Project title:	Understanding and developing methods for managing spotted wing drosophila (SWD) in the UK: Vital research to maintain the viability of the UK fruit industry
Project number:	SF145
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Previous report:	Years 1 and 2
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Location of project:	NIAB EMR
Industry Representative:	Marion Regan, Hugh Lowe Farms
Date project commenced:	01 April 2013
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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

Headline

- A greater understanding of the biology and control of SWD in the UK has been achieved with findings directly relevant to UK soft and stone fruit growers.

Background and expected deliverables

Spotted wing drosophila (*Drosophila suzukii*, SWD) is a new invasive pest to the UK, but has caused considerable losses in fruit crops in Europe and the USA. The overall aim of the project is to monitor the spread of *D. suzukii* within the UK and to develop measures for its control. To this end five objectives have been set for the project:

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.
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.
3. To develop and evaluate sampling and extraction methods for quantifying *D. suzukii* infestations in different soft and stone fruits.
4. To develop a synthetic lure and attract and kill technology for *D. suzukii* for incorporation into IPM programmes.
5. To obtain evidence for the effectiveness of different plant protection products including biopesticides to aid developing an insecticide resistance management strategy for SWD.

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 wild hosts and overwintering.

National monitoring

In 2015, the national monitoring of adult *D. suzukii* numbers was continued at a network of 15 sites across the UK using modified Biobest traps with Cha-Landolt bait: 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.

Numbers of *D. suzukii* caught were considerably higher in 2015 than in previous years. The largest catches were in the south east of England, but *D. suzukii* were found at all sites in 2014. In a similar pattern to previous years the numbers caught in crops peaked in August before declining and then rose again in late October, with adults migrating to woodlands and hedgerows where numbers remained high throughout November and December, reaching a peak of over 20,000 per trap per week at one site. It is estimated that numbers in the winter of 2015 are at least 3 times higher than at the same time in 2014, although the winter has been quite mild, probably resulting in a higher activity and trapping efficacy.

Habitat survey

The distribution of *D. suzukii* on two farms, including NIAB EMR was studied throughout the year. Over 50 traps were deployed on each farm in a range of crops and in neighbouring wild areas and woodlands. *D. suzukii* were recorded in the traps every week in 2015, in contrast to 2014 when for 5 weeks in the spring none were recorded.

The trap catch throughout 2015 showed a similar pattern to previous years. Activity increased in early spring from 14 April to 8 June, with more pronounced activity towards the end of the summer from 27 July until the winter. Numbers peaked in late autumn and winter with considerably higher catches than in 2014. Higher numbers of *D. suzukii* were caught in cherry orchards than in other crops surveyed. The number of *D. suzukii* decreased in the cherry orchards once all the leaves had fallen.

All reproductive stages were seen during the fruit growing season on both farms in 2015. *D. suzukii* from Farm 2 appeared to have a longer period of fertility than Farm 1 (April through to

November), a trend that was found in the previous year. This may be related to the later removal of polytunnels and/or the removal of raspberry waste at Farm 1.

Population modelling

A computer model was constructed using fertility studies, climatic data and biological parameters derived from this project and from published literature, to predict *D. suzukii* population changes. This model is being validated against population data collected during the course of this project and gives a good estimate of the time of first egg laying and the start of population growth. Future research should continue to optimise the programme, especially for population prediction later in the year.

Monitoring SWD larval infestations in early, mid and late season cherry varieties

In the first trial, seven commercial varieties of cherries were assessed covering early to late cropping. Brix (sugar content), hardness and *D. suzukii* emergence were monitored for each crop. In general there was a positive relationship between Brix and the softening of the cherry fruits, especially Sweetheart, Korvic and Merchant.

D. suzukii larvae were not found in the early variety Simone (picked first week of July) and although they emerged from the other early variety, Merchant, this was only after the optimal time for harvest had passed and the fruits were very soft. If no plant protection products were applied eggs could be laid in Sweetheart and Penny very early.

In a second survey of *D. suzukii* occurrence in four commercial orchards in Southern England, no *D. suzukii* emerged from fruits harvested between 14 May and 8 June, however small numbers were recorded after this time.

UK host plants for D. suzukii and overwintering sites.

Black Bryony and Yew berries were shown to be potential oviposition sites towards the end of the year. However, Cotoneaster, Snowberry, Guelder Rose, Dogwood, Hawthorn, Red Bryony and Rose do not appear to support *D. suzukii* development. All of these fruits are rather dry and fibrous. Raspberry cuttings and leaf litter did not appear to be significant sheltering sites for *D. suzukii* in the late autumn and winter in this limited study.

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.

Treatment in anaerobically sealed Dolav bins was shown to kill all *D. suzukii* in soft fruit waste, as long as the ambient temperature was over 18^{o}C. 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 16^{o}C ambient temperature.
Oxygen depletion was very rapid for each fruit type. It was 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 of 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.

Mixing treated waste with at least 90% (w/w) organic matter such as manure or slurry was shown to prevent re-inoculation, as was rotavation 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 not considered to be financially attractive due to the high moisture content and low calorific value of fruit wastes, along with transport and gate fee costs.

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

Low cost methods were trialled to detect *D. suzukii* larvae (both early and late stage) in samples of blueberries, cherries, raspberries and strawberries. The methods included immersion of crushed fruit in strong sugar or salt solutions, in a weak detergent solution or freezing whole fruit overnight. These methods were compared to emergence testing (keeping fruits in boxes at room temperature for 3 weeks and counting adult emergence) and dissecting the fruits open to count the numbers of larvae directly by hand. Sugar and salt immersion were the 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 and a standard protocol has been prepared in conjunction with AHDB Horticulture for growers. Training videos and training posters on how to undertake a flotation test using sugar solution have been published by AHDB Horticulture and are available on the dedicated SWD site of the AHDB Horticulture website.

Evaluating intra species competition for egg laying sites

D. suzukii appear to prefer to oviposit into media that has not been exposed to *D. melanogaster.* The latter do not seem affected by previous exposure to *D. suzukii* egg laying. This may be related to the niche that *D. suzukii* occupies; they do not need to compete with other *Drosophila* species for egg laying sites, whereas *D. melanogaster* need to compete for egg laying sites with other UK species. The absence of competition may also explain the relatively long development time of *D. suzukii*.

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

Testing commercially available and experimental baits

A comparison of various commercially available *D. suzukii* lures and traps was made, comparing *D. suzukii* catch, bycatch and ease of use. A detailed breakdown of the results is provided in the Science Section of this report. It was also found that topping up precision monitoring traps with DrosAttract rather than replacing the whole unit was as effective and cheaper, whilst producing less bycatch and waste. Chemical analysis of the successful commercial baits suggested that ethanol might not be as necessary as previously believed. In contrast, 3-Methylbutanol was released from all baits tested and might be a useful component of future lures.

Develop target device and identify suitable insecticide(s) for attract and kill formulation

An ‘attract and kill’ device is being developed in conjunction with the NRI. Miniature, dry versions of the Cha Landolt lures have been shown to be effective in attracting *D. suzukii* and deltamethrin (Decis) appears to be a good candidate choice for an insecticide. This work will be continued in 2016.

The evaluation of components of Cha-Landolt baits for the efficiency of trapping

Delivery systems for the components of the Cha Landolt trap system, ethanol, acetic acid, acetoin and methionol were assessed. Sachets for acetoin and methionol developed by NRI were found to be as effective as the vials previously used and much easier to use. However, ethanol and acetic acid were more effective in the drowning solution than in sachets or vials. This is being investigated further.

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.

Evaluate the efficacy of approved and emerging products against adults and other life stages in crops

In a replicated field experiment, with a population of *D. suzukii*, crop protection products were assessed for effectiveness of *D. suzukii* control on cherries. The efficacy of *D. suzukii* control varied with the plant protection product applied and time post spraying. Spinosad, lambda cyhalothrin, cyantraniliprole and a coded product gave good control over the duration of the study, whilst deltamethrin, acetamiprid and another coded product gave good initial protection, but by day 14 were beginning to lose effectiveness. In contrast, one application of lime and a pyrethrins mixture gave relatively poor control.

Evaluate the use of sugar as an adjuvant for enhancement of insecticide treatments in the control of D. suzukii

Sugar was investigated as a way of enhancing the effectiveness of plant protection products against *D. suzukii*. A literature review found sugar to be effective in a number of cases. In our trials, sugar significantly enhanced adult mortality from chlorantraniprole. However, no significant effect of sugar on adult mortality or subsequent emergence was found with spinosad, lambda cyhalothrin or deltamethrin.

Additional research

Efficacy of Jet 5 or Lime for the control of egg laying of Drosophila suzukii

Jet 5 and lime were assessed for usefulness in *D. suzukii* control. Blueberries were dipped in the treatments and adult flies were added. Direct mortality, egg laying and subsequent emergence of adults from the fruit were measured. Jet 5 caused mortality to flies added to the fruit, but this may be due to vapour action within the experimental arena. Lime reduced the number of flies emerging from treated fruit indicating a repellent effect on egg laying

Population dynamics of Drosophila suzukii in relation to other Drosophila species in the UK 2014-17

Six *Drosophila* species and the species group *Drosophila obscura* were regularly found in the national monitoring traps. These species may interact with *D. suzukii* via competition, the transmission of pathogens or spread of parasitoids. The commonest bycatch was the *D. obscura* group. These were found in all sites and all time periods. In terms of monitoring, bycatch of other *Drosophila* can slow assessment considerably and several species can look like *D. suzukii* to an inexperienced observer.

Financial benefits

D. suzukii poses a clear threat to the fruit industry and has had a commercial impact on UK grown fruit since 2014. Growers have reported significant financial losses in cherry and some soft fruit crops.

Action points for growers

- Monitor adults in susceptible crops and wild areas around crops from February onwards to predict the onset of egg laying by *D. suzukii*. Use a recommended trap and bait.

- Consider winter trapping and deploy perimeter trapping around vulnerable crops before fruit begins to ripen, potentially delaying movement of *D. suzukii* into the crop.

- Monitor for the presence of larvae in the crop. The flotation technique using sugar solution is recommended for rapid detection of larvae, but growers should consider emergence testing (boxes of fruit at room temperature) for early season detection.

- Crop hygiene is one of the best methods of population control. All waste fruit should be removed from the crop during and after harvest and be treated in sealed vessels and then disposed of responsibly by incorporating into the surface of field soils or mixed with organic matter such as manure or slurry.

- Consult BASIS trained advisers for the latest approvals for effective plant protection products and use the comprehensive information on SWD in the dedicated SWD site of the AHDB Horticulture website http://horticulture.ahdb.org.uk/.

SCIENCE SECTION

Objective 1. To determine the distribution and seasonal population dynamics of all life stages of SWD 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 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.

Materials and methods

Sites

Fourteen fruit farms selected in year one (2013) were used, with an additional farm that was added in the West Midlands from 2014 (Table 1.1.1). The distribution of the farms were as follows; five in Kent (including East Malling Research), one in Surrey, three in the West Midlands (Herefordshire and Staffordshire), two in Eastern England (Northamptonshire and Norfolk) and four in Scotland (including the James Hutton Institute). 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.

Table 1.1.1. Summary of fruit farms involves in the national monitoring survey (2015)

Region and crops	
South East England (46 traps)	
Farm 1	Raspberry, strawberry
Farm 2	Raspberry, strawberry
Farm 3	Cherry
Farm 4	Raspberry
Farm 5	Cherry, strawberry, plum, grape
Farm 6	Blueberry, redcurrant, strawberry
Eastern England (20 traps)	
Farm 7	Blueberry, raspberry
Farm 8	Raspberry, strawberry
West Midlands (27 traps)	
Farm 9	Blackberry, blueberry, raspberry, redcurrant, strawberry
Farm 10	Blueberry, cherry, raspberry, strawberry
Farm 10a	Cherry, raspberry
Scotland (40 traps)	
Farm 11	Blackcurrant, blueberry, raspberry, strawberry
Farm 12	Blueberry, cherry, strawberry
Farm 13	Blackberry, blueberry, raspberry, strawberry
Farm 14	Blackberry, blueberry, raspberry, strawberry

D. suzukii traps

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 wooded areas 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 EMR on 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*. Flies were caught in a drowning solution, which also 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. In strawberry fields, traps were hung off the ground to prevent slugs entering the traps, but low enough so that they passed under the sprayer.

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

Results

The results for England in 2015 are summarised in Fig. 1.1.1, in comparison to 2013 and 2014. The results for mid-February to mid-August are shown in more detail in Fig. 1.1.2.

Overall, numbers in January 2015 were far higher than the previous year, before falling to low levels in early February. Catches of *D. suzukii* adults continued at low levels, although higher than 2014, until early August, at which point catches increased rapidly. This increase corresponded closely to the pattern for 2014, including a temporary drop in September, but from September onwards numbers in 2015 were considerably higher than the previous year.

There was considerable variation between sites within the UK regions, but some trends are evident (Fig. 1.1.3). As in 2014, the largest catches were in the south east of England. Here adult *D. suzukii* were caught in woodland areas in January and before falling to very low numbers in all areas and habitats in early-February. In March, April, May and June only low numbers of *D. suzukii* appeared in the traps, though there was evidence of a slight peak in woodland traps in March. The numbers caught in crops rose to a peak in August, before falling and then rising again in September. At the time of writing (early November) numbers are still increasing.

Catches in traps in the East of England and the West Midlands remain, on average, low compared to the South East. After January, almost no adult *D. suzukii* were caught until August. From then on, trap catches in both regions followed a similar pattern to the South East until October, though at much lower numbers and without a discernible peak in August. From October onwards numbers declined.

No *D. suzukii* had been caught in the National Monitoring scheme in Scotland in 2013, and the first were detected at the end of July 2014. Numbers in 2015 remained very low compared to the rest of the country, although catches were starting to increase in October.

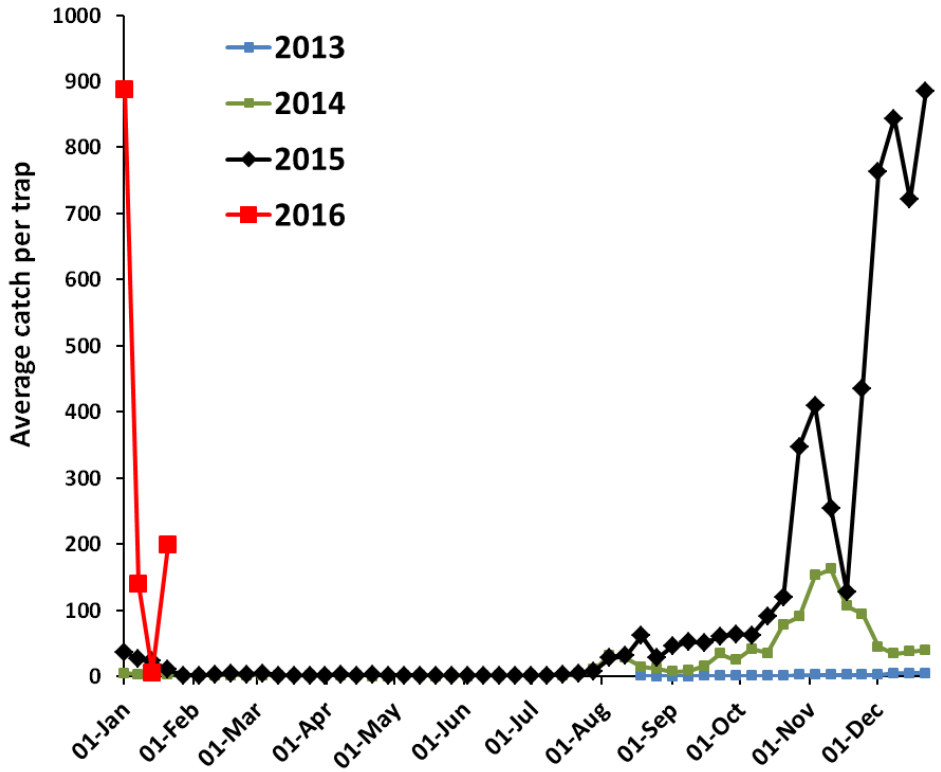

Figure 1.1.1. Comparison of average adult *D. suzukii* catch per trap in 2013, 2014, 2015 and 2016.

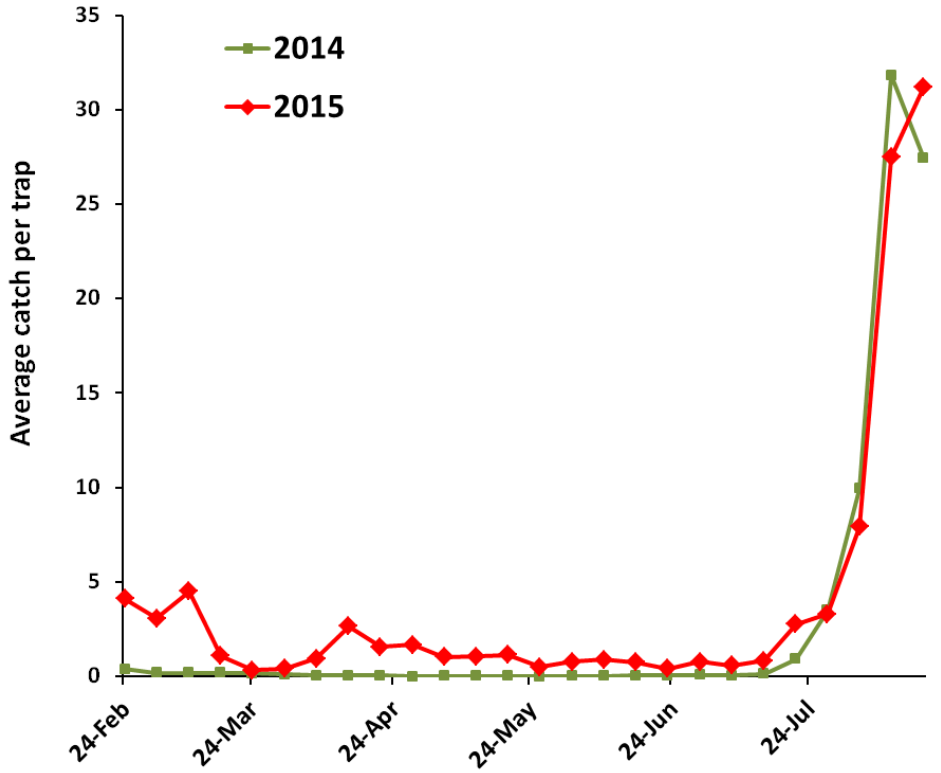

Figure 1.1.2. Comparison of average adult *D. suzukii* catch per trap from mid-February to mid-August 2014 and 2015

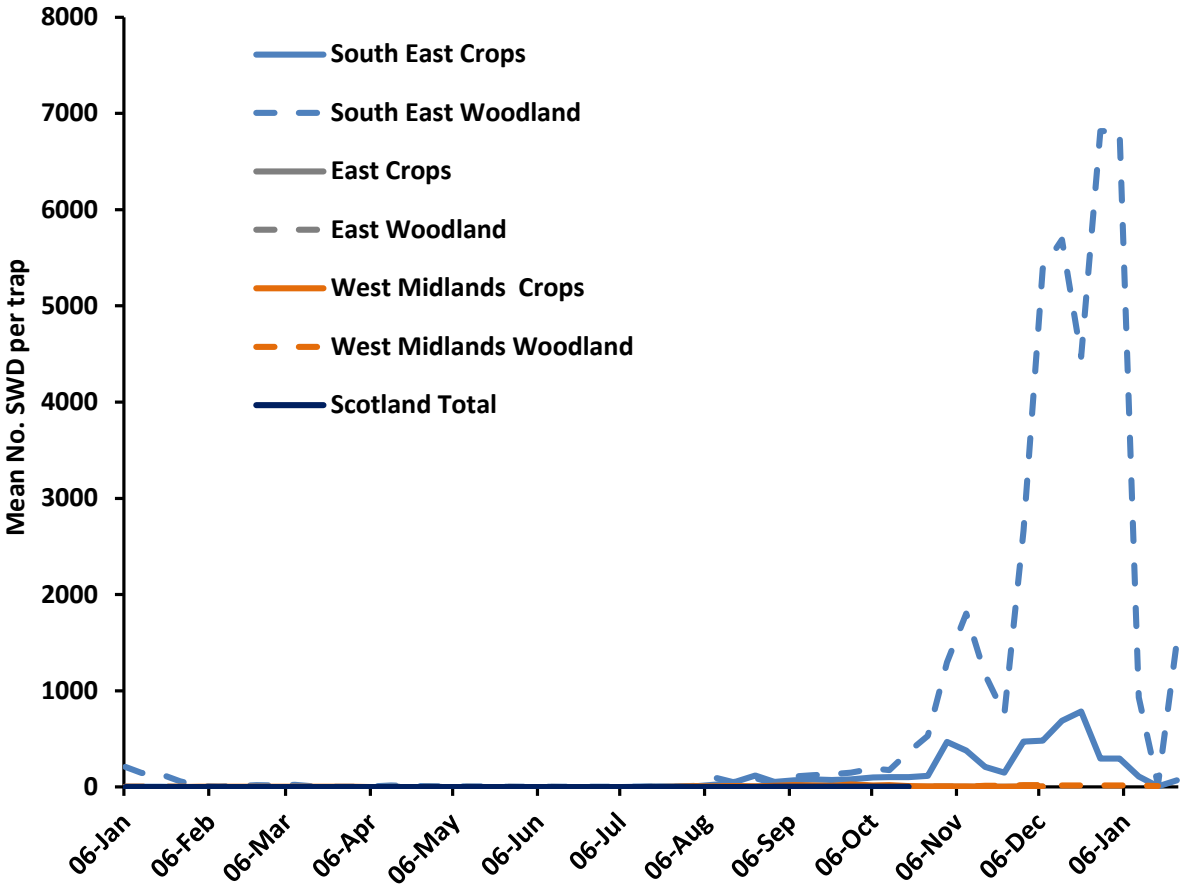

Figure 1.1.3. Mean numbers of *D. suzukii* adults per trap in the main habitat types and regions during 2015

Discussion

Catches of *D. suzukii* were relatively high in January, at least in the South East. This may be a consequence of the warm November and December the previous year and populations rising over time since introduction and becoming more established (Fig. 1.1.4). It could also be a consequence of higher woodland cover and hence a greater ability to overwinter in the South East (Fig. 1.1.5).

As in 2014, catches of adult *D. suzukii* in the traps were very low in the UK until late July. It was anticipated, given the higher overwintering population and geographical spread in 2015, that numbers in the spring and summer might be very high. However, because traps are in competition with fruits during the summer the traps are probably not representative of 'real' in-field populations.

There was a sharp increase in the numbers caught in woodland in the late autumn of 2015. This pattern was also found in 2013 and 2014, as well as other temperate regions at the northern limits of the *D. suzukii* range, and presumably reflects a migration to more sheltered areas. The numbers

trapped were at least 3 times higher than the previous year. In one trap, in one week, in a woodland, at one farm there were 15,000 *D. suzukii* in December 2015.

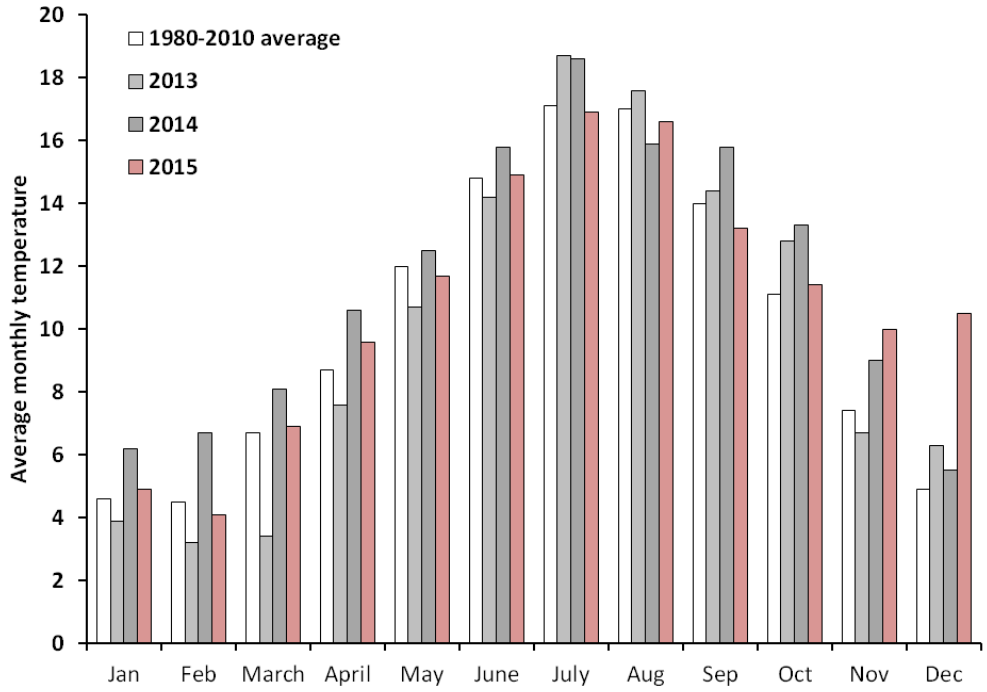

Figure 1.1.4. Comparison of the weather in the South East and Central South region of the UK in 2013, 2014 and 2015 with the 1980-2010 average (Met Office, UK)

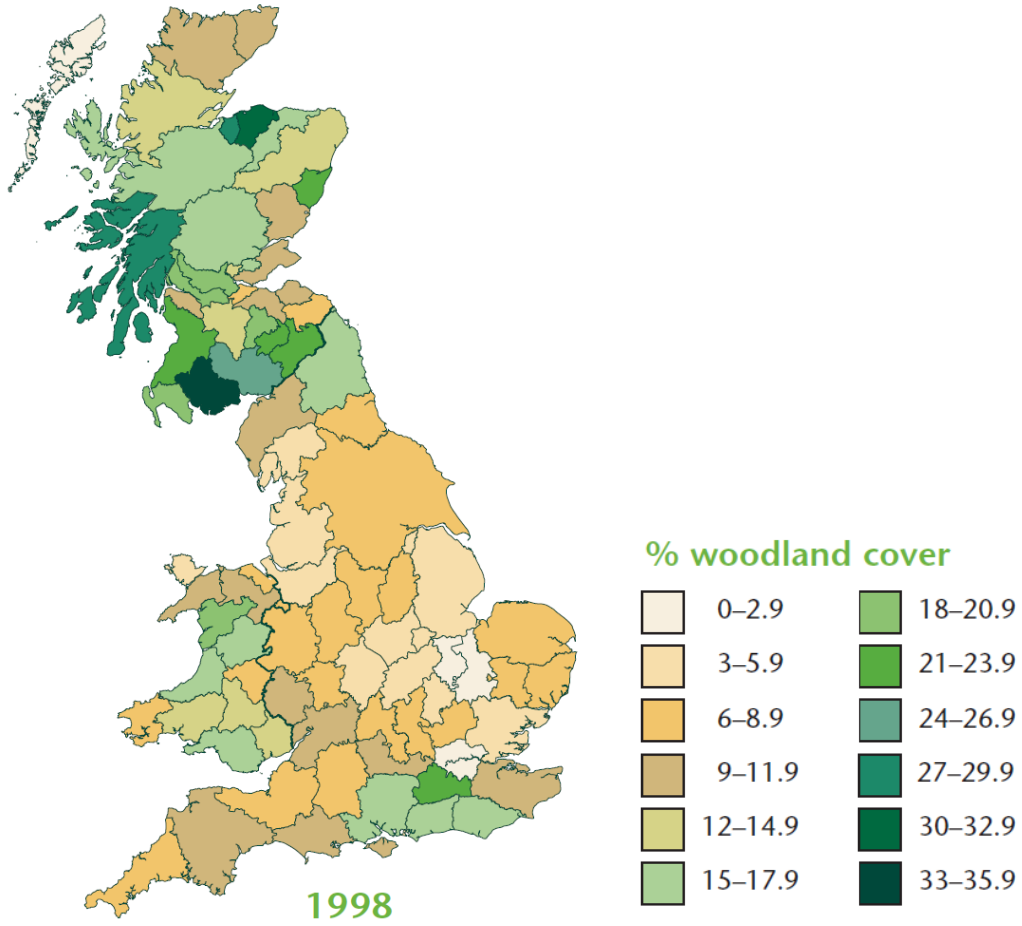

Figure 1.1.5. Percentage woodland cover (1998) from;
http://www.forestry.gov.uk/pdf/nigreatbritain.pdf/$FILE/nigreatbritain.pdf

Summary

- Numbers of adult *D. suzukii* caught in traps were higher in 2015 than previous years
- All trap sites caught *D. suzukii*
- Largest numbers were caught in late autumn and winter, especially in woodland areas.

Task 1.2. Determine the phenology, population dynamics and spatial distributions of SWD on two fruit farms in SE England, including one polytunnel cherry, raspberry and strawberry crop, throughout the year for four successive years (EMR; 1-4).

Materials and Methods

Adult trapping

Commercial polytunnel crops (cherry, raspberry and strawberry) were assessed at two farms in Kent. In addition, a variety of surrounding habitats were included for analysis including woodland, hedgerow and compost heaps. 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. The strawberry crop was removed in October 2014 resulting in the traps being moved to a strawberry crop on Farm 1. In 2015, the number of traps were reduced to twenty three as key areas of interest had been identified, reducing the need for duplicate locations. Traps were removed from wasteland and pear and apple orchards leaving the sites described in Table 1.2.1. 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 (Fig. 1.2.1). Each location was allocated two traps which were spaced 10 metres apart.

Figure 1.2.1. Modified Drosotraps with synthetic bait

Trap assessments were completed weekly from February until December in 2015. Trap contents were taken back to the laboratory to be assessed, where male and female *D. suzukii* were counted plus any other *Drosophila* trapped in the solution. From each trap two male and two female *D. suzukii* were kept and stored in 70% alcohol for further analysis.

Table 1.2.1. Numbered pairs of traps at Farm 1 and 2 and associated habitat.

Farm 1		Farm 2	
Trap pair no.	Habitat	Trap pair no.	Habitat
1	Cherry orchard	113	Raspberry
2	Cherry orchard	114	Raspberry
4	Woodland	115	Raspberry
5	Cherry orchard	116	Woodland
9	Cherry orchard	118	Woodland
10	Cherry orchard	119	Raspberry
13	Cherry orchard	127	Raspberry
14	Cherry orchard	128	Raspberry
16	Hedgerow		
19	Strawberry		
24	Compost heap		
25	Strawberry		
26	Strawberry		
27	Strawberry		
28	Strawberry		

Habitat assessments

Trap catches were assessed in groups defined by habitat type. Records of plant species diversity and abundance were taken from the areas surrounding the paired traps. Abundance was calculated using the Total Estimate Scale (Table 1.2.2). The growth stage of each crop was recorded using definitions published by the European and Mediterranean Plant Protection Organisation (EPPO). Wild fruits were assessed by observing percentage of ripeness, divided into under ripe, ripe and over ripe.

Results from the adult trapping were correlated with their habitat type. Data was correlated weekly. Monthly habitat assessments recorded the presence of wild and cropping fruiting plant abundance and growth stage.

Table 1.2.2. The Total Estimate Scale

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

Fruit samples

Samples of fruit were taken weekly from both the edges and centre of each focus crop, from the green fruit stage until the end of fruiting. Within each soft fruit crop 50 fruit were sampled between the pair of traps. Fruits picked were low in the canopy (raspberry) and overripe or damaged looking (where possible). For the cherries, a sample was taken from each of the six main cherry varieties (confidential), adjacent to where the traps were placed. Fruit samples were put in ventilated Perspex boxes at 26°C for three weeks with 16:8 h light: dark regime. The boxes were checked twice weekly for three weeks to test for the presence of *D. suzukii* adults (emergence testing).

An additional ten cherries were sampled from each crop and measurements of colour (comparison with Ctfil colour chart), hardness/softness (measurements with penetrometer/durometer) and sugar content (refractometer measurements of °Brix) made weekly from June until the end of harvest in July.

Fruit samples were checked weekly for the presence of *D. suzukii* adults (emergence testing). Flies that did emerge were removed and counted.

Reproductive stage of females

Trapped female *D. suzukii* were stored in 70% alcohol and refrigerated. Females were dissected under a dissecting microscope with light source. Measurements of the body length, and wing length and depth were taken using a graticule within the eye piece. General observations of the colouration and banding depth were recorded using a similar system to that of G. Petavy *et al.* (2002) which looks at the number of darkened tergites. The abdomens of the females were removed by grasping the thorax in one set of forceps and the ovipositor in another and pulling apart.

Assessments of females were made weekly for those dates in which females were collected from the two farms in the habitat assessments. Five females per sample were randomly taken and floated in 70% alcohol within a petri dish. The state of reproductive maturity is derived from a visual assessment of ovary and egg development using stage definitions published by Beverly S. Gerdeman, Washington State University.

Results

Trapping

Trapping continued through the winter of 2014/15. *D. suzukii* were recorded in the traps every week of 2015. The previous year in comparison had five weeks where no *D. suzukii* were recorded in the traps. Farm 1 had three weeks (07/02/14, 07/03/14 &13/06/14) and Farm 2 had two weeks (14/02/14 & 06/06/14) where the traps were completely devoid of *D. suzukii* (Fig 1.2.2). However, in 2014 and 2015 trap catches show a distinct pattern whereby the number of *D. suzukii* are considerably less at the beginning of the year, suggesting that the population of *D. suzukii* active at this time is most likely in their overwintering forms laying the first generation eggs for when habitat conditions are more favourable (Fig 1.2.2).

