Research Report

Monitoring pyrethroid resistance (kdr) and genetic diversity in UK populations of the grain aphid, *Sitobion avenae* during 2015

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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. Rothamsted Research examined these resistant populations and found they contained the kdr mutation (L1014F) in the sodium channel gene, with bioassays confirming a Resistance Factor of ~40 to lambda cyhalothrin (Foster *et al*., 2014). Additional testing of suction trap- and field-collected samples showed that this resistance mechanism was reasonably widespread in the *S. avenae* population.

The current project has measured the distribution and frequency of kdr aphids in English and Scottish populations collected from suction traps and has used these to examine in more detail the genetic composition of pyrethroid-resistant aphids compared to their pyrethroid-susceptible (SS) counterparts. Work carried out for AHDB in 2013 and 2014 showed that pyrethroid-resistant *S. avenae* were all heterozygotes (i.e. they carried the kdr gene only in the heterozygous form: SR) and were comprised of one clonal type (named SA3). These forms are likely to be less resistant than kdr homozygotes (RR), should they arise. It is therefore important to continue to monitor the UK population for kdr genotype and frequency, particularly in Scotland where the majority of the seed potato industry is found, and look for any potential 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 2015 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 (April) through to autumn (October).

1.3. Key findings

A total of 1,003 individual *S. avenae* collected from the 16 UK suction traps (12 in England and 4 in Scotland) were tested for the kdr mutation. Overall frequencies in the English trap samples were similar to those reported in 2013 and 2014, ranging from just over 76% at York to 0% at Wye. As previously, the higher frequencies tended to be recorded at traps in more intensive cereal growing areas (Kirton 42% and Broom's Barn 46%) and this probably reflects a higher pyrethroid selection pressure in these areas. Having said this, the highest kdr frequencies were found at York and Preston (76% and 53 %, respectively). In Scotland, kdr frequencies were similar to those found in 2014 with Dundee having the highest frequency (45%). All of the aphids that tested positive for the kdr mutation, both from England and Scotland, were heterozygotes (SR). As in previous years, no kdr homozygotes (RR) were identified in any of the samples.

A subset of *S. avenae* samples (150 from England and 125 from Scotland) were successfully tested for their genetic variability by microsatellite analysis. As in 2013 and 2014, there was considerable genetic variation within these samples with a total of 102 different genotypes being detected. There were 9 genotypes that were only found once in 2015 but had been found in the UK in previous years and two individuals that had identical genotypic profiles to *S. avenae* individuals that had been collected in Slovenia in 2014. There were 72 individuals (39 from England and 33 from Scotland) with unique profiles that had not been detected previously. Clone SA3 was once again the dominant type occurring in 64 of the English aphids tested and 42 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 and 2014 analysis, and all 106 SA3 aphids scored as kdr-SR in the mutation testing. Unlike the study in 2014, there were no individuals identified with the kdr mutation that did not belong to the SA3 genotype. In 2014, two individuals were detected that had the kdr-SR mutation but were not SA3 genotypes. One of these had a unique genotype profile (named as 'new R genotype') and one was an example of the SA8 genotype, which is normally susceptible. It was thought that these genotypes could have a selective advantage and would therefore increase in frequency. However, our current 2015 study has demonstrated that this did not occur. Three representatives of the SA8 genotype were found in 2015 and all were the SS insecticide sensitive genotype. No 'new R genotype' individuals were detected.

Analysis of historical *S. avenae* DNA (collected in England in 1997 and 1998) did not identify the resistant SA3 clone, suggesting that it was not present (or present at a very low frequency) in the UK at that time. Several genotypes were identified that are still represented in current populations. Interestingly three very common clones found in 1997 and 1998 (A, C and E) are no longer found. The reasons for their demise are not known but the finding indicates that successful clones of *S. avenae*, like successful clones of UK *M. persicae*, are subject to clonal turnover processes (Fenton *et al*., 2005; Kasprowicz *et al.,* 2008).

1.4. Practical recommendations

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 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 vulnerable 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, as a result, pyrethroid insecticides have been used extensively to control this important vector. Spray failures with these compounds were first detected in 2011 on cereal crops in England and resistant populations were found to contain aphids carrying the classic kdr mutation (L1014F) in their sodium channel gene. This has been found in other pests and is consistent with resistance to pyrethroids. Previous monitoring of the *S. avenae* populations caught in the English and Scottish trap networks from 2012-2014 (see AHDB Reports for 2013 and 2014,) have shown high frequencies (<50%) of kdr aphids in some of the English traps and in the Edinburgh and Dundee traps for Scotland in 2014. The *S. avenae* 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 is important that we continue to monitor populations for kdr

genotype and frequency, particularly in Scotland where the majority of the seed potato industry is found.

In this study we report the results of the analysis of the frequency of the kdr mutation and microsatellite genotyping of the UK *S. avenae* population in 2015 and compare the genotypic composition and frequency of the kdr resistant genotype to that found in previous years.

3. MATERIALS AND METHODS

3.1. Insect samples

Sitobion avenae were collected from sixteen 12.2m high suction traps across the UK in 2015 (12 in England and 4 in Scotland) (Figure 1). English trap samples were provided by the Rothamsted Insect Survey; Scottish trap samples were provided by Fiona Highet 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, thereby giving a good measure of population diversity for kdr and microsatellite genotype.

3.2. Historical aphid material from 1997 and 1998

Historical DNA extractions from *S. avenae*, which had been analysed as part of a previous study (Llewellyn *et al*., 2003), had been maintained in storage at -20^{o}C at Rothamsted Research and these were re-analysed using our microsatellite markers. A total of 121 aphids, originally collected from the Rothamsted suction trap in July 1997 and June and July 1998 plus field collections near Hemel Hempstead (Hertfordshire), were tested.

