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Evaluating the impact of pyrethroid insecticide resistance on reproductive fitness in *Sitobion avenae*

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Abstract

Resistance to insecticides used to control pests is an issue of increasing concern for agriculture. The grain aphid, *Sitobion avenae*, is a pest of cereals and grasses worldwide, and one of growing concern due to the evolution of resistance to certain insecticides. Resistance confers benefits to insects by enabling them to survive exposure to insecticide compounds; however, the mutations conferring resistance may also penalise the insect in pesticide-free environments due to fitness costs associated with the new phenotype. Here we tested the hypothesis of a reproductive penalty linked to the knock-down resistance mutation (*kdr*) to pyrethroid insecticides. The mutation occurs predominantly in a single SA3 clone. To date, only heterozygous-resistant forms (*kdr*-SR) have been detected in populations in Ireland and the UK, and this suggests that a fitness penalty may preclude the formation of both male and female heterozygous-resistant sexual forms. By designing an experiment which included a resistant and a non-resistant clone, we were able to simulate reduced daylight and temperature conditions which, in nature, trigger sexual reproduction and therefore study the responses of each clone. This allowed us to detect the switch from asexual females to sexual females and males and report on the conditions associated with the production of sexual forms. The results showed that both aphid clones were able to produce sexual forms with no difference in the onset of sexual reproduction, although reproductive strategies differed between clones. The later onset of male forms in the SA3 clone may decrease the likelihood of mating interactions to create fully resistant (*kdr*-RR) genotypes and this may constitute a fitness penalty due to pyrethroid resistance.

KEYWORDS
fitness penalty, grain aphid, L1014, pyrethroid resistance, sexual reproduction, *Sitobion avenae*

1 | INTRODUCTION

Sitobion avenae (Fabricius), the grain aphid, is an important pest of cereal grains and grasses worldwide. It causes crop losses through direct feeding damage and the transmission of plant viruses, including Barley Yellow Dwarf Virus (BYDV), impacting photosynthesis and crop development and ultimately reducing crop yield (Fiebig, Poehling, & Borgemeister, 2004).

There has been extensive research into the biology and ecology of *S. avenae* (Dedryver, Le Gallic, Gauthier, & Simon, 1998; Helden & Dixon, 2002; Llewellyn et al., 2003; Papura et al., 2003) and grain aphids are known to exhibit intricate life cycles in response to environmental stimuli, particularly temperature and daylight. Typically, four reproductive behaviours are exhibited. Holocyclic clones or cyclical parthenogenetic lineages are fully dedicated to sexual reproduction, or producing only mating males and females (oviparae) once a year in winter. Anholocyclic clones also known as obligate parthenogenetic lineages produce only parthenogenetic females (viviparae) throughout the year. A third intermediate clone can produce either parthenogenetic females or oviparous mating females along with males at the onset of winter conditions. Finally, androcyclic clones produce males only which can then mate with holocyclic female oviparae, providing an opportunity for gene flow between the different reproductive clones.

Sexual reproduction predominates under a colder continental climate while asexual lineages prevail under warmer, oceanic climates (Papura et al., 2003), although other selection pressures may interact with winter climate to regulate local life cycle polymorphism (Dedryver, Hullé, Le Gallic, Caillaud, & Simon, 2001). In colder regions and at higher latitudes, the production of cold-hardy eggs facilitates overwintering survival (Loxdale & Lushai, 2007). Overwintering is simply defined as the way an organism passes the winter. During mild winters and at lower latitudes aphid populations are largely anholocyclic, continuing to reproduce parthenogenetically throughout the year, even when exposed to periodic sub-zero temperatures (Figueroa et al., 2005; Knight & Bale, 1986).

It is known that fitness costs are associated with resistance mutations (Kliot & Ghanim, 2012). This is because adaptation is, nearly always, biologically expensive to an organism, if not lethal, and results in deleterious pleiotropic effects on fitness in the absence of insecticide exposure. This occurs either directly by affecting important life functions such as metabolic processes, or indirectly by diverting resources away from energy production for development, reproduction and ultimately, survival.

Establishing if fitness costs are linked to resistance mutations is not straightforward. Where fitness costs in this context have been studied, for example, in the diamondback moth *Plutella xylostella* in Chile (Castañeda et al., 2011), there was no evidence of an energy cost, reduced reproductive fitness, or reduced metabolism in resistant genotypes. Whereas, in highly resistant strains of the same species, which were collected in Japan and the Philippines, differences were apparent in fitness. These included lengthier development times, reduced weight at immature stages and reduced fecundity (Steinbach, Moritz, & Nauen, 2017). Research on the peach-potato aphid, *Myzus persicae*, has shown behavioural side effects to insecticide resistance, including an inability to respond to the aphid alarm pheromone leading to greater vulnerability to wasp parasitism (Foster et al., 2007).