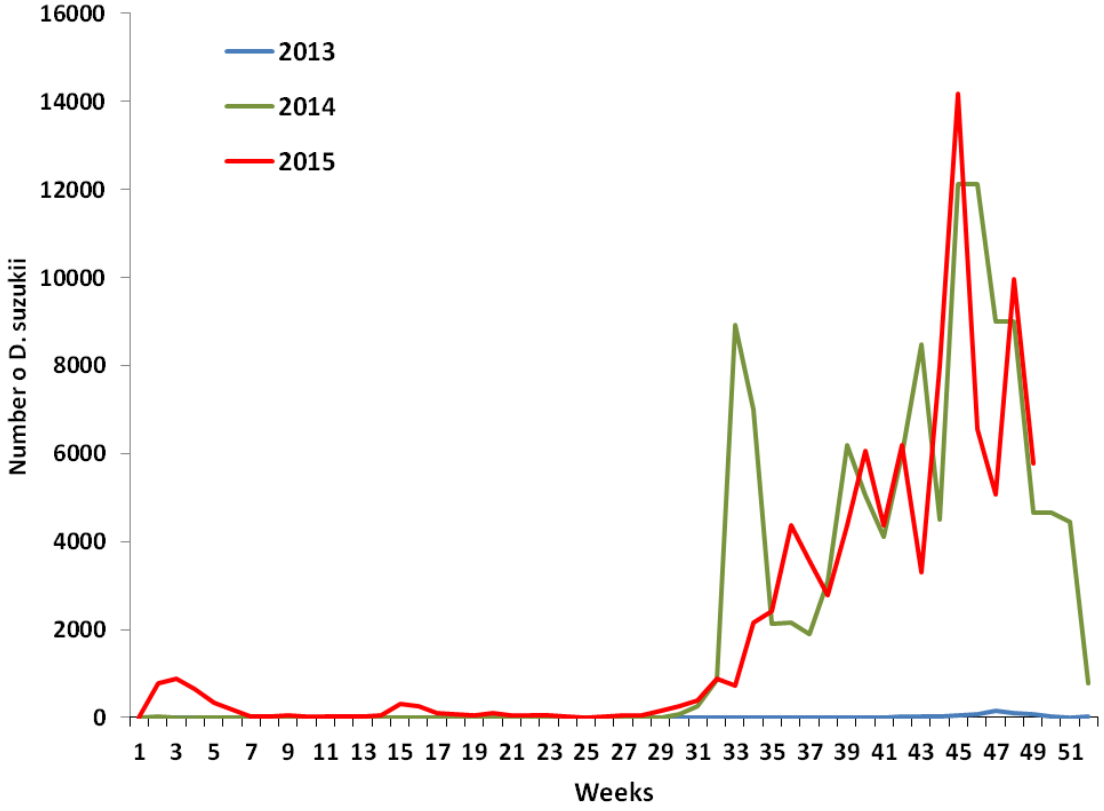

Figure 1.2.2. Adult *D. suzukii* trap catches at farms 1 and 2 in 2013, 2014 and 2015.

The rapid increase in activity between week 31 and 35 observed in 2014 did not occur in 2015. This could be due to improvements in the spray programmes leading to delayed capture. However effectiveness of the spray programme or perhaps the reduction in fruit availability increased the desirability of the traps leading to an increase in *D. suzukii* from August onwards. Numbers peaked at the beginning of November, resulting in a higher trap catches than in 2014 (Fig. 1.2.2).

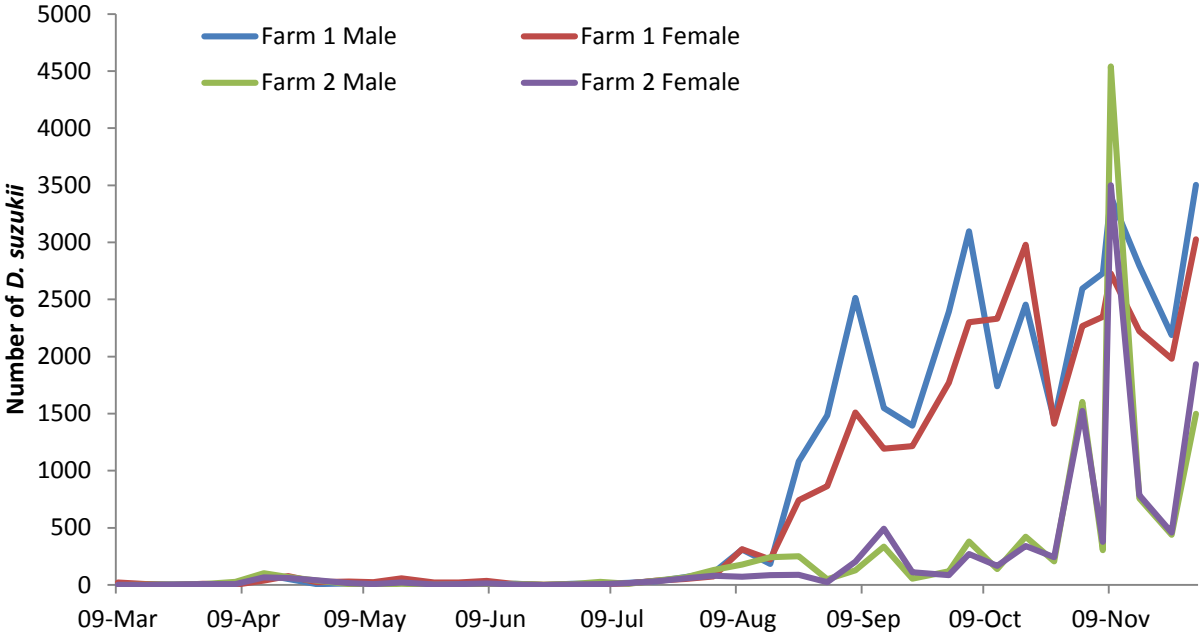

Figure 1.2.3. Number of male and female *D. suzukii* trap catches at farms 1 and 2 in 2015.

The number of *D. suzukii* in both Farm 1 and 2 followed a similar activity pattern with the first signs of activity found at the beginning of the year. This activity increased in early spring from 14 April - 8 June. More pronounced activity occurred towards the end of the summer from 27 July and this continued into the winter (Fig. 1.2.3). The peak of activity at both farms occurred at the beginning of November. The larger winter morphs of *D. suzukii* were still found in May.

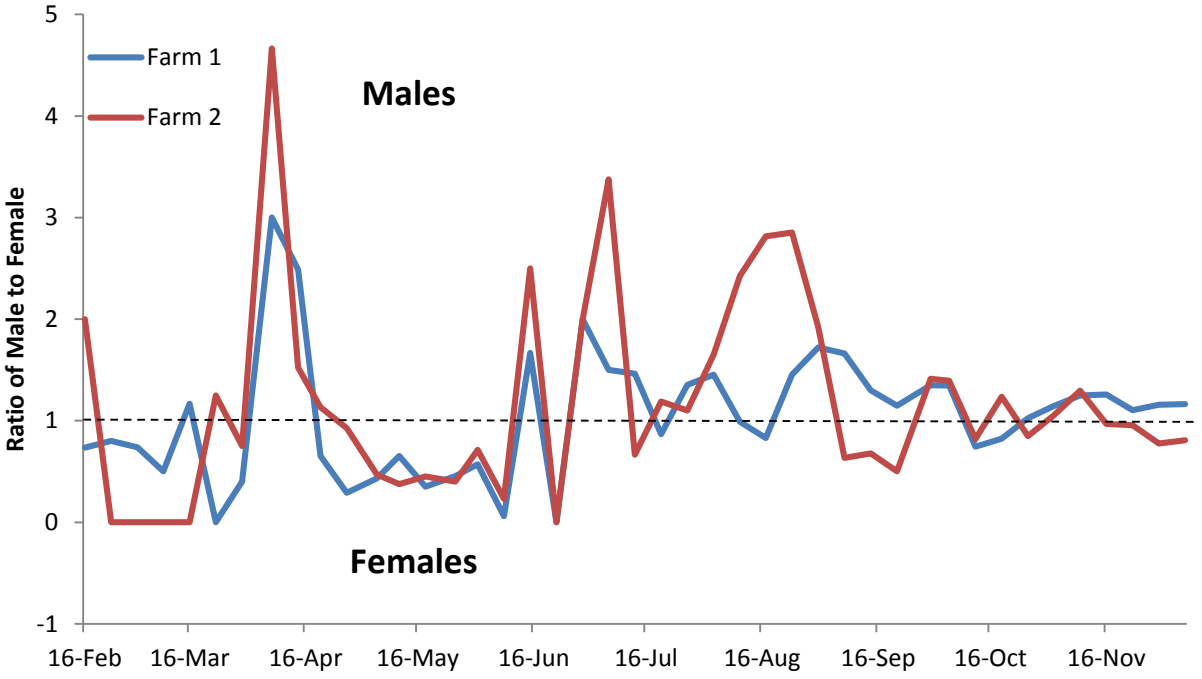

Figure 1.2.4. Ratio of adult male to female *D. suzukii* collected from Farm 1 and Farm 2.

The combined ratio of males to females in 2015 for both farms was 1.14 male to female, comparable to the ratio of 1.13 found last year. When analysed individually the numbers remain fairly similar, with ratios of 1.15 for Farm 1 and 1.09 for Farm 2 (Fig. 1.2.4). Therefore, males were to some extent more prevalent overall in the current trapping devices.

In 2014, there were generally higher trap catches of males in the winter months. In addition, males were captured first in both 2013 (13 August for males compared to 17 September for females) and 2014 (in 2014 males Farm 1 on 18 February compared to females on 25 February). In 2015 this trend continued with more males being caught in the winter and early spring (Fig. 1.2.4). The increased proportion of females to males in the first week of April coincided with the appearance of the first fecund females (Fig. 1.2.11).

Habitat assessments

D. suzukii were caught in all trap locations within Farm 1 and 2 in 2015 (Fig. 1.2.5 & 1.2.6). The highest numbers of *D. suzukii* were found within the woodland areas on both farms. Numbers were highest from mid-September and peaked in November but woodland was also shown to be the preferred habitat in the spring, from late March until mid-May.

On the crops that were sampled, much higher numbers of *D. suzukii* were caught in the cherry orchard. In the cherry orchard the ratio of male to female *D. suzukii* showed a preference towards the males. Numbers of *D. suzukii* increased from 24 August and peaked 9 September (Fig. 1.2.5). On the 5 October numbers of *D. suzukii* caught in the traps in the cherry orchard were overtaken by those in the woodland habitat and increased exponentially from the beginning of November and peaked on 9 November. The number of *D. suzukii* decreased in the cherry orchards once all the leaves had fallen.

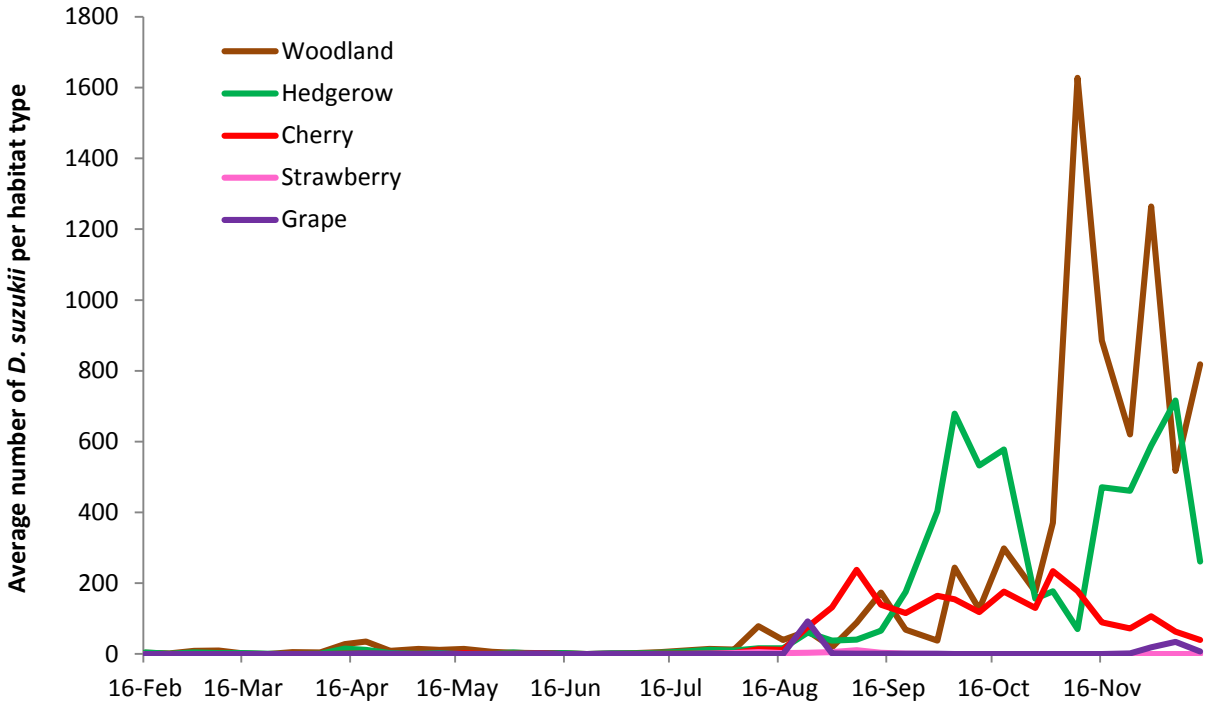

Figure 1.2.5. Mean numbers of *D. suzukii* captured per habitat type at Farm 1.

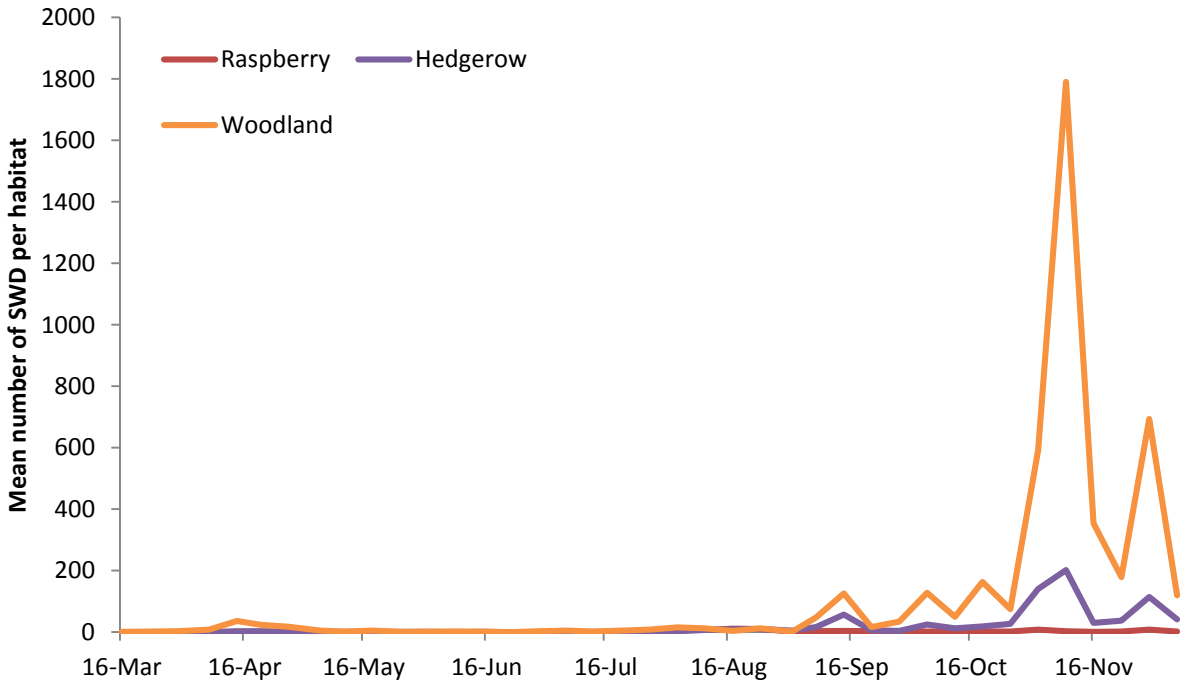

Figure 1.2.6. Mean numbers of *D. suzukii* captured per habitat type at Farm 2.

Other drosophila

The combined ratio of *D. suzukii* to other drosophila on both farms was 0.46. However, at the peak of SWD activity on Farm 2 the ratio climbed to 5.48 *D. suzukii* to other drosophila, so that *D. suzukii* were the dominant catch (Fig. 1.2.7).

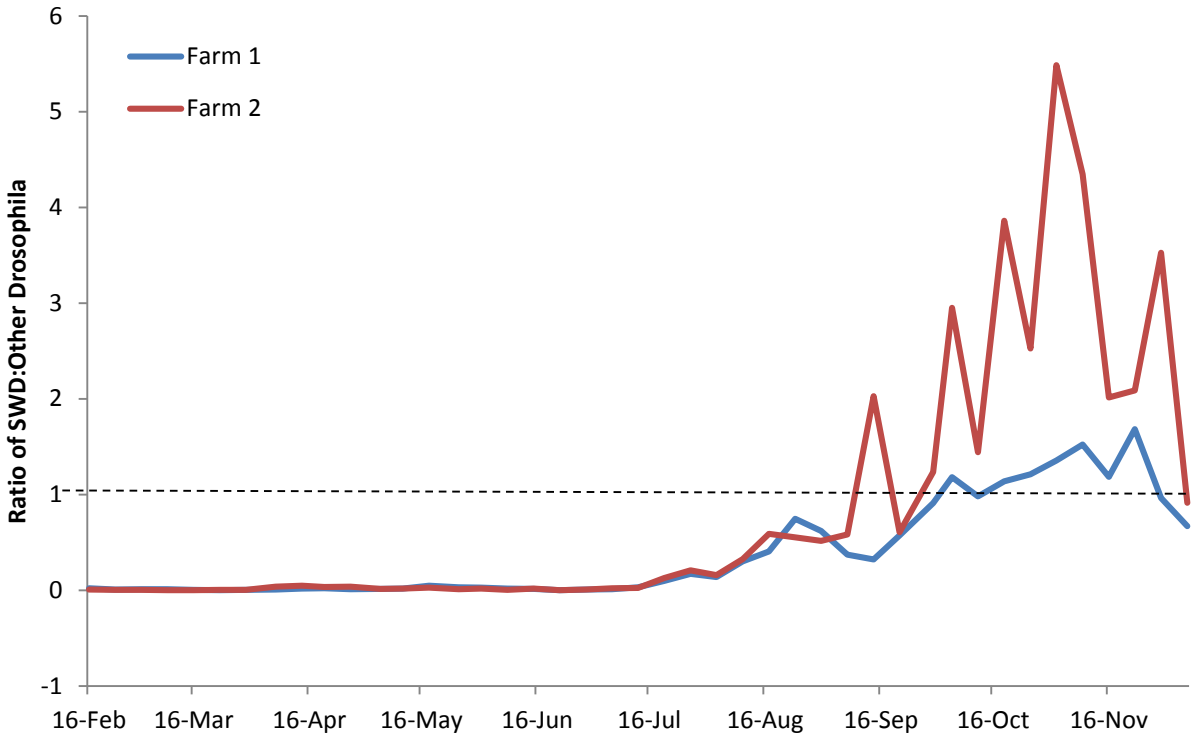

Figure. 1.2.7. Ratio of *D. suzukii* to other drosophila

Reproductive stage of females

Measurements of body length and wing length were taken for all individuals at both farms (Fig 1.2.8 and Fig 1.2.9). At both Farms 1 and 2 the average body length remained high up until May. However, throughout the summer months the average body length decreased on both farms. This corresponded with a reduced average wing length on both farms from May onwards. This reduced size was maintained up until the most recent sampling date (02 November 2015). In 2014, a similar trend was observed in the average body length on Farm 1. However, at Farm 2 the size increased throughout 2014. All reproductive stages were seen during the growing season on both farms in 2015 (Fig 1.2.10 and 1.2.11). The period of greatest reproduction (egg laying) was between April and October. This suggests a months delay in egg laying compared to the previous year (March and September).

D. suzukii from Farm 2 appeared to have a longer period of fecundity than Farm 1 (April through to November), a trend also found in the previous years results. At Farm 1 the polytunnels on cropping areas were removed on 10th August 2015, whereas the polytunnels at Farm 2 continue to remain in place. Furthermore, at Farm 2 waste raspberry fruits had not been removed. This may explain the continued fecundity late into the season.

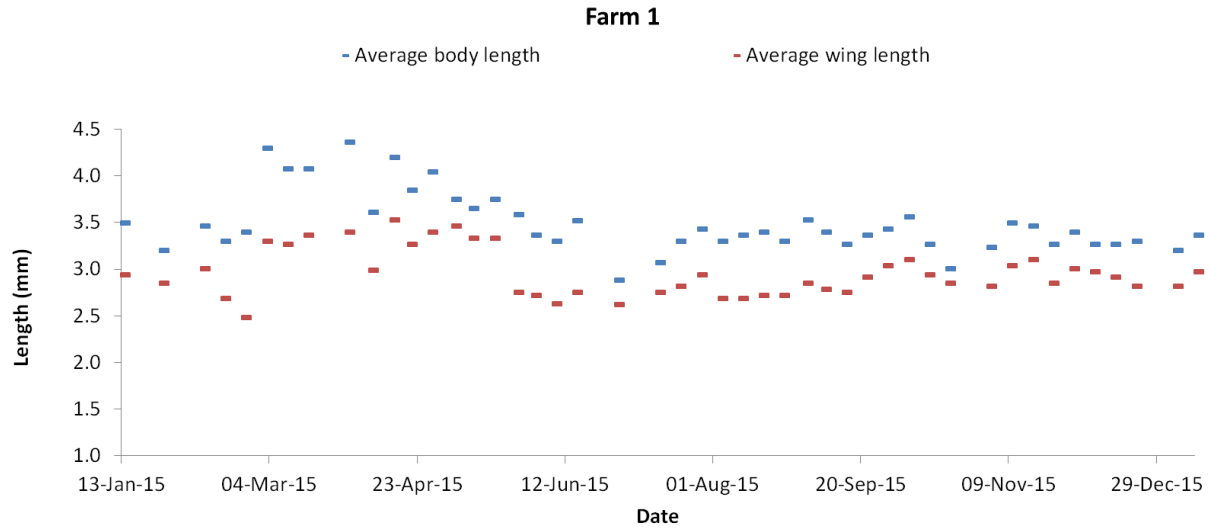

Fig. 1.2.8. Body and wing length measurements from Farm 1

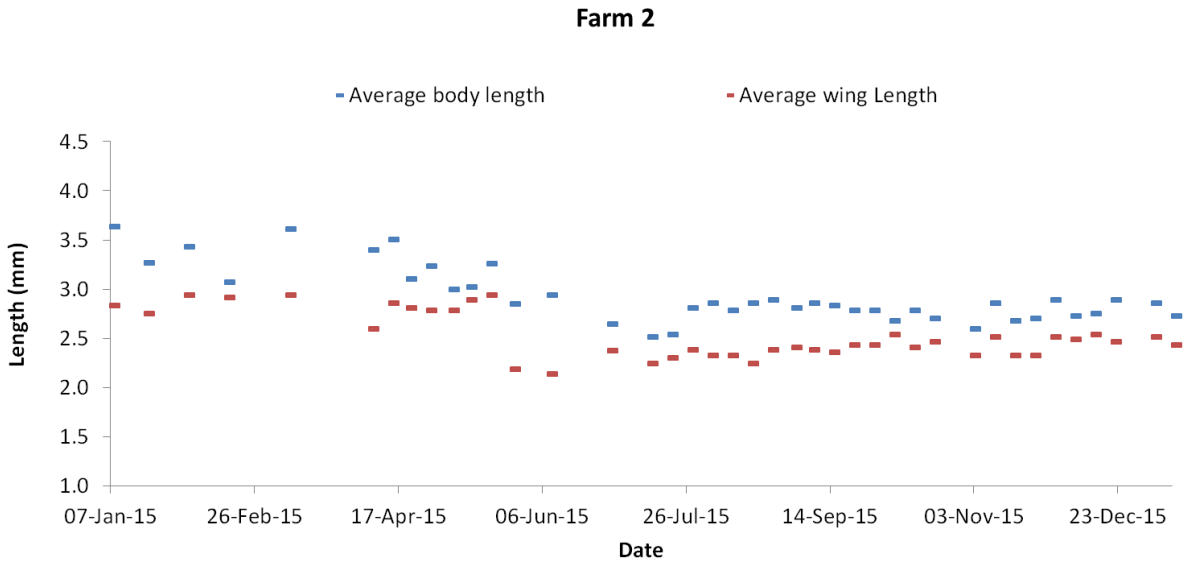

Fig. 1.2.9. Body and wing length measurements from Farm 2

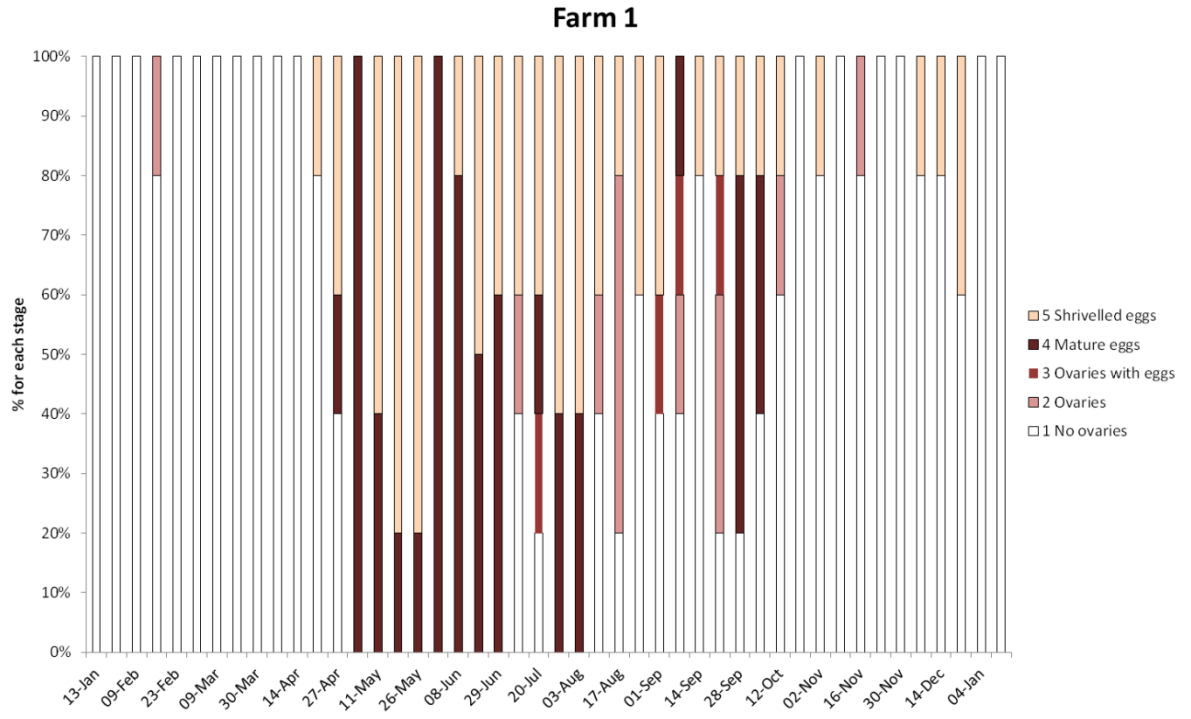

Fig. 1.2.10. Fecundity of *D. suzukii* at Farm 1.

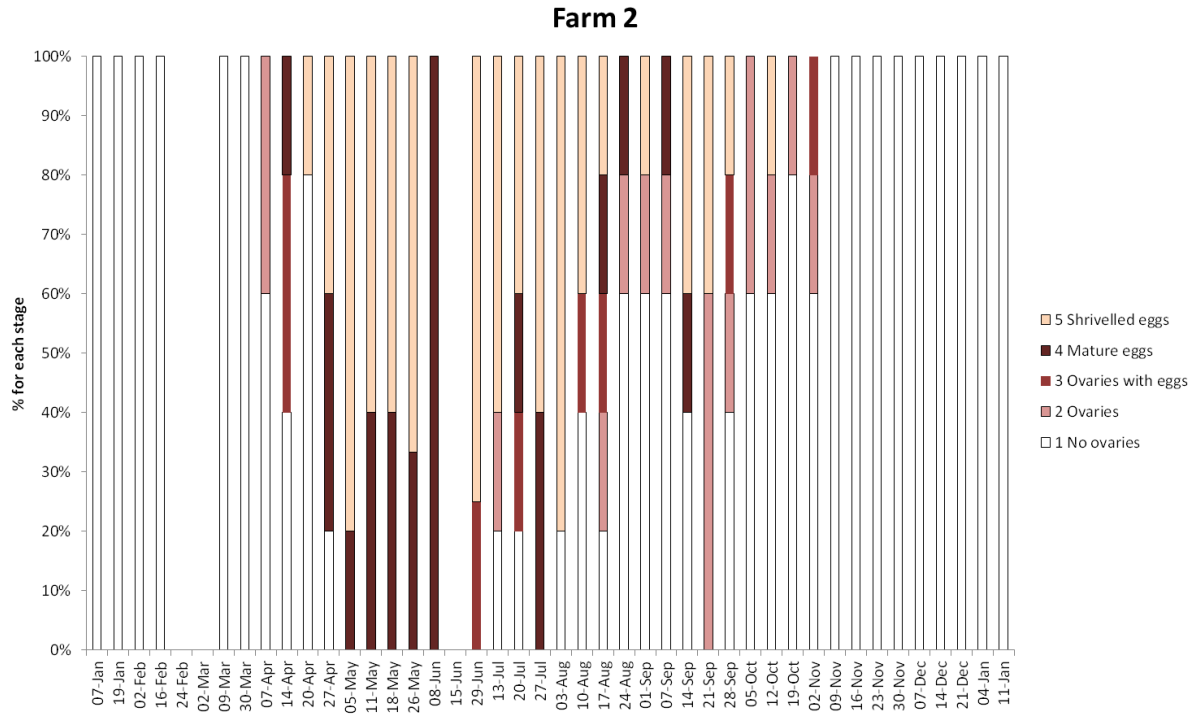

Fig. 1.2.11. Fecundity of *D. suzukii* at Farm 2.

Summary

- Adult *D. suzukii* were caught thoughout the year and were the most abundant insect caught in the traps from September onwards, outnumbering all the other bycatch combined.
- Higher numbers of *D. suzukii* were caught in cherry orchards than in other crops surveyed. The number of *D. suzukii* decreased in the cherry orchards once all the leaves had fallen.
- There is a high ratio of females in the traps from the beginning of April, which also corresponded to an increase in fertility.
- The winter morphs are still found until May.

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Task 1.3. Establishing a population model for *Drosophila suzukii*

Introduction

In order to have a better understanding of *Drosophila suzukii* as a new invasive pest to the UK, knowledge of the population dynamics throughout the season of this insect is important. Currently it is unclear how *D. suzukii* populations behave throughout the year and at which time of the season the adults are utilising different crops, such as cherries, strawberries and raspberries. A population model would help aid the understanding and prediction of *D. suzukii* population dynamics. This model could be used to predict the first egg laying of female *D. suzukii* and identify different intervals with a high egg laying pressure. These intervals would be useful to enable growers to time the application of crop protection product spray programmes. Furthermore, a population model could be used for a better understanding of the life cycle of *D. suzukii* and to develop a more accurate prediction tool for a more sustainable integrated pest management.

Materials and methods

The population model uses an algorithm to calculate population size. These calculations were done in R (R Core Team, 2015) combined with R Studio (RStudio Inc, Version 3.2.1, 18-06-2015) as an integrated development environment. A mathematical package, 'stringi' (Gagolewski and Tartanus, 2015), was also used to execute the algorithm.

Weather Data

Before an algorithm can be calculated it needs input values. One of the key parameters which influence the outcome of our algorithm, *D. suzukii* population growth, is the weather. The algorithm uses weather data as a basis for almost all calculations. The weather data used in this study was provided by Agrii (Agrii intelligence, MetQuest Weather Stations). All imported weather data were collected from different weather stations and were checked for errors made by the electronics of the weather stations. The files were also checked for consistent measurements during 15 minute intervals. In preparation for further calculations each file with weather data was provided with cumulative degree days (DD) based on the temperature. The calculation with cumulative DD was used to eliminate temperature variances during the different seasons and across multiple years and places. It is a tool used to compare different climatological circumstances. For this data the DD were calculated with 10 and 30°C as a lower and upper limit, above and below which *D. suzukii* reproduction is ceases (Equation 1). The range between 10 and 30°C corresponds with the normal development of *D. suzukii* (Kinjo and Kunimi, 2014). Each degree above the lower limit was multiplied by the interval time between two measurements. If the temperature rose above the upper limit of 30°C or dropped below the lower limit of 10°C there were no DD added. The cumulative DD is the sum of the previous measuring points and the DD calculated at a given time.

This method of calculating cumulative DD was more accurate than using a single or double sine method with the average daily temperatures, more frequently used in population models.

Equation 1. Calculation of DD. With T_x= air temperature on a given $Time_x$, T_{lower}=10°C, T_{upper}=30°C. Because of the 15 minute intervals there was a conversion factor needed to convert the DD in minutes to a DD in days by multiplying by 1440.

$$DD\ (\text{days}) = [(T_x - T_{lower}) * (Time_x - Time_{x-1})] * 1440$$
$$if\ T_{lower} \leq T_x \leq\ T_{upper}\ else\ DD(days) = 0$$

The weather data consisted of 15 minute intervals and included air temperature, relative humidity, rain and solar radiation. Currently the model only uses the air temperature to model the population dynamics. Under the assumption that temperature has the major influence on the development of this invasive insect this parameter will have the greatest impact in the model. In a later phase relative humidity and rain could be implemented as these are known to affect activity of the adults (Tochen *et al.* , 2015). Potentially rain and dry conditions may decrease activity and prevent mating and thereby effecting the next generation.

Estimation of first egg laying

For a population to grow, eggs need to be laid and larvae develop through all of the development stages. Therefore, the time of the first egg laying is a critical time point for the algorithm. The estimation of the first egg laying in spring was found by comparing the calculated cumulative DD with ovary development. The time points of general ovary development were selected as points of the first egg laying.

