is most likely to be due to increasing levels of enhanced metabolism in contrast to increasing levels of ACCase target site resistance with Islip/clodinafop. The relatively modest increases in resistance to flupyr sulfuron seemed to mask any effects that cultivations may have imposed.

Islip results

- Islip Plough and Islip Shallow cults. both showed very high, and similar levels of resistance to clodinafop (RI = 16.7 and 18.4) (Table 13). There was no significant difference between the two cultivations. The vigour scores on plants back this up: For Islip Plough and Islip Shallow cults. respectively, there were 39/48 and 41/48 plants rated in categories 1 & 2 (unaffected/slightly affected) for the three doses, 120 – 480 g a.i./ha.
- The glasshouse dose response results contrast with the annual plant counts done on the

containers which showed a *substantial* difference in clodinafop efficacy between cultivations. For Islip Plough and Islip Shallow respectively, % reduction in plant numbers for the 3 years were: yr 1 90%/90%; yr 2 85%/42%; yr 3 64%/4% (S.E. \pm 5.44). This apparent contradiction can be partly explained by the fact that glasshouse resistance tests were conducted on seed samples which take no account of how many resistant plants were present. Thus it appears that there was very high selection for resistance even in the Plough samples which were 90% baseline population (Islip 2000) each year, but little additional selection after the first year. However, this does not fully explain the progressive decline in control of plants on the Shallow cults. treatments, which indicate a *steady* increase in resistance over the three years.

- There was a big difference in the response of Islip, as compared with Faringdon. In Faringdon the modest increase in resistance to flupyr-sulfuron was most likely due to increasing levels of enhanced metabolism. In contrast, the much greater increase in resistance to clodinafop with the Islip population, was probably due to increasing levels of ACCase target site resistance. The relative increases were very similar to those recorded in the other container experiments detailed in Section 1.2.
- Overall the results do highlight that in assessing the impact of cultivations and resistance, two aspects must be considered. Firstly, the effects cultivations have on the weed infestation (plant population) and secondly, their effects on the degree of resistance within the population (proportion of resistant individuals). Thus different cultivations/herbicides could result in a high weed infestation with a low proportion of resistant plants, or conversely, a low weed infestation with a high proportion of resistant plants.

Objective 4. To integrate the new information into a decision model framework, to allow resistance risks to be better evaluated.

4.1 Decision model framework

An outline decision model framework for the production of herbicide resistance risk profiles was devised in conjunction with Alice Milne at Silsoe for incorporation into Weed Manager (WMSS), (Sustainable LINK Ref. LK0916). It now forms the herbicide resistance module within the latest version of WMSS. See www.wmss.net. The computer interface for this module is shown in Figure 6.

It encompasses the following criteria: herbicide mode of action, weed species, mechanism of resistance, herbicide mixtures/sequences, crop rotation, cultivations, infestation level. The model has the potential to be extended to include other parameters if necessary. The model framework estimates an overall risk value based on a historical evaluation of the past five cropping years. The risk factors are largely based on the results of the HeRMES project.

Five species of weeds are considered in the system for potential resistance to herbicides. These are black-grass, wild-oats, Italian rye-grass, chickweed and poppy. The three grass-weeds can be inputted by the user as susceptible, or as exhibiting metabolic resistance, ACCase target site resistance or mixed resistance (exhibiting both mechanisms). Chickweed and poppy are inputted as either susceptible or resistant (ALS target site). The system calculates risk of developing/increasing target site and/or metabolic resistance. Time of sowing, cultivation type, and size of weed population are assumed to affect the risk of resistance. These factors are incorporated into the system by scaling the risk value.

Figure 6. The herbicide resistance module screen in WMSS (Weed Management Support System)

Likelihood of resistance

Resistance test results
 Weed name: black-grass Last tested negative to resistance: Not tested

Field data
 Season: 2000/2001 2001/2002 2002/2003 2003/2004 2004/2005
 Cropping: Winter Winter Spring Winter Winter
 Cultivation: Inversion Non-inversion Inversion Non-inversion Inversion

Herbicide HRAC group

Group	2000/2001	2001/2002	2002/2003	2003/2004	2004/2005
Group A	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Group B targeting broad-leaved and grass	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Group B targeting broad-leaved only	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
None	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Risk of resistance

Weed name	Enhanced metabolic resistance	Target site resistance
black-grass	Medium	Medium
poppy		Low

Unfortunately you have not provided sufficient details of previous herbicide use to make an assessment - the estimates given above are therefore based on worst case.

