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Evidence Project Final Report

Department for Environment Food & Rural Affairs

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Project identification

1. Defra Project code: PS2721

2. Project title:
   Combating herbicide resistance by developing and promoting more sustainable grass-weed control strategies

3. Contractor organisation(s):
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4. Total Defra project costs (agreed fixed price): £ 524,518

5. Project: start date ..... 01 April 2012
   end date ..... 31 March 2015

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Executive Summary

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

The main objective was to gain a better understanding of the factors influencing the evolution of herbicide resistance so that more integrated weed management strategies could be developed and promoted.

Evolved herbicide resistance is a major problem worldwide, affecting control of 246 weed species in 66 countries. In the UK, and in the rest of western Europe, black-grass (*Alopecurus myosuroides*) is the major resistant weed of arable crops so this was the focus for most studies in this project.

There were four specific objectives and the main research findings are presented under each one below:

Specific objective 1. To quantify the relationship between dose rate ('low' v 'high') and evolution of herbicide resistance using flufenacet (an oxyacetamide), pendimethalin (a dinitroaniline) and mesosulfuron+iodosulfuron (mixture of two sulfonylureas) as model herbicides.

Research studies elsewhere suggest that reduced rates of herbicides may hasten the evolution of enhanced metabolic resistance. Our research, conducted in outdoor containers, found:

- Clear evidence of selection for resistance to flufenacet, but no significant effect of dose over 4 years.
- Clear evidence of selection for resistance to pendimethalin with the higher dose selecting more than the lower dose.
- Clear evidence for selection for resistance to mesosulfuron+iodosulfuron with the higher doses selecting more than lower dose.
- Whenever statistically significant differences were found, higher doses had always selected more than lower doses. There was no evidence in any of these studies that lower doses were selecting for resistance more than higher doses.

Several criticisms might be levelled at these studies: 1.The populations were already partially resistant to the herbicides; 2. The population sizes in containers were insufficient and that different results might be obtained with higher infestations in the field; 3. The levels of control were unrealistic; 4. The same population size was used every year despite differences in selection. None of these criticisms stand up to detailed scrutiny, except possibly the last one. We would not claim that these studies are totally comprehensive, but we do consider them very relevant to the debate about dose and herbicide resistance.

We are convinced that the dose rate debate in relation to herbicide resistance in black-grass in the UK is a distraction, with no evidence that reduced rates encourage resistance to herbicides used within crops – in fact all the evidence from our research points to the opposite view. Some herbicides pose a higher risk than others and over-reliance on such herbicides is the critical factor and much more important than dose

rate in determining resistance risk. There may be specific cases where low rates encourage resistance (e.g. glyphosate), but it is the generalisation that is dangerous, as agrochemical companies are keen to promote this concept yet appear to have little or no information to support this from their own research.

Specific objective 2. To determine whether non-target site resistance (enhanced metabolism) reaches a stable, peak level at which adequate, if reduced but sustainable, levels of weed control are achieved.

Selection for resistance usually results in a progressive decrease in herbicide efficacy. With non-target site resistance such as enhanced metabolism, it is possible that a resistance 'plateau' is reached, where no further decline in efficacy occurs and continued use results in adequate, if reduced, levels of weed control in the long-term. We investigated this concept by imposing very high selection regimes on black-grass populations with flufenacet and mesosulfuron+iodosulfuron. There was clear evidence that selection for enhanced metabolic resistance to mesosulfuron+iodosulfuron can occur more rapidly, and have a much bigger impact on efficacy than is the case with flufenacet. Although it is not possible to say whether a 'resistance plateau' was reached with either herbicide, it was clear that resistance to mesosulfuron+iodosulfuron can confer totally inadequate levels of control, regardless of mechanism.

A related study showed that selection for non-target site resistance (enhanced metabolism) to mesosulfuron+iodosulfuron conferred cross-resistance to other ALS herbicides but not flufenacet, which has a different mode of action. The ALS herbicides studied belonged to four different ALS sub-groups – mesosulfuron+iodosulfuron and flupyrsulfuron (sulfonylureas), pyroxsulam (triazolopyrimidines), imazamox (imidazolinones), propoxycarbazone (sulfonyl aminocarbonyl triazolinones).

The lack of cross-resistance to flufenacet implies that mesosulfuron+iodosulfuron was not selecting for a broad-spectrum mechanism, but to something more specific to ALS inhibitors. If such ALS resistance was herbicide specific then, in theory, changing to a different ALS herbicide could have potential benefits. The results of this study do not support this theory, and indicate that enhanced metabolic resistance is compromising the efficacy of other ALS inhibiting herbicides too, even if they have never been used. From a practical perspective, while the very intensive use of mesosulfuron+iodosulfuron during the last 10 years has resulted in widespread resistance, this does not appear to be compromising the efficacy of flufenacet, now the mainstay of pre-emergence herbicide programmes. This *may* also use be true for other pre-emergence herbicides, but requires verification. In this study, selection for resistance was intensive, but not totally unrealistic, so we have no reason to think that what we have recorded is not relevant in the field, although the selection process is likely to take longer.

Specific objective 3. To maintain a 'watching brief' for potential new cases or types of herbicide resistance in weeds of the arable, horticultural, industrial and amenity sectors.

An update on cases of herbicide resistance in the UK was carried out and published in the Aspects of Applied Biology **127** in 2014 (Hull *et al*., 2014). The main points can be summarised:

- Black-grass is the major herbicide-resistant weed problem and, by 2013, occurred on virtually all of the estimated 20,000 farms in 35 counties where herbicides are applied regularly for its control. Resistance to mesosulfuron + iodosulfuron, first used in the UK in autumn 2003, has now been detected in black-grass on >700 farms in 27 counties in England. Resistance is conferred by both ALS target site (Pro-197 & Trp-574 mutations) and non-target site mechanisms.
- Resistant Italian rye-grass (*Lolium multiflorum*) occurs on >475 farms in 33 counties. The first cases of ALS target site resistance in UK populations of Italian rye-grass were detected in 2012.
- Resistant wild-oats (*Avena* spp.) were confirmed on >250 farms in 28 counties of England. One population of wild-oats highly resistant to both mesosulfuron+iodosulfuron and pinoxaden was found.
- ALS-resistant common chickweed (*Stellaria media*) was found on >50 farms in 13 counties in England, Scotland and Northern Ireland and ALS-resistant common poppy (*Papaver rhoeas*) on >40 farms in nine counties of England.
- ALS-resistant scentless mayweed (*Tripleurospermum inodorum*) was found on five farms in three counties (Yorkshire, Norfolk and Angus). These included the first recorded case in Scotland where the ALS mutation responsible (Pro-197-Gln) was determined, making this the first UK population of mayweed to have ALS target site resistance confirmed.

Two populations of sterile brome (*Bromus sterilis*) showing partial resistance to glyphosate were identified. The degree of insensitivity was modest, with resistance indices of only approx. 2.0 compared with a susceptible standard. However, the fact that this originated as a farmer complaint and samples collected from nearby areas never treated with glyphosate remained fully susceptible, supports this interpretation.

Specific objective 4. To conduct Knowledge Transfer (KT) initiatives to inform CRD, suppliers and users of herbicides of the risks posed by herbicide-resistance and to promote more rational pesticide use through Integrated Weed Management (IWM).

- Key messages highlighted in KT initiatives were the increasing threat posed by herbicide resistance, the absence of new herbicide modes of action, the importance of early detection in new areas and quantifying the control from non-chemical methods, which can reduce the reliance on herbicides.

- **153** KT initiatives were undertaken comprising **81** articles in the popular farming press, **51** presentations to farmers, agronomists and industry technical personnel, **21** formal publications in scientific journals, conferences, reports and technical information sheets.

Implications of this research

Black-grass is a resistance-prone species, but herbicide dose appears to have little relevance to the rate of development of resistance. Some herbicides are also more resistance prone than others – resistance to ALS herbicides, such as mesosulfuron+iodosulfuron, was shown to evolve quickly, even in populations never previously treated. Black-grass selected with these sulfonylurea herbicides also showed cross-resistance (by enhanced metabolism) to three other ALS sub-groups (triazolopyrimidines, imidazolinones, sulfonylamino carbonyl triazolinones) – a particularly significant finding. Similar results were obtained with ACCase inhibitors in past projects. The implication is that resistance to these post-emergence herbicide classes will increase and control failures are likely to occur widely. ACCase inhibitors are now not commonly used for black-grass control, largely as a consequence of resistance, and ALS herbicides are likely to suffer the same fate. What is harder to predict is the timescale for their demise in the field.

In contrast, the implications of studies with flufenacet, which are probably also applicable to other pre-emergence herbicides, were more encouraging. While it was possible to select for resistance to flufenacet, resistance was partial and tended to increase slowly, even under very intensive selection. Significantly, there was no evidence that enhanced metabolic resistance selected by mesosulfuron+iodosulfuron had any adverse impact on flufenacet activity. The implication is that the efficacy of pre-emergence herbicides, such as flufenacet, is likely to be more durable, so maintaining the availability of these will be critical for effective black-grass management longer-term. In addition, it is vital that farmers place less reliance on herbicides and make greater use of non-chemical control methods. The extensive KT initiatives have made farmers much more receptive to these messages than in the past.

Further work required on herbicide resistance

Suggestions are: 1. Monitor the efficacy of pre-emergence herbicides in order to determine the impact of resistance longer-term. 2. Utilise material generated within this and previous projects in more fundamental studies into the mechanisms of non-target site resistance. 3. Investigate the ongoing threat posed by resistance in other grass-weeds, such as rye-grass and wild-oats. 4. Evaluate and quantify the threat posed by glyphosate resistance in the UK – especially in black-grass. 5. Refine non-chemical control methods to improve their efficacy and reliability at an individual field scale.

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).

PS2721: Combating herbicide resistance by developing and promoting more sustainable grass-weed control strategies

Background

Evolved herbicide resistance is a major problem worldwide, affecting control of 246 weed species in 66 countries (Heap, 2015). In the UK, and in the rest of western Europe, black-grass (*Alopecurus myosuroides*) is the major resistant weed of arable crops so this was the focus for most studies in this project, although some work was done on other weeds too. 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.

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.

Scientific objectives

The overall objective was to gain a better understanding of the factors influencing the evolution of herbicide resistance so that more integrated weed management strategies could be developed and promoted.

There were four more specific objectives:

1. To quantify the relationship between dose rate and evolution of herbicide resistance using flufenacet and mesosulfuron+iodosulfuron as model herbicides.
2. To determine whether non-target site resistance (enhanced metabolism) reaches a stable, peak level at which adequate, if reduced but sustainable, levels of weed control are achieved.
3. To maintain a 'watching brief' for potential new cases or types of herbicide resistance in weeds of the arable, horticultural, industrial and amenity sectors.
4. To conduct Knowledge Transfer (KT) initiatives to inform CRD, suppliers and users of herbicides of the risks posed by herbicide-resistance and to promote more rational pesticide use through Integrated Weed Management (IWM).

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 relationship between dose rate and evolution of herbicide resistance using flufenacet, pendimethalin and mesosulfuron+ iodosulfuron as model herbicides.

The aim was to investigate the impact of herbicide dose ('low' v 'high') on herbicide resistance development using two contrasting herbicides which are of major importance in control of black-grass; flufenacet (an oxyacetamide, HRAC class K3) and mesosulfuron+iodosulfuron (mixture of two sulfonylureas, HRAC class B)).

