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 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

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[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.

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Deputy Head of Pest and Pathogen Ecology

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

Signature .....M Fountain..Date 02 Apr 2020

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Signature .... Date .

[Name]

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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 - SWD, *D. suzukii*) first invaded the UK in 2012 and immediately became a key pest of soft and stone fruits. Numbers have increased from year to year, causing severe fruit damage and increases in crop management and 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). There is also 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).

In Europe and America, research projects on *D. suzukii* are coming to an end (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 in 2019 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.

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

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

Task 1.2. Modelling of the 7-year National Monitoring dataset (Peter Skelsey JHI)

Headline

- *D. suzukii* numbers at NIAB EMR in 2019, overall, were slightly higher than 2017 and 2018.

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. More frequent reports have been made both nationally and in Ireland. In the West Midlands and East Anglia the numbers are slightly lower than some of the fruit growing regions of England. In contrast to the general UK trend, populations in Scotland have been low since the pest was first detected there in 2014.

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

Monitoring traps were deployed in pairs, one in the centre of each crop and one at the edge. Pairs of traps were also deployed in a wooded area on each farm. The modified Biobest trap design and Cha-Landolt bait was used. Activity-density of adult *D. suzukii* in the monitoring traps was lower in the spring (Mar-May) of 2017 compared to 2018 due to the cold weather. However, the overall tally of *D. suzukii* for 2018 was lower than 2017. Variation in inter-annual trap catches appeared to be largely dependent upon temperature. Despite higher than average temperatures recorded in Scotland during the summer months of 2018 the number/activity levels of *D. suzukii* remained low.

Additionally, 2018 data from all three Scottish monitoring groups showed similar trends suggesting that the national monitoring data set is representative of the *D. suzukii* density/activity in Scotland. The density/activity was lower in 2018 than in 2017. The lack of potential egg laying sites detected may have partially contributed to the reduction in overall catch.

Summary of the project and main conclusions following 2019 monitoring

In 2019, following consultation with the project steering group, monitoring in England was reduced with only 10 traps at NIAB EMR maintained. A warmer spring resulted in higher trap catches in comparison to 2018, and 2019 saw an unprecedented peak in June, which coincided with above average temperatures during this time. In September, the largest peak trap catch occurred (since monitoring began in 2013); during a period of increased temperatures. There continues to be a year on year increase in annual mean trap catch at East Malling, indicating we have not yet reached carrying capacity.

In Scotland, average peak trap catches from the three monitoring traps increased to 130 per trap, surpassing 89 per trap from 2014. The total number of *D. suzukii* caught during peak season, August-November (weeks 33-47), reached a mean of ~120; surpassing peak catches in 2014.

In the 12 m high Rothamsted suction trap network, *D. suzukii* were identified between August and November, 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). Trap catches from 2019 will be analysed in spring 2020. NIAB EMR now hosts a suction trap replacing the trap that was removed from Rye. Rothamsted have agreed to share the Scottish suction trap catches from 2014, 2017 and 2019. Results are expected to be reported in the fourth Annual Report.

A predictive model is being developed at the James Hutton Institute using historic trap catch data coupled with environmental information. The model has been successful in predicting percentage cumulative abundance of historic data with 72-99% accuracy. Flight prediction has also been successful with 92% accuracy. This will be further developed in 2020.

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

Financial benefits

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.

Action points for growers

- Continue to monitor adult *D. suzukii* in hedgerow and cropping areas.
- Monitor for fruit damage throughout the cropping period 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 (NRI and Rory Jones)

Headline

- Work has been initiated to collect and identify volatile compounds from yeast species associated with SWD as a route to discover new attractants for SWD.

Background and expected deliverables

Drosophila species have evolved strong mutualistic associations with yeast communities that best support their growth and survival, and it is reported that flies recognise these yeasts by the rich repertoire of volatile organic compounds produced by the yeasts.

Rory Jones of University of Lincoln is undertaking an AHDB PhD Studentship (CP171) to investigate the attractiveness of a range of yeast species to SWD, including those associated with SWD and exotic species exclusive to Lincoln University. To date, he has tested several species in a laboratory bioassay and field trapping tests. The aim of this work was to identify the chemicals produced and investigate whether there is any correlation between these chemicals and attractiveness to SWD. This work could lead to identification of new attractants for SWD.

Summary of the project and main conclusions

- Compounds produced by five strains of yeast grown on sterile strawberry juice were identified. These results will be correlated with bioassays of attractiveness of the yeasts in laboratory and field bioassays.
- Having established the methodology for collection and analysis of yeast volatiles, the work will be repeated with the same yeast cultures grown on a more neutral medium.
- No obvious new candidate attractants for SWD have been identified, although ethyl acetate, 2-phenylethanol and isoamyl acetate could be re-examined.

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Action points for growers

- This work has not resulted in any direct action points for growers to date.

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

Headline

- Preliminary findings indicate that 'precision monitoring' in natural habitats reduces overwintering *D. suzukii* populations in woodlands and neighbouring crops.

Background and expected deliverables

In addition to 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. The UK *D. suzukii* national monitoring survey (Objective 1) 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* populations 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 8 metre intervals were deployed in isolated pockets of woodlands on six soft fruit farms in the South East of 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 the respective neighbouring crop. These were checked fortnightly to monitor numbers of *D. suzukii.* In addition, a transect of precision monitoring traps were also checked for *D. suzukii* catches.

So far it is too early to conclude if precision monitoring can prevent invasions of *D. suzukii* into the neighbouring crop. However, six weeks after precision monitoring traps were deployed, numbers of *D. suzukii* in the RIGA monitoring traps in woodlands with precision monitoring and respective neighbouring crops decreased. Numbers of *D. suzukii* in the untreated control equivalents continued to rise (not statistically analysed). Thereafter, *D. suzukii* numbers have remained consistently lower in the precision monitoring trap treated areas.

To determine if precision monitoring can prevent or reduce *D. suzukii* numbers invading the neighbouring crop, in spring 2020, sentinel traps containing raspberries will be deployed in the woodlands and respective neighbouring crops to attract females to lay eggs. *D. suzukii* are being dissected weekly to test for the onset of fecundity. Subsequent numbers of adult *D. suzukii* emerging from these raspberries will be compared.

Habitat assessments around each of the precision monitoring traps are underway to identify why some traps consistently capture more flies than other traps. This will help to identify optimum locations for future trapping and inform growers on optimum trap positioning. To date there is some evidence that traps positioned on the woodland perimeter catch more *D. suzukii.* However, a more thorough investigation is required later in the season to determine the best place to concentrate traps. Aspect may also be playing an important role.

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

Financial benefits

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Action points for growers

- This work has not resulted in any direct action points for growers to date.

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

Headline

- Weekly applications of Benevia at 30 ml in 40L per ha, combined with *H. uvarum* or Combi-protec baits, 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 91% with the same *D. suzukii* control effect).

Background and expected deliverables

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

In 2018, the baits included 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 insecticides, the baits did not affect *D. suzukii* mortality. With spinosad (Tracer), cyantraniliprole (Exirel) and lambda-cyhalothrin (Hallmark), the baits caused higher mortality of *D. suzukii* summer morphs, under summer conditions, compared with using the insecticides in water. The efficacy of insecticides, in terms of increased mortality and reduced egg laying, was greater with *H. uvarum*, FSJ + *H. uvarum* and Combi-protec treatments than with 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.

Phytotoxicity on cherry and strawberry leaves in the field was observed in treatments including cyantraniliprole, both with and without baits, but was not seen in any other insecticide and/or bait combinations.

Phagostimulant baits improved the insecticidal control of *D. suzukii* summer and winter morphs by increasing mortality and reducing oviposition. The relative phagostimulant effect of the baits did not fully correspond with their olfactory attractiveness to *D. suzukii* determined using the chronophysiology equipment.

With insecticide treatments, *D. suzukii* mortality was lower using raspberry leaves than using blackberry, blueberry, cherry or strawberry leaves but the effect of leaf type on *D. suzukii* mortality was small (up to 12% difference) compared with the effects of baits and insecticides (up to 90% difference).

Summary of the project in 2019 and main conclusions

In 2019, baits were tested in mini tunnels containing strawberry plants in grow bags. Bands of Benevia 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 £112.50/ha. Application time was reduced by 75% in the bait combined with Benevia treatments compared to Benevia alone.

In 2020 Combi-protec will be tested for efficacy on raspberry in mini tunnels at NIAB EMR.

Financial benefits

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.

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Action points for growers

- At the time of writing, Combi-protec was an approved adjuvant for use with *D. suzukii* control plant protection products.
- Growers should consider using Combi-protec to enhance *D. suzukii* control in strawberry.

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

Task 4.2. Investigate the impact of different spray methods on cherry.

Headline

- Good spray coverage on cherry crops at two farms ensured minimum fruit damage from *D. suzukii* on a fortnightly spray programme.

Background and expected deliverables

In 2018 field trials were carried out to test the effects of increasing spray intervals for control of *D. suzukii* at two cherry farms in East Kent (see details outlined under Task 4.3 below). Fortnightly spray programmes gave equal efficacy of *D. suzukii* control to the grower's standard spray programme. In addition, very few fruits were damaged by *D. suzukii* egg laying in both spray programmes, even though adults were clearly in the crop and around the perimeter. Where insect excluding mesh was employed there were fewer *D. suzukii* adults in the crop.

The trials in 2018 recorded effects on insect populations, fruit damage and length of time of effectiveness of the spraying, but did not measure spray deposition.

Summary of the project in 2019 and main conclusions

In June 2019, the farms were re-visited, and the same tunnels were sprayed in the same way as in 2018. The spray deposition was measured using the handheld imaging fluorometer (developed in an IUK project) to quantify spray coverage and fluorescence intensity (a proxy for spray liquid volume on the leaf surface). The two farms had different spray application methods, using different spray machines, water volumes, and forward speeds. Using high water volumes generally provided much greater spray coverage on the target but was slower and more costly to spray. Using higher forward speeds can make navigating the orchard rows more challenging but may also improve deposition into the canopy by reducing the volume of air per tree. With a faster forward speed, the droplets' perpendicular momentum is reduced and they are more likely to deposit into the canopy rather than be pushed through and out the other side.

Although these trials were relatively small assessments of spray deposition, the results indicate that both farms achieved a good level of spray deposition overall. However, at Farm 1 there was very little spray deposition at the 'inner' canopy area, and the spray plume was seen to spray over the tops of the trees. Farm 2 had quite low spray coverage (due to the lower water application volume used), but still managed a good level of spray deposition (measured by fluorescence intensity). The faster forward speed and better targeted spray plume at Farm 2 are likely resulting in improved spray deposition.

The 2-row beds used at Farm 2 may result in very low spray deposition on the leeward side of the trees. This area was not assessed in these trials due to time constraints.

Financial benefits

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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.

Action points for growers

- Spray intervals under protected cherry can be extended to two weeks from white fruit stage in combination with insect exclusion mesh and rigorous crop hygiene.
- Good spray coverage is essential to protect the fruit. Thorough coverage allows SWD to pick up the product and achieve further control.
- Continue to monitor adult SWD both inside and outside the mesh to ensure spray programmes are effective.
- Make regular inspections of fruits to ensure populations are not building inside the crops.

Task 4.3. Investigate the consequence of extending the spray interval from 1 to 2 weeks in raspberry

Headline

- Unlike cherry, a fortnightly spray programme in raspberry was not as successful at controlling *D. suzukii* as weekly applications of plant protection products.

Background and expected deliverables

The aims of this objective were to determine the length of time that cherry extrafloral resources were available to *D. suzukii* in a cherry orchard and to investigate the length of time that PPPs targeted against *D. suzukii* in spray programmes were active in order to prolong the spray intervals beyond 7-10 days.

In 2017 we picked leaves weekly from the cherry varieties `Penny` and `Sweetheart’ and developed laboratory trials to observe behaviour. The number of *D. suzukii* that landed and fed, the time to find the extrafloral nectaries and the length of feeding time over a five-minute period was recorded. As the season progressed the time taken to locate nectaries in the leaves tended to increase, but demonstrated that there was a food source available to *D. suzukii* until after fruit harvest. There appeared to be less feeding after a period of rain, indicating that potentially nectar and beneficial microbes could have been washed from the surface of the leaves making the extra floral nectaries less attractive to *D. suzukii*.

In the early years of the project, it was found that fortnightly sprays of effective rotated plant protection products (PPPs) on protected cherry were very successful at controlling *D. suzukii* in cherry fruit. Two small cherry trials were established in 2017; 1) Commercial trial on emergence of *D. suzukii* from fruit from netted tunnels, 2) Semi-field trial at NIAB EMR on mortality of adult *D. suzukii* in contact with residues. Either a weekly or fortnightly commercially approved spray programme was employed at both sites. Monitoring traps were in place at both sites on the perimeter and inside the crop. At the commercial site, the numbers of adult *D. suzukii* captured inside the insecticide treated tunnels (peak 11), was lower than outside the insect exclusion mesh (peak 70). Only 2 female *D. suzukii* emerged from fruits throughout the growing season; 1 from the weekly and 1 from the fortnightly spray programme.

In the semi-field leaf bioassay there was significantly higher *D. suzukii* mortality in the weekly and fortnightly spray programmes compared to the untreated control, but no difference between the two spray programmes while applications were made. Following the cessation of sprays, the effects of the insecticides declined over time (7-28 Aug).

In 2018, field trials were carried out to test the effects of increasing spray intervals for control of *D. suzukii* at two commercial farms in East Kent. Fortnightly spray programmes gave equal efficacy of *D. suzukii* control to the grower's standard spray programme on cherry. In addition, very few fruits were damaged by *D. suzukii* egg laying in both spray programmes even though adults were clearly in the crop and around the perimeter. Where insect excluding mesh was employed there were fewer *D. suzukii* adults in the crop.

Also in 2018 we began to pilot test extending the spray interval from one to two weeks in raspberry, but only on two primocane raspberry crops. This was expanded to eight raspberry crops in 2019.

Summary of the project and main conclusions

In 2019, trials investigated whether extending spray intervals on protected raspberry could adequately control *D. suzukii* damage to fruit. The trial employed fortnightly spray intervals in comparison to weekly spray intervals. The incidence of *D. suzukii* in fruit, adult mortality in contact with leaves and adult presence in the crop were assessed.

Fortnightly spray intervals were not as effective at protecting fruit from *D. suzukii* as a weekly programme. Hence, the fortnightly programme was not as successful in raspberry as it was for cherry production. In addition, the fortnightly sprayed plots were located on the edge of the fields and under higher pressure of *D. suzukii* immigration from wild host habitats, particularly later in the trial when fruit was fading.

Main conclusions

- Unlike cherry, a fortnightly spray programme in raspberry was not as successful at controlling *D. suzukii* as weekly applications of plant protection products.

- A fortnightly spray programme on raspberry for *D. suzukii* control was more challenging, partly because the fortnightly sprays were applied to the perimeter of the crop where the *D. suzukii* pressure is greatest.
- However, the weekly spray programme on raspberry was more effective at reducing numbers of *D. suzukii* in fruit and resulted in higher mortality of adults that were exposed to treated leaves compared to a fortnightly spray programme.

Financial benefits

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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.

Action points for growers

- *D. suzukii* control on raspberry is challenging and research will now focus on raspberry.
- Until then, monitoring adults and fruit damage is key to tracking the progress of current control methods.
- Good spray coverage is essential to protect the fruit but also leave a residue for contact of adult flies on the foliage.
- Crop hygiene and insect mesh are critical to prevent build-up of numbers inside the crop and migration of new *D. suzukii* into crops.
- Precision monitoring in hedgerows around the perimeter may also reduce numbers entering the crop.
- It is essential to rotate modes of action of plant protection products to prevent insect resistance developing to these products.
- It is also vital to make sure that spray drift does not contact hedgerows and woodlands therefore preserving natural enemies of *D. suzukii* (parasitic wasps and a range of generalist predators).

Objective 5. Integrating exclusion netting with other successful controls

A decision was made to defer work under this objective until a later year, as a new Waitrose CTP PhD student will be working on this in collaboration with BerryWorld, the University of Reading and NIAB EMR from 2019.

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

Headline

- AHDB and the scientists leading this project at NIAB EMR and the James Hutton Institute promoted the results of this project and a year-round strategy through five peer reviewed publications and contributions to 16 industry and scientific communication events over the past year.

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 through publications and events. Over 14 presentations and courses were delivered in 2017, and 10 in 2018.

Summary of the project and main conclusions

In 2019, five peer reviewed manuscripts were published and 16 industry/scientific communications/presentations were given. This does not include all of 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.

Financial benefits

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.

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

Headlines

- In Scotland, using sentinel *Drosophila melanogaster* larvae and pupae, potential *D. suzukii* parasitoid activity has begun to be identified.
- In England, attempts to identify the percentage parasitism in the wild has been thwarted by technical issues and squirrels! Methodology has been improved and data collected in 2019.

Background and expected deliverables

A Worshipful Company of Fruiterers funded project linked to SF/TF 145a, aimed to identify species of parasitic wasps parasitizing *D. suzukii* in the South East of England. Field surveys also aimed to monitor for the presence of the SWD parasitoid *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.

Summary of the project and main conclusions

In 2019, parasitoid surveys were conducted in Scotland using *D. melanogaster* baited traps from the end of July. From the numbers of parasitoids emerging from baited traps it indicates that parasitoid populations were already established prior to the deployment of traps. Due to staff shortages at NHM, species have not yet been identified, although it appears that there are two distinct morphotypes.

To determine the percentage of parasitism in the field, known numbers of *D. suzukii* larvae were deployed in areas with known parasitoid populations, as identified in 2018. Only one *D. suzukii* parasitoid was identified in 2019. It is likely that changes in trapping method reduced the numbers of parasitoids observed in 2019 compared to previous years.

Financial benefits

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.

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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.

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 to use crop hygiene measrues and insect exclusion mesh to reduce the need for plant protection products.

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

Headline

- Variation in susceptibility level to three commonly used plant protection products between wild populations of *D. suzukii* was identified in comparison to an isolated laboratory culture.
- Baseline susceptibility from 2019 will be used as a comparison for future assessments to monitor resistance development.

Background and expected deliverables

Since its arrival in the UK in 2012, the use of plant protection products has played a vital role in supressing *D. suzukii* numbers in vulnerable fruit crops. In 2018, an increased tolerance to spinosad was detected in Californian organic raspberries by Gress and Zalom (2018). Flies from spinosad treated areas required 4.3-7.7 times higher dose of spinosad for control than those from untreated areas. In 2019, laboratory trials were established to identify a baseline level of susceptibility in wild populations of *D. suzukii.*

Summary of the project and main conclusions

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 to an unsprayed laboratory strain, which has been in culture since 2013 and is expected to have a very low tolerance to plant protection products (PPP). Between the three wild populations, there were varying levels of susceptibility to three tested PPPs; lambda-cyhalothrin (Hallmark), cyantraniliprole (Exirel) and spinosad (Tracer). Although there does not currently seem to be resistance in the populations we tested, there was an increased level of tolerance in some of the populations to one or more of the insecticide products tested. Annual baseline testing should be employed to monitor tolerance levels over seasons so that spray programmes can be adjusted in response.

Financial benefits

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.

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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.

Action points for growers

- Employ as many non-PPP *D. suzukii* controls (precision monitoring, mesh, crop hygiene, proper waste fruit disposal) as feasible, to reduce reliance on sprays and reduce the incidence of resistance.
- When applying plant protection products, ensure that there is good coverage, and that equipment is calibrated and set up correctly, ensuring the protection of the surrounding environment.
- Rotate modes of actions of products to avoid resistance in the future.
- Consult your BASIS qualified agronomist for the latest approvals.

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, to enable more resource to be focused on control measures the monitoring in England was reduced to the NIAB EMR site and trap catches in this report are annual catches for this site and the Scotland sites only, so that annual trends can be followed.

All data was supplied to the James Hutton Institute for modelling for modelling populations with climatic conditions in each year. Data was also supplied to PC Fruit (with a collaboration agreement) in Belgium for inclusion in their model. In 2020, data will also be 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. Originally there were 57 traps on nine farms in England and 40 traps on four farms in Scotland. From 2019 there were 10 traps at NIAB EMR (cherry, strawberry, grape, and woodland) in England and 3 traps at the James Hutton Institute (blackberry, raspberry, wild) in Scotland. One wild area was monitored at each farm.

Monitoring traps were generally deployed in pairs, one in the centre and one at the edge of each crop. For continuity, within the National Monitoring Survey we continued to use the modified Biobest trap design and Cha-Landolt bait used from 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. Methionol 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.

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

Results

In 2017, *D. suzukii* numbers in monitoring traps continued to rise with inter-annual variation in trap catches, at least in the late autumn, probably dependent upon temperature (Tochen et al. 2013) and humidity (Tochen et al. 2015). 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 (September - 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*.

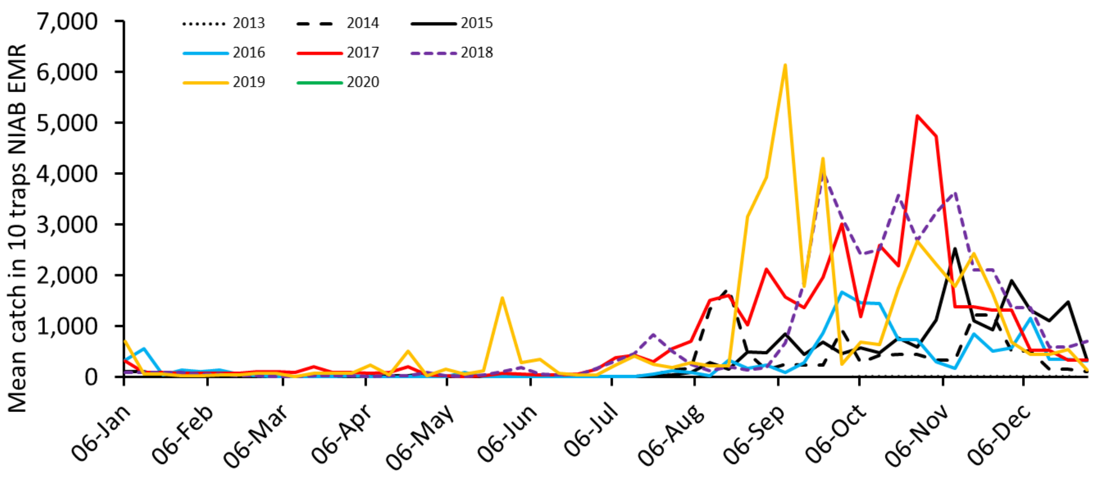
The activity-density of adult *D. suzukii* in the monitoring traps was lower in the spring 2018 (March - May) compared to 2017. This was likely caused by a prolonged, cold, spring in 2018 (Fig. 1.1.2) decreasing the opportunity for *D. suzukii* to be active, and hence, captured in the monitoring traps.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

In 2018, 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).

In 2019 (yellow line), monitoring at NIAB EMR only, demonstrated higher catches in the spring compared to the previous year (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 East Malling in September, again correlating to higher than average temperatures in that month. October was 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 East Malling, although influenced by temperature, gradually rose until 2019; Mean per trap; 2013 = 1, 2014 = 229, 2015 = 362, 2016 = 280, 2017 = 806, 2018 = 789, and 2019 = 814, Fig. 1.1.1).

a)



b)

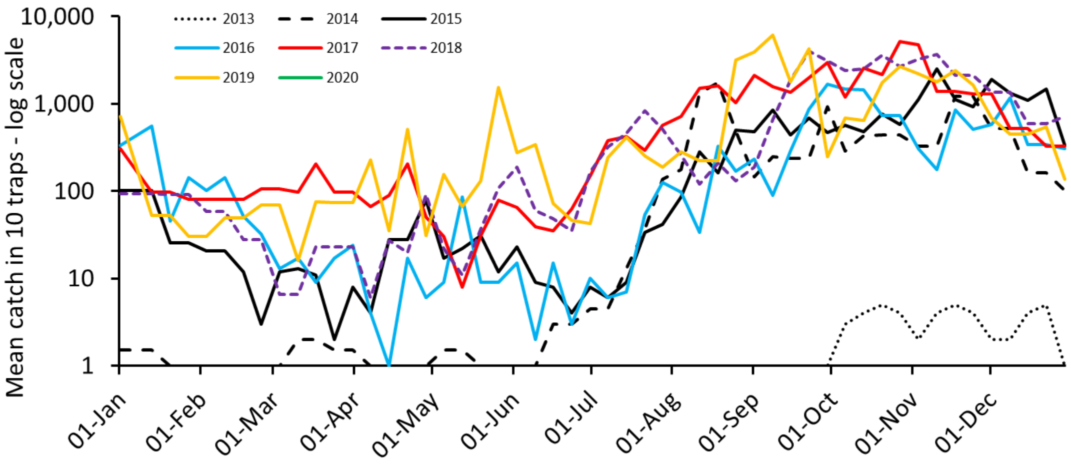


Figure 1.1.1. a) Comparison of average adult *D. suzukii* catch per trap in 2013, 2014, 2015, 2016, 2017, 2018 and 2019, and b) plotted on a log_{10} (n + 1) scale on the Y axis

The highest peaks in England occur during the late autumn – winter months when the flies are in reproductive diapause in their winter-form. The leaves have fallen from deciduous trees at this time giving less shelter and there is also a reduced availability of commercial and wild fruit.

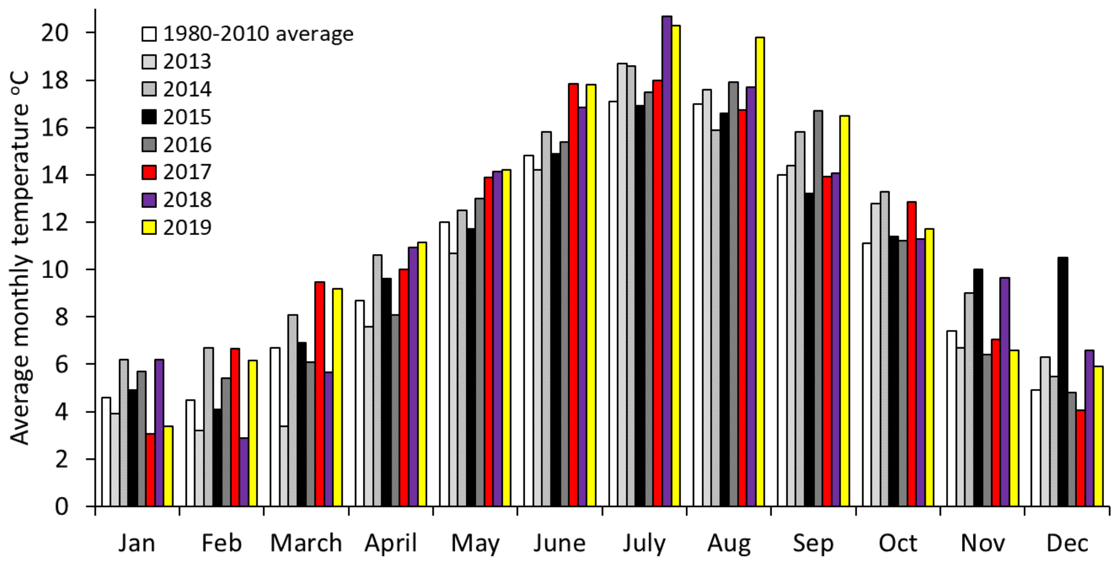


Figure 1.1.2. Comparison of the mean monthly temperatures between years

In Scotland, in general, catches of adult *D. suzukii* in the three traps followed previous years (Fig. 1.1.3). However, peak catches in 2019 for the 3 traps were higher (average

130 per trap) than the previous highest recording (average 89 per trap) for these traps, at this site, in December 2014. The total number of *D. suzukii* caught in the three monitoring traps from week 33-47 (peak activity) was also higher than in any of the previous monitoring years (Fig. 1.1.4). This agrees with other monitoring data from Scotland for 2019 (personal communication).

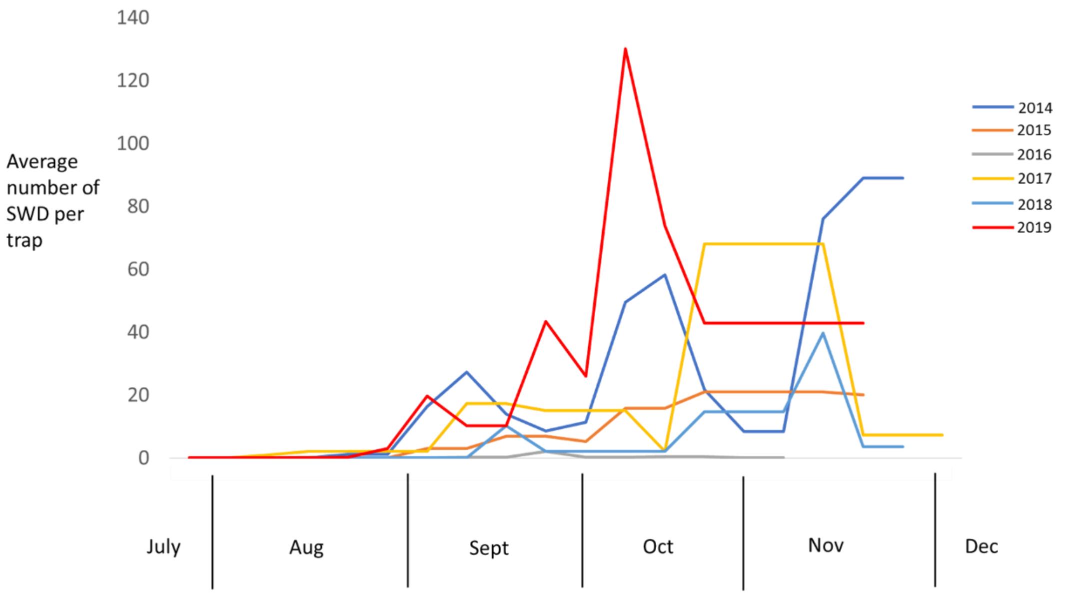


Figure 1.1.3. Average number of *D. suzukii* caught per trap at site 1300 in 2014-2019

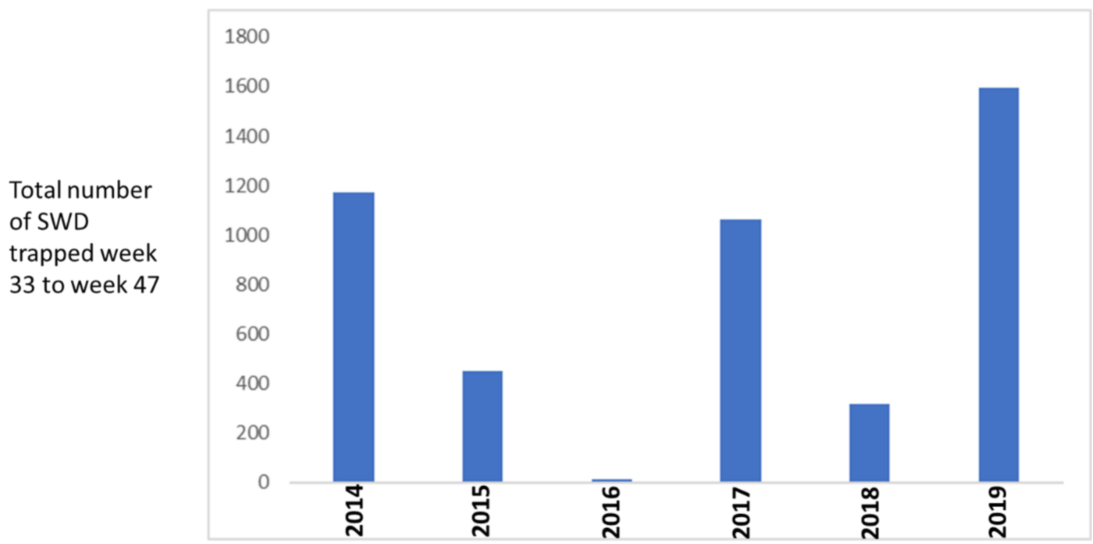


Figure 1.1.4. Total number of *D. suzukii* caught in three traps at site 1300 in week 33 (mid-August) to week 47 (end November) for 2014-2019

For the Scotland monitoring site (1300) we rely on the goodwill of the grower to service the traps and collect the catch. Monitoring was not carried out from 29 November 2018 until 4 February 2019. Therefore, we do not have data from this site for this important overwintering period. However, the catch numbers were very low from 4 Feb 2019 when monitoring commenced until late August 2019 when numbers started to rise. Winter/spring numbers are shown in Table 1.1.1.

Table 1.1.1. Winter spring catches of *D. suzukii* (3 traps) site 1300 in 2019.

Week beginning	14 Feb	20 Mar	20 Aug
Number of male *D. suzukii*	4	0	1
Number of female *D. suzukii*	1	1	0

Figure 1.1.5 to 1.1.7 demonstrate the variability between catches in the same regions in different years. Data from Yorkshire was only collected at one site from 2016. In Scotland, the numbers remain low at the national monitoring sites possibly because the available period of activity of *D. suzukii* to reproduce over a season is more restricted. Figure 1.1.8 shows variation in trap catches for the NIAB EMR site only from 2013 to the 2020.

In addition, NIAB EMR staff visited Rothamsted Research and sorted through samples that were positive for *D. suzukii,* collected from suction traps as part of the Rothamsted Insect Survey (RIS) (Figure. 1.1.9). 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 2013-18). Further counts and confirmation will be done in spring 2020. Traps in Scotland are still to be checked (Fig. 1.1.10).