OK Cancel

4.2 Resistance risk profiles for different weed/herbicide/cultural scenarios

One required output of the HeRMES project was to integrate the research results into a decision making tool for farmer stakeholders. In summer 2004, a technical fact sheet was produced which was published by Crops magazine with funding from DEFRA and PSD.

This fact sheet ('Herbicide-resistant grass-weeds: are you at risk?') took the form of a herbicide resistance audit in which farmers and advisors could assess the herbicide resistance risk factors for individual fields by considering six cultural (e.g. crop rotation, cultivation system) and six chemical (e.g. frequency of use of herbicides, use of different modes of action) factors. Each factor could be scored as low, medium or high risk according to descriptions given on the fact sheet. A total score could then be calculated indicating what the overall herbicide resistance risk was for individual fields. Summarised advice on how the resistance risk imposed by the current cultural/herbicide system could be reduced, and reference to the latest WRAG Guidelines, was also presented.

30,000 copies of the fact sheet were printed and over 25,000 circulated in 'Crops' magazine and an electronic version (Figure 7) has also been made available on the UK Weed Resistance Action Group's website hosted by PSD (<http://www.pesticides.gov.uk/rags.asp?id=714>).

Figure 7. A screen shot of the online version of the 'Herbicide-resistant grass-weeds: are you at

risk' technical fact sheet.

http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/RAGs/22june04_Resistance_Fact_8_QXD.pdf - Microsoft Internet Explorer

Address http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/RAGs/22june04_Resistance_Fact_8_QXD.pdf

68%

YOUR HERBICIDE RESISTANCE AUDIT *Herbicide Resistance: What's your rating?*

- Photocopy this sheet, before completing, if you wish to use the audit on one more than one field or part of the farm
- Decide whether the descriptions low, medium or high best suit the field history for each of the individual risk factors
- Write the relevant score (1, 3 & 5) for each risk factor in the right hand column (use the intermediary values 2 and 4 if necessary)
- Calculate the sub-totals for cultural and chemical control and add them together for the final score to find out how big a risk herbicide resistance is on your farm

Risk Factor (Score)	Low (1)	Medium (3)	High (5)	Your score
Cultural				
1 Crop rotation	More than 3 different crops in the rotation	2-3 different crops in the rotation	Single crop - no rotation	
2 Spring sown crops/set-aside	Comprise more than 25% of crops in the rotation	Comprise 25% or less of crops in the rotation	None	
3 Cultivation system	Plough used more than non-inversion tillage	Non-inversion tillage and ploughing used to about the same extent	Non-inversion tillage used more than ploughing	
4 Autumn cereal drilling date	After mid October	Mid September-mid October	Before mid September	
5 Other cultural control methods (see other side)	Many	Some	None	
6 Weed infestation	Low	Medium	High	
				Sub-total (A) =
Chemical				
7 Grassweed herbicides used	Less than 1 per year	1-2 per year	3 or more per year	
8 Fops' and 'dims' (see other side)	Used less than 1 in every 3 years	Used 1 to 2 times in every 3 years	Used every year	
9 Grassweed sulfonylureas (SUs)	Used less than 1 in every 3 years	Used 1 to 2 times in every 3 years	Used every year	
10 Other modes of action effective against grass weeds comprise	More than 50% of your herbicide programme	About 50% of your herbicide programme	Less than 50% of your herbicide programme	
11 General herbicide efficacy	Good	Mediocre/variable	Poor	
12 Resistance in locality	None	Some	Common in similar cropping systems	
				Sub-total (B) =
				Grand Total (A+B) =

Max grand total score = 12 x 5 = 60; Minimum score = 12 x 1 = 12
 Interpretation of herbicide resistance risk score: 12-24 = Low; 25-48 = Medium; 49-60 = High

● Aim to reduce the risk of herbicide resistance imposed by both your cultural system and your current herbicide strategy
 ● Consult the Weed Resistance Action Group's Resistance Guidelines

Weed Resistance Action Group's Guidelines
 Copies are available from: WERA, Calderian House, 213 Parkside Road, London N11 9HF. Tel: 020 7502 3020. E-mail: publications@wera.com
 An online version (pdf) is available on the Weed Resistance Action Group's website: <http://www.pesticides.gov.uk/rags/wera/71674>
 This site also has a pdf version of the risk audit document.