Table 1.3.1. 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

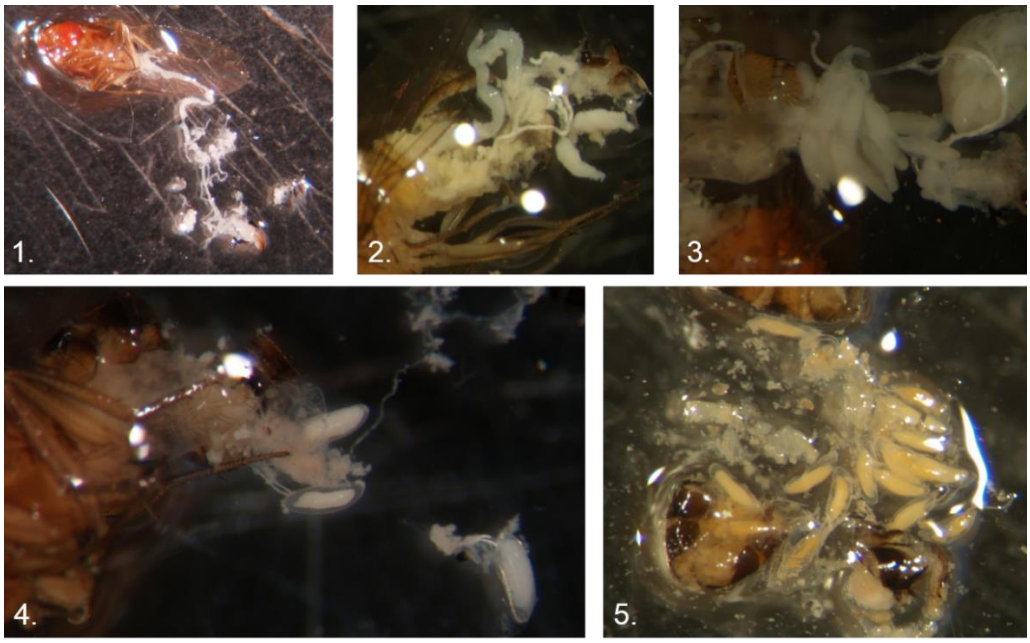

Figure 1.3.1. The five different ovary stages as described in Table 1 (photographs by Bethan Shaw)

The ovary development was monitored by EMR, at the two sites used in the habitat monitoring, by dissecting five female *D. suzukii* from each site each week and assessing their ovaries. The habitats were monitored with modified drosotraps (Biobest) with Cha-Landolt bait. Each ovary received a number based on the stage of development (developed by Beverley Gerdeman USA, Table 1.3.1).

To establish the time of the first egg laying after winter, stage three and four were used (Figure 1.3.1). These stages represented the most likely start of laying eggs in the monitored week. The ovary data were collected over a period of two years.

Population calculations

The population model requires different inputs, shown in Figure 1.. The imported source file, “DD_year_name.csv” data file, contained the audited weather data file combined with the calculated cumulative DD for each 15 minute interval. This data file was the base for all further calculations. Next to the weather data additional parameters were required. The second data file was a fixed data frame which consisted of the development and mortality rate of the adult flies for a range of temperatures (“Development-MortalityRateMin.csv”).

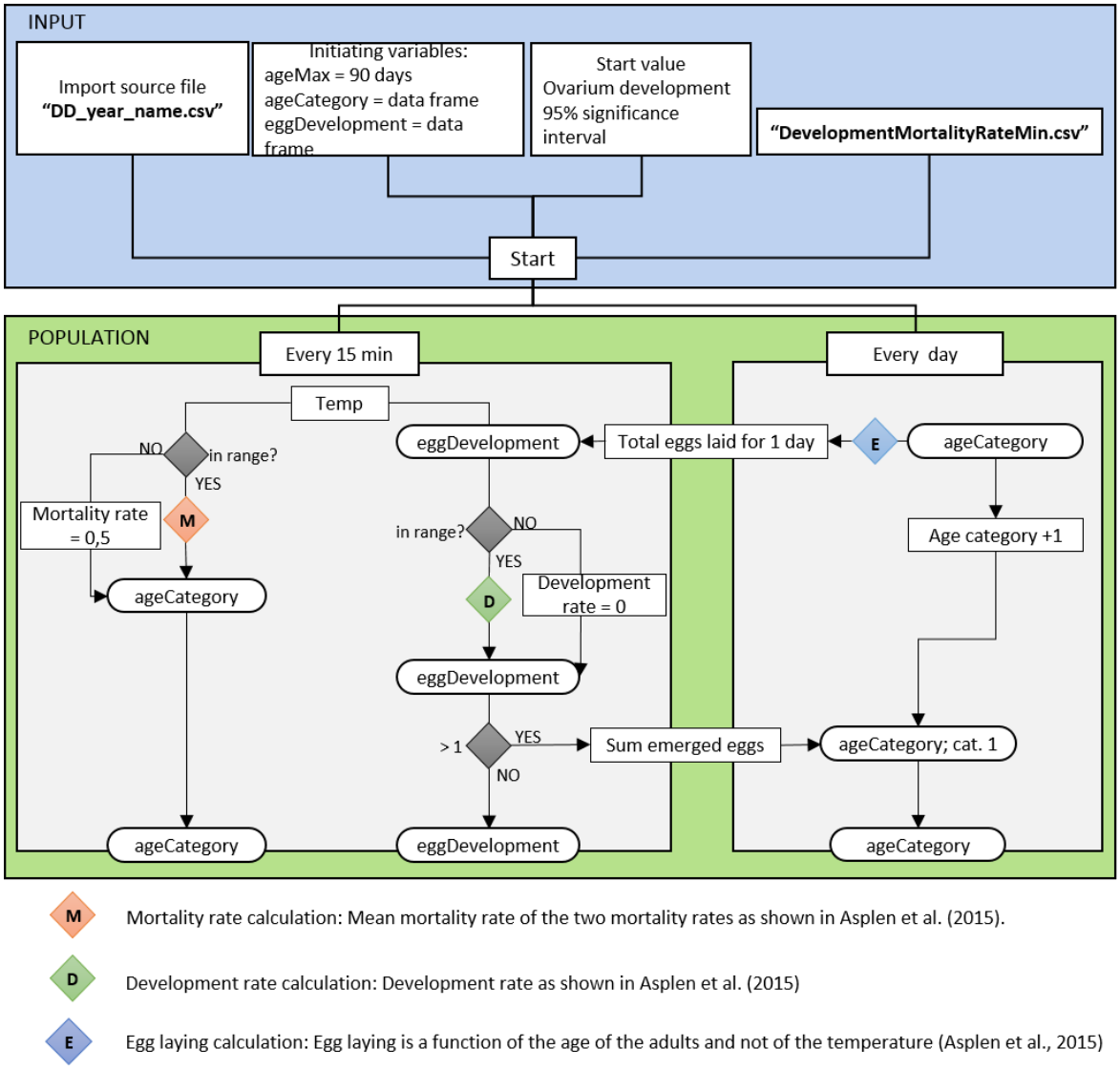

Figure 1.3.2. Calculation diagram to predict the *D. suzukii* population. On top of the diagram the different input data sets are shown to start the model. The actual population calculations are split up in two groups. The first set of calculations has to be done every 15 minutes. The other group of calculations has to be done each day. The different algorithms to calculate mortality rate and development rate are based on Asplen *et al.* (2015) as well as the age based egg laying.

The development rate of the eggs and pupae are dependent on temperature and their calculation is based on a function from Asplen *et al.* (2015). The mortality rate is also temperature based.

Due to the lack of information about the age specific oviposition the assumption was made that the temperature had no influence on this parameter. Therefore all calculations were executed with age specific egg laying at 21°C found in Asplen *et al.* (2015). The maximum age was set at 90 days for the population model.

The first egg estimation (Table 1.3.2) was used as a starting point for the actual algorithm to calculate the *D. suzukii* population (green area in Figure 1.). The calculation algorithm was divided into two types of calculations. The first set of calculations was done every 15 minutes and calculated the development of each egg for a given temperature. The mortality rate for each age category was also calculated every 15 minutes. The second calculation set used the age category of the female adults to calculate the eggs laid during the day and placed the emerged adults in at age one of the “ageCategory” data frame.

Throughout the calculations there were a couple of assumptions made in order to simplify the model. All the eggs laid by *D. suzukii* females were assumed to emerge, because currently there is no information available about how many eggs survive the three larval stages and pupation before becoming an adult. There is also a lack of information about the pre-oviposition period after females emerge from the egg. In the model it was assumed that there was no ripening period of the ovaries for egg laying after female emergence. Therefore when the females emerge we assumed they could lay eggs immediately. A third assumption was made, based on field data collected over three years in the UK at EMR, about the proportion of male / female adults. The proportion was assumed to be 50/50. Therefore, half of all the eggs laid will become females.

Results

Estimation of first egg laying compared to cumulative degree day

Table 1.3.2 shows the calculated estimation of the possible first and second egg laying in cumulative degree days (DD). These results are based on the ovarian development dissection done by EMR. The estimation for the second spike of egg laying has a high variance, making it difficult to pinpoint the exact cumulative DD around which female *D. suzukii* lay their first eggs. Furthermore, the lower threshold of the 95% significance interval was also too early compared to the assessments done by the dissections of the ovaries. The earliest record of developed ovaries was found at 25 cumulative DD.

Table 1.3.2. Estimation of first and second egg laying in cumulative degree days (DD). The model used the 95% significance interval of the first egg laying to establish the time range of the first egg laying.

DD (day)	Mean	S.E.	68% significance		95% significance	
			lower threshold	upper threshold	lower threshold	upper threshold
1st egg laying	36,88	13,12	23,76	50,01	10,64	63,13
2nd egg laying	88,82	42,42	46,40	131,25	3,975	173,67

The 95% significance interval was further used to calculate the start of the female population of *D. suzukii*; the three intersections between the mean, upper- and lower threshold of the estimated first egg laying and the cumulative DD for each farm used in the national monitoring (Figure 1.3.2) were used to establish the starting points.

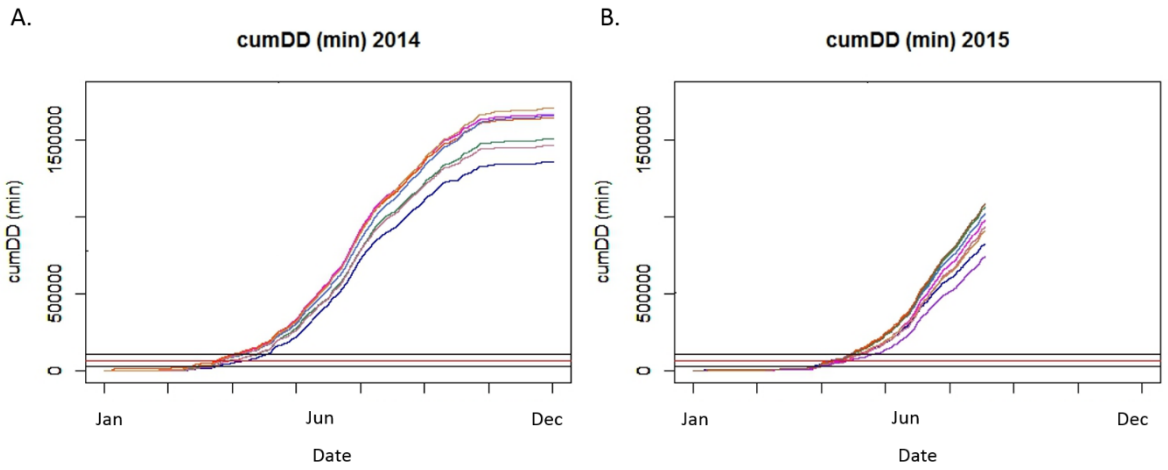

Figure 1.3.2. First *D. suzukii* egg laying estimation compared to cumulative degree days. The two black horizontal lines are the upper and lower thresholds of the 95% significance interval (Table 1.3.2), the red horizontal line is the mean (Table 1.3.2). Graph A. shows the calculated cumulative DD (minutes) of 2014 for seven farms. Graph B. shows the calculated cumulative DD (minutes) of 2015 for nine farms. Those farms are part of the national monitoring of *D. suzukii*.

Population estimation

With the input data and calculation explained in the previous paragraphs an output could be generated. More precisely, the population growth over the year could be modelled. Figure 1.3.3 shows the female population of *D. suzukii* throughout the year. The graph compares the three different start points as calculated in Table 1.3.2. The earliest start point at 10.64 cumulative DD shows the highest number of female *D. suzukii* at the end of the year.

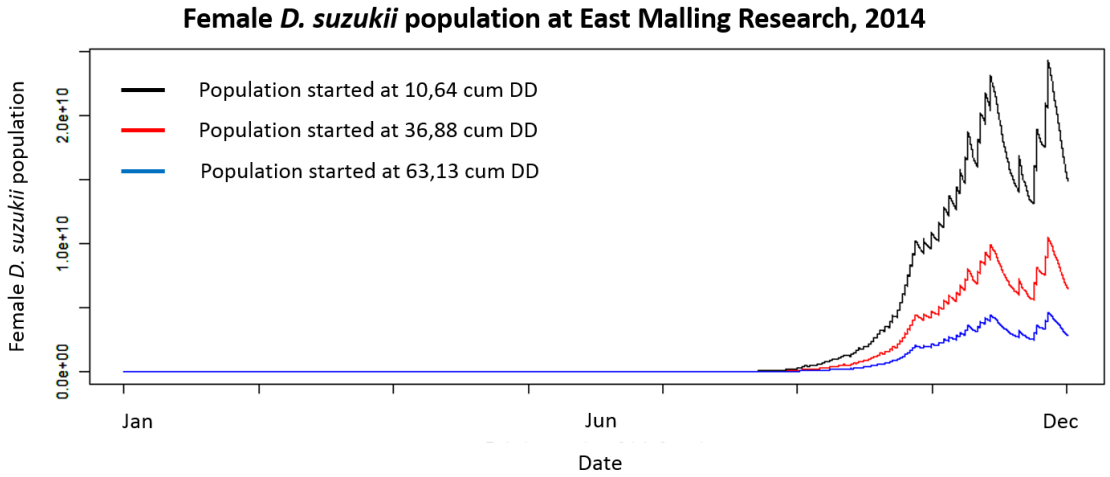

Figure 1.3.3. Female *D. suzukii* population graph during 2014 at EMR. The different lines show the female population at different starting points for the algorithm. The start points were based on the 95% significance interval of the estimation of the first egg laying (Table 1.3.2)

The calculation algorithm used to calculate the population had a very large output of female flies. As shown in Figure 1.3.3 the population reached the number of 20 billion female *D. suzukii*. Although not visible in Figure 1.3.3 this exponential growth starts at the beginning of June which is visible when looking at a smaller time range (Figure 1.3.4). The calculations for 2015 will follow later when the complete weather data set is available.

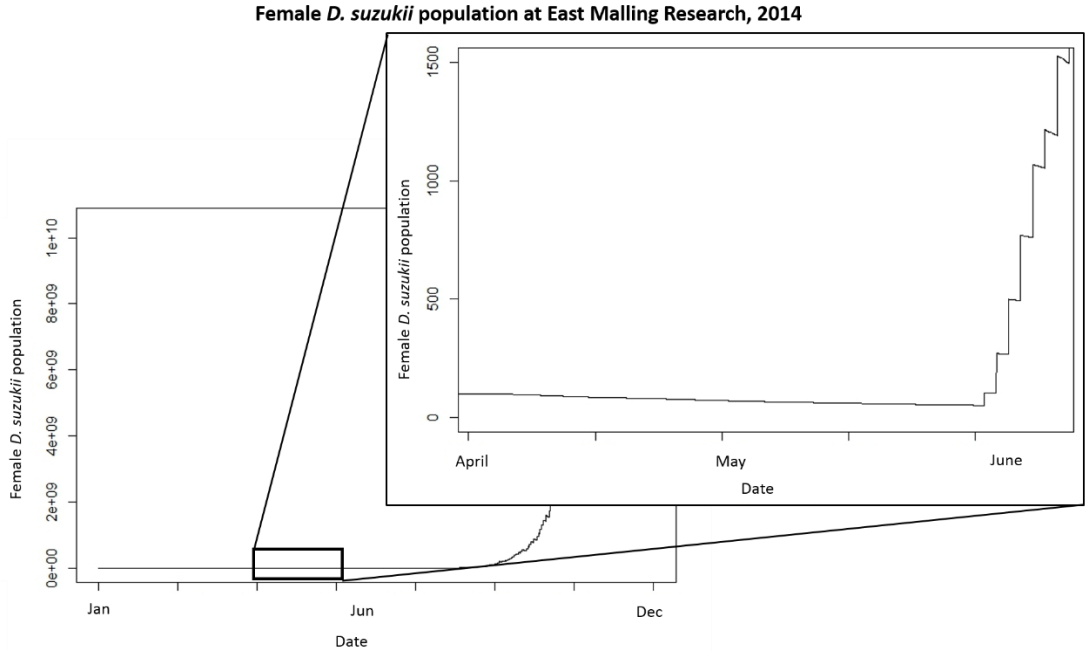

Figure 1.3.4. Female *D. suzukii* population at EMR in 2014 focused in on April to June. The population is calculated with 36.88 cumulative DD as a starting point.

Discussion

The population model constructed in this study serves as a good starting point for the population estimation of *D. suzukii*. It succeeds in pinpointing the time of egg laying and the start of the population growth. By making various assumptions, based on the literature, the model is a simple representation of the reality. The cumulative DD are a good parameter to start the calculations and estimate the first egg laying.

The moment of rapid growth can be pinpointed, giving important information to the farmers. Based on weather data the start of the exponential growth of a given year can be extracted. By providing this information to the farmers they could use control measurements at the right time to 'knock back' *D.* suzukii populations before they grow exponentially. Furthermore the model is an important step towards a clear life cycle follow-up of *D. suzukii*, providing a useful tool for research.

Despite several good points, there is still room for some improvements. The current population model gives a good representation of the population dynamics but is less accurate in the estimation of the actual population. The population model gives really high numbers of the female *D. suzukii* population. Those high numbers are probably a result of the assumed low mortality rate and 100% survival rate from laid eggs to emerged adults. The high numbers of *D. suzukii* could also be explained by the assumption that the females lay eggs immediately after they emerge from their pupae. The important thing to conclude so far is the start of the exponential growth of the

population around the beginning of June for 2014. By ameliorating the assumptions of mortality and survival rate the model will also be applicable for absolute numbers. Other factors such as parasitism and predation will be more difficult to incorporate in the population model because there is currently no data available.

The calculation algorithm is currently based on temperature input with corresponding mortality and development rates. These rates are based on the research of Asplen *et al.* (2015). The different rates discussed in this article are concise. To improve the basis of this population model it is important to do further research on the influence of temperature, relative humidity and light radiation on the life cycle of *D. suzukii*.

The estimation of the first egg laying consists of four data points (two farms over two years). Although these values give a good indication, the sample size of ten flies for each week is relatively small to draw firm conclusions. This means that the standard error and significance intervals are relatively large and can be reduced by extending the research about the ovary development.

A comparison with true data is necessary to validate the model, this could be done with national and habitat monitoring data. However the traps currently used are not optimal to accurately measure the population. Later in the season, during the ripening of different crops, the trap catches are less accurate because of competition between the traps and the ripening fruit. In this situation, *D. suzukii* is probably more attracted to the ripening fruit than to the traps. Until April it is possible to use currently available trap catches as a key to estimate the actual population with the help of a conversion factor. The current traps measure mainly the activity of adult *D. suzukii* flies and less so the actual population. Therefore further research to improve the attractants and traps needs to be continued. They are especially necessary to get a more accurate reading during the ripening season of the different crops and to establish a more accurate prediction of the infection pressure during ripening of the crops. This is the time when an accurate prediction is most needed for the growers. The ultimate goal of the model is to predict the fruit damage pressure with the help of available weather data at a given time during the year.

By using the same traps across the UK all flies behave the same towards the traps. Therefore the trap catches can be compared and can be used to validate the population model. In order to continue the construction of this model it is best to continue with the current traps to have consistency across multiple years. Better traps can be supplemented next to the current traps as to generate data which can be also used in this model in the future.

Further research

The population model in this form can give useful information. There are some possible improvements which can make it even more relevant. In the current model there is only an implementation of the temperature. Further on, other weather parameters have to be implemented. To further improve the population model lab test, field tests are needed to establish more detailed information about the mortality and development rate of *D. suzukii* in different weather conditions.

In a next step, the model has to be verified by the trap catches of the national monitoring and habitat monitoring. The habitat monitoring is especially useful because this is more detailed than the national monitoring and also provides more information about the displacement during the year from the woods to the crops and back.

There is not much knowledge about the survival rate during the winter period and the trigger of the female ovary development in spring. It would be useful to know if female *D. suzukii* winter forms have spermathecae (receptacula seminis) to store male sperm before or after the winter. At the moment the assumption was made that females do go in winter form without ovaries to save some energy to survive the winter. But what mechanism do male *D. suzukii* winter forms have to survive winter conditions?

To conclude it can be said that the generated population model is a good starting point for estimating population dynamics. This gives valuable information for growers to start their pest management of *D. suzukii* at the right time. Together with the above mentioned improvements this model can become even more relevant and result in huge advantages for the agricultural sector.

Summary

- A population model was constructed using fertility studies, climatic data and biological parameters
- This model gave good estimates for the start of egg laying and population growth early in the year.
- Work is continuing to optimise this model for use later in the growing season.

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Task 1.4. Monitoring SWD larval infestations in early, mid and late season cherry varieties

Objective

To characterize seven sweet cherry cultivars ('Merchant', 'Simone', 'Kordia', 'Kordic', 'Penny', 'Regina' and 'Sweetheart') by phenological and fruit quality aspects to determine the 'window' of exposure to *D. suzukii* egg laying in the fruit. We aimed to identify the time taken from white fruit to picking.

Experiment 1

Seven commercial varieties were studied: 'Merchant', 'Simone', (early cropping); 'Kordia', 'Kordic', 'Penny', (mid-late cropping); 'Regina' and 'Sweetheart', (late cropping). Fruits were sampled from a commercial plot (Univeg, 4 years old) and the strategic, unsprayed, plot of cv. Penny and Sweetheart in 'Rookery' field (6-7 years old) at EMR by kind permission of Graham Caspell (EMR Farm Manager) (Table 1.4.1).

Harvest in a commercial orchard can start anywhere from late June to mid-July depending on preceding temperatures. We began phenological records in the third week of April and the fruit quality assessment was in the second week of June. There were five trees of each variety. All of the trees were in the same block in the Univeg or strategic orchard with the same planting distance and pruning system. The stage of flower development was determined once a week from 23 April until fruit set. After this the fruit development stage was checked twice a week. The following quality traits were measured;

- Size (diameter measured using Vernier callipers in mm)
- Colour using colourimeter (Chroma meter CR-400/410, Konica-Minolta) and the Ctfil colour chart (allowing us to avoid a possible error to quantify the fruit colour and the difference among varieties).
- Firmness. *The FirmTech1* (BioWorks, Stillwater, Okla).
- Brix (where possible) (Palette 7 digital refractometer).
- *D. suzukii* emergence. Five intact fruit from each variety.

We also used other EMR data on emergence and larval extraction. The extraction method used for the larval extraction was the standard crush test with sugar solution.

Preliminary studies monitoring emergence of *D. suzukii* from the fruits showed that in almost all cases the adults emerged within 2 weeks of picking. Using a model of *D. suzukii* development time published by Tochen & Walton (Oregon University) we predicted approximately when the eggs were laid in the cherries. With a regression analysis based on this model we estimated the number of days from egg to adult for each temperature. We used the temperature in the field for one week and the lab temperature for the week before the emergence for each variety. Once we had the average temperature for those two weeks, we were able to predict the days from egg to adult and relate this to the fruit quality traits on the day of egg laying. We used the temperature data collected from the data loggers in the protected cherry crop and the EMR meteorological data for the two varieties which were not grown under plastic.

Table 1.4.1. Stage and physical attributes of cherry varieties as they develop

Variety	Date	Stage	Firmness (g/mm)	Size (mm)	Colour (key)	Colour	Brix
Kordia	24-Apr	Stamens/Open flower					
	06-May	Open flower					
	29-Jun	Open flower					
	13-May	Petal fall					
	20-May	Calyx fall					
	26-Jun	Green fruit	498	25.0	1.0	1.2	9.1
	03-Jul	Egg laying	405	26.3	1.4	1.6	11.0
	21-Jul	Emergence	305	28.5	5.4	5.8	11.2
	24-Jul	Harvest	267	28.3	6.4	6.6	10.3
Korvic	24-Apr	Open flower					
	06-May	Petal fall					
	13-May	Fruit set					
	20-May	Calyx fall					
	16-Jun	Green fruit	400	24.7	1.3	Green/1	6.4
	30-Jun	Egg laying	326	27.1	2.4	2.2	13
	17-Jul	Emergence	291	25.6	5.4	5.8	9.3
	21-Jul	Harvest	239	26.3	6.2	6.6	14.3
Merchant	24-Apr	Open flower					
	06-May	Petal fall					
	13-May	Fruit set					
	20-May	Calyx fall					
	23-Jun	Emergence (other farms)	263	23.0	2.4	2.4	10.5
	30-Jun	Egg laying (EMR)	202	29.3	4.4	5.4	11.0
	14- Jul	Harvest	174	25.7	6.0	6.8	16.0
	17-Jul	Emergence (EMR)	177	24.3	6.0	6.2	16.8
Penny	24-Apr	Flower buds /Stamens					
	29-Apr	Stamens					
	06-May	Open flower					
	13-May	Fruit set					
	20-May	Calyx fall					
	16-Jun	Green fruit	979	25.3	Green	Green	
	30-Jun	Egg laying	402	25.3	1.2	Green/1	12.0
	07-Jul	Larvae (other farms)	314	27.5	3.4	4.2	19.5
	10-Jul	Larvae (EMR)	327	24.3	3.8	4.8	19.6
	17-Jul	Harvest (emergence EMR)	326	26.7	5.6	6.0	16.4

Table 1.4.1 cont... Stage and physical attributes of cherry varieties as they develop

Variety	Date	Stage	Firmness (g/mm)	Size (mm)	Colour (key)	Colour	Brix
Simone	24-Apr	Open flower					
	06-May	Open flower/Petalfall					
	13-May	Fruit set					
	20-May	Calyx fall					
	09-Jun	Green fruit	601	21.6	White/1	Green	
	03-Jul	Harvest	188	24.5	6.0	6.0	12.1
Sweethe							
art	24-Apr	Open flower					
	29-Apr	Petalfall					
	13-May	Calyx fall					
	23-Jun	Green fruit	681	22.5	1.0	Green	
	03-Jul	Egg laying (EMR)	465	27.4	1.0	1.0	14.3
	21-Jul	Emergence (EMR)	360	26.4	3.6	4.8	18.5
	24-Jul	Optimal timing of harvest	298	28.5	4.6	4.6	14.8
Regina	24-Apr	Stamens					
	29-Apr	Stamens/Open flower					
	06-May	Open flower					
	13-May	Petalfall					
	20-May	Calyx fall					
	30-Jun	Green fruit	460	22.8	Green/1	Green/1	7.2
	24-Jul	Harvest	218	29.0	5.6	6.8	15.1

Results

No *D. suzukii* were found in Simone (early variety) and Regina (late variety) during this trial. *D. suzukii* emerged from the other 5 varieties. The average firmness and Brix from all varieties where *D. suzukii* was able to lay eggs was 356 (g/mm) and 12.1 degrees respectively (see Table 1.4.1 and 1.4.2 for individual varieties). There was a positive relationship between Brix and the softening of the cherry fruits (Fig. 1.4.1). *D. suzukii* was not found in the early variety Simone (picked first week of July), but was found in Merchant. However it was only found in Merchant fruits after the optimal time for harvest had passed and hence the fruits were very soft. For Regina it was possible that this variety was well protected because it was in a commercial orchard receiving plant protection products.

Table 1.4.2. Firmness and Brix when *D. suzukii* was first able to lay eggs in cherry

Variety		Firmness	Brix	Colour	Sprayed
Kordia	mid-late	405	11	1.4	Yes
Korvic	mid-late	326	12.7	2.4	Yes
Merchant	early	202	11	4.4	Yes
Penny	mid-late	402	12	Green-1.2	No
Sweetheart	late	465	14.3	1.0	No

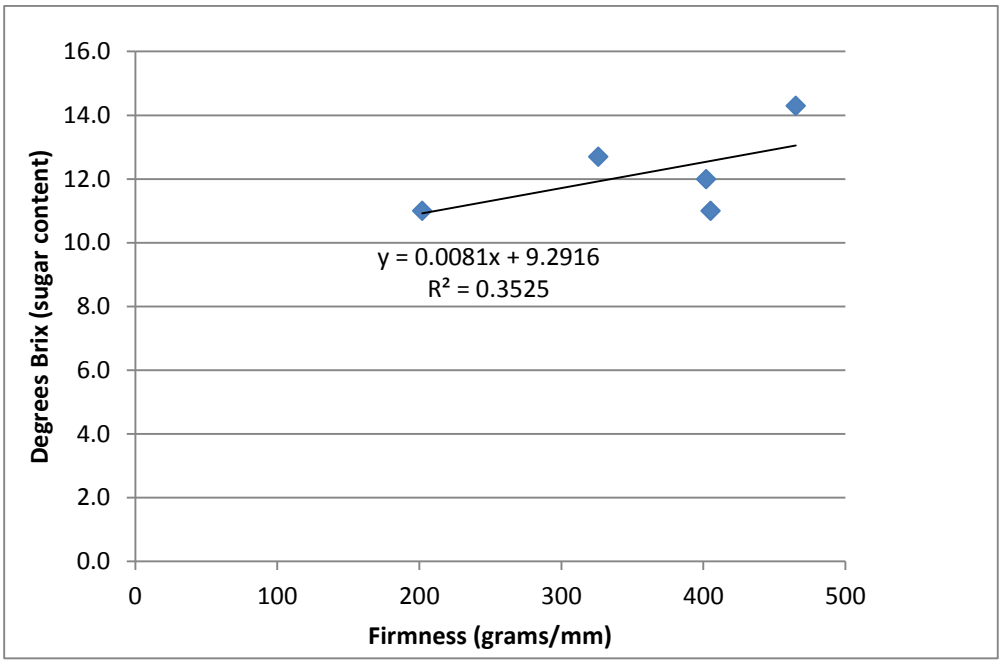


Figure 1.4.1. Relationship between firmness and Brix in the cherry varieties and when *D. suzukii* was first able to lay eggs.

By combining all of the data collected and comparing firmness and colour (chart) for each variety and each assessment day we could predict the cherry colour at the mean firmness for first egg laying. For a firmness value of 356 grams/mm the colour was almost 3 in the Ctfil colour chart. This is a red, however, the data is somewhat misleading as the crops which were treated with plant protection products had eggs laid in them much later than varieties which were not sprayed (Table 1.4.1). For example, Sweetheart and Penny had eggs in them at the green to chart colour 1 stage. Hence applying the plant protection products delayed the ability of *D. suzukii* to lay viable eggs in cherry fruits.

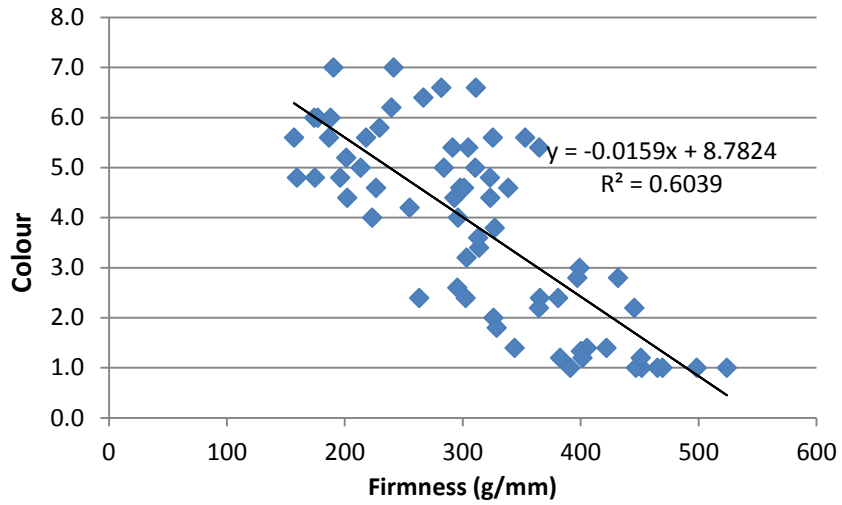


Figure 1.4.2. Relationship between colour and firmness of all tested varieties in two different orchards.

