Final Report

Pyrethroid sensitivity in UK cereal aphids

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1. SUMMARY

1.1. Aim

The project aimed to gain contemporary information of any reduced sensitivity to pyrethroids (which is an early sign of the evolution of resistance that can cause control failures) or stronger pyrethroid resistance (which would compromise aphid control after spray applications) in two important UK cereal aphid pests: The English grain aphid (*Sitobion avenae*) and the bird cherry-oat aphid (*Rhopalosiphum padi*).

1.2. Methodology

Aphids were collected from cereal crops between November 2019 and November 2020. The sample sites of a current AHDB project (21120077) "Management of aphid and BYDV risk in winter cereals" were used to collect aphid samples from across the UK**,** including Northumberland (NE), Yorkshire (N), Devon (SW), Suffolk (E), and Hereford (W). Where possible, aphids were also sourced from independent growers, agronomists, or researchers in Scotland and Wales, in order to ensure samples were representative of the wider UK cereal aphid population.

Aphid screening bioassays were done using insecticide-coated glass vials. The vials were coated internally with technical lambda-cyhalothrin dissolved in technical grade acetone. The responses of the samples were compared, using statistical analysis, to insecticide-susceptible and –resistant baselines in *S. avenae* and insecticide-susceptible and –reduced sensitivity baselines in *R. padi*. These had been gained previously as part of another project. There was a period needed between sample collection and testing in order to rear up sufficient aphid numbers for bioassay.

1.3. Key findings

We have gained contemporary information on the resistance status of two important virus-transmitting aphid pests of UK cereal crops. In *S. avenae*, there is no evidence of the evolution or selection of pyrethroid resistance above and beyond that already known in this species. Five samples were tested. Four samples showed statistically significant higher responses than the insecticide-susceptible baseline clone. Two of these carried moderate resistance levels (over 20-fold) associated with the kdr (knock-down resistance) mechanism that is known to be present in this species. However, and importantly, greater levels of resistance were not seen than those previously reported in 2015.

Twenty-one *R. padi* samples were tested. All of these showed bioassay responses similar to those seen in pyrethroid-susceptible aphids in this species and there was no evidence of any shift in sensitivity from that seen in recent years.

1.4. Practical recommendations

Our findings strongly suggest that, at the time of application, pyrethroids should prove to be effective against these two aphid pests, as long as sprays are applied at the full recommended rate for aphid control and good spray contact is made.

2. Introduction

Cereal aphids, including the English grain aphid (*Sitobion avenae*) and the bird cherry-oat aphid (*Rhopalosiphum padi*) are herbivorous insect pests of global importance (Van Emden & Harrington, 2017; Vickerman & Wratten, 2009). Cereal aphids can cause extensive damage to economically important arable crops, including wheat and barley (Vickerman & Wratten, 2009; Perry *et al*., 2000). Yield loss can be caused via direct feeding damage (losses of up to 20%; Valenzuela & Hoffmann, 2014) and, more significantly, by the transmission of plant viruses (yield losses can be up to and in excess of 80%; Perry *et al*., 2000). The most important virus transmitted by cereal aphids is barley yellow dwarf virus (BYDV), although other economically important cereal viruses, alongside viruses of non-host plants (e.g. PVY in potato), are also transmitted by cereal aphids (Vickerman & Wratten, 2009; Masterman et al., 1994; Katis & Gibson, 1985).

Management and control of aphids is routinely achieved through the application of insecticides, with pyrethroids representing the most common class applied for aphid control. However, over recent years resistance against pyrethroids has appeared in many aphid species of agricultural and horticultural importance, including the broad-spectrum pest, the peach-potato aphid (*Myzus persicae*) and *S. avenae* (Walsh *et al*., 2020a; Fenton *et al*., 2002). Recent Irish-based insecticide resistance surveys have shown decreased sensitivity to pyrethroids in *S. avenae* populations (Walsh *et al*., 2020a) and in the single *R. padi* clone that was tested (Walsh *et al*., 2020b). The withdrawal of approval for the use of neonicotinoid seed treatments in cereals has increased the reliance on pyrethroid applications, increasing the concern that pyrethroid-resistant cereal aphid populations will become more abundant across Europe (McNamara *et al.*, 2020) and virus levels will rise as a result of reduced efficacy of pyrethroid sprays.

Decreased sensitivity to insecticides is an early indicator that insecticide resistance is emerging within a population (Bass *et al*., 2014; Feyereisen, 1995). Regular screening of aphid populations ensures that the efficacy of the compounds used to control aphid populations remain effective and identifies regions/localities where sensitivity/resistance may be present. The use of insecticide resistance management practices aims to reduce the likelihood of resistance evolving (Sparks & Nauen, 2015), although, when resistance, or decreased sensitivity, towards an active ingredient starts to emerge, alternative chemistries, with a different Mode of Action, can, if available, be used to control the aphid population. Alternative means of pest control (e.g. biocontrol via natural enemies) could be deployed to suppress aphid populations. Interestingly, insecticide resistance, through associated fitness costs, can sometimes increase the effectiveness of natural biocontrol as recent research has shown that resistance to pyrethroids (*kdr*-based resistance) in *S. avenae* is associated with heightened aphid susceptibility to the natural enemy parasitoid wasp, *Aphidius ervi* (Jackson *et al.,* 2020).

In this project, we investigated the status of pyrethroid sensitivity/resistance in UK cereal aphid populations, testing for resistance phenotype in a total of successfully-reared 26 cereal aphid samples (comprising 5 *S. avenae* and 21 *R. padi* samples; with 1 *R. padi* sample being lost after a few weeks of rearing). These were collected from some of the key cereal production regions of the UK: Yorkshire, East Anglia, Tayside, Herefordshire, Northumberland, Devon, and Tayside.