July 2004

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4.3 Validation of resistance risk profiles

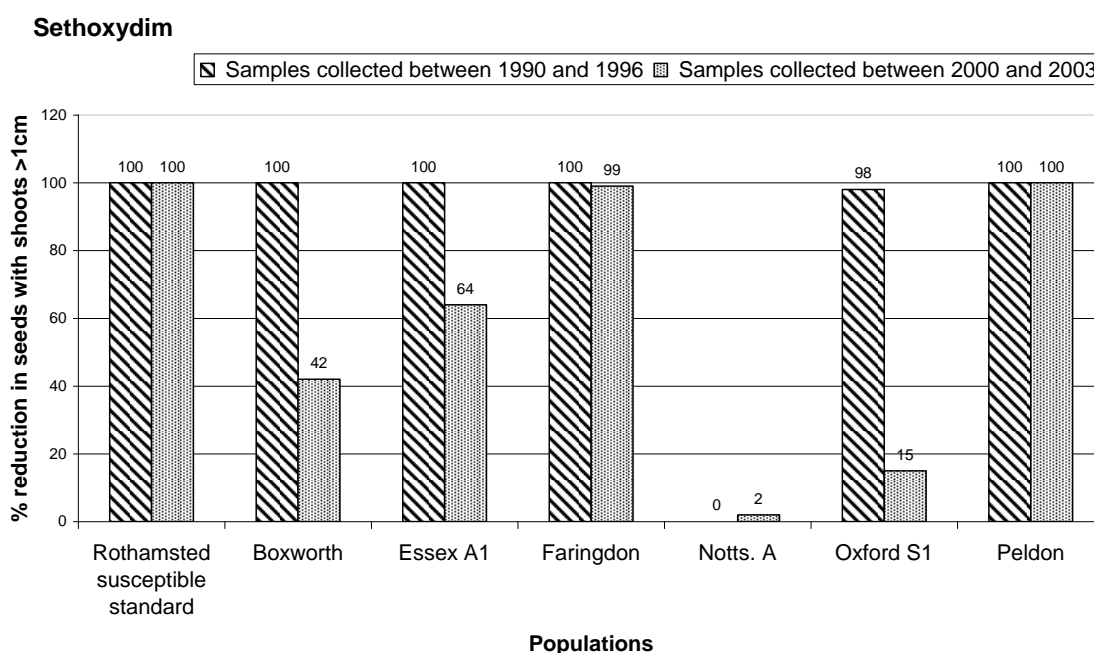
To put the results of the research conducted within HeRMES into a broader perspective, re-sampling and testing of black-grass seed samples collected from farms where resistant grass-weeds had previously been identified was undertaken. Field histories were also obtained so that predicted changes in resistance level could be compared with actual changes in relation to herbicide/cultural practices.

Six fields originally sampled between 1990 and 1996 were revisited in 2000 or 2003 and black-grass samples collected from the same areas. There was a 5 – 13 year time span between samplings, with a mean of 8 years. The original and new samples were tested for resistance to sethoxydim (10 ppm) in either Petri-dish tests (for ACCase fop/dim target site resistance (=TSR)) or in glasshouse pot screening experiments for resistance to fenoxaprop (68.75 g a.i./ha), chlorotoluron (2.75 kg a.i./ha) and flupyrsulfuron (20 g a.i./ha). A susceptible standard (Rothamsted 2000 & 2003 seed) was included in all tests.

Herbicide histories were also obtained for each field and in summary: winter cereals were grown in 40 – 100% crop years (mean = 62%); ploughing in 0 – 100% years (mean 50%); number of grass-weed herbicide active ingredients applied was 1.7 – 4.4 per year (mean= 2.7). Of all grass-weed active ingredients applied, fops/dims comprised 8 – 38% (mean = 24%), sulfonylureas 0 – 14% (mean = 7%), dinitroanilines 17 – 32% (mean 25%), ureas 5 – 25% (mean 15%), others 13 – 38% (mean 29%).

