Project title:	**New approaches to aphid control in strawberries combining botanicals and natural enemies.**
Project number:	CRP_RES_0148
Project leader:	Dr Tom Pope, Harper Adams University
Report:	Annual report, December 2021
Previous report:	Annual report, October, 2020
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Location of project:	Harper Adams University
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Date project commenced:	23rd September, 2019

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Initial laboratory bioassays indicate that physically-acting pesticide products demonstrate high efficacy against three aphid pests of strawberry (the potato aphid, the strawberry aphid and the cotton-and-melon aphid).

Background

Control of strawberry-feeding aphid pests has historically relied upon conventional synthetic insecticides. Several of these insecticides, however, have recently been withdrawn from use amid concerns about their impact on human and environmental health whilst insect resistance to the remaining insecticides is becoming increasingly widespread. Additionally, pressure from consumers and retailers to reduce the use of synthetic pesticides is leading growers to consider alternative control options.

Summary

Bioassays were completed to determine the effect of a conventional synthetic insecticide (Batavia) and three physically acting insecticides (AHDB9811, AHDB9810 and FLiPPER) on the mortality and reproduction of three aphid pests of strawberry, *Macrosiphum euphorbiae* (potato aphid), *Chaetosiphon fragaefolii* (strawberry aphid) and *Aphis gossypii* (melon-and-cotton aphid). Each bioassay utilised leaflets taken from strawberry plants, *Fragaria* × *ananassa*, which had been infested with one of the target aphid species. Each infested leaflet was directly sprayed to 'run-off' with one of the products being tested. Control leaflets were sprayed with water to 'run-off' (water control) or were not sprayed at all (unsprayed control).

Bioassays demonstrated an increase in mean aphid mortality following the application of each of the four insecticide products tested compared to controls. This research demonstrates that under laboratory conditions these insecticides show comparable efficacy against strawberry aphid pests. However further work, including field trials and bioassays using natural enemies, is required to fully understand how effective these products may be within an integrated pest management system.

Financial Benefits

According to figures from DEFRA (2021), in 2020 the UK strawberry industry was worth £429.1 million domestically, with exports amounting to a further £10 million. Improved control of strawberry pests, including aphids, provided by a wider range of control products will lead to a reduction in crop damage.

Action Points

There are no grower action points at this stage.

SCIENCE SECTION

Introduction

Strawberry, *Fragaria x ananassa* Duchesne (Rosales: Rosaceae), is an economically important soft fruit crop. According to figures from Defra (2020), in 2019 the value of strawberry marketed in the UK was £347.8 million with exports worth an additional £4 million. In total, 4,774 hectares were utilised for strawberry production in the UK in 2019. Strawberry crops, however, are subject to losses due to a variety of pests and diseases (Ridley *et al.*, 2020). Strawberry is a host plant for 30 aphid species across 16 genera, of which 18 are economically important pests (Mitchell and Karley, 2020) and aphid damage is estimated to cost growers at least 1% of the marketed value of crops annually (Hodgkiss, Brown and Fountain, 2019). Damage to the plant occurs either directly from the feeding activity (Nalam, 2021), which leads to the excretion of honeydew, encouraging mould growth (Moir *et al.*, 2018) and making the fruit unsaleable (European Commission, 2011), or indirectly, by the transmission of viruses (Ng and Perry, 2004; Kwon *et al.*, 2018) such as strawberry mottle virus (Thompson and Jelkmann, 2003), strawberry vein banding virus (EFSA Panel on Plant Health (PLH) 2014a), strawberry crinkle virus (EFSA Panel on Plant Health (PLH), 2014b) and strawberry mild yellow edge virus (EFSA Panel on Plant Health (PLH), 2014c). Control of these aphid pests has relied on the use of conventional synthetic insecticides, however aphid resistance to pesticides such as carbamates, organophosphates and pyrethroids is becoming more widespread, especially in *Aphis gossypii* (melon-and-cotton aphid) populations (Furk and Hines, 1993; Marshall *et al.*, 2012; Gong *et al.*, 2014). More recently, Gong et al. (2016) suggested that CarE, the detoxifying enzyme conferring organophosphate resistance in *A. gossypii*, may play a role in conferring potential spirotetramat resistance. Additionally, there are concerns that use of conventional synthetic insecticides may result in environmental contamination and harm human health, leading to pressure from consumers and retailers for alternatives (Chandler *et al.*, 2011; Mitchell and Karley, 2020).