3. Materials and methods

3.1. Aphid sampling and rearing procedure

84 winter wheat and barley fields, covering 62 localities, were visited on up to four occasions between September and December in 2019 and 2020 (see Appendix 1 and Appendix 2 for the full list of sampling sites, visit dates, and locations). Field sampling efforts were supplemented with samples provided by independent growers, agronomists, and researchers. At each site, the crop and field margins were searched and, where present, cereal aphids (*S. avenae* and *R. padi*) were collected (see Figure 1 and Table 1 for details of the locations aphids were successfully sampled from). Aphids were sampled by removing infested plant tissue (either by excising an infested leaf at the petiole or by removing an infested shoot from the soil) and placing the infested plant tissue into either a small box-cage (Blackman box), an Austin tube, or a sampling cup. Sampling cups were created from two plastic water-sampling cups (e.g. Kartell™

Polypropylene containers, Fischer Scientific) by boring a hole in an 'inner' cup, perforating the lid of the sampling container, and placing the 'inner' cup inside an 'outer' cup; this enabled a water source to be included in the 'outer' to create a suitable environment for sampling and transporting aphids. Each sample was established from an individual field-collected aphid.

Each aphid sample was reared under laboratory conditions using two methods. Initially, a viable population was produced at ADAS by rearing aphids on wheat shoots grown in compost in pots under aerated plastic mesh before transporting to Rothamsted Research for bioassay. This approach proved to be problematical as aphids did not multiply up well. As a result, a new rearing method was used for the latter, 2020, samples, which involved the initial small samples being posted to Rothamsted where they were then reared up, again in the laboratory, in compost in pots of barley shoots under aerated plastic cloches. In both approaches, each aphid sample was subsequently screened for its response in glass vial, topical insecticide bioassays (see section 3.2 for the bioassay methodology).

Figure 1: Cereal aphid sampling site locations. (Sa1, Sa2, Sa3, Sa4, Sa5 represent the samples coded as Sav 1, Sav 2, Sav 3, Sav 4 and Sav 5 in Table 1 below).

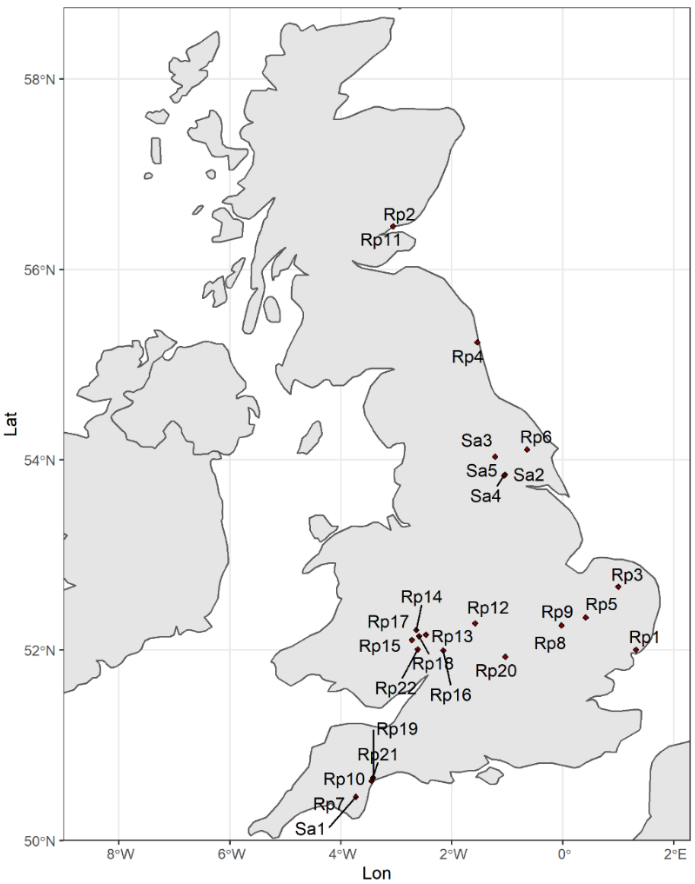


Table 1: Sampling location, collection date, date bio-assays were complete, aphid species, and host plant collected from in the field for the 26 aphid samples screened as part of this survey.

Population name	Locality collected (location on map)	Date collected	Bioassay reference number	Aphid species	Host plant
DCP-1 [Ŧ]	Kirton, Suffolk (1)	15/12/2019	Rp 1	*R. padi*	Unknown
JHI-01-A	Dundee, Tayside (2)	15/11/2019	Rp 2	*R. padi*	Winter barley
M Ramsden	Norfolk (3)	Winter 2019	Rp 3	*R. padi*	Unknown
HM-02-01-A	Cresswell, Northumberland (4)	18/11/2019	Rp 4	*R. padi*	Winter barley