3.3. 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 1,003 aphids were tested from the 16 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 the James Hutton institute for microsatellite analysis. Historical DNA had been extracted

by Llewellyn *et al.* (2003) using the salting out procedure described by Sunnucks & Hales (1996).

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 (SS) or resistant (SR, 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 an Applied Biosystems 7900HT real-time PCR cycler using cycling conditions of 10 min at 95ºC, followed by 40 cycles of 95ºC for 10s and 60ºC for 45s.

Figure 1. UK suction trap sites 2015

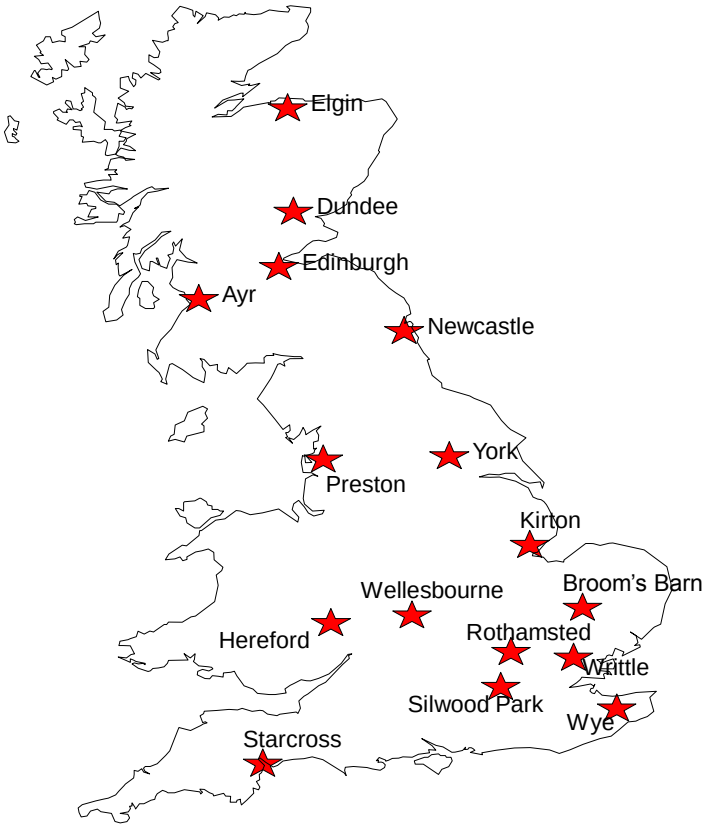


Table 1. Collections of *Sitobion avenae* from suction traps used for kdr genotyping

Location	Months of collection	No. of Insects tested
Dundee	June - August 2015	78
Edinburgh	June - August 2015	51
Elgin	June - August 2015	242
Ayr	June - August 2015	31
Kirton	June - August 2015	19
Rothamsted	June- August 2015	39
Broom's Barn	May - August 2015	24
Hereford	May - August 2015	92
Starcross	May - August 2015	99
Preston	June - August 2015	47
Writtle	May - August 2015	28
Wellesbourne	June - August 2015	31
Wye	June - July 2015	18
Silwood Park	June - August 2015	36
York	May - August 2015	123
Newcastle	June - August 2015	45
Total		1,003

3.4. Microsatellite genotyping

A sub-sample of 287 *S. avenae* were selected and analysed for their microsatellite genotype. These aphids were collected from the twelve traps in England and four traps in Scotland during 2015 (Table 2). Twelve individuals failed to produce a trace that was of high enough quality for scoring. These were removed from the analysis so that, in total, 150 English aphids and 125 Scottish aphids were successfully genotyped.

Genotypes of individual *S. avenae* were examined at five microsatellite loci: Sm10, Sm12, Sm17, Sa□4 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 Sa□4 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) (Table 3).

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

Location	Months of collection	No. of Insects tested	No. of resistant insects
Dundee	June-August 2015	60	28
Edinburgh	July- August 2015	35	12
Elgin	July-August 2015	32	2
Ayr	July-August 2015	8	0
Kirton	June- August 2015	7	2
Rothamsted	June- August 2015	12	3
Broom's Barn	July - August 2015	7	1
Hereford	July - August 2015	19	9
Starcross	July - August 2015	12	3
Preston	July - August 2015	18	16
Writtle	July - August 2015	9	2
Wellesbourne	June-July 2015	6	0
Wye	July 2015	4	0
Silwood Park	June- August 2015	8	0
York	June- August 2015	26	22
Newcastle	June- August 2015	24	6

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

4.1. *Sitobion avenae* kdr genotypes in England and Scotland in 2015

This analysis used an allelic discrimination (TaqMan) PCR diagnostic test to detect the presence of the kdr mutation (L1014F) in individual aphids which had been previously developed for *S. avenae*. 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.

4.2. kdr-SR frequency

A total of 1,003 individual *S. avenae* from the 16 UK suction traps (601 from England and 402 from Scotland) were tested for the kdr mutation. The numbers and frequency of resistant (kdr-SR) and sensitive (kdr-SS) *S. avenae* from each trap are shown in Figs. 2-5. Overall frequencies in the English trap samples were similar to those reported in 2013 and 2014, ranging from just over 76% at York to 0% at Wye. As previously, the higher frequencies tended to be recorded at traps in more intensive cereal growing areas (Kirton 42% and Broom's Barn 46%) and this probably reflects a higher pyrethroid selection pressure in these areas. Having said this, the highest kdr frequencies were found at York (76%) and Preston (53%). The level of kdr at Preston has doubled from that reported in 2014 (27%); while the relative increase at York is not known since this trap was not operational in 2014. In Scotland, kdr frequencies were also similar to those found in 2014 with Dundee having the

highest frequency at 45% (up from 33% in 2014), compared to 39% at Edinburgh (down from 55% in 2014). No resistant aphids were found at Ayr (of 31 tested), while the frequency at Elgin was just 3% of the 242 that were tested (down from 6% in 2014). The increased numbers tested at Elgin were a consequence of the higher catches at this trap, particularly during August when numbers were more than 10 fold higher than those recorded at Dundee and Edinburgh. All of the aphids that tested positive for the kdr mutation, both from England and Scotland, were heterozygotes (SR). As in previous years, no kdr homozygotes (RR) were identified in any of the samples.

