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Date project commenced:	01 April 2021
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DISCLAIMER

[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

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GROWER SUMMARY

Objective 1. Continued National Monitoring of the populations of D. suzukii in Scotland and England

Task 1.1. National Monitoring in England and Scotland (NIAB, JHI, NRI)

Headline

- *D. suzukii* numbers at NIAB EMR in 2021 overall, were similar to the catch numbers of 2015 and 2016 (Jan-Oct). 2021 did not express an activity trend closely associating to any other years until late July; 2017 (from Jul-Oct) and 2020 (Jul-Aug, and Nov).
- As with previous years at NIAB EMR, unprecedented peaks in trap catches occurred in conjunction with uncharacteristic peaks in temperature.
- *D. suzukii* numbers in Scotland in 2021 were higher than previous years.

Background and expected deliverables

Since the first detection of *D. suzukii* in the UK in 2012, populations of the pest have continued to rise in most regions of England. In contrast, populations in Scotland, in which the pest was first detected in 2014, have been slow to increase. To monitor the pest, modified Biobest traps using the Char Landolt bait system were deployed in a range of commercial and wild crops in 2013 at 14 sites across the UK.

In collaboration with Berry Gardens, in 2017 and 2018, the main fruit growing regions of England were monitored by 57 traps across 9 farms (Kent, Surrey, Herefordshire, Staffordshire, Northamptonshire, Yorkshire and Norfolk) and 40 traps on 4 farms in Scotland.

In 2019, monitoring was reduced to maintaining 10 traps in England at NIAB EMR and 3 traps in Scotland at JHI, both including one wild area. Monitoring data is summarized monthly from both institutes and reported to the project team at project meetings and is disseminated to growers and other stakeholders at regular intervals. Although there is a reduction in the number of monitoring traps, NIAB EMR and JHI were still able to provide the AHDB with updates on pest dynamics which in turn are used to alert growers to key SWD population events.

Predictive models have been developed using historic trap catch data collected within this objective coupled with environmental information. The models have been successful in predicting first spring female peak (93.3% accuracy), SWD presence / absence (90.2%

accuracy), first summer peak (83.1% accuracy), and female fecundity (76.1% accuracy). Modelling can also predict female activity based on male activity (83-87% accuracy) and time required to reach a % value of SWD population size (72-99% accuracy). These weather-dependent predictive tools could be further improved with the addition of more SWD data, in particular fecundity.

Summary of the project and main conclusions

At NIAB EMR, the activity-density of adult *D. suzukii* in the monitoring traps was lower in the spring (March - May) 2021 (Figure 1.1.1 pink line) compared to 2019 and 2020. This was likely caused by a prolonged cold winter and spring. Numbers of *D. suzukii* caught in the traps were lowest during the period of peak fruit production and in 2021 which is consistent with previous years. In July, 2021's trap catch trend more closely followed 2020's and 2017's (Figure 1.1.1 red line) until mid-August, and continuing from then, 2021 only continues to follow 2017's trend through to the midst of autumn (mid-October), where it has sharply risen in November.

In Scotland, average peak trap catches have varied between years, and are typically 10-40-fold lower than numbers collected at NIAB EMR. The pattern of abundance is similar between years, with insects appearing in traps in August-September, increasing to a peak in October-November, then decreasing to low values December-January. Winter/spring catches are low with very few insects trapped. Highest peak catches were obtained in 2021(*c.* 35 per trap). There is an indication that trap catches at the Hutton site might be increasing in 2019-2021 compared with earlier years. However, this may be a local finding.

For both Scotland and England, there continues to be a general year-on-year increase in annual mean trap catch, except for the year 2020 where a decrease of ~14 SWD per trap was recorded at JHI compared to 2019. At NIAB EMR trap catches rose year-on-year until 2019.

Data has been collated throughout the reporting period and regularly sent to AHDB.

Action points for growers

- Be aware of AHDB communications with alerts to key SWD monitoring events.
- Continue to monitor adult SWD in hedgerow and cropping areas.
- Monitor for adult *D. suzukii* presence and fruit damage throughout the season, particularly in Sept/Oct when abundance is highest

Objective 2. Develop and optimise a push-pull system using repellents, and attract and kill strategies

Task 2.1. Evaluating the efficacy of repellents to protect cherry and raspberry fruit from SWD oviposition. (NIAB & NRI)

Headline

- Repellent 129/08, formulated in slow-release dispensers, has been shown previously to reduce emergence in polytunnels.
- The same formulation was tested for efficacy against *D. suzukii* in a strategic cherry orchard and a commercial raspberry crop in a replicated field trial in 2021
- There was no significant difference between numbers of *D. suzukii* emerging from treated and control fruit at any time point in either crop.

Background and expected deliverables

Push–pull is a strategy for controlling agricultural pests, typically using a repellent plant to "push" the pest out of the target crop towards an attractant acting as the "pull". Work conducted by NIAB EMR and NRI CTP student, Christina Conroy, led to identification of several compounds which might repel *D. suzukii*. Two of the compounds tested reduced numbers of larval *D. suzukii* emerging from fruit at distances of over 6 m in polytunnels. The objective of the trials described below was to test one of these repellents, coded 129/08, in open field trials. If effective, the expected deliverable from this work would be a repellent formulation that could be incorporated into a push-pull system, alongside existing attractants, to reduce damage by *D. suzukii* in commercial crops.

Summary of the project and main conclusions

The repellent that was most effective over the largest distance in Christina Conroy's work (129/08) was taken forward to tests efficacy within a strategic cherry orchard and a commercial raspberry crop. Dispensers were deployed within blocks of cherry trees or raspberry canes at flowering, a minimum of 1 month prior to first fruit assessment. For cherry, the first assessment was taken at white fruit stage and for raspberry at 1st commercial pick. Fruit samples were collected from the central area of treated and untreated blocks, where no 129/08 dispensers were deployed. The number of larvae were extracted using the sugar water method and larval counts compared between treated and untreated blocks.

For the cherry trial, there was an interaction between assessment number and treatment on number of larvae recovered. However, there was no significant difference at any assessment in either crop.

An effective repellent has been identified in small-scale field trials, but this needs to be optimised before it can be implemented in commercial crops. Additional work is required to confirm correct densities of deployment, release rates and timing of deployment. The efficacy of the repellent in combination with an effective ‘pull’ device, such as a trap, should also be a priority for investigation.

Action points for growers

There are no actions at this point.

Task 2.2. Investigating the potential of precision monitoring to reduce fruit damage in the neighbouring crop by reducing numbers of overwintering D. suzukii (NIAB).

Headline

- From September 2019 to March 2022 we investigated whether implementation of precision monitoring in winter refuges can reduce the winter form of *D. suzukii* and numbers migrating into the neighbouring soft fruit crop during the subsequent cropping season.
- Sentinel fruit traps were deployed spring 2020 and 2021 of the trial and showed some evidence to suggest precision monitoring can reduce the incidence of *D. suzukii* egg laying in the neighbouring soft fruit crop.
- Data also showed where there was precision monitoring (both woodlands and neighbouring soft fruit crops), fewer *D. suzukii* were caught in RIGA monitoring traps compared to untreated (control) equivalents most assessments during the two and a half year trial. However this difference was only statistically significant when catches of female *D. suzukii* were analysed and only on 4 assessments out of the 41 made.
- Analysis of precision monitoring trap position in 2020 found traps positioned on the woodland perimeter nearest the crop caught significantly more male *D. suzukii* than within the main woodland during summer, autumn and winter.
- We found evidence to suggest the more favourable vegetation surrounding traps is to *D. suzukii* and the more coverage, the more *D. suzukii* were caught.
- Bramble and ivy were the only species found to have a significant positive influence on catches of *D. suzukii*, during summer and autumn assessments respectively.

Background and expected deliverables

Alongside commercially grown fruit, *D. suzukii* utilises wild fruits and habitat where it can find food and a shelter year-round (Grassi et al, 2011). Such habitats provide a source of *D. suzukii* at the beginning (winter form) and throughout the crop growing season (summer form), which migrate into crops. This is supported by the institute monitoring (Objective 1), which shows high activity peaks of *D. suzukii* in woodlands at NIAB EMR during late autumn/early-winter when there is reduced availability of commercial and wild fruit.

From October 2019 to March 2020 we investigated whether implementation of precision monitoring in winter refuges can reduce the winter form of *D. suzukii* and numbers migrating into the neighbouring soft fruit crop during the subsequent cropping season.

The main aims were to determine whether:

- Precision monitoring for the *D. suzukii* winter morph can reduce the incidence of fruit damage in the neighbouring crop in spring.
- Continued precision monitoring in woodland winter refuge habitat during the growing season can maintain protection against *D. suzukii* fruit damage in the neighbouring crop.
- Traps can be positioned more strategically to optimise catches of *D. suzukii.*

Summary of the project and main conclusions

In October 2019, a grid of 64 precision monitoring traps, spaced at 8 metre intervals, were deployed in a small isolated pocket of woodland on 6 soft fruit farms in Southeast England. Also on each farm, a second similar sized pocket of woodland with no precision monitoring traps, serving as an untreated control was assessed. A commercial RIGA monitoring trap was deployed in each woodland and respective neighbouring crop to monitor and compare *D. suzukii* population numbers throughout the trial. In addition, sentinel fruit was deployed in spring 2020 and 2021 to monitor *D. suzukii* egg laying. The trial also investigated whether precision monitoring traps can be positioned more strategically according to surrounding host vegetation and abiotic factors, to optimise *D. suzukii* catches, hence establishing a more targeted approach which would reduce labour in the maintenance of the traps.

Sentinel fruit deployments showed some evidence to suggest precision monitoring for the *D. suzukii* winter morph can reduce the incidence of fruit damage in the neighbouring crop. For 6 out of the 8 sentinel fruit deployments (April to June 2020 and 2021 combined) fewer *D. suzukii* were counted emerging from fruit deployed in treated woodlands and neighbouring soft fruit crops compared to untreated (control) equivalents. However, this difference was only statistically significant on 2 occasions. *D. suzukii* numbers emerging from fruit was low in general, probably due to competition from other Drosophila spp. which emerged from the same fruit in much higher numbers both years.

Overall fewer male and female *D. suzukii* were caught in monitoring traps in woodlands treated with precision monitoring and their neighbouring soft fruit crops compared to untreated equivalents, but the difference was inconclusive. Approximately half the number of adult *D. suzukii* (males and females) and adult female *D. suzukii* were caught by RIGA monitoring traps in treated woodlands and neighbouring crops compared to control equivalents, however this difference was not statistically significant. For 31 out of the 41 trap counts made at regular intervals during the trial, fewer adult *D. suzukii* were caught in treated crops compared to control crops, but the differences were not statistically significant. The difference was only statistically significant for catches of female *D. suzukii* at 4 trap counts, each made late-winter/early-spring (2020 and 2021). Between June and October 2021, fewer adult *D. suzukii* were caught in treated crops compared to control equivalents 5 out of the 6 assessments. During the same period in 2020, fewer adult *D. suzukii* were also caught in treated crops 5 out of 7 assessments.

Fewer *D. suzukii* were caught in the 2nd year of the trial (2021) compared to the 1st (2020), however it is difficult to conclude if this was due to continued precision monitoring. The annual catches between treated woodlands and their neighbouring soft fruit crops compared to untreated equivalents was not statistically significant. Other factors also influence *D. suzukii* population levels. These include winter temperatures affecting overwintering survival of adults, which were slightly lower in 2021 compared to 2020, particularly the first half of the year.

Analysis of precision monitoring trap position in 2020 found traps positioned on the woodland perimeter nearest the crop caught significantly more male *D. suzukii* than within the main woodland during summer, autumn and winter. We also found if traps were positioned amongst bramble and ivy, more *D. suzukii* were caught.

Action points for growers

- Monitor for *D. suzukii* in and around soft fruit crops year-round to predict potential incursions.
- When deploying monitoring trap placement, growers should consider *D. suzukii*'s preference for wild host species such as bramble and ivy.

Objective 3. Develop bait sprays for control of *D. suzukii*

Task 3.4_1 Determine the effect of baits in combination with reduced dose insecticides on SWD control in cherry

Headline

- In cherries, weekly alternating dilute applications of Tracer at 10 ml in 40L per ha and Exirel at 36 ml in 40L per ha, combined with Combi-protec or molasses baits, were as effective in controlling *D. suzukii* numbers (number of larvae extracted from fruit) as full field rates of the same insecticides applied at 250 ml (Tracer) or 900 ml (Exirel) in 500L per ha.
- This was a reduction in insecticide application of 96%, with the same *D. suzukii* control effect.
- Control of *D. suzukii* was equally good with the full field and new reduced Tracer rates without bait.
- If molasses bait with low insecticide rates in low volume applications are used instead of new Tracer and existing Exirel field rates in high volume applications, the savings in materials and total spray application costs are around 50%.

Background and expected deliverables

D. suzukii phagostimulatory baits could improve the efficacy of insecticides or minimise the dose of insecticide required. The use of baits is expected to improve *D. suzukii* control efficacy of insecticides with the potential to reduce application rates and improved efficacy of a wider range of insecticide types, leading to reduced risk of pesticide residues and resistance. In a series of laboratory- and field-based assays we tested commercially available and novel baits for attractiveness to *D. suzukii*, toxicity when combined with a low dose of insecticide, and finally, ability to prevent egg laying.

In 2020, small-scale replicated field trials were performed on raspberry held within insect proof mini poly tunnels. In these trials, artificial inoculations of *D. suzukii* were made and treatment efficacy was assessed on the number of *D. suzukii* larvae recovered from fruit. We found that dilute applications of Tracer at 8 ml in 40L per ha and Exirel at 36 ml in 40L per ha, combined with Combi-protec or molasses baits, were as effective in controlling *D. suzukii* numbers as full field rates of the same insecticides applied at 200 or 900 ml in 500L per ha.

Summary of the project and main conclusions

The aims of the 2021 work were to compare the efficacy of weekly alternating applications of Tracer and Exirel in cherries under semi-field conditions when used:

- at current full field rate applications
- at the new reduced rate for Tracer
- in low or reduced concentrations with and without Combi-protec or molasses.

Treatments were applied to cherry trees within a strategic orchard at NIAB EMR. Compartments were constructed to prevent treatment drift between plots. Treatments were applied from white fruit stage and efficacy was assessed on numbers of larvae and adults extracted from fruit sampled from each plot.

Weekly alternating dilute applications of Tracer at 10 ml in 40L per ha and Exirel at 36 ml in 40L per ha, combined with Combi-protec or molasses baits, were as effective in controlling *D. suzukii* numbers as full field rates of the same insecticides applied at 250 or 900 ml in 500L per ha (i.e. a reduction in insecticide application of 96% with the same *D. suzukii* control effect). Control of *D. suzukii* was equally good with the molasses and Combi-protec bait spray treatments. Control of *D. suzukii* was equally good with the full field rate and new reduced Tracer rates without bait. This new rate is expected to reflect upcoming changes in approvals for Tracer. The above treatments maintained good control of *D. suzukii* during the first three assessment weeks of the crop; by the fourth week, the majority of the fruit was over-ripe, resulting in very high level of *D. suzukii* infestation and reduced treatment efficacy.

The application time for the bait sprays was 10% of the full field rate application of insecticide sprays. Compared with untreated control plots, the dilute rates of insecticides reduced *D. suzukii* numbers by about 50%; the inclusion of baits significantly improved this control effect. *D. suzukii* numbers determined from adult emergence in boxes corresponded with larvae flotation tests, although the latter were only 30% of the former. Residues of spinosad and cyantraniliprole in fruit samples taken from the full field rate, new field rate and Combi-protec medium spray plots were below the EU MRLs. No spinosad or cyantraniliprole residues were found in any of the fruit from the bait spray + low rate treatments. Spray deposition coverage was between 5 and 134 times higher in corresponding positions for the full rate application (500 L/ha) than for the Combi-protec + low rate application (40 L/ha), except on leaves furthest from the spray nozzle at the middle tree height (both leaf surfaces) and top of trees (upper leaf surface).

If molasses bait with low insecticide rates in low volume applications are used instead of new Tracer and existing Exirel field rates in high volume applications, the savings in materials and total spray application costs are around 50%.

Action points for growers

- Adjuvants such as Combi-protec can only be used if in combination with approved plant protection products and varies from crop to crop.
- Growers should discuss the use of approved adjuvants in combination with plant protection products with their agronomy provider and adhere to approvals.

Objective 8. *Drosophila suzukii* tolerance to plant protection products

Task 8.1. Investigating the susceptibility of D. suzukii to approved plant protection products (NIAB)

Headline

- Wild strains have been tested for insecticide resistance since 2019.
- There are some differences between 2019 and 2020 in levels of susceptibility, however there is no indication that resistance has developed to the three products tested.
- In 2020, early and late season strains were established from the field in to assess differences in susceptibility.
- Generally, early season strains were more susceptible to the products tested than late season strains.

Background and expected deliverables

Since its arrival to the UK in 2012, PPP control has played a vital role in supressing *D. suzukii* numbers in vulnerable fruit crops. In 2018, an increased tolerance to spinosad was detected in Californian organic raspberries by Gress and Zalom (2018). Flies from spinosad treated areas required 4.3-7.7 times higher dose of spinosad for control than those from untreated areas.

In 2019, laboratory trials were established to identify a baseline level of susceptibility in wild populations of *D. suzukii.* Three wild populations were collected from soft and stone fruit farms in the South-East of England and mass reared in the laboratory. They were established from crops with a known insecticidal input and included two commercial crops and one with minimal inputs. These were compared an unsprayed laboratory strain, which has been in culture since 2013 and is expected to have a very low tolerance to PPP. There were varying levels of susceptibility to three PPPs (lambda-cyhalothrin (Hallmark), cyantraniliprole (Exirel) and spinosad (Tracer)) tested between the three wild populations. Although there was no detection of resistance in the populations we tested, there was an increased level of tolerance in some of the populations to one or more of the insecticides tested.

Summary of the project and main conclusions

In 2020 early season strains were collected from fruit at the end of July. Due to the logistical operations being affected by the pandemic, the early season wild strains took several months to build-up enough flies to execute the bioassays. When looking at the survival probability of the wild strains between years, there was a significant difference between 2019 and 2020 with lower survival in 2020 from all three strains when treated with spinosad and for WS1 when treated with lambda-cyhalothrin. If resistance had been developing in the field populations, we would expect 2020 to have higher survival than 2019. It may be that due to these early season populations being collected early in the growing season they have not been as exposed to insecticides as those collected towards the end of the season, like the 2019 strains.

In 2020 there were some differences in susceptibility between the early and late season strains, often with those collected earlier in the season having a lower tolerance to the PPP. This indicates that an increase in tolerance develops through the season. However, it is likely that cold winters reduce the survival of the tolerant lines as the result of a fitness cost, often associated with resistance mechanisms.

Analysis of the LC50 values from 2019-2021 has highlight some changes over time in susceptibility but to date resistance has not been detected at the three sites. In many of the interactions, the later years have a higher susceptibility than the earlier years; the opposite of what would be observed if resistance had developed.

Action points for growers

- Growers should consult their agronomist for up-to-date approvals prior to making insecticide applications.
- Where possible, growers should rotate between different modes of action to prevent insecticide resistance build-up.
- If growers suspect resistance has occurred on their farms, please alert researchers at NIAB EMR.

SCIENCE SECTION

Objective 1. Continued National Monitoring of the populations of *D. suzukii* in Scotland and England

Task 1.1. National Monitoring in England and Scotland (Yrs. 1-4; NIAB, JHI, NRI)

Introduction

Since the first detection of *D. suzukii* in the UK in 2012, populations of the pest have continued to rise in most regions of England. In addition there are more frequent reports of the pest being detected nationally and in Ireland. In contrast to the general UK trend, populations in Scotland have been slow to rise, and only since 2017 and 2018 have some sites seen an increase in incidence since 2014. It is not known if populations in Scotland will increase or whether factors, including climatic conditions, weather patterns and agricultural practices will adversely affect the *D. suzukii* population there. In the West Midlands and East Anglia, the numbers were reasonably low, but locally *D. suzukii* impacted fruit production and fruit damage in the latter regions.

To enable the industry to assess risk of fruit damage, distribution, and dynamics of the pest, we have continued to monitor how *D. suzukii* populations respond over time (since 2013) in Scotland (at the James Hutton Institute) and England (at NIAB EMR). Monitoring began at 14 fruit farms in 2013 in project SF145. In 2017 monitoring was reduced to 57 traps on 9 farms in England, and 40 traps on 4 farms in Scotland. From 2019 the number of traps was reduced again to 10 traps at NIAB EMR in England and 6 traps in Scotland at two sites. One wild area was monitored at NIAB EMR and one in Scotland. This change occurred as it was agreed we had gained a good understanding of the pest dynamics in different crops, which was the original objective, but wished to continue monitoring at a reduced rate. In Scotland during 2019 and 2020, there were issues with trap catch continuity at site 1300, which resulted in data being used solely from JHI (site 1100) for the analysis from 2019 onwards.

In 2020, England data was analysed by a NIAB EMR PhD student to model the effect of proximity of wild populations to crops on trap catches. Annual monitoring data is supplied to the JHI for modelling populations with climatic conditions. Data was provided for 2021 but analysis is yet to be performed and required additional funding. Once these models are available, they would be hosted on the AHDB legacy web site for growers' use.

The data from the reduced monitoring has continued to be used to alert growers of the pest dynamics and contribute towards future pest modelling.

Methods

As of 2021, the 10 traps at NIAB EMR were deployed in the following locations: 4 in cherry, 2 in strawberry, 2 in vineyard grape, and 2 in woodland. Monitoring traps were generally deployed in pairs, one in the centre and one at the edge of each crop and woodland. In Scotland 2021, monitoring data was collected from 3 traps hosted at JHI, deployed in the following locations: 1 in blackcurrant, 1 in blueberry and 1 in a wild area near blackberry. As of July 2021, the blackberry and blackcurrant traps were moved to another location within the same farm and crop type.

For continuity within the national monitoring survey, we have used the modified Biobest trap design and Cha-Landolt bait since 2013. Droso-traps (Biobest, Westerlo, Belgium) were modified with 20 extra 4 mm holes drilled into the top portion of the body of the trap to maximise catches of *D. suzukii*. Adults were captured in a drowning solution, which included ethanol (7.2%) and acetic acid (1.6%) as attractants, and boric acid to inhibit microbial growth. Methional and acetoin (diluted 1:1 in water) were placed into two polypropylene vials (4 ml) with a hole (3 mm diameter) in the lid (to allow the chemical to release and be refilled), attached near the fly entry holes within the trap. The traps were positioned at the height of the main crop (±1 metre).

Adult *D. suzukii* counts were taken weekly during the cropping season and fortnightly during the winter.

Results and discussion

England

At NIAB EMR the mean weekly trap catch has generally risen year on year since data collection began in 2013, however reductions did occur in 2016, 2018, 2020 and have continued to decline until 2022 (up to March) (Figure 1.1.1a). The seasonal variations in trap catches (Figure 1.1. 1b & c) continues to be greatly influenced by temperature fluctuations (Figure 1.1.2). The period of late-autumn to winter months coincides with a depletion in egg laying resources and defoliation of trees. Decreases in trap catches during the summer months are likely due to traps being less attractive than the crop and not because there is a decrease in the numbers of *D. suzukii*. Figure 1.1.3 shows the variation in trap catches for the NIAB EMR site from 2013 to the 2022 (March) between the cropping and wild areas. Since data collection began in 2013, trap catches in the wild habitats continue to exceed those in cropping areas significantly, as demonstrated by the yearly continuation of peaks in autumn-winter periods.

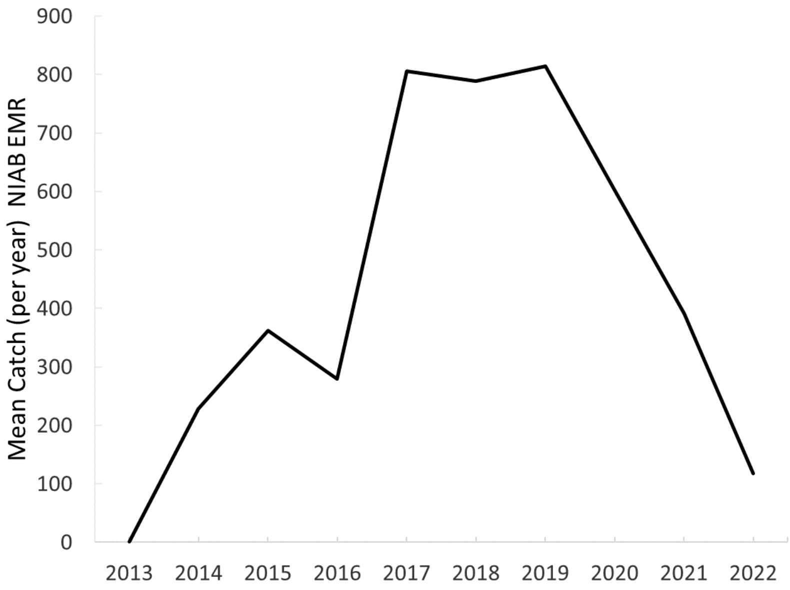
At NIAB EMR in 2018 (purple dotted line), patterns of adult *D. suzukii* catches in the traps followed previous years. Catches in the winter of 2017/18 (red line) were 50% lower than 2015/16; potentially explained by a milder November and December in 2015 (black line). Peaks in the winter of 2018 were lower than the previous year, 2017, but higher than all other years (Figure 1.1.1 b & c).

In 2019 (yellow line) and 2020 (green line), monitoring at NIAB EMR showed higher catches in the spring (March-May) compared to all other years (likely due to a warmer spring), and had both displayed a peak in late May, which coincided with high temperatures in that month (Figure 1.1.2). September 2020 recorded the highest trap catch to date, again correlating to higher than average temperatures in that month. October 2019 and 2020 were relatively cold leading to a drop in trap catches with the usual activity peaks in November as *D. suzukii* returned to overwintering habitat. Trap catch peaks during autumn and winter at NIAB EMR in 2020 occurred when the flies were in a reproductive diapause in their winter-form (winter morph). The leaves had fallen from deciduous trees, providing less shelter and a reduced availability of commercial and wild fruit.

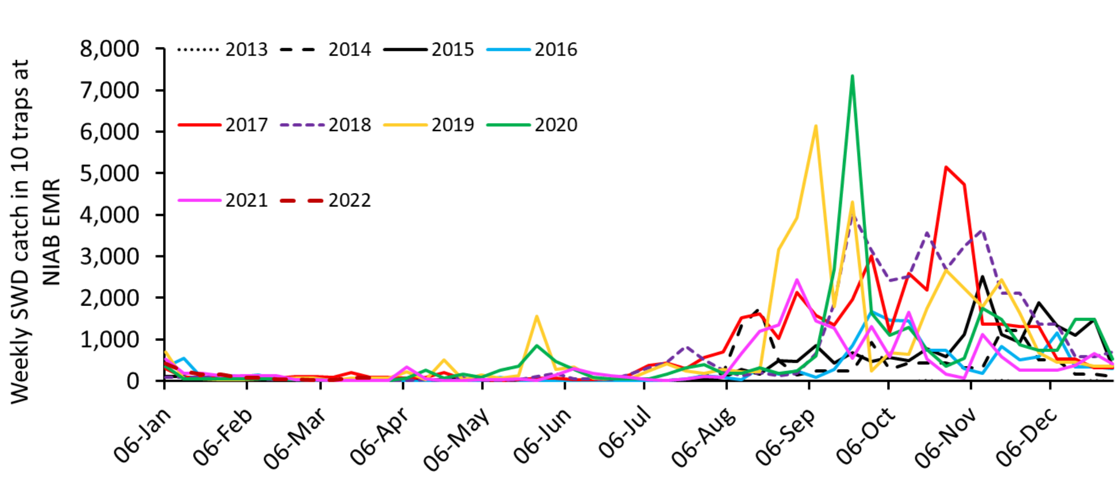
The activity-density of adult *D. suzukii* in the monitoring traps was lower in the spring (March - May) 2021 (pink line) compared to 2019 and 2020. This was likely caused by a prolonged cold winter and then also spring, similar to spring 2018 & 2020 (Figure 1.1.2). These cooler temperatures decrease the opportunity for *D. suzukii* to be active, and hence, captured in the monitoring traps. Numbers of *D. suzukii* caught in the traps (Figure 1.1.1 b & c) were lowest during the period of peak fruit production and in 2021 during this time, it did not express an activity trend closely associating to any other years until late July. Trap catches did increase to an accumulative volume *of D. suzukii* similar to 2020 and 2018 (end of August) and overall similar to 2015 and 2016 (November) (Figure 1.1.1a). In July, 2021's trap catch trend more closely followed 2020's and 2017's (red line) until mid-August. 2021 only continues to follow 2017's trend through to mid-October, where it has sharply risen in November much like 2020 and 2016. The highest peaks of activity for autumn (September – November) compared over the recorded years, was seen in late October of 2018, in early September of 2019, and the highest peak yet, in late September of 2020 (Figure 1.1.1b & c). From November to December the highest peak occurred in 2017, which is over 20% more than 2018 and almost double the trap catch compared to 2020 (Figure 1.1.1b & c).

Annual means per trap at NIAB EMR, although influenced by temperature, gradually rose until 2019 with intermittent peaks and troughs; Mean per trap; 2013 = 1, 2014 = 229, 2015 = 362, 2016 = 280, 2017 = 806, 2018 = 789, and 2019 = 815, 2020 = 603, 2021 = 392, 2022 = 118 (Jan-Mar) (Figure 1.1.1a).

a)



b)



c)

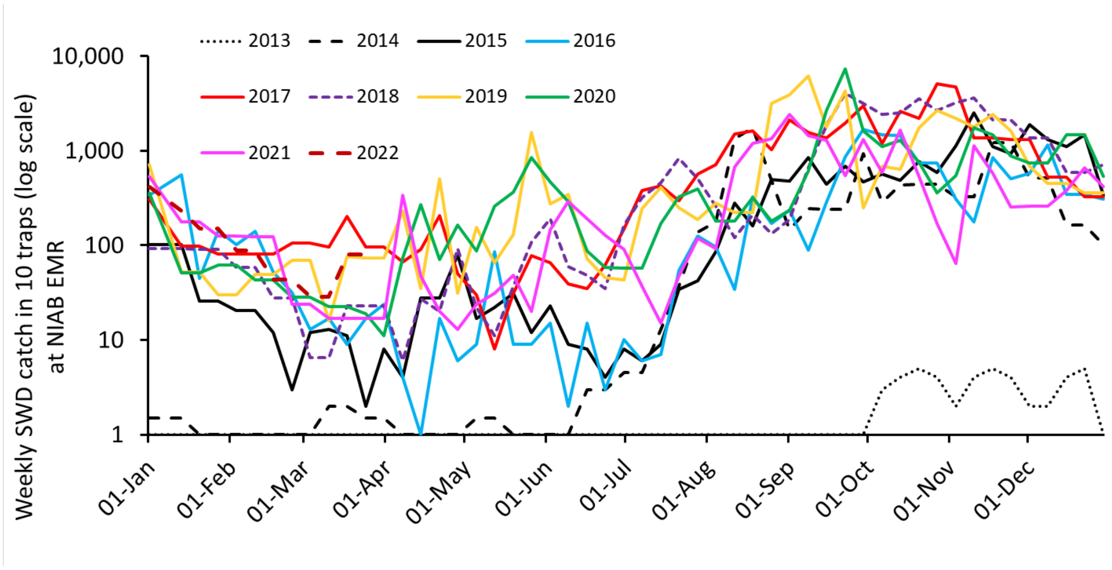


Figure 1.1.1: a) Mean weekly trap catch per trap each year (2013-2022) in NIAB EMR traps. b) mean numbers of adult *D. suzukii* catches per trap in 2013-2022 raw data and c) same data, plotted on a $\log_{10}$ (n + 1) scale. Please note that y-axis varies in graduation and maximum values.

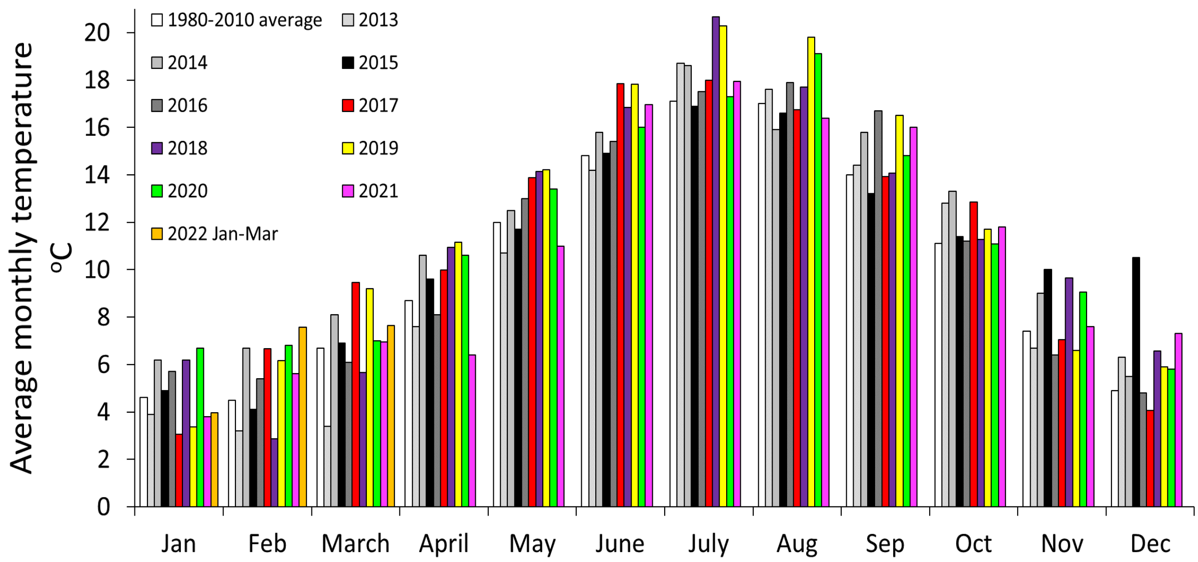


Figure 1.1.2. Comparison of the mean monthly temperatures between years at NIAB EMR.

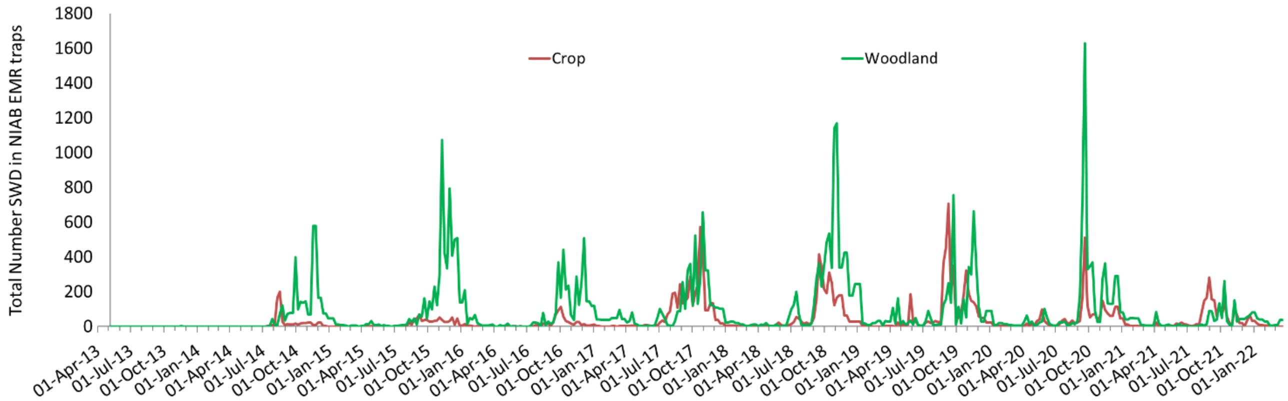


Figure 1.1.3**.** Mean numbers of *D. suzukii* adults per trap in crop (brown line) and woodland (green line) at the NIAB EMR site from 2013 to 2022.

Scotland

From 2019 onwards, it was necessary to change the selected indicator traps that are used to represent the monitoring data for Scotland. This was due to unavoidable logistical challenges experienced by the grower at site 1300. Therefore, three existing traps at site 1100 were selected to represent the Scottish SWD monitoring. These were the traps that consistently caught the highest abundance of *D. suzukii* at this site. Figure 1.1.4 shows the mean catch per trap using data from the 40 original monitoring traps at four Scottish sites for years 2013 to 2018 and from the three indicator traps at site 1100 for years 2019-2021.

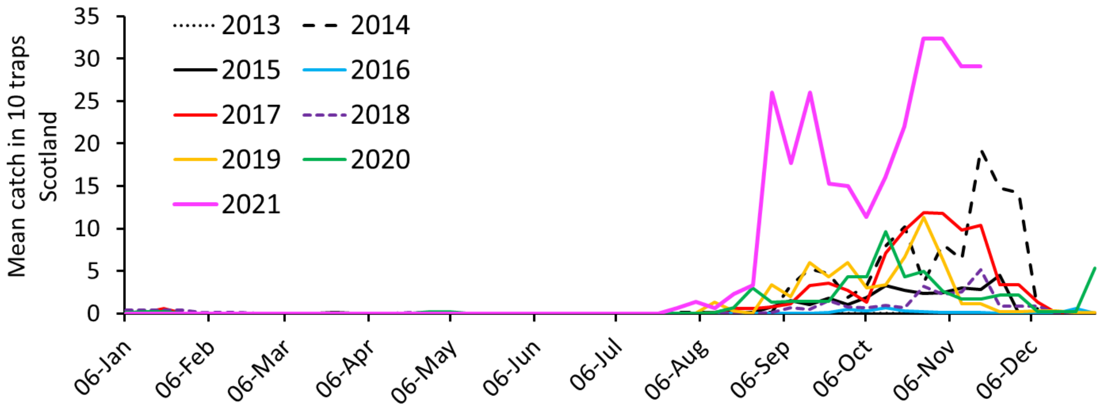


Figure 1.1.4. Mean catch of *D. suzukii* in Scotland from all four Scottish sites for years 2014 to 2018 and 3 traps from site 1100 from 2019-2021.

In general, catches of adult *D. suzukii* in the three traps followed previous years with very small catches of SWD from January until late July (Figure 1.1.4). However, the monitoring results from site 1100 suggest that abundance is increasing at that site (Figure 1.1.4 and Figure 1.1.5), particularly in 2021, when numbers increased earlier and reached up to 100x higher when compared with the lowest catch year (in 2016). This increase might have related to higher-than-average temperatures in September and October 2021 (Figure 1.1.6). Unfortunately, we do not have comparable data for the other monitoring sites for 2019 onwards to determine if this is a local increase at a single site or if this increase is found at other Scottish sites. The abundance of *D. suzukii* in Scotland is still low in comparison to the South of the UK. There is no winter/ spring catch data for 2020/21 as no SWD were trapped from the 24th December 2020 until the 26th July in 2021.

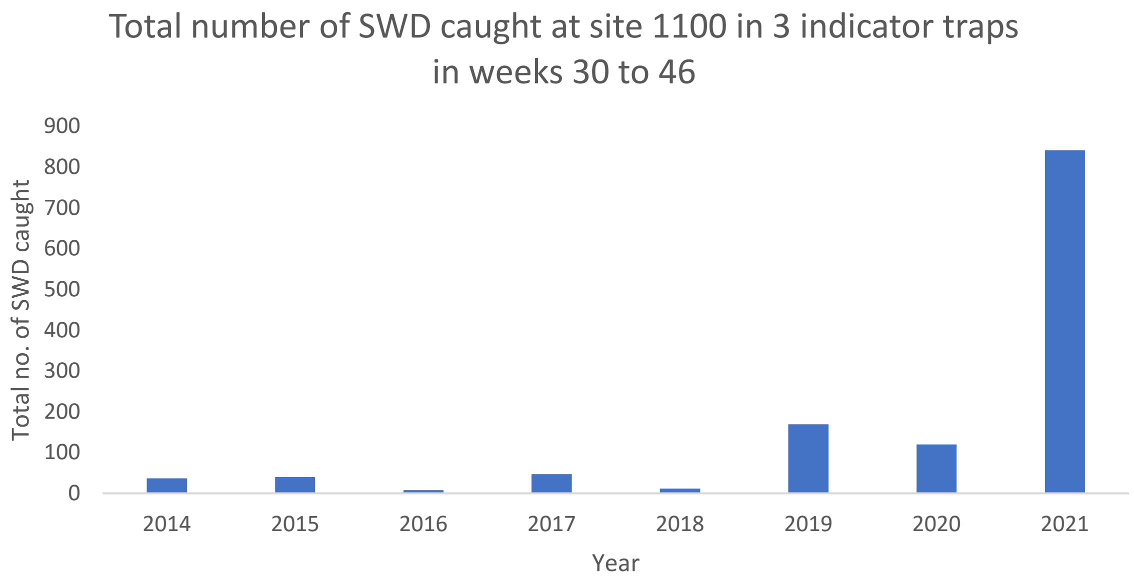


Figure 1.1.5 Total number of *D. suzukii* caught in the three indicator traps at site 1100 in Scotland for the period 2014-2021 during the peak catch period (end of July to mid-November).

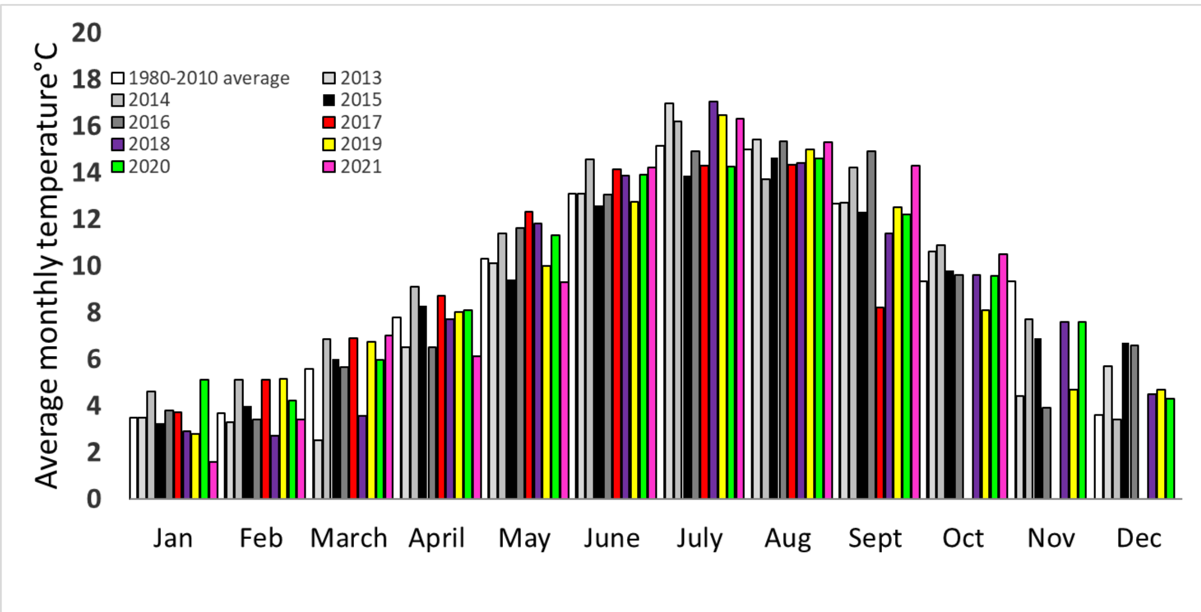


Figure 1.1.6. Comparison between years of the mean monthly temperatures at site 1100.

Conclusions

- *D. suzukii* numbers at NIAB EMR in 2021 overall, were similar to the catch numbers of 2015 and 2016 (as of October). 2021 did not express an activity trend closely associating to any other years until late July. From July, 2021's trend closely related to 2017 (from July-October) and 2020 (July-August, and November).
- *D. suzukii* in Scotland appeared in late July and increased more rapidly than previous years, with peaks in mid-September and again in early November. Average trap catches in the peak period were between four- and 100-fold higher than previous years.
- There continues to be variation in interannual trap catches, at least in the late autumn, probably largely dependent upon temperature.
- September–November coincides with the emergence of the winterform adults, a depletion in egg laying resources (fruit) and defoliation of trees (reduced refugia).
- Decreases in trap catches during the summer months are likely to be due to traps being less attractive than crops and not due to a decrease in the number of *D. suzukii.*

Objective 2. Develop and optimise a push-pull system using repellents, and attract and kill strategies

Task 2.1. Evaluating the efficacy of repellents to protect cherry and raspberry fruit from SWD oviposition.

Introduction

Push–pull is a strategy for controlling agricultural pests (Cook et al., 2007), typically using a repellent plant to "push" the pest out of the target crop towards an attractant acting as the "pull". To develop push-pull against *D. suzukii,* knowledge of the chemical ecology of the pest is required. Since 2008, researchers have gained a better understanding of *D. suzukii' s* attraction to specific odours from fermentation, yeast, fruit, and leaf sources, and the visual cues that elicit long-range attraction (Cloonan et al., 2018). Promising results were reported for a *D. suzukii* push-pull strategy in raspberry, where findings showed an 87.6% reduction of oviposition on raspberry fruit under laboratory conditions and a 57.4% reduction in egg deposition compared to control blocks in the field (Wallingford et al., 2018).

Potential repellents to deter *D. suzukii* laying eggs in fruits or the discourage adults entering the cropping area were investigated in the previous SF 145 project. Other research has focused on geosmin (Wallingford et al., 2016), plant essential oils (Renkema et al., 2016), lime (Dorsaz et al., 2017) and 1-octen-3-ol (Wallingford et al., 2015). To date, only the latter two potential repellents were reported to show efficacy in field tests (Dorsaz et al., 2017, Wallingford et al., 2016). In previous work in SF 145, four compounds, including geosmin and 1-octen-3-ol, were shown to reduce egg-laying by SWD when released next to sentinel fruit in small block, single tree experiments. However, in subsequent experiments, 25 dispensers per cherry tree did not deter *D. suzukii* egg laying.

Based on the above findings and reports in the literature, studies by NIAB EMR and NRI CTP student, Christina Conroy, were conducted between 2017-2020 on several synthetic compounds suggested to be repellent to *D. suzukii.* From electrophysiological studies, bioassays, and field experiments three compounds were shown to be repellent to *D. suzukii* and were taken forward for semi-field testing 2019. In subsequent trials on strawberries in 2020 in experimental polytunnels, two of the three repellents significantly reduced egg-laying by *D. suzukii* at distances of up to 6 m.

The aim of the current work within SF/TF 145a was to test the repellent that was most effective over the largest distance in Christina Conroy's work (coded 129/08) within a strategic cherry orchard and a commercial raspberry crop.

Methods

Cherry

The trial was deployed within cv. Merchant within the 'Carves Leys' strategic cherry orchard at NIAB EMR, Kent (Figure 2.1.1). This orchard received no pest or disease management throughout this trial. The Carves Leys orchard is a mixed orchard consisting of 3 cultivars (Merchant, Simone and Samba) however only Merchant trees were used within this trial. Trees were double rows within 8 m tunnels, with 4 m between rows and 2 m between trees. Ten trees were included in each block (five rows of two trees) and a buffer zone of sixteen trees (eight rows of two trees) were left between blocks. In treated blocks, eight repellent dispensers were hung using paper clips on each of the ten trees. Dispensers were distributed evenly around the tree at varying heights to ensure good coverage. Repellents were deployed within the treated blocks on 07/04/21. The deployment was made prior to bud bust, before *D. suzukii* move into the orchard to feed on extra floral nectaries. In control blocks, no dispensers were deployed.

The crop was monitored weekly to ensure the first assessment was taken at white fruit. The first assessment was collected 07/06/21 with 30 fruits collected per block. Weekly fruit assessments were taken until 28/06/21 at which point the fruit was overripe.

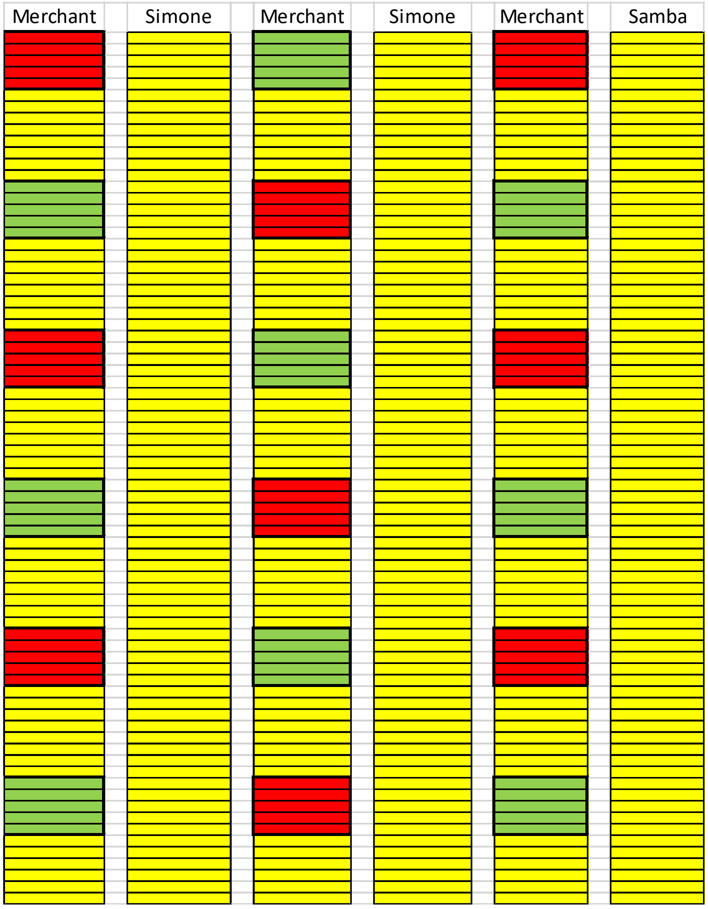


Figure 2.1.1. Varieties (top of figure) and tunnel arrangement in Carves Leys cherry orchard. Trees are assigned to treatment (red) and control (green) blocks of ten trees each. Each lateral line indicates one row of two trees. Eight 129/08 dispensers were be deployed in each tree in test blocks at the start of the trial. Trees not used in experiments (yellow) formed a buffer between test and control blocks.

Raspberry

The trial was deployed in a commercial raspberry crop cv. Nobility at Hugh Lowe Farm, Kent (Figure 2.1.2). Treated and control blocks included 4 rows of potted raspberry, covering 16 x 16 m area, spanning two tunnels wide. Buffer zones of 16 m were left between blocks. Dispensers were hung on support wires with paper clips roughly 1 m above the ground. Eighty dispensers were deployed per treated block with 10 dispensers deployed on each side of the four rows of raspberry. Dispensers were evenly distributed with one dispenser every 1.6 m along the row. Dispensers were deployed 05/07/21 when the first fruit was setting. No dispensers were deployed in the control blocks.

The first assessment was collected 18/08/21 with 20 ripe fruits picked per block. Weekly assessments were taken until 14/09/21.

Figure 2.1.2. Bird's eye view of the tunnels to be used in this trial. Black box indicates the blocks. Smaller green and blue boxes show the control and treated areas respectively.

Fruit collections and assessment- both crops

Fruit was collected from the centre of the blocks and transferred to a ventilated Perspex box (20 x 12 x 8 cm) which was lined with blue laboratory roll. The lids were taped on to prevent contamination of the fruit post collection from the field. After collection, the fruit was transferred to a control temperature room which was maintained at 20°C on a 16:8 light:dark cycle for two days. This ensured that any *D. suzukii* eggs within the fruit had time to hatch into larval stages making larval extraction possible. Fruit was then transferred to the lab, the blue roll removed, and the fruits gently squished to split the fruit skin. This has been found to improve larval detection by facilitating exit from the fruit during extraction. Larvae were extracted using a sugar water solution (170 g white sugar to 1 L of tepid tap water) which was poured over the fruit and allowed to stand for 30 minutes, after which fruit was removed with forceps. The liquid was filtered through a fine mesh to strain the larvae from the liquid. The mesh was then inspected under the microscope and the number of larvae were counted.

Data analysis

Cherry

Numbers of larvae extracted from cherries were entered as the dependent variable in a negative binomial (log link) mixed effects model (Bates et al., 2015). Treatment (129/08 or control) and Assessment Week (1-4) were entered as fixed factors in the model, alongside a Treatment by Assessment Week interaction. Block was entered as a random effect in the model. Significance of terms within the model was assessed through stepwise deletion. Significant differences between individual factor levels of fixed effects were assessed using Tukey's tests on estimated marginal means (Lenth, 2021). All analyses were performed in R V 4.1.0.

Raspberry

Numbers of larvae and pupae extracted from raspberries were summed and entered as the dependent variable in a negative binomial (log link) mixed effects model. Treatment (129/08 or control) and Assessment Week (1-5) were entered as fixed factors in the model, alongside a Treatment by Assessment Week interaction. Block was entered as a random effect in the model. Significance of terms within the model was assessed through stepwise deletion. All analyses were performed in R V 4.1.0.

Results

Cherry

There was an indication of an interaction between Assessment Week (1-4) and Treatment (repellent and control) on numbers of *D. suzukii* larvae emerging from cherries (test of change of residual deviance: $\chi^2 = 7.8$, df = 3, P = 0.051). However, there were no significant differences between numbers of larvae emerging from treated or control cherries in each of the assessment weeks (Tukey's test, not significant at $P<0.05$, Figure 2.1.3). There was no overall difference between numbers of larvae emerging from cherries picked from trees treated with repellents or controls ($\chi^2 < 0.01$, df = 1, P = 0.94).

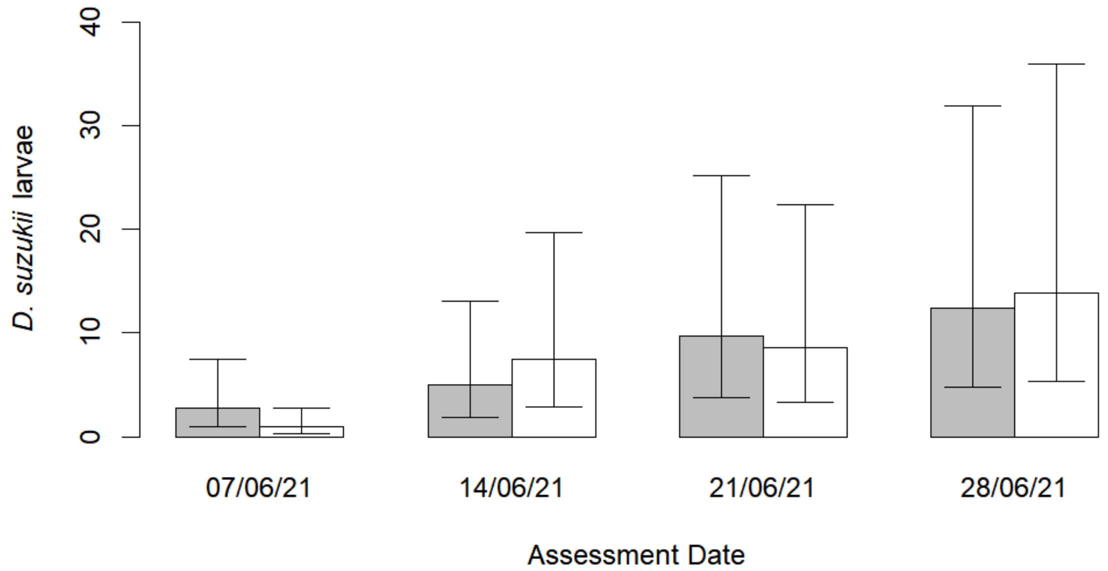


Figure 2.1.3. Mean (± 95% confident intervals) estimated number of *D. suzukii* larvae emerging from cherries picked from control trees (grey bars) and trees treated with 129/08 dispensers (white bars). Estimates of fixed effects have been back-transformed from the generalized linear model.

Raspberry

There was no evidence of an interaction between assessment week (1-5) and treatment on numbers of *D. suzukii* extracted from raspberries (Test of change of residual deviance: χ^2 = 2.0, df = 4, P = 0.73; Fig. 2.1.4). There was also no overall difference between numbers of larvae emerging from raspberries treated with repellents and controls (χ^2 = 0.02, df = 1, P = 0.86).

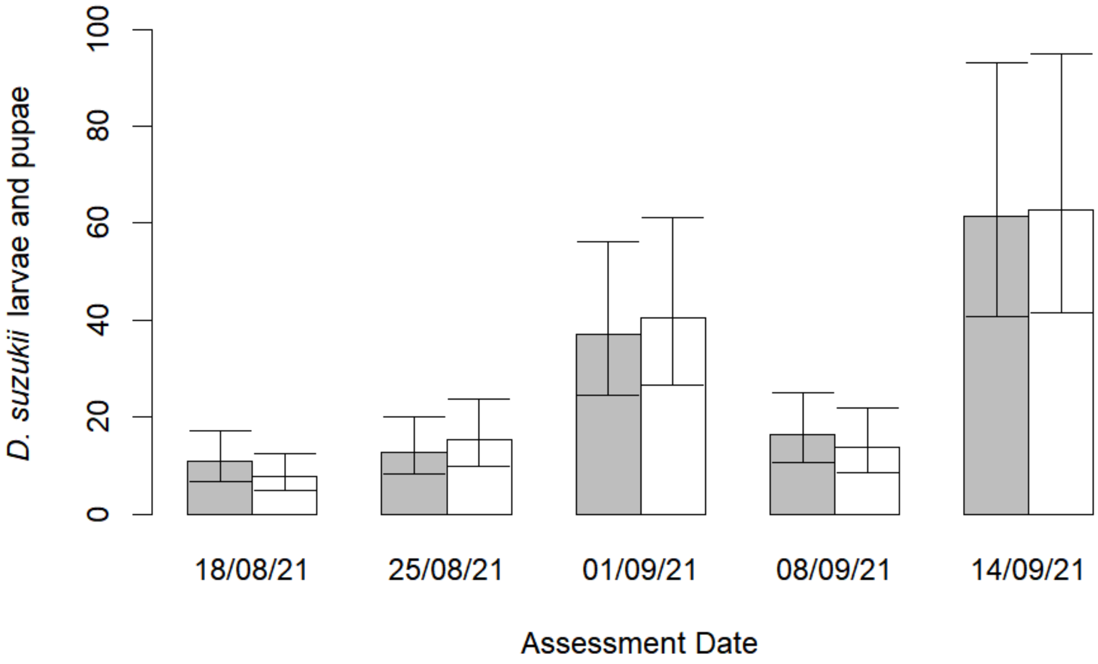


Figure 2.1.4. Mean (± 95% confident intervals) estimated number of *D. suzukii* larvae emerging from raspberries picked from control bushes (grey bars) and bushes treated with 129/08 dispensers (white bars). Estimates of fixed effects have been back-transformed from the generalized linear model.

Discussion

While the efficacy of the 129/08 repellent used has been found to significantly reduced *D. suzukii* oviposition in previous polytunnel semi-field trials, there was no reduction within these field trials. This result may also be due to the high attraction of *D. suzukii* to cherry and raspberry which were used in this trial. In the previous small-scale trials, repellents were deployed within the vicinity of strawberry, which is known to be less attractive to *D. suzukii.* It may be that in the presence of less attractive fruit, the repellent effect is strong enough to repel *D. suzukii* but in the presence of more attractive fruit it is not.

The results gathered by Christina Conroy indicate that 129/08 can repel *D. suzukii* egg laying for a minimum of 6 m in a linear, small canopy strawberry crop. The canopy of cherry trees and the wall-like structure of raspberry canes are very different structures to strawberry crops and overall surface area is significantly bigger. It may be that the density or location of deployment was not optimum for these crop structures. Additional research is required to fully optimise deployment density, location, and release rate of 129/08 dispensers in these crops.

Furthermore, these repellents have been developed to be integrated into a push-pull system and paired with a pull element. There have been several objectives within the SF/TF SWD projects and from PhD student Rory Jones, which have found attractive compounds and trapping attractants that could be paired with repellents to produce an effective push-pull system. It may be that when combined with an attractive pull device, the efficacy of the repellent is enhanced.

Conclusion

- There was no significant reduction in *D. suzukii* larval counts in blocks treated with the 129/08 repellent compared to an untreated control.
- Optimum density, deployment position and repellent release are yet to be determined.
- The efficacy of 129/08 is yet to be tested as part of a push-pull system and this should be prioritised in future research.

Task 2.2. Investigating the potential of precision monitoring to reduce fruit damage in the neighbouring crop by reducing numbers of overwintering D. suzukii (NIAB).

Introduction

Since September 2019 we have been investigating whether implementation of precision monitoring in winter refuges can reduce the winter form of *D. suzukii* and numbers migrating into the neighbouring soft fruit crop during the subsequent cropping season. In October 2019, a grid of 64 precision monitoring traps, spaced at 8 metre intervals, were deployed in a small isolated pocket of woodland on 6 soft fruit farms in Southeast England. Also on each farm was a second similar sized pocket of woodland with no precision monitoring traps, serving as an untreated control. A commercial RIGA monitoring trap was deployed in each woodland and respective neighbouring crop to monitor and compare *D. suzukii* population numbers throughout the trial.

So far data has shown where there is precision monitoring, fewer *D. suzukii* have been caught in RIGA monitoring traps (both woodlands and neighbouring soft fruit crops). Two weeks after precision monitoring trap deployment (early-October 2019) to mid-April 2020, consistently fewer adult *D. suzukii* were caught in RIGA monitoring traps in treated woodlands (and neighbouring crops), compared to untreated equivalents. This difference was statistically significant between respective woodlands. Following redeployment of precision monitoring traps, July 2020 to December 2020, consistently fewer *D. suzukii* were caught in monitoring traps in treated plots compared to control.

To establish whether precision monitoring can also reduce the *D. suzukii* egg laying potential, sentinel fruit was deployed spring, summer and autumn 2020. On 5 occasions in spring 2020 (initiated when the first fecund *D. suzukii* females were caught in monitoring traps), sentinel fruit traps, carrying defrosted raspberries, were deployed in treated and control woodlands and respective neighbouring crops. This was repeated summer and autumn. Numbers of adult *D. suzukii* and other Drosophila species emerging from sentinel fruit were recorded. Results showed very few adult *D. suzukii* emerging from fruit every deployment spring summer and autumn, and much higher numbers of other Drosophila spp. emerging from the same fruit, suggesting other Drosophila spp. were deterring *D. suzukii* egg laying (Shaw et al. 2018) and/or predating *D. suzukii* larvae (Ahmad et al. 2015).

Based on findings from the precision monitoring trial, the purpose of this year's trial was to investigate whether:

- A consecutive year of precision monitoring for the *D. suzukii* winter morph can further reduce the incidence of fruit damage in the neighbouring crop in spring 2021. Using ripening strawberries enable *D. suzukii* egg laying exclusively
- Continued precision monitoring in woodland winter refuge habitat during the 2021 growing season can maintain protection against *D. suzukii* fruit damage in the neighbouring crop

Methods

Trial sites*:* the trial was set up in September 2019 at 6 commercial soft fruit farms (blocks) in Kent and West Sussex. Crops tested include strawberry, raspberry, cherry and one wine grape. Since June 2021, the trial ended at block 4 (wine grape).

Treatments: Each block was divided into two plots (Figure 2.2.1):

1. A treatment plot, consisting of a small isolated pocket of woodland (*D. suzukii* winter refuge habitat) containing a grid of 64 precision monitoring traps, spaced at 8 metre intervals (shape dependent on woodland topography), adjacent to a soft fruit crop.
2. A control plot (>50 m from the treated plot), consisting of a second small isolated pocket of woodland, containing no precision monitoring traps, adjacent to a separate soft fruit crop.

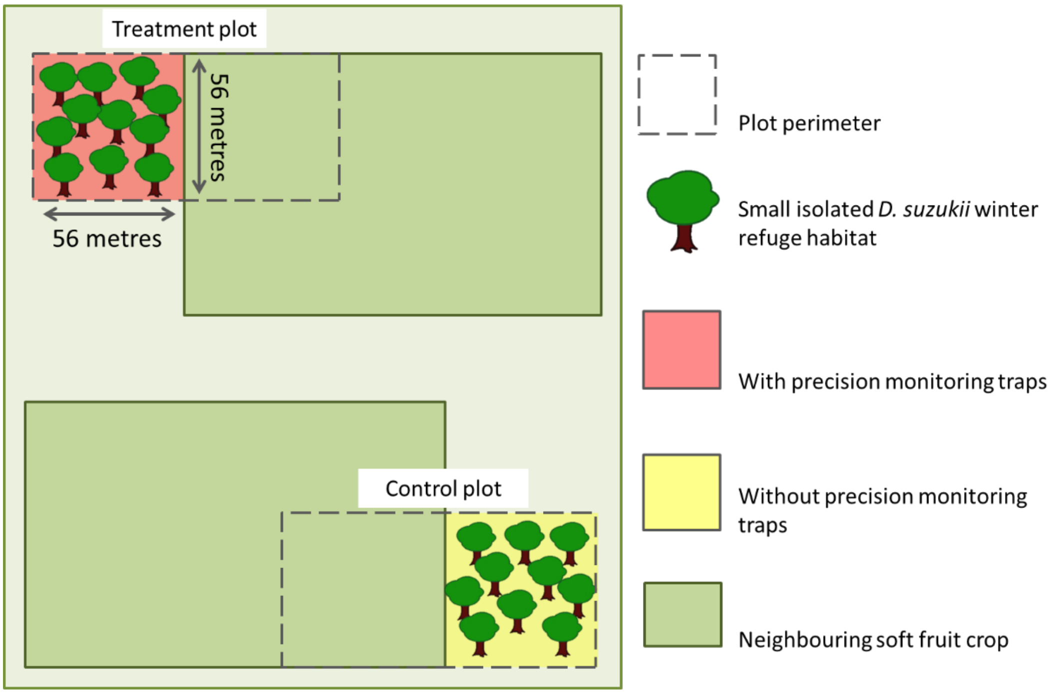


Figure 2.2.1. Diagrammatic representation of an experimental block of the precision monitoring trial September 2019 to March 2022. Each block consists of 1) a treatment plot, comprising of a small isolated pocket of woodland (red square) containing precision monitoring traps, adjacent to a soft fruit crop (darker green square), and 2) a control plot, comprising of a second small isolated pocket of woodland (yellow square) without precision monitoring traps, adjacent to a separate soft fruit crop.

Assessments

To monitor the effectiveness of this strategy of precision monitoring for controlling *D. suzukii,* assessments were made since the original trial start (2019). See Table 2.2.1 for dates and assessments 2021/22 and Appendix 2.2.1 for dates and assessments 2019/20. RIGA and transect traps assessment frequency was dictated by adult *D. suzukii* activity.

Monitoring background numbers of D. suzukii: To compare numbers of adult *D. suzukii* between treated and control plots, a RIGA trap (Agralan) was placed in each woodland and respective neighbouring crop (Figure 2.2.2). RIGA traps were first deployed September 2019, 2 weeks before precision monitoring traps were first deployed (as a pre-assessment), then regularly since. Each assessment, the contents of each RIGA trap was filtered and the trap renewed. Then numbers of male and female *D. suzukii* were counted at NIAB EMR.

Monitoring D. suzukii catches in precision monitoring traps: A transect of 8 precision monitoring traps per treated woodland (Figure 2.2.2) was assessed regularly since traps were first deployed October 2019. At each assessment, the contents of each transect trap was emptied onto a white tray and the number of male *D. suzukii* (spots on wings) counted, then the trap returned to the original position. In 2021, lures in all precision monitoring traps were renewed early April, and all precision monitoring traps were renewed mid-May and again in late November (Table 2.2.1).

Sentinel fruit traps: To compare egg laying between treated and control plots, red Delta traps (Agralan) containing ~100g of ripening strawberries (Figure 2.2.3), were deployed in each woodland and respective neighbouring crop (Figure 2.2.2) during spring 2021. Deployments started 19 April, when average temperature was warm enough for *D. suzukii* activity and ovaries of caught females were dissected and confirmed fecund. A total of 9 deployments were made at each block (6 at block 4). Each deployment, sentinel fruit was left in position an equal number of days at each block (4-7 days depending on temperature), then fruit was brought back to NIAB EMR and incubated (at ~22C, >40 % RH, 16 h light: 8 h dark) for 14 days (to avoid mating and 2nd generation adult emergence). During incubation, numbers of emerged adult *D. suzukii* and other adult Drosophila spp. were counted.

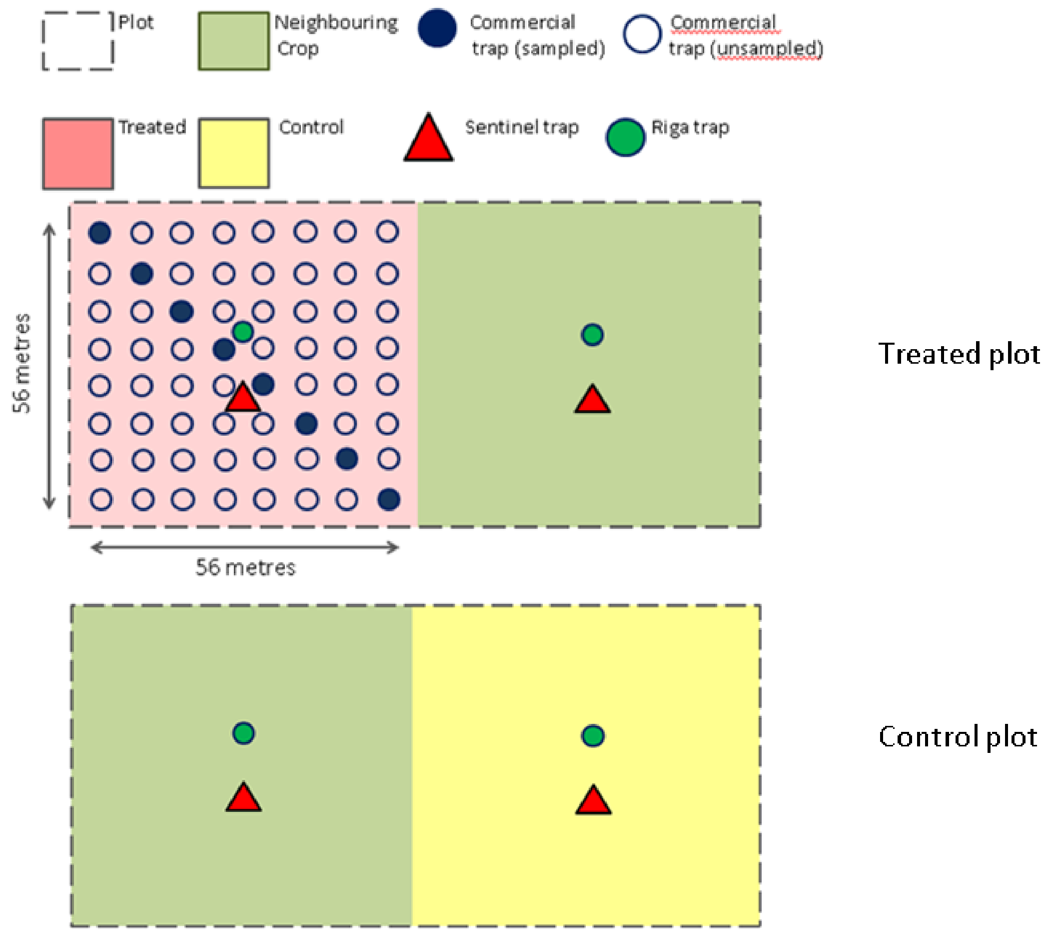


Figure 2.2.2. Diagram of an experimental block (Fig. 2.2.1) and positions of the 3 types of *D. suzukii* monitoring trap used during the precision monitoring trial. Precision monitoring traps = blue outline circles, transect traps = blue fill circles, RIGA traps = green fill circles and sentinel fruit traps = red fill triangles.

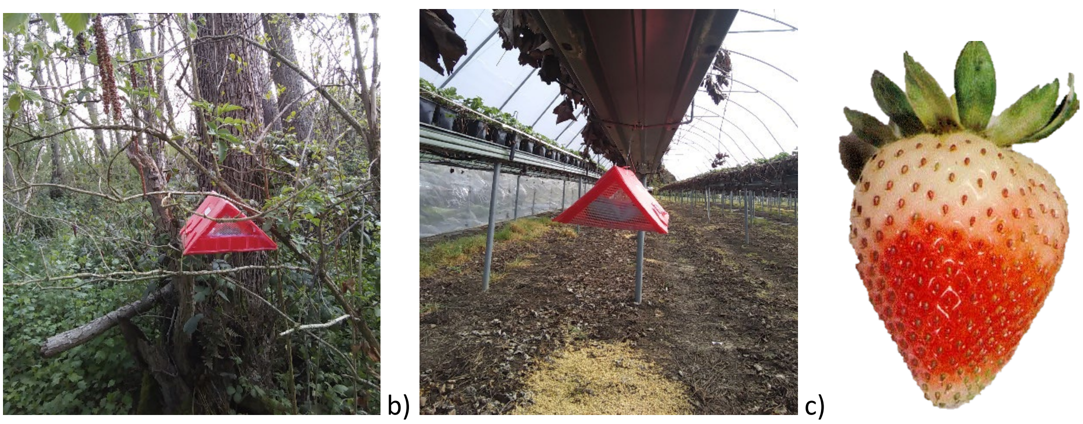

a) b) c)

Figure 2.2.3. Examples of sentinel fruit trap positions and sentinel fruit during the precision monitoring trial; a) woodland position, b) soft fruit crop position, and c) stage of ripening strawberry placed in traps.

Table 2.2.1. Dates of precision monitoring trial assessments and trap replacements at each block, 2021. *Assessment 26 was the last at block 4, also RIGA traps were not assessed all blocks.

Week beginning	Assessment No.	Block(s) RIGA traps	Transect traps	Precision monitoring lure renewal	Sentinel fruit traps	Precision monitoring trap renewal
08-Feb-21	21	1	1			
15-Feb-21		2, 3	2, 3			
22-Feb-21		4	4			
01-Mar-21		5, 6	5,6			
08-Mar-21	22	1,2,3,	1,2,3,			
15-Mar-21		4,5,6	4,5,6			
22-Mar-21	23	1,2,3	1,2,3			
29-Mar-21		4,5,6	4,5,6			
05-Apr-21	24	1,2,3	1,2,3	1,2,3		
12-Apr-21		4,5,6	4,5,6	4,5,6		
19-Apr-21					All	
26-Apr-21	25	All	All		All	
03-May-21					All	
10-May-21					All	
17-May-21	26	*	All		All	1,2,3,5,6
23-May-21					All	
31-May-21	*27	All	All		1,2,3,5,6	
07-Jun-21					1,2,3,5,6	
14-Jun-21					1,2,3,5,6	
21-Jun-21	28	All	All			
26-Jul-21	29	All	All			
16-Aug-21	30	All	All			
20-Sep-21	31	All	All			
11-Oct-21	32	All	All			
01-Nov-21	33	All	All			
15-Nov-21	34	All	All			
22-Nov-21						1,2,3,5,6
06-Dec-21	35	All	All			
18-Dec-21	36	All	All			
03-Jan-22	37	All	All			
17-Jan-22	38	All	All			
31-Jan-22	39	All	All			
14-Feb-22	40	All	All			
07-Mar-22	41	All	All			

Meteorological records: Temperature and humidity was taken using 2 USB data loggers positioned near each RIGA trap at all sites (48 total).

