Project title:	Development and implementation of season long control strategies for *Drosophila suzukii* in soft and tree fruit
Project number:	SF145a
Project leader:	Michelle Fountain, NIAB EMR, New Road, East Malling, Kent ME19 6BJ
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Key staff:	Adam Walker, Bethan Shaw, Charles Whitfield, Francesco Maria Rogai, Celine Silva, Jonah Budd, Umberto Rosalina, Molly Perry-Clark, Zoe Clarke, Greg Deakin, Jacob Lowe, Dave Shaw (NIAB EMR); David Hall, Dudley Farman (NRI); Ralph Noble, Andreja Dobrovin-Pennington (Microbiotech), Alison Karley, Alison Dolan, Gaynor Malloch, Peter Skelsey (JHI)
Key collaborators	Berry Gardens Growers and Angus Soft Fruits
Location of project:	NIAB EMR
Industry Representative:	Marion Regan, Hugh Lowe Farms
Date project commenced:	01 April 2017
Date project completed (or expected completion date):	31 March 2021

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.]

CONTENTS

AUTHENTICATION

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

Michelle Fountain

Deputy Head of Pest and Pathogen Ecology

NIAB EMR, New Road, East Malling, Kent ME19 6BJ

Signature .....M T Fountain   Date 03 March 2021

Report authorised by:

Marion Regan

Industry Representative

Hugh Lowe Farms

Signature ....   Date .

[Name]

[Position]

[Organisation]

Signature ....   Date ....

GROWER SUMMARY

Introduction

Native to eastern and south-eastern Asia (Walsh et al. 2011), the Asiatic vinegar fly *Drosophila suzukii* Matsumura (Spotted Wing Drosophila, *D. suzukii*) recently invaded the UK, immediately becoming a key pest of stone and soft fruit crops. Numbers have increased from year to year, causing severe fruit damage and increases in production costs. The invasion of *D. suzukii* across Europe has strongly disrupted existing and developing integrated pest management IPM control strategies, as currently crops are being protected against the pest with programmes of multiple sprays of plant protection products (PPPs) including broad spectrum products. This causes a deterioration of beneficial arthropod populations disrupting their ecological contribution in keeping pests below economic threshold values. In the EU there has also been an ongoing review and phase-out of chemical PPPs since the 1980s (pan-europe.info. 2008), including a recent restriction on neonicotinoid applications (eur-lex.europa.eu. 2013). Along with this there is a continuing trend to reduce the risks and impacts of chemical PPP use and to promote the use of non-chemical alternatives (eur-lex.europa.eu. 2009). Internationally, the need for insecticide-based management programmes to control *D. suzukii* close to harvest has become problematic too, because of inconsistencies among export markets regarding maximum residue limits (MRLs) that are allowed for different insecticides on imported fruit (Haviland et al. 2012). Moreover, there is now evidence for *D. suzukii* resistance to spinosad which is commonly used to control this pest, further presenting a need for the development of alternative management strategies (Gress et al. 2018).

In Europe and America, research projects on *D. suzukii* have ended (projects IPMDROS, DROSKII and DROPSA). The aim of these projects was to create new knowledge and understanding of the damage and losses on fruit crops resulting from *D. suzukii* activity, by studying its biology and evaluating control methods. This project builds on progress internationally and on the AHDB project SF145 but focuses on practical development and elaboration of new control technologies that can be used by UK growers within the short to medium term.

The specific objectives within this AHDB funded project were:

1. Continue to monitor *D. suzukii* in England and Scotland with additional habitat evaluation in Scotland

2. Develop and optimise a push/pull system using repellents and attract and kill strategies

3. Further develop, optimise and test bait sprays

4. Investigate prolonging spray intervals for maximum effect but minimal applications

5. Integrate exclusion netting with other successful controls

6. Integrate approaches for season long control

7. Identification and quantification of D. suzukii parasitism in the UK

8. Identification of Drosophila suzukii tolerance to plant protection products

This Grower summary reports on the results of each of these objectives in turn.

Financial benefits of this research

Gaining control of spotted wing drosophila does not just require additional crop protection sprays, it also requires good crop management and hygiene, which incurs additional labour costs.

Growers producing susceptible crops incur additional labour to monitor for the presence of the pest using monitoring traps and flotation testing for the presence of SWD larvae in the fruit. They incur additional labour costs to remove old and damaged fruit from the plantation floor (to stop attracting SWD into the crop). They also incur additional labour costs to pick and remove late ripening fruits, which continue to develop several weeks after the main harvest has been picked.

Some growers employ narrow mesh netting to prevent SWD ingress into the crop to reduce population numbers in and around the developing fruits. This incurs expenditure for the netting and additional labour to erect it.

Typical additional costs incurred for all of this, coupled to the additional sprays required to control the pest are listed in the table below.

	SWD cost per hectare
Strawberries	£4,344
Raspberries	£6,557
Blackberries	£11,074

The continuing programme of research in this and other SWD projects, aim to develop novel and sustainable control methods, which will become available for growers to adopt in the short to medium term to reduce reliance on the use of conventional spray control and reduce the typical costs being incurred in the crops listed above.

Objective 1. Continue to monitor *D. suzukii* in England and Scotland

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

Task 1.2. Modelling of the 17-year National Monitoring dataset (JHI)

Headlines

- *D. suzukii* numbers at NIAB EMR in 2020, overall, were similar to the catch numbers of 2017 and 2018, but 2020's trend most closely relates to 2019's profile.
- As with previous years at NIAB EMR, unprecedented peaks in trap catches occurred in conjunction with uncharacteristic peaks in temperature.
- In Scotland 2020 total trap catches for the year were lower than 2019.
- Predictive models using monitoring trap catches have been successful in predicting key SWD events including first spring female peak with 93.3% accuracy.

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 Cha Landolt bait system were deployed in a range of commercial and wild crops in 2013 at 14 sites across the UK.

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

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

Since 2018, NIAB EMR has been hosting one of the 12 m high Rothamsted suction traps and has been given access to historic trap catches from other locations. In 2018 *D. suzukii* was identified between August and November at NIAB EMR, which is consistent with previous years. Adults were detected at 12 m from the ground during the main flight/dispersal period which coincides with the emergence of the winter-form adults, a depletion in egg laying resources (fruit) and defoliation of trees (reduced refugia). Suction trap catches from 2019 and 2020 will be analyzed in summer 2021. Rothamsted have also agreed to share the Scottish suction trap catches from 2014, 2017 and 2019. Access to these samples has been delayed by COVID-19 restrictions.

Summary of the project and main conclusions

At NIAB EMR, a cooler spring in 2020 resulted in lower trap catches compared to 2019. In addition to 2019, 2020 also saw an unprecedented activity peak in June, which coincided with above average temperatures during this time. In September 2020, the largest peak trap catch occurred (since monitoring began in 2013), a 20% increase from 2019.

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 and to NIAB EMR’s trend, 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 2014 (c. 20 per trap). There is an indication that trap catches at the JHI site might be increasing in 2019 and 2020 compared with earlier years. However, this may be a local finding.

For both sites, there continues to be a general year-on-year increase in annual mean trap catches, except for the year 2020 where a decrease of ~200 SWD per trap was seen at NIAB EMR and ~14 SWD per trap at JHI.

Predictive models have been developed using historic trap catch data 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.

Action points for growers

- Continue to monitor adult SWD in hedgerow and cropping areas.
- Be aware of AHDB communications with alerts to key SWD monitoring events.
- Monitor for fruit damage throughout the cropping season to inform control measures.

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

Task 2.1. Analyses of fermentation products from yeasts attractive to D. suzukii (Rory Jones and NRI)

Headline

- Yeast species of ecological relevance to D. suzukii can be separated from one another by the relative amounts of volatile chemicals which they produce.

Background and expected deliverables

Yeast species are known to play vital roles in the ecology of *D. suzukii* in terms of nutrition and insect behaviour. Rory Jones of University of Lincoln has been undertaking an AHDB PhD Studentship (CP171) to investigate the attractiveness of a range of exotic yeast species to SWD. NRI scientists are assisting him in collection and analysis of volatiles produced by yeast species with the aim of finding new attractants for *D. suzukii*.

Summary of the project and main conclusions

Volatiles were collected from four exotic yeast species associated with *D. suzukii* and a commercial yeast species. Collections were analysed by gas chromatography coupled to mass spectrometry and over 34 compounds were detected and identified. Results of Principle Component Analyses (PCA) indicated that the five yeast species could be separated according to amounts of the different volatiles produced. However, these differences could not be correlated with differences in behaviour of *D. suzukii* in an activity bioassay.

More work would be required to test whether differences in yeast volatile profiles may render some yeast species more attractive than others to *D. suzukii* in the field. This information could be used to reduce movement of *D. suzukii* into crops and increase catches in precision monitoring traps.

Action points for growers

- Currently there are no action points for growers.

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

Headlines

- In woodlands (and neighbouring crops) where trapping 'precision monitoring' has been applied to control the wild source of *D. suzukii,* fewer *D. suzukii* have been recorded compared to untreated (control) equivalents.
- Preliminary findings show traps positioned on the woodland perimeter caught significantly more male *D. suzukii* than within the main woodland.
- Summer habitat assessments show more *D. suzukii* were caught in traps surrounded by vegetation favoured by the pest.

Background and expected deliverables

Alongside commercially grown fruit, *D. suzukii* utilises wild fruits and habitats 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 UK *D. suzukii* national monitoring survey (Objective 1), which shows high activity peaks of *D. suzukii* in woodlands during late autumn/early-winter when there is reduced availability of commercial and wild fruit. A trial was established in 2019 to investigate whether the deployment of precision monitoring traps in wild habitats has the potential to reduce *D. suzukii* numbers and minimise the impact in crops in the early spring.

Summary of the project and main conclusions

In September 2019, a grid of 64 precision monitoring traps spaced at eight metre intervals were deployed in isolated pockets of woodlands on six soft fruit farms in South East England. These were compared to a second woodland on each farm with no traps (untreated control).

A RIGA monitoring trap was positioned in the centre of each woodland and respective neighbouring crop and checked fortnightly to monitor numbers of *D. suzukii.* So far, this data shows fewer *D. suzukii* were caught in RIGA monitoring traps in woodlands treated with precision monitoring (and neighbouring crops) than untreated (control) equivalents.

To determine if precision monitoring can prevent or reduce *D. suzukii* numbers invading the neighbouring crop, in spring, summer and autumn 2020, sentinel traps containing raspberries were deployed in the woodlands and respective neighbouring crops to attract females to lay eggs. Subsequent numbers of adult *D. suzukii* emerging from these raspberries were compared. To date, low numbers of *D. suzukii* have emerged from all sentinel fruit deployments. This is likely the result of competition from other Drosophila spp. egg laying in the same fruit. Sentinel fruit deployments in spring 2021 will need a method to allow *D. suzukii* egg laying exclusively. Ripening fruit instead of ripe fruit is being considered.

The trial is also investigating findings that some precision monitoring traps catch more *D. suzukii* than others. This information should help growers decide where best to position precision monitoring to optimise *D. suzukii* control. Catches of *D. suzukii* in traps were investigated in relation to surrounding host vegetation, temperature, humidity, light level and trap position.

To date, findings show significant positive correlations between *D. suzukii* catches in traps and vegetation that is favoured by the pest, during summer. There was also a positive correlation in autumn, though not significant. Statistical analysis of the winter assessment is underway. Further investigation of the influence of specific host vegetation on trap catches is recommended.

Our investigation also found traps positioned on the woodland perimeter catch more *D. suzukii* than those on the woodland interior spring, summer, and autumn.

Assessment of microclimate conditions at traps has so far revealed negative correlations between numbers of male *D. suzukii* caught in traps and temperature, but only in summer. So far, we've found no significant correlations with humidity or light intensity.

This trial will continue into 2021, to see if long-term placement of these traps can suppress local *D. suzukii* populations over time.

Action points for growers

- Monitor for *D. suzukii* in and around soft fruit crops year-round to predict potential incursions.

Task 2.3. Development of a push-pull system for control of Drosophila suzukii (Christina Conroy and NRI)

Headline

- Two repellent compounds have been demonstrated to reduce egg-laying by *D. suzukii* in strawberries at over 6 m from the source.

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’. In previous work, several compounds were found to repel *D. suzukii* in small-scale trapping experiments. NRI are working with CTP student, Christina Conroy, to develop a push-pull approach for control of *D. suzukii* using synthetic repellents and attractants. From electrophysiological studies, bioassays, and field experiments three compounds were shown to be repellent to *D. suzukii*. These were taken forward into field trials to test their efficacy in preventing egg laying on strawberries.

Summary of the project and main conclusions

The three candidate repellents were formulated in polyethylene sachets. In trials on strawberries in experimental polytunnels, two repellents significantly reduced egg-laying by *D. suzukii* at distances over 6 m. These should be taken forward into larger-scale field trials.

Action points for growers

- Currently there are no action points for growers.

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

Task 3.4A Determine the effect of baits in combination with reduced dose insecticides on D. suzukii control in raspberry (Microbiotech, NIAB EMR)

Headlines

- Weekly alternating dilute applications of Tracer and Exirel combined with Combi-protec or molasses baits, were as effective in controlling *D. suzukii* as full field rates (i.e. a reduction in insecticide application of 96% with the same *D. suzukii* control effect)
- Residues of spinosad and cyantraniliprole were at least x11 higher in fruit samples taken from plots sprayed with the full field rates of insecticides than from plots sprayed with the dilute rates with baits

Background and expected deliverables

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

In 2018, the baits were; fermented strawberry juice (FSJ), a suspension of the yeast *Hanseniaspora uvarum*, a combination of the two and Combi-protec, a proprietary mixture of protein, yeast and sugars. Experiments were done in the laboratory in jar microcosm bioassays. Chronophysiology assays (activity counts) using the activity of *D. suzukii*, in the presence of different baits, was the more useful screening method of attractant baits than the large arena test. Without plant protection products, the baits did not affect *D. suzukii* mortality. For Tracer (spinosad), Exirel (cyantraniliprole) and Hallmark (lambda-cyhalothrin), the baits caused higher mortality of *D. suzukii* summer morphs, under summer conditions, compared with using the products in water. The efficacy of products, in terms of increased mortality and reduced egg laying, was greater with *H. uvarum*, FSJ + *H. uvarum* and Combi-protec treatments than with the FSJ only bait. In addition, *H. uvarum* and FSJ baits increased the mortality of *D. suzukii* winter morphs held under winter conditions when used with spinosad or cyantraniliprole but not with lambda-cyhalothrin. When used with cyantraniliprole, *H. uvarum* reduced the egg laying of winter morphs that were transferred to summer conditions after three days of exposure to treatments under winter conditions.

In 2019, baits were tested in mini tunnels containing strawberry plants in grow bags. Bands of Benevia (cyantraniliprole) combined with either *H. uvarum* or Combi-protec were applied as 30 ml per hectare in 40 L, twice during the experiment to the crown of the strawberry plants. This was compared to a water control (untreated) and a positive control (Benevia at maximum field rate). Male and female *D. suzukii* were released into the tunnels on several occasions to inoculate the fruit. Both baits, in combination with Benevia, significantly reduced *D. suzukii* in fruit compared to the water control. There was no significant difference between the positive control, Benevia at full field rate (750 ml in 500L/ha) and the two baits combined with Benevia (30 ml in 40L/ha). The cost of Benevia applied in the bait treatments amounted to £77.50/ha, a reduction from the full rate of £112.5/ha. Application time was reduced by 75% in the bait combined with Benevia treatments compared to Benevia alone.

Summary of the project and main conclusions

The aims of the work in 2020 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, against full field application rates of the same products in raspberries under semi-field conditions.

Weekly alternating 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 products applied at 200 or 900 ml in 500L per ha (i.e. a reduction in product application of 96% with the same *D. suzukii* control effect). The products used at the full field rates or dilute rates with bait sprays remained as effective in controlling *D. suzukii* numbers in the two weeks after they were applied as they were during the four weeks when they were being applied. Control of *D. suzukii* was equally good with the molasses spray treatment as with the Combi-protec or full field rate spray treatments but at only 21% or 17% of the product costs. The application time for the bait sprays was 10% of the full field rate application of product sprays.

Residues of spinosad and cyantraniliprole were at least x11 higher in fruit samples taken from plots sprayed with the full field rates of products than from plots sprayed with the dilute rates with baits. Residues in fruit from the dilute insecticide rates + bait spray plots were not detectable or lower in samples taken from the bottom of plants than in samples from the top and middle of plants. None of the product or product + bait treatments caused phytotoxicity symptoms and there was no mould growth on the bait spray droplets. The spray coverage of the low rate sprays was approximately 8-times lower than the full rate spray. Despite the larger droplet sizes used for the low rate applications, there was no evidence of any extremely large deposits that could breach MRLs.

Action points for growers

- Growers should discuss the use of approved adjuvants in combination with plant protection products with their agronomy provider and adhere to approvals.

Objective 5. Integrating exclusion netting with other successful controls

A decision was made to defer this until a later year as a new Waitrose CTP PhD student will be working on this in collaboration with Berry World, the University of Reading and NIAB EMR, with 10 replicate tunnels of meshed versus unmeshed raspberry crops in 2020.

Objective 6. Develop, design and communicate a year-round strategy for *D. suzukii* control in UK crops

Headline

- One peer reviewed publication and 15 oral presentations were disseminated in 2020.

Background and expected deliverables

In collaboration with the AHDB communications team, we are producing recommendations for year-round control of *D. suzukii* that targets all life stages and habitats to reduce year on year populations, damage to fruit and the use of plant protection products used for control. Results have been disseminated – over 14 presentations and courses were delivered in 2017, 10 in 2018. In 2019, five peer reviewed manuscripts were published and 16 industry/scientific communications/presentations were given.

Summary of the project and main conclusions

In 2020, one peer reviewed manuscript was published and 15 oral presentations given at both national and international events. This does not include all the one-to-one discussions on *D. suzukii* control with individual agronomists and growers.

NIAB EMR monitoring data was regularly communicated to the AHDB and SWD Working Group, for dissemination to growers.

Action points for growers

- Keep abreast of the latest *D. suzukii* control strategies and research through AHDB communications.

Objective 7. Identification and quantification of *D. suzukii* parasitism in the UK

Task 7.1. Screening Scottish habitats for the presence of Drosophila suzukii parasitoids (JHI)

Headline

- In Scotland, using sentinel *Drosophila melanogaster* larvae and pupae, potential *D. suzukii* parasitoid activity has been identified.

Background and expected deliverables

A Worshipful Company of Fruiterers funded project linked to SF 145 aimed to identify species of parasitic wasps parasitizing *D. suzukii* in South East England. Field surveys also aimed to identify *Trichopria drosophilae*, and to investigate potential interactions of *D. suzukii* with native UK parasitoid species that may contribute to *D. suzukii* control. Field surveys were conducted across several fruit growing and wild sites in the South East of England in two consecutive years (2017 and 2018). Five species of hymenopteran parasitoids were collected using *D. suzukii* larvae/pupae sentinel traps. Two species of larval parasitoids and three pupal parasitoids were recorded in 2018. All five species are generalist parasitoids of *Drosophila.* Habitat surveys highlighted how landscape diversity could influence parasitoid presence.

In 2019, parasitoid surveys were conducted in Scotland using *D. melanogaster* baited traps from the end of July (2019). The numbers of parasitoids emerging from baited traps in 2019 indicated that parasitoid populations were already established prior to the deployment of traps. Parasitoids in 2019 were identified as the larval parasitoid *Asobara tabida*. Parasitoids were trapped in highest numbers in July-September in 2019.

Summary of the project and main conclusions

In Scotland in 2020, parasitoid surveys were conducted using *D. melanogaster* baited traps deployed at the end of May. The numbers of parasitoids emerging from baited traps in 2019 indicated that populations were already established prior to the deployment of traps in July, and so traps were deployed earlier in 2020. Parasitoids were trapped in highest numbers in August and October in 2020, compared to July-September in 2019, and the numbers were up to four-fold higher in 2020 compared with 2019. In 2020 *Asobara tabida* was confirmed as the species detected in the baited traps. There was regional variation in total catch size but no clear link between parasitoid presence/abundance and the suitability of the surrounding vegetation for their SWD hosts.

Action points for growers

- Currently there are no action points for growers

Task 7.2. Investigating the proportion of Drosophila suzukii pupae in sentinel traps parasitized by UK parasitoids (NIAB EMR)

Headlines

- In England, we were able to calculate the rate (%) by which some of the native parasitoids, previously identified in our surveys, parasitize *D. suzukii* in the field.
- Our survey also recorded the parasitoid *Trichopria modesta* for the first time since the survey began in 2017.
- To date, the survey has recorded six native parasitoids associated with *D. suzukii* in England.
- *Trichopria drosophilae,* remains unidentified in the UK and hence cannot be released as a biocontrol agent.

Background and expected deliverables

In 2017 and 2018, a Worshipful Company of Fruiterers funded project linked to SF 145, aimed to identify species of Hymenoptera parasitizing *D. suzukii* in South East England that may contribute to *D. suzukii* control. Field surveys were conducted across several fruit growing and wild sites, also aiming to identify *Trichopria drosophilae*; a pupal parasitoid commercially available in Europe for use in biological control.

Five species of hymenopteran parasitoids were collected using *D. suzukii* larvae/pupae sentinel traps. This included two species of larval parasitoids (*Asobara tabida* and *Leptopilina heterotoma*) and three pupal parasitoids (*Spalangia erythromera*, *Pachycrepoideus vindemmiae* and *Trichopria prema*) all of which are generalist parasitoids of *Drosophila.*

The presence and abundance of these parasitoid species varied greatly among the sites and across the season. At sites where parasitoids were active, small numbers were recovered in May, but the main period of activity was from June to October, with no parasitoids present from November onwards.

Habitat surveys also highlighted how landscape diversity could influence parasitoid presence.

Laboratory tests were performed to calculate the rate by which collected parasitoids parasitize *D. suzukii*. Results showed two pupal parasitoids produced most offspring per parent on cultures of *D. suzukii.* However, the rate of *D. suzukii* parasitism by these species and potential others could not be calculated accurately in UK populations in the field.

The objectives of the survey in 2020 were to:

- Calculate *D. suzukii* parasitism rate under field conditions.
- Determine if parasitism rates change throughout the year.

- Continue to search for the pupal parasitoid *T. drosophilae*, to confirm its presence in the UK.

Summary of the project and main conclusions

To determine the percentage of parasitism in the field, in 2020 two types of sentinel traps containing fruit (control and treated) were infested with equal numbers of *D. suzukii* and deployed in areas with known parasitoid populations, as identified in 2018. A control trap was designed to enable normal *D. suzukii* development, using a lid which prevented parasitoid entry, whilst a treatment trap was designed to enable parasitoids to enter and lay eggs in developing *D. suzukii*. Equal numbers of these traps were deployed on five occasions between July and September, then collected and incubated for 6 weeks. During incubation, adult *D. suzukii* and parasitoids emerging within traps were identified and counted. Mean percent parasitism was calculated as number of parasitoids emerging in treatment traps as a percent of number of *D. suzukii* emerging in control traps.

The most common species recorded in 2020 was the pupal parasitoid *Spalangia erythromera,* with a mean parasitism rate of 1.1% (range 0 to 6%), which peaked in August. *S. erythromera* has been recorded in consistent numbers at the same two sites every survey year. It has also shown promise for *D. suzukii* biocontrol; being active from May to October and completing development to adulthood in lab cultures of *D. suzukii* with mean offspring per parent 0.2. It also does not hyperparasitize.

Surprisingly the pupal parasitoid *P. vindemmiae* was not recorded in 2020, despite it being the most common parasitoid recorded in sentinel traps at the same sites 2017 and 2018. In laboratory tests, this species recorded the highest mean offspring per parent 3.6.

The survey also identified *T. modesta* for the first time since surveys began in 2017, bringing the total number of native parasitoid species recorded in sentinel traps containing *D. suzukii* up to six.

Larval parasitoids *L. heterotoma*, *A. tabida*, *T. prema,* and *T modesta* are less successful at parasitising *D. suzukii*.

To date, *T. drosophilae* has not been identified during these surveys.

Action points for growers

- Ensure that spray drift does not contact hedgerows and woodlands to preserve parasitic wasps of *D. suzukii*; and a range of other generalist predators.
- Continue with crop hygiene and insect exclusion mesh measures to reduce the need for plant protection products.

Task 7.3. Investigating UK waste fruits as a potential source of parasitoids to control Drosophila suzukii in neighbouring crops (NIAB EMR)

Headline

- Due to the low recovery of parasitoids, waste fruit is not worth pursuing as a source of parasitoids.

Background and expected deliverables

Improved hygiene practices are known to help reduce *D. suzukii* pressure in commercial crops. This includes removing waste and unmarketable fruit which may be the result of larval feeding damage. It is possible that the waste fruit collected at UK soft fruit farm is a potential source of parasitoids which could be used for biological control of *Drosophila suzukii.* If managed effectively, waste fruit could be used to provide a source of parasitoids to control *D. suzukii* without releasing *D. suzukii* into the crop.

Summary of the project and main conclusions

Waste fruit was collected from sites known to have *D. suzukii* parasitoids, which were identified during the 2018 surveys (see Obj 7.2). Fruit was collected during the period that parasitoids were known to be active; July-September. These samples were collected on a minimum of two occasions and maintained for six weeks. Most parasitoids have been previously recorded to emerge within one to five weeks post collection.

D. suzukii emerged from all waste fruit collections, but numbers varied between collections. The highest number that emerged (per kg of waste fruit) was from raspberry collected on 17 July and the lowest from cherry collected on 6 July (total = 351.5 and 0.1 respectively). The raspberry collection from which the highest number of *D. suzukii* emerged (17 July) was also the only waste fruit collection from which a parasitoid emerged (0.2 parasitoid kg^{-1}). The parasitoid was identified to be in the Braconidae family.

Other *Drosophila* spp. emerged from all waste fruit collections, but these numbers also varied between collections. The highest number emerged (per kg of waste fruit) was from strawberry collected on 9 September and the lowest from cherry collected on 21 July (total = 353.3 and 0.1 respectively). Other Drosophila species that emerged included: *Drosophila melanogaster, Drosophila simulans* and *Drosophila subobscura*. The species of parasitoids that have been identified in the UK to date are generalist parasitoids and so in cases where *D. suzukii* emergence was low, parasitoids could have emerged from the other species of *Drosophila.*

Where there was low insect emergence from waste fruit, the use of chemical plant protection product (PPP) application and crop hygiene could have potentially been the cause. PPPs are known to affect parasitoids regardless of species (Schlesener et al., 2019) and crop hygiene measures reduce the opportunity for *D. suzukii* egg laying and subsequent parasitism.

This pilot study demonstrated that waste fruit is unlikely to be a significant source of parasitoids, although the one collection with parasitoid emergence was collected in July, which is the time of year when highest numbers of parasitoids emerged during the 2018 wild habitat survey.

Action points for growers

- Currently there are no action points for growers.

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

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

Headline

- Early season strains of SWD collected in 2020 appear to be more sensitive to spinosad and lambda-cyhalothrin than late season strains collected in 2019.

Background and expected deliverables

Since its arrival in the UK in 2012, use of Plant Protection Products (PPP) has played a vital role in supressing *D. suzukii* numbers in vulnerable fruit crops. In 2018, an increased tolerance to spinosad (Tracer) was detected in organic raspberries in California 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 tested between the three wild populations; lambda-cyhalothrin (Hallmark), cyantraniliprole (Exirel) and spinosad (Tracer). 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 products 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 control products as those collected towards the end of the season, like the 2019 strains.

Action points for growers

- Growers should consult their agronomist for up-to-date approvals prior to using spray applications for control.
- Where possible, growers should rotate between different product modes of action to prevent build-up of resistance.
- 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 and 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 in the last 2 years are some sites seeing an increase in incidence since 2014. In the West Midlands and East Anglia, the numbers have been reasonably low, but locally *D. suzukii* can impact fruit production and fruit damage in the latter regions is increasingly reported. 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.

To enable the industry to assess risk of fruit damage we have continued to monitor how *D. suzukii* populations respond over time (since 2013). In 2019 the monitoring was reduced to NIAB EMR in England and the James Hutton Institute (JHI) in Scotland. Trap catches in this report are annual catches for both sites, so that annual trends can be followed.

All data was supplied to the JHI for modelling populations with climatic conditions each year. In 2020, England data was analysed by a NIAB EMR PhD student to model the effect of proximity of wild populations to crops. Once these models are available the aim would be to host them on the AHDB web site for growers to use.

Methods

Monitoring began at 14 fruit farms in 2013 in project SF145. In 2017 monitoring was reduced to 57 traps on nine farms in England and 40 traps on 4 farms in Scotland. From 2019 the number of traps were 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 have gained a good understanding of the pest dynamics in different crops, which was the original objective, but wished to continue a reduced monitoring. The data from the reduced monitoring would continue to be used to alert growers of the pest dynamics and to be used in future pest modelling. Issues with trap catch continuity at site 1300 in Scotland in 2019 and 2020 resulted in data being used solely from JHI (site 1100) for the analysis from 2019 onwards.

As of 2020, the 10 traps at NIAB EMR were deployed in the following locations: 4 in cherry, 2 in strawberry, 1 in grape, 2 in woodland and 1 in plum. The trap in the plum demonstration orchard was relocated from the vineyard at the end of 2019. In Scotland in 2020, monitoring data was collected from 3 traps hosted at JHI, deployed with 1 in blackcurrant, 1 in blueberry and 1 in a wild area near blackberry.

For continuity within the National Monitoring Survey, we use the modified Biobest trap design and Cha-Landolt bait used 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 released from two polypropylene vials (4 ml) with a hole (3 mm diameter) in the lid, attached near the fly entry holes within the trap. The traps were deployed at the height of the main crop (±1 metre).

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

Results

England

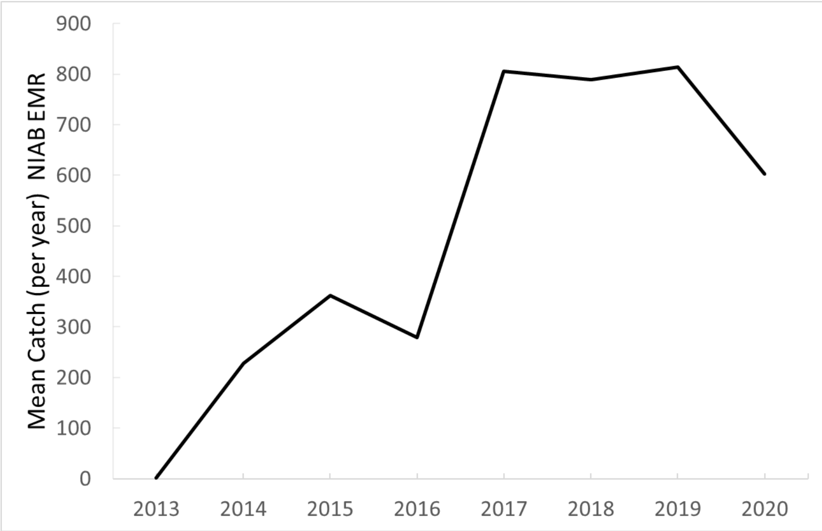
At NIAB EMR the mean weekly trap catch has generally risen year on year since data collection began in 2013, however reductions did occur in 2016, 2018 and 2020 (Fig. 1.1.1a). The seasonal variations in trap catches (Fig 1.1. 1b & c) continues to be greatly influenced with temperature fluctuations (Fig 1.1.2). In addition, it was confirmed that *D. suzukii* can be detected at 12 m height (Rothamsted suction traps) during the main period when the flies are captured in the traps in cropping and woodland areas (August - November). This period 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 crop and not because there is a decrease in the numbers of *D. suzukii*.

The activity-density of adult *D. suzukii* in the monitoring traps was lower in the spring 2020 (March - May) compared to 2019. This was likely caused by a prolonged, cold, spring in 2020 like that of 2018 (Fig. 1.1.2) decreasing the opportunity for *D. suzukii* to be active, and hence, captured in the monitoring traps. Numbers, as usual, in the traps, were lowest during the period of peak fruit production but increased to levels very similar to 2017 and 2018 by the end of July. The highest peak of activity for October was seen in 2018 compared to other years (Fig.1.1.1b & c). From November to December 2017 there was almost double the trap catch (>800) compared to the previous highest recording in 2015/16 (Fig. 1.1.1b & c). Peaks in November - December have not reached the levels of 2017 (Fig.1.1.1b).

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 2015/16 (black line). Peaks in the winter of 2018 were lower than the previous year (Fig. 1.1.1 b & c).

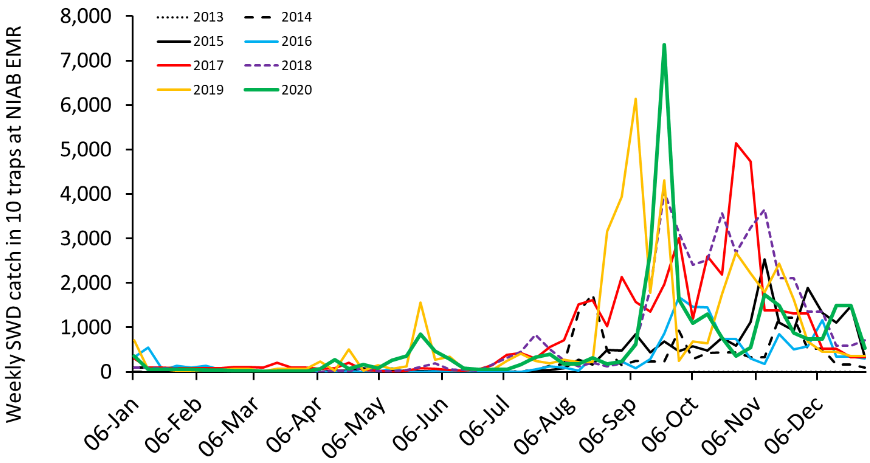
In 2019 (yellow line) and 2020 (green line), monitoring at NIAB EMR only, showed higher catches in the spring (March-May) compared to all previous years (warmer spring) and a peak in June which coincided with higher temperatures in that month (Fig. 1.1.2). There was the highest trap catch peak, thus far, at NIAB EMR in September 2020, 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. 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 = 814, 2020 = 603 (Fig. 1.1.1a).

The highest peaks in England in 2020 occurred during the late autumn to winter months when the flies are in reproductive diapause in their winter-form (winter morph). The leaves have fallen from deciduous trees at this time, giving less shelter and a reduced availability of commercial and wild fruit. Fig. 1.1.3 shows variation in trap catches for the NIAB EMR site only from 2013 to the 2020 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.



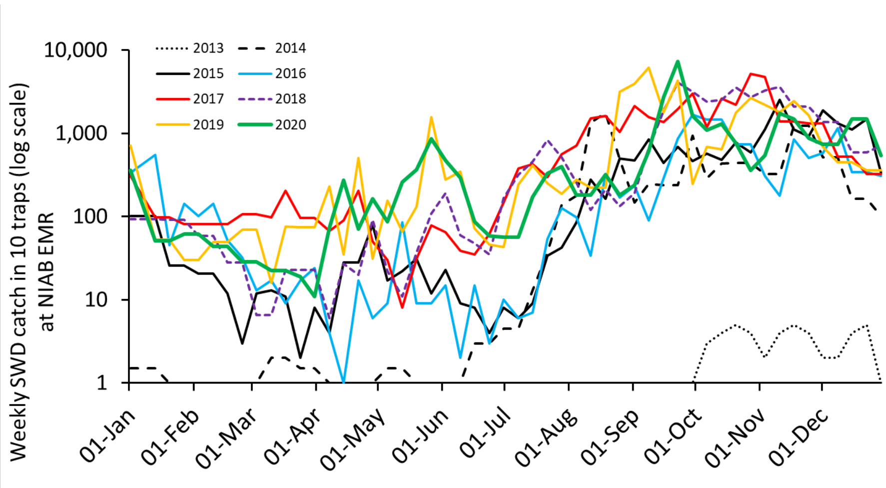
Mean Catch (per year) NIAB EMR
0
100
200
300
400
500
600
700
800
900
2013
2014
2015
2016
2017
2018
2019
2020

a)



Weekly SWD catch in 10 traps at NIAB EMR
0
1,000
2,000
3,000
4,000
5,000
6,000
7,000
8,000
2013
2014
2015
2016
2017
2018
2019
2020
06-Jan
06-Feb
06-Mar
06-Apr
06-May
06-Jun
06-Jul
06-Aug
06-Sep
06-Oct
06-Nov
06-Dec

b)



Weekly SWD catch in 10 traps (log scale) at NIAB EMR
1
10
100
1,000
10,000
2013
2014
2015
2016
2017
2018
2019
2020
01-Jan
01-Feb
01-Mar
01-Apr
01-May
01-Jun
01-Jul
01-Aug
01-Sep
01-Oct
01-Nov
01-Dec

c)

Figure 1.1.1: a) Mean weekly trap catch per trap each year (2013-2020) in NIAB EMR traps. b) mean numbers of adult *D. suzukii* catches per trap in 2013-2020 raw data and c) same data plotted on a $\log_{10}$ (n + 1) scale.

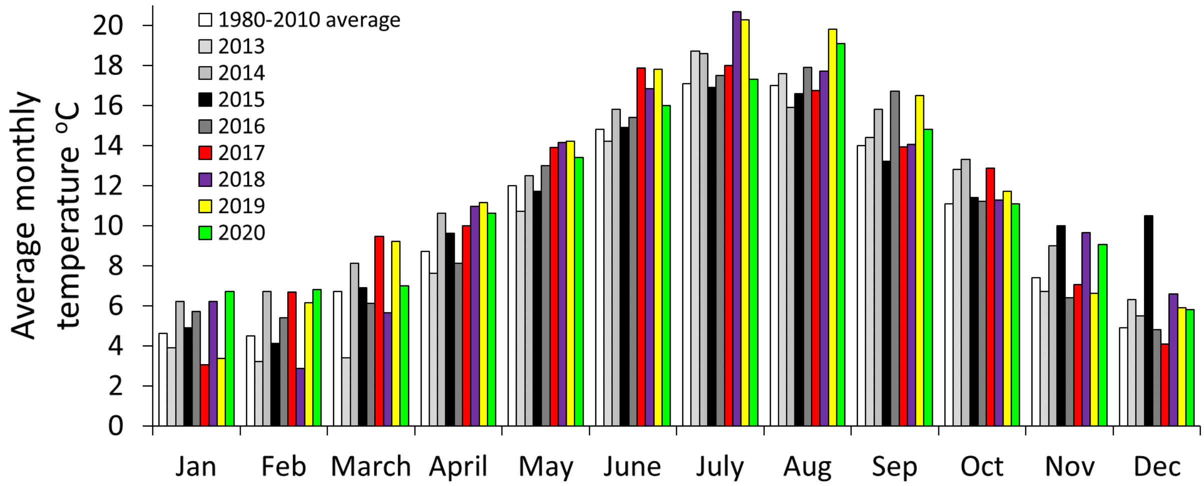


Figure 1.1.2. Comparison of the mean monthly temperatures between years at NIAB EMR.