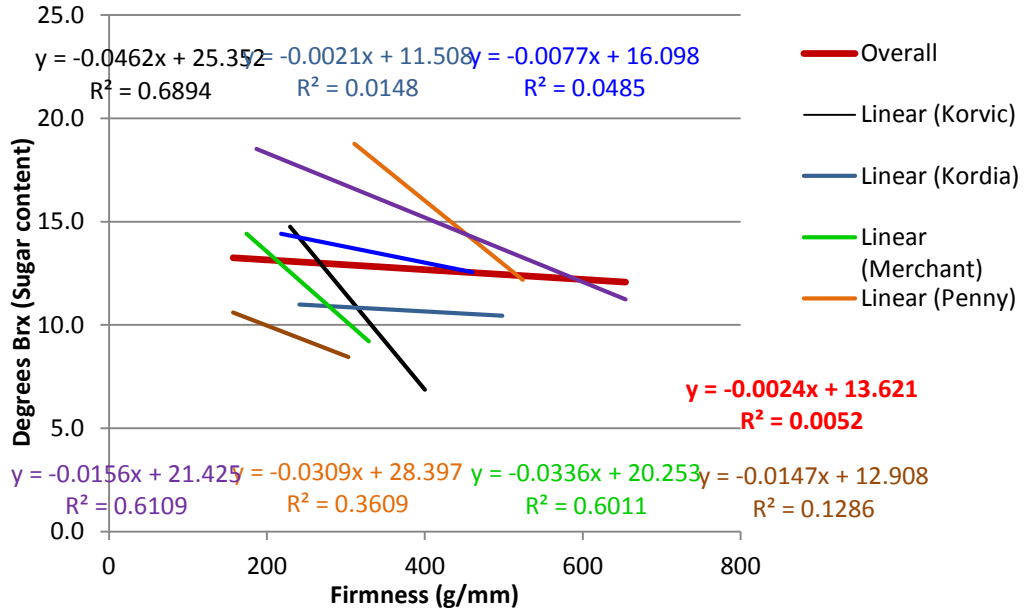
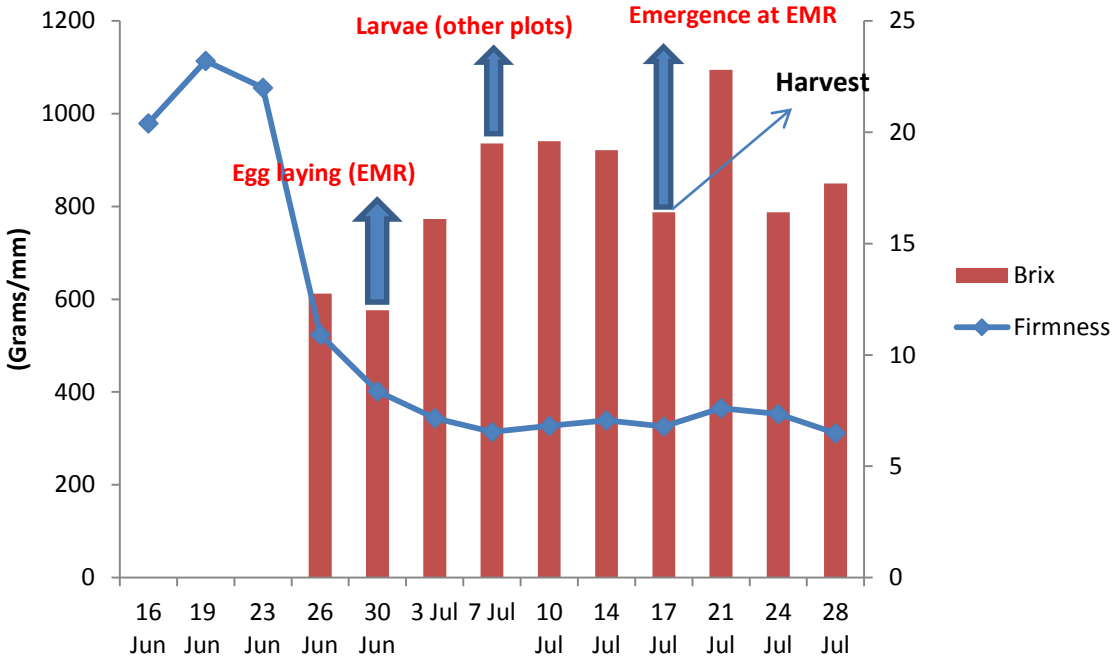


Figure 1.4.3. Regression analysis between Brix and firmness for each variety.

If all varieties are plotted together for firmness and Brix there is no clear relationship. However some varieties e.g. Sweetheart, Korvic and Merchant showed a stronger relationship between these two variables than the other varieties tested. Eggs were laid from a Brix level of 11 in most varieties (Table 1.4.2). Brix levels are highly dependent on cultivar, annual climatic conditions, cultivation system and rootstock (Gongalves *et al.* ,2006: Usenik *et al.* ,2010) so we can conclude that protecting fruits from the white stage is a recommended option for reducing fruit damage by *D. suzukii.*

The following illustrations give the time of *D. suzukii* egg laying and appearance of each variety in 2015;

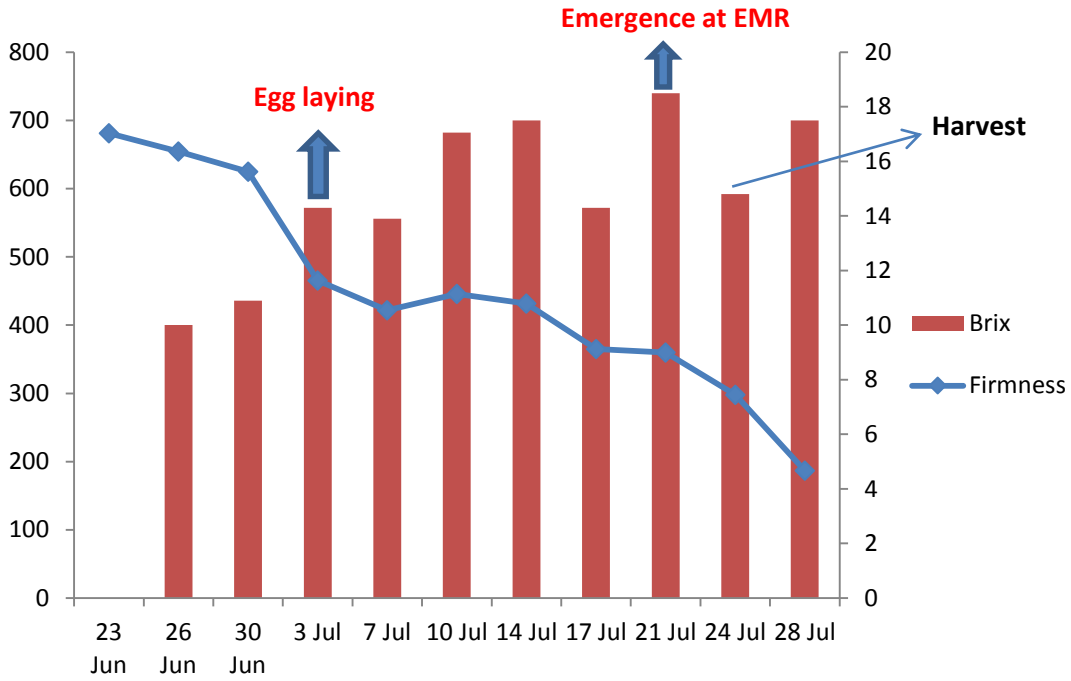
a)



b)

c)

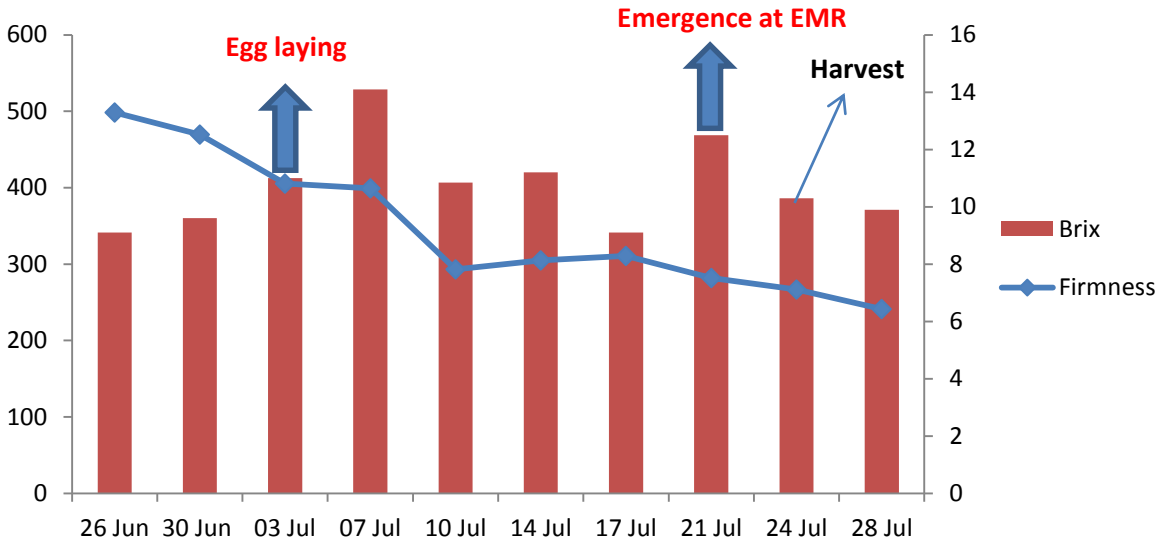
Figure 1.4.4. a) Phenology and *D. suzukii* activity on cv. Penny in relation to Brix and firmness and b) photographs of when *D. suzukii* was able to lay eggs (30 June) and c) 28 July, past harvest, when there was obvious damage to the fruits.



a)

b)

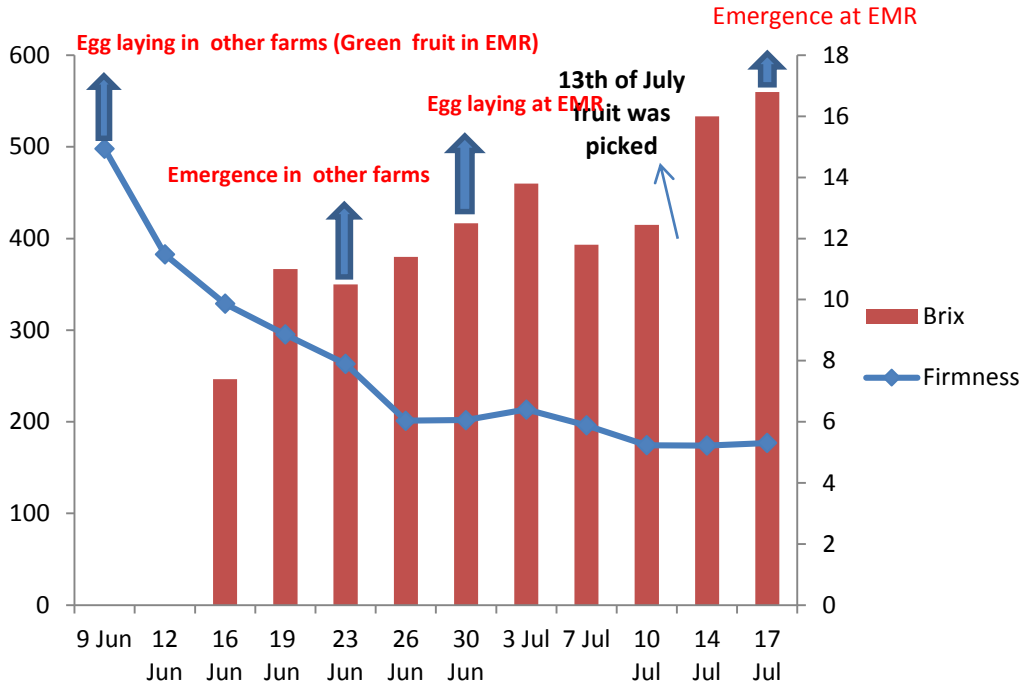
Figure 1.4.5. a) Phenology and *D. suzukii* activity on cv. Sweetheart in relation to Brix and firmness and b) photographs of when *D. suzukii* was able to lay eggs (3 Jul).



a)

b)

Figure 1.4.6. a) Phenology and *D. suzukii* activity on cv. Kordia in relation to Brix and firmness and b) photographs of when *D. suzukii* was able to lay eggs (3 Jul).



a)

b)

Figure 1.4.7. a) Phenology and *D. suzukii* activity on cv. Merchant in relation to Brix and firmness and b) photographs of when *D. suzukii* was able to lay eggs (30 Jun).

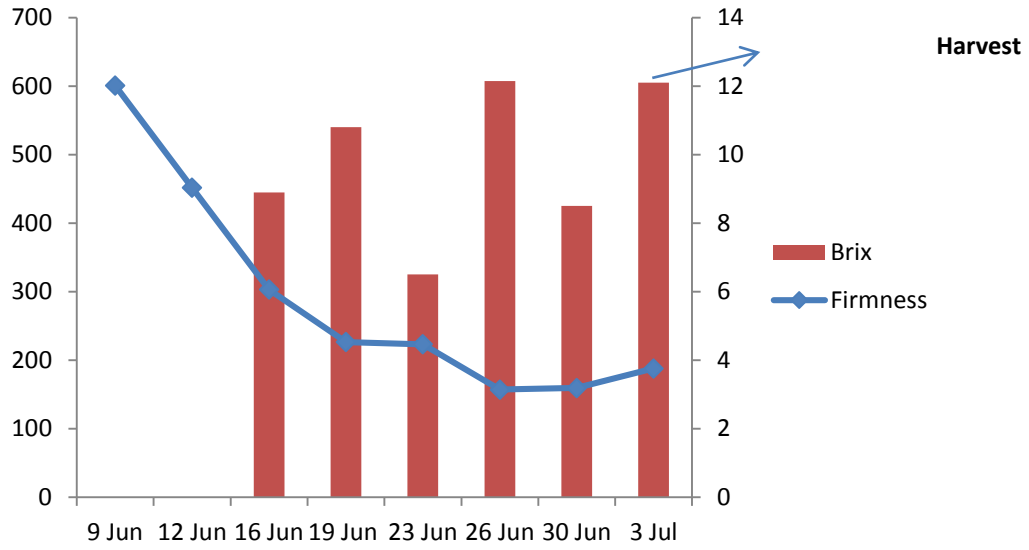


Fig. 1.4.8. Phenology and *D. suzukii* activity on cv. Simone in relation to Brix and firmness

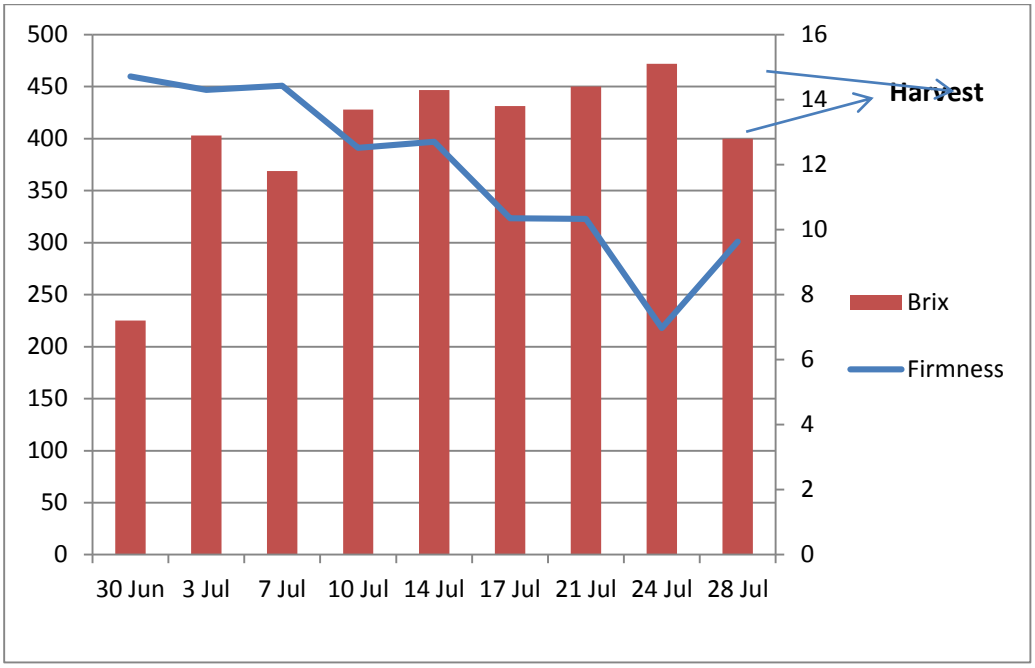


Fig. 1.4.9. Phenology and *D. suzukii* activity on cv. Regina in relation to Brix and firmness

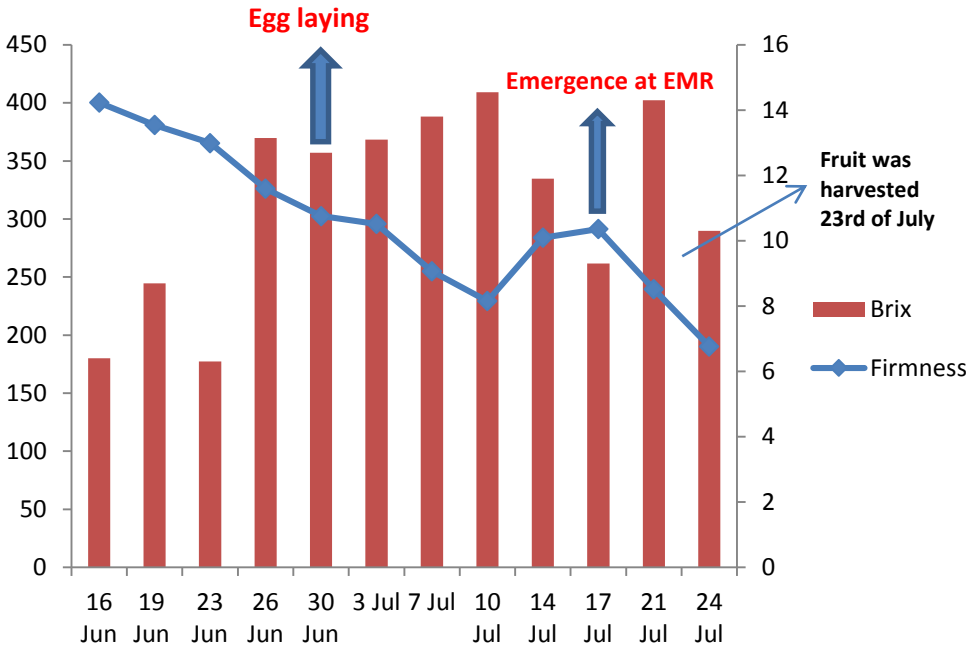


Fig. 1.4.10.a) Phenology and *D. suzukii* activity on cv. Korvic in relation to Brix and firmness and b) photographs of when *D. suzukii* was able to lay eggs (28 Jun)

Conclusions

- The average firmness and Brix from all varieties when *D. suzukii* was first able to lay eggs was 356 (g/mm) and 12.1 degrees respectively.
- No larvae were found in the early varieties when harvested at optimal time.

References

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Experiment 2

Objective

To monitor the occurrence of *D. suzukii* larvae in early, mid and late season cherry crops at 4 commercial farms in Kent UK as the fruit ripens. Weekly samples were taken from an early, mid and late variety at each site from the early white to fully ripe fruit stages.

Methods

Four farms were chosen in the South East of England. There are no experimental treatments. The samples of fruit were taken from the growers commercially sprayed cherry orchards.

Adult activity

Two standard *D. suzukii* adult monitoring traps (Biobest modified traps with Cha-Landolt lure system) were deployed in each orchard, one in the middle one at the edge near to possible sources of infestation, at the end of blossom (late April/early May). These were monitored weekly by grower staff for numbers of male and female *D. suzukii* in each trap each week. Trap contents were strained from the drowning solution through a paint filter, which was then transferred to a polythene bag, sealed and labelled with pencil (label in bag) and sent to East Malling for confirmation of counts.

Larval infestation of fruits

Four replicate samples of 50 fruits were taken from each variety weekly from early white fruit to harvest and beyond if possible. Samples were held in ventilated plastic boxes (supplied by EMR). Two samples were taken from the edge of the orchard close to woodland or sources of natural infestation and two samples were taken from the centre of the orchard. Samples were taken randomly, selecting the ripest (most forward) at the time.

Crush testing

Standard sugar method (see Objective 3).

Emergence testing

Each sample was held in a ventilated Perspex plastic box (228x121x66 mm) covered with fine mesh (300 mmx400 mm) held tightly in place with electrical tape. Fruit was placed on paper towel to absorb liquid. Boxes were held at room temperature at the host farm then delivered to reception at EMR where they were held at 20°C for 3 weeks. They were assessed weekly for adult

emergence. Communication of results was by email between the nominated person at each site and NIAB EMR.

Results

No *D. suzukii* emerged from fruits between 14 May and 8 June (Table 1.4.3). Low numbers were detected in early varieties on all but Farm 3 (Merchant and Burlat) from 15 June (<5/ 50 cherries). Higher numbers of *D. suzukii* emerged from a mixed variety orchard known to have received only one spray at the beginning of the season. Spraying was abandoned at this site as the coverage was not considered sufficient because of the size of the trees.

Table 1.4.3. Table of infested samples of 50 cherries from commercial cherry farms. E= adult *D. suzukii* emerging from fruit within 3 weeks. L= numbers of larvae extracted using sugar flotation method. Grey areas indicate picking had ceased.

Site	Harvest	Variety	14 May -08 Jun		15 Jun		22 Jun		29 Jun		06 Jul		13 Jul		20 Jul		27 Jul		03 Aug		16 Aug		22 Aug	
			L	E	L	E	L	E	L	E	L	E	L	E	L	E	L	E	L	E	L	E	E	L
1	Mixed	Heritage	0	0	0	0	0	0	4	11	0	0	5	13					25	0				
	Early	Merchant	0	0	0	4	0	0	0	0	0	0	0	0					15	28				
	Mid	Lapins	0	0	0	0	0	0	0	0	0	0	0	0					2	45				
	Latest	Penny	0	0	0	0	0	0	0	0	0	0	0	0					70	13				
2	Early	Burlat	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mid	Kordia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Latest	Sweetheart	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Early	Merchant	0	0	0	0	0	0	0	0	0	0	0	0										
	Mid	Kordia	0	0	0	0	0	0	0	0	0	0	0	0										
	Latest	Penny	0	0	0	0	0	0	0	0	0	0	0	0										
4	Early	Merchant	0	0	0	5	0	0	0	0	0	0	0	0										
	Mid	Kordia	0	0	0	0	0	0	0	0	0	0	0	0										
	Latest	Regina	0	0	0	0	0	0	0	0	0	0	0	0										

Task 1.5. Identify the common wild host plants of *D. suzukii* adults and larvae in the UK. Years 2-3.

See year 2 report task 1.3 for materials and methods.

Results

No choice emergence

D. suzukii that were introduced into boxes with fruits had a second generation emerge from several species.

Table 1.5.1. Field collected fruits exposed to adult male and female *D. suzukii* and resulting second generation emergence (no choice).

Description	**Total *D. suzukii* (per g fruit)**
Wall Cotoneaster (*Cotoneaster horizontalis*)	2.9
Fig (*Ficus carica*)	2.3
Mistletoe (*Viscum album*)	1.5
Honeysuckle (*Lonicera)*	0.7
Elderberry (*Sambucus*)	0.7
Japanese rose (*Rosa rugosa*)	0.7
Nightshade (*Solanum*)	0.6
Yew (*Taxus)*	0.5
Rowan (*Sorbus* sp.),	0.3
Pink Pagoda *(Sorbus hupehensis)*	0.3
Spindle (*Euonymus europaeus*)	0.2
Guelder Rose (*Viburnum opulus*)	0.1
Hawthorn (*Crataegus* sp.)	0
Ivy (*Hedera helix*)	0
Damson (*Prunus domestica* subsp. *insititia*)	0
Dogwood (*Cornus sanguinea*)	0
Holly (*Ilex aquifolium)*	0
Pyracantha (*Pyracantha* sp.)	0
Rubella *(Skimmia japonica)*	0
Mahonia	0

Natural emergence

In 2014 *D. suzukii* naturally emerged from cherry, strawberry, blackcurrant, black bryony and yew. It did not emerge from cotoneaster, snowberry, guelder rose, dogwood, hawthorn, red bryony and rose in the samples we collected (Table 1.5.2). Natural emergence was not observed from the strawberry tree plant berries (*Arbutus unedo*), natural emergence tests on mistletoe (*Viscum album*) were not possible because wild fruits have not been found (birds). Emergence was observed from mistletoe (*Viscum album*) fruit collected from the wild (Briem 2015) and may be a first spring host in Central Europe. Lee *et al.* (2015) provide further information on wild hosts of *D. suzukii.*

Table 1.5.2. First date of collection resulting in natural emergence.

Fruit	Date	Number emerged
Cherry	30 May	10
Blackberry	24 Jul	1
Strawberry	04 Aug	2
Raspberry	07 Aug	2
Blackcurrant	22 Aug	1
Elderberry	05 Sep	1
Black Bryony	13 Oct	8
Yew	04 Nov	6

Conclusions

Black bryony and yew berries may provide egg laying sites towards the end of the year. Cotoneaster, snowberry, guelder rose, dogwood, hawthorn, red bryony and rose do not appear to support *D. suzukii* development.

References

Briem M., Breuer K., Köppler H., Vogt F., 2015 Phenology and occurrence of Spotted Wing Drosophila in Germany and case studies for its control in berry crops. IOBC Bull. Working Group “Integrated Protection of Fruit Crops, Subgroup Soft Fruits”, Vol. 109: 233-237.

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Task 1.6. Explore SWD overwintering and determine whether SWD overwinters in UK fruit crops, including dead plant material and polytunnel structures (EMR; [JHI]; Years 3-4)

Raspberry cuttings were collected at 4 different sites. They were placed in polythene bags with a precision monitoring trap for 3 weeks (Fig. 1.6.1). The traps were checked on a weekly basis for *D. suzukii*. None were found on any of the sites, however small numbers of anthocorids were observed to emerge. We are currently trying different methods (bags and cages) of extracting from woodlands with very high populations of *D. suzukii.* To date *D. suzukii* has not been found in leaf litter. This does not mean it is not using this resource.

Figure 1.6.1. Polythene bags containing raspberry cuttings and a precision monitoring trap

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. (Years 1-4)

Task 2.2. Laboratory determination of environmental conditions needed to eliminate SWD and the pest attractiveness from the waste

Previous results showed that anaerobic treatment of soft fruit waste in 200-500L sealed vessels for 24 hours at ambient summer air temperatures of 15°C and above was sufficient to eradicate *Drosophila melanogaster* from the waste. However, for stone fruit, and at ambient air temperatures below 15°C for both soft and stone fruit, a longer period may be needed. Work in Year 3 has examined the effect of anaerobic treatment of soft and stone fruit wastes at different temperatures on *D. melanogaster* and *D. suzukii.*

Materials and methods

Fruit waste anaerobic treatment in sealed vessels

The method for treating fruit waste in sealed vessels and measuring the gas composition and waste temperature in the vessels was described in the Year 1 and Year 2 reports. Eggs, larvae and pupae of *D. melanogaster* and *D. suzukii* on separate infested fruit samples (300 g) were inserted in the soft and stone fruit waste batches at the start of each test in sealed bins. The tests were conducted at GH Dean and Lower Hope Farm (cherries), Pershore Centre (plums), and EMR (strawberries).

Figure 2.2.1. Anaerobic treatment of cherry and strawberry wastes in sealed vessels

Testing for eradication of Drosophila species

The samples of infested waste fruit from the above vessels were placed in plastic containers and covered with a fine mesh to exclude any *Drosophila*. Similar batches of untreated *D. melanogaster* or *D. suzukii* infested fruit waste were prepared. After three weeks, the containers were checked for any *D. melanogaster* or *D. suzukii* adults that may have emerged from the waste and the numbers counted.

Results

Fruit waste anaerobic treatment in sealed vessels

The batches of soft fruit waste degraded more rapidly than the stone fruit waste batches. Within two days, the soft fruit waste had started to soften and exude liquid, whereas the stone fruit waste batches remained largely intact.

Oxygen concentration in the headspace of the bins rapidly became depleted and was not detectable in any of the soft or stone fruit batches after 6 h (Fig. 2.2.2). In soft fruit at 18-25°C, there was a corresponding increase in CO_2 concentration which exceeded 20% v/v within 6 h and reached 80% v/v within 24 h. In some of the stone fruit batches at 14-16°C, 3 days were required to reach 80% CO_2, the maximum detection limit of the CO_2 detection tubes (Fig. 2.2.3). Ambient air temperatures were 2 to 5°C lower than those of the wastes.

Adult *D. melanogaster* subsequently emerged from all untreated fruit waste samples, and from soft fruit samples taken from bins treated for up to 24 h after the bins were sealed (Fig.2.2.4). A small proportion of adults, about 5% of the numbers emerging from untreated waste, emerged from stone fruit wastes treated for 3 days (Fig. 2.2.3).

The results for emergence of *D. suzukii* from treated fruit wastes were similar to those for *D. melanogaster* (Fig. 2.2.5). No adult *D. suzukii* emerged from soft fruit waste treated in sealed bins for 2 days at 18-25°C, but two adult *D. suzukii* emerged from stone fruit waste treated for 2 days at 14-16°C, compared with 75 adults emerging from untreated waste.

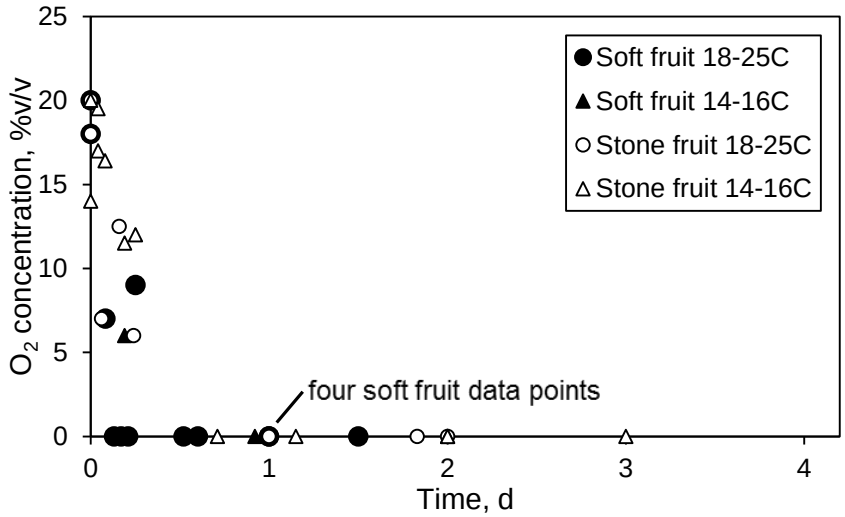

Figure. 2.2.2. Oxygen concentration in the headspace of sealed bins containing fruit waste

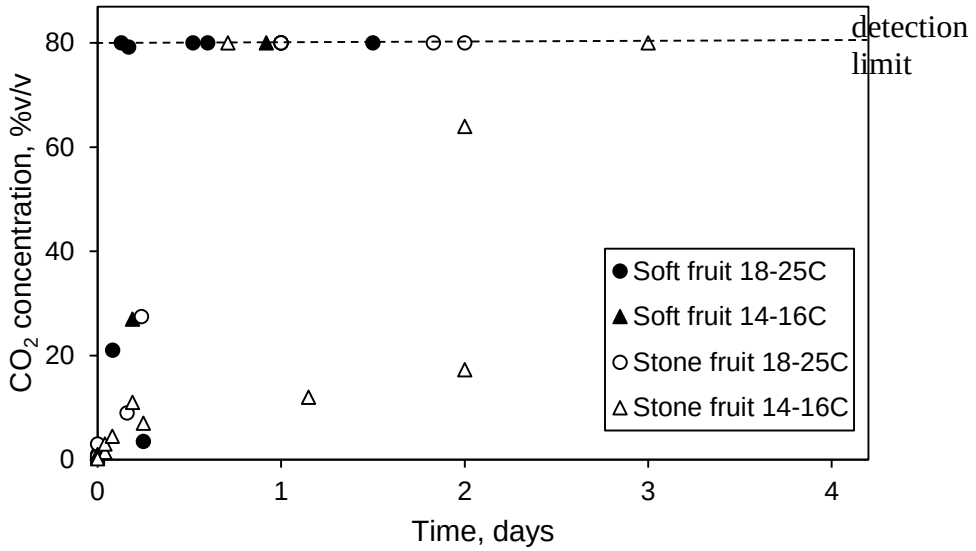

Figure 2.2.3. Carbon dioxide concentration in the headspace of sealed bins containing fruit waste

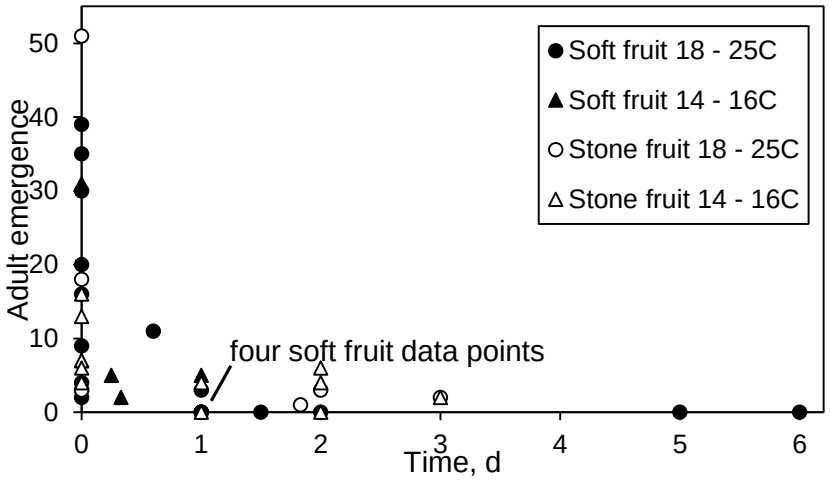

Figure 2.2.4. *Drosophila melanogaster* emergence after different durations in sealed bins

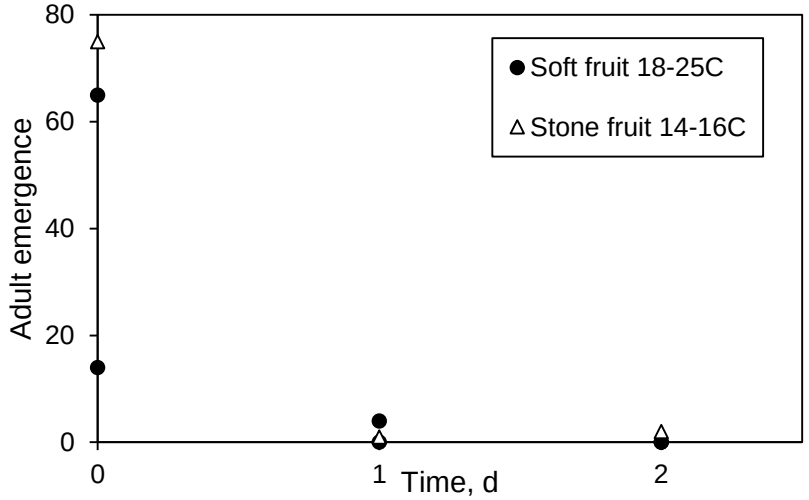

Figure 2.2.5. *D. suzukii* emergence after different durations in sealed bins

Task 2.3. Composting, digestion and other processing of fruit wastes

Further enquiries were made into the feasibility of composting and digestion of treated and untreated wastes. Due to the high moisture content and low calorific value of fruit wastes, disposal to composting or digestion plants is not a financially attractive option due to transport and gate fee costs. One fruit farm disposed of fruit wastes to a yoghurt factory but due to unreliable demand and low prices, this disposal route became unviable.