Research studies elsewhere in the world suggest that reduced rates of herbicides may hasten the evolution of enhanced metabolic resistance by allowing more partially resistant individuals to survive, cross with other individuals with partial resistance, producing progeny with higher resistance as a consequence of stacking of minor genes (Renton *et al*., 2014). Consequently, there can be a progressive increase in the overall level of resistance over a period of years. However, this concept is controversial and others consider this a dangerous generalisation, which has been used by companies to discourage growers from using lower than recommended rates. Reduced doses are more consistent with an EU policy aimed at reducing pesticide usage and there have been both reductions (e.g. pendimethalin, metazachlor) and increases (flupyrsulfuron, flufenacet (split application) in the maximum approved rates in the UK. Consequently studies were done to determine the relationship between herbicide dose and rate of development of resistance as this has a direct impact on the longevity and efficacy of herbicides.

1.1: Does dose rate of flufenacet affect the rate of evolution of resistance?

Outdoor container experiments. Two populations of black-grass (Peldon 2003 and Colsterworth 2005) with partial resistance conferred by enhanced metabolism were used. Containers (29x19x13 cm) were filled with a Kettering loam soil (4% O.M.), seeds (0.5g/container) sown in the surface 2 cm of soil in late September and treated pre-emergence with flufenacet at 60 g/ha (= 'low rate') and 180 g/ha (= 'high rate') 6 – 7 days later. Untreated containers were included along with a susceptible reference population (Rothamsted) and there were three replicates. Surviving plant numbers were assessed 2 – 3 months later as a measure of herbicide efficacy (Table 1). Treated containers were isolated in glasshouses in spring to prevent cross-pollination and seeds collected in summer. These were subsequently re-sown into new containers in the following autumn and the procedure repeated for four years. An average of 120 plants was present in each untreated container.

Table 1. Control of black-grass by flufenacet in outdoor containers treated with two rates of flufenacet over a four year period

		% reduction in number of black-grass plants relative to untreated			
		Year 1	Year 2	Year 3	Year 4
Population	Flufenacet g/ha	2010/11	2011/12	2012/13	2013/14
Rothamsted	60	100	97	98	-
	180	100	100	100	-
Peldon	60	81	54	69	38
	180	95	72	83	75
Colsterworth	60	95	56	67	41
	180	99	83	92	60

The results confirm that both the Peldon and Colsterworth baseline populations used in year 1 had partial resistance to flufenacet as levels of control were slightly lower than for the Rothamsted susceptible standard. Over the four years, the control at the higher rate of 180 g flufenacet/ha was consistently greater than at the lower rate of 60 g flufenacet/ha, as would be expected. With Peldon, the mean control over the four years was 81% at the higher and 61% at the lower rate, a 20% differential. The corresponding values for Colsterworth were 84% and 65%, a 19% differential. This meant that, on average, just over twice as many plants survived at the lower compared with the higher dose. Control was poorer in Year 4 compared with Year 1 with both populations but there was not a progressive decline in efficacy with control in Year 3 being better than Year 2. Efficacy of flufenacet is affected by environmental conditions, and year to year variation should be expected. The assessments show that neither excessively high, or excessively low levels of control occurred, and that a good differential between higher and lower doses was achieved consistently.

Glasshouse assay. Glasshouse dose response assays were conducted to quantify any differences in response to flufenacet as a consequence of selection at different dose rates. One assay was conducted after two years of selection, but as the differences were small (resistance indices relative to baseline populations <2) the experiment was continued for an additional two years and another glasshouse assay conducted. Baseline and 4-year selected populations were used together with a susceptible standard (Rothamsted). Ten pre-germinated seeds were sown in each 9 cm pot and flufenacet applied at eight doses from 7.5 to 960 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 48 – 49 days after spraying as a measure of herbicide efficacy (Table 2).

Table 2. Glasshouse dose response analysis for black-grass populations selected with low (60 g/ha) and high (180 g/ha) doses of flufenacet for four years in outdoor containers

Population	Log_{10} ED_{50}	**ED_{50} g/ha**	RI	Population	Log_{10} ED_{50}	**ED_{50} g/ha**	RI
Rothamsted	0.602	**4.0**	-	Rothamsted	0.602	**4.0**	-
Peldon baseline	1.515	**32.7**	**1.0**	Colsterworth baseline	1.272	**18.7**	**1.0**
Peldon 4 years @ 60 g/ha	1.853	**71.3**	**2.18** (1.12)	Colsterworth 4 years @ 60 g/ha.	1.697	**49.8**	**2.64** (1.76)
Peldon 4 years @ 180 g/ha	1.771	**59.0**	**1.80** (1.53)	Colsterworth 4 years @ 180 g/ha	1.890	**77.6**	**4.15** (1.68)
L.S.D. (*P≤0.05*)	0.191				0.288		

ED_{50} = estimated dose required to reduced foliage fresh weight by 50% relative to untreated

RI = Resistance Index, ratio of ED_{50} values relative to the ***baseline*** populations

Figures in brackets are from the assay done after 2 years selection. Full data not presented.

The Rothamsted susceptible standard was well controlled with an ED_{50} value of **4.00** (1.7% field rate). Both the Peldon and Colsterworth baseline populations were significantly more resistant to flufenacet than the Rothamsted susceptible standard, with resistance indices ***relative to the susceptible Rothamsted*** of 8.2 and 4.7 respectively. This confirms a modest level of resistance in both baseline populations.

After four years of selection in containers at 60 (low dose) and 180 (high dose) g flufenacet/ha, the ED_{50} values were respectively **71.3** and **59.0** g/ha for the Peldon populations and **49.8** and **77.6** g/ha for the Colsterworth population. With both populations, these values were significantly higher than the baselines (**32.7** Peldon; **18.7** Colsterworth), which was not the case after only two years selection. This continued, low level, selection for resistance at both doses is also evident from the resistance indices after two years (in brackets in table) which were always lower than those recorded after four years selection. However, with both Peldon and Colsterworth, there was no significant difference between the ED_{50} values obtained for the two dose rates used for selection. Although there were no significant differences between the two selecting doses, lower dose selection conferred slightly greater resistance in Peldon whereas higher dose selection conferred slightly greater resistance in Colsterworth. Meaned over both populations, and relative to the baselines, resistance indices were **2.41 for low dose selection and 2.98 at higher rate selection**.

The results indicate that Peldon was more resistant than Colsterworth initially, but that subsequent selection in Colsterworth was slightly faster. Thus, whereas the Peldon baseline ED_{50} was 1.7 x the Colsterworth value, the mean values for the two levels of selection for four years were very similar for the two populations (65.1 v 63.7). This supports previous studies.

- **These results confirm that resistance to flufenacet can increase following annual treatment.**
- **These results did not support the view that low doses of flufenacet increase selection for resistance. Dose rate had no significant impact on the degree of resistance observed after four years of selection.**
- **The results support other work showing that it takes three to four years of selection with flufenacet to detect a statistically significant increase in resistance**.
- **Flufenacet can be considered a 'low resistance risk' herbicide, but is clearly not a 'no resistance risk' herbicide.**

1.2: Variability in control by flufenacet using black-grass samples from random surveys.

Flufenacet was the most widely used herbicide for control of black-grass in 2012, being applied to over 1.8 million ha of arable land (Pesticide Usage Survey, 2014). Selection experiments conducted in containers as part of previous projects (PS2714: Developing and promoting more sustainable grass-weed control strategies to combat herbicide resistance), and those described in section 1.1 above, show that selection for resistance to flufenacet tends to be slow. However, much less information is available from true field samples. The black-grass samples collected in 2009-2011 from random farms in England and used in project PS2714 represent a good resource for further evaluation of variability in efficacy of flufenacet.

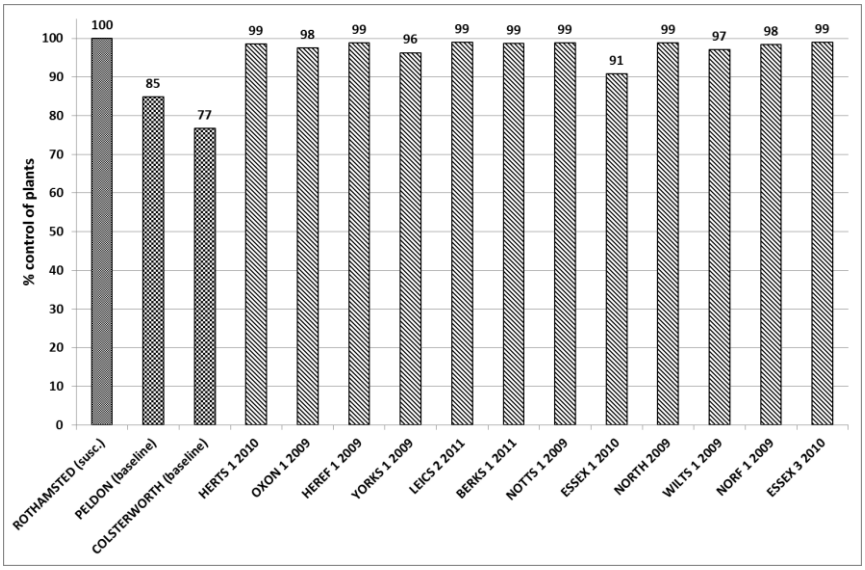
Outdoor container experiment. Seventeen black-grass populations were used: 12 of the black-grass samples collected from random farms; a susceptible standard (Rothamsted); Peldon 2003 and Colsterworth 2005 baseline populations (as used in 1.1 above); and Peldon 2014 and Colsterworth 2014 populations which had been selected for 8 years with 180 g flufenacet/ha in containers.

The same procedure as described in section 1.1 above was used, with flufenacet at 60 g and 180 g/ha applied pre-emergence on 7 October 2014, 7 days after sowing. Surviving plants were assessed on 16 December 2014, with an average of 110 plants in each untreated container. Conditions were very favourable for herbicide efficacy in autumn 2014 and, at 180 g flufenacet/ha, almost complete (>98.3%) control was achieved of all populations except the 8 year selected Peldon (90.3%) and Colsterworth (91.8%) populations. At the 60 g flufenacet/ha dose, control of these two populations was much poorer (56.6% and 60.6% respectively). The reductions in plant numbers of the other populations, including the Peldon and Colsterworth baselines, relative to untreated containers, are shown in Figure 1.

Complete control of the Rothamsted susceptible standard was achieved, even at 60 g flufenacet/ha which is only 25% of the field recommended rate, highlighting the favourable conditions for herbicide activity. Good control (91 – 99%) of all 12 random populations was also achieved. The lowest control (91%) was with Essex 1, a field near Colchester. The control of both the Peldon 2003 (85%) and Colsterworth 2005 (77%) baseline populations was significantly poorer. These were collected 10 – 12 years ago and the fact that all the more recently (2009 – 2011) collected random populations were better controlled indicates that resistance to flufenacet has not developed widely, despite its extensive use. The results highlight that the Peldon and Colsterworth populations are somewhat atypical in their degree of insensitivity to flufenacet.

- **These results indicate that resistance to flufenacet is not widespread, although conditions in autumn 2014 were favourable for its activity which could have masked marginal differences.**
- **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.**
- **While the Peldon and Colsterworth populations appear slightly atypical in their degree of insensitivity to flufenacet, they represent good reference populations for any future work on evolution of resistance to flufenacet.**

Figure 1. Control of 12 random and three other black-grass populations treated with 60 g flufenacet/ha in outdoor containers (L.S.D. (*P≤0.05*) = 2.86**).**



1.3: Does dose rate of pendimethalin affect the rate of evolution of resistance?

