Research Report

Analysis of grain aphid (*Sitobion avenae)* populations – genetic composition and the frequency of pyrethroid resistance

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1. SUMMARY

1.1. Aim

The overall aim of the project was to determine i) the distribution and frequency of the kdr mutation (conferring resistance to pyrethroids) in UK suction trap-collected samples of the grain aphid, *Sitobion avenae*, and ii) the level of genetic variation within these samples using high resolution microsatellite markers.

The grain aphid, *S. avenae,* can transmit destructive potato viruses such as PVY. Preventing crop losses due to significant virus accumulation normally requires some control and pyrethroid insecticides are most extensively used in this role. Pyrethroid spray failures were first noted in June 2011 against this pest on cereals in England. We examined these resistant populations and found they contained the kdr mutation in the sodium channel gene, with bioassays confirming a Resistance Factor of ~40 to lambda cyhalothrin. Additional testing of suction trap and field specimens showed that this resistance mechanism was reasonably widespread in the *S. avenae* population in England.

This project has measured the distribution and frequency of kdr aphids in UK populations collected from suction traps and used these to examine in more detail the genetic composition of resistant aphids compared to their sensitive counterparts. Each aphid has two matching sets of genes and, in all of the samples tested to date, only one set of the genes carries the knockdown resistance (kdr) mutation which confers reduced sensitivity to pyrethroids (the aphids are referred to as heterozygous; kdr-SR [Susceptible Resistant]). Work done for the Potato Council and HGCA in 2013 showed that the pyrethroid resistant *S. avenae* were comprised of one clonal type (named SA3). It is assumed that kdr-SR aphids are less resistant than if both sets of genes carried the mutation (homozygous; kdr-RR [Resistant Resistant]). It was considered important to continue monitoring grain aphids for kdr genotype and frequency, particularly in Scotland where the majority of the seed potato industry is found, and look for any significant changes in kdr zygosity or population genetic structure.

1.2. Methodology

The distribution and frequency of pyrethroid resistant *S. avenae* samples from England and Scotland in 2014 were established using a high throughput PCR-based TaqMan assay for detecting the kdr mutation in individual aphids. DNA extracted for kdr genotyping at Rothamsted was sent to the James Hutton Institute for microsatellite

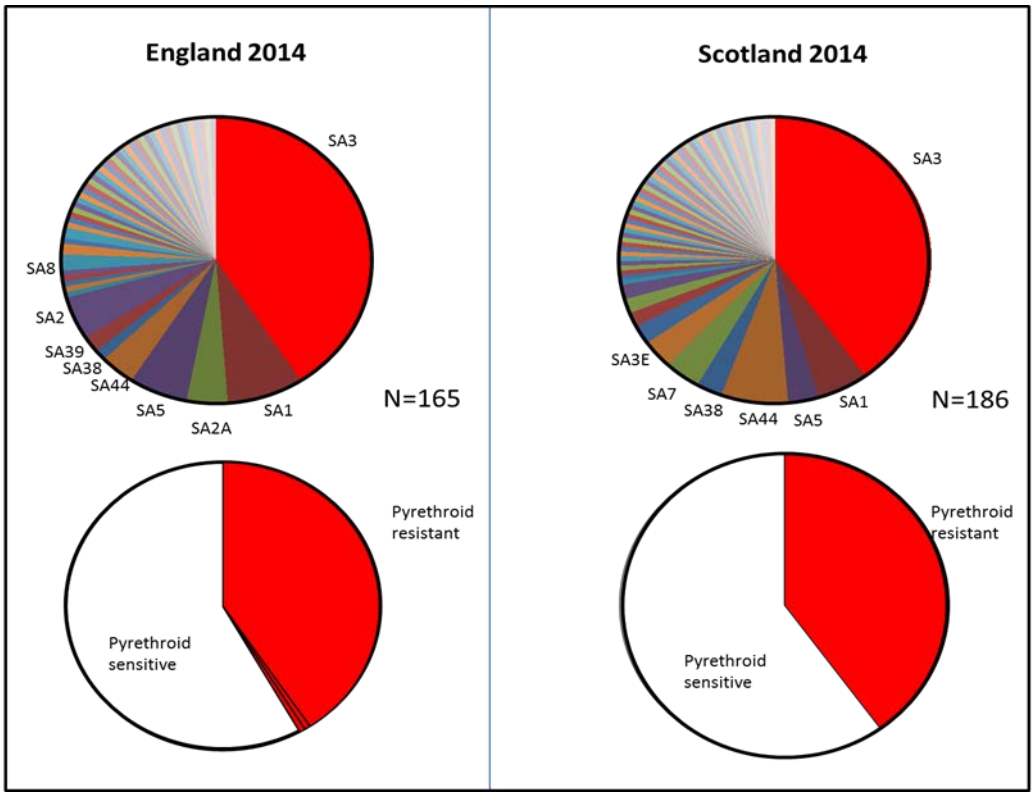
analysis. Suction trap samples were tested in real time from spring (early May) through to autumn (early September).

1.3. Key findings

A total of 1638 individual *S. avenae* from the 15 UK suction traps were tested for the kdr mutation. Overall frequencies in the English trap samples were similar to those reported in 2013, ranging from just over 60% at Kirton to 0% at Silwood Park. As previously, the highest frequencies were recorded at traps in more intensive cereal growing areas (Kirton 61%, Broom's Barn 53%, Writtle 43%) and this probably reflects a higher pyrethroid selection pressure in these areas. More dramatic changes were observed for the Scottish traps in 2014, particularly Edinburgh where the overall frequency of kdr-SR aphids increased from 8% in 2013 to 53% in 2014. Increases were also recorded for Dundee (from 27% to 33%) and Elgin (0% to 6%). Only 23 aphids were received from the trap at Ayr, two of which contained the kdr mutation. All the aphids that tested positive for the mutation, both English and Scottish, were SR heterozygotes. No RR homozygotes were identified in any of the samples.

