Project title:	Development and implementation of season long control strategies for *Drosophila suzukii* in soft and tree fruit
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Project leader:	Michelle Fountain, NIAB EMR, New Road, East Malling, Kent ME19 6BJ
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Location of project:	NIAB EMR
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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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GROWER SUMMARY

Development and implementation of season long control strategies for *Drosophila suzukii* in soft and tree fruit

Headlines

- Trap catches of SWD continued to increase in traps in most regions of the UK
- The components of a 'Push-Pull' system have shown promise and will be tested in 2018
- Advances have been made with a feeding bait which increases mortality and reduces egg laying when combined with a low dose of crop protection product.
- A fortnightly spray programme was as effective at controlling SWD in cherry as weekly sprays when combined with insect mesh.
- The potential for SWD to feed on the extra-floral nectaries of cherry leaves lasts until the leaves senesce in late summer.

Background and expected deliverables

The Asiatic vinegar fly *Drosophila suzukii* (spotted wing drosophila, SWD) invaded the UK in 2012. It has increased in numbers from year to year and has become a key pest of stone and soft fruits. It has the potential to cause significant fruit damage and growers incur significant increases in production costs in gaining control. The spread of *D. suzukii* across Europe has strongly disrupted existing and developing IPM control strategies in all countries. Growers currently control the pest with sprays of plant protection products (PPPs), some of which are broad spectrum. This causes a reduction in beneficial arthropod populations, disrupting their ecological contribution in keeping pests below economic threshold values. Repeated use of a limited number of active ingredients to control SWD could accelerate resistance development.

In Europe and North America, initial 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, studying its biology and evaluating control methods. This AHDB project builds on international progress and on AHDB funded work in project SF145. It focuses on practical development and elaboration of new control technologies that can be used by UK farmers within the short to medium term.

To this end, six project objectives have been set up and this Grower Summary will report on these objectives worked upon in Year 1.

1. Continue to monitor populations of *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 using minimal applications

5. Integrate exclusion netting with other successful controls

6. Integrate approaches for season long control

This work is being led by Michelle Fountain and her entomology team at NIAB EMR in Kent in collaboration with Alison Dolan at the James Hutton Institute who is working with growers in the East of Scotland.

Summary of the project and main conclusions in Year 1

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

Since the first detection of *D. suzukii* in the UK in 2012, populations of *D. suzukii* have continued to rise in most regions of England and there are more frequent reports of the pest being detected nationally and also in Ireland. In contrast to the general UK trend, populations in Scotland have been low since the pest was first detected in 2014.

In collaboration with Berry Gardens, in 2017, we continued to monitor in the main fruit growing regions with 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 and one at the edge of each crop. 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 higher in the spring (Mar-May) and late summer (Jul-Aug) of 2017 than in previous years. This correlated with increases in reported damage to early forced June bearer strawberry and cane fruits respectively, by the industry. The first peak autumn catch was almost a month earlier with catches in Nov-Dec almost double the trap catch (>800) from the previous highest recording in 2015/16. Peaks in Nov-Dec in 2015 corresponded with mild weather and a similar pattern was observed in Oct 2017. These autumn – winter peaks in activity are when the flies are in reproductive diapause in their winter-form. At this time, *D. suzukii* can be detected at 50 m height in suction traps (Rothamsted Research). This period coincides with depletion in egg laying resources and defoliation of trees. Decrease in trap catches during the summer months can be attributed to several causes. Traps tend to be less attractive than ripening crops. Numbers can be reduced by crop protection products and warmer weather can influence catches.

Additional study sites, in Scotland, in 2017, caught very few *D. suzukii* although ‘hotspots’ were identified. Data showed similar trends suggesting that the national monitoring data set is representative of the *D. suzukii* density in Scotland. Very few *D. suzukii* emerged from unsprayed wild hosts suggesting that populations are still very low. Indeed no *D. suzukii* were detected until 9 August, with fecund females present through until November.

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

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

Potential repellents to deter *D. suzukii* laying eggs in fruits or discouraging adults from entering the cropping area were investigated in the previous project (SF 145). These were further investigated in 2017 alone or as a blend. Repellent methods are likely to be more effective in combination with other methods, such as 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. Therefore, further optimised the NIAB EMR / NRI prototype Falcon tube device including the design and the attractant formulation and compared this to a commercial trap currently undergoing approval. The control component in the prototype is enclosed within the inner surface of the device to minimise human exposure and environmental contamination including adverse effects on beneficial insects. Unlike ‘mass traps’, the A&K device is open ended and does not become saturated with dead flies which reduces high labour costs.

Two repellent experiments were done in an unsprayed cherry orchard at NIAB EMR. All six treatments were synthetic semio-chemical compounds and were coded. Repellents were dispensed from polyethylene sachets or rubber septa. Twenty sachets/septa were suspended evenly throughout each cherry tree (a plot) on 12 May and again on 13 July. Sentinel fruits were then deployed within the tree canopy and incubated for two weeks in a laboratory to test for the presence of *D. suzukii*. There were five replicates of each treatment in a randomised block design. Sentinel fruit were deployed on 15 and 22 May for the first experiment and 14 and 21 July for the second experiment. Only one *D. suzukii* emerged from sentinel strawberries in the blend treatment in the first experiment suggesting that a blend may be more effective than single components. However, *D. suzukii* was aggregated in only two blocks in the first experiment, removing the possibility of detecting a significant effect. *D. suzukii* was present throughout the cherry orchard in July but numbers were too high and plots probably too small to detect repellent effects.

In work to improve the A&K Falcon tube device (Figure A) we compared the NIAB / NRI device and attractant to a commercial standard. In a series of experiments set up in semi-field cages, 10 male and 10 female (3 – 12 days old), mated *D. suzukii*, from a laboratory culture, were introduced with the prototypes and mortality assessed 24 hours later. The lures used in the prototype were separate half size sachets containing ethanol/ acetoin, acetic acid and methionol (provided by NRI) and referred to as mini Cha-Landolt. Experimental prototypes, with the exception of the untreated controls, were coated on the inside with a formulation of Decis (deltamethrin) or a field formulation of spinosad (Tracer). All experiments had seven replicates and manipulated the number or positioning of the entry holes and/or red colouration on the prototype devices.

Figure A. Prototype Falcon tube devices compared to a commercial standard

The prototype Falcon tube devices, with Decis as killing agent, were as effective as the commercial trap in controlling *D. suzukii*. The devices give up to 30% kill of *D. suzukii* within 24 hours in these semi-field cage trials. The devices with eight holes on the red sections were more effective than devices with four holes on the clear part of the trap. However, increasing the number of holes on the device from eight to sixteen did not increase the efficacy.

In a third piece of work we aimed to improve and miniaturise the standard Cha-Landolt bait which is composed of the fermenting volatiles: ethanol, acetic acid, acetoin and methionol into a dry formulation removing the need for a liquid killing agent.

All tested formulations were compared to the standard Cha-Landolt lure; ethanol and acetic acid were dispensed from the drowning solution (300 ml) and/or the commercial Biobest “Dros’Attract” solution (300 ml). Dry formulations were dispensed in polyethylene sachets. Release of the four components of the Cha-Landolt blend from polyethylene sachets provides a practical “dry” alternative to the conventional liquid bait, as required for development of devices for control of *D. suzukii* by attract-and-kill and, particularly, lure-and-infect approaches. The standard sachet lure developed originally released ethanol and acetic acid at 1% and 10%, respectively, of the rates from the liquid Cha-Landolt lure and require changing every six weeks rather than weekly.

The attractiveness of the standard sachet lure was not affected by increasing the release rates of ethanol or acetic acid, or by reducing the release rate of ethanol to one quarter. However, the attractiveness of the standard sachet lure can be increased by increasing the release rate of acetoin by four times to approximately 32 mg/d. Further increase in release rate of acetoin did not increase catches significantly.

In most experiments, removing the methionol did not affect catches of *D. suzukii*, but in other experiments catches were reduced. The requirement for methionol needs further investigation.

In some experiments catches with the optimised sachet lure were at least as great as those with the liquid Cha-Landolt and Dros’Attract lures, but in others they were significantly lower. The reason for this is not fully understood.

A MiniLure has been developed for use in the Falcon tube attract-and-kill devices and shown to be effective under semi-field conditions. This should have a lifetime of at least 6 weeks and probably longer in the confines of the Falcon tube. Although release rates of ethanol, acetic acid and methionol are probably adequate, there is scope to increase attractiveness by increasing the release rate of acetoin from the MiniLure nearer to the optimum level. Longevity can be increased by increasing the loading of the compounds once the release rates have been optimised.

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

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

Results from a jar microcosm bioassay were aligned with chronophysiology (activity counts) methods in comparison to large arena tests. Chronophysiology assays using the activity of *D. suzukii*, in the presence of different baits was, therefore, a more useful screening method of attractant baits than the large arena test.

Attractant baits significantly enhanced the efficacy of Tracer (spinosad) when used at 3.3% of the recommended field rate for protected strawberries. When used in combination with Tracer at the above rate, a suspension of the yeast *Hanseniaspora uvarum* in sugar solution was more effective in killing *D. suzukii* than fermented waste strawberry juice and sugar or Gasser bait. When used with Tracer at 3.3%, fermented strawberry juice and sugar, Gasser liquid or a suspension of *H. uvarum* in sugar solution were all effective in reducing egg laying to a low level. A suspension of *H. uvarum* in sugar solution or fermented strawberry juice and sugar improved the efficacy of Tracer, Hallmark (lambda-cyhalothrin) and Exirel (cyantraniliprole) in terms of *D. suzukii* mortality. Calypso (thiacloprid) was not effective at 50% of the field rate for protected strawberries, either with or without attractant baits. This work shows good promise for the use of feeding stimulant baits to improve the mortality and reduce egg laying of *D. suzukii* in crops.

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

The aim of the studies in this objective were to determine the length of time that cherry extrafloral resources were available to *D. suzukki* 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.

For the first aim, each week we picked leaves from \`Penny\` and \`Sweetheart\` orchards at NIAB EMR. The trees were not under polythene and therefore exposed to rain. From 5 April to 14 September, five leaves from each variety were collected and introduced, individually, onto on the floor of a culture cage of *D. suzukii*. 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.

The first fecund *D. suzukii* was found on 6 April, then a week later more than half (57%) of the female *D. suzukii* in the traps were fecund; this coincided with flowering. 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 was a weak link with less feeding after a period of rain, indicating that nectar and beneficial microbes could possibly have been washed from the surface of the leaves making the extra floral nectaries less attractive to *D. suzukii*.

To investigate spray intervals on cherry, two small trials were established; 1) Commercial trial with two replicate tunnels, 2) Semi-field trial at NIAB EMR in one tunnel. In the commercial trial, all plots were insect meshed but no untreated control was used. In the semi-field trial no insect mesh was installed and an untreated control was included.

Either a weekly or fortnightly commercially approved spray programme was employed at both sites. At the commercial site, 50 fruits were collected weekly. At the semi-field site leaves were collected weekly, just before the next spray was applied and a laboratory bioassay done to test the mortality of *D. suzukii* that came into contact with the leaves. In the commercial trial on fruit, there were two replicates of two cherry fruit varieties (Kordia and Regina) and in the semi-field trial there were four replicates of five leaves. Fruits collected from the commercial trial were incubated to record emerging *D. suzukii*. 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 number, 11), inside the mesh, was lower than in the perimeter (peak 70), outside the insect mesh. Only two female *D. suzukii* were found in all of the fruits sampled throughout the growing season; one from the weekly and one from the fortnightly spray programme.

In the semi-field leaf bioassay the mortality in the untreated control plots was usually less than 10%. There was significantly more *D. suzukii* mortality in the weekly and fortnightly spray programmes compared to the untreated control, but no difference between the two spray programmes until the spray applications ceased. Following the cessation of sprays, the effects of the insecticides declined over time (7-28 August). Hence, in this study, either weekly or fortnightly applications of insecticides to cherry leaves gave significantly higher mortality (~90%) compared to untreated leaves (up to 10%) 48 hours after exposure.

Objective 5. *Integrating exclusion netting with other successful controls*

Work on this objective will begin in 2019.

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. In 2017, over 14 presentations and courses were delivered in 2017 by the entomology team in both Scotland and England. National Monitoring data was regularly communicated to the AHDB and SWD Working Group for dissemination to growers.

Financial benefits

In 2014, there were 9,440 hectares of soft fruit grown in the UK, producing 143,000 tonnes worth a total of £393 million. There were 752 hectares of plums producing 11,700 tonnes worth £27.5 million 600 hectares of cherries, producing 4,500 tonnes of fruit worth a value of £22.5 million (Sources: Defra Horticulture Statistics and British Summer Fruits).

SWD is capable of causing total crop loss in cherry crops and potentially up to 75% crop loss in other soft fruit and plum crops, so is seen as an existential threat to the industry.

All new management and control methods developed by research work such as that being funded in this project will therefore protect against losses of the magnitude listed above.

Action points for growers

- Use a range of control measures to control SWD on affected fruits.
- Prevent migration of SWD into the crop in the spring by using insect mesh and maintain the barrier.
- Protect fruits with applications of approved products. Consult your BASIS qualified agronomist for the latest approvals.
- Good spray coverage is essential to gain effective control.
- Preliminary data shows that fortnightly sprays in protected and insect meshed cherry give comparable efficacy to weekly sprays.
- Continue to monitor for adult SWD inside the mesh and outside the mesh to ensure spray programmes are effective.
- Make regular inspections of fruits using flotation testing in the lead up to and during harvest to ensure populations are not developing in fruit.
- Consult past reports (SF 145) for guidance on crop hygiene and product efficacy.

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 low since the pest was first detected in 2014. In the West Midlands and East Anglia, the numbers have been reasonably low, but 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. To further enhance and understand the trap catches in Scotland, JHI are monitoring more of the main soft fruit growing area and additional monitoring data from two growers groups is included.

In addition the distribution of *D. suzukii* in Scotland and the seasonal population dynamics of its different life stages in relation to wild hosts are unknown. Hence, the incidence and distribution of known common UK wild hosts of *D. suzukii* adults and larvae in the fruit growing area of Scotland are being assessed and the places where it may overwinter determined. This information may help us determine some of the factors required for *D. suzukii* to become established. It will assist in the prediction of the severity and onset of future attacks and increase our understanding of the spatial dynamics and colonisation patterns of this damaging pest.

Methods

Monitoring began at 14 fruit farms in 2013 in project SF145. Currently there are 57 traps on nine farms in England and 40 traps on four farms in Scotland that make up the National Monitoring Dataset. The distribution of the farms is; three in Kent (including NIAB EMR), 1 in Surrey, two in the West Midlands (Herefordshire and Staffordshire), two in eastern England (Northamptonshire and Norfolk), one in Yorkshire and four in Scotland (including the James Hutton Institute) (Table 1.1.1). Many of the traps are serviced by Berry Gardens field staff. Farms were chosen to give good geographical coverage and to ensure that a full range of vulnerable soft and stone fruit crops were assessed. At least one wild area was also assessed at each farm.

Table 1.1.1. Summary of fruit farms in the National Monitoring Survey. An area of woodland was also included at each farm with the exception of one farm in the east which was reinstated in 2017

Farm No. / Region	No. traps	Crops
3 / SE	2	Cherry
3/ SE	2	Wild
4 / SE	4	Raspberry
4 /SE	2	Wild
5 / SE	6	Cherry, wine grape, table grape
5/ SE	2	Wild
6 / SE	8	Blueberry, redcurrant, strawberry
6/ SE	2	Wild
7 / East	4	Blueberries
7/ East	1	Wild
8 / East	4	Raspberries, strawberries
8 /East	2	Wild
9 / WM	4	Raspberries, strawberries
9 / WM	2	Wild
10 / WM	8	Blueberry, cherry, raspberry, strawberry
10 / WM	2	Wild
10b / NE	1	Strawberry
10b /NE	1	Wild
11 / Scotland	8	Blackcurrant, blueberry, raspberry, strawberry
11/ Scotland	2	Wild
12 / Scotland	8	Blueberry, cherry
12 / Scotland	2	Wild
13 / Scotland	8	Blackberry, blueberry, raspberry, strawberry
13 / Scotland	2	Wild
14 / Scotland	8	Blackberry, blueberry, raspberry, strawberry
14 / Scotland	2	Pack house
	97	

Monitoring traps were generally deployed in pairs, one in the centre and one at the edge of each crop. Pairs of traps were also deployed in a wooded area on each farm. 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 dia.) in the lid, attached near the fly entry holes within the trap. The traps were deployed at the height of the main crop.

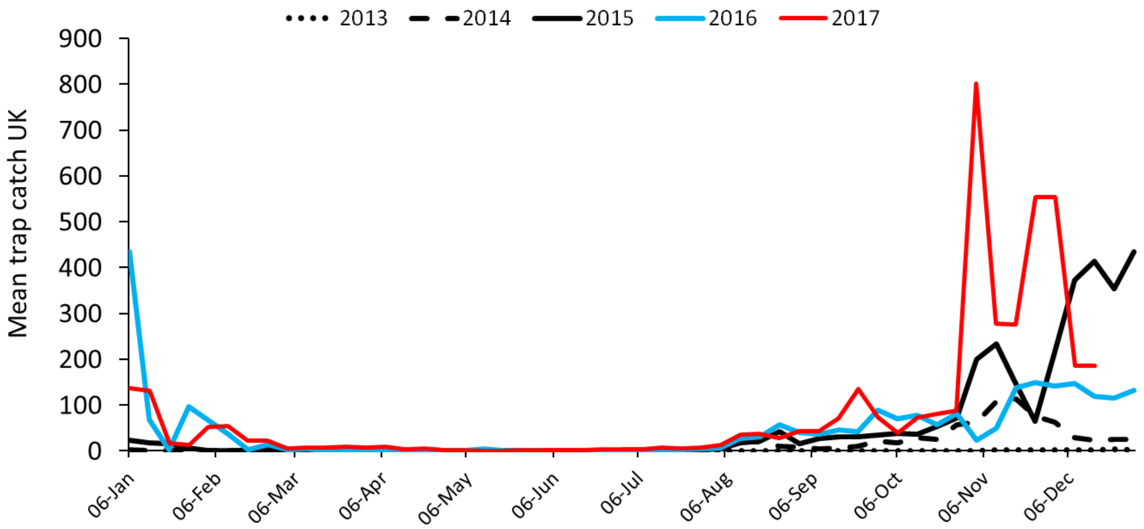
Trapping has been continuous at most sites since May 2013 with new sites being added and some sites ceasing to be monitored. Adult *D. suzukii* counts were done weekly during the cropping season and biweekly during the winter.

Results

At the time of writing the activity-density of adult *D. suzukii* in the monitoring traps was higher in the spring (Mar-May) and late summer (Jul-Aug) of 2017 than in previous years (Figure.1.1.1). This correlates with increases in reported damage to early forced June bearers and cane fruits respectively, by the industry. The first peak autumn catch was almost a month earlier with catches in Nov-Dec almost double the trap catch (>800) from the previous highest recording in 2015/16 (Figure. 1.1.1). Peaks in Nov-Dec in 2015 corresponded with mild weather (Figure.1.1.2). A similar pattern is observed in Oct 2017 (Figure.1.1.2).

In general, patterns of adult *D. suzukii* catches in the traps followed previous years. Catches in the winter of 2016/17 (blue line) were two thirds lower than the previous winter (potentially explained by a milder Nov and Dec in 2015/16).

a)



b)

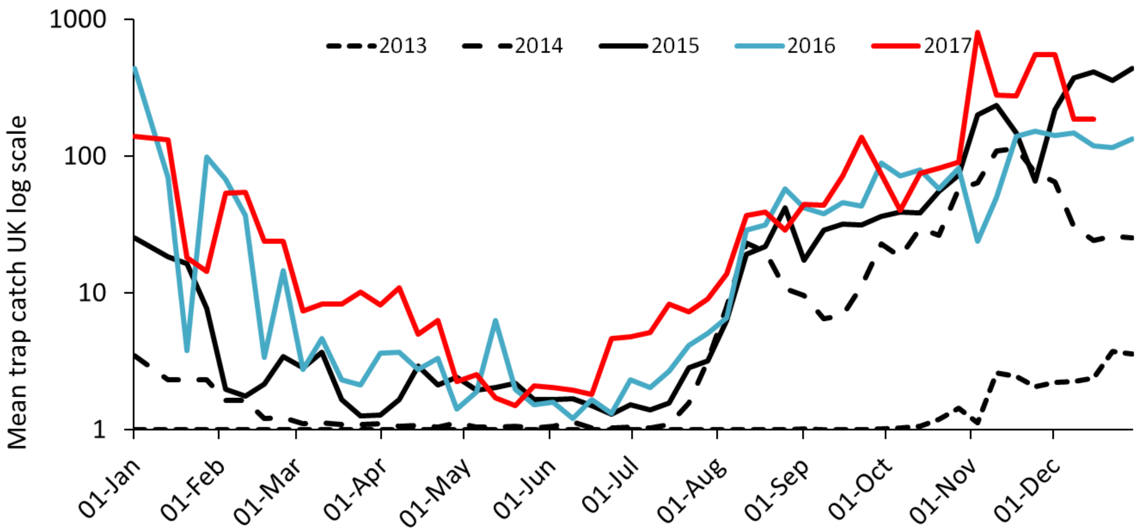


Figure 1.1.1. a) Comparison of average adult *D. suzukii* catch per trap in 2013, 2014, 2015, 2016 and 2017 and b) plotted on a $\log_{10}$ (n + 1) scale on the Y axis

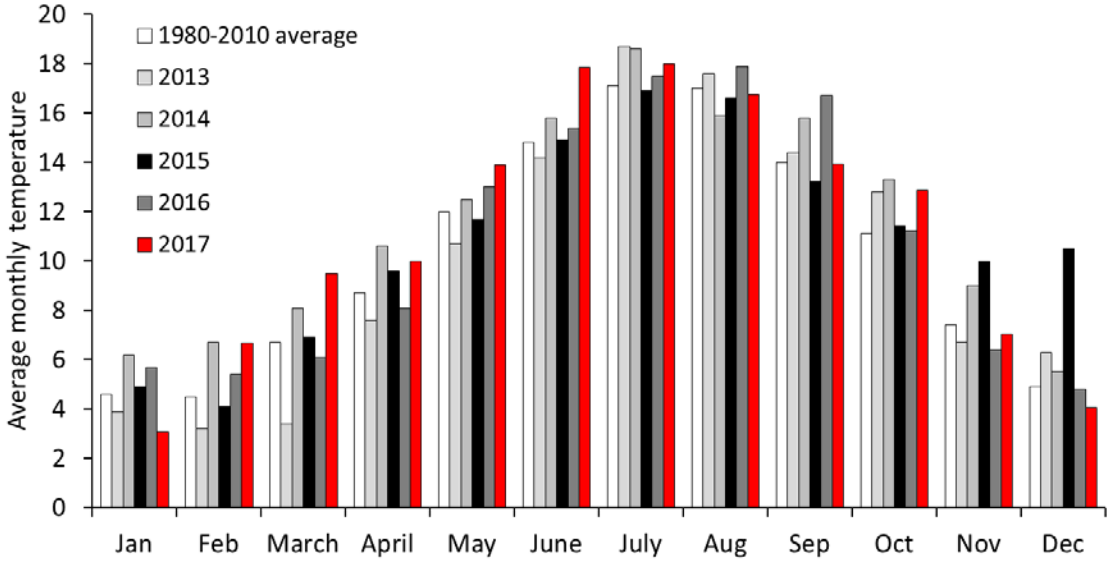


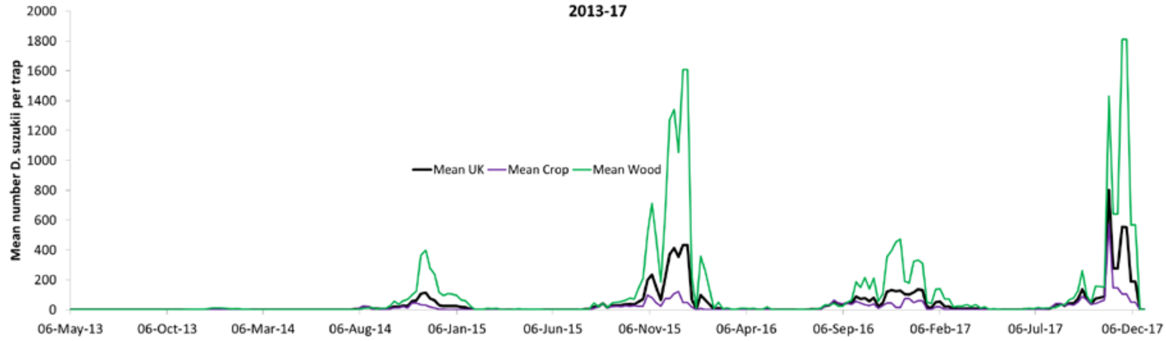
Figure 1.1.2. Comparison of the temperatures between years.

The peaks in trap catches are primarily driven by catches in wild areas and follow a similar pattern to the catches in the South East of England which are several fold higher at one farm (Figure. 1.1.3). The highest peaks 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 1.1.4 and 1.1.5 demonstrate the variability between catches in the same regions in different years. Data from Yorkshire has only been collected at one site since 2016 so more time is needed to see inter-annual trends. In general numbers have increased over time in all regions with the exception of Scotland. It is possible that the available period of activity of *D. suzukii* to reproduce over a season is more restricted.

