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Integrated Management of Herbicide Resistance

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1. ABSTRACT

Research was conducted to improve our understanding of herbicide resistance in the grass-weed, black-grass (*Alopecurus myosuroides*). Research aims were to: Quantify the effectiveness of resistance mitigation strategies; Develop robust tests for resistance to ALS (acetolactate synthase) inhibitors; Investigate sampling strategies to improve resistance detection; Quantify the impact of population dynamics; Develop more sustainable resistance management strategies for individual fields.

Target site resistance (TSR) to ALS herbicides (e.g. mesosulfuron+iodosulfuron, 'Atlantis') can build up rapidly following repeated annual use of this herbicide. Use of other modes of action in combination with ALS herbicides improved weed control, but did not reduce selection for ALS TSR. There was also evidence for development of enhanced metabolic resistance to mesosulfuron+iodosulfuron, as well as ALS TSR.

Robust and reliable resistance tests were developed. Glasshouse pot assays are more robust than Petri-dish assays, but take longer. Testing showed that resistance to ALS inhibiting herbicides occurs in at least 21 counties in England. Improved advice for farmers/agronomists on collecting representative seed samples for resistance testing was obtained; sampling from several patches improves the assessment for the whole field, but sampling from a single field can give a highly misleading representation in terms of the resistance status of the whole farm.

Resistance to ALS herbicides was shown to increase faster in minimum tillage systems compared with ploughing. ALS TSR did not decline when ALS herbicides were not used for 3 years, so there was no loss of resistance in the absence of herbicides. Modelling studies showed that: Pre-emergence herbicides can compensate, to some degree, for the declining performance of post-emergence herbicides; Modifiers in the form of alternative herbicides or non-chemical methods slowed, but did not prevent, the build-up of resistance; Non-chemical control methods are increasingly important in combating resistance by reducing the reliance on post-emergence herbicides.

Key aspects of more sustainable resistance management strategies are: greater use of non-chemical control methods; less reliance on high resistance risk post-emergence

herbicides; greater use of pre-emergence herbicides; more critical monitoring of herbicide performance in individual fields; regular testing for resistance.

2. SUMMARY

2.1 Introduction

Herbicide-resistant black-grass (*Alopecurus myosuroides*) is very widespread in the UK and has been confirmed on over 2,000 farms in 31 counties of England. Grass weed control is critically dependant on only four herbicide classes - 80% of all grass weed herbicides applied are phenylureas, dinitroanilines, ACCase (acetyl-CoA carboxylase) or ALS inhibitors.

With little prospect of new herbicides in the near future, maintaining the efficacy of existing herbicides, in the face of increasing resistance, must be a priority.

The major risk is now associated with the potential increase of herbicide resistance to ALS herbicides (includes sulfonylureas such as mesosulfuron+iodosulfuron, 'Atlantis'). Mesosulfuron+iodosulfuron is being used extensively in many countries in Europe and, in 2006, was applied to 551,000 ha in the UK making it the fifth most widely used herbicide (after glyphosate, isoproturon, pendimethalin and trifluralin). Recently, ALS (sulfonylurea) target site resistance has been confirmed in UK black-grass populations. Selection for resistance is likely to increase and the challenge is to develop sound strategies, based on good scientific principles, to minimise the risk.

Major research aims of the project were to:

- Quantify the effectiveness of resistance mitigation strategies through a better understanding of the selection pressure imposed by herbicides.
- Develop robust tests for resistance to ALS inhibitors in grass-weeds.
- Investigate and develop sampling strategies to improve resistance detection and monitoring at a local level.
- Quantify the impact on population dynamics of cultural and herbicidal mitigation strategies.
- Develop more sustainable and appropriate resistance management strategies for individual fields.

An integrated experimental programme was conducted involving laboratory, glasshouse, outdoor container and field studies utilising black-grass populations well characterised for resistance. The research was conducted under three main scientific objectives, and the relevant results are presented under each objective below.

2.2 Objective 1: To quantify the ability of resistance mitigation strategies to moderate or prevent herbicide resistance in grass-weeds, with particular emphasis on ALS inhibiting and dinitroaniline herbicides.

2.2.1 Outdoor container experiments.

The aim in two container experiments was to grow black-grass populations with known resistance status and compare selection conferred by non-ALS herbicides with ALS herbicides used alone, or in mixture, sequence or rotation, with herbicides with other modes of action. Subsequent glasshouse tests on seeds collected from surviving plants each year assessed changes in the proportion of resistant individuals.

Mesosulfuron+iodosulfuron (an ALS herbicide) selected very rapidly for ALS resistance, resulting in an appreciable and rapid loss of efficacy (annual decline rates of 19 – 23%). The use of non-ALS herbicides (e.g. clodinafop, flufenacet, isoproturon, pendimethalin) in mixture or sequence with mesosulfuron+iodosulfuron increased overall levels of weed control. However, the most significant finding was that the use of non-ALS herbicides in mixture or sequence with mesosulfuron+ iodosulfuron did *not* reduce selection for ALS resistance (Figure 2.1).

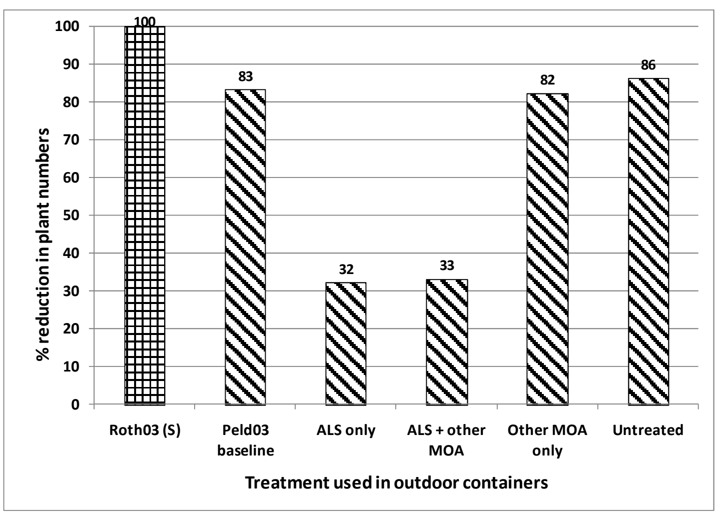


Figure 2.1 Control of black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in a glasshouse evaluation of seeds collected from outdoor containers. (Note: The ALS only, ALS+other modes of action (MOA) and Other MOA values are means of four, six and four treatments respectively).

Plants of the Roth susceptible standard were all killed confirming that this population was susceptible. Control of the Peld03 baseline, a population with about 18% individuals with ALS target site resistance, was 83% confirming that this population was partially resistant to mesosulfuron+iodosulfuron. Control of the seed samples from containers originally sown with this baseline population but treated with this herbicide alone was much poorer (32%). Where mesosulfuron+iodosulfuron was used in mixture or sequence with other modes of action (MOA) control (33%) was almost identical to where it was used alone (32%). There was no evidence that using mesosulfuron+iodosulfuron in mixture or sequence with other MOA reduced selection for ALS resistance, compared to applying it alone.

In contrast, seeds derived from the other MOA, non-ALS treatments, gave very similar results to the Peld03 baseline population (mean difference = 1%). Consequently, there was no evidence that the non-ALS herbicides used (clodinafop, flufenacet, isoproturon, pendimethalin) were selecting for increased resistance to mesosulfuron+ iodosulfuron (e.g. by enhanced metabolism) over the two years of these trials. The untreated population showed no major loss of resistance compared with the baseline, which would have indicated a fitness penalty with ALS target site resistant plants.

The main practical implication of these studies was that the use of herbicides with other modes of action, in mixture or sequence with mesosulfuron+iodosulfuron, should be viewed as a useful method of increasing overall weed control, but not as a resistance prevention or mitigation strategy. Non-ALS herbicide treatments do not appear to select very actively for resistance to mesosulfuron+iodosulfuron (e.g. by enhanced metabolism) over the limited time scale (2 yrs) studied in these experiments. This conclusion was supported by results from another container experiment which showed considerable year to year variation in herbicide efficacy of pre-emergence herbicides, making it difficult to detect changes in resistance.

2.2.2 Field experiments

The two sites, at ADAS Boxworth and Rothamsted, were existing long-term field experiments, which provided an opportunity to investigate the sustainability of different herbicidal mitigation strategies based on the use of ALS inhibiting herbicides in mixture and/or sequence with other modes of action. Seed samples from other field experiments conducted by the agrochemical industrial partners, were also tested to quantify the influence of different herbicide regimes on the development of resistance to ALS inhibiting herbicides.

There was no clear association between the efficacy of mesosulfuron+iodosulfuron in the field and past use of herbicides, irrespective of whether they were ALS or other modes of action. Efficacy tended to vary from one year to the next at both sites, but not in any systematic way. However, a glasshouse assay, using seeds collected from field plots, indicated that marginal resistance to mesosulfuron+iodosulfuron, due to probable enhanced metabolism, could develop within only four years. Molecular assays on 28 plants surviving in the glasshouse assay, found that only three had a Pro-197-Thr mutation conferring ALS target site resistance. The remaining 25 plants had no detectable ALS mutations. This indicates that mesosulfuron+iodosulfuron was selecting primarily for enhanced metabolic resistance in the Rothamsted field experiment, rather than ALS target site resistance.

However, the degree of resistance was relatively modest and would be very difficult to detect in a true field situation, especially if the herbicide was used in mixture or sequence with other modes of action, as is recommended. Consequently, a container experiment aimed to show whether these relatively marginal levels of resistance would impact on the efficacy of mesosulfuron+iodosulfuron outdoors (Table 2.1).

Table 2.1 Outdoor container experiment using black-grass seeds collected from the Rothamsted field experiment in 2008. A baseline population comprising seeds collected from the identical plots in 2003 and a susceptible reference population (Roth05) were also included.

Seed source	Mesosulfuron+ iodosulfuron dose (g a.i. ha^{-1})	Plant counts	Fresh weight
		% reduction in plant numbers	% reduction compared to untreated
Baseline 2003 seeds	12+2.4	97	94
	6 + 1.2	86	91
2008 seeds	12+2.4	54	61
	6 + 1.2	51	56
Roth05 (susceptible)	12+2.4	100	98
	6 + 1.2	98	94
S.E. ±		3.5	3.2
L.S.D. $P \leq 0.05$		11.1	10.1

The susceptible standard (Roth05) was well controlled by both doses, as expected (Table 2.1). The 2003 baseline population was slightly less well controlled than the susceptible standard. In contrast, plants grown from the 2008 seeds, collected from the same plots as the baseline 2003 seeds, were controlled much less well. Whereas control of the baseline 2003 population ranged from 86 – 97%, control of the 2008 population (no herbicide up to 2003/04, but subsequently four annual applications of mesosulfuron+iodosulfuron), was only 51 - 61%. The results based on plant number and foliage fresh weight were similar, and there was relatively little difference between full and half rate of mesosulfuron+iodosulfuron. Further comparative studies are needed to fully address the relative occurrence, rate of increase and risks posed by both ALS target site and enhanced metabolic resistance.

2.2.3 Company samples from field experiments

Herbicide manufacturers treated additional plots, alongside existing trials they were conducting. The aim was to treat the plots with four different herbicide regimes in order to determine whether shifts in resistance to mesosulfuron+iodosulfuron could be detected after a single year. It was hoped such studies would validate the more controlled container experiments conducted as part of this project, and could provide additional information on selection pressures. In total, seed samples were sent in from 26 sites across Eastern England. All seed samples were tested against mesosulfuron+iodosulfuron in a glasshouse pot assay.

Degree of resistance varied greatly between sites but, in the majority of samples, there was little or no evidence of differences in the level of resistance between samples treated with different herbicides. There were, however, four sites where either one or both of the mesosulfuron+iodosulfuron treatments were one resistance category, or more, higher than the untreated or non-ALS treatments. In contrast, there was only a single case where the ALS treated plots had a lower resistance rating. Although trends were small, the results supported the conclusions of the container experiments, that the use of other modes of action in mixture or sequence with mesosulfuron+iodosulfuron will not reduce selection for resistance. This work has assisted in the validation process by demonstrating that, over a cross section of sites, it is unlikely that resistance to mesosulfuron+iodosulfuron will be generally detectable after just a single application of mesosulfuron+iodosulfuron.

2.3 Objective 2: To establish the incidence of existing and novel mechanisms of herbicide resistance in grass-weeds, with particular emphasis on ALS inhibiting and dinitroaniline herbicides, in order to refine resistance sampling and monitoring procedures.

2.3.1 Developing robust tests for resistance to ALS inhibitors.

The aim was to develop reliable tests using populations already characterised for mechanism of resistance. Studies showed that glasshouse pot assays could reliably detect resistance to mesosulfuron+iodosulfuron ('Atlantis') and sulfometuron ('Oust') in black-grass plants grown from seeds. High organic matter growing media (e.g. peat based compost) must be avoided, as they can reduce herbicide activity and give misleading results. The best single doses to use in glasshouse pot assays, are the recommended field rate of mesosulfuron+iodosulfuron (12 + 2.4 g ha^{-1}) and 50 – 100 g ha^{-1} sulfometuron as an indicator of ALS target site resistance. Lower doses of sulfometuron should be avoided. Herbicides should be applied to plants at the 3 leaf stage and assessments of foliage fresh weight made 3 – 4 weeks later. This methodology was found to give consistent results.

Petri-dish germination assays were investigated as a more rapid method of detecting resistance to ALS herbicides, such as mesosulfuron+iodosulfuron.

Table 2.2. Petri-dish 'ring' test: % reduction in shoot length compared to untreated, using total shoot length per dish averaged over all testing centres

Herbicide	Mesosulfuron+iodosulfuron 0.1 ppm		Sulfometuron 1.0 ppm	
Population	% reduction in shoot length	Resistance 'R' rating	% reduction in shoot length	Resistance 'R' rating
WILTS	39	RR	58	RR
EAST	20	RRR	25	RRR
Peld 05 SS	-5	RRR	-1	RRR
LONG C	-1	RRR	-8	RRR
BIG F	57	S	76	S
FLAW	54	S	73	S
Roth04 (Susc.)	58	S	73	S
S.E. ±	4.96	*	5.55	*
LSD (*P≤0.05*)	13.93		15.60	

The best discriminatory concentrations were found to be 0.1 ppm of mesosulfuron+ iodosulfuron ('Atlantis') and 1.0 ppm sulfometuron ('Oust'). A 'ring' test, involving 10 companies/organisations, was conducted to evaluate the robustness of the Petri dish protocol for detecting resistance to ALS inhibiting herbicides. On the basis of measured shoot lengths, both herbicides could discriminate between highly resistant and susceptible populations (Table 2.2).

Petri-dish assays, which take 2 weeks, have potential for identifying ALS target site resistance. However pot tests, which typically take 8 weeks, are more robust, as they have the potential for detecting resistance conferred by non-target site mechanisms as well as ALS target site resistance.

2.3.2 Cross-resistance studies with different classes of ALS inhibitors.

Glasshouse dose response studies were conducted to determine the cross-resistance patterns to different classes of ALS inhibitors. Three black-grass populations were used: a susceptible standard (Roth 04); a population with confirmed Pro197 ALS mutation (LongC06) and a population with confirmed Trp574 ALS mutation (R30). These populations were treated with a range of doses of commercial formulations of the sulfonylureas mesosulfuron+iodosulfuron and sulfometuron-methyl, the sulfonylaminocarbonyltriazolinone propoxycarbazone and the imidazolinone imazapyr.

Both the LongC (Pro197) and R30 (Trp574) populations showed very high degrees of resistance to mesosulfuron+iodosulfuron (Figure 2.2) and sulfometuron, and to propoxycarbazone. With all three herbicides, the susceptible standard Roth04 was well controlled at 12.5% or less of the field recommended dose. In contrast, even at the highest doses used, 400% of the field rates, there was little effect on growth of both resistant populations. There was no indication that the two different mutations involved affected the outcome. Resistance indices were very high, from 218 – 3613.

Response to imazapyr differed markedly between populations (Figure 2.2). Again the susceptible standard, Roth04, was well controlled, even at low doses, with an ED_{50} value of 9.6 g a.i. ha^{-1}. The LongC population was much less resistant than the R30 population, with ED_{50} values of 146 and 2000 g a.i. ha^{-1} respectively. In contrast to the other ALS classes, the specific mutation present had a very big impact on degree of resistance to the imidazolinone, imazapyr, with Trp574 in R30 conferring much greater resistance than Pro197 in LongC.

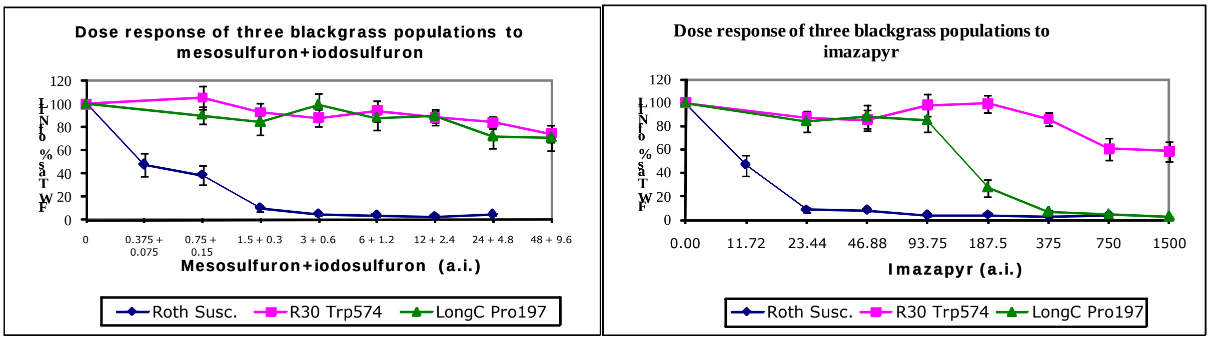


Figure 2.2 Response of three black-grass populations to mesosulfuron+iodosulfuron and imazapyr.

The two, most common, ALS mutations responsible for target site resistance, Pro197 & Trp574, appeared to confer equally high degrees of resistance to sulfonylurea and sulfonylaminocarbonyltriazolinone herbicides. The specific mutation was much more important in determining the degree of resistance to the imidazolinone, imazapyr, with Trp574 conferring much greater resistance than Pro197. However, it is important to note that other imidazolinones may respond differently to imazapyr.

2.3.3 Refining assays for dinitroaniline herbicides to enable detection of novel mechanisms of resistance.

Dinitroanilines, such as pendimethalin and trifluralin, are major components of risk mitigation strategies due to their perceived lower resistance risk. Pendimethalin is vulnerable to enhanced metabolism, but no resistance to trifluralin has so far been detected in black-grass in the UK. A range of 14 UK populations were assayed in Petri-dishes in order to quantify any changes in resistance to pendimethalin and trifluralin. The control of both susceptible standards (Roth 04 and Herb06) by both trifluralin and pendimethalin was good (84 – 100% reduction). Ten of the 14 populations showed resistance (RRR or RR) to pendimethalin. In marked contrast, all 14 populations were susceptible to trifluralin. This supports past findings that trifluralin is not vulnerable to enhanced metabolism, in contrast to pendimethalin. However, resistance to pendimethalin tends to be partial, so it can still provide useful levels of control in many situations. From the 2008/09 cropping season trifluralin can no longer be used in the UK due its failure to acquire Annex 1 listing in the EU review of pesticides. These results highlight the importance of the loss of a herbicide for which resistance has never been detected in black-grass and the need to continue to monitor development of resistance to pendimethalin, which is still available.

2.3.4 To develop a sampling strategy, involving spatial and temporal elements, in order to improve resistance detection and monitoring at the local level.

Weeds are less mobile than many pests or pathogens so herbicide-resistant populations of weeds tend to be more localised in distribution both within, and between, farms. Most samples for resistance testing comprise a single bulked seed sample from a restricted part of a field. We investigated sampling strategies for black-grass on a range of scales with the objective of determining spatial distribution of resistance both on a field and farm scale, with the aim of developing better methods for detecting and monitoring resistance. Over four cropping seasons, from July 2005 to July 2008, a range of fields in the east of England were identified that contained distinct patches of black-grass. In total, 179 patches were sampled on 30 fields on 16 farms. The fields had all received an application of mesosulfuron+ iodosulfuron in that cropping season. The testing methodology developed within the overall project was used to evaluate resistance status of each sample.

The incidence of resistance detected in the patch samples was high, but this was expected as fields were not selected at random and samples were biased towards black-grass 'survivors' in the field. Seventy-five percent of the black-grass samples collected between 2006 and 2008 showed resistance to the ALS inhibiting herbicide mesosulfuron + iodosulfuron. Ninety-eight percent of the samples collected showed resistance to fenoxaprop, 91% showed resistance to sethoxydim and 72% to pendimethalin. Because the incidence of resistance was so high, the consistency of the resistance test results for fenoxaprop and sethoxydim between different patches in the same field, and between fields, was very good. The lower incidence of resistance to pendimethalin and mesosulfuron+ iodosulfuron resulted in greater variability between fields.

Resistance testing of the patch samples demonstrates that one sample taken from within a *single* patch is likely to be representative of the whole patch, due to the very good consistency in the resistance levels for all herbicides tested. The consistency levels of the resistance test results from samples taken from *different* patches within the same field were good, but not as high as for the within patch samples. Where resistance is just starting to develop in a field, there can be more variation in the amount of resistance present within a single field, but as resistance develops further, the ratings become increasingly consistent (Figure 2.3).

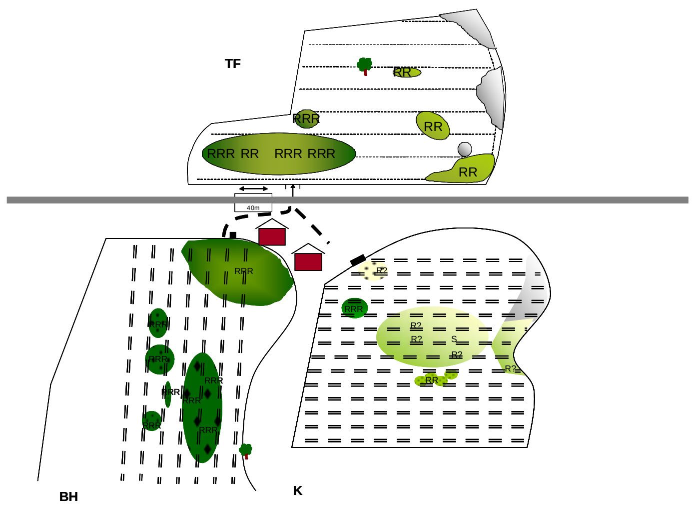


Figure 2.3. Distribution of resistance to mesosulfuron+iodosulfuron on neighbouring fields on Farm 1.

There was considerable variability between black-grass resistance test results from different fields on the same farm, and between different farms. Neighbouring farm results should not be used as an indication of the level of resistance on another farm and weed density alone is not a good indicator of resistance. Table 2.3 summarises a sampling strategy based on the findings of these sampling studies.

Table 2.3 A summary of the consistency of resistance test results for each unit of assessment, and the implications of these for black-grass resistance sampling strategies.

Unit of assessment	Consistency	Implications for sampling
Within a patch	Very good	One sample likely to be representative of that patch
Between patches within a field	Good/variable	Collect seed from a number of patches across the field
Between fields	Variable/poor	Consider carefully how to approach sampling and be prepared to take samples from several fields on each farm
Between farms	Variable/poor	Do not rely on the results at one farm to predict those on another

2.4 Objective 3: To quantify the impact of the population dynamics of grass-weeds on cultural and herbicidal resistance mitigation strategies by utilising existing knowledge and generating new information where this is lacking.

2.4.1 Influence of different cultivation systems on development of ALS resistance

Different cultivation systems can affect the rate of resistance development by changing the proportion of the weed population that is derived from recently shed seeds, as compared with older, less selected seeds, from the seedbank. An experiment was conducted in outdoor containers over three years with two populations of black-grass, ALS resistant (Peld03) and susceptible (Roth03). Cultivations were simulated each autumn by either resowing seeds comprising 90% collected from the same treatment that summer plus 10% original baseline seeds (‘non-inversion tillage’), or with 10% seed collected from the same treatment that summer plus 90% original baseline seeds (‘ploughing’). Mesosulfuron+iodosulfuron 12+2.4 g a.i. ha^{-1} was applied each autumn to treated containers.

The susceptible (Roth03) plants were killed by all treatments in each year (100% reduction in plant numbers) confirming that this population was susceptible. In contrast, poorer control of the ALS resistant (Peld03) population was achieved by mesosulfuron+iodosulfuron in all years, confirming resistance (Figure 2.4).

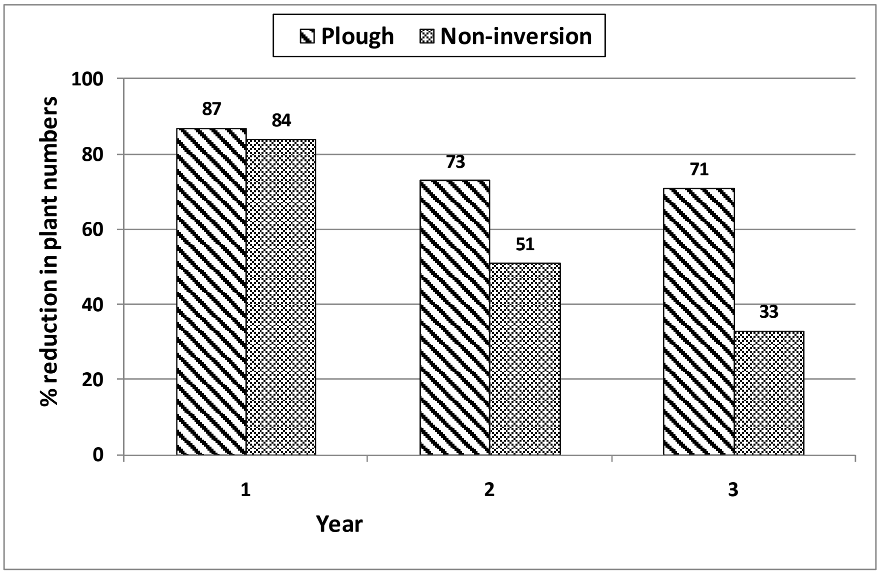


Figure 2.4 Effect of simulated cultivations on control of ALS resistant (Peld03) black-grass by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) over 3 years.

Herbicide performance declined over the three years in both cultivation systems, but to a much greater extent in the non-inversion tillage treatment. With ploughing, the decline was from 87% to 71% control over the three years, but with non-inversion tillage control declined from 84% to 33%. Although these cultivations were simulated, and may have exaggerated the difference in seed distribution caused by cultivations, we believe the results highlight the increased risk of more rapid development of resistance under non-inversion cultivation systems compared with ploughing.

2.4.2 Fitness/deselection studies on ALS target site resistant black-grass

We aimed to investigate whether a decline in the degree of ALS resistance occurred in the absence of herbicide selection. Seeds of six populations of black-grass, known to be resistant to ALS inhibitors, were sown with wheat (cv. Hereward) in outdoor containers, but no herbicides were applied. Seeds were collected in July and containers were re-sown each autumn with seeds collected from the same population. The effect of deselection on the proportion of seeds produced with resistance to mesosulfuron+iodosulfuron was evaluated in a glasshouse assay using the original baseline populations and seeds collected in 2008, after 1 – 3 years deselection.

Control of the baseline populations varied considerably, as would be expected from their resistance profiles, whereas plants of a susceptible standard (Roth05) were all killed, confirming that this population was susceptible (Figure 2.5).

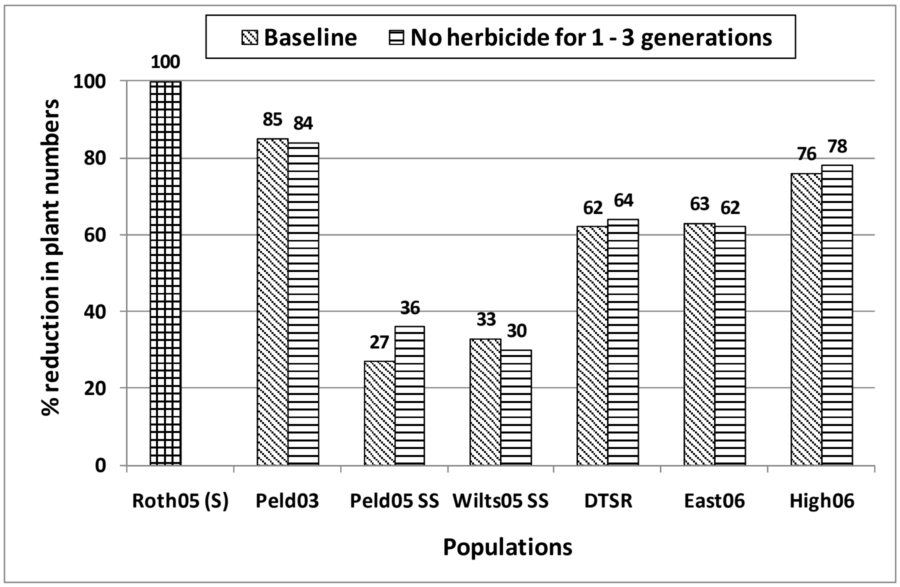


Figure 2.5 Control of black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in a glasshouse evaluation of seeds collected from the fitness/deselection outdoor containers.

There was no evidence in any of the six populations of any major change in the level of control by mesosulfuron+iodosulfuron following 1 or 3 years deselection. Meaned over all populations, the % control of the baseline and untreated populations averaged 57.7% and 59.0% respectively. If lack of herbicide treatment had caused resistance to mesosulfuron+ iodosulfuron to be deselected as a consequence of a fitness penalty associated with resistance mutations, then the level of control should have increased. The fact that no substantial increases were recorded is good evidence that any fitness penalty associated with ALS target site resistance is minimal, and is unlikely to have a significant impact over an agronomically relevant timescale of 5 – 10 years.

Both ALS and ACCase resistance selection appear to be very much one-way processes – potentially increasing rapidly, but not declining in the absence of selection. Farmers need to aim to maintain ALS and ACCase resistance at as low a frequency as possible, as there seems little chance of ‘turning the clock back’ with these types of resistance.

2.4.3 Modelling the effects of grass-weed population dynamics on herbicide resistance mitigation strategies

Models will not reliably predict the rate of development of resistance on any individual field as so many variables are involved. However, models have value as advisory tools showing what could happen in a typical situation and this can aid the practical decision making process at the individual farm level. Consequently, we modelled different resistance scenarios to assess the effectiveness of different risk mitigation strategies. It is important to recognise two distinct components to the management of herbicide-resistant weeds: the *number* of weeds per unit area (the infestation level) and the *proportion* that are resistant (resistance level). Both of these factors were incorporated into the modelling process as both are relevant to the farm situation.

The existing black-grass population model in winter wheat, produced in 1990, was updated. There were just over twice as many heads per plant (8.5) at low densities of up to 10 black-grass plants m^{-2}, compared with the original model (3.9). The main reason for this is probably earlier sowing of winter cereals resulting in a longer vegetative phase leading to more tillering of black-grass plants in the autumn. The practical implications are that each black-grass plant is now relatively more competitive and also produces about twice as much seed per plant.

Twelve different scenarios were modelled, primarily in winter wheat crops, and some of the main conclusions are summarised below:

- Higher levels of control are now required to prevent populations increasing (97% with tine/disc cultivation 20 cm deep; 90% with ploughing to 20–25 cm).
- Ploughing (annual or rotational), pre-emergence herbicides and non-chemical weed control methods can help maintain low weed populations where resistance to post-emergence herbicides is increasing – but only in the short-term.
- In deep tine/disc systems in which a pre-emergence herbicide is applied, the overall level of control will be insufficient to prevent black-grass populations increasing when post-emergence herbicide efficacy drops below about 90% (Table 2.4).

- Where non-chemical methods are used in combination with deep tine/discs and pre-emergence herbicides, post-emergence efficacy can decline to 60%-70% before overall control becomes insufficient (Table 2.4).

Table 2.4 The % kill of black-grass plants needed from pre-emergence herbicides to compensate for declining activity of post-emergence herbicides for three overall levels of control from pre/post herbicide sequences.