A subset of *S. avenae* samples (165 from England, 186 from Scotland) were tested for genetic variability by microsatellite analysis. As in 2013, there was considerable genetic variation within these samples with a total of 29 different clonal types (defined as 2 or more individuals showing the same genotype profile) and a further 80 individuals (36 from England, 46 from Scotland) showing unique profiles. Of the different clones, SA3 was once again the dominant type occurring in 67 of the English aphids tested and 75 of the Scottish aphids. This was as expected since SA3 was known to be the major clone type carrying the kdr mutation from our 2013 analysis, and once again all 142 of the SA3 aphids were scored as kdr-SR in the mutation testing. Interestingly, two individuals were identified in England with the kdr mutation that did not belong to the SA3 genotype. One of these had a unique genotype profile (named as 'new R genotype') and one showed the clone SA8 genotype, which is normally susceptible. The discovery of these two non-SA3 aphids carrying the kdr mutation indicates that it is possible for the mutation to move/arise in other genetic backgrounds.

2014: Genotypic composition of the *Sitobion avenae* UK population in relation to pyrethroid resistance.



In Scotland the genotypic diversity of the grain aphid population has decreased compared to 2013. In 2013 only ~10% of the population was derived from a clone. This contrasts with ~75% of the population by 2014. Current hypotheses suggest that genotypic diversity in *S. avenae* increases moving north where the conditions favour the sexual cycle and more individuals are derived from sexual reproduction. However, the results suggest that selection pressure imposed by pyrethroid based insecticide usage maybe altering the genotypic composition of the northern UK *S. avenae* populations.

1.4. Further Information

There are several sources of information on the occurrence of aphids during the growing season. These include information from the suction trap network, which provides information on the numbers of individual species including *S. avenae*:

http://www.rothamsted.ac.uk/insect-survey/bulletins

There is also information based on the numbers of aphids (which are vectors of potato viruses) caught in yellow water traps:

http://www.potato.org.uk/online-toolbox/aphid-monitoring

These can be used to indicate if/when numbers of *S. avenae* are present or increasing at susceptible crop stages. Consult a BASIS-registered advisor regarding the aphid management options that may need to be applied.

2. INTRODUCTION

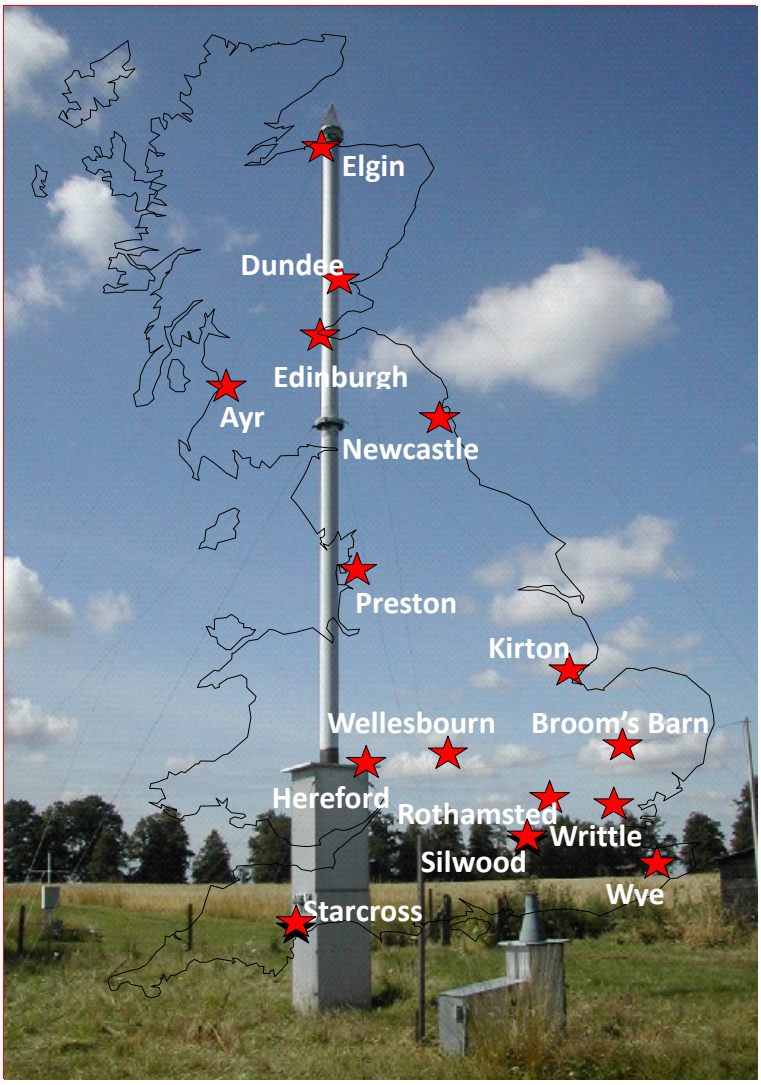
The grain aphid, *Sitobion avenae,* transmits potato viruses such as PVY and pyrethroid insecticides are used extensively to control this important vector. Spray failures were first detected in 2011 on cereal crops in England and the resistant populations were found to have the kdr mutation in their sodium channel gene that is consistent with resistance to pyrethroids. Monitoring of the grain aphid populations caught in the English suction trap network in 2013 and the Scottish trap network in 2012 and 2013 was carried out during the summer/autumn of 2013 (see HGCA/Potato Council report for 2013). The results indicated high frequencies of the resistant aphids in some of the English traps and kdr aphids were also caught for the first time in the Scottish traps at Dundee and Edinburgh. The *S. avenae* populations were analysed using high resolution microsatellite markers and the initial results suggested that the kdr mutation is only found in one clonal type (named SA3). However resistance mutations could occur in other genotypes and it was important that monitoring for the kdr genotype and frequency was continued, particularly in Scotland where the majority of the seed potato industry is found. In this study we continued to carry out resistance testing and genotyping of the UK *S. avenae* population in 2014 and compare the genotypic composition and frequency of the kdr resistant genotype to that found in 2013.