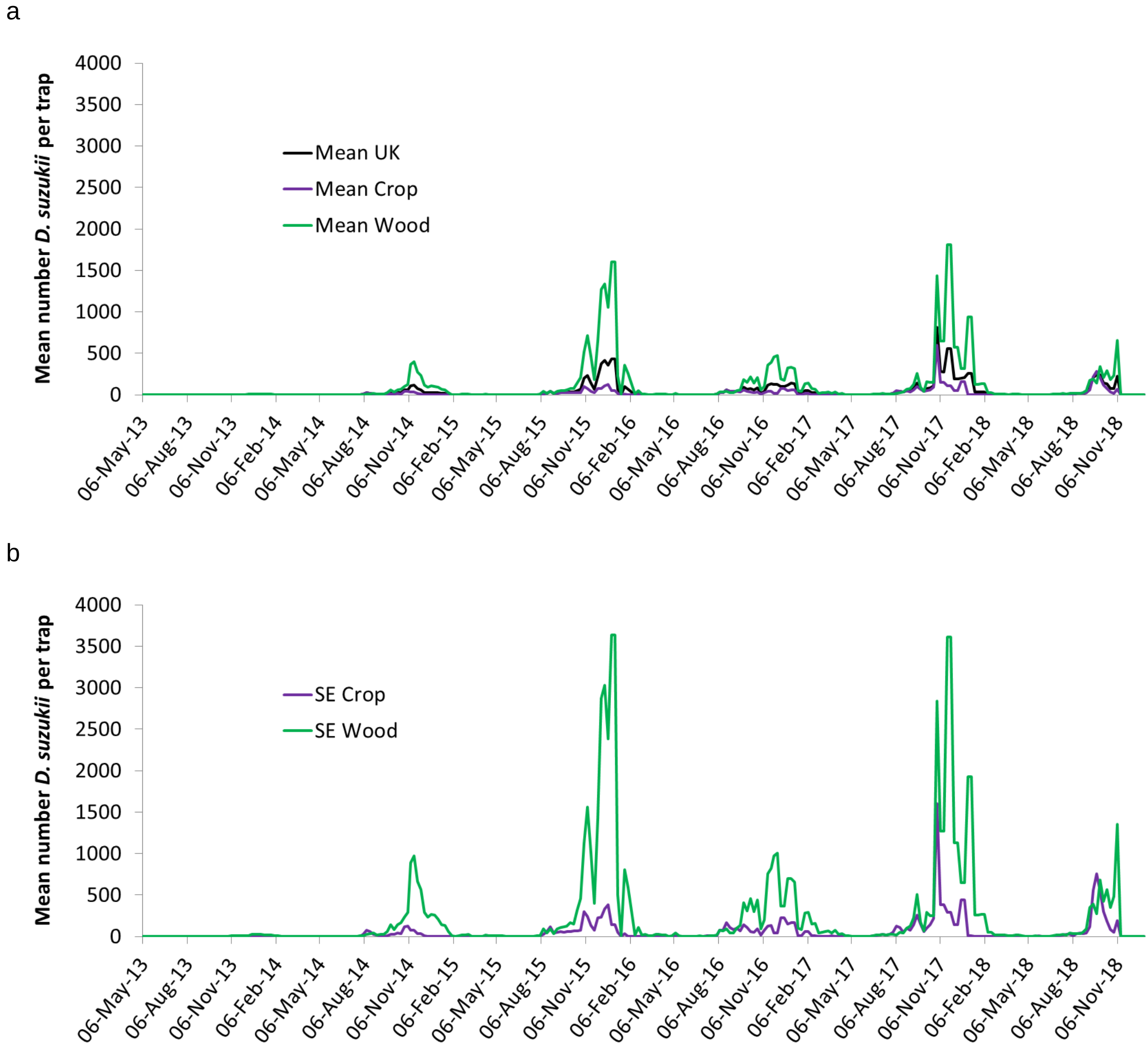
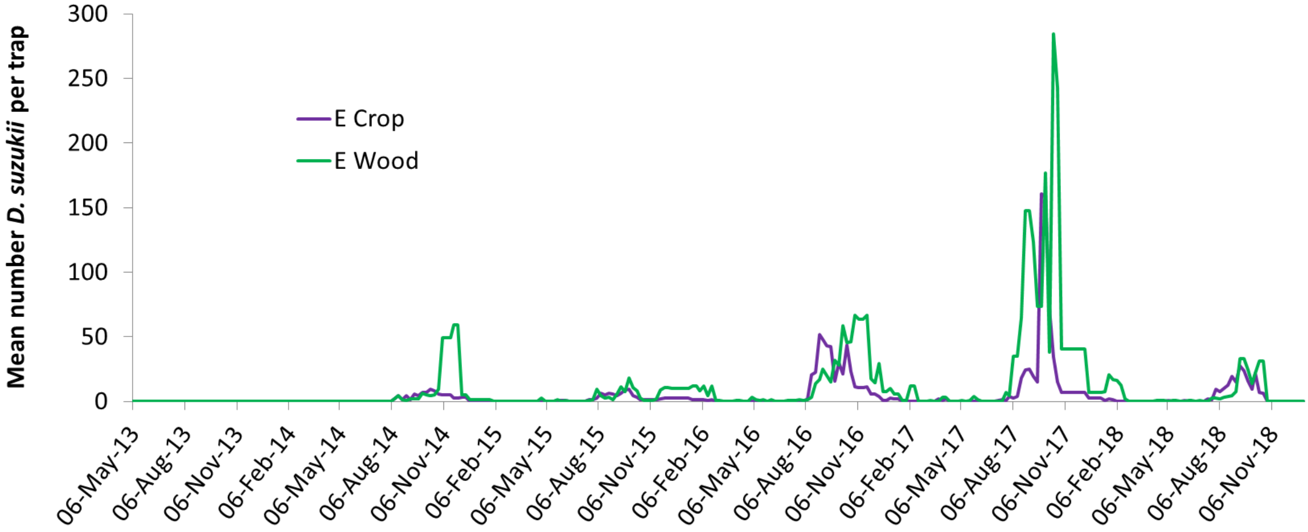


Figure 1.1.5. Mean numbers of *D. suzukii* adults per trap a) in the UK and b) in the South East of England (SE) from 2013 to 2018

a



b

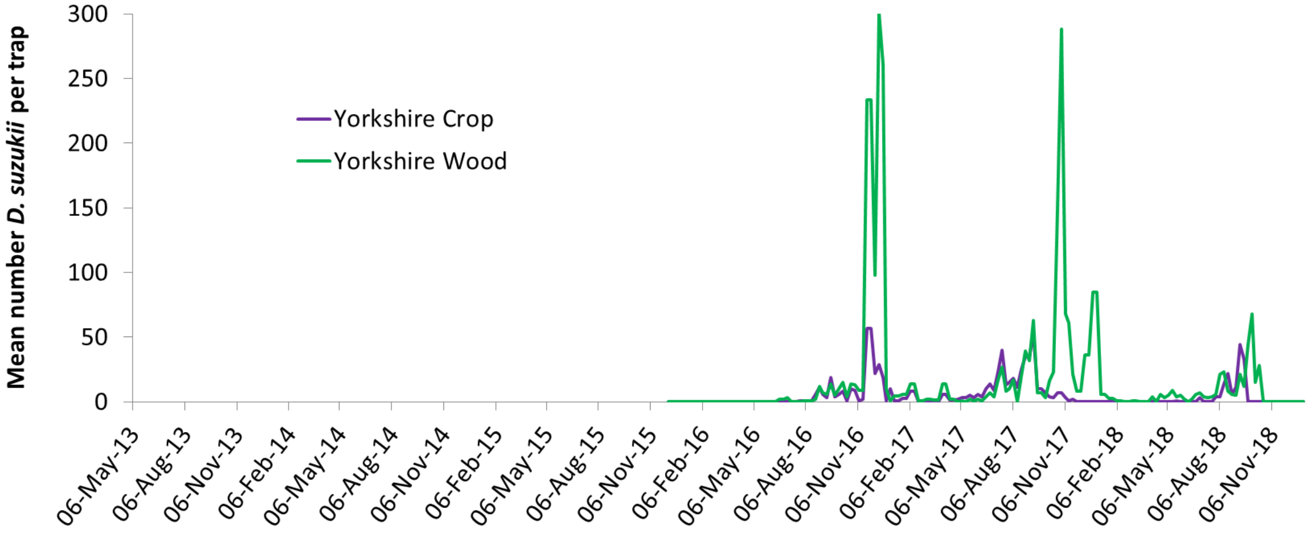
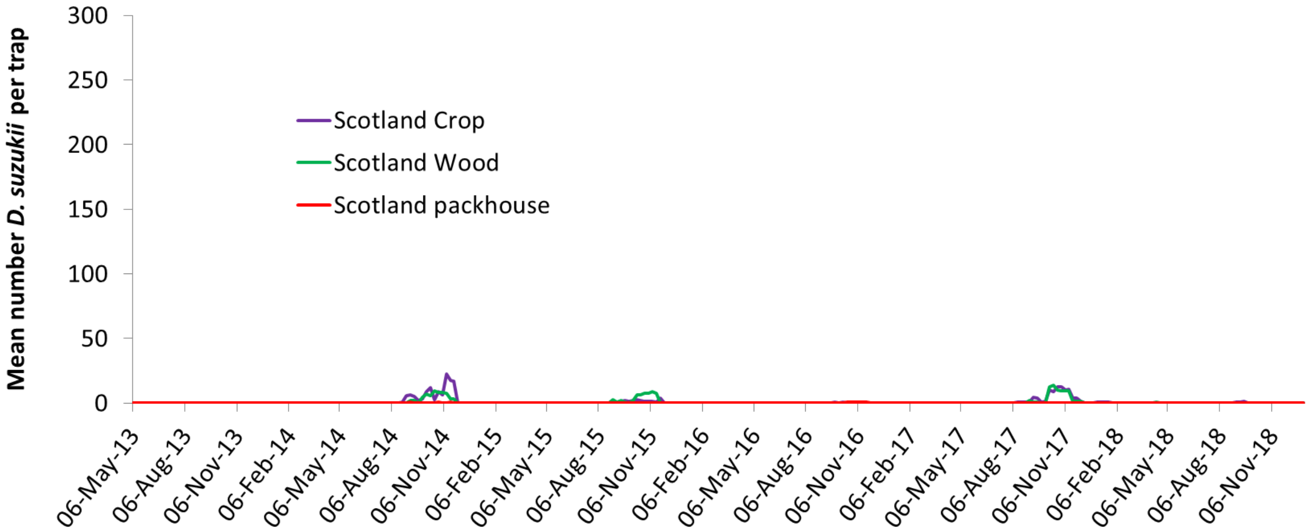


Figure 1.1.6. Mean numbers of *D. suzukii* adults per trap in a) East England (E) and b) Yorkshire (NB monitoring only began in January 2016) from 2013 to 2018

c



d

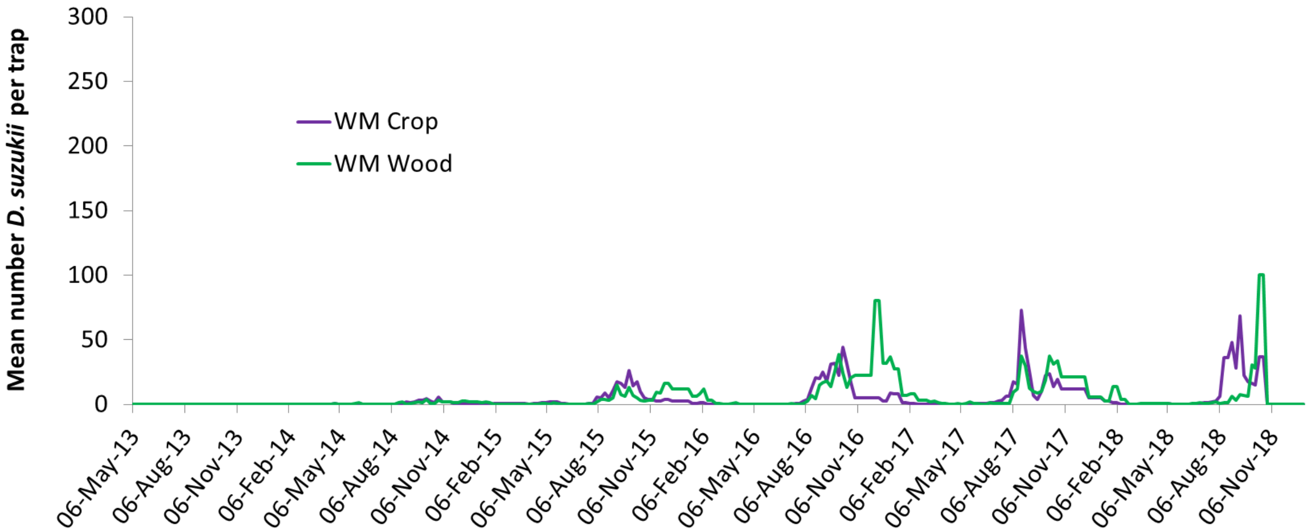


Figure 1.1.7. Mean numbers of *D. suzukii* adults per trap in c) Scotland and d) the West Midlands (WM) from 2013 to 2018

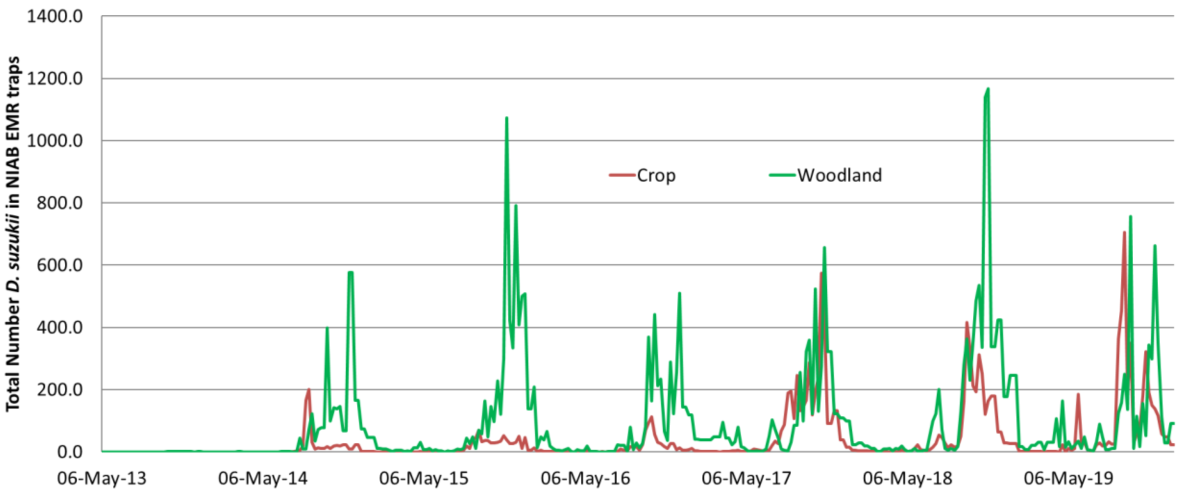


Figure 1.1.8. Mean numbers of *D. suzukii* adults per trap at the NIAB EMR site from 2013 to 2019

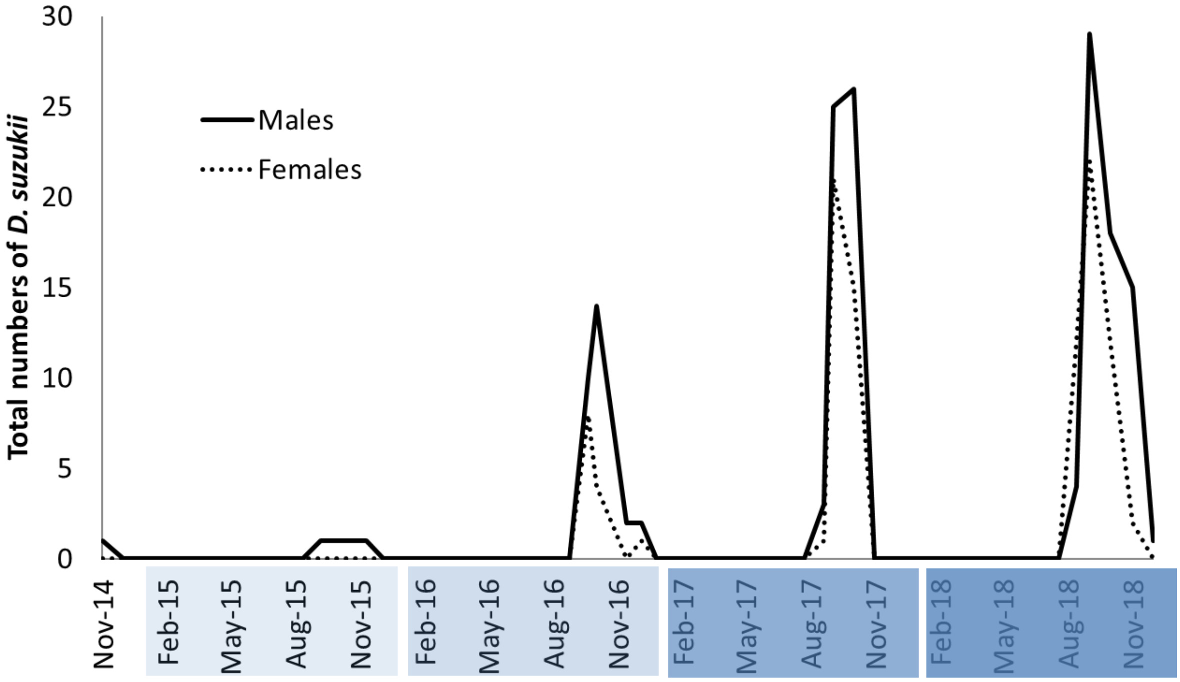


Figure 1.1.9. Total numbers of *D. suzukii* adults in 12 m height suction traps (Rothamsted Research) from 2013 to 2017. First catches were in 2014. 2019 samples will be assessed later in 2020

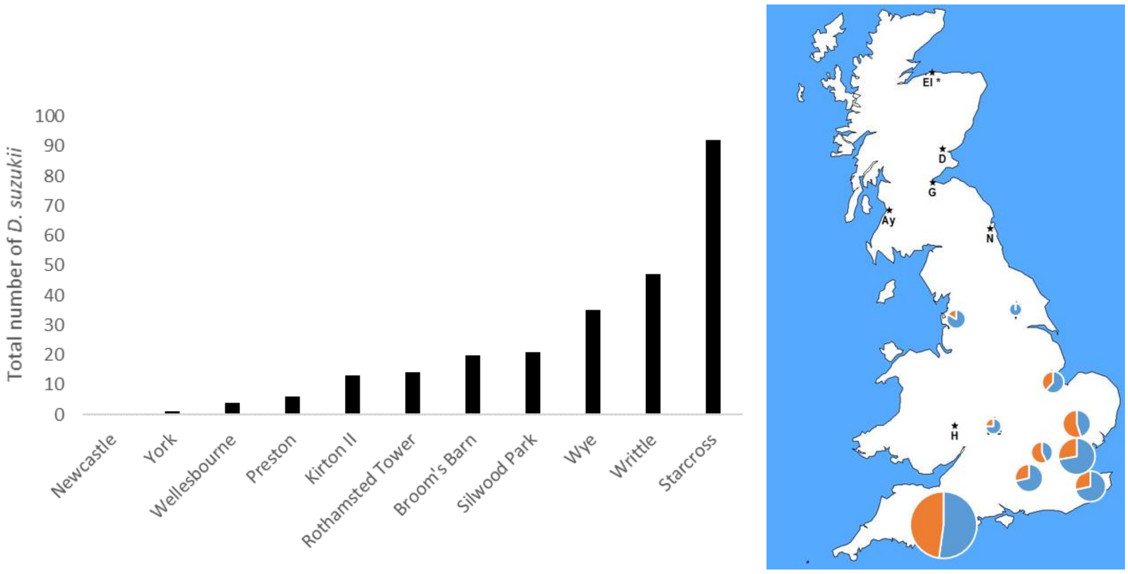


Figure 1.1.10. Total numbers of *D. suzukii* adults from 12 m height Rothamsted Suction Trap samples (2014-18) at different locations. NB: traps from Scotland have not yet been checked. Orange = females, Blue = males

An enquiry was made by JHI to Fiona Highet and Mairi Carnegie at SASA, Edinburgh on 17 October 2019 regarding the monitoring of *D. suzukii* in the Scottish suction traps. Currently, the system is only being used to monitor aphids and psyllids.

All by-catch material is kept and stored at SASA. A request was made for any by-catch material caught during September/October in either 2014, 2017 or 2019 to be made available to researchers at JHI. These periods represent the peak periods of *D. suzukii* activity as assessed by the National Monitoring data and, therefore, these catches would be the most likely to contain *D. suzukii* individuals. Provisionally the exchange of material has been agreed, however, due to seasonal changes and workload priorities, it may be some time before SASA staff would be able to process the 'unsorted' catches for sending to JHI. By-catch insect material will be provided on condition that it be returned to SASA once the data collection is complete.

Conclusions

- *D. suzukii* numbers at NIAB EMR in 2019, overall, were slightly higher than 2017 and 2018.
- There continues to be variation in interannual trap catches, at least in the late autumn, probably largely dependant upon temperature.

- *D. suzukii* can be detected at 12 m during the main flight/dispersal period when the flies are captured in the traps in cropping and woodland areas (September - 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 7-year National Monitoring dataset (Peter Skelsey JHI)

Objectives

1. Develop a model to predict percentage emergence (abundance) of *D. suzukii* populations.
2. Develop a model that can predict mean weekly *D. suzukii* capture patterns.

Data

Mean abundance (male + female) data from 16 locations for 2013-2018 were used for analysis. This made a total of 96 datasets (16 sites x 6 years). UK hourly synoptic Met. Office observations for temperature, precipitation, wind speed, wind direction, wind gust, relative humidity, and sunshine duration were used.

Modelling

Abundance model

Determination of the temporal patterns of *D. suzukii* abundance has potential application for pest management because it can help ensure that phenologies of candidate agents for biocontrol or chemical applications are synchronous with those of the pests they are targeted to control. To develop a distribution model of *D. suzukii*, proportional abundance of *D. suzukii* for each site-year dataset was calculated, and then abundance by sampling dates was cumulated. This was done from April 1 to Mar 31 in the following year to account for the seasonality in trap catches (peak abundance in Autumn-Winter and decline in Spring-Summer). Degree days (DD) were calculated as the sum of daily average temperatures minus a baseline temperature for *D. suzukii* development.

Proportional abundance data for each site-year was analysed relative to accumulated degree days using five nonlinear regression models: sigmoid, logistic, Weibull, exponential-Weibull, and Gompertz. The logistic model had the highest R^2 values and was therefore selected as the preferred model (Fig. 1.2.1).

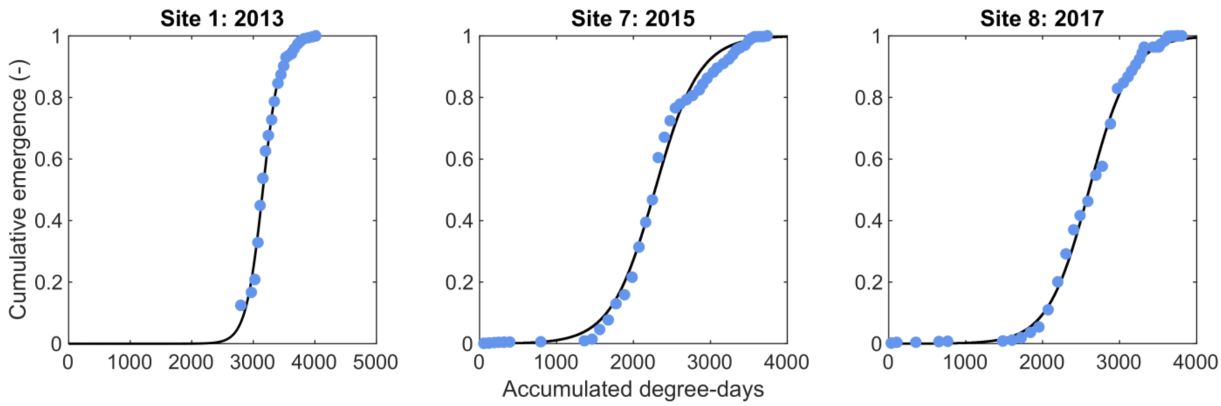


Fig. 1.2.1. Comparison between observed and estimated cumulative abundance of D. suzukii at three representative site-year combinations

Then the required DD (i.e. time) to reach any proportion of cumulative population abundance can easily be predicted using the fitted parameters for any monitoring site. This is useful if a control procedure is required at or prior to a certain level of population abundance; the DD required to reach that level can be predicted at the start of the season, and then degree days can be accumulated throughout the season using temperature observations. The model 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 highly successful in predicting percentage cumulative abundance, with R^2 values of 0.72, 0.86, 0.97, 0.99, 0.99, and 0.92 for 5, 10, 25, 50, 75, and 95% emergence, respectively (Fig. 1.2.2).

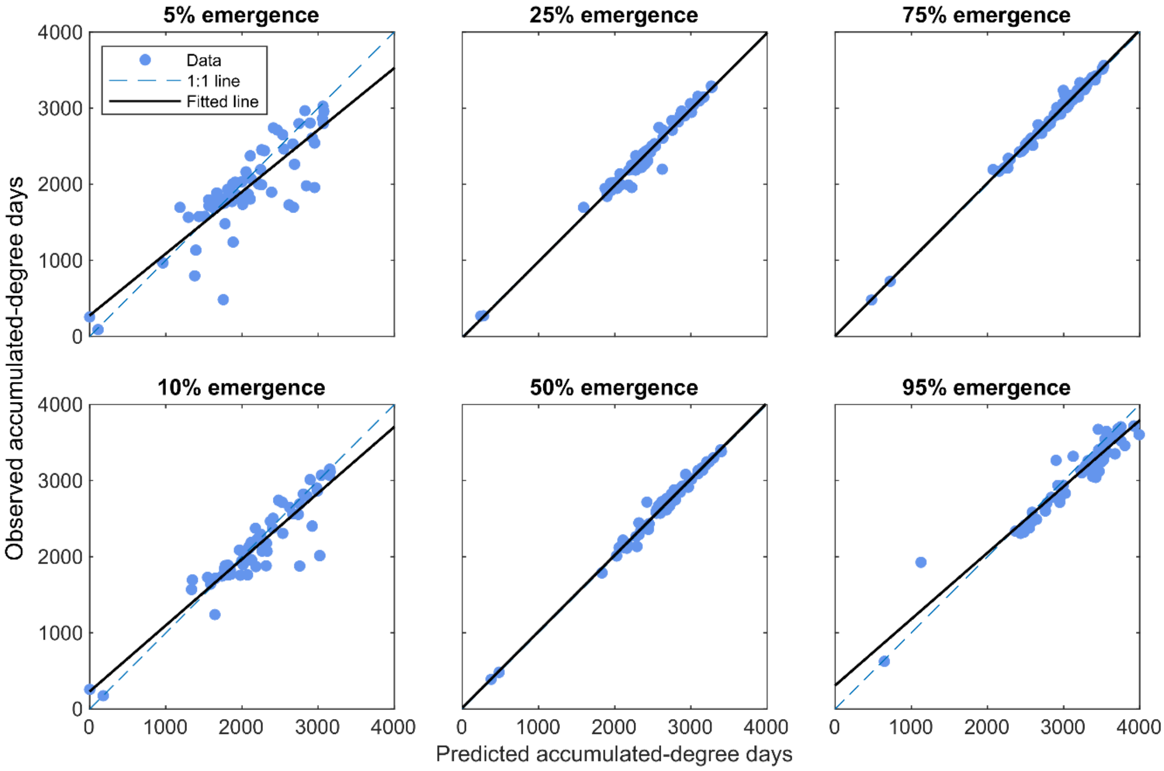


Fig. 1.2.2. Observed vs. predicted regression scatter plots for percentage emergence (abundance) of *D. suzukii* at all site-year combinations

Flight activity model

The ability to forecast the presence/absence of *D. suzukii* (i.e. flight activity) at any location on any given day based on weather conditions would provide another useful tool for decision-making. Machine learning techniques were used to develop a classification model that predicts if *D. suzukii* will be present or absent (1 = presence, 0 = absence).

Trap counts 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 (typically 7 days), and the latitude and longitude of the postcode district centroid were also included as predictor variables. The data were then split into a 'training' dataset, used to learn the parameters of a model, and a 'test' dataset, used to provide an unbiased estimate of how well a model would generalize to unseen data. There were 3,888 site-date observations in the training data, and 848 in the test data.

A suite of 30 different machine learning techniques were applied to the training data. The most successful algorithm misclassified a total of 68 site-date trap values (1 = presence, 0 = absence of *D. suzukii*) out of the 848 examples in the test dataset, giving it a classification accuracy of 92% (Fig. 1.2.3).

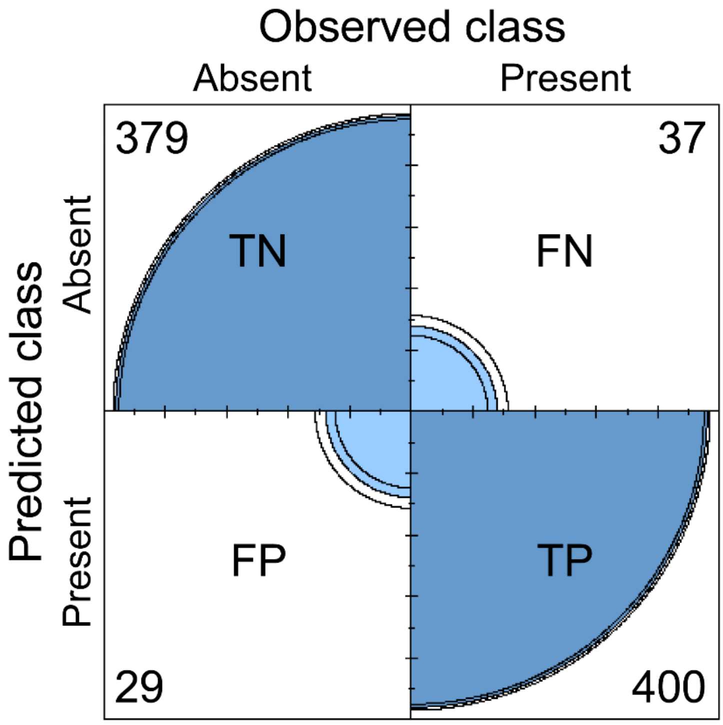


Fig. 1.2.3. Confusion matrix for binary classification of D. suzukii trap catches (*D. suzukii* are absent or present). TN = true negative result, FN = false negative, FP = false positive, and TP = true positive, where the numbers in each quadrant refer to the number of test examples

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

Push–pull technology 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), increasing numbers of natural enemies in the crop and increasing 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 discouraging 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).

Four compounds, including geosmin and 1-octen-3-ol, have shown some efficacy in small plot (single tree) experiments with fruit as bait for egg laying females at NIAB EMR. In more recent experiments (SF145), 25 sachets per cherry tree did not deter *D. suzukii* egg laying. Since these initial studies, a NIAB EMR CTP student (Christina Conroy) in collaboration with NRI has identified 3 repellent compounds using a range of laboratory and semifield experiments. One pilot study at the end of 2019 showed a linear relationship between the point source repellent and a declining incidence of *D. suzukii* egg laying. These PhD studies will continue into 2020 with the range of 3 compounds to determine potency of repellence and distance from point source in unsprayed strawberry crops.

In addition, larger scale trials will be needed on formulations to ensure that repellents are long lasting and remain effective. Work is needed on the best time to apply repellents and discover if they cease to become effective once *D. suzukii* is already in the crop. Pest repellents for other horticultural crops have recently been developed in an Innovate UK project and formulation testing as emulsifyable or micro-encapsulated sprays or sachets has been completed.

Although none of the compounds proposed here are on Annex 1, repellents may need to be registered in the same way as for attractants - using the new semiochemical guidance as a framework, but, as the compounds involved are Generally Regarded as Safe (GRAS) this should speed the availability for use.

Repellents are more likely to be effective if used in combination with other control methods, especially, with Attract and Kill (A&K) technology to form a Push-Pull strategy; pushing away from the crop and pulling towards an attractant which would contain a distracting or fatal component (Eigenbrode et al. 2016).

Although earlier work in SF 145 did not show a convincing deterrent we did develop a prototype A&K device which gave up to 25% kill of *D. suzukii* every 24 hours in semi-field cage in the absence of fruit. However, in the presence of ripe fruit, the efficacy of this and a commercial device decreased substantially killing up to only 15% of flies within 24 hours. This suggests that these A&K devices can have the highest impact in the winter when food resources are scarce.

Trials in this project are currently studying the effects of precision monitoring away from the crop. It is anticipated that these two approached can be combined for year-round depression of *D. suzukii* populations.

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

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 a laboratory bioassay and field trapping tests. The aim of this work was to identify the chemicals produced and investigate whether there was any correlation between these chemicals and attractiveness to SWD.

Similar work was reported previously by Scheidler et al. (2015), and the methodology used here was essentially the same in order to allow comparison with the results obtained.

Materials and Methods

Yeast samples

Preliminary studies were done on a sample of sourdough yeast provided by Prof Hall. Subsequent studies were done on yeast strains provided by Rory Jones as in Table 2.1.1.

Table 2.1.1. Origin, source and strain of yeast species used in choice tests. Yeasts were grown in sterile strawberry juice.

Yeast Strain	Origin	Source	Reference
EC-1118	France	Commercial wine yeast	Lallemand Inc.
218	New Zealand	Pinot noir ferment	Goddard culture collection
164	New Zealand	Chardonnay ferment	Anfang *et al.,* 2009.
190	New Zealand	Sauvignon Blanc ferment	Goddard culture collection

Sample collection and analysis

Yeast suspensions (5 ml) were placed in 10 ml glass sample vials and sealed with aluminium foil. Volatiles were sampled by solid-phase microextraction (SPME) for 30 min at room temperature (20-22ºC). In the preliminary studies, SPME needles coated with PDMS (red) or with divinylbenzene/Carboxen/PDMS (grey) (Supelco, Gillingham, Dorset) were evaluated. In the main studies only the latter were used.

Collections were analysed by gas chromatography coupled to mass spectrometry (GC-MS) on a Varian CP3700 GC coupled to a Saturn 2200 mass spectrometer operated in EI mode (Varian, now Agilent, Manchester). The GC was fitted with fused silica capillary columns (30 m x 0.25 mm i.d. x 0.25 µm film thickness) coated with non-polar VF5 (Varian) or polar DBWax (Agilent) with a column switching system. Carrier gas was helium (1 ml/min for theVF5 column and 1.5 ml/min for the DBWax) and both injectors were at 220ºC and operated in splitless mode with the split opening after 1 min. The oven temperature was programmed from 40ºC for 2 min then at 10ºC/min to 250ºC and held for 5 min.

Data were captured and processed with MS Data Station software (Varian). Retention Indices (RI) of compounds were calculated by comparison of their retention times (RT) with those of a series of *n*-alkanes, and compounds were identified by matching their mass spectra with those in the NIST library and confirmed by comparison of their retention indices and mass spectra with those of authentic standards. The latter were available at NRI, mostly purchased from SigmaAldrich (Gillingham, Dorset, UK).

Results

Preliminary studies

Initial studies were done on the sourdough sample. Volatiles were sampled with PDMS (red) and divinylbenzene/Carboxen/PDMS (grey) SPME fibres and analysed by GC-MS on both non-polar (Figure 2.1.1) and polar GC columns (Figure 2.1.2).

For analyses on both GC columns, visual comparison showed that larger quantities and larger number of compounds were collected with the grey fibre than the red fibre. Visual comparison also indicated that peaks were sharper and better separated on the polar column (Figure 2.1.2) than the non-polar column (Figure 2.1.1).

Compounds identified in collections with the two types of SPME fibre and analysed on the polar GC column are shown in Table 2.1.2. Total areas were 52,606,021 and 8,739,381 for the grey and red fibres, respectively, confirming the larger amount collected by the former. The selectivity of adsorption by the different fibre coatings is markedly different, as illustrated by the greater amount of acetic acid (9.26 min in Figure 2.1.2) collected on the red fibre than the grey.

Table 2.1.2. Compounds identified in collections with PDMS/divinylbenzene/Carbopack (grey) and PDMS (red) SPME fibres and analysed on the polar DBWax GC column

			Area %	
RT (min)	**RI**	**Compound**	**Grey**	**Red**
2.30		ethyl acetate	21.6	8.9
2.70		ethanol	20.1	29.9
4.26	1071	hexanal	0.4	0.0
4.38	1080	hydrocarbon	0.2	0.0
4.54	1092	hydrocarbon	0.4	0.0
4.82	1113	2/3-methylbutyl acetate	1.4	1.2
5.49	1163	silicon impurity	0.4	0.0
6.14	1212	2/3-methylbutanol	16.1	8.6
6.44	1234	ethyl hexanoate	1.4	0.0
6.72	1255	styrene + alcohol?	1.8	0.0
7.01	1276	silicon	3.2	5.9
7.97	1347	ethyl lactate	0.9	0.0
8.17	1362	hexanol	15.2	3.5
9.20	1442	ethyl octanoate	1.3	2.7
9.26	1446	acetic acid	5.9	30.7
9.39	1457	1-octen-3-ol?	1.0	0.0
9.49	1465	heptanol?	2.4	0.0
9.99	1505	silicon impurity	0.3	1.0
10.76	1566	octanol	1.9	0.0
13.94	1848	hexanoic acid	0.6	0.0
14.71	1922	2-phenylethanol	3.4	7.7
		Total integration (K)	52,606	8,739

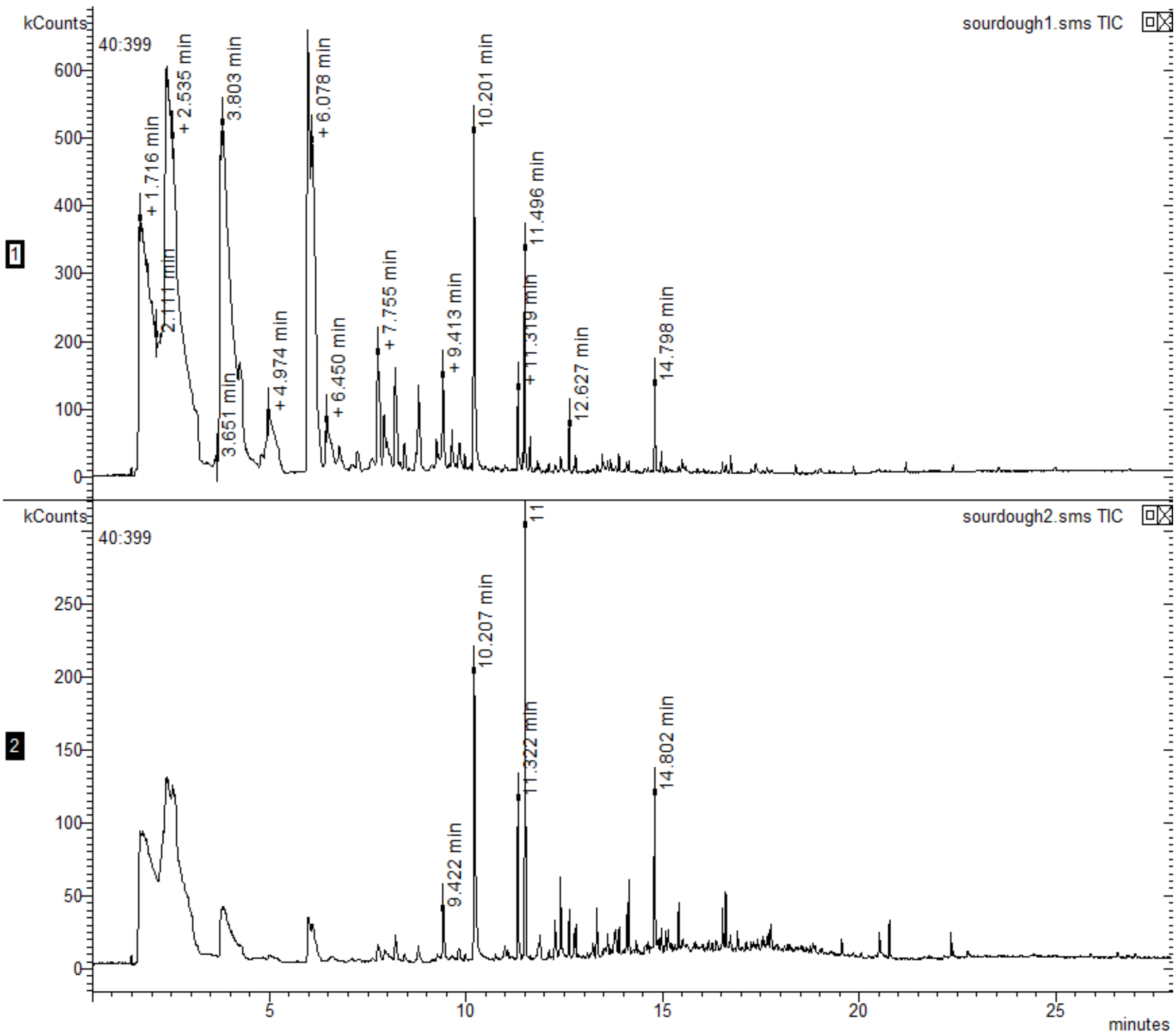


Fig. 2.1.1. GC-MS Analyses of sourdough volatiles on non-polar VF5 GC column collected with divinylbenzene/Carboxen/PDMS fibre (upper) and PDMS fibre (lower)

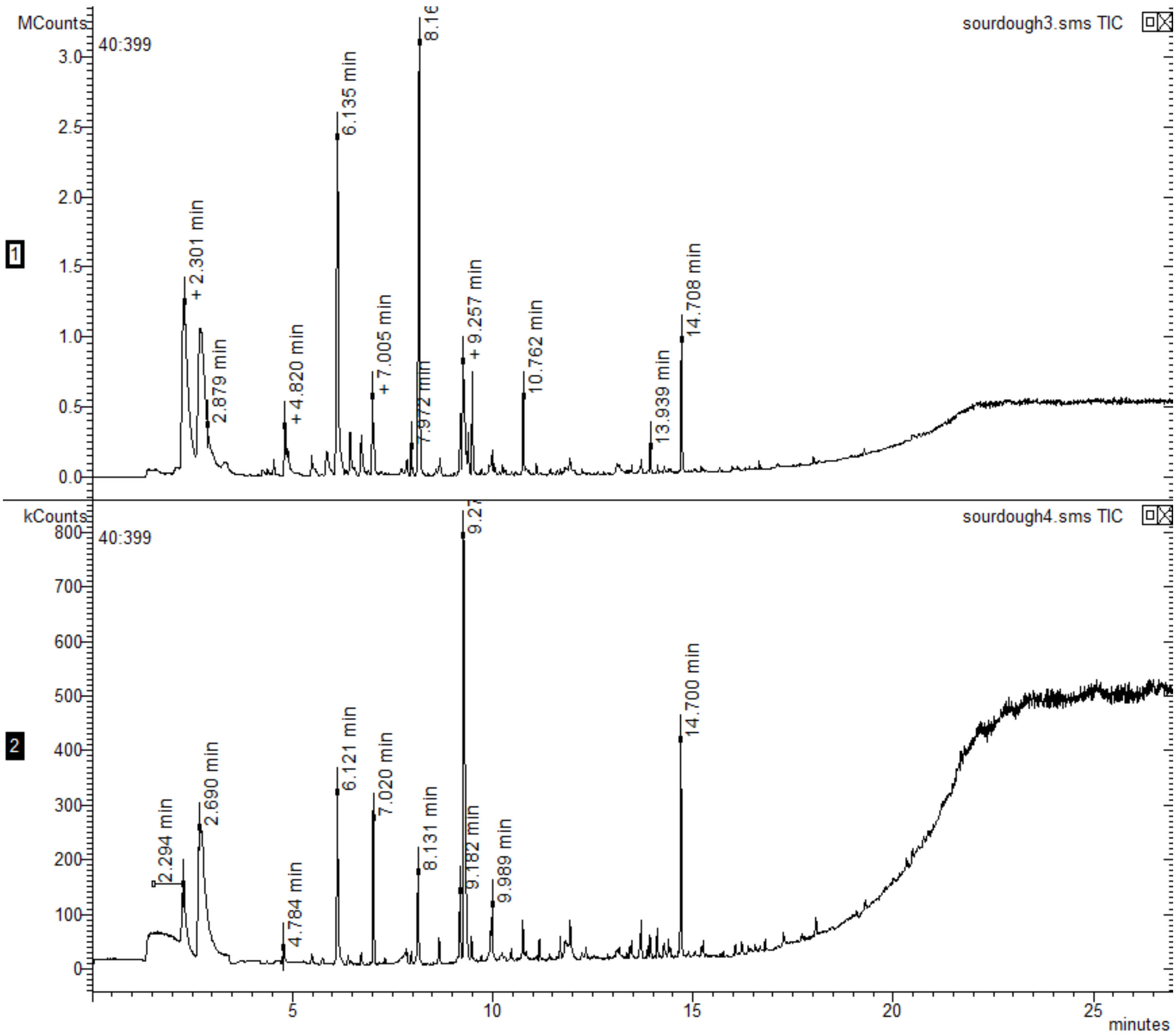


Fig. 2.1.2. GC-MS Analyses of sourdough volatiles on polar DBWax GC column collected with divinylbenzene/Carboxen/PDMS fibre (upper) and PDMS fibre (lower)

In view of these preliminary findings, subsequent collections were done with the grey fibre and analysed on the polar column. Replicate analyses (*N* = 3 or 4) were done on each sample, and results were consistent between replicates. For example, replicate analyses on the 218 sample are shown in Figure 2.3.