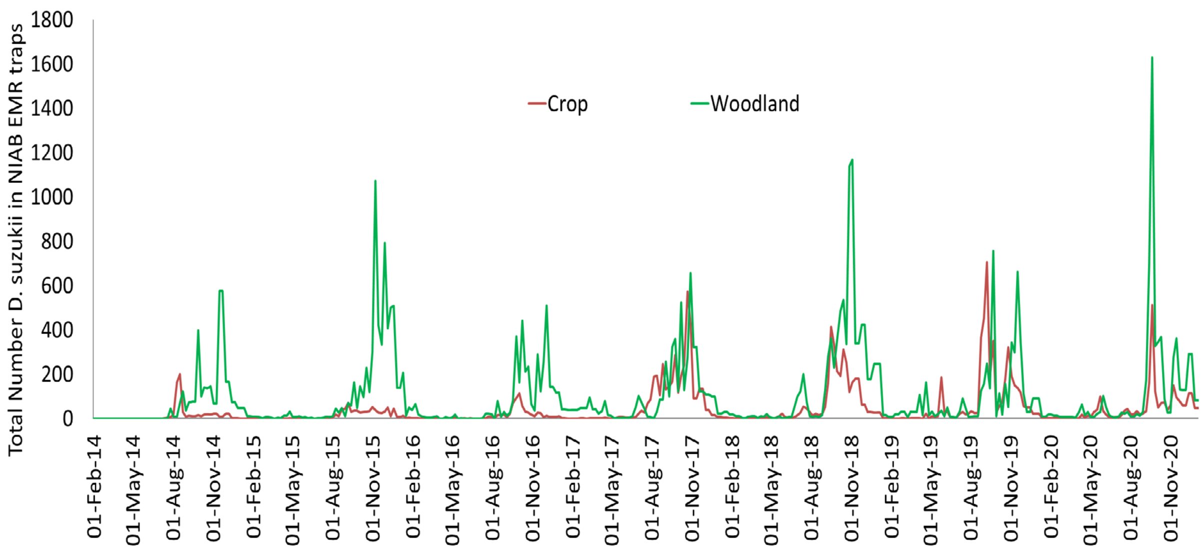
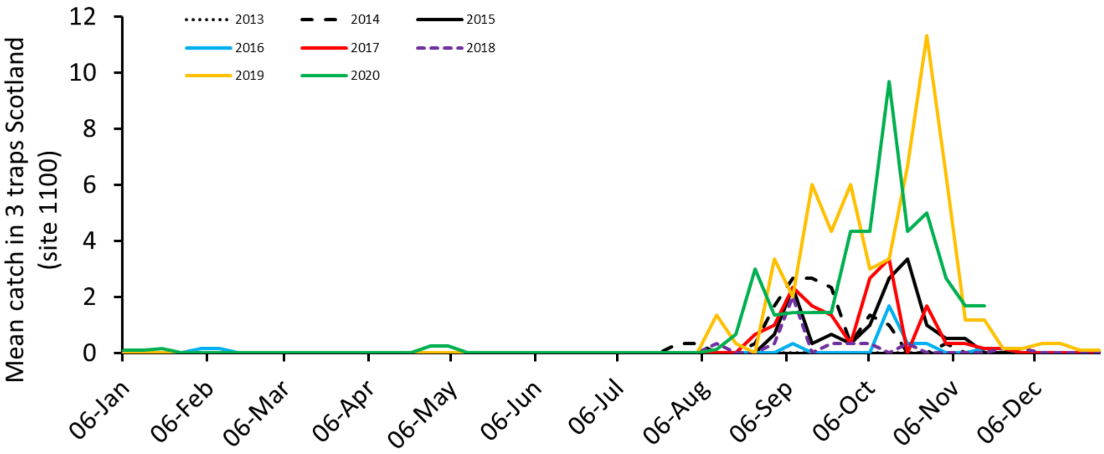


Figure 1.1.3. Mean numbers of *D. suzukii* adults per trap in crop (orange line) and woodland (green line) at the NIAB EMR site from 2013 to 2020.

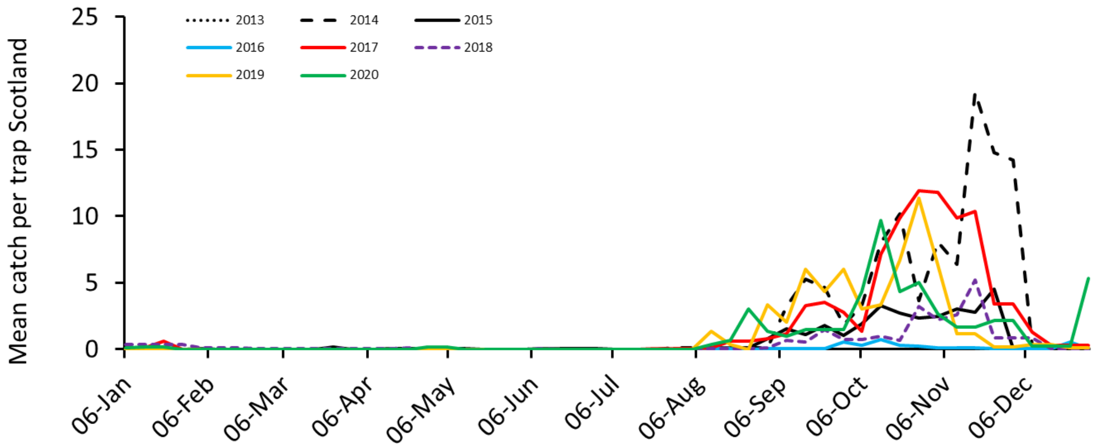
Scotland

In Scotland in 2019, 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 and it was these three traps that produced the 2019 and 2020 data. These were the traps that consistently caught the highest abundance of *D. suzukii* at this site. While traps are still deployed at site 1300, data will not be collected from them for the foreseeable future. To make it possible to directly compare monitoring data from previous years, comparative data from the three indicator traps at site 1100 traps are shown Figure 1.1.4a. For comparison, Figure 1.1.4b shows the mean catch for 40 original monitoring traps at four Scottish sites for years 2014 to 2018 in addition to the mean catch for the three indicator traps at site 1100 for years 2019 and 2020. Note that *D. suzukii* abundance is lower at site 1100 than at site 1300.

In general, catches of adult *D. suzukii* in the three traps followed previous years (Fig.1.1.4a). However, the monitoring results from site 1100 suggest that abundance may be increasing at that site (Fig 1.1.5) with the total number of SWD caught during the peak time (weeks 30-46) increasing from between 10-45 between 2014-2018 to 130-170 in 2019-2020. Unfortunately, we do not have comparable data for the other monitoring sites for 2019 and 2020 to determine if this is a local increase at a single site or if this increase is found at other Scottish sites. Winter/spring catches in 2020 were low (Table 1.1.1). Figure 1.1.6 displays the average monthly temperatures recorded at JHI. The abundance of *D. suzukii* in Scotland is still low in comparison to the South of the UK.



a)



b)

Figure 1.1.4. a) mean catch in 3 indicator traps at site 1100 Scotland 2013-2020, and b) mean catch of *D. suzukii* in Scotland from four Scottish sites for years 2014 to 2018 and one site (1100) for 2019 and 2020.

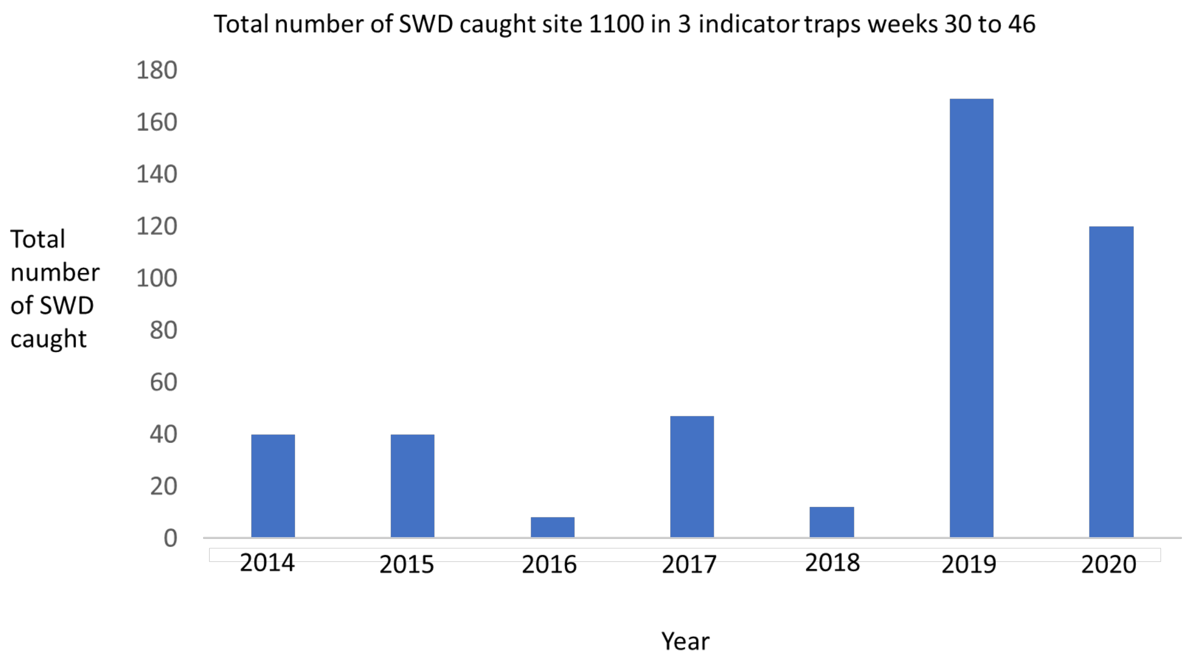


Figure 1.1.5. Total number of *D. suzukii* caught in the three indicator traps at site 1100 in Scotland for the period 2014-2020 during the peak catch period (end of July to mid-November).

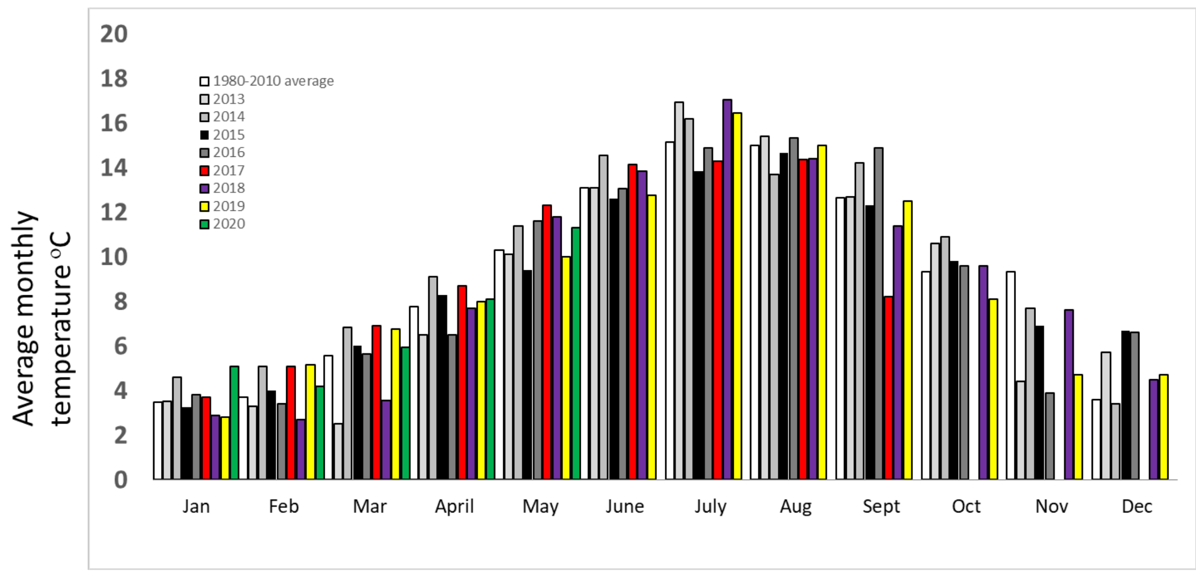


Figure 1.1.6. Comparison of the mean monthly temperatures between years at JHI.

Table 1.1.1 Winter and spring catches of *D. suzukii* (3 traps) site 1100 in Scotland

Week beginning	**23 Jan 2020**	**20 April 2020**
Number of male *D. suzukii*	1	1
Number of female *D. suzukii*	0	0

Suction trap

NIAB EMR staff visited Rothamsted Research in 2018 and sorted through samples that were positive for *D. suzukii,* collected from suction traps as part of the Rothamsted Insect Survey (RIS) (Figure. 1.1.7). The first visit was made in 2013 when no *D. suzukii* were found in samples. However from 2014 onwards male and female *D. suzukii* have been captured at a height of 12 m. This is correlated with the highest trap catches in the late autumn at crop and woodland level (Sep-Nov 2013-20). Traps in Scotland are still to be checked (Fig. 1.1.8). Unfortunatley, due to Covid restictions preventing visits to Rothamsted in 2020 the winter 2019 samples and winter 2020 samples are pending and we hope to be able to assess these in summer 2021.

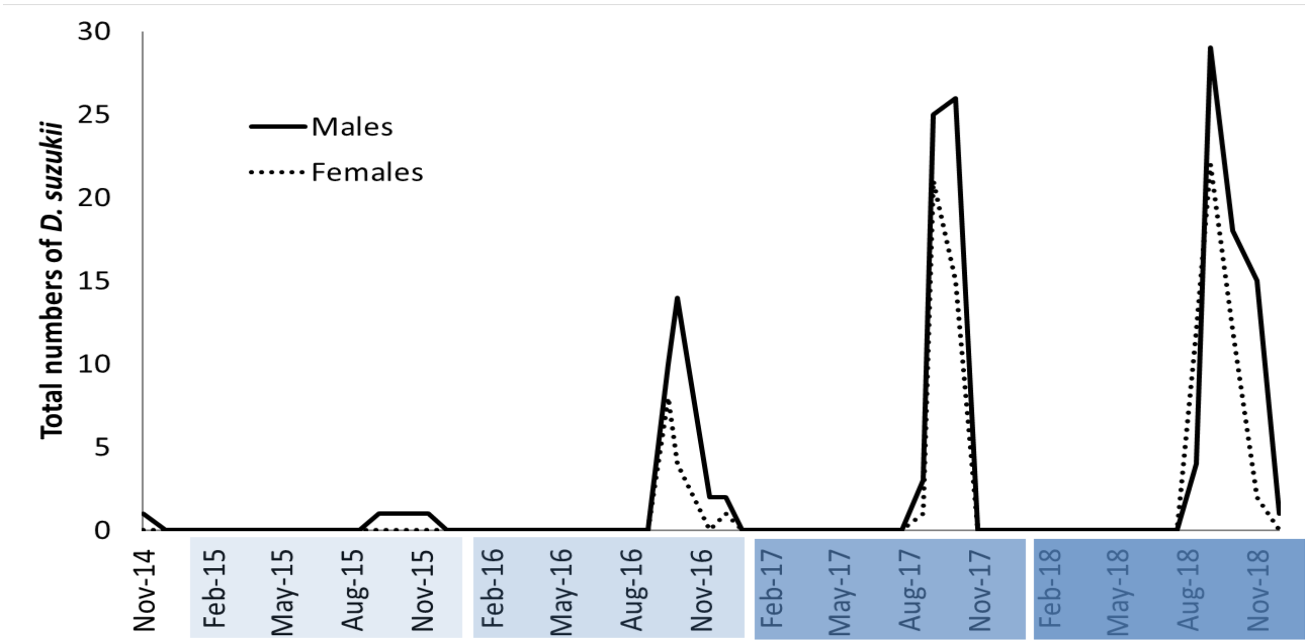


Figure 1.1.7. Total numbers of *D. suzukii* adults in 12.2 m height suction traps 2013 to 2018 from the Rothamsted Research suction trap survey. First catches were in 2014. 2019 and 2020 samples will be assessed in 2021.

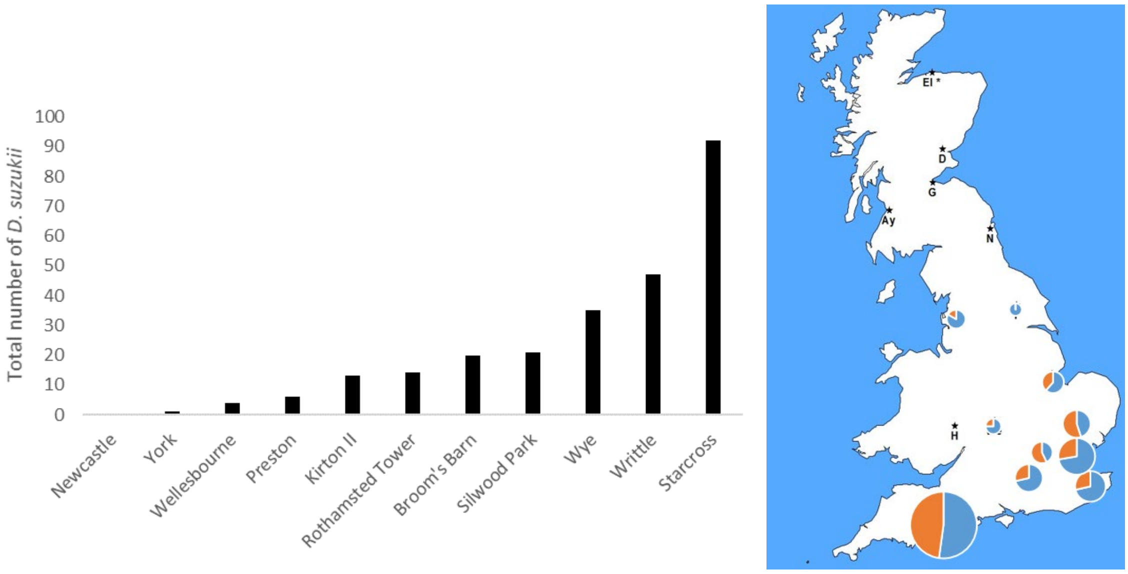


Figure 1.1.8. Total numbers of *D. suzukii* adults from 12 m height Rothamsted Suction Trap samples (2014-18) at different locations. NB: traps from Scotland have not yet been checked. Orange = females, Blue = males

Conclusions

- *D. suzukii* numbers at NIAB EMR in 2020, overall, were similar to the catch numbers of 2017 and 2018, but 2020's trend most closely relates to 2019's profile.
- Peaks of trap catches in Scotland occur between the end of July to November.
- There continues to be variation in interannual trap catches, at least in the late autumn, probably largely dependent upon temperature.
- *D. suzukii* can be detected at 12 m by the suction trap during the main flight/dispersal period when the flies are captured in the traps in cropping and woodland areas (August - November).
- 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.*
- Data is communicated to the AHDB each month or on request.

Task 1.2. Modelling of the 6-year National Monitoring dataset (JHI)

Objectives

1. Predict the first peak of summer morph flies
2. Analyse trap catch data for males and females separately to determine if males are a good enough indicator of female activity/density
3. Predict female spring activity
4. Predict onset of fecundity of overwintered SWD
5. Predict of SWD abundance in crops and woodlands
6. Predict SWD activity in crops (not including wild areas)

1: Predict the first peak of summer morph flies

The aim is to develop a predictive tool that can use short-term weather forecast data to predict whether the first peak of summer morph flies will occur in the upcoming week. Total crop counts (male + female) from 16 sites over 6 years (2013–2018) were analysed to find local maxima (peaks) in capture values (Fig. 1.2.1). A local peak was defined as a data sample that is larger than its two neighbouring samples. It was further stipulated that local peaks must have a value greater than 1 (as each data point is an average of multiple trap captures, giving some fractional values) and first summer local peaks were the first peaks that occurred between May 1 and August 31. The prediction task was framed as a binary classification problem, where the day of the first summer local peak was labelled as class 1, and another day in the year was randomly selected to serve as class 0. Out of a total of 96 site-year combinations, first summer local peaks were identified in 53 cases, due to either an absence of summer captures (e.g., 2013) or missing data. This gave a total of 106 instances (53 of each class) for model development. Weather variables were summarised over the week prior to the date of each instance using data from the nearest UKMO weather station: minimum temperature, maximum temperature, mean temperature, average relative humidity, average wind speed, cumulative incoming shortwave radiation, total precipitation, and degree-days. Degree-days were calculated for SWD according to Tochen et al. (2014) using a base temperature of 7.2 °C and an upper threshold of 30 °C.

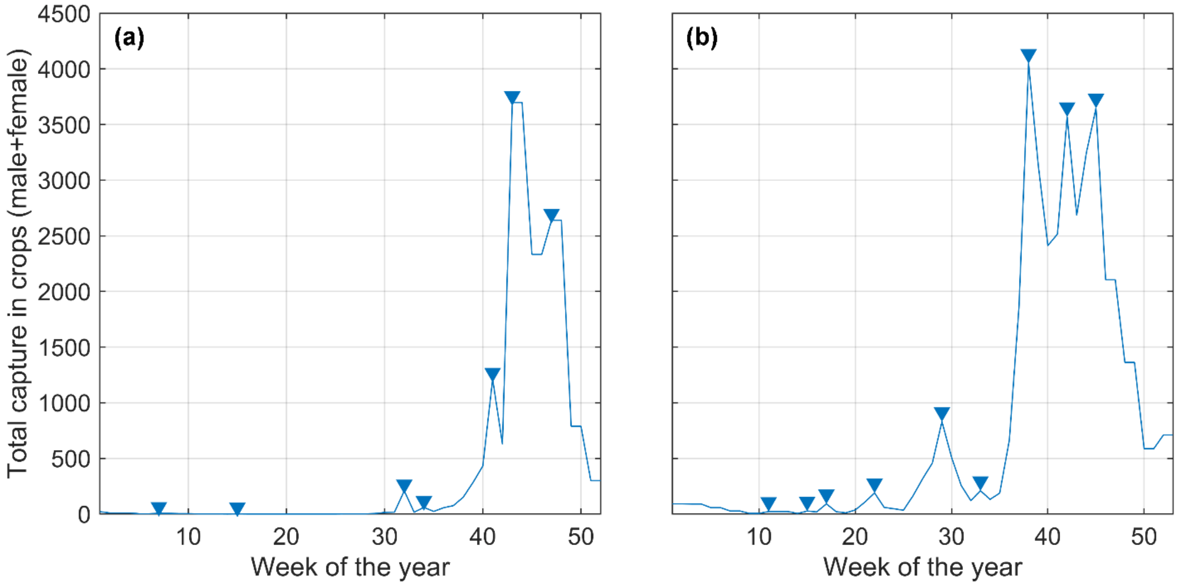


Fig. 1.2.1. Local peaks in SWD trap captures for two example site-year combinations: (a) Site 1, 2014; (b) Site 5, 2018.

The point-biserial correlation coefficient was used to test the correlation between weather variables and the first peak of summer morph flies (peak/non-peak) (Fig. 1.2.2). Note that this is mathematically equivalent to the Pearson (product moment) correlation if the predictor variables are continuous and the categorical variable is dichotomous. Values for the three temperature variables and degree-days were all highly significant ($P < 0.005$). There was also strong evidence of correlation among predictor variables; the mean variance inflation factor was 22.58, indicating excessive or serious multicollinearity.

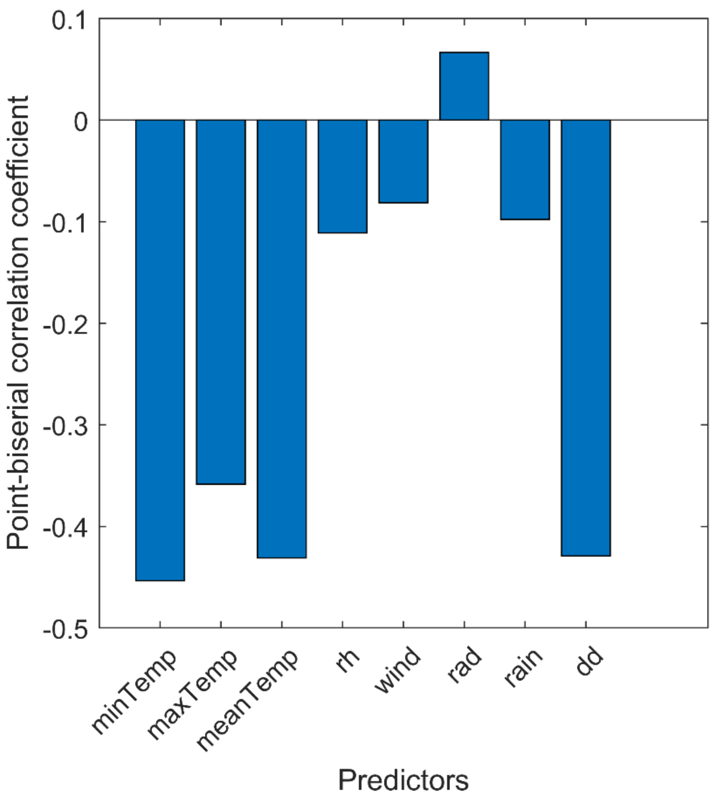


Fig. 1.2.2. Correlation of weather variables with first peak of summer morph flies. The length of the bars should be viewed relative to one another – temperature variables were far more strongly correlated to observed peaks in captures than the other weather variables. This is not a modelled result; it is correlation between observed peaks and weather.

A suite of 37 different machine learning classification algorithms were applied to the data. The most promising algorithm for further optimisation was a discriminant analysis classifier (DAC). This was implemented using the MATLAB procedure FITCDISCR. The performance of the algorithm depends on the tuning of hyperparameter values; hyperparameters are parameters that cannot be learned from the data and must be set prior to model training. A nested *k*-fold cross-validation procedure was used to train and tune the algorithm and obtain an unbiased estimate of the generalization accuracy of the entire model-building process to unseen data. This consisted of a 5-fold cross-validation inner loop for hyperparameter optimization and a 5-fold cross-validation outer loop for model assessment. Hyperparameter optimization in the inner loop was conducted using the Bayesian optimization approach. Principal components analysis was applied to the data within the nested *k*-fold design to combine the weather features into a smaller number of uncorrelated variables and to improve the execution time and accuracy of the classifiers. Prediction performance was computed by averaging the results of the 5-fold cross-validation in the outer loop.

Bayesian optimisation did not improve predictive performance due to the small size of the dataset, and default values for FITCDISCR were used: hyperparameter DISCRIMTYPE = QUADRATIC, GAMMA = 0, and DELTA = 0. The first three principal components explained over 95% of the variability in the data and were retained as predictors. The resulting DAC achieved a training accuracy of 80.4% (*SD*=2.5%) and a test accuracy of 83.0% (*SD*=2.5%). Figure 1.2.3 shows the results on the outer test folds for the first two principal components only, for ease of visualisation. A confusion matrix was also generated using the predicted responses on the outer test folds (Fig. 1.2.4). This is a technique for summarising and visualising the performance of a classification algorithm. A confusion matrix is a cross-tabulation formed by the overall agreement-disagreement, where the row and column labels of the matrix represent observed and predicted classes, respectively, and each cell contains the corresponding number of test cases. Moving clockwise from the top left-hand corner cell provides the number of true negative, false positive, true positive, and false negative results. The row summary to the right of the matrix displays the number of correctly (first column) and incorrectly (second column) classified observations for each true class as percentages of the number of observations of the corresponding true class, i.e., the true positive rates and false positive rates. The column summary displays the number of correctly (first row) and incorrectly (second row) classified observations for each predicted class as percentages of the number of instances of the corresponding predicted class, i.e., the positive predictive values and false discovery rates. The confusion matrix showed that first summer local peak days were classified with 75.5% accuracy, and non-peak days with 90.6% accuracy. The positive predictive value indicates that 88.9% of the positive predictions were true positives.

Note that the approach defined here for identifying peaks in trap counts can also be used to identify overlapping generations later in the season. The ability to predict peaks in traps catches is a measure of SWD activity and does not necessarily show the first summer generation however, a rise in numbers from June with lighter-coloured SWD in traps is a good indicator of the first generation.

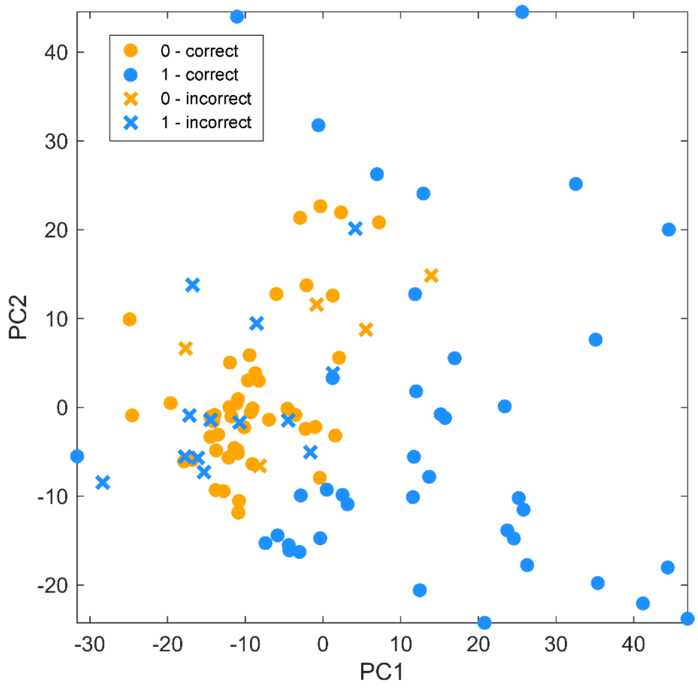


Fig. 1.2.3. Classification of first summer local peak days (1) and non-peak days (0) using a quadratic discriminant analysis algorithm and nested k-fold cross-validation. Values are shown for the first and second principal components only.

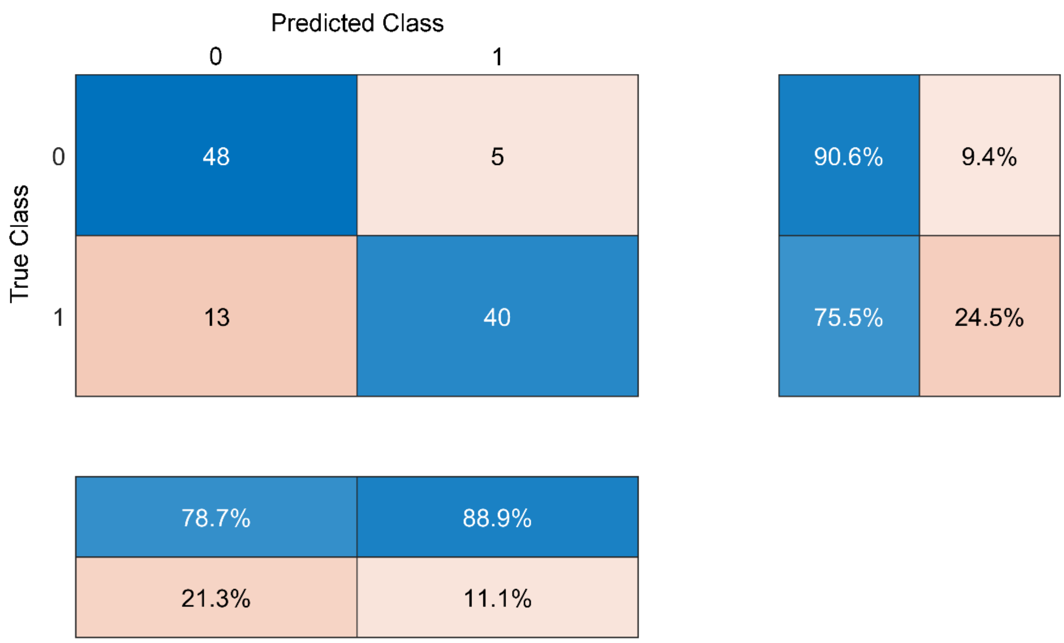


Fig. 1.2.4. Confusion matrix for binary classification of first summer local peak days (1) and non-peak days (0) using a quadratic discriminant analysis algorithm and nested k-fold cross-validation. Blue colours are used for 'correctness' and pink for 'incorrectness'.

2: Analyse trap catch data for males and females separately to determine if males are a good enough indicator of female activity/density

The pairwise correlation between male and female counts was 0.93, 0.96 and 0.97 for crop, woodland, and total counts, respectively. This indicated that male counts could be a good predictor of female counts. It would be useful to run the data in the spring when numbers of SWD are low and the survival of males over the winter is known to be lower than females to determine if the correlation is as accurate for year-round numbers.

Male and female counts from the different sites and years were pooled together to produce three datasets: crop counts (n = 1294), woodland counts (n = 1353), and total counts (crop + woodland, n = 1583). Any instance where the male or female count was less than 1 or missing was omitted from the datasets. There were sufficient data to set aside hold-out test sets, therefore each dataset was randomly shuffled (by rows) to remove any temporal correlations and then split into a training dataset (80%) and a test dataset (20%). Simple linear regression was applied to the training data for crop, woodland, and total counts to produce three models: $Y = a + Bx$. The fitted models were then used to predict female counts in the corresponding held-out data (crop, woodland, or total) as a test of how well the modelling approach can generalize to unseen data. The models were then refit to all the data (training + test) to report the fitted coefficients.

Note that more complex Generalized Linear Models and Generalized Linear Mixed Effects models were also fit to the data, but this did not significantly improve performance. Similarly, random forests were also fit to the data, using a low resample rate to further remove any effect of temporal correlations, but the performance on the held-out test data was again very similar. The simple regression approach was therefore deemed as preferable for use in the industry by practitioners with limited modelling experience.

Performance of the fitted models on the held-out test data is given in Table 1.2.1, estimated coefficients in Table 1.2.2, and the fitted lines in Fig. 1.2.5. The results show that male counts can be used to predict female counts with a reasonable degree of confidence.

Table 1.2.1. Performance of the models on the held-out test data

Model	MAE	NMAE	RMSE	R^2
Crop	58.57	0.94%	215.65	0.83
Wood	104.1	1.60%	289.75	0.87
Total	162.9	0.98%	932.26	0.86

MAE = mean absolute error

NMAE = MAE normalised by the range in observed values

RMSE = root mean square error

Table 1.2.2 Estimated coefficients for the models fit to all the data

Model	Coefficient	Estimate	SE	t	P	95% CI	
Crop	*a*	21.9520	10.8200	2.0289	0.04268	0.7258,	43.1788
	B	1.1654	0.0126	92.2090	.0000	1.1406,	1.1902
Wood	*a*	43.8980	29.4930	1.4884	0.1369	- 13.9590,	101.756
	B	1.2505	0.0159	78.6970	.0000	1.2194,	1.2817
Total	*a*	46.5500	27.1900	1.7120	0.0871	-6.7830,	99.8821
	B	1.2414	0.0124	100.5300	.0000	1.2172,	1.2657

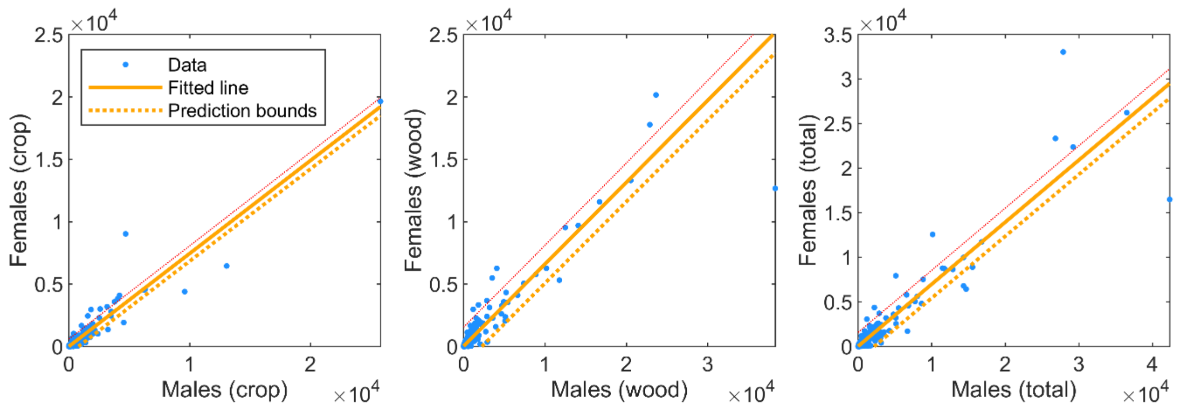


Fig. 1.2.5. Regression of SWD female counts on male counts and 95% prediction intervals.

3: Predict female spring activity

The approach described in Objective 1 for identifying local peaks in the data was used to identify the first spring local peaks in female counts (crop + woodland) and to set up a binary classification task, where the day of the first spring local peak was labelled as class 1 and another day in the year was randomly selected to serve as class 0. This resulted in a much smaller dataset than in Objective 1 due to lower trap counts in the spring than the summer, with 30 first spring local peaks identified across all site-year combinations, giving a combined dataset of 60 instances (30 first peak days, 30 non-peak days). Predictor variables were generated as described in Objective 1. Figure 1.2.6 shows the correlation between weather variables and the first spring local peak (peak/non-peak). Values for the three temperature variables and degree-days were again all highly significant ($P < .001$). A suite of 37 different machine learning classification algorithms were applied to the data. The most promising algorithm for further optimisation was a fine *k*-nearest neighbor (fKNN) algorithm. fKNN was implemented using the MATLAB FITCKNN procedure with hyperparameters NUMNEIGHBORS = 1, and DISTANCE = EUCLIDEAN. The nested *k*-fold cross-validation procedure described above was used to train, tune, and test the algorithm.

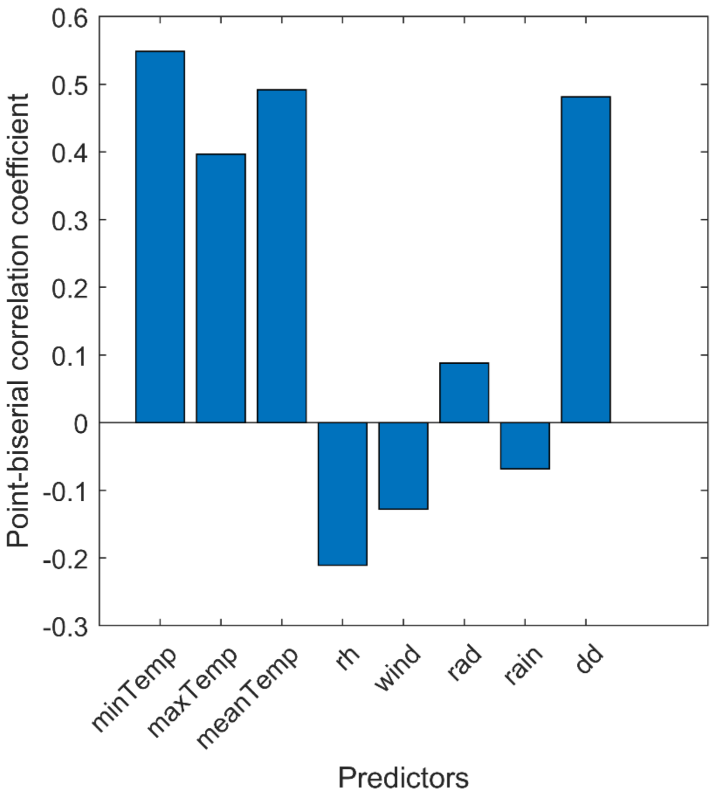


Fig. 1.2.6. Correlation of weather variables with first spring local peak. A model was built to predict the peaks in trap counts in the spring. This figure shows the correlation between weather variables and those actual spring peaks in observed captures. The length of the bars should be viewed relative to one another – temperature variables were more strongly correlated to observed peaks in captures than the other weather variables.

The optimized hyperparameter was DISTANCEWEIGHT = SQUAREDINVERSE. The first three principal components explained over 95% of the variability in the data and were retained as predictors. The resulting fKNN classifier achieved a training accuracy of 91.7% (*SD*=2.5%) and a test accuracy of 93.3% (*SD*=6.9%) (Fig. 1.2.7). The confusion matrix showed that 96.7% of peak days were classified correctly and 90% of non-peak days (Fig. 1.2.8). Hence, the peak of the first winter morph SWD activity in the spring can be predicted with over 96% accuracy.

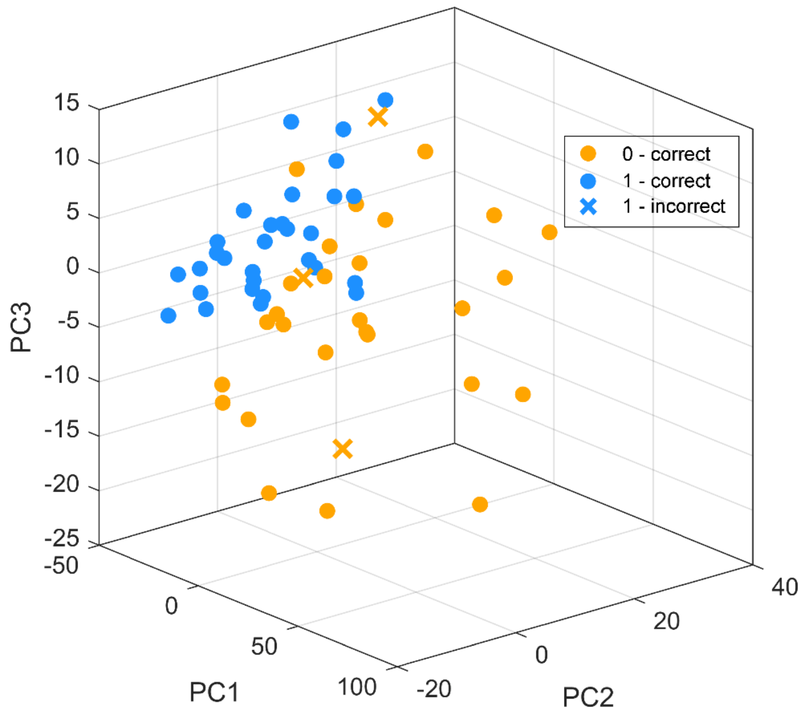


Fig. 1.2.7. Classification of first spring local peak days (1) for female SWD counts and non-peak days (0) using a fine KNN classifier and nested k-fold cross-validation. Here, 3 principal components explained 95% of total variation and were used as predictors in the model.

