Project title:	Developing a ‘push-pull’ strategy for the management of *Drosophila suzukii*
Project number:	CTP_FCR_2017_1
Project leader:	David Hall, Daniel Bray (University of Greenwich), Charles Whitfield, Michelle Fountain (NIAB EMR)
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Location of project:	Greenwich University, Medway Campus and NIAB EMR.
Industry Representative:	Harriet Duncalfe
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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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CONTENTS

GROWER SUMMARY

Headline

- Through a series of laboratory experiments, the *D. suzukii* summer morphs were repelled seven chemicals, and the winter morphs were repelled by five repellents. Both morphs were repelled by four chemicals.
- When these coded chemicals were tested in a semi-field strawberry crop, two chemicals (129/04, 129/13) were identified that reduced the overall number of emerging *D. suzukii*, and two chemicals pushed egg-laying away from the chemical dispensers (129/08, 129/13).
- The questionnaire results indicated that training opportunities should be given to early adopters of novel IPM strategies, with a secondary focus on how the strategy works.

Background

Drosophila suzukii, also known as spotted wing Drosophila, is the major insect pest threatening European fruit production (Asplen *et al.*, 2015; Cini *et al.*, 2012). This invasive fruit fly was first found in the UK in 2012 (Harris and Shaw, 2014) and within three years had established. *Drosophila suzukii* lay their eggs in ripening fruit (Goodhue *et al.*, 2011), and damage is caused by larvae feeding inside the fruit and where pathogens enter the egg insertion hole (Walsh *et al.*, 2011; Calabria *et al.*, 2012). Currently, the pest is controlled through a combination of plant protection products (PPPs), monitoring, crop hygiene, integrated pest management (IPM), and exclusion netting (Cotes *et al.*, 2018; HSE, 2020; AHDB, 2021).

There are two distinct forms of *D. suzukii.* Summer morphs are primarily situated in the crop, and the winter morphs are generally located in woodland and hedgerows (Tochen *et al.*, 2016; Pelton *et al.*, 2016). Larvae develop into the winter morph in response to lower temperatures and reduced exposure to light (Toxopeus *et al.*, 2016). *Drosophila suzukii* winter morphs are adapted to survive these conditions and were identified as the primary source of fruit crop infestation at the start of the growing season (Panel *et al.*, 2018). To date, most research has focused on the control of *D. suzukii* summer morphs. However, preventing the winter morph from entering a crop early in the fruit growing season may help to prevent escalations in population growth and fruit damage.

The aim of the project was to develop a commercial repellent that could be used as part of a push-pull control strategy. Here, repellents are used to ‘push’ an insect pest from the crop, and attractants are employed to ‘pull’ an insect into a trap or onto a non-target crop (Cook *et al.*, 2007; Fountain *et al.*, 2021). Electrophysiological assays (EAG) were conducted in year one to identify chemicals detected by the antenna of *D. suzukii* summer and winter morphs. In year two, laboratory bioassays were undertaken at three chemical concentrations to identify chemicals that function as repellents against both *D. suzukii* morphs. Successful chemicals were placed in outdoor polytunnels to determine if they could reduce the numbers of *D. suzukii* caught in traps and eggs laid in sentinel fruit. Then the experiment was reproduced using high and low-dose sachets. In year three, repellents were trialled in the presence of a strawberry crop, and the distance the repellents reduced oviposition was measured. It was hypothesised that living in contrasting environmental niches may cause *D. suzukii* summer and winter morphs to respond differently to chemical stimuli (Cini *et al.*, 2012; Kirkpatrick *et al.*, 2018; Panel *et al.*, 2018). Finally, novel controls are frequently not implemented despite technological advancements. Therefore, a questionnaire was undertaken to determine the barriers to adopting new strategies.

Summary

EAG was undertaken to identify chemicals that were detected by the antenna of *D. suzukii*. The chemicals were puffed over the adult summer and winter morphs antenna, and the antennal response was recorded. Fourteen chemicals were detected by the antenna of both morphs, and a difference in antennal response was detected between *D. suzukii* summer and winter morphs in response to three of these chemicals. Behavioural bioassays were conducted to establish which of the 14 chemicals repelled *D. suzukii* summer and winter morphs from a fruit and yeast bait. The bioassays were composed of a two-way choice test at three chemical concentrations and replicated ten times. Overall, the number of *D. suzukii* summer and winter morphs entering gated traps containing a repellent was reduced when four chemicals were presented individually. The four most effective repellents were tested in small outdoor polytunnels.

Semifield experiments were undertaken to determine the repellents able to reduce the numbers of *D. suzukii* caught in traps and eggs laid in sentinel fruit. One red Drosotrap was placed at either end of 12 netted polytunnels (12 m in length). Fresh raspberries were placed into the traps to act as an attractive odour and egg-laying substrate. One of the traps in each tunnel was surrounded by five repellent dispensers, and the second trap was encircled with untreated control dispensers. Laboratory reared *D. suzukii* were released into the centre of the tunnels. The traps were removed after 48 hours, and adult flies were counted. The fruit

was incubated for 14 days to assess *D. suzukii* emergence. Three chemicals reduced the number of *D. suzukii* attracted into traps and subsequent egg-laying in raspberry fruits. High-dose sachets (used in previous experiments) were compared to low dose sachets. The experiment used the same methodology as above. One chemical was effective when using a low-dose sachet (129/08). A final experiment was undertaken in a crop to determine 1) repellents effective at reducing overall *D. suzukii* emergence and 2) estimate the distance the repellents were effective. Successful repellents from year two were placed 1 m from one end of each tunnel, and *D. suzukii* were released in the centre of each tunnel. Fruit samples (0, 1, 3, 5, 7, 9, and 11 m from the chemical dispensers) were taken after one week, and *D. suzukii* emergence was recorded. Two chemicals (129/04, 129/13) were identified that reduced the overall number of emerging *D. suzukii*, and two chemicals pushed egg-laying away from the chemical dispensers (129/08, 129/13).

A questionnaire was conducted to determine barriers to adopting novel IPM strategies. A pilot study was conducted with the help of three growers and two academics. These results were fed back into the final questionnaire and distributed by the Knowledge Exchange Manager at the AHDB. The questionnaire results indicated that training opportunities should be given to early adopters of novel IPM strategies, with a secondary focus on how the strategy works. In addition, grower-led training and workshops should be used once the strategy has become established on other farms.

Main Conclusions

- Fourteen chemicals were detected by the antenna of *D. suzukii* summer and winter morphs when using EAG.
- In the laboratory, summer morph *D. suzukii* were repelled by seven repellents, and winter morphs were repelled by five repellents. Both *D. suzukii* morphs were repelled by four chemicals.
- In semi-field experiments, three chemicals reduced the numbers of *D. suzukii* caught in traps and eggs laid in sentinel fruit, and one chemical 129/08 was effective when a low-dose chemical dispenser was used.
- In a strawberry crop, two chemicals were observed that reduced the overall number of emerging *D. suzukii*, and two chemicals were identified that pushed egg-laying away from chemical dispensers.
- In the questionnaire, it was ascertained that training opportunities should be given to early adopters of novel IPM strategies, and how the strategy works should be focused upon.

- Grower-led training should occur once novel IPM strategies have been established on a small number of farms.
- The education of individuals in the horticultural industry was determined to reduce the time between strategy design and application.

Financial Benefits

This project will help meet a need within the soft and stone fruit industry to reduce *D. suzukii* crop damage using a novel push-pull approach that can be used in Integrated Pest Management (IPM).

Action Points

There are no grower action points at this stage of the project.

SCIENCE SECTION

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae), also known as spotted wing Drosophila (SWD), is the major insect pest threatening European horticulture (Asplen *et al.*, 2015; Wiman *et al.*, 2014). This invasive fruit fly was first reported in the UK in 2012 in a wild blackberry monitoring trap (*Rubus fruticosus*) L.) (Rosales: Rosaceae) (Harris and Shaw, 2014) and has quickly spread. Yield loss associated with *D. suzukii* was estimated to be between 20-80% of a crop (Lee *et al.*, 2011; Walsh *et al.*, 2011). Commercial losses from crop damage have been estimated to be between £20-£30 million per annum in the UK (R. Harnden, pers. comms. 2019) and $443.8 million per annum in the USA (Bolda *et al.*, 2010; Yeh *et al.*, 2020).

Drosophila suzukii is one of two *Drosophila* species with a highly serrated ovipositor (organ used by the female to lay eggs) which lays their eggs in ripening fruit (Goodhue *et al.*, 2011; Polidori and Wurdack, 2019). Damage is caused by larvae feeding inside the fruit and pathogens entering the fruit by the hole created by female egg-laying (Walsh *et al.*, 2011; Calabria *et al.*, 2012). *Drosophila subpulchrella* Takamori and Watabe (Diptera: Drosophilidae) was the second reported *Drosophila* species to lay eggs in ripening fruit. However, *D. subpulchrella* are not found in the UK and are not considered pests in their native range (China and Japan) (Takamori *et al.*, 2006).

Two phenotypically distinct forms of *D. suzukii* were identified in the crop. *Drosophila suzukii* summer morphs are primarily reported in fruit crops and feed on blossom pollen and yeasts (Tochen *et al.*, 2016). The winter morph is found in woodland and hedgerows and feeds on fungi and bacteria in water droplets (Pelton *et al.*, 2016; Fountain *et al.*, 2018). *Drosophila suzukii* winter morphs have significantly larger wings, exhibit delayed ovary development, and have darker abdominal melanisation (Shearer *et al.*, 2016; Tran *et al.*, 2020). A larger range of environmental conditions are tolerated by *D. suzukii* winter morphs compared to summer morphs as they have a higher expression of stress-related genes (Toxopeus *et al.*, 2016). Most experimental research has focused on the control of *D. suzukii* summer morphs; however, the winter morphs were reported to be coming into the crop in early spring and infesting the crop (Panel *et al.*, 2018). Current control methods cannot keep *D. suzukii* under a threshold that is not economically disruptive to growers (Cini *et al.*, 2012). Living and feeding in different environmental niches could lead to behavioural differences when *D. suzukii* are exposed to chemicals. A novel control method may be needed if behavioural differences are detected between morphs.

Push-pull was first conceived in 1987 by Pyke et al. (1987) and later advanced by Miller and Cowles (1990). Repellents were used to 'push' an insect from the crop, and attractants were applied to 'pull' the pest into a trap (Bruce *et al.*, 2005; Cook *et al.*, 2007; Khan *et al.*, 2008; Midega *et al.*, 2018). Plants are employed as attractants and repellents in traditional push-pull systems (Khan *et al.*, 2008; Hooper *et al.*, 2009; Peoples *et al.*, 2009; Midega *et al.*, 2018). In the UK, space is a limiting factor, and intensive growing systems are designed for monocrops. If synthetic chemicals can provide the same benefits as plants, space could be maximised, and disruption to production minimised. However, growers frequently do not implement novel pest management strategies despite financial, ecological, and technological benefits (Mottaleb, 2018). Understanding growers' knowledge, attitudes, and practices towards current and novel IPM strategies should help reduce the time between pest management design and implementation.