Compounds identified and their relative peak areas in the GC-MS analyses are shown in Table 2.3. Unfortunately, the basic strawberry juice and hence all the yeast samples contained large amounts of a silicon impurity eluting around 3.5 min (e.g. Figure 2.3). This did not seem to conceal any other peaks of interest and was excluded from the analyses, as were several other small peaks due to silicon impurities from the SPME fibres.

Compounds present are shown with their peak areas in the analyses in Figure 2.3 and the relative amounts in the analyses in Table 2.4. Compounds present in the sterile strawberry juice are shaded. Additional compounds or those apparently present in greater quantities in the volatiles from the yeast ferments are shown in red.

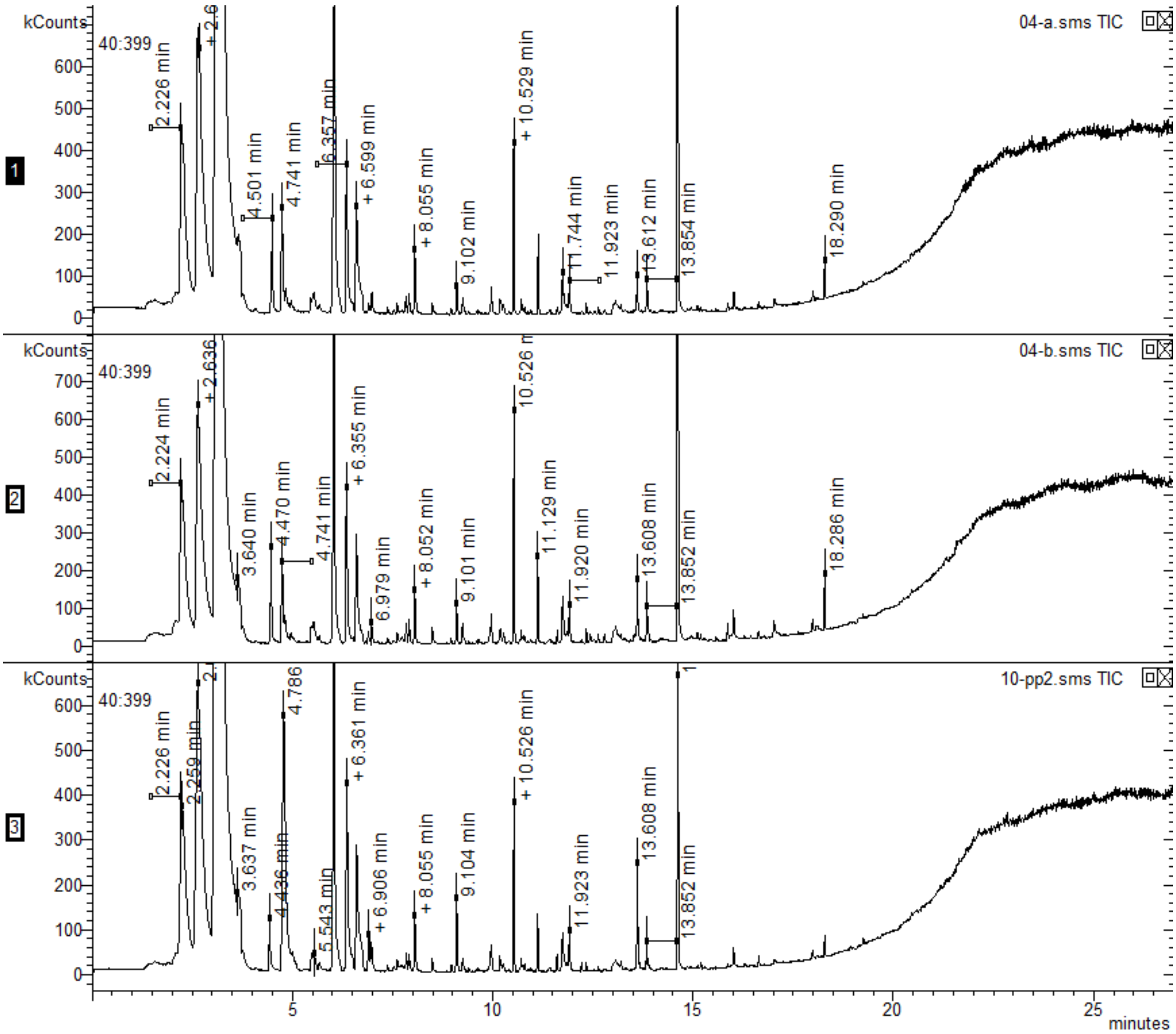


Fig. 2.1.3. GC-MS Analyses of replicate collections from yeast sample 218 collected with grey SPME fibre and analysed on the polar GC column

Table 2.1.3. Compounds identified in volatiles from sterile strawberry juice (SSJ) and yeast strains collected on grey PDMS/divinylbenzene/Carboxen SPME fibre and analysed by GC-MS on polar DBWax column and their peak areas in the analyses; compounds in SSJ shaded, additional to SSJ in red (RT retention time; RI retention index relative to RT of *n*-alkanes; SE standard error).

RT			SSJ (N=3)		201 (N=4)		190 (N=4)		218 (N=4)		164 (N=3)		EC-1118 N=3)	
(min)	RI	Compound	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
2.22		ethyl acetate	0	0	9127	551	1492	241	3235	134	0	0	147	147
2.65		ethanol	1152	194	3733	302	2080	371	6207	286	5980	868	5858	744
3.78	1039	m/z105 silicon impurity?	0	0	4049	361	0	0	0	0	2162	438	0	0
4.42	1087	hydrocarbon	0	0	47	17	238	36	321	121	105	17	339	28
4.78	1114	2/3-methylbutyl acetate	0	0	231	84	0	0	1606	602	0	0	420	161
5.39	1160	alcohol?	0	0	0	0	0	0	0	0	389	62	114	114
5.68	1182	methyl hexanoate	484	111	0	0	0	0	0	0	0	0	0	0
6.03	1208	2/3-methylbutanol	18	18	1836	138	1334	181	3324	229	684	100	2603	226
6.35	1232	ethyl hexanoate	424	101	64	37	0	0	1220	89	163	10	1096	22
6.60	1251	styrene	0	0	0	0	0	0	1055	38	0	0	1513	144
6.88	1272	hexyl acetate	15	15	0	0	0	0	109	65	311	30	0	0
6.99	1280	alcohol?	36	36	164	26	13	13	96	37	904	13	78	39
7.07	1286	acetoin	0	0	0	0	0	0	0	0	0	0	0	0
7.75	1337	(E)-2-hexenyl acetate	57	34	0	0	0	0	0	0	0	0	0	0
7.92	1349	ethyl 2-hexenoate	0	0	0	0	0	0	0	0	0	0	0	0
8.05	1359	hexanol	0	0	278	11	356	30	277	21	242	21	306	22
8.75	1412	(E)-2-hexenol	18	18	0	0	0	0	0	0	0	0	0	0
8.97	1430	di-tertbutyl-benzene	0	0	0	0	0	0	0	0	0	0	0	0
9.10	1440	ethyl octanoate	0	0	0	0	0	0	226	46	0	0	199	18
9.25	1452	acetic acid	0	0	276	32	0	0	0	0	43	43	122	61
10.21	1529	benzaldehyde	632	138	353	33	308	55	0	0	160	80	174	87
10.53	1554	linalool	780	192	649	80	624	75	749	99	630	13	754	88
11.14	1604	strawberry furan	309	59	267	36	257	32	289	37	254	20	303	27

11.60	1644	ethyl decanoate	0	0	0	0	0	0	0	0	0	0	0	0
11.75	1658	4-methylphenyl-glyoxal?	197	33	168	63	143	51	133	79	148	74	314	56
11.91	1672	2/3-methyl butanoic acid	58	58	140	8	142	13	149	11	146	91	169	8
12.34	1710	terpineol	0	0	0	0	0	0	0	0	0	0	0	0
12.65	1738	benzyl acetate	18	18	0	0	0	0	0	0	0	0	0	0
13.62	1826	2-phenylethyl acetate	0	0	0	0	0	0	351	106	0	0	72	36
13.86	1849	hexanoic acid	138	22	82	31	87	9	167	24	120	8	143	11
14.63	1923	2-phenylethanol	0	0	228	30	201	43	1925	250	268	23	976	116
15.88	2048	nerolidol	0	0	0	0	0	0	0	0	0	0	0	0
16.01	2061	octanoic acid	0	0	0	0	0	0	44	44	0	0	27	27
16.78	2130	ethyl cinnamate	0	0	0	0	0	0	0	0	0	0	0	0
		Total integration (K)	4,337		21,693		7,275		21,483		12,708		15,726	

Table 2.1.4. Compounds identified in volatiles from sterile strawberry juice (SSJ) and yeast strains collected on grey PDMS/divinylbenzene/Carboxen SPME fibre and analysed by GC-MS on polar DBWax column and their relative percentage amounts; compounds in SSJ shaded, additional to SSJ in red (RT retention time; RI retention index relative to RT of *n*-alkanes; SE standard error).

RT			SSJ (N=3)		201 (N=4)		190 (N=4)		218 (N=4)		164 (N=3)		EC-1118 (N=3)	
(min)	RI	Compound	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
2.22		ethyl acetate	0.00	0.00	41.99	1.67	20.33	0.77	15.07	0.69	0.00	0.00	0.87	0.87
2.65		ethanol	27.35	4.01	17.16	1.11	28.27	3.02	28.89	1.17	46.67	5.16	37.03	3.33
3.78	1039	m/z105 silicon impurity?	0.00	0.00	18.72	1.79	0.00	0.00	0.00	0.00	17.04	3.31	0.00	0.00
4.42	1087	hydrocarbon	0.00	0.00	0.21	0.08	3.25	0.14	1.52	0.58	0.85	0.18	2.15	0.10
4.78	1114	2/3-methylbutyl acetate	0.00	0.00	1.08	0.41	0.00	0.00	7.33	2.66	0.00	0.00	2.64	1.02
5.39	1160	alcohol?	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.11	0.62	0.74	0.74
5.68	1182	methyl hexanoate	11.15	1.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6.03	1208	2/3-methylbutanol	0.38	0.38	8.53	0.85	18.34	0.81	15.53	1.29	5.38	0.74	16.59	1.56
6.35	1232	ethyl hexanoate	9.83	1.41	0.31	0.18	0.00	0.00	5.67	0.36	1.29	0.09	7.00	0.37
6.60	1251	styrene	0.00	0.00	0.00	0.00	0.00	0.00	4.92	0.21	0.00	0.00	9.68	1.10
6.88	1272	hexyl acetate	0.34	0.34	0.00	0.00	0.00	0.00	0.49	0.29	2.48	0.36	0.00	0.00
6.99	1280	alcohol?	0.65	0.65	0.76	0.14	0.19	0.19	0.45	0.17	7.15	0.41	0.49	0.25
7.07	1286	acetoin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.75	1337	(E)-2-hexenyl acetate	1.10	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.92	1349	ethyl 2-hexenoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.05	1359	hexanol	0.00	0.00	1.29	0.08	4.97	0.23	1.29	0.11	1.90	0.08	1.94	0.07
8.75	1412	(E)-2-hexenol	0.62	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.97	1430	di-tertbutyl-benzene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9.10	1440	ethyl octanoate	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.20	0.00	0.00	1.26	0.08
9.25	1452	acetic acid	0.00	0.00	1.27	0.14	0.00	0.00	0.00	0.00	0.37	0.37	0.80	0.40
10.21	1529	benzaldehyde	14.31	0.77	1.63	0.14	4.27	0.66	0.00	0.00	1.32	0.67	1.15	0.58
10.53	1554	linalool	17.68	1.71	2.99	0.34	8.84	1.31	3.50	0.48	4.98	0.21	4.83	0.66
11.14	1604	strawberry furan	7.11	0.33	1.23	0.15	3.63	0.51	1.35	0.18	2.00	0.16	1.94	0.22

11.60	1644	ethyl decanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11.75	1658	4-methylphenyl-glyoxal?	4.56	0.06	0.76	0.28	1.92	0.72	0.62	0.37	1.21	0.61	2.02	0.40
11.91	1672	2/3-methyl butanoic acid	1.27	1.27	0.65	0.03	2.01	0.21	0.70	0.05	1.18	0.70	1.08	0.06
12.34	1710	terpineol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.65	1738	benzyl acetate	0.39	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13.62	1826	2-phenylethyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	1.62	0.48	0.00	0.00	0.45	0.23
13.86	1849	hexanoic acid	3.25	0.32	0.38	0.13	1.24	0.20	0.78	0.11	0.95	0.07	0.91	0.06
14.63	1923	2-phenylethanol	0.00	0.00	1.05	0.13	2.74	0.38	9.02	1.29	2.13	0.29	6.28	0.96
15.88	2048	nerolidol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.01	2061	octanoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.20	0.00	0.00	0.16	0.16
16.78	2130	ethyl cinnamate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Great care should be taken in drawing quantitative conclusions from analyses of SPME collections. As noted above, the selectivity of the fibres for trapping different compounds varies greatly according to the coating, although the divinylbenzene/Carboxen/PDMS fibres used here are probably the most generalised adsorbents available. The amount of any given substance adsorbed onto the fibre is a function of the concentration in the static atmosphere above the sample liquid, not necessarily the amount produced, and this can be affected by the presence of large amounts of other compounds present – e.g. the ethanol and ethyl acetate present in these analyses.

The collections and analyses done here were all done under identical conditions and over two days of analyses, so it is probably valid to consider presence and absence of particular compounds and, with the above provisos, to compare relative amounts present.

Total amounts of volatiles present in analyses of the yeast ferments were all higher than in those from the sterile strawberry juice, particular strains 201, 218 and EC-1118 (Table 2.1.3). The main compounds in volatiles from the strawberry juice were ethanol, methyl hexanoate, ethyl hexanoate, benzaldehyde, linalool, 2,5-dimethyl-4-methoxy-2,3-dihydro-3-furanone (strawberry furanone), 4-methylphenyl-glyoxal (?) and hexanoic acid (Tables 2.1.3 and 2.1.4). The most noteworthy additional compounds present in volatiles from the yeast ferments were ethyl acetate and 2-phenylethanol, while amounts of 2/3-methylbutanol (isoamyl alcohol) in volatiles from all the yeast ferments seemed to be greater than those from the strawberry juice. 2/3-Methylbutyl acetate (isoamyl acetate), styrene, hexanol and acetic acid were also present in significant quantities in some of the yeast ferments,

Discussion

In this study, compounds produced by five strains of yeast grown on sterile strawberry juice were identified. These results will be correlated with bioassays of attractiveness of the yeasts in laboratory and field bioassays. It is planned to repeat this work with the same yeast cultures grown on a more neutral medium as in Scheidler et al. (2015)

The most noteworthy additional compounds present in volatiles from the yeast ferments were ethyl acetate and 2-phenylethanol. Cha et al. (2012) reported that addition of ethyl acetate to a mixture of ethanol and acetic acid significantly decreased catches of SWD in field trapping tests, while 2-phenylethanol had no effect. 2-Phenylethanol seems to be a fairly ubiquitous compound in fermentations, but recent EAG studies showed no electroantennogram (EAG) responses from SWD to this compound (Dan Bray, unpublished).

2/3-Methylbutanol was detected in increased amounts from the yeast fermentations. This compound is a significant component of commercial SWD lures based on wine/vinegar mixes. It was extensively tested earlier in this project SF145, but did not show any attractiveness for SWD itself and could not replace the ethanol in a Cha-Landolt lure.

Of the other compounds identified from the yeast fermentations, acetic acid is a component of commercial SWD lures, and addition of hexanol to a mixture of ethanol and acetic acid significantly reduced the attractiveness to SWD in field trapping tests (Cha et al. 2010). 2/3-Methylbutyl acetate (isoamyl acetate) was reported to be attractive to SWD in a laboratory bioassay by Revadi et al. (2015).

Conclusions

- Compounds produced by five strains of yeast grown on sterile strawberry juice were identified. These results will be correlated with bioassays of attractiveness of the yeasts in laboratory and field bioassays.
- Having established the methodology for collection and analysis of yeast volatiles, it is planned to repeat this work with the same yeast cultures grown on a more neutral medium.
- No obvious candidate attractants for SWD were identified, although ethyl acetate, 2-phenylethanol and isoamyl acetate could be re-examined.

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

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 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. Traps have been deployed in crops and adjacent wild host refuges and mean numbers of *D. suzukii* compared fortnightly throughout the year. To date findings show highest peaks in mean numbers of *D. suzukii* to occur in wooded areas during the late autumn-winter months when there is reduced availability of commercial fruit. Subsequently, lowest numbers are recorded in late winter-early spring when overwintered flies are seeking food and hosts. The aim of this study was to determine:

- Whether the 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 the following spring.

Materials and Methods

Trial sites*:* The trial was set up at 6 commercial soft fruit crops (blocks) in Kent and Sussex. Crops tested included five strawberry 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.

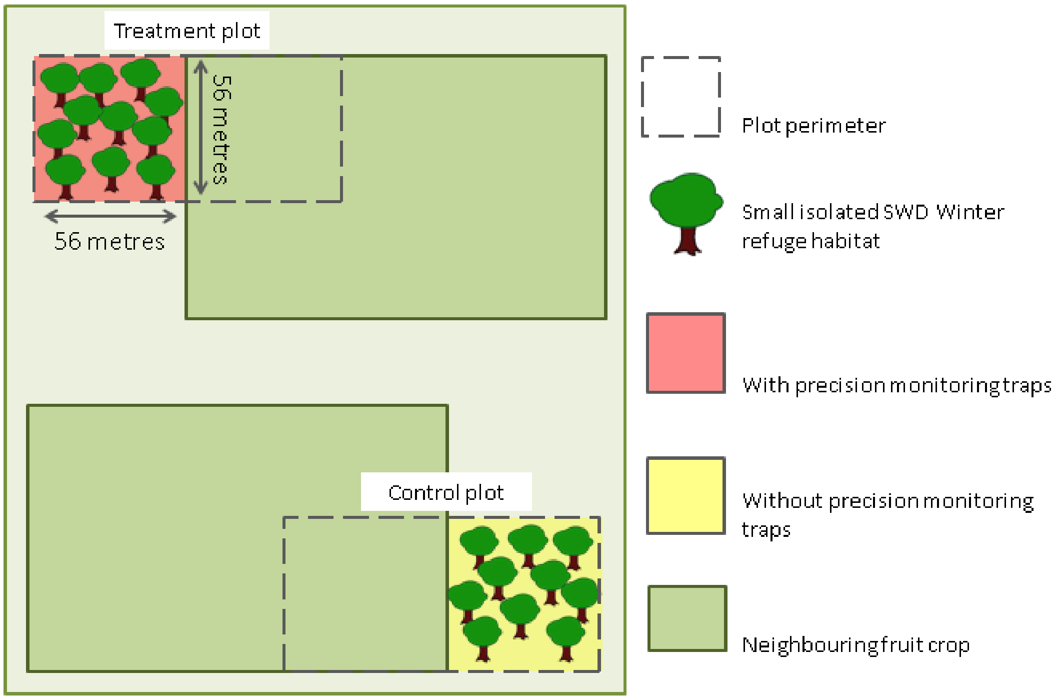


Figure 2.2.1. Diagrammatic representation of an experimental block for the precision monitoring trial 2019/20. Each block consisted of a treatment woodland plot (red square) containing precision monitoring traps and a control woodland plot (yellow square) without precision monitoring traps. Beside each woodland was a neighbouring soft fruit crop (darker green squares)

Assessments were conducted fortnightly at each block. Blocks were divided into 2 groups of 3; assessed on alternate weeks for practical reasons. See Table 2.2.3 for assessment dates.

D. suzukii monitoring – 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. Treated winter refuge habitat adjacent crop
3. Control winter refuge habitat
4. Control winter refuge habitat adjacent crop

Riga traps were deployed 2 weeks before the trial start (pre-assessment), then collected and renewed every two weeks 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 was sampled every two weeks at each block (Fig. 2.2.2). 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, Delta traps containing sentinel fruit were deployed centrally at each block on 4 to 5 occasions in the spring when climate conditions were considered warm enough for *D. suzukii* activity (Fig. 2.2.2). To confirm that sentinel fruit was not toxic to *D. suzukii* before deployment (from insecticide residues), 5 male and 5 female *D. suzukii* from cultures at NIAB EMR were applied to 3 fruit for 48 hours. *D. suzukii* mortality was then counted within this period and fruit was incubated at ~22 °C, >40 % RH, 16 h light: 8 h dark for 14 days. During this period, emerged adult *D. suzukii* were counted to confirm egg-laying.

At each deployment, sentinel fruit was left in the field for 3-7 days, 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.

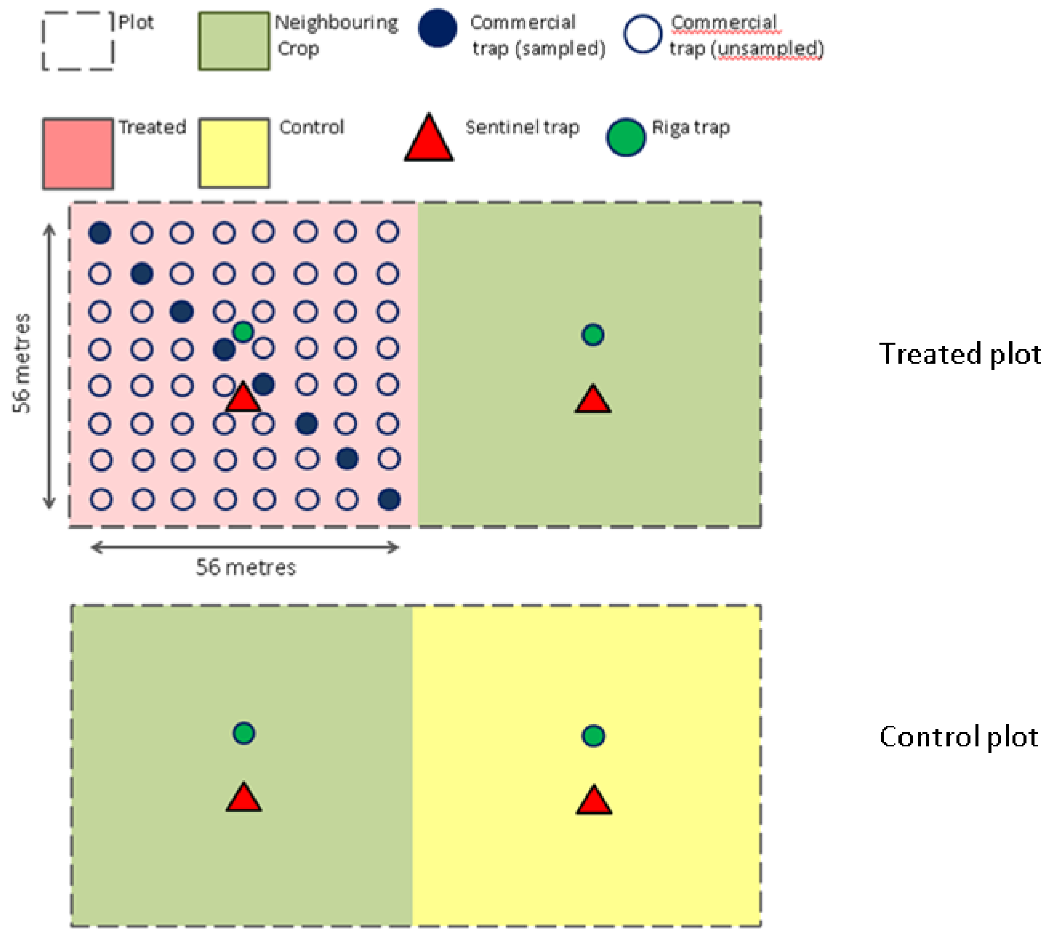


Figure 2.2.2. Diagrammatic representation of trap positions in an experimental block during the precision monitoring trial 2019. Treated woodlands contained 64 precision monitoring traps (blue outline circles). Of these, a transect of 8 traps (blue fill circles) were sampled. A RIGA trap (green fill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

Habitat assessments: In December 2019, the habitat within a 1 metre radius of each transect trap was assessed for *D. suzukii* 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 development and feeding score (Table 2.2.3) in order to calculate the *D. suzukii* plant host score in each evaluated habitat.

Table 2.2.1. Dates for precision monitoring trial assessments at each block, 2019. NB *Habitat and Sentinel fruit trap data will be presented in the 2020 report.

		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	

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
4	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

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

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 – RIGA traps: From RIGA trap counts between treated and control plots, mean numbers of *D. suzukii* fluctuated comparably at the beginning of the trial, but from assessment 3 were consistently lower in treated woodlands and neighbouring crops compared to untreated control counterparts (Fig. 2.2.3). At the pre-assessment (before precision monitoring traps were deployed) there were fewer *D. suzukii* per RIGA trap in treated compared to control woodlands (mean =209.8 and 309.7 respectively), also in treated compared to control neighbouring crops (mean = 21.9 and 64.7).

At the first assessment (2 weeks after precision monitoring trap deployment) mean *D. suzukii* per RIGA trap was still lower in treated compared to control woodlands (mean = 182.7 and 256.1 respectively), but now higher in treated compared to control neighbouring crops (167 and 108 respectively).

At the second assessment, mean *D. suzukii* per RIGA trap peaked in treated woodlands but was still similar to control woodlands (mean = 1714.1 and 1624.5 respectively); the same trend occurred in treated and control neighbouring crops (mean = 238.0 and 93.8 respectively).

Then at the third assessment, mean *D. suzukii* per RIGA trap decreased in treated but continued to increase to a peak in control woodlands (mean = 1136.2 and 2436.0 respectively); neighbouring crops followed the same trend (mean = 67 and 192.2 respectively). Thereafter, mean *D. suzukii* per RIGA trap decreased in general, but remained consistently lower in treated compared to control plots at assessment 4 (mean = treated woodland 699, control woodland 848.8, treated crop 64.3, control crop 99.7) and 5 (mean = treated woodland 99.7, control woodland 696, treated crop 2, rising again slightly in the control crop 217.7, Fig. 2.2.3).

D. suzukii monitoring – precision monitoring traps: Precision monitoring trap counts in treated woodlands followed a similar trend to RIGA trap counts in treated woodlands, except were consistently lower (see mean numbers of *D. suzukii* per RIGA trap above) (Fig. 2.2.4). At assessment 1 mean numbers of *D. suzukii* per 8 precision monitoring traps were 46.4. Numbers increased to a peak at assessment 2 (mean = 258.4), then continued to decrease at assessment 3 (mean = 119.2), 4 (mean = 45.5) and 5 (mean = 19.9). Assuming a 1:1 sex ratio, Grand mean male and female *D. suzukii* per precision monitoring trap from assessments 1 to 5 was 195.1 compared to 778.2 per RIGA trap (in treated woodlands only and excluding the pre-assessment).

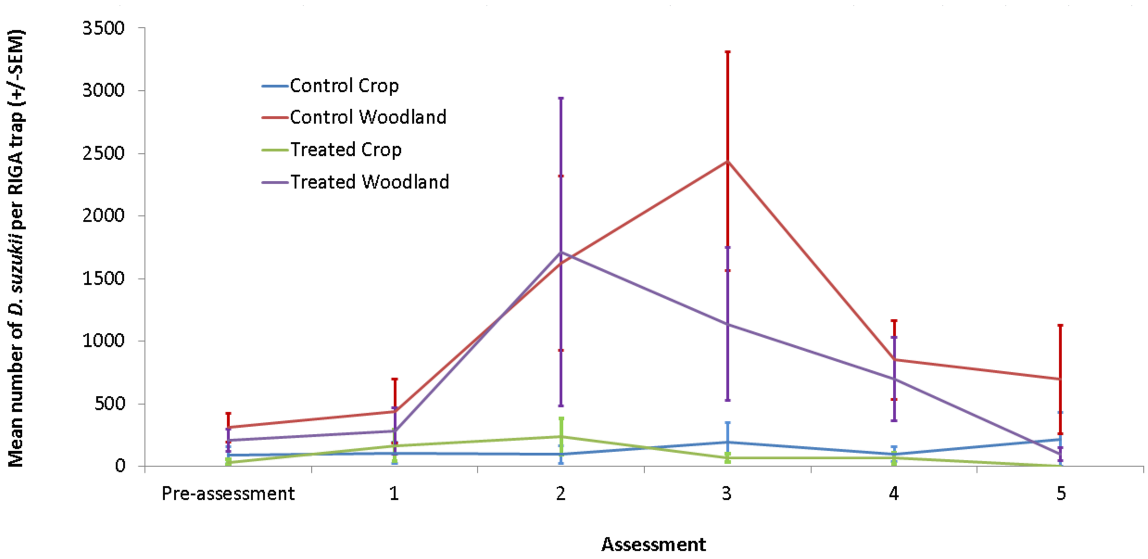


Figure 2.2.3. Mean number of *D. suzukii* per RIGA trap at different plot positions during precision monitoring trial assessments autumn/winter 2019

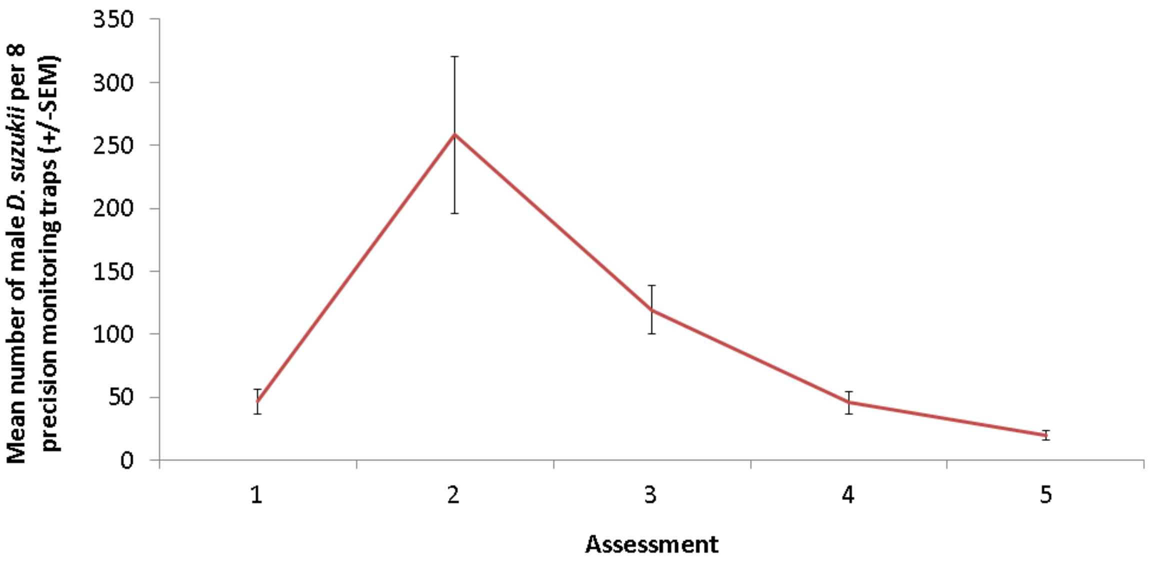


Figure 2.2.4. Mean numbers of *D. suzukii* males per 8 precision monitoring transects traps at treated woodlands during precision monitoring trial assessments autumn/winter 2019

Habitat assessments: From the assessment of *D. suzukii* wild hosts in treated woodlands, there was no clear correlation between habitat score and mean number of *D. suzukii* in trap catches. When the assessment was made (mid-December 2019), mean host score was highest at Site 5 (mean = 12.8) (Fig. 2.2.5), owing mainly to bramble, but most males per 8 precision monitoring traps were caught at Site 4 (mean = 310.7) - the only wine grape site - compared to 95.5 at Site 5.

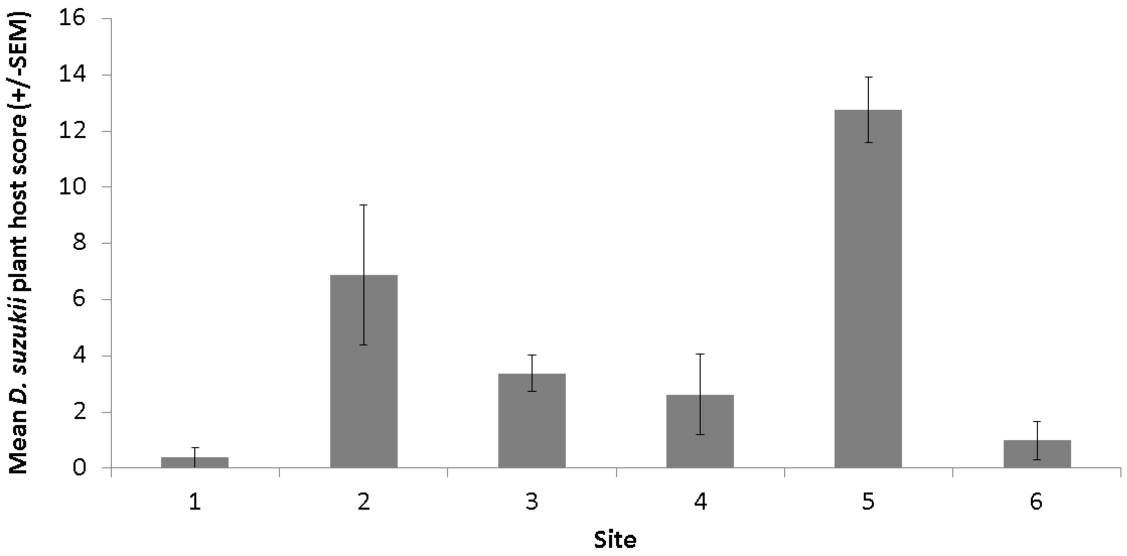


Figure 2.2.5. Mean scores of *D. suzukii* plant hosts in a 1 m radius of the 8 transect traps at each site of the precision monitoring trial, recorded during assessment 5, mid-December 2019

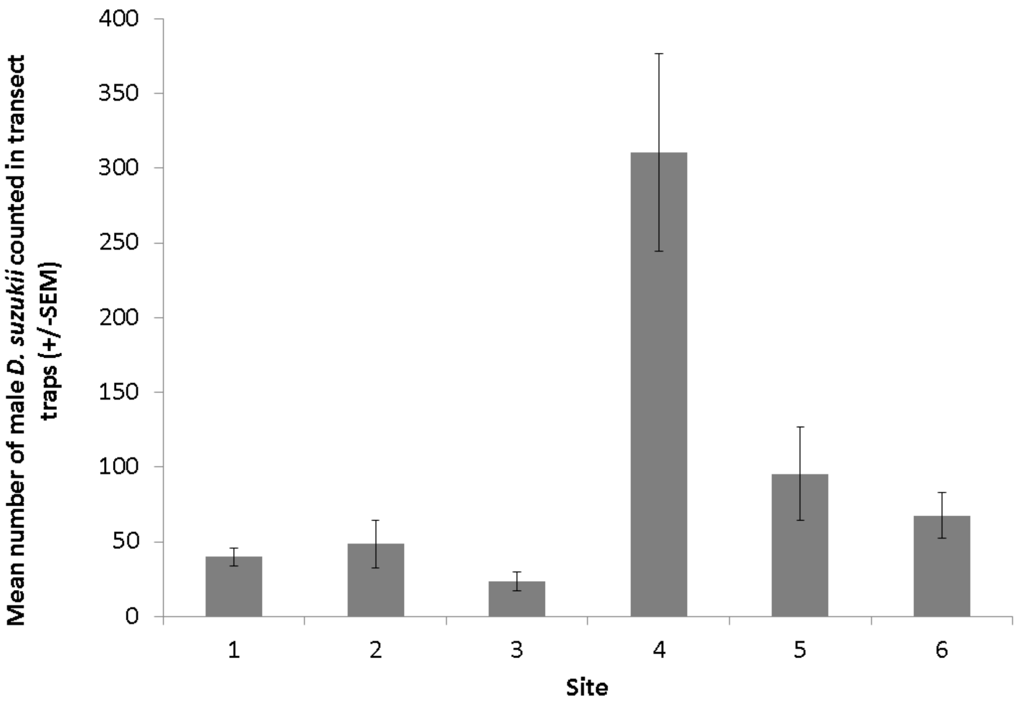


Figure 2.2.6. Mean numbers of *D. suzukii* males per 8 transect traps at each site of the precision monitoring trial assessments 1 to 5, 2019

Sentinel fruit traps: Sentinel fruit traps will be deployed spring 2020.

