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Evidence Project Final Report

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Project identification

1. Defra Project code: PS2714

2. Project title:
   Developing and promoting more sustainable grass-weed control strategies to combat herbicide resistance

3. Contractor organisation(s):
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4. Total Defra project costs (agreed fixed price): £ 656,982

5. Project: start date .... 01 January 2009
   end date .... 31 March 2012

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(a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

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(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

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

The overall scientific objective was: To promote more sustainable grass-weed control practices in UK agriculture by investigating the problems posed by use of 'high resistance risk' herbicides (ALS and ACCase inhibitors) and their potential replacement by 'lower resistance risk' alternatives.

Companies making submissions to CRD (Chemicals Regulation Directorate) for registration of herbicides are required to undertake a resistance risk assessment and, if appropriate, propose a resistance management strategy. This project should assist regulators formulate what they require from companies while providing information on which to base any future regulatory changes. Herbicide resistance threatens the sustainability of arable farming and outputs from this project should help growers manage resistance. Black-grass (*Alopecurus myosuroides*) is the major resistant weed of arable crops in the UK, so this was the focus for most studies, although some work was also conducted with another major annual grass-weed, Italian rye-grass (*Lolium multiflorum*).

There were five specific objectives, and the main research findings are present under each one:

Specific objective 1. To quantify the changes in frequency and degree of resistance that have occurred since 2002 in black-grass and rye-grass in relation to 'high' and 'low' resistance risk herbicides, in order to better assess the longer-term risks associated with use of different herbicide classes.

- Tests on black-grass samples collected from random fields showed a consistent, and statistically significant, trend for increasing resistance between 2002 and 2009/10/11 for all the five herbicides evaluated (mesosulfuron-methyl+iodosulfuron-methyl, clodinafop-propargyl, chlorotoluron, cycloxydim, pendimethalin).
- As the fields were randomly selected, this provides the best evidence we have that resistance to all these herbicides has increased more generally in the UK between 2002 and 2009/10/11.
- Increases in resistance varied between herbicides, with the smallest changes being recorded with the urea, chlorotoluron, and the dinitroaniline, pendimethalin. Larger changes were recorded with the ALS inhibitors, mesosulfuron-methyl+iodosulfuron-methyl, and the ACCase inhibitors, clodinafop-propargl and cycloxydim.
- This finding supports the view that ALS and ACCase inhibiting herbicides pose a bigger resistance risk than other modes of action (MOA), but these other MOA are not immune from increasing resistance.
- Herbicide resistance had increased despite the use of a wide range of different MOA – the minimum number of MOA used on any sampled field between 2002 and 2009/10/11 was four, the maximum 11.
- ALS target site resistance conferred by two mutations (P197T & W574L) and probable enhanced ALS metabolism resistance were shown to be roughly equally important mechanisms in reducing the efficacy of mesosulfuron-methyl+iodosulfuron-methyl against black-grass.

- Tests on Italian rye-grass samples collected from random fields showed only a small loss in efficacy between 2006/07 and 2010/11 for the five herbicides evaluated (diclofop-methyl, mesosulfuron-methyl+iodosulfuron-methyl, cycloxydim, pinoxaden, pendimethalin). The time span between the baseline and more recent collections was shorter than for the black-grass study, which may explain the smaller changes.
- ACCase target site resistance was commonly recorded in rye-grass, with six of the seven known ACCase mutations detected, but it was the main mechanism in only 32% of the resistant populations.
- A compilation exercise showed that black-grass is the most important resistant species, occurring on an estimated 16,000 farms in the UK. Resistance in rye-grass and wild-oats needs continual monitoring, as major problems occur on some farms. Resistance to mesosulfuron-methyl+iodosulfuron-methyl, first used in the UK in autumn 2003, was confirmed in black-grass on over 400 farms in 26 counties.

Specific objective 2. To identify the occurrence of stacked resistance mechanisms, such as 'double' ACCase and ALS target site resistance (DTSR), and assess their impact on grass-weed populations.

- The presence of 'double' target site resistance to both ALS and ACCase inhibiting herbicides in individual plants of four black-grass populations was demonstrated.
- In plants with 'double' target site resistance, sequential or rotational use of ACCase and ALS inhibitors was ineffective. In contrast, sequential or rotational use of these two MOA was effective when the ACCase and ALS mutations were present in *different* individual plants within a population.
- The presence of 'double' target site resistance is likely to increase as black-grass is a predominantly cross-pollinating species.

Specific objective 3. To assess the significance of non-target site resistance mechanisms (principally enhanced metabolism) on ALS inhibitors used for grass-weed control.

- Experiments showed that enhanced metabolic resistance to mesosulfuron-methyl+iodosulfuron-methyl can build up progressively, even in black-grass populations with little previous exposure to ALS graminicides.
- The degree of resistance to mesosulfuron-methyl+iodosulfuron-methyl conferred by enhanced metabolism can approach that conferred by ALS target site resistance, with both mechanisms capable of reducing herbicide efficacy in the field, regardless of application timing (autumn or spring).
- Both ALS target site mutations (P197T and W574L) conferred a high degree of resistance to sulphonylurea (e.g. mesosulfuron-methyl+iodosulfuron-methyl), triazolopyrimidine (e.g. pyroxsulam) and sulfonylaminocarbonyl-triazolinone (e.g. propoxycarbazone) herbicides used for grass-weed control in the UK. The specific mutation present had a bigger impact on determining the degree of resistance to the imidazolinone, imazapyr, with W574L conferring much greater resistance than P197T.

Specific objective 4. To determine the impact and consequences of non-target site resistance mechanisms (principally enhanced metabolism) on the potential long term sustainability of use of 'low resistance risk' herbicides.

- Experiments in outdoor containers showed that resistance to the pre-emergence herbicides flufenacet, pendimethalin and prosulfocarb occurs in black-grass and can increase following annual treatment.
- Annual increases in resistance, most likely due to enhanced metabolism, were relatively small and resulted in losses of efficacy of 4 – 7% per year for all three herbicides.
- Two fields, the source of seeds for the container studies, were resampled after six years but, surprisingly, showed no evidence of any increase in resistance to flufenacet during that period. This occurred despite the heavy use of herbicides, comprising 7 to 9 different MOA , including flufenacet.
- There was less selection for resistance to flufenacet in the field than in outdoor containers, where flufenacet alone was used for 5 years. This apparent contradiction indicates that the use of multiple modes of action was effective at reducing the increase in resistance to flufenacet, despite the 4.6 – 7 seven-fold greater use of herbicides overall (23 or 35 a.i. applications in the field over six years v 5 a.i. applications (annual flufenacet only) in containers). This important finding requires validating.
- Under field conditions, increases in resistance to all three herbicides are likely to be difficult to detect due to the confounding effects of other factors affecting activity (e.g. soil moisture).
- Good diagnostic assays for detecting resistance and well characterised reference populations will be essential for monitoring the impact of resistance on flufenacet, pendimethalin and prosulfocarb.

Specific objective 5. To conduct Knowledge Transfer (KT) initiatives to inform PSD, suppliers and users of herbicides of the future risks posed by herbicide-resistance and to promote appropriate resistance prevention and management strategies and more rational herbicide use.

- Key elements highlighted in KT initiatives were: the threat posed by herbicide resistance, the loss of alternative herbicides, the importance of early detection of resistance, the high resistance risk posed by ACCase and ALS inhibiting herbicides, the value and limitations of alternative, lower resistance risk herbicides and the essential role of cropping and cultural alternatives to herbicides.

- **131** KT initiatives were conducted comprising **46** articles in the popular farming press, **64** presentations (including 9 conferences in UK, Belgium, Iran, New Zealand, USA, 16 technical presentations to agronomists/technical personnel; 18 talks to farmers; 5 student training courses, 14 discussion meetings relating to research and management of resistance, 2 press briefings), **21** formal publications in scientific journals, conferences, reports and technical information sheets.

Implications of the research

The results show that, with black-grass, resistance to a range of different herbicides is increasing, despite the use of many different modes of action (MOA). The findings support the view that ALS and ACCase inhibiting herbicides pose a bigger resistance risk than most other MOA. Resistance to the ALS inhibitor, mesosulfuron-methyl+iodosulfuron-methyl, introduced into the UK in autumn 2003, is now widespread. Resistance is due to multiple resistance mechanisms, including both target site resistance (TSR) (two different mutations) and enhanced metabolism. In addition, 'double' target site resistance, ALS and ACCase TSR within the same individual plant occurs, and can be expected to increase. A major finding was that ALS enhanced metabolism can impact on herbicide efficacy almost as much as ALS target site resistance and can increase rapidly. The high resistance risk associated with ALS inhibiting herbicides, combined with the widespread occurrence of resistance to ACCase inhibitors, means greater reliance will be placed on herbicides with other MOA, especially pre-emergence herbicides. Resistance to all three pre-emergence herbicides investigated, flufenacet, pendimethalin and prosulfocarb, occurs, and can increase with continued use. However, the rate of increase in resistance was modest, even where each herbicide was used annually. The lack of further increase in resistance to flufenacet in field samples, despite heavy use of a range of herbicides, was a finding with important implications. This supports the view that use of many different MOA can slow up the development of resistance in herbicides that are commonly used as alternatives to ALS and ACCase inhibitors. This important result requires validation on additional samples. With a lack of new MOA, it is clear that widespread and increasing resistance means that reliance on herbicides is unlikely to give sustainable control of black-grass. One major issue, as demonstrated by these research results, is that resistance rarely results in complete herbicide failure and can be difficult to detect in the field. Consequently farmers compensate for declining herbicide performance by the use of more herbicides (e.g. pre-emergence 'stacking'). It is vital that non-chemical control measures are actively promoted and used in order to reduce this reliance on herbicides.

Project Report to Defra

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

PS2714: Developing and promoting more sustainable grass-weed control strategies to combat herbicide resistance

Background

Companies making submissions to CRD (Chemicals Regulation Directorate) for registration, or re-registration of herbicides are required to consider the risk that resistance poses to their active ingredients and, if appropriate, propose a resistance management strategy. For CRD to properly evaluate such strategies, data are required to design, test and validate the resistance management strategies proposed by registrants, and to provide a comparative assessment of different assumptions, so that resistance risk profiles can be formulated. This project should assist regulators formulate what they require from companies in relation to resistance mitigation strategies for both new, and existing, herbicides, while providing information on which to base any regulatory changes that may be needed to maintain the effectiveness of herbicides in the longer term.

In addition, herbicide resistance threatens the sustainability of arable farming in the UK due to an increased reliance on the higher resistance risk ACCase and ALS inhibitor herbicide classes. This is partly a consequence of the recent EU Review of Pesticides which has reduced the availability of alternative herbicides. Black-grass (*Alopecurus myosuroides*) is the major resistant weed of arable crops in the UK and other western European countries, so this was the focus for most studies in this project, although some work was done with on another major grass weed, Italian rye-grass (*Lolium multiflorum*).

Scientific objectives

The overall scientific objective was: To promote more sustainable grass-weed control practices in UK agriculture by investigating the problems posed by use of 'high resistance risk' herbicides (ALS and ACCase inhibitors) and their potential replacement by 'lower resistance risk' alternatives.

There were five more specific objectives:

1. To quantify the changes in frequency and degree of resistance that have occurred since 2002 in black-grass and rye-grass in relation to 'high' and 'low' resistance risk herbicides, in order to better assess the longer-term risks associated with use of different herbicide classes.
2. To identify the occurrence of stacked resistance mechanisms, such as 'double' ACCase and ALS target site resistance (DTSR), and assess their impact on grass-weed populations.
3. To assess the significance of non-target site resistance mechanisms (principally enhanced metabolism) on ALS inhibitors used for grass-weed control.
4. To determine the impact and consequences of non-target site resistance mechanisms (principally enhanced metabolism) on the potential long term sustainability of use of 'low resistance risk' herbicides.
5. To conduct Knowledge Transfer (KT) initiatives to inform PSD, suppliers and users of herbicides of the future risks posed by herbicide-resistance and to promote appropriate resistance prevention and management strategies and more rational herbicide use.

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

Objective 1. To quantify the changes in frequency and degree of resistance that have occurred since 2002 in black-grass and rye-grass in relation to 'high' and 'low' resistance risk herbicides, in order to better assess the longer-term risks associated with use of different herbicide groups.

In the absence of regular, comprehensive and expensive surveys**,** monitoring the development of resistance at a range of random sites gives a good indication of changes in resistance at a national level.

1.1 Monitoring changes in resistance in black-grass

In 2002, black-grass seed samples were collected from 25 *randomly* selected farms in England. The fact that they were *randomly* selected is important, as the vast majority of seed samples tested for resistance during the last 25 years have been from farms where herbicides have worked poorly ('complaints'), and so are not necessarily representative of the majority of fields. Nineteen of these 25 fields were re-sampled in 2009, 2010 or 2011 and tested alongside the 2002 samples, seeds of which have been stored at Rothamsted. Samples were from 13 counties and farm size ranged from 48 to 735 ha. (Of the remaining 6 fields, two had been abandoned, two were in grass, one was in maize and one was weedfree). Black-grass head densities were assessed at the same time as seed sampling. Most fields were impressively clean - twelve of the 19 fields had low (<10 heads/m^2) populations of black-grass surviving treatment but seven had higher density patches of up to 350 heads/m^2. These results support the view that these fields are a good representative set, and are not generally fields with major, uncontrollable populations of black-grass.