In this PhD, I am developing the push component of the push-pull control strategy and discerning the barriers to adopting novel IPM strategies. In year one, I conducted EAG assays to identify chemicals detected by the antenna of *D. suzukii* summer and winter morphs. In year two, laboratory bioassays were conducted at three chemical concentrations. Moreover, semi-field experiments were undertaken at two concentrations to identify chemicals that function as repellents against both *D. suzukii* summer and winter morphs. In years three and four, semi-field experiments in polytunnels were conducted to identify chemicals effective at repelling *D. suzukii* in the presence of a crop. A questionnaire was also undertaken to ascertain sensitive grower information and understand the barriers to adopting new pest management strategies. EAG, bioassays, and semi-field research (years one and two) are detailed in the previous reports. This report discusses the methods and results of the third and fourth years of my PhD project.

Objectives:

Efficacy of repellents on *D. suzukii* in closed-ended polytunnels planted with strawberry crops

1. Identify if repellent dispensers can significantly reduce the total number of emerging *D. suzukii.*
2. Estimate the distance that chemical dispensers can deter *D. suzukii* oviposition.

Questionnaire of growers and farm managers

1. To understand respondents' knowledge of *D. suzukii* summer and winter morphs.
2. Determine currently used *D. suzukii* IPM strategies and respondents' relationship with their agronomic advisors.
3. Identify respondents requirements for novel IPM strategies

Materials and Methods

Cultures of D. suzukii

An unsuccessful attempt to rear a UK *D. suzukii* strain was attempted in 2017. Therefore, an Italian strain was used in all laboratory and semi-field trials to ensure comparable data. *Drosophila suzukii* were reared in the Natural Resources Institute (NRI), University of Greenwich, Kent, UK. The colony was established from an Italian strain, caught in Trento, 2013. In 2017 they were brought into NRI from the National Institute of Agricultural Botany, East Malling Research (NIAB EMR) as larvae and pupae. The *D. suzukii* summer and winter morphs were reared following the method described by Shaw et al. (2018).

Putative Repellents

Fourteen potential repellents were selected based upon their reported repellent effects on *D. suzukii* summer morphs, other closely related Diptera species, or from other ongoing projects at NIAB EMR and NRI. The test repellents were coded 129/01 to 129/14.

Screening fruit for toxicity before D. suzukii semi-field experiments

Two whole raspberries were selected at random from each supermarket purchased punnet and placed into a 1 L plastic cup to check that the fruits used in the experiments did not have a lethal dose of insecticides. Ten female *D. suzukii* were introduced into the cup and left for 48 hours in a temperature-controlled environment (25 ± 1∘C, 16:8 h L: D, 60 ± 5% R.H). The whole punnet was disposed of if over 25% of the *D. suzukii* died.

Efficacy of repellents on D. suzukii in closed-ended polytunnels planted with strawberry crops

Twelve polytunnels (12 m x 2 m x 1.5 m) were set up at NIAB EMR. Twenty coir grow bags (500 mm x 200 mm) were positioned in a row down the centre of the polytunnel. Eight strawberry plants (*cv. amesti)* were planted in each coir bag (Figure 1).

Three chemicals (129/04, 129/08, 129/13) and a control were used in the experiment. The chemical was dispensed onto a cotton dental roll and held within a heat-sealed polyethylene bag (5 cm x 5 cm, 120 µm thick). These test sachets were placed in a grid at one end of the polytunnel (4 repellent sachets per tunnel). Fifteen mated females and fifteen males were released at the central 6 m point and left for one week.

On day zero, the fruit was sampled to check for any prior infestation, and a second sample was taken on day seven. Six fruit were picked from the following sampling points (distance from the sachet): 0, 1, 3, 5, 7, 9, and 11 m. The fruit was placed into ventilated emergence boxes in a temperature-controlled room (~22 ± 2°C, 16:8 L: D, and > 40 % R.H).

The number of adult *D. suzukii* emerging after fourteen days were counted. *Drosophila suzukii* identification was confirmed using a *D. suzukii* key (Markow and O'Grady, 2006). Twelve replicates of each repellent were undertaken in total, and the treatments were run simultaneously. The location and polytunnel end the repellent or control was assigned to changed ends between replications.

Polytunnels were cleaned between replications. All ripe fruit was picked one and five days after the end of the experiment to remove *D. suzukii* larvae. A red Drosotrap trap was placed into the centre of the polytunnel to trap surviving adult *D. suzukii*. The next replicate was set up seven days after the end of the last replicate.

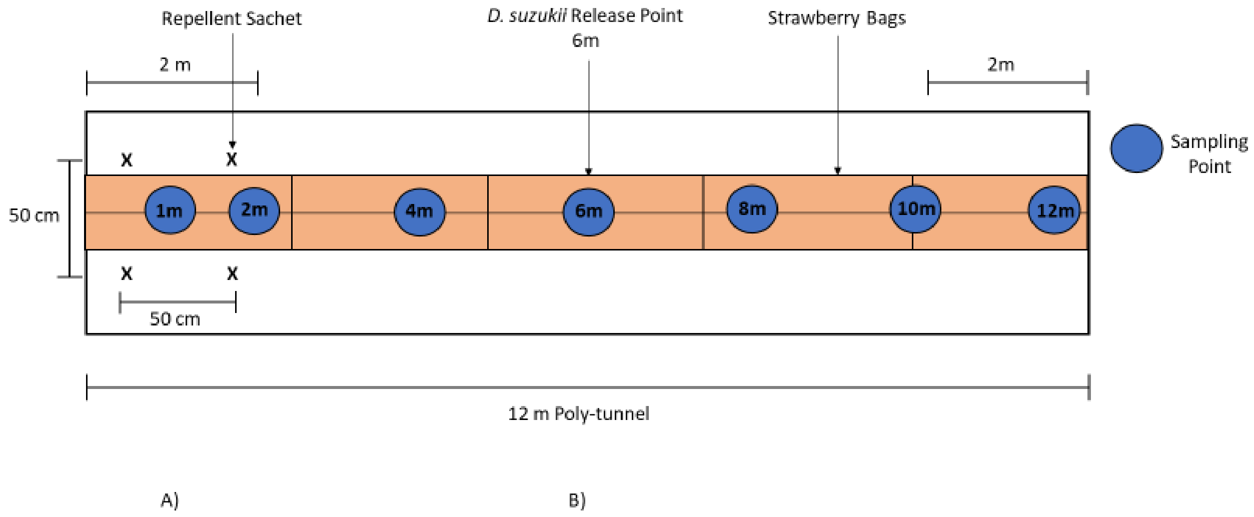

Figure 1: The experiment was conducted in 12 m closed-ended polytunnels and composed of two main areas: **A)** Experimental repellents: four repellent sachets **B)** Release point of laboratory-reared *D. suzukii.* Blue dots indicate five sampling points.

Construction of grower questionnaire

The Bristol-online survey tool was used to construct two questionnaires (BOS, 2021). A pilot study was conducted on a test panel, and the gathered data were fed back into the final questionnaire. The final questionnaire was completed by growers and managers. The questionnaire had eight sections with 31 questions and took approximately 30 minutes to complete. The eight sections were: I) creating a unique identification code II) you and the spotted wing Drosophila, III) agronomists, IV) knowledge exchange, V) currently employed management strategies, VI) using a push-pull strategy and adopting new control methods, VII) the winter morph, and VIII) cross-population data.

An introduction was placed at the start of each section to help participants focus (Aarons, 2020). In addition, sensitive data were placed at the end of the questionnaire to increase the number of respondents (Foddy, 2003). Finally, the questionnaire was distributed from a place of trust to increase survey completion, and precise language was used to reduce bias (Tabachnick *et al.*, 2019).

Four question types were used I) categorical, II) continuous data, III) scale data, and IV) ordinal data (Tabachnick *et al.*, 2019). Six-point Likert scales were used as respondents were forced to select a position (Nemoto and Beglar, 2013). The scales were also modified to help respondents select a position (Frey, 1994; Foddy, 2003).

Ethical Approval

A consent form and information sheet were signed before respondents completed the pilot study. However, the consent form and information sheet were combined into an online page in the final questionnaire. After reading the information sheet, respondents were redirected if they were under 18 or did not wish to participate in the questionnaire. Data were treated following the Data Protection Act 1998, the General Data Protection Regulation (2018), and ethical approval was granted by the University of Greenwich University Research Ethics Committee on 21/06/18. Participants could opt out of the questionnaire until 31/05/19.

Distribution of questionnaires and feedback

Three growers and two academics were selected to undertake the pilot study. These individuals were selected based on their industry knowledge or expertise in working on questionnaires. A hyperlink was given to participants, and feedback was requested at the end of the survey.

The final questionnaire was distributed by the Knowledge Exchange Manager at the AHDB and circulated in monthly newsletters by the AHDB, Berry Gardens Ltd, Totalberry Ltd, SoloBerry Ltd, BerryWorld Ltd, Worldwide Fruits Ltd, and Avalon Produce Ltd.

Statistical Analysis

Efficacy of repellents on D. suzukii in closed-ended polytunnels planted with strawberry crops

Data were recorded in an Excel spreadsheet, and statistical analyses were carried out using R (R Core Team, 2018) and R Studio (R Studio Team, 2018). In addition, the data were visually checked for normality using histograms.

The number of emerging *D. suzukii* was log+1 transformed and entered as the dependent variable in a linear mixed model. In addition, replicate was entered as a random factor, and distance was entered as a fixed factor (Lenth, 2019). The fixed effect was assessed using $\chi^{2,}$ and a Tukey's test was used to compare numbers of *D. suzukii* emerging from each distance.

Chemical effect on the total number of emerging D. suzukii in a polytunnel containing strawberries

The number of emerging *D. suzukii* was log+1 transformed and entered as the dependent variable in a general linear model. Next, the chemicals or control were entered as a four-level factor and entered as the independent model and assessed using an F-test. Finally, a Dunnett's post hoc test ($P < 0.05$) was used to compare the three repellents and the control (IBM Corp, 2019; Bates and Maechler, 2018).

Questionnaire data cleaning and analysis

Data were downloaded into Microsoft Excel (Jisc, 2021; BOS, 2021), imported into IBM SPSS Statistics for Windows version 26 and analysed (IBM Corp, 2019).

Data were cleaned to ensure results were representative of respondents. Data were removed if I) data were inconsistent, II) the same response was selected consistently in Likert scale questions, and III) the completion time of the questionnaire was too short (<15 minutes) (Gitlin, 2021; Ilyas and Chu, 2019). Descriptive statistics were used when examining data outliers.

Data were analysed using either a Spearman's Rank test or a Fisher's Exact test. Spearman's Rank tests are nonparametric and were used to determine the direction and strength of monotonic relationships. Fisher's Exact tests were used to analyse independent categorical variables. Adjusted residuals (> 2) were used to find combinations of frequencies that occurred more often than expected when using a χ^2 distribution (Kim, 2017).