Control from main post-emergence treatment	Control required from pre-emergence herbicides to achieve three different overall target levels of control		
	90% (Control needed in ploughing systems)	93% (Control needed in deep tine systems + cultural control)	97% (Control needed in deep tine systems)
99%	0	0	0
89%	9%	36%	73%
79%	52%	67%	86%
69%	68%	77%	90%
59%	76%	83%	93%

Note: unshaded, lighter and heavier shading = respectively achievable (<60%), potentially achievable (60 – 80%) and unlikely to be achievable (>80%) routinely.

- Target site resistance can increase rapidly – it took only 4 years for resistant plants to increase from 1% to 100% of the population in non-inversion tillage.
- Close monitoring of herbicide performance in association with regular testing for resistance could help as an early warning of resistance problems ahead.
- The most powerful message from the modelling studies was that modifiers in the form of alternative herbicides or non-chemical methods can slow the build-up of resistance. They may not stop resistance developing, but can help maintain black-grass populations at tolerable levels, at least in the short term.

2.5 Key outcomes by objective

Integrated Management of Herbicide Resistance

Objective 1: Quantify the effectiveness of resistance mitigation strategies (especially in relation to ALS and dinitroaniline herbicides)

- Target site resistance (TSR) to ALS herbicides (e.g. sulfonylureas) can build up quickly in black-grass as a result of repeated annual use of this chemistry alone
- ALS in mixture or sequence with herbicides with different modes of action led to:
    - improved weed control due to lower black-grass numbers
    - no reduction of selection pressure for ALS TSR

- Non-ALS herbicides did not select for ALS TSR
- Effective pre-emergence herbicides were vital to:
    - Reduce black-grass numbers
    - Reduce reliance on post-emergence herbicides (higher resistance risk)
- In most cases 2+ years of selection pressure are needed to positively identify resistance risks of ALS herbicides (1 year in some cases)

Objective 2: Establish the incidence of different mechanisms of resistance and develop improve detection methods at the local level

- The number of cases of resistance to ALS inhibiting herbicides in black-grass is increasing throughout England – confirmed in 21 counties
- Robust and reliable tests were developed and are available to farmers/advisors to detect resistance to ALS inhibiting herbicides
- Improved advice for farmers/agronomists on collecting representative seed samples for resistance testing:
    - Sampling from a single patch does not consistently reflect the resistance status of all patches in the same field
    - Sampling from a single field on a farm definitely does not represent the whole farm in terms of resistance status

Objective 3: Quantify the impact of population dynamics of grass-weeds in relation to resistance mitigation strategies

- Resistance to ALS herbicides increases faster in minimum tillage systems compared with ploughing
- ALS TSR did not disappear or even decline when ALS herbicides were not used for 3 years – there is no loss of resistance in the absence of the selecting herbicide
- Pre-emergence herbicides can compensate, to some degree, for the declining performance of post-emergence herbicides due to increasing resistance
- Modifiers in the form of alternative herbicides or non-chemical methods slowed, but did not prevent, the build-up of resistance
- Non-chemical cultural control methods are increasingly important in combating resistance by reducing the reliance on herbicides

2.6 Guidelines for more sustainable resistance management strategies

The research highlights key factors that can contribute to better management of herbicide-resistant black-grass. These are:

- **Greater use of non-chemical control methods to reduce reliance on herbicides.** It must be recognised that many non-chemical methods are less effective than herbicides, more complex to manage and can have negative environmental attributes. Non-chemical methods cannot replace herbicides on most farms, but reduced reliance on herbicides will be necessary both from a practical (increasing resistance, lack of new herbicides) and political aspect (complying with new EU legislation).
- **Less reliance on high resistance risk post-emergence herbicides.** Research studies clearly indicate that the regular use of ACCase and ALS inhibiting herbicides is associated with a high risk of herbicide resistance. Moderating this risk is vital if the effectiveness of these herbicides is to be maintained in the longer term. These herbicides will continue to be very important in controlling black-grass, but their use needs to be integrated with other control measures, both cultural and chemical.
- **Greater use of pre-emergence herbicides**. Resistance to the pre-emergence herbicides used for black-grass control tends to be only partial and builds up relatively slowly. Consequently, pre-emergence herbicides appear to be a lower resistance risk than some post-emergence options, especially ACCase and ALS inhibiting herbicides, and can substitute for them to some degree.
- **More critical monitoring of herbicide performance in individual fields.** Resistance in black-grass can vary considerably between and, to a lesser extent, within different fields. Management strategies need to take account of this inter-field variation. Close monitoring of variations in herbicide performance both within, and between, fields can act as an early warning of potentially greater problems ahead.
- **Regular testing for resistance.** While the factors responsible for the evolution of herbicide resistance are well established, predicting the risk at an individual field scale is imprecise. Consequently, actual testing of seeds or plants from fields provides a more robust indicator of the degree of herbicide resistance. This needs to be done regularly, at least once every 2 – 3 years if changes in resistance are to be detected reliably.

3. TECHNICAL DETAIL

3.1 Introduction

Herbicide-resistant grass-weeds are very widespread in the UK. By 2005, herbicide-resistant black-grass (*Alopecurus myosuroides*) had been confirmed on 2,085 farms in 31 counties, resistant Italian rye-grass (*Lolium multiflorum*) on 324 farms in 28 counties and resistant wild-oats (*Avena* spp.) on 218 farms in 26 counties of England (Moss *et al*., 2005a). Failure to combat herbicide resistance poses a major threat to the sustainability of current cropping practices. Resistance can result in increased use of herbicides, limits cropping and herbicide choices, and may encourage inversion tillage rather than minimum tillage practices that have environmental benefits such as a reduced risk of soil erosion and diffuse pollution, and lower energy requirements.

The PSD/CSL Pesticide Usage Survey of Arable Crops in Great Britain in 2006 (Garthwaite *et al*., 2007) showed that herbicides were the largest group of pesticides applied, accounting for 57% by weight of all pesticides used. In addition, grass weed control is critically dependant on only four herbicide classes - 80% of all grass weed herbicides applied were phenylureas, dinitroanilines, ACCase or ALS inhibitors. The withdrawal of approved products or uses as a consequence of the EU Pesticide Authorisation Directive 91/414/EEC and the fact that there is little prospect of any new modes of action likely to become available in the near future, is of major concern in relation to sustainable resistance management. In addition, the new EU Thematic Strategy for Pesticides and the Water Framework Directive could have a major impact on availability of herbicides, especially those used for grass-weed control (Clarke *et al*., 2009).

As a result of past research, there is now a good base of evidence on the risks of development of resistance to ACCase herbicides ('fops' and 'dims') in grass-weeds (Moss *et al.*, 2005b). The major risk is now associated with the potential increase of herbicide resistance to ALS herbicides (includes sulfonylureas such as mesosulfuron+iodosulfuron, 'Atlantis') in grass weeds. This was also rated as the most important future herbicide-resistance issue in a recent European survey (Moss, 2004). Worldwide more weed species have developed resistance to ALS inhibiting herbicides than to any other herbicide class (Heap, 2009). Resistance to ALS inhibitors has been less common in Europe than elsewhere, although there are indications that this situation is changing. ALS inhibiting herbicides have mainly been targeted at broad-leaved weeds in the past, and consequently resistance has developed in 101 species. ALS resistance has also evolved in 22 grass-weed species and this number is steadily increasing as a consequence of greater use of newer ALS herbicides with better grass-weed activity.

A considerable amount of research has been conducted on ALS target site resistant *broad-leaved* weeds at the biochemical and molecular level. Several different mutations have been documented in many publications (see review by Tranel and Wright, 2002). In contrast, the first report of a point mutation in the ALS of a grass-weed (*Bromus tectorum*) was published only five years ago (Park and Mallory-Smith, 2004). Past studies at Rothamsted have demonstrated that some UK populations of black-grass have an enhanced ability to metabolise the ALS inhibiting herbicide flupyrsulfuron. More recently, ALS target site resistance has been confirmed in black-grass populations in the UK making this one of the first verified cases of ALS target site resistance in a grass-weed in Europe (Marshall, 2007; Marshall & Moss, 2008). The sulfonylurea grass-weed herbicide mesosulfuron+iodosulfuron ('Atlantis') is being used very widely in many countries in Europe. In 2006 it was applied to 551,000 ha in the UK making it the fifth most widely used herbicide (after glyphosate, isoproturon, pendimethalin and trifluralin) (Garthwaite *et al*., 2007). Consequently, selection for resistance is likely to increase and the challenge is to develop sound strategies, based on good scientific principles, to minimise the risk.

A key element requiring investigation is the effect of weed population dynamics on herbicide risk mitigation strategies, such as the use of mixtures or sequences, the rotational use of different modes of action and cultural control measures. Industry, advisers and regulators need to know whether selection pressure lessons from ACCase herbicides can be applied to ALS herbicides in grass weed species (black-grass, Italian rye-grass and wild-oats). If this is possible, it will provide much better information on the resistance risk of both existing and new modes of action and the potential value of risk mitigation strategies.

Major research aims of the project were to:

- Quantify the effectiveness of resistance mitigation strategies through a better understanding of the selection pressure imposed by herbicides.
- Develop robust tests for resistance to ALS inhibitors in grass-weeds.
- Investigate and develop sampling strategies to improve resistance detection and monitoring at a local level.
- Quantify the impact of population dynamics in relation to cultural and herbicidal mitigation strategies.
- Develop more sustainable and appropriate resistance management strategies for individual fields.

3.1.1 Objectives

The three main scientific objectives of this project were:

Objective 1: To quantify the ability of resistance mitigation strategies to moderate or prevent herbicide resistance in grass-weeds, with particular emphasis on ALS inhibiting and dinitroaniline herbicides.

Objective 2: To establish the incidence of existing and novel mechanisms of herbicide resistance in grass-weeds, with particular emphasis on ALS inhibiting and dinitroaniline herbicides, in order to refine resistance sampling and monitoring procedures so that resistance management strategies at the local level can be optimised.

Objective 3: To quantify the impact of the population dynamics of grass-weeds on cultural and herbicidal resistance mitigation strategies by utilising existing knowledge and generating new information where this is lacking.

These objectives were studied primarily in black-grass, but the principles should also be relevant to other grass-weeds. An integrated experimental programme was conducted involving laboratory, glasshouse, outdoor container and field studies utilising populations well characterised for resistance to different modes of action. Each of these elements has its own advantages and disadvantages, but integrating all of these has been a very successful approach in past resistance studies.

Details of the experimental programme and results for each of these objectives are presented in the following three sections.

3.2 Objective 1: To quantify the ability of resistance mitigation strategies to moderate or prevent herbicide resistance in grass-weeds, with particular emphasis on ALS inhibiting and dinitroaniline herbicides.

The herbicidal elements of current risk mitigation strategies are based largely on the use of mixtures and sequences of different modes of action. However, there is little evidence to quantify the benefits in terms of preventing or delaying resistance development. Grass-weed control in cereals is heavily dependent on just four major herbicide modes of action: ACCase and ALS inhibitors, phenyl-ureas and dinitroanilines, and no major new modes of action are likely to become available in the near future. The former two can be considered 'higher risk' based on worldwide experience whereas the latter two can be considered 'lower risk'. However resistance to all four modes of action has been demonstrated in the UK, so none can be considered 'no risk'. Increasing dependence on ACCase and ALS inhibitors means that a better understanding of risk mitigation strategies is essential. We aimed to subject grass-weed populations with contrasting resistance characteristics to mitigation strategies that were likely to impose different degrees of selection. Changes in resistance were assessed with the aim of developing more robust strategies for resistance prevention and management.

3.2.1 Container experiments: To compare selection pressure imposed by the individual components of mixtures, sequences or rotations of herbicides.

Outdoor containers successfully mimic field conditions but allow more controlled studies to be made on rate of development of resistance. The aim in the first two container experiments was to grow populations with known resistance status and compare selection conferred by non-ALS herbicides with ALS herbicides used alone, or in mixture, sequence or rotation, with herbicides with other modes of action. Seed samples were collected each summer and re-sown each year with the emphasis on establishing both herbicide efficacy and changes in proportion of resistant individuals. Changes in proportion of resistant individuals were assessed in subsequent glasshouse tests on seeds collected from surviving plants each year. A third container experiment was conducted with the aim of detecting any shifts in resistance to the pre-emergence herbicides pendimethalin, trifluralin and flufenacet+pendimethalin.

Materials & Methods

Container experiment 1. Three black-grass populations were used: Rothamsted 2003 (= Roth), a susceptible standard; Peldon 2003 (Peld03), a population collected from Essex with proven ALS target site resistant (Pro-197-Thr) present in about 18% of seeds (Marshall, 2007); Wiltshire 2005 (Wilts05), a population showing partial resistance to sulfonylurea graminicides (in a glasshouse pot screen, 31% plants survived treatment with mesosulfuron+iodosulfuron ('Atlantis') and sulfometuron). Resistance in Wilts05 is not conferred by either of the two ALS mutations (Pro-197-Thr; Trp-574-Leu) found in other ALS target site resistant black-grass in the UK (Marshall & Moss, 2008). In each container (40 x 33 x 16 cm deep), 250 black-grass (500 Yr 1 Peld03) and 21 wheat (cv. Hereward) seeds were sown into the surface 2.5 cm of a Kettering loam soil. There were four replicates in a randomised block design. Each population was evaluated for two years: Peld03 Yr 1 = 2004/05, Yr 2 = 2006/07; Wilts05 Yr1 = 2006/07, Yr2 = 2007/08). Seeds were sown in late September or early October each year and containers kept outdoors on a sandbed at Rothamsted. Herbicides were applied using a track sprayer delivering 240 L spray solution ha^{-1} at 245 kPa through a single 'Teejet' TP110015VK flat fan nozzle. Recommended adjuvants were used;' Biopower' @ 0.5% with mesosulfuron+ iodosulfuron and 'Actipron' @ 0.5% with clodinafop+trifluralin.

The seven herbicide treatments used in all years were:

1. ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) applied in autumn post emergence at the 3 leaf stage to 2 tiller stage **(= ALS x1).**
2. ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) applied twice, autumn post emergence and in spring (= **ALS x2**).
3. ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) applied autumn post-emergence in mixture with clodinafop+trifluralin (30+960 g a.i. ha^{-1}) (=**ALS+other MOA mixture**). (Note: MOA = mode of action)
4. ALS herbicide (mesosulfuron+iodosulfuron 12+2.4 g a.i. ha^{-1}) applied autumn post-emergence in mixture with pendimethalin (1320 g a.i. ha^{-1}) following a pre-emergence herbicide (flufenacet+pendimethalin, 180+900 g a.i. ha^{-1}) (=**ALS+other MOA sequence**).

5. Rotation of treatment 1 (ALS alone) in first year followed by treatment 6 (other MOA) in second year (**Herbicide rotation**).
6. Non-ALS herbicides used in sequence (isoproturon+pendimethalin, 2500+1320 g a.i. ha^{-1} applied autumn post-emergence followed by clodinafop+trifluralin, 30+960 g a.i./ha) (= **other MOA sequence**).
7. Untreated (= **Untreated**).

The number of individual and % ALS active ingredients for the seven treatments listed above were respectively: Tr.1 2, 100%; Tr.2 4, 100%; Tr.3 4, 50%; Tr.4 5, 40%; Tr.5 2, 100% 1st yr/ 4, 0% 2nd yr.; Tr.6 4, 0%; Tr.7 0, 0%. Consequently the treatments encompassed a range of intensities of use of ALS inhibiting herbicides (0%, 40%, 50%, 100%).

Pre-emergence herbicides were applied 7 days after sowing and autumn post-emergence treatments between late October and December when black-grass was at the 3 leaf to 2 tiller stage. Spring treatments of mesosulfuron+iodosulfuron were applied between February and April when black-grass was well tillered. Plants per container were assessed prior to spraying and survivors recorded in the spring between January and May depending on treatment. Containers for each treatment were isolated in individual small glass-houses in early May to prevent cross-pollination. Seeds were collected as they matured from each individual container between June and August. Containers were re-sown each autumn with seeds collected from the same treatment that summer, except for the Roth susceptible standard which was sown each year with seed from the same original sample to act as reference. The effect of each treatment on the proportion of seeds produced with ALS resistance was evaluated in a glasshouse assay for seeds collected from each individual container, by sowing 60 pre-germinated seeds in germination trays (38 x 22 x 5 cm deep) containing Kettering loam soil. There were four replicates and the original baseline populations (Peld03, Wilts05) and the Roth susceptible standard were also included. The number of plants established in each tray was counted and then mesosulfuron+iodosulfuron at the field rate (12 + 2.4 g a.i./ha) plus 'Biopower' adjuvant (@0.5%) was applied at the three leaf stage using a track sprayer delivering 240 L spray solution ha^{-1} at 245 kPa through a single 'Teejet' TP110015VK flat fan nozzle. The number of plants surviving with little or no damage was recorded after 4 weeks as a measure of resistance. Twenty-four surviving plants grown from seeds derived from the containers originally sown with Peld03 seeds were assayed at the molecular level using the protocol of Delye & Boucansaud, (2008).

Container experiment 2. A second container experiment was set up at Rothamsted in order to evaluate a more comprehensive range of herbicide timings and sequences. Two populations were used: the Rothamsted 2003 (= Roth) susceptible and Peldon 2003 (Peld03) resistant populations as used in container experiment 1. The same methodology as described above was used and the experiment was conducted for one year, 2005/06.

The 15 herbicide treatments (Tr.) used were:

1. & 2. ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) applied post emergence in autumn (Tr. 1) or spring (Tr. 2) **(= ALS x1).**

3. ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) applied twice, both autumn post emergence and in spring (= **ALS x2**).

4. ALS herbicide (mesosulfuron+iodosulfuron, 6+1.2 g a.i. ha^{-1}) applied at half field rate post emergence in autumn **(= ALS half rate).**

5. & 6. ACCase herbicide (clodinafop, 30 g a.i. ha^{-1}) applied post-emergence in autumn with an ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) applied sequentially post emergence in spring (Tr. 5), or the order reversed (Tr. 6) **(= ACCase / ALS) & (= ALS / ACCase).**

7. & 8. Tank mix of an ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) with an ACCase herbicide (clodinafop, 30 g a.i. ha^{-1}) applied post-emergence in autumn (Tr. 7) or spring (Tr. 8) **(= ALS+ACCase).**

9. Tank mix of an ALS herbicide (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) with pendimethalin (1320 g a.i. ha^{-1}) applied autumn post-emergence (= **ALS + Pend**).

10. A sequence consisting of a pre-emergence herbicide (flufenacet+ pendimethalin, 180+900 g ha^{-1}) followed by (mesosulfuron+iodosulfuron, 12+2.4 g a.i. ha^{-1}) applied in spring post-emergence (= **Pre / ALS**).

11. & 12. ACCase herbicide (clodinafop, 30 g a.i. ha^{-1}) applied post emergence in autumn (Tr. 11) or spring (Tr. 12) **(= ACCase).**

13. Pendimethalin (1320 g a.i. ha^{-1}) applied autumn post-emergence (= **Pend**).

14. Non-ALS herbicide sequence consisting of a pre-emergence herbicide (flufenacet+pendimethalin, 180+900 g a.i. ha^{-1}) followed by isoproturon+pendimethalin, 2500+1320 g a.i. ha^{-1} applied autumn post-emergence (= **other MOA sequence**).
15. Untreated (= **Untreated**).

MOA = mode of action. The number of individual and % ALS active ingredients for the 15 treatments listed above were respectively: Tr.1 2, 100%; Tr.2 2, 100%; Tr.3 4, 100%; Tr.4 2, 100%; Tr.5 3, 66%; Tr.6 3, 66%; Tr.7 3, 66%; Tr.8 3, 66%; Tr.9 3, 66%; Tr.10 4, 50%; Tr.11 1, 0%; Tr.12 1, 0%; Tr.13 1, 0%; Tr.14 4, 0%; Tr.15 0, 0%. Consequently the treatments encompassed a range of intensities of use of ALS inhibiting herbicides (0%, 50%, 66% or 100%).

Containers were sown on 27 September 2005. Pre-emergence herbicides were applied 10 days after sowing, autumn post-emergence treatments on 29 November, when black-grass had 3 – 4 leaves, and spring post-emergence treatments on 23 January 2006, when black-grass had 4 tillers. Recommended adjuvants were used; 'Biopower' @ 0.5% with mesosulfuron+ iodosulfuron and 'Actipron' @ 0.5% with clodinafop. Plants per container were assessed prior to spraying and survivors recorded in the spring on 30 March 2006. Containers for each treatment were isolated in individual small glass-houses in early May and seeds were collected. These were tested in a glasshouse assay as described for Container experiment 1 above.

Container experiment 3. A third container experiment was set up, at Boxworth, in order to evaluate changes in resistance to the pre-emergence herbicides pendimethalin, trifluralin and flufenacet+pendimethalin. Three black-grass populations were used, Roth03, Wilts05 and Peld03 as described in container experiment 1 above. The same containers, soil and sowing methods were used with 1.25g (approx 500) of seeds incorporated into the surface 2 cm of soil and 24 wheat seeds sown in each container. Containers were sited outside at ADAS Boxworth in a randomised block design with four replicates and one untreated container per population per replicate. The following herbicides were applied pre-emergence, 6-7 days after sowing: pendimethalin (900 g a.i./ha); trifluralin (960 g a.i./ha); flufenacet+pendimethalin (180+900 g a.i./ha). Herbicides were applied at 200 l ha^{-1} water volume using an Oxford Precision knapsack spray and hand boom (2m) at 200kPa.

Details of sowing, herbicide application and assessment dates for all years are presented in Table 3.2.1. The number of plants per container was counted in the autumn and spring of each season to assess black-grass emergence and herbicide effects by comparing to the untreated control. In late spring (from pre- flowering) the containers were separated (by treatment), by at least 20m to reduce the likelihood of cross-pollination between the plants from different treatments. A black-grass head count was carried out on all containers in late spring/early summer of each season. In June/July black-grass seed was collected from each container, by sampling every 3-4 days over a period of approximately 2-3 weeks to ensure all seed was collected. Seeds were air dried and cleaned, then stored in paper envelopes. Seeds collected in the previous summer from the Wilts05 and Peld03 populations were re-sown in the following autumn, but the same original Roth03 seed was sown each year as susceptible standard.

Table 3.2.1 Container experiment 3: Details of sowing, herbicide application and assessment dates for all seasons.

Activity	**Date of activity in each season**		
	2005/06	**2006/07**	**2007/08**
Sowing	03/10/05	19/09/06	16/10/07
Herbicide application	10/10/05	25/09/06	22/10/07
Autumn count	15/11/05	06/11/06	27/11/07
Spring count	*none*	30/04/07	27/02/08
Isolation date	17/05/06	30/04/07	08/05/08
Head count	17/05/06	*none*	11/06/08
Seed collection	July 06	July 07	June/July 08

Results & Discussion

Container experiment 1. Plants established well in the containers each year giving the following plant densities in untreated containers: Peld03 Yr 1 179, Yr 2 107; Wilts05 Yr 1 135, Yr 2 100. The Roth plants were killed by all treatment in both years (100% reduction in plant numbers) confirming that this population was susceptible and that the application methodology and conditions at time of application were conducive to good control. In contrast, poorer control was achieved by all treatments applied to the Peld03 and Wilts05 populations, confirming resistance (Table 3.2.1.1). The fact that reduced control occurred with non-ALS herbicides (treatment 6) with both populations, indicates that resistance was not confined just to ALS inhibiting herbicides. This was almost certainly due to presence of broad spectrum enhanced metabolic resistance in addition to the more specific ALS target site resistance.

Table 3.2.1.1 Control of black-grass plants in outdoor container experiment 1

		% reduction in plant numbers compared to untreated containers			
		Peldon03		Wilts05	
	Treatment	Yr1	Yr2	Yr 1	Yr 2
1	ALS x1	69	54	76	30
2	ALS x2	76	43	75	42
3	ALS + other MOA mixture	84	59	83	48
4	ALS + other MOA sequence	94	60	87	64
5	Herbicide rotation	69	50	76	95
6	Other MOA sequence	57	45	82	85
	S.E. ±	3.08	6.50	2.63	3.59
	L.S.D. ($P \leq 0.05\%$)	9.27	19.59	7.92	10.83

Key: 1&2 = ALS only treatments. 3&4 = ALS in mixture or sequence with non-ALS herbicides.
5 = rotational treatment (ALS used in Yr 1 only). 6 = Other MOA treatments in containers.

Meaned over all six herbicide treatments, control declined significantly from a mean 75% to 52% (S.E. ± 2.09) with Peld03 and from 80% to 61% (S.E. ± 1.26) with Wilts05. These 19 – 23% declines represent appreciable declines in herbicide efficacy in one year. Activity for the four treatments (Treatments 1 – 4) which included an ALS herbicide (mesosulfuron+iodosulfuron) declined more than the non-ALS treatment (Treatment 6). Meaned over treatments 1 – 4, control declined by 27% with Peld03 (81% to 54%) and by 34% (80% to 46%) with Wilts05 – appreciably greater than the 12% and -3% recorded with the other MOA sequence (Treatment 6).

Applying mesosulfuron+iodosulfuron twice (Treatment 2, mean control 59%) did not greatly increase control over the single application (Treatment 1, mean control 57%). The use of herbicides with other modes of action in mixture or sequence with mesosulfuron+iodosulfuron (Treatments 3 & 4) gave a useful, but modest, increase in overall level of control compared with mesosulfuron+iodosulfuron alone (Treatment 1). This trend was consistent with both populations in both years. The ALS + other MOA sequence (Treatment 4) consistently gave slightly better control than the ALS + other MOA mixture (Treatment 3), demonstrating the benefit of including a pre-emergence herbicide component in a sequence.

Significantly, the use of other modes of action in mixture or sequence with mesosulfuron+iodosulfuron did not prevent declines in herbicide efficacy. The mean declines for the ALS alone treatments (Treatments 1 & 2) versus ALS in mixture or sequence with other MOA (Treatments 3 & 4) were respectively: 24% v 30% for Peld03 and 40% v 29% for Wilts 05, or 32% v 30% meaned over both populations.

The rotational treatment, effectively treatment 1 in first year and treatment 6 in the second, gave slightly better control in the second year than the other MOA sequence (Treatment 6) with both Peld03 and Wilts 05. It gave the highest control of all treatments with Wilts05 in the second year, but not with Peld03. However, many other climatic and environmental factors can affect herbicide activity in addition to resistance, so trends between years based on herbicide efficacy in outdoor conditions should be treated with caution, although the container system does minimise these effects to a large extent. Hence tests on seeds in the glasshouse allow changes in resistance to ALS inhibiting herbicides to be quantified more critically.

The results for the glasshouse tray assay in which plants grown from seeds collected each year from each container were treated with mesosulfuron+iodosulfuron are presented in Table 3.2.1.2 and Figure 3.2.1.1. Plants of the Roth susceptible standard were all killed (100% reduction in plant numbers) confirming that this population was susceptible and that the application methodology was conducive to good control. Control of the Peld03 and Wilts05 baseline populations was 82% and 76% respectively, which was similar to predictions (82% and 69%) based on plant survival in previous glasshouse screening assays. Control of the samples from containers treated with mesosulfuron+iodosulfuron alone (Treatments 1 & 2) was much poorer and declined progressively, with a mean of only 26% control of Peld03 and 41% control of Wilts 05 after two years, representing a 56% and 35% decline in activity. Applying mesosulfuron+iodosulfuron twice (Treatment 2) resulted in significantly poorer control after two years with Peld03, but not with Wilts 05, compared with the single application (Treatment 1). Where mesosulfuron+iodosulfuron was used in mixture or sequence with other MOA (Treatments 3 & 4, mean Peld03 = 37%, Wilts05 = 47%), control was similar to where it was used alone (Treatments 1 & 2, mean Peld03 = 41%, Wilts05 = 45%). Consequently, there was no evidence that using mesosulfuron+iodosulfuron in mixture or sequence with other MOA reduced selection for ALS resistance, compared to applying mesosulfuron+iodosulfuron alone. In both cases, resistance increased substantially over the two years.

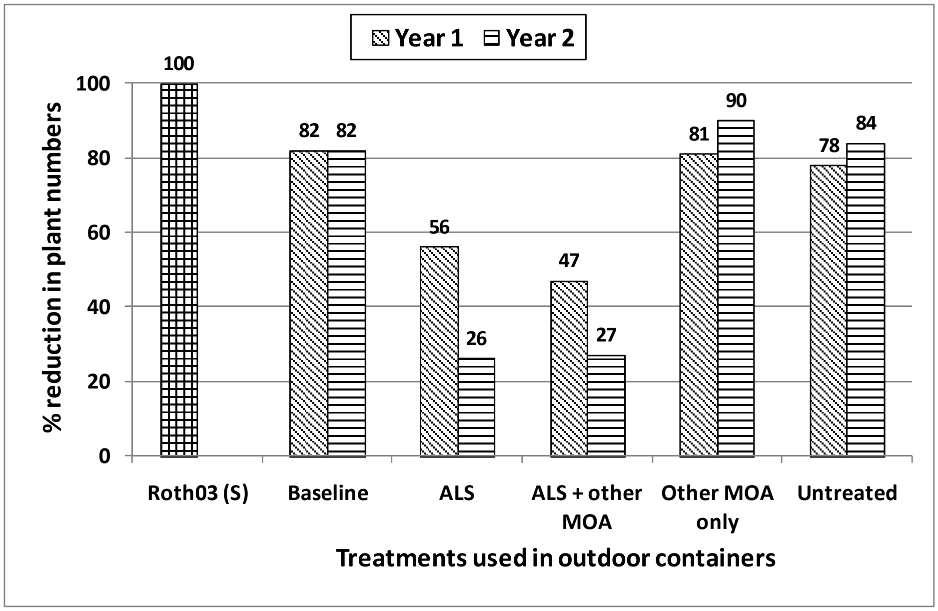
Table 3.2.1.2 Control of black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in a glasshouse evaluation of seeds collected from outdoor container experiment 1. (Note: Plants tested were grown from seeds obtained from the outdoor containers receiving the treatments listed in the left columns, but all the values in the table relate to control by mesosulfuron+iodosulfuron in this glasshouse tray assay).

		% reduction in plant numbers by mesosulfuron+iodosulfuron (12+2.4 g a.i./ha) in glasshouse assay			
		Peldon03		Wilts05	
	Container Treatment	Yr1	Yr2	Yr 1	Yr 2
1	ALS x1	57	35	49	42
2	ALS x2	54	16	49	39
3	ALS + other MOA mixture	39	28	52	47
4	ALS + other MOA sequence	55	26	39	48
5	Herbicide rotation	57	50	49	31
6	Other MOA sequence	81	90	75	75
7	Untreated	78	84	82	78
	Original baseline seeds	82	82	76	76
	S.E. ±	6.16	5.99	3.41	4.71
	L.S.D. (P≤0.05%)	18.30	17.62	10.12	13.86

Key: 1&2 = ALS only treatments. 3&4 = ALS in mixture or sequence with non-ALS herbicides.
5 = rotational treatment (ALS used in Yr 1 only). 6 = Other MOA treatments in containers.

The rotational treatment (Treatment 5, mesosulfuron+iodosulfuron in yr 1; other MOA sequence in yr 2) showed a marked reduction in control by mesosulfuron+iodosulfuron after one year, as would be expected. There was little further reduction in control following the second year's non-ALS treatment with Peld03, but a larger reduction with Wilts05. One possible explanation for this apparently anomalous result is that the non-ALS treatment gave very good (95%) control of Wilts05 in the outdoor containers in the second year, leaving a mean of only five plants/container. If these happened, by chance, to be particularly ALS resistant, then that could explain the further apparent selection for resistance. Certainly the use of a non-ALS treatment in the second year did not prevent a high degree of ALS resistance being maintained in both populations.

