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Crop Production in Southern Britain: Precision Decisions for Profitable Cropping

Current status of herbicide-resistant weeds in the UK

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Summary

Alopecurus myosuroides (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 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 *Lolium multiflorum* (Italian rye-grass) occurs on >475 farms in 33 counties and resistant *Avena* spp. (wild-oats) on >250 farms in 28 counties of England. The first cases of ALS target site resistance (Pro-197) in UK populations of *L. multiflorum* were detected in 2012. ALS-resistant *Stellaria media* (common chickweed) was found on >50 farms in 13 counties in England, Scotland and Northern Ireland and ALS-resistant *Papaver rhoeas* (common poppy) on >40 farms in nine counties of England. ALS-resistant *Tripleurospermum inodorum* (scentless mayweed) 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 *Tripleurospermum inodorum* to have ALS target site resistance confirmed.

Key words: Herbicide-resistance, survey, *Alopecurus myosuroides*, *Lolium*, *Avena*, *Papaver rhoeas*, *Stellaria media*, *Tripleurospermum inodorum*

Introduction

Herbicide resistance in the UK continues to be a significant problem and the last compilation exercise was published in 2011, which included results for screening assays conducted between 2005 and 2010 (Moss *et al*., 2011). The key herbicide-resistant weed species of arable crops in the United Kingdom are: *Alopecurus myosuroides* (black-grass), *Lolium multiflorum* (Italian rye-grass), *Avena* spp. (wild-oats), *Papaver rhoeas* (common poppy), *Stellaria media* (common chickweed) and *Tripleurospermum inodorum* (scentless mayweed).

Molecular assays are now cheaper and more readily available, especially for identifying ACCase and ALS target site mutations, and their use is likely to increase in future. However, whole-plant assays generate results that are closest to plant responses in the field and have the advantage of detecting resistance regardless of mechanism (Burgos *et al*., 2013). For this reason, they are likely to remain the main method for identifying herbicide resistance.

Since the last compilation exercise, additional testing has been done by many research organisations and agrochemical companies. The aim of this paper is to provide an update on the current status of herbicide-resistant weeds in relation to the major herbicide classes used for control of arable weeds in the UK.

Alopecurus myosuroides (Black-grass)

In the UK, herbicide-resistant *A. myosuroides* was first found in 1982 and is the most important herbicide-resistant weed in Europe, occurring in at least 12 countries (Heap, 2014). It was estimated that, in 2010, resistant *A. myosuroides* occurred on at least 80% of the 20,000 farms that spray regularly for control of this weed (Moss *et al*., 2011). In the UK, populations show resistance to a wide range of different modes of action, with ACCase and ALS target site resistance (TSR) and non-target site resistance (NTSR), especially enhanced metabolism, now widespread. Combined TSR, in which both ACCase and ALS target site resistance mutations occur within the same plant, has been demonstrated and must be expected to increase (Marshall *et al*., 2013).

Resistance screening assays

In 2013, ADAS tested 122 non-random samples, supplied by BASF, by treating with mesosulfuron-methyl + iodosulfuron-methyl-sodium (abbreviated to mesosulfuron + iodosulfuron in rest of paper; 12+2.4 g a.i. ha^{-1}) in a glasshouse pot assay and cycloxydim (5 mg L^{-1}) and pendimethalin (5 mg L^{-1}) in petri-dishes using standard techniques (Clarke *et al*., 1994; Moss, 2000). The % reductions in foliage fresh weight or shoot length were used to assign resistance 'R' ratings using the system described by Moss *et al*. (2007). This system assigns populations to four categories, S (susceptible), R? (marginally resistant), RR (resistant), RRR (highly resistant), depending on the degree of resistance relative to a susceptible standard. Of the 122 populations, 98% showed resistance (RR or RRR) to at least one herbicide, with 75% resistant to mesosulfuron + iodosulfuron, 84% resistant to cycloxydim, 66% resistant to pendimethalin and 46% resistant to all three herbicides (Fig. 1). Cycloxydim was used as an indicator of broad spectrum ACCase TSR mutations, such as Ile-1781-Leu, the most common type in the UK, but will not detect the

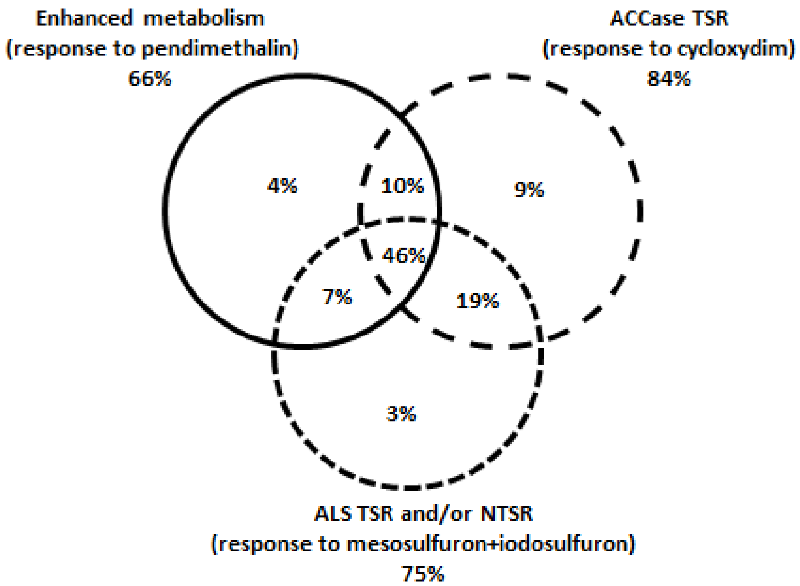

Fig. 1. Proportion of *A. myosuroides* samples that were resistant (RR or RRR) to three herbicides based on assays with mesosulfuron + iodosulfuron, pendimethalin and cycloxydim. A total of 122 non-random populations, collected in 2013, were tested.