The classic L1014F mutation (known as knockdown resistance or *kdr*) is conferred by a simple point mutation on one allele of the voltage-gated sodium channel (VGSC) gene which affects the binding ability of pyrethroid compounds within the channel protein transmembrane region (Davies & Williamson, 2009; Martinez-Torres, Foster, Field, Devonshire, & Williamson, 1999). This mutation was identified in samples of *S. avenae* in the UK for the first time in 2014 (Foster et al., 2014). Subsequent molecular assays and genotyping in the Irish environment confirmed the presence of this *kdr* mutation in a single *S. avenae* superclone (SA3), with more intensive resistance screening of 621 grain aphids over a 4-year period in Ireland discovering repeated instances of *kdr*-heterozygotes in cereal fields (Walsh et al., 2020).

While some aphids have lost their ability to produce sexual forms, we know that *S. avenae* retains this ability (Papura et al., 2003), including within the resistant SA3 clone where oviparae have previously been reported (Walsh et al., 2019). However, the prevalence of *kdr*-heterozygotes in the population and the absence of *kdr*-homozygotes in extensive sampling efforts so far suggest that there may indeed be some kind of fitness penalty to the production of sexual forms. This may prevent genetic crossing, including bringing together the resistance mutation in the homozygous form as a potentially fully resistant *kdr*-homozygote.

This research set out to build on previous work (Walsh et al., 2019) by comparing the (heterozygous-resistant, *kdr*-SR) SA3 clone with a non-resistant (homozygous-susceptible, *kdr*-SS) SA27 clone and studying the population structure and reproduction ability in both genotypes under reduced daylight and temperature conditions. Barley leaves were sampled weekly to record the total number of aphids and the frequency of sexual forms produced over a 12-week period. This allowed for a comparison of aphid numbers and reproductive timings between genotypes in order to detect the switch from production of parthenogenetic (asexual) females to sexual oviparae and males, and report on the conditions associated with the production of sexual mating forms. The main objective of the study was to determine if a reproductive impact may be linked to the resistant allele causing *kdr*, by testing the hypothesis of no difference in reproductive strategy and productivity between genotypes.

2 | MATERIALS AND METHODS

A 6-week experiment was initially designed to measure and observe reproduction in the two genotypes. At week 5, when a switch from asexual to sexual reproduction was observed, the decision was made to extend the experiment into a second phase in order to make pilot observations of asexual and sexual reproduction. A description of each phase is provided in the flow diagram (Figure 1) and explained in greater detail in this section.

2.1 | Background

Several successful studies have been conducted to understand cereal aphid reproduction (Helden & Dixon, 2002; Kati et al., 2013), and these were carried out on aphids isolated in small tubes (Austin, Tatchell, Harrington, & Bale, 1991). There is also evidence that aphid

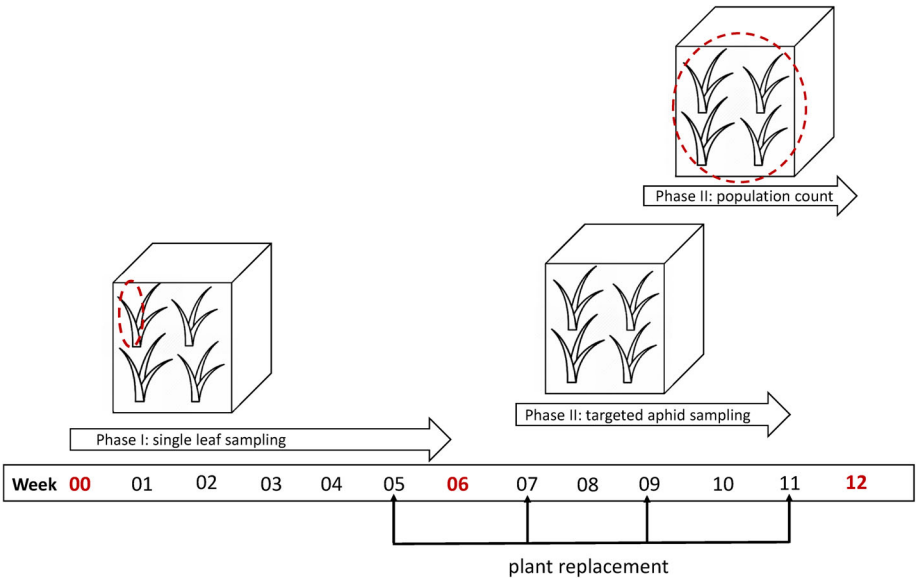

FIGURE 1 A flow diagram indicating the experimental phases, the sample unit, and the experimental timeline. Key time-points are highlighted: start (week 0), end of phase I (week 6) and end of phase II (week 12). The time-points when plants were replaced and aphids transferred to new plant material took place on four occasions, in weeks 5, 7, 9 and 11. During phase I a single leaf was sampled. The cage population was randomly sampled from week 7 until week 11. Whole cage population counts took place weekly from week 9, focusing on one replicate each week, until the experiment ended in week 12

density, as well as reduced daylight and temperature, may be involved in triggering sexual morph production (Leather, Walters, & Bale, 1995). It is also known that aphids respond to chemical cues in their environment (Pickett & Glinwood, 2007), from the broader colony (e.g., pheromones) or from other species (e.g., allelochemicals) which can lead to behavioural changes (Boullis & Verheggen, 2016). Therefore, the experiment was designed to proceed as a cage-based experiment with the view that density and chemical cues from the broader colony may trigger sexual forms.

