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Location of project:	NIAB EMR
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Date project commenced:	01 April 2013
Date project completed (or expected completion date):	31 March 2017

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

CONTENTS

AUTHENTICATION

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

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GROWER SUMMARY

Understanding and developing methods for managing spotted wing drosophila (SWD) in the UK: Vital research to maintain the viability of the UK fruit industry

Headline

- Through this project a greater understanding of the biology and control of SWD in the UK has been achieved with findings directly relevant to UK soft fruit, stone fruit and vine growers.

Background and expected deliverables

Spotted wing drosophila (*Drosophila suzukii*, SWD) is a new invasive pest and was first identified in the UK in 2012. This pest which is of Asian origin, has caused considerable losses in fruit crops in mainland Europe and the USA. The overall aim of this project was to monitor the spread of *D. suzukii* within the UK, and to develop measures for its control. To this end, five objectives were set to;

1. Determine the distribution and seasonal population dynamics of all life stages of *D. suzukii* in different cropping situations and especially polytunnel crops on fruit farms in the UK.
2. Develop economically and environmentally sustainable treatment and disposal strategies for soft and stone fruit waste to eliminate it as a source of *D. suzukii* infestation and attraction on fruit farms.
3. Develop and evaluate sampling and extraction methods for quantifying *D. suzukii* infestations in different soft and stone fruits
4. Develop a synthetic lure and attract and kill technology for *D. suzukii* for incorporation into IPM programmes.
5. Obtain evidence for the effectiveness of different plant protection products including biopesticides to aid developing an insecticide resistance management strategy for *D. suzukii*.

Summary of the project and main conclusions

Objective 1. To determine the distribution and seasonal population dynamics of all life stages of *Drosophila suzukii* in different cropping situations and especially polytunnel crops on fruit farms in the UK and investigate its wide range of wild hosts and overwintering sites.

Task 1.1. Determine the population dynamics of adult SWD in vulnerable polytunnel and outdoor grown fruit crops at 13 sites in the different fruit growing regions of England and Scotland throughout the year for four successive years

In 2013, 130 modified biobest traps with Cha-Landolt synthetic bait were deployed at 14 sites in wild and cropping areas across five regions of the UK (Kent, Surrey, West Midlands, East England and Scotland) and monitored from 20 May 2013 onwards. The first *D. suzukii* adult was captured and identified at NIAB EMR in August 2013. Since then adult *D. suzukii* trap catches have increased year on year, with the following peaks in mean adult *D. suzukii* trap catches of ~5, ~163, ~885 and 530 in 2013, 2014, 2015 and 2016 respectively. A pattern in adult *D. suzukii* trap catches has been observed each year with very low numbers caught from March through to July, before increasing rapidly from early August onwards. Counts of *D. suzukii* trap catches comparing wild and cropping areas has also been recorded. *D. suzukii* trap catches consistently remained low throughout the beginning of the fruiting season. Then from late July onwards trap catches in fruit cropping areas started to increase and often reached a peak in October before decreasing at which point *D. suzukii* trap catches in woodland areas increased rapidly.

In 2016, the national monitoring of adult *D. suzukii* numbers was continued at a network of 15 sites across the UK: 5 in Kent, including NIAB EMR, 1 in Surrey, 3 in the West Midlands, 2 in East England and 4 in Scotland, including the James Hutton Institute. A further site was added in Yorkshire in 2016.

Catches of *D. suzukii* going into January 2016 increased two fold (~530 *D. suzukii* per trap) from the previous season (~200 *D. suzukii* per trap) in wild areas. This may have been aided by the warm November and December in 2015 potentially increasing the activity of the adult flies. As in 2014 and 2015, catches of adult *D. suzukii* in the traps were very low in England until late July. It was anticipated, given the high overwintering population and geographical spread in 2015, that numbers in the spring and summer of 2016 might be very high. However, the low catches might be explained by the trap lures competing with commercial and wild fruits during the summer and so they are probably not representative of 'real' in-field populations. Catches of *D. suzukii* adults continued at low levels, similar to 2015, until early August, at which point catches increased rapidly.

D. suzukii numbers in Scotland remained low – typically less than a peak of 20 per trap, and no adults were captured until April, with only one individual up to August at the four sites. Fruit damage has been reported in all regions, thus far, with the exception of Scotland.

Task 1.2. Estimation of fecundity of D. suzukii throughout the year

Following studies in 2014 and 2015, the fecundity of adult female *D. suzukii* at two farms in Kent was assessed at the start and end of the growing season in 2016. Mature eggs were detected from 18 April at both sites. Overall, the period of fertility was similar to 2015, with a later start in the season compared to 2014, in which the first mature eggs were detected in March.

Farm 2 appeared to have a longer period of fecundity than Farm 1, as found in the previous two years. Mature eggs were still detected in the last recorded samples (24 October) whilst mature eggs were not detected at Farm 1 after 10 October. The two farms are geographically close (~ 5 km apart), and so this difference is unlikely to be climatic. However, the polytunnels at Farm 2 remained in place longer than Farm 1, which may explain the slightly longer period of fecundity.

Task 1.3. Determine the phenology, population dynamics and spatial distributions of SWD on two fruit farms in SE England, throughout the year for four successive years (EMR; 1-4)

More intensive monitoring was undertaken on two farms using the same design of traps as the national monitoring scheme continued through 2016. The trap catch pattern was similar to that nationally with a large increase in adult catches in woodland in the autumn and early winter. As in 2015 it was noticeable that catches also increased in the cherry orchard in early autumn, presumably as it resembled woodland in terms of shelter. A similar pattern was found in the plum orchard, although in both cases catches fell after leaf fall.

The ratio of males to females in 2016 for both farms was 1.15 male to female, comparable to the ratio of 1.13 and 1.14 found in 2014 and 2015. A similar pattern to 2015 was found throughout the year, with higher female numbers at the start of the year reflecting the overwintering population and then a peak of males in late March/ early April before higher female numbers in the early summer and more males thereafter.

Task 1.4. Determine the attractiveness of cherry and plum extrafloral nectaries to D. suzukii

The attractiveness of extrafloral nectaries on leaves of cherry and plum was assessed. *D. suzukii* adults were attracted to the nectaries and were observed feeding on the nectar in the laboratory. *D. suzukii* fed on cherry extrafloral nectaries for longer than plum, possibly reflecting their higher nectar content. This information has commercial significance as it may account for the early activity of *D. suzukii* in cherry orchards, before fruit is ripening.

Task 1.5. Assessment of the susceptibility of six of the main blackcurrant varieties grown in the UK to Drosophila suzukii (SWD) and the potential future risk to the crop

Six UK-grown blackcurrant varieties (Ben Gairn, Ben Vane, Ben Starav, Ben Hope, Ben Klibreck and Ben Tirran) were tested for vulnerability to *D. suzukii.* A comparison was made between harvested fruit, which was either incubated to assess natural infestation in the field in relation to trap catches or inoculated with a laboratory culture of *D. suzukii*.

D. suzukii naturally emerged from three of the varieties; Ben Vane, Ben Hope and Ben Tirran. The plantations of Ben Hope and Ben Tirran had significantly higher natural emergence of adult *D. suzukii* than the other varieties. There was also a significantly higher *D. suzukii* trap catch (field pressure) at the site growing Ben Tirran compared to all other varieties.

In the laboratory, *D. suzukii* was able to lay eggs and develop in all varieties. However, Ben Hope and Ben Tirran were significantly more vulnerable to egg laying and adult emergence.

D. suzukii was present at all sites tested and could potentially lay eggs and develop into adults in all varieties screened. Preliminary data suggests that skin strength and thickness at time of harvest may be important but this needs to be confirmed.

Objective 2. To develop economically and environmentally sustainable treatment and disposal strategies for soft and stone fruit waste to eliminate it as a source of *D. suzukii* infestation and attraction on fruit farms.

This objective was completed in year 3 of the project with an accompanying AHDB factsheet (19/16 *Disposing of fruit waste affected by spotted wing drosophila*). Treatment in anaerobically sealed pallet bins was shown to kill all *D. suzukii* in soft fruit waste, as long as the ambient temperature was over 18ºC. However, if the ambient temperature was lower, such as might be found at the end of the season, then three days would be necessary to kill eggs and larvae.

Stone fruit was shown to take a longer period of storage to remove all *D. suzukii*, so that four days of storage are required, or five days if temperatures are below 16ºC ambient temperature.

Oxygen depletion was very rapid for each fruit type, non-detectable after 6 hours and it is possible that this is the crucial factor in killing eggs and larvae. There was a rapid increase in CO_2 levels in soft fruit waste, but this was much slower in stone fruit waste, possibly because of the firmer nature of the fruit and the presence of air pockets between fruits.

Mixing treated waste with at least 90% (w/w) organic matter such as manure or slurry was shown to prevent re-inoculation, as was incorporation in the surface of field soils to a depth of 20 cm. The rate of application of treated waste to land should not exceed 125 tonnes/ha to prevent exceeding EU directives on nitrate addition.

Disposal of fruit waste via digestion plants was considered less attractive due to the high moisture content and low calorific value of fruit wastes, while transport and gate fee costs were high.

Objective 3. To develop and evaluate sampling and extraction methods for quantifying *D. suzukii* infestations in different soft and stone fruits.

This objective was completed in year 3 of the project with an accompanying AHDB wallchart and training DVD (both available on the dedicated SWD pages of the AHDB Horticulture website).

The researchers assessed low cost methods of detecting all stages of *D. suzukii* larvae in ripe and ripening fruit (blueberries, cherries, raspberries, blackberries and strawberries). The fruits were immersed in solutions of strong sugar (180 g per l water), salt (75 g per l water) or a weak detergent. Freezing whole fruit overnight was also assessed. These methods were compared to both emergence testing (keeping fruits in boxes at room temperature for 3 weeks and counting adult emergence) and dissecting the fruits to directly count the numbers of larvae.

Immersion in sugar and salt solutions were most successful in detecting *D. suzukii* larvae, whether late or early stage, with sugar solution slightly more effective. No method gave 100% recovery of the larvae.

Flotation with a strong sugar solution was the most practical way to determine the infestation levels of fruits.

Objective 4. To develop a synthetic lure and attract and kill technology for *D. suzukii* for incorporation into IPM programmes

Task 4.2. Develop target device and identify suitable insecticide(s) for attract and kill formulation (EMR, NRI, yrs 2-3)

A non-saturating attract and kill (A&K) device was further developed with field and laboratory tests to optimise efficacy. Decis (deltamethrin) was confirmed as effective in the field over a season. Initial comparisons of two prototype A&K devices with a commercial alternative found lower catches of *D. suzukii* but improvements in design and orientation of clear and red areas improved relative efficacy. The numbers of entrance/exit holes influenced the kill of *D. suzukii*. Now these advances have been made, future tests should estimate the efficacy of the device when the insects are allowed to enter and leave the device or die by dropping from the open bottom of the device.

Task 4.3. The evaluation of pheromone components of Cha-Landolt baits for the efficiency of trapping D. suzukii

In previous work, 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. Adjusting the release rate of ethanol did not appear to improve the trap catch of *D. suzukii*. Preparation and maintenance of large numbers of the Cha-Landolt lures is inconvenienced by difficulties associated with producing acetic acid, ethanol and methionol sachets.

A series of experiments were done in 2016 that aimed to optimise the release rate, ratio and component compounds used, to produce a convenient lure combination with a high *D. suzukii* catch and low by-catch.

Adult *D. suzukii* trap catch was significantly higher from traps where acetic acid and ethanol was released from the drowning solution. Varying the release rate of acetic acid or ethanol over an 8 fold range in the sachets did not improve catches of *D. suzukii*. However, through doubling the number of sachets thus, the release of acetic acid and acetoin components to 36 mg/d and 16mg/ day, respectively, it was possible to double the number of adult *D. suzukii* caught in the traps. Replacing ethanol with 3-Methyl-1-butanol did not increase the *D. suzukii* trap catch.

Objective 5. To obtain evidence for the effectiveness of different plant protection products including biopesticides and for developing an insecticide resistance management strategy for *D. suzukii*.

Task 5.1. Addition of bait attractants to increase insecticide efficacy for control of D. suzukii

Novel and commercial baits were tested for strength of attractiveness to *D. suzukii* adults in a range of laboratory tests. The relative *D. suzukii* attractiveness of different substances differed between laboratory methods and duration. A 30-60 minute Petri droplet test showed that sugar + yeast suspensions were the most attractive substances whereas Gasser at 5 to 100% was relatively unattractive; however, the reverse trend occurred in a 12-hour choice test in a large arena. Solutions of molasses at 5-50% were moderately attractive in both systems and the most attractive substance in a 3-day chronophysiology apparatus test. A species of yeast found in the gut of *D. suzukii* (*Hanseniaspora uvarum*) was more attractive to *D. suzukii* than bakers' yeast (*Saccharomyces cerevisiae*) when used with the same concentration of sugar and at an equivalent yeast cell concentration. Further work is needed to optimise sugar + yeast suspensions, to test the relative attractiveness of the above substances in the field and their effect on *D. suzukii* control with pesticides.

Task 5.2. Determine compounds that repel D. suzukii and prevent egg laying

In small scale field trials five compounds placed close to fruit in delta traps in a *D. suzukii* infested cherry orchard prevented *D. suzukii* laying eggs in fruits compared to an untreated control. These compounds are worthy of further investigation. Repellent compounds as large point sources (sachets) in trees did not deter egg laying in fruits. However, it should be noted that the latter trial was done in the autumn when populations of *D. suzukii* were high and did not have alternative feeding or egg laying resources in the orchard – hence pest pressure would have been high. Further studies should investigate the early use of repellents, in the spring, before *D. suzukii* migrates from wild habitats and the use of smaller, higher numbers of point sources.

Financial benefits

D. suzukii poses a clear threat to the fruit industry and has had a commercial impact on UK grown fruit since 2014. The impact not only includes damage to fruits and the cost of quality and control, but increased labour due to removing unmarketable fruits from the crop and then handling and treating the waste material. Growers have reported significant financial losses in cherry and soft fruit crops. The soft fruit growing sector is increasing by 10-15% p.a. with increases in cherry and blueberry production in Scotland.

Action points for growers

- Use a recommended trap and bait to monitor adults in susceptible crops and wild areas year round. This will help to establish the pest pressure from year to year.
- Monitor for larval infestation in the crop. The flotation technique using sugar solution is recommended for rapid detection of larvae and dissecting and inspecting fruits in the crop whilst crop walking.
- Consider winter trapping and deploy perimeter trapping around vulnerable crops before fruit begins to ripen to potentially delay the movement of *D. suzukii* into the crop.
- Use barriers to help prevent ingress of the pest from wild areas to crops. Netting of ~0.9 mm gauge is recommended. Complete netting gives best control but monitoring should still take place within the netted area.
- Ensure that other pests are well controlled using biological methods from early in the season, before sprays need to be targeted against *D. suzukii*.
- Estimate the risk to crops on the farm by assessing vulnerability of each crop and adjacent sources of *D. suzukii*.
- Keep humidity to a minimum, with good control of irrigation, where possible as *D. suzukii* prefers high humidity.
- Ensure that fruit picking staff are well trained and understand the impact of the pest and the need to remove all fruit from the crop.
- Consider crop canopy management to reduce humidity and ensure that fruits in the centre and lower down in the canopy are not missed during picking.
- Remove all damaged and unmarketable fruit from the crop every 2-3 days where possible in soft fruit. Remove waste fruit from cherry crops.
- Crop hygiene is one of the best measures of population control and should be maintained by waste fruit being treated to eliminate SWD by containing it in sealed vessels and then disposing of it responsibly.
- Ensure saleable fruit is cold stored to prevent further development of *D. suzukii*.
- Consult BASIS trained advisers for the latest approvals for effective plant protection products and contact the AHDB for further advice.
- Control damage by *D. suzukii* by employing year round multiple control strategies which consider all developmental stages of the pest – Integrated Pest Management.

SCIENCE SECTION

Objective 1. To determine the distribution and seasonal population dynamics of all life stages of *D. suzukii* in different cropping situations and especially polytunnel crops on fruit farms in the UK and investigate its wild hosts and overwintering.

Task 1.1. Determine the population dynamics of adult D. suzukii in vulnerable polytunnel and outdoor grown fruit crops at 13 sites in the different fruit growing regions of England and Scotland throughout the year for four successive years.

Materials and methods

Fourteen fruit farms were selected in year one (2013) of the project with an additional farm that was added in the West Midlands from 2014 and a site from the North East added in 2016 (Table 1.1.1). The distribution of the farms was as follows; five in Kent (including NIAB EMR), one in Surrey, three 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). 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; blackberry, blueberry, cherry, raspberry, redcurrant and strawberry crops were included in the trial sites. At least one woodland area was also assessed at each farm.

At the end of the growing season it was decided to concentrate efforts on traps which gave the most information and reduced effort on behalf of the Berry Gardens and NIAB EMR teams, but which still provided continuous data across the main growing regions (Table 1.1.1).

Table 1.1.1. Summary of fruit farms in the national monitoring survey.

	Region and Crops				
	Farm	Pre Nov 2016		Post Nov 2016	
		No. traps	Crops*	No. traps	Crops*
	1 SE	6	Raspberry, strawberry	Removed	
	2 SE	10	Raspberry, strawberry	Removed	
	3 SE	4	Cherry	4	Cherry
	4 SE	6	Raspberry	6	Raspberry
	5 SE	8	Cherry, plum, grape	8	Cherry, plum, grape
	6 SE	10	Blueberry, redcurrant, strawberry	10	Blueberry, redcurrant, strawberry
	7 East	8	Blueberries	4	Blueberries
	8 East	12	Raspberries, strawberries	6	Raspberries, strawberries
	9 WM	14	Blackberry, blueberry, raspberry, redcurrant, strawberry	6	Raspberries, strawberries
	10 WM	10	Blueberry, cherry, raspberry, strawberry	10	Blueberry, cherry, raspberry, strawberry
	10a WM	3	Cherry, raspberry	Removed	
	10b NE	2	Strawberry	2	Strawberry
	11 Scotland	10	Blackcurrant, blueberry, raspberry, strawberry	10	Blackcurrant, blueberry, raspberry, strawberry
	12 Scotland	10	Blueberry, cherry	10	Blueberry, cherry
	13 Scotland	10	Blackberry, blueberry, raspberry, strawberry	10	Blackberry, blueberry, raspberry, strawberry
	14 Scotland	10	Blackberry, blueberry, raspberry, strawberry	10	Blackberry, blueberry, raspberry, strawberry
	TOTAL	**143**		**96**	

*Note: An area of woodland was also included at each farm

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. For continuity, within the National Monitoring scheme we continued to use the modified Biobest trap design and Cha-Landolt bait used at the end of 2013. Trials at NIAB EMR comparing and improving lure and trapping technology for *D. suzukii* are described in detail under Objective 4.

Droso-traps (Biobest, Westerlo, Belgium) were modified with 20 extra 4 mm holes drilled into the top portion of the body of the trap to maximise catches of *D. suzukii*. Adults were captured in a drowning solution, which included ethanol (7.2%) and acetic acid (1.6%) as attractants, and boric acid to inhibit microbial growth. Methionol and acetoin (diluted 1:1 in water) were released from two polypropylene vials (4 ml) with a hole (3 mm dia.) in the lid, attached near the entry holes within the trap. The traps were deployed at the height of the main crop.

Trapping began in May 2013 and was continued with weekly counts during the cropping season and biweekly counts during the winter.

Results

The results for England in 2016 are summarised in Figure 1.1.1, in comparison to 2013, 2014 and 2015.

Overall, numbers caught in the late autumn and early winter of 2015-16 were far higher than the previous year, before falling to low levels in mid-January 2016. There was a peak in trap catches in early February. Catches of *D. suzukii* adults continued at low levels, similar to 2015, until early August, at which point catches increased rapidly (~500 average *D. suzukii* per trap). There appears to be a relationship with catches of *D. suzukii* adults and temperature measurements. For example in December 2016 catches of *D. suzukii* adults were lower (~500 average *D. suzukii* per trap) than in 2015 (~1000 average *D. suzukii* per trap*).* In December 2016 there were nine days where the temperature fell below 0°C whereas in December 2015 there were no days where the temperature fell below 0°C (Figure 1.1.2A). Furthermore, a peak in catches of *D. suzukii* adults was observed in May. This corresponded with seven days of temperatures above 20°C (Figure 1.1.2B) suggesting that *D. suzukii* trap catches are related to *D. suzukii* activity which is responsive to temperature fluctuations. This is observed in other insect trapping systems, for example, pit fall trapping of ground dwelling invertebrates where trap catch is not only a representation of populations but also activity.

Throughout the majority of 2016, catches of *D. suzukii* adults were higher than in 2014. The number of days where the temperature fell below 0°C was higher in 2016 (39 days) than in 2014 (25 days) and the number of days above 20°C was 93 in 2016 compared to 85 in 2014.

Even so, catches of *D. suzukii* adults were higher throughout most of 2016. There was considerable variation between sites within the UK regions, but some trends are evident (Figure 1.1.3). As in 2014 and 2015, the largest catches were in South East England. Trap catches continued to rise in December in the wild areas (particularly woodlands). Catches in traps in the East of England and the West Midlands remained, on average, lower compared to the South East, on the farms monitored within the survey.

Numbers of *D. suzukii* adults at the one site monitored in Yorkshire were very low until late August, numbers continued to increase in the woodland. No adults were caught in Scotland until April and then only one individual until August at the four sites.

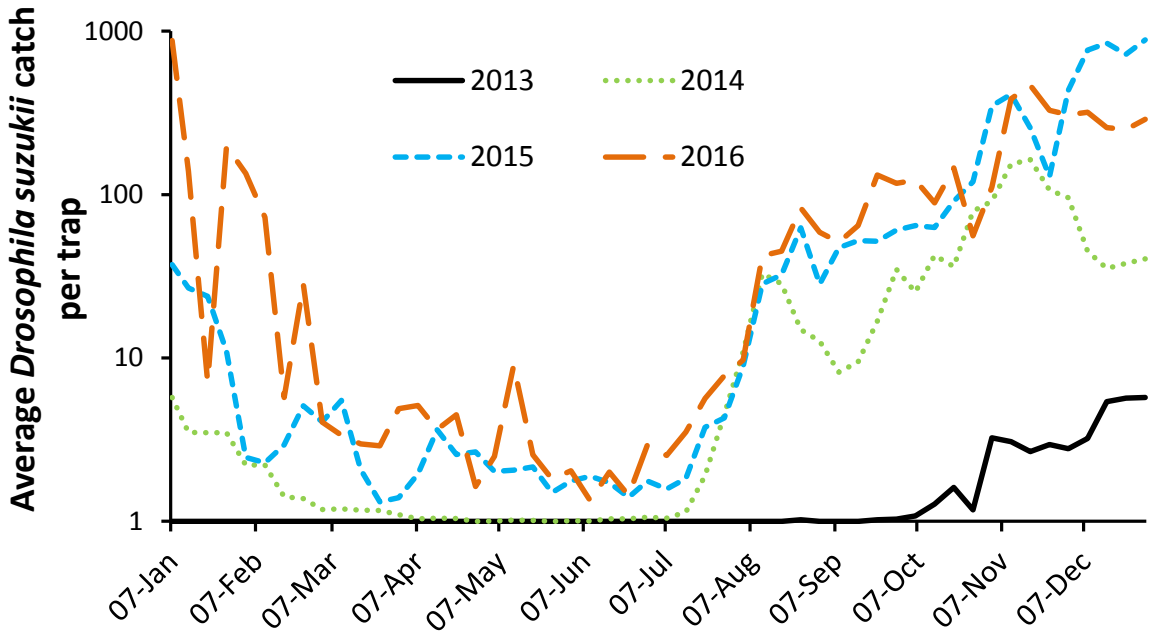


Figure 1.1.1. Comparison of average adult *D. suzukii* catch per trap in 2013, 2014, 2015 and 2016, plotted on a log (n + 1) 10 scale on the Y axis.