less common mutations which confer resistance to aryloxyphenoxypropionate ('fop') herbicides only (Kaundun, 2014; Delye *et al*., 2010), so the true incidence of ACCase TSR in these populations is likely to be even higher. Pendimethalin was used as an indicator of broad spectrum enhanced metabolism, which is likely to affect the activity of other pre-emergence herbicides too (Moss & Hull, 2009*a*). Mesosulfuron + iodosulfuron activity is affected by both ALS TSR and enhanced metabolic NTSR mechanisms (Moss *et al*., 2014).

In a survey of 19 randomly selected fields, sampled between 2009 and 2011, resistance (RR or RRR) to mesosulfuron + iodosulfuron was detected in 47%, to clodinafop in 100%, to chlorotoluron in 47%, to cycloxydim in 58% and to pendimethalin in 79% of fields (Moss *et al*., 2014). The incidence of resistance in the 122 non-randomly collected samples in 2013 was higher, except for pendimethalin, probably because samples were more likely to come from fields where there were problems in controlling *A. myosuroides.* In both studies resistance to at least one herbicide was recorded in 98 – 100% of fields, supporting the view that some level of herbicide resistance occurs on virtually all of the estimated 20,000 farms where herbicides are used routinely for the control of this weed. The 35 counties where herbicide resistance has been confirmed to at least one herbicide are shown in Fig. 2. Derbyshire is the only new addition since the last compilation exercise in 2011.

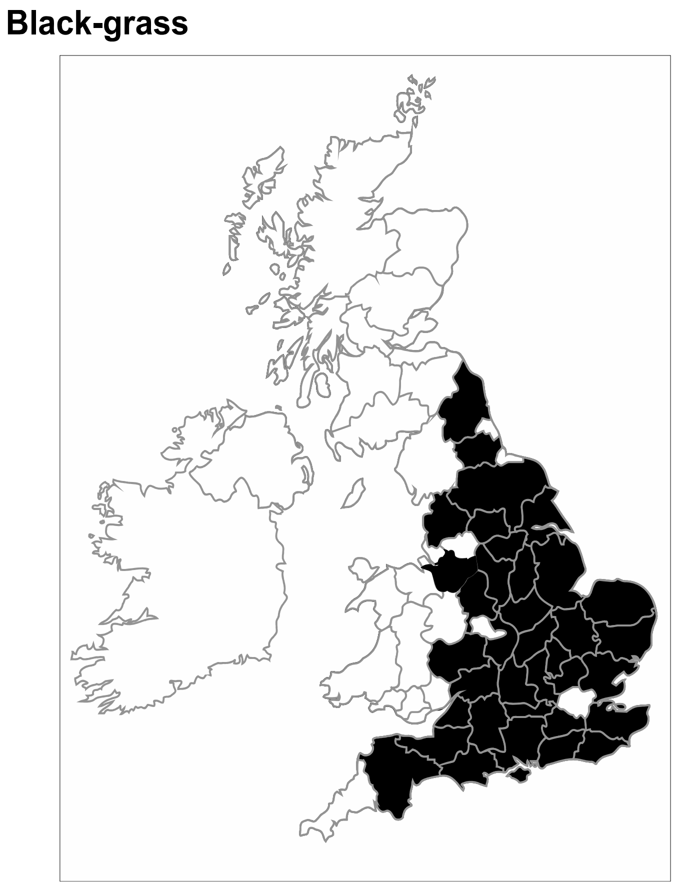

Fig. 2. Counties (35) with herbicide-resistant *Alopecurus myosuroides* (black-grass).

ALS resistance occurrence and mechanisms

Globally, resistance to ALS inhibitors is of major concern, with over 140 weed species having evolved resistance, the highest number for any class of herbicide (Heap, 2014). The formulated mixture of two sulfonylurea (ALS) herbicides, mesosulfuron-methyl + iodosulfuron-methyl-sodium, combined with the safener mefenpyr-diethyl, was introduced into the UK market in autumn 2003 as 'Atlantis WG' (Bayer CropScience). Resistance to this herbicide was detected soon after its introduction and, by 2010, resistance had been confirmed on >400 farms (Moss *et al*., 2011). Updating this information we found that resistance to mesosulfuron + iodosulfuron had been confirmed on >700 farms in 27 counties in England by 2013. ALS TSR was shown to be responsible for at least some of the early cases of resistance (Marshall & Moss, 2008). In more recent studies, the Pro-197-Thr and Trp-574-Leu ALS TSR mutations were each recorded in 7% of the 570 plants treated with mesosulfuron + iodosulfuron from 19 random populations collected between 2009 and 2011 (Moss *et al*., 2014). An additional 20% of plants survived treatment but did not possess any ALS mutation. These results indicate that both non-target site and ALS target site resistance mechanisms are important, but the former is more common. Cross-resistance to other ALS herbicides, such as pyroxsulam and flupyrsulfuron, also occurs.