Sethoxydim (ACCase target site resistance)

Figure 8. Petri-dish results using sethoxydim as an indicator of ACCase TSR. (S.E. ± = 7.2)



- The Rothamsted susceptible standards were well controlled as expected.
- For the 1990's samples, high levels of control were obtained with all populations except Notts A, indicating that only this field had a high level of TSR. Oxford S1 had a marginal level of TSR in 1993 (2%).
- In the 2000/03 samples appreciably lower levels of control were achieved with the Essex A1, Boxworth and Oxford S1 populations indicating that TSR had increased. Peldon showed no TSR, Faringdon showed marginal TSR (1%) while the Notts A population was still highly resistant.
- In the 1990's, only one out of the six populations (Notts A) had a high level of TSR, but by 2000-2003, four out of the six populations showed clear evidence of TSR.
- These sites were originally sampled because resistance (not specifically TSR) was suspected, so they were not randomly chosen. However, if they are representative of other fields, these results indicate that TSR has increased substantially during the past 10 years, which supports the results of other surveys.
- There was a correlation between use of fops and dims and increase in TSR

ACCase (fop/dim) TSR

	Increase in ACCase TSR (Petri-dish assay)	Fop/dim use as % of all herbicides applied between sampling dates
Oxford S1	85%	24%
Boxworth	58%	38%
Essex A1	36%	31%
Peldon	0%	19%
Faringdon	0%	8%
(Notts. A)	(-3%)	(24%)

- Generally the higher the proportion of fops/dims used in relation to all herbicides, the greater the increase in TSR recorded. The very high increase at Oxford S1 was probably a consequence of the initial low level of TSR already detectable in 1993 and the use of a fop and a dim in 2003, the year of seed collection. The use of fops and dims maintained a very high level of TSR in the Notts A population.

Fenoxaprop (S.E. ± 6.6 for all comparisons)

- The Rothamsted susceptible standards were well controlled (87 – 90%) as expected.
- For the 1990's samples a good level of control was obtained with the Boxworth population only (83%), and mediocre control of Faringdon (62%) and Essex A1 (42%). There was virtually no control of the other three populations (0 – 9%).
- In the 2000/03 samples, control of all six sampled populations was extremely poor (0 – 7%).
- Resistance had either increased substantially between the 1990's and 2000/2003 (Faringdon, Essex A1, Boxworth), or had been maintained at a high level (Peldon, Oxford S1, Notts A).
- Fenoxaprop's efficacy is affected by both enhanced metabolism (EM) and TSR. For this reason it is difficult to relate changes in fenoxaprop's activity to the use of any specific type of herbicide.

Flupyrulfuron (*S.E. ± 5.8 for all comparisons*)

- The Rothamsted susceptible standards were well controlled as expected (85 – 89%).
- For the 1990's samples, a high level of control (similar to the susceptible standard) was obtained with the Essex A1 (86%), Boxworth (86%) and Notts A (87%) populations. There was mediocre control of Faringdon (66%) and poor control of Peldon (21%) and Oxford S1 (27%).
- Resistance to flupyrulfuron in populations collected in the early 1990's must have been a consequence of resistance selected by other herbicides, as flupyrulfuron was not used in the UK until autumn 1997.
- In the 2000/03 samples, control of all populations was significantly poorer than the Rothamsted susceptible standards. Control of three populations was mediocre, Faringdon (72%), Boxworth (61%) and Notts A (57%), and poor for the other three, Peldon (0%), Essex A1 (14%), Oxford S1 (18%).
- Resistance had increased in five of the six populations between the 1990's and 2000/2003. This change was statistically significant in four populations (Peldon, Essex A1, Boxworth, Notts A). Faringdon was the only population where there appeared to have been no change in degree of resistance, but there was only a 5 year time span between samplings at this site.

Chlorotoluron (*S.E. ± 6.7 for all comparisons*)

- The Rothamsted susceptible standards were well controlled as expected (91 -94%).
- For the 1990's samples, a high level of control (similar to the susceptible standard) was obtained with the Boxworth (90%) and Notts A (87%) populations. There was mediocre control of Faringdon (61%) and Essex A1 (58%) and poor control of Peldon (0%) and Oxford S1 (29%).
- In the 2000/03 samples, control of only one population (Essex A1) had declined significantly (to 17%) compared with the 1990's results. This population also showed the greatest change in resistance to flupyrulfuron. Smaller, non-significant reductions had occurred at three other sites (Faringdon (to 55%), Boxworth (81%), Notts A (74%). The moderate to high levels of resistance at Peldon and Oxford S1 recorded in the 1990's had been maintained, with little or no change.
- Resistance to chlorotoluron varied considerably between sites in the 1990's, and this level had generally not changed appreciably by 2000-2003, except at Essex A1 where there was a 13 year time span between samplings (the greatest for any site). High resistance to chlorotoluron was first detected at Peldon in 1984, and that situation still exists. At four of the five other sites changes, albeit often small, were always towards poorer control.