Results

Repellent in the crop

Efficacy of repellents on D. suzukii in closed-ended polytunnels planted with strawberry crops

Overall a difference was identified between the total number of *D. suzukii* emerging from fruit in the polytunnels containing the four chemical treatments ($F = 4.9$, df = 3, 44, $P < 0.01$). Fewer *D. suzukii* emerged from fruits with chemical dispensers containing 129/04 and 129/13 compared to the control dispensers (Tukey's test; $P < 0.05$). No difference from the control was found when 129/08 was placed into polytunnels compared to the control (Tukey's test; $P = 0.16$; Figure 2).

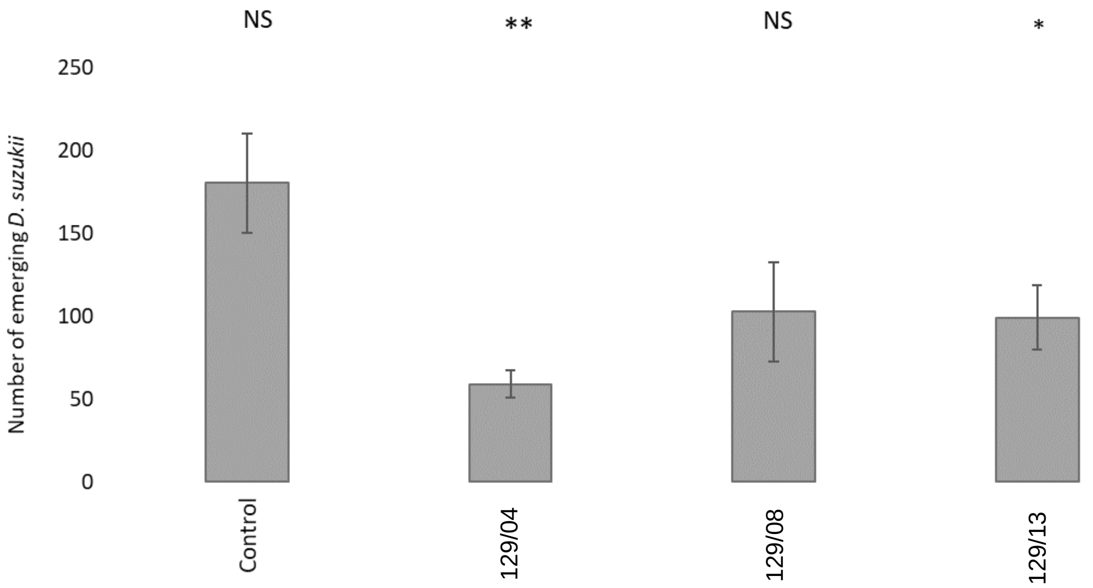

Figure 2: Mean (± SEM) number of *Drosophila suzukii* summer morphs that emerged from fruit taken from polytunnels (n = 12 per treatment) containing strawberry plants (*cv.* Amesti). The polytunnels had dispensers containing either 129/04, 129/08, 129/13 or control dispensers. Statistical significance from the control = '*' (n = 12; NS = not significantly different, statistical significance, *P <0.05, **P <0.01, ***P < 0.001).

Chemical effect on the total number of emerging D. suzukii in a polytunnel containing strawberries

129/04 significantly affected the number of *D. suzukii* emerging from the seven sampling points ($\chi^2 = 80.83$, df = 6, $P < 0.0001$; Figure 3). The fewest *D. suzukii* emerged between 0-2 m, with the highest emergence at 5, 7, and 9 m. 129/08 significantly affected the number of *D. suzukii* emerging from the seven sampling points ($\chi^2 = 122.25$, df = 6, $P < 0.001$; Figure 4). The fewest *D. suzukii* emerged between 0-4 m, with the highest emergence at 7-11 m. 129/13 significantly affected the number of *D. suzukii* emerging from the seven sampling points ($\chi^2 = 14.99$, df = 6, $P < 0.05$; Figure 5). No difference in emergence was found between 0, 1, 3, 5, and 9 m. Eleven metres was significantly lower compared to the other distances. The number of *D. suzukii* emerging from the seven sampling points did not change in polytunnels containing a control ($\chi^2 = 0.65$, df = 6, $P = 0.99$; Figure 6).

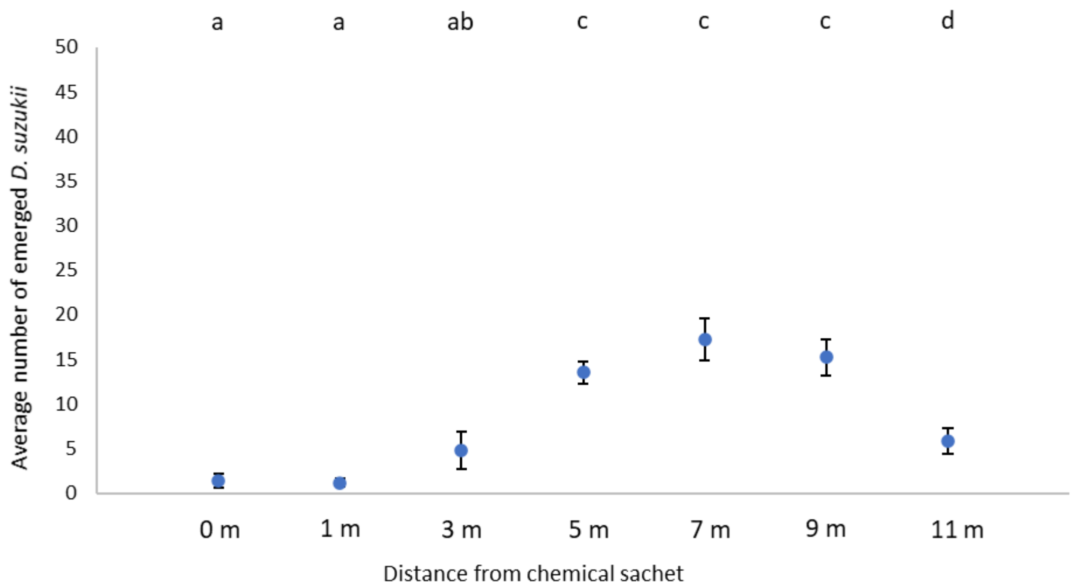

Figure 3: 129/04: Mean (± SEM) number of *Drosophila suzukii* emerging from strawberries (*cv.* Amesti) taken at seven sampling points (0 m, 1 m, 3 m, 5 m, 7 m, 9 m, and 11 m) starting from the polytunnel end (n = 12 per treatment). Mean values are represented, and error bars show the standard error mean. Means denoted by a different letter indicate significant differences between treatments (*P* < 0.05).

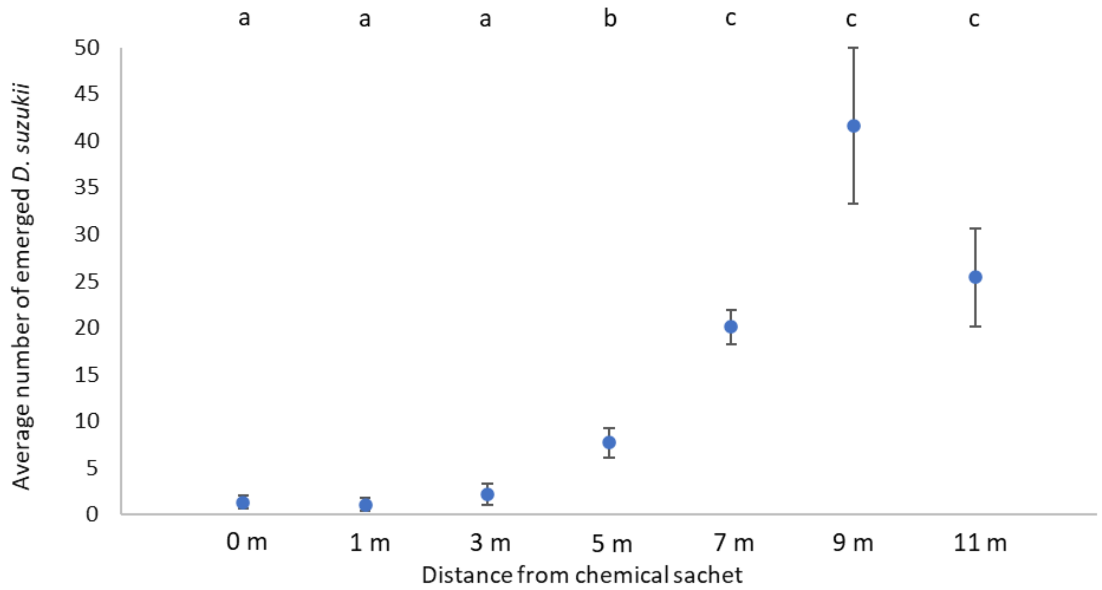

Figure 4: 129/08: Mean (± SEM) number of *Drosophila suzukii* emerging from strawberries (*cv.* Amesti) taken at seven sampling points (0 m, 1 m, 3 m, 5 m, 7 m, 9 m, and 11 m) starting from the polytunnel end (n = 12 per treatment). Mean values are represented, and error bars show the standard error mean. Means denoted by a different letter indicate significant differences between treatments ($P < 0.05$).

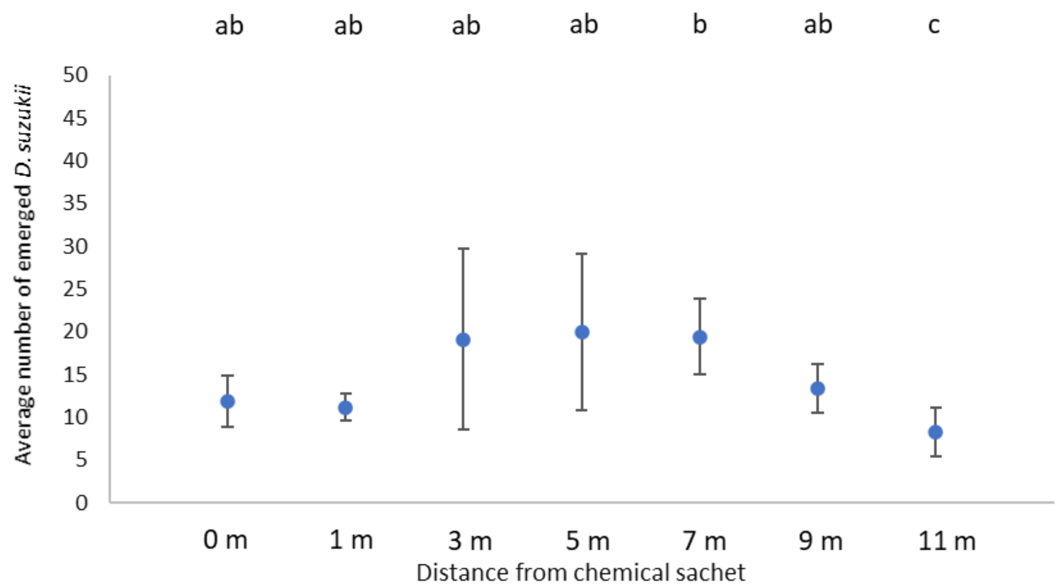

Figure 5: 129/13: Mean (± SEM) number of *Drosophila suzukii* emerging from strawberries (*cv.* Amesti) taken at seven sampling points (0 m, 1 m, 3 m, 5 m, 7 m, 9 m, and 11 m) starting from the polytunnel end (n = 12 per treatment). Mean values are represented, and error bars show the standard error mean. Means denoted by a different letter indicate significant differences between treatments ($P < 0.05$).

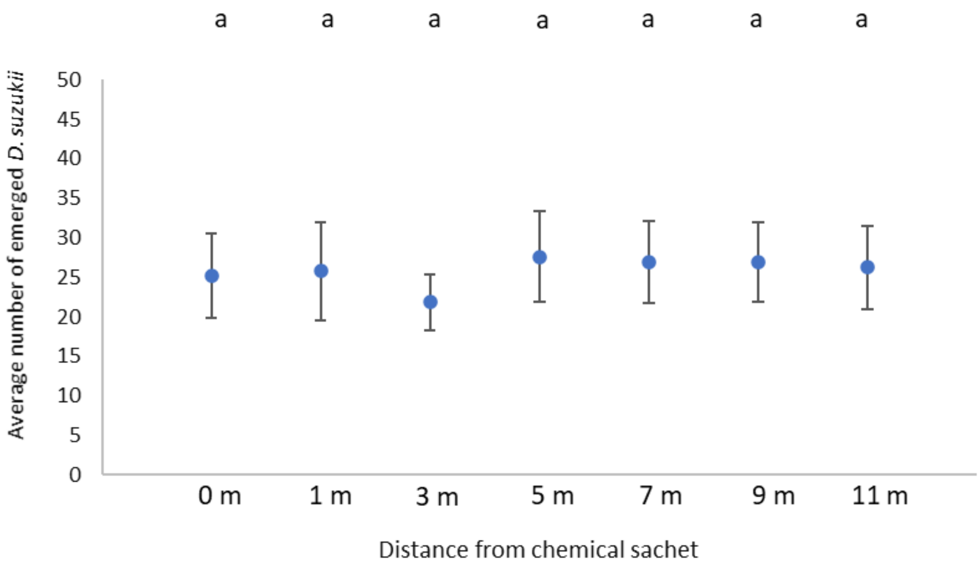

Figure 6: Control: Mean (± SEM) number of *Drosophila suzukii* emerging from strawberries (*cv.* Amesti) taken at seven sampling points (0 m, 1 m, 3 m, 5 m, 7 m, 9 m, and 11 m) starting from the polytunnel end (n = 12 per treatment). Mean values are represented, and error bars show the standard error mean. Means denoted by a different letter indicate significant differences between treatments ($P < 0.05$).

Questionnaire

Pilot study

Three areas of the pilot questionnaire were identified for improvement. Firstly, some terminology was identified as overly complex for the target demographic. Secondly, the Likert scales were determined as too broad, making it difficult for respondents to select a position. Finally, opening paragraphs and additional questions were needed to help with questionnaire flow and ensure all respondents were basing their answers on the material provided.

Respondents knowledge of D. suzukii summer and winter morphs

No difference was identified between the number of respondents who were aware of *D. suzukii* summer and winter morphs (Fishers Exact Test = 0.3, df = 1, $P = 0.9$). Moreover, no relationship was reported between respondents knowledge of *D. suzukii* summer morphs (yes/no) and: farm location (Fishers Exact Test = 13.8, df = 10, $P = 0.78$), the number of *D. suzukii* vulnerable hectares (Fishers Exact Test = 4.41, df = 4, $P = 0.3$), and the total number of fruit crops grown (Fishers Exact Test = 1.2, df = 2, $P = 1$).

Similarly, no relationship was identified between respondents knowledge of *D. suzukii* winter morphs (yes/no) and farm location (Fishers Exact Test = 11.92, df = 10, $P = 0.16$). However, a significant relationship was observed between respondents knowledge of *D. suzukii* winter morphs (yes/no) and respondents' number of *D. suzukii* vulnerable hectares (Fishers Exact Test = 8.56, df = 4, $P < 0.01$), the overall number of crops grown (Fishers Exact Test = 7.21, df = 2, $P < 0.01$), and having heard of *D. suzukii* winter morphs. Farms were less likely to have heard of *D. suzukii* winter morphs if they grew seven or more crop species on their farm (Adjusted residual > 2, table 1) or if the respondent had the smallest number of *D. suzukii* vulnerable hectares (Adjusted residual > 2, Table 1).

The frequency respondents saw summer and winter morphs in their crop and how it changed the IPM strategies implemented

A significant relationship was identified between the frequency of *D. suzukii* summer morph encounters and the IPM strategies implemented on farms (Fishers Exact Test = 10.98, df = 6, $P < 0.01$, table 2). Participants that reported seeing *D. suzukii* summer morphs on their farm once a month were more likely to use non-pesticide IPM control (Adjusted residuals > 2). In contrast, no relationship was reported between *D. suzukii* winter morph encounter and type of implemented IPM strategies (Fishers Exact Test = 5.6, df = 6, $P = 0.11$, Table 2).

A significant relationship was found between participants encountering *D. suzukii* summer morphs and the number of IPM strategies implemented (Fishers Exact test = 22.6, df = 6, $P < 0.001$, Table 2). Participants that identified *D. suzukii* summer morphs in their crops daily were more likely to use eight or more IPM strategies (Adjusted residual > 2). In contrast, the frequency of *D. suzukii* winter morph encounters did not vary the number of IPM strategies implemented (Fishers Exact Test = 10.49, df = 6, $P = 0.06$, Table 2).

Grower Interest in novel IPM strategies based on their knowledge of D. suzukii summer and winter morphs

New IPM strategies were requested by 100 % of the participants (27/27), and 70.4 % were interested in using a year-round control strategy (19/27). Participants believed there was room to improve current IPM strategies if *D. suzukii* summer morphs (Correlation Coefficient = 0.44, df = 15, P < 0.05, Figure 7) and winter morphs (Correlation Coefficient = 0.43, df = 15, P < 0.05, Figure 8) were a problem on their farm. Participants were more likely to implement year-round control strategies if they believed the winter morph would be a problem in the UK over the next ten years (Correlation coefficient = 0.52, df = 15, P < 0.01, Figure 9).

Table 1: Summary of covariation between farm characteristics and participants' knowledge of *Drosophila suzukii* summer (SM) and winter morphs (WM) (Objective 1).

Hypothesis Tested	Variable 1	Variable 2	Fishers Exact Test	Degrees of Freedom	*P*-value	Relationship
The proportion of growers aware of the SM and WM will vary based on farm characteristics	Growers awareness of the SM	Growers awareness of the WM	0.3	1	0.9	NA
		Number of hectares of *D. suzukii* vulnerable crops	4.41	4	0.16	NA
		The overall number of crops grown per farm	1.2	2	1	NA
		Farm location	13.8	10	0.78	NA
	Growers awareness of the WM	Farm location	11.92	10	0.16	NA
		Number of hectares of *D. suzukii* vulnerable crops	8.56^{+}	4	**	Growers with the smallest farms were less likely to have heard of the WM.
		The overall number of crops grown per farm	7.21^{+}	2	**	Farms that grew seven or more crops were less likely to have heard of the WM.

Fishers Exact Test results. Statistical significance indicating a relationship between two variables was denoted using an '*' (statistical significance; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). The final column indicates the relationship between the two variables. No relationship was denoted using an 'NA'.

Table 2: Summary of the frequency participants see *Drosophila suzukii* summer (SM) and winter morphs (WM) in their crop and how that changes the IPM strategies implemented (Objective 1).

Hypothesis Tested	Variable 1	Variable 2	Fishers Exact Test	Degrees of Freedom	*P*-value	Relationship
The frequency growers see *D. suzukii* SM, and WM in their crop changes the IPM strategies implemented by growers	Frequency SMs were encountered by participants	Type of IPM strategy implemented	10.98⁺	6	**	Participants were more likely to report seeing *D. suzukii* summer morphs on their farm monthly when using non-pesticide control
		The overall number of implemented IPM strategies	22.6⁺	6	***	Participants that saw *D. suzukii* summer morphs on their crops daily were more likely to use eight or more IPM strategies
	Frequency WMs were encountered by participants	Type of IPM strategy implemented	5.6	6	0.11	NA
		The overall number of implemented IPM strategies	10.49	6	0.06	NA

Fishers Exact Test results. Statistical significance indicating a relationship between two variables was denoted using an '*' (statistical significance; *P < 0.05, **P < 0.01, ***P < 0.001). The final column indicates the relationship between the two variables. No relationship was denoted using an 'NA.'

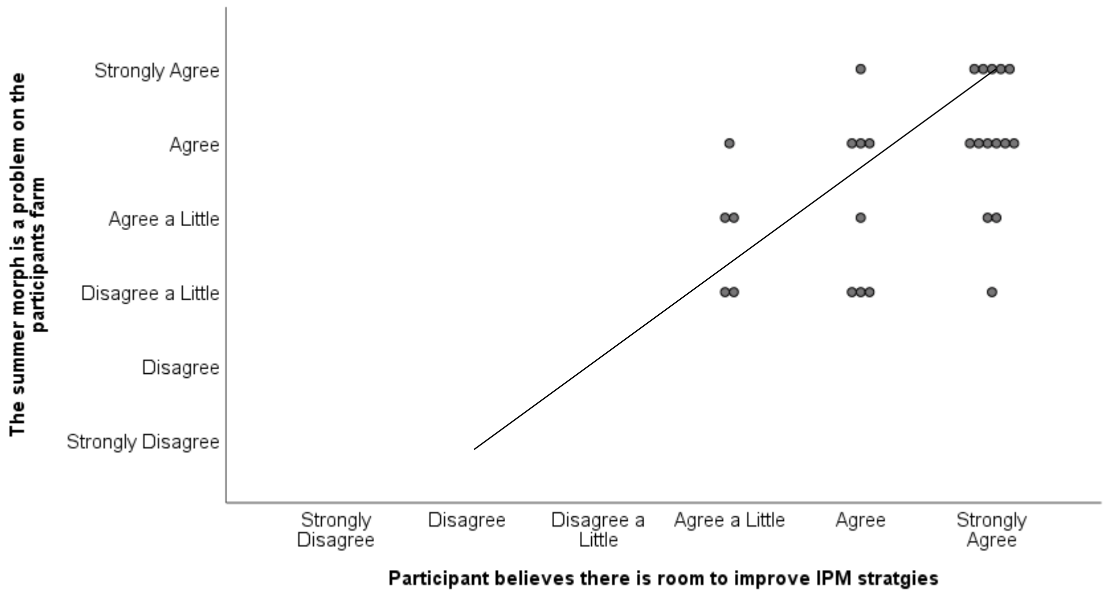

Figure 7: The graph shows the correlation between participants that believed there was room to improve *Drosophila suzukii* IPM strategies and respondents that believed the summer morphs were a problem on their farm. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.44, df = 15, P < 0.05)**.**

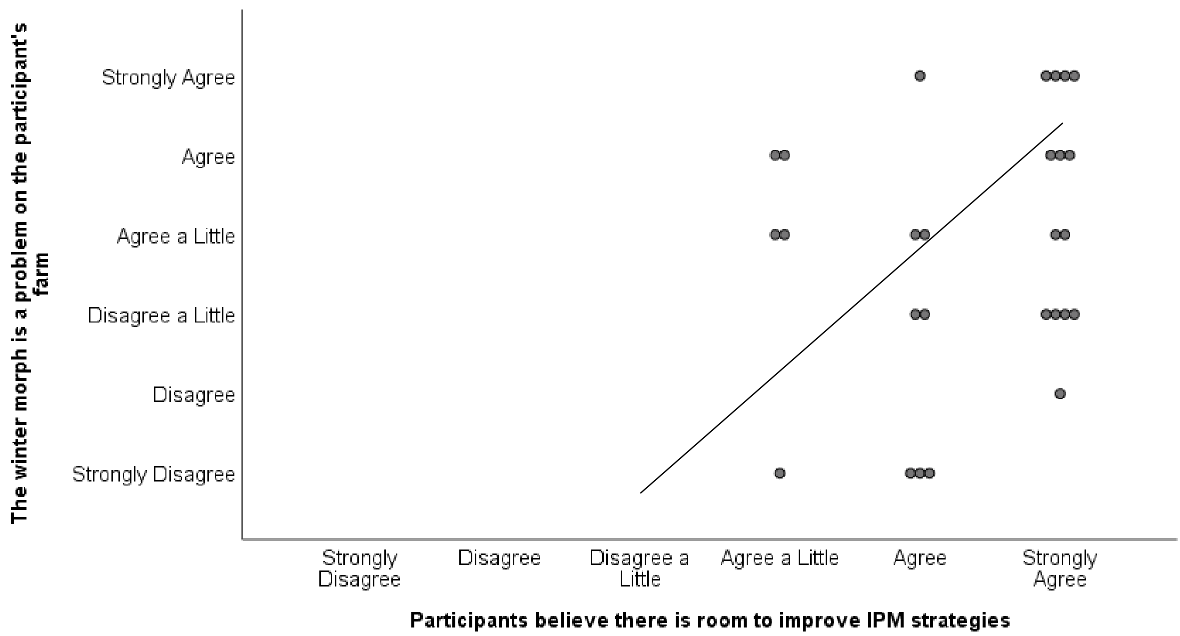

Figure 8: The graph shows the correlation between participants that believed there was room to improve current *Drosophila suzukii* IPM strategies and the respondents that thought the winter morphs were a problem on their farm. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.43, df = 15, P < 0.05)**.**

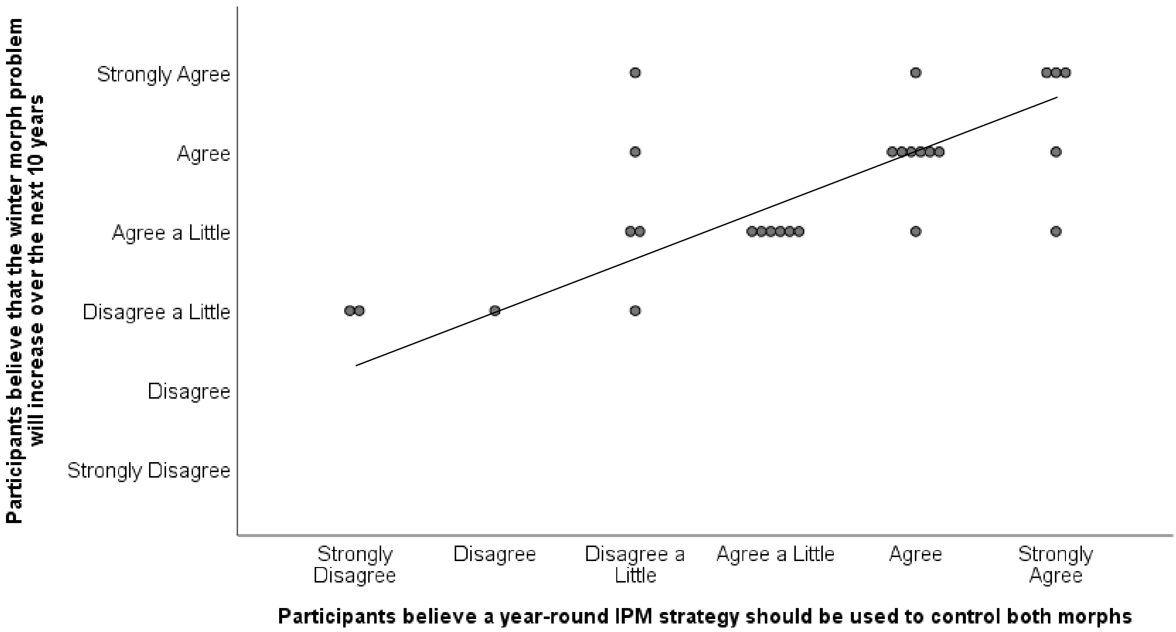

Figure 9: The graph shows the correlation between participants who believed a year-round control IPM strategy should be used to control both Drosophila suzukii morphs and the respondents who believe that the winter morph problem will increase next ten years. The line of best fit is represented using a solid black line (n = 27, Correlation coefficient = 0.52, df = 15, P < 0.01).

Determine currently used D. suzukii IPM strategies, respondents relationship with their agronomic advisors, and IPM satisfaction

The three most commonly implemented IPM strategies were good hygiene (96.3 %, 26/27), PPPs (77.8 %, 21/27), and *D. suzukii* monitoring devices (62.9 %, 17/27). However, the most money was spent on labour (48.1 %, 13/27), PPPs (37 %, 10/27), and good hygiene (7.5 %, 2/27).

Participants that spoke to their agronomic advisor more frequently were also more likely to use more IPM strategies (Correlation Coefficient = 0.45, df = 8, $P < 0.05$, Figure 10) or non-pesticide control strategies (Correlation Coefficient = 0.45, df = 8, $P < 0.05$, Figure 11). Interestingly, a significant relationship was found between where growers that sourced their agronomic advisor and the number of IPM strategies implemented Fishers Exact Test = 8.55, df = 4, $P < 0.05$). Participants were more likely to use less than five implemented IPM strategies if their agronomist was sourced as an independent contractor (Adjusted residuals > 2, Table 3).

A respondent was more likely to believe there was room to improve current *D. suzukii* IPM strategies if the respondents speak with other growers (Correlation coefficient = 0.48, df = 11, $P < 0.05$, Figure 6.12). No relationships were reported between participants wanting to improve IPM strategies and respondents attending public engagement events (Correlation Coefficient = -0.89, df = 11, $P > 0.05$), attending networking events (Correlation Coefficient = 0.18, df = 11, $P > 0.05$), reading industry produced magazine (Correlation Coefficient = 0.16 , df = 11, $P > 0.05$), using the internet to collect information (Correlation Coefficient = 0.09 , df = 11, $P > 0.05$), or seeing their agronomists (Correlation Coefficient = -0.1, df = 11, $P > 0.05$).

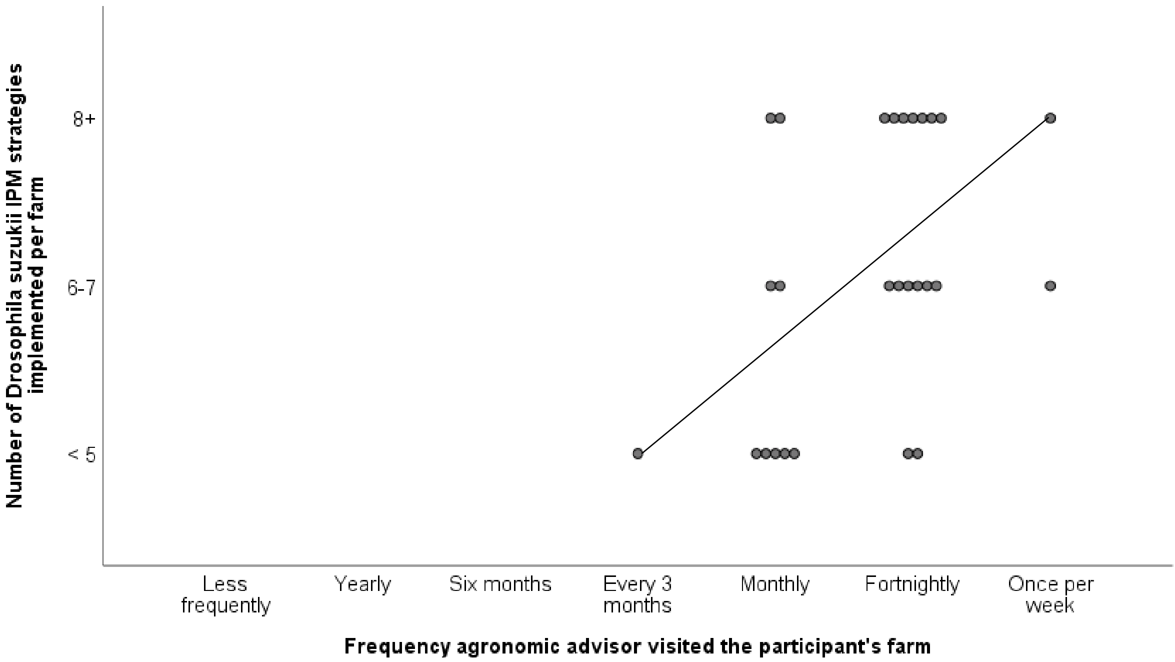

Figure 10: The graph shows the correlation between the frequency an agronomic advisor visited the participant's farm and the number of *Drosophila suzukii* integrated pest management strategies (IPM) implemented on each farm. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.45, df = 8, $P < 0.05$).

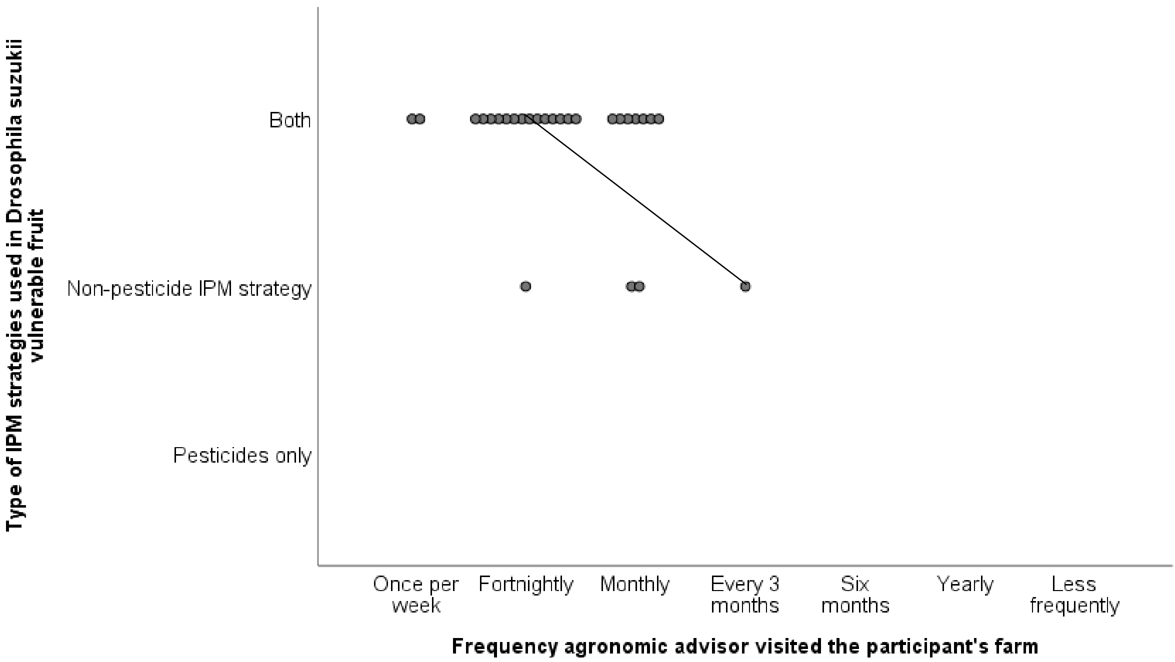

Figure 11: The graph shows the correlation between the frequency an agronomic advisor visited the participant's farm and the type of *Drosophila suzukii* integrated pest management strategy (IPM) used on their farm. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.45, df = 8, $P < 0.05$).