(a) Peld03



(b) Wilts05

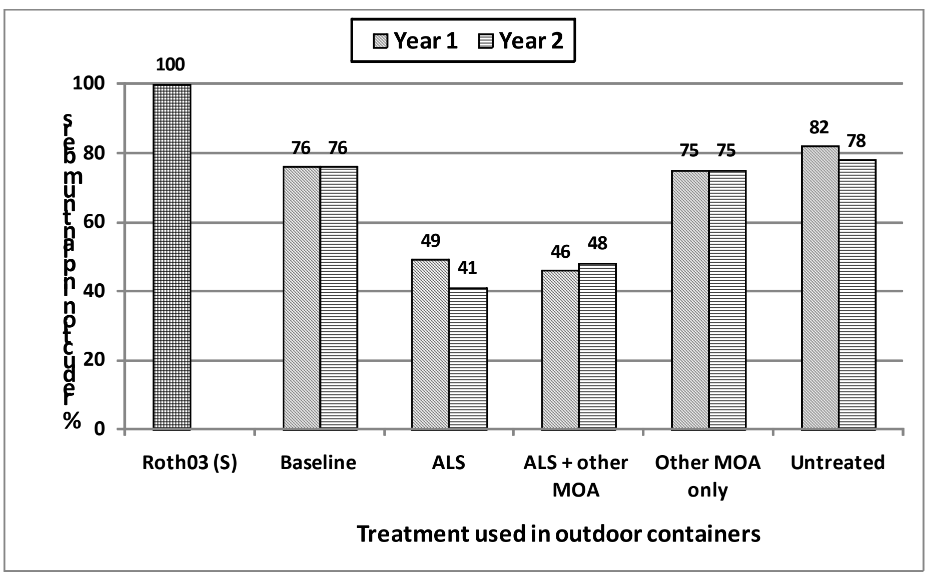


Figure 3.2.1.1 Control of black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in a glasshouse evaluation of seeds collected from outdoor container experiment 1. (Note: The ALS and ALS + other MOA values are each the mean of two treatments. See Table 3.2.1.2 for full data)

With both populations, seeds derived from the other MOA sequence, non-ALS treatment (Treatments 5) gave very similar results to the baseline populations (mean difference = +1.3%). Consequently, there was no evidence that the non-ALS herbicides used in Treatment 5 (isoproturon, pendimethalin, clodinafop, trifluralin) were selecting for increased resistance to mesosulfuron+iodosulfuron (e.g. by enhanced metabolism) over the two years of the container trial. The untreated (Treatment 7) populations showed no evidence of loss of resistance, compared with the baseline (mean difference = +1.5%), which might have indicated a fitness penalty with ALS target site resistant plants.

The molecular assays on the plants derived from the Peld03 population which survived mesosulfuron+iodosulfuron in the glasshouse tray assay, showed that all were resistant (9 homozygous, 15 heterozygous) due to the Pro-197-Thr mutation conferring ALS target site resistance. This confirmed that with the Peld03 population, mesosulfuron+iodosulfuron was selecting primarily for ALS target site, rather than enhanced metabolic, resistance. The selection target in the Wilts05 population is unknown, but is neither of the two commonest ALS mutations. It could be enhanced metabolism, but we believe it is more likely to be a less common ALS target site resistance mutation. However, difference in the resistance mechanisms between Peld03 and Wilts05 do not affect the overall conclusions.

Container experiment 2. Plants established well in the containers with a mean of 135 plants in untreated containers. The Roth plants were killed by most treatments (100% reduction in plant numbers) except for Treatment 10 (pendimethalin) 87.5%, Treatment 11 (clodinafop applied in spring) 98.6% and Treatment 13 (mesosulfuron+iodosulfuron applied at half field rate in spring) 98.6%. This confirmed that this population was susceptible and that the application methodology and conditions at time of application were conducive to good control.

In contrast, poorer control was achieved by virtually all treatments applied to the Peld03 population, confirming resistance (Table 3.2.1.3). As in container experiment 1, the fact that reduced control occurred with non-ALS herbicides (treatment 11-14) confirmed that resistance was not confined just to ALS inhibiting herbicides.

Table 3.2.1.3 Control of Peld03 black-grass plants by a range of treatments in outdoor container experiment 2, and control by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in the glasshouse evaluation of seeds collected from surviving plants from each container treatment.

	Treatment	% reduction in plant numbers	
		In outdoor containers	By mesosulfuron+ iodosulfuron in glasshouse assay
1	ALS x1 autumn	82	26
2	ALS x1 spring	88	32
3	ALS x2 autumn and spring	85	30
4	ALS half rate spring	77	40
5	ACCase autumn/ALS spring	97	42
6	ALS autumn/ACCase spring	94	27
7	ALS+ACCase autumn	79	31
8	ALS+ACCase spring	92	35
9	ALS+pend autumn	83	26
10	Pre-em./ALS spring	88	34
11	ACCase autumn	89	79
12	ACCase spring	78	87
13	Pendimethalin autumn	40	79
14	Other MOA sequence	74	82
15	Untreated	(135 plants)	86
-	Peld03 baseline	-	83
	S.E. ±	3.46	4.46
	L.S.D. ($P \leq 0.05$)	9.91	12.70

Key: 1-4 = ALS only treatments. 5-10 = ALS in mixture or sequence with non-ALS herbicides. 11-14 = Other MOA treatments in containers.

Control from mesosulfuron+iodosulfuron applied alone in autumn (Treatment 1) was slightly better (82%) in this experiment than in the first year of container experiment 1 (69%), even though the same seeds (Peld03) had been sown. This was probably due to slightly colder winter conditions which may have increased mortality of damaged plants (mean temp at 10 cm under bare soil for Dec - March = 4.4 °C 2004/05; 3.7°C 2005/06). Overall, there was little difference in control between autumn and spring, or two sequential applications of mesosulfuron+iodosulfuron (Treatments 1 – 3). However, half rate applied in spring (Treatment 4) gave significantly poorer control (77%) than full rate (88%) at the same timing. Mesosulfuron+iodosulfuron (=ALS) used in mixture or sequence with other MOA (clodinafop = ACCase; pendimethalin= pend; flufenacet+pendimethalin = Pre-em) (Treatments 5 – 10) gave generally quite high levels of control, between 79% and

97% (mean 89%). Overall, this was slightly better than the mean (83%) of the four mesosulfuon+iodosulfuron treatments alone (Treatments 1 – 4), supporting the results of the container 1 experiment. Applied alone, the ACCase herbicide, clodinafop, tended to work slightly better in autumn than spring whereas the reverse was true with mesosulfuron+iodossulfuron (= ALS) (See results for Treatments 1, 2, 11 & 12). This trend was supported by the results for the herbicide sequences with clodinafop (=ACCase) in autumn followed by mesosulfuron+iodosulfuron (=ALS) in spring (Treatment 5) giving the highest control (97%) overall. The sequences (Treatments 5, 6, 10) gave slightly better control than the mixtures (Treatment 7, 8, 9) overall (mean 93% v 85%). Pendimethalin alone gave mediocre (40%) control of Peld03 and added very little when used in mixture with mesosulfuron+iodosulfuron (see Treatments 1, 13 & 9). However, the sequence of flufenacet+pendimethalin (= Pre-em.) applied pre-emergence followed by mesosulfuron+iodosulfuron (= ALS) in spring (Treatment 10), gave the same control (88%) as the ALS herbicide alone (Treatment 2). The non-ALS herbicide treatment (Other MOA sequence, Treatment 14) gave only 74% control. This was the poorest treatment of all (except for pendimethalin alone), but slightly better than the equivalent treatment in container experiment 1, which gave only 57% control, although not with identical herbicides. These results highlight the difficulty of achieving adequate control of the Peld03 population with herbicides that are not ALS or ACCase inhibitors.

The results for the glasshouse tray assay in which plants grown from seeds collected each year from each container were treated with mesosulfuron+iodosulfuron are presented in Table 3.2.1.3 and Figure 3.2.1.2. Plants of the Roth susceptible standard were all killed (100% reduction in plant numbers) confirming that this population was susceptible and that the application methodology was conducive to good control. Control of the Peld03 baseline population was 83% which was very similar to that obtained (82%) in the equivalent test in container experiment 1. This provides good validation of the technique used. Control of the samples from containers treated with mesosulfuron+iodosulfuron alone (Treatments 1 - 4) was much poorer, with a mean of only 32% control. Applying mesosulfuron+iodosulfuron at half field rate resulted in slightly better control (40%) than where the full or repeated applications had been made (mean 29%). Compared with full rate, using half rate had slightly reduced the control of black-grass in the outdoor containers but had also slightly reduced selection for resistance. So, there is no evidence that a reduced rate increased selection for resistance – in fact the reverse was true.

Where mesosulfuron+iodosulfuron was used in mixture or sequence with other MOA (Treatments 5 – 10, mean 33%) control was almost identical to where it was used alone (Treatments 1-4, mean 32%) (Figure 3.2.1.2). So, as with container experiment 1, there was no evidence that using mesosulfuron+iodosulfuron in mixture or sequence with other MOA reduced selection for ALS resistance, compared to applying mesosulfuron+iodosulfuron alone. Selection for resistance was identical in both cases.

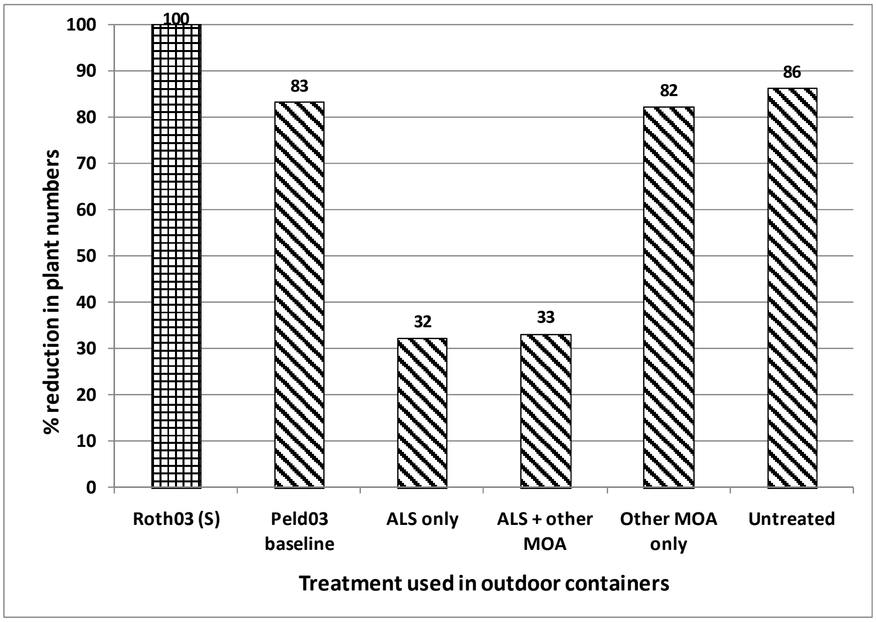


Figure 3.2.1.2 Control of black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in the glasshouse evaluation of seeds collected from outdoor container experiment 2. (Note: The ALS only, ALS + other MOA and Other MOA values are means of four, six and four treatments respectively. See Table 3.2.1.3 for full data).

In contrast, seeds derived from the other MOA, non-ALS treatments, (Treatments 11 - 14) gave very similar results to the Peld03 baseline population (mean difference = 1%). Consequently, as with container experiment 1, there was no evidence that the non-ALS herbicides used (clodinafop, pendimethalin, isoproturon, flufenacet) were selecting for increased resistance to mesosulfuron+iodosulfuron (e.g. by enhanced metabolism) over the one year of this container trial. The untreated (Treatment 15) populations showed no clear evidence of loss of resistance, compared with the baseline (mean difference = +3%), which might have indicated a fitness penalty with ALS target site resistant plants.

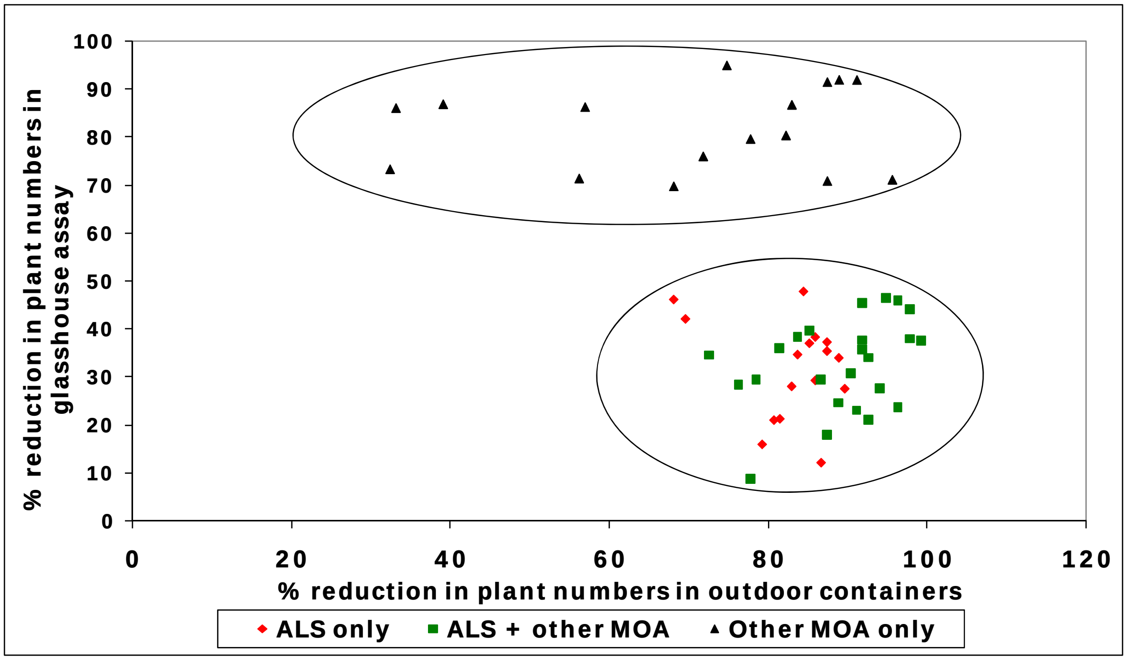


Figure 3.2.1.3 % reduction values for each individual container in terms of control of plants outdoors (x axis) and in the glasshouse tray assay (y axis) for container experiment 2.

The results for each individual container, both in terms of control of plants outdoors and in the glasshouse tray assay are presented in Figure 3.2.1.3. The points fall into two distinct groups, as indicated. Points for ALS treatment alone, or in mixture or sequence with other MOA, form a single and distinct group. In marked contrast, points for the other MOA (non-ALS) treatments form a distinctly separate group. In this group, the degree of control of plants outdoors (x axis) is clearly not related to the degree of resistance to mesosulfuron+iodosulfuron recorded in the glasshouse assay (y axis). There might have been a positive relationship if enhanced metabolic resistance had been more significant. This scatter plot highlights the high selection for resistance that occurs with mesosulfuron+iodosulfuron, irrespective of whether it is used alone or in mixture or sequence with other modes of action.

Generally, control of Peld03 black-grass in the outdoor containers was better, and control in the glasshouse trays poorer, in container experiment 2 compared with container experiment 1. This was certainly true for identical treatments, which were applied in different calendar years. Mesosulfuron+iodosulfuron applied in autumn gave 69% and 82%, and the repeat application treatment 76% and 85% reductions in plant numbers in outdoor container experiments 1 & 2 respectively. The respective values for control in glasshouse tray assay were 57% and 26%, and 54% and 30%. However, the fact that the control of the Peld03 baseline population was very consistent in both glasshouse tray assays indicates that these differences were real. So, as with the results for the reduced rate treatment of mesosulfuron+iodosulfuron, poorer control of plants in containers was associated with lower levels of resistance selection, and vice-versa. The trends and overall conclusions of both experiments were entirely consistent.

Container Experiment 3. In 2006, high levels of control were achieved using trifluralin. All populations had 90% or more control (Figure 3.2.1.4). The Wilts05 population had 98% control, which left virtually no plants to go through to flowering and produce seed for the following year's trial. The seed that was produced was very small and of poor quality, so it was not possible to proceed with the Wilts05 population, treated with trifluralin in the following year.

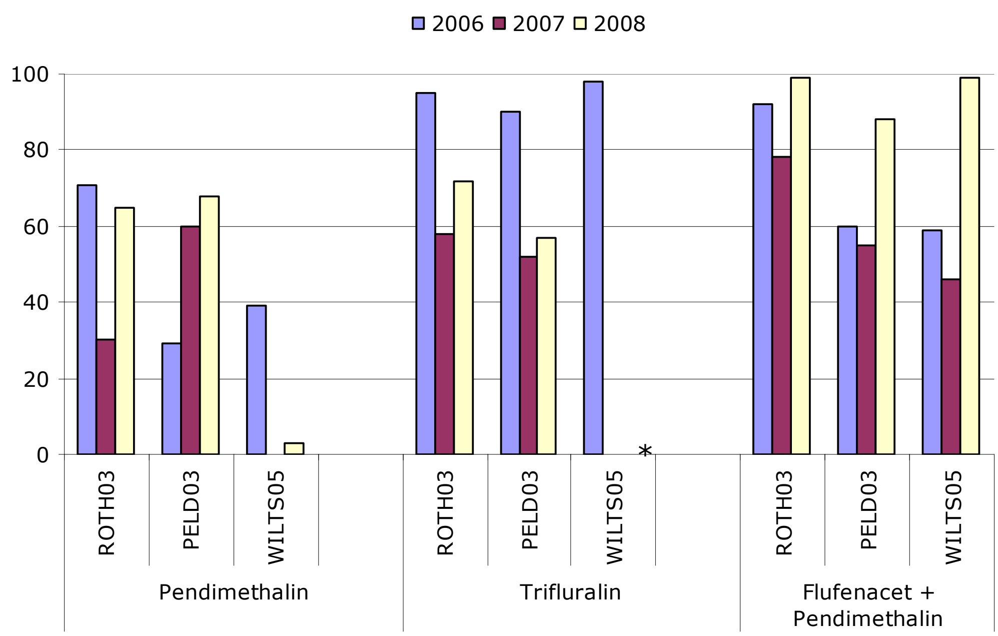


Figure 3.2.1.4. Percentage reduction in plant numbers achieved for each population, treated with each treatment over the three years of the experiment.

It can be seen from Table 3.2.1.4 that in the majority of cases the level of control achieved in 2008 was greater than, or similar too, that seen in 2006. The only population treatment combinations that showed a poorer level of control were Wilts05 treated with pendimethalin and Peld03 treated with trifluralin. The Roth populations treated with pendimethalin or trifluralin also showed a reduction in the level of control, but the seed used in 2008 was the same original seed as that used in 2006, and was not collected from containers previously treated with either active ingredient. If all the treatments are averaged together it can be seen that overall there was no evidence of repeated applications of a single pre-emergence herbicide causing a shift in the resistance levels of a black-grass population.

Table 3.2.1.4 Percentage reduction in plant numbers in 2006 and 2008 showing change in level of control.

	% reduction in plant numbers								
	Roth			Peld			Wilts		
	2006	2008	change	2006	2008	change	2006	2008	change
pendimethalin	71	65	-6	29	68	+39	39	3	-36
trifluralin	95	72	-23	90	57	-33	98	100	+2
flufenacet + pendimethalin	92	99	+7	60	88	+28	59	99	+40
Mean reduction	86	79	-7	60	71	+11	65	67	+2

The variable results in this trial make it difficult to determine whether a sufficiently long period of assessment has occurred to detect any changes in resistance level. The Peld03 population, treated with trifluralin seems to have shown a reduction in the level of control, but there was also a reduction (although not as large) in the level of control achieved in the Roth susceptible control population, indicating that there may have been external factors that influenced the result. The Wilts05 population showed a reduction in the level of control when treated with pendimethalin but, conversely, the Peldon population showed a similar increase in level of control. The percentage reduction in plant numbers seen in the control Roth susceptible population showed little change. All other combinations of population and pre-emergence herbicide showed no change or an increase in the level of control between 2006 and 2008, making it difficult to draw any conclusions about selection for resistance.

Conclusions of the three container experiments:

- **Mesosulfuron+iodosulfuron can very rapidly select for ALS resistance, resulting in an appreciable and rapid loss of efficacy.**
- **Results were similar with both populations studied despite different resistance mechanisms in the two populations.**
- **The use of non-ALS herbicides in mixture or sequence with mesosulfuron+iodosulfuron can increase levels of weed control.**
- **The use of non-ALS herbicides in mixture or sequence with mesosulfuron+iodosulfuron does *not* reduce selection for ALS resistance.**
- **The use of herbicides with other modes of action, in mixture or sequence with mesosulfuron+iodosulfuron, should be viewed as a useful method of increasing overall weed control, but not as a resistance prevention or mitigation strategy.**
- **Non-ALS herbicide treatments do not appear to select very actively for resistance to mesosulfuron+iodosulfuron (e.g. by enhanced metabolism), or at least not over the limited time scale (2 yrs) studied in these experiments.**
- **Resistance to mesosulfuron+ iodosulfuron does not appear to decline to any significant extent if it ceases to be used.**
- **Non-ALS pre-emergence herbicides, such as pendimethalin, trifluralin and flufenacet+pendimethalin, did not appear to select very actively for resistance over the three year time scale studied in these experiments.**
- **There was considerable year to year variation in herbicide efficacy of the pre-emergence herbicides studied, making it difficult to detect changes in resistance.**

3.2.2 Field experiments: To quantify the influence of herbicide history on the development of ALS resistance.

The two sites were existing long-term field experiments, at Boxworth and Rothamsted, with plots which had received contrasting herbicide treatments (Moss *et al*, 2005b). At the Boxworth site, in the three cropping years between 2000/01 and 2002/03, clodinafop had been used alone or in mixture with flupyrsulfuron in the two wheat crops, and either fluazifop or propyzamide used in the oilseed rape break. In 2003/04 all plots were treated with mesosulfuron + iodosulfuron and seeds were collected annually for resistance testing. This site provided an opportunity to investigate the sustainability of different herbicidal mitigation strategies based on the use of ALS inhibiting herbicides in mixture and/or sequence with other modes of action.

At the Rothamsted site, treatment between 2000/01 and 2002/03 with different intensities (from 0 – 100%) of 'fop'/'dim' herbicides (clodinafop, fluazifop, cycloxydim, tepraloxydim), used in mixture or sequence with flupyrsulfuron and propyzamide, resulted in plots with widely differing levels of ACCase target site resistance (from 20 – 90%). The increase in ACCase target site resistance, based on annual seed sampling and testing, was very well correlated with intensity of fop/dim use. These plots provided an ideal opportunity to determine whether the development of target site resistance to one mode of action (e.g. ACCase inhibitors) increased the risk of target site resistance to another *different* mode of action (e.g. ALS inhibitors).

Seed samples from other field experiments conducted by the agrochemical industrial partners, were also tested to quantify the influence of different herbicide regimes on the development of resistance to ALS inhibiting herbicides.

Materials & Methods

Boxworth field experiment. This was a continuation of one of the field experiment used in the HeRMES resistance project, from 2000 to 2005 (Moss *et al*, 2005b). It was located in Extra Close Field at ADAS Boxworth. The trial area was grown as a commercial crop with all fertiliser and spray applications (except herbicides) made according to local farm practice. Plots were 18m x 24m wide with a 12m cropped strip between blocks; in the original HeRMES trial there were four replicates of six treatments, including two different cultivations (plough and min-till). Seed samples were collected from the central 6m x6m section of each plot to minimise cross pollination.

Repeated annual cultivations remained the same in HeRMES as was used in the previous trial, as either plough or minimum cultivation. Plots were then drilled with either winter wheat or winter oilseed rape as a rotation (Table 3.2.2.1 below)

Table 3.2.2.1 Cropping history for Boxworth field experiment

Year	2000-01	01-02	02-03	03-04	04-05	05-06	06-07	07-08
Crop	WW	wOSR	WW	WW	wOSR	WW	WW	wOSR

(ww = winter wheat, wOSR = winter oilseed rape)

During the four years of the previous trial the area had a history of herbicide applications based around 'fops' alone, or in sequence with 'amides', or in mixture with 'SU' (Table 3.2.2.2).

Table 3.2.2.2 Treatments in HeRMES experiment (Defra PSD) Boxworth 2000-2004

Treat	Cultivation	Herbicide treatment	
		Wheat	Winter Oilseed Rape
1	Min till	Clodinafop-propargyl 30 g/ha **(fop)**	Fluazifop-P-butyl 94 g/ha **(fop)**
2	Plough	Clodinafop-propargyl 30 g/ha **(fop)**	Fluazifop-P-butyl 94 g/ha **(fop)**
3	Min till	Clodinafop-propargyl 30 g/ha **(fop)**	Propyzamide 700g a.i/ha **(amide)**
4	Plough	Clodinafop-propargyl 30 g/ha **(fop)**	Propyzamide 700g a.i/ha **(amide)**
5	Min till	Clodinafop-propargyl 30 g/ha + Flupyrsulfuron-methyl 10g/ha **(fop + SU)**	Fluazifop-P-butyl 94 g/ha **(fop)**
6	Min till	Clodinafop-propargyl 30 g/ha + Flupyrsulfuron-methyl 10g/ha **(fop + SU)**	Propyzamide 700g a.i/ha **(amide)**

In the project described in this report, all plots were treated with the same herbicide, either mesosulfuron+iodosulfuron (12 + 2.4 g a.i. ha $^{-1}$ + 'Biopower' @ 1 L ha $^{-1}$) on wheat or propyzamide (850 g a.i. ha $^{-1}$) on oilseed rape.

For each year of this trial seed was collected from each plot, this was cleaned dried and stored. In 2003 seed samples from before the first mesosulfuron+iodosulfuron application was made and after the third mesosulfuron+iodosulfuron application (2007) were tested in a glasshouse pot test for ALS resistance. The 2007 seed was also tested using the Rothamsted Rapid Resistance Test (RRRT) for ACCase resistance and pendimethalin resistance.

The number of black-grass plants m^{-2} were counted in the autumn and spring of each season, using 10 x 0.1m^2 quadrats per plot, to assess black-grass emergence and herbicide effects. A black-grass head count was carried out on all plots in late spring/early summer of each season, using the same method. In June/July of each season black-grass seed was collected from each plot using the standard black-grass resistance testing method. Seeds were air dried and stored in paper envelopes ready to be cleaned and tested for resistance in the final year of the project.

Table 3.2.2.3 Details of sowing and herbicide application dates at Boxworth

Application and date	2005/06	2006/07	2007/08
Cultivations	04/10/05– 07/10/05	19/09/06- 04/10/06	14/09/07- 20/09/07
Plough	28/09/05	19/09/06	14/09/07
Min tillage	29/09/05	27/09/06	18/09/07
Drilling date	06/10/05	04/10/06	19/09/07
(cv)	(Consort)	(Consort)	(Nijinsky)
Herbicide application	05/04/06	22/11/06	29/01/07

Table 3.2.2.4 Details of assessment dates for all seasons at Boxworth

Assessment and date	2005/06	2006/07	2007/08
Autumn plant count	29/11/05	06/12/06	21/12/07
Spring plant count	29/03/06	02/03/07	-
Summer head count	03/07/06	15/06/07	11/06/08
Seed collection	July 2006	July 2007	July 2008

Rothamsted field experiment. This was a continuation of one of the field experiment used in the HeRMES resistance project, from 2000 to 2005 (Moss *et al*, 2005b). It was located 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. There were four replicates and cultivations in all years were by shallow tine/discs to 10 cm maximum depth. 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.

Winter oil-seed rape had been sown in 2000/01 and separate plots were treated with cycloxydim (200 g a.i./ha) + oil (C), tepraloxydim (50 g a.i./ha) (T), fluazifop-P-butyl (187.5 g a.i./ha) + 'Partna' (two plots per rep) (F1, F2), propyzamide (700 g a.i./ha) (two plots per rep) (P1, P2), propyzamide+cycloxydim + oil (P+C) and untreated (Nil). In 2001/02 & 2002/03 cropping years, the cycloxydim, tepraloxydim, 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 duplicate plots treated with fluazifop and propyzamide in 2000/01 were sown with winter wheat in 2001/02 &

2002/03, with the pairs of plots treated with clodinafop (30 g a.i./ha) + mineral oil alone (F1, P1) or in mixture with flupyrsulfuron (10 g a.i./ha) (F2, P2). All plots were uncultivated and uncropped during 2003/04 and regrowth was cut to prevent black-grass seed return.

For the next 4 cropping years (2004/05, 2005/06, 2006/07, 2007/08) all plots were sown with winter wheat between 13 October and 2 November following shallow tine/disc tillage, and all plots treated with mesosulfuron+iodosulfuron (12+2.4 g a.i./ha) each spring between 25 February and 3 April. Consequently some plots (C, T, F1, P1, P+C, Nil) had received no grass-weed ALS inhibitors for four years prior to the use of mesosulfuron+iodosulfuron, whereas the F2 and P2 plots had been treated with an ALS inhibitor (flupyrsulfuron) in mixture with clodinafop in two of the preceding four years.

Weed populations were assessed by counting plants or heads in 10 x 0.1 m^2 random quadrats per plot. In addition, seed samples were collected from surviving plants in July each year. Seed samples collected from each plot in 2007 were evaluated for response to mesosulfuron+iodosulfuron in a glasshouse tray assay using the method described for the container experiments (see section 3.2.1 above). Seed samples from the summer 2003 Nil plots (3 years without herbicide) were also included as a baseline population together with the Roth04 susceptible standard. Twenty-eight surviving plants grown from seeds collected in 2007 were assayed at the molecular level using the protocol of Delye & Boucansaud (2008).

To confirm that the findings of the glasshouse tray assay were applicable to outdoor conditions, seeds collected from the summer 2008 'Nil' plots (by this time treated with mesosulfuron+iodosulfuron for 4 successive years) were tested alongside samples previously collected from the same plots in summer 2003 (Nil plots, 3 years without herbicide) in an outdoor small container assay using methods described by Moss & Hull (2009). A susceptible reference population (Roth05) was also included. Seeds (250 per container) were sown in outdoor containers (28.5 x 18.5 x 13 cm deep) containing a Kettering loam soil on 25 September 2008 at Rothamsted. Mesosulfuron+iodosulfuron at 12+2.4 g a.i. ha (field rate) and 6+1.2 g a.i. ha (half field rate) was applied with the recommended adjuvant ('Biopower' at 0.5% spray volume) on 6 November 2008 when black-grass plants were at the 2 – 3 leaf stage, using a laboratory sprayer delivering 236 litres water/ha at 238 kPa through a single

‘Teejet’ flat fan 110015VK nozzle. The experiment comprised a randomised block design with four replicates, with one untreated container per population per replicate. The number of black-grass plants present before spraying was assessed, as was the number of survivors and foliage fresh weights on 11 March 2009.

Results

Boxworth field experiment. In the first season of the field trial the autumn counts showed that the initial levels of black-grass present in the plots tended to be highest on those plots that were minimally tilled and which had received a high proportion of ‘fop’ use in recent history (i.e. on both winter wheat and winter oilseed rape). These plots averaged 315-379 plants m^{-2} (Table 3.2.2.5). The next highest levels of black-grass were in those plots that had been ploughed with between 211-243 plants m^{-2}. The lowest level of black-grass present was in those plots that had a previous history of minimum tillage and amide usage during the oilseed rape crop, with between 115-133 plants m^{-2}.

(See overleaf for table 3.2.2.5)

Table 3.2.2.5 Boxworth autumn plant counts for three seasons – comparing against cultivations, historic herbicide applications and current herbicide applications.

	HeRMES (2001-2004) treatments		Plants m^{-2} – autumn (pre spray application)		
			2005/06	2006/07	2007/08
	Winter wheat	Winter Oilseed Rape	Mesosulfuron+ iodosulfuron 12+2.4g ha^{-1} **(SU)**	Mesosulfuron+ iodosulfuron 12+2.4g ha^{-1} **(SU)**	Propyzamide 850g a.i ha^{-1} **(amide)**
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	379	167	35
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	243	61	108
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700g a.i ha^{-1} **(amide)**	115	84	16
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700g a.i ha^{-1} **(amide)**	211	46	163
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10g ha^{-1} **(fop + SU)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	315	213	28
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10g ha^{-1} **(fop + SU)**	Propyzamide 700g a.i ha^{-1} **(amide)**	133	86	22

The second treatment with mesosulfuron+iodosulfuron (2005/06) resulted in some moderate levels of control on the minimally tilled plots, especially in plots where there had been less black-grass to start with (previous history of propyzamide applications). About 40% control was achieved on ploughed plots (Table 3.2.2.6). Mesosulfuron+iodosulfuron application in this year was late (April) due to poor weather conditions and it is likely that the black-grass plants were on the large side at time of application especially on min-till plots. Those plants on the ploughed plots would potentially have been growing from depth, resulting in a proportion being of smaller size and therefore easier to control with this late application of mesosulfuron+iodosulfuron.