Collating the results – bringing it all together

The HeRMES project involved a wide range of experiments at laboratory, glasshouse and field level. The aim of this section is to summarise the main findings and then comment on their implications.

Black-grass

1. The field experiments showed that there was a correlation between the use of ACCase inhibitors and the level of ACCase target site resistance as measured by response to sethoxydim. The greater the use of ACCase inhibiting herbicides, the greater the level of ACCase target site resistance.
2. The most widely promoted mitigation strategy, the use of mixtures and sequences of ACCase inhibitors in combination with herbicides with other modes of action, did not prevent an increase in ACCase target site resistance, but did tend to reduce the increase relative to use of ACCase inhibitors alone.
3. The reductions in the increase in resistance achieved by mixtures and sequences were relatively modest, and certainly could not be considered as a resistance prevention strategy. Consequently, the main implication is that, while mixtures and sequences have a useful role in mitigation strategies, this is likely to have relatively short term benefits and they should not be considered a sustainable solution in the longer term.
4. In container studies, resistance to both clodinafop and flupyr-sulfuron increased over the three years. Resistance to clodinafop increased more rapidly than resistance to flupyr-sulfuron, probably reflecting rapid selection for ACCase target site resistance by clodinafop and slower selection for enhanced metabolism by flupyr-sulfuron.
5. An important finding was that the value of mixtures was dependent on the herbicide under evaluation. Mixtures of clodinafop+flupyr-sulfuron had not reduced the otherwise low level of selection for resistance to flupyr-sulfuron. In contrast, mixtures of clodinafop+flupyr-sulfuron had substantially reduced the otherwise high rate of selection to clodinafop. The results with mixtures were a reflection of the interaction between the herbicide and resistance mechanisms.
6. The container studies supported the findings of the field experiments. Mixture had not prevented an increase in resistance, but in some cases had slowed the process, although not always to a degree that was reflected in adequate herbicide efficacy.
7. There was no consistent effect of low v high rates on resistance development with either flupyr-sulfuron or clodinafop.
8. Rotational use of herbicides sometimes resulted in marginally lower levels of resistance after three years, but overall showed only a modest benefit at reducing resistance development.
9. In the absence of herbicides there was little decline in ACCase target site resistance, indicating little or no fitness penalty to this form of resistance. The implication is that resistance management strategies need to be effective enough to prevent, or at least minimise, increases in ACCase target site resistance because the process cannot easily be reversed.
10. In contrast, there was evidence of a loss of resistance to urea herbicides, in the absence of selection. This was probably due to changes in degree of enhanced metabolism. However, the degree of deselection was relatively modest and probably of limited significance on an agronomically relevant timescale.
11. Mesosulfuron+iodosulfuron, (a mixture of two sulfonyleureas, ALS inhibitors), was shown to be very effective and capable of giving good control of populations resistant to both ACCase inhibiting herbicides and another sulfonyleurea, flupyr-sulfuron. However, mesosulfuron+iodosulfuron was not immune from resistance, and there were indications that its efficacy could be reduced following use of a range of other herbicides.
12. An important finding was that the use of one sulfonyleurea herbicide (flupyr-sulfuron) could

select for probable enhanced metabolism resistance to another sulfonylurea (mesosulfuron+iodosulfuron) to a greater extent than non-sulfonylureas such as isoproturon and cycloxydim, and to a lesser extent the ACCase inhibitor clodinafop. Although the differences in efficacy were relatively small and may not impact significantly on efficacy in the field in the short term, this finding has implications for the value of different herbicide mixtures and warrants further investigation.