BW20-055-01-B	Isleham, Suffolk (5)	27/11/2019	Rp 5	*R. padi*	Winter barley
HM01-01-A	Duggleby, North Yorkshire (6)	11/11/2019	Rp 6	*R. padi*	Winter barley
SX-01-A Ŧ	Riverford Bridge, Devon (7)	16/12/2019	Sav 1	*S. avenae*	Winter barley
SX-02-B Ŧ	Riverford Bridge, Devon (7)	16/12/2019	Rp 7	*R. padi*	Winter barley
BW20-045-01-A	Boxworth, Cambridgeshire (8)	04/12/2019	Rp 8	*R. padi*	Winter barley
BW20-045-02-B	Boxworth, Cambridgeshire (8)	16/12/2019	Rp 9	*R. padi*	Winter barley
SX-03-B Ŧ	Riverford Bridge, Devon (7)	16/12/2019	Rp 10	*R. padi*	Winter barley
JH 02-01A	Dundee, Tayside (2)	15/11/2019	Rp 11	*R. padi*	Winter barley
Corteva Ŧ	Warwick, Warwickshire (9)	08/06/2020	Rp 12	*R. padi*	Grass weed
SE 63029 39093	Stockton on Forest (10)	28/10/20	Sav 2	*S. avenae*	Winter wheat
SE52157-600094	Newton-on-Ouse, North Yorkshire (11)	22/10/2020	Sav 3	*S. avenae*	Winter wheat
SE63029-39093-A	Riccall, North Yorkshire (12)	28/10/2020	Sav 4	*S. avenae*	Winter wheat
Stanford Bishop	Stanford Bishop, Herefords (13).	30/10/20	Rp13*	*R. padi*	Winter wheat
Bannut-N10	Bredenbury, Herefordshire (14)	30/10/2020	Rp 14	*R. padi*	Winter wheat
Centre field	Moreton on Lugg, Herefordshire (15)	03/11/2020	Rp 15	*R. padi*	Winter wheat
RM21-0007-540	Tewkesbury, Gloustershire (16)	10/11/2020	Rp 16	*R. padi*	Winter wheat
Docklow	Docklow, Herefordshire (17)	10/11/2020	Rp 17	*R. padi*	Winter wheat
Ullswick	Ullingswick, Herefordshire (18)	15/11/2020	Rp 18	*R. padi*	Winter barley
Wheelwash	Exton, Devon (19)	18/11/2020	Rp 19	*R. padi*	Winter wheat
SE63029-39093-B	Riccall, North Yorkshire (12)	16/11/2020	Sav 5	*S. avenae*	Winter wheat
DCP-2 Ŧ	Twyford, Berkshire (20)	26/11/2020	Rp 20	*R. padi*	Shepherd's purse
1A	Starcross, Devon (21)	24/11/2020	Rp 21	*R. padi*	Winter barley
RM 21-007 S10	Fownhope, Herefordshire (22)	24/11/2020	Rp 22	*R. padi*	Winter wheat

ŦThese samples were provided externally, therefore the sampling procedure might differ for these aphid lines.

*This sample (Rp13) succumbed to mummification during the lab-rearing period at Rothamsted, so it could not be screened in the bioassay.

3.2. Pyrethroid sensitivity/resistance bioassays

The screening bioassays were done using insecticide-coated glass vials with the methods based on IRAC (Insecticide Resistance Action Committee) Method 031, described in Zimmer *et. al.* (2014). The vials were coated internally with technical lambda-cyhalothrin dissolved in technical grade acetone, spun on a roller in a fume hood, allowed to dry for at least 24 h before being stored at 4^{o}C in the dark before use. A range of doses were used (equivalent to 0.1 up to 150 ng cm^{2}) plus acetone alone, in the control vials.

For each sample, aphids were brushed gently from the cereal shoots onto a white tray and up to 25 adult aphids added to each vial using a fine paint brush and the cap replaced. Only adult, mobile aphids were chosen. Two replicates were done at each of up to seven doses plus a control.

The vials containing the aphids were then stored under standardised conditions at 20°C and a 16:8 h light:dark regime. The bioassays were then scored, using a binocular microscope, after 5 h with the aphids being poured onto a white tissue. Individuals were categorised as being either: 'mobile' (capable of moving in a coordinated way), 'affected' (incapable of coordinated movement but not dead) or 'dead' (this last category was very rarely seen). There were no affected or dead aphids seen in the control, untreated vials.

3.3. Statistical analysis

LC_{50} Values, Confidence Limits and Response Slopes were calculated by Probit Analysis using the POLO PLUS Program (Leora Software, Petaluma, California) comparing fully mobile aphids versus affected plus dead aphids (with the latter two categories pooled).

4. RESULTS

Figures 2a,b,c and 3 show the raw data (black circles) respectively for the *S. avenae* and *R. padi* samples compared to baseline data (coloured circles) gained previously in these species. Each circle represents a bioassay replicate (one vial test) within each sample.

Figure 2a: Response of Sa1 (2019) sample of *Sitobion avenae* showing susceptibility to lambda-cyhalothrin versus fully susceptible (kdr-SS) and kdr-SR baseline standards.

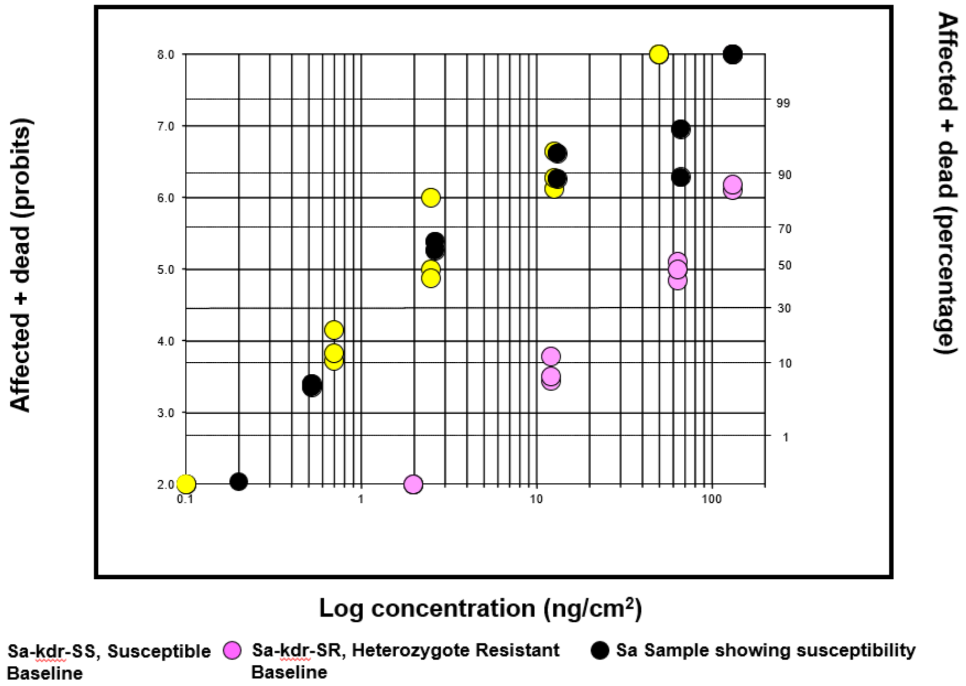


Figure 2b: Response of Sav 3 (2020) sample of *Sitobion avenae* showing reduced sensitivity to lambda-cyhalothrin versus fully susceptible (kdr-SS) and kdr-SR baseline standards.