Task 2.4. Development of fruit waste collection and storage strategies and Task 2.5. Optimisation of large-scale waste treatment systems for Standard Operating Procedure

Several fruit farms were visited to examine large-scale treatment and disposal of treated fruit wastes. At one site, soft fruit waste was filled into 10 m^3 vessels for 4 days. The waste was then emptied with a slurry tanker (Fig. 2.4.1 left) and emptied into a slurry lagoon (Fig. 2.4.1 right). There were several plum trees around the edge of the lagoon. The plums were sampled and tested for the emergence of *D. suzukii* in late August and mid-September as described above. No *D. suzukii* emerged from the samples, indicating that the slurry lagoon was not attracting *D. suzukii* and no *D. suzukii* were emerging from the waste emptied into it.

At a second site, cherry waste was treated in sealed pallet bins for 4-5 days and then mixed with straw+ cattle manure (Fig. 2.4.2) before field spreading. At a third site, strawberry waste was treated in pallet bins for 4 days and then spread on to bare soil with a tractor mounted rotavator (Fig. 2.4.3). No Drosophila were observed in the treated areas on the field. The maximum amount of organic waste that can be applied to land is restricted by the EU Nitrates Directive in Nitrate Vulnerable Zones to 250 kg N/ha. Fruit wastes contain about 0.2% N fresh weight, so that up 125 tonnes fruit waste can be applied per hectare each season. This must not be done during the 'closed season' for land-spreading (October to March) and a record must be kept of the amount of waste applied.

At two further sites, large quantities of coir were available from spent grow bags, and at one of the sites this was used to cover fruit waste. The depth of the coir covering layer was about 0.2 m. There was some seepage of brown liquid from heaps after several weeks but this was not attractive to Drosophila. After a sufficient time period (not established), the coir/fruit waste mix should be suitable as an organic soil amendment.

Figure 2.4.1. Treatment of soft fruit waste in 10m^3 vessel (left) and disposal using slurry tanker into slurry lagoon containing cattle slurry (right).

Figure 2.4.2. Treatment of cherry waste in pallet bins (left) and cattle manure used for disposal (right).

Figure 2.4.3. Field area with treated strawberry waste.

Summary

- Treatment of soft fruit waste in sealed bins for 2 days at waste temperatures of at least 18°C will ensure eradication of *Drosophila* species including *D. suzukii*.
- If waste temperatures are below 16°C, a 3-day sealed bin treatment should be used for soft fruit.
- There can be a low level of survival of *D. melanogaster* and *D. suzukii* (5% of the original population) in stone fruit waste treated for 3 days, particularly if the waste temperature is below 16°C.
- A 4-day treatment should be used for stone fruit; a 5-day treatment may be needed at waste temperatures of 16°C or less, but this requires further investigation.
- Oxygen depletion in the sealed bins occurred within 6 hours, irrespective of the type of fruit waste or waste temperature. There was also a rapid increase in CO_2 concentration in sealed bins of soft fruit waste at 18-25°C. However, achieving a high concentration (80% v/v) of CO_2 occurred more slowly in stone fruit at temperatures below 16°C. Further CO_2 measurements are required of soft fruit below 16°C, and stone fruit at 18-25°C.
- The higher risk of *D. suzukii* survival in batches of stone fruit waste, particularly at lower ambient temperature, may be explained by the greater stability of the waste, and slower increase in CO_2 concentration than in soft fruit waste.
- Mixing the treated waste with at least 90% w/w with other manures, slurries or other organic wastes is a suitable disposal route.
- Rotavation of treated waste into soil to a depth of 20 cm is also a suitable disposal route. The rate of application of treated waste to land should not exceed 125 tonnes/ha.

Objective 3. To develop and evaluate sampling and extraction methods for quantifying *D. suzukii* infestations in different soft and stone fruits. (Years 1-3).

Task 3.2. Optimise fruit sampling methods for quantifying numbers of SWD larvae in field crops and harvested fruit (Years 2 and 3)

Materials and methods

Infestation of fruit

Supermarket bought fruit (100g of blueberry, cherry, raspberry or strawberry) was added to the base of a plastic box (228 x 121 x 66 mm) (Fig. 3.2.1) after being washed. Only healthy, undamaged, fruit were used. Data was normalised against results from dissection of raspberry fruit infested at the same time.

Figure 3.2.1. Fruit incubation box.

D. suzukii (10 females and 5 males per box) were added to infest the fruit, the boxes were sealed with electrical tape to prevent escape, and incubated at 20°C for 24 hours. After 24 hours the adult flies were removed and the fruit incubated at 20°C for 7 days (for 3rd instar larvae) or 4 days (for 1-2 instars).

Treatments

For assessment of third instar *D. suzukii* larvae three methods were compared to two controls, i) manual dissection of fruit and counting of larvae, and ii) counts of adult emergence. Each treatment was replicated 6 times and repeated on two separate occasions. The methods of larval assessment were;

1. *Sugar immersion:* Fruit (100g) was placed in a clear plastic bag and gently crushed as this increases larval extraction by 50% compared to use of whole fruit (Dreves *et al.,* 2013). Fruit was then covered with a sugar solution (180g /l water) and 1-2 drops spray tank de-foamer and observed for 20 mins, with gentle mixing at 10 m.
2. *Salt immersion:* As above, but fruit were covered with a salt solution (75g /l water) and 1-2 drops spray tank de-foamer and observed for 20 mins, with gentle mixing at 10 m.
3. *Freezing:* Fruit (100g) was placed in a clear plastic bag and frozen overnight. Reportedly, large larvae will exit the fruit and die on the surface. Fruit was examined visually next day.
4. *Direct observation*: Fruit (100g) was dissected under a binocular microscope and larvae observed directly.
5. Adult emergence: Fruit (100g) was incubated at 20°C until any adults emerged and these were counted.

NB: Strawberries were quartered before immersion and freezing.

Visual counts were made of swimming larvae in the salt and sugar treatments, of emerged, dead larvae for the frozen fruit and under a binocular microscope for the larvae in dissected fruit. Counts were made of adults for emergence treatments. The data was normalized against the raspberry dissection results. The two trials for each fruit were combined and analysed by General ANOVA. When the two trials were statistically different to each other they could not be combined and were analysed separately.

For later trials on small larvae it was decided to discard freezing as ineffective and replace it with a method recommended by Catherine Baroffio (Agroscope Centre de recherche Conthey, Switzerland) consisting of 5 minutes immersion in a weak detergent mixture followed by filtering through a mesh and counting under a microscope.

Note that a further method in the literature involves boiling the berries in 150 ml of water for three minutes and then crushing them over a screen with the back of a spoon and rinsing the fruit with cold water (Issacs, 2011). This method appeared to be difficult to apply in the field and so was not trialled here, but it remains an option.

Results

3rd instar larvae

In general, sugar solution gave the highest recovery of larvae; salt solution second and freezing gave the lowest counts (Fig. 3.2.2). However, there were no statistically significant differences between sugar and salt solutions for third instar larval extraction. Sugar solution immersion and freezing were only significantly different for blueberries and cherries.

The two cherry trials were significantly different to each other, and although combined for the purposes of Fig. 3.2.2, they were also analysed separately. In each, the same trend was observed, but in the first trial sugar solution immersion gave significantly higher recovery compared to the salt solution and freezing, whereas in the second trial the differences between the treatments were not significant.

Immersion in carbonated water was also investigated, but abandoned after one trial as there were major difficulties in seeing the larvae (data not shown).

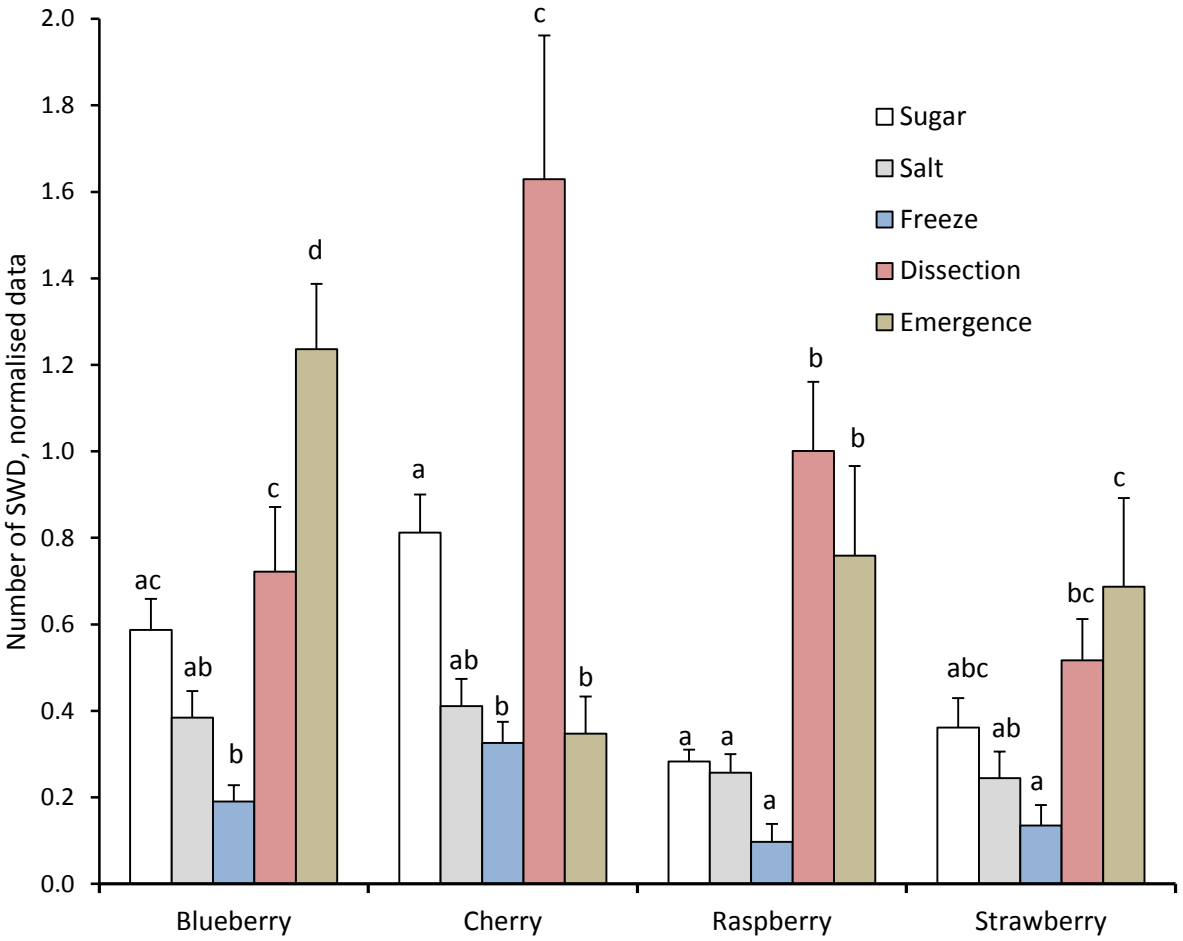

Figure 3.2.2. Comparison of methods for recovery of 3rd instar *D. suzukii* from fruit. Data normalised by comparison to raspberry infestation in the same trial. Values per fruit with the same letter are not significantly different (P<0.05)

1st and 2nd instar larvae

For blueberries, the sugar and salt methods were not significantly different in either of the two trials. The detergent method gave consistently lower numbers of *D. suzukii* than salt in the first trial and sugar in the second trial (Fig. 3.2.3).

The first trial on cherries with sugar gave significantly higher *D. suzukii* larvae with sugar than salt and both were significantly higher than using detergent and filtering. The second trial on cherries showed no significant difference between the three methods.

The first trial on raspberries showed a similar pattern to cherries, with sugar yielding significantly higher *D. suzukii* than salt in cherries and both were significantly higher than using detergent and filtering. A second raspberry trial found no larvae in the fruit. A third found no significant difference between the treatments.

All three trials on strawberries showed no, or very low, infestation despite the strawberries being quartered. The reasons for this are not known

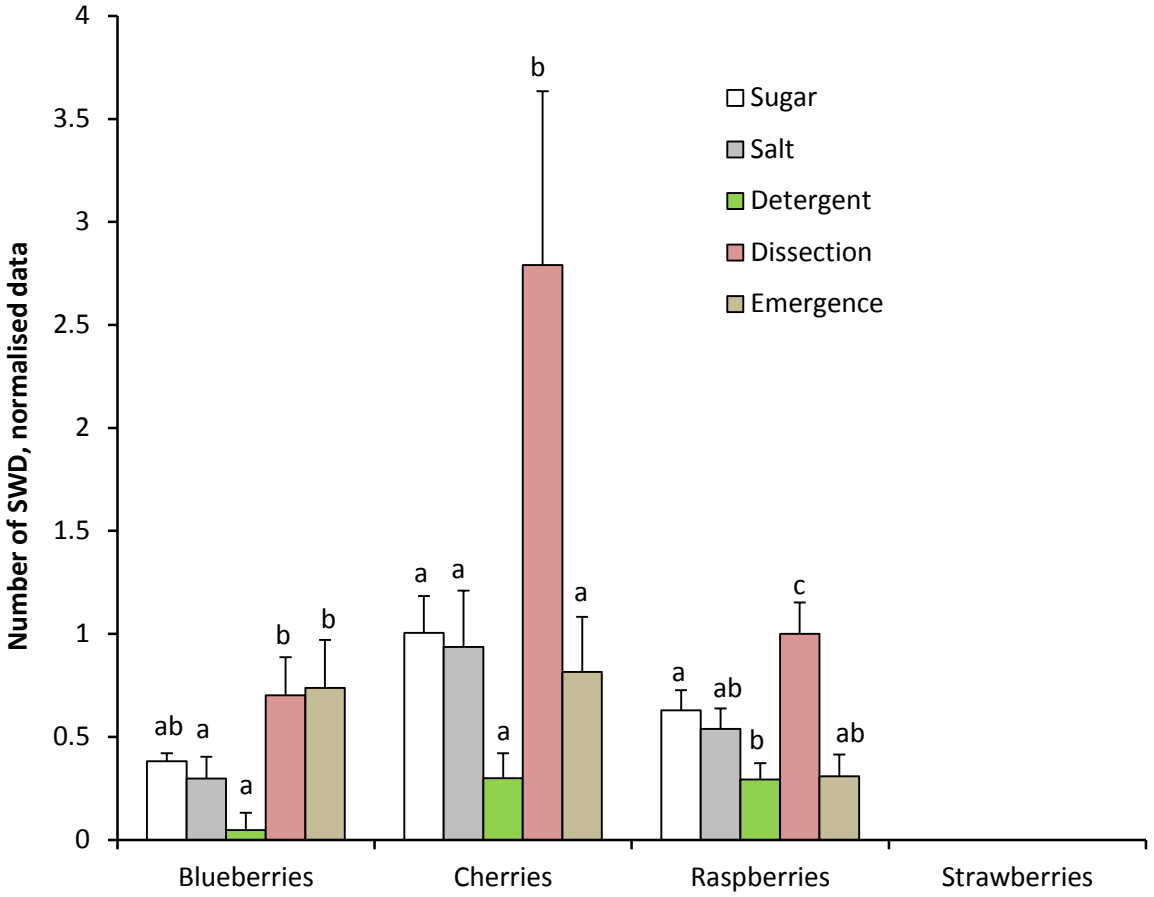

Figure 3.2.3. Comparison of methods for recovery of 1st and 2nd instar *D. suzukii* from fruit. Data normalised by comparison to raspberry infestation in the same trial. Values per fruit with the same letter are not significantly different (P<0.05)

Summary

- Sugar immersion gave the highest recovery of third instar *D. suzukii* larvae in blueberry, cherry raspberry and strawberry, although the recovery in raspberry was less efficient (~25%). In general, this method gave better recovery than salt solution immersion or freezing, although the difference between sugar and salt immersion was not statistically significant. The situation with smaller larvae is less clear cut, but there was again a trend for sugar to give higher counts.
- The concentration of sugar trialled was relatively high, but corresponded to suggested concentrations in the literature. Although lower concentrations have been recommended (eg. 56g/l, Issacs, 2011), they have been found to be less effective in comparison (Hueppelsheuser, 2010). Salt solution immersion also gave good results in our trials, and this method has been recommended by some authors (for example, Fisher, 2015). It has the additional advantage of being cheaper.

References

Dreves, A.J., Ohrn, A., Cave, A., Coop, L., Bruck, D., Lee, J., Little, R. (2013). Prevention: From crisis management to IPM programming, lessons and challenges. Annual Pacific Northwest insect management conference, January 7th, 2013.

Fisher, P. (2015). Salt water assessments for SWD larvae in fruit. Ontario Ministry of Agriculture, Food and Rural Affairs. http://www.omafra.gov.on.ca/english/crops/facts/swd-saltwater.htm

Hueppelsheuser, T. (2010). Assessment of solutions used for the purpose of determining Spotted Wing Drosophila larval infestation in blueberry fruit. British Colombia Ministry of Agriculture, August 2010 http://whatcom.wsu.edu/ipm/swd/documents/LavalExtraction.pdf

Issacs, R. (2011). Comparison of fruit sampling methods for spotted wing Drosophila in blueberries. http://msue.anr.msu.edu/news/comparison_of_fruit_sampling_methods_for_spotted_wing_drosophila_in_blueber

Task 3.3. Produce protocols giving standard extraction and sampling methods for use in different crops in the UK (year 3)

The data from section 3.2 was used to prepare a protocol, in conjunction with Scott Raffle of the Agriculture and Horticulture Development Board, for distribution to UK growers (Fig. 3.3.1).

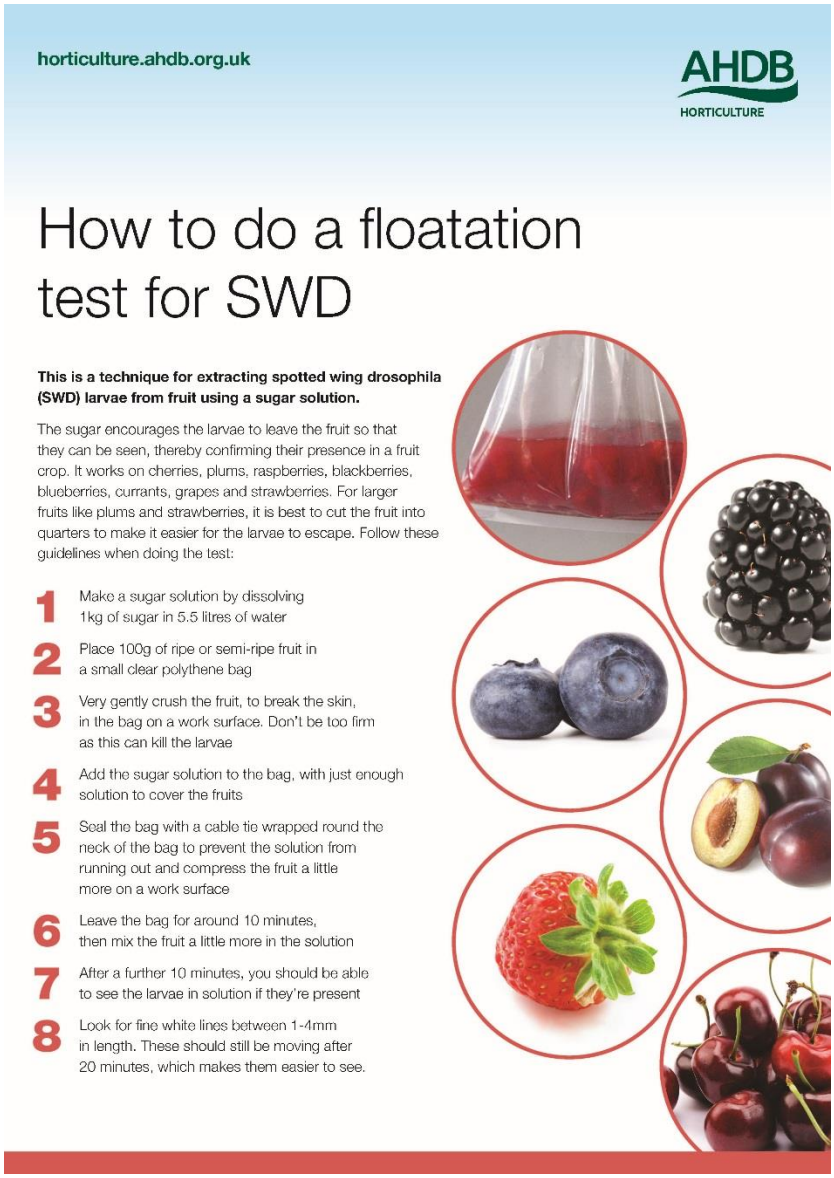

Figure 3.3.1. Protocol for extracting SWD from fruit using a sugar solution

Task 3.3. Evaluating intra species competition for egg laying sites

Materials and methods

The trial was repeated twice; once with a cornmeal diet and once on raspberry fruit ('Autumn Treasure' and 'Autumn Amber' both grown at East Malling Research with minimal sprays applied).

A standard cornmeal diet (600 ml dH_2O, 6.5 g Fisher agar, 52.5 g table sugar, 52.5 g precooked ground maize, 11.5 g baker's yeast, 1 g methylparaben (dissolved in 10 ml 70% ethanol)) was offered as an egg laying resource. 5 ml was decanted into 5 cm petri dishes using a syringe and left to set and cool within a fume hood for 1 hour before having the lids replaced.

For the raspberry experiment, fruit was washed under distilled water for 30 seconds and then left to dry within a quarantine facility at East Malling Research for 30 minutes. The berries were then frozen for 48 hours to kill any existing eggs or larvae that may have been in the fruit. The fruit was defrosted 24 hours before being used in the trial.

Either a cornmeal petri dish or 5 berries per 5 cm petri dish were put into 12 x 7 x 7cm ventilated Perspex boxes.

Treatments

For Treatment 1, 5 female and 2 male *D. melanogaster* were introduced to a box for 48 hours. Treatment 2 was the same except using *D. suzukii* adults. After this period the adults were removed and a 'blank' petri dish of cornmeal/fruit was added. Five female and 2 male *D. suzukii* for Treatment 1 and the same number of *D. melanogaste*r for Treatment 2 were introduced to the Perspex boxes for a further 48 hours. At this stage Treatment 3 was inoculated with 5 female and 2 male *D. suzukii*. After the 48 hour period all flies were removed from the boxes and Petri dishes were transferred into individual ventilated boxes. These were kept at 25°C for the remainder of the experiment (Table 3.3.1).

Table 3.3.1. Treatments

Treatment Number	1st Inoculation	2nd Inoculation
1	*D. melanogaster*	*D. suzukii*
2	*D. suzukii*	*D. melanogaster*
3		*D. suzukii*

Assessments

Petri dishes were frozen 16 days after the inoculation of *D. melanogaster* (see discussion) for 48 hours before assessment. Assessments were exact counts of male and female *D. suzukii* and *D. melanogaster* under a microscope so species and sex could be identified.

Results

Significantly more *D. suzukii* emerged from blank Petri dishes than dishes previously exposed to *D. melanogaster*, in both repeats of this trial. There was no difference between the numbers of *D. melanogaster* that emerged from dishes where either blank or *D. suzukii* had laid eggs (both trials). There was no significance between the numbers of *D. suzukii* that emerged from dishes that had *D. suzukii* eggs and the controls (first trial). In the second trial, on raspberry, more eggs were laid in the blank dish. However, numbers of *D. suzukii* emerging in this trial, overall, were very low. Hence this is more likely to be a density dependence effect. i.e. in the first trial the availability of egg laying was probably at its maximum, but in the second trial there were 2 opportunities to lay eggs in the blank dishes. Furthermore the dishes were not saturated with eggs (Figs. 3.3.1a & 3.3.1b).

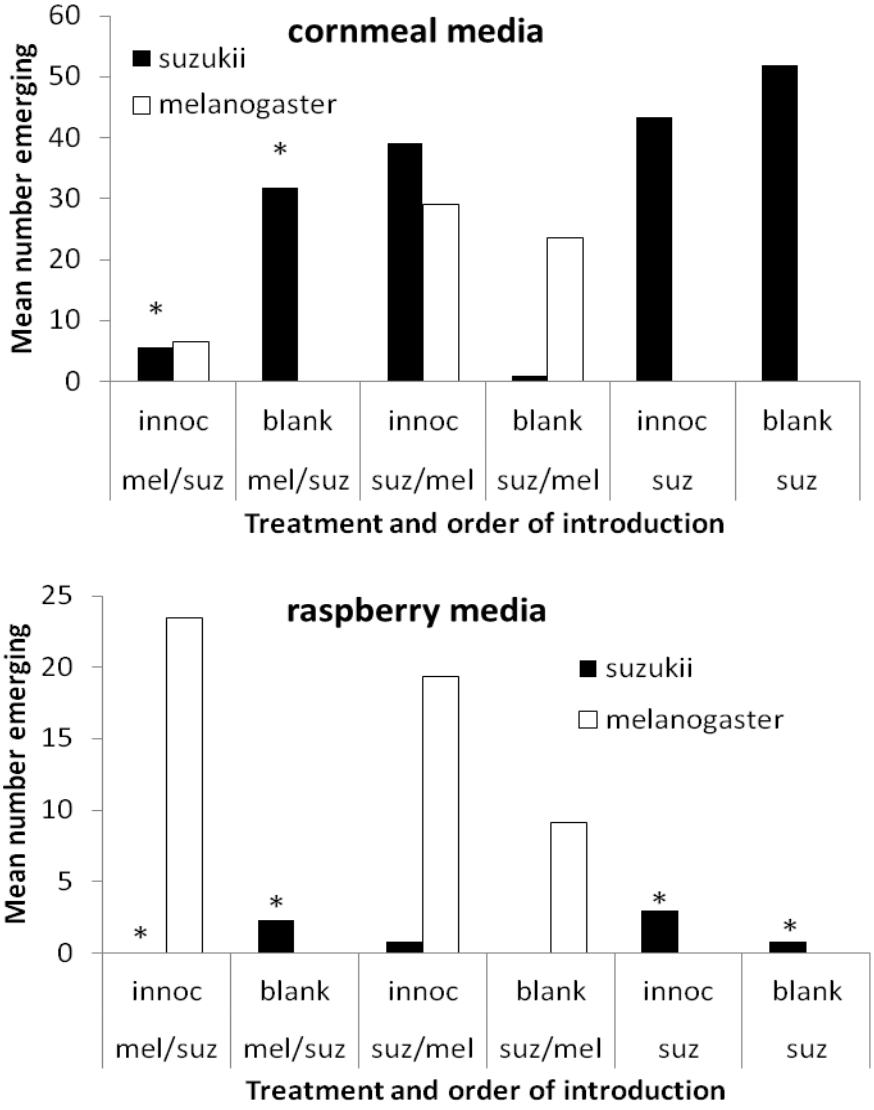

Figure 3.3.1. Mean number of *Drosophila* that emerged from cornmeal and raspberry. *indicates significant difference between pre-inoculated and blank dishes within treatments (order of *Drosophila* introduction).

Conclusions

The results indicate that given a choice *D. suzukii* prefer to oviposit into a resource that has not been exposed to *D. melanogaster.* The latter do not seem affected by previous exposure to *D. suzukii* egg laying.

The niche that *D. suzukii* occupies means that they do not need to compete with other *Drosophila* species for egg laying sites. Their invasion success is due to the development of targeting ripening and under ripe fruit. As *D. melanogaster* compete for egg laying with other UK species, it appears that the females oviposit into fruit that has already been inoculated with the eggs of *D. suzukii*. It is not known whether this is true for other species.

As *D. melanogaster* and *D. suzukii* have different development times the duration of the trials was critical. For *D. melanogaster*, at 25 degrees it takes a minimum of 7 days from egg laying to next generation emergence and a further 48 hours before newly emerged females are able to oviposit viable eggs (Tyler 2000). Hence, it would mean that a third generation would be emerging after a minimum of 16 days. The minimum development time for *D. suzukii* at 25 degrees is 11 days (personal observation). To prevent counting the third generation of *melanogaster* but to allow enough time for *D. suzukii* to emerge, Petri dishes were frozen 16 days after the first inoculation. Newly emerged *D. melanogaster* were not included in the analysis.

References

Tyler, M.S. (2000) Developmental Biology, A Guide for Experimental Study, Second Edition, 2000 Sinauer Associates, Inc. Publishers, Sunderland, MA ISBN 0-87893-843-5

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

Task 4.1. Develop synthetic lure for SWD (EMR, NRI, Years 1-2)

Testing commercially available and experimental baits

Objective: To determine the efficiency of bait and trap designs for trapping *D. suzukii*

Materials and Methods

The trap comparisons were deployed in Rookery field at EMR. Traps were greater than 10 m apart and hung from a branch on the cherry trees (mainly cv. Penny). There were 6 replicates of each treatment. Assessments were counts of male, female and 'other' <5 mm and >5 mm insects.

Test 1: Treatments were a combination of different trap designs, lures and liquid baits (Table 4.1.1). NRI baits were acetoin, ethanol, acetic acid and methionol sachets. Traps were deployed on 28th July and checked weekly for 3 consecutive weeks (4th, 11th, 19th August). Liquid was filtered from the traps each week through a paint filter and replaced into the trap. On 11th August the DrosAttract was replaced as per label instructions. Diatomaceous earth was sieved through a metal sieve. Agralan and Sentomol supplied many of the baits and traps needed for this study. Others were sourced directly from the manufacturers. Data was analysed using ANOVA on log10 transformed data in Genstat.

Test 2: Two treatments were deployed; Treatment 1: Precision monitoring traps (Fig. 4.1.1) renewed every 4 weeks and Treatment 2: Precision monitoring traps emptied and refreshed with DrosAttract liquid every 4 weeks. The precision monitoring traps were set up on 9th September and placed in two rows of the Rookery Field cherry orchard at EMR. On the 07th October the traps were replaced or refreshed with DrosAttract. Traps were then filtered on the 4th November and 2nd December. The liquid was emptied onto the ground near the trap.

Figure 4.1.1 Precision monitoring trap from Riga (supplied by Agralan) containing DrosAttract liquid.

Table 4.1.1. Table of treatments for testing commercially available traps and baits. ACV = apple cider vinegar.

Trap	Lure	Liquid bait	Company
New Droso trap (Biobest) 	-	Fruit Fly Attractant 	Koppert
	Pherocon 	ACV 	Trécé
	Russel IPM solid bait 	Water and detergent	Russel IPM
	Dry lure NRI 	Water and detergent	-
		Diatomaceous earth 	-
	-	DrosAttract 	Biobest
	-	Super Gasser 	Riga

Scentry trap 	Scentry lure 	Water and detergent	Scentry
Pherocon trap 	Pherocon 	ACV 	Trécé

Assessment of volatiles from the baits tested

Volatiles from the various baits (1 ml) were collected by drawing charcoal-filtered air over the sample (2 litre/min) for 1 hr at 20-22°C and trapping the volatiles on Porapak Q (200 mg; 50-80 mesh). Trapped volatiles were desorbed with dichloromethane (1 ml) and decyl acetate (5 µg) added as internal standard. The samples were analysed quantitatively relative to the decyl acetate by GC/FID using a polar DBWax column (30 m x 0.32 mm i.d. x 0.125 µ film thickness), splitless injection (220°C), helium carrier gas (2.4 ml/min) and oven temperature programmed at 50°C for 2 min then at 10°C/min to 250°C. Compounds were identified by Gas Chromatography /Mass Spectrometry (GC/MS) on a CP3800 GC coupled directly to a Saturn 2200 MS using a similar column and temperature programme. Compounds were identified from their retention times, mass spectra and comparison with synthetic standards.

For measurement of the amounts of ethanol, acetic acid, acetoin and 3-methylbutanol in the baits, the solution (100 µl) was dissolved in distilled water (5 ml) and acetone (5 mg) added as internal standard. The solutions were analysed by Gas Chromatography – Flame Ionisation Detector on a capillary column (10 m x 0.32 mm i.d.) coated with Poraplot Q. Injection was splitless (200°C) and the oven temperature was programmed from 60°C for 2 min then at 10°C/min to 200°C.

Results

Test 1: The Drosotrap design was effective at capturing significant numbers of *D. suzukii* over the 3 week period of the test when baited with Super Gasser, Koppert or DrosAttract liquid or the NRI dry bait with a water and detergent drowning solution. The next group of traps catching approximately half the number of *D. suzukii* compared to the Super Gasser in the Drosotrap were the dry Pherocon bait with apple cider vinegar (ACV) in either the Pherocon trap or the Drosotrap and the dry Scentry lure with water and detergent as a drowning solution. Using diatomaceous earth instead of water and washing up liquid was not effective at capturing *D. suzukii* (Fig. 4.1.2, Table 4.1.3).