In addition, NIAB EMR staff visited Rothamsted Research and sorted through samples thought to be positive for *D. suzukii,* collected from suction traps as part of the Rothamsted Insect Survey (RIS) (Figure. 1.1.6). The first visit was made in 2013 when no *D. suzukii* were found in samples. However from 2014 male and female *D. suzukii* have been captured at a height of 50 m and this is correlated with the highest trap catches in the late autumn at crop and woodland level (Sep-Nov 2013-17).

a



b

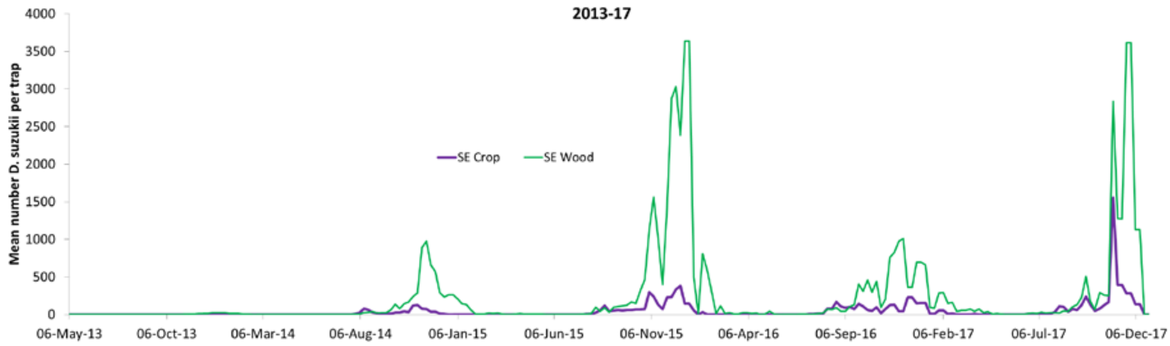
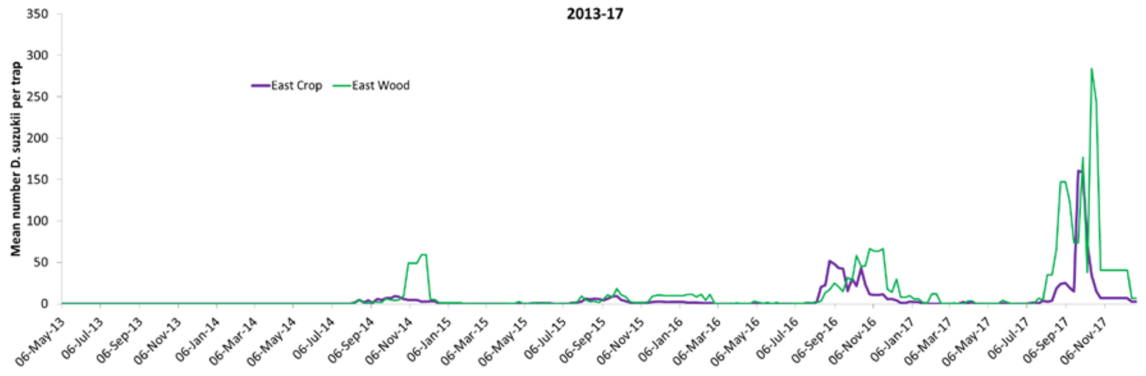


Figure 1.1.3. Mean numbers of *D. suzukii* adults per trap a) in the UK and b) in the South East of England from 2013 to 2017

a



b

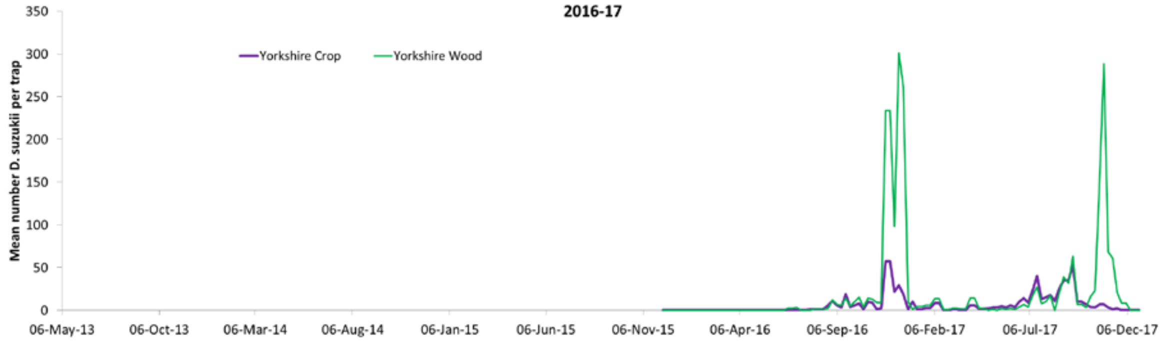
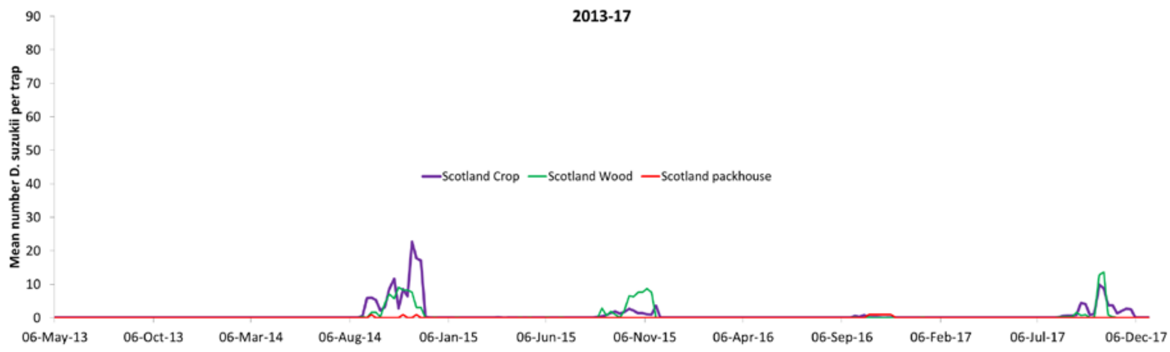


Figure 1.1.4. Mean numbers of *D. suzukii* adults per trap in a) East England and b) Yorkshire (NB monitoring only began in January 2016) from 2013 to 2017

c



d

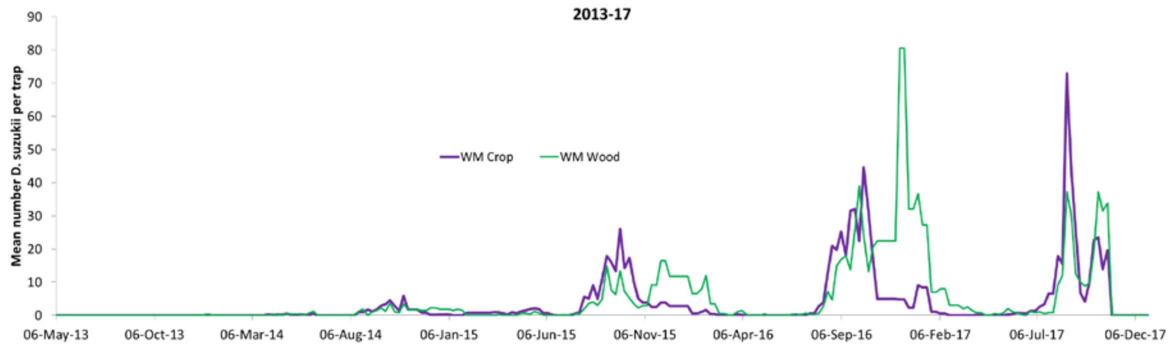


Figure 1.1.5. Mean numbers of *D. suzukii* adults per trap in c) Scotland and d) the West Midlands from 2013 to 2017

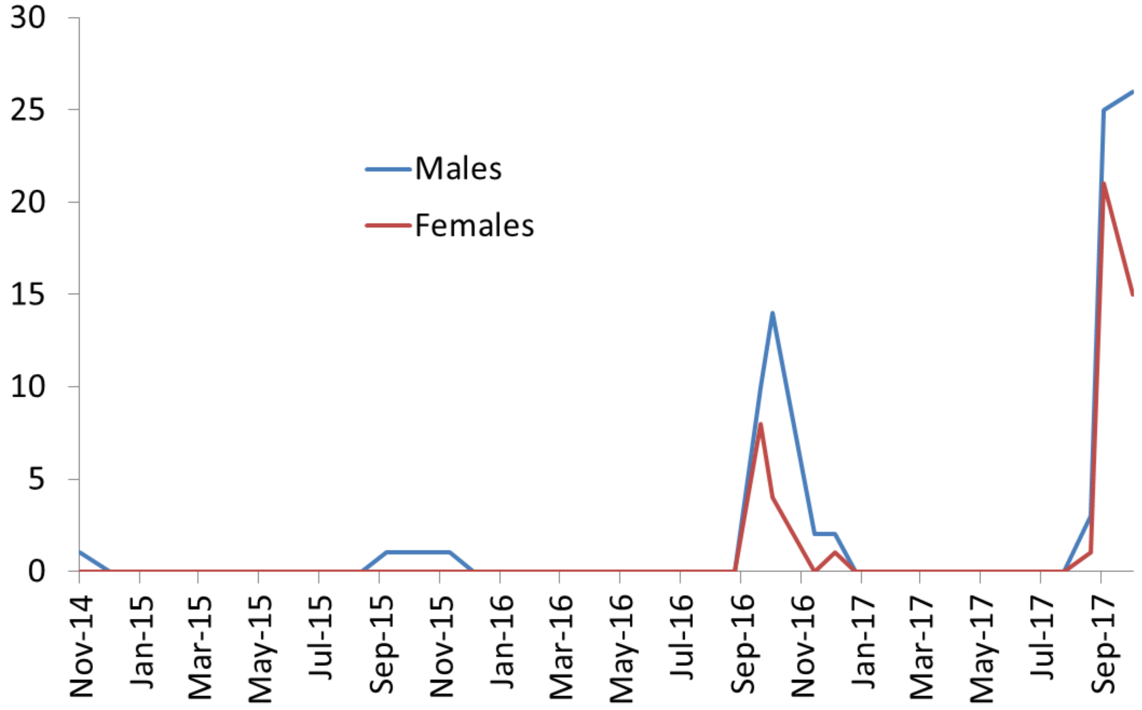


Figure 1.1.6. Total numbers of *D. suzukii* adults in 50 m height suction traps (Rothamsted) from 2013 to 2017. First catches were in 2014

Conclusions

- *D. suzukii* numbers in monitoring traps continue to rise with interannual variation in trap catches, at least in the late autumn, probably dependant upon temperature as temperature (Tochen *et al*., 2013) and humidity (Tochen *et al*. 2015) are known to affect the activity of *D. suzukii*.
- In addition, it has been confirmed that *D. suzukii* can be detected at 50 m during the main period when the flies are captured in the traps in cropping and woodland areas (Sep-Nov).
- This period coincides with a depletion in egg laying resources and defolation of trees.
- Decrease in trap catches during the summer months likely due to trap being less attractive than crop and not a decrease in the number of *D. suzukii.*

Task 1.2. Additional Sites in Scotland (Yrs. 1-4; JHI, NIAB, NRI)

Introduction

To provide a more comprehensive picture of the density of *D. suzukii* in Scotland and to determine if the existing monitoring data was representative, catch data were collected and collated from two growers' groups in Scotland and compared to the results from the National Monitoring study (NM -Grower group 1) in Scotland, comprising of data collected from 4 sites.

Methods

Grower group 2 provided data from 40 traps at ten sites in 2015, 41 traps in 2016 and 50 traps at 12 growers' sites in 2017. The sites represent the main fruit production area including farms in Fife, Perthshire, Dundee, Angus and Aberdeenshire. Drosotraps from Agralan were used with Dros'Attract bait and were sampled from on a weekly basis from March to October. The bait used is different to the national monitoring traps as the bait is commercial bait with no vials.

Growers' group 3 provided catch data from eight sites. Their records began in 2015 and each year they monitored from the beginning of March until the end of October using a Biobest Drosotrap modified with a mesh to reduce bycatch and using Riga Gasser attractant. The bait is changed every two weeks throughout the season, and the traps are assessed weekly on a total catch basis of males plus the same number of females.

Results

In all three monitoring groups the numbers of *D. suzukii* caught in Scotland in 2016 were lower than in 2015 (Figure 1.2.1). One of the sites in group 1 and another from group 2 consistently record higher numbers than the other sites and these 'hotspots' have a strong influence on the data set. The possible parameters that might be responsible for the higher numbers at these sites have not been evaluated. Catches in 2017 are slightly higher than in previous years in all 3 groups.

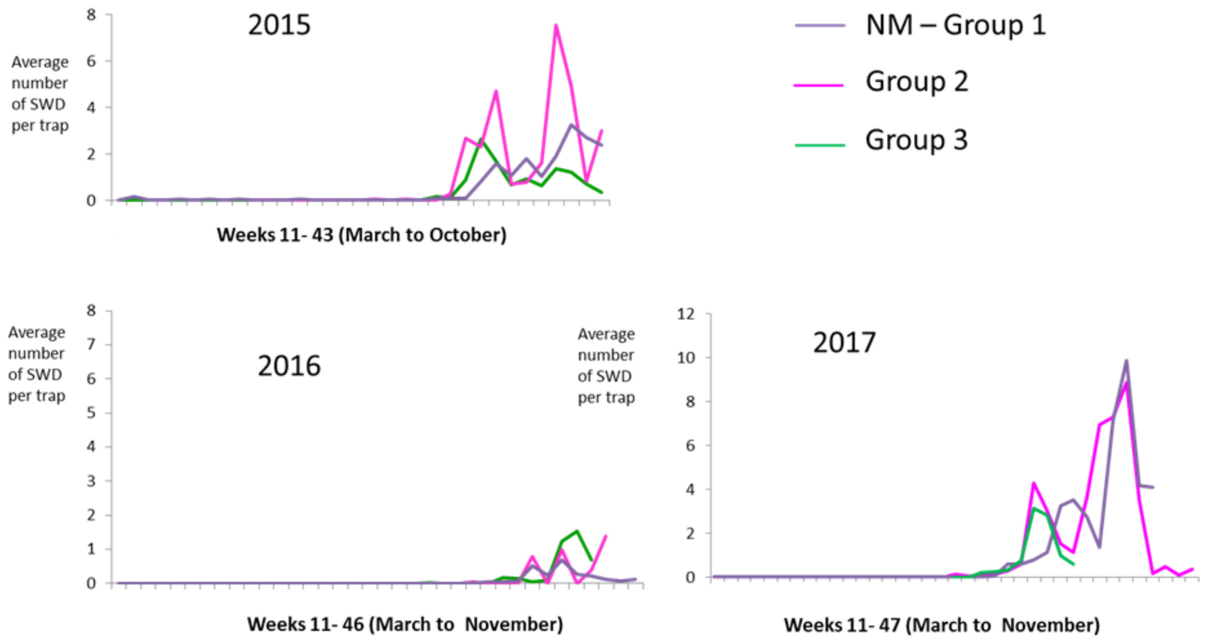


Figure 1.2.1. Comparison of the monitoring data from the Scottish monitoring organisations (NM-Group 1, Group 2 and Group 3) in Scotland in 2015, 2016 and 2017

Conclusions

- The data from all three Scottish monitoring groups show similar trends suggesting that the national monitoring data set is representative of the *D. suzukii* density in Scotland.
- This data gives information about trap catches but not abundance of *D. suzukii*. It is likely the prevalence of *D. suzukii* may be underestimated in summer due to the presence of the crop. This has been further explored in task 1.3.

Task 1.3. Egg laying sites for D. suzukii in Scotland (Years 1-2; JHI)

Introduction

Attention was focused on identifying possible egg laying and early and late nectar sources of wild hosts of *D. suzukii* in Scotland. Sampling was done to determine the length of the fruiting stage in possible hosts and to identify those that continue to provide fruit over winter and therefore may provide suitable early hosts for oviposition.

Methods

At monthly intervals from August 2017, samples of wild berries were collected from a wide range of hedgerow and woodland plants from the grounds at the James Hutton Institute. Targets of 100 ripe and overripe fruits per sample were collected; however this was not always possible due to availability. In addition, a small number of samples from the wild hosts in the habitat study at site 1400 were collected. The samples were examined visually and incubated for adult emergence to determine whether *D. suzukii* was developing in the fruits.

Results

Samples of berries from hedgerow and woodland plants (Berberis, Cotoneaster, Rowan, wild Blackberry, Choke berry, Rosehip, Sloes and Hawthorn were collected on 25 August, 21 September and 20 October 2017 at site 1100 (the James Hutton Institute). There was insufficient fruit on wild cherry to test. One female *D. suzukii* emerged from wild blackberry collected on 21 September but none were detected from either the flotation or emergence tests from fruit collected on 25 August or 20 October.

On 16 November wild Blackberry and Chokeberry were no longer fruiting and were therefore not sampled. Viburnum and Ivy were added to the wild hosts sampled previously and flotation and emergence tests were carried out. No *D. suzukii* were detected.

Two female *D. suzukii* emerged from wild blackberry collected on 23 August 2017 from the habitat study (site 1400) close to trap 1428.

Three female and four male *D. suzukii* emerged from wild blackberry collected on 21 September 2017 from the habitat study (site 1400) close to trap 1416. Fruit from hawthorn was also collected but no *D. suzukii* were found. No *D. suzukii* were found from either the wild blackberry or hawthorn fruit that was collected from site 1400 on 19 October.

Conclusions

Although data is currently limited, at both sites, wild Blackberry appears to be the preferred egg laying host.

Task 1.4. Habitat preference and fecundity in Scotland (Years 1-2; JHI, NRI)

Introduction

The distribution, habitat preference and fecundity of *D. suzukii* is being monitored fortnightly at one of the Scottish monitoring sites using an additional 20 Biobest traps with the same bait used as for the National Monitoring. The location of the additional traps include a variety of surrounding habitats e.g. woodlands, hedgerows and wasteland. Reproductive stages of trapped female *D. suzukii* were assessed by dissection under a microscope. The stages of ovary and egg development were determined using stage definitions published by Beverly S. Gerdeman, Washington State University (Table 4.1.2) and training was provided by NIAB EMR.

Methods

Habitat trap catches were collected fortnightly and counted in the laboratory (total numbers were divided by two as the trapping was fortnightly). Where possible five females from each trap were chosen at random and dissected to assess fecundity (Table 4.1.2).

Records of species diversity and abundance were taken from areas surrounding the traps. Abundance was calculated using the Total Estimate Scale. Assessments were carried out monthly and plant abundance and growth stage were recorded.

Results

Monitoring began on 14 June 2017. No *D. suzukii* were detected until 9 August. Initial findings suggest that, as with previous studies, more *D. suzukii* were caught in the traps located in the wild (habitat) than the traps located in the fruit crop (National Monitoring) (Figure 1.4.1). Initial findings also indicate two ‘hotspots’ (Trap 1416 and 1428, Figure 1.4.2). The habitat surrounding the hotspots includes blackberries, cherries, nettle, goosegrass and grasses. Interestingly two traps located very close to trap 1416 (1415 and 1417) only caught small numbers of *D. suzukii* suggesting that ‘hotspots’ can be very localised.

The percent of females at different stages of reproductive state is shown in Figure 1.4.3. No *D. suzukii* were caught in the traps from 12 June- 24 July.

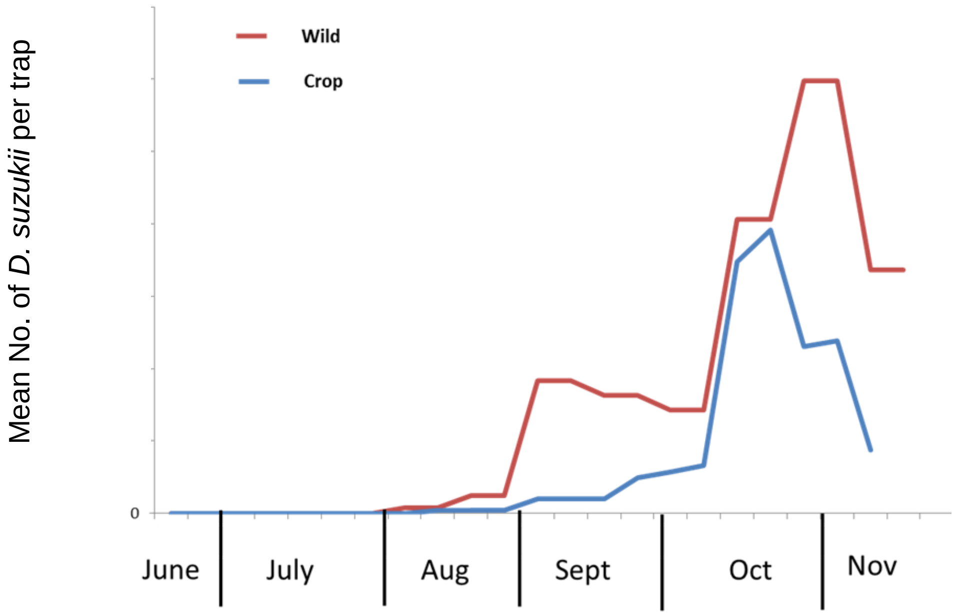


Figure 1.4.1. Mean weekly numbers of *D. suzukii* per trap at habitat study site in 2017

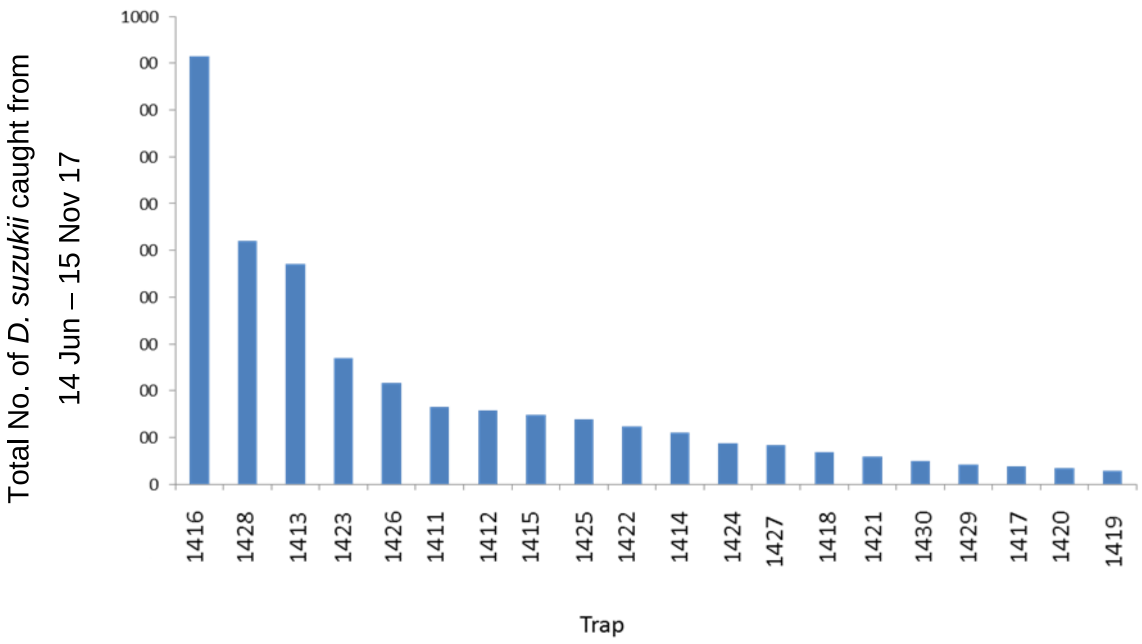


Figure 1.4.2. Total numbers of *D. suzukii* per trap at one site between 14 June and 15 Nov 2017. Number on the x axis referes to the trap code

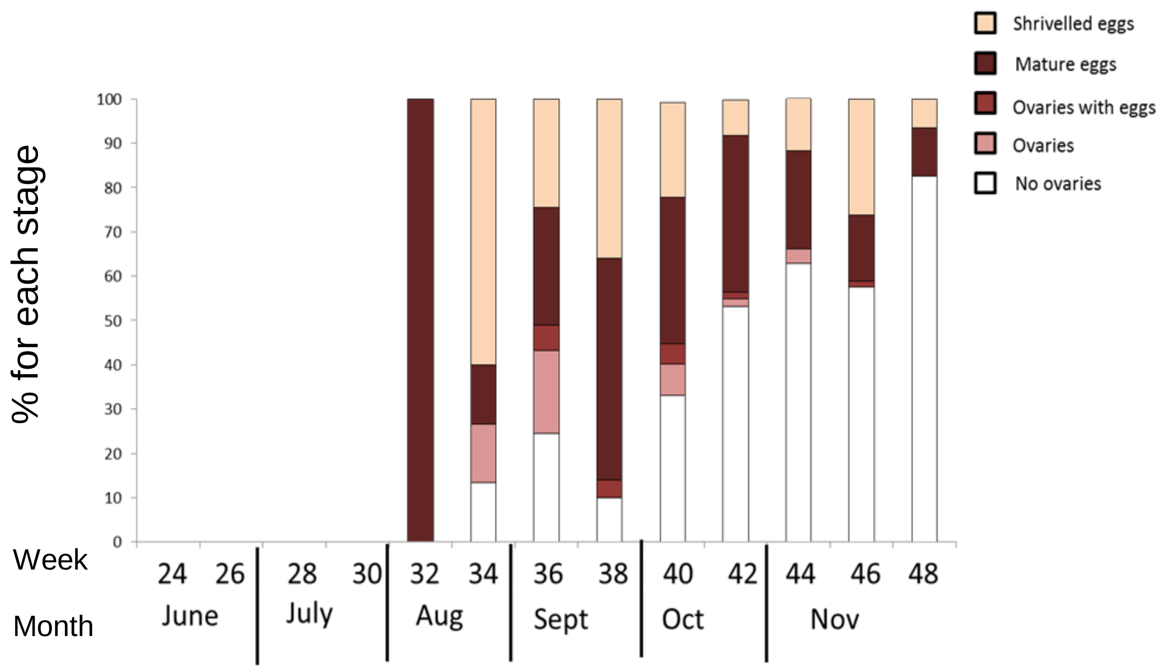


Figure 1.4.3. Percentate of female *D. suzukii* at different fecundity stages (Table 4.1.2) at habitat study site 1400

Conclusions

- No *D. suzukii* were captured until August in 2017
- Fecund females were present through until at least November
- Collated data from 3 Scottish growers groups indicate similar *D. suzukii* trap catches and patterns from year to year
- All Scottish data sets indicate that *D. suzukii* trap catches in Scotland have increased in 2017 in comparison to data from 2015 and 2016. However the 2017 peak is lower than that reached in the Scottish National Monitoring data for 2014
- In Scotland *D. suzukii* were not detected in the traps from January until mid-July
- As with previous studies, *D. suzukii* catches at the habitat study were higher in the traps located in the wild locations than the traps located in the crop
- Emergence tests carried out on wild and crop hosts indicated that *D. suzukii* was detected late in the growing season in wild brambles and only found in fruit crops at one growers site

Task 1.5. Data collation and dissemination (Yrs. 1-4; JHI, NIAB, NRI)

This project will generate basic, strategic and applied knowledge on the control of *D. suzukii* in a practical field setting and provide innovative solutions for UK growers. All results will be effectively disseminated in a timely manner (See knowledge exchange section of report). The findings of the analysis of the monitoring data and the most up to date information on the pest and its control measures will be disseminated at various knowledge transfer soft fruit and tree fruit industry events. Regular updates will be given to Scottish Government (SG) by the James Hutton Institute.