Discussion

It is too early to conclude if precision monitoring is reducing the *D. suzukii* population in the treated plots, despite RIGA trap data (not statistically analysed) showing a reduction in *D. suzukii* numbers in treated woodlands and neighbouring crops compared to the control. Before assessment 2, mean numbers of *D. suzukii* caught in RIGA traps was comparable between treated and control plots. However, at assessment 3 (6 weeks after precision monitoring traps were deployed) mean numbers of *D. suzukii* per RIGA trap decreased in treated woodlands and neighbouring crops but continued to increase to a peak in control woodlands and neighbouring crops. This could suggest up to assessment 2 precision monitoring traps were not noticeably impacting the *D. suzukii* population, but between 2 and 3 traps had reduced population numbers enough that fewer were present to trap. Alternatively, from assessment 1, crop and wild host fruit were decreasing in availability so traps were becoming increasingly attractive, but when traps were most attractive (between assessments 2 and 3), RIGA trap catches in treated plots were diluted by precision monitoring trap catches. After assessment 3 there was a decline of mean *D. suzukii* per RIGA trap in both treated and control plots matching the normal decline of trap catches witnessed early to mid-November in the UK *D. suzukii* National Monitoring survey. During this period *D. suzukii* numbers were consistently lower in treated plots; again, this might be due to a population reduction in treated woodlands or a dilution effect of precision monitoring traps.

RIGA traps are potentially more effective for mass trapping *D. suzukii*, though less practical. By doubling the overall mean number of *D. suzukii* males caught per precision monitoring trap to include uncounted females, the number was approximately a quarter of that caught per RIGA trap in treated woodlands (mean = 195.1 and 778.2, respectively). Manufacturer recommendations state “RIGA traps should be placed every 2 metres” whereas our traps were spaced 8 metre based on mark and recapture studies and mathematical model studies of trap attraction to *D. suzukii*. The RIGA manufacturer also recommends “after 3 weeks, new traps should be placed between existing traps. The traps retain their effectiveness until all the liquid in the cup has dried up. The cup containers in installed traps should only be replaced once this has occurred” (becherfalle.ch 2019). The precision monitoring traps, used in this trial during winter could potentially be left unattended for longer periods, months. Moreover, from our experience RIGA traps also need replacing once liquid bait is saturated with dead *D. suzukii*.

As yet, the habitat assessment has not been instructive as to the most effective habitats to concentrate *D. suzukii* traps for highest population reduction, possibly due to survey timing. Our survey and calculations found no clear correlation between surrounding habitat and mean number of *D. suzukii* in trap catches (Fig. 2.2.5 and 2.2.6). However, the assessment (5) was made mid-December 2019 in the absence of wild host vegetation and fruit, and when numbers of *D. suzukii* in traps was declining (Fig. 2.2.3 and 2.2.4). A correlation might have been found if the survey was conducted earlier in the trial when wild host plants are finishing fruiting and *D. suzukii* trap catches are generally highest and potentially looking at a wider area (4 m radius) and the aspect of individual traps (e.g. in shade or northerly aspect). Most males were caught at Site 4 - the only wine grape site (Fig. 2.2.6). Grapes were harvested early in the trial, whilst strawberry was still growing at the other sites and attracting *D. suzukii*. Site 4 treated woodland also had a low wild host score, so traps were probably more attractive to *D. suzukii* here than other sites.

Sentinel fruit traps are due to be deployed in treated and control plot areas in early spring when overwintered *D. suzukii* females are active and fecund. To avoid precision monitoring traps competing with sentinel fruits for *D. suzukii* females, precision monitoring traps will be removed or inactivated before/each sentinel fruit trap deployment.

Conclusions

- Although trap data shows a reduction in *D. suzukii* numbers in treated woodlands and neighbouring crops compared to the control, it is too early to conclude if precision monitoring is reducing the *D. suzukii* population in the treated plots (not statistically analysed).
- Sentinel fruit traps in spring 2020 should indicate if the technique can reduce pest invasion into the neighbouring crop
- RIGA traps seem more attractive to *D. suzukii* than our precision monitoring traps, but the commercial precision monitoring traps required less attention (hence labour).
- To determine the best winter hosts to concentrate precision monitoring traps, habitat assessments should be made when autumn wild host plants stop fruiting and *D. suzukii* trap catches are generally highest.

- Trap microclimate, aspect, and a wider habitat assessment are also advised as part of a study to inform growers of the best location for *D. suzukii* trapping, to maximise catch whilst minimising labour.

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

Introduction

A full review of bait sprays for control of *D. suzukii* has been prepared in Noble et al. (2019). The review indicated that the most promising phagostimulant baits for insecticidal control of *D. suzukii* summer morphs are those based on yeasts (particularly *H. uvarum*), fermentation products, plant extracts and sugars. This has been confirmed in previous work in SF145 in laboratory bioassays showing that a suspension of the yeast *Hanseniaspora uvarum*, fermented strawberry juice or the commercial product Combi-protec (based on plant extracts, proteins and sugars) were all effective phagostimulant baits for *D. suzukii*. They all increased the efficacy, in terms of *D. suzukii* mortality and reduced oviposition (egg laying), of dilute doses of Tracer (spinosad), Exirel (cyantraniliprole) and Hallmark (lambda-cyhalothrin). *H. uvarum* and Combi-protec also improved the efficacy of Calypso (Thiacloprid). Since this work was completed, there have been approvals in the UK for the use of Combi-protec as an adjuvant, and for cyantraniliprole (Benevia) against *D. suzukii* on strawberries. The aim of this work was to compare the *D. suzukii* control efficacy of dilute rates of Benevia when used with *H. uvarum* or Combi-protec, against full field application rates of Benevia under semi-field conditions. Currently, there is approval for two applications of Benevia per strawberry crop. However, the recommendation from Combi-protec is to spray with insecticides weekly. Since the quantity of Benevia in four applications with phagostimulant baits is less than 10% of the amount in two full field applications, the application of four dilute sprays of Benevia was considered justified in terms of a potential application for approval. Application of low doses of insecticides normally increases the risk of pesticide resistance, but in combination with baits, the ingested dose of insecticide may be increased.

Task 3.4. Determine the effect of the optimum bait on the D. suzukii *efficacy of insecticides applied in the field alone or in combination with other controls (NIAB EMR, Microbiotech)*

Materials and 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 3.4.1. Plug plants of an everbearing variety of strawberry (cv. Amesti) were planted in the polytunnel compartments in March. Ten coir grow bags (Dutch Plantin), each containing eight plants were set out in two adjacent rows on 10 cm height plastic crates. The cropping area in each compartment measured 5 × 0.8 m. 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.

<u>Treatments and experimental design</u>

1. Unsprayed positive control; no spray application to plants during the experimental period. The remaining plants were sprayed at first white fruit stage (week 1) and again at intervals; the total number of sprays depending on the treatment. Sprays were applied with a motorised knapsack sprayer (Birchmeier 14 REC ABC) at a maximum pressure of 3 bar.
2. Two high volume applications of Benevia (750 ml in 500L/ha; 0.075 g a.i. /litre) were applied with through an Orange Albuz hollow cone nozzle @ 12.55 ml spray per plant. The spray was applied with the above sprayer together with a motorised mist blower (Solo Inc.) to the entire crop surface. The BCPC droplet spectra size was fine to very fine (154 to 225 microns). Spray applications were made in weeks 1 and 2 only.
3. Four weekly low volume applications of Benevia (30 ml in 40L/ha; 075 g a.i./litre) were applied with *H. uvarum* suspension. The spray was applied at a nominal 1 ml

per plant through a Lechler nozzle type IDK 120-015 rotated through 90° to spray a 200 mm band down the centre of each double row of the plants in grow bags (calibrated nozzle output 0.408 litre/min). The BCPC droplet spectra size was coarse, ~340 microns. The fine filter in the sprayer was removed to avoid filtering out yeast cells. *H. uvarum* strain 1-382 from the Phaff Yeast Culture Collection, Food Science, UC Davis, CA was used for the experiment. The *H. uvarum* suspension was prepared in yeast YPD broth (Fisher Scientific, 50g/L) and incubated at 20 °C for 48 h on a shaker. The yeast cell counts in the *H. uvarum* suspension were determined on samples taken before spraying and after passing through the coarse filter in the sprayer. Sprays were applied in weeks 1, 2, 3 and 4.

4. Four weekly low volume applications of Benevia (30 ml in 40L/ha; 075 g a.i./litre) with Combi-protec (2 litres in 40 litres/ha). Combi-protec was prepared by pre-mixing in warm (30 °C) water, as per Combi-protec recommendations. The application method was the same as for treatment 3 (sprays applied in weeks 1, 2, 3, and 4).

The volumes of spray per plant were determined from the initial and final volumes in the spray tank. Each compartment was artificially infested with 10 female and 10 male adult summer morph *D. suzukii*, one day after the timing of the first, second and third sprays. Ripe fruits (4 to 16 in week 1 due to unavailability, and 24 in weeks 2, 3 and 4, at least two collected from all grow bags along the tunnel where possible) from each compartment were picked six days after each spraying and introduced into four 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 20 °C, in 16h:8h light:dark. Ripe fruit not used for *D. suzukii* emergence testing was also picked at regular intervals. Temperature and humidity 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.

Table 3.4.1. Time schedule of Task 3.4.

Date	Expt Day	Activity
29 March		Set out strawberry plants in tunnels
26 April		Remove first flowers for last time
13 June	0	Spray 1, Benevia, Combi-protec, *H. uvarum*
14 June	1	Introduce 10 ♀ and 10 ♂ *D. suzukii* in tunnels
19 June	6	Sample fruit 1
20 June	7	Spray 2, Benevia, Combi-protec, *H. uvarum*
21 June	8	Introduce 10 ♀ and 10 ♂ *D. suzukii* in tunnels
26 June	13	Sample fruit 2
27 June	14	Spray 3, Combi-protec, *H. uvarum*
28 June	15	Introduce 10 ♀ and 10 ♂ *D. suzukii* in tunnels
3 July	20	Sample fruit 3
4 July	21	Spray 4, Combi-protec, *H. uvarum*
10 July	27	Sample fruit 4

Experimental design and statistical analyses

There were six replicates of each treatment. Treatments were allocated to half polytunnels so that each treatment was paired with the other three treatments twice, once in the north end and once in the south end of the tunnels. Each treatment was in every row once and in every column once or twice. *D. suzukii* emergence data were analysed by ANOVA.

Results

Polytunnel environment

Diurnal fluctuations in air temperature and relative humidity among the polytunnel strawberry plants are shown in Figure 3.4.1. During the experiment, average temperature was 18.9 °C; the maximum and minimum temperatures recorded were 31.5 °C and 9.9 °C respectively. The average relative humidity was 74.7%.

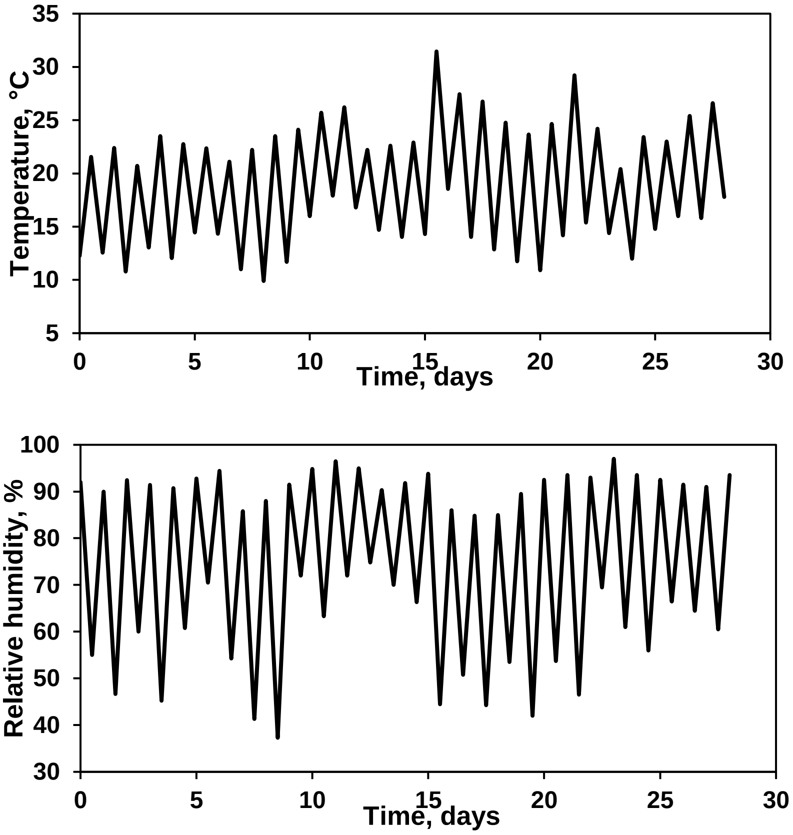


Figure 3.4.1. Temperature and relative humidity in among polytunnel strawberry plants

Spray applications

Full foliar application took 40 seconds per half tunnel compared with 6 seconds for bait band sprays. Spray applications measured from the start and end tank volumes were about 10% higher than the target values (Table 3.4.2). Small losses in spray at the start and end of each application and the residual amount of spray left in the spray lines between cleaning for each treatment meant that the actual and target applications were very close. The cumulative total amount of Benevia applied per plant in the full field rate, high volume application (Treatment 3.4.2) was more than x11 the amount of Benevia applied to the Combi-protec and *H. uvarum* treatments (Table 3.4.2). This was equivalent to a reduction of at least 91% in the amount of Benevia applied to the bait spray treatments. Yeast cell counts were slightly higher (about 5%) in the samples taken from the prepared suspension than after passing through the sprayer. The population of *H. uvarum* yeast cells in the applied broth suspensions was consistently around 3 × 10^{-9} cells per ml in all four weeks. This may have been due to settlement or filtering in the sprayer since work in Year 2 of SF145 showed that at the concentration used, cyantraniliprole is not toxic to *H. uvarum*.

Droplet application patterns on the crop are shown in Figure 3.4.2. The full field rate, fine spray of Benevia resulted in a uniform film over the leaves whereas the Combi-protec and *H. uvarum* treatments were applied as distinct droplets. No phytotoxicity symptoms were observed on any of the plants.

Table 3.4.2. Target and actual measured quantities of sprays and Benevia applied, and concentration of yeast cells in Treatment 3 before and after spraying.

Treatment	**weeks**	**Spray, ml/plant**		**Benevia, actual µl/plant**		**Yeast cells, ×10⁹/ml**	
		target	actual	weekly	cum. total	before	after
1 Control	1,2,3,4	0	0	0	0	-	-
2 Benevia	1	12.55	13.44	20.2	20.2	-	-
	2	12.55	13.90	20.9	41.1	-	-
	3,4	0	0	0	41.1	-	-
3 *H. uvarum*	1	1	1.15	0.9	0.9	3.67	2.83
	2	1	1.25	0.9	1.8	3.25	2.93
	3	1	1.31	1.0	2.8	3.48	2.49
	4	1	1.13	0.8	3.6	3.51	2.97
4. Combi-protec	1	1	1.13	0.8	0.8	-	-
	2	1	1.19	0.9	1.7	-	-
	3	1	0.94	0.7	2.4	-	-
	4	1	1.08	0.8	3.2	-	-

Figure 3.4.2. Spray application for Benevia (left), Combi-protec (centre) and *H. uvarum* (right)

<u>*D. suzukii* assessments</u>

There were significant (*P*<0.001) effects of Benevia applications, with or without baits, on the numbers of *D. suzukii* adults, but no significant differences between Benevia treatments. This trend was the same, irrespective of whether the numbers of flies were expressed per mesocosm, per berry or per kg of fruit (Fig. 3.4.2). The number of flies per kg of fruit was significantly higher in week 1 than in the following weeks (Fig. 3.4.2c); this was due to the smaller average berry number (10 SD±4 per tunnel) and weight (mean 19 g) in the first week compared with >24 berries per tunnel and berry weight (means 45 to 62 g) in the following weeks. The full rate Benevia treatment remained effective in weeks 3 and 4, even though it was only applied in weeks 1 and 2 and new cohorts of flies continued to be introduced in the tunnels until week 3. In all the treatments and weeks, there were about equal proportions of females and males that emerged in the mesocosms (48 to 55% females).

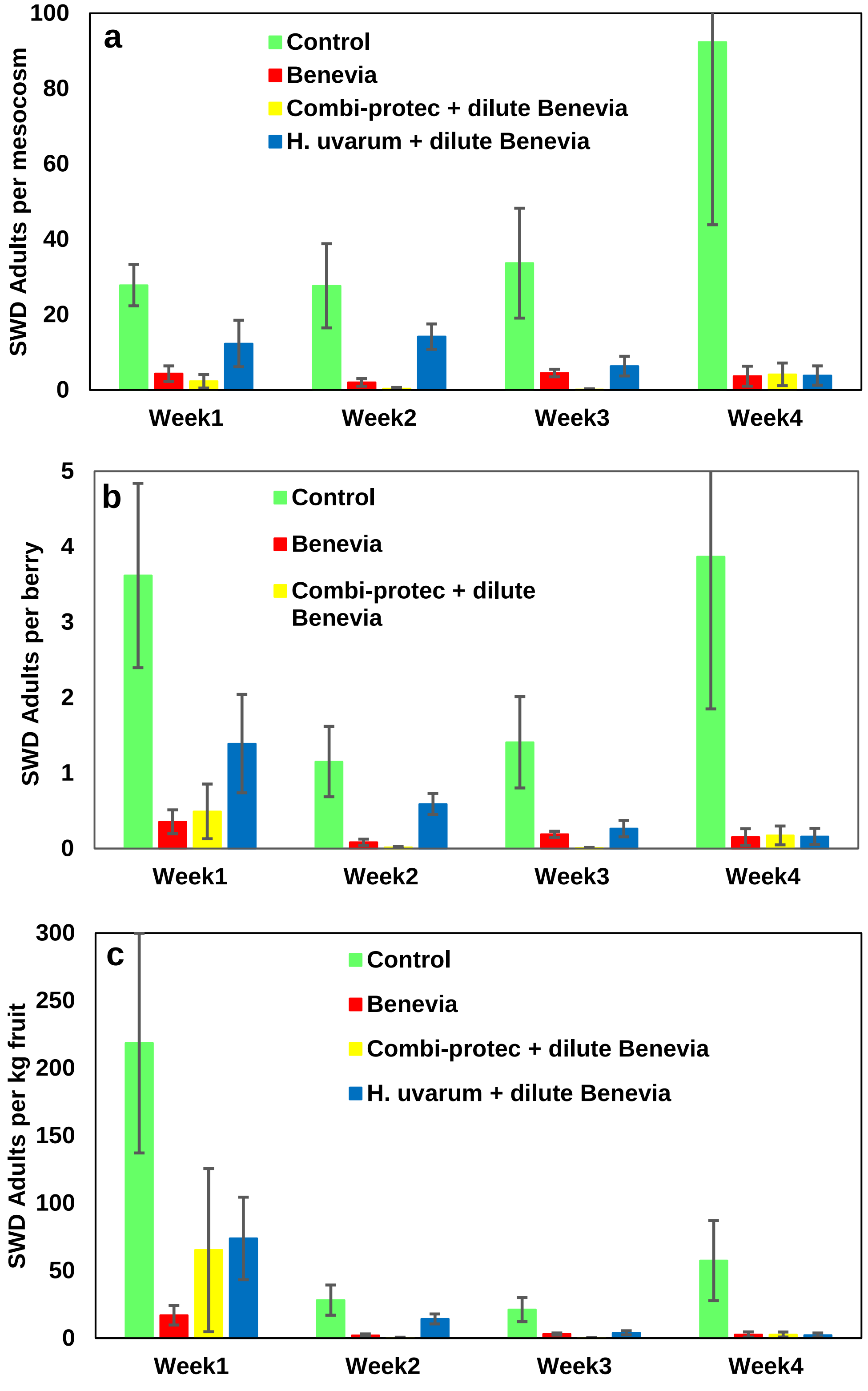


Figure 3.4.3. Effect of full rate Benevia applications (750 ml in 500L per ha) in weeks 1 and 2, and bait + dilute Benevia (30 ml in 40L per ha) applications in weeks 1 to 4 on numbers of *D. suzukii* adults expressed per (a) mesocosm (b) berry and (c) kg fruit. Mean values (±SE), n = 6

Cost of treatments

The application time for the bait band sprays was only 15% of the full foliar spray applications. The cost of Benevia is £150 per litre and Combi-protec £36.50 per litre. At 750 ml per hectare (full field rate), the cost of the Benevia product was £112.50/ha per spray or £225/ha for two sprays, excluding the application cost. The product cost of the Combi-protec + dilute Benevia (30 ml per hectare) treatment was £77.50/ha per spray or £298/ha for four sprays, excluding the application cost.

Conclusions

- Weekly applications of Benevia at 30 ml in 40L per ha, combined with *H. uvarum* or Combi-protec baits, 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 91% with the same *D. suzukii* control effect).
- Benevia at the full field rate remained as effective in controlling *D. suzukii* numbers in the two weeks after it was applied as it was in the two weeks when it was applied.
- Control of *D. suzukii* was at least as good with Combi-protec as with *H. uvarum* in all four weeks; the Combi-protec treatment is easier to prepare than *H. uvarum* and is already commercially available in the UK.
- There were similar proportions of male and female *D. suzukii* in all the mesocosms from the unsprayed, Benevia and Benevia + bait treatments.
- The spray equipment with a Lechler nozzle type IDK 120-015 produced a uniform application of the bait spray treatments in a 200 mm band on the strawberry plants.
- There was a small reduction in the *H. uvarum* cell counts after mixing and passing through the sprayer; the applied counts were about 3×10^9 cells/ml.
- 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.
- The application time for the bait band spray was 15% of the full field rate application of Benevia.
- None of the Benevia or Benevia + bait treatments caused phytotoxicity symptoms.

Future Research

The Combi-protec treatment should be tested with other insecticides and on other fruit crops (raspberries and cherries) where control of *D. suzukii* may be more challenging than in strawberries. For raspberries, a different method of application will need to be developed, for example application to lower foliage or to the surface of the pots.

Results from jar bioassays in Year 2 indicate that Spinosad, Hallmark and Calypso (as well as Exirel or Benevia) are the most promising insecticide treatments with Combi-protec for *D. suzukii* control. Semi-field trials with Combi-protec by Helsen & van der Sluis (2017) also showed that Combi-protec with Hallmark, as well as Decis and Pirimicarb, gave good control of *D. suzukii* in strawberries.

The longevity of the control efficacy of two Benevia applications should be examined beyond the two weeks that were tested here. Similarly, the longevity of the four Combi-protec + Benevia applications should also be examined.

To determine the contribution of the bait to the control efficacy of the band spray application, the effect of band spraying the same dilute Benevia rate (30 ml in 40L per ha) without bait should be tested.

There is evidence that the growth medium for *H. uvarum* can affect its attractiveness to *D. suzukii* (Lasa et al., 2019). The effect of different *H. uvarum* strains and growth media on their phagostimulant effect should be tested with insecticides in jar bioassays.

Other phagostimulant baits (e.g. molasses) may give the same control efficacy of Combi-protec; this should also be tested in jar bioassays. The cost of molasses is less than £1/litre, which would substantially reduce the cost of the bait spray, if effective.

Bioassay work in Year 2 of SF145 showed that phagostimulant baits are also effective in improving insecticidal control of winter morph *D. suzukii*. The potential for controlling overwintering populations of *D. suzukii* using phagostimulant baits should be investigated further.

An application should be made for approval of four dilute applications of Benevia or Exirel per season. The current approval limit is for two applications per season, but this is for the full field rate.

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

Task 4.2. Investigate the impact of different spray methods on cherry.

Introduction

In 2018, field trials were carried out to test the effects of increasing spray intervals for control of *D. suzukii* at two farms cherry farms in East Kent. Fortnightly spray programmes gave equal efficacy of *D. suzukii* control as the grower’s standard spray programme. In addition, very few fruits were damaged by *D. suzukii* egg laying in both spray programmes even though adults were clearly in the crop and around the perimeter. Where insect excluding mesh was employed there were fewer *D. suzukii* adults in the crop.

The trials in 2018 recorded effects on insect populations, infestation, and length of time of effectiveness of the spraying but did not measure spray deposition.

Objective

In June 2019, the farms were re-visited, and the same tunnels were sprayed in the same way as in 2018. The spray deposition was measured using the handheld imaging fluorometer to quantify spray coverage and fluorescence intensity (a proxy for spray liquid volume on the leaf surface).

Materials and Methods

At Farm 1, the polytunnels had two rows of trees per tunnel and the trees were approximately 2.5 – 3 m tall. At Farm 2, the polytunnels had a single row with 2 beds of trees which were approximately 2.5 – 3 m tall (Figs. 4.2.1).

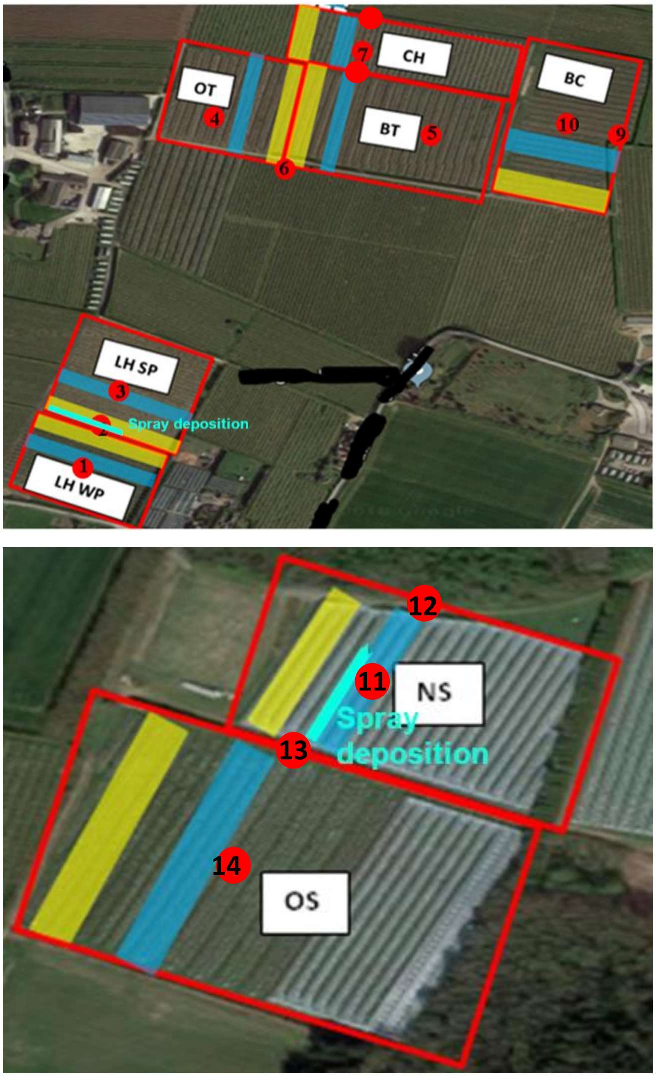


Figure 4.2.1. Location of the study at each farm. The cyan coloured lines show where the spray deposition assessment was completed

At each farm the spray machines were set up using the farm's standard procedure and settings. Fluorescent tracer dye (5 L) was added to 200 L of water in the spray tank agitated. A 30 s spray onto bare ground was done to ensure the tracer dye was thoroughly mixed and flowing to the nozzles (Table 4.2.1). A 20 m spray plot was marked out on both alleyways in the polytunnel. The trees were sprayed from both sides with a 5 m sprayed buffer before and after the plot. Spray deposition was measured using the handheld imaging fluorimeter (developed in a NIAB EMR IUK project) by randomly selecting leaves from the sprayed trees (Table 4.2.1).

The same procedure was used for both farms. Spray deposition was measured using handheld imaging fluorimeters. The trees' canopy was divided into 8 zones (4.2.2) by canopy height from the ground and leaf side (upper/adaxial and lower/abaxial). Two people, using two imaging fluorimeters, randomly selected leaves within the plot to measure the spray deposition, each person measuring on one side of the row. For most of the canopy zones leaves were sampled from the outer region of the branches which was between the edge of the canopy up to about 0.4 m inwards (‘arm’s length’). For the ‘middle-inner’ zones leaves were sampled close to the trunk to assess how spray droplets penetrate the canopy. For each zone at least 60 leaves were sampled.

The data were assessed with descriptive statistics using R (R Core Team, 2018) and R-Studio (RStudio Team, 2016). The spray deposition on the leaf samples was measured using the handheld imaging fluorometer and within each zone the means, percentage coefficient of variation (CV%), max and min values were calculated.

Table 4.2.1. Details of farm sprayers and operations.

	Farm 1	**Farm 2**
Speed (km/h)	3	7-8
Nozzles	Albuz ATR 80 (3 blue/6 orange– 9 per side) Hollow cone	Albuz ATR 80 Orange (8 per side) Hollow cone
Bar pressure	10-12	12
Droplet size	Blue: Fine (>159um/<231um)	Very fine (<159um)
	Orange: Very Fine (<159um)	
Spray height (m)	4	3
Air induction	Fan full speed	Fan full speed
Spray volume (l/ha)	750	300
Sprayer model	BAB A206EP MOS. Single frame with single tower, single fan and mower. A-frame	Munkoff – half tower
Tank size (l)	2000	1500
Other factors	Not winter precision monitoring	Winter precision monitoring
Photo of the spray machines		
Spraying		

Table 4.2.2. Leaf deposition measurements

Canopy position and approximate height from ground	**Leaf side**	**Minimum number of samples (30 / imaging device)**
Top (> 2 m)	Upper	60
Top (> 2 m)	Lower	60
Middle (1 – 2 m)	Upper	60
Middle (1 – 2 m)	Lower	60
Inner (1 – 2 m) (leaves sampled closed to trunk)	Upper	60
Inner (1 – 2 m) (leaves sampled closed to trunk)	Lower	60
Bottom (< 1 m)	Upper	60
Bottom (< 1 m)	Lower	60
Total		**480**

Results and Discussion

The CV% shows the level of variation in the data regardless of the measurement unit. Higher CV% indicates that there is variability in the spray coverage of that canopy zone, with some leaves receiving very high levels of spray and some leaves receiving very little. The percentage area of each leaf sample that was covered by spray deposits was measured (Table 4.2.3).

At each farm the spray coverage on the upper and lower leaf sides was similar. Slightly more coverage was measured on the underside of leaves for some canopy sections. This often occurs when spraying trees with axial fan spray machines with air-assistance as the droplets' trajectory is from below the leaves.

The spray coverage achieved on Farm 1 was much greater than at Farm 2. Overall, the whole canopy Farm 1 had a mean coverage of 53.1 % coverage compared to 18.1 % at Farm 2. The spray coverage on Farm 2 was therefore 34% that of Farm 1. This fits well with the application water volume used at Farm 1 which was 750 L/ha compared to 300

L/ha at Farm 2. If all else was equal between the two farms, based on the different water volumes used we would expect that the spray coverage at Farm 2 would be approximately 40% that of Farm 1.

Table 4.2.3. Percentage area of each leaf sample that was covered by spray deposits was measured for each zone. The percentage coefficient of variation, and the maximum and minimum values were also calculated.

Farm	Canopy	Leaf side	N	Coverage %	CV%	Max	Min
Farm1	Top	Upper	60	67.1	38.9	100.0	5.6
Farm1	Top	Lower	61	73.6	29.2	98.4	2.5
Farm1	Middle	Upper	62	58.8	48.2	99.4	10.7
Farm1	Middle	Lower	65	79.4	29.4	100.0	16.6
Farm1	Inner-middle	Upper	60	22.4	95.3	83.5	0.9
Farm1	Inner-middle	Lower	60	19.6	124.1	98.3	0.3
Farm1	Bottom	Upper	60	44.5	57.7	97.6	6.5
Farm1	Bottom	Lower	63	56.2	44.8	99.7	2.8
Farm 1	***Total mean***		***491***	***53.1***	***60.5***	***100***	***0.3***
Farm2	Top	Upper	60	17.0	94.8	68.1	1.2
Farm2	Top	Lower	61	23.9	91.1	82.3	0.9
Farm2	Middle	Upper	61	17.7	92.3	77.1	0.7
Farm2	Middle	Lower	66	30.0	75.8	81.4	0.9
Farm2	Inner-middle	Upper	61	9.8	87.7	33.7	0.4
Farm2	Inner-middle	Lower	61	8.2	115.2	59.4	0.1
Farm2	Bottom	Upper	61	15.8	78.9	55.5	0.6
Farm2	Bottom	Lower	60	23.2	88.3	83.6	0.6
Farm 2	***Total mean***		***491***	***18.1***	***98.9***	***83.6***	***0.1***

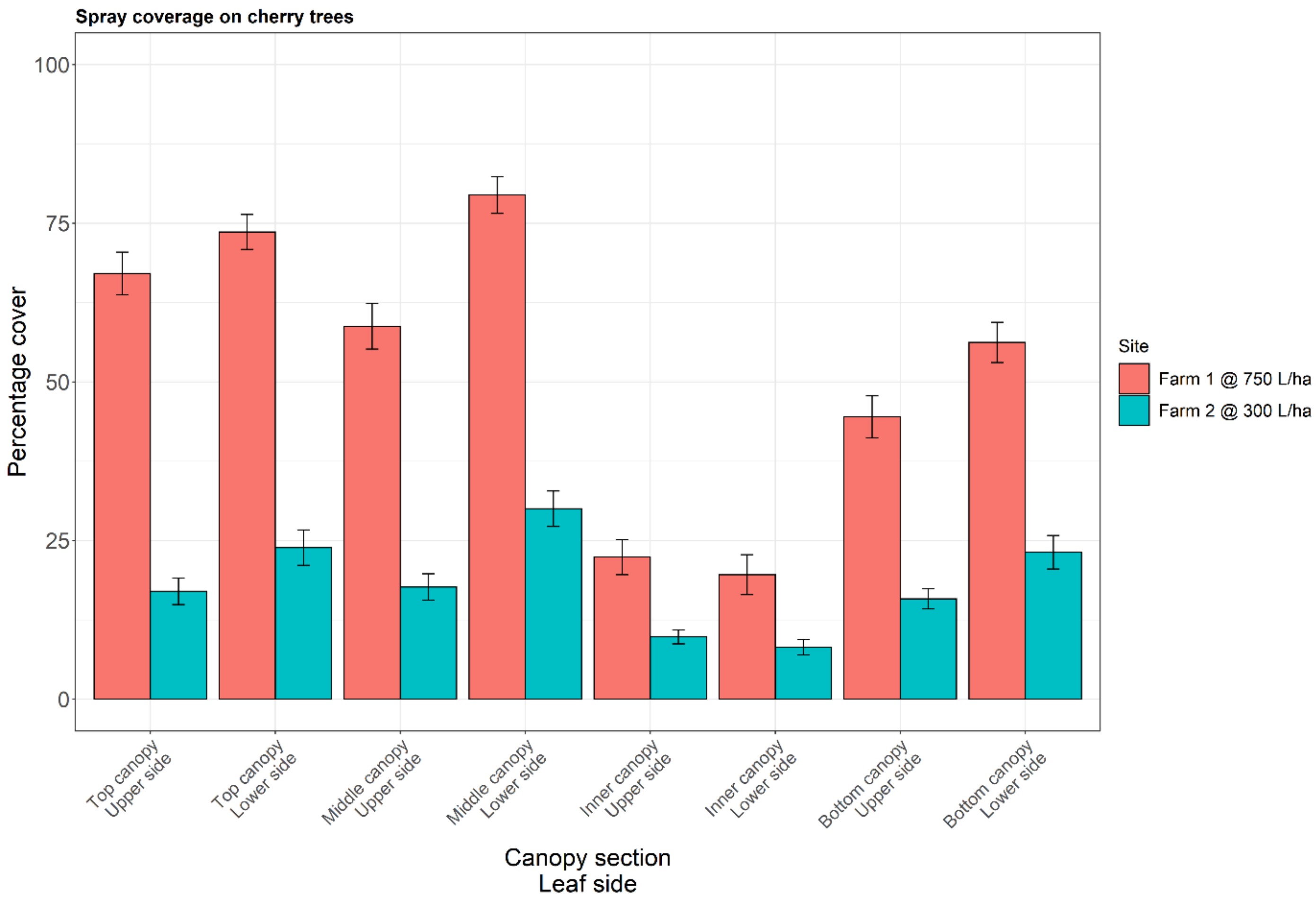


Figure 4.2.2. Percentage of leaf area covered by spray deposits at the two farms. The trees were divided into 8 zones by canopy and leaf side (Table 4.2.3)

At Farm 1 spray coverage was greatest at the top and middle canopy sections with a large reduction in spray coverage measured at the inner canopy section. At Farm 2 the pattern of spray across the canopy was smoother, with moderate spray coverage across all canopy zones although coverage at the 'inner canopy' zone was still lower than for other zones. This may indicate that the spray machine at Farm 2 is set up well and is penetrating the canopy slightly better than at Farm 1.