In the third year of treatment with mesosulfuron+iodosulfuron, the level of control was greater at an average 68.5% control. Mesosulfuron+iodosulfuron was applied in the autumn (22/11/06) to smaller plants. The highest levels of control were achieved on ploughed plots or those that had a history of high 'fop' applications. Slightly lower levels of control were achieved on plots that had a history of amide applications. Previous SU usage did not appear to affect the level of control achieved.

Table 3.2.2.6 Boxworth: percentage reduction in black-grass numbers for two seasons (not done in winter oilseed rape) – comparing against cultivations, historic herbicide applications and current herbicide applications.

	HeRMES (2001-2004) treatments		% control	
	Winter wheat	Winter oilseed rape	2005/06 Mesosulfuron+ iodosulfuron 12+2.4g ha^{-1} **(SU)**	2006/07 Mesosulfuron+ iodosulfuron 12+2.4g ha^{-1} **(SU)**
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	24	75
Plough	Clodinafop-propargyl 30 ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	43	75
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700g a.i ha^{-1} **(amide)**	-3	64
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700g a.i ha^{-1} **(amide)**	41	69
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10 g ha^{-1} **(fop + SU)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	9	71
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10 g ha^{-1} **(fop + SU)**	Propyzamide 700g a.i ha^{-1} **(amide)**	-92	57

Note: mesosulfuron+iodosulfuron also applied to all plots in 2004/05

Despite apparently better levels of control in the third year of mesosulfuron+iodosulfuron use (2006/07) the number of heads present in the plots at the end of that season tended to be higher than were present at the end of 2005/06 (Table 3.2.2.7).

Table 3.2.2.7 Boxworth: head counts for three seasons – comparing against cultivations, historic herbicide applications and current herbicide applications.

	HeRMES (2001-2004) treatments		Heads m^{-2}		
	Winter wheat	Winter oilseed rape	2005/06 Mesosulfuron + iodosulfuron 12+2.4 g/ha **(SU)**	2006/07 Mesosulfuron +iodosulfuron 12+2.4 g/ha **(SU)**	2007/08 Propyzamide 850g a.i/ha **(amide)**
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	127	182	597
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	156	112	1196
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700g a.i ha^{-1} **(amide)**	48	118	584
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700g a.i ha^{-1} **(amide)**	93	112	1212
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10g ha^{-1} **(fop + SU)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	235	225	534
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10g ha^{-1} **(fop + SU)**	Propyzamide 700g a.i ha^{-1} **(amide)**	56	114	604

The head counts from 2007/08 show a large increase in heads compared to the previous year. This is despite relatively low plant numbers seen during the autumn plant counts. The oilseed rape crop that was planted in the plots in this season was drilled into a dry seed bed. A lack of autumn rainfall and high pressure from pigeon and rabbit grazing resulted in the crop failing. Additional seed was broadcast onto the trial site on 07/11/07 to try and establish a crop. This remained poor and non-competitive through out the duration of the trial. Initial indications from the propyzamide applications were that the level of control on the min-till plots was very good – with obvious brown areas where the black-grass was dying. The control on the ploughed areas appeared poor with large areas of green marking the ploughed plots. Propyzamide is known to be very effective in shallow rooted black-grass, however, the lack of competition from a viable winter oilseed rape crop resulted in the few surviving black-grass on the min-till plots being able to recover and produce heads. The propyzamide slowed its growth, and reduced the number of tillers compared to the plough plots, but without crop competition failed to completely kill many black-grass plants. It is likely that in a commercial situation this field would have been re-sown in the spring, never allowing the black-grass to reach such high levels.

Black-grass seed samples collected at the end of the HeRMES project in 2003 and also after the second mesosulfuron+iodosulfuron application in the current trial (2007) were tested in a glass house pot trial to determine whether or not a shift in the level of resistance to mesosulfuron+iodosulfuron had occurred. All samples tested from the 2003 batch of seed were susceptible to Atlantis, irrespective of the previous herbicide applications that had occurred, with all samples showing a 98-99% reduction in biomass compared to untreated controls (Table 3.2.2.8).

Table 3.2.2.8 Glasshouse assay of seeds collected from the Boxworth field experiment. Reduction in biomass resulting from treatment with mesosulfuron+ iodosulfuron, compared to untreated controls.

	HeRMES (2001-2004) treatments		% reduction in biomass	
	Winter Wheat	Winter oilseed rape	2003	2007
			Baseline	Mesosulfuron+iodosulfuron 12+2.4 g ha^{-1} **(SU)** (x 3 years)
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 94 g ha^{-1} **(fop)**	98	97
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 94 g ha^{-1} **(fop)**	99	97
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700 g ha^{-1} **(amide)**	98	97
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700 g ha^{-1} **(amide)**	98	97
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10 g ha^{-1} **(fop + SU)**	Fluazifop-P-butyl 94 g ha^{-1} **(fop)**	98	96
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10 g ha^{-1} **(fop + SU)**	Propyzamide 700g g ha^{-1} **(amide)**	98	91
Untreated			99	
Roth 06 susceptible				98

In 2007 the majority of the seed samples remained highly susceptible to mesosulfuron+iodosulfuron, with the average reduction in biomass ranging from 96-97% for most treatments. The exception was the minimum tillage treatment with a history of 'fop', SU and amide applications. One of the plots sampled, from four reps, showed RR resistance with a percentage reduction in biomass of 75%. This reduced the overall average reduction in biomass for this treatment to 91%. This is still considered to be susceptible, but may indicate that resistance is starting to appear, where there has been a previous history of SU use.

The RRRT, in petri dishes for the 2006 and 2007 seed batches showed consistent results for sethoxydim between the years. The plots with the highest levels of resistance to sethoxydim were those plots that had received 'fop' applications in both winter wheat and winter oilseed rape with five out of six samples being highly resistant (RRR), and % reductions averaging 32% (range 26 – 39%). Those plots that had 'fop' applications only in winter wheat with an amide in the oilseed rape crops tended to be slightly less resistant (RR), with % reductions averaging 53% (range 50 – 56%) (Table 3.2.2.9).

Table 3.2.2.9 Petri-dish assay of seeds collected from the Boxworth field experiment. Response to sethoxydim for 2006 and 2007 seed.

	HeRMES (2001-2004) treatments		2005/06 seed		2006/07 seed	
	Winter Wheat	Winter oil-seed rape	% reduction	resistance rating	% reduction	resistance rating
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	22	RRR	41	RR
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	25	RRR	36	RRR
Min till	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700 g ha^{-1} **(amide)**	45	RR	56	RR
Plough	Clodinafop-propargyl 30 g ha^{-1} **(fop)**	Propyzamide 700 g ha^{-1} **(amide)**	56	RR	55	RR
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10 g ha^{-1} **(fop + SU)**	Fluazifop-P-butyl 188 g ha^{-1} **(fop)**	32	RRR	40	RRR
Min till	Clodinafop-propargyl 30 g ha^{-1} + Flupyrsulfuron-methyl 10 g ha^{-1} **(fop + SU)**	Propyzamide 700 g ha^{-1} **(amide)**	49	RR	56	RR

The pendimethalin RRRT from 2007 indicated that all of the plots were susceptible to pendimethalin.

Rothamsted field experiment. The results for the assessments in the field are presented in Table 3.2.2.10. Black-grass plant populations were quite variable between treatments as a consequence of past herbicide history. However, the main interest was whether past herbicide history would influence subsequent infestation levels as a consequence of differential activity of mesosulfuron+iodosulfuron. Meaned over all plots, control by mesosulfuron+iodosulfuron was mediocre (66%) in 2004/05, very good (98%) in 2005/06, poor (34%) in 2006/07 and mediocre (54%) in 2007/08. These variable levels of control had an influence on the populations in the subsequent year. So, the excellent control in 2005/06 resulted in a low black-grass infestation in 2006/07, but the poor control in that year meant that populations increased to high levels in 2007/08.

Table 3.2.2.10 Rothamsted field experiment: Black-grass plant populations m^{-2} before and after treatment with mesosulfuron+iodosulfuron (12+2.4 g a.i./ha). Treatment codes relate to herbicides used prior to 2004/05 – see text.

Treatment Codes	Experiment year (mesosulfuron+iodosulfuron applied to all plots each year) 2004/05		2005/06		2006/07		2007/08	
	9/3/05 Pre-spray	11/5/05 Survivors	16/2/06 Pre-spray	25/5/06 Survivors	21/2/07 Pre-spray	1/5/07 Survivors	7/3/08 Pre-spray	8/5/08 Survivors
C	144	48	59	0.3	19	9	223	124
T	94	47	63	2.0	25	13	276	95
P+C	117	36	69	0.5	20	11	209	79
'Nil'	130	42	70	2.5	22	16	186	112
F1	81	17	59	0.3	19	12	228	115
P1	86	24	53	0.3	16	11	282	144
F2 (ALS)	59	23	54	1.5	21	15	285	132
P2 (ALS)	90	38	67	1.0	19	21	402	164
S.E. ±	22.2	9.8	9.9	0.72	4.2	3.4	52.8	21.7
L.S.D (P≤0.05)	65.3	28.8	29.0	2.10	12.2	10.1	155.4	63.8
Overall mean	100	34	61	1.1	20	13	261	121
% reduction	-	66%	-	98%	-	34%	-	54%
Mean of 6 treatments C to P1	109	36	62	1.0	20	12	234	112
Mean F2 & P2	75	31	61	1.3	20	18	344	148

However, there was no clear association between past herbicide treatment and efficacy of mesosulfuron+iodosulfuron. The two treatments, F2 and P2, which included the sulfonylurea flupyrsulfuron in the mixture (an ALS inhibitor), consistently had a higher number of black-grass plants surviving mesosulfuron+iodosulfuron than the F1 and P2 treatments, which did not include an ALS inhibitor. This was true in all four years, but the differences were generally small and never statistically significant. In addition, the 'Nil' treatment, which had received no herbicide for 4 years prior to 2004/05, was not conspicuously associated with better levels of control by mesosulfuron+iodosulfuron than the other seven herbicide treatments.

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 outdoors (e.g. environmental conditions). Consequently changes in the resistance status of seeds collected from survivors are alternative, and potentially better, indicators of longer term trends. The results for the glasshouse tray assay of seeds collected from the field experiment are presented in Table 3.2.2.11.

Table 3.2.2.11 Glasshouse tray assay: Response of black-grass plants grown from seeds collected from the Rothamsted field experiment in 2007. The baseline population comprising seeds collected from the 'Nil' plots in 2003 and a susceptible reference population (Roth04) were also included. Treatment codes relate to herbicides used prior to 2004/05 – for details see Material and Methods (Rothamsted field experiment).

	% reduction by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in glasshouse assay			
Treatment codes	% reduction in plant numbers	mean	% reduction in foliage weight*	mean
C	92		80	
T	93		88	
P+C	97	91	83	77
'NIL'	90		72	
F1	84		66	
P1	90		74	
F2 (ALS)	74	79	64	68
P2 (ALS)	84		71	
Baseline 2003	100		93	
Roth04 (Susc.)	100		94	
S.E. ±	5.8		5.6	
L.S.D. *P≤0.05*	16.9		16.4	

* Relative to mean weight of Baseline 2003 Nil unsprayed trays.

Both the susceptible standard, Roth04, and the baseline population derived from the 2003 Nil plots were well controlled (>93%) showing that herbicide application methodology and conditions were conducive to good control. There was some variation between treatments but no overall significant reductions in terms of % reduction in plant numbers. There were some significant reductions, (treatments F1, F2, P1, P2, 'Nil'), relative to the baseline 2003 Nil, in terms of % reductions in foliage fresh weights. The 'Nil' plots (no herbicide up to 2003/04, but subsequently three annual applications of mesosulfuron+iodosulfuron) gave results that were significantly lower (72%) than samples collected from the identical plots in 2003 (93%), indicating that resistance had developed between 2003 and 2007.

The lowest levels of control tended to be with the F2 and P2 treatments, which had received the sulfonylurea, flupyrsulfuron, for two years prior to the three years of treatment with mesosulfuron+iodosulfuron. As with the field assessments, control of F2 was consistently lower than F1, and P2 lower than P1. However, these effects were generally marginal and not statistically significant.

The molecular assays on 28 plants surviving mesosulfuron+iodosulfuron in the glasshouse tray assay, found that only three had a Pro-197-Thr mutation conferring ALS target site resistance (1 homozygous, 2 heterozygous). The remaining 25 plants had no detectable ALS mutations. This indicates that in the Rothamsted field experiment, mesosulfuron+iodosulfuron was selecting primarily for enhanced metabolic resistance, rather than ALS target site resistance, although this needs validating. This contrasts with the mechanism selected for in containers with the Peld03 population, which was principally ALS target site (see section 3.2.1).

This glasshouse tray assay showed that marginal resistance to mesosulfuron+iodosulfuron, due to probable enhanced metabolism, can develop within only three years. However, the degree of resistance was relatively modest and would be very difficult to detect in a true field situation, especially if the herbicide was used in mixture or sequence with other modes of action, as is recommended. The outdoor container experiment aimed to show whether these relatively marginal levels of resistance would impact on the efficacy of mesosulfuron+iodosulfuron in outdoor conditions. Results are shown in Table 3.2.2.12 (see overleaf).

Table 3.2.2.12 Outdoor container experiment using black-grass seeds collected from the Rothamsted field experiment in 2008 ('Nil' plots). A baseline population comprising seeds collected from the 'Nil' plots in 2003 and a susceptible reference population (Roth05) were also included.

Seed source	Mesosulfuron+ iodosulfuron dose (g a.i. ha^{-1})	Plant counts		Fresh weight	
		Plants surviving per container	% reduction in plant numbers	Foliage fresh weight	% reduction compared to untreated
Baseline 2003 seed (Nil plots)	12+2.4	5	97	8	94
	6 + 1.2	17	86	13	91
	Untreated	128	-	134	-
2008 seeds	12+2.4	31	54	46	61
	6 + 1.2	36	51	52	56
	Untreated	74	-	116	-
Roth05 (susc.)	12+2.4	0	100	3	98
	6 + 1.2	2	98	7	94
	Untreated	114	-	128	-
S.E. ±		-	3.5	-	3.2
L.S.D. *P ≤0.05*		-	11.1	-	10.1

The Roth05 susceptible standard was well controlled by both doses, as expected. Fewer plants from the 2008 seeds established compared with the 2003 baseline seeds, probably because of greater innate dormancy in the more recently collected seeds. Despite this, large differences in herbicide efficacy were recorded. The 2003 baseline population was slightly less well controlled than the susceptible standard, Roth05. In contrast, plants grown from the 2008 seeds, collected from the same plots as the baseline 2003 seeds, were controlled much less well. Whereas control of the baseline 2003 population ranged from 86 – 97%, control of the 2008 population (no herbicide up to 2003/04, but subsequently four annual applications of mesosulfuron+iodosulfuron), was only 51 - 61%. The results based on plant number and foliage fresh weight were similar, and there was relatively little difference between full and half rate of mesosulfuron+iodosulfuron.

These results from the container experiment support those from the glasshouse assay above. In both experiments, mesosulfuron+iodosulfuron at full rate gave 93 – 100% control of the 2003 baseline population in terms of plant numbers and foliage weight.

With the 2007 population in the glasshouse, 90% (numbers) and 72 % (weight) control was achieved whereas with the 2008 seeds in containers, the control levels were 54% and 61% respectively. The better control in the glasshouse was probably partly the result of one less year's selection, and partly the generally more favourable conditions for herbicide efficacy in that environment.

Conclusions of the two field experiments:

- **Mesosulfuron+iodosulfuron can select for partial resistance conferred by probable enhanced metabolic resistance, as well as ALS target site resistance.**
- **Enhanced metabolism resistance to mesosulfuron+iodosulfuron may be detected in controlled conditions after only three applications.**
- **There was no very clear association between past non-ALS herbicide treatments and efficacy of mesosulfuron+ iodosulfuron.**
- **Previous use of sulfonylurea graminicides may increase the risk of enhanced metabolic resistance to mesosulfuron+iodosulfuron, but only marginal effects were detected in these experiments.**
- **Detecting changes in efficacy of mesosulfuron+iodosulfuron due to increasing degrees of enhanced metabolism is likely to be difficult in the field, due to the many other factors affecting activity.**
- **The changes recorded in the field experiments occurred where mesosulfuron+iodosulfuron was applied alone, and not in mixture or sequence with other modes of action, as is recommended.**
- **Although the use of mixtures or sequences appears to have little effect on the increase in ALS target site resistance (see section 3.2.1), this may not be the case with ALS enhanced metabolic resistance.**
- **Increasing frequency of use of 'fops' was associated with higher frequencies of ACCase target site resistance. This supported the conclusions of the previous HeRMES project.**
- **Further comparative studies are needed to fully address the relative occurrence, rate of increase and risks posed by both ALS target site and enhanced metabolic resistance.**

3.2.3 Company seed samples from field experiments: To determine whether shifts in resistance to mesosulfuron+iodosulfuron could be detected after one year.

Agrochemical companies treated additional plots, alongside existing trials they were conducting. The aim was to treat the plots with four different herbicide regimes in order to determine whether shifts in resistance to mesosulfuron+iodosulfuron ('Atlantis') could be detected after a single year. It was hoped such studies would validate the more controlled container experiments conducted as part of this project (see section 3.2.1)

Materials & Methods

There were five companies involved in this project (BASF, Bayer, Dow, DuPont and Syngenta). Each of these companies set up four trial plots associated with existing black-grass trials on sites that were known to have populations of black-grass that were difficult to control. Each trial consisted of four plots measuring at least 6m x 12m, although in some cases wider plots of 12m were used where these fitted in with existing trials.

On each site four treatments were applied;

1. Untreated control – no grass-weed herbicides applied ('UTC')
2. Mesosulfuron+iodosulfuron ('Atlantis alone')
3. A non ALS treatment ('Non-Atlantis') – e.g. pre-em diflufenican + flufenacet followed by post-em clodinafop-propargyl+trifluralin and isoproturon
4. Mesosulfuron+iodosulfuron in mixture and/or sequence with non-ALS herbicides ('Atlantis mixture') – e.g. pre-em diflufenican + flufenacet followed by mesosulfuron+iodosulfuron and trifluralin

In July of each year the companies collected black-grass seeds from the survivors of these treatments. Seed samples were sent to ADAS Boxworth in paper envelopes. ADAS dried and cleaned the seed and then ran it through a standard glasshouse pot test, similar to that described in section 3.3.1, to determine whether or not a single application of mesosulfuron+iodosulfuron could cause a shift in resistance.

For each seed sample, six plants were established in 20 pots containing a Kettering loam:grit mix (~4% organic matter). Once seedlings reached the 3 leaf stage, a single dose (12+2.4 g a.i. ha^{-1}) of a commercial formulation of mesosulfuron+ iodosulfuron was applied to 16 replicate pots per sample, with four replicate pots per sample left untreated. Spraying was done using a Mardrive pot sprayer at 200kPa, 200 l ha^{-1}, with 'Biopower' adjuvant at 0.5% spray volume. Assessments occurred 5-7 weeks after treatment, when the susceptible standard was clearly dead. All pots were assessed with a visual vigour score (4, healthy – 1, dead). Once visual assessments were complete, all plants from each pot were cut off at soil level and foliage fresh weight per pot recorded. The fresh weights of the treated company samples were then compared with the weights of the corresponding untreated sample, with the percentage reduction in weight used to determine the effect of the herbicide.

Results & Discussion

In total, seed samples were sent in from 26 sites across Eastern England. Some of these samples were incomplete due to very effective control from the herbicides (especially mesosulfuron+iodosulfuron) or poor quality of the seed sample, leaving 17 complete sets. All seed samples were tested against mesosulfuron+iodosulfuron in the glasshouse pot assay (Table 3.2.3.1).

Degree of resistance varied greatly between sites but, in the majority of samples, there was little or no evidence of differences in the level of resistance between samples treated with different herbicides. There were, however, four sites where either one or both of the 'Atlantis' treatments were one resistance category or more higher than the untreated or non-Atlantis treatments (Bayer 3, Dow 2, DuPont 6 & 7). In contrast, there was only a single case where the 'Atlantis' treated plots had a lower resistance rating (DuPont 5).

The graph in Figure 3.2.3.1 shows the results for 22 sets, with the number of sites in each category graphed against the resistance rating and herbicide regime.

Table 3.2.3.1 Resistance ratings for 22 (complete or mostly complete) company samples and % reduction in biomass relative to untreated.

Site	Treatment	Resistance Rating	% reduction in biomass
ADAS 1 - 2007	Atlantis alone	RRR	3
ADAS 1 - 2007	Atlantis mixture	RRR	-26
ADAS 1 - 2007	Non-Atlantis	RRR	-13
ADAS 1 - 2007	UTC	RRR	-36
BASF 1 - 2007	Atlantis alone	RRR	2
BASF 1 - 2007	Atlantis mixture	RRR	-24
BASF 1 - 2007	Non-Atlantis	R?	76
BASF 1 - 2007	UTC	RRR	-37
BASF 2 - 2007	Atlantis alone	RRR	4
BASF 2 - 2007	Atlantis mixture	RRR	21
BASF 2 - 2007	Non-Atlantis	RRR	-5
BASF 2 - 2007	UTC	RRR	29
BASF 3 - 2008	Atlantis alone	RR	44
BASF 3 - 2008	Atlantis mixture	RRR	38
BASF 3 - 2008	UTC	RR	42
BASF 4 - 2008	Atlantis alone	S	99
BASF 4 - 2008	Atlantis mixture	S	99
BASF 4 - 2008	UTC	S	99
Bayer 1 - 2006	Atlantis alone	RRR	4
Bayer 1 - 2006	Atlantis mixture	RRR	8
Bayer 1 - 2006	Non-Atlantis	RRR	-35
Bayer 1 - 2006	UTC	RRR	32
Bayer 2 - 2006	Atlantis alone	S	85
Bayer 2 - 2006	Atlantis mixture	S	85
Bayer 2 - 2006	Non-Atlantis	S	83
Bayer 2 - 2006	UTC	S	88
Bayer 3 - 2008	Atlantis alone	RR	55
Bayer 3 - 2008	Atlantis mixture	RR	64
Bayer 3 - 2008	Non-Atlantis	S	96
Bayer 3 - 2008	UTC	S	91
Bayer 5 - 2008	Atlantis alone	S	93
Bayer 5 - 2008	Atlantis mixture	S	95
Bayer 5 - 2008	Non-Atlantis	S	95
Bayer 5 - 2008	UTC	S	97
Dow 1 - 2008	Atlantis alone	RR	65
Dow 1 - 2008	Atlantis mixture	RR	74
Dow 1 - 2008	Non-Atlantis	RR	62
Dow 1 - 2008	UTC	RR	65

Table 3.2.3.1 (continued)

Site	Treatment	Resistance Rating	% reduction in biomass
Dow 2 - 2008	Atlantis alone	R?	86
Dow 2 - 2008	Atlantis mixture	R?	89
Dow 2 - 2008	Non-Atlantis	S	95
Dow 3 - 2008	Atlantis mixture	S	98
Dow 3 - 2008	Non-Atlantis	S	98
Dow 3 - 2008	UTC	S	98
Dupont 1 - 2006	Atlantis alone	S	86
Dupont 1 - 2006	Atlantis mixture	S	94
Dupont 1 - 2006	Non-Atlantis	S	90
Dupont 1 - 2006	Untreated	S	92
DuPont 3 - 2007	Atlantis alone	RRR	30
DuPont 3 - 2007	Atlantis mixture	RRR	27
DuPont 3 - 2007	Non-Atlantis	RRR	-12
DuPont 3 - 2007	UTC	RRR	24
DuPont 4 - 2007	Atlantis alone	RRR	7
DuPont 4 - 2007	Atlantis mixture	RRR	12
DuPont 4 - 2007	Non-Atlantis	RR	49
DuPont 4 - 2007	UTC	RRR	33
DuPont 5 - 2007	Atlantis alone	RR	45
DuPont 5 - 2007	Atlantis mixture	RRR	2
DuPont 5 - 2007	Non-Atlantis	RRR	7
DuPont 5 - 2007	UTC	RRR	6
DuPont 6 - 2008	Atlantis alone	R?	86
DuPont 6 - 2008	Atlantis mixture	S	93
DuPont 6 - 2008	Non-Atlantis	S	99
DuPont 6 - 2008	UTC	S	96
DuPont 7 - 2008	Atlantis alone	RRR	37
DuPont 7 - 2008	Atlantis mixture	RR	50
DuPont 7 - 2008	Non-Atlantis	RR	50
DuPont 7 - 2008	UTC	RR	62
DuPont 8 - 2008	Atlantis alone	S	96
DuPont 8 - 2008	Atlantis mixture	S	95
DuPont 8 - 2008	Non-Atlantis	S	99
DuPont 8 - 2008	UTC	S	96
Syngenta 1 - 2006	Atlantis alone	S	94
Syngenta 1 - 2006	Non-Atlantis	S	87
Syngenta 1 - 2006	UTC	S	96
Syngenta 2 - 2008	Atlantis alone	S	90
Syngenta 2 - 2008	Atlantis mixture	S	97
Syngenta 2 - 2008	Non-Atlantis	S	98
Syngenta 2 - 2008	UTC	S	97
Syngenta 3 - 2008	Atlantis alone	S	94
Syngenta 3 - 2008	Atlantis mixture	S	96
Syngenta 3 - 2008	Non-Atlantis	S	97
Syngenta 3 - 2008	UTC	S	97

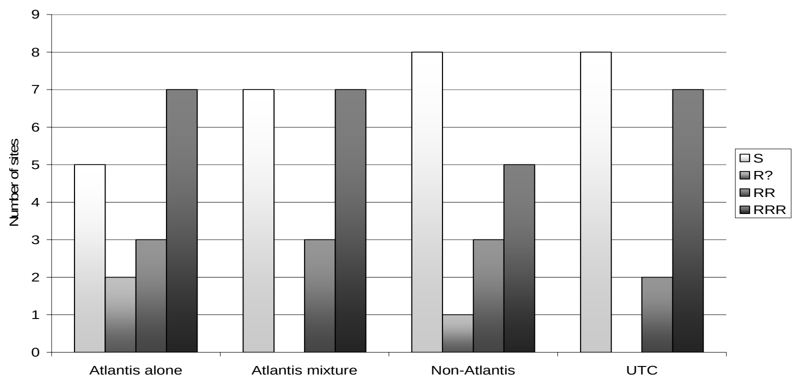


Figure 3.2.3.1 Number of sites in each resistance category for 4 herbicide regimes.

If each of the resistance ratings is given a value (S=0, R?=1, RR=2 & RRR=3) and the values for the 17 complete sets meaned, the resistance rating of the 'Atlantis' alone and 'Atlantis mixture' treatments are slightly higher than the 'non-Atlantis' and untreated treatments (Table 3.2.3.2). Differences are not statistically significant.

Table 3.2.3.2 Average resistance rating (across 17 sites)

Treatment	Average resistance score
'Atlantis alone'	1.7
'Atlantis mixture'	1.6
'Non-Atlantis'	1.3
'UTC'	1.5
S.E.D. (d.f.)	0.476 (64)

Conclusions from the company seed samples collected from field experiments:

- **There was evidence of a shift towards greater resistance to mesosulfuron+iodosulfuron, as a consequence of its use in the field, in four (18%) of the 22 trials. Such shifts were relatively small, and were not detectable over a single year on the other 18 fields.**
- **Although trends were small, the results support the conclusions of the container experiments, that the use of other modes of action in mixture or sequence with mesosulfuron+iodosulfuron will not reduce selection for resistance.**
- **This work has assisted in the validation process by demonstrating that over a cross section of sites it is unlikely that resistance to**

mesosulfuron+iodosulfuron will be generally detectable after just a single application of 'Atlantis'.

3.3 Objective 2: To establish the incidence of existing and novel mechanisms of herbicide resistance in grass-weeds, with particular emphasis on ALS inhibiting and dinitroaniline herbicides, in order to refine resistance sampling and monitoring procedures so that resistance management strategies at the local level can be optimised.

Accurate diagnosis of resistance is critical to resistance prevention and management. Resistance must be detected reliably so that farmers have confidence in the results and techniques must also be suitable for subsequent monitoring in order to assess the long-term success of management strategies. We assessed the robustness of conventional glasshouse pot tests in comparison with quicker Petri-dish and similar assays, with the specific aim of discriminating between mechanisms conferring different degrees of resistance (e.g. ALS target site resistance and enhanced metabolism) in black-grass seed samples. Detailed field sampling was conducted to help devise the best sampling strategy for detecting and monitoring resistance. Cross-resistance studies with other classes of ALS inhibiting herbicides were also conducted to establish whether resistance is specific to sulfonylureas, or whether it extends to sulfonylcarbonyltriazolinones (e.g. propoxycarbazone) and imidazolinones. Tests were also done with the dinitroaniline herbicides pendimethalin and trifluralin.

3.3.1 Developing robust tests for resistance to ALS inhibitors.

The aim was to develop reliable glasshouse pot tests using populations already characterised for mechanism of resistance, so results could be related to their known resistance profiles. Once a methodology had been developed, additional populations from fields and experiments were assayed in order to validate the procedure. Petri-dish assays were also investigated as these are potentially much quicker and cheaper to conduct than glasshouse pot assays.

The ultimate aim was to produce a protocol suitable for routine use for both resistance detection and monitoring purposes. A "ring test" using appropriate standards was conducted involving all participants, in order to validate the robustness of the tests and to establish the consistency of results between different testing organisations/companies.

Materials & Methods

Glasshouse assays. The three populations used were: Rothamsted S, a susceptible standard; Peldon EM, a population collected from a site in Essex in 1996 which shows resistance due to an enhanced ability to metabolise herbicides (Cocker *et al*., 1999; Hall *et al*., 1995); Peldon TS, a population from the same site in Essex, collected in 2004, in which 25% - 40% of individual plants show ALS target site resistance (Marshall, 2007). These populations were treated with a range of doses of commercial formulations of mesosulfuron+iodosulfuron ('Atlantis WG' 30+6 g a.i. kg^{-1} plus 90 g mefenpyr-diethyl safener kg^{-1}, Bayer CropScience) and sulfometuron-methyl ('Oust' 750 g a.i. kg^{-1}, DuPont) in order to determine the best single dose to use for detecting herbicide resistance in glasshouse screening assays. Two different growing media were used, a peat based compost and a loam soil (~4% organic matter), in order to determine whether type of growing media affected herbicide efficacy. A randomized block design was used with five replicates. Six plants were established in each 9cm diameter pot containing either a peat based compost or a Kettering loam:grit mix (~4% organic matter). A commercial formulation of mesosulfuron+iodosulfuron was applied at six or eight doses between 0.75+0.15 and 96+19.2 g a.i. ha^{-1} and sulfometuron was applied at six or seven doses between 1.56 and 100 g a.i. ha^{-1} to plants at the three-leaf stage using a track sprayer delivering 240 L spray solution ha^{-1} at 225 kPa through a single 'Teejet' TP110015VK flat fan nozzle. The recommended adjuvant was used with mesosulfuron+iodosulfuron (surfactant at 0.5% spray solution, Biopower, Bayer). Ten untreated pots per population were included in the experimental design.

Pots were kept in a glasshouse with a 14-h, 16°C day and a 10-h, 8°C night phase and watered from above daily. Plants were visually rated for herbicidal effects and foliage weight (g) per pot recorded 28 days after spraying as a measure of herbicide activity. Data were analysed by fitting a four parameter logistic curve using MLP v3.09 (Ross, 1987) and $\log_{10}ED_{50}$ values calculated. ED_{50} values were derived by de-transforming the $\log_{10}$ data and represent the herbicide dose required to decrease foliage weight by 50% relative to the no-herbicide controls. ED_{50} resistance indices

(RI) were defined as the ratio of ED_{50} values relative to the susceptible standard, Rothamsted.