Data has been collected at the James Hutton Institute, BGG and NIAB EMR collated at NIAB EMR and sent to AHDB communications so that growers can be informed of risk to crops. All growers' details within the project remain confidential.

At the Fruit For the Future Event held in July at the James Hutton Institute stakeholders were reminded to remain vigilant for the presence of *D. suzukii* and given advice on identification and testing methods they could use on their farms to look for the pest in traps and fruit. Free testing of fruit was provided at the drop-in clinic to help the fruit industry with early detection of the pest in the crop. Results were provided confidentially.

Glasshouse and estate staff at the James Hutton Institute attended a presentation in November 2017 providing feedback on the density of *D. suzukii* at the Hutton and in the UK National Monitoring sites and reminded to destroy unwanted fruit wherever possible.

The three growers in the monitoring project in Scotland were updated regularly on their confidential catch data and in October were given a more comprehensive verbal report on the Scottish and UK monitoring results. Scottish Government has also received a verbal update.

A poster is being produced for display at the SSCR/Bulrush Horticulture Ltd joint winter meeting to be held near the James Hutton Institute in February 2018.

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

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, but this could have resulted from the wrong formulation to dispense repellents or that the sachets were applied too late in the season, once *D. suzukii* was already in the crop. Although promising, more work was required to test compounds singly and in blends in the spring to give them a better chance of success. 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 four 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).

NIAB EMR, with NRI, has developed a small A&K device which needs further evaluation. It attracts the adult flying stage of the pest to a device which currently contains a lethal dose of an insecticide, but there is potential to exploit already approved biological control agents. The control component is enclosed within the inner surface of the trap to minimise human exposure and environmental contamination including adverse effects on beneficial insects. Unlike ‘mass traps’, the A&K device is open ended and does not become saturated with dead flies which reduces high labour costs. Preliminary data (Kirkpatrick and Gut 2016) shows that attractant baited traps catch for a distance of 4 m, so that if devices were used without repellents within the crop they would need to be a minimum of 8 m apart around the perimeter of a crop as part of the Push-Pull system. Findings from a recently completed Innovate *D. suzukii* attractants project (NIAB EMR, NRI, BGG, Real IPM) could be employed to enhance the traps with long (Cha *et al*. 2013) and short range (for retention of *D. suzukii* in the trap) compounds not typical of fermenting fruit volatiles exploited in current commercial traps. Ideally the lure would last a whole season and this needs to be optimised. Servicing and replacing trap contents is a high labour cost hence attractant longevity and prevention of saturation with dead flies is critical to reducing cost. The trap could be designed with alternative killing agents to Decis, currently being supported and registered and commercialised by an industrial company. For example, entomopathogenic fungi whilst they have a slower kill time (Cuthbertson *et al*. 2014; 2016; Haye *et al*. 2016), could enable horizontal transfer and wild population build-up during the season. New strains of fungi are being developed by industry and some are already registered for use. Currently the Decis trap used for MedFly is 4.5 Euros per trap at 100 traps per ha, although the price is likely to be lower for *D. suzukii*.

Task 2.1. Further test blends, efficacy and frequency of application of repellents (Yrs. 1-2; NIAB, NRI)

Introduction

In 2016 experiments, where six individual compounds were deployed in the field directly above fruit in red delta traps, *D. suzukii* egg laying decreased. However, when the experiment was repeated later in the season, post cherry harvest, the repellent effect was less effective. It was hypothesised that this could have been because the numbers of *D. suzukii* in the crop canopy were very high. In 2017 we repeated the experiment earlier in the season before the first generation of *D. suzukii*.

Methods

Two experiments were done in 'Rookery' cherry (cvs. Penny and Sweetheart) orchard at NIAB EMR (Table 4.1.1.). All treatment compounds are synthetic semio-chemical compounds that have been coded. There were seven treatments (six compound treatments + an untreated control) in each experiment. The same compounds were tested in each and included a blend of compounds 2017-070/B, 2017-070/C and 2017-070/D (Table 2.1.1). Each treatment was dispensed from a sachet, except for 2017-070/A which was released from a septum in the first and a sachet in the second experiment, respectively.

Table 2.1.1: Treatments

NRI code	Sachet/Septum dimensions
2017-070/A	4mg septum (Exp 1) 1 ml in sachet 25 mm x 50 mm x 120µm (Exp 2)
2017-070/B	1ml in Sachet 50mm x 50mm x 120µm.
2017-070/C	1ml in Sachet 50mm x 50mm x 250µm.
2017-070/D	1ml in Sachet 50mm x 50mm x 120µm.
2017-070/E	1ml in Sachet 50mm x 50mm x 120µm.
2017-070/F (blend)	1ml in Sachet 50mm x 50mm x 120µm.
Untreated control	1ml in Sachet 50mm x 50mm x 120µm.

Twenty sachets/septa were suspended – dispersed evenly throughout each tree (small plot) on 12 May 2017 and 20 more on 13 July 2017 (sachets/septa left in tree from experiment 1). Prior to deploying the fruit, *D. suzukii* from cultures at NIAB EMR were applied to the fruit for 48 hours to ensure there was no mortality from insecticide residues. In both experiments, no laboratory *D. suzukii* died within 48 hours and *D. suzukii* emergence was observed two weeks later. Treatments were placed into five rows (blocks) with each tree as a plot. Plots were 12 m apart and each row was divided by a single guard row. Three strawberries were placed in each petri dish, hung in two green delta traps. One with a sachet/septum of the same treatment compound/compounds hung from the lid of the delta trap and one without. There were five replicates of each treatment in a complete randomised block design with sub plots of the tree plots (delta traps).

The sentinel fruit was deployed on 15 and 22 May for the first experiment and 14 and 21 July for the second experiment. Fruit was collected in three days after being deployed. The strawberries were collected into ventilated boxes and incubated at 22°C, 16 h: 8 h light dark, for three weeks. The number of emerged male and female *D. suzukii* and other drosophila were then recorded.

Emergence data was SQRT transformed and each variate analysed at each of the start dates using ANOVA in GENSTAT.

Results

There was no significant difference in emergence of male or female, adult *D. suzukii* or other drosophila between any of the treatments or between traps with the compound/compounds in the lid and without, in Experiment 1 or 2 (Table 2.1.2, $P > 0.05$).

In the first Experiment one, there was very low emergence of *D. suzukii* with emergence only observed in the first two plots of Block 1. In the second replicate run more *D. suzukii* emerged however only in Blocks 4 and 5. A total of 105, 21, 50, 15, 35, 1 and 74 adult *D. suzukii* emerged from fruit in the 2017-070/A, 2017-070/B, 2017-070/C, 2017-070/D, 2017-070/E, 2017-070/F (Blend) and the untreated control, respectively (Table 2.1.3).

In Experiment 2 in both replicate tests there was *D. suzukii* emergence from fruit in all of the blocks. A total of 1539, 1287, 769, 1356, 826, 995 and 929 adult *D. suzukii* emerged from fruit in the 2017-070/A, 2017-070/B, 2017-070/C, 2017-070/D, 2017-070/E, 2017-070/F (Blend) and the untreated control, respectively (Table 2.1.3).

Table 2.1.2. Mean emergence from sentinel fruit (bold data). Emergence from fruit placed in a delta trap with the corresponding sachet in the lid and without the corresponding sachet in the lid (first and second data points in brackets, respectively) ($P > 0.05$).

		2017-070/A	2017-070/B	2017-070/C	2017-070/D	2017-070/E	Blend	Untreated
Exp 1	Male	**3.83** (4.1, 3.6)	**0.75** (0.3, 1.2)	**2.1** (2.25, 1.95)	**0.45** (0, 0.9)	**0.78** (0.7, 0.9)	**0** (0,0)	**2.7** (2.4, 3.0)
	Female	**3.15** (3.2, 3.2)	**0.83** (0.6, 1.05)	**1.65** (1.8, 1.5)	**0.675** (0.3,1.1)	**0.95** (0.1,1.8)	**0**.08 (0,0.15)	**2.85** (2.1, 3.6)
	Other Drosophila	**0** (0,0)	**0.15** (0, 0.3)	**0** (0,0)	**0** (0,0)	**0** (0,0)	**0** (0,0)	**0** (0, 0)
	Adult *D. suzukii*	**6.68** (7.2, 6.2)	**1.58** (0.9, 2.25)	**3.75** (4.1, 3.5)	**1.13** (0.3, 1.9)	**1.73** (0.75, 2.7)	**0.08** (0,0.15)	**5.55** (4.5, 6.6)
Exp 2	Male	**35.9** (35.1, 36.8)	**29.6** (33.1, 26.1)	**17.6** (14, 21.2)	**30.9** (26.4, 35.4)	**19** (14.4, 23.6)	**22.9** (15.1, 30.7)	**19.9** (20.8, 19)
	Female	**41** (40.1, 41.9)	**34.8** (37.9, 31.6)	**20.9** (16.8, 24.9)	**36.9** (32.8, 41)	**22.3** (17.5,27.1)	**26.9** (16.9, 36.8)	**26.6** (30.7, 22.4)
	Other Drosophila	**15.1** (10.2, 20)	**12.4** (18.1, 6.7)	**19.2** (29.6,8.8)	**9.9** (6,13.8)	**6.35** (7.5, 5.2)	**5.7** (1.8, 9.6)	**12.6** (12.3, 12.8)
	Adult *D. suzukii*	**77.0** (75.2, 78.7)	**64.4** (71, 57.7)	**38.5** (30.8,46.1)	**67.8** (59.2, 76.4)	**41.3** (31.9,50.7)	**49.75** (32, 67.5)	**46.5** (51.5, 41.4)

Table 2.1.3. Total adult *D. suzukii* emergence from all sentinel strawberry fruit

Treatment compound	Exp 1	Exp 2
2017-070/A	105	1539
2017-070/B	21	1287
2017-070/C	50	769
2017-070/D	15	1356
2017-070/E	35	826
2017-070/F (blend)	1	995
Untreated	74	929

Conclusions

- Only one *D. suzukii* emerged from sentinel strawberries in the blend treatment in the first experiment suggesting that a blend may be more effective than single components.
- *D. suzukii* was aggregated in only 2 blocks in the first experiment (mid-May) removing any possibility of detecting a significant effect.
- *D. suzukii* was present throughout the cherry orchard in July but numbers were potentially too high and the repellent effect suppressed.

Future work

- Test repellents in a less challenging crop, e.g. strawberry potentially in combination with pull strategy e.g. mass traps or A&K devices.
- Use tunnels or wind tunnel experiment to look at distance of repellence with sentinel fruits.
- Test different blends of the six compounds.

Task 2.2. Establish efficacy of A&K device (Yrs. 1-2; NIAB, NRI)

Introduction

After preliminary development trials, in 2015 and 2016 a prototype “attract and kill” (A&K) device was designed based on the following principles:

1. Low cost, as the commercial version would need to be deployed in large numbers at a labour cost to the grower
2. Relatively small size;
3. Lures should be attractive to *D. suzukii*, but small-sized enough to fit inside the device;
4. Killing agent used should be fatal to *D. suzukii* after a low time of contact;
5. Drowning solutions are not part of this design as the device will be left unattended for weeks. A small device becomes saturated with rain and dead insects; hence a drainage/escape hole is used at the bottom of the device.

This year we conducted three trials to optimize the trap design and assess its efficacy for *D. suzukii* control.

Methods

All prototypes were compared to a commercial standard. All trials were set up in wire framed cages with insect proof mesh, 43 x 43 x 95 cm, weighed down with two bricks in a shady, humid, outside area at NIAB EMR. Cages were set up vertically, with devices hung from straps at the top of the cage (Figure. 2.2.1). Cages were spaced 50 cm apart and had one device (one plot). Wet paper and a feeding station with 5% sugar solution on a sponge in an AA cup were added to each cage to ensure a high humidity and provide a food source for *D. suzukii*, respectively.

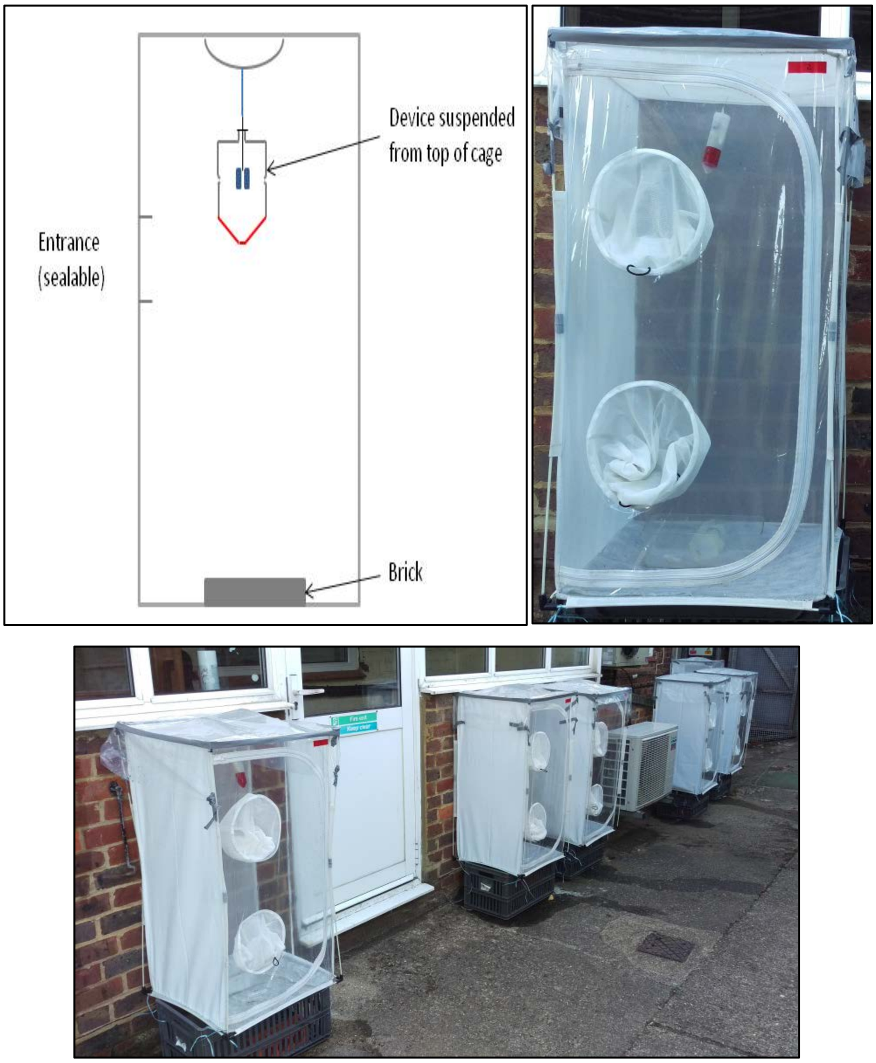


Figure. 2.2.1. Graphic and photographs of experimental set up; device hanging from top of cage

The lures used in the prototype were separate half size sachets of ethanol/ acetoin, acetic acid and methionol (provided by NRI) and referred to as mini Cha-Landolt (Figure. 2.2.2). The commercial trap contained its own bait (Coded B), but was also tested in combination with the mini-lure. The commercial trap was much larger than the prototype and did not allow insects to leave. All experimental prototypes, with the exception of the untreated controls, were coated on the inside with the Decis formulation (deltamethrin) unless stated otherwise (Trial 1). All devices were orientated so that the red part of the device was facing downward with the clear part at the top.

Figure. 2.2.2. Mini-Lures provided by NRI: ethanol/ acetoin, acetic acid and methionol

In every trial 10 male and 10 female, 3 – 12 days old, mated *D. suzukii,* from a laboratory culture, were introduced at time zero to each cage. After 24 hours the devices were removed and the numbers of live and dead *D. suzukii* counted. There were seven replicate days in total for each trial.

Statistical analysis: Data were analysed using a generalised analysis of variance on count data in GENSTAT.

Semi-Field Cage Trial 1:

The first trial (in June) prototypes were 50 ml Falcon tubes with 4 x 0.5 cm holes on the clear section towards the top of the device with 1 x 0.6 cm hole in the bottom and painted red on the base (Figure.2.2.3A). Five cages were kept in the same position and traps were re-randomised after each replicate run (a replicate was 24 hours).

Two Falcon tube devices with the NRI dry mini-lure were coated on the inside with either Decis or spinosad. These were compared to a Falcon tube device with a lure but no insecticide coating and to two commercial traps with either the NRI or commercial trap lures inside (Table 2.2.1).

Table 2.2.1. Devices tested in Trial 1.

Trap design	Lure	Insecticide	Insecticide applied to	Surface area (cm^2)	Application rate (mg/cm^2)	Insecticide applied to device in 1 ml of distilled water
50 ml falcon tube (A)	NRI (mini cha-landolt)	DECIS WG	Inside of device	101.38	0.63	64 mg/ 0.064 g
50 ml falcon tube (A)	NRI (mini cha-landolt)	Spinosad	Inside of device	101.38	0.63	64 mg/ 0.064 g
50 ml falcon tube (A)	NRI (mini cha-landolt)	None	None	None	None	None
Commercial trap (B)	NRI (mini cha-landolt)	DECIS WG	Lid	95	0.63	60 mg/ 0.060 g
Commercial trap (B)	Commercial lure	DECIS WG	Lid	95	0.63	60 mg/ 0.060 g

Semi-Field Cage Trial 2:

The second trial (in July) prototypes were either 50 ml Falcon tubes with 4 x 0.5 cm holes on the clear section towards the top of the device with 1 x 0.6 cm hole in the bottom and painted red on the base (Figure.2.2.3A) or 50 ml Falcon tubes with 8 x 0.5 cm holes on the red part and 1 x 0.6 cm hole in the bottom, painted red in the middle and base clear (Figure.2.2.3C). Six cages were kept in the same position and traps were re-randomised after each replicate run (a replicate was 24 hours).

Two Falcon tube devices with the NRI dry mini-lure were coated on the inside with Decis. These were compared to two controls, the same as above, but with no Decis coating and to two commercial traps with either the NRI or commercial trap lures inside (Table 2.2.2).

Table 2.2.2. Devices tested in Trial 2.

Trap design	Lure	Insecticide	Insecticide applied to	Surface area (cm^2)	Application rate (mg/cm^2)	Amount of insecticide applied to device in 1 ml of distilled water
50 ml falcon tube (A)	NRI (mini cha-landolt)	DECIS WG	Inside of device	101.38	0.63	64 mg/ 0.064 g
50 ml falcon tube (New design; 8 holes)(C)	NRI (mini cha-landolt)	DECIS WG	Inside of device	101.38	0.63	64 mg/ 0.064 g
50 ml falcon tube (A)	NRI (mini cha-landolt)	None	None	None	None	None
50 ml falcon tube (New design; 8 holes)(C)	NRI (mini cha-landolt)	None	None	None	None	None
Commercial trap (B)	Commercial lure	DECIS WG	Lid	95	0.63	60 mg/ 0.060 g
Commercial trap (B)	NRI (mini cha-landolt)	DECIS WG	Lid	95	0.63	60 mg/ 0.060 g

Semi-field cage trial 3:

The third trial (in August) prototypes were either 50 ml Falcon tubes with 8 x 0.5 cm holes on the red part and 1 x 0.6 cm hole in the bottom, painted red in the middle and base clear (Figure.2.2.3C) or 50 ml Falcon tubes with 16 x 0.5 cm holes on the red part and 1 x 0.6 cm hole in the bottom, painted red in the middle and base clear (Figure. 2.2.3D) or 50 ml Falcon tubes with 8 x 0.5 cm holes on the red part, 1 x 0.6 cm hole in the bottom, painted red from the middle to the base (Figure.2.2.3E) or 50 ml Falcon tubes with 16 x 0.5 cm holes on the red part, 1 x 0.6 cm hole in the bottom, painted red from the middle to the base (Figure.2.2.3F). Six cages were kept in the same position and traps were re-randomised after each replicate run (24 hours).

Four Falcon tube devices with the NRI dry mini-lure were coated on the inside with Decis. These were compared to a cage with no device inside and to a commercial trap with commercial trap lures inside (Table 2.2.3).

Table 2.2.3. *Devices tested in trial 3.* *Industry sensitive

Trap design	Lure	Insecticide	Insecticide applied to	Surface area (cm^2)	Application rate (mg/cm^2)	Amount of insecticide applied to device in 1 ml of distilled water
50 ml falcon tube (8 holes full red)(E)	NRI (mini cha-landolt)	DECIS WG	Inside of device	101.38	0.63	64 mg/ 0.064 g
50 ml falcon tube (8 holes red band)(C)	NRI (mini cha-landolt)	DECIS WG	Inside of device	101.38	0.63	64 mg/ 0.064 g
50 ml falcon tube (16 holes full red)(F)	NRI (mini cha-landolt)	DECIS WG	Inside of device	101.38	0.63	64 mg/ 0.064 g
50 ml falcon tube (16 holes red band)(D)	NRI (mini cha-landolt)	DECIS WG	Inside of device	101.38	0.63	64 mg/ 0.064 g
Commercial trap (B)	Commercial lure	DECIS WG	Lid	*	0.63	60 mg/ 0.060 g
No trap	None	None	None	None	None	None

Figure. 2.2.3. Prototype Falcon tube devices from left to right: A), C), D), E), F)

Results

Semi-Field Cage Trial 1:

Significantly more *D. suzukii* died in the cages which contained Decis or Spinosad coated devices and the commercial traps than in the cage which contained the uncoated device. However, Decis was a more effective killing agent than the formulation of spinosad. Within 24 hours in the commercial and prototype Falcon tube devices up to 30% of the flies were dead at the bottom of the cage (Decis treated devices minus control mortality). There was no significant difference between the Falcon tube and the commercial traps in efficacy of D. suzukii control in this test. (Fprob <0.001, sed. 6.17, lsd. 12.46, Figure.2.2.4).

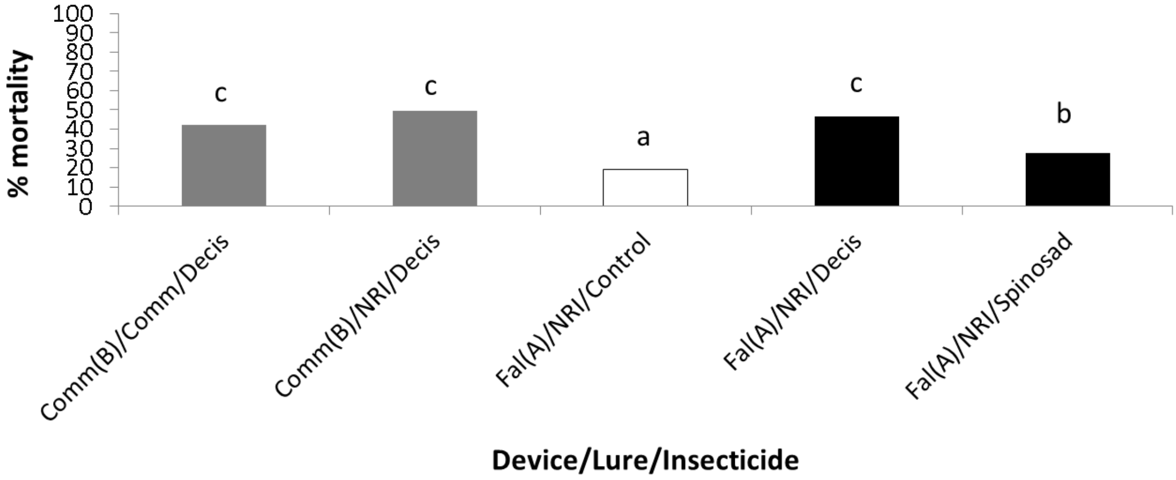


Figure. 2.2.4. Mean percentage mortality of *D. suzukii* in field cages containing prototype attract and kill devices with 4 holes on the clear part of the trap (A) coated either with Decis or Spinosad compared to two commercial traps (B) and an untreated control. Axis x label: Fal= Falcon tube with 4 holes on the clear section towards the top of the device, Letter: trap code – refer to Figure. 2.2.3, NRI = NRI lure, Comm: commercial trap and lure

Semi-Field Cage Trial 2:

Significantly more *D. suzukii* died in the cages which contained Decis coated devices with either four or eight holes and the commercial traps than in the cages which contained the uncoated devices. Within 24 hours in the commercial and new prototype Falcon tube device (C) up to 30% of the flies were dead at the bottom of the cage (commercial and prototype Falcon tube (C) devices minus control mortality). There was no significant difference between the Falcon tubes or the commercial devices (B) in efficacy of *D. suzukii* control in this test. However, significantly more *D. suzukii* were killed by the new device with eight holes on the red band (C) compared to four holes on the clear section (A) (Fprob <0.001, sed. 4.18, lsd. 8.48, Figure. 2.2.5).