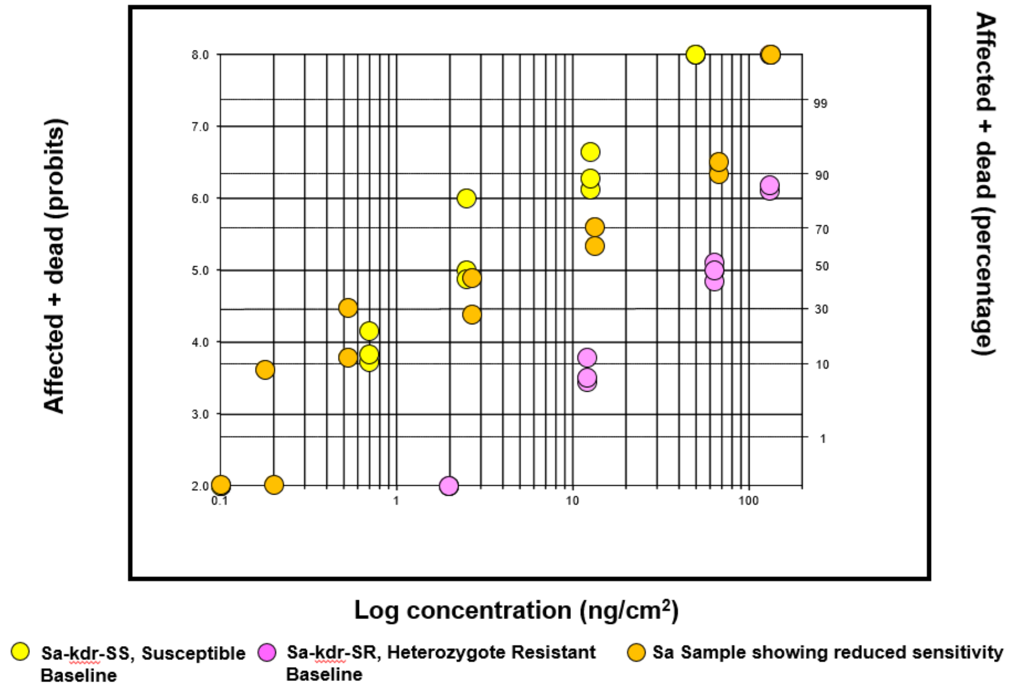


Figure 2c: Response of Sav 2, Sav 4 and Sav 5 (2020) samples of *Sitobion avenae* showing kdr-SR-type resistance to lambda-cyhalothrin versus fully susceptible (kdr-SS) and kdr-SR baseline standards.

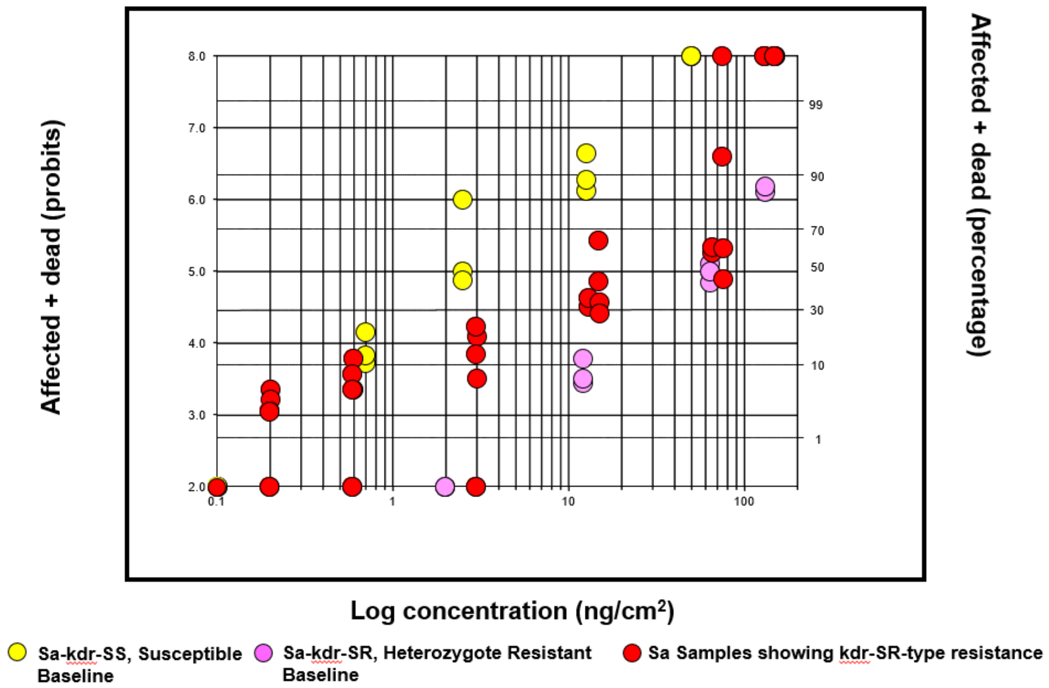
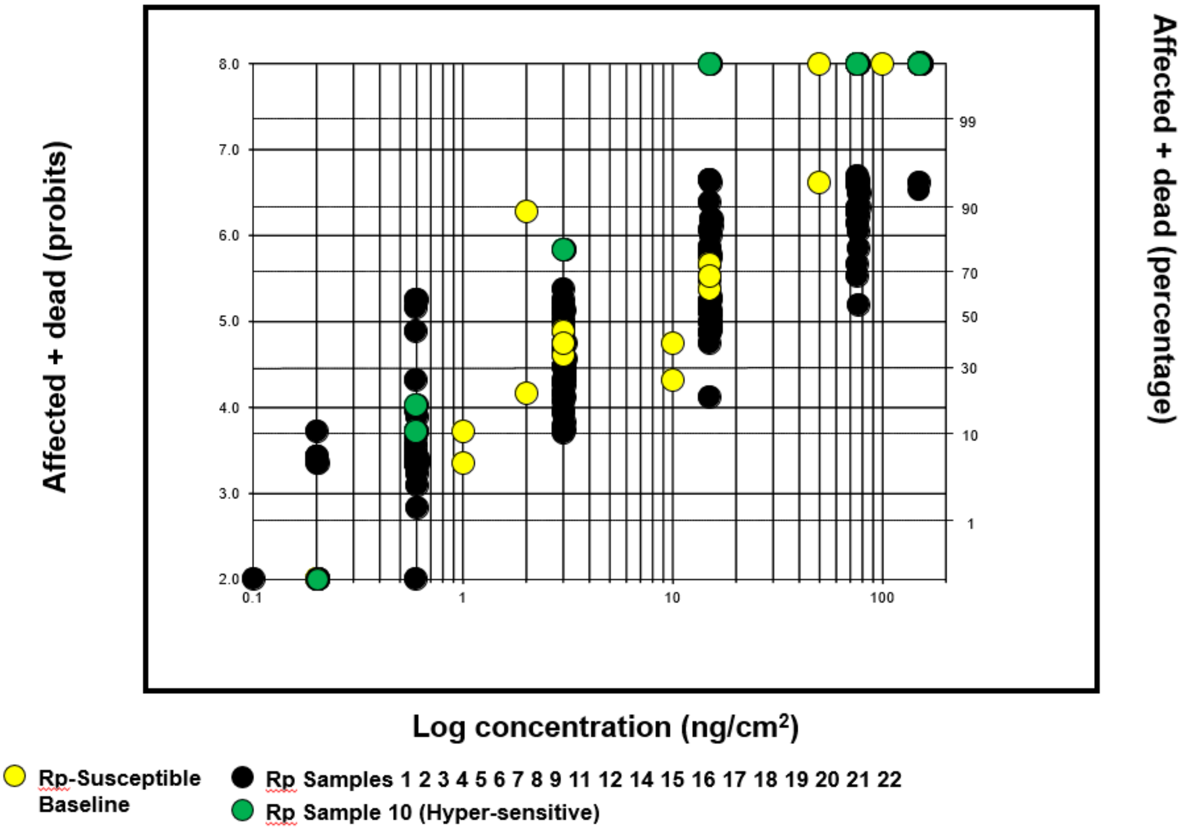