ACCase resistance occurrence and mechanisms

In a previous compilation exercise, out of 291 populations showing resistance to mesosulfuron + iodosulfuron, 231 (79%) were also resistant to cycloxydim (Moss *et al.,* 2009b). This proportion is very similar to that (84%) recorded in the 2013 screening experiment outlined above. These results indicate a very high incidence in ACCase target site resistance in England.

The first ACCase target site mutation was identified in the early 2000's and research since then has identified mutations at seven ACCase codon positions (Ile-1781, Trp-1999, Trp-2027, Ile-2041, Asp-2078, Cys-2088, Gly-2096) with 14 allelic variants so far identified (Kaundun, 2014; Delye, 2005; Powles & Yu, 2010). Marshall ***et al*.,** (2013) found the following frequencies of ACCase target site mutations in the five populations studied: 80% Ile-1781; 16% Ile-2041; 4% Asp-2078. This result, showing the predominace of ACCase mutations at the Ile-1781 codon position in *A. myosuroides*, supports the findings of much more extensive testing by Delye *et al*. (2010).

Summary

- Herbicide-resistant *A.myosuroides* occurs on virtually all of the estimated 20,000 farms where herbicides are used routinely for the control of this weed.
- Resistance has been confirmed in 35 counties in England (Fig. 2). Derbyshire is the only new county added since the last compilation exercise.
- ACCase target site resistance is present in most populations, but at varying frequencies.
- ACCase TSR is conferred most frequently by mutations at the Ile-1781 codon position, but NTSR enhanced metabolic resistance is also common.
- ALS target site resistance is also widespread but enhanced metabolic NTSR appears to be at least as common.
- ALS TSR is conferred by mutations at both the Pro-197 and Trp-574 codon positions, which appear to occur at approximately equally frequency.
- Resistance to pre-emergence herbicides is widespread but resistance tends to be partial and does not increase very rapidly (Hull *et al*., 2014).

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

In the UK, herbicide resistant *L. multiflorum* was first found in 1990. Relatively little additional testing has been done since 2010, but updated information indicates that by 2013 the number of confirmed cases of resistance had increased to >475 in the same 33 counties (Fig. 3).

In 2006 and 2007, 55 populations were collected on a semi-random basis from 50 farms in 22 counties in England (Alarcon-Reverte, 2010). Resistance (RR or RRR) to at least one herbicide was detected in 70% of the populations. Resistance was detected on the following proportion of farms for each herbicide tested: diclofop-methyl 62%; tralkoxydim 60%; fluazifop-P-butyl 36%; cycloxydim 20%; and pinoxaden 18%. Molecular studies detected mutations at six of the seven known codon positions associated with ACCase target site resistance (TSR) (Alarcon-Reverte *et al.,* 2012). The six mutations found, and their frequencies as a proportion of the total number of resistant plants assayed (384), were: Asp-2078-Gly (24.5%), Ile-1781-Leu (13.3%), Ile-2041-Asn (2.1%), Cys-2088-Arg (1.8%), Trp-2027-Cys (1.0%) and Trp-1999-Cys (0.3%). No plants with the Gly-2096-Ala mutation were found. Even though an ACCase TSR mutation was found in 40% of the 384 resistant plants studied, the main mechanism conferring resistance, in 68% of populations with confirmed resistance, was non-target site resistance (NTSR). There is no evidence that this situation has changed in the last 6 years.

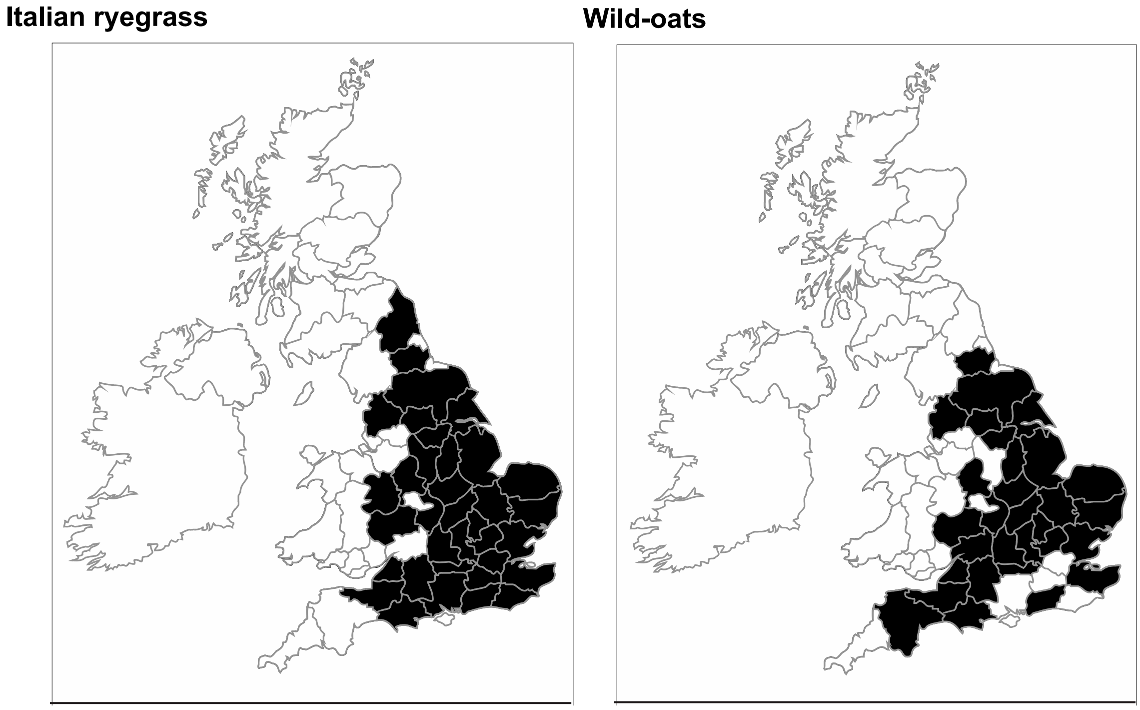

Fig. 3. Counties with herbicide-resistant *Lolium multiflorum* (Italian rye-grass) (33 counties, > 475 farms in total) and *Avena* spp. (wild-oats) (28 counties, >250 farms in total).