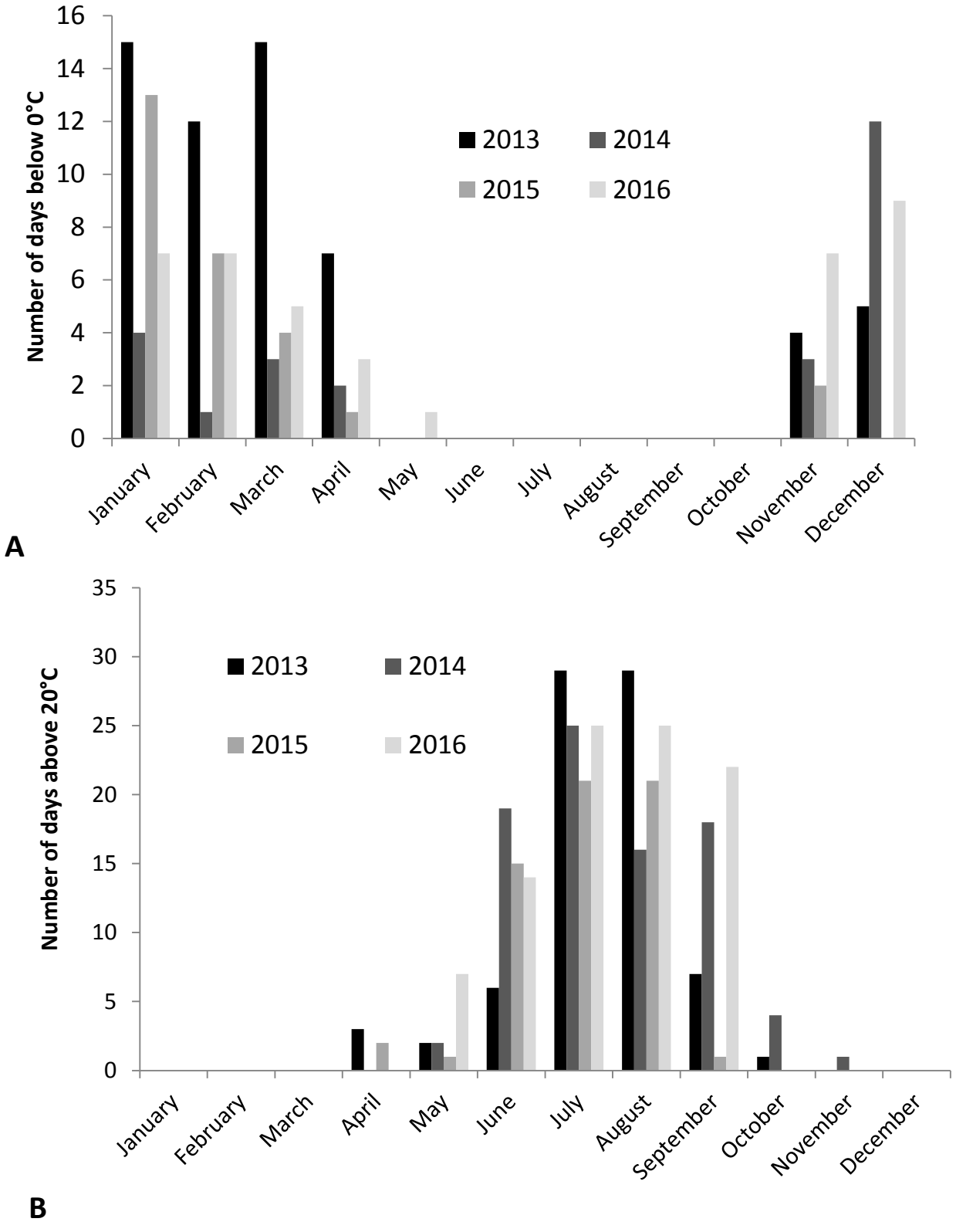


Figure 1.1.2. Meteorological data from weather station at NIAB EMR based on maximum and minimum air temperature per day. **A.** Number of days below 0°C throughout 2013, 2014, 2015 and 2016. **B.** Number of days above 20°C in 2013, 2014, 2015 and 2016.

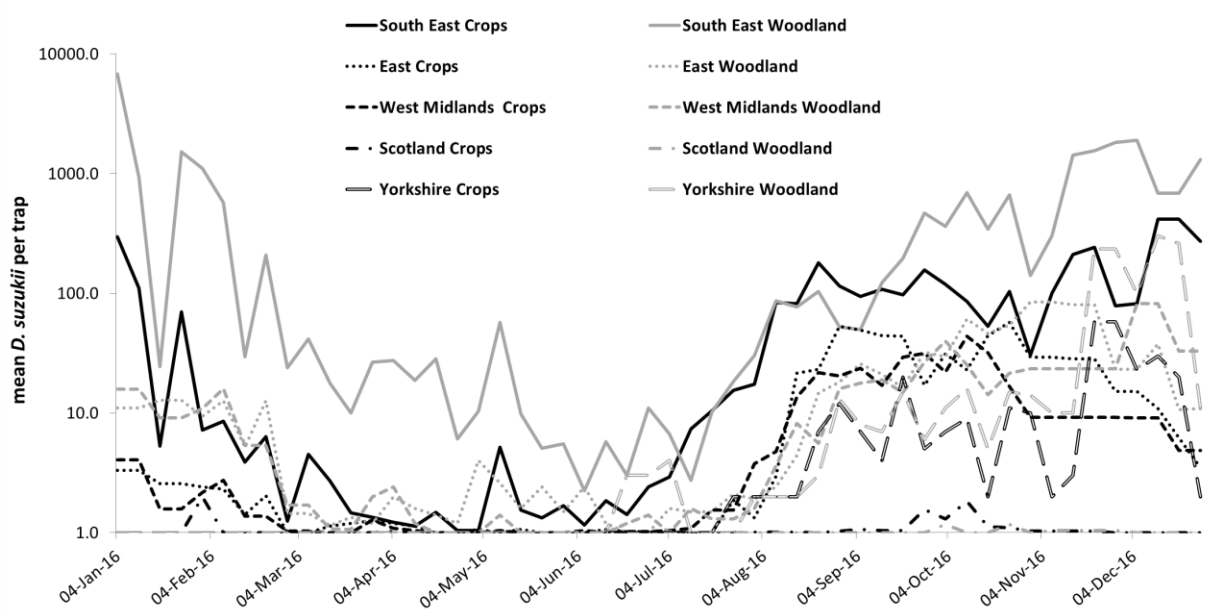


Figure 1.1.3. Mean numbers of *D. suzukii* adults per trap in the main habitat types and regions during 2016. Plotted on a log (n + 1) 10 scale on the Y axis.

Discussion

Catches of adult *D. suzukii* in 2016 reached almost 7,000 per trap in January in one woodland in the South East. This may have been aided by the warm November and December the previous year (Figure 1.1.2A & 1.1.4) and populations becoming established.

As in 2014 and 2015, catches of adult *D. suzukii* in the traps were very low in England until late July. It was anticipated, given the high overwintering population and geographical spread in 2015, that numbers in the spring and summer of 2016 might be higher than the previous year. However, possibly, the catches remained low as trap catch is not only a representation of populations but also activity as in other insect trapping systems, for example, pit fall trapping of ground dwelling invertebrates.

It is unclear if *D. suzukii* has established in Scotland as numbers are very low and no winter-morphs have been captured.

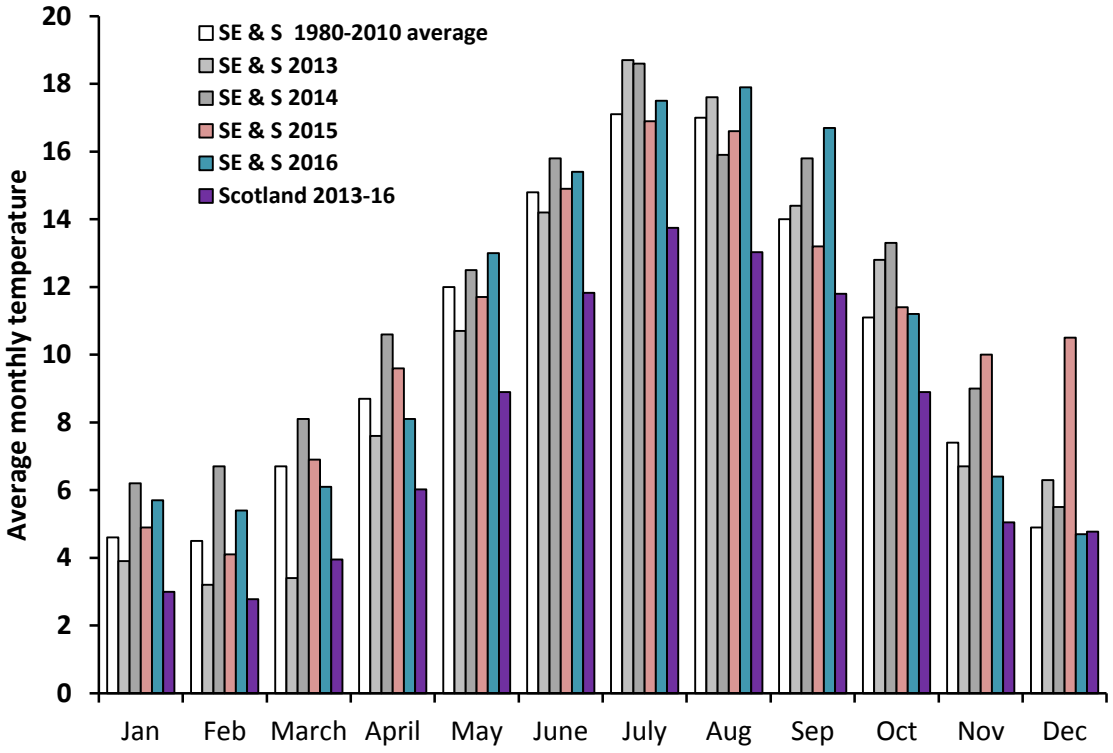


Figure 1.1.4. Comparison of the temperatures in Scotland, the South East (SE) and Central South (S) region of the UK in 2013, 2014, 2015 and 2016 with the 1980-2010 average (Met Office, UK)

Task 1.2. Estimation of fecundity of D. suzukii throughout the year

Materials and Methods

The same two farms and trap sites were sampled as in previous years of the project. Adult *D. suzukii* were caught using traps identical to those described in Section 1.1. Instead of monitoring for the whole growing season as in 2015, only the predicted beginning and end of the female fertile period were monitored.

Assessments of females were made weekly. Five females per sample were taken randomly and floated in 70% alcohol within a Petri dish. The reproductive maturity was derived from a visual assessment of ovary and egg development using stage definitions published by. Gerdeman *et al.* (2013) (Table 1.2.1 and Fig. 1.2.1).

Table 1.2.1. Stage of ovarian development determination key

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

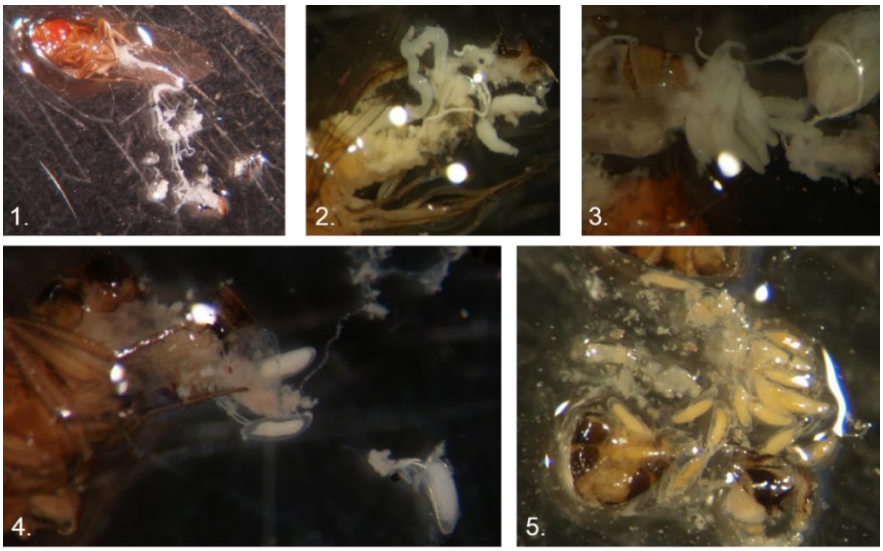


Figure 1.2.1. The five different ovary stages as described in Table 1.2.1

Results

Fecund female *D. suzukii* with mature eggs were found in farms in Kent from mid April to October in 2016.

Mature eggs were detected from 18 April at both sites (Figures 1.2.2 and 1.2.3). Overall the period of fertility was similar to 2015, with a later start in the season compared to 2014, in which mature eggs were detected in March.

Farm 2 had a longer period of fecundity than Farm 1, as was found in the previous two years. Mature eggs were still detected in the last recorded samples (24 October) whilst mature eggs were not detected at Farm 1 after 10 October. The two farms were geographically close, ~ 5 km apart, and so this difference is unlikely to be temperature driven. The polytunnels at Farm 1 were removed before the polytunnels at Farm 2.

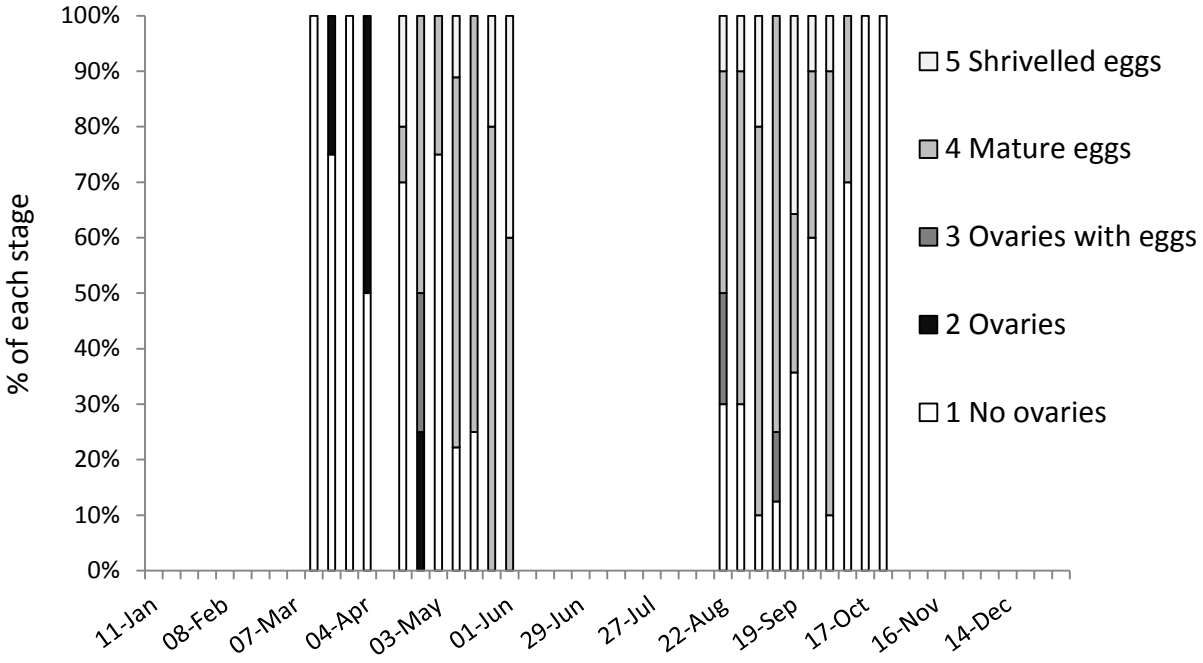


Fig. 1.2.2. Fecundity of *D. suzukii* at Farm 1

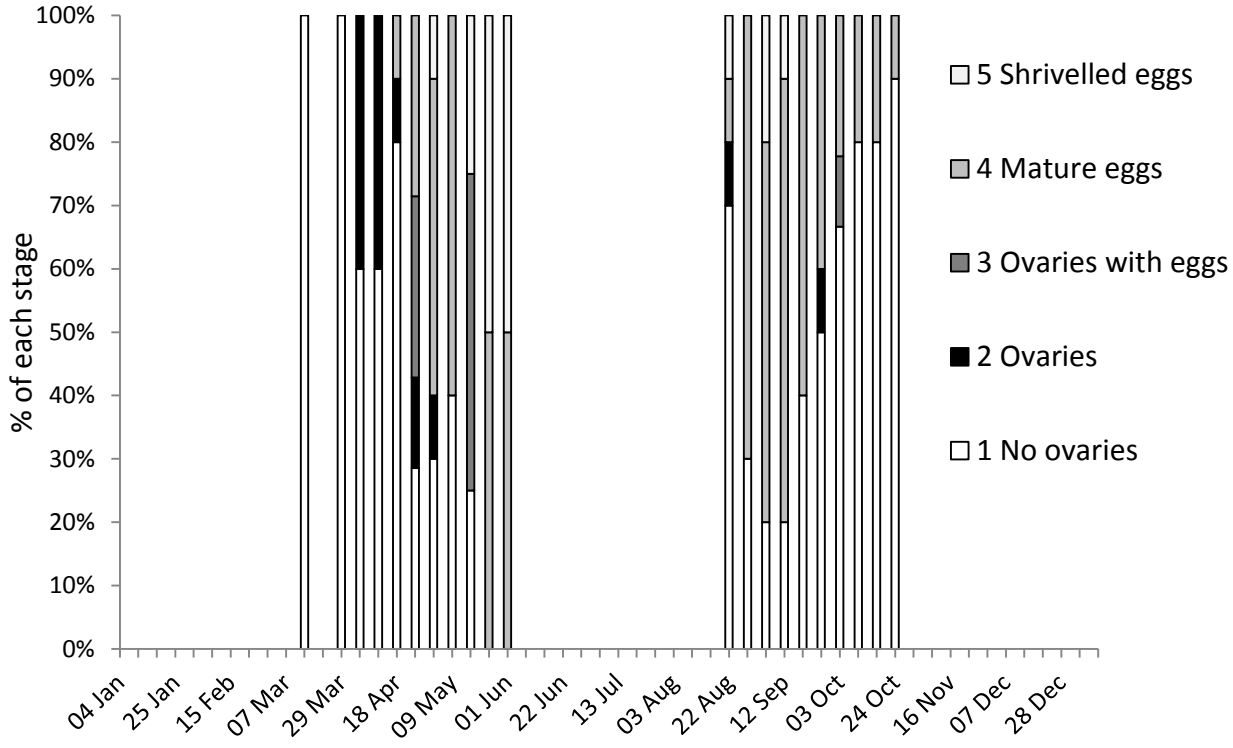


Fig. 1.2.3 Fecundity of *D. suzukii* at Farm 2

Task 1.3. Determine the phenology, population dynamics and spatial distributions of D. suzukii on two fruit farms in SE England, throughout the year for four successive years (EMR; 1-4).

Materials and Methods

Continuing from work in Years 1, 2 and 3 commercial crops (cherry and raspberry) were monitored for adults at two farms in Kent. In addition grape and plum crops were also assessed. Traps were distributed to cover the edges and centre of the focus crops.

Twenty seven pairs of traps were originally deployed on Farms 1 and 2 in 2013. In 2016, the number of traps was reduced to fourteen as key areas of interest had been identified, and plums and grapes were added. The traps used were based on the Cha-Landolt system (as used in the National Monitoring scheme) consisting of two polypropylene vials, one acetoin and one methionol, and a synthetic bait (ethanol, acetic acid, boric acid and detergent) which was also used as a drowning solution to capture insects entering the trap (Figure 1.3.1). Each location was allocated two traps which were spaced 10 metres apart.

Trap assessments were completed weekly during the cropping season and biweekly outside this period. Trap contents were taken back to the laboratory to be assessed, where male and female *D. suzukii* were counted.

Figure 1.3.1. Modified Drosotraps with synthetic bait.

Results

Trapping continued through 2016 (Figures 1.3.2 and 1.3.3). The pattern was similar to that nationally with a large increase in adult catches in woodland in the autumn and early winter. As in 2015 it was noticeable that catches also increased in the cherry orchard in early autumn, presumably as it resembled woodland in terms of shelter. A similar pattern was found in the plum orchard, although in both cases catches fell after leaf fall.

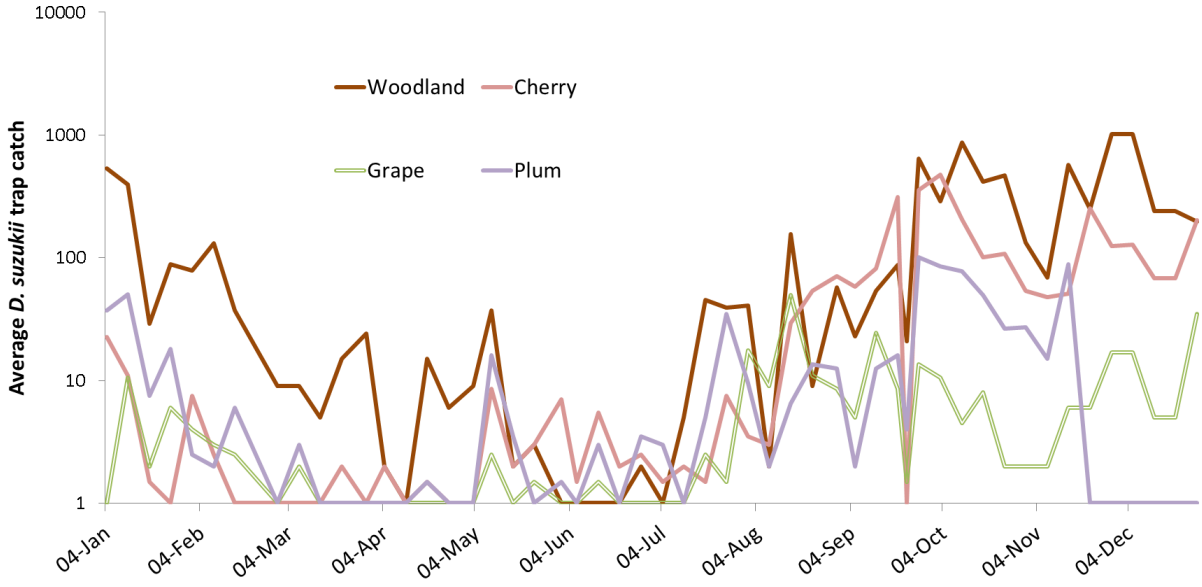


Figure 1.3.2. Adult *D. suzukii* trap catches at Farm 1 in 2016 by habitat. Plotted on a log (n + 1) 10 scale on the Y axis.

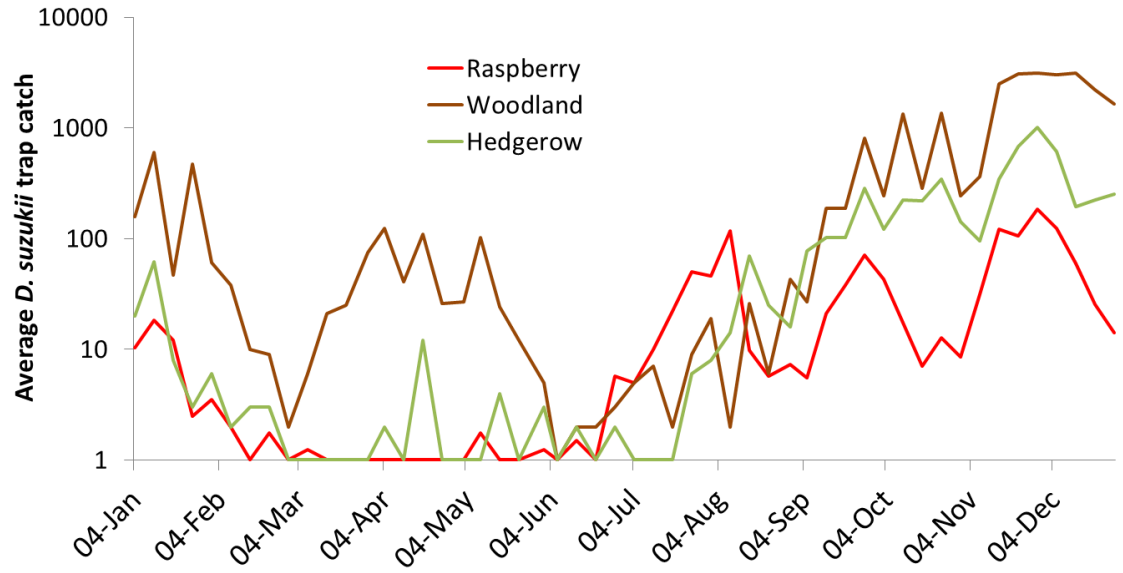


Figure 1.3.3. Adult *D. suzukii* trap catches at Farm 2 in 2016 by habitat. Plotted on a log (n + 1) 10 scale on the Y axis.

The ratio of males to females in 2016 (Figure 1.3.4) for both farms was 1.15 male to female, comparable to the ratio of 1.13 and 1.14 found in 2014 and 2015. A similar pattern to 2015 was found throughout the year, with higher female numbers at the start of the year reflecting the overwintering population and then a peak of males in late March/ early April before higher female numbers in the early summer and more males thereafter.

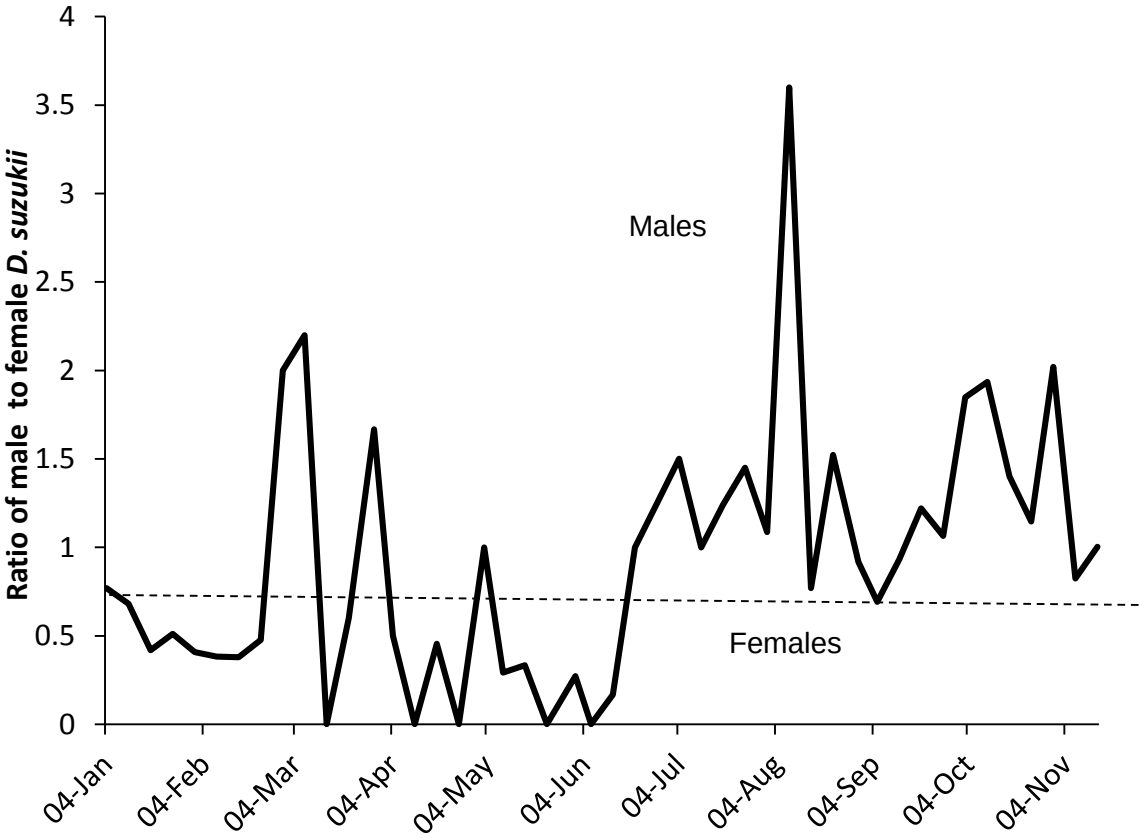


Figure 1.3.4. Ratio of adult male to female *D. suzukii* collected from Farm 1 in 2016

Task 1.4. Determine attractiveness of cherry and plum extrafloral nectaries to D. suzukii

Drosophila suzukii are found in cherry orchards before fruit become available for oviposition. One possible explanation is that the adults are feeding on extrafloral nectaries found on the leaf petioles of both these species. These nectaries are potential sources of both sugars and proteins.

Materials and Methods

Fresh cherry and plum leaves with visible nectaries were picked from orchards at NIAB EMR in mid May 2016 and kept with their stems in water to maintain flow of nectar. Individual leaves were then placed in 30 cm square Bugdorm mesh cages in the laboratory.

Male and female 3-7 day old *D. suzukii* from a laboratory culture were added to the cage and the feeding times were measured. Each batch of *D. suzukii* was assessed for 30 minutes and any leaves without visible nectar were replaced.

Three nectar samples were collected from cherry leaves taken from 'Deadmans' orchard at NIAB EMR on 18 May and 14 June using capillary tubes. The nectar was diluted 10 fold with water to prevent the analytical column becoming overloaded. A 5 µl injection of the dilution was made. The sugar in the 5 µl injection was separated by a Phenomenex Luna NH_2 LC column (250 x 4.6 mm) in a high performance liquid chromatogram (Water 2690 HPLC). The sugars were then detected on a differential refractometer (Waters 410). Each sample was injected three times. A calibration of 20-100 µg for fructose, glucose and sucrose was run and 3 injections were made per calibration point, so the nectar samples could be quantified.

Results and Discussion

D. suzukii were observed feeding on both cherry and plum leaves (Figure 1.4.1). The length of time female *D. suzukii* spent feeding on cherry extrafloral nectaries was significantly longer than for plums ($p<0.05$) (Figure 1.4.2). A similar tendency was found for males, but the result was not statistically significant. This could be a reflection of the larger volume of nectar found in cherry nectaries compared to plum or differences in nutritional composition, not determined in this preliminary study. The nectar from the cherry leaves contained fructose, glucose and sucrose. Higher concentrations of each of these sugars were observed per in nectar collected on the 14 June. There was approximately a two-fold higher mean concentration at both collection dates (18 May and 14 June) of sucrose (221 and 549 µg μl^{-1}, respectively) than fructose (114 and 194 µg μl^{-1}, respectively) and glucose (118 and 192 µg μl^{-1}, respectively) (Table 1.4.1). There has been little published literature on the importance of extra floral nectaries on *D. suzukii* populations. However, literature has found that some fruit fly species

(e.g. *Rhagoletis indifferens* (Western cherry fruit fly), *Grammicomya* sp. and *Mimegralla* sp. as well as ants are attracted to and feed on nectar or sugars on the plant leaves (Heil et al. 2003; Wee et al. 2008). Further studies have also found that early spring cherry blossoms may also provide a food source to *D. suzukii* (Tochen et al. 2016).

Figure 1.4.1. *D. suzukii* feeding on cherry extrafloral nectaries

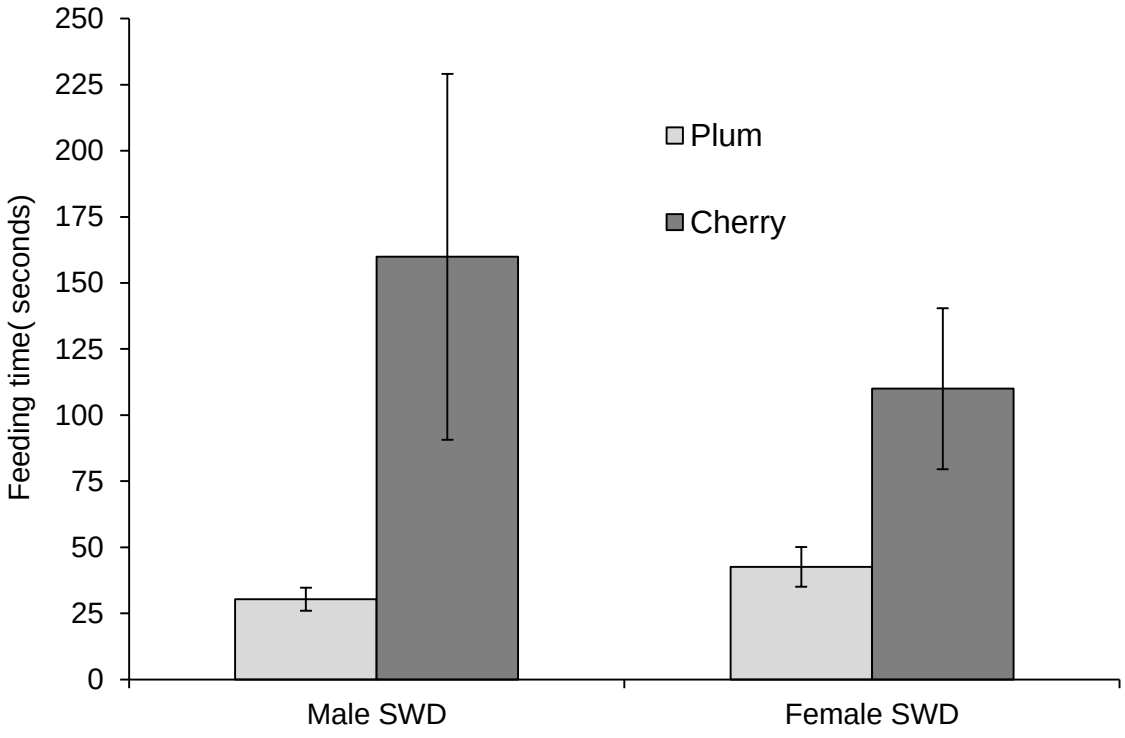


Figure 1.4.2. Average feeding time of *D. suzukii* on plum (n=23) and cherry (n=24) nectaries. Standard deviations **Plum;** Male (4.3333), Female (7.50382), **Cherry;** Male (69.1836) Female (30.47122).

Table 1.4.1. Mean concentration of fructose, glucose and sucrose from the three samples of nectar collected on the 18 May and 14 June

Date sample taken	Concentration µg $µl^{-1}$ nectar			
	Fructose	Glucose	Sucrose	Total
18 May	114	118	221	454
14 June	194	192	549	935

Task 1.5. Assessment of the susceptibility of six of the main blackcurrant varieties grown in the UK to D. suzukii and the potential future risk to the crop

Introduction

D. suzukii has been found to be a significant pest of soft and stone fruit crops throughout Europe (Walsh et al. 2011, Lee et al. 2011). However, damage on blackcurrants has yet to be documented even though potential risk was indicated to blackcurrants in the first year of the AHDB funded *D. suzukii* project SF145. This trial looked to establish a greater understanding of the potential risk *D. suzukii* poses to blackcurrants and determine the susceptibility of 6 of the main blackcurrant varieties.

Methods and Materials

Varieties screened: Six of the main blackcurrant varieties were selected at 3 farms located in the South East of the UK (Figure 1.5.1).

Figure 1.5.1. Six blackcurrant varieties (Harvest dates; **Ben Gairn** 15 July, **Ben Vane** 21 July, **Ben Starav** 28 July, **Ben Hope** 22 July, **Ben Klibreck** 4 August, **Ben Tirran** 11 August)

Fruit collection and emergence counts: Twenty Perspex boxes of fruit (on strigs) of each variety were collected as close to harvest as possible (6 varieties x 20 boxes = 120 boxes) from 3 sites in the South East of the UK (Figure 1.5.2). Each box of fruit was weighed and a Brix measurement taken from a subsample of ten fruit from each variety using a refractometer. Ten of the boxes were inoculated, for three days, with 1-12 day old *D. suzukii* cultured at NIAB EMR, providing five males and ten females for each box. The other ten boxes were not inoculated and were tested for natural emergence of *D. suzukii*. All boxes were incubated for 3 weeks in the CT rooms at NIAB EMR (20-25°C; D:L 8:16 h). The number of male and female *D. suzukii* and other drosophila that emerged were then counted on a weekly basis for 3 weeks. The *D. suzukii* emergence per 100 grams of fruit was calculated for each box using the formula;

D. suzukii emergence per 100 g of fruit =

100 x (*D. suzukii* emergence count from box/ weight of fruit in box (g))

Figure 1.5.2. Collection boxes

Egg counts: From the inoculated fruit a random subsample of 12 fruit was taken from each box and weighed (10 subsamples of each variety). The number of eggs was then counted per berry under a light microscope. Eggs were identified by counting the breathing tubes on the surface of the blackcurrants. A count of larvae and eggs on the surface of the fruits was also made. The number of eggs laid per 100 grams for each box was then calculated using the formula;

Eggs per 100 g of fruit =

100 x (number of eggs counted on 12 currants/subsample weight (g))

There was some difficulty when making egg counts, due to presence of hairs on the blackcurrant surface, however, breathing tubes could be identified in the centre of an indent in the fruit and were stained pink/purple by the juices in the fruit.

Trap monitoring: A week after fruit collection standard red Drosotraps containing a Dros'Attract bait were deployed at the edge and centre of the crop to determine *D. suzukii* field pressure (Figure 1.5.3). Traps were deployed for three weeks, the bait was replaced and the traps were sampled on a weekly basis. Samples were assessed for male and female *D. suzukii* and other drosophila.

Figure 1.5.3. Standard Drosotrap deployed in blackcurrant plantation (left) and being sampled from (right)

Statistical analysis: Data was analysed using a general analysis of variance on square root transformed data in GENSTAT.

Results

Field populations: D. suzukii naturally emerged from three of the varieties that were assessed (Ben Vane, Ben Hope and Ben Tirran, Figure 1.5.4). Significantly more adult *D. suzukii* emerged from Ben Hope (17 *D. suzukii* per 100 g) and Ben Tirran (22 *D. suzukii* per 100 g) than all other varieties.

There were significantly higher adult *D. suzukii* trap catches at the site where Ben Tirran was grown compared to all other sites, including the site where Ben Hope was grown (Figure 1.5.5). Damage was clearly visible within 2 weeks after the fruit was inoculated with *D. suzukii* (Figure 1.5.6).

Laboratory inoculations: D. suzukii could lay eggs and develop and emerge as adults from all of the blackcurrant varieties tested (Figures 1.5.7. and 1.5.8). Ben Hope and Ben Tirran were significantly more vulnerable to egg laying and hence had higher adult emergence per 100 g of fruit. Ben Klibreck had significantly lower numbers of eggs laid and adult emerge per 100 g of fruit than all the other varieties tested.

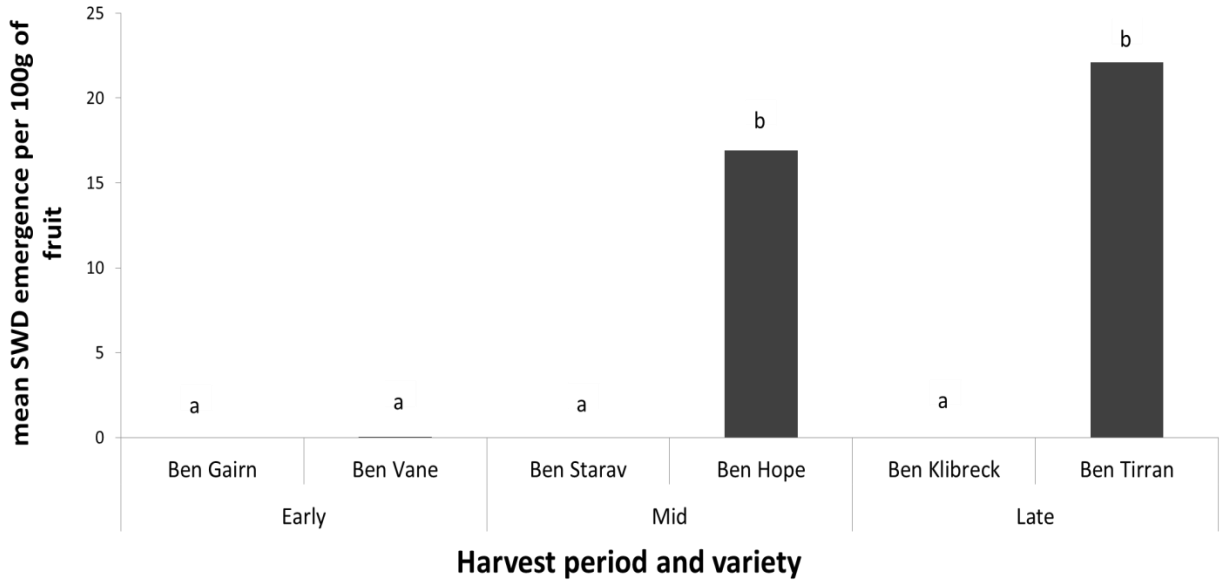


Figure 1.5.4. Mean numbers of *D. suzukii* that naturally emerged per 100g of fruit. Significant differences indicated by different letters above varieties (Fprob < 0.001, s.e.d. 0.48, l.s.d. 0.962)

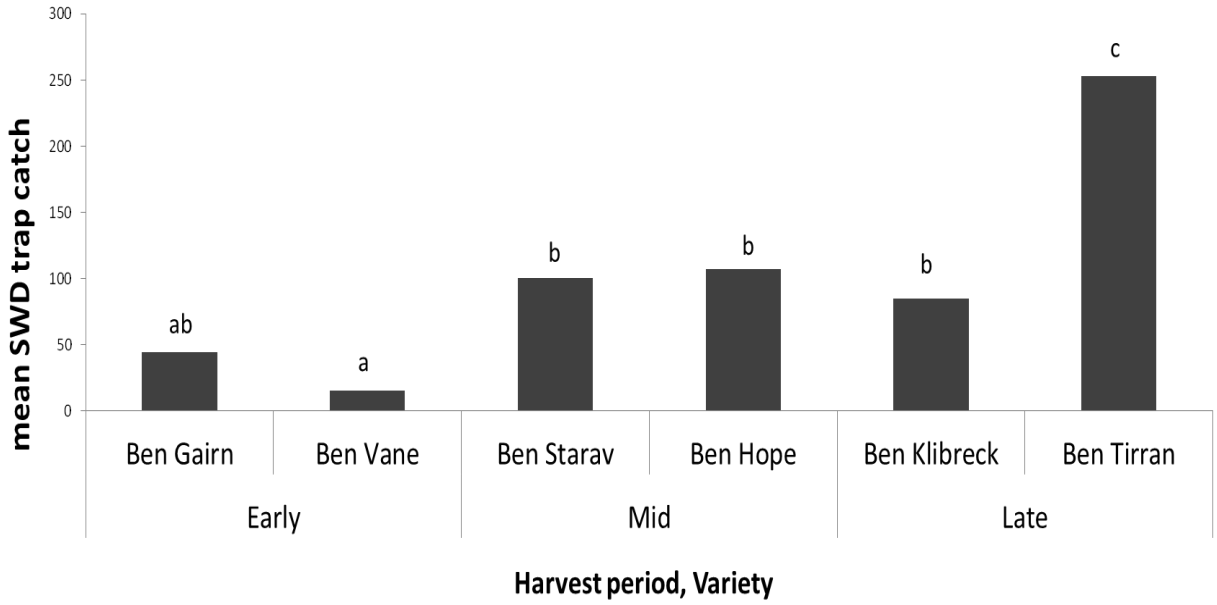


Figure 1.5.5. Mean *D. suzukii* trap catch over 3 weeks at each of the 6 plantations. Significant differences indicated by different letters above varieties (Fprob < 0.001, s.e.d. 2.201, l.s.d. 4.542)

Figure 1.5.6. Damage observed in Ben Gairn 2 weeks after fruit was inoculated

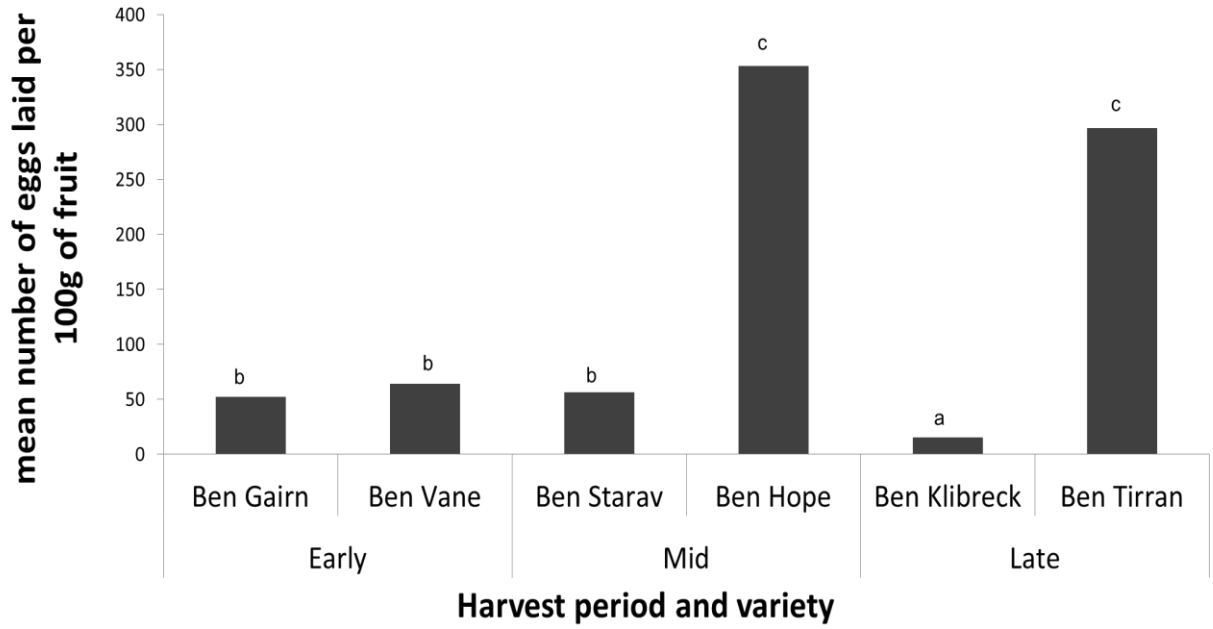


Figure 1.5.7. Mean number of *D. suzukii* eggs laid per 100 g of inoculated fruit**.** Significant differences indicated by different letters above varieties (Fprob < 0.001, s.e.d. 1.46, l.s.d. 2.928)

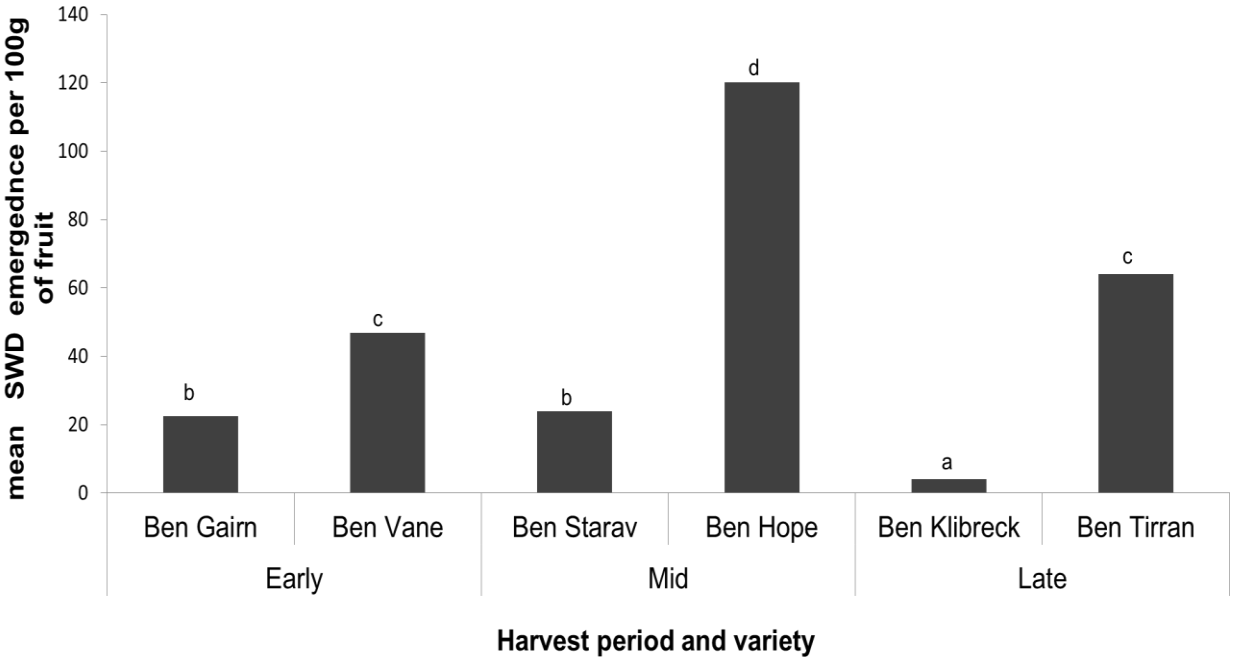


Figure 1.5.8. Mean *D. suzukii* emergence per 100 g of inoculated fruit. Significant differences indicated by different letters above varieties (Fprob < 0.001, s.e.d. 0.652, l.s.d. 1.307)

Discussion

All of the blackcurrant plantations sampled in 2016 had *D. suzukii* present in the field.

Both Ben Hope and Ben Tirran had significantly more adult *D. suzukii* naturally emerge from the field collected fruit than all other varieties. This may be due to a varietal characteristic expressed in these two varieties that incurs susceptibility to *D. suzukii* egg laying and emergence. This is more likely to be the case for Ben Hope which had statistically the same field population as Ben Gairn, Ben Starav and Ben Klibreck yet *D. suzukii* did not emerge from these varieties. Natural emergence in Ben Tirran may have occurred in response to a significantly higher *D. suzukii* field pressure.

In the laboratory, when given no choice *D. suzukii* laid eggs, developed and emerged from all of the varieties tested.

Data from JHI in the HortLINK Blackcurrant IPDM project calculated the force in Newton's (N) required to pierce the skin of a range of blackcurrant genotypes as a measure of blackcurrant skin strength. Varieties which had the greatest number of eggs laid and *D. suzukii* emerge in the current study (Ben Hope and Tirran) were also varieties which required the least amount of force to penetrate the skin. Ben Klibreck, which had significantly lower numbers of eggs laid and *D. suzukii* emerge, required the greatest amount of force to pierce the skin from the 6 varieties tested for *D. suzukii* emergence in 2016 (Fig 1.5.9), suggesting there may be a link between varietal vulnerability to *D. suzukii* egg laying and skin resilience to piercing, thus, skin strength. However, the data on skin strength is from 2012 and cannot be directly linked to the fruit collected in 2016. Furthermore varietal characteristics are not the only factor that can influence skin strength in blackcurrants, environmental factors and harvest date can influence skin strength (AHDB, 2012). Further work would be needed to confirm the role of skin thickness.

We have established that *D. suzukii* can develop on blackcurrant varieties screened thus highlighting a potential future risk if populations are left to continue to rise in blackcurrant crops. Importantly, another field season of data would be needed to confirm this.

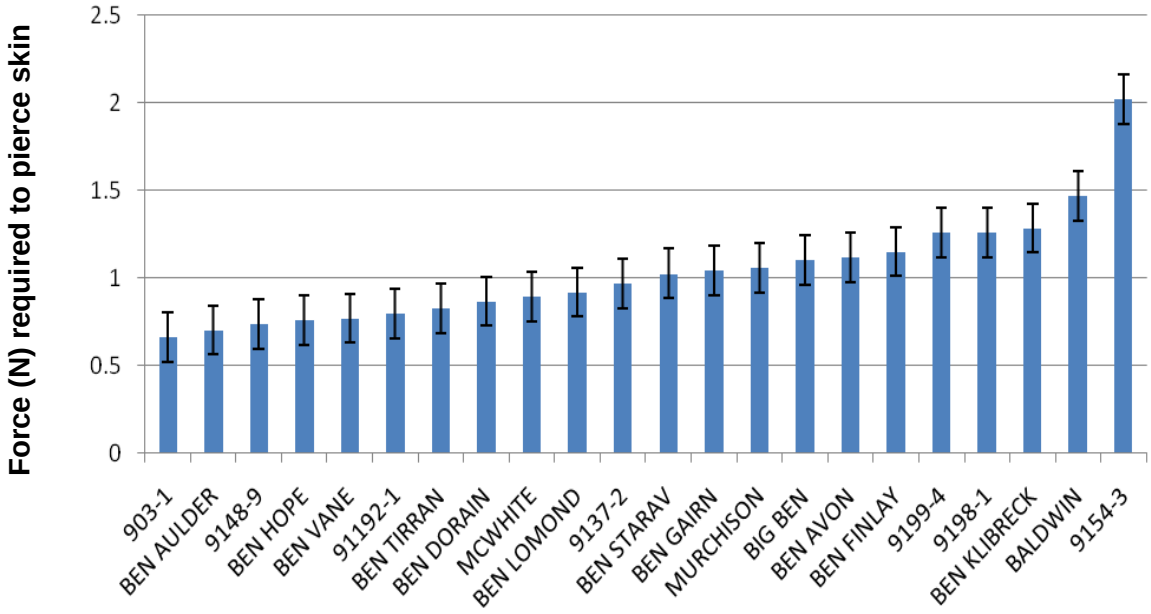


Figure 1.5.9. Force (N) required to pierce skin of blackcurrant genotypes (AHDB, 2012, JHI data)

Objective 4. To develop a synthetic lure and attract and kill technology for *D. suzukii* for incorporation into IPM programmes (Yrs 1-4).

Task 4.2. Develop target device and identify suitable insecticide(s) for attract and kill formulation (EMR, NRI, yrs 2-3)

After preliminary development trials in 2015 a prototype attract and kill (A&K) device was designed based on the following principles was produced;

a) Low cost, as the commercial version would need to be deployed in large numbers
b) Relatively small size
c) Lures should be attractive to *D. suzukii*, but of sufficiently small size to fit inside the device
d) Insecticide used should be fatal to *D. suzukii* after a low time of contact
e) Drowning solutions should not be used as the aim was to have a trap which could be left unattended for weeks if necessary. A small trap could fill with rain in this time and thus be rendered ineffective, and therefore it would need a drainage hole in the bottom of the device.

This year we conducted a series of trials to optimise the trap design and assess its efficacy for *D. suzukii* control.

Field trial 1

Aim: Confirmation that Decis was an effective killing agent in a field setting

Site: Woodland in East Kent known to be a hotspot for *D. suzukii*

Date: February 2016

Lures: Separate half size sachets of ethanol + acetoin, acetic acid, and methionol (NRI).

Device design: Attract and kill devices were 50 ml plastic Falcon tubes, as these satisfied criteria (a) and (b) above (Figure 4.2.1). Tubes were painted red. Half of the tubes had Decis (deltamethrin) coated on the inside at 0.63 mg/cm^2 corresponding to the dose on commercially available attract and kill traps for other species, the other tubes were without Decis (control).

Experimental design: 6 replicates of each treatment. Traps deployed in a randomised design in a line 10 m apart in woodland.

Assessment: Counts of the numbers of *D. suzukii* in the bottom of the bag.

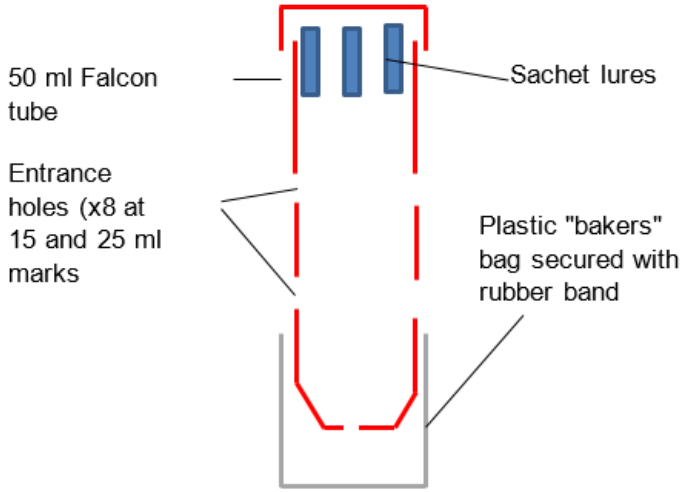


Figure 4.2.1. Device design

Results: Higher numbers of *D. suzukii* were found in the bag at the bottom of the trap containing Decis than no Decis. This indicated that *D. suzukii* entering the device may be contacting the Decis causing higher mortality than the control traps (Figure 4.2.2).

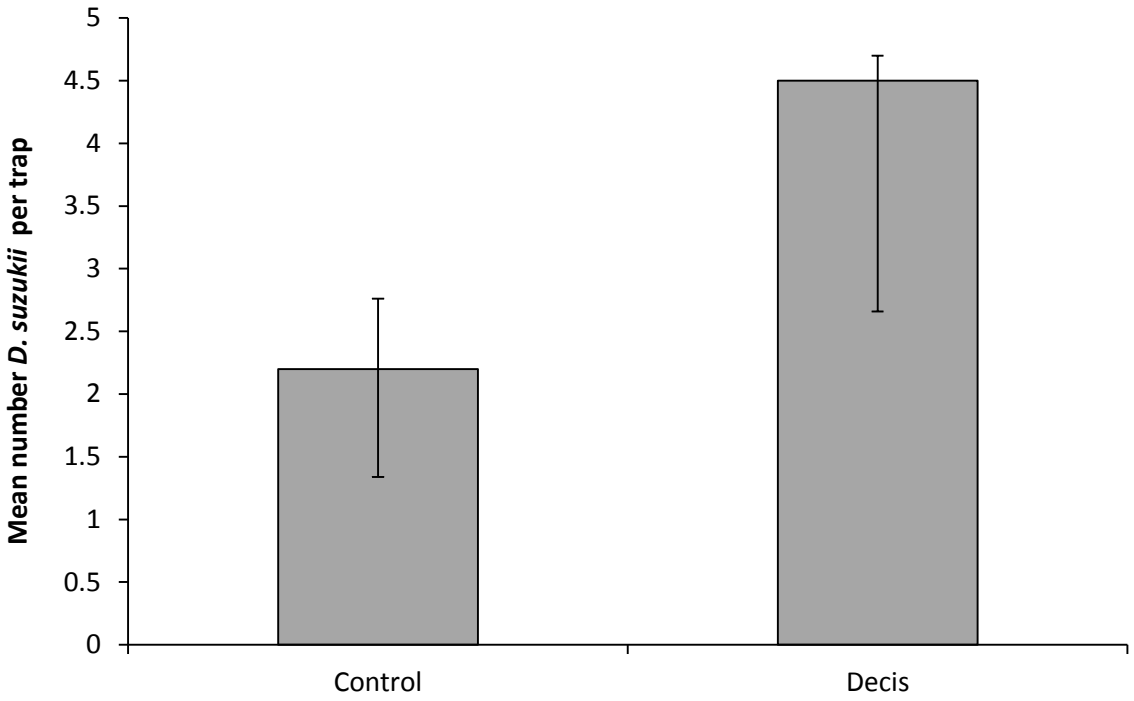


Figure 4.2.2. Numbers of *D. suzukii* caught in red painted A&K devices with or without an internal Decis coating. Fprob 0.264, s.e.d. 1.894, l.s.d. 4.479.

Field trial 2

Aim: Comparison of device designs

Site: Cherry orchard RF181 (NIAB EMR)

Date: June/ July 2016

Lures: Separate half size sachets of ethanol/ acetoin, acetic acid, and methionol (NRI)

Device design: 50 ml Falcon tubes compared to other potential A&K designs;

A) Clear plastic 50 ml Falcon tube with 8 x 4.5 mm entrance holes at the 45 ml marks and red tape around the holes. The internal surface of the lid and walls were coated with Decis at 0.63 mg/cm^2. No hole was placed in the bottom of the tube, and flies caught were assessed by emptying the tube (Figure 4.2.3).

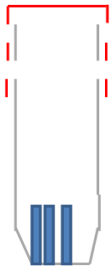
B) Drosal Cup Traps (from Sentomol Ltd). Clear plastic frustum base (r 5.5 x R 7.1 x h 6 cm) with 12 x 0.3 mm entrance holes at the top of the trap beneath the flat coned red lid (d 7.5 x h 1.5 cm). Internal base surface area of 499.2 cm^2 and volume of 752 cm^3. Internal lid surface area of 44.2 cm^2 (Figure 4.2.3).

C) Drosal Cup Traps (from Sentomol Ltd, same dimensions as above) coated on the inside with Decis (Figure 4.2.3).

D) Commercial dry trap (Coded) which is supplied with Decis on the lid (Figure 4.2.3). Red plastic hemisphere base (r 7 x h 7.2 xm) and clear plastic frustum lid (r 11.3 x R 12.7 x 4.7 h cm). Internal base surface area of 307 cm^2 and volume of 102.6 cm^3. Internal lid surface area of 1277 cm^2 and volume of 2128 cm^3.

Experimental design: 8 replicates of each treatment. Traps deployed in a line 10 m apart in cherry in randomised order.

A



B and C

D

CONFIDENTIAL

COMMERCIAL TRAP

Figure 4.2.3. Devices used in Figure 4.2.1. A) Falcon tube, B and C) Drosal Cup Trap, D) Commercial dry trap (Coded)

Results: The Coded Decis trap captured more *D. suzukii.* However it was noted that these traps had larger holes with cones inside to prevent escape of the flies (Figure 4.2.4).

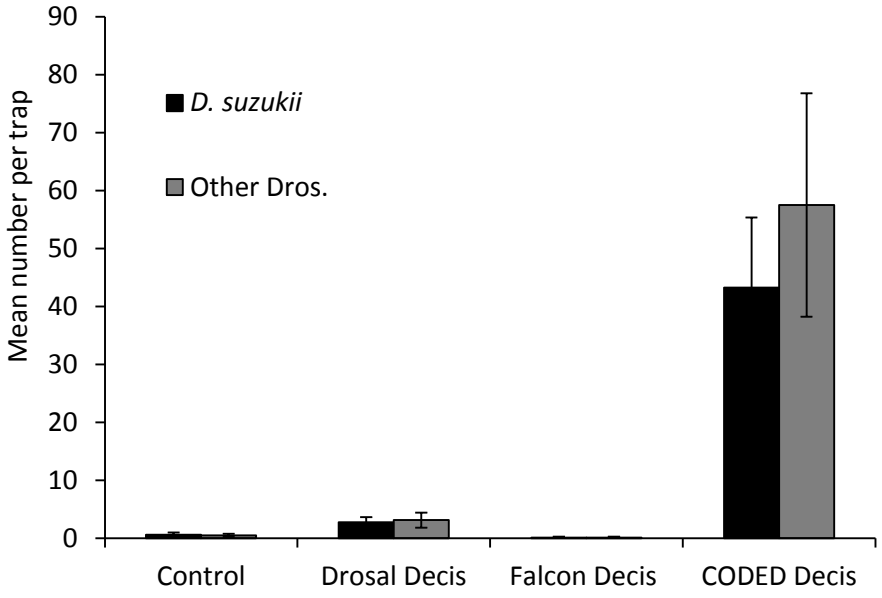


Figure 4.2.4. Catches of adult *D. suzukii* (Fprob < 0.001, s.e.d. 8.46, l.s.d. 17.6) and other Drosophila (Fprob < 0.001, s.e.d. 13.57, l.s.d. 28.22) in the different devices.

Field trial 3

Aim: To compare devices with identical sized holes

Site: Cherry orchard RF181 (NIAB EMR)

Date: August 2016

Lures: Separate half size sachets of ethanol/ acetoin, acetic acid, and methionol supplied by NRI *Device design*: As Section 4.2.2 (Figure 4.2.1 and 4.2.3) except that each device had 2 x 5mm holes. In the case of the Commercial dry trap (Coded) this required reducing the size of the existing holes.

Experimental design: 8 replicates of each treatment. Traps randomised in a line 10 m apart.

Results: No difference was found between the Drosal and Falcon tubes, but again the Commercial dry trap (Coded) captured more *D. suzukii* (Figure 4.2.5).

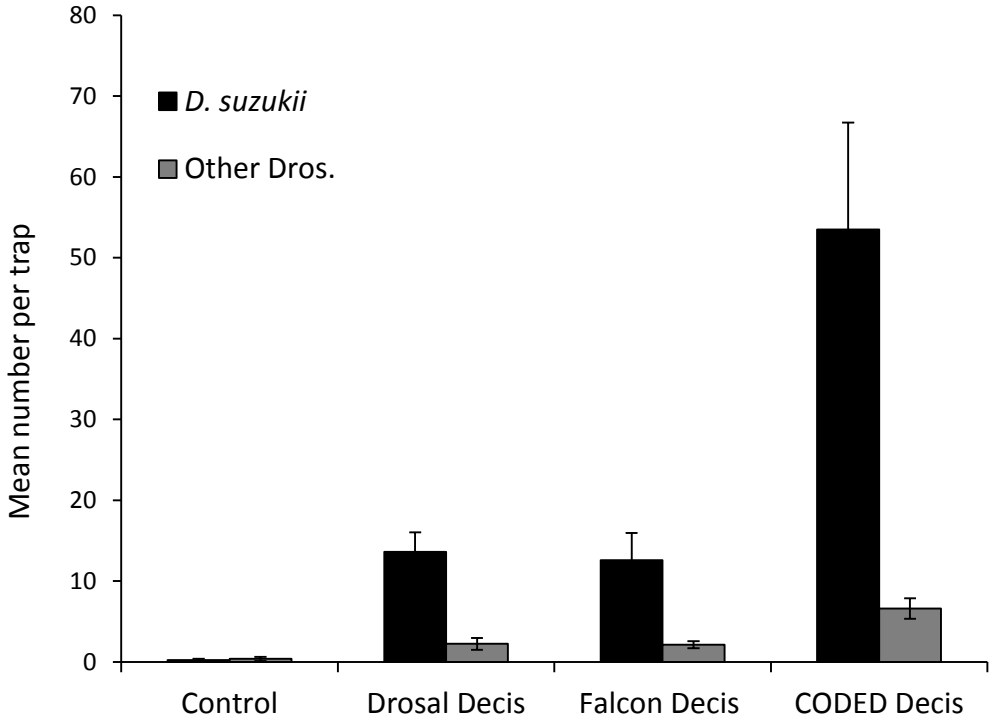


Figure 4.2.5. Catches of adult *D. suzukii* (Fprob < 0.001, s.e.d. 9.76, l.s.d. 20.3) and other Drosophila (Fprob < 0.001, s.e.d. 1.112, l.s.d. 2.313) in devices with two 0.5 cm holes.

Field trial 4

Aim: Another explanation for the higher *D. suzukii* catches of the Commercial dry trap (Coded) was the orientation of red colouration versus clear plastic in the device. *D. suzukii* are known to be attracted to red, but conversely, within the device they orientate to light through the clear plastic lid (coated with Decis). This orientation of red and clear plastic was another difference between the Commercial dry trap (Coded) and the prototypes tested (Figure 4.2.3).

Site: On the edge of scrubland by the railway line at NIAB EMR

Date: September 2016

Lures: Separate half size sachets of ethanol/ acetoin, acetic acid, and methionol (NRI)

Device design: As for trial 3 with each device having 2 x 5 mm holes. The vertical orientation of the Drosal and Falcon devices was reversed so that all devices had red on the lowest portion of the device and clear plastic at the top.

Experimental design: 8 replicates of each treatment. Traps randomised in a line 10 m apart.

Results: All of the Decis coated devices contained more *D. suzukii* than the 'no Decis' controls ($p<0.05$), but there was no significant differences between the Decis coated devices.

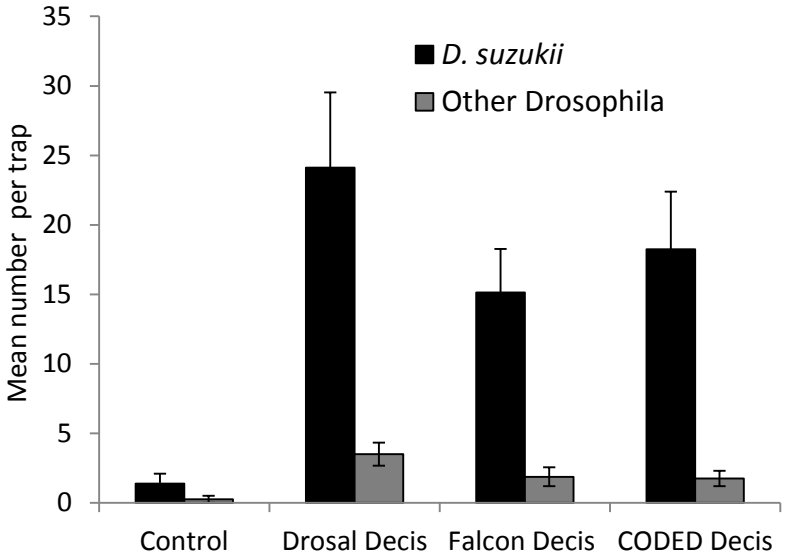


Figure 4.2.6. Catches of adult *D. suzukii* (Fprob < 0.001, s.e.d. 4.84, l.s.d. 10.06) and other Drosophila (Fprob < 0.05, s.e.d. 0.855, l.s.d. 1.778) in the devices with the light section orientated at the top

Field trial 5

Aim: To produce an enhanced attract and kill device using key factors discovered so far.

a) Half size sachet lures which have been shown to be as effective as the full size sachets, but allow more space within a small device. Following work showing that methionol may not be necessary (Section 4.3.1) this was excluded, thus saving both cost and also substance handling.

b) Incorporating red within the design as a visual attractant, but on the lower surface, with a clear plastic upper surface allowing the entrance of light.

c) Decis coating on the inside of the device at 0.63 mg/cm^2.

d) Inclusion of 175 ml Falcon tube for size comparison.

Site: On the edge of scrubland by the railway line at NIAB EMR and woodland in East Kent.

Date: October / November 2016

Lures: Separate half size sachets of ethanol/acetoin and acetic acid (NRI)

Device design: All devices had two 0.5 cm holes

A) Clear plastic 175 ml Falcon graduated tube (Falcon 352076) with the lower surface painted red (Figure 4.2.7).

B) Clear plastic 175 ml Falcon tube with the lower surface painted red (Figure 4.2.7) and Decis on the inner surface.

C) Clear plastic 50 ml Falcon tube with red lid (supplied) facing downwards and Decis on the inner surface (Figure 4.2.7).

d) Commercial dry trap (Coded) supplied with Decis on the inner surface of lid (Figure 4.2.7).

Experimental design: 4 replicates of each treatment. Traps randomised in a line 10 m apart.

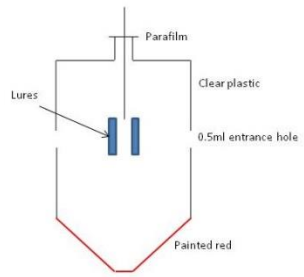
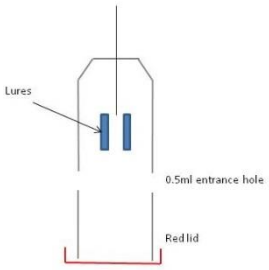
A and B	C	D
		CONFIDENTIAL COMMERCIAL TRAP

Figure 4.2.7. Devices used A and B) 175 ml Falcon tube, C) 50 ml Falcon tube, D) Commercial dry trap (Coded)

Results: Significantly more *D. suzukii* were caught in the coded commercial trap than the other prototypes. However there was a significantly higher by-catch in the coded trap. All of the Decis coated devices contained more *D. suzukii* than the 'no Decis' control.

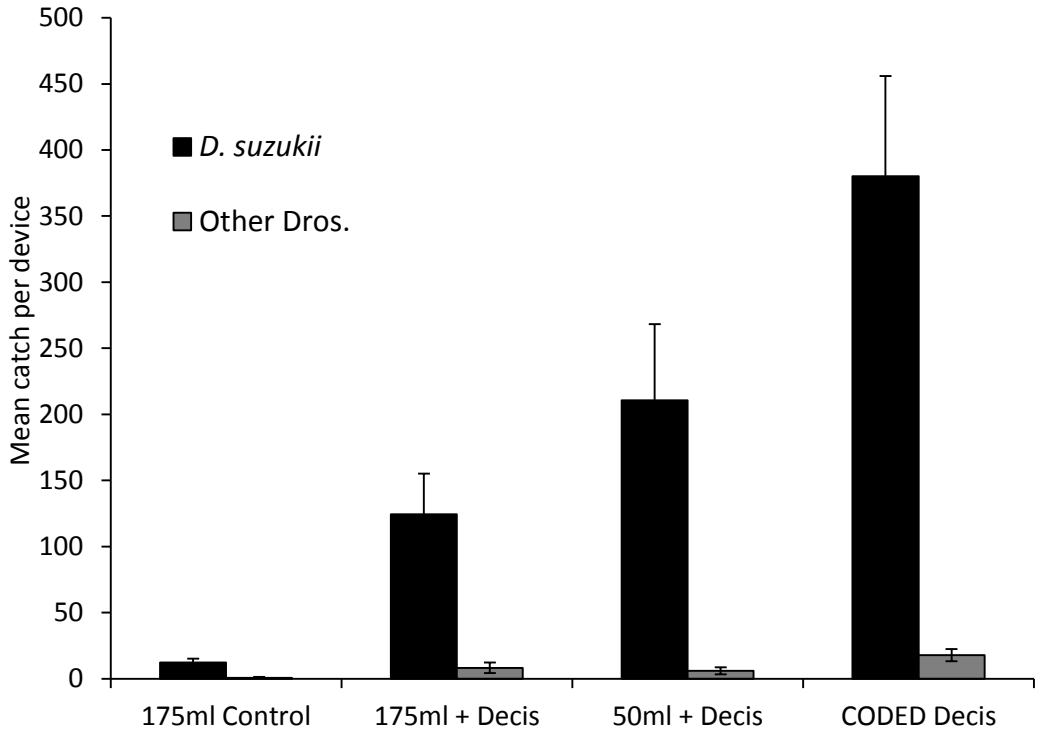


Figure 4.2.8. Catches of adult *D. suzukii* (Fprob 0.001, s.e.d. 60.6, l.s.d. 137.0) and other Drosophila (Fprob < 0.05, s.e.d. 5.05, l.s.d. 11.43) in the devices in Figure 4.2.7.

Field trial 6

Aim: To determine if the number of entrance holes on a device has an effect on *D. suzukii* catch.

Site: Woodland in East Kent

Date: November 2016

Lures: Separate half size sachets of ethanol/acetoin and acetic acid (NRI)

Device design: All treatments were based on the 175 ml Falcon tube coated internally with Decis and painted red on the lower surface (Figure 4.2.7). However, devices had 2, 4, 8 or 16 x 0.5 cm holes. No controls were included as these were already shown to be less effective at *D. suzukii* A&K.

Experimental design: 4 replicates of each treatment. Traps randomised in a line 10 m apart.

Results: Significantly more adult *D. suzukii* were caught in the traps with four 0.5 cm holes (336 per trap) than all the other treatments (<221 per trap) (Fprob < 0.001). There was a higher catch of other drosophila in the traps containing 4 holes. However, this was not significantly different from the traps containing 2 and 8 holes.

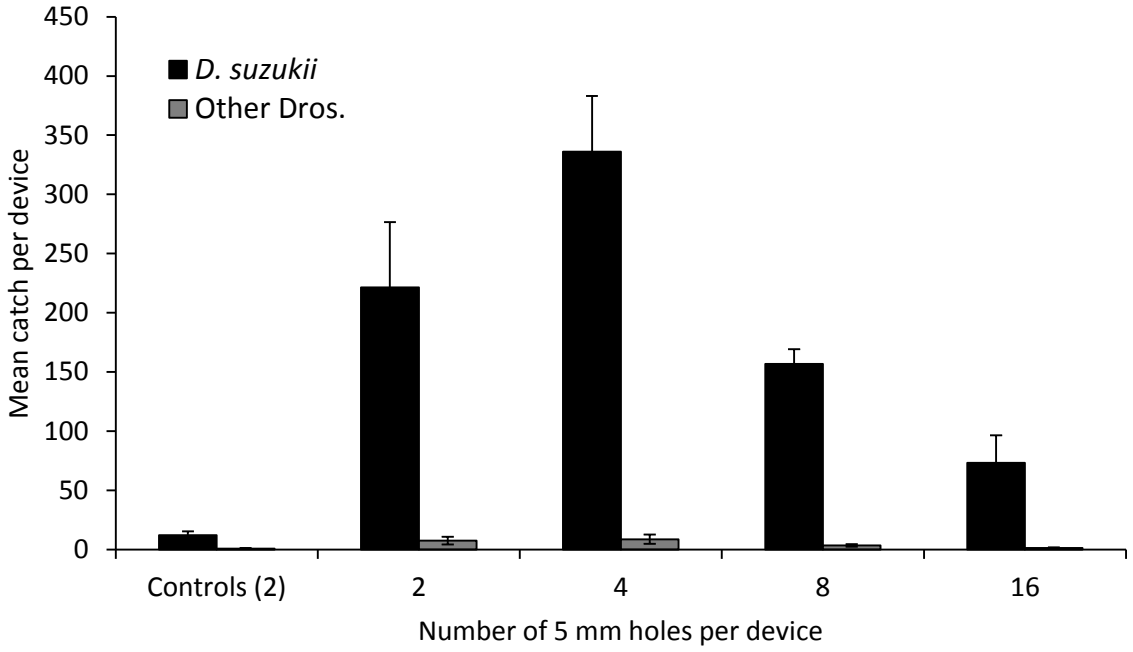


Figure 4.2.9. Influence of entrance hole number in devices on catches of adult *D. suzukii* (Fprob < 0.001, s.e.d. 37.2, l.s.d. 84.1) and other Drosophila (Fprob 0.149, s.e.d. 3.27, l.s.d. 7.39).

Semi field cage trial optimisation – pilot study

Aim: To develop a method for assessing attract and kill devices in cages. Laboratory studies can be difficult with attract and kill devices as climatic conditions are unrealistic and lure concentrations can build up in a confined space. In addition, it is challenging to determine mortality in field studies as flies may leave the device and die elsewhere. The aim of this trial was to develop and assess a method for assessing attract and kill devices in cage trials.

Site: NIAB EMR. Cages within an open polytunnel and glasshouses.

Date: November 2016

Cage design: Wire framed cages with insect proof mesh, 43 x 43 x 95 cm weighed down with 2 bricks for stability. Cages were set up vertically, with devices hung from straps integral to the cage (Fig. 4.2.10). Each cage had one device. Wet paper was added to each cage to ensure a high level of humidity.

Lures: Separate half size sachets of ethanol/acetoin and acetic acid supplied by NRI

Device design: 175 ml Falcon tubes with 2 x 0.5 cm holes and painted red on the base.

Experimental design: Two cages were placed in either an open ended polytunnel or one of two glasshouses (glasshouses N and O, NIAB EMR). Each site had a control device and a Decis treated device in separate cages, one device per cage; one device per cage.

Ten male and ten female adult *D. suzukii* from the laboratory culture were introduced at time zero. Each cage and device was assessed after 24 and 48 hours. At this point the devices were removed and a pot of apple cider vinegar placed in each cage to catch remaining live flies.

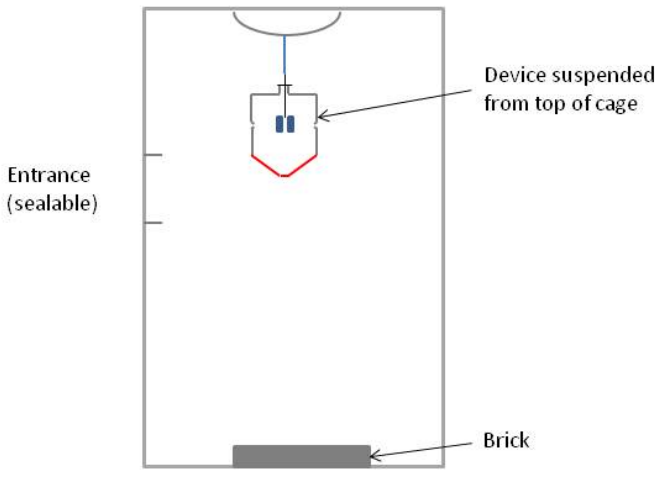


Figure 4.2.10. Experimental set up

Results: Recovery of *D. suzukii* at the end of the trial was 97.5% in the cages in tunnels and 99% in the cages in glasshouses.

Mortality is given in Table 4.2.1. The control devices within the glasshouses had a low mortality after 24 hours, but this had risen considerably after 48 hours, suggesting that 24 hours is the maximum time suitable for this type of study. Differences between the control and Decis treated devices were evident after 24 and 48 hours in the glasshouses. Mortality was greater in the cages within the polytunnels likely due to low humidity making it difficult to determine any effect from the Decis.

Table 4.2.1. Mortality (%) within cages in either polytunnel or glasshouses. Cages with either a control or Decis treated device as described in the text.

	Tunnel		Glasshouse 1		Glasshouse 2	
	Control	Decis	Control	Decis	Control	Decis
24 hour	30	40	10	20	5	20
48 hour	70	70	45	50	30	75

Conclusions

- An improved attract and kill device is being developed.
- Field and laboratory trials have been run to optimise the device.
- Decis was confirmed as effective in the field, and was found to maintain efficacy over a season.
- Light at the top of the device improves kill of *D. suzukii* likely due to orientation towards the insecticide coated lid.
- A semi-field cage trial protocol has been developed to assess efficacy of decis.

Task 4.3. Evaluation of components of Cha-Landolt baits for the efficiency of trapping Spotted Wing Drosophila (SWD)

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.

In previous work it was shown that the open vial dispensers for acetoin and methionol could be replaced by sealed polyethylene sachets without loss of attractiveness. However, lures with the ethanol and acetic acid also dispensed from polyethylene sachets were generally not as attractive as the Cha-Landolt lure. The release rates of ethanol and acetic acid were much lower from the sachets than from the drowning solution, but increasing the release rate of ethanol from the sachets did not increase attractiveness.

In other work at NRI it was shown that 3-methyl-1-butanol is a significant component of several of the fermentation baits available commercially. This compound is more convenient to purchase, handle and dispense than ethanol and it would be a significant advance if this could be used to enhance the attractiveness of the Cha-Landolt lure or even to replace the ethanol.

The objective of this Task was thus to make the Cha-Landolt lure more convenient to use for monitoring traps and to minimise the number and size of dispensers for use in lure-and-kill devices. The effectiveness of lures was determined in respect of both maximising their attractiveness to *D. suzukii* and reducing by-catch of other *Drosophila* species.

Materials and Methods

A series of experiments were carried out during 2016. Modified Drosotraps with a drowning solution or yellow sticky cards were used in experiments, and the basic dispensers tested are listed in Table 4.3.1.

Table 4.3.1. Specification of basic sachets used in experiments

Sachet type	Abbreviation	Specification	Image	Release rate (mg/day 22°C)
Standard Ethanol	E	2 ml ethanol on 2 dental rolls in 79 mm x 54 mm x 50 µ "baggie"		38
Half rate ethanol	E-half rate	2 ml ethanol on 2 dental rolls in 50 mm x 50 mm x 120 µ standard sachet		19
Quarter rate ethanol	E-quarter rate	2 ml ethanol on 2 dental rolls in 50 mm x 50 mm x 250 µ thick sachet		9
Standard Acetic acid	AA	1 ml on dental roll in 25 mm x 50 mm x 120 µ sachet		18
Standard Acetoin	Ac	1 ml of 1:1 mix in water on dental roll in 79 mm x 54 mm x 50 µ "baggie"		8
Standard Methionol	M	1 ml on dental roll in 25 mm x 50 mm x 120 µ sachet		1.3
Standard Methyl butanol	Mb	2 ml 3-methyl-1-butanol on dental roll in 50 mm x 50 mm x 120 µ sachet		2.3

Experiment 1

This experiment aimed to investigate whether changing the release rate of acetic acid could improve attractiveness.

Table 4.3.2. Table of treatments in Experiment 1

Treatment	Acetic acid	Ethanol	Acetoin	Methionol	Drowning Solution	Acetic acid release rate (mg/d 22°C)
1	Vial 3mm hole	Sachet	Sachet	Sachet	Water, detergent boric acid	53.2
2	Vial 6mm hole	Sachet	Sachet	Sachet	Water, detergent boric acid	212.9
3	Vial 9mm hole	Sachet	Sachet	Sachet	Water, detergent boric acid	479.0
4	7.2% in drowning solution	1.6% in drowning solution	Vial 3mm	Vial 3mm	Ethanol, acetic acid, detergent, boric acid	170
5	Sachet	Sachet	Sachet	Sachet	Water, detergent, boric acid	18
6	Super Gasser	Super Gasser			Super Gasser	

Treatments are listed in Table 4.3.2. To establish different release rates of acetic acid, lids with holes with diameters of 3 mm (53.2 mg/d), 6 mm (212.9 mg/d) and 9 mm (479 mg/d) were used with vials containing acetic acid and a cigarette filter. The remaining three components were hung in sachets from the lid. These treatments were compared with Super Gasser, the Cha-Landolt system and all four components in sachet form. There were 8 replicates of each treatment that were deployed in a randomised design in the woodland. The total counts after

10 days in each trap for male, female and total *D. suzukii*, insects <5 mm and insects >5 mm was then established. The blocks were re-randomised each week by moving the position of each trap down one place. The data were analysed using ANOVA on square root and angular transformed data in GENSTAT.

Experiment 2

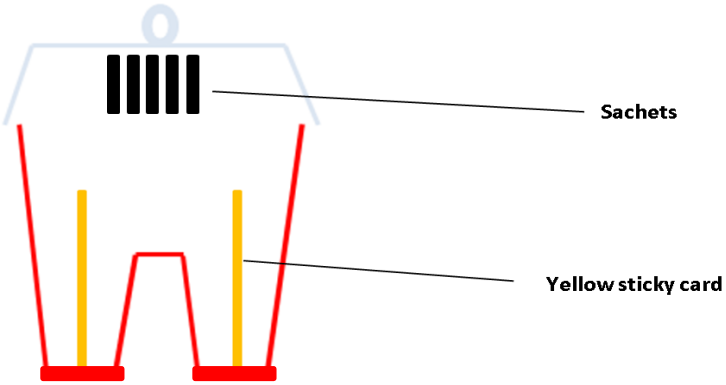
This experiment aimed to investigate whether 3-methyl-1-butanol can replace ethanol and if methionol is a necessary component.

There were six replicates of seven treatments (Table 4.3.3). The sachets were hung in the lid of a red standard Drosotrap containing two yellow sticky cards (7.5 x 10 cm) (Figure 4.3.3 and 4.3.4). Traps were spaced 10 m apart. Each block was re-randomised each week by moving the position of each trap down one position.

Table 4.3.3. Table of treatments in Experiment 2 with standard sachets containing acetoin (Ac), ethanol (E), acetic acid (AA), methionol (M) and 3-methyl-1-butanol (Mb).

Treatment code	Treatment
1	E, AA, Ac, M, Mb
2	E, AA, Ac, M
3	AA, Ac, M, Mb
4	E, AA, Ac, Mb
5	AA, Ac, Mb
6	E, AA, Ac
7	Blank

The trial was replicated twice at two different sites in the South East. The first trial ran 9 March - 4 April in dense woodland located near cherry and raspberry cropping and the second trial 12 August - 30 September in the 'Rookery' cherry orchard at NIAB EMR. The lures containing acetoin, acetic acid, ethanol and methylbutanol were replaced every four weeks where necessary. The drowning solution containing ethanol and acetic acid and the Super Gasser solution were replaced weekly. The traps were sampled and assessed on a weekly basis and the number of male and female *D. suzukii*, other drosophila and insects greater than 5 mm was counted (Figure 4.3.4).



A 

B 

C 

D 

Figure 4.3.4. Trap at field site 1 (A), Trap hanging in field (B), Inside of trap with sticky cards and sachet (C), Sticky card collected in from field (D).

Experiment 3

This experiment aimed to investigate whether doubling the release rate of acetoin and acetic acid improves *D. suzukii* catch, whether altering the release rate of ethanol has a significant effect on trap catch, and if methionol is necessary in an effective attract and kill system.

The standard release rates of acetic acid, ethanol or acetoin were quartered, halved or doubled for some treatments by adjusting the bag specifications or by adding more sachets (Table 4.3.4). There were eight treatments and six replicates of each treatment.

The sachets were hung from the lid of the red standard Drosotraps located along the edge of the woodland where the National Monitoring Trap No. 4 is located. A drowning solution of water, detergent and boric acid was used and replaced on a weekly basis. Traps were sampled each week for five weeks (3 November - 5 December 2016) and assessed for male and female *D. suzukii*, other Drosophila and insects greater in length than 5 mm. Traps were spaced 10 metres apart. Each block was re-randomised each week by moving the position of each trap down one position. The data were analysed using ANOVA on square root and angular transformed data in GENSTAT.

Table 4.3.4. Table of treatments in Experiment 3 using sachets containing acetoin (Ac), methionol (M), acetic acid (Ac) and ethanol (E)

Treatment code	Components
1	Standard E, AA, Ac, M
2	E, AA, Ac
3	E, AA, AA, Ac, Ac, M
4	E, AA, AA Ac, Ac
5	E, E, AA, Ac
6	E-half rate, AA, Ac,
7	E-quarter rate, AA, Ac,
8	Blank

Experiment 4

This experiment aimed to investigate why the *D. suzukii* trap catch is lower when acetic acid and ethanol are in sachets rather than in the drowning solution.

There were three treatments (Table 4.3.5), using standard Drosotraps. A grid was placed over the drowning solution to stop flies getting to it and a sticky card was placed within the trap.

Table 4.3.5. Treatments in Experiment 4 using sachets containing acetoin (Ac), methionol (M), acetic acid (AA) and ethanol (E)

Treatment code	**Components and their positions**
1	E, AA, Ac, M in sachets with water, detergent and boric acid drowning solution
2	Ac and M (sachets) with ethanol, acetic acid, water, boric acid and detergent drowning solution
3	Water and detergent in drowning solution with no lure

Results

Experiment 1

There were no significant differences in the mean number of *D. suzukii* when acetic acid was released at different rates from the vials (Treatments 1-3) or the standard sachet (5, Figure 4.3.5). When the acetic acid was released in a drowning solution (Cha- Landolt drowning solution 4 and Super Gasser 6) the mean number of *D. suzukii* was significantly higher than when released from the sachet or vials (Figure 4.3.5), even though the release rate of acetic acid was lower than in some of the vial treatments (170 mg/d).

The results indicate that treatments with acetic acid in the drowning solution had significantly lower specificity to *D. suzukii* than treatments with acetic acid released from sachets and vials (Figure 4.3.6).

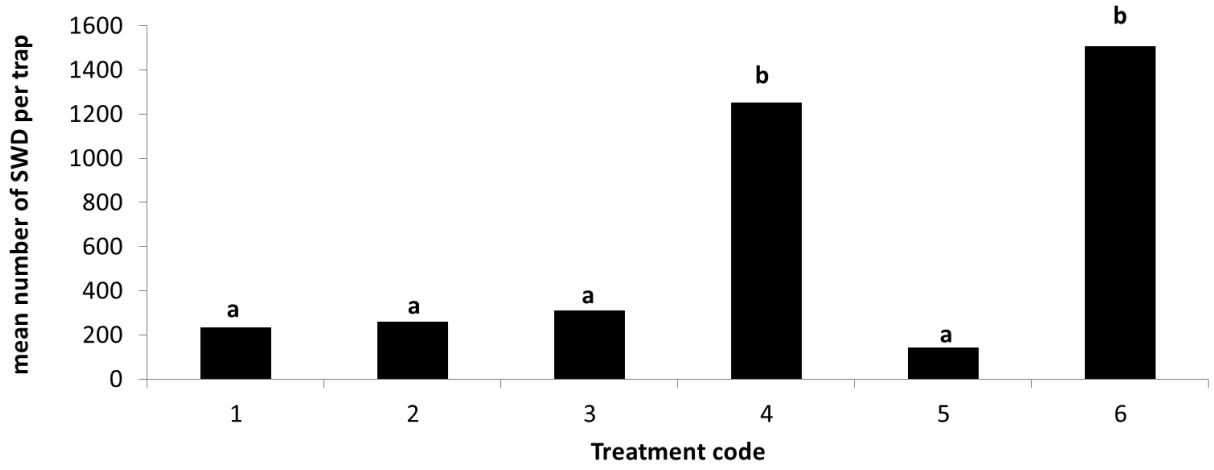


Figure 4.3.5. Mean number of *D. suzukii* captured in the traps over 10 days in Experiment 1. Treatment **1.** acetic acid 53 mg/d **2.** 213 mg/d **3.** 479 mg/d **4.** Cha-Landolt 170 mg/d **5.** sachet 18 mg/d **6.** Super Gasser. Significant differences indicated by different letters above treatments ($P < 0.001$, s.e.d. 3.339, l.s.d. 6.739)

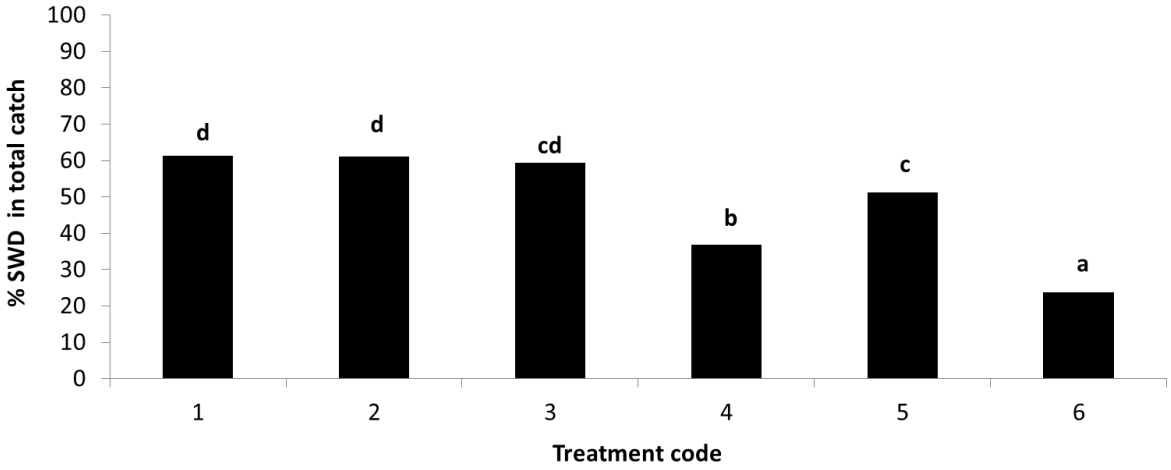


Figure 4.3.6. Mean % of *D. suzukii* caught in the traps over 10 days in Experiment 1. Treatment **1.** acetic acid 53 mg/d **2.** 213 mg/d **3.** 479 mg/d **4.** Cha-Landolt 170 mg/d **5.** sachet 18 mg/d **6.** Super Gasser. Significant differences indicated by different letters above treatments ($P < 0.001$, s.e.d. 2.769, l.s.d. 5.587)

Experiment 2

In the March (woodland) trial, there were no significant differences between treatments in the mean number of *D. suzukii* per trap other than the unbaited control (Figure 4.3.7). Replacing ethanol by 3-methyl-1-butanol (Treatment 3) gave the highest catch, and removing methionol did not affect catches (Treatments 4, 5 and 6).

These traps used sticky cards to retain insects. The national monitoring trap containing the lure in vials and drowning solution located in the same woodland had an average of 47 *D. suzukii* per trap over 4 weeks. Numbers caught in the dry traps were relatively low (<15 per trap).

Treatment 5 (AA, Ac, Mb) had a lower percentage of *D. suzukii* caught in the traps than the other treatments, but it was only significantly lower than Treatment 1 (E, AA, Ac, M, Mb). There was no significant difference in the percentage of *D. suzukii* caught between the remaining Treatments (1, 2, 3, 4 and 5). A similar by-catch was observed in all treatments and no treatment was significantly more specific to *D. suzukii* (Figure 4.3.8) ($P < 0.001$, l.s.d. 4.499).

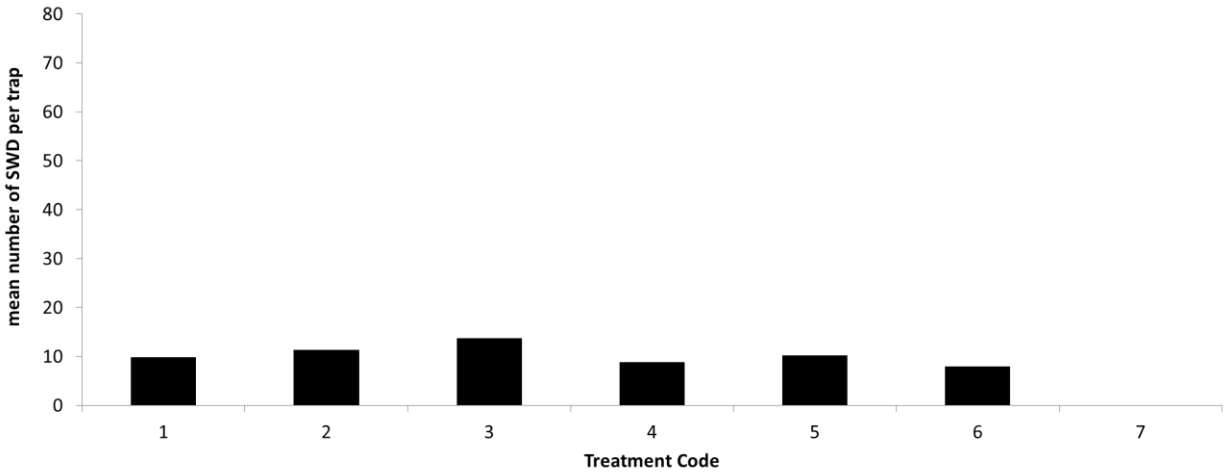


Figure 4.3.7. Mean number of *D. suzukii* per trap after 4 weeks in Experiment 2 March trial. Treatment **1.** E, AA, Ac, M, Mb **2.** E, AA, Ac, M **3.** AA, Ac, M, Mb **4.** E, AA, Ac, Mb **5.** AA, Ac, Mb **6.** E, AA, Ac **7.** Un-baited. Significant differences indicated by different letters above treatments ($P < 0.001$, s.e.d. 0.595, l.s.d. 1.209)

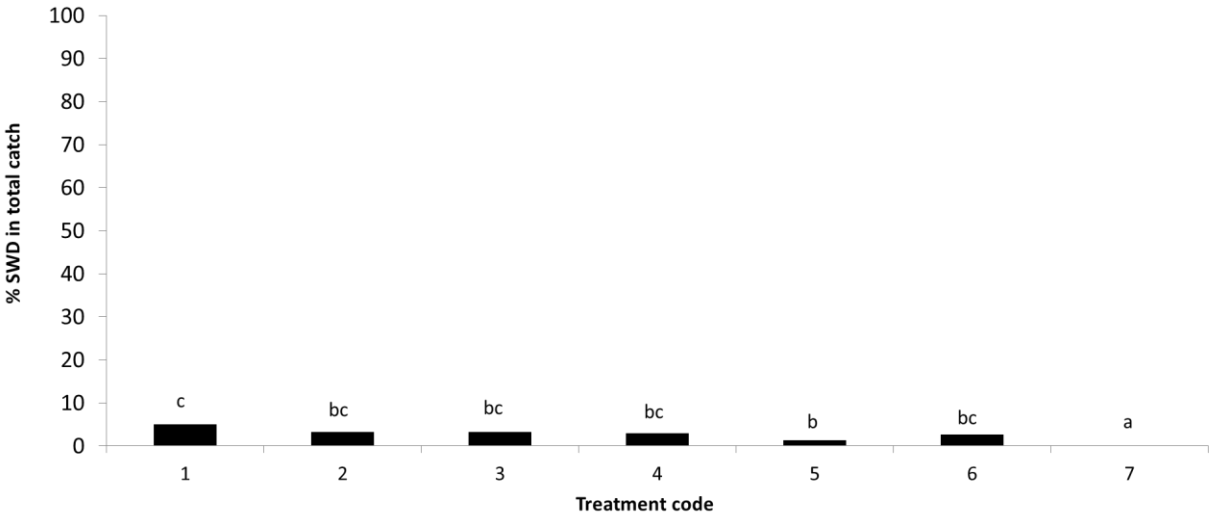


Figure 4.3.8. Mean % of *D. suzukii* caught in the traps after 4 weeks in Experiment 2 March trial. Treatment **1.** E, AA, Ac, M, Mb **2.** E, AA, Ac, M **3.** AA, Ac, M, Mb **4.** E, AA, Ac, Mb **5.** AA, Ac, Mb **6.** E, AA, Ac **7.** Un-baited. Significant differences indicated by different letters above treatments ($P < 0.001$, s.e.d. 2.216, l.s.d. 4.499).

In the August (cherry orchard) trial, the mean number of *D. suzukii* per trap was significantly higher than in the woodland in March (35 and 8.8 *D. suzukii* per trap respectively ($P < 0.001$, s.e.d. 0.515, l.s.d. 1.025, Figure 4.3.9). Furthermore the mean numbers of *D. suzukii* in the traps with sticky cards was closer to those in the liquid Gasser traps in the field which had a mean of 107 *D. suzukii* per trap. Therefore, comparisons between treatments can be made. Treatment 6 (E, AA, Ac) had a significantly higher mean number of *D. suzukii* per trap than all the treatments containing methyl butanol (Treatments 1, 3, 4, 5). Additionally Treatment 2 (E, AA, M, Ac) had a higher *D. suzukii* trap catch than all treatments containing methyl butanol, although this was not significant. When methionol was removed from Treatments 1, 2 and 3 to give Treatments 4, 6 and 5, respectively, the mean number of *D. suzukii* per trap was either not significantly different or the treatment with methionol removed had significantly higher mean numbers of *D. suzukii* per trap (Fig. 4.3.9, $P < 0.001$, l.s.d. 2.181).

The mean numbers of other Drosophila per trap was significantly lower in August compared with the trial in March; 27 and 456 other drosophila per trap, respectively ($P < 0.001$, l.s.d. 3.289). Therefore, there was a higher mean percentage of *D. suzukii* in the trap catches over the seven weeks. There was no significant difference in the percentage of *D. suzukii* caught in the traps between treatments (P value < 0.001, l.s.d. 1.629, Figure 4.3.10).

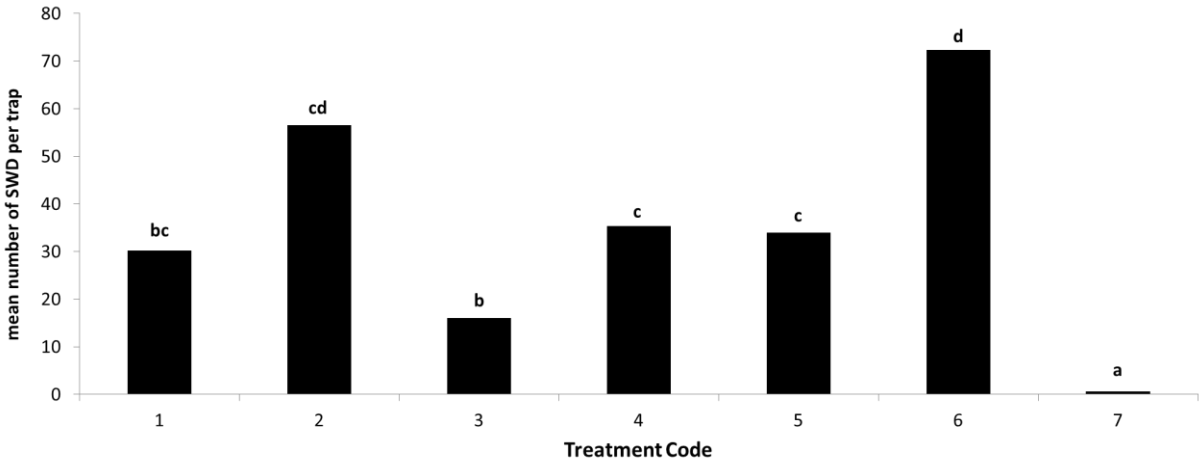


Figure 4.3.9. Mean numbers of *D. suzukii* per trap after seven weeks in Experiment 2 August trial. Treatment **1.** E, AA, Ac, M, Mb **2.** E, AA, Ac, M **3.** AA, Ac, M, Mb **4.** E, AA, Ac, Mb **5.** AA, Ac, Mb **6.** E, AA, Ac **7.** Un-baited. Significant differences indicated by different letters above treatments ($P < 0.001$, s.e.d. 1.068, l.s.d. 2.181).

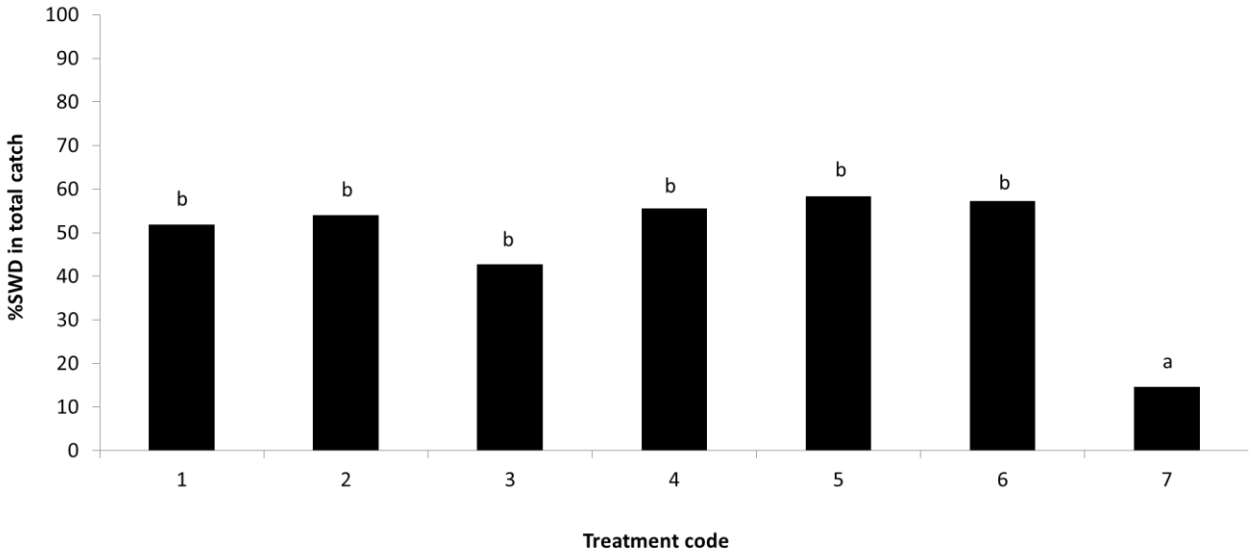


Figure 4.3.10. Mean % of *D. suzukii* caught in the traps after 7 weeks in Experiment 2 August trial. Treatment **1.** E, AA, Ac, M, Mb **2.** E, AA, Ac, M **3.** AA, Ac, M, Mb **4.** E, AA, Ac, Mb **5.** AA, Ac, Mb **6.** .E, AA, Ac **7.** Unbaited. Significant differences indicated by different letters above treatments ($P < 0.001$, s.e.d. 5.92, l.s.d. 12.09)

Experiment 3

As shown in Figure 4.3.11, Treatment 3 (double acetoin (32 mg/d) and double acetic acid (16mg/d) with methionol) had significantly higher *D. suzukii* per trap than all other treatments (Fprob < 0.001, l.s.d. 3.555). Removal of the methionol from this treatment to give Treatment 4 caused a significant reduction in catch, whereas removal of methionol from the standard lure (Treatment 1) to give 2 did not affect catch significantly, as previously. Doubling (76 mg/d), halving (19 mg/d) or quartering (9 mg/d) the release of ethanol in Treatments 5, 6 and 7, respectively, had no significant effect on the catch of *D. suzukii* when compared to Treatment 2 containing just the standard ethanol sachet (38 mg/d).

The average trap catch from the national monitoring trap (Cha-Landolt system with vials and drowning solution) located inside the woodlands was 741 *D. suzukii* per trap, approximately double the catches in this experiment using all-sachet lures.

There was no significant difference in the % *D. suzukii* caught between the treatments except the untreated control (Fprob < 0.001, l.s.d.16.63, Figure 4.3.12)**.**

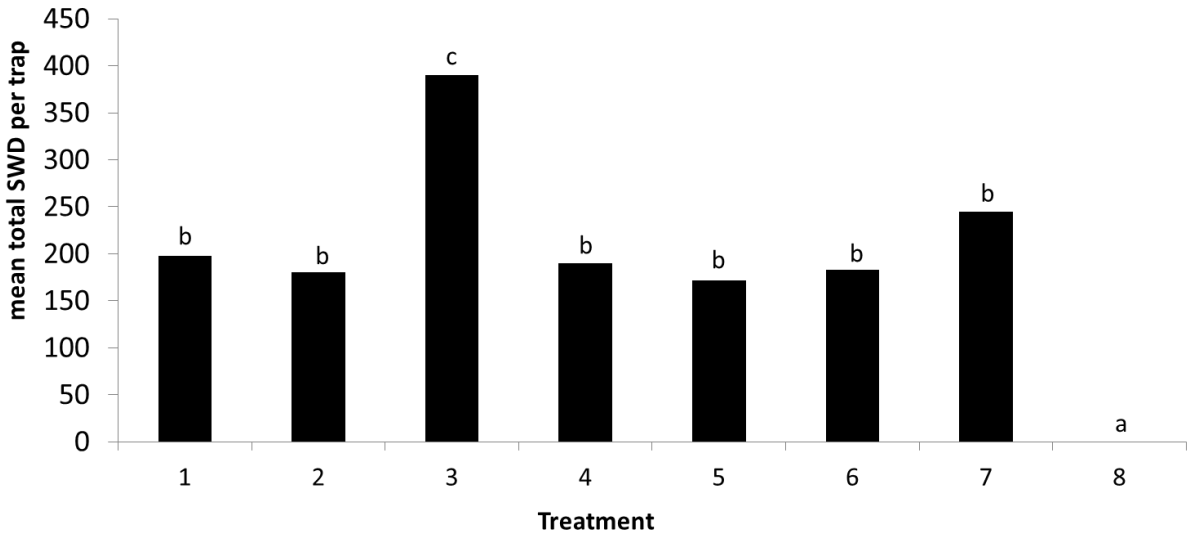


Figure 4.3.11. Mean numbers of *D. suzukii* per trap after 5 weeks in Experiment 3. Treatment **1.** E, AA, Ac, M **2.** E, AA, Ac **3.** E, AAx2, Acx2, M **4.** A, AAx2, Acx2 **5.** Ex2, AA, Ac **6.** E half-rate, AA, Ac **7.** E quarter rate, AA, Ac **8.** Un-baited. Significant differences indicated by different letters above treatments (Fprob < 0.001, s.e.d.1.751, l.s.d. 3.555)

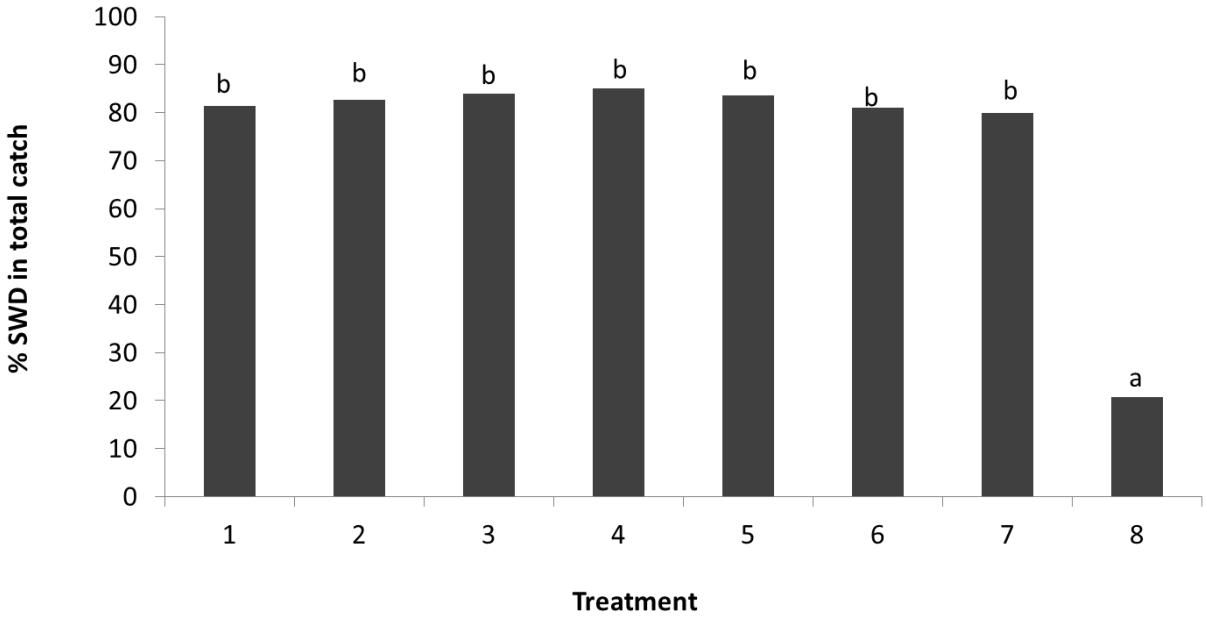


Figure 4.3.12. Mean % of *D. suzukii* caught in the traps after 5 weeks in Experiment 3. Treatment **1.** E, AA, Ac, M **2.** E, AA, Ac **3.** E, AAx2, Acx2, M **4.** A, AAx2, Acx2 **5.** Ex2, AA, Ac **6.** E half-rate, AA, Ac **7.** E quarter rate, AA, Ac **8.** Unbaited. Significant differences indicated by different letters above treatments (Fprob < 0.001, s.e.d. 8.190, l.s.d. 16.63)

Experiment 4

There were significantly more adult *D. suzukii* caught on the sticky base in the traps where acetic acid and ethanol were released from the drowning solution than when released from sachets (F prob<0.001, Figure 4.3.13, l.s.d.1.411). There was no significant difference in the % *D. suzukii* caught between treatments 1 and 2 (Fprob < 0.001, l.s.d. 16.75, Figure 4.3.14)

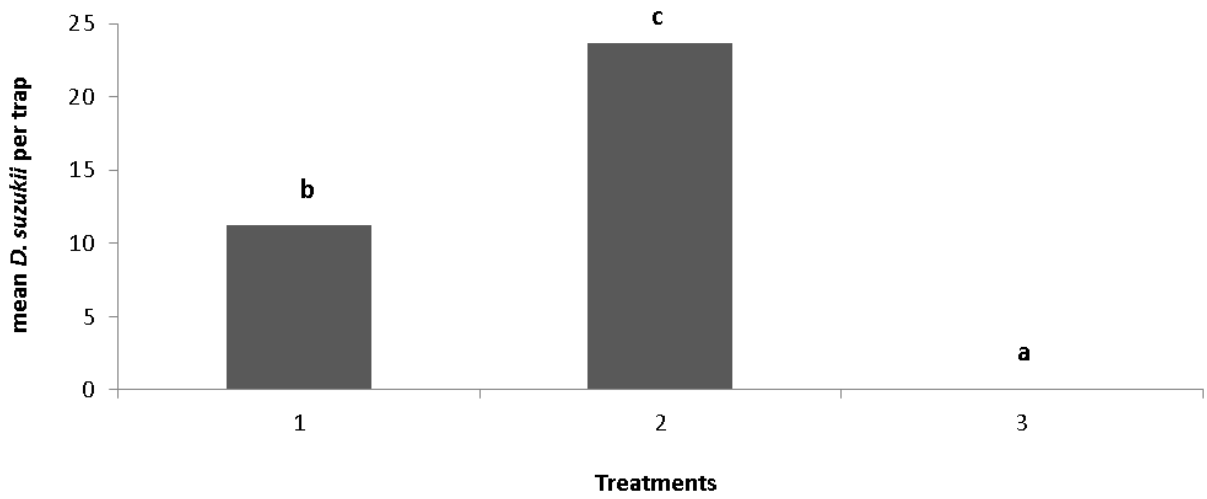


Figure 4.3.13. Mean numbers of *D. suzukii* per trap after 3 weeks in Experiment 4. Treatment **1.** Acetic acid and ethanol released from sachets **2.** Acetic acid and ethanol released from drowning solution. Significant differences indicated by different letters above treatments (F prob<0.001, s.e.d. 0.633, l.s.d. 1.411)

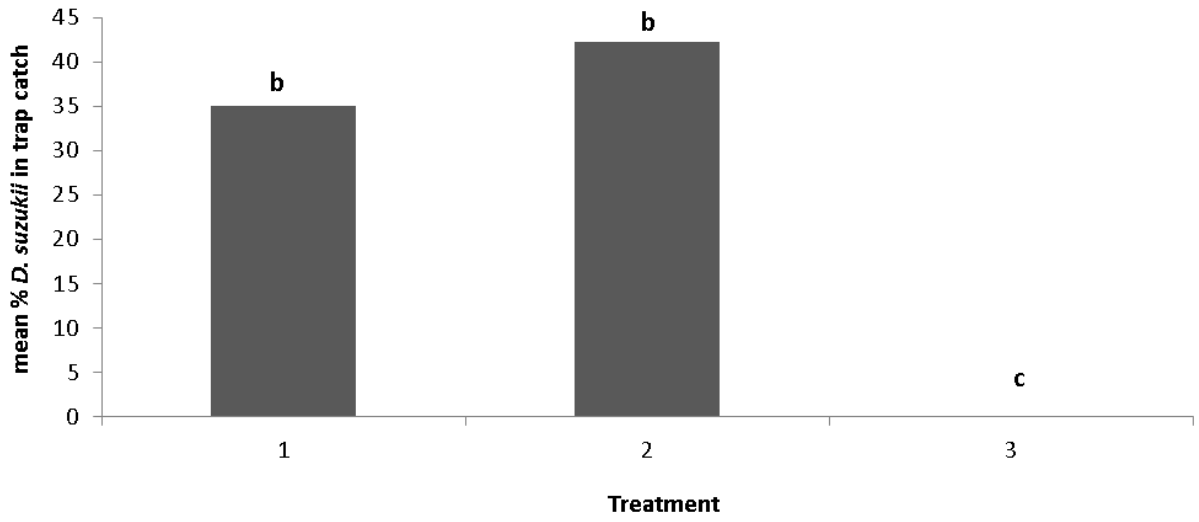


Figure 4.3.14. Mean % of *D. suzukii* in the traps after three weeks in Experiment 4. Treatment **1.** Acetic acid and ethanol released from sachets **2.** Acetic acid and ethanol released from drowning solution. Significant differences indicated by different letters above treatments (Fprob < 0.001, s.e.d. 7.52, l.s.d. 16.75)

Conclusions

- Varying the release rate of acetic acid (Experiment 1) or ethanol (Experiment 3) over an 8-fold range did not significantly affect catches of *D. suzukii*.
- Doubling release rates of both acetic acid and acetoin almost doubled catches of *D. suzukii* (Experiment 3).
- Removal of methionol from the standard all-sachet lure did not seem to affect catches of *D. suzukii* (Experiments 2 and 3), but removal of methionol from the lure with double acetic acid and acetoin significantly reduced catches (Experiment 3).
- 3-Methyl-1-butanol probably cannot replace ethanol or increase its attractiveness to *D. suzukii* (Experiment 2).
- Adult *D. suzukii* trap catch was significantly higher from traps where acetic acid and ethanol was released from the drowning solution (Experiment 4).

Objective 5. To obtain evidence for the effectiveness of different plant protection products including biopesticides for control of *D. suzukii* and for developing an insecticide resistance management strategy.

Task 5.1. Addition of bait attractants to increase insecticide efficacy for control of Drosophila suzukii

Objectives

1. Develop a bioassay for testing bait and bait/pesticide attractiveness in the laboratory

2. Determine the most attractive baits for Attract & Kill strategy, to increase *D. suzukii* pesticide contact and uptake

Background

Several commercial baits are available for control of *D. suzukii* (e.g. Bioiberica, Combi-protec, Gasser, Suzukii Trap, Dros'Attract). However, there is little information available on the relative *D. suzukii* attractiveness of different commercial baits and natural substances. Previous studies have shown that combinations of substances (e.g. vinegar, wine, sugar, yeast) are more attractive to *D. suzukii* than individual substances. It is an additional requirement that baits should not be attractive to managed or wild bees.

Materials and Methods

Three methods were used for testing the attractiveness of potential bait substances under laboratory conditions: (1) Petri dish droplet test (2) large arena test (3) chronophysiology.

Test substances: Test substances were used at between 0.2 and 100% w/w depending on the individual experiment and the manufacturers recommended rate for use in traps (Table 5.1.1). Concentrations of sugar (0.36-16%w/w), yeast powder inoculum (0.4-4%w/w) and flour (8%) were based on previous research (Cowles 2012, 2015; Anon. 2014; Burrack et al. 2015; Iglesias et al 2014). Some of the commercial products (Attracker, Bioiberica, Gasser) were examined at 100% in the first Petri dish test; in subsequent tests, the maximum rate for commercial products was reduced to 50% because the 100% rate was not considered to be economically viable in an bait spray programme. Due to the number of commercial products (5), substances (7) and yeasts (2), it was not possible to examine all of the treatments and at different concentrations with all of the test methods in Table 5.1.1. It was also not possible to compare solids (sugar, yeast powder, flour) at high concentrations (50-100%w/w) as for liquid substances and commercial products with unknown concentrations of unknown solutes. A saturated sugar solution at 20 °C contains 20%w/w sugar.

Table 5.1.1. Substances, concentrations (%w/w in water), and methods used in the experiments. A *Hanseniaspora uvarum* (yeast H) suspension was added so that the cell concentrations in the mixtures (*2×10^8/ml and **1×10^9/ml) were the same as for *Saccharomyces cerevisiae* (yeast S) when added at 0.4% and 2% inoculum respectively. Brewery wastes were obtained from Kent (K) and Warwickshire (W).

Subst. Method	Petri-dish experiment				Large arena		Chronophysiology	
	a	b	c	d	a	b	a	b
Apple cider vinegar	-	-	-	-	50	50	-	-
Attracker	0.2 100	50	-	-	50	5	50	-
Bioiberica	5 100	-	-	-	50	5	-	-
Brewery waste (W)	-	50	50	-	50	5	50	-
Brewery waste (K)	-	50	-	-	50	-	-	-
Combi-Protec	5	50	50	5	50	5	50	5
Dros'Attract	-	-	-	-	50	5	-	-
Gasser	100	-	50	5	50	5	-	5
Molasses	5	50	50	-	50	5	50	-
Molasses+yeast (S)	-	-	50+4	-	50+4	-	-	-
Sugar	0.36	16	-	-	-	-	16	-
Yeast (S)	0.36	4	-	-	-	-	-	-
Sugar+yeast (S)	0.36	16+4	16+4	8+2 1.6+0.4	16+4	8+2	16+4	8+2 1.6+0.4
Sugar+yeast (S)+flour	-	16+4+8	-	-	-	-	16+4+8	-
Sugar+yeast (H)	-	-	-	8** 1.6*	-	8**	-	8** 1.6*
Ferm. strawb. juice	-	-	100	50	50	50	-	50
Ferm. plum juice	-	-	-	-	50	-	-	-
Strawberry juice	-	100	100	100	100	100	100	100

Strawberries (chopped or juice) or whole blueberries were used as a control treatment in place of bait drops in some of the experiments. Fermented strawberry and plum juices were obtained from sealed containers of fruit wastes. Dried bakers' yeast inoculum and two sources of brewery waste, from Kent and Warwickshire, containing yeast (different strains of *Saccharomyces cerevisiae*) were used in suspensions with water or water + sugar. The suspensions were kept at 20 °C for at least 1 hour before use to ensure an active yeast culture was used. An isolate of a yeast species (*Hanseniaspora uvarum*) found in the gut of *D. suzukii* (Hanby et al. 2012) was obtained from the University of California, Davis. It was cultured on plates of potato dextrose agar and used in a water suspension with sugar. Freshly prepared, 7-day old *H. uvarum* cultures were used to produce the suspensions to ensure that they contained actively growing cells. Cell counts in the suspensions of bakers' yeast and *H. uvarum* were determined with a haemocytomer to calculate the amount of inoculum required in the sugar solution. All the tests were conducted at 20 °C.

Petri dish test: The attractiveness of bait droplets to *D. suzukii* was tested against a water or water + 16% sugar droplet for 30 minutes (time increased to 60 mins in later experiments). Two 20 µL droplets, (i) test substance (ii) water or water + sugar, were placed in 9 cm Petri dishes 45 mm apart. In later tests, the droplets were positioned 25 mm apart on apple or bramble leaves. After 30 minutes, except in the initial test, a whole blueberry or chopped strawberry was also placed into the Petri dish to test the relative attractiveness of the fruit and bait droplets, and the activity of *D. suzukii* placed in the Petri dish.

D. suzukii (mated males and females, three to nine days old) were starved for 18 hours with access to water on paper tissue. Equal numbers of males and females were used for the experiments. Depending on the experiment, one or two *D. suzukii* of the same sex were introduced in each Petri dish, 45 mm from the droplets, and observed at five minute intervals for 30 or 60 minutes, and the following noted:

- whether *D. suzukii* moved to the test substance, to only water or water + sugar but not to the test substance, or to neither during the initial 30 minutes
- whether the *D. suzukii* moved to the fruit, test substance or water after the fruit was introduced, for a further 30 minutes.

The substances and concentrations used in replicated experiments are shown in Table 5.1.1.

Petri dish experiment (a): Substances and fruit (blueberry or chopped strawberry) were each tested with between 16 and 24 individual *D. suzukii* on two or three different occasions depending on the treatment. The test duration was 30 minutes. Water droplets were used as a control.

Petri dish experiment (b): Six replicate tests were conducted with eight treatments. Each replicate test for each treatment consisted of four Petri dishes, each with a pair of male or female *D. suzukii* (48 *D. suzukii* per treatment). Water + 16% w/w sugar was used as a control. Individual blueberries were introduced into the Petri dishes after 30 minutes. The test duration was 60 minutes.

Petri dish experiment (c): Seven replicate tests were conducted with eight treatments. Droplets were placed on apple leaves. Each replicate test for each treatment consisted of four Petri dishes, each with a pair of male or female *D. suzukii* (56 *D. suzukii* per treatment). Water was used as a control. Individual chopped strawberries were introduced into the Petri dishes after 30 minutes. The test duration was 60 minutes.

Additional Petri dishes were also set up in which blue food dye was added to three of the attractant baits (Combi-protec, sugar + yeast, strawberry juice). The gut of the *D. suzukii* was then examined for the blue dye after 12 hours to record if feeding had occurred.

Petri dish experiment (d): Eleven replicate tests were conducted with eight treatments. Droplets were placed on bramble leaves. Other details were the same as Petri dish experiment (c) (88 *D. suzukii* per treatment).

Large arena test: A laboratory wind tunnel with the air flow turned off was used for a choice test for the *D. suzukii* attractiveness of 20 ml samples of test substances in 50 ml open plastic containers (Table 5.1.1; Figure 5.1.1). A single container of each substance with a drop of Triton detergent was randomly positioned on the bench top, spaced 15 – 20 cm apart. Starved *D. suzukii* males and mated females (between 100 and 200 per replicate run) were introduced into the chamber which had a controlled humidity. The numbers of *D. suzukii* attracted to and drowning in the containers of test substances was recorded after 24 hours. The positioning of the test substance treatments was re-randomised between replicate runs of experiments. Two experiments were conducted with the test substances and concentrations differing between experiments (Table 5.1.1). Experiment (a) consisted of four replicate runs with 13 treatments; Experiment (b) consisted of three replicate runs of 12 treatments.

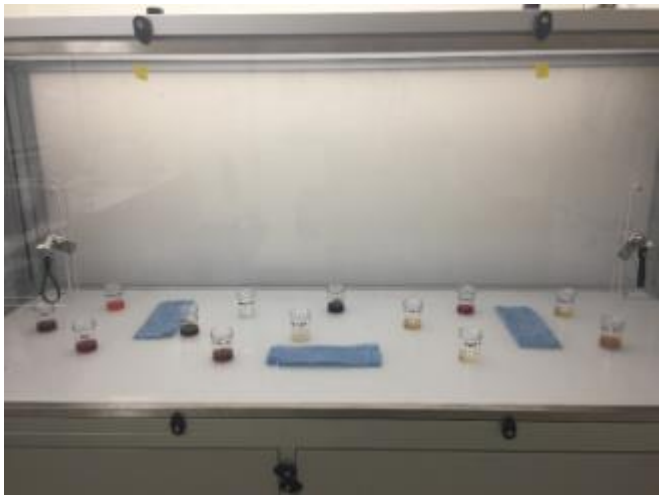


Figure 5.1.1. Large arena bait test showing positioning of open containers with test substances

Chronophysiology: A 32 channel Trikinetics *Drosophila* Activity Monitoring (DAM) (Figure 5.1.2) placed inside a BugDorm cage in which *D. suzukii* were allowed to fly in and out of small tubes containing 0.2 ml of test substances was used to compare their relative attractiveness. An infra-red beam at the entrance of each tube enabled the number of entries and exits by the flies to be recorded electronically over three days. Two experiments were conducted; each experiment consisted of four replicate tubes of eight test substances (Table 5.1.1).

Figure 5.1.2. Chronophysiology DAM apparatus

Results

Petri dish experiments: In all four experiments, generally less than 10 percent of *D. suzukii* were attracted to the water or water + sugar control droplets, and this was usually less than the proportion attracted to the test substances. Generally more than half of the SWD remained inactive, even after the introduction of a blueberry into the Petri dish. There was no significant difference between the behaviour of males and females in the Petri dish tests.

In Petri dish Experiment (a), a high percentage of *D. suzukii* were attracted to whole blueberries (Figure 5.1.3). However, in subsequent experiments, only a small proportion of previously unresponsive *D. suzukii* moved to the blueberry when it was introduced after 30 minutes (Figures 5.1.4 to 5.1.6). A 5% molasses solution was the most attractive test substance in Petri dish experiment (a) (Figure 5.1.3). A dilute mixture of sugar and yeast was more attractive than the individual materials, and at least as attractive as the commercial products tested at 5 or100% v/v.

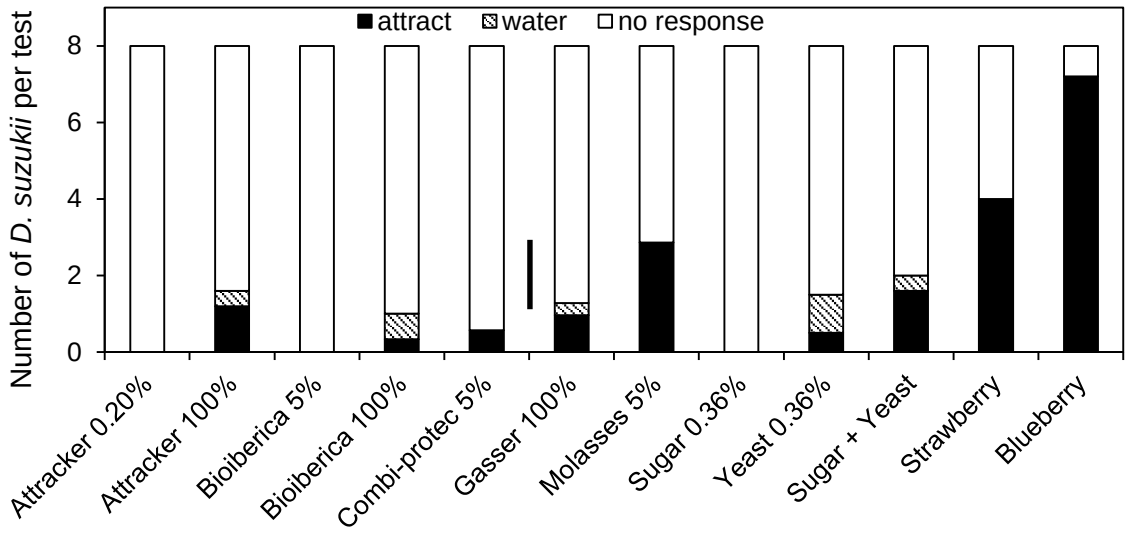


Figure 5.1.3. Petri dish experiment (a). Each column represents the movement or no response of *D. suzukii* introduced individually into Petri dishes containing droplets of the test substances (or fruit) or water, and left for 30 minutes. Each value is the mean of two or three replicate tests; bar represents LSD (P = 0.05)

In Petri dish experiment (b), more than half of the *D. suzukii* remained unresponsive after 60 minutes, even after the introduction of a blueberry (Figure 5.1.4). The test substances, except Attracker (50%), which was the least attractive substance to *D. suzukii*, attracted more *D. suzukii* than the droplet of 16% sugar solution. The attractiveness to *D. suzukii* of other test substances was not significantly different to that of strawberry juice. Two sources of brewery

waste used at 50% v/v had a similar attraction to *D. suzukii* than sugar solution with baker's yeast. The addition of flour to sugar + yeast did not improve the attractiveness.

In Petri dish experiment (c), a suspension containing 80 g sugar and 20 g bakers' yeast per litre was the most attractive substance to *D. suzukii* (Figure 5.1.5). Combi-protec (50%) was the least attractive substance. The addition of yeast to molasses did not increase its attractiveness. Tests with the blue dye showed that after 12 h, all *D. suzukii* with the sugar + yeast droplets and 95% or more of *D. suzukii* with Combi-protec (50%) or strawberry juice droplets had blue dye in their gut.

In Petri dish Experiment (d), the attraction of *D. suzukii* to sugar + yeast suspension was lower than in Experiment (c), although the substances were only used at 10% and 50% of the concentrations used in Experiment (c). The mixtures containing 80 g sugar + 20 g yeast per litre were more attractive to *D. suzukii* than the 5% v/v Combi-protec and Gasser solutions (Fig. 5.1.6). Reducing the concentration of the sugar + yeast suspension reduced its attractiveness for bakers' yeast (*S. cerevisiae)* but not for *H. uvarum*.

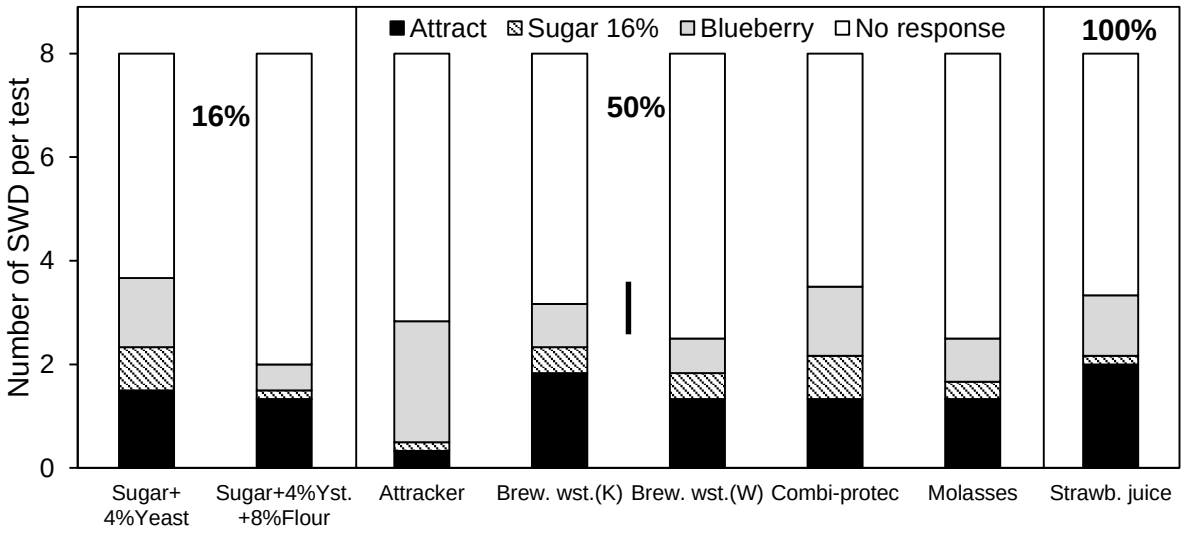


Figure 5.1.4. Petri dish experiment (b). Each column represents the movement or no response of *D. suzukii* introduced in pairs into Petri dishes containing droplets of the test substances or sugar solution. The tests were left for 30 minutes, before a blueberry was introduced for 30 minutes. Each value is the mean of six replicate tests; bar represents LSD (P = 0.05)

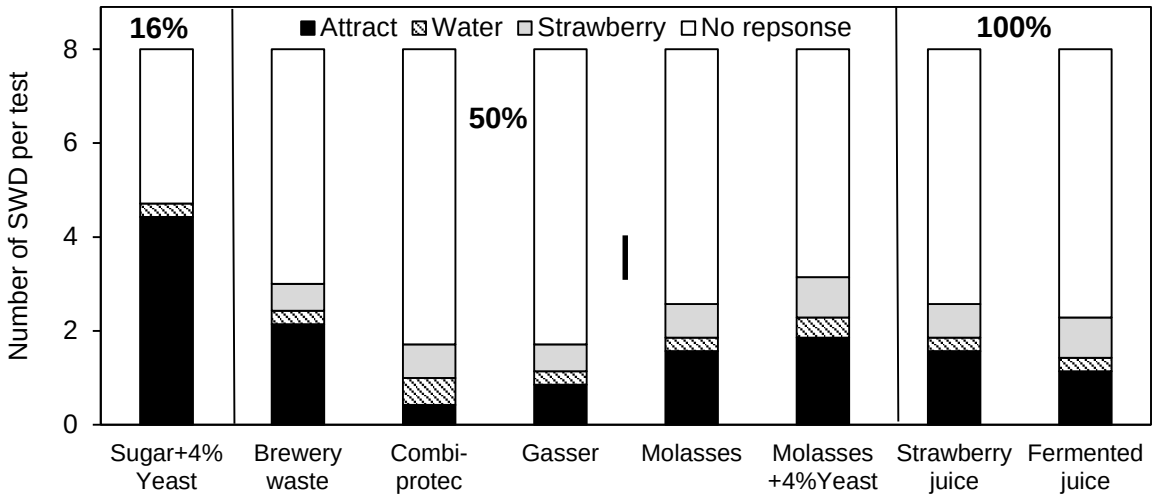


Figure 5.1.5. Petri dish experiment (c). Each column represents the movement or no response of *D. suzukii* introduced in pairs into Petri dishes containing droplets of the test substances or water placed on apple leaves. The tests were left for 30 minutes, before a strawberry piece was introduced for 30 minutes. Each value is the mean of seven replicate tests; bar represents LSD (P = 0.05)

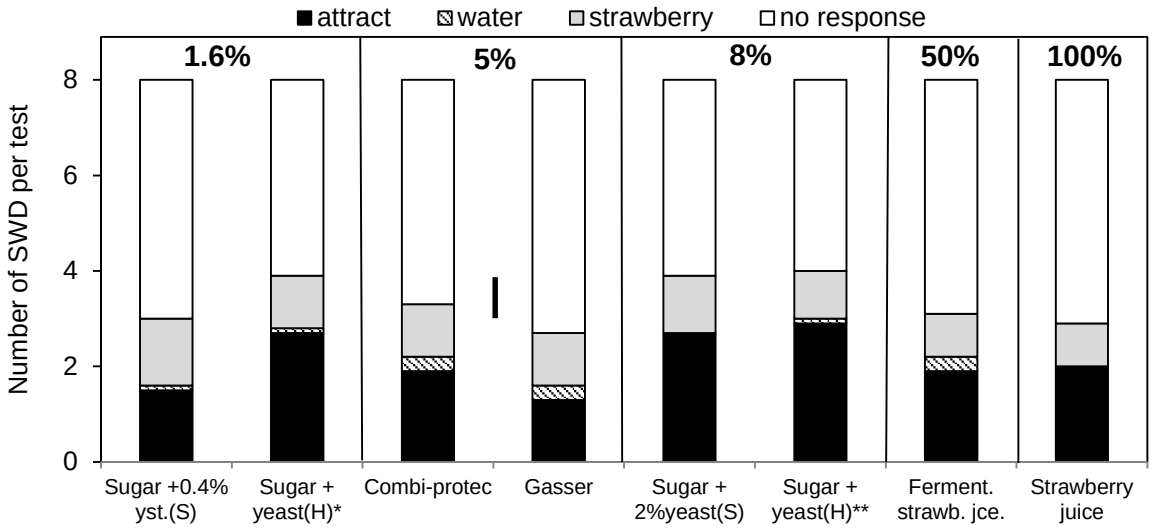


Figure 5.1.6. Petri dish experiment (d). Each column represents the movement or no response of *D. suzukii* introduced in pairs into Petri dishes containing droplets of the test substances or water placed on bramble leaves. A *Hanseniaspora uvarum* (yeast H) suspension was added so that the cell concentrations in the mixtures (* 2×10^8/ml and ** 1×10^9/ml) were the same as for *Saccharomyces cerevisiae* (yeast S) when added at 0.4% and 2% inoculum respectively. The tests were left for 30 minutes, before a strawberry piece was introduced for 30 minutes. Each value is the mean of eleven replicate tests; bar represents LSD (*P* = 0.05)

Large arena experiments: In large arena experiment (a), strawberry juice and 50% Gasser were the most attractive substances over 24 hours. Molasses and fermented strawberry juice at 50% dilution were significantly more attractive than the other test substances (Figure 5.1.7).

Large arena experiment (b) also showed that strawberry juice (100% fresh or 50% fermented) were the most attractive substances (Fig. 5.1.8). There was no significant difference between Combi-protec, Gasser or molasses at 5% dilution, or a suspension of sugar (80g/L) + *H. uvarum*. The brewery waste at 50% dilution, which was similar in attractiveness to *D. suzukii* as the baker's yeast and *H. uvarum* treatments, also had a similar yeast cell concentration (1.9×10^8 cells/ml^{-1}). There was no significant interaction between attractiveness of test substances and *D. suzukii* sex in the large arena experiments.

As well as different odours and volatiles, the different bait substances also differed in colour (Fig. 5.1.1). Red, purple and black are more attractive to *D. suzukii* than white or clear (Kirkpatrick et al. 2016). The attractiveness of some baits (e.g. strawberry juice) in the large arena test, may at least partly be due to colour.

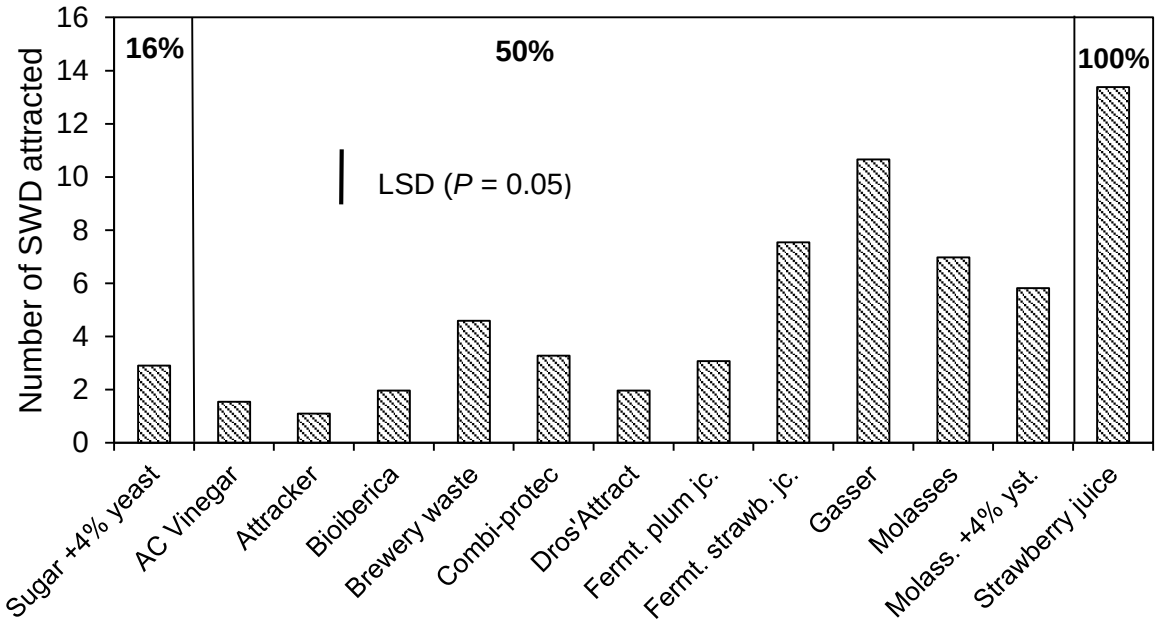


Figure 5.1.7. Numbers of *D. suzukii* attracted to different test substances and concentrations (%w/w) in large arena experiment (a). Each bar is the mean of four replicate test runs.

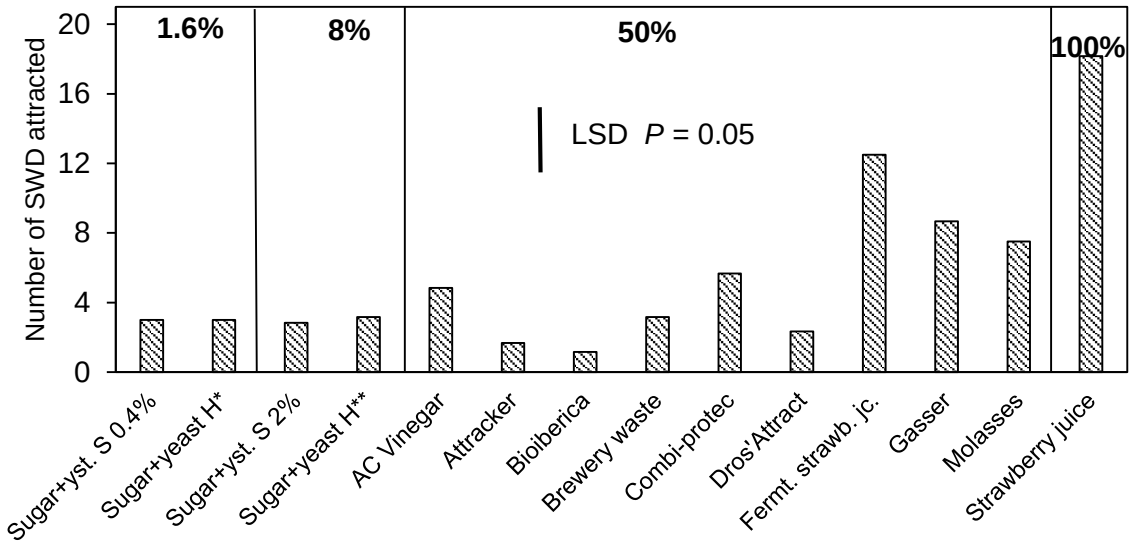


Figure 5.1.8. Numbers of *D. suzukii* attracted to different test substances in large arena experiment (b). Each bar is the mean of two replicate test runs. A *Hanseniaspora uvarum* (yeast H) suspension was added so that the cell concentrations in the mixtures (* 2×10^8/ml and **1×10^9/ml) were the same as for *Saccharomyces cerevisiae* (yeast S) when added at 0.4% and 2% inoculum respectively. Each bar is the mean of three replicate test runs.

Chronophysiology: Chronophysiology experiment (a) showed that strawberry juice (100%) and molasses (50%) were more attractive than and sugar (16%, with or without baker's yeast), Attracker, brewery waste and Combi-protec (50%) over 3 days (Figure 5.1.9).

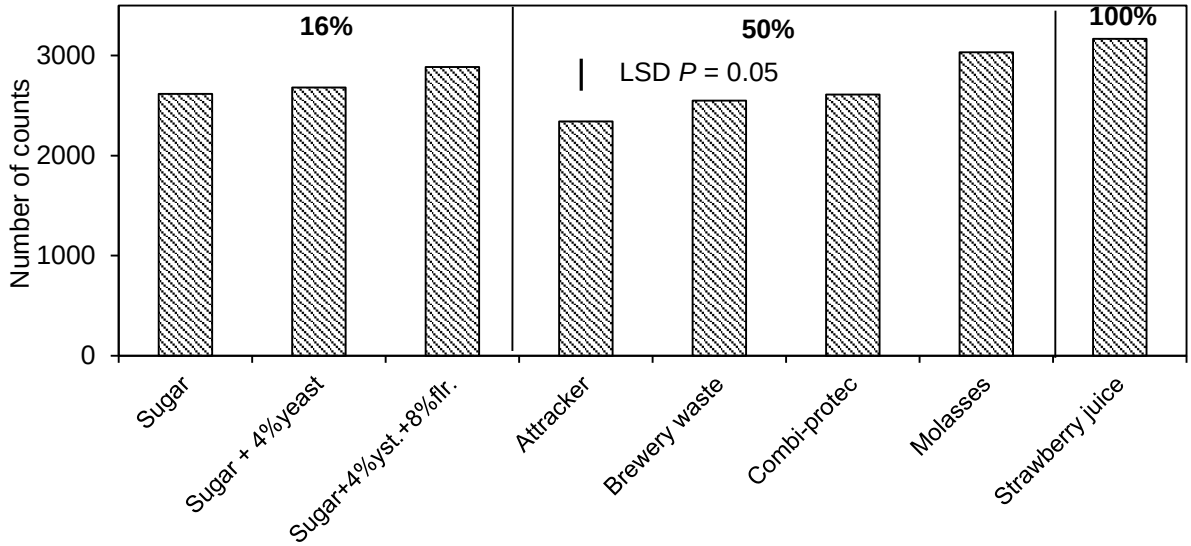


Figure 5.1.9. Number of *D. suzukii* counts in tubes containing test substances at different concentrations over a 3-day period in chronophysiology experiment (a). Each bar is the mean of four replicate tubes.

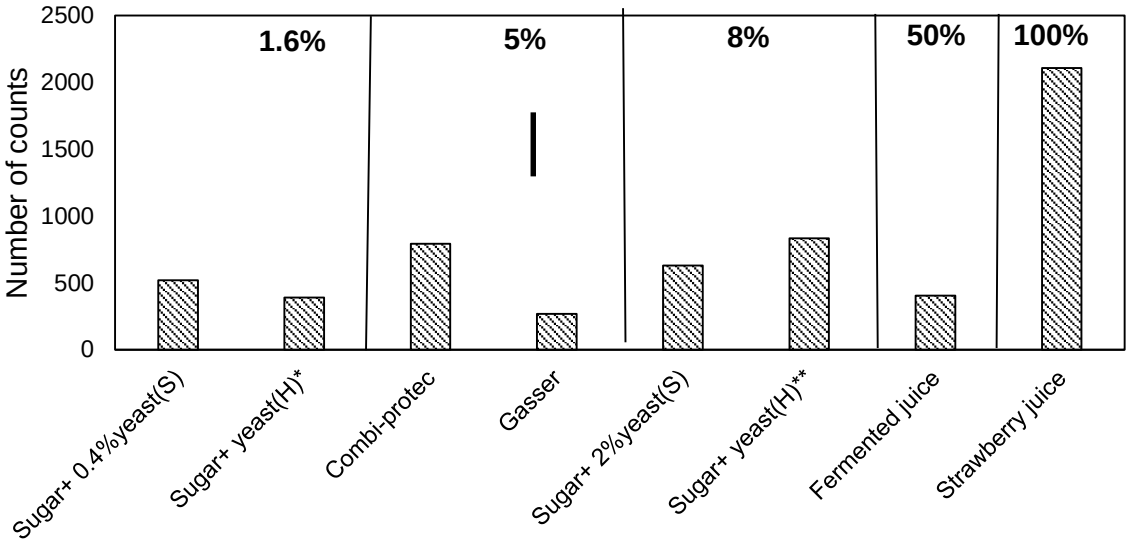


Figure 5.1.10. Number of *D. suzukii* counts in tubes containing test substances at different concentrations over a 3-day period in chronophysiology experiment (b). A *Hanseniaspora uvarum* (yeast H) suspension was added so that the cell concentrations in the mixtures (* 2×10^8/ml and ** 1×10^9/ml) were the same as for *Saccharomyces cerevisiae* (yeast S) when added at 0.4% and 2% inoculum respectively. Each bar is the mean of four replicate tubes.

Chronophysiology Experiment (b) showed that strawberry juice (100%) was more attractive than the other diluted substances or products tested (Fig. 5.1.10). Combi-protec (5%) and sugar (8%) + yeast H solutions were more attractive than Gasser (5%); there were no other significant differences between treatments.

Conclusions

A direct comparison of solid and undiluted substances such as sugar and molasses with equivalent concentrations of commercial products containing unknown solutes of unknown concentrations was not possible. However, the following conclusions provide an indication of the relative *D. suzukii* attractiveness of specific treatments (substances or products used at known dilutions with water). Due to the large number of available products, it was not possible to test all of the products with all three methods or some of the products at a range of concentrations (e.g. Dros'Attract was not tested in the Petri dish or chronophysiology bioassays). Some of the more promising treatments from different experiments have not been compared in the same experiment (e.g a 5% molasses solution from Petri dish experiment [a] was not compared with a 16% sugar + yeast (S) solution from experiment [c]). In the remaining part of the project it is planned to fill in some of these knowledge gaps.

- Some consistent differences in the relative attractiveness of substances and commercial products were recorded across different test methods:
  - Strawberry juice and 50% molasses or Combi-protec solutions were more attractive than a 50% Attracker solution in Petri dish experiment (b), large arena experiments (a) and (b) and chronophysiology experiment (a)
  - There was no significant difference in attractiveness of baker's yeast (*S. cerevisiae*) and *H. uvarum* when present at the same cell concentration in 8% sugar solution in any of the test methods.

- In other instances, the relative attractiveness of different substances and commercial products differed between test methods:
  - In Petri dish bioassays (b) and (c), there were no significant differences between Combi-protec and Gasser at 5% or 50%. However, in large arena experiments (a) and (b), a 50% Gasser solution was significantly more attractive than a 50% Combi-protec solution, whereas in chronophysiology test (b), Combi-protec was more attractive than Gasser at 5%
  - Brewery waste and fermented strawberry juice at 50% were less attractive than 100% fresh strawberry juice in both large arena experiments (a) and (b) and in chronophysiology Experiments (a and b respectively) but there was no significant difference between these treatments in Petri dish experiments (b) (c) and (d)
  - Sugar + yeast (S) solutions at 8-16% were more attractive than 50% Gasser in Petri dish Experiments (c) and (d) but less attractive in large arena experiments (a) and (b)
  - A 16% sugar + yeast (S) solution was at least as attractive as strawberry juice in Petri dish experiments (b) and (c) and in chronophysiology experiment (a) but was less attractive in large arena experiments (a) and (b)
  - A 16% sugar + yeast (S) solution was at least attractive as a 50% molasses solution in Petri dish experiments (b) and (c) but significantly less attractive in large arena experiments (a) and (b) and in chronophysiology experiment (a).

- The addition of bakers' yeast (*S. cerevisiae*) increased the attractiveness of a sugar solution in the Petri dish test but not in the chronophysiology test; a sugar + *S. cerevisiae* or *H. uvarum* suspension was not attractive in the large arena test.
- Reducing the concentration of sugar + yeast from 16% reduced its SWD attractiveness in the Petri dish and chronophysiology tests:
  - In Petri dish experiment (a), 0.36% solution was less attractive than strawberry juice and in Experiment (d), a 1.6% solution was less attractive than an 8% solution

- In chronophysiology test (b), an 8% solution was less attractive than strawberry juice.

- At 5 or 50% dilutions, none of the commercial products were consistently more attractive than all of the test substances (50% molasses or fermented strawberry juice and 16% sugar + 4% yeast) across the test methods used.
- After 12 hours, blue food dye was found in the gut of at least 95% of *D. suzukii* that were placed in the Petri dish droplet test
- No differences between male and female SWD were recorded in the Petri dish or large arena experiments; SWD gender was not recorded in the chronophysiology experiments.

Task 5.2. Determine compounds that repel D. suzukii and prevent egg laying.

Introduction

Potential repellents to deter *D. suzukii* laying eggs in fruits or discouraging adults entering the cropping area are being investigated, for example, 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 were reported to be useful in field tests (Dorsaz and Baroffio 2016; Wallingford et al 2016b).

Table 5.2.1 Table of potential repellents for *D. suzukii*

Potential repellent *	**Notes**
geosmin	Fungal metabolite (Wallingford et al 2016a)
1-octenol-3-ol	Fungal metabolite (Wallingford et al 2016a)
butyl anthranilate	DEET analogue (Pham & Ray 2015)
methyl N,N-dimethylanthranilate	DEET analogue (Pham & Ray 2015)
ethyl anthranilate	DEET analogue (Pham & Ray 2015)
hexyl butyrate	Strawberry HortLINK project. Repellent to Lygus
(*R)*-actinidine	*Leptopilina boulardi* odours (Ebrahim et al. 2015)
nepetalactol	*Leptopilina boulardi* odours (Ebrahim et al. 2015)
(-)-iridomyrmecine	*Leptopilina boulardi* odours (Ebrahim et al. 2015)
2016-091-G	IUK project
2016-091-F	IUK project
2016-091-F	IUK project

Pham and Ray (2015) reported that the DEET-replacements butyl anthranilate, ethyl anthranilate and methyl N,N-dimethylanthranilate deterred *D. suzukii* from egg-laying on grape juice in a laboratory bioassay and coating cherries with the first compound also reduced oviposition in the laboratory. Wallingford et al. (2016a) tested the fungal metabolites 1-octen-3-ol and geosmin and found both compounds reduced attraction of *D. suzukii* to fruit juice in a laboratory bioassay. The former compound also reduced oviposition by *D. suzukii* when dispensed in the middle of fruit clusters in the field (Wallingford et al., 2016a) and also from a SPLAT formulation in 2 m long plots of red raspberries over 4 d (Wallingford et al. 2016b). Ebrahim et al. (2015) showed that *D. suzukii* avoided sites visited by parasitic *Leptopilina*

wasps in laboratory bioassays, and reported the compounds responsible are (*R*)-actinidine, isomers of nepetalactol and (-)iridomyrmecine.

Essential oils are interesting as repellents for *D. suzukii* as many of these are registered for use in food and medical applications and so their registration as plant-protection products should be simplified. Erland et al. (2015) tested nine essential oils and the pure compounds 1,8-cineole, linalool and 3-carene for toxicity and oviposition deterrence towards *D. suzukii.* Linalool had good fumigant toxicity and 1,8-cineole good contact toxicity, but none of the oils or compounds deterred oviposition (Erland et al. 2015). Renkema et al. (2016) tested 12 essential oils for their ability to deter *D. suzukii* from feeding on raspberry juice in a laboratory bioassay and found peppermint oil was the most effective while potassium metabisulphite had no effect. Effects on oviposition were not tested.

NRI and NIAB EMR are partners in an Innovate UK project to investigate generalised repellents for agricultural pests. There were three key compounds from this project investigated.

Dorsaz and Baroffio (2016) tested lime as a physical barrier to oviposition by *D. suzukii*. Although there was some effect in laboratory tests, there was no useful effect in the field (Dorsaz and Baroffio 2016).

The aim of this task is to screen likely candidate compounds for their ability to prevent oviposition by *D. suzukii* on fruit in a field situation.

Materials and Methods

Two experiments were carried out in cherry orchards at NIAB EMR.

Experiment 1

In this experiment, repellents were placed next to host fruits in delta traps and the effect on oviposition by *D. suzukii* in the fruits was measured relative to fruits without the repellent.

Treatments were single compounds in polythene sachets or rubber septa (Table 5.2.2). Release rates were measured at NRI by weight loss under laboratory conditions at 22°C. A dispenser was hung inside the top of a red delta trap and containing a Petri dish of fruit (18-22 July, 6 raspberries and 2 strawberries; 25-28 July, 5 strawberries; 2-4 August, 6 raspberries per Petri dish). Delta traps were hung from a lower branch, approximately 50-100 cm from the ground, 5 trees apart in each row with a guard row in-between in ‘Rookery Field’. The experiment was begun on 18 July and there were 10 replicates of each treatment. For the final run of the test, newly manufactured devices were made and hung in the traps alongside the old devices to renew the release of repellents.

Experiment 2

The four most successful compounds from Experiment 1 were tested in cherry trees to determine their effect on oviposition by *D. suzukii* in fruit baits placed in the tree.

Compounds tested were geosmin, methyl anthranilate, 2016-091-G and 1-octen-3-ol (Table 5.2.3). Twenty sachets were suspended – dispersed evenly throughout each tree ('Deadman' Orchard). Ripening fruit was picked from a crop which had not received a spray for at least seven days (raspberry). Sachets were 5 cm x 5 cm rather than the half sized sachets used in the attract and kill devices. The geosmin was dispensed from a septa (Table 5.2.3). Treatments were placed into five rows with each tree as a plot. Plots were 10 trees (20 m) apart and each row was divided by three guard rows. Eight raspberries were placed in each petri dish, hung in a two delta traps in each tree. Fruit was deployed on 20 and 26 September and collected in on 23 and 29 September, respectively. There were five replicates of each treatment in a complete randomised design with an untreated control.

Assessments

Fruit was collected into ventilated boxes and incubated for two weeks. Numbers of emerging *D. suzukii* and other Drosophila were recorded.

Statistics

Data on *D. suzukii* counts was skewed and, therefore, SQRT transformed and analysed using ANOVA in GENSTAT.

Table 5.2.2. Potential repellents tested in Experiment 1

NRI Code	**Compound***	**Lure**
2016-091-A	Geosmin	4 mg septum
2016-091-B	Butyl anthranilate	100µl in sachet 25mm x 25mm x 120 µm
2016-091-C	Methyl anthranilate	100µl in sachet 25mm x 25mm x 120 µm
2016-091-D	Methyl N-methyl-anthranilate	100µl in sachet 25mm x 25mm x 120 µm
2016-091-E	Methyl N,N-dimethyl-anthranilate	100µl in sachet 25mm x 25mm x 120 µm
2016-091-F	2016-091-F	50µl each in sachet 25mm x 25mm x 120 µm
2016-091-G	2016-091-G	100µl in sachet 25mm x 25mm x 120 µm
2016-091-H	1-octen-3-ol	100µl in sachet 25mm x 25mm x 120 µm
2016-091-I	No compound	Sachet 25mm x 25mm x 120 µm

Table 5.2.3. Potential repellents tested in Experiment 2

NRI Code	compound*	Lure
2016-108-A	Geosmin	4 mg septum
2016-108-C	Methyl anthranilate	1ml in sachet 50mm x 50mm x 120µm
2016-108-G	2016-108-G	1ml in sachet. 50mm x 50mm x 250µm
2016-108-H	1-octen-3-ol	1ml in sachet 50mm x 50mm x 120µm
2016-108-I	No compound	Sachet 50mm x 50mm x 120µm

Results

Laboratory release rates of the seven compounds tested in the Test 1 demonstrated a rapid loss of methyl salicylate for the first 6 days. Other compounds were released more gradually with butyl anthranilate, methyl N,N-dimethyl anthranilate and 1-octen-3-ol released more slowly and consistently (Figure 5.2.1).

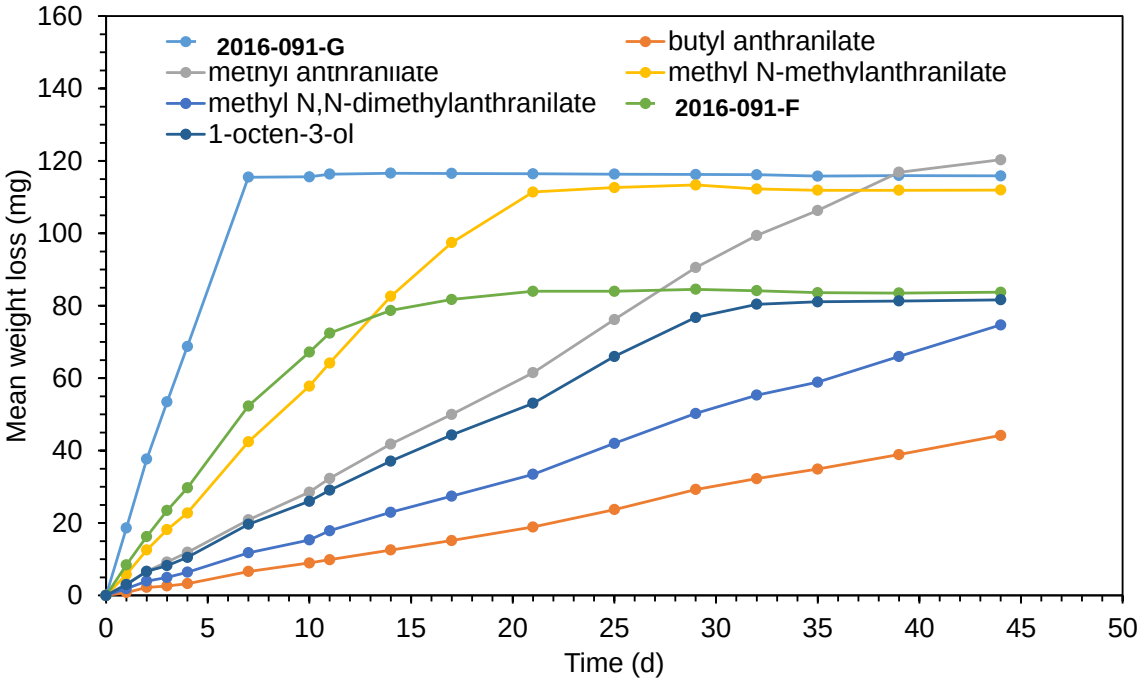


Figure 5.2.1. Release rates of candidate repellents (100 µl) from sachets at 22 °C as measured by weight loss (sachets 5 cm x 5 cm x 120 µ thick; mean of two)

Release of geosmin from the rubber septa lasted for about 10 days at 27 °C (Figure 5.2.2), so would have been much longer under field conditions. When geosmin was dispensed from a polyethylene vial, it took several days for the material to penetrate through the wall of the vial and the release rate then increased slowly, persisting for at least 30 d at 27 °C (Figure 5.2.2).

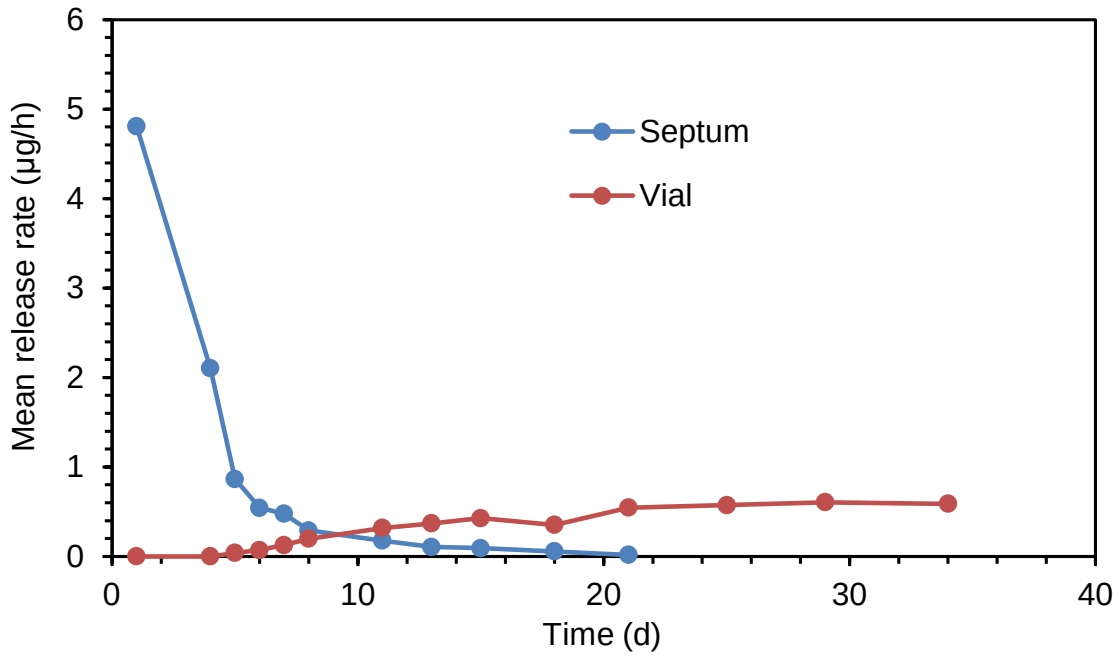


Figure 5.2.2. Release of geosmin (4 mg) from rubber septa or polyethylene vials at 27 °C as measured by collection of volatiles (mean of two replicates)

Experiment 1

In the first four days of the test with the lures inside the red delta traps adjacent to the fruit there was no significant difference in emergence of *D. suzukii* between the strawberry fruit and raspberry fruit and no interaction between fruit and treatment. Hence data were combined. Significantly fewer *D. suzukii* emerged from fruit adjacent to 2016-091-G, 1-octen-3-ol, methyl anthranilate and geosmin compared to the untreated control fruits (F=1.93$_{8,64}$, lsd=1.37, P=0.07). Other compounds did not significantly reduce *D. suzukii* egg laying compared to the untreated control (Figure 5.2.3). There was no significant effect on other Drosophila (data not shown).

By the second assessment, eight days after repellent deployment, 2016-091-G, 1-octen-3-ol, geosmin, methyl anthranilate and methyl N-methylanthranilate continued to repel *D. suzukii* from strawberry fruits, placed inside the delta traps, compared to the control (F=4.54$_{8,32}$, lsd=2.024, P=0.001, Figure 5.2.4). There was no significant effect on other Drosophila.

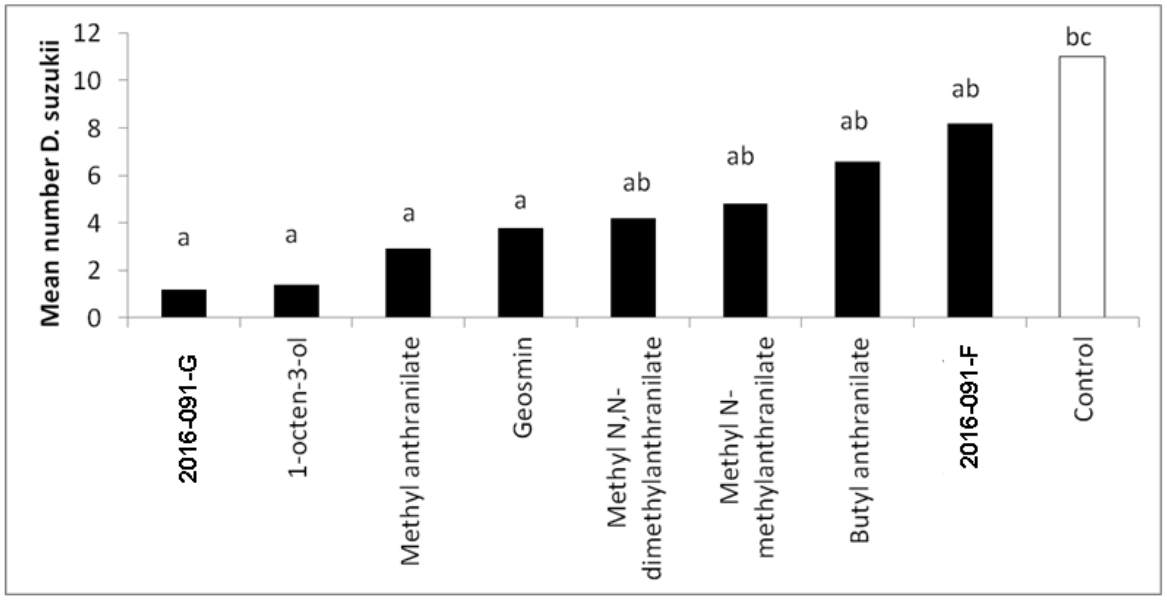


Figure 5.2.3. Mean numbers of *D. suzukii* emerging from fruit placed in red delta traps next to different compounds up to four days after deployment (18-22 July, six raspberries and two strawberries)

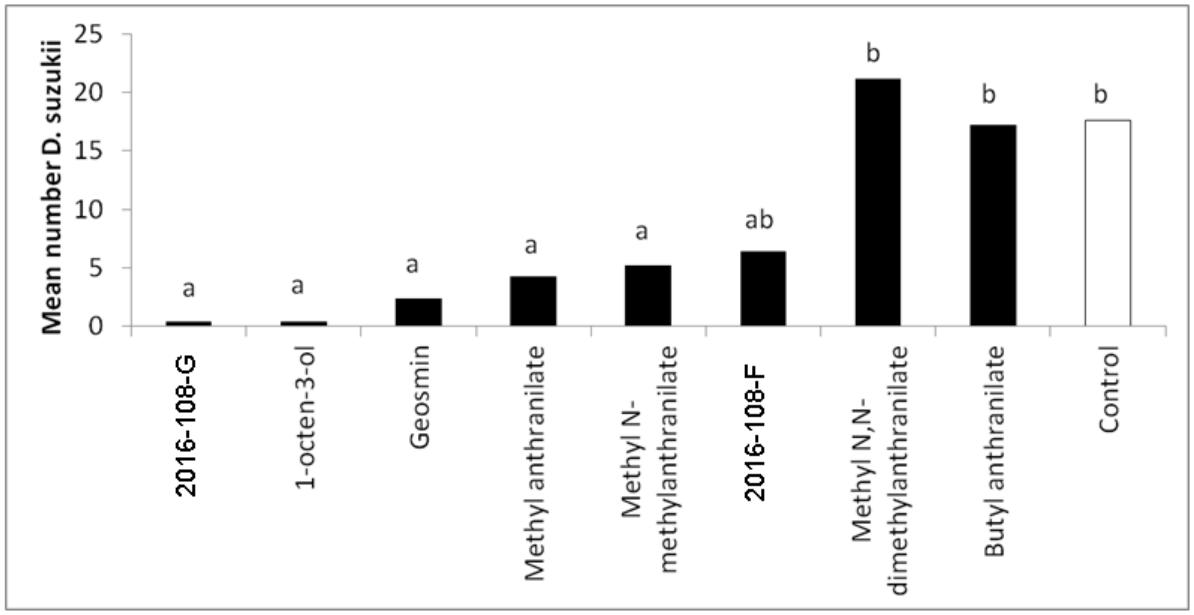


Figure 5.2.4. Mean numbers of *D. suzukii* emerging from fruit placed in red delta traps next to different compounds up to four days after deployment (25-28 July, five strawberries)

Because of the highly variable numbers of *D. suzukii* between plots on the third assessment, Day 15 (2-4 August), on raspberry, there were no significant effects of *D. suzukii* egg laying deterrent from any of the compounds (F=1.08$_{8,32}$, lsd=2.015, P=0.402). This was even though the dispensers had been renewed. However the mean numbers of *D suzukii* emerging from fruits treated with geosmin and methyl salicylate were approximately 5 compared to over 20 in the untreated control plots (data not shown).

Experiment 2

There were no significant differences in the numbers of adult *D. suzukii* that emerged between all treatments from the raspberries deployed in Week 1 and 2 (overall mean 19.6 per 8 fruit) in the test where geosmin, methyl anthranilate, 2016-108-G or 1-octen-3-ol were released via 20 point sources dispersed throughout a tree (20-23 September). In addition significantly more *D. suzukii* emerged in Week 2 (mean 29.1 per eight fruit) than in Week 1 (mean 10.0 per eight fruit) (F=34.32$_{,1,76}$, lsd=1.109, P<0.001, data not shown).

Conclusions

- Five tested compounds show promise for repelling *D. suzukii* away from fruits, namely; 2016-108-G, 1-octen-3-ol, methyl anthranilate, geosmin and methyl N-methylanthranilate. These compounds are worthy of further investigation.
- There was no significant effect on oviposition by other Drosophila.
- Repellent devices places from large point sources (sachets) in trees did not deter egg laying in fruits. However, it should be noted that the latter trail was done in the autumn when populations of *D. suzukii* were high and did not have alternative feeding or egg laying resources in the orchard, hence pest pressure would have been high.
- Further studies should investigate the early use of repellents in the spring before *D. suzukii* migrates from wild habitats and the use of smaller, higher numbers of point sources or a sprayable formulation.

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Knowledge and Technology Transfer

23 November 2016 – Fountain, Cannon - EMRA/AHDB Horticulture Soft Fruit Day

12-13 Jan 2017 – Fountain - Bioline AgroSciences – Paris. SWD research at NIAB EMR

16 Feb 2017 – Fountain - Soft Fruit Information Day, Winter Meeting, Spotted Wing Drosophila – an update on research in the UK

28 Feb 2016 – Fountain, Cannon - EMR Association/AHDB Horticulture Tree Fruit Day, Technical Up-Date on Tree Fruit Research, East Malling, Kent

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Annex 1. Milestones

Table showing original overview of progress against milestones for project as a whole. Please note that other work packages and objectives have also been funded and met during the course of this project.

Target date (31/03/2016)		No. of months from start date	Description of milestone	Progress
1	01/05/2014	13	Identify 12 commercial sites for task 1.1, secure grower cooperation, deploy traps	✓
2	31/03/2015	24	Report seasonal adult dynamics from 2014	✓
3	31/03/2016	36	Report seasonal adult dynamics from 2015	✓
4	31/03/2017	48	Report seasonal adult dynamics from 2016	✓
5	01/05/2014	13	Identify commercial sites for task 1.2, secure grower cooperation, deploy traps	✓
6	31/03/2015	24	Phenology and population dynamics of each life stage of *D. suzukii* and their changing spatial distributions determined for 2014	✓
7	31/03/2016	36	Phenology and population dynamics of each life stage of *D. suzukii* and their changing spatial distributions determined for 2015	✓
8	31/03/2017	48	Phenology and population dynamics of each life stage of *D. suzukii* and their changing spatial distributions determined for 2016	✓
9	31/03/2015	24	Common wild host plants of *D. suzukii* adults and larvae in the UK identified	✓

10	31/03/2016	48	SWD overwintering sites investigated and whether *D. suzukii* overwinters in UK fruit crops, including dead plant material and polytunnel structures determined	✓
11	31/03/2014	12	Seasonal soft and stone fruit waste types and quantities produced from different commercial scales established	✓
12	31/03/2014	12	Conditions needed for eradication of SWD, indicators and attractiveness to *D. suzukii* from fruit wastes established in bench-scale facilities	✓
13	31/03/2015	24	Large-scale methods for in-vessel composting, digestion and other processing of fruit wastes established and evaluated	✓
14	31/03/2015	24	Temporary storage conditions and facilities for soft fruit waste developed and evaluated	✓
15	31/03/2015	24	Attractiveness of treated soft fruit waste to *D. suzukii* and indicator *Drosophila* species tested	✓
16	31/03/2017	48	Collection and disposal optimised for different types and scales of fruit waste; sanitization and loss of attractiveness confirmed	✓
17	31/03/2017	48	Economics of treatment options for different types of fruit waste and scales of production quantified	✓
18	31/03/2017	48	Standard Operating Procedure and final report submitted	✓
19	31/03/14	12	Efficacy of detection and economic costs of different methods of quantifying larval infestations in different fruits	✓

20	31/03/16	36	Sampling methods for quantifying numbers of *D. suzukii* larvae in field crops and harvested fruit determined and protocols produced	✓
21	31/03/15	24	Synthetic lure for *D. suzukii* developed	✓
22	31/03/16	36	Target device and identify suitable insecticide(s) for attract and kill formulation developed	ongoing
23	31/03/17	48	Attract and kill treatment and methods of application in the field optimized and commercialisation initiated	ongoing
24	31/03/17	48	Efficacy of approved and emerging products against adults and other life stages in polytunnel protected crops evaluated	✓
25	31/03/14	12	Bioassay methodology for determining the susceptibility of adults to insecticides and baseline lethal concentration established	✓
26	31/03/17	48	Study on variation in susceptibility of *D. suzukii* populations to 3 insecticides in 3 successive years completed	✓