Pendimethalin is a widely used dinitroaniline herbicide (Class K1) with a different mode of action to flufenacet. We initiated (in autumn 2012) container experiments looking at the effect of dose rate of pendimethalin, on the degree of selection for resistance. The baseline population used, which has no previous treatment with any herbicide, is a particularly interesting population in relation to potential future molecular studies on enhanced metabolism resistance mechanisms.

Outdoor container experiments. Black-grass seeds collected in 2005 from Section 8 of Broadbalk field at Rothamsted were used in a container experiment set up using the same procedure as described in section 1.1 above. Pendimethalin was applied at 900 g/ha ('low dose') and 2000 g/ha ('high dose') pre-emergence for two successive years, with seeds being collected from surviving plants each summer after isolation in separate glasshouse to prevent cross-pollination. Pendimethalin was applied pre-emergence in early October, 5 – 7 days after sowing. An average of 111 plants established in each untreated container in the first year and a rather lower number (58), due to poorer seed quality, in the second year. Surviving plants were assessed on 7 February 2013 (year 1) and 9 December (Year 2).

The effect of dose rate on selection was evaluated in a container experiment in autumn 2014 in which the original baseline population and those selected for two years at 900 and 2000 g pendimethalin/ha were included. Note that all populations were treated with both rates of herbicide in this evaluation, not just the dose used for selection. Pendimethalin was applied pre-emergence on 7 October 2014, 7 days after sowing, and surviving plants assessed on 16 December 2014.

In year 1, 900 and 2000 g pendimethalin achieved respectively 91.9% and 98.2% reduction in black-grass plants, relative to the untreated. This meant that, on average, 9 and 2 plants per container survived treatment with the lower and higher rate respectively, or 4.5 as many at the lower dose. In the second year, 86% reduction occurred at 900 g pendimethalin/ha but poorer control, only 57%, at the higher rate of 2000 g pendimethalin/ha. This apparently anomalous result was actually a consequence of greater selection for resistance at the higher rate.

There were large differences between populations in the number of plants establishing in untreated containers so the % reduction values relative to the untreated values for the same population are more meaningful (Table 3).

Table 3. Evaluation of black-grass populations selected with low (900 g/ha) and high (2000 g/ha) doses of pendimethalin for two years in outdoor containers.

	Container treatment		
Population	**Pendimethalin**	**Surviving plants per container**	**% reduction relative to untreated**
Broadbalk 2005 (= baseline)	Untreated	**91**	**-**
	900 g/ha	**11**	**88**
	2000 g/ha	**5**	**94**
Selected for 2 years @ 900 g pendimethalin/ha (= 'low' dose)	Untreated	**50**	**-**
	900 g/ha	**9**	**82**
	2000 g/ha	**6**	**89**
Selected for 2 years @ 2000 g pendimethalin/ha (= 'high' dose	Untreated	**57**	**-**
	900 g/ha	**37**	**35**
	2000 g/ha	**32**	**43**
L.S.D. (*P≤0.05*)		**13.2**	**9.7**

Control in the population selected at the higher dose was significantly poorer, by 46 – 47%, than that at the lower dose. The control achieved at the lower dose of 900 g pendimethalin/ha, was not significantly different to the baseline population. This is consistent with previous studies (See project PS2714) with selection with pendimethalin where it has taken at least three years of selection at this rate to achieve a statistically significant effect, A factorial analysis of the selected populations showed that there was a highly significant (*P≤ 0.05*) effect of dose on selection for resistance.

- **These experiments confirm that resistance to pendimethalin, almost certainly due to enhanced metabolism, can evolve in a population never previously treated with herbicide.**
- **The relatively small starting population, of only 333 plants (across the 3 reps), was no limitation to the evolution of resistance, which demonstrates just how common is the genetic capacity to evolve resistance.**
- **The higher dose (2000 g pendimethalin/ha) selected for resistance to a much greater degree than the lower dose (900 g/ha).**
- **These results did not support the view that low doses of pendimethalin increase selection for resistance – in fact quite the opposite.**

1.4: Does dose rate of mesosulfuron+iodosulfuron affect the rate of evolution of resistance?

Outdoor container experiments. Three populations of black-grass which did not have ALS target site resistance were used. These were: Main 1999 (a field sample from Woburn); Suffolk 2002 (a field sample from a random survey); and Highfield 2007 (a sample from a field trial sown with Main 1999 seed and sprayed for four successive years with mesosulfuron+iodosulfuron). Neither Main 1999 nor Suffolk 2002 had been treated previously with mesosulfuron+iodosulfuron. Container experiments were set up in late September 2011 using the same procedure as described in section 1.1 above and four rates of mesosulfuron+iodosulfuron (3+0.6; 6+1.2; 12+2.4; 24.48 g a.i./ha + Biopower adjuvant@0.5%) were applied post emergence at the 2-3 leaf stage in early November. Plant numbers were assessed prior to spraying and survivors were assessed in January 2012 so that degree of control could be calculated for each individual container (Table 4). Those treated containers which appeared to be of most interest were isolated in glasshouses in spring to prevent cross-pollination and seeds collected in summer. These were subsequently re-sown into new containers in the following autumn and the procedure repeated for a second year (2012/13) with final plant assessments made in March 2013. Seeds collected in summer 2013 were used in a glasshouse dose response assay to quantify changes in response to mesosulfuron+iodosulfuron.

Table 4. Control of black-grass by mesosulfuron+iodosulfuron in outdoor containers treated with different rates of mesosulfuron+iodosulfuron over a two year period

		Year 1	Year 2	
		2011/12	2012/13	
Population	Meso.+Iodo. g/ha	% reduction in number of black-grass plants	% reduction in number of black-grass plants	Loss of efficacy Yr2 – Yr1
Main 1999	6+1.2	85	61	24
	12+2.4	99	58	41
Suffolk 2002	6+1.20	78	46	32
	12+2.4	96	62	34
Highfield 2007	6+1.20	56	37	19
	12+2.4	71	41	30
	24+4.8	87	49	38

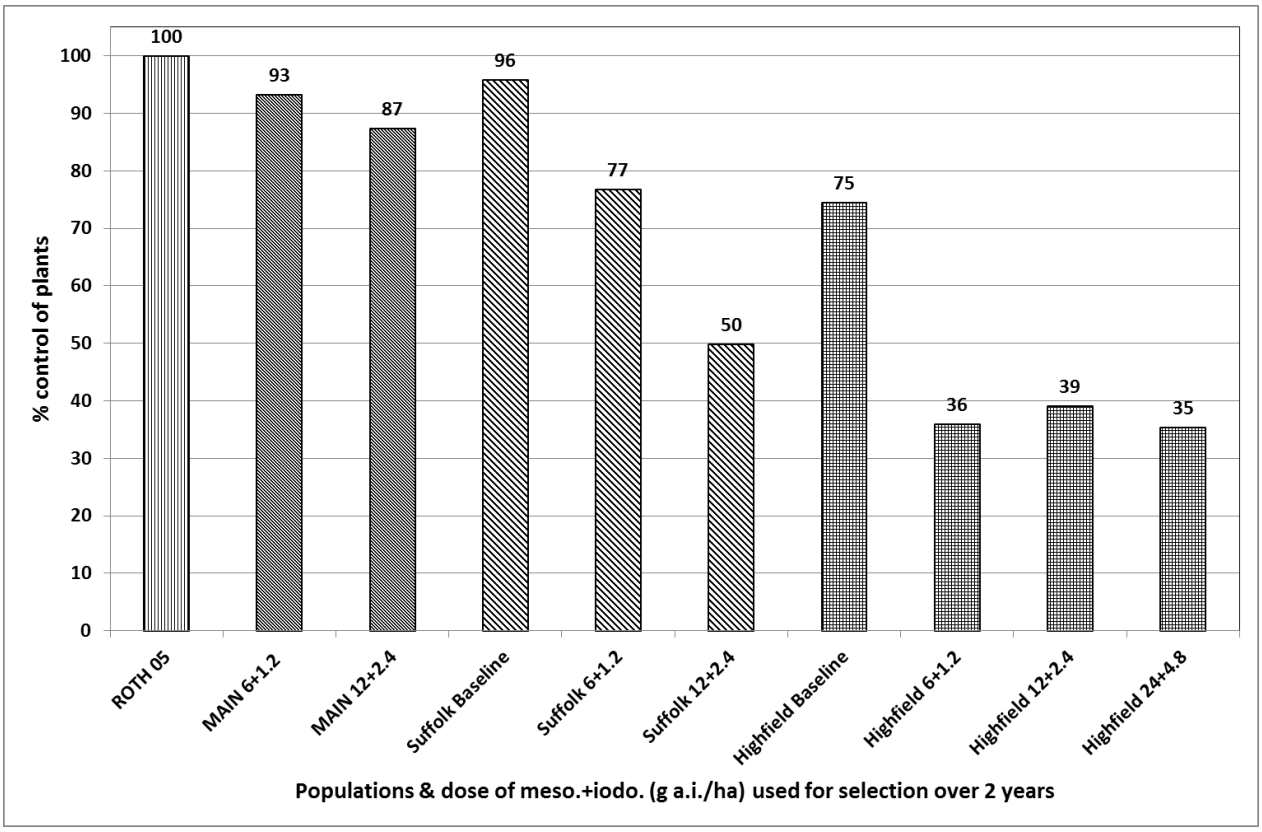
The mean number of black-grass plants established per container pre-spraying varied somewhat being, for year 1 and 2 respectively: Main 1999 - 112, 116; Suffolk 2002 – 106, 139; Highfield 2007 – 67, 99. Such differences are not unexpected so the % reduction values, relative to the numbers pre-spraying for the same container, are more meaningful.

A good differential was achieved in terms of herbicide efficacy at the different doses. In Year 1, with both Main 1999 and Suffolk 2002, the field rate of mesosulfuron+iodosulfuron (12+2.4 g a.i./ha) gave good control (96 – 99%) while the half rate gave lower, but still reasonable, control (78 – 85%) (Table x). The Highfield 2007 population, which had already been treated with mesosulfuron+iodosulfuron for four years, was controlled less well by the field rate (71%) – poorer control than the half rate on the other two populations. The half rate gave mediocre control (56%) of Highfield 2007 and even the double rate (24+4.8 g a.i./ha) achieved less than 90% control (87%).

In Year 2, control was consistently poorer by an average of 31%. Averaged over doses, this loss of efficacy was similar for all three populations – 33% for Main 1999, 29% for Highfield 2007 and 33% for Suffolk 2002. The losses tended to greater at the higher herbicide rates. This might be associated with increasing resistance but could also be due to poorer environmental conditions for herbicide activity although all plants of a susceptible reference population (Rothamsted) were killed by the field rate. The subsequent container and glasshouse dose response evaluation assays helped to clarify this issue.

Outdoor container evaluation assay. A container experiment was set up on 30 September 2013 to quantify the response to mesosulfuron+iodosulfuron in the three populations after selection at different doses for two years. Baseline populations (except Main 1999 as insufficient seeds) and a susceptible standard reference population (Rothamsted) were included. The same procedure as described in section 1.1 above was used and two test rates of mesosulfuron+iodosulfuron (12+2.4 & 24+4.8 g a.i./ha + Biopower adjuvant@0.5%) were applied post emergence at the 2-3 leaf stage on 6 November 2013. Plant numbers were assessed prior to spraying and survivors on 10 February 2014. Foliage fresh weight per container was determined on 12 March 2014 and the degree of control calculated relative to untreated containers for the same population (Table x). An average 50 plants (range 34 – 71) established and mean foliage fresh weights was 55.37 g (range 41.42 – 77.09g) each untreated container. Results based on both assessment methods were similar and there was no population x test dose interaction. Consequently, only results based on plant numbers meaned over both test doses are presented (Figure 2).