Petri-dish assays. A series of preliminary experiments was conducted to determine the best discriminatory concentration to use in Petri-dish germination assays for detection of resistance to ALS inhibiting herbicides (data not shown). A test was undertaken to validate the methodology in which 11 black-grass populations were tested, including three reference populations (Roth04, susceptible; Peld EM, enhanced metabolism standard, as used in glasshouse assay above; Peld05SS, a population showing a high degree of ALS target site resistance). There were two replicates of the following three treatments: 0.1 ppm of mesosulfuron+iodosulfuron ('Atlantis'); sulfometuron ('Oust') at 1.0 ppm; untreated controls (KNO_3 solution only). There were 50 seeds per dish and three cellulose + one glassfibre filter paper per dish. Dishes were placed in an incubator with a 14 hour 17 ºC day and a 10 hour 11 ºC night phase. Shoot length of every germinated seed was assessed after 14 days. In addition a visual assessment of % reduction in shoot growth, relative to untreated for the same population, was made by six different assessors for the mesosulfuron+iodosulfuron dishes only.

'Ring' test. A ring test was conducted to evaluate the robustness of the Petri dish protocol, described in the previous section, for detecting resistance to ALS inhibiting herbicides. The same two herbicides were used in this test, 0.1 ppm mesosulfuron+iodosulfuron ('Atlantis') and 1 ppm sulfometuron ('Oust'). Sulfometuron was included because, even though not available in the UK, it is generally accepted that it is unaffected by metabolic resistance. Ten companies/organisations conducted the test either in an incubator (ADAS, BASF, Dow, DuPont, Rothamsted, Syngenta, UAP), controlled environment room (Bayer), greenhouse (Agrochemex) or window sill (Oxford Plant Sciences). The resistance status of all samples had been established in glasshouse pot experiments at Rothamsted and a standard protocol was provided. All participants used the same seven coded seed samples (Table 3.3.1.1) with only the susceptible standard (S) identified.

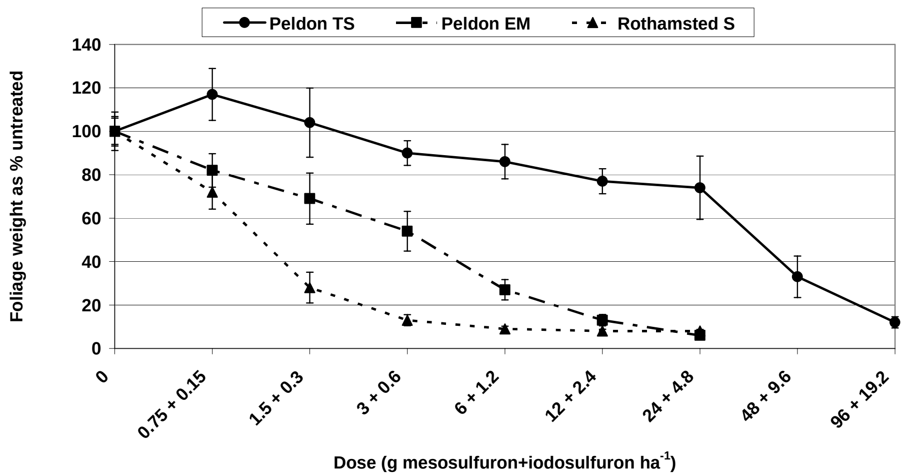
Table 3.3.1.1 Seed samples used in the 'ring test' to evaluate the robustness of a Petri-dish assay for detecting resistance to ALS inhibiting herbicides.

Code	Name	ALS resistance profile based on glasshouse and molecular assays
A	WILTS05	Resistant, but not ALS TSR due to Pro197 or Trp574 mutations
B	EAST06 (Lincs)	ALS TSR conferred by Pro197 mutation (low germination)
C	Peld SS 05 (Essex)	ALS TSR conferred by Pro197 mutation (ALS target site (TSR) resistance standard)
D	LONGC 06 (Oxon.)	ALS TSR conferred by Pro197 mutation (Highly resistant)
E	BIG F 05 (Wilts)	Susceptible (same farm, different field, as WILTS05)
K	FLAW 06 (Notts)	Marginal resistance
S	Roth04	Susceptible (Standard reference population)

Results

Glasshouse assays. There were large differences in the response of the three populations (Figure 3.3.1.1). In both growing media, the ED_{50} values for the Peldon TS population were much higher than those for the Rothamsted susceptible standard, indicating a high degree of resistance to mesosulfuron+iodosulfuron (Table 3.3.1.2). The resistance indices (RI) were high, ranging from 22 to 38. The Peldon EM population showed a much smaller degree of insensitivity (RI 3.6 to 4.1) although the ED_{50} values were significantly higher ($P \leq 0.05$) than the Rothamsted susceptible standard. Mesosulfuon+iodosulfuron was more active in loam than compost. The ratios of compost:loam ED_{50} values were 2.4, 2.7 and 1.4 for the three populations respectively, with a mean of 2.2. At the recommended field rate of 12 g mesosulfuron + 2.4 g iodosulfuron ha^{-1} in loam, there was a 92% reduction in foliage fresh weight with Rothamsted, an 87% reduction with Peldon EM and only a 23% reduction with Peldon TS. At twice this rate (24+4.8 g a.i. ha^{-1}) the equivalent values were little different at 92%, 94% and 26% respectively. However at half the field rate (6+1.2 g a.i. ha^{-1}) the equivalent values were 91%, 73% and 14% respectively. Consequently, over these three rates the response of Peldon EM varied much more (by 21%) than Rothamsted (1%) and Peldon TS (12%).

Figure 3.3.1.1 Response of three black-grass populations to mesosulfuron+ iodosulfuron in a glasshouse assay with plants grown in a loam soil (4% O.M.).



(*See overleaf for table 3.3.1.2)*

Table 3.3.1.2 Response of three black-grass populations to mesosulfuron+ iodosulfuron in a glasshouse dose response assay using two different growing media

	Log_{10} ED_{50} values		Detransformed ED_{50} values g mesosulfuron+iodosulfuron ha^{-1} (RI ratios in brackets)	
Population	Loam	Compost	Loam	Compost
Rothamsted S	-0.0035	0.3785	0.99 (1.0)	2.39 (1.0)
Peldon EM	0.5500	0.9882	3.55 (3.6)	9.73 (4.1)
Peldon TS	1.5799	1.7291	38.02 (38.4)	53.59 (22.4)
S.E. ±	0.1060	0.0886	-	-
L.S.D. *(P≤0.05)*	0.2998	0.2507	-	-

The control of the Peldon TS population increased substantially at the two highest rates (Figure 3.3.1.1), which represent 4x and 8x field recommended rate, indicating that ALS target site resistance does not confer absolute resistance. The best single dose of mesosulfuron+iodosulfuron for use in resistance screening assays appears to be the recommended field rate of 12 g mesosulfuron + 2.4 g iodosulfuron ha^{-1} (400 g 'Atlantis WP' ha^{-1}) applied to plants growing in loam soil.

With sulfometuron, there were large differences in the response of the three populations, and the growing media had more influence on herbicide efficacy than with mesosulfuron+iodosulfuron (Figure 3.3.1.2; Table 3.3.1.3). Due to the high activity of sulfometuron on the Rothamsted susceptible standard in loam, and low activity of the same herbicide on Peldon TS in both types of media, precise ED_{50} values could not be calculated for these population/media combinations. Peldon TS was much more resistant than the Rothamsted susceptible standard (RI >77 - >144). Peldon EM showed an intermediate response between the Rothamsted susceptible standard and Peldon TS and growing media had a big influence on herbicide efficacy (Table 20). The ED_{50} value for Peldon EM was much higher in compost than in loam, with a compost:loam ED_{50} ratio of 11.1.

Figure 3.3.1.2 Response of three black-grass populations to sulfometuron in a glasshouse assay with plants grown in a loam soil (4% O.M.).

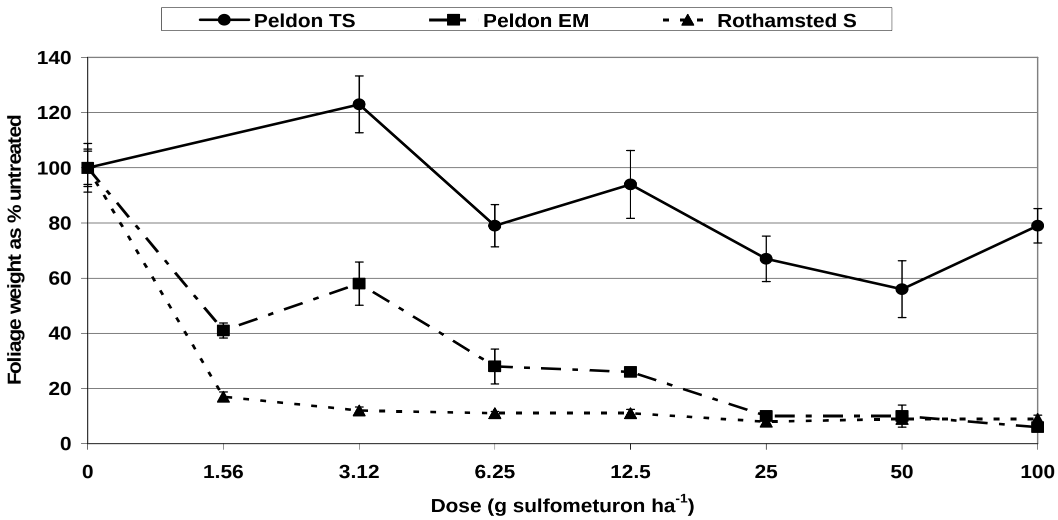


Table 3.3.1.3 Response of three black-grass populations to sulfometuron in a glasshouse dose response assay using two different growing media.

	Log_{10} ED_{50} values		Detransformed ED_{50} values g sulfometuron ha^{-1} (RI ratios in brackets)	
Population	Loam	Compost	Loam	Compost
Rothamsted S	<0.1938	0.1132	<1.56 (1.0)	1.30 (1.0)
Peldon EM	0.3165	1.3629	2.07 (>1.3)	23.06 (17.8)
Peldon TS	2.3519	>2.00	224.87 (>143.9)	>100 (>77)
S.E. ±	0.5506	0.1083	-	-
L.S.D. *(P≤0.05)*	1.5574	0.3064	-	-

In loam, Peldon EM was controlled much better than Peldon TS at all doses, and 59% reduction was achieved even at the lowest dose used (1.56 g sulfometuron ha^{-1}). In contrast in compost, less than 18% reductions in foliage weight were achieved with both Peldon EM and Peldon TS at all doses up to 12.5 ha^{-1}. Doses of 50 or 100 g sulfometuron ha^{-1} were needed to achieve moderately good control (81%) of Peldon EM in compost. Consequently, for single dose assays, the growing medium used is critical for detecting resistance to sulfometuron, and potential ALS target site resistance. At doses of 25 – 100 g sulfometuron ha^{-1}, Peldon TS can clearly be distinguished from Peldon EM in loam. In compost, clear differences occurred at 50 and 100 g/ha, but not at lower doses. Consequently, the best single dose of sulfometuron for use in resistance screening assays appears to be in the range 25 – 100 g sulfometuron ha^{-1} applied to plants growing in loam soil. High organic matter compost should be avoided, as this substantially reduces the activity of sulfometuron and gives potentially misleading results, especially with partially resistant populations.

Petri-dish assays. The % reductions in total shoot length per dish, relative to the mean of the two untreated dishes, were calculated for each treated dish. 'R' resistance ratings were calculated as described by Moss *et al*., (1999) and the results are summarised in Table 3.3.1.4. With both mesosulfuron+iodosulfuron and sulfometuron there was some growth of shoots in the susceptible standard (Roth04) but moderate reductions (64-66%) relative to untreated dishes. In contrast there was very little reduction in shoot growth in treated dishes of the Peld05SS ALS target site resistant (TSR) standard (6-9%). With mesosulfuron+iodosulfuron, the Peld-EM enhanced metabolism standard gave similar results to the Roth04 susceptible standard, indicating that no enhanced metabolism resistance was being detected in the Petri-dishes at the dose used. These results for mesosulfuron+iodosulfuron on the standard populations are exactly what would be expected, and indicate that this Petri dish assay is detecting ALS target site resistance rather than enhanced metabolism resistance.

Table 3.3.1.4 Response of 11 black-grass populations to mesosulfuron+iodosulfuron and sulfometuron in a Petri-dish assay

	Mesosulfuron+iodosulfuron 0.1 ppm				Sulfometuron 1.0 ppm	
	Measured % reduction in shoot length	'R' rating	*Visual* % reduction in growth	'R' rating	*Measured* % Reduction in shoot length	'R' rating
Roth4 Susceptible	66	S	77	S	64	S
Peld EM E. M. standard	66	S	72	S	46	RR
Peld05 SS ALS TS standard	9	RRR	2	RRR	6	RRR
Bayer GBR06-03 (Cambs)	75	S	76	S	61	S
BIGF 2005 (Wilts)	67	S	72	S	48	RR
FLAW 2006 (Notts)	52	RR	65	R?	49	RR
EAST 2006 (Lincs)	10	RRR	17	RRR	-13	RRR
COCK 2006 (Essex)	6	RRR	9	RRR	-4	RRR
Syngenta 30/06 (Lincs)	-1	RRR	5	RRR	-10	RRR
LONGC 2006 (Oxford)	-6	RRR	3	RRR	-22	RRR
BSH 2006 (Oxford)	-12	RRR	4	RRR	1	RRR
S.E. ±	8.51		4.87		9.44	
L.S.D. *($P \leq 0.05$)*	26.83		15.33		29.73	

Resistance to mesosulfuron+iodosulfuron was detected in BSH, LONGC, Syngenta 30/06, COCK and EAST samples, as % reductions relative to untreated were very low, or even negative. It is probable that ALS target site resistance is responsible. The Bayer GBR06-03 and BIGF 2005 samples were clearly susceptible to mesosulfuron+ iodosulfuron. FLAW gave intermediate results and further studies to confirm its resistance status are needed.

The data for the visual assessments are the mean of six recorders. Generally visual estimates were slightly greater than those based on measurements, but overall agreement was good. It should be borne in mind that *all* the dishes in the experiment can be assessed visually in 15 minutes by one recorder. Measuring shoot length on each of the 3300 seeds in the 66 dishes takes about 1 day, and at least another half day to collate and analyse the data.

The results for sulfometuron based on measured shoot length were broadly in agreement with the mesosulfuron+iodosulfuron results, especially with the Roth susceptible standard, Peldon05SS ALS TSR standard, the five highly resistant (BSH, LONGC, Syngenta, COCK and EAST) and one intermediate (FLAW) populations. Peld96 and BIGF appeared to show some resistance to sulfometuron, but this was fairly marginal.

This Petri-dish method appears to be capable of detecting probable ALS target site resistance affecting the activity of mesosulfuron+iodosulfuron ('Atlantis') and sulfometuron. Results can be obtained by early September for seed samples collected in the previous July. It is not as robust a test as the Petri-dish test used for identifying ACCase target site resistance, so is perhaps better used as an indicator of ALS resistance. Highly resistant and susceptible populations will probably be detected reliably, whereas those with only a small proportion of resistant individuals will probably give indeterminate results and require a pot test for more accurate diagnosis of resistance. The Petri-dish test is unlikely to reliably detect resistance conferred by enhanced metabolism. Visual assessments are quick and appear to be satisfactory.

Ring test. The results below do not include those for Bayer, Oxford Plant Science (OPS) or Agrochemex. OPS and Agrochemex did not conduct the test under fully controlled conditions, and the results obtained were inconsistent. The filter papers used by Bayer were of German origin and were 40% heavier than the Whatman ones used at Rothamsted (weight per paper: cellulose, 0.759g v 0.544g; glassfibre, 0.481g v 0.343g). Hence it appears likely that the suggested 7 ml solution per dish with three cellulose and one glassfibre paper was insufficient to maintain the correct degree of seed imbibition with the German filter papers. Pro-rata, 9.8 ml would have been needed.

Two assessment methods were carried out during the test. Firstly, a visual assessment was conducted by five centres sites looking at the reduction in shoot growth for the treatments compared to the untreated for the same population. This took on average 10 – 15 minutes to assess ***all*** 42 dishes. Secondly, and much more time consuming, all centres measured the shoot length (mm) for each germinated seed in each dish. This took on average 10 – 15 minutes ***per dish*** and substantially more time to process the data.

On the basis of measured shoot lengths, both herbicides could discriminate between the highly resistant and susceptible populations (Table 3.3.1.5). Sulfometuron gave better control of the Roth04 standard susceptible population (73.1% compared to 57.5% for mesosulfuron+iodosulfuron). Three highly resistant and three susceptible populations were identified by both herbicides, with the 'R' ratings identical for both herbicides when the data was averaged, but this was not the case for each individual centre.

Table 3.3.1.5 Ring test: % reduction in shoot length compared to untreated using ***measured data*** (total shoot length per dish) averaged over seven testing centres

Herbicide	Mesosulfuron+iodosulfuron 0.1 ppm		Sulfometuron 1.0 ppm	
Population	% reduction in shoot length	R Rating	% reduction in shoot length	R Rating
WILTS	39	RR	58	RR
EAST	20	RRR	25	RRR
Peld 05 SS	-5	RRR	-1	RRR
LONG C	-1	RRR	-8	RRR
BIG F	57	S	76	S
FLAW	54	S	73	S
Roth04 (Susc.)	58	S	73	S
S.E. ±	4.96	*	5.55	*
LSD ($P \leq 0.05$)	13.93		15.60	

The 'R' rating across all seven centres varied more for mesosulfuron+iodosulfuron than sulfometuron. The three highly resistant populations (EAST, Peld 05SS and LONGC) were identified by all collaborators using both herbicides, with one exception, but there was greater variation amongst the susceptible populations using mesosulfuron+iodosulfuron, especially with Big F. Sulfometuron not only gives better control of the susceptible populations at the concentrations used, but the results were more consistent across the test sites. The 'R' Rating for the WILTS population ranged from S – RRR for both herbicides. This is a population that has showed resistance in pot and container tests but the mechanism has not been identified, although it does not have Pro197 or Trp574 mutations. It appears that the Petri-dish test does not reliably detect resistance in populations such as WILTS.

The results from the visual assessment were very similar to those based on shoot lengths. The three highly resistant populations (EAST, Peld 05SS and LONGC) were all identified and the 'R' ratings are identical to those obtained from the shoot lengths data. The visual score data also showed that sulfometuron controls the susceptible populations better than mesosulfuron+iodosulfuron, as found using the shoot length data. Therefore, it appears that not only can the visual assessment pick out the differences between the populations, but is also about 20 times faster than measuring each shoot.

Figure 3.3.1.3 shows the relationship between the measured shoot length data and visual assessment for the five centres that obtained both data sets. There is a very strong correlation between both methods. The regression is slightly skewed by the fact that most visual assessors did not go below zero when scoring the dishes, even though there might have been greater shoot length than in the untreated dishes (i.e. negative control).

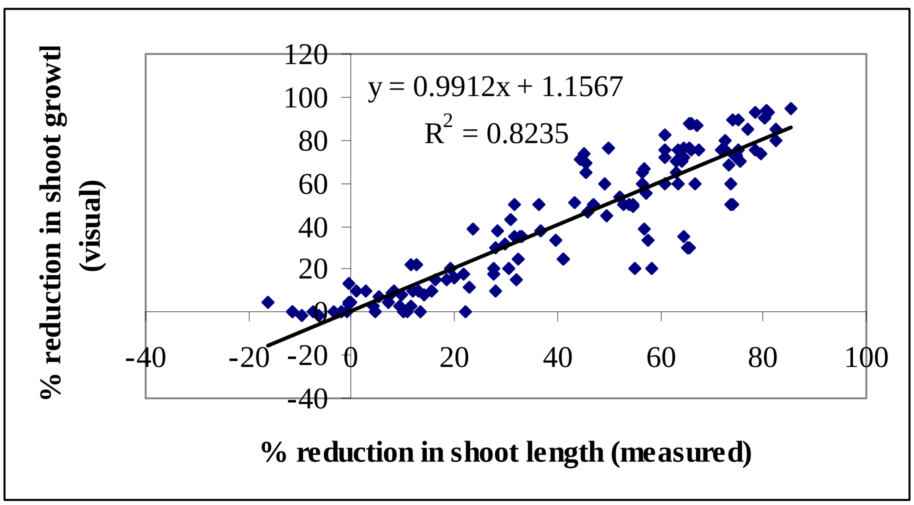


Figure 3.3.1.3 Ring test: relationship between % reduction in shoot length (***measured***) vs. % reduction in shoot growth (***visual***). Each point represents a single dish and data are for both mesosulfuron+iodosulfuron and sulfometuron.

Conclusions of the glasshouse and Petri-dish assays:

- **Glasshouse pot assays can reliably detect resistance to mesosulfuron+iodosulfuron ('Atlantis') and sulfometuron ('Oust') in black-grass plants grown from seeds.**
- **High organic matter growing media (e.g. peat based compost) should be avoided, as they can reduce herbicide activity and give misleading results. Low organic matter (4%) growing media is preferred.**
- **The best single doses to use in glasshouse pot assays, are the recommended field rate of mesosulfuron+iodosulfuron (12 + 2.4 g ha^{-1}) and 50 – 100 g sulfometuron g ha^{-1} as an indicator of ALS target site resistance. Lower doses of sulfometuron should be avoided.**
- **Herbicides should be applied to plants at the 3 leaf stage and assessments of foliage fresh weight made 3 – 4 weeks later.**
- **Petri-dish assays, using mesosulfuron+iodosulfuron (0.1 ppm) and sulfometuron (1 ppm) have potential for identifying ALS target site resistance within two weeks, using black-grass seed populations.**
- **Petri-dish tests are not as robust as pot tests, and need to be conducted in controlled conditions, such as an incubator. Attention to detail is important, as even the type of filter paper used can affect results.**
- **Sulfometuron at 1.0 ppm gives more robust results than mesosulfuron+iodosulfuron at 0.1 ppm.**
- **The test will not reliably detect resistance conferred by non-target site mechanisms.**
- **The test is only likely to detect resistance in samples in which a relatively high proportion of individual seeds is resistant.**
- **Visual assessments are about 20 times quicker than assessments based on measuring shoot length but give very similar results.**

3.3.2 Cross-resistance studies with different classes of ALS inhibitors.

ALS target site resistance conferred by Pro197 and Trp574 mutations has been indentified in UK black-grass populations (Marshall & Moss, 2008). By 2009, black-grass with the Pro197 mutation had been identified on 10 farms and the Trp574 mutation on 1 one farm based on molecular studies at Rothamsted (R Marshall, pers. comm.). On another farm, both mutations had been identified in different fields. Initial resistance screening studies were conducted with the sulfonylureas mesosulfuron+iodosulfuron and sulfometuron. Further studies were conducted to determine the cross-resistance patterns to other classes of ALS inhibitors.

Materials & Methods

The three black-grass populations used were: Rothamsted S, a susceptible standard; LongC06, a population from Oxfordshire with confirmed Pro197 mutation; R30, a population from Cambridgeshire with confirmed Trp574 mutation (Marshall & Moss, 2008). These populations were treated with a range of doses of commercial formulations of the sulfonylureas mesosulfuron+iodosulfuron ('Atlantis WG') and sulfometuron-methyl ('Oust'), the sulfonylaminocarbonyltriazolinone propoxycarbazone ('Attribute') and the imidazolinone imazapyr ('Arsenal'). Single plants were established in 5 cm square pots containing a Kettering loam:grit mix (~4% organic matter). A fully randomized design was used with 15 replicate treated pots per dose and 40 untreated pots per population. Commercial formulations were applied at eight doses of mesosulfuron+iodosulfuron (0.375+0.075 to 48+9.6 g a.i. ha^{-1}), sulfometuron (0.781 – 400 g a.i. ha^{-1}), propoxycarbazone (2.188 – 280 g a.i. ha^{-1}) and imazapyr (5.86 - 1500). Recommended adjuvants were used with mesosulfuron+iodosulfuron ('Biopower' @ 0.5% spray volume) and propoxycarbazone ('Comulin' mineral oil @ 1 L ha^{-1}). Plants were treated at the three-leaf stage using a track sprayer delivering 225 L spray solution ha^{-1} at 238 kPa through a single 'Teejet' TP110015VK flat fan nozzle. Pots were kept in a glasshouse with a 14-h, 16°C day and a 10-h, 8°C night phase and watered from above daily. Foliage weight (g) per pot was recorded 29-30 days after spraying as a measure of herbicide activity. Data were analysed in the same manner as in the glasshouse assays described in the previous section.

Results & Discussion

Both the LongC (Pro197) and R30 (Trp574) populations showed very high degrees of resistance to the sulfonylureas, mesosulfuron+iodosulfuron and sulfometuron, and to the sulfonylaminocarbonyltriazolinone, propoxycarbazone (Figure 3.3.2.1). With all three herbicides, the susceptible standard Roth04 was well controlled at 12.5% or less of the field recommended dose. In contrast, even at the highest doses used, 400% of the field rates, there was little effect on growth of both resistant populations. LongC and R30 gave very similar responses to all three herbicides, with no indication that the two different mutations involved affected the outcome. Resistance indices were very high, from 218 – 3613.

Response to imazapyr differed markedly between populations (Figure 3.3.2.2). Again the Roth04 susceptible standard was well controlled, even at low doses, with an ED_{50} value of 9.6 g a.i. ha^{-1}. The LongC population was much less resistant than the R30 population, with ED_{50} value of 146 and 2000 g a.i. ha^{-1} respectively. Thus, the resistance index for R30 was 209, but only 15 for LongC. Clearly, in contrast to the other ALS classes, the specific mutation present had a very big impact on degree of resistance to the imidazolinone, imazapyr, with Trp574 in R30 conferring much greater resistance than Pro197 in LongC. Resistance to imazapyr was totally dependent on the dose used for comparison, so at 93.75 g imazapyr ha^{-1} or less, LongC and R30 appeared equally resistant. In contrast, at doses of 375 g imazapyr ha^{-1} or more, only R30 appeared resistant, with LongC appearing as susceptible as the Roth susceptible standard.

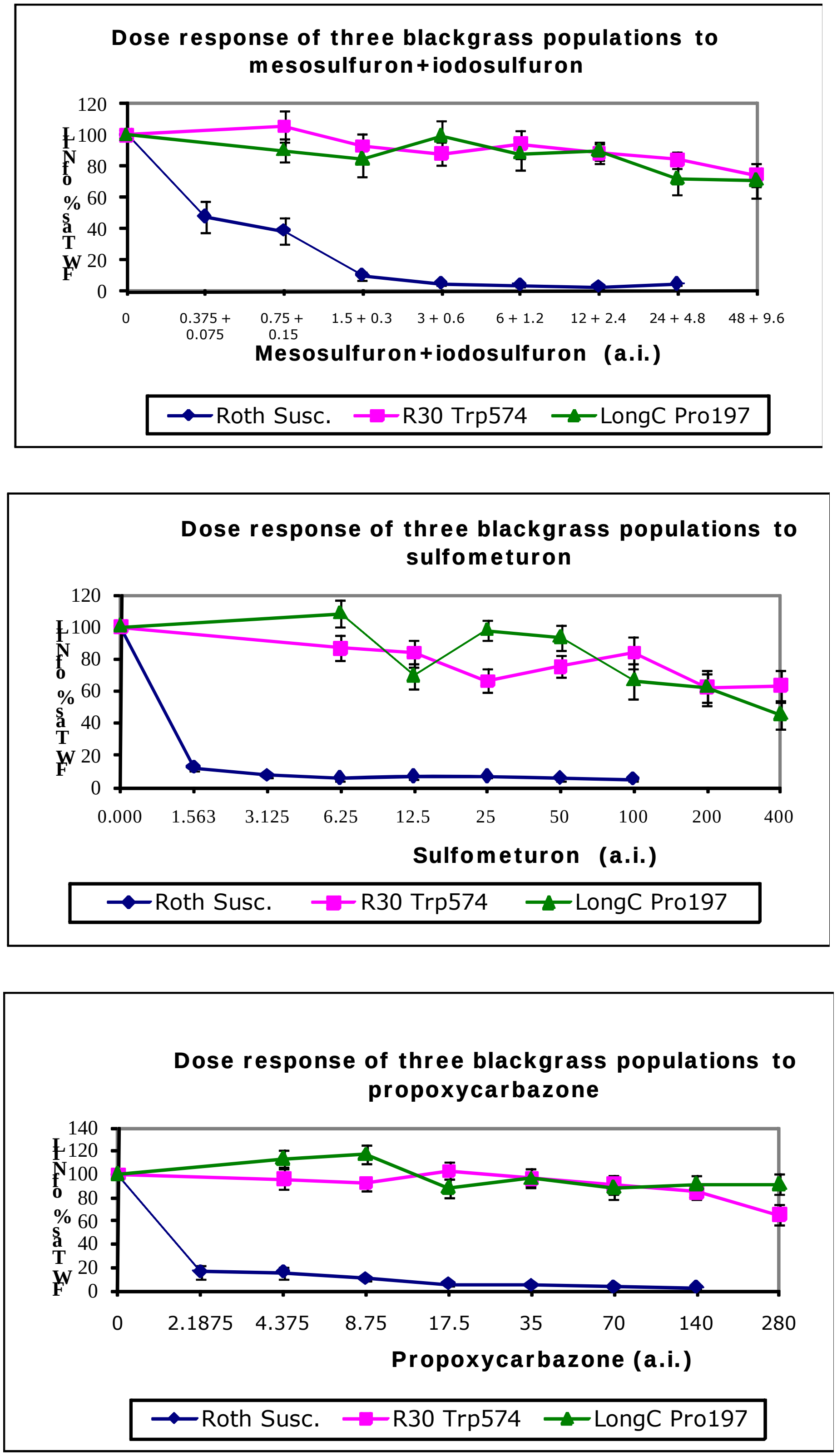


Figure 3.3.2.1 Response of three black-grass populations to mesosulfuron+ iodosulfuron, sulfometuron and propoxycarbazone.

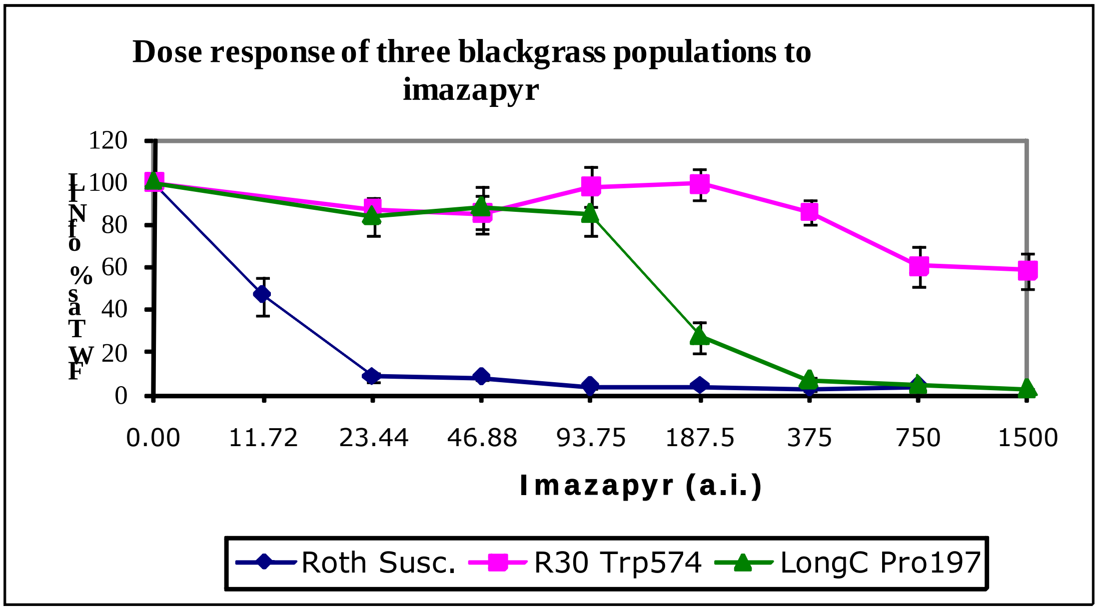


Figure 3.3.2.2 Response of three black-grass populations to imazapyr.

Conclusions of the cross-resistance studies with different classes of ALS inhibitors.