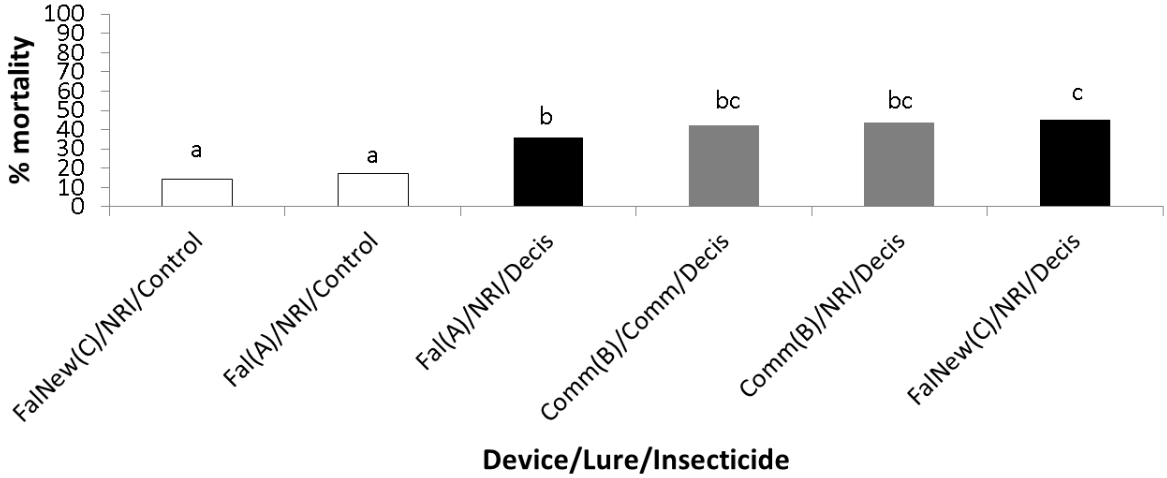


Figure. 2.2.5. Mean percentage mortality of *D. suzukii* in field cages containing prototype attract and kill devices with either four holes on the clear part of the trap (A) or eight holes on the red band of the trap (C) compared to two commercial traps (B) and two untreated controls (with four holes or eight holes). Axis x label: Fal= Falcon tube with four holes on the clear section towards the top of the device, FalNew = Falcon tube with eight holes on the red band, Letter: trap code – refer to Figure. 2.2.3, NRI = NRI lure, Comm: commercial trap and lure

Semi-Field Cage Trial 3:

Significantly more *D. suzukii* died in the cages which contained Decis coated devices with either eight or sixteen holes and the commercial trap than in the cage which contained no device. Within 24 hours in the commercial and prototype Falcon tube devices up to 35% of the *D. suzukii* had died (commercial and prototype Falcon tube devices minus control mortality). There was no significant difference between all prototype Falcon tubes or the commercial device in efficacy of *D. suzukii* control in this test. The prototype falcon tube device was confirmed to be as effective as the commercial trap in killing *D. suzukii* adults. (Fprob <0.001, sed. 7.46, lsd. 15.13, Figure. 2.2.6).

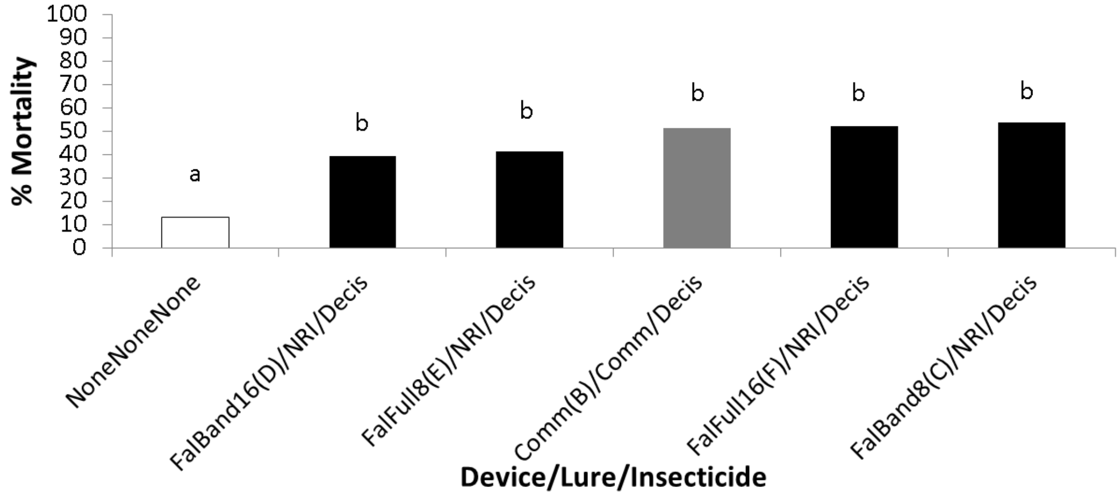


Figure. 2.2.6. Mean percentage mortality of *D. suzukii* in field cages containing prototype attract and kill devices with eight and sixteen holes compared to a commercial trap and a cage with no device inside. *Axis x label:* Fal = Falcon tube, Full= painted red from the middle to the base, Band= painted red in the middle and base clear, number= number of holes, Letter: trap code – refer to Figure. 2.2.3, NRI = NRI lure, Comm: commercial trap and lure.

Analysis of D. suzukii males and females mortality:

In the semi-field trials 1, 2 and 3 both male and female mortality of *D. suzukii* adults was analysed (Figure. 2.2.7) (Figure. 2.2.8) (Figure. 2.2.9). In all three trials, the efficacy of each device in killing males and females essentially mirrors the percentage of mortality found in the overall analysis.

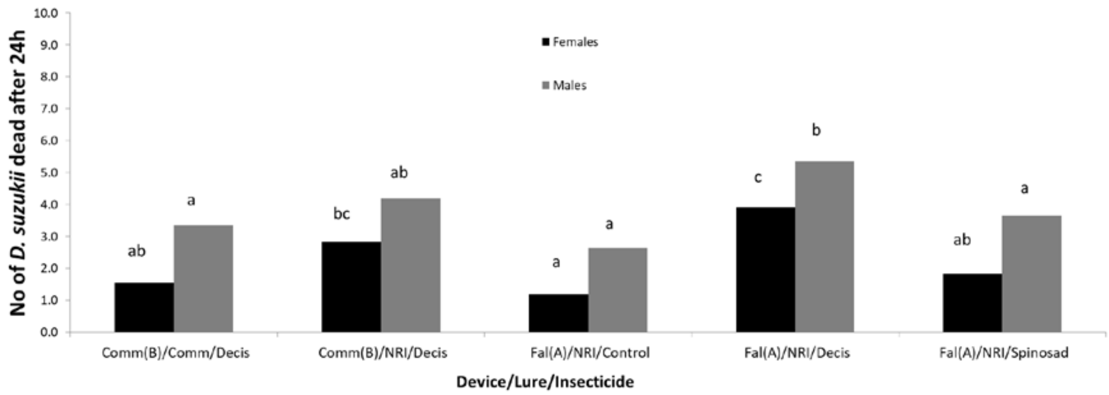


Figure. 2.2.7. Number of dead male and female *D. suzukii* in field cages (from 10 adults of each sex) containing prototype attract and kill devices with four holes on the clear part of the trap (A) coated either with Decis or Spinosad compared to two commercial traps (B) and an untreated control. (Females: Fprob <0.001, sed. 0.62, lsd. 1.26) (Males: Fprob <0.001, sed. 0.8, lsd. 1.62). Axis x label: Fal= Falcon tube with four holes on the clear

section towards the top of the device, Letter: trap code – refer to Figure. 2.2.3, NRI = NRI lure, Comm: commercial trap and lure.

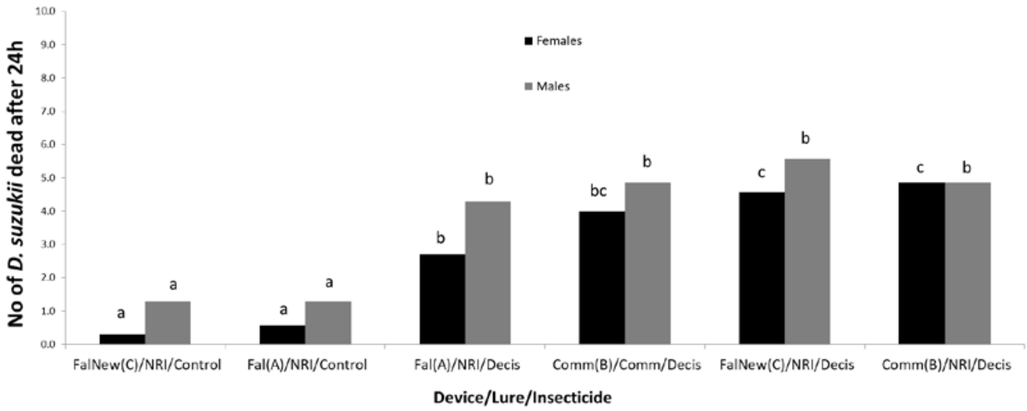


Figure. 2.2.8. Number of dead male and female *D. suzukii* in field cages (from 10 adults of each sex) containing prototype attract and kill devices with either four holes on the clear part of the trap (A) or eight holes on the red band of the trap (C) compared to two commercial traps (B) and two untreated controls (with four holes or eight holes) (Females: Fprob <0.001, sed. 0.67, lsd. 1.37) (Males: Fprob <0.001, sed. 0.93, lsd. 1.88). Axis x label: Fal= Falcon tube with four holes on the clear section towards the top of the device, FalNew = Falcon tube with eight holes on the red band, Letter: trap code – refer to Figure. 2.2.3, NRI = NRI lure, Comm: commercial trap and lure.

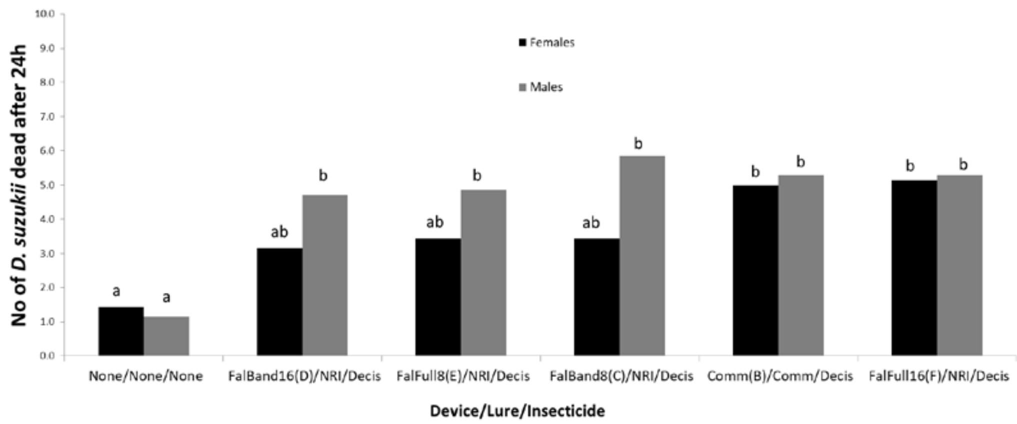


Figure. 2.2.9. Number of dead male and female *D. suzukii* in field cages (from 10 adults of each sex) containing prototype attract and kill devices with eight and sixteen holes compared to a commercial trap and a cage with no device inside (Females: Fprob <0.001, sed. 1.10, Lsd. 2.23) (Males: Fprob <0.001, sed. 1.26, Lsd. 2.56). *Axis x label:* Fal = Falcon tube, Full= painted red from the middle to the base, Band= painted red in the middle and base clear, number= number of holes, Letter: trap code – refer to Figure. 2.2.3, NRI = NRI lure, Comm: commercial trap and lure.

Conclusions

- An improved attract and kill device has been developed.
- The prototype Falcon tube devices, with Decis as killing agent, were as effective as the commercial trap in controlling *D. suzukii.*
- The devices give up to 30% kill of *D. suzukii* within 24 hours in these semi-field cage trials.
- The devices with eight holes on the red sections were more effective than devices with four holes on the clear part of the trap.
- Increasing the number of holes in the device from eight to sixteen did not increase the efficacy.

Future work

  - Could include increasing the attraction of the device by adjusting the ratio of the components in the mini-lure; increasing the acetoin.
  - Repeat experiments with fruit in the cages to see if the devices are as effective in the presence of fruit.
  - Large scale semi-field or field trials with combination of push-pull.

Task 2.3. Extend the life and further reduce the size of the dry lure (Yr. 1-2 NRI)

Introduction

Cha *et al*. (2012) found that attraction of *D. suzukii* to wine vinegar depends upon four compounds: ethanol (E), acetic acid (AA), acetoin (Ac) and methionol (M). These authors developed the Cha-Landolt bait for *D. suzukii* consisting of a solution of ethanol and acetic acid as the drowning solution and acetoin and methionol dispensed from separate polyterephthalate vials with a hole in the lid (Cha *et al*. 2013).

Purchase and use of large quantities of ethanol requires approval from HM Revenue and Customs and acetic acid is caustic. Methionol is relatively expensive and unpleasant to handle, and so preparation and maintenance of large numbers of the Cha-Landolt lures is not particularly convenient. Furthermore, studies at NRI indicated that the ethanol was lost from the solution within a few days.

For development of approaches to controlling *D. suzukii* by attract-and-kill where large numbers of devices are required, use of 300 ml of drowning solution requiring replacement each week is not practicable. A “dry” lure that lasts much longer under field conditions is required. This is even more imperative for control of *D. suzukii* by lure-and-infect approaches in which the flies are attracted to a device that transfers an entomopathogenic fungus and then releases them.

In previous work it was shown that the open vial dispensers for acetoin and methionol could be replaced by sealed polyethylene sachets without loss of attractiveness. However, lures with the ethanol and acetic acid also dispensed from polyethylene sachets were generally not as attractive as the Cha-Landolt lure.

The release rates of ethanol and acetic acid were much lower from the sachets than from the drowning solution, but previous results during 2015-2016 showed that increasing the release rate of ethanol or the acetic acid did not increase attractiveness. In fact further reducing the release rate of ethanol to 25% of the rate from the “standard” sachet did not reduce attractiveness to *D. suzukii*. However, the final trial during 2016 indicated that doubling the release rate of both acetic acid and acetoin doubled catches of *D. suzukii*.

The objectives of this Task are to optimise the attractiveness of the “dry” version of the Cha-Landolt lure further, to minimise its size and to increase its longevity in the field.

Methods

Dispensers and release rates

For the standard Cha-Landolt lure, ethanol and acetic acid were dispensed from the drowning solution (300 ml) at 7.2% and 1.6% respectively. Boric acid (1%) was added to reduce fungal growth. Acetoin (1 ml of 1:1 solution in water) and methionol (1 ml) were dispensed separately from cotton dental rolls in PET (4 ml) vials with a hole (3 mm diameter) in the lid. Biobest "Dros'Attract" solution (300 ml) was used as supplied.

Sachets used were made by heat sealing low-density polyethylene lay-flat tubing (50 mm width; thickness 205 μ, 120 μ, 60 μ or 30 μ; Transpack, Southampton, UK). Alternatively press-seal "Baggies" were used (79 mm x 54 mm x 50 μ thick; Fisher Scientific, Loughborough, UK).

Release rates were measured from dispensers maintained in a laboratory fume hood at 20-22°C by periodic weighing and/or by trapping of volatiles emitted on Porapak resin followed by quantitative GC analysis. Release rates of ethanol and acetic acid from the drowning solution were measured with the solution in the Biobest trap by quantification of the amounts remaining at intervals by GC analysis.

Field trapping tests

Four trapping experiments were carried out at NIAB EMR using modified red Biobest traps with extra holes drilled in the sides. Traps were deployed at least 10 m apart in randomised complete block designs and catches were recorded weekly. The drowning solution (ethanol/acetic acid or Dros'Attract solution) was renewed each week but the sachets were not.

Catches were sorted into male and female *D. suzukii*, other *Drosophila* species and insects >5 mm in size. Catch data were transformed to square root or log(x+1) and subjected to analysis of variance (ANOVA). Differences between mean catches were tested for significance ($P < 0.05$) by the Least Significant Difference (LSD) test.

Results

Dispensers and release rates

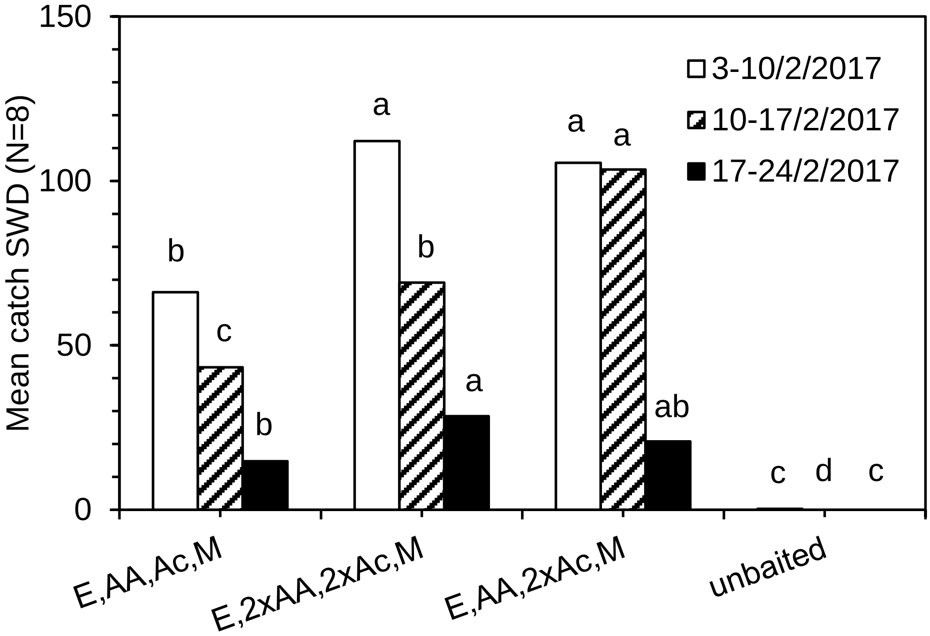
Release rates of dispensers used in these studies are summarised in Table 2.3.1. The "standard" NRI sachet lure consisted of ethanol in a Baggie sachet, acetic acid in a 25 mm x 50 mm x 120 μ thick sachet, acetoin as a 1:1 solution in water in a Baggie and methionol in a 25 mm x 50 mm x 120 μ thick sachet. The release rates of ethanol and acetic acid from this system were 1% and 10%, respectively, of that from the liquid Cha-Landolt system. The release rates of acetoin and methionol were similar to those from the liquid Cha-Landolt (Table 2.3.1).

Table 2.3.1. Dispensers used in these studies with release rates and estimated lifetimes measured at 20-22°C

Compound	Dispenser	Release rate (mg/d)	Lifetime (d)
Cha-Landolt in Biobest trap			
Ethanol	300 ml drowning solution (7.2%)	3,100	7
Acetic acid	300 ml drowning solution (1.6%)	170	28
Acetoin	1 ml 1:1 vial 3 mm hole	7.0-15.9	31
Methionol	1 ml vial 3 mm hole	0.4	2,500
NRI standard sachet lure			
Ethanol	2 ml Baggie (79 mm x 54 mm x 50 μ)	38	42
Acetic acid	1 ml sachet (50 mm x 25 mm x 120 μ)	18	55
Acetoin	1 ml 1:1 Baggie (79 mm x 54 mm x 50 μ)	7.9	63
Methionol	1 ml sachet (50 mm x 25 mm x 120 μ)	1.3	770
Acetoin (thin)	1 ml 1:1 sachet (50 mm x 50 mm x 30 μ)	9	55
MiniLure			
Ethanol	1 ml half Baggie (40 mm x 54 mm x 50 μ)	19	42
Acetic acid	1 ml sachet (25 mm x 25 mm x 120 μ)	9	111
Acetoin	1 ml 1:1 half Baggie (40 mm x 54 mm x 50 μ)	4	125
Methionol	1 ml sachet (25 mm x 25 mm x 120 μ)	0.7	1,400

Field trapping trials

Experiment 1 (3-24 February 2017). During 2016 it was shown that doubling the release rates of both acetic acid and acetoin from the "standard" NRI sachet lure doubled catches of *D. suzukii*. As previous work had shown that increasing the release rate of acetic acid only did not affect catches, the effect of doubling the release rate of acetoin was investigated.



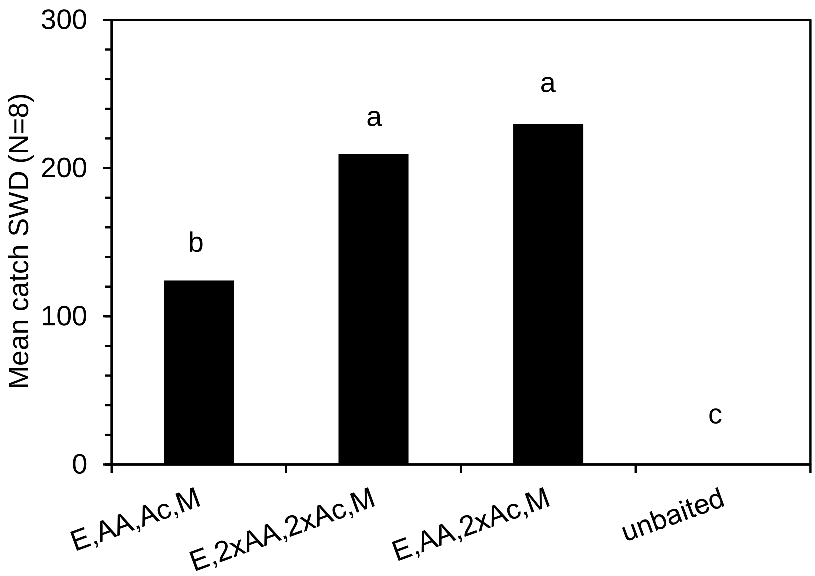


Figure. 2.3.1. Effect of doubling release rates of acetic acid (AA) and/or acetoin (Ac) with standard rates of ethanol (E), acetic acid (AA) and methionol (M) on catches of *D. suzukii* by week (upper) and overall (lower); means with different letters are significantly different ($P<0.05$)

As previously, doubling the release rate of both acetic acid and acetoin doubled catches, but a similar effect was obtained by doubling the release rate of acetoin only (Figure. 2.3.1). The proportion of *D. suzukii* in the catch of all insects was not affected by the different lures (Figure. 2.3.2).

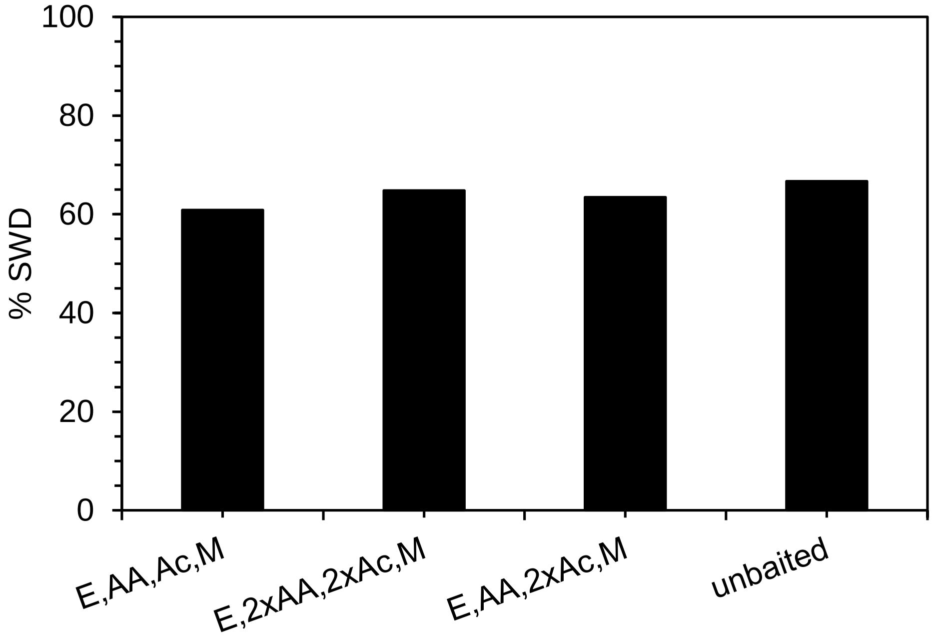


Figure. 2.3.2. Catch of *D. suzukii* as a percentage of all insects caught in first experiment (3-24 February 2017) (E ethanol; AA acetic acid; Ac acetoin; M methionol)

Experiment 2 (24 February – 9 March 2017). The effect of increasing the release rate of acetoin further was investigated in the second experiment. A further doubling of the release rate of acetoin increased catches of *D. suzukii*, although not significantly so (Figure. 2.3.3). As in the previous experiment, the percentage of *D. suzukii* in the catch was not affected by treatment (Figure. 2.3.4).

