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ABSTRACT

A concerning development for Irish agriculture is the detection of partial resistance in the main aphid pest (*Sitobion avenae*) of cereal crops to the most widely used pyrethroid insecticide compound. The mechanism of this resistance, termed 'knockdown resistance' (*kdr*), affects pyrethroid binding and enables *S. avenae* to survive insecticide exposure. This partial resistance to insecticide means that *S. avenae* can continue to inflict feeding damage and transmit barley yellow dwarf virus (BYDV), which may carry a significant yield penalty. The incidence and persistence of resistant *S. avenae* in the Irish population is currently unknown. To address this knowledge gap, in-field sampling of *S. avenae* was carried out from 2016 to 2018 in the 11 major cereal-growing counties, and 621 cases of *S. avenae* were screened. Genotyping was used to screen *S. avenae* for *kdr* and to determine the diversity of clones in the resistant and susceptible genotypes, thus testing the hypothesis of resistance in a single dominant super-clone. The data were statistically analysed to determine annual variation in resistance levels. Findings revealed that resistant *S. avenae* are widespread across Ireland, occurring in all the major cereal-growing counties. Despite an initial high prevalence of resistant *S. avenae* (54%), matching levels detected in the UK, prevalence in Irish field populations appeared not to be increasing over the duration of this study, suggesting that pyrethroids remain largely effective at managing aphid populations. Resistance was detected in a single dominant SA3 super-clone, which may be explained by the loss of cyclical parthenogenesis as a potential impact of resistance alleles.

INTRODUCTION

*Sitobion avenae* (Fabricius), commonly known as the grain aphid, is a significant pest of cereals worldwide, including in Ireland where it is considered to be the main pest of spring and winter barley and the main vector of barley yellow dwarf virus (BYDV), which can reduce yield by up to 62% in extreme cases (Riedell et al. 2003). Control of BYDV depends on successfully inhibiting infection by viruliferous *S. avenae*, which to date is mainly achieved by means of foliar insecticide sprays applied after crop emergence (Kennedy and Connery 2012). Insecticide options for aphid pest management between 2016 and 2018 in Ireland included four insecticidal compounds, with only two permitted for use at early crop emergence stage. A neonicotinoid insecticide was approved for application as a seed dressing, a pyrethroid insecticide is permitted as an early spray at crop emergence, and a sulfoxaflor insecticide (sulfoximine) is restricted to a single late foliar insecticide spray after the booting growth stage. Additionally, an organophosphate compound is permitted for use only on wheat as a late head spray. Since the European Union’s decision in May 2018 to ban outdoor use of the three main neonicotinoid compounds (EU 2018), only the pyrethroid compound remains for BYDV control in cereals after crop emergence, reducing the options growers have to manage BYDV risk. The detection of partial resistance to pyrethroids, which are now the most widely used insecticide for grain aphid control (Dewar and Foster 2017), in combination with the loss of the neonicotinoid seed dressing for winter cereals, increases the risk of yield loss that may hinder development of Ireland’s tillage sector.

Reported instances of poor aphid control and the detection of BYDV outbreaks across the south-east of Ireland coincided with the detection of *S. avenae* heterozygous for the *kdr* mutation in Ireland for the first time (Walsh et al. 2019). This spurred efforts to understand the extent of resistance in Ireland’s main cereal pest population in order to determine how best to alter crop protection strategies to prevent widespread yield reductions.
INSECTICIDE RESISTANCE

When a pest develops the capacity to survive pesticide exposure through rapid adaptive evolution and mutation, it is considered to be resistant (Ranson et al. 2011). The consequence of resistance is a rapid increase in the prevalence of resistant forms in the pest population, particularly when parthenogenesis is prolonged, which increases concern for negative impacts to crop production and, potentially, even food security (Barres et al. 2016). The first indications of pyrethroid resistance in S. avenae in the British Isles coincided with reports of pyrethroid spray failures in 2011 in England (Dewar and Foster 2017).

Molecular, nucleic acid-based assays are established methods of detecting genes or mutations involved in resistance (Barres et al. 2016) once genotyping has characterised the target site mutation. The mutation that was first characterised in S. avenae by Foster et al. (2014) led to the development of an allele-specific polymerase chain reaction (PCR) assay for the crude screening and rapid detection of kdr in samples of S. avenae following DNA amplification.