2.2 | Description of clonal lineages

Colonies from a single female in 2017 were maintained on barley, *Hordeum vulgare*, var Propino at 20 ± 1°C under a 16 L:8 D hour cycle until experimentation in May 2018. DNA extraction and testing for the L1014F mutation using a Taqman PCR assay was carried out to determine the pyrethroid (*kdr*) resistance status of each colony. The clonal genotype was confirmed using microsatellite genotyping at the James Hutton Institute in Scotland, UK. The clonal genotypes were identified as the SA3 clone (pyrethroid-resistant genotype), confirmed to be a *kdr*-heterozygote (*kdr*-SR), collected from winter wheat in Co. Carlow, Ireland, and the SA27 clone (non-pyrethroid-resistant genotype) which was confirmed to be susceptible (*kdr*-SS), originated from spring barley in Co. Kildare, Ireland. Further details of these methods are available in Walsh et al. (2020), which also showed that the SA27 *kdr-SS* and the SA3 *kdr-SR* microsatellites were the most prevalent *S. avenae* clones recovered within a survey of Irish cereal fields.

2.3 | Preparation of plants and cages

Black plastic plant pots (7 cm × 7 cm × 7 cm) were filled halfway with a peat compost substrate containing a specialised slow release fertiliser made of fractionated sphagnum peat moss <14 mm with 8–10% air filled porosity (Bord Na Mona potting substrate+), and planted with *H. vulgare* seeds 14 days ahead of commencement of the experiment. Five equally spaced seeds were placed in each pot and allowed to germinate and develop to the two leaf growth stage GS12 (Tottman, 1987). Four pots, each with five plants, were numbered and placed in a white mesh nylon netted cage (21 cm × 21 cm × 21 cm) in the same order. Each plant was inoculated with a single fourth instar nymph taken from long-day length cultures (20°C, 16 L:8 D hours) with an initial sample size of $n = 20$ in each cage. There was a total of eight cages included in the experiment, with four replicate cages for each clone. The placement of cages was randomised across two shelves of a light temperature-controlled incubator at short-day conditions (16°C, 12 L:12 D, RH [35–52%]), and cages retained their position for the duration of the experiment.

Additional plant pots of the same specifications were planted with barley 14 days ahead of the experiment. These pots were used during the experiment for colony maintenance, to provide fresh plant material which was at the same plant growth stage. The removal and replacement of plant pots was standardised fortnightly across cages and commenced in week 5 (35 days after inoculation [DAI]). Live aphids were transferred from old to new plant material using a fine paintbrush, and this was performed to keep the colony intact. Based on results of a preparatory experiment, each plant pot received 50 mL of water twice a week.

2.4 | Phase 1: Analysis of population structure

2.4.1 | Plant harvesting

Cages were sampled once a week for the first 6 weeks at 7, 14, 21, 28, 35 and 42 DAI, with all live aphids being removed and counted. A single barley leaf was randomly harvested from a different plant

pot weekly, sampling the same pot number in each cage replicate. This was performed by carefully cutting the shoot at the base. Following leaf removal, pots were rotated in a clockwise direction in each cage. Leaf area and dry leaf measurements were carried out on barley leaves.

2.4.2 | Leaf area and dry leaf measurements

Leaf area and dry leaf weights were measured in order to account for the mediating effects of plant quality on population structure. Immediately after harvesting, plant material was flattened and scanned using a Bizhub C287 scanner. Scans were analysed for leaf area using the Easy-Leaf-Area software (Easlon & Bloom, 2014) which quickly measures the leaf area of digital images (in cm^2). Plant material was then transferred, individually, to labelled brown paper bags and placed in an oven, uninterrupted for 24 hr at 70°C, before recording dry leaf weight (g) on a fine-scale balance (OHAUS Pioneer with accuracy to three decimal places).

2.4.3 | Aphid collection and classification

Grain aphids progress through four age stages (instars) until they reach the adult reproductive stage. The appearance of adults is notably different to instars, as they are greater in size with well-developed appendages. The different adult forms or morphs, either male/female, and asexual/sexual, differ in their morphology with clear visual characteristics that are associated with finding suitable mating partners in the field (e.g., distinct anatomical sensory structures) (Blackman, 1987, 2010).