Statistical analyses

RIGA trap catches: D. suzukii count data was analysed using repeated measures ANOVA, with RIGA trap as random effect to account for the repeated measures. Assessments 0 and 26 were removed before analysis because each had instances of 0 *D. suzukii* counts.

Sentinel fruit emergence: D. suzukii count data was analysed using repeated measures ANOVA, with sentinel trap as random effect to account for the repeated measures.

Results

Sentinel fruit emergence:

Statistical analysis of adult *D. suzukii* emergence from sentinel fruit deployed during spring 2020 and 2021, found 2 significant differences. In weeks beginning 20th April 2020 and 31st May 2021, significantly fewer adult *D. suzukii* were counted emerging from sentinel fruit collected from treated plots (woodlands and neighbouring crops combined) compared to control equivalents (mean = 0.04 and 1.11, P = 0.02 and mean = 0.04 and 1.17, P = 0.03 respectively). In 6 out of the 8 assessments across both years, when numbers of adult *D. suzukii* emerged from sentinel fruit were high enough for statistical analysis, fewer adult *D. suzukii* were counted emerging from sentinel fruit collected from treated plots. There were no occasions when significantly fewer adult *D. suzukii* were counted emerging from sentinel fruit collected from control plots (Figure 2.2.4). Numbers of adult *D. suzukii* emerging from sentinel fruit were low all assessments (grandmean = 0.8). Numbers of other adult Drosophila spp. emerging from the same fruit were higher (grandmean = 17.7). Other Drosophila spp. were not identified to species.

RIGA trap catches:

Overall approximately half the number of adult *D. suzukii* (males and females) and adult female *D. suzukii* were caught by RIGA monitoring traps in treated woodlands and neighbouring crops compared to control equivalents (Table 2.2.2), however statistical analysis found this difference was not statistically significant. For 31 out of the 41 assessments made during the trial, fewer adult *D. suzukii* were caught in treated crops compared to control crops, but the differences were not statistically significant (Figure 2.2.5) and for 34 assessments, fewer adult *D. suzukii* were caught in treated woodlands compared to control woodlands, but the differences were not statistically significant (Figure 2.2.6). For 29 assessments fewer adult female *D. suzukii* were caught in treated plots (woodlands and neighbouring crops) compared to the control, this difference was statistically significant on 4 occasions, all during late-winter/early-spring (Figure 2.2.7). Between June and October, fewer *D. suzukii* were caught in treated crops compared to control 5 out of the 7 assessments 2020 and 5 out of 6 assessments 2021, but the differences were not statistically significant.

Comparing years, statistical analysis of numbers of *D. suzukii* caught in RIGA traps found significantly fewer *D. suzukii* (males and females) were caught year 2 (2021) than year 1 (2020) (mean = 1573 and 2956 respectively, $P < 0.001$) and significantly fewer female *D. suzukii* were caught year 2 than year 1 (mean = 869 and 1369 respectively, $P = 0.004$) (Figure 2.2.8). There were no significant interactions between year and RIGA trap position.

Average annual temperature was slightly lower in 2021 compared to 2020 (mean = 11.4 °C and 12.2 °C respectively). Average quarterly temperature was slightly lower quarters 1 (January to March) and 2 (April to June) of 2021 compared to the same quarters 2020 (mean = 6.4 °C and 12.3 °C compared to 7.2 °C and 14.1 °C respectively). Average temperature was similar 2020 and 2021 quarters 3 (July to September) and 4 (October to December) (mean = 17.2 °C and 8.6 °C compared to 17.2 °C and 8.9 °C respectively).

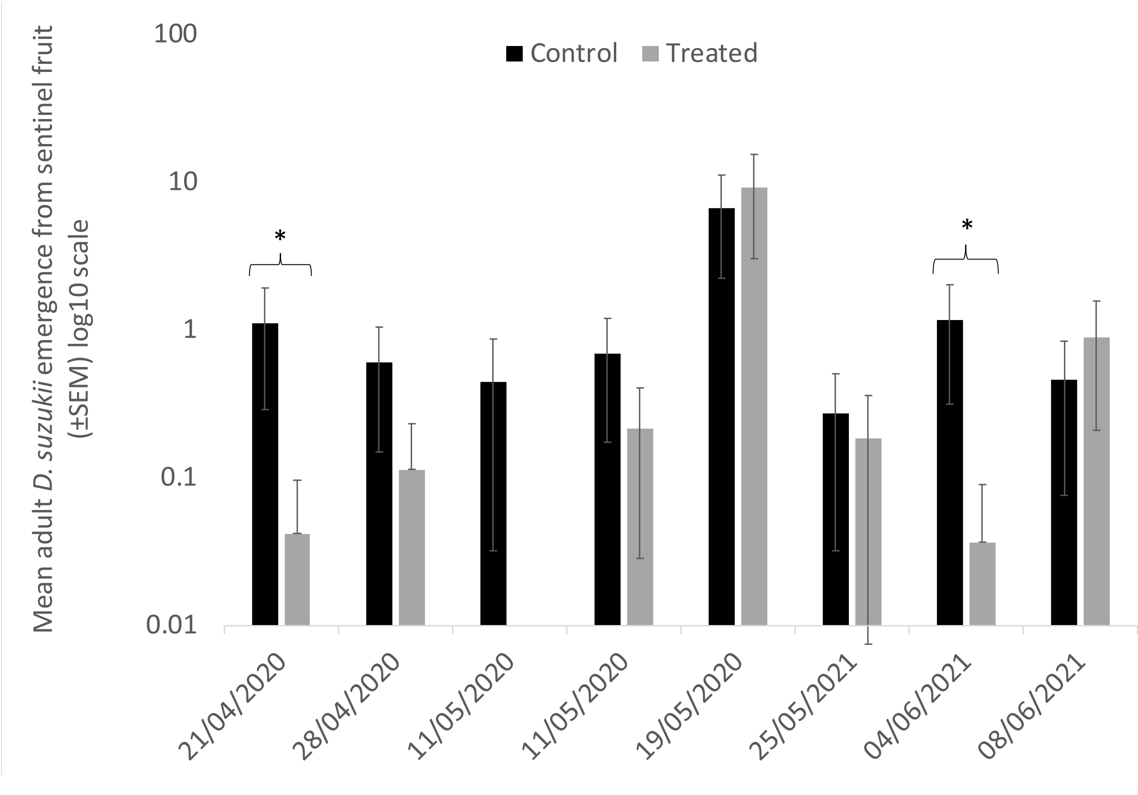


Figure 2.2.4. Mean numbers of adult *D. suzukii* emerged from sentinel fruit deployed at control and treated plots during spring 2020 and 2021 assessments of the precision monitoring trial. * indicates significant differences at $P = 0.05$, n = 6.

Table 2.2.2. RIGA trap position and mean catches of adult *D. suzukii* (males and females) and adult female *D. suzukii* throughout the precision monitoring trial, October 2019 to March 2022.

RIGA trap position	Mean adult *D. suzukii* caught	Mean adult female *D. suzukii* caught
Control Crop	20.7	12.75
Treated Crop	11.1	7.54
Control Woodland	190.9	95.08
Treated Woodland	89.8	51.84

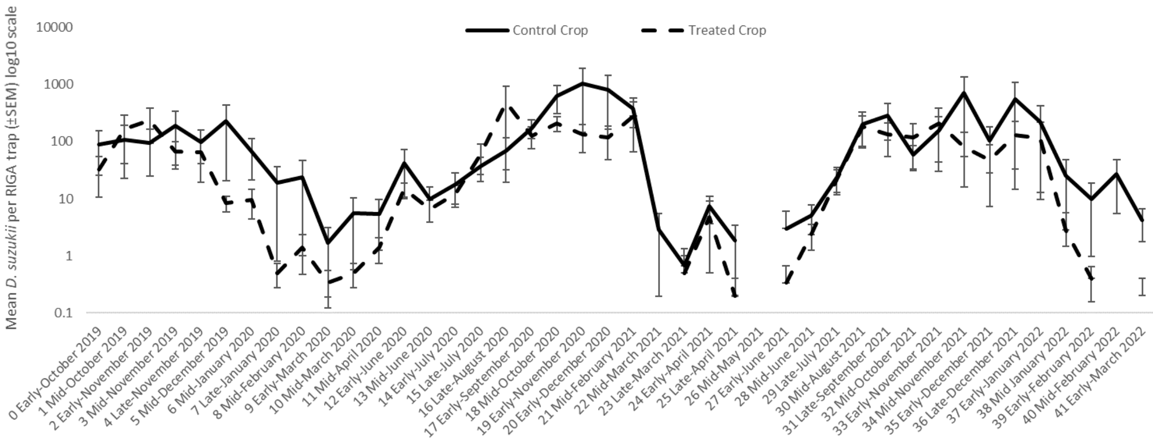


Figure 2.2.5. Mean numbers of adult *D. suzukii* caught per RIGA monitoring trap in control and treated crops from assessments 0 (early-October 2019) to 41 (early-March 2022) of the precision monitoring trial. Precision monitoring traps were removed from treated woodlands mid-April to mid-June 2020 during sentinel fruit deployments. No RIGA traps were assessed mid-May 2021.

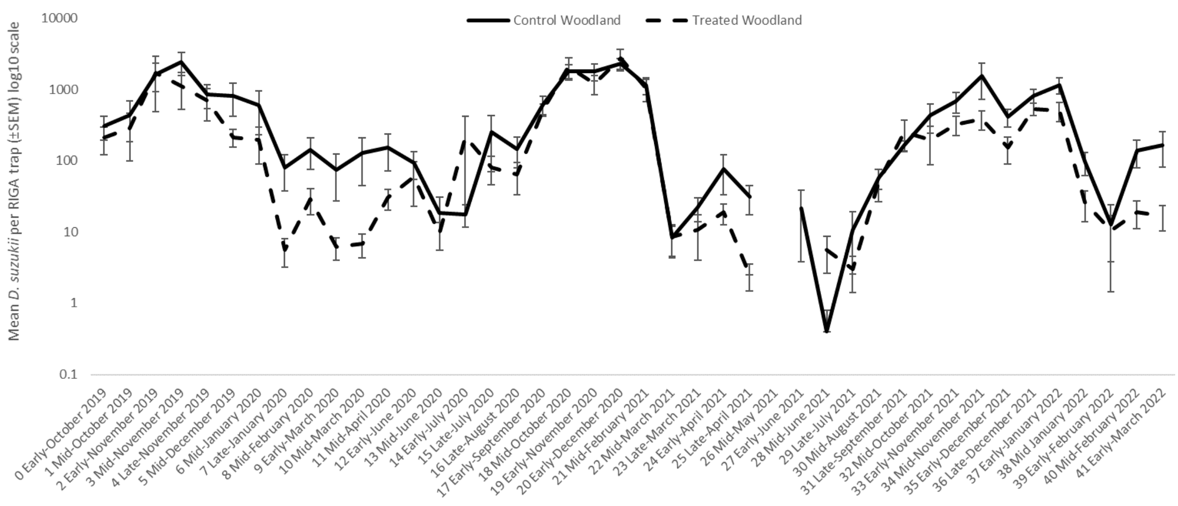


Figure 2.2.6. Mean numbers of adult *D. suzukii* caught per RIGA monitoring trap in control and treated woodlands from assessments 0 (early-October 2019) to 41 (early-March 2022) of the precision monitoring trial. Precision monitoring traps were removed from treated woodlands mid-April to mid-June 2020 during sentinel fruit deployments. No RIGA traps were assessed mid-May 2021.

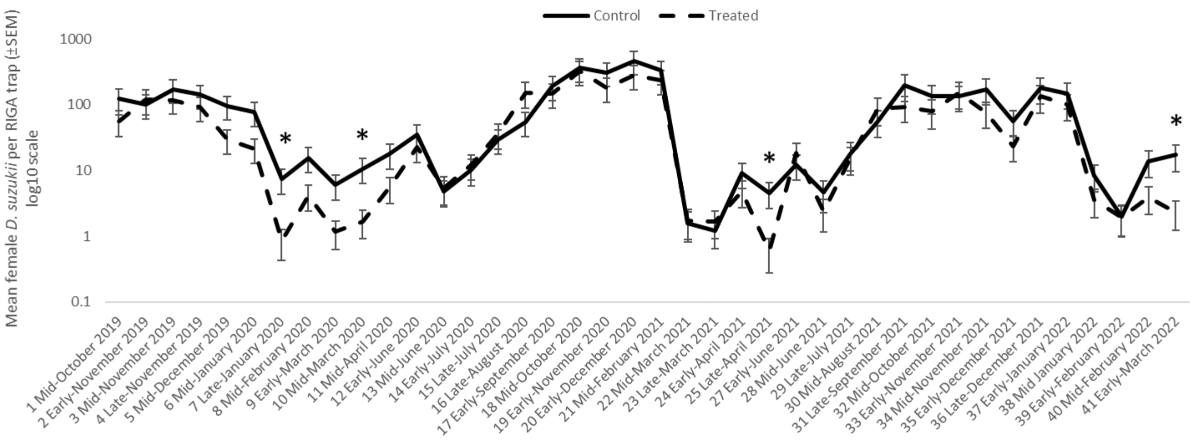


Figure 2.2.7. Mean numbers of adult female *D. suzukii* caught per RIGA monitoring trap in control and treated plots (crops and woodlands combined) from assessments 1 (mid-October 2019) to 41 (early-March 2022) of the precision monitoring trial. Precision monitoring traps were removed from treated woodlands mid-April to mid-June 2020 during sentinel fruit deployments. No RIGA traps were assessed mid-May 2021. * indicate significant differences at $P = 0.05$, n= 6.

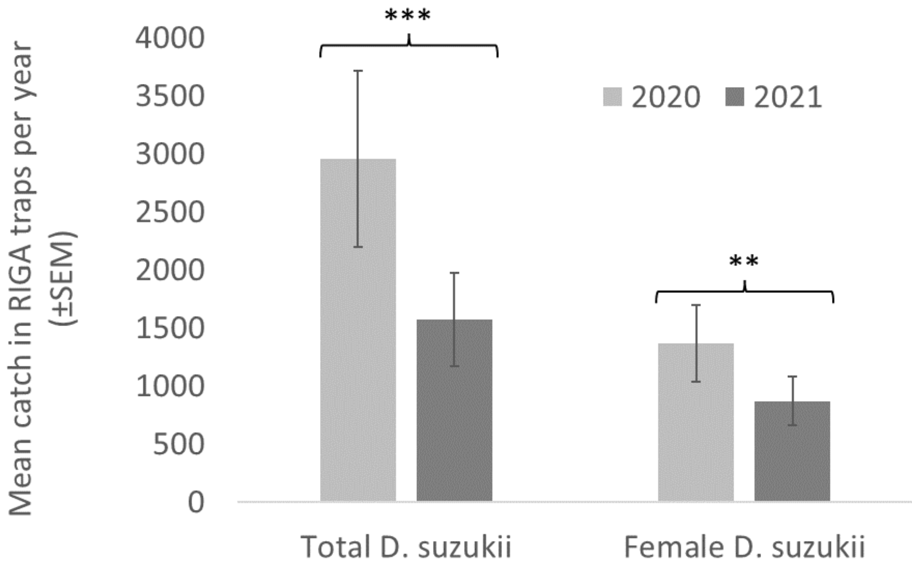


Figure 2.2.8. Mean numbers of total *D. suzukii* (males and females) and female only *D. suzukii* caught in RIGA monitoring traps year 1 (January 2020 to December 2020) and year 2 (January 2021 to December 2021) of the precision monitoring trial. ** indicate significant differences at *P* = 0.01 and *** at *P* = 0.001, n = 6.

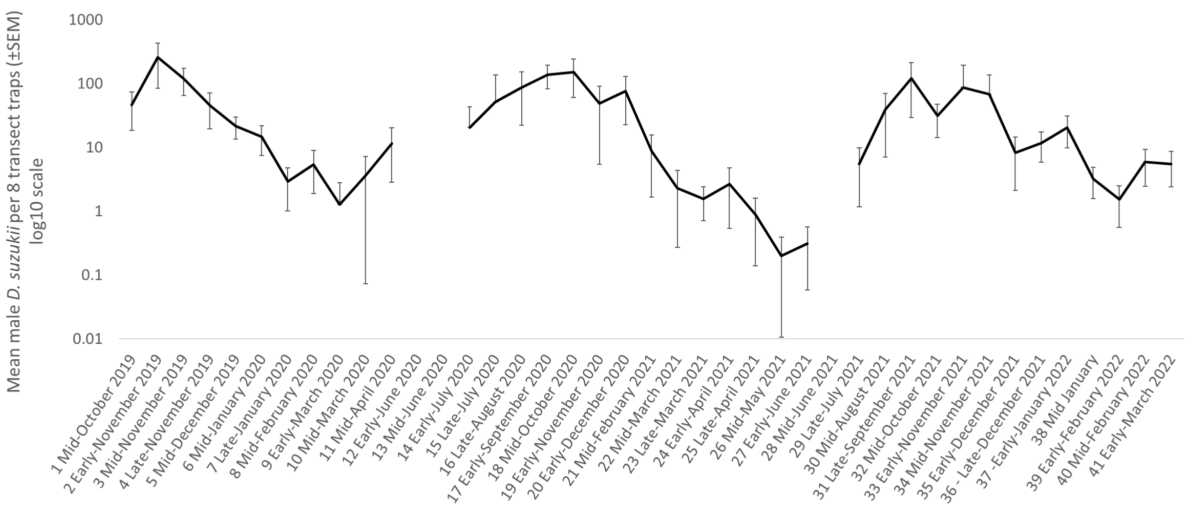


Figure 2.2.9. Mean numbers of male *D. suzukii* caught per 8 transect traps in treated woodlands of the precision monitoring trial.

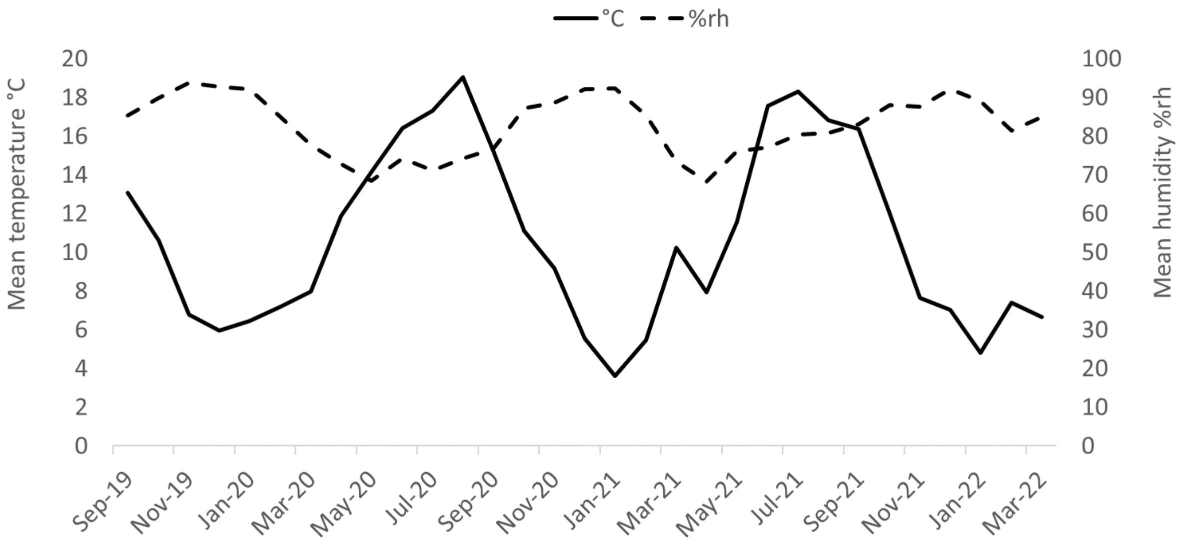


Figure 2.2.10. Mean monthly temperature (°C) and humidity %rh during the precision monitoring trial.

Discussion

From October 2019 to March 2022, the precision monitoring trial investigated whether applying precision monitoring traps in habitats where *D. suzukii* overwinter, can reduce winter morphs and then subsequent incursion into the adjacent soft fruit crop from spring.

A grid of 64 precision monitoring traps were deployed in a small isolated pocket of woodland on 6 soft fruit farms (5 since June 2021) in Kent and West Sussex. A second small isolated pocket of woodland on each farm was designated as a control with no precision monitoring traps. Single RIGA monitoring traps were positioned in each woodland and respective neighbouring soft fruit crop to monitor numbers of *D. suzukii* regularly throughout the trial. In addition, sentinel fruit was deployed in spring 2020 and 2021 (when catches of female *D. suzukii* were increasing and dissected flies first fecund) ~ 8 m from RIGA traps (avoiding interference), to monitor *D. suzukii* egg laying. The trial also investigated whether precision monitoring traps can be positioned more strategically according to surrounding host vegetation and abiotic factors, to optimise *D. suzukii* catches, hence establishing a more targeted approach which would reduce labour in the maintenance of the traps (SF TF145a annual report 2020).

Sentinel fruit deployments showed some evidence to suggest precision monitoring for the *D. suzukii* winter morph can reduce the incidence of fruit damage in the neighbouring crop. Overall adult *D. suzukii* emergence from sentinel fruit deployed spring 2020 and 2021 (April through June) was low, however statistical analysis showed that on 2 occasions *D. suzukii* egg laying was significantly lower in plots (woodlands and neighbouring soft fruit crops) treated with precision monitoring than control plots. For weeks beginning 20th April 2020 and 31st May 2021, significantly fewer adult *D. suzukii* were counted emerging from sentinel fruit collected from treated plots compared to control equivalents (mean = 0.04 and 1.11, P = 0.02 and mean = 0.04 and 1.17, P = 0.03 respectively, Figure 2.2.4). In support, analysis of RIGA trap catches found significantly fewer adult female *D. suzukii* were caught in treated plots (woodlands and neighbouring crops) compared to control during the assessment immediately preceding these two sentinel fruit deployments, potentially explaining the lower egg lay in sentinel fruit (Figure 2.2.7). Overall fewer adult *D. suzukii* emerged from sentinel fruit collected from treated plots compared to control 6 out of the 8 deployments (although the differences were not statistically significant) and there were no occasions when significantly fewer adult *D. suzukii* emerged from sentinel fruit collected from control plots. Numbers of adult *D. suzukii* emerging from sentinel fruit were low all assessments (grand mean = 0.8), whereas numbers of other adult Drosophila spp. emerging from the same fruit were higher (grand mean = 17.7). Ripening strawberries were deployed spring 2021 to promote *D. suzukii* egg laying and larval development, but ripening during deployment may have attracted other Drosophila spp. to egg lay. Other Drosophila are known to compete with *D. suzukii.* For example, a deterrent effect of egg laying in fruit was demonstrated in studies by Shaw et al. (2018) and is the focus of a BBSRC project led by NIAB EMR with NRI. *Drosophila melanogaster* larvae can also predate other fruit fly larvae (Ahmad et al. 2015). A more reliable method to monitor *D. suzukii* egg laying might be to capture live adult female *D. suzukii* using unripe strawberry deployed for a short period, then to assess fecundity under controlled conditions in the laboratory, but this method would need to be verified.

Statistical analysis of numbers of adult *D. suzukii* caught in RIGA monitoring traps could not conclude whether precision monitoring can maintain protection against *D. suzukii* fruit damage in the neighbouring crop during the 2021 growing season, despite there being consistently lower catches of adult *D. suzukii* in treated plots. Between June and October 2021, fewer adult *D. suzukii* were caught in treated crops compared to control equivalents 5 out of the 6 assessments (Figure 2.2.5), but the difference was not statistically significant. During the same period in 2020, fewer adult *D. suzukii* were also caught in treated crops 5 out of 7 assessments (Figure 2.2.5), but again this difference was not statistically significant. Analysis of adult female *D. suzukii* found significantly fewer were caught in treated plots (woodlands and neighbouring crops) compared to the control on 4 occasions during the entire trial. These were all made late-winter/early spring when adult *D. suzukii* catches were low on average (Figure 2.2.7).

Investigation of trap catches between years found significantly fewer *D. suzukii* (males and females) were caught in the 2nd year of the trial (2021) compared to the 1st (2020) (mean = 1573 and 2956 respectively, *P*< 0.001) and of these, significantly fewer female *D. suzukii* were caught in the 2nd year of the trial compared to the 1st (mean = 869 and 1369 respectively, *P* = 0.004). However there were no significant interactions between year and RIGA trap position. It is therefore difficult to conclude if the decrease in *D. suzukii* catches in the 2nd year of the trial can be attributed to precision monitoring because other factors also influence *D. suzukii* population levels. These include winter temperatures affecting overwintering survival of adults. Average annual temperature was slightly lower in 2021 compared to 2020 (mean = 11.4 °C and 12.2 °C respectively). This was mainly owed to the first two quarters being cooler on average in 2021 (mean = 6.4 °C and 12.3 °C) compared to 2020 (mean = 7.2 °C and 14.1 °C). Plant resources for reproduction and intrinsic rate of growth also influence *D. suzukii* population levels as does predation and parasitism by natural enemies (Drummond et al. 2019).