3. MATERIALS AND METHODS

Insect samples

Sitobion avenae were collected from fifteen 12.2m high suction traps across the UK; eleven in England and four in Scotland (Fig 1). English trap samples were provided by Richard Harrington and colleagues at the Rothamsted Insect Survey; Scottish trap samples were provided by Fiona Highet and Helen Payne at SASA, Edinburgh. All samples were stored in 90% ethanol prior to DNA extraction. Collection from suction traps ensures that the aphid population is sampled randomly and thereby gives a good measure of population diversity for kdr and microsatellite genotype.

Figure 1. UK suction trap sites



DNA extraction and kdr genotyping

DNA was extracted from adult aphids using a modification of the sodium hydroxide method described by Malloch *et al*. (2006). A total of 1,638 aphids were tested from the 15 traps (Table 1). Individual aphids were homogenised in the wells of a 96 well immunoplate with 20 µl of 0.25 M NaOH. The homogenates were heated at 99^{o}C for 3 min and neutralised with 10 µl of 0.25 M HCl, 5 µl of 0.5 M Tris HCl and 5 µl of 2 % Triton X-100. Samples were heated again at 99^{o}C for 3 min and the plates centrifuged at 4000 rpm for 5 min. Aliquots of the DNA supernatants were initially taken for kdr genotyping, with selected samples sent to James Hutton institute for microsatellite analysis.

A PCR-based allelic discrimination assay (TaqMan) was used to detect the presence/absence of a mutation (kdr) in individual aphids and genotype them as susceptible (kdr-SS) or resistant (kdr-SR, kdr-RR). The technique uses short

fluorescent dye-labelled DNA probes that are selective for either the normal (susceptible) gene or the kdr (resistant) gene sequence. TaqMan PCR reactions were run on a Rotor-Gene 6000TM real-time PCR cycler using cycling conditions of 10 min at 95^{o}C, followed by 40 cycles of 95^{o}C for 10s and 60^{o}C for 45s.

Table 1 Collections of *Sitobion avenae* from suction traps used for kdr genotyping

Location	Months of collection	No. of insects
Dundee	May-August 2014	220
Edinburgh	May-August 2014	209
Elgin	May-August 2014	202
Ayr	June-July 2014	23
Kirton	April-August 2014	74
Rothamsted	April-August 2014	139
Broom's Barn	April-August 2014	116
Hereford	April-August 2014	82
Silwood Park	April-August 2014	44
Starcross	April-August 2014	135
Newcastle	April-August 2014	60
Preston	April-August 2014	88
Writtle	April-August 2014	129
Wye	April-August 2014	31
Wellesbourne	April-August 2014	86
	Total tested	1638

Microsatellite genotyping

A sub-sample of 378 *S. avenae* were selected and analysed for microsatellite genotype. These aphids were collected from the four traps in Scotland and nine traps in England in 2014 (Table 2). Twenty seven individuals failed to produce a trace that was of high enough quality for scoring. These were removed from the analysis and in total, 165 English aphids and 186 Scottish aphids were successfully genotyped.

Table 2 Collections of *Sitobion avenae* from suction traps used for microsatellite analysis

Location	Months of collection	No. of insects	No. of resistant insects
Dundee	May- July 2014	62	29
Edinburgh	May -July 2014	66	37
Elgin	May-July 2014	41	7
Ayr	June-July 2014	17	2
Kirton	June-July 2014	21	14
Rothamsted	June 2014	19	7
Broom's Barn	June-July 2014	33	24
Hereford	June 2014	15	3
Starcross	June 2014	15	1
Preston	May-June 2014	21	6
Writtle	June 2014	20	13
Wellesbourne	June 2014	14	0
Silwood Park	April – May 2014	7	0

Genotypes of individual *S. avenae* were examined at five microsatellite loci: Sm10, Sm12, Sm17, Sa4 and S16b. Sm10, Sm 12 and Sm17 isolated from *Sitobion miscanthi* and described by Wilson *et al*., 1997 and Sunnucks *et al.*, 1996, 1997. Primer sequences are reported for the first time in Simon *et al*., 1999. Primer Sa4 was cloned from *S. avenae* (Simon *et al.*, 1999) and primer S16b was isolated from *S. miscanthi* and its sequence is published in Wilson *et al*., 2004, see Table 3.

Table 3 Primer sequences

Primer	Sequence	Repeat	Size range published	Size range observed	Reference
Sm10f	TCT GCT GCA TTA CTG TTG GC	(CA)23	152-240	149-197	SIMON ET AL 1999
Sm10r	TCG TCT ACT TCG CCG TCA	(CA)23	152-240	149-197	SIMON ET AL 1999
Sm12 f	CAC CAT CGC GTT TCA TCT TA	(CA)33	127-177	112 (133)-154(175)	Llewellyn et al 2003
Sm12r	ACT CCC AAC CTC TGA TGA GC	(CA)33	127-177	112 (133)-154(175)	Llewellyn et al 2003
S16bf	ATA AAA CAA AGA GCA ATT CC	(CA) 14	166-206	158-281	Wilson et al 2004
S16br	GTA AAA GTA AAG GTT CCA CG	(CA)14	166-206	158-281	Wilson et al 2004
Sm17f	TGG ACA TTT CAT CGT TCG C	(TC)14AC(TC)3	174-185	88-97	Simon et al., 1999
Sm17r	ATG CGT TCG AGT TTA CCT GC	(TC)14AC(TC)3	174-185	88-97	Simon et al., 1999
SA4ΣF	GTG ACG TAT AAC GCG ATG CG	(AC)5TT(AC)16	162-176	155-213	Simon et al 1999
SA4ΣR	GAC GTC GAT ATT AGC CTA GCC	(AC)5TT(AC)16	162-176	155-213	Simon et al 1999

PCR was carried out in 8ul volumes using Illustra^{TM} Ready to Go PCR beads (GE Healthcare). When the bead is reconstituted the concentration of each dNTP is 200uM in 10mM Tris-HCl 50mM KCl and 1.5mM $MgCl_2$. Each bead contains 2.5U of *Taq* DNA polymerase. PCR was carried out on a Biometra T Personal thermal cycler using the Touchdown programme described in Sloane *et al*., (2001).