In a subsequent experiment, samples from the same 50 farms sampled in 2006 or 2007 were treated with mesosulfuron + iodosulfuron (12 + 2.4 g a.i. ha^{-1}) in a glasshouse screening assay. Nine (18%) showed partial resistance with a % reduction in foliage fresh weight of 52 – 66% compared with 92% for a susceptible standard and 39% for an Australian (WLR1) ALS TSR *Lolium rigidum* standard and 34% for a French *L. multiflorum* population. Leaves of surviving plants of the nine UK populations, WLR1 and the French population were assayed for ALS TSR mutations (Deepak Kaundun, Syngenta, pers. comm.). The Pro-197-Thr mutation was detected in WLR1 and Trp-574-Leu in the French population, but none of the nine UK populations had an ALS TSR mutation. It was concluded that partial resistance to mesosulfuron + iodosulfuron in the semi-random UK populations was due to NTSR, most probably enhanced metabolism.

In a later glasshouse screening experiment, one population from Essex, collected by Rothamsted in 2012 (WALL12), showed resistance to mesosulfuron + iodosulfuron with a 52% reduction in foliage fresh weight compared with 93% for six other populations. Sixteen of the 30 treated WALL12 plants survived, most with few herbicidal symptoms. Leaves from these 16 plants were assayed for ALS TSR mutations (Deepak Kaundun, Syngenta pers. comm.) and a Pro-197-Thr mutation was detected in two plants.

In a separate investigation by Bayer, eight leaves from each of six *L. multiflorum* populations with confirmed resistance to mesosulfuron + iodosulfuron, collected and tested in 2012, were subject to molecular analysis. Pro-197 ALS TSR mutations (both Pro-197-Leu and Pro-197-His) were detected in four and six leaves respectively of two populations, one from Lincolnshire and one from Yorkshire, but not in the other four populations. These populations, from Essex, Lincolnshire and Yorkshire, collected in 2012 by Rothamsted and Bayer, represent the first confirmed cases of ALS target site resistance in *L. multiflorum* in the UK. ALS TSR was detected in samples collected in France in 2006 or 2007 but, as in the UK, only in a minority of the ALS-resistant populations tested (Delye *et al*., 2009). ALS TSR in *Lolium* spp. is considered to be still relatively rare in France where it has been detected in only three out of 34 populations assayed between 2008 and 2012 (Christophe Delye, INRA, pers. comm). It appears that NTSR, especially enhanced metabolism, as found by Cocker *et al*., (2001) is the main resistance mechanism conferring resistance in UK populations of *L. multiflorum*.

Summary

- Herbicide-resistant *L. multiflorum* occurs on >475 farms in 33 counties of England (Fig. 3). No new counties were added since the last compilation exercise in 2010.
- There is some evidence of resistance 'hotspots', such as parts of Essex, Kent and Yorkshire, where particular problems occur.
- ACCase TSR occurs, but less commonly than in *A. myosuroides*.
- ACCase TSR mutations at the Asp-2078 codon position are much commoner than in *A.myosuroides*, where mutations at the Ile-1781 position predominate.
- In 2012, ALS TSR (several different amino acid substitutions at the Pro-197 codon position) was confirmed for the first time in UK populations of *L. multiflorum* in three counties, Essex, Lincolnshire and Yorkshire.
- ALS TSR appears to be much less common in *L. multiflorum* than in *A. myosuroides.*
- At present, NTSR enhanced metabolic resistance appears to be the major resistance mechanism, and relatively much commoner than both ACCase and ALS TSR.

***Avena spp.* (Wild-oats)**

Herbicide resistant *Avena spp* populations were first found in the UK in 1994. Few additional samples have been tested since 2010 so our knowledge of the occurrence and distribution is unchanged, with resistance being found on >250 farms in 28 counties (Fig. 3). Resistance has been identified in both *Avena fatua* (common wild-oat) and *Avena sterilis* spp*. ludovociana* (winter wild-oat), with resistance in the latter species being detected relatively more frequently than its more limited occurrence as a weed would suggest (Preston *et al*., 2002).

Cocker *et al.* (2000) showed that resistance in UK populations of *Avena* spp. was confered by both ACCase TSR and enhanced metabolic NTSR. However, 'fop' specific mutations appear to be much more common in *Avena* spp. than in *A. myosuroides* and *L. multiflorum,* meaning that most 'dims' (e.g. cycloxydim) and 'dens' (e.g. pinoxaden) remain effective.

The continuing threat posed by resistant *Avena* spp. can be illustrated by results of a recent glasshouse screening experiment at Rothamsted. Two populations (HALL and OSB) with suspected resistance to mesosulfuron + iodosulfuron, collected in 2013, were tested alongside susceptible (LLUD), enhanced metabolism (EM) (T/11) and ACCase TSR ('fop' specific) reference populations. Plants (5 pot^{-1}; 5 reps) were sprayed, with recommended adjuvants, at the three leaf stage and foliage fresh weight recorded 28 days later (Table 1).