Figure 3: Response of UK samples of *Rhopalosiphum padi* to lambda-cyhalothrin versus fully susceptible standard clone.



For each aphid sample, Bioassay LC_{50} values, Confidence Limits, Response Slopes and Response Ratios are shown in Table 2.

The five *S. avenae* samples showed either full susceptibility to lambda-cyhalothrin (Sav 1), resistance similar to aphids carrying kdr (knock-down resistance) in the heterozygous (SR) form (Sav 2, Sav 4 and Sav 5) or reduced (< 5-fold) sensitivity (Sav 3). None of the samples showed response ratios greater than that seen in kdr-SR aphids.

All 21 *R. padi* samples showed responses to lambda-cyhalothrin that were statistically the same as the fully-susceptible (S) baseline standard clone, although 6 of these samples showed responses that also overlapped with a UK sample (Rp (reduced)), collected in 2017, which showed a 5-fold reduced sensitivity to this compound.

Table 2: LC_{50} responses to lambda-cyhalothrin (ng/cm^2 after 5h in coated glass vials) of UK samples of *Sitobion avenae* and *Rhopalosiphum padi* versus standard aphid references (shown in red font).

Standard Baseline Clone/ UK Sample	*N*[a]	*LC_{50}*[b]	*95% CL*[c]	*Slope*	*Response Ratio*[d]
Sa-kdr-SS	230	1.14	$0.660\text{-}1.869_{a}$	1.6	1
Sa-kdr-SR	201	39.39	$17.74\text{-}75.74_{c}$	2.7	35
Sav 1 (2019)	215	3.13	$0.885\text{-}9.850_{a}$	1.8	2.8
Sav 2 (2020)	226	33.34	$11.20\text{-}73.45_{c}$	2.1	29
Sav 3 (2020)	184	5.17	$3.353\text{-}8.008_{b}$	1.3	4.5
Sav 4 (2020)	192	24.96	$7.065\text{-}177.7_{c}$	1.0	22
Sav 5 (2020)	209	8.30	$3.617\text{-}20.58_{c}$	1.4	7.3
Rp (S)	251	6.33	$2.449\text{-}15.98_{a}$	1.4	1
Rp (reduced)[e]	126	31.80	$16.46\text{-}72.98_{b}$	1.3	5.0
Rp1 (2019)	164	11.03	$3.035\text{-}38.89_{ab}$	1.5	1.7
Rp2 (2019)	186	5.30	$3.134\text{-}8.608_{a}$	1.6	0.8
RP3 (2019)	217	4.63	$3.645\text{-}5.865_{a}$	2.0	0.7
Rp4 (2019)	242	7.02	$4.300\text{-}11.39_{a}$	1.7	1.1
Rp5 (2019)	249	5.11	$2.375\text{-}10.76_{a}$	1.8	0.8
Rp6 (2019)	250	8.18	$3.741\text{-}17.87_{ab}$	1.6	1.3
Rp7 (2019)	231	3.01	$2.596\text{-}3.494_{a}$	2.3	0.5
Rp8 (2019)	250	8.59	$4.883\text{-}14.56_{a}$	1.7	1.4
Rp9 (2019)	245	6.87	$3.741\text{-}12.45_{a}$	2.0	1.1
Rp10 (2019)	197	1.51	1.294-1.767*	3.0	0.3
Rp11 (2019)	247	8.51	$0.649\text{-}110.7_{ab}$	1.1	1.3
Rp12 (2020)	236	19.19	$7.969\text{-}49.07_{ab}$	1.2	3.0
RP14 (2020)	231	2.63	$1.324\text{-}5.164_{a}$	1.3	0.4
RP15 (2020)	221	9.30	$4.629\text{-}18.08_{ab}$	1.2	1.5
RP16 (2020)	231	4.80	$1.492\text{-}15.80_{a}$	1.0	0.8
RP17 (2020)	186	4.20	$1.680\text{-}10.02_{a}$	1.5	1.4
RP18 (2020)	202	8.97	$4.593\text{-}17.33_{ab}$	1.4	1.4
RP19 (2020)	180	4.12	$1.057\text{-}15.50_{a}$	1.2	0.7
RP20 (2020)	203	4.85	$2.488\text{-}9.546_{0}$	1.7	0.8
RP21 (2020)	197	3.30	$2.811\text{-}3.880_{a}$	2.1	0.5
RP22 (2020)	221	7.72	$6.212\text{-}9.582_{a}$	1.6	1.2