4. RESULTS

An allelic discrimination (TaqMan) PCR diagnostic test which detects the presence of the kdr mutation (L1014F) has been developed for *S. avenae* (see Materials and Methods). The TaqMan assay is a PCR method that uses oligonucleotide probes that are dual labelled with a fluorescent reporter dye and a quencher molecule. Amplification of the probe-specific product causes cleavage of the probe, generating an increase in reporter fluorescence as the reporter dye is released from the quencher. By using different reporter dyes (VIC and FAM), cleavage of the allele-specific probes can be detected in a single PCR reaction. Comparison of control DNA from *S. avenae* of known genotypes allows discrimination of the wild-type and resistant (kdr) alleles. The assay uses two probes, and an increase in fluorescence indicates whether the individual is a homozygous wild type individual (kdr-SS), a heterozygous mutant type (kdr-SR) or a homozygous mutant type (kdr-RR). To help assign the genotypes, software is used to plot fluorescence values for the two dyes on bidirectional scatter plots.

kdr-SR frequency

A total of 1,638 *S. avenae* were tested from the 15 suction traps; 984 from the 11 English traps and 654 from the 4 traps in Scotland (Table 1). Only 23 aphids were available from the trap at Ayr, however over 200 were tested from each of the other 3 Scottish traps at Dundee, Edinburgh and Elgin. The numbers and frequency of resistant (kdr-SR) and sensitive (kdr-SS) *S. avenae* from each trap are shown in Figs. 2-5. The frequency of kdr-SR in the English trap samples were broadly similar to those reported in 2013, ranging from just over 60% at Kirton to 0% at Silwood Park. Kirton has consistently shown the highest kdr-SR frequency, closely followed by Broom's Barn (53%), which probably reflects the higher pyrethroid selection pressure in these cereal growing areas. There were more dramatic changes in Scotland, and particularly Edinburgh, where the overall frequency of kdr-SR aphids increased from 8% in 2013 to 55% in 2014. There were also modest increases in kdr frequency at Dundee (from

27% in 2013 to 33% in 2014) and Elgin (from 0% to 6%). Of the 23 aphids tested from Ayr, two were found to carry the kdr mutation. As in previous years, only kdr-SR heterozygote aphids were found; no kdr-RR homozygotes were identified in any of the samples tested.

Figure 2. Number of kdr-SR and kdr–SS *S. avenae* in English suction traps in 2014

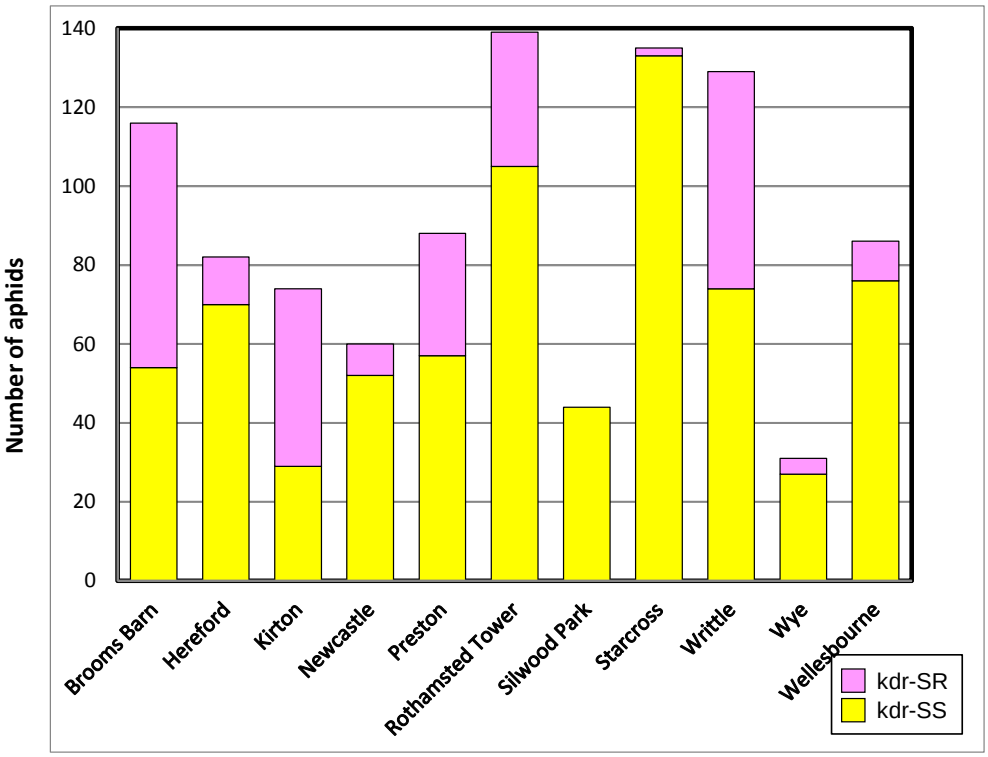


Figure 3. Frequency of kdr-SR and kdr–SS *S. avenae* in English suction traps in 2014