APHID CLONES

The prevalence of kdr-resistant S. avenae is reported to be high in the UK, with up to 50% of grain aphids collected in suction traps testing positive for kdr (Dewar and Foster 2017). Initial work indicated that resistant grain aphids are from a single, dominant and established SA3 clone in England and Scotland (Malloch et al. 2016), which is sometimes referred to as a ‘super-clone’ (Wash et al. 2019), particularly when it becomes the prevalent clone within the overall species population. Other work to characterise the diversity of multi-locus genotypes (MLG) in S. avenae (Loxdale et al. 2010) found that S. avenae in the UK were consistently comprised mostly of single (i.e. unique) or low numbers of repeats rather than larger multiple copy (clonal) MLG repeats. This may be attributed to prolonged asexual reproduction and resistance mechanisms that create a favourable genetic background for survival in cold, wet winters (Foster et al. 2017), analogous to the success of two dominant resistant—although non-kdr—superclones in Myzus persicae (Fenton et al. 2010).

ESTABLISHING CLONAL DIFFERENCES

Microsatellites, or single sequence repeats (SSRs), are widely employed as molecular markers in population genetic studies (Guichoux et al. 2011). Microsatellites are comprised of motifs of 1 to 10 nucleotides recurring several times as tandem repeats (with >10 repeats for [A/T] n and > 6 repeats for [GT/CA] n). These microsatellites have distinguishing mutation behaviours (Kelkar et al. 2010). Microsatellite genotyping is used here in order to categorise at the clonal level, S. avenae of the kdr-SS and kdr-SR genotypes, and in doing so detect variation in clones within S. avenae in Ireland. The hypothesis of lower genetic diversity in the kdr-SR genotype is tested to establish if this corresponds to the population structure of UK S. avenae.

Cereals are the most widely produced tillage crop, taking up 8% (approximately 300,000ha) of Ireland’s agricultural land; yet despite this, monitoring of cereal aphid pests has been low. The prevalence of S. avenae resistant to pyrethroid insecticides in field and the clonal profile of the resistant genotype in Ireland is currently unknown, hampering progressive efforts to tailor crop protection strategies. Within this study the aim was to (1) quantify the prevalence of resistant S. avenae with the kdr mechanism in Ireland at several spatial and temporal scales, and (2) investigate the clonal profile of resistant grain aphids with kdr. Efforts to understand the population genetics of resistance would help to contextualise further research and control efforts aimed at resistance management. Stratified random sampling for S. avenae was carried out in cereal fields to collect S. avenae over a three-year period. DNA was extracted from individual S. avenae, tested and scored for kdr.

METHODS AND MATERIALS

GRAIN APHID SAMPLING

Sampling was carried out in 70 cereal fields that encompassed four insect pest management approaches across 11 counties (Fig. 1) where cereal acreage was high, based on data obtained from Teagasc advisory services. Fields in this study were categorised as (i) untreated, (ii) treated with pyrethroid only, (iii) neonicotinoids only and (iv) pyrethroid + neonicotinoid insecticides. These fields were sampled to represent the standard approaches used by Irish growers, and an equal number of fields were targeted (although not always accessed) in each county.

Sampling was carried out in the summer of 2016, 2017 and 2018 in a three-month period between May and July. S. avenae were collected from the heads of cereals where grain aphids are known to locate at this time (Phillips 1916) and samples were transported back to the laboratory for processing and identification using Blackman’s key (Blackman 2010). Aphid collections were undertaken by actively searching a total of 50 tillers (50 sample points), at 20m intervals in each field. Where S. avenae were detected, they were inspected using a handheld lens and collected off the crop into a 1.5ml tube using a fine paintbrush. Up to 50 S. avenae were targeted in each field, although aphid abundance was highly variable. Once identification was confirmed samples were placed in 90% ethanol and at -20°C for DNA extraction.
In-field prevalence of resistant grain aphid *Sitobion avenae* (Fabricius)

**Fig. 1**—The location of Irish counties where sampling was carried out to collect grain aphids, screened for *kdr*.

**IDENTIFICATION OF RESISTANT PHENOTYPES AND MICROSATELLITE ANALYSIS**

**DNA extraction and preparation**

Genomic DNA (gDNA) was extracted and purified from individual adult grain aphids using a sucrose buffer extraction method similar to Louis (1997) where whole *S. avenae* were suspended in 50µl of 300mM extraction buffer (0.3M sucrose, 0.3M NaCl, 60mM Tris HCL pH 8) in a single well of a flat bottom 96-well plate and gently homogenised. The plate was sealed and placed in a shallow water bath for nine minutes at 95°C, then centrifuged for two minutes at 3000rpm before being placed on ice, in order to collect the excess cellular debris (the pellet) in the centre of the well. Subsequently, 30µl of the supernatant was transferred, carefully avoiding the pellet, to a new 96-well storage plate, and stored at -20°C. For microsatellite genotyping DNA quality and concentration was measured using a Nanodrop® ND-1000 Spectrophotometer (Labtech Int. UK), prior to storage.

**Knock down resistance (***kdr***) screening and scoring**

Each grain aphid was screened for *kdr* resistance using a Taqman Polymerase Chain Reaction (PCR) assay developed by Rothamsted Research, UK using forward (ATTCTTCTTGCTACCGTTGTCAT),
and reverse (CCTCCTAAATTCTTGACGCAGTAGTACATATTATAA) outer primer, and dye-labelled probe sequences (VIC: ACCACGATTTACCCG and FAM: ACCACGAAATTTACCG), to detect the presence of the kdr mutation L1014F. A single reaction mix was prepared using 1.5µl of aphid DNA, 0.375µl of forward and reverse primers and VIC and FAM probes prepared with sterilised distilled water, 7.5µl Sensifast™ Hi-Rox master mix and 5.625µl sterilised distilled water. Probe and primer sequences were provided by Rothamsted Research, where the analysis was carried out on an ABI 7900 HT RT-PCR system in 2016. Testing was carried out at Teagasc Ashtown Research Centre, Ireland in 2017 and 2018 on an ABI 7500 HT RT-PCR system. Genomic DNA was extracted from heterozygous resistant and susceptible clones obtained from Rothamsted Research, which served as positive and negative controls in the diagnostic assay.

In a simple model of resistance development conferred by a mutation in one gene—in this instance the voltage gated sodium channel (VGSC) gene with two alleles—three potential genotypes may be generated based on R (resistance) and S (susceptibility): (i) kdr-RR homozygous resistant DNA with the mutation detected on both alleles of the VGSC gene (ii) kdr-SR heterozygous resistant DNA with the mutation detected on only a single allele of the VGSC gene, (iii) kdr-SS homozygous susceptible DNA with the mutation undetected on both alleles of the VGSC gene.

Microsatellite genotyping

Microsatellite genotyping of preserved DNA samples was carried out at the James Hutton Institute (JHI) in Invergowrie, Scotland. Genotypes from the study were examined at five microsatellite loci: Sm10, Sm12, Sm17, Sa24 and S16b using published primer pair sequences (Llewellyn et al. 2003, Simon et al. 1999; Wilson et al. 2004) and a published protocol (Malloch et al. 2016). PCR was carried out on a Biometra T Personal thermal cycler using the Touchdown programme described in Sloane et al. (2001), after annealing temperatures were calculated and checked for each primer pair. Extracted DNA and primers were diluted approximately to 1:10 ahead of PCR to equalise quantities of DNA in each reaction. To save time and resources multiplexing was used to combine Sm10 NED labelled, Sm12 VIC labelled and Sm17 6-FAM labelled primers in a first PCR and Sa24 VIC and S16b 6-FAM in a second PCR, and 24µl of PCR mastermix cocktail (1µl primer + sterilised distilled water) was added to a single illustra bead (GE Healthcare life sciences with ~2.5 units of recombinant Phusion DNA polymerase, dATP, dCTP, dGTP, dTTP stabilizers, BSA and reaction buffer) reconstituted according to manufacturer’s instructions. Ahead of genotyping, samples were centrifuged at 3000rpm for 2 minutes. For genotyping 12µl of GeneScan™ 1200 LIZ™ dye Size Standard (Thermofisher Scientific, cat no. 4379950) and 9µl of Hi-Di™ Formamide (Thermofisher scientific, cat no. 4440753) was added to each well of a 96 well plate along with 1µl of PCR product. The multiplex sequencing was carried out on an ABI 3730 DNA analyser with the results interpreted using Genemapper® (Applied Biosystems 2005) based on the sizing of peaks against those of the five published loci, and in reference to the James Hutton Institute reference dataset.