Figure 2. Number of kdr-SR and kdr–SS *Sitobion avenae* in English suction traps in 2015

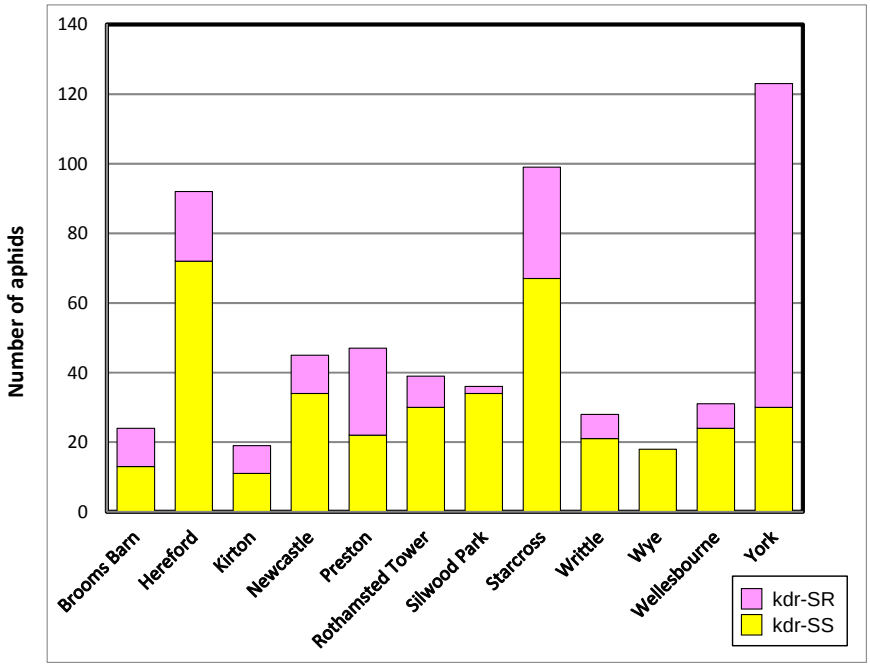


Figure 3. Frequency of kdr-SR and kdr–SS *Sitobion avenae* in English suction traps in 2015

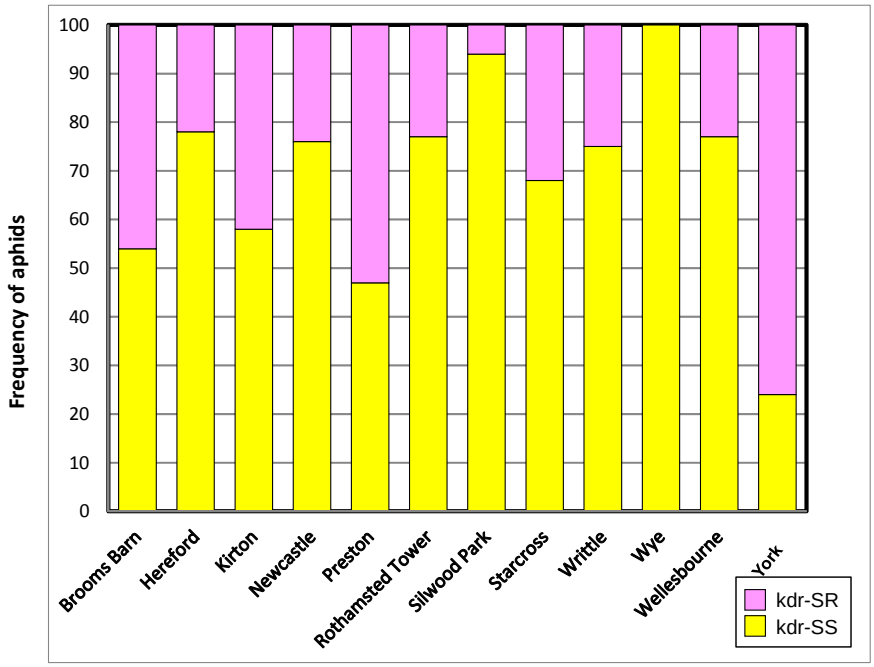


Figure 4. Number of kdr-SR and kdr–SS *Sitobion avenae* in Scottish suction traps in 2015