Conclusions

- Sentinel fruit deployments showed a some evidence to suggest precision monitoring for the *D. suzukii* winter morph can reduce the incidence of egg laying in the neighbouring soft fruit crop up to June.
- However analysis of numbers of adult *D. suzukii* caught in RIGA monitoring traps could not conclude whether precision monitoring can maintain protection against *D. suzukii* fruit damage in the neighbouring crop during the growing season. This is despite there being consistently lower catches of adult *D. suzukii* (males and females) in crops next to woodlands treated with precision monitoring than those without (control crops).
- Significantly fewer adult *D. suzukii* were caught year 2 (2021) of the trial than year 1 (2020), but we cannot conclude if this was due to precision monitoring because there was no significant difference in annual catches between treated and control areas. Other factors might have had an influence, including average overwintering temperature, which was lower in 2021.
- Analysis of precision monitoring trap position in 2020 found traps positioned on the woodland perimeter nearest the crop caught significantly more male *D. suzukii* than within the main woodland during summer, autumn and winter (SF TF145a annual report 2020).
- Also in summer 2020 there was a significant positive correlation between vegetation score in a 4 m radius around traps and numbers of *D. suzukii* caught in respective

traps, i.e., the more favourable the vegetation to *D. suzukii* and the more coverage, the more *D. suzukii* were caught. In autumn, the correlation was also positive, but not significant.

- Bramble and ivy were the only species found to have a significant positive influence on catches of *D. suzukii*, during summer and autumn assessments respectively.

Objective 3. Develop bait sprays for control of *D. suzukii*

Task 3.4_1 Determine the effect of baits in combination with reduced dose insecticides on SWD control in cherry

Introduction

Strawberry and raspberry experiments in 2019 and 2020 showed that weekly dilute applications of spinosad (product Tracer) and/or cyantraniliprole (product Benevia or Exirel) combined with Combi-protec in 40 L/ha applications, were as effective in controlling *D. suzukii* as full field rate sprays in 500 L/ha (i.e. a reduction in pesticide application of more than 95% with the same *D. suzukii* control effect). In the 2020 raspberry experiment, molasses was also used in a bait spray and control of *D. suzukii* was equally good as with Combi-protec but at significantly lower cost. Combi-protec is authorised and commercially available as a sticker adjuvant in the UK whereas molasses was not authorised for use at the time of the experiment. Tracer and Exirel are currently approved for use on soft and stone fruit under emergency authorisations but the approved rate for Tracer is expected to be reduced from 250 to 100 mL/ha in 2022. The aims of this work were to compare the *D. suzukii* control efficacy of weekly applications of dilute rates of Tracer and Exirel when used with and without Combi-protec or molasses in 40 L/ha band sprays, against full field rates in 500L/ha applications of the same insecticides in cherry under semi-field conditions. Additionally, the *D. suzukii* control efficacy of the existing and new reduced field rates of Tracer were compared when used alternating with (i) the full 100% field rate of Exirel in 500 L/ha applications or (ii) a 16% rate of Exirel in 80 L/ha Combi-protec band sprays.

Methods

The semi-field experiment at NIAB EMR was conducted in a 2008 planted cherry orchard, cv. Penny with cv. Sweetheart pollinators in every fourth row. The trees were planted at 2 m spacing within the row and 3 m between rows orientated north - south, and had an average height of 3.4 m and width of 2 m, without any canopy below 0.5 m. The time schedule of tasks is shown in Table 3.4.1. In April 2021, after flower set, two central rows, each of 76 cv. Penny trees, including guards at each end, were covered under a polythene tunnel for the experiment. The tunnel had a central height of 3.7 m, side walls of 1.2 m height, and width of 7.9 m. The side walls and adjacent rows of trees were covered with 1 cm square netting in place of polythene. The tunnel was divided into 35 plots by polythene dividing walls spaced at approximately 3.4 m intervals so that each plot contained four trees and had an average area of 26.5 m^2.

The trees were irrigated through a drip irrigation system. No pesticide sprays were applied to the trees other than the experimental treatments and a single application of Gazelle (a.i. acetamiprid) at 375 g/ha on 26 May 2021 for control of black cherry aphid.

Table 3.4.1. Time schedule of Task 3.4_1 - Cherry

Month	Expt Day	Activity
February		Pruning of trees
April		Polythene cover on tunnel
May 26	-22	Spray of Gazelle at 375 g ha^{-1} for control of black cherry aphid
June 14	-3	Pre-assessment of white fruit stage to check background SWD levels
June 17	0	Spray 1, Tracer
June 18	1	Introduce SWD cohort in compartments, 20♀ 10♂
June 23	6	Sample fruit for SWD 1
June 24	7	Spray 2, Exirel
June 30	13	Sample fruit for SWD 2
July 1	14	Spray 3, Tracer; Select fruit for residue testing
July 7	20	Sample fruit for SWD 3;Take tap samples for beneficials
July 8	21	Spray 4 Exirel; Select fruit for residue testing
July 14	27	Sample fruit for SWD 4
July 15	28	Spray with fluorescent dye (replicate 1 only)

The trees were sprayed in mid-June at white fruit stage, and three times again at weekly intervals with the treatments below (Figure 3.4.1 and 3.4.2). All sprays were applied from the alleyway between the two rows of trees, which had canopy sections designated as ‘near’, ‘mid’ and ‘far’ from the spray nozzle. These sections corresponded to depths across the canopy from one side of the tree to the other of 0-0.6, 0.7-1.3 and 1.4-2.0 m.

Each compartment was artificially infested with adult summer morph *D. suzukii*; 20 females and 10 males were introduced one day after the first spray. Approximately equal numbers of flies were deployed either side of the tunnel compartments. Samples of 20 cherries from the near, mid and far canopy sections of the entire height of all four trees in each compartment were picked three days before the first spray and six days after each of four sprays. Fruits were incubated for 48 hours at 20°C and then each fruit was flotation tested and the numbers of *D. suzukii* larvae in each individual fruit counted. A further sample of 24 cherries from the entire height and depth of all four trees in each compartment was also picked for *D. suzukii* adult emergence testing. The fruit was introduced into clear Perspex boxes (27 × 15 × 10 cm). The boxes had a mesh covered ventilation hole in the lid and were lined with tissue paper to absorb excess moisture. Adult male and female *D. suzukii* emergence was recorded from each box during a 15-day incubation at 22ºC, in 16h:8h light:dark. Temperature and humidity among the trees were recorded by Grant sensors and data loggers. Tree foliage was assessed for phytotoxicity symptoms on a 0 no damage to 3 severe damage scale, one week after the timing of each spraying.

Immediately after the second applications of Tracer and Exirel, 1 kg samples of cherries from the near, mid and far canopy sections of the trees from treatments 1, 2, 3, 4 and 6 (pooled samples from all replicates) were analysed for pesticide residues. Samples were analysed by QTS, Sittingbourne, Kent, using liquid chromatography – mass spectrometry (LC-MS). The detection limit for pesticide residues was 0.01 mg/kg fruit. The majority of the fruit ripened to a harvestable stage by weeks 2 and 3 of the experiment. Ripe fruit not used for *D. suzukii* testing or residue analysis was not removed from the trees, so that by week 4, there was a large quantity of over-ripe fruit in the compartments. Although over-ripe fruit was not used for *D. suzukii* testing, together with the surrounding unsprayed trees in the orchard, it contributed substantially to pest pressure, particularly by the end of the experiment.

Figure 3.4.1. White fruit stage for first spray (left) and ripening fruit for second spray (right) applications

Figure 3.4.2. Rookery field cherry orchard (left) and method of spray application to trees inside polytunnel compartments (right)

Treatments, experimental design and statistical analysis

1. Unsprayed positive control; no spray application to trees during the experimental period.

The remaining trees were sprayed with a motorised knapsack sprayer (Birchmeier 14 REC ABC) at a maximum pressure of 3 bar. Weekly alternating sprays of Tracer and Exirel were applied at the full field rate, or at reduced field (medium) or dilute (low) rates with and without baits (Tables 3.4.2 and 3.4.3).

2. The full field rate of insecticides and new reduced rate for Tracer were applied without baits as a high volume, fine spray with a motorised mist blower (Solo Inc.) and hollow-cone nozzle (Albuz ATR 80 orange) over the entire sprayed side of the trees at a rate of 1332 ml spray per plot which is equivalent to 500 L/ha. The BCPC droplet spectra size was fine to very fine (154 to 225 microns).

3. The medium and low rate sprays (with and without baits) were applied as a medium or low volume spray, in 340 micron droplets with a Lechler IDK 120-015 green nozzle to spray a 1 m width swath with the centre of spray aimed at the middle of sprayed side of the trees at a rate of 106.4 or 213.2 ml per plot which is equivalent to 40 or 80 L/ha.

The estimated volumes of spray to be applied per tree were based on an approximate industry standard number of 1500 trees/ha. The actual volumes of spray applied per tree were determined from the initial and final volumes in the spray tank.

There were five replicate compartments of each treatment arranged in a randomised block design.

Table 3.4.2. Bait and insecticide rate spray treatments and application timeline

Treatment number and name	Bait, %v/v	Tracer & Exirel, % full field rate*	Spray 1	Spray 2	Spray 3	Spray 4
1 Full field rate	None	100	Tracer	Exirel	Tracer	Exirel
2 New Tracer rate	None	40 & 100	Tracer	Exirel	Tracer	Exirel
3 Combi-protec Low	Combi-protec, 5	4	Tracer	Exirel	Tracer	Exirel
4 Molasses Low	Molasses, 5	4	Tracer	Exirel	Tracer	Exirel
5 No Bait Low	None	4	Tracer	Exirel	Tracer	Exirel
6 Combi-protec Medium	Combi-protec, 5	40 & 16	Tracer	Exirel	Tracer	Exirel
7 Untreated	None	None	None	None	None	None

* Full field (100%), medium (40% or 16%) and low (4%) rates of insecticides are in Table 3.4.3.

Table 3.4.3. Full field, medium and low rates of insecticide sprays

Insecticide	Active ingredient	Rate, % of full field	ml/ha	g a.i. /ha	Spray volume
Tracer	spinosad 480 g/l	Full field, 100	250	120	500 L/ha
Tracer	spinosad 480 g/l	Medium, 40	100	48	500 L/ha or 80L/ha
Tracer	spinosad 480 g/l	Low, 4	10	4.8	40 L/ha
Exirel	cyantraniliprole 100 g/L	Full field, 100	900	90	500 L/ha
Exirel	cyantraniliprole 100 g/L	Medium, 16	144	14.4	80 L/ha
Exirel	cyantraniliprole 100 g/L	Low, 4	36	3.6	40 L/ha

Spray deposition methodology

The spray deposition of an application of treatments 1 Full field rate and 3 Combi-protec Low was assessed using a handheld imaging fluorometer and fluorescence tracer dye. The dye was mixed into a stock solution at 2% v/v. From this stock, each of the sprayed treatments was mixed with the appropriate adjuvant (Combi-protec or nothing added). Compartments in block 1 were sprayed using the appropriate spray settings for each treatment (Table 3.4.4).
The cherry tree canopy was divided into nine sections: near, mid and far distances from the sprayer nozzle (as previously described) × top (above 2.5 m), middle (1.5 – 2.5 m), and bottom (below 1.5 m). Within each canopy section, both sides of the leaves were sampled. For each leaf side, 25 readings were taken.

Table 3.4.4. Treatments applied for the spray deposition analysis

Treatment name	Adjuvant	Water volume rate, L/ha
Full field rate	None	500
Combi-protec Low	Combi-protec	40
Combi-protec Medium	Combi-protec	80

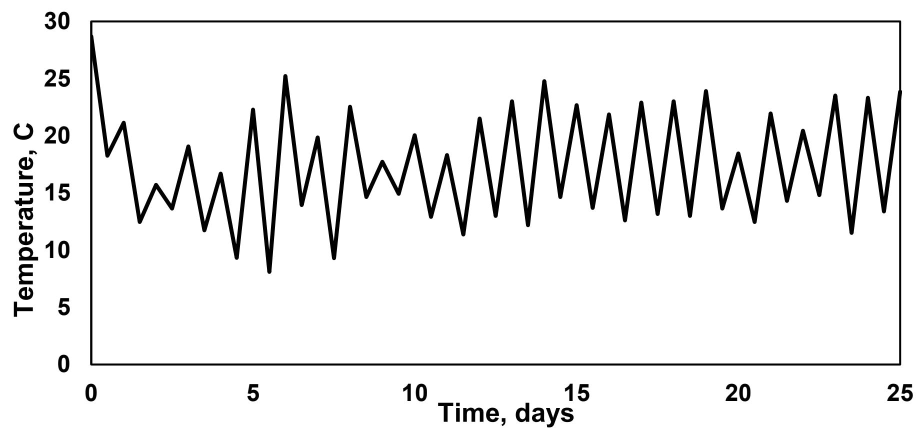
<u>Statistical analysis</u>

Emergence, larvae flotation and spray deposition data were analysed using GLM. A logit transformation was used for spray deposition data.

Results

<u>Polytunnel environment</u>

Diurnal fluctuations in air temperature and relative humidity among the polytunnel cherry trees are shown in Figure 3.4.4. During the experiment, average daily maximum and minimum temperatures were 21.6ºC and 12.9 ºC; average daily maximum and minimum relative humidities were 97.5% and 74.5%.



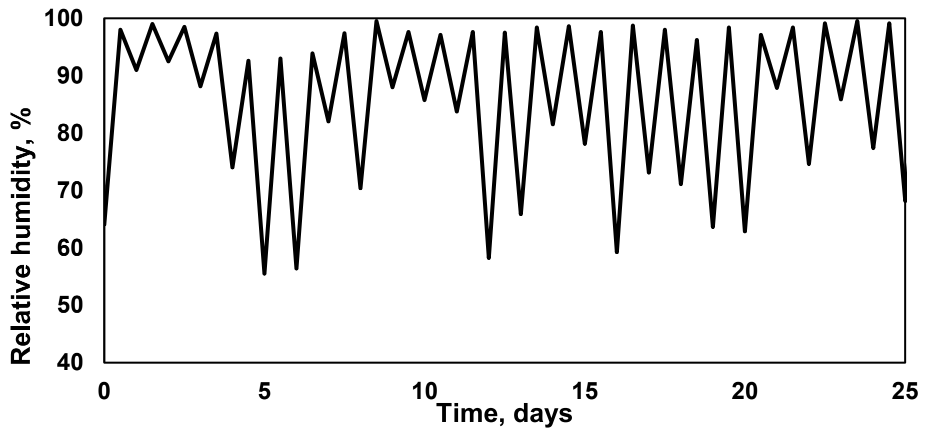


Figure 3.4.3. Temperature and relative humidity among polytunnel cherry trees

Spray applications

Full field and new rate applications took 97 seconds per plot (4 trees) compared with 20 and 10 seconds for medium and low rate bait sprays respectively. Spray applications measured from the start and end tank volumes were 85% to 108% of the target values (Table 3.4.5). Amounts of active ingredients applied per tree are shown in Table 3.4.6.

No phytotoxicity symptoms were observed on any of the trees and there was no mould growth on the bait spray droplets.

Table 3.4.5. Target and actual measured quantities of sprays applied

Treatment	Spray	Insecticide	Spray vol., ml/tree		Actual/
			target	actual	Target
1. Full field rate	1	Tracer	333.0	333.7	100.2%
	2	Exirel	333.0	342.7	102.9%
	3	Tracer	333.0	352.6	105.9%
	4	Exirel	333.0	338.7	101.7%
2. New Tracer rate	1	Tracer	333.0	327.3	98.2%
	2	Exirel	333.0	342.7	102.9%
	3	Tracer	333.0	339.3	101.9%
	4	Exirel	333.0	338.3	101.6%
3. Combi-Protec Low	1	Tracer	26.6	27.5	103.4%
	2	Exirel	26.6	27.5	103.4%
	3	Tracer	26.6	27.2	102.3%
	4	Exirel	26.6	28.8	108.1%
4. Molasses Low	1	Tracer	26.6	25.0	94.0%
	2	Exirel	26.6	27.0	101.5%
	3	Tracer	26.6	26.5	99.6%
	4	Exirel	26.6	26.8	100.6%
5. No Bait Low	1	Tracer	26.6	22.5	84.6%
	2	Exirel	26.6	25.0	94.0%
	3	Tracer	26.6	24.5	92.1%
	4	Exirel	26.6	26.0	97.7%

6. Combi-Protec Medium	1	Tracer	53.3	50.0	93.8%
	2	Exirel	53.3	51.6	96.9%
	3	Tracer	53.3	51.1	95.8%
	4	Exirel	53.3	50.0	93.8%

Table 3.4.6. Amounts of active ingredients applied per tree in individual sprays and in total

Treatment	Spray	Insecticide	Active ingredient mg/tree	
			spinosad	cyantraniliprole
1. Full field rate	1	Tracer	80.1	0
	2	Exirel	0	61.7
	3	Tracer	84.6	0
	4	Exirel	0	61.0
	Total		**164.7**	**122.7**
2. New Tracer rate	1	Tracer	31.4	0
	2	Exirel	0	61.7
	3	Tracer	32.6	0
	4	Exirel	0	60.9
	Total		**64.0**	**122.6**
3. Combi-Protec Low	1	Tracer	3.3	0
	2	Exirel	0	2.5
	3	Tracer	3.3	0
	4	Exirel	0	2.6
	Total		**6.6**	**5.1**
4. Molasses Low	1	Tracer	3.0	0
	2	Exirel	0	2.4
	3	Tracer	3.2	0
	4	Exirel	0	2.4
	Total		**6.2**	**4.8**
5. No Bait Low	1	Tracer	2.7	0
	2	Exirel	0	2.3
	3	Tracer	2.9	0

	4	Exirel	0	2.3
	Total		**5.6**	**4.6**
6. Combi-Protec Medium	1	Tracer	30.0	0
	2	Exirel	0	9.3
	3	Tracer	30.7	0
	4	Exirel	0	9.0
	Total		**60.7**	**18.3**

D. suzukii adult emergence assessments

The first adults emerged 5-8 days after placing the fruit in the emergence boxes, with the majority emerging by day 13. Boxes were discarded after 17 days so there was no possibility of a second generation of flies. Similar proportions of female and male *D. suzukii* emerged in all the boxes from the unsprayed, insecticide and insecticide + bait treatments; overall, the ratio of *D. suzukii* females to males was 54:46. About three times as many *D. suzukii* adults emerged from the control boxes in week 4 than in weeks 2 and 3. This corresponded with a higher level of *D. suzukii* control with insecticide treatments in weeks 2 and 3 than in week 4. However, by week 4, the majority of fruit was over-ripe. The low rate insecticide sprays without baits resulted in a significant reduction in *D. suzukii* adult emergence, about 50% of those in controls, in all assessment weeks (Figure 3.4.4). Overall, the full rate, new rate and molasses bait spray applications resulted in further reductions in these numbers. Differences between insecticide treatments in individual assessment weeks were not significant.

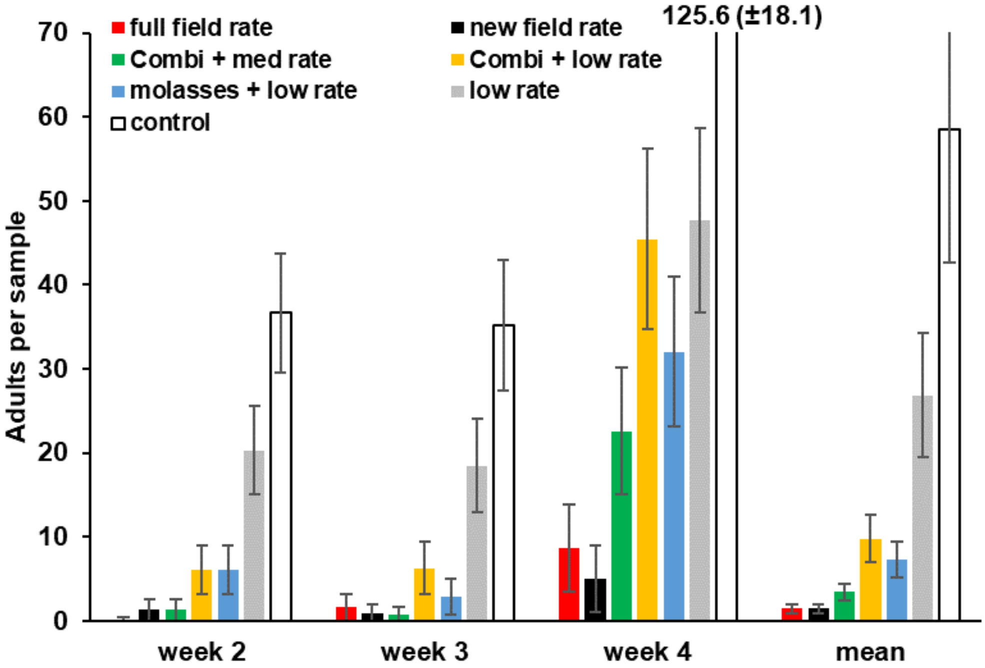


Figure 3.4.4. Effect of full field rate and new approved rate applications of Tracer in weeks 1 and 3 and Exirel in weeks 2 and 4, and reduced or dilute applications of the same insecticides applied in the same weeks with and without baits (Combi-protec or molasses) on the weekly numbers of emerged *D. suzukii* adults from boxes containing 24 cherries. Mean values (±SE), n = 5.

Larvae flotation assessments

The lower *D. suzukii* numbers determined from larvae flotation tests than from adult emergence in boxes may be due to the non-detection of eggs in flotation tests. There was an average of 1.5 larvae per fruit in samples taken for flotation tests three days before spraying commenced, which indicated a moderate background *D. suzukii* infestation of the crop before the introduction of laboratory reared *D. suzukii* cohorts had commenced. In assessment week 1, the new approved rate of Tracer resulted in a significant reduction in the number of larvae in flotation tests; differences between other treatments were not significant (Figure 3.4.5). The numbers of larvae in flotation tests in assessment weeks 2, 3 and 4 followed a similar trend to the emergence tests but the larvae flotation numbers per fruit were only 30% of the emergence numbers (Figures 3.4.4 and 3.4.5). *D. suzukii* eggs in the sample fruits may have developed into adults in the emergence tests (up to 17 days) but may have only reached 1st instar larvae which may not be extracted by the flotation tests (2 days). In week 4 and averaged across all weeks, the low rate insecticide resulted in a significant reduction in the number of larvae in flotation tests on fruit samples compared with the untreated control (Figure 3.4.5 and 3.4.6). In weeks 2, 3 and 4, the full field rate and new approved Tracer rate resulted in significantly fewer larvae than the low rate sprays (Figure 3.4.5 and 3.4.6). Averaged across all weeks, the full rate, new approved Tracer rate and bait sprays were not significantly different but all resulted in fewer larvae than the low rate sprays without bait (Figure 3.4.6).

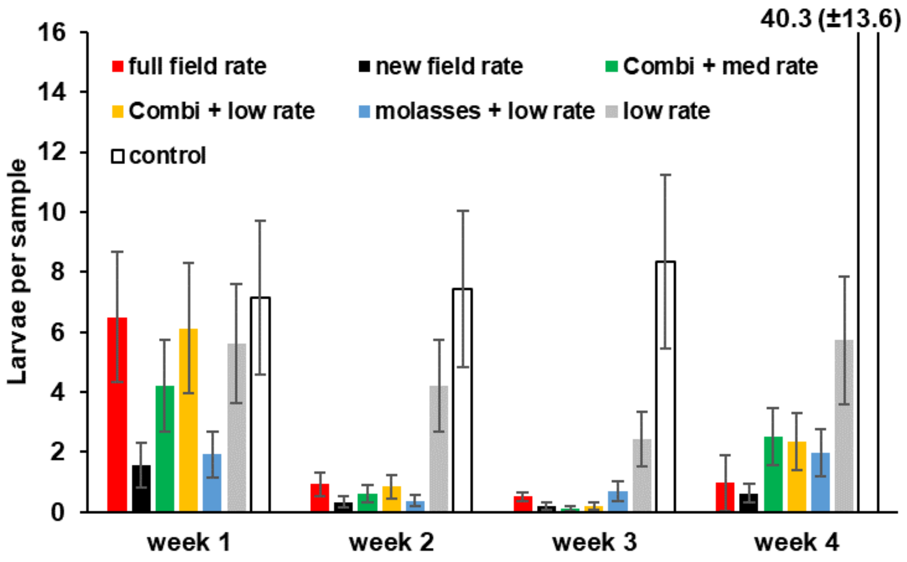


Figure 3.4.5. Effect of full and new rate applications of Tracer in weeks 1 and 3 and Exirel in weeks 2 and 4, and reduced or dilute applications of the same insecticides applied in the same weeks with and without baits (Combi-protec or molasses) on the weekly numbers of larvae from flotation tests on samples of 20 cherries. Mean values of samples taken from different distances from the sprayer nozzle (±SE), n = 15.

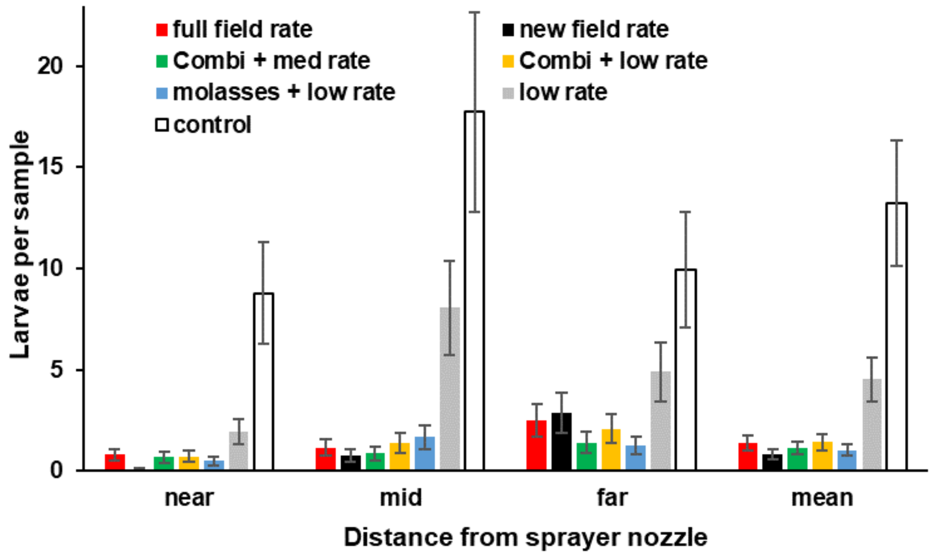


Figure 3.4.6. Effect of full and new rate applications of Tracer in weeks 1 and 3 and Exirel in weeks 2 and 4, and reduced or dilute applications of the same insecticides applied in the same weeks with and without baits (Combi-protec or molasses) on the numbers of larvae from flotation tests on samples of 20 cherries taken from different distances from the sprayer nozzle (±SE). Mean values of samples taken in four weeks (±SE), n = 20; overall mean, n = 60.

The difference between the low rate sprays and other insecticide treatments was only significant for samples taken from the mid distance from the spray nozzle, and for samples averaged across all three spray nozzle distances (Figure 3.4.6).

Although the *D. suzukii* numbers from flotation tests in untreated control plots were highest in the middle of the tree canopy, control with full and new field rate insecticide treatments decreased with increasing distance from the spray nozzle (Figure 3.4.6). The effect of distance from the spray nozzle on the efficacy of the bait sprays was less pronounced.

Residue analysis

All the residue concentrations were within the EU MRLs for spinosad, cyantraniliprole and acetamiprid in cherries. Where insecticide residues were detected, these were highest in fruit samples taken from the sprayed sides of the trees (Tables 3.4.7, 3.4.8 and 3.4.9). Acetamiprid residues of <0.025 mg kg^{-1} were found in some of the fruit samples taken from the near and mid tree positions, although this insecticide was sprayed for control of black cherry aphid at least 36 days before fruit sampling. No spinosad or cyantraniliprole residues were found in any of the fruit samples taken from the far sides of trees or from any tree canopy positions in the low rate bait sprayed treatments. Residues of spinosad and cyantraniliprole in samples taken from the full field rate, new field rate and Combi-protec medium spray plots corresponded with the insecticide application rates for these insecticides (Tables 3.4.7 and 3.4.8). Residues of spinosad were higher after the second Exirel spray than after the second Tracer spray (Table 3.4.7). Residues of cyantraniliprole were higher after the second Exirel spray than after the second Tracer spray (Table 3.4.8).

Table 3.4.7. Residues of spinosad in fruit samples taken from different tree canopy positions immediately after the second spray of Tracer (mg/kg fruit)

Sample timing	After 2nd Tracer			After 2nd Exirel		
Canopy position	Near	Mid	Far	Near	Mid	Far
1 Full field rate	0.07	<0.01	<0.01	0.13	0.03	<0.01
2 New Tracer rate	0.03	<0.01	<0.01	0.05	<0.01	<0.01
3 Combi-protec Low	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
4 Molasses Low	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
6 Combi-protec Medium	0.06	<0.01	<0.01	0.06	<0.01	<0.01

EU MRL 1.5 mg/kg fruit

Table 3.4.8. Residues of cyantraniliprole in fruit samples taken from different tree canopy positions immediately after the second sprays of Tracer and Exirel (mg/kg fruit)

Sample timing	After 2nd Tracer			After 2nd Exirel		
Canopy position	Near	Mid	Far	Near	Mid	Far
1 Full field rate	0.28	0.04	<0.01	0.20	0.03	<0.01
2 New Tracer rate	0.34	0.11	0.02	0.21	0.03	<0.01
3 Combi-protec Low	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
4 Molasses Low	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
6 Combi-protec Medium	0.07	0.02	<0.01	0.07	0.02	<0.01

EU MRL 0.9 mg/kg fruit

Table 3.4.9. Residues of acetamiprid in fruit samples taken from different tree canopy positions immediately after the second sprays of Tracer and Exirel (mg/kg fruit)

Sample timing	After 2nd Tracer			After 2nd Exirel		
Canopy position	Near	Mid	Far	Near	Mid	Far
1 Full field rate	0.02	0.01	<0.01	0.02	0.01	<0.01
2 New Tracer rate	<0.01	0.01	<0.01	0.01	<0.01	<0.01
3 Combi-protec Low	0.01	<0.01	<0.01	0.02	<0.01	<0.01
4 Molasses Low	0.02	<0.01	<0.01	0.01	0.01	<0.01
6 Combi-protec Medium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

EU MRL 1.5 mg/kg fruit

Spray deposition analysis

The spray deposition data was divided by canopy section and leaf side into 18 surfaces for each treatment (Figure 3.4.7). The results show that for the full rate spray (500 L/ha), coverage was >20% for leaf surface nearest the spray nozzle but <10% further from the nozzle. The lower leaf surfaces nearest the spray nozzle at the middle height and top of trees received the highest coverage, and the upper surfaces of leaves furthest from the nozzle at the middle height and bottom of trees the lowest. Coverage on lower leaf surfaces furthest from the nozzle was <0.5%. Spray deposition coverage was significantly higher (between 5 and 134 times higher in corresponding positions) for the full rate application (500 L/ha) than for the Combi-protec + low rate application (40 L/ha), except on leaves furthest from the spray nozzle at the middle tree height (both leaf surfaces) and top of trees (upper leaf surface) (Figure 3.4.7). For the bait spray, only leaf surfaces nearest the spray nozzle at the middle height and top of trees received 1-4% spray coverage. In all other canopy positions, spray coverage was <0.4%.

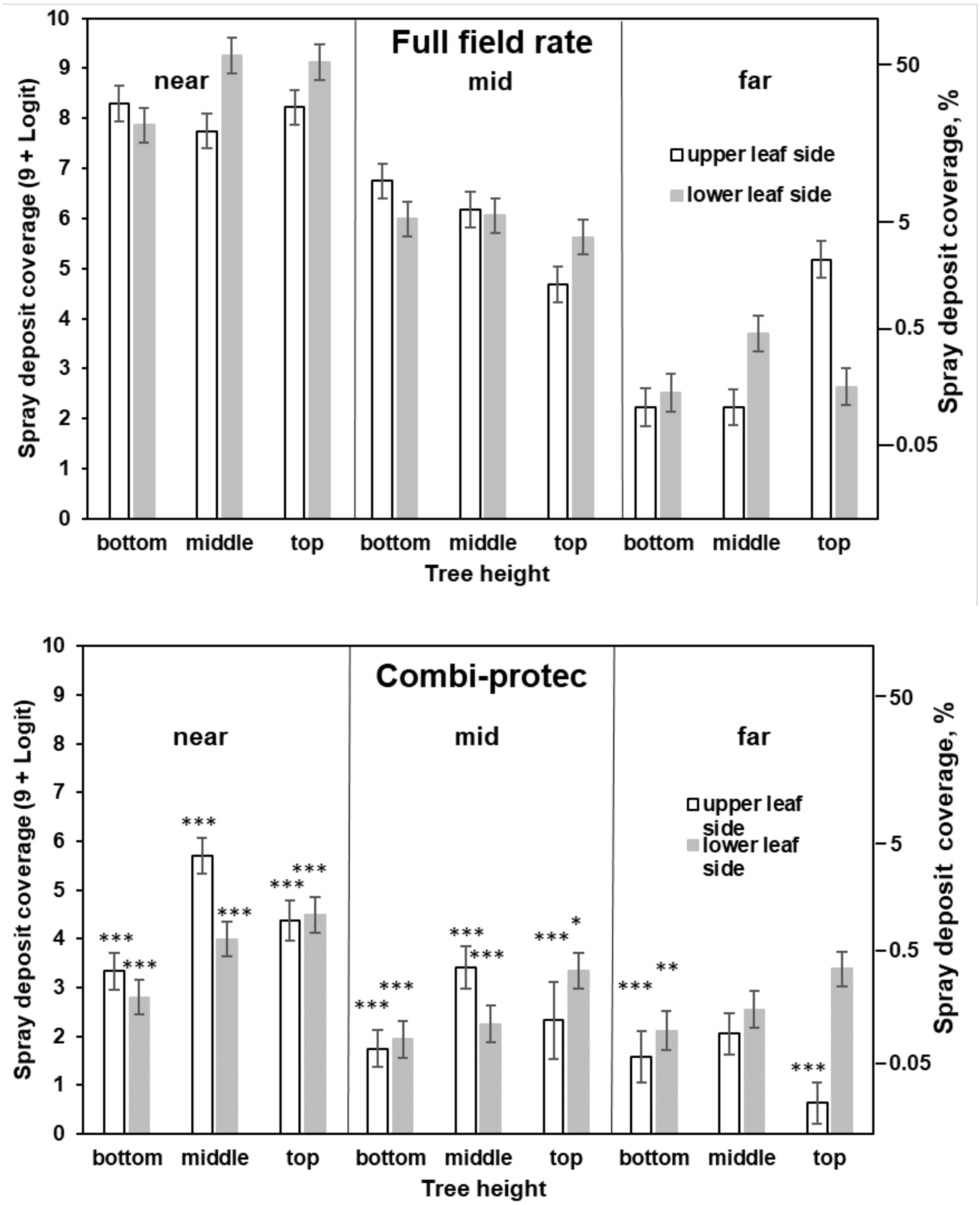


Figure 3.4.7. Spray deposition as percentage coverage on the leaves of cherry trees across all canopy sections and leaf side (Logit transformation). Trees were sprayed at the full field rate or at the low rate with Combi-protec (Table 3.4.4). Mean values (±SE), n = 25. Asterisks denote leaf surfaces where spray deposit coverage was significantly lower for the Combi-protec +low rate spray than for the full rate spray.

For full the rate spray, the decreasing spray coverage on leaves with increasing distance from the spray nozzle corresponded with the decreasing control efficacy of *D. suzukii*. However, for the bait sprays, despite significant differences in spray coverage, differences in *D. suzukii* control efficacy between canopy positions were less pronounced. This indicates that for bait sprays, the pest was attracted to spray droplets from other parts of the tree canopy, and that uniform spray coverage is less important that with full rate sprays.

Spray costs

Estimated materials and labour costs of the treatments are based on the tree planting density (1600 trees/ha) and spray application times used in this report (Table 3.4.10). The materials costs are based on prices per litre for Tracer (£340), Exirel (£150), and Combi-protec (£40). No price is yet available for an approved adjuvant form of molasses but this assumed to be £25/Litre.

Labour costs are based on £13/hour. The low and medium volume bait spray applications are made with a knapsack sprayer. The spraying time is 1.1 hours/ha with an additional 1 hour for three refills of a 15 litre spray tank (total 2.1 hours/ha for labour). A nominal £3/ha has been added for the on-going costs of the knapsack sprayer. Application of high volume, full rate sprays with a knapsack sprayer would not be viable, based on the application times in this report (the application time per hectare would be several hours for each spray). Using a knapsack sprayer, there is also greater risk of operator exposure to insecticides using full rate sprays than to low rate sprays. It was therefore assumed that high volume, full rate sprays would be made with an air assisted sprayer trailed by a tractor. The cost of four 500L/ha full rate sprays with an air assisted sprayer trailed by a tractor was estimated from costs for apple orchard spraying by the Australian Apple and Pear Levy (Manktelow, 2014) using an annual inflation of 3%. The labour requirement is 1.8 hours/ha, with additional costs for fuel and depreciation of machinery.

Table 3.4.10. Materials and application costs of four sprays of insecticide or insecticide + bait treatments.

Treatment number and name	Application method	Materials £/ha	Application £/ha	Total £/ha
1 Full field rate	Tractor + air sprayer	440	134	574
2 New Tracer rate	Tractor + air sprayer	338	134	472
3 Combi-protec Low	Knapsack sprayer	178	124	302
4 Molasses Low	Knapsack sprayer	118	124	242
6 Combi-protec Medium	Knapsack sprayer	431	124	555
Molasses Medium	Knapsack sprayer	311	124	435

The materials costs of the low rate insecticide + bait sprays (Treatments 3 and 4) are lower than the high volume, new Tracer with existing Exirel rate sprays (Treatment 2). If Combi-protec is used in medium insecticide rate sprays (Treatment 6), the cost is higher than the new Tracer with existing Exirel rate sprays (Treatment 2). If molasses is used in the medium rate sprays (bottom row), the materials cost is similar to Treatment 2.

A small cost saving in applying low volume sprays with a knapsack sprayer compared with high volume sprays with a tractor mounted air assisted sprayer was calculated (Table 3.4.10). Overall, the total cost of applying four low volume, low rate insecticide with molasses bait sprays (Treatment 4) would be around 50% of the high volume, new Tracer with existing Exirel field rate sprays (Treatment 2).

Conclusions

1. Weekly alternating dilute applications of Tracer at 10 ml in 40L per ha and Exirel at 36 ml in 40L per ha, combined with Combi-protec or molasses baits, were as effective in controlling *D. suzukii* numbers as full field rates of the same insecticides applied at 250 or 900 ml in 500L per ha (i.e. a reduction in insecticide application of 96% with the same *D. suzukii* control effect).
2. Control of *D. suzukii* was equally good with the molasses and Combi-protec bait spray treatments.
3. Control of *D. suzukii* was equally good with the full field rate and new reduced Tracer rate spray treatments without bait.
4. The above treatments maintained good control of *D. suzukii* during the first three assessment weeks of the crop; by the fourth week, the majority of the fruit was over-ripe, resulting in very high level of *D. suzukii* infestation and reduced treatment efficacy.
5. The application time for the bait sprays was 10% of the full field rate application of insecticide sprays.
6. Compared with untreated control plots, the dilute rates of insecticides reduced *D. suzukii* numbers by about 50%; the inclusion of baits significantly improved this control effect.
7. There were similar proportions of male and female *D. suzukii* in all the boxes from the unsprayed, insecticide and insecticide + bait treatments.
8. *D. suzukii* numbers determined from adult emergence in boxes corresponded with larvae flotation tests, although the latter were only 30% of the former.
9. Residues of spinosad and cyantraniliprole in fruit samples taken from the full field rate, new field rate and Combi-protec medium spray plots were below the EU MRLs.

10. No spinosad or cyantraniliprole residues were found in any of the fruit from the low rate bait spray treatments.
11. None of the insecticide or insecticide + bait treatments caused phytotoxicity symptoms and there was no mould growth on the bait spray droplets.
12. Spray deposition coverage was between 5 and 134 times higher in corresponding positions for the full rate application (500 L/ha) than for the Combi-protec + low rate application (40 L/ha), except on leaves furthest from the spray nozzle at the middle tree height (both leaf sides) and top of trees (upper leaf side).
13. If molasses bait with low insecticide rates in low volume applications are used instead of new Tracer and existing Exirel field rates in high volume applications, the savings in materials and total spray application costs are around 50%.

Objective 8. *D. suzukii* and insecticide tolerance

Task 8.1. Investigating the susceptibility of D. suzukii to approved plant protection products (NIAB)

Introduction

Since its arrival to the UK in 2012 chemical plant protection product (cPPP) control has played a vital role in supressing *D. suzukii* numbers in affected crops. Although there are other control options which are effective in providing protection, very few are a fast acting and as quick to show an effect. However, in 2018 the first report of insecticide resistance was published. An increased tolerance to spinosad was detected in Californian organic raspberries by Gress and Zalom (2018) who found flies from treated areas required 4.3-7.7 times higher dose than those from untreated areas. The dose in treated-area populations was also 11-22 times higher than the susceptible population baseline identified a few years previous. There is now widespread resistance in Californian raspberry to spinosad (personal communication R. Isaacs). *D. suzukii* was first detected in California in 2008 (Bolda et al. 2010) meaning this increased tolerance has developed within 10 years. It is therefore not unreasonable to predict resistance could be detected within UK populations within the next few years, since *D. suzukii* was found in 2012 (Harris and Shaw 2014).

Although organic growers are limited to very few insecticides, spinosad is used within conventional spray programs and has been regarded as one of, if not the most effective active against *D. suzukii.* It is likely that resistance to spinosad has been driven by a lack of rotation of modes of action in organic growing. If so, then conventional growers need to ensure they are not relying on any one single product and use the range of products available to them. With the PhD project by Shaw (2019), it became apparent that there were variations in tolerances to cPPP within laboratory populations, with some females surviving high doses of products and then continuing to egg lay, with no detrimental effect on offspring survival.

In 2019, laboratory strains were established from wild populations of *D. suzukii* to identify a baseline level of susceptibility to commonly used PPP. Three wild populations were collected from soft and stone fruit farms in the South-East of England and mass reared in the laboratory. They were established from crops with a known insecticidal input and included two commercial crops and one with minimal inputs. These were compared an unsprayed laboratory strain, which has been in culture since 2013 and is expected to have a very low tolerance to PPP. There were varying levels of susceptibility to three PPPs, lambda-cyhalothrin (Hallmark), cyantraniliprole (Exirel) and spinosad (Tracer) tested between the three wild populations and the laboratory strain.

In 2019 there were several differences in susceptibility between the lab and wild strains at various doses. While there have, to date, been no reports of resistance developing to cyantraniliprole and lambda-cyhalothrin, within this objective we found varying tolerances to the products tested.

In 2020, this study aimed to determine if insecticide tolerance is occurring in UK populations of *D. suzukii* and to see if there are differences in susceptibility between seasons and time of the year (2020 and 2020B strains) and between years (2019 and 2020). Due to issues with the rearing of flies we were unable to full report the 2020 results in the previous project and so some of the 2020 results are included below.

A comparison of the past three years of data collection is on-going at present and results gathering of this project should be complete by March 2022.

Materials and Methods

Wild strain collection for 2020, 2020-B and 2021: Ripe waste fruit was collected from commercial field sites in Kent (Table 8.1.1) in July 2020 and 2021 (early season populations) and at the beginning of November 2020 (end of season populations). Fruit was transferred to standard emergence boxes (a ventilated, Perspex box lined with blue roll, stored at 20°C). Fruit was stored for three weeks and checked weekly for the emergence of adult Drosophila. Any flies that emerged were collected and sedated with CO_2 for species to be identified under a microscope. All *D. suzukii* were transferred to 25 mm x 90 mm glass vials containing Drosophila media (cornmeal, sugar, yeast, malt, soya flour and agar, with propionic acid and nipagin for anti-microbial and anti-fungal properties) and labelled with a farm and crop identification. Vials were closed with cotton wool. After three weeks the fruit was frozen and disposed of.

Culturing of strains: Once transferred to culture vials, wild strain flies were stored at 20°C, 16:8 light:dark cycle. Flies were tipped into new vials once a week and offspring were mixed between vials to prevent genetic bottlenecks. Vials were labelled with generation number. Once enough numbers had developed (generation 8-10) laboratory bioassays were performed.

Direct spray bioassay: A 9 cm filter paper (Whatman 5) was placed in the base of a 9 cm plastic Petri dish. A cigarette filter (Swan, slim filter tip) soaked in a distilled water solution (10 g granulated table sugar in 100 ml distilled water), was added to the filter paper. Three to seven-day old *D. suzukii* from mix sex populations were anaesthetised on a CO_2 pad (FlyStuff). Six males and six females were transferred to the Petri dish. The Petri dish (spray arena) was then covered with a 4 mm metal mesh to prevent flies escaping. Flies could recover for a minimum of 10 minutes before and after spray treatments were applied.

Table 8.1.1. Collection and spray information of strains of *D. suzukii.*

Grower/ Adviser	Farm ID	Crop	Spray exposure 2020
Graham Caspell	WS1	NIAB EMR Breeder plot cherries (mixed varieties)	Minimal: Calypso: 27/04 Tracer: 19/06, 03/07, 16/07, 05/08 Exirel: 11/06, 26/06, 24/07, Hallmark/Liadir: 15/07
Confidential	WS2	Raspberry (Maravilla)	Commercial: Decis: 21/04, 29/04 Pyrethrum: 06/05, 29/05 Calypso: 07/06, 18/06 Naturalis: 14/07
Confidential	WS3	Raspberry (Kweli)	Commercial: Calypso: 20/05, 02/08 Hallmark: 02/07 Tracer: 18/09

Grower/ Adviser	Farm ID	Crop	Spray exposure 2021
Graham Caspell	WS1	NIAB EMR Breeder plot cherries (mixed varieties)	Minimal: Exirel: 29/07, 01.07 Tracer: 22/07, 12/07 Batavia: 01/11
Confidential	WS2	Strawberry (mixed varieties)	

Confidential	WS3	Raspberry (Paris, Bella, Rafiki, Kweli) Strawberry (Murano)	Commercial: Calypso: 23/01, 26/01, 27/01 Decis: 03/05 Hallmark: 03/05, 01/06 Tracer: 14/09

The maximum field rate (FR) dose for cherry, strawberry or raspberry of lambda-cyhalothrin (Hallmark), cyantraniliprole (Exirel) and spinosad (Tracer) were prepared in line with 2018 label rates. This was to ensure years could be compared even if recommended rates or amount of A.I. in the product changed over time. Serial dilutions were then produced to include % rates in Table 8.1.2. The dose range was dictated by results from the 2019 bioassay. Dilutions were prepared no more than 30 minutes before direct application by a Burkhard benchtop computer-controlled sprayer (used on all laboratory, 2019, 2020 and 2020B strains; except WS3-20B treated with spinosad) or a measured spray bottle (WS3-20B treated with spinosad and all 2021 strains). A control of distilled water was applied for comparison to each insecticide. Applications of rate were made in ascending order starting with the water control. After application, flies could recover for 10 minutes within the arena, after which the flies were transferred to a glass vial containing *Drosophila* media and returned to the previously stated environmental conditions.

Application of treatment was modified during the treatment of the WBC2020B strain and for all 2021 strains, this was due to an unforeseen malfunctioning with the Burkard sprayer (Figure 8.1.1). An alternative method was devised, where a spray bottle (Figure 8.1.2) with a calibrated output was mounted and used to apply treatments. All other processes and equipment remained unchanged. Both Burkard and spray bottle were calibrated prior to spraying treatments to flies.

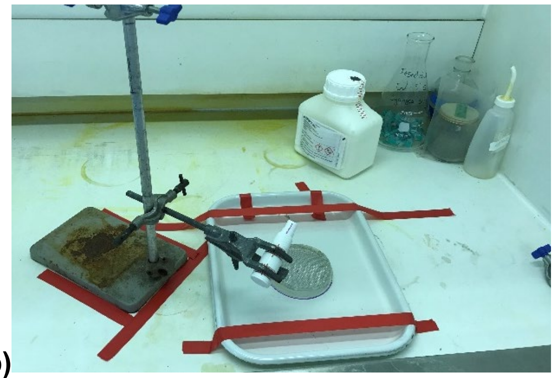
a)  b) 

Figure 8.1.1a Burkard sprayer used to apply treatments to all strains and PPP excluding WS3-20B spinosad. **b** calibrated spray bottle used to apply treatments to WS3-20B spinosad due to malfuction of burkard sprayer.

Table 8.1.2. Products and % rates tested in bioassays.

Active ingredient (% active ingredient in formulation)	**Trade name and (company)**	**Maximum field rate ml/ha**	**% Active Ingredient in maximum field rate ml/ha**	**Dilution range of % FR**
Cyantraniliprole (10)	Exirel (DuPont)	1125	112.5	1.5, 3, 6, 12, 25 +Water control
Lambda-cyhalothrin (10)	Hallmark Zeon® (Syngenta)	75	7.5	6, 12, 25, 50, 100 +Water control (used for Lab)
Spinosad (44.03)	Tracer® (Dow AgroSciences)	150	66	3, 6, 12, 25, 50 +Water control

Flies were assessed 24 hrs after application and were categorised as:

- Dead
- Heavily moribund (individuals are those flies that are on their back or side with one or more legs twitching. These are flies that are clearly almost dead, but still technically alive)
- Lightly moribund (flies that are clearly suffering effects of the insecticide but are still able to move around. Characteristics to look for in lightly moribund individuals include: 1) Flies walking in a slow, staggering manner, clearly affected by the insecticide. Sometimes flies will walk around in circles, while other times flies will walk slowly sideways. 2) Flies unable to hold on to the vial surface when vial is moved. Very often these flies will also have a hard time righting themselves when they fall off and are on their backs. 3) Individuals will often exhibit wing and leg cleaning behaviour as well.
- Alive

The results were analysed by fitting a dose response curve and Probit analysis. For this 'dead' and 'heavily moribund' are classed as total dead counts and 'lightly moribund' and 'alive' are classed as 'total alive'. Each wild strain and insecticide combination were analysed individually. Comparison of LC50s between years were made for each site. For the analysis probit analysis was performed on 'live' and 'dead' counts. Tukey post hoc analysis was performed with adjusted p values for a family of 3 estimates.