All samples were evaluated for resistance in three glasshouse pot assays using mesosulfuron-methyl+iodosulfuron-methyl ('Atlantis WG'), clodinafop-propargl ('Topik') and chlorotoluron ('Tolugan 700') (chlorotoluron), and in three Petri-dish assay with cycloxydim ('Laser') and pendimethalin ('Stomp 400 SC'). See Tables 1 & 2 for doses used. In addition to the random samples, two pairs from organic farms (STEP 2002 & 2009; WELSH 2002 & 2010); one pair of susceptible standards (ROTH 2002 & 2009), one reference population for mesosulfuron-methyl+iodosulfuron-methyl resistance due to both ALS target site and enhanced metabolism (PELDON SS 05), and one reference population for clodinafop-propargyl and cycloxydim resistance due to ACCase target site resistance (NOTTS 05) were also included. In the glasshouse assay, plants (6 per pot) were treated at the 3 leaf stage in a randomised block design with five replicates. Plant survivorship and foliage fresh weight per pot was recorded 3 - 4 weeks after spraying as a measure of herbicide activity. In the Petri-dish assays, 50 seeds per dish were used with two replicates per population and numbers of seeds with shoots >10mm recorded after 14 days. The % reductions in foliage fresh weight (pots), or reduction in shoots >10mm (petri-dishes) relative to untreated pots/dishes for the same population, were calculated**.** After assessment, leaves from plants treated with mesosulfuron-methyl+iodosulfuron-methyl were subject to a molecular analysis to determine whether either of two ALS target site mutations (P197T & W574L), known to confer target site resistance, were present, using methods described by Marshall & Moss (2008).

Glasshouse pot assays (See Table 1)

The ROTH susceptible samples were well controlled by all herbicides (92.5 – 97.7 % reduction in foliage fresh weight and all plants killed) confirming that the herbicide application and test conditions were conducive to good herbicidal activity. The samples from the two organic farms, which received no herbicides between 2002 and 2009/10, were also well controlled by all herbicides (91.4 – 96.0% reduction in foliage weight (except for WELSH10 clodinafop-propargyl – 84.4%). The PELD SS05 reference was very poorly controlled by all three herbicides, confirming it as a good resistant reference population (Results for 2009, 2010 & 2011 tests respectively: mesosulfuron-methyl+iodosulfuron-methyl 15.5%, 11.7%, 9.4%, mean 1.9%; clodinafop-propargyl 32.6%, 57.4%, 34.8%, mean 42%; chlorotoluron 29.1%, 46.2%, 16.9%, mean 31%). In PELD SS05, resistance to mesosulfuron-methyl+iodosulfuron-methyl was largely due to ALS targets site resistance whereas resistance to clodinafop-propargyl and chlorotoluron was due to enhanced metabolism. The NOTTS 05 population showed high resistance to clodinafop-propargyl only, due to ACCase target site resistance. (Results for 2009, 2010 & 2011 tests respectively: mesosulfuron-methyl+iodosulfuron-methyl 94.7%, 92.8%, 93.9%, mean 94%; clodinafop-propargyl 23.3%, 21.3%, 26.7%, mean 24%; chlorotoluron 97.1%, 95.7%, 93.1%, mean 95.3%). NOTTS 05 does *not* have a high degree of enhanced metabolism (in contrast to PELD SS05), and hence was as well controlled by mesosulfuron-methyl+iodosulfuron-methyl and clodinafop-propargyl as the susceptible reference populations (ROTH). The results for the susceptible and resistant reference populations are *exactly* as predicted and fully validate the accuracy and repeatability of this test methodology, and justify combining the three experiments into a single analysis.

Mesosulfuron-methyl+iodosulfuron-methyl

All the 2002 samples from the 19 *random* fields were fully susceptible to mesosulfuron-methyl+iodosulfuron-methyl, which was not introduced into the UK until autumn 2003**. However, by 2009/10/11, resistance (RR/RRR) was detected in nine populations (47% of the total) and partial resistance (R?) in two more.** It is important to note that black-grass head infestations were low (≤10 heads/m^2) on six of these fields, and seeds were collected from survivors of herbicide application. The mean % reduction in foliage weight of the nine populations showing resistance was 52%. Thus the detection of resistance does not mean complete failure of mesosulfuron-methyl+iodosulfuron-methyl is likely in the field, as many black-grass plants will continue to emerge from the soil seedbank, which will contain many older, still susceptible, seeds. However, it is likely that resistance will increase if this herbicide continues to be used regularly, so these results should act as an early warning of greater resistance problems ahead. Of the 570 plants from the 2002 random samples (19 populations x 30 plants) treated with mesosulfuron-methyl+iodosulfuron-methyl, only two (<1%) survived (one from each of OXON1 02 and LEICS2 02). However, of the 570 plants from the 2009/10/11 collections, 197 (34%) survived treatment with mesosulfuron-methyl+iodosulfuron-methyl, albeit often stunted and severely damaged (59 (10%) were little affected; 138 (24%) were seriously damaged).

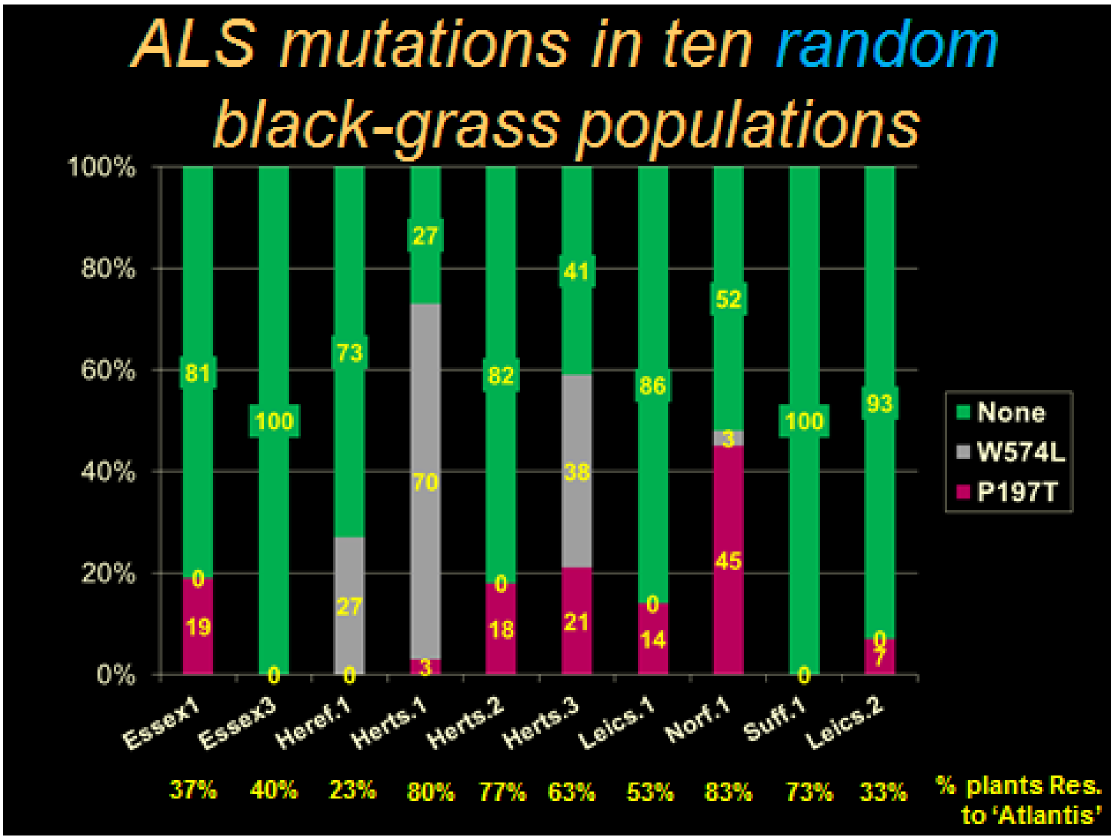
Table 1.

Testing of 2002 & 2009/10/11 survey samples in glasshouse pot assays										
Averages										
Treatment	Nil	**Atlantis (400 g/ha)**			**Topik (125 ml/ha)**			**Tolugan (3.93 l/ha)**		
		12+2.4 g/ha meso+iodo				**30 g/ha clodinafop**		**2.75 kg/ha chlorotoluron**		
Population	FWT	FWT	% reduction in FWT (compared to Nil)	R Rating	FWT	% reduction in FWT (compared to Nil)	R Rating	FWT	% reduction in FWT (compared to Nil)	R Rating
KENT 02	5.42	0.33	93.92	S	4.01	26.0	RRR	4.23	22.0	RRR
KENT 09	7.21	0.43	94.05	S	4.44	38.4	RR	1.98	72.6	RR
YORKS 02	6.27	0.49	92.12	S	4.47	28.8	RRR	0.51	91.9	S
YORKS 09	6.92	0.66	90.47	S	6.12	11.6	RRR	1.40	79.8	R?
NOTTS1 02	7.91	0.56	92.91	S	0.63	92.0	S	0.21	97.4	S
NOTTS1 09	6.85	0.43	93.79	S	1.94	71.7	RR	0.56	91.9	S
HERTS2 02	6.18	0.46	92.63	S	3.96	35.8	RRR	1.45	76.5	RR
HERTS2 09	5.96	4.96	16.76	RRR	4.30	27.9	RRR	2.17	63.6	RR
HERTS3 02	6.54	0.42	93.58	S	0.57	91.2	S	0.31	95.3	S
HERTS3 09	6.27	3.32	47.06	RR	2.77	55.8	RR	1.35	78.5	R?
NORTHANTS1 02	7.35	0.36	95.08	S	6.65	9.5	RRR	0.35	95.2	S
NORTHANTS1 09	6.88	0.65	90.57	S	6.10	11.4	RRR	0.65	90.6	S
SUFFOLK1 02	7.34	0.61	91.74	S	5.95	18.9	RRR	0.93	87.3	S
SUFFOLK1 09	6.83	2.97	56.48	RR	5.28	22.7	RRR	1.58	76.9	RR
ESSEX2 02	6.50	0.34	94.72	S	3.46	46.8	RR	1.69	74.0	RR
ESSEX2 09	6.30	1.41	77.59	R?	3.80	39.7	RR	3.38	46.4	RR
WILTS1 02	7.30	0.51	92.96	S	4.85	33.5	RRR	2.04	72.1	RR
WILTS1 09	7.74	0.44	94.28	S	6.82	11.9	RRR	2.81	63.7	RR
HEREF1 02	7.80	0.55	92.91	S	1.20	84.7	S	0.28	96.4	S
HEREF1 09	7.75	1.56	79.87	R?	3.03	60.9	RR	0.93	88.1	S
NORF1 02	6.50	0.38	94.13	S	5.17	20.4	RRR	0.55	91.6	S
NORF1 09	7.07	3.99	43.61	RR	5.96	15.7	RRR	0.83	88.3	S
OXON1 02	6.32	0.67	89.35	S	5.72	9.4	RRR	1.84	70.8	RR
OXON1 09	5.16	0.46	91.06	S	5.76	-11.5	RRR	1.94	62.4	RR
ESSEX 1 02	9.17	0.65	92.93	S	6.4	30.18	RRR	1.91	79.16	R?
ESSEX 1 10	9.25	2.47	73.27	RR	5.25	43.21	RR	1.90	79.51	R?
ESSEX 3 02	9.42	0.52	94.31	S	2.88	68.61	RR	1.89	79.43	R?
ESEEX 3 10	9.21	2.63	71.47	RR	5.73	37.74	RRR	1.53	83.38	R?
HERTS 1 02	9.53	0.60	93.74	S	3.41	64.21	RR	1.94	79.65	R?
HERTS 1 10	9.71	6.96	28.38	RRR	7.88	18.87	RRR	1.15	88.20	S
LEICS 1 02	8.88	0.48	94.55	S	1.41	84.10	R?	0.46	94.86	S
LEICS 1 10	10.05	3.78	62.38	RR	7.45	25.91	RRR	0.87	91.37	S
LECIS2 02	9.298	0.728	92.17	S	5.754	38.1	RRR	4.093	56.0	RR
LEICS2 11	9.855	2.704	72.56	RR	7.431	24.6	RRR	8.119	17.6	RRR
BERKS1 02	8.634	0.499	94.22	S	6.467	25.1	RRR	2.791	67.7	RR
BERKS1 11	9.764	1.324	86.44	S	5.906	39.5	RR	3.126	68.0	RR
OXON2 02	10.453	0.487	95.34	S	6.294	39.8	RR	3.156	69.8	RR
OXON2 11	9.372	1.094	88.32	S	6.918	26.2	RRR	4.862	48.4	RR
d.f.	148				148			148		
S.E.	0.591	0.406	4.780	*	0.524	6.95	*	0.500	6.20	*
L.S.D.≤0.05	1.652	1.134	13.358		1.463	19.43		1.397	17.33	

Resistance 'R' ratings: *RRR* = Resistance confirmed, highly likely to reduce herbicide performance; *RR* = Resistance confirmed, probably reducing herbicide performance; *R?* = Early indications that resistance may be developing, possibly reducing herbicide performance.
***S* = Susceptible, no clear evidence of resistance.**

The results for the molecular assays conducted on the nine 2009/10/11 populations showing resistance (RR/RRR) and one showing R? resistance to mesosulfuron-methyl+iodosulfuron-methyl are shown in Figure 1.

Figure 1. Molecular assays on 10 black-grass populations showing resistance to mesosulfuron-methyl+iodosulfuron-methyl in glasshouse pot assays (30 plants assayed per population)



The results for the presence of ALS target site resistance mutations are presented as a % of the 30 plants treated with mesosulfuron-methyl+iodosulfuron-methyl for each population in the glasshouse assay (6 plants per pot x 5 replicates). All plants were assayed, including both live and dead plants, and the % of plants which survived treatments are shown as '% plants resistant to 'Atlantis'. No ALS target site mutations were detected in any dead plant, which provides a good validation of the methodology employed.