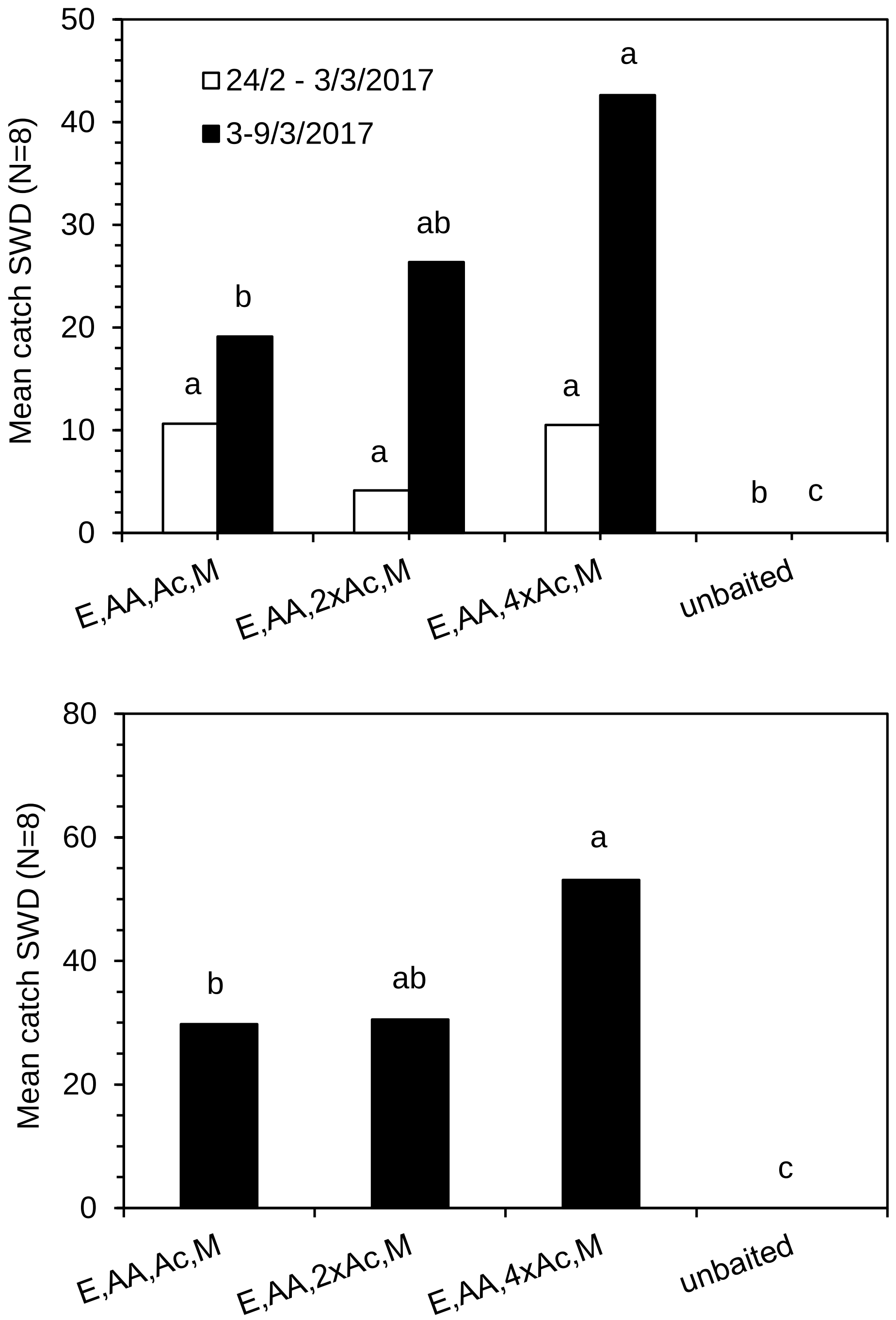


Figure. 2.3.3. Effect of increasing release rate of acetoin (Ac) in the presence of standard release rates of ethanol (E), acetic acid (AA) and methionol (M) on catches of *D. suzukii* by week (upper) and overall (lower); means with different letters are significantly different (P<0.05)

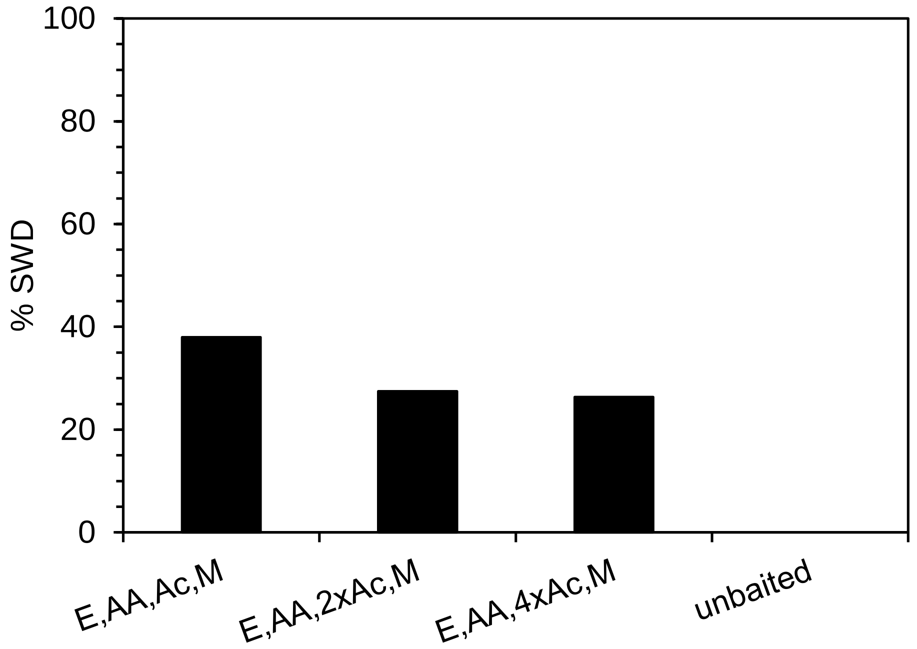
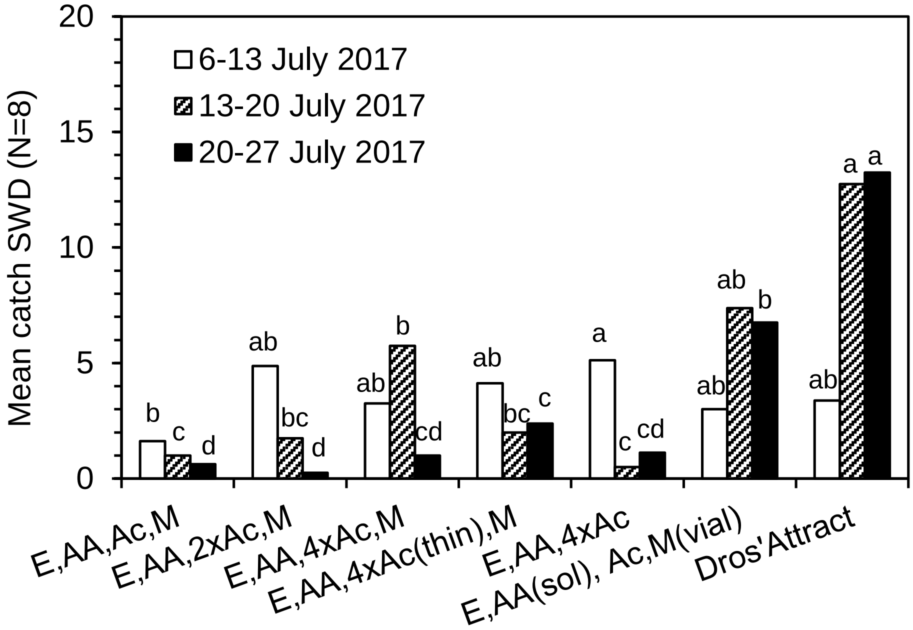


Figure. 2.3.4. Catch of *D. suzukii* as a percentage of all insects caught in second experiment (24 February – 9 March 2017) (E ethanol; AA acetic acid; Ac acetoin; M methionol)

Experiment 3 (6-27 July 2017). In the previous experiments, the release rate of acetoin was increased by increasing the number of sachets. In this experiment, the release rate of acetoin was increased further by using very thin sachets. The effect of leaving out the methionol was also investigated, and catches with the "dry" sachet lures were compared with those with the liquid Cha-Landolt lure and Biobest Dros'Attract.

Results in Figure 2.3.5 indicate that increasing the release rate of acetoin further does not significantly increase catches of *D. suzukii*. Leaving out methionol from the most attractive "dry" lure did not significantly decrease catches. In the first week, catches with the sachet lure were at least as high as those with the liquid Cha-Landolt and Dros'Attract lures, although over the three weeks they were significantly lower. It should be noted that the liquid lures were renewed each week while the sachet lure was not.

The relative proportions of *D. suzukii* in the total insect catches by the different treatments were not significantly different (Figure. 2.3.6).



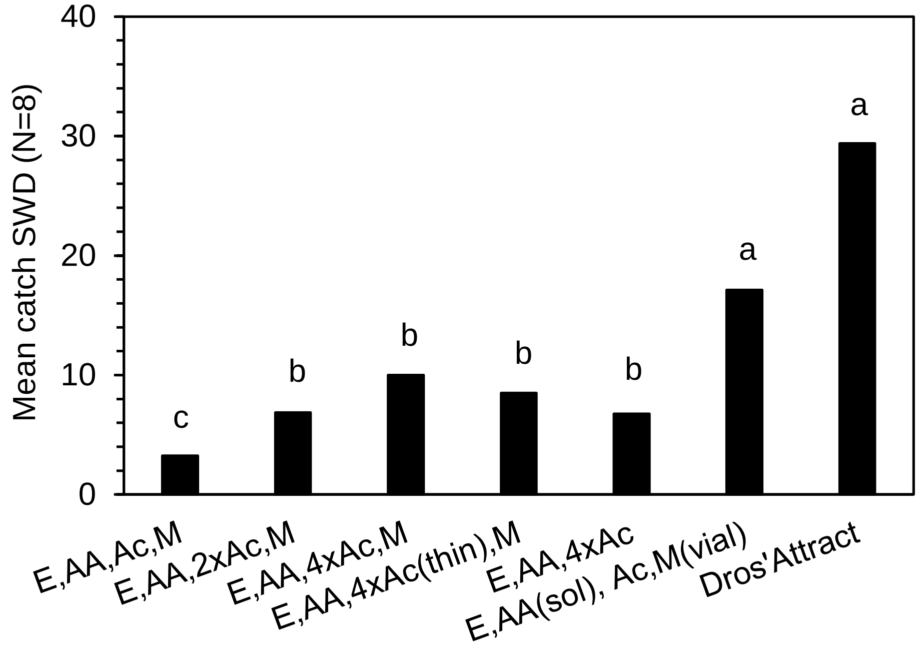


Figure. 2.3.5. Effect of increasing release rate of acetoin (Ac) in the presence of standard release rates of ethanol (E), acetic acid (AA) and methionol (M) on catches of *D. suzukii* by week (upper) and overall (lower); the effect of leaving out the methionol was also investigated, and catches with the "dry" sachet lures were compared with those with the liquid Cha-Landolt lure and Biobest Dros'Attract; means with different letters are significantly different (P<0.05)

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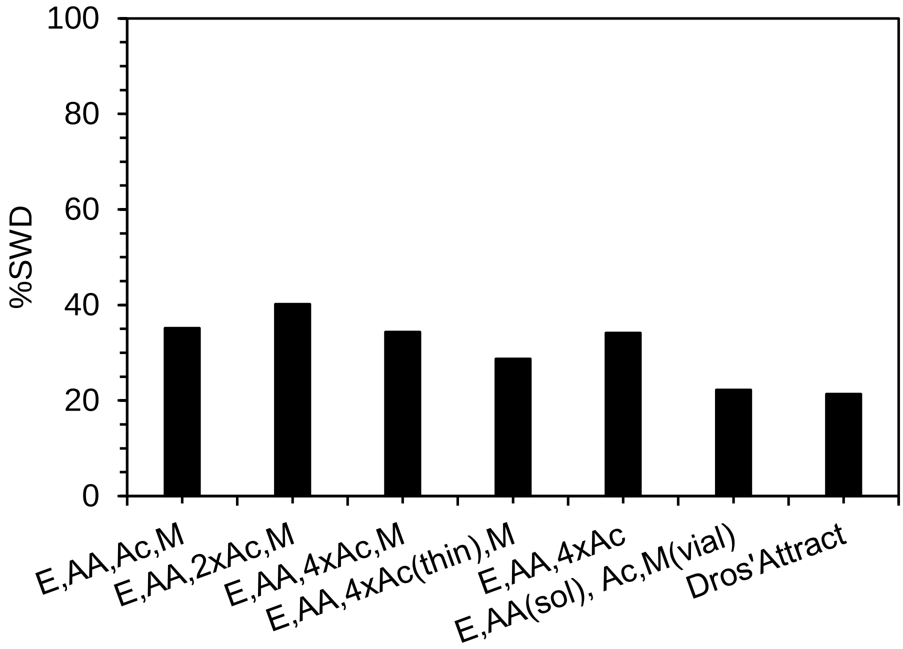


Figure. 2.3.6. Catch of *D. suzukii* as a percentage of all insects caught in third experiment (3-27 July 2017) (E ethanol; AA acetic acid; Ac acetoin; M methionol)

Experiment 4 (5 October – 2 November 2017). As catches were not particularly high in Experiment 3, this experiment was repeated when numbers of *D. suzukii* had increased in October 2017. During the first week, results confirmed that increasing the release rate of acetoin increased catches of *D. suzukii* up to around four times the standard release rate. However, in this experiment removing the methionol significantly decreased catches. Catches with the sachet lures were similar to catches with Dros'Attract, but lower than those with the Cha-Landolt mixture (Figure. 2.3.7).

Results in the second and third weeks were similar, but in the fourth week catches in traps baited with the dry lures were higher in relation to catches in those baited with the Cha-Landolt lure (Figure. 2.3.7). Doubling the release rate of acetoin from that in the standard lure significantly increased catches, but increasing it further did not increase catches further. Omitting methionol did not affect catches significantly. Catches with the dry lures were similar to those with the Dros'Attract, and at least half the catches with the Cha-Landolt.

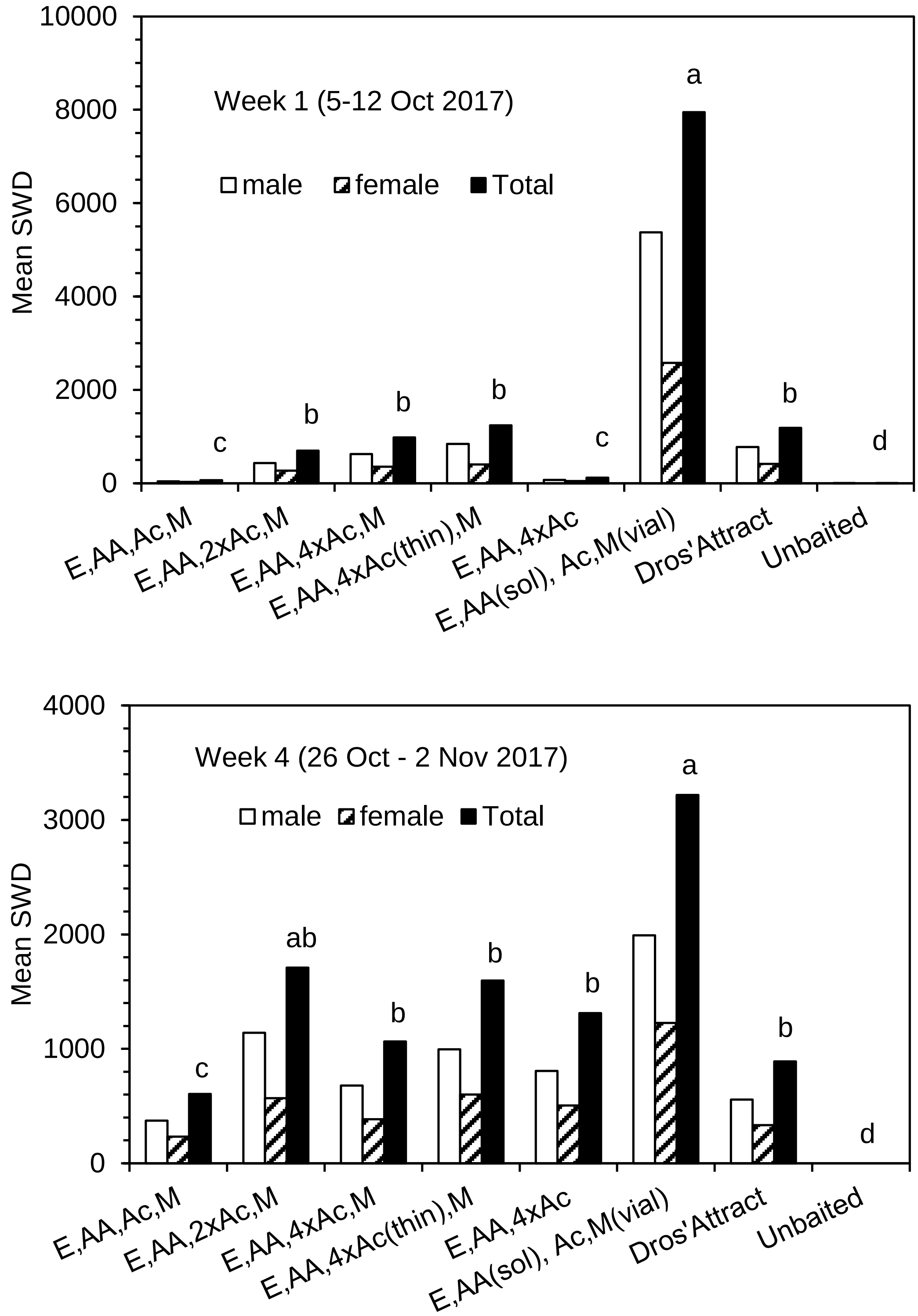


Figure. 2.3.7. Effect of increasing release rate of acetoin (Ac) in the presence of standard release rates of ethanol (E), acetic acid (AA) and methionol (M) on catches of *D. suzukii* in Weeks 1 (upper) and 4 (lower) of Experiment 4; the effect of leaving out the methionol was also investigated, and catches with the “dry” sachet lures were compared with those with the liquid Cha-Landolt lure and Biobest Dros’Attract; means with different letters are significantly different (*P*<0.05)

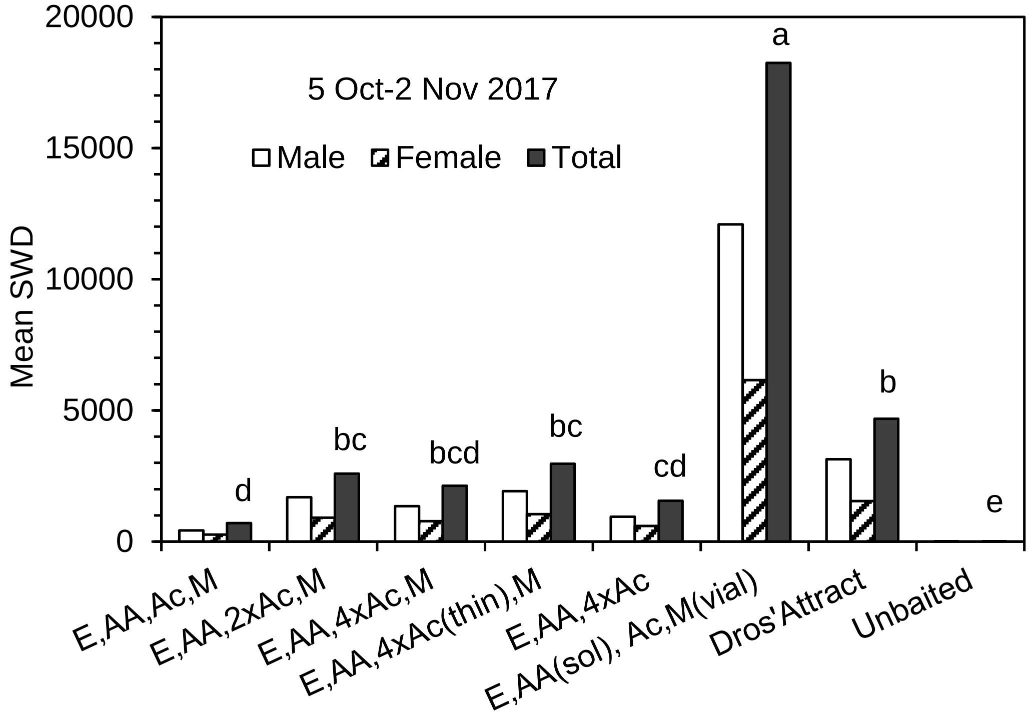


Figure. 2.3.8. Effect of increasing release rate of acetoin (Ac) in the presence of standard release rates of ethanol (E), acetic acid (AA) and methionol (M) on catches of *D. suzukii* in Experiment 4 (5 October – 2 November 2017); the effect of leaving out the methionol was also investigated, and catches with the “dry” sachet lures were compared with those with the liquid Cha-Landolt lure and Biobest Dros’Attract; means with different letters are significantly different (P<0.05).

Over the four weeks of the experiment, results were similar to those above. Catches in traps baited with the dry lures were not significantly different from those in traps baited with the Dros’Attract, although lower than those in traps baited with the Cha Landolt lure. It should be noted that the liquid Dros’Attract and Cha-Landolt lures were renewed each week whereas the dry lures were not changed during the four-weeks of the experiment. The dry lures were clearly still attractive during the fourth week of the experiment.

The proportion of *D. suzukii* in the total catch was not affected by treatment (Figure. 2.3.9), and catches of drosophila were almost exclusively *D. suzukii* at this time.

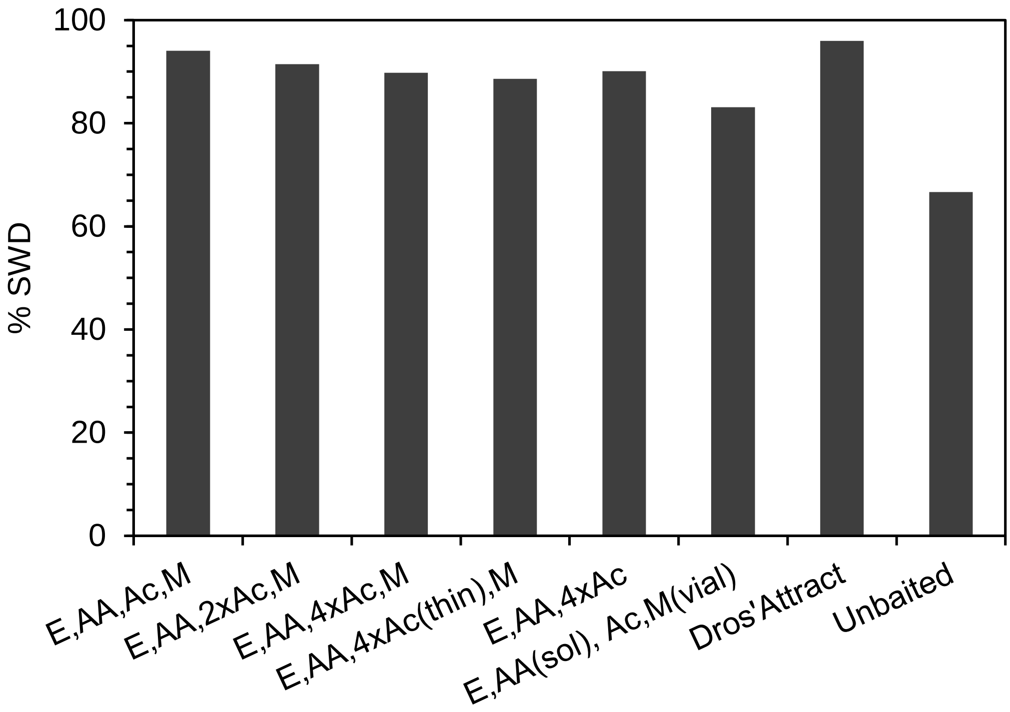


Figure. 2.3.9. Catch of *D. suzukii* as a percentage of all insects caught in fourth experiment (5 October – 2 November 2017) (E ethanol; AA acetic acid; Ac acetoin; M methionol)

Development of MiniLure. The standard NRI lure consisting of four sachets did not fit in the Falcon tubes being developed as "attract-and-kill" devices for *D. suzukii*. Accordingly a smaller version was developed by halving the sizes of all four sachets (Table 2.3.1) and this was used in effectively in the tests described under Task 2.2. It was estimated that these lures should last at least six weeks from laboratory release rate studies. However, it should be noted the latter measurements were made from completely exposed lures and that release in the confines of the Falcon tube device would probably be much slower giving a longer effective life.

According to results obtained in field trapping trials during 2015-2017, the resulting reduction in release rates of ethanol, acetic acid and methionol should not have affected catches of *D. suzukii* significantly. However, the results above indicated that the optimum release rate of acetoin was four times the standard, i.e. approximately 32 mg/d, such that the release of 4 mg/d from the MiniLure needs to be increased for maximum catches.

Conclusions

- Release of the four components of the Cha-Landolt blend - ethanol, acetic acid, acetoin and methionol – from polyethylene sachets provides a practical "dry" alternative to the conventional liquid bait, as required for development of devices for control of *D. suzukii* by attract-and-kill and, particularly, lure-and-infect approaches.
- The standard sachet lure developed originally released ethanol and acetic acid at 1% and 10%, respectively, of the rates from the liquid Cha-Landolt lure and requires changing every six weeks rather than weekly.
- The attractiveness of the standard sachet lure is not affected by increasing the release rates of ethanol or acetic acid, or by reducing the release rate of ethanol to one quarter.
- The attractiveness of the standard sachet lure can be increased by increasing the release rate of acetoin by four times to approximately 32 mg/d. Further increase in release rate does not increase catches significantly.
- In most experiments removing the methionol did not affect catches of *D. suzukii*, but in others catches were reduced. The requirement for methionol needs further investigation.
- In some experiments catches with the optimised sachet lure were at least as great as those with the liquid Cha-Landolt and Dros'Attract lures, but in others they were significantly lower. This requires further investigation.
- A MiniLure has been developed for use in the Falcon tube attract-and-kill devices and shown to be effective under laboratory conditions (Task 2.2). This should have a lifetime of at least 6 weeks and probably longer in the confines of the Falcon tube. Although release rates of ethanol, acetic acid and methionol are probably adequate, there is scope to increase attractiveness by increasing the release rate of acetoin from the MiniLure nearer to the optimum level. Longevity can be increased by increasing the loading of the compounds once the release rates have been optimised.
- Recent results presented by Cha *et al*. at a meeting in 2015 and published by Cha *et al*. (2017) claimed that simultaneous increase in release rates of acetic acid and acetoin are necessary to increase catches of *D. suzukii*. These studies still used ethanol and acetic acid in the drowning solution with acetoin and methionol in vials or sachets, but their optimum value for release rate of acetoin was 34 mg/d, remarkably close to the 32 mg/d found here.

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

D. suzukii phago-stimulatory baits could improve the efficacy of insecticides. Cowles *et al*. (2015) used sucrose to improve efficacy of spinosyn, spinetoram and acetamiprid in the field against *D. suzukii*. However, recent results from Michigan State University (P. Fanning) and by NIAB EMR in the previous project did not show a clear benefit of adding sucrose to insecticides. Andreazza *et al*. (2016) found that Suzukii Trap improved the insecticidal activity of treatments applied to fruits in the laboratory. Van Steenwyk *et al*. (2016) used 50% Suzukii Trap to improve *D. suzukii* control with spinosad in the field. A mixture of 40% Monterey insect bait, 30% apple cider vinegar and 30% wine was also effective but the acid vinegar caused foliage damage. Dederichs (2015) used 5% Combi-protec to improve *D. suzukii* control with spinosad and acetamiprid. Suzukii Trap, Combi-protec or sugar solution were not very attractive to *D. suzukii* in laboratory tests in AHDB project SF145. Costing at least £5/L, commercial attractants would only be viable in low volume spray applications.

Baker's or brewers' yeast (*Saccharomyces cerevisiae*) and a yeast species found in the gut of *D. suzukii*, *Hanseniaspora uvarum* are known to be attractive to Drosophila species (Palanca *et al*. 2013). Knight *et al*. (2016) used a mixture of sugar and *S. cerevisiae* to improve control of *D. suzukii* with spinosad in the field. However, in their laboratory tests, the addition of *S. cerevisiae* to sugar did not significantly reduce egg densities in fruit compared with sugar alone. P. Fanning did not show a clear benefit of adding sugar and yeast to insecticides in laboratory tests or in the field. Mori *et al*. (2017) found that application of both *H. uvarum* and spinosad to leaves increased feeding and mortality and reduced oviposition of *D. suzukii* compared with using only spinosad. Tests in SF145 showed that the addition of yeast to a sugar solution increased its attractiveness to *D. suzukii* but there was no significant difference between *S. cerevisiae* and *H. uvarum* at the same cell concentration. *H. uvarum* as an attractant for *D. suzukii* is to be investigated in an AHDB studentship.

Tests in SF145 showed that solutions containing molasses or fermented strawberry waste liquor were at least as attractive to *D. suzukii* as a range of commercial drosophila or *D. suzukii* attractants. Fermented strawberry (or other fruit) liquor is widely available on farms from sealed disposal bins of fruit waste, enabling high volume application (1000 L/ha). It contains natural yeasts and may support introduced cultures of S. cerevisiae or other yeasts.

The use of baits is expected to improve *D. suzukii* control efficacy of insecticides with the potential to reduce application rates and improved efficacy of a wider range of insecticide types, leading to reduced risk of pesticide residues and resistance. The recycling of on-farm waste to a beneficial use will cost less than commercial drosophila bait products, thereby allowing applications of 1000L/ha.

Task 3.1. Develop D. suzukii bait with all-round attractiveness in different laboratory bioassays (Yrs. 1-2; NIAB EMR)

Introduction

Previous work in SF 145 has shown that the relative attractiveness to *D. suzukii* between test substances depended on the bioassay used. These bioassays were: Petri dish [short term and short distance to test substance], large arena [medium term and distance] and chronophysiology [long term and medium distance] bioassays. The Petri dishes were too confined and here the method was replaced by a larger volume vessel, similar to that used by Mori *et al* (2017). However, the system used by Mori *et al*. (2017) which involved using cherry leaves and fruit, would have been difficult to use year-round, particularly using materials not sprayed with insecticides.

The aims of this task were (1) to compare the relative attractiveness of test substances in different bioassays to identify an all-round attractive bait (2) to modify the bioassay used by Mori *et al*. (2017) so that it can be used year-round using unsprayed materials.

Methods

1. Large arena test. A laboratory wind tunnel with the air flow turned off was used for a choice test for the *D. suzukii* attractiveness of samples of test substances in open plastic containers. Starved *D. suzukii* males and mated females (between 100 and 200 per replicate run) were introduced into the chamber which had a controlled humidity. The numbers of *D. suzukii* attracted to and drowning in the containers of test substances were recorded after 24 hours. To reduce the effect of colour differences between substances, opaque containers were used and red dye was added to pale or colourless substances. Water and sugar solutions with red dye were used as controls. The test substances and mixtures used in the tests are shown in Table 3.1.1. Eight further mixtures containing two or three of the following substances were also prepared: molasses, sugar, fermented strawberry juice, *H. uvarum* suspension or baker's yeast suspension. The positioning of the test substance treatments was re-randomised between four replicate runs of experiments. *H. uvarum* was not tested with 100% Gasser (which contains acetic acid/vinegar) as spraying these in a tank mix is not commercially viable.

2. Chronophysiology. A 32 channel Trikinetics Drosophila Activity Monitoring (DAM) rig placed inside a BugDorm cage in which *D. suzukii* were allowed to fly in and out of small tubes containing 0.2 ml of test substances was used to compare their relative attractiveness. An infra-red beam at the entrance of each tube enabled the number of entries and exits by the flies to be recorded electronically over three days. The test substances and mixtures used in the tests are shown in Table 3.1.1. Two replicate runs of the experiment were conducted, with the positioning of the tubes re-randomized between replicate runs. The large arena tests showed

that a combination of *H. uvarum* and sugar and fermented strawberry juice and sugar was not significantly more attractive to *D. suzukii* than the individual treatments. Therefore the treatments were tested individually.

Table 3.1.1. Test substances used on *D. suzukii* in the wind tunnel and chronophysiology bioassays and target populations of yeasts. *Manufacturers recommended rate

Substance	Large arena	Chronophysiology
Water	100%	-
Combi-Protec	5%*	5%*
Fermented strawberry juice	100%	100%
Strawberry juice	100%	100%
Sugar	160 g/L	8 g/L 160 g/l
Molasses	5%	-
Gasser	100%	100%
Baker's yeast suspension + sugar	10^8 cells/ml +160 g/L	10^8 cells/ml +160 g/L
H. uvarum suspension + sugar	10^8 cells/ml + 160 g/L	10^8 cells/ml + 160 g/L

3. Jar microcosm bioassay for *D. suzukii* mortality and egg laying. Four bait treatments (and no bait control) from the preliminary screening were tested for *D. suzukii* attraction and mortality using a mortality bioassay based on the method of Mori *et al*. (2017). The baits were: (a) Combi-Protec (5%) (b) *H. uvarum* suspension + 160 g/L sugar (c) Fermented strawberry juice + 160 g/L sugar (d) Gasser 100%. The *D. suzukii* attractiveness of six 10 µL droplets applied to two bramble leaves was tested in closed 500 mL plastic containers. The containers had a 10 mm hole in the lid, covered with a fine mesh, to allow ventilation but prevent escape of *D. suzukii*. The droplets contained bait and spinosad (0% or 3.3% permitted application rate). This produced the following treatments: five bait treatments (including no bait control) x (+/- spinosad) x six replicates = 60 containers. The attractiveness of the bait droplets to *D. suzukii* was tested against six 10 µL sugar solution droplets as an alternative food source, applied to a third bramble leaf in the jars. A 50 mm Petri dish containing a grape juice agar (+Nipogen) was placed in each jar as an egg laying medium. Seven mated females and five males were introduced in each container and mortality was recorded after one and three days. More females than males were used to optimise egg laying and ensure all females had been mated with; additional males

were deemed unnecessary and may have interfered with egg laying females. The number of eggs laid in the Petri dishes was recorded after three days.

The cell populations of *H. uvarum* and *S. cerevisiae* yeasts in water, the sugar solution and fermented fruit waste liquor were determined by plating on potato dextrose agar (PDA) and measurements of cell numbers taken with a haemocytometer.

Results

1. Large arena tests. Differences in numbers of *D. suzukii* trapped in containers with various attractants were significant at P <0.001. Significantly (P = 0.008) more females were trapped (average 8.8 flies per container) than males (average (6.3 flies per container). However, there was no significant interaction between *D. suzukii* sex and attraction to different baits. The Gasser liquid attracted significantly more *D. suzukii* than any of the other substances or mixtures in the large arena tests (Figure. 3.1.1). Water, with red dye, attracted the fewest *D. suzukii*. Molasses (5%) was less attractive than sugar (160g/L), either in water or fermented strawberry juice. Fermented strawberry juice with sugar or *H. uvarum* was more attractive than fermented strawberry juice alone or with molasses. However, the inclusion of both *H. uvarum* and sugar in fermented strawberry juice did not significantly increase the attractiveness compared with using only using two of these three substances. Suspensions of *H. uvarum* in sugar solution or fermented strawberry juice (alone or with sugar or molasses) were more attractive than the respective mixtures with bakers' yeast suspension. The attractiveness of Combi-Protec (5%) and mixtures of fermented strawberry juice, sugar and *H. uvarum* suspension and were not significantly different.

2. Chronophysiology. Differences in numbers of *D. suzukii* visits between attractants were significant at P = 0.036. Tubes containing a suspension of *H. uvarum* and sugar or strawberry juice attracted significantly more visits than any of the other test substances (Figure. 3.1.2). Gasser liquid attracted significantly more *D. suzukii* visits than sugar solution (160g/L). There were no significant differences in attractiveness between Gasser (100%), Combi-Protec (5%), fermented strawberry juice or baker's yeast + sugar.

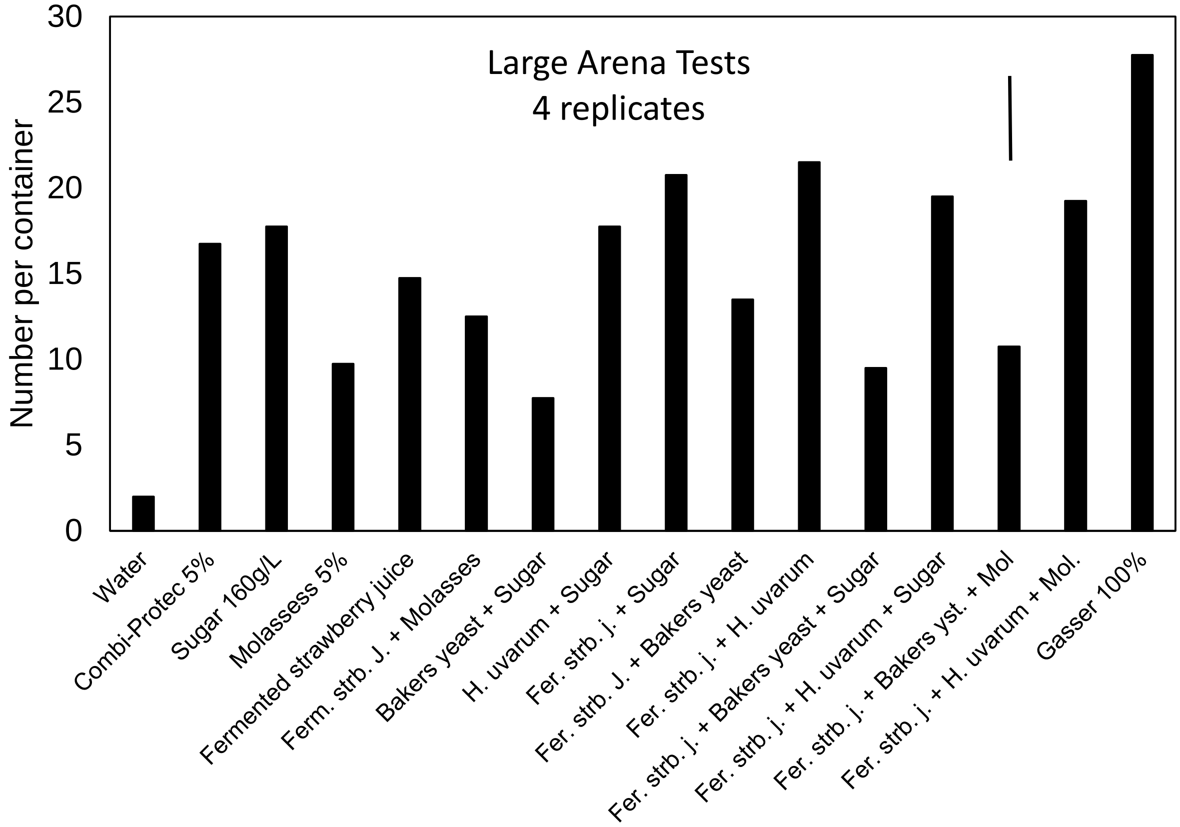


Figure. 3.1.1. Numbers of *D. suzukii* trapped in containers with different test substances after 24 hours. Vertical bar represents LSD (P = 0.05)

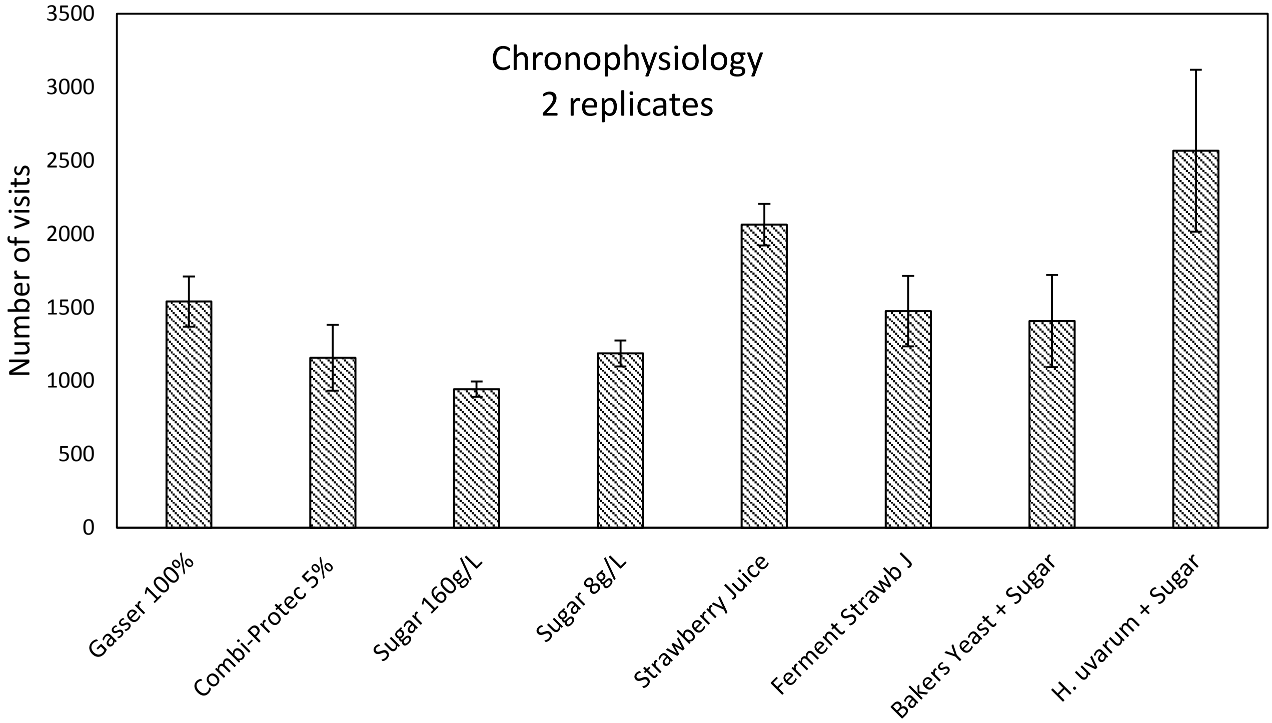


Figure. 3.1.2. Numbers of *D. suzukii* visits to tubes with different test substances after 3 days. Values are means of eight replicate tubes and two replicate experiments (± SE)

3. Jar microcosm bioassay for *D. suzukii* mortality and egg laying. When spinosad (Tracer) was used at 10% of the rate recommended for strawberries (Table 3.1.1), there was no survival of *D. suzukii* or egg laying after three days in any of the jars, irrespective of whether it was combined with a bait attractant (data not shown). The effects of Tracer at 3.3% rate and attractants on *D. suzukii* mortality were significant at $P < 0.001$. There was also a significant interaction ($P < 0.001$) between the effects of Tracer and attractant on *D. suzukii* mortality. None of the attractants baits, when used without Tracer, affected the mortality of *D. suzukii* compared with the water control (Figure. 3.1.3). When used at 3.3% of the rate recommended for strawberries, Tracer was ineffective in reducing the number of live *D. suzukii* compared with the water control. However, Tracer at this rate was effective in killing *D. suzukii* when used in combination with the attractants. When used in combination with Tracer, a suspension of *H. uvarum* in sugar solution was more effective in killing D. SUZUKII than fermented strawberry juice and sugar or Gasser.

Results from the jar microcosm bioassay were more aligned with the chronophysiology results (*H. uvarum* + sugar more effective than Gasser) than the large arena results (Gasser more effective *H. uvarum* + sugar).

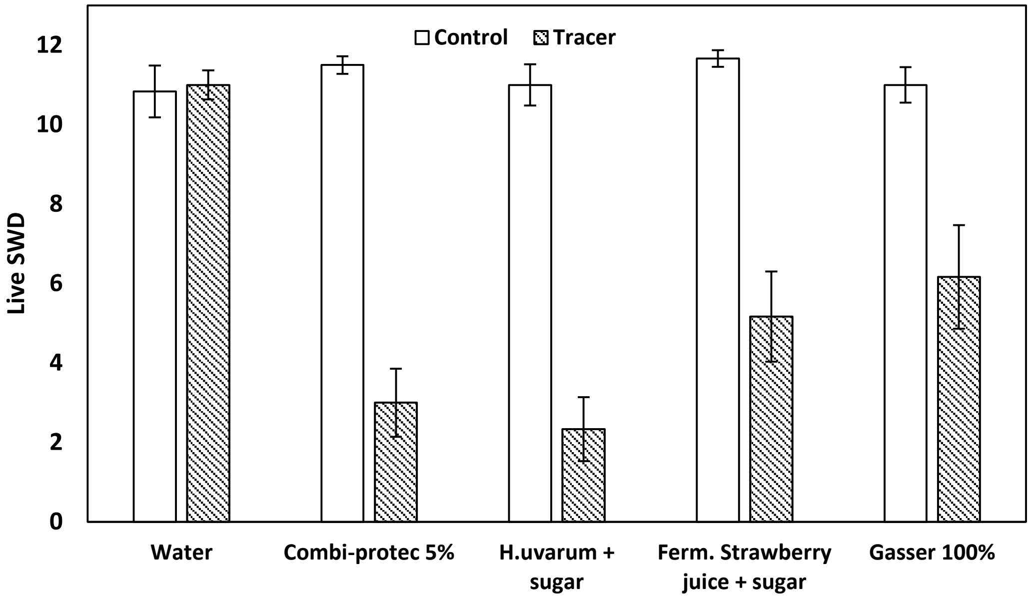


Figure. 3.1.3. Number of live *D. suzukii* (out of 12) after three days in jar microcosms; Tracer used at 3.3% of field rate for strawberries. Each value is the mean of six replicate tests (± SE)

When used at 3.3% of the rate recommended for strawberries, Tracer was effective in reducing the number of eggs laid in Petri dishes containing egg laying media (Figure. 3.1.4). Due to the large variability in egg laying between replicates, differences between attractant bait treatments were not statistically significant at P = 0.05. However, when used with Tracer, fermented strawberry juice, with sugar at 160 g/L, or Gasser liquid supressed egg laying to a very low level and there some evidence that these treatments were more effective than Combi-Protec in reducing egg laying. A suspension of *H. uvarum* in sugar solution was also effective in reducing egg laying to a low level.

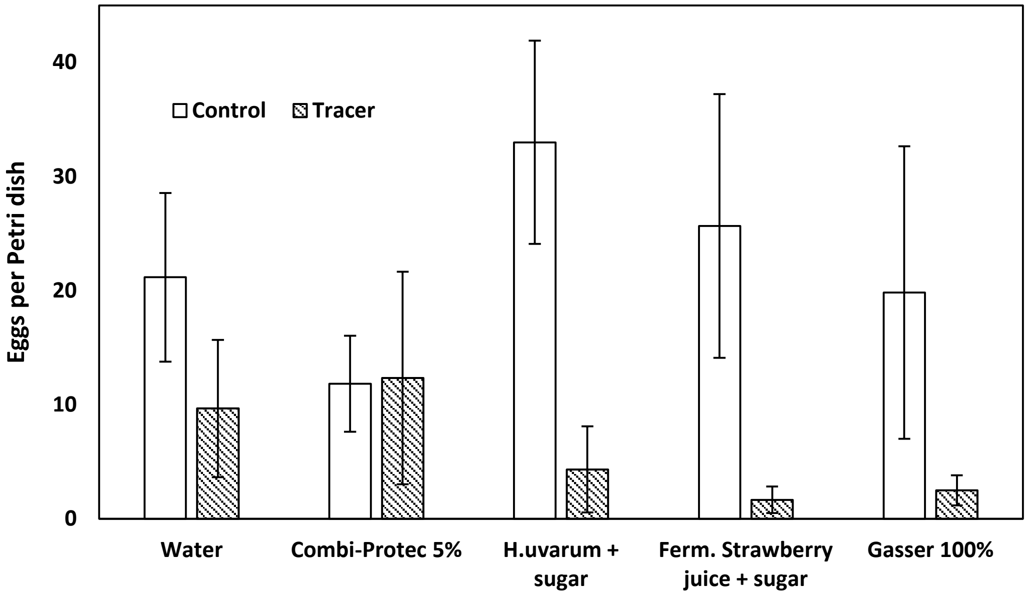


Figure. 3.1.4. Number of eggs laid in Petri dishes containing grape juice agar after three days in jar microcosms; Tracer used at 3.3% of field rate for strawberries. Each value is the mean of six replicate tests (± SE)

Yeast populations. The cell populations of baker's yeast (*S. cerevisiae*) were higher than those of *H. uvarum* in the same test substances used in the above tests (Table 3.1.2). The effects of sugar and fermented strawberry juice on the cell counts were not significant. This indicates that there was sufficient nutrition for the yeast in the initial water suspensions of nutrient cultures.

Table 3.1.2. Cell populations of yeasts in different test substances, cells/ml, mean (±SE)

Test substance	***S. cerevisiae***	***H. uvarum***
Water	0.95 (± 0.11) x 10^9	0.76 (±0.29) x 10^9
Sugar solution (160 g/L)	1.37 (± 0.12) x 10^9	1.12 (±0.41) x 10^9
Fermented strawberry juice	1.38 (± 0.30) x 10^9	0.77 (±0.41) x 10^9
Fermented strawberry juice + sugar (160 g/L)	1.47 (± 0.46) x 10^9	0.86 (±0.49) x 10^9

Conclusions

- Gasser liquid was the most attractive substance to *D. suzukii* in the large arena test whereas strawberry juice or a suspension of *H. uvarum* with sugar were the most attractive substances in the chronophysiology tests
- Results from the jar microcosm bioassay were more aligned with the chronophysiology results than the large arena results. Chronophysiology is therefore a more useful screening method of attractant baits than the large arena test
- Attractant baits significantly enhanced the efficacy of Tracer when used at 3.3% of the rate recommended for protected strawberries ($P<0.001$)
- When used in combination with Tracer at the above rate, a suspension of H. uvarum in sugar solution was equivalent to 5% Combi-protec and more effective in killing D. suzukii than fermented strawberry juice and sugar or Gasser
- When used with Tracer at the above rate, fermented strawberry juice and sugar or Gasser liquid significantly reduced egg laying compared with a Tracer + water control. The effects of a suspension of *H. uvarum* in sugar solution or Combi-protec on egg laying were not significant
- The cell counts of yeasts (*S. cerevisiae* and *H. uvarum*) washed from culture plates into water were not significantly different to those washed into sugar solution (160g/L) or fermented strawberry juice. The data is from Table 3.1.2

Task 3.2. Assess the effect of the optimum bait on the **D. suzukii** *control efficacy of different insecticides and concentrations in laboratory bioassays (Yrs. 1-2; NIAB EMR)*

Introduction

The results of 3.1 showed that a suspension of *H. uvarum* or fermented strawberry juice (both with sugar added at 160 g/L) were at least as effective as Combi-Protec or Gasser in improving the efficacy of Tracer. Fermented strawberry juice is a by-product which could be sprayed at high volume (e.g. 1000 L/ha), unlike the commercial products, which would only be economically viable at low application volumes (e.g. 50 L/ha). Two other insecticides have an EAMU for use on protected strawberries, and Exirel is approved for use in Canada and the USA (Table 3.2.1).

The aim of this task was to compare the efficacy of three other insecticides with Tracer when applied with and without attractant baits.

Table 3.2.1. Insecticide rates for protected strawberries. * Pending approval, ** Assuming application at 1000 L/ha

Product	Active ingredient	g/l	Rate	Source	Rate in Experiment (% of field rate**)
Tracer	spinosad	480	150ml/ha	Label	0.005 mL/L (3.3%)
Calypso	thiacloprid	480	250 ml/ha	EAMU 20142132	0.125 mL/L (50%)
Hallmark	lambda-cyhalothrin	100	75 ml/ha	EAMU 20111705	0.0375 mL/L (50%)
Exirel	cyantraniliprole	100	1500ml/ha*	Label (Canada)	0.375 mL/L (25%)

Methods

The same jar microcosm bioassay system described in Section 3.1 was used with the insecticides and concentrations shown in Table 3.2.1. Insecticides were tested with and without the following attractants described in 3.1: (a) *H. uvarum* suspension + sugar (b) fermented strawberry juice + sugar. Water droplets, without insecticide or attractant, were used as controls. There were three replicate jars of each treatment.

Results

The effects of insecticides and attractants on *D. suzukii* mortality were significant at *P* <0.001. There was also a significant interaction (*P* <0.001) between the effects of insecticide and attractant on *D. suzukii* mortality. Tracer (3.3% rate), Calypso (50% rate) and Hallmark (50% rate) were all ineffective in reducing the number of live *D. suzukii* after three days compared with the water control (Figure. 3.2.1). Exirel (25% rate) significantly reduced *D. suzukii* survival. The addition of *H. uvarum* suspension in sugar solution or fermented strawberry juice + sugar improved the efficacy of Tracer (3.3% rate), Hallmark (50% rate) and Exirel (25% rate) but not of Calypso (50% rate).

Due to the variability in egg laying, the effects of the insecticides (*P* = 0.07) and insecticide x attractant interaction (*P* =0.09) were not quite significant (Figure. 3.2.2). However, no eggs were laid in insecticide treatments combined with the *H. uvarum* + sugar suspension. No eggs were laid in the Tracer (3.3% rate) or Exirel (25% rate) treatments combined with fermented strawberry juice + sugar. There was some evidence, also in Figure. 3.1.4, that egg laying was increased where *H. uvarum* suspension + sugar was used without insecticide.

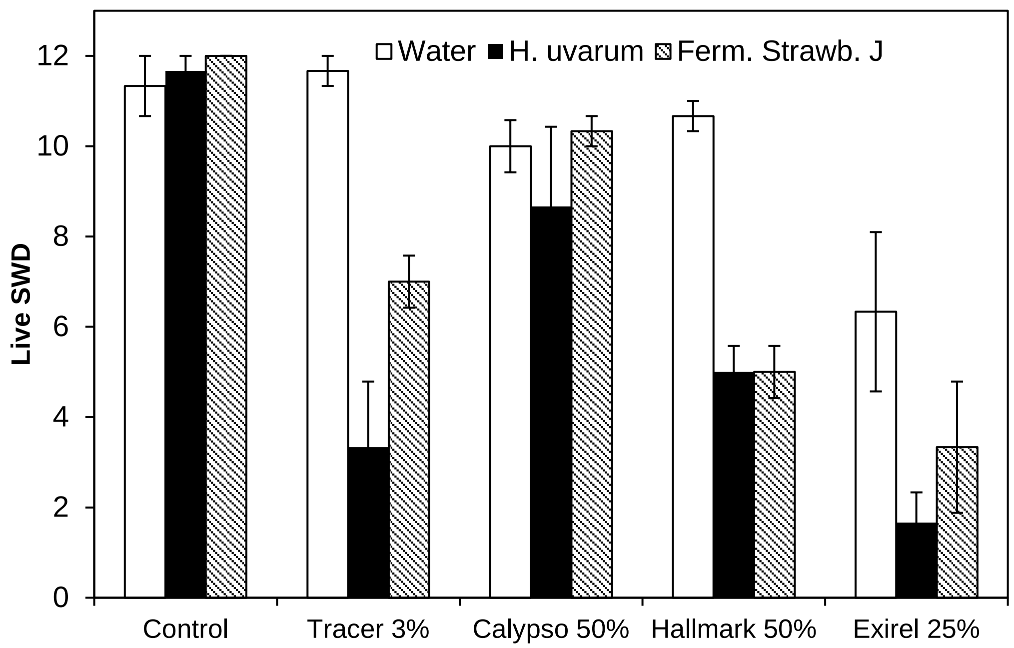


Figure. 3.2.1. Number of live *D. suzukii* (out of 12) after three days in jar microcosms, with or without attract baits and insecticides applied to blackberry leaves. Each value is the mean of three replicate tests (± SE)

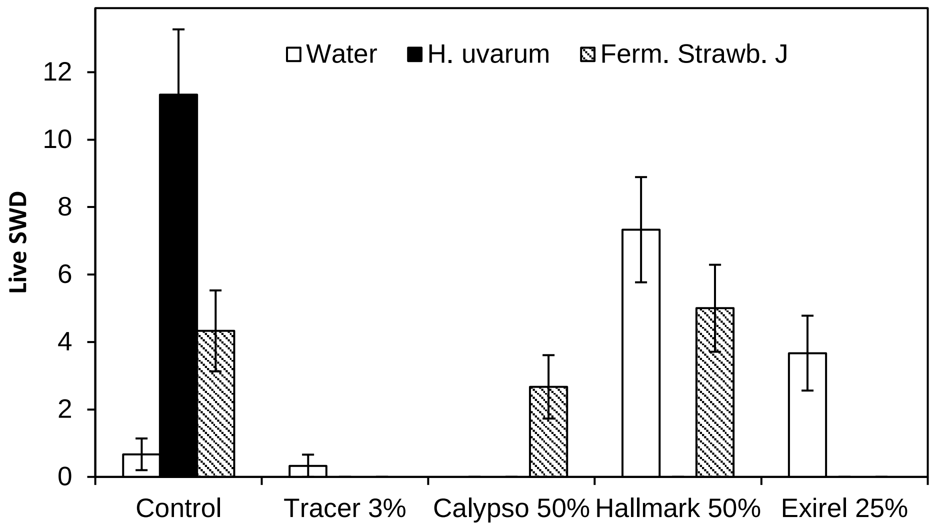


Figure. 3.2.2. Number of *D. suzukii* eggs laid in Petri dishes containing grape juice agar after three days in jar microcosms. Each value is the mean of three replicate tests (± SE)

Conclusions

- A suspension of *H. uvarum* in sugar solution or fermented strawberry juice + sugar improved the efficacy of Tracer, Hallmark and Exirel in terms of *D. suzukii* mortality
- Calypso was not effective at 50% of the field rate for protected strawberries, either with or without attractant baits
- No eggs were laid when a combination of insecticide and *H. uvarum* + sugar were used. However, there was some evidence that a suspension of *H. uvarum* + sugar without insecticide increased egg laying

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

Introduction

Currently the main method of *D. suzukii* control, with the exception of crop hygiene, is routine applications of insecticides to kill the adult flies or eggs as they are laid. Because the risk of damage is high there is currently a reluctance to leave the fruit unprotected for longer than a week. However, spray trials in SF 145 showed that in cherry, at least, some products are effective for longer. In addition it was observed in laboratory tests in the same project that adult *D. suzukii* can feed on the extra-floral nectaries of cherry leaves and this may explain why they enter the orchards early, before the fruits are developing. Other research has shown that *D. suzukii* adults also feed on cherry flower nectaries (Tochen and Walton 2016). Potentially an early spray post petalfall would reduce adult populations in the crop followed by protection of early developing fruits with alternative products. Preliminary data from SF 145 and other researchers (Dorsaz and Baroffio 2016) has also demonstrated that the Ds-mix, a spray programme which combines DS lime, Cuprum and ManZincum, and other novel 'alternative' products deter egg laying in fruits by *D. suzukii*. Rigorous testing of spray intervals of different products on the main crops under protection in combination with 'softer' products (e.g. Ds-mix, approved as a fertiliser) are needed to extend the spray interval or delay the onset of conventional applications. This will help to reduce the frequency and numbers of applications made and hence residues. A laboratory test to determine the egg laying repellence of alternative products is planned for the next SCEPTRE PLUS call. The research in this objective will field test extending the spray intervals in vulnerable ripening crops and investigate the longevity of nectar in cherry leaves.

Task 4.1. Further investigate the timing of cherry extrafloral leaf feeding by D. suzukii (Yr 1, NIAB EMR)

Methods

This experiment aimed to give an understanding of the nectar sources available to *D. suzukii* in cherry orchards and how this relates to migration and phenology The trial was done in the strategic plot at Rookery field of cvs. \`Penny\` and \`Sweetheart\` by kind permission of Graham Caspell (Table 4.1.1). The trees were not under polythene and therefore exposed to rain.

Table 4.1.1. Site details

Grower/ adviser	Farm	Crop	ha	Crop details		
				Row space	**Tree space**	**Age (yrs)**
Graham Caspell	Rookery Field	Cherry varieties: \`Sweetheart\` and \`Penny\`	1,5	4 m	2 m	7-8 years old

Crop stage in the orchard was determined twice a week for the two varieties including the opening of blossoms and nectar availability. Monitoring traps were assessed weekly for the numbers of adult females and males. USB-502 data loggers (inside a Stevenson's screen) were used to take hourly temperature and humidity readings inside the orchard.

D. suzukii adult monitoring traps were used to assess migration and activity into the orchard. Two modified Biobest Droso-traps were deployed in the orchard, one at the edge of the orchard, the other 20 m into the crop. The vial and liquid Cha Landolt bait was used (Objective 1).

All trapped females (maximum 10) were kept in ethanol in an Eppendorf tube, the onset of fecundity was assessed using the egg/ovary development stage. (Table 4.1.2.). Once >50% of the females had ovarian development stage 4 and 5, the fecundity monitoring was ceased as this would be the beginning of the egg laying period (Table 4.1.3).

Table 4.1.2. *D. suzukii* oviposition development

1	No distinguishable ovaries when opened
2	Ovaries are distinguishable when abdomen opened but no eggs within
3	Ovaries distinguishable full of eggs without filaments when opened
4	Matures eggs with filaments
5	Ovaries with few mature eggs, many wrinkled, may look slightly yellow

From 05 Apr to 14 Sep, weekly, 10 representative leaves (five from each variety) were collected from within the same row the traps were deployed. The size of the leaves was representative of the orchard foliage and free

from pest or disease damage. Because `Sweetheart` trees flower and produce leaves before `Penny` trees, the first assessments were made only with ‘Sweetheart’ leaves (Table 4.1.1).

Photographs were taken of the leaves next to a ruler to estimate the leaf size and extra-floral nectary development (Figure 4.1.1).

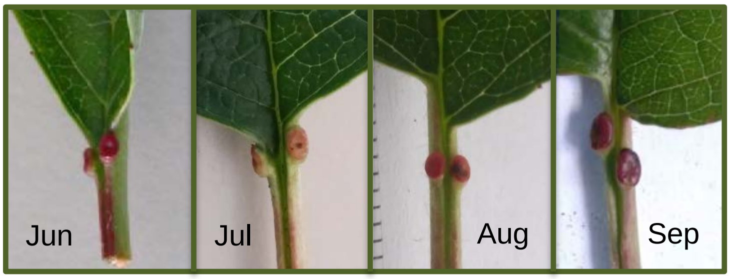


Figure 4.1.1a. Extrafloral nectary development on `Penny`

Figure 4.1.1b. Extrafloral nectary development on `Sweetheart`

Five leaves from each variety after collection from the field, in turn, were placed on the floor of a cage of an established culture of *D. suzukii* (Figure. 4.1.2) containing a mixed sex and age, non-starved *D. suzukii, D. suzukii* were not starved so that they had a choice to feed on the nectaries or the diet in their cages. The number of *D. suzukii* that landed and fed, the time for *D. suzukii* to find the extrafloral nectaries and the length of feeding time over a 5 minute period was recorded.

Figure 4.1.2. Experimental set up; established *D. suzukii* culture + leaf inside the cage

Results

Figure 4.1.3 shows how the numbers of *D. suzukii* (summary of males + females) changed over the time of the assessments in the orchard. The ovarian development of the female *D. suzukii* is shown in Table 4.1.3. The first fecund *D. suzukii* was found on 6 Apr, then a week later more than half (57%) of the female *D. suzukii* in the traps were fecund (Figure 4.1.3).

Table 4.1.3. Summary table of trap catch and ovarian development of *D. suzukii*

Date	No. of *D. suzukii* Edge trap		No. of *D. suzukii* Centre trap		Total No. ♀ *D. suzukii*	No. of ♀ *D. suzukii* ovarian development stage				
	♂	♀	♂	♀		**1**	**2**	**3**	**4**	**5**
15 Mar	2	5	2	1	**6**	6				
27 Mar	1	2	0	1	**3**	3				
30 Mar	1	4	1	0	**4**	3	1			
03 Apr	0	1	0	0	**1**					
06 Apr	0	2	0	0	**2**			1	1	
10 Apr	0	0	0	2	**2**	1			1	
13 Apr	**0**	**7**	**0**	**0**	**7**	**2**		**1**	**3**	**1**

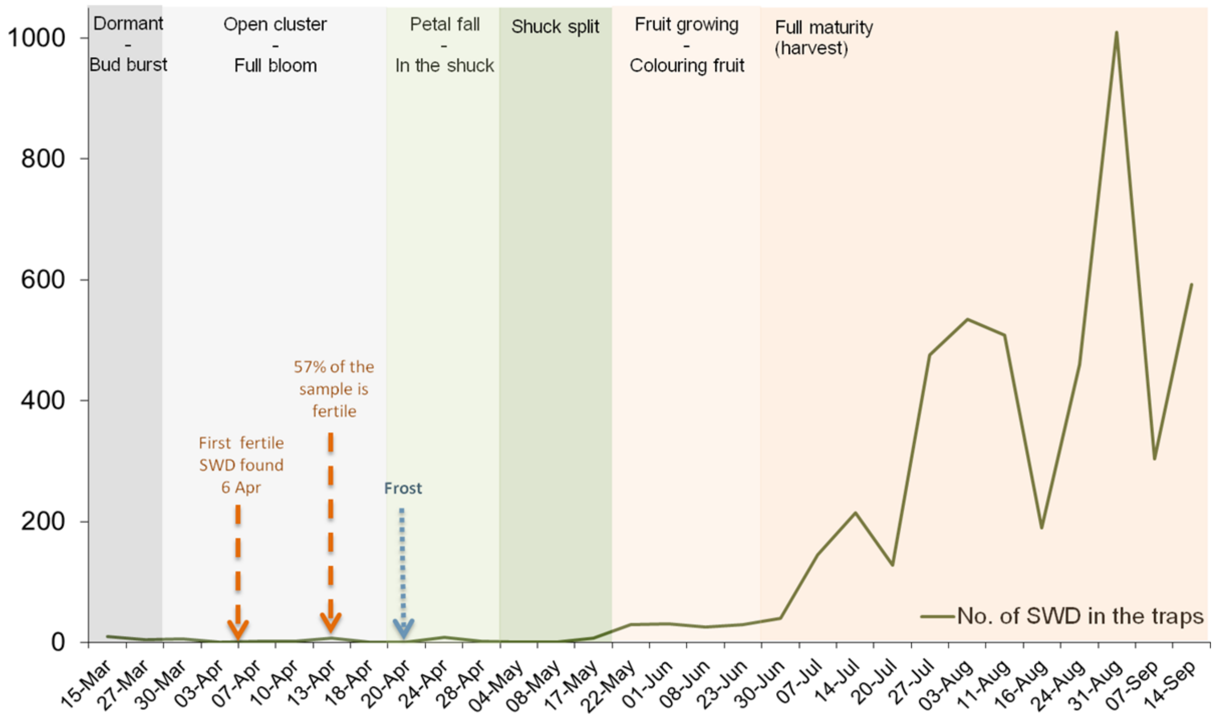


Figure 4.1.3. Number of *D. suzukii* found in at Rookery Field throughout the experiment

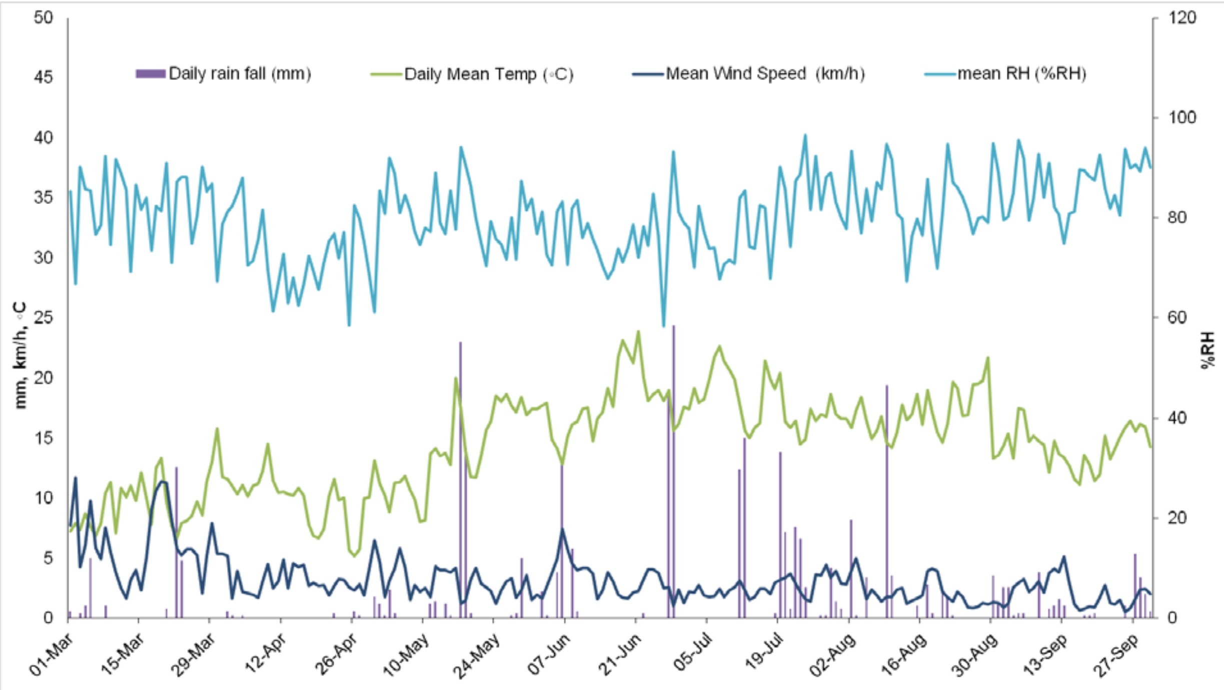
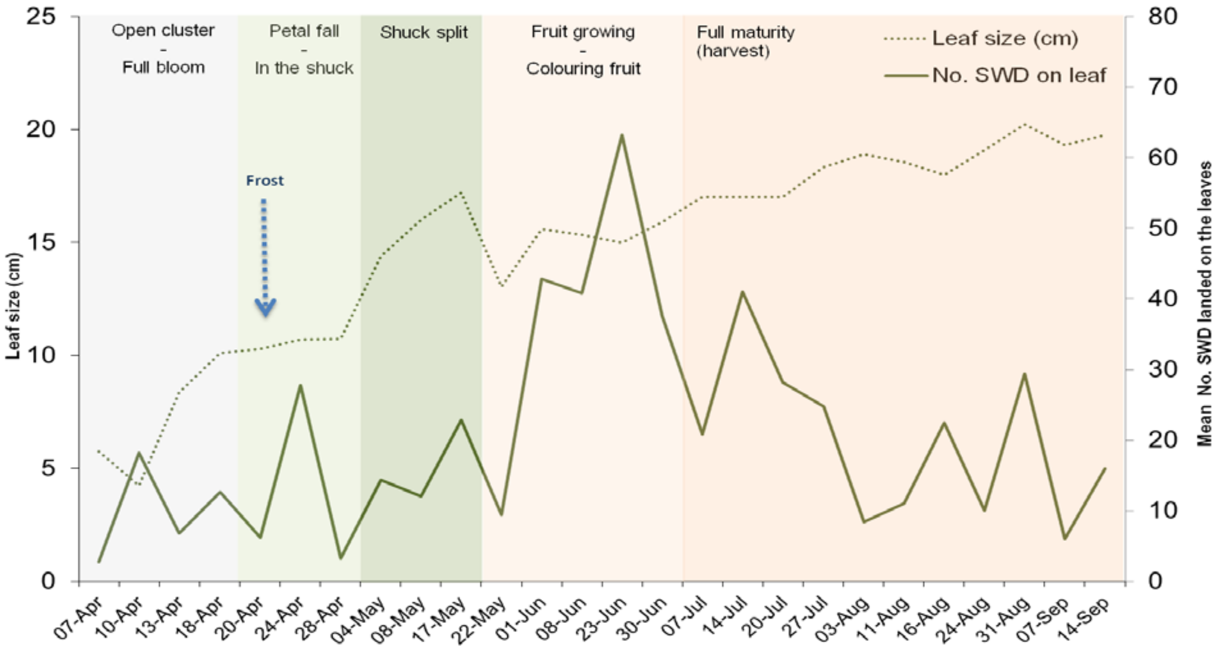


Figure 4.1.4. Meteorological records

Leaves continued to expand until September although small decrease in size in June was due to newly emerging leaves. In both `Sweetheart` and `Penny` there were large fluctuations in the mean numbers of *D. suzukii* landing on leaves introduced to culture cages throughout the season, with peaks in June and beginning of July (Figure 4.1.5a,b).

a)



b)

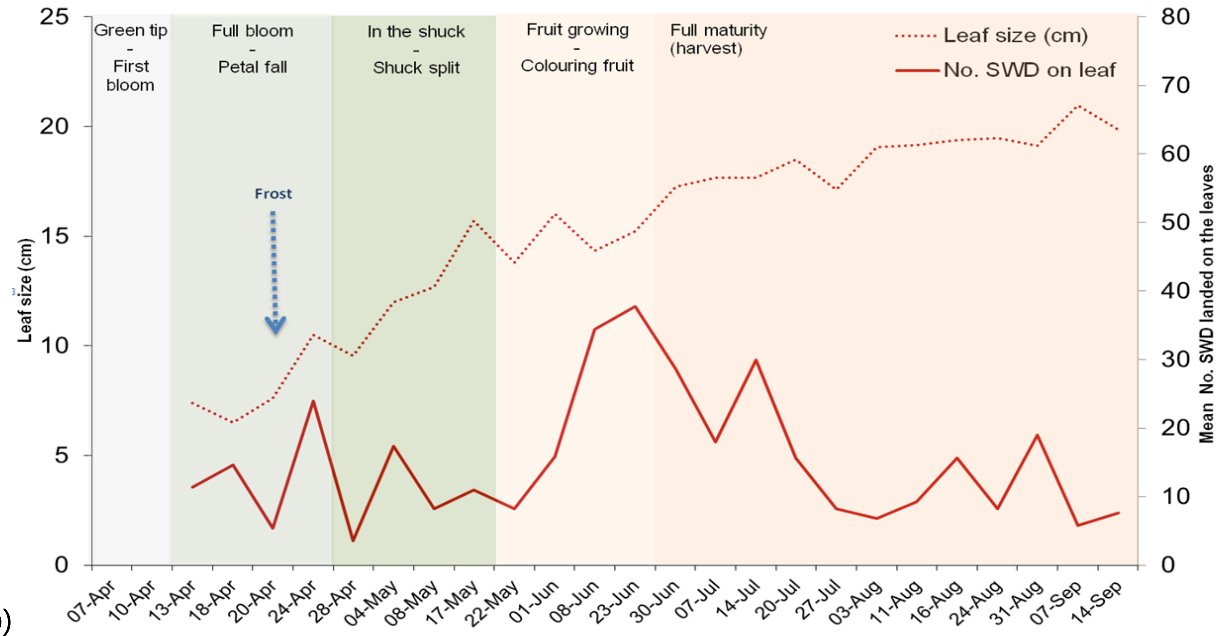
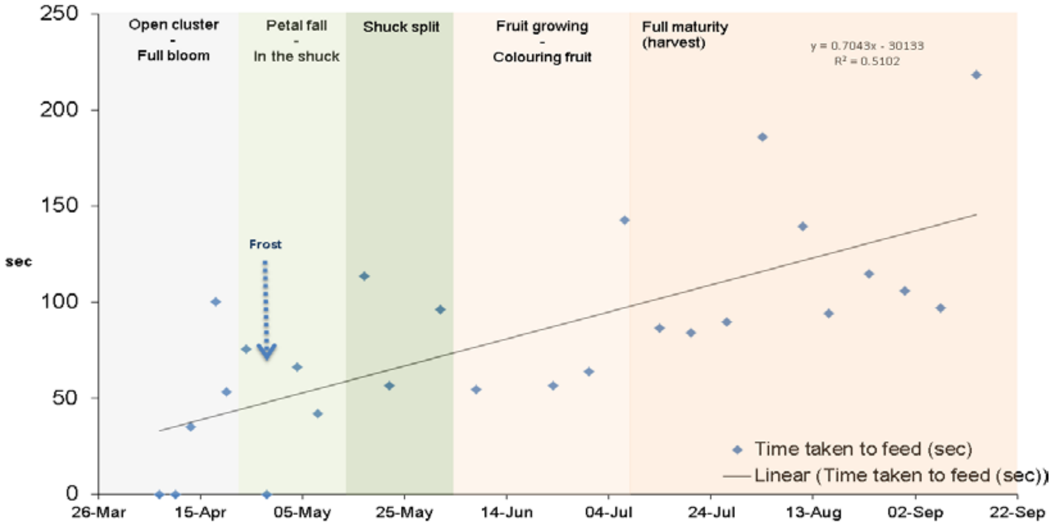


Figure 4.1.5. Changes in leaf size over the season and mean number of *D. suzukii* landing on a) 'Sweetheart' and b) `Penny` leaves

The mean time taken for *D. suzukii* to first start feeding on extrafloral nectaries placed into the cage fluctuated from week to week (Figure 4.1.6a,b). Although the general trend was for a decrease in the time taken to start feeding in both varieties over time. Potentially the older the leaves, and extra floral nectaries, the less attractive they became to *D. suzukii*.

a)



b)

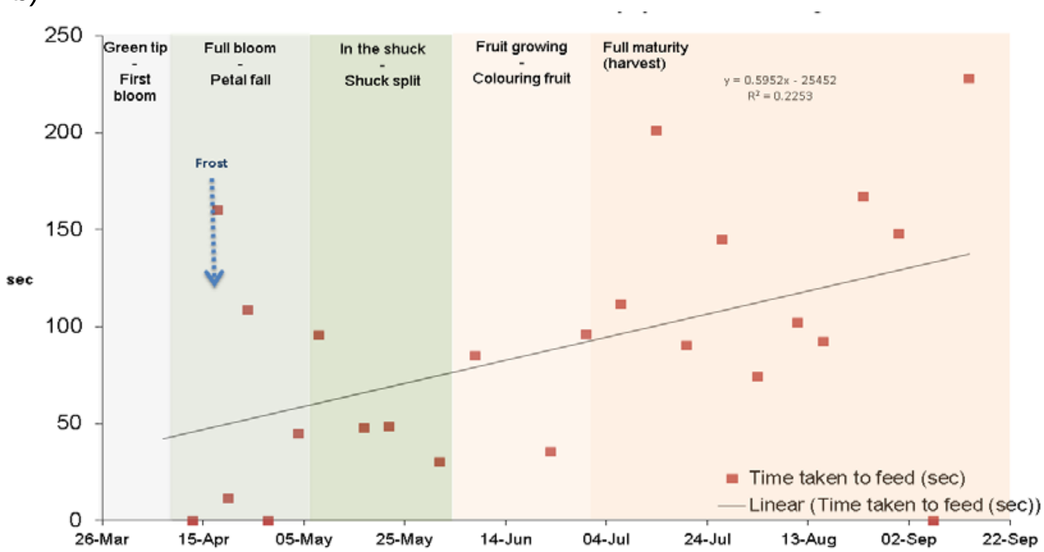
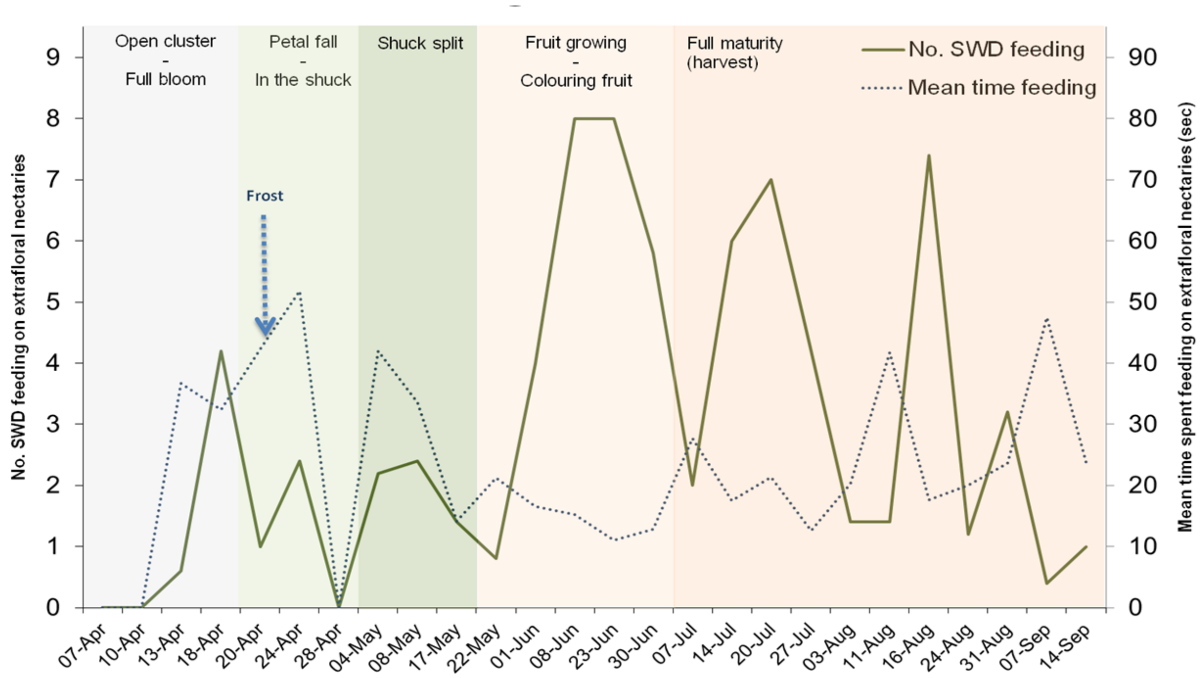