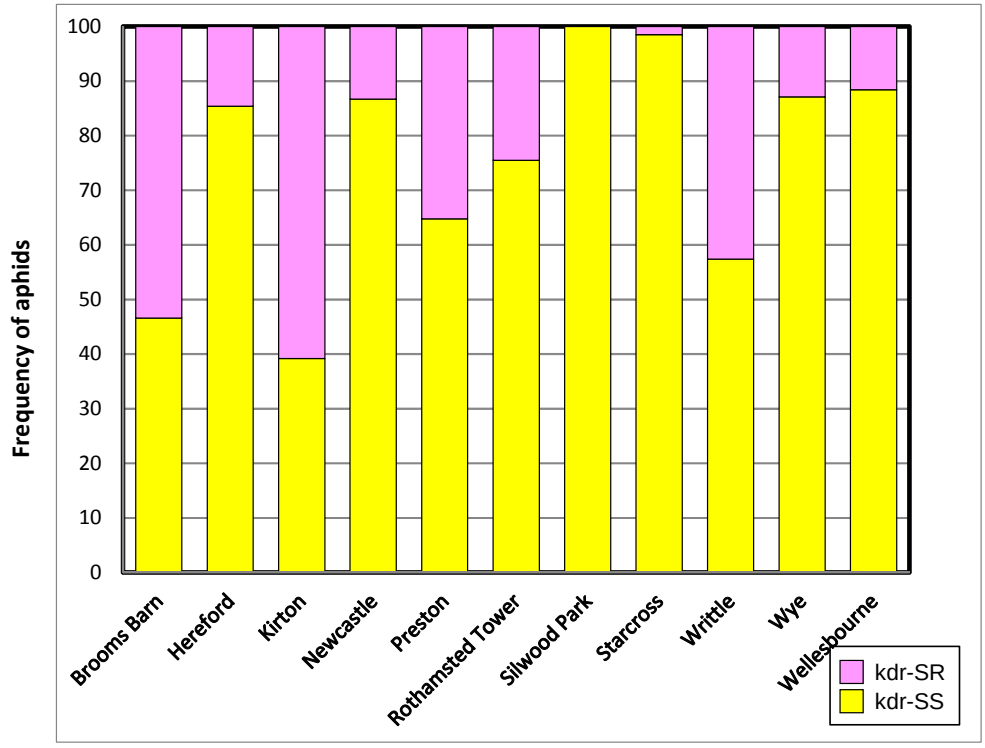


Figure 4. Number of kdr-SR and kdr–SS *Sitobion avenae* in Scottish suction traps in 2014

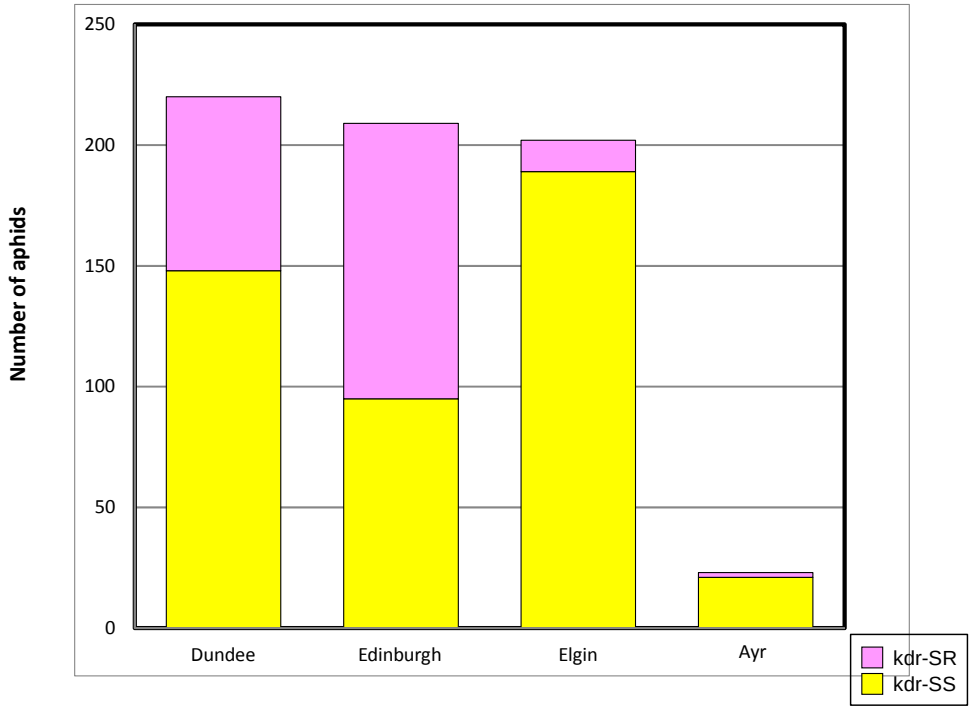
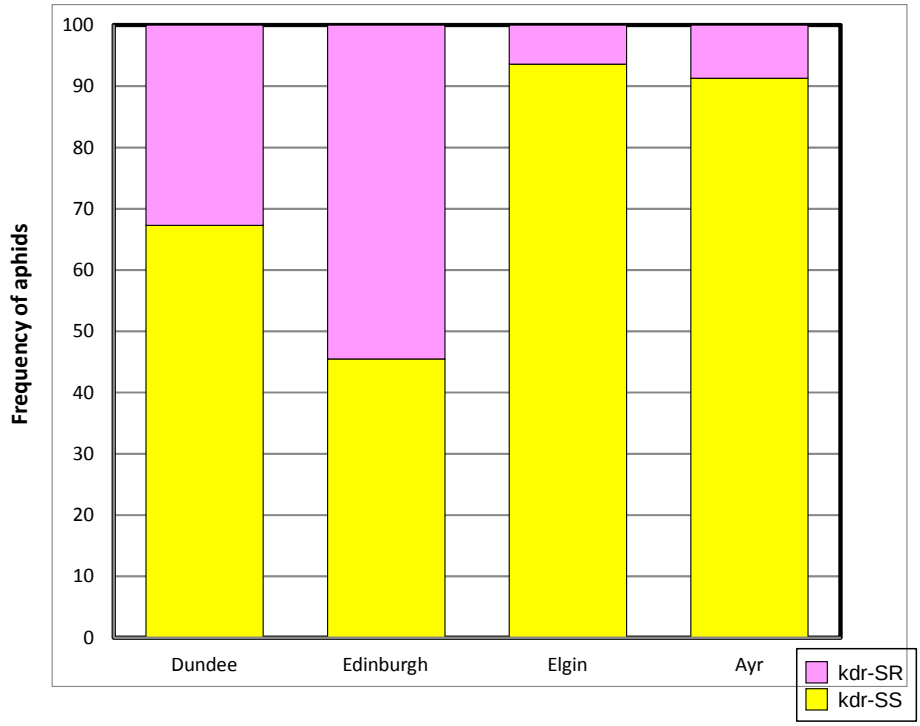


Figure 5. Frequency of kdr-SR and kdr–SS *Sitobion avenae* in Scottish suction traps in 2014



Genotypic composition of the UK population

A total of 351 aphid DNAs were successfully scored by microsatellite analysis (186 from Scotland and 165 from England). Several common insecticide susceptible clones were found in both England and Scotland and many of these had also been found in samples analysed in 2012 and 2013 (Table 4).

Table 4 Common *Sitobion avenae* genotypes

Genotype	Resistance	England 2013	England 2014	Scotland 2012	Scotland 2013	Scotland 2014	UK 2013	UK 2014
SA3	SR	32	67	9	7	75	39	142
SA1	SS	6	13	5	2	10	8	23
SA2A	SS	6	7	1	0	0	6	7
SA2	SS	2	8	0	0	0	2	8
SA5	SS	3	10	0	0	6	3	16
SA44	SS	14	6	0	2	13	16	19
SA16	SS	4	0	0	0	0	4	0
SA6	SS	2	1	0	0	0	2	1
SA7	SS	1	0	1	2	7	3	7
CLONE SCOT1	SS	0	0	0	2	0	2	0
SA11	SS	1	1	1	1	3	2	4
SA1D	SS	0	1	2	0	4	0	5
SA38	SS	1	2	1	1	5	2	7
SA39	SS	1	3	1	0	0	1	3
CLONE SCOT 2	SS	0	0	0	2	0	2	0
UNIQUE	SS	24	34	39	84	46	108	80
NEW R GENOTYPE	SR	0	1	0	0	0	0	1
SA3E	SS	0	1	1	0	6	0	7
SA8	SS SR	0	3	0	0	1	0	4
SA44C	SS	0	0	0	0	3	0	3
SA10	SS	1	1	0	0	0	1	1
CLONE F	SS	0	2	0	0	0	0	2
SA AYR 13 1	SS	0	2	0	1	0	1	2
SA AYR 13 2	SS	0	1	0	1	1	1	2
CLONE 2014 1	SS	0	0	0	0	3	0	3
scot 4	SS	0	0	1	0	1	0	1
sa14	SS	0	0	1	0	1	0	1
clone 2014 2	SS	0	1	0	1	0	1	1
clone 2014 3	SS	0	0	1	1	0	1	0
clone 2014 4	SS	0	0	0	1	1	1	1
TOTAL		98	165	64	108	186	206	351
FAILS			21			6		27
BLANKS AND CONTROLS			6			0		6
TOTAL TESTED			192			192		384

Table 5 Allele sizes of common clones

Genotype	S16b	S16b	Sm12	Sm12	Sm10	Sm10	Sm17	Sm17	sa Σ4	saΣ4	Location
SA3	173	211	115	146	161	163	92	96	162	163	UK
SA8	173	211	144	146	161	160	92	93	162	163	UK and France
New SR Genotype	173	250	143	144	160.66	162	92	93	167	169	England
SA2A	173	211	115	126	160	163	92	96	163	172	UK
SA2	173	211	115	126	149	163	92	93	162	172	England
SA3E	173	211	115	115	160	160	92	96	163	172	UK
SA7	173	217	115	115	160	160	92	96	163	172	UK
SA11	173	215	115	126	161	163	92	93	162	163	UK
SA1	173	266	115	115	157	161	92	96	163	172	UK
SA5	173	266	115	134	160	161	92	96	163	172	UK
SA1D	211	217	115	115	161	161	92	93	163	172	UK
SA38	211	241	115	136	160	163	92	96	161	163	UK
SA39	211	279	115	115	160	161	92	93	161	163	UK
SA44	266	279	115	128	160	161	92	96	161	164	UK
SA44C	266	279	113	113	160	160	95	95	161	163	Scotland
2014 1	233	258	115	126	160	161	96	97	161	163	Scotland

Results of the previous project carried out in 2013 indicated that the kdr mutation was found in only one clonal genotype (SA3). This was set against a background of high genetic diversity within a general susceptible population consisting of many genotypes (Figure 6B). In Scotland in 2013, this resistant genotype (SA3) was only found in trap samples from Dundee and Edinburgh at a low level (~10%). Results from the current study (2014) indicate that the frequency of the resistant genotype in Scotland has increased to more than 40% (Fig 6A) and in addition to the Dundee and Edinburgh traps, the resistant clone was also found in small numbers in trap catches from Ayr and Elgin (Table 2).

Evidence of kdr mutation in two additional genotypes

In 2014, there is also evidence that the kdr mutation has been found in two additional genotypes caught in the English suction traps (Preston and Rothamsted). One of these genotypes (caught in the Rothamsted trap) had some unusual allele sizes (termed 'New SR genotype', Table 5). The other individual, found at Preston, was a representative of a common clone SA8. This genotype shares many alleles with the common resistant SA3 genotype (Table 5). Other representatives of this clone (SA8) were found in field collected samples from the UK in 2013 and also in a collection of *S. avenae* from France in 2013. However, kdr resistance testing has always indicated that the other representatives of this clone are susceptible to insecticides (SS). The DNA from the kdr resistant individual was re-tested for both the kdr mutation and the genotype profile and the SR result and SA8 genotype was