Aphids were therefore counted and visually classified into instars or adult morphs based on their size and development of features (Figure 2).

To improve visualisation of structures, adults were mounted in 85% lactic acid solution (ACS reagent, ≥85%, Sigma–Aldrich 252476) and gently heated for up to 2 hr to display key structures following standard procedures for clearing genitalia (Blahnik et al., 2007; Mazzucconi, 2011). They were then classified as either alatae or apterae and as either viviparous females, oviparous females or males, based on their morphological features (described in Table 1).

2.5 | Phase 2: Observation of sexual forms

2.5.1 | Frequency and incidence of sexual morphs

Cage replicates were maintained for a further 6 weeks until week 12 (84 DAI) to record the incidence and absolute frequency (the number of male and female forms) per replicate.

FIGURE 2 Grain aphid instars used to determine population structure in cage replicates. Aphid size, cornicle (sensory structures on abdomen) and cauda (tail) length and colour were used to assign aphid instars. The image is magnified to a zoom range of 6.3× using a digital microscope

TABLE 1 Description of morphological features used to determine the sexuality of adult *S. avenae*

Feature[a]	Viviparous female	Oviparous female	Males
Form	Apterae/Alate	Apterae	Alate
Reproductive features	Evidence of nymphs	Evidence of eggs	No evidence of nymphs/eggs. Presence of male genitalia
Sensory features	Absence of pseudosensoria on the hind meta-tibia	Presence of pseudosensoria on the hind meta-tibia	High number of secondary rhinaria on third antennal segment

[a]Morphological references are based on two aphid identification keys (Blackman, 2010; Favret & Miller, 2014).

Cage replicates were sampled weekly until week 11, randomly selecting 6 alate and 6 apterous aphids to detect the presence of sexual forms. In order to evaluate the entire cage population, matched replicates were terminated weekly from weeks 9–12. All live adult aphids were recorded and categorised as viviparous females, oviparous females or males to compare the frequency and proportion of each morph in the cage population on four occasions.

2.6 | Statistical analysis

Statistical analysis and modelling were performed in SAS (SAS, 2014), with data visualisation carried out using the SAS ODS graphic editor and Excel graphics.

2.6.1 | Logistic regression modelling

To establish if there were significant differences in the frequency of the total number of aphids and the frequency of age cohorts (instars) between the *kdr*-SR (SA3: resistant genotype) and the *kdr*-SS (SA27: susceptible genotype), aphid counts and frequency of age cohorts (instars) were transposed using the PROC TRANSPOSE function and analysed by fitting a PROC LOGISTIC generalised linear model. The odds ratio was calculated in order to explore the size effect for each variable. Response variables were based on frequency of age cohorts and frequency of aphids, and the explanatory variables (e.g., leaf area and dry weight) were allocated to microsatellite clonal genotype. Cage replicates, from where aphids were sampled on a weekly-basis, were treated as having fixed effects in the model.

2.6.2 | Fisher's χ^2 tests

The incidence and frequency of live aphid morphs, sampled on a weekly basis (weeks 7–11) from cage replicates, as well as harvested from the full cage population in weeks 9–12, were analysed using the Fisher's χ^2 test, often used for independent samples to test for an association between factors. Alate apterous forms were analysed in 2×2 contingency table based on resistance status. Adult form (viviparous, oviparous or male) was analysed in a 2×3 contingency table based on resistance status, as well as in a 2×2 contingency table (form: sexual asexual). This helped establish if the use of two instead of three categories, changed the significance of results as the numbers of male aphids were overall very low.

3 | RESULTS

3.1 | Phase 1: Frequency of total aphids

The number of aphids recovered each week increased weekly with a significantly greater number in the *kdr-SR* genotype at 21 DAI (week 3) only (Figure 3), despite efforts to balance leaf area by matching plant numbers and growth stage across cages prior to commencing experimentation. The explanatory factors: week ($F(20.29)$, $df = 5$, $p < .0001$) and leaf area ($F(20.12)$, $df = 1$, $p < .0001$) were significant in the model, affecting the frequency of aphids. However, insecticide resistance and dry leaf weight were not significant.

The variables: resistance ($\chi^2 = 9.70$, $df = 1$, $p = .0018$), week ($\chi^2 = 174.26$, $df = 5$, $p < .0001$) and their interaction ($\chi^2 = 38.68$, $df = 5$, $p < .0001$) were found to be significant in the second LOGISTIC regression model influencing aphid frequency in a cage replicate. The interaction was only significant in the *kdr*-SR (resistant) genotype, and only in weeks 3, 4, and 5 ($p < .0001$). The odds were greater ($1.91\times$) of a live aphid being the *kdr*-SR genotype ($Z = 6.73$, $p < .0001$).