Table 3: Summary of participant interactions with agronomic advisors and how that changes the number of IPM strategies implemented on a respondent's farm (Objective 2, hypothesis 1).

Hypothesis Tested	Variable 1	Variable 2	Fishers Exact Test	Degrees of Freedom	*P*-value	Relationship
Identify current control strategies used by participants against *D. suzukii* in the UK, their relationship with their agronomic advisors, and their satisfaction with IPM availability	Number of implemented IPM strat geis	Where participants sourced their agronomic advisor	8.55	4	*	Participants were more likely to use less than five implemented IPM strategies if their agronomist was sourced as an independent contractor

Fishers Exact Test results. Statistical significance indicating a relationship between two variables was denoted using an '*' (statistical significance; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). The final column indicates the relationship between the two variables. No relationship was denoted using an 'NA.'

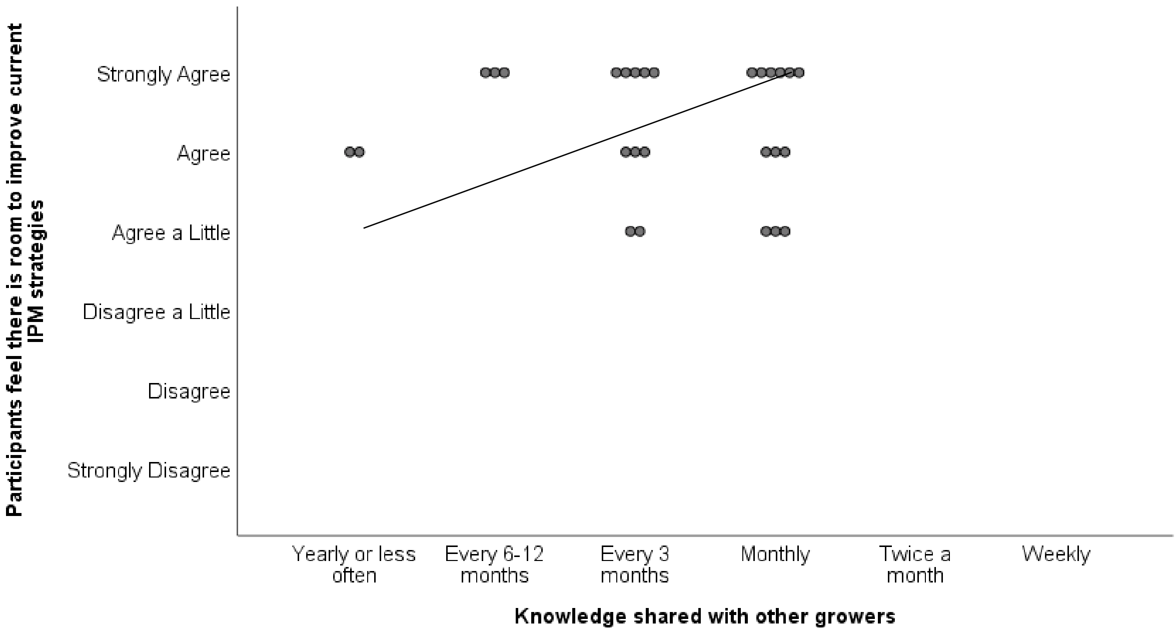

Figure 12: The graph shows the correlation between participants that feel there is room to improve current *Drosophila suzukii* IPM strategies and if the respondents shared their knowledge with other growers. The line of best fit is represented using a solid black line (n = 27, Correlation coefficient = 0.48, df = 8, $P < 0.05$).

Identify respondents requirements for novel IPM strategies

Novel control strategies that cost less than currently implemented IPM plans were more likely to be implemented if additional training opportunities were available (Correlation Coefficient = 0.44, df = 12, P < 0.05; Figure 13). Novel control strategies that cost the same as currently implemented IPM strategies were more likely to be implemented if another grower explained how to apply the new strategy (Correlation Coefficient = 0.58, df = 12, P < 0.01, Figure 14), and if the participant understood the science behind the new IPM strategy (Correlation Coefficient = 0.47, df = 12, P < 0.05, Figure 15). When a more significant number of resources were required, participants were more likely to implement the new approach if another grower explains how to apply the new strategy (Correlation Coefficient = 0.55, df = 12, P < 0.01, Figure 16). However, negative interactions with growers reduced grower acceptance of novel IPM strategies that cost the same (Correlation Coefficient = 0.47, df = 12, P < 0.05, Figure 17) or more than those currently implemented (Correlation Coefficient = 0.39, df = 12, P < 0.05, Figure 18).

Respondents were more likely to be the first to implement new IPM strategies if there were training opportunities available (Correlation Coefficient = 0.44, df = 12, P < 0.01, Figure 6.19).

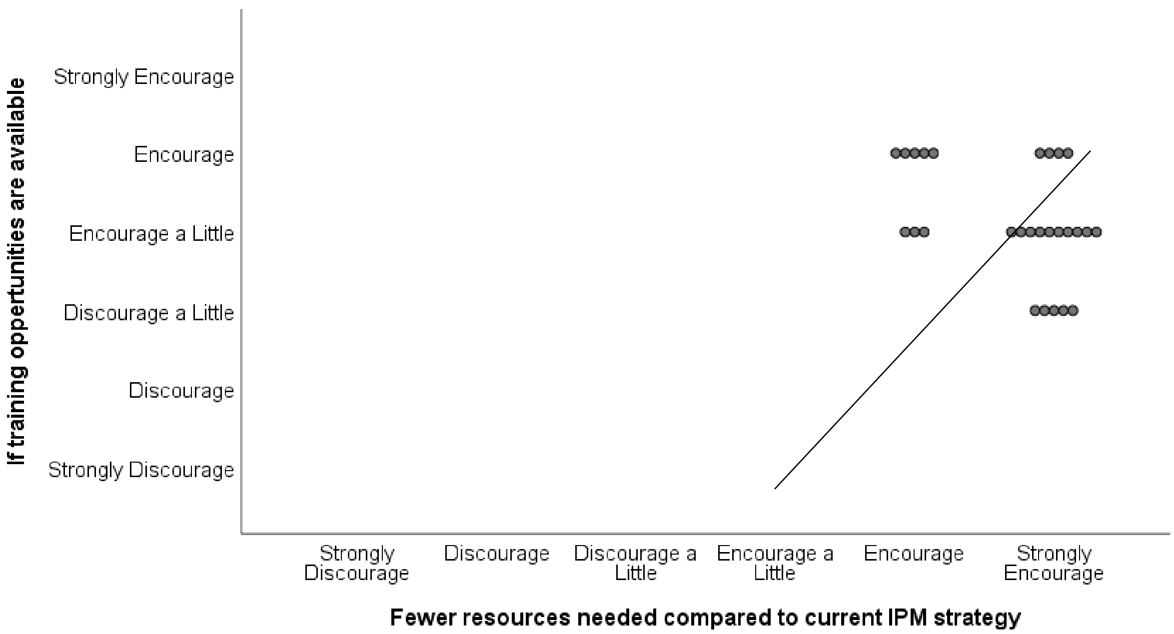

Figure 13: The graph shows the correlation between participants who were more likely to implement a new IPM strategy that required fewer resources than their current IPM approach and if there were plenty of training opportunities available. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.44, df = 12, *P* < 0.05).

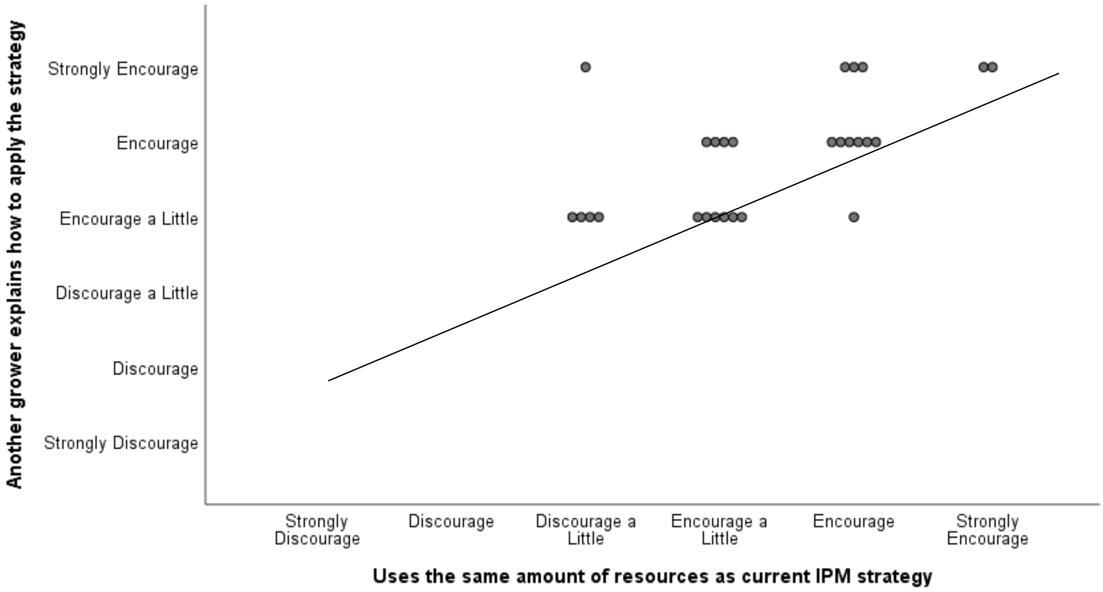

Figure 14: The graph shows the correlation between participants who were more likely to implement a new IPM strategy that required fewer resources than their current IPM approach and if another grower explained how to implement the novel control method. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.58, df = 12, $P < 0.01$).