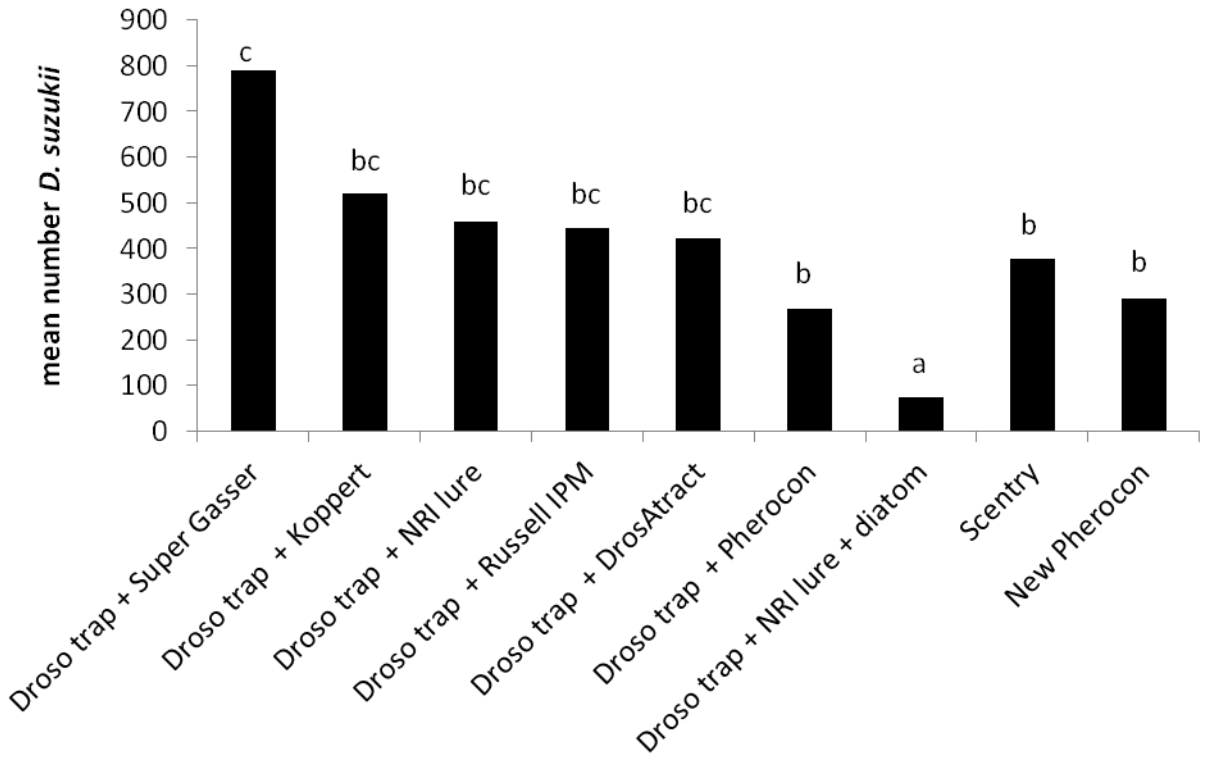

Figure 4.1.2. Mean numbers of *D. suzukii* captured in the traps over 3 weeks

Because the Drosotrap has larger holes there is the potential for large insects (>5 mm) to get into the traps (Fig. 4.1.3, Table 4.1.3). There were significantly larger insects in the Drosotraps baited with Super Gasser, Koppert, Russel IPM, and DrosoAttract lures than the NRI dry lure or the Pherocon dry lure in the Drosotrap. The Diatomaceous earth tended to capture many earwigs. The Pherocon dry bait in the Drosotrap or the Pherocon trap and the Scentry lure in the Scentry trap caught fewer large insects.

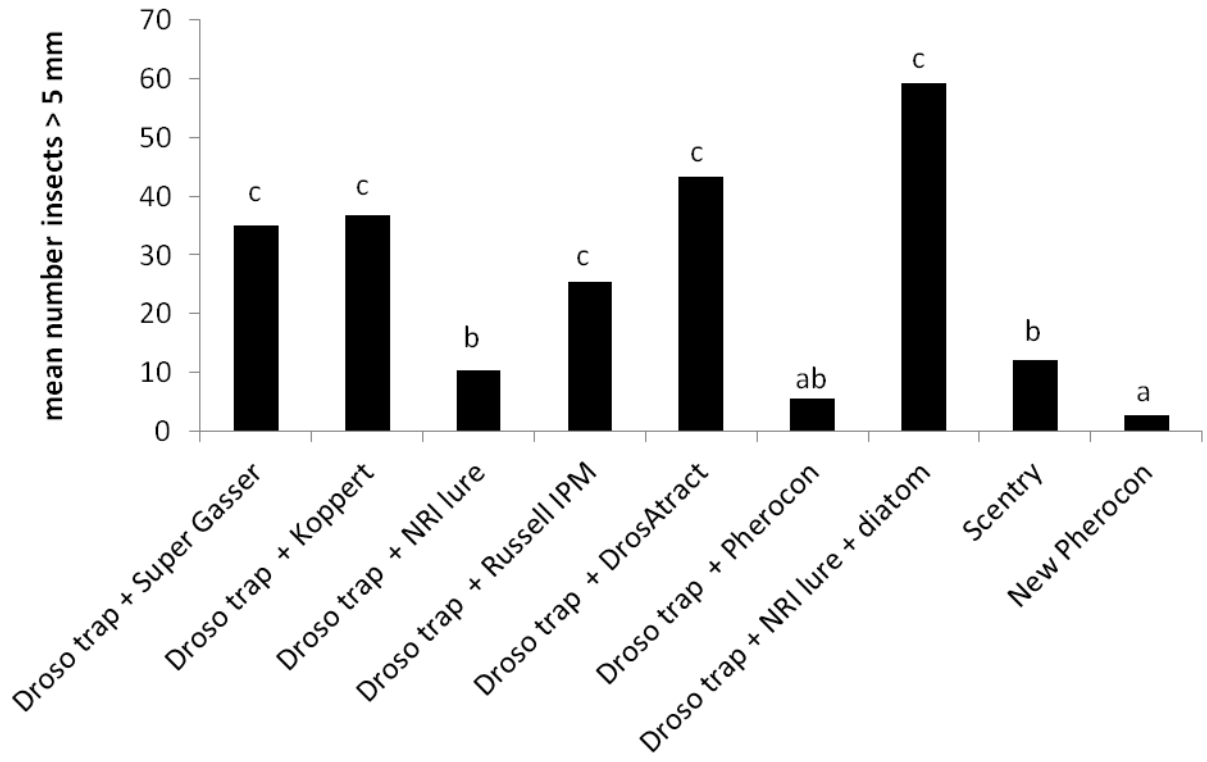

Figure 4.1.3. Mean numbers of large insect trapped in *D. suzukii* traps

Figure 4.1.4 shows the species specificity of the traps. Traps catching a larger proportion of *D. suzukii* compared to 'other' insects were the Super Gasser, the NRI dry bait and the DrosAttract in the Drosotrap and the Pherocon bait in the Pherocon trap. This is important when monitoring for the pest as sorting through 'other' insects is time consuming. The NRI dry bait captured almost 3.5 times the number of *D. suzukii* compared to 'other' insects. The Scentry trap and bait were less *D. suzukii* specific capturing double the amount of 'other' insects compared to *D. suzukii* (Table 4.1.3).

Traps also differed in their ease of use and comments on this are made in Table 4.1.2.

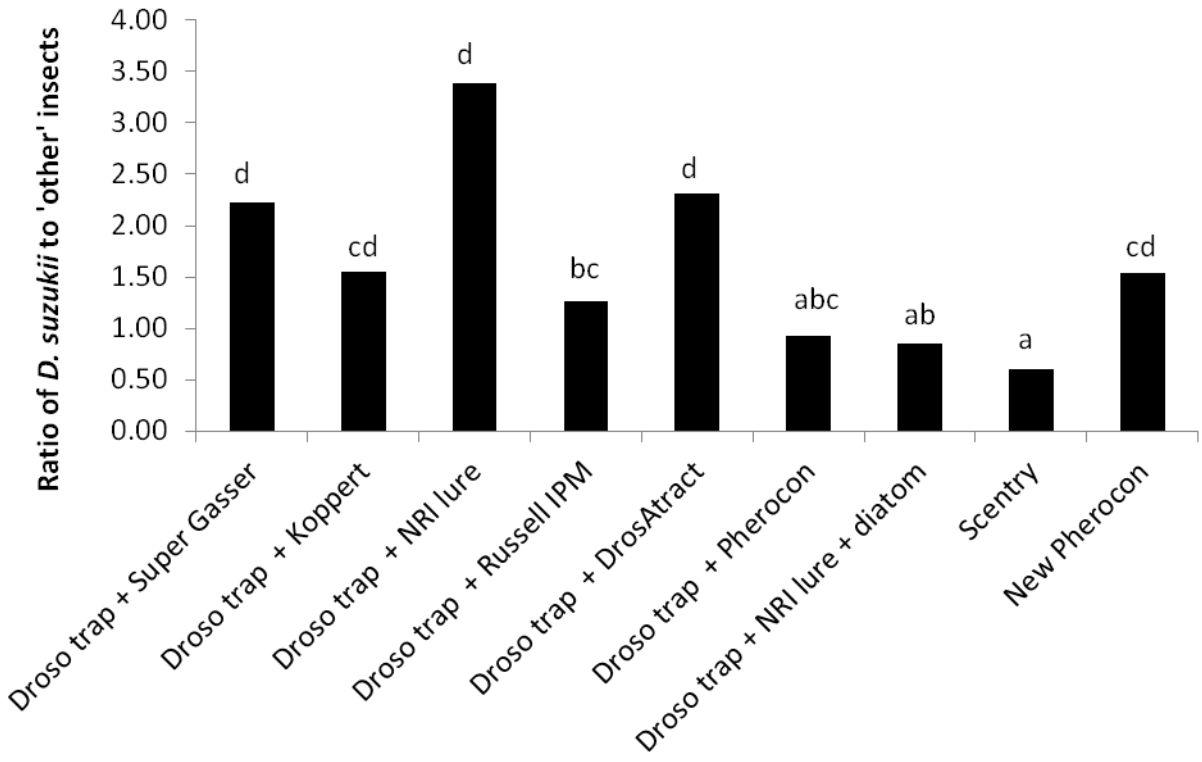

Figure 4.1.4. The ratio of *D. suzukii* to 'other' insects in each trap and bait tested

Table 4.1.2. Comments on the ease of use of the traps for field trapping

Trap	Lure	Liquid bait	Comments
New Droso trap (Biobest)	-	Koppert	Liquid requires refrigeration. Viscosity increases in field after 3 weeks.
	Pherocon	ACV	New hole and hook required to attach lure.
	Russel IPM solid bait	Water and detergent	New hole and tape required to attach lure.
	Dry lure NRI	Water and detergent	New hole and hook required to attach lure.
	Dry lure NRI	Diatomaceous earth	Impractical. Insects dry out. Many earwigs in traps.
	-	DrosAttract	Could be difficult if liquid congealed.
	-	Super Gasser	Samples difficult to examine when liquid becomes congealed.
Sentry trap	Sentry lure	Water and detergent	Easy to use with screw top lid. Can see flies through trap. Few large insects. Not as specific for *D. suzukii*.
Pherocon	Pherocon	ACV	Difficult to open lid. Needs larger hole to attach lure.

Table 4.1.3. ANOVA table of the mean numbers of male SWD, female SWD, Overall SWD, insects larger and less than 5mm which were not *D.suzukii* and the ratio of *D. suzukii* to by- catch. Significant differences are shown as different letters in the same column.

	Female			Male			Total			Insects >5mm			Insects <5mm			Ratio Of SWD		
Trap and Bait	mean	Log10		mean	Log10		mean	Log10		mean	Log10		mean	Log10		mean	Log10	
Droso trap + Super Gasser	238	2.31	c	554	2.69	c	791	2.84	c	35	1.51	c	318	2.47	bc	2.23	0.5	d
Droso trap + Koppert	157	2.02	bc	363	2.41	bc	520	2.56	bc	36.8	1.49	c	283	2.37	bc	1.55	0.39	cd
Droso trap + NRI lure	220	2.3	c	240	2.32	b	460	2.61	bc	10.3	1.03	bc	144	2.1	b	3.39	0.62	d
Droso trap + Russell IPM	256	2.38	c	190	2.24	b	445	2.61	bc	25.5	1.29	c	582	2.53	c	1.26	0.34	bc
Droso trap + DrosAtract	173	2.23	bc	248	2.37	bc	421	2.61	bc	43.3	1.49	c	146	2.12	b	2.31	0.52	d
Droso trap + Pherocon	107	1.98	b	162	2.12	b	269	2.36	b	5.5	0.76	ab	354	2.41	bc	0.93	0.28	abc
Droso trap + NRI lure + diatom	26	1.39	a	48	1.62	a	74	1.82	a	59.2	1.58	c	44	1.52	a	0.85	0.25	ab
Scentry	124	2.03	bc	253	2.3	b	377	2.49	b	12	0.966	b	1062	2.85	c	0.6	0.19	a
New Pherocon	108	2.02	bc	183	2.26	b	290	2.45	b	2.7	0.54	a	228	2.31	bc	1.54	0.38	cd
F. prob		<.001			<.001			<.001			<.001			<.001			<.001	
rep.		6			6			6			6			6			6	
d.f.		40			40			40			40			40			40	
s.e.d.		0.144			0.159			0.148			0.196			0.18			0.063	
l.s.d.		0.29			0.321			0.3			0.396			0.364			0.127	

Test 2: On average more *D. suzukii* were caught in the precision monitoring traps that were refreshed with new liquid (100.25) than the ones that were completely replaced (93.3) but the difference was not statistically significant (Table 4.1.4). Results also indicated that the percentage of *D. suzukii* compared to by-catch was significantly higher when the liquid in the traps was refreshed (43.3%) than when the traps were completely replaced (31.6%) (Table 4.1.4). This is important when monitoring for the pest as sorting through 'other' insects is time consuming. When analysing costings we found that 5l of DrosAttract costing £22 would be enough to refresh 71 precision monitoring traps. Meanwhile a box of 40 new precision monitoring traps cost £35 (Agralan, 2015). Therefore using DrosAttract to refresh the precision monitoring traps instead of replacing them is a much more cost effective and specific method of catching *D. suzukii*. In addition it also reduces waste from many hundreds of plastic and foil devices.

Table 4.1.4. ANOVA table of mean numbers in precision monitoring traps that were completely replaced or had the liquid refreshed with DrosAttract. Male, female and total *D. suzukii* (SWD), insects larger or less than 5mm which were not SWD and the mean percentage of SWD Significant differences are shown as different letters in the same column.

Treatment	Male			Female			Average SWD			Other			>5mm			% SWD in catch		
	mean	$\sqrt{n}$		mean	$\sqrt{n}$		mean	$\sqrt{n}$		mean	$\sqrt{n}$		mean	$\sqrt{n}$		mean	angular	
Replaced	40	5.76	a	54	6.2	a	93	8.52	a	302	14.8	a	3	1.67	a	29	31.6	a
Refreshed	52	6.52	a	49	6.4	a	100	9.19	a	83	8.7	b	3	1.41	a	47	43.3	b
F. prob		0.5			0.89			0.71			0.05			0.46			<.001	
rep.		12			12			12			12			12			12	
d.f.		10			10			10			10			10			10	
s.e.d.		1.08			1.4			1.75			2.69			0.34			2.11	
l.s.d.		2.4			3.11			3.9			5.99			0.75			4.7	

Assessment of volatiles produced by baits tested

Laboratory Assessments

The main volatile components from the bait solutions detected in volatiles trapped on Porapak were 3-methylbutanol, acetic acid and acetoin (Fig. 4.1.5). Ethanol would not have been effectively trapped or analysed in this approach. The release of acetic acid from the Biona Apple Cider Vinegar was, as expected, high, but otherwise amounts of 3-methylbutanol were generally higher than those of acetic acid or acetoin, the latter being used as components of the Cha-Landolt lure. Methionol, another component of the Cha-Landolt lure, could not be detected from any of the bait solutions. Nor could β-cyclocitral which has been proposed as an attractant by Keesey *et al.* (2015).

The Russell IPM sachet released acetic acid, acetoin and methionol at 13.8, 2.2 and 0.9 mg/d respectively. Significant amounts of allyl methylsulphide (0.09 mg/d) were also released. We previously showed this is the result of interaction of the acetic acid and methionol in the same lure.

Analysis of the actual solutions of the baits showed approx. 3% ethanol in the two Riga Gasser baits, 1% in the Biobest DrosAttract and barely detectable amounts in the Koppert attractant and the apple cider vinegar. Acetic acid was present in all the solutions and highest in the apple cider vinegar. Acetoin was at the limit of detection in all solutions and 3-methylbutanol would not be reliably quantified (<0.01%) (Table 4.1.5).

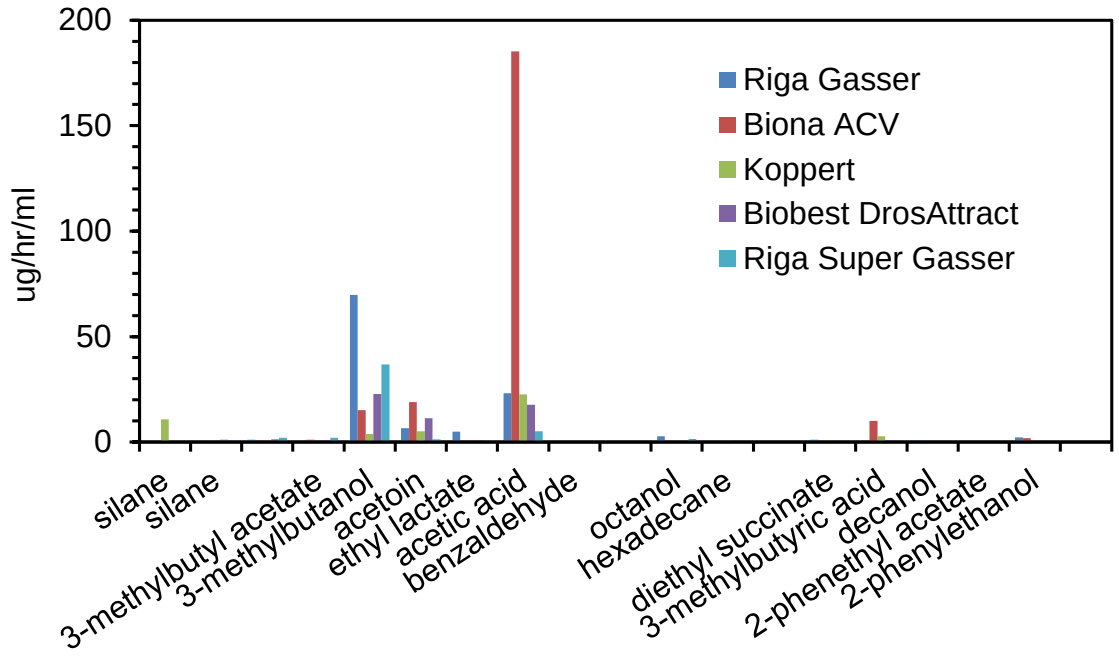

Fig. 4.1.5. Volatiles released from bait solutions and trapped on Porapak (1 ml for 1 hr; mean of 2 replicates)

Table 4.1.5 Analysis of bait solutions on Poraplot column (mean of 2 replicates)

Material	Ethanol %	Acetic Acid %	Acetoin %
Riga "precision monitoring fluid"	3.78	2.25	0.01
Biona Organic "apple cider vinegar"	0.02	5.40	0.01
Koppert "Fruit Fly Attractant"	0.02	4.78	0.01
Biobest "Dros' Attract" New Formula	1.03	2.20	0.01
Riga "Super Gasser"	3.31	1.83	0.00

Discussion

The Cha-Landolt lure for SWD was developed by analysis of volatiles from wine vinegar and red wine and subsequent field testing of the 13 compounds which elicited EAG responses from SWD (Cha *et al.*, 2012). The resultant lure consists of a drowning solution of ethanol (7%) and acetic acid (1.6%) with acetoin and methionol released from vials with a hole.

In the above field test the Koppert bait with no ethanol was not significantly different in attractiveness from baits with ethanol. Although the bait solutions produced significant amounts of acetoin initially, previous tests have shown that this only lasts for a few days, yet these baits performed as well as the NRI lure with a sustained release of acetoin. Furthermore, none of the bait solutions contained detectable amounts of methionol but were as attractive as the NRI lure with methionol.

3-Methylbutanol was released from all the bait solutions. It has not been previously tested as an attractant for SWD, apparently because it did not elicit an EAG response (e.g. Cha *et al.*, 2012), but should be tested as an additional component in the Cha-Landolt lure or possibly as a replacement for the ethanol.

Summary

- Various commercially available *D. suzukii* lures and traps were compared for attractiveness, bycatch and ease of use.
- Topping up precision monitoring traps is as effective as replacing them.
- 3-Methylbutanol might be a useful component in future lures.

References

Agralan (2015) Pheromone traps [online]. Available at: http://www.agralan-growers.co.uk/pheromone-traps-10-c.asp [Accessed 08 December 2015].

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

After preliminary development trials, a prototype attract and kill device was designed based on the following principles;

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.

4.2.1. Field trial 1

The aim of this trial was to evaluate the effectiveness of two Decis (Deltamethrin WG 25W) treated trap and lure designs in SWD control. Two trap designs were compared and Decis was tested as a potential insecticide.

Materials and Methods

Site

Cherry trees (cv. Penny) in Rookery Field RF 181 at NIAB EMR.

Lures

The lures used were separate sachets of ethanol, acetic acid, acetoin and methionol as discussed in section 4.1.

Trap design

The traps compared are summarised in Table 4.2.1. Two trap bodies were tested, the modified Biobest trap used for national monitoring (section 1.1) and a red trap supplied by Bayer (Fig. 4.2.1). Decis was chosen as the insecticide due to it's successful use in attract and kill traps for other Diptera. The Bayer traps were supplied by the manufacturer with lids pre-treated with Decis. The lids and sides of the Biobest traps were coated with the same formulation to give an equivalent dose per cm^2. Biobest traps with lures, but without Decis, were used as controls.

Figure 4.2.1. Red Bayer trap

Following concerns in preliminary trials that flies were not entering the traps, or were entering and leaving, an identical set of traps were used with drowning solution (water and detergent).

Table 4.2.1. Traps used in Field trial 1.

Trap	Lure	Active ingredient	Product and placement	Drowning solution
Bayer	Dry bait	Deltamethrin	Decis Top	Yes
Bayer	Dry bait	Deltamethrin	Decis Top	No
Biobest	Dry bait	Deltamethrin	Decis Top and sides	Yes
Biobest	Dry bait	Deltamethrin	Decis Top and sides	No
Biobest	Dry bait	None	None	Yes
Biobest	Dry bait	None	None	No

Experimental design

A block system with 5 replicates of 6 treatments, traps placed in rows at least 10 m from another trap. Trap catches were assessed on days 3, 6 and 10 after placement.

Results

At each time point the Decis coated traps without solution contained significantly more *D. suzukii* than the controls ($P<0.05$, Fig. 4.2.2), although there was no difference between the two trap designs. However, for each time point and trap design more *D. suzukii* were found in drowning solution than the dry traps, although the difference was not always significant. There was no significant difference between the traps containing drowning solution at any point.

Decis caused *D. suzukii* mortality, though there was no difference between the trap designs used. It was noticeable that more *D. suzukii* were entering the traps than were found dead in the dry traps, as was revealed by the drowning solution catches. Dry catches were therefore an underestimation. There had been concern that Decis, a pyrethroid, might have had a repellent effect but this does not appear to be the case.

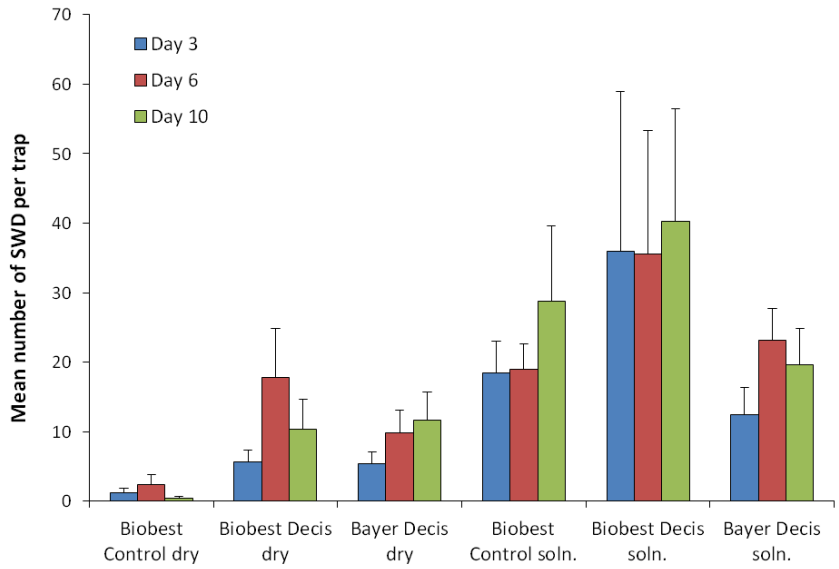

Figure 4.2.2. Mean number of *D. suzukii* caught in each trap at 3, 6 and 10 days after trap placement. Traps emptied of flies at each time point.

4.2.2. Field trial 2

Following from the results in Field Trial 1 Decis appeared an appropriate insecticide to use. However, the Biobest monitoring trap was far too large for use as an attract and kill device, and so a smaller prototype was developed. This however necessitated using smaller versions of the sachet lures above, of approximately half size. Therefore a field trial was run to assess the effectiveness of the half size lures and the new trap design.

Device development

The initial device design was based on a plastic 50 ml Falcon tube (Fig. 4.2.3), as these are cheap and easily available. This was painted red on the outside, as red has been shown to be attractive to *D. suzukii* by several studies (for example Kirkpatrick *et al.* 2015). Eight 4 mm holes were made in the lower part of the tube to allow lure emission and fly entry. The inside of the tube was coated with Decis.

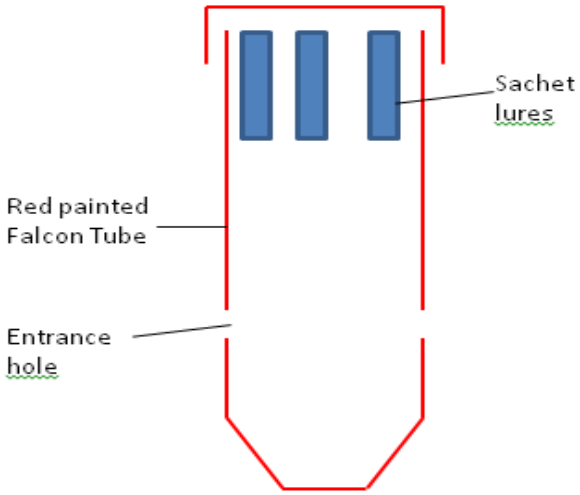

Figure 4.2.3. Prototype device

Lures

Sachet lures were supplied by the NRI. In addition they supplied sachets of half the size, which also combined ethanol and acetic acid in the same sachet.

Treatments

In total five lure/ trap combinations were assessed, summarised in Table 4.2.2. Red Biobest traps were combined with full or half sized sachet lures (both with a drowning solution of water, boric acid and detergent) or with the Cha-Landolt system used for national monitoring (vials of acetoin and methionol with ethanol, acetic acid, boric acid and detergent as a drowning solution). The Falcon prototype was combined with either full of half size sachets.

Table 4.2.2. Attract and kill devices used in Field trial 2.

Trap body	Lure	Solution
Biobest	Half sized sachets	Drowning solution
Biobest	Full sized sachets	Drowning solution
Biobest	Vials	Ethanol, acetic acid and detergent
Falcon	Half sized sachets	None
Falcon	Full sized sachets	None

Site: Cherry trees (cv. Penny) in Rookery Field RF 181 at East Malling Research.

Experimental design: A block system with 8 replicates of 5 treatments, traps placed in rows at least 20 m from another trap.

Assessment: Trap catches were assessed on days 5 and 10 after placement. Solutions changed at each assessment.

Results

There was no significant difference ($P<0.05$, Fig. 4.2.4) between the half size and full size lures at either time point. At day 10 the Cha-Landolt system caught more *D. suzukii* ($P<0.05$) than the half size lures, but otherwise there was no significant different between the Biobest traps. However, no *D. suzukii* were found in any of the Falcon prototypes (data not shown). Possible explanations included; a) the Decis was insufficiently toxic, b) insufficient volatiles were emitted from the traps to have an effect, c) some physical feature of the traps was unattractive to the flies.

The half size lures, a more appropriate size for an attract and kill device, were shown to be as effective as the full size lures.

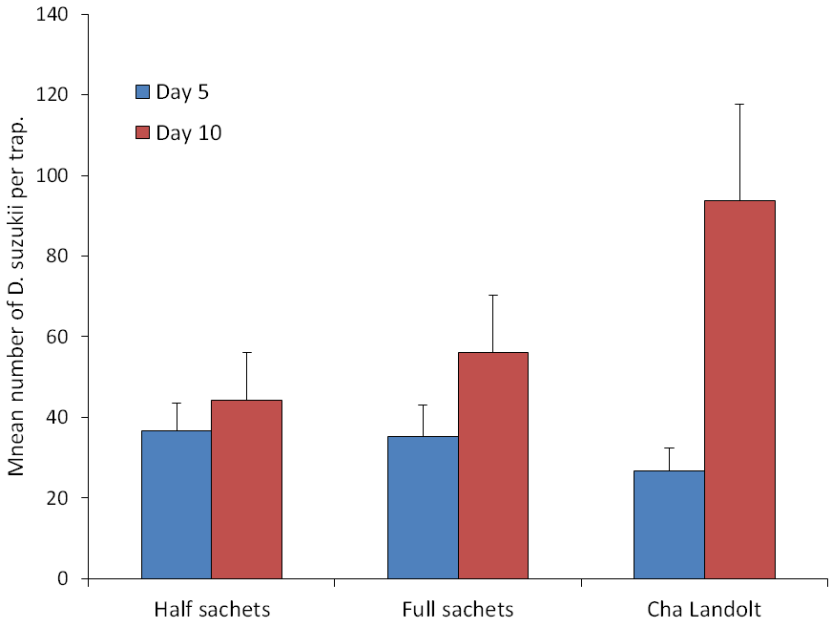

Figure 4.2.4. Mean number of *D. suzukii* caught in each trap at 5 and 10 days after trap placement. Traps emptied of flies at each time point.

4.2.3 Laboratory trials

Following the results from 4.2.1 and 4.2.2 it was decided to assess various aspects of the trap design using cultured *D. suzukii* in the laboratory.

Materials and methods

Population and conditions

A population of *D. suzukii* maintained at EMR since 2013 were used. All trials took place in 30cm square mesh cages at 20 °C in 16hr light/ 8 hr dark conditions.

Decis toxicity test

Falcon traps identical to the one shown in Fig 4.2.3 were prepared, but with or without Decis and without lures. Five male *D. suzukii* were introduced into the traps for 10 seconds and then transferred to an empty culture cage for a recovery period of one hour, at which time mortality was assessed. There were 3 replicates.

Decis trap bioassay

Falcon traps as in Figure 4.2.3 were prepared with or without Decis and with a full set of lures. These were introduced into cages of 20 male and 20 female *D. suzukii* and mortality was assessed after 24 hours. There were 2 replicates.

Lure attractively test

Twenty *D. suzukii* (10 males and 10 females) were introduced into a cage and after 5 minutes a Falcon tube with lures was introduced. This tube had holes as above, but no Decis and was clear plastic except for a region 1 cm either side of the holes which was covered with red tape. The cage was monitored for twenty minutes. Four replicates.

Cage activity test

A cage was set up as above but with a piece of cotton wool soaked in DrosAttract in the middle of the cage. The cage was monitored as above. Four replicates.

Results

Bioassays

All *D. suzukii* exposed to Decis coated tubes for 10 seconds were dead after 1 hour. However, mean mortality from the cage trial was 22.5% and 12.5% for males and females respectively compared to 12.5 and 15% in the controls.

Attractively tests

On average only 6.25% of the flies in the cage flew to the Falcon traps within 20 minutes despite the presence of lures. The same proportion flew to cotton wool soaked in DrosAttract. The Falcon trap design appeared initially to be unattractive to the laboratory *D. suzukii* population. However, a known *D. suzukii* attractant also failed to elicit a response over the same time period. It is therefore possible that laboratory tests are an ineffective method for monitoring attractants, possibly because the air in the cage gets rapidly swamped with attractant, or the flies are sufficiently well fed to ignore any lures.

Further development trials will therefore focus on outdoor trials and natural populations.

4.2.4 Field trials

The model used in 4.2.3 was used to test various aspects of the deign in a woodland known to have high numbers of *D. suzukii* in early winter 2015/16.

Materials and methods

Site

A woodland in Kent known to have high levels of D. suzukii.

Lure attraction and trap colour tests

Falcon tubes as in Figure 4.2.3 were used as the basis for the trap. Four trap variations (n=6) were arranged in replicated blocks along a track within the woodland. Traps were left for one week, and then the order was reversed over the 2nd week.

The four variants were;

Clear tube with lures

Clear tubes without lures

Red painted tubes with lures

Red painted tubes without lure.

All tubes were coated with Decis on the inner surface. Numbers of flies within the tube and the bag suspended below were counted.

Results

Results are displayed in Figure 4.2.5. Population levels were different in the two weeks and so they are considered separately. The lures are clearly attractive to *D. suzukii*. More expectantly, the clear tubes appear more attractive to *D. suzukii*, despite previous reports that red was attractive to *D. suzukii* (for example Kirkpatrick et al 2016). It should be noted that the "clear tubes" did have red plastic lids.

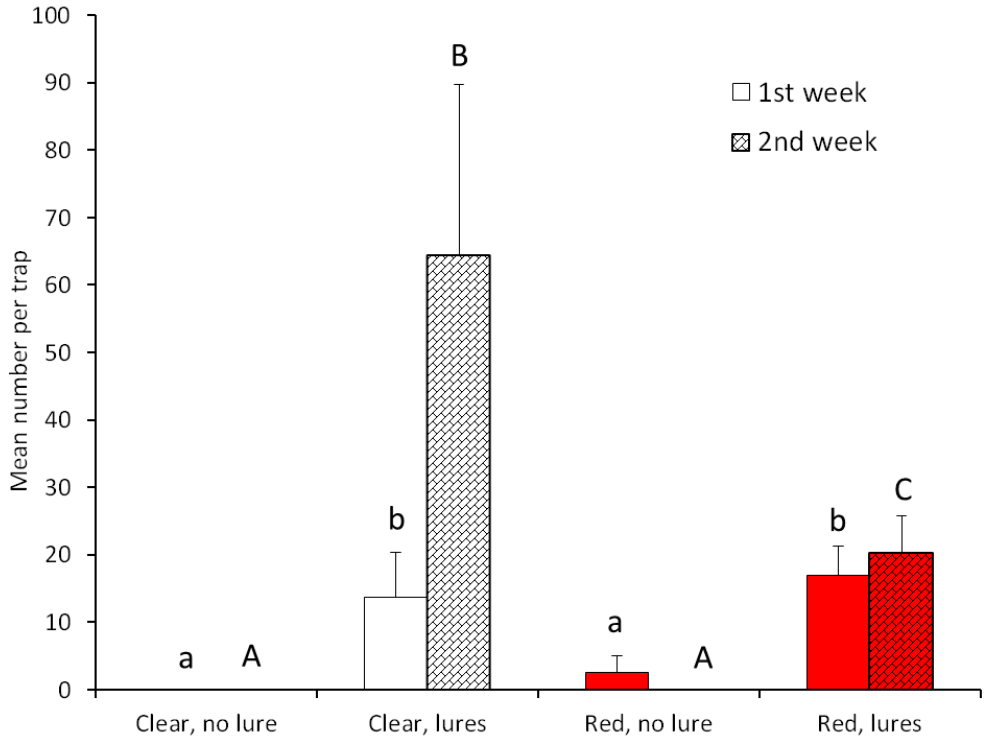

Figure 4.2.5. Mean number of *D. suzukii* caught in variants of the attract and kill prototype. Letters refer to statistical significance within each week, columns with different letters are statistically different (P<0.05%).