[a] Total number of aphids tested (including controls).
[b] Concentration resulting in 50% dead or irreversibly poisoned (in ng/cm^2).
[c] Confidence limits at 95%; values followed by the same letter do not differ significantly, i.e. they overlap for the sample with the standard aphid references.
[d] Within-species Response Ratio calculated from LC_{50} sample/LC_{50} for standard baseline Sa-kdr-SS or Rp (S) clones.
[e]Rp (reduced): UK sample collected in 2017 that showed reduced sensitivity to lambda-cyhalothrin.
*Rp sample showing hyper-sensitivity.

5. DISCUSSION

In total, 84 winter cereal fields were visited: 35 in 2019 with an additional 49 fields visited in 2020. Each site was visited up to four times resulting in 299 direct field site visits carried out as part of this project across both years. Our direct field sampling efforts were supplemented with samples received from independent farmers, agronomists, and researchers; resulting in 26 cereal aphid populations successfully tested in the pyrethroid sensitivity bioassays. In 2019, aphid sampling was impeded by adverse weather conditions across the country. Adverse weather, including excessive rainfall, can cause aphids to take shelter at the base of the stem closer to the soil, making them more difficult to identify under field conditions. Furthermore, the adverse weather in the winter of 2019 also had a direct impact on the seed drilling plans of winter cereal growers, with some farmers opting to delay drilling until conditions improved, reducing the number of field sites that could be visited in the winter of 2019 aphid sampling period. Aphid samples also failed to establish in culture, this was in most part due to adult mortality from exposure to parasitoid wasps or entomopathogens under field conditions before the sample was collected. The number of aphid populations tested in this study is similar to the number of populations tested in other insecticide resistance monitoring schemes (Menger *et al*., 2020; Walsh *et al*., 2020b; Hanson *et al*., 2017; Kati *et al*., 2014; Margaritopoulos *et al*., 2009).

The bioassays showed that there is no evidence of the evolution of greater resistance levels in *S. avenae* than are conferred by the kdr mechanism reported previously (Malloch *et al.,* 2016). The kdr mechanism is known in many pests to give moderate pyrethroid (20-40 fold) resistance. This finding strongly suggests that aphids carrying kdr in the homozygous (RR) form or a super-kdr mechanism, which confers very strong pyrethroid resistance in other aphids (such as *Myzus persicae*), are not present in the UK *S. avenae* population. Therefore, there is no evidence of resistance levels that would cause control failures of pyrethroids when they are applied at the recommended field rate.

None of the *R. padi* samples showed evidence of any increased selection of reduced sensitivity beyond that seen in a sample collected in 2017, which was 5-fold higher than the insecticide-susceptible (Rp S) standard clone. Reduced sensitivity to pyrethroids has been reported for a sample collected in Ireland (Walsh et al., 2020b). In the current project, one sample (Rp10), which was collected from winter wheat in Devon in December 2019, showed significant hyper-sensitivity compared to the Rp S clone, i.e., it had a phenotype that was extremely susceptible to pyrethroids.

The loss of neonicotinoid seed treatments on cereals, imposed by legislation, is inevitably putting extra selection pressure on the remaining pyrethroid insecticides and this situation may lead to the evolution of strong resistance that will cause control failures and an increase in virus levels. Therefore, it is important that continued monitoring for any shifts in sensitivity or strong pyrethroid resistance is carried out.

6. CONCLUSIONS

The project has gained contemporary information on the resistance status of two important virus-transmitting aphid pests of UK cereal crops. In *S. avenae*, there is no evidence of the evolution or selection of pyrethroid resistance above and beyond that already known in this species. A couple of samples showed moderate resistance levels associated the kdr (knock-down resistance) mechanism that is known to be present in this species. However, and importantly, greater levels of resistance were not seen. All of the *R. padi* samples showed bioassay responses similar to those seen in pyrethroid-susceptible aphids and there was no evidence of any shift in sensitivity from that seen previously.

In conclusion, our findings strongly suggest that, at the time of application, pyrethroids should continue to work against these two aphid pests, as long as sprays are applied at the full recommended rate for aphid control and good spray contact is made.