3.2 | Phase 1: Frequency of age cohorts (instars)

The variables: resistance ($F(9.45)$, $df = 1$, $p = .002$), week ($F(35.28)$, $df = 5$, $p < .0001$) and their interaction ($F(7.85)$, $df = 5$, $p < .0001$) were significant in determining the frequency of instars. This means aphid frequency in each genotype was mediated by time (week). The odds of viviparous aphids being the *kdr*-SR genotype was therefore $1.41\times$ greater than the *kdr*-SS genotype; a consistent trend observed across all weeks. Although this was notably greater in week 5 than in any other week, being $2.49\times$ more likely in this week.

3.3 | Phase 1: First incidence of sexual forms

Sexual female oviparae were first detected in both lineages at a low frequency in week 5 (35 DAI) based on sampling leaf material (Figure 4). Oviparae were present in all four cage replicates of the *kdr*-SS genotype. Oviparae numbers ranged between 1 and 10, averaging 4.5 aphids

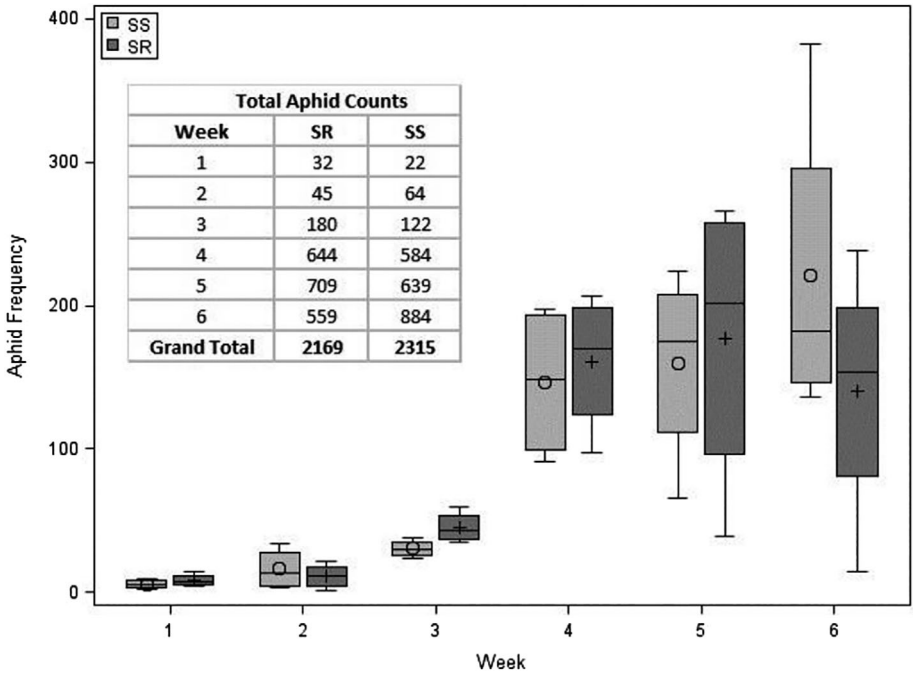

FIGURE 3 The median number of aphids across replicate cages recorded on harvested leaves during phase 1 of experimentation in the *kdr*-SR (resistant) and *kdr*-SS (susceptible) genotypes. Comparative boxplots represent the weekly number of aphids pooled across the four cage replicates. There was a significant difference in aphid numbers in week 3 ($F(20.29)$, $p < .0001$, $n = 4$); however, differences were not significant in any other week. A table showing the cumulative total number of aphids recorded in each genotype is also displayed

across replicates (making up 44% of the adult aphids sampled). Only leaf material from one cage replicate of the *kdr*-SR genotype in week 5 yielded oviparae ($n=11$), averaging 2.75 oviparae across replicates (making up 24% of adult aphids sampled). This increased significantly in the susceptible *kdr*-SS genotype only . In week 6, oviparae numbers ranged from 8 to 81, averaging 34.25 aphids across replicates (making up 93% of the adult aphids sampled). In comparison, oviparae numbers in the *kdr*-SR genotype ranged from 0 to 5, averaging 2.25 aphids across replicates (making up 20% of the adult aphids sampled). As a percentage of total aphids sampled (adults + nymphs), we noted an increase from 3% in week 5 to 13% in week 6 in the susceptible *kdr*-SS genotype, while the frequency remained consistent at 1% in week 5 and 2% in week 6 in the *kdr*-SR genotype. Male aphids were detected at low frequency for the first time in week 6, but only in a single cage replicate of the *kdr*-SS genotype. There was no significant difference in sexual morph production in week 5; however, there were significant differences in week 6.

3.4 | Phase 2: Incidence of sexual forms—Cage samples

Following the onset of sexual morph production in the *kdr*-SS genotype, significantly more sexual forms were detected, on a weekly basis in the *kdr*-SS genotype. By week 7, viviparous forms were no longer detected in the *kdr*-SS genotype during weekly sampling. All alate aphids sampled were males and all apterae were female. In contrast, in the *kdr*-SR genotype, all alate aphids were viviparous and apterae were either oviparous or viviparous.