13. Laboratory studies with flupyr-sulfuron confirmed that enhanced metabolism is a mechanism of resistance to this sulfonylurea herbicide in black-grass in the UK. A new LINK resistance project will aim to determine whether ALS target site resistance or enhanced metabolism poses the greater threat to the efficacy of this class of herbicides.
14. The validation exercise involving sampling on six farms supported the conclusion from the field and container experiments that ACCase target site resistance can increase rapidly, and that the greater the use of these herbicides, the greater the increase in resistance. The use of other modes of action in mixtures and sequences did not prevent ACCase target site resistance increasing.
15. This exercise also showed that resistance to flupyr-sulfuron appears to have increased significantly since the 1990's. This is probably due to selection by many other herbicides, (not just flupyr-sulfuron), for increasing levels of enhanced metabolism. Increased levels of enhanced metabolism, and ALS target site resistance, are likely to have serious implications not only for flupyr-sulfuron, but also for herbicides with the same mode of action (e.g. mesosulfuron+iodosulfuron).
16. The exercise also showed that a high degree of resistance to fenoxaprop is very widespread. This could be due to enhanced metabolism and/or ACCase target site resistance, possibly including forms specific to 'fops'.
17. In contrast, resistance to chlorotoluron (and *probably* isoproturon and pendimethalin too), appeared to increase quite slowly with time. Varying degrees of resistance occurred, but these were maintained at a fairly constant level in the medium term. Such herbicides appear to be 'lower risk' from a resistance development perspective. They are emphatically not 'no risk', especially in the longer term – as testified by the reductions in efficacy, albeit small, recorded at several sites.
18. Ploughing had benefits, not only at reducing weed populations by roughly half, but also by reducing the increase in ACCase target site resistance. This was probably due to the 'diluting' effect of bringing older, less selected, buried seeds back to the soil surface. However, as with mixtures and sequences, the additional use of ploughing, did not prevent an increase in ACCase target site resistance, so again is not in itself a long term solution.
19. Overall the results do highlight that in assessing the impact of cultivations and resistance, two aspects must be considered. Firstly, the effects cultivations have on the weed infestation (plant population) and secondly, their effects on the degree of resistance within the population (proportion of resistant individuals). Thus different cultivations/herbicides could result in a high weed infestation with a low proportion of resistant plants, or conversely, a low weed infestation with a high proportion of resistant plants.
20. An outline decision model framework for the production of herbicide resistance risk profiles was devised for incorporation into Weed Manager (WMSS), (Sustainable LINK Ref. LK0916). It now forms the herbicide resistance module within the latest version of WMSS. See www.wmss.net
21. The research results were integrated into a decision making tool for farmer stakeholders. A technical fact sheet ('Herbicide-resistant grass-weeds: are you at risk?') was produced which was published by Crops magazine with funding from DEFRA and PSD. This fact sheet took the form of a herbicide resistance audit in which farmers and advisors could assess the herbicide resistance risk factors for individual fields by considering six cultural (e.g. crop rotation, cultivation system) and six chemical (e.g. frequency of use of herbicides, use of different modes of action) factors.

Wild-oats

1. Container experiments showed that, as with black-grass, very rapid selection for ACCase target site resistance could occur. This was in marked contrast to the lack of selection recorded with a population with enhanced metabolism in previous experiments. The implications of this, and previous studies, are that the mechanism of resistance in wild-oats can have a huge influence on the risk of selection for greater resistance in the longer term.
2. Studies showed that early herbicide timing is of critical importance in achieving maximum control of resistant wild-oats.
3. No fitness differences were detected between resistant and susceptible wild-oat populations, regardless of whether resistance was conferred by enhanced metabolism or ACCase target site insensitivity.

Rye-grass

1. Detailed studies on the biochemical basis of resistance in populations of Italian rye-grass were undertaken. These showed that enhanced metabolism of diclofop-methyl existed in three populations from Essex, Lincolnshire and Wiltshire but ACCase target site resistance occurred in a population in Yorkshire. It was concluded that enhanced metabolism is currently the major mechanism of resistance in Italian rye-grass, but ACCase target site resistance also occurs.
2. Studies showed that early herbicide timing is of critical importance in achieving maximum control of resistant rye-grass.

Broad-leaved weeds

1. Resistance in broad-leaved weeds to ALS inhibiting herbicides was demonstrated for the first time in the UK. The relationship between resistance to different ALS inhibitors and insensitivity to herbicides with different modes of action (such as mecoprop and fluroxypyr) needs further investigation as some unexpected relationships were identified in this study.