The aphid species thought to have the greatest economic impact as pests of strawberry, are *Macrosiphum euphorbiae* (the potato aphid), *Chaetosiphon fragaefolii* (the strawberry aphid) and *Aphis gossypii* (the cotton-and-melon aphid) (Mitchell and Karley, 2020). The potato aphid, *Macrosiphum euphorbiae* Thomas is a heteroecious species of aphid that feeds on more than 20 plant families and has been documented as a vector of over 100 plant viruses (Blackman and Eastop, 2006b). Whilst usually anholocyclic, populations in north-eastern USA have been documented exhibiting a sexual phase on *Rosa* species. The strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell) is primarily an autoecious species, feeding on strawberry (Cédola and Greco, 2010) but some populations have been recorded on silverweed (*Potentilla anserina)* (Blackman and Eastop, 2006a). It is an important vector of strawberry viruses, including mild yellow-edge virus (Lavandero *et al.*, 2012; Fránová, Přibylová and Koloniuk, 2019). Nymphs of *C. fragaefolii* are most frequently found on leaves whilst adults are predominantly found on buds (Rondon *et al.*, 2005). The cotton-and-melon aphid, *Aphis gossypii* Glover is heteroecious (Wu *et al.*, 2013), and is found feeding on a wide range of crops. This species is predominantly anholocyclic (Blackman and Eastop, 2006b). Like other aphid species, *A. gossypii* is an important vector of over 50 plant viruses (Blackman and Eastop, 2006b; Kim, 2007). Furk and Hines (1993) discussed the role of this species as a major pest of cotton, cucurbits and ornamentals, and its role as a strawberry pest was outlined by Rondon et al. (2005). This pest was historically controlled by the carbamate insecticide pirimicarb, but in 1987 it was reported that neither pirimicarb nor the organophosphates diazinon or heptenophos would control infestations (Furk and Vedjhi, 1990; Furk and Hines, 1993) in the UK. Subsequently, resistance to neonicotinoids (Steinkraus et al. 2002) as well as pyrethroids (Amad, Arif and Denholm, 2003) has been reported and more recently work has found susceptibility to anthranilic diamide insecticides (Foster et al. 2012). Control of this pest has been achieved with chemical controls in combination with biological control agents, particularly in cotton crops in China (Xia, 2018; Jiang et al. 2020).

The use of conventional synthetic insecticides is becoming more closely regulated for several reasons. Firstly, there is increasingly strict health and safety legislation leading to the withdrawal of conventional synthetic insecticide products (Chandler et al., 2011; Hillocks, 2012, 2013). Between 1993 and 2011, there was a decrease of around 75% in the number of approved active substances, from around 1,000 to around 250 (Chapman, 2014) following the introduction of Directive 91/414/EEC (Hillocks, 2013). In the same period, around 180 new products came to the market, although the majority of these were simply variants of products that had already been approved. As of 2021, there were 478 active substances approved for use within the EU with a further 5 pending approval (European Commission, 2020). Of the 454 approved products, 82 are insecticides and specific to the UK, there are 262 active substances approved for use, 42 of which are insecticides. Prophylactic use of insecticides can lead to pest resurgence or secondary pest problems, and can also lead to development of resistance in pest populations to these insecticide products (Edwards *et al.*, 2008; Sparks and Nauen, 2015). Insecticide resistance develops as a result of strong, unambiguous selection pressures (Foster *et al.*, 2011) and examples of resistance mechanisms include changes in the insect’s cuticle (Zhang, Goyer and Pelletier, 2008), mutations in insecticide target genes (Li, Schuler and Berenbaum, 2007), and increased production of metabolic enzymes that break down insecticides (Puinean *et al.*, 2010). Once organophosphate and carbamate resistance became widespread, these products were replaced by neonicotinoids and Bass et al. (2014) suggested that this led to a change in selection pressure. *Aphis gossypii* in particular is known to show resistance to neonicotinoids (Shi et al. 2010; Herron & Wilson, 2011; Matsuura & Nakamura, 2014).