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8. Appendices

8.1. Appendix 1: Location of the 84 field sites and date of sampling visits

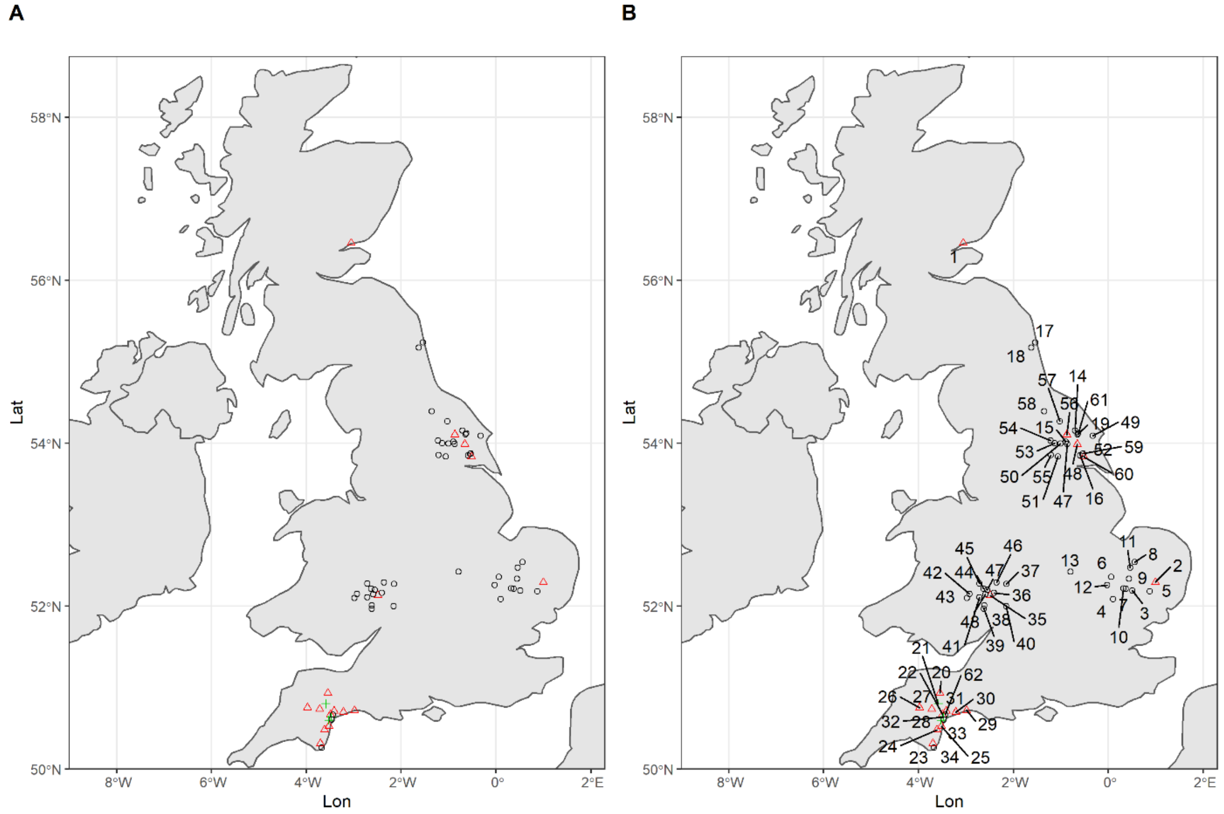
Appendix 1: Location of the 84 sampling sites visited during this project alongside dates of the sampling visits. Each site represents a unique field with up to three fields visited in a single locality. Location of the 62 sampling locations is displayed in Appendix 2.

Site number	Number of the locality on Appendix 2	Locality	Date of first visit	Date of second visit	Date of third visit	Date of fourth visit
1	1	Dundee, Tayside	15/11/2019			
2	1	Dundee, Tayside	15/11/2019			
3	2	Westhorpe	05/11/2019	28/11/2019	03/12/2019	
4	3	Lidgate, Suffolk	05/11/2019	28/11/2019	03/12/2019	
5	4	Thriplow, Cambridgeshire	05/11/2019	28/11/2019	03/12/2019	
6	5	Rattlesden, Suffolk	05/11/2019	28/11/2019	03/12/2019	
7	6	Earith, Cambridgeshire	04/11/2019	27/11/2019	01/12/2019	
8	7	Newmarket, Suffolk	05/11/2019	28/11/2019	03/12/2019	
9	8	Methwold, Norfolk	04/11/2019	27/11/2019	01/12/2019	
10	9	Isleham, Suffolk	04/11/2019	27/11/2019	01/12/2019	
11	10	Newmarket, Suffolk	05/11/2019	28/11/2019	03/12/2019	
12	11	Feltwell, Norfolk	04/11/2019	27/11/2019	01/12/2019	
13	12	Boxworth, Cambridgeshire	27/11/2019	04/12/2019	11/12/2019	17/12/2019
14	13	Desborough	27/11/2019	04/12/2019	11/12/2019	17/12/2019
15	14	Rillington, North Yorkshire	21/11/2019	28/11/2019	04/12/2019	12/12/2019
16	15	Buttercrambe, North Yorkshire	21/11/2019	28/11/2019	04/12/2019	12/12/2019
17	16	Sancton, North Yorkshire	21/11/2019	28/11/2019	04/12/2019	12/12/2019
18	17	Cresswell, Northumberland	14/11/2019	18/11/2019	30/11/2019	04/12/2019
19	18	Bothal, Northumberland	14/11/2019	18/11/2019	30/11/2019	04/12/2019
20	19	Dugleby, Malton	05/11/2019	12/11/2019	19/11/2019	26/11/2019
21	20	Tiverton, Devon	07/11/2019	11/11/2019	25/11/2019	02/12/2019
22	21	Crediton, Devon	04/11/2019	21/11/2019	25/11/2019	
23	22	Exminster, Devon	07/11/2019	11/11/2019	25/11/2019	02/12/2019
24	23	East Allington, Devon	07/11/2019	11/11/2019	25/11/2019	02/12/2019
25	24	Teignbridge, Devon	25/11/2019	02/12/2019	05/12/2019	09/12/2019
26	25	Stokeinteignhead, Devon	04/11/2019	21/11/2019	25/11/2019	
27	26	Okehampton, Devon	04/11/2019	21/11/2019	25/11/2019	
28	27	Tedburn St Mary, Devon	04/11/2019	21/11/2019	25/11/2019	
29	28	Starcross, Devon	11/18/2019	18/11/2019	05/12/2019	09/12/2019
30	29	Rousdon, Devon	07/11/2019	11/11/2019	05/12/2019	09/12/2019
31	30	Sidford, Devon	25/11/2019	02/12/2019		
32	31	Clyst Honiton, Devon	04/11/2019	21/11/2019	25/11/2019	09/12/2019
33	32	Kenton, Devon	18/11/2019	25/11/2019	02/12/2019	09/12/2019
34	33	Dawlish, Devon	18/11/2019	25/11/2019	02/12/2019	09/12/2019
35	34	Stokenham, Devon	18/11/2019	25/11/2019	02/12/2019	09/12/2019