Figure 2. Container study: % reduction in number of black-grass plants averaged over two test doses of mesosulfuron+iodosulfuron (12+2.4 & 24+4.8 g/ha) after two years selection with different doses rates in outdoor containers (L.S.D. (*P≤0.05*) = 9.21)**.**



Complete control of the Rothamsted susceptible standard was achieved, but none of the baseline populations was completely controlled, indicating a degree of resistance despite, in the case of Suffolk 2002, never having been previously treated with mesosulfuron+iodosulfuron. With both the Main and Suffolk populations, selection with higher rates of mesosulfuron+iodosulfuron was associated with decreasing control in this test assay, indicating higher degrees of resistance. With Highfield, all three rates used during the selection process resulted in significantly poorer control in this test assay relative to the baseline, but there was no difference between the three rates.

A more detailed factorial analysis was conducted using data for just the 6+1.2 and 12+2.4 selection doses and omitting the data for the Rothamsted susceptible standard and the baselines, which was absent for Main anyway. There was a significant effect of selection dose (*P≤0.001*), population (*P≤0.001*), and test dose (*P≤0.028)* but the only interaction that was significant (*P≤0.001*) was for selection dose x population (Table 5).

Table 5. Container study: % reduction in number of black-grass plants averaged over two test doses of mesosulfuron+iodosulfuron (12+2.4 & 24+4.8 g/ha) after two years selection with different doses rates in outdoor containers.

	Population			
Selection dose g a.i./ha	Main	Suffolk	Highfield	mean
6+1.2	93.2	76.8	35.9	68.6
12+2.4	87.4	49.8	39.1	58.8
	n.s.	sig	n.s.	sig
	L.S.D. (*P≤0.05*) = 6.71			L.S.D. (*P≤0.05*) = 3.87

- **With both the Suffolk and Highfield populations there was clear evidence of greater resistance to mesosulfuron+iodosulfuron after only two years of selection. Significantly, the Suffolk population had never previously been treated with this herbicide.**
- **The relatively small starting population of Suffolk 2002, of only 318 plants (across the 3 reps), was no limitation to the evolution of enhanced metabolic resistance, which demonstrates just how common is the genetic capacity to evolve resistance to mesosulfuron+iodosulfuron.**
- **The higher dose (12+2.4 g a.i./ha), which is the field recommended rate, selected for resistance at either a significantly greater (Suffolk 2002), a higher, but not significantly greater, (Main) or similar degree (Highfield) to the half rate (6+1.2 g a.i./ha).**
- **These results did not support the view that low doses of mesosulfuron+iodosulfuron increase selection for resistance – in fact quite the opposite.**

Glasshouse evaluation assay. A glasshouse dose response assay was conducted to quantify any differences in response to mesosulfuron+iodosulfuron as a consequence of selection at different dose rates. The same baseline (but including Main) and 2-year selected populations as used in the container evaluation assay, together with a susceptible standard (Rothamsted), were included. Six plants per 9 cm pot were established and mesosulfuron+iodosulfuron applied at eight doses in the range 0.188+0.038 to 48+9.6 g a.i./ha (+ Biopower @0.5%) at the three leaf stage. There were five replicates and 10 untreated pots for each population. Foliage fresh weight per pot was assessed 28 days after spraying as a measure of herbicide efficacy (Table 6).

Table 6. Glasshouse dose response analysis for black-grass populations selected with lower (6+1.2 g/ha) and higher (12+2.4 or 24+4.8 g/ha) doses of mesosulfuron+iodosulfuron for two years in outdoor containers.

Population	*Log_{10} ED_{50}	*ED_{50} meso.+iodo. g/ha	De-transformed Resistance index (relative to baseline)
ROTH05 susceptible	1.1162	0.392+0.078	-
Main99 Baseline	1.5935	1.177+0.235	**1.0**
Main 2 yr selection @ 6+1.2 g/ha	1.7288	1.607+0.321	**1.4**
Main 2 yr selection @ 12+2.4 g/ha	1.8586	2.166+0.433	**1.8**
L.S.D. (*P≤0.05*)	0.2388		
Suffolk02 Baseline	1.9136	2.459+0.492	**1.0**
Suffolk 2yr selection @ 6+1.2 g/ha	2.5712	11.177+2.235	**4.6**
Suffolk 2yr selection @ 12+2.4 g/ha	3.0256	31.823+6.365	**12.9**
L.S.D. (P≤0.05)	0.2374		
Highfield07 Baseline	2.3079	6.096+1.219	**1.0**
Highfield 2 yr selection @ 6+1.2 g/ha	3.0361	32.598+6.520	**5.4**
Highfield 2 yr selection @ 12+2,4 g/ha	3.0932	37.179+7.436	**6.1**
Highfield 2 yr selection @ 24+4.8 g/ha	3.0219	31.549+6.310	**5.2**
L.S.D. (P≤0.05)	0.2694		

* For convenience in the MLP statistical analysis, rates of the commercial product ('Atlantis') were used, where 400 g product/ha (field rate) = 12+2.4 g mesosulfuron+iodosulfuron. The detransformed ED_{50} values have been re-converted to g mesosulfuron+iodosulfuron/ha.

ED_{50} = estimated dose required to reduced foliage fresh weight by 50% relative to untreated.

Resistance Index = ratio of ED_{50} values relative to the ***baseline*** populations.

For each of the seven selected populations, eight leaves from survivors of mesosulfuron+iodosulfuron at 12+2.4 g/ha were sent to PlantaLyt in Germany on 3 January 2014 for molecular testing to confirm absence of any ALS target site mutations. No 197, 574, 122 or 376 ALS mutations were detected. Samples of all three baseline populations have also been tested by Bayer in 2010, 2011 & 2012 and no ALS target site mutations found. However, elevated levels of mesosulfuron metabolism were identified relative to the susceptible standard, Rothamsted, which supports the view that enhanced metabolism, and not ALS target site resistance, is the primary mechanism of resistance in all three populations.

The mean foliage weights for untreated pots 28 days after spraying were similar for all populations, being in the range 5.558 – 6.824 g/pot. The resistance indices for the three baseline populations, ***relative to the Rothamsted susceptible*** **standard**, were 3.0 (Main99), 6.27 (Suffolk02) and 15.6 for Highfield 07. This shows that all three populations had some partial resistance to mesosulfuron+iodosulfuron, despite the first two of these never having been previously treated with this herbicide. This was almost certainly due to the selection for broad spectrum enhanced metabolic resistance as a consequence of the previous exposure to other herbicide modes of action.

The ED_{50} value for the Main population selected at the higher dose of mesosulfuron+iodosulfuron (12+2.4 g/ha) for two successive years was significantly higher than the Main baseline value, whereas that for the lower dose selection (6+1.2 g/ha) was not. Thus there had been some selection for resistance to mesosulfuron+iodosulfuron after only two years of selection, although this was modest (RI values1.4 – 1.8 relative to baselines), and some evidence of greater selection at the higher dose.

With the Suffolk population there was substantial, and significant, selection for resistance to mesosulfuron+iodosulfuron with two years of selection at both doses, and greater selection at the higher (12+2.4 g/ha) dose (RI = 12.9 relative to baseline) than at the lower (6+1.2 g/ha) dose (RI = 4.6). This difference between doses was statistically significant.

The Highfield baseline population had a higher degree of resistance to mesosulfuron+iodosulfuron than the other two baseline populations because, unlike them, it had been treated for four years previously with this herbicide in field plots. There was substantial, and significant, further selection for resistance with two years of selection at all three doses. However, there was no difference in degree of selection between the three doses used, with similar RIs relative to the baseline (5.2 – 6.1). Thus, the three doses appeared to have selected for resistance equally strongly.

- **The results from this glasshouse dose response experiment are entirely consistent with those from the outdoor container assay above (Figure x).**
- **The higher dose (12+2.4 g a.i./ha), which is the field recommended rate, selected for resistance at either a significantly greater (both Main and Suffolk) or a similar degree (Highfield) to the half rate (6+1.2 g a.i./ha).**
- **These results did not support the view that low doses of mesosulfuron+iodosulfuron increase selection for resistance – in fact, quite the opposite.**

Implications of this research

The results of the studies into the effect of dose rate on evolution of resistance can be summarised:

- Flufenacet – clear evidence of selection for resistance but no significant effect of dose over 4 years.
- Pendimethalin – clear evidence of selection for resistance and higher dose selecting more than lower dose.
- Mesosulfuron+iodosulfuron – clear evidence of selection for resistance with higher doses selecting more than lower dose.
- Whenever statistically significant differences were found, higher doses had always selected more than lower doses.
- There was no evidence that lower doses were selecting for resistance more than higher doses.

The following criticism might be levelled at these studies:

1. **The populations were already partially resistant to the herbicides which might have affected the results**. This was true for some, but not all populations. The Broadbalk population had never been treated with any herbicides, yet there was still more selection with higher rates. In addition, two of the mesosulfuron+iodosulfuron treated populations had never previously been treated with this herbicide, yet greater selection at higher rates occurred. Given that herbicide resistance occurs in virtually all sprayed black-grass populations in the UK, proponents of this view would have to accept that the 'low doses encourage resistance' argument would not be relevant in situations where a low level of resistance already exists, as with black-grass in the UK.
2. **The population sizes in containers were insufficient and that different results might be obtained in the field where much greater populations would occur.** This might have some validity but it is significant that it was possible to select for resistance in a population (Broadbalk) never previously treated with herbicide despite an initial population size of only 333 plants. There might be greater genetic diversity in a larger population but clearly there was sufficient within the populations and sizes studied to allow selection for resistance to all three herbicides.
3. **The levels of control were unrealistic.** An aspect too often missing from the 'dose debate' is what is meant by a 'low dose' in terms of efficacy relative to a 'high rate'. With 99% control only one plant survives in every 100, but at 90% control, a 9% drop, 10 survive – a 10 fold increase. In contrast, with 80% control, even a 20% drop in control to 60% means only a doubling of number of survivors from 20 to 40 in every 100 treated. This would be expected to have very substantial implications in relation to selection. This is clearly relevant in relation to the control achieved by a full dose, but this aspect is often ignored in the generalised view that reduced doses encourage resistance. In the studies reported here, levels of control appeared appropriate for the herbicide used, with a good differential between doses, and not unrealistically high, or unrealistically low control.
4. **The same population size was used every year despite differences in selection.** In the field, use of different doses is likely to result in varying levels of weed control which will result in different amounts of seed return with implications for the infestation size in subsequent years. In the container studies, the same amount of seed was sown each year regardless of the control achieved in the previous year. This is a legitimate concern, but it is difficult to fully understand its validity in relation to the results achieved especially as the potential for the evolution of resistance was evidently present in a relatively small population size. It might have been possible to adjust the amount of seeds sown according to the control achieved in the previous year, but this could have caused a confounding effect, especially if there was a need for substantial difference in the amount of seed sown, and consequently population size, between containers.

We would not claim that these studies are totally comprehensive, but we do consider them very relevant to the debate about dose and herbicide resistance. There may well be specific cases where it can be shown that low rates encourage resistance, but it is the generalisation that is dangerous, as agrochemical companies are keen to promote this concept, for obvious reasons. Despite this, companies have produced little, if any, scientific information to support this view themselves.