Strawberry growers are presently reliant on Batavia following the withdrawal of other insecticides, and the incompatibility of pyrethroids with natural enemies (Bielza *et al.*, 2020). However, there is some suggestion that resistance to spirotetramat, the active ingredient in Batavia, could be exhibited by certain aphid pests, particularly *A. gossypii* through the same metabolic processes that confer resistance to organophosphate insecticides (Gong *et al.*, 2016). The present study aimed to investigate the efficacy of a range of physically active insecticides against three aphid pest species on strawberry to determine if they offer comparable protection against aphids to Batavia. Bioassays were completed to determine lethal effects in the form of mortality as well as sublethal effects in the form of reproduction. The results from these bioassays could help inform growers of the feasibility of using these products within an integrated pest management (IPM) system.

Materials and methods

Strawberry plants, *Fragaria* × *ananassa* Duchesne, of the Elsanta variety were grown from cold-stored crowns (R. W. Walpole, UK) and were potted up in 13 cm diameter plant pots using John Innes No. 2 compost (J. Arthur Bower's, UK) in glasshouse conditions at Harper Adams University. Glasshouse conditions were a 16:8 hour photoperiod at 15°C during daylight hours and 5°C during night hours, and a constant 85% relative humidity (RH). The plants were kept in W60 x D60 x H60 cm insect cages (BugDorm-4S4545, MegaView Science Co. Ltd, Taichung, Taiwan) and watered from beneath twice weekly.

Three aphid species were selected due to their importance to UK strawberry growers (Mitchell & Karley, 2020): the strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell), the potato aphid, *Macrosiphum euphorbiae* Thomas, and the melon-and-cotton aphid, *Aphis gossypii* Glover. *Macrosiphum euphorbiae* were collected from commercial strawberry crops (variety unknown) grown in Kent, UK, in 2019. The aphids were kept on strawberry plants in a BugDorm-4S4545 in a growth room (Fitotron, Weiss Technik, Loughborough, UK) set to a constant 20°C, 60% (RH) and 16:8 photoperiod. *Chaetosiphon fragaefolii* were collected from strawberry plants (variety Amesti) grown in polytunnels at NIAB EMR in Kent, UK, in July, 2019 and *Aphis gossypii* were collected from strawberry plants (variety Malling Centenary) grown in a polytunnel at NIAB EMR in Kent, UK, in June, 2021. Collected *A. gossypii* were checked to ensure that aphids were free from parasitoids or diseases by initially culturing small cohorts of adult aphids in Blackman boxes (W47 x D21 x H79 mm) before using the nymphs collected to establish a laboratory culture.

Approximately 100 adult aphids were transferred from laboratory cultures using a wet paint brush (size 000) onto fresh, clean strawberry plants two weeks after the plants had been potted up. These aphid-infested plants were then placed in a BugDorm-4S4545 within a growth room (20°C, 60% RH, 16:8 L:D). After 48 hours, the adults were removed from the plants and the nymphs they had produced formed a standardised cohort of approximately 600 nymphs for use in experiments. These nymphs were then reared for 2-3 days on the same plants to reach third instar. Daily observations were made to follow aphid nymph development, checking for shed cuticles.

The pesticide products selected for use in the experiment (Table 1) included the two-way systemic insecticide Batavia (Bayer Crop Science, UK), widely used for the control of aphids in strawberries grown in the UK. The physically acting insecticides were AHDB9811, AHDB9810 and FLiPPER (Alphabio Control, UK). all physically-acting pest control products marketed as alternatives to systemic insecticides. All three physically acting products claim efficacy against numerous pest species including aphids, and little impact on non-target organisms such as natural enemies. These products are, therefore, potentially useful alternatives to Batavia for the control of aphid pests of strawberry. There is, however, a current lack of data comparing the efficacy of each physically acting product against Batavia.

Table 1. *The products selected, their mode of action, active ingredients, and the concentration at which they were applied. Products were prepared to the highest concentration of the range recommended by their respective manufacturers.*

Product	Mode of action	Active ingredient(s)	Concentration (% v/v)
Insecticides			
Batavia	Lipid biosynthesis inhibition	Spirotetramat (100g/L)	0.1
AHDB9811	Insects become fixed to leaves	Sodium lauryl ether sulphate (10 – 20%)	0.1
AHDB9810	Silicone polymers immobilise insects	Polyalkyleneoxide modified heptamethyltrisiloxane (<75% w/w)	0.1
FLiPPER	Blocks insect detoxification enzymes	Carboxylic acid potassium salts C7 – C20 (479.8g/L)	1.6
Negative controls			
Water	N/A	N/A	N/A
Unsprayed	N/A	N/A	N/A