The 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, 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, but excellent control of OSB. The HALL population represents the most resistant *Avena* population ever detected in tests at Rothamsted. The level of resistance detected to ALS inhibitors, such as mesosulfuron + iodosulfuron, and

Table 1. *Response of three reference (LLUD, T/11, T/41) and two test populations of* Avena *spp. from Essex treated with field recommended rates of five herbicides in a glasshouse pot assay. Values are percentage reductions in foliage weight, relative to untreated pots of the same population, recorded 28 days after spraying*

		mesosulfuron +iodosulfuron	pyroxsulam +florasulam	fenoxaprop	pinoxaden	cycloxydim
	g a.i. ha^{-1}	12+2.4	18.8+3.7	55	30	100
LLUD	Susc. ref.	79	77	87	86	90
T/11	EM ref.	71	83	49	86	92
T/41	ACCaseTSR ref.	76	84	-12	77	91
HALL	Essex	4	46	36	26	95
OSB	Essex	20	62	24	90	91
SE ±		4.6	3.1	4.4	3.4	0.7

Note: The ACCase TSR reference population has 'fop' specific resistance (Cocker *et al*., 2000).

ACCase inhibitors such as pinoxaden is worrying, as very few other effective modes of action are available for use in cereals.

Leaf samples from eight plants of the T/11, T/41, HALL and OSB populations were subject to molecular analysis by Bayer. The analysis and interpretation of results is difficult because *Avena* spp. are hexaploid, having six sets of homologous chromosomes, rather than the two sets found in diploid species such as *A. myosuroides* and *Lolium* spp. (Yu *et al*., 2013). This means that from one to six alleles can be mutated in *Avena* spp. The conclusions were:

- All four populations had some plants with Pro-197 ALS TSR mutations, but mostly with only one or two mutated alleles. No Trp-574 ALS TSR mutations were detected.
- The frequency of Pro-197 ALS TSR mutations did not appear any higher in HALL and OSB compared with T/11 and T/41 and at least 50% of plants had no mutated Pro-197 alleles.
- All four populations had a high frequency of plants with Ile-1781-Leu ACCase TSR mutations, but again with mostly only one or two mutated alleles.
- Only the T/41 population had a Trp-2027-Cys ACCase TSR mutation, with two mutated alleles in every plant. This mutation confers resistance to 'fops', such as fenoxaprop, but not to 'dims' such as cycloxydim (Powles & Yu, 2010), which helps explain the responses seen at the whole plant level.
- No other ACCase TSR mutations (1999, 2041, 2078, 2088, 2096) were found, except for an Asp-2078-Gly mutation in one allele of a single HALL plant.

These results highlight the difficulty in interpreting resistance in hexaploid species such as *Avena* spp. The Ile-1781-Leu ACCase TSR mutation confers very high resistance to cycloxydim in *A. myosuroides* but, despite its presence in most plants of all four populations, there was no evidence of resistance at the whole plant level in the glasshouse assay. In addition, resistance to mesosulfuron + iodosulfuron was not directly correlated with the presence of Pro-197 ALS TSR mutations. It seems probable that enhanced metabolic NTSR may play a bigger role than TSR in determining resistance to both ACCase and ALS inhibiting herbicides, and further studies are in progress.

Summary

- Herbicide-resistant *Avena spp.* occur on >250 farms in 28 counties of England (Fig. 3). No new counties were added since the last compilation exercise in 2010.
- ACCase TSR occurs, but this tends to be 'fop' specific.

- No ALS TSR associated with herbicide failure in the field has been identified in the UK so far. The hexaploid nature of *Avena* spp. makes molecular characterisation difficult.
- Enhanced metabolic NTSR may play a bigger role than TSR in determining resistance to both ACCase and ALS inhibiting herbicides, although this requires confirmation.

Stellaria media (Common chickweed)

ALS resistant *S. media* occurs in seven European countries and was first detected in the UK in 2000 (Heap, 2014). Relatively little additional testing has been done since 2010, partly due to the difficulty of seed collection, but updated information indicates that, by 2013, the number of confirmed cases of resistance had increased to >50 in the same 13 counties (Fig. 4). These comprise seven counties in Scotland, five in England and one in Northern Ireland. Studies showed that resistance was conferred by two ALS gene point mutations (Pro-197-Gln or Trp-574-Leu) (Marshall *et al*., 2010). The first of these mutations appears more common and was associated with resistance to the sulfonylurea, metsulfuron-methyl, but not to the triazolopyrimidine, florasulam. In contrast, the latter mutation was associated with resistance to both herbicides.

The *S. media* samples tested over the last three years were resistant to ALS inhibiting herbicides only, with alternative herbicides, such as fluroxypyr and mecoprop-P, remaining effective. Although we have no evidence of resistance to mecoprop-P in these samples, some farmers and agronomists in both Scotland and Ireland are convinced that the efficacy of this herbicide on *S. media* has declined in the field. As resistance to synthetic auxins has been found in *S. media* in the past (Lutman & Snow, 1987), further investigations are warranted. We have observed in several glasshouse assays that some test plants treated with mecoprop take longer to die than those of susceptible standards. In a glasshouse pot assay at Rothamsted with two Scottish samples collected from the same farm in 2013, five out of 24 plants treated with mecoprop at 600g a.i. ha^{-1} (half field rate) survived, with two plants reaching maturity and producing seed. These await further evaluation.

Papaver rhoeas (Common poppy)

Herbicide resistant *P. rhoeas* is the most important resistant broad-leaved weed in Europe, occurring in eight countries, and was first detected in the UK in 2000. Updated information indicates that, by 2013, the number of confirmed cases of resistance had increased to >40 in the same nine counties of England as recorded in 2010 (Fig. 4). No cases have been confirmed other parts of the UK. Most cases of resistance in Europe have been to ALS inhibiting herbicides, but resistance has been found to the synthetic auxin herbicides in Italy and Spain (Cirujeda *et al.,* 2003).

Studies on UK populations have shown that resistance was conferred by ALS TSR mutations (Pro-197-Leu or Pro-197-His) (Marshall *et al*., 2010), although additional mutations have been found in populations from Italy (Delye *et al.*, 2011). There is increasing evidence that NTSR may play a more important role in resistance in *P. rhoeas*, and other broad-leaved weeds, than previously thought (Delye *et al.,* 2011). However, alternative herbicides, such as pendimethalin, MCPA and ioxynil + bromoxynil, were effective in controlling ALS-resistant populations from the UK (Marshall *et al*., 2010). A study is in progress investigating the best way to manage ALS-resistant *P. rhoeas* in different cropping rotations (Tatnell *et al*., 2014).

Tripleurospermum inodorum (Scentless mayweed)

ALS-resistant *T. inodorum* has been found in three European countries (Denmark, Germany, UK) and was first detected in England in samples collected in 2002 (Moss *et al*., 2011). ALS-resistance

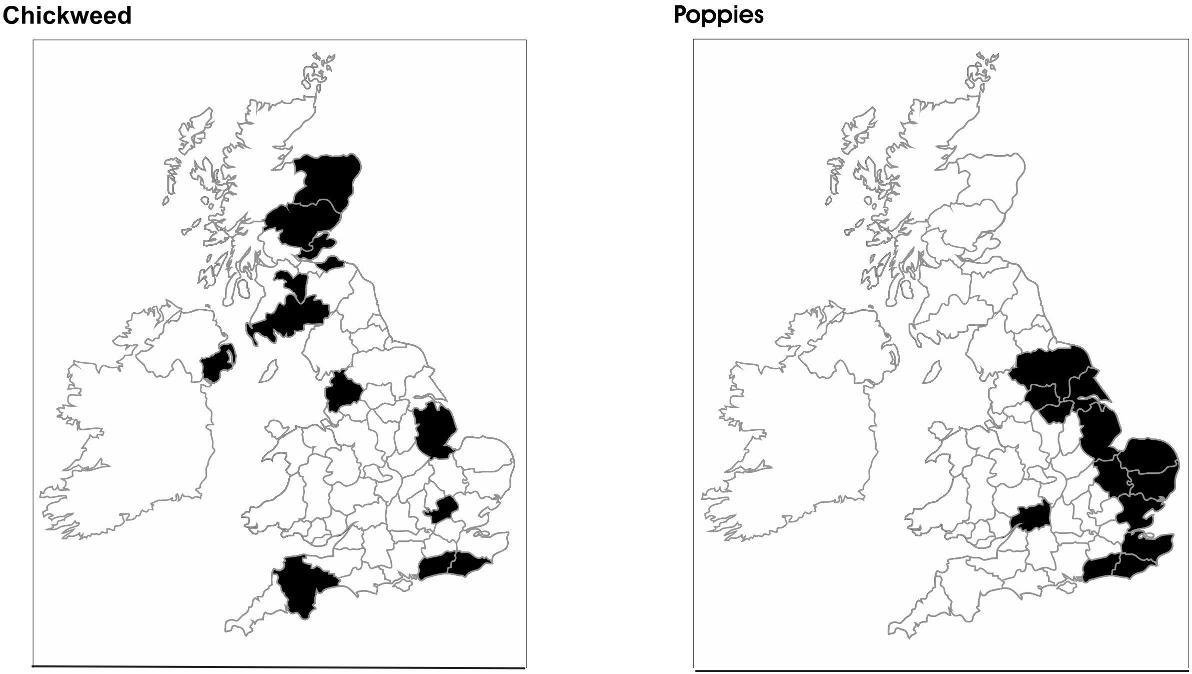

Fig. 4. Counties with herbicide-resistant *Stellaria media* (chickweed) (13 counties, >50 farms in total) and *Papaver rhoeas* (poppy) (9 counties, >40 farms in total).

has also been confirmed in the closely related *Matricaria recutita* (scented mayweed) in Belgium and Germany (Heap, 2014). Resistance may be under-reported due to the difficulty of seed collection in the field and few additional UK samples have been tested. By 2013, ALS-resistant *T. inodorum* had been identified on a total of five farms in three counties, Yorkshire and Norfolk in England and Angus in Scotland.

The *T. inodorum* population from Forfar, Angus, collected in 2013, was tested in a glasshouse assay at Rothamsted using metsulfuron-methyl (6 g a.i. ha^{-1}), florasulam (5 g a.i. ha^{-1}) and ioxynil + bromoxynil (400+400 g a.i. ha^{-1}) using the method previously described (Moss *et al.*, 2011). Resistant (AMC) and susceptible standards were included. The Forfar and AMC populations were poorly controlled by metsulfuron-methyl (0 – 45% reduction in foliage weights) with 12 of the 13 treated Forfar plants surviving, whereas all plants of the susceptible standard were killed. All plants of all populations were killed by florasulam and ioxynil + bromoxynil.