3.5 | Phase 2: Incidence of sexual forms—Cage populations

The population structure of the *kdr*-SS and *kdr*-SR genotypes was significantly different across all weeks (Table 2). More alates were recorded in the *kdr-SR* genotype. Viviparous aphids were not present in cage replicates of the *kdr*-SS genotype in weeks 9, 10 and 12, indicating these populations were comprised only of sexual forms. Viviparous aphids were detected at a low frequency (5%) in week 11 in the *kdr*-SS genotype, although the population remained significantly different to the *kdr*-SR genotype (Table 2). In contrast, viviparous aphids made up >50% of live aphids recovered in weeks 9, 10 and 11 in the *kdr*-SR genotype, and 38% of live aphids recovered in week 12.

Oviparous aphids were the most abundant form recovered in the *kdr*-SS genotype in weeks 9, 10 and 11, making up over 90% of live aphids recovered. In contrast, oviparous aphids made up a smaller proportion of the population in the *kdr*-SR genotype, between 10 and 50% of live aphids recovered in weeks 9–12.

Male aphids were detected in the *kdr*-SS genotype although their abundance in the population was low, making up only between 2 and 5% of live aphids recovered in weeks 9–12. Only three males (the entire cage population of live aphids) were recovered in week 12, when an abundance of aphid eggs were observed to remain in the cage. A low frequency of males was recovered from the *kdr*-SR genotype ranging from 0 to 2%. In this observation, males were first detected in the *kdr*-SR genotype in week 9 of the experiment.

TABLE 2 The number of aphid morphs in cage populations, recorded in weeks 9–12, are provided as whole numbers and as a percentage (%) of total aphids (n) recovered from each replicate

Week	Genotype	Replicate	*n**	Alate	Viviparous (%)	Oviparous (%)	Male (%)	2×*n***	*df*	χ^2	*p*-value
9	SS(SA27)	4	718	16	0	702 (98)	16 (2)	1050	2	505.151	<.0001
9	SR(SA3)	4	332	54	191 (57)	139 (42)	2 (1)				
10	SS(SA27)	3	535	9	0	526 (98)	9 (2)	940	2	764.117	<.0001
10	SR(SA3)	3	405	124	355 (88)	42 (10)	8 (2)				
11	SS(SA27)	2	204	11	10 (5)	183 (90)	11 (5)	444	2	115.013	<.0001
11	SR(SA3)	2	240	56	120 (50)	120 (50)	0				
12	SS(SA27)	1	3	3	0	0	3 (100)	277	2	164.987	<.0001
12	SR(SA3)	1	274	108	104 (38)	168 (61)	2 (1)				

Note: The proportion of aphid morphs was calculated from the total number of live aphids' recovered each week (2×*n***). This total number of live aphids refers to all aphids in each replicate (i.e., the sum of two cages, one being SA27 and the other being SA3).

3.6 | Confirmation of *kdr* status of sexual males and oviparae detected in *kdr*-SR lineage

DNA was extracted from two male aphids of each genotype produced in week 11 in order to confirm that the resistance genotype matched the original cage lineage. All four aphids were of the correct lineage. The methodology used is further described in Walsh et al. (2020).

4 | DISCUSSION

For the first time, males of the SA3 clone were discovered, alongside female oviparae. The frequency of males detected was significantly less than that of oviparae in both the resistant and susceptible genotypes and this is likely linked to the high biological cost associated with producing males (Helden & Dixon, 2002).

4.1 | Phase 1: Significant differences in population structure and aphid abundance

Greater asexual reproductive output was associated with the *kdr*-SR genotype in weeks 1, 3, 4 and 5. The population structure across age stages was significantly different between the two aphid genotypes. Oviparae were detected in both genotypes at week 5, indicating the onset of sexual reproduction was harmonised in both genotypes, under matching environmental conditions, although the significantly greater incidence of sexual forms in week 6 in the *kdr-SS* genotype was the first indication that overwintering strategy may be different across genotype.