An 85 mm diameter disc of qualitative filter paper (Grade 601, Fisher Scientific, Loughborough, UK) was placed inside each of the ventilated Petri dishes. Single leaflets from two-week old unfurled trifoliate leaf clusters (BBCH stage 12) were taken from strawberry plants grown in a glasshouse by cutting the petiole with a scalpel and removing the side leaflets from the trifoliate leaf cluster to leave just the middle leaflet. There was a risk that removal of the leaflets from the plant stimulated the release of volatile organic compounds (VOCs) (Smith and Beck, 2013). Small holes were pierced in the Parafilm covering the water-filled Eppendorf tubes and the petioles of the leaflets were then inserted through these holes and into the tubes before being placed into their Petri dishes on top of the filter paper. This provided the leaflets with a source of water to prevent wilting during the experiment. Ten third-instar aphid nymphs were placed onto each leaflet using a damp paint brush (Figure 1). For each treatment 10 leaflets were sprayed twice by firstly removing the leaflet from the Petri dish and then spraying once on the adaxial surface of the leaflet and once on the abaxial surface, using a hand-held atomiser (2 x 0.75 mL). This ensured thorough coverage of the leaflet, and whilst this is not representative of a field situation it allows product efficacy to be determined when optimal coverage is achieved. Once sprayed the leaflets were immediately returned to their respective Petri dishes whilst still wet and returned to the laboratory where they were arranged in a fully randomised order using a random sequence generator in R (version 3.6.2) in the growth room (20° C, 60% RH, 16:8 L:D).

Mortality of the aphids was recorded daily for five days. Observations were made using a stereoscopic microscope (Microtec HM-3, Tec Microscopes Ltd, Somerset, UK). If signs of life were observed, for example walking, movement of limbs or antennae, or reproduction, the aphid was counted as alive. Aphids were counted as dead if they showed no signs of life, and did not respond to the physical stimulus of a paint brush. In addition to recording mortality of any of the aphids introduced to the leaflets, any reproduction during the five days of observation were recorded. Nymphs were counted and subsequently removed from the Petri dishes each day. Disposable nitrile gloves were worn when handling the dishes and new gloves were worn after checking all Petri dishes of each treatment, before checking the Petri dishes in the subsequent treatment.

Results

Macrosiphum euphorbiae

All of the treatments caused higher mortality five days after application to the leaflets infested with *M. euphorbiae* (Figure 2a) (Batavia: $t = 12.008$; AHDB9811: $t = 14.160$; AHDB9810: $t = 14.418$; FLiPPER: $t = 10.932$; $p < 0.001$ in each case) when compared to the controls (water: $t = 1.205$; $p > 0.1$). Batavia was the most effective product tested against this species of aphid (99% mortality), followed by AHDB9810 (96% mortality), AHDB9811 (94% mortality) and FLiPPER (80% mortality).

Chaetosiphon fragaefolii

Results for *C. fragaefolii* populations (Figure 2b) were similar to those for *M. euphorbiae*. All treatments again caused higher mortality compared to controls five days after treatment application (Batavia: $t = 14.438$; AHDB9811: $t = 17.581$; AHDB9810: $t = 15.468$; FLiPPER: $t = 16.989$; $p < 0.001$) compared to controls (water: $t = 0.638$; $p > 0.1$). As with the *M. euphorbiae*, Batavia was the most effective product tested, causing 100% mortality after five days. Results for AHDB9811 were similar to those recorded to those recorded against *M. euphorbiae* at 95% mortality, FLiPPER was the next most effective (89% mortality), then AHDB9810 (86% mortality), which was the least effective of the products tested against *C. fragaefolii*. Mortality of *C. fragaefolii* was the lowest of the three aphid species after five days when treated with the water control or when left unsprayed (20% and 15% mortality, respectively). This is compared to *M. euphorbiae* (45% mortality for both negative controls) and *A. gossypii* (water control: 70%; unsprayed: 64%).

Aphis gossypii

AHDB9810 was the most effective of the products tested against *A. gossypii* (100% mortality) (Figure 2c). AHDB9811 was the next most effective product (94% mortality), followed by Batavia (91% mortality) and FLiPPER (87% mortality). This was the lowest level of mortality exhibited by Batavia within the experiment. All of the treatments tested caused higher mortality after application to leaflet infested with *A. gossypii* (Batavia: t = 6.321; AHDB9811: t = 9.006; AHDB9810: t = 13.705; FLiPPER: t = 7.608; p < 0.001) when compared to the controls (water: t = 1.063; p > 0.1).

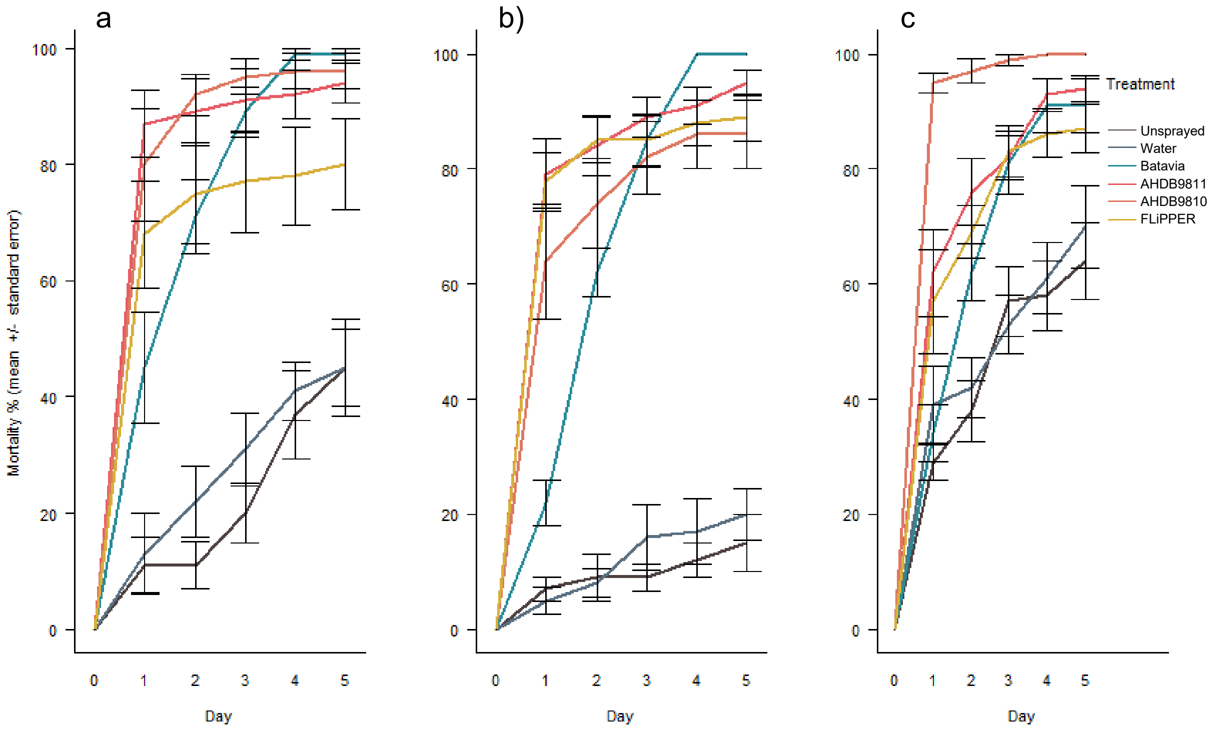

Figure 1. Aphid mortality recorded each day following treatment application (mean +/- standard error, n = 10) for the species a) M. euphorbiae*; b)* C. fragaefolii*; c)* A. gossypii.

Comparison of treatments

Following treatment with Batavia, there was a significant difference in mortality between the *M. euphorbiae* populations and the other two species (*C. fragaefolii*: t = -2.256; p < 0.05; *A, gossypii*: t = -2.919; p < 0.01) (Figure 3). following AHDB9810 treatment, there was no significant difference between the *M. euphorbiae* and *A. gossypii* populations (t = 1.910; p > 0.05) but there was a significant difference between the *M. euphorbiae* and *C. fragaefolii* populations (*C. fragaefolii*: t = -3.998; p < 0.01). This was also the case following treatment with FLiPPER in that while there was a significant difference between the *M. euphorbiae* and *C. fragaefolii* populations (t = 2.343; p < 0.05) there was no significance in the difference between the *M. euphorbiae* and *A. gossypii* populations (t = 0.199; p > 0.05). Conversely, after AHDB9811 application there was no significant difference between the *M. euphorbiae* and *C. fragaefolii* populations (t = -0.987; p > 0.05) but there was a significant difference between the *M. euphorbiae* and the *A. gossypii* populations (t = -3.026; p < 0.01). Both of the negative control treatments showed differences between all three of the species. In the water-sprayed populations, there was a significant difference between the *M. euphorbiae* populations and both the *C. fragaefolii* and *A. gossypii* populations (*C. fragaefolii*: t = -4.564; p < 0.01; *A. gossypii*: t = 5.996; p < 0.01) and this was also the case in the unsprayed controls (*C. fragaefolii*: t = -3.922; p < 0.01; *A. gossypii*: t = 6.646; p < 0.01).