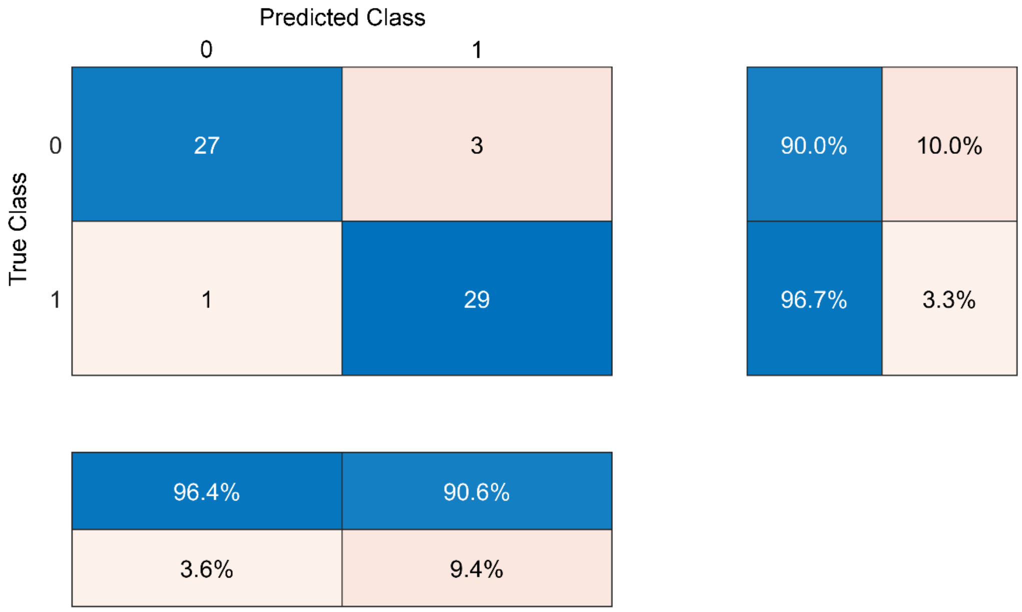


Fig. 1.2.8. Confusion matrix for binary classification of first spring local peak days (1) for female SWD counts and non-peak days (0) using a fine KNN classifier and nested k-fold cross-validation. Blue colours are used for 'correctness' and pink for 'incorrectness'.

4: Predict onset of fecundity of overwintered SWD

Fecundity data for sites 3, 4, and 5 for the years 2014–2017 were used to develop a model to predict the stage (5 classes) of fecundity. These data were extremely problematic, however, as the sample size was typically less than 5 females on any given date. The distribution of weather variables per fecundity stage was summarised using boxplots to explore patterns in the data and identify potential predictor variables (Fig. 1.2.9). The data were also submitted to one-way ANOVA followed by Tukey's-HSD test ($P < 0.05$). The results showed no evidence of an association between weather variables and fecundity stages.

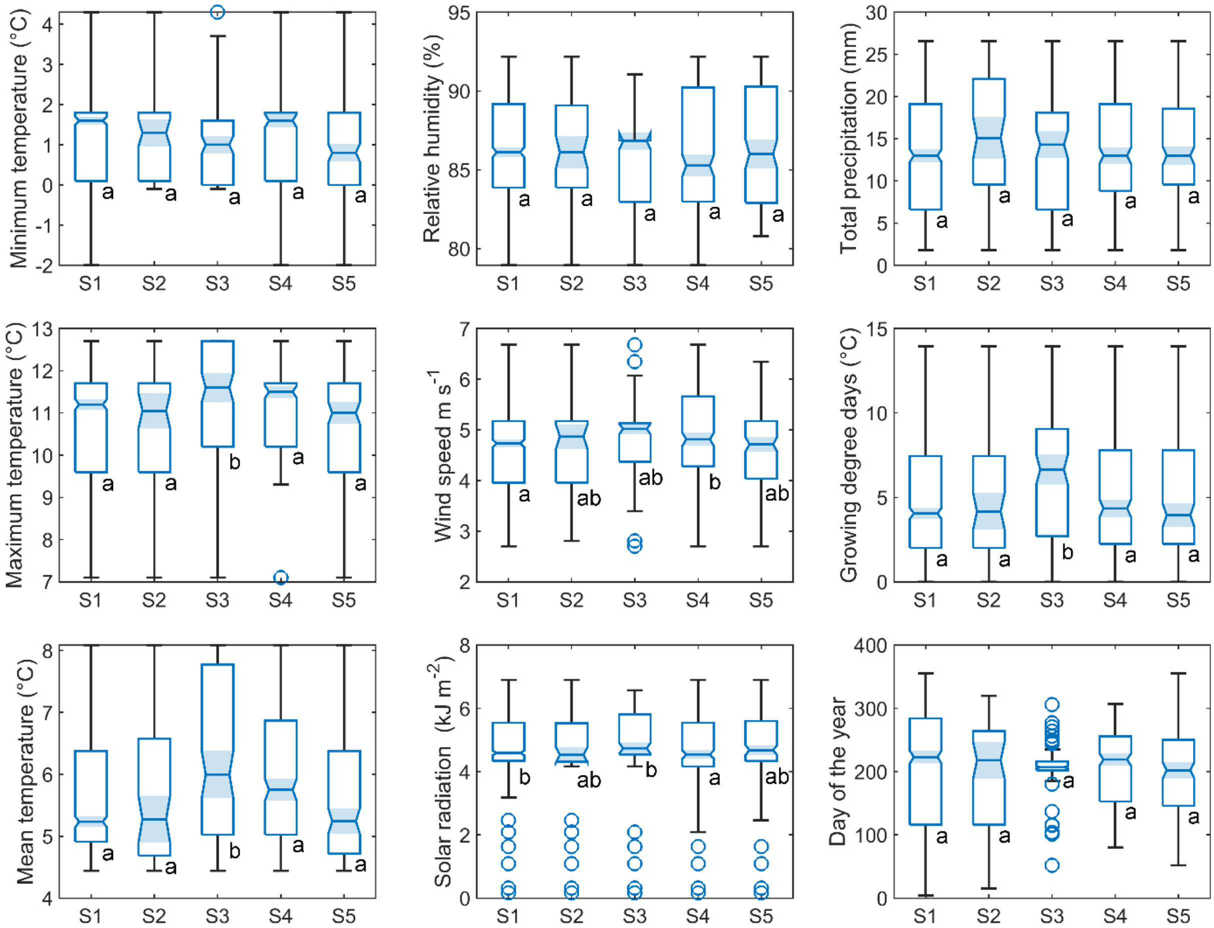


Fig. 1.2.9. Boxplots of weather variables grouped by SWD fecundity stage. Letters represent significance groupings based on one-way ANOVA followed by Tukey's HSD post-hoc analysis ($P<0.05$).

A suite of 37 different machine learning classification algorithms, including soft- and hard-clustering techniques, were applied to the data. The results were poor, with an adaptively boosted decision tree (ABDT) algorithm achieving the highest accuracy of 59%. This was in part due the lack of association of fecundity stage with available predictors and to a very large imbalance between the classes: the percentage of instances in each fecundity stage were 52.96, 4.7, 9.64, 20.49, and 12.22% for Stages 1-5 (1. no ovaries; 2. unripe ovaries 3. ripening eggs in ovarioles; 4. mature eggs in ovarioles; and 5. mature eggs in the abdomen (Grassi et al, 2018)), respectively. Most classification machine learning algorithms work best when the number of instances of each class is roughly equal. Adding latitude, longitude, Site and Year as predictors did not improve results. An attempt was made to balance the data using upsampling and downsampling techniques, but this did not lead to any improvements.

As just over half the data were Stage 1, the prediction task was again framed as a binary classification problem, where Stage 1 instances were labelled as class 1 and all other fecundity stages were labelled as class 0. This led to a marked improvement in predictive accuracy. ABDT was selected as the superior algorithm and implemented using the MATLAB procedure FITCENSEMBLE with hyperparameters METHOD = ADABOOSTM1, and NUMVARIABLESTOSAMPLE = ALL. The data was randomly shuffled (by rows) and split into a training dataset (80%) and a test dataset (20%). The nested *k*-fold cross-validation scheme was used to train, tune, and test the algorithm. The optimized hyperparameters were: MINLEAFSIZE = 26, MAXNUMSPLITS = 18, SPLITCRITERION = DEVIANCE, NUMLEARNINGCYCLES = 18, and LEARNRATE = 0.1187. The resulting ABDT classifier achieved a training accuracy of 70.8% (*SD*=1.1%) and a test accuracy of 69.6% (*SD*=1.6%). The confusion matrix showed that 78.4% of fecundity stage 1 instances were classified correctly and 73.4% of non-stage 1 instances (Fig. 1.2.10). Performance on the held-out test data was 76.1% accuracy (Fig. 1.2.10). Note that ABDT performs its own internal feature selection as an integral part of the procedure.

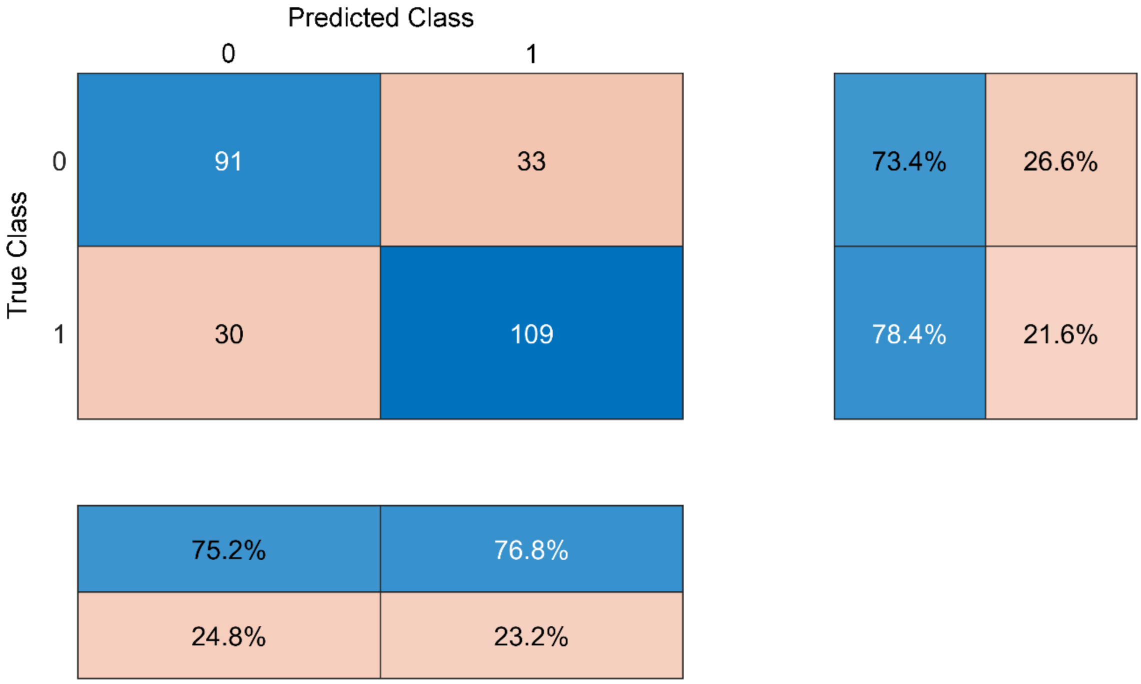


Fig. 1.2.10. Confusion matrix for binary classification of SWD fecundity stage on held-out test data using a fine decision tree algorithm, where Stage 1 = class 1 and Stages 2-5 = class 0. Blue colours are used for 'correctness' and pink for 'incorrectness'.

As the dimensionality of the predictor data was not reduced using PCA, it is useful to quantify the most important variables for prediction according to the algorithm; these were incoming shortwave radiation, followed by maximum temperature, and day of the year (Fig. 1.2.11).

Note that the ABDT algorithm is a very powerful and currently popular machine learning technique that combines the performance of many 'weak' learners to produce a powerful ensemble of models. Decision trees can, however, be sensitive and small changes in the training data can result in a very different output model. It is therefore recommended that more samples are obtained for assessment of fecundity on each sampling date if this or any other approach is to be developed further; ~5 samples appeared to be insufficient to obtain a balanced dataset. There was not enough time to include the Scottish fecundity data and this may lead to improved performance, depending on its quality. There may also be utility in exploring cost-sensitive learning approaches for imbalanced data in the future, as well as the inclusion of additional predictor variables. Currently the data set available only predicts onset of fecundity vs not fecund with ~74% accuracy using the weather data from the previous weeks. It is likely that this is because of the limited dataset available on dissected female flies.

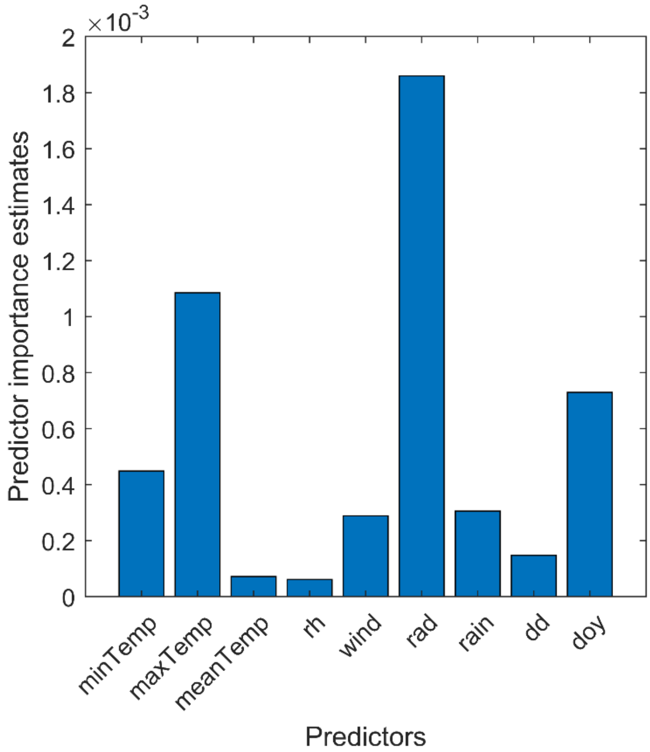


Fig. 1.2.11. Predictor importance of weather variables in SWD fecundity stage estimation using an adaptively boosted decision tree classifier. The length of the bars should be viewed relative to one another.

5: Predict of SWD abundance in crops and woodlands

The model that was developed for predicting SWD abundance (crop + woodland) in the previous report was fit to data for crops only. To recap, the model is a logistic function of degree-days that was fit separately to total SWD captures for each site-year combination. It predicts population abundance on a scale from 0 to 1 and can be rearranged to predict the degree-days required to reach a certain population level, e.g., 50% of the maximum abundance.

Degree-days (DD) were calculated as described above, and the model again achieved a good fit to the trap data, despite only half as many datapoints being available for fitting (crops only) (Fig. 1.2.12).

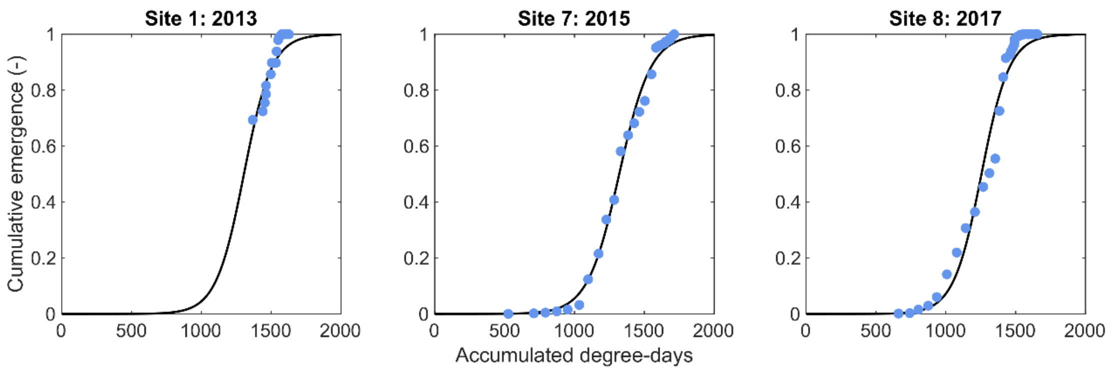


Fig. 1.2.12. Comparison between observed and estimated cumulative abundance of SWD (male+female) in crops at three representative site-year combinations.

The required DD (i.e. time) to reach any proportion of cumulative population abundance, *P*, can easily be predicted by rearranging the logistic model and plugging in the fitted parameters (a, b) for any monitoring site:

$$DD = -\log\left(\frac{\frac{1}{P} - 1}{a}\right)/b$$

The above formula was used to predict 5, 10, 25, 50, 75, and 95% cumulative abundance in each site-year dataset. Results for all datasets were grouped together by percentage abundance and the model evaluated by fitting a straight line to observed vs. predicted values and comparing the slope and intercept parameters against the 1:1 line. In simple terms, if the model is successful in predicting percentage cumulative abundance then the fitted line should closely match the 1:1 line. Results indicate the model was successful in predicting mid-to-high percentage cumulative abundance, with R^2 values of 0.39, 0.46, 0.81, 0.94, 0.93, and 0.87 for 5, 10, 25, 50, 75, and 95% emergence, respectively (Fig. 1.2.13). It can be concluded that this approach can be used to predict 25-100% abundance in crops with reasonable confidence.

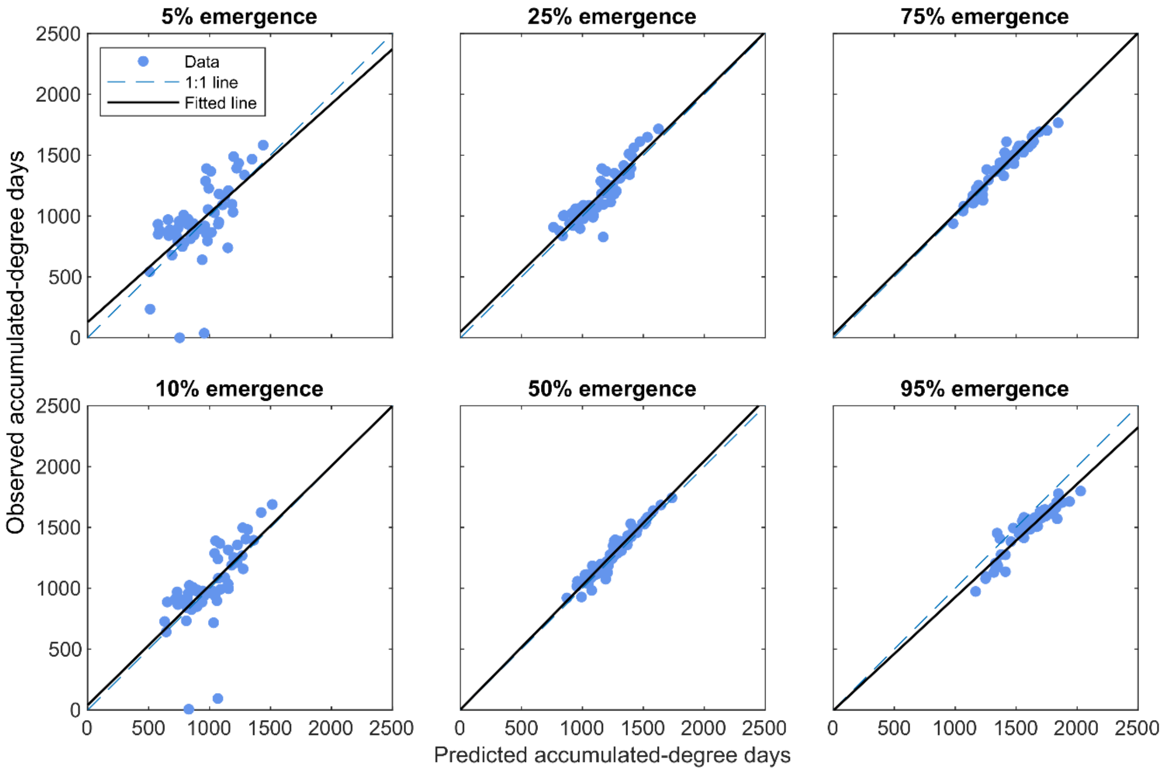


Fig. 1.2.13. Observed vs. predicted regression scatter plots for percentage emergence (abundance) of SWD (male+female) in crops at all site-year combinations.

6: Predict SWD activity in crops (not including wild areas)

The model that was developed in the previous report to forecast the presence/absence of SWD (i.e. flight activity) at any location on any given day based on weather conditions was trained and tuned using data for crops only. The model is an adaptively boosted decision tree model.

Trap counts in crops (male+female) for each site-date were converted to a binary response (1 = presence, 0 = absence). UKMO weather data were summarized over the periods from the setting of the traps to sample collection. The data was randomly shuffled (by rows) and split into a training dataset (80%) and a test dataset (20%). The time in days after the first capture event (tafe), time in days after the last capture event (tae) and degree-days were used as predictor variables. The model was implemented using MATLAB's FITCENSEMBLE procedure with hyperparameters METHOD = ADABOOSTM1 and NUMVARIABLESTOSAMPLE = ALL. The nested *k*-fold cross-validation scheme was used to train, tune, and test the algorithm.

The optimized hyperparameters were: MINLEAFSIZE = 28, MAXNUMSPLITS = 14, SPLITCRITERION = DEVIANCE, NUMLEARNINGCYCLES = 12, and LEARNRATE = 0.0022. The resulting ABDT classifier achieved a training accuracy of 92.0% (*SD*=0.37%) and a test accuracy of 91.8% (*SD*=1.6%). The confusion matrix showed that 90.1% of SWD presence instances were classified correctly and 90.3% of absence instances (Fig. 1.2.14). Performance on the held-out test data was also excellent at 90.2% accuracy (Fig. 1.2.10).

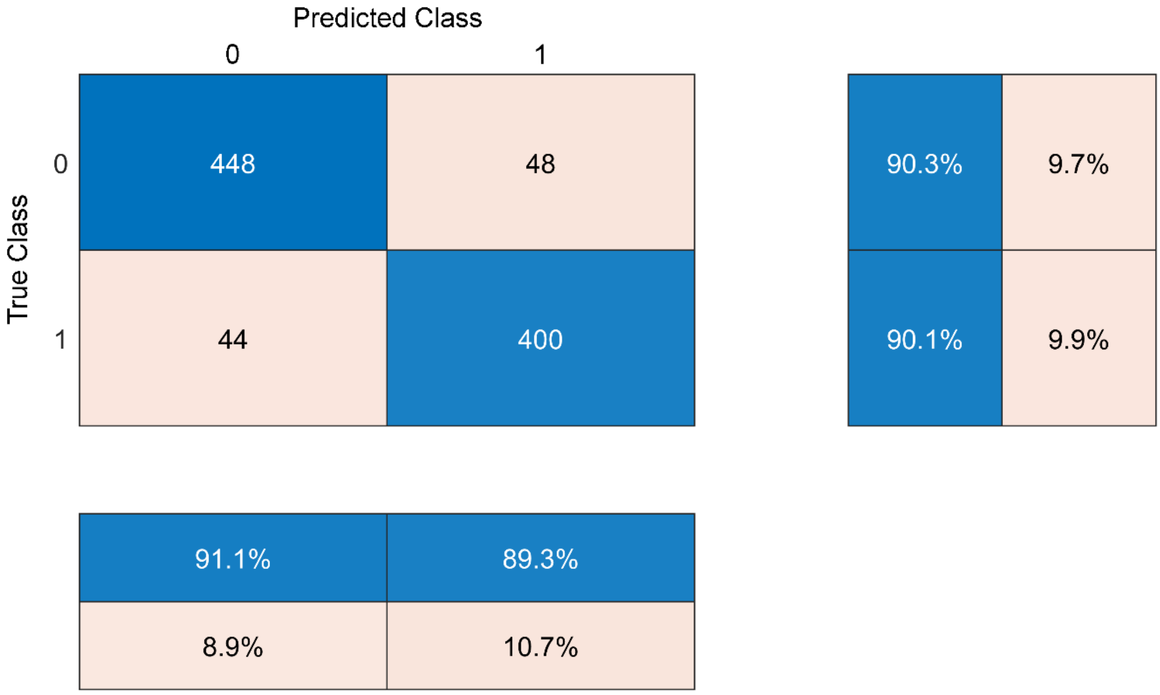


Fig. 1.2.14. Confusion matrix for binary classification of SWD presence/absence (flight activity) on held-out test data using an adaptively boosted decision tree algorithm. Blue colours are used for 'correctness' and pink for 'incorrectness'.

Conclusions

- Machine learning can predict first spring female peak with 93.3% accuracy, SWD presence / absence with 90.2% accuracy, first summer peak with 83.1% accuracy, and fecundity with 76.1% accuracy.
- Regression can predict female activity from male activity with 83-87% accuracy and time required to reach a % value of SWD population size with 72-99% accuracy.
- These weather-dependent predictive tools could be further improved with the addition of more SWD data, in particular fecundity.
- These prediction models can be used to highlight periods of crop vulnerability or threat of SWD pressure and can help growers make informed control decisions.

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

Task 2.1. Analyses of fermentation products from yeasts attractive to D. suzukii (Rory Jones and NRI)

Introduction

Rory Jones of University of Lincoln is undertaking an AHDB PhD Studentship (CP171) to investigate the attractiveness of a range of exotic yeast species to SWD. To date, he has tested several species in laboratory bioassays and field trapping experiments. The aim of this work was to identify the chemicals produced by yeasts and investigate whether there was any correlation between these chemicals and the attractiveness of yeasts to SWD.

During 2019, NRI provided assistance and facilities to Rory Jones in developing methods for collection of volatiles released by yeast cultures using solid-phase microextraction (SPME) and analysis of these chemicals by gas chromatography coupled to mass spectrometry. Volatiles from four proprietary yeast species, and a commercial wine yeast, grown on sterile strawberry juice were collected and analysed. In all, 34 compounds were identified, but the analyses were complicated by eight major compounds from the sterile strawberry juice.

Progress during 2020/2021

The collections and analyses were repeated by Rory Jones at NRI with the same five yeast species grown on yeast potato dextrose which produces few contaminating volatiles. As previously, amounts of some components such as ethanol, 3-methylbutanol and 2-phenylethanol initially appeared similar from all species, but amounts of others such as ethyl acetate, acetoin and acetic acid varied (Fig. 2.2).

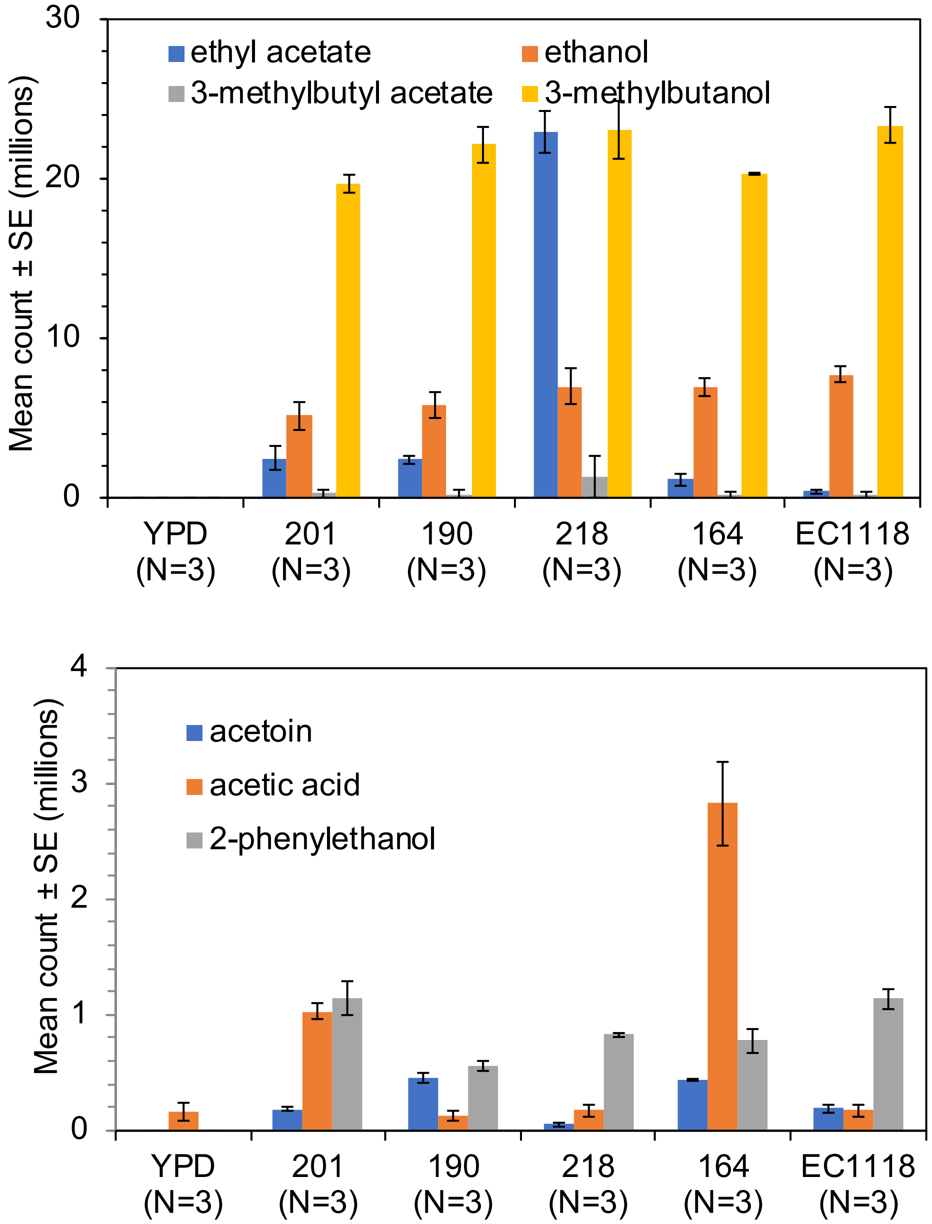


Fig. 2.2 Relative amounts of selected components emitted by five yeast species collected by SPME and analysed by GC-MS.

With assistance from Dan Bray (NRI), Rory Jones has carried out a Principle Component Analysis to identify if there are consistent differences in the volatile profiles of different yeast species. To test whether volatile profile might influence behaviour of *D. suzukii* towards yeasts, strength of correlation was measured between principle component scores derived from volatile analysis and previously collected measures of behavioural activity.

Two principle components (PC1, PC2) extracted from scaled GC-MS data accounted for 21% and 17% (Total: 38%) of variation in yeast volatile profiles. PC1 separated volatile collections from yeast potato dextrose media controls from those made from yeasts (Fig 2.3). Most yeast species were separated along PC2, although overlap occurred between collections made from *S. cervisiae* and *H. uvarum* (Fig 2.3).

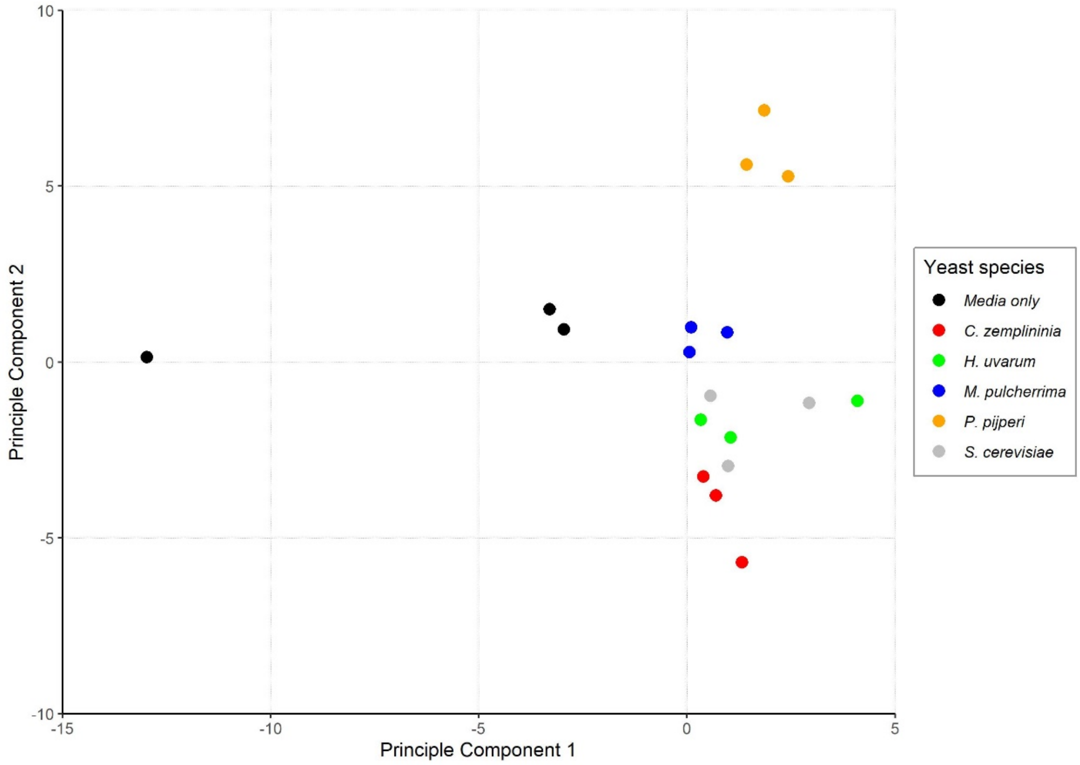


Fig 2.3 PCA plot illustrating partial separation of five yeast species (coloured dots) and a yeast potato dextrose media control (black dots) by scores on two Principle Components derived from volatile analysis. PC1 separated media only controls from the yeasts, while PC2 separated yeast species except *H. uvarum* and *S. cerevisiae*.

PC1 was positively correlated (Pearson's correlations, $P<0.05$) with relative amounts of ethanol, 3-methylbutanol and 2-phenylethanol recovered from yeast volatile collections (Fig 2.4). PC2 was positively correlated ($P<0.05$) with relative amounts of ethyl acetate, ethyl butanoate and 3-methylbutyl acetate recovered (Fig. 2.5).

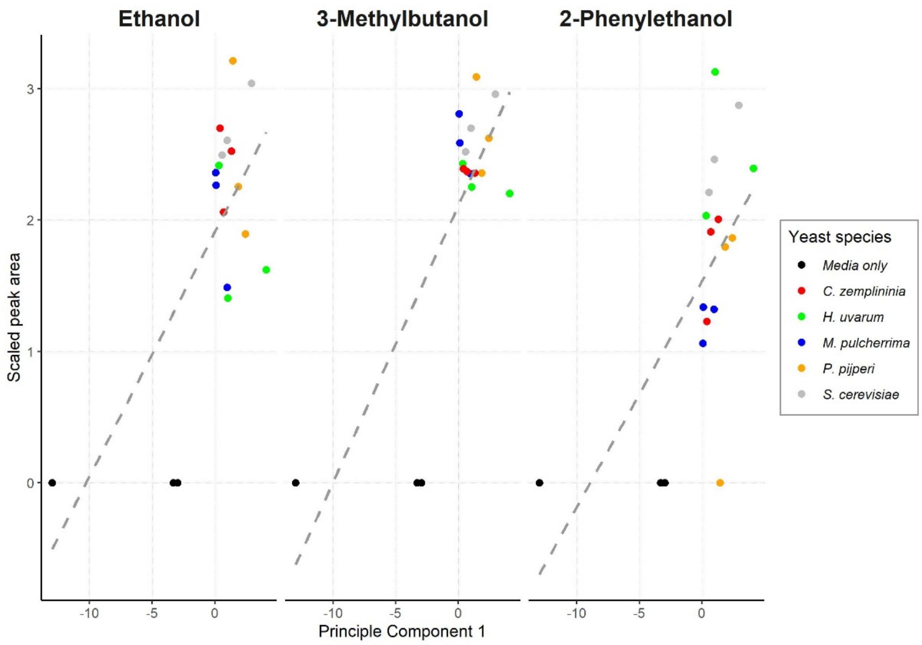


Fig 2.4 Correlations (dotted line) between scores for Principle Component 1 and amounts of volatile chemicals recovered from five different yeast species (coloured dots) and media only controls (black dots).

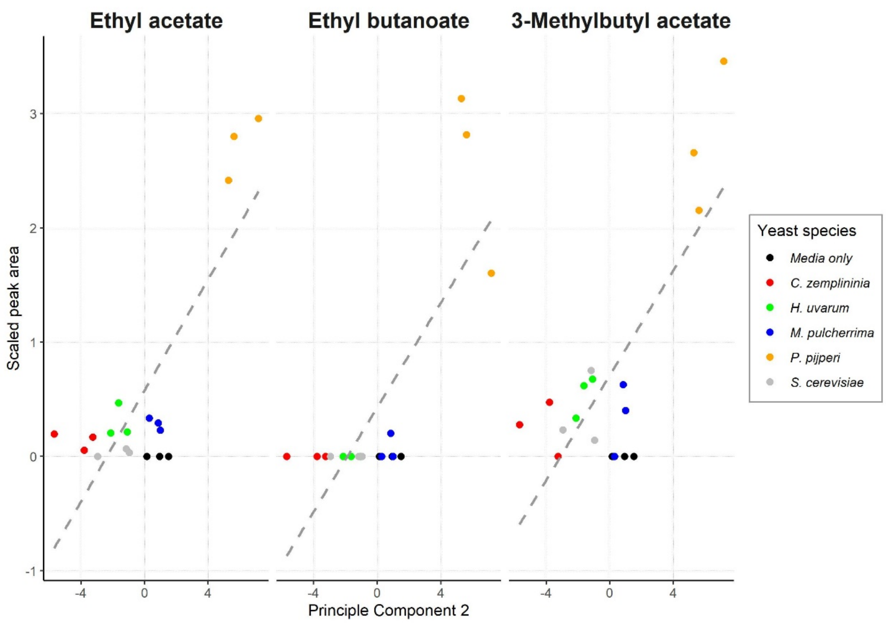


Fig 2.5 Correlations (dotted line) between scores for Principle Component 2 and amounts of volatile chemicals recovered from five different yeast species (coloured dots) and media only controls (black dots).

No correlation (Pearson's correlation, Not Significant at $P<0.05$) was found between PC1 and activity of summer morph *D. suzukii*, as measured at 4h and 24h previously in the laboratory (Fig 2.6). Similarly, no correlation was found between PC1 and activity of winter morph *D. suzukii* at 4h or 24h (Fig 2.6).

There was also no correlation found between PC2 and activity of summer morph *D. suzukii*, as measured at 4h and 24h (Fig 2.7). No correlation was found between PC2 and activity of winter morph *D. suzukii* at 4h or 24h (Fig 2.7).