The %CV across both farms was reasonable compared to previous orchard spray trials, indicating that the spray deposition is evenly dispersed on the leaves within each canopy zone. On both farms the 'inner' zone had high variability indicating some leaves received extremely little spray.

The fluorescence intensity of the spray deposits on the leaves is a proxy for the volume of liquid of spray deposit on the leaf surface (Table 4.2.4).

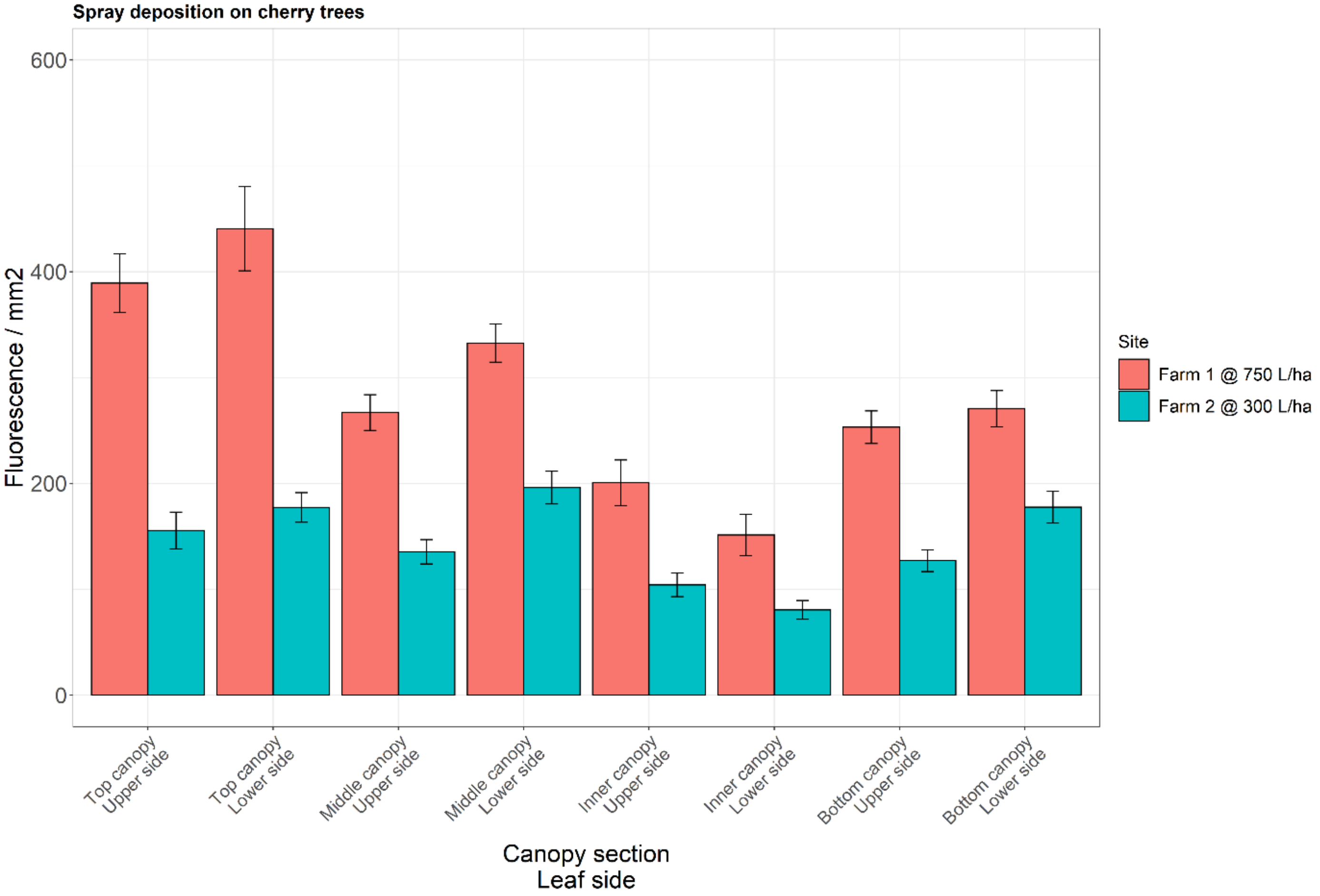


Figure 4.2.2. Fluorescence intensity / mm^2 of leaf provides an indication of the volume of sprayed liquid on the leaf surface. Higher fluorescence intensity / mm^2 on the leaf surface means greater amounts of spray liquid on the leaf compared to leaves with lower fluorescence intensity. The trees were divided into 8 zones by canopy and leaf side. Farm 1 was sprayed at 750 L/ha with a fluorescent tracer concentration at 2.5 %v/v. Farm 2 was sprayed at 300 L/ha with a fluorescent tracer concentration at 2.5 %v/v

Table 4.2.4. Fluorescence intensity per mm^2 on each leaf sample was measured for each zone. The percentage coefficient of variation, and the maximum and minimum values were also calculated. Fluorescence intensity provides an indication of the volume of spray deposited on the leaf surface, with higher intensity equating to high volumes of liquid.

Farm	Canopy	Leaf side	N	Fluorescence intensity / mm^2	CV%	Max	Min
Farm1	Top	Upper	60	389.3	55.0	927.7	71.1
Farm1	Top	Lower	61	440.7	70.6	1927.0	27.8
Farm1	Middle	Upper	62	267.0	49.6	673.6	93.8
Farm1	Middle	Lower	65	332.5	43.6	893.9	115.0
Farm1	Inner-middle	Upper	60	200.7	83.2	717.8	11.8
Farm1	Inner-middle	Lower	60	151.4	99.8	560.3	2.2
Farm1	Bottom	Upper	60	253.2	47.1	621.3	69.4
Farm1	Bottom	Lower	63	270.6	50.1	773.6	34.1
Farm 1	***Total mean***		***491***	***288.7***	***69.5***	***1927***	***2.2***
Farm2	Top	Upper	60	155.6	86.6	646.0	12.3
Farm2	Top	Lower	61	177.3	61.6	503.1	8.3
Farm2	Middle	Upper	61	135.5	65.9	506.2	4.5
Farm2	Middle	Lower	66	196.1	64.2	548.6	9.8
Farm2	Inner-middle	Upper	61	104.2	83.2	361.7	4.3
Farm2	Inner-middle	Lower	61	80.7	86.2	338.4	0.1
Farm2	Bottom	Upper	61	127.2	63.3	345.3	5.5
Farm2	Bottom	Lower	60	177.7	65.6	574.5	6.1
Farm 2	***Total mean***		***491***	***144.7***	***75.9***	***646***	***0.1***

The fluorescence intensity broadly followed the trends seen in the spray coverage, with higher deposition at the top of the canopy at Farm 1 and more even deposition across the canopy at Farm 2. The fluorescence intensity at Farm 2 is also higher than expected relative to Farm 1. At the water volume rates used on the two farms the fluorescence at Farm 2 should be around 40% that of Farm 1, yet in many canopy zones the fluorescence intensity at Farm 2 is considerably more than 40% that of Farm 1, particularly at the 'middle' and 'inner' canopy zones.

These data are an indication of the amount of spray liquid on the leaf surface. As Farm 2 is using a lower water volume than Farm 1 during their spray schedule the pesticide active ingredient concentration will be higher (approximately 150%). In this spray deposition trial, the concentration of the fluorescent tracer was kept the same between the two farms (due to technical practicalities). Therefore, although the fluorescence intensity at Farm 2 is half that of Farm 1 (Table 4.2.4), when the Farm 2 is spraying with a pesticide the actual amount of pesticide a.i. would be around 150% higher. An estimation of what the fluorescence intensity / mm^2 might look like on the two farms when the data is corrected for the water volume used is shown in Fig. 4.2.3.

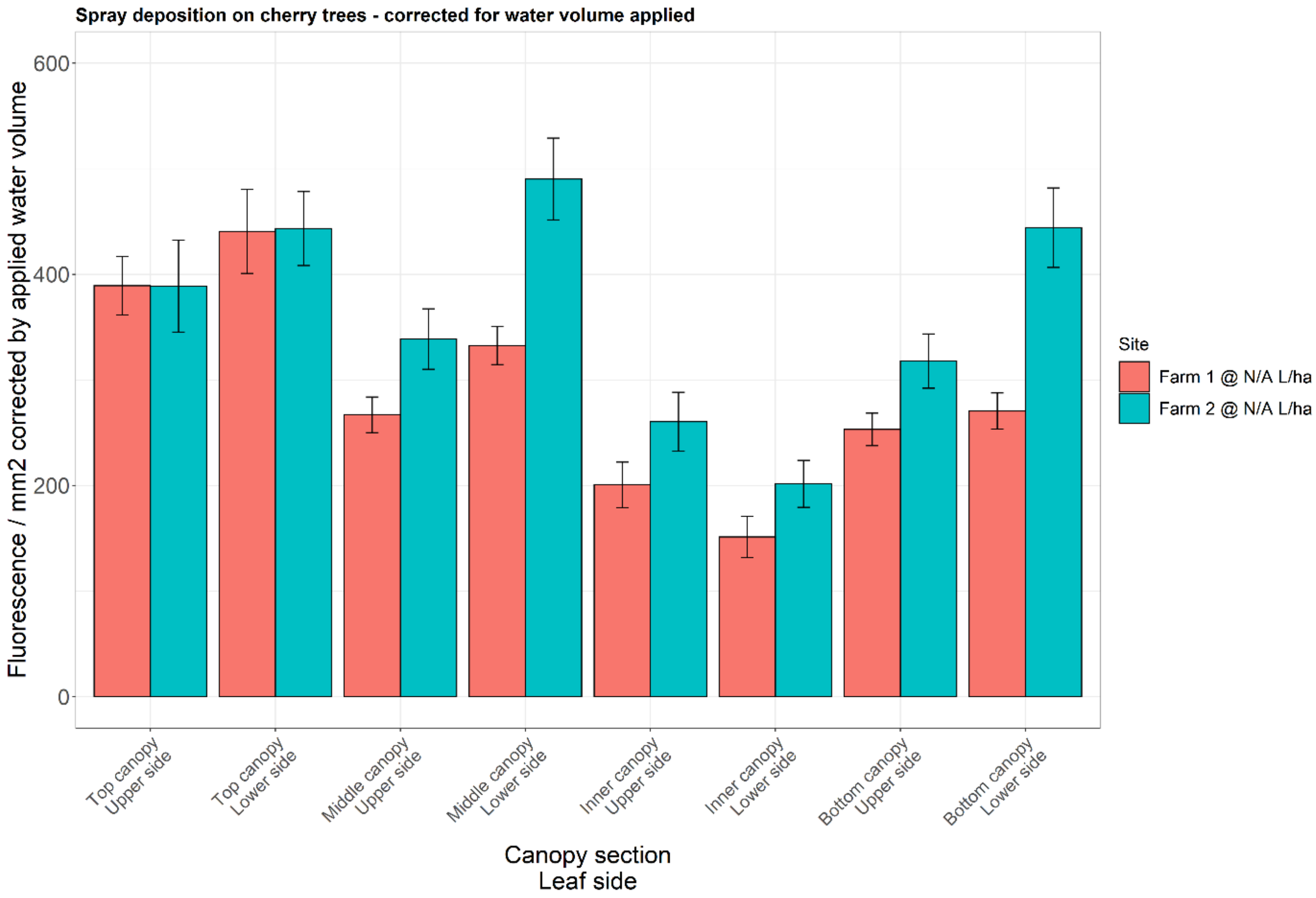


Figure 4.2.3. Fluorescence intensity / mm^2 of leaf corrected by the water volume each farm uses. This is an estimate of what the relative amounts of active ingredient deposited across the tree canopy could be between the two farms

Conclusions

The spray coverage on Farm 1 was good. Farm 1 spraying at 750 L/ha achieved a very high level of coverage over most of the canopy, except for the 'inner' zone. Considering Farm 2 was spraying at 300 L/ha it also achieved a reasonable level of coverage. The spray coverage and deposition on the farms could be even better by checking and adjusting the air-assistance plume shape and volume to better fit the tree canopy shape.

The forward speed used at the two farms were very different. Farm 1 spraying at 750 L/ha with a forward speed of 3 km/h, whereas Farm 2 spraying at 300 L/ha with a forward speed of 7 – 8 km/h. With similar fan output, increasing forward speed results in less cubic meters of air being pushed through the tree canopy per tree. Spray deposition can be improved by ensuring that the air stream matches with the forward speed, fan power, and plume shape for the tree canopy. More air per tree does not necessarily result in better deposition and may blow droplets and deposits out of the canopy.

During the spray trials it was noted that at Farm 1 there was a high amount of spray plume going over the top of the trees' canopy and hitting the ceiling of the polytunnel. The spray plume was reaching approximately 1 – 2 m over the top of the trees. At Farm 2 the spray plume was reaching just over the top of the trees' canopy. Relative to the volume of water and the difficulties of spraying 2-row beds, Farm 2 achieved reasonable coverage and excellent deposition.

Task 4.3. Investigate the consequence of extending the spray interval from 1 to 2 weeks in raspberry

Introduction

Extending the interval between sprays of insecticides on raspberry has the potential to offer equal protection against *D. suzukii* compared to standard spray practices, whilst reducing the number of insecticides used. *D. suzukii* Matsumura, native to eastern and south-eastern Asia, is a pest insect that causes economic damage to commercially grown soft and stone fruits. Control includes insecticide application (Walsh et al. 2011), however in the EU there has been an ongoing review and phase-out of chemical plant protection products cPPPs (pan-europe.info. 2008) limiting the number of effective products available to protect a crop throughout the growing season.

In 2018, NIAB EMR investigated whether the interval for applying insecticides to raspberry could be extended to 2 weeks in meshed commercial raspberry to protect against *D. suzukii* whilst reducing the number of applications. Two insect meshed primocane raspberry varieties in 2 plantations were used. Treatments were either a fortnightly spray program of approved products; Exirel and Tracer or the growers' spray program. Findings showed more adult *D. suzukii* were caught inside the crops where the growers' spray program was applied compared to the fortnightly spray program, even though the fortnightly plots were under higher *D. suzukii* immigration pressure from the surrounding habitat. However, there were only 2 replicates of each treatment so it was not possible to do statistical analyses on pest emergence from fruit (an indicator of egg-laying) or the mortality of *D. suzukii* that came into contact with raspberry leaves collected from plots under each spray program. Nevertheless, fewer *D. suzukii* emerged from fruit and more adults died in contact with leaves in the crop in the fortnightly applied spray program compared to the growers' program (where more sprays were applied), indicating that a fortnightly application of approved insecticides is effective for protecting commercially grown raspberry under cladding. The purpose of the 2019 trial was to:

- Statistically confirm the 2018 findings by comparing a fortnightly spray program of approved insecticides to a weekly spray program in 8 replicate protected raspberry crops.
- Determine if fortnightly spray intervals on protected raspberry reduce the incidence of *D. suzukii* in fruit, adult mortality in contact with leaves and adult presence in the crop comparably to the weekly program.

Materials and Methods

Trial sites: 8 raspberry sites (blocks) split between 2 farms (4 blocks each farm) were used for the trial. All raspberries were polytunnel grown varieties including, Grandeur, Kweli, Paragon and Ovation. Polytunnels were approximately 7 m wide, each with 3 rows of raspberry canes spaced 2.5 m apart (Image 4.3.1). Insect exclusion mesh covered polytunnel ends to prevent migration of *D. suzukii* into the crop (Image 4.3.2).

Figure 4.3.1. Polytunnel used for the raspberry spray trial 2019, with end labelled to indicate fortnightly spray program

Figure 4.3.2. Polytunnels with insect exclusion mesh covering ends as used for the raspberry spray trial 2019

Block layout: Each block was sub-divided into 2 plots; a fortnightly spray program plot (yellow) and a weekly spray program plot (blue) (Fig. 4.3.3). To prevent spray drift, plots employing the different spray programs were isolated using a polythene barrier. NB: for ease of spraying the fortnightly plots were always on the ends on the polytunnel blocks and usually nearer to a wild source of *D. suzukii.*

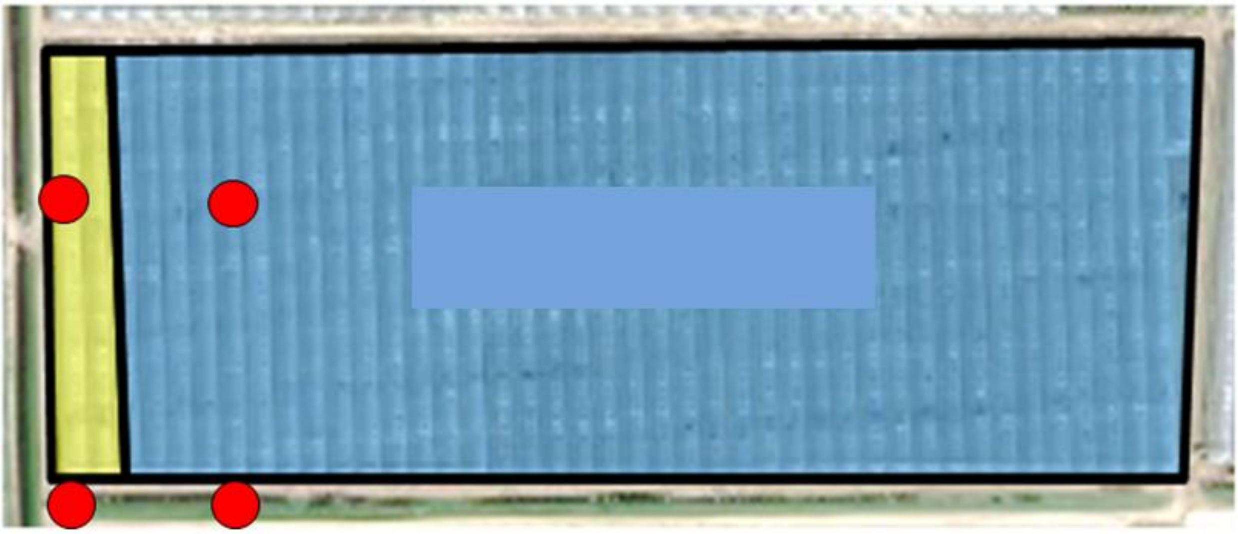


Figure 4.3.3. Plantation map of experimental block of raspberry spray trial 2019 divided into 2 plots; a fortnightly spray program plot (yellow) and a weekly spray program plot (blue). Red dots indicate locations of Droso traps with commercial bait, inside and outside each plot

Treatments: Treatments were either a fortnightly spray program of approved products; rotating Tracer and Exirel from 22 August at Farm 1 (blocks 1 to 4) and 29 August at Farm 2 (blocks 5 to 8), or a weekly spray program (Table 4.3.1). Exirel 10SE was granted emergency approval. The growers' standard spray equipment was used on all plots and other pests and disease treatments were the same across all plots.

Assessments: 6 assessments were made over the trial period; 1 pre-assessment and 5 during spray programs. Assessments were made fortnightly in each plot per block, the day before spraying if a spray was planned (Table 4.3.3).

D. suzukii damage to fruits: 50 ripe raspberry fruits were picked per plot (800 per fortnight). Over ripened fruits were picked from the centre of the row and lower down in the canopy to give the best chance of detecting *D. suzukii*. Fruit was incubated for 2 weeks (~22 °C, >40 % RH, 16 h light: 8 h dark) in a Perspex box (20 x 10 x 10 cm) with a mesh lid and the numbers of male and female *D. suzukii* emerging from fruit were counted. Results were compared to the weekly spray program to confirm whether a fortnightly spray program gives comparable protection against *D. suzukii*.

Table 4.3.1. Date and spray application for *D. suzukii* at Blocks 1 to 8 in weekly and fortnightly spray programs during the raspberry spray trial 2019.

	Blocks 1 to 4				Blocks 5 to 8			
Date	Activity	Spray	Week-ly plots	Fortnight-ly plots	Activity	Spray/ frequency	Week-ly plots	Fortnight-ly plots
22-Aug	Spray 1	Tracer 1	Yes	Yes				
29-Aug	Spray 2	Pyrethrin 1	Yes	No	Spray 1	Tracer 1	Yes	Yes
05-Sep	Spray 3	Exirel 1	Yes	Yes	Spray 2	Pyrethrin 1	Yes	No
12-Sep	Spray 4	Calypso 1	Yes	No	Spray 3	Exirel 1	Yes	Yes
19-Sep	Spray 5	Tracer 2	Yes	Yes	Spray 4	Calypso 1	Yes	No
26-Sep	Spray 6	Pyrethrin 2	Yes	No	Spray 5	Tracer 2	Yes	Yes
03-Oct	Spray 7	Exirel 2	Yes	Yes	Spray 6	Pyrethrin 2	Yes	No
10-Oct	Spray 8	Calypso 2	Yes	No	Spray 7	Exirel 2	Yes	Yes
17-Oct	Spray 9	Tracer 3	Yes	Yes	Spray 8	Calypso 2	Yes	No
24-Oct	Spray 10	Pyrethrin 3	Yes	No	Spray 9	Tracer 3	Yes	Yes
31-Oct					Spray 10	Pyrethrin 3	Yes	No

Longevity and efficacy of sprays on raspberry leaves: 20 leaves per plot and an additional two weekly batches of 20 leaves from a wild raspberry bush growing at NIAB EMR as an unsprayed comparison (control) were picked (360 leaves per fortnight). Leaves were divided into 4 groups of 5 and placed into deli cups with moist filter paper and a feeder containing 5% dextrose solution (as for Task 4.2). 5 male and 5 female *D. suzukii* were introduced into each pot and then *D. suzukii* mortality counted at 48 hours.

Monitoring D. suzukii inside and outside the crop perimeter: A DrosoTrap was placed within each plot and one outside the perimeter of the plot. DrosoTraps were baited with commercial bait (Biobest Dros' attract new formulation); 4 per block (Fig. 4.3.1). The traps were filtered fortnightly and numbers of male and female *D. suzukii* counted. The perimeter of the polytunnels was insect meshed (Fig. 4.3.2).

Regular communication was made between growers and staff at NIAB EMR. All samples were collected by staff at NIAB EMR.

Data loggers were installed within Delta Traps; 2 per block (1 per plot), 21 August 2019 to record temperature and humidity throughout the experimental period (Appendix 4.3).

The insecticides in Table 4.3.2. were recommended by the AHDB in 2018. The insecticides for 2019 were a maximum of 3 x Decis (deltamethrin/pyrethroid), 3 x Tracer (spinosad/spinosyn) and 2 x Exirel (cyantraniliprole/diamide).

Table 4.3.2. Products approved for application against *D. suzukii* on raspberry canes in 2018 for the use in 2018 mid-August through to December 2019.

Crop Situation	Active	Typical Product	Approval	Max. Applications	Max. Rate	Harvest Interval
Outdoor	cyantraniliprole	Exirel 10 SW	Emergency 120-day authorisation	2	900 ml/ha	3 days
	deltamethrin	Decis	Full	None listed	0.5 l/ha (Max Dose: 1.5 l/ha)	7 days
	lambda-cyhalothrin	Hallmark with Zeon Technology	EAMU	4	0.075 l/ha (Max Dose: 0.15 l/ha)	28 days
	pyrethrins*	Pyrethrum 5 EC	Full	No limit	0.02 l per 5 litres	1 day
	spinosad	Tracer	EAMU	2	0.2 l/ha	3 days
	thiacloprid*	Calypso	EAMU	None listed	0.25 l/ha (Max Dose: 0.75 l/ha)	3 days
Protected	abamectin*	Dynamec	EAMU	None listed	0.5 l/ha (Max Dose: 1 l/ha)	3 days
	cyantraniliprole	Exirel 10 SW	Emergency 120-day authorisation	2	900 ml/ha	3 days
	deltamethrin	Decis	Full	None listed	0.5 l/ha (Max Dose: 1.5 l/ha)	7 days
	pyrethrins*	Pyrethrum 5 EC	Full	No limit	0.02 l per 5 litres	None stated
	spinosad	Tracer	EAMU	3	200 ml/ha	1 day
	thiacloprid*	Calypso	EAMU	None listed	0.25 l/ha (Total Dose: 0.75l/ha)	3 days

***Denotes limited effect**

Table 4.3.3. Dates of *D. suzukii* assessments at each block during the raspberry spray trial 2019. Assessments per plot included; counting *D. suzukii* in 4 DrosoTraps (1 inside polytunnel and 1 outside), counting *D. suzukii* emerged from 50 raspberry fruit samples and counting *D. suzukii* mortality after 48 hr contact with 20 leaf samples.

Assess. No.	Week	Blocks 1 to 4	Blocks 5 to 8	4 Droso traps	50 raspberries	20 raspberry leaves
1	Pre-assess	21-Aug	28-Aug	X	X	X
2	2	04-Sep	11-Sep	X	X	X
3	4	18-Sep	25-Sep	X	X	X
4	6	02-Oct	09-Oct	X	X	X
5	8	16-Oct	23-Oct	X	X	X
6	10	30-Oct	06-Nov	X	X	X

Statistical Analyses

All statistical analyses were carried out in R 3.51 (RSTUDIO).

D. suzukii damage to fruits: To compare adult *D. suzukii* emergence from raspberries sampled at weekly and fortnightly spray plots, numbers of adult *D. suzukii* emerged after 2 weeks incubation were analysed using a likelihood ratio test.

Longevity and efficacy of sprays on raspberry leaves: To compare adult *D. suzukii* mortality after 48 hr contact with raspberry leaves sampled at weekly, fortnightly and control plots, percent of dead adult *D. suzukii* were analysed using a likelihood ratio test.

Monitoring D. suzukii inside and outside the crop perimeter: To compare adult *D. suzukii* caught in DrosoTraps inside and outside weekly and fortnightly spray plots, numbers of adult *D. suzukii* in traps were analysed using a likelihood ratio test.

Results

D. suzukii damage to fruits: On average, significantly more adult *D. suzukii* emerged per 50 raspberries sampled from fortnightly spray program plots compared to weekly (Grand mean = 193.6 and 41.9 respectively, *P*<.001). This was the case on 4 of the 5 assessments during spray programs, weeks 2, 6, 8 and 10 (*P*<0.001, Fig. 4.3.4, Table 4.3.4). Following all 3 Tracer applications on fortnightly and weekly plots, a follow-up application of Pyrethrin on weekly plots a week later significantly reduced adult *D. suzukii* emergence from fruit in weekly spray program plots compared to fortnightly. Whereas from only 1 of the 2 Exirel applications on fortnightly and weekly plots, a follow-up application of Calypso on weekly plots a week later significantly reduced adult *D. suzukii* emergence in weekly spray program plots compared to fortnightly (Table 4.3.4). In comparison to pre-assessment numbers, the fortnightly spray program only significantly reduced adult *D. suzukii* emergence from fruit once, whereas the weekly spray program significantly reduced it 4 times (Table 4.3.5).

Table 4.3.4. Significant means between treatments and within assessments.

Assessment	Treatment	Previous spray	Treatment	Previous spray	P-value
Week 2	Fortnightly	Tracer	Weekly	Pyrethrin	P<0.001
Week 6	Fortnightly	Tracer	Weekly	Pyrethrin	P<0.001
Week 8	Fortnightly	Exirel	Weekly	Calypso	P<0.001
Week 10	Fortnightly	Tracer	Weekly	Pyrethrin	P<0.001

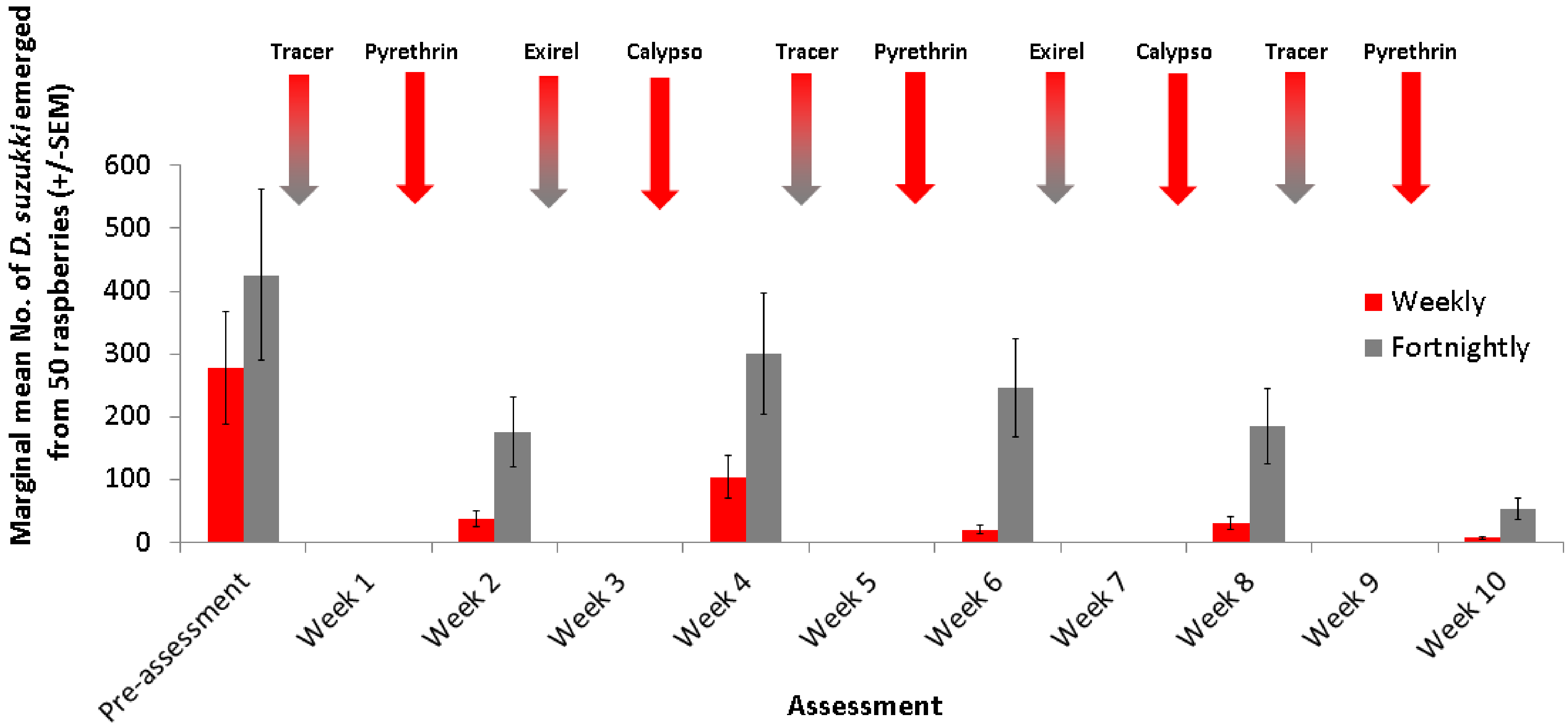


Figure 4.3.4. Marginal means of adult *D. suzukii* emerged from 50 raspberries sampled from blocks 1 to 8, according to spray programme (fortnightly or weekly). Arrow and colour (red = Weekly, and grey = Fortnightly) represent spray and application timing

Table 4.3.5. Significant means between assessments with treatments.

Assessment	Previous spray	Treatment	Assessment	Previous spray	P-value
Pre-assessment	No	Fortnightly	Week 10	Tracer	P<0.001
Week 4	Exirel	Fortnightly	Week 10	Tracer	P<0.001
Week 6	Tracer	Fortnightly	Week 10	Tracer	P<0.001
Pre-assessment	No	Weekly	Week 2	Pyrethrin	P<0.001
Pre-assessment	No	Weekly	Week 6	Pyrethrin	P<0.001
Pre-assessment	No	Weekly	Week 8	Calypso	P<0.001
Pre-assessment	No	Weekly	Week 10	Pyrethrin	P<0.001
Week 2	Pyrethrin	Weekly	Week 10	Pyrethrin	P<0.001
Week 4	Calypso	Weekly	Week 6	Pyrethrin	P<0.001
Week 4	Calypso	Weekly	Week 10	Pyrethrin	P<0.001

Longevity and efficacy of sprays on raspberry leaves: Out of 40 adult *D. suzukii* applied to 20 raspberry leaves sampled from weekly and fortnightly spray program plots and the unsprayed control, a significantly higher percent died in contact with weekly sprayed leaves than fortnightly sprayed and unsprayed control leaves (mean % = 24.5, 17.5 and 10.2 respectively, $P = .006$, Fig. 4.3.5). Treatment post hoc tests revealed overall mean % *D. suzukii* mortality in contact with sampled leaves was not significantly different between fortnightly sprayed and unsprayed control plots.

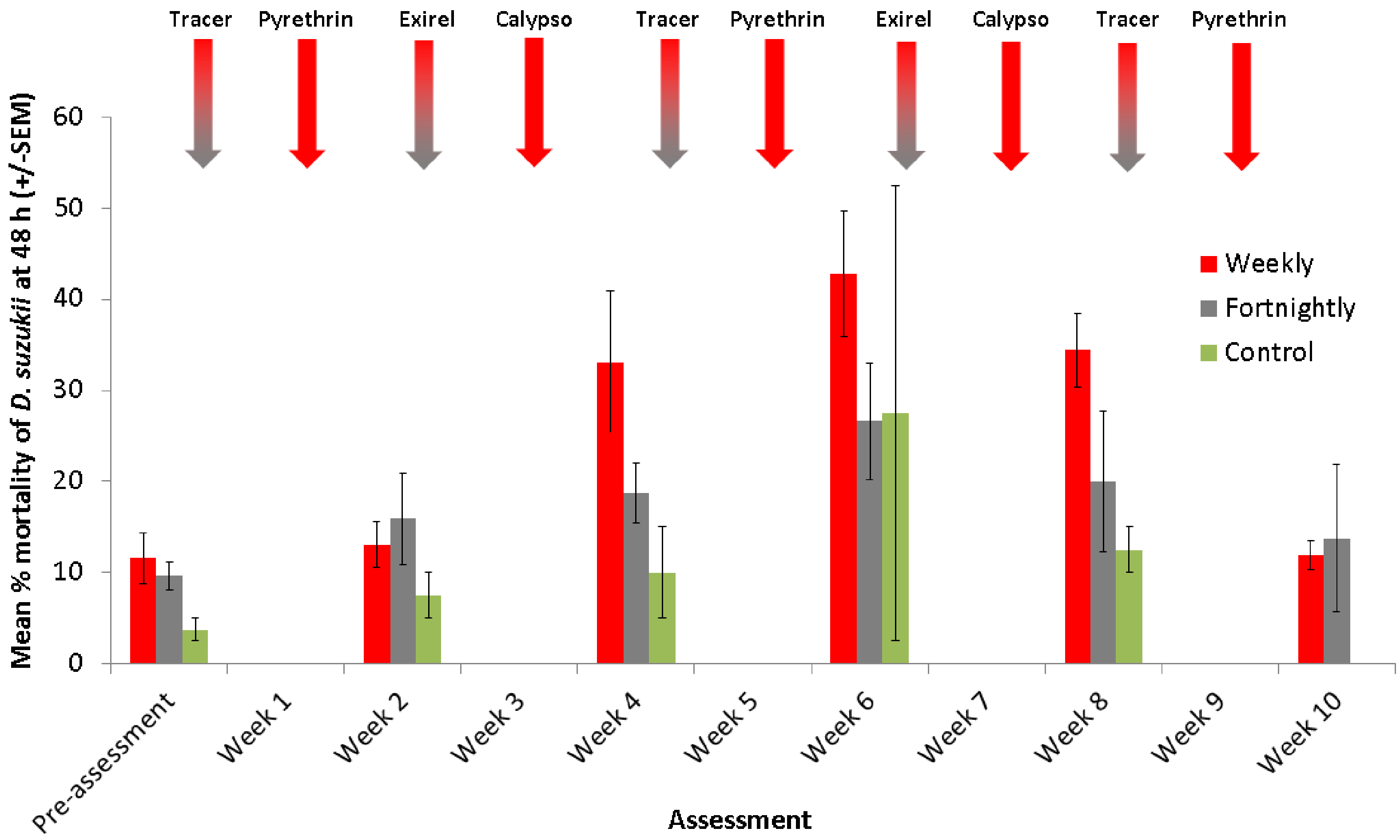


Figure 4.3.5. Mean percent of adult *D. suzukii* that had died after 48 hours contact with insecticide treated (fortnightly or weekly program) raspberry leaves compared to unsprayed raspberry leaves (green bars).

Monitoring D. suzukii inside and outside the crop perimeter: There was no significant difference in mean numbers of *D. suzukii* caught in DrosoTraps between spray programs, but there was between the inside and outside of the polytunnels.

Post hoc analysis found no significant difference between mean numbers of *D. suzukii* caught in DrosoTraps between weekly and fortnightly sprayed plots when comparing respective trap counts inside polytunnels (Grand mean = 703.5) and outside polytunnels (Grand mean = 4933.5), despite consistently lower counts at weekly plots than fortnightly (both inside and outside) from assessment week 6 (Fig. 4.3.6). However, ignoring spray program, treatment post hoc test results comparing spray programs and DrosoTraps positions found significant differences; all between mean numbers of *D. suzukii* caught inside and outside polytunnels (*P*<.001, Table 4.3.6). Comparing assessments and DrosoTrap positions, up to assessment week 6 there was no significant difference between mean numbers of *D. suzukii* caught in DrosoTraps inside and outside polytunnels, thereafter the difference was significant (*P*<.001).