- **Very high degrees of resistance to two sulfonylureas (mesosulfuron+iodosulfuron, sulfometuron) and one sulfonylaminocarbonyltriazolinone herbicide (propoxycarbazone) were recorded in two black-grass populations with ALS target site resistance.**
- **The two mutations responsible, Pro197 & Trp574, appeared to confer equally high degrees of resistance to these two ALS classes.**
- **The specific mutation was much more important in determining the degree of resistance to the imidazolinone, imazapyr, with Trp574 conferring much greater resistance than Pro197.**
- **While the results indicate that activity of sulfonylureas and sulfonylaminocarbonyltriazolinone herbicides would be minimal against ALS target site resistant black-grass, the situation with imidazolinones against Pro197 resistant black-grass is harder to predict. Other imidazolinones may respond differently to imazapyr.**

3.3.3 Refining assays for dinitroaniline herbicides to enable detection of novel mechanisms of resistance.

Dinitroanilines, such as pendimethalin and trifluralin, are major components of risk mitigation strategies due, at least partly, to their perceived lower resistance risk (e.g. mixtures with dinitroanilines are actively promoted with both the sulfonylureas flupyrsulfuron and mesosulfuron+iodosulfuron). Pendimethalin is vulnerable to enhanced metabolism, but no resistance to trifluralin has so far been detected in the UK. However, resistance to *both* herbicides has been found in grass-weeds in other countries (e.g. *Eleusine indica* and *Setaria viridis* in North America) so the potential for resistance conferred by other mechanisms may exist in UK grass-weeds. We assayed a range of UK populations that have received regular applications of dinitroaniline herbicides in order to quantify resistance to pendimethalin and trifluralin. Initial studies were conducted in Petri dish assays.

Materials & Methods

Petri-dish assay. The responses of 14 black-grass populations to pendimethalin and trifluralin were assessed in a Petri-dish assay. The populations used included two susceptible standards (Roth04, Herb06), one with moderate levels of enhanced metabolism (Far00, from Oxfordshire), two with high levels of enhanced metabolism (Peld96 from Essex, HB-Peld04 bulked by Herbiseeds from Peld96 original stock), two with ALS target site resistance (Pro197) (Peld05 SS from Essex, Maid05 from Berkshire), one with ALS resistance but not conferred by either the Pro197 or Trp574 mutations (Wilts T+B, from Wiltshire), and six other populations collected from cereal fields in England (Colstw05, from Lincolnshire, A10-05 from Essex, A12-05 from Norfolk, Lucas02 from Oxfordshire, U05-A05 and U05-A09).

Seeds (50 per dish) were germinated in Petri-dishes containing 5 ppm of pendimethalin or trifluralin using the 'Rothamsted Rapid Resistance Test' methodology (Moss, 1999). There were two replicates and untreated controls for each population. Dishes were sealed in polythene bags in an incubator with a 17 °C 14 hour day and 11 °C 10 hour night. The number of shoots over 10 mm was recorded for each dish after two weeks as an indicator of resistance to pendimethalin and trifluralin.

Results and Discussion

The % germination in untreated dishes averaged 90% (range 65 – 98%). This is an excellent level of germination for black-grass seeds and showed that seed quality was very good. To avoid the effects of herbicide activity being confounded with differences in germination capacity between populations, data were converted to % reduction values relative to the untreated dishes for the same population. These % reductions in number of shoots over 10 mm values can be used as indicators of resistance to each herbicide, and are presented in Table 3.3.3.1. Resistance 'R' ratings were calculated as described by Moss *et al.*, (1999) which assign a rating of RRR, RR, R? or S (susceptible) depending on the degree of resistance.

Table 3.3.3.1 Petri-dish Experiment: Response of 14 black-grass populations to pendimethalin and trifluralin. (Populations listed in descending order of susceptibility to pendimethalin).

	Herbicide			
	Pendimethalin (5ppm)		Trifluralin (5ppm)	
Population	% reduction in number of shoots >10 mm compared to Nils	'R' rating	% reduction in number of shoots >10 mm compared to Nils	'R' rating
Roth04 (Susc.)	100	S	84	S
Herb06 (Susc.)	94	S	98	S
U05-A05	93	S	83	S
Far00	85	R?	94	S
Maid05	73	RR	89	S
U05-A09	71	RR	83	S
A12-05	64	RR	90	S
Lucas02	61	RR	89	S
Wilts T+B	45	RR	95	S
A10-05	39	RRR	83	S
Peld05 SS	24	RRR	100	S
HB-Peld04	19	RRR	83	S
Colstw05	3	RRR	87	S
Peld96	2	RRR	86	S
S.E. ±		7.8		
L.S.D ($P \leq 0.05$)		22.7		

The control of both susceptible standards (Roth 04 and Herb06) by both trifluralin and pendimethalin was good (84 – 100% reduction), indicating that the concentrations used in the assay were appropriate. Ten of the 14 populations showed resistance (RRR or RR) to pendimethalin and one population was rated R? (Far00). The Peldon populations, which comprised three of the five RRR ratings, are known to possess a high degree, and the Far00 population a lower degree of enhanced metabolism resistance. Thus the pendimethalin results are entirely consistent with previous findings. The HB-Peld04 Herbiseeds bulked sample gave similar results to the Peld96 parent field sample, and appears to be a good substitute, given that the original sample is now 13 years old and losing viability.

The Maid05 and Peld05 SS are both ALS target site resistant (Marshall & Moss, 2008) but these results indicate that both have differing degrees of enhanced metabolism. The Peld96 sample was also used in the study on resistance diagnostics (see section 3.3.1) and the Maid05 field was one of the three fields at Maidenhead which were intensively sampled as part of the resistance distribution study. Wilts T+B seeds were from the same field from where seeds were obtained for the container study (see section 3.2.1). The Wilts population shows resistance to mesosulfuron+iodosulfuron, but does not possess either of the two ALS target site mutations identified in other UK populations (Marshall & Moss, 2008). It is not clear whether enhanced metabolism alone can explain the response of the Wilts T+B population to mesosulfuron+ iodosulfuron. This population was rated RR to pendimethalin compared with RRR for Peld96, and the % reduction values differed substantially (45% v 2%). However, Wilts T + B shows more resistance to mesosulfuron+iodosulfuron than Peld96, which tends to be fully controlled at the field rate, although not at reduced rates (see section 3.3.1). This indicates that Wilts T + B could possess an ALS target site mutation, as yet unidentified, as well as a moderate level of enhanced metabolism. Alternatively, it could have a form of enhanced metabolism that is fundamentally different to that of Peld96, and capable of greater impact on the efficacy of ALS inhibiting herbicides, such as mesosulfuron+iodosulfuron.

In marked contrast to the response to pendimethalin, all 14 populations were susceptible to trifluralin (Table 3.3.3.1). While there was some variation in the % reduction values, there was no indication of any resistance. Indeed, some of the populations that were highly resistant to pendimethalin had % reduction values for trifluralin that were higher than those of the susceptible standards. This supports past findings that trifluralin is not vulnerable to enhanced metabolism. Resistance to trifluralin (and other dinitroanilines) does occur elsewhere in the world in other weed species, due to other mechanisms (Heap, 2009). The results of the Petri-dish assay indicate that such mechanisms were not present in any of the 14 populations studied. No resistance to trifluralin has ever been detected in any UK population of black-grass. The explanation appears to be that the ring CF_3 groups in trifluralin's molecular structure are more resilient to oxidation than the ring CH_3 groups present in pendimethalin's molecular structure (James *et al.*, 1995). Trifluralin can no longer be used in the UK after the 2008/09 cropping season due to its failure to acquire Annex 1 listing in the EU review of pesticides. These results highlight the importance of the loss of a herbicide for which resistance has never been detected in black-grass.

It is important to recognise that while the Petri-dish test can effectively detect quantitative differences in responses of different populations to herbicides, trying to relate these results directly to efficacy in the field is difficult. However, other studies have shown that resistance in the Peld96 and Colstw05 populations (both RRR in the Petri-dish assay) can result in poor control by pendimethalin under outdoor conditions (Moss & Hull, 2009). In the same experiment a susceptible population (Roth) was well controlled. This indicates that while Petri-dish assays may have their limitations, their results should not be ignored.

However, Peld96 and Colstw05 are two of the two of the most pendimethalin-resistant populations found so far in the UK, and are not typical of the majority of resistant populations. The same studies showed that on partially resistant populations, similar to those giving RR ratings in the Petri-dish test, pendimethalin can give useful levels of control of black-grass, especially when used in mixtures.

Conclusions of the dinitroaniline Petri-dish experiment:

- **Response to pendimethalin varied considerably, from very good to very poor control, indicating a variable degree of resistance in the 14 populations studied.**
- **The results highlight the need to continue to monitor development of resistance to pendimethalin, and to relate this to its overall contribution to weed control and hence resistance management, when used in mixture or sequence with other herbicides.**

- **In marked contrast to the response to pendimethalin, no population was resistant to trifluralin.**
- **The lack of resistance to trifluralin highlights the importance of its recent loss due to its failure to acquire Annex 1 listing in the EU review of pesticides.**

3.3.4 To develop a sampling strategy, involving spatial and temporal elements, in order to improve resistance detection and monitoring at the local level.

Weeds are less mobile than many pests (e.g. aphids) or pathogens (e.g. *Septoria*), so herbicide resistant populations of weeds such as black-grass, rye-grass and wild-oats are more localised in distribution both within, and between, farms. Most samples for resistance testing comprise a single bulked seed sample from a restricted part of a field, typically about 100 m x 2-3 tramlines. Consequently resistance tests provide little information on the distribution of resistance, and may under or over estimate the problem at a field or farm scale. Target site resistance (ACCase or ALS), if derived from initially rare resistant individuals, might be expected to be more 'patchy'. We investigated sampling strategies for black-grass on a range of scales with the objective of determining spatial distribution of resistance both on a field and farm scale. Existing testing methods were available for ACCase target site resistance which was used as a model system, and procedures developed within sub-objective 3.3.1 above were used for ALS resistance.

Materials & Methods

Over four-cropping seasons from July 2005 to July 2008, a range of fields in the east of England were identified that contained distinct patches of black-grass, measuring at least 2m x 2m in size, with ideally eight patches per field, with a distance of at least 10m between patches. The total number of patches, fields and farms are summarised in Table 3.3.4.1. In three fields a grid sample was taken on a smaller scale from within a patch, by sampling every 5m in a grid pattern (Figure 3.3.4.1) and collecting 20 samples per patch.

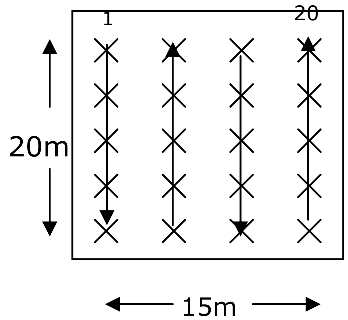


Figure 3.3.4.1 A grid design used for sampling 20 points, 5 m apart within a black-grass patch.

Where possible more than one field per farm was sampled for comparison. The fields had all received an application of mesosulfuron+iodosulfuron in that cropping season.

Table 3.3.4.1 The number and location of samples collected over four years

Unit of assessment	Total number (all years)
Counties	6
Farms	16
Fields	30
Patches	179
Grids (detailed patches*)	3

(20 sampling points in each 5m apart)*

Initially a sketch map of the field was drawn to mark the approximate size and shape of each patch and then black-grass seeds were collected from each patch individually using the standard method described in HGCA/WRAG Guidelines (Moss and Orson, 2003), in July of each season. Seeds were then tested for resistance using either the standard Rothamsted Rapid Resistance Test (RRRT) (Moss, 1999) in petri-dishes using fenoxaprop (10 ppm), sethoxydim (10 ppm) or cycloxydim (5 ppm) and pendimethalin (5 ppm) to identify ACCase target site and enhanced metabolism resistance, or the ALS pot test method developed as part of this project (see section 3.3.1) to identify ALS resistance. In the pot method mesosulfuron+iodosulfuron was applied at the field recommended rate (12+2.4 g a.i. ha^{-1}) to plants at the 3 leaf stage and foliage weight recorded after 4 weeks as a measure of herbicide efficacy. In 2005 it was only possible to use the ACCase RRRT as the pot test method had not yet been developed and validated. In 2006 and 2007 black-grass seed were tested in both

Petri-dish and pot tests. In 2008 black-grass seeds were only tested using the ALS pot test method due to the large quantity of seed being tested from other aspects of this project in the final season.

In 2008 three fields on the same farm in Berkshire that had been sampled in 2006 were re-visited and sampled again to assess any changes in the patches over that time period. From these same fields in 2008 a head count of black-grass plants m^{-2} was recorded to provide an assessment of patch density.

Results & Discussion

Resistance 'R' ratings were calculated for each black-grass sample as described by Moss *et al*., (1999) which assign a rating of RRR, RR, R? or S (susceptible) depending on the degree of resistance. Table 3.3.4.2 provides an overall summary and Tables 3.3.4.3 & 3.3.4.4 include more detailed information on some of the samples, such as variation within a patch and between distinct patches in the same field.

(See overleaf for table 3.3.4.2)

Table 3.3.4.2 The proportion (%) of black-grass samples allocated to each herbicide resistance 'R' rating category for each individual year.

		Resistance test method and herbicide			
		Petri-dish test			ALS Pot test
Year (*no. of patches*)	Resistance rating	Fenoxaprop (10 ppm)	Sethoxydim (10 ppm)	Pendimethalin (5ppm)	Mesosulfuron+ iodosulfuron
2005	RRR	100	18	46	-
(*11*)	RR	0	64	36	-
	R?	0	9	18	-
	% Resistant	**100**	**91**	**100**	**-**
	% Susceptible	***0***	***9***	***0***	-
2006	RRR	32	39	0	16
(*31*)	RR	55	45	13	16
	R?	7	0	16	13
	% Resistant	**94**	**84**	**29**	**45**
	% Susceptible	***6***	***16***	***71***	***55***
2007	RRR	85	51	10	40
(*100*)	RR	15	41	57	40
	R?	0	6	21	13
	% Resistant	**100**	**98**	**88**	**93**
	% Susceptible	***0***	***2***	***12***	***7***
2008	RRR	-	-	-	35
(*37*)	RR	-	-	-	51
	R?	-	-	-	0
	% Resistant	-	-	-	**87**
	% Susceptible	-	-	-	***13***
Sum of	RRR	72	36	19	30
all years	RR	23	50	35	36
(*179*)	R?	2	5	18	9
	% Resistant	**98**	**91**	**72**	**75**
	% Susceptible	***2***	***9***	***28***	***25***

(- not tested)

The overall proportion of black-grass seed samples that were resistant to all herbicides tested in each year has generally been very high (84%), although lower levels of resistance to pendimethalin (29%) and mesosulfuron + iodosulfuron (45%) were recorded for the samples collected in 2006. However, samples are relatively biased as distinct patches of black-grass were targeted so high levels of resistance were likely.

Across the three years of testing against ACCase resistance (2005-2007) 98% of the black-grass samples were resistant to fenoxaprop and 96% of these were in the highest RRR or RR resistant categories. Over 90% of the black-grass samples were resistant to sethoxydim, with 86% of these in the highest RRR or RR resistant categories. Resistance to pendimethalin was slightly lower, with 72% of black-grass samples showing resistance to this herbicide (54% RRR or RR and 18% R?). In the three years of testing ALS resistance (2006-2008), 75% of the black-grass samples showed resistance to mesosulfuron+iodosulfuron (66% RRR or RR and 9% R?).

Table 3.3.4.3 presents the results for the detailed patch sampling (20 sample grid) conducted in three fields, on two different farms in Cambridgeshire (2005 & 2008) and one in Oxfordshire (2008).

Table 3.3.4.3 The proportion (%) of the black-grass samples in each 'R' resistance rating category **within a patch**, (grid of 20 sampling points taken within one patch*).

	Herbicide group			
Resistance 'R' rating	ALS Mesosulfuron+ iodosulfuron	ACCase Fenoxaprop	ACCase Sethoxydim	Dinitroaniline Pendimethalin
RRR	100	100	100	0
RR	0	0	0	95
R?	0	0	0	5
S	0	0	0	0

*(*from a total of 60 sampling points in 3 field patches)*

The results in Table 3.3.4.3 show that there is very good consistency between the resistance ratings of the black-grass samples collected within patches, for all herbicides tested. For all herbicides 100% of the samples were in a single resistance category alone, except for pendimethalin, where 95% were in one category.

Table 3.3.4.4 presents results for the 120 black-grass samples collected from distinct patches within fields. In total eight patches were sampled in each of 15 fields, with one seed sample per patch.

Table 3.3.4.4 The proportion (%) of black-grass samples in each resistance rating category from distinct **patches within a field***, summarised over all four years.

Resistance 'R' rating	Herbicide group ALS Mesosulfuron+ iodosulfuron	ACCase Fenoxaprop	ACCase Sethoxydim	Dinitroaniline Pendimethalin
RRR	36	72	58	10
RR	33	24	38	46
R?	11	2	2	16
S	14	2	2	28

(from 8 sampling patches per field, from 15 individual fields on 5 farms).*

The results in Table 3.3.4.4 show very good consistency between resistance ratings for the herbicides fenoxaprop (72% in one category) and sethoxydim (58% in one category) with 95% of the samples either RRR or RR resistant. However, for the other herbicides there was more variability, so with mesosulfuron+iodosulfuron and pendimethalin less than 50% were in any one resistance category. This summary provides a good overview of the relative frequency of resistance to the different herbicides from the resistance tests conducted on the individual 120 samples.

However, to better demonstrate variability in resistance between patches, both within and between farms, data are presented for each individual field sampled (Table 3.3.4.5). This table summarises the data that was collected for the 15 individual fields from five different farms. In all cases eight samples were collected from different patches in each field and the resistance rating to mesosulfuron+iodosulfuron determined in a glasshouse pot assay.

Table 3.3.4.5 The number of patches (out of 8) in 15 fields for each resistance 'R' rating, based on response to mesosulfuron+iodosulfuron in a glasshouse pot assay, summarised over all four years.

Farm (County)	Field	Mesosulfuron+iodosulfuron 'R' resistance rating			
		RRR	RR	R?	S
1	WH – 2006	1	0	3	4
(Berks)	WH – 2008	4	4	0	0
	BD – 2006	0	0	1	7
	BD – 2008	1	6	0	1
	TF – 2006	4	4	0	0
	TF - 2008	4	4	0	0
	BH – 2007	8	0	0	0
	K – 2007	1	1	5	1
2	Field 1	1	6	1	0
(Beds)	Field 2	4	4	0	0
	Field 3	7	1	0	0
3	Field 1	0	0	4	4
(Oxon)					
4	Field 1	5	3	0	0
(Lincs)	Field 2	1	7	0	0
5	Field 1	4	4	0	0
(Cambs)					

Fields WH, BD and TF were assessed on two separate occasions once in 2006 and again in 2008. In TF there was a large patch near the gate which had four sampling points taken from it in 2006 and then four additional patches were also sampled. In 2008 the large patch near the gate was used for one of the within patch grid assessments. The four original separate patches were sampled, plus an additional four new patches (hence 10 results on the map). In 2006 the black-grass populations in WH and BD were very high, with some patches almost merging together, so samples were taken by moving up the centre of a tramline sampling and moving up 40m and sampling again. In 2008 the level of control achieved in these fields was far greater with more distinct patches present. Where possible samples were take from close to previous sampling points, however in some cases no black-grass was present so additional patches elsewhere in the field were sampled.

In both 2006 and 2008 the level of resistance to mesosulfuron+iodosulfuron in field TF was high, with all samples having a resistance rating of RR or higher. In contrast, in both WH and BD the level of resistance 2006 was low, with just one patch in WH rated RRR, and all the remaining samples either being R? or susceptible. However, by 2008, all eight patches in WH were rated RRR or RR, whilst on BD, seven patches were rated RR or RRR with just one patch still susceptible. This implies that resistance to mesosulfuron+iodosulfuron was increasing in these fields. If just one or two sampling points were assessed in the first year it is likely that the early signs of resistance might have been missed. By sampling in eight locations we were able to improve the ability to detect the first indications of developing resistance. However, once resistance was well established across a field, the amount of variation between the samples was much reduced, as seen in the TF and BH fields on Farm 1. The results also highlight the importance of regular sampling and resistance testing. Relying on results from 2006 to plan herbicide strategies in 2008 in the WH and BD fields would have been misleading.

The ratings for the other seven fields on farms 2 – 5 support the main conclusions from the more intensive sampling on farm 1. Generally there was less variation in resistance ratings between patches within a field than might have been expected. In 14 of the 15 fields, at least 88% of the samples in each field (i.e. 7 out of 8) were rated as either RR/RRR or R?/S. In 11 of these fields, 100% of the samples (8 out of 8) were rated as either RR/RRR or R?/S. This is an excellent level of consistency. The one exception was K-2007, and even in this field 75% of samples (6 out of 8) gave a consistent result (R?/S).

For each field a patch map was drawn and the resistance 'R' ratings of each sampling point included (Figure 3.3.4.2).

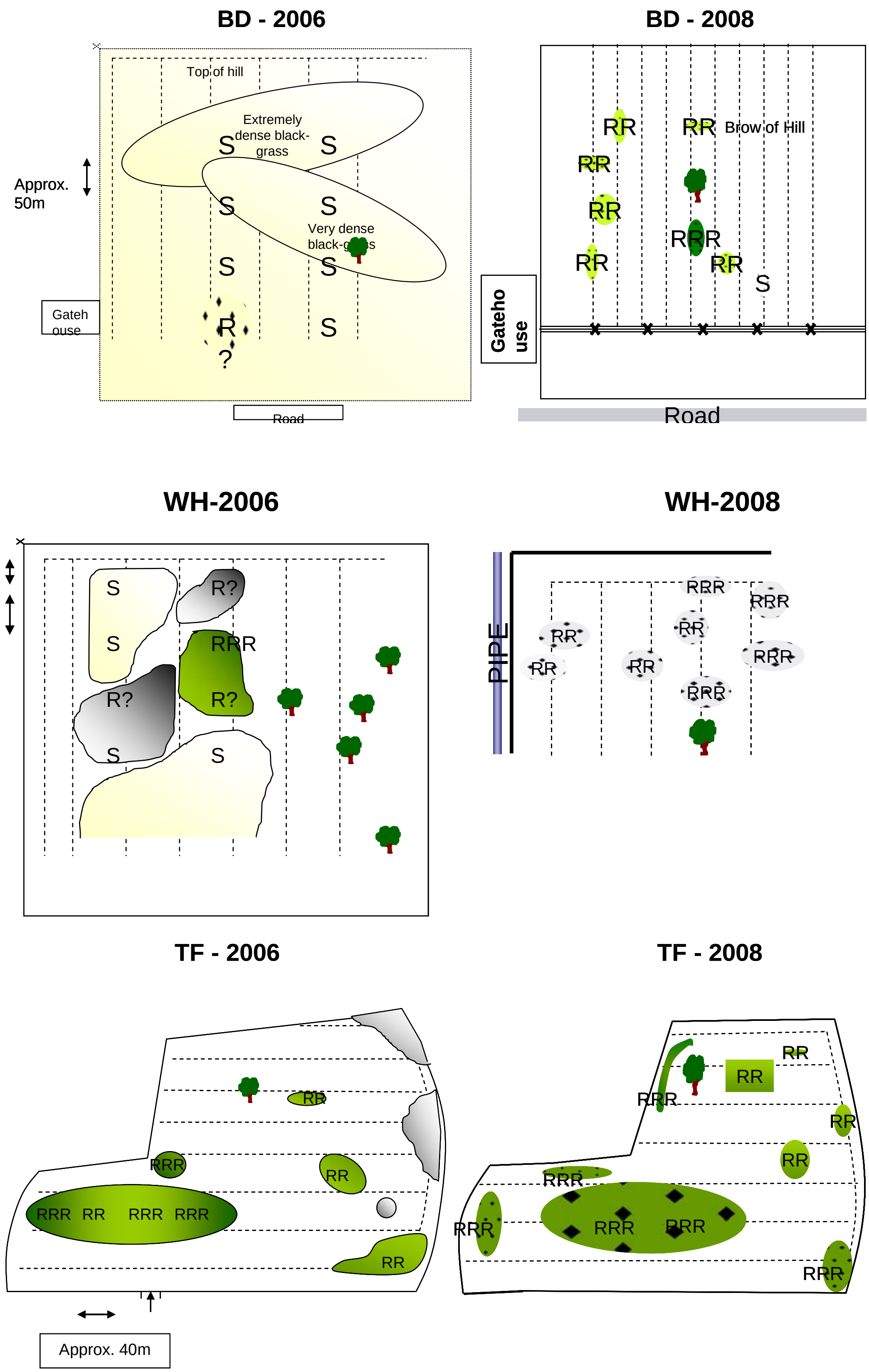


Figure 3.3.4.2 Three fields on the same farm sampled in two different years. Maps show the distribution of patches and variation in resistance level to mesosulfuron+iodosulfuron within and between years, patches and fields.

TF, BH and K fields were all close to one another on the same part of the Farm 1 (Figure 3.3.4.3). BH had high levels of resistance (8 x RRR) detected in it, the neighbouring field K has resistance starting to appear with one RRR patch on the side of the field neighbouring BH. TF has the highest level of resistance in the gateway and then moving into the field.

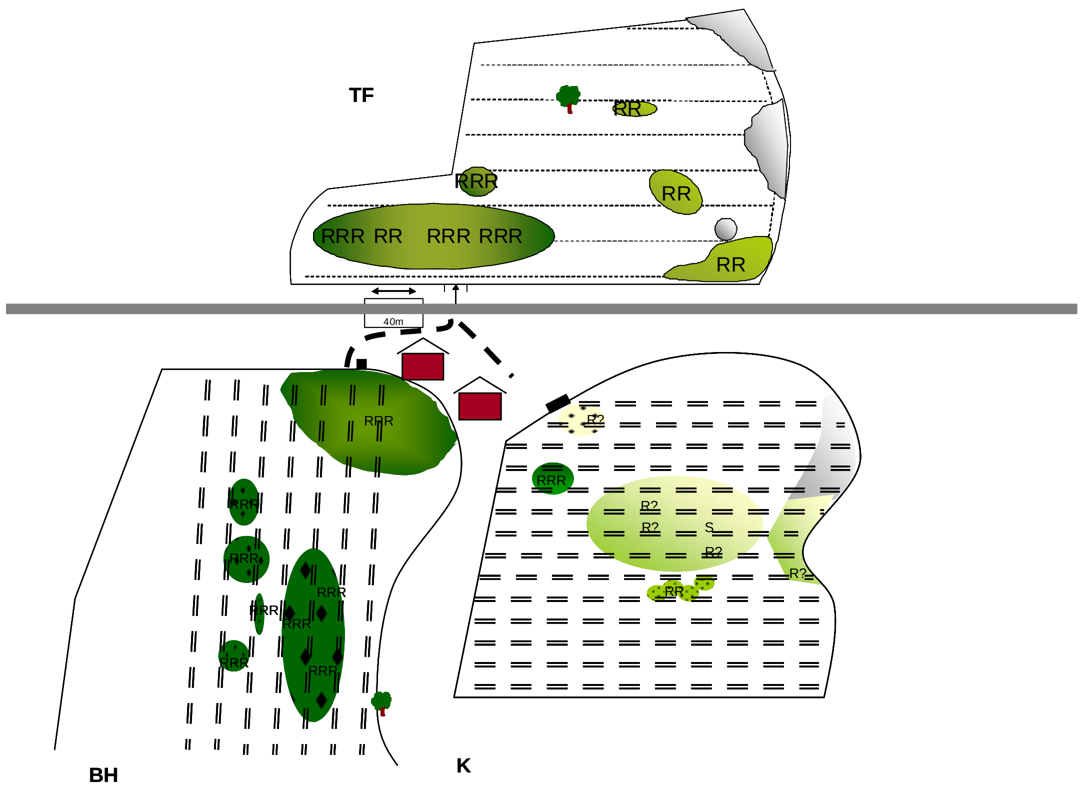


Figure 3.3.4.3 Distribution of resistance to mesosulfuron+iodosulfuron on neighbouring fields on Farm 1.

It is clear that while there was a good level of consistency in resistance test results within a single field, there can be considerable differences between fields, even on the same farm. However, a single sample from any field may give a misleading result if it is collected from one unrepresentative area. For example, if a single sample had been collected from the gateway into field K (Figure 3.3.4.3) – which was the most easily accessible patch from the farm yard - an under-estimation of the overall level of resistance present in the three fields around the farm would have resulted.

Conclusions of studies on seed sampling strategies, involving spatial and temporal elements.

- **The incidence of resistance detected in the patch samples was high, but this was expected as fields were not selected at random and samples were obviously biased towards black-grass 'survivors' in the field.**
- **75% of the black-grass samples collected (from a total of 168 samples) between 2006 and 2008 showed resistance to the ALS inhibiting herbicide mesosulfuron + iodosulfuron, with the majority of these samples in the highest resistance rating categories.**
- **98% of the samples collected showed resistance to fenoxaprop, 91% showed resistance to sethoxydim and 72% to pendimethalin.**
- **Because the incidence of resistance was so high, the consistency of the resistance test results for fenoxaprop and sethoxydim between different patches in the same field, and between fields, was very good.**
- **The lower incidence of resistance to pendimethalin and mesosulfuron+iodosulfuron resulted in greater variability between fields.**
- **The results from the resistance testing of the patch samples demonstrate that one sample taken from within a patch is likely to be representative of the whole patch due to the very good consistency in the resistance levels for all herbicides tested.**
- **The consistency levels of the resistance test results from samples taken from different patches within the same field were good, but not as high as for the within patch samples.**
- **Where resistance is just starting to develop in a field, there can be more variation in the amount of resistance present within a single field, but as resistance develops further, the ratings become increasingly consistent.**
- **There was considerable variability between black-grass resistance test results from different fields on the same farm, and between different farms. Neighbouring farm results should not be used as an indication of the level of resistance on another farm.**
- **Weed density alone is not a good indicator of resistance.**
- **Table 3.3.4.6 summarises a sampling strategy based on the findings of these sampling studies.**

Table 3.3.4.6 A summary of the consistency of resistance test results for each unit of assessment and the implications of these for black-grass resistance sampling strategies.

Unit of assessment	Consistency	Implications for sampling
Within a patch	Very good	One sample likely to be representative of that patch
Between patches within a field	Good/variable	Collect seed from a number of patches across the field
Between fields	Variable/poor	Consider carefully how to approach sampling and be prepared to take samples from several fields on each farm
Between farms	Variable/poor	Do not rely on the results at one farm to predict those on another

Sampling strategies for black-grass resistance should be on a small scale and, when more than one patch is present in the field, multiple samples should ideally be taken for maximum accuracy. However, from a practical level when multiple patches are present, a bulk of samples from a geographic cross-section of the patches could potentially provide a good indication of the overall resistance status of that field.

3.4 Objective 3: To quantify the impact of the population dynamics of grass-weeds on cultural and herbicidal resistance mitigation strategies by utilising existing knowledge and generating new information where this is lacking.

The risk of resistance and its rate of development are dependent on a matrix of interacting biological and management factors, each of which is driven by other variables. Understanding these relationships is vital to improving the ability to predict the risks associated with both existing and new incidences of herbicide resistance and the potential effectiveness of resistance mitigation strategies. Conducting experiments to investigate all possible mitigation strategies is clearly impractical, although it is essential that the principles are investigated and validated using realistic data. We aimed to model different resistance scenarios using experimental data to evaluate the principles and scope for improving the ability to assess the effectiveness of different risk mitigation strategies. Initially, we also investigated two specific aspects (effects of different cultivations and fitness/deselection) in relation to ALS resistance, where information of relevance to modelling was lacking.

3.4.1 Influence of different cultivation systems on development of ALS resistance

Materials & Methods

This experiment was conducted in outdoor containers over three years (2005-2008) with two populations of black-grass, Peld03 (ALS resistant) and Roth03 (susceptible) (see Section 3.2.1 above for details). In the third year only, additional containers were sown with the Peld03 baseline population. 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). The sets of treated and untreated containers were sown in the same manner. The herbicide mesosulfuron+iodosulfuron 12+2.4 g a.i. ha^{-1} + 'Biopower' adjuvant at 0.5% spray volume was applied each year to treated containers (22 Nov 2005, black-grass at 1 tiller stage; 17 October 2006, 3 leaf; 27 November 2007, 1-2 tillers). Methodology, replication, container isolation and seed collection procedures were otherwise the same as in Section 3.2.1 except that a total of 400 seeds were sown in each container each year. Black-grass plants were counted in each container prior to spraying and survivors assessed between late January and March each year.