It is evident that resistance to mesosulfuron-methyl+iodosulfuron-methyl is complex and conferred by multiple mechanisms. In some populations, resistance was conferred by the P197T mutation (Essex 1, Herts 2, Leics. 1, Leics. 2), in others by the W574L mutation (Heref. 1), in others by both mutations (Herts 1, Herts 3, Norf.1) and in others, no mutations were detected (Essex 3, Suffolk 1). It is also clear that the correlation between the % of resistant plants and the % of plants with a confirmed ALS mutation was poor. It is likely that other mechanisms, almost certainly enhanced metabolism, are responsible for resistance in populations such as Essex 3 and Suffolk 1, which did not possess any ALS target site mutations and also in plants of other populations where a much higher % of plants survived than possessed an ALS target site mutation.

In total, 570 plants from the 19 resampled populations were assessed for resistance to mesosulfuron-methyl+iodosulfuron-methyl (30 plants per population). The glasshouse and associated molecular assays indicated that, overall, 7% of these were resistant due to the P197T ALS mutation, 7% were resistant due to the W574L mutation and 21% were resistant due to non-target site mechanisms, most probably enhanced metabolism.

- **Resistance to mesosulfuron-methyl+iodosulfuron-methyl has increased since 2002 and is now widespread in England.**
- **Resistance does not tend to affect the whole population with useful, if reduced, levels of control still likely to be achieved, at least in the short term.**
- **Both ALS target site resistance (two mutations) and probable enhanced ALS metabolism resistance are important mechanisms in reducing efficacy of mesosulfuron-methyl+iodosulfuron-methyl, but the latter appears to be relatively more important at present.**

Clodinafop-propargyl

Only three of the 19 random samples collected in 2002 were susceptible to clodinafop-propargyl showing that resistance to this herbicide was already widespread. **By 2009/10/11, all 19 samples were resistant (RR or RRR) to clodinafop-propargyl**. Of the 570 plants from the 2002 and 2009/10/11 samples treated with clodinafop-propargyl, respectively 351 (62%) and 437 (77%) survived treatment, although often stunted and damaged. In contrast, no plants of the ROTH02 or 09 susceptible standards survived treatment in any year, but six (5%) of the 120 plants from the two organic farms (STEP & WELSH) did survive This may have been due to them having being farmed conventionally previously or due to the spread of resistance via pollen. The fact that both the PELD SS 05 (enhanced metabolism but no ACCase target site resistance) and the NOTTS 05 (ACCase target site resistance but no enhanced metabolism) populations showed resistance to clodinafop-propargyl (see above) shows that it is not possible to conclude which of these two mechanisms is responsible for resistance in the random fields, although it is likely that both mechanisms are important.

- **Resistance to clodinafop-propargyl is now very widespread and some level of resistance is likely to occur in most arable fields in England.**

Chlorotoluron

Eight of the 19 random 2002 samples were resistant (RR/RRR) to chlorotoluron and three were rated R? **By 2009/10/11, nine samples were resistant (RR or RRR) to chlorotoluron, the same eight as in 2002 plus Suffolk1, and four were rated R?** Of the 570 plants from the 2002 and 2009/10/11 samples treated with chlorotoluron, respectively 272 (48%) and 346 (61%) survived treatment, although often stunted and damaged. In contrast, no plants of the ROTH02 or 09 susceptible standards survived treatment in any year, and only two (2%) of the 120 plants from the two organic farms (STEP & WELSH) did survive.

- **Resistance to chlorotoluron has changed relatively little between 2002 and 2009/10/11, with little sign of any substantial change in resistance overall, although there were bigger changes in individual fields.**

Petri-dish assay (See Table 2)

The ROTH susceptible reference populations were well controlled by both herbicides in all tests (cycloxydim 100% reduction; pendimethalin 90.8 - 100 % reduction in number of seeds with shoots > 1 cm), confirming that the test methodology was conducive to good herbicidal activity. The samples from the two organic farms, which received no herbicides between 2002 and 2009/10, were also well controlled by both herbicides (cycloxydim 95.2 – 100% reduction; pendimethalin 83.9% – 98.7 % reduction). The PELD SS05 reference was well controlled by cycloxydim (100% in all three assays) but poorly controlled by pendimethalin (3.5% – 34.4% reductions), as expected. In this population, the absence of ACCase target site resistance meant that cycloxydim worked well, but the high level of enhanced metabolism severely reduced pendimethalin activity, (cycloxydim is not affected by enhanced metabolism). The NOTTS 05 population showed high resistance to cycloxydim only (1.1% – 6.5% reductions), due to ACCase target site resistance. This population does *not* have a high degree of enhanced metabolism (in contrast to PELD SS05), and hence was as well controlled by pendimethalin as the ROTH susceptible reference populations (96.9 – 97.9%). These results for the susceptible and resistant reference populations are exactly as predicted and fully validate the accuracy of the Petri-dish test methodology.

Cycloxydim

Twelve of the 19 random samples collected in 2002 were rated susceptible to cycloxydim and one was rated R?. However, seven of the susceptible populations had % reductions less than the 100% that was consistently obtained for the ROTH susceptible standard, indicating the presence of a low proportion of (≤6%) ACCase target site resistant individuals. Consequently, while ACCase target site resistance was only confirmed in six (=32%) of the 2002 populations, it was likely to have been present in 14 (74%) of the 2002 samples. **By 2009/10/11, 11 samples (=58%) were resistant (RR or RRR) and two were rated R? to cycloxydim.** This included five of the seven 2002 samples rated as susceptible but which had % reductions less than the 100%. In addition, all six of the 2009/10/11 populations rated susceptible had % reductions of less than the 100% that was consistently obtained for the ROTH susceptible standard. The % reduction values had declined between 2002 and 2009/10/11 for 18 out of 19 of the samples, albeit sometimes by only a small amount.

- **Resistance to cycloxydim had increased between 2002 and 2009/10/11, and there were indications that most populations in England now contain at least a small proportion of ACCase target site resistant individuals.**
- **The proportion of cycloxydim resistant individuals on many fields was relatively low, so useful levels of control would still be expected, even on some fields rated as resistant.**

Table 2.

Testing of 2002 & 2009/10/11 survey samples in petri-dish assays				
Averages				
Population	cycloxydim (5ppm)		pendimethalin (5ppm)	
	% reduction in number of seeds with shoots >1cm relative to NILs	R Rating (compared to ROTH 02 & 09	% reduction in number of seeds with shoots >1cm relative to NILs	R Rating (compared to ROTH 02 & 09
KENT 02	100.0	S	17.6	RRR
KENT 09	90.1	S	85.9	S
YORKS 02	80.8	R?	78.2	R?
YORKS 09	-8.7	RRR	71.0	RR
NOTTS1 02	94.1	S	97.1	S
NOTTS1 09	86.5	R?	80.8	R?
HERTS2 02	98.8	S	71.3	RR
HERTS2 09	90.4	S	79.5	R?
HERTS3 02	100.0	S	100.0	S
HERTS3 09	92.0	S	67.8	RR
NORTHANTS1 02	31.4	RRR	68.6	RR
NORTHANTS1 09	18.2	RRR	60.6	RR
SUFFOLK1 02	47.1	RR	85.1	S
SUFFOLK1 09	39.5	RRR	38.4	RR
ESSEX2 02	98.2	S	90.9	S
ESSEX2 09	94.0	S	84.3	R?
WILTS1 02	98.7	S	64.9	RR
WILTS1 09	28.2	RRR	64.1	RR
HEREF1 02*	100.0	S	11.1	RRR
HEREF1 09*	98.7	S	64.9	RR
NORF1 02	96.5	S	80.0	R?
NORF1 09	87.0	R?	58.7	RR
OXON1 02	37.3	RRR	57.3	RR
OXON1 09	28.9	RRR	17.1	RRR
ESSEX 1 02	71.4	RR	59.7	RR
ESSEX 1 10	18.8	RRR	40.0	RR
ESSEX 3 02	100.0	S	48.7	RR
ESEEX 3 10	92.6	S	46.3	RR
HERTS 1 02	100.0	S	67.6	RR
HERTS 1 10	27.5	RRR	68.8	RR
LEICS 1 02*	97.5	S	87.7	S
LEICS 1 10*	0.0	RRR	36.4	RRR
LECIS2 02	76.4	RR	72.7	RR
LEICS2 11	68.8	RR	68.8	RR
BERKS1 02	68.5	RR	69.7	RR
BERKS1 11	78.2	RR	72.4	RR
OXON2 02	95.4	S	67.8	RR
OXON2 11	23.1	RRR	49.5	RR
d.f	33		33	
S.E.	8.25	*	5.367	*
L.S.D.≤0.05	23.75		15.441	
* omitted from ANOVA as low germination				

Resistance 'R' ratings: *RRR* = Resistance confirmed, highly likely to reduce herbicide performance;
***RR* = Resistance confirmed, probably reducing herbicide performance; *R?* = Early indications that resistance may be developing, possibly reducing herbicide performance. *S* = Susceptible, no clear evidence of resistance.**

Pendimethalin

Twelve of the 2002 populations were rated resistant (RRR/RR) to pendimethalin and two were rated R? showing that resistance to this herbicide was already widespread. **By 2009/10/11, 15 samples (=79%) were resistant (RR or RRR) and two were rated R? to pendimethalin.** The % reduction values had declined between 2002 and 2009/10/11 for 14 out of 19 of the samples, albeit sometimes by only a small amount. However, moderate levels of control were achieved with pendimethalin even with the resistant populations, and very few samples were as resistant as the PELDSS 05 reference population (see above).

- **Resistance to pendimethalin is widespread, but tends to be partial and has not increased dramatically in most fields since 2002.**
- **Pendimethalin is likely to give useful, if reduced, levels of control in the field, even on resistant populations, especially if applied in mixtures pre-emergence, or to small plants.**

Overall trends with black-grass

The overall trends can be summarised by averaging the % reduction results for the 19 samples for each collection period. This gives the best indication of likely changes more generally.

Table 3. Mean % reductions for 19 black-grass populations collected in both 2002 and 2009/10/11

Treatment	**2002**	**2009/10/11**	**Loss of efficacy**	L.S.D. (*P≤0.05*)	
Meso.+iodosulfuon (12+2.4 g/ha)	93.3 %	71.5 %	**21.8%**	3.07	SIG
Clodinafop-propargyl (30 g/ha)	44.6 %	30.1 %	**14.5%**	4.46	SIG
Ch orotoluron (2.75 kg/ha)	78.8 %	72.6 %	**6.2%**	3.98	SIG
Cycloxydim (5 ppm)	82.3 %	56.2	**26 1%**	5. 6	SIG
Pendimethalin (5p m	70.4 %	62.0 %	**8.4%**	3.75	SIG

These results show that, overall, there has been a statistically significant decline in activity of all five herbicides evaluated (Table 3). The decline has been greater with mesosulfuron-methyl+iodosulfuron-methyl, clodinafop-propargyl and cycloxydim than with chlorotoluron and pendimethalin, even though the baseline level of resistance in 2002 varied with herbicide. However, there was a significant interaction (P<0.001) between population and year with all five herbicides indicating, as expected, that changes in resistance were not consistent between fields. These are average values of course, and larger and smaller changes have occurred with all herbicides on individual fields. Resistant is not absolute, and these results were obtained using a single herbicide dose in all assays, so care is needed in relating these results on the likely impact on herbicide activity in the field.

- **With all five herbicides, there was a consistent, and significant, trend for herbicide performance to be poorer in the more recently collected samples, compared with the 2002 baseline samples from the same fields.**
- **As the fields were randomly selected, this provides the best evidence we have that resistance to all these herbicides has increased more generally between 2002 and 2009/10/11.**
- **Resistance appears to be increasing more rapidly to some herbicides than others, with the smallest changes being recorded with chlorotoluron and pendimethalin, and larger changes being recorded with mesosulfuron-methyl+iodosulfuron-methyl, clodinafop-propargyl and cycloxydim.**
- **Seed samples were collected from plants surviving herbicides in the field, which could potentially distort the results to some degree, so care is needed in interpretation.**

Field histories

Records were obtained for the period 2002 to 2009/10/11 for 14 of the resampled fields. These included cropping, cultivations and grass-weed herbicide use. In total, information for 98 cropping years was obtained. Autumn sown crops were grown in 87% of years, with winter cereals accounting for 66% of crops. Ploughing was done in 52% of years. 1.85 grass-weed herbicide products and 2.74 grass-weed a. i./year were applied on average. ACCase herbicide use was low, accounting for only 10% of all the grass-weed a.i.s applied. This is almost certainly a reflection of widespread resistance to this MOA. Use of mesosulfuron-methyl+iodosulfuron-methyl was very common, with 73% of all wheat crops receiving this herbicide and 82% of the last three wheat crops grown. ALS herbicides accounted for 44% of all grass-weed a.i.s applied on fields with confirmed resistance to mesosulfuron-methyl+iodosulfuron-methyl, but only 20% of a.i. use on fields still susceptible to this herbicide. This difference was statistically significant (L.S.D = 14.8%). A very wide range of other MOA was used, and these accounted for 48% of all grass-weed a.i.s applied on fields with confirmed resistance to mesosulfuron-methyl+iodosulfuron-methyl, and 67% of a.i. use on fields still susceptible to this herbicide.