We are not aware of any evidence that indicates that use of low rates has been responsible for the current extent of resistance in grass-weeds in the UK, or elsewhere in Europe. However, it is possible that past, widespread use of ALS broad-leaved weed herbicides, which also give some control of susceptible grass-weeds (e.g. metsulfuron) have impacted on resistance in weeds such as black-grass and rye-grass. But even if this low level selection has happened, this is not a dose issue, but rather an efficacy issue – selection conferred by full rates of herbicide on weeds which are not very susceptible, or on the label.

In the UK, use of many different herbicide active ingredients is common practice on black-grass, such as use of flufenacet, diflufenican, pendimethalin, triallate, mesosulfuron+iodosulfuron on a single crop, often costing the value of 1 tonne of wheat or more (>£100). This is financially feasible when wheat crops yield 8 or more t/ha, as in UK, but totally unrealistic in parts of the world (e.g. USA, Australia) where 2 t/ha are more common. In such situations, use of reduced rates of a single active ingredient may well have implications in terms of herbicide efficacy and possibly also resistance.

A critical argument in the dose rate debate is that if a herbicide is not giving very good control, either because weeds are 'naturally' tolerant or a lower than recommended dose is used, many farmers will use an additional herbicide – often of a different mode of action. This is certainly true in the UK and this is generally considered a 'good' anti-resistance strategy. Use of flufenacet in the UK is a good example. This has relatively low efficacy on black-grass (<70% control) when used alone so is only used in mixtures. All the available evidence indicates that resistance increases only slowly. In research conducted for project PS2714, we found that while it was possible to select for resistance with annual applications of flufenacet in containers over a five year period, little selection was recorded in the field from where the original samples were collected (Peldon and Colsterworth). This was despite much

greater use of herbicides in the field over 6 years - 35 active ingredients at Peldon, including 6 flufenacet (7 different modes of action) and 23 active ingredients at Colsterworth, including 3 flufenacet (9 different modes of action). This lack of development of resistance seemed more to do with it being a 'low resistance risk' herbicide, and use in mixtures as a direct consequence of its low efficacy. In contrast, resistance to mesosulfuron+iodosulfuron has increased rapidly in the UK, which is nothing to do with use of low doses, but more to do with it being a 'high resistance risk' ALS herbicide. Previous projects have shown that mixing with other herbicides has had little effect at reducing resistance build-up.

We are convinced that the dose rate debate in relation to herbicide resistance in black-grass in the UK is a distraction from more important issues, with no evidence that reduced rates encourage resistance to herbicides used within crops – in fact all the evidence from our research points to the opposite view. The situation with glyphosate may well be different, and is certainly worthy of study. Some herbicides are higher risk than others, some weeds are higher risk than others, some cultural systems (e.g. over-reliance on herbicides) are higher risk than others and reduced doses result in poorer weed control. These are the critical factors and much more important than dose rate in determining resistance risk. **Dose rate is largely an irrelevance.**

Objective 2. To determine whether non-target site resistance (enhanced metabolism) reaches a stable, peak level at which adequate, if reduced but sustainable levels of weed control are achieved.

Selection for resistance usually results in a progressive decrease in herbicide efficacy. With target site resistance a point is often reached where continued use of the herbicide is pointless due to totally inadequate control. However, with non-target site resistance such as enhanced metabolism, it is possible that a resistance plateau is reached, where no further decline in efficacy occurs and continued use results in adequate, if reduced, levels of weed control in the long-term. There is circumstantial evidence that this applied to isoproturon which, despite being used for over 25 years often on an annual basis, still gave useful levels of control of black-grass on most fields in the UK despite clear evidence of resistance.

2.1: Does non-target site resistance to different herbicides reaches a stable, peak level?

Our approach was to impose, for two successive years, a very high selection ('super-selection') on populations with resistance to mesosulfuron+iodosulfuron and flufenacet already selected in previous studies (Project PS2714). The aim was to see to what degree resistance could be further increased under extreme conditions of selection.

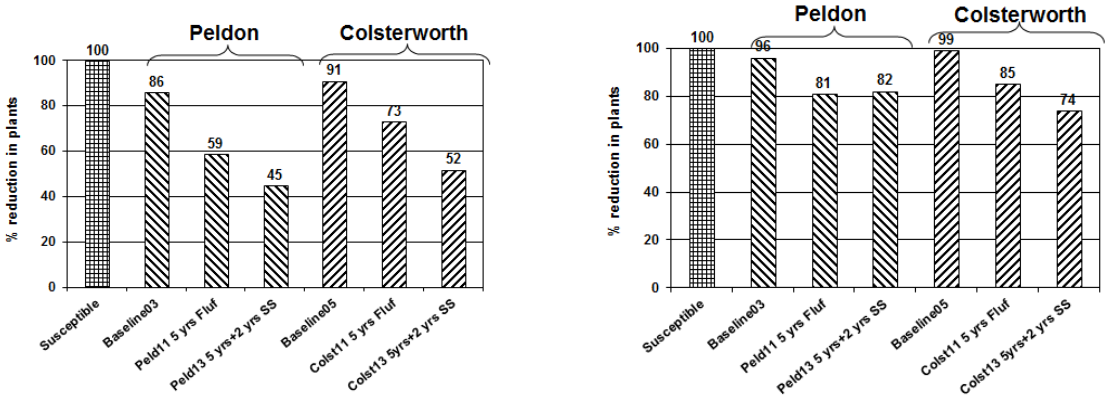
Flufenacet

Black-grass seeds of two populations already selected with flufenacet at 180 g/ha for five years were used, **Peldon 5yrs Fluf**. and **Colsterworth 5yrs Fluf** (both 2011). These had been produced from the original field collected baselines, Peldon 2003 and Colsterworth 2005. A container experiment was set up using the same procedure as described in section 1.1 above with flufenacet applied at 360 g/ha pre-emergence on 4 October 2011, six days after sowing. An average of 96 plants established in each untreated container and the healthiest 15.6% (Peldon) and 14.4% (Colsterworth) of treated plants were retained for seed production. In autumn 2012, these seeds were sown in new containers and treated with 480 g flufenacet/ha pre-emergence on 2 October 2012, six days after sowing. An average of 136 plants established in each untreated container and the healthiest 6.0% Peldon) and 7.8% (Colsterworth) of treated plants were retained for seed production. Hence selection had been at 1.5 x (Year 1) and 2x (Year 2) the field recommended rate (= 240 g/ha).

Container evaluation assay. This outdoor study was conducted in autumn 2013 and included the original field baseline (Peldon 2003 & Colsterworth 2005), the Peldon 5yrs Fluf. & Colsterworth 5yrs Fluf and the 'superselected' Peldon & Colsterworth populations. Flufenacet was applied at 180 & 480 g/ha pre-emergence on 8 October 2013, seven days after sowing, and surviving plants assessed on 28 January 2014. An average of 121 plants established in each untreated container.

Figure 3. Flufenacet 'super-selection' container study: % reduction in number of black-grass plants in baseline populations, those selected for five years with 180 g flufenacet/ha and 'super-selected'(SS) populations subjected to two additional years of selection (L.S.D. (*P≤0.05*): Peldon = 11.6; Colsterworth = 8.6)**.**

180 g flufenacet/ha **480 g flufenacet/ha**



The Rothamsted susceptible standard was completely controlled, in contrast to all the other populations, confirming some degree of resistance to flufenacet (Figure 3). The results for the five-year selected populations show clearly that it is possible to select for greater resistance to flufenacet. Relative to the baselines, the 27% and 18% declines in control for the Peldon and Colsterworth population treated with 180 g flufenacet/ha, an average loss of 4.5% per year, are similar to those recorded in assays conducted as part of project PS2714, where a 5.5% annual decline was recorded. The addition 'super-selection' over two years increased resistance and there was a further decline in efficacy of 14% and 21% for the two populations at the 180 g/ha test rate, or an average loss of efficacy of 8.75%.

Meaned over both test doses, the % reductions for the baseline, five-year selected and 'super-selected' populations were: Peldon 91%, 70%, 63% (L.S.D. (*P≤0.05*) 8.2); Colsterworth 95%, 79%, 63% (L.S.D. (*P≤0.05*) 6.1).

Thus there was some evidence that applying a higher degree of selection had increased the rate of selection and that a 'resistance plateau' had not yet been reached. At the 480 g/ha test rate, twice the recommended field rate, levels of control were higher, as expected, but there was still evidence of resistance relative to the baselines. However, the values for the 'super-selected' populations were either no different (Peldon) or only 11% lower (Colsterworth) than for the five-year selected material.

The results show clearly that resistance to flufenacet occurs and selection can increase the degree of resistance progressively. However, this increase is relatively small, and flufenacet still gave a useful, if reduced, level of control even after an enhanced degree of selection over two years high. It is not possible to say whether a 'resistance plateau' had been reached, even after a total of seven years selection, although this seems unlikely.

The results do support the view that flufenacet should have greater longevity than many other herbicides, in that resistance build-up is relatively slow. The methodology used here could be considered a 'worst case scenario' for several reasons. Firstly, only flufenacet was used and previous studies (project PS2714) have indicated that use of flufenacet in mixture and sequence with other herbicides reduces selection for resistance. Secondly, as containers were re-sown with each year, there was no buffering from older, less selected seeds, which would happen in true field conditions. Thirdly, the conditions for herbicide activity were good, with a uniform soil, seeds in a shallow (2 cm) surface layer, adequate soil moisture and uniform herbicide application (laboratory sprayer). All of these factors are likely to favour selection.

From a regulatory, end-user and agchem industry viewpoint, the 'longevity' (how many years it gives effective control in the field) of a herbicide is becoming increasingly important, especially in the absence of new modes of action. With black-grass this is particularly relevant, as there is overwhelming evidence for the rapid decline in efficacy of many herbicides, especially ACCase (e.g. fenoxaprop) and ALS (e.g. mesosulfuron+iodosulfuron) herbicides. With herbicides such as flufenacet, the longer-term situation is harder to predict, but we believe the methodology used in our studies has good potential for clarifying this aspect with many herbicides with similar characteristics (e.g. pre-emergence herbicides, modest and variable control, not prone to target site resistance).

Detecting resistance to flufenacet, and similar residual herbicides, in the field is very difficult, due to their

intrinsic variability in efficacy as a consequence of varying environmental factors, especially soil moisture. Resistance also tends to be partial, so detecting changes in resistance over a period of years within the background 'noise' imposed by differing environmental conditions is virtually impossible in the field. The container approach we have used, by standardising soil and environmental factors, permits more critical comparisons to be made in respect of response of different populations to herbicides. Consequently, this procedure should have value for monitoring resistance to such herbicides.

Mesosulfuron+iodosulfuron

Black-grass seeds of three populations already selected with mesosulfuron+iodosulfuron for five or six years were used: **Highfield 2009** (a sample from a field trial sown with Main99 Woburn seed and sprayed for four successive years in the field and then one year in containers with mesosulfuron+iodosulfuron @12+2.4 g/ha); **Highfield 2011** (a sampled derived from Highfield 2009 by spraying containers once with mesosulfuron+iodosulfuron @ 12+2.4 g/ha); **Highfield 2010 x4** (derived from the Highfield 2009 by spraying containers with mesosulfuron+iodosulfuron @24+4.8 g/ha twice – thus surviving plants had received four times the recommended field dose of this herbicide); Resistance in these populations was by enhanced metabolism, not ALS target site resistance. This was confirmed in biochemical and molecular assays – see below.

A container experiment was set up using the same procedure as described in section 1.1 above with mesosulfuron+iodosulfuron applied @24+4.8 g/ha post-emergence at the three-leaf stage on 3 November 2011. The adjuvant biopower @0.5% was used with all applications. The Highfield 2010 x4 population was re-sprayed with the same dose on 23 February 2012. An average of 73 plants established in each container and the healthiest 13.7% Highfield 2009, 19.1% Highfield 2010 x4 and 23.4% Highfield 2011 of treated plants were retained for seed production. In autumn 2012, these seeds were sown in new containers and treated with mesosulfuron+iodosulfuron @24+4.8 & 48+9.6 g/ha post-emergence at the two to three-leaf stage on 5 November 2012. An average of 106 plants established in each untreated container and the healthiest 20.4% Highfield 2009, 21.6% Highfield 2010 x4 and 24.3% Highfield 2011 of plants treated with ***the higher dose (48+9.6 g/ha) only*** were retained for seed production.

On 18 February 2013, 16 leaves from survivors of the Highfield '2009' and Highfield '2010 x4' populations treated with mesosulfuron+iodosulfuron at 24+4.8 g/ha in containers were tested by Bayer, Frankfurt for mesosulfuron metabolism. The higher dose (48+9.6 g/ha) survivors were retained for seed production. Note that by time of sampling, these populations (grown from 2012 seed) had been treated with mesosulfuron+iodosulfuron on seven and eight occasions respectively. Leaves from untreated plants of the Rothamsted 2005 susceptible standard were also tested. In both Highfield populations only 38 – 40% unmetabolised mesosulfuron was present after 16 hours compared with 86% still present in Rothamsted. This provided good evidence that enhanced metabolism, and not ALS target site resistance, is the primary mechanism of resistance in the Highfield populations (see confirmatory testing undertaken as part of the glasshouse evaluation assay below).

Container evaluation assay. This was conducted in autumn 2013 and included the Highfield 2009 population (5 years selection), the three populations from containers after two additional years of intense selection with mesosulfuron+iodosulfuron (total of 7 or 8 years selection), HOR08 (a reference population with ALS target site resistance, 574 mutation) and the Rothamsted 2005 susceptible standard.

An average of 70 plants established in each container. Mesosulfuron+iodosulfuron was applied at 12+2.4 (field rate) and 48+9.6 (4x field rate) g/ha post-emergence on 6 November 2013 at the 2 – 3 leaf stage, surviving plants were assessed on 9 January 2014 and foliage fresh weights per container determined on 21 February 2014.

Complete control of the Rothamsted standard was achieved demonstrating that it was fully susceptible and that spraying procedures were conducive to good control (Table 7). With black-grass plant numbers, only modest control (32 – 55%) of the Highfield 2009 ('baseline') population was achieved, even at the higher rate. In contrast almost no control of plants was achieved with all the other populations, despite using a dose four times that recommended in the field. Thus there was evidence of substantial further selection for resistance as a consequence of two further years of intense selection. However, it was evident that many plants were damaged, although not killed, and the results based on foliage weight reflect this.

On a foliage weight basis, again very good control of Rothamsted was evident, but some control (20 – 31%) of the HOR08 reference population was achieved, even though this is known to possess a high degree of ALS target site resistance in every plant (Marshall *et al*., 2013). A fair degree of control of the Highfield 2009 ('baseline') population was achieved, especially at the higher dose (81%). Control of all three populations receiving two additional years of intense selection was poorer, but better than on a plant basis.

Table 7. Mesosulfuron+iodosulfuron 'super-selection' study – container evaluation: % reduction in number of black-grass plants and foliage weight per container for reference populations and those 'super-selected' for two additional years.

Population 'Year' is for original sample, pre extra selection	Meso.+Iodo. g/ha	% reduction in number of black-grass plants	% reduction in foliage weights
Rothamsted (susc.)	12+2.4	100	98
	48+9.6	100	98
HOR08 (ALS TSR)	12+2.4	1.3	20
	48+9.6	2.6	31
Highfield 2009	12+2.4	31.8	58
5 yrs selection (='baseline')	48+9.6	55.1	81
Highfield '2009'	12+2.4	0	48
5+2 yrs extra selection	48+9.6	0	71
Highfield '2011'	12+2.4	4.8	45
6+2 yrs extra selection	48+9.6	0.5	62
Highfield '2010 x4'	12+2.4	0	15
6+2 yrs extra selection	48+9.6	1.4	25
L.S.D. (*P≤0.05*)		5.14	21.1

There was a direct correlation between intensity of selection and decreasing control. Meaned over both doses, % foliage fresh weight reductions were: Highfield 2009 5 yrs selection (='baseline') – **70%**; Highfield '2009' 5+2 yrs extra selection – **60%**; Highfield '2011' 6+2 yrs extra selection – **53%**; Highfield '2010 x4' 6+2 yrs extra selection – **20%** (L.S.D. (*P≤0.05*) = 14.9%). This is very compelling evidence for a progressive increase in enhanced metabolic resistance conferring ever greater reductions in herbicide efficacy over a period of a few years.

Glasshouse evaluation assay. A glasshouse dose response assay was conducted to better quantify the effect of intense selection with mesosulfuron+iodosulfuron on enhanced metabolic resistance. The same six populations as used in containers (see previous section) were used. Six plants per 9 cm pot were established and mesosulfuron+iodosulfuron applied at eight doses in the range 0.188+0.038 to 192+38.4 g a.i./ha (+ Biopower @0.5%) at the three leaf stage on 20 November 2013. There were five replicates and 10 untreated pots for each population. Foliage fresh weight per pot was assessed 26 days after spraying as a measure of herbicide efficacy (Table 8).

For each of the populations, eight leaves from survivors of mesosulfuron+iodosulfuron at 12+2.4 or 24+4.8 g/ha were sent to PlantaLyt in Germany for molecular testing to confirm the absence of any ALS target site mutations. No 197, 574, 122 or 376 ALS target site mutations were detected in any population except HOR08, in which the known presence of the 574 ALS mutation was confirmed.

The mean foliage weights for untreated pots 26 days after spraying were similar for all populations, being in the range 5.085 – 6.148 g/pot. The resistance indices, ***relative to the Rothamsted susceptible* standard,** were >196 for the HOR08 ALS TSR reference population, 13.2 for Highfield 2009 5 yrs selection **(='baseline')** and 42.4, 56.1 and 141.5 for the three selected Highfield populations (Table 8). This shows that all populations had high resistance to mesosulfuron+iodosulfuron.

As in the containers, there was a direct correlation between intensity of selection and increasing resistance (Table 8). Hence the resistance indices, relative to the baseline, increase progressively from 3.2 to 4.2 to 10.7 in the most resistant Highfield population. There was evidence that the HOR08 ALS target site resistant population was more resistant than the Highfield population with the highest level of enhanced metabolic resistance (Highfield '2010 x4' 6+2 yrs extra selection). This contrasts with the containers where there appeared to be little difference in degree of resistance. However, this appears to be largely of academic interest as the actual level of resistance were very high regardless of mechanism responsible – the ED_{50} value for the Highfield '2010 x4' 6+2 yrs extra selection population was over 11 x the field recommended rate of 12+2.4 g/ha. In addition, as in the containers, few plants of any of the three selected Highfield populations were killed by 48+9.6 g/ha, which is four times the recommended rate.

Table 8. Glasshouse dose response analysis for black-grass populations 'super selected' with mesosulfuron+iodosulfuron for two years in outdoor containers.

Population 'Year' is for original sample, pre extra selection	***Log_{10} ED_{50}**	***ED_{50} meso.+iodo. g/ha**	**De-transformed Resistance index (relative to baseline)**
Rothamsted 2005 susceptible	1.5134	0.979+0.196	**-**
HOR08 (ALS TSR) reference	>3.806	>192+38.4	**>14.8**
Highfield 2009 5 yrs selection (**='baseline'**)	2.6347	12.937+2.587	**1.0**
Highfield '2009' 5+2 yrs extra selection	3.1405	41.455+8.291	**3.2**
Highfield '2011' 6+2 yrs extra selection	3.2625	54.902+10.980	**4.2**
Highfield '2010 x4' 6+2 yrs extra selection	3.6644	138.513+27.703	**10.7**
L.S.D. (*P≤0.05*)	0.4527		

* For convenience in the MLP statistical analysis, rates of the commercial product ('Atlantis') were used, where 400 g product/ha (field rate) = 12+2.4 g mesosulfuron+iodosulfuron. The detransformed ED_{50} values have been re-converted to g mesosulfuron+iodosulfuron/ha.

ED_{50} = estimated dose required to reduced foliage fresh weight by 50% relative to untreated

Resistance Index = ratio of ED_{50} values relative to the ***baseline*** populations

Implications of this research

The biochemical and molecular assays confirmed the absence of ALS target site resistance in the Highfield populations and that enhanced metabolic resistance was responsible. The fact that this mechanism conferred degrees of resistance comparable with ALS target site resistance high resistance is an important finding. This is not the case with most other herbicides where enhanced metabolic resistance tends to confer only partial resistance which usually has less of an impact on efficacy than target site resistance. This implies that the underlying mechanism of enhanced metabolic resistance to mesosulfuron+iodosulfuron may be different to that affecting flufenacet. It is possible that it is the same, but that one herbicide molecule is simply more vulnerable to enhanced metabolism due to its different molecular structure. However, the results of the studies conducted in the next section do not support this idea and provide weight to the view that different mechanisms are responsible.

In these studies, there was clear evidence that selection for enhanced metabolic resistance to mesosulfuron+iodosulfuron can occur more rapidly, and have a bigger impact on efficacy, than is the case with flufenacet. Although it is not possible to say whether a 'resistance plateau' was reached with either herbicide, it was clear that resistance to mesosulfuron+iodosulfuron can confer levels of control that are totally inadequate, regardless of mechanism.

Although the experimental procedures used here are likely to have speeded up the process of selection compared to true field conditions, we have no reason to doubt that the same process will occur in the field, but probably over a slightly longer time frame. On the evidence of these studies, flufenacet, although a herbicide with lower intrinsic efficacy than mesosulfuron+iodosulfuron against black-grass, is likely to have much greater longevity.

2.2: Does non-target site resistance to mesosulfuron+ iodosulfuron confer cross-resistance to other ALS herbicides, or herbicides with other modes of action?

The selection experiments detailed above produced seed material that is highly resistant to mesosulfuron+iodosulfuron. Molecular and biochemical assays detailed above show that resistance is due to non-target site mechanisms, principally enhanced metabolism. Enhanced metabolism often confers cross-resistance to a wide range of other herbicides, but this is not invariably the case. Our selected material provides an ideal opportunity for evaluating cross-resistance to both other ALS inhibiting herbicides, and other modes of action. This will help answer the important question: to what degree has the very widespread use of mesosulfuron+iodosulfuron during the last 10 years compromised the activity of other ALS inhibiting herbicides and other modes of action?

Glasshouse evaluation assay. A glasshouse dose response assay was conducted using five populations of black-grass. See section 2.1 under mesosulfuron+iodosulfuron for details of the Highfield populations and section 1.1 for details of the Suffolk populations. **Rothamsted 2009** was a susceptible

standard; **Highfield 2009** had been selected for five years and **Highfield '2010 x4'** (seed actually collected in 2013) for eight years with mesosulfuron+iodosulfuron, including three years of intense selection with high rates; **Suffolk 2002** was a field collected sample and **Suffolk 2013** was produced from this after two years treatment with 12+2.4 g mesosulfuron+iodosulfuron/ha in containers. Pre-germinated seeds were sown and one plant was established in each 5 cm square pot. The following six herbicides were applied at eight doses within the ranges shown at the three leaf stage on 29 October 2014.