Results

Plants established well in the containers each year giving the following plant densities in untreated containers: Yr 1 Non inversion 147, Plough 162; Yr 2 Non inversion 116, Plough 135; Yr 1 Non inversion 171, Plough 210. The Roth03 plants were all killed by herbicide treatment each year (100% reduction in plant numbers), confirming that this population was susceptible and that the application methodology and conditions at time of application were conducive to good control each year.

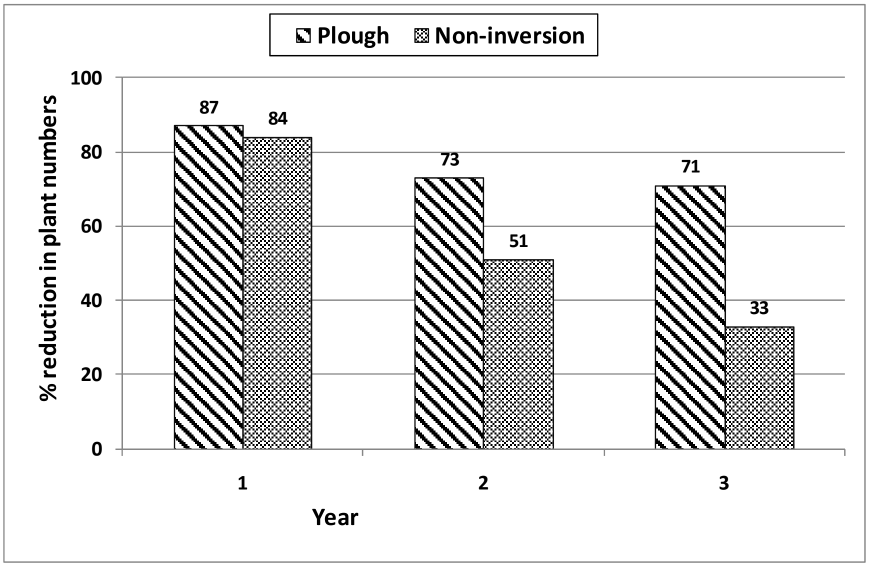


Figure 3.4.1.1 Effect of different simulated cultivations on control of Peld03 black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) over a three year period.

In contrast, poorer control of the Peld03 population was achieved by mesosulfuron+iodosulfuron in all years, confirming resistance (Figure 3.4.1.1). Statistical analysis showed a highly significant effect (F pr. <0.001) of both year and cultivation, and a significant (F pr. <0.016) interaction between year and cultivation. Herbicide performance declined over the three years in both cultivation systems, but to a much greater extent in the non-inversion tillage. With ploughing, the decline from 87% to 71% control over the three years was not quite statistically significant. In contrast, a highly significant reduction from 84% to 33% occurred with non-inversion tillage, as a consequence of a larger proportion of plants being derived from freshly shed seeds.

Table 3.4.1.1 Effect of different simulated cultivations on control of Peld03 black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1})

Cultivation treatment	% reduction in plant numbers compared to untreated containers Yr1	Yr2	Yr 3
'Plough'	86.5	72.8	71.0
'Non-inversion tillage'	84.0	50.6	32.8
Peld03 Baseline	-	-	74.8
S.E. ±		5.4	
L.S.D. (P≤0.05%)		16.3	

Control in year 3 with non-inversion tillage was over 40% lower than the Peld03 baseline population included in that year (Table 3.4.1.1). Control of the Peld03 baseline was somewhat lower than the control in year 1 (84% - 87%). Control of the same population with the same herbicide treatment in container experiments 1 & 2 was 69% and 82% respectively (see Tables 3.2.1.1 & 3.2.1.3, section 3.2.1). This demonstrates the variability that can occur in efficacy between different years, even in containers where many of the variables that occur in true field conditions are eliminated. Such year to year variability is likely to be even greater under true field conditions making detection of small declines in efficacy conferred by resistance difficult to detect. We believe these results demonstrate that containers provide a good model system for investigating these complex interactions under conditions that closely simulate true field conditions.

Although these cultivations were simulated, and may have exaggerated the difference in seed distribution caused by cultivations, we believe the results highlight the increased risk of more rapid development of resistance under non-inversion cultivation systems compared with ploughing.

Conclusions of the simulated cultivations experiment:

- **Mesosulfuron+iodosulfuron can very rapidly select for ALS resistance in situations where there is little 'buffering' from older, less selected seeds, resulting in an appreciable loss of efficacy.**
- **Resistance is likely to build up more rapidly under non-inversion tillage systems than where ploughing is the primary cultivation.**

- **Predicting the rate of build up will be difficult in any specific field due to the numerous variables that exist (e.g. the proportion and numbers of resistant seeds in the freshly shed component as compared with the soil seedbank and the efficiency of the cultivation system at moving seeds within the soil profile)**

3.4.2 Fitness/deselection studies on ALS target site resistant black-grass

We aimed to investigate whether deselection, or a decline in the degree of ALS resistance, occurred in the absence of herbicide selection. This is a potentially important factor to consider in any modelling exercise as it is critical to the rate of evolution of resistance.

Materials and Methods

This experiment was conducted in outdoor containers with six populations of black-grass:

1. Peldon 2003 (**Peld03**), a population collected from Essex with proven ALS target site resistant (Pro-197-Thr) present in about 18% of seeds (Marshall, 2007).
2. Peldon 2005 SS (**Peld05 SS**), a population produced from plants of Peldon 2003 that had survived treatment with sulfometuron in the glasshouse.
3. Wiltshire 2005 SS (**Wilts05 SS**), a population derived from plants of Wilts04 that had survived treatment with sulfometuron in the glasshouse. Wilts 04 seeds were collected from the same field as the Wilts05 population used in container experiment 1 (See Section 3.2.1 above for details). Resistance in Wilts05 SS is *not* conferred by either of the two ALS mutations (Pro-197-Thr; Trp-574-Leu) found in other ALS target site resistant black-grass in the UK (Marshall & Moss, 2008).
4. 'Double' target site resistant population (**DTSR**), a population derived from crossing Peld03 plants surviving sulfometuron treatment with Notts01 plants surviving sethoxydim treatment in the glasshouse. Notts01 has proven ACCase resistance conferred by the Isl-1781-Leu mutation (Brown *et al.*, 2002). The DTSR population was obtained from Peld mother plants and in glasshouse screening tests 42% of plants were only resistant to ALS herbicides, 18% only resistant to

ACCase herbicides, 22% resistant to both ALS and ACCase herbicides, with 17% susceptible.

5. Lincolnshire 2006 (**East06**), a field population that showed resistance to mesosulfuron+iodosulfuron in a glasshouse screening assay and proven ALS target site resistant (Pro-197-Thr) (Marshall & Moss, 2008).
6. Highfield 2006 (**High06**), a population from a field plot sown at Rothamsted in autumn 2003 with seeds collected from a field at Woburn and sprayed for three successive years with mesosulfuron+iodosulfuron. Glasshouse screening tests indicate partial resistance in this seed sample, probably due to enhanced metabolism and not ALS target site resistance.

In each container (40 x 33 x 16 cm deep), 400 black-grass and 21 wheat (cv. Hereward) seeds were sown into the surface 2.5 cm of a Kettering loam soil. There were two replicates in a randomised block design. Populations 1 – 4 were each grown for three successive years (2005/06 - 2007/08) and populations 5 & 6 for one year only (2007/08). Seeds were sown in late September each year and containers kept outdoors on a sandbed at Rothamsted. The number of plants was assessed in each container in late October or early November each year and no herbicides were applied. Containers for each population were isolated in individual small glass-houses in late April or early May to prevent cross-pollination. Seeds were collected as they matured from each individual container between June and August. Containers were re-sown each autumn with seeds collected from the same treatment that summer.

The effect of deselection on the proportion of seeds produced with ALS resistance was evaluated in a glasshouse assay for seeds collected in 2008 from each individual container by sowing 60 pre-germinated seeds in germination trays (38 x 22 x 5 cm deep) containing Kettering loam soil. There were four replicates comprising the six original baseline populations plus a susceptible standard (Roth05). The number of plants established in each tray was counted and then mesosulfuron+iodosulfuron at the field rate (12 + 2.4 g a.i. ha^{-1}) plus 'Biopower' adjuvant (@0.5%) was applied at the three leaf stage using a track sprayer delivering 240 l spray solution ha^{-1} at 245 kPa through a single 'Teejet' TP110015VK flat fan nozzle. The number of plants surviving with little or no damage was recorded after 4 weeks as a measure of resistance.

A Petri-dish assay was also conducted with the same populations to determine whether any changes in response to cycloxydim had occurred. This herbicide is an ACCase inhibitor (a 'dim') and a good indicator of ACCase target site resistance (Brown *et al*., 2002; Moss *et al*., 2003). Seeds were exposed to 5 ppm cycloydim and the number of germinated seeds with shoots over 10 mm long assessed after two weeks as an indicator of ACCase target site resistance (Moss, 1999).

Results

Plants established well with 104 – 157 plants per container in 2005/06, 75 – 105 in 2006/07 and 177 – 232 in 2007/08. The results for the glasshouse tray assay in which plants, grown from seeds collected in 2008 from each container, were treated with mesosulfuron+iodosulfuron are presented in Table 3.4.2.1 and Figure 3.4.2.1. Plants of the Roth05 susceptible standard were all killed (100% reduction in plant numbers) confirming that this population was susceptible and that the application methodology was conducive to good control.

(See overleaf for table 3.4.2.1)

Table 3.4.2.1 Glasshouse assay on the activity of mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) on six original baseline seed sources and the same populations after 1 or 3 years (generations) of no herbicide treatment in outdoor containers.

Population	Years without herbicide	Number of plants per tray		% reduction
		Pre-treatment	Surviving treatment	
Peld03	Baseline	57	9	85
	3 years	60	10	84
Peld05 SS	Baseline	58	43	27
	3 years	55	35	36
Wilts 05 SS	Baseline	60	41	33
	3 years	58	41	30
DTSR	Baseline	56	22	62
	3 years	58	21	64
East06	Baseline	59	22	63
	1 year	60	23	62
High06	Baseline	56	13	76
	1 year	59	13	78
Roth05 (susceptible)		57	0	100
S.E. ±		1.0	2.5	4.2
L.S.D. ($P \leq 0.05$)		2.8	7.3	12.1

Control of the baseline populations varied considerably, as would be expected from their resistance profiles. Thus, control of the Peld03 field sample was much higher than Peld05 SS which had been exposed to further selection for resistance in the glasshouse. The 85% control of the Peld03 baseline population was very similar to the control achieved with the same population in the glasshouse tray assays used in container experiments 1 (82%) and 2 (83%), which shows the excellent reproducibility of this technique (see Tables 3.2.1.2 & 3.2.1.3, section 3.2.1) .

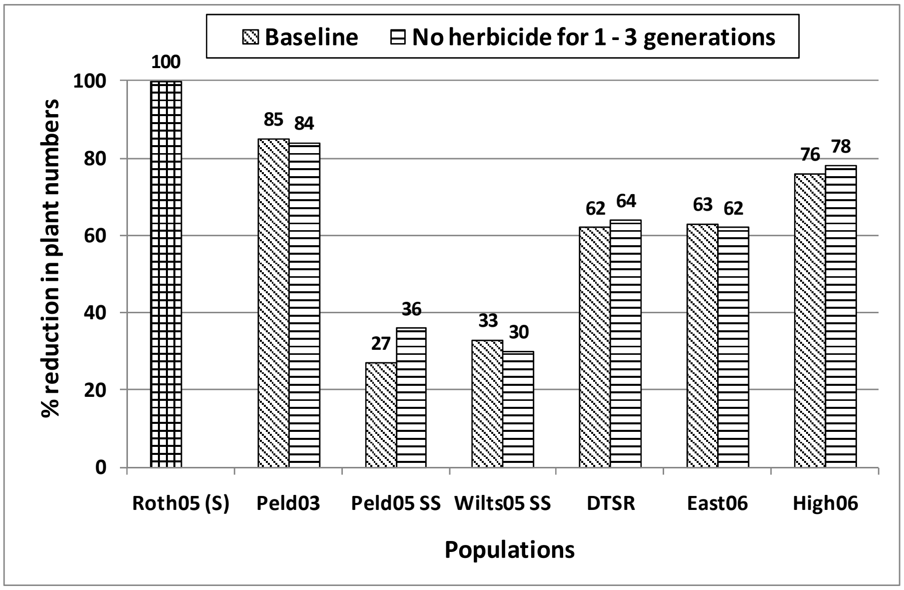


Figure 3.4.2.1 Control of black-grass plants by mesosulfuron+iodosulfuron (12+2.4 g a.i. ha^{-1}) in the glasshouse evaluation of seeds collected from the fitness/deselection outdoor containers.

There was no evidence in any of the six populations of any significant change in level of control by mesosulfuron+iodosulfuron following 1 or 3 years deselection. In five of the six populations, the levels of control in the unselected populations were remarkably similar to the baseline populations, within 3%. In three populations, control increased marginally, and in the other three populations control decreased marginally. The biggest change was with the Peld05 SS population, where control increased by 9% after three years without herbicide, but this was not statistically significant.

Meaned over all six populations, the % control of the baseline and untreated populations averaged 57.7% and 59.0% respectively, a 1.3% increase in control. If lack of herbicide treatment had caused resistance to mesosulfuron+iodosulfuron to be deselected as a consequence of a fitness penalty associated with the ALS resistance mutations, then the level of control should have increased. The fact that no substantial increases were recorded is very strong evidence that any fitness penalty associated with ALS target site resistance is minimal or non-existent. Fitness penalties of even 1% might certainly have an impact on selection for resistance over evolutionary timescales of hundreds of generations, but are extremely unlikely to have a significant impact over an agronomically relevant timescale of 5 – 10 years.

The populations tested included two with known ALS target site resistance conferred by the Pro-197-Thr mutation, one with an unknown mechanism (but not Pro-197-Thr or Trp-574-Leu mutations) and one with probable enhanced metabolic resistance. Despite this range of mechanisms, the results were very consistent, indicating that the conclusions drawn from this study should be robust and widely applicable, regardless of the precise ALS resistance mechanism responsible in any situation.

Table 3.4.2.2 Petri-dish assay using cycloxydim (5 ppm) as an indicator of ACCase target site resistance to 'fops' and 'dims' in six original baseline seed sources and the same populations after 1 or 3 years (generations) of no herbicide treatment in outdoor containers.

Population	Years without herbicide	% reduction in number of seeds with shoots >10mm relative to untreated
Peld03	Baseline	100
	3 years	100
Peld05 SS	Baseline	100
	3 years	100
Wilts 05 SS	Baseline	96
	3 years	96
DTSR	Baseline	56
	3 years	65
East06	Baseline	57
	1 year	55
High06	Baseline	90
	1 year	92
S.E. ±		4.2
L.S.D. *($P \leq 0.05$)*		12.8

There was no evidence of any ACCase target site resistance in the two Peldon populations, but variable amounts in the other four populations (Table 3.4.2.2). This is consistent with past screening studies. This also provides validation that the isolation system used to prevent cross-pollination between populations with different resistance status worked very well. There was no evidence in any of the six populations of any significant change in level of control by cycloxydim following 1 or 3 years deselection. In five of the six populations, the levels of control in the unselected populations were remarkably similar to the baseline populations, within 2%. The biggest change was with the DTSR population, where control increased by 9% after three years without herbicide, but this was not statistically significant.

Meaned over all six populations, the % control of the baseline and untreated populations averaged 83.2% and 84.7% respectively, a 1.5% increase in control. The fact that no substantial increases were recorded is very strong evidence that any fitness penalty associated with ACCase target site resistance is minimal or non-existent. This is entirely consistent with the results of the HeRMES project, which concentrated on ACCase resistance in black-grass and came to the same conclusion (Moss *et al.,* 2005b).

Thus the results of both the glasshouse tray test with mesosulfuron+ iodosulfuron and the Petri-dish assay with cyloxydim provide very strong evidence that any fitness penalties associated with both ALS and ACCase target site resistance are minimal or non-existent, and are extremely unlikely to have a significant impact over an agronomically relevant timescale of 5 – 10 years.

Conclusions of the fitness/deselection studies:

- **If mesosulfuron+iodosulfuron ceases to be used, the proportion of ALS resistant plants in the population is likely to remain at the same frequency.**
- **Consequently, ALS resistance is unlikely to decline even if herbicides of this class cease to be used. This appears to be true regardless of ALS resistance mechanism.**
- **There appears to be no significant fitness penalty associated with ALS resistance, or at least not one that is likely to be important over an agronomically relevant timescale.**
- **There also appears to be no significant fitness penalty associated with ACCase target site resistance, with these results validating past studies.**
- **Both ALS and ACCase resistance selection appear to be very much one-way processes – increasing rapidly given favourable conditions**

for selection, but not appearing to decline at all in the absence of selection pressure.

- **Farmers and advisors need to be aware of these findings and aim to maintain ALS and ACCase resistance at as low a frequency as possible, as there seems little chance of 'turning the clock back' with either of these types of resistance.**

3.4.3 Modelling the effects of grass-weed population dynamics on herbicide resistance mitigation strategies

The existing black-grass population model (Moss, 1990) is based on a considerable amount of field derived data collected in the 1970's and 80's, but does not include any herbicide resistance parameters. Consequently, one aim of this project was to update and refine the existing model in order to add a greater resistance component. Specifically, the resistance modelling component of this project investigated resistance selection imposed by different herbicidal and cultural strategies. This included the impact of different mechanisms of resistance, herbicide mixtures, sequences and rotations, and cultural control measures.

It is important to recognise two distinct components to the management of herbicide-resistant weeds: the *number* of weeds per unit area (the infestation level) and the *proportion* that are resistant (resistance level). Both of these factors were incorporated into the modelling process as both are relevant to the farm situation. In the short term, most farmers want to minimise the number of weeds (low infestation level) to protect crop yield and quality. If this is achieved by intensive use of herbicides, one consequence may be an increasing proportion of the population being resistant (high resistance level), which may compromise control and resistance management in the longer term.

Black-grass population model update

The existing black-grass population model in winter wheat (Moss, 1990) includes the parameters shown in Table 3.4.3.1, based on a considerable number of field investigations. The equation for the density dependent relationship between plant and head densities was:

$$y = B x / (1 + C x)$$

where:

y = numbers of heads m^{-2}

x = number of plants m^{-2}

B = number of heads per plant under conditions of no intra-specific competition (3.88)

C = a constant governing the response rate to increasing plant density, calculated as B/the y axis asymptote (= maximum numbers of heads m^{-2} = 2155). C = 0.0018.

This equation was based on data from 131 field experiments conducted in the UK and Germany between 1975 and 1988. However, in more recent field experiments it has been observed that there tend to be more black-grass heads per plant than the existing model would predict. There are three probable reasons for this. Firstly, earlier sowing of winter cereals in September, rather than October which was more common in the past, results in a longer vegetative phase leading to more tillering of black-grass plants in the autumn. Consequently, there is the potential for more heads per plant once the reproductive phase starts in spring. Secondly, milder winter weather may encourage more growth of black-grass during the winter than was formerly the case. Thirdly, a trend for sowing low cereal seed rates means less inter-specific competition during the vegetative phase of black-grass, which also favours tillering and greater head production.

Table 3.4.3.1 Parameters used in the black-grass population model (Moss, 1990).

Factor	Value	Parameter used in model
Seed survival in soil	30% annual survival	0.3
Seedling emergence from seeds in surface 5 cm soil	15% of new seeds 30% of old seeds	0.15 0.3
Herbicide efficacy	0 – 100%	0 to 1
Heads per plant	See text above	See text above
Seeds per head	100	100
Viability of seeds	55%	0.55
Survival of seeds on stubble	45%	0.45
Movement of seeds in the soil by ploughing	95% seeds within surface 5 cm are buried to over 5cm by ploughing; 35% of buried seeds are returned to the surface 5 cm layer by ploughing 25 cm deep.	Surface retention = 0.05 Burial = 0.95 Return to surface = 0.35 Depth retention = 0.65
Movement of seeds in the soil by tine/disc cultivations	100%, 80% and 60% of seeds within 5 cm of the soil surface are retained there by cultivations carried out to a depth of 5cm, 10cm or 20 cm respectively.	Very shallow tine or Direct drill = 1.0 10 cm tine/disc = 0.8 20 cm tine/disc = 0.6

Consequently, black-grass plant and head data from 462 plots in 16 winter wheat field experiments conducted between 1996 and 2008 was used to update the relationship between black-grass plant and head numbers. This is shown in Figure 3.4.3.1 overleaf.

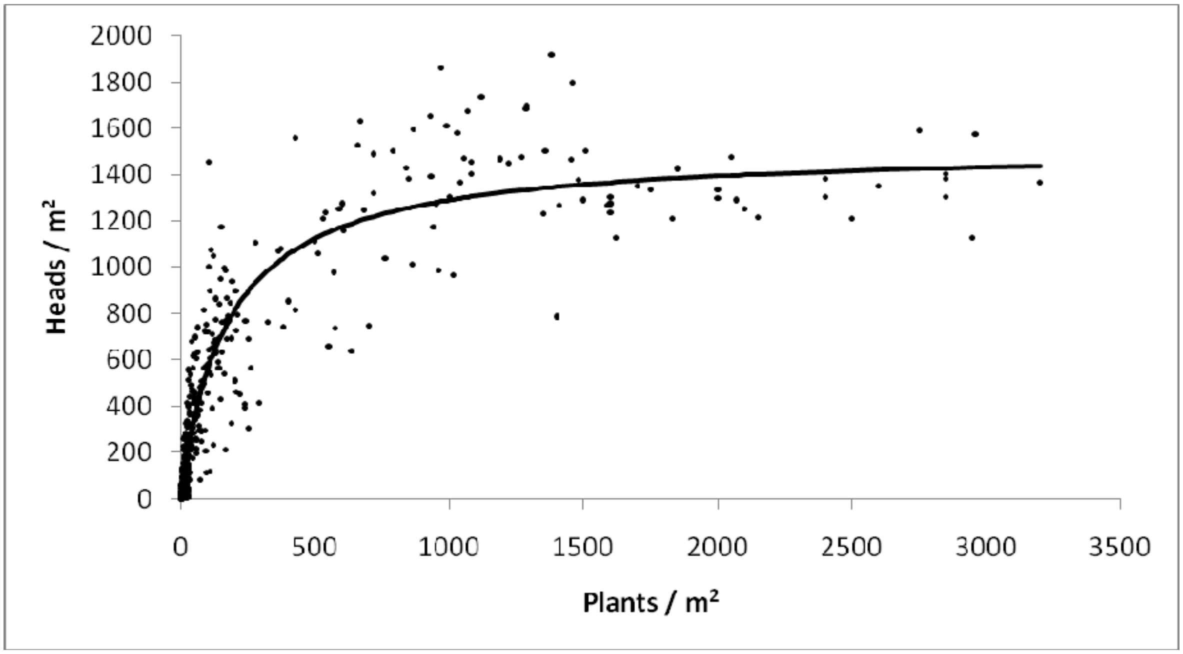


Figure 3.4.3.1 Relationship between black-grass plant and head densities based on data from 462 plots in 16 field experiments conducted between 1996 and 2008.

The line fitted to this new data shown in Figure 3.4.3.1 is based on the same equation as in the original model, but with updated parameters. A high proportion (88%) of the variance is accounted for in the updated equation. Thus the updated relationship between black-grass plants and heads is:

$$y = B\,x / (1 + C\,x)$$

where:

y = numbers of heads/m^2

x = number of plants/m^2

B = number of heads per plant under conditions of no intra-specific competition (8.71)

C = a constant governing the response rate to increasing plant density, calculated as B/the y axis asymptote (= maximum numbers of heads/ m^2 = 1517). C = 0.005741.

Predicted head densities and heads per plant for a range of black-grass plant densities for both the revised and original model (Moss, 1990) are presented in Table 3.4.3.2.

Table 3.4.3.2 Predicted head densities and heads per plant for a range of black-grass plant densities for both the revised and original model (Moss, 1990).

Black-grass Plants m^{-2}	Revised model		*Original model (Moss, 1990)*	
	Heads m^{-2}	Heads per plant	*Heads m^{-2}*	*Heads per plant*
1	8.7	8.7	*3.9*	*3.9*
10	82	8.2	*38*	*3.8*
25	190	7.6	*92*	*3.7*
50	338	6.8	*178*	*3.6*
100	553	5.5	*329*	*3.3*
250	894	3.6	*669*	*2.7*
500	1125	2.3	*1021*	*2.0*
1000	1292	1.3	*1386*	*1.4*
5000	1466	0.3	*1940*	*0.4*

With the revised model, there are just over twice as many heads per plant at densities of up to about 25 black-grass plants m^{-2}, compared with the original model. At higher densities, differences between the predictions of the two models are much smaller.

Modelling herbicide resistance

A series of modelling exercises were performed using the revised black-grass population model and incorporating various herbicide resistance components. These are detailed below with a brief explanation of the rationale behind each exercise, a summary table and comments on the outcomes. Unless otherwise stated, the initial seed population in the soil was assumed to be 100 seeds m^{-2} distributed evenly to a depth of 25 cm. No fitness penalty was attributed to ALS resistant plants as a consequence of studies reported in the previous section. The overall conclusions of the modelling exercises are presented at the end of this section.

(a). Increase in black-grass populations in winter cereals grown under different cultivation systems in the absence of herbicides.

The aim was to estimate the uncontrolled increase in black-grass that would occur in the absence of herbicides.

Table 3.4.3.3 Estimates of black-grass plant populations m^{-2} in winter cereals grown under different cultivation systems in the absence of herbicides.

Cultivation system	Years 1	2	3	4	*5*	*Maximum annual rate of increase*
Tine/disc (5 cm deep)	3	97	2075	6424	8970	*32*
Tine/disc (10 cm deep)	3	78	1427	4692	6435	*26*
Tine/disc (20 cm deep)	3	58	868	3124	4270	*19*
Plough (20 – 25 cm deep)	3	7	31	93	283	*4.4*

The output (Table 3.4.3.3, Figure 3.4.3.2) shows that, if uncontrolled, black-grass populations increase much more rapidly under non-inversion tillage than under ploughing. The less intensive the cultivation, the greater the potential increase. These very high potential population increases are about twice those predicted by the original model, and are a direct consequence of the greater seed production per plant at low densities resulting from a greater number of heads per plant. These predictions highlight the threat posed by this weed.

(See overleaf for figure 3.4.3.2)

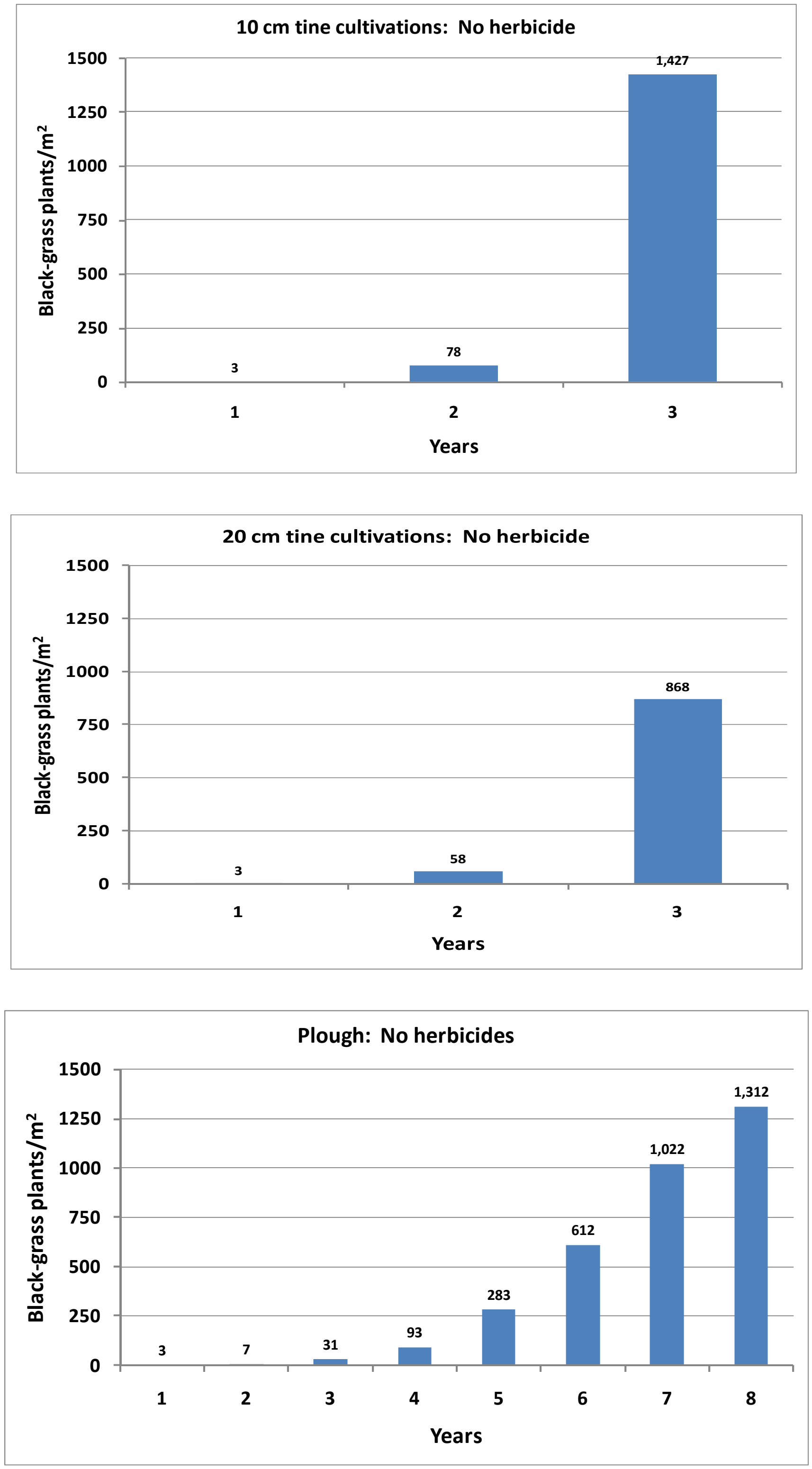


Figure 3.4.3.2 Estimated population increases for black-grass in winter cereal crops where no herbicides were used

(b). The annual percentage kill of black-grass plants needed within the crop to prevent the weed increasing in winter cereals.

The aim was to estimate the annual control needed from herbicides to maintain black-grass populations at a constant level. The values derived from the original model are presented for comparison.

Table 3.4.3.4 The annual percentage kill of black-grass plants needed within the crop to prevent the weed increasing in winter cereals.

Cultivation system	% kill of plants	
	Revised figures (2009)	*Moss (1990)*
Very shallow tine/disc cultivation (5 cm)	99%	*97%*
Shallow tine/disc cultivation (10 cm deep)	98%	*95%*
Tine/disc cultivation (20 cm deep)	97%	*93%*
Plough (20 – 25 cm deep)	90%	*78%*
Tine/disc cultivation (20 cm deep) + cultural control measures	93%	-

Note: Cultural control measures assumed to reduce heads per plant by 50% at low weed densities as a consequence of use of higher cereal seed rates, later drilling and more competitive varieties.

The output (Table 3.4.3.4) shows that high levels of control of black-grass are needed, especially in non-inversion tillage systems. The values are higher than the comparable values derived from the original model. This is a direct consequence of the greater seed production per plant at low densities resulting from a greater number of heads per plant. If heads per plant, and consequently black-grass seed production, could be reduced by encouraging greater crop competition, lower % kills should be acceptable. The additional use of cultural options was investigated, as indicated in the footnote to the table, in the 20 cm tine/disc cultivation system. This did decrease the % control needed from 97% to 93%. This may not appear very substantial, but is the difference between allowing 3 and 7 plants out of every 100 to survive, or allowing over twice as many escapes.

(c). The effect of different levels of weed control on black-grass plant and head populations in winter cereals grown under a deep tine/disc (20 cm) cultivation system.

The aim was to demonstrate the implications of levels of control below those needed to prevent populations increasing and also the impact of higher weed seedbank populations. Three levels of weed control were modelled, 85%, 90% and 95%.