- **Herbicide resistance had increased despite the use of a wide range of different MOA – the minimum number used any one field between 2002 and 2009/10/11 was four, the maximum was 11.**
- **Use of ACCase inhibiting herbicides ('fops'/''dims'/'dens') was low, and mainly confined to oil-seed rape crops, largely reflecting the widespread incidence of resistance.**
- **Resistance to mesosulfuron-methyl+iodosulfuron-methyl was associated with a greater usage of ALS inhibiting herbicides.**
- **Mesosulfuron-methyl+iodosulfuron-methyl was by far the most frequently used ALS inhibiting herbicide being applied to 82% of the last three wheat crops.**
- **This high frequency of use of mesosulfuron-methyl+iodosulfuron-methyl is likely to result in increasing problems of resistance, both in terms of number of fields affected and the proportion of resistant plants within individual fields.**

1.2 Monitoring changes in resistance in rye-grass

Resistant Italian rye-grass (*Lolium multiflorum*) occurs in 33 counties in the UK (Moss *et al*., 2011) and a recent (2006/07) semi-random survey found resistance to at least one herbicide in 70% of the 50 semi-random fields sampled (Alarcon-Reverte, 2010). Fifteen of the fields sampled originally in 2006/07 were re-sampled in 2010 or 2011, and tested alongside the 2006/07 samples, seeds of which have been stored at Rothamsted. In addition, standard reference populations were included: two susceptible standards (Trajan11 and Trajan PRG); one enhanced metabolism resistance standard (CLEV97); one 1781 ACCase target site resistant standard (LBAN98); one 2078 ACCase target site resistant standard (PYL99); one 197 ALS target site resistant standard (ISK10). The aim was to see what changes in resistance have occurred in these fields, as this should give a good indication of what is happening in arable fields in England more generally. Two screening experiments were done: a glasshouse pot assay using diclofop-methyl-methyl ('Hoegrass'), cycloxydim ('Laser'), pinoxaden ('Axial') and mesosulfuron-methyl+iodosulfuron-methyl ('Atlantis'); and a Petri-dish assay with pendimethalin ('Stomp'). The methods used were similar to those for black-grass, as detailed in the previous section. Samples of resistant plants from the 50 farms were screened for single nucleotide polymorphisms (SNPs) or mutations to ACCase inhibitors were undertaken using the methods of Alarcon-Reverte (2010).

Glasshouse pot assay

The Trajan susceptible samples were well controlled (>87%) by all herbicides confirming that the herbicide application and test conditions were conducive to good herbicidal activity. The CLEV99 enhanced metabolism standard was very poorly controlled by diclofop-methyl (0%), but well controlled by cycloxydim and pinoxaden (98 – 99%), which are not vulnerable to enhanced metabolism. The LBAN98 and PYL99 ACCase target site resistant (TSR) standard populations were generally poorly controlled by the ACCase inhibitors diclofop-methyl, cycloxydim and pinoxaden (<66%). The ISK10 ALS target site resistant (TSR) population was very poorly controlled (12%) by mesosulfuron-methyl+iodosulfuron-methyl, whereas all other standard populations were well controlled (>87%). The results for the susceptible and resistant reference populations were very largely as predicted and fully validate the test methodology.

Diclofop-methyl

Thirteen of the 15 pairs of populations were rated RR or RRR for resistance to diclofop-methyl, using both older (2006/07) and newer (2010/11) seeds. This highlights how common resistance to the once widely used herbicide, diclofop-methyl, is in rye-grass. Diclofop-methyl is vulnerable to both enhanced metabolism and ACCase TSR, so while it is a good indicator of general resistance, it does not provide useful information on the precise mechanism responsible.

Cycloxydim & Pinoxaden

The response to these two herbicides was similar, with the same four samples showing resistance to both herbicides in both the 2006/07 and 2010/11 samples. One additional sample (HAYSHED11) had changed from being susceptible to cycloxydim in 2006/07 to resistant (RR) in 2010/11, but this population was still susceptible to pinoxaden in 2010/11. Also, one other population was rated susceptible to pinoxaden in 2006/07 but R? in 2010/11. Consequently, there was no evidence of a substantial increase in resistance to either cycloxydim or pinoxaden between 2006/07 and 2010/11. However, a small number of plants did survive treatment with both herbicides even in populations rated susceptible. In the 2006/07 samples, only two plants survived cycloxydim or pinoxaden in the 11 non-resistant populations but, by 2010/11, 24 plants survived in three populations which were still rated S or R?. Thus there are indications of resistance to cycloxydim and pinoxaden at a low frequency in some populations rated as susceptible, so carefully monitoring of such sites is essential.

Mesosulfuron-methyl+iodosulfuron-methyl

Efficacy of this herbicide was lower than expected, for reasons that cannot be fully explained. It did give good control (>87%) of the susceptible and all three ACCase resistance standards (CLEV99, LBAN98, PYL99). Thus, control of the standards was exactly as expected. The ISK ALS TSR population was very poorly controlled (12%) by mesosulfuron-methyl+iodosulfuron-methyl as expected, but well controlled by diclofop-methyl (85%). In addition, good control (81%) by mesosulfuron-methyl+iodosulfuron-methyl was achieved of the only two populations from 2010/11 rated as susceptible to diclofop-methyl (20AC 11 & KEN11). However, control of the other 13 pairs of semi-randomly collected populations by both mesosulfuron-methyl+iodosulfuron-methyl and diclofop-methyl was generally poor to mediocre (4 – 73%), with most populations being rated as resistant (RR or RRR). This correlation between resistance to diclofop-methyl and mesosulfuron-methyl+iodosulfuron-methyl, except where explained by presence of either ACCase or ALS target site mutations, strongly suggests that enhanced metabolism is responsible for partial resistance to both herbicides, although we consider that this assay overstates the likely impact on performance of mesosulfuron-methyl+iodossulfuron in the field.

Petri-dish assay

A Petri-dish assay was used to determine the responses of 13 pairs of rye-grass populations, as used in the glasshouse assay, to pendimethalin (‘Stomp’) which is used as an indicator of enhanced metabolism resistance. Two of the populations used in the glasshouse had to be omitted due to insufficient seeds. The TRAJAN 11 susceptible, the PYL99 ACCase TSR and the ISK10 ALS TSR reference populations were well controlled (100%) confirming the robustness of the test methodology and that ACCase and ALS target site resistance (TSR) has no impact on pendimethalin activity. Four populations were rated susceptible (S) and three population resistant (RR) in relation to both the 2006/07 and 2010/11 seed samples. Resistance in two populations had increased from R? to RR and resistance had developed (from S), in DAIRY and PARSON (to RR), and in THORNHAM (to R?). In the remaining population (HAYSHED), resistance appeared to have declined from R? to S. Consequently, the susceptibility or resistance status had stayed broadly constant (ignoring R?/RR differences) in nine (69%) of the 13 paired samples tested, had increased slightly (from S to R?) in one population (THORNHAM) and had decreased slightly (from R? to S) in another (HAYSHED). In only two populations, had there been a substantial increase in resistance, from S to RR (DAIRY and PARSON). However, the impact of these differences on the efficacy of pendimethalin in the field is harder to predict as this has not been investigated.

Molecular assays

The six ACCase mutations found, and their frequencies as a proportion of the total number of resistant plants assayed (384), were: Asp-2078-Gly (24.5%), Ile-1781-Leu (13.3%), Ile-2041-Asn (2.1%), Cys-2088-Arg (1.8%), Trp-2027-Cys (1.0%) and Trp-1999-Cys (0.3%). No plants with the Gly-2096-Ala mutation were found. Even though a point mutation was found in 40% of the 384 resistant plants studied, the main mechanism conferring resistance in 68% of the fields with confirmed resistance to at least one of the herbicides assayed was a non-target site resistance mechanism, most probably enhanced metabolism.

Overall trends with rye-grass

The overall trends can be summarised by averaging the % reduction results for the 15 samples for each collection period. This gives the best indication of likely changes more generally.

Table 4. Mean % reductions for 15 rye-grass populations collected in both 2006/7 and 2010/11

Treatment	**2006/07**	**2010/11**	**Loss of efficacy**	L.S.D. ($P \leq 0.05$)	
Meso.+iodosulfuon (12+2.4 g/ha	54.9 %	36.0 %	**18.0%**	5.92	SIG
Diclofop-methyl (567 g/ha)	19.5 %	17.2 %	**2.3%**	4.51	N.S.
Cycloxydim (75 g/ha)	83.6 %	81.1 %	**2.5%**	2.50	SIG
Pinoxaden (22.5 g/ha)	82.5 %	81.7 %	**0.8%**	2.23	N.S.
Pendimethalin (5ppm)	86.9 %	78.3 %	**8.6%**	3.08	SIG

These results show that, overall, there has been little change in the degree of resistance to diclofop-methyl, cycloxydim and pinoxaden between 2006/07 and 2010/11 (Table 4). There were modest, but statistically significant, declines in the efficacy of pendimethalin and mesosulfuron-methyl+iodosulfuron-methyl between 2006/07 and 2010/11. However, some of these statistically significant differences were small from an agronomic viewpoint. There were statistically significant interaction between population and year indicating that, as expected, changes in resistance were not consistent between fields. Resistant is not absolute, so care is needed in relating these results on the likely impact on herbicide activity in the field.

- **With all five herbicides, there was a trend for herbicide performance to be slightly poorer in the more recently collected samples, compared with the 2006/07 baseline samples.**
- **The declines in efficacy were mainly small, and unlikely to have a big impact in the field.**
- **The time span between the baseline and more recent collections was shorter than for the black-grass study, so this may be one reason why smaller changes were recorded.**
- **Seed samples were collected from plants surviving herbicides in the field, which could potentially distort the results to some degree, so care is needed in interpretation.**
- **Currently, the most common mechanism of resistance to ACCase-inhibiting herbicides in UK Italian rye-grass populations appears to enhanced metabolic resistance.**
- **Although ACCase target site resistance is also common, and six of the seven known ACCase mutations were detected, it was the main mechanism of resistance in only 32% of resistant populations.**

1.3 Outdoor container experiments

Glasshouse and Petri-dish assays are a convenient method for quantifying changes in herbicide efficacy resulting from increasing resistance, but it is important to demonstrate that such changes significantly impact on herbicidal activity in the field. Outdoor container experiments enable the effects of resistance on the efficacy of different herbicides at field recommended doses to be assessed under conditions which closely mimic the field environment.

Pairs of samples (2002 baseline v 2009/10 resampled) of four black-grass populations from the monitoring exercise detailed in section 1.1 above, were evaluated in outdoor container experiments. Seeds were sown in early October 2010 or 2011 and plants treated post-emergence at the 2 – 3 leaf stage in early November. A susceptible standard (ROTH) was also included and foliage fresh weight per container was assessed in Feb/March. The populations and herbicides tested are shown in Table 5 along with the results from the glasshouse assay conducted as part of the resistance monitoring exercise (see Table 1).

Table 5. % reductions in foliage fresh weight for black-grass populations collected in two years from the same fields and evaluated in outdoor container experiments in 2010/11 or 2011/12 (results for previous glasshouse assays also presented – taken from Table 1).

		ROTH	Herts2		Suffolk1		L.S.D. ($P \leq 0.05$)
Herbicide		**Susceptible**	**2002**	**2009**	**2002**	**2009**	
meso.+iodo. 12+2.4 g/ha	**containers**	**96**	**91**	**27**	**90**	**31**	**13.5**
	glasshouse	93	93	17	92	57	13.4
	-	**ROTH**	**Herts1**		-		
	-	**Susceptible**	**2002**	**2010**	-	-	
	containers	**98**	**89**	**15**	-	-	**15.2**
	glasshouse	96	94	28	-	-	13.4
clodinafop-propargyl 30 g/ha	-	**ROTH**	**Notts1**		**Yorks1**		
	-	**Susceptible**	**2002**	**2009**	**2002**	**2009**	
	containers	**100**	**99**	**61**	**48**	**24**	**25.5**
	glasshouse	93	92	72	29	12	19.4
	-	**ROTH**	**Herts1**		-		
	-		**2002**	**2010**	-	-	
	containers	**98**	**90**	**31**	**-**	**-**	**15.2**
	glasshouse	98	64	19	-	-	19.4
chlorotoluron 2.75 kg/ha	-	**ROTH**	**Essex2**		**Norfolk1**		
	-	**Susceptible**	**2002**	**2009**	**2002**	**2009**	
	containers	**100**	**43**	**41**	**60**	**44**	**27.0**
	glasshouse	97	74	46	92	88	17.3

The results for the outdoor container experiment validated those obtained from the glasshouse assay. There was a decline in efficacy of both mesosulfuon+iodosulfuron-methyl and clodinafop-propargyl between 2002 and 2009/10 for the five populations tested, with similar levels of control in both experimental conditions. This shows that glasshouse pot tests give a reliable indication of the likely impact of resistance on the efficacy of both herbicides. The poor control of the Suffolk 1 population demonstrated that, even in a population without ALS target site resistance (see Figure 1 above), probable enhanced metabolic resistance could result in a substantial loss of efficacy. With chlorotoluron, there was more variability between the experimental conditions. Control was consistently poorer in the outdoor containers by an average of 28%, but the results do support the view that the decline in efficacy with chlorotoluron had been less than with the other two herbicides. Chlorotoluron tends to be more variable under glasshouse conditions as, being a photosynthetic inhibitor, light intensity has a large effect on its efficacy. Consequently, care is needed in predicting the likely impact of resistance to chlorotoluron in the field based on glasshouse assay results.

- **Results from the outdoor container studies provided good supporting evidence that the losses in efficacy documented in the resistance monitoring exercise in the glasshouse (see Section 1.1) do impact substantially on herbicide efficacy in the field.**

1.4 'Watching brief' on potential new cases of resistance

A very useful component of this, and previous projects, has been to maintain a 'watching brief' on potential new cases of resistance. This has involved undertaking initial screening evaluations in new species or investigating new forms of resistance. In addition, a periodic update on the current status of herbicide resistance in the UK has been undertaken and published (see Moss *et al*., 2011 for the latest update). Key aspects are summarised in Table 6.