Leaf samples from eight Forfar plants surviving treatment with metsulfuron-methyl were sent for molecular analysis to IDENTXX GmbH in Germany (Cornelia Kocher, pers. comm.). All eight plants had the Pro-197-Gln mutation, with no mutations detected at the 574 codon position. The Forfar population represents the first confirmed case of resistant *T. inodorum* in Scotland and the first UK population to have ALS TSR confirmed using molecular techniques.

Discussion

This update confirms *A. myosuroides* as the major herbicide-resistant weed problem of arable crops in England, with multiple-herbicide resistance a particular issue. Both ACCase and ALS target site resistance (TSR) occur widely, but non-target site resistance (NTSR), mainly enhanced metabolism, appear to be the commonest mechanism. One consequence of widespread resistance to ACCase inhibiting herbicides, and increasing resistance to ALS herbicides such as mesosulfuon + iodosulfuron, is that farmers are becoming ever more dependent on pre-emergence herbicides. However, all pre-emergence herbicides are affected by resistance to some degree, but resistance tends to be partial and build up slowly (Moss & Hull, 2009a; Hull *et al.,* 2014). However, their efficacy is dependent on adequate soil moisture and it is doubtful whether reliance on pre-emergence herbicides alone for control of *A. myosuroides* is sustainable in the longer-term.

Resistance in *L. multiflorum* and *Avena* spp. is less severe generally, and both ACCase and ALS TSR less common than in *A. myosuroides*. However, this update includes the first confirmed cases of ALS TSR in *L. multiflorum* in the UK and evidence of high resistance to mesosulfuron+iodosulfuron and pinoxaden in *Avena* spp. Resistance in these grass-weeds is likely to increase, but may take longer to reach the level of severity currently experienced with *A. myosuroides*. Resistance in *Avena* spp. needs monitoring as, apart from tri-allate, farmers are almost totally dependent on post-emergence ACCase and ALS herbicides for weed control. In addition, pre-emergence herbicides are unlikely to control spring emerging plants and non-chemical methods of control are less effective than with *A. myosuroides*.

The number of cases of confirmed resistance in the broad-leaved weeds, *S. media, P. rhoeas* and *T. inodorum*, has increased since the last update in 2010 but is still relatively low, at ≤50 cases per species. This may partly reflect the greater difficulty in collecting seed samples compared with the grass-weeds. In marked contrast to the grass-weeds, resistance is largely restricted to the ALS inhibiting herbicides and is conferred predominately by target site resistance. However, NTSR in broad-leaved weeds may be more prevalent than previously thought and requires more research.

Currently in the UK we have no species with resistance to glyphosate and the few cases elsewhere in Europe are mainly in perennial crops, such as orchards and vineyards. However, the first case of a glyphosate-resistant weed (*Lolium* spp. in Italy) in a European annual arable cropping system has recently been reported (Collavo & Satin, 2014). In the UK, the widespread use of cultivations as an additional means of weed control has probably reduced the risk of glyphosate-resistance in grass-weeds. However, we should not be complacent, and the trend towards using shielded sprayer to apply glyphosate between the rows of crops such as oilseed rape and sugar beet may increase the resistance risk in future, even in the absence of GM glyphosate-resistant crops.

Worldwide, no new modes of action have been marketed for over 20 years (Duke, 2012), which is why herbicide resistance poses an increasing threat. Weeds with resistance to several different modes of action occur, which means that resistance cannot be countered simply by using different types of herbicide. Increasingly there is recognition that farmers have become over-reliant on herbicides in recent decades and that this ‘dependency culture’ will have to change. Greater use of non-chemical methods of weed control, as recently reviewed for *A. myosuroides* (Lutman *et al*., 2013), in combination with herbicides, will be essential.

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References

Alarcon-Reverte R. 2010. *Understanding and combating the threat posed by* Lolium multiflorum *as a weed of arable crops*. PhD Thesis. University of Reading. 240 pp.

Alarcon-Reverte R, Hanley S, Kaundun S S, Karp A, Moss S R. 2012. A SNaPshot assay for the rapid and simple detection of known point mutations conferring resistance to ACCase-inhibiting herbicides in *Lolium* spp. *Weed Research* **53**:12–20.

Burgos N R, Tranel P J, Streibig J C, Davis V M, Shaner D, Norsworthy J K, Ritz, C. 2013. Review: Confirmation of resistance to herbicides and evaluation of resistance levels. *Weed Science* **61**:4–20.

Cirujeda A, Recasens J, Taberner A. 2003. Dormancy cycle and viability of buried seeds of *Papaver rhoeas*. *Weed Research* **46**:327–334.

Clarke J H, Blair A M, Moss S R. 1994. The testing and classification of herbicide resistant *Alopecurus myosuroides* (black-grass). *Aspects of Applied Biology* **37**, *Sampling to Make Decisions*, pp. 181–188.

Cocker K M, Coleman J O D, Blair A M, Clarke J H, Moss S R. 2000. Biochemical mechanisms of cross-resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in populations of *Avena* spp. *Weed Research* **40**:323–334.

Cocker K M, Northcroft D S, Coleman J O D, Moss S R. 2001. Resistance to ACCase inhibiting herbicides and isoproturon in UK populations of *Lolium multiflorum*: mechanisms of resistance and implications for control. *Pest Management Science* **57**:587–597.