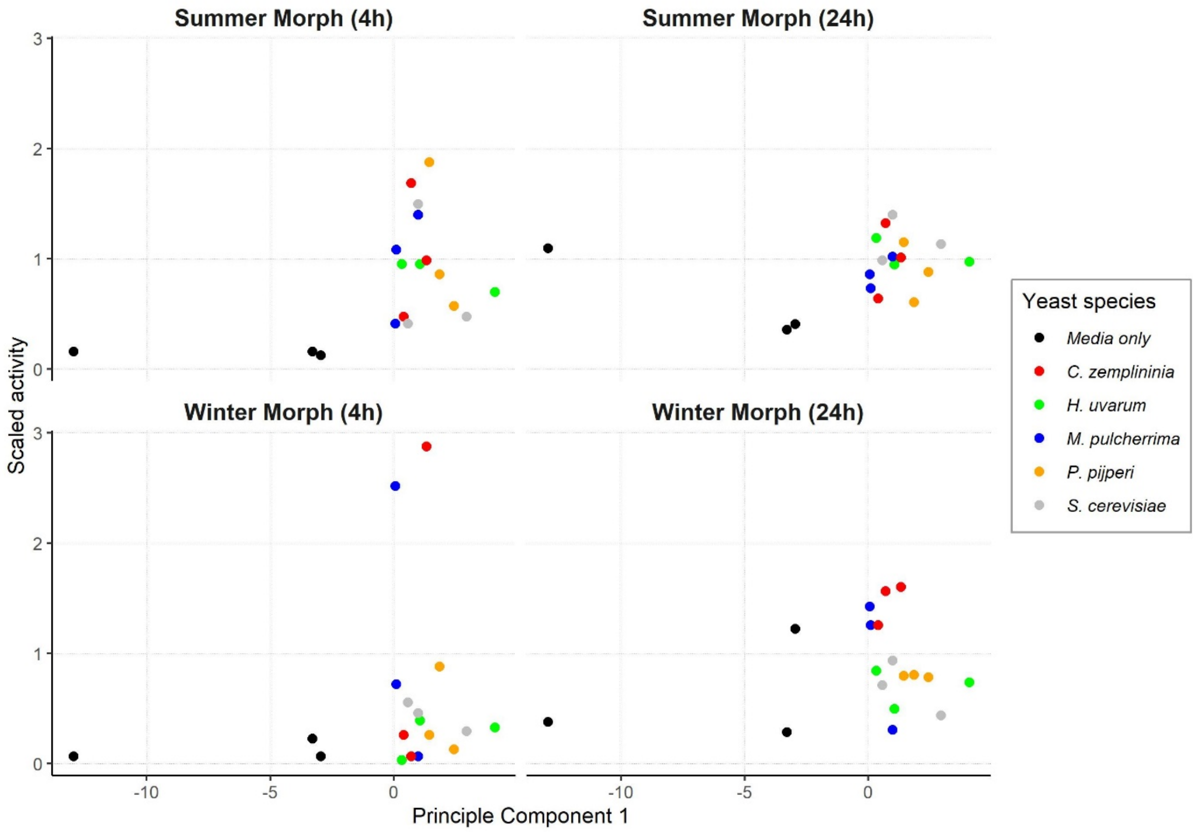


Fig 2.6 Scores for Principle Component 1 derived from volatile collections from five yeast species (coloured dots) and yeast potato dextrose media controls (black dots) and *D. suzukii* behavioural activity measured in response to the same yeasts. No correlation was found between PC1 and the activity of summer or winter morph *D. suzukii* after 4h or 24h.

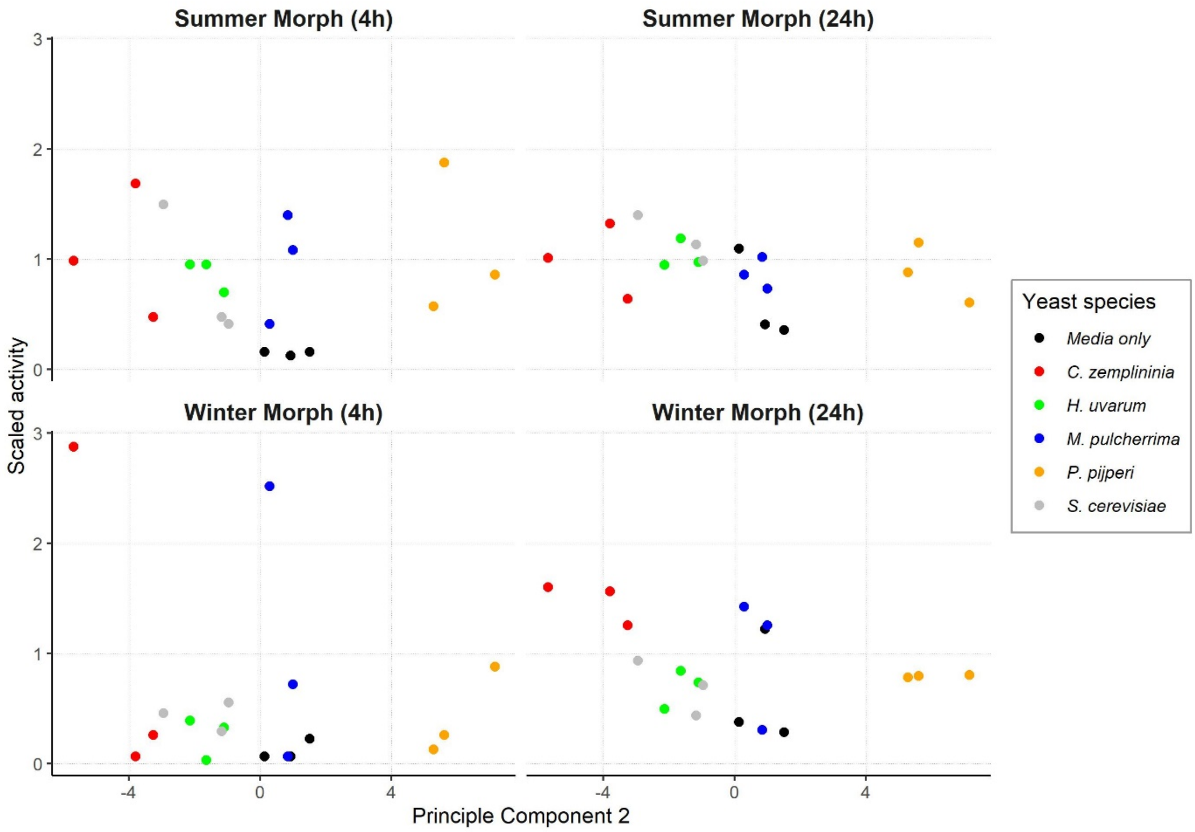


Fig 2.7 Scores for Principle Component 2 derived from volatile collections from five yeast species (coloured dots) and yeast potato dextrose media controls (black dots) and *D. suzukii* behavioural activity measured in response to the same yeasts. No correlation was found between PC2 and the activity of summer or winter morph *D. suzukii* after 4h or 24h.

Conclusions

The results of these analyses indicate that yeast species of ecological relevance to *D. suzukii* can be separated from one another by the relative amounts of volatile chemicals which they produce. However, underlying differences in yeast volatile profiles was not found to be related to *D. suzukii* responses to yeasts in activity bioassays. More work would be required to test whether differences in yeast volatile profiles may render some yeast species more attractive than others to *D. suzukii* in the field. This information could be used to reduce movement of *D. suzukii* into crops and increase catches in precision monitoring traps.

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

Deployment of Attract and Kill (A&K) devices in habitats adjacent to commercial crops where *D. suzukii* are known to overwinter has the potential to reduce crop infestation the following growing season. Besides commercially grown soft fruit, *D. suzukii* development is also fostered by susceptible wild fruits where it can find food and a suitable microclimate year-round (Grassi et al, 2011). Such wild hosts are known to grow in woodland habitats adjacent to commercially grown crops (Pelton et al, 2016), providing a source of *D. suzukii* at the beginning and throughout the crop growing season. Since 2013, NIAB EMR has monitored the distribution of *D. suzukii* in the UK to determine seasonal population dynamics in relation to crop ripeness and wild hosts (objective 2 National survey). Traps have been deployed in crop and adjacent winter refuges and mean numbers of *D. suzukii* compared fortnightly throughout the year. To date findings show highest peaks in mean numbers of *D. suzukii* occur in wooded areas during late autumn-early winter when there is reduced availability of commercial and wild fruit. Subsequently, lowest numbers are recorded in late winter-early spring.

Since September 2019 we have been investigating whether implementation of precision monitoring in winter refuges can reduce the winter form of *D. suzukii*. A grid of 64 precision monitoring traps spaced at 8 metre intervals were deployed in an 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 numbers of *D. suzukii* throughout the trial. Using a single monitoring trap avoids the possibility of multiple monitoring traps in the control acting as a ‘pull’, retaining *D. suzukii* in wild areas and making them less likely to venture into crops. After six weeks, data showed (to be statistically analysed) where there is precision monitoring, mean numbers of *D. suzukii* in RIGA traps (woodlands and neighbouring crops) decreased, but increased to a peak in untreated control equivalents. Thereafter *D. suzukii* numbers remained consistently lower where there is precision monitoring.

In spring 2020, traps containing sentinel fruit were deployed in treated and control woodlands and respective neighbouring crops to attract *D. suzukii* females to egg lay. Results comparing numbers of *D. suzukii* emerging from this fruit will indicate if this method of precision monitoring can reduce/prevent spring infestations of the pest.

A pilot habitat assessment was also made during winter 2019 to indicate the most effective habitat(s) to concentrate precision monitoring traps to catch most *D. suzukii*. Vegetation in a 1 m radius around a transect of 8 precision monitoring traps at each woodland was scored according to *D. suzukii* preference and coverage. The pilot study was inconclusive when correlating host plant score to *D. suzukii* catches; possibly due to it being a snapshot analysis of a small radius of a low number of precision monitoring traps. However, there was evidence to suggest that precision monitoring traps positioned on the woodland perimeter catch more *D. suzukii*. It is possible therefore that aspect could play a role in trap catches, e.g. shady or North-West facing positions. Furthermore, research elsewhere shows host vegetation surrounding crops can contribute to elevated *D. suzukii* catches (Klick et al. 2016). Temperature can also have a significant influence on *D. suzukii* (Tochen et al. 2014) and ambient humidity levels result in decreased trap captures (Tochen et al. 2016), so there is scope for further investigation.

Continuing from the initial winter precision monitoring trial 2019/20, this study will investigate whether:

- Implementation of precision monitoring in winter refuges from October to April for the winter form of *D. suzukii*, can reduce the incidence of fruit damage in the neighbouring crop in the spring.
- Continued precision monitoring in woodland winter refuge habitat during the 2020 growing season can maintain protection against *D. suzukii* fruit damage in the neighbouring crop.
- Precision monitoring traps can be positioned strategically according to surrounding host vegetation and abiotic factors, e.g. temperature, humidity, light intensity to optimise *D. suzukii* catches.
- 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.

Materials and Methods

Trial sites*:* The trial was set up at 6 commercial soft fruit crops (blocks) in Kent and West Sussex. Crops tested included strawberry, raspberry, cherry and one wine grape.

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

1. A treatment plot consisting of a woodland winter refuge containing a grid of 64 precision monitoring traps spaced at 8 metre intervals (shape dependent on woodland topography), alongside a soft fruit crop.
2. A control plot consisting of a woodland winter refuge containing no precision monitoring traps beside a separate soft fruit crop.

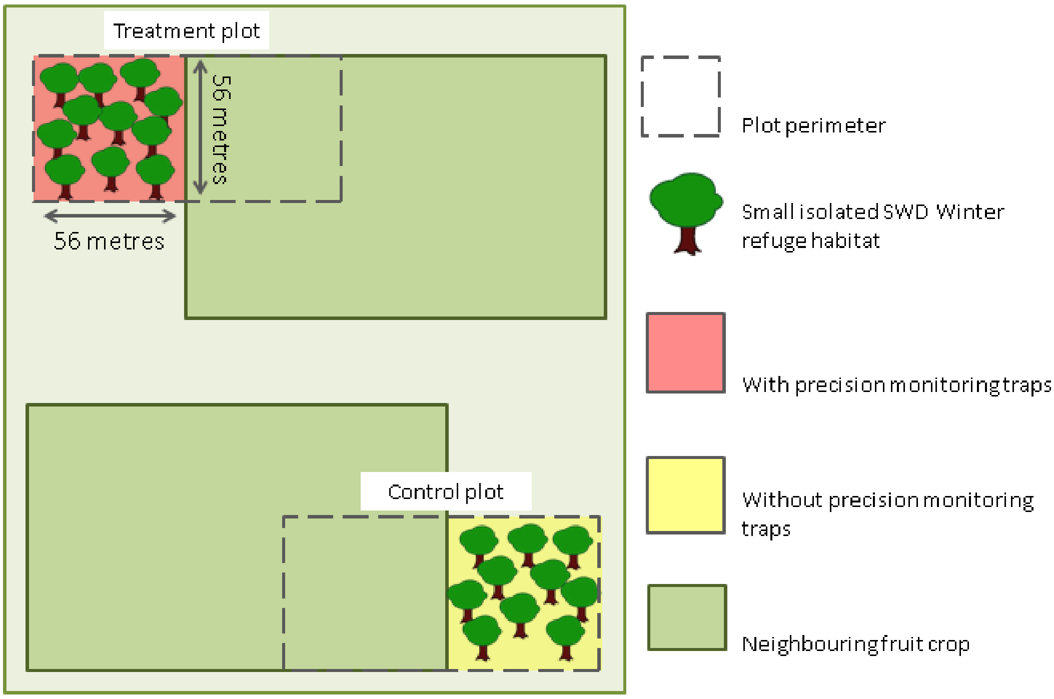


Figure 2.2.1. Diagrammatic representation of an experimental block for the precision monitoring trial 2019 to 2021. Each block consisting of a treatment woodland plot (red square) containing precision monitoring traps and a control woodland plot (yellow square) without precision monitoring traps. Beside each woodland is a neighbouring soft fruit crop (darker green squares).

Assessments were conducted at regular intervals at each block; fortnightly during periods of high *D. suzukii* caught activity and monthly during periods of low *D. suzukii* caught activity. Blocks were divided into 2 groups of 3; assessed on alternate weeks for practical reasons. See Table 2.2.1 for assessment dates.

D. suzukii monitoring using RIGA traps: To compare numbers of *D. suzukii* between treated and control plots over the trial period, a RIGA trap was placed centrally in the following positions at each block (Fig. 2.2.2):

1. Treated winter refuge habitat
2. Crop adjacent to treated winter refuge habitat
3. Control winter refuge habitat
4. Crop adjacent to control winter refuge habitat

Riga traps were deployed 2 weeks before the trial start (pre-assessment), then collected and renewed at regular intervals until the end of the trial. During each collection, the content of each RIGA trap was filtered and male and female *D. suzukii* were counted.

D. suzukii monitoring – precision monitoring traps: To monitor *D. suzukii* numbers in precision monitoring traps over the course of the trial, an 8 trap transect (Fig. 2.2.2) was sampled at regular intervals at each block. All traps were also sampled during summer and autumn habitat assessments. During sampling, the content of each trap was emptied onto a white tray and the numbers of males (spots on wings) were counted.

Sentinel fruit traps: To compare *D. suzukii* egg-laying between treated and control plots, red Delta traps containing 240g of sentinel fruit protected from larger animals by an exclusion grid were deployed centrally at each block (Fig. 2.2.2) on 5 occasions in the spring (when climate conditions were warm enough for *D. suzukii* activity and females were confirmed fecund by dissection) and twice during the growing season. At each deployment, sentinel fruit was left in the field for an equal number of days at each block (3-7 days depending on temperature), after which, fruit was incubated at ~22°C, >40 % RH, 16 h light: 8 h dark for 14 days at NIAB EMR. During this period, emerged adult *D. suzukii* were counted.

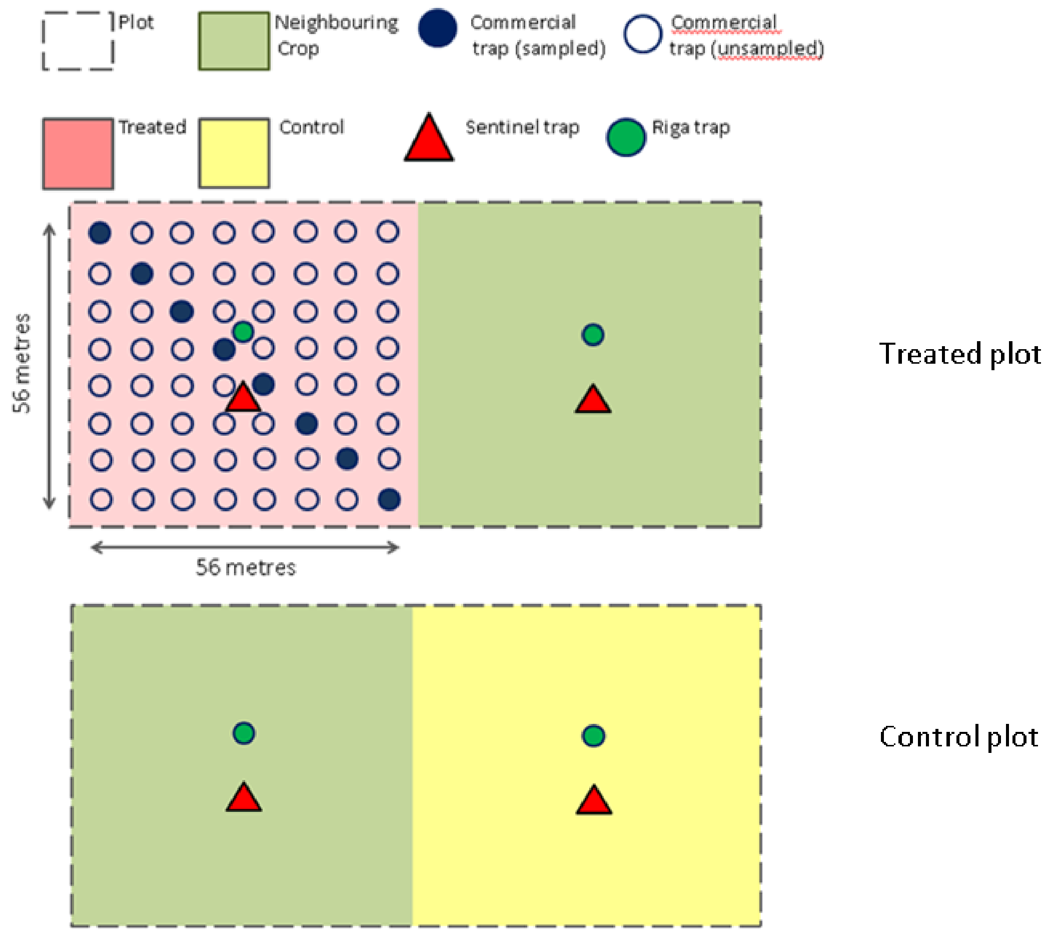


Figure 2.2.2. Diagrammatic representation of trap positions in an experimental block during the precision monitoring trial 2019 to 2021. Treated woodlands contained 64 precision monitoring traps (blue outline circles). Of these, a transect of 8 traps (blue fill circles) were sampled. A RIGA trap (green fill circle) and sentinel fruit trap (red fill triangle) were deployed in treated and control woodlands and respective neighbouring crops.

Habitat assessments: To correlate trap catches of male *D. suzukii* with surrounding vegetation, in summer and autumn 2020 a 4-metre radius of all traps was assessed for plant hosts. Using the semi-quantitative coverage and abundance index (Total Estimate Scale) (TES) of Braun-Blanquet (Braun-Blanquet 1983; Mueller-Dombois and Ellenberg 1974; Smith 1996, Table 2.2.2), records of plant species diversity, abundance and percentage cover were taken. By combining scientific indications, a score to evaluate alternative plant hosts of *D. suzukii* was developed (Kenis et al. 2016; Ardin, 2017). The score ranked the potential of wild plants to host and feed *D. suzukii* adults and larvae (Table 2.2.3). The plant coverage score, obtained using TES (Table 2.2.2), was then multiplied by each single plant species *D. suzukii* development, and feeding score (Table 2.2.3) to calculate an overall *D. suzukii* preference score in each evaluated habitat.

To correlate trap catches of male *D. suzukii* with potential abiotic influences, from assessment number 12, a temperature and humidity logger was attached to the three transect traps which up to that assessment had caught lowest, median and highest numbers of male *D. suzukii* at each block (18 total). Light intensity (Photosynthetically Active Radiation, μmol m^{-2} s^{-1}) was measured at the same traps in summer and autumn 2020. During the summer assessment, two light wands were used at midday; one at a trap, the other outside the woodland to correct for changing light conditions between traps and blocks. This was repeated for each trap. In autumn, four light sensors (Apogee instruments, original quantum sensor) and temperature and humidity data loggers were deployed simultaneously between 10:00 and 16:00; one at each trap and one outside the woodland. The loggers recorded PAR intensity every 5 minutes for approximately 6 hours (10:00 and 16:00). The average light intensity at each trap was calculated as a percentage of the outside level.

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 data was analysed using a negative binomial generalised linear mixed effect model. Negative binomial was used due to high levels of over dispersion. These results include the pre assessment. Assessments were analysed in two groups;1) 1 to 11; 2) 12 to 18.

Habitat assessment: Summer and autumn habitat score assessments were analysed separately using a GLM mixed model regression with habitat score as a continuous variable.

Individual species coverage:

Trap position: The pilot assessment of the 8 transect traps (December 2019) was analysed using a negative binomial regression. Full assessments, summer and autumn 2020 were analysed using a mixed model to take account of the repeated measures of the traps.

Temperature and humidity: temperature and humidity data was analysed using a negative binomial generalised linear mixed effect model. Negative binomial approach was used due to high levels of over dispersion. Summer and Autumn assessment were analysed separately and temperature and humidity data was also analysed separately.

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

Table 2.2.2. Total estimate scale, abundance plus coverage (modified from Smith (1996); Braun-Blanquet 1983; Mueller-Dombois and Ellenberg 1974). Solitary species conventionally assigned an "r," were combined with those assigned a "+" (cross) rating in our study.

Score	**Description**
r	Solitary, one observation, coverage very small
+	Individuals of a species sparsely present in the stand; coverage very small
1	Individuals plentiful, but coverage small
2	Individuals very numerous if small; if large, covering at most 5% of area
3	Individuals few or many, collectively covering 6-25% of the area
	Individuals few or many, collectively covering 26-50% of the area
5	Plants cover 51-75% of the area
6	Plants cover 76-100% of the area

Table 2.2.3. *D. suzukii* development and feeding score for each host plant recorded in the habitat assessment Each is given a score according to *D. suzukii* food and larval development source: Very good = 3, Good = 2, Low = 1, No food or development source = 0.

Plant species	Common name	Score
Rubus fruticosus	Bramble	3
Sambucus nigra	Elderberry	3
Cornus mas	Dogwood	3
Solanum dulcamara	Nightshade	3
Viscum album	Mistletoe	2
Ruscus aculeatus	Butcher's Broom	1
Hedera elix	Ivy	1
Crataegus sp.	Hawthorn	1
Ilex aquifolium	Holly	1
Fagus sylvatica	Beech	0
Betula pendula	Birch	0
Fraxinus sp.	Ash	0
Corylus avellana	Hazelnut	0
Quercus sp.	Oak	0
Castanea sativa	Chestnut	0
Urtica dioica	Nettle	0
Tilia sp.	Lime	0
Alnus sp.	Alder	0

Results

D. suzukii monitoring using RIGA traps: During assessments 1 to 11 (October 2019 to April 2020), there were fewer *D. suzukii* in RIGA monitoring traps in treated plots compared to control (mean = 70.4 and 102.8 respectively). The difference was significantly lower for treated woodlands compared to control (mean = 82.8 and 247.5 respectively, *P* = 0.0079, Fig. 2.2.3) and lower in treated compared to control crops, but not significantly (mean = 12.5 and 18.4

respectively). Following redeployment of precision monitoring traps, assessment 13 to 19 (July 2020 to early-December 2020), there was no overall significant difference between treated and control plots (grand mean = 362.3) (Fig. 2.2.4 and 2.2.5).

Sentinel fruit emergence: Numbers of *D. suzukii* emerging per 100g of sentinel fruit were low in all assessments; spring, summer, and autumn (means = 3.07, 0.03 and 2.26 respectively). Numbers of other Drosophila spp. emerging from fruit were higher than *D. suzukii* at all assessments (means = 280.79, 91.21 and 477.17 respectively). Other Drosophila spp. were not identified to species.

Habitat assessments: During the summer habitat assessment, there was a significant positive correlation between mean numbers of male *D. suzukii* caught in traps and the surrounding habitat score (P = <0.001). A parameter value for habitat score was calculated; 0.0333. This is the expected log increase in *D. suzukii* counts for a 1 unit increase in habitat score and was best illustrated by plotting a range of habitat scores and the predicted number of *D. suzukii* at each block (Fig 2.2.6). There was variation between blocks, with block 1 having the highest predicted male *D. suzukii* trap counts and block 3 the lowest, but all with a positive correlation. For the autumn habitat assessment, the correlation was not significant. An expected log increase of 0.016 *D. suzukii* counts was found for a 1 unit increase in habitat score (data not shown). Data from the winter assessment is awaiting statistical analysis.

Individual species coverage: During the summer assessment, there was a significant positive correlation between the coverage of *Rubus fruticosus* (Bramble) around traps and the number of male *D. suzukii* caught in traps (P= <0.001). During the autumn assessment, there was a significant positive correlation between the coverage of ivy growing on trees and bushes around traps and the number of male *D. suzukii* caught in traps ($P < 0.001$).

Trap position: Statistical analysis of pilot habitat assessment data (8 transect traps, December 2019) found transect traps positioned on the perimeter of treated woodlands (nearest crop and general perimeter) caught significantly more male *D. suzukii* than those positioned inside the woodlands (means = 82.2, 59 and 33.7 respectively, P= 0.003) (Fig 2.2.7). There was no significant difference between perimeter traps nearest the crop and general perimeter traps.

Data from the full assessments (all 64 traps per treated woodland) summer, autumn and winter 2020, found significantly more male *D. suzukii* were caught in traps in the perimeter nearest the crop, than general perimeter and inside woodlands during summer (mean = 43.9, 17.5 and 12.4 respectively, P= <0.001 respectively) (Fig. 2.2.8a). During autumn significantly more male *D. suzukii* were caught in traps in the perimeter nearest the crop and general perimeter then inside woodlands (mean = 327.1, 275.4 and 171.9 respectively, P= <0.001 and 0.023

respectively) (Fig. 2.2.8b). During winter, significantly more male *D. suzukii* were also caught in traps in the perimeter nearest the crop and general perimeter then inside woodlands (mean = 78.6, 89.8 and 26.5 respectively, *P*= <0.001 respectively) (Fig. 2.2.8c).

Temperature and humidity: In the summer assessment there was a significant negative correlation between average daytime and average night-time temperatures and numbers of male *D. suzukii* caught in traps (k = -1.986 and -6.835 respectively, P = 0.017 and < 0.001 respectively). There was no significant correlation between average humidity and numbers of male *D. suzukii* caught in traps. In the autumn assessments there were also no significant correlations between temperature and humidity and numbers of male *D. suzukii* caught in traps. A more detailed analysis is underway.

Light intensity: Data is awaiting statistical analysis.

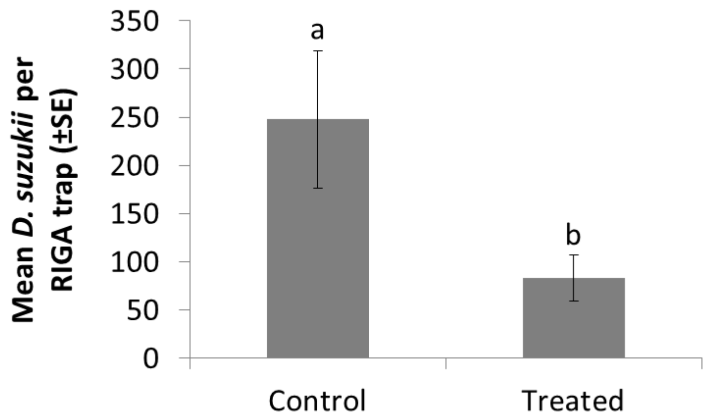


Figure 2.2.3. Mean numbers of *D. suzukii* caught per RIGA monitoring trap in control and treated woodlands up to assessment 11 (October 2019 to April 2020). Letters denote significant differences at P = <0.05.

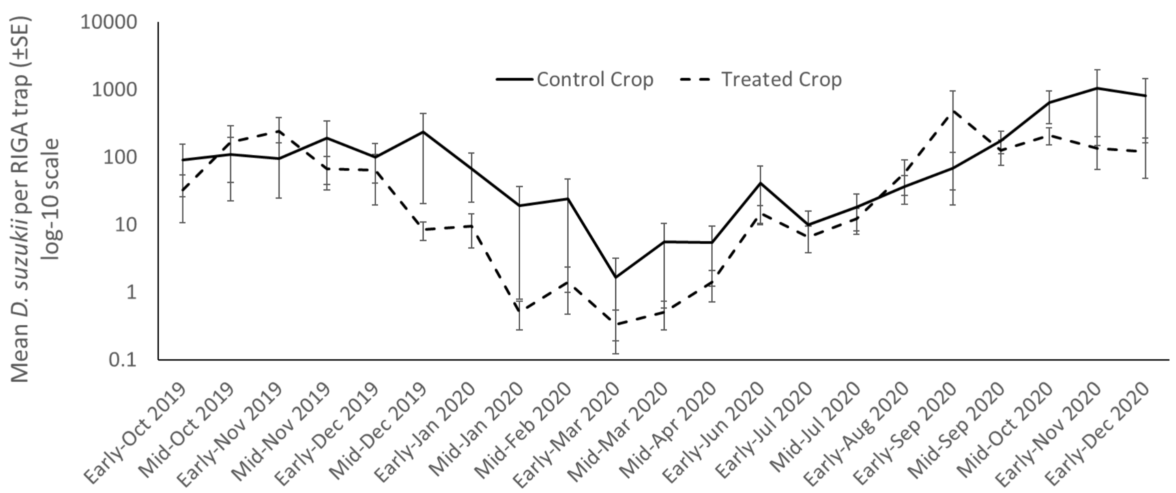


Figure 2.2.4. Mean numbers of *D. suzukii* caught per RIGA monitoring trap in control and treated crops from assessments 1 to 19 of the precision monitoring trial. Precision monitoring traps were removed from treated woodlands mid-April to mid-June 2020 during sentinel fruit deployments.

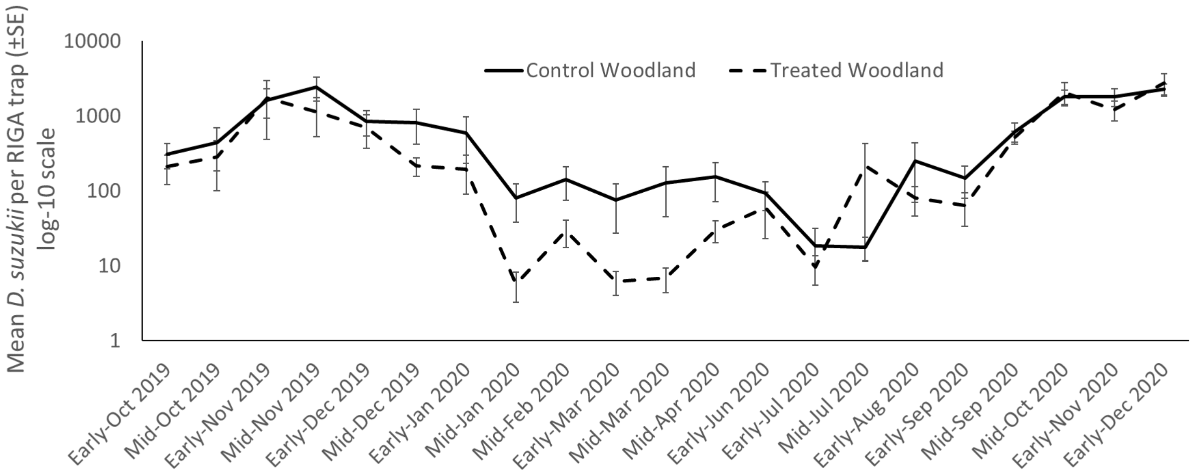


Figure 2.2.5. Mean *D. suzukii* caught per RIGA monitoring trap in control and treated woodlands from assessments 1 to 19 of the precision monitoring trial. Precision monitoring traps were removed from treated woodlands mid-April to mid-June 2020 during sentinel fruit deployments.

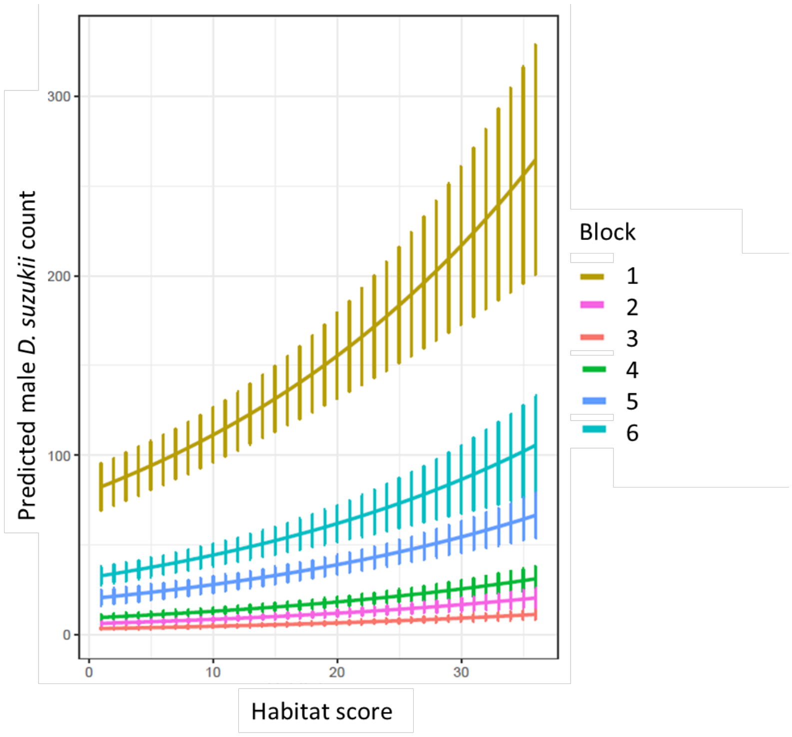


Figure 2.2.6 Predicted male *D. suzukii* catches per trap according to habitat score, using data from the summer habitat assessment which scored vegetation in a 4 m radius around all 64 precision monitoring traps at all 6 blocks according to *D. suzukii* preference.

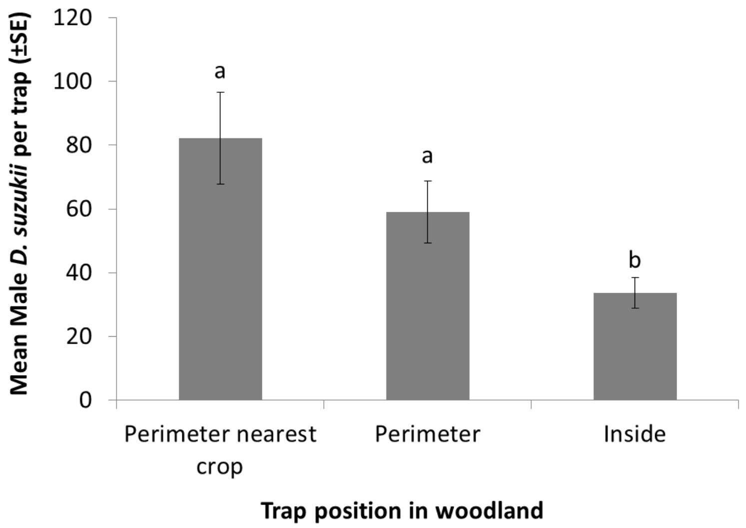


Figure 2.2.7. Mean male *D. suzukii* catches according to trap position in treated woodlands during the pilot habitat assessment December 2019. Data is from the 8 transect traps per block. Letters denote significant differences at *P* = <0.05.

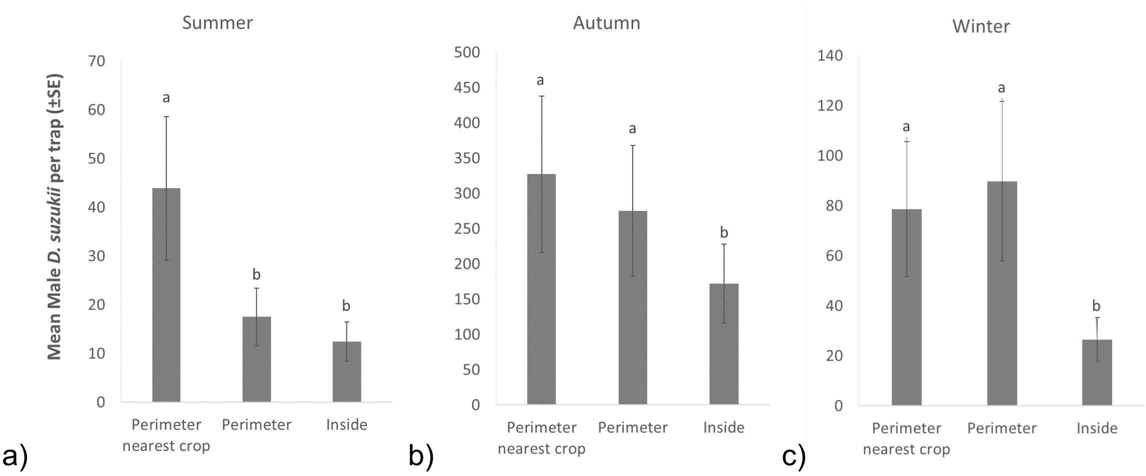


Figure 2.2.8 Mean male *D. suzukii* catches according to trap position in treated woodlands during full habitat assessments in a) summer, b) autumn, and c) winter, 2020. Data is from all 64 precision monitoring traps per block. Letters denote significant differences at *P*=0.05.

Discussion

Since October 2019, a grid of 64 precision monitoring traps has been deployed in 6 isolated pockets of woodland in Kent and West Sussex and numbers of adult *D. suzukii* compared to woodland with no precision monitoring traps. The aim was to reduce winter morphs in their overwintering habitats and then subsequent incursion into the adjacent crop in the spring. Single RIGA monitoring traps in each plot were used to regularly monitor numbers of *D. suzukii* in both treatments in the woodland and neighbouring crop. In addition, sentinel fruit was deployed to monitor *D. suzukii* egg laying in spring 2020, then twice during the fruit growing season. The trial also explored 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.

From the first assessment, 2 weeks after precision monitoring trap deployment (early-October 2019,) to assessment 11 (mid-April 2020), fewer *D. suzukii* adults were counted in RIGA monitoring traps in treated plots compared to control (mean = 70.4 and 102.8 respectively). Overall, significantly fewer were counted in treated woodlands compared to the untreated control (mean = 82.8 and 247.5 respectively, $P = 0.0079$) and fewer in treated crops compared to control, but not significantly (mean = 12.5 and 18.4 respectively). It is likely that the network of traps in the treated woodland are either acting by catching large numbers of adult *D. suzukii* or that they are 'confusing' adults and hence fewer are locating the RIGA monitoring trap. Based on findings that large numbers of adults are found in the precision monitoring traps, the former explanation seems more likely

Following redeployment of precision monitoring traps, at assessment 13 to 19 (July 2020 to early-December 2020), there was no overall significant difference between treated and control plots (grand mean = 362.3).

The deployment of sentinel fruit in treatment and control plots and subsequent emergence tests to compare *D. suzukii* egg-laying did not give conclusive results to date. Numbers of *D. suzukii* emerging per 100g of fruit were consistently low in spring, summer, and autumn 2020 (means = 3.07, 0.03 and 2.26 respectively), and there was no clear difference between treatment and control plots. The reason for low *D. suzukii* emergence from fruit could be competition from other Drosophila species which emerged in much higher numbers from the same sentinel fruit (means = 80.79, 91.21 and 477.17 respectively). 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 larvae (Ahmad et al. 2015). Sentinel fruit is due to be deployed spring 2021, but a change in methodology is needed to exclude other Drosophila species, ripening fruit is an option.

The relationship between male *D. suzukii* catches in precision monitoring traps and surrounding vegetation was explored. There were positive correlations in both the summer (*P* = <0.001), and autumn (NSD) assessments (i.e. the more preferable vegetation around traps is to *D. suzukii,* the more male *D. suzukii* were caught). Specific host plant species were also analysed. A significant positive correlation was found with bramble coverage and catches of male *D. suzukii* during the summer assessment, but there was no significant correlation autumn. Blackberry fruit is known to support *D. suzukii* development. In the autumn there was a significant positive correlation with ivy coverage and catches of male *D. suzukii*. Ivy is a potential host during the autumn/winter period. During the autumn assessment, many Ivy bushes had well-developed berries.

