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Crop Protection in Southern Britain

Current status of herbicide-resistant weeds in the United Kingdom

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Summary

This paper updates information on the status of resistant weeds of arable crops in the UK, last compiled in 2005. It is estimated that resistant *Alopecurus myosuroides* (black-grass) occurs on at least 80% of the 20,000 farms that spray regularly for control of this weed. Resistance to mesosulfuron+iodosulfuron, first used in the UK in autumn 2003, has been detected on > 400 farms in 26 counties of England. *Lolium multiflorum* (Italian rye-grass) resistant to at least one herbicide mode of action has been found on > 450 farms in 33 counties and resistant *Avena* spp. (wild-oats) on > 250 farms in 28 counties of England. ALS-resistant *Stellaria media* (common chickweed) was found on > 40 farms in 13 counties in England, Scotland and Northern Ireland. This represents the first documented case of a herbicide-resistant weed in Northern Ireland. ALS-resistant *Papaver rhoeas* (common poppy) was found on > 25 farms in nine counties of England. ALS-resistance in *Tripleurospermum inodorum* (scentless mayweed) was documented for the first time in England, with resistant populations being found on three farms in Yorkshire. Partial resistance to the triazinone herbicides, metribuzin and metamitron, was also documented for the first time in England in *Senecio vulgaris* (groundsel), which also showed complete resistance to triazine herbicides.

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

Introduction

The last compilation exercise for herbicide-resistant weeds in the UK was published in 2005 (Moss *et al*., 2005). At that time, results for screening assays conducted between 2000 and 2004 by 13 organisations/companies were collated in order to update the information on the status of resistant arable weeds of arable crops in Great Britain previously compiled in 1999 (Moss *et al*., 1999).

Now that herbicide-resistant weeds occur so widely, it has become impractical to maintain an accurate record of the number of farms affected. All agrochemical companies, research and advisory organisations recognise that herbicide resistance is a major issue, and the precise number of cases has little relevance. However, it is useful to document changes in resistance distribution, new cases and the occurrence of resistance to new herbicides. The aim of this paper is to present an update on the current status of herbicide resistant weeds in relation to the major herbicide classes used for weed control in the UK.

Alopecurus myosuroides (Black-grass)

Alopecurus myosuroides (black-grass) is the most important herbicide-resistant weed in Europe occurring in at least 10 countries (Moss *et al*., 2007). In the UK, herbicide resistant *A. myosuroides* was first found in 1982 and, by 2004, resistance had been found on 2085 farms in 31 counties in Great Britain (Moss *et al*., 2005). Resistance occurs to a wide range of different modes of action, including acetolactate-synthase (ALS) inhibitors. Resistance to ALS inhibitors is an increasing problem worldwide with 107 species now showing resistance (Heap, 2011). A formulated mixture of two sulfonylurea herbicides, mesosulfuron-methyl and iodosulfuron-methyl sodium combined with the safener mefenpyr-diethyl was introduced into the UK market in autumn 2003 as 'Atlantis WG' (Bayer CropScience). Mesosulfuron+iodosulfuron controls *A. myosuroides* and many other grass weeds and has been introduced and used very extensively in several European countries (Kotting, 2005; Borrod, 2005). Concern that the performance of mesosulfuron+iodosulfuron would be affected by resistance has resulted in a considerable amount of research to develop and validate diagnostic tests (Hull *et al*., 2008).

Based on glasshouse screening assays conducted by Bayer, Syngenta, ADAS, OPS and Rothamsted, resistance (RR or RRR) to mesosulfuron+iodosulfuron was confirmed on > 400 farms in 26 counties of England by 2010. These counties include all those where *A. myosuroides* commonly occurs as a weed of arable crops. In addition, 231 (79%) out of 291 mesosulfuron+iodosulfuron resistant populations tested were also resistant to cycloxydim (Moss *et al*., 2009). This very high frequency of resistance to the cyclohexanedione herbicide cycloxydim, indicates a very high incidence of ACCase (acetyl Co-A carboxylase) target site resistance in England.

Today, it is generally agreed that some level of herbicide resistance occurs on most of the estimated 20,000 farms where herbicides are used routinely for the control of *A. myosuroides*. The high incidence of resistance, in about 80% of samples, detected in both random and non-random sampling (Moss *et al*., 2005; Moss & Perryman, 2007) indicate that it is not unreasonable to suggest that at least 16,000 farms with *A. myosuroides* are affected by some level of herbicide resistance in the UK. Of course, this does not mean that all plants within a field are resistant or that the efficacy of all herbicides used on those farms will be reduced. The 34 counties where herbicide resistance has been confirmed to at least one herbicide are shown in Fig. 1.

Black-grass

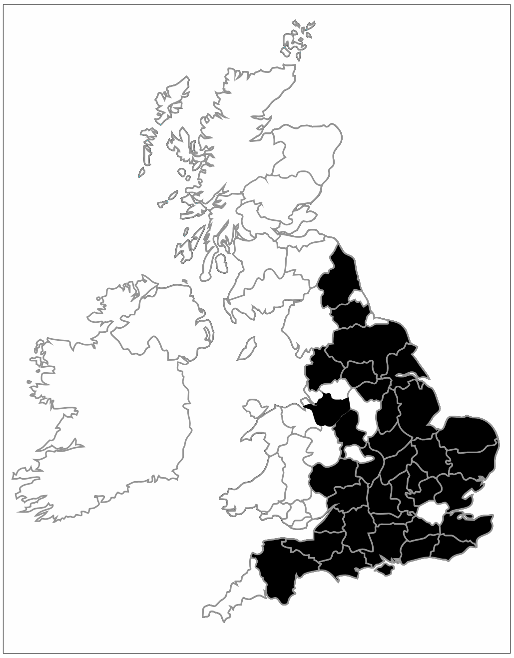

Fig. 1. Counties (34) with herbicide-resistant *Alopecurus myosuroides* (black-grass).

Lolium multiflorum (Italian rye-grass)

Herbicide-resistant populations of *Lolium* spp. (*L. multiflorum, L. perrene, L. persicum, L. rigidum*) have been found in 17 countries worldwide (Heap, 2011). In the UK, herbicide resistant

L. multiflorum was first found in 1990 and, by 2004, resistance had been found on 324 farms in 28 counties in Great Britain (Moss *et al*., 2005). More recently, in 2006 and 2007, a semi-random survey of resistance in *L. multiflorum* was conducted (Alarcon-Reverte, 2010). In this survey, 55 populations were collected on a semi-random basis from winter wheat fields from 50 farms in 22 counties of England. The populations were collected principally for evaluating seed dormancy and were not chosen because herbicide control had been poor. The aim was to determine the extent of resistance to ACCase inhibiting-herbicides (diclofop-methyl, fluazifop-P-butyl, tralkoxydim, cycloxydim, pinoxaden) in a glasshouse pot assay.

Resistance (RRR/RR) to at least one herbicide was detected in *L. multiflorum* samples from 35 (70%) of the 50 farms semi-randomly sampled. Resistance to diclofop-methyl and tralkoxydim was detected on 31 (62%) and 30 (60%) of farms respectively from 16 counties. Resistance to fluazifop-P-butyl was detected on 18 farms (36%), and to cycloxydim and pinoxaden on 10 (20%) and 9 (18%) farms respectively. Hence, resistance to diclofop-methyl, tralkoxydim and, to a slightly lesser extent, fluazifop-P-butyl appears to be widespread in England, but resistance to both cycloxydim and pinoxaden is much less common.

Molecular studies detected six of the seven known ACCase target site mutations in resistant plants (Alarcon-Reverte, 2010). 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 point mutation was found in 40% of the 384 resistant plants studied, the main mechanism conferring resistance in 68% of populations with confirmed resistance was a non target site resistance mechanism, most probably enhanced metabolism.

In a subsequent glasshouse assay, partial resistance to mesosulfuron+iodosulfuron was detected in samples from nine (18%) of these 50 farms surveyed on a semi-random basis in 2006 and 2007. A molecular assay found no evidence of any ALS target site mutations in surviving plants (Deepak Kaundun, pers. comm.), and it was concluded that partial ALS resistance was due to non target site resistance mechanism, most probably enhanced metabolism.

Italian ryegrass

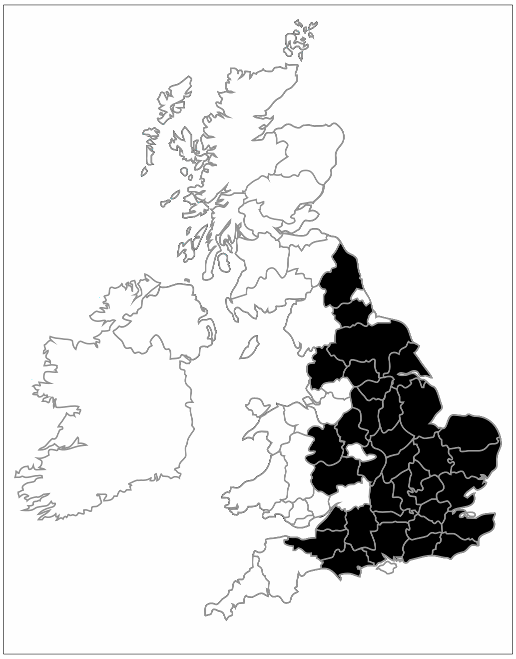

Fig. 2. Counties (33) with herbicide-resistant *Lolium multiflorum* (Italian rye-grass).

ACCase and ALS target site resistance currently appear to be much less common in *L. multiflorum* than in *A. myosuroides*. However, in the previous compilation exercise, resistance to cycloxydim or sethoxydim was recorded in only 9% of the non-random samples tested (Moss *et al*., 2005). The higher incidence (20%) in the 2006/07 semi-random sampling indicates that the frequency of ACCase target site resistance is increasing.

Additional testing of samples has been done since the last compilation exercise and, by 2010, resistance had been found on > 450 farms in 33 counties in Great Britain (Fig. 2).

Avena spp. (Wild-oats)

Herbicide-resistant populations of *Avena spp*. (*A. fatua and A. sterilis* ssp. *ludoviciana*) have been found in 13 countries worldwide (Heap, 2011). In the UK, herbicide resistant *Avena* spp. were first found in 1993 and, by 2004, resistance had been found on 218 farms in 26 counties in Great Britain (Moss *et al*., 2005). About 67% of cases of resistance involved *Avena fatua* (common wild-oats), the others being *Avena sterilis* ssp. *ludoviciana* (winter wild-oat). This indicated that resistance was relatively more common in the latter species, which generally comprises much less than 33% of *Avena populations* (Preston *et al*., 2002).

Despite the widespread occurrence of resistant *Avena* spp., they appear to cause much less problem than *A. myosuroides* and *L. multiflorum,* and relatively few additional samples have been tested since 2004. Past work showed that resistance in *Avena* spp. was conferred by both ACCase target site and enhanced metabolic resistance (Cocker *et al*., 2000). In contrast to *A. myosuroides* and *L. multiflorum*, where ACCase target site mutations affecting activity of 'fop', 'dim' and 'den' herbicides tend to be most prevalent, 'fop' specific mutations are commoner in resistant *Avena* spp. (Deepak Kaunden, pers. comm.). The reasons for this difference are not clear, but one consequence is that 'dim' and 'den' herbicides are still effective on many 'fop' resistant populations.

Additional testing of samples has been done since the last compilation exercise and, by 2010, resistance had been found on > 250 farms in 28 counties in Great Britain (Fig. 3).

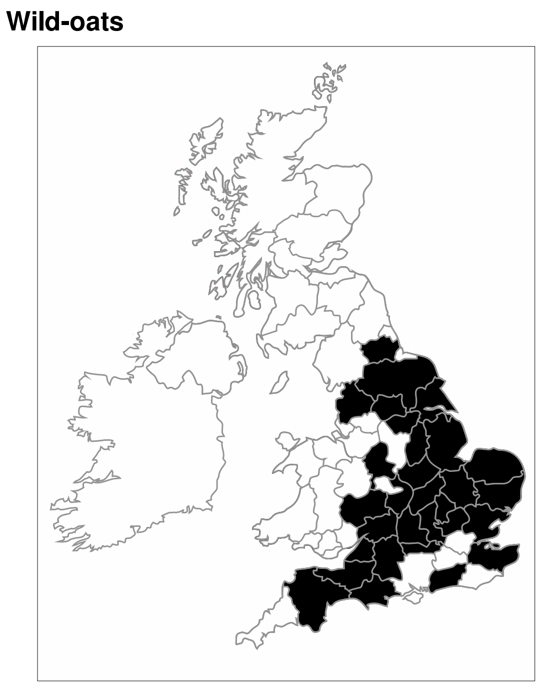

Fig. 3. Counties (28) with herbicide-resistant *Avena* spp. (wild-oats)

Stellaria media (Common chickweed)

Herbicide-resistant populations of *S. media* have been found in 10 countries worldwide (Heap, 2011). Most cases involve resistance to ALS inhibiting herbicides, but resistance to the synthetic auxin herbicides (e.g. mecoprop) was detected in the UK in 1985 (Lutman & Snow, 1987). In the UK, ALS-resistant *S. media* was first detected in 2000 and, by 2004, had been found on 15 farms in 11 counties in England and Scotland (Moss *et al*., 2005). 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 target site resistance mutations appears more common, and was associated with resistance to the sulfonylurea metsulfuron, but not to the triazolopyrimidine florasulam. In contrast, the latter was associated with resistance to both herbicides. No evidence of resistance conferred by enhanced metabolism was detected, and this remains a mechanism much less commonly found in broad-leaved weeds compared with grass-weed.

In addition, a sample of *S. media*, collected in 2008 from County Down in Northern Ireland, was tested at Rothamsted in a standard glasshouse pot assay. This population was controlled very poorly by metsulfuron at 6 g a.i. ha^{-1} compared with a susceptible standard (31% *v* 95%; reduction in foliage weight, SE ± 2.9), confirming that it was resistant and representing the first documented case of a herbicide resistant weed in Northern Ireland. However, florasulam, fluroxypyr and mecoprop-P at field rates gave equally good (96–98%) control of both populations. Although mecoprop (1200 g a.i. ha^{-1}) gave very high reductions in fresh weight, plants of the Northern Ireland population took longer to die and three plants were not completely dead when assessed. In the English and Scottish populations of *S. media* studied by Marshall *et al*. (2010), no evidence of resistance to mecoprop-P or fluroxypyr was detected. However, some farmers and agronomists in both Scotland and Ireland are convinced that the efficacy of mecoprop 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 investigation may be warranted.

Additional testing of samples has been done since the last compilation exercise and, by 2010, ALS-resistant *S. media* had been identified on a total of > 40 farms in 13 counties, seven in Scotland (Aberdeen, Angus, Dumfries and Galloway, East Lothian, Fife, Perth & Kinross, South

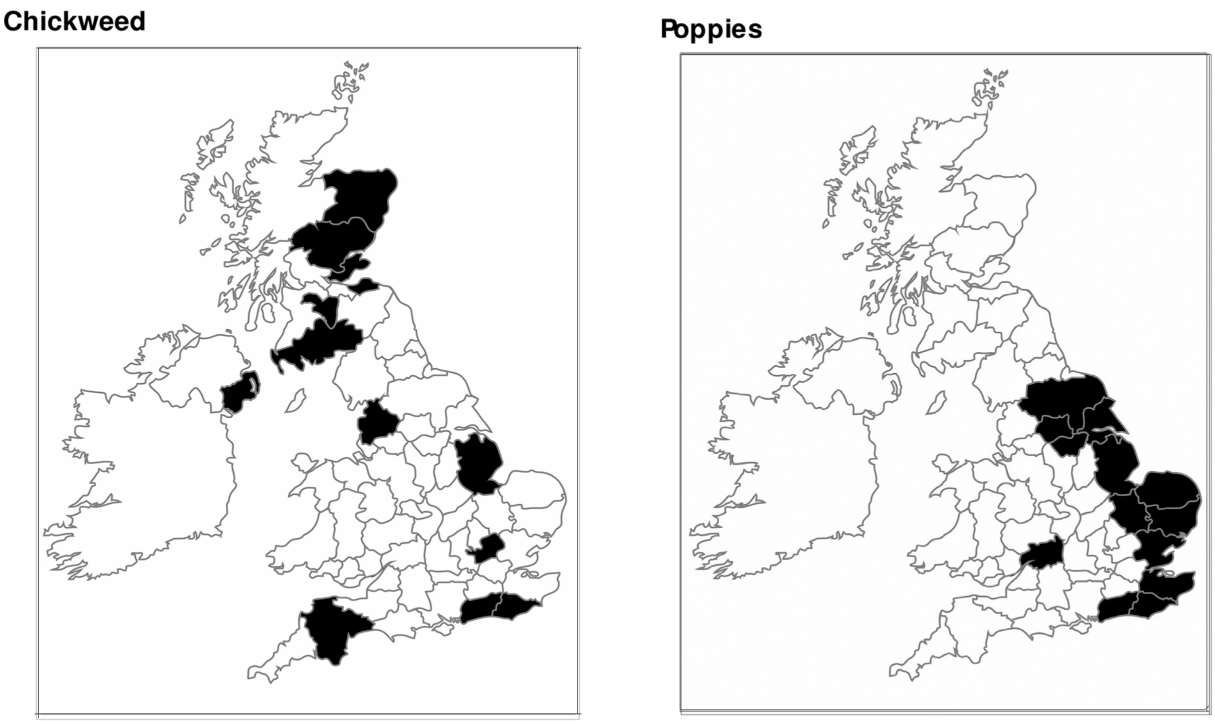

Fig. 4. Counties (13 and 9) with herbicide-resistant *Stellaria media* (chickweed) and *Papaver rhoeas* (poppy).

Lanarkshire), five in England (Devon, Hertfordshire, Lancashire, Lincolnshire, Sussex) and one in Northern Ireland (County Down) (Fig. 4).

Papaver rhoeas (Common poppy)

Herbicide-resistant populations of *P rhoeas;* have been found in six countries (Heap, 2011) and it is the most important herbicide-resistant broad-leaved weed in Europe (Moss *et al*., 2007). Most cases involve resistance ALS inhibiting herbicides, but resistance to the synthetic auxin herbicides has also been found in Spain (Cirujeda *et al.*, 2003). In the UK, ALS-resistant *P. rhoeas* was first detected in 2000 and, by 2004, had been found on 11 farms in seven counties in England (Moss *et al*., 2005). Studies into the mechanisms of resistance showed that resistance was conferred by ALS gene point mutations (Pro-197-Leu or Pro-197-His) (Marshall *et al*., 2010), although additional point mutations have been found in populations from Italy (Delye *et al.*, 2011). Resistance in the UK seems to be due to target site resistance as no evidence of resistance conferred by enhanced metabolism was detected. Alternative herbicides, such as MCPA and ioxynil+bromoxynil, were effective at controlling ALS-resistant populations (Marshall *et al*., 2010).

Additional testing of samples has been done since the last compilation exercise and, by 2010, ALS-resistant *P. rhoeas* had been identified on > 25 farms in nine counties of England (Fig. 4).

Tripleurospermum inodorum (Scentless mayweed)

Resistance in various 'mayweed' species had been recorded in a few countries worldwide (Heap, 2011). In the UK and France, there were reports of resistance to synthetic auxin herbicides in *T. inodorum* as long ago as the mid 1970s (Ellis & Kay, 1975). However, Gasquez (See Heap, 2011) maintains, "*Matricaria perforata* (=*T. inodorum*) resistant to synthetic auxins is a very old mistake. It has been impossible to confirm this report. After a large investigation there is no proof that there is today a resistant population of this species." More recently, in 2008, ALS-resistance was found in *Matricaria recutica* (Scented mayweed) in Germany (listed as *Matriacaria chamomilla* in Heap, 2011).

A sample (coded AMC) of *T. inodorum*, first collected in 2002 from a cereal field in Yorkshire, was tested alongside a susceptible standard population (Herbiseed09) in a glasshouse pot assay (Marshall *et al*., 2010). Plants (16 plants per treatment) were sprayed with field rates of metsulfuron, florasulam, and ioxynil + bromoxynil at the 1–4 leaf stage using a track sprayer delivering 224 L water ha^{-1} at 231 kPa through a single 'Teejet' flat fan 110015 VK nozzle. Untreated pots were also included for each population and foliage fresh weights were recorded 27 days after spraying (Table 1).

Table 1. *Response of two* T. inodorum *populations to three herbicides in a glasshouse assay*

Population	Mean % reduction in foliage fresh weight compared to untreated		
	Metsulfuron 6 g a.i. ha^{-1}	Florasulam 5 g a.i. ha^{-1}	Ioxynil + bromoxynil 400+400 g a.i. ha^{-1}
Susceptible standard	95.2	96.4	99.7
AMC (Lincs.)	0	96.2	99.7
		SE ± 2.2	

The AMC population was highly resistant to the sulfonylurea metsulfuron with all plants surviving treatment whereas the susceptible standard was well controlled. In contrast, there was no evidence of cross-resistance to the triazolopyrimidine herbicide florasulam, or to ioxynil/bromoxynil, which gave equally good (> 96%) control of both populations. This is the first documented case of ALS-resistant *T. inodorum* in the United Kingdom.

Testing of additional samples has been done and, by 2010, ALS-resistant *T. inodorum* had been identified on a total of three farms in one county (Yorkshire) in England.

Senecio vulgaris (Groundsel)

Senecio vulgaris was the first triazine-resistant weed species to be identified (Ryan, 1970). Triazine resistance became the most common type of resistance recorded worldwide between 1970 and 1990 being identified in over 65 species (Heap, 2011). Triazine-resistant populations of eight weed species, including *S. vulgaris*, have been recorded in the UK to date (Heap, 2011; Holliday & Putwain, 1977). EU regulatory action meant that the triazine herbicides, atrazine, cyanazine and simazine could no longer be used in the UK after 2007. Consequently, triazine resistance might be viewed as being of only academic interest now in the UK. However, triazinone herbicides, such as metribuzin and metamitron, are still widely used and have the same mode of action as the triazines, being inhibitors of photosynthesis at PSII. Both triazines and triazinones are in the same (C1) HRAC mode of action class.

Table 2. *Response of* Senecio vulgaris *populations to three herbicides in a glasshouse assay*

Population	% reduction in foliage fresh weight relative to untreated			
	Simazine g a.i. ha^{-1}			
	500	1000	2000	4000
Susceptible standard	99	99	99	99
Mean of four populations	3	1	2	3
(Range)	(0–8)	(0–3)	(0–9)	(0–13)
	SE ± 7.2			
	Metribuzin g a.i. ha^{-1}			
	350	700	1400	2800
Susceptible	99	100	100	100
Mean of four populations	45	76	98	99
(Range)	(43–47)	(70–78)	(98–99)	(99–99)
	SE ± 3.2			
	Metamitron g a.i. ha^{-1}			
	875	1750	3500	7000
Susceptible	99	99	99	100
Mean of four populations	68	90	98	99
(Range)	(62–71)	(88–92)	(96–99)	(99–99)
	SE ± 2.6			

Following reports of poor control by metribuzin, samples of *S. vulgaris* seeds collected in 2010 from four asparagus fields in Worcestershire and Warwickshire (coded HU10, HU11, T43, T44) were tested alongside a susceptible standard population (Herbiseeds06) in a standard glasshouse pot assay. Plants (10 plants per herbicide treatment) were sprayed with four rates of simazine, metribuzin and metamitron (+ 'Fyzol' @ 1% spray volume) at the 2–4 true leaf stage using a track sprayer delivering 217 L water ha^{-1} at 231 kPa through a single 'Teejet' flat fan 110015 VK nozzle.

Twenty untreated pots per population were also included and foliage fresh weights were recorded 24 days after spraying (Table 2).

The susceptible standard was completely controlled by all rates of all three herbicides, with no plants surviving. The four test populations (HU10, HU11, T43, T44) gave similar responses to each herbicide, so mean results are presented in Table 2. Simazine gave no control of any of the four test populations, even at the highest rate used, with 159 of the 160 treated plants surviving. This confirms a very high degree of triazine resistance in all four populations. The triazinones, metribuzin and metamitron, showed greater activity, but herbicide efficacy was significantly reduced at the lower two rates of both herbicides relative to the susceptible standard. At the lowest rate of herbicide applied, the proportion of plants which showed no, or only slight damage, was 98% for metribuzin and 65% for metamitron. At the second dose, equivalent to the field recommended rate, the respective proportions were 23% for metribuzin and 3% for metamitron. At the third rate, a small proportion of plants survived treatment (5% metribuzin; 15% metamitron) but all showed severe herbicide damage. All plants of all populations were killed at the highest dose applied of both herbicides.

These results confirm triazine resistance and partial cross-resistance to triazinone herbicides in these four populations of *Senecio vulgaris*. This is the first documented case of resistance to triazinone herbicides in the UK. Target site resistance is the most likely mechanism, being a consequence of a point mutation of the *psbA* gene resulting in a $Ser_{264} \rightarrow Gly$ substitution. This needs to be confirmed, but is the mechanism of triazine target site resistance in virtually all cases that have evolved in the field worldwide, and is well documented as conferring very high resistance to triazine herbicides and moderate cross-resistance to the triazinones (Gronwald, 1994). On the affected fields, triazinone herbicides had been applied annually in asparagus for at least the past 4–5 years, in sequence with other herbicides such as isoxaben and pendimethalin. Clearly, the use of other modes of action had not prevented evolution of triazine/triazinone site resistance.

Discussion

This update shows that the herbicide-resistant grass-weeds, *A. myosuroides, L. multiflorum* and *Avena* spp., occur widely in the main arable areas of England, but remain uncommon in other parts of the UK. *A. myosuroides* represents the major problem, and the increasing number of confirmed cases of resistance to mesosulfuron+iodosulfuron, the most widely used herbicide for its control, is worrying, especially as no new modes of action are likely to become available in the near future. Herbicide-resistant *L. multiflorum* and *Avena* spp. occur widely, but ACCase and ALS target site resistance appear to be less common than in *A. myosuroides*, which probably explains why control of these two species seems less problematic. However, increasing dependence on ACCase and ALS inhibiting herbicides, and lack of alternative modes of action, is likely to increase selection for resistance, so greater control problems may lie ahead.

Since the last compilation exercise in 2005, the number of cases of herbicide-resistant *S. media* and *P. rhoeas* has increased, and ALS-resistant *T. inodorum* has been documented in the UK for the first time. Despite the lack of resistant grass-weeds in Scotland, ALS-resistant *S. media* is widespread in the arable areas there, and was also detected in Northern Ireland for the first time. This may be due to a greater dependence on ALS inhibiting herbicides for broad-leaved weed control, a consequence of growing more spring cereals and less use of alternative modes of action for grass-weed control.

The International Survey of Herbicide Resistant Weeds lists 15 weed species as being resistant in the UK (Heap, 2011). However, eight of these are triazine–resistant weeds first documented in the 1970's and 1980's. There have been virtually no studies on the extent of triazine resistance in the UK for over 20 years, although it was thought to occur commonly in weeds in orchard, horticultural and amenity situations where triazines were used repeatedly. Although atrazine and simazine can no longer be used in the UK, the detection of partial resistance to triazinone herbicides shows that triazine/triazinone resistance is still relevant today. There may be a particular problem in perennial crops, such as asparagus, which are grown on the same land for many successive years thus limiting the opportunity for rotating herbicides with different modes of action.

Most of the seed samples of all species tested were not collected at random, but from fields where herbicides had given poorer than expected control. Thus, there is likely to be a bias towards fields with control problems which are more likely to be resistant. However, the proportion (70%) of *L. multiflorum* populations found to be resistant in the semi-random survey of 2006 and 2007 (Alarcon-Reverte, 2010), was identical to that recorded for non-randomly sampled farms (Moss *et al*., 2005). In addition, the incidences of resistance in *A myosuroides* to fenoxaprop (83%) and sethoxydim/cycloxydim (41%) in the 2005 non-random compilation exercise (Moss *et al*., 2005) was very similar to the results (80% and 40% respectively) from the smaller random survey conducted in 2002 (Moss & Perryman, 2007). The only other random surveys for resistance in England were conducted between 1988 and 1991 when the incidence of resistance was much lower (Clarke *et al*., 1994). These recent results indicate that non-random compilation exercises, which tend to be much easier and cheaper to conduct, are not always as biased as might be expected, and can provide an accurate indication of the current status of herbicide resistance. This has important logistical and financial implications for the detection and monitoring of resistance, but would benefit from additional validation with other resistant weeds in other countries.

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