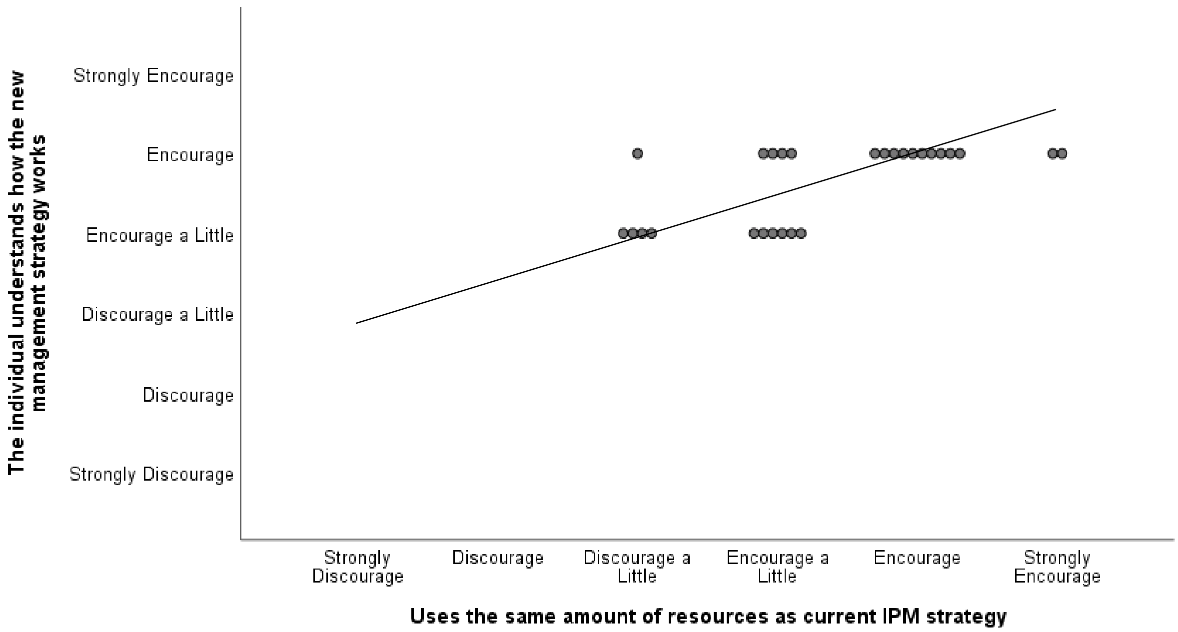

Figure 15: The graph shows the correlation between participants who were more likely to implement a new IPM strategy that required fewer resources than their current IPM approach and if the participant understands how the new IPM strategy works. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.47, df = 12, $P < 0.05$).

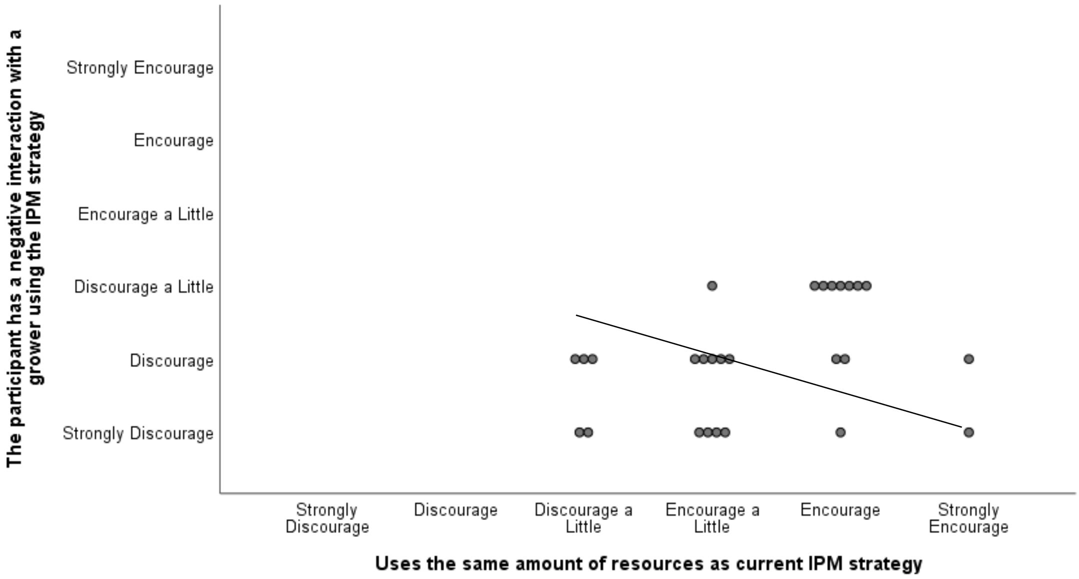

Figure 16: The graph shows the correlation between participants who were more likely to implement a new IPM strategy that required fewer resources than their current IPM approach and if the participant had a negative interaction with a grower using the same IPM strategy. The line of best fit is represented using a solid black line (n = 27, Correlation coefficient = 0.47, df = 12, $P < 0.05$).

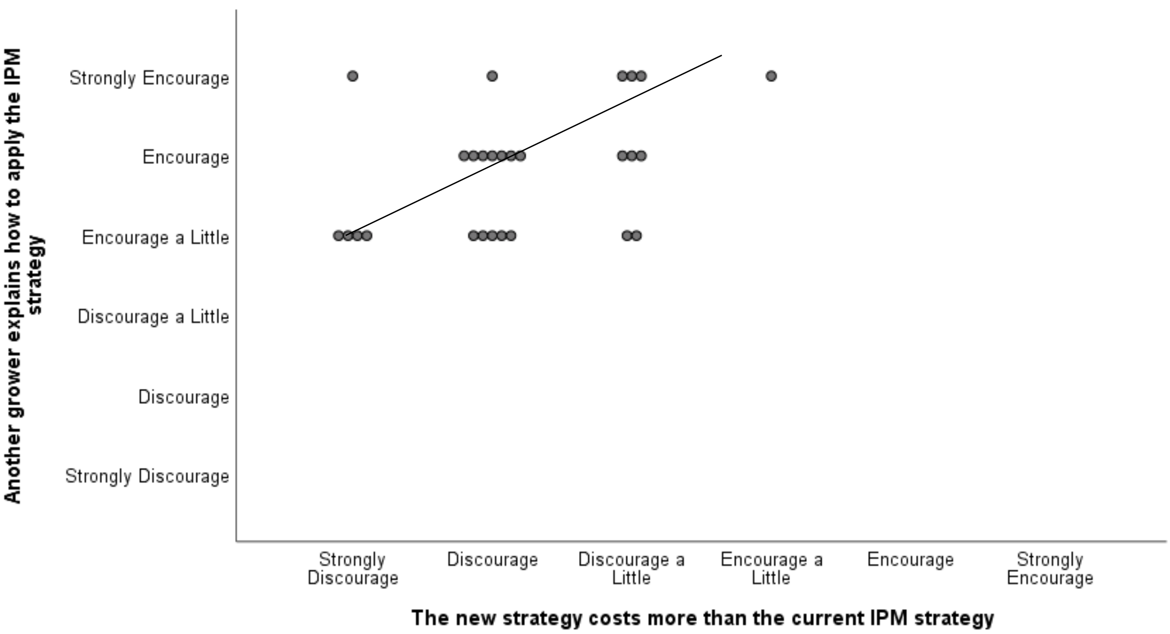

Figure 17: The graph shows the correlation between participants who were more likely to implement a new IPM strategy that required a larger number resources than their current IPM approach and another grower explaining how the new IPM strategy works. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.55, df = 12, $P < 0.01$).

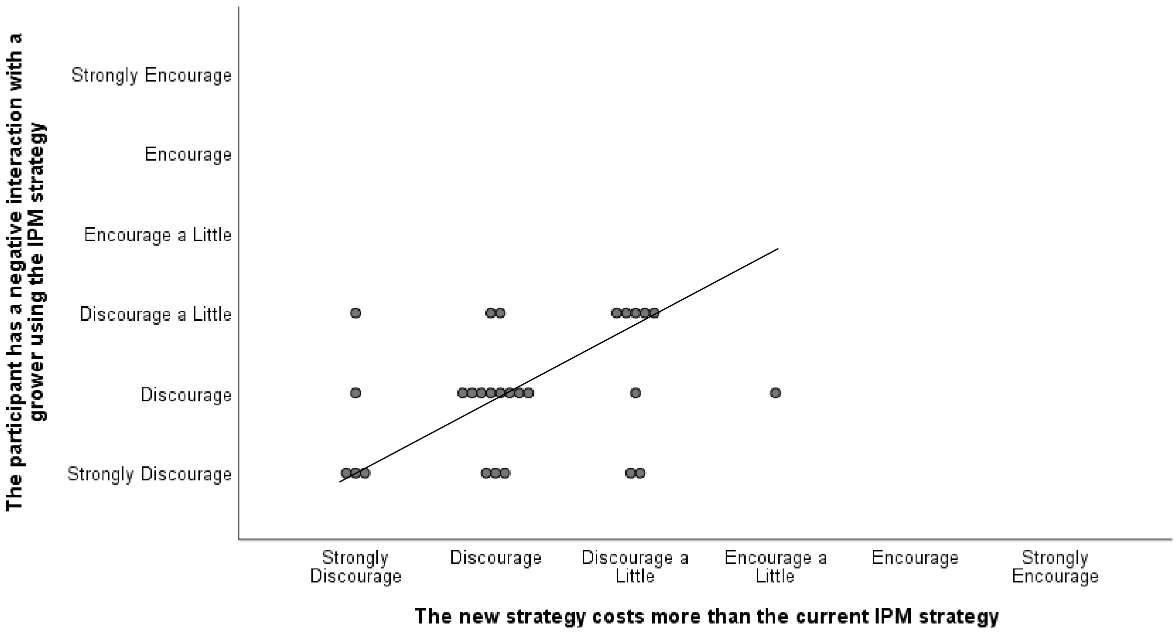

Figure 18: The graph shows the correlation between participants who were more likely to implement a new IPM strategy that required a larger number of resources than their current IPM approach and if a participant has a negative interaction with a grower using the new approach. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.39, df = 12, $P < 0.05$).

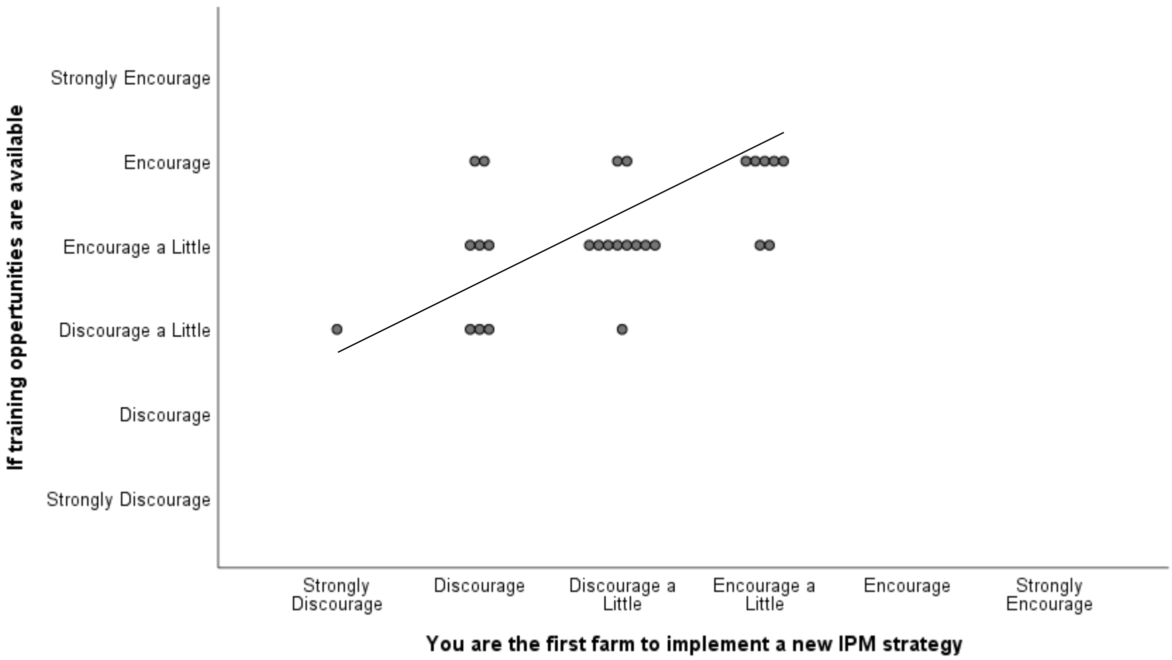

Figure 19: The graph shows the correlation between participants who were more likely to be first to implement a novel IPM strategy and if there were training opportunities available. The line of best fit is represented using a solid black line (n = 27, Correlation Coefficient = 0.44, df = 12, $P < 0.01$).