Table 6. Summary of current status of resistant weeds of arable crops in the UK

	Farms with confirmed resistance	Counties	Comments
Black-grass	>16,000	34	England, status in Scotland uncertain
Italian rye-grass	>450	33	England, status in Scotland uncertain
Wild-oats	>250	28	England, status in Scotland uncertain
Chickweed	>40	13	7 in Scotland, 5 in England, 1 in Northern Ireland
Poppy	>25	9	All in England, status in Scotland uncertain
Mayweed	4	2	Yorks. and Norfolk, status in in Scotland uncertain

- **Black-grass is the most important resistant species by far, but resistance in rye-grass and wild-oats needs continual monitoring, as major problems occur on individual farms.**
- **Resistance to mesosulfuron-methyl+iodosulfuron-methyl, first used in the UK in autumn 2003, was confirmed in black-grass on over 400 farms in 26 counties by 2010. Resistance continues to increase widely.**
- **Resistance in chickweed, poppy and mayweed is mainly to the ALS inhibitors, especially sulfonylureas. Alternative MOA generally remain effective.**
- **Resistant groundsel, selected by the triazinone herbicide metribuzin, was identified for the first time in the UK in asparagus fields in 2010.**
- **The potential of resistance in an increasingly common weed, Rat's-tail fescue (*Vulpia myuros*), was investigated but no evidence of evolved resistance was found. Flufenacet and meso.+iodosulfuron-methyl gave effective control (Hull *et al*., 2011).**
- **A case of potential glyphosate resistance in sterile brome (*Bromus sterilis*) on an arable farm was investigated. The suspect population showed 'marginal' resistance and further studies are in progress, including the activity of different glyphosate formulations.**

Objective 2. To identify the occurrence of stacked resistance mechanisms, such as 'double' ACCase and ALS target site resistance (DTSR), and assess their impact on grass-weed populations.

The proven presence of both ACCase and ALS target site resistance in grass-weeds means that there is increasing likelihood of double target site resistance (DTSR) where *both* resistance mechanisms occur. If the different mechanism are present in *different* plants within a field, then rotations or mixtures of ACCase and ALS herbicides should give good control overall, as the ALS will kill the ACCase resistant plants and vice-versa. The presence of both mechanisms in the *same* plant greatly increases the problem of management as such plants will be resistance to both herbicides.

2.1 Detection and implication of 'Double' Target site resistance (DTSR) in grass-weeds

The objective was to confirm the presence of 'combined' (= 'double') ACCase and ALS target site resistance (TSR) in individual black-grass plants due to separate mutations conferring resistance to each mode of action (MOA).

Glasshouse and molecular assays

Individual plants (20-30 plants of seven populations) were split into three parts and individual clones treated with either the ALS inhibitor mesosulfuron-methyl+iodosulfuron-methyl (12 + 2.4 g/ha), the ACCase inhibitor cycloxydim (200 g/ha) or left untreated. Following screening and phenotype characterisation, leaf samples were taken from each individual untreated clone and tested for the presence of six ALS and seven ACCase single nucleotide changes known to confer ALS and ACCase target site resistance in weed species (Marshall & Moss, 2008; Powles & Yu, 2010). The assay was performed using the ABI PRISM® SNaPshot® Multiplex Kit (Applied Biosystems). The populations used and results are summarised in Table 7.

Table 7. Plant responses and molecular characterisation of ALS and ACCase target site resistance (TSR) mutations in seven black-grass populations.

			% plants with mutations					
	% plants resistant in assay of cloned plants		ALS TSR mutations*		ACCase TSR mutations*			% plants with 'double' TSR
	meso+iodo. (ALS)	cycloxydim (ACCase)	197	574	1781	2041	2078	ALS+ACCase
Roth05	0	0	0	0	0	0	0	**0**
Peld05	100	0	100	0	0	0	0	**0**
Notts05	5	90	0	0	90	0	0	**0**
R30-08	90	95	0	90	95	10	5	**85**
Hor08	100	100	0	100	100	0	0	**100**
Velc08	100	60	0	65	52	63	11	**50**
LongC08	90	33	84	0	31	0	3	**27**

* data for homozygous and heterozygous mutations combined in this summary table

The Roth05, Peld05 and Notts05 populations were used as susceptible, ALS TSR and ACCase TSR reference populations respectively. The results for those populations are exactly as predicted, with no 'double' target site resistant in any plants. The four other populations were chosen as they had shown high levels of resistance in previous screening experiments. A high proportion (90 – 100%) of plants in all four populations showed resistance to the ALS inhibitor, mesosulfuron-methyl+iodosulfuron-methyl, and molecular assays showed that this was due partly to the 574 ALS mutation in three populations and the 197 ALS mutation in the other. All four populations showed resistance to the ACCase inhibitor cycloxydim, but the proportion of resistant plants varied from 33 – 100%. Resistance was conferred mainly by the 1781 ACCase mutation in three populations but the Velc08 population possessed three different ACCase mutations. The proportion of plants containing both an ALS and a ACCase target site mutation ('double target site resistance') varied from 27% in the LongC08 population to 100% in the Hor08 population.

Outdoor container validation

To demonstrate that 'double' target site resistance is likely to impact on herbicide performance in the field, an outdoor container experiment was conducted using three of the populations used in the glasshouse and molecular study above (Roth05, Hor08, LongC08). In addition, a mixture of Peld05 and Notts05 seeds was used to represent a population with both ALS and ACCase TSR, but with no *individual* plants possessing both forms of TSR. Seeds were sown in containers and plants treated with either the ALS inhibitor sulfometuron, or the ACCase inhibitor cycloxydim, or a sequence of both herbicides, or untreated. These herbicides were used, as both are considered to be unaffected by enhanced metabolic resistance, which could otherwise have confounded the results.

Table 8. The % reduction in black-grass plants, relative to untreated, for four populations treated with herbicides in outdoor containers (2010/11)

	Herbicide		
Population	**cycloxydim ACCase**	**sulfometuron ALS**	**cycloxydim + sulfometuron**
Roth05	100	100	100
Hor08	0	0	0
LongC	6	72	72
Peld05 + Notts05	-	-	100
	L.S.D. ($P \leq 0.05$) = 5.9		

The results fully validated the glasshouse and molecular studies (Table 8). The Roth05 susceptible standard and the mixed Peld05 ALS TSR + Notts05 ACCase TSR were completely controlled by the sequence of the two herbicides. In contrast, no control of the Hor08 population was achieved, which was consistent with the molecular assay demonstrating 100% 'double' target site resistance. The 72% control of LongC08 was also consistent with the presence of 27% of plants with 'double' target site resistance.

- **These experiments proved the presence of 'double' target site resistance to ALS and ACCase inhibiting herbicides in four black-grass populations collected from fields.**
- **The sequential or rotational use of these two MOA was shown to be effective when the ACCase and ALS mutations were present in *different* individual plants within a population.**
- **With population with 'double' target site resistance, such sequential or rotational use of ACCase and ALS inhibitors was ineffective.**
- **The presence of 'double' target site resistance is likely to increase as black-grass is a predominantly cross-pollinating species.**

Objective 3. To assess the significance of non-target site resistance mechanisms (principally enhanced metabolism) on ALS inhibitors used for grass-weed control.

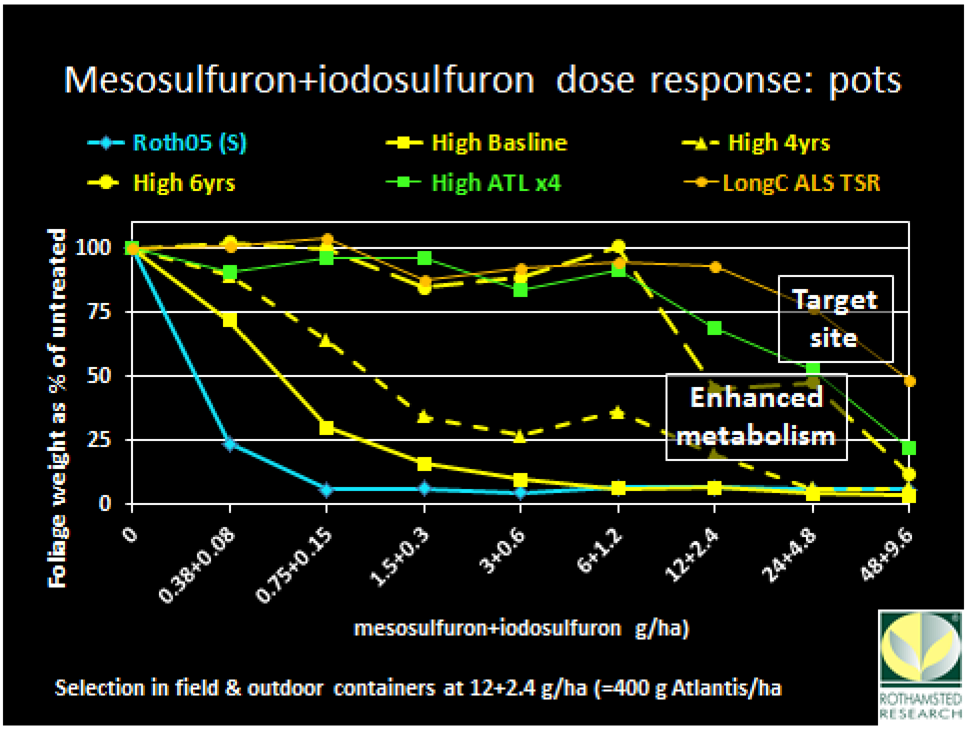
The ALS herbicide mesosulfuron-methyl+iodosulfuron-methyl was introduced into the UK market in autumn 2003 and is now the most widely used herbicide for grass-weed control. Black-grass populations resistant to this herbicide were confirmed on over 400 farms by 2010 (Moss *et al*., 2011). ALS target site resistance conferred by two mutations (Proline-197-Threonine or Trytophan-574-Leucine) has been shown to be responsible in some populations (Marshall & Moss, 2008), but non-target site mechanisms (probably enhanced metabolism) were also suspected. The results of the black-grass monitoring exercise outlined in Section 1.1 strongly support this view. However, the relative importance of ALS target site resistance (TSR) and ALS enhanced metabolism resistance (EMR) on herbicide efficacy and rate of development of resistance is unclear, but is an important factor in weed management.

3.1 Implications of non-target site resistance on ALS inhibiting herbicides used against grass-weeds – laboratory and glasshouse studies

The seven black-grass populations used in this experiment were: Roth05 (susceptible), LongC08 (ALS TSR standard), Highfield baseline (=Main99), Highfield 4 yrs, Highfield 5 yrs, Highfield 6 yrs, Highfield ATL x4. Seeds of the Highfield baseline population, collected from a field at Woburn, had been sown in plots, or subsequently in containers, at Rothamsted and treated annually with the recommended rate of mesosulfuron-methyl+iodosulfuron-methyl (12+2.4 g a.i./ha). Seeds from surviving plants were collected after 4, 5 & 6 years of selection. Highfield ATL x4 was identical to Highfield 6 yrs except that in the final year of selection (2009/10) plants were treated with 2x the recommended in both autumn and spring (total dose = 48+9.6 g a.i./ha). Molecular assays showed that ALS target site resistance (P197T) was present in a high proportion of plants of LongC08 (Marshall & Moss; Marshall & Moss, 2012) but neither of the common ALS target site mutations (P197T, W574L) could be detected in leaves taken from the Highfield 5 yr population. Plants of this population (taken from the container experiments described in Section 3.2 below) were also assayed by Bayer CropScience in Frankfurt in 2009 and 2010 and no ALS mutations were detected, but 23% and 61% of the 31 plants assayed with radiolabelled mesosulfuron-methyl were rated as having an intermediate or high ability to metabolise that herbicide respectively. Consequently, the populations used in this experiment had been well characterised at the molecular and biochemical level.

Seeds of these seven populations were sown in individual pots and plants treated with eight doses of mesosulfuron-methyl+iodosulfuron-methyl (0.375+0.075 to 48+9.6 g a.i./ha) at the 3 leaf stage in a dose response experiment in a glasshouse. There were 16 replicates and 32 untreated pots per population. Foliage fresh weight per pot was assessed 28 days after spraying as a measure of herbicide efficacy (Figure 2).

Figure 2. Glasshouse dose response experiment with mesosulfuron-methyl+iodosulfuron-methyl ('Atlantis')



Note: 'Atlantis' = 3% mesosulfuron-methyl + 0.6% iodosulfuron-methyl;
Field rate = 400g Atlantis/ha = 12+2.4 g a.i./ha

The Roth05 susceptible standard was well controlled (>90%) by 0.75+0.15 g/ha, or 6.25% of the field rate. The Highfield baseline had received only one ALS inhibiting grass-weed herbicide (imazamethabenz 21/11/95) prior to the start of this selection experiment and was slightly, but not significantly more resistant to mesosulfuron-methyl+iodosulfuron-methyl than the Roth05 susceptible standard, with an ED50 of 0.36+0.07 g a.i./ha and an Resistance Index (RI) of 1.48. After four, five and six years of selection with mesosulfuron-methyl+iodosulfuron-methyl, there was evidence of increasing selection for resistance in the Highfield populations. ED50 and RI values were respectively 0.79+0.16 g/ha and 3.2 after 4 yrs, 1.57+0.31 g/ha and 6.4 after 5 yrs and 13.2+2.6 g a.i./ha and 54.1 after 6 yrs. These shifts were statistically significant relative to both the Roth05 susceptible standard and the Highfield baseline populations.

Clearly, selection with annual applications of mesosulfuron-methyl+iodosulfuron-methyl had resulted in a progressive increase in resistance and, while initial changes were quite small, ultimately, after 6 years of selection, black-grass was clearly showing high resistance. Some control was being achieved and % reductions in foliage weights for the Highfield 6 yrs selection were 55%, 52% and 88% for the 12+2.4, 24+4.8 and 48+9.6 g/ha doses respectively. However, it only took 3+0.6 g/ha (25% of the field rate) to achieve 90% control of the Highfield baseline population, so there had clearly been a substantial shift which was of a scale likely to impact on herbicide performance. Control of the Highfield baseline population by 12+2.4 g/ha (field rate) was 93% and, after 6 years selection, control was only 55% at the same rate, indicating an average mean decline in efficacy of 6% per year.

Even higher levels of resistance were detected in the Highfield ATL x4 population that had received double field rate in autumn and spring 2009/10, with ED50 value of 22.1+4.4 g/ha and RI of 90.6. This indicates that resistance conferred by enhanced metabolism had not peaked in the Highfield 6 yr selection as a consequence of annual herbicide applications, and that there was the potential for selection for greater resistance. The highest level of resistance was, not surprisingly, in the LongC ALS target site (P197T) resistant standard, where the ED50 value was 47.5+9.5 g/ha (almost 4 x the field rate), and a RI of 194 relative to the Roth05 susceptible standard. However, this level of resistance was not statistically greater than that in the Highfield ATL x4.

These results indicate that enhanced metabolic resistance to mesosulfuron-methyl+iodosulfuron-methyl (‘Atlantis’) can build up rapidly, even in a population with little previous exposure to ALS graminicides. In addition, the degree of resistance to mesosulfuron-methyl+iodosulfuron-methyl conferred by enhanced metabolism approaches that of ALS TSR. This conclusion is supported by the results of the container experiments detailed in Section 1.3 above. This result was somewhat unexpected, as enhanced metabolism often confers partial resistance and builds up slowly, as was the experience with isoproturon in the past. What is unclear is whether the probable enhanced metabolic resistance recorded here, is specific to ALS inhibiting herbicides, or is more generic. This should be a focus for future research.

- **These experiments showed that enhanced metabolic resistance to mesosulfuron-methyl+iodosulfuron-methyl (‘Atlantis’) can build up progressively and rapidly, even in a population with little previous exposure to ALS graminicides.**
- **The degree of resistance to mesosulfuron-methyl+iodosulfuron-methyl conferred by enhanced metabolism approaches that of ALS target site resistance.**
- **There was evidence that the maximum level of enhanced metabolism of mesosulfuron-methyl+iodosulfuron-methyl had not been reached. Material from this experiment is being used in a new project which will investigate the potential for further selection.**

3.2 Implications of non-target site resistance on ALS inhibiting herbicides used against grass-weeds – container and field studies

It is important that differences in herbicide efficacy recorded in glasshouse studies are validated in outdoor conditions in order to assess the likely impact in field conditions. Studies on the timing of herbicide applications are important in order to determine whether earlier applications can overcome, to some extent at least, the impact of resistance on efficacy.

Three container experiments were conducted in which black-grass seeds were sown in late September and plants treated post-emergence. Foliage fresh weight per container was assessed in the following February to April period as a measure of herbicide efficacy. Untreated containers were included and there were three replicates.

Experiment 1 2009/10: impact of ALS target site resistance on herbicide efficacy

Three black-grass populations were used: Roth05 (susceptible standard), LongC08 (ALS target site resistant (TSR) standard for the P197T mutation), R30 08 (ALS target site resistant (TSR) standard for the W574L mutation). These populations had all been characterised using molecular techniques (Marshall & Moss, 2008). Four different groups of ALS herbicides were applied: mesosulfuron-methyl+iodosulfuron-methyl at 12+2.4 g a.i./ha (= sulphonylureas), pyroxsulam at 18.75 g a.i./ha (= triazolopyrimidine), propoxycarbazone at 70 g a.i./ha (= sulfonylaminocarbonyl-triazolinone), imazapyr at 125, 375 & 1,125 (= imidazolinone). Herbicides were applied at the 2 – 3 leaf stage on 2 November 2009.

The Rothamsted susceptible standard was well controlled (>88%) by all treatments (Table 9). In contrast, both the LongC08 ALS (P197T) and R30 08 ALS (W574L) TSR populations were very poorly controlled (<17%) by mesosulfuron-methyl+iodosulfuron-methyl, pyroxsulam and propoxycarbazone. However, the two populations responded differently to imazapyr. R30 08 was controlled very poorly (<29%) by all doses but control of LongC08 varied from mediocre (65%) at 125 g a.i./ha to almost as good (89%) as the susceptible standard (93%) at the 1125 g a.i./ha dose.

These results fully validate previous glasshouse dose response experiments with the same populations conducted as part of a previous Sustainable Arable Link project (Moss *et al*., 2010). In that study, very poor control of R30 08 was achieved with imazapyr at all doses up to 1500 g a.i./ha whereas control of LongC08 increased considerably as dose increased from 93.75 to 375 g a.i./ha.

Table 9. Experiment 1. The % reduction in foliage weights for three black-grass populations with different forms of ALS target site resistance treated with different ALS inhibiting herbicides

		Populations and resistance mechanism		
Herbicide	Dose	**Roth05**	**LongC08**	**R30 08**
	g a.i./ha	**Susceptible**	**ALS TSR P197T**	**ALS TSR W574L**
mesosulfuron-methyl+ iodosulfuron-methyl	12+2.4	**89**	**17**	**15**
pyroxsulam	18.75	**89**	**15**	**4**
propoxycarbazone	70	**88**	**1**	**4**
imazapyr	125	**91**	**65**	**19**
	375	**92**	**89**	**16**
	1125	**93**	**89**	**29**
		L.S.D. (*P≤0.05*) = 13.1		

This study confirmed that both of the common ALS target site mutations (P197T and W574L) confer a high degree of resistance to sulphonylurea, triazolopyrimidine and sulfonylaminocarbonyl-triazolinone herbicides used for grass-weed control in the UK. Activity of mesosulfuron-methyl+iodosulfuron-methyl, pyroxsulam and propoxycarbazone would be expected to be minimal on black-grass plants with ALS target site resistance, regardless of mutation. However, it should not be assumed that other herbicides from these same classes will necessarily respond in the same manner. The specific mutation present was much more important in determining the degree of resistance to the imidazolinone, imazapyr, with W574L conferring much greater resistance than P197T. Other imidazolinones may respond differently to imazapyr.

Experiment 2 2009/10: impact of different types of ALS resistance on herbicide efficacy

Four black-grass populations were used: Roth05 (susceptible standard), LongC08 (ALS target site (P197T) resistant (TSR) standard), Highfield09 (ALS enhanced metabolism resistant (EMR) standard – this is the same as Highfield 5 yrs in Section 3.1 above), Warren09 (ALS resistant – enhanced metabolism suspected). A range of ALS inhibiting herbicides as shown in Table 9 was applied to these four populations at the 2 – 3 leaf stage on 5 November 2009. In addition, the Highfield baseline (=Main99) and Warren03 baseline populations were treated with mesosulfuron-methyl+iodosulfuron-methyl only.

The Rothamsted susceptible standard was well controlled (>89%) by all herbicides (Table 10). In contrast, the LongC08 population was very poorly (<20%) controlled by all the ALS inhibiting herbicides used, indicating that the P197T target site mutation confers resistance, not only to sulphonylurea herbicides such as mesosulfuron-methyl+iodosulfuron-methyl and flupyrsulfuron, but also to the triazolopyrimidine herbicide pyroxsulam. The Highfield09 population was poorly controlled (<55%) by all ALS inhibiting herbicides, indicating that enhanced metabolic resistance can also significantly reduce activity of ALS inhibiting herbicides - both sulphonylurea and triazolopyrimidines.

Control of the Highfield baseline population by mesosulfuron-methyl+iodosulfuron-methyl was 91%. This compared with the 55% control achieved for the Highfield09 population that had been selected with this herbicide for 5 years, representing an annual 7% loss in efficacy due to increasing enhanced metabolic resistance. Control by ALS inhibitors of the LongC ALS TSR population was always poorer than for the Highfield09 EMR population, which validates the results found in the glasshouse dose response detailed in section 3.1 above. The Warren09 population was poorly controlled by all ALS herbicides.

Molecular analyses conducted at Rothamsted and by Bayer showed that Warren09 was resistant by both ALS target site resistance (P197T in 44% and W574L in 25% of plants) and enhanced metabolism (intermediate and high mesosulfuron-methyl metabolism in 50% and 12% of plants respectively). This highlights the multiple ALS resistance mechanisms present also recorded in the survey samples (see Section 1.1).

Table 10. Experiment 2. The % reduction in foliage weight for four black-grass populations with different resistance mechanisms treated with ALS inhibiting herbicides

		Populations and resistance mechanism			
Herbicide	g a.i./ha	**Roth05**	**LongC08**	**Highfield09**	**Warren09**
		Susceptible	**ALS TSR**	**ALS EMR**	**ALS resistant**
mesosulfuron-methyl+ iodosulfuron-methyl	12+2.4	**93**	**12**	**55**	**24**
pyroxsulam	18.75	**94**	**19**	**23**	**10**
flupyrsulfuron	10	**89**	**4**	**27**	**10**
meso.+iodo. + flupyrsulfuron	12+2.4 + 10	**95**	**13**	**48**	**43**
meso.+iodo. + flufenacet+DFF	12+2.4 + 120+30	**93**	**66**	**66**	**65**
		L.S.D. (*P≤0.05*) = 9.3			

Control of the Warren03 baseline population by mesosulfuron-methyl+iodosulfuron-methyl was 95%. This compared with the 24% control achieved for the Warren09 population that had been selected with this herbicide for five years, representing an annual 14% loss in efficacy due to increasing multiple resistance

The mixture of mesosulfuron-methyl+iodosulfuron-methyl and flupyrsulfuron was no better than mesosulfuron-methyl+iodosulfuron-methyl alone on both the LongC08 ALS TSR and the Highfield09 ALS EMR populations. The addition of flufenacet+DFF at half field rate to mesosulfuron-methyl+iodosulfuron-methyl substantially increased overall performance relative to mesosulfuron-methyl+iodosulfuron-methyl alone. The smaller benefit seen on the Highfield09 ALS EMR population indicates that the increased level of metabolism *might* be affecting flufenacet+DFF, which have different MOA. However, this needs verifying.

Experiment 3 2010/11: impact of different types of ALS resistance and time of application on herbicide efficacy

Four black-grass populations were used: Roth05 (susceptible standard), LongC08 (ALS target site (P197T) resistant (TSR) standard), Highfield09 (ALS enhanced metabolism resistant (EMR) standard – (this is the same as Highfield 5 yrs in Section 3.1 above), Highfield baseline (=Main99). Mesosulfuron-methyl+iodosulfuron-methyl at 12+2.4 g a.i./ha was applied to these four populations at the 2 – 3 leaf stage on 3 November and at the 3 – 4 tiller stage on 9 February.

The Rothamsted susceptible standard was well controlled (>93%) at both timings (Table 11). In contrast, both the LongC08 ALS TSR and the Highfield09 ALS EMR population were poorly (<26%) controlled at both timings. There was little evidence that size of plant (2-3 leaf v 3-4 tillers) and time of application (November v March) had any appreciable influence on the impact of either resistance mechanism on herbicide efficacy. Regardless of mechanism, small resistant plants were poorly controlled by mesosulfuron-methyl+iodosulfuron-methyl.

Control of the Highfield baseline population was better in November (94%) than in February (50%) supporting the view that autumn applications to small susceptible plants tend to be more effective. Control of the Highfield09 population was poorer (26%) in this experiment than in Experiment 2 (55%). The corresponding estimated annual loss in efficacy of mesosulfuron-methyl+iodosulfuron-methyl due to increasing metabolic resistance was 14% in this experiment compared with 7% in Container experiment 2 and 6% in the glasshouse dose response experiment (See Section 3.1 above), giving an overall mean of 9%.

Table 11. Experiment 3. The % reduction in foliage weight for four black-grass populations with different resistance mechanisms treated with mesosulfuron-methyl+iodosulfuron-methyl at two timings.

		Populations and resistance mechanism			
Herbicide		**Roth05**	**LongC08**	**Highfield09**	**Highfield baseline**
		Susceptible	**ALS TSR**	**ALS EMR**	**-**
mesosulfuron-methyl+ iodosulfuron-methyl	**Autumn (Nov)**	**100**	**14**	**26**	**94**
mesosulfuron-methyl+ iodosulfuron-methyl	**Spring (Feb)**	**93**	**12**	**3**	**50**
		L.S.D. (*P≤0.05*) = 10.8			

- **These three outdoor container experiments confirmed that both ALS target site mutations (P197T and W574L) confer a high degree of resistance to sulphonylurea (e.g. mesosulfuron-methyl+iodosulfuron-methyl), triazolopyrimidine (e.g. pyroxsulam) and sulfonylaminocarbonyl-triazolinone (e.g. propoxycarbazone) herbicides used for grass-weed control in the UK.**
- **The specific mutation present had a bigger impact on determining the degree of resistance to the imidazolinone, imazapyr, with W574L conferring much greater resistance than P197T. Other imidazolinones may respond differently to imazapyr.**
- **Efficacy of both mesosulfuron-methyl+iodosulfuron-methyl and pyroxsulam was reduced by both ALS target site resistance and enhanced metabolic resistance and size of plant at application had little effect.**
- **The addition of herbicides with other modes of action to mesosulfuron-methyl+iodosulfuron-methyl can improve overall control and help compensate for reduced efficacy due to resistance.**
- **These container studies fully validated the results of the glasshouse dose response assay confirming that enhanced metabolic resistance to mesosulfuron-methyl+iodosulfuron-methyl can build up rapidly and that the degree of resistance conferred by enhanced metabolism approaches that of ALS target site resistance.**

Objective 4. To determine the impact and consequences of non-target site resistance mechanisms (principally enhanced metabolism) on the potential long term sustainability of use of ‘low resistance risk’ herbicides.