Results

The early season strains were collected from fruit at the end of July 2020. The end of season populations were collected from fruit at the beginning of November 2020.

Due to the logistical operations being affected by the pandemic, the early season wild strains took several months to build-up enough flies to execute the bioassays and provide full analysis of the results. The statistical analysis is now complete for the entire 2020 strains (early and late seasons) and the 2021 strains.

Of the 2021 strains WS3 was unable to be treated due to a population crash over the winter. The strain was unable to be re-established due to wild flies being in reproductive dormancy and so a new population was not able to be established.

WS1 – Early (20) and late (20B) season population mortality

WS1 is the wild population established from flies collected at East Malling from a cherry orchard that receives minimal insecticide applications. Large numbers of SWD emerged from the WS1-20 waste fruit collected in July 2020 which enabled rapid population increase in the lab. Roughly 25% of WS1-20 flies and ~31% of WS1-20B flies treated with the highest dose (25% of field rate) of cyantraniliprole survived 24 hours post application (Figure 8.1.3). This is consistent with the results from 2019 for this insecticide and strain. From treatment concentrations 1.5-25% for strains WS1-20&20B, there is a significant difference in survival, with WS1-20B, the later season strain having higher survival.

For WS1-20 flies treated with Spinosad, 100% mortality occurred in the highest dose (50% of field rate), whereas 33% of WS1-20B flies survived the same dose (Figure 8.1.4). From treatment concentrations 3-50% there was a significant difference in survival, where WS1-20B, the later season strain having higher survival.

Roughly 8% of WS1-20 flies and 27% of WS1-20B flies treated with 100% field rate of lambda-cyhalothrin (the highest dose) survived 24 hours post application (Figure 8.1.5). From treatment concentrations 6-100% there was a significant difference in survival, where WS1-20B had higher survival. Overall there was higher than expected survival in the WS1-20B strain in the three highest doses with no obvious explanation.

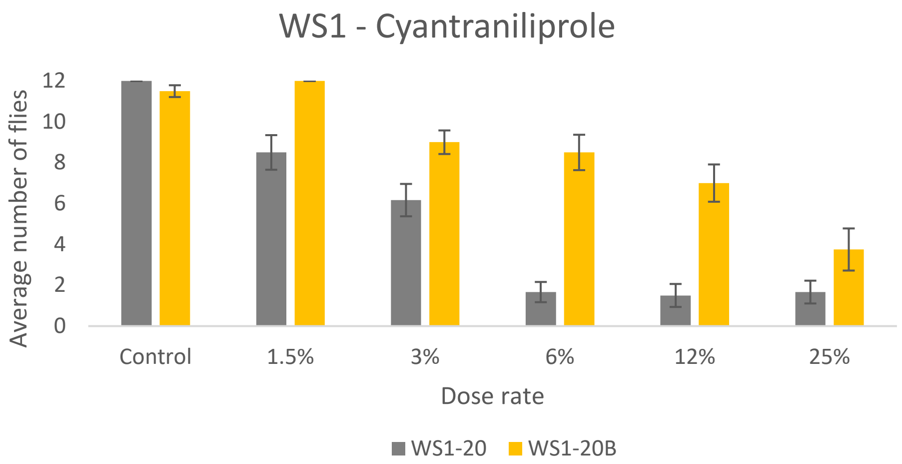


Figure 8.1.3. Average number of live WS1-20 (early season strain) (grey) and WS1-20B (late season strain) (orange) after 24 hours post spray treatment (+/- standard error) with Cyantraniliprole. Treatments are displayed as a percentage of the recommended field rate.

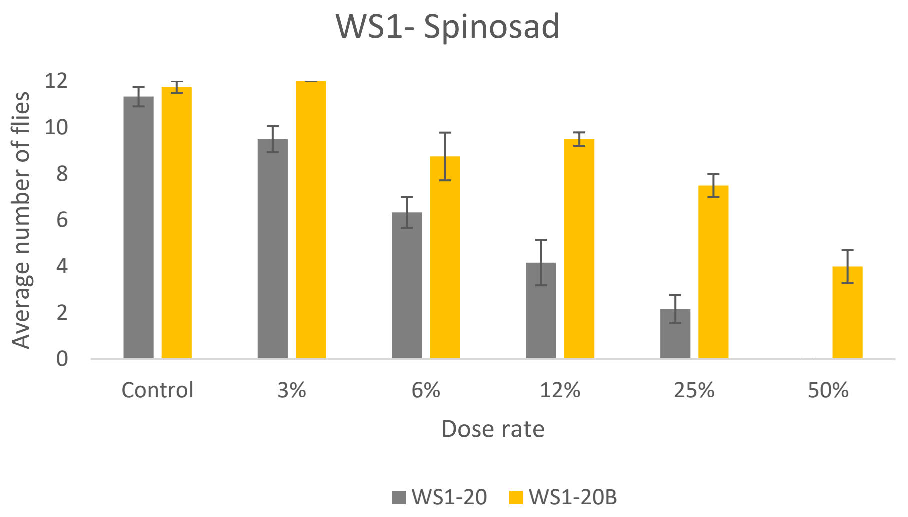


Figure 8.1.4. Average number of live WS1-20 (early season strain) (grey) and WS1-20B (late season strain) (orange) after 24 hours post spray treatment (+/- standard error) with spinosad. Treatments are displayed as a percentage of the recommended field rate.

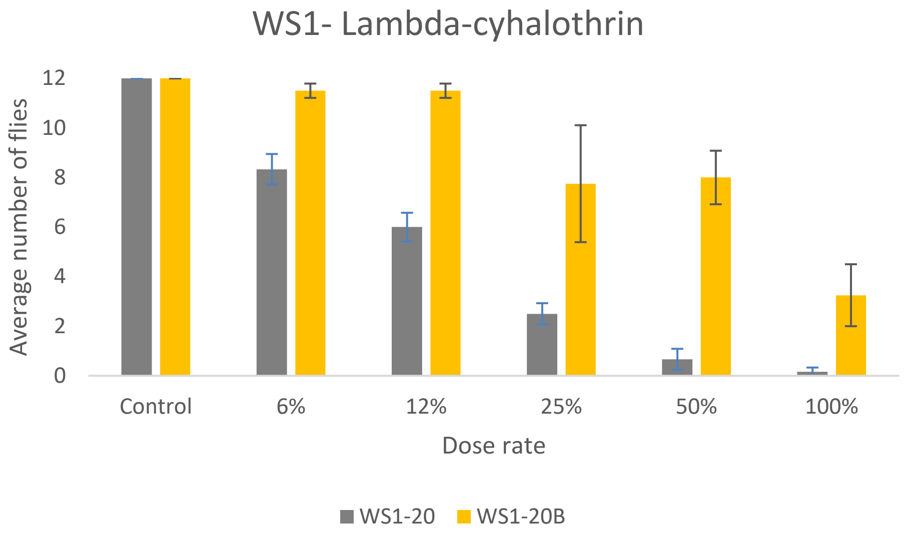


Figure 8.1.5. Average number of live WS1-20 flies alive (grey) and WS1-20B (orange) after 24 hours post spray treatment (+/- standard error) with Lambda-cyhalothrin. Treatments are displayed as a percentage of the recommended field rate.

WS2- 2020 Early (20) and late (20B) season population mortality

WS2 is the wild population established from flies collected from a raspberry crop that receives commercial insecticide applications (Table 8.1.1).

21% of WS2-20 flies and 33% of WS2-20B flies treated with the highest dose (25% of field rate) of cyantraniliprole survived 24 hours post application (Figure 8.1.6). All treatment concentrations (excluding Control and 50%) show a significant difference in survival, with WS2-20B flies displaying higher survival.

For WS2 flies treated with spinosad, 8% of WS2-20 flies and 4% of WS2-20B flies with the highest dose (50% of field rate) survived (Figure 8.1.7). From treatment concentrations 3, 12 & 25% there was a significant difference in survival. On average, WS2-20B had a higher survival at treatments 3% and 6%, then WS2-20 had higher survival at 12, 25 and 50%.

1% of WS2-20 flies and 54% of WS2-20B flies treated with 100% field rate of lambda-cyhalothrin (the highest dose) survived 24 hours post application (Figure 8.1.8). At treatment concentration 25% WS2-20B flies had a significant decreased survival average of 42% and increased back to 69% at treatment concentration 50%. From treatment concentrations 6-100% there was a significant difference in survival, where WS2-20B had the higher survival per treatment. For WS2-20B flies sprayed with lambda-cyhalothrin, the trend shown in Figure 8.1.8 is unexpected and does not follow other treatments, and again there is no obvious justifications for this result. It may be that resistance is developing in field populations and analysis from the 2021 wild populations will aid in confirming this.

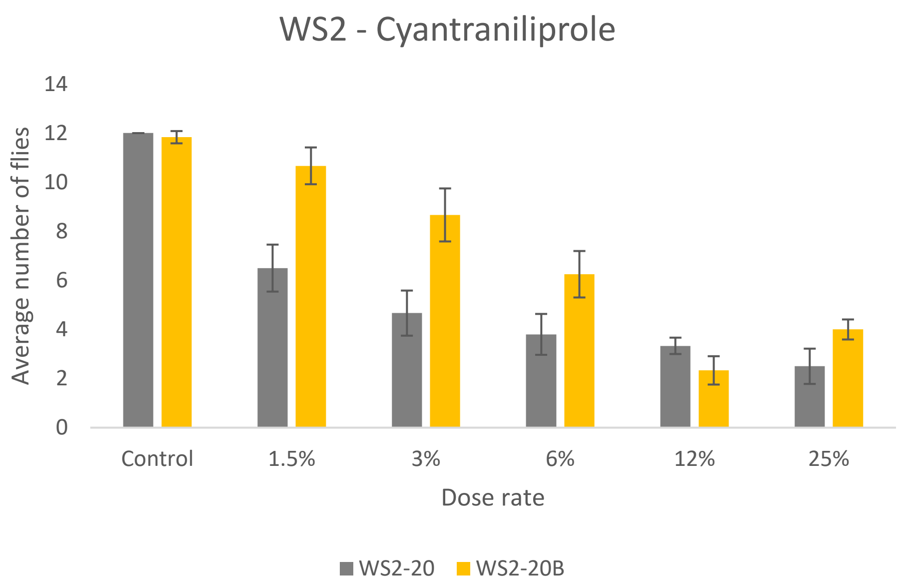


Figure 8.1.6. Average number of live WS2-20 (early season strains) (grey) and WS2-20B (late season strains) (orange) after 24 hours post spray treatment (+/- standard error) with Cyantraniliprole. Treatments are displayed as a percentage of the recommended field rate.

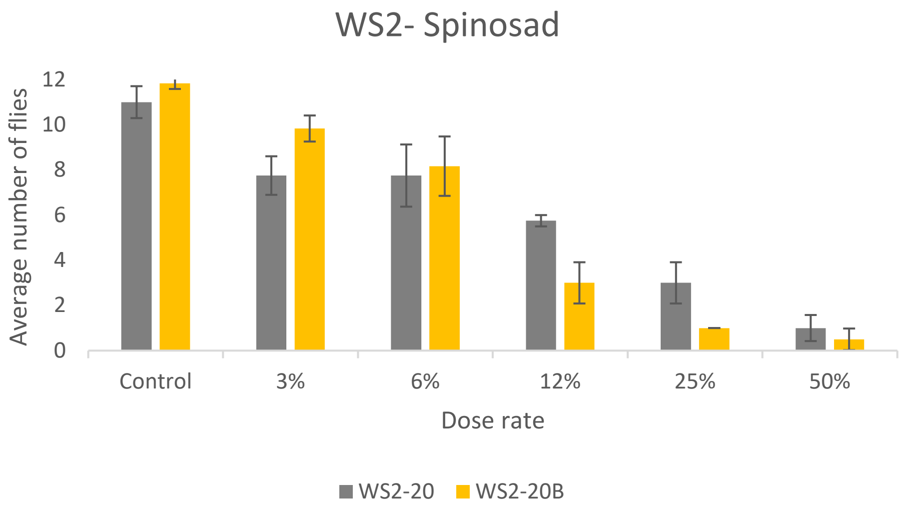


Figure 8.1.7. Average number of live WS2-20 (early season strains) (grey) and WS2-20B (late season strains) (orange) after 24 hours post spray treatment (+/- standard error) with spinosad. Treatments are displayed as a percentage of the recommended field rate.

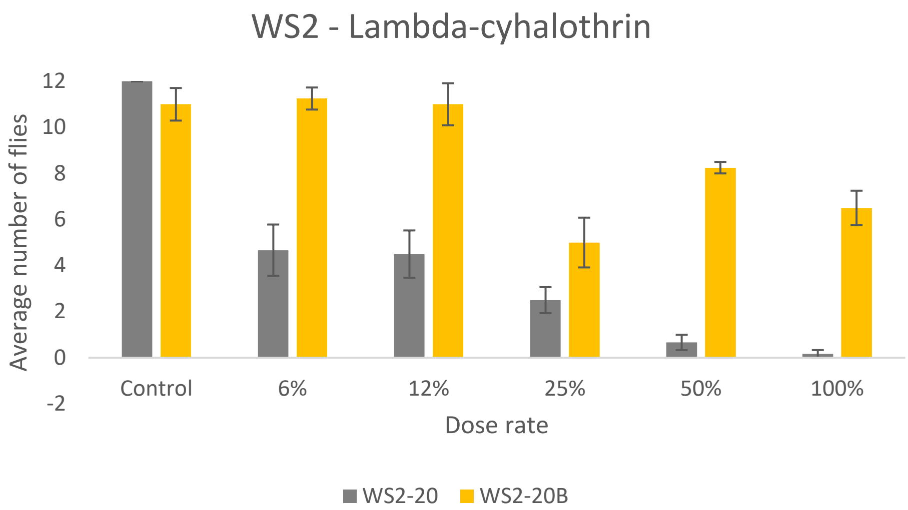


Figure 8.1.8. Average number of live WS2-20 (early season strains) (grey) and WS2-20B (orange) after 24 hours post spray treatment (+/- standard error) with Lambda-cyhalothrin. Treatments are displayed as a percentage of the recommended field rate.

WS3- 2020 Early (20) and late (20B) season population mortality

WS3 is the wild population established from flies collected from a raspberry crop that receives commercial insecticide applications (Table 8.1.1). WS3-20 (early season) populations struggled to establish initially due to low number of SWD in the collected fruit.

19% of WS3-20 flies and 67% of WS3-20B flies treated with the highest dose (25% of field rate) of cyantraniliprole survived 24 hours post application (Figure 8.1.9). All treatment concentrations (excluding control) show a significant difference in survival, with WS2-20B flies displaying consistently higher survival. The survival trend for WS3-20 flies display a minor significant increase in survival from 12 to 25% survival.

For WS3 flies treated with spinosad, 63% of WS2-20 flies and 42% of WS2-20B flies with the highest dose (50% of field rate) survived (Figure 8.1.10). From treatment concentrations 6 and 12% there was a significant difference in survival, showing higher survival with WS3-20B flies. At treatment concentration 50%, WS3-20 shows a significantly higher survival than WS3-20B flies.

4% of WS3-20 flies and 35% of WS3-20B flies treated with 100% field rate of lambda-cyhalothrin (the highest dose) survived 24 hours post application (Figure 8.1.11). Treatment concentration 6 and 12% there was a significant difference in survival, where WS3-20 had the higher survival. For 12, 25 and 100% concentrations, there are no significant differences between the average survival for WS3-20B strain. WS3-20B flies sprayed with 25% lambda-cyhalothrin, display a rise in survival (rather than decrease) at this treatment concentration.

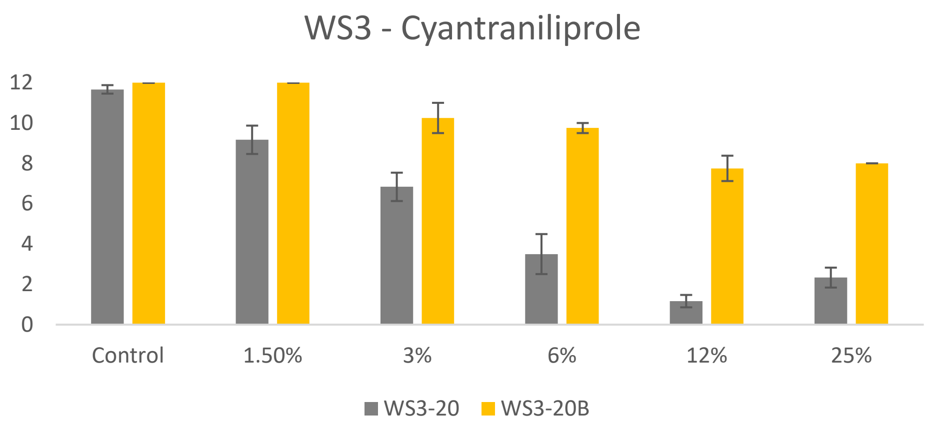


Figure 8.1.9. Average number of live WS3-20 (early season strains) (grey) and WS3-20B (late season strain) (orange) after 24 hours post spray treatment (+/- standard error) with Cyantraniliprole. Treatments are displayed as a percentage of the recommended field rate.

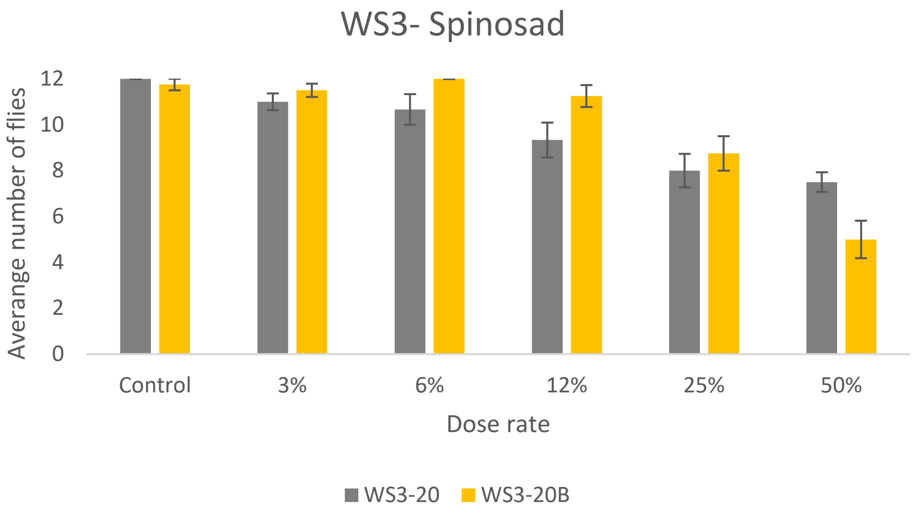


Figure 8.1.10. Average number of live WS3-20 (early season strain) (grey) and WS3-20B (late season strain) (orange) after 24 hours post spray treatment (+/- standard error) with spinosad. Treatments are displayed as a percentage of the recommended field rate.

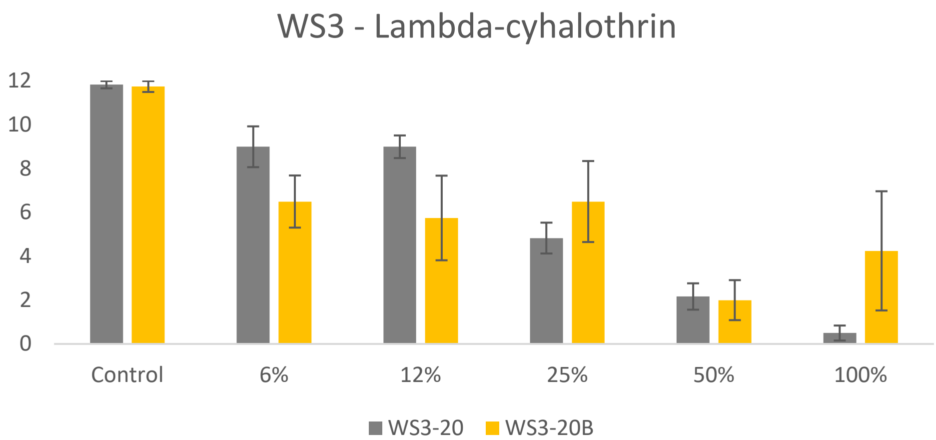


Figure 8.1.11. Average number of live WS3-20 (early season strain) (grey) and WS3-20B (late season strain) (orange) after 24 hours post spray treatment (+/- standard error) with Lambda-cyhalothrin. Treatments are displayed as a percentage of the recommended field rate.

Spinosad- between years

There were significant differences in the overall survival of the strains collected in 2020 and 2019 from all three locations (all $p<0.0001$) when treated with spinosad. There was a significant reduction in survival in 2020 compared to 2019 (Figure 8.1.12). There were no significant differences between years for specific doses.

In overall survival only WS1-20 and WS1-20B were significantly different from one another when treated with Spinosad (Figure 8.1.13).

At the dose level, WS3-20 showed significantly higher survival from 3-50% spinosad compared to WS1-20 and WS2-20 (Figure 8.1.14). There was no significant difference in survival between WS1-20B and WS3-20B at any dose of spinosad. WS2-20B has a significantly lower survival probability at 3-50% doses compared to WS3-20B and 12-50% doses to WS1-20B (Figure 8.1.14).

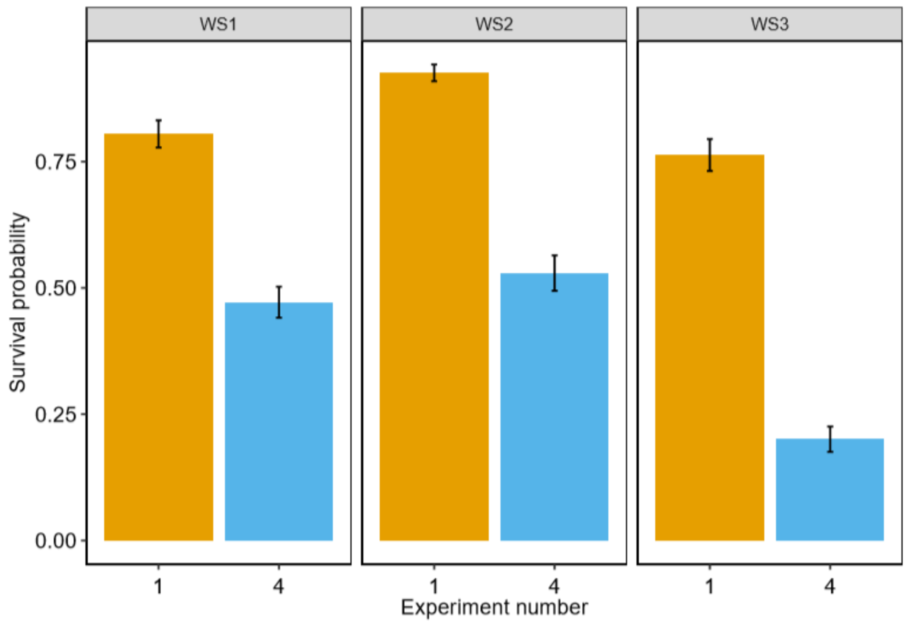


Figure 8.1.12. Survival probability of strains collected in 2019 (orange bars) and strains collected in 2020 (blue bars) from the three locations (WS1, WS2 and WS3) treated with spinosad.

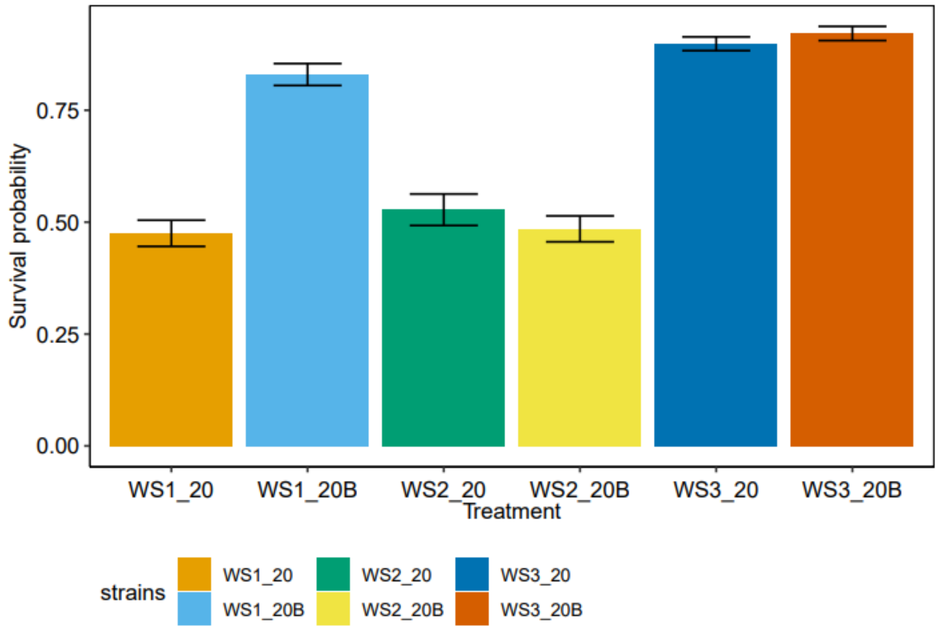


Figure 8.1.13. Survival probability of strains collected at the beginning-of-the-season of 2020 (light orange, green, dark blue bars) and strains collected at the end-of-the-season of 2020 (light blue, yellow, dark orange bars) from the three locations (strain WS1, WS2 and WS3) treated with spinosad.

