Final Project Summary

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

Project number	214-0004	**Final Project Report**	PR535
Start date	April 2013	**End date**	May 2014
HGCA funding	£10,892	**Total cost**	£21,785

What was the challenge/demand for the work?

Preventing crop losses due to significant virus accumulation normally requires some control during the seedling stage. Pyrethroid insecticides are most extensively used in this role. In 2010, cypermethrin and lambda cyhalothrin were the most commonly used insecticides on the wheat crop, accounting for 63% of the sprayed area. The vast majority of these sprays were targeted at aphid control (79%). Pyrethroid spray failures were first noted in June 2011 for some field populations of the grain aphid. Testing of suction trap and field specimens found the resistance mechanism was reasonably widespread in the English grain aphid population.

The pyrethroid resistant *S. avenae* have not been fully characterised and it is not yet known if this resistance is found in a single clone (as the species can reproduce asexually all year round in the UK) or if the resistant gene has spread to different genotypes. By understanding the population genetic structure, it will be possible to model the likely trajectory of the evolving insecticide resistance in this species.

How did the project address this?

This project measured the frequency and spread of knockdown resistance (kdr) forms in the English population (using samples collected in suction traps and from field crops) and used these to examine the more detailed genetic composition of resistant aphids in the population compared to their sensitive counterparts. Currently, all resistant grain aphid individuals carry the kdr gene in the heterozygous form (kdr-SR) which means they are most probably less resistant than if they were to become homozygous (kdr-RR). By monitoring the population for kdr and understanding the population genetic structure, it will be possible to model the likely trajectory of the evolving insecticide resistance in this species, as has been possible for *Myzus persicae* (peach–potato aphid).

The specific objectives of the project were:

1. To establish the frequency and distribution of pyrethroid resistant *S. avenae* in the UK in 2013.
2. To generate microsatellite profiles from DNA extracted from individual *S. avenae* known to carry pyrethroid resistance conferred by kdr and compare these to the remaining population.
3. To establish if the resistance has been acquired by one or a small number of successful

lineages (clones) that have undergone clonal expansion and whether this has occurred in local clones

4. To establish if kdr has been inherited and spread by sexual reproduction into numerous lineages.

A high throughput PCR-based TaqMan assay for detecting the kdr mutation in individual *S. avenae* had been developed prior to the start of the project (HGCA-funded summer bursary; Rothamsted Research, 2012). DNA extracted for kdr genotyping at Rothamsted was sent to James Hutton Institute for microsatellite analysis. Occasional sequencing of kdr gene fragments was also carried out in order to identify any novel mutations.

What outputs has the project delivered?

Overall, the frequency of *S. avenae* carrying kdr-SR collected in the English suction traps was similar in 2012 and 2013 suggesting that this form of resistance may have stabilised. The frequency of kdr-SR varied between English sites in 2013 from 0% (at Starcross) to a maximum of just over 50% (Kirton). Kirton consistently showed the highest kdr-SR frequency which may reflect a higher pyrethroid selection pressure in that area.

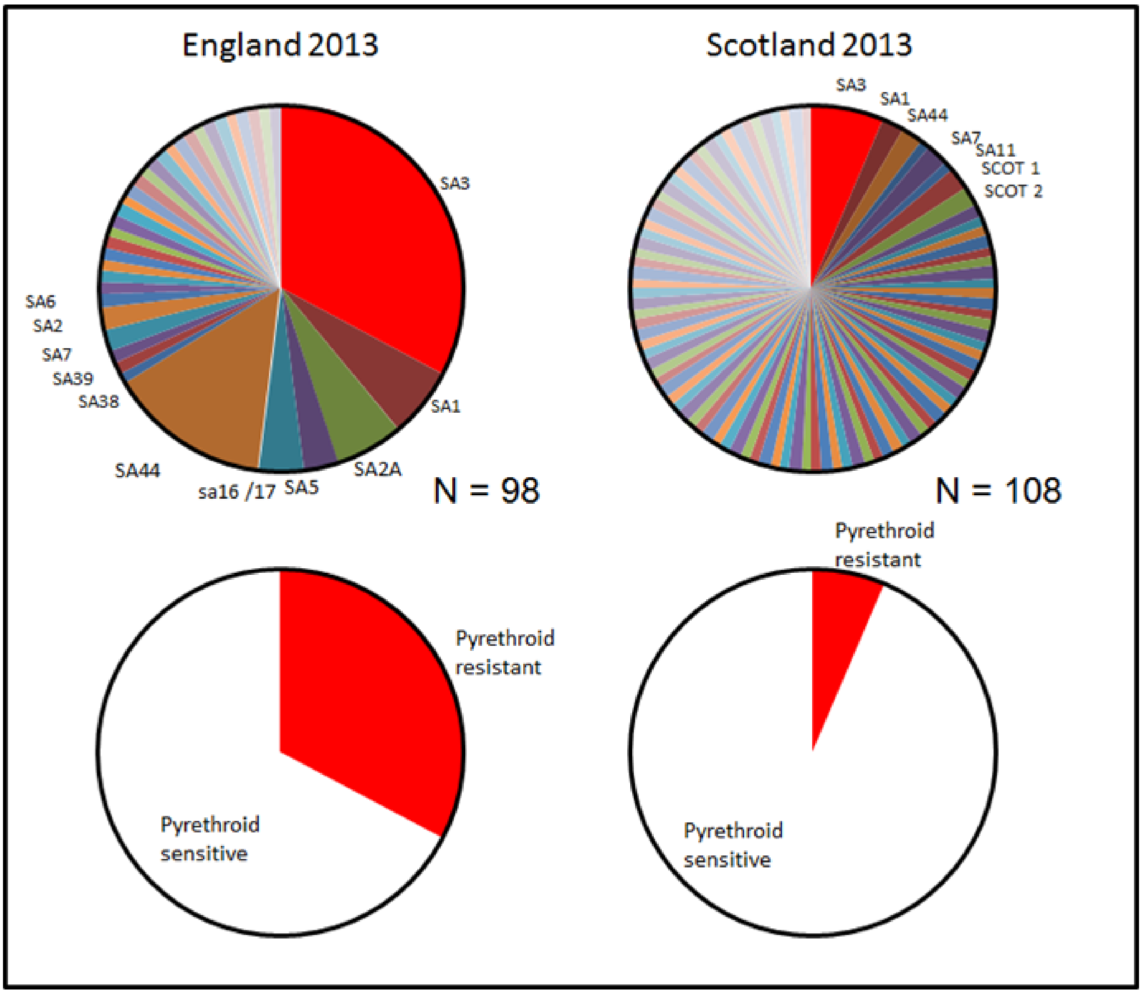
The kdr-SR frequency in Scottish *S. avenae* was overall lower than in the English population with 8% and 27% in the Edinburgh and Dundee traps, respectively, in 2013. No kdr-SR aphids were recorded from the Elgin and Ayr traps in 2013.

A subset of the *S. avenae* samples tested for the presence of the kdr mutation was also tested to understand their genetic make-up. If a genotype appears more than once during the analysis, this is considered as evidence of a clone (resulting from asexual reproduction). If a particular genotype appears to be unique then this suggests that the individual aphid has arisen from sexual reproduction.

So far, all of the *S. avenae* shown to have the kdr mutation (and therefore reduced sensitivity to pyrethroids) belong to a single genotype or clone. In addition to this clone, there were also at least seven other clones detected. These are all sensitive to pyrethroids (i.e. do not possess the kdr mutation). There were also many individual *S. avenae* that were a unique genotype.

England and Scotland differ in the proportion of the population that is clonal. In the areas of England sampled ~75% of the population was from one clone or another, whereas in Scotland, only ~10% was derived from a clone (see below). This is consistent with the hypothesis that the prevalence of individuals which have been derived from sexual reproduction increases at more northerly locations, where the conditions favour the sexual cycle.

Potato Council (PCL) is funding continuation of the survey of the frequency of the kdr-SR genotype and analysis of the genetic diversity of *S. avenae* populations in 2014. This will provide further information to determine if/how the *S.avenae* populations and frequency of the kdr mutation vary over time and across regions.



Who will benefit from this project and why?

The initial beneficiaries of this research are the cereal growers requiring methods to prevent the spread of viruses by aphids in their cereal crops. Pyrethroids are one of the cheapest and most effective insecticides available and growers will wish to continue to use these products if resistant *S. avenae* are not a problem but other aphid sensitive species, such as *Rhopalosiphum padi,* are.

In addition to cereal growers, a second group affected by resistant *S. avenae* are likely to be seed potato producers. Recent work funded by the Potato Council has suggested that *S. avenae* is much more efficient in spreading potato virus Y than was thought previously. Despite the presence of strong and widespread resistance in the peach–potato aphid (*M. persicae*), pyrethroids are still widely used on potato crops. Part of this strategy has been due to the scientific understanding of when to switch chemical classes to avoid resistance from *M. persicae*. A potato farmer will be affected by pyrethroid control failures on neighbouring cereal crops. If these cereal crops are under the farmer's control, then new agronomy may require they intervene with an alternative product on cereal crops close to the seed potatoes. Grain aphids will not multiply on the potato crop, but large numbers of pyrethroid resistant *S. avenae* arising from cereal crops will actively probe and move through the potato canopy, whereas they would have otherwise been removed by the pyrethroids.

Overall, the frequency of *S. avenae* carrying kdr-SR collected in the English suction traps was similar in 2012 and 2013, suggesting that this form of resistance may have stabilised (at a maximum of just over 50% of the aphids collected at several sites) since its increase between 2009 and 2012. The frequency of kdr was overall lower in Scotland in the year it was tested (2013). This may reflect differences in climate, ecology or pyrethroid selection pressure affecting this species.

Where a pyrethroid is applied, full rate should be used. If control failures are experienced, the grower should switch to an alternative mode of action.

If the challenge has not been specifically met, state why and how this could be overcome

There is evidence of the survival of asexual clones over extended periods of time in the absence of any insecticide selection. There is also evidence that genotypes can turnover, i.e. common clones can become less common and even disappear. The long term fate of the SA3 clone could be determined by natural turnover processes, such as winter hardiness. This may be more important to its long term survival than its selective advantage of containing the kdr mutation. Should the SA3 clone be found as a historically common clone, then this would suggest that it is robust and the mutation occurred in this background because it was a common clone. Conversely, if it had not been detected before 2009, then it is most likely that a mutation occurred in a suitable genetic background in an ecologically less successful asexual clone and there is a reasonable chance that SA3 will slowly die out. Further work covering the year 2013-2014 is being funded by Potato Council.

Lead partner	Brian Fenton, JHI
Scientific partners	Rothamsted Research
Industry partners	Potato Council (£10,892)
Government sponsor	