Analysis of pilot habitat assessment data (8 transect traps, December 2019) found transect traps positioned on the perimeter of treated woodlands (nearest crop and general perimeter) caught significantly more male *D. suzukii* than those positioned inside the woodlands (means = 82.2, 59 and 33.7 respectively, *P*= 0.003) (Fig 2.2.7) (Fig 2.2.12). There was no significant difference between perimeter traps nearest the crop and other perimeter traps. Data from the full assessments (all 64 traps per treated woodland) summer, autumn and winter 2020, found significantly more male *D. suzukii* were caught in traps in the perimeter nearest the crop, than general perimeter and inside woodlands during summer (mean = 43.9, 17.5 and 12.4 respectively, *P*= <0.001 respectively. During autumn significantly more male *D. suzukii* were caught in traps in the perimeter nearest the crop and general perimeter then inside woodlands (mean = 327.1, 275.4 and 171.9 respectively, *P*= <0.001 and 0.023 respectively). During winter, significantly more male *D. suzukii* were also caught in traps in the perimeter nearest the crop and general perimeter then inside woodlands (mean = 78.6, 89.8 and 26.5 respectively, *P*= <0.001 respectively).

A preliminary analysis investigated potential relationships between selected abiotic factors and numbers of *D. suzukii* caught in traps. During the summer assessment there was a significant negative correlation between average daytime and average night-time temperatures and numbers of male *D. suzukii* caught in traps (k = -1.986 and -6.835 respectively, P = 0.017 and < 0.001 respectively). There was no significant correlation between average humidity and numbers of male *D. suzukii* caught in traps. In the autumn assessments there were also no significant correlations between temperature and humidity and numbers of male *D. suzukii* caught in traps. A more detailed analysis is underway.

a)  b) 

Figure 2.2.12 Example habitats where precision monitoring traps have been deployed since October 2019 of the precision monitoring trial; a) woodland perimeter beside soft fruit crop, b) interior of the same woodland.

Conclusions

- Fewer *D. suzukii* were caught in monitoring traps in woodlands with precision monitoring (and neighbouring crops) than untreated (control) equivalents, October 2019 to April 2020. There was no overall significant difference July to early-December, but the analysis is awaiting data from spring 2021.
- To date, low numbers of *D. suzukii* have emerged from sentinel fruit deployments spring, summer, and autumn 2020. This is likely the result of competition from other Drosophila spp. egg laying in the same fruit. Deployments 2021 will need a method to allow *D. suzukii* egg laying exclusively, ripening fruit instead of ripe fruit is being considered.
- The analysis of precision monitoring trap position during the pilot assessment (Dec 2019) found traps positioned on the woodland perimeters caught significantly more male *D. suzukii* than within the main woodland. There is a similar trend summer, autumn and winter 2020 assessments.
- In summer 2020 there was a significant positive correlation between vegetation 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 so far to have a significant positive influence on catches of *D. suzukii*, during summer and autumn assessments respectively. Further investigation is recommended.
- Preliminary analysis looking for relationships between abiotic factors and numbers of *D. suzukii* caught in traps so far has found a significant negative correlation with temperature, summer, but not autumn, and no significant correlation with humidity. A more detailed analysis is underway.

Task 2.3. Development of a push-pull system for control of Drosophila suzukii (Christina Conroy and NRI)

Introduction

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" (Cook et al. 2007). The approach has been used to control several insect pest species, including the crucifer flea beetle, *Phyllotreta cruciferae*, a pest of broccoli (Parker et al. 2016). Besides pest control, additional benefits of push-pull include reduced need for chemical plant protection products (PPPs), increased numbers of natural enemies in the crop and increased numbers of beneficial soil organisms (Kelemu 2015).

To develop push-pull against *D. suzukii* knowledge of the chemical ecology of the pest is required. However, prior to 2008 little was known about its courtship and host-seeking behaviours or chemical ecology. Since then, researchers have gained a better understanding of the pest'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). Recently 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 plots in the field (Wallingford et al. 2017).

Potential repellents to deter *D. suzukii* laying eggs in fruits or discourage adults entering the cropping area were investigated in the previous project. Other research has focused on geosmin (Wallingford et al. 2016a), plant essential oils (Renkema et al. 2016), lime (Dorsaz and Baroffio 2016) and 1-octen-3-ol (Wallingford et al. 2016a). To date, only the latter two products were reported to show efficacy in field tests (Dorsaz and Baroffio 2016; Wallingford et al. 2016b).

In previous work in SF145, 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 plot, single tree experiments. However, in subsequent experiments, 25 sachets per cherry tree did not deter *D. suzukii* egg laying.

Since these initial studies, CTP student Christina Conroy and staff at NIAB EMR and NRI (University of Greenwich) have identified three compounds which repelled SWD summer and winter morphs in laboratory and semi-field experiments.

In addition, Christina conducted a survey in 2018-2019 of UK growers' knowledge of SWD and their attitudes towards current management practices. The aim was to identify potential barriers to uptake of any new technology for SWD monitoring and control.

Progress in 2020/2021

Development of Repellents

In 2020 three repellents were tested individually in 12-metre polytunnels in the presence of strawberry plants. The objectives were to determine whether these treatments could reduce overall numbers of *D. suzukii* emerging from fruit compared to controls, and the maximum distance from the point of release over which the repellents were active.

Two of the three repellents significantly reduced total numbers of SWD emerging from fruit in polytunnels compared to a control treatment. Two of the repellents reduced numbers of emerging larvae from fruit even at more than 6 m from the source (Fig. 2.1). The researchers are working with AHDB towards future approval of these products and field testing in commercial crops.

Grower Survey

In total 27 growers completed the survey. The results included data on the costs to growers of management of SWD, and the most frequently used approaches.

Data have been pre-processed and outliers removed prior to formal analysis. Statistical analyses will be conducted to examine how grower attitudes and knowledge about SWD influence control measures applied. A full report will be available from the AHDB on completion of the PhD in 2021.

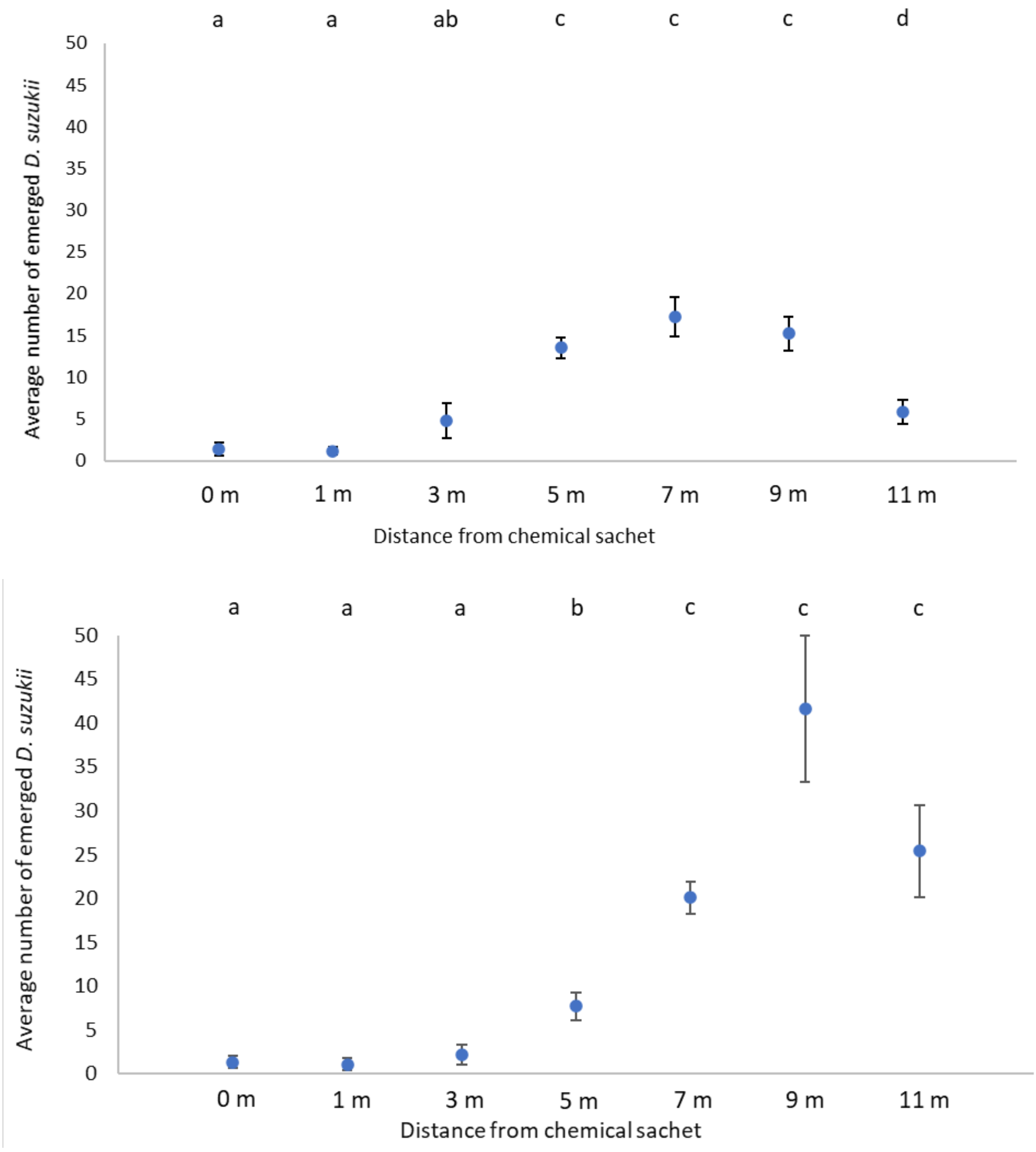


Fig. 2.1 Mean (± SEM) number of *Drosophila suzukii* emerging from strawberries (cv. Amesti) taken at seven sampling points from release dispensers (n = 12). Top: Formulation 129/04, Bottom: Formulation 129/08. Means denoted by a different letter indicate significant differences between sampling points ($P < 0.05$).

Conclusions

Through this research chemical formulations have been developed which repel both winter and summer morph *D. suzukii* away from fruit in the laboratory and field. These formulations can reduce oviposition in protected crops up to 7m from the point of release. This new technology has potential for use in reducing fruit losses to *D. suzukii* and could be applied with commercially available attractants in a “push-pull” system to further reduce numbers of *D. suzukii* around ripening fruit. Future research will address the effectiveness of these formulations in protecting other high-value fruit crops from *D. suzukii*.

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

Task 3.4A Determine the effect of baits in combination with reduced dose insecticides on D. suzukii control in raspberry (Microbiotech, NIAB)

Introduction

A strawberry experiment in 2019 showed that weekly applications of Benevia (cyantraniliprole) at 30 ml in 40L per ha, combined with Combi-protec, were as effective in controlling *D. suzukii* numbers as two sprays of Benevia at 750 ml in 500L per ha (i.e. a reduction in Benevia application of more than 95% with the same *D. suzukii* control effect). Combi-protec is authorised and commercially available as a sticker adjuvant in the UK. The product costs per spray were £112.50/ha for the full field rate application of Benevia and £77.50/ha for the Combi-protec + dilute dose of Benevia. Preliminary studies in bioassay jars (Rory Jones, personal communication) have indicated that molasses is as effective as Combi-protec as a phagostimulant bait in control of *D. suzukii* but at significantly lower cost, although it is not authorised for use. Currently, under emergency authorisation, only two sprays of either Exirel or Benevia are permitted per season. If four weekly insecticide sprays are to be applied, two sprays of an alternative insecticide are also required. 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, against full field application rates of the same insecticides in raspberries under semi-field conditions.

Methods

The experiment at NIAB EMR was conducted in 12 small tunnels (12 × 1.5 × 2 [high] m), each covered and divided in half with fine mesh to prevent entry or exit of flies. The roofs and upper sides of the tunnels were covered with standard commercial polythene leaving the ends of the tunnels and the lower 1 m of the side walls covered only in mesh. There was a 26 m gap between adjacent tunnels, which were arranged in a 4 × 3 grid.

The schedule of tasks is shown in Table 1.3.1. Unsprayed long cane, cv. Paragon raspberry plants in 4.5L pots containing coir substrate were re-potted in 6L pots containing coir substrate. The plants were introduced into the tunnels in three batches (~80 per batch) in late March, mid-April, and mid-May to ensure continuous fruiting. Ten potted plants with two canes per plant were set out in a single row at 0.6 m centre to centre on 10 cm height plastic crates in each compartment. The plants were irrigated with a nutrient solution through a drip irrigation system and the electrical conductivity of the substrate measured twice weekly, with the strength of the nutrient solution adjusted accordingly. No pesticide sprays were applied to the plants, other than the experimental treatments.

Table 1.3.1. Time schedule of Task 3.4A - Raspberry

Month	Expt Day	Activity
March		First 80 plants in tunnels
April		Second 80 plants in tunnels
May		Third 80 plants in tunnels
2 July	0	Spray 1, Tracer
3 July	1	Introduce first SWD cohort in tunnels 10♀ 10♂
8 July	6	Sample fruit for SWD 1
8 July	7	Spray 2, Exirel
9 July	8	Introduce second SWD cohort in tunnels 20♀ 10♂
15 July	13	Sample fruit for SWD 2
15 July	14	Spray 3, Tracer; Select fruit for residue testing
16 July	15	Introduce third SWD cohort in tunnels 20♀ 10♂
22 July	20	Sample fruit for SWD 3
22 July	21	Spray 4, Exirel; Select fruit for residue testing
28 July	26	Sample fruit for SWD 4
28 July	26	Spray with fluorescent dye (replicate 5 only)
5 Aug	33	Sample fruit for SWD 5
20 Aug	48	Sample fruit for SWD 6
2 Sep	61	Sample fruit for SWD 7

The plants were sprayed in early July when there were at least 20 ripe fruit in each compartment, and three times again at weekly intervals with the treatments below. Each compartment was artificially infested with adult summer morph *D. suzukii*; 10 females and 10 males were introduced one day after the first spray, and 20 females and 10 males were introduced one day after the second and third sprays. Samples of 20 berries from the top (above 1 m), middle (0.6-1 m) and bottom (below 0.6 m) of all plants along the tunnel from each compartment were picked 6 days after each spraying. Two further samples of berries were collected at 2-week intervals from each compartment after spraying had ended. 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 25 berries from the entire height of all plants along the tunnel was also picked for *D. suzukii* adult emergence testing. The fruit was introduced into clear plastic mesocosms (27 × 15 × 10 cm). The mesocosms 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 mesocosm during a 19-day incubation at 22ºC, in 16h:8h light:dark. Ripe fruit not used for *D. suzukii* emergence or larvae flotation testing was also picked at regular intervals. Temperature and humidity among the plants in the polytunnels were recorded by Grant sensors and data loggers. Plants were assessed for phytotoxicity symptoms on foliage 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, 150 g samples of berries from the top, middle and bottom of the plants from treatments 1, 2, 3 and 5 (pooled samples from all replicates) were analysed for cyantraniliprole and spinosad 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.

<u>Treatments, experimental design and statistical analysis</u>

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

The remaining plants 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 dilute rates with and without baits using an electric motorised knapsack sprayer (Orange Albuz) along each side of the row of plants (Tables 3.1.2 and 3.1.3).

2. The full field rates were applied as a high volume, fine spray with a motorised mist blower (Solo Inc.) and hollow-cone nozzle over the entire plants at a rate of 103 ml spray per plant which is equivalent to 500 L/ha. The BCPC droplet spectra size was fine to very fine (154 to 225 microns).

3. The low-rate sprays (with and without baits) were applied as a low volume spray, in 340 micron droplets with a Lechler IDK 120-015 nozzle with the centre of spray aimed at the centre of the canopy of the plants at a rate of 8.26 ml per plant which is equivalent to 40 L/ha.

The estimated volumes of spray to be applied per plant were based on an approximate industry standard number of 4840 plants/ha. The actual volumes of spray applied per plant were determined from the initial and final volumes in the spray tank.

There were five replicates of each spray treatment and four replicates of the untreated control. Treatments were allocated to half polytunnels so that each treatment was paired with the other four treatments once or twice, once in the north end and/or once in the south end of the tunnels. Each treatment appeared in every column once or twice, and each row contained four different treatments. *D. suzukii* emergence data were analysed by ANOVA.

Table 3.1.2. Bait and insecticide spray treatments

Treatment	Bait, %v/v	Insecticide rate *	Spray 1	Spray 2	Spray 3	Spray 4
1 Full field rate	None	Full field	Tracer	Exirel	Tracer	Exirel
2 Combi-protec	Combi-protec 5%	Low	Tracer	Exirel	Tracer	Exirel
3 Molasses**	Molasses 5%	Low	Tracer	Exirel	Tracer	Exirel
4 No bait spray	None	Low	Tracer	Exirel	Tracer	Exirel
5 Untreated	None	None	None	None	None	None

* Full field and low rates of insecticides are shown in Table 3

** Holland & Barrett

Table 3.1.3. Full field rate and low-rate sprays of insecticides

Insecticide	Active ingredient	Rate	ml/ha	Spray volume	Harvest interval	MAPP NO. EAMU
Tracer	spinosad 480 g/l	Full field	200	500 L/ha	1 day	12438
Tracer	spinosad 480 g/l	Low	8	40 L/ha	1 day	
Exirel	cyantraniliprole 100 g/L	Full field	900	500 L/ha	3 days	
Exirel	cyantraniliprole 100 g/L	Low	36	40 L/ha	3 days	

Spray deposition methodology

The spray deposition of each of the sprayed treatments (Full field rate, Combi-protec, Molasses, No bait spray) 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 mixed with the appropriate adjuvant (Combi-protec, molasses, or nothing added). Four tunnels in block 5 were sprayed using the appropriate spray settings for each treatment (Table 3.1.4).

Table 3.1.4. Treatments applied to four tunnels for the spray deposition analysis on 28th July 2020

Treatment name	Adjuvant	Water volume rate	Fluorescent tracer dye (% v/v)
Full rate	None	500 L/ha	2
No bait	None	40 L/ha	2
Combi-protec	Combi-protec	40 L/ha	2
Molasses	Molasses	40 L/ha	2

To measure spray deposition, a handheld imaging fluorometer was used to take readings from the surface of the sprayed leaves. The raspberry canopy was divided into three sections: top, middle, and bottom, which were approximately one third each of the total height of the canes. Within each canopy section, both sides of the leaves were sampled. For each leaf side, 40 readings were taken. Pictures of spray deposition were taken immediately after spraying and can be seen in the results (Figure 3.1.8.).

Results

Polytunnel environment

Diurnal fluctuations in air temperature and relative humidity among the polytunnel raspberry plants are shown in Figure 3.1.1. During the experiment, average temperature was 18.3ºC; the maximum and minimum temperatures recorded were 33.2 ºC and 6.8 ºC. The average relative humidity was 73.1%.

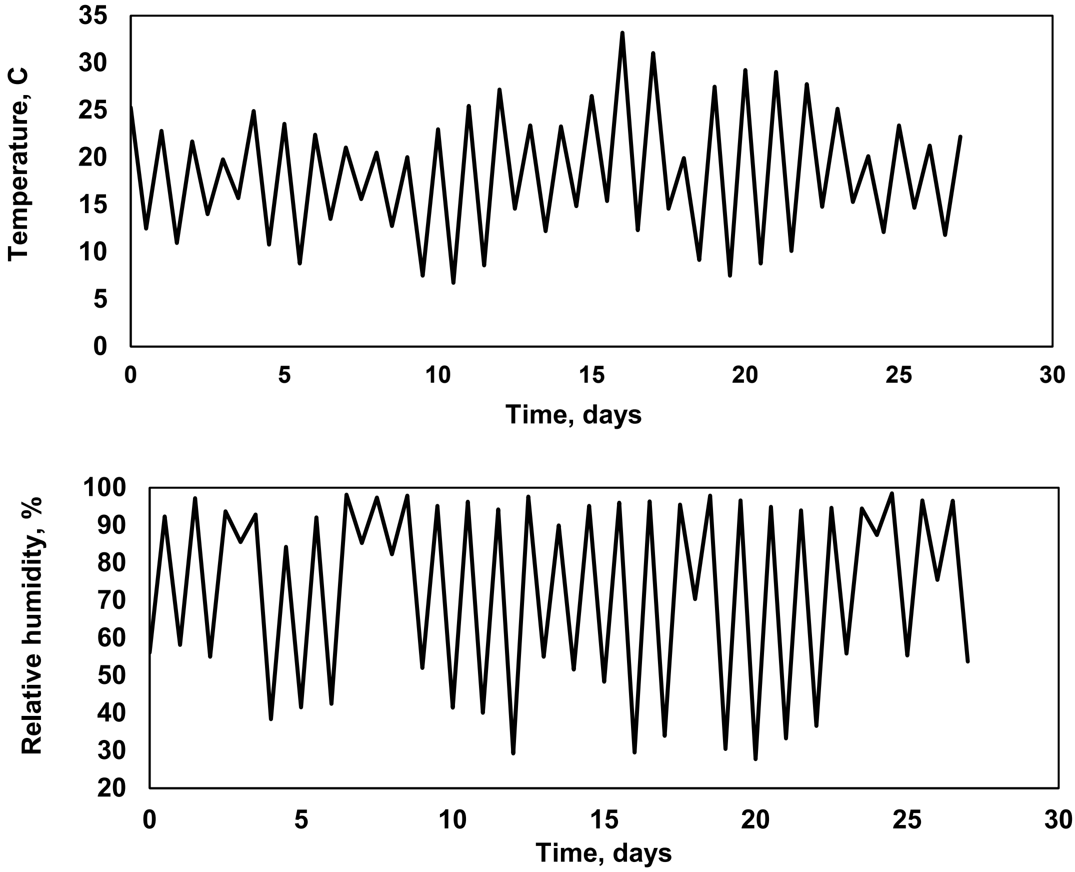


Figure 3.1.1. Temperature and relative humidity among polytunnel raspberry plants

Spray applications

Full foliar application took 80 seconds per half tunnel compared with 8 seconds for bait sprays. Spray applications measured from the start and end tank volumes were about 10% lower than the target values (Table 3.1.5).

Droplet application patterns on the crop are shown in Figure 3.1.2. The full field rate, fine spray resulted in a uniform wetted film over the leaves whereas the Combi-protec and molasses treatments were applied as distinct droplets. No phytotoxicity symptoms were observed on any of the plants and there was no mould growth on the bait spray droplets.

Table 3.1.5. Target and actual measured quantities of sprays applied

Treatment	Spray	Insecticide	Spray vol., ml/plant	
			target	actual
Full field rate	1	Tracer	103.0	97.0
	2	Exirel	103.0	89.3
	3	Tracer	103.0	90.8
	4	Exirel	103.0	89.2
Combi-Protec	1	Tracer	8.3	8.0
	2	Exirel	8.3	5.8
	3	Tracer	8.3	8.7
	4	Exirel	8.3	7.3
Molasses	1	Tracer	8.3	8.2
	2	Exirel	8.3	7.7
	3	Tracer	8.3	7.3
	4	Exirel	8.3	7.5
Low rate, no bait	1	Tracer	8.3	7.3
	2	Exirel	8.3	7.3
	3	Tracer	8.3	7.5
	4	Exirel	8.3	7.3

Figure 3.1.2. Spray applications for Combi-Protec (left) and full field rate (right)

D. suzukii adult emergence assessments

The first adults emerged 7-9 days after placing the fruit in the emergence boxes, with the majority emerging by day 13. All boxes were discarded after 19 days so there was no possibility of a second generation of flies. In the week 1 assessment, less than four *D. suzukii* were present in control fruit samples and no *D. suzukii* adults emerged from samples taken from insecticide sprayed plots; these samples were therefore omitted from the analysis. Of flies that emerged in the subsequent mesocosms, 78%, 55% and 82% were *D. suzukii* in weeks 2, 3 and 4, and there were about equal proportions of *D. suzukii* females and males (54% females overall). In these weeks, averages of between 22 and 37 *D. suzukii* adults emerged from the control mesocosms. Other flies emerging in the mesocosms were predominantly *D. melanogaster*. 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 weeks (Figures 3.1.3 and 3.1.4). The full rate and bait spray applications resulted in significant further reductions in these numbers, with very few or no *D. suzukii* adults emerging in these mesocosms. There was no significant interaction between the effects of spray treatments and week number on the emergence of *D. suzukii* adults in the mesocosms.

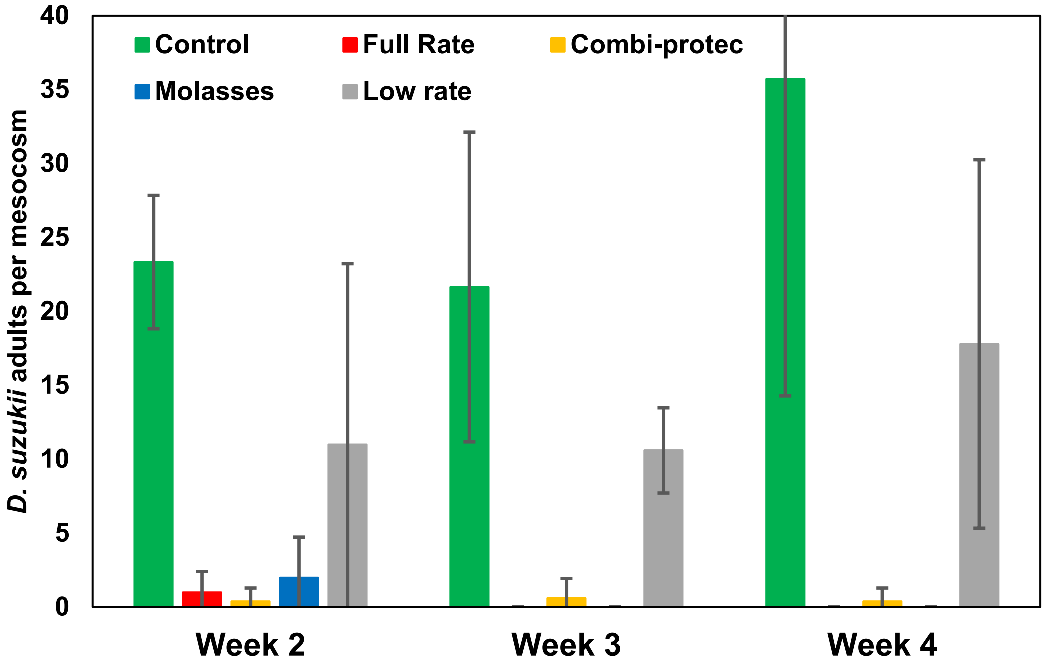


Figure 3.1.3. Effect of full rate applications of Tracer in weeks 1 and 3 and Exirel in weeks 2 and 4, and dilute applications of the same insecticides applied in the same weeks with and without baits (Combi-protec or molasses) on the weekly numbers of emerged *D. suzukii* adults from mesocosms containing 25 raspberries. Mean values (±SE), n = 5.

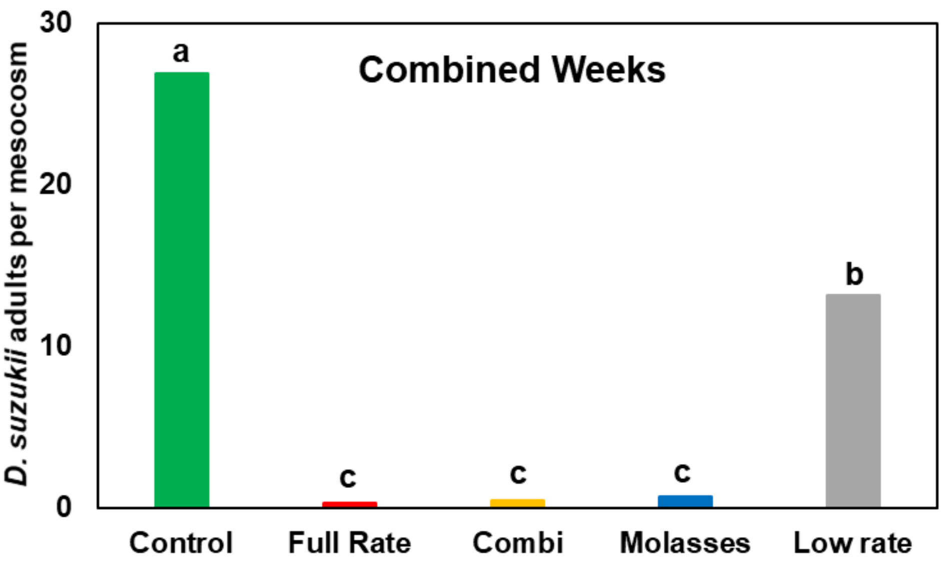


Figure 3.1.4. Effect of full rate applications of Tracer in weeks 1 and 3 and Exirel in weeks 2 and 4, and dilute applications of the same insecticides applied in the same weeks with and without baits (Combi-protec or molasses) on the average number of emerged *D. suzukii* adults from mesocosms containing 25 raspberries in weeks 2, 3 and 4. Bars with same letters are not significantly different (*P* = 0.05); n = 5.

Larvae flotation assessments

The numbers of larvae in flotation tests followed a similar trend to the emergence tests but the numbers were significantly lower in the flotation tests in the corresponding weeks 2,3 and 4 (Figures 3.1.3 and 3.1.5). This is partly explained by the smaller number of sample fruits (20 instead of 25). *D. suzukii* eggs in the sample fruits may have developed into adults in the emergence tests (up to 19 days) but may not have developed into larvae for the flotation tests (2 days).

The insecticides at the full field rates or dilute rates with bait sprays remained as effective in controlling *D. suzukii* numbers in the two weeks after they were applied as they were during the four weeks when they were being applied. However, the experiment was conducted in enclosed tunnels and conditions in the open, where wet weather and/or infestations of *D. suzukii* from outside, may be more challenging.

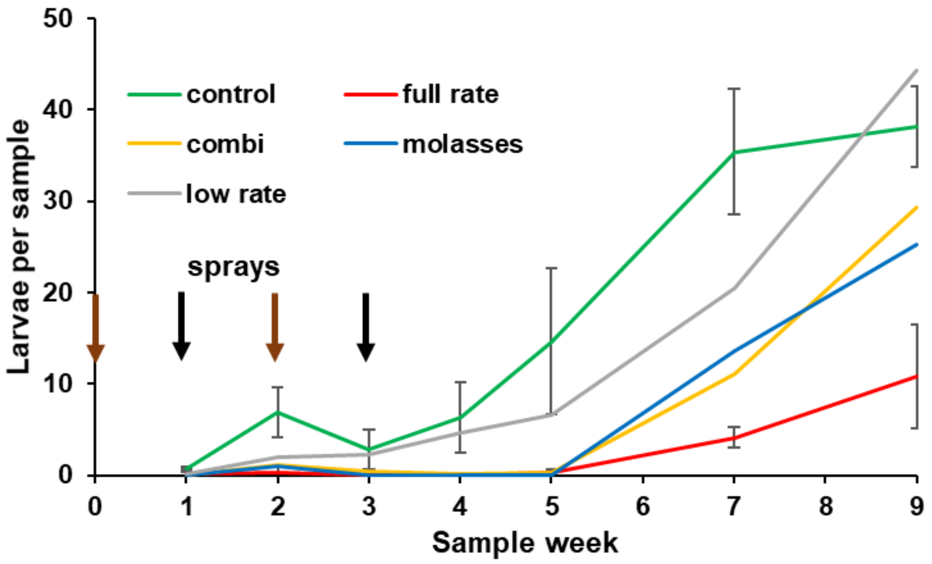


Figure 3.1.5. Effect of full rate applications of Tracer in weeks 1 and 3 (brown arrows) and Exirel in weeks 2 and 4 (black arrows), and dilute applications of the same insecticides applied in the same weeks with and without baits (Combi-protec or molasses) on the weekly numbers of larvae from flotation tests on samples of 20 raspberries. Mean values of top, middle and bottom of plants (±SE), n = 5.

Residue analysis

No pesticide residues were found in any of the untreated control fruit samples, and no spinosad residues were found in any of the fruit samples taken after the second Exirel spray. All the residue concentrations were within the EU MRLs for spinosad and cyantraniliprole in raspberries, except in one sample taken from the top of plants given the full rate spray of Exirel, which slightly exceeded the MRL. However, the samples were taken immediately after spraying and not after the three-day harvest interval. Residues of spinosad and cyantraniliprole were at least x11 higher in samples taken from the full field rate spray plots than from bait spray plots (Tables 3.1.6 and 3.1.7). There were no differences in residue concentrations between the Combi-protec and molasses treatments. Residues of cyantraniliprole were higher after the second Exirel spray than one week after the first Exirel spray (Table 3.1.7). Residues of spinosad and cyantraniliprole were generally lower in fruit samples taken from the bottom of plants than in samples taken from the top and middle; the exception was in the first samples taken from the full rate spray treatment where cyantraniliprole residues slightly increased down the plant (Table 3.1.7).

If pesticide residues in flowers follow a similar pattern to residues in fruit, it is possible that bait sprays would reduce the risk of pesticide contact with bees compared with the full rate pesticide applications. However, attractiveness of bait sprays to bees and beneficial arthropods requires further investigation.

Table 3.1.6. Residues of spinosad in fruit samples taken from different plant positions immediately after the second spray of Tracer (mg/kg fruit)

Plant position	**Top**	**Middle**	**Bottom**
Full field rate	0.69	0.59	0.45
Combi-protec	0.025	0.021	<0.01
Molasses	0.017	0.017	<0.01

EU MRL 1.5 mg/kg fruit

Table 3.1.7. Residues of cyantraniliprole in fruit samples taken from different plant positions immediately after the second sprays of Tracer and Exirel (mg/kg fruit)

Insecticide spray	**2nd Tracer**			**2nd Exirel**		
Plant position	**Top**	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**
Full field rate	0.29	0.38	0.42	1.10	0.83	0.75
Combi-protec	0.02	0.024	<0.01	0.039	0.06	<0.01
Molasses	0.025	0.017	<0.01	0.044	0.048	0.011

EU MRL 0.9 mg/kg fruit

Spray deposition analysis

The spray deposition of each sprayed treatment was assessed using a handheld imaging fluorometer and fluorescent tracer dye. Where appropriate the data were analysed using R and R-Studio. The spray coverage for each treatment was compared using Kruskal-Wallis, with *post-hoc* analysis using the Dunn Test (Figure 3.1.6).

The results show there was a highly significant difference in spray deposition coverage between the full rate application (500 L/ha) and low-rate applications (40 L/ha), but not between the different baits. The difference in spray coverage between the full rate and the low rate was approximately 8-times lower (62.2 % versus 7.8 %). There was no evidence of any difference in deposition between the Combi-protec and the molasses treatment, nor the treatment without any bait added.

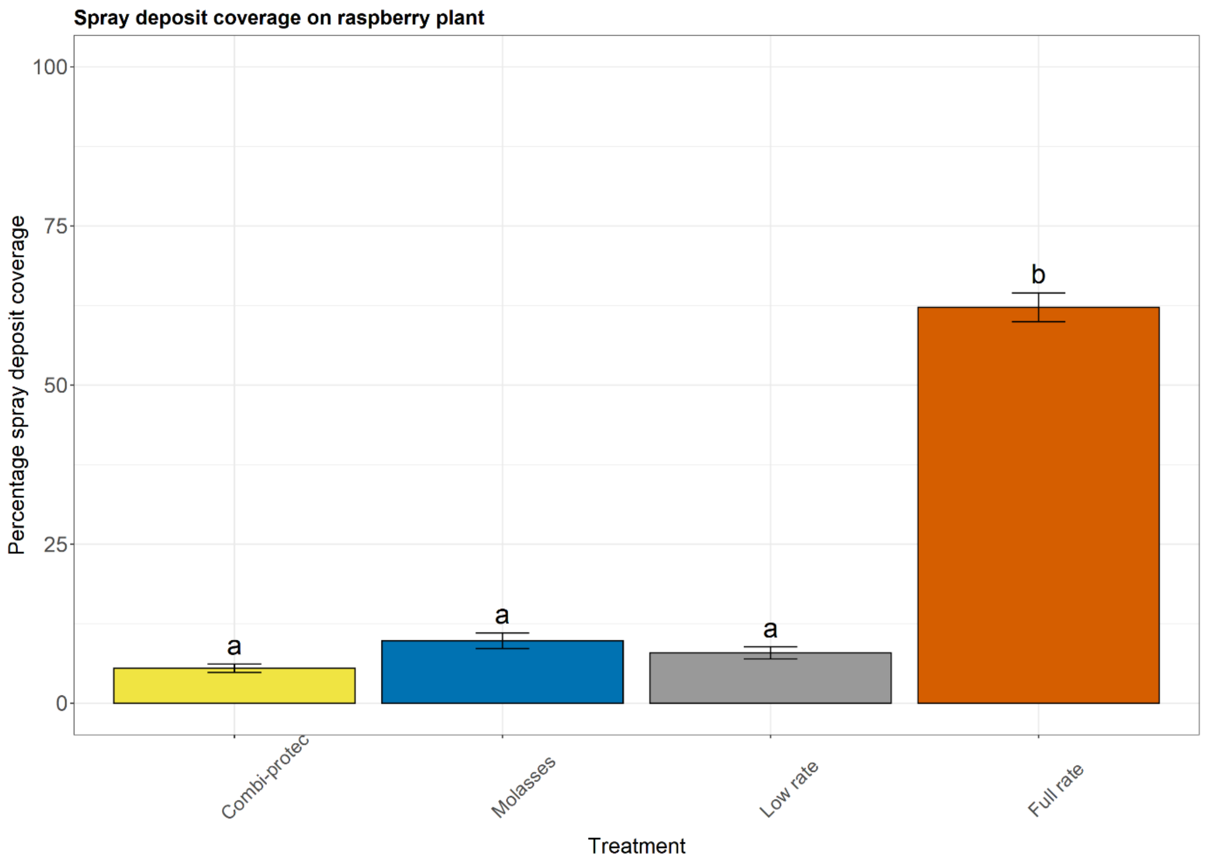


Figure 3.1.6. The spray deposition as percentage coverage on the leaves of raspberry plants across all canopy sections and leaf side. The plants were sprayed with the treatments adjuvants (Combi-protec, or Molasses), or no adjuvants at either low rate or full rate (table 4). Mean values (±SE), n = 240 per treatment. Letters denote significant differences ($p < 0.05$).

The spray deposition data was divided by canopy section and leaf side (Figure 3.1.7). The results show that for the standard spray rate (500 L/ha) the spray coverage is extremely high at between 50 – 75 % coverage across the leaf sides and canopy sections.

In contrast, the low-rate applications (40 L/ha), either with or without a bait adjuvant, provided extremely low levels of spray coverage. For these low-rate applications, the middle canopy section-upper leaf side received the most spray coverage with between 17 – 37 % spray coverage, compared to 57 % for the full rate application. The higher level of spray coverage to middle canopy sections is consistent with the spray application method, which directed the spray towards this section of the canopy. For all other canopy and leaf sections the low water volume rate applications provided between 0 – 12 % spray coverage, with the majority being around 5 % spray coverage. This level of spray coverage is extremely low and would normally not provide sufficient control of pests or diseases unless the active ingredient is systemic.