Active ingredient	Herbicide class	Lowest dose	Highest dose	Field rate	Adjuvant
Mesosulfuron +iodosulfuron	ALS–sulfonylureas	0.0938 +0.0188	96+19.2	12+2.4	Biopower@0.5%
Flupyrsulfuron	ALS–sulfonylurea	0.625	160	10	None
Pyroxsulam	ALS–triazolopyrimidine	0.146	150	18.75	Biosyl @1%
Propoxycarbazon -sodium	ALS–sulfonylamino -carbonyl-triazolinone	0.5466	560	70	Actirob @0.5%
Imazamox	ALS–imidazolinone	2.188	280	35	None
Flufenacet	Oxyacetamide	7.5	960	240	None
		g a.i./ha	g a.i./ha	g a.i./ha	

There were 14 replicates per dose and 16 untreated pots for each population. Foliage fresh weight per pot was assessed 28 days after spraying as a measure of herbicide efficacy. The mean foliage weights for untreated pots 28 days after spraying were similar for all populations, being in the range 2.392 – 2.598 g/pot. Summarised results for all herbicides are presented in Table 9 and full results for three of the herbicides in Table 10.

Following selection with mesosulfuron+iodosulfuron, both populations showed a large and significant increase in resistance to this herbicide, with resistance indices of 9.7 – 14.1 (Tables 9 & 10). These are similar to those recorded in previous experiments, 10.7 to 12.9 (see Tables 6 & 8 above). The substantial selection for resistance in the Suffolk population after only two years selection is especially noteworthy, as the baseline population had never previously been treated with this herbicide, unlike the Highfield baseline.

Both mesosulfuron+iodosulfuron selected populations also showed substantial, and significant, increases in resistance to **all** the other ALS inhibiting herbicides tested (Tables 9 & 10): flupyrsulfuron (RIs = 4.0 – 10.4); pyroxsulam (RIs = 5.5 – 33.3); propoxycarbazone (RIs = 2.5 – 10.8); imazamox (RIs = 2.2 – 8.1). Neither selected population had ever been treated with any of these herbicides, so clearly selection with mesosulfuron+iodosulfuron had conferred cross-resistance, almost certainly by enhanced metabolism, to these other ALS herbicides. The degree of resistance to mesosulfuron+iodosulfuron and other ALS herbicides in the Suffolk 2002 baseline population was lower than in Highfield 2009 baseline,. This explains why the increases in resistance tended to be greater in the Suffolk population – there was more potential for increase in this population.

In marked contrast, these same mesosulfuron+iodosulfuron selected populations showed **no** evidence of any increase in resistance to the non-ALS herbicide flufenacet, with RIs of 1.0 and 0.8 (Tables 9 & 10). Consequently selection for higher levels of non-target site resistance to mesosulfuron+iodosulfuron was not associated with an increase in resistance to flufenacet, a herbicide with a different mode of action.

Table 9. Resistance indices, relative to baseline populations, for six herbicides evaluated in a cross-resistance study with two populations selected with mesosulfuron+iodosulfuron.

	Herbicide					
Selected population	**Meso+iodo**	**Flupyr -sulfuron**	**Pyroxsulam**	**Propoxy -carbazone**	**Imazamox**	**Flufenacet**
Suffolk 2yr selected	**14.1**	**10.4**	**33.3**	**10.8**	**8.1**	**1.0**
Highfield 8yr selected	**9.7**	**4.0**	**5.5**	**>2.5**	**2.2**	**0.8**

Resistance Index = ratio of ED_{50} values relative to the ***baseline*** populations

Table 10. Glasshouse dose response analysis for cross-resistance study with populations selected with mesosulfuron+iodosulfuron. (data for only three of six herbicides used presented here)

Population 'Year' is for original sample, pre extra selection	***Log_{10} ED_{50}**	***ED_{50} meso.+iodo. g/ha**	**De-transformed Resistance index (relative to baseline)**
Mesosulfuron+iodosulfuron			
Rothamsted 2009 susceptible	0.3200	0.063+0.013	**-**
Suffolk 2002 (**baseline**)	1.1466	0.421+0.084	**1.0**
Suffolk 2013 2yrs selection	2.2957	5.928+1.186	**14.1**
Highfield 2009 5 yrs selection (**baseline**)	2.8101	19.377+3.875	**1.0**
Highfield '2010 x4' 8 yrs selection	3.7961	187.596+37.519	**9.7**
L.S.D. (*P≤0.05*)	0.7082		
Pyroxsulam			
Rothamsted 2009 susceptible	n.d.	<0.146	-
Suffolk 2002 (**baseline**)	-0.4494	0.355	**1.0**
Suffolk 2013 2yrs selection	1.0742	11.86	**33.3**
Highfield 2009 5 yrs selection (**baseline**)	1.6607	45.79	**1.0**
Highfield '2010 x4' 8 yrs selection	2.3977	249.9	**5.5**
L.S.D. (*P≤0.05*)	0.4206		
Flufenacet			
Rothamsted 2009 susceptible	1.2415	17.4	
Suffolk 2002 (**baseline**)	2.5989	397.1	**1.0**
Suffolk 2013 2yrs selection	2.6134	410.6	**1.0**
Highfield 2009 5 yrs selection (**baseline**)	2.4412	276.2	**1.0**
Highfield '2010 x4' 8 yrs selection	2.3646	231.6	**0.8**
L.S.D. (*P≤0.05*)	0.2556		

* For convenience in the MLP statistical analysis, rates of the commercial product containing mesosulfuron+iodosulfuron ('Atlantis') were used, where 400 g product/ha (field rate) = 12+2.4 g mesosulfuron+iodosulfuron. The detransformed ED_{50} values have been re-converted to g/ha.

ED_{50} = estimated dose required to reduced foliage fresh weight by 50% relative to untreated

Resistance Index = ratio of ED_{50} values relative to the ***baseline*** populations

Implications of this research

This study provides clear evidence that selection for non-target site resistance (enhanced metabolism) to mesosulfuron+iodosulfuron confers cross-resistance to other ALS inhibiting herbicides but not flufenacet, a herbicide with a different mode of action. It should be noted that the ALS inhibiting herbicides studied belong to four different ALS sub-groups – mesosulfuron+iodosulfuron and flupyrsulfuron (sulfonylureas), pyroxsulam (triazolopyrimidines), imazamox (imidazolinones), propoxycarbazone (sulfonylamino carbonyl triazolinones).

Enhanced metabolism is more dependent on the molecular structure of an individual herbicide than its mode of action. A good example is past work which showed that enhanced metabolic resistance to pendimethalin did not result in cross-resistance to trifluralin as the former has ring-methyl groups in its molecular structure (vulnerable to metabolic degradation) whereas the latter does not (James *et al*., 1995).

Thus, there is no reason why enhanced metabolic resistance to mesosulfuron+iodosulfuron should automatically confer cross-resistance to other ALS inhibitors, especially those which are structurally different. The fact that there was clear evidence of cross-resistance to all the ALS inhibiting herbicides implies that there is some common aspect to their molecular structure that makes them all vulnerable. This is an important finding which is relevant to studies on mechanisms of enhanced metabolic resistance.

The fact that there was very clear evidence for a lack of cross-resistance to flufenacet implies that mesosulfuron+iodosulfuron was not selecting for a broad-spectrum mechanism, but to something more

specific to ALS inhibitors. It might be argued that flufenacet is simply not vulnerable to enhanced metabolism, in the same way that cycloxydim, sethoxydim, trifluralin and propyzamide appear unaffected by this mechanism. However, the baseline populations used in this study possessed partial resistance to flufenacet when compared with the Rothamsted susceptible standard (Table 10), and resistance increased progressively, if slowly, in the selection experiments described in section 1.1 of this report.

From a practical perspective, while the very intensive use of mesosulfuron+iodosulfuron during the last 10 years has resulted in widespread resistance, this does not appear to be compromising the efficacy of flufenacet, now the mainstay of pre-emergence herbicide programmes. This *may* also use be true for other pre-emergence herbicides, but requires verification. If resistance to mesosulfuron+iodosulfuron was very specific then, in theory, changing to a different ALS inhibiting herbicide could have potential benefits in terms of greater efficacy. The results of this study do not support this theory, and indicate that increasing enhanced metabolic resistance is compromising the efficacy of other ALS inhibiting herbicides too, even if they have never been used. This will be in addition to ALS target site resistance (both 197 & 574), which is also a common mechanism. With ALS target site resistance the degree of cross-resistance to herbicides belonging to different ALS sub-groups may vary according to the mutation present (197 v 574). However, this will be of little use, practically, if non-target site resistance mechanisms are also present in the same plants, which seems highly likely given that black-grass is a cross-pollinating species.

In this study, selection for resistance was quite intensive, but not totally unrealistic, so we have no reason to think that what we have recorded is not relevant in the field, although the selection process is likely to take longer there due to the buffering effect of the soil seedbank.

Objective 3. To maintain a 'watching brief' for potential new cases or types of herbicide resistance in weeds of the arable, horticultural, industrial and amenity sectors.

3.1: 'Watching brief' on potential new cases of resistance

Status of herbicide-resistance in the UK.

An update on cases of herbicide resistance in the UK was carried out and published in the Aspects of Applied Biology **127** in 2014 (Hull *et al*., 2014a). This included results for screening assays conducted at Rothamsted.as part of this objective. The main points can be summarised:

- Black-grass is the major herbicide-resistant weed problem and, by 2013, occurred on virtually all of the estimated 20,000 farms in 35 counties where herbicides are applied regularly for its control.
- Resistance to mesosulfuron + iodosulfuron, first used in the UK in autumn 2003, has now been detected in black-grass on >700 farms in 27 counties in England. Resistance is conferred by both ALS target site (Pro-197 & Trp-574 mutations) and non-target site mechanisms.
- Resistant Italian rye-grass (*Lolium multiflorum*) occurs on >475 farms in 33 counties***. The first cases of ALS target site resistance in UK populations of Italian rye-grass were detected in 2012.***
- ALS-resistant common chickweed (*Stellaria media*) was found on >50 farms in 13 counties in England, Scotland and Northern Ireland and ALS-resistant common poppy (*Papaver rhoeas*) on >40 farms in nine counties of England.
- ALS-resistant scentless mayweed (*Tripleurospermum inodorum*) was found on five farms in three counties (Yorkshire, Norfolk and Angus). ***These included the first recorded case in Scotland where the ALS mutation responsible (Pro-197-Gln) was determined, making this the first UK population of mayweed to have ALS target site resistance confirmed.***
- Resistant wild-oats (*Avena* spp.) were confirmed on >250 farms in 28 counties of England.

The continuing threat posed by resistant wild-oats can be illustrated by results of a glasshouse screening experiment at Rothamsted. Two populations (HALL and OSB) with suspected resistance to mesosulfuron + iodosulfuron, collected from Essex in 2013, were tested alongside a susceptible (LLUD) reference population. Poor control (≤ 20%) of both the HALL (*A. fatua*) and OSB (*A. sterilis* ssp. *ludoviciana*) populations provide clear evidence of resistance to mesosulfuron + iodosulfuron. With both populations, over 85% of plants survived treatment. Control by pyroxsulam + florasulam was slightly better (46 – 62%) but still significantly poorer than the LLUD susceptible reference population. Fenoxaprop gave poor control of all populations, except LLUD, whereas cycloxydim gave excellent control. Pinoxaden gave poor control of HALL (26%), but excellent control of OSB. The HALL population represents the most resistant wild-oat population ever detected in tests at Rothamsted.