Discussion

Fourteen chemicals were detected by the antenna of both *D. suzukii* morphs when using EAG. In the laboratory, summer morph *D. suzukii* were repelled by seven repellents, and winter morphs were repelled by five repellents. Consequently, both *D. suzukii* morphs were repelled by four chemicals. In semi-field experiments, three chemicals reduced the numbers of *D. suzukii* caught in traps and eggs laid in sentinel fruit, and 129/08 was effective when a low-dose chemical dispenser was used. In a strawberry crop, two chemicals were found that reduced the overall number of emerging *D. suzukii*, and two chemicals were identified that pushed egg-laying away from chemical dispensers. These results are one of the first semi-field demonstrations of a synthetic formulation that reduced *D. suzukii* oviposition.

Synthetic repellents are historically used for protection against blood-feeding insects (Panthawong *et al.*, 2020). In sub-Saharan Africa, plants are used in push-pull control strategies (D'Annolfo *et al.*, 2020). Plant heterogeneity is employed to reduce pest numbers (Root, 1973; Willis and McElwain, 2014); however, large areas of growing space are taken up by plants. Synthetic chemicals could offer growers an alternative strategy when growing space and land costs are at a premium. Furthermore, synthetic chemicals were shown to be effective at reducing the numbers of *Lygus rugulipennis* Poppius (Heteroptera: Miridae) in strawberry crops (Fountain *et al.*, 2021). Currently, there are no commercial repellents available for horticultural pests (Nilsson *et al.*, 2012).

Chemicals were discussed in the previous 2020 AHDB report.

Two chemicals were reported that reduced oviposition in the strawberry crop. However, additional research should be conducted on the chemical's mode of action. The chemicals may act as 'true' repellents where a pest species is orientated away from an odour source (Dethier *et al.*, 1960). However, adult *D. suzukii* would need to be tracked to determine if the chemical was a true repellent. For example, the software EthoVision could be used (Noldus, 2021). Alternatively, the chemicals may act as oviposition deterrents. This mode of action would reduce *D. suzukii* oviposition but not push the pest from the crop (Kennedy, 1947). Lastly, reduced emergence could be caused by embryo lethality; however, embryo lethality and lengthened development can appear similar in the short term (Bräcker *et al.*, 2020).

The results gathered from the semi-field experiments may offer growers short- and long-term advantages. In the short term, growers would be more conscious of the obstacles surround *D. suzukii* winter morphs. In the long term, new technology could be available to growers.

Although, basic substance approval would need to be sought by the Chemical Regulation Division for commercialisation (HSE, 2020; European Commission, 2021).

Additional experiments should be undertaken to determine the effect of these repellents on pollinators, natural enemies, and beneficial predators. Furthermore, careful consideration should be given to the deployment of the repellents. *Drosophila suzukii* vulnerable crops are grown in a range of environments, structures, and landscapes. For example, strawberry crops are grown outside, in polytunnels, or glasshouses (Schöneberg *et al.*, 2021). In addition, chemical plume dispersion is affected by temperature, humidity, light, and wind (Fares *et al.*, 1980). Therefore, repellent dispensers should be placed in areas that maximise *D. suzukii* contact with odour plumes.

The questionnaire was conducted to identify respondents knowledge, attitudes, and practices on UK farms. Previous questionnaires have been undertaken and have focused on push-pull strategies or *D. suzukii*. However, these data were collected in Africa, making them difficult to compare to the UK market. Moreover, the *D. suzukii* surveys were broad and did not focus on the implications of novel control strategies which is often slow despite technical advancement (Mottaleb, 2018). However, IPM risk was shown to becomes acceptable when there are no effective control strategies (von Helversen and Rieskamp, 2020). Understanding grower motivation is therefore critical for product development (World Bank, 2008). The results gathered in the questionnaire represent a framework for determining the best methods of overcoming difficulties in adopting novel technology in both *D. suzukii* and could be applied to other horticultural pests.

Reduced awareness of *D. suzukii* winter morphs was reported on smaller farms or farms with eight or more crops. To create a workable income, farms often diversify their portfolios (McElwee and Bosworth, 2010). For example, pick your own, farm tourism, or growing multiple crop species. Consequently, growers have a broader knowledge, and less educational time is spent per crop (Larson *et al.*, 2016). Despite this, diversified farms were reported to produce more food and had larger profit margins (per acre) (Montgomery, 2012). However, this broad knowledge can lead to unfamiliarity with upcoming novel IPM strategies (Creissen *et al.*, 2021). For example, participants were more interested in a year-round control strategy if they were aware of *D. suzukii* winter morphs and highlighted the importance of grower education.

Grower interest in control strategies increased when training was given (Meijer *et al.*, 2015). However, a decrease in IPM uptake also occurred when growers fully understood the risks associated with the strategy (Grieshop *et al.*, 1988). Alternatively, the method and type of grower education may be more important. Social learning (education from peers) was reported to reduce barriers to adopting novel IPM strategies (Garbach and Morgan, 2017; Chen *et al.*, 2021). Furthermore, combining social learning and discussions with scientific advisors was determined to aid grower understanding and reduce the time between IPM development and implementation (Garbach and Morgan, 2017). For example, peer teaching reduced organophosphate use by 75 % in Californian pear growers (Warner, 2006). However, no standardised methods were used to calculate the role of education in the augmentation of agricultural output, and the role of agronomists are infrequently mentioned.

In this questionnaire, participant willingness to uptake a new IPM strategy was not affected by agronomists. However, promoting unproven IPM strategies could be considered unethical (Paudel, 2008), and rigorously tested strategies will be promoted instead. Larger numbers of IPM strategies were reported on farms that spoke to their agronomist more frequently. Agronomists have social and environmental responsibilities, which may explain the increased number of IPM strategies implemented (Mukhametzhanova, 2019). Conversely, five or fewer IPM strategies were implemented if their agronomist was sourced as an independent contractor. Therefore, it is plausible that independent contractors spend longer educating their growers (Paudel, 2008). Alternatively, independent contractors could have been employed on farms with low pest populations; however, the analysis could not confirm this due to low replication numbers.

PPPs and good hygiene were the two IPM strategies most frequently used by questionnaire respondents. However, good hygiene is often ineffective when used alone, and emergency approval of PPPs consistently occurs in the UK when *D. suzukii* cannot be controlled with traditional authorised products (Haye *et al.*, 2016; HSE, 2020). These responses may explain why 73 % of questionnaire respondents were interested in an alternative push-pull control strategy that does not use PPPs. However, whilst alternative non-pesticide strategies may be introduced, a good IPM strategy may include PPPs.

Future Work

The work package for this PhD has been completed, and two repellents were identified. However, a repellent should not be used alone. Commercially available attractants and repellents identified in this PhD could be combined to create a push-pull control strategy. Field trials could be undertaken, emulating Fountain *et al.* (2021), to compare repellents used alone, attractants used alone, a control plot, and a push-pull control strategy. Additional research could be conducted on the effect of the repellents on beneficial insects. These may include multiple natural predators and pollinators.

Conclusions

- In the laboratory, fourteen chemicals were detected by the antenna of *D. suzukii* summer and winter morphs, and four chemicals were identified in laboratory bioassays that repelled *D. suzukii* summer and winter morphs. These chemicals were taken forward for semi-field testing.
- Three chemicals reduced the numbers of *D. suzukii* caught in traps and eggs oviposited in sentinel fruit in semi-field experiments with no competing fruit. One chemical 129/08 was effective when a low-dose chemical dispenser was used. It was determined that *D. suzukii* summer and winter morphs exhibit similar behavioural choices but can behave differently in response to some chemicals.
- In a strawberry crop, two chemicals were reduced the overall number of emerging *D. suzukii*, and two chemicals were identified that pushed egg-laying away from chemical dispensers.
- The questionnaire ascertained that training opportunities should be given to early adopters of novel IPM strategies. Furthermore, the mechanics of a novel strategy were shown to be essential. Moreover, grower-led training should occur once novel IPM strategies have been established on a small number of farms.
- The education of individuals in the horticultural industry was determined to reduce the time between strategy design and application.

Knowledge and Technology Transfer

Knowledge Gained – Year 4 2020-2021

- November 2020: How to be a critical reader
- November 2020: English Grammer into context
- November 2020: Preparing for your PhD final viva
- June 2021: Statistical analysis using SPSS
- July-August 2021: How to undertake a cost-benefit analysis

Events Attended – Year 4 2020-2021

- September 2020: Online Presentation – Royal Entomological Society – The Smell of Success: Semiochemical Manipulation of Insect Pests
- November 2020: Online Presentation – AHDB Soft Fruit Day
- January 2021: Crops PhD Conference 2021
- February 2021: Online Presentation – Tree Fruit Day
- July 2021: Online presentation – CTP Summer Event
- September 2021: Online Presentation – Chemical Ecology and Sustainable Development South Africa Conference
- Ongoing: NIAB EMR Seminar Series
- Ongoing: University of Greenwich Seminar Series

Awards – Year 4 2020-2021

- January 2021: Second place for the 'Best Final Year Presentation' at the AHDB Crops PhD Conference
- April 2021: Highly Commended Poster (Postgraduate Researcher Excellence Awards)

Other – Year 4 2020-2021

- 2020: Working as part of the technical team for RES special interest group: The Smell of Success: Semiochemical Manipulation of Insect Pests
- 2018-2021: Sitting on the research and Enterprise as the PhD student representative at Greenwich University
- 2020: Attended Buckingham Palace as part of the University Group (The Queens Anniversary Prizes for Higher and Further Education)
- 2020: Taken part in CTP-FRC advertising campaign
- 2021: Completed assessment for PA9

- 2021: Featured in ‘The Grower,’ The Harper Adams Blog, and the University of Greenwich website

- 2020: Work Placement at Berry Gardens
    - Logistics
    - Sales
    - Category Management and Marketing
    - Technical Team
    - Warehouse

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