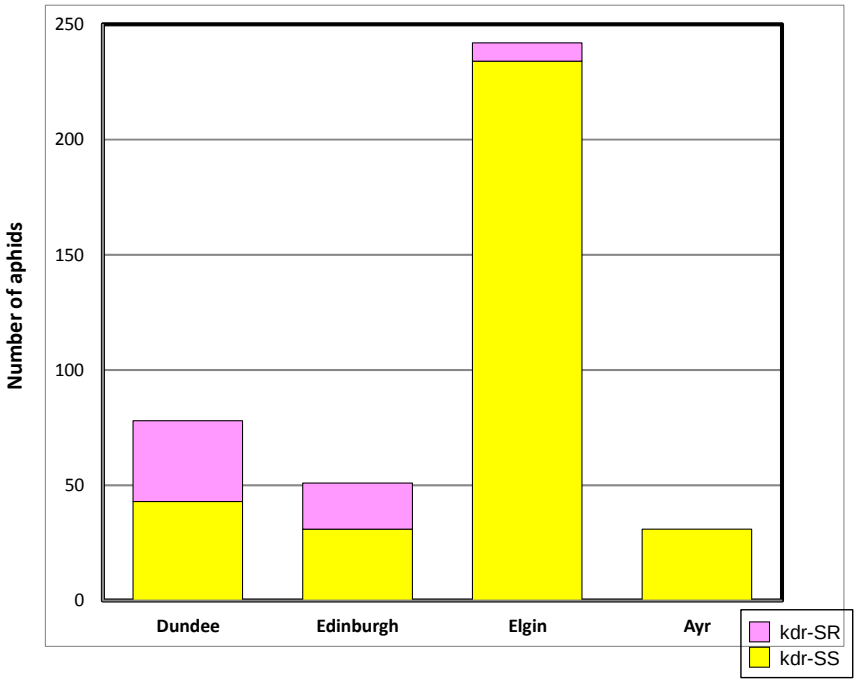
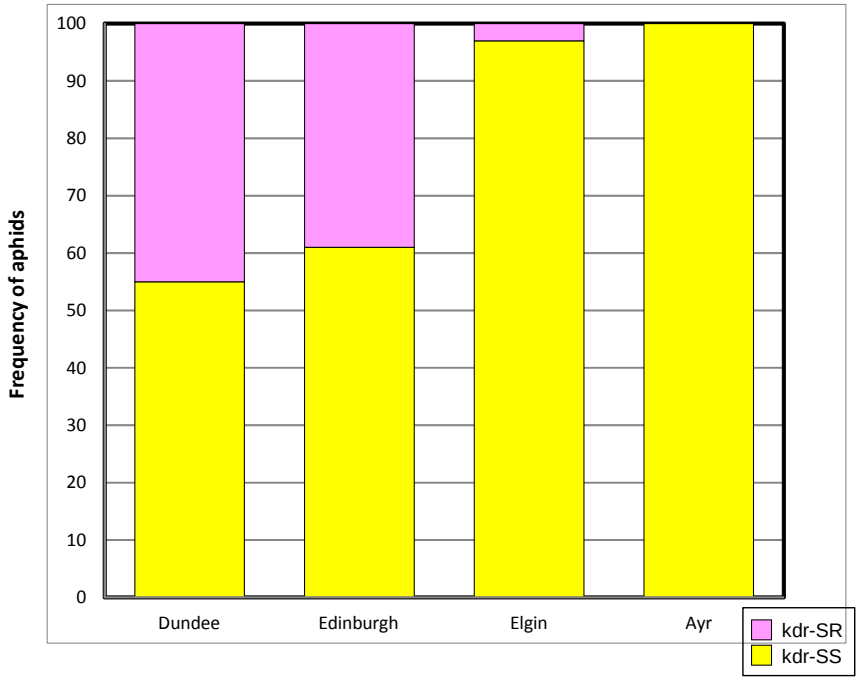


Figure 5. Frequency of kdr-SR and kdr–SS *Sitobion avenae* in Scottish suction traps in 2015



4.3. *Sitobion avenae* micro-satellite genotypes in England and Scotland in 2015

A total of 275 *S. avenae* aphid DNAs were successfully scored by microsatellite analysis (125 from Scotland and 150 from England). Wherever a genotype appeared more than once, it was considered as evidence of a clone. In addition to the SA3 genotype, numerous common insecticide susceptible clones were found in both England and Scotland and many of these had previously been identified in samples collected and analysed in 2013 and 2014 (Tables 4 and 5).

Table 4. Common *Sitobion avenae* genotypes

Genotype	Resistance	UK 2013	UK 2014	England 2015	Scotland 2015	UK 2015
SA3	SR	39	142	64	42	106
SA1	SS	8	23	1	4	5
SA2A	SS	6	7	1	0	1
SA2	SS	2	8	2	0	2
SA5	SS	3	16	2	1	3
SA44	SS	16	19	11	7	18
SA16	SS	4	0	0	0	0
SA6	SS	2	1	1	0	1
SA7	SS	3	7	0	4	4
CLONE SCOT1	SS	2	0	0	0	0
SA11	SS	2	4	5	12	17
SA1D	SS	0	5	0	0	0
SA38	SS	2	7	0	2	2
SA39	SS	1	3	0	3	3
CLONE SCOT2	SS	2	0	0	0	0
NEW R GENOTYPE	SS	0	1	0	0	0
SA3E	SS	0	7	0	2	2
SA8	SS SR	0	4	3	0	3
SA44C	SS	0	3	0	0	0
SA10	SS	1	1	0	0	0
CLONE F	SS	0	2	0	0	0
SA AYR 13 1	SS	1	2	0	0	0
SA AYR 13 2	SS	1	2	0	0	0
CLONE 2014 1	SS	0	3	0	0	0
SCOT 4	SS	0	1	1	0	1
SA14	SS	0	1	0	0	0
CLONE 2014 2	SS	1	1	0	0	0
CLONE 2014 3	SS	1	0	0	1	1
CLONE 2014 4	SS	1	1	0	1	1
CLONE 1514 1	SS	0	1	1	0	1
CLONE 1514 2	SS	0	1	8	0	8
CLONE 1514 3	SS	0	1	4	0	4
2015-1	SS	0	0	1	1	2
SLOV 3	SS	0	0	1	1	2
SLOV 13	SS	0	0	0	1	1
SA27	SS	1	0	1	3	4
SA45	SS	1	0	1	0	1
2015-4	SS	0	0	0	2	2
2015-2	SS	0	0	0	2	2
2015-3	SS	0	0	0	2	2
CL1315S1	SS	1	0	0	1	1
SA3D	SS	0	0	2	0	2

CLONE D	SS	0	0	1	0	1
Genotype	**Resistance**	**UK 2013**	**UK 2014**	**England 2015**	**Scotland 2015**	**UK 2015**
UNIQUE	SS	105	77	39	33	72
TOTAL		206	351	150	125	275
FAILS			27	2	10	12
BLANKS/ CONTROLS			6	0	1	1
TOTAL TESTED			384	152	136	288

Table 5. Allele sizes of common *Sitobion avenae* clones

Genotype	S16b	S16b	Sm12	Sm12	Sm10	Sm10	Sm17	Sm17	sa Σ 4	saΣ 4	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	161	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
Hist clone F	173	211	115	115	160	164	96	97	163	172	England
Hist clone C	173	211	126	146	160	160	96	97	162	163	England
Hist clone D	177	211	115	134	160	163	96	97	161	163	England
SA7	173	217	115	115	160	160	92	96	163	172	UK
SA11	173	215	115	126	161	163	92	93	162	163	UK
Hist clone A	173	190	131	134	149	163	92	93	157	174	England
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
cl 1514 2	171	173	127	128	161	163	92	93	161	163	UK
cl 1514 3	158.68	266.5	126	146	161	161	95	97	161	163.6	England
SA27	177	279	115	146	160	161	91	96	163	172	UK

Results of the previous projects, carried out in 2013 and 2014, indicated that the kdr mutation is found mainly in one clonal genotype (SA3). This was set against a background of high genetic diversity within a general susceptible population consisting of many genotypes (Figure 6C). In Scotland in 2013, the resistant genotype (SA3) was only found in trap samples from Dundee and Edinburgh at a low level (~10%). The resistant genotype (SA3) made up more than 40% of the total sample genotyped in 2014 (Figure 6B) and results from the current study suggest that this may have stabilised (~34%, Figure 6A). Once again, in Scotland the resistant clone (SA3) was mainly found in trap catches from Dundee and Edinburgh and was only found in small numbers in trap catches from Elgin. The sub-sample genotyped using microsatellite markers in 2015 are not a proportional representation of the entire catch. A peak in the number of *S. avenae* caught in the Elgin trap occurred in August 2015 and these were tested for the kdr mutation and were found to

be kdr-susceptible (SS). The main aim of the current project was to look for novel SR or RR genotypes, therefore the additional SS samples from Elgin were not genotyped using the microsatellite markers. No Sa3 individuals were found in the catch from Ayr (Figure 4).

4.4. No further evidence of the kdr mutation in additional genotypes

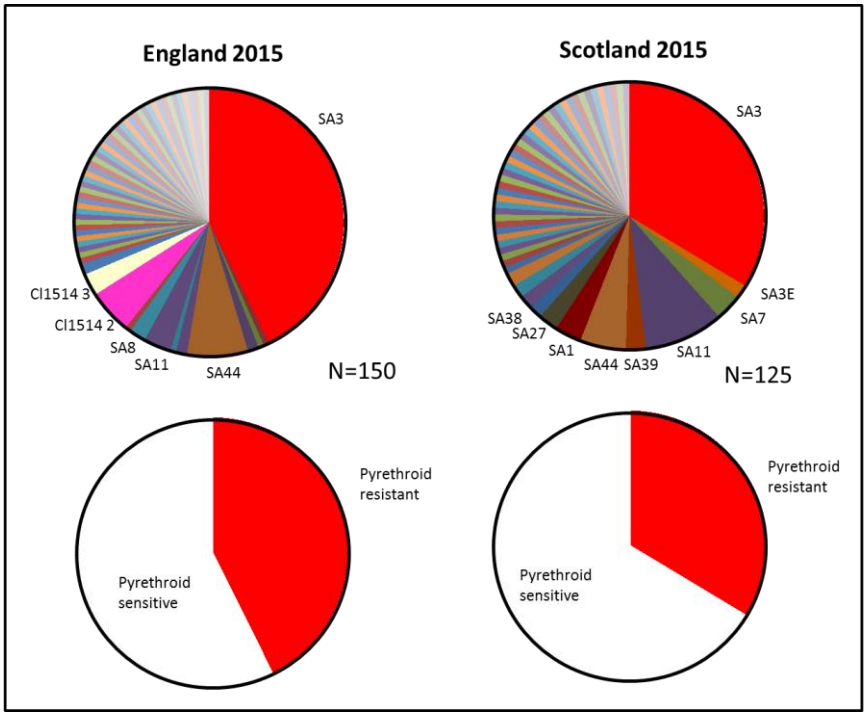
Results in 2014 provided evidence that the kdr mutation had been found in two additional *S. avenae* genotypes caught in the English suction traps (Preston and Rothamsted). One of these genotypes (caught in the Rothamsted trap) had some unusual allele sizes (termed 'New SR genotype', Table 5). The other 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 and four suction trap individuals in 2014. kdr testing has always indicated that the other representatives of this clone are susceptible to pyrethroids (SS). Three individuals of the SA8 clone were identified in the current 2015 study and, once again, all were susceptible to pyrethroids (SS). No representatives of the new SR genotype were detected in 2015.