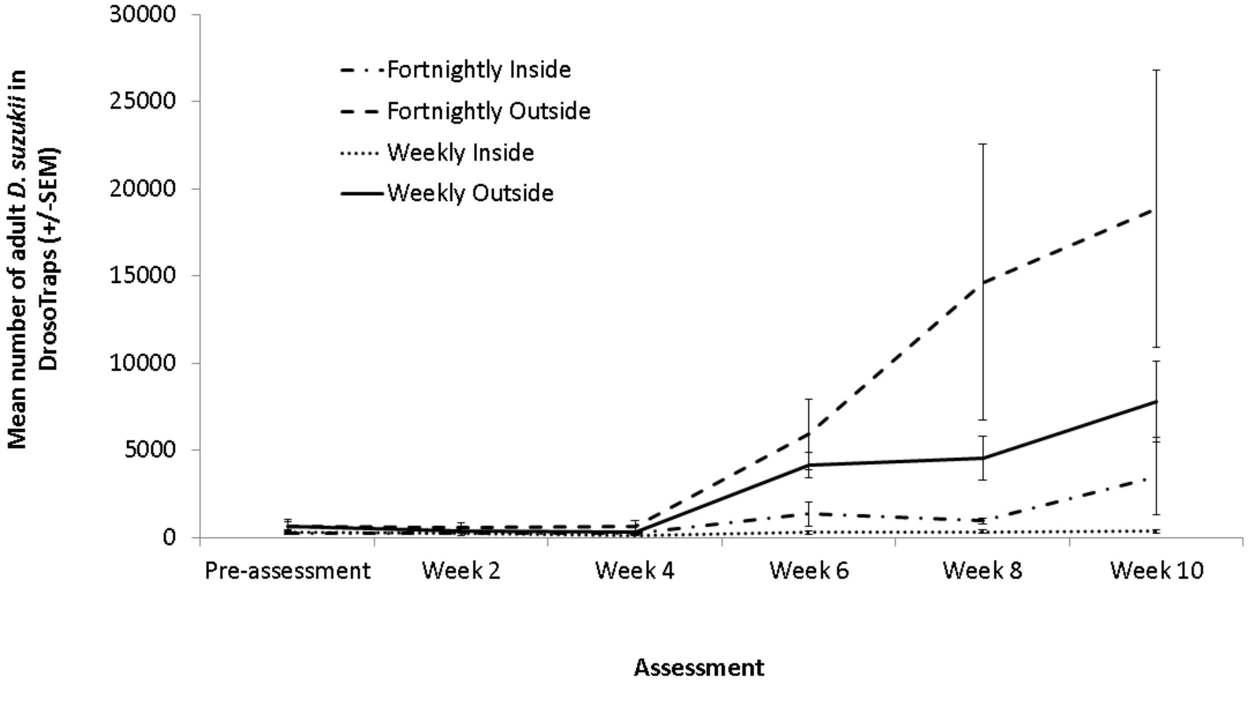


Figure 4.3.6. Date and mean numbers of total *D. suzukii* caught in DrosoTraps at blocks 1 to 8, according to trap position: **Fortnightly Inside** = fortnightly spray programme with trap inside raspberry polytunnel, **Fortnightly Outside** = fortnightly spray programme with trap outside polytunnel, **Weekly Inside** = weekly spray programme with trap inside polytunnel, **Weekly Outside** = weekly spray programme, trap outside polytunnel

Table 4.3.6. Treatment post hoc test results comparing spray plots and respective DrosoTraps positions and showing significant differences between mean numbers of *D. suzukii* caught during the raspberry spray trial 2019.

Contrast	P-value
Fortnightly Inside / Fortnightly Outside	<.0001
Fortnightly Inside / Weekly Inside	0.2506
Fortnightly Inside / Weekly Outside	0.0002
Fortnightly Outside / Weekly Inside	<.0001
Fortnightly Outside /Weekly Outside	0.9615
Weekly Inside / Weekly Outside	<.0001

Estimated costs of respective spray programs: Using costs supplied by the grower for tractor, operator and insecticide, the respective costs of spray programs applied during this trial were calculated. The fortnightly spray program was cheaper at a total of £423.80 per hectare compared to £722.74 per hectare for the weekly spray program (Table 4.3.7).

Table 4.3.7. Estimated costs of weekly and fortnightly spray programs of insecticides tested against *D. suzukii* during the spray trial in protected raspberry 2019. Costs were provided by the grower and include tractor, operator and insecticides.

Spray	**Weekly plots (cost per Ha.)**	**Fortnightly plots (cost per Ha.)**
Tracer 1	£74.60	£74.60
Pyrethrin 1	£65.18	No spray
Exirel 1	£100	£100
Calypso 1	£51.70	No spray
Tracer 2	£74.60	£74.60
Pyrethrin 2	£65.18	No spray
Exirel 2	£100	£100
Calypso 2	£51.70	No spray
Tracer 3	£74.60	£74.60
Pyrethrin 3	£65.18	No spray
TOTAL	**£722.74**	**£423.80**

Discussion

Overall, in 2019 the weekly spray program for raspberry was more effective at reducing numbers of *D. suzukii* compared to the fortnightly spray program in contrast to findings from the 2018 pilot study. From samples of 50 raspberries collected at weekly and fortnightly sprayed crops, significantly more adult *D. suzukii* emerged from raspberries sampled from fortnightly sprayed plots compared to weekly on 4 of the 5 assessments during spray programs ($P<.001$). For 3 of these, the weekly program applied Tracer followed by Pyrethrin a week later, on the other occasion Exirel was applied followed by Calypso a week later. The only occasion there was no significant difference was when Exirel was applied followed by Calypso a week later, suggesting Tracer followed by Pyrethrin is more effective at reducing *D. suzukii*. However, mean *D. suzukii* emergence was still lower in weekly compared to fortnightly on that occasion (mean = 113.3 and 386.3 respectively). In 2018, excluding 16 Oct (which followed 2 weeks after a less effective Spruzit application), more *D. suzukii* emerged from raspberries sampled at growers' spray program plots compared to fortnightly (mean = 12.7 and 7.8 respectively). However, this was possibly because the growers' spray program in 2018 used fewer Tracer and Exirel applications than the fortnightly program. Previous research has concluded both Tracer and Exirel give good control of *D. suzukii* emerging from fruits (AHDB SF 145, Pavlova et al. 2017; Van Timmeren 2013).

In agreement with fruit emergence findings in 2019, significantly more adult *D. suzukii* died in contact with raspberry leaves sampled from weekly spray program plots compared to fortnightly and the unsprayed control (mean % = 24.5, 17.5 and 10.2 respectively, $P=.006$). During the 2018 raspberry trial there was higher *D. suzukii* mortality in contact with leaves sampled from fortnightly spray program plots compared to growers' (mean % = 40.5 and 20.8 respectively). Although not statistically validated, again this was probably attributable to fewer Tracer and Exirel applications in the growers' spray program. Findings in cherry 2017 however showed similar adult *D. suzukii* mortality in fortnightly and weekly spray program plots after the same number and timing of Tracer and Exirel applications (Shaw et al. 2019).

There was no significant difference in mean numbers of *D. suzukii* caught in DrosoTraps between spray programs, but there was between DrosoTraps inside and outside of the polytunnels. Despite statistical analysis finding no significant difference between mean numbers of *D. suzukii* caught at fortnightly and weekly spray program plots, overall more were caught at fortnightly plots than weekly plots (mean = 3982.3 and 1619.9 respectively). This was mainly influenced by 2 DrosoTraps in habitat neighbouring fortnightly plots at 2 separate blocks which caught most *D. suzukii* during a period when competition from the crop and wild host fruit was diminishing (Fountain et al. 2017); fortnightly plots were always at the edge of blocks and therefore under higher *D. suzukii* immigration pressure. Added to this, DrosoTraps inside fortnightly spray program polytunnels caught more *D. suzukii.* Significantly more *D. suzukii* were caught inside polytunnels compared to outside ($P<.001$). This agrees with cherry spray trial findings (Shaw et al. 2019), supporting the use of insect exclusion mesh to protect against *D. suzukii* immigration. From assessment week 6 to 10 (early-October to early-November), mean numbers of *D. suzukii* caught in DrosTraps rose significantly compared to earlier assessments in nearly all plot positions, except the weekly spray program inside the polytunnel. The increase in *D. suzukii* numbers caught in DrosoTraps during this period follows the natural trend observed during the UK *D. suzukii* national monitoring survey; avoidance of this in the weekly spray program polytunnel supports a more effective spray program.

At present we cannot recommend a fortnightly spray program of PPPs over weekly intervals to control *D. suzukii* in protected raspberry. However, considering ongoing PPP restrictions, benefits to natural enemies, and reduced spray application costs calculated during this trial (Table 4.3.7), this approach should be further explored with additional alternative treatments.

Conclusions

- The trial set out to compare weekly and fortnightly spray intervals of *D. suzukii* targeted PPPs on protected raspberry.
- The weekly spray program was more effective at reducing fruit damage and adult mortality in contact with sampled leaves compared to the fortnightly spray program.
- This is contrary to findings in cherry (AHDB SF TF 145a report 2017; Shaw et al. 2019).
- Fortnightly sprayed plots were at the edge of blocks and under higher pressure from *D. suzukii* immigration from wild host habitats, particularly later in the trial when fruit was scarce.
- In agreement with cherry spray trial findings (Shaw et al. 2019), 2019 raspberry trial findings suggest insect exclusion mesh offers good protection against *D. suzukii* immigration.

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.

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 but also via the AHDB website and a wallchart and factsheets.

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

Introduction

Pesticide use has led to resistance developing in *D. suzukii* (Gress and Zalom (2019)) and thus alternative methods for control are needed, e.g. use of biological control agents for example parasitic wasps (Lee et al. 2019). *Trichopria drosophilae* is a generalist parasitoid that also parasitizes *D. suzukii* and is commercially available in Europe for use in biological control. However, this parasitoid species has not yet been identified in the UK and as such cannot be released as a biocontrol agent; therefore, native species need to be identified.

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 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. 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 (*Asobara tabida* and *Leptopilina heterotoma*) and three pupal parasitoids (*Pachycrepoideus vindemmiae*, *Spalangia erythromera* and *Trichopria prema*) were recorded from *D. suzukii* in South East England. All five species are generalist parasitoids of *Drosophila*.

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 very poor ability to develop from *D. suzukii* larvae in the sentinel traps in our survey, probably because of the high immune response produced by *D. suzukii* larvae.

The presence and abundance of these 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.

Aims

The aims of the studies in 2019 were to identify if the same parasitoids that parasitize *D. suzukii* in South East England were also present further north, in Scotland. The second aim was to determine what level of parasitism occurs outside the cropping area.

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

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*.

Following the NIAB EMR Standard Operating Procedure for the previous studies, traps were deployed fortnightly between July and October/November 2019 at each site, 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. 7.1.1). 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). 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 *Drosophila melanogaster* which was cultured on the medium provided by the insect supplier (Blades Biological Ltd, UK). 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 JHI 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.

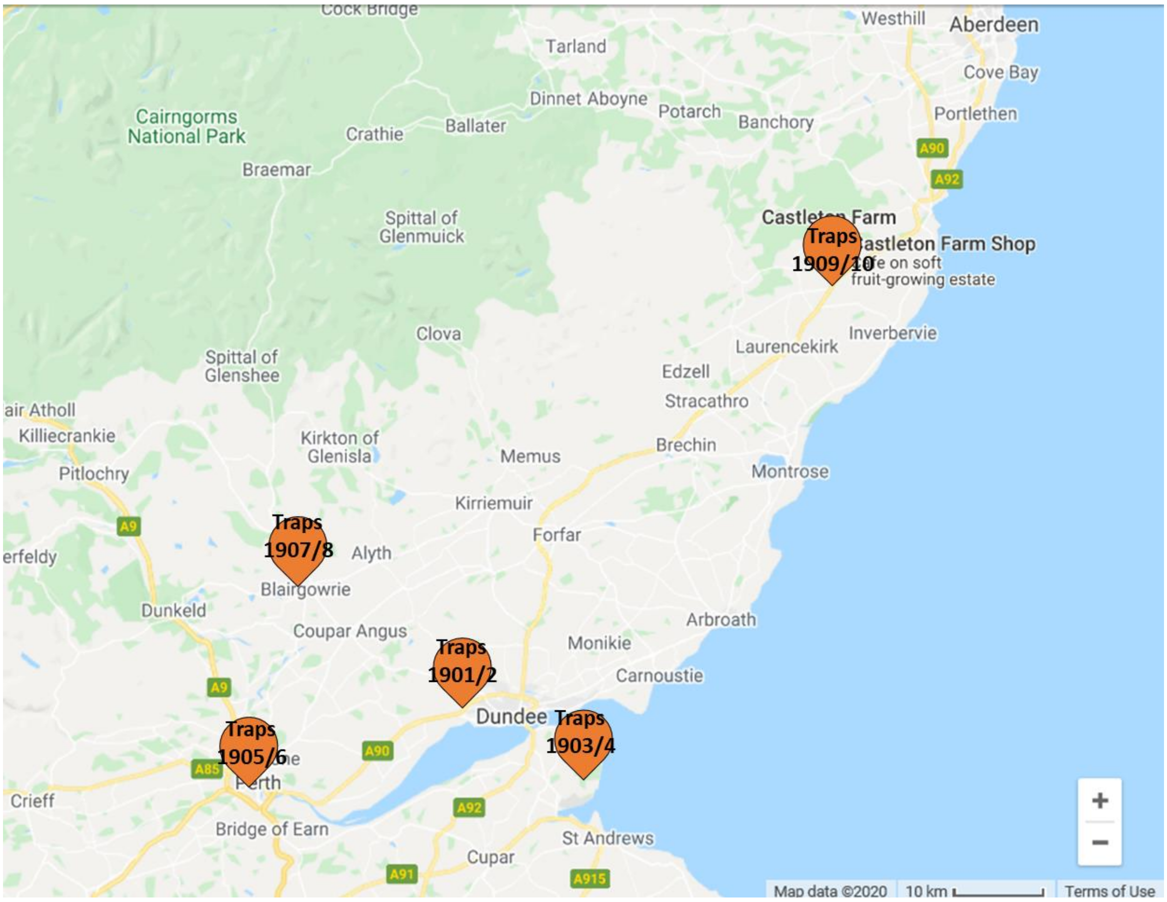


Figure 7.1.1. Map showing location of sites used for SWD parasitoid sampling in Eastern Scotland in 2019

Results

Most parasitoids emerged within 1-5 weeks of trap collection. Two morphotypes have been detected that differ in size and wing venation (approx. two-thirds of the trapped individuals belong to the larger morphotype) and are currently awaiting taxonomic identification to species level. Contact has been made with NHM who are currently unable to identify the samples due to staff absence; we are investigating whether samples can be identified with molecular methods. Parasitoids were trapped in highest numbers in late

July/early August (Fig. 7.1.2), although there was another small peak in abundance in September, particularly at one trap location at the institute.

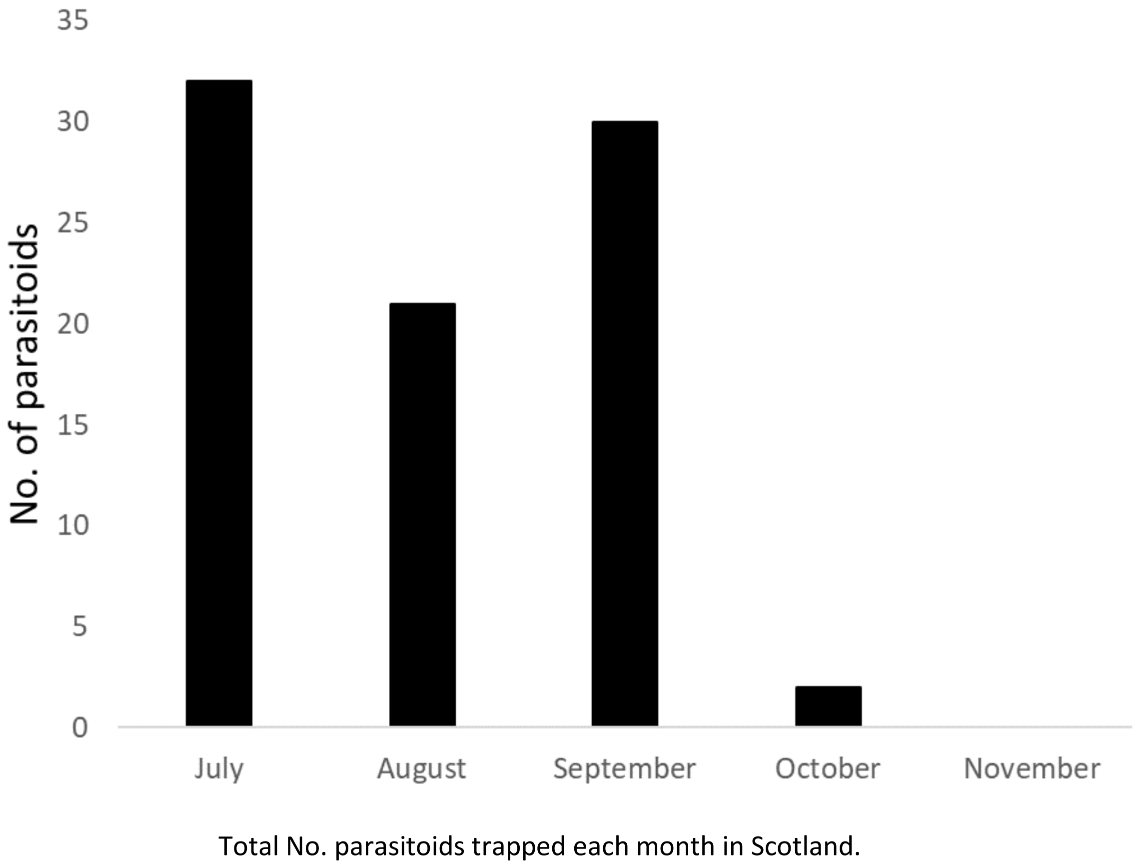


Figure 7.1.2. Phenology of parasitoids captured in the sites in Scotland

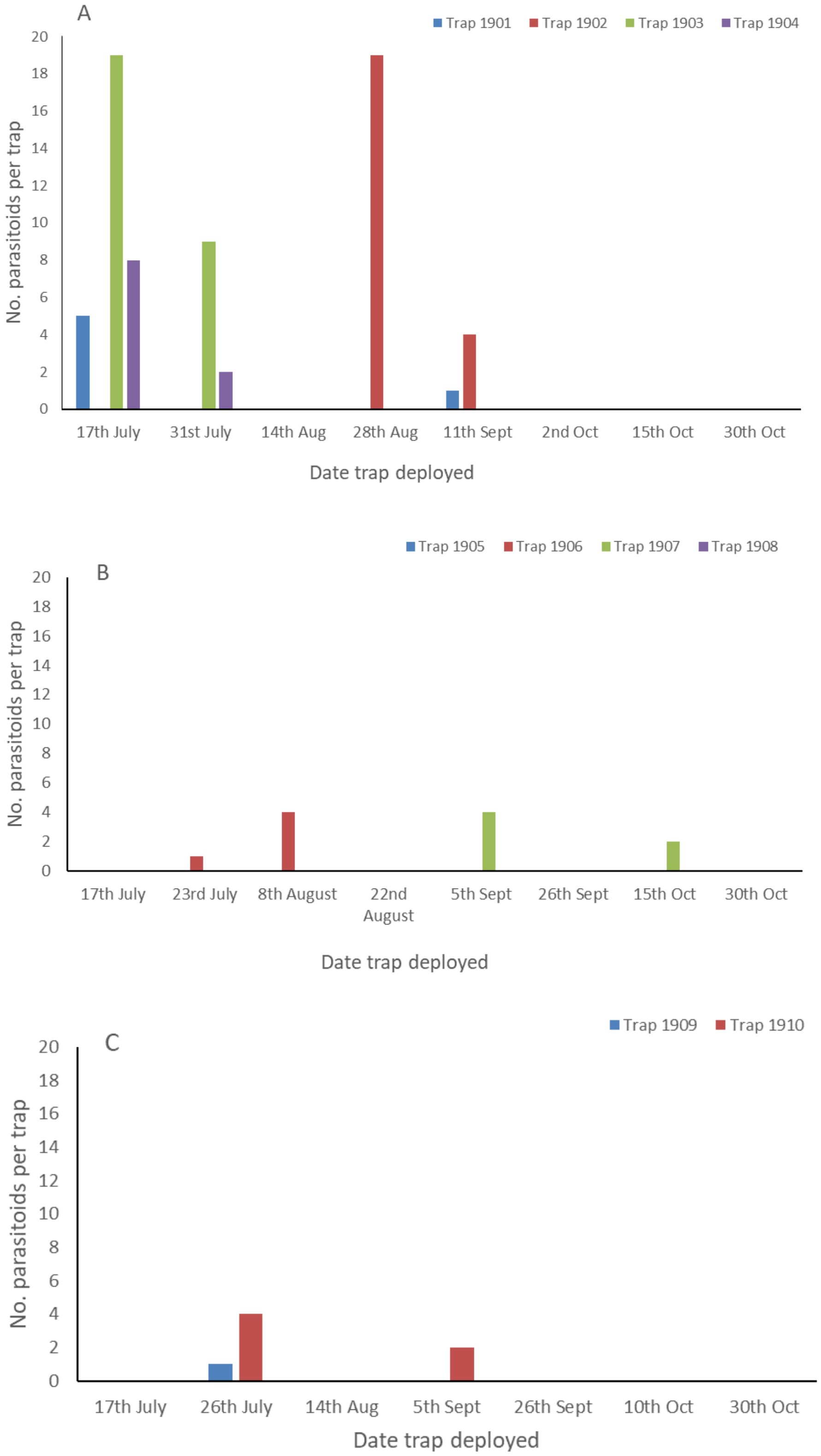


Figure 7.1.3. Numbers of parasitoids emerging from sentinel traps at ten sites in (A) western, B) central-southern and (C) northern areas of Eastern Scotland

There was regional variation in total catch size of parasitoids (Fig. 7.1.3), with highest numbers at two sites (traps 1901/2 at the institute and traps 1903/4 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 1902 and 1903 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. 1905, 1907) did not have high parasitoid trap catches.

Conclusions

- The *D. suzukii* sentinel trap method was successfully adapted for *D. melanogaster* and deployed in Eastern Scotland in 2019.
- Within-season variation in parasitoid abundance suggested that parasitoids were present earlier in the summer than anticipated (before mid-July).
- It is unclear, at present, 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.

Aims

1. To test parasitoid efficiency in field conditions.
2. To test if parasitism rates change throughout the year.
3. Continue to search for the pupal parasitoid *T. drosophilae* in the UK.

Materials and Methods

Sites: Two sites were surveyed for parasitoids in 2019; one woodland (Site 1) and one hedgerow next to an abandoned cherry orchard (Site 2). Sites chosen were where highest numbers of parasitoids were captured in the 2017/2018 trials.

Parasitoid traps: 7 traps were deployed at each site; 6 treatment and 1 control. Treatment traps were composed of Perspex boxes (10 x 10 x 20cm) containing: a bed of blue paper roll; dampened for humidity, 1 Petri dish containing grape agar and 3 slices of fresh banana on the agar, fresh fruit contained in a small (4 x 4 x 6 cm) ventilated Perspex pot, 50 third instar *D. suzukii* larvae were individually transferred into clean media in the boxes. The boxes were covered with a netted lid with 2 mm diameter holes which would allow parasitoids to enter while preventing larger insects and more *D. suzukii* from entering/exiting. The control trap was the same as the treatment except the netted lid had 0.4 mm holes to prevent parasitoid entry. These traps were deployed between 4 and 19 June 2019.

The first traps deployed were destroyed by rodents. Between 02 July and 10 September 2019 an iron lid with holes was added to allow parasitoids to enter but exclude rodents.

From these samples very few parasitoids were emerging. We compared the method (used by scientists from Edmund Mach Foundation, Italy) to our original technique and then deduced that the only element missing was the degraded food material (media) fed on by the *D. suzukii* larvae. On 24 September 2019, an additional small ventilated Perspex pot was added to each trap, which contained previously de-frosted fly culture media to include the odour of larvae waste. Two additional traps were also put out at each site following a similar method to previous years: Perspex boxes (10 x 10 x 20 cm) containing: a bed of blue paper roll, previously frozen strawberries, fresh fruit contained in a small (4 x 4 x 6 cm) ventilated Perspex pot and adult *D. suzukii*, these didn't have a lid. The iron lid was also replaced with aluminium mesh.

Between 8 October and 5 November 2019 a new method was applied: traps were composed of Perspex boxes (10 x 10 x 20cm) containing: a bed of blue paper roll; dampened for humidity, 1 Petri dish containing grape agar and 2-4 (size dependant) previously frozen strawberries, fresh fruit (strawberries or raspberries depending upon what was available) and some live adult flies (5-6) contained in a small ventilated Perspex pot, 50 third instar *D. suzukii* larvae, small ventilated Perspex pot containing previously frozen fly culture media. All of these changes were made to counteract the poor results from emerging parasitoids.

Deployment and assessment: Traps were replaced fortnightly; from 4 June 2019. Upon collection traps were returned to NIAB EMR where the number of adult *D. suzukii* males and females per trap were counted and recorded. Traps were then incubated at 20-25 °C, ~50% relative humidity and 16: 8 hours light: dark photoperiod. The 2 mm hole lids were changed for fine mesh lids (~ 0.4 mm hole diameter) to prevent the escape of newly emerged parasitoids. Traps were examined weekly for a period of six weeks to ensure all emerging parasitoids were recorded. Parasitoids were captured and stored in 70% ethanol for later identification.

Results

Parasitoids identified: From 145 undamaged traps deployed throughout the trial, 10 parasitoids were collected; nine *A. tabida* (90%) and one *Leptacis sp* (10%) (Table 7.1.1). Parasitoids were collected from three separate deployments; *Leptacis sp* from Site 1 on 16 July and all *A. tabida* from Site 2, eight on 24 September and one on 8 October. Of the parasitoids collected, 8 adults emerged after three weeks of incubation, while 2 adults were present in traps on the day of collection from the field. Parasitoids were only present in a single trap each collection. *A. tabida* has been recorded previously and is a known parasitoid of *D. suzukii*, *Leptacis sp* was identified by Dr David Notton of the Natural History Museum, but according to Dr Christina Fisher, is not a parasitoid of *D. suzukii* (Appendix 7.2). Total numbers of parasitoids emerging as well as number per trap are far below previous years (Table 7.2.1). *T. drosophilae* was not detected in any traps.

Of the 166 traps deployed at both sites, seven were destroyed at Site 1 (second set of traps deployed on 19 June 2019) and fourteen were destroyed at Site 2 (first two sets of traps deployed 4 and 19 June 2019). Rodents were the suspected cause. Between 2 July

2019 and 10 September 2019 an iron lid was added to protect the traps, however during that time only 1 parasitoid (at Site 1, 0 at Site 2) was collected. In response the iron lid was changed to an aluminium lid on 24 September 2019. After this time, a further 9 parasitoids were collected, however 8 of these were in the trap following the 2017/18 method meaning a lid was not used for that trap.

Table 7.2.1. Total numbers of parasitoids collected on three collection dates (omitting dates where nothing was collected) in 2019 and how long the trap had been incubated prior to the parasitoid being collected.

Date trap was collected from field	Parasitoid	Location	Total number	Weeks incubated
16 July 2019	*Leptacis sp*	Woodland	1	0
24 September 2019	*A. tabida*	Cherry Orchard	8	3
08 October 2019	*A. tabida*	Cherry Orchard	1	0

Table 7.2.2. The total parasitoid emergence and average per trap for the sites surveyed in each of the three trial years (2017-2019) showing poor recovery in 2019.

Habitat	Year	Total emergence	Total traps deployed in habitat	Average number/ trap
Woodland	2017	410	42	9.7
Woodland	2018	364	76	4.8
Woodland	2019	0	76	0
Wild Cherry Orchard	2017	98	38	2.6
Wild Cherry Orchard	2018	361	38	9.5
Wild Cherry Orchard	2019	8	69	0.11594

Parasitism rates: The number of adult *D. suzukii* in the traps upon collection at Site 1 fluctuated over time in the control, while the number in the treatment were more consistent, however only a single control trap was deployed each time so it is less reliable than the treatment of which there were six per deployment. The numbers in both decreased sharply 24 September 2019. Thereafter few to zero adult *D. suzukii* were present (Fig. 7.2.1). This decrease coincides with decreasing temperatures (Fig. 7.2.2). The proportion of *D. suzukii* emerging as adults in the treatment traps ranged between 60 and 74%, while the proportion in the control traps ranged between 42 and 82% before the decline at the assessment on 24 September 2019.

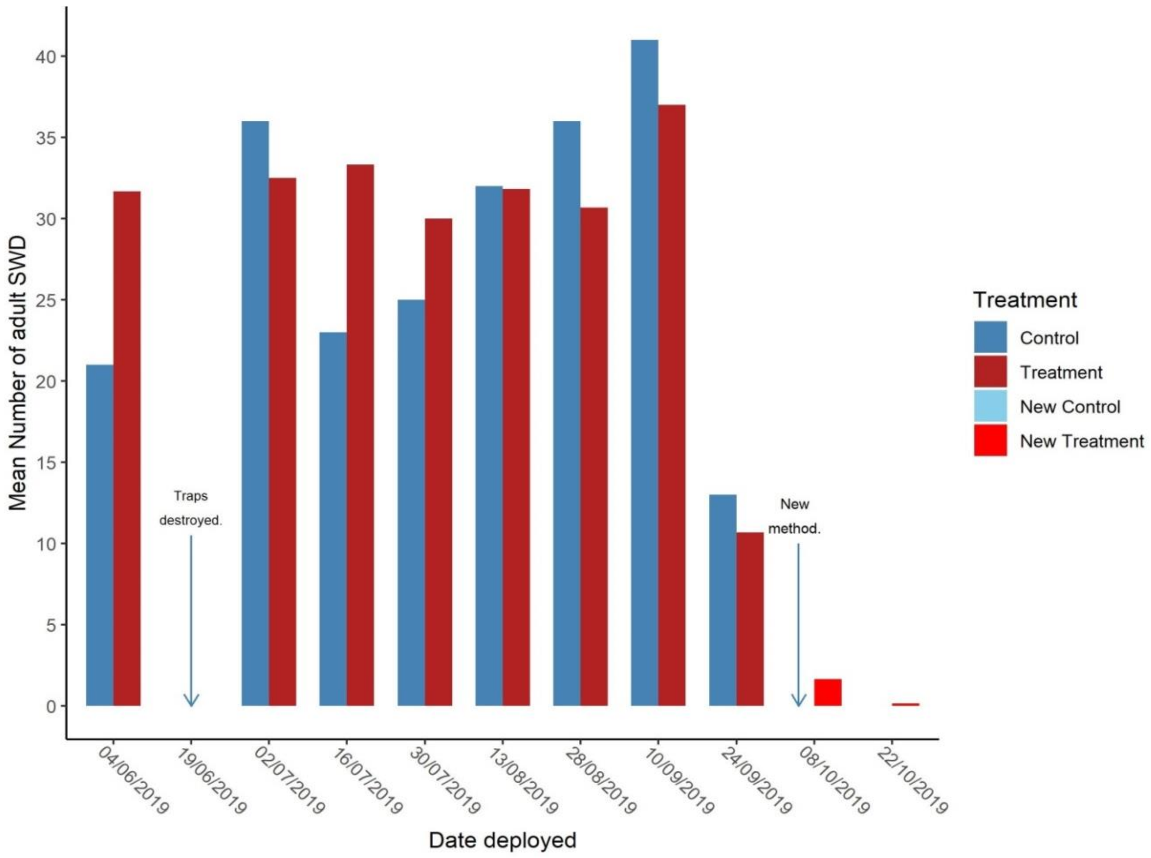


Figure 7.2.1. Mean number of adult *D. suzukii* in the sentinel traps on the day they were collected from the field for Site 1. Control = traps that parasitoids were unable to enter (0.4 mm holes). Treatment = traps parasitoids could enter (2 mm holes). New control and new treatment are as above but with strawberry rather than banana and 5-6 live adult flies in the fresh fruit pot (not counted towards the mean number of adult *D. suzukii*)

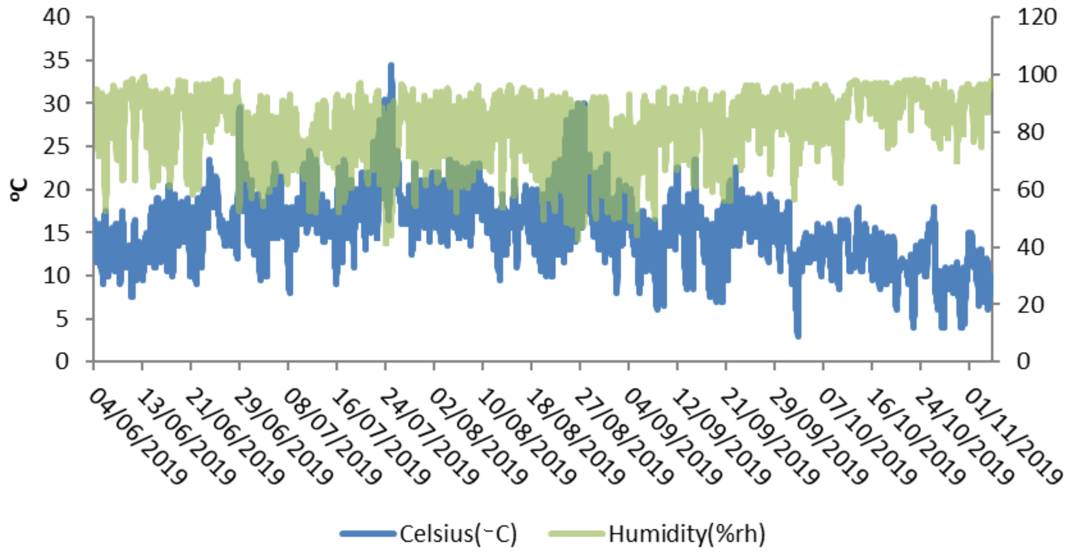


Figure 7.1.2. Temperature and relative humidity data for the trial period from a data logger at site 1

The number of adult *D. suzukii* present in the traps upon collection from site 2 fluctuated more in the control traps than the treatment traps until the 24 September 2019 at which point the numbers decreased (Fig. 7.2.3), although not as sharply as site 1. As before only one control trap was deployed each time while six treatments were deployed. As with site 1 the decrease in adult *D. suzukii* coincides with a decrease in temperature (Fig. 7.2.4). The proportion of *D. suzukii* emerging as adults in the treatment traps ranged between 46 and 67%, while the proportion in the control traps ranged between 48 and 80% before the decline at the assessment on the 24 September 2019.