Enhanced metabolic (EM) resistance affects many of the alternatives to the ‘high resistance risk’ ACCase and ALS inhibitors. Although the enzymatic basis of enhanced metabolism is complex, current work indicates that there is greater consistency than might be expected in the degree of resistance between populations and herbicide modes of action (i.e. the ‘pecking order’ remains the same). However, a better understanding of the longer term implications of EM on such herbicides is needed. Two complementary experimental approaches were used to gather information on the dynamics of resistance in black-grass treated with the pre-emergence herbicides flufenacet, pendimethalin and prosulfocarb which are affected primarily by enhanced metabolism.

4.1 Impact of enhanced metabolic resistance on black-grass in containers

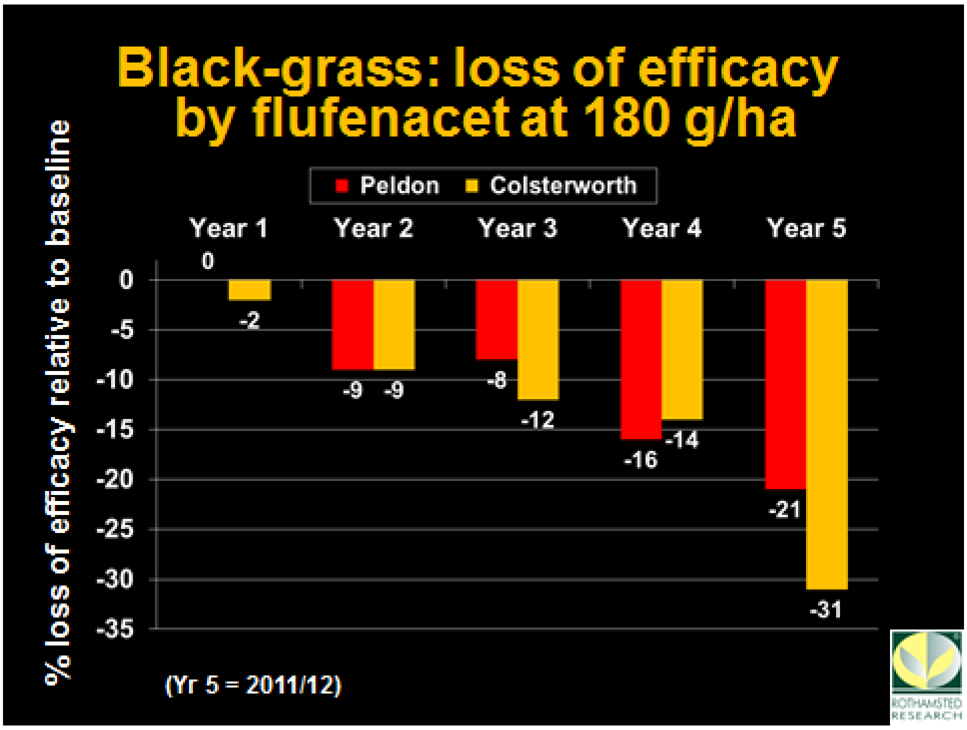
A series of outdoor container experiments were conducted in which black-grass seeds were sown in late September and treated pre-emergence 6 days after sowing. The soil was watered prior to herbicide application but subsequent watering was by natural rainfall. Surviving plants numbers were assessed 2 – 3 months later as a measure of herbicide efficacy. Baseline and untreated containers were included and there were three replicates. Treated containers were isolated in glasshouses in spring, to prevent cross-pollination, and seeds collected in the summer. These were subsequently re-sown into new containers in the following autumn. A glasshouse dose response assay was also conducted with flufenacet.

Flufenacet

Outdoor container experiments. Two baseline populations of black-grass (Peldon03 and Colsterworth05) were used as both had shown relatively high resistance to pendimethalin in screening assays, which is indicative of a relatively high ability to metabolise herbicides. Seeds collected in 2011 after five yearly cycles of selection with flufenacet at 180 g a.i./ha were evaluated at different doses in outdoor containers. In addition, samples collected in 2009 or 2011 from the fields where the baseline samples had been originally collected were also included in the container assay.

The differences in % reduction in plant numbers between the baseline population and the selected populations in the container experiment are shown in Figure 3. It is important to note that the actual % reduction figures for the baseline seeds varied considerably between years, despite the same baseline seed samples being used each year. For Peldon03, control varied from 71% (2011/12) to 95% (2010/11) and for Colsterworth05 from 93% (2007/08) to 99% (2010/11). This shows that containers mimic field conditions well, as 2010/11 (damp autumn) and 2011/12 (dry autumn) were noted for respectively excellent and poor performance of pre-emergence herbicides in many fields throughout England.

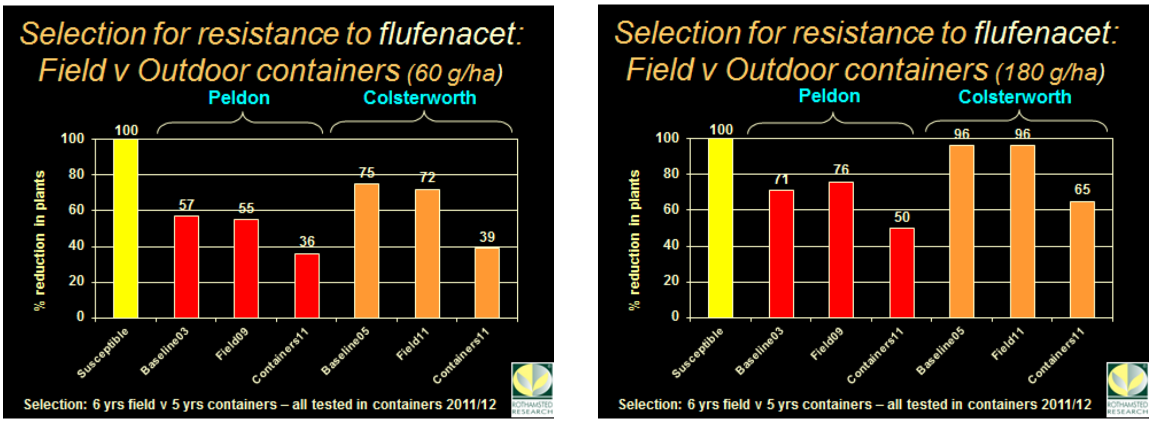
Figure 3. Loss of efficacy of flufenacet relative to the baseline population for two populations of black-grass treated annually with flufenacet in outdoor containers for 5 years (L.S.D. (*P≤0.05*) = 12.37 (Peldon) and 9.33 (Colsterworth)).



There was a progressive loss of efficacy during the five year selection process with both populations (Figure 3). These reductions were not generally statistically significant until after 3-4 years of selection.

The actual % reduction in plants for both populations treated with flufenacet in 2011/12, after selection for 5 years, and for the baseline and field samples collected from those fields after 6 years, are shown in Figure 4.

Figure 4. Efficacy of flufenacet at 60 and 180 g a.i./ha in containers using baseline (Peldon03 and Colsterworth 05), selected for 5 years (= Containers 11) and field populations (collected from the baseline fields after 6 years) (L.S.D. (*P≤0.05*) = 9.19 (Peldon) and 10.83 (Colsterworth)).



A susceptible standard (Roth05) was completely controlled by both 60 and 180 g a.i./ha showing that the conditions for flufenacet activity were very good (Figure 4). With both populations there was a reduction in flufenacet activity following selection in containers for 5 years. For Peldon and Colsterworth respectively, this was 21% and 36% at the 60 g a.i./ha test rate and 21% and 31% at the 180 g a.i./ha test rate (same as shown in Figure 3). However, control of both the field populations at both test rates was very similar (<5% difference) to the baseline populations, indicating no increase in resistance in the field. Note that the results show that partial resistance to flufenacet occurred, as control was poorer than the susceptible standard, but no evidence of an increase in resistance.

The fields at Peldon and Colsterworth received respectively 35 and 23 active ingredient applications in the 6 harvest years 2004-09 and 2006-11 respectively, an average of 5.8 or 3.8 / year. Winter wheat was sown every year at Peldon and in three years at Colsterworth in rotation with oil-seed rape. The herbicides used comprised seven (Peldon) or nine (Colsterworth) different modes of action including flufenacet (oxyacetamide), which was applied each year at Peldon and in three years at Colsterworth. Other MOA were: dinitroanilines, thiocarbamates, phenylureas, inhibitors of pigment synthesis, ACCase and ALS inhibitors, carbamates and benzamides). So, despite this much greater use of herbicides in the field, there was less selection for resistance to flufenacet than in outdoor containers, where flufenacet alone was used for 5 years. This apparent contradiction indicates that the use of multiple modes of action has been effective at reducing the increase in resistance to flufenacet, despite the 4.6 – 7 seven-fold greater use of herbicides overall (23 or 35 a.i. in field v 5 a.i. (all flufenacet) in containers).

Glasshouse assay. A dose response assay was conducted to better quantify the differences in response to flufenacet recorded in containers. Populations comprised samples collected from containers after two and four years selection, samples after two years selection in Petri-dishes (see section 4.2 below) and baseline and susceptible standards (see Table 12). Ten pre-germinated seeds were sown in each 9 cm pot and flufenacet applied at eight doses from 3.75 to 480 g a.i./ha pre-emergence 24 hours later. There were 5 replicates and untreated pots were included for each population. Pots were kept in an unheated glasshouse to better mimic outdoor conditions and foliage fresh weight per pot was assessed 39 – 40 days after spraying as a measure of herbicide efficacy (Table 12).

Table 12. Glasshouse dose response analysis for black-grass populations selected with flufenacet in outdoor containers and in Petri-dishes

Population		$logED_{50}$	**ED_{50} g/ha**	RI	Population		$logED_{50}$	**ED_{50} g/ha**	RI
Roth. 05	Susc	0.316	**2.1**	-	Roth. 05	Susc	0.316	**2.1**	-
Peldon03	B/L	1.207	**16.1**	**1.0**	Colstw.05	B/L	0.886	**7.7**	**1.0**
Peldon conts.	2 yrs	1.442	**27.7**	**1.7**	Colstw conts.	2 yrs	1.106	**12.8**	**1.7**
Peldon conts.	4yrs	1.749	**56.1**	**3.5**	Colstw conts.	4 yrs	1.710	**51.3**	**6.7**
Peldon petris	2 yrs	1.287	**19.4**	**1.2**	Colstw. petris	2 yrs	1.311	**20.5**	**2.7**
L.S.D. (*P≤0.05*)		0.252					0.334		

Note: B/L = baseline. ED_{50} = estimated dose required to reduced foliage fresh weight by 50%
RI = Resistance Index, ratio of ED_{50} values relative to the ***baseline*** populations

The Roth05 susceptible standard was quite well controlled (70%) by even the lowest rate used (3.75 g a.i./ha) and the ED_{50} value of 2.1 is less than 1% of the field rate (240 g a.i./ha). Both the Peldon and Colsterworth baseline populations were significantly more resistant to flufenacet than the Roth05 susceptible standards, with RI (resistance indices) ***relative to Roth05*** of 7.8 and 3.7 respectively. After 4 years selection in containers, both the Peldon and Colsterworth populations were significantly more resistant to flufenacet than the baseline populations, with RI values of 3.5 and 6.7 respectively, ***relative to the baselines*** (Table 12)***.*** Thus, compared with Colsterworth, Peldon had a higher initial level of resistance but after four years selection, the ED_{50} values were similar (56.1 v 51.3). The same trend was evident in the container experiments described in the previous section. Selection for two years in Petri-dishes gave given broadly similar results to selection in containers for two years, with selection in dishes being slightly greater for Colsterworth and slightly less for Peldon.

Meaned over the three doses, 30, 60 & 120 g a.i./ha, the % reductions in foliage weight were 78% and 87% for the baselines, and 52% and 55% after four years selection for the Peldon and Colsterworth populations respectively, representing a mean loss in flufenacet efficacy of 7.3% per year. This is similar to the mean annual 5.5% decline per year recorded in the outdoor container experiments (see Figure 4, meaned over both populations and doses). Consequently, the glasshouse dose response data complements the container experiment results indicating that selection with flufenacet can result in a reduction in efficacy of 5 – 7% per year.

The container and associated glasshouse assay demonstrate that both baseline populations show clear evidence of resistance to flufenacet in comparison with a susceptible standard. There was a progressive further loss of flufenacet efficacy in both populations during the five year selection process in outdoor containers. These reductions were not generally statistically significant until after 3-4 years of selection. The relatively slow rate of development of resistance, the big year to year variability in herbicide efficacy due to soil moisture differences and availability in the UK of flufenacet only in mixtures, means that it will be very hard to detect resistance to flufenacet in the field. Hence good diagnostic assays for detecting resistance in seed samples and well characterised reference populations will be essential for monitoring the impact of resistance on flufenacet longer term.

Surprisingly, samples taken from the baseline fields six years later showed no evidence of change in resistance status during that period, despite annual use of flufenacet and many other herbicides. This has important implications for resistance management although caution is needed in drawing broader conclusions. Resampling additional fields, and testing baseline and recent samples for response to flufenacet, would help to fully validate the robustness of this finding.