4.5. New *Sitobion avenae* clones and examples of long-lived genotypes

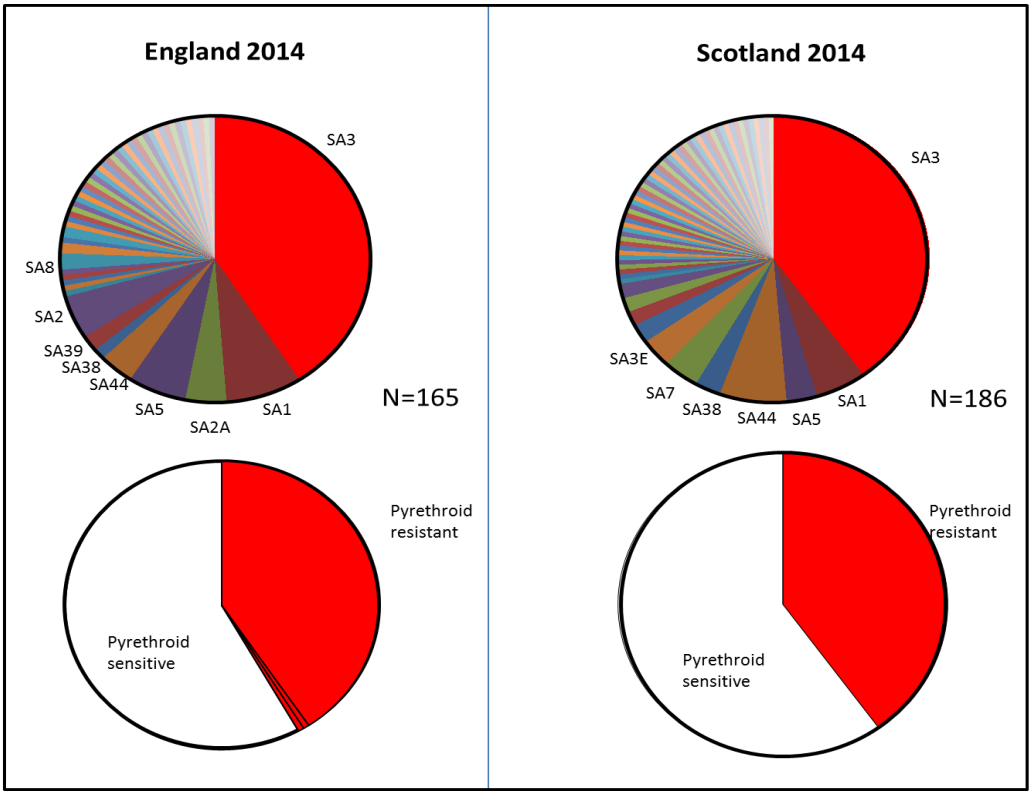
In addition to the resistant clone (SA3), examples of other common clones, SA1, SA2, SA2A, SA5, SA6, SA7, SA8, SA11, SA38, SA39, SA44, SA3E, and scot 4 were all found in the 2015 suction trap samples, with clone SA11 showing the largest clonal expansion in comparison with data from previous years (Table 4 and Figure 6). Four new clones that had not been detected previously were found in 2015 (2015-1, 2015-2, 2015-3 and 2015-4). These new clones were not found in high numbers (Table 4). Six genotypes were detected in 2015 that had been found as single genotypes in previous years (clone 13 15 s1, clone 15 14 1, clone 15 14 2, clone 15 14 3, SA27 and SA45) with clone 15 14 2 found in reasonably high numbers (Table 4 and Figure 6). Interestingly, two individuals were found that had the same genotype as a clone collected in Slovenia in 2014 (Slov3) and one individual that shared its genotype with another Slovenian clone (Slov13) (results not shown).