Collavo A, Sattin M. 2014. First glyphosate-resistant Lolium spp. biotype found in a European annual arable cropping system also affected by ACCase and ALS resistance. *Weed Research* **54**:325–334.

Delye C. 2005. Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. *Weed Science* **53**:728–746.

Delye C, Boucansaud K, Pernin F, Le Corre V. 2009. Variation in the gene encoding acetolactate-synthase in *Lolium* species and proactive detection of mutant herbicide-resistant alleles. *Weed Research* **49**:326–336.

Delye C, Michel S, Bernard A, Chauvel B, Brunel D, Guillemin J-P, Dessaint F, Le Corre V. 2010. Geographical variation in resistance to acetyl-coenzyme A carboxylase-inhibiting herbicides across the range of the arable weed *Alopecurus myosuroides* (black-grass). *New Phytologist* **186**:1005–1017.

Delye C, Pernin F, Scarabel L. 2011. Evolution and diversity of the mechanisms endowing resistance to herbicides inhibiting acetolactate-synthase (ALS) in corn poppy (*Papever rhoeas* L.). *Plant Science* **180**:333–342.

Duke S O. 2012. Why have no new herbicide modes of action appeared in recent years? *Pest Management Science* **68**:505–512.

Heap I. 2014. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org.

Hull, R, Clark S J, Westrup L, Moss S R. 2014. The efficacy of flufenacet based herbicides on *Alopecurus myosuroides* (black-grass): analysis of data from 375 field trials. *Aspects of Applied Biology* **127**, *Crop Production in Southern Britain, Precision decisions for profitable cropping,* pp. 49–55.

Kaundun S S. 2014. Resistance to acetyl-CoA carboxylase-inhibiting herbicides. *Pest Management Science* **70**:1405–1417.

Lutman P J W, Snow H S. 1987. Further investigations into the resistance of chickweed (*Stellaria media*) to mecoprop. *Proceedings of the 1987 British Crop Protection Conference – Weeds*. pp. 901–908.

Lutman P J W, Moss S R, Cook S, Welham S J. 2013. A review of the effects of crop agronomy on the management of *Alopecurus myosuroides*. *Weed Research* **53**:299–313.

Marshall R, Moss S R. 2008. Characterisation and molecular basis of ALS inhibitor resistance in the grass weed *Alopecurus myosuroides*. *Weed Research* **48**:439–447.

Marshall R, Hull R, Moss S R. 2010. Target site resistance to ALS inhibiting herbicides in *Papaver rhoeas* and *Stellaria media* biotypes from the UK. *Weed Research* **50**:621–630.

Marshall R, Hanley S, Hull R, Moss S R. 2013. The presence of two different target site resistance mechanisms in individual plants of *Alopecurus myosuroides* Huds., identified using a quick molecular test for the characterisation of six ALS and seven ACCase SNPs. *Pest Management Science* **69**:727–737.

Moss S R. 2000. The “Rothamsted Rapid Resistance Test” for detecting herbicide-resistance in annual grass-weeds. *Weed Science Society of America Annual Meeting, Toronto* **40**, Abstract 102.

Moss S R, Perryman S A M, Tatnell L V. 2007. Managing herbicide-resistant black-grass (*Alopecurus myosuroides*): Theory and practice. *Weed Technology* **21**:300–309.

Moss S R, Hull R. 2009a. The value of pre-emergence herbicides for combating herbicide-resistant *Alopecurus myosuroides* (black-grass). *Aspects of Applied Biology* **91**, *Crop Protection in Southern Britain* pp. 79–86.

Moss S R, Hull R, Marshall R. 2009b. Control of *Alopecurus myosuroides* (black-grass) resistant to mesosulfuron + iodosulfuron. *Communications in Agricultural and Applied Biological Sciences (University of Gent, Belgium)* **74**(2):479–488.

Moss S R, Marshall R, Hull R, Alarcon-Reverte R. 2011. Current status of herbicide-resistant weeds in the United Kingdom. *Aspects of Applied Biology* **106,** *Crop Protection in Southern Britain*, pp. 1–10.

Moss S R, Hull R I, Marshall R, Perryman S A M. 2014. Changes in the incidence of herbicide-resistant *Alopecurus myosuroides* (black-grass) in England, based on sampling the same random fields on two occasions. *Aspects of Applied Biology* **127**, *Crop Production in Southern Britain, Precision decisions for profitable cropping,* pp. 39–48.

Powles S B, Yu Q. 2010. Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology* **61**:317–347.

Preston C D, Pearman D, Dines T D. 2002. *New atlas of the British & Irish flora: an atlas of the vascular plants of Britain, Ireland, the Isle of Man and the Channel Islands.* Oxford: Oxford University Press.

Tatnell L V, Clarke J H, Moss S R. 2014. Preventing a wide-scale increase in ALS resistant broad-leaved weeds through effective management in a cereal/oilseed rape cropping rotation in the UK. *Aspects of Applied Biology* **127**, *Crop Production in Southern Britain, Precision decisions for profitable cropping,* pp. 65–70.

Yu Q, Ahmad-Hamdani M S, Han H, Christoffers M J, Powles S B. 2013. Herbicide resistance-endowing ACCase gene mutations in hexaploid wild oat (*Avena fatua)*: insights into resistance evolution in a hexaploid species. *Heredity* **110**:220–231.