The bycatch (insects other than *D. suzukii*) were also assessed (data not shown). A similar pattern was found, except that in the first week the red traps with and without lures were not significantly different.

Summary

- An attract and kill trap is being developed
- Miniature lures developed by the NRI have been shown to be attractive to *D. suzukii*
- Decis appears to be a good candidate for the kill component.

References

Kirkpatrick, D.M., McGhee, P.S., Hermann, S.L., Gut, L.J., Miller, J.R. (2015). Alightment of Spotted Wing Drosophila (Diptera: Drosophilidae) on odorless disks varying in color. Environ Entomol., 45(1), 185-91.

Task 4.3. Optimise the attract and kill treatment and methods of application in the field (NRI, EMR, Years 3-4)

Task 4.3.1.The evaluation of pheromone components of Cha-Landolt baits for the efficiency of trapping Spotted Wing Drosophila (SWD)

Introduction

Attraction of spotted wing drosophila (SWD) to wine and vinegar is stimulated by key olfactory cues which consist of: acetoin (Ac), acetic acid (AA), ethanol (E) and methionol (M). These components can be utilised to form a synthetic attractant which can then be used in an attract and kill trap system. This provides an effective way of detecting and managing *D. suzukii* (Cha *et al.* 2013). A recent trial found that the synthetic bait alone had a reduced *D. suzukii* catch when compared with the standard Cha-Landolt system. This trial aimed to decipher which elements of the bait system were the most effective.

Figure 4.3.1. Modified Drosotrap.

Materials and Methods

The traps used were modified red Drosotraps (Fig.4.3.1) and were deployed in a cherry orchard (mainly cv. Penny) in Rookery Field at EMR. They were spaced greater than 10 m apart and hung from a branch on the cherry trees. There were 6 replicates of each of the treatments. The treatments consisted of a combination of the different lure components compared against an industry standard and an un-baited control (Table 4.3.1).

Table 4.3.1. Table of treatments. Acetoin (Ac), acetic acid (AA), ethanol (E) and methionol (M)

Treat code	Colour	Lure	Liquid bait
A	Green	Un-baited	Water +detergent
B	Blue	E + AA + Ac + M (sachets)	Water +detergent
C	Grey	E + AA (sachets), Ac + M (vials)	Water +detergent
D	Red	Ac + M in sachets	E + AA in drowning solution
E	Black	Ac + M (vials)	E + AA in drowning solution
F	Yellow	Gasser	Gasser

The traps were deployed on 12th August and checked weekly by sieving through a paint filter for 8 consecutive weeks (19th August, 26th August, 2nd September, 9th September, 16th September, 23th September, 20th September, 7th October). The liquid baits were replaced weekly. The acetoin vials were topped up every four weeks. All sachets, except methionol, were replaced every four weeks. Assessments were counts of male, female and 'other' drosophila. Data was analysed using ANOVA on $\log_{10}$ transformed data in Genstat.

Release rates

The release rates of the four components in the different forms that were used in this experiment were assessed by NRI.

Results

The treatments containing the ethanol and acetic acid in the drowning solution were significantly more effective at capturing *D. suzukii* than the 'dry' treatments. This is shown with the 'dry' treatments having approximately half the *D. suzukii* trap catch of the treatments containing ethanol and acetic acid in the drowning solution. Additionally, there was no significant difference in the mean *D. suzukii* catch between the traps that contained the methionol and acetoin in sachet or vial form (Fig. 4.3.2, Table 4.3.3).

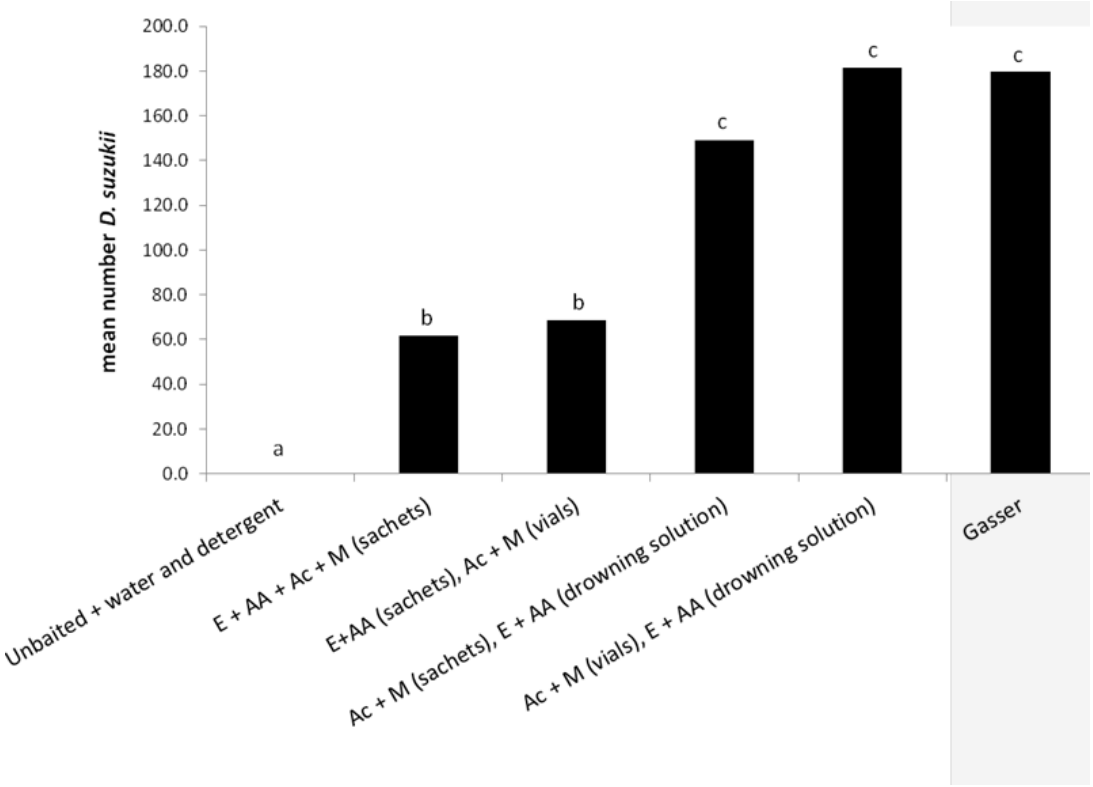

Figure 4.3.2. Mean numbers of *D. suzukii* captured in the traps over 8 weeks

The ratio of *D. suzukii* to other drosophila was significantly higher in the Gasser than all the other treatments. The ratio in the treatment using all four sachets was significantly higher than the remaining treatments. Additionally, there was no significant difference in the ratio of *D. suzukii* to 'other' drosophila between the traps that contained the methionol and acetoin in sachet or vial form (Fig. 4.3.3, Table 4.3.3).

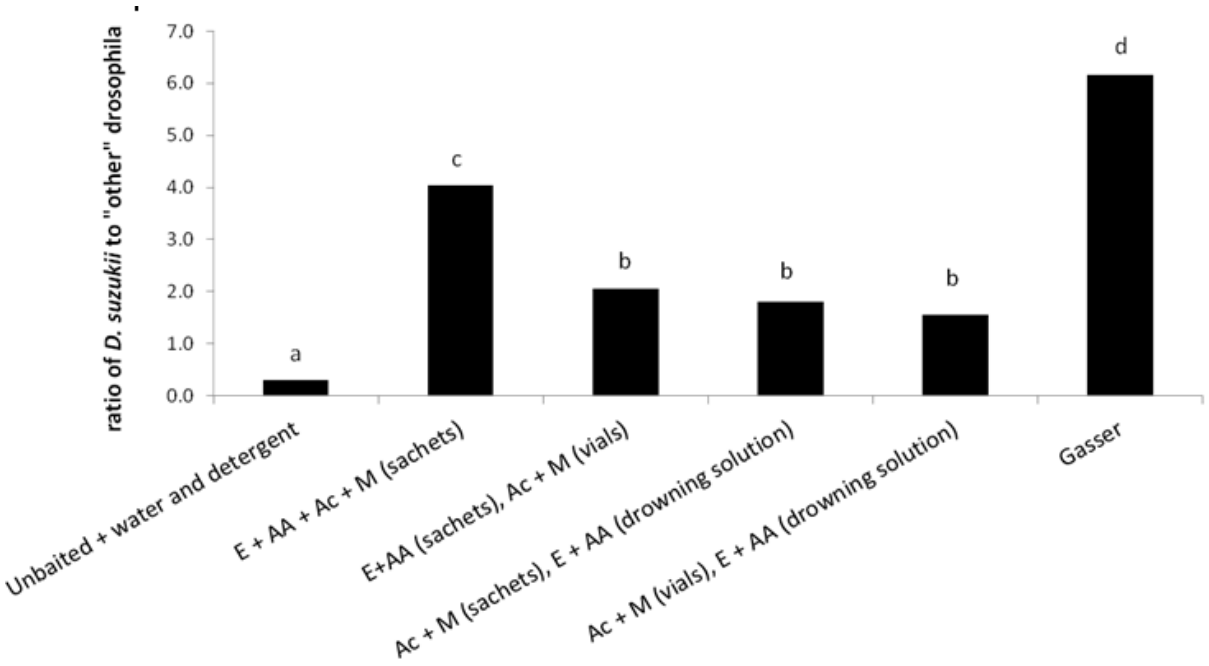

Figure 4.3.3. The ratio of *D. suzukii* to 'other' drosophila in each of the trap and bait

Release rates

Table 4.3.2. Details of lures used in trapping experiments with release rates at 22°C in brackets().

Compound	Drowning solution	Sachet	Vial
Ethanol	300 ml 7% ethanol in water (3.1 g/d for 7 d)	2 ml on dental roll in 78 x 58 mm x 50 µ thick sachet (25 mg/d for 60 d)	
Acetic acid	300 ml 1.6% acetic acid in water (0.17 mg/d for > 10 d)	1 ml on dental roll in 50 x 25 mm x 120 µ thick sachet (18 mg/d for >30 d)	
Acetoin		1 ml 1:1 acetoin/water on dental roll in 78 x 58 mm x 50 µ sachet (7.9 mg/d for > 60 d)	4 ml polypropylene vial with 3 mm diameter hole (7.0-15.9 mg/d)
Methionol		1 ml on dental roll in 50 x 25 mm x 120 µ thick sachet (1.3 mg/d for >30 d)	4 ml polypropylene vial with 3 mm diameter hole (0.37 mg/d)

Discussion

Ethanol and acetic acid in drowning solutions were significantly more effective at capturing *D. suzukii* than the 'dry' treatments. There was no difference in trap catch of *D. suzukii* whether acetoin and methionol were in sachets or vials. This suggests that the low catches may be a result of acetic acid or ethanol not releasing at an optimal rate over the course of the trial when in sachets. Results from NRI indicated that ethanol in the sachet form was being released at a rapid rate (Table 4.3.2). The results from this experiment led us to conduct a further trial to determine a release rate of ethanol from a sachet that will allow for an optimal *D. suzukii* catch.

References

Cha, D.H., Adams, T., Werle, C.T., Sampson, B.J., Adamczyk, J.J., Rogg, H., Landolt, P.J. (2014) A four-component synthetic attractant for Drosophila suzukii (Diptera: Drosophilidae) isolated from fermented bait headspace. *Pest Management Science* 70 (2) pp.324-331.

Table 4.3.3. ANOVA table of the mean number of male, female and overall *D. suzukii* (SWD), other drosophila and the ratio of *D. suzukii* to other drosophila. Significant differences are shown as different letters in the same column.

Treatment	Male			Female			Total SWD			Other			Ratio SWD		
	Mean	Log10		Mean	Log10		Mean	Log10		Mean	Log10		Mean	Log10	
Unbaited + water and detergent	0.2	0.089	a	0.1	0.055	a	0.3	0.125	a	0.3	0.114	a	0.3	0.114	a
E + AA + Ac + M (sachets)	35.5	1.562	b	24.6	1.409	b	61.5	1.796	b	18.6	1.292	b	4	0.702	c
E+AA (sachets), Ac + M (vials)	41.8	1.631	b	24.9	1.414	b	68.8	1.844	b	40.6	1.619	c	2	0.484	b
Ac + M (sachets), E + AA (drowning solution)	85.1	1.935	c	62	1.799	c	149	2.176	c	94.9	1.982	d	1.8	0.449	b
Ac + M (vials), E + AA (drowning solution)	99.5	2.002	c	77.9	1.897	c	181.4	2.261	c	129.6	2.116	d	1.6	0.409	b
Gasser	110.2	2.046	c	66.9	1.832	c	179.7	2.257	c	31.1	1.507	bc	6.2	0.855	d
F prob.		<.001			<.001			<.001			<.001			<.001	
rep.		6			6			6			6			6	
s.e.d.		0.1327			0.1091			0.1231			0.1481			0.0618	
d.f.		30			30			30			30			30	
l.s.d.		0.271			0.2228			0.2514			0.3025			0.1262	

Task 4.3.2. To determine the release rate of ethanol from a sachet that will allow for an optimal *D. suzukii* catch.

Materials and Methods

Treatments consisted of the four components (Ethanol, Acetic acid, Acetoin and Methionol) applied in different forms compared against an untreated control (Table 4.3.4). The size of the hole in the ethanol vial determined the release rate of ethanol; the larger the diameter of the hole the greater the release rate. This meant that the vials with 9mm, 6mm, 3mm and 1mm diameter holes had to be topped up four times a week, twice a week, once a week and every other week, respectively. The acetoin was topped up and the ethanol, acetic acid and acetoin sachets were replaced every 4 weeks. There were 8 replicates of each treatment and the traps were spaced 10 m apart. The traps were located along the hedgerow running next to the railway line located behind the glasshouses at EMR. The data analysed in the results is from 3 weeks sampling conducted on the 1 December, 8 December and 15 December. The data was analysed using ANOVA on square root and angular transformed data in GENSTAT.

Table 4.3.4. Table of treatments

Treatment code	Ethanol	Acetic acid	Acetoin	Methionol	Solution	Ethanol Release rates
A	Vial 1mm hole	Sachet	Sachet	Sachet	Water, detergent and boric acid	38.3 (>37d)
B	Vial 3mm hole	Sachet	Sachet	Sachet	Water, detergent and boric acid	285 (10d)
C	Vial 6mm hole	Sachet	Sachet	Sachet	Water, detergent and boric acid	1,280 (2d)
D	Vial 9mm hole (no lid)	Sachet	Sachet	Sachet	Water, detergent and boric acid	2, 415 (1d)
E (Cha Landolt)	Drowning solution	Drowning solution	Vial 3mm hole	Vial 3mm hole	Ethanol, Acetic acid, detergent and boric acid	3,100 (7d)
F	Sachet	Sachet	Sachet	Sachet	Water, detergent and boric acid	25 (60d)
G	Blank	Blank	Blank	Blank	Water, detergent and boric acid	

Results and discussion

The results show no significant difference in the mean number of *D. suzukii* captured in the traps between the four treatments containing 1 mm, 3 mm, 6 mm or 9 mm diameter holes in the ethanol vials. This suggests that the comparably lower *D. suzukii* catch observed when the four components (AA, M, Ac, E) are in sachet form is not due to the release rate of ethanol from the sachet. Additionally, no significant difference was observed between these treatments and the treatment containing all four components in sachet form. The Cha-Landolt system, where the ethanol was released from the drowning solution had a significantly higher mean number of *D. suzukii* captured in the traps (Fig.4.3.4, Table 4.3.5). The results further show that there was no significant difference in the percentage of *D. suzukii* in the trap catches between the treatments except for the untreated control (Fig.4.3.5, Table 4.3.5).

From previous experiments we know that there is no difference in trap catch when the acetoin and methionol are in vial or sachet form. Hence the high *D. suzukii* catch observed in the Cha- Landolt system is due to the presence of ethanol and acetic acid in the drowning solution. We are now testing the position of the ethanol and/or acetic acid in the trap.

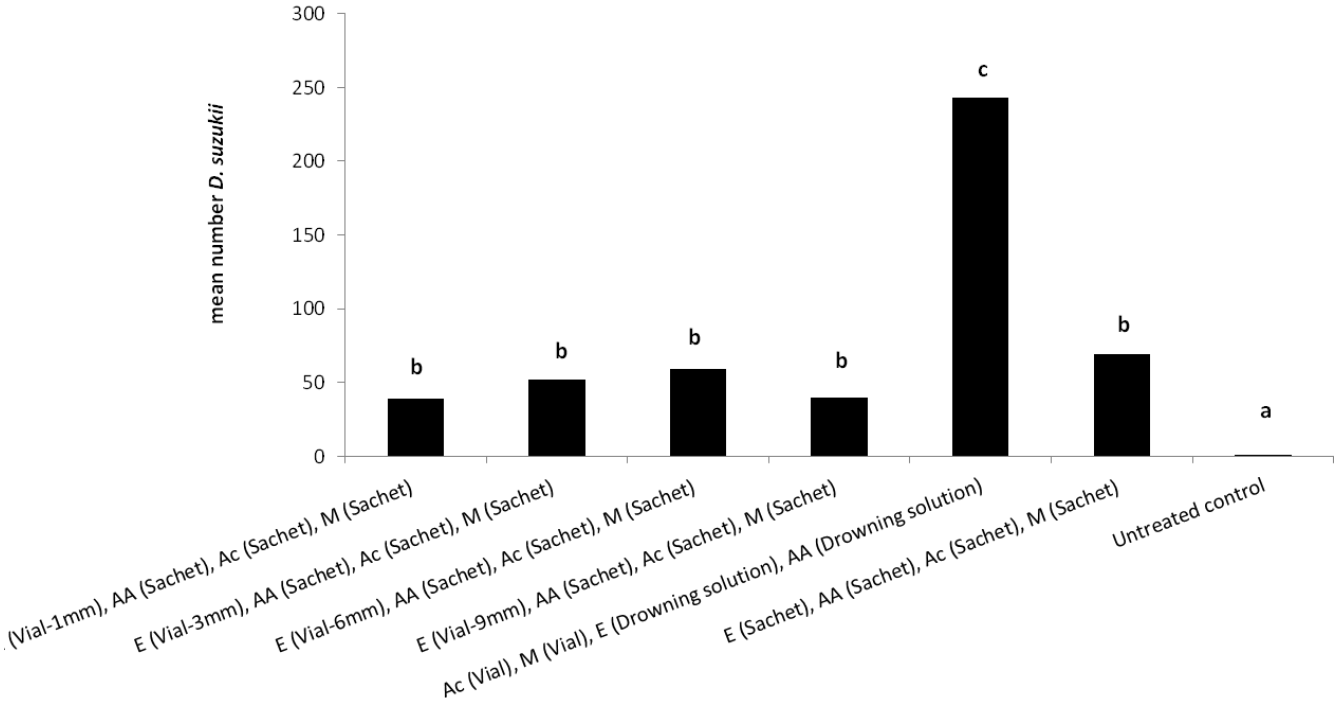

Figure 4.3.4. Mean number of *D. suzukii* captured in the traps over 3 weeks. E-Ethanol, AA-Acetic acid, Ac-Acetoin and M- Methionol.

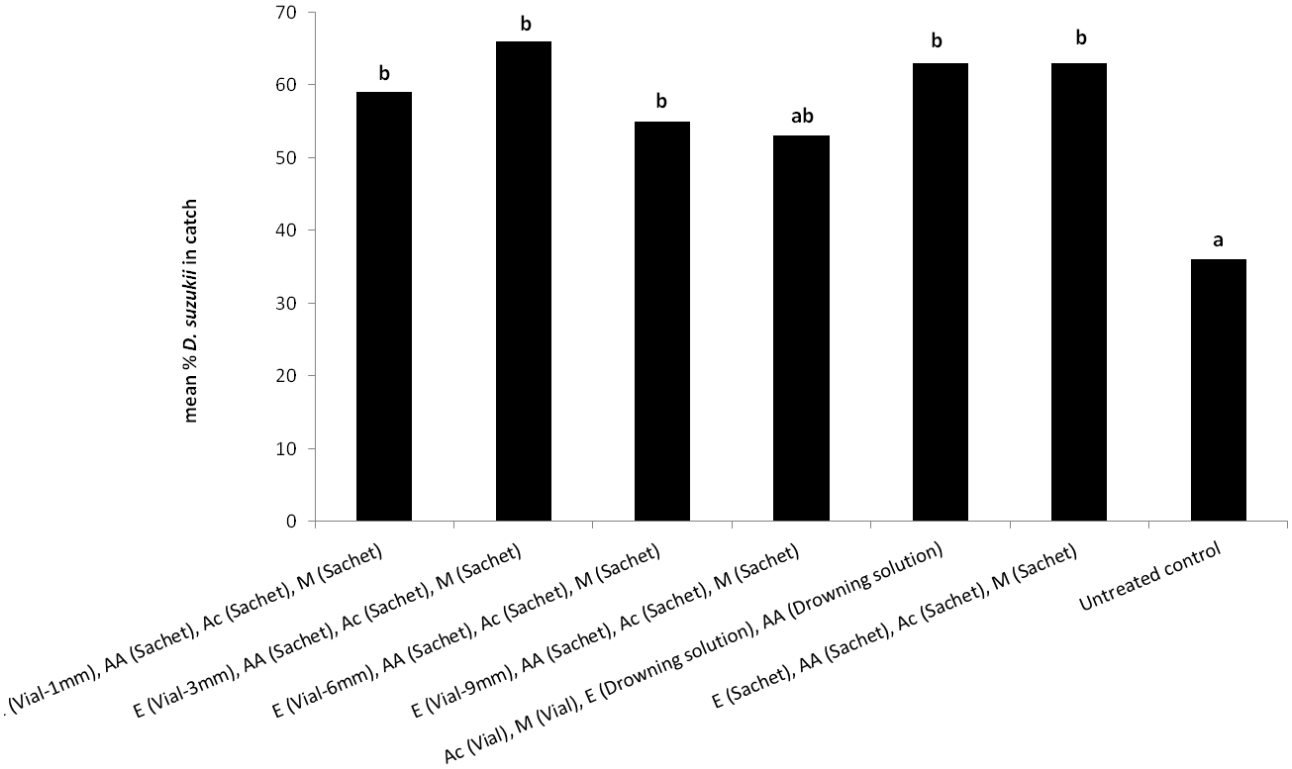

Figure 4.3.5. Mean percentage of *D. suzukii* captured in the traps over 3 weeks. E-Ethanol, AA-Acetic acid, Ac-Acetoin and M- Methionol.

Summary

- No difference in trap catch of *D. suzukii* whether acetoin and methionol were in sachets or vials. Sachets are much easier to handle.
- Ethanol and acetic acid in drowning solutions were significantly more effective at capturing *D. suzukii* than sachets.
- Ethanol in vials with different size openings had the same trap catch, and so ethanol release rate does not appear to be a factor

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Table shown

Table 4.3.5. ANOVA of the mean number of male, female, overall SWD, other drosophila and the average percentage of *D. suzukii* in catch. Significant differences (p<0.05) indicated by different letters in columns.

Treatr

	code	mean	√n		mean	√n		mean	√n		mean	√n		mean	angular	
E (Vial-1mm), AA (Sachet), Ac (Sachet), M (Sachet)	A	23	4.5	b	16	3.8	b	39	5.9	b	34	5.2	b	59	51.3	b
E (Vial-3mm), AA (Sachet), Ac (Sachet), M (Sachet)	B	29	4.9	b	22	4.3	b	52	6.6	b	39	5.2	b	66	55.5	b
E (Vial-6mm), AA (Sachet), Ac (Sachet), M (Sachet)	C	32	5.4	b	27	5	b	59	7.3	b	56	6.9	b	55	48.2	b
E (Vial-9mm), AA (Sachet), Ac (Sachet), M (Sachet)	D	22	4.4	b	18	4.1	b	40	6	b	40	5.8	b	53	47.2	ab
Ac (Vial), M (Vial), E (Drowning solution), AA (Drowning solution)	E	128	10.9	c	115	10.3	c	243	15	c	283	13.3	c	63	53.1	b
E (Sachet), AA (Sachet), Ac (Sachet), M (Sachet)	F	40	5.6	b	29	5	b	69	7.5	b	34	5.5	b	63	52.8	b
Untreated control	G	0	0.3	a	1	0.4	a	1	0.6	a	0	0.1	a	36	32.3	a
F prob.			<.001			<.001			<.001			<.001			<.001	
rep.			24			24			24			24			24	
s.e.d.			0.9312			0.7529			1.174			2.04			7.76	
d.f.			49			49			49			49			49	
l.s.d.			1.8712			1.5131			2.359			4.099			15.58	

Task 4.4. Determine whether intercropping camphor basil plants with commercially grown raspberries is an effective way of repelling SWD and therefore reducing egg laying.

A trial was conducted on a commercial fruit farm in a raspberry field grown under polytunnels. The objective was to determine whether placing potted camphor basil (*Ocimum kilimanscharicum*) around commercially grown raspberry plants would act as a repellent and reduce the number of *D. suzukii* eggs laid in the fruit. To establish this, a comparison was made between plots containing camphor and those without by collecting fruit over a 5 week period and assessing emergence (17 September, 24 September, 01 October, 08 October and 15 October). Emergence occurred 2 weeks after each of the sampling dates. The data was analysed using ANOVA on untransformed data in GENSTAT.

The results found that there was no significant difference in the number of *D. suzukii* that emerged between the plots with camphor basil and those without ($P>0.05$). The average over the 5 weeks for the plots with camphor was 2.7 and those without was 3.27. It is important to point out that the numbers of *D. suzukii* at the trial site were very low due to the use of plant protection products. Thus the effectiveness of the treatments could not be observed completely. A repetition of the trial in cropping areas where there are higher populations of *D. suzukii* would be recommended for future investigations.

Summary

- In this limited study planting camphor basil adjacent to raspberry plants did not appear to reduce *D. suzukii* damage to raspberry fruit.

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. Evaluate the efficacy of approved and emerging products against adults and other life stages in crops (Years 1-4).

A cherry crop at EMR was treated with field rates of plant protection products. Fruit was picked at a series of time points after spraying and subsequent emergence of adult *D. suzukii* from the fruit was monitored to assess the effectiveness of residues over time. This follows trials on strawberries in 2013 and on raspberries in 2014. Products tested were selected in consultation with the industry.

Materials and methods

Plants and site

Cherry trees (cv. Penny) in 2 rows under tunnels on plot Rookery Field RF 181 at East Malling Research.

Figure 5.1.1. Experimental site at EMR.

Experimental design

A randomised block design with 6 replicates of 10 treatments including untreated controls. The plots in each block were arranged end to end in a row, each plot was one tree with a guard tree in-between and there was a guard row between the two treatment rows.

Insecticide application

Sprays were applied (July 9th 2015) as a single application with a motorised knapsack sprayer set to a fine spray quality. Sprays applied at a volume of 1000 l/ha (Table 5.1.1).

Table 5.1.1. Treatments

Active ingredient	Product name	Ai/l	Product rate/ha (spray volume 1000 l/ha)
Acetamiprid	Gazelle	20%w/w	375g
Deltamethrin	Decis	25g/l	200 ml
Cyantraniliprole	-		
HDCI 094	-		
HDCI 095	-		
Lambda cyhalothrin	Hallmark	100g/l	0.09l
Lime	DsLime	-	2 kg
micro Copper	Cuprum	-	1000 ml
micro Manganese and micro-Zinc	ManZincum	-	250ml
Pyrethrins	Spruzit	4.59g/l	12l
Spinosad	Tracer	480g/l	250ml
Untreated	-	-	-

Assessments

Fruits (20 cherries) were removed from each plot on each sample day (Days 0, 1, 4, 7 and 14) (July 9th, 10th, 13th, 16th and 23rd 2015) and placed into ventilated Perspex plastic boxes (228 x 121 x 66 mm). Boxes were kept for 3 weeks at 20 °C and assessed for adult emergence. Towards the end of the experiment some trees had less than 20 cherries available, in which case all suitable fruit were picked and data was analysed using the numbers of *D. suzukii* per fruit.

Statistical analysis

The data was analysed by Generalized Linear Model Regression using Genstat (VSN International Ltd., version 13.1.0.4470) following statistical advice from Phil Brain. To allow for variation along the rows the model fitted was "Treatment + Row + Spline(D;4)" where D = Distance from the end of the row and Spline(*;4) was a smoothing spline vs D on 4 d.f. To allow for trees where 50 cherries were not available a Poisson distribution was used with log-link and log(# Cherries) as an offset.

Comparisons vs. the controls were made by fitting nested models for the Treatments. Model 1 used a treatment factor with a single level for Control & Treatment i (i=1…9), and Model 2 used the full treatment factor. The increase in the deviance when fitting Model 2 after fitting Model 1 was then used to test for the significance of Control vs i. This was carried out for all nine values of i. For days 0, 1, 4 there was no evidence of overdispersion, so all significances were tested using Chi-Squared tests. For Days 7 & 14 there was evidence of overdispersion so all significances were tested using the F-distribution.

Natural infestation increased over the course of the trial, as can be seen from the "untreated" row (Fig. 5.1.2). The numbers of adults emerging from the untreated cherries harvested on Days 0 and 1 were too low for meaningful statistics. Statistical analysis of emergence from Days 4, 7 and 14 are presented in Table 5.1.2.

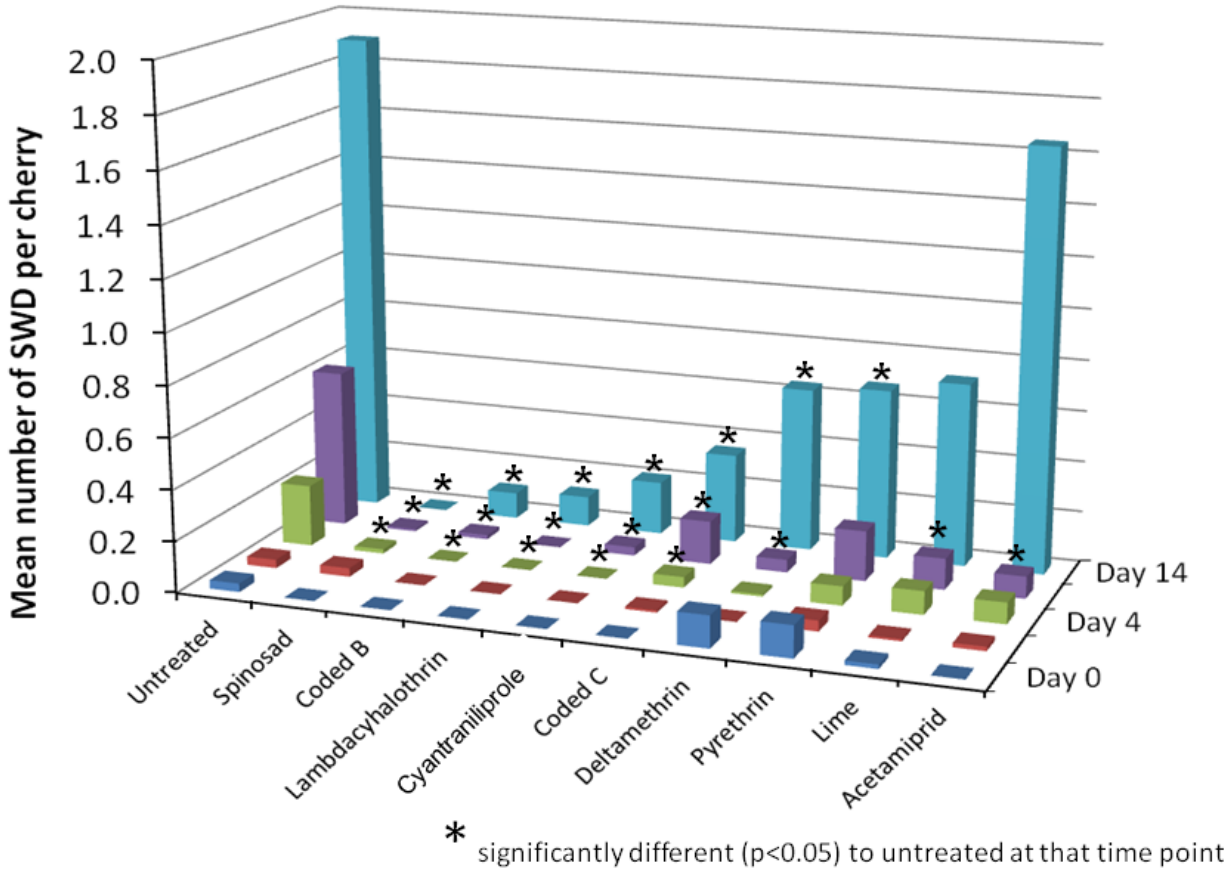

Figure 5.1.2. Adult emergence of *D. suzukii* from cherries at various time points after spraying. Mean number of adults per cherry.

The efficacy of *D. suzukii* control varied with the plant protection product applied and time post spraying. Spinosad, lambda cyhalothrin, cyantraniliprole and HDCI 094 gave good control over the course of the study, whilst deltamethrin and HDCI 095 gave good initial protection, but by Day 14 were starting to lose effectiveness, and a similar pattern was seen with acetamiprid. In contrast, lime and the pyrethrin mixture gave relatively poor control.