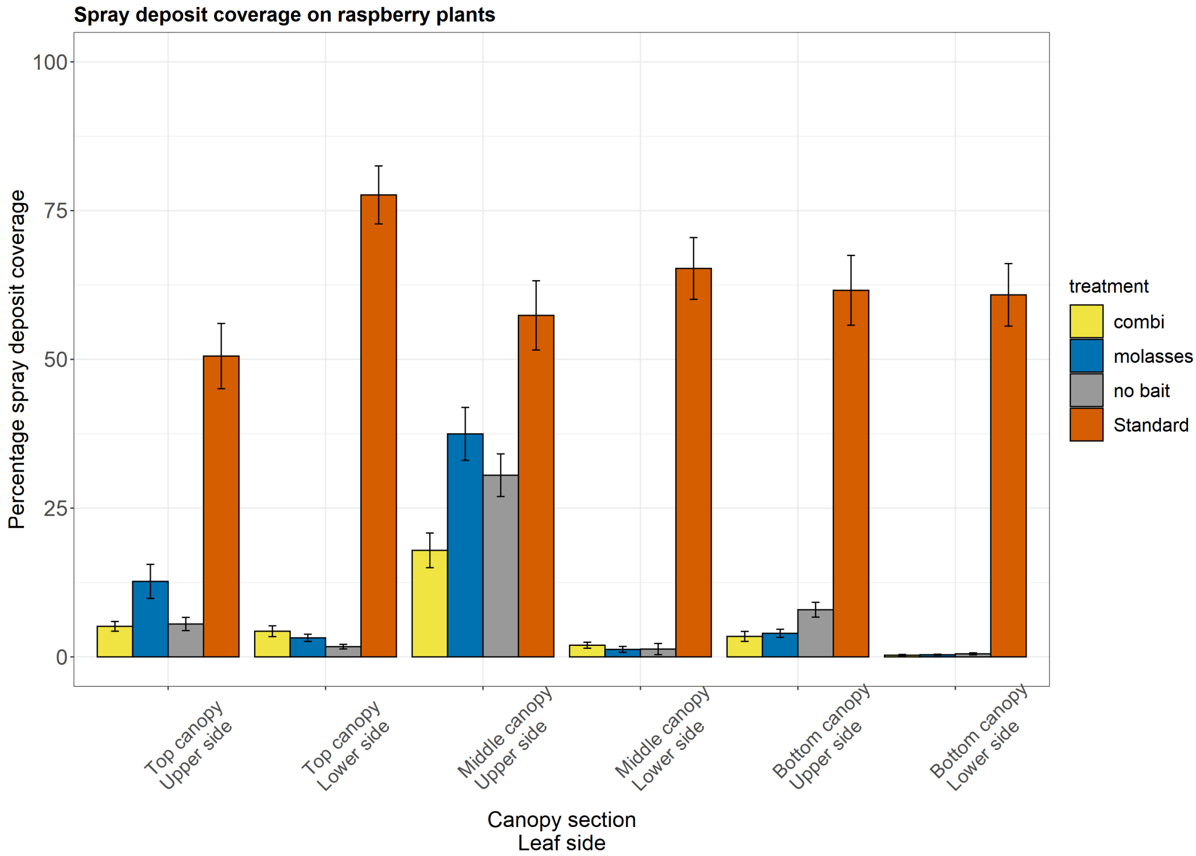
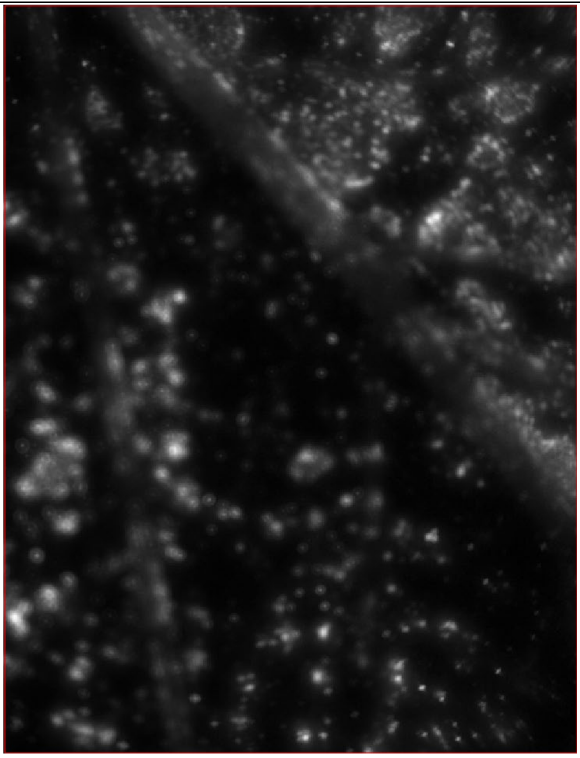


Figure 3.1.7. The spray deposition as percentage coverage on the leaves of raspberry plants for each canopy section and leaf side. The plants were sprayed with the treatments adjuvants (Combi-protec, or Molasses), or no adjuvants at either low rate or full rate (table 4). Mean values (±SE), n = 40 per leaf side.

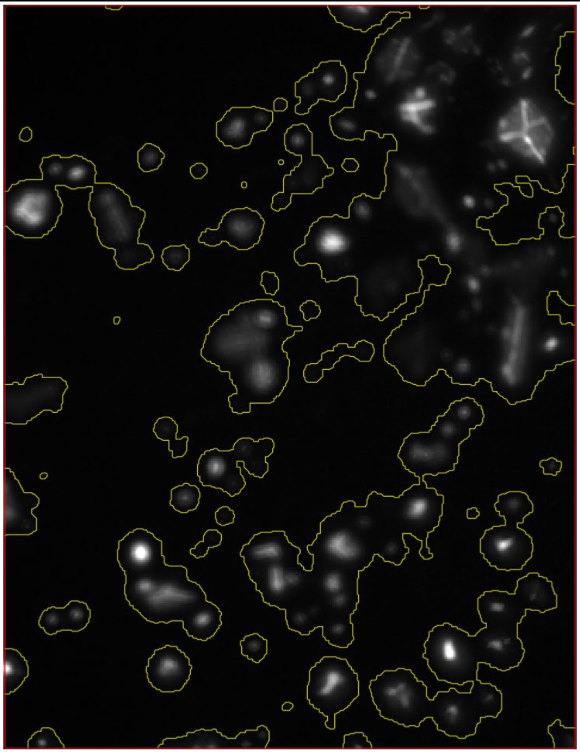
Example images of spray deposits from the trial are presented in Figure 3.1.8. Images A, B, C, and D, show what the different amounts of spray coverage look like on the leaves. The deposits often coalesce and follow the contours of the leaf. The discreet deposits shown in the images E and F indicate the size of the deposits for the full rate fine spray (E) compared to the low-rate medium size spray droplets (F). Many of the deposits in E are approximately 20-40 microns diameter, compared to the large deposits of 100-200 microns in image F.

Deposits greater than 200 microns diameter are common in horticultural spray application. The concentration of the active ingredient in the treatments was kept within the product label parameters, therefore the spray deposits measured in this trial confirm that there is no risk of breaching MRLs. The images confirm that for all treatments both the fine droplets and the larger droplets are dispersed across the leaves and do not form very large deposits.



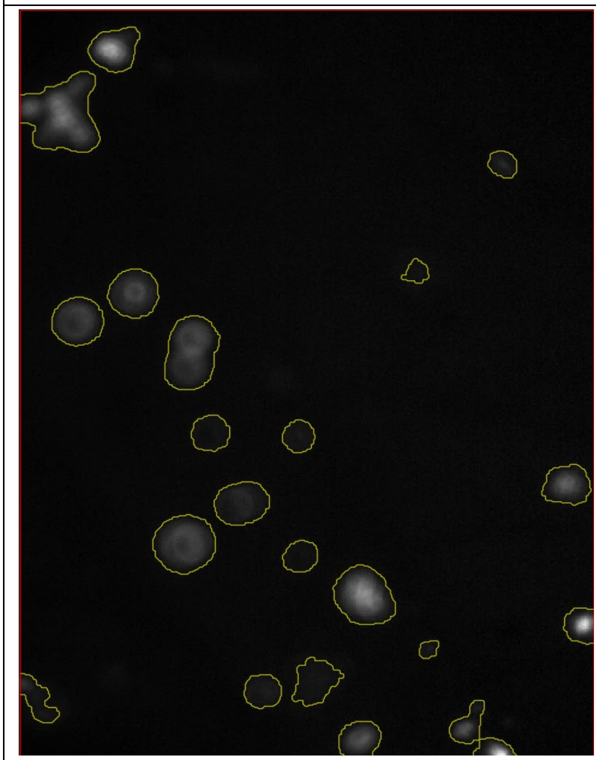
A) Full rate application.

Middle canopy, upper leaf side. 100 % spray coverage.



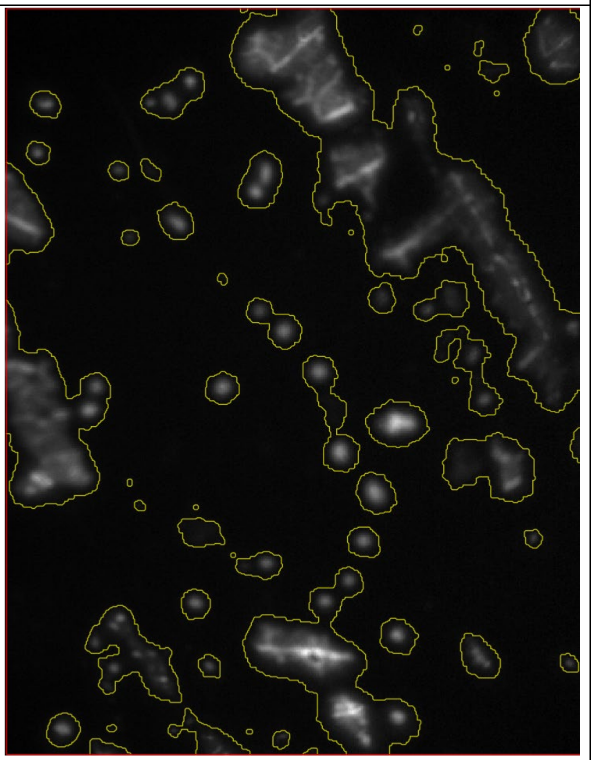
B) No bait.

Middle canopy, upper leaf side. 39 % spray coverage.



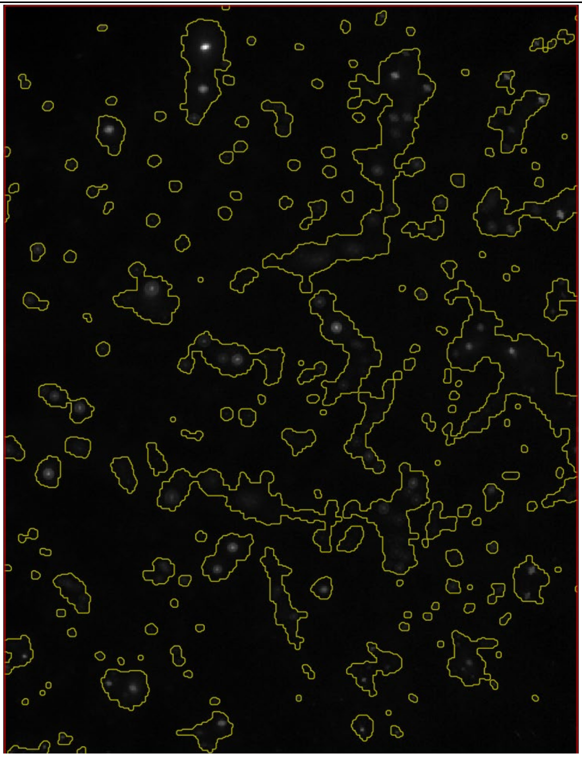
C) Combi-protec.

Middle canopy, upper leaf side. 8.4 % spray coverage



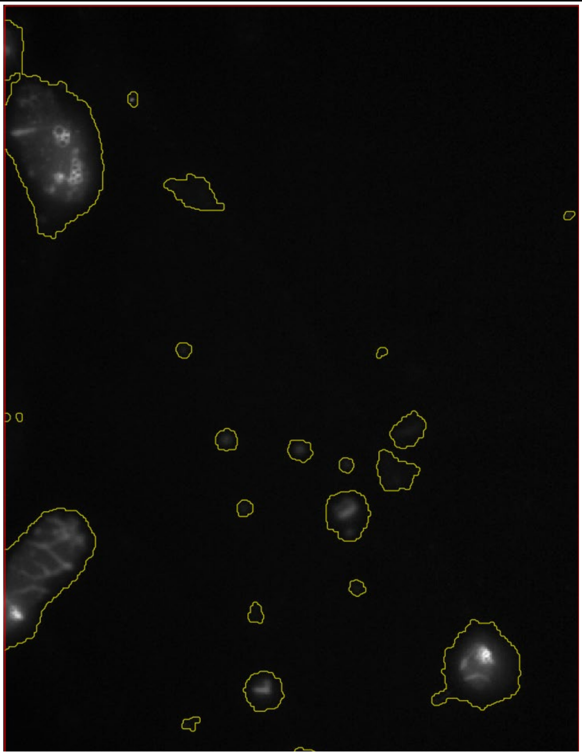
D) Molasses.

Middle canopy, upper leaf side. 28 % spray coverage



E) Full rate application.

Bottom canopy, lower leaf side. 20 % spray coverage.



F) Molasses.

Bottom canopy, upper leaf side. 8.3 % coverage

Figure 3.1.8. Example images of spray deposition on sampled leaves from the middle canopy section, upper leaf side. A) Full rate treatment; B) no bait; C) combi-protec; D) molasses; E) full rate; F) molasses. The spray deposits are highlighted in yellow around the perimeter. Each image is 9.8 x 7.4 mm.

<u>Cost of treatments</u>

The application time for the bait sprays was only 10% of the full foliar spray applications. The cost of Exirel is £150 per litre, Tracer £330 per litre, Combi-protec £36.50 per litre and molasses £6.64 per litre. At 900 (full field rate) or 36 (dilute rate) ml per hectare, the costs of the Exirel product was £135/ha or £5.40/ha per spray, excluding the application cost. The costs for the full field and dilute rates of Tracer were £66/ha and £2.64/ha per spray. The product cost of the Combi-protec or molasses (2 litres per hectare) were £77.50/ha or £13.28/ha per spray, excluding the application cost. The product costs for the four insecticide treatments are shown in Table 3.1.8. The product cost of the four sprays with molasses was only 21% of Combi-protec and 17% of the full field spray rate spray treatments. Bulk sources of molasses are available that are lower in cost than the source used here.

Table 3.1.8. Product costs of spray treatments, £/ha

Treatment	Spray 1	Spray 2	Spray 3	Spray 4	Total
Insecticide	Tracer	Exirel	Tracer	Exirel	
Full field rate	66.00	135.00	66.00	135.00	402.00
Combi-protec	80.14	82.90	80.14	82.90	326.08
Molasses	15.92	18.68	15.92	18.68	69.20

Conclusions

1. Weekly alternating 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 molassess 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 (i.e. a reduction in insecticide application of 96% with the same *D. suzukii* control).
2. The insecticides at the full field rates or dilute rates with bait sprays remained as effective in controlling *D. suzukii* numbers in the two weeks after they were applied as they were during the four weeks when they were being applied; the experiment was conducted in enclosed tunnels and conditions in the open, where wet weather and/or infestations of *D. suzukii* from outside, may be more challenging.
3. Control of *D. suzukii* was equally good with the molasses spray treatment as with the Combi-protec or full field rate spray treatments but at only 21% or 17% of the product costs.
4. The application time for the bait sprays was 10% of the full field rate application of insecticide sprays.
5. 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.
6. There were similar proportions of male and female *D. suzukii* in all the mesocosms from the unsprayed, insecticide and insecticide + bait treatments.
7. Residues of spinosad and cyantraniliprole were at least x11 higher in fruit samples taken from plots sprayed with the full field rates of insecticides than from plots sprayed with the dilute rates with baits.
8. Residues in fruit from the dilute insecticide rates + bait spray plots were not detectable or lower in samples taken from the bottom of plants than in samples from the top and middle of plants; some readjustment of the height and distance of spraying from plants may be needed to give a more uniform application.

9. None of the insecticide or insecticide + bait treatments caused phytotoxicity symptoms and there was no mould growth on the bait spray droplets.
10. The spray coverage of the low-rate sprays was approximately 8-times lower than the full-rate spray. This level of coverage without the use of baits would not provide sufficient control of pests.
11. Despite the larger droplet sizes used for the low-rate applications, there was no evidence of any extremely large deposits that could breach MRLs. The spray deposits were dispersed on the leaf surfaces.

Recommendations for Future Work on Baits

1. Bait sprays should be trialled in cherry orchards to enable the reduction of insecticide to these crops.
2. The bait treatments should be tested with other insecticides. Results from jar bioassays in Year 2 indicate that Hallmark (lambda-cyhalothrin) is the most promising other insecticide treatment with Combi-protec for *D. suzukii* control. Semi-field scale trials with Combi-protec by Helsen & van der Sluis (2017) also showed that Combi-protec with Hallmark, as well as Decis (deltamethrin) and Pirimicarb, gave good control of *D. suzukii* in strawberries.
3. The effect of using bait sprays at fortnightly intervals could be compared with the effect of using weekly sprays on *D. suzukii* control.
4. Baits should be tested with entomopathogenic fungi biocontrol products such as *Metarhizium*, *Beauveria* and *Lecanicillium*. However, Helsen & van der Sluis (2017) found that Combi-protec did not give control of *D. suzukii* with *Bacillus thuringiensis.*
5. Molasses should be tested with higher spray volumes – 80 or 100L/ha, particularly on cherries, where 40L/ha may not give good crop spray coverage.
6. The uniformity of bait spray application from top to bottom of plants should be improved by adjusting the height and distance of spraying.
7. The relative effects of bait and full rate pesticide sprays on bees and other beneficial insects should be investigated.
8. An application should be made for approval of molasses as a sticker adjuvant.

Objective 5. Integrating exclusion netting with other successful controls

Progress is being made on this objective in a NIAB EMR, University of Reading and Berry World, Waitrose CTP PhD studentship which has just concluded its first year. Results will be reported to the AHDB steering committee and the SWD Working Group. A short summary of insect exclusion mesh efficacy and side effects is currently being prepared by the PhD student and M. Fountain for publication by AHDB. Due to Covid in 2020 the PhD student field studies were delayed but are planned for 2021.

Objective 6. Develop, design, and communicate a year-round strategy for UK crops for *D. suzukii* control

In collaboration with the AHDB communications team we are producing recommendations for year-round control of *D. suzukii* that targets all life stages and habitats to reduce year on year populations, damage to fruit and the use of plant protection products used for control. Results would be disseminated via processes outlined in Section 3.1 of the proposal but also via the AHDB website and a wallchart and factsheets, see also Knowledge and Technology Transfer, below.

Objective 7. Identification and quantification of *D. suzukii* parasitism in the UK

Task 7.1. Screening Scottish habitats for the presence of D. suzukii parasitoids (JHI)

Materials and Methods

To detect parasitoids capable of parasitizing *D. suzukii*, sites in Scotland with *D. suzukii* populations and/or host plants and low pesticide pressure were selected to capture the main fruit farming areas in Eastern Scotland. *Drosophila melanogaster* baited traps were placed at two separate locations at each of five sites, including at the institute. Due to the relatively low abundance of *D. suzukii* in Scotland, the bait in the traps was created using strawberry fruits infested with larvae/pupae of *D. melanogaster* which was cultured on the medium provided by the insect supplier (Blades Biological Ltd, UK).

Following the NIAB EMR Standard Operating Procedure for the previous studies, traps (Fig 7.1.1) were deployed fortnightly at each site between July and October/November in 2019, and between June and November in 2020, then returned to the institute and maintained under controlled conditions. Traps were placed at two separate locations, between 0.5-1 km apart, at each of five sites across Eastern Scotland, including at the institute (Fig. 7.1.2). Traps were typically located in wooded/hedge and field margin vegetation adjacent to fruit-growing tunnels (at grower sites: traps 1903, 1904, 1907, 1908, 1909, 1910) or fruit plots (at institute/garden sites: traps 1901, 1902, 1905, 1906). The location, date of deployment and removal, and characteristics of the surrounding habitats were recorded for each trap. Once collected from the field, all sentinel boxes were returned to the institute and incubated at 20-25 °C, ~50% relative humidity and 16:8 hours light:dark photoperiod for a minimum of 6 weeks (parasitoid emergence time). Sentinel boxes were examined weekly for a period of up to six weeks to ensure all emerging parasitoids were recorded. All parasitoids were recorded and stored in 70% ethanol for later identification.

Figure 7.1.1. Example location of the trap used for SWD parasitoid sampling in Eastern Scotland in 2019 and 2020.

Figure 7.1.2. Map showing location of sites used for SWD parasitoid sampling in Eastern Scotland in 2019 and 2020.

Results

Most parasitoids emerged within 1-5 weeks of trap collection. In 2019 samples, two morphotypes were detected that appeared to differ in size and wing venation (approx. two-thirds of the trapped individuals belong to the larger morphotype) but molecular barcoding of representative samples confirmed they belonged to a single species, the larval parasitoid, *Asobara tabida*. Samples collected in 2020 were also identified as *Asobara tabida*. Parasitoids were trapped in highest numbers in July-September in 2019 and in August and October in 2020, and the numbers were up to four-fold higher in 2020 compared with 2019 (Fig. 7.1.3).

There was regional variation in total catch size of parasitoids (Fig. 7.1.4), with highest numbers at two sites (traps 1 and 2 at the institute and traps 3 and 4 at site 1300), which are in the south-eastern part of the region being used for monitoring. *D. suzukii* is present at these sites (see Scotland National Monitoring), but there is no *D. suzukii* monitoring data for the remaining sites to allow comparison with regional variation in potential host abundance for these parasitoids. Habitat assessment indicated that traps 2 and 3 were sited amongst vegetation that included elder, *Rubus* spp., cherry, and ivy, which are suitable host plants for *D. suzukii*. However, other traps sited amongst suitable host plants (e.g. traps 5 and 7) did not have high parasitoid trap catches.

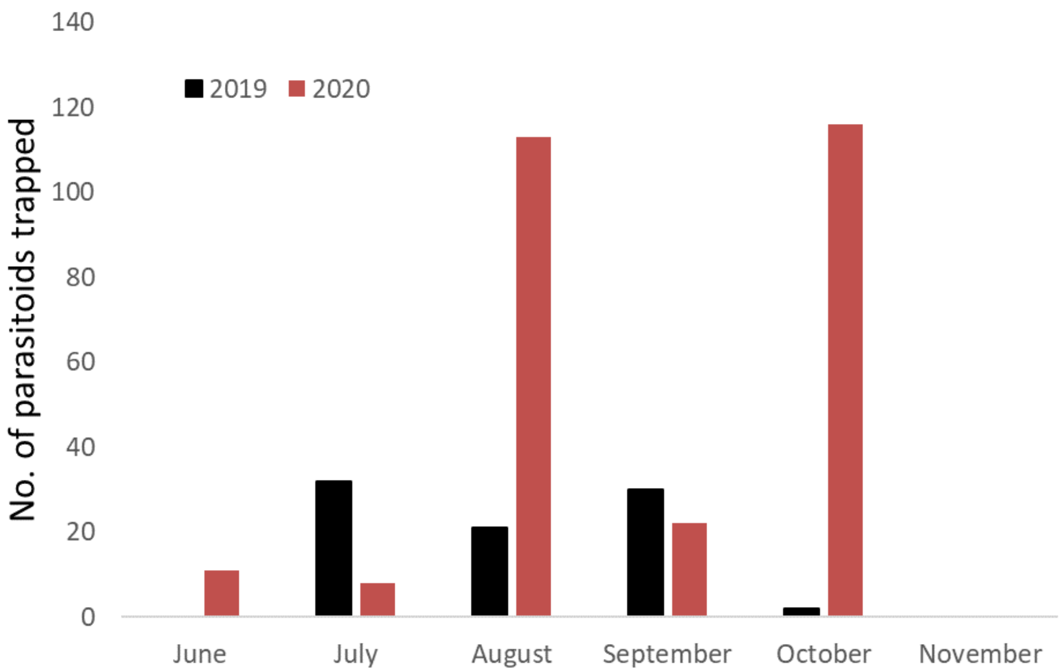


Figure 7.1.3. Numbers of parasitoids emerging from sentinel traps placed at ten sites across Eastern Scotland during the summer and autumn months of 2019 and 2020. Trap catches are shown per month based on when the traps were in the field.

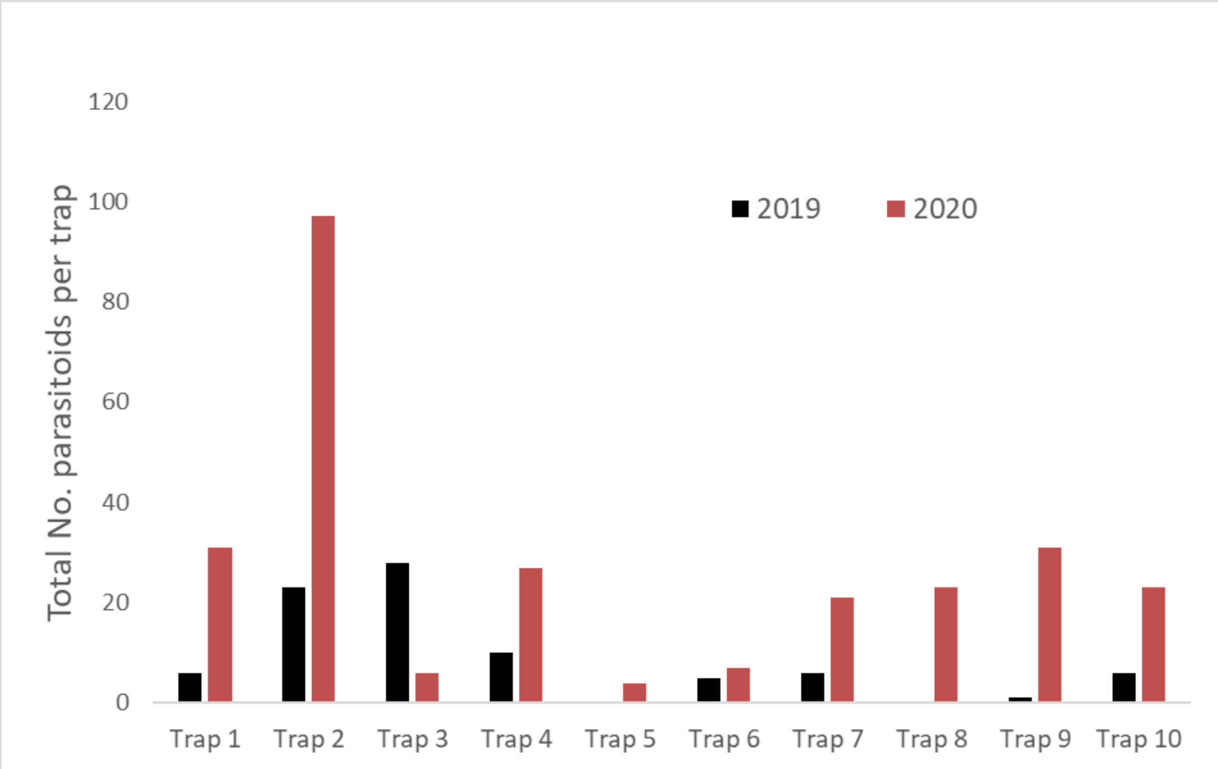


Figure 7.1.4. Total numbers of parasitoids emerging from sentinel traps at ten sites across Eastern Scotland in 2019 and 2020.

Conclusions

- The SWD sentinel trap method was successfully adapted and deployed in Eastern Scotland in 2019 and 2020.
- Parasitoids collected in 2019 were confirmed as *Asobara tabida*
- Parasitoids collected in 2020 were also confirmed as *Asobara tabida*.
- Within-season variation in parasitoid abundance suggested that parasitoids were present earlier in the summer than anticipated (before mid-July), which was confirmed in 2020.
- There were significant differences between years in the abundance of emerging parasitoids.
- It is unclear if variation in parasitoid abundance within season and between sites is linked with variation in SWD abundance and SWD host plant availability.

Task 7.2. Investigating the proportion of **Drosophila suzukii** *pupae in sentinel traps parasitized by UK parasitoids (NIAB)*

Introduction

Some species of parasitic Hymenoptera target *Drosophila* spp. and have potential as biocontrol agents of *Drosophila suzukii* in and around UK crops. A number of parasitoids have been reported in association with *D. suzukii* in its native area and newly invaded production regions in Pacific North America and northern Italy (Rossi-Stacconi et al., 2013), including *Trichopria drosophilae*. *T. drosophilae* is a pupal parasitoid commercially available in Europe for use in biological control; however, at the time of writing, it has not been formally identified in the UK, making approval for commercial use more problematic.

In 2017 and 2018, a Worshipful Company of Fruiterers funded project linked to SF 145, aimed to identify species of Hymenoptera parasitizing *D. suzukii* in South East England. In addition, field surveys were conducted across several fruit growing and wild sites in the South East of England aimed to identify *T. drosophilae*, and to investigate potential interactions of *D. suzukii* with native UK parasitoid species that may contribute to *D. suzukii* control.

Five species of native parasitoid were identified from *D. suzukii* larvae/pupae sentinel traps. Two species of larval parasitoids (*Asobara tabida* and *Leptopilina heterotoma*) and three pupal parasitoids (*Pachycrepoideus vindemmiae*, *Spalangia erythromera* and *Trichopria prema*) were recorded from *D. suzukii*. All five species are generalist parasitoids of Drosophila spp. *P. vindemmiae* was the most common, in both agricultural and semi-natural habitats. *S. erythromera* was collected in relatively high numbers in 2018 from the sentinel traps from all habitats and its occurrence was consistent over the cropping season. Unlike *P. vindemmiae*, this species does not hyperparasitize and therefore may provide a more viable tool in controlling *D. suzukii*. Unfortunately, very little is known about *T. prema* and there is no literature evidence confirming that this species could parasitize *D. suzukii*. The larval parasitoids *L. heterotoma* and *A. tabida* were found in low numbers. In contrast to the pupal parasitoids, *P. vindemmiae* and *S. erythromera*, they exhibited a poor ability to develop from *D. suzukii* larvae in our studies, probably because of the high immune response produced by *D. suzukii* larvae.

The presence and abundance of these parasitoid species varied greatly among the sites and across the season. At sites where parasitoids were active, small numbers were recovered in May, but the main period of activity was from June to October, with no parasitoids present from November onwards.

The habitat assessment showed how landscape diversity could influence the parasitoid presence. The surveys demonstrated that native parasitoids may interact with *D. suzukii* and should be considered when implementing pest control measures.

A laboratory test was also done to validate parasitism. *P. vindemmiae*, *S. erythromera*, *L. heterotoma* were able to parasitize and develop from *D. suzukii* pupae and larvae in our laboratory cultures, though only one *L. heterotoma* emerged. *A. tabida* failed to parasitize *D. suzukii* probably because only 3 female individuals were recovered. The pupal parasitoid *P. vindemmiae* produced most offspring per parent in all laboratory cultures (mean = 3.6) compared to the pupal parasitoid *S. erythromera* (mean = 0.2) and *L. heterotoma* (mean = 0.1). *T. prema* could not be tested because these died soon after emergence. The rate of *D. suzukii* parasitism by these species and potential others is yet to be calculated accurately in UK populations in the field.

Aims

- Calculate *D. suzukii* parasitism rate under field conditions.
- Determine if parasitism rates change throughout the year.
- Continue to search for the pupal parasitoid *T. drosophilae*, to confirm its presence in the UK.

Materials and Methods

Trial sites: The trial was set up at 2 soft fruit farms in Kent. Sites were selected which had the highest catches of parasitoids during the 2018 survey. Site 1 was a hedgerow beside an abandoned cherry orchard and Site 2 a woodland next to a strawberry crop.

Treatments: Each site had 12 Delta traps (alternating paired treatment and control traps, 6 of each) spaced at 8 m intervals (Fig. 7.1.2). Delta traps contained a large Perspex sentinel box (10 x 10 x 20 cm), holding 160g of *D. suzukii* inoculated fresh strawberries on dampened blue roll in a Petri dish base (Fig. 7.1.1a). Strawberries were inoculated with 5 male and 10 female adult *D. suzukii* and incubated at ~22 °C, ~50% relative humidity and 16:8 hours light:dark photoperiod for 96 hrs prior to deployment in the field. A box of fresh fruit (not inoculated) was incubated at NIAB EMR to confirm that no *Drosophila* spp. were already present in the commercial fruit. Content of treatment and control boxes were the same, but lid mesh was different:

1. Treatment: large Perspex sentinel boxes had a lid with mesh holes 2 mm diameter, to prevent adult *D. suzukii* passage, but permit entry of parasitoids to egg lay in sentinel *D. suzukii* larvae and pupae.

2. Control: large Perspex sentinel boxes had a lid with mesh holes <0.5 mm diameter, to prevent parasitoid entry.

Delta traps remained in position for the whole trial. Large Perspex sentinel boxes were collected every 10 days and replaced fortnightly, 6 times from July to September, falling in the period (June to September) parasitoid numbers were highest during the 2017 and 2018 screening trials. See table 7.1.1 for collection dates. Lids of large Perspex sentinel boxes were protected with metal gauze to prevent animal damage to underlying lid mesh (Fig. 7.1.1b).

Assessments: On the collection day, large Perspex sentinel boxes were transported to NIAB EMR's quarantine facility. Any specimens found in the boxes were transferred to vials of 70% ethanol for later identification and counting. Treatment box mesh lids were replaced with a fine mesh lid <0.5 mm diameter to prevent the escape of adult parasitoids. Subsequently, all boxes were incubated under ambient laboratory conditions ~22 °C, ~50% relative humidity and 16:8 hours light:dark photoperiod and examined weekly. *D. suzukii* and parasitoids were collected and counted during the entire 6-week period.

a) 

b) 

Figure 7.1.1. Sentinel traps used to attract parasitoids during the *D. suzukii* parasitoid survey 2020 a) Large Perspex sentinel boxes each consisting of large Perspex box containing fresh strawberries inoculated with *D. suzukii* larvae and pupae and b) Large Perspex sentinel box with metal gauze to prevent animal damage to underlying lid mesh.

Figure 7.1.2. Delta trap containing large Perspex sentinel box during the *D. suzukii* parasitoid survey in 2020.

Table 7.1.1. Dates large Perspex sentinel boxes were collected from both sites during the parasitoid survey 2020.

Collection 1	Collection 2	Collection 3	Collection 4	Collection 5	Collection 6
17-Jul-20	31-Jul-20	10-Aug-20	21-Aug-20	01-Sep-20	21-Sep-20

Statistics A GLM was used to compare *D. suzukii* emergence between treatment and control boxes

Mean percent parasitism was calculated as the mean of:

$$\frac{\text{Parasitoids emerged per treatment box}}{\textit{D. suzukii}\text{ emerged in paired control box}} \times 100$$

Results

From 6 sentinel trap deployments between July and September 2020, 4 parasitoid species emerged, including a first record of *Trichopria modesta* (total = 2). Other species were *Spalangia erythromera* (total = 65), *Asobara tabida* (total = 2), and *Leptopilina heterotoma* (total = 2). Interestingly, the parasitoid most prevalent in previous years, *P. vindemmiae*, was not recovered. Out of 72 treatment boxes deployed, *L. heterotoma* and *T. modesta* only emerged from 1 box each, *S. erythromera* emerged from 6 and *A. tabida* emerged from 2. *D. suzukii* parasitism rate was variable, ranging from 0 to 43% for *S. erythromera* and 0 to 6% for *A. tabida* (Fig. 7.1.3); *S. erythromera* (mean% = 1.1), followed by *A. tabida*.

Overall, *D. suzukii* emergence was significantly lower in treatment boxes than control (mean = 68.9 and 87.5 respectively (P= 0.029), (Fig. 7.1.5).

During the period of deployments, the rate of *D. suzukii* parasitism varied for all parasitoid species recorded. Peaking on 10 August for *S. erythromera* and *A. tabida* (6.2 and 1% respectively) (Fig. 7.1.4). *L. heterotoma* and *T. modesta* were only found on one collection date (01 and 21 September respectively, data not shown).

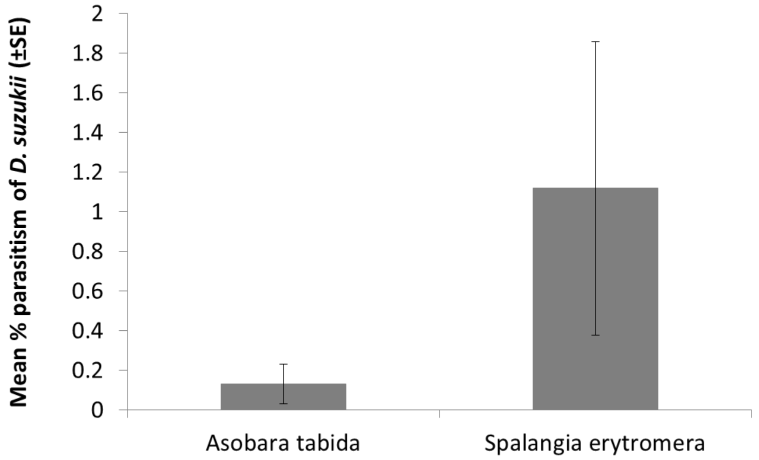


Figure 7.1.3. Overall rate (mean%) of *D. suzukii* parasitism calculated for the two parasitoid species recorded on separate occasions during the 2020 survey. Rate was calculated as parasitoids emerged in treatment boxes (lid mesh permitting parasitoid entry) as a percent of *D. suzukii* emerged in control boxes (lid mesh preventing parasitoid entry).

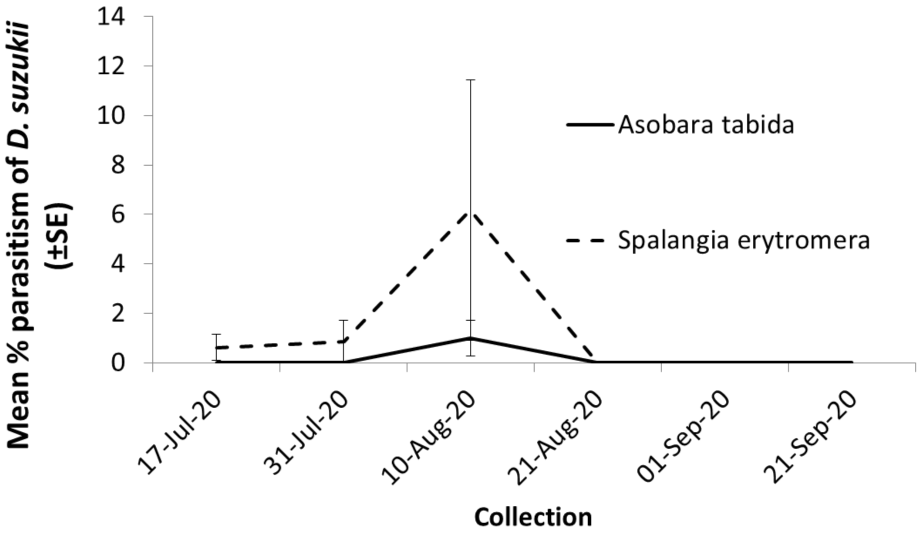


Figure 7.1.4. Rate (mean%) of *D. suzukii* parasitism at different collection dates calculated for the two parasitoid species recorded on separate occasions during the 2020 survey. Rate was calculated as parasitoids emerged in treatment boxes (lid mesh permitting parasitoid entry and egg laying) as a percent of *D. suzukii* emerged in control boxes (lid mesh preventing parasitoid entry).

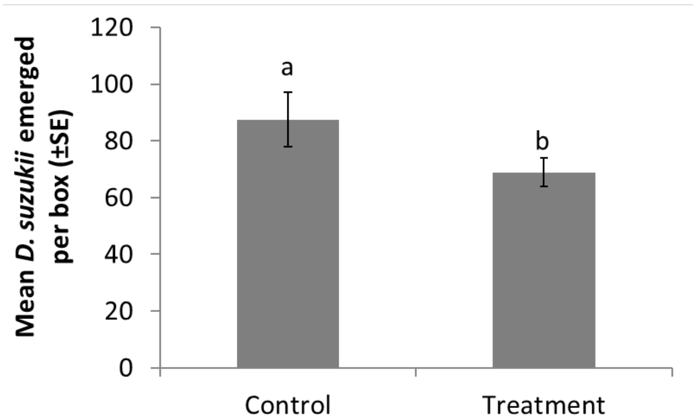


Figure 7.1.5. Mean *D. suzukii* emergence per sentinel trap for control (lid mesh preventing parasitoid entry) and treatment (lid mesh permitting parasitoid entry and egg laying) during the 2020 survey. Letters denote significant differences at *P*=0.05.

Table 7.1.2 Parasitoid species recorded and mean emergence per sentinel trap at the same sites 2017, 2018 and 2020.