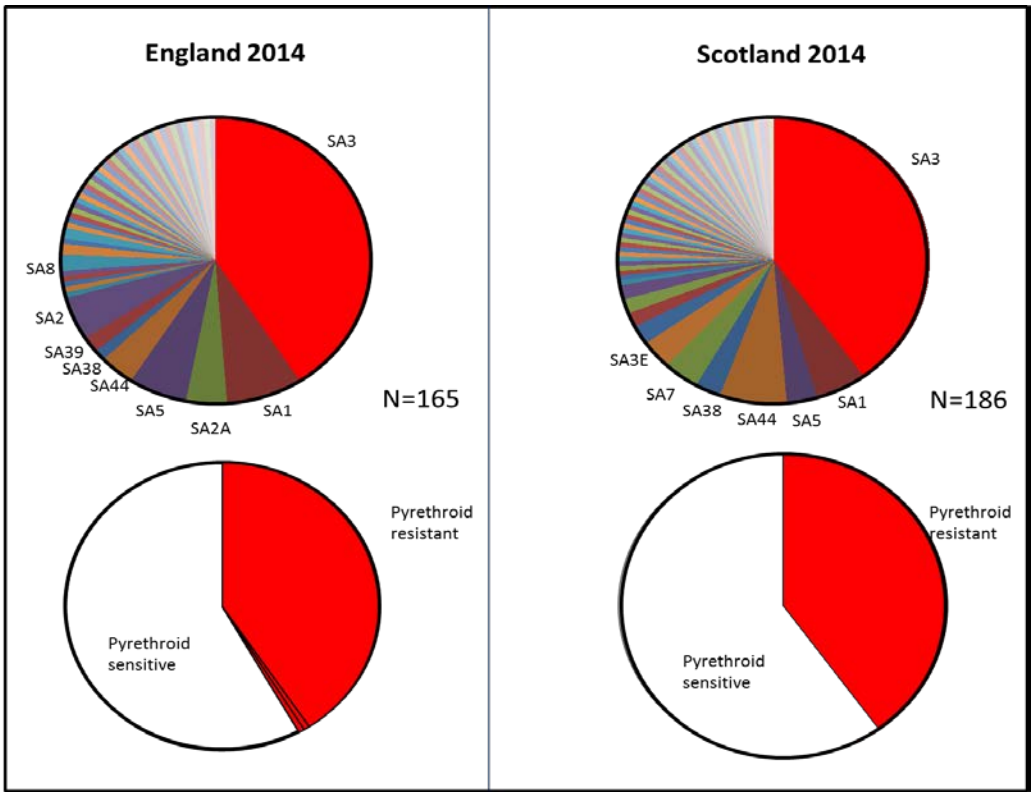
confirmed. No other representatives of the New SR genotype were detected but three other SA8 individuals were found in the suction trap material in 2014 and all scored as (SS) susceptible.

Seasonal and geographical variation in clones

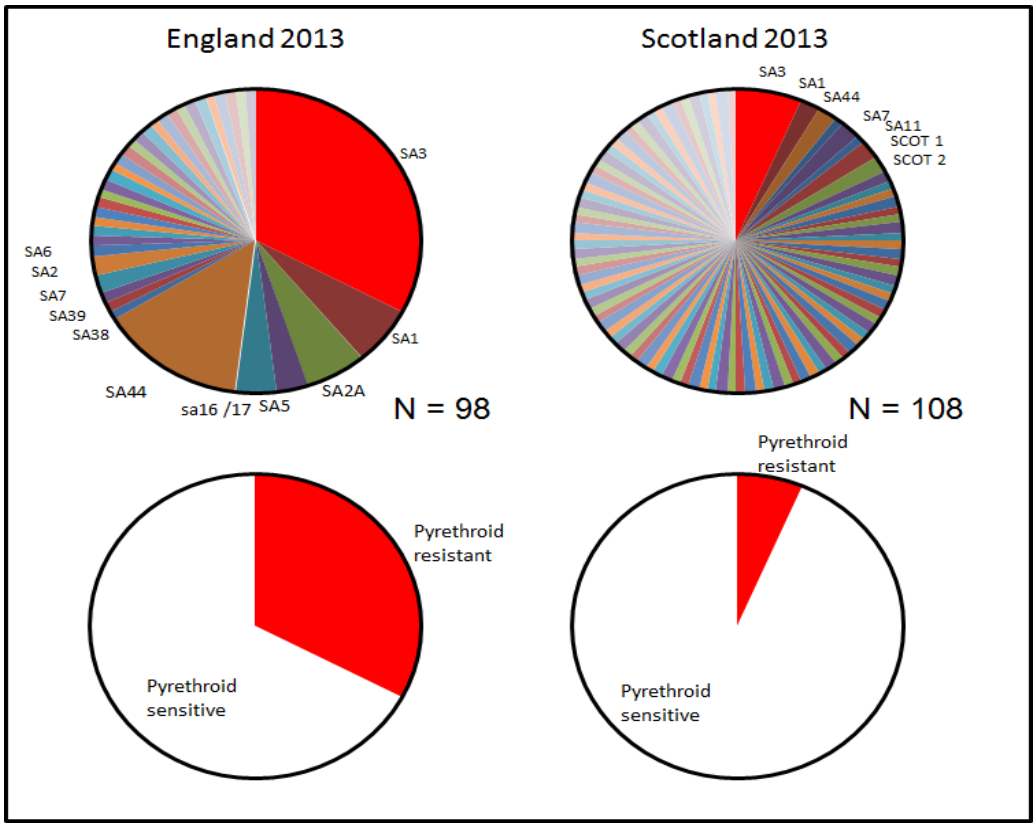
Wherever a genotype appears more than once, then this is considered as evidence of a clone. A simple measure of genotypic diversity can be calculated using the formula k=G/N where G is the number of different genotypes present in a sample and N is the sample size. In both England and Scotland in 2014 there were a total of 105 genotypes of *S. avenae* from a sample of 351 k = 0.3 (Table 4). This represents a decrease in the diversity as in 2013 158 genotypes were found from a total sample of 270 k = 0.58. In Scotland this reduction in the genotypic diversity of the population is quite dramatic as in 2013 only ~10% of the population was derived from a clone and this contrasts with ~75% of the population by 2014 (Figs 6A and 6B). If we compare the genotypic diversity in England in 2014 (k=0.32) to that of Scotland in 2014 (k= 0.33) we find that the levels are now very similar. Current hypotheses suggest that genotypic diversity in *S. avenae* increases moving north where the conditions favour the sexual cycle and more individuals are derived from sexual reproduction. However, selection pressure imposed by pyrethroid based insecticide usage appears to be dramatically altering the genotypic composition of the northern UK *S. avenae* populations.

Figure 6. Genotypic composition of the *Sitobion avenae* UK population in relation to pyrethroid resistance.