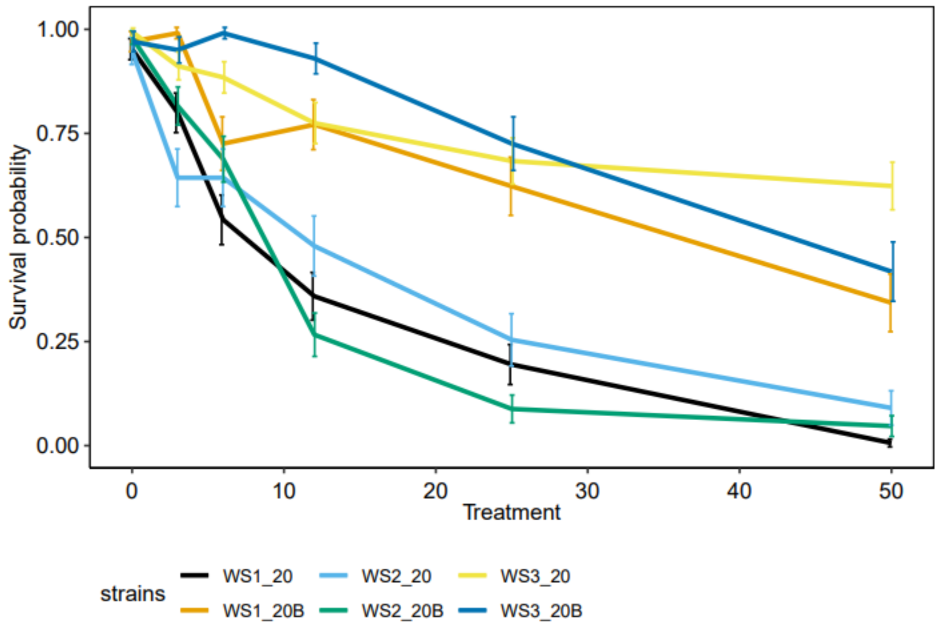


Figure 8.1.14. Survival probability of WS1, WS2 and WS3 strains established at the beginning-of-season 2020 (black, light blue, and yellow line) and end-of-season 2020 (orange, green and dark blue line) when treated with percentage doses of spinosad field rate.

Cyantraniliprole- between years

For WS1 (collected from NIAB EMR) there was no overall difference in survival between 2019 and 2020 when treated with cyantraniliprole. However, there was a significant difference in survival between the two years at 6% (p=0.045) and 12% (p=0.024) of the field rate, with 2020 having higher survival than 2019 (Figure 8.1.15).

In relation to early (WS1,2&3-20) and late (WS1,2&3-20B) 2020 strains, WS1-20B, WS2-20B and WS3-20B show a significant increase in survival probability compared to WS1-20, WS2-20 and WS3-20 (Figure 8.1.16).

Comparing between locations, WS1-20B and WS3-20B display no significant differences in survival, apart from at 1.5 and 25% doses (Figure 8.1.17). WS2-20B has a significantly lower survival probability at 1.5, 6 and 12% doses compared to WS1-20B, and 6-25% doses compared to WS3-20B. All strains and years follow the predicted decreasing survival probability with increasing dose concentration.

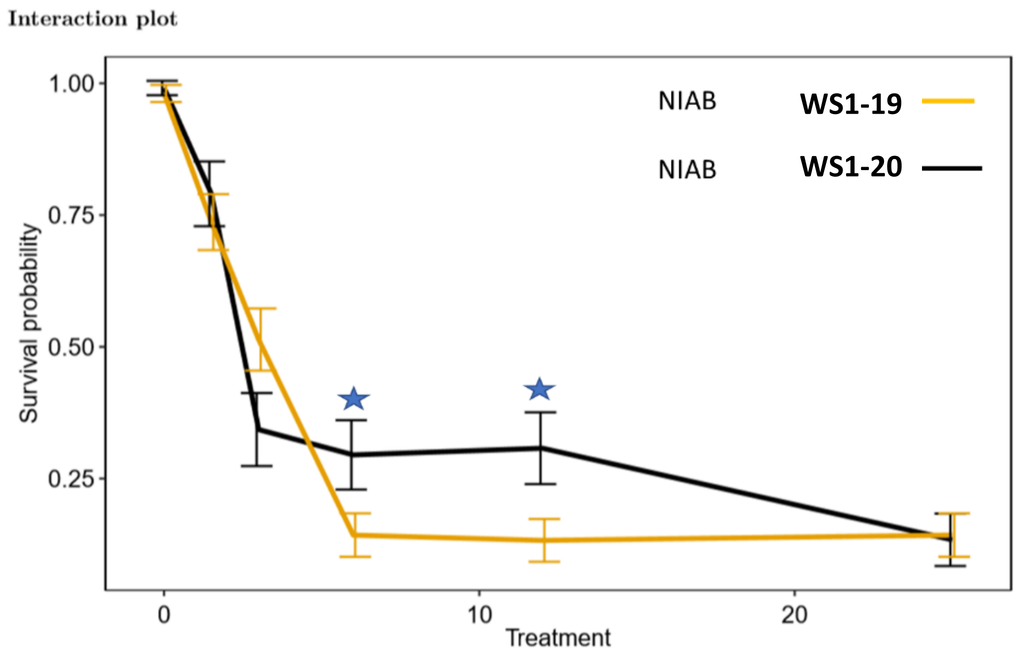


Figure 8.1.15. Survival probability of NIAB EMR (WS1) strains established in 2019 (yellow line) and 2020 (black line) when treated with percentage doses of Cyantraniliprole field rate.

* indicates significant differences.

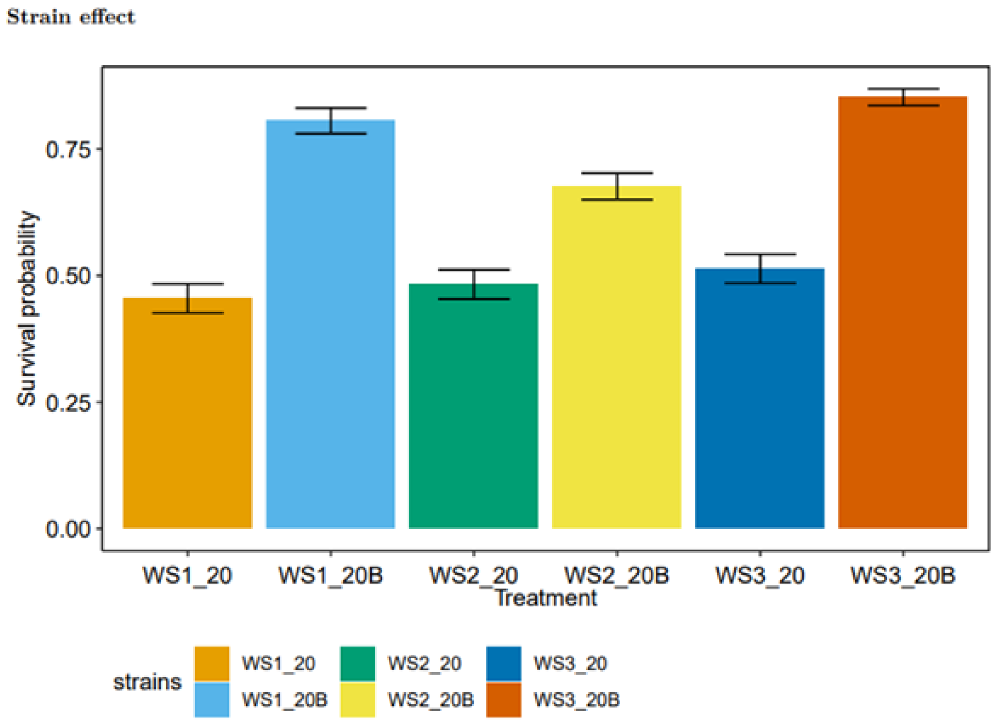


Figure 8.1.16. Survival probability of strains collected at the beginning-of-the-season of 2020 (light orange, green, dark blue bars) and strains collected at the end-of-the-season of 2020 (light blue, yellow, dark orange bars) from the three locations (strain WS1, WS2 and WS3) treated with Cyantraniliprole.

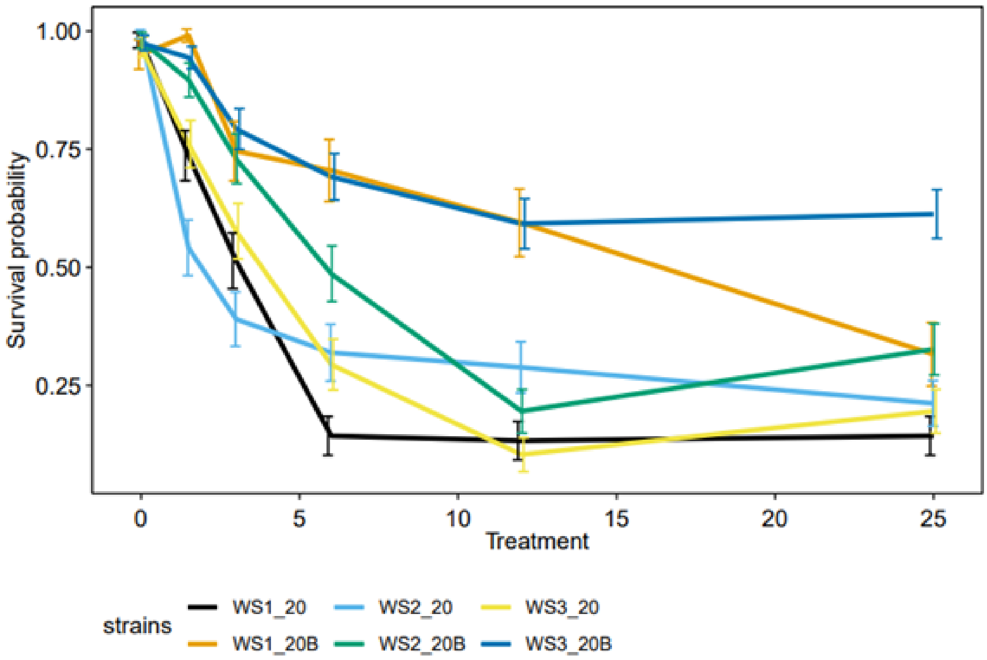


Figure 8.1.17. Survival probability of WS1, WS2 and WS3 strains established at the beginning-of-season 2020 (black, light blue, and yellow line) and end-of-season 2020 (orange, green and dark blue line) when treated with percentage doses of cyantraniliprole field rate.

Lambda-cyhalothrin- between years

For NIAB EMR (WS1) strains there was a significant difference between 2019 and 2020 in survival, with 2020 survival lower than 2019 ($p<0.0001$) (Figure 8.1.18) when treated with lambda-cyhalothrin. There was no significant difference between years based on doses of lambda-cyhalothrin.

Both WS1-20B and WS2-20B had significantly higher survival probability compared to their early season strains (WS1-20 and WS2-20 respectively) (Figure 8.1.19). There was no difference between WS3-20 and WS3-20B.

Between location, WS3-20 showed significantly higher survival from WS1-20 at 12-50% doses and WS2-20 at 6-50% doses (Figure 8.1.20). Locations WS1-20B and WS2-20B were significantly different in survival at 25 and 100% doses. WS3-20B has a significantly lower survival probability at all doses apart from at 25%, where WS2-20B has decreased significantly compared to 12 and 50 % doses within WS3-20B.

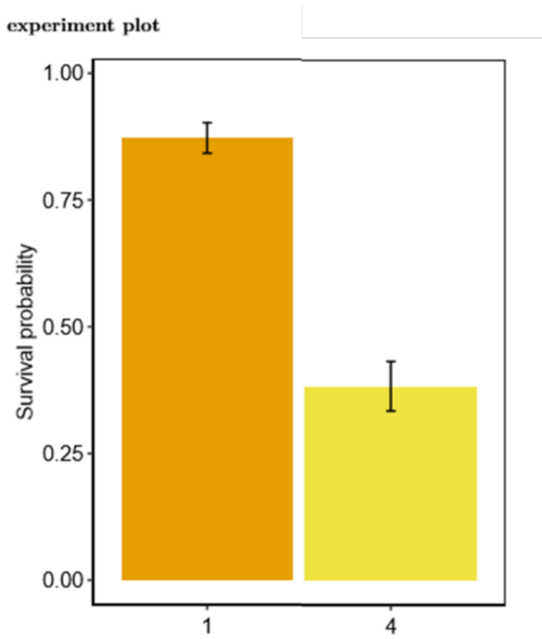


Figure 8.1.18. Survival probability of NIAB EMR (WS1) collected in 2019 (orange bar) and strains collected in 2020 (yellow bar) when treated with lambda-cyhalothrin.

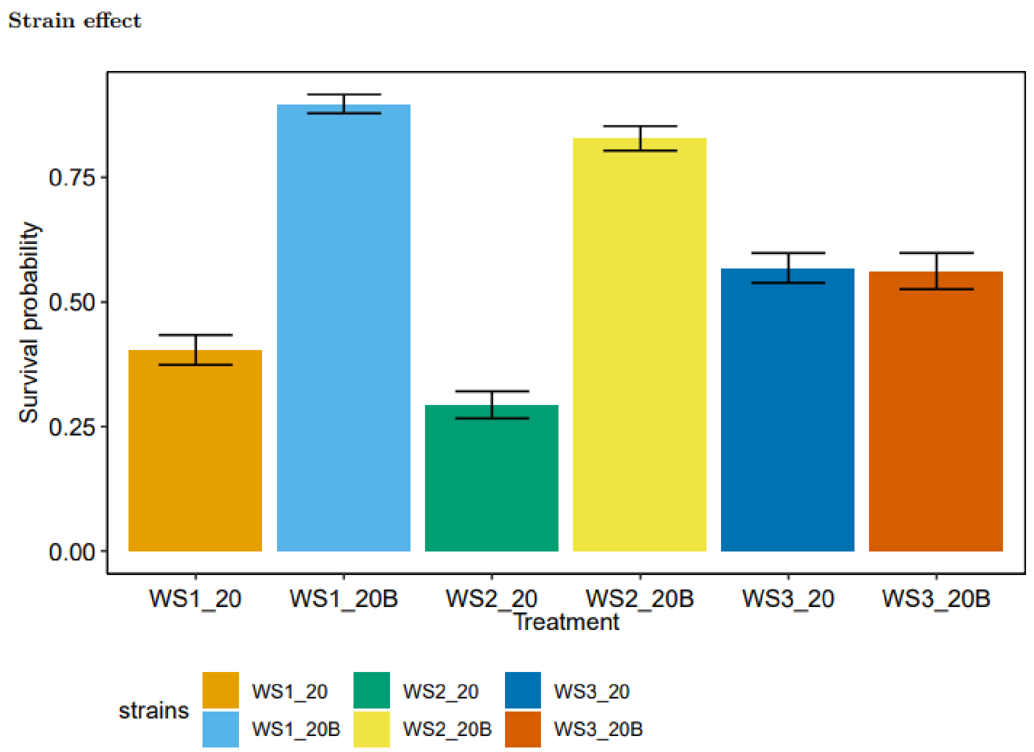


Figure 8.1.19. Survival probability of strains collected at the beginning-of-the-season of 2020 (light orange, green, dark blue bars) and strains collected at the end-of-the-season of 2020 (light blue, yellow, dark orange bars) from the three locations (strain WS1, WS2 and WS3) treated with lambda-cyhalothrin.

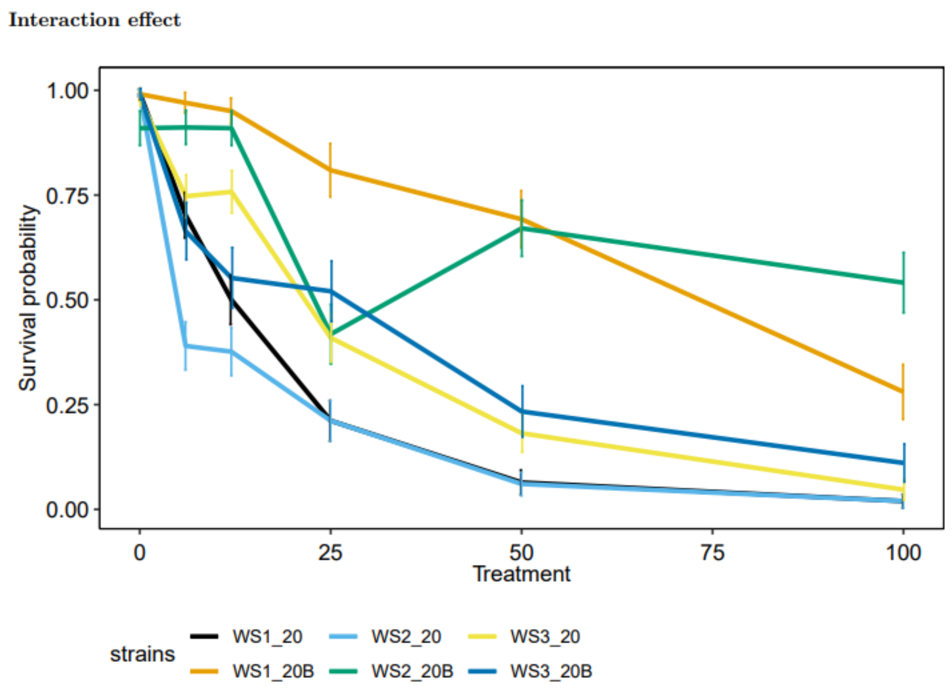


Figure 8.1.20. Survival probability of WS1, WS2 and WS3 strains established at the beginning-of-season 2020 (black, light blue, and yellow line) and end-of-season 2020 (orange, green and dark blue line) when treated with percentage doses of lambda-cyhalothrin field rate.

Comparisons of LC50's 2019-2021

As wild fly lines have been collected from the same locations since 2019-2021 we are able to compare the LC50 (the dose required to kill 50% of the population; a standard tool for comparison) between years. Interactions between years for each active ingredient and for each individual strain can be seen in Table 8.1.3.

For cyantraniliprole there was a significate difference in the LC50 values between the early and late season strains for all three sites, as indicated by the analysis above. There was also a significant different in the LC50 values for Spinosad for WS1 and WS3 and for lambda-cyhalothrin for WS1. In addition there were several difference between the strains over the years which can be seen in more detail in Table 8.1.3.

To establish if there is an increase in tolerance to any of the active ingredients we would expect the 2021 LC50 values to be significant higher than the 2019 values. For cyantraniliprole there were no significantly higher LC50 values for any of the three stains in 2021 in comparison to 2019. For Spinosad the LC50 value for WS1-19 was significantly higher than that of WS1-21 indicating an increased susceptibility to this active ingredient at this site. For Spinosad WS2-21 the LC50 value was significantly higher than WS2-20B but there were no other differences between the years. For WS3 analysis of the 2021 strain was not possible due to the death of the cultures, however, WS2-20B had a significantly higher LC50 value than the strains collected in 2019 and 2020 indicating a steady increase in tolerance. For lambda-cyhalothrin the WS1-19 and WS2-19 strains had a significantly higher LC50 value than their respective 2021 strains. In addition, the WS3-19 also had a significantly higher LC50 value than the WS3-20 and WS20B strains.

Table 8.1.3. Table of summary of analysis comparing LC50 values. P values are colour coded to reflect a significant increase in value (red) or a significant decrease (green) between years.

Cyantraniliprole

	WS1-20	WS1-20B	WS1-21
WS1_19	NSD	<0.001	NSD
WS1-20		<0.001	NSD
WS1-20B			NSD
WS1-21			

	WS2-20	WS2-20B	WS2-21
WS2-19	<0.001	<0.038	NSD
WS2-20		<0.001	0.006
WS2-20B			NSD
WS2-21			

	WS3-20	WS3-20B	WS3-21
WS3-19	NSD	NSD	*
WS3-20		0.05	*
WS3-20B			*
WS3-21			*

Spinosad

	WS1-20	WS1-20B	WS1-21
WS1_19	<0.001	NSD	<0.001
WS1-20		<0.001	NSD
WS1-20B			<0.001
WS1-21			

	WS2-20	WS2-20B	WS2-21
WS2-19	NSD	NSD	NSD
WS2-20		NSD	NSD
WS2-20B			0.048
WS2-21			

	WS3-20	WS3-20B	WS3-21
WS3-19	0.017	<0.001	*
WS3-20		<0.001	*
WS3-20B			*
WS3-21			

Lambda-cyhalothrin

	WS1-20	WS1-20B	WS1-21
WS1_19	<0.001	NSD	<0.001
WS1-20		<0.001	NSD
WS1-20B			<0.001
WS1-21			

	WS2-20	WS2-20B	WS2-21
WS2-19	*	NSD	<0.001
WS2-20		*	*
WS2-20B			0.013
WS2-21			

	WS3-20	WS3-20B	WS3-21
WS3-19	0.015	0.006	*
WS3-20		NSD	*
WS3-20B			*
WS3-21			

Discussion

When looking at the survival probability of the wild strains between years, there was a significant difference between 2019 and 2020 with lower survival in 2020 from all three strains when treated with spinosad and for WS1 when treated with lambda-cyhalothrin. If resistance had been developing in the field populations, we would expect 2020 to have higher survival than 2019. It may be that due to these early season populations being collected early in the growing season they have not been as exposed to insecticides as those collected towards the end of the season, like the 2019 strains.

In 2020, there were some differences in susceptibility between the early and late season strains, often with those collected earlier in the season having a lower tolerance to the PPP. This indicates that an increase in tolerance develops through the season. However, it is likely that cold winters reduce the survival of the tolerant lines as the result of a fitness cost, often associated with resistance mechanisms.

When assessing LC50 values over the four years of this objective, there are several significant differences that have been detected. Luckily there are no significant differences between the 2019 strains and 2021 strains which would indicate an increase in tolerance. While there are several interactions between other years it does not appear that for these locations and for these active ingredients that insecticide resistance has developed up to 2021.

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Conclusions

- In general, the 2020 early season strains were significantly more susceptible to products than the strains collected later in the season from all three sites.
- 2020 strains were more susceptible to spinosad (WS1, WS2 and WS3) and lambda-cyhalothrin (WS1) than the 2019 collected strains. If resistance was developing, we would expect the opposite to occur.
- There were variations in susceptibility between locations.
- There are variations in LC50 values between the years for each site.
- To date the results gathered do not indicate the occurrence of insecticide resistance to these active ingredients at these sites.

Acknowledgments

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Knowledge transfer

Presentations in 2021/22

Walker. A. Reducing over-wintering populations of SWD. NIAB EMR Soft Fruit Day.

Shaw, B. Native UK parasitoids of SWD. NIAB EMR Soft Fruit Day.

Shaw, B. The use of repellents to control SWD. NIAB EMR Soft Fruit Day.

Shaw, B. SWD control strategies- Results from AHDB projects. Berry Garden Grower Agronomy conference.

Shaw, B. SWD control strategies- Results from SWD repellents project. Berry Garden Grower Agronomy conference.Shaw, B. Native UK parasitoids of SWD. NIAB EMR Tree Fruit Day.

Shaw, B. The use of repellents to control SWD. NIAB EMR Tree Fruit Day.

Project presentation to steering group at summer meeting and at end of project.

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Appendix

Appendix 2.2.1. Dates for precision monitoring trial assessments at each block, 2019/20.

		Blocks 1 to 3				Blocks 4 to 6			
Week beginning	Assess No.	RIGA traps	Transect traps	Habitat	Sentinel fruit traps	RIGA traps	Transect traps	Habitat	Sentinel fruit traps
30-Sep-19	Pre	X	X						
07-Oct-19	Pre					X	X		
14-Oct-19	1	X	X						
21-Oct-19	1					X	X		
28-Oct-19	2	X	X						
04-Nov-19	2					X	X		
11-Nov-19	3	X	X						
18-Nov-19	3					X	X		
25-Nov-19	4	X	X						
02-Dec-19	4					X	X		
09-Dec-19	5	X	X	X					
16-Dec-19	5					X	X	X	
06-Jan-20	6	X	X						
13-Jan-20	6					X	X		
20-Jan-20	7	X	X						
27-Jan-20	7					X	X		
17-Feb-20	8	X	X						
24-Feb-20	8					X	X		
02-Mar-20	9	X	X						
09-Mar-20	9					X	X		
16-Mar-20	10	X	X						
23-Mar-20	10					X	X		
20-Apr-20	11	X	X		X				X
27-Apr-20	11				X	X	X		X
04-May-20					X				X

11-May-20					X				X
18-May-20					X				X
01-Jun-20	12	X							
08-Jun-20	12					X			
15-June-20	13								
22-June-20	13								
06-Jul-20	14	X	X						
13-Jul-20	14					X	X		
27-Jul-20	15	X	X	X	X				
03-Aug-20	15					X	X	X	X
24-Aug-20	16	X	X						
01-Sep-20	16					X	X		
07-Sep	17	X	X						
14-Sep	17					X	X		
12-Oct-20	18	X	X	X	X				
19-Oct-20	18					X	X	X	X
02-Nov-20	19	X	X						
09-Nov-20	19					X	X		
30-Nov-21	20	X	X						
14-Dec-21	20					X	X		