Project title:	Development and implementation of season long control strategies for *Drosophila suzukii* in soft and tree fruit
Project number:	SF145a
Project leader:	Michelle Fountain, NIAB EMR, New Road, East Malling, Kent ME19 6BJ
Report:	Annual report, Year 2, March 2019
Previous report:	Annual report, Year 1, March 2018
Key staff:	Glen Powell, Adam Walker, Francesco Maria Rogai, Rosa Blanco Fernandez, Gabriele Antoniella, Alastair Gibbons, Molly Perry-Clark, Chris Coyne, Phil Brain (NIAB EMR); David Hall, Dudley Farman (NRI); Dr Ralph Noble, Andreja Dobrovin-Pennington (Microbiotech), Alison Dolan, Gaynor Malloch (JHI)
Key collaborators	Berry Gardens Growers and Angus Soft Fruits
Location of project:	NIAB EMR
Industry Representative:	Marion Regan, Hugh Lowe Farms
Date project commenced:	01 April 2017
Date project completed (or expected completion date):	31 March 2021

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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

CONTENTS

AUTHENTICATION

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

Michelle Fountain

Deputy Head of Pest and Pathogen Ecology

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

Signature .....M Fountain..Date 27 Mar 19

Report authorised by:

Marion Regan

Industry Representative

Hugh Lowe Farms

Signature .... Date .

[Name]

[Position]

[Organisation]

Signature .... Date ....

GROWER SUMMARY

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

Headline

- A number of novel techniques are being developed to manage and control SWD in soft and stone fruit crops

Background and expected deliverables

The Asiatic vinegar fly *Drosophila suzukii* Matsumura (Spotted Wing Drosophila - SWD) first appeared in the UK in 2012 and has increased in numbers ever since. It has become a key pest of soft and stone fruits, causing significant damage and an increase in production costs. Growers are forced to implement a number of additional cultural and management practices to monitor for the pest, maintain crop hygiene and exclude it from developing fruit. Additional crop protection sprays are also necessary to achieve complete control and these can disrupt IPM programmes deployed for other insect pests.

Concerted European and North American research projects on SWD are coming to an end (projects IPMDROS, DROSKII and DROPSA). The aim of these projects was to develop new knowledge and understanding of the damage and losses on fruit crops resulting from SWD activity, studying its biology and evaluating control methods. This AHDB funded project builds on international progress and a previous AHDB project (SF145), but focuses on the practical development and elaboration of new control technologies that can be used by UK growers within the short to medium term.

SWD is native to eastern and south-eastern Asia (Walsh et al. 2011) and is a potential target for push-pull control strategies. Growers are still highly reliant on plant protection products to gain complete control. However the number of approved products continues to decline and consumer pressure to reduce their use is increasing. More reliance is placed on those approved products that remain and this can result in the development of pest resistance to these products.

This project therefore aims to develop novel management and control techniques which will reduce our reliance on the remaining approved products.

Specific objectives are to;

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

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

3. Further develop, optimise and test bait sprays

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

5. Integrate exclusion netting with other successful controls

6. Integrate approaches for season long control

Summary of the project and main conclusions

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

Since SWD was first detected in the UK in 2012, populations 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 these regions is increasingly being reported.

In collaboration with Berry Gardens, in 2017, monitoring continued 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 the East of 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 SWD in the monitoring traps was lower in the spring (Mar-May) of 2018 compared to 2017 and is typical of findings in a cold spring. The overall tally for SWD in 2018 was therefore lower than 2017. Trap catches in the late autumn vary each year and are largely dependent upon temperature. SWD adults were again detected (Rothamsted Research) at 12 m from the ground during the main flight/dispersal period from September to November. This timing coincides with the emergence of the winter-form adults, a depletion in egg laying resources (fruit) and defoliation of trees (reduced refugia). Despite higher than average temperatures recorded in Scotland during the summer months of 2018 the number/activity levels of SWD remained low.

Data from all four Scottish monitoring sites showed similar trends to those in England, suggesting that the national monitoring data set is representative of the SWD density/activity in Scotland. The density/activity was lower in 2018 than in 2017. The lack of potential egg laying sites detected at the site may have partially contributed to the reduction in the overall catch.

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 entering the cropping area were investigated in the previous project (SF 145). These were further investigated in 2017 either 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. To this end, we further optimised the NIAB EMR / NRI prototype 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 the high labour costs which can be associated with A&K.

Repellents

In 2017, two repellent experiments were done in an unsprayed cherry orchard at NIAB EMR. All six treatments used synthetic semio-chemical compounds which were coded. Repellents were dispensed from polyethylene sachets or rubber septa. Twenty sachets/septa were suspended evenly throughout each cherry tree (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 SWD emerged from sentinel strawberries in a blend treatment in the first experiment suggesting that a blend may be more effective than single components. However, SWD was aggregated in only two blocks in the first experiment removing the possibility of detecting a significant effect. SWD was present throughout the cherry orchard by July but numbers were too high and plots probably too small to detect repellent effects.

In 2018, it was decided to use the blend of potential repellent compounds as part of a push pull strategy. Repellent sachets were placed in 25 x 25 m plots and surrounded with a commercial, coded, trap, the aim being to push SWD out of the crop and towards the traps to remove adult flies from the cropping area.

The trial was repeated at four commercial strawberry plantations in Kent and a randomised block design was used. Each block was sub-divided into four plots which had one of four treatments 1) **Push** - a central square of polyethylene sachets containing the repellent every 2 m, 2) **Pull** - sixteen coded traps positioned around the plot perimeter 3) **Push-Pull** - repellents and traps 4) **Control** – no repellents or traps.

Despite repeated attempts to adjust the methodology to gain information, the trial was unsuccessful. Following statistical analysis, push, pull, or push-pull appeared to have no significant effect on SWD.

In 2019, a more focused semi-field study will be done to determine the repellent activity of the blend. Some new potential repellents will be assessed as part of the Berry Gardens CTP PhD studentship. Once this finer detailed study is complete, and if successful, we may follow this with re-testing on commercial crops.

Attract and kill device

Work has been developing an A&K Falcon Tube at NIAB EMR. In 2017, we compared the device and attractant to a commercial standard. Laboratory cultured SWD were introduced with the prototypes and mortality assessed 24 hours later. 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. Experimental prototypes, with the exception of the untreated controls, were coated on the inside with Decis formulation (deltamethrin) or a field formulation of spinosad (Tracer). The colour, position and number of holes in the prototype were manipulated in the replicated trial.

The prototype Falcon tube devices, with Decis as killing agent, were as effective as the commercial trap in controlling SWD. The devices give up to 30% kill of SWD 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 2018, with a similar experimental set up we tested the Falcon tube device in comparison to two other commercial standards; one with insecticide coating and one without. The Falcon tubes had 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. This experiment was done with and without fresh strawberries in the cage to determine whether the presence of fruit affected the kill of *D. suzukii*.

In a second trial we only introduced either mated or unmated female SWD to determine whether the traps were more likely to kill unmated rather than mated female flies.

The prototype Falcon tube A&K device gave up to 25% kill of SWD within 24 hours in these semi-field cage trials in the absence of fruit. Compared to the same trial in 2017, the efficacy of the device declined by 5%. The Falcon tube A&K device was confirmed to be as effective as the commercial trap in causing mortality of SWD. With no insecticide coating, neither the commercial trap (B) nor the Falcon tube was effective in controlling SWD. Importantly, in the presence of ripe fruit, the efficacy of both the Falcon tube device and the commercial trap (A), with Decis, decreased substantially killing up to only 15% of flies within 24 hours. This suggests that these trap/devices should be deployed in early spring when there is no competition with ripening fruit and SWD populations are at their lowest. A&K devices should be used within an IPM context and be deployed in large numbers around the outside of crop perimeters and combined with insect meshing to prevent migration into the crop. There was no difference in effectiveness of the devices at controlling mated or unmated females. Our study confirmed that mated females are motivated to spend more time on fruit than away from fruit.

Work in 2019 will focus on using the devices in wild habitat in order to reduce populations in the spring adjacent to cropping areas.

Improving the Cha-landolt bait

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 SWD by attract-and-kill and, particularly, lure-and-infect approaches.

In 2017, 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 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 the release rate of acetoin did not increase catches significantly.

In most experiments removing the methionol did not affect catches of SWD, but in other experiments catches were reduced. 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.

A MiniLure was 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 was scope to increase attractiveness by increasing the release rate of acetoin from the MiniLure nearer to the optimum level.

In 2018, the aims were to further reduce the size of the mini-lure, evaluate commercial versions of the mini-lures developed by Russell IPM for use in attract-and-kill devices and optimise the attractiveness of the mini-lure relative to those of Cha-Landolt and commercial wine/vinegar mixture standards.

Using repeated field trapping tests, it was confirmed that catches with the sachet lure could be at least doubled by increasing the release rate of acetoin, making it similar in attractiveness to the Cha-Landolt mixture currently used in the UK SWD National Monitoring Survey. Methionol was found to be unnecessary in either the sachet lures or the Cha-Landolt. This is an important result as methionol is the most expensive component and the most unpleasant and hazardous. In addition, it should also be noted that we have never detected methionol in any of the commercial wine/vinegar lures.

The greater attractiveness of lures with attractants in the drowning solution over "dry" lures is probably due to large differences in release rate rather than some specific effect of having attractants in the drowning solution. In this year's experiments, the Cha-Landolt lure was less attractive than the current Biobest Dro'Attract, even though previous work had shown them to be comparable in attractiveness.

The Russell IPM lures need further improvement, at least in part due to low release rates of ethanol and acetoin.

In 2019 we will aim to determine the volatile attractants in the yeast ferments of attractive yeast species from a recent CTP PhD on attractive yeast strains.

Objective 3. Develop bait sprays for control of SWD in vitro

SWD phagostimulatory baits could improve the efficacy of control products or minimise the dose of sprays required. The use of baits is expected to improve SWD control efficacy of products, potentially reducing application rates. They could also improve the efficacy of a wider range of product types, leading to reduced risk of residues and resistance. In a series of laboratory assays we tested commercially available and novel baits for attractiveness to SWD. We also assessed toxicity when combined with a low dose of product, and finally, their ability to prevent egg laying.

The baits tested included fermented strawberry juice (FSJ), a suspension of the yeast *Hanseniaspora uvarum*, a combination of the two and Combi-protec, a proprietary mixture of protein, yeast and sugars. Experiments were done in the laboratory in jar microcosm bioassays. Chronophysiology assays (activity counts) using the activity of SWD in the presence of different baits, was a more useful screening method of attractant baits than the large arena test.

Without control products, the baits did not affect SWD mortality. For spinosad, cyantraniliprole and lambda-cyhalothrin, the baits caused higher mortality of SWD summer morphs, under summer conditions, compared with using the products in water. The efficacy of products, in terms of increased mortality and reduced oviposition, was greater with *H. uvarum*, FSJ + *H. uvarum* and Combi-protec treatments than with FSJ only bait. In addition, *H. uvarum* and FSJ baits increased the mortality of SWD winter morphs held under winter conditions when used with spinosad or cyantraniliprole but not with lambda-cyhalothrin. When used with cyantraniliprole, *H. uvarum* reduced the oviposition of winter morphs that were transferred to summer conditions after three days of exposure to treatments under winter conditions.

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

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

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

This work will now progress to semi-field testing in tunnels at NIAB EMR in 2019. Baits will be tested with strawberry plants containing SWD.

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 SWD in a cherry orchard and to investigate the length of time that control products targeted against SWD in spray programmes were active in order to prolong the spray intervals beyond 7-10 days.

For the first aim, in 2017 we picked leaves weekly from the varieties \`Penny\` and \`Sweetheart\`. From 05 April to 14 September, five leaves from each variety were collected and introduced, individually, onto the floor of a culture cage of SWD. The number of SWD 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 SWD was found on 6 April, then a week later more than half (57%) of the female SWD 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 SWD until after fruit harvest. There was a weak link with less feeding after a period of rain, indicating that potentially nectar and beneficial microbes could have been washed from the surface of the leaves making the extra floral nectaries less attractive to SWD.

To investigate spray intervals on cherry, two small trials were established in 2017; 1) Commercial trial with 2 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 the two 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 SWD 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 calculate emerging SWD. Monitoring traps were in place at both sites on the perimeter and inside the crop.

At the commercial site, the numbers of adult SWD captured inside the insecticide treated tunnels (peak 11) inside the mesh, was lower than in the perimeter (peak 70), outside the insect exclusion mesh. Only two female SWD 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 SWD mortality in the weekly and fortnight 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 products declined over time (7-28 Aug). 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.

We repeated the cherry spray trial in 2018, but on eight orchards across two grower sites. The findings were similar to the smaller commercial trial (two orchards) in 2017. Fortnightly spray programmes gave equal efficacy of SWD control as the grower's standard spray programme. In addition, very few fruits were damaged by SWD egg laying in both spray programmes, even though adults were clearly in the crop and around the perimeter. Where mesh was employed, there were fewer SWD adults in the crop. Hence for cherry under protection, even on mid and late season varieties, as long as insect exclusion mesh is employed and good crop hygiene measures are used, the current recommendations for the number of SWD sprays appears adequate under current SWD populations.

Also in 2018, we began to test extending the spray interval from one to two weeks in raspberry, but only on two primocane raspberry crops. At a grower site, two tunnels in each of the two crops were treated with the fortnightly spray programme and compared to the growers' standard programme.

Assessments were made weekly, again the day before spraying (if a spray was planned). More SWD were caught in monitoring traps outside the raspberry tunnels than inside the insect meshed tunnels.

More adult SWD were also caught inside the crops where the growers spray programme was applied, on three occasions, compared to the fortnightly spray programme, even though the fortnightly plots were under higher SWD immigration pressure from the surrounding habitat.

Because there were only two replicates of each treatment it was not possible to do statistical analyses on pest emergence from fruit (an indicator of egg laying) or the numbers of SWD that came into contact with raspberry leaves. However, in most weeks, fewer SWD emerged from fruit and more adults died in contact with leaves in the crop in the fortnightly applied spray programme compared to the growers' conventional programme.

As with the cherry spray trial, a fully replicated spray trial in 2019 will help to confirm the beneficial findings of the fortnightly spray programme in raspberry.

Objective 5. Integrating exclusion netting with other successful controls

A decision was made to defer this until a later year as a new CTP PhD student will be working on this in collaboration with Berry World. Initial results will be communicated in late summer 2019.

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

In collaboration with the AHDB communications team, we are producing recommendations for year round control of SWD that targets all life stages and habitats to reduce year on year populations, damage to fruit and the use of plant protection products used for control. Results have been disseminated – over 14 presentations and courses were delivered in 2017 and 10 in 2018, by the team. National Monitoring data was regularly communicated to the AHDB and *D. suzukii* Working Group for dissemination to growers.

Main conclusions

- SWD numbers 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 2019.
- Advances have been made with a feeding bait which increases mortality and reduces egg laying when combined with a low dose of spray control product.
- A fortnightly spray programme was as effective as a 7-day spray programme at controlling SWD in cherry 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.

Financial benefits

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

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

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

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

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

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

Action points for growers

- Use a range of control measures to control SWD on affected fruits.
- Prevent SWD migration into the crop in the spring by using insect mesh, ideally in combination with precision monitoring around the perimeter.
- Continue to use precision monitoring throughout the winter when the traps are more attractive due to the lack of fruit.
- Protect fruits with applications of approved products. Consult your agronomist for the latest approvals.
- Spray intervals under protected cherry can be extended to 2 weeks from white fruit stage in combination with insect exclusion mesh and rigorous crop hygiene.
- Good spray coverage is essential to protect the fruit. Thorough coverage allows SWD to pick up the product and achieve further control.
- Continue to monitor adult SWD both inside and outside the mesh to ensure spray programmes are effective.
- Make regular inspections of fruits to ensure populations are not building inside the crops.
- Consult AHDB Factsheet 06/17 ‘Management and control of spotted wing drosophila’ for full guidance on current management and control practices.

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), one 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 were 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 diameter) 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.

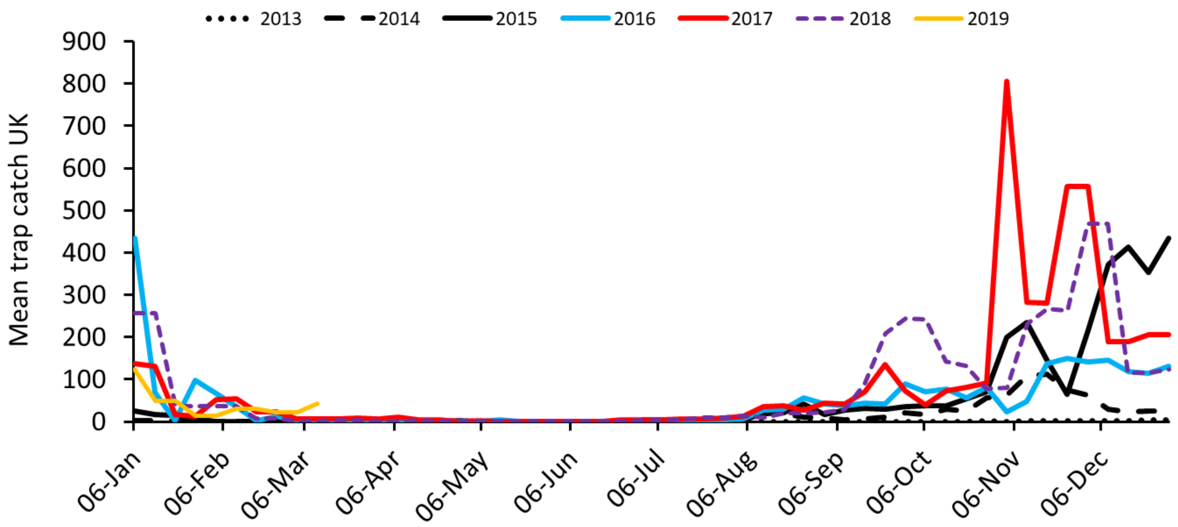
In 2017, *D. suzukii* numbers in monitoring traps continued to rise with interannual variation in trap catches, at least in the late autumn, probably dependent upon temperature (Tochen et al., 2013) and humidity (Tochen et al. 2015). In addition, it was confirmed that *D. suzukii* can be detected at 50 m during the main period when the flies are captured in the traps in cropping and woodland areas (September - November). This period coincides with a depletion in egg laying resources and defoliation of trees. Decreases in trap catches during the summer months are likely due to traps being less attractive than crop and not because there is a decrease in the numbers of *D. suzukii*.

Results

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

In general, patterns of adult *D. suzukii* catches in the traps followed previous years. Catches in the winter of 2017/18 (red line) were 50% lower than 2015/16 (potentially explained by a milder November and December in 2015/16 (black line). Peaks in the winter of 2018 were lower than the previous year. However, annual means per trap, although influenced by temperature, continued to rise until 2018; 2013 = 0.4, 2014 = 774, 2015 = 2951, 2016 = 2430, 2017 = 4587 and 2018 = 4121 (data from 2018 still being collated). Numbers in March 2019, to date, look higher than previous years.

a)



b)

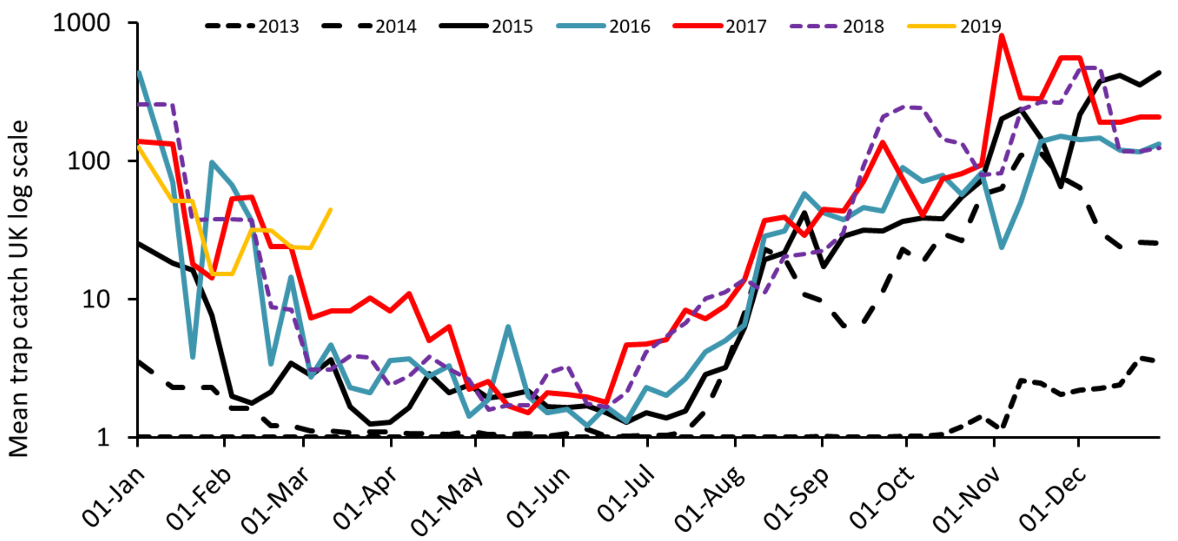


Figure 1.1.1. a) Comparison of average adult *D. suzukii* catch per trap in 2013, 2014, 2015, 2016 and 2017 and b) plotted on a log_{10} (n + 1) scale on the Y axis

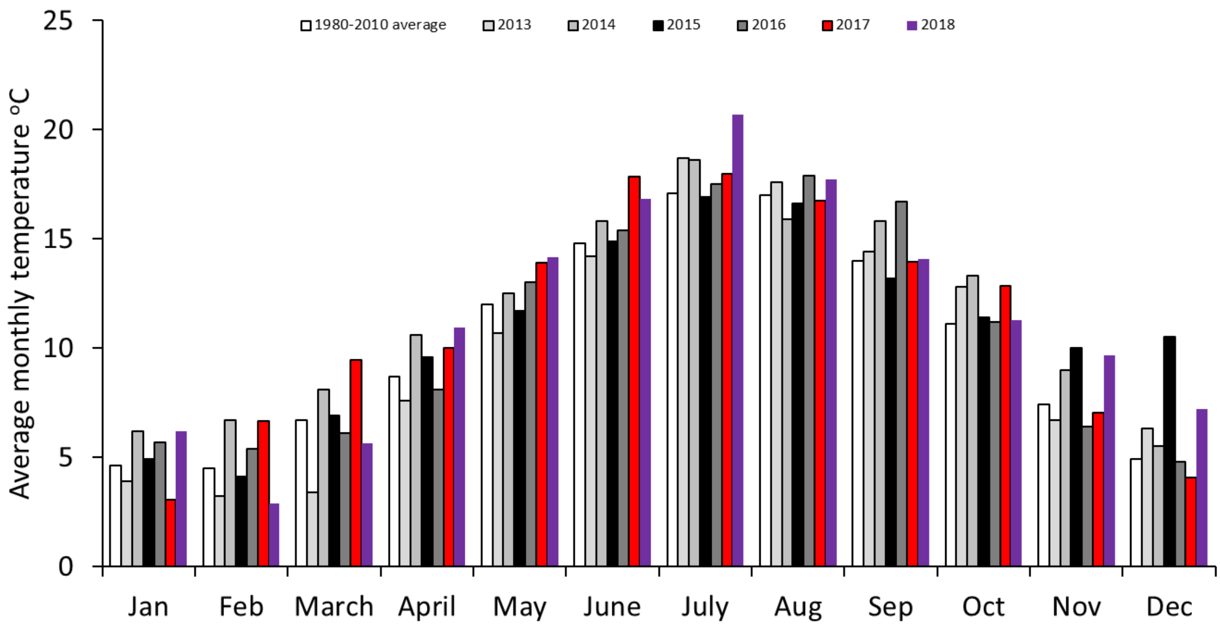


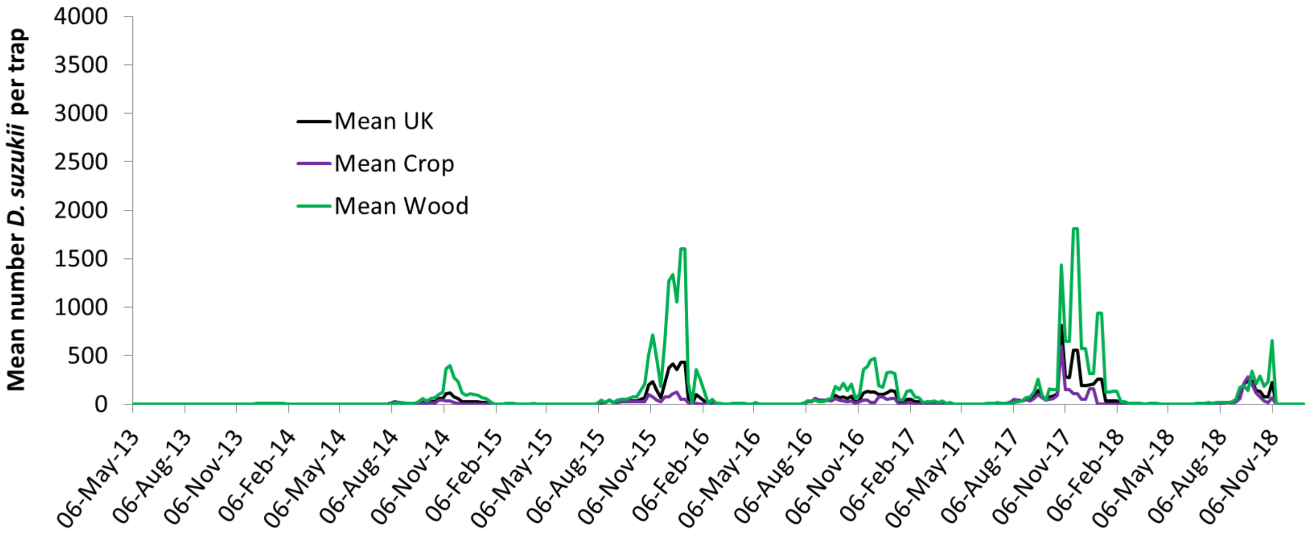
Figure 1.1.2. Comparison of the mean monthly temperatures between years

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 to 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 1.1.3 to 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. It is possible that peak numbers have been reached but data from the remaining years of this project will confirm if this is the case. In Scotland the numbers remain low at the national monitoring sites possibly because the available period of activity of *D. suzukii* to reproduce over a season is more restricted.

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 onwards male and female *D. suzukii* have been captured at a height of 12 m. This is correlated with the highest trap catches in the late autumn at crop and woodland level (Sep-Nov 2013-17). Further counts and confirmation will be done in spring 2019.

a



b

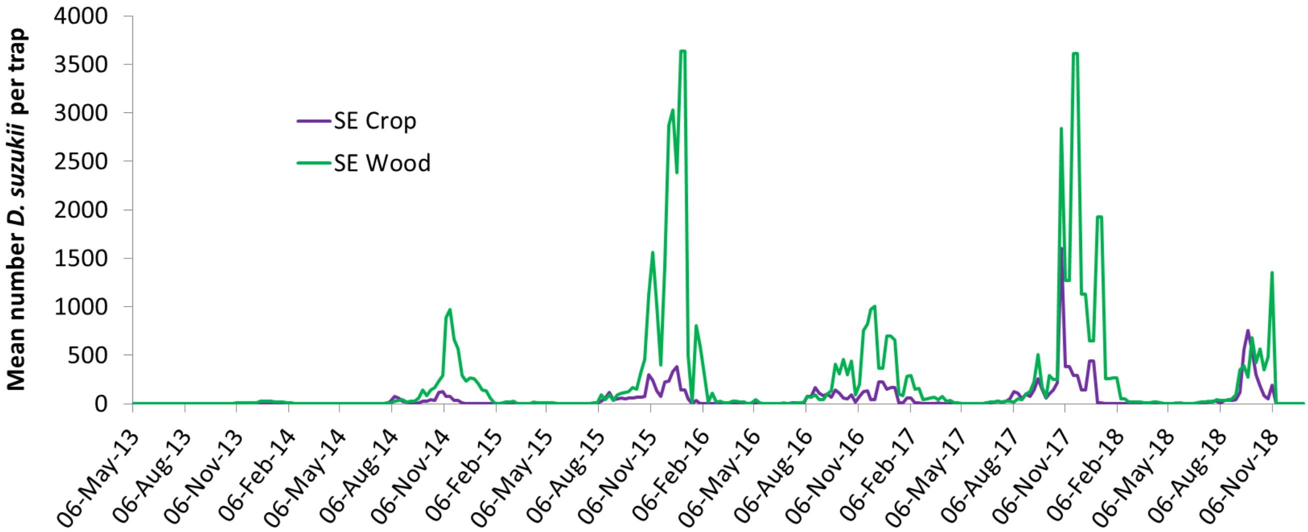
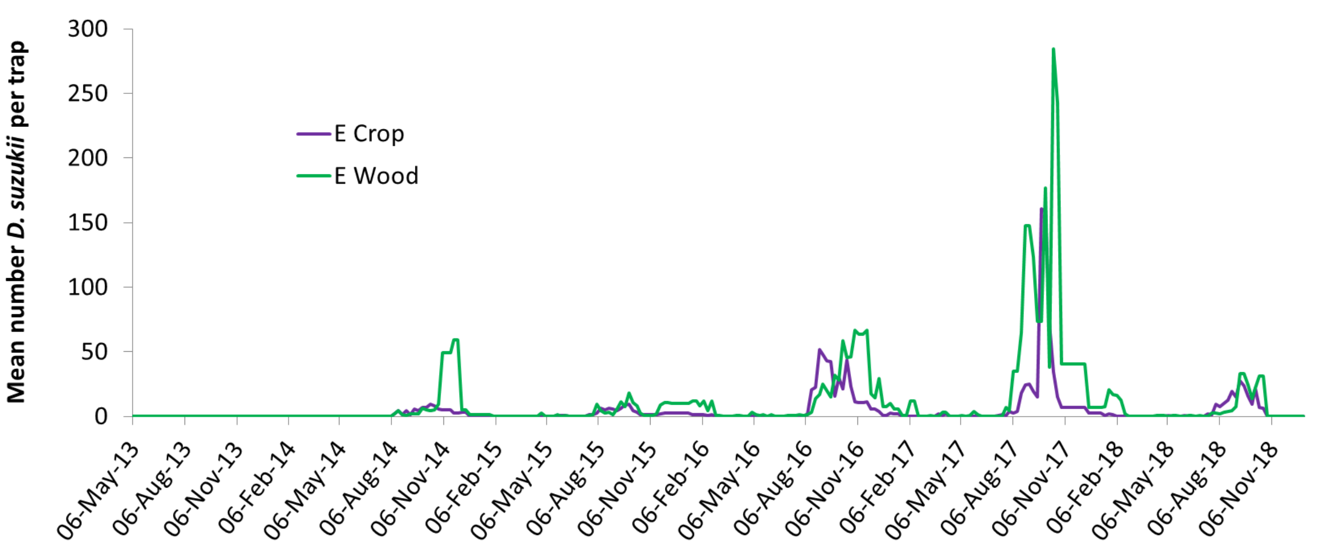


Figure 1.1.3. Mean numbers of *D. suzukii* adults per trap a) in the UK and b) in the South East of England (SE) from 2013 to 2018

a



b

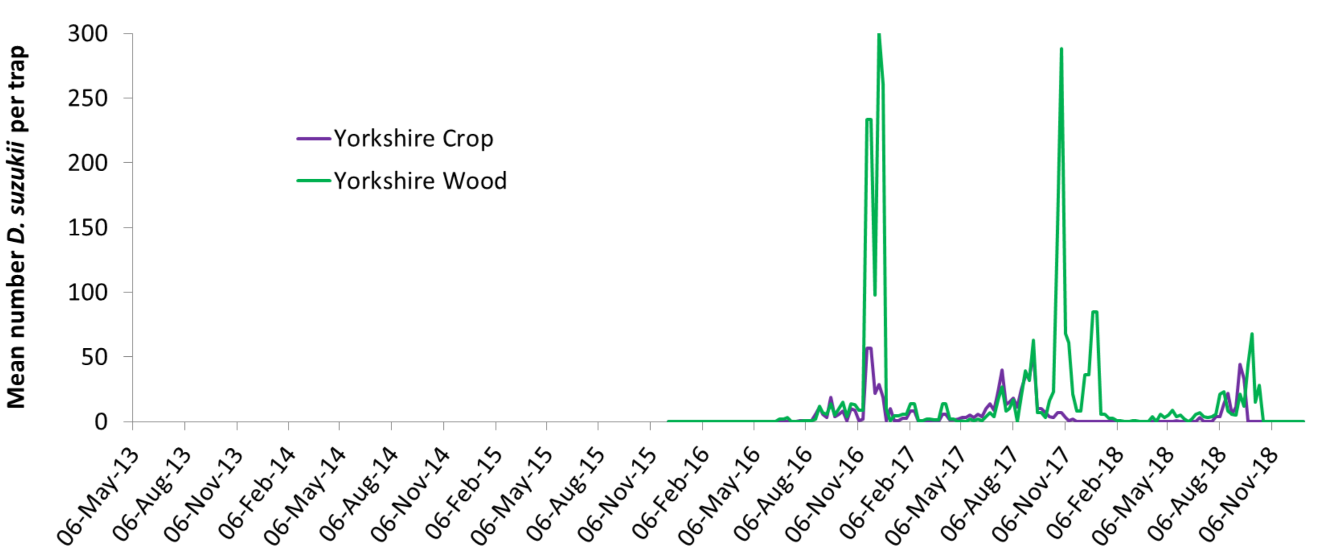
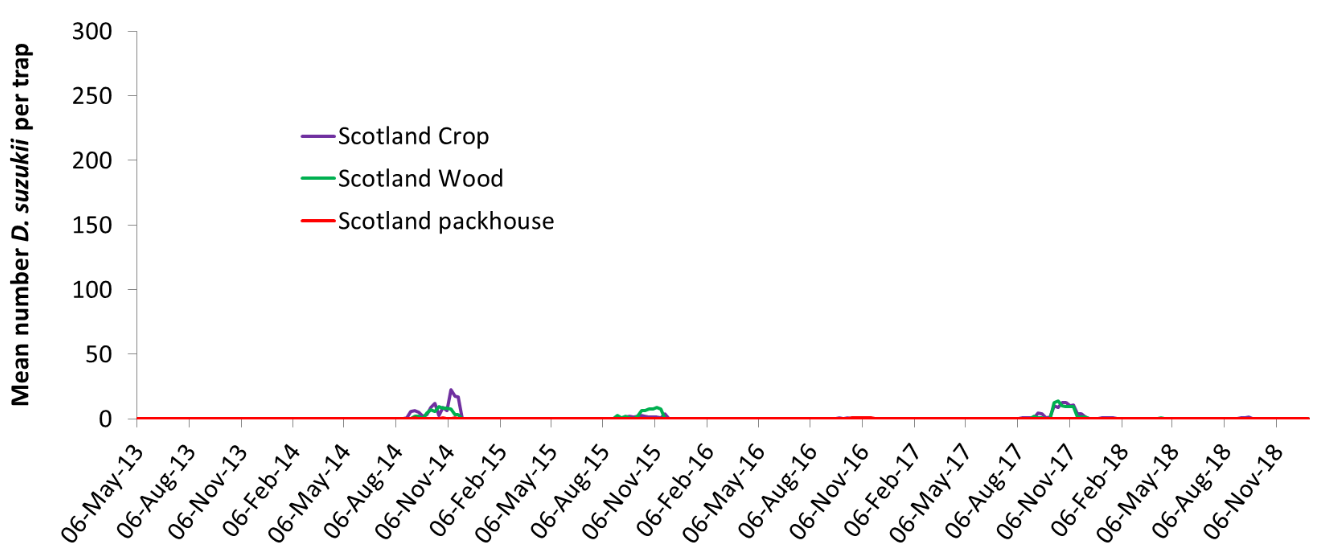


Figure 1.1.4. Mean numbers of *D. suzukii* adults per trap in a) East England (E) and b) Yorkshire (NB monitoring only began in January 2016) from 2013 to 2018

c



d

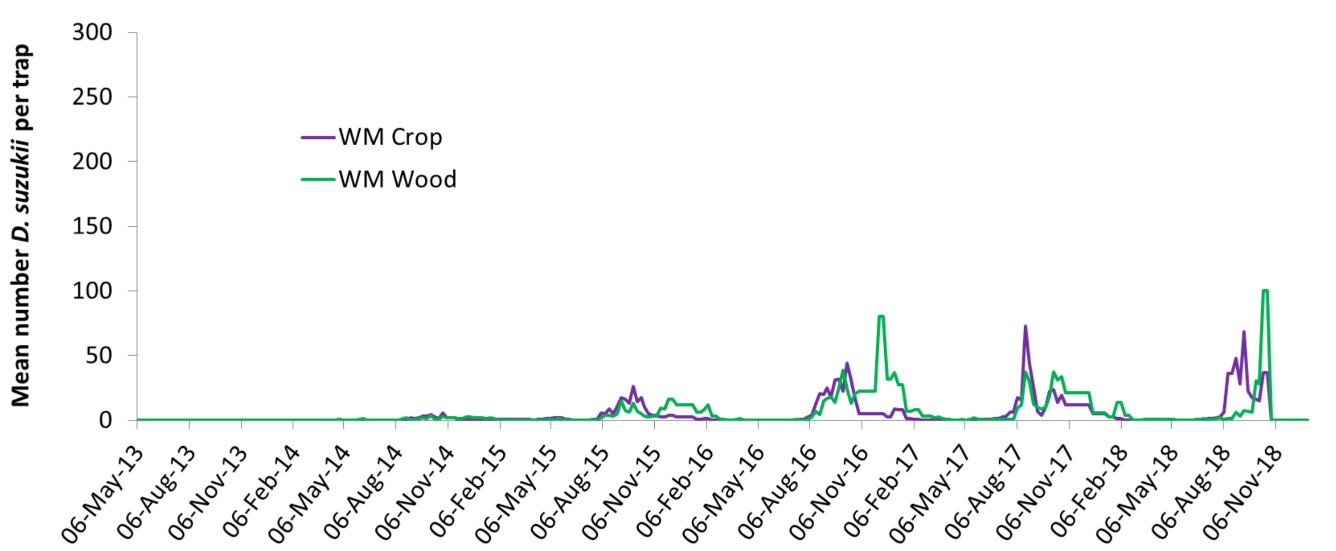


Figure 1.1.5. Mean numbers of *D. suzukii* adults per trap in c) Scotland and d) the West Midlands (WM) from 2013 to 2018

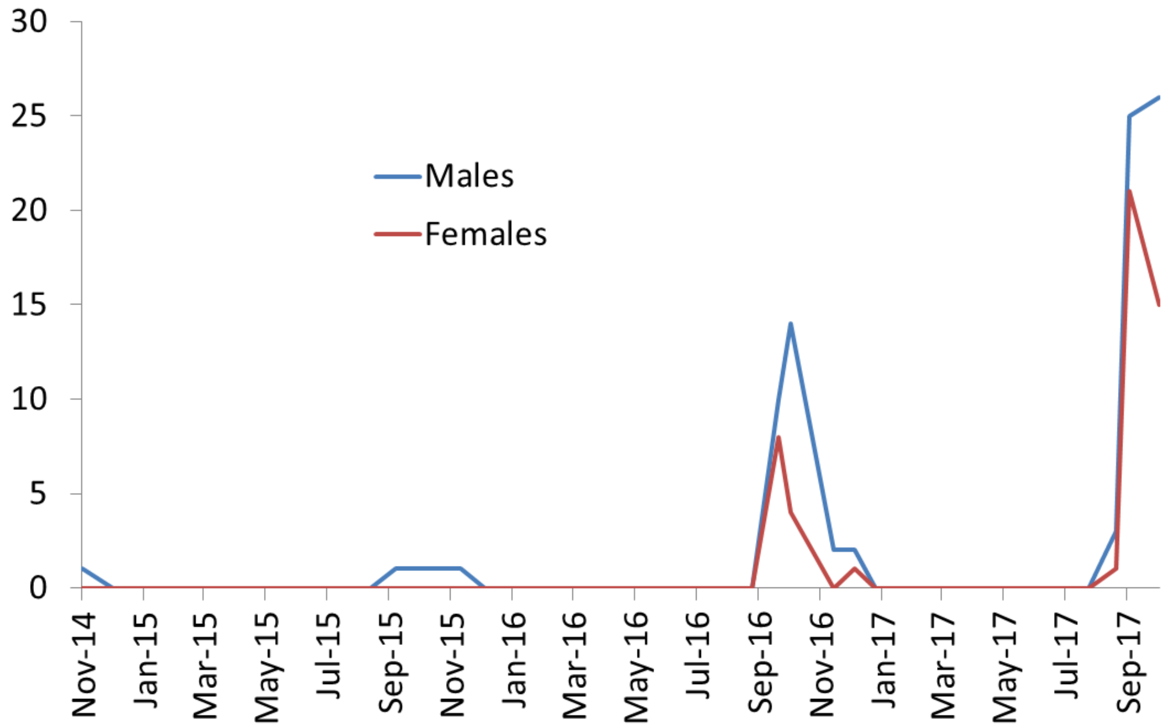


Figure 1.1.6. Total numbers of *D. suzukii* adults in 50 m height suction traps (Rothamsted Research) from 2013 to 2017. First catches were in 2014. Further data will be reported in the 2020 report

Conclusions

- *D. suzukii* numbers in 2018, overall, were slightly lower than 2017, probably due to a cold spring and therefore delayed start to the first summer generations.
- There continues to be variation in interannual trap catches, at least in the late autumn, probably largely dependant upon temperature.
- *D. suzukii* can be detected at 12 m during the main flight/dispersal period when the flies are captured in the traps in cropping and woodland areas (September to November).
- September to November coincides with the emergence of the winterform adults, a depletion in egg laying resources (fruit) and defolation of trees (reduced refugia).
- Decrease in trap catches during the summer months are likely to be due to traps being less attractive than crop and not a decrease in the number of *D. suzukii.*
- Despite higher than average temperatures recorded in Scotland during the summer months of 2018 the number/activity levels of *D. suzukii* remained low.

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

Data from the two additional growers' groups in Scotland are only available from 2015 onwards. Therefore, it is not possible to make a comparison with data collected in the initial year of detection in Scotland (2014). However, the results from 2015-2018 generated from the additional grower's groups are broadly similar to those reported in the National Monitoring study for Scotland (Fig. 1.2.1).

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/activity in Scotland.

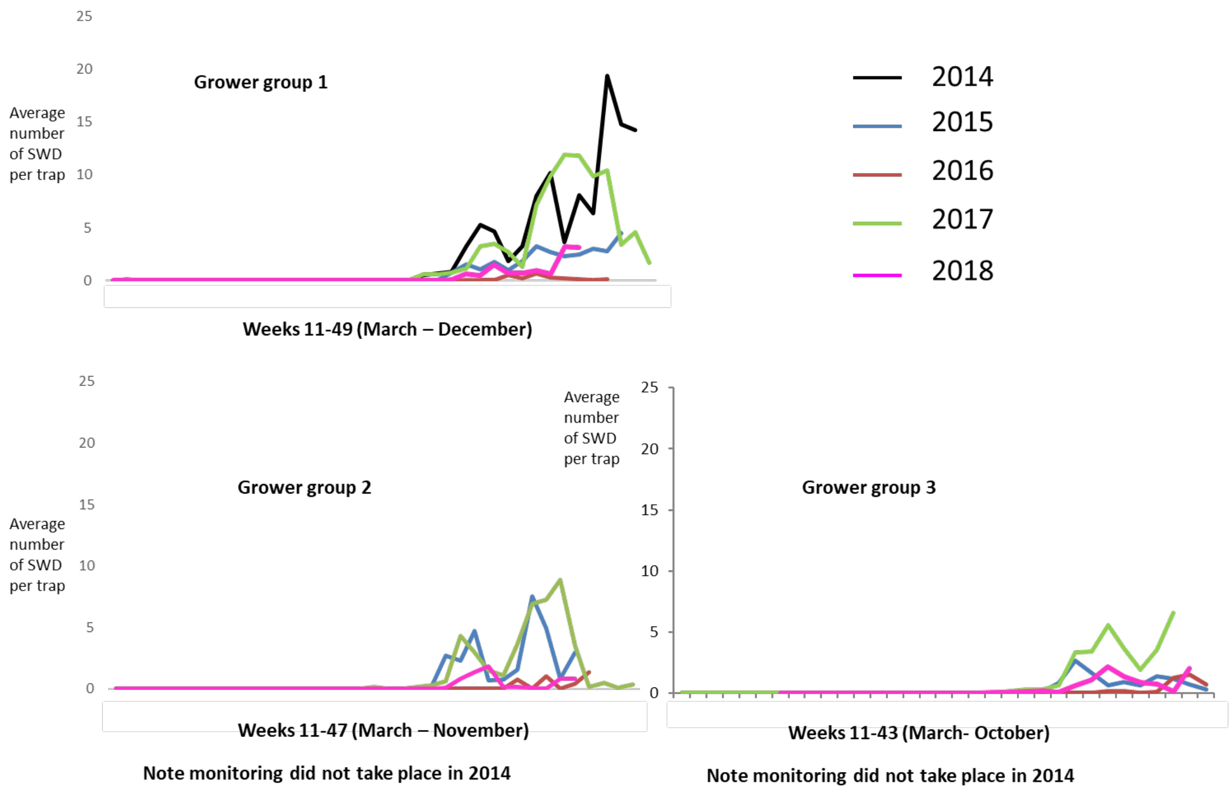


Figure 1.2.1. *Drosophila suzukii* monitoring data from 3 grower's groups in Scotland 2014-2018. Grower Group 1 is part of the National Monitoring

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 in 2018, 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. Samples were taken in January, February, August, September, October, November and December. No wild fruit was available from March to July in 2018. In addition, five samples from wild hosts in the habitat study at site 1400 were taken from wild blackberry, blueberry and hawthorn in August, September, October and November Table 1.3.1. The samples were examined visually and incubated for adult emergence to determine whether *D. suzukii* was developing in the fruits.

Table 1.3.1. Samples taken for flotation and emergence tests from potential wild hosts at two sites (1100 and 1400) in 2018 (Year 2)

Wild Hosts Tested	2018
Berberis	1
Cotoneaster	6
Ivy	3
Sea Buckthorn	5
Cherry Laurel	3
Viburnum	2
Rose Hip	10
Sloes	6
Blackberries	10
Rowan	4
Chokeberry	2
Hawthorn	5
Elderberry	1
High Bush Cranberry	1
wild blueberry	1
Total no. of samples	**60**

Results

Samples of berries from hedgerow and woodland plants (Berberis, Cotoneaster, Rowan, wild Blackberry, Choke berry, Rosehip, Sloes, Sea Buckthorn, Cherry Laurel, Ivy, Viburnum, Elderberry, Highbush Cranberry and Hawthorn) were found and collected on seven occasions from January until November at site 1100 (the James Hutton Institute). No *D. suzukii* adults emerged from any of the samples. Four larvae were detected in the November sample of Hawthorn berries in the flotation test, but the morphology suggests that they were not *D. suzukii* larvae. The specimens will be DNA barcoded to confirm. In addition, in studies by other researcher's hawthorn is not a favoured host of *D. suzukii (*Lee *et al., 2015;* Poyet *et al.,* 2015; Kenis *et al.,* 2016*.).*

Very little wild fruit was available in the habitat study (site 1400) in 2018, possibly due to the warmer and drier than average summer conditions in Scotland in 2018. Fruit was collected from hawthorn, wild blackberry and blueberries from August to November. No *D. suzukii* were found from either the flotation or emergence test.

Conclusions

The *D. suzukii* density/activity was lower in 2018 than in 2017. The lack of potential egg laying sites detected at the site may have partially contributed to the reduction in overall catch.

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 includes 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 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).

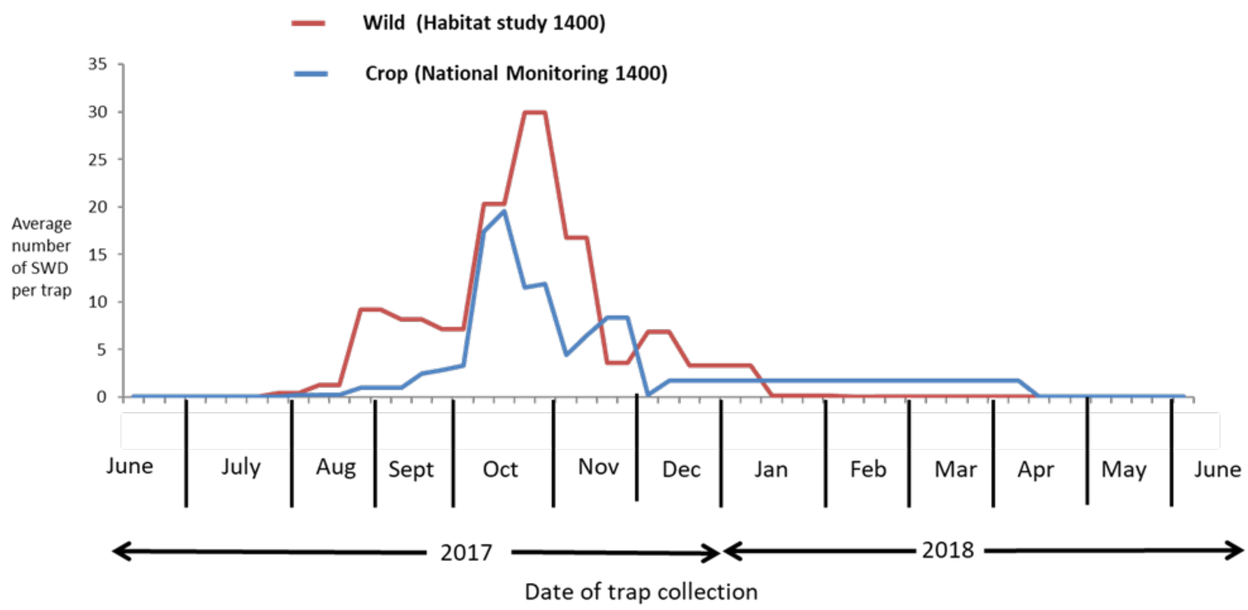
Records of species diversity and abundance were taken from areas surrounding the traps. Abundance was calculated using the Total Estimate Scale. Assessments were carried out monthly and plant abundance and growth stage were recorded.

Results

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 mainly in the fruit crop (National Monitoring) (Figure 1.4.1).

As with the Scottish National Monitoring figures, catches from the wild traps at the habitat study site are lower in 2018 than in 2017. In the wild traps in 2017 *D. suzukii* numbers peaked at approximately 30 per trap whereas in 2018 the peak was approximately 10 per trap (Figure 1.4.1). *D. suzukii* catches were not evenly distributed throughout the site and several 'hotspots' occurred (Figure 1.4.2). Findings in Year 1 indicated 2 'hotspots' (Trap 1416 and 1428 at site 1400). The plant species surrounding the hotspots include blackberries, cherries, nettle, goose grass and grasses. Preliminary analysis of the data does not suggest that the hotspots were linked to the abundance of the plant species. Year 2 hotspots identified in this report were based on total catch in each trap from June to November 2018. A more accurate analysis has since been carried out over a longer period and using a calculation of proportion of the catch (see comments and Figure 1.4.2 below). To date, these traps are still collecting relatively high numbers of adults, however in Year 2 traps 1411 and 1414 also look like potential hotspots at this site (Figure 1.4.2). The plant species surrounding traps 1411 and 1414 include Sycamore, Red Campion, grasses, goose grass, Willow, Spruce, thistle and Poplar. Traps 1416, 1411 and 1412 are in larger sheltered patches of woodland near to a body of water. It was interesting to note that the hotspots in Year 1 of the study were not the same as in Year 2. This finding suggests that year to year variables such as weather patterns may have a large influence on the distribution of the catch. We also noted that hotspots varied throughout the study period in both years and were probably influenced by which plants were fruiting at the time.

Fecundity monitoring of the *D. suzukii* caught in traps located in the wild (site 1400) indicate that female adults with mature eggs were only caught from August to November 2017 (Figure 4.1.3). Apart from one individual with mature eggs trapped in June 2018, the overall proportion and distribution of females with mature eggs appears to be similar for both years (Figure 4.1.3).



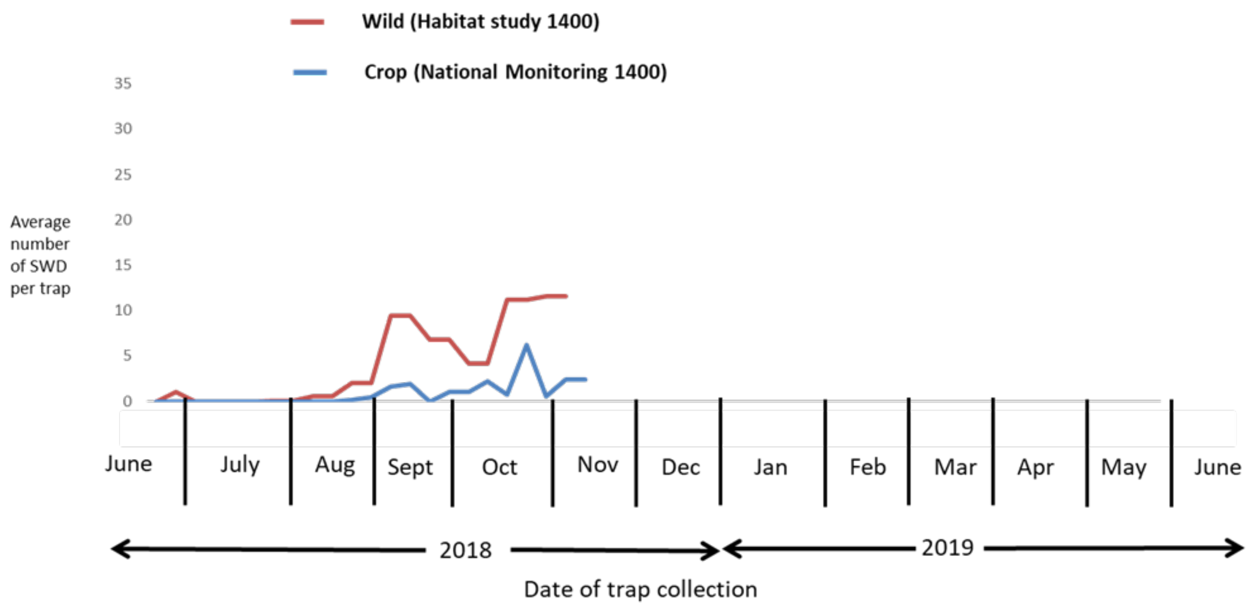


Figure 1.4.1. *D. suzukii* habitat preference at site 1400 in Scotland. NB: Met data was not included in this study

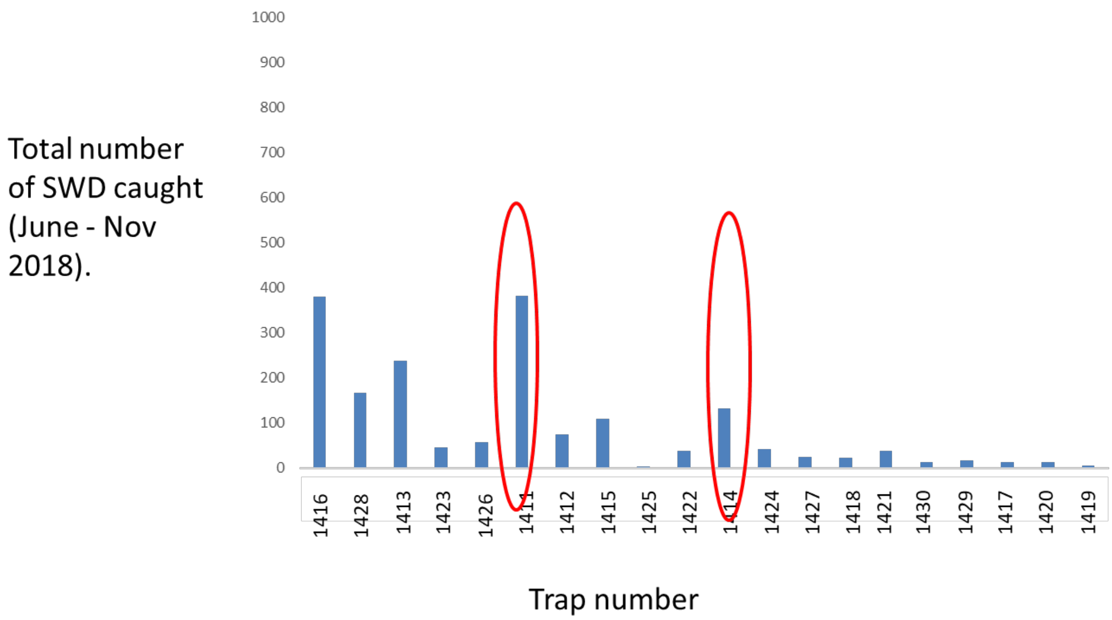


Figure 1.4.2. ‘Hot spots’ of *D. suzukii* trap catches at the habitat site 2018 to 2019 Year 2

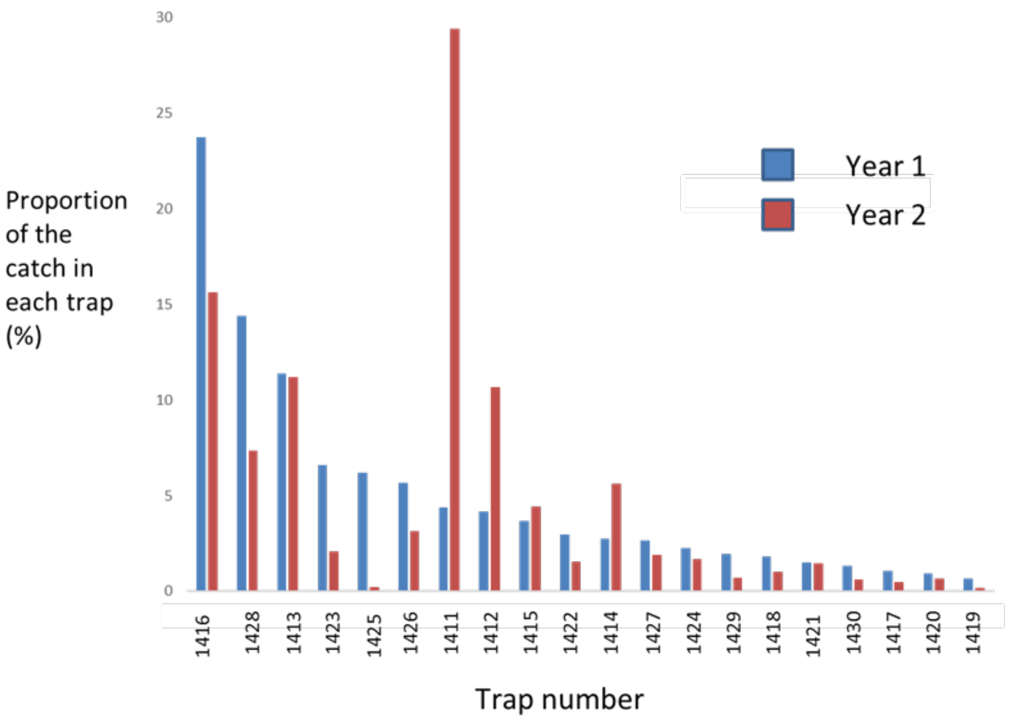


Figure 1.4.2. ‘Hot spots’ of *D. suzukii* trap catches at the habitat site Year 1: June 2017 to January 2018 and Year 2: June 2018 to January 2019

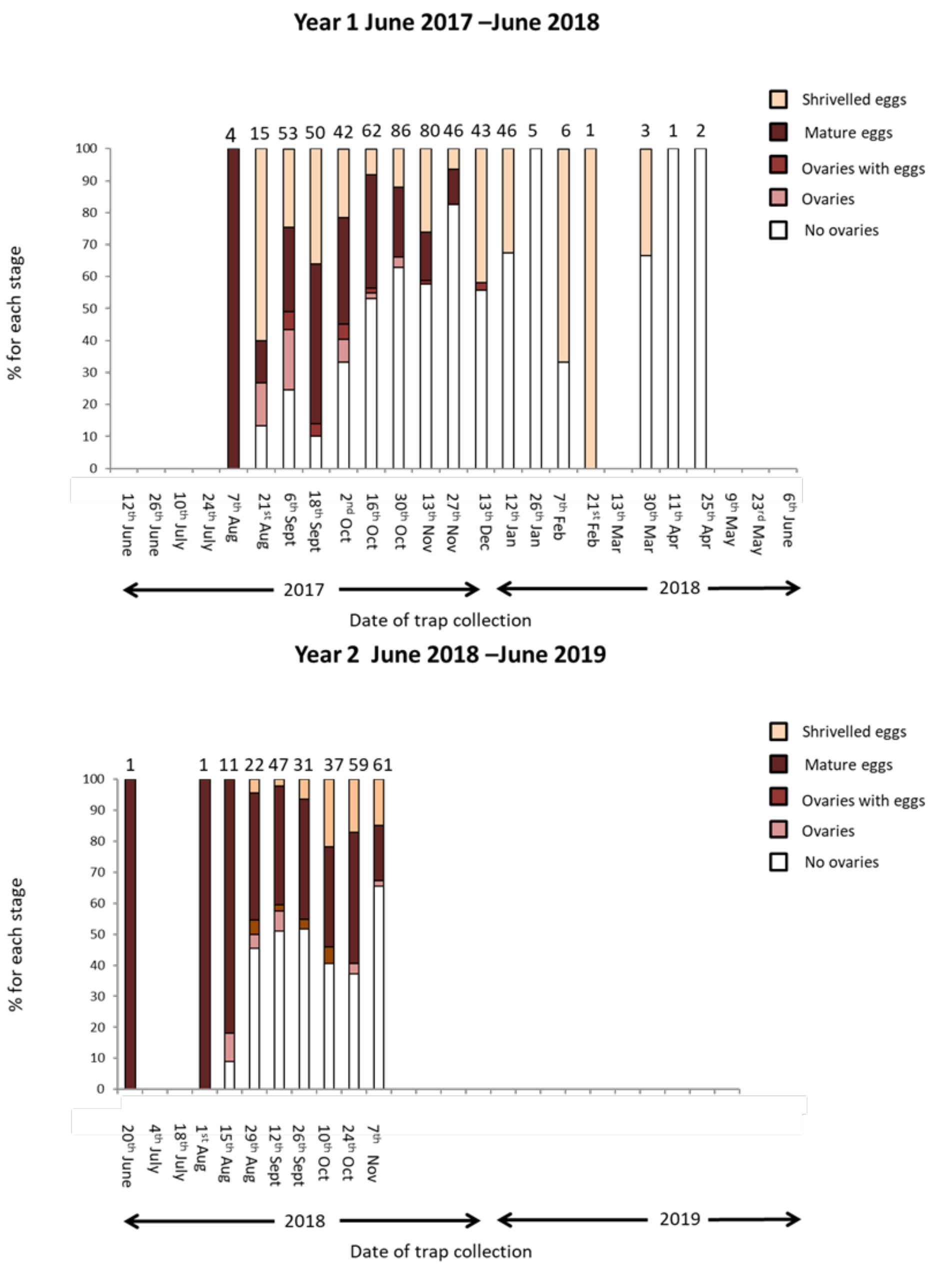


Figure 4.1.3. Percent of females at different stages of reproductive state in Scotland habitat study, Years 1 and 2. Numbers above the bar indicate the total number of females dissected not the number of females that were caught (i.e. 46 females were dissected from the total catch collected during the period from the 13 December 2017 to the 12 January 2018). A maximum of five females from each of the 20 traps.

Conclusions

D. suzukii density/activity and reproduction rate is very low during the late winter/spring months in Scotland.

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. 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 KT soft 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, 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 2018 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. There was a marked increase in samples received from 6 in 2016 to 21 in 2017 and up to 38 in 2018. The samples, submitted by growers, covered a range of fruit crops. They were assessed for the presence of SWD using the Flotation and Emergence Test and the results were returned confidentially. All were negative.

The three growers in the monitoring project in Scotland were updated regularly on their catch data. Scottish Government has also received a verbal update.

A presentation is being produced for the SSCR soft fruit winter meeting to be held at the James Hutton Institute in February 2019.

Please see Knowledge and Technology Transfer section for more information.

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).

In 2017 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 (plot) on 12 May and again on 13 July. Sentinel fruits were then deployed within the tree canopy an incubated for 2 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.

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 the high labour costs which can be associated with A&K. 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 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. To investigate the potential of a push-pull system for control of SWD in strawberry (Yrs. 1-2; NIAB, NRI)

Introduction

Push–pull technology is a strategy for controlling agricultural pests, typically using a repellent plant to "push" the pest out of the target crop towards an attractant acting as the "pull" (Cook et al. 2007). The approach has been used to control several insect pest species, including the crucifer flea beetle, *Phyllotreta cruciferae*, a pest of broccoli (Parker et al. 2016). Besides pest control, additional benefits of push-pull include, reduced need for chemical plant protection products (PPPs), increasing numbers of natural enemies in the crop and increasing beneficial soil organisms (Kelemu S. 2015).

To develop push-pull against *D. suzukii* knowledge of the chemical ecology of the pest is required. However prior to 2008 little was known about its courtship and host-seeking behaviours or chemical ecology. Since then, researchers have gained a better understanding of the pest's attraction to specific odours from fermentation, yeast, fruit, and leaf sources, and the visual cues that elicit long-range attraction (Cloonan et al. 2018). Recently promising results were reported for a *D. suzukii* push-pull strategy in raspberry, where findings showed an 87.6% reduction of oviposition on raspberry fruit under laboratory conditions and a 57.4% reduction in egg deposition compared to control plots in the field (Wallingford et al. 2017).

In 2016 and 2017, the potential for a push-pull system against *D suzukii* was investigated at NIAB EMR focussing on repellents. In 2016, trials in cherry varieties Penny and Sweetheart showed a decrease of egg laying where six individual repellent compounds were deployed directly above sentinel fruit in delta traps hung within the tree canopy. However, when the experiment was repeated later in the season, post cherry harvest, this 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, so in 2017 the experiment was repeated earlier in the season before the first generation of *D. suzukii*. Repellent sachets or septa of each treatment were randomly dispersed throughout a tree and two delta traps were hung at the same height in the middle of each tree, one with a repellent sachet placed above sentinel fruit (shown to be effective in spring 2016) and one with no sachet above the fruit. Following deployment, sentinel fruit (strawberry) were collected after three days. Findings showed that in the treatment where a blend of repellents was used, only one adult *D. suzukii* emerged from sentinel strawberries, suggesting that a repellent blend may be more effective than single components.

In 2018, the main objective was to investigate the potential of a push-pull system for controlling *D. suzukii*, this time in commercially grown strawberry. During the trial, the repellent blend, showing most promise in 2017, was tested in combination with a pull using a lure and trap (coded) (Figure 2.1.1), to test whether:

- *D. suzukii*, could be repelled from a strawberry crop using a blend of compounds in sachets (push system).
- A perimeter semiochemical trapping system (pull system) could be used in conjunction with the repellent system for improved efficacy.
- *D. suzukii* damage, to the fruit and presence in egg laying media, could be reduced where treatments were applied.

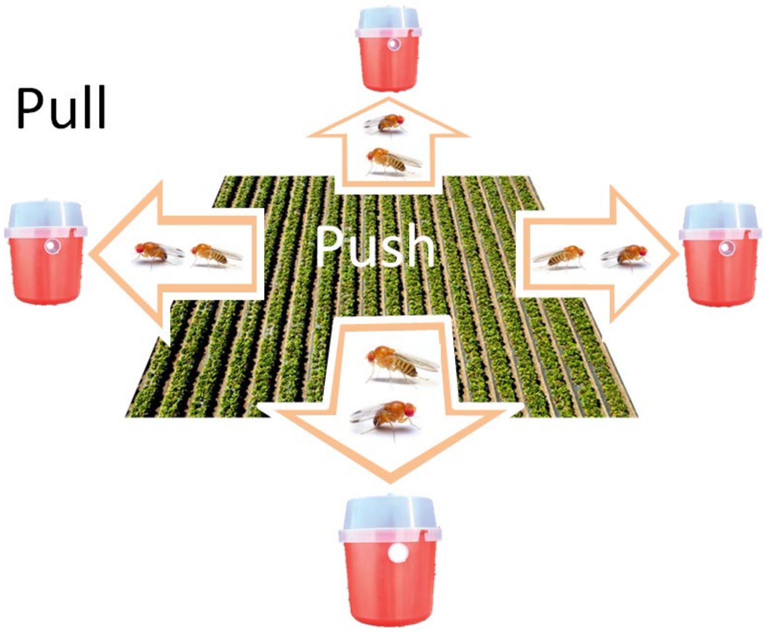


Figure 2.1.1. Schematic diagram of a potential push-pull system against *D. suzukii*, where a repellent is deployed in the centre of the crop creating the push, and an attractant is deployed in traps around the crop perimeter, creating the pull. *D. suzukii* are killed in the traps

Methods

Location: The trial was done at four commercial strawberry plantations (sites) in Kent. Between sites, the shortest distance was 281 m, the longest distance was 57.24 km. Strawberries were grown in tunnels on standard height table tops, with the exception of Site 4 which used low table tops.

Treatments: A randomised block design was used, with each strawberry plantation (site) acting as a replicate block. Each block was sub-divided into four plots (Figure 2.1.2.) with plot positions randomized each replicate. Plots were 25 m x 25 m (3 or 4 tunnels wide depending on the tunnel span at each site, i.e. 8 or 6 m tunnel spans) and were set up either at the corners of the crop, or along the edge of the crop, depending on pest pressure. Plots were spaced ≥50 m apart to avoid interaction between the treatments.

Treatments were:

- **Push** - a central square (14 x 14 m) with 8 rows of 8 polyethylene sachets containing the repellent, stapled to strawberry growbags, 1 approximately every 2 m. The central square of repellents was 5.5 m from the plot perimeter on all sides.
- **Pull** - Sixteen coded traps containing the lure (coded), positioned at crop height around the plot perimeter – 4 per side, spaced every 5 m. No repellents.
- **Push-Pull** - a central square (14 x 14 m) with 8 rows of 8 polyethylene sachets containing the repellent, stapled to strawberry growbags, 1 approximately every 2 m. The central square of repellents was 5.5 m from the plot perimeter on all sides. Sixteen coded traps containing the lure, positioned at crop height along the plot perimeter – four per side, spaced every 5 m. Traps were positioned 5.5 m away from the repellents.
- **Control** - no push or pull.

To assess *D. suzukii* egg laying (Table 2.1.2.), 6 delta traps containing a Petri dish with egg laying bait were deployed in the central 2 rows of all plots, 5.5 m in from each end, and spaced approximately 7 m apart in each row.

Four Droso traps were also deployed around each block – 1 adjacent to each plot, approximately 20 m away, hanging at crop height in the surrounding hedgerow.

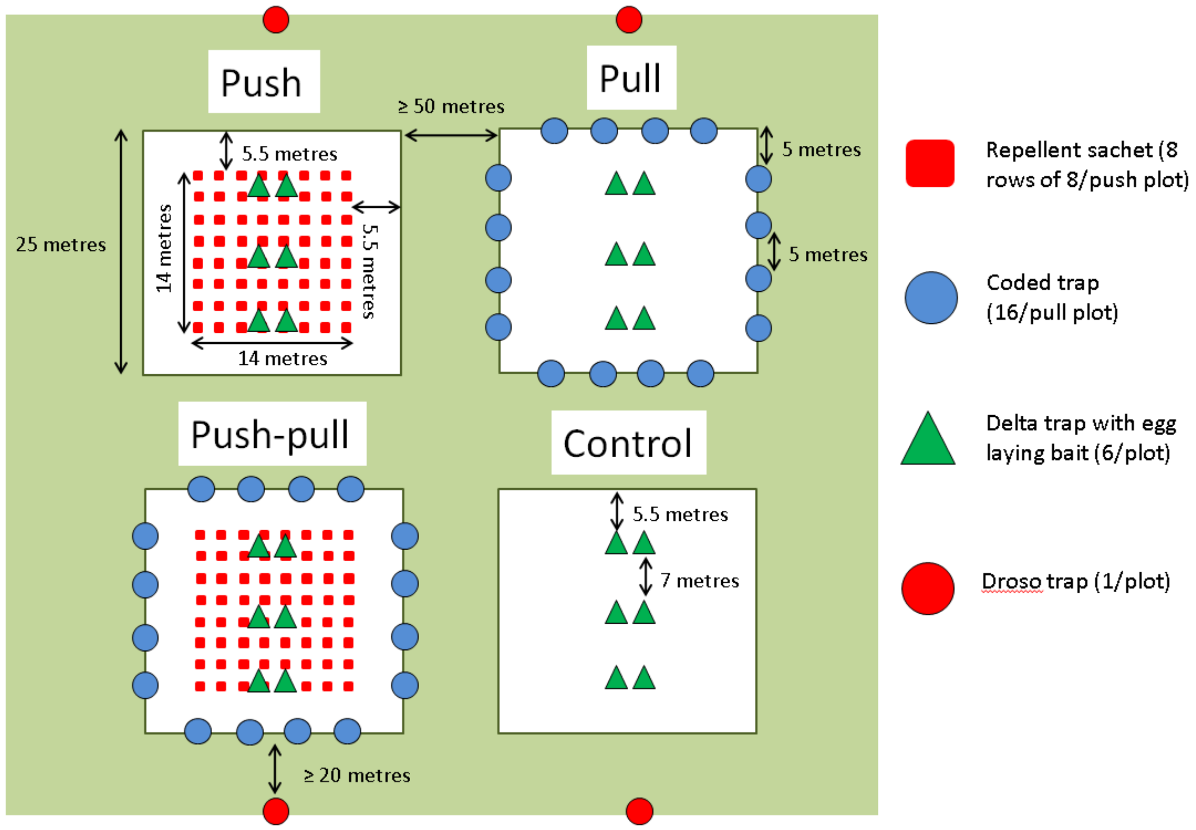


Figure 2.1.2. Diagrammatic representation of an experimental block of the push-pull trial, showing: **Push** plot with 14 x 14 m of repellent sachets in centre, **Pull** plot with no repellents and 16 coded traps every 5 m around the perimeter, **Push-Pull** plot with 14 x 14 m of repellent sachets in centre and 16 coded traps every 5 m around the perimeter of the plot, **Control** plot with no push or pull. Plots were spaced ≥ 50 m apart. Six delta traps with egg laying bait were deployed in the central two strawberry rows of all plots. A Droso trap was also positioned ≥ 20 m on one side of the perimeter of each plot

Crop husbandry involved the standard grower practices, including the grower's standard spray programme. The grower was advised that non-essential insecticide sprays should be avoided to prevent target pests and beneficials being affected. A copy of the spray programme was provided to NIAB EMR after the trial (APPENDIX 2.1.1).

Table 2.1.1. Date of trial setup, repellent renewal and trial end at all four sites during the *D. suzukii* push-pull trial. Coded trap lures in the pull traps lasted a season so were not replaced.

Date	**Site 1**	**Site 2**	**Site 3**	**Site 4**
11 May	Trial setup		Trial setup	Trial setup
23 May		Trial setup		
8 June	Repellents replaced			
10 July	Repellents replaced			
10 Aug	Repellents replaced			
18 Sep	Repellents replaced			
4 Oct	End of trial			

Assessments: To determine whether the push, pull and push-pull treatments could significantly reduce the numbers of *D. suzukii* in the crop, the following assessments were done at all 4 trial sites throughout the trial (Table 2.1.2.);

1. Droso trap count – all plots

To compare numbers of *D. suzukii* around each of the plots, a Droso trap containing Dros'Attract, was positioned in the perimeter hedgerow adjacent to each plot. Every two weeks following the trial start, *Drosophila* were removed from the traps and retained and Dros'Attract replaced. *D. suzukii* were identified, sexed and counted at NIAB-EMR. Other *Drosophila* were also counted.

2. Commercial (coded) trap count – pull and push-pull plots only

To find out if the push component could be improved when combined with a pull, 16 commercial (coded) traps were set up for A&K along the perimeter of pull only and push-pull treatment plots. Every two weeks following the trial start, *Drosophila* were removed from all traps. From eight of these traps (the two middle traps on each side of the plot) *Drosophila* were retained and returned to NIAB EMR where *D. suzukii* were identified, sexed and counted. Other *Drosophila* were also counted.

3. Strawberry emergence count – all plots

To compare *D. suzukii* egg laying within the crop, between treatments, samples of 100 strawberries were collected within a central 14 x 14 m square, 5.5 m from the plot perimeter on all sides. This occurred on four occasions. Fruit was stored in Perspex boxes (25 fruit per box) with mesh lid and incubated at approximately 23 °C for two weeks at NIAB EMR. During this period, *D. suzukii* adults were removed, sexed and counted. Other Drosophila were also counted.

4. Egg laying bait count – all plots

To further compare *D. suzukii* egg laying within the crop between treatments, an egg laying bait developed at NIAB EMR, comprising grape agar with yeast, was deployed on three occasions. During the trial, crops in all plots received PPP sprays to control *D. suzukii.* Sprays were predicted to prevent egg laying into fruit. The purpose of the egg laying bait was to enable Drosophila egg, *D. suzukii* and other Drosophila numbers to be measured without insecticide residues. On the first two deployments (5 May and 22 June), three green delta traps, each containing a Petri dish with egg laying bait, were placed in the central row of each plot at crop height, 10 m apart. However, due to large variations in egg numbers between replicate plots, from the 3rd deployment (19 July) onwards, six green delta traps, each containing a Petri dish with egg laying bait, were deployed. These were positioned in the central two rows of each plot at crop height, 10 m apart per row. Petri dishes with egg laying bait remained in each delta trap for a maximum of 48 hours before removal. Subsequently, the numbers of eggs laid within, and on the surface of the grape agar with yeast were counted using a light microscope (x6 magnification). As *D. suzukii* eggs were sometimes difficult to distinguish from those of other Drosophila, following egg counting, Petri dishes with egg laying bait were stored in Perspex boxes (one per box) and surrounded by three frozen strawberries in which *D. suzukii* and other Drosophila larvae could develop to adult for identification. The Perspex box was sealed with a mesh lid and incubated at approximately 23 °C for two weeks. During this period, emerged *D. suzukii* adults were removed, sexed and counted. Other Drosophila adults were also counted.

Because of the time taken to assess each site it was not possible to visit each site on the same day and hence site visits were rotated (Table 2.2.1).

Table 2.1.2. Assessment dates at all four sites during the *D. suzukii* push-pull trial.

Date	Droso traps	Commercial (coded) traps	100 Strawberry sample	Egg laying bait
25 May	X	X		
6 Jun	X	X		
8 Jun				
20 Jun	X	X		
22 Jun			X	X
4 Jul	X			
11 Jul		X		
17 Jul	X			
19 Jul			X	X
20 Jul		X		
31 Jul	X	X		
16 Aug	X	X	X	X
4 Sep	X	X		
13 Sep				
18 Sep	X	X		
20 Sep			X	
4 Oct	X	X		

Statistical analysis

1. Droso trap count – all plots: ADULT SWD - Analysed as a GLMM with Distribution = Poisson & log-link. Fixed effects: Block +Treatment*Date_sampled. Random effects: Block.Treatment. Dispersion = Estimate. Other Drosophila - Analysed as a GLM with Distribution = Poisson & log-link. Fixed effects: Block + Push*Pull*Date_sampled, Dispersion = Estimate (GLMM not used as Block.Treat Error term -ve).

2. Commercial (coded) trap count – pull and push-pull plots only: ADULT SWD and Other Dropsophila - Analysed as a GLMM with Distribution = Poisson & log-link. Fixed effects: Block

+ Treatment*Date_sampled. Random effects: Block.Treatment/Trap_position, Dispersion = Estimate.

3. Strawberry emergence count – all plots: ADULT SWD and Other Dropsophila - Analysed as a GLMM with Distribution = Poisson & log-link. Fixed effects: Block + Treatment*Date_fruit_collected. Random effects: Block/Treatment, Dispersion = Estimate.

4. D. suzukii egg laying and adult count – all plots: Eggs - Analysed as a GLMM with Distribution = Poisson & log-link. Fixed effects: Block + Treatment*Date_collected. Random effects: Block.Treatment/Replicate, Dispersion = Estimate. Last date missing.

Total SWD - very few values - Analysed as a GLM with Distribution = Poisson & log-link. Fixed effects: Block + Treatment*Date_collected, Dispersion = 1.

Other Drosophila - Analysed as a GLMM with Distribution = Poisson & log-link. Fixed effects: Block + Treatment*Date_collected. Random effects: Block.Treatment/Replicate. Dispersion = Estimate

*For all statistical analyses data was log transformed.

Results

Droso trap count – all plots

Following statistical analysis of mean numbers (log transformation) of adult *D. suzukii* caught in the Drosotraps on the perimeters of the plots found no significant difference between the treatments (Grand mean = 357) and other adult Drosophila (Grand mean = 93).

Experiment site did have a significant effect on mean numbers of adult *D. suzukii* (P = 0.001, s.e.d. = 0.903, l.s.d. = 2.051) and other adult Drosophila (P<0.001, s.e.d. = 0.314, l.s.d. = 0.622), with significantly more caught at Sites 2 and 4 (Fig. 2.1.3).

Assessment date also impacted numbers of adult *D. suzukii* (P = 0.001, s.e.d. = 0.903, l.s.d. = 2.051) and other Drosophila (P<0.001, s.e.d. = 0.314, l.s.d. = 0.622), with mean numbers of *D. suzukii* remaining comparatively low between 25 May and 4 September (Actual mean = 20 per trap) then increasing significantly to the last sample on 4 October (Actual mean = 3159 per trap) (Fig. 2.1.8). Overall, mean numbers of other adult Drosophila were lower than *D.*

suzukii (Grand mean = 91 and 349 respectively), remaining unchanged between 25 May and 4 September (Actual mean = 19 per trap), then increasing significantly to the last sample on 4 October (Actual mean = 717 per trap) (Fig. 2.1.4).

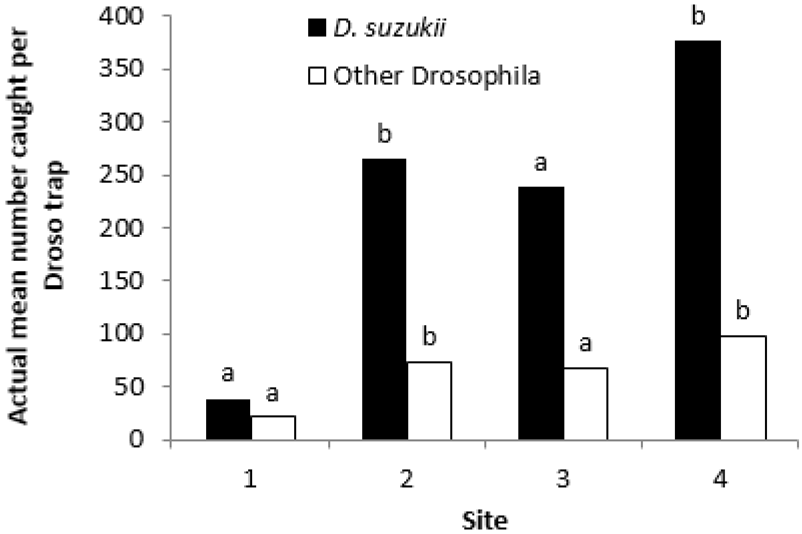


Figure 2.1.3. Actual mean numbers of adult *D. suzukii* and other Drosophila caught per Drosotrap between sites, averaged across all plots

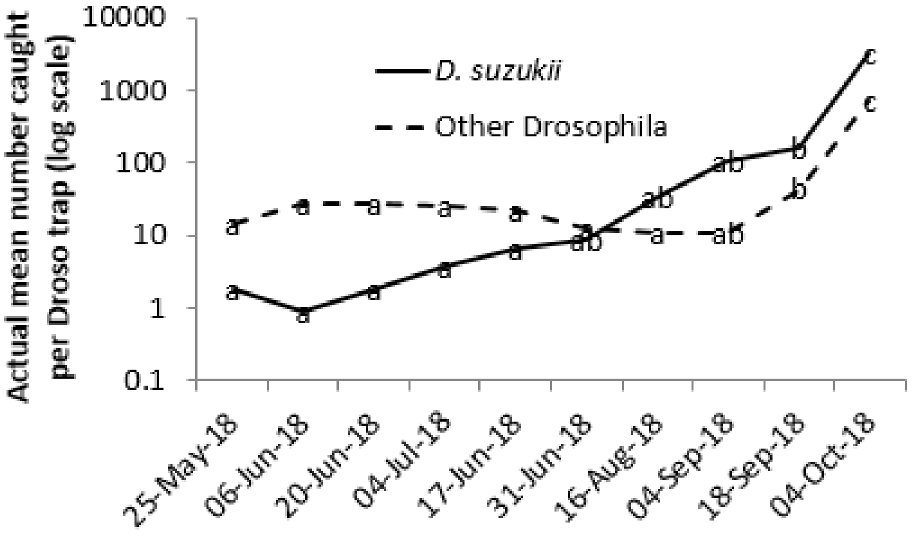


Figure 2.1.4. Actual mean numbers of adult *D. suzukii* and other Drosophila caught per Droso trap between assessment dates, averaged across all sites

Commercial (coded) trap count – pull and push-pull plots only

Following statistical analysis of log mean numbers of Drosophila caught in the coded traps throughout the trial, the push-pull treatment was found to have a significant effect on mean numbers of other Drosophila (P = 0.012, s.e.d. = 0.204, l.s.d. = 0.409), whereby mean numbers caught in coded traps were significantly higher when a semiochemical repellent was deployed in the centre of the plot (Back transformed mean = 12.7) than without the push (back transformed mean = 5.166) (Figure 2.1.5). Treatment had no significant effect on adult *D. suzukii* (Grand mean = 92).

Experiment site had a significant effect on mean numbers (on a log scale) of other adult Drosophila (P<0.001, Av. s.e.d. = 0. 0.232, Av. l.s.d. = 0.463), with significantly more caught at Sites 1 and 2 and most at Site 2 (Figure 2.1.6). However there was no significant effect on adult *D. suzukii* (Grand mean = 92).

Assessment date had a significant effect on mean numbers (on a log scale) of adult *D. suzukii* (P<0.001, Av. s.e.d. = 0.945, Av. l.s.d. = 1.857) and other Drosophila (P<0.001, s.e.d. = 0.314, l.s.d. = 0.622), with actual mean numbers of *D. suzukii* remaining comparatively low between 25 May and 4 July (Actual mean = 2.2 per trap) then increasing significantly to a peak on 4 September (Actual mean = 337.9 per trap), before decreasing significantly to the last sample on 4 October (Actual mean = 173.8 per trap) (Figure 2.1.8). Overall, mean numbers of other adult Drosophila were lower than *D. suzukii* (Grand mean = 29.8 and 90 respectively), increasing significantly from the trial start to a peak on 4 September (Actual mean = 63.2 per trap), before decreasing to the last sample on 4 October (Actual mean = 50.7 per trap) (Figure 2.1.4).

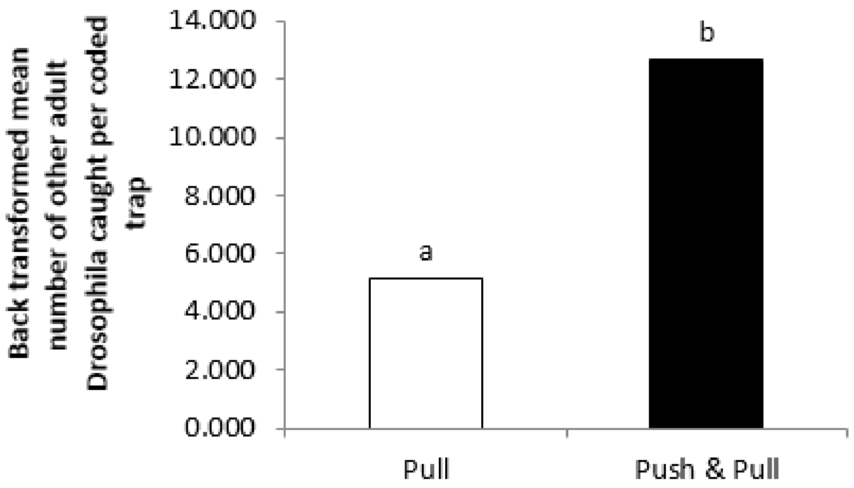


Figure 2.1.5. Back transformed mean numbers of 'other' adult Drosophila caught per coded trap between treatments, averaged across all plots

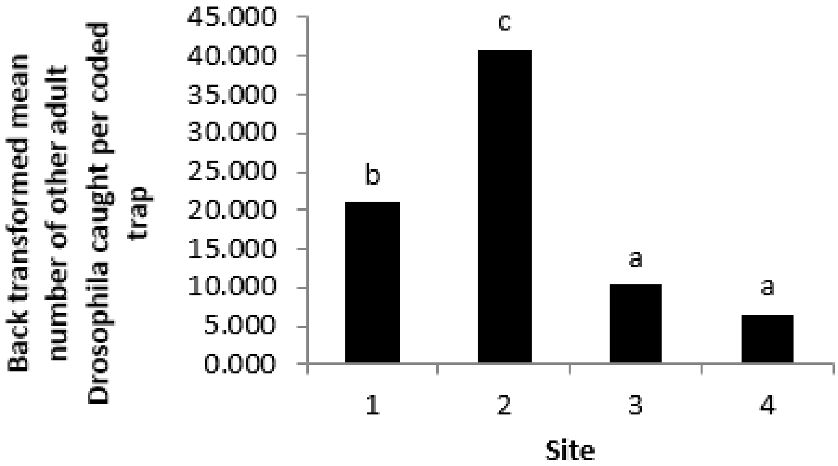


Figure 2.1.6. Back transformed mean numbers of other adult Drosophila caught per coded traps between sites, averaged across all plots

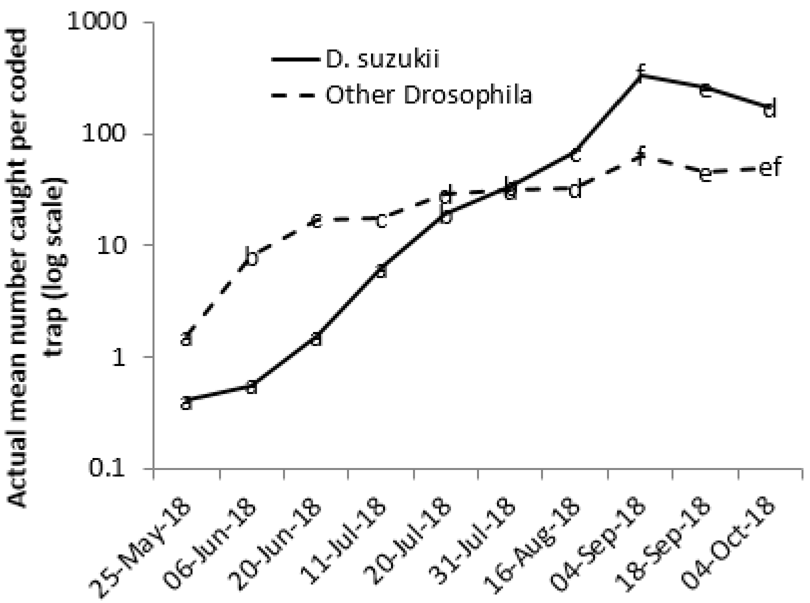


Figure 2.1.7. Mean numbers of adult *D. suzukii* and other Drosophila caught per coded trap between assessment dates, averaged across all sites

Emergence from fruit – all plots

Treatment had no impact on the numbers of 'other' Drosophila (Grand mean = 5.9) or *D. suzukii* (Grand mean = 16.175) that emerged from samples of 100 strawberries throughout the trial.

Experiment site also had no significant effect on mean numbers (on a log scale) of adult *D. suzukii* and other Drosophila.

Assessment date was significant. Mean numbers of *D. suzukii* (P<0.001) and other Drosophila (P<0.001), remained comparatively low between 16 June and 19 July (Actual mean = 2.6 per 100 fruit) before increasing significantly to a peak on 4 September (Actual mean = 39.4 per 100 fruit), then decreasing slightly – but not significantly to the last sample on 13 September (Actual mean = 33.6 per 100 fruit) (Figure 2.1.8). Overall, numbers of other Drosophila were lower than *D. suzukii* (Grand mean = 5.9 and 16.2 respectively), remaining unchanged between 16 June and 16 August (Actual mean = 0.125 per 100 fruit), then increasing significantly to the last sample on 13 September (Actual mean = 21.3 per 100 fruit) (Figure 2.1.8).

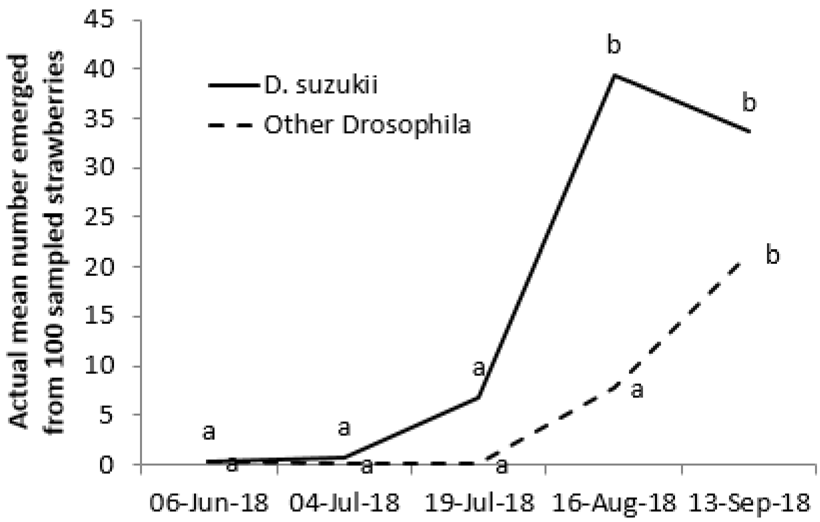


Figure 2.1.8. Mean numbers of adult *D. suzukii* and other Drosophila emerged from 100 sampled strawberries between assessment dates, averaged across all sites

Drosophila egg laying and adult count – all plots

Overall, there were too few adult *D. suzukii* for statistical analysis (Total number = 4).

Following statistical analysis of mean numbers of ‘other’ Drosophila counted from egg laying bait throughout the trial, treatment was found to have no significant effect on eggs (Grand mean = 11) or ‘other’ adult Drosophila (Grand mean = 18).

Experiment site had a significant effect on numbers of Drosophila eggs in the bait (P = 0.045, s.e.d. = 0.521, l.s.d. = 1.171) with significantly more at Site 2 (Figure 1.9). However, site had no significant effect on mean numbers of ‘other’ adult Drosophila deriving from the egg laying bait (Grand mean = 18).

Assessment date had a significant effect on numbers of Drosophila eggs laid in bait (P<0.001, Av. s.e.d. = 0.773, Av. l.s.d. = 4.193) and ‘other’ adult Drosophila deriving from egg laying bait (P<0.001, Av. s.e.d. = 0.485, Av. l.s.d. = 0.956), with fewest Drosophila eggs on 19 July (Actual mean = 2.6) and most on 16 August (Actual mean = 21) (Fig. 2.1.10). Overall actual mean numbers of ‘other’ adult Drosophila was higher than Drosophila eggs (Grand mean = 15.4 and 10.1 respectively), remaining significantly unchanged between 22 June and 19 July (Actual mean = 0.7 per egg laying bait), then significantly increasing to a peak on 16 August (Actual mean = 48.4 per egg laying bait), before decreasing significantly to the last sample on 20 September (Actual mean = 11.8 per egg laying bait) (Figure 2.1.10).

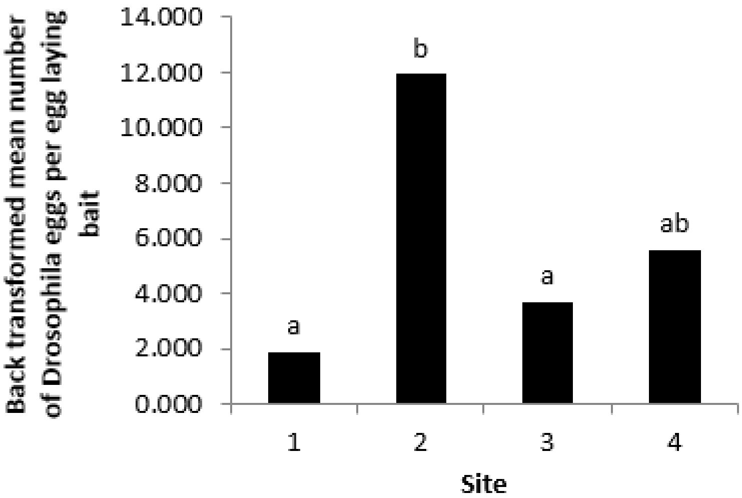


Figure 2.1.9. The back transformed mean numbers of Drosophila eggs counted in egg laying bait between sites, averaged across all plots

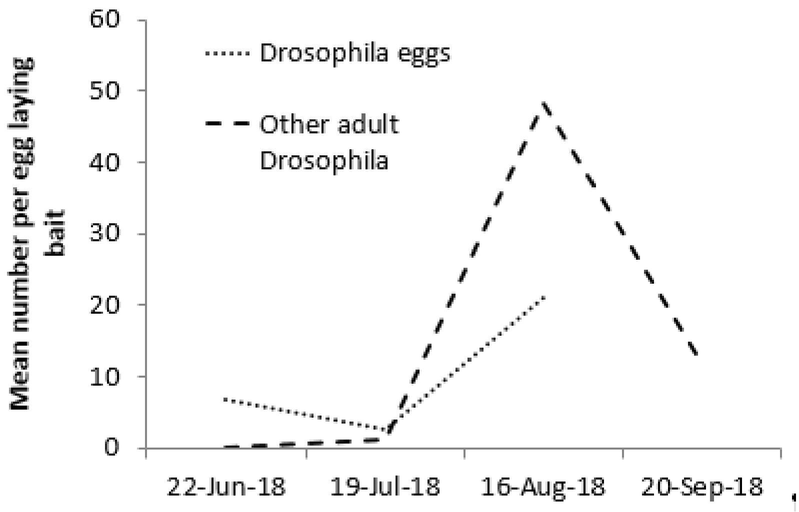


Figure 2.1.10. Actual mean numbers of Drosophila eggs and other adult Drosophila counted per egg laying bait between assessment dates, averaged across all sites

Discussion

Following encouraging findings in 2016 and 2017 for a potential repellent blend to deter *D. suzukii* from egg laying within a commercial crop, in 2018 we set out to further investigate the potential of a push-pull system for controlling *D. suzukii* by conducting field trials in commercially grown strawberry.

A Droso trap containing Dros'Attract, was positioned in the perimeter hedgerow adjacent to each plot to compare numbers of *D. suzukii* and other adult Drosophila. A comparison of Droso trap counts during the trial suggests that pest pressure was equal between plots. Following statistical analysis of mean numbers (on a log scale) of Drosophila caught in the Droso traps throughout the trial, plot type did not have a significant effect on adult *D. suzukii* (Grand mean = 357) and other adult Drosophila (Grand mean = 93).

In general, numbers of Drosophila remained very low between 25 May and 4 September before a significant increase from 18 September to 4 October, when the trial finished. Low Drosophila trap catches between 25 May and 4 September can be attributed to competition from the strawberry crop. During that period the fruit would have been more attractive to Drosophila than the Drosotraps. After 4 September when the strawberry growing period drew to a close, traps had less competition from fruit hence the increase in Drosophila numbers.

To test whether the repellent blend of compounds in sachets (push) in combination with an attractant (pull) could deter *D. suzukii* egg laying within the strawberry crop, on four occasions, samples of 100 strawberries were taken and on three occasions egg laying bait was deployed within each plot. Subsequently, mean numbers of *D. suzukii* and other adult Drosophila deriving from both types of sample were compared. Following statistical analysis treatment (push, pull, or push-pull) had no significant effect on *D. suzukii* (Grand mean = 16.175) and other Drosophila (Grand mean = 5.9) numbers. From egg laying bait, treatment had no significant effect on mean numbers of Drosophila eggs (Grand mean = 11) or adults (Grand mean = 18). Overall, too few adult *D. suzukii* were found for statistical analysis (Total number = 4).

There is insufficient evidence from the 2018 trial that the combination of a repellent and a commercially available attractant trap reduced number of *D. suzukii* in the strawberry crops. The trial was hampered by an inability to be able to assess the numbers of *D. suzukii*, either because routine sprays for *D. suzukii* were applied to the fruit diluting any potential effect or because *D suzukii* was outcompeted from laying eggs on the insecticide free egg laying bait deployed within the crop by 'other' Drosophila (Total number = 4 compared to 5898). When nutritionally challenged, *Drosophila melanogaster* larvae, are known to consume a diet composed of conspecifics and even eggs of their own species (Ahmad et al. 2015). In future trials, deployment of sentinel fruit, free of insecticide residue, that favour *D. suzukii* egg laying, might overcome this shortcoming.

Despite push-pull treatments failing to reduce mean numbers of *D. suzukii* in the crop, they did have a significant effect on mean numbers of 'other' adult Drosophila (P = 0.012, s.e.d. = 0.204, l.s.d. = 0.409). Throughout the trial, mean numbers caught in coded traps were significantly higher when the push was used in conjunction with a pull (Back transformed mean = 12.7) than when the pull was used alone (back transformed mean = 5.166). However, the mean numbers of other Drosophila within the crop was not affected so this finding should be treated with caution.

There was a significant difference in the mean number of Drosophila caught in traps between sites. Significantly more adult *D. suzukii* and other adult Drosophila were found at Sites 2 and 4. Numbers of 'other' adult Drosophila were higher at Sites 1 and 2, with most at Site 2. It is possible that areas surrounding sites with higher mean Drosophila numbers had a greater occurrence of natural hosts such as blackberry, elderberry and varieties of current, which increase the natural population size, however this was not scored.

Future work

- In 2019 it is anticipated a more focused semi-field study will be done to determine the repellent activity of the blend and some new potential repellents as part of the Berry Gardens CTP PhD studentship.
- Once this finer detailed study is complete, and if successful, we will follow this with re-testing on a commercial crop scale.

Task 2.2. Verify efficacy of Attract and Kill device in presence of fresh fruit (Yrs. 1-2; NIAB, NRI)

Introduction

After preliminary development trials, in 2015, 2016 and 2017 a prototype attract and kill (A&K) prototype device (hereon in referred to as just ‘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 affordable to the grower;
2. Relatively small size;
3. Lures should be attractive to *D. suzukii*, but small 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.

The optimal characteristics (shape, colour, size, lure and killing agent), of the final “attract and kill” device were done in 2017. Based on these findings, this year, we conducted two trials to establish if:

- in the presence of fresh fruit, the A&K device was less effective at killing *D. suzukii,*
- the A&K device was more effective at killing mated or unmated *D. suzukii* females in the presence of fresh fruit.

Methods

Our A&K device was compared to two other commercial standards one with and insecticide coating and one without. An untreated, control, trap was used for comparison. All trials were set up in fibreglass framed cages with insect proof mesh, 43 x 43 x 95 cm (Bugdorm). Cages were located 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). Humidity was maintained by spraying the experimental area with tap water and adding wet paper to each cage.

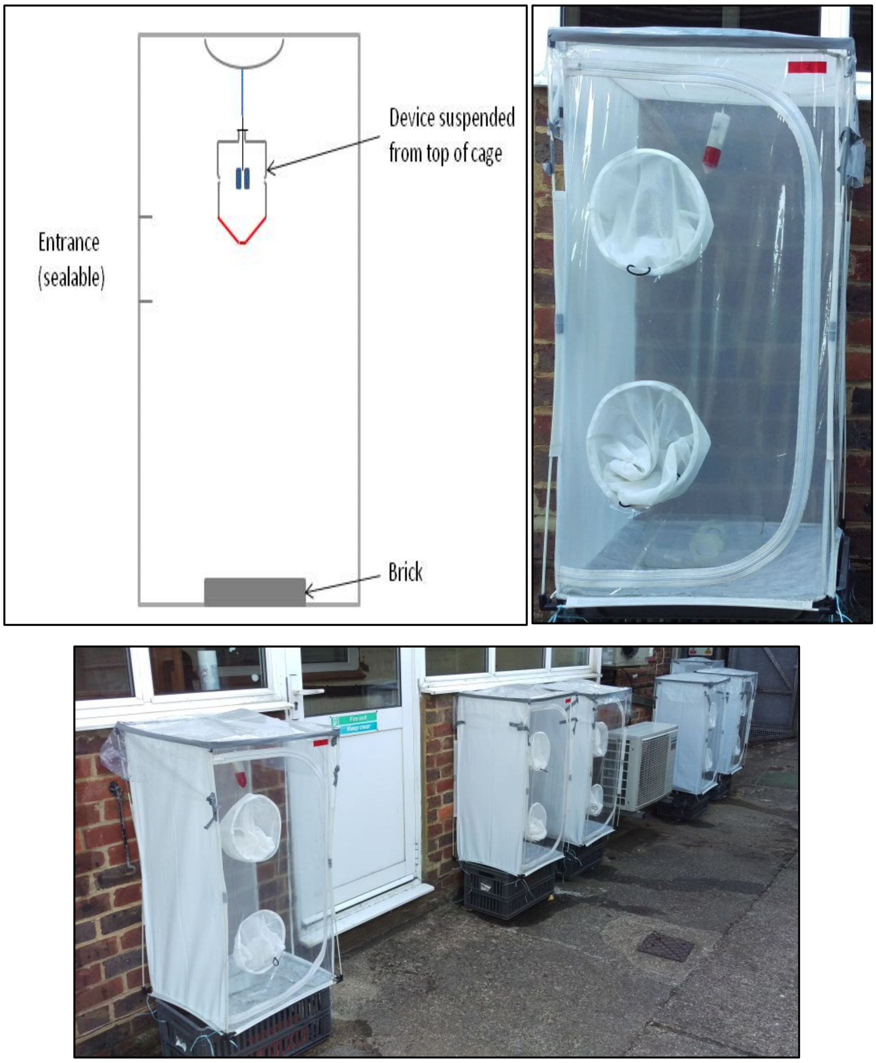


Figure 2.2.1. Graphic and photographs of experimental set up; device hanging from top of cage

The lures used in the device 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). Commercial Trap A (coded) contained its own bait and Commercial Trap B contained the mini Cha-Landolt lure. Both commercial traps were much larger than the prototype device and did not allow insects to leave. All experimental devices, with the exception of the untreated controls, were coated on the inside with the Decis formulation (deltamethrin, 64 mg per 1 ml of distilled water). 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: polyethylene sachets containing ethanol/ acetoin, acetic acid and methionol

In Trial 1, 10 male and 10 female, 3 to 12 day old, mated *D. suzukii,* from a laboratory culture, were introduced at time zero (each day at 16:30) to each cage. After 24 hours the devices were removed and numbers of live and dead *D. suzukii* were counted. Also Perspex boxes (10 x 10 x 20 cm) each containing a moist paper towel and either with or without 10 ripe strawberries (approx. same size), were placed in the bottom of the cage (Table 2.2.1). To ensure strawberries used in the trial were free of insecticide, prior to the experiment 10 male and 10 female *D. suzukii* were released in the Perspex boxes with 10 fruits. Flies were then evaluated for mortality after 48 hours (Beers et al., 2011). No dead flies were recorded after the bioassay indicating fruit was suitable for the trial. Devices were 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 2.2.3). The eight cages remained in the same position and traps were re-randomised after each replicate run (a replicate run was 24 hours).

The following four devices were tested:

1. A Falcon tube with the NRI dry mini-lure coated on the inside with Decis
2. A Falcon tube with the NRI dry mini-lure but no insecticide coating (control)
3. A commercial trap with lure and Decis coating on the lid (coded A)
4. A commercial trap with NRI lure and no insecticide coating (coded B).

The four devices were compared with and without fresh strawberries placed in the bottom of the cage (Table 2.2.1). The experiment took place in September.

In Trial 2, 10 unmated 0 to 1 day old *D. suzukii* females, or 10 mated 3 to 12 day old females were introduced at time zero (each day at 16:30) to each cage (Table 2.2.2). Unmated females were collected every hour from a laboratory culture set up the same day as each replicate. Females were separated from the males to ensure no mating had occurred (Wong et al, 2017). After 24 hours the devices were removed and numbers of live and dead *D. suzukii* were counted. Perspex boxes (10 x 10 x 20 cm) containing a moist paper towel and 10 ripe strawberries were placed in all cages. The position of *D. suzukii* within the cage (on strawberries or cage) was also recorded during the assessment and after the mortality assay females were kept and dissected to assess ovary development. Devices were as for the Trial 1 but fresh strawberries were placed in all cages. In this experiment the efficacy of the devices at killing mated or unmated *D. suzukii* female was tested (Table 2.2.2). The experiment took place in October when *D. suzukii* is known to still be active. There were seven replicate days, in total, for each experiment.

Statistical analysis: In both trials data were analysed in GENSTAT using a generalised analysis of variance, following a SQRT transformation.

Table 2.2.1. Traps, attractants and killing agents used in semi-field cage trial 1. NRI = mini Cha-Landolt bait

Treatment	Trap design	Lure	Presence of fresh fruit Y/N	SWD mated (Y/N)	Insecticide	Insecticide applied to	Surface area (cm^2)	Application rate (mg/cm^2)	Insecticide per ml distilled water
1	50 ml falcon tube	NRI	Y	Y	DECIS WG	Inside surface	101.38	0.63	64 mg/ 0.064 g
2	Commercial trap A	Commercial lure	Y	Y	DECIS WG	Lid	95	0.63	60 mg/ 0.060 g
3 CTRL	50 ml falcon tube	NRI	Y	Y	None	Inside surface	N/A	N/A	N/A
4	Commercial trap B	NRI	Y	Y	None	Lid	N/A	N/A	N/A
5	50 ml falcon tube	NRI	N	Y	DECIS WG	Inside surface	101.38	0.63	64 mg/ 0.064 g
6	Commercial trap A	Commercial lure	N	Y	DECIS WG	Lid	95	0.63	60 mg/ 0.060 g
7 CTRL	50 ml falcon tube	NRI	N	Y	None	Inside surface	N/A	N/A	N/A
8	Commercial trap B	NRI	N	Y	None	Lid	N/A	N/A	N/A

Table 2.2.2. Traps, attractants and killing agents used in semi-field cage trial 2. NRI = mini Cha-Landolt bait

Treatment	Trap design	Lure	Presence of fresh fruit Y/N	SWD mated (Y/N)	Insecticide	Insecticide applied to	Surface area (cm^2)	Application rate (mg/cm^2)	Insecticide per ml distilled water
1	50 ml falcon tube	NRI	Y	N	DECIS WG	Inside device	101.38	0.63	64 mg/ 0.064 g
2	Commercial trap A	Commercial lure	Y	N	DECIS WG	Inside device	95	0.63	60 mg/ 0.060 g
3 CONTROL	50 ml falcon tube	NRI	Y	N	None	N/A	N/A	N/A	N/A
4	Commercial trap B	NRI	Y	N	None	N/A	N/A	N/A	N/A
5	50 ml falcon tube	NRI	Y	Y	DECIS WG	Inside device	101.38	0.63	64 mg/ 0.064 g
6	Commercial trap A	Commercial lure	Y	Y	DECIS WG	Inside device	95	0.63	60 mg/ 0.060 g
7 CONTROL	50 ml falcon tube	NRI	Y	Y	None	N/A	N/A	N/A	N/A
8	Commercial trap B	NRI	Y	Y	None	N/A	N/A	N/A	N/A

Figure 2.2.3. A&K Falcon tube device, containing NRI minilure, used in both trials

Results

Semi-Field Cage Trial 1:

Significantly more *D. suzukii* died in the cages which contained Decis coated devices and the Commercial Trap A than in the cages which contained the uncoated devices. In the absence of ripe strawberries, the Falcon tube device was as effective as the Commercial Trap A, killing up to 25% of the flies within 24 hours (Decis treated devices minus control mortality). However when ripe fruit were present in the cages, the efficacy of the devices significantly declined with only 15% of flies found dead at the bottom of the cage (Decis treated devices minus control mortality). There was no significant difference between the Commercial Trap A (22% of mortality) and the other Decis coated devices (16% and 12% mortality for Commercial Trap A and the Falcon tube, respectively, in presence of strawberries). There was no significant difference between Commercial Trap B and the control, either with or without ripe strawberries (Fprob <0.001, sed. 3.48, lsd. 6.98, following SQRT transformation analysis of means, Figure 2.2.4).

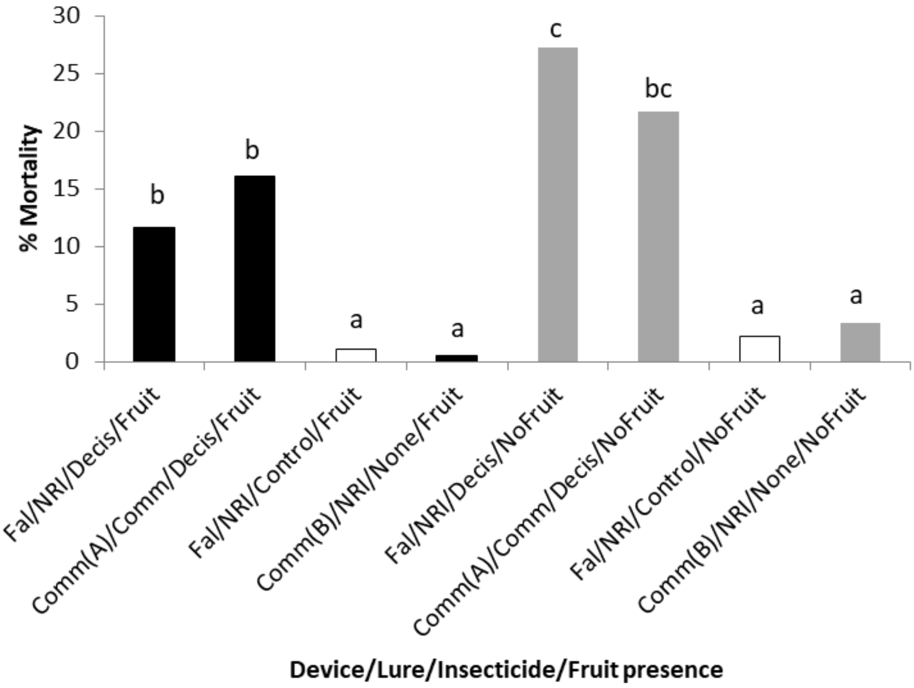


Figure 2.2.4. Mean percentage mortality of *D. suzukii* within 24 hours in field cages containing the prototype attract and kill device compared to two commercial traps (A) (B) and an untreated control either in the presence or absence of ripe strawberries. Axis x label: Fal= Falcon tube, Letter: trap code, NRI = NRI lure, Comm: commercial trap and lure, Fruit/NoFruit = ripe strawberries presence/absence

Semi-Field Cage Trial 2:

Significantly more *D. suzukii* died in cages which contained Decis coated devices and Commercial Trap A than in the cages which contained the uncoated devices (control and Commercial Trap B. This aligns with results obtained in Trial 1 in presence of ripe strawberries. However in this trial, within 24 hours, in the presence of commercial and Falcon tube devices, up to 17% of the flies died. Interestingly there was no significant difference in mortality between mated and unmated *D. suzukii* females between all the Decis coated devices (Fprob <0.001, sed. 3.22, lsd. 6.50, following SQRT transformation analysis of means, Figure 2.2.5).

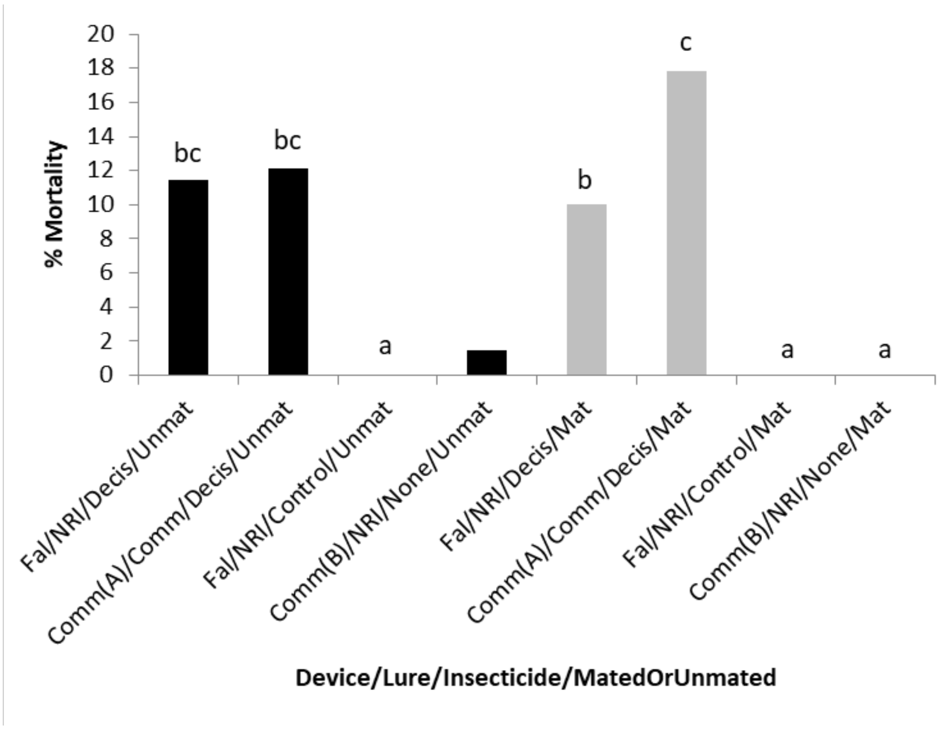


Figure 2.2.5. Mean percentage mortality of *D. suzukii* females within 24 hours either mated or unmated in field cages containing the A&K device compared to two commercial traps (A) (B) and an untreated control. Axis x label: Fal= Falcon tube, Letter: trap code, NRI = NRI lure, Comm: commercial trap and lure, Mat/Unmat = Mated or Unmated.

Analysis of the position of mated or unmated D. suzukii females:

The mating status of the *D. suzukii* females had a significant effect on the position of the flies within the cages. Significantly more (overall mean number = 11) mated females were found on the ripe strawberries than unmated females (overall mean number = 5.37) for all treatments (Fprob <0.001, sed. 0.32, lsd. 0.67, following SQRT transformation analysis of means, Figure 2.2.6).

D. suzukii female ovary development analysis:

All of the unmated dissected flies were at ovarian development 1, therefore no ovaries were distinguishable, and the unmated status was confirmed.

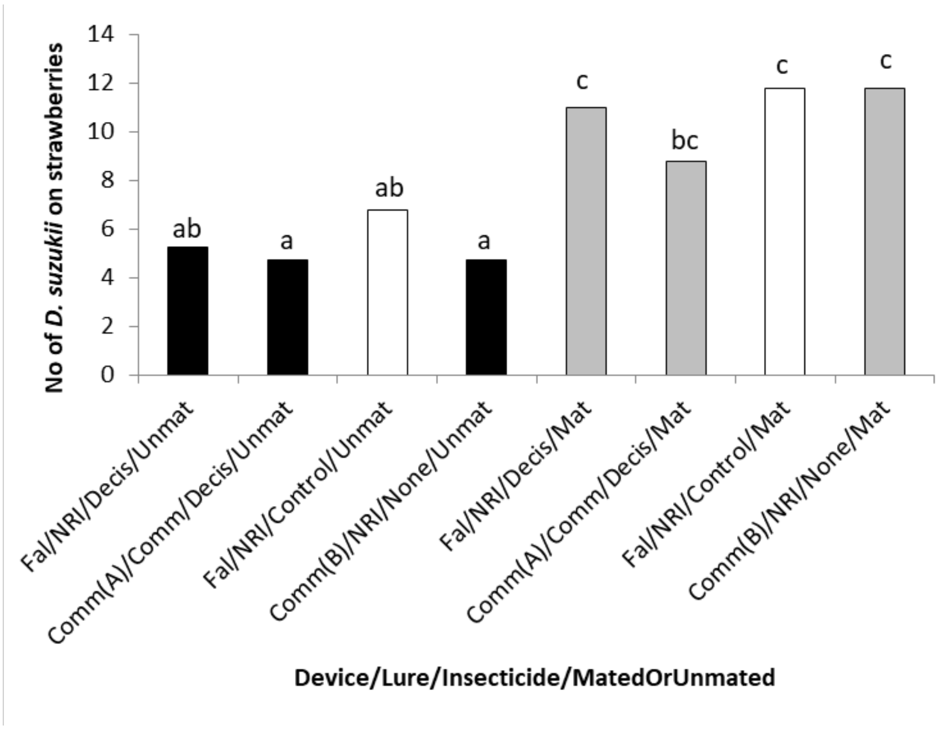


Figure 2.2.6. Mean number of live mated or unmated *D. suzukii* females found on strawberries after 24 hours in field cages containing the attract and kill device compared to two commercial traps (A) (B) and an untreated control. Axis x label: Fal= Falcon tube, Letter: trap code, NRI = NRI lure, Comm: commercial trap and lure, Mat/Unmat = Mated or Unmated

Conclusions

- The prototype Falcon tube A&K device gave up to 25% kill of *D. suzukii* within 24 hours in these semi-field cage trials in the absence of fruit. Compared to the same trial last year, the efficacy of the device declined by 5%.
- The Falcon tube A&K device is confirmed to be as effective as the commercial trap in causing mortality of *D. suzukii*.
- Neither the commercial trap (B) nor the Falcon tube with no insecticide coating were not effective in controlling *D. suzukii* in this trial.
- Importantly, in the presence of ripe fruit, the efficacy of both the Falcon tube device and the commercial trap (A) decreased substantially killing up to only 15% of flies within 24 hours.
- This suggests that these trap/devices should be deployed in early spring. At this time there is no competition with ripening fruit and *D. suzukii* population are at their lowest. A&K devices should be

used within an IPM context and be deployed in large numbers around the outside of crop perimeters and combined with insect meshing the crop to prevent immigration into the crop.

- There was no difference in effectiveness of the devices at controlling mated or unmated females. However, in a recently published study (Wong et al., 2017) unmated flies were more attracted by fermentation odours, as our NRI lure, than fruit odours (Cha et al., 2012), compared to mated females.
- Our study also confirmed that mated females are motivated to spend more time on fruit than away from fruit.
- The presence of fruit with current trap baits based on fermenting volatile odours reduced trap catch and hence killing efficacy.

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, probably because release rates of ethanol and acetic acid from the sachets were 1% and 10% of those from the Cha-Landolt solution respectively.

A mini-lure using smaller polyethylene sachets has been developed for use in Falcon-tube attract-and-kill devices shown to be effective at killing SWD under laboratory conditions. This should have a lifetime of at least six weeks and probably longer in the confines of the Falcon tube.

Previous work showed that increasing the release rate of ethanol or acetic acid did not greatly increase attractiveness to SWD, although increasing the release rate of acetoin could do. Furthermore there were indications that omission of methionol from the lures did not decrease attractiveness.

Aims

The aims of this year's work were:

- To further reduce the size of the mini-lure;
- To evaluate commercial versions of the mini-lures developed by Russell IPM for use in attract-and-kill devices;

- To further optimise the attractiveness of the mini-lure relative to those of Cha-Landolt and commercial wine/vinegar mixture standards.

Methods

Measurement of release rates in the laboratory

Devices were maintained in a laboratory fume hood at 20-22 °C. Release rates were measured by regular weighing. Release of acetoin was measured by collecting volatiles from the device onto Porapak resin and quantitative GC analysis as described previously.

Field trapping tests

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 in either unsprayed cherry orchards or woodlands known to have high populations of *D. suzukii* and catches were recorded weekly. Traps in each block were moved on one place each week to remove bias of positioning. The drowning solution (ethanol/acetic acid, Biobest Dros'Attract solution or 1% boric acid) was renewed each week but the sachets were not.

Catches were sorted, weekly, 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

Experiment 1

The mini-lure consisted of four sachets containing ethanol, acetic acid, 1:1 acetoin/water, and methionol, respectively. This experiment was designed to test:

- whether the acetoin could be dispensed as a solution in ethanol, reducing the number of sachets to three;
- whether the methionol sachet was necessary, potentially reducing the number of sachets to two;
- whether a lure provided by Russell IPM consisting of double blister pack, containing acetic acid in one and acetoin/ethanol in the other was effective (Table 2.3.1).

Release rates from the Russell lure were measured in the laboratory. Results are shown in Figure 2.3.1 and 2.3.2.

Table 2.3.1. Treatments evaluated in Experiment 1 (5 October to 2 November 2018)

Code	Dispenser	Compartments	ethanol	acetic acid	1:1 acetoin: water	methio-nol	1:1 ethanol: acetoin
Minilure standard	Mini sachet	4	x	x	x	x	
Minilure Ac/EtOH	Mini sachet	3		x		x	x
Minilure-met	Mini sachet	3	x	x	x		
Minilure Ac/EtOH-met	Mini sachet	2		x			x
Standard	Stand sachet	4	x	x	x	x	
Standard Ac/EtOH	Stand sachet	3		x		x	x
Standard-met	Stand sachet		x	x	x		
Standard Ac/EtOH-met	Stand sachet	2		x			x
RIPM dry lure	Blister pack	2		x			x
Biobest	Liquid blend	1	Biobest Dros'Attract (300 ml) replaced weekly				
Unbaited	Unbaited trap	-	-				

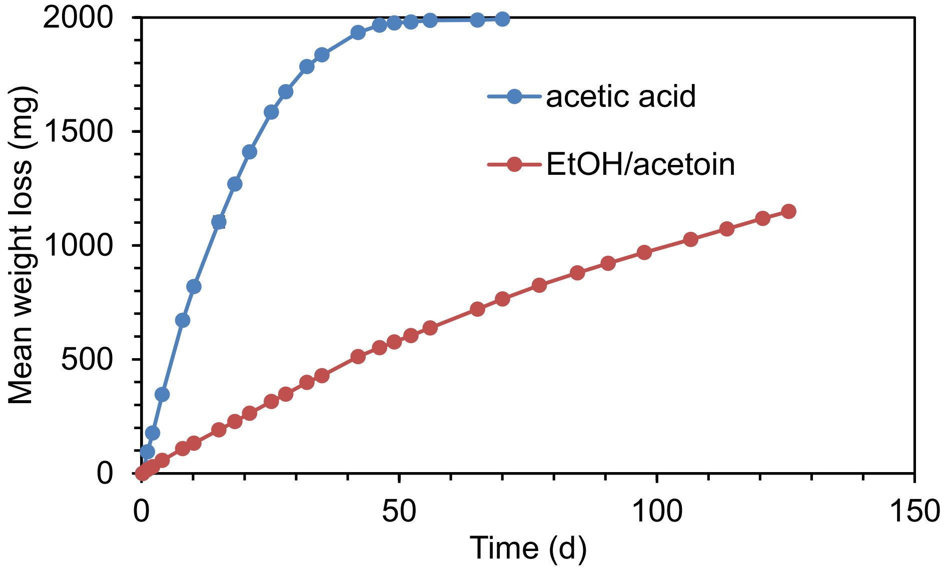


Figure 2.3.1. Release rates from of acetic acid and acetoin in ethanol Russell IPM blister packs measured by weight loss (20-22 °C)

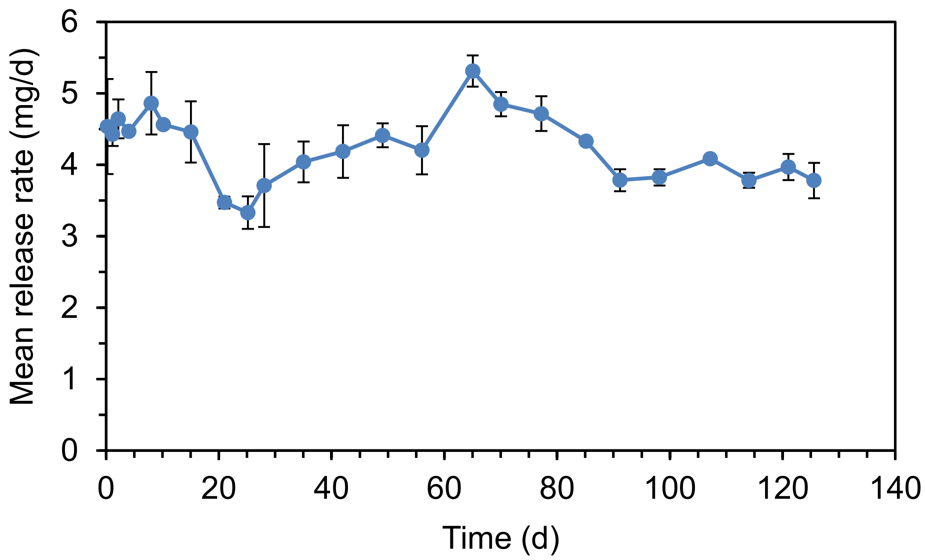


Figure 2.3.2. Release rate of acetoin from Russell IPM blister pack measured by collection of volatiles (20-22 °C)

The release rate of acetic acid was approximately 70 mg/d and lasted for approximately 40 d at 20-22 °C. Release of acetoin and ethanol was approximately 12 mg/d by weight loss and release of acetoin was approximately 4 mg/d by collection of volatiles, so release of ethanol was approximately 8 mg/d. This lasted for over 120 d at 20-22 °C. These figures should be compared with rates for ethanol, acetic acid and acetoin of 38, 18 and 8 mg/d respectively from the standard sachet lure and 19, 9 and 4 mg/d from the mini-lure.

In field trapping tests (Figures 2.3.3, 2.3.4), with both the mini-lure and standard sachet lure replacement of two sachets containing ethanol and acetoin respectively with one sachet containing acetoin in ethanol did not significantly reduce catches. For both devices, with separate ethanol and acetoin sachets and with the combined sachet, omitting the methionol did not significantly reduce catches.

Catches with the mini-lure, standard lure and Russell IPM blister packs were not significantly different.

However, catches with all the "dry" lures were significantly lower than with the Biobest lure. The latter was renewed each week, and the performance of the dry lures relative to the Biobest lure decreased week on week (Table 2.3.2). Even so, the catch with the best dry lure during the first week was only approximately 25% of that with the Biobest lure.

Catches of 'other' *Drosophila* spp. in all traps were > 80% *D. suzukii* and 67% male overall with no obvious consistent differences between treatments.

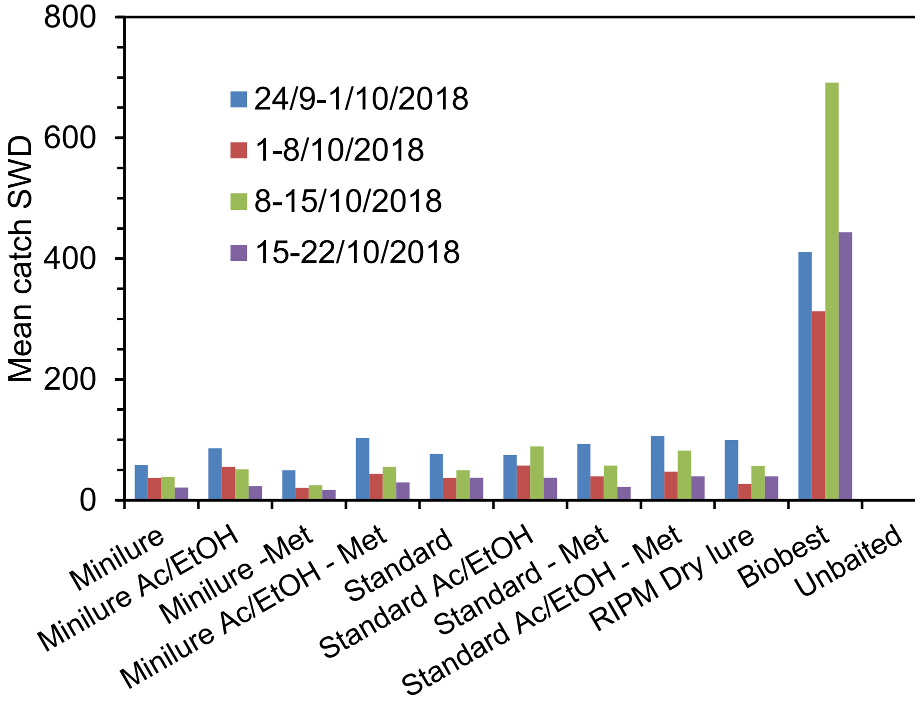


Figure 2.3.3. Mean catches of total *D. suzukii* in Experiment 1 over four weeks

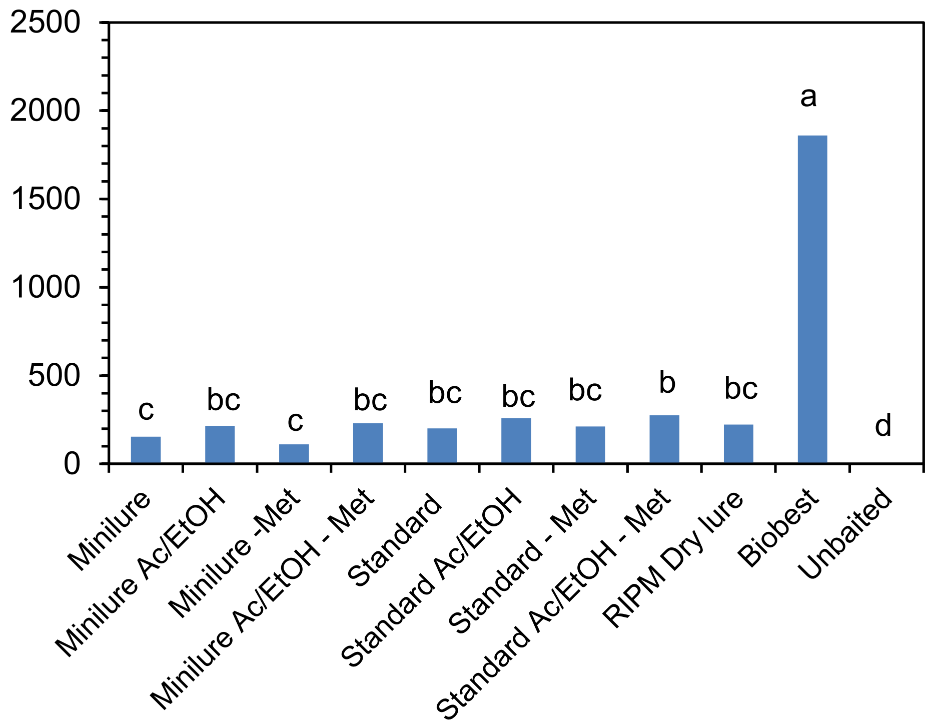


Figure 2.3.4. Overall mean catches of *D. suzukii* in Experiment 1 (5 October to 2 November 2018; *N* = 8; means with the same letter are not significantly different LSD test $P < 0.05$ after ANOVA on data transformed to log(x+1)).

Table 2.3.2. Comparison of catches in all dry lures combined (not renewed) and Biobest (renewed every week) in Experiment 1. It should be noted that the release rate from the Biobest lure was magnitudes higher due to the volume of liquid recommended in the traps.

	Week 1	**Week 2**	**Week 3**	**Week 4**	**Overall**
Dry lures	746.75	364.25	504.5	266.625	1882.125
Biobest	411.125	312.75	691.5	443.75	1859.125
Ratio	1.8	1.2	0.7	0.6	1.0

Experiment 2

In this experiment, two lures from Russell IPM were compared. These were a new version of the double blister pack containing acetoin in ethanol in one and acetic acid in the other, and two pastes containing the same chemicals.

Measurement of release rates in the laboratory showed release from the paste was extremely rapid and essentially complete within 5 d. Release of ethanol/acetoin from the blister pack was slower than the earlier model at approximately 10 mg/d overall with acetoin at approximately 1 mg/d at 20-22 °C (Figures 2.3.5 and 2.3.6).

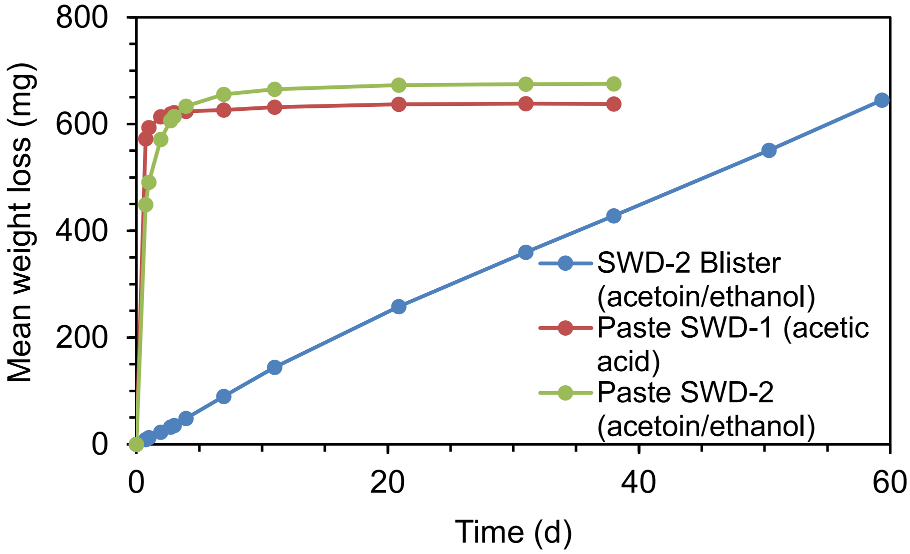


Figure 2.3.5. Release rates from Russell IPM blister pack and pastes as measured by weight loss at 20-22°C

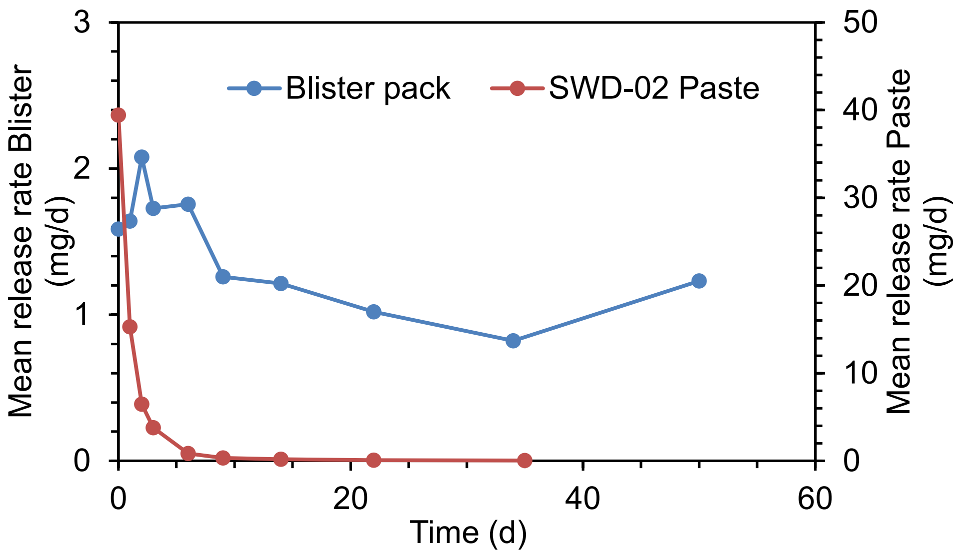


Figure 2.3.6. Release of acetoin from Russell IPM blister pack and paste as measured by collection of volatiles at 20-22 °C

In field tests, traps were baited with (a) two Russell blister packs containing acetoin/ethanol and acetic acid, (b) 2 g of each paste containing acetoin/ethanol and acetic acid respectively, or (c) Biobest Dros'Attract. Traps baited with (a) or (b) contained a drowning solution of 1% boric acid in water only.

Results in Figures 2.3.7 and 2.3.8 show both Russell lures were significantly less attractive than the Biobest mixture and the attractiveness of the paste declined rapidly with time, in line with the laboratory release rate measurements.

Overall, in Experiment 2, catches were 56% male and 62% of *Drosophila* were *D. suzukii*, with no obvious consistent differences between treatments.

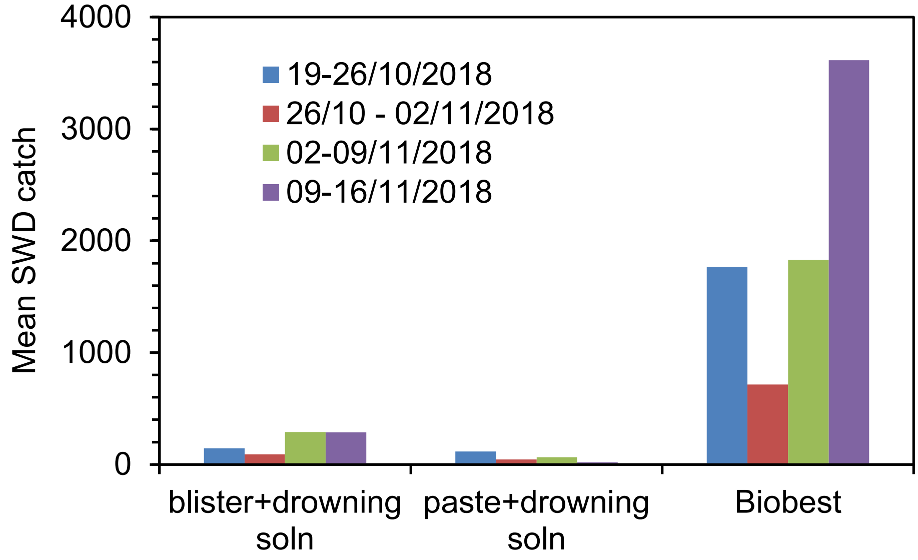


Figure 2.3.7. Mean catches of *D. suzukii* over four weeks in Experiment 2 (N = 10)

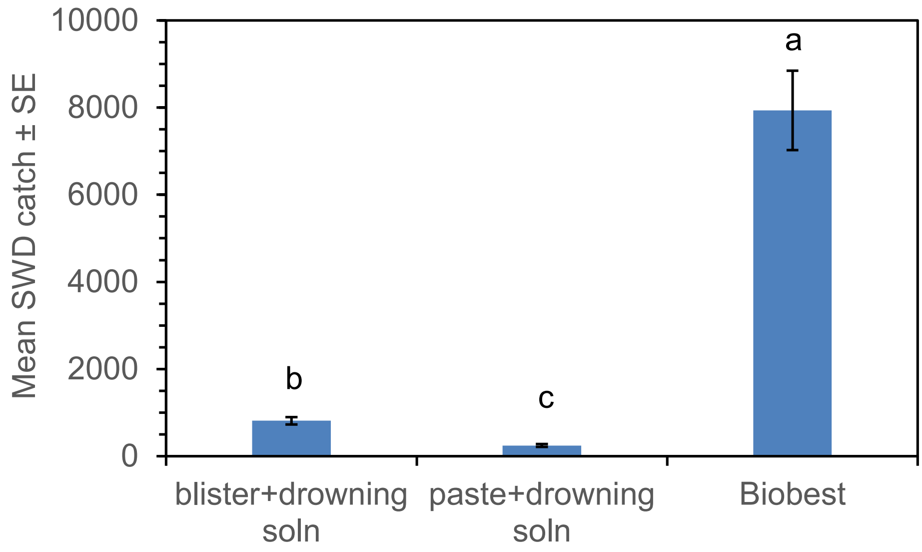


Figure 2.3.8. Overall mean catches of *D. suzukii* in Experiment 2 (19 October to 11 November 2018; N = 10; means with the same letter are not significantly different by LSD test $P < 0.05$, after ANOVA on data transformed to log(x+1))

Experiment 3

In the previous experiments, the dry sachet lures were much less attractive to *D. suzukii* than the Biobest Dros'Attract. A third experiment was conducted to determine whether this was entirely due to differences in chemicals present and/or release rates, or whether the difference was due to the attractants being actually in the drowning solution.

For this the Cha-Landolt lure was used consisting of a drowning solution (300 ml) containing 7.2% ethanol and 1.6% acetic acid and acetoin and methionol dispensed from separate vials with 3 mm diameter holes in the lids. In other treatments the ethanol, acetic acid or acetoin were replaced with standard sachet dispensers (Table 2.3.3)

Table 2.3.3. Treatments evaluated in Experiment 3 (14 November to 11 December 2018)

Code	Treatment
Biobest	Biobest Dros'Attract (300 ml) replaced weekly
Cha-Landolt	Drowning solution (300 ml) containing 7.2% ethanol and 1.6% acetic acid and acetoin and methionol dispensed from separate vials with 3 mm diameter holes in the lids. Drowning solution renewed weekly
C-L EtOH sachet	Cha-Landolt with ethanol in drowning solution replaced by ethanol (2 ml) in Baggie sachet (79 mm x 54 mm x 50 µ)
C-L HOAc sachet	Cha-Landolt with acetic acid in drowning solution replaced by acetic acid (1 ml) in sachet (50 mm x 25 mm x 120 µ)
C-L Acetoin sachet	Cha-Landolt with acetoin vial replaced by sachet (79 mm x 54 mm x 50 µ) containing 2 ml 1:1 acetoin/water
C-L no Met	Cha-Landolt without methionol vial
Sachet	Standard sachet lure with ethanol (2 ml) in Baggie sachet (79 mm x 54 mm x 50 µ), acetic acid (1 ml) in sachet (50 mm x 25 mm x 120 µ), sachet (79 mm x 54 mm x 50 µ) containing 2 ml 1:1 acetoin/water, methionol (1 ml) in sachet (50 mm x 25 mm x 120 µ)
Unbaited	Unbaited trap

The number of *D. suzukii* trapped with the Cha-Landolt lure was only about 20% of that trapped with the Biobest Dros'Attract even though previous tests had shown they were comparable in attractiveness (Figures 2.3.9 and 2.3.10).

Replacing the ethanol or acetic acid in the drowning solution of the Cha-Landolt with these compounds in a sachet reduced catches. Overall the differences were significant but were less so within weeks, at least in part as the drowning solution was renewed each week. This is probably due to the orders-of-magnitude lower release rates: ethanol 3,100 mg/d and 38 mg/d, acetic acid 170 mg/d and 18 mg/d from drowning solution and sachets respectively.

Replacing the acetoin in the vial with the sachet gave an increase in catches, even though the release rates are similar (7-16 mg/d and 8 mg/d respectively).

Removing the methionol from the Cha-Landolt lure did not affect attractiveness.

The sachet lure was less attractive than the Cha-Landolt lure but this is consistent with the much lower release rate of ethanol and acetic acid.

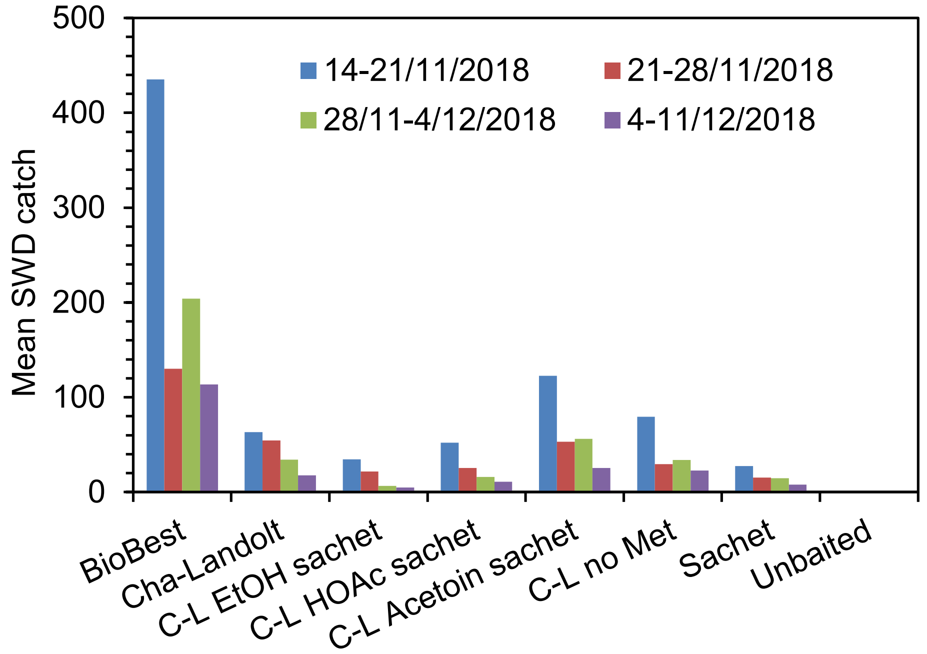


Figure 2.3.9. Mean catches of *D. suzukii* over four weeks in Experiment 3 (N = 8)

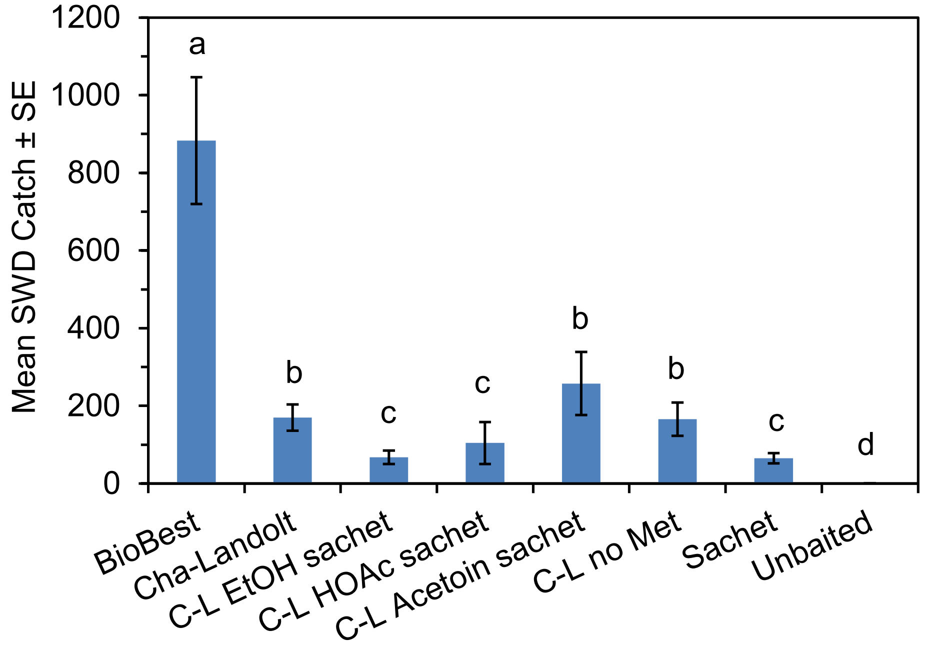


Figure 2.3.10. Overall mean catches of *D. suzukii* in Experiment 3 (14 November – 11 December 2018; *N* = 8; means with the same letter are not significantly different LSD test, $P < 0.05$, after ANOVA on data transformed to log(x+1))

Conclusions

- The mini-lure can be simplified to consist of two sachets containing acetoin/ethanol and acetic acid respectively.
- Previous work has shown that catches with the sachet lure can be at least doubled by increasing the release rate of acetoin, making it similar in attractiveness to the Cha-Landolt mixture currently used in the UK *D. suzukii* National Monitoring Survey.
- Methionol is not necessary in either the sachet lures or the Cha-Landolt. This is an important result as methionol is the most expensive component and the most unpleasant and hazardous. It should also be noted that we have never detected methionol in any of the commercial wine/vinegar lures.
- The greater attractiveness of lures with attractants in the drowning solution over "dry" lures is probably due to large differences in release rate rather to some specific effect of having attractants in the drowning solution.
- In this year's experiments, the Cha-Landolt lure was less attractive than the current Biobest Dro'Attract, even though previous work had shown them to be comparable in attractiveness.
- The Russell IPM lures need further improvement, at least in part due to low release rates of ethanol and acetoin.

Future work

- In 2019 we will aim to determine the volatile attractants in the yeast ferments of attractive yeast species from a recent CTP PhD on attractive yeast strains.

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

Introduction

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.

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) Assess the effect of the optimum bait on the *D. suzukii* control efficacy of different insecticides and concentrations in laboratory bioassays

(2) Assess the effect of the optimum baits on the SWD control efficacy of different insecticides on different leaf surfaces in laboratory bioassays

(3) Assess the effect of the optimum baits on the SWD control efficacy of different insecticides on different leaf surfaces in laboratory bioassays

Task 3.2a. Assess the effect of the optimum bait on the D. suzukii control efficacy of different insecticides and concentrations in laboratory bioassays

Methods

A jar bioassay developed by Mori et al. (2017) and adapted in SF145 was used to test the effect of baits in combination with insecticides to control *D. suzukii.* For the testing, insecticides had to be used at sub-lethal doses in order to detect differences, otherwise, at field rates, all flies died and no comparisons could be made (Table 3.2.1). The concentrations in Table 1 are expressed as a percentage of the recommended rate for strawberries or for cherries (Gazelle), based on 1000 litres spray/ha. Four of the insecticides were used at concentrations that have been shown to be discriminatory between baits in Year 1. Two further insecticides, Gazelle and Pyrethrum, were used at 50% of the recommended rates for cherries and strawberries respectively. The following bait treatments were used with each insecticide or water (control):

(a) *Hanseniaspora uvarum* suspension + sugar 16 g/L (*H. uvarum* applied in a suspension containing 10^9 cfu/ml)
(b) Fermented strawberry juice (produced using a standard method, Noble et al. 2017) + sugar 16 g/L
(c) Fermented strawberry juice + *H. uvarum* suspension + sugar 16 g/L
(d) Combi-protec 5%
(e) Water control

This produced the following 5 bait treatments x 7 insecticide treatments, including a zero control, = 35 treatments. There were four replicates per factorial treatment = 140 containers. The experiment was set up in a randomised block design.

The clear plastic jars (103 mm diameter, 95 mm height) had a 10 mm diameter ventilation hole covered with fine mesh in the opaque screw-on lid and were lined with a moist filter paper in the base (see Image below). The jars contained three blackberry leaves, about 25 × 20 mm; insecticide and/or bait were placed as six 10 µl droplets on two of the leaves, the third leaf had six 10 µl sugar solution (160 g/l) droplets only. A 30 mm Petri dish with grape juice agar was placed as an egg laying medium. Seven mated females and five males of summer *D. suzukii* morphs were placed in each jar. The isolate of *H. uvarum* for the above tests was obtained from Kelly Hamby, UC Davis, California and has been has been shown to be attractive to *D. suzukii* in Year 1 of this project. The filter paper base was rewetted with 1 ml water after one day.

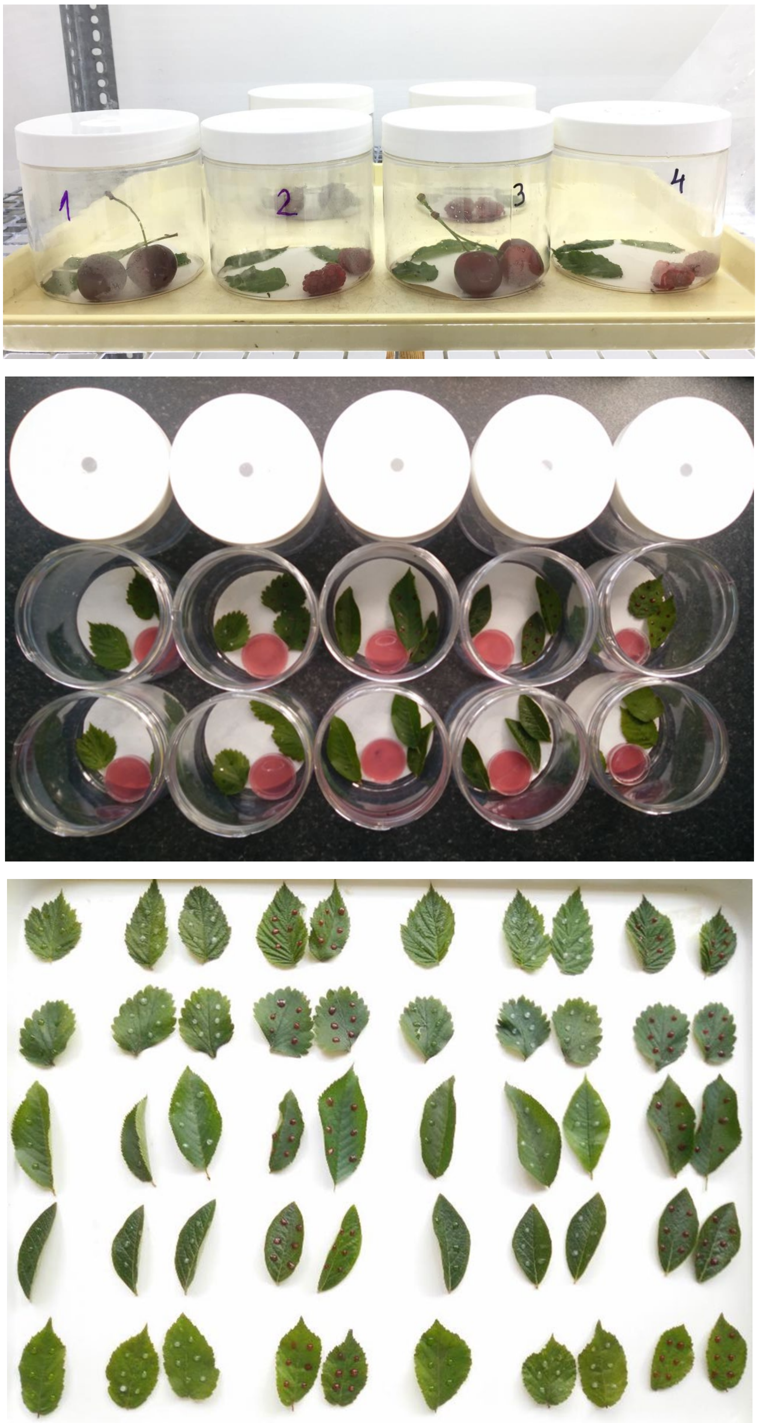
D. suzukii mortality was recorded after one and three days. The number of eggs laid in the egg-laying medium, and any that had hatched into larvae, in each jar was recorded after three days. The Petri dishes were removed from the bioassay jars, covered and kept at 20 °C. Subsequent development of eggs into larvae was recorded seven days after removal from the bioassay jars.

Temperature and relative humidity in the jars were measured by inserting a probe (Vaisala, Finland) connected to a data logger. The jars were kept in natural daylight (16 h: 8h light: dark) but out of direct sunlight.

Results for the bioassays were analysed by ANOVA. A square root transformation was used to homogenise the variances in the treatment means in the oviposition data.

Table 3.2.1. Insecticides, recommended rates for protected strawberries and percentages of recommended rates used for the jar bioassays

Product	Active ingredient	g/l	Strawberry, protected rate/1000 litres	% of rate used in bioassay
Tracer	spinosad	480	150 ml	3.3
Exirel	cyantraniliprole	100	1500 ml	25
Calypso	thiacloprid	480	250 ml	50
Gazelle	acetamprid	200	375 g (cherries)	50
Hallmark	lambda-cyhalothrin	100	75 ml	50
Pyrethrum	pyrethrum	50	2400 ml	50



a)

b)

c)

Images a) laboratory bioassay set up, b) bioassay with egg laying media viewed from the top, c) droplets of baits on different fruit leaf surfaces

Results

Percentage rates used in the bioassays refer to percentages of the recommended rates for protected strawberries (cherries for Gazelle). Temperature and relative humidity in the jars were 20.9 (± 1.9) °C and 98 (±1.5) % respectively.

D. suzukii mortality and oviposition

Without insecticides, and compared with the water control, none of the baits had a significant effect on *D. suzukii* mortality (Figure 3.2.1), although FSJ + *H. uvarum* did increase oviposition (t_{105} = 5.38; p < 0.001) (Figure 3.2.2). Without baits, and compared with the water control, none of the diluted doses of insecticides affected oviposition (Table 3.2.1, Figure 3.2.2) although mortality was increased by cyantraniliprole, acetamiprid, pyrethrum (t_{105} ≥ 3.37; p <0.001) and thiacloprid (t_{105} = 2.12; p = 0.036) but not significantly by spinosad or lambda-cyhalothrin (Figure 3.2.1). For spinosad, cyantraniliprole and lambda-cyhalothrin, the baits resulted in higher mortality than using the insecticides in water alone (t_{105} > 2.91; p = 0.005). However, the baits did not improve the efficacy of acetamiprid, thiacloprid or pyrethrum. Averaged across all six insecticides, there were no significant differences in mortality between the *H. uvarum*, FSJ + *H. uvarum* and Combi-protec treatments (average results not shown). However, mortality was higher when insecticides were combined with FSJ (t_{105} > 4.25; p < 0.001), than using insecticides in water (t_{105} = 4.92; p < 0.001). Averaged across insecticide treatments, oviposition was lower with the *H. uvarum* and Combi-protec baits than with FSJ and FSJ + *H. uvarum* baits (average results not shown, t_{105} = 2.39; p = 0.019). However, the difference in oviposition between bait and water treatments within individual insecticides was only significantly lower for lambda-cyhalothrin and *H. uvarum* (t_{105} = 2.53; p = 0.013) (Figure 3.2.2).

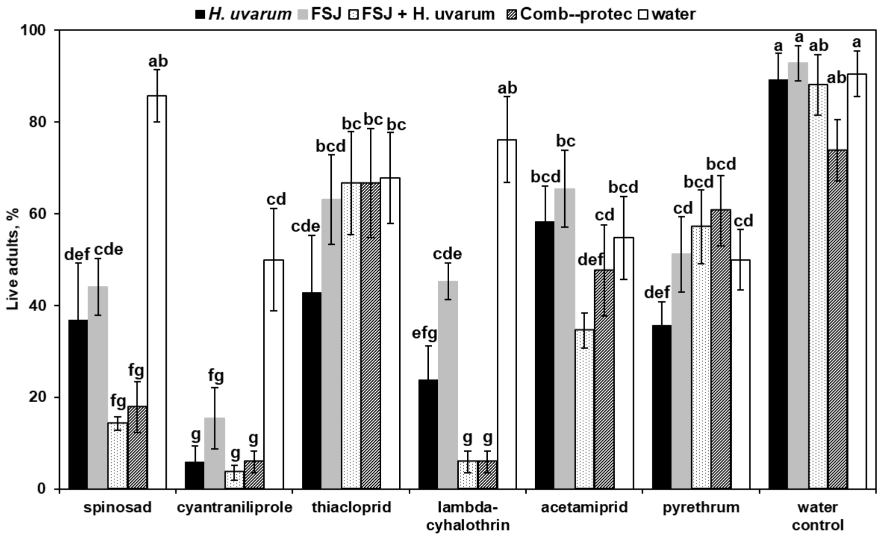


Figure 3.2.1 Effect of bait treatments (*Hanseniaspora uvarum* and/or FSJ fermented strawberry juice, Combi-protec) and insecticides on mortality of *Drosophila suzukii*; mean values (±SE), n = 4. Bars with the same letter are not significantly different (p = 0.05)

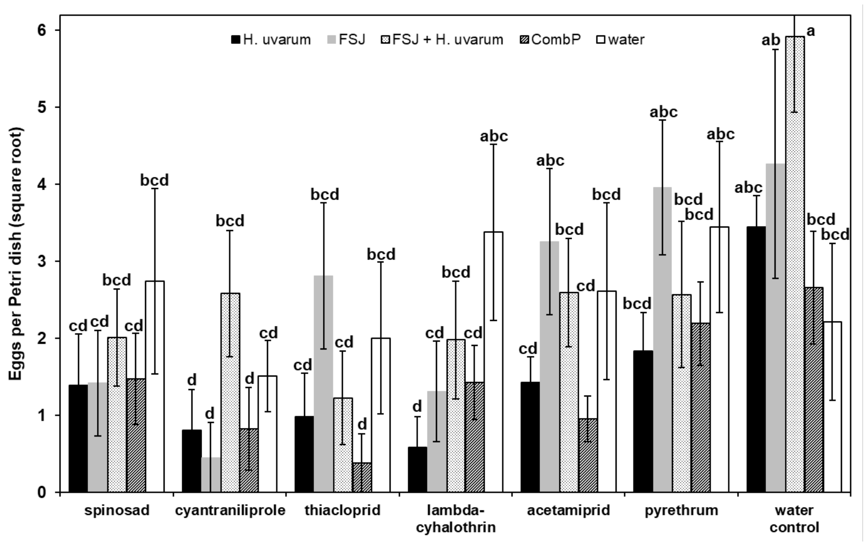


Figure 3.2.2 Effect of bait treatments (*Hanseniaspora uvarum* and/or FSJ fermented strawberry juice, Combi-protec) and insecticides on oviposition of *Drosophila suzukii*; mean values (±SE), n = 4. Bars with the same letter are not significantly different (p = 0.05)

Conclusions

- Without baits, none of the insecticides used at the specified rates had a significant effect on *D. suzukii* mortality or egg laying, except Exirel (25% rate) which increased mortality and reduced oviposition compared with a water control.
- Without insecticides, none of the baits had a significant effect on *D. suzukii* mortality or oviposition compared with a water control.
- A *H. uvarum* suspension, Combi-protec and fermented strawberry juice were all effective in increasing the efficacy of spinosad (Tracer, 3.3% rate), cyantraniliprole (Exirel, 25% rate) and lambda-cyhalothrin (Hallmark, 50% rate) in terms of *D. suzukii* mortality. The *H. uvarum* suspension also increased the efficacy of lambda-cyhalothrin (Hallmark, 50% rate) in terms of *D. suzukii* oviposition.
- Within individual insecticide treatments, the effects of a *H. uvarum* suspension and combined *H. uvarum* + fermented strawberry juice treatments on *D. suzukii* mortality and oviposition were not significantly different.
- Averaged across all insecticide treatments, the *H. uvarum* suspension and Combi-protec treatments were more effective than fermented strawberry juice in increasing insecticide efficacy when applied at the same volumetric applications. The recommended application rate for Combi-protec is 50 litres per hectare. The abundance of fruit waste means that fermented strawberry juice could potentially be applied at a much higher application rate.

- The feasibility and costs of producing *H. uvarum* suspension on a commercial scale require further investigation. This will determine the economically viable application rate.

Task 3.2b. Assess the effect of the optimum baits on the SWD control efficacy of different insecticides on different leaf surfaces in laboratory bioassays

Methods

A jar bioassay method was used to test the effect of *H. uvarum* and fermented strawberry juice (FSJ) baits on the *D. suzukii* control efficacy of Exirel and Tracer when used at discriminatory lethal concentrations (Table 3.2.2) on different leaf surfaces. The concentrations in Table 3.2.2 are expressed as a percentage of the recommended rate for strawberries, based on 1000 litres spray/ha. Using the *D. suzukii* control efficacy results from Task 3.2a, the concentration for Tracer was slightly increased from 3.3 to 5.0%, and the concentration for Exirel reduced from 25 to 12.5%. This was because the % mortality in Experiment 3.2a was slightly too low for Tracer and slightly too high for Exirel to be able to clearly discriminate the effect of the baits (if most flied are killed by the insecticide alone, it is not possible to determine the effect of the bait). Information on the bioassay jars, environmental conditions and monitoring are provided in the report for Task 3.2a. The following bait treatments were used with each insecticide or water (control):

(f) *Hanseniaspora uvarum* suspension + sugar 16 g/L (*H. uvarum* applied in a suspension containing 10^9 cfu/ml)
(g) Fermented strawberry juice + sugar 16 g/L
(h) Water control.

Each of the above bait x insecticide treatments was tested on the following leaf surfaces:

(a) Blackberry
(b) Blueberry
(c) Cherry
(d) Raspberry
(e) Strawberry.

Wild blackberry leaves were used for treatment (a); unsprayed fruit crops were used for treatments (b) to (e). This produced the following; 3 bait treatments (including a water control), 3 x insecticide treatments (including a water control) x 5 leaf types = 45 treatments (see Image, above). There were 3 replicates per factorial treatment = 135 containers. The experiment was set up in a randomised block design.

Table 3.2.2. Insecticides, recommended rates for protected strawberries and percentages of recommended rates used in the jar bioassays. The rates for Tracer and Exirel were adjusted from Experiment 1 to be able to clearly discriminate the effect of the bait treatments.

Product	Active ingredient	g/l	Strawberry, protected rate/1000 litres	% of rate used in bioassay
Tracer	spinosad	480	150 ml	5.0
Exirel	cyantraniliprole	100	1500 ml	12.5

The experimental method was the same as in Task 3.2a except that three leaves of types (b) to (e) above were used in place of three blackberry leaves (a) where appropriate. Mature leaves were selected that were sufficiently small (about 25-30 x 20-25 mm) to fit inside the jars without overlapping each other or the Petri dish with grape juice agar. For all leaves, the insecticide/bait or sugar solution droplets were placed on the adaxial surface (upper side) which were placed in the jars facing upwards.

Results for the bioassay were analysed on untransformed data by ANOVA using Excel.

Results

Temperature and relative humidity in the jars were 21.6 (± 1.3) °C and 98.5 (±1.3) % respectively.

Averaged across all insecticide and bait treatments, *D. suzukii* mortality was lower using raspberry leaves than using blueberry or strawberry leaves (average results not shown; $t_{135} > 2.49$; $p < 0.014$). However, the effect of leaf type on mortality was small (means for different leaves 46.8 to 60.2 %) when compared with the effect of bait × insecticide treatments (7.1 to 96.7 %). Oviposition was not affected by leaf type and there were no significant interactions between the effects of bait, insecticide and leaf type on *D. suzukii* mortality or oviposition. Results averaged across all five leaf types are therefore presented in Figures 3.2.3 and 3.2.4. When used in water, without baits, cyantraniliprole at 37.5 mg l^{-1} resulted in greater *D. suzukii* mortality than spinosad at 3.6 mg l^{-1} ($t_{135} = 4.63$; $p < 0.001$); both insecticides applied at these diluted doses resulted in greater mortality than the water control ($t_{135} = 7.98$ or 3.37; $p < 0.001$) but did not significantly affect oviposition. Baits in water, without insecticide, did not significantly affect mortality or oviposition compared with the water control. Averaged across both insecticides, *H. uvarum* increased mortality (95.6 %) compared with FSJ (86.7 %) ($t_{135} = 2.43$; $p = 0.016$). Mortality was higher following the use of insecticides with FSJ than in water (44.2 %) (t_{135} = 11.52; p < 0.001) (Figure 3.2.3). Compared with using insecticides in water, both FSJ and *H. uvarum* resulted in similar reductions in oviposition (t_{135} = 5.82 or 5.84; p < 0.001) (Figure 3.2.4).

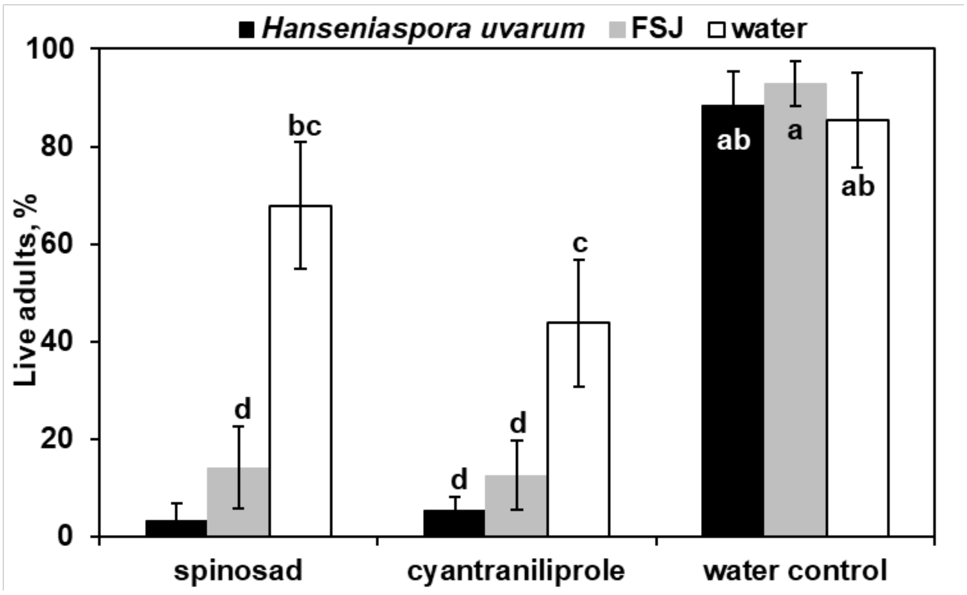


Figure 3.2.3 Effect of bait treatments (*Hanseniaspora uvarum* or FSJ fermented strawberry juice) and insecticides on mortality of *Drosophila suzukii*; mean values of five leaf types (±SE), n = 4. Any of the bars with the same letter are not significantly different (p = 0.05)

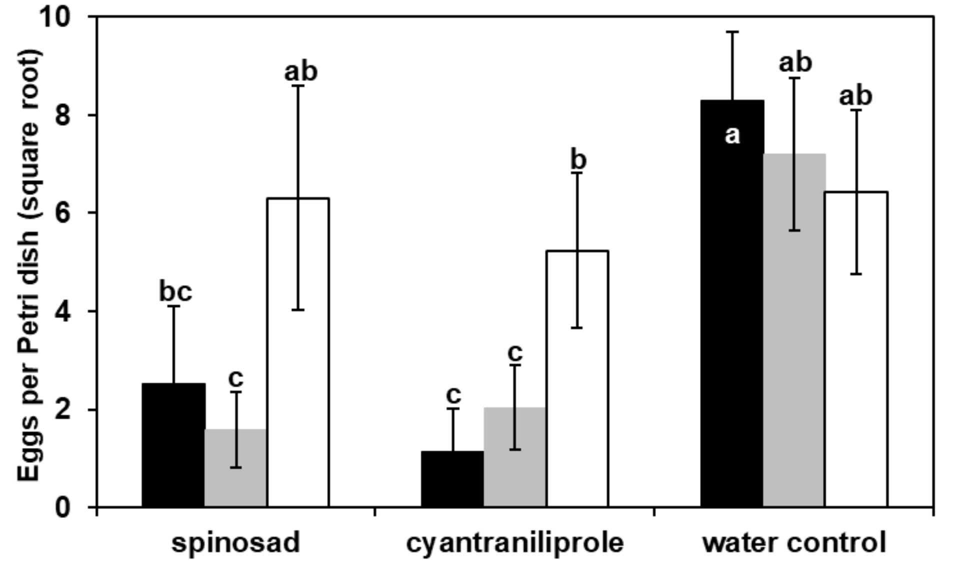


Figure 3.2.4 Effect of bait treatments (*Hanseniaspora uvarum* or FSJ fermented strawberry juice) and insecticides on oviposition of *Drosophila suzukii*; mean values of five leaf types (±SE), n = 4. Any of the bars with the same letter are not significantly different (p = 0.05)

Conclusions

- Without baits, cyantraniliprole (Exirel, 12.5 % field rate) and spinosad (Tracer, 5.0 % field rate) significantly increased *D. suzukii* mortality but did not affect oviposition compared with a water control.
- Without insecticides, fermented strawberry juice or *H. uvarum* suspension did not significantly affect *D. suzukii* mortality or oviposition compared with a water control.
- When used with cyantraniliprole (Exirel, 12.5 % field rate) or spinosad (Tracer, 5.0 % field rate) fermented strawberry juice or a *H. uvarum* suspension resulted in a significant increase in *D. suzukii* percentage mortality (30-59 %) and reduction in oviposition (76-94 %) compared with using the insecticides in water.
- With insecticide treatments, *D. suzukii* mortality was lower using raspberry leaves than using blackberry, blueberry, cherry or strawberry leaves but the effect of leaf type on *D. suzukii* mortality was small (up to 12 % difference) compared with the effects of baits and insecticides.
- There were no interactions between the effects of bait, insecticide and leaf type on *D. suzukii* mortality.
- Oviposition was unaffected by leaf type.
- The feasibility and costs of producing fermented strawberry juice and *H. uvarum* suspension on a commercial scale require further investigation. This will determine the economically viable application rate.

Task 3.2c. Assess the effect of the optimum bait on the winter morph Drosophila suzukii control efficacy of different insecticides in laboratory bioassays

Methods

A jar bioassay developed in SF145 was used to test the winter morph *D. suzukii* control efficacy of different insecticides when used with and without baits. The jars, the same as those described in 3.2a, contained two blackberry leaves with bait/insecticide droplets and one leaf with sugar solution droplets. Winter morphs were produced using conditions and method similar to those developed by Shearer et al. (2016) and Wallingford and Loeb (2016). Preliminary tests showed that no eggs were laid if winter morph *D. suzukii* remained in winter conditions for up to four weeks. However, oviposition commenced five to seven days after winter morph *D. suzukii* were transferred into summer conditions, and then continued to increase after a further three days. Larvae reared under summer conditions (20 °C, day length 16 h) were transferred after a maximum of 10 days to an incubator at 12-14 °C, day length 8 hours. Winter morph *D. suzukii* (seven females and five males – these numbers resulted in sufficient oviposition for analysis, adding more males increased interference between flies) were introduced in each jar. The jars were kept at 12-14 °C with day length 8 h for three days. The leaves with insecticide/bait droplets were then removed and replaced with two further leaves with sugar solution and a Petri dish with grape juice agar. The jars were then transferred to summer conditions (20 °C, day length 16 h) for 11 days.

Four insecticides at a percentage of their recommended field rate for strawberry (the same as used in Experiment 3.2b; Tracer 5%, Exirel 12.5%, Hallmark 50%, Calypso 50%) and a no insecticide control, were used with and without baits (*H. uvarum* and fermented strawberry juice).

This produced the following treatments:

3 bait treatments x 5 insecticide treatments incl. zero control = 15 jars.

Four replicates were used per factorial treatment = 60 jars.

D. suzukii mortality was recorded after one and three days (winter conditions) and 14 days (3 days winter + 11 days summer conditions). The number of eggs laid in the egg-laying medium, and any that had hatched into larvae, in each jar was recorded after 14 days (3 days winter + 11 days summer conditions). The Petri dishes were removed from the bioassay jars, covered and kept at 20 °C. Subsequent development of eggs into larvae was recorded seven days after removal from the bioassay jars. Temperature and relative humidity in the jars were measured by inserting a probe (Vaisala, Finland) connected to a data logger.

Results

Percentage insecticide rates used in the bioassays refer to percentages of the recommended rates for protected strawberries. Temperature and relative humidity in the jars were 13.0 (± 0.8) °C and 98.5 (±0.5) % during winter conditions and 20.2 (± 0.9) °C and 98 (±1.5) % during summer conditions.

Summer morphs. The mortality and oviposition results for summer morphs (Figures 3.2.5a and 3.2.6a) resembled those of the corresponding treatments in Experiment 3.2a (where the diluted doses for spinosad and cyantraniliprole were different), and in Experiment 3.2b (where the diluted doses were the same (Table 1)). Without insecticides, *H. uvarum* and FSJ had no significant effect on summer morph *D. suzukii* mortality compared with the water control, although unlike Experiment 3.2a, *H. uvarum* did not significantly affect oviposition. In water without baits, cyantraniliprole and thiacloprid, again, increased mortality (t_{58} = 3.62 or 2.58; p <0.001 or p = 0.012), while none of the four insecticides in water significantly affected oviposition, as in Experiment 1. Averaged across all four insecticides, *H. uvarum* resulted in greater mortality than FSJ (average results not shown; t_{58} = 2.67; p = 0.010). Mortality was higher and oviposition lower following the use of insecticides with FSJ than in water (t_{58} = 5.08 and 2.71; p <0.001 or p = 0.009) (Figures 3.2.5a and 3.2.6a). *H. uvarum* increased *D. suzukii* mortality with all four insecticides (t_{58} > 2.93; p < 0.005) and reduced oviposition (t_{58} > 2.12; p < 0.036) with all except thiacloprid (Figures 3.2.4a and 3.2.6a), compared with using the insecticides in water. FSJ increased mortality with spinosad and cyantraniliprole (t_{58} = 4.82 or 2.93; p < 0.00 or p = 0.005) and reduced oviposition with cyantraniliprole and lambda-cyhalothrin (t_{58} = 2.21 or 2.41; p = 0.031 or 0.019), compared with using the insecticides in water.

Winter morphs. After three days, average mortality across all insecticide and bait treatments for winter morph *D. suzukii* (35.1 %) was lower than for summer morphs (68.1 %) (t_{58} = 9.05; p < 0.001) (Figures 3.2.5a and 3.2.5b). Without insecticides, mortality after three days was also lower for winter morphs (2.1 %) than for summer morphs (22.2 %) (t_{58} = 3.32; p = 0.002). Without baits, winter morph mortality after three days was increased by cyantraniliprole and lambda-cyhalothrin (t_{58} = 2.54 or 2.20; p = 0.014 or 0.032) but not by spinosad or thiacloprid, when applied at the diluted doses (Table 3.2.1). After transfer of these three-day treated winter morphs to 11 days of summer conditions without insecticide and/or bait droplet leaves in the jars, the final mortality was not significantly different to that of the summer morphs after three days (Figures 3.2.5a and 3.2.5c) although oviposition was lower (t_{58} = 3.42; p = 0.001) (Figures 3.2.6a and 3.2.6b). Without insecticides, neither of the bait treatments significantly affected final mortality or oviposition compared with the water control (Figures 3.2.5b, c and 3.2.6b, c). Averaged across bait treatments, the mortality of winter morphs increased during the 11 days of summer conditions in the water controls (t_{58} = 2.84; p = 0.006) and in the spinosad, cyantraniliprole and lambda-cyhalothrin treatments (t_{58} > 3.44; p = 0.001) but not thiacloprid (average results not shown). Cyantraniliprole or lambda-cyhalothrin in water increased final mortality of winter morphs (t_{58} = 3.10 and 2.93; p = 0.003 and 0.005) but the effects of spinosad and thiacloprid in water on final mortality were not significant, and none of the insecticides in water affected winter morph oviposition compared with the water control (Figs. 3.2.5b, c and 3.2.6b, c). Averaged across insecticide treatments applied at diluted doses, there was no significant difference between baits in mortality of winter morphs, either three days after exposure or after a further 11 days without the treated leaves present (Figures 3.2.5b and 3.2.5c). Both baits increased winter morph mortality (t_{58} > 2.24; p < 0.029) when used with spinosad or cyantraniliprole, but not with thiacloprid or lambda-cyhalothrin (Figures 3.2.5b,c and 3.2.6b). *H. uvarum* reduced oviposition when used with cyantraniliprole (t_{58} = 2.19; p < 0.033). The effect of the insecticide + bait treatments on winter morph mortality and oviposition in subsequent summer conditions therefore persisted beyond the initial three days of winter conditions in which they were present.

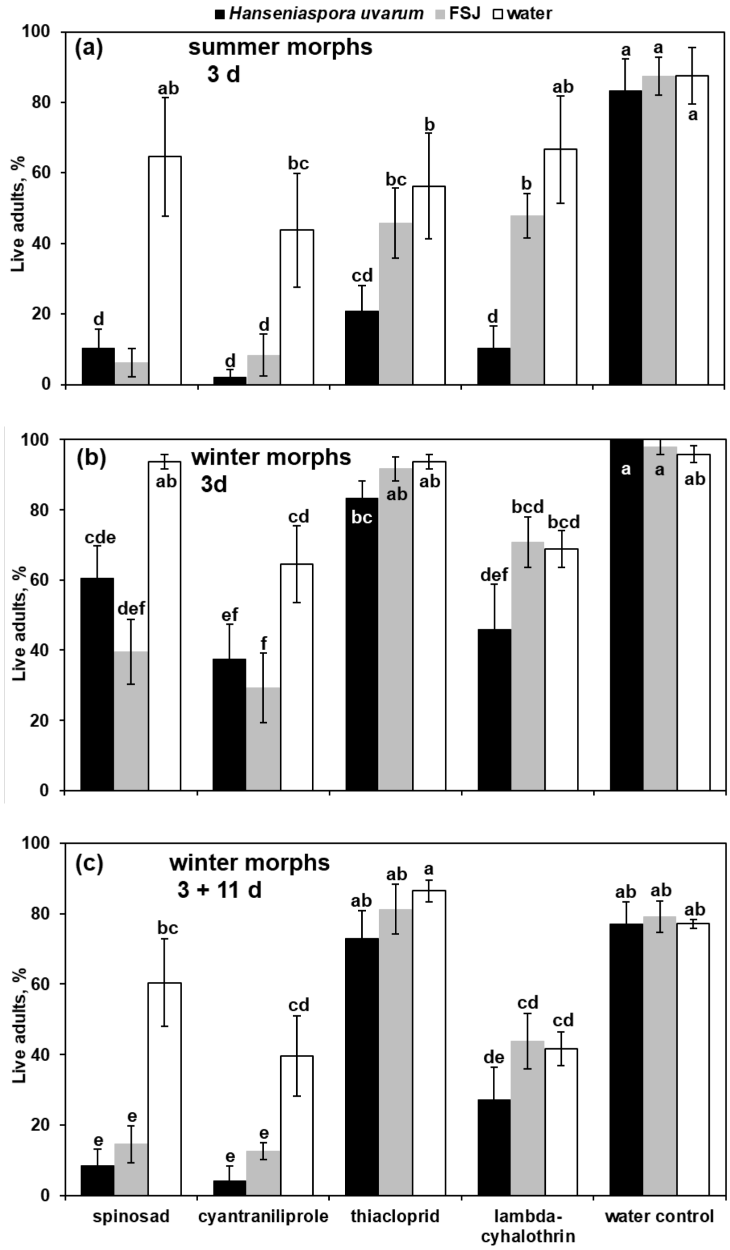


Figure 3.2.5 Effect of bait treatments (Hanseniaspora uvarum or FSJ fermented strawberry juice) and insecticides on *Drosophila suzukii* mortality of (a) summer and (b) winter morphs after three days and (c) winter morphs after three days followed by 11 days of summer conditions; mean values (±SE), n = 4. Within the same graphs, bars with the same letter are not significantly different (p = 0.05)

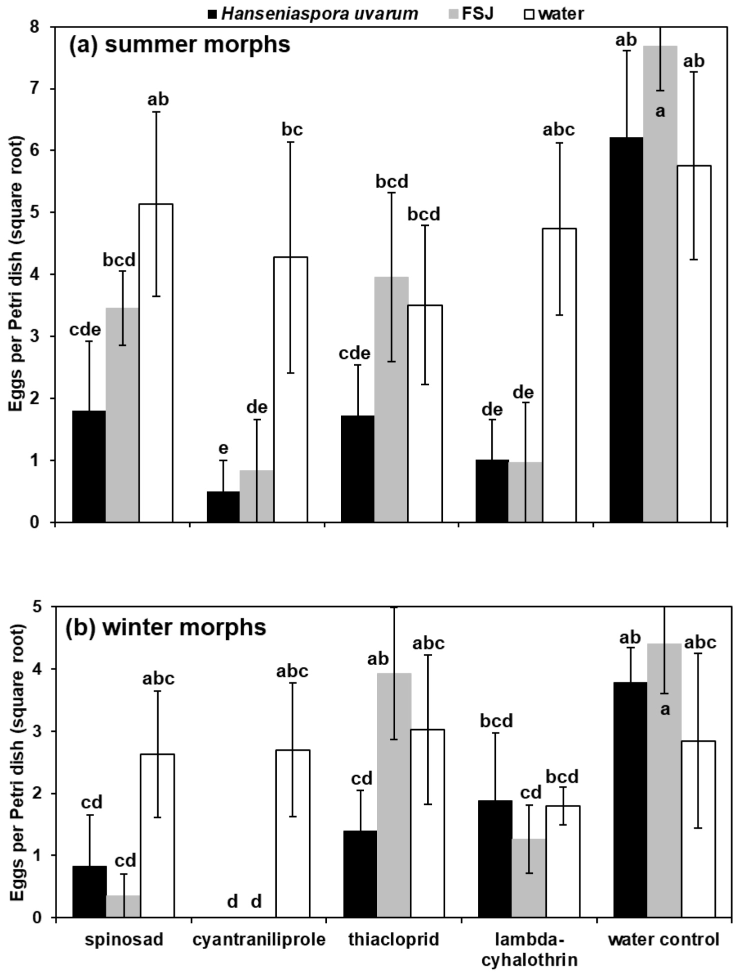


Figure 3.2.6 Effect of bait treatments (*Hanseniaspora uvarum* or FSJ fermented strawberry juice) and insecticides on *Drosophila suzukii* oviposition of (a) summer morphs after three days and (b) winter morphs after three days followed by 11 days of summer conditions; mean values (±SE), n = 4. Within the same graphs, bars with the same letter are not significantly different (p = 0.05)

Discussion

The effects of *H. uvarum* suspension and fermented strawberry juice on the efficacy of Tracer and Exirel, in terms of increased mortality and reduced oviposition, were similar for both winter and summer morph *D. suzukii* (Experiments 3.2a and 3.2b). However, the baits only increased the efficacy of lambda-cyahalothrin (Hallmark, 50 % field rate) for summer morph *D. suzukii* and not for winter morphs. For the same treatments, the overall average mortality after three days was significantly higher for summer morphs (6.9 adults) than for winter morphs (3.4 adults); significant at $p < 0.001$. However, after transfer of these winter morphs into 11 days of summer conditions without further exposure to insecticides or baits, the average mortality (6.2 adults) was similar to that recorded in the summer morphs after three days. Winter morph *D. suzukii* produced fewer eggs after transfer into 11 days of summer conditions (overall average 4.2 per Petri dish) than summer morph *D. suzukii* after three days (overall overage 11.8 eggs per Petri dish); significant at $p < 0.001$.

Conclusions

- Winter morph *D. suzukii* commenced oviposition five to seven days after transfer into summer conditions.
- When used without insecticides, *H. uvarum* suspension and fermented strawberry juice did not affect winter morph *D. suzukii* mortality or oviposition in subsequent summer conditions.
- *H. uvarum* suspension and fermented strawberry juice increased the efficacy of Tracer (5% rate) and Exirel (12.5% rate) in terms of increased winter morph *D. suzukii* mortality (by 68 to 89%) and reduced or eliminated oviposition (by 68 to 100%) compared with using the insecticides in water (after 14 days, egg numbers from winter morphs were not significantly different from summer morph numbers after three days).
- Unlike summer morph *D. suzukii*, for winter morphs the effect of the baits on the efficacy of Calypso or Hallmark was either small or not statistically significant.
- After exposure to the same treatments for three days, average mortality was higher in summer morphs than in winter morphs; however, after a further 11 days of summer conditions without further exposure to insecticides or baits, mortality in the winter morphs had increased to that recorded after three days in the summer morphs.

Task 3.3. Measure the effect of bait + insecticide mixtures on the viability of yeast, and on phytotoxicity to crop plants

Methods

The concentrations in Table 3.3.1 are based on the recommended rates per hectare for protected strawberries or cherries. Where products were not approved for use on strawberries in the UK, either the recommended rate for use in Canada (Exirel) or the rate for cherries (Gazelle) was used. The following bait treatments were used with insecticides or water (control):

(i) *Hanseniaspora uvarum* suspension + sugar 16 g/L (applied in a suspension containing 10^9 cfu/ml)(produced in shaking flasks for 16 h using a 50 g/L solution of yeast broth)

(j) Fermented strawberry juice (produced using a standard method, Noble et al. 2017) + sugar 16 g/L

Table 3.3.1. Insecticides, recommended rates for protected strawberries and cherries, and concentration based on an application volume of 1000 litres/ha

Product	Active ingredient	g/l	Strawberry, protected		Cherry	
			rate/h	conc.	rate/h	conc.
Tracer	spinosad	480	150 ml	0.15 ml/L	250 ml	0.25 ml/L
Exirel	cyantraniliprole	100	1500 ml*	1.5 ml/L	900 ml	0.9 ml/L
Calypso	thiacloprid	480	250 ml	0.25 ml/L	312.5 ml	0.3125 ml/L
Gazelle	acetamprid	200	-	-	375 g	0.375 g/L
Hallmark	lambda-cyhalothrin	100	75 ml	0.075 ml/L	90 ml	0.09 ml/L
Pyrethrum 5EC	pyrethrum	50	2400 ml	2.4 ml/L	2400 ml	2.4 ml/L

* Canada rate. Benevia also contains 100 g/l and is recommended at 750 ml/ha.

Yeast viability

In Experiment 3.3, the effect of six insecticides (strawberry rates except cherry rate for Gazelle) on the total yeast populations (natural and inoculated *H. uvarum*) in the above bait treatments was examined. This produced the following treatments: 6 insecticide treatments and a zero control x 2 bait solutions = 14 treatments; 3 replicates per factorial treatment = 42 containers.

The effect of reduced rates of insecticides on the yeast populations in sugar solution and fermented strawberry juice was examined in Experiment 3.2a. *H. uvarum* suspension was added to both sugar solution (16 g/L) and fermented strawberry juice. The following percentages of the above recommended rates of insecticides were used: Tracer 3.3 %, Exirel 25 %, Calypso, Hallmark and Pyrethrum 5EC, 50 % (strawberry rates); Gazelle 50 % (cherry rate). This produced the following treatments:

7 insecticide treatments including zero control x 3 solutions = 21 treatments

2 replicates per factorial treatment = 42 containers

The containers of *H. uvarum* suspension or fermented strawberry juice were kept at 20 °C for three weeks and samples taken from each container at the start and after 3, 7, 14 and 21 days.

The inoculated *H. uvarum* and naturally occurring *Saccharomyces cerevisiae* yeast cell populations in (a) suspension of *H. uvarum* and (b) fermented strawberry juice were determined by serial dilutions and measurements of cell numbers taken with a haemocytometer.

Phytotoxicity

The phytotoxicity of insecticides, baits and mixtures were assessed by spraying on to strawberry and cherry plant foliage and checking for subsequent leaf damage and comparing with water sprayed controls. Sprays (50 ml) were applied to the upper (adaxial) and lower (abaxial) leaf surface of five mature plants (strawberry) or two 0.7 m length branches (cherry).

The following insecticides were selected, based on *D. suzukii* control efficacy in 3.2a: Tracer, Exirel, Calypso and Hallmark. Insecticides were used at the recommended field application rates (Table 3.3.1). This produced the following treatments: 5 insecticide treatments including a zero control x 3 bait treatments including a water control x 2 leaf types = 30 treatments, with 2 replicates per factorial treatment.

Phytotoxicity was assessed on a scale of 0 (no damage) to 3 (severe) (EPPO 2014), 7 and 14 days after application of sprays. Photographs were taken of each treatment at each time point.

Unsprayed, one year old strawberry plants (cv. Korona) and 15-year old cherry trees (cv. Sunburst) at the Pershore Centre, Worcestershire were used for the tests which were conducted between 6 and 20 August 2018, immediately after harvesting had been completed.

Results

Yeast viability

In both Experiments 3.2a and 3.3, the populations of inoculated *H. uvarum* were higher than those of naturally occurring *S. cerevisiae* (Figures 3.3.1 and 3.3.3).

In Experiment 3.2a, there was no significant effect of any of the insecticides on the populations of *H. uvarum* or *S. cerevisiae* when added to sugar solution or fermented strawberry juice at the above reduced rates (Figure 3.3.2). There were 50 % decreases in the populations of *H. uvarum* in sugar solution and *S. cerevisiae* in fermented strawberry juice over three weeks, but no decrease in the population of *H. uvarum* in fermented strawberry juice (Figure 3.3.1).

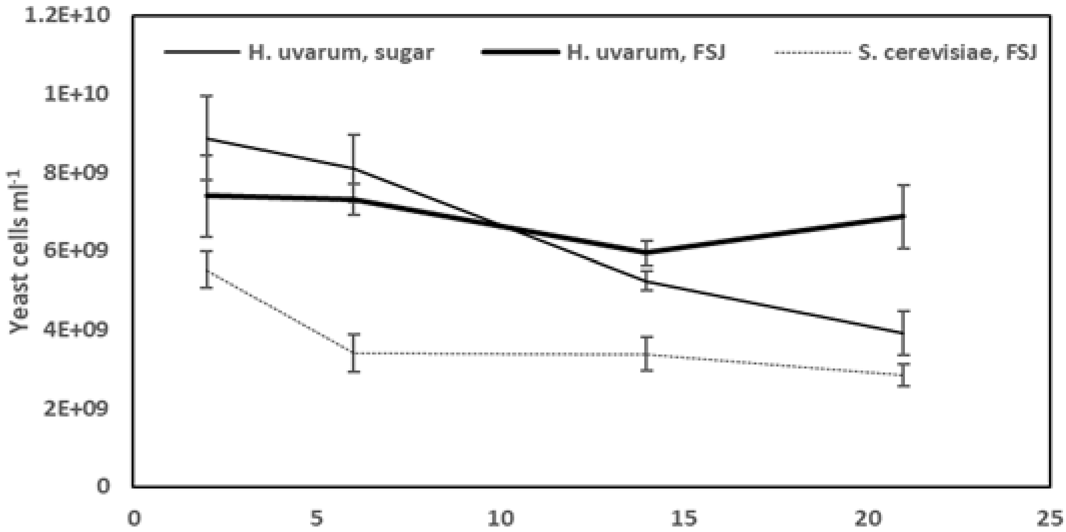


Figure 3.3.1. Populations of yeast (inoculated *H. uvarum* or naturally occurring *S. cerevisiae*) cells in sugar solution and fermented strawberry juice in Experiment 3.2a over a period of days. Each value is the mean (± SE) of six insecticides (there was no significant difference between insecticides) at reduced field rates and an untreated control

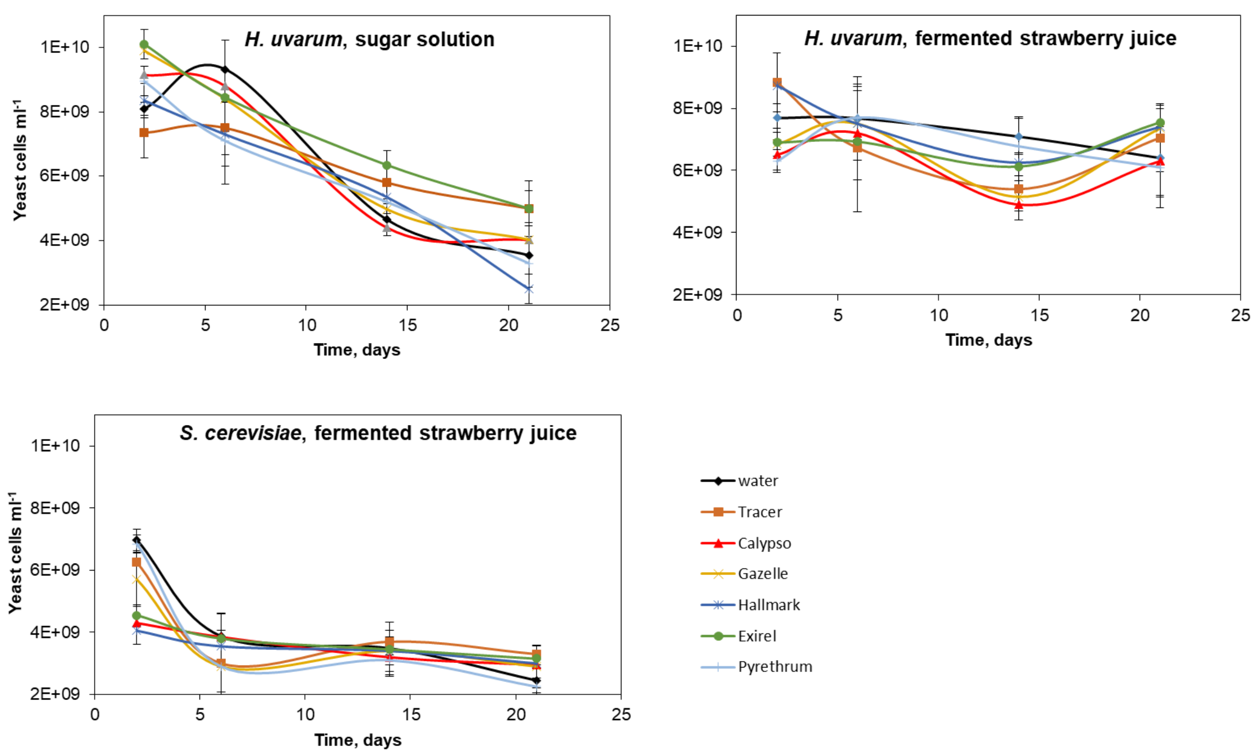


Figure 3.3.2. Populations of yeast (inoculated *H. uvarum* or naturally occurring *S. cerevisiae*) cells in sugar solution and fermented strawberry juice containing water or different insecticides at reduced rates in Experiment 3.2a. Each value is the mean of three replicate samples, and three determinations per sample

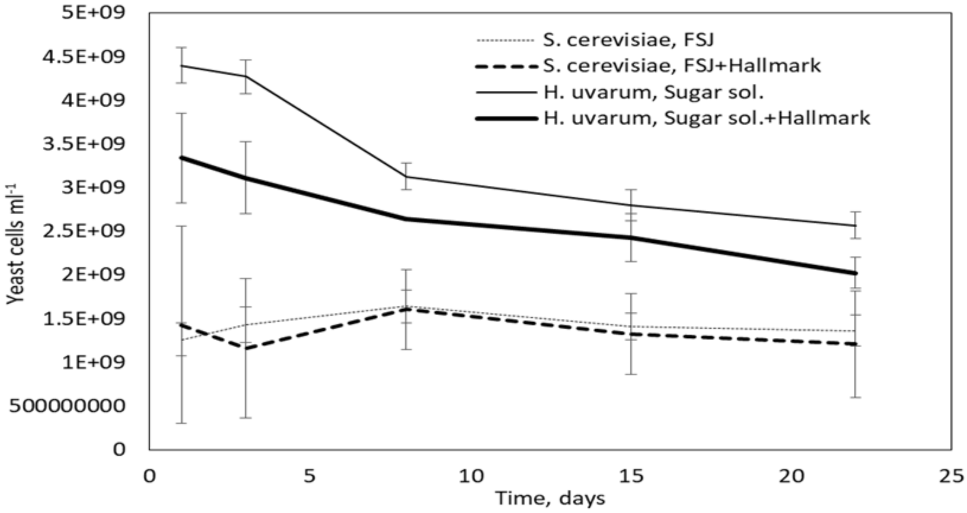


Figure 3.3.3. Populations of inoculated *H. uvarum* in sugar solution and naturally occurring *S. cerevisiae* in fermented strawberry juice in Experiment 3.3. Each value (FSJ or sugar solution) is the mean (± SE) of five insecticides (there were no significant differences) at recommended field rates and an untreated control, or of the Hallmark treatments

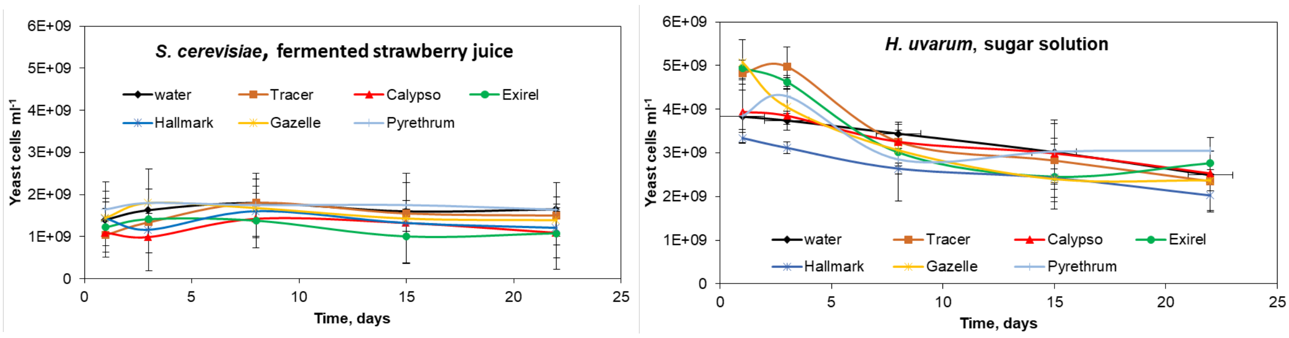


Figure 3.3.4. Populations of inoculated *H. uvarum* in sugar solution and naturally occurring *S. cerevisiae* in fermented strawberry juice containing water or different insecticides at recommended field rates in Expt. 3.3. Each value is the mean of two replicate samples, and three agar plate determinations per sample.

In Experiment 3.3 the starting population of *H. uvarum* in sugar solution was slightly lower where Hallmark was added (Figure 3.3.3); the decline in *H. uvarum* in sugar solution was then similar with or without Hallmark. There were no other effects of any of the insecticides on the populations of *H. uvarum* or *S. cerevisiae* when added to sugar solution or fermented strawberry juice at the recommended field rates (Figure 3.3.3).

Phytotoxicity

During the tests, average temperature was 18.3 (±6.6) °C. No rainfall was recorded during the period of the experiment.

No phytotoxicity was observed on strawberry or cherry plants one and two weeks after spraying with water, Tracer, Calypso or Hallmark, with or without either of the baits, or with baits alone, (Table 3.3.2) (Figures 3.3.3 to 3.3.6). One week after spraying with Exirel, about 10 % of the strawberry and cherry leaves had pale brown speckles on the under (abaxial) surface (Figures 3.3.3 and 3.3.5). Slight bronzing was also observed on the upper (adaxial) surface of strawberry leaves. This was irrespective of whether Exirel was sprayed with water, fermented strawberry juice or *H. uvarum* suspension. The phytotoxicity symptoms remained the same after two weeks (Table 3.3.2).

Table 3.3.2. Phytotoxicity scores of plants two weeks after spraying with different insecticides and/or baits. 0 = no symptoms, 1= slight, 2 = medium, 3 = strong symptoms.

Product	Conc.	Crop	Water	Ferm. strawberry Juice + sugar	*H. uvarum* 16g/L+ 16g/L sugar
Water	-	strawberry	0	0	0
Water	-	cherry	0	0	0
Tracer	0.15 ml/L	strawberry	0	0	0
Tracer	0.25 ml/L	cherry	0	0	0
Calypso	0.25 ml/L	strawberry	0	0	0
Calypso	0.3125 ml/L	cherry	0	0	0
Hallmark	0.075 ml/L	strawberry	0	0	0
Hallmark	0.09 ml/L	cherry	0	0	0
Exirel	1.5 ml/L	strawberry	1.0	1.0	0.5
Exirel	0.9 ml/L	cherry	1.0	1.0	1.0

Discussion

There was no evidence from this work that five insecticides (Calypso, Exirel, Gazelle, Pyrethrum 5EC and Tracer), if used at recommended field application rates in 1000 litre/ha, have a detrimental effect on the populations of yeasts in baits. When used at 50 % rate, no effect of Hallmark on *H. uvarum* was observed and the bait remained effective when used with Hallmark in jar bioassays in Tasks 3.2a and 3.2c. However, if the spray volume is significantly reduced from 1000 litres per hectare but the same amount of insectide applied per hectare, this will increase the insecticide concentration in the spray tank. If low volume applications per hectare are to be used with *H. uvarum* bait, the effect of higher concentrations of insecticides on the yeast population needs to be first examined. This is not an issue with fermented strawberry juice which can, if necessary be applied in high volume applications. It is also not established whether the naturally occuring yeast population in fermented strawberry juice (predominantly *S. cerevisiae*) has any significance in the attractiveness of the bait.

The longer persistence of *H. uvarum* in fermented strawberry juice than in sugar solution may be of interest if used with insecticides which are effective for more than 10 days, or if used in repeated applications. Fermented strawberry juice may also be suitable with *H. uvarum* in a trapping solution.

De Lury et al. (2008) found that a fruit fly bait GF-120 NF Naturalyte resulted in some phytotoxicity on sweet cherry leaves; this was not affected by the inclusion of spinosad at 0.04 g a.i. L^{-1}. No phytotoxicity was observed on a range of plant species when spinosad was applied at 0.072 a.i. L^{-1} (Durkin 2016) which agrees with the results obtained here for strawberry and cherry leaves when spinsoad was applied with or without baits. No phytotoxic effects were found when cyantraniliprole was applied to cotton plants at 1.5 g a.i. L^{-1} or 0.64 g a.i. L^{-1} (Anon. 2013; Kartik et al 2017). Here, slight phytotoxicity was observed when Exirel was applied to cherry leaves at 0.09 g a.i. L^{-1} and to strawberry leaves at 0.15 g a.i. L^{-1}. This was unaffected by the inclusion of bait treatments. The recommended application rate of Benevia (100 g L^{-1} cyantraniliprole) for strawberry is 750 ml ha^{-1} (0.075 g a.i. L-1 if applied in 1000 L ha^{-1}) which may avoid the phytotoxicity observed for cyantraniliprole at 0.15 g a.i. L^{-1}.

No disease (mildew or grey mould) symptoms were observed on any of the sprayed strawberry or cherry plants. Although strawberry fruit waste was used, this was fermented before use so it is possible that anaerobic conditions and fermentation products (organic acids and alcohols) may destroy any pathogen spores in the waste. The impact of sucrose and other phagostimulants as baits on other dipterans such as syrphids, non-dipetran natural enemies and pollinators has not been studied in fruit crops but requires further investigation. Sprays of sugar solutions have not affected the incidence of fruit rots and fermented liquids or compost teas are capable of plant disease suppression which may provide an additional benefit of spraying FSJ on to fruit crops.

Conclusions

- Five insecticides (Calypso, Exirel, Gazelle, Pyrethrum 5EC and Tracer) at recommended field and reduced rates had no effect on the populations of *H. uvarum* in sugar solution or of *S. cerevisiae* in fermented strawberry juice.
- There were 50 % decreases in the populations of *H. uvarum* in sugar solution and *S. cerevisiae* in fermented strawberry juice over three weeks, but no decrease in the population of *H. uvarum* in fermented strawberry juice.
- No phytotoxicity or disease was observed on strawberry or cherry leaves one and two weeks after spraying with fermented strawberry juice or *H. uvarum* suspension baits.
- At recommended field application concentrations, Tracer, Calypso and Hallmark, did not cause phytotoxicity either with or without the baits.
- Slight phytotoxicity was observed when Exirel was applied to cherry leaves at 0.09 g a.i. L^{-1} and to strawberry leaves at 0.15 g a.i. L^{-1}. This was unaffected by the inclusion of bait treatments, and may

be avoided if cyantraniliprole is applied to strawberries as Benevia at 0.075 g a.i. L^{-1} (this requires confirmation).

Water Control Fermented Strawberry Juice *H. uvarum* suspension

Exirel in water Exirel in fermented strawberry juice Exirel in *H. uvarum* suspension

Figure 3.3.5. Cherry phytotoxicity tests, water, bait and Exirel treatments

Calypso in water | Calypso/ fermented strawberry juice | Calypso in *H. uvarum* suspension

Hallmark in water | Hallmark/ fermented strawberry juice | Hallmark/ *H. uvarum* suspension

Figure 3.3.6. Cherry phytotoxicity tests, water, bait, Calypso and Hallmark treatments

Water Control | Fermented strawberry juice | Exirel in *H. uvarum* suspension

Exirel in water | Exirel in fermented strawberry juice | Exirel in *H. uvarum* suspension

Figure 3.3.7. Strawberry phytotoxicity tests, water, bait and Exirel treatments

Calypso in water Calypso in fermented strawberry juice Calypso in *H. uvarum* suspension

Hallmark in water Hallmark in fermented strawberry juice Hallmark in *H. uvarum* suspension

Figure 3.3.8. Strawberry phytotoxicity tests, water, bait, Calypso and Hallmark treatments

Future Work

- In 2019 baits will be field tested with fruiting plants inoculated with *D. suzukii.*

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 and mesh barriers, 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. *D. suzukii* adults also feed on cherry flower nectaries (Tochen and Walton 2016). Potentially an early spray post petal fall in cherry 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. Preliminary laboratory tests found at least two promising egg laying repellent alternative products in AHDB SCEPTRE PLUS.

The research in this objective, in 2017, field tested extending the spray intervals in vulnerable ripening cherry crops and investigated the longevity of nectar in cherry leaves.

D. suzukii fed on extrafloral cherry nectaries in the laboratory throughout the season but as the season progressed the time taken to locate nectaries tended to increase. This study demonstrated that there is a food source available to *D. suzukii* from flowering until 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.

In the same year, on cherry, insect exclusion 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 (only two emerged from all of the 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.

Task 4.2a. Further investigate the consequence of extending the spray interval from one to two weeks in cherry

Aim

To further investigate whether the interval for applying insecticides to cherry can be extended to two weeks in meshed and un-meshed commercial cherry orchards to protect against SWD.

Methods

Two farm sites, five insect meshed orchards and three orchards without insect mesh were selected. At farm Site 1, there were five orchards with insect mesh and one without insect mesh. At Farm Site 2, there were two orchards without insect mesh.

Figure 4.2.1. Orchards selected at Farm Site 1 and 2. Red circles are adult monitoring traps. Yellow plots were sprayed with a fortnightly spray programme. Blue plots will received the growers spray programme

Treatments were a fortnightly spray programme of approved effective insecticides tested against the grower spray programme in cherry orchards (Table 4.2.1). The insecticides in Table 4.2.2 were recommended by the AHDB in 2017. The products, Exirel 10 SE and Tracer, were granted emergency approval. Applications for the emergency approval of these products were submitted to CRD and gained approval for 2018. Approval, max applications, max rate and harvest interval shown in Table 4.2.2 were correct at the time of writing the protocol (19 February 2018). The spray programmes were adapted in response to the presence of other pests or weather (Table 4.2.3).

Table 4.2.2. Products approved for SWD on cherry in 2017. Protected indicates crops under polythene / glass

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**

Table 4.2.3. Actual fortnightly *D. suzukii* spray programmes and dates applied by the growers to the fortnightly spray plots. At Site 1 there was also a spray of Calypso on 15 April and 2 May and then Batavia on 15 May for aphid and capsid. At Site 2 there was also a spray of Calypso on 13 April for capsid and Batavia on 15 May for aphid control.

Fortnightly Site 1		**Fortnightly Site 2**	
Hallmark	15 - May	Calypso + Tracer	30 - May
Tracer	12 - Jun	Exirel	19 - Jun
Exirel	26 - Jun	Tracer	28 - Jun
Tracer	10 - Jul	Exirel	07 - Jul
Exirel	24 - Jul		

Table 4.2.1. Specifications of orchards at both grower sites. Note where polythene is used one side of the tunnel is vented and only bird netting is used. Insect mesh has a diameter of 0.8 x 0.8 or 0.9 x 0.9. Posts at the corners of the orchards were sprayed with coloured paints for particular varieties Kordia = blue, Regina = Red, Ruby = blue, Georgia = green.

Site	Field	Insect mesh (Y/N)	Polythene or Voen covers	Plot colour	Spray programme	Varieties assessed	Other varieties in rows	Tunnels (T) or rows (R) sprayed	Tunnels (T) or rows (R) sampled
1	LH SP	Y	Voen	Yellow	Fortnightly	Kordia, Regina		R24 - 26	Kordia, Regina; R25
				Blue	Grower	Kordia, Regina	Ruby, S/S, Stella, Sunburst, Van	Other rows	Kordia, Regina; R16,R19,R22
	LH WP	Y	Voen	Yellow	Fortnightly	Merchant	Burlatt, Giorgia	R1- 4	Merchant; R3
				Blue	Grower	Merchant	Burlatt, Giorgia	Other rows	Merchant; R7, R9
	OT	Y	Polythene	Yellow	Fortnightly	Kordia, Regina	Karina	R24 -26	Regina; R24, R25, Kordia; R25
				Blue	Grower	Kordia, Regina	Karina	Other rows	Regina; R18, Kordia; R19
	B10	Y	Polythene	Yellow	Fortnightly	Kordia, Regina	Merchant	R1-4	Kordia; R2, Regina; R3
				Blue	Grower	Kordia, Regina	Merchant	Other rows	Kordia; R6, Regina; R7
	BC	Y	Polythene	Yellow	Fortnightly	Kordia, Regina		R1- 4	Regina; R1, R2, Kordia; R2
				Blue	Grower	Kordia, Regina		Other rows	Regina;R7, Kordia; R9
	CH	N	Voen	Yellow	Fortnightly	Van	Early River	R1- 2	Van; R1, R2
				Blue	Grower	Van	Early River	Other rows	Van; R4, R5
2	NS	N	Polythene	Yellow	Fortnightly	Skena		T 19	Skeena; T19
				Blue	Grower	Skena		Other rows	Skeena; T16
	OS	N	Polythene	Yellow	Fortnightly	Skena, Penny		T28 - 29	Skeena; T29, Penny; T28
				Blue	Grower	Skena, Penny		Other rows	Penny; T25, Skeena; T26

In each of the eight orchards there were two plots. One plot was treated with the growers spray programme and the other with a fortnightly spray programme. The growers spray equipment will was used. Otherwise the orchard plots received the same management for other pests and diseases.

Assessments included adult trap catches; one trap placed within each orchard and one outside the perimeter of the orchard. Biobest traps with Dros'attract as the liquid bait were used. The traps were filtered weekly and assessed for male and female SWD.

Figure. 4.2.2. Bioassay for testing the efficacy of spray residue on cherry leaves for *D. suzukii* mortality. A sugar feeder was included to maintain the flies

The incidence of *D. suzukii* damage to the cherry fruits was assessed each week from white fruit (BBCH growth stage 81). Forty, non-damaged well-shaped cherries were collected from each plot (20 of each variety). Cherries were picked from the central 10 trees in each of the 16 plots. In orchards with two row spacing, fruit was sampled from the inside of both rows. In orchards with single row spacing fruit was sampled from the central row (Table 4.2.1).

Fruit was incubated for 2 weeks (~22C, >40 % RH, 16 h light: 8 h dark) in Perspex boxes (20 x 10 x 10 cm) with a mesh lid and the numbers of male and female *D. suzukii* emerging from fruit counted. All samples were labelled with treatment (weekly or fortnightly), orchard name, date, variety.

In addition orchards coded NS and LH-SP (Table 4.2.1) were sampled weekly pre spraying from the fortnightly and weekly sprays (5 deli cups (http://www.reptilesupplyco.com/281-insect-deli-cups-lids) per plot x 4 = 20, plus 5 deli cups of unsprayed cherry leaves collected from NIAB EMR = 25 cups, Figure 4.2.2). Five medium size leaves were placed into each cup. Therefore 25 leaves were collected per plot. Five male and five female *D. suzukii* were introduced into each pot and then mortality recorded at 48 hours. Leaf samples were collected by staff at NIAB EMR. Continued communication was made between growers and staff at NIAB EMR.

Data was analysed using ANOVA on SQRT transformed data and between treatment differences differentiated using the least significant difference.

Results

Mean numbers of *D. suzukii* captured in the monitoring traps during the fruit ripening period were generally low, as is normally the case as the fruit is often more attractive to flies than the bait in the traps. At Site 1 there were always more *D. suzukii* in the traps in the perimeter of the crop compared to inside the mesh. At Site 2, even though this site has a dense woodland where large numbers of *D. suzukii* are known to inhabit, there were low trap catches throughout the trial (Figure 4.2.3).

From 3,000 collected for natural emergence only six adult *D. suzukii* emerged from the two farms over the whole trial; two and one *D. suzukii* from the grower and fortnightly programme from Site 1, respectively, and three *D. suzukii* from the fornightly programme at Site 2.

Analyses of the 48 hour leaf contact mortality test revealed that both the grower and fortnightly spray programme gave significant mortality of *D. suzukii* adults compared to the untreated control at both sites (Figure 4.2.4). Spraying ceased after 23 July at Site 1 and 03 Jul at Site 2. Up to this time the efficacy of spray programmes varied between fortnightly and grower, but always gave a higher adult fly mortality than unsprayed cherry leaves. After spraying ceased the adult *D. suzukii* mortality after 48 hours contact with leaves was very similar to contact with unsprayed leaves (Figure 4.2.4).

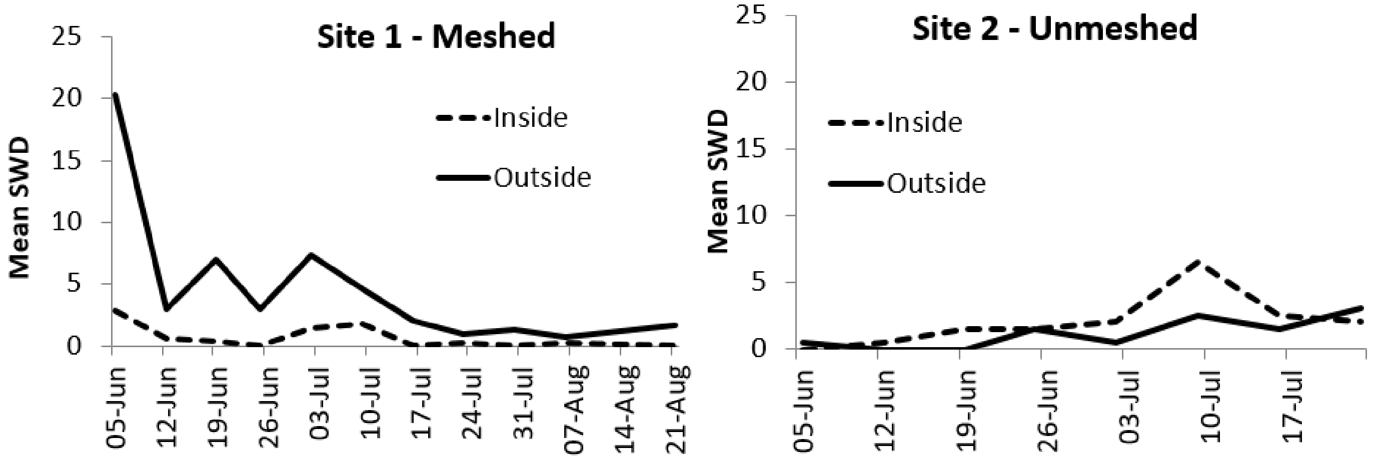


Figure 4.2.3. Mean numbers of *D. suzukii* adults captured each week in DrosTraps insdie and outside the crop perimeter

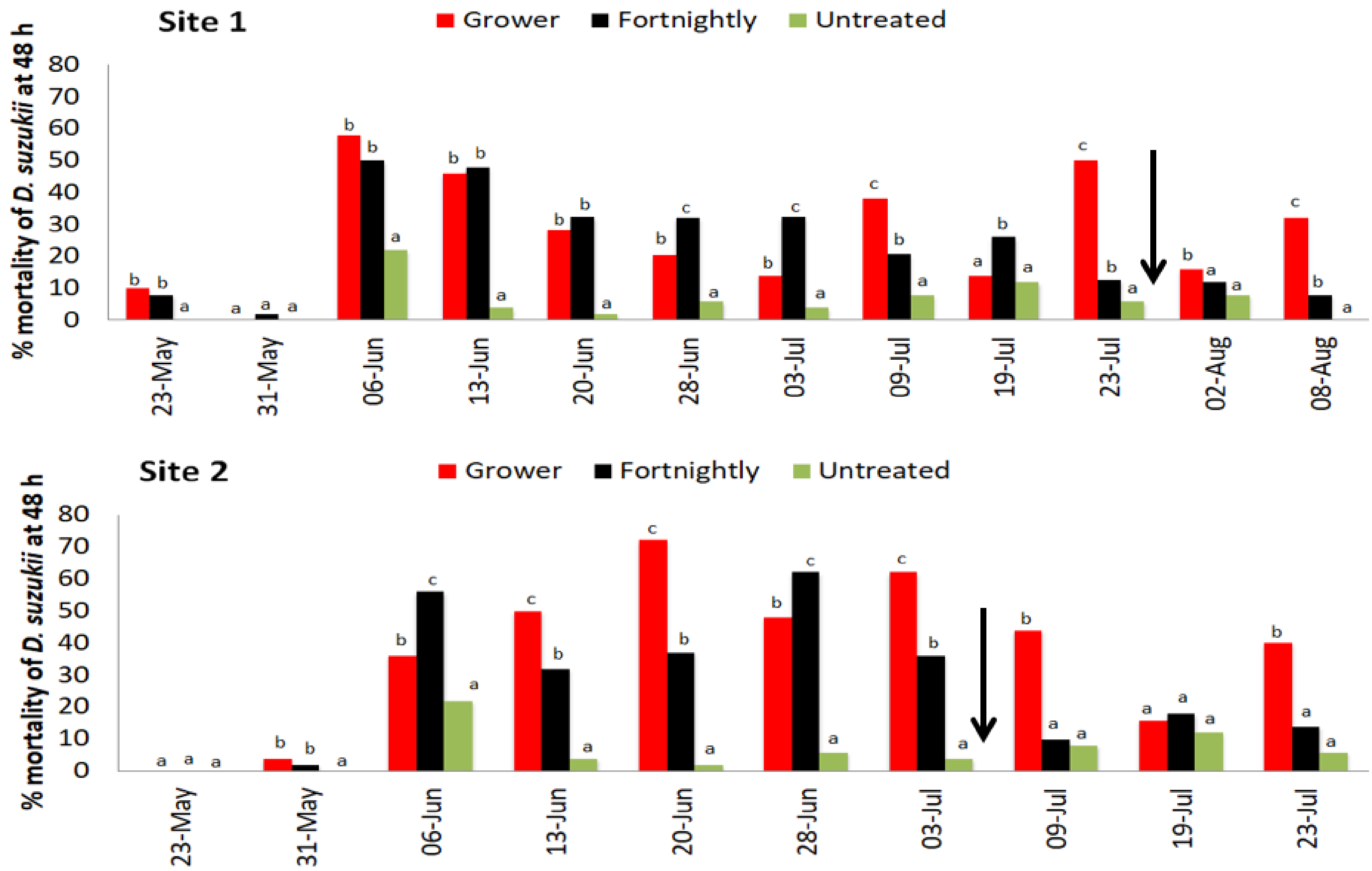


Figure 4.2.4. Mean numbers of adult *D. suzukii* that had died after 48 hours contact with insecticide treated (fortnightly or growers programme) treated leaves compared to unsprayed cherry leaves. Black arrow indicates last spray application before harvest of cherry fruits

The two growers involved in the trial used different spray equipment and application speeds and volumes. Grower 2 also carried out winter precision monitoring (Table 4.2.2).

Table 4.2.2. Growers spray equipment and spray strategies in the cherry orchards.

	Farm 1	Farm 2
Speed km/h	3.2	7-8
Nozzles	Ablbuz ATR 80 (3 blue /6 orange– 9 each side)	Yellow albert (8 per side)
Air induction	Fan full speed	Fan full speed
Spray volume (l/ha)	750	200
Plantings	2 row beds about 7x7 m and 4.5 m in tunnel	2 row beds about 7x7 m and 4.5m in tunnel
Sprayer model	Bap single frame with single tower, single fan and mower A frame with more nozzles	Munkoff – half tower
Tank size (l)	2000	1500
Other factors	Not winter precision monitoring	Winter precision monitoring
		

Conclusions

- The trial in 2018 (eight orchards) had similar findings to the smaller commercial trial (two orchards) in 2017.
- Fortnightly spray programmes gave equal efficacy of *D. suzukii* control as the grower’s standard spray programme.

- In addition, very few fruits were damaged by *D. suzukii* egg laying in both spray programmes even though adults were clearly in the crop and around the perimeter.
- Where mesh was employed there were fewer *D. suzukii* adults in the crop. Site 2 may benefit from using mesh as three *D. suzukii* emerged from the fortnightly sprays compared to none in the grower programme – although this result could not be tested statistically.

Task 4.2b. Investigate the consequence of extending the spray interval from one to two weeks in raspberry

Aim

To further investigate whether the interval for applying insecticides to raspberry can be extended to two weeks in meshed commercial raspberry plantations to protect against *D. suzukii*.

Methods

Two primocane raspberry varieties were used for this trial, Grandeur and Kweli (Table 4.2b.1). Tunnels were eight metres wide. Each tunnel had three rows of raspberry (Table 4.2b.2) with 2.5 m between the rows. The blue areas, in Figure 4.2b.1, were treated by the growers programme and the yellow tunnels were treated with a fortnightly programme of sprays known to be effective against *D. suzukii*. To prevent spray drift, areas employing the different spray programmes were kept separate using a polyene barrier.

D. suzukii were monitored inside the crop and outside the perimeter using DrosoTraps baited with commercial bait (Biobest Dros' attract new formulation); four per site (Figure 4.2b.1). The perimeter of the tunnels was insect meshed (see Image, below). Data loggers were installed, two in each site, one in each plot on 21 Aug.

Image. Meshed tunnels used in trial and labelled ends of tunnels

Treatments were either a fortnightly spray programme of approved products; rotating Exirel and Tracer (Table 4.2b.2) from 22 August (yellow), or a grower spray programme (blue) (Table 4.2b.3). The insecticides in Table 4.2b.2 were recommended by the AHDB in 2017. The products, Exirel 10 SE and Tracer, were granted emergency approval. The spray programmes were adapted in response to the presence of other pests or weather.

The experimental design was two replicate areas for each of the two treatments. All tunnels were managed by the grower except the fortnightly tunnels where the spray programme was dictated by NIAB EMR staff in response to trap catches of *D. suzukii*.

The growers standard spray equipment was used on all plots and other pests and disease treatments were the same across all plots.

Key:
Red spot = Droso trap
Orange spot = Data logger 1 (SH7) and 3 (SH8)
Green spot = Data logger 2 (SH7) and 4 (SH8)

SH7. Trap Numbers from left to right: 4,3,2,1

Trap position and number

SH7F IN	1
SH7F OUT	2
SH7W IN	3
SH7W OUT	4
SH8F IN	5
SH8F OUT	6
SH8W IN	7
SH8W OUT	8

SH8. Trap Numbers from left to right 5,6,7,8

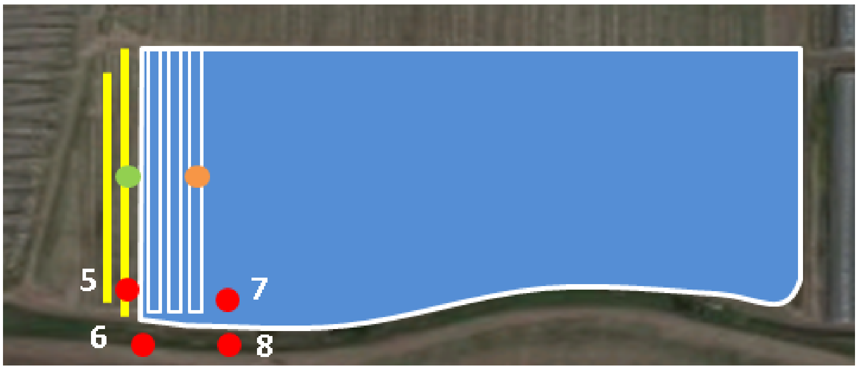


Figure 4.2b.1. SH7 and SH8 plantation maps with rows and treatment positions. Red dots indicate locations of Droso traps with commercial bait. Orange and green dots are locations of temperature and humidity data loggers

Table 4.2b.1. Specifications of raspberry plantations. Crops were planted as bare root in March 18 in soil. They were expected to flower in mid-July with first fruit in mid-August through to September.

Field	Insect mesh (Y/N)	Cover type	Plot colour	Spray programme	Varieties to be assessed	Other varieties in rows	Tunnels (T) or rows (R) to be sprayed	Tunnels (T) or rows (R) to be sampled from
SL7	Yes	Polythene	Yellow	Fortnightly	Grandeur	None	South West T: 31,39 & 49,38 and South East T: 38 & 37	South West T: 31,39 & 49,38 and South East T: 38 & 37
SL7	Yes	Polythene	Blue	Grower	Grandeur	None	Other T	
SL8	Yes	Polythene	Yellow	Fortnightly	Kweli	None	West T: 42,33 and 56,32	West T: 42,33 and 56,32
SL8	Yes	Polythene	Blue	Grower	Kweli	None	Other T	

Table 4.2b.2. Products approved for SWD on raspberry in 2017

RASPBERRY: Products currently approved with activity against SWD

Crop Situation	Active	Typical Product	Approval	Max. Applications	Max. Rate	Harvest Interval
Outdoor	deltamethrin	Decis	Full	None listed	0.5 l/ha (Max Dose: 1.5 l/ha)	7 days
	lambda-cyhalothrin	Hallmark with Zeon Technology	EAMU	4	0.075 l/ha (Max Dose: 0.15 l/ha)	28 days
	pyrethrins*	Pyrethrum 5 EC	Full	No limit	0.02 l per 5 litres	1 day
	spinosad	Tracer	EAMU	2	0.2 l/ha	3 days
	thiacloprid*	Calypso	EAMU	None listed	0.25 l/ha (Max Dose: 0.75 l/ha)	3 days
Protected	abamectin*	Dynamec	EAMU	No limit	0.05 l per 100 litres (Max Dose: 1 l/ha)	3 days
	deltamethrin	Decis	Full	None listed	0.5 l/ha (Max Dose: 1.5 l/ha)	7 days
	pyrethrins*	Pyrethrum 5 EC	Full	No limit	0.02 l per 5 litres	None stated
	spinosad	Tracer	EAMU	3	200 ml/ha	1 day
	thiacloprid*	Calypso	EAMU	None listed	0.25 l/ha (Total Dose: 0.75l/ha)	3 days

***Denotes limited effect**

Assessments were made weekly (Table 4.2b.4), the day before spraying (if a spray was planned). To assess the populations of *D. suzukii*, one DrosoTrap was placed within each plantation and one outside the perimeter of the plantation. The traps were filtered weekly and assessed for male and female *D. suzukii*.

To assess *D. suzukii* damage to fruits, each week from ripening, 50 ripe raspberry fruits were picked from each plot (200 fruits per week). Fruits were picked from the centre of the row and lower down in the canopy to give the best chance of detecting any damage. Fruit was incubated for two weeks (~22 °C, >40 % RH, 16 h light: 8 h dark) in a Perspex box (20 x 10 x 10 cm) with a mesh lid and the numbers of male and female *D. suzukii* emerging from fruit were counted. All samples were labelled with treatment (grower or fortnightly), field name (SH7 or 8) and date. Results were compared to the growers spray programme to confirm whether a fortnightly spray programme gives comparable protection against *D. suzukii*.

In order to assess the longevity and efficacy of sprays on raspberry leaves, at each weekly assessment, 20 leaves from each of the four plots were picked. An additional 20 leaves were picked from a wild raspberry bush growing at NIAB EMR as an unsprayed comparison (control). Five leaves were placed into deli cups with moist filter paper and a feeder containing 5 % dextrose solution (as for Task 4.2). Five male and five female *D. suzukii* were introduced into each pot and then *D. suzukii* mortality recorded at 48 hours.

Continued communication was made between growers and staff at NIAB EMR via a WhatsApp group. All samples were collected by staff at NIAB EMR.

Table 4.2b.3. Date and spray application for *D. suzukii* in tunnels SH7 and SH8 in the fortnightly programme.

Week	Date	Spray applied
24	10 Jun	Hallmark
32	6-11 Aug	Exirel
34	22 Aug	Exirel
36	6 Sep	Tracer
40	3 Oct	Spruzit
42	17 Oct	Tracer
44	31 Oct	Spraying ended

Table 4.2b.4. Date that *D. suzukii* assessments were done; including eight DrosoTraps (one inside and one outside each of the four plots), raspberry fruit for emergence testing and leaf samples for contact mortality assessments.

Date	8 Droso traps	50 raspberries	20 raspberry leaves
9 Aug		X (pre assessment)	
21 Aug	X	X	X
28 Aug	X	X	X
5 Sep	X	X	X
12 Sep	X	X	X
17 Sep	X	X	X
25 Sep	X	X	X
2 Oct	X	X	X
9 Oct	X	X	X
16 Oct	X	X	X
23 Oct	X	X	X
30 Oct	X	X	X
6 Nov	X	X	X
14 Nov	X	X	X
20 Nov	X	X	X

Statistical analysis

DrosoTraps: GLM with poisson distribution & logarithm link. Because there were only two replicates of each treatments (fortnightly and grower programme it was not appropriate to do statistics on this data – hence trends only are reported.

Results

Droso traps

Following statistical analysis of mean numbers of total *D. suzukii* caught in DrosoTraps throughout the trial (Figure 4.2b.2), significant differences were found between blocks (SH7 and SH8), trap position (inside or outside the tunnel) and spray programme applied (fortnightly or grower) (see Table 4.2b.5 for P values). From the 14 assessments, on four occasions there was a significant difference between blocks, whereby significantly more *D. suzukii* were caught in SH7 compared to SH8. On 13 occasions there was a significant difference between trap positions, whereby significantly more *D. suzukii* were caught outside the raspberry tunnels than inside. Importantly, on three occasions, there was a significant difference between spray programmes, whereby significantly more *D. suzukii* were caught where the growers spray programme was used compared to the fortnightly spray programme.

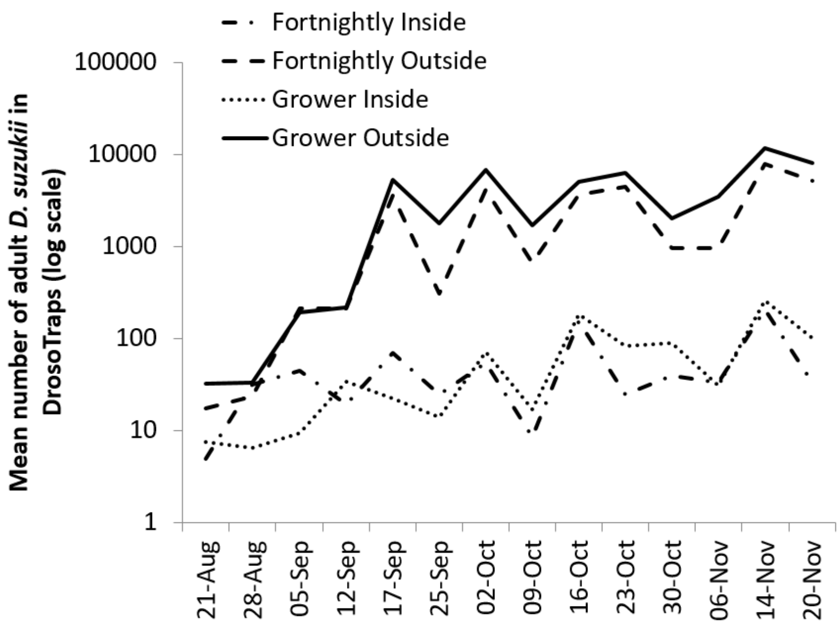


Figure 4.2b.2. Date and mean numbers of total *D. suzukii* caught between DrosoTraps at both sites (SH7 and 8), according to trap position: **fortnightly inside** = fortnightly spray programme with trap inside raspberry tunnel, **fortnightly outside** = fortnightly spray programme with trap outside tunnel, **grower inside** = grower spray programme with trap inside tunnel, **grower outside** = grower spray programme, trap outside tunnel.

Table 4.2b.5. DrosoTrap sampling dates and significant differences. Left side: *p* values and significant differences between predicted mean numbers of total *D. suzukii* caught in DrosTraps, according to block (SH7 or 8), trap position (inside or outside the tunnel) and plot (fortnightly or grower spray programme). Right side: Estimated mean values where there are significant differences according to trap position and spray programme.

	P value			Estimated mean values, formed on scale of linear predictor			
				Trap position		Spray programme	
Date	Block	Trap position	Spray programme	Inside	Outside	Fortnightly	Grower
21-Aug	<.001	0.022	0.002	0.628	1.988	1.052	1.564
28-Aug	-	-	-	-	-	-	-
05-Sep	-	0.009	-	3.004	5.278	-	-
12-Sep	-	0.013	-	3.255	5.364	-	-
17-Sep	0.005	<.001	-	3.542	8.254	-	-
25-Sep	0.021	0.008	0.026	2.537	6.21	4.077	4.671
02-Oct	-	0.005	-	4.01	8.45	-	-
09-Oct	-	0.047	-	2.345	6.83	-	-
16-Oct	0.049	0.006	-	5.001	8.235	-	-
23-Oct	-	0.002	-	3.804	8.559	-	-
30-Oct	-	0.053	-	4.048	7.205	-	-
06-Nov	-	0.005	-	4.494	7.744	-	-
14-Nov	-	0.036	-	4.655	7.131	-	-
20-Nov	-	0.018	0.054	3.157	6.167	4.294	5.029

Fruit emergence

In general, in most weeks, only half the number of *D. suzukii* adults emerged from the fortnightly compared to grower spray programme. It is important to note that the fortnightly plots were under higher *D suzukii* pressure as they were closer to the border of overwintering habitat (see Figure 4.2b.2).

Residue on leaf bioassay

The mortality of *D. suzukii* that came into contact with leaves at least two weeks after the last application of an effective spray was applied was, in general, higher in the fortnightly spray programme compared to the growers spray programme. The mortality in contact with the unsprayed leaves was generally 5-10 % after 48 hours. In the fortnightly plots mortality was between 15-80 % and 15-50 % in the fortnightly and grower sprayed plots respectively (Figure 4.2b.4).

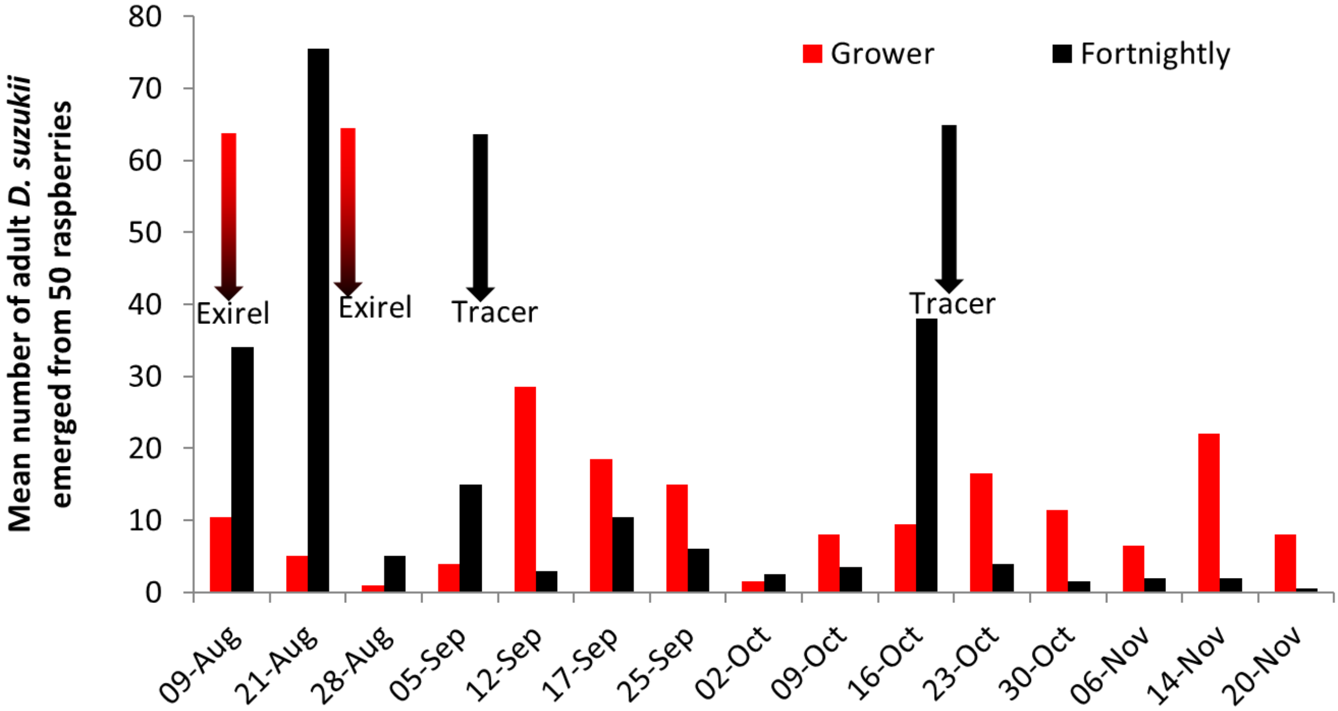


Figure 4.2b.3. Mean numbers of adult *D. suzukii* emerged from 50 raspberries sampled from sites SH7 and 8, according to spray programme (fortnightly or grower). Arrow and colour (red = Grower, and black = Fortnightly) represent spray and application timing

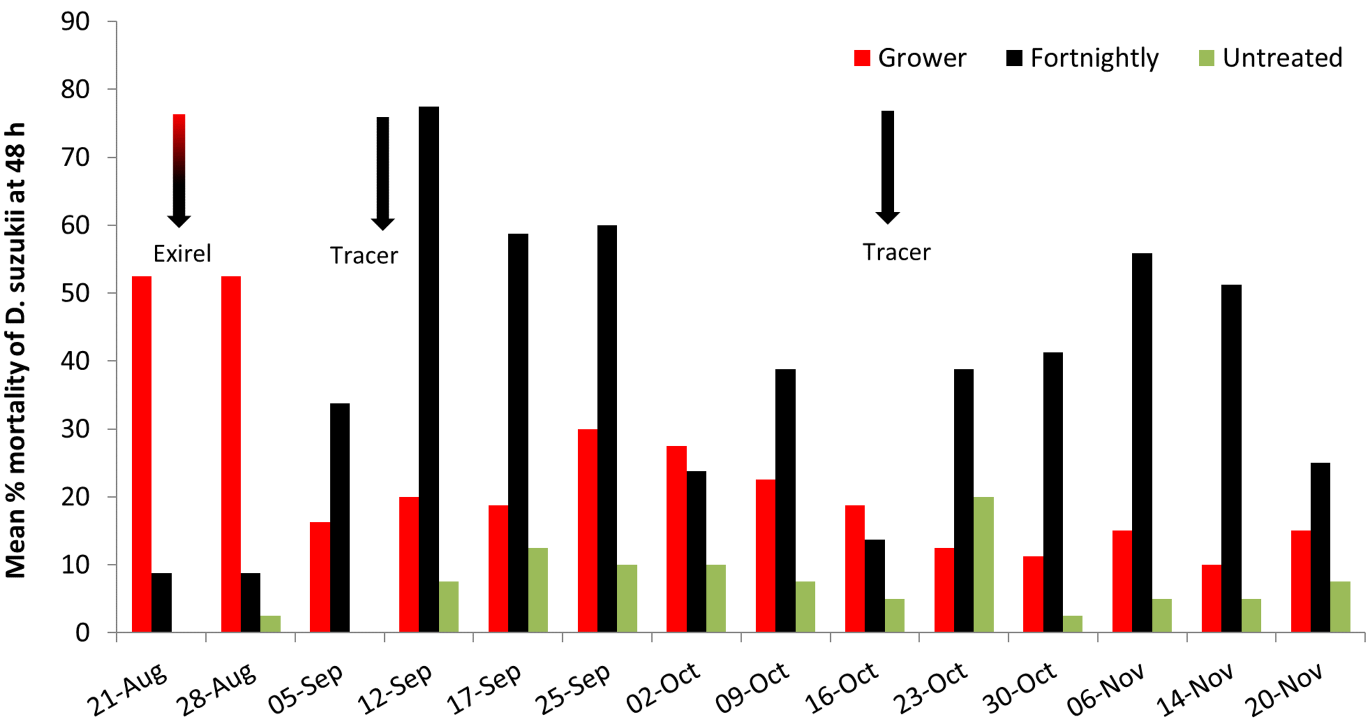


Figure 4.2b.4. Mean numbers of adult *D. suzukii* that had died after 48 hours contact with insecticide treated (fortnightly or growers programme) treated leaves compared to unsprayed raspberry leaves (green bars)

Conclusions

- We investigated whether the interval for applying insecticides to raspberry could be extended to two weeks in meshed commercial raspberry to protect against D*. suzukii.*
- Two insect meshed primocane raspberry varieties in two plantations were used.
- Treatments were either a fortnightly spray programme of approved products; Exirel and Tracer or a grower spray programme.
- Significantly more *D. suzukii* were caught in monitoring traps outside the raspberry tunnels than inside the insect meshed tunnels.
- More adult *D. suzukii* were caught inside the crops where the growers spray programme was applied, on three occasions, compared to the fortnightly spray programme, even though the fortnightly plots were under higher *D suzukii* immigration pressure from surrounding habitat.
- Because there were only two replicates of each treatment it was not possible to do statistical analyses on pest emergence from fruit (an indicator of egg laying) or the numbers of *D. suzukii* that came into contact with raspberry leaves.
- However, in most weeks, fewer *D. suzukii* emerged from fruit and more adults died in contact with leaves in the crop in the fortnightly applied spray programme compared to the growers' conventional programme.
- More work and a fully replicated trials is needed to confirm this.

Future Work

- As with the cherry spray trial research it is recommended that this work is repeated on at least two farms on a number of raspberry crops to confirm the beneficial findings of the fortnightly spray programme.

Objective 5. Integrating exclusion netting with other successful controls

A decision was made to defer this until a later year as a new CTP PhD student will be working on this in collaboration with Berry World. Initial results will be communicated in late summer 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 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.

Acknowledgements

We would like to thank the funders of the research, AHDB Horticulture, for their support. We would also like to thank all growers for the use of their crops and Berry Gardens for continued support in sourcing sites and help with the National Monitoring. We would also like to thank Angus Soft Fruits for their contribution to the Scotland Monitoring data. We also thank the technicians at NIAB EMR for help with treatment application and data gathering and Phil Brain for his advice on the statistics used.

Knowledge and Technology Transfer

2017

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

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

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

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

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

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

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

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

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

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

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

2018

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

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

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

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

Fountain: 19 Jan 18 Talk to Tracey Crouch MP on SWD

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

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

Fountain: 17 Oct 18 RHS Wisley, SWD talk to professionals at RHS

Fountain: 06 Dec 18 Berry Gardens Research and Agronomy Conference, RESEARCH AND AGRONOMY CONFERENCE Latest SWD Research

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

References

Ahmad, M., Chaudhary, S. U., Afzal, A. J., & Tariq, M. 2015. Starvation-Induced Dietary Behaviour in Drosophila melanogaster Larvae and Adults. Scientific reports, 5, 14285.

Andreazza F, Bernardi D, Baronio CA, Pasinato J, Nava DE, Botton M. (2016). Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae). Pest Manag Sci 2016 Jun 29. doi: 10.1002/ps.4348.

Anonymous (2013) Public Release Summary on the Evaluation of the New Active Constituent Cyantraniliprole in the Product DuPont Exirel Insecticide. APVMA Product Number 6410. Australian Pesticides and Veterinary Medicines Authority.

Beers, E. H., Van Steenwyk, R. A., Shearer, P. W., Coates, W. W. and Grant, J. A. (2011), Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest. Manag. Sci., 67: 1386-1395.

Cha D, Landolt PJ, Adams TB (2017). Effect of chemical ratios of a microbial-based feeding attractant on trap catch of *Drosophila suzukii* (Diptera: Drosophilidae). Env Entomol 46:907-915.

Cha D. H., Adams T., Rogg H. and P. J. Landolt. (2012). Identification and Field Evaluation of Fermentation Volatiles from Wine and Vinegar that Mediate Attraction of Spotted Wing Drosophila, *Drosophila suzukii*. *J. Chem. Ecol*. 38: 1419-1431.

Cha DH, Hesler SP, Cowles RS, Vogt H, Loeb GM, Landolt PJ. (2013). Comparison of a synthetic chemical lure and standard fermented baits for trapping *Drosophila suzukii* (Diptera: Drosophilidae). Environmental Entomology 42:1052-1060.

Cini A., Ioriatti C., Anfora G. 2012. A review of the invasion of *Drosophila suzuki*i in Europe and a draft research agenda for integrated pest management. Bulletin of Insectology, 65(1): 149-160.

Cloonan, K., Abraham, J., Angeli, S., Syed Z., Rodriguez-Saona C. 2018. Advances in the Chemical Ecology of the Spotted Wing Drosophila (*Drosophila suzukii*) and its Applications. Journal of Chemical Ecology 44: 922–939

Cook S, Khan Z, Picket J (2007) The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52:375–400

Cowles RS, Rodriguez-Saona C, Holdcraft R, Loeb GM, Elsensohn JE, Hesler SP. (2015). Sucrose improves insecticide activity against *Drosophila suzukii* (Diptera:Drosophilidae). J Econ Entomol 108:640-653.

Cuthbertson A, Audsley N. (2016). Further Screening of Entomopathogenic Fungi and Nematodes as Control Agents for *Drosophila suzukii*. Insects 2016 Jun 9;7(2). pii: E24. doi: 10.3390/insects7020024.

Cuthbertson A, Collins D, Blackburn L, Audsley N, Bell H. (2014). Preliminary Screening of Potential Control Products against *Drosophila suzukii*. Insects 5:488-498.

Dederichs U (2015) Using the bait spray method to control the spotted-wing drosophila. European Fruit Magazine No. 2015-04: 6-9.

Dorsaz M, Baroffio C. (2016). Efficacy of lime treatments against *Drosophila suzukii* in Swiss berry fruit. IOBC WPRS 9th International Conference on Integrated Fruit Production, 4th-8th September 2016, Thessaoloniki, Greece, Presentation & Abstract Book, page 82.

Durkin PR (2016) Spinosad: Human Health and Ecological Risk Assessment Final Report SERA TR-056-16-03b. Syracuse Environmental Research Associates, Inc. Manlius, New York 13104.

Eigenbrode, S. D., Birch, A. N. E., Lindzey, S., Meadow, R. and Snyder, W. E. (2016), REVIEW: A mechanistic framework to improve understanding and applications of push-pull systems in pest management. J Appl Ecol, 53: 202–212. doi:10.1111/1365-2664.12556

EPPO (2014) EPPO Standard PP 1/135 (4) Phytotoxicity assessment.

eur-lex.europa.eu. 2009. Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides (Text with EEA relevance); [accessed 2018 Nov 12]. https://eur-lex.europa.eu/eli/dir/2009/128/2009-11-25

eur-lex.europa.eu. 2013. European Commission, Commission implementing regulation (EU) No 485/2013 of 24 May 2013 amending Implementing Regulation (EU) No 540/2011, as regards the conditions of approval of the active substances clothianidin, thiamethoxam and imidacloprid, and prohibiting the use and sale of seeds treated with plant protection products containing those active substances. Off J Eur Union 139:12–26 (2013); [accessed 2018 Nov 12]. https://eur-lex.europa.eu/eli/reg_impl/2013/485/oj

Gress B., Zalom F. 2018. Identification and risk assessment of spinosad resistance in a California population of Drosophila suzukii. Pest Management Science. doi: 10.1002/ps.5240

Haviland D., Beers E. 2012. Chemical Control Programs for Drosophila suzukii that Comply With International Limitations on Pesticide Residues for Exported Sweet Cherries. Journal of Integrated Pest Management, 3: 1 – 6

Haye T, Girod P, Cuthbertson AGS, Wang XG, Daane KM, Hoelmer KA, Baroffio C, Zhang JP, Desneux N. (2016). Current *D. suzukii* IPM tactics and their practical implementation in fruit crops across different regions around the world. J Pest Sci 89: 643-651. doi:10.1007/s10340-016-0737-8.

Kelemu S. 2015. The ‘Push–Pull’ Farming System: Climate-smart, sustainable agriculture for Africa.

Kenis M, Tonina L, Eschen R, van der Sluis B, Sancassani M, Mori N, Haye T and Helsen H (2016). Non-crop plants used as hosts by Drosophila suzukii in Europe. Journal of Pest Science 89: 735-748.

Kirkpatrick D, Gut L. (2016). Improving monitoring tools for Spotted Wing Drosophila, Drosophila suzukii. IOBC WPRS 9th International Conference on Integrated Fruit Production, 4th-8th September 2016, Thessaoloniki, Greece, Presentation & Abstract Book, page 77.

Knight AL, Basoalto E, Yee W, Hilton R, Kurtzman CP. (2016). Adding yeasts with sugar to increase the number of effective insecticide classes to manage *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in cherry Pest Manag Sci 72:1482-1490 doi:10.1002/ps.4171

Lee J C, Dreves A J, Cave A M, Kawai S, Isaacs R, Miller J C, Van Timmeren S and Bruck D J (2015). Infestation of wild and ornamental noncrop fruits by Drosophila suzukii (Diptera:Drosophilidae). Ann.Entomol.Soc.Am 108:117-129

Molina JJ Harisson MD Brewer JW. 1974. Transmission of Erwinia carotovora var. atropeptica by Drosophila melanogaster Meig. American Potato Journal 51: 245–250

Mori BA et al (2017) Enhanced yeast feeding following mating facilitates control of the invasive fruit pest Drosophila suzukii. J. Applied Ecology 54, 170–177.

Noble R et al (2017) Fermentation for disinfesting fruit waste from Drosophila species (Diptera: Drosophilidae) Environmental Entomology 46, 939–945.

Palanca, L., Gaskett, A.C., Günther, C.S., Newcomb, R.D. & Goddard, M.R. (2013). Quantifying variation in the ability of yeasts to attract *Drosophila melanogaster*. PLoS ONE, 8, e75332.

Pan-europe.info. 2008. Which Pesticides are Banned in Europe? [accessed 2018 Nov 12]. https://www.pan europe.info/old/Resources/Links/Banned_in_the_EU.pdf

Parker J., Crowder D., Eigenbrode S., Snyder W. 2016. Trap crop diversity enhances crop yield. Agric Ecosys Environ 232: 254–262

Poyet M, Le Roux V, Gilbert P, Meirland A, Prevost G, Elsin P and Chabrerie O (2015). The wide potential trophic niche of the Asiatic fruit fly Drosophila suzukii: The key of its invasion success in temperate Europe? PLoS ONE 10: e10142785

Renkema JM, Wright D, Buitenhuis R, Hallett RH (2016). Plant essential oils and potassium metabisulfite as repellents for *Drosophila suzukii* (Diptera: Drosophilidae). Sci Rep. 6:21432 doi:10.1038/srep21432.

Shearer J et al. (2016) Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. BCM Ecology 16: 2-18.

Van Steenwyk RA, Wise CR, Caprile JL. (2016a). Control of spotted wing drosophila, *Drosophila suzukii*, in cherry using a new low volume, reduced-risk technique. Integrated Protection of Fruit Crops Subgroups “Pome fruit arthropods” and “Stone fruits”. IOBC-WPRS Bulletin 112:15-20.

Wallingford A, Loeb G (2016) Spotted wing drosophila winter biology. New York Fruit Quarterly 24 (3):11-13.

Wallingford A., Cha D., Linn J., Wolfin M., Loeb G. 2017. Robust manipulations of pest insect behaviour using repellents and practical application for integrated pest management. Environ Entomol 46: 1041–1050

Wallingford AK, Connelly HL, Brind'Amour GD, Boucher MT, Mafra-Neto A, Loeb GM. (2016b). Field Evaluation of an Oviposition Deterrent for Management of Spotted-Wing Drosophila, *Drosophila suzukii*, and Potential Nontarget Effects. Journal of Economic Entomology May 2016, tow116; DOI: 10.1093/jee/tow116.

Wallingford AK, Hesler SP, Cha DH, Loeb GM. (2016a). Behavioral response of spotted-wing drosophila, *Drosophila suzukii* Matsumura, to aversive odors and a potential oviposition deterrent in the field. Pest. Manag. Sci. 72:701–706. doi:10.1002/ps.4040.

Wong, J. S., Wallingford, A. K., Loeb G. M. and J. C. Lee. (2017). Physiological status of *Drosophila suzukii* (Diptera: Drosophilidae) affects their response to attractive odours. *Journal of applied entomology*. 2018:1-10.

APPENDIX 2.1.1.

Site 1.

Field name / planting	Product	Application Date	Application Time	Harvest Interval	First available harvest Date	First available harvest Time
CHERRY GROUND	Corbel	12/3	11:30	14	26/3	11:30
	[illegible] Signum	10/4	17:45	14	24/4	17:45
	[illegible] Tracer	13/4	12:30	3	16/4	12:30
	[illegible]	17/4	09:00	35	15/5	09:00
	[illegible] Hortiphyte	24/4	13:00	3	27/4	13:00
	TALIUS	29/4	12:00	3	2/5	12:00
	Maxicrop [illegible]	13/5	05:00	3	16/5	05:00
	Hortiphyte Signum	16/5	05:00	3	19/5	05:00

Field name / planting	Product	Application Date	Application Time	Harvest Interval	First available harvest Date	First available harvest Time
CHERRY GROUND						
	[illegible] Hortiphyte	21/5	13:10	3	24/5	13:10
	[illegible] Hortiphyte	29/5	17:30	3	1/6	17:30
	Fupro Hortiphyte	5/6	13:00	3	8/6	13:00
	[illegible] Maxicrop	13/6	23:00	0	13/6	23:00
	[illegible] Maxicrop	21/6	21:00	0	21/6	21:00

Field name / planting	Product	Application Date	Application Time	Harvest Interval	First available harvest Date	First available harvest Time
CHERRY GROUND						
	LUNA Sensation	26/6	23:00	1	27/6	23:00
	[illegible]	11/7	23:50	1	11/7	23:50
	[illegible] Maxicrop	17/7	23:00	0	17/7	23:00
	Hortiphyte CHARM	1/8	22:30	1	2/8	22:30
	LUNA Sensation	19/8	18:30	1	20/8	18:30

Field name / planting	Product	Application Date	Application Time	Harvest Interval	First available harvest Date	First available harvest Time
CHERRY GROUND	SP058	1/9	22:50	0	1/9	22:50

Site 2.

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2233

Tunnelled Straw

DATE: 14/5/18

LOCATION	FIELD NAME	VARIETY	Ha.
Quarry	K1	Amesti	1.5
"	K2	"	2.5
"	R27	Ladoria	2.08

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000L	Pyrethrum EC	Pyrethrum	2.4l

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~HARDI/LLL DIGGER~~	
KUBOTA TRACTOR	✓
LOUTMAN SPRAYER	✓

Ref 95

Steve Lambe

PEST LEVEL & JUSTIFICATION RSM

HARVEST INTERVAL 0 EARLIEST PICK DATE 14/5/18

SPECIAL INSTRUCTIONS

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Doran WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED

UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD K1 HI 0 PICK DATE 31/5

K2

KELSEY FARMS

No. 2232

CROP MANAGEMENT SYSTEM

Tunnelled Strawberry

DATE: 11/5/18

LOCATION	FIELD NAME	VARIETY	Ha.
Queens	K1	Amesti	1.5
"	K2	"	2.5
"	RH7	Katrina	2.0

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000l.	Dynamec	Abamectin	500ml
	Maxicrop.	Seaweed	2.0

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~HARDI WHEELED SPRAYER~~	
KUBOTA TRACTOR	✓
LOCHMAN SPRAYER	✓

Ref OK
Steve Lembo

PEST LEVEL & JUSTIFICATION Red Spider

HARVEST INTERVAL 3d. EARLIEST PICK DATE 14/5/18

SPECIAL INSTRUCTIONS

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Darren WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD K1 HI PICK DATE 31/5/18
K2 5/6/-
RH7

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2239

Tunnelled strawberry

DATE: 20/6/18

LOCATION	FIELD NAME	VARIETY	Ha.
Quarries	K1	Amort	1.5
"	K2	"	2.5

Jack Shix

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000L	Tracer	Spinosad	2

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~HARDI SELECTOR~~	
KUBOTA TRACTOR	✓
LOCHMAN SPRAYER	✓

Ref
S Leader

PEST LEVEL & JUSTIFICATION Thrip

HARVEST INTERVAL 1d EARLIEST PICK DATE 21/6/18

SPECIAL INSTRUCTIONS

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Barnes WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD	HI	PICK DATE
K1	1d	22/6
K2	1d	22/6

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2241

Tunnelled Strawberries

DATE: 4/7/18

LOCATION	FIELD NAME	VARIETY	Ha.
Queens	K1	Amest	1.5
	K2	Amest	2.5
	KK7	Salsa	2.0

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000	Naturalis-L	Beauveria Bassiana	3.0L

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~HARDI WEED KILLER~~	
KUBOTA TRACTOR	✓
LOCHMAN SPRAYER	✓

PEST LEVEL & JUSTIFICATION Red Spider Mite

HARVEST INTERVAL 0 EARLIEST PICK DATE 5/7/18

SPECIAL INSTRUCTIONS After picking

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR C. Barnes WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD	HI	PICK DATE
K1	0	6/7
K2	0	6/7
	0	5/7

KELSEY FARMS No. 2243
CROP MANAGEMENT SYSTEM

Tunnelled Strawberry

DATE: 7/7/18

LOCATION	FIELD NAME	VARIETY	Ha.
Quaver	K1	Amesti	1.5
Quaver	K2	Amesti	2.5

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000L	Takumi	Cyflufenamid	150ml
	Calypso	Thiacloprid	250ml

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~HARDI / WEEDKILLER~~	
KUBOTA TRACTOR	✓
LOCHMAN SPRAYER	✓

Rob S. Lewis

PEST LEVEL & JUSTIFICATION Mildew / Aphid

HARVEST INTERVAL 3d EARLIEST PICK DATE 20/7/18

SPECIAL INSTRUCTIONS Follow pickers into field, 6-30pm

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Burnes WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD	HI	PICK DATE
K1	3d	20/7
K2	3d	21/7

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2226

Tunnelled Strawbs

DATE: 7/4/18

pm J.Pix

LOCATION	FIELD NAME	VARIETY	Ha.
Quarven	K1	Avert	1.5
.	K2	Avert	2.5

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA	
1000L	Switch	Cyprodinil	800g	
		+ Fludioxonil		
	Maxicrop	seaweed	2.0L	

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
HARDI WEEDKILLER	✓
KUBOTA TRACTOR	✓
LUK TIMAN SPRAYER	✓

Ref S. Kemlar

PEST LEVEL & JUSTIFICATION Botrytis, Foliar Feed

HARVEST INTERVAL 3 EARLIEST PICK DATE 10/4/18.

SPECIAL INSTRUCTIONS

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G Isoman WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK
FIELD K1, K2 HI 3d PICK DATE 31/5

KELSEY FARMS No. 2229

CROP MANAGEMENT SYSTEM

Tunnelled Strawberry

DATE: 9/5/18

LOCATION	FIELD NAME	VARIETY	Ha.
Queens	K1	Amesti	1.5
..	K2	Amesti	2.5
..	RH7	Katrina	2.0

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000L	Fortress	Quinoxyfen	250ml
	Maxicrop	Seaweed	2.0L

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
HARDI WEEDKILLER	
KUBOTA TRACTOR	✓
LOCHMAN SPRAYER	✓

Plan 93

Steve Leah

PEST LEVEL & JUSTIFICATION Mildew

HARVEST INTERVAL 14d EARLIEST PICK DATE 23/5/18

SPECIAL INSTRUCTIONS

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Baines. WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD K1 K2 HI 14d PICK DATE 31/5

RH7 5/6

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2235

Tunnelled Strawbs

DATE: 6/6/17

Jun
J Rix

LOCATION	FIELD NAME	VARIETY	Ha.
Quarry	K2	Amesti	2.5

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000l	Topas	Penconazole	500ml

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~[illegible]~~	
KUBOTA TRACTOR	✓
LAHTIMAN SPRAYER	✓

S. Lewis

PEST LEVEL & JUSTIFICATION Mildew

HARVEST INTERVAL 3d EARLIEST PICK DATE 9/6/18

SPECIAL INSTRUCTIONS

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Kamer WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK
FIELD K2 HI 3d PICK DATE 11/6

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2237

Tunnelled Strawberry

DATE: 9/6/18

JRix

LOCATION	FIELD NAME	VARIETY	Ha.
Quarrie	K1	Amesti	1.5
"	K2	"	2.5

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000	Scala	Pyrimethanil	2.0L

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
HAND WEED KILLER	
KUBOTA TRACTOR	✓
LOWMAN SPRAYER	

Ref S. Lewton

PEST LEVEL & JUSTIFICATION Botrytis

HARVEST INTERVAL 3d EARLIEST PICK DATE 12/6/18

SPECIAL INSTRUCTIONS Follow pickers into field K1 - 7.30

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Barnes WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD K1 HI 3d PICK DATE 12/6/18

K2 3d

KELSEY FARMS No. 2238

CROP MANAGEMENT SYSTEM

Tunnelled Strawberry

DATE: 17/6/18

Jun SRix

LOCATION	FIELD NAME	VARIETY	Ha.
Quarries	RH7	Kalvina	2.0
"	K1	Amesti	1.5
	K2	"	2.5

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000			
	Luna Sensation	Fluopyram	800ml
		Trifloxystrobin	

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~[illegible]~~	
KUBOTA TRACTOR	✓
LOCHMAN SPRAYER	✓

Rob
S. Lander

PEST LEVEL & JUSTIFICATION Mildew

HARVEST INTERVAL 1d EARLIEST PICK DATE 18/6/18

SPECIAL INSTRUCTIONS Do RH7 before 5.00 then K1, K2

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G. Barnes WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD	HI	PICK DATE
RH7	1d	18/6/18
K1	1d	18/6/18

KELSEY FARMS No. 2244

CROP MANAGEMENT SYSTEM

Tunnelled Strawberries

DATE: 18/7/18

LOCATION	FIELD NAME	VARIETY	Ha.
Craven	K2	Amesti	2.5

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
600L	Takumi	Cyflufenamid	900ml

BOOTS	
OVERALLS	
RESPIRATOR	
FACE MASK	
GLOVES	
HARDI WELDKILLER	
KUBOTA TRACTOR	
LOCHMANN SPRAYER	

Re. S. Kowler

PEST LEVEL & JUSTIFICATION Mildew

HARVEST INTERVAL 3d EARLIEST PICK DATE 21/7/18

SPECIAL INSTRUCTIONS Do at 5.00 am

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR J. Tamm WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED

UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD HI PICK DATE

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2249

Tunnelled Strawberry

DATE: 23/8/18

pm

Glx

LOCATION	FIELD NAME	VARIETY	Ha.
Quaver	K1	Amesti	1.5
"	K2	"	2.0

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000l	Frupica	Mepanipyrim	800ml
	Maxicrop	Seaweed	3.0l

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
~~[illegible]~~	
KUBOTA TRACTOR	✓
LOCHMAN SPRAYER	✓

Col steiner.

PEST LEVEL & JUSTIFICATION Mildew Scab

HARVEST INTERVAL 3d EARLIEST PICK DATE 26/8

SPECIAL INSTRUCTIONS Do K1 on 23rd & K2 at 12.00 on 24th

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR G Barnes WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD K1, K2 HI 3d. PICK DATE 27/8

27/8

KELSEY FARMS
CROP MANAGEMENT SYSTEM

No. 2253

DATE: 14/9/18

Tunnelled Strawberry

LOCATION	FIELD NAME	VARIETY	Ha.
Quinnes	K1	Amesti	1.5
"	K2	Amesti	2.5
"	RH7	Kalimna	2.0

WATER VOLUME	CHEMICAL OR FERTILISER	ACTIVE INGREDIENTS	RATE HA
1000L	AQ10	Ampelomyces quisqualis 58%	70g

BOOTS	✓
OVERALLS	✓
RESPIRATOR	✓
FACE MASK	✓
GLOVES	✓
HAND WEEDKILLER	✓
KUBOTA TRACTOR	✓
LOCHMANN SPRAYER	✓

PEST LEVEL & JUSTIFICATION Mildew Greater

HARVEST INTERVAL 0 EARLIEST PICK DATE N/A

SPECIAL INSTRUCTIONS

ALWAYS READ THE LABEL AND CHECK PROTECTIVE CLOTHING REQUIRED

OPERATOR C Barnes WEATHER & WIND SPEED

DATE: START TIME: END TIME:

ACTUAL QUANTITY/OF CHEMICAL APPLIED
UNITS FERTILISER

HARVEST INTERVAL CHECK

FIELD	HI	PICK DATE
K2	0	15/9
RH7	0	15/9
K1		

MANAGER/SUPERVISOR

Site 3.

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Roundup 2.9700 ha 5.000 L/ha
MAPP:12645, Active Ingredients:Glyphosate, Manufacturer:Monsanto UK Limited, Expires:30/06/2018

? Reference: Plan 04892 Mini T-tops Inter-row 06/11 Job 1 — Issued by: John Longley (05/11/2018)
Target growth stage: Post production-grubbing out
Implement: Micron 200L, Volume rate: 500.000 L

Roundup 1.4850 ha 4.000 L/ha
MAPP:12645, Active Ingredients:Glyphosate, Manufacturer:Monsanto UK Limited, Expires:30/06/2018

Stomp Aqua 1.4850 ha 3.000 L/ha
MAPP:14664, Active Ingredients:Pendimethalin, Manufacturer:BASF plc.

Devrinol (09374) 1.4850 ha 7.000 L/ha
MAPP:09374, Active Ingredients:Napropamide, Manufacturer:UPL Europe Limited, Expires:31/07/2018

Earliest safe harvest date: 20/09/2018 11:45

Townfield.Sweet eve 2 — Working ha: 2.6982
Variety: Sweet eve 2 — Map sheet:
Crop: Strawberry (Protected) — NG number:

? Reference: Plan 04229 Slugs Jan/Feb 2018 Job 1 — Issued by: John Longley (05/02/2018)
Implement: Vicon

Derrex 2.6982 ha 7.000 kg/ha
MAPP:15351, Active Ingredients:Ferric phosphate, Manufacturer:Certis, Expires:30/06/2033

Reference: Plan 04280 Townfield 04/04 Job 1 — Issued by: John Longley (04/04/2018)
Target growth stage: 16: Sixth leaf
Job Harvest Interval: 07 Days 00 Hours
Start: 04/04/2018 08:30 Finish: 04/04/2018 13:00 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 10 Wind speed/direction: 1 SE Soil: Dry
Implement: 6 row t-top, Volume rate: 1000.000 L, Spray quality: Medium

Borneo 2.6982 ha 0.350 L/ha
MAPP:13919, Active Ingredients:Etoxazole, Manufacturer:Interfarm (U.K.) Ltd.