Leaf samples from eight plants were subject to molecular analysis by Bayer, Frankfurt. The analysis and interpretation of results is difficult because wild-oats are hexaploid, having six sets of homologous chromosomes, rather than the two sets found in diploid species such as black-grass and rye-grass (Yu *et al*., 2013). This means that from one to six alleles can be mutated in wild-oats. Resistance to ALS and ACCase inhibitors was not directly correlated with the presence of target site mutations, although these were detected in some plants. It seems probable that enhanced metabolic non-target site resistance may play a bigger role in determining resistance, although this needs verifying. The level of resistance detected to ALS inhibitors and ACCase inhibitors in the HALL population is worrying, as very few other effective modes of action are available for use in cereals, apart from tri-allate, and cultural control options (delayed autumn drilling, spring cropping, ploughing) are less effective than with black-grass and rye-grass.

Sterile brome response to glyphosate

In 2010, poor control of sterile brome (*Bromus sterilis*) by glyphosate on stubbles was reported on a farm in Leicestershire (LEICS) and initial screening experiments confirmed some insensitivity. This was marginal and describing this as 'resistance' was debatable. A second partially resistant population, from Oxfordshire, was detected in 2012 (OXON). In a glasshouse screening assay, conducted in February 2013, 540 g glyphosate/ha (the field recommended rate) gave only 39% (LEICS) and 27% (OXON) reductions in foliage weight relative to untreated after 33 days, whereas 88% control of a susceptible standard from ADAS Boxworth (SUSC) was achieved. There was equally good control of eight other populations collected from throughout England (80% – 94%).

Additional pot screening experiments were conducted in spring 2014 using 40 sterile brome populations collected by ADAS from 17 counties of England, together with the SUSC, LEICS and OXON as reference populations. In addition, roadside and hedgerow samples from areas never treated with glyphosate on the Leicestershire (ROAD) and Oxfordshire (PATH) farms were included. The OXON and LEICS populations were again the most insensitive to glyphosate, although an additional population (09D118), collected in Leicestershire in 2009, showed comparable insensitivity. The resistance indices (ratio of ED_{50} values relative to the susceptible (SUSC) population) for these three populations were similar (2.0 to 2.1). The ROAD and PATH populations were significantly more sensitive than the LEICS and OXON samples, providing good evidence that the partial resistance detected in the field samples was a consequence of selection by glyphosate.

A glasshouse dose response assay involving the SUSC, LEICS, OXON and ROAD and three different glyphosate formulations gave the following results (Table 11). These confirm significant partial resistance to glyphosate in both the LEICS and OXON populations, with a mean resistance index of 2.1, and the susceptibility of the ROAD populations (untreated area on same farm as OXON). There were also significant differences between formulations, with 'Clinic Ace' (a tallow amine formulation) the most effective. Ironically, this was the formulation used which prompted the original farmer complaint. In a total of four studies with different formulations, 'Clinic Ace' gave consistently better control than the other two formulations, with 'Touchdown' giving better control than 'Roundup' in three of the four studies. However, differences between formulations were often small and not always statistically significant. In a separate study, the use of deionised water, in place of moderately hard tap water, increased mean control from glyphosate by 15% despite mixing up and spraying being done within two minutes.

Table 11. Results for four sterile brome populations treated with three different commercial glyphosate formulations in a glasshouse dose response experiment.

	All formulations combined					***All populations combined***		
	Population					**Glyphosate formulation**		
	SUSC.	**ROAD**	**LEICS**	**OXON**		**'Clinic Ace'**	**'Roundup'**	**'Touchdown'**
$Log_{10}ED_{50}$	2.508	2.555	2.786	2.867		2.555	2.714	2.630
	L.S.D. (*P≤0.05*) = 0.110					L.S.D. (*P≤0.05*) = 0.113		
ED_{50} g/ha	322.2	359.1	611.1	736.1		358.7	517.9	426.5
RI	**1.0**	**1.01**	**1.90**	**2.28**		**1.0**	**1.44**	**1.19**

ED_{50} = estimated dose required to reduced foliage fresh weight by 50% relative to untreated

Resistance Index (RI) = ratio of ED_{50} values relative to the susceptible (SUSC) population

These results support the view that partial resistance to glyphosate has evolved in sterile brome in the UK. This could be considered the first case of evolved resistance to glyphosate to be recorded in the UK. The fact that this originated as a farmer complaint and the samples collected from areas never treated with glyphosate on both farms remain fully susceptible supports this interpretation. However, caution is needed. The dose response to glyphosate is very steep, with double rate (1080 g/ha) killing all populations and half rate (270 g/ha) allowing many plants to survive, even of susceptible standards. Thus the 540 g/ha dose, recommended for control of all annual weeds on stubbles, appears marginal for sterile brome control and even a slight loss of efficacy, however caused, is likely to result in survivors. As well as partial resistance, adverse environmental conditions, poor application, use of hard water and use of less effective formulations could all contribute to inadequate control.

The differences we found between commercial formulations were surprising and, perversely, may reflect the better activity of older tallow amine formulations which are likely to be phased out in future due to regulatory concerns. This is a sensitive issue, as companies don't want to give the impression that newer, and often more expensive formulations, are less effective. However, this issue should not be ignored, especially where the recommended dose is only marginally effective – as is the case with sterile brome. It is possible that this marginal dose is responsible for the evolution of partial resistance in sterile brome by allowing some plants to survive which would not have occurred at a higher dose, (e.g. 1080 g/ha). This could be considered as support for the 'low dose favours resistance' argument, but is better considered as a comment on glyphosate efficacy. Higher doses of glyphosate than currently recommended (540 g/ha) for control of sterile brome on stubbles appear fully justified primarily from an efficacy aspect, but might also reduce the risk of resistance.

Specific Objective 4. To conduct Knowledge Transfer (KT) initiatives to inform CRD, suppliers and users of herbicides of the risks posed by herbicide-resistance and to promote more rational pesticide use through Integrated Weed Management (IWM).

Knowledge Transfer initiatives were given a high priority with the two-fold aim of: (1) minimising ineffective and wasteful herbicide use by early detection of resistance and avoidance of high risk active ingredients and (2) promoting greater use of non-chemical methods to reduce reliance on herbicides.

Sub-objective 4.1: Active Knowledge Transfer (AKT) initiative

The key elements highlighted in AKT initiatives were:

- The increasing threat posed by herbicide resistance as a consequence of loss of alternative herbicide solutions.
- The importance of early detection of resistance, and how this is best achieved, to avoid wasteful and ineffective use of herbicides.
- The essential role that non-chemical methods (cropping and cultural) can play in weed control with consequential reduced reliance on 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 other objects of this project, and additional information from other current projects on cultural control, were incorporated into the KT package.

The following **153** knowledge transfer initiatives were conducted by Stephen Moss and Richard Hull during the duration of this project:

- Contributed to **81 articles in the farming press** (2012 – 29; 2013 – 29; 2014 – 23).
  These included 'Farmers Weekly', 'Crops', 'Farmers Guardian' and 'CPM magazine'. Between 1990 and December 2014, there was a specific reference to practical outputs of herbicide-resistance research conducted at Rothamsted in **334 popular articles**, an average of 13 per year since 1990, with an average of 25 per year for the last three years.
- Made **51 presentations at conferences and meetings** (2012 – 13; 2013 – 20; 2014 – 18)
  These included: Conference presentations (e.g. EWRS, AAB, Danish Agronomy Conference, HGCA, BCPC), technical presentations to independent agronomists (e.g. AICC) and those working for agrochemical companies and distributors, talks to farmers and managers (including HGCA roadshows, Cereals event), training presentations to students, discussion meetings relating to research and management of resistance both in UK and internationally (e.g. WRAG, EWRS Resistance Working Group, EPPO).
- Wrote, or contributed to, **21** formal publications (see references in next section).

In summer 2013, a summer student (Louise Westrup, University of Lincoln) conducted a project aimed at developing a Petri-dish test for detecting resistance to flufenacet. A series of experiments was conducted and the conclusion was that any single dose was likely to give misleading results, but use of three concentrations, 0.1, 0.5 and 1 ppm, could give a useful indication of resistance. At 1 ppm, reductions in number of shoots over 10 mm for the resistant Peldon 2010 and Colsterworth 2010 populations, selected for five years with flufenacet, were only 33% and 27% respectively. In contrast, over 90% reductions were obtained with the Rothamsted susceptible population. However, control of three more typical field populations was only marginally less (74% - 87%) than the susceptible standard, which is probably a fair reflection of the current degree of resistance in most populations. It was concluded that this petri-dish test would be useful for detection of high degrees of resistance to flufenacet, but might not give robust results where resistance was marginal.

In addition, the student assisted in the collation and analysis of data from 375 field trials on the efficacy of flufenacet based herbicides on black-grass (Hull *et al*., 2014b). This data had been kindly provided by Bayer, BASF, DuPont and Syngenta. Control of black-grass by pre-emergence flufenacet + pendimethalin and flufenacet + diflufenican did not vary significantly between the mixtures (mean 71%), but varied considerably within individual years (0–100%) and between years (49–87%). Control of heads was not only lower, by an average of 16.5% in three recent drier autumns, but also more variable. A fitted model predicted a small decline in mean herbicide efficacy over the 12 years between 2001 and 2013, averaging less than 1% per year, demonstrating that the efficacy of flufenacet based herbicides is not declining rapidly as a consequence of increasing resistance, as has happened with post-emergence ACCase and ALS inhibitors. This conclusion was entirely consistent with the results obtained from the container studies detailed in objectives 1 & 2 above. The most important outcome was clear evidence that the performance of flufenacet based pre-emergence herbicides is likely to be increased if drilling of winter cereals, and consequently herbicide application, is delayed until October. The model fitted predicted a potential additional 29% control from pre-emergence herbicides by delaying drilling from mid-September to mid-October in average rainfall conditions. However, in years with lower than average autumn rainfall, reduced flufenacet efficacy and greater variation in control is likely, regardless of timing.

Sub-objective 4.2: Production, distribution and promotion of a leaflet specifically on non-chemical weed control.

A four-page leaflet ('***Black-grass: the potential of non-chemical control'***) aimed at farmers and their advisors, was produced in-house at Rothamsted. This was based on a review by Lutman, Moss, Cook & Welham (2013), originally funded by Syngenta, and published in Weed Research journal. An accompanying article and 14,000 copies were distributed in CPM magazine, with Syngenta funding the printing and insertion fee. An electronic version was also produced and this is available on both the WRAG and Rothamsted websites (http://www.rothamsted.ac.uk/black-grass-and-herbicide-resistance). In additional, several agrochemical companies' websites host electronic versions and leaflets have also been distributed at farmer meetings.

The leaflet explained why there is a need to place less reliance on herbicides, quantified the likely effectiveness of a range of non-chemical methods on grass-weeds and highlighted the advantages and disadvantages for each method. This leaflet was very well received and the information has been reproduced in many other articles and publications, including many from the agrochemical industry. This initiative supports CRD's responsibility for ensuring compliance with the Sustainable Use Directive (2009/128/EC) which requires that priority be given, wherever possible, to non-chemical methods of crop protection.

Sub-objective 4.3: Production of final project report.

This is the final project report which has been produced according to the specified requirements detailing the methodologies, results and implications of the entire research project.

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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