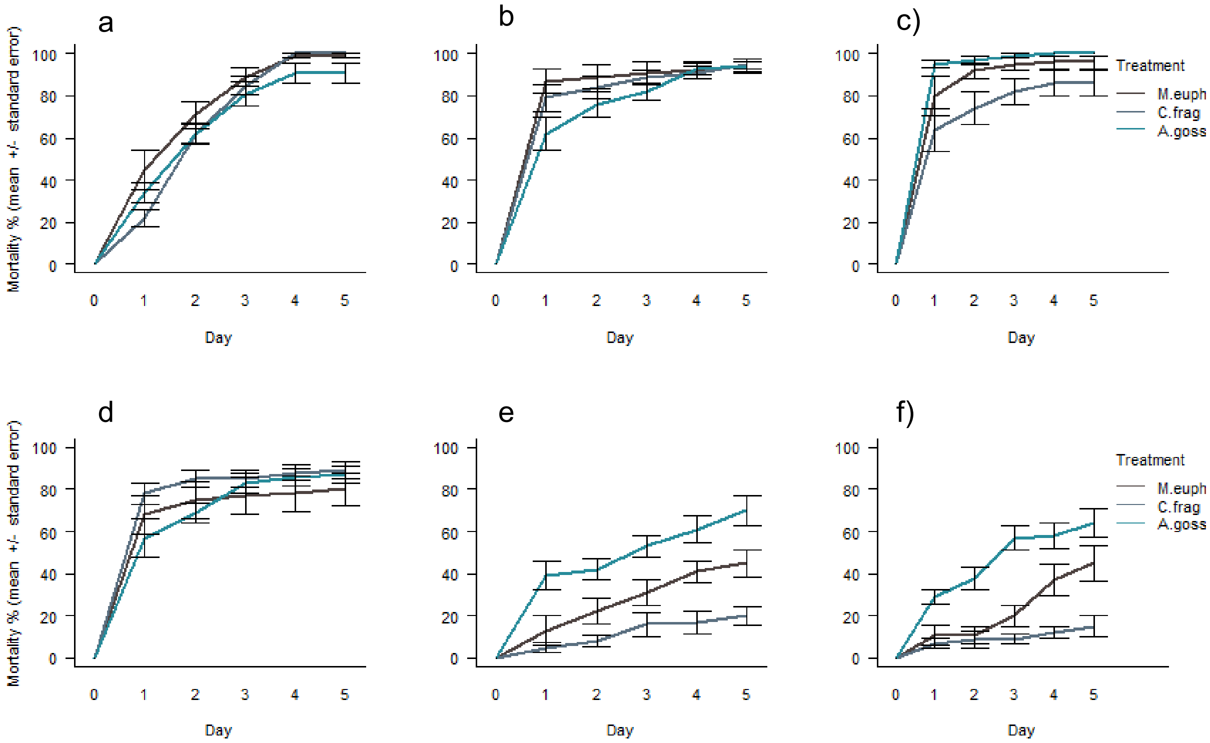

Figure 2. Aphid mortality recorded each day following treatment application (mean +/- standard error, n=10) for the treatments a) Batavia; b) AHDB9811; c) AHDB9810; d) FLiPPER; e) water; f) unsprayed.

Discussion

As growers move away from conventional synthetic insecticides, such as Batavia, alternative controls will need to be identified and the results from this experiment show that AHDB9811, AHDB9810 and FLiPPER may be viable alternatives. All of the products tested in this study caused mean aphid mortality of 80% or more, and in the case of AHDB9810 against *A. gossypii*, 100% mortality was recorded. However, Batavia exhibited lower levels of mortality on exposed *A. gossypii* populations when compared with the other species. This may be linked to the CarE detoxification enzyme suggested by (Gong *et al.*, 2016) to be responsible for conferring resistance to organophosphate insecticides in this species. It was suggested that this enzyme may play a role in conferring resistance to spirotetramat, the active ingredient in Batavia.