Dynamec 2.6982 ha 0.500 L/ha
MAPP:18316, Active Ingredients:Abamectin, Manufacturer:Syngenta UK Ltd

Stroby WG 2.6982 ha 0.300 kg/ha
MAPP:17316, Harvest interval:07 Days 00 Hours, Active Ingredients:Kresoxim-methyl, Manufacturer:BASF plc., Expires:30/06/2024

SP058 2.6982 ha 0.400 L/ha

Reference: Plan 04304 Cornel spraying 13/04 Job 1 — Issued by: John Longley (13/04/2018)
Target growth stage: 61: Early Flower, 10% flowers open
Job Harvest Interval: 03 Days 00 Hours
Start: 13/04/2018 08:00 Finish: 13/04/2018 10:30 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 10 Wind speed/direction: 1 SE Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Switch 2.6982 ha 1.000 kg/ha
MAPP:15129, Harvest interval:03 Days 00 Hours, Active Ingredients:Fludioxonil, Cyprodinil, Manufacturer:Syngenta UK Ltd, Expires:31/10/2020

Amistar 2.6982 ha 1.000 L/ha
MAPP:18039, Harvest interval:03 Days 00 Hours, Active Ingredients:Azoxystrobin, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024

Reference: Plan 04334 Cornel Spraying 24/04 !!!! Job 2 — Issued by: John Longley (24/04/2018)
Job Harvest Interval: 03 Days 00 Hours
Start: 24/04/2018 14:00 Finish: 24/04/2018 17:00 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 12 Wind speed/direction: 1 SE Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Topas 2.6982 ha 0.500 L/ha
MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024

Sinpro 2.6982 ha 1.500 L/ha
MAPP:16236, Harvest interval:02 Days 00 Hours, Active Ingredients:Iprodione, Manufacturer:Sinon EU Corporation, Expires:05/06/2018

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Job Harvest Interval: 02 Days 00 Hours
Start: 09/05/2018 14:00 Finish: 09/05/2018 16:00 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 12 Wind speed/direction: 1 N Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Sinpro 2.6982 ha 1.500 L/ha
MAPP:16236, Harvest interval:02 Days 00 Hours, Active Ingredients:Iprodione, Manufacturer:Sinon EU Corporation, Expires:05/06/2018

Charm 2.6982 ha 0.600 L/ha
MAPP:18396, Harvest interval:01 Days 00 Hours, Active Ingredients:Fluxapyroxad, Difenoconazole, Manufacturer:BASF plc., Expires:30/06/2021

Reference: Plan 04409 Cornel Spraying 17/05 Job 1 — Issued by: John Longley (17/05/2018)
Target growth stage: Harvest
Start: 17/05/2018 10:00 Finish: 17/05/2018 13:30 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 12 Wind speed/direction: 1 SE Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Luna Sensation 2.6982 ha 0.800 L/ha
MAPP:15793, Active Ingredients:Fluopyram, Trifloxystrobin, Manufacturer:Bayer CropScience Limited

Reference: Plan 04419 Cornel Bicarb week 21 Job 2 — Issued by: John Longley (18/05/2018)
Target growth stage: Harvest
Start: 21/05/2018 13:00 Finish: 21/05/2018 15:30 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 15 Wind speed/direction: 1 N Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Wetcit 2.6982 ha 0.500 L/ha

Potassium Bicarbonate 2.6982 ha 5.000 kg/ha

Reference: Plan 04432 Cornel spraying 31/05 Job 1 — Issued by: John Longley (31/05/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 31/05/2018 10:00 Finish: 31/05/2018 13:00 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 18 Wind speed/direction: 1 SW Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Topas 2.6982 ha 0.500 L/ha
MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024

Reference: Plan 04446 Cornel Spraying 05/06 Job 1 — Issued by: John Longley (05/06/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 05/06/2018 17:00 Finish: 05/06/2018 18:30 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 18 Wind speed/direction: 1 SW Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Maxicrop+Iron 2.6982 ha 3.000 kg/ha

Fast copper 2.6982 ha 3.000 L/ha

Signum 2.6982 ha 1.800 kg/ha
MAPP:11450, Harvest interval:03 Days 00 Hours, Active Ingredients:Boscalid, Pyraclostrobin, Manufacturer:BASF plc., Expires:31/07/2021

Reference: Plan 04469 Cornel Spraying 12/06 Job 2 — Issued by: John Longley (12/06/2018)
Target growth stage: Harvest
Start: 12/06/2018 14:00 Finish: 12/06/2018 16:00 Operator: Cornel Anghel
Weather: Partly cloudy Temp °C: 17 Wind speed/direction: 1 S Soil: Dry
Implement: 6 row t-top, Volume rate: 500.000 L, Spray quality: Medium

Potassium Bicarbonate 2.6982 ha 5.000 kg/ha

Wetcit 2.6982 ha 0.500 L/ha

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Reference: Plan 04528 Cornel Spraying 28/06 Job 1 — Issued by: John Longley (28/06/2018)
Target growth stage: Harvest
Job Harvest Interval: 07 Days 00 Hours
Start: 28/06/2018 18:30 — **Finish: 28/06/2018 20:30** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 18 — Wind speed/direction: 1 S — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Fast Manganese	2.6982 ha	3.000 L/ha
Stroby WG	2.6982 ha	0.300 kg/ha

MAPP:17316, Harvest interval:07 Days 00 Hours, Active Ingredients:Kresoxim-methyl, Manufacturer:BASF plc., Expires:30/06/2024

Reference: Plan 04555 Cornel Spraying 05/07 Job 1 — Issued by: John Longley (05/07/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 05/07/2018 20:30 — **Finish: 05/07/2018 20:30** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 18 — Wind speed/direction: 1 SE — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Takumi SC	2.6982 ha	0.150 L/ha

MAPP:16000, Harvest interval:03 Days 00 Hours, Active Ingredients:Cyflufenamid, Manufacturer:Certis, EAMU:2055/16, Expires:30/09/2022

Reference: Plan 04575 Townfield 11/07 Job 1 — Issued by: John Longley (10/07/2018)
Target growth stage: Harvest
Start: 11/07/2018 17:00 — **Finish: 11/07/2018 19:00** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 19 — Wind speed/direction: 1 SW — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Potassium Bicarbonate	2.6982 ha	5.000 kg/ha
Wetcit	2.6982 ha	0.500 L/ha

Reference: Plan 04601 Townfield 16/07 Job 1 — Issued by: John Longley (16/07/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 16/07/2018 16:30 — **Finish: 16/07/2018 18:30** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 19 — Wind speed/direction: 1 SE — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Topas	2.6982 ha	0.500 L/ha

MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd., Expires:30/06/2024

Reference: Plan 04621 Townfield 24/07 Job 1 — Issued by: John Longley (24/07/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 24/07/2018 18:00 — **Finish: 24/07/2018 20:30** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 20 — Wind speed/direction: 1 SW — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Amistar	2.6982 ha	1.000 L/ha

MAPP:18039, Harvest interval:03 Days 00 Hours, Active Ingredients:Azoxystrobin, Manufacturer:Syngenta UK Ltd., Expires:30/06/2024

Reference: Plan 04646 Townfield 31/07 Job 1 — Issued by: John Longley (31/07/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 01/08/2018 17:30 — **Finish: 01/08/2018 19:30** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 22 — Wind speed/direction: 1 SE — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Charm	2.6982 ha	0.600 L/ha

MAPP:18396, Harvest interval:01 Days 00 Hours, Active Ingredients:Fluxapyroxad, Difenoconazole, Manufacturer:BASF plc., Expires:30/06/2021

Hallmark With Zeon Technology	2.6982 ha	0.075 L/ha

MAPP:12629, Harvest interval:03 Days 00 Hours, Active Ingredients:Lambda-cyhalothrin, Manufacturer:Syngenta UK Ltd, EAMU:1705/11

To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Implement: 6 row I-Iop, Volume rate: 1000.000 L, Spray quality: Medium

Tracer	2.6982 ha	0.150 L/ha

MAPP:12438, Harvest interval:01 Days 00 Hours, Active Ingredients:Spinosad, Manufacturer:Landseer Ltd., EAMU:1238/17, Expires:15/10/2018

Reference: Plan 04711 Cornel Spraying 23/08 Job 1 — Issued by: John Longley (23/08/2018)
Target growth stage: Harvest
Start: 23/08/2018 08:00 — **Finish: 23/08/2018 11:00** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 19 — Wind speed/direction: 1 S — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Pyrethrum 5 EC	2.6982 ha	1.100 L/ha

MAPP:18532, Active Ingredients:Pyrethrins, Manufacturer:Pelgar International Ltd

Reference: Plan 04730 Cornel spraying 30/08 Job 1 — Issued by: John Longley (30/08/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 30/08/2018 09:00 — **Finish: 30/08/2018 12:00** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 19 — Wind speed/direction: 1 SW — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Decis	2.6982 ha	0.100 L/ha

MAPP:16124, Harvest interval:03 Days 00 Hours, Active Ingredients:Deltamethrin, Manufacturer:Bayer CropScience Limited, EAMU:1643/13, Expires:30/04/2021

Topas	2.6982 ha	0.500 L/ha

MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd., Expires:30/06/2024

Switch	2.6982 ha	1.000 kg/ha

MAPP:15129, Harvest interval:03 Days 00 Hours, Active Ingredients:Fludioxonil, Cyprodinil, Manufacturer:Syngenta UK Ltd., Expires:31/10/2020

Reference: Plan 04845 Cornel spraying 01/09 Job 1 — Issued by: John Longley (01/10/2018)
Target growth stage: Harvest
Start: 01/09/2018 10:30 — **Finish: 01/09/2018 13:15** — Operator: Cornel Anghel
Weather: Clear — Temp °C: 17 — Wind speed/direction: 2 SW — Soil: Moist
Implement: 6 row I-Iop, Volume rate: 1000.000 L, Spray quality: Medium

Potassium Bicarbonate	2.6982 ha	10.000 kg/ha
Kumulus DF	2.6982 ha	2.000 kg/ha

MAPP:04707, Active Ingredients:Sulphur, Manufacturer:BASF plc., Expires:31/12/2021

Reference: Plan 04753 Cornel Spraying 04/09 Job 2 — Issued by: John Longley (04/09/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 04/09/2018 13:00 — **Finish: 04/09/2018 15:00** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 11 — Wind speed/direction: 1 S — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Amistar	2.6982 ha	1.000 L/ha

MAPP:18039, Harvest interval:03 Days 00 Hours, Active Ingredients:Azoxystrobin, Manufacturer:Syngenta UK Ltd., Expires:30/06/2024

Hallmark With Zeon Technology	2.6982 ha	0.075 L/ha

MAPP:12629, Harvest interval:03 Days 00 Hours, Active Ingredients:Lambda-cyhalothrin, Manufacturer:Syngenta UK Ltd, EAMU:1705/11

? Reference: Plan 04806 Cornel Spraying 19/09 Job 1 — Issued by: John Longley (18/09/2018)
Target growth stage: Harvest
Job Harvest Interval: 01 Days 00 Hours
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Benevia 10OD	2.6982 ha	0.750 L/ha

MAPP:99992, Harvest interval:01 Days 00 Hours, Active Ingredients:Cyantraniliprole, Manufacturer:Headland Agrochemicals Ltd., EAMU:1382/18, Expires:30/09/2018

Kindred	2.6982 ha	0.600 L/ha

MAPP:13891, Active Ingredients:Meptyldinocap, Manufacturer:Landseer Ltd.

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Activator 90	2.6982 ha	0.400 L/ha

Reference: Plan 04872 Cornel Spraying 19/10 Job 2 — Issued by: John Longley (19/10/2018)
Job Harvest Interval: 03 Days 00 Hours
Start: 20/10/2018 09:00 — **Finish: 20/10/2018 12:00** — Operator: Cornel Anghel
Weather: Partly cloudy — Temp °C: 15 — Wind speed/direction: 1 SE — Soil: Dry
Implement: 6 row I-Iop, Volume rate: 500.000 L, Spray quality: Medium

Calypso	2.6982 ha	0.250 L/ha

MAPP:11257, Harvest interval:03 Days 00 Hours, Active Ingredients:Thiacloprid, Manufacturer:Bayer CropScience Limited, EAMU:2131/14, Expires:31/10/2020

Teldor	2.6982 ha	1.500 kg/ha

MAPP:11229, Harvest interval:01 Days 00 Hours, Active Ingredients:Fenhexamid, Manufacturer:Bayer CropScience Limited

Nimrod (13046)	2.6982 ha	1.400 L/ha

MAPP:13046, Harvest interval:01 Days 00 Hours, Active Ingredients:Bupirimate, Manufacturer:Adama Agricultural Solutions UK Ltd., Expires:30/11/2019

Site 4.

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Curlings./02.Sweet eve 2 — Working ha: 2.9700
Variety: Sweet eve 2 — Map sheet:
Crop: Strawberry (Protected) — NG number:

Reference: Plan 04223 Curlings Inter-row 26/01 Job 1 — Issued by: John Longley (25/01/2018)
Target growth stage: 00: Winter Dormancy
Start: 26/01/2018 08:00 — Finish: 26/01/2018 16:00 — Operator: Cornel Anghel
Weather: Cloudy — Temp °C: 7 — Wind speed/direction: 1 NE — Soil: Dry
Implement: Micron 200L, Volume rate: 500.000 L

Quit	0.9900 ha	2.000 L/ha
MAPP:14874, Active Ingredients:Diquat, Manufacturer:Belchim Crop Protection Limited, Expires:31/12/2020		
Shark	0.9900 ha	0.330 L/ha
MAPP:17256, Active Ingredients:Carfentrazone-ethyl, Manufacturer:Headland Agrochemicals Ltd., EAMU:0378/17, Expires:31/01/2021		
Stomp Aqua	0.9900 ha	2.900 L/ha
MAPP:14664, Active Ingredients:Pendimethalin, Manufacturer:BASF plc.		

? Reference: Plan 04229 Slugs Jan/Feb 2018 Job 1 — Issued by: John Longley (05/02/2018)
Implement: Vicon

Derrex	2.9700 ha	7.000 kg/ha
MAPP:15351, Active Ingredients:Ferric phosphate, Manufacturer:Certis, Expires:30/06/2033		

Reference: Plan 04264 Curlings 21/03 Job 1 — Issued by: John Longley (20/03/2018)
Target growth stage: 61: Early Flower, 10% flowers open
Job Harvest Interval: 14 Days 00 Hours
Start: 21/03/2018 10:00 — Finish: 21/03/2018 14:00 — Operator: Tyanko Zapryanov
Weather: Bright sun — Temp °C: 10 — Wind speed/direction: 1 SW — Soil: Moist
Implement: 4 Bed Munkhof, Volume rate: 1000.000 L

Fortress	2.9700 ha	0.250 L/ha
MAPP:08279, Harvest interval:14 Days 00 Hours, Active Ingredients:Quinoxyfen, Manufacturer:Dow AgroSciences Ltd., EAMU:1923/04, Expires:31/10/2020		
Switch	2.9700 ha	1.000 kg/ha
MAPP:15129, Harvest interval:03 Days 00 Hours, Active Ingredients:Fludioxonil, Cyprodinil, Manufacturer:Syngenta UK Ltd, Expires:31/10/2020		
Hallmark With Zeon Technology	2.9700 ha	0.075 L/ha
MAPP:12629, Harvest interval:03 Days 00 Hours, Active Ingredients:Lambda-cyhalothrin, Manufacturer:Syngenta UK Ltd, EAMU:1705/11		

Reference: Plan 04306 Curlings 14/04 Job 1 — Issued by: John Longley (13/04/2018)
Target growth stage: 61: Early Flower, 10% flowers open
Job Harvest Interval: 03 Days 00 Hours
Start: 14/04/2018 12:00 — Finish: 14/04/2018 14:20 — Operator: Tyanko Zapryanov
Weather: Bright sun — Temp °C: 14 — Wind speed/direction: 1 SW — Soil: Moist
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Topas	2.9700 ha	0.500 L/ha
MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024		
Calypso	2.9700 ha	0.250 L/ha
MAPP:11257, Harvest interval:03 Days 00 Hours, Active Ingredients:Thiacloprid, Manufacturer:Bayer CropScience Limited, EAMU:2131/14, Expires:31/10/2020		
SP058	2.9700 ha	0.300 L/ha
Teldor	2.9700 ha	1.500 kg/ha
MAPP:11229, Harvest interval:01 Days 00 Hours, Active Ingredients:Fenhexamid, Manufacturer:Bayer CropScience Limited		

Reference: Plan 04335 Curlings 24/04 Job 1 — Issued by: John Longley (24/04/2018)
Target growth stage: 63: 30% Flowering
Job Harvest Interval: 03 Days 00 Hours
Start: 24/04/2018 15:00 — Finish: 24/04/2018 18:30 — Operator: Tyanko Zapryanov
Weather: Partly cloudy — Temp °C: 10 — Wind speed/direction: 1 SW — Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 1000.000 L

Scala	2.9700 ha	2.000 L/ha
MAPP:15222, Harvest interval:03 Days 00 Hours, Active Ingredients:Pyrimethanil, Manufacturer:BASF plc., Expires:31/10/2020		
Pyrethrum 5 EC	2.9700 ha	1.100 L/ha
MAPP:18532, Harvest interval:01 Days 00 Hours, Active Ingredients:Pyrethrins, Manufacturer:Pelgar International Ltd		

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Target growth stage: 63: 30% Flowering
Job Harvest Interval: 07 Days 00 Hours
Start: 04/05/2018 07:00 — Finish: 04/05/2018 11:40 — Operator: Tyanko Zapryanov
Weather: Clear — Temp °C: 16 — Wind speed/direction: 1 SE — Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Sinpro	2.9700 ha	1.500 L/ha
MAPP:16236, Harvest interval:02 Days 00 Hours, Active Ingredients:Iprodione, Manufacturer:Sinon EU Corporation, Expires:05/06/2018		
Stroby WG	2.9700 ha	0.300 kg/ha
MAPP:17316, Harvest interval:07 Days 00 Hours, Active Ingredients:Kresoxim-methyl, Manufacturer:BASF plc., Expires:30/06/2024		

Reference: Plan 04375 Curlings 07/05 Job 1 — Issued by: John Longley (07/05/2018)
Target growth stage: 75: Fruits half final size
Job Harvest Interval: 02 Days 00 Hours
Start: 07/05/2018 08:00 — Finish: 07/05/2018 11:00 — Operator: Tyanko Zapryanov
Weather: Clear — Temp °C: 14 — Wind speed/direction: 2 SW — Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Charm	2.9700 ha	0.600 L/ha
MAPP:18396, Harvest interval:01 Days 00 Hours, Active Ingredients:Fluxapyroxad, Difenoconazole, Manufacturer:BASF plc., Expires:30/06/2021		
Sinpro	2.9700 ha	1.500 L/ha
MAPP:16236, Harvest interval:02 Days 00 Hours, Active Ingredients:Iprodione, Manufacturer:Sinon EU Corporation, Expires:05/06/2018		

Reference: Plan 04410 Ivo spraying 17/05 Job 2 — Issued by: John Longley (17/05/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 17/05/2018 12:30 — Finish: 17/05/2018 15:15 — Operator: Ivaylo Duganov
Weather: Partly cloudy — Temp °C: 12 — Wind speed/direction: 1 SW — Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Topas	2.9700 ha	0.500 L/ha
MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024		
Switch	2.9700 ha	1.000 kg/ha
MAPP:15129, Harvest interval:03 Days 00 Hours, Active Ingredients:Fludioxonil, Cyprodinil, Manufacturer:Syngenta UK Ltd, Expires:31/10/2020		

Reference: Plan 04416 Ivo spraying bicarb Week 21 Job 1 — Issued by: John Longley (18/05/2018)
Target growth stage: Harvest
Start: 22/05/2018 06:30 — Finish: 22/05/2018 09:30 — Operator: Ivaylo Duganov
Weather: Partly cloudy — Temp °C: 13 — Wind speed/direction: 1 N — Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Potassium Bicarbonate	2.9700 ha	5.000 kg/ha
Wetcit	2.9700 ha	0.500 L/ha

Reference: Plan 04425 Ivo spraying 30/05 Job 2 — Issued by: John Longley (30/05/2018)
Target growth stage: Harvest
Start: 30/05/2018 15:30 — Finish: 30/05/2018 18:30 — Operator: Ivaylo Duganov
Weather: Partly cloudy — Temp °C: 15 — Wind speed/direction: 1 S — Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Potassium Bicarbonate	2.9700 ha	5.000 kg/ha
Wetcit	2.9700 ha	0.500 L/ha

Reference: Plan 04454 Tyanko Spraying 07/06 Job 1 — Issued by: John Longley (07/06/2018)
Target growth stage: Harvest
Start: 07/06/2018 08:00 — Finish: 07/06/2018 10:00 — Operator: Tyanko Zapryanov
Weather: Clear — Temp °C: 15 — Wind speed/direction: 1 SW — Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Potassium Bicarbonate	2.9700 ha	5.000 kg/ha
Wetcit	2.9700 ha	0.500 L/ha

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Weather: Bright sun | Temp °C: 17 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Scala 2.9700 ha 2.000 L/ha
MAPP:15222, Harvest interval:03 Days 00 Hours, Active Ingredients:Pyrimethanil, Manufacturer:BASF plc., Expires:31/10/2020
Topas 2.9700 ha 0.500 L/ha
MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024

Reference: Plan 04485 Curlings 18/06 Job 1 | Issued by: John Longley (18/06/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 18/06/2018 16:00 | Finish: 18/06/2018 18:15 | Operator: Tyanko Zapryanov
Weather: Bright sun | Temp °C: 17 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Amistar 2.9700 ha 1.000 L/ha
MAPP:18039, Harvest interval:03 Days 00 Hours, Active Ingredients:Azoxystrobin, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024
Maxicrop+Iron 2.9700 ha 3.000 kg/ha

Reference: Plan 04531 Curlings 29/06 Job 1 | Issued by: John Longley (28/06/2018)
Target growth stage: Harvest
Start: 29/06/2018 07:00 | Finish: 29/06/2018 10:30 | Operator: Ivaylo Duganov
Weather: Partly cloudy | Temp °C: 14 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Potassium Bicarbonate 2.9700 ha 5.000 kg/ha
Wetcit 2.9700 ha 0.500 L/ha

Reference: Plan 04559 Curlings 06/07 Job 1 | Issued by: John Longley (05/07/2018)
Job Harvest Interval: 01 Days 00 Hours
Start: 06/07/2018 16:00 | Finish: 06/07/2018 18:50 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 18 | Wind speed/direction: 1 N | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Charm 2.9700 ha 0.600 L/ha
MAPP:18396, Harvest interval:01 Days 00 Hours, Active Ingredients:Fluxapyroxad, Difenoconazole, Manufacturer:BASF plc., Expires:30/06/2021

Reference: Plan 04517 Curlings 10/07 Job 1 | Issued by: John Longley (10/07/2018)
Target growth stage: Harvest
Start: 10/07/2018 08:00 | Finish: 10/07/2018 14:00 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 16 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 1000.000 L

Potassium Bicarbonate 2.9700 ha 10.000 kg/ha
Wetcit 2.9700 ha 1.000 L/ha

Reference: Plan 04585 Curlings 12/07 Job 1 | Issued by: John Longley (12/07/2018)
Target growth stage: Harvest
Start: 19/07/2018 17:00 | Finish: 19/07/2018 20:00 | Operator: Ivaylo Duganov
Weather: Partly cloudy | Temp °C: 15 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 1000.000 L

Potassium Bicarbonate 2.9700 ha 10.000 kg/ha
Wetcit 2.9700 ha 1.000 L/ha

Reference: Plan 04622 Curlings 24/07 Job 1 | Issued by: John Longley (24/07/2018)
Target growth stage: Harvest
Start: 25/07/2018 17:00 | Finish: 25/07/2018 19:30 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 17 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 1000.000 L

Potassium Bicarbonate 2.9700 ha 10.000 kg/ha
Wetcit 2.9700 ha 1.000 L/ha

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Weather: Partly cloudy | Temp °C: 15 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Stroby WG 2.9700 ha 0.300 kg/ha
MAPP:17316, Harvest interval:07 Days 00 Hours, Active Ingredients:Kresoxim-methyl, Manufacturer:BASF plc., Expires:30/06/2024

Reference: Plan 04668 Ventsi spraying 10/08 Job 1 | Issued by: John Longley (09/08/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 10/08/2018 06:30 | Finish: 10/08/2018 12:00 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 12 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 1000.000 L

Nimrod 2.9700 ha 1.400 L/ha
MAPP:18522, Harvest interval:01 Days 00 Hours, Active Ingredients:Bupirimate, Manufacturer:Adama Agricultural Solutions UK Ltd
Teldor 2.9700 ha 1.500 kg/ha
MAPP:11229, Harvest interval:01 Days 00 Hours, Active Ingredients:Fenhexamid, Manufacturer:Bayer CropScience Limited
Hallmark With Zeon Technology 2.9700 ha 0.075 L/ha
MAPP:12629, Harvest interval:03 Days 00 Hours, Active Ingredients:Lambda-cyhalothrin, Manufacturer:Syngenta UK Ltd, EAMU:1705/11

Reference: Plan 04595 Ventsi spraying 20/08 Job 1 | Issued by: John Longley (20/08/2018)
Target growth stage: Harvest
Job Harvest Interval: 01 Days 00 Hours
Start: 20/08/2018 10:00 | Finish: 20/08/2018 13:00 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 17 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Benevia 10OD 2.9700 ha 0.375 L/ha
MAPP:99992, Harvest interval:01 Days 00 Hours, Active Ingredients:Cyantraniliprole, Manufacturer:Headland Agrochemicals Ltd., EAMU:1382/18, Expires:30/09/2018

Reference: Plan 04725 Ventsi spraying 28/08 Job 1 | Issued by: John Longley (24/08/2018)
Target growth stage: Harvest
Job Harvest Interval: 03 Days 00 Hours
Start: 28/08/2018 10:00 | Finish: 28/08/2018 13:00 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 15 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Decis 2.9700 ha 0.100 L/ha
MAPP:16124, Harvest interval:03 Days 00 Hours, Active Ingredients:Deltamethrin, Manufacturer:Bayer CropScience Limited, EAMU:1643/13, Expires:30/04/2021
Topas 2.9700 ha 0.500 L/ha
MAPP:16765, Harvest interval:03 Days 00 Hours, Active Ingredients:Penconazole, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024
Switch 2.9700 ha 1.000 kg/ha
MAPP:15129, Harvest interval:03 Days 00 Hours, Active Ingredients:Fludioxonil, Cyprodinil, Manufacturer:Syngenta UK Ltd, Expires:31/10/2020

Reference: Plan 04731 Ventsi Spraying 30/08 Job 2 | Issued by: John Longley (03/09/2018)
Job Harvest Interval: 01 Days 00 Hours
Start: 30/08/2018 13:00 | Finish: 30/08/2018 15:00 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 16 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Pyrethrum 5 EC (18210) 2.9700 ha 1.100 L/ha
MAPP:18210, Harvest interval:01 Days 00 Hours, Active Ingredients:Pyrethrins, Manufacturer:Pelgar International Ltd, Expires:31/05/2020

Reference: Plan 04755 Ventsi Spraying 04/09 Job 1 | Issued by: John Longley (04/09/2018)
Target growth stage: Harvest
Start: 04/09/2018 09:00 | Finish: 04/09/2018 13:00 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 15 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Potassium Bicarbonate 2.9700 ha 5.000 kg/ha
Wetcit 2.9700 ha 0.500 L/ha

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

Weather: Partly cloudy | Temp °C: 14 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Tracer 2.9700 ha 0.150 L/ha
MAPP:12438, Harvest interval:01 Days 00 Hours, Active Ingredients:Spinosad, Manufacturer:Landseer Ltd., EAMU:1395/18, Expires:15/10/2018
Amistar 2.9700 ha 1.000 L/ha
MAPP:18039, Harvest interval:03 Days 00 Hours, Active Ingredients:Azoxystrobin, Manufacturer:Syngenta UK Ltd, Expires:30/06/2024

Reference: Plan 04795 Curlings 14/9 Job 1 | Issued by: John Longley (13/09/2018)
Target growth stage: Harvest
Start: 14/09/2018 07:50 | Finish: 14/09/2018 14:00 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 15 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 1000.000 L

Potassium Bicarbonate 2.9700 ha 10.000 kg/ha
Kumulus DF 2.9700 ha 2.000 kg/ha
MAPP:04707, Active Ingredients:Sulphur, Manufacturer:BASF plc., Expires:31/12/2021

Reference: Plan 04807 Ventsi spraying 19/09 Job 1 | Issued by: John Longley (18/09/2018)
Target growth stage: Harvest
Job Harvest Interval: 01 Days 00 Hours
Start: 19/09/2018 08:30 | Finish: 19/09/2018 11:45 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 15 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Nimrod (13046) 2.9700 ha 1.400 L/ha
MAPP:13046, Harvest interval:01 Days 00 Hours, Active Ingredients:Bupirimate, Manufacturer:Adama Agricultural Solutions UK Ltd, Expires:30/11/2019
Benevia 10OD 2.9700 ha 0.750 L/ha
MAPP:99992, Harvest interval:01 Days 00 Hours, Active Ingredients:Cyantraniliprole, Manufacturer:Headland Agrochemicals Ltd., EAMU:1382/18, Expires:30/09/2018

Reference: Plan 04831 Curlings 26/09 Job 1 | Issued by: John Longley (25/09/2018)
Target growth stage: Harvest
Start: 28/09/2018 10:30 | Finish: 28/09/2018 13:30 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 14 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Potassium Bicarbonate 2.9700 ha 5.000 kg/ha
Kumulus DF 2.9700 ha 1.000 kg/ha
MAPP:04707, Active Ingredients:Sulphur, Manufacturer:BASF plc., Expires:31/12/2021

Reference: Plan 04846 Ventsi spraying 01/10 Job 1 | Issued by: John Longley (01/10/2018)
Target growth stage: Harvest
Start: 01/10/2018 10:15 | Finish: 01/10/2018 16:15 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 14 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Kumulus DF 2.9700 ha 1.000 kg/ha
MAPP:04707, Active Ingredients:Sulphur, Manufacturer:BASF plc., Expires:31/12/2021
Potassium Bicarbonate 2.9700 ha 5.000 kg/ha

Reference: Plan 04861 Curlings 05/10 Job 1 | Issued by: John Longley (04/10/2018)
Target growth stage: Harvest
Start: 05/10/2018 13:30 | Finish: 05/10/2018 16:15 | Operator: Ventsislav Buzov
Weather: Partly cloudy | Temp °C: 17 | Wind speed/direction: 1 SW | Soil: Dry
Implement: 4 Bed Munkhof, Volume rate: 500.000 L

Potassium Bicarbonate 2.9700 ha 5.000 kg/ha
Wetcit 2.9700 ha 0.500 L/ha

Year: 2018
To: 15/11/2018
Headings: Pesticides, Seed / Plants, Nutrition
Area: ha

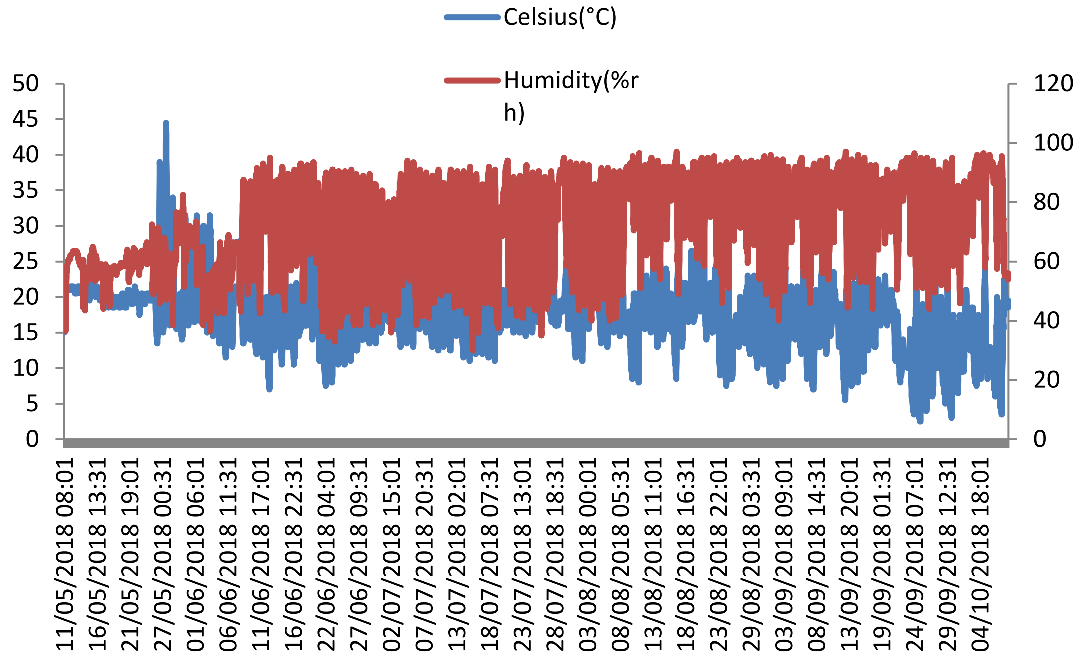
Roundup 2.9700 ha 5.000 L/ha
MAPP:12645, Active Ingredients:Glyphosate, Manufacturer:Monsanto UK Limited, Expires:30/06/2018

? Reference: Plan 04892 Mini T-tops Inter-row 06/11 Job 1 | Issued by: John Longley (05/11/2018)
Target growth stage: Post production-grubbing out
Implement: Micron 200L, Volume rate: 500.000 L

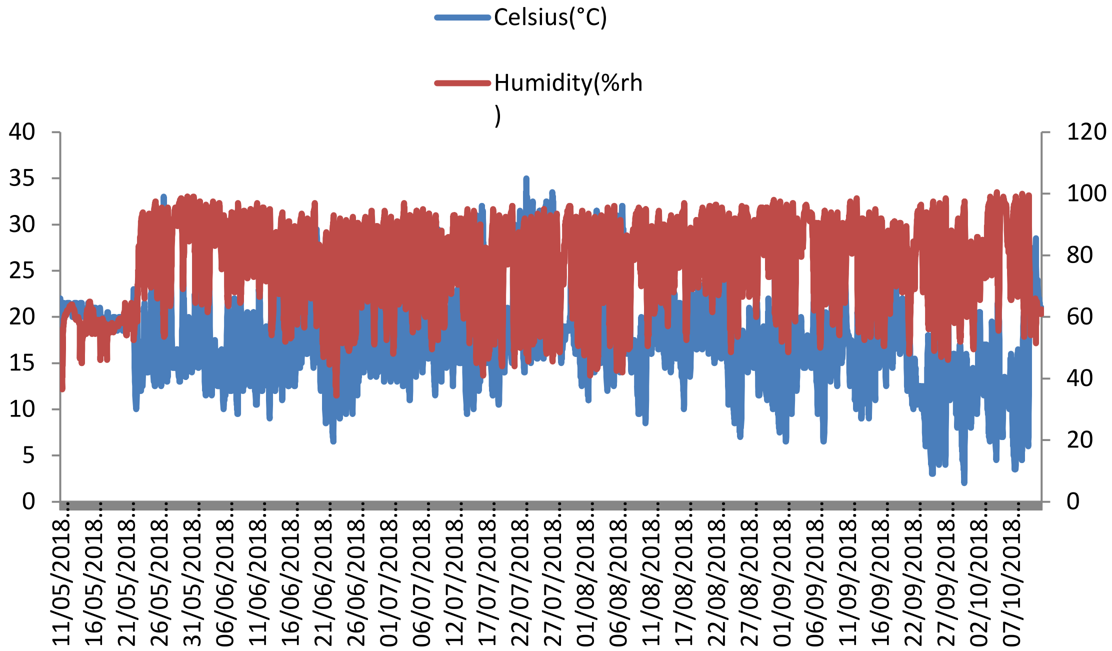
Roundup 1.4850 ha 4.000 L/ha
MAPP:12645, Active Ingredients:Glyphosate, Manufacturer:Monsanto UK Limited, Expires:30/06/2018
Stomp Aqua 1.4850 ha 3.000 L/ha
MAPP:14664, Active Ingredients:Pendimethalin, Manufacturer:BASF plc.
Devrinol (09374) 1.4850 ha 7.000 L/ha
MAPP:09374, Active Ingredients:Napropamide, Manufacturer:UPL Europe Limited, Expires:31/07/2018

APPENDIX 2.1.2.

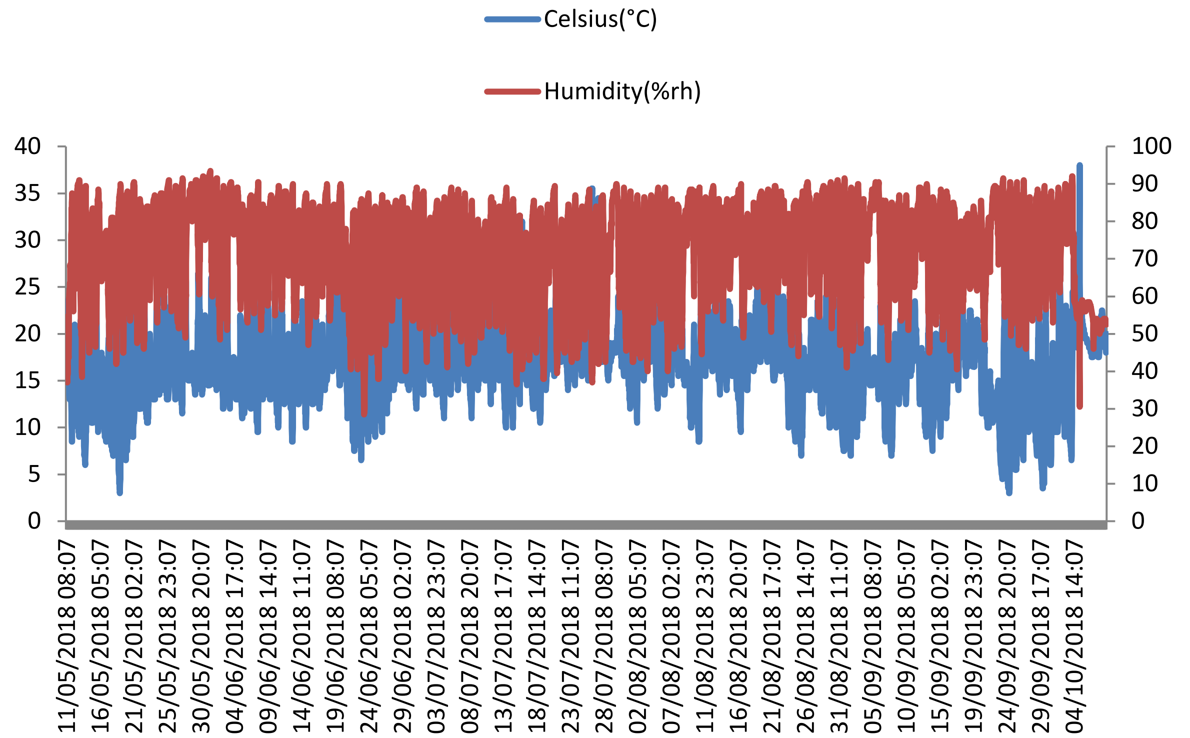
Site 1.



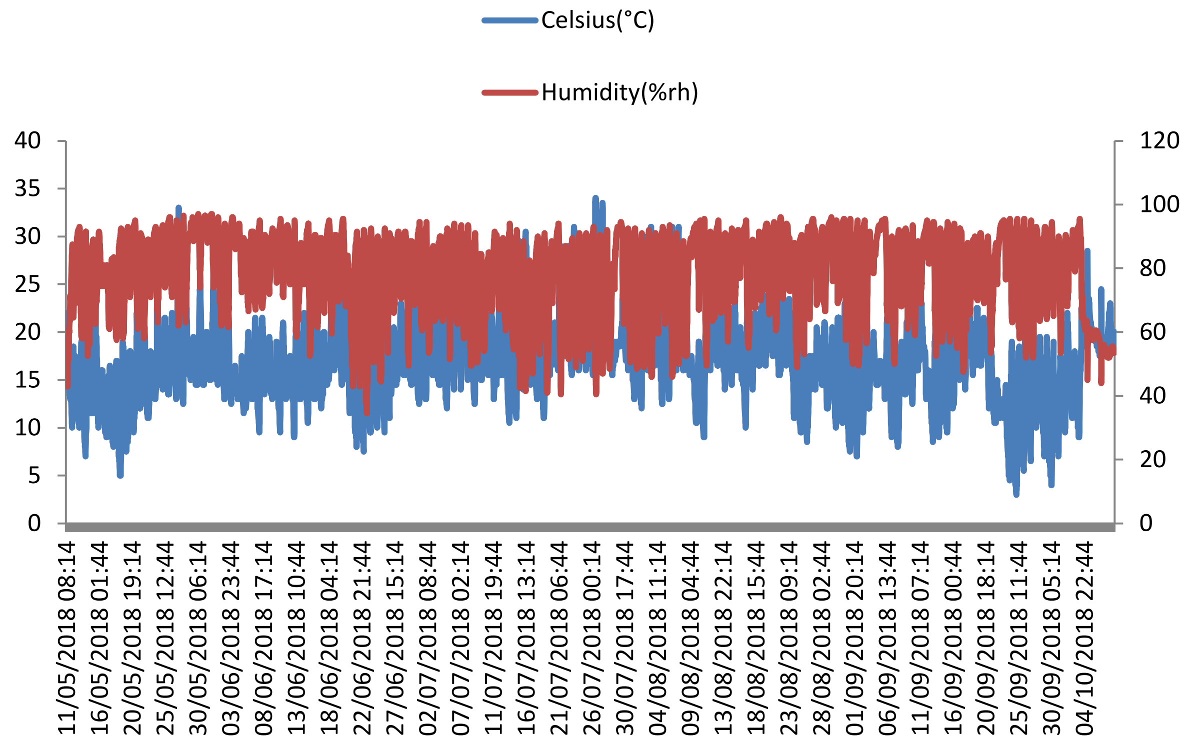
Site 2.



Site 3.


Celsius(°C)
Humidity(%rh)
40
35
30
25
20
15
10
5
0
100
90
80
70
60
50
40
30
20
10
0
11/05/2018 08:07
16/05/2018 05:07
21/05/2018 02:07
25/05/2018 23:07
30/05/2018 20:07
04/06/2018 17:07
09/06/2018 14:07
14/06/2018 11:07
19/06/2018 08:07
24/06/2018 05:07
29/06/2018 02:07
03/07/2018 23:07
08/07/2018 20:07
13/07/2018 17:07
18/07/2018 14:07
23/07/2018 11:07
28/07/2018 08:07
02/08/2018 05:07
07/08/2018 02:07
11/08/2018 23:07
16/08/2018 20:07
21/08/2018 17:07
26/08/2018 14:07
31/08/2018 11:07
05/09/2018 08:07
10/09/2018 05:07
15/09/2018 02:07
19/09/2018 23:07
24/09/2018 20:07
29/09/2018 17:07
04/10/2018 14:07

Site 4.


Celsius(°C)
Humidity(%rh)
40
35
30
25
20
15
10
5
0
120
100
80
60
40
20
0
11/05/2018 08:14
16/05/2018 01:44
20/05/2018 19:14
25/05/2018 12:44
30/05/2018 06:14
03/06/2018 23:44
08/06/2018 17:14
13/06/2018 10:44
18/06/2018 04:14
22/06/2018 21:44
27/06/2018 15:14
02/07/2018 08:44
07/07/2018 02:14
11/07/2018 19:44
16/07/2018 13:14
21/07/2018 06:44
26/07/2018 00:14
30/07/2018 17:44
04/08/2018 11:14
09/08/2018 04:44
13/08/2018 22:14
18/08/2018 15:44
23/08/2018 09:14
28/08/2018 02:44
01/09/2018 20:14
06/09/2018 13:44
11/09/2018 07:14
16/09/2018 00:44
20/09/2018 18:14
25/09/2018 11:44
30/09/2018 05:14
04/10/2018 22:44