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

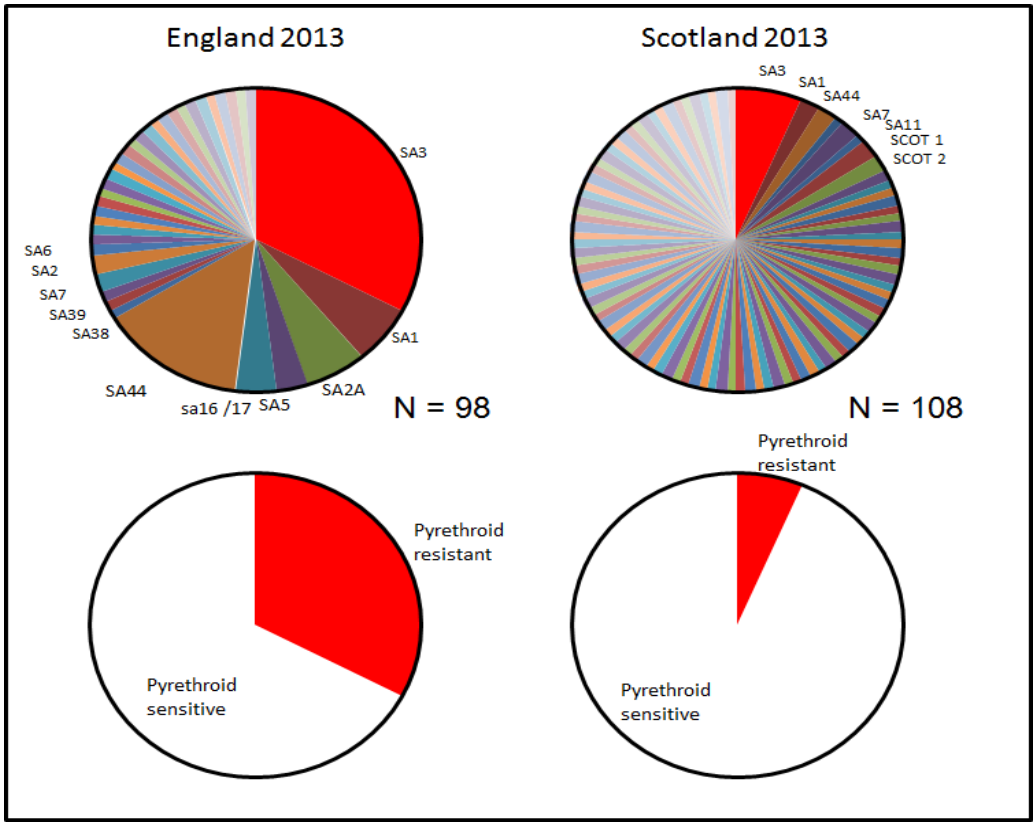
A. England vs Scotland 2015



B. England vs Scotland 2014.



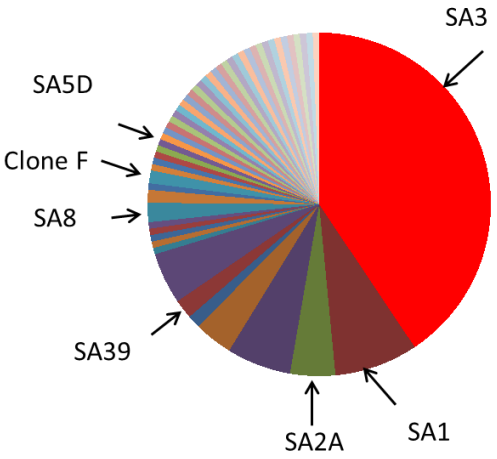
C. England vs Scotland 2013.



Using DNA extracted by Llewellyn *et al.* (2003), we also screened 121 individual *S. avenae* collected from Rothamsted suction trap and from field sites around Hemel Hempstead in 1997 and 1998) using the five microsatellite loci (Sm10, Sm12, Sm17, Sb16 and Sa□4).

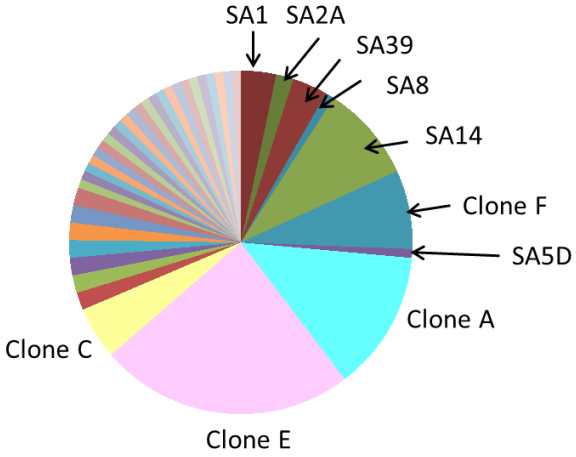
We compared the genotypic profiles of the historical DNA (1997 and 1998) to those we found in 2014 and 2015 (Figure 7A and 7B). It was interesting to note that there were many clones that have survived and are still represented in the current population (SA1, SA2A, SA39, SA8, SA14, clone D, CLONE F, SA5D). However, there were no representatives of the commonest clones found in 1997 and 1998 (Clones A, clone C and clone E) in the current UK populations collected 2013-2015. Furthermore, the resistant genotype (SA3) was not found in 1997 or 1998, suggesting that it did not originate from an historical UK clone that had acquired the kdr mutation. Having said this, a wide UK geographical area was not covered and we cannot exclude the possibility that this clone was not present elsewhere.

Figure 7A. Comparison of genotypic composition of the 2014 English *Sitobion avenae* population with those sampled in 1997/1998.



England suction trap catches 2014

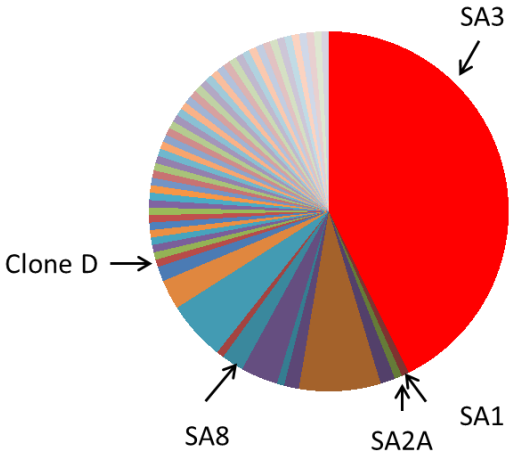
n= 165



Rothamsted and Hemmel Hempsted 1997 & 1998

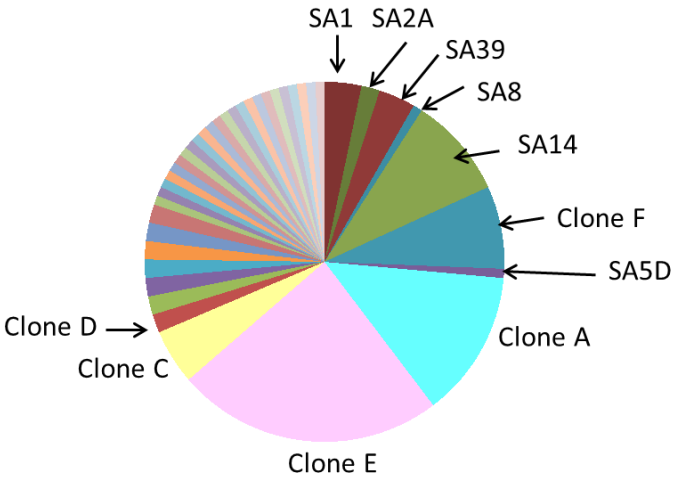
n= 121

Figure 7B. Comparison of genotypic composition of the 2015 English *S. avenae* population with those sampled in 1997/1998.