Year	Trap position	*Pachycrepoideus vindemmiae*	*Spalangia erythromera*	*Leptopilina heterotoma*	*Asobara tabida*	*Trichopria prema*	*Trichopria modesta*
2017	Woodland	8.9	0.4	0.4	0.1	0	0
	Hedgerow	2.7	2.2	0	0	0	0
2018	Woodland	2.87	1.87	0.04	0.01	0	0
	Hedgerow	2.82	0.14	0	0	0.04	0
2020	Woodland	0	1.0	0.06	0.03	0	0
	Hedgerow	0	0.8	0	0.03	0	0.03

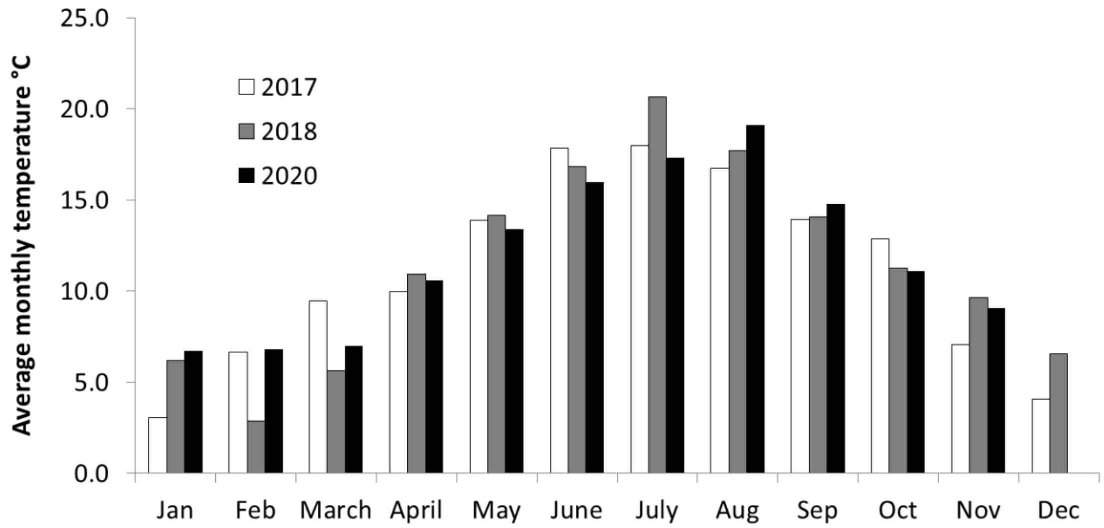


Figure 7.1.6. Mean monthly temperature recorded from March to November at NIAB EMR weather station in 2017, 2018 and 2020.

Discussion

In 2020, NIAB EMR set out to calculate the rate of *D. suzukii* parasitism by UK parasitoids in the field. From 7 July to 21 September, sentinel traps were deployed at the two sites surveyed which caught high numbers of parasitoids during the same period in 2017 and 2018. Two types of sentinel traps were deployed (treatment and control), each inoculated with equal numbers of *D. suzukii.* Treatment traps were equipped with a lid to permit parasitoid entry and egg laying in *D. suzukii* larvae and pupae, whereas control traps had a lid preventing parasitoid entry. Mean rate of *D. suzukii* parasitism was calculated per parasitoid species, by using the number of parasitoids emerged per treatment box as a percent of *D. suzukii* emerged per corresponding control box.

Overall, 4 different parasitoid species emerged in sentinel traps, including NIAB EMRs first record of *T. modesta* (total = 2). The most common species was *S. erythromera* (total = 65), followed jointly by *A. tabida*, *L. heterotoma,* and *T. modesta* (total = 2 of each). *Trichopria drosophilae* is yet to be recorded in the UK.

The rate of *D. suzukii* parasitism in the field was variable owing to the small proportion of traps in which parasitoids emerged. Of the parasitoids which emerged in separate boxes, rate was highest for *S. erythromera* (mean% = 1.1) compared to *A. tabida* (mean % = 0.13) and ranged from 0 to 43% for *S. erythromera* and 0 to 6% for *A. tabida* (Fig. 7.1.3). *L. heterotoma* and *T. modesta* only emerged in 1 sentinel trap each out of 72, *S. erythromera* emerged from 6 and *A. tabida* emerged from 2. For comparison, during laboratory experiments testing *T. drosophilae* parasitization of *D. suzukii* pupae, the daily mean parasitization rate was recorded at 9.47 % per female when 30 host pupae were provided daily. In that instance though, the parasitization rate was calculated as the total number of pupae used by parasitoids (including those successfully emerging and failing to emerge) divided by the total number of host pupae available (Cheng et al., 2017).

Over the period of collections, July to September, the rate of *D. suzukii* parasitism varied for all parasitoid species recorded, peaking 10 August for *S. erythromera,* and *A. tabida* (6.2 and 1% respectively). In 2018, *S. erythromera* and *A. tabida* were found from June to August. In 2020 *L. heterotoma* and *T. modesta* were only found once, on separate collection dates (01 and 21 September respectively). In 2018, *L. heterotoma* was also recorded once; 18 June.

D. suzukii emergence was significantly lower overall in treatment boxes compared to control (mean = 68.9 and 87.5 respectively (P= 0.029). Fruit was in a similar condition upon collection and both types of boxes were maintained under the same conditions during the 6-week emergence period. It is possible that the higher *D. suzukii* mortality than expected by emerged parasitoids was due to parasitoids that failed to emerge (or be recovered), or stings from parasitoids attempting to lay eggs. More research is needed to confirm these interactions.

Comparing the same sites between survey years, the most common species at both sites in 2017 and 2018 was *P. vindemmiae*, but surprisingly none were found in 2020. Previously numbers of this species at the woodland site varied between 2017 and 2018 (8.9 and 2.82 respectively) but remained consistent at the hedgerow. Average regional temperature during this period was similar between years (Fig. 7.1.6). The cause of *P. vindemmiae* absence in 2020 is unclear. The next most abundant species per trap in 2020 has been *S. erythromera*, then *L. heterotoma* and *A. tabida*, with numbers per trap of all three species similar between years. *S. erythromera* was found at both sites every year, whereas *L. heterotoma* was only in the woodland. 2020 was the first year *A. tabida* was found in the hedgerow. The only previous recording of *T. prema* was in the hedgerow 2018. *T modesta* has only been found in the hedgerow 2020.

Of the species recorded and studied during our surveys, so far *P. vindemmiae* and *S. erythromera* have shown most promise for biocontrol against populations of *D. suzukii* in the UK. Both species were shown to be active from May to October. With *P. vindemmiae* the most frequently found parasitoid in both agricultural and semi-natural habitats. *S. erythromera* was collected in relatively high numbers in 2018 from the sentinel traps from all habitats and its occurrence was consistent over the cropping season. In laboratory cultures of *D. suzukii, P. vindemmiae* produced most offspring per parent (mean = 3.6) compared to *S. erythromera* (mean = 0.2) and *L. heterotoma* (mean = 0.1). *T. prema* could not be tested because these died soon after emergence. Unlike *P. vindemmiae*, *S. erythromera* does not hyperparasitize other parasitoid wasps and therefore may provide a more viable tool in controlling *D. suzukii*.

The two *T. modesta* found in 2020 were females. *T. modesta* is a west European species, native to Britain and found locally in England as far north as Lancashire and known to be a parasitoid of *Drosophila* spp. Elsewhere *T. modesta* is reported to be less adapted to parasitize *D. suzukii* and has a markedly longer developmental time than *T. drosophilae* (Trivellone et al., 2020).

T. drosophilae is commercially available in Europe to be used in IPM contexts but remains, at the time of writing, unidentified in the UK and hence cannot be released as a biocontrol agent.

Conclusions

- During the NIAB EMR survey in 2020, which looked for native parasitoids with potential for *D. suzukii* biocontrol, four parasitoid species were identified from *D. suzukii* sentinel traps.
- These included three found by NIAB EMR in previous survey years and the first recording of *T. modesta.*
- Since the surveys began in 2017, six native parasitoid species have been recorded emerging from *D. suzukii.*
- The most common species in 2020 was the pupal parasitoid *S. erythromera* with a mean parasitism rate of 1.1% (range 0 to 6%), which peaked August. At the same two sites, *S. erythromera* has been recorded in consistent numbers per trap every survey year. In the laboratory the parasitization rate of *T. drosophilae* has been calculated at 9.47 % per female when 30 *D. suzukii* pupae were provided daily (Cheng et al., 2017). However, this calculation includes pupae from which parasitoids failed to emerge which could not be confirmed and therefore included in our calculation.
- In 2017 and 2018, the pupal parasitoid, *P. vindemmiae,* was most common, but none were recorded in 2020.
- So far *P. vindemmiae* and *S. erythromera* have shown most promise for *D. suzukii* biocontrol; both are active from May to October and completed development to adulthood in lab cultures of *D. suzukii* with mean offspring per parent 3.6 and 0.2, respectively. Unlike *P. vindemmiae, S. erythromera* does not hyperparasitize.
- Larval parasitoids *L. heterotoma*, *A. tabida*, *T. prema,* and *T modesta* are less successful at parasitising *D. suzukii*.
- *T. drosophilae* is commercially available in Europe to be used in IPM contexts but remains, at the time of writing, unidentified in the UK and hence cannot be released as a biocontrol agent.

Task 7.3. Investigating UK waste fruits as a potential source of parasitoids to control Drosophila suzukii in neighbouring crops (NIAB)

Introduction

Waste fruit collected at UK soft fruit farms offers a potential source of parasitoids which could be used for biological control of *Drosophila suzukii* in and around the crop. In 2017 and 2018, NIAB EMR conducted field surveys in ten fruit crops and wild sites across South East England to record native parasitoids capable of parasitizing *D. suzukii.* Using sentinel traps containing fruit infested with *D. suzukii* larvae and pupae to attract parasitoid egg laying, five native species of parasitoid Hymenoptera were recorded from *D. suzukii*: two species of larval parasitoids (*Asobara tabida* and *Leptopilina heterotoma*) and three pupal parasitoids (*Pachycrepoideus vindemmiae*, *Spalangia erythromera* and *Trichopria prema*). All five species are generalist parasitoids of *Drosophila* spp. If managed effectively, waste fruit could be used to provide a source of parasitoids to control *D. suzukii* without releasing *D. suzukii* into the crop.

Aims

- Investigate whether UK waste fruit bins are a source of *D. suzukii* parasitoids
- If so, calculate the number of each parasitoid species per kg of waste fruit
- Report findings of the parasitoid, *Trichopria drosophilae* to the AHDB

Materials and Methods

Trial sites: Waste fruit was collected from soft fruit farms in Kent; including those where relatively high numbers of parasitoids were recorded during the 2017 and 2018 surveys. Three varieties of waste fruit were collected; raspberry, cherry, and strawberry. Raspberries and strawberries were grown in ~8 metre polytunnels with insect-proof mesh at either end, and cherries were grown in tunnels with anti-bird mesh. Crop husbandry involved standard grower practices including spray schedules for pests (Appendix A 7.3.1).

Waste fruit collections: For each soft fruit variety, waste fruit was collected on at least two separate occasions between June and September 2020; the period when highest numbers of parasitoids emerged in sentinel boxes during the 2017 and 2018 surveys (Table 7.3.1). Upon collection, waste fruit was combined from multiple waste bins (when possible) and brought back to NIAB EMR. Up to 8 kg was evenly distributed into a single layer (to facilitate adult emergence), originally in trays (42 x 42 x 4 cm, at ~2kg / tray) (Fig 7.3.1), then in Perspex boxes (10 x 10 x 20 cm, at ~320g / box) from 08 September where humidity could be maintained more easily (Fig. 7.3.2). Both were lined with a layer of blue roll, kept damp by adding water regularly. Trays were transferred to a Bugdorm (50Lx50Wx50H cm) with mesh holes <0.5mm and large Perspex boxes were sealed with a lid with mesh holes <0.5mm, to prevent parasitoid escape. Both were covered in ventilated plastic bagging to maintain humidity.

Table 7.3.1 Soft fruit variety and waste fruit collection date during the parasitoid waste fruit survey 2020.

Soft fruit variety (cultivars)	**Waste fruit collection date**
Raspberry (Kweli & Driscoll's Maravilla)	19-Jun-20
Cherry (Merchant, Simone, Summit, Canada Giant, Korvic, Kordia, Skeena, Regina, Karina)	06-Jul-20
Raspberry (Driscoll's Riviera, Driscoll's Maravilla)	17-Jul-20
Cherry (Merchant, Simone, Summit, Canada Giant, Korvic, Kordia, Skeena, Regina, Karina)	21-Jul-20
Raspberry (Driscoll's Maravilla)	08-Sep-20
Strawberry (Driscoll's Katrina)	09-Sep-20
Raspberry (Driscoll's Maravilla)	16-Sep-20
Strawberry (Driscoll's Katrina)	16-Sep-20

Figure 7.3.1 Bugdorm emergence cages used during the parasitoid waste fruit trial 2020.

Bugdorm (50 x 50 x 50 cm) contains tray (42 x 42 x 4 cm) with a single layer of waste fruit (~2kg) on dampened blue roll. Humidity was maintained by storing Bugdorms in ventilated plastic bagging and dampening blue roll under fruit and on Bugdorm floor regularly.

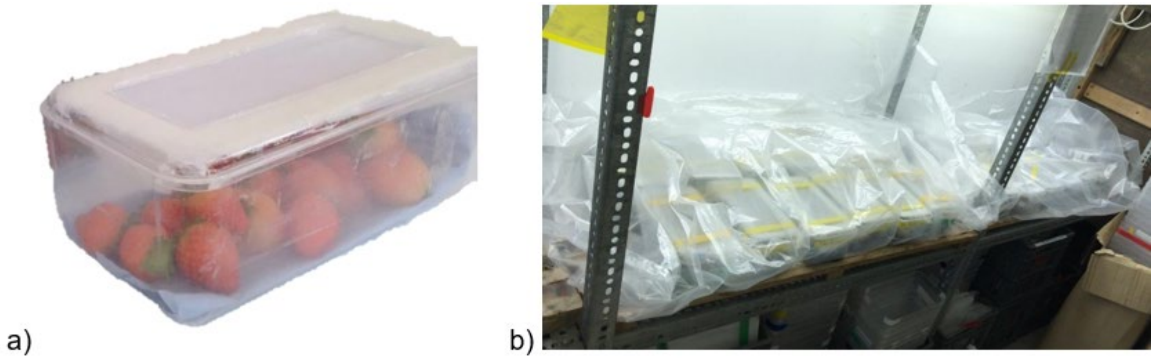


Figure 7.3.2 a) Perspex box emergence cage used during the parasitoid waste fruit trial 2020. Perspex box (10 x 10 x 20 cm), contains a single layer of waste fruit (~320g) on dampened blue roll; b) Humidity was maintained by storing boxes in ventilated plastic bagging and dampening blue roll regularly.

Assessments: Emergence cages and respective waste fruit were inspected the day of collection for parasitoids, adult *D. suzukii* and other fruit fly species. If present, insects were transferred to tubes containing ethanol, then later identified and numbers recorded.

After the initial inspection, emergence cages were incubated under ambient laboratory conditions ~22 °C, ~50% relative humidity and 16:8 hours light:dark photoperiod and examined weekly. Parasitoids, adult *D. suzukii* and other fruit fly species were collected, identified, and recorded during the six-week period.

Results

From the eight waste fruit collections, only a single parasitoid emerged (Family: Branconidae) from a raspberry sample collected on 17 July; 0.2 parasitoid kg^{-1} (Table 7.3.2).

D. suzukii emerged from all waste fruit collections, but numbers varied between collections (Table 7.3.2). The highest number emerged (per kg of waste fruit) was from raspberry collected 17 July and the lowest from cherry collected 06 July (total = 351.5 and 0.1 respectively). The raspberry collection from which the highest number of *D. suzukii* emerged (17 July) was also the only waste fruit collection from which a parasitoid emerged.

Other *Drosophila* spp. also emerged from all waste fruit collections, but numbers varied between collections (Table 7.3.2). The highest number emerged per kg of waste fruit was from strawberry collected 09 September and the lowest from cherry collected 21 July (total = 353.3 and 0.1 respectively). Other Drosophila species emerged included: *Drosophila melanogaster, Drosophila simulans* and *Drosophila subobscura*.

Table 7.2.2 Waste fruit collection and total number of adult parasitoids, *D. suzukii* and other *Drosophila* spp. emerged per kg during the parasitoid waste fruit survey 2020.

Collection	Waste fruit	Date collected	Total parasitoids / kg	Total *D. suzukii* / kg	Total other Drosophila spp. / kg
1	Raspberry	19.06	0.0	29.4	1.6
1	Cherry	06.07	0.0	0.1	0.2
2	Raspberry	17.07	0.2	351.0	0.9
2	Cherry	21.07	0.0	0.3	0.1
3	Raspberry	08.09	0.0	0.5	0.3
1	Strawberry	09.09	0.0	9.7	353.3
4	Raspberry	16.09	0.0	0.5	5.3
2	Strawberry	16.09	0.0	3.9	71.0

Discussion

From this pilot study, we have shown that waste fruit is unlikely to be a significant source of parasitoids. The one collection with parasitoid emergence was collected in July; the time of year when highest numbers of parasitoids emerged during the 2018 wild habitat survey. The parasitoid that emerged was from the Braconidae family. Waste fruit collections with no parasitoid emergence included raspberry (three other collections), cherry (two collections) and strawberry (two collections). *T. drosophilae* was not found during this waste fruit survey.

D. suzukii emerged from all waste fruit collections, but numbers varied. The highest number that emerged per kg of waste fruit was from raspberry collected 17 July (total = 350.97), corresponding with emergence of the only parasitoid found (total = 0.2). Less than 10 *D. suzukii* emerged from each of the other seven waste fruit collections when there was also no parasitoid emergence.

Other *Drosophila* spp. also emerged from all waste fruit collections, but numbers varied between collections. The highest number that emerged was from strawberry in September (total = 353.3) and the lowest from cherry in July (total = 0.1). Other *Drosophila* species that emerged included: *Drosophila melanogaster, Drosophila simulans* and *Drosophila subobscura*.

Low insect emergence from waste fruit during this survey is likely due to chemical plant protection product (PPP) application and crop hygiene. Chemical PPPs (including Exirel and Tracer) were applied at some sites to control *D. suzukii* (Appendix 7.3.1) and are known to affect parasitoids. For example, spinosyns cause high *Pachycrepoideus vindemmiae* mortality rates. Neonicotinoids (Calypso), organophosphates, and pyrethroids (Pyrethrum) also cause high mortality rates regardless of parasitoid species (Schlesener et al., 2019). Farms were also following current Best Practice hygiene methods / Good Agricultural Practices (Hooper and Grieshop, 2020) by regular removal and disposal of waste fruit, reducing opportunity for *D. suzukii* egg laying and subsequent parasitism of larvae and pupae.

Conclusion

Considering the low recovery of parasitoids from waste fruit during this survey, currently waste fruit is not worth pursuing as a method to boost parasitoids on farms.

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), sub-lethal doses of commonly used plant protection products were applied to laboratory strains of *D. suzukii* and the impact these had on mortality, oviposition rate and offspring survival evaluated. The Lethal Concentration to kill 50% of the population (LC50) were identified for each of the products, ensuring future comparisons could be made: a vital tool in resistance monitoring. It was also apparent that there were variations in tolerance within laboratory populations, with some females surviving high doses of products and then continuing to egg lay, with no detrimental effect on offspring survival. For females treated with 100% field rate of spinosad there was low survival with minimal egg laying however these eggs did not survive through to next generation emergence. As this work was performed on laboratory strains established in 2013, the survival response is expected to be lower than that of wild *D. suzukii* populations, which would have had some contact with plant protection products and therefore can develop a tolerance. Within this objective, results of both the laboratory strain (from Shaw, 2019) and wild populations established within 2019 and 2020 are discussed in relation to the variation in tolerance between strains and to different pesticides.

Year 1

In year 1 (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.

Cyantraniliprole treated flies had the fewest significant differences between laboratory and field *D. suzukii* strains. To date there has been no reports of resistance or tolerance increases to cyantraniliprole. However it has not been used to control *D. suzukii* for as long as the other two products, with emergency approval granted in 2016. There were mininal difference between the lab and wild strains with only WS2 having significanlty higher survival than the lab strain at 6, 12 and 25% of the field rate of cyantraniliprole. There were several differences between the wild strains, with WS2 having a higher tolerance than the other two.

There was no significant difference in survival between the laboratory strain and WS3 when treated with doses of spinosad. WS1 had significanlty higher survival than the lab strain at 12% of the field rate. WS2 high higher survival than the lab at 12-50% and than WS3 at 25 and 50% field rate: the two highest doses applied to the field strains. From the spray records we would have expected WS3 to have the higher survival due to higher insecticidal inputs however this was not the case.

For lambda-cyhalothrin there were several differences in survival between the laboraotry and wild strains at several doses but in all cases survival was lower in the lab strain than the wild. Between 30 and 50% survival occurred in WS1 when treated with the full field rate of lambda-cyhalothrin. To our knowledge this is the first documentation of an increased tolerance to lambda-cyhalothrin. There were significant differences in the susecptibilty of the wild strains with lower survival in WS2 and WS3 than WS1 strain when treated with 50% of the field rate.

This was unexpected as the WS1 population had low insecicidal input prior to strain establishment than the other two strains.

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,

Aim

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.

Materials and Methods

Wild strain collection 2020: Ripe waste fruit was collected from commercial field sites in Kent (Table 8.1.1) in July 2020 (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) 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 sugar 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. Six males and 6 females were transferred to the Petri dish. The Petri dish (spray arena) was then covered with a 4 mm mesh to prevent flies escaping. Flies could recover for a minimum of 10 minutes before 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,
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

The maximum field rate (FR) dose for cherry or strawberry 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. 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, flies were transferred to a glass vial containing *Drosophila* media and returned to the previously stated environment conditions.

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) Lightly moribund 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. Comparisons will be made between the LC50 value of each strain between years and early vs. end season populations.

Results

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

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. However, the raw data is presented for the morality results of the early season strains; statistical analysis is still to be done but cannot be finalised until the end of season populations have been assessed.

The end-of-season population has been established and are in their 2-3 generations within the lab (as of Feb 2021). It is estimated that lab trials on these strains will be executed in March as originally predicted.

WS1- early 2020 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 waste fruit collected in July which enabled rapid population increase in the lab. Roughly 25% of WS1 flies treated with the highest dose (25% of field rate) of cyantraniliprole survived 24 hours post application (Figure 8.1). This is consistent with the results from 2019 for this insecticide and strain. For WS1 treated with spinosad 100% mortality occurred in the highest dose (50% of field rate) (Figure 8.2). 8% of WS1 flies treated with 100% field rate of lambda-cyhalothrin (the highest dose) survived 24 hours post application (Figure 8.3).

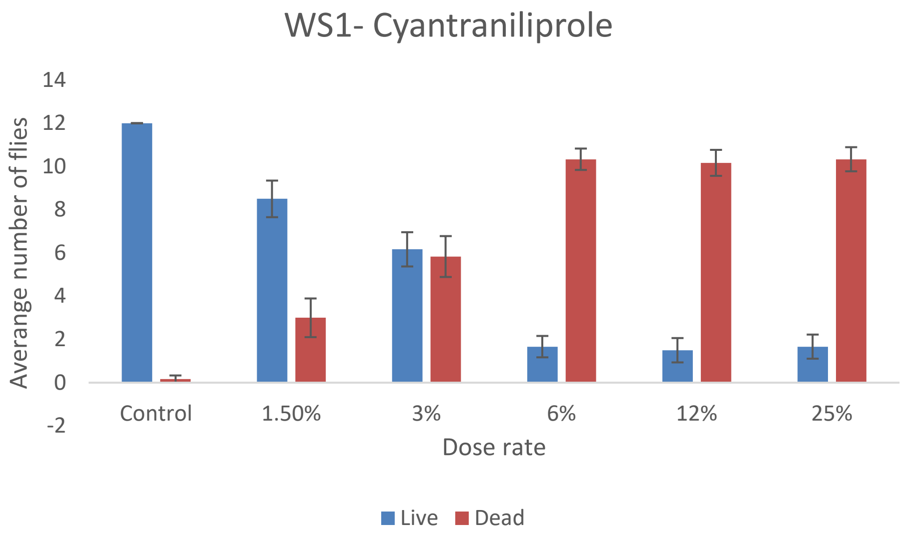


Figure 8.1. Average number of WS1 flies live (blue) or dead (orange) after 24 hours post spray treatment (+/- standard error) with cyantraniliprole. Treatments are displayed as a percentage of the recommended field rate.

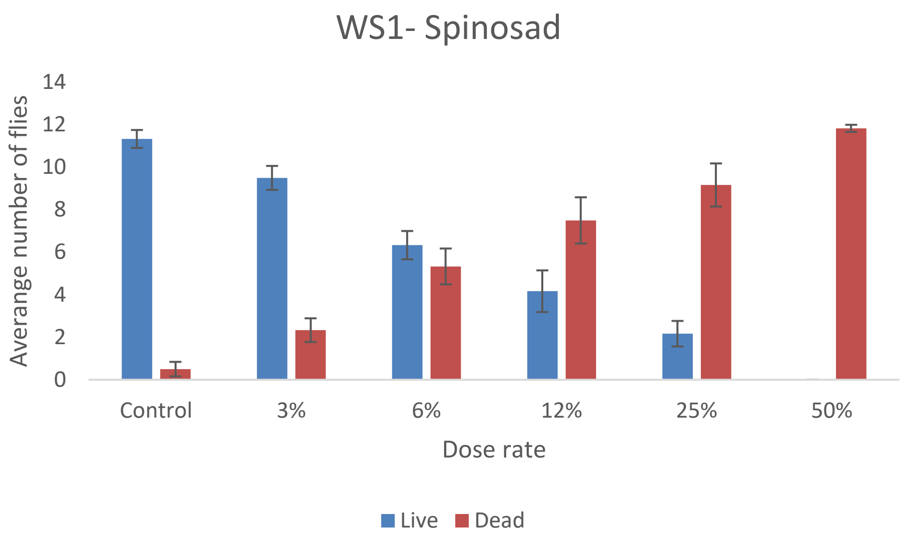


Figure 8.2. Average number of WS1 flies live (blue) or dead (orange) after 24 hours post spray treatment (+/- standard error) with spinosad. Treatments are displayed as a percentage of the recommended field rate.

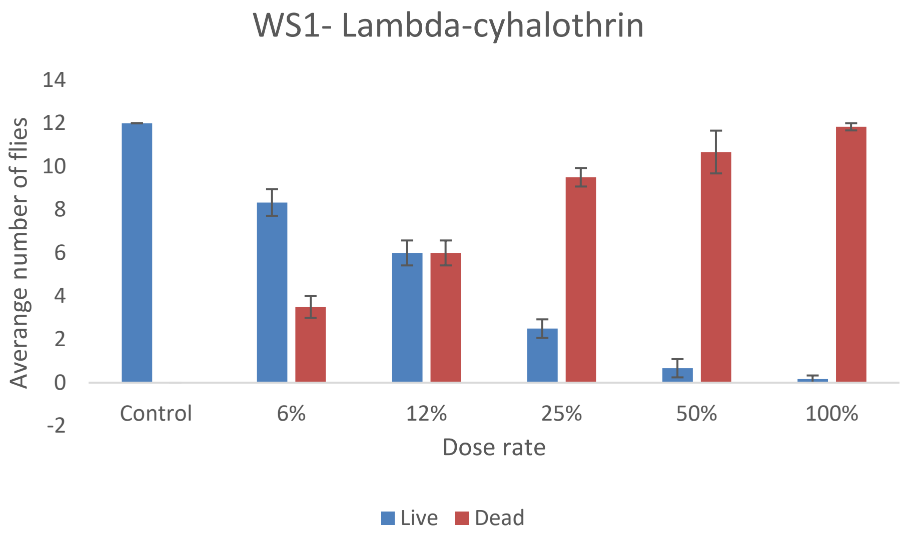


Figure 8.3. Average number of WS1 flies live (blue) or dead (red) after 24 hours post spray treatment (+/- standard error) with lambda-cyhalothrin. Treatments are displayed as a percentage of the recommended field rate.

WS2- 2020 early season population mortality

The wild strain established from WS2 displayed a small level of survival when flies were treated with the highest dose of spinosad (50% of the field rate).

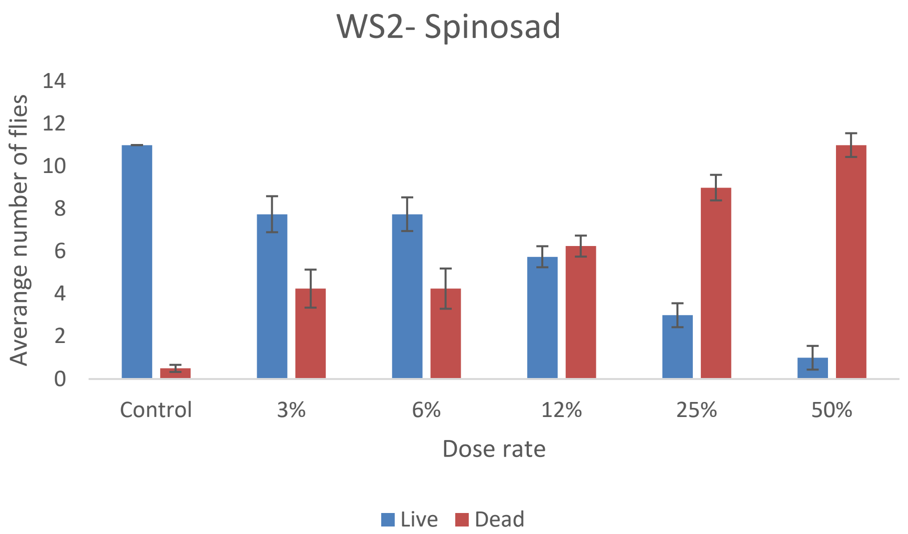


Figure 8.4. Average number of WS2 flies live (blue) or dead (red) after 24 hours post spray treatment (+/- standard error) with spinosad. Treatments are displayed as a percentage of the recommended field rate.

WS3- 2020 early season population mortality-

WS3 early season populations struggled to establish initially due to low number of SWD in the collected fruit. Due to this limitation in population size only one insecticide has been applied in the lab to date. A figure based on the raw data is displayed below for WS3 flies treated with spinosad (Figure 8. 5). The high control mortality in this trial (25%) indicated that the fly health may have not been optimum. When assessing the trial, it was noted that there were high numbers of flies with wings stuck onto the media. This can be as the result of the media not setting correctly or humidity being too high in the control temperature cabinet. If the flies get stuck on their backs by the wings this results in starvation and death. Due to the high control mortality in this bioassay, it will be repeated and the data presented below will not be included in future analysis.

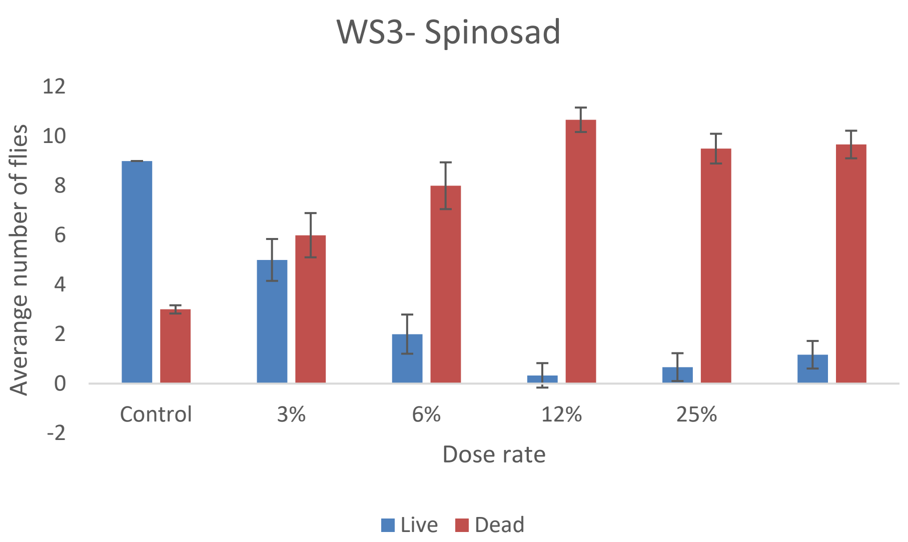


Figure 8.5. Average number of WS3 flies live (blue) or dead (red) after 24 hours post spray treatment (+/- standard error) with spinosad. Treatments are displayed as a percentage of the recommended field rate.

Spinosad- between years

When looking at the difference in mortality, there were significant differences in the survival of the strains collected in 2020 and 2019 from all three locations (all $p<0.0001$) (Figure 8.6) when treated with spinosad. There was a significant reduction in survival in 2020 compared to 2019. There were no significant differences between years for specific doses.

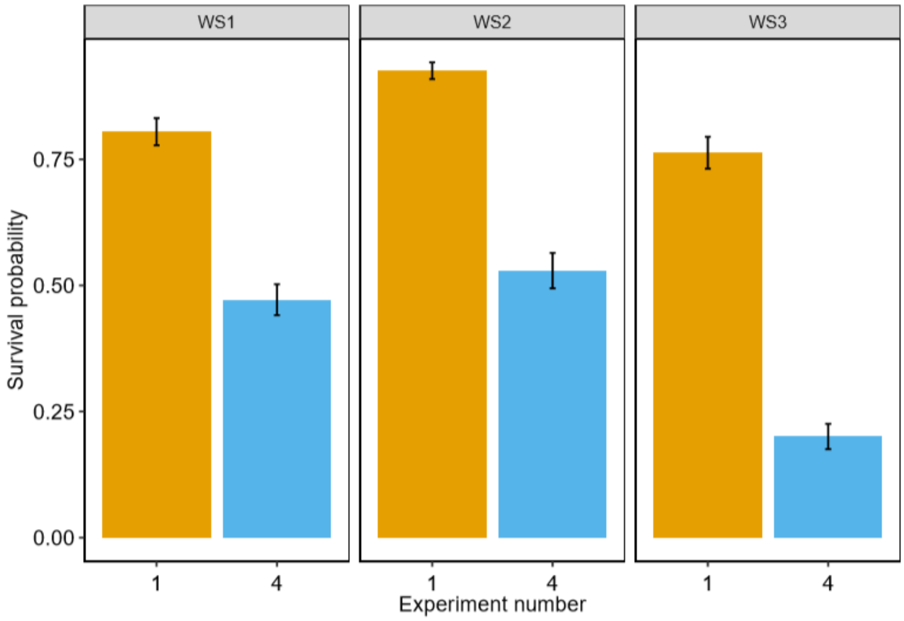


Figure 8.6. Survival probability of strains collected in 2019 (orange bars) and strains collected in 2020 (blue bars) from the three locations (NIAB EMR =WS1, WS2 and WS3) treated with Spinosad.

Cyantraniliprole- between years

For the NIAB EMR strain (WS1) there was no overall difference in survival between the two years (Figure 8.7) 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.

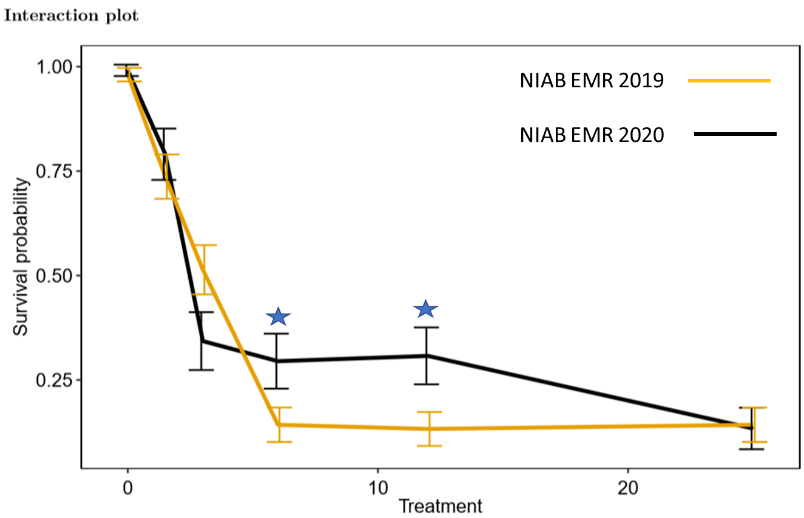


Figure 8.7. Survival probability of NIAB EMR (WS1) strains established in 2019 (yellow line) and 2020 (black line) when treated with percentage doses of cyantraniliprole field rate. * indicate significant differences.

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 8.8.) when treated with lambda-cyhalothrin. There was no significant difference between years based on doses of lambda-cyhalothrin.

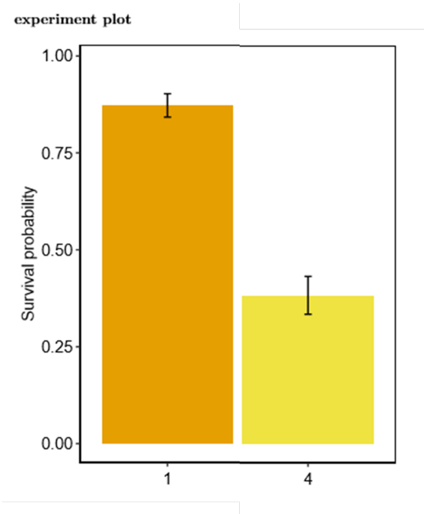


Figure 8.8. Survival probability of NIAB EMR (WS1) collected in 2019 (orange bar) and strains collected in 2020 (yellow bar) when treated with lambda-cyhalothrin.

Discussion

The survival probability of the wild strains between years, showed 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. The results of the 2020 'end of season' strain mortality assessments should provide more evidence to support or disprove this theory. Due to the time needed to generate sufficient populations to execute the bioassay this is expected to be completed by March end 2021.

For the stains established from NIAB EMR (WS1) there was a significant difference in survival at 6 and 12% of the field rate of cyantraniliprole. The strain established in 2020 had a higher survival than the 2019 established strain. Although this does not confirm resistance development, it does appear there is a slight increase in tolerance. The LC50 analysis will indicate if this is the case. This will be performed once the end of season strains have been analysed. To date there have been no reports of resistance developing to cyantraniliprole for SWD in other parts of the world.

As for all these insecticides, a greater understanding of any variation in tolerance/ susceptibility is likely to be highlighted once the end of season strains have been treated. At this point the LC50 analysis will be performed and will give a clearer summary of any changes between years and season.

Conclusions

- To date no reports of insecticide resistance have been detected outside the USA
- Our data shows the first record of variation in susceptibility to cyantraniliprole

Acknowledgements

We would like to thank the funders of the research, AHDB Horticulture, for their support. We would also like to thank all growers for the use of their crops and Berry Gardens for continued support in sourcing sites. We also thank the technicians at NIAB EMR for help with treatment application and data gathering and Greg Deakin for his advice on the statistics used.

Knowledge and Technology Transfer

2017

Fountain: 12-13 Jan 2017 - Bioline AgroSciences – Paris. *D. suzukii* research at NIAB EMR

Fountain: 16 Feb 2017 - Scottish Society for Crop Research, James Hutton Institute, Soft Fruit Information Day, Winter Meeting - Spotted Wing Drosophila – an update on research in the UK

Fountain: 28 Feb 2016 - EMR Association/AHDB Horticulture Tree Fruit Day, Technical Up-Date on Tree Fruit Research, East Malling, Kent, Year-round IPM for *D. suzukii*

Fountain: 6-7 June 2017, 1-day *D. suzukii* meeting in Belgium: invitation: *D. suzukii* Workshop

Fountain: 16-20 July 17 - The Fourth International Horticultural Research Conference, NIAB EMR UK – Poster: Winterform *Drosophila suzukii* gut contents

Fountain: 25 Jul 2017 - Research update to the BGG Grower Research Advisory Panel

Dolan: July 2017 - Fruit for the Future Event at the James Hutton Institute Presentation on *D. suzukii,* identification and testing methods

Cannon & Rogai: 13 Sep 2017 - AHDB Agronomist day at NIAB EMR, Update on *D. suzukii* research

Fountain: 6 Sep 17 - Tomato Growers Association Technical Committee meeting - Integrated Pest Management

Fountain: 16 Nov 17 - Berry Gardens Growers Ltd Annual Technical Conference, - Latest *D. suzukii* research and Reducing insect populations through new generation polythene tunnel

Fountain: 21 Nov 2017 - EMR Association/AHDB Soft Fruit Day, Technical Up-Date on Soft Fruit Research, Orchards Events Centre, NIAB EMR, Kent, The latest research into *D. suzukii* control

2018

Publications

Shaw, B. Brain, P. Wijnen, H. Fountain, M. T. (2018). "Reducing Drosophila suzukii emergence through inter-species competition." Pest management science 74(6): 1466-1471.

Shaw, B. Wijnen, H. Fountain, M. T. (2018). "Recording and reproducing the diurnal oviposition rhythms of wild populations of the soft- and stone- fruit pest Drosophila suzukii." PLoS ONE 13(10): e0199406.