Logistic regression modelling predicted that significantly more aphids are produced by the *kdr*-SR genotype over the *kdr*-SS genotype. This corresponds with research in other insecticide-resistant gastropods and arthropods. For example, in *Biomphalaria glabrata* snails resistant to the parasite *Schistosoma mansoni*, the numbers of offspring produced in susceptible genotypes were fewer (Webster & Woolhouse, 1999), and in *Myzus persicae*, the peach-potato aphid, clones with R1 or R2 esterase (metabolic-based resistance to organophosphate insecticides) had higher reproductive performance than non-resistant clones (Eggers-Schumacher, 1983). Later work on *M. persicae* by Fenton, Kasprowicz, Malloch, and Pickup (2010) found no clear pattern between the offspring count of lineages of sensitive and resistant clones, and a clone with MACE (modified acetylcholinesterase giving resistance to di-methyl carbamates) and *kdr* resistance displayed outstanding reproductive performance across three different host plants (potato, oilseed rape and radish) compared to other clones in the study. One explanation is that in clones which have evolved increased reproductive potential, there is an advantage to insecticide resistance alleles as a form of direct compensation for other fitness costs (Fenton et al., 2010), although another potential explanation, based on this experimental design, may be due to the early switch to sexual reproduction observed in the susceptible *kdr*-SS genotype.

Biologically, the development of oocytes into live nymphs or eggs is associated with varied development times, indicating that there may be a biological basis for model predictions of greater aphid numbers in the *kdr-SR* genotype. Research on *Megoura viciae* (the vetch or green aphid) indicates that ovulations progress rapidly in embryos destined to be viviparae, while growth stagnates in oocytes of future oviparae until after birth (Blackman, 1987) and this would explain the model prediction of more aphids in *kdr*-SR genotype where viviparae production were sustained. While there is evidence both in support and opposition (Eggers-Schumacher, 1983; Castañeda et al., 2011) of a reproductive penalty in resistant aphids, even within the same species, this appears to be associated with other mediating factors such as temperature, host plant and field ecology (Fenton et al., 2010).

4.2 | Phase 2: Low incidence of males and significant difference in reproductive strategy between genotypes

The observation of sampled aphids in weeks 7–11, as well as population observations of all live adults in cage replicates in weeks 9–12, provides evidence of reproductive strategy differences between genotypes.

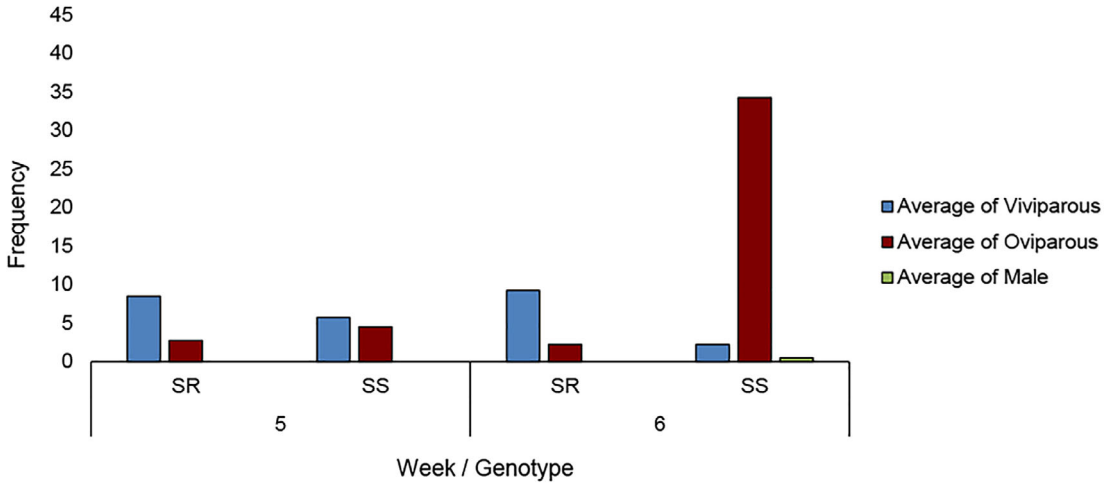

FIGURE 4 The average (mean) number of viviparous, oviparous and male adult grain aphids recorded at the onset of sexual reproduction in weeks 5 and 6. This is shown for the *kdr*-SR (resistant) "SR" clone and *kdr*-SS (susceptible) "SS" clone. Differences were significant between genotypes in week 5 ($\chi^2 = 3.6343$, $p = .0566$) and week 6 ($\chi^2 = 107.2524$, $p < .001$)

A noteworthy observation of the data is significant variation in the reproductive strategies of susceptible (*kdr*-SS) and resistant (*kdr*-SR) clones. The *kdr-SR* SA3 clone committed partially to both reproductive strategies ("hedging its bets" in an evolutionary sense), and we observed that the occurrence of viviparae, oviparae and males overlapped in the *kdr-SR* genotype. Research on *S. avenae* in France found this to be the more common strategy in 43% of intermediate clones and 24% of androcyclic clones, and generally in clones from milder climates (Dedryver et al., 2001; Rispe, Pierre, Simon, & Gouyon, 1998; Simon et al., 1999).

Sexual reproduction is perceived to be more costly in resource terms requiring energy and nutritional contributions to mating and egg production (De Loof, 2011; Williams, 2005), although it ensures better survival in variable environments such as extreme winter conditions (Simon, Rispe, & Sunnucks, 2002). In our study the *kdr*-SS genotype appears to commit fully to the more costly form of sexual reproduction opting to overwinter in the egg phase, and we recovered significantly more sexual forms each week in the *kdr*-SS genotype. There was a complete switch to sexual reproduction in this genotype by the end of the experiment (week 12) when only three males and hundreds of eggs were observed to remain in the final cage replicate. This could be explained by later onset male production as suggested in other research (Helden & Dixon, 2002). This would also diminish mating opportunities to create fully homozygous resistant (*kdr-RR*) aphids in the *kdr*-SRgenotype. This poor overlap in the production of males and female oviparae to provide mating opportunity may offer insight into why *kdr*-RR genotypes have not been detected in the environment.

Our findings have potential implications for cereal crop production. According to one hypothesis by Cooper and Kaplan (1982), genotypes with "mixed" outputs will be more successful, over deterministic genotypes in variable environments, such as changing winter climates (Dedryver et al., 2001). Based on our data it seems likely that *kdr*-SR genotypes are more likely to persist in the environment over winter periods, continuing to feed and reproduce asexually, and increasing the opportunities for transmitting BYDV in the crop.

A smaller number of male aphids were recovered across genotypes consistently during this study and this may be linked to the biological cost of their production. While males make an important contribution to life-cycles, balancing polymorphism through the transfer of alleles for parthenogenetic overwintering and generating genetic variation in aphids, which normally reproduce parthenogenetically (Helden & Dixon, 2002; Rispe et al., 1998), the cost of offspring production in terms of lower fecundity and total offspring biomass can be high. The timing of male production is thought to be delayed, with a time gap between the end of female production and the first males, and is likely to result in slower growth and development time for male embryos. Research showed this gap has occurred in two androcyclic clones, although not in a third clone where fewer males were produced, intermixed with female births (Helden & Dixon, 2002). Both scenarios are observed in the *kdr*-SR SA3 clone where the production of males is delayed and is intermingled among female births of viviparae and oviparae. The low incidence of males, even in the SA27 clone may also be explained by the sudden switch from the long day, warm temperature conditions to the short day, cooler conditions. In a field situation, as autumn progresses this change would be gradual, and perhaps this gradual change is important in the generation of males and oviparae.

5 | CONCLUSIONS AND IMPLICATIONS

This research set out to test the hypothesis of no difference in reproductive effort and strategy between the *kdr*-SR SA3 (partially pyrethroid-resistant) clone and the *kdr*-SS SA27 (fully-pyrethroid-susceptible) clone, in order to assess if a reproductive penalty may be associated with the *kdr* genotype. We observed no obvious preclusion to the production of sexual forms linked to *kdr*. Indeed, there was a

significant difference in the overwintering strategy between the two genotypes. Significantly more viviparous aphids were produced by the *kdr*-SR genotype, indicating a higher reproductive output and fecundity with regards to asexual reproduction. There was no difference in the onset of sexual reproduction as the presence of oviparae was detected at 5 weeks in both genotypes. However the *kdr*-SR genotype (SA3 clone) produced significantly fewer sexual forms, opting to use both asexual and sexual reproductive strategies. This research provides the first evidence that the capacity to produce sexual males is retained in the *kdr* SA3 clone, and builds on research reporting on the production of oviparous sexual forms in this clone (Walsh et al., 2019).

While there was no obvious reproductive penalty to the production of sexual forms observed in *kdr*-SR SA3 clones, fecundity may be impacted by poor overlap in the timing of oviparae and males to provide mating opportunities. Other possibilities may be unviable eggs or poor longevity of *kdr*-RR homozygotes, for example due to a reduced alarm pheromone response seen in another aphid species, *M. persicae* (Foster et al., 2007), or by being more prone to mummification (Jackson, Malloch, McNamara, & Little, 2020).

The observation of oviparous females and males produced by the SA3 clone has important implications for pest management in cereals. With evidence now of the potential to generate homozygous *kdr* (RR) genotypes through sexual crossing between *kdr*-heterozygote males and oviparous females, the adoption of an active resistance management strategy (Sparks & Nauen, 2015) becomes critically important. However, in the current near-absence of alternative pesticide chemistry, it is essential to explore alternative, non-chemical control options, as part of a wider integrated pest management strategy. In this regard novel technologies such as the use of bio-pesticides, the exploration of cultivar-bred resistance traits in cereals (Ferry & Gatehouse, 2010; Stoger, Williams, Christou, Down, & Gatehouse, 1999; Xu et al., 2014) supported by controlling aphids by encouraging beneficial insects in the environment, and possibly drilling crops later, could significantly reduce aphid colonisation of newly emerging cereals.

5.1 | Limitations

The soil compost depth used in plant pots may have impacted plant growth and development over time. However, this was standardised across all pots, cage replicates and genotypes and therefore this variable was controlled.

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