England suction trap catches 2015

n= 150



Rothamsted and Hemmel Hempsted 1997 & 1998

n= 121

4.6. Seasonal variation in *Sitobion avenae* clones and changes in diversity in the Scottish population

S. avenae has a range of lifecycle types from obligate parthenogens which never recombine, to holocyclic forms (with an annual sexual phase), to androcyclic forms which can produce intermediates (Lewellyn *et al.,* 2004; Delmotte *et al.,* 2001; Simon *et al.,* 2002). Current hypotheses suggest that genotypic diversity in the *S. avenae* population increases from south to north where northern climate conditions favour the sexual cycle and a greater proportion of individuals are derived from the survival of the cold-hardy egg stage (Helden and Dixon, 2002). 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 sample size. Data from the Dundee and Rothamsted traps in 1997 and 1998 were reported previously (Llewellyn et al., 2003). The authors of this study observed a change in diversity between the catch from the Dundee population collected in 1997 (k = 0.92) and from the same suction trap in 1998 (k=0.52). However, the diversity of the catch from the Rothamsted trap in these two years remained broadly similar (Table 6). The authors suggested that the reduction in diversity in the Dundee trap was a function of the mild temperatures experienced in the preceding winter (1997-1998) and increased survival and reproductive success of asexual genotypes. Our results support this finding, as we demonstrate considerable variation in the k value from year to year and in general the diversity is greater at the traps in the north (Dundee, Elgin) than the diversity found at the Rothamsted trap. This is apparent when the data from the SA3 clone is included in the calculation and when it is not (Table 6). It is interesting to note that despite high numbers of *S. avenae* being caught at the Elgin trap in 2015, the SA3 (pyrethroid resistant) clone was not highly represented in the catch (3%). All evidence suggests that SA3 is an asexual genotype and the conditions near Elgin, which is the most northerly trap in the UK, may not favour the survival of aphid clones with this form of reproduction. This northerly region may therefore represent a threshold limit for the over-winter survival of asexual clones.

Table 6. Changes in genotypic diversity in *Sitobion avenae* populations collected from suction traps located at Dundee, Rothamsted and Elgin.

Dundee trap				
Year	**k**	**k (sa3 removed)**	**no. tested**	**% SA3**
1997	0.92	0.92	53	0
1998	0.49	0.49	51	0
2013	0.93	1	46	9
2014	0.48	0.88	62	47
2015	0.28	0.54	54	52
Rothamsted trap				
Year	**k**	**k (sa3 removed)**	**no. tested**	**% SA3**
1997	0.52	0.52	54	0
1998	0.5	0.5	50	0
2013	0.5	0.77	19	33
2014	0.58	0.77	19	31.6
2015	0.54	0.62	11	27
Elgin trap				
Year	**k**	**k (sa3 removed)**	**no. tested**	**% SA3**
2013	1	1	45	0
2014	0.6	0.69	40	17.5
2015	0.86	0.88	29	6.8

5. DISCUSSION

Overall, the frequency of *S. avenae* carrying the kdr mutation collected in the English suction traps was similar in 2013, 2014 and 2015, suggesting that this form of resistance may have stabilised. In contrast, the frequency of kdr dramatically increased in some traps in Scotland between 2013 and 2014 but remained at a similar level in 2015, suggesting that it may too have now also stabilised in that region.

Results from the study in 2014 suggested that the kdr mutation may exist in a new (non-SA3) genetic background. One individual of a genotype closely related to SA3 (SA8) appeared to carry the kdr mutation. However, all other individuals of this clone were kdr insecticide sensitive (SS). In addition to this single kdr resistant SA8 individual, a single example of a second new genotype, which has not been found previously, was also found

in England in 2014 (new R genotype). One aim of the current study was to monitor the population in 2015 for any increase in the frequency of these new genotypes as it is likely that their insecticide resistance properties would give them a selective advantage. Although three individuals with the SA8 genotype were found in 2015, all were shown to be pyrethroid susceptible (kdr-SS), and no examples of the new R genotype were detected. This suggests that the frequency of these new genotypes, identified in 2014, has not increased and it is likely that they were not suited to the UK environment or they may have suffered from fitness costs associated with carrying insecticide resistance.

Previous studies suggest that clonal diversity of *S. avenae* varies, with greater diversity being found in northern regions. The results from 2014 and 2015 indicate that the overall levels of genotypic diversity in Scotland and in England are currently similar. Measurements of diversity in Scotland appear to vary quite extensively from year to year and between traps. This is in contrast to the population in England which appears to be more stable. This observation was first reported by Llewellyn *et al.* (2003) who compared the diversity of *S. avenae* caught in the Dundee suction trap in 1997 with those caught in Dundee in 1998 and found that the diversity dropped dramatically. The authors of the 2003 publication suggested that this is due to the severe temperatures experienced in the winter preceding 1997 followed by mild temperatures in the winter preceding the 1998 collection. Our data provides further support for this, as the diversity appeared to increase in Scotland when the preceding winter was colder than average.

Results in 2015 provided further support for the hypothesis that some common *S. avenae* genotypes, e.g. SA1, SA3, SA44, SA5, SA7, SA11 etc., are entirely asexual and have lost the ability to produce sexual forms as they have been found consistently over a three year period. Many of these genotypes were also present in 1997/98 demonstrating that some asexual clones are robust and well suited to UK conditions and are capable of long-term survival. Interestingly, no representatives of three of the most common clones found in 1997/98 (A, C and E) were found more recently in the UK population in 2013-2015. The reasons for their demise are not clear although it is possible that these were holocyclic clones and they have undergone sexual reproduction. Further monitoring will determine whether the SA3 clone, with its insecticide resistance selective advantage, will continue to dominate the UK population or if it will be subject to clonal turnover and decline and die out in a similar way to some *Myzus persicae* genotypes (Fenton *et al.*, 2005) and some historical *S. avenae* genotypes such as clones A, C and E.

Finally, the apparent absence of homozygous kdr-RR aphids in the UK continues to be an interesting observation. This is most likely due to the fact that all heterozygous (SR) aphids are a single asexual clone (Sa3) and so the R allele is not available for sexual crossing to form RR individuals. This is an hypothesis that can be tested in the laboratory by taking SR aphids through a period of short day/low temperature to see if they are able to produce sexual forms.

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7. ACKNOWLEDGEMENTS

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