Table 3.4.3.5 Effect of different levels of weed control on black-grass plant and head population in winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm, and also 100 times this population).

	Years							
	1	2	3	4	5	6	7	8
% weed control	Surviving black-grass plants m^{-2}							
85%	0.5	1.5	4.7	15	45	120	248	379
90%	0.3	0.7	1.6	3.5	7.9	17	36	70
95%	0.2	0.2	0.3	0.3	0.4	0.5	0.7	0.9
95% (initial seedbank population 100 x greater)	15	19	22	26	30	34	39	43

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely.

The output (Table 3.4.3.5) shows that a level of control slightly below that needed to prevent populations increasing (95% instead of 97%) can maintain populations at a low level, providing the initial population is low. However, at poorer levels of weed control (90% or 85%) or at higher initial seedbank populations, the populations soon reach levels which are likely to seriously impact on yields. The lower the level of control and the higher the initial seedbank level, the sooner this occurs. It should be noted that the higher initial seedbank modelled here (10,000 seeds m^{-2}) is not wildly excessive in practice, as seedbanks of over 50,000 black-grass seeds m^{-2} have been recorded on commercial farms. The conclusion is clear – higher seedbanks will make sustainable control very difficult in non-inversion tillage systems unless very high levels of control from herbicides can be maintained. Increasing herbicide resistance makes this an increasingly challenging objective.

(d). The percentage kill of black-grass plants needed to reduce populations to 3 target densities at 5 different pre-spraying infestation levels.

On average, a density of 12 black-grass plants m^{-2} will cause a 5% reduction in winter wheat yield. At this density, the cost of control roughly equals the cost of herbicide treatment and can be considered the economic threshold. Most farmers and advisors would not be satisfied with black-grass densities this high, due to the seed production potential and threat to future crops. A density of about 5 plants m^{-2} has been suggested as a more appropriate long term threshold (Doyle, Cousens & Moss, 1986). Such thresholds are averages, so greater yield losses and seed production will often occur at lower weed densities. Hence many farmers and advisors would aim to reduce black-grass populations to as low a level as possible, 1 plant m^{-2} or less. These three target black-grass densities (1, 5 and 12 plants m^{-2}) were used to calculate the % control needed at five different pre-spraying infestation levels (Table 3.4.3.6).

Table 3.4.3.6 The percentage kill of black-grass plants needed to reduce populations to 3 target densities at 5 different pre-spraying infestation levels.

Black-grass plants m^{-2} pre-spraying	Target surviving black-grass plants m^{-2}		
	12 (=98 heads m^{-2})	5 (=42 heads m^{-2})	1 (=8.7 heads m^{-2})
	% kill of plants required		
25	52.0%	80.0%	96.0%
50	76.0%	90.0%	98.0%
100	88.0%	95.0%	99.0%
250	95.2%	98.0%	99.6%
1000	98.8%	99.5%	99.9%

Note: unshaded, lighter and heavier shading = respectively achievable (<90%), potentially achievable (90 – 95%) and unachievable (>95%) with resistant weed populations.

The higher the pre-spraying population, and the more ambitious the target, the higher the level of control required. At low to moderate populations adequate control is potentially achievable, even with resistant populations, but at high pre-spraying populations achieving a consistent reduction to meet any of the three targets is unlikely, especially if black-grass is resistant. These results highlight the problem of reducing black-grass to acceptable levels, especially with high infestations of resistant black-grass.

(e). The percentage kill of black-grass plants needed from pre-emergence herbicides to compensate for declining activity of post-emergence herbicides.

If control from post-emergence treatments is inadequate to contain black-grass, either through lack of effective herbicides or increasing resistance, activity from pre-emergence herbicides can compensate, at least to some degree. This exercise aims to evaluate the robustness of this approach for three overall levels of control.

Table 3.4.3.7 The percentage kill of black-grass plants needed from pre-emergence herbicides to compensate for declining activity of post-emergence herbicides for three overall levels of control from pre/post herbicide sequences (90%, 93% and 97% reduction in plant numbers. See Table 3.4.3.4 above).

Control from main post-emergence treatment	Control required from pre-emergence herbicides to achieve three different overall target levels of control		
	90% (Control needed in ploughing systems)	93% (Control needed in deep tine systems + cultural control)	97% (Control needed in deep tine systems)
99%	0	0	0
94%	0	0	50%
89%	9%	36%	73%
84%	38%	56%	81%
79%	52%	67%	86%
74%	62%	73%	88%
69%	68%	77%	90%
64%	72%	81%	92%
59%	76%	83%	93%
54%	78%	85%	93%
49%	80%	86%	94%

Note: unshaded, lighter and heavier shading = respectively achievable (<60%), potentially achievable (60 – 80%) and unlikely to be achievable (>80%) routinely.

Where post-emergence treatments give high levels of control, black-grass populations can be contained without the additional use of a pre-emergence herbicide (Table 3.4.3.7). However, with decreasing efficacy from the post-emergence herbicide, increasing reliance on good activity of the pre-emergence partner is required to achieve sustainable levels of control from the pre/post sequence. It is unrealistic to expect any pre-emergence herbicide, used alone or in combination, to achieve over 80% control consistently. In deep tine systems, the model predicts that overall control will be insufficient to prevent black-grass populations increasing when post-emergence efficacy drops below about 90%. Where cultural control measures are used in combination with deep tine tillage, post-emergence efficacy can decline to 60% - 70% before overall control will be insufficient. This 20% - 30% difference is quite a substantial loss of efficacy, and demonstrates that the use of cultural control measures could potentially make a valuable contribution to more sustainable weed control in situations where post-emergence herbicide performance is declining. With ploughing, lower overall levels of control are acceptable, and the model predicts that post-emergence efficacy has got to decline very substantially, to less than 50%, before overall control with a pre/post sequence becomes inadequate.

One practical conclusion is that farmers and consultants need to get a better idea of exactly what control is being achieved by each component of any pre/post sequence, and how this is changing with successive years, in order to better evaluate the sustainability of their overall herbicide strategy.

(f). Effect of declining herbicide performance due to enhanced metabolic resistance on black-grass plant and head population in winter cereals grown under a deep tine/disc (20 cm) cultivation system.

Enhanced metabolic resistance tends to confer partial resistance, with a continuum in response from minor to major effects on herbicide performance. Gradually increasing levels of enhanced metabolic resistance can be modelled by assuming that herbicide efficacy reduces progressively with time. In this modelling exercise an annual reduction in herbicide performance of 5% was assumed.

In this scenario (Table 3.4.3.8), black-grass populations were well controlled and maintained at levels that are unlikely to seriously impact on yield for about five years. In practice, the decline in herbicide performance up to this point would probably not be noticed.

Table 3.4.3.8 Effect of declining herbicide performance on black-grass plant and head population in winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds/m^{-2} distributed evenly to a depth of 25 cm).

	Years							
	1	2	3	4	5	6	7	8
	% weed control by herbicide (declining by 5% per year)							
Post-em. herbicide efficacy	99%	94%	89%	84%	79%	74%	69%	64%
	Black-grass plants m^{-2}							
Plants pre-spraying m^{-2}	3.0	1.7	2.3	5.8	20	85	410	1568
Plants surviving m^{-2}	0.03	0.1	0.3	0.9	4.1	22	127	564
Heads m^{-2}	0.3	0.9	2.2	8.0	35	171	640	1159

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely.

However, as efficacy continued to decline below 80%, there was a rapid increase in weed infestation level and, within two years, populations had reached a level that would very substantially impact on yield. These declines in efficacy are not unrealistic and highlight the risk of complacency where the black-grass population appears to be well under control. Such populations may respond very quickly to inadequate control caused by increasing levels of enhanced metabolic resistance.

Close monitoring of herbicide performance in association with regular seed testing for resistance could help to act as an early warning of resistance problems ahead.

(g). Effect of declining herbicide performance (5% per year) and rotational ploughing on black-grass plant and head population in winter cereals. Ploughing done at start of years 5 and 8. Deep tine/disc (20 cm) cultivation system used in other years.

This exercise was similar to the previous one, except that rotational ploughing was included to evaluate its value at reducing population increases. In this scenario (Table 3.4.3.9), ploughing after five years prevented the rapid increase in black-grass populations that occurred in the previous exercise, where deep tine/disc cultivation was used annually. However, populations did continue to increase in years 6 and 7 as a consequence of a return to non-inversion tillage and declining herbicide performance.

Table 3.4.3.9 Effect of declining herbicide performance (5% per year) and rotational ploughing on black-grass plant and head populations in winter cereals. Ploughing done at start of years 5 and 8. Deep tine/disc (20 cm) cultivation system used in other years. (Assuming an initial seed population of 100 seeds m^{-2} distributed evenly to a depth of 25 cm).

	Years							
	1	2	3	4	5	6	7	8
	DT	DT	DT	DT	Plough	DT	DT	Plough
	% weed control by herbicide (declining by 5% per year)							
Post-em. herbicide efficacy	99%	94%	89%	84%	79%	74%	69%	64%
	Black-grass plants m^{-2}							
Plants pre-spraying m^{-2}	3.0	1.7	2.3	5.8	2.6	9.7	47	31
Plants surviving m^{-2}	0.03	0.1	0.3	0.9	0.5	2.5	15	11
Heads m^{-2}	0.3	0.9	2.2	8.0	4.3	22	118	87

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely

A second ploughing at the start of year 8 again reduced the population but to a limited degree, as herbicide performance had declined to a level which was inadequate for sustainable control, even in a ploughing system. The surviving plant population in year 8 was less than 2% of that in the previous exercise (f) showing the potential benefit of rotational ploughing at containing resistant black-grass.

(h). Effect of using a pre-emergence herbicide (giving 60% control) when post-emergence herbicide performance is declining by 5% per year. Values are black-grass plant and head population in winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm. Pre-emergence herbicide used from Yr 2 onwards).

Farmers are increasingly using pre-emergence herbicides in sequence with post-emergence herbicides to improve overall control, partly because of reductions in efficacy due to resistance and partly due to the loss of some post-emergence options. Consequently, in this modelling exercise, it was assumed that the efficacy of the pre-emergence herbicide applied annually did not decline with time, whereas the efficacy of the post-emergence herbicide applied in sequence did decline, by 5% per annum.

Table 3.4.3.10 Effect of using a pre-emergence herbicide (giving 60% control) when post-emergence herbicide performance is declining by 5% per year. Values are black-grass plant and head population in winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm. Pre-emergence herbicide used from Yr 2 onwards).

	Years							
	1	2	3	4	5	6	7	8
	% weed control by herbicide (declining by 5% per year)							
Post-em. herbicide efficacy	99%	94%	89%	84%	79%	74%	69%	64%
Pre + post em. efficacy	99%	98%	96%	94%	92%	90%	88%	86%
	Black-grass plants m^{-2}							
*Plants pre-spraying m^{-2}	3.0	1.7	1.2	1.4	2.1	4.1	9.6	26.3
Plants surviving m^{-2}	0.03	0.04	0.05	0.09	0.18	0.43	1.2	3.8
Heads m^{-2}	0.3	0.3	0.5	0.8	1.5	3.7	10.3	32.2

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely. * = potential population as pre-emergence herbicide would reduce this number in practice.

In this scenario (Table 3.4.3.10), the additional use of a pre-emergence herbicide helped greatly in maintaining low weed populations compared with the situation modelled in exercise f above, where no pre-emergence herbicide was used. The main contribution of the pre-emergence herbicide was to maintain a high overall level of weed control despite the declining performance of the post-emergence herbicide. In year 8, the overall control was 86% compared with only 64% in exercise f. This higher overall level of control meant that, even after 8 years, there were only 3.8 black-grass plants m^{-2} surviving treatment compared with 564 plants m^{-2} where no pre-emergence herbicide was used (see Table 3.4.3.8 in exercise f above). However, the number of surviving plants was increasing, so the use of the pre-emergence herbicide was delaying, not preventing, resistance impacting on infestation level. In addition, resistance may progressively reduce the efficacy of the pre-emergence herbicide too, which would be expected to have an increasingly negative impact on overall weed control.

(i). Effect of rotating a herbicide with declining performance (5% every 2 years) with one giving a consistent 90% control. Values are black-grass plant and head population in winter cereals grown under a deep

tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm).

With resistance prone herbicides, one approach could be to use these only in alternate years, and rely on a different herbicide in the intervening years. The hope would be that resistance would build up more slowly, and populations maintained at a lower level, than where the high risk herbicide was used annually. This was modelled assuming that the alternative herbicide gave 90% control consistently, and so was unaffected by resistance.

Table 3.4.3.11 Effect of rotating a herbicide with declining performance (5% every 2 years) with one giving a consistent 90% control. Values are black-grass plant and head populations in winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm).

	Years							
	1	2	3	4	5	6	7	8
	% weed control by herbicide							
Herbicide efficacy	99%	*90%*	94%	*90%*	89%	*90%*	84%	*90%*
	Black-grass plants m^{-2}							
Plants pre-spraying m^{-2}	3.0	1.7	3.6	5.4	12.3	30.1	67.5	220
Plants surviving m^{-2}	0.03	0.17	0.2	0.5	1.4	3.0	10.8	22.0
Heads m^{-2}	0.3	1.4	1.9	4.7	11.7	25.8	88.6	170

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely.

In this scenario (Table 3.4.3.11), populations built up more slowly than in exercise f, where alternative herbicides were not used, but more rapidly than in exercises g & h where either rotational ploughing was practiced or pre-emergence herbicides used annually. Thus, after 8 years, the numbers of surviving plants in exercises f – i were 564, 11, 4 and 22 respectively. Consequently, in these modelling exercises there was not a large difference in benefit between these three modifiers, namely rotational ploughing, pre-emergence herbicides and use of alternative herbicides unaffected by resistance. However, care is needed when drawing conclusions from such exercises as different assumptions on herbicide efficacy could greatly affect the outcomes. For example, the benefit of the rotational use of an alternative herbicide, as modelled in exercise i, depends critically on its efficacy and consistency in controlling black-grass. If the alternative herbicide was itself affected by resistance, then the benefit from its use would be short lived.

In all these scenarios, the number of surviving plants was increasing over the 8 year period. So the problem of increasing infestation level, and consequent impact on yields and seed production, was simply delayed, not prevented.

(j). Increase in target site resistance (e.g. ALS or ACCase) in response to annual use of a post-emergence herbicide selecting for that resistance type (e.g. sulfonylurea or 'fop') in winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm with an initial frequency of 1% of seeds target site resistant).

Target site resistance to either ACCase or ALS inhibitors normally gives a high degree of resistance in plants containing mutant isoforms, but only to herbicides of that specific class. This contrasts with enhanced metabolism resistance which tends to give partial resistance, but to a wide range of herbicides. Target site resistance is normally monogenic, with the resistance trait dominant and follows simple Mendelian genetic principles. Consequently heterozygous resistant plants (RS) can produce some seeds that are susceptible (SS), if they cross with either susceptible (50% seed will be susceptible) or other heterozygous plants (25% seed susceptible). At low frequencies of resistant plants, these scenarios are likely. At higher frequencies, more homozygous resistant plants (RR) are likely to exist, which will produce 100% resistant seeds (RR or RS) regardless of whether they cross with a homozygous resistant (RR), heterozygous resistant (RS) or susceptible (SS) plant. Incorporating such genetic components into models can be done, but relies on assumptions that are hard to enumerate, such as relative density of plants with different resistance traits, flowering periodicity of resistant and susceptible plants, effective pollen movement distances and ability to self pollinate. In addition, target site resistant plants may not be totally immune to herbicides, and some control may be achieved of small plants or of heterozygous plants if the resistance trait has incomplete dominance.

Consequently in this modelling exercise, we have assumed that the herbicide gives 97% control of susceptible plants and 25% control of plants with target site resistance. In many of the other studies within this project, much lower levels of control of resistant plants have been achieved with field collected samples, so we consider this to be quite an optimistic level of control.

Table 3.4.3.12 Increase in target site resistance (e.g. ALS or ACCase) in response to annual use of a post-emergence herbicide selecting for that resistance type (e.g. sulfonylurea or 'fop') in winter cereals grown under a **deep tine/disc** (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm with **an initial frequency of 1% of seeds target site resistant**).

	Years							
	1	2	3	4	5	6	7	8
Post-em. herbicide efficacy	% weed control by herbicide 97% control of susceptible; 25% control of target site resistant plants							
	Black-grass m^{-2}							
Plants pre-spraying m^{-2}	3.0	3.3	9.2	100	1039	3130	4206	4526
% target site resistant	1%	14%	73%	98%	100%	100%	100%	100%
Plants surviving m^{-2}	0.11	0.4	5.1	74	778	2346	3155	3394
Actual % control of plants	96%	87%	44%	27%	25%	25%	25%	25%
Heads m^{-2}	1.0	3.7	44	451	1241	1412	1438	1443

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely.

Surviving black-grass plant populations increased rapidly, as did the % of plants that were target site resistant (Table 3.4.3.12). It took only four years for the proportion of resistant plants to increase from 1% to 100% of the population, and the control of plants decreased equally rapidly. 1% was used as the initial frequency as this is about the level that could be detected in resistance screening tests. These results highlight the importance of taking action as soon as resistance is detected, as while good levels of control may be maintained for one or two years, control is likely to decline rapidly thereafter if a herbicide selecting for that form of target site resistance continues to be used as the sole means of control.

In the container experiment in which different cultivations were simulated, (see section 3.4.1 above), control declined from 84% to 33% over a three year period with non-inversion tillage, or a 51% decline overall. In this modelling exercise with deep tine/disc tillage, control declined from 87% to 27% between years 2 and 4, a 60% decline overall. Thus there was good agreement between the container experiment and this modelling exercise on the likely rate of loss of efficacy if a herbicide selecting for that form of target site resistance is applied annually as the sole means of black-grass control.

(k). Increase in target site resistance (e.g. ALS or ACCase) in response to annual use of a post-emergence herbicide selecting for that resistance type (e.g. sulfonylurea or 'fop') in winter cereals grown under a plough based (20 -25 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm with an initial frequency of 1% of seeds target site resistant).

This exercise used the same parameters as those in exercise j above except that ploughing was used as the primary cultivation instead of a deep tine/disc system.

In this plough based scenario (Table 3.4.3.13), surviving black-grass plant populations increased much less rapidly than under the deep tine/disc system used in exercise j above. There was also a slower increase in the % of plants that were target site resistant. It took about twice as long for the proportion of resistant plants to increase from 1% to 100% of the population, and the control of plants decreased more slowly too. However, ultimately the increase in resistance impacted severely on infestation level regardless of cultivation system, it simply took about twice as long under a ploughing regime. These results highlight the importance of taking action as soon as resistance is detected regardless of cultivation system, although under a ploughing regime one gains a few extra years before the agronomic system breaks down.

Table 3.4.3.13 Increase in target site resistance (e.g. ALS or ACCase) in response to annual use of a post-emergence herbicide selecting for that resistance type (e.g. sulfonylurea or 'fop') in winter cereals grown under a **plough based** (20 -25 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm with **an initial frequency of 1% of seeds target site resistant**).

	Years							
	1	2	3	4	5	6	7	8
Post-em. herbicide efficacy	% weed control by herbicide 97% control of susceptible; 25% control of target site resistant plants							
	Black-grass plants m^{-2}							
Plants pre-spraying m^{-2}	3.0	2.8	2.0	1.6	2.6	6.0	17	49
% target site resistant	1%	2.3%	11%	39%	75%	94%	99%	100%
Plants surviving m^{-2}	0.11	0.13	0.22	0.50	1.5	4.3	12.7	36
Actual % control of plants	96%	95%	89%	69%	43%	29%	26%	25%
Heads m^{-2}	1.0	1.1	2.0	4.3	13	36	103	262

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely.

In the container experiment in which different cultivations were simulated, (see section 3.4.1 above), control declined from 87% to 71% over a three year period with ploughing, or a 16% decline overall. In this modelling exercise with ploughing, control declined from 95% to 69% between years 2 and 4, a 26% decline overall. Thus there was generally good agreement between the container experiment and the modelling exercises for both a non-inversion tillage system (Exercise j) and a ploughing system (Exercise k) on the likely rate of loss of herbicide efficacy. In all cases the assumption was that a herbicide selecting for that form of target site resistance was applied annually as the sole means of black-grass control. Both the experimental and modelling approaches support the view that target site resistance will build up more rapidly in a non-inversion than in a ploughing system.

(l). Increase in target site resistance (e.g. ALS or ACCase) in response to annual use of a post-emergence herbicide selecting for that resistance type (e.g. sulfonylurea or 'fop') in sequence with a pre-emergence herbicide. Crop is winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm with an initial frequency of 1% of seeds target site resistant. Pre-emergence herbicide used from Yr 2 onwards).

This exercise used the same parameters as those in exercise j above except that a pre-emergence herbicide was used in addition to the post-emergence herbicide. In practice, many farmers apply a pre-emergence herbicide prior to use of an ALS or ACCase inhibiting herbicide post-emergence. In this modelling exercise, it was assumed that the efficacy of the pre-emergence herbicide applied was unaffected by resistance and consequently was equally effective on all black-grass plants regardless of whether they were target site resistant or not. Pre-emergence herbicides are generally less effective than post-emergence herbicides, in the absence of resistance, so a value of 60% control was used for the pre-emergence application.

In this scenario (Table 3.4.3.14), in which both pre- and post-emergence herbicides were used, surviving black-grass plant populations increased less rapidly than where a post-emergence herbicide alone was used, as in exercise j. However, the inclusion of a pre-emergence herbicide in exercise l was less beneficial than ploughing, as in exercise k. Thus, in exercise j (deep tine/disc, post-emergence herbicide only) populations reached levels (74 plants m^{-2}) that would have major effects on yields after 4 years, but the additional use of a pre-emergence herbicide, as in exercise l, meant that this infestation level was reached two years later, after 6 years. However, with ploughing and use of post-emergence herbicides only, as in exercise k, infestation levels had only reached 36 plants m^{-2}, even after 8 years.

Table 3.4.3.14 Increase in target site resistance (e.g. ALS or ACCase) in response to annual use of a post-emergence herbicide selecting for that resistance type (e.g. sulfonylurea or 'fop') in sequence with a **pre-emergence herbicide.** Crop is winter cereals grown under a deep tine/disc (20 cm) cultivation system. (Assuming an initial seed population in the soil of 100 seeds m^{-2} distributed evenly to a depth of 25 cm with **an initial frequency of 1% of seeds target site resistant**. Pre-emergence herbicide used from Yr 2 onwards).

	Years							
	1	2	3	4	5	6	7	8
	% weed control by herbicide							
Pre-em. herbicide efficacy	60% control of both susceptible and target site resistant plants							
Post-em. herbicide efficacy	97% control of susceptible; 25% control of target site resistant plants							
	Black-grass m^{-2}							
*Plants pre-spraying m^{-2}	3.0	1.9	2.0	7.7	44	250	1105	2598
% target site resistant	1%	10%	58%	92%	98%	100%	100%	100%
Plants surviving m^{-2}	0.05	0.08	0.36	2.15	13	75	332	779
Actual % control of plants	99%	96%	82%	72%	70%	70%	70%	70%
Heads m^{-2}	0.4	0.7	3.1	18	106	457	995	1240

Note: unshaded, lighter and heavier shading = respectively minimal, minor and major effects on crop yield likely. * = potential population as pre-emergence herbicide would reduce this number in practice.

A significant feature about exercises k & l, was that the number of plants pre-spraying declined initially for a few years, but subsequently increased. In a field situation, it might well have appeared that the herbicide programme was driving down black-grass infestations. What was happening was that, although the pre-spraying populations were declining, the proportion of resistant individuals was increasing steadily, with the consequence that the number of plants surviving herbicide treatment actually increased each year. We believe this is a very realistic scenario that may well be occurring in many fields at the present time. This highlights the fact that a decreasing black-grass population should not automatically be assumed to indicate a successful weed management strategy. If the residual population is increasingly resistant, then control is likely to decline with a consequent revival in weed infestation. This highlights the need for screening populations for resistance, even when they appear to be declining.

Conclusions of the resistance modelling exercises with black-grass:

Models cannot, or at least should not, be used to try to predict the rate of development of resistance in any individual field. Such an approach is doomed to failure, as so many variables exist between individual fields and farms. However, models have value as educational and advisory tools showing what could happen in a typical farm situation. In particular, they can demonstrate the potential development of resistance over longer time periods than could realistically be studied in practice. Consequently, they can highlight the key aspects that are critical to the prevention and management of resistance in the longer term, and this can aid the practical decision making process at the individual farm level. Models can highlight the 'early warning signs' of greater resistance problems ahead, which should persuade farmers and their advisors to take action sooner, before a major resistance problem has developed, rather than later, when options may be much more limited. In addition, models can identify weaknesses in the available information and management procedures, leading to a more focussed approach to future research.

- **At low black-grass populations, plants now produce about twice as many heads (8.7 v 3.9) and seeds per plant than was typical in the 1970's and early 1980's. This is probably a consequence of earlier drilling of winter cereals allowing a longer period of vegetative growth.**
- **Higher seed return means higher potential population increases in the absence of herbicides, especially in non-inversion tillage systems (up to a 30 fold annual increase with very shallow tillage compared with a 4 fold increase with ploughing).**
- **Higher levels of control are now required to prevent populations increasing (97% with tine/disc cultivation 20 cm deep; 90% with ploughing to 20 – 25 cm).**
- **Both the level of weed control achieved and the weed population density have a highly significant impact on the sustainability of any weed management policy – the lower the level of weed control and the higher the initial weed seedbank, the sooner an infestation level is reached that seriously impacts on yields. Consequently, keeping infestation levels as low as possible is vital. It is important to consider the whole rotation and plan long term strategies.**

- **The higher the pre-spraying population and the more ambitious the target level for an acceptable numbers of survivors, the higher the level of control required. Achieving very high levels of control from herbicides alone may become an unrealistic objective in the face of increasing resistance.**
- **The use of ploughing (annual or rotational), pre-emergence herbicides and non-chemical weed control methods can help maintain low weed populations in situations where resistance to post-emergence herbicides is increasing. Using several modifiers in combination will help, even if each alone gives only modest control.**
- **In a deep tine/disc (20 cm) system in which a pre-emergence herbicide is applied in winter cereals, modelling predicts that the overall level of control will be insufficient to prevent black-grass populations increasing when post-emergence herbicide efficacy drops below about 89%.**
- **Where non-chemical methods are used in combination with deep tine/discs and pre-emergence herbicides, post-emergence efficacy can decline to 69% before overall control becomes insufficient.**
- **Target site resistance can increase very rapidly if a herbicide selecting for that resistance type is used annually as the sole means of control – it took only four years for the proportion of resistant plants to increase from 1% to 100% of the population in a deep tine/disc system.**
- **In a ploughing system, target site resistant weed populations increased less rapidly than under deep tine/disc systems – it took about twice as long for the proportion of resistant plants to increase from 1% to 100% of the population, and herbicide performance decreased more slowly too.**
- **Both the experimental and modelling approaches supported the conclusion that target site resistance will build up more rapidly in non-inversion than in ploughing systems.**
- **The use of a pre-emergence prior to the use of a post-emergence herbicide slightly delayed the build up of target site resistant weed populations, but this was less beneficial than ploughing.**
- **Perhaps the most powerful message from the modelling studies was that modifiers in the form of alternative herbicides or non-chemical**

methods can slow the build-up of resistance. They may not prevent resistance developing in the longer term, but can help maintain black-grass populations at tolerable levels, at least in the short term.

- **The studies highlight the importance of long term planning in order to maintain black-grass populations at as low a level as possible using all appropriate weed control measures, chemical and non-chemical. This is essential, even where black-grass populations appear to be well under control, as low black-grass populations have the potential to increase rapidly if control measures are relaxed.**
- **Farmers and advisors need to get a better idea of exactly what control is being achieved by each component of their grass-weed management strategy (both from herbicides and non-chemical methods), and how this is changing with successive years, in order to better evaluate the sustainability of their agronomic system.**
- **Close monitoring of herbicide performance within individual fields, in association with regular seed testing for resistance, can help as an early warning of resistance problems ahead.**
- **Assumptions are unavoidable in modelling exercises, but we believe the assumptions made here are realistic. It is important to recognise that, while these modelling exercises highlight critical issues in resistance prevention and management, they have not been fully validated in the field. However, we believe they provide useful pointers to better management and that ongoing studies will help validate many of the outcomes.**

4. KEY OUTCOMES BY OBJECTIVE

Integrated Management of Herbicide Resistance

Objective 1: Quantify the effectiveness of resistance mitigation strategies (especially in relation to ALS and dinitroaniline herbicides)

- Target site resistance (TSR) to ALS herbicides (e.g. sulfonylureas) can build up quickly in black-grass as a result of repeated annual use of this chemistry alone
- ALS in mixture or sequence with herbicides with different modes of action led to
  - improved weed control due to lower black-grass numbers
  - no reduction of selection pressure for ALS TSR
- Non-ALS herbicides did not select for ALS TSR
- Effective pre-emergence herbicides were vital to
  - Reduce black-grass numbers
  - Reduce reliance on post-emergence herbicides (higher resistance risk)
- In most cases 2+ years of selection pressure are needed to positively identify resistance risks of ALS herbicides (1 year in some cases)

Objective 2: Establish the incidence of different mechanisms of resistance and develop improved detection methods at the local level

- The number of cases of resistance to ALS inhibiting herbicides in black-grass is increasing throughout England – confirmed in 21 counties
- Robust and reliable tests were developed and are available to farmers/advisors to detect resistance to ALS inhibiting herbicides
- Improved advice for farmers/agronomists on collecting representative seed samples for resistance testing:
  - Sampling from a single <u>patch</u> does not consistently reflect the resistance status of all patches in the same field
  - Sampling from a single <u>field</u> on a farm definitely does not represent the whole farm in terms of resistance status

Objective 3: Quantify the impact of population dynamics of grass-weeds in relation to resistance mitigation strategies

- Resistance to ALS herbicides increases faster in minimum tillage systems compared with ploughing
- ALS TSR did not disappear or even decline when ALS herbicides were not used for 3 years – no loss of resistance in absence of selecting herbicide
- Pre-emergence herbicides can compensate, to some degree, for the declining performance of post-emergence herbicides due to increasing resistance
- Modifiers in the form of alternative herbicides or non-chemical methods slowed, but did not prevent, the build-up of resistance
- Non-chemical cultural control methods are increasingly important in combating resistance by reducing the reliance on herbicides

5. GUIDELINES FOR MORE SUSTAINABLE RESISTANCE MANAGEMENT STRATEGIES

The research highlights key factors that can contribute to better management of herbicide-resistant black-grass. These are:

- **Greater use of non-chemical control methods to reduce reliance on herbicides.** It must be recognised that many non-chemical methods are less effective than herbicides, more complex to manage and can have negative environmental attributes. Non-chemical methods cannot replace herbicides on most farms, but reduced reliance on herbicides will be necessary both from a practical (increasing resistance, lack of new herbicides) and political aspect (complying with new EU legislation).
- **Less reliance on high resistance risk post-emergence herbicides.** Research studies clearly indicate that the regular use of ACCase and ALS inhibiting herbicides is associated with a high risk of herbicide resistance. Moderating this risk is vital if the effectiveness of these herbicides is to be maintained in the longer term. These herbicides will continue to be very important in controlling black-grass, but their use needs to be integrated with other control measures, both cultural and chemical.
- **Greater use of pre-emergence herbicides.** Resistance to the pre-emergence herbicides used for black-grass control tends to be only partial and builds up relatively slowly. Consequently, pre-emergence herbicides appear to be a lower resistance risk than some post-emergence options, especially ACCase and ALS inhibiting herbicides, and can substitute for them to some degree.
- **More critical monitoring of herbicide performance in individual fields.** Resistance in black-grass can vary considerably between and, to a lesser extent, within different fields. Management strategies need to take account of this inter-field variation. Close monitoring of variations in herbicide performance both within, and between, fields can act as an early warning of potentially greater problems ahead.
- **Regular testing for resistance.** While the factors responsible for the evolution of herbicide resistance are well established, predicting the risk at an individual field scale is imprecise. This needs to be done regularly, at least once every 2 – 3 years if changes in resistance are to be detected reliably.

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