The physically acting products were faster acting than Batavia, which is known to be a slow acting insecticide (Pavela and Benelli, 2016). Contact activity does, however, mean that it is essential that good coverage is achieved (Copping & Menn, 2000). It is possible that these factors would necessitate multiple applications in real-world scenarios (Ikbal and Pavela, 2019) similar to the terpene-based biopesticides discussed by Smith et al. (2018) which were applied twice (azadirachtin A), three times (*Chenopodium ambrosioides*) or five times (orange oil) to control aphids on ornamentals in both glasshouse and polytunnel conditions.

There was a notable difference in survivability between the aphid species within the control groups. This was most apparent between the strawberry aphid (15% and 20% mortality on the unsprayed and water-sprayed leaflets respectively) and the melon-and-cotton aphid (64% and 70% respectively). *Chaetosiphon fragaefolii* nymphs are most frequently found on strawberry leaves (Rondon *et al.*, 2005) and whilst *A. gossypii* nymphs are also often found on the leaves of strawberry plants, more mature aphids migrate to the buds (Rondon *et al.*, 2005). Douglas (1993) reported that the amino acid content of the plant phloem sap is highest when foliage is growing or senescing in spring and autumn respectively, and comparatively low in mature leaves during summer. The two-week-old strawberry leaflets that the aphids were maintained on throughout this experiment may therefore have been sub-optimal for the *A. gossypii* populations, which could explain the higher mortality in the controls than was recorded for *C. fragaefolii*. This difference also draws attention to the fact that across the aphid species in this study, populations that were unsprayed showed lower levels of mortality and higher levels of reproduction than the control populations sprayed with water. This suggests that even the act of spraying, simulating strong rainfall, could have some impact (Mann *et al.*, 1995). Stoyenoff (2001) investigated the effect of plant washing on aphid populations and found a significant reduction following 30 seconds of spraying the plants with tap water, although the difference in mortality between water-sprayed and unsprayed aphid populations was not found to be statistically significant in the present study.

There was no difference in aphid fecundity following treatment with the exception of FLiPPER treatment on *A. gossypii populations.* This may, however, was likely to be due to the high levels of mortality before the aphids matured to reproductive age. It is therefore important to carry out dose-response experiments in order to establish the concentrations of each insecticide product causing only low levels of mortality (Gong *et al.*, 2016; Yousaf *et al.*, 2018). Having established sub-lethal concentrations of each product it would be possible to investigate the effects of these physically acting products more effectively, for example, aphid settling behaviour, feeding and reproduction (Petrakis *et al.*, 2014; Gong *et al.*, 2016) (Shi et al., 2010).

This study suggests that the physically acting insecticides AHDB9811, AHDB9810 and FLiPPER are comparable to the widely used conventional synthetic insecticide Batavia in their efficacy against *M. euphorbiae* and *C. fragaefolii*. In addition, against *A. gossypii* these physically acting insecticides were more effective than the conventional synthetic insecticide Batavia.

Pavela and Benelli (2016) have noted the lack of field-based experiments in comparison to the volume of laboratory-based studies published on the topic of alternatives to conventional synthetic insecticides in combination with natural enemies. In addition to a lack of efficacy data under commercial crop conditions, there remain concerns around of the availability of affordable products and a lack of persistence after application (Chapman, 2014). It remains important, therefore, to perform field-realistic studies to better inform growers of the efficacy of these products, and how best to use them within IPM programmes. Field experiments are being prepared at Harper Adams University to mirror the present laboratory study, utilising whole plants in place of excised leaflets. These plants will be grown in coir growbags and will be artificially infested with a single aphid pest species, the population of which will then be allowed to increase for two weeks before treatment application. This will give a more realistic insight into the effects of these products which can then be used to further inform growers of their suitability.

Dose response experiments completed with each of the physically acting insecticides against the three aphid pest species should also be completed in order to better understand how concentration determines efficacy and how this relates to label recommendations for each product. In addition, an understanding of sub-lethal concentrations for each product would enable studies to be completed that investigate effects on feeding, settling and reproduction.

In order to be successfully integrated into an IPM programme it will be essential to understand the compatibility of each of the physically acting products against a range of biological controls used aphids these strawberry aphid pests (Foster *et al.*, 2011). This work should explore the effects of different concentrations of each product as well as the persistence of the products on non-target organisms, such as aphid parasitoids.

Conclusions

Three physically acting pesticide products offer comparable efficacy to a conventional synthetic pesticide for controlling three aphid pests on strawberry.

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