Site number	Number of the locality on Appendix 2	Locality	Date of first visit	Date of second visit	Date of third visit	Date of fourth visit
36	35	Bishops Frome, Herefordshire	30/10/2020	03/11/2020	13/11/2020	17/11/2020
37	36	Suckley, Worcestershire	06/11/2020	10/11/2020	20/11/2020	24/11/2020
38	37	Droitwich, Worcestershire	06/11/2020	10/11/2020	20/11/2020	24/11/2020
39	38	Fownhope, Herefordshire	06/11/2020	10/11/2020	20/11/2020	24/11/2020
40	39	Fawley, Herefordshire	06/11/2020	10/11/2020	20/11/2020	24/11/2020
41	40	Tewkesbury, Gloustershire	06/11/2020	10/11/2020	20/11/2020	24/11/2020
42	41	Moreton on Lugg, Herefordshire	30/10/2020	03/11/2020	13/11/2020	17/11/2020
43	42	Sarnesfield, Herefordshire	30/10/2020	03/11/2020	13/11/2020	17/11/2020
44	43	Bredwardine, Herefordshire	30/10/2020	03/11/2020	13/11/2020	17/11/2020
45	44	Docklow, Herefordshire	06/11/2020	10/11/2020	20/11/2020	24/11/2020
46	45	Luston, Herefordshire	30/10/2020	03/11/2020	13/11/2020	17/11/2020
47	46	Great Witley, Worcester	06/11/2020	10/11/2020	20/11/2020	24/11/2020
48	47	Bredenbury, Herefordshire	30/10/2020	03/11/2020	13/11/2020	17/11/2020
49	48	Ullingswick, Herefordshire	15/11/2020			
50	20	Tiverton, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
51	21	Crediton, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
52	22	Exminster, Devon	06/11/2020	09/11/2020	20/11/2020	23/11/2020
53	23	East Allington, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
54	24	Teignbridge, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
55	25	Stokeinteignhead, Devon	31/10/2020	02/11/2020	13/11/2020	16/11/2020
56	26	Okehampton, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
57	27	Tedburn St Mary, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
58	28	Starcross, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
59	29	Rousdon, Devon	13/11/2020	16/11/2020	27/11/2020	30/11/2020
60	30	Sidford, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
61	31	Clyst Honiton, Devon	23/10/2020	27/10/2020	06/11/2020	09/11/2020
62	47	Full Sutton, East Yorkshire	23/10/2020	27/10/2020	13/11/2020	16/11/2020
63	48	Huggate, East Yorkshire	23/10/2020	27/10/2020	13/11/2020	16/11/2020
64	49	Rudston, East Yorkshire	24/10/2020	28/10/2020	14/11/2020	17/11/2020
65	50	Stockton on the Forest, North Yorkshire	24/10/2020	28/10/2020	14/11/2020	17/11/2020
66	51	Riccall, North Yorkshire	24/10/2020	28/10/2020	14/11/2020	17/11/2020
67	52	Gardham, East Yorkshire	24/10/2020	28/10/2020	14/11/2020	17/11/2020
68	53	Skelton, North Yorkshire	23/10/2020	27/10/2020	13/11/2020	16/11/2020
69	54	Newton on Ouse, North Yorkshire	23/10/2020	27/10/2020	13/11/2020	16/11/2020
70	55	Ulleskelf, North Yorkshire	24/10/2020	28/10/2020	14/11/2020	17/11/2020
71	56	Huttons Ambo, North Yorkshire	24/10/2020	28/10/2020	14/11/2020	17/11/2020
72	57	Pockley, North Yorkshire	23/10/2020	27/10/2020	13/11/2020	16/11/2020

Site number	Number of the locality on Appendix 2	Locality	Date of first visit	Date of second visit	Date of third visit	Date of fourth visit
73	58	East Harlsey, North Yorkshire	23/10/2020	27/10/2020	13/11/2020	16/11/2020
74	59	Cherry Burton, East Yorkshire	23/10/2020	26/11/2020	16/12/2020	
75	60	Bishop Burton, East Yorkshire	19/11/2020	26/11/2020	16/12/2020	
76	61	Rayslack, North Yorkshire	22/10/2020	29/10/2020	06/11/2020	13/11/2020
77	56	Huttons Ambo, North Yorkshire	24/10/2020	29/10/2020	06/11/2020	13/11/2020
78	48	Huggate, East Yorkshire	29/10/2020	06/11/2020	13/11/2020	19/11/2020
79	21	Crediton, Devon	08/09/2020	15/09/2020	22/09/2020	29/09/2020
80	62	Exton, Devon	18/09/2020	25/09/2020	02/10/2020	09/10/2020
81	32	Kenton, Devon	28/09/2020	05/10/2020	12/10/2020	19/10/2020
82	28	Starcross, Devon	16/11/2020			
83	32	Kenton, Devon	24/11/2020			
84	2	Westhorpe, Suffolk	26/11/2020			

8.2. Map of sampling site localities



Appendix 2: Location of the 62 sampling localities. The colour of the points represents the number of sampling sites in each locality: black = one site, red = two sites, green = three sites. Righthand map contains locality number overlay.

9. Knowledge Exchange Activities

Crop Protection in Northern Britain conference, Dundee, February 2020.

Croprotect Webinar, hosted by Rothamsted Research, February 2021

ADAS Spring 2021 technical update, March 2021

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