Encouragingly, two compounds currently approved for *D. suzukii* control on cherries spinosad (Tracer) and lambda cyhalothrin (Hallmark) gave good control over the course of the study, as did the coded product B and cyantraniliprole. Deltamethrin and the biological product HDCI 095 gave good control for the first week.

This trial follows studies on strawberries and raspberries in 2013 and 2014. Cyantraniliprole consistently gave good protection over all three studies. Spinosad and lambda cyhalothrin were also effective, although it is noticeable that both were less effective in the 2014 trial, possibly because the raspberry crop was not under cover. The pyrethrin mixture was relatively ineffective over all three studies.

Given the likely emergence of insecticide resistance in *D. suzukii*, rotation of insecticides with different modes of action to prevent insecticide resistance is important. It is therefore useful that products representing several different modes of action are effective here under field conditions and in combination with crop hygiene and other non-chemical control measures should help avoid resistance building.

Summary

- Spinosad, lambda cyhalothrin, cyantraniliprole and HDCI 094 gave good control over two weeks
- Deltamethrin, acetamiprid and HDCI 095 gave good initial protection, but by Day 14 were starting to lose effectiveness.

Table 5.1.2. Statistical analysis of adult *D. suzukii* emergence from cherries with various residues. Days 4, 7 and 14 after spraying. Numbers indicate significant difference to the untreated controls (P<0.05)

	Probability of significant difference to untreated controls		
	Day 4	Day 7	Day 14
Acetamiprid	NS	0.001	NS
Cyantraniliprole	0.017	0.016	0.002
Deltamethrin	0.050	0.016	0.037
HDCI 095	0.001	0.002	0.006
Lambda cyhalothrin	0.043	0.003	0.000
Lime	NS	0.006	NS
Pyrethrin	NS	NS	0.011
HDCI 094	0.012	0.004	0.018
Spinosad	0.000	0.000	0.000

Task 5.1.2. Evaluate the use of sugar as an adjuvant for enhancement of insecticide treatments in the control of *D. suzukii*

It has been suggested that sugars can enhance insecticide effectiveness by acting as a feeding stimulant (Jiang & Mulla, 2006), and that this can be an effective control against *D. suzukii* (for example, Loeb *et al.*, 2014). The aim of this trial was to assess possible sugar enhancement for four insecticides, chlorantraniprole, spinosad, lambda cyhalothrin and deltamethrin using fruit dipped in insecticide solutions with or without sugar. Both mortality of adult *D. suzukii* and larval development to emergence from the fruit were assessed.

Materials and Methods

Purchased blueberries were dipped in insecticide with or without sugar (2.4g/l, equivalent to 2 pounds per 100 US gallons, Cowles, 2012; Agnello *et al.* 2014) and allowed to air dry for 2 hours. Insecticides used were chlorantraniprole (Coragen, 0.0875 ml/l), lambda cyhalothrin (Hallmark, 0.013ml/l), spinosad (Tracer, 0.0015ml/l) and deltamethrin (Decis 0.025 ml/l).

Fruit (6 or 8 per replicate) were then placed in a plastic 5 cm weigh boat within a 14 cm plastic petri dish over filter paper and exposed for 24 hours to female *Drosophila suzukii* (1 female per fruit) at 20°C within a plastic bag.

Mortality of adults was assessed after 24 hours and all flies removed. Fruit was maintained to assess emergence.

Statistical analysis: The data (mortality or emergence) was analysed by General ANOVA. Analysis was performed following an angular transformation (for % mortality) or square root transformation (for emergence counts). As experiments were replicated on two separate occasions, the data was first checked to ensure the days could be combined by running a General ANOVA with treatment as trt*day, and if this was >0.05 the data was considered suitable for combination.

Results and discussion

Sugar significantly enhanced adult mortality from chlorantraniprole (Fig. 5.1.3). However, no difference in emergence from chlorantraniprole treated fruit was observed (data not shown).

In contrast, no significant effect of sugar on adult mortality or subsequent emergence was found with spinosad, lambda cyhalothrin or deltamethrin (data not shown).

Sugar did not cause any statistically significant mortality beyond that of water alone (combination of all trials, n=29 per treatment) and there was no significant increase in emergence from sugar compared to the control (combination of all trials, n=29 per treatment).

There are several reports in the literature suggesting that sugar may have a useful role in enhancing insecticide action against *D. suzukii* (reviewed in Table 5.1.3). This study adds three new compounds to the list, chlorantraniprole, lambda cyhalothrin and deltamethrin, though only in the case of chlorantraniprole did sugar appear to have a significant effect on adult mortality.

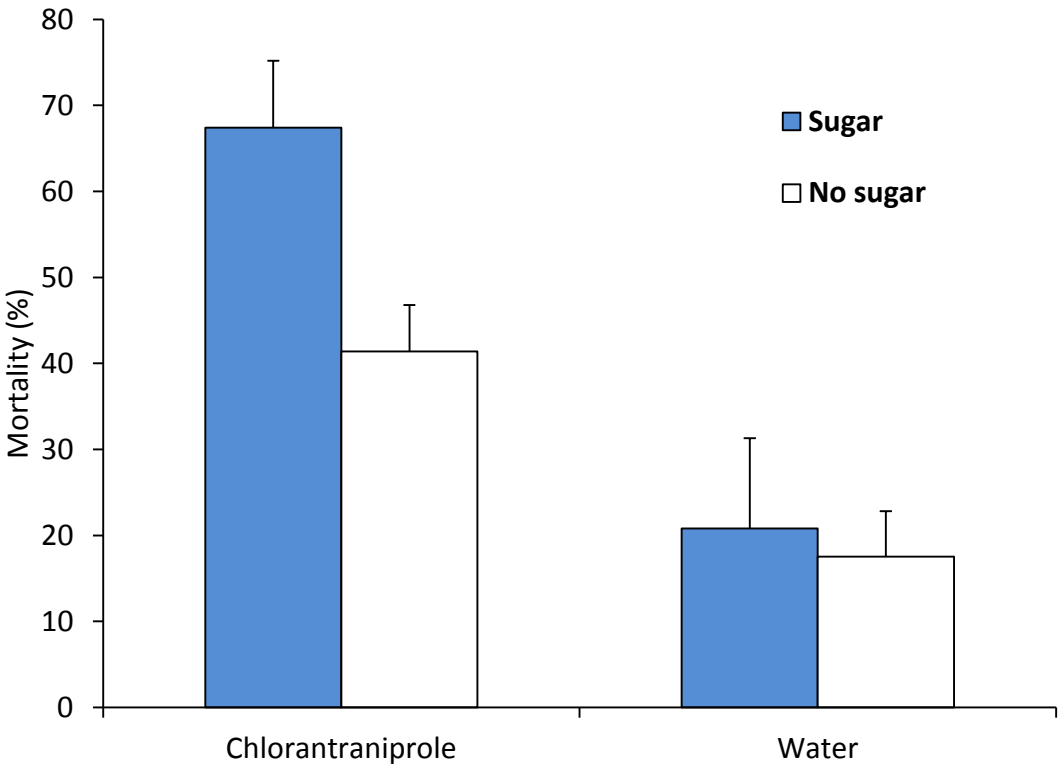

Figure 5.1.3. Mortality of adult *D. suzukii* after exposure to blueberries with and without chlorantraniprole (Coragen, 0.0875 ml/l) and sugar (2.4g/l)

The interaction of sugar with spinosad is unclear from the literature. Our results agree with Loeb *et al.* (2014) in suggesting no effect, but Cowles *et al.* (2015) did find an increase in mortality. It should be borne in mind that in the field sugar may be more important in maintaining contact of *D. suzukii* with the sprayed surface, which could account for some of the results of Cowles *et al.* (2015).

There is also a slight, but consistent, suggestion that sugar increases the success of egg laying in fruit, which might counteract, or at least reduce, any benefit from adult mortality, but the results in our tests were not significant. This may become important if UK growers follow a practice found in the USA of spraying sugar as a feeding deterrent for birds such as starlings, which lack the capacity to digest high sugar concentrations (Clark & Mason, 1993, Knight *et al.* 2015).

Summary

- Sugar significantly increased the effectiveness of chlorantraniprole against adult *D. suzukii*.

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Loeb, G., Elsenhohn, J., Hesler, S., Cowles, R. (2014). Enhancing insecticide efficacy with phagostimulants. New York Berry News (Cornell University Dept. of Horticulture), 12, 14-15.

Table 5.1.3. A review of the effect of sugar on insecticide efficacy against *D. suzukii*

Insecticide	Study	Sugar (%)	Type trial	Mortality	Emergence
Acetamiprid	Loeb *et al* 2014	0.24	Residue, laboratory	Increased (16 to 50%)	-
	Cowles *et al* 2015	0.3	Residue, laboratory	50% reduction in LD_{50}	-
		0.12	Residue, laboratory	Increased (12 to 51%)	No change
Bifenthrin	Cowles *et al.* 2015	0.12	Residue, laboratory	Increased (18 to 67%)	No change
Chlorantraniprole	EMR	0.24	Residue, laboratory	Increased (41 to 67%)	No change
Cyantraniprole	Loeb *et al.* 2014	0.24	Residue, laboratory	No change	-
	Cowles *et al.* 2015	0.12	Residue, laboratory	Increased (13 to 49%)	No change

	Knight *et al.* 2015	0.36	Residue, laboratory	Increased (31 to 78%)	-
Deltamethrin	EMR	0.24	Residue, laboratory	No change	No change
Fenpropathrin	Cowles *et al.* 2015	0.12	Residue, laboratory	No change	No change
Imidacloprid	Cowles *et al.* 2015	0.24	Residue, laboratory	Increased (58 to 85%)	-
Lambda cyhalothrin	EMR	0.24	Residue, laboratory	No change	No change
Phosmet	Cowles *et al.* 2015	0.12	Residue, laboratory	No change	No change
Spinetoram	Loeb *et al.* 2014	0.24	Residue, laboratory	No change	-
	Cowles *et al.* 2015	0.12	Residue, laboratory	Increased (46 to 93%)	No change
Spinosad	EMR	0.24	Residue, laboratory	No change	No change
	Loeb *et al.* 2014	0.24	Residue, laboratory	No change	-
	Cowles *et al.* 2015	0.3	Residue, laboratory	Faster mortality	-
	Cowles *et al.* 2015	0.24	Residue, laboratory	No change	-
	Knight *et al.* 2015	0.36	Residue, laboratory	Increased (69 to 84%)	-
Spirotetramat	Cowles *et al.* 2015	0.12	Residue, laboratory	No change	No change

Task 5.2. Monitor the susceptibility (LC_{50} values) of *D. suzukii* populations in the UK to the three insecticide groups used to control *D. suzukii* (the OP chlorpyrifos, a synthetic pyrethroid (e.g. lambda cyhalothrin), and a spinosyn (e.g. spinosad) and to monitor how susceptibility changes over time (Years 1-4).

Materials and methods

Insect populations

Populations were sampled from 5 sites in the south east of England, including East Malling Research, from crops and wild areas. These sites included the three sampled in 2014. A modified Biobest Droso trap (Agralan, UK) was used (Fig. 5.2.1). Bait consisted of cherries or blueberries which had been previously frozen at least 48 hr to kill any contaminating *D. suzukii*. Collected traps were placed in plastic bags for transport back to the laboratory where individual flies were caught by pooter and later cooled and sorted on a cool table. Single female flies were separated and raised as isofemale lines on an agar diet. Using cultures in this way has the dual advantages of providing sufficient numbers (as live traps typically produce low numbers compared to drowning traps) and reducing the effect of environmental variation in the history of individuals.

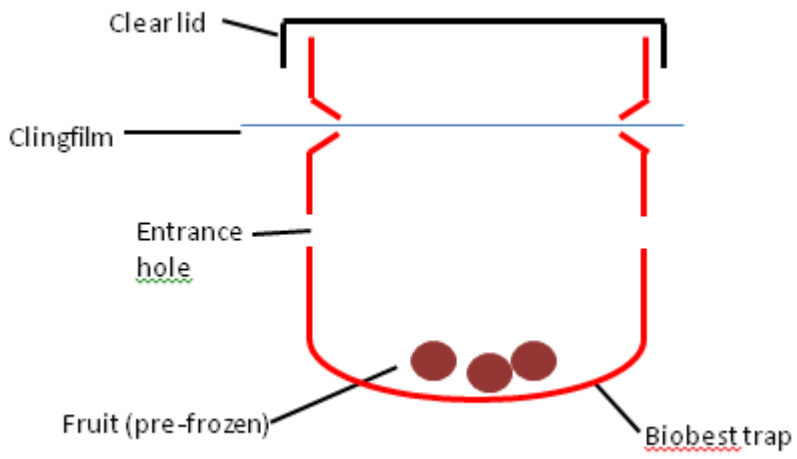

Figure 5.2.1. The trap used for catching live *Drosophila suzukii*.

Bioassays

Females will be assessed as the genders have different insecticide tolerances (P. Shearer, personal communication). In addition, care will be taken to apply insecticides at a similar time of day, as circadian variation has been shown to have a marked effect on *D. suzukii* insecticide tolerance (Hamby *et al.* 2013, and J. Chiu, personal communication).

The bioassay apparatus is a plastic Petri dish (9 cm diameter) with a filter paper disk placed in the bottom and a gridded lid. Eight female *D. suzukii* to be added by pooter and the Petri dish halves joined and sealed with parafilm and the dish stored in a refrigerator for 2 hours. Each concentration assessed with three replicates.

Insecticide application

Insecticide to be applied using a Burkhard sprayer and diluted to give the desired rate, assuming a uniform flat surface. The insecticides assessed are spinosad (Tracer), lambda cyhalothrin (Hallmark) and chlorpyrifos (Equity). After spraying, flies will be transferred to a new petri dish with wet filter paper and 1 cm^2 piece of *Drosophila* food. Petri dishes will be placed in plastic bags to maintain humidity and incubated at 20°C. Mortality assessed after 24 hours. A range of concentrations will be used to estimate the LD_{50} value.

Results and discussion

The results from 2014 are shown in Tables 5.2.1 and 5.2.2.

Table 5.2.1. Estimated LD_{50} values of the laboratory population

Active ingredient	Product	LD_{50} (ml/l)
Spinosad	Tracer	0.078
Lambda cyhalothrin	Hallmark	0.019
Chlorpyrifos	Equity	0.0052*

* It should be noted that the vapour action of chlorpyrifos would tend to increase mortality in enclosed laboratory environments, and the results here may be an over estimation.

Table 5.2.2. Estimated LD_{50} values for spinosad of various UK populations

Farm	Habitat	LD_{50} (ml/l)
1	Woodland	0.097
1	Woodland	0.066
1	Strawberry	0.095
1	Strawberry	0.094
2	Cherry orchard	0.103
3	Cherry orchard	0.101

Sixty traps have been set out in 2015. Trap catches are at various stages of culturing to provide sufficient flies for bioassay.

It is too early yet to draw conclusions about the *D. suzukii* populations in 2015, as the populations in culture have not reached sufficient numbers for assessment. In 2014, there appeared to be little difference between the wild UK populations, and laboratory population, which has not been exposed to insecticides for over 18 months. These values will be a useful baseline for determination of any growth in insecticide resistance in 2015.

References

Hamby, K.A., Kwok, R.S., Zalom, F.G., Chiu, J.C. (2013) Integrating circadian activity and gene expression profiles to predict chronotoxicity of *Drosophila suzukii* response to insecticides. PLoS ONE 8, e68472.

6. Additional research

6.1 Efficacy of dipping fruit in Jet 5 or Lime for the control of egg laying of *Drosophila suzukii*

To determine the efficacy of Jet 5 and Lime as a crop protection product against *D. suzukii* at all life stages: egg, larvae and adult. Results from Jet 5 and Lime were compared with spinosad (a proven control).

Materials and Methods

Treatments

Four treatments were applied to blueberries; water (as the control), Jet 6, Lime (plus Cuprum (micro Copper), 1ml/l/ha and ManZincum (micro Manganese and micro-Zinc) 250 ml/ha) and spinosad (positive control). 50 blueberries were used per sample with six replicates per treatment type. Each sample had two periods of exposure to the *D. suzukii,* the details are as follows:

- 1st inoculation: Before the first treatment was applied, the blueberries were exposed to adult *D. suzukii.* The fruit were placed in 7.5 cm^3 ventilated square boxes (Transpack) with ten females and five males. Boxes were stored for 24 hours at 25°C (the optimum temperature for egg laying) to insure pre-inoculated fruit contained eggs and not larvae.

- 2nd inoculation: After the storage period the fruit were dipped in one of the four treatments and left to dry in the fume cupboard. Fruit was then placed into ventilated boxes with five female and two male *D. suzukii* for inoculation which were then removed after 24 hours.

Fruit

The blueberry variety used was Star imported from Spain. Treatments were completed on fruit collected from the supplier on 1st June 2015. Before experimentation could take place, all fruit was cleaned, which involved removal of flowers and washing to attempt to remove any chemical residues left on the fruit. This was completed by emptying the punnets onto flat white trays and running water over the fruit for one minute. After the water was drained the blueberries were spread onto blue laboratory roll and left for 30 minutes in an air conditioned room set to 25°C to dry and raise them to inoculation temperature.

Dipping

Fruit was dipped in the treatments at the recommended label rate (Table 6.1.1) to obtain full coverage and to limit variation between application in a laboratory setting in comparison to spraying. To dip, the fruit was placed into Nylon mesh bags (10 cmx15 cm) then submerged into the treatment, for a maximum of five seconds per treatment. In order to dry fruit, the bags were then hung in the fume cupboard with the extractor fan running (Fig. 6.1.1). In addition, a control of undipped fruit and non-inoculated fruit was kept to monitor for natural emergence of larvae that may already be present in the fruit before any inoculation.

Storage

After treatment the fruit was stored as before in ventilated boxes at a temperature of 25°C. Two weeks after the adult *D. suzukii* inoculation the boxes were removed from storage and emerged flies identified and counted.

Figure 6.1.1. Fruit in bags hanging after being dipped in treatments.

Figure 6.1.2. Emergence testing: fruit was stored in ventilated boxes at a temperature of 25ºC

Table 6.1.1. Treatments.

Treatment no	Product name or code	Dose rate	Timing
1	Water	-	Inoculate fruit/dip 1 days after
2	Water	-	Dip fruit/infest once fruit dry
3	Spinosad	(1000 l/ha) 250 ml/ha	Inoculate fruit/dip 1 days after
4	Spinosad	(1000 l/ha) 250 ml/ha	Dip fruit/infest once fruit dry
5	Jet5	(400 l/ha) 1 l/125 l water	Inoculate fruit/dip 1 days after
6	Jet5	(400 l/ha) 1 l/125 l water	Dip fruit/infest once fruit dry
7	Lime**	(1000 l/ha) 2 kg / ha	Inoculate fruit/dip 1 days after
8	Lime**	(1000 l/ha) 2 kg / ha	Dip fruit/infest once fruit dry

@400 l/ha and use a rate of 1 l/125 l water

** Plantosys recommend adding Cuprum (micro Copper1 ml/l/ha) and ManZincum (micro Manganese and micro-Zinc 250 ml/ha).

Results

All results are shown in Fig. 6.1.3. Statistical analyses are in Table 6.1.2.

There was a low mortality of *D. suzukii* in the water only control <12% (Fig 6.1.3, Table 6.1.2).

The only treatment that affected the emergence of *D. suzukii* was spinosad (positive control) with a mean of <1 *D. suzukii* per plot compared to 16 in the controls. Although *D. suzukii* could lay eggs in the fruit before treatment the no adults subsequently emerged from the fruit. 100% of adult *D. suzukii* were killed by spinosad.

There was no significant difference between the water only control and Jet 5 for egg laying or emergence. However Jet 5 significantly increased mortality of adults (almost 50%) when introduced after dipping. This could be due to vapour action in the vessels used and may not be relevant to field conditions.

Lime did not reduce the numbers of eggs laid, but dipping in lime before eggs could be laid appeared to reduce the numbers of *D. suzukii* emerging. As mortality was not affected this may be because *D. suzukii* were deterred from laying eggs in the fruits.

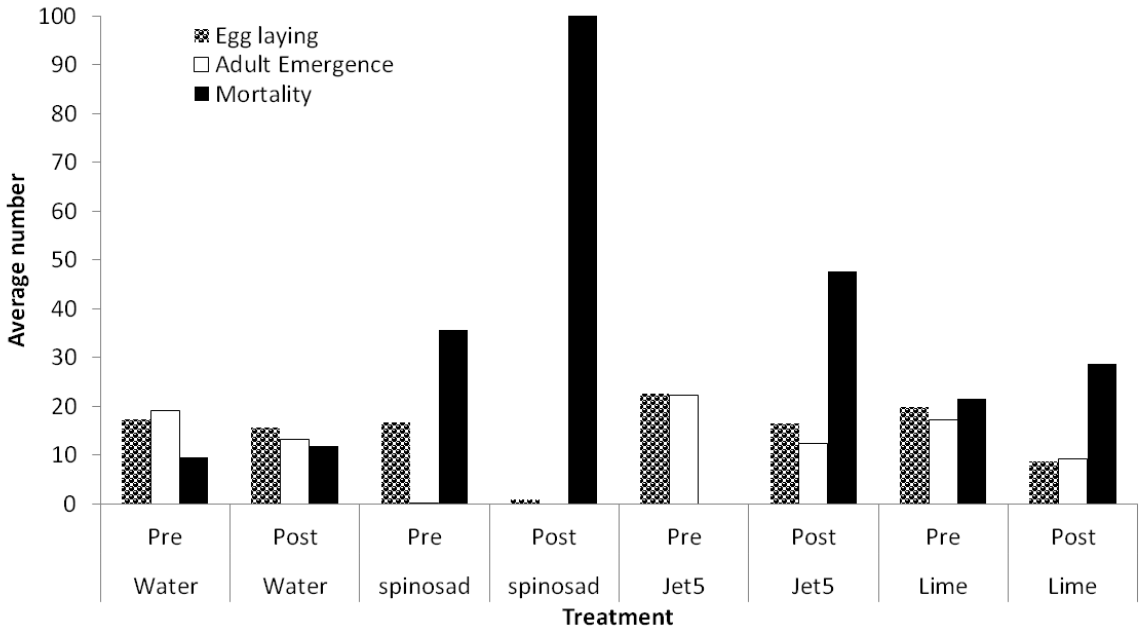

Figure 6.1.3. Mean numbers of *D. suzukii* eggs, adult emergence and percentage mortality from fruit treated with either lime, spinosad of Jet 5 compared to an untreated control pre or post egg laying.

Summary

- Jet5 increased mortality of adult flies but this may be due to vapour action in the bioassay
- Lime reduced the number of adult *D. suzukii* emerging from fruit.

Reference

Cuthbertson, A. G. S., Collins, D. A., Blackburn, L. F., Audsley, N. & Bell, H. A. (2014). Preliminary Screening of Potential Control Products against *Drosophila suzukii*. *Insects*. 5 (2), 488-498.

Table 6.1.2. Analysis of variance on square root (SQRT) and ANG transformed egg laying, emergence and mortality of *D. suzukii* exposed to products on fruit. Significant differences between rows in lower case and between columns in capital letters

Egg laying - no treatment / egg laying timing interaction MEANS					
Jet5	19.5		Post dipping	10.4	
Lime	14.2		Pre dipping	19	
Spinosad	8.8				
Water	16.3				
SQRT MEAN					
Jet5	4.02	b	Post dipping	2.7	a
Lime	3.42	ab	Pre dipping	4.11	b
Spinosad	2.26	a			
Water	3.92	b			
F pr.	0.02			0.002	
s.e.d.	0.592			0.418	
l.s.d.	1.201			0.849	

Adult emergence - no treatment / egg laying timing interaction - MEANS					
Jet5	17.2		Post dipping	8.7	
Lime	13.2		Pre dipping	14.7	
Spinosad	0.1				
Water	16.2				
SQRT MEAN					
Jet5	3.63	b	Post dipping	2.28	
Lime	3.2	b	Pre dipping	3.07	
Spinosad	0.08	a			
Water	3.77	b			
F pr.	<.001			NSD	
s.e.d.	0.656				
l.s.d.	1.331				

Percentage mortality - MEANS				
	Jet5	Lime	Spinosad	Water
	47.6	28.6	100	11.9
	0	21.4	35.7	9.5
ANG MEAN				

	Jet5		Lime		Spinosad		Water	
Post dipping	45.9	bA	26.6	aA	90	bB	11.9	bA
Pre dipping	0	aA	17.8	aAB	36.3	aB	8.2	bA
F pr.	0.017							
s.e.d.	12.88							
l.s.d.	26.15							

6.2. Population dynamics of *Drosophila suzukii* in relation to other *Drosophila* species in the UK 2014-17

There are many species of *Drosophila* found in the UK, some of which are found in the same habitats as *Drosophila suzukii*. These species could potentially interact in a number of ways, such as competition for resources, or distribution of diseases. In addition, *Drosophila* species are frequently found as by-catch in SWD traps, and can be confused for *D. suzukii* by inexperienced operators.

However, very little is known of *Drosophila* species phenology in the UK. This study uses trap catches from the SWD National Monitoring scheme to assess population changes in common *Drosophila* species through the year.

Methods

The study is restricted to wild (woodland) sites and nearby crop (strawberry) fields, and to quarterly samples. Two farms in England, EMR and a site near Hereford, 140 miles apart by direct line, were assessed and four farms in Scotland. National monitoring trap catches for the selected traps were stored at -20°C and then assessed by EMR (England) or JHI (Scotland) staff. Following consultation with Darren Obbard (University of Edinburgh) it was decided to limit identification to the following categories, based on which species would be likely to come to the *D. suzukii* traps and ease of identification;

Drosophila suzukii

Drosophila busckii

Drosophila funebris

Common both inside and outside of buildings (Basden, 1951), notably stables and toilets (Baechli et al 2004).

Drosophila hydei

Susceptible to cold, but heat tolerant and closely associated with human habitation (Spencer, 1940). Associated with rotting fruit (Baechli et al 2004).

Drosophila immigrans

Reportedly more tolerant of cold temperatures than *D. hydei*, *melanogaster* or *simulans* (Spencer, 1941). Females possess a conspicuous ovipositor which can be mistaken for that of *D. suzukii*.

Drosophila melanogaster

Common, and superficially similar to *D. suzukii* females in general coloration. Not very cold tolerant. Closely associated with rotting fruit (Baechli et al 2004).

Drosophila obscura group

As a group of species these are very difficult to distinguish physically and they are therefore grouped together here. *D. subobscura* is common and widespread, especially near woodlands, though, unusually, it can be found in wide open spaces (Basden, 1951).

Drosophila simulans

Superficially very similar to *melanogaster*, with slightly different ecological characteristics (for example Montchamp-Moreau, 1983).

It was further decided to limit traps to those in wild areas or in strawberry crops.

Results and discussion

Numbers of each species in each habitat are given in Table 6.2.1, with numbers converted to percentages of totals in table 6.2.2.

Table 6.2.1. Numbers of individuals per trap caught in each habitat and time point.

	Drosophila species							
	buskii	funebis	hydei	immigrans	melanogaster	simulans	obscura gp.	suzukii
Strawberry								
Jun/July 2014	0	4.8	0	1.25	0.75*	na*	20	0
October 2014	0	3.3	0.25	4.0	3.75	1.5	10.8	0
January 2015	0	0.0	0	1.0	0	0	19.8	0
Apr/May 2015	0	0.3	0	2.0	0	0	6.3	0
Scot April 2015	0	0	0	0	0	0	5.4	0
Woodland								
Jun/July 2014	0.5	19.5	0	10.3	2.3*	na*	188.8	0
October 2014	2.0	2.0	0.5	10.8	1.5	0.25	22.8	44.8
January 2015	0	0	0	1.5	0	0	434	39.8
Apr/May 2015	0	0	1.25	3.8	0.5	0	163	4.3
Scot April 2015	0	1.2	0	3.7	0	0	110	0.2

NB. N=4 for the traps in England, N=6 for Scottish woodland traps and N=5 for the Scottish strawberry traps

* combined *Drosophila melanogaster* and *simulans*

Table 6.2.2. Percentage of individuals caught in each habitat and time point.

	Drosophila species							
	buskii	funebis	hydei	immigrans	melanogaster	simulans	obscura gp.	suzukii
Strawberry								
Jun/July 2014	0.0	17.8	0.0	4.7	2.8*	na*	74.8	0.0
October 2014	0.0	13.8	1.1	17.0	16.0	6.4	45.7	0.0
January 2015	0.0	0.0	0.0	4.8	0.0	0.0	95.2	0.0
Apr/May 2015**	0.0	3.8	0.0	23.1	0.0	0.0	73.1	0.0
Scot April 2015	0	0	0	0	0	0	100	0
Woodland								
Jun/July 2014	0.2	8.9	0.0	4.7	1.0*	na*	85.2	0.0
October 2014	2.4	2.4	0.6	12.7	1.8	0.3	26.9	53.0
January 2015	0.0	0.0	0.0	0.3	0.0	0.0	91.3	8.4
Apr/May 2015	0.0	0.0	0.7	2.2	0.3	0.0	94.3	2.5
Scot April 2015	0	1	0	3.2	0	0	95.7	0.1

* combined *Drosophila melanogaster* and *simulans*

** missing one trap count

Numbers of each species per trap at each site are given in table 6.2.3, with numbers converted to percentages of totals in table 6.2.4.

Table 6.2.3. Numbers of individuals per trap caught in each site and time point.

	Drosophila species							
	buskii	funebis	hydei	immigrans	melanogaster	simulans	obscura gp.	suzukii
Kent								
Jun/July 2014	0	23.3	0	9.0	2.0*	na*	160	0.5
October 2014	2.0	2.3	0.5	9.0	0.8	0.25	13	44
January 2015	0	0	0	1.8	0	0	405	35.5
Apr/May 2015	0	0.3	1.7	2.7	0.7	0	175	5

Hereford								
Jun/July 2014	0.5	1.0	0	2.5	1.0*	na*	47.8	0
October 2014	0	3.0	0.3	5.8	4.5	1.5	20.1	0.8
January 2015	0	0	0	0.8	0	0	48.5	4.25
Apr/May 2015	0	0	0	3.3	0	0	35.6	0.5
Scotland								
April 2015	0.0	0.6	0.0	2.0	0.0	0.0	62.6	0.1

* combined *Drosophila melanogaster* and *simulans*

Table 6.2.4. Percentage of individuals caught in each site and time point.

	Drosophila species							
	buskii	funebis	hydei	immigrans	melanogaster	simulans	obscura gp.	suzukii
Kent								
Jun/July 2014	0.0	11.9	0.0	4.6	1.0*	NA*	82.2	0.3
October 2014	2.8	3.1	0.7	12.5	1.0	0.3	18.1	61.3
January 2015	0.0	0.0	0.0	0.4	0.0	0.0	91.6	8.0
Apr/May 2015	0.0	0.2	0.9	1.4	0.4	0.0	94.4	2.7
Hereford								
Jun/July 2014	0.9	1.9	0.0	4.7	1.9*	NA*	90.5	0.0
October 2014	0.0	8.3	0.7	15.9	12.4	4.1	56.6	2.1
January 2015	0.0	0.0	0.0	1.4	0.0	0.0	90.7	7.9
Apr/May 2015	0.0	0.0	0.0	8.2	0.0	0.0	90.5	1.3
Scotland								
April 2015	0.0	1.0	0.0	3.1	0.0	0.0	95.8	0.1

* combined *Drosophila melanogaster* and *simulans*

Although *Drosophila suzukii* are unique amongst the UK *Drosophila* population in being able to attack ripening fruit, the other *Drosophila* species present may be having a significant effect on *D. suzukii* in a number of ways. One is through competition, not in ripening fruit, but in media such as rotting fruit. It is well established that larval density has a detrimental effect on adult fly size and survivability (Atkinson 1979), and other competition effects have been illustrated in this report. As

this trial was based on lures resembling rotting fruit, all the species caught are presumably attracted to the same oviposition media or feeding site. In addition, there is the increased chance of disease or parasite transfer when the invasive *D. suzukii* comes into contact with native Drosophalids. And, in practical terms, the presence of large numbers of non-target *Drosophila* in the trap catch makes counting more difficult and time consuming.

The data for *D. suzukii* is derived from traps within the national monitoring scheme, and shows a pattern described before, with higher numbers in the site in Kent than the one in Hereford, and in wooded areas compared to the strawberry fields. As expected, there was a peak in wild area catches in October and January, during a particularly mild winter.

The highest catches in the traps were from the *obscura* group, a combination of at least four species in the UK. As a group they were found in high numbers all through the year, although both sites showed an unexplained dip in numbers in October. As with *suzukii*, there was an increase in numbers in wild areas in January. If competition or pathogen transfer exists between these two species, this may have a significant effect on *D. suzukii* populations as there is no time of year when *D. suzukii* do not encounter members of this group in large numbers. In practical terms, because of their dark coloration they are relatively easy to distinguish from *D. suzukii*.

One species found in significant numbers was *Drosophila immigrans*, important due to the potential for confusion of females with *D. suzukii*, especially as it was caught in July when *D. suzukii* was less available for comparison. Unlike the majority of species, some *D. immigrans* were still found in January. They are reportedly more cold tolerant than average (Spencer, 1940), perhaps because of their relatively large size.

Five other *Drosophila* species were all caught in the traps at one time or another, including *D. melanogaster* which have been shown to compete with *D. suzukii*. It should be noted that the trap bait was optimised to resemble rotting fruit, and future use of bait resembling ripening fruit is likely to considerably reduce catches of species other than *D. suzukii*.

Summary

- Six *Drosophila* species and the species group *Drosophila obscura* were regularly found in the national monitoring traps
- These species may interact with *D. suzukii* via competition, the transmission of pathogens or spread of parasitoids and predators.
- The commonest bycatch was the *D. obscura* group. These were found in all sites and all time periods.
- In terms of monitoring, bycatch of other *Drosophila* can slow assessment considerably, and several species can look like *D. suzukii* to an inexperienced observer.

References

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Annex 1

Table showing overview of progress against milestones for project as a whole

	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**	**In progress**
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**	**started**
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**	