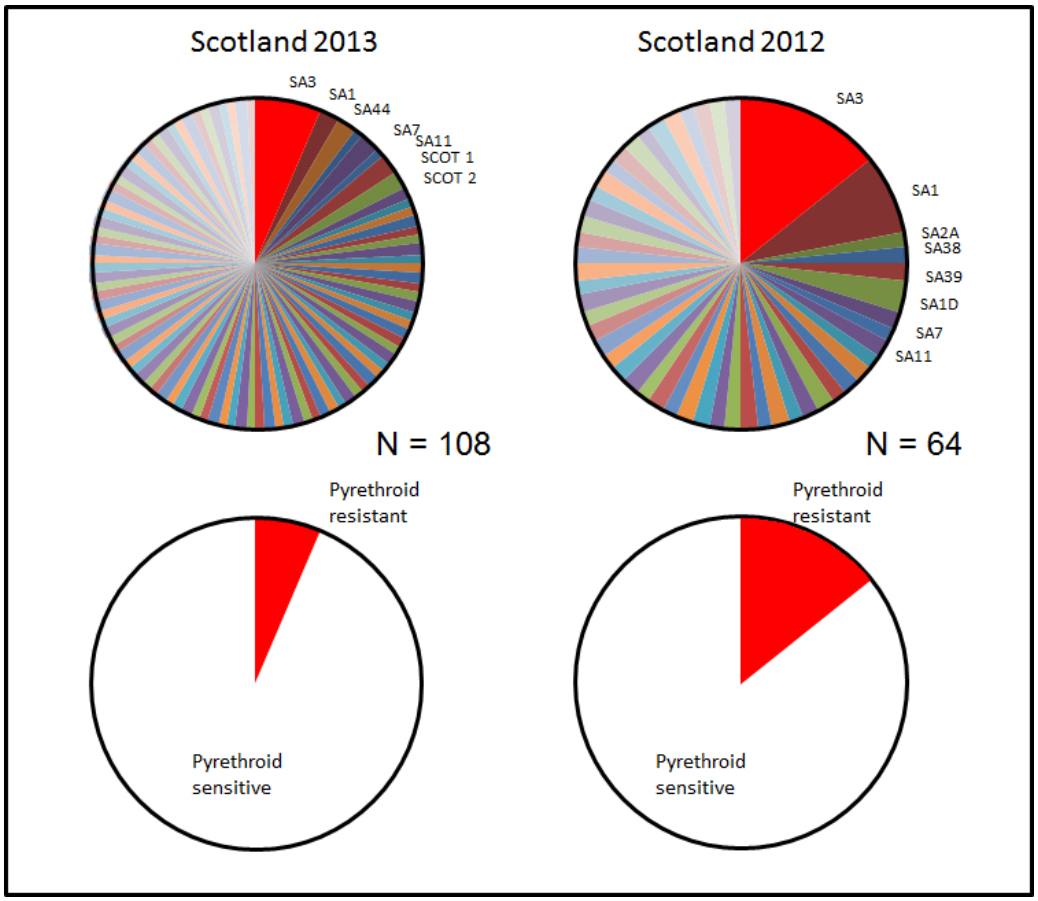
A. England vs Scotland 2014.



B. England vs Scotland 2013.



C. Scotland 2012 vs Scotland 2013.



5. DISCUSSION

Overall, the frequency of *S. avenae* carrying the resistant kdr mutation collected in the English suction traps was similar in 2012 and 2013 and has increased a little in 2014 suggesting that this form of resistance may have stabilised. However, the frequency of kdr has dramatically increased in Scotland between 2013 and 2014. Previous studies suggest that clonal diversity of *S. avenae* varies, with more diversity in northern regions. However, the results from 2014 indicate that the levels of genotypic diversity in Scotland and in England are now very similar.

Common clones of *S. avenae* were present and many of these were the same as those identified in the study carried out in 2013 supporting the hypothesis that common *S. avenae* clones e.g. SA1, SA3 and SA44 are asexual and have lost the ability to produce sexual forms. The candidate asexual clones include the resistant clone SA3 which survives from one year to the next. If this clone was capable of undergoing sexual reproduction to produce overwintering eggs then the kdr mutation would be found in new genetic backgrounds the following season (assuming that

there are no strong fitness costs for kdr-RRs). We have some evidence that this may be occurring as the results suggest one individual of a closely related genotype SA8 appears to carry the kdr mutation. However, other individuals of this clone were found to be kdr insecticide sensitive (kdr-SS). Further monitoring of the population would determine if this finding is significant. In addition, to the kdr resistant SA8 individual, a single example of a second new genotype which has not been found previously, was found in England in 2014 (new R genotype). If the kdr mutation has spread to new genetic backgrounds and those backgrounds can tolerate any negative fitness costs we would expect the numbers of these genotypes to increase under insecticide resistance selection pressure. Furthermore, if these clones can produce sexual forms the kdr mutation could be brought together in kdr-RR (homozygote) aphids which would probably be more resistant to pyrethroids than heterozygotes that currently predominate in some areas.

Technically it is possible to screen historical *S. avenae* samples for the presence of resistant genotypes should funding be available. Should any of the pyrethroid resistant genotypes be found as a historically common clone then this would suggest that they are robust (well suited to UK survival) and are likely to continue to be dominate the population.

6. REFERENCES

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7. KNOWLEDGE TRANSFER ACTIVITIES

Presentations

- B Fenton, G Malloch, M Williamson & S Foster. *Sitobion avenae* – the grain aphid: could it become a threat to your seed potato crop? *Potatoes in Practice Seminar*, Dundee, August 2014.
- S Foster. Insecticide resistance in aphids. *European Congress of Entomology*, York, August 2014.
- S Foster. Pyrethroid resistance in the grain aphid, *Sitobion avenae. Bayer Press Meeting*, Cambridge, May 2014.
- G Malloch. *Sitobion avenae* – the grain aphid: is it a new vector threat to your potato crop? *Scottish Society for Crop Research: Potato Committee: Winter Meeting*, James Hutton Institute, Dundee, March 2014.

Articles in Farming and Popular Press

- Resistant aphids pose BYDV threat (*CPM Magazine*, July 2014)
- How is the grain aphid overcoming pyrethroid insecticide sprays? (*Crops*, July 2014)
- BYDV likely to increase (*Farmers Guide*, May 2014)
- BYDV set to increase (*Anglian Farmer*, May 2014)
- Aphids on the march (*Farm Business*, May 2014)
- BYDV likely to become more important for Scottish farmers (*Scottish Farmer*, May 2014)
- *Sitobion avenae* and pyrethroid resistance (*Farmers Weekly*, July 2014).
- Controlling aphids and virus diseases in cereals and oilseed rape (*HGCA e-newsletter*, October 2014)

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