Figure 4.1.6. Mean time taken for *D. suzukii* to find the extrafloral nectaries on a) 'Sweetheart' leaves and b)`Penny` leaves

The mean numbers of *D. suzukii* feeding on extrafloral nectaries and the mean time they spent feeding also fluctuated over the season in both varieties (Figure 4.1.7a,b).

a)



b)

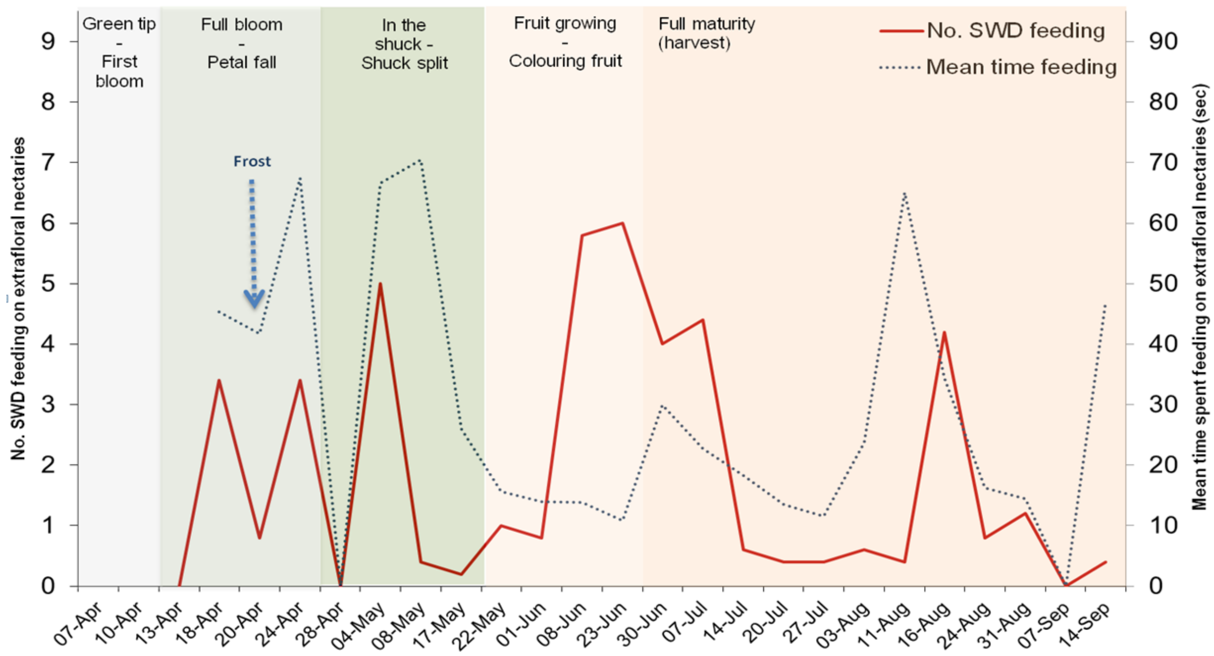


Figure 4.1.7. Mean number of *D. suzukii* feeding on a) ‘Sweetheart’ leaves or b) \`Penny\` leaves and mean time spent feeding on extrafloral nectaries

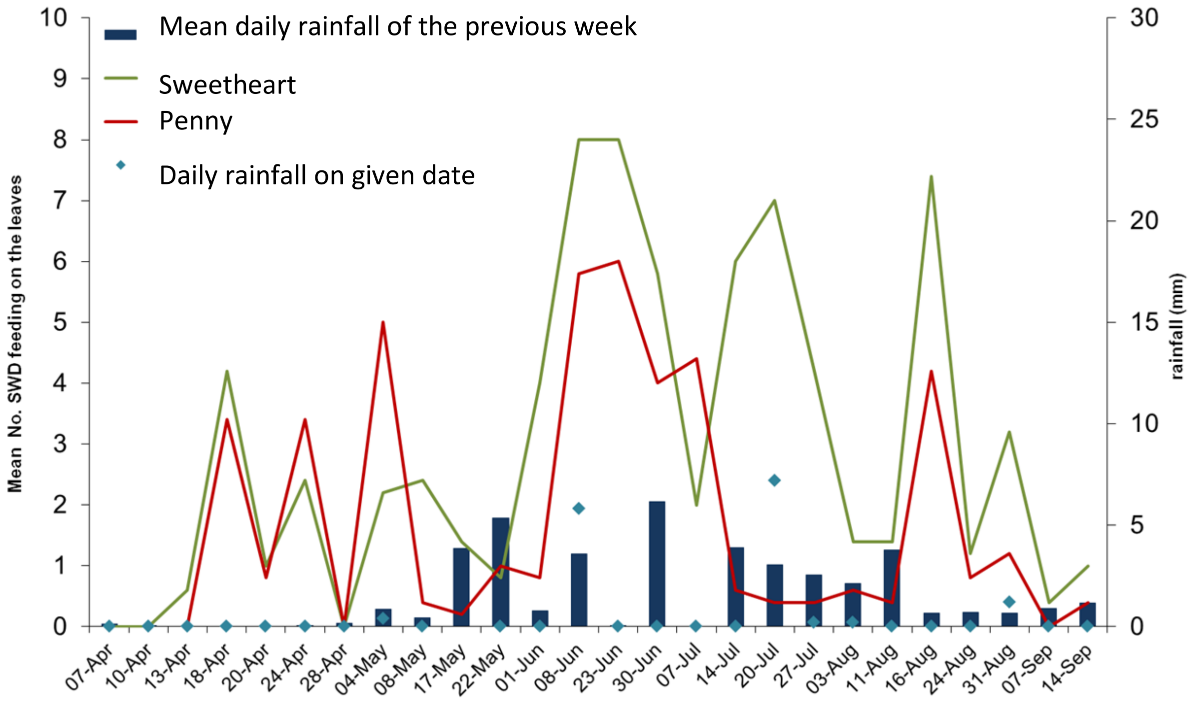


Figure 4.1.8. Mean number of *D. suzukii* feeding on the extra floral nectaries (`Sweetheart` and `Penny`) plotted with the previous weeks rainfall

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* (Figure. 4.1.8).

Conclusions

As the season progressed the time taken to locate nectaries tended to increase. This study demonstrates that there is a food source available to *D. suzukii* after fruit harvest in cherry orchards until the cherry leaves senesce and fall from the trees. After this time more *D. suzukii* are captured in traps.

Task 4.2. Investigate the consequence of extending the spray interval from 1 to 2 weeks in single tunnel comparisons (Yr. 1; NIAB EMR)

Introduction

The aim of this task was to determine whether cherry fruits need to be sprayed on a weekly or fortnightly programme to protect them from *D. suzukii* damage.

Methods

Two small trials were established; 1) Commercial trial with 2 replicate tunnels, 2) Semifield trial at NIAB EMR in one tunnel.

The commercial trial was done in collaboration with Tom Hulme (AC Hulme and Sons, Hoaden Court, Hoaden, Ash Canterbury CT3 2LG, Table 4.2.1) where all plots were insect meshed but no untreated control was used.

The semifield trial was done at NIAB EMR. No insect mesh was installed and an untreated control was included. The orchard was Rookery (RF 181/182), a 2008 planting of cv. Penny with cv. Sweetheart pollinators (every 4 trees in every 4th row, Table 4.1.1). Row No. 12, under polythene, was used. The planting distance was 2 m in the row and 4 m between the rows. Each plot contained 3 trees (24 m^2 per plot, 2.4 l /plot) and was separated from adjacent plots by a single guard tree. The rows were orientated north-south. The trees were 3.5 m tall and 2 m wide, centre leader type trees, with no canopy below 0.5 m from the ground.

Table 4.2.1. Varieties and spray programme used at AC Hulme and Sons, Hoaden Court

Orchard name	Varieties	Row label	Spray programme	Spacing age	Growing system
Little Hoaden Steel Posts	Kordia, Regina	25 (41)	Fortnightly	Single row	Voen netting and insect mesh
Little Hoaden Steel Posts	Kordia, Regina	19 and 22	Weekly	Single row	Voen netting and insect mesh
Blackcurrants	Kordia, Regina	1 (33 and 34)	Fortnightly	Double row bed	Polythene and insect mesh
Blackcurrants	Kordia, Regina	4 and 5	Weekly	Double row bed	Polythene and insect mesh

Either a weekly or fortnightly spray programme was employed at the 2 sites (Table 4.2.2). Sprays and rates were applied within the current approvals for cherry at the time of writing (Table 4.2.3).

At the commercial site, 2 replicate protected orchards (Table 4.2.1) were employed. Each orchard had one tunnel sprayed on a fortnightly programme. Samples of 50 fruit were collected from the centre of 4 tunnels; 2 from the fortnightly and 2 from the weekly spray programme. Each tunnel contained the 2 same cherry varieties cvs. Kordia (mid-season ripening) and Regina (late-season ripening). Trees were sprayed using the growers own spray equipment.

The NIAB EMR trees were under polythene. Three trees were sprayed every week, 3 sprayed every fortnight and 3 trees not sprayed (untreated control, Table 4.4.2). Treatments were applied with a Birschmier B245 air assisted Knapsack mist blower. Each week 4 replicates of 5 leaves were collected at random from the mid canopy of the central tree of each of the 3 treatments for the weekly bioassay.

Table 4.2.2. Timing and spray programme applied on orchard trees at NIAB EMR.

Weekly	Fortnightly	sprayed	pre-spray sample taken	bioassay set up
Tracer	Tracer	09-Jun	09-Jun	14-Jun
Hallmark	NA	16-Jun	16-Jun	20-Jun
Exirel	Exirel	23-Jun	23-Jun	27-Jun
Gazelle	NA	30-Jun	30-Jun	04-Jul
Tracer	Tracer	07-Jul	07-Jul	11-Jul
Hallmark	NA	14-Jul	14-Jul	18-Jul
Exirel	Exirel	21-Jul	21-Jul	24-Jul
Pyrethrin	NA	31-Jul	31-Jul	01-Aug
NA	NA	NA	07-Aug	09-Aug
NA	NA	NA	14-Aug	15-Aug
NA	NA	NA	21-Aug	22-Aug
NA	NA	NA	28-Aug	28-Aug

Table 4.2.3. Products and rates used in trial at NIAB EMR. Below: table from AHDB grower guidelines.

Product	Active ingredient	Conc	Rate /ha	Number of applications	Harvest interval (days)	Approval
Tracer	spinosad	480g/l	250 ml	2	7	20171201
Hallmark	lambda cyhalothrin	100g/l	90 ml	2	7	20131273
Exirel	cyantraniliprole	100g/l	900 ml	2	7	20171134
Gazelle	acetamiprid	20% w/w	375 g	1	14	20170260
Pyrethrum 5EC	Pyrethrin	5% w/v	4 L	No Limit	0	On Label

CHERRIES: Products currently approved with activity against SWD

Crop Situation	Active	Typical Product	Approval	Max. Applications	Max. Rate	Harvest Interval
Outdoor	acetamiprid	Gazelle	Full	1	0.375 kg/ha	14 days
	cyantraniliprole	Exirel 10 SE	Emergency 120-day authorisation	2	0.9 l/ha	7 days
	lambda-cyhalothrin (including crops under rain covers)	Hallmark with Zeon Technology	EAMU	2	90 ml/ha	7 days
	pyrethrins*	Pyrethrum 5 EC	Full	No limit	0.02 l per 5 litres	1 day
	spinosad (including crops under rain covers)	Tracer	Emergency 120-day authorisation	2	0.25 l/ha	7 days
	thiacloprid* (outdoor crops under rain covers)	Calypso	EAMU	2	0.313 l/ha	14 days
Protected	acetamiprid*	Gazelle	Full	1	0.375 kg/ha	14 days
	cyantraniliprole	Exirel 10 SE	Emergency 120-day authorisation	2	0.9 l/ha	7 days
	pyrethrins*	Pyrethrum 5 EC	Full	No limit	0.02 l per 5 litres	Not stated

***Denotes limited effect**

At the commercial site 2 traps (modified Drosotrap and Droski-drink solution) per orchard were deployed and numbers of *D. suzukii* monitored. One was placed inside the insect mesh and one on the perimeter outside the mesh. The grower filtered the traps weekly and *D. suzukii* counts were done by NIAB EMR.

Each week, from pink fruit, 50 cherries of each variety were picked from the central 10 trees in each of the 4 plots by the grower and sent to NIAB EMR. All samples were labelled with; treatment (weekly or fortnightly), orchard name, date and variety. Fruits were examined for egg laying and then incubated for 2 weeks and the numbers of male and female *D. suzukii* emerging were counted.

In the semifield experiment *D. suzukii* adult populations were being monitored as part of the National Monitoring scheme (Objective 1) using liquid and vial ChaLandolt bait. Before spray application 20 leaves from each of the 3 treatments were picked and placed into deli cups (http://www.reptilesupplyco.com/281-insect-deli-cups-lids). Five leaves (4 replicates of each treatment each week) were suspended from the lid inside the pot with moist filter paper and a sugar feeder (Figure. 4.2.1). 5 male and 5 female *D. suzukii* were introduced into each pot and then observed at 4, 24 and 48 hours for mortality.

In addition, all cherries remaining on the 3 plot trees were removed and tested for emergence of *D. suzukii*.

Figure. 4.2.1. Bioassay for testing the efficacy of spray residue on cherry leaves for *D. suzukii* mortality. A sugar feeder was included to maintain the flies

Results

At the commercial site the numbers of adult *D. suzukii* captured inside the tunnels (peak 11), inside the insect mesh barrier, were lower than around the perimeter of the crop (peak 70), outside the insect mesh (Figure. 4.2.2). Fruits were sampled from Kordia and Regina from 9 Jun to 8 Aug (~3,500 fruits). Only 2 female *D. suzukii* were found in all of the fruits sampled; 1 from Regina on 7 Jul and 1 from Kordia on 14 Jul, weekly and fortnightly spray programme respectively .

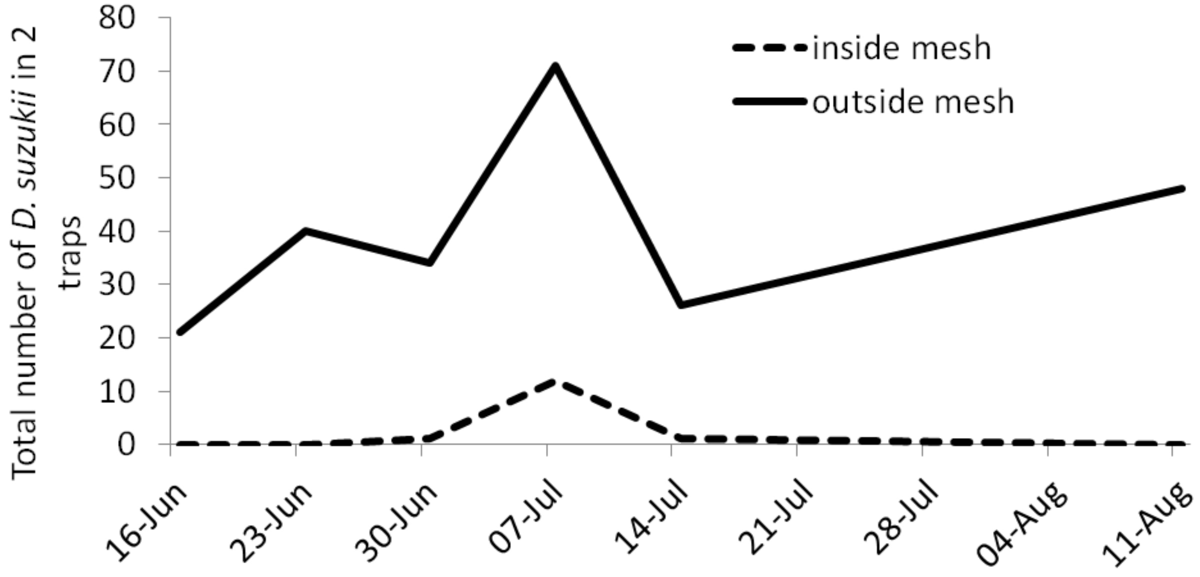


Figure. 4.2.2. Total numbers of *D. suzukii* in 2 traps inside the insect mesh (orchard area) and outside the mesh (in the hedgerow)

In the semifield leaf bioassay there was no significant difference in mortality of *D. suzukii* exposed to leaves from the untreated, weekly or fortnightly programme trees before the sprays began. Hence the co-variate time zero (T0) was removed from the analyses. The mortality in the untreated control plots was usually less than 10%. There was significantly more *D. suzukii* mortality in the weekly and fortnight spray programmes compared to the untreated control once spray applications began (repeated measures ANOVA, P=0.007, F=2.11, lsd=17.572, Figure.4.2.3).

D. suzukii adults were generally more likely to die from contact exposure with leaves after applications of Tracer, Exirel and Hallmark (Figure. 4.2.4). Gazelle, sprayed on 30 Jun, did not appear to give lasting mortality effects. Following the cessation of sprays the effects of the insecticides declined over time (7-28 Aug). Results were variable depending on the time the assessment was done post *D. suzukii* exposure to the leaves but, in general, either weekly or fortnightly applications of insecticides to cherry leaves gave significantly higher mortality (~90%) compared to untreated leaves (up to 10%) 48 hours after exposure. There was very little difference between leaves that had been sprayed either 7 or 14 days previously up to 31 Jul, when the fruits would have been commercially harvested.

The total numbers of *D. suzukii* per fruit emerging from the fortnightly, weekly and unsprayed fruits in high *D. suzukii* pressure ‘Rookery’ orchard at NIAB EMR were 0.8, 3.3 and 19.6, respectively.

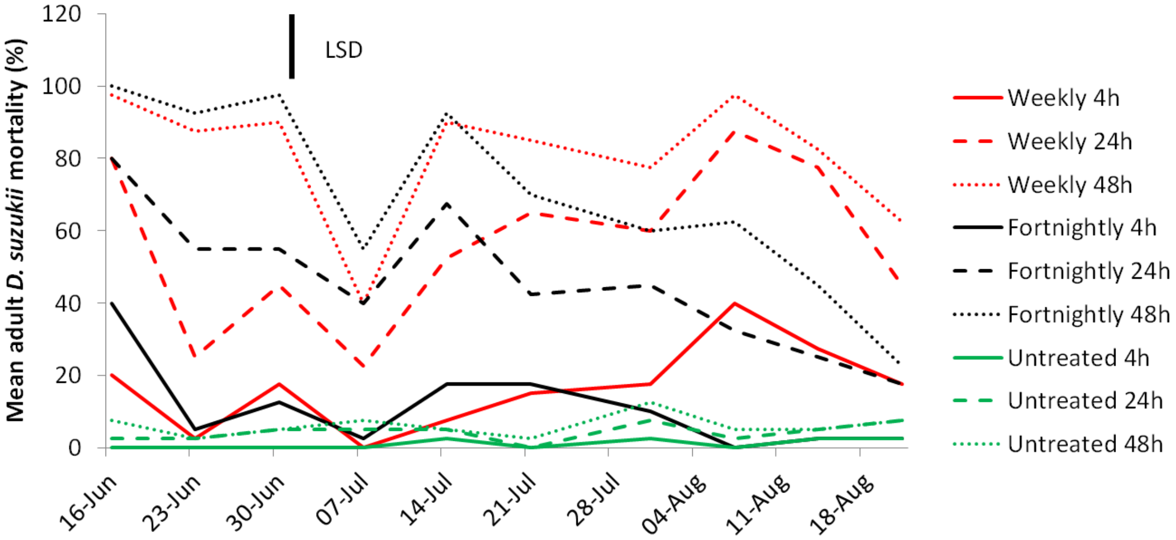


Figure. 4.2.3. Percentage adult mortality of *D. suzukii* at 4, 24 or 48 hours after exposure to cherry leaves sprayed on a weekly or fortnight programme compared to an unsprayed control

Conclusions

- Insect mesh was effective at reducing the numbers of *D. suzukii* in the crop, but not fully effective
- The incorporation of mesh and either weekly or fortnightly spray programmes resulted in virtually no *D. suzukii* emerging from cherry fruits in this small trial (two emerged from all fruit collected)
  Either weekly or fortnightly applications of insecticides to cherry leaves gave significantly higher mortality (~90%) compared to untreated leaves (up to 10%)
- There was no difference in mortality of adult *D. suzukii* exposed to leaves from the weekly or fortnightly spray programmes until spraying ceased
- Tracer, Exirel and Hallmark were effective compared to Gazelle.

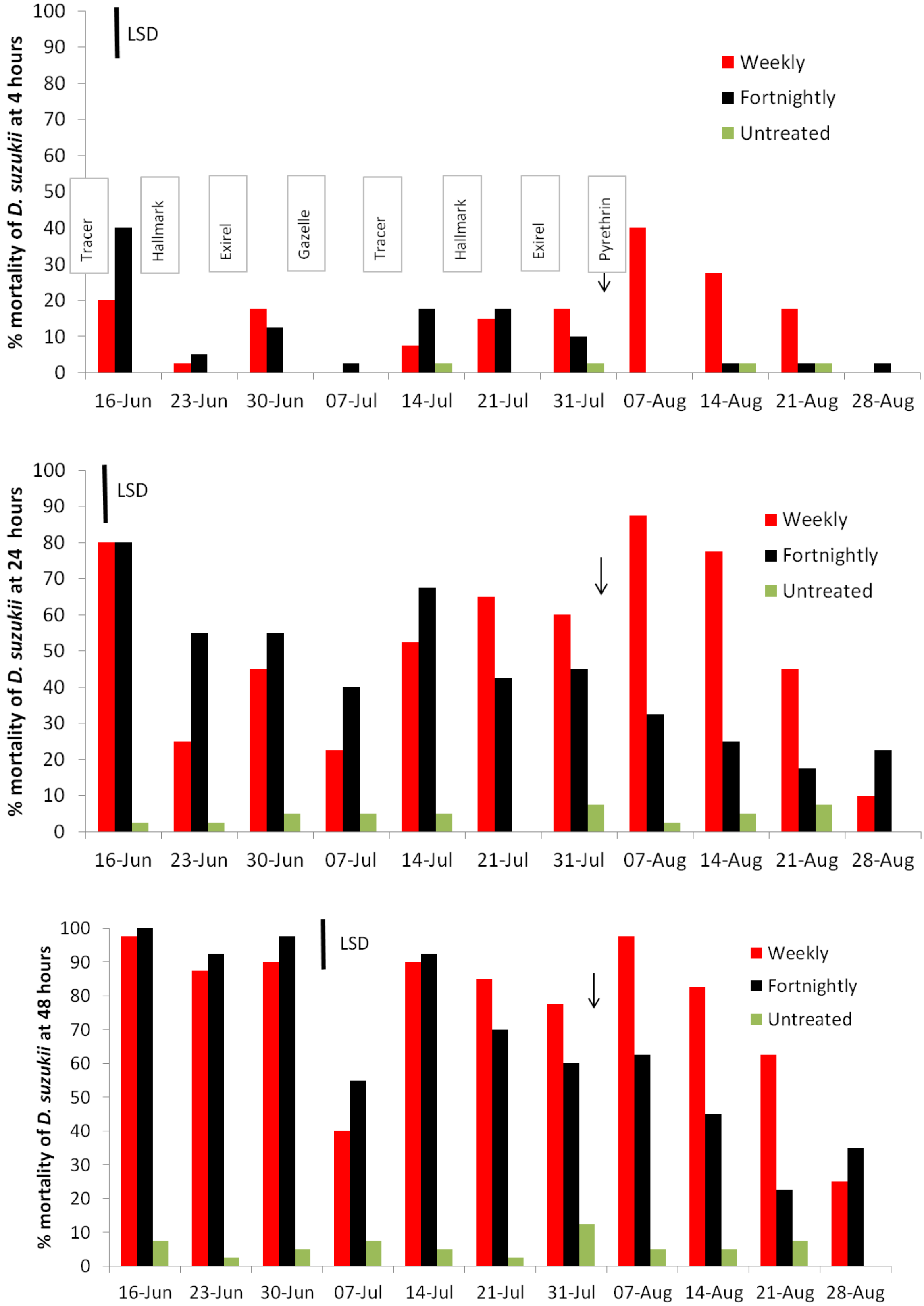


Figure. 4.2.4. Percentage mortality of *D. suzukii* 4 (P<.001, sed=7.06, lsd=14.02), 24 (P<.001, sed=8.8, lsd=17.46) and 48 (P<.001, sed=7.67, lsd=15.23) hours after exposure to sprayed leaves (weekly or fortnightly) or unsprayed leaves. Arrow indicates last spray application

Objective 5. Integrating exclusion netting with other successful controls

To begin in year 3

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 will produce 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 or factsheet.

Proposed research for year 2

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)

Task 1.2. Additional Sites in Scotland (Yrs. 1-4; JHI, NIAB, NRI)

Task 1.3. Egg laying sites for *D. suzukii* in Scotland (Years 1-2; JHI).

Task 1.4. Habitat preference and fecundity in Scotland (Years 1-2; JHI, NRI).

Task 1.5. Data collation and dissemination (Yrs. 1-4; JHI, NIAB, NRI)

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

This objective has been amended as more progress than expected has been made;

- Investigate the field application of a push pull system utilising a blend of repellents and an attract and kill device
- Test the A&K devices in the presence of fruit to determine if efficacy remains at 30% in 24 hours

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

Task 3.1. Develop *D. suzukii* bait with all-round attractiveness in different laboratory bioassays (Yrs. 1-2; NIAB EMR)

Task 3.2. Assess the effect of the optimum bait on the *D. suzukii* control efficacy of different insecticides and concentrations in laboratory bioassays (Yrs. 1-2; NIAB EMR)

Task 3.3. Measure the effect of bait + insecticide mixtures on the viability of yeasts, and on phytotoxicity to crop plants (Yrs. 2-3; NIAB EMR)

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

This task has been amended to a fuller study in cherry with a pilot study in raspberry

Task 4.3. Investigate the use of extended spray intervals to reduce *D. suzukii* in raspberry (Yr. 2; NIAB EMR)

Acknowledgements

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Knowledge and Technology Transfer

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

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 – ARTIS course, training the vine industry on *D. suzukii* management in vineyards

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