STATISTICAL ANALYSIS

Data was statistically analysed using IBM SPSS Statistics 24. The margin of error for each sample size was calculated at the 95% confidence interval. Analysis of variance (ANOVA) with a Bonferroni post hoc test was used to establish a significant difference between years in the number of S. avenae with kdr and a 2x7 contingency table was formulated to display clonal types. A Pearson’s X² test was performed to test for a relationship between variables with the Cramer’s V post-test to determine the association between the variables resistance and microsatellite clone. The susceptible genotype (kdr-SS) is abbreviated as SS and the partially resistant genotype (kdr-SR) as SR in results.

RESULTS

PREVALENCE OF RESISTANT S. AVENAE IN IRISH CEREALS

S. avenae numbers varied across fields. In 2016, n=200 S. avenae were collected in 19 fields each with 50 sample points, leading to a mean detection rate of 21% and a margin of error of 3.2% at a confidence level of 95%. In 2017, n=339 S. avenae were collected in 45 fields each with 50 sample points, with a mean detection rate of 15% and margin of error of 2.1% at a confidence level of 95%. In 2018 n=82 were collected in 6 fields each with 50 sample points, leading to a mean detection rate of 27% with a margin of error of 5.7% at a confidence level of 95%. The detection rate of S. avenae was less in 2017 than in 2016, and more in 2018 than in 2017, with the lowest number of S. avenae detected for the sample effort in 2017. The numbers of S. avenae detected were significantly different in each year, as evidenced by the absence of overlap in the confidence limits at the 95% confidence level.

In 2016 the prevalence of resistant kdr-heterozygotes (kdr-SR) was 108 out of a total of 200 S. avenae. The prevalence of kdr-SS susceptible homozygotes was lower at 92 out of the total 200 S.
*In-field prevalence of resistant grain aphid *Sitobion avenae* (Fabricius)

*aavenae* tested and scored. The prevalence of resistant heterozygotes was 54% in 2016 (Fig. 2, pie chart, 2016), with a margin of error of 5.7% at a confidence level of 95%. In 2017 the prevalence of resistant *kdr*-heterozygotes was 85 out of a total of 339 (25%) while a greater prevalence of *kdr*-SS susceptible homozygotes recorded at 254 out of the total 339 *S. avenae* tested and scored, with a margin of error of 5.3% at a confidence level of 95% (Fig. 2, pie chart 2017). In 2018 the prevalence of resistant *kdr*-heterozygotes (*kdr*-SR) was 16 out of a total of 82 *S. avenae* (20%), while the prevalence of *kdr*-SS susceptible homozygotes was lower at 66 out of the total 82 *S. avenae* tested, with a margin of error of 10.8% at a confidence level of 95% (Fig. 2, pie chart 2018). The overlap in the confidence limits indicates that the proportion of *S. avenae* heterozygous for *kdr* was significantly different in 2016, although it was not significantly different in 2017 and 2018.

**INTER-ANNUAL VARIATION IN THE PREVALENCE OF RESISTANT S. AVENAE**

*S. avenae* were collected from seven Irish counties in 2016 and the proportion of *S. avenae* with *kdr* (SR) in relation to the proportion without *kdr* (SS) is presented in the 2016 bar chart in Fig 2. In county Louth, *n*=53 *S. avenae* were detected with a 75% prevalence of *S. avenae* with *kdr*. In county Wexford, *n*=50 *S. avenae* were detected with a 40% prevalence of *S. avenae* with *kdr*. In county Wicklow, *n*=1 *kdr* *S. avenae* was detected. In county Carlow, *n*=64 *S. avenae* were detected with a 50% prevalence of *S. avenae* with *kdr*. In county Kilkenny, *n*=6 *S. avenae* were detected, none with *kdr*, and in county Meath, *n*=12 *S. avenae* were detected with a 58% prevalence of *S. avenae* with *kdr*.

*S. avenae* were collected from nine Irish counties in 2017 and the proportion of *S. avenae* with *kdr* (SR) in relation to the proportion without *kdr* (SS) is presented in the 2017 bar chart in Fig 2. In county Louth, *n*=34 *S. avenae* were detected with a 41% prevalence of *S. avenae* with *kdr*. In county Wexford, *n*=25 *S. avenae* were detected with an 8% prevalence of *S. avenae* with *kdr*. In county Wicklow, *n*=7 *S. avenae* were detected with no detection of *S. avenae* with *kdr*. In county Carlow, *n*=40 *S. avenae* were detected with an 18% prevalence of *S. avenae* with *kdr*. In county Meath, *n*=22 *S. avenae* were detected with a 32% prevalence of *S. avenae* with *kdr*. In county Kildare, *n*=60 *S. avenae* were detected with a 20% prevalence of *S. avenae* with *kdr*. In county Tipperary, *n*=30 *S. avenae* were detected with a 13% prevalence of *S. avenae* with *kdr*. In county Laois, *n*=20 *S. avenae* were detected with a 65% prevalence of *S. avenae* with *kdr*.

*S. avenae* were collected from four Irish counties in 2018 and the proportion of *S. avenae* with *kdr* (SR) in relation to the proportion without *kdr* (SS) is presented in the 2018 bar chart in Fig 2. In county Wexford, *n*=6 *S. avenae* were detected with a 17% prevalence of *S. avenae* with *kdr*. In county Cork, *n*=3 *S. avenae* were detected with a 33% prevalence of *S. avenae* with *kdr*. In county Kildare, *n*=24 *S. avenae* were detected with a 13% prevalence of *S. avenae* with *kdr*. In county Waterford, *n*=49 *S. avenae* were detected with a 22% prevalence of *S. avenae* with *kdr*.

A significant difference was detected in the number of *S. avenae* with *kdr* using ANOVA (*F*=30.387, df=2, *P*< 0.0001) and a multiple comparison was undertaken using the Bonferroni post hoc test to establish where there were significant differences between years. Overall there is significant inter-annual variability in the number of *S. avenae* with *kdr*, although differences were not significant between 2017 and 2018 (Table 1).

**CLONAL DIVERSITY WITHIN S. AVENAE GENOTYPES**

A subsample of 44 *S. avenae* collected in 2016 were genotyped to determine their clonal identity, which led to the recognition of seven clonal categories with reference to the dataset at the JHI. Six new un categorised clones were detected in the *kdr*-SS genotype (Table 2). A unique, previously undetected resistant clone, the New 145 clone was detected in the *kdr*-SR genotype for the first time in the British Isles. The SA27 clone was the most dominant of all documented clones detected in the *kdr*-SS genotype. The *kdr*-SS genotype displays a greater diversity of clones with five clones identified from 15 *S. avenae* DNA, while only two clones are detected out of 29 *S. avenae* DNA tested and scored in the *kdr*-SR genotype. The dominant resistant clone detected in the *kdr*-SR genotype is the SA3 clone.

A Pearson’s X² test was performed to test for a relationship between variables. The results of the crosstabs (X²=39.703, df=6, *P*< 0.0001) and the Cramer’s V post-test (0.950) indicate a significantly strong association between the variables resistance and microsatellite clone.

**DISCUSSION**

The study set out to identify the prevalence of *S. avenae* in Irish fields. Unlike the UK where an established suction trap network exists, supporting aphid sampling and resistance detection across space and time (Macaulay et al. 2009; Dedryver et al. 2011), Ireland relies on in-field, ad-hoc sampling of grain aphids since a suction trap network is lacking.
Fig. 2—Bar Chart: the prevalence of heterozygous resistant \( kdr \)-(SR) and non-resistant, pyrethroid susceptible \( kdr \)-(SS) \( S. avenae \) in Irish counties between 2016 - 2018 shown as a proportion of total aphids analysed. The X-axis indicates the county name and the number of fields sampled each year. Pie Chart: Overall percentage of \( kdr \)-(SR) and \( kdr \)-(SS) aphids screened during three years of stratified random sampling in Irish cereal fields, 2016 (\( n = 200 \)), 2017 (\( n = 339 \)) and 2018 (\( n = 82 \)).
In-field prevalence of resistant grain aphid Sitobion avenae (Fabricius)

Table 1—Multiple comparisons of the average number of aphids heterozygous for kdr between years using ANOVA with a Bonferroni post hoc test

<table>
<thead>
<tr>
<th>(I) Year</th>
<th>(J) Year</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>2017</td>
<td>0.289*</td>
<td>0.040</td>
<td>0.000</td>
<td>0.19 - 0.39</td>
</tr>
<tr>
<td>2018</td>
<td>2017</td>
<td>0.345*</td>
<td>0.059</td>
<td>0.000</td>
<td>0.20 - 0.49</td>
</tr>
<tr>
<td>2016</td>
<td>2018</td>
<td>-0.289*</td>
<td>0.040</td>
<td>0.000</td>
<td>-0.39 - -0.19</td>
</tr>
<tr>
<td>2017</td>
<td>2018</td>
<td>-0.056</td>
<td>0.056</td>
<td>0.953</td>
<td>-0.08 - 0.19</td>
</tr>
<tr>
<td>2016</td>
<td>2017</td>
<td>-0.056</td>
<td>0.056</td>
<td>0.953</td>
<td>-0.19 - 0.08</td>
</tr>
</tbody>
</table>

*The mean difference is significant at the 0.05 level.

Table 2—A 2x7 contingency table of variables kdr-resistance x microsatellite genotype as categorised based on the reference genotype database at the James Hutton Institute (JHI)

<table>
<thead>
<tr>
<th>kdr-resistance</th>
<th>Microsatellite clone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>New 145</td>
</tr>
<tr>
<td></td>
<td>Ay 100816</td>
</tr>
<tr>
<td></td>
<td>Clone13-15-s1</td>
</tr>
<tr>
<td></td>
<td>New Irish</td>
</tr>
<tr>
<td></td>
<td>SA27</td>
</tr>
<tr>
<td></td>
<td>SA3</td>
</tr>
<tr>
<td></td>
<td>SA44</td>
</tr>
<tr>
<td>SR</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
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<td></td>
<td>0</td>
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<td>0</td>
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<tr>
<td></td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>SS</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
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<tr>
<td></td>
<td>1</td>
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<td></td>
<td>6</td>
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<td>4</td>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Although as a result of studies such as this, a limited network of suction traps are now set to be installed. All results refer to S. avenae being heterozygous for the kdr mutation as no homozygous individuals were detected in the three years of sampling undertaken, matching findings coming out of the UK suction trap monitoring network (Malloch et al. 2016).

The annual prevalence of S. avenae in crops was found to vary at a detection rate between 15–27%. This leads to the expectation that the number of S. avenae will vary in the crop.

The observation in this study of inter-annual variation in the prevalence of resistant kdr-heterozygotes supports the conclusion reached by Winder et al. (1999) about this species. High prevalence was observed in 2016, with significantly lower prevalence in 2017 and 2018. The levels of kdr in 2016 more closely match the prevalence of S. avenae with kdr detected in UK suction traps in that same year (Dewar and Foster 2017). The over reliance on a single insecticide compound, mainly pyrethroid insecticides, against resistant S. avenae is the most likely explanation for the high prevalence (Dewar 2016; Dewar and Foster 2017) and has been associated with pest resurgence (Hardin et al. 1995). In fact this would have the effect of lowering the proportion of susceptible individuals in the population (Denholm and Rowland 1992) and the literature provides further supporting evidence. A study in Ireland by Walsh et al. (2019) reported that S. avenae are able to survive pyrethroid exposure up to twice field rate applications and continue reproducing at a comparable rate to unexposed S. avenae. One potential explanation for this is the hormesis effect of insecticide where low doses of insecticide which do not inhibit resistant insects, in fact have a stimulus effect. Resistant individuals have shown evidence of increases in life history traits such as lifespan, fecundity or shorter generation times (Guedes and Cutler 2014). This has even been documented in response to pyrethroid insecticides in the southern red mite Oligonychus ilicis and in response to low doses of permethrin in the stinkbug Podisus distinctus, where female survival and reproductive rates were greater (Cordeiro et al. 2013; Zanuncio et al. 2013). One other explanation is that incidence of outbreaks may be cyclical in multivoltine insects like S. avenae, with high incidence correlated with high temperatures and declines associated with decreasing temperature. This has been shown in a model of tortrix moth outbreaks by Nelson et al. (2013).

The cause of a decline in S. avenae detected to carry kdr in 2017 and 2018 may also be for other reasons entirely. Firstly, a shift by growers to the use of neonicotinoid seed dressing as the dominant pest management approach would provide a different insecticide mode of action against pyrethroid resistant S. avenae, in effect a resistance management strategy (Sparks and Nauen 2015), which would increase in-field control of kdr-SR genotypes. Secondly, a fitness penalty may be linked
to the kdr mutation preventing the persistence of kdr forms under variable environmental conditions such as high predator pressure or extreme winter conditions as experienced in Ireland in 2017/2018 (Met Éireann 2018). Supporting research from Scottish suction trap data shows variability in resistant aphid clones between years (Malloch et al. 2016), likely linked to fitness characteristics. Another, equally likely explanation is that alternative mechanisms may be manifesting in the resistant S. avenae population which are not the kdr mechanism, and would therefore be undetected by the Taqman assay used in this study. For example, research on Myzus persicae in Scotland showed that two non-kdr clones came to predominate in the population at the apparent expense of kdr. These two clones which carry super-kdr (an alternative mutation) coupled with MACE (modified acetylcholinesterase) confers stronger resistance to pyrethroid and carbamate insecticides (Fenton et al. 2010). Resistance evolves in response to stressful conditions (Chen et al. 2013) and at a molecular level, the response may be the development of new genes or the occurrence of gene duplication events. Different genotypes can also assemble resistant phenotypes in reaction to environmental drivers (Silva et al. 2012). Interactions become important in how resistance evolves and in this way different forms of multigene resistance are upheld in the field (Hardstone and Scott 2010).

The outcome of microsatellite genotyping is the observation of a single dominant SA3 clone in the resistant group. The detection of an alternative clone with kdr (New 145) indicates that other grain aphid clones may also carry the L1014F mutation conferring kdr. This new clone was detected for the first time in Ireland, although there have been observations in Scottish suction traps of other kdr carrying clones (Malloch et al. 2016). Like the New 145 clone, these clones were only detected on a single occasion suggesting that their prevalence in the population is low. A greater diversity of clones was detected in the susceptible group more likely the result of cyclical parthenogenesis with a sexual phase that facilitates genetic crossing, which may not be the case for the resistant SA3 clone that instead opts for greater investment in parthenogenetic overwintering strategies, similar to the strategy of S. avenae in the warmer southern regions of France (Dedryver et al. 2001). This would explain the absence of fully resistant homozygotes in this study as well as in monitoring efforts in the UK (Dewar 2016; Dewar and Foster 2017; Malloch et al. 2016). Investigating this link between resistance alleles and altered reproductive traits becomes important as an area of further research, in order to shed light on the evolutionary and ecological dynamics of resistance in S. avenae.

This study provides the first attempt to quantify the prevalence of S. avenae resistant to pyrethroids at a field-level in Ireland. A suction trap network, as it exists in the UK, elsewhere in Europe and in the US, is absent in Ireland and therefore in-field assessments currently provide the only manner in which to assess the extent of resistance in the population.

Grain aphid populations are shown to be affected by several ecological factors and with resistance in the mix, further penalties may manifest at a genetic and molecular level that impact the survival of resistant S. avenae. It therefore becomes important to assess, at a field/crop interface where S. avenae are having the most impact, and what level of resistance can be expected in field populations. As such, this study indicates that the prevalence of S. avenae varies spatially and temporally in the field, matching the observations of other studies on this species (Vialatte et al. 2007; Winder et al. 1999). Likewise, the prevalence of resistant S. avenae with kdr also varies and this may be underpinned by husbandry factors that in turn may explain patterns of resistance observed in-field.

This study indicates that the prevalence of kdr is lower than levels reported in the UK, although the differences in sample collection methodology needs to be acknowledged. The results suggest that as the prevalence of the kdr-SR genotype, in particular the SA3 clone appears not to be increasing in the S. avenae population, pyrethroids therefore seem to remain effective in controlling a significant proportion of the grain aphid population and the cereal aphid population in general. However, with the withdrawal of the neonicotinoid seed dressings from 2019 onwards, the extent to which alternating insecticide compounds with differing modes of action played in the overall management of resistant genotypes in Irish cereal crops remains to be seen. This study will provide a useful baseline in quantifying the prevalence of kdr-SR S. avenae in Ireland, and in time will allow further assessment of the impact pyrethroids will have in continuing to manage resistant S. avenae in Irish crops.

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