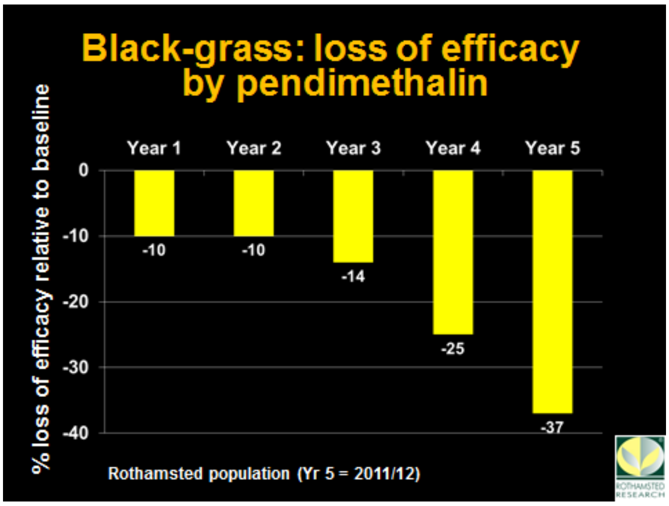
- **These experiments showed that resistance to flufenacet occurs and can increase following annual treatment.**
- **Annual increases in resistance, most likely due to enhanced metabolism, were relatively small and resulted in losses of efficacy of 5 – 7% per year.**
- **Under field conditions, increases in resistance to flufenacet are likely to be difficult to detect due to the confounding effects of other factors affecting activity.**
- **Two fields, each resampled after six years, showed no evidence of any increase in resistance to flufenacet despite heavy annual use of this and other herbicides. This important finding requires validating.**

Pendimethalin

Outdoor container experiment. A susceptible population of black-grass from Broadbalk field (Rothamsted05), never previously treated with herbicide, was used. The initial population comprised 200 plants (total of 3 reps). Pendimethalin at 900 g a.i./ha was applied pre-emergence in each of five years and surviving plants assessed and containers then isolated to prevent cross-pollination prior to seed collection. The differences in % reduction in plant numbers between the baseline population and the selected populations in the container experiment are shown in Figure 5. It is important to note that the actual % reduction figures for the baseline seeds varied considerably between years from 62% (2010/11) to 88% (2009/10), despite the same baseline seed sample being used each year.

There was a progressive reduction in pendimethalin activity following selection in containers for 5 years, averaging about 7% per year. This reduction was statistically significant after 3 years of selection (Figure 5).

Figure 5. Loss of efficacy of pendimethalin relative to the baseline population of black-grass treated annually with this herbicide outdoor containers for 5 years (LSD = 13.6).



Petri-dish assay. This was conducted to help validate the container results. Seed samples of the Rothamsted 05 baseline and the five year selected material were germinated in the presence of pendimethalin at 0, 1, 5 and 25 ppm. The % reduction in number of seeds with shoots > 1cm relative to control dishes (water only) after 14 days was determined. The values for the baseline and 5 year selected material were respectively: 1 ppm 0%, - 6%; 5 ppm 88%, 25%; 25 ppm 99%, 69% (LSD = 16.3). The results at both 5 and 25 ppm showed significantly poorer activity of pendimethalin on the selected material supporting the container results.

These results showed that, even a small black-grass population of only 200 plants, derived from a population never previously treated with herbicide, had the capacity to evolve resistance. The resistance mechanism is almost certainly enhanced metabolism, as this has been demonstrated previously (James *et al*., 1995). The implication is that selection has operated on genetic processes present in susceptible plants, and not by selection of rare resistant individuals, which is the explanation usually given for the evolution of resistance. This helps explain how readily and how widely resistance to pendimethalin, and in all probability some other herbicides, can evolve.

It should not be assumed that this process will apply to all weed species or to all herbicides. However, grass-weeds such as black-grass and rye-grass appear particularly vulnerable to enhanced metabolism compared with broad-leaved weeds, and enhanced metabolic resistance has been demonstrated to herbicides with a wide range of modes of action. Herbicides are likely to vary in the ease to which they can be metabolised, and consequently the impact on herbicide efficacy is likely to be unpredictable. A key question is whether the degree of resistance conferred by enhanced metabolism continues to increases, or reaches a plateau level where useful, if reduced, levels of control can be expected to be maintained long-term.

- **These experiments showed that resistance to pendimethalin can develop in populations never previously treated with herbicides.**
- **Increases in resistance, most likely due to enhanced metabolism, resulted in losses of efficacy of about 7% per year, very similar to that recorded with flufenacet.**
- **Under field conditions, increases in resistance to pendimethalin are likely to be difficult to detect due to the confounding effects of other factors affecting activity.**

Prosulfocarb

Outdoor container experiment. A susceptible population of black-grass from Broadbalk field (Rothamsted05), and two other field populations (LongC08 & Peldon03) were used. Prosulfocarb at 3000 g a.i./ha was applied pre-emergence in each of three years and surviving plants assessed and containers then isolated to prevent cross-pollination prior to seed collection.

In the first year, the % reductions in plant numbers were: Rothamsted05 96%; LongC08 62%; Pelon03 30%. This showed that LongC08 was partially, and Peldon03 highly resistant to prosulfocarb. Poor control of the Peldon03 population in the second year indicated that there was little potential for further selection for resistance so it was omitted. A container evaluation of the other two baseline populations and three year selected material was conducted in which two doses of prosulfocarb were applied and surviving plants assessed after 8 weeks (Table 13).

Table 13. Evaluation of two black-grass populations selected with prosulfocarb in containers for three years

		1500 g a.i./ha		3000 g a.i./ha	
Population		% reduction	% loss in efficacy	% reduction	% loss in efficacy
Rothamsted05	Baseline	87	-	93	-
Rothamsted selected	3 yrs	67	20	82	11
LongC08	Baseline	30	-	45	-
LongC selected	3 yrs	29	1	30	15
L.S.D. *P≤0.05*		15.1		15.1	

There was a modest reduction in prosulfocarb efficacy following selection in containers for three years, averaging about 4% per year at the 3000 g a.i./ha rate, which is somewhat lower than that recorded with flufenacet and pendimethalin above. However, most of the reductions in efficacy recorded were not statistically significant. However, selection with prosulfocarb was conducted for only three years, in contrast to the five years with flufenacet and pendimethalin. With both flufenacet and pendimethalin statistically significant reductions only became evident after 3 – 4 years of selection, so prosulfocarb appears to be behaving in a similar fashion.

- **These experiments showed that resistance to prosulfocarb occurs and can increase following annual treatment.**
- **Increases in resistance, most likely due to enhanced metabolism, resulted in losses of efficacy of about 4% per year, slightly less than that recorded with flufenacet and pendimethalin.**
- **Under field conditions, increases in resistance to prosulfocarb, as with flufenacet and pendimethalin, are likely to be difficult to detect due to the confounding effects of other factors affecting activity.**
- **Good diagnostic assays for detecting resistance in seed samples and well characterised reference populations will be essential for monitoring the impact of resistance on flufenacet, pendimethalin and prosulfocarb in the longer term.**

4.2 Impact of enhanced metabolic resistance on black-grass in nutrient culture

The same baseline populations (Peldon03 and Colsterworth05) as used in 4.1 above were used with the aim of achieving a more precise assessment of the potential for black-grass plants to evolve resistance to flufenacet. 500 pre-germinated seeds per population were placed in Petri-dishes containing 10 ppm flufenacet in a 2 g/L KNO_3 solution used to promote seedling growth. The 30 – 40 seedlings per population with the greatest visible shoot growth after 11 days were transferred to 5 cm pots of compost and the most vigorous 25 plants (= 5% of original seed used) subsequently transferred to 25 cm pots (6 or 7 plants per pot), isolated to prevent cross-pollination, and seeds collected. A second cycle of selection was conducted using the same method on seeds produced from the first cycle of selection, but only the best 15 plants (= 3% of original seed used) were grown on for seed production.

These two approaches for selection of resistance to flufenacet (4.1 in containers & 4.2 in Petri-dishes) complement each other, as the first is more ‘realistic’ while the second is more ‘scientific’. The expectation was that selection for resistance would be more rapid in Petri-dishes as all seeds were exposed to the same herbicide concentration, whereas exposure would be expected to be more variable in containers given that seeds were distributed throughout the top 2 cm soil.

After two cycles of selection, seeds were tested in a glasshouse dose response assay with flufenacet alongside seeds collected after two and four year’s selection in containers. These results are presented in Table 12 above. Selection for two years in Petri-dishes gave given broadly similar results to selection in containers for two years, with selection in dishes being slightly greater for Colsterworth and slightly less for Peldon. Thus, rather surprisingly, there was little evidence that selection in Petri-dishes was more rapid than in the outdoor containers. This conclusion was supported by results from a container experiment in which the response of seeds collected after two cycles of selection in Petri-dishes was compared with seeds after four years of selection in containers. The % reductions in number of surviving plants averaged over three rates of flufenacet (60, 120 & 180 g a.i./ha), were 8% (Peldon) and 12% (Colsterworth) lower for the four year container compared with the two year Petri-dish selected material.

- **Selection for resistance to flufenacet was demonstrated using a Petri-dish assay technique, but the results were broadly similar to those in a container assay.**

Objective 5. To conduct Knowledge Transfer (KT) initiatives to inform PSD, suppliers and users of herbicides of the future risks posed by herbicide-resistance and to promote appropriate resistance prevention and management strategies and more rational herbicide use.

A key requirement for minimising problems associated with herbicide resistance is to persuade pesticide users to take action proactively (i.e. before major resistance problems develop) rather than reactively (i.e. after major resistance problems have evolved). This is recognised as a considerable challenge worldwide, partly because herbicides are seen as an 'easier' option than cultural control measures and partly because of misplaced expectations that new herbicides will provide a solution. In a situation of increasing resistance and absence of new herbicide modes of action in the UK, KT initiatives were given a high priority during the course of this project.

5.1 'Active Herbicide Resistance Management (AHRM) – a knowledge transfer initiative

The key elements highlighted in KT initiatives were:

- The threat posed by herbicide resistance, not only in black-grass but with other grass and broad-leaved weeds too, and the on-going loss of alternative herbicidal solutions.
- The importance of early detection of resistance and how this is best accomplished.
- The high resistance risk posed by ACCase and ALS inhibiting herbicides.
- The value and limitations of alternative, lower resistance risk herbicides.
- The essential role of cropping and cultural alternatives to herbicides.
- The importance of monitoring to assess the effectiveness of any resistance management strategy.

A considerable amount of information has been generated on these elements in previous projects. In addition, outputs from the four previous objectives of this project, and additional information from other current projects on cultural control, were incorporated into the KT package.

The following **131** knowledge transfer initiatives were conducted during the duration of this project:

- Contributed to **46** articles in the popular farming press (2009 – 13; 2010 – 12; 2011 – 21). These included 'Farmers Weekly', 'Crops', 'Farmers Guardian' and 'CPM magazine'.
- Made **64** presentations at meetings (2009 – 19; 2010 – 23; 2011 – 19; 2012 – 3) comprising:
    - **9** conference presentations (BCPC, Glasgow; AAB, Cambridge; Ghent, Belgium; Babolsar, Iran; Christchurch, New Zealand; Miami, USA)
    - **16** technical presentations to independent agronomists (e.g. AICC) and those working for agrochemical companies and distributors.
    - **18** talks to farmers and managers (including HGCA roadshows and Cereals events)
    - **5** training presentations to students
    - **14** discussion meetings relating to research and management of resistance both in UK and internationally (e.g. CRD, WRAG, EWRS Resistance Working Group, BCPC)
    - **2** specific press briefings (in addition to press attendance at many of the above)
- Wrote, or contributed to, **21** formal publications (see references in next section).

Two of the most significant farmer orientated publications were:

1. 'Managing weeds in arable rotations – a guide.' *HGCA Technical publication.* 24pp. This included updated Weed Resistance Action Group (WRAG) Guidelines on preventing and managing herbicide resistance. 15,000 copies were produced and distributed.

2. 'Black-grass (*Alopecurus myosuroides*): Everything you really wanted to know about black-grass but didn't know who to ask.' *Rothamsted technical publication.* 4 pp. This was a summary of over 30 years research on black-grass aimed at farmers and advisors. 28,000 copies were produced and distributed via Crops magazine (14,000 copies, funded by Syngenta) and directly by agrochemical companies, distributors and advisory organisations.

Implications of research

The results show that, with black-grass, resistance to a range of different herbicides is increasing, despite the use of many different modes of action (MOA). The findings support the view that ALS and ACCase inhibiting herbicides pose a bigger resistance risk than most other MOA. Resistance to the ALS inhibitor, mesosulfuron-methyl+iodosulfuron-methyl, introduced into the UK in autumn 2003, is now widespread. Resistance is due to multiple resistance mechanisms, including both target site resistance (TSR) (two different mutations) and enhanced metabolism. In addition, 'double' target site resistance, ALS and ACCase TSR within the same individual plant occurs, and can be expected to increase. A major finding was that ALS enhanced metabolism can impact on herbicide efficacy almost as much as ALS target site resistance, and can increase rapidly. The high resistance risk associated with ALS inhibiting herbicides, combined with the widespread occurrence of resistance to ACCase inhibitors, means greater reliance will be placed on herbicides with other MOA, especially pre-emergence herbicides. Resistance to all three pre-emergence herbicides investigated, flufenacet, pendimethalin and prosulfocarb, occurs, and can increase with continued use. However, the rate of increase in resistance was modest, even where each herbicide was used annually. The lack of further increase in resistance to flufenacet in field samples, despite heavy use of a range of herbicides, was a finding with important implications. This supports the view that use of many different MOA can slow up the development of resistance in herbicides that are commonly used as alternatives to ALS and ACCase inhibitors. This important result requires validation on additional samples. With a lack of new MOA, it is clear that widespread and increasing resistance means that reliance on herbicides is unlikely to give sustainable control of black-grass. One major issue, as demonstrated by these research results, is that resistance rarely results in complete herbicide failure and can be difficult to detect in the field. Consequently farmers compensate for declining herbicide performance by the use of more herbicides (e.g. pre-emergence 'stacking'). It is vital that non-chemical control measures are actively promoted and used in order to reduce this reliance on herbicides.

References to published material

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

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