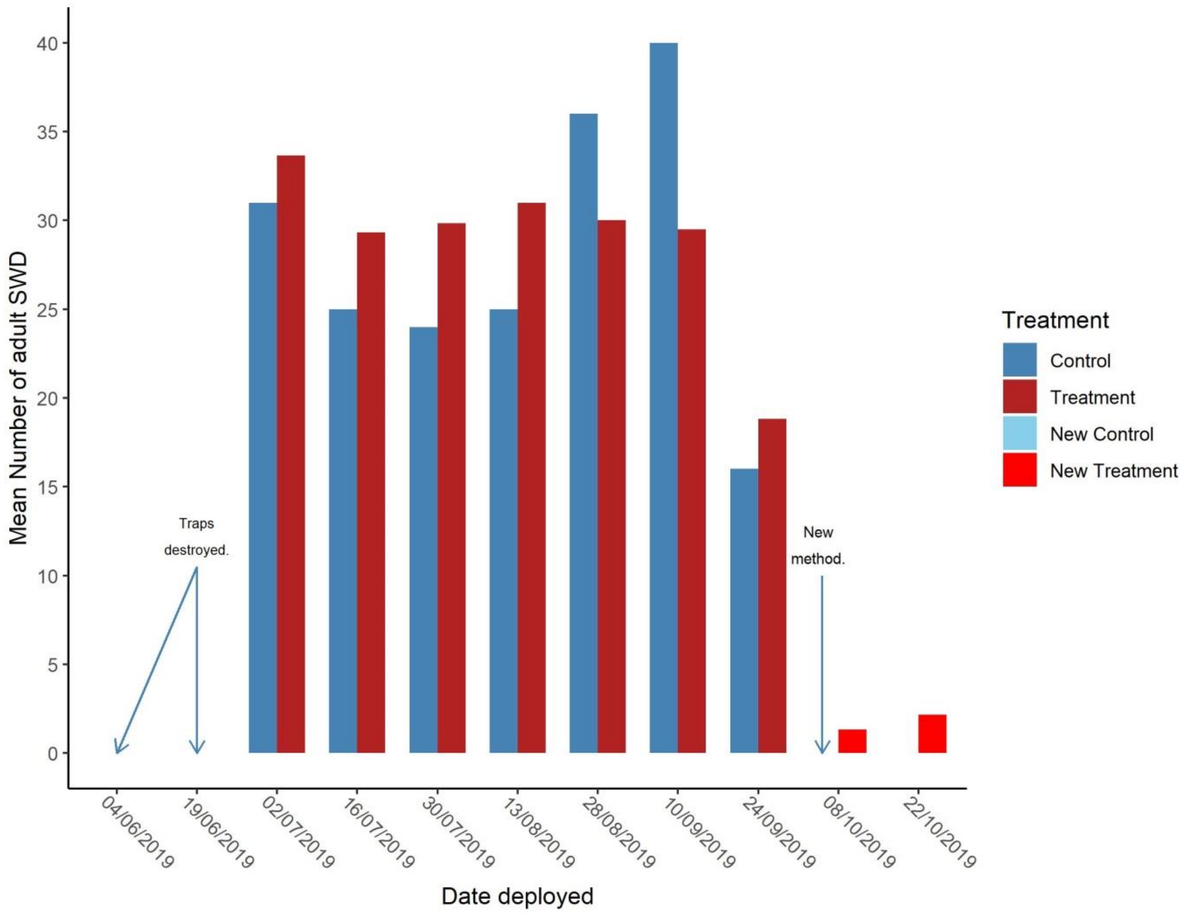


Figure 7.1.4. Mean number of adult *D. suzukii* in the sentinel traps on the day they were collected from the field for site 2. Control = the traps that parasitoids were unable to enter (0.4 mm holes). Treatment = traps parasitoids could enter (2 mm holes). New control and new treatment are as above but with strawberry rather than banana and 5-6 live adult flies in the fresh fruit pot (not counted towards the mean number of adult *D. suzukii*)

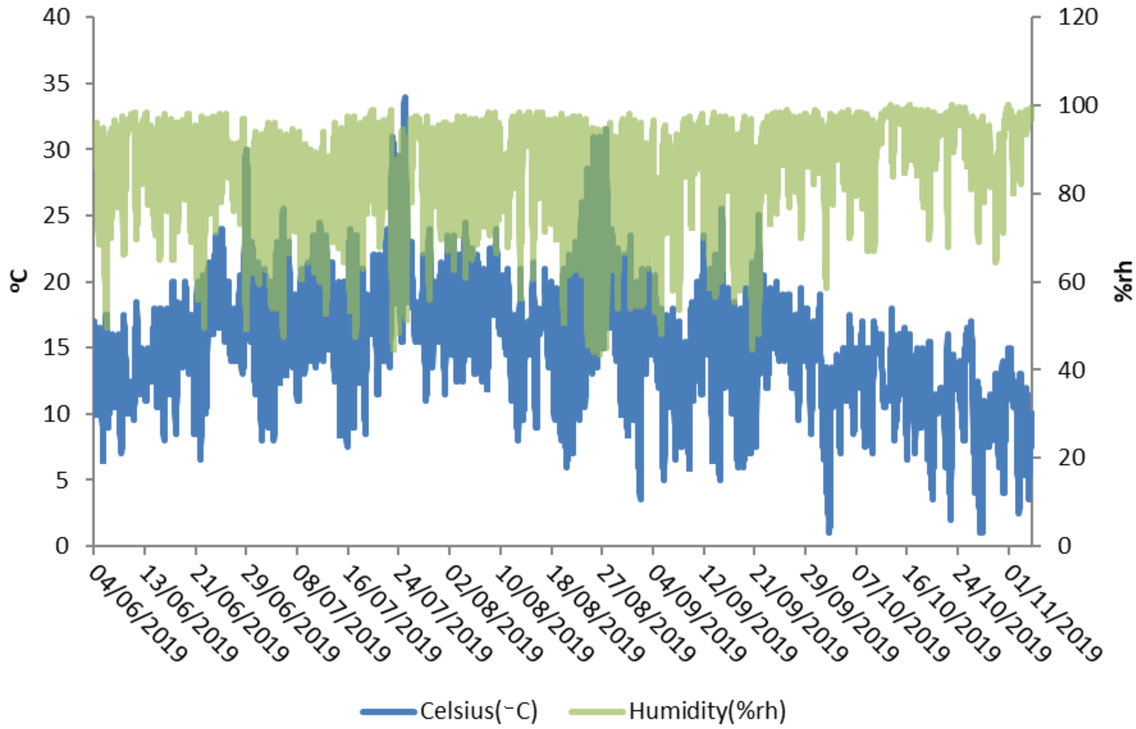


Figure 7.1.4. Temperature and relative humidity data for the trial period from a data logger at site 2

Discussion

Only one *D. suzukii* parasitoid was observed in 2019 (*A. tabida*) so efficiency of *D. suzukii* parasitism by known UK parasitoids could not be calculated.

It is likely the absence of parasitoids was due to a lack of attempted parasitism rather than failed attempts of emergence. The number of emerged (and therefore unparasitized) adult *D. suzukii* in both trap types was similar across all deployments and remained a relatively high percentage of the 50 larvae initially in each trap. The decrease in adult *D. suzukii* numbers in collected boxes at the last three deployments, particularly the last two, is most likely the effect of temperature on *D. suzukii* life cycle. Ryan et al. (2016) states there will be no pupal or adult emergence at temperatures below 9°C and time for development from egg to adult and mortality increases at lower temperatures. The temperature at both sites regularly dropped below this threshold after 24 October 2019 and was often below 15°C after the 29 September 2019, thus the cool weather towards the end of the study is likely responsible for the decrease in adult *D. suzukii* numbers recorded in traps upon collection rather than parasitoid activity.

The absence of larval (*L. heterotoma*) and pupal parasitoids (*P. vindemmiae* and *S. erythromera*) was unexpected as these were the most abundant in previous years and the sites used in this study. It is likely that the chance in methodology and attack by rodents were the primary causes.

Potentially, the perforated iron lid to prevent rodent ingression prevented the parasitoids from accessing the *D. suzukii*. During this time a single parasitoid was recorded: *Leptacis sp*.. This species is not known to parasitize *D. suzukii* and was in the trap on the day of collection from the field. After the iron lid was changed to a mesh aluminium lid (24 September 2019) to improve access, again only a single parasitoid was collected.

It is possible the trap used at the beginning of the 2019 trial reduced the amount of *D. suzukii* and fruit volatiles released. In previous years, traps contained multiple adults which would have produced more *D. suzukii* volatiles. Benelli et al. (2013) found that parasitoids are attracted to host faeces, while Tumlinson et al. (1993) found parasitoids are attracted by host volatiles and those produced by the plants the host is feeding upon. It is possible that in 2019 the sentinel traps were not attractive enough to parasitoids while *D. suzukii* were pupating. To correct this, pots containing *D. suzukii* waste media were added from 24 September 2019. After this date only a single *A. tabida* was observed, however in previous years parasitoid numbers were lower by this time.

The other main difference was the use of banana and grape agar rather than strawberries. It is possible that the banana used in the traps may not have attracted the parasitoids. Biondi et al. (2017) found that *Asobara japonica* sometimes prefers *D. suzukii* from fruit of the same species on which the parasitoid was raised. Benelli et al. (2013) used banana in a study in Italy and successfully captured *P. vindemmiae* however Miller et al. (2015) found traps with banana captured fewer *P. vindemmiae* than traps with raspberry in Italy but significantly more in America. Godfray (2007) suggests parasitoids can prefer volatiles experienced during oviposition or after pupating, as such native parasitoids may prefer native fruits. Parasitoids learn to find their host partially through the volatiles present when they pupate (Tumlinson et al. 1993).

Nine *A. tabida* were recorded in total, one in a freshly collected trap from site 2 on 8 October and eight in another trap at site 2, deployed 24 September 2019, which used the same method as previous years.

Parasitoid numbers have been shown to fluctuate between years and between sites (Mazzetto et al. 2016). Parasitoid numbers can also vary greatly between two years of a study for example one species was observed 23 times one year then 319 the next

(Wermelinger 2002), while Miller et al. (2015) found *P. vindemmiae* numbers varied between years and site.

Conclusions

- It is likely that changes in trapping method, to achieve percentage parasitism calculations, reduced the numbers of parasitoids observed in 2019 compared to previous years.
- The most likely explanation, other than destruction by rodents, was the removal of larvae during counting onto clean media and the use of banana instead of strawberry.

Future research

- This study should be repeated based on learnings from 2019.
- The search for *T. drosophilae* should continue to be able to gain approval for release of this species for contribution to control.

Objective 8. *D. suzukii* and insecticide tolerance

Task 8.1. Investigating the susceptibility of D. suzukii *to approved plant protection products*

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. *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 have the opportunity to develop a tolerance. Within this objective, results of both the laboratory strains (from Shaw, 2019) and wild populations (established within this project) will be compared.

Aim

This study aimed to determine if insecticide tolerance was occurring in UK populations of *D. suzukii.*

Materials and Methods

Wild strain collection: Ripe fruit was collected from commercial field sites in Kent (Table 8.1.1) on in July 2019, and 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 bi-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 twice a week and offspring were mixed between vials to prevent genetic bottlenecks. Vials were labelled with generation number. Once enough numbers had developed (generation 5-6) laboratory bioassays were performed. The laboratory strains initially established in 2013 from Italian collected fruit were cultured in the same manor although generation is not known.

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
Graham Caspell	WS1	NIAB EMR Breeder plot cherries (mixed varieties)	Minimal: Hallmark (lambda-cyalothrin): 30/05/19, 21/06/19. Tracer (spinosad): 14/06/19.
Confidential	WS2	Raspberry (mixed varieties)	Commercial: Calypso (thiacloprid): 22/05/19, 27/06/19. Tracer (spinosad): 05/07/19
Confidential	WS3	Blackberry (karaka black)	Commercial: Dates not provided or frequency but have been exposed too: Dynamec (abamectin), Calypso (thiacloprid), Tracer (spinosad), Hallmark (lambda-cyalothrin), Exirel (cyantraniliprole), Spruzit (pyrethrins)
NA-original Italian strain	Lab_1	NA	None

The maximum field rate (FR) dose for cherry or strawberry of lambda-cyhalothrin (Hallmark), cyantraniliprole (Exirel) and spinosad (Tracer) were prepared. Serial dilutions were then produced to include % rates in Table 8.1.2. 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. *The lambda-cyhalothrin was repeated due to low mortality in initial bioassay. ** Was repeated again with a different batch of formulated product.

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) 3, 6, 12, 25, 50 +Water control (used for WS1-1, WS2 and WS3) *50, 75, 100 +Water control (used for WS1-2) ** 25, 50, 75, 100 + Water control (used for WS1-3)
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.

Results

Wild strains took several generations to build-up enough flies to execute the bioassays. Results are discussed by active ingredient.

<u>Cyantraniliprole</u>

There was no significant difference in survival between the lab strain and WS1 (EMR) or WS3 at any rate. WS2 had significantly higher survival than the lab strain at 6, 12 and 25%, and WS1 at 3, 6 and 25% and WS3 at 1.5, 6 and 25%. There was no significant difference between WS1 and WS3. (Fig. 8.1.1).

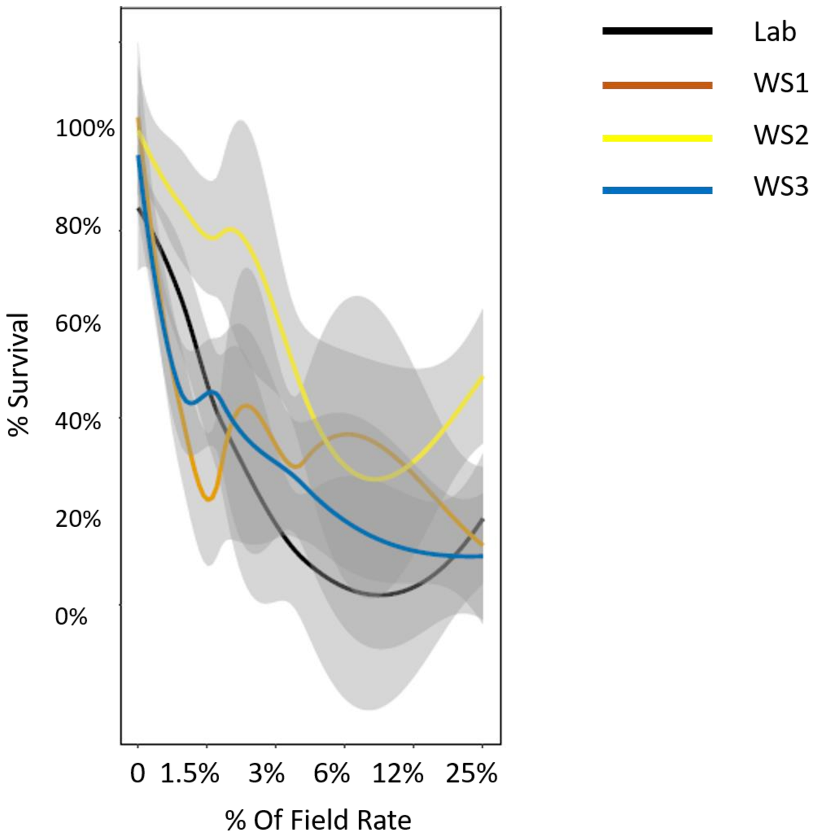


Figure 8.1.1. Survival of wild and lab strains of *D. suzukii* directly treated with doses of cyantraniliprole 24 hours after application. Different colours indicate the different strains of *D. suzukii*. The grey shaded area is the standard deviation. Lab is original 2013 Italian strain (Black). WS1 is EMR strain (Orange). WS2 (Yellow) and WS3 (Blue) are two other wild lines from confidential locations

<u>Spinosad</u>

There was no significant differences between the lab strain and WS3. Only one difference occurred between WS1 (EMR) and the lab strain and this was at 12% of the field rate. WS2 had significantly higher survival than the lab strain when treated with 12-50% of the field rate. WS2 also had higher than WS3 at 25 and 50% field rate (Fig. 8.1.2). At 100% of the field rate there was 6% survival in the lab strain.

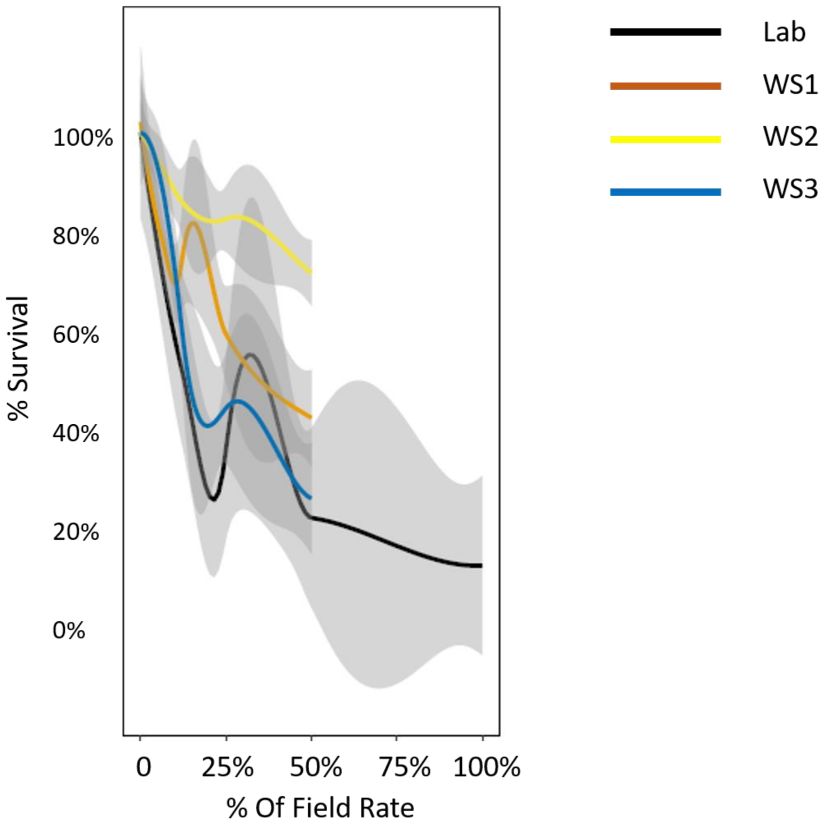


Figure 8.1.2. Survival of wild and lab strains of *D. suzukii* directly treated with doses of spinosad 24 hours after application. Different colours indicate the different strains of *D. suzukii*. The grey shaded area is the standard deviation. Lab is original 2013 Italian strain (Black). WS1 is EMR strain (Orange). WS2 (Yellow) and WS3 (Blue) are two other wild lines from confidential locations

Lambda-cyhalothrin

In the first WS1 (-1) bioassay, high survival occurred and so was repeated with higher treatment doses (WS1-2) and once again with a new batch of formulated product. This was to ensure the high survival was not due to problems with the product itself. There was a reduction in survival in the repeated bioassay (WS1-3) however it was only significant at 50 and 75%. This indicates that it could have been an issue with the product. There was significantly higher survival in WS1-1, WS2 and WS3 at 6-50% of field rate in comparison to the lab strain. There was also significantly higher survival between the lab strain and WS1-3 at 25%. Between WS1-2 there was a different to the lab strain at 50 and 100% field rate with only 50% mortality occurring in the wild strain at the full field dose. There was also a difference between the wild strains with WS1-2 having higher survival than WS2 and WS3 at 50% field rate.

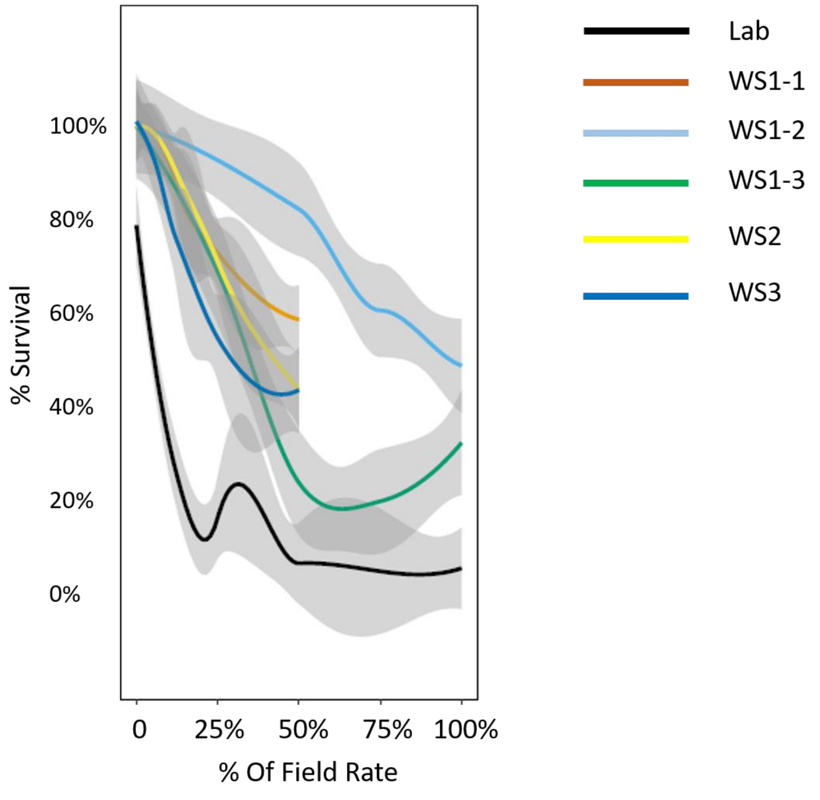


Figure 8.1.3. Survival of wild and lab strains of *D. suzukii* directly treated with doses of Lambda-cyhalothrin 24 hours after application. Different colours indicate the different strains of *D. suzukii*. The grey shaded area is the standard deviation. Lab is original 2013 Italian strain (Black). WS1-1 is EMR strain (Orange). WS1-2 is EMR strain treated with an increased doses. (Light Blue). WS1-3 is EMR strain treated with increased doses and a different batch of formulated product (Green). WS2 (Yellow) and WS3 (Blue) are two other wild lines from confidential locations.

Discussion and Conclusions

Cyantraniliprole, of the three products, tested 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. Although there was a different between the lab strain and WS2 at 6 12 and 25% of the field rate, this was the only difference between the lab and wild strains. There was a difference between the wild strains however, with WS2 having higher survival than WS1 at 3, 6 and 25% field rate and WS3 at 1.5, 6 and 25% field rate respectivley.

For the lab strain there was a 6% survival when treated with the field rate of spinosad. Unfortunately there were not enough resources to increase treatment number in the wild lines to investigate how they compared to the lab strain at the field rate. It would be expected that the wild lines would have a much higher tolerance than the lab strain based on the trend seen within this project. There were no significant differences between the lab strain and WS3. 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.

As there was high survival in the EMR strain in the first lambda-cyhalothrin experiment it was decieded to repeat it with an increase in doses and different batch of formulated product. This was to ensure that the high survival was not due to a problem with the insecticide and was infact a high tolerance of the wild populations. On the repeat of the bioassay with the increased doses we actually had higher survival than the first experiment, significant at 50 and 75% field rate. This indicates it could have been an issue with the formulated product however we did not see this difference at 25 and 100% rates. Between 30 and 50% survival occurred in the EMR wild lines when treated with the full field rate. To our knowledge this is the first documentation of an increased tolerance to lambda-cyhalothrin. There were also differences in the susecptibilty of the wild strains with lower survival in WS2 and WS3 than the EMR strain (WS1-2) when treated with 50% of the field rate. This was unexpected as the EMR population had low insecicidal input prior to strain establishment.

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, The development of resistance to commonly used plant protection products is a realistic threat facing growers globally. It is vital to monitor increases in tolerance so development can be mitigated. Annual monitoring would be beneficial form many locations as it is clear it varies greatly between farms, even from the same county.

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 and Angus Soft Fruits for their contribution to the Scotland Monitoring data. 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

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

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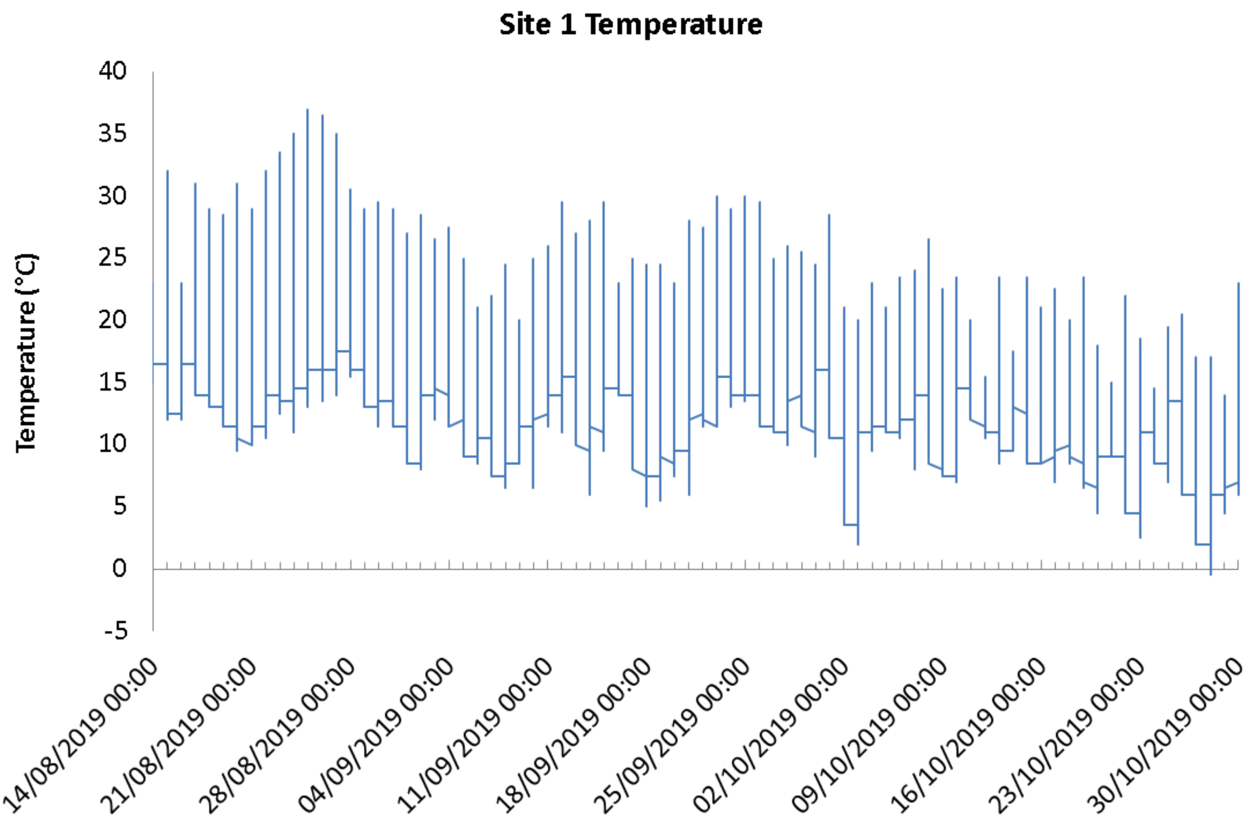
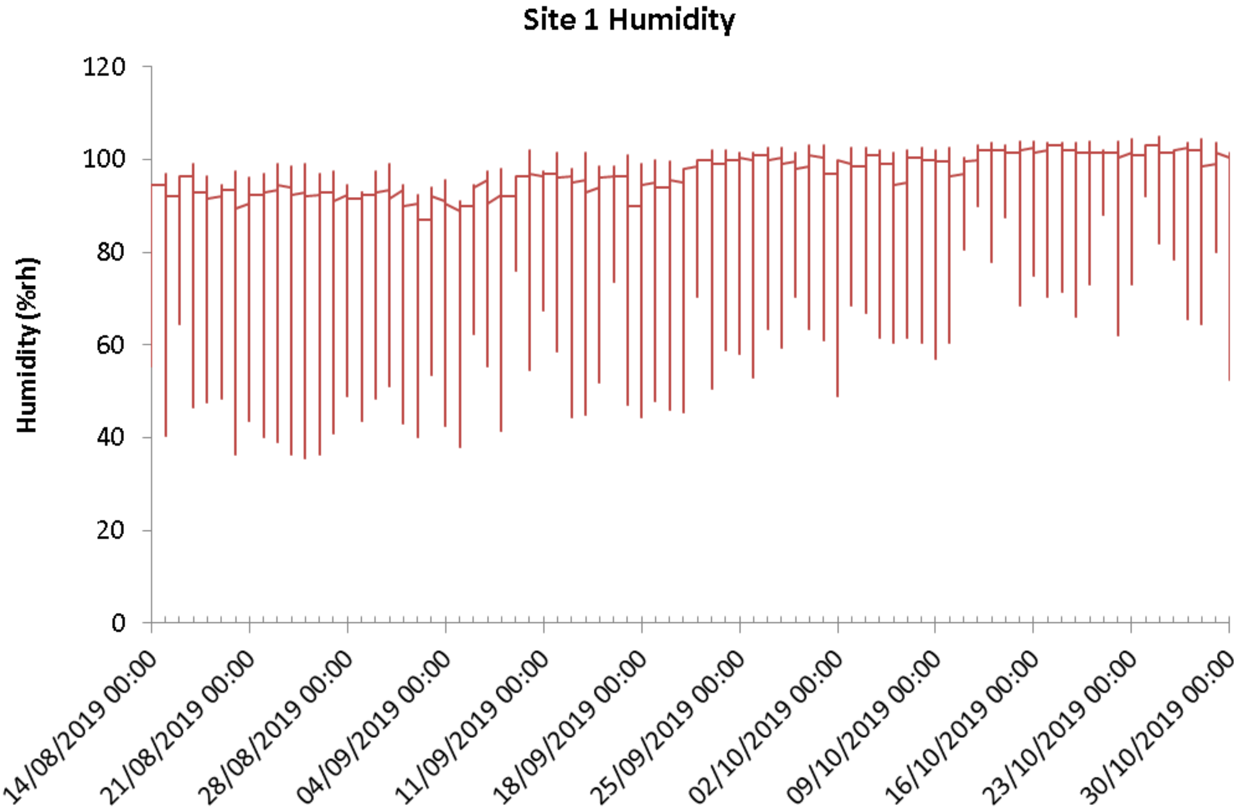
APPENDIX 4.3.

Husbandry raspberry spray trial 2019

	HOMEFIELD 1,4,7,8						SKINNERS1,2,3,4				
APLLY DATE	Week	Date	Activity	Spray	Grower plots	Fortnightly plots	Activity	Spray	Grower plots	Fortnightly plots	APLLY DATE
22/08/2019	34	22-Aug	Spray 1	Tracer 1	Yes	Yes					
29/08/2019	35	29-Aug	Spray 2	Pyrethrin 1	Yes	No	Spray 1	Tracer 1	Yes	Yes	29/08/2019
05/09/2019	36	05-Sep	Spray 3	Exirel 1	Yes	Yes	Spray 2	Pyrethrin 1	Yes	No	05/09/2019
12/09/2019	37	12-Sep	Spray 4	Calypso 1	Yes	No	Spray 3	Exirel 1	Yes	Yes	12/09/2019
19/09/2019	38	19-Sep	Spray 5	Tracer 2	Yes	Yes	Spray 4	Calypso 1	Yes	No	19/09/2019
26/09/2019	39	26-Sep	Spray 6	Pyrethrin 2	Yes	No	Spray 5	Tracer 2	Yes	Yes	26/09/2019
03/10/2019	40	03-Oct	Spray 7	Exirel 2	Yes	Yes	Spray 6	Pyrethrin 2	Yes	No	03/10/2019
10/10/2019	41	10-Oct	Spray 8	Calypso 2	Yes	No	Spray 7	Exirel 2	Yes	Yes	10/10/2019
17/10/2019	42	17-Oct	Spray 9	Tracer 3	Yes	Yes	Spray 8	Calypso 2	Yes	No	17/10/2019
24/10/2019	43	24-Oct	Spray 10	Pyrethrin 3	Yes	No	Spray 9	Tracer 3	Yes	Yes	24/10/2019
	44	31-Oct	Spray 11				Spray 10	Pyrethrin 3	Yes	No	31/10/2019
	45	07-Nov	Spray 12				Spray 11				
	46	14-Nov					Spray 12				
	47	21-Nov									

Temperature and Humidity data raspberry spray trial 2019

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Humidity (%rh)
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80
60
40
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0
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21/08/2019 00:00
28/08/2019 00:00
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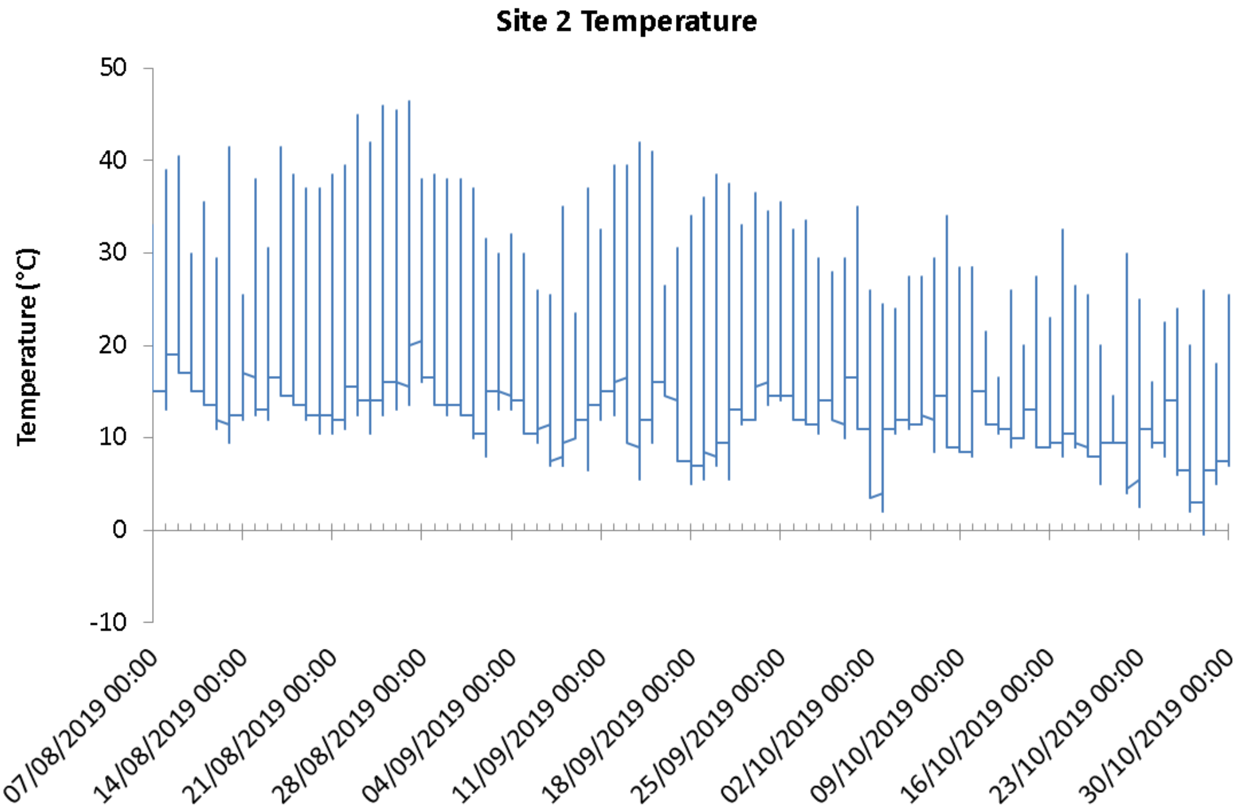
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Temperature (°C)
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10
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-10
07/08/2019 00:00
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21/08/2019 00:00
28/08/2019 00:00
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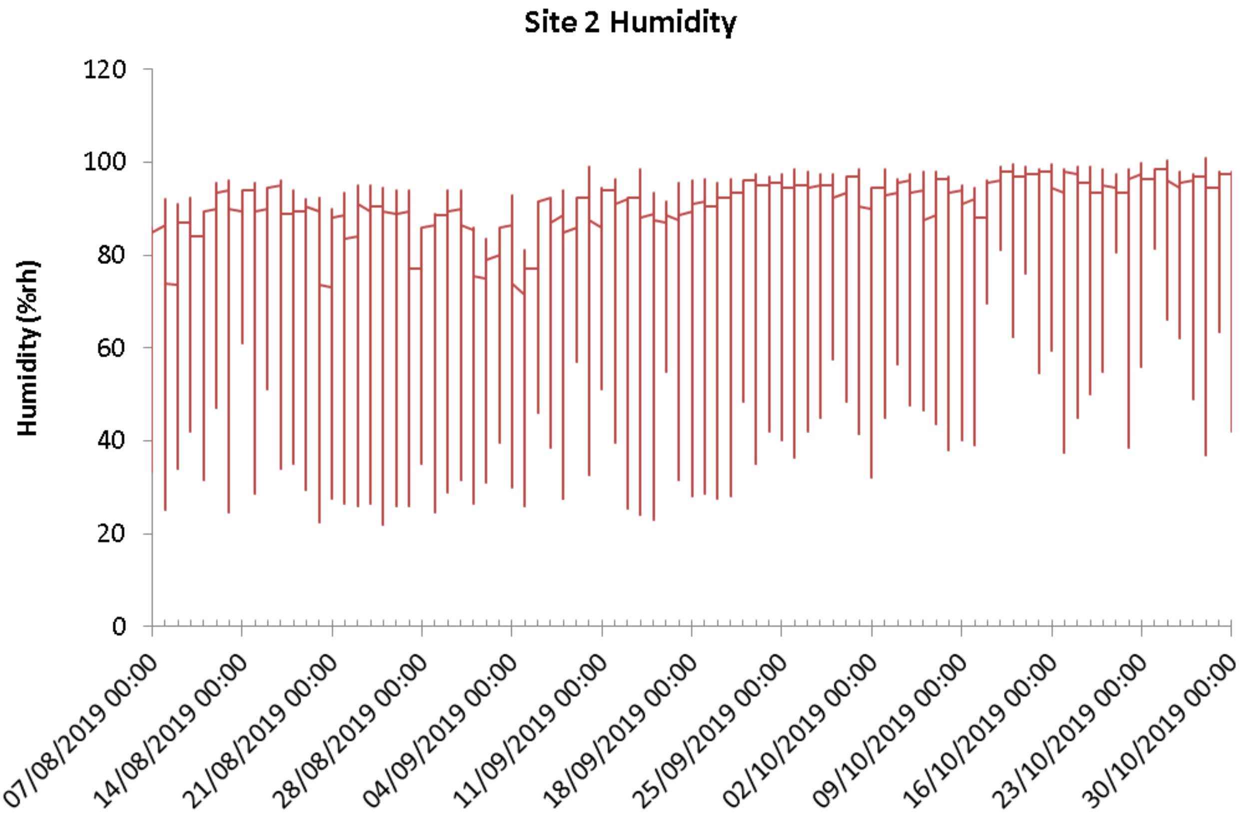
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Humidity (%rh)
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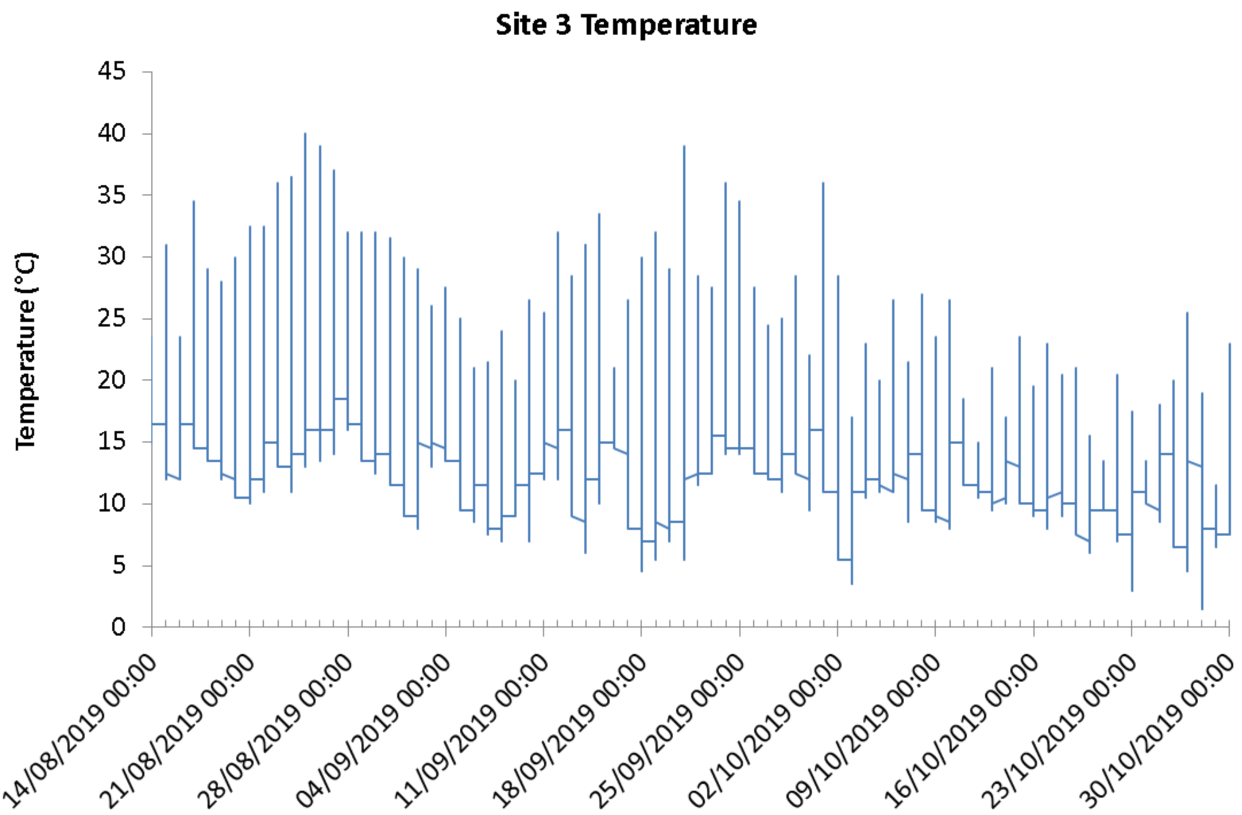
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Temperature (°C)
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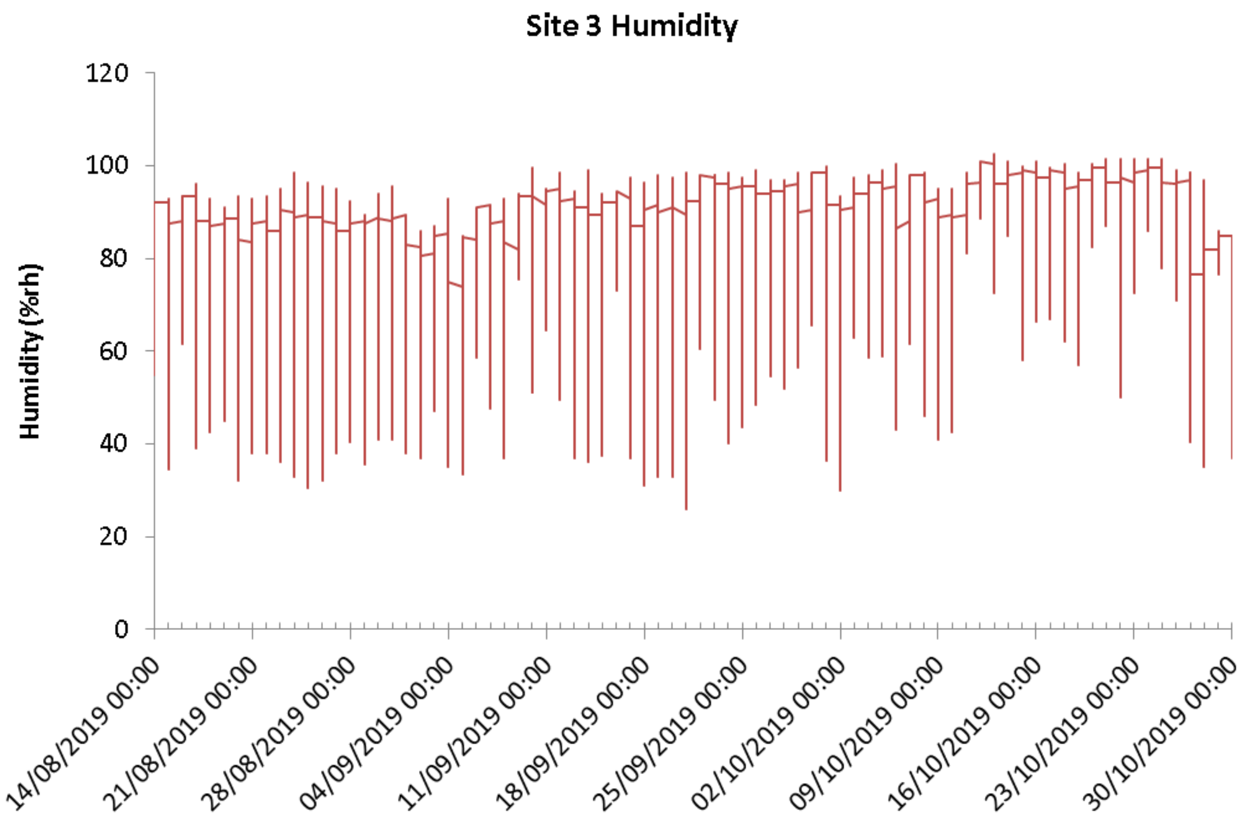
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Humidity (%rh)
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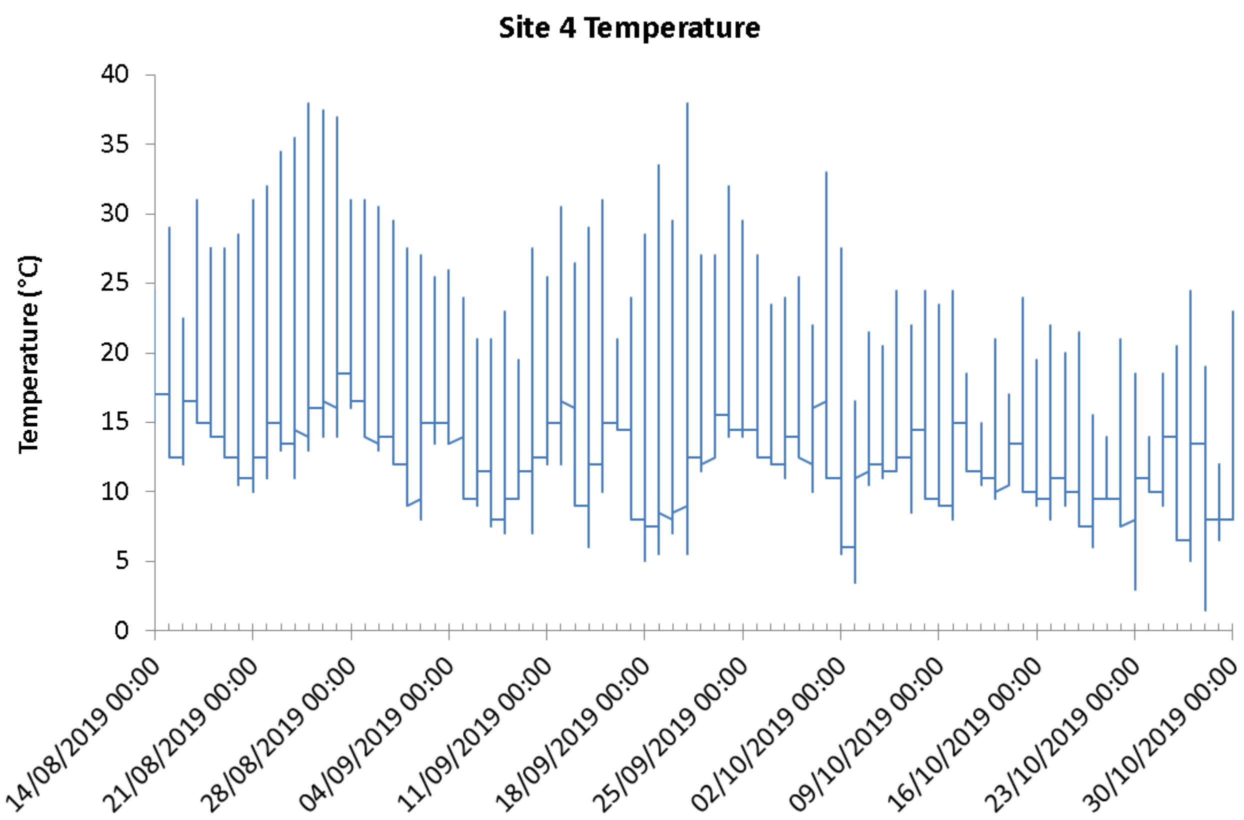
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Temperature (°C)
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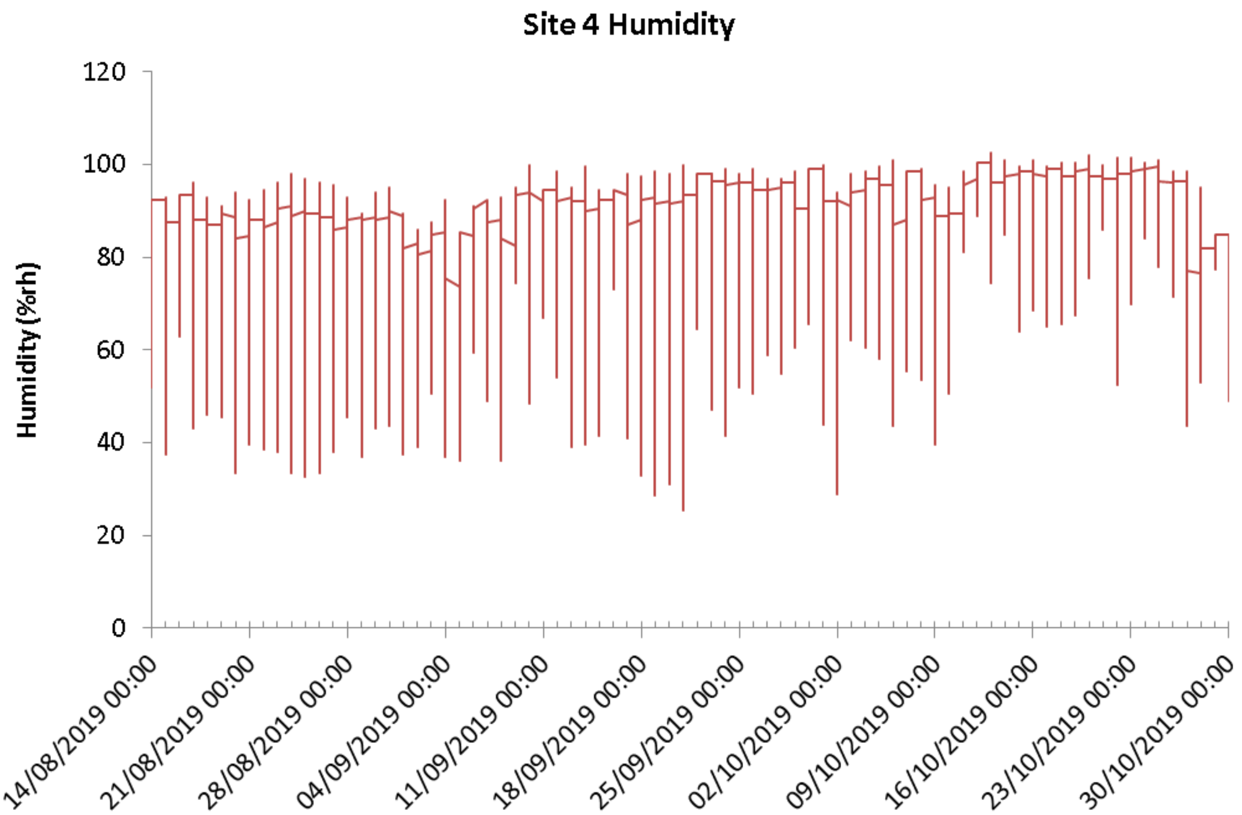
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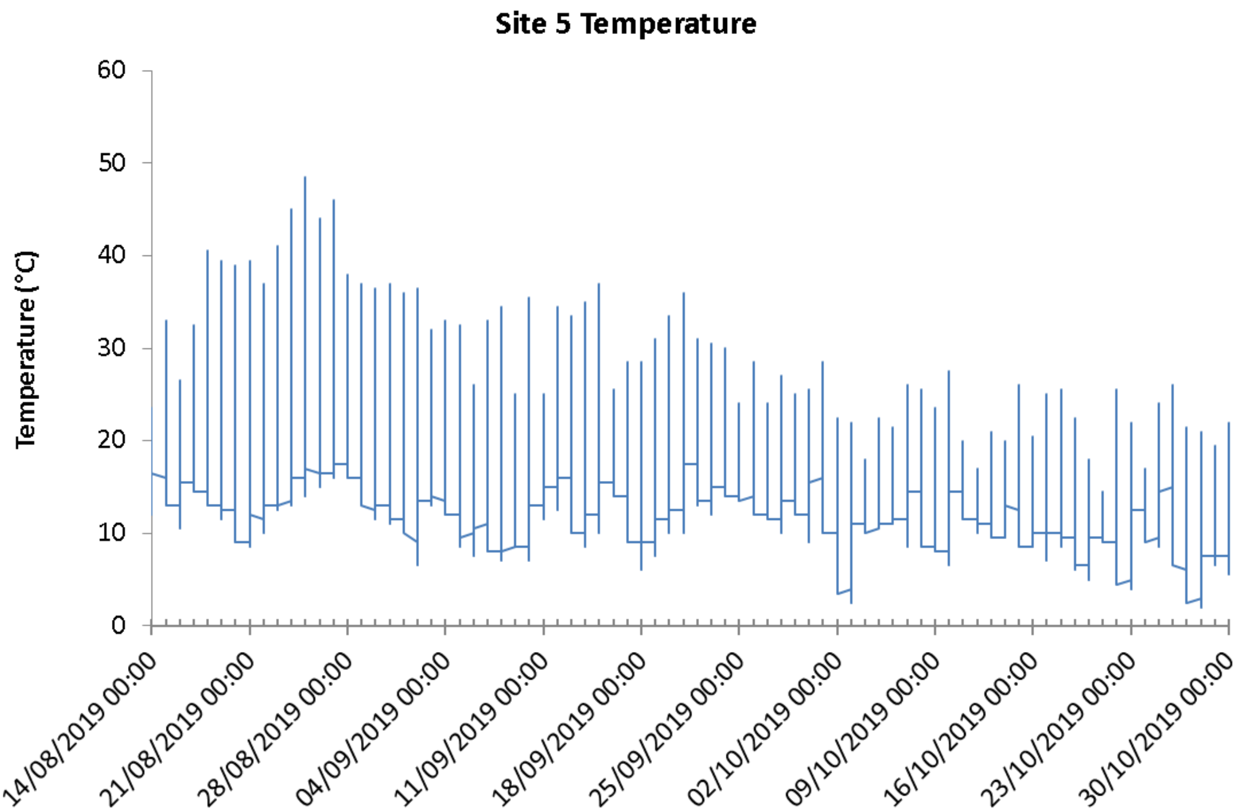
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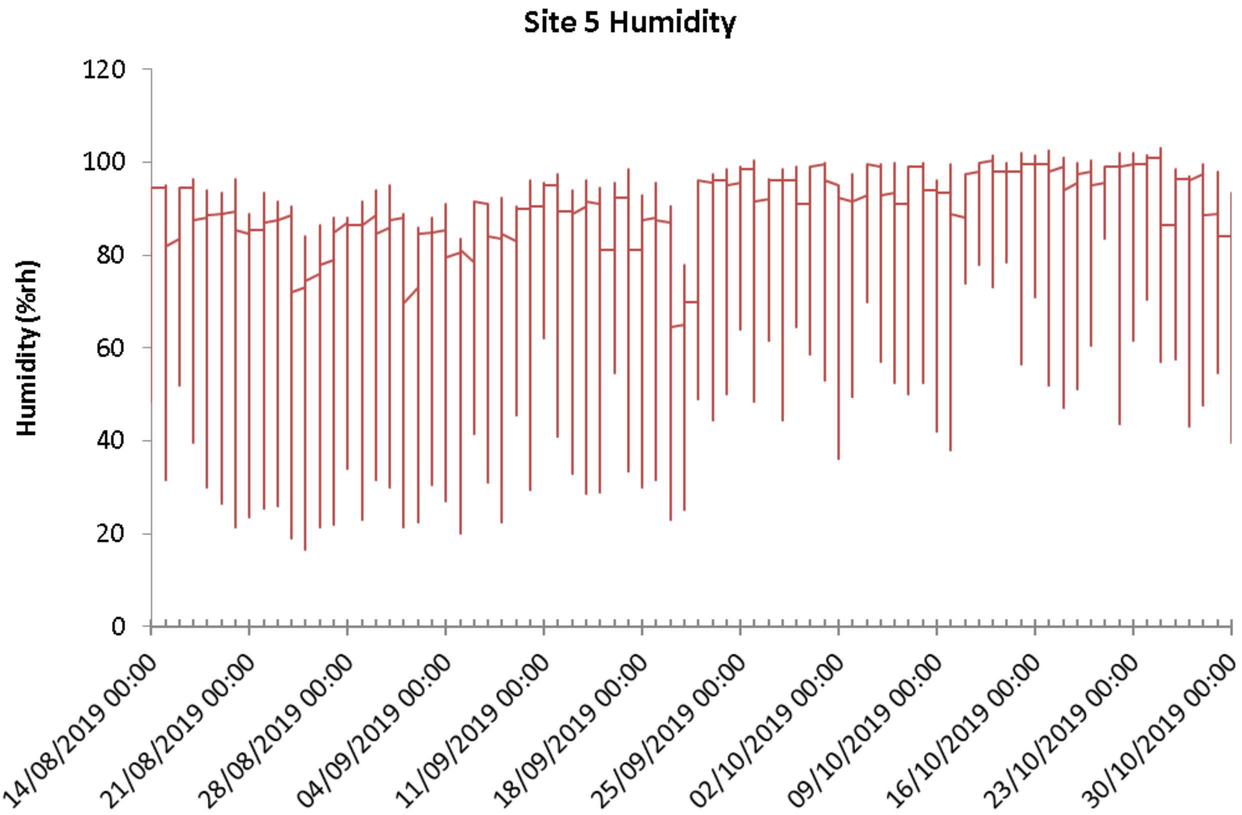
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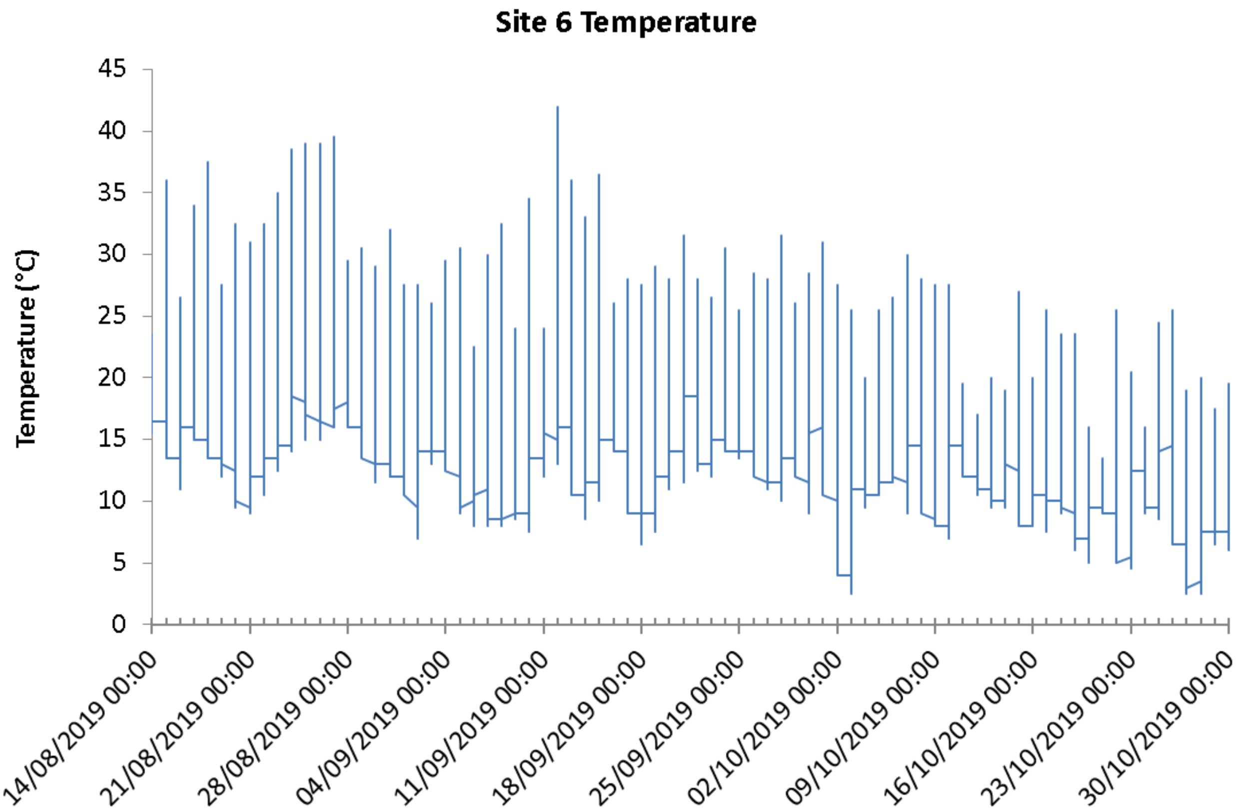
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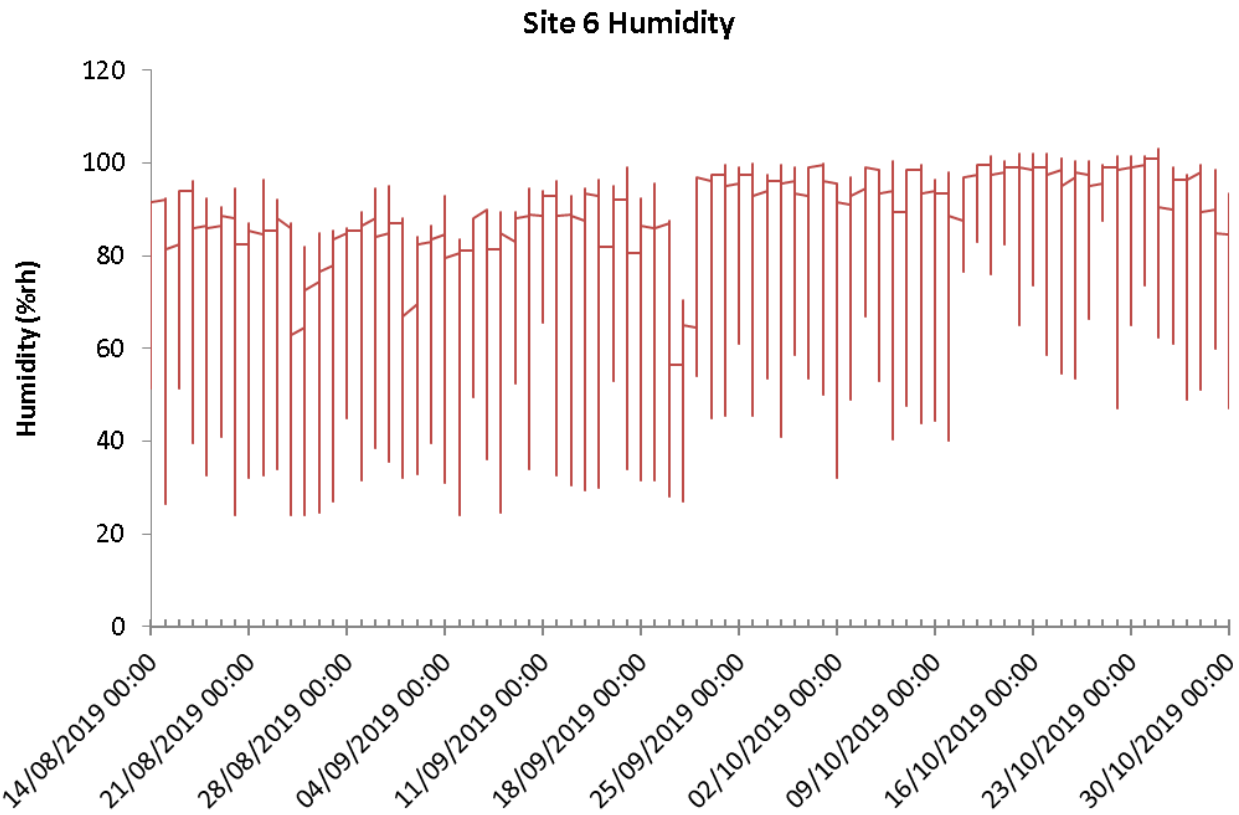
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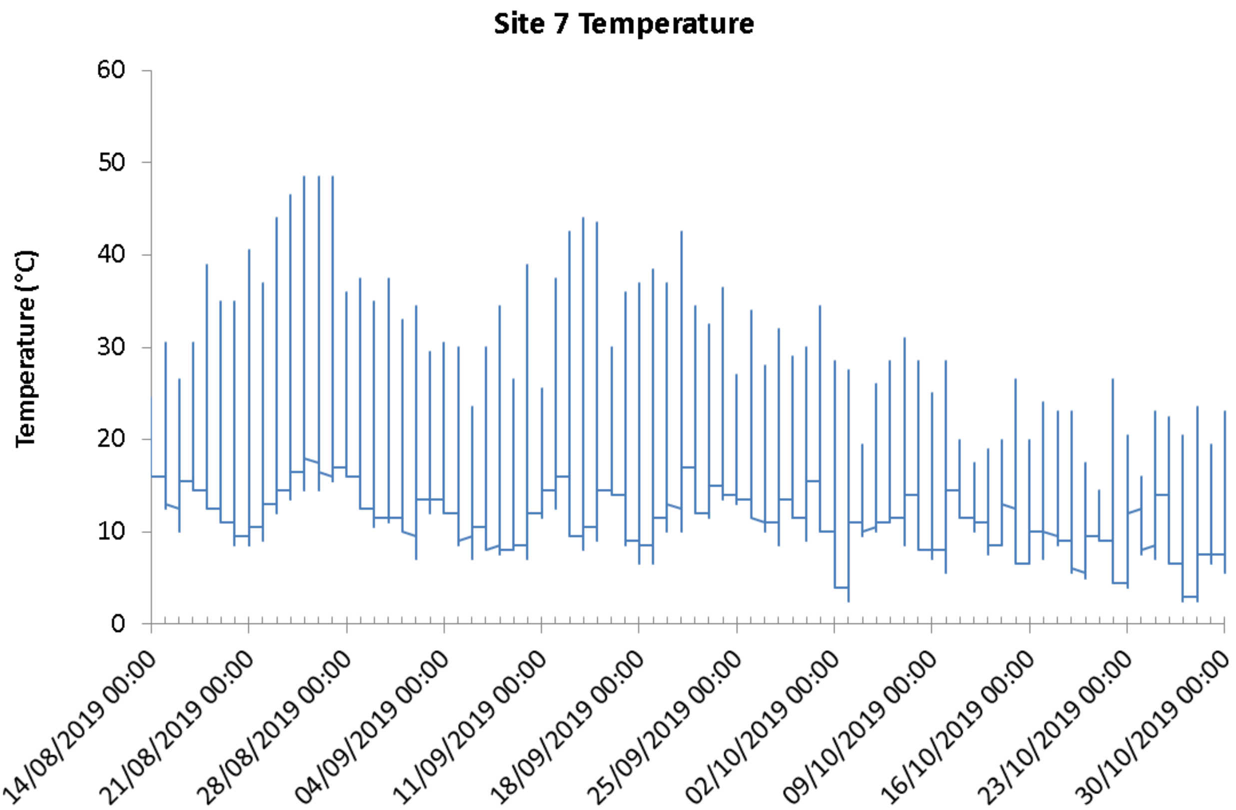
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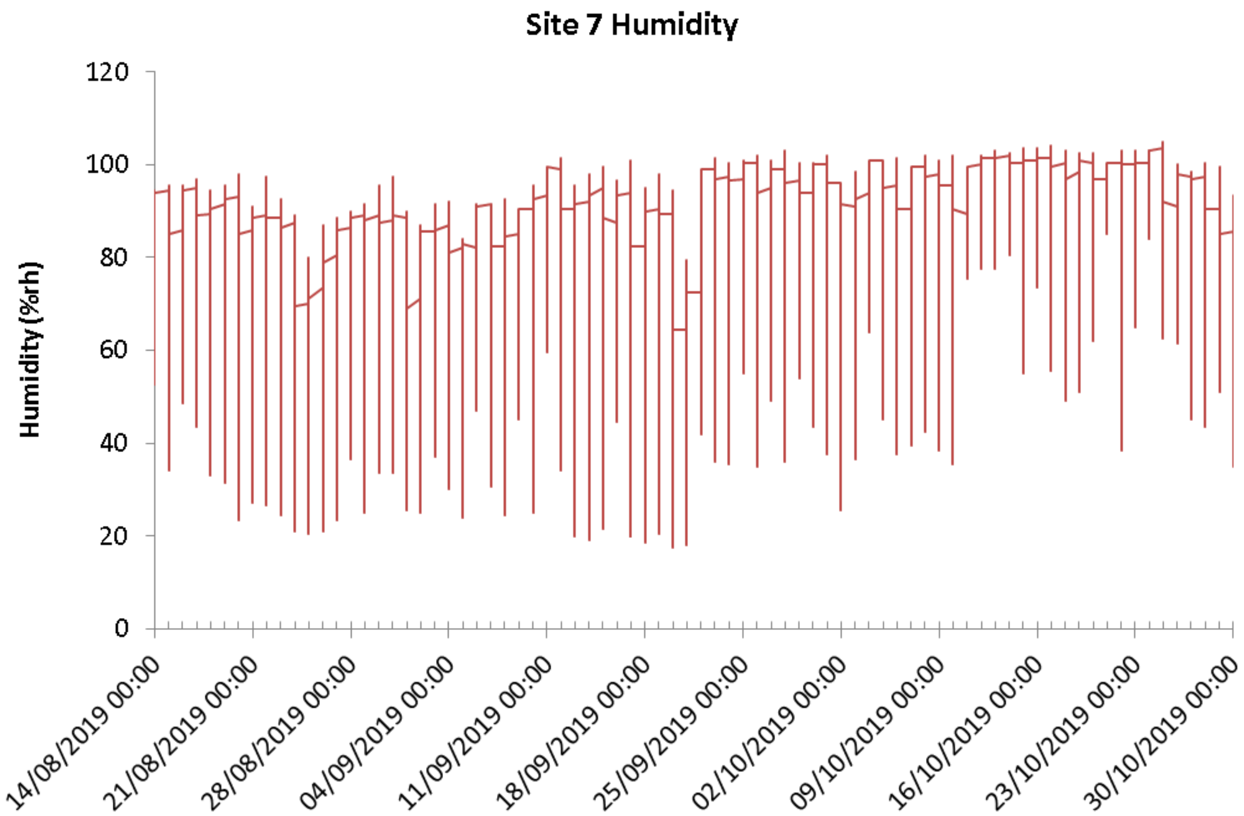
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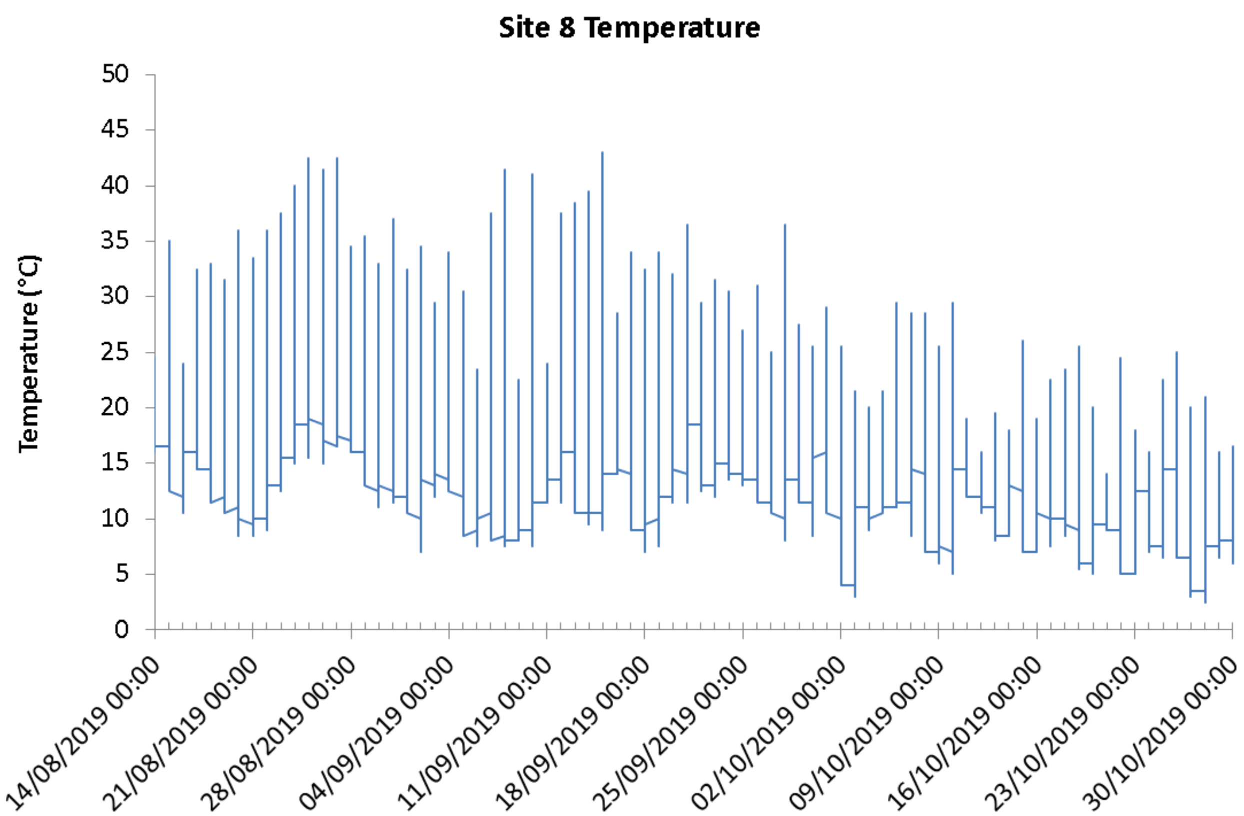
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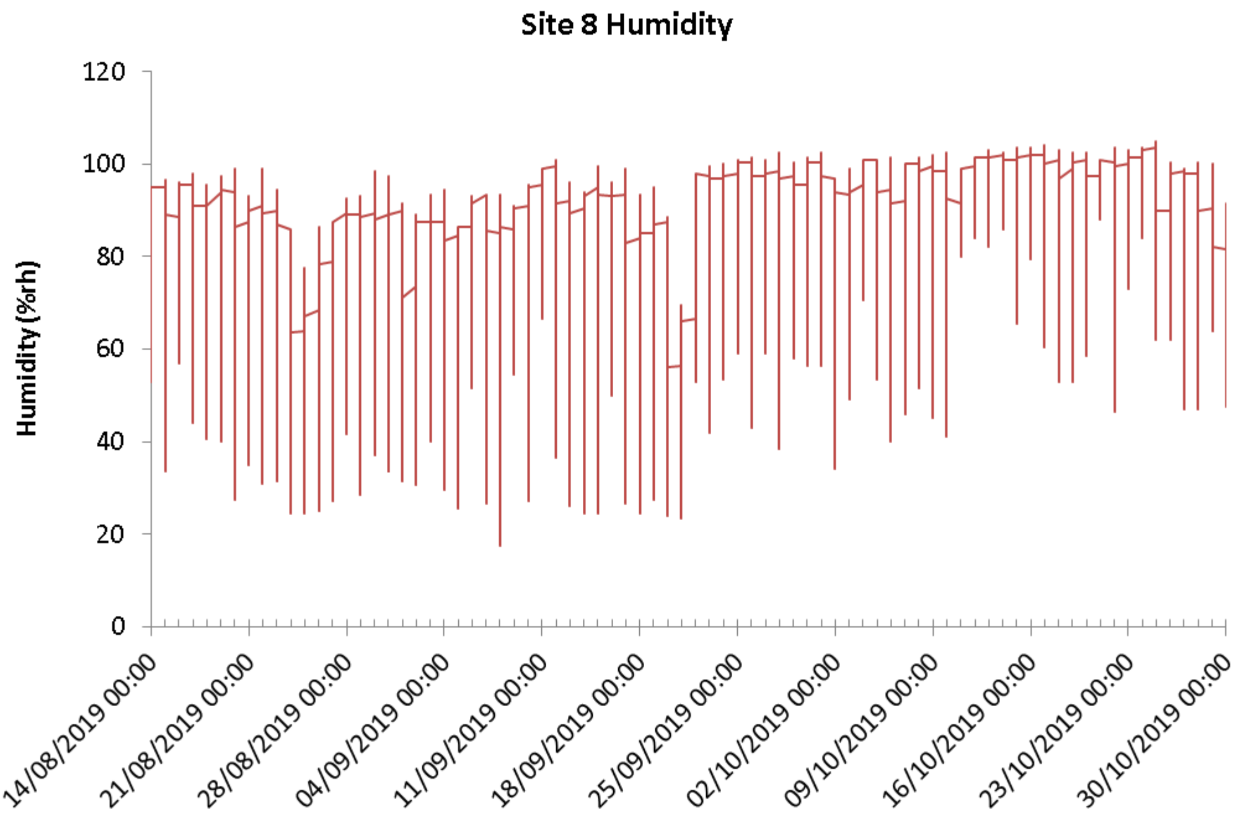
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APPENDIX 7.1.

NATURAL HISTORY MUSEUM

Francesco Maria Rogan
NIAB EMR
New Road
East Malling
Kent
ME19 6BJ

August 23rd 2019

Dear Francesco,

Re: Parasitoid Identification (101) **Our Ref: IAS 2019-7428**

Our specialist, Dr. David Notton, has examined your specimens and he has provided the following information:

The specimens have been identified as, ***Leptacis sp***. (Platygastridae)

This is a parasitoid of gall midges (Cecidmyidae) unfortunately, and not a parasitoid of ***Drosophila suzukii.***

I hope this information is useful to you.

Kind regards,

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