Implications

The main implication is that mitigation strategies for ACCase target site resistance based on the use of mixtures and sequences are unlikely to be effective in the long-term. It is probable, but needs to be confirmed, that this will also be true for target site resistance to other herbicide classes sharing similar characteristics (e.g. high resistance risk). Mixtures and sequences do have short term benefits, so their use is still recommended. However, the evidence is that ACCase target site resistance has increased, despite the widespread use of mixtures and sequences. A sensible practical aim would be to limit the use of ACCase inhibitors ('fops' and 'dime') to less than 25% of all the herbicide active ingredients used over a period of years to reduce the risk of an increase in ACCase. However, on the evidence of these studies, even this more limited use would not prevent a build up of resistance.

With regards enhanced metabolism resistance, the situation is more complex. This mechanism appears to generally develop more slowly, confer partial resistance but affect a wider range of modes of action. There is some evidence of loss of resistance in the absence of herbicide use, although this is probably of limited significance on an agronomically relevant timescale. There was some evidence that different herbicide classes do not select for enhanced metabolism resistance in the same manner. Consequently the use of mixtures and sequences of as wide a range of different herbicide modes of action appears a valid means of reducing overall selection for enhanced metabolism resistance. However, it is still not clear whether there is a 'peak' level of enhanced metabolism at which little or no further increase occurs. Herbicides differ in their

vulnerability to enhanced metabolism, so it is possible that those which are less affected may still continue to give useful (if reduced) levels of control despite repeated use. However, as enhanced metabolism is so complex, clear evidence would be needed from the use of individual herbicides at many different locations to substantiate this effect.

Perhaps the main implication is to highlight the importance of monitoring the status of resistance on individual fields or farms. The highly variable nature of black-grass in particular, means that accurate predictions about the rate of development of resistance are always going to be difficult. Hence the need to monitor individual fields so that success of any resistance management strategy can be measured. This will be particularly important in relation to ALS target site resistance, and the possibility of 'double target site resistance' - ACCase + ALS target site resistance in the same population. It is also vital that cultural control measures are used in order to reduce reliance on herbicides.

Possible future work

- **Resistance to ALS inhibitors in grass-weeds.** The increasing use of herbicides with this mode of action is likely to result in new resistance problems. This has been recognised, and this topic forms the focus for a new Arable Sustainable LINK project (from 1/04/05).
- **Herbicide-resistant broad-leaved weeds.** Chickweed and poppy resistant to ALS-inhibiting herbicides were identified with variable degrees of resistance and different cross-resistance patterns. There were indications that resistance extended to other modes of action apart from ALS inhibitors. This was unexpected and further work is required to fully characterise resistant populations and assess the likely impact of resistance
- **Herbicide-resistant rye-grass.** Rye-grass is increasing as a weed of arable crops. This increase is probably driven, at least in part, by the evolution of herbicide-resistant populations. The competitive nature of this weed, the limited knowledge available on its agro-ecology and the limited number of herbicides available for controlling rye-grass are important issues in relation to dealing with resistant populations.
- **'Watching brief' for new forms of resistance.** It is important that new cases of resistance are detected at an early stage and that there is the capability to investigate new types of resistance in weeds e.g. glyphosate resistance, dinitroaniline resistance.
- **Loss of herbicide activity due to antagonism.** Antagonism between herbicides may cause reductions in efficacy that are blamed (wrongly) on resistance, and may reduce the chances of obtaining adequate control of partially resistant populations. It may also result in larger amounts of herbicides being used in order to counteract antagonistic effects. This is incompatible with pesticide minimisation policies.
- **Loss of herbicide activity due to enhanced degradation in the soil.** Enhanced microbial degradation of isoproturon in the soil can occur. No attempt has been made to determine whether this affects on herbicide performance and other residual herbicides may also be affected. Such loss of activity may be attributed to resistance, and also make it harder to achieve adequate control of partially resistant populations.
- **Regulatory issues.** Providing support to regulators in interpreting data submitted for registration purposes is important. Herbicide-resistance needs to be considered in relation to: (a) Maintaining a wide range of different modes of action. (b) Baseline monitoring and EU data requirements. (c) Monitoring after introduction of a new active ingredient to detect changes in resistance. (d) Possible withdrawal of approval for herbicides affected by resistance. (e) Environmental implications of a restricted number of effective herbicides. (f) Certification of rye-grass seed in relation to resistance contamination.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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