Presentations

Fountain: 31 Jan 18 - Rothamsted Research BCPC Pests and Beneficials Review - Successful application of biocontrols in outdoor horticultural crops

Dolan: February 2018 - Poster presentation at the SSCR/Bulrush Horticulture Ltd joint winter meeting held near the James Hutton Institute in Scotland

Cannon: 22 Feb 18 - AHDB/EMR Association Tree Fruit Day - *D. suzukii* Research up-date on 2017

Cannon, Rogai & Fountain Feb 18 ARTIS course, training the vine industry on *D. suzukii* management in vineyards

Fountain: 19 Jan 18 Talk to Tracey Crouch MP on *D. suzukii*

Fountain: 09 Feb 18 Hutchinson's Annual Conference. Whittlebury Hall in Northamptonshire. Led an open floor discussion on *D. suzukii*

Fountain: 14 Aug 18 East Kent Fruit Society. WALK OF THE WINNING TOP FRUIT ORCHARD AT A C HULME & SONS ON TUESDAY *D. suzukii* update

Shaw: 8-12 Oct 18 SWD awareness tour of Australia. Talk titled 'SWD: lessons from the UK'.

Fountain: 17 Oct 18 RHS Wisley, *D. suzukii* talk to professionals at RHS

Fountain: 06 Dec 18 Berry Gardens Research and Agronomy Conference, RESEARCH AND AGRONOMY CONFERENCE Latest *D. suzukii* Research

Rogai, Noble, Shaw, Faulder, Jones: 21 Nov 2018 EMR ASSOCIATION/AHDB SOFT FRUIT DAY, Technical Up-Date on Soft Fruit Research, *D. suzukii* – National monitoring and spray intervals, *D. suzukii* – The use of bait sprays for control, *D. suzukii* – Exploiting activity patterns for its control, *D. suzukii* – Optimising attractants and repellents for use in control strategies, *D. suzukii* – Developing attractive yeast strains for attraction and control.

2019

Publications

Noble R, Dobrovin-Pennington A, Phillips A, Cannon MFL, Shaw B, Fountain MT (2019) "Improved insecticidal control of spotted wing drosophila (Drosophila suzukii) using yeast and fermented strawberry juice baits." Crop Protection doi.org/10.1016/j.cropro.2019.104902

Shaw, B., Brain, P. Wijnen, H. Fountain, M. T. (2019). "Implications of sub-lethal rates of insecticides and daily time of application on Drosophila suzukii lifecycle." Crop Protection 121: 182-194.

Shaw, B., Hemer, S. Cannon, M. F. L. Rogai, F. Fountain, M. T. (2019). "Insecticide Control of Drosophila suzukii in Commercial Sweet Cherry Crops under Cladding." Insects 10(7): 196.

Shaw, B., Cannon, M. F. L. Buss, D. S. Cross, J. V. Brain, P. Fountain, M. T. (2019). "Comparison of extraction methods for quantifying Drosophila suzukii (Diptera: Drosophilidae) larvae in soft- and stone-fruits." Crop Protection 124: 104868.

Shaw, B. Fountain, M. T. Wijnen, H. (2019). "Control of Daily Locomotor Activity Patterns in Drosophila suzukii by the Circadian Clock, Light, Temperature and Social Interactions." Journal of Biological Rhythms 34(5): 463-481.

Presentations

JHI: Fruit for the Future event held in July 2019 at The James Hutton Institute, stakeholders were reminded to remain vigilant for the presence of *D. suzukii*. Advice and practical demonstrations on identification and testing methods were provided.

JHI: Testing of fruit was provided at a drop-in clinic and results were fed-back confidentially.

JHI: Regular updates on the monitoring data were given to Scottish Government via RESAS reporting.

Shaw/Powell: 29/30 Jan 19 Agrovista grower seminar 'Update on SWD control'

Shaw 11-13 Nov 19 travelled to Lepe, southern Spain to provide D. suzukii advice to the grower consortium Onubafruit.

Shaw 25-29 Nov 19 Attended IOBC conference in Serbia and presented poster 'Implications of sub-lethal rates of insecticides on Drosophila suzukii life stages'

Fountain 20 May 19, 25 Dutch companies, NIAB EMR, WET Centre, SWD and pollinators

Fountain (invited speaker) 26-28 Jul 19, "IV Berries Festival" SERIDA Villaviciosa (Principality of Asturias, Spain) "Control strategies for *Drosophila suzukii*"

Fountain 23 May 19, NIAB EMR WET Centre, NIAB Board Meeting, SWD research

Fountain 11 Sep 19, AHDB Fruit Agronomists' Day, NIAB EMR, Bait v overall sprays (SF/TF 145a)

Fountain 08 Oct 19, Agrii Fruit, Throws Farm Essex. SWD, aphid control and forest bug

Fountain 14 Nov 19, Berry Gardens Growers Conference 'New advances in SWD management and controls',

Fountain 20 Nov 19, AHDB/EMR Association Soft Fruit Day at East Malling.

2020

Publications

Jones R, Fountain MT, Günther C, Eady P, Goddard M (2021) Separate and combined volatile profiles produced by *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* yeasts are attractive to *Drosophila suzukii* in the laboratory and field", Scientific Reports

Presentations

Fountain 13 Jan 20, Agrovista Grower Day, Black Horse Inn, Pilgrims Way, Thurnham, Maidstone, SWD, aphid control and forest bug

Fountain 28 Jan 20, Agrovista Grower Day, White Lion, The Street, Selling, Faversham, SWD, aphid control and forest bug

Fountain 27 Feb 20, AHDB/NIAB EMR Tree Fruit Day, East Malling, Kent, SWD – The search for new repellents and SWD – Protecting natural enemies

Fountain 29 Jul 20 Katrina Hayer's visit BBSRC – Entomology research at NIAB EMR

Fountain 11 Nov 20 Integrated pest management control update to Berry Gardens Annual Research and Agronomy Conference

Fountain 15-18 Nov 20 Entomological Society of America 2020 Symposium Proposal Orlando, FL WEBINAR - "Interspecific signals to deter oviposition by spotted wing drosophila (Drosophila suzukii, SWD),"

Shaw 14 Aug 20 Cesar and HortInnovate Australia "SWD- lessons from the UK"

Shaw 6 Nov 20 AHDB tree-fruit panel " SF 145a Research Update" and "Future research"

Shaw 2 Dec 20 AHDB soft-fruit panel " SF 145a Research Update" and "Future research"

18 Nov 20 AHDB "Soft Fruit Technical Day"

- SWD – The search for new repellents (Christina Conroy, NIAB EMR and Greenwich NRI)
- SWD – Developing the use of bait sprays (Ralph Noble, Microbiotech)
- SWD – Reducing overwintering populations (Adam Walker, NIAB EMR)

25 Feb 20 AHDB "Tree Fruit Technical Day"

- SWD – The search for new repellents (Christina Conroy, NIAB EMR and Greenwich NRI)
- SWD – Developing the use of bait sprays (Ralph Noble, Microbiotech)
- SWD – Reducing overwintering populations (Adam Walker, NIAB EMR)

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Appendix 7.3.1

Spray Schedule raspberry 1st collection

BD7 Raspberries Kweli 3.30 ha Projected Harvest Date: 31/12/2020 EHD: 11/10/2020 14:30

Activity Date / Timing	Operation	Product	HI	Rate	Reason	Water	Area	Operator	Machine
09/01/2020	Spraying #67	Kerb Flo (13716) Propyzamide (400 g/l)	42 days	1.500 lts/ha	Weed control	400.00 lts/ha	3.30 ha		Weed killing
		Flexidor (18042) Isoxaben (500 g/l)		0.500 lts/ha	Weed control				
27/04/2020	Spraying #72	Switch (15129) Cyprodinil (37.5 % w/w); Fludioxonil (25 % w/w)	14 days	1.000 kgs/ha	Botrytis	1,000.00 lts/ha	3.30 ha		DUO PROP
20/05/2020	Spraying #77	Calypso (11257) Thiacloprid (480 g/l) EAMU: 2139/14	3 days	0.250 lts/ha	Leaf curling midge	1,000.00 lts/ha	3.30 ha		DUO PROP
		Omex SW7 (MBS119) Silicate		0.400 lts/ha	Adjuvant				
02/07/2020	Spraying #91	Hallmark With Zeon Technology (12629) Lambda-cyhalothrin (100 g/l) EAMU: 0728/06	28 days	0.075 lts/ha	Leaf Curling Midge	1,000.00 lts/ha	3.30 ha		DUO PROP
		Omex SW7 (MBS119) Silicate		0.400 lts/ha	Adjuvant				
02/08/2020	Spraying #92	Calypso (11257) Thiacloprid (480 g/l) EAMU: 2139/14	3 days	0.250 lts/ha	Leaf curling midge	1,000.00 lts/ha	3.30 ha		Antis 1000
		Omex SW7 (MBS119) Silicate		0.400 lts/ha	Adjuvant				
08/09/2020	Spraying #94	Signum (11450) Pyraclostrobin (6.7 % w/w); Boscalid (26.7 % w/w) EAMU: 2102/10	3 days	1.250 kgs/ha	Botrytis	1,000.00 lts/ha	3.30 ha		Antis 1000
18/09/2020	Spraying #96	Tracer (12438) Spinosad (480 g/l) EAMU: 1207/18	1 days	0.200 lts/ha	SWD	1,000.00 lts/ha	3.30 ha		DUO PROP
		Teldor (11229) Fenhexamid (50 % w/w)	1 days	1.000 kgs/ha	Botrytis				
10/10/2020	Spraying #97	Teldor (11229) Fenhexamid (50 % w/w)	1 days	1.000 kgs/ha	Botrytis	1,000.00 lts/ha	3.30 ha		DUO PROP

HARVEST INTERVAL CHECK FORM

Field name / planting	Product	Application		Harvest Interval	First available harvest		Actual harvest		Picking authorised by
		Date	Time		Date	Time	Date	Time	
Jubilee 2	PARAAT	19/02	09:00	35	26/03	09:00			
	Decis	26/4	16:30	7	5/5	16:30			
	Hortiphyte Maxicrop Decis	29/4	16:00	7	6/5	16:00			
	Pyrethrum Hortiphyte Maxcrop	6/5	13:30	0	6/5	13:30			
	Pyrethrum 5EC	29/5	08:30	0	29/5	08:30			
	Calypso Maxicrop	7/6	04:20	3	10/6	04:20			

Doc no: QA107 | Issue No: 19 | Issue date: 31/01/2020 | Authorised by:

HARVEST INTERVAL CHECK FORM

Field name / planting	Product	Application		Harvest Interval	First available harvest		Actual harvest		Picking authorised by
		Date	Time		Date	Time	Date	Time	
Jubilee 2									
	Maxicrop SP05B Calypso	18/6	8:00	3	21/6	08:00			
	NATU RALIS	14/7	5:30	0	14/7	05:30			

Doc no: QA107 | Issue No: 19 | Issue date: 31/01/2020 | Authorised by:

Spray Schedule cherry collections

HORTICULTURAL SERVICES – PEST & DISEASE CONTROL APPLICATION FORM

APPLICATION DATE	CHEMICAL		RATE	UNIT	WATER	REASON	HID	LOT COD	TOTAL AREA TO SPRAY (ha)
07/04/2020	Signum Pollibuild Fortify	Boscalid +Pyraclostrobin 0 Copper	0.75 2.5 1	Kg Kg Litres	500 500 500	Blossom Wilt Nutrition Nutrition	7 0 0	DM 187	0.74
14/04/2020	Switch/Clayton Gear Urea Sprayguard	Cyprodinil +fludioxonil Urea di-l-p-menthene	0.2 10 0.5	Kg Kg Litres	500 500 500	Blossom Wilt Nutrition Frost Porduction	7 0 0	DM 187	0.74
27/04/2020	Switch/Clayton Gear Calypso Kristalon Red Transact	Cyprodinil +fludioxonil Thiacloprid 0 0	0.6 0.25 3 0.5	Litres Litres Kg Litres	500 500 500 500	Blossom Wilt Aphid Nutrition Water Conditioner	7 14 0 0	DM 187	0.74
29/05/2020	Gazelle/Vault Fortify Transact Oxyleze	Acetamiprid Copper 0 0	0.375 1 1 0.5	Litres Litres Litres Litres	500 500 500 500	Aphid Nutrition Wetter Water Conditioner	14 0 0 0	DM 187	0.01
11/06/2020	Exirel Switch/Clayton Gear Bio Chel Cal Oxyleze Fortify	0 Cyprodinil +fludioxonil Calcium 0 Copper	0.9 0.6 3 0.5 0.5	Litres Kg Litres Litres Litres	500 500 500 500 500	SWD Brown Rot Nutrition Water Conditioner Nutrition	7 7 0 0 0	DM 187	0.74
11/06/2020	Exirel Bio Chel Cal Oxyleze Fortify	0 Calcium 0 Copper	0.9 3 0.5 0.5	Litres Litres Litres Litres	500 500 500 500	SWD Nutrition Water Conditioner Nutrition	7 0 0 0	DM 187	0.01
19/06/2020	Tracer	Spinosad	0.25	Litres	500	SWD	7	DM 187	0.74 0.01
26/06/2020	Exirel H20 Opte Oxyleze	0 Water conditioner 0	0.9 2 1	Litres Litres Litres	500 500 500	SWD Water Conditioner Water Conditioner	7 0 0	DM 187	0.74 + 0.01
03/07/2020	Tracer Bio Chel Cal Oxyleze H20 Opte	Spinosad Calcium 0 Water conditioner	0.6 0.25 2 0.5	Litres Litres Litres Litres	500 500 500 500	SWD Nutrition Water Conditioner Water Conditioner	7 0 0 0	DM 187	0.5 + 0.01
07/07/2020	Switch/Clayton Gear Lepinox Opte Cal Oxyleze Brix Builder	Cyprodinil +fludioxonil Baccillus thuringgiensis 0 0 0	0.6 0.5 3 1 0.5	Kg Kg Litres Litres Kg	500 500 500 500 500	Brown Rot Caterpillar Nutrition Water Conditioner Nutrition	7 0 0 0 0	DM 187	0.5
16/07/2020	Tracer	Spinosad	2	Litres	500	SWD	7	DM 187	0.3
24/07/2020	Exirel	0	0.9	Litres	500	SWD	7	DM 187	0.264
05/08/2020	Tracer	Spinosad	0.25	Litres	500	SWD	7	DM 187	0.74 0.01
06/08/2020	Envidor SPO58	0 0	0.6 1	Litres Litres	500 500	Red Spider Wetter	0 0	DM 187	0.74 + 0.01

Spray Schedule raspberry 2nd to 4th collections

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
19/03/2020 14:20 - 15:00 11:First leaves separating	Spraying #17		Signum (11450) EAMU: 2110/10 Pyraclostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	1.500 kgs/ha	Cane diseases and botrytis	1,000.00 lts/ha	0.70 ha	5 kph / N 7 °C / _		Vanguard1000L, VOLCAN
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.000 lts/ha	iron and biostimulant					
			YaraVita Bud Builder FL (MBS517) Boron (30 g/l); Zinc (100 g/l); Magnesium (144 g/l); Phosphorus (50 g/l); nitrogen (69 g/l)		5.000 lts/ha	Improve bud micro nutrition to aid fruit set					
			Notes: Nozzle blue x 8, 5 bar, 6 kph, PPE full.								
26/03/2020 13:45 - 14:35 11:First leaves separating	Spraying #21		Codacide Oil (ADJ0011) Rapeseed Oil (95 % w/w); Soya Oil (95 % w/w)		0.100 lts/ha	Sticker	500.00 lts/ha	0.70 ha	5 kph / NE 11 °C / _		mistral, Farmer 400
			Shark (18700) EAMU: 0622/19 Carfentrazone-ethyl (60 g/l)	21 days	0.600 lts/ha	Spawn control					
			Notes: Oc20 x 2, 0.5 bar, 1h, PPE full.								
24/04/2020 12:10 - 13:00	Spraying #45		Shark (18700) EAMU: 0622/19 Carfentrazone-ethyl (60 g/l)	21 days	0.800 lts/ha	Spawn control	400.00 lts/ha	0.70 ha	4 kph / NE 18 °C / _		mistral, Farmer 400
			SP058 (ADJ0793) trisiloxane organosilicone copolymers (64 % w/w)		0.500 lts/ha	Adjuvant					
			Notes: Oc20 x 2, 0.4 bar, 3M 1500 rpm, PPE full.								
18/05/2020 08:05 - 08:45 57:Single flower buds nodding partly red in colour	Spraying #65		Calypso (11257) EAMU: 2139/14 Thiacloprid (480 g/l)	3 days	0.250 lts/ha	aphid	800.00 lts/ha	0.70 ha	5 kph / SW 13 °C / _		Vanguard1000L, mistral
			Maxicrop Triple (MBS003)		1.000 lts/ha	Foliar Feed					
			Fortify 30-20 (MBS1028) P (30 % w/w); K (20 % w/w)		2.000 lts/ha	Foliar Feed					
			Signum (11450) EAMU: 2102/10 Pyraclostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	1.250 kgs/ha	Cane diseases and botrytis					
			Notes: Nozzle green x 10, 6 kph, 8 bar, PPE full.								

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
10/01/2020 10:00 - 17:00 98:Dormancy	Spraying #5		Paraat (15445) Dimethomorph (50 % w/w)	90 days	0.250 kgs/100L	Phytophthora	400.00 lts/ha	1.01 ha	1 kph / N 3 °C / _		mistral, Farmer 400
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		0.500 lts/100L	Nutritional- with bio-stimulant effects					
			Notes: Nozzle hand Lance.								
20/03/2020 14:15 - 15:00 11:First leaves separating	Spraying #17		Signum (11450) EAMU: 2110/10 Pyraclostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	1.500 kgs/ha	Cane diseases and botrytis	1,000.00 lts/ha	1.01 ha	5 kph / N 7 °C / _		Vanguard1000L, VOLCAN
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.000 lts/ha	iron and biostimulant					
			YaraVita Bud Builder FL (MBS517) Boron (30 g/l); Zinc (100 g/l); Magnesium (144 g/l); Phosphorus (50 g/l); nitrogen (69 g/l)		5.000 lts/ha	Improve bud micro nutrition to aid fruit set					
			Notes: Nozzle 8 blue, 5 bar, 6 kph, Full PPE.								
27/03/2020 10:50 - 11:50 11:First leaves separating	Spraying #21		Codacide Oil (ADJ0011) Rapeseed Oil (95 % w/w); Soya Oil (95 % w/w)		0.100 lts/ha	Sticker	500.00 lts/ha	1.01 ha	5 kph / NE 9 °C / _		mistral, Farmer 400
			Shark (18700) EAMU: 0622/19 Carfentrazone-ethyl (60 g/l)	21 days	0.300 lts/ha	Spawn control					
			Notes: Nozzle 0c20 x 2, 0.5 bar, Full PPE .								
24/04/2020 10:05 - 11:00	Spraying #45		Shark (18700) EAMU: 0622/19 Carfentrazone-ethyl (60 g/l)	21 days	0.800 lts/ha	Spawn control	400.00 lts/ha	1.01 ha	4 kph / NE 14 °C / _		mistral, Farmer 400
			SP058 (ADJ0793) trisiloxane organosilicone copolymers (64 % w/w)		0.500 lts/ha	Adjuvant					
			Notes: Nozzle 0c20 x 2, 0.9 bar, 3M 1500 rpm, PPE full.								
18/05/2020 09:00 - 10:05 57:Single flower buds nodding partly red in colour	Spraying #65		Calypso (11257) EAMU: 2139/14 Thiacloprid (480 g/l)	3 days	0.250 lts/ha	aphid	800.00 lts/ha	1.01 ha	5 kph / SW 14 °C / _		Vanguard1000L, mistral
			Maxicrop Triple (MBS003)		1.000 lts/ha	Foliar Feed					
			Fortify 30-20 (MBS1028) P (30 % w/w); K (20 % w/w)		2.000 lts/ha	Foliar Feed					
			Notes: Nozzle green x 10, 8 bar, 6 kph, PPE full.								
14/08/2020 08:10 - 08:55 49:Nine or more leaves unfolded	Spraying #149		Apollo 50 SC (17187) EAMU: 0620/18 Clofentezine (500 g/l)	26 days	0.400 lts/ha	Spider mite control	900.00 lts/ha	1.01 ha	5 kph / S 16 °C / _		Vanguard1000L, VOLCAN
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.000 lts/ha	iron and biostimulant					
			Notes: Nozzle blue x 10, 5 bar, 6 kph, PPE full.								

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
15/05/2020 13:50 - 14:30	Spraying #66		Calypso (11257) EAMU: 2139/14 Thiacloprid (480 g/l)	3 days	0.250 lts/ha	aphid	900.00 lts/ha	0.65 ha	5 kph / S 14 °C / _		Vanguard1000L, VOLCAN
			Pyrethrum 5 EC (12685) Pyrethrins (50 g/l)		2.400 lts/ha	Leaf curling midge					
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.500 lts/ha	iron and biostimulant					
			Poliverdol (MBS289) phosphorous pentoxide (8 %); potassium oxide (6 %); nitrogen (8 %)		2.000 lts/ha						
			Notes: Nozzle blue x 2, 5 bar, 6 kph, PPE full.								
11/06/2020 14:40 - 15:15	Spraying #96		Calypso (11257) EAMU: 2139/14 Thiacloprid (480 g/l)	3 days	0.250 lts/ha	Leaf curling midge	1,000.00 lts/ha	0.65 ha	5 kph / NE 15 °C / _		Vanguard1000L, VOLCAN
			Maxicrop Triple (MBS003)		3.000 lts/ha	Foliar Feed					
			Notes: Nozzle yellow x 6 , 5 bar, 6 kph, PPE full.								
14/07/2020 13:35 - 14:05 39:Canes about final length	Spraying #122		Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.500 lts/ha	iron and biostimulant	900.00 lts/ha	0.65 ha	5 kph / SW 18 °C / _		Vanguard1000L, VOLCAN
			optE-Mag (MBS577) Magnesium (8 % w/v); nitrogen (9 % w/v)		2.000 lts/ha	Magnesium and nitrogen					
			NorTrace Phyte P Plus (MBS572) phosphorus pentoxide (392 g/l); potassium oxide (266 g/l)		3.000 lts/ha						
			Notes: Nozzle blue x 8, 6 kph, 5 kph, PPE full.								
30/07/2020 10:50 - 11:25 31:Canes 10% of final length	Spraying #111		YaraVita Bud Builder FL (MBS517) Boron (30 g/l); Zinc (100 g/l); Magnesium (144 g/l); Phosphorus (50 g/l); nitrogen (69 g/l)		5.000 lts/ha	Improve bud micro nutrition to aid fruit set	800.00 lts/ha	0.65 ha	5 kph / SW 14 °C / _		Vanguard1000L, VOLCAN
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		2.000 lts/ha	Nutritional- with bio-stimulant effects					
			Maxicrop Concentrate (SA006) Seaweed Extract		1.500 lts/ha	BIOSTIMULANT					
			Notes: Nozzle blue x 6, 5 bar, 6 kph, PPE full.								
14/08/2020 13:15 - 13:50	Spraying #147		Naturalis-L (17526) Beauveria bassiana ATCC-74040 (71.6 g/l)		3.000 lts/ha	Two-Spotted Mite	900.00 lts/ha	0.65 ha	5 kph / S 18 °C / _		Vanguard1000L, VOLCAN
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.000 lts/ha	iron and biostimulant					
			Notes: Nozzle blue x 10, 5 bar, 6 kph, PPE full.								
04/11/2020 14:10 - 14:50 98:Dormancy	Spraying #159		Codacide Oil (ADJ0011) Rapeseed Oil (95 % w/w); Soya Oil (95 % w/w)		0.100 lts/ha	Sticker	400.00 lts/ha	0.26 ha	5 kph / S 10 °C / _		mistral, Farmer 400
			Shark (18700) Carfentrazone-ethyl (60 g/l)		0.330 lts/ha	Spawn control					
			Kerb Flo (13716) Propyzamide (400 g/l)	42 days	2.500 lts/ha	AMG grass small broadleaved weed control					
			Notes: Oc20 x2, 0.5 bar, 3 m 1500rpm, PPE full.								
05/11/2020 09:00 - 11:30 91:Leaves begin to discolour	Spraying #160		Paraat (15445) Dimethomorph (50 % w/w)	90 days	3.000 kgs/ha	Phytophthora	350.00 lts/ha	0.65 ha	5 kph / S 10 °C / _		mistral, Farmer 400
			NorTrace Phyte P Plus (MBS572) phosphorus pentoxide (392 g/l); potassium oxide (266 g/l)		3.000 lts/ha	Phytophthora					

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
27/05/2020 16:00 - 17:00 55:Flower petals elongating (single flower buds separating)	Spraying #73		Shark (18700) EAMU: 0622/19 Carfentrazone-ethyl (60 g/l)	21 days	0.400 lts/ha	Spawn control	400.00 lts/ha	1.06 ha	5 kph / NE 21 °C / _		mistral, Farmer 400
			Codacide Oil (ADJ0011) Rapeseed Oil (95 % w/w); Soya Oil (95 % w/w)		0.100 lts/ha	Sticker					
			Notes: Speed 3H/1500rpm, nozzle oc20 x2, pressure 0.4 bar, PPE FULL.								
30/06/2020 08:10 - 09:00 39:Canes about final length	Spraying #110		Signum (11450) EAMU: 2102/10 Pyradostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	1.250 kgs/ha	Powdery Mildew	900.00 lts/ha	1.06 ha	5 kph / SW 14 °C / _		Vanguard1000L, VOLCAN
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		2.000 lts/ha	Nutritional- with bio-stimulant effects					
			Maxicrop Concentrate (SA006) Seaweed Extract		1.500 lts/ha	BIOSTIMULANT					
			Notes: 6kph, blue x8 , 5bar, PPE full.								

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
27/05/2020 16:00 - 17:00 55:Flower petals elongating (single flower buds separating)	Spraying #73		Shark (18700) EAMU: 0622/19 Carfentrazone-ethyl (60 g/l)	21 days	0.400 lts/ha	Spawn control	400.00 lts/ha	1.02 ha	5 kph / NE 20 °C / _		mistral, Farmer 400
			Codacide Oil (ADJ0011) Rapeseed Oil (95 % w/w); Soya Oil (95 % w/w)		0.100 lts/ha	Sticker					
			Notes: Nozzle 0c20 x 2, 0.4 bar, 3M 1500 rpm, PPE full.								
30/06/2020 09:25 - 10:20 39:Canes about final length	Spraying #110		Signum (11450) EAMU: 2102/10 Pyradostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	1.250 kgs/ha	Powdery Mildew	900.00 lts/ha	1.02 ha	5 kph / SW 14 °C / _		Vanguard1000L, VOLCAN
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		2.000 lts/ha	Nutritional- with bio-stimulant effects					

			Maxicrop Concentrate (SA006) Seaweed Extract		1.500 lts/ha	BIOSTIMULANT					
			Notes: Nozzle blue x 8, 5 bar, 6 kph, PPE full.								

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
09/01/2020 10:00 - 17:00 98:Dormancy	Spraying #5		Paraat (15445) Dimethomorph (50 % w/w)	90 days	0.250 kgs/100L	Phytophthora	400.00 lts/ha	0.95 ha	5 kph / S 8 °C / _		mistral, Farmer 400
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		0.500 lts/100L	Nutritional- with bio-stimulant effects					
			Notes: Root drench, 200ml per pot.								
19/03/2020 15:35 - 16:25 11:First leaves separating	Spraying #17		Signum (11450) **EAMU: 2110/10** Pyradostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	1.250 kgs/ha	Cane diseases and botrytis	1,000.00 lts/ha	0.95 ha	5 kph / N 7 °C / _		Vanguard1000L, VOLCAN
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.000 lts/ha	iron and biostimulant					
			YaraVita Bud Builder FL (MBS517) Boron (30 g/l); Zinc (100 g/l); Magnesium (144 g/l); Phosphorus (50 g/l); nitrogen (69 g/l)		5.000 lts/ha	Improve bud micro nutrition to aid fruit set					
			Notes: Nozzle 8 blue, 5 bar, 6kph, full PPE.								
27/03/2020 12:05 - 13:00 11:First leaves separating	Spraying #21		Codacide Oil (ADJ0011) Rapeseed Oil (95 % w/w); Soya Oil (95 % w/w)		0.100 lts/ha	Sticker	500.00 lts/ha	0.95 ha	5 kph / NE 9 °C / _		mistral, Farmer 400
			Shark (18700) **EAMU: 0622/19** Carfentrazone-ethyl (60 g/l)	21 days	0.300 lts/ha	Spawn control					
			Notes: Nozzle 0c 20 x 2, 0.5 bar, Full PPE.								
24/04/2020 09:15 - 10:00	Spraying #45		Shark (18700) **EAMU: 0622/19** Carfentrazone-ethyl (60 g/l)	21 days	0.800 lts/ha	Spawn control	400.00 lts/ha	0.95 ha	4 kph / NE 14 °C / _		mistral, Farmer 400
			SP058 (ADJ0793) trisiloxane organosilicone copolymers (64 % w/w)		0.500 lts/ha	Adjuvant					
			Notes: Speed 1500 rpm 3M, 0.4 bar, 0c 20 x 2, PPE full.								
18/05/2020 12:35 - 13:30 57:Single flower buds nodding partly red in colour	Spraying #65		Calypso (11257) **EAMU: 2139/14** Thiacloprid (480 g/l)	3 days	0.250 lts/ha	aphid	800.00 lts/ha	0.95 ha	6 kph / SW 20 °C / _		Vanguard1000L, mistral
			Maxicrop Triple (MBS003)		1.000 lts/ha	Foliar Feed					
			Fortify 30-20 (MBS1028) P (30 % w/w); K (20 % w/w)		2.000 lts/ha	Foliar Feed					
			Signum (11450) **EAMU: 2102/10** Pyradostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	1.250 kgs/ha	Cane diseases and botrytis					
			Notes: Nozzle green x 8, 8bar, 6 kph, Full PPE.								
14/08/2020 11:10 - 11:50 49:Nine or more leaves unfolded	Spraying #149		Apollo 50 SC (17187) **EAMU: 0620/18** Clofentezine (500 g/l)	26 days	0.400 lts/ha	Spider mite control	900.00 lts/ha	0.95 ha	5 kph / S 18 °C / _		Vanguard1000L, VOLCAN
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.000 lts/ha	iron and biostimulant					
			Notes: Nozzle blue x 10, 5 bar, 6 kph, Full PPE.								

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
16/01/2020 - 17/01/2020 10:00 - 17:00 98:Dormancy	Spraying #5		Paraat (15445) Dimethomorph (50 % w/w)	90 days	0.250 kgs/100L	Phytophthora	400.00 lts/ha	1.83 ha	1 kph / N 3 °C / _		mistral, Farmer 400
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		0.500 lts/100L	Nutritional- with bio-stimulant effects					
			Notes: Hand Lance, 200ml/pot.								
15/04/2020 14:30 - 16:00 11:First leaves separating	Spraying #30		Centurion Max (17911) **EAMU: 3640/19** Clethodim (120 g/l)	30 days	0.800 lts/ha	Grass Weed Control	400.00 lts/ha	1.83 ha	6 kph / SE 15 °C / _		mistral, Farmer 400
			Notes: Oc20 x2, 0.4 bar, 3M 1600 rpm, PPE full.								
15/05/2020 11:00 - 12:30	Spraying #66		Calypso (11257) **EAMU: 2139/14** Thiacloprid (480 g/l)	3 days	0.250 lts/ha	aphid	900.00 lts/ha	1.83 ha	5 kph / S 10 °C / _		Vanguard1000L, VOLCAN
			Pyrethrum 5 EC (12685) Pyrethrins (50 g/l)		2.400 lts/ha	Leaf curling midge					
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.500 lts/ha	iron and biostimulant					

			Poliverdol (MBS289) phosphorous pentoxide (8 %); potassium oxide (6 %); nitrogen (8 %)		2.000 lts/ha						
			Notes: 6 kph, 5 bar, blue x 2, 5 bar.								
30/06/2020 12:40 - 14:05 31:Canes 10% of final length	Spraying #111		YaraVita Bud Builder FL (MBS517) Boron (30 g/l); Zinc (100 g/l); Magnesium (144 g/l); Phosphorus (50 g/l); nitrogen (69 g/l)		5.000 lts/ha	Improve bud micro nutrition to aid fruit set	800.00 lts/ha	1.83 ha	5 kph / SW 16 °C / _		Vanguard1000L, VOLCAN
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		2.000 lts/ha	Nutritional- with bio-stimulant effects					
			Maxicrop Concentrate (SA006) Seaweed Extract		1.500 lts/ha	BIOSTIMULANT					
			Notes: Nozzle blue x 6, 5 bar, 6 kph, PPE full.								
14/07/2020 09:30 - 12:00 39:Canes about final length	Spraying #122		Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.500 lts/ha	iron and biostimulant	900.00 lts/ha	1.83 ha	5 kph / SW 17 °C / _		Vanguard1000L, VOLCAN
			optE-Mag (MBS577) Magnesium (8 % w/v); nitrogen (9 % w/v)		2.000 lts/ha	Magnesium and nitrogen					
			NorTrace Phyte P Plus (MBS572) phosphorus pentoxide (392 g/l); potassium oxide (266 g/l)		3.000 lts/ha						
			Notes: Nozzle blue x 8, 6 kph, 5 bar, PPE full.								
24/07/2020 10:05 - 13:15 89:Majority of fruits harvested	Spraying #133		ProTac SF (MBS1220) Silicone Polymers; Siloxanes; Organic Antioxidants		0.500 lts/ha	Mite Control	500.00 lts/ha	1.83 ha	5 kph / S 17 °C / _		Vanguard1000L, VOLCAN
			Notes: Nozzle Blue x 6, 6 kph, 5 bar, PPE full.								

Activity Date / Timing	Operation	Advisor	Product	HI	Rate	Reason	Water	Area	Wind Spd / Dir Temp / Hum	Operator	Machine
30/01/2020 - 31/01/2020 10:00 - 16:00 98:Dormancy	Spraying #5		Paraat (15445) Dimethomorph (50 % w/w)	90 days	0.250 kgs/100L	Phytophthora	400.00 lts/ha	2.35 ha	2 kph / N 8 °C / _		mistral, Farme 400
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		0.500 lts/100L	Nutritional- with bio-stimulant effects					
			Notes: Hand Lance, 200ml/pot.								

15/05/2020 10:05 - 11:00	Spraying #66		Calypso (11257) Thiacloprid (480 g/l) **EAMU: 2139/14**	3 days	0.250 lts/ha	aphid	900.00 lts/ha	2.35 ha	5 kph / S 10 °C / _		Vanguard1000L, VOLCAN
			Pyrethrum 5 EC (12685) Pyrethrins (50 g/l)		2.400 lts/ha	Leaf curling midge					
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.500 lts/ha	iron and biostimulant					
			Poliverdol (MBS289) phosphorous pentoxide (8 %); potassium oxide (6 %); nitrogen (8 %)		2.000 lts/ha						
			Notes: Nozzle blue x 2, 5 bar, 6 kph, PPE full.								
03/07/2020 12:45 - 14:30 31:Canes 10% of final length	Spraying #111		YaraVita Bud Builder FL (MBS517) Boron (30 g/l); Zinc (100 g/l); Magnesium (144 g/l); Phosphorus (50 g/l); nitrogen (69 g/l)		5.000 lts/ha	Improve bud micro nutrition to aid fruit set	900.00 lts/ha	2.35 ha	10 kph / SW 17 °C / _		Vanguard1000L, VOLCAN
			HortiPhyte (MBS110) Potassium (8 %); nitrogen (4 %); Phosphorous (30 %)		2.000 lts/ha	Nutritional- with bio-stimulant effects					
			Maxicrop Concentrate (SA006) Seaweed Extract		1.500 lts/ha	BIOSTIMULANT					
			Notes: Nozzle blue x4, 5 bar, 6 kph, PPE full.								
14/07/2020 09:30 - 12:00 39:Canes about final length	Spraying #122		Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		1.500 lts/ha	iron and biostimulant	900.00 lts/ha	2.35 ha	5 kph / SW 17 °C / _		Vanguard1000L, VOLCAN
			optE-Mag (MBS577) Magnesium (8 % w/v); nitrogen (9 % w/v)		2.000 lts/ha	Magnesium and nitrogen					
			NorTrace Phyte P Plus (MBS572) phosphorus pentoxide (392 g/l); potassium oxide (266 g/l)		3.000 lts/ha						
			Notes: Nozzle blue x 8, 6 kph, 5 bar, PPE full.								
17/07/2020 12:45 - 14:30	Spraying #130		Tracer (12438) Spinosad (480 g/l) **EAMU: 1207/18**	1 days	0.200 lts/ha	Thrips	900.00 lts/ha	2.35 ha	5 kph / NW 19 °C / _		Vanguard1000L, VOLCAN
			Apollo 50 SC (17187) Clofentezine (500 g/l) **EAMU: 0620/18**	26 days	0.400 lts/ha	Two Spotted Spider Mite Egg					
			Calypso (11257) Thiacloprid (480 g/l)		0.250 lts/ha	aphid					

			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		**1.500 lts/ha**	iron and biostimulant					
			Notes: Nozzle blue x 6, 5 bar, 6 kph, PPE full.								
30/07/2020 10:00 - 13:15 51:First flower buds visible	**Spraying #135**		Signum (11450) **EAMU: 2110/10** Pyraclostrobin (6.7 % w/w); Boscalid (26.7 % w/w)	3 days	**1.250 kgs/ha**	Botrytis and cane diseases	800.00 lts/ha	2.35 ha	5 kph / S 18 °C / _		Vanguard1000L, VOLCAN
			Calypso (11257) **EAMU: 2139/14** Thiacloprid (480 g/l)	3 days	**0.250 lts/ha**	aphid					
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		**1.000 lts/ha**	iron and biostimulant					
			Notes: Nozzle blue x 8, 5 bar, 6 kph, PPE full.								
04/08/2020 12:50 - 14:40 61:10% of flower open	**Spraying #141**		NorTrace Phyte P Plus (MBS572) phosphorus pentoxide (392 g/l); potassium oxide (266 g/l)		**3.000 lts/ha**		300.00 lts/ha	2.35 ha	5 kph / S 19 °C / _		Vanguard1000L, VOLCAN
			optE-Mag (MBS577) Magnesium (8 % w/v); nitrogen (9 % w/v)		**4.000 lts/ha**	Magnesium and nitrogen (Solid Mag Nitrate would be an alternative)					
			Maxicrop Plus Sequestered Iron (MBS138) Seaweed Extract; Iron		**1.500 lts/ha**	iron and biostimulant					
			Notes: Nozzle yellow x 8, 6 kph, 7 bar, PPE full.								
17/11/2020 09:00 - 17:00 91:Leaves begin to discolour	**Spraying #160**		Paraat (15445) Dimethomorph (50 % w/w)	90 days	**3.000 kgs/ha**	Phytophthora	350.00 lts/ha	2.35 ha	10 kph / S 10 °C / _		Farmer 400
			NorTrace Phyte P Plus (MBS572) phosphorus pentoxide (392 g/l); potassium oxide (266 g/l)		**3.000 lts/ha**	Phytophthora					
			Notes: 3 low 1300rpm, 1.5 bar, hand lance, 100ml/pot. PPE full.								

Spray Schedule strawberry collections

HARVEST INTERVAL CHECK FORM

Product	Application		Harvest Interval	First available harvest		Actual harvest		Picking authorised by
	Date	Time		Date	Time	Date	Time	
LUNA	1/6	0700	1	2/6	07:00			
						6/6	10:00	P.Y
CALMAX CHARM	20/6	09:45	1	21/6	09:45			
NATURALIS SP058	30/6	21:00	0	30/6	21:00			
LUNA	6/7	22:30	1	7/7	22:30			
NATURALIS SP058	7/7	21:30	0	7/7	21:30			

Issue No: 19 | Issue date: 31/01/2020 | Authorised by: ...

HARVEST INTERVAL CHECK FORM

Product	Application		Harvest Interval	First available harvest		Actual harvest		Picking authorised by
	Date	Time		Date	Time	Date	Time	
NATURALIS SP058	14/7	23:00	0	14/7	23:00			
NATURALIS SP058	20/7	23:30	0	20/7	23:30			
ROBIN TRACER	2/9	21:00	1	3/9	21:00			

Issue No: 19 | Issue date: 31/01/2020 | Authorised by: