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GROWER SUMMARY

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

Headline

- 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 the development of 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 *D. suzukii.* This was subdivided into two tasks; monitoring of the UK population throughout the year and a habitat survey.

National monitoring

In 2014, the national monitoring of adult *D. suzukii* numbers was continued at a network of 15 sites across the UK (one more than in 2014) using modified Biobest traps with Cha-Landolt bait: five in Kent, including East Malling Research, one in Surrey, three in the West Midlands, two in East England and four in Scotland, including the James Hutton Institute.

Numbers of *D. suzukii* were considerably higher in 2014 than in 2013. The largest catches were in the south east of England, but *D. suzukii* was found at all sites in 2014. The numbers caught in crops peaked in August before falling and then rising again in late October. Numbers then rose in November in woodlands and dense hedgerows, corresponding to a decrease in numbers in the cropping areas.

Habitat survey

The distribution of *D. suzukii* on two farms, including EMR, was studied throughout the winter and fruit growing seasons. Fortnightly or weekly trap samples were taken. Over 50 traps were deployed on each farm in a range of crops and in neighbouring wild areas and woodlands. *D. suzukii* was detected throughout the farms, but especially associated with particular woodlands and hedgerows. Adult *D. suzukii* continued to be trapped throughout the winter. Only two weeks were absent of catches in February, when cold weather probably reduced adult flight activity on both farms. November and December 2014 saw the highest trap catches in hedgerows and wild areas suggesting the movement of flies to denser sheltered areas for the winter.

One very important finding of the habitat surveys was that the traps did not detect *D. suzukii* in cherry crops before eggs were laid in the ripening fruits. This has implications for spray timings and product rotation and will be further investigated in 2015.

To determine if *D. suzukii* could utilise other plants, fruit was collected from potential hosts in areas of known *D. suzukii* activity. Fruit was maintained in ventilated boxes at room temperature and the numbers of emerging adult *D. suzukii* recorded. In 2013, *D. suzukii* were using wild elder and blackberry. In 2014, elder and blackberry were confirmed as breeding hosts, along with yew and black bryony.

By keeping adult *D. suzukii* on fruits collected from a range of plants and monitoring their reproduction, several more possible hosts have been identified including dogwood, sloe, snowberry, red bryony, spindle, rosehip, Guelder rose, cotoneaster, rowan, honeysuckle and nightshade. *D. suzukii* was also found to successfully reproduce in the ornamental plant, Pink Pagoda (*Sorbus hupehensis)*.

Additional research, tracking the development of the ovaries in the female flies, has enabled us to track the reproductive stage of females through the season, indicating times when females are reproductively active and therefore when crops are vulnerable.

In laboratory tests, it was demonstrated that where a choice of different stages of fruit development is available, *D. suzukii* 'prefer' to lay eggs in ripe fruits compared to over ripe fruits, highlighting the need for effective crop hygiene.

Preliminary results from the field (where sentinel fruits were placed in the crops; cherry and raspberry) and laboratory experiments to examine the time of egg laying, suggest diurnal female egg laying activity, with more eggs being laid in two peaks; late morning and in the evening, before dark. However, small numbers of eggs could be laid over the whole 24 hour period.

Objective 2

Researchers in other EU countries highlight the importance of crop hygiene for *D. suzukii* control. Consultations with UK soft fruit growers indicated that ~20% of the strawberry crop and 10-15% of the raspberry crop is currently waste, mainly disposed of in a 'compost heap' which rots down over several months. Cherry and plum waste is not usually collected from under the trees. Quantities of fruit waste produced by individual companies can range from <1 tonne to >100 tonnes per week during peak season.

In replicated tests, fruit fermentation in Dolav bins was effective at killing *Drosophila* larvae. Bins needed to be sealed with plastic sheeting and with the lid in place. This has the effect of

depleting the oxygen within the bins and thus killing larvae within 48 hours at ambient temperatures of 16^{o}C. Longer storage temperatures may be needed at cooler ambient temperatures. The waste product was still found to be attractive to *D. suzukii* after this treatment and hence waste material spread onto land should be incorporated into the soil. Future tests will look at composting with other farm waste materials.

Objective 3

Three low cost methods were trialled to detect *D. suzukii* late stage larvae in fruit (blueberries, cherries, raspberries and strawberries); immersion of crushed fruit in strong sugar or salt solutions 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 to directly count the numbers of larvae.

Sugar and salt immersion were the most successful in detecting *D. suzukii* larvae, with sugar solution slightly more effective. No method gave 100% recovery of the larvae. Freezing overnight generally gave lower counts of *D. suzukii* larvae. Hence, flotation with a strong sugar solution is the most practical way for growers to determine the infestation levels of fruits. A regular programme of emergence testing, although taking longer, could give growers additional information on fruit infestation early in the season and is generally more sensitive than flotation testing.

Objective 4

A wide variety of traps and baits have been developed around the world for *D. suzukii* recording. Work at EMR compared the most promising of the commercially available traps for efficiency and ease of use. The results from 2014 suggest that the most practical of those tested for grower use is the 2014 Biobest trap combined with Dros'Attract liquid bait. However, it was shown that because of the larger entry hole sizes in this trap, a significantly greater number of >4 mm insects are captured making identification of adult *D. suzukii* more time consuming.

For scientific use, the modified Biobest trap used in conjunction with the Cha-Landolt bait system provided high catches of *D. suzukii* and the lowest bi-catch.
A dry bait produced by NRI containing the same 4 components as the Cha-Landolt bait was trialled in a cherry crop and shown to be attractive to *D. suzukii* and more selective for this species, with less by-catch of other insects.

Development of a lure and kill formulation using pesticides combined with an attractant is underway and has positive initial results.

Objective 5

Fruit from unprotected raspberry plants sprayed with field doses of insecticide were assessed using a laboratory culture to determine efficacy and any residual effects. Insecticides tested were: abamectin (Dynamec), acetamiprid (Gazelle), chlorantraniliprole (Coragen), chlorpyrifos (Equity), deltamethrin (Bandu), lambda cyhalothrin (Hallmark), pyrethrins (Spruzit), spinosad (Tracer), thiacloprid (Calypso) and a coded product. These were compared to an untreated control.

The insecticides which caused greatest direct mortality to adults introduced into the boxes of fruit were chlorpyrifos (100%), spinosad (57%) and the coded product (47%). These were also amongst the most successful in reducing subsequent larval emergence, with good control of emergence up to 1-2 weeks after spraying (chlorpyrifos; 1% emergence compared to controls after 14 days, coded product 11% emergence compared to controls after 14 days). This was broadly in agreement with the trial on strawberries in 2013, although spinosad efficacy had declined after the first week in 2014 (from 29% emergence compared to controls at 7 days to 67% at 14 days), potentially because the trial in 2014 was on unprotected raspberry, as opposed to protected strawberry the year before.

Abamectin, acetamiprid, and thiacloprid also significantly reduced larval emergence from treated fruit exposed to *D. suzukii* (<20% emergence compared to controls, on fruit from day of spraying, and next day), even though they had a limited toxicity to adults, especially abamectin (3%).

Of the other tested products, chlorantraniliprole, deltamethrin, lambda cyhalothrin, and deltamethrin gave a degree of control of adults, but no significant control of emergence from fruit.

In 2013, a baseline for resistance (LD_{50}) was set with the laboratory culture. In 2014, field *D. suzukii* were sampled from crops known to have been treated with insecticides and nearby wild areas. There is no evidence of resistance to spinosad from these populations thus far. In addition, sugar is being tested as a possible adjuvant to increase the efficacy of insecticides.

Additional research

Survival of eggs and larval stages through the cold store chain, and the use of hypospectral imaging to detect *D. suzukii* eggs in fruit, was investigated. Further details (Objective 6) can be found in the science section of this report.

Financial benefits

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

Action points for growers

- Monitor adults in susceptible crops and wild areas around crops from February onwards so that they can predict the onset of egg laying by *D. suzukii*. Use the recommended trap and bait.
- Deploy perimeter trapping around vulnerable crops before fruit begins to ripen, to delay movement of *D. suzukii* into the crop.
- Monitor for larval infestation in the crop. The floatation 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 should be maintained and waste fruit treated by containing in sealed vessels and then disposed of responsibly.
- Consult BASIS trained advisors for the latest approvals for effective plant protection products.
- Growers can find comprehensive information about spotted wing drosophila (including useful videos on trapping and monitoring) on the dedicated SWD pages of the HDC website www.hdc.org.uk/swd.

SCIENCE SECTION

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

Task 1.1 Population dynamics of adult *D. suzukii* in vulnerable polytunnel and outdoor grown fruit crops at 11 sites in England (EMR + 10 farms) and four sites in Scotland (JHI + three farms)

Materials and methods

Sites: The same 14 fruit farms from Year 1 were used as field sites for 2014 and an additional farm in the West Midlands was added from April. 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 based on the growers' willingness to participate, cooperate and share data, and to ensure that a full range of vulnerable soft and stone fruit crops (blackcurrant, blueberry, cherry, raspberry, and strawberry) were included. A brief summary of the farms is given in Table 1.1.

D. suzukii traps: D. suzukii 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. Trials at EMR on comparing and improving lure and trapping technology for *D. suzukii* are described in detail under Objective 4. For continuity, within the National Monitoring Programme the trap design used at the end of 2013 was retained.

Droso traps (BioBest, Westerlo, Belgium) were modified with 20 extra 4 mm holes drilled into 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. To enhance attraction further, 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.

Table 1.1 Summary of fruit farms involves in the National Monitoring Programme (2014)

Region and crops*	
South East England (46 traps)	
Farm 1	Raspberry, strawberry
Farm 2	Raspberry, strawberry
Farm 3	Cherry
Farm 4	Raspberry, strawberry
Farm 5	Blackcurrant, cherry, raspberry, strawberry
Farm 6	Blueberry, redcurrant, strawberry
Eastern England (20 traps)	
Farm 7	Blueberry, raspberry, strawberry
Farm 8	Raspberry, strawberry
West Midlands (27 traps)	
Farm 9	Blackberry, blackcurrant, 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, redcurrant, strawberry
Farm 13	Blackberry, blueberry, raspberry, strawberry
Farm 14	Blackberry, blueberry, raspberry, strawberry

* One woodland area was also assessed at each farm

The traps were deployed at the height of the main crop. In strawberry fields, traps were hung so as to be 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 continued throughout the year, with weekly counts during the cropping season and biweekly counts during the winter.

Results

The results for England in 2014 are summarised in Fig. 1.1.1, in comparison to 2013. Numbers of *D. suzukii* were considerably higher in 2014.

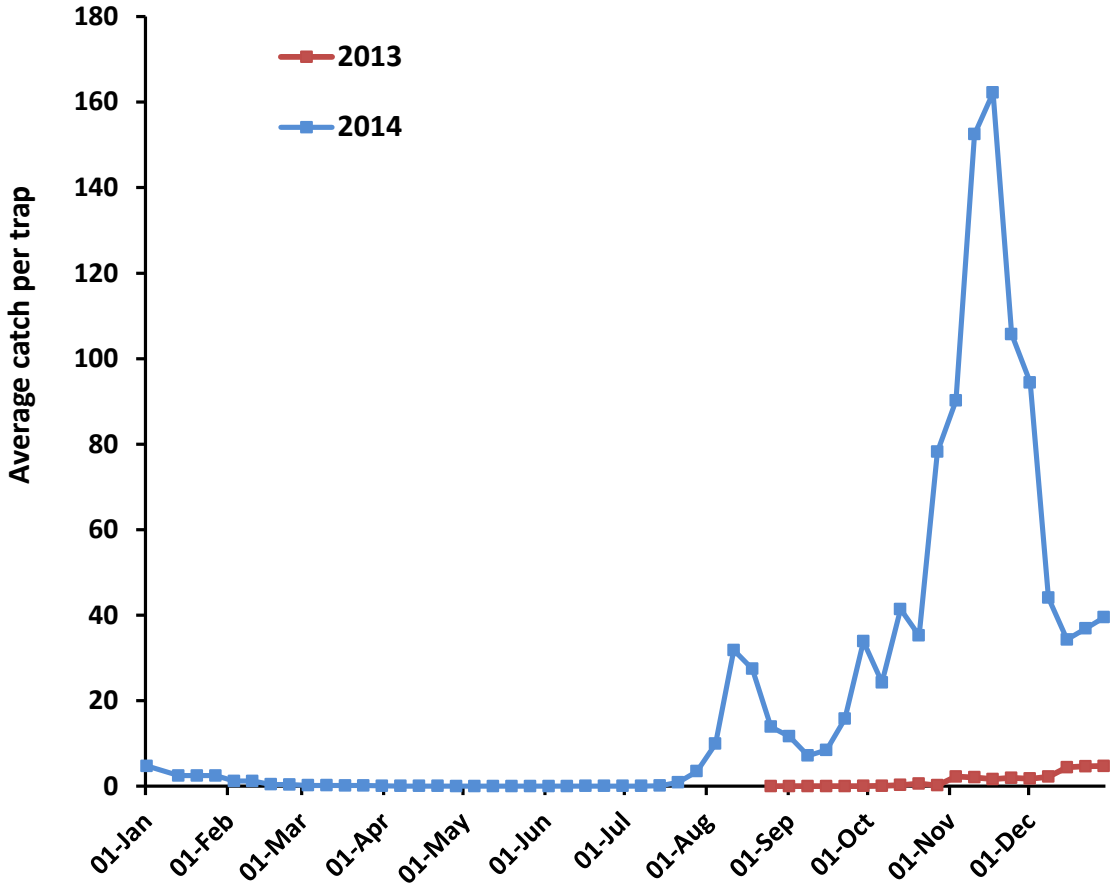

Figure 1.1.1 Comparison of average total adult *D. suzukii* catches in 2013 and 2014

There was considerable variation between sites within regions, but some trends are evident (Fig. 1.1.2). The largest catches were in the South East of England. Here adult *D. suzukii* were caught in woodland areas in January and February before falling to very low numbers in all areas in mid-February. In April, May and June almost no *D. suzukii* adults were caught, before numbers started to increase in July. The numbers caught in crops rose to a peak in August, before falling and then rising again to another peak at the end of October/ beginning of November. Numbers remained relatively high into December. The numbers in South Eastern wild areas did not have the August peak, but increased steadily, until mid-October, from when large numbers (for the UK) were caught, before a decline at the end of November to very low numbers in February 2015.

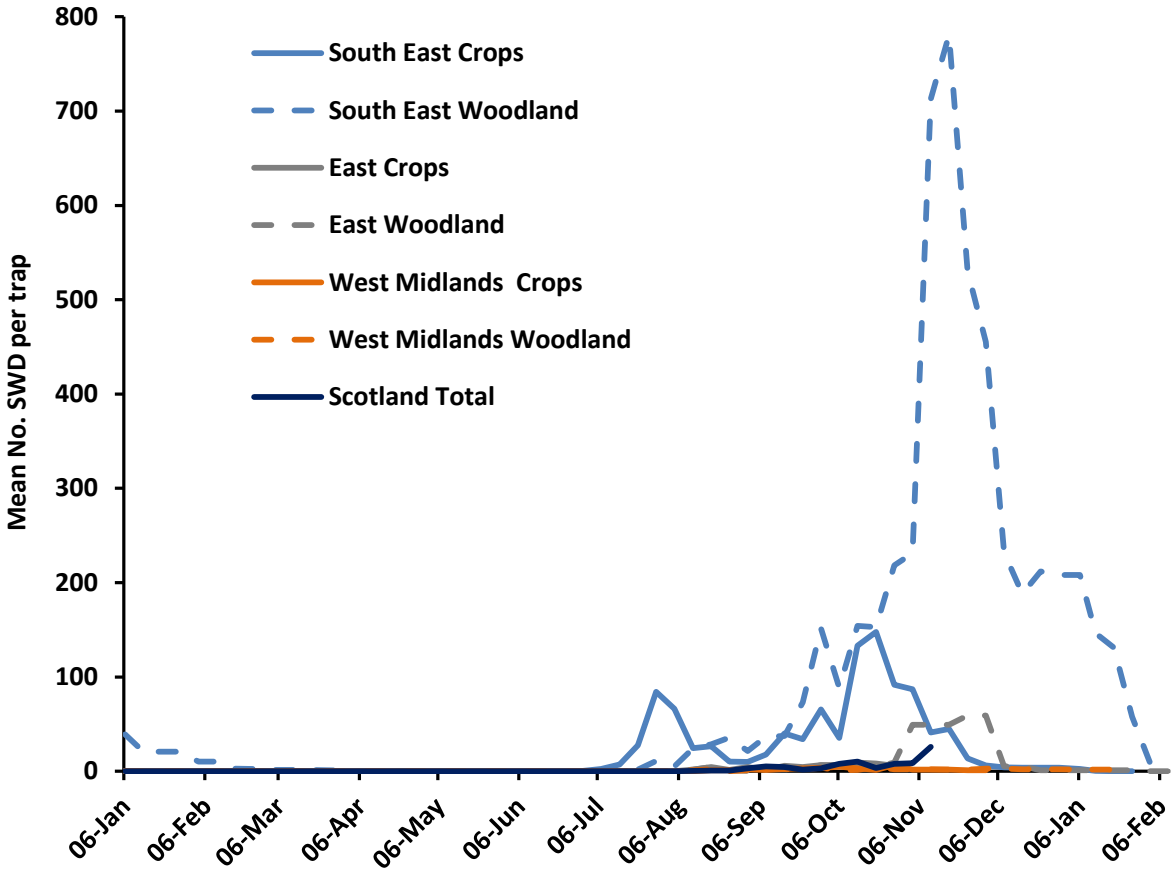

Figure 1.1.2 Mean numbers of *D. suzukii* adults per trap in the main habitat types during 2014

No adult *D. suzukii* were caught in 2014 at the sites in the East of England or the West Midlands until mid-July, apart from one individual at the start of January. Crop catches in Eastern England followed a similar pattern to the South East, though at much lower numbers and without a discernible peak in August. The numbers caught in wild areas increased dramatically at the end of October, and remained high at the start of December. Numbers in the West Midlands remained, on average, relatively low compared to the rest of the country.

No *D. suzukii* had been caught in the National Monitoring Programme in Scotland in 2013. The first *D. suzukii* were detected at the end of July 2014, and numbers increased to a slight peak in early September before declining and then increasing through October and November.

Discussion

Catches of adult *D. suzukii* in the traps were very low in 2014 in the UK until July, after which numbers increased in all parts of the country, with considerably greater numbers than in 2013. The difference between the two years presumably reflects an interaction between the establishment of the pest and the UK climate.

D. suzukii was first detected in the UK in 2012. This was followed by an unusually cold winter and colder than average spring and summer (Fig. 1.1.3). This probably delayed the reproduction of *D. suzukii*. The extremely mild 2013/14 winter caused favourable conditions resulting in a greater number of over wintering *D. suzukii.* The unusually warm spring, early summer and autumn provided ideal conditions for rapid population growth early on in the season.

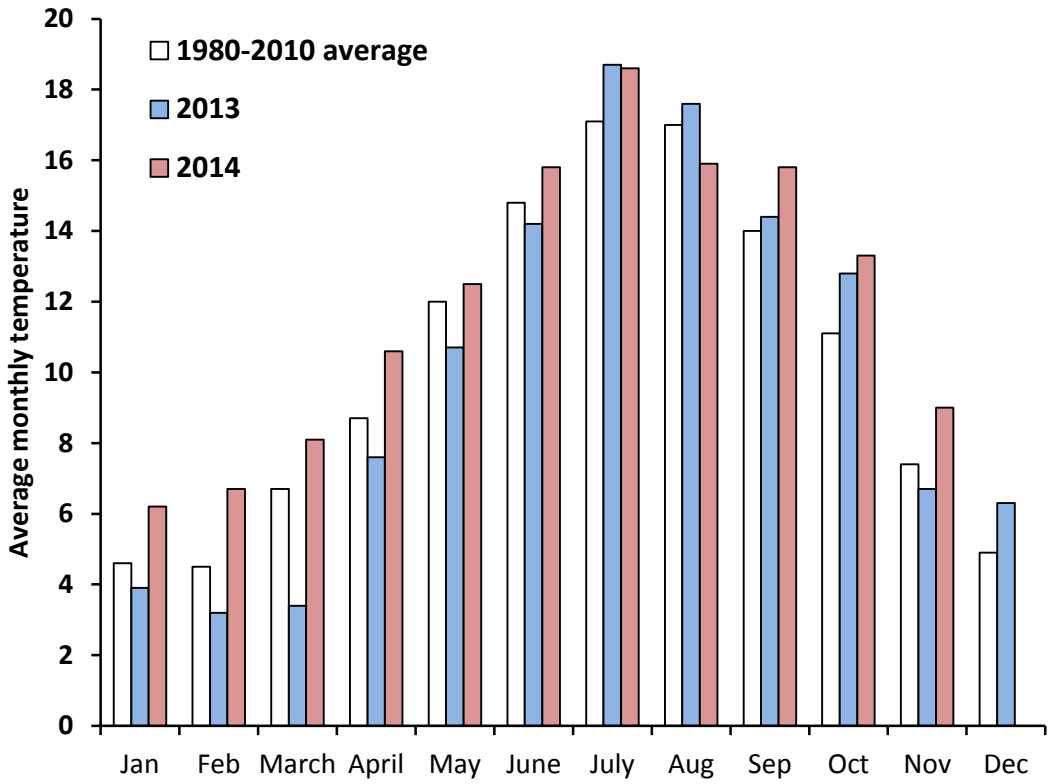

Figure 1.1.3 Comparison of the weather in the South East and Central South region of the UK in 2013 and 2014 with the 1980-2010 average (Met Office, UK)

As in 2013, there was a sharp increase in the numbers caught in woodland in the late autumn of 2014, with a corresponding decrease in crops. This pattern was also found in

other temperate regions at the northern limits of the *D. suzukii* range, and presumably reflects a migration to more sheltered areas.

Task 1.2 Phenology, population dynamics and spatial distributions of each life stage of *D. suzukii* on two fruit farms in SE England, including one polytunnel cherry, raspberry and strawberry crop, from May 2013 to March 2015 inclusive

Materials and methods

Adult trapping: One commercial polytunnel cropping area each of, cherry (0.62 ha), raspberry (1.6 ha) and strawberry (2.0 ha) in Kent were studied, in addition to a variety of surrounding habitats, including woodlands, hedgerows, compost heaps, wasteland and other fruiting crops. Within the focus crops, traps were distributed covering the edges and the centre of the crop.

Twenty seven pairs of traps were deployed on Farms 1 and 2 in 2013. In October 2014 the strawberry crop was removed from Farm 2 and traps were moved to a strawberry crop at Farm 1 (Table 1.2.1). Adults were trapped with the same traps as for the National Monitoring Programme (Fig. 1.2.2) (See Objective 4 for details of baits.). Traps within a pair were spaced 10 m apart.

Weekly trap catch records for *D. suzukii* males and females, and other flies were taken from March 2014 until November, when catches were counted fortnightly. Trap catches were taken back to the laboratory to be counted.

Figure 1.2.2 Synthetic bait trap with liquid bait trapping media

Table 1.2.1 Numbered pairs of traps at Farms 1 and 2 and associated habitat. *traps removed **traps redistributed

Farm 1		Farm 2	
Trap pair no.	**Habitat**	**Trap pair no.**	**Habitat**
1	Cherry orchard	101	Strawberry*
2	Cherry orchard	102	Strawberry*
3	Hedgerow	103	Hedgerow
4	Woodland	104	Hedgerow
5	Cherry orchard	105	Strawberry*
6	Waste ground	106	Woodland
7	Soft fruit	107	Raspberry
8	Hedgerow	108	Raspberry
9	Cherry orchard	109	Strawberry*
10	Cherry orchard	110	Woodland
11	Pear orchard	111	Hedgerow
12	Hedgerow	112	Strawberry*
13	Cherry orchard	113	Raspberry
14	Cherry orchard	114	Raspberry
15	Apple orchard	115	Raspberry
16	Hedgerow	116	Woodland
17	Apple orchard	117	Raspberry
18	Hedgerow	118	Woodland
19	Strawberry	119	Raspberry
20	Hedgerow	120	Hedgerow
21	Compost heap	121	Hedgerow
22	Hedgerow	122	Woodland
23	Hedgerow	125	Raspberry
24	Compost heap	126	Raspberry
25	Strawberry**	127	Raspberry
26	Strawberry**	128	Raspberry
27	Strawberry**		
28	Strawberry**		
29	Strawberry**		

Habitat assessments: Trap catches were assessed in groups defined by habitat type. Records of species diversity and abundance were taken from the areas surrounding the pairs of traps. Abundance was calculated using the Total Estimate Scale (Table 1.2.3). 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 (for the winter trapping the total numbers were divided by two as trapping was fortnightly). Monthly habitat assessments recorded the presence of wild and cropping fruiting plant abundance and growth stage.

Table 1.2.3 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 covering 76-100% of the area

Fruit samples: Samples of fruit were taken weekly from both the edges and centre of each focus crop and wild plants near to monitoring traps, from the green fruit stage until the end of fruiting. Within each soft fruit crop 50 fruit from between each pair of traps were sampled. Fruits were picked which 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), where the traps were placed. The fruit samples were put in ventilated Perspex boxes at 26^{o} C for three weeks with 16:8 h light: dark regime. The boxes were checked weekly for the presence of *D. suzukii* adults (emergence testing).

An additional 10 fruits were sampled from each crop and weekly measurements were made of colour (comparison with Ctfil colour chart), hardness/softness (measurements with penetrometer/durometer) and sugar content (refractometer measurements of °Brix) until the end of harvest.

Fruit samples were checked weekly for the presence of *D. suzukii* adults (emergence testing). Flies that did emerge were removed and counted. Fruit measurements were made using a Ctfil colour chart, penetrometer/durometer for the firmness and a refractometer to measure the sugar content in °Brix. These were taken weekly until the end of harvest.

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 was derived from a visual assessment of ovary and egg development. The stage definitions were published by Beverly S. Gerdeman, Washington State University.

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Egg laying preference

In the laboratory: Fresh raspberries were either stored at 3°C or 26°C, depending on treatment, for five days. *Drosophila* 'Quickmix' (Blades Biological) was used as the control. 40 g of each of the three media were put into 5 cm Petri dishes within a large bug dorm (30 cm x 30 cm x 30 cm). Twenty female and 10 male *D. suzukii* were introduced to the cages. The Petri dishes were removed from the cages after 48 hours and put into individual boxes for adult emergence (Fig. 1.2.4). The boxes were then stored at 20°C for three weeks in the quarantine facility at East Malling Research for adult emergence. For both trials assessments were counts of emerging adults up to three weeks after inoculation.

Figure 1.2.4 Petri dishes of the different media divided into individual emergence boxes

On the plant: Six strawberry plants; three cv. Evie and three cv. Finesse, were put into large bug cages (40 cm x 40 cm x 100 cm cages, Fig. 1.2.5) which zip closed. The cages were inoculated with 20 female and 10 male *D. suzukii* adults. Two days after inoculation all fruit was removed from the plants and divided into ventilated boxes depending on ripeness stage (Fig. 1.2.6). The boxes were then stored at 20°C for three weeks in the quarantine facility at East Malling Research.

Figure 1.2.5 Cage set up with strawberry plants

Figure 1.2.6 Strawberries divided up into boxes depending on ripeness stage

Time of egg laying: Traps baited with ripe fruit were distributed throughout either a cherry crop (unprotected) or a raspberry crop (protected) at EMR. Traps were red blackcurrant leaf midge traps (East Malling Research) with a Petri dish of 100 grams of raspberries in the centre. The fruit was changed every 1.5 or 2 hours depending on the crop and then stored at 26^{o}C for three weeks.

Counts of emerging adult *Drosophila* were made after three weeks. This included several other common Drosophilid species.

Results

Trapping: Trapping continued through the winter of 2013/14 and only three weeks were devoid of *D. suzukii* on both farms (Fig. 1.2.7a) in 2014; two weeks in February and one week in June. The active population in February and March is likely to be the overwintering forms laying first generation eggs (see later).

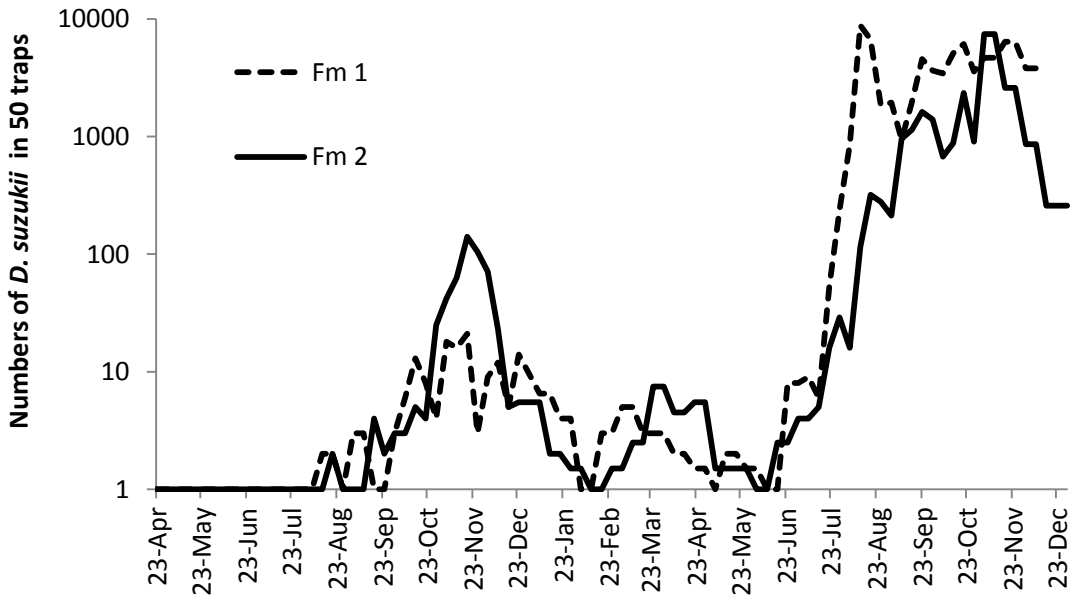

Figure 1.2.7a Adult *D. suzukii* trap catches at Farms 1 and 2 in 2013 and 2014. NB: on a log scale

The numbers of male and female *D. suzukii* on each farm was relatively even although there was a slight bias of males at Farm 1 and females at Farm 2 in the adult traps (Fig. 1.2.7b).

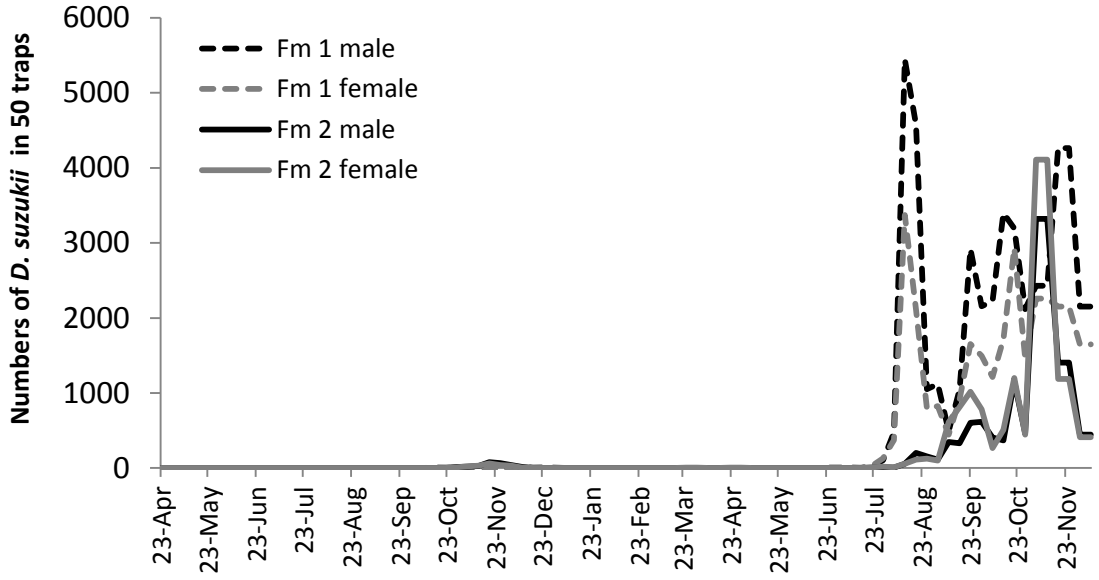

Figure 1.2.7b Numbers of male and female *D. suzukii* trap catches at Farms 1 and 2 in 2013 and 2014

The ratio of males to females throughout the study was 1.13 male to female. Hence males were slightly more prevalent overall in the current trapping devices (Fig. 1.2.7c). In general there were 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 at Farm 1 on 18 Feb compared to females on 25 Feb).

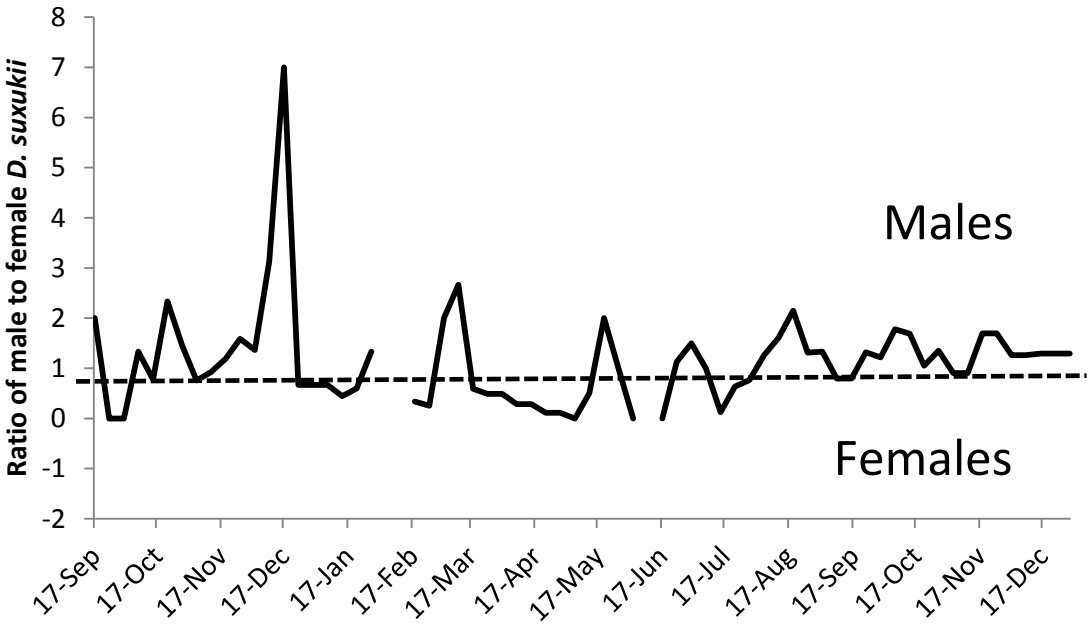

Figure 1.2.7c Ratio of male to female adult *D. suzukii* trap catches over the study at the two farms

Habitat assessments: D. suzukii were caught in all trap locations on the two farms in 2014 (Figs. 1.2.8 and 1.2.9a). Numbers of adult trap catches increased rapidly from July to August in crop traps. Adult *D. suzukii* tended to be caught in areas that contained wild blackberry and elderberry and became more abundant in crops once the wild fruits were diminishing around the end of September. From October onwards the majority of *D. suzukii* were trapped in neighbouring woodlands and this peaked in December. Numbers captured declined once overnight frosts began. There were particular woodlands or hedgerows on both farms where *D. suzukii* were caught almost every week through the year.

There was a peak of *D. suzukii* at Farm 1 in the cherry orchard in August. This coincided with the end of the cherry harvest (Fig. 1.2.9b) and the disappearance of fruit from the ground and remaining on the trees. This suggests that there is competition between the remaining fruits after harvest and the traps.

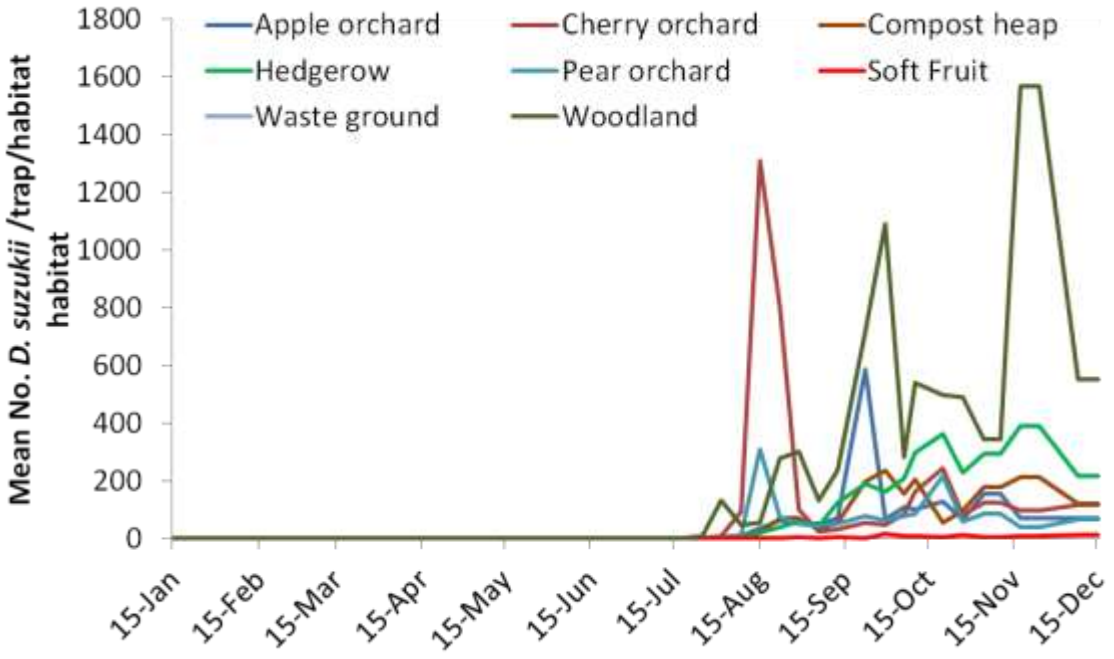

Figure 1.2.8 Mean numbers of *D. suzukii* captured per habitat type at Farm 1

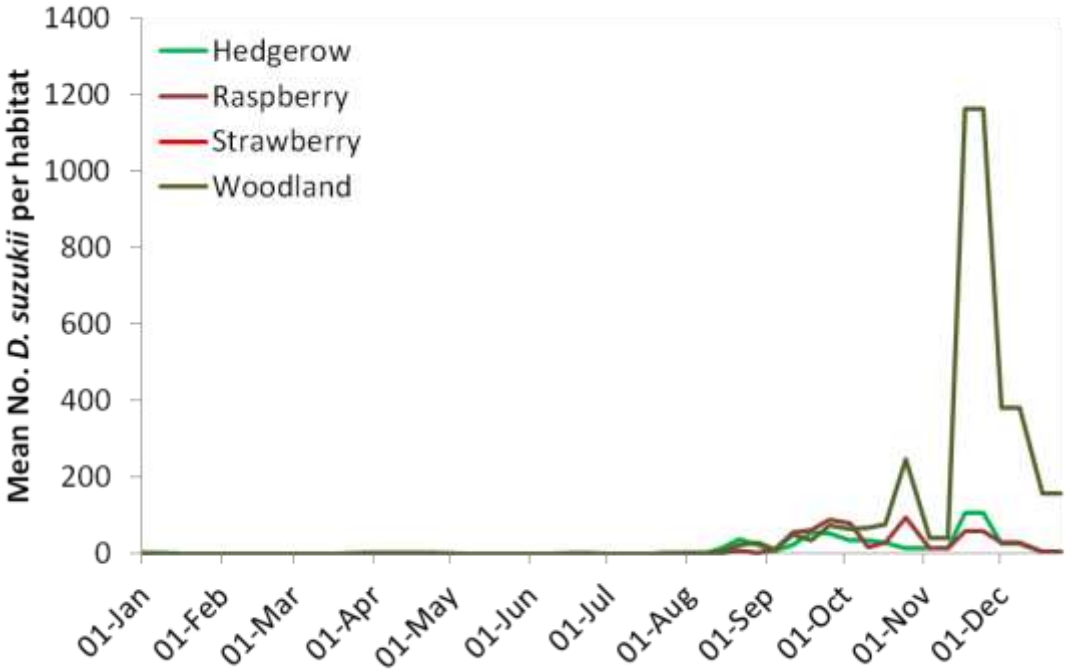

Figure 1.2.9a Mean numbers of *D. suzukii* captured per habitat type at Farm 2

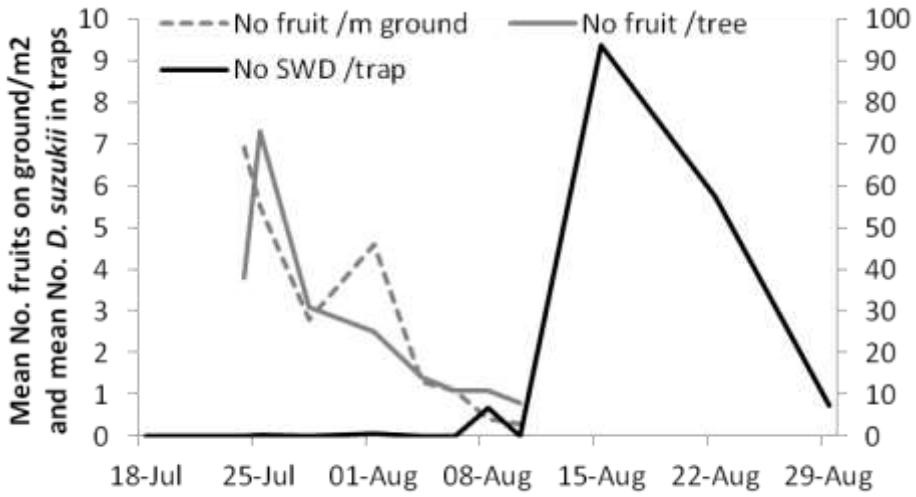

Figure 1.2.9b The relationship between *D. suzukii* in the traps and fruits remaining in the orchard

Figure 1.2.10 *D. suzukii* male on an old cherry fruit

Spray programmes in crops: No insecticides were applied against *D. suzukii* at Farm 1 and there was a low incidence of larvae in the fruits. This is believed to be because it was a strawberry breeder's crop which was being picked very regularly and which had all fruit removed (crop hygiene). Only one spray of lambda-cyhalothrin was applied to the cherry orchard on 19 June for *D. suzukii* control. At Farm 2 several products were applied to the raspberry crops:

Date	**Product**
10 February	Hallmark Zeon
23 April	Alpha Chlorpyrifos 48EC
01 May	Codacide oil
03 Jun	Alpha Chlorpyrifos 48EC
25 Jun	Dynamec/Apollo
27 Jun	SP057
19 Aug	Tracer
08 Sep	Pyrethrum
24 Sep	Pyrethrum
08 Oct	Alpha Chlorpyrifos 48EC

Fruit samples: Records were made of fruit quality, but are not reported here. This work needs to be optimised for cherry varieties in 2015. The first emergence from fruit was from an early variety of cherry (confidential) that was collected at the end of May (Table 1.2.10) weeks before adults were caught in monitoring traps. *D. suzukii* continued to emerge from cherry, with the highest numbers emerging from fruit collected at the end of harvest. *D. suzukii* emerged from all the three focus crops: cherry, raspberry and strawberry, throughout the season. It also emerged from cropping blackcurrant. In wild fruits, emergence occurred from elderberry, blackberry and black bryony. The variety of fruit that *D. suzukii* were found in was greater in 2014 than 2013 (Table 1.2.10). This is probably due to population increase. Emergence in 2014 was also much earlier for all of the fruits than in 2013. The first emergence was from an early variety of cherry, probably because of the preceding mild winter and spring. *D. suzukii* were found in the crop two weeks before the trap catches in both cherry and raspberry.

Table 1.2.10 Date *D. suzukii* first in fruit and first in traps

Fruit	First found in fruit 2013	First found in fruit 2014	First found in trap	In trap before fruit
Cherry	N/A	30 May	27 Jun	No
Blackberry	NA	24 Jul	04 Jul	Yes
Strawberry	01 Oct	04 Aug	04 Aug	Same
Elderberry	N/A	07 Aug	23 May	Yes
Raspberry	05Nov	07 Aug	30 Jul	Yes
Blackcurrant	N/A	22 Aug	10 Jul	Yes
Yew*	N/A	04 Nov	N/A	N/A

* Yew berries not collected at either of the farms in this trial

Reproductive stage of females: For weeks where no females were collected sample dates are blank. Measurements of body length and wing length were taken for all individuals at both farms (Fig 1.2.11 and Fig 1.2.12). The average body length dipped in the summer months at Farm 1 and rose in the colder months as we would expect. At Farm 2, body length increased gradually through the year. As all of the cropping sites at Farm 2 are under tunnels, the flies may not have experienced true abiotic factors. The wing length underwent little seasonal change at either farm.

All reproductive stages were seen during the growing season on both farms (Fig. 1.2.13 and Fig. 1.2.14). The main period of reproduction (egg laying) was between March and September. However, at Farm 2 there was a longer period of fecundity (March through to the beginning of November) which may be due to the conditions in the polytunnels. Tunnels were removed during October at Farm 1. As there was no commercial fruit available in March it is unclear what resource the females would have been using to lay eggs in. Cherry was in flower at this time and fruit setting began two weeks later.

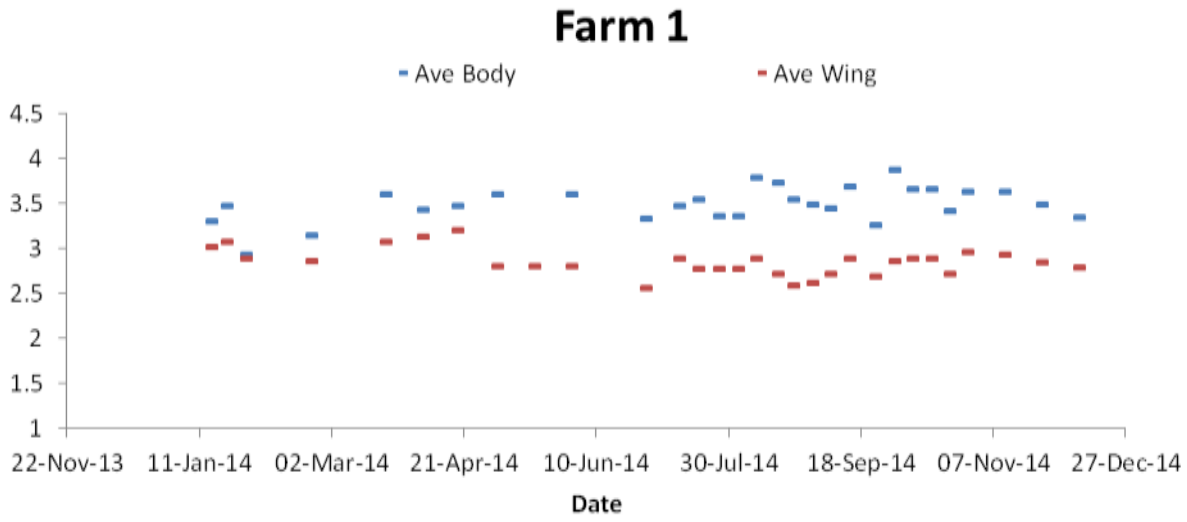

Figure 1.2.11 Body and wing length measurements from Farm 1

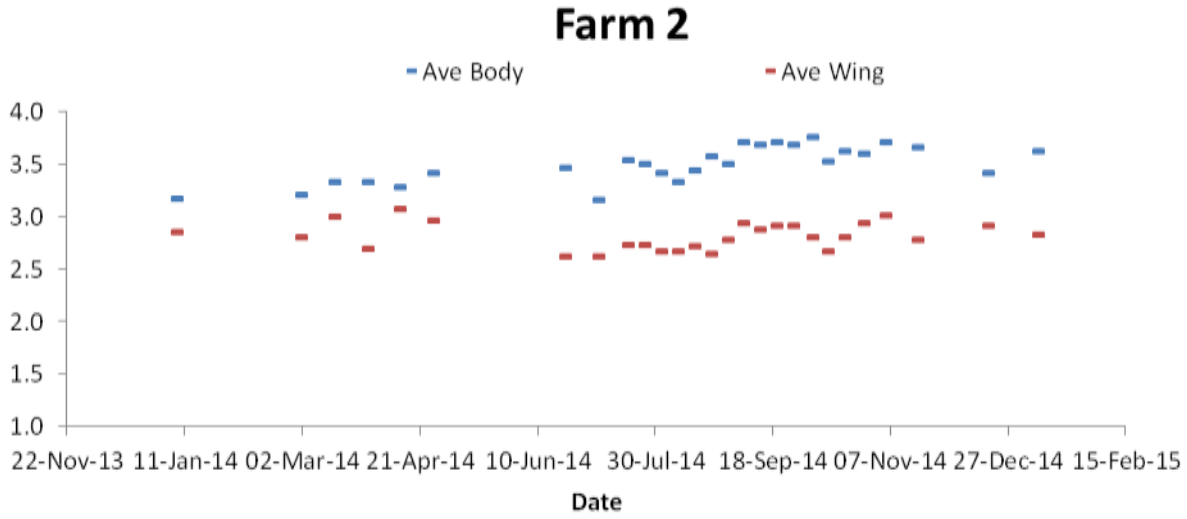

Figure 1.2.12 Body and wing length measurements from Farm 2

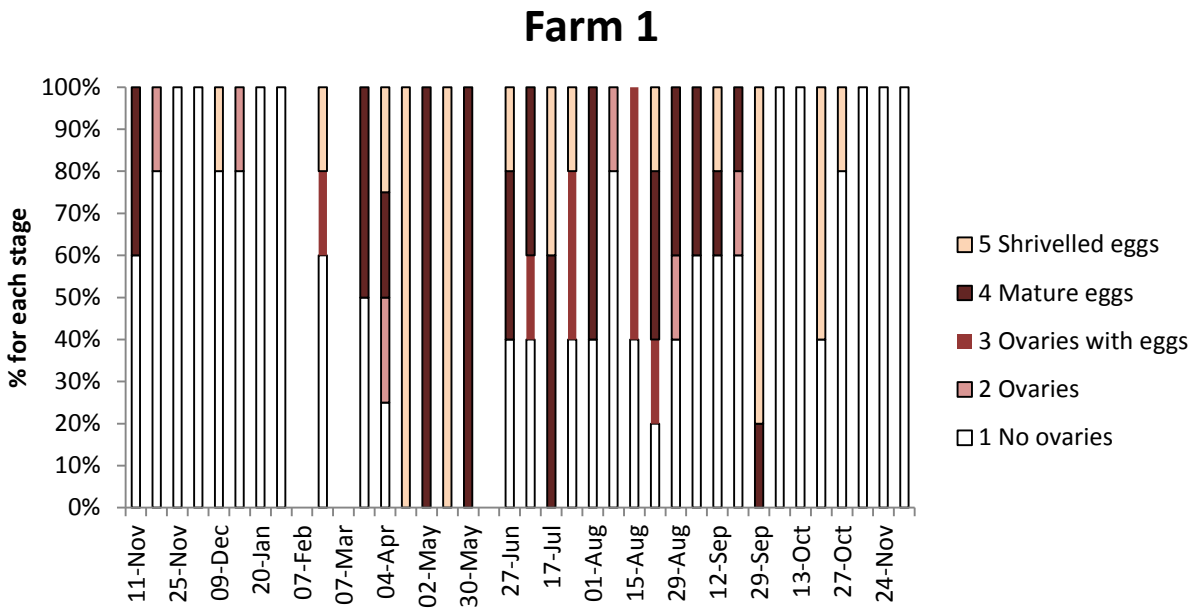

Figure 1.2.13 Percent of females at different stages of reproductive state at Farm 1

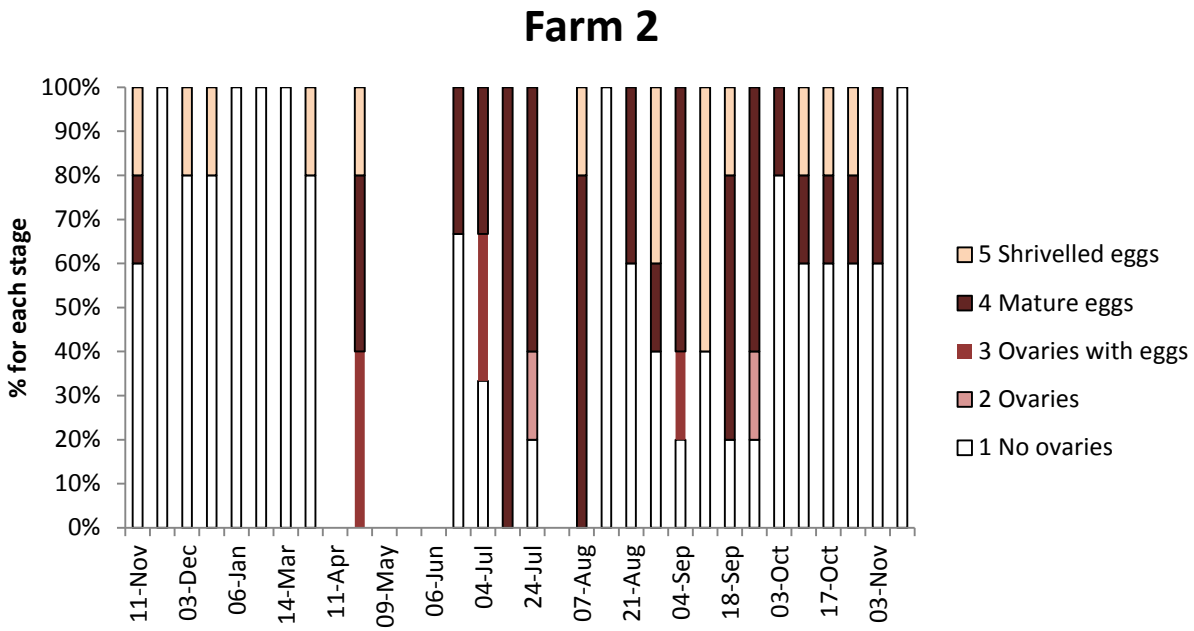

Figure 1.2.14 Percent of females at different stages of reproductive state at Farm 2

Egg laying preference

In the laboratory: Significantly more *D. suzukii* emerged from ripe- than over-ripe raspberry and the *Drosophila* 'Quickmix' diet (Fig. 1.2.15). This nonetheless highlights the consequences of leaving overripe fruit on the crop (population emergence).

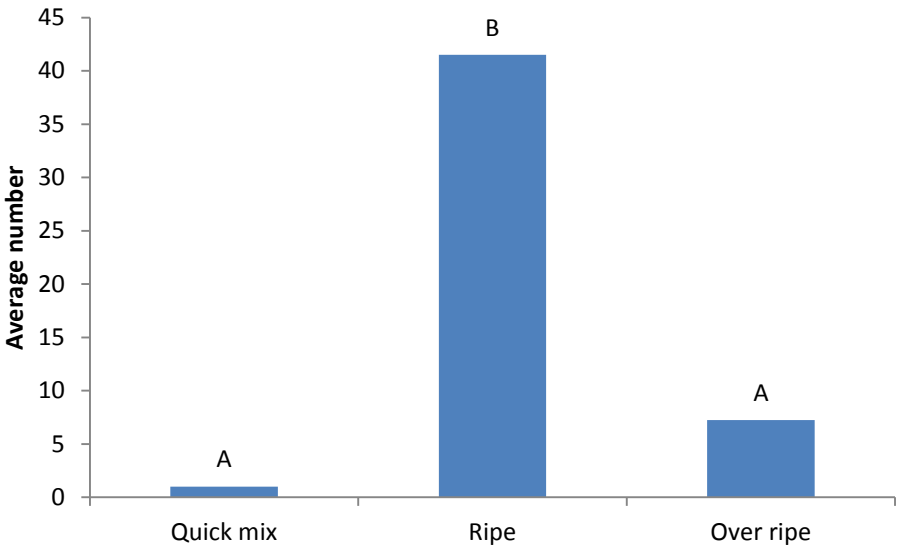

Figure 1.2.15 The average number of *D. suzukii* that emerged from egg laying media and raspberry fruits

On the plant: There was no difference between the numbers of *D. suzukii* that emerged for fruit of different stages of ripeness (under ripe (green-pink), ripe and overripe) when strawberries were on the plant (Fig. 1.2.16). This could be because there was a small overlap between the stages and ripeness of the fruit or competition between females for egg laying sites was higher than would be expected in the crop.

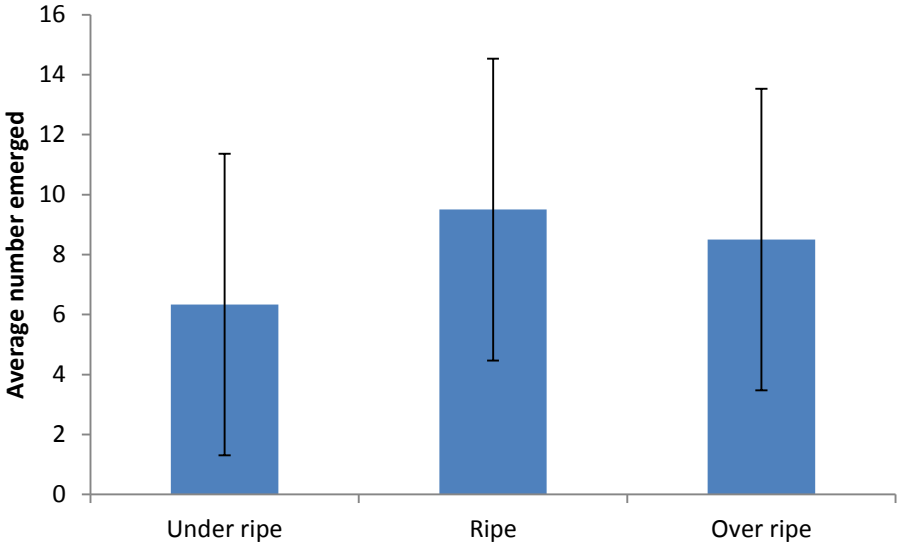

Figure 1.2.16 The average number of *D. suzukii* that emerged from the different ripeness stages of strawberry fruits on the plants

Time of egg laying: Female *D. suzukii* laid eggs at all times of the day and night in both trials (protected and unprotected crops). In the unprotected cherry (Fig. 1.2.17) there were peaks of egg laying late morning and late evening, before dark, for both days that data was collected. This trend was not as clear in the protected raspberry (Fig. 1.2.18), although there

was a peak of activity on the first day in early evening. These trends correlate with laboratory research of Dr. Ezio Rosato from Leicester University. It is currently unclear how temperature and humidity affect the times of egg laying. In both trials, temperatures did not drop below 10°C, which is the lowest temperature for oviposition in *D. suzukii* females (Sakai, 2005).

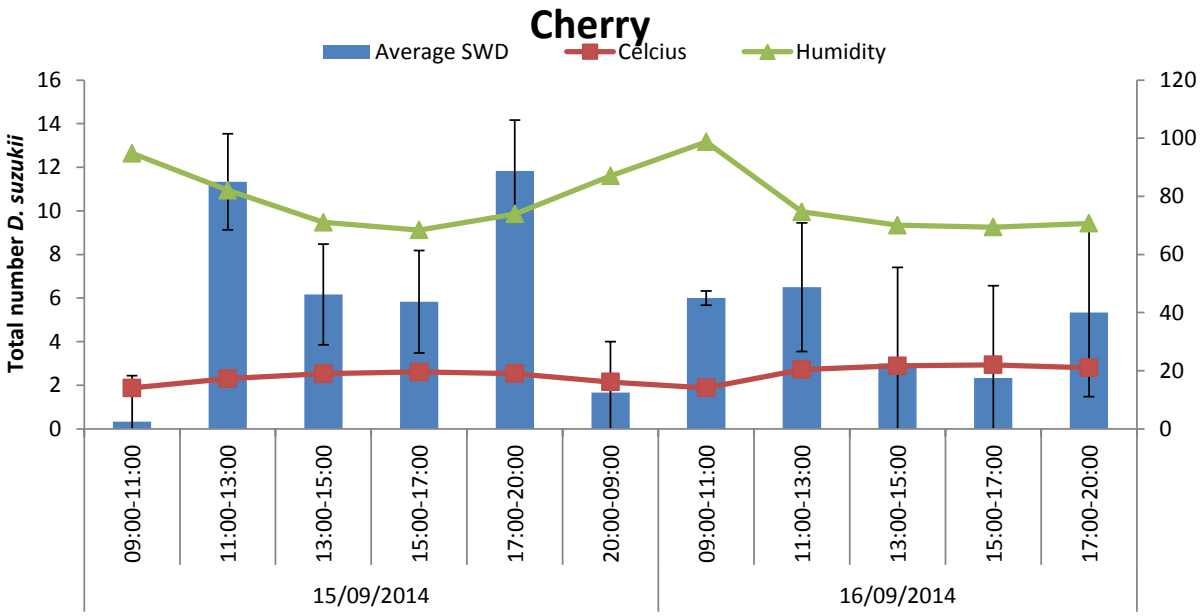

Figure. 1.2.17 Time of egg laying activity of *D. suzukii* in unprotected cherry

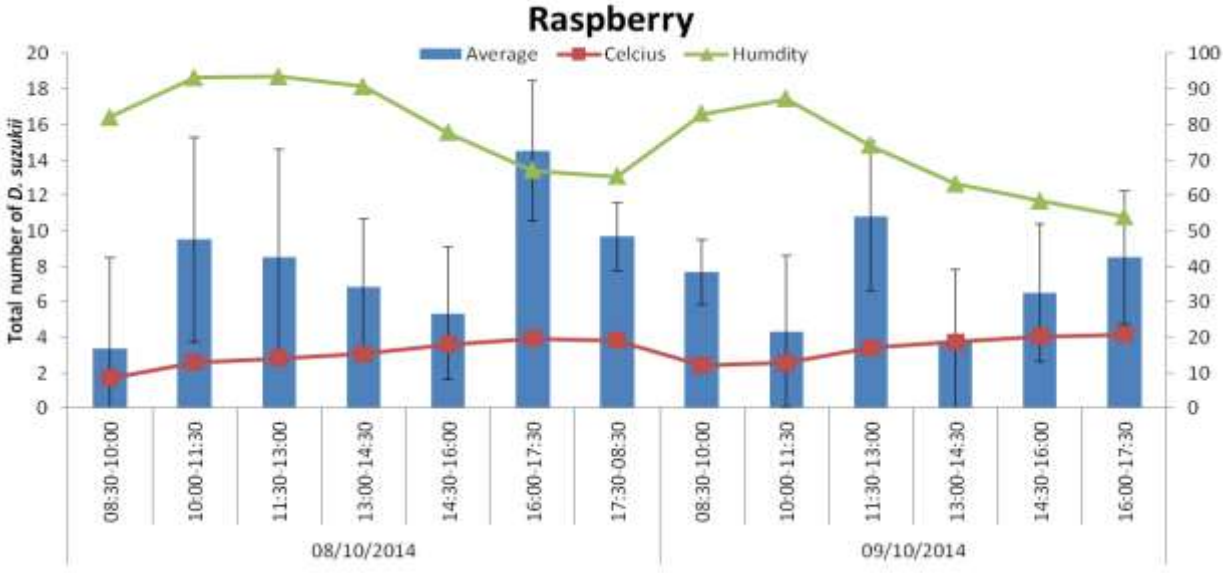

Figure. 1.2.18 Time of egg laying activity of *D. suzukii* in protected raspberry

Conclusions

- *D. suzukii* was widespread on the farms in 2014;

- Populations peaked in December in woodlands;
- There is evidence of trap–fruit competition; trap catches go up when commercial and wild fruits are less abundant;
- There were particular woodlands or hedgerows on both farms where *D. suzukii* were caught almost every week;
- *D. suzukii* did not cause financial damage to commercially cropped fruit at Farms 1 and 2 but commercial damage was reported for other growers in the region;
- The mild 2013/14 winter resulted in *D. suzukii* being caught throughout the year;
- *D. suzukii* emerged from cropped fruit throughout harvest;
- Female *D. suzukii* were able to lay mature eggs from March to November if fruit was available and environmental conditions favourable;
- Females 'prefer' to lay eggs in ripe fruit, but under-ripe and overripe fruit is also used;
- Females will egg lay throughout the day and night as long as temperatures do not drop below 10°C (Sakai, 2005).

References

Sakai M, 2005, Cherry *Drosophila suzukii*, in Primary Colour Pest and Disease encyclopedia.Vol.2. Apples, Cherries, European Pears and Walnuts, 2nd edition. Rural Culture Association Japan, Tokyo, Japan, pp. 489–492.

Task 1.3 Identify the common wild host plants of *D. suzukii* adults and larvae in the UK

Objective

Identify the common wild host plants of *D. suzukii* adults and larvae in the UK. By identifying the wild host plants that *D. suzukii* can breed on it may be possible to highlight problem areas and possible 'hot spots' or reservoirs of populations. This could result in the removal of wild hosts from the vicinity of commercial crops and, in turn, could decrease the availability of breeding habitats. Ripening and ripe fruits were collected from the field and then tested for either natural emergence, or whether *D. suzukii* would lay eggs and develop in fruits in the laboratory (no choice tests). No choice tests consisted of introducing males and females into a ventilated Perspex box along with a sample of fruit. This made it possible to determine

whether the plant was a potential host for *D. suzukii*, but not whether it was a preferred host. The natural emergence tests examined whether female *D. suzukii* were utilising fruit in the wild as a resource for reproduction.

Materials and methods

No choice emergence: Small samples of ripe or ripening fruit were collected from natural or semi-natural habitats, gardens or commercial crops. They were put into 5.5 cm x 3.5 cm x 2.5 cm ventilated Perspex insect rearing boxes with one sheet of paper towel sprayed with distilled water (Fig. 1.3.1). Five females and two males were added to the fruit and the box was sealed with electrical tape. The boxes were maintained at 26°C and sprayed with distilled water weekly. The five females and two males were removed after one week. The boxes were resealed and left for a further three weeks. At the end of this time the total numbers of emerged male and female *D. suzukii* were recorded.

Figure 1.3.1 No choice emergence set up

Natural emergence: Samples of ripe and ripening fruit were collected (as above), but from locations with known *D. suzukii* populations. Perspex boxes (10 cm x 5 cm x 3.5 cm) were filled with fruit, along with one sheet of paper towel sprayed with distilled water. The Perspex box was stored within a large bug dorm within a polythene bag to maintain humidity (Fig. 1.3.2) and maintained at 20°C for three weeks. The boxes were kept damp by spraying the paper towel with distilled water weekly. After this period of time the numbers of adult *D. suzukii* were recorded. Keeping the fruit in culture for longer periods risked a second generation emergence.

Figure 1.3.2 Large bug dorm with a sample of fruit within a Perspex box, stored within a large polythene bag to prevent drying out

Results

No choice emergence: D. suzukii that were introduced into boxes with fruits had a second generation emerge from several species; Wall Cotoneaster (*Cotoneaster horizontalis*), Fig (*Ficus carica*), Honeysuckle (*Lonicera* sp), Elderberry (*Sambucus*), Japanese rose (*Rosa rugosa*), Yew (*Taxus* sp), Rowan (*Sorbus* sp.), Pink Pagoda (*Sorbus hupehensis*), Spindle (*Euonymus europaeus*), Guelder Rose (*Viburnum opulus*) and Nightshade (*Solanum* sp). Species tested in which *D. suzukii* did not appear to develop are shown in Table 1.3.3.

Natural emergence: D. suzukii naturally emerged from raspberry, elderberry and blackberry in the previous year of this study. In 2014 we also had natural emergence from cherry, strawberry, blackcurrant, black bryony and yew (yew was collected from a different location) (Table 1.3.4). It did not emerge from cotoneaster, snowberry, guelder rose, dogwood, hawthorn, red bryony and rose in the samples we collected. From those fruits where natural emergence occurred, several separate incidences indicated that these hosts were being utilised regularly in 2014 for egg laying.

Table 1.3.3 Field collected fruits exposed to adult male and female *D. suzukii* and resulting second generation emergence (no choice).

Description	Total *D. suzukii* (per gram fruit)
Wall Cotoneaster (*Cotoneaster horizontalis*)	2.9
Fig (*Ficus carica*)	2.3
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	0
Dogwood	0
Holly (*Ilex aquifolium)*	0
Pyracantha (*Pyracantha* sp.)	0
Rubella *(Skimmia japonica)*	0
Mahonia	0

Table 1.3.4. 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

Several wild, commercial and garden species were shown to be potential hosts for *D. suzukii*. Berries in which *D. suzukii* did not develop were quite firm e.g. rose, hawthorn and ivy. Studies in the literature comparing cultivars of commercial fruit have found a strong negative correlation of vulnerability to *D. suzukii* and fruit firmness (eg. Kinjo et al 2012, Lee et al., 2011) and this presumably is one consideration of host preference for the females looking for egg laying sites. Several species that are being used by *D. suzukii* for reproduction were identified. Competition from higher populations could be intense, forcing females to utilise a wider variety of hosts for reproduction.

Conclusion

- *D. suzukii* were found in more host plants in commercial and wild fruits in 2014;
- Cherries and early fruiting crops are vulnerable as they are the first available resource after the overwintering period.

References

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Lee, J. C., D. J. Bruck, H. Curry, D. Edwards, D. R. Haviland, R. A. Van Steenwyk, and B. M. Yorgey. 2011a. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Manag. Sci*. 67: 1358-1367.

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 2.1 Establish the types and production quantities of soft and stone fruit wastes

Consultations with soft fruit growers in 2013 indicated that about 20% and 10-15% of the strawberry and raspberry crop is waste, respectively. Cherries grown under polythene protection produce about 5% crop waste whereas cherries grown unprotected produce between 10 and 100% waste depending on the season. Quantities of fruit waste produced by individual companies can range from <1 tonne to >100 tonnes per week during peak season.

Until now, soft fruit waste has mainly been collected and stored in various open vessels or double-skinned plastic bags (Fig. 2.1). The waste is then disposed of in a ‘compost heap’ which gradually rots down over several months or waste is taken to off-site composting or anaerobic digestion facilities. The main weakness of this disposal method is that the bags

can leak or burst due to bird pecking or microbial activity, and the exposed waste remains as a potential *D. suzukii* source and attractant until it is degraded.

Figure 2.1 Disposal of soft fruit waste in plastic bags and open vessel

Stone fruit waste is not usually collected and is left in the orchard where it also remains a potential source of *D. suzukii* (Fig. 2.2).

Figure 2.2 Plum waste on orchard ground

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

A review of literature and preliminary results described in the Year 1 report indicated that killing *Drosophila* species in high moisture content fruit waste using depletion of oxygen in sealed vessels was more feasible than obtaining sufficiently high temperatures in a

composting system. Further tests were conducted in Year 2 to determine the minimum treatment times needed to eradicate *Drosophila* species from infested soft and stone fruit wastes.

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 report. Eggs, larvae and pupae of *Drosophila melanogaster* on infested fruit were inserted in the soft and stone fruit waste batches at the start of each test in sealed bins.

Testing for eradication of Drosophila *species*: Litre samples of the surface fermented waste fruit from the above vessels was placed in five litre plastic containers and covered with a fine mesh to exclude any *Drosophila*. Similar batches of untreated *Drosophila* infested fruit waste were prepared. After three weeks, the containers were checked for any *Drosophila* adults that may have emerged from the waste and the numbers counted.

Attractiveness to D. suzukii *of treated waste and mixtures with soil or coir*: The attractiveness of fermented fruit waste from the above sealed vessels was tested by placing 10g samples in Petri dishes in plastic cages with four-six female and two-four male *D. suzukii* adults. In the first test, strawberry and apple waste from sealed vessels obtained from two different sites was compared with fresh untreated strawberry waste. 'Quickmix' *Drosophila* rearing medium was used as a control. In the second test, treated waste was mixed with 0, 67, 80 or 90% soil or spent coir from grow bags, and the mixtures were tested similarly in 10 g samples. Four replicate containers of each treatment were used. The adults were removed after six days and the cages tested for the presence of further adults after three weeks.

Results

Fruit waste anaerobic treatment in sealed vessels: Oxygen concentration in the headspace of the bins rapidly depleted and was not detectable in any of the stone or soft fruit batches after 24 and 36 hours respectively (Fig. 2.3). There was a corresponding increase in carbon dioxide concentration which rapidly exceeded 20% v/v. The temperature of the waste was 18 - 26°C in all except in two of the soft fruit waste batches and one batch of stone fruit waste which were filled in mid-October (mean waste temperature 14 - 16°C). Ambient air temperatures were 2 - 5°C lower than that of the waste.

Testing for eradication of Drosophila *species*: Adult *Drosophila* subsequently emerged from all untreated fruit waste samples, and from soft fruit samples taken from bins treated for up to 14 hours after the bins were sealed (Fig. 2.4). No adult *Drosophila* emerged from soft fruit samples taken at least 24 hours after the bins were sealed, except from one batch that was at 16°C (Fig. 2.4). There was also a large decline in emergence of *Drosophila* with increasing duration of treatment of stone fruit waste, although a single adult emerged after 44 hours (Fig. 2.4).

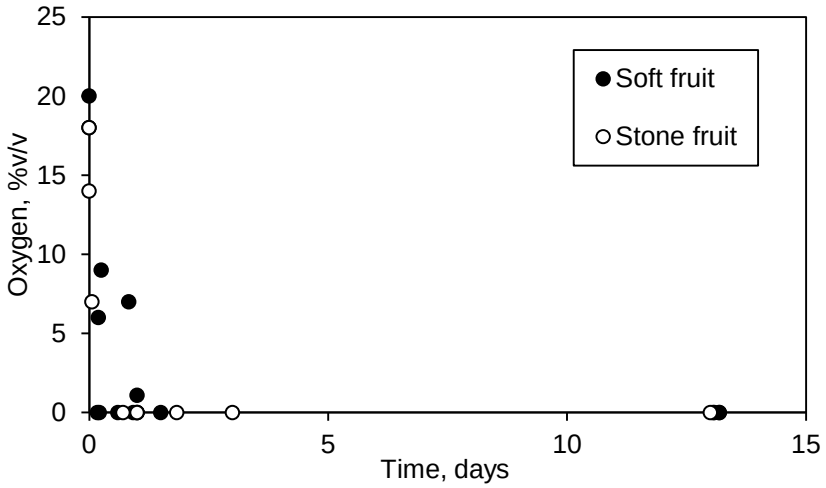


Fig. 2.3 Oxygen concentration in the headspace of sealed vessels containing fruit waste

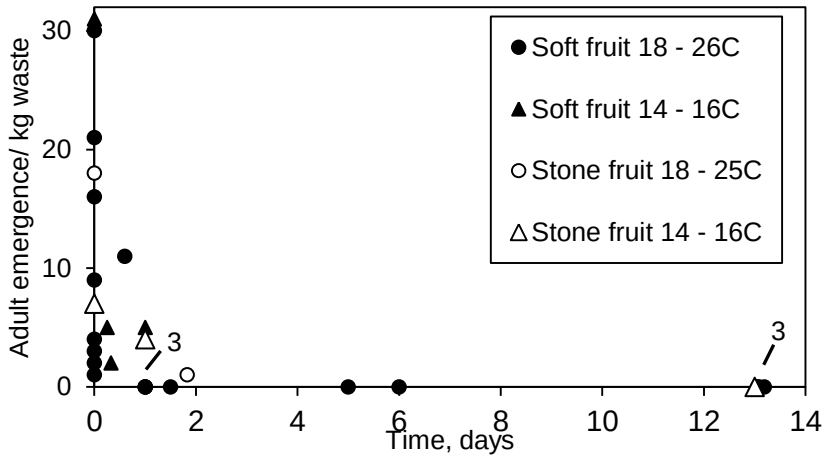


Fig. 2.4 *D. melanogaster* emergence after different durations in sealed bins at different temperatures of the waste. 3 = three data points overlapping.

Attractiveness to D. suzukii *of treated waste and mixtures with soil or coir:* The treated fruit waste remained attractive for *D. suzukii* egg laying and subsequent *D. suzukii* development into adults, and the difference between treated and untreated was not significant. *D. suzukii* emergence from the 'Quickmix' medium was greater but more variable than from the fruit waste samples (Fig. 2.5).

Addition of soil or waste coir to the treated fruit waste reduced the emergence of *D. suzukii* adults from samples of mixtures. *D. suzukii* emergence was eliminated when treated fruit waste was mixed with 90% soil or waste coir (Fig. 2.6).

Further tests

This work has shown that treatment of soft fruit waste for 24 hours in sealed 200-500 L vessels at ambient summer air temperatures of 15°C (waste is normally 3°C above) and above is sufficient to eradicate *D. melanogaster* from the waste. At lower temperatures, early and late in the season, a longer period of up to 48 hours may be needed for both soft and stone fruit waste. This will be investigated in tests in 2015. Larvae of *D. melanogaster* are well adapted to survival at low oxygen and it is unlikely that *D. suzukii* larvae are better adapted since they have a 'breathing tube' for living in fruit. However, the eradication conditions for *D. melanogaster* will be tested on *D. suzukii* in 2015.

Mixing treated fruit waste with 90% soil or waste coir removes its suitability for development of *D. suzukii*; it is possible that 85% soil or coir or covering the treated waste with a layer of soil or coir are also effective. This will also be tested in 2015.

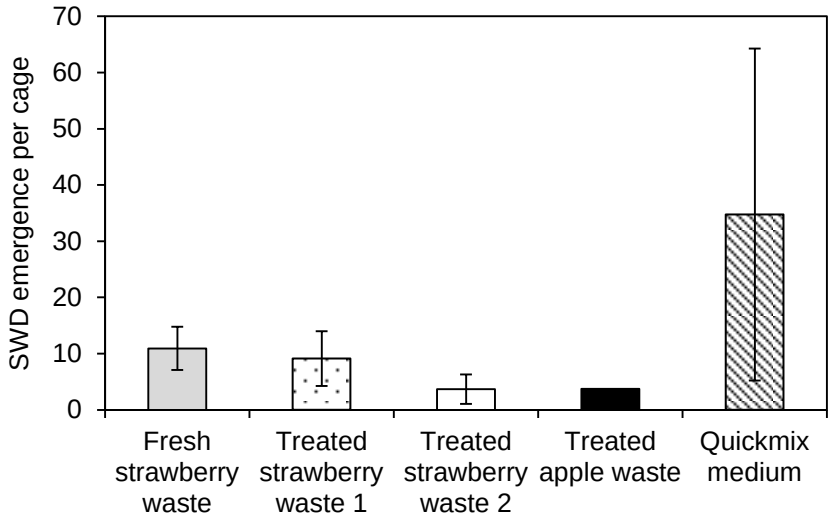


Fig. 2.5 Emergence of *D. suzukii* from fruit waste before and after treatment in sealed bins and from 'Quickmix' medium following egg laying in cage tests. Fresh is before anaerobically treated, treated is after anaerobic (1 and 2 = different sources)

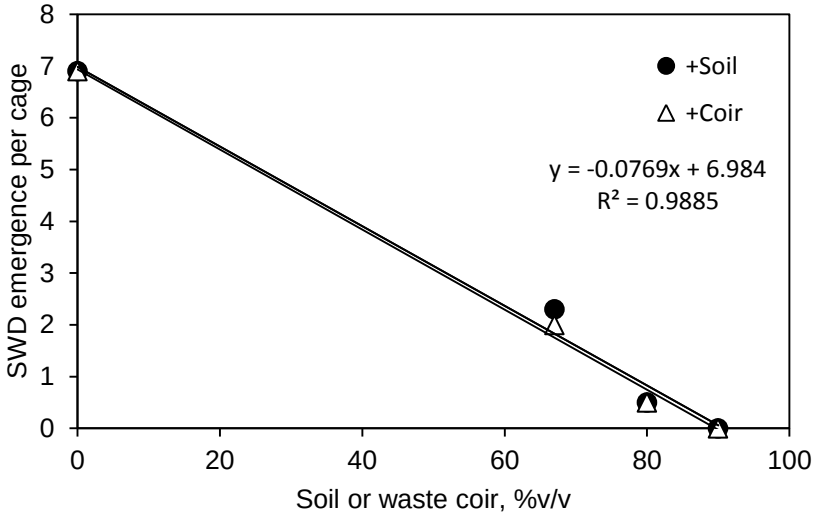


Fig. 2.6 Emergence of *D. suzukii* from mixtures of treated soft fruit waste and soil or waste coir, following egg laying tests in cage tests

Task 2.3 Composting, digestion and other processing of fruit wastes

Visits were made to three anaerobic digestion (AD) facilities to determine the feasibility of AD as a means of disposal of fruit wastes. These were constructed between 2011 and 2013 by R&L Holt Ltd, Wyre Piddle, Worcestershire, G's Fresh Vegetables, Littleport, Cambridgeshire, and GWE Bioenergy, Driffield, Yorks. Two of these facilities use predominantly maize silage as the main feedstock, together with sugar beet and vegetable crop residues. The maize and sugar beet are stored from a single harvest in clamps throughout the rest of the year. Vegetable wastes are stored for several days before use. The third facility uses predominantly packaged food wastes. None of the sites currently use fruit production waste. Due to the high moisture content and low energy value of fruit waste in AD, this would be charged a gate fee. Charges were not supplied by the companies.

The construction of an AD facility solely to dispose of fruit waste is therefore not a viable option, although existing AD facilities offer a potential disposal route. Due to the delay in transporting and treating fruit waste batches, these would need to be stored in sealed containers before use.

Fig. 2.7 Anaerobic digestion plants at GWE Bioenergy and G's Fresh

Task 2.4 Development of fruit waste collection and storage strategies

The method for anaerobically treating pallet-based bins of fruit waste developed in 2013 was improved by modifying the procedure for sealing the bins (Fig. 2.8). This involved first covering the pallet-based bins with shrink-wrap polythene followed by covering with the lid, which was then kept in place with polythene. No pressure release valve is needed with this method. Using this method, the oxygen concentration in the headspace air of the bins rapidly declined and was not detectable 24 hours after filling with soft fruit waste batches. The carbon dioxide concentration in the headspace air increased to above 20% v/v.

Fig. 2.8 Procedure for treating fruit waste in 500 litre plastic pallet-based bins

Conclusions

- Treatment of soft fruit waste in 200-500 L sealed vessels for 24 hours at ambient summer air temperatures of 15 °C and above is sufficient to eradicate *D. melanogaster* from the waste;
- For stone fruit, and at ambient air temperatures below 15°C for both soft and stone fruit, a longer period of up to 48 hours may be needed; this will be determined in 2015;
- Fruit waste can be anaerobically treated and stored in pallet-based bins which are first covered with shrink-wrap polythene, before the lid is secured with polythene;
- Fermented fruit waste remains attractive for *D. suzukii* egg laying and subsequent development. More work needs to be done on cherry waste from EMR in 2015;
- Mixing treated fruit waste with 90% soil or waste coir removes this capability for *D. suzukii* reproduction. Covering fruits with coir/compost to mask the smell will be investigated. Care needs to be taken with this method and nitrogen leaching into soil;
- Construction of anaerobic digestion facilities for disposal solely of fruit waste is not a viable option, but AD plants are a possible disposal route; the waste needs to be kept sealed during collection, transport and storage before AD.

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

Objectives

Determine the efficacy of detection of low cost methods of quantifying *D. suzukii* larval infestations in different fruits.

Materials and methods

Infestation of fruit: Supermarket bought fruit (100 g of blueberry, cherry, raspberry or strawberry) was added to the base of a (228 x 121 x 66 mm) plastic box (Fig. 3.1) 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 3.1. Fruit incubation box

D. suzukii (10 females and five males per box) were added to infest the fruit, the boxes were sealed with electrical tape to prevent escape, and incubated at 20 ^{o}C for 24 hours. After 24 hours the adult flies were removed and the fruit incubated at 20 ^{o}C for seven days; this period of incubation having been shown previously to produce late instar larvae.

Treatments: Three methods of *D. suzukii* larval (third instar) assessment were compared to two controls, 1) manual dissection of fruit and counting of larvae, and 2) counts of adult emergence. Each treatment was replicated six times and repeated on two separate occasions. The methods of larval assessment were:

1. Sugar immersion: Fruit (100 g) 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 (180 g /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 (75 g /l water) and 1-2 drops spray tank de-foamer and observed for 20 mins, with gentle mixing at 10 m;

3. Freezing: Fruit (100 g) 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 (100 g) was dissected under a binocular microscope and larvae observed directly;

5. Adult emergence: Fruit (100 g) was incubated at 20^{o}C until any adults emerged and these were counted.

N.B.: 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.

Results

In general, sugar solution gave the highest recovery, salt solution second and freezing gave lowest counts (Fig. 3.2). However, there were no statistical differences between sugar and salt solution third instar larval extraction. Sugar solution 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, they were also analysed separately. In each, the same trend was observed, but in the first trial sugar solution gave significantly higher recovery compared to 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).

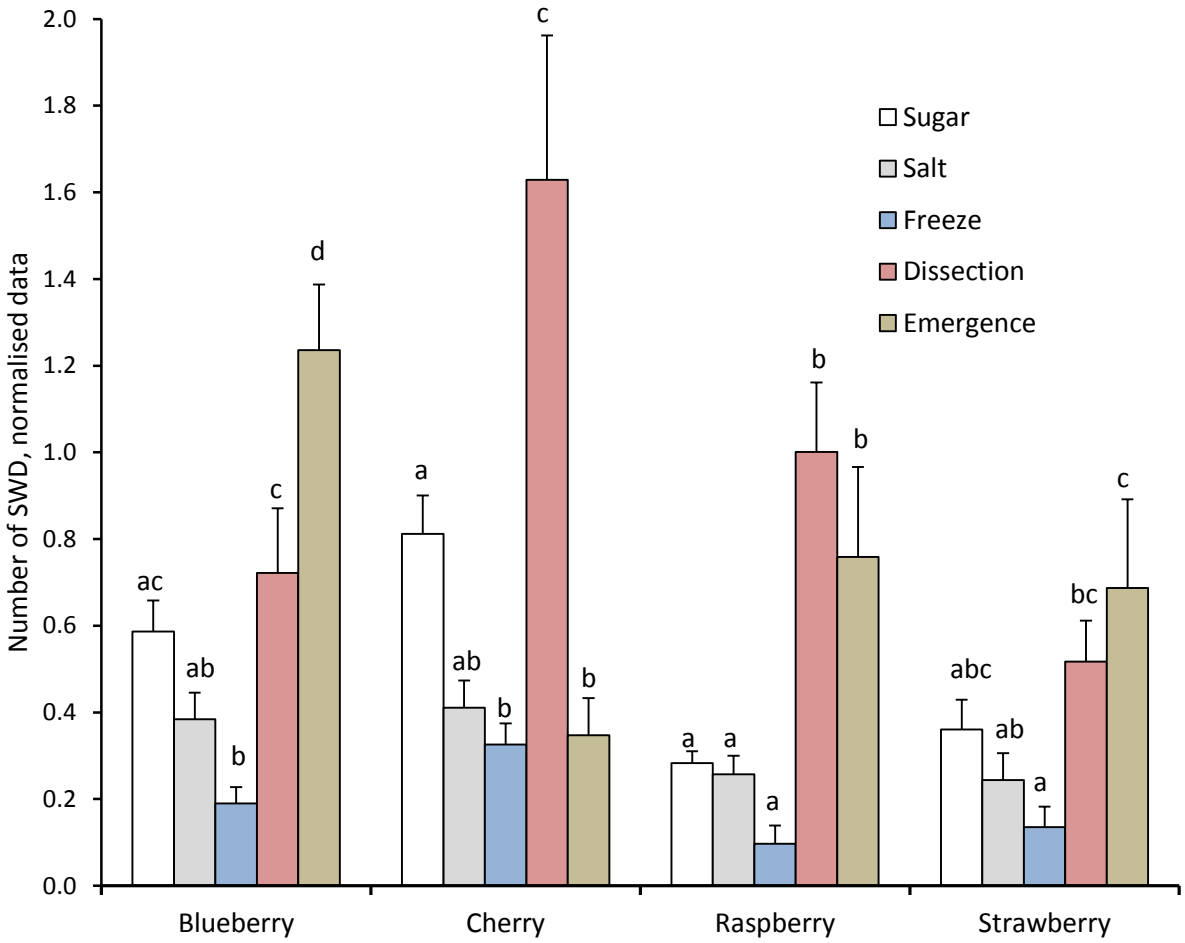


Figure 3.2 Comparison of methods for recovery of 3rd instar *D. suzukii* from fruit. Data normalised by comparison to raspberry infestation in the same trial. Values per fruit with the same letter are not significantly different (P<0.05)

Conclusions

Sugar immersion gave at least 50% recovery of third instar *D. suzukii* larvae in blueberry, cherry and strawberry. The recovery in raspberry was less efficient (~25%). In general, this method gave better recovery than salt solution immersion and freezing. These trials need to be repeated on younger instar larvae to determine if the same situation applies.

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, 7 January 2013.

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

Introduction

A wide variety of traps and lures are available for monitoring for the presence of *D. suzukii*. The aims of this work were to identify the most effective trap and bait combination to suit the needs of growers, agronomists and scientists and to investigate whether lure-and-kill approaches can be used for controlling *D. suzukii* as part of IPM programmes against the pest.

Previous work involved lures based on various natural fermentation products such as vinegar, apple cider and wine (e.g. Landolt et al., 2012a; 2012b). More recently a four-component blend of synthetic chemicals was reported to be attractive to *D. suzukii* (Cha et al., 2012, 2013a). This consisted of ethanol and acetic acid dispensed from an aqueous drowning solution and acetoin and methionol dispensed from polypropylene vials with a hole in the lid, and was shown to be as attractive as standard fermentation baits (Cha et al, 2013b). The availability of a reliable, long-lasting synthetic lure is a pre-requisite for development of cost-effective control methods based on attract-and-kill.

Work during the first year of this project aimed to investigate the requirements for development lures which are convenient to use and long-lasting in performance and to evaluate the attractiveness of synthetic lures in the field. As a result the above four-component “Cha-Landolt” lure and modified Biobest trap were adopted as standard for the EMR trap network during 2014.

However, the Cha-Landolt lure is not particularly convenient to use in the field. It was shown that the ethanol is all released from the drowning solution within a few days so that this requires replacement on a weekly basis and uses large amounts of ethanol and acetic acid. The vial dispensers for the acetoin and methionol are long-lived, but they are expensive and spillage and blockage can occur during field use. During the second year of the project, release rate studies were carried out on a range of different dispensers in the laboratory with the aim of developing more practical, sealed dispensers for the lure components. These, and a range of new trap designs, were evaluated in field trapping tests.

Attract-and-kill formulations would be a valuable resource for growers. The combination of pesticides and attractant would provide chemical protection without direct spray application to the crop.

Materials and methods

Measurement of release rates: Lures were maintained in a laboratory fume hood at 20-22°C. Release rates were measured by weight loss, analysis of residual material and/or by trapping and analysis of released volatiles as appropriate. Typically two replicates of each sample were measured.

Weight loss was determined by regular weighing of replicate samples.

Release rates of ethanol and acetic acid from drowning solutions were determined by gas chromatographic (GC) analysis of the amount remaining using a Poraplot column and acetone as internal standard.

For collection of volatiles, dispensers were placed in a 1-litre round-bottomed flask. Air was drawn into the flask at 2 litre/min through an activated charcoal filter and out through a collection filter containing 200 mg Porapak Q (50-80 mesh). Trapped volatiles were removed with dichloromethane and analysed by GC on a polar DBWax column using decyl acetate (5 µg) as internal standard. The identity of volatiles was checked by gas chromatography coupled to mass spectrometry (GC-MS) also on a polar GC column.

Trap and bait design field trial: Three trap designs were tested (Fig. 4.1): the red Biobest trap modified by drilling an additional 56 4 mm diameter holes in the wall used as standard for the EMR trap network (supplied by Agralan); the new red Biobest trap with larger 10 mm holes and rounded bottom (Agralan); the Pherocon cup trap from Trece (supplied by Sentomol) which has two mesh entrance areas of 4 mm holes.

Combinations of lures and liquid baits/drowning solutions were tested (Fig. 4.1) (Table 4.1). The Cha-Landolt system consisted of polypropylene vial dispensers (4 ml) with a hole (3 mm diam.) in the lid. Methionol or a 1:1 mixture of acetoin and water (1 ml) were deposited on a cotton dental roll (40 mm x 8 mm) in the vial. Also assessed were dispensers for acetoin and methionol from Trécé which were sealed plastic capsules (approx. 15 mm x 7 mm) and supplied by Sentomol. The EMR drowning solution was an aqueous solution containing 7.2% ethanol and 1.6% acetic acid (v/v) with 1% boric acid to prevent microbial growth.

Dros'Attract is a combination of red wine, vinegar and sugar developed by Biobest. Apple cider vinegar with a drop of detergent to break surface tension was also tested.

There were five replicates and the experiment ran from 28 August – 18 September 2014. Trap catches were counted on 1, 4, 8, 11 and 18 September 2014. Liquid baits were filtered back into the traps and baits were not refreshed during this trial as duration was also to be tested. Catches of organisms over 5 mm in size were also recorded.

Figure 4.1 Trap designs used in trial. From left to right: the modified Biobest trap, the new Biobest trap and the Pherocon trap

Table 4.1 Table of trap and bait treatments

Treat Number	Trap	Lure	Liquid bait/drowning solution
A	Red Biobest	Char-Landolt	EMR bait
B	Red Biobest	Trécé	EMR bait
C	Red Biobest	Trécé	Apple cider vinegar
D	Red Biobest	Dros'Attract	Dros'Attract
E	New Biobest	Dros'Attract	Dros'Attract
F	Pherocon *D. suzukii* trap	Trécé	Apple cider vinegar

Lure component field trial: The modified Biobest trap was used to compare the attractiveness of different combinations of the four components in the Cha-Landolt lure in different dispensing systems. Ethanol was dispensed as 2 ml on two cotton dental rolls in a self-seal polyethylene sachet (74 mm x 50 mm x 50 µ) with a release rate of 38 mg/d at 22°C. Acetic acid was dispensed as 1 ml on a cotton dental roll in a polyethylene sachet (50 mm x 50 mm x 250 µ) prepared by heat sealing lay-flat LDPE tubing (Transatlantic Plastics, Southampton, UK) giving a release rate of 18 mg/d at 22°C. Methionol and acetoin were dispensed from

polypropylene vial dispensers (4 ml) with a hole (3 mm diam.) in the lid. Methionol or a 1:1 mixture of acetoin and water (1 ml) were deposited on a cotton dental roll (40 mm x 8 mm) in the vial giving release rates of 0.4 mg/d and 8 mg/d respectively (Fig. 4.2). Traps with the ethanol and acetic acid in sachets had water and detergent as drowning solution. The 'standard' trap had acetoin and methionol dispensed from vials and ethanol and acetic acid in the drowning solution as above.

There were six replicates and the experiment ran from 28 August – 25 September 2014. Trap catches were counted on 29 August, 1, 4, 8, 11, 18 and 25 September 2014. The lures with ethanol and acetic acid in sachets were not changed during the course of the experiment. The drowning solution in the standard lure was replaced on 18 September 2014.

Figure 4.2 Synthetic baits: from left to right methionol, acetoin, acetic acid, ethanol.

Attract-and-kill formulations

Release rates: A slow-release formulation for pheromones based on castor oil (confidental) was combined with methional and acetoin. Samples of 1 g containing 2% of each component were taken. Release rates of acetoin and methionol from this were measured in the laboratory.

Attraction in the field: Modified Biobest traps were baited with the castor oil formulation either with or without methional and acetoin combined within it. Traps were deployed in the field for a week. Trap catches were counted after this time.

Combination with pesticides: The formulation was combined with deltamethrin at different dose rates: 0.125, 0.375, 1.25 and 3.75. The mixture was then spread on all the inside

surfaces of a 5 cm Petri dishes. Flies were deposited with a pooter within the Petri dish and the lid was put on. Flies were exposed for 5 seconds before being removed with soft forceps. Behaviour was recorded at 5 minute intervals, ending once the fly had died.

Results

Release rates from Dros'Attract solution: The Gasser Lure wine vinegar mixture from Biobest, was adopted as the standard for monitoring by Berry Gardens during 2014. Release rates of ethanol and acetic acid from this were measured using the standard Biobest trap under laboratory conditions at 20-22°C by GC analysis of the amounts remaining at intervals. Results were similar to those found for the "synthetic" drowning solution during 2013 with rapid release of the ethanol over a few days and more sustained release of the acetic acid (Fig. 4.3). The initial ethanol concentration was 1.4% and acetic acid 0.7%. A trace of acetoin (0.15%) was detected in the initial solution but this could not be detected after 1 day.

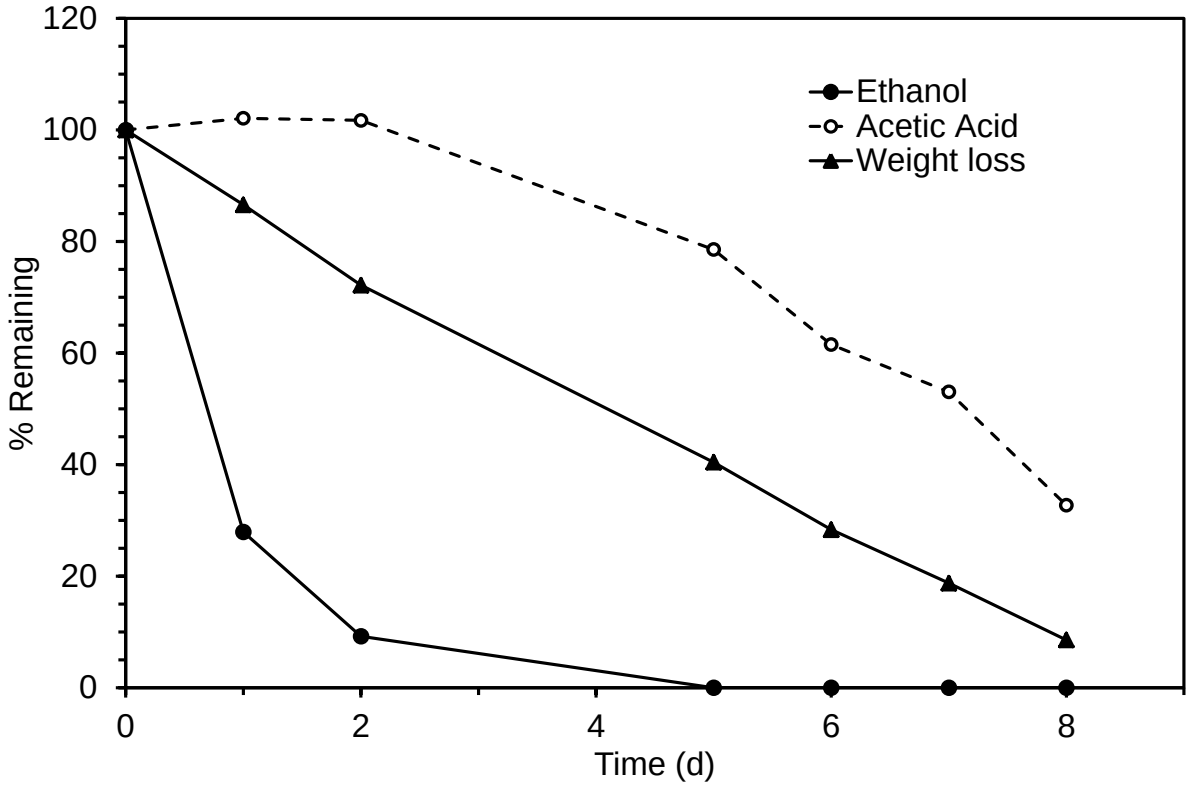


Figure 4.3 Total weight loss and loss of ethanol and acetic acid from the Dros'Attract wine vinegar mixture in a Biobest trap in the laboratory at 20-22°C

Release rates of methionol and acetoin from vials: Measurement of release rates of acetoin and methionol from the polypropylene vials with a hole in the lid, as used in the Cha-Landolt

lure, were started in 2013 and continued in 2014. Acetoin was applied as a 1:1 solution in water and the release rate increased from 7 mg/d to 13 mg/d, presumably as the water evaporated. The lure was exhausted after 50 days at 20-22°C (Fig. 4.4). Release of methionol continued at approximately 0.4 mg/d for over 250 days (Fig. 4.4)

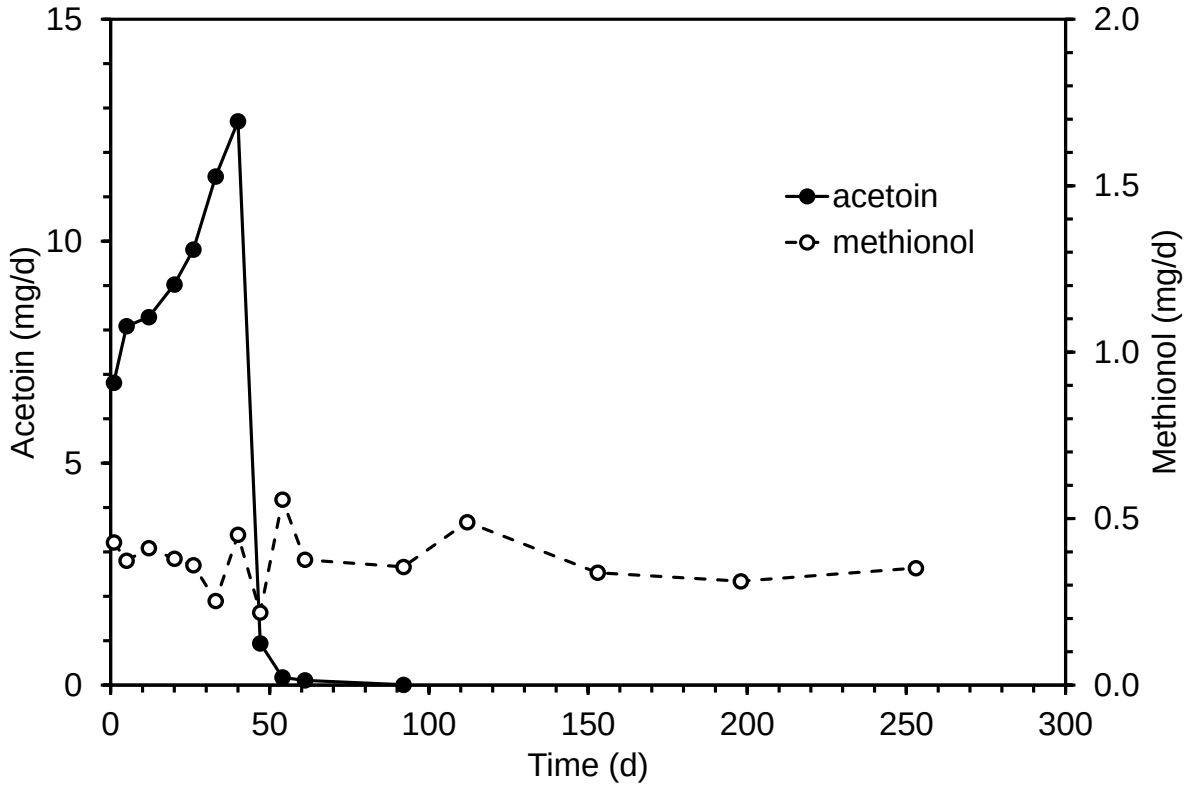


Figure 4.4 Release of acetoin and methionol from 4 ml polypropylene vials with a 3 mm hole in the lid under laboratory conditions at 20-22°C, as measured by trapping volatiles.

Release of acetoin and methionol from Trécé lures: In 2013 Trécé lures were provided by the UK agents, Sentomol. These were two plastic "blister-pack" type dispensers marked A and B (Fig 4.5). Collection of volatiles from these showed that one contained acetoin and the other methionol respectively. Release rates were 1.5 mg/d and 0.5 mg/d respectively (Fig. 4.6).

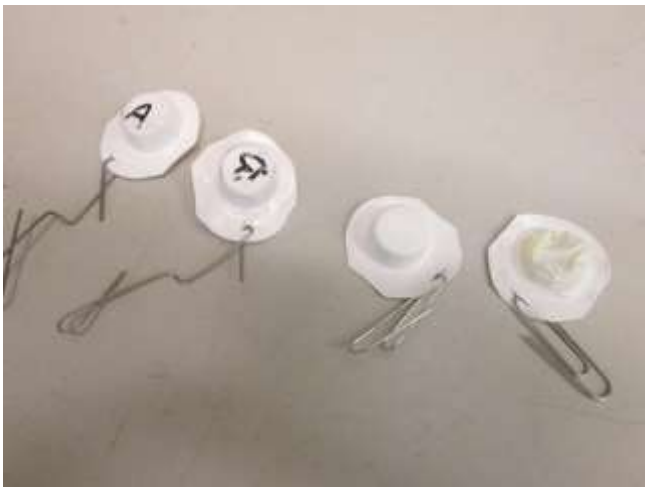


Figure 4.5 Trécé lures: left 2013 A and B; right 2014

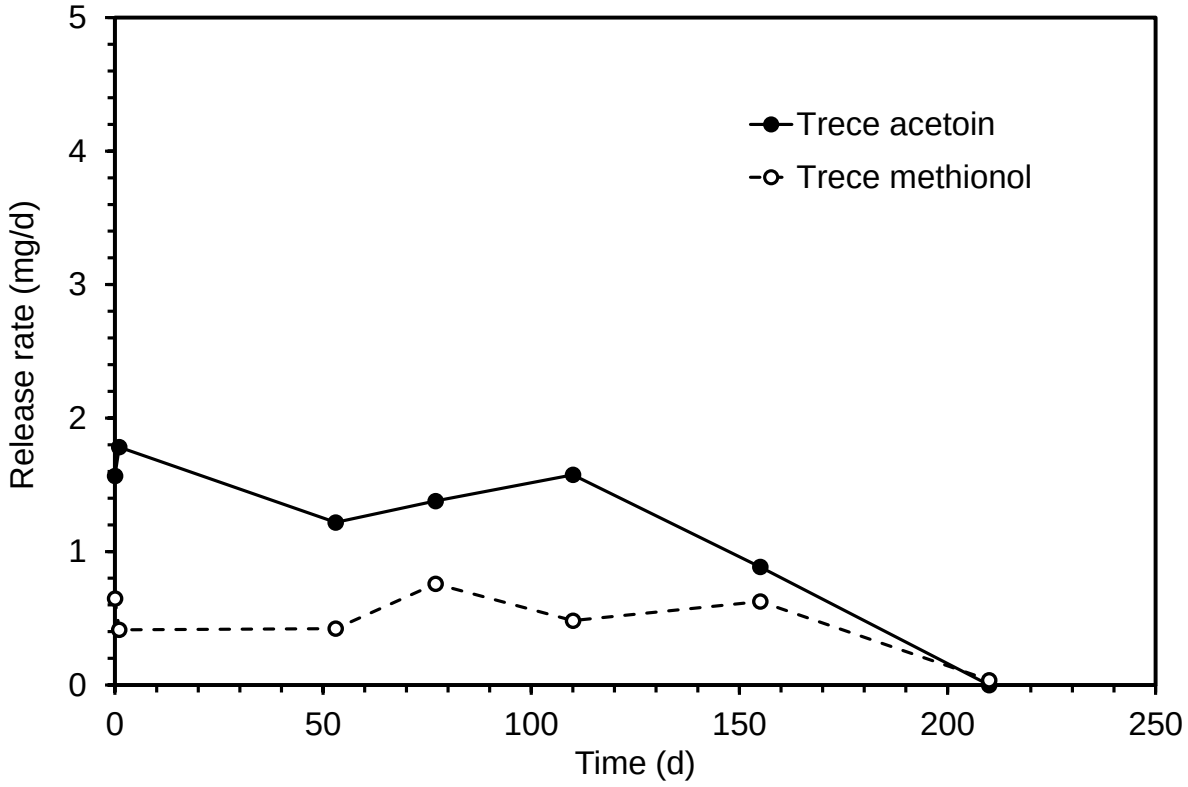


Figure 4.6 Release rates of acetoin and methionol from Trécé 2013 lures

Lures supplied in 2014 were apparently similar although one seemed to be made of a thinner plastic (Fig. 4.5). The lures were packaged in pairs and analysis of the contents or the volatiles released indicated considerable cross-contamination. The major component released from the 2014 lure was identified as allyl methylsulphide from its mass spectrum and GC retention time (Fig. 4.7). This was thought to be the product of dehydration of methionol (Fig. 4.8 and 4.9) and mixing methionol with acetic acid did indeed cause surprisingly rapid dehydration to the allyl methylsulphide (Fig. 4.10).

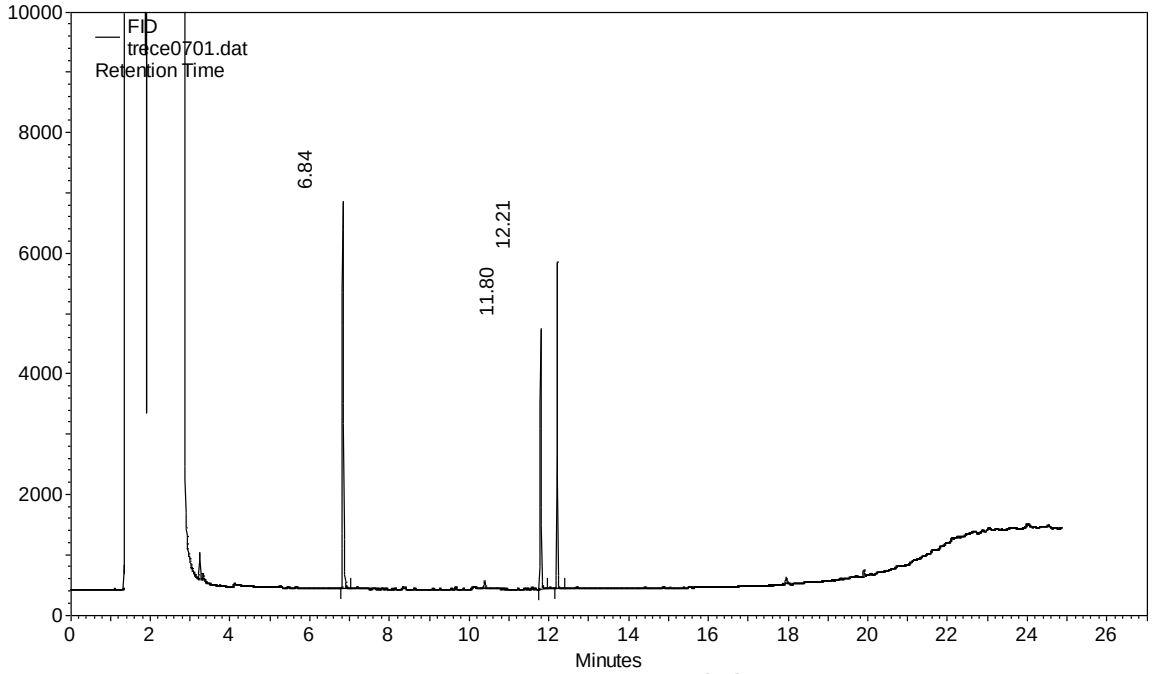


Figure 4.7 GC Analysis of volatiles from both 2013 Trécé lures at once (6.84 min acetoin; 11.80 min decyl acetate (10Ac) internal standard; 12.21 methionol)

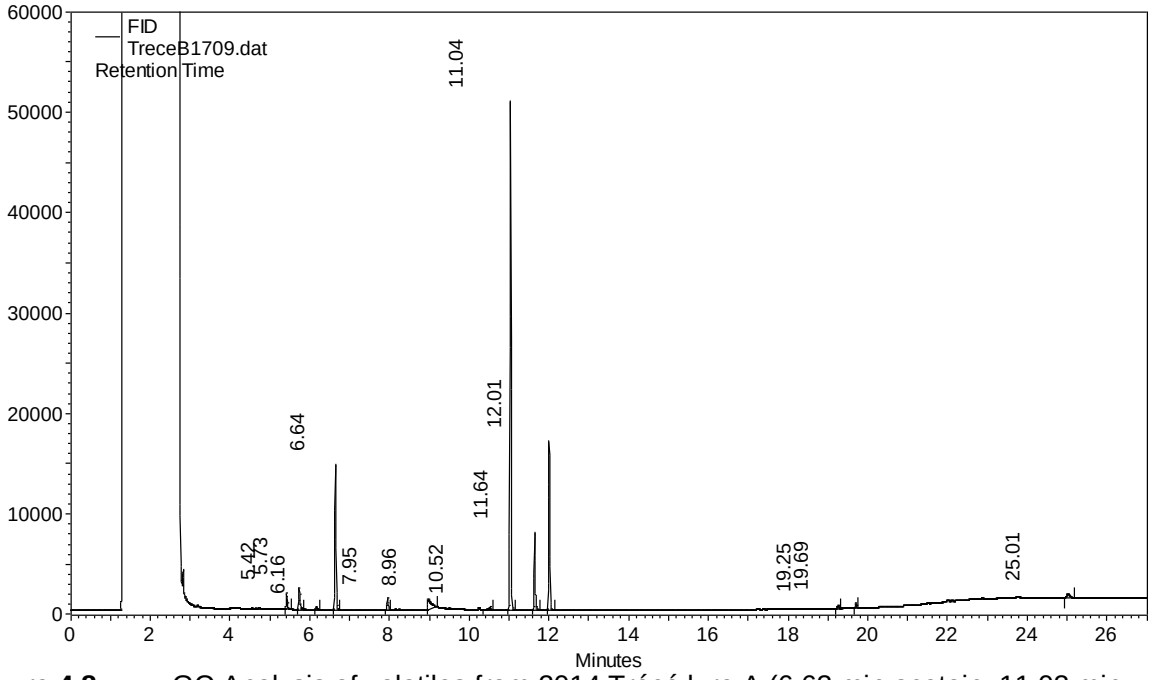
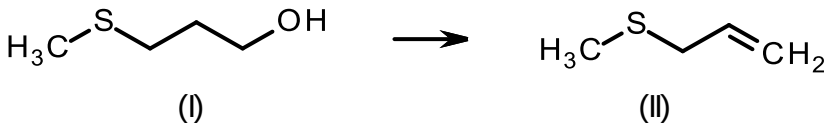


Figure 4.8 GC Analysis of volatiles from 2014 Trécé lure A (6.63 min acetoin; 11.03 min allyl methylsulphide; 11.64 10Ac)



(I) (II)

Figure 4.9 Degradation of methionol (I) to allyl methylsulphide (II)

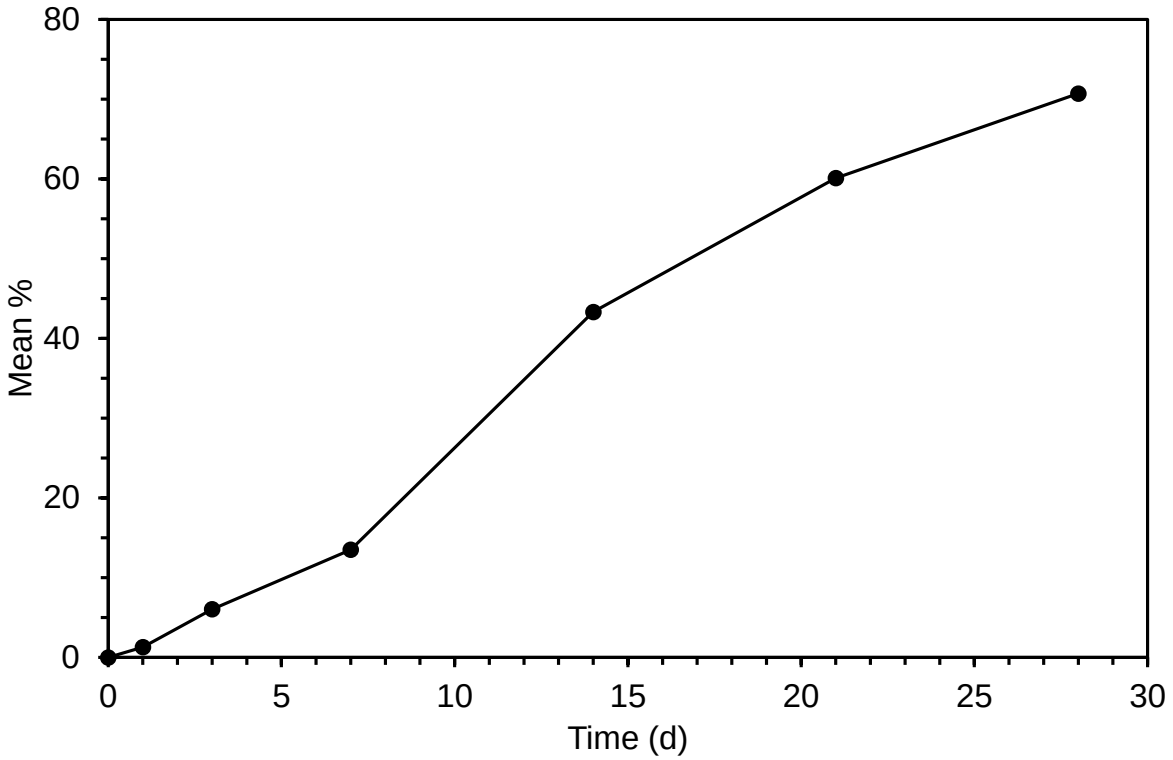


Figure 4.10 Percentage of allyl methylsulphide in 1:1 mixture of methionol and acetic acid maintained at room temperature

Dispensers for ethanol and acetic acid: Sealed dispensing systems for ethanol and acetic acid were investigated. During 2013, release rates of these two compounds from standard polyethylene sachets (50 mm x 50 mm x 120 µ thick) were measured as 7 and 36.5 mg/d respectively. These were considered to be too slow and too fast respectively. The release rate of ethanol from a thinner sachet (50 mm x 74 mm x 50 µ thick resealable) was measured at 38 mg/d at 20-22°C (Fig. 4.11). Acetic acid was released at 18 mg/d from a thick sachet (50 mm x 50 mm x 250 µ thick).

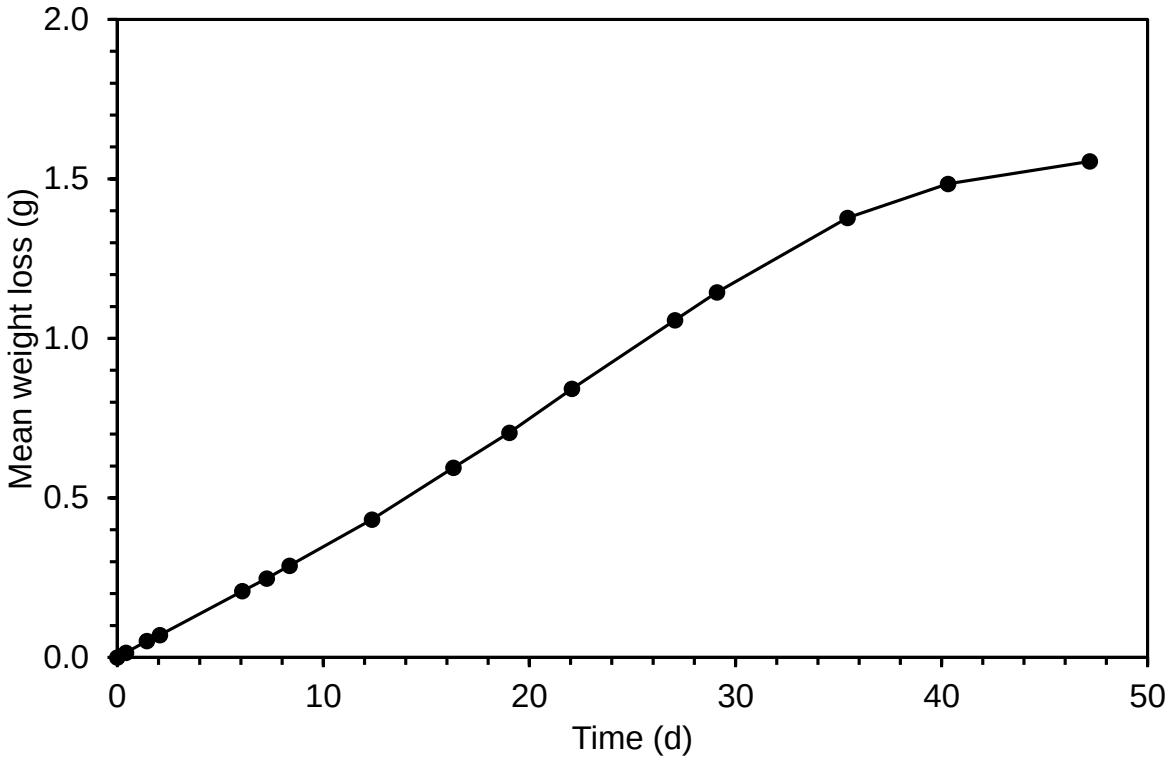


Figure 4.11 Release of ethanol (2 ml) from a resealable sachet (50 mm x 74 mm x 50 µ thick) at 20-22°C

Trap and bait design field trial: In the field comparison of different traps and baits, the trap that caught the highest mean number of *D. suzukii* was the new Biobest trap using Dros'Attract as the lure and drowning solution. However, this also had the highest amount of bi-catch over 5 mm in size (Fig. 4.12).

The modified Biobest trap with the Cha-Landolt lure and EMR bait or the Dros'Attract caught similar numbers of *D. suzukii*, but the Trécé lures performed very poorly in this trap. This prompted investigation of the 2014 Trécé lures. As described above, they apparently contained all four components of the Cha-Landolt blend, but degradation of the methionol to allyl methylsulphide had occurred.

The Trécé lures performed better in the Pherocon trap with apple cider vinegar.

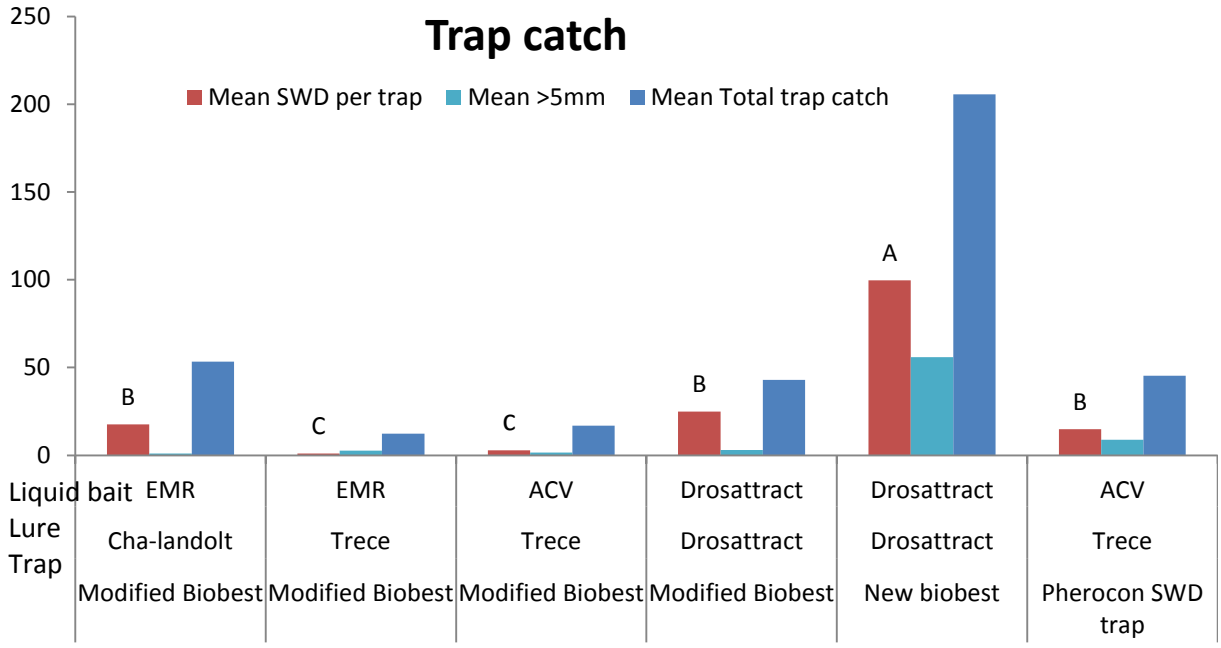


Figure 4.12 Mean numbers of *D. suzukii* (SWD), total *Drosophila* spp. and insects > 5mm captured in the trap and bait design trial (28 August – 18 September 2014; 5 replicates; catches with different letters are significantly different at 5% level after ANOVA on data transformed to log(x+1) and LSD test)

Lure component field trial: Catches of *D. suzukii* in modified Biobest traps baited with different combinations of the four components of the Cha-Landolt lure in sachet and vials dispensers were compared with those in traps baited with "standard" formulation of acetoin and methionol in vials and ethanol and acetic acid in the drowning solution. Over the three-week period 28 August – 18 September 2014 lure components were not renewed and total catches in the traps baited with the four components dispensed from sachets and vials were not significantly different from those in the standard trap (Fig. 4.13).

Catches of *D. suzukii* in the standard traps were greater than those in the traps baited with the sachet and vial dispensers during the first day of the experiment (Fig. 4.14) as expected with the observed initial high release rate of the ethanol and acetic acid from the drowning solution. However, when the drowning solution was refreshed after the initial three-week period (18/09/2014), catches in the following week with the two baited were similar (Fig. 4.15).

Over the three-week period, catches in traps baited with two- or three-component lures were significantly lower than those in traps baited with the four components (Fig. 4.14). However, it should be noted that catches in traps baited with acetoin and methionol only were significantly higher than those in unbaited traps.

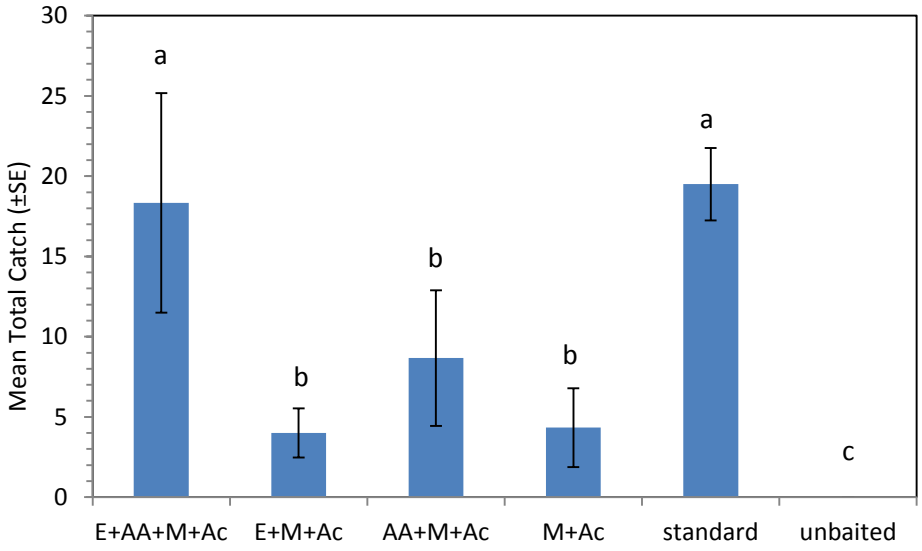


Figure 4.13 Mean total catches of *D. suzukii* in modified Biobest traps baited with different combinations of the Cha-Landolt lure: E ethanol, AA acetic acid, Ac acetoin, M methionol in sachets and vials (28 August –25 September 2014; 6 replicates; catches with different letters are significantly different at 5% level after ANOVA on data transformed to log(x+1) and LSD test)

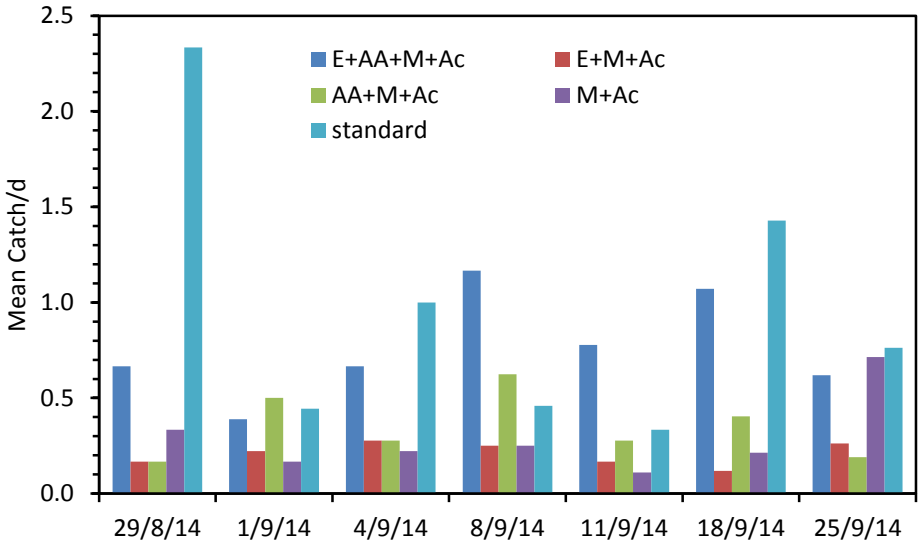


Figure 4.14 Mean daily catch of *D. suzukii* in modified Biobest traps baited with different combinations of the Cha-Landolt lure: E ethanol, AA acetic acid, Ac acetoin, M methionol in sachets and vials (28 August – 25 September 2014; six replicates).

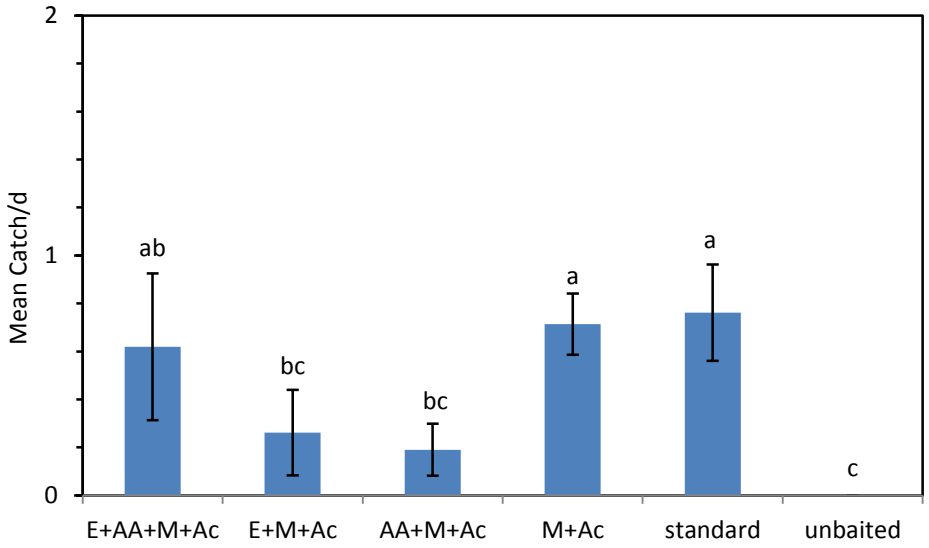


Figure 4.15. Mean total catches of *D. suzukii* in modified Biobest traps baited with different combinations of the Cha-Landolt lure after refreshing of drowning solution in standard traps: E ethanol, AA acetic acid, Ac acetoin, M methionol in sachets and vials (18-25 September 2014; 6 replicates; catches with different letters are significantly different at 5% level after ANOVA on data transformed to log(x+1) and LSD test)

Attract-and-kill

Release rates: Release of both components was very rapid, although measurable release continued for at least 10 d at 22°C (Fig. 4.16).

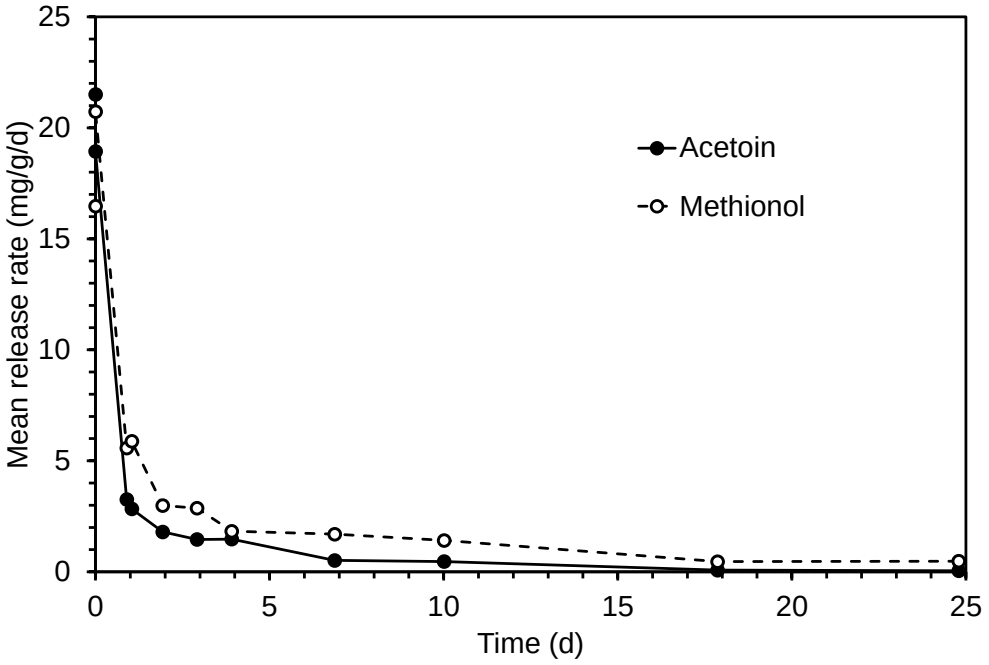


Figure 4.16 Release rates of acetoin and methionol from formulation (2% each component; mean of two 1 g samples; 22°C)

Attraction in the field: Although population numbers were low at the time of this trial, data was still obtained. No *D. suzukii* were caught in traps that did not have the attractants combined with the formulation (4.17).

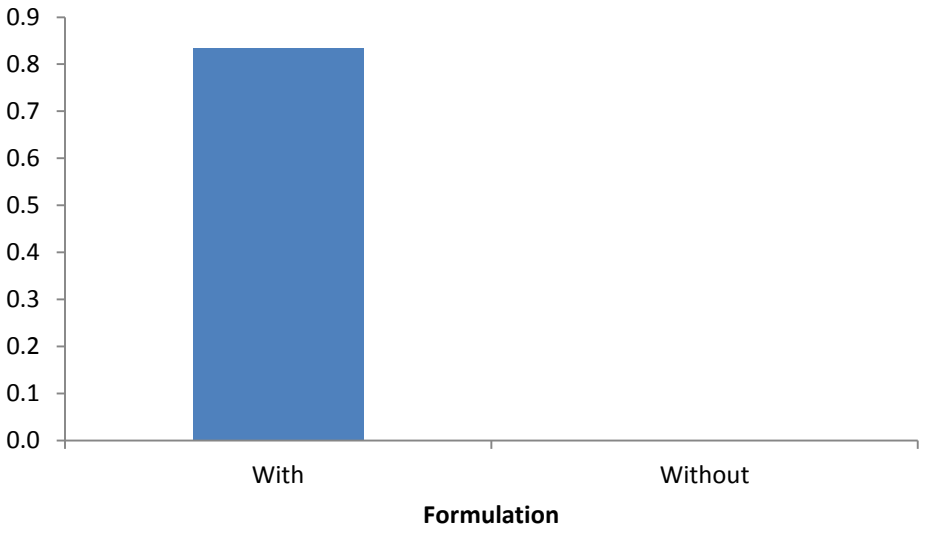


Figure 4.17 Mean numbers of *D. suzukii*

Combination with pesticides: There were no differences in time to mortality between males and females (Fig. 4.18). Higher dose rates generally had quicker times to mortality.

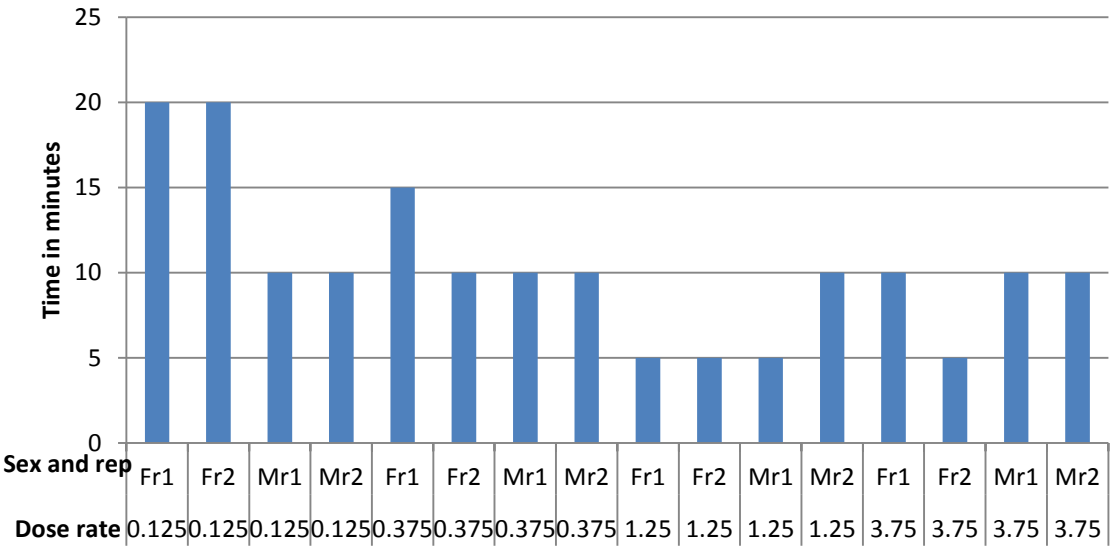


Figure 4.18 The time to mortality for male and female *D. suzukii* after exposure to different dose rates of deltamethrin

Discussion

Dispensers: Currently many different trap designs are used for trapping *D. suzukii* and lures are normally a combination of wine and vinegar or the synthetic Cha-Landolt lure. The latter consists of acetoin and methionol dispensed from polypropylene vials with a hole in the lid and ethanol and acetic acid dissolved in the drowning solution. The wine/vinegar mixtures are variable in composition and require replacing frequently. The Cha-Landolt lure provides a standard blend, but uses large amounts of ethanol and acetic acid and the drowning solution requires frequent replacement. During 2014, the modified Biobest trap baited with the Cha-Landolt lures was adopted as standard for the EMR network of traps and the Biobest with Dros'Attract wine/vinegar mixture by Berry Gardens Growers.

In the first year of this project it was shown that the ethanol is released very rapidly within a few days from the synthetic drowning solution in traps, although the acetic acid is more persistent. A similar result was obtained this year for the Dros'Attract solution. The release rate of ethanol from the aqueous solution was up to 3-4 g/d and that of acetic acid was 170-420 mg/d in the laboratory at 20-22°C. During 2014 it was shown that replacing the drowning solution with ethanol dispensed from a thin-walled, polyethylene sachet at 38 mg/d and acetic acid dispensed from a thick-wall sachet at 18 mg/d caused no reduction in catch of *D. suzukii* over a three-week period. The ethanol sachet loaded with 2 ml should last for at least one month in the field, compared with 22 ml in the drowning solution which is typically changed weekly or fortnightly. Subsequent discussions with Dr Cha (USDA) confirmed that he had also found the release rate of ethanol could be significantly reduced up to at least fifty-fold without loss of attractiveness.

In the Cha-Landolt lure the acetoin and methionol are dispensed from separate polypropylene vials with a hole in the lid. Measurements of release rates in the laboratory showed that these lasted for approximately 40 d and >250 d respectively at 20-22°C, with release rates of 7-13 mg/d and 0.4 mg/d respectively. Investigation of sealed dispensing systems showed that acetoin could be dispensed at 10 mg/d from a thin-walled polyethylene sachet and methionol at 1.4 mg/d from a standard sachet, with both capable of being loaded to last for several months in the field. These would seem to provide good candidates for evaluation in the field.

During 2013, dispensers from Trécé for acetoin and methionol were assessed and found to release the compounds at 1.5 mg/d and 0.5 mg/d respectively. Although the rate for acetoin was a little low, these seemed very convenient and lasted for at least four months under field

conditions. However, apparently similar lures provided in 2014 gave very different results. It transpired that these contained mixtures of all four components of the Cha-Landolt lure and that mixing of methionol with acetic acid caused dehydration to give allyl methylsulphide. These lures performed very poorly in field tests.

Trap design: Selection of the most appropriate trap design is dependent on how the trap will be used. The trap and bait that caught the highest numbers of *D. suzukii* was the new Biobest trap combined with the Dros'Attract liquid bait. The trap has bigger holes (10 mm) than the other traps and this also results in a high amount of unwanted bi-catch most of which are larger than 5 mm in size. This trap would be useful as a precision monitoring device or in situations where analysis of the contents and counts of *D. suzukii* are not needed. However, the trap is not very user-friendly because of the rounded base and is unlikely to be able to withstand frequent handling.

The modified Biobest trap with either the Dros'Attract liquid bait or the Cha-Landolt system caught good numbers of *D. suzukii* and had a low amount of bi catch. It caught fewer insects over 5 mm than the new Biobest trap, making checking the contents of the trap much easier.

The Pherocon trap with the Trécé lure and apple cider vinegar liquid bait caught the same amount of *D. suzukii* as the modified biobest trap. It did catch a higher percentage of other drosophila which could make identification of *D. suzukii* more difficult, but a low numbers of 5 mm bi-catch due to entry hole size.

Attract-and-kill: Ethanol and acetic acid would be very difficult to incorporate in a long-lived attract-and-kill formulation due to their quick dissipation. However, the trapping trials showed that a blend of acetoin and methionol with the trialled substance was attractive to *D. suzuk*ii in a small field trial. The addition of deltamethrin to the formulation did kill the flies in the laboratory in all the dose rates tested although it was quicker in the higher dose rates.

Conclusions

- The new Biobest trap with Dros'Attract bait could be a good precision monitoring device for growers, catching the most *D. suzukii* in this year's trials;
- A trap with smaller holes (4 mm) should be used when higher sensitivity and accurate counts are needed;

- It is likely that the Dros'Attract and Cha-Landolt baits can be replaced with a lure made up of four totally-sealed components which will last for at least one month under field conditions;
- Trécé lures were unreliable this year and manufacturers are modifying them for 2015;
- Lures containing only acetoin and methionol were significantly attractive to *D. suzukii* and these two compounds might be used in an attract-and-kill formulation.

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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* (Years 1-4)

Task 5.1 Evaluate the efficacy of approved and emerging products against adults and other life stages in crops (Years 1-4)

Introduction

It is important to establish chemical control for *D. suzukii.* In a farm situation, *D. suzukii* will encounter plants treated with insecticide, which could cause mortality directly, act as a deterrent (especially in the case of pyrethroids) or impact on larval development. Each of these factors could be important commercially.

A raspberry crop at EMR was treated with field doses of insecticides and adult *D. suzukii* in the laboratory were exposed to harvested fruit to determine any insecticide residue efficacy. This followed a trial on strawberries in 2013. In 2014 an extended trial was run, with a greater selection of insecticides reflecting the wider range of approved products available to growers. In addition an experimental (coded) product was assessed.

Materials and methods

Plants and site: Raspberry plants (cv. Autumn Treasure, primocane) at East Malling Research. The rows were 3 m apart (Fig. 5.1.1). Plants were provided with an overall spray programme of fungicides.

Figure 5.1.1 Experimental site at EMR

Experimental design: A randomised block design with five replicates of 11 treatments: plots in each block were arranged end to end in a row. Each plot was 4 m long with a 1 m space between each. Barrier rows were left between each treated row.

Insecticide application: Sprays were applied as a single application with a hand lance from a hand pump knapsack sprayer. A fine spray quality application at a volume of 1000 l/ha was used (Table 5.1.1).

Table 5.1.1 Insecticide treatments used in 2014 raspberry spray efficacy trial

Trt No.	Active ingredient	Product name	Ai/l	Product rate/ha (spray volume 1000 l/ha)
1	Spinosad	Tracer	480 g/l SC	200 ml †
2	Chlorpyrifos	Equity	480 g/l EC	1.5 l
3	Lambda cyhalothrin	Hallmark	100 g/l CS	75 ml ‡
4	Chlorantraniliprole	Coragen	200 g/l SC	175 ml
5	Coded		100 g/l OD	750 ml
6	Deltamethrin	Bandu*	0.2 ml / l	200 ml
7	Pyrethrins	Spruzit	4.59 g/l EC	20 l
8	Thiacloprid	Calypso	480g/l	750 ml @
9	Abamectin	Dynamec	18g/l	0.5 l #
10	Acetamiprid	Gazelle	20%w/w	375 ml $
11	Untreated	-	-	-

† SOLA 1291, ‡ SOLA 1705, * EAMU 1643, @ SOLA 20060336, # SOLA 20072290, $ SOLA 20082857

Assessments: Fruit (100g) was removed from each plot on each sample day (0, 1, 2, 4, 8, and 14). Fruits were placed on paper towel in ventilated Perspex plastic boxes (228 x 121 x 66 mm). *D. suzukii* (four female, two male) were transferred by pooter and then removed after 24 hours, at which point mortality was assessed. Boxes were kept for at least three weeks at 20^{o}C in the EMR quarantine facility and assessed for adult emergence. Results were analysed in Genstat by General ANOVA with a square root transformation of the data.

Results

Initial adult mortality (from fruit on day of spraying) is presented in Fig. 5.1.2. Adult emergence from fruit is presented in Figs. 5.1.3 and 5.1.4.

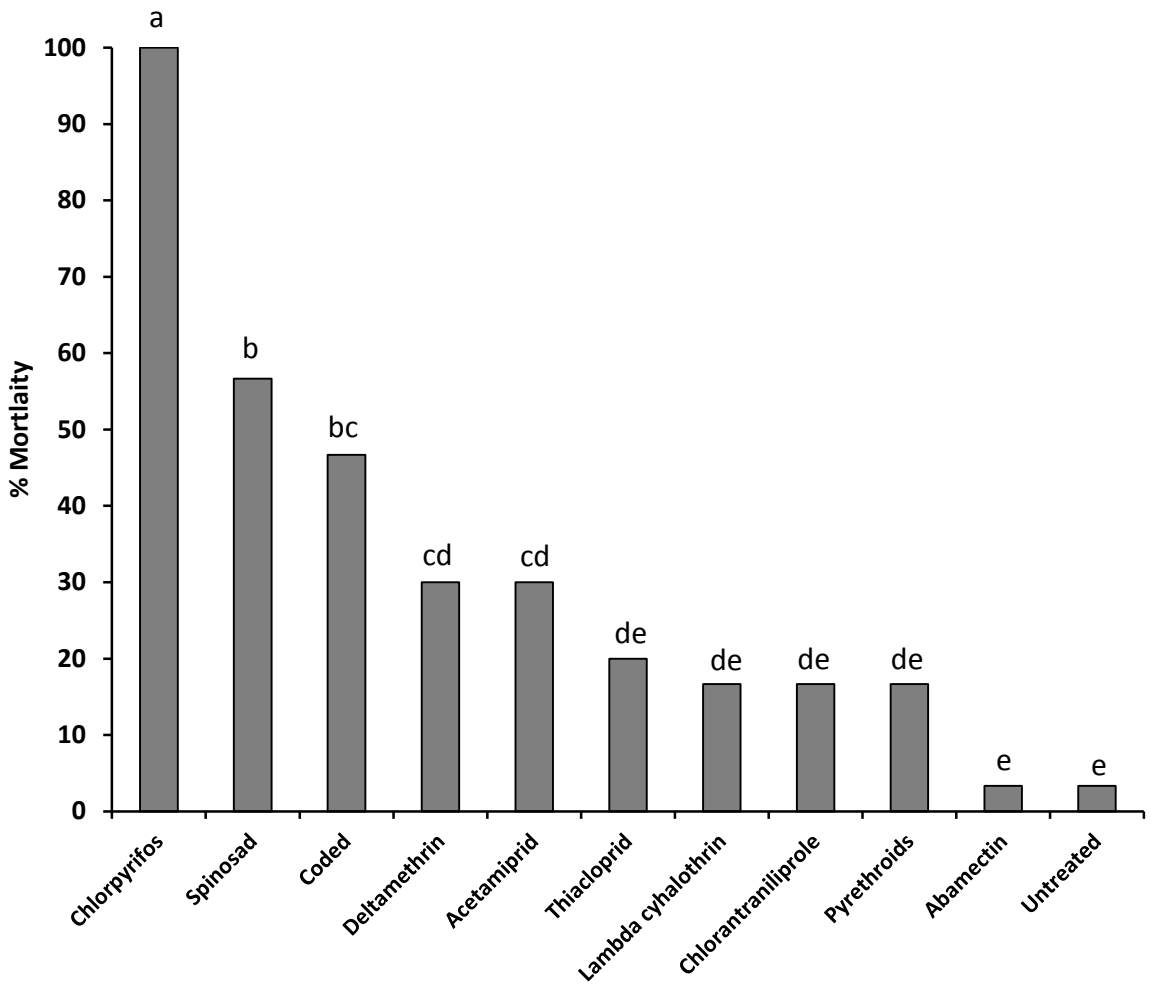

Figure 5.1.2 Mortality of adult *D. suzukii* exposed to sprayed fruit on day of spraying. Bars marked with the same letter are not significantly different to each other (P<0.05).

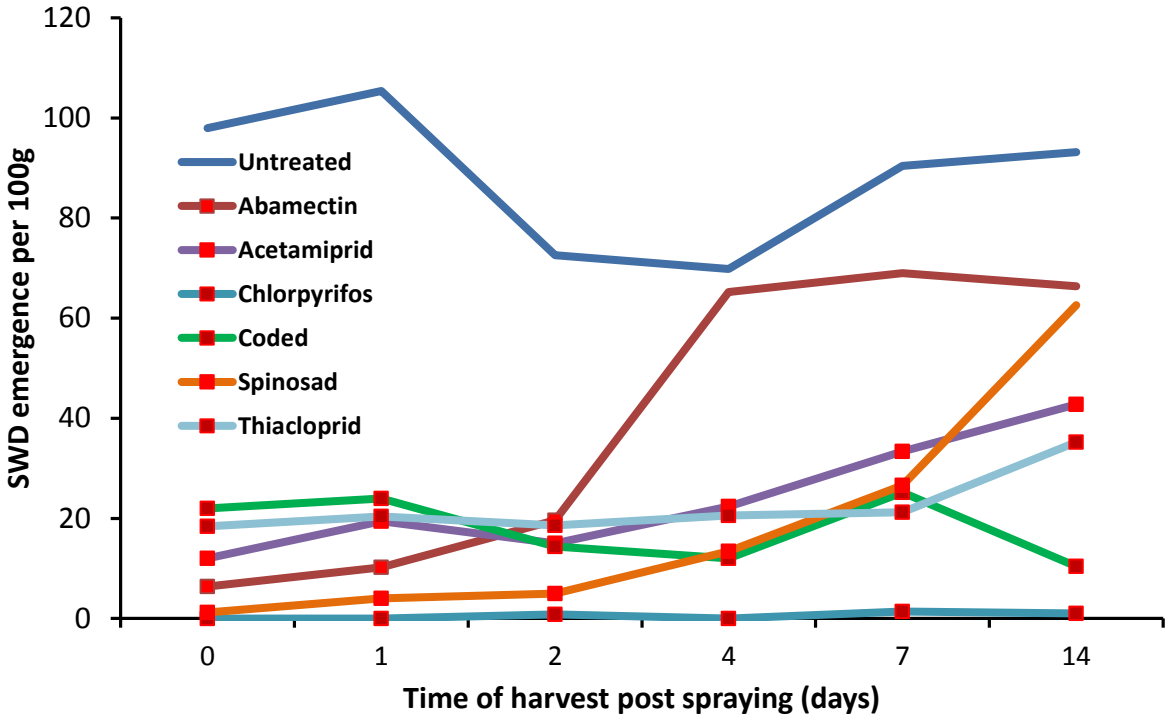

Figure 5.1.3 Adult emergence from raspberry fruit exposed to *D. suzukii* at various days after spraying, abamectin, acetamiprid, chlorpyrifos, coded product, spinosad and thiacloprid. Red marks indicate significant difference to control (P<0.05)

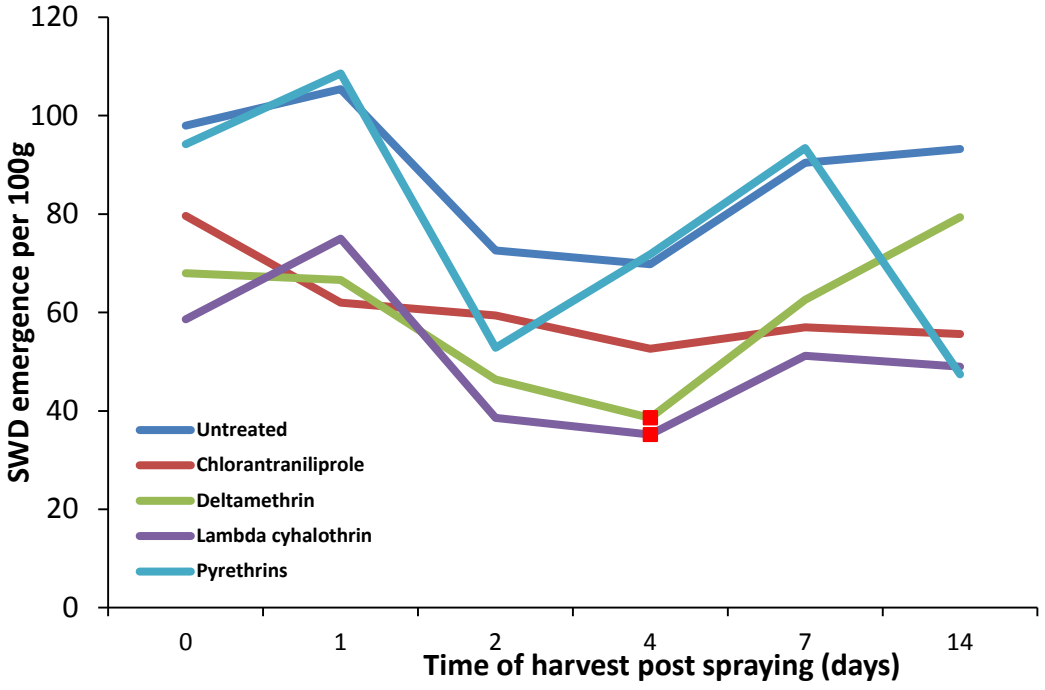

Figure 5.1.4 Adult emergence from raspberry fruit exposed to *D. suzukii* various days after spraying, chlorantraniliprole, deltamethrin, lambda cyhalothrin and pyrethrins. Red marks indicate significant difference to control (P<0.05)

The effectiveness of *D. suzukii* chemical control varied with insecticide and time post spraying. The most effective in terms of direct mortality to adults added to the sprayed fruit on day of spraying were chlorpyrifos, spinosad and the coded product (Fig. 5.1.2). Perhaps not surprisingly, these were amongst the most successful in reducing subsequent larval emergence (Fig. 5.1.3). However, abamectin, acetamiprid, and thiacloprid also significantly reduced larval emergence from treated fruit when exposed to *D. suzukii* up to two days after spraying, even though they had a limited toxicity to adults, especially abamectin.

Several insecticides lost their effectiveness over the course of this trial. Four–day-old abamectin on raspberries had no significant effect on emergence compared to the controls, and nor did 14-day-old spinosad. However, acetamiprid, chlorpyrifos, the coded product and thiacloprid still had a significant effect on emergence 14 days after spraying.

Of the other four products, chlorantraniliprole, deltamethrin, lambda cyhalothrin and the pyrethrin mixture, only deltamethrin had a significant effect on adult mortality and none had a significant effect on emergence, except for deltamethrin and lambda cyhalothrin at Day 4.

Conclusions

The emergence trial in raspberry in 2014 was broadly in agreement with the trial on strawberry in 2013. Spinosad, chlorpyrifos and the coded product gave good control of emergence up to a week after spraying, though spinosad had declined after the second week, possibly because the trial in 2014 was on unprotected crops and it rained within 24 hours of application, which could have affected the efficacy of some products. 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. Encouragingly, the three products approved for *D. suzukii* control this year, abamectin, acetamiprid and thiacloprid all gave significant control of emergence, although the effectiveness of abamectin declined after two days and all three had limited effects on direct adult mortality.

Of the other tested products, chlorantraniliprole, deltamethrin, lambda cyhalothrin and the pyrethrin mixture, deltamethrin gave a degree of control of adults but all were of limited use in preventing emergence from fruit.

D. suzukii develops insecticide resistance easily and rotation of insecticides with different modes of action to prevent insecticide resistance is likely to be important. Fortunately,

several are now available, but they should be combined with good crop hygiene and other non-chemical control.

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

Introduction

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 two insecticides, chlorantraniliprole and spinosad, using fruit dipped in insecticide solutions with or without sugar. Both mortality to adult *D. suzukii* and consequent emergence from the fruit, were assessed.

Materials and methods

Trial 1: Purchased blueberries were dipped in standard field rates of chlorantraniliprole (200g/l, 175ml/ha) and spinosad (480g/l, 200 ml/ha) with or without sugar (2.4 g/l, equivalent to 2 lbs per 100 US gallons, Cowles, 2012; Agnello et al., 2014) and allowed to air dry for two hours. Six blueberries were then placed in a 14 cm plastic Petri dish over filter paper. Six female *D. suzukii* were added and the dish was incubated at 20^{o}C for 48 hours within a plastic bag to reduce desiccation. Four replicates per treatment. Mortality of adults was assessed after 24 hours and all *D. suzukii* were removed. Fruit was maintained at 20^{o} C to assess emergence.

Trial 2: As in Trial 1 except that half standard field rates were used, and fruit was exposed to adult *D. suzukii* for 24 hours. Three replicates per treatment.

Trial 3: Given high mortality to spinosad in the first two trials, the trial was repeated with only spinosad, at 0.1 x standard field rate, with mortality assessed at 2, 19 and 24 h (3a). Three replicates per treatment. Additionally, an identical trial was established (3b), with fruit infested for 24 hours, the *D. suzukii* were removed and then the fruit was dipped.

Statistical analysis: The data (mortality or emergence) was subjected to a square root transformation and then analysed by General ANOVA.

Results

Sugar significantly enhanced adult mortality from chlorantraniliprole at standard and half field rate (Fig. 5.1.5a). However, no difference in emergence from chlorantraniliprole treated fruit was observed in either trial (Fig. 5.1.5b).

Sugar significantly increased emergence from control (water treated) fruit in Trials 1 and 3a, but Trials 2 and 3b did not give statistically significant differences (Figs. 5.1.5 b and d). Sugar did not cause any statistically significant mortality beyond that of water alone (5.1.5c).

In both Trials 1 and 2 mortality from spinosad was too high for meaningful statistics. A time course using a lower dose found a significant enhancement of direct mortality using sugar at 19 hours, though not at 2 or 24 (Fig. 5.1.5c). There was no significant difference in emergence (Fig. 5.1.5d).

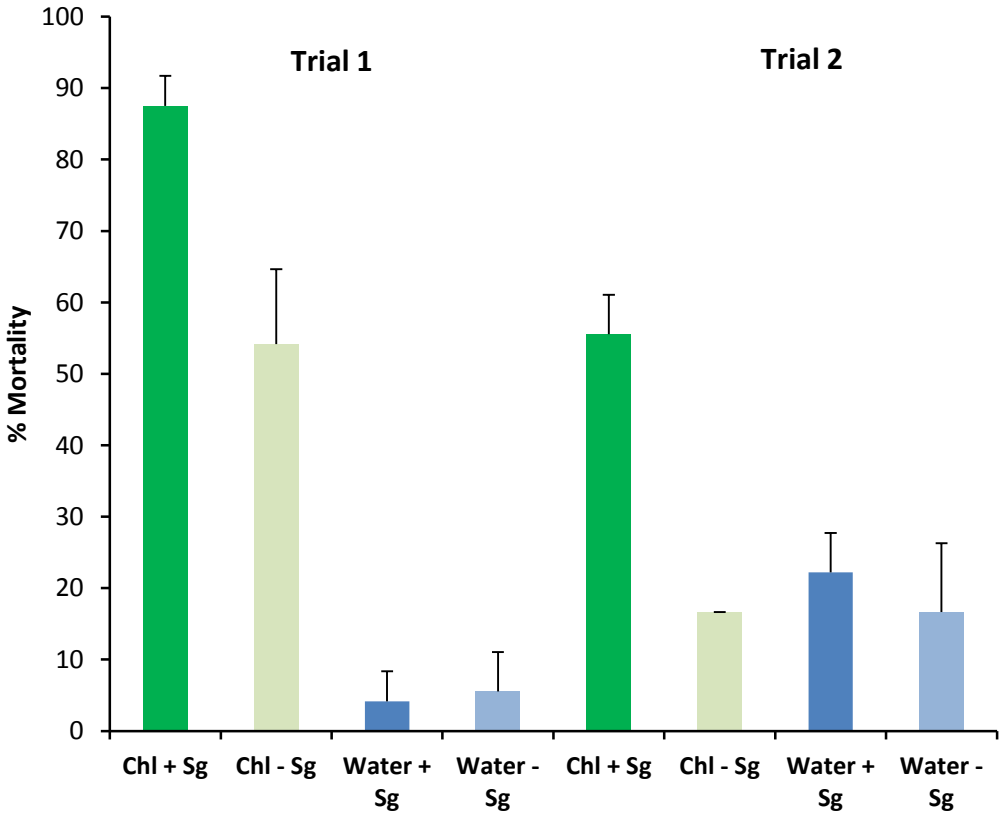

Figure 5.1.5a Mortality of adult *D. suzukii* after exposure to blueberries with and without chlorantraniliprole and sugar residues. Trial 1 (field rate chlorantraniliprole, 2.4g/l sugar, 48 hour exposure), Trial 2 (half field rate chlorantraniliprole, 2.4g/l sugar, 24 hour exposure). Chl = chlorantraniliprole, Sg = sugar

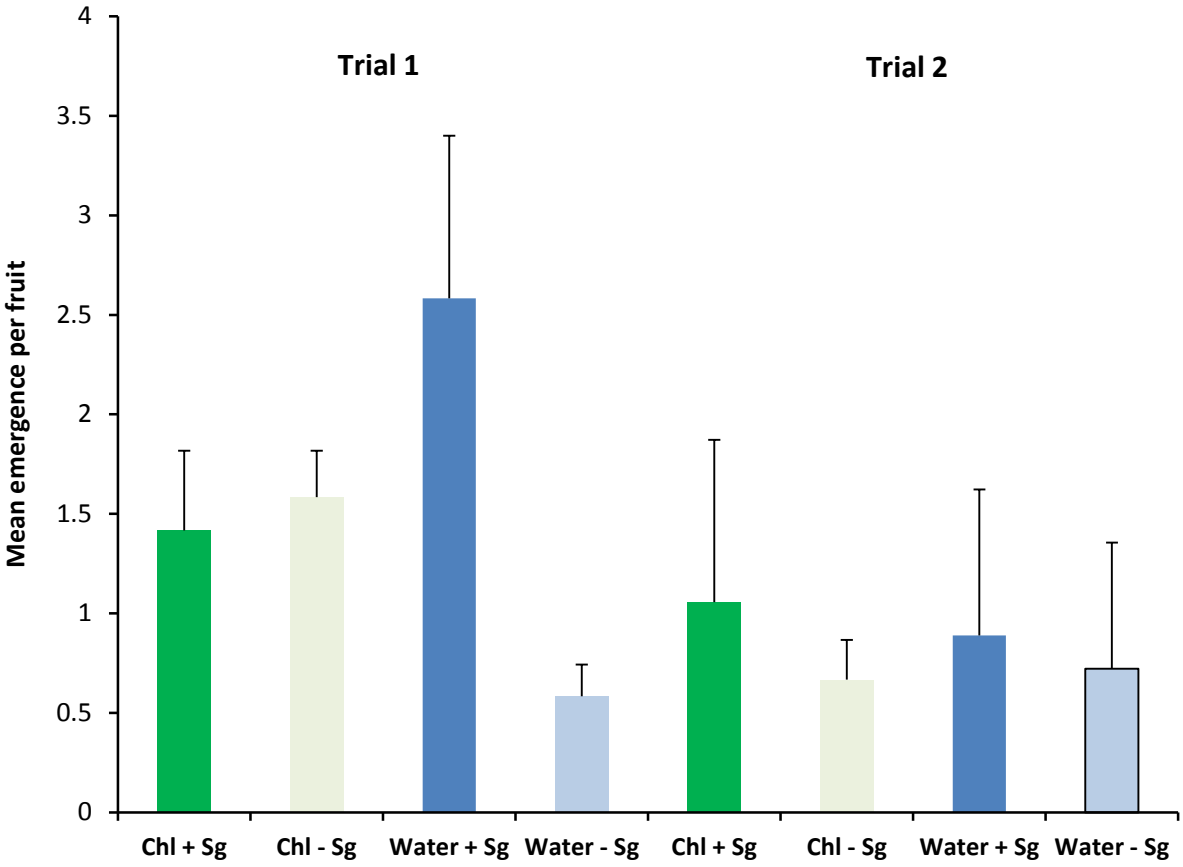

Figure 5.1.5b Emergence of adult *D. suzukii* from fruit after exposure to blueberries with and without chlorantraniliprole and sugar residues. Trial 1 (field rate chlorantraniliprole, 2.4g/l sugar, 48 hour exposure), Trial 2 (half field rate chlorantraniliprole, 2.4g/l sugar, 24 hour exposure). Chl = chlorantraniliprole, Sg = sugar

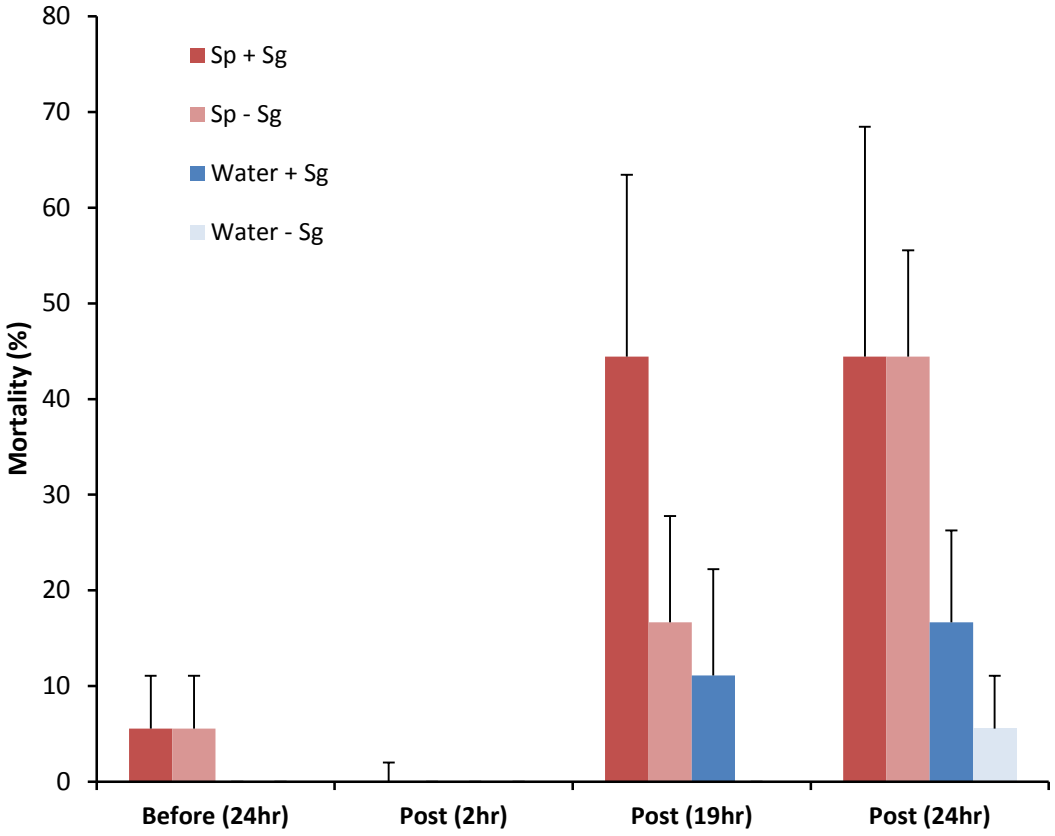

Figure 5.1.5c Mortality of adult *D. suzukii* after exposure to blueberries with and without spinosad and sugar residues (0.1x field rate spinosad, 2.4g/l sugar). Fruit either infested for 24 hours before treatment with reagents, or infested after treatment and assessed at various time points. Sp = chlorantraniliprole, Sg = sugar

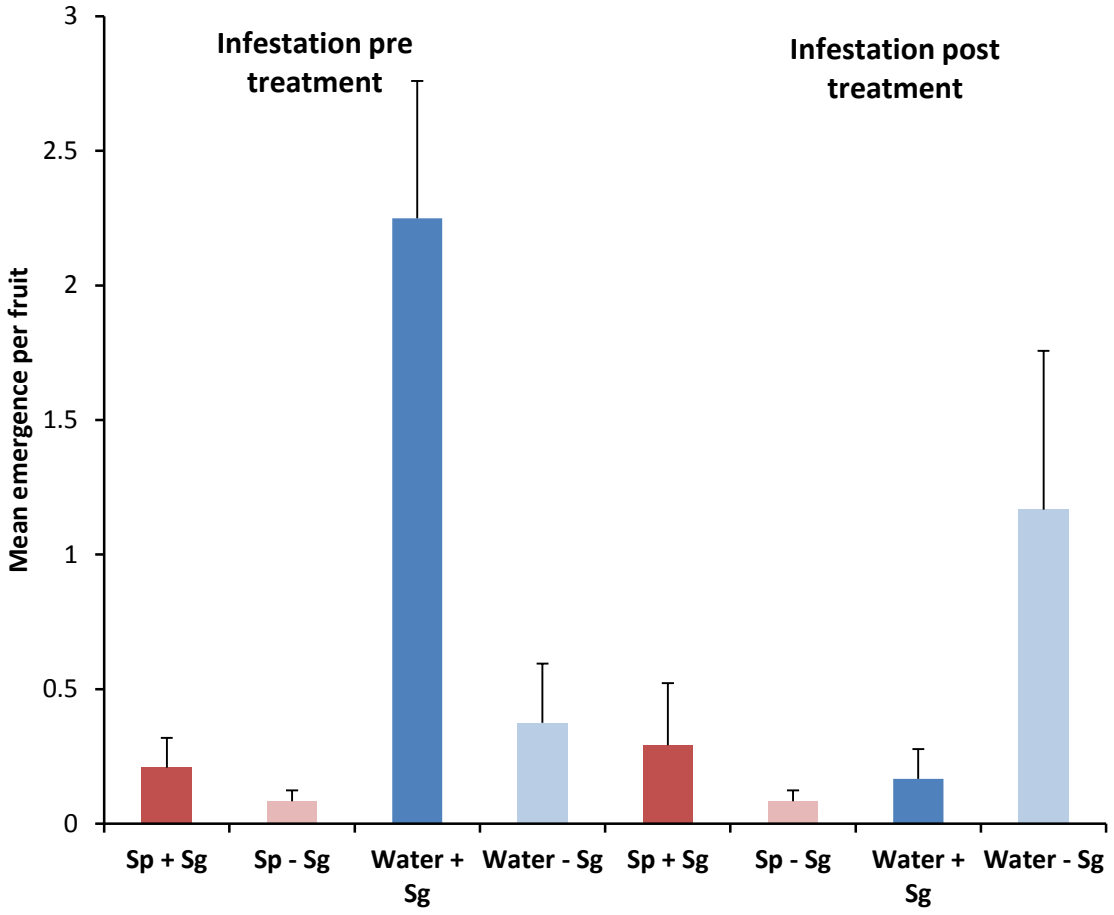

Figure 5.1.5d Emergence of adult *D. suzukii* from fruit after exposure to blueberries with and without spinosad and sugar residues (0.1x field rate spinosad, 2.4g/l sugar). Fruit either infested for 24 hours before or after treatment with reagents. Sp = chlorantraniliprole, Sg = sugar

Conclusions

Preliminary data suggests that, at least for chlorantraniliprole, sugar could be a useful adjuvant, increasing mortality. However, there is also a suggestion that sugar increases the success of egg laying in fruit, which might counteract, or at least reduce, any benefit from adult mortality. This study is ongoing.

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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: Initial bioassays used the laboratory *D. suzukii* population at East Malling Research. To sample UK populations, individuals were trapped using the standard *D. suzukii* trap, but with cotton wool soaked in Dros'Attract (Agralan, UK) as an attractant, and with no drowning solution. Flies were removed 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.

The first wild populations sampled did not survive in culture, but a second sampling of nine populations from three sites in the South East has been established. Members of these populations have been stored at -80^{o}C for later genetic analysis.

Females were assessed separately as they have different insecticide tolerances (P. Shearer, personal communication). In addition, care was 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).

Bioassay apparatus: The bioassay apparatus was a plastic Petri dish (9 cm diameter) with a filter paper disk placed in the bottom and a gridded lid. Eight female flies were added by

pooter and the Petri dish halves joined and sealed with parafilm and the dish stored in a refrigerator for two hours. Each concentration was assessed with three replicates.

Insecticide application: Insecticide was applied using a Burkhard sprayer and diluted to give the desired rate, assuming a uniform flat surface. The insecticides assessed were spinosad (Tracer), lambda cyhalothrin (Hallmark) and chlorpyrifos (Equity).

After spraying, flies were transferred to a new Petri dish with wet filter paper and 1 cm^2 piece of *Drosophila* food. Petri dishes were placed in plastic bags to maintain humidity and incubated at 20^oC. Mortality was assessed after 24 hours.

Initial bioassays used recommended field and 0.1 x field doses to relate to grower experience and give an estimate of the treatments required for more precise LD_{50} value, i.e. the concentration of insecticide required to kill 50% of the population. Further bioassays used a range of concentrations to estimate the LD_{50} value.

Results

Estimated LD_{50} values of the laboratory strain to spinosad, lambda cyhalothrin and chlorpyrifos are given in table 5.2.1.

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 overestimation

Estimated LD_{50} values of six strains derived from populations in the South East of England are given in table 5.2.2.

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

Conclusions

The laboratory population at EMR has been assessed and wild populations taken from pesticide treated crops and wild areas in 2014 have been cultured for comparison. It should be noted that the chlorpyrifos value obtained by this technique is extremely low. This possibly relates to the vapour action of chlorpyrifos, which might enhance mortality in enclosed laboratory environments.

The values given here will be a useful baseline for the determination of any growth in insecticide resistance in 2015. At this time there appears to be little difference between the wild UK populations, and laboratory population, which has not been exposed to insecticides for over 18 months.

There also appears to be little difference between the different populations, although Farms 1 and 2, for example, are 49 km apart. Further monitoring of the situation at these sites will continue 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.

Additional research

Objective 6.1 To determine whether *D. suzukii* eggs and larvae are able to withstand periods of cold in relation to the cold store chain of commercial fruit

Cold store chain: With the difficulty of detecting larvae and eggs in commercial fruit it is important to put in place measures to reduce the chance of infested fruit reaching consumers. As most soft fruit and stone fruit spend some period in cold store before sale it would be a practical time to target any undetected eggs and larvae.

Materials and methods

Raspberry: Ripe (store bought) raspberries, cv. Glen Ample grown by WB Chambers, were introduced to a *D. suzukii* culture cage for one week. They were divided between 20 ventilated push-fit Perspex boxes and sealed with electrical tape. These were then divided between five bug dorms (four in each) and contained within a large polythene bag to control humidity. Two bug dorms were then transferred to the 1.7°C cold store and two to the 4.8°C cold store. The control was stored at 20°C. After three days one bug dorm from both the 1.7°C and 4.8°C cold stores were transferred to 20°C. After five days the remaining bug dorms were transferred to 20°C.

Cherry: This trial was done with the assistance of Oliver Doubleday (GH Dean). Ripe cherries (origin confidential) were weighed out into boxes with 200g in each. Five females and two males were introduced to each box for 24 hours before being removed. Boxes then went straight into cold store at 0.5-1.5°C (egg stage) or were left for five days to develop into larvae before going into cold store. The positive control was also inoculated with *D. suzukii* and an untreated control had no *D. suzukii* inoculation. Both treatments spent five days in the cold store before being kept at ambient temperatures for three weeks for emergence to occur. The positive control and an untreated control were kept at ambient temperature.

For both trials counts of emerged adults were taken after three weeks.

CO_2 measurements: Evidence to suggest that infested fruit produced higher levels of CO_2 compared to uninfested fruit had been suggested from a brief pilot study. As part of the cherry assessment, readings of O_2 and CO_2 were taken from samples that had been inoculated with *D. suzukii* and the control. The fruit was sealed within an Asda Ziplock food

bag with approximately 900 ml of headspace. A rubber septum was struck to the outside of the bags, through which a syringe needle was inserted to withdraw gases for analysis (approx. 30 ml gas per measurement). Percentages of O_2 and CO_2 were measured with an Ipos handheld gas analyser.

Results

Raspberry: There was a significant difference between the control (20°C) and the five days at 1.7°C. None of the other treatments significantly reduced the numbers of *D. suzukii* (Fig. 6.1.1).

Cherry: There was a significant difference between the control (20°C) and the egg treatment stored at cold store at 0.5-1.5°c. The larvae treatment was the same as the control and there was no emergence from the untreated control (Fig. 6.1.2.).

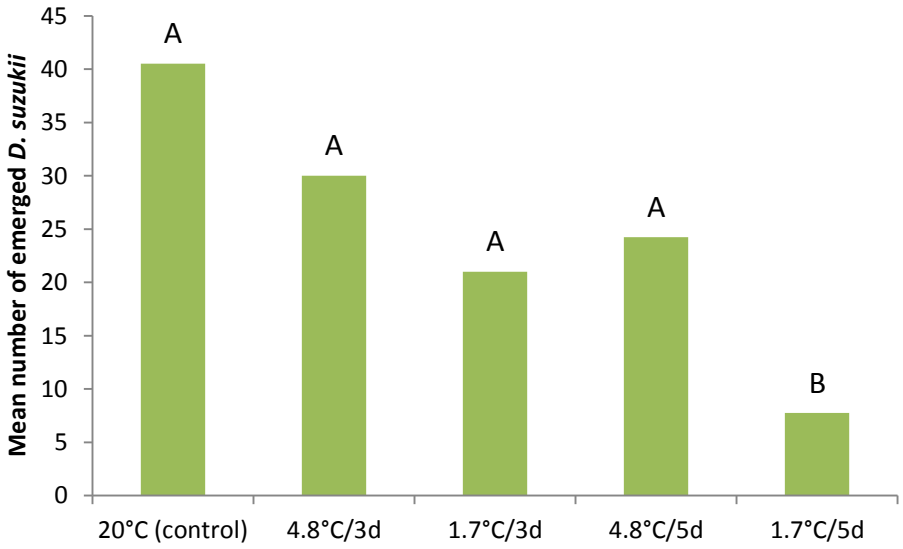


Figure 6.1.1 Mean number of emerged *D. suzukii* from raspberry cold store trial. Storage temperature and length of time at that temperature

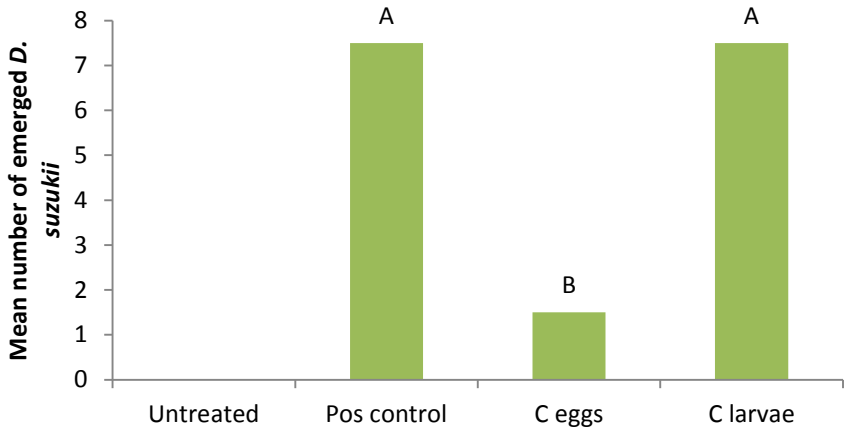
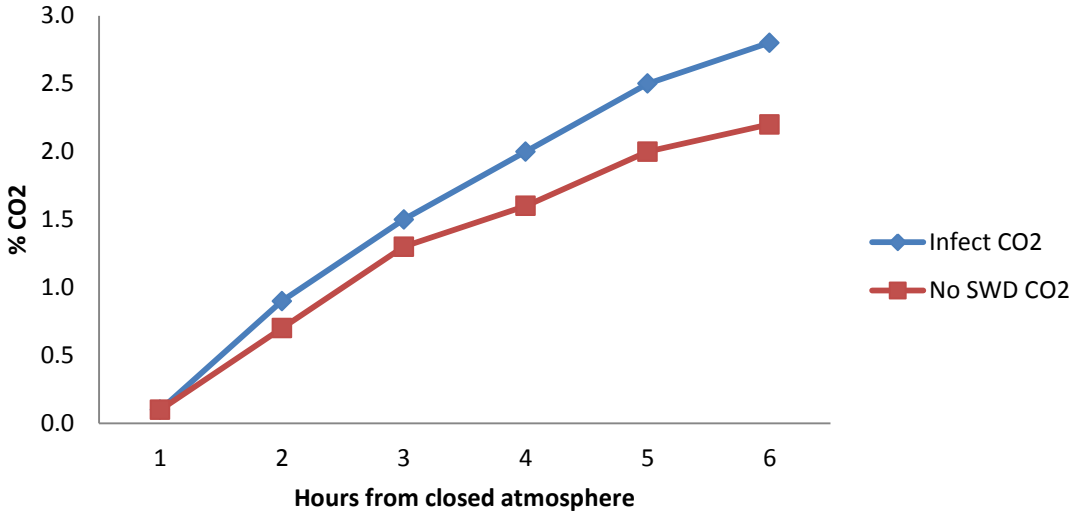


Figure 6.1.2 Mean number of emerged *D. suzukii* from cherry cold store trial

CO_2 measurements: The percentage of carbon dioxide increased more rapidly in the samples that had been inoculated with *D. suzukii* than in the controls (Fig 6.1.3) and differences could be detected two hours after the environment was sealed.



Discussion

Temperature and duration had an effect on the number of *D. suzukii* that emerged from fruits. Numbers of *D. suzukii* were only significantly reduced if cold stored at 1.7°C for five days in raspberry and 0.5-1.5°C in cherry. There were no effects on larval mortality in this trial in comparison with the control.

For the CO_2 measurements, inoculated fruits had higher respiration rates than the controls. This would be a useful tool in detecting *D. suzukii* without using destructive methods to find the egg and larval stages. CO_2 readings were taken hourly and so differences may well be detected earlier if sample interval times were shorten.

Objective 6.2 To determine whether *D. suzukii* eggs can be detected in fruit by using hyperspectral imagining

Preliminary investigation into the use of hyperspectral imaging to detect *D. suzukii* eggs in fruit has begun. This produces an image that highlights imperfections on the surface of fruit that are difficult to see with the naked eye. Areas of the fruit can then be selected their intensity and wavelength readings are plotted – hence a model could be built to predict damage to fruits.

Materials and methods

Fresh blueberries were inoculated with adult *D. suzukii* for 24 hours. They were then secured to a board and images were taken at 804 nm with a hyperspectral imaging program and camera. Visual assessments were made of the fruit to see if impurities on the hyperspectral image could be identified under a compound microscope.

Results

The compound microscope image shows the breathing tubes of the *D. suzukii* eggs protruding from the surface of the blueberry (left image Fig 6.2.1). These can be correlated with the darker patches on the hyperspectral image (right image Fig. 6.2.1). The highlighted areas on the hyperspectral image can then be selected and the intensity and wavelength of the area is plotted. Differences in readings can be distinguished by the trend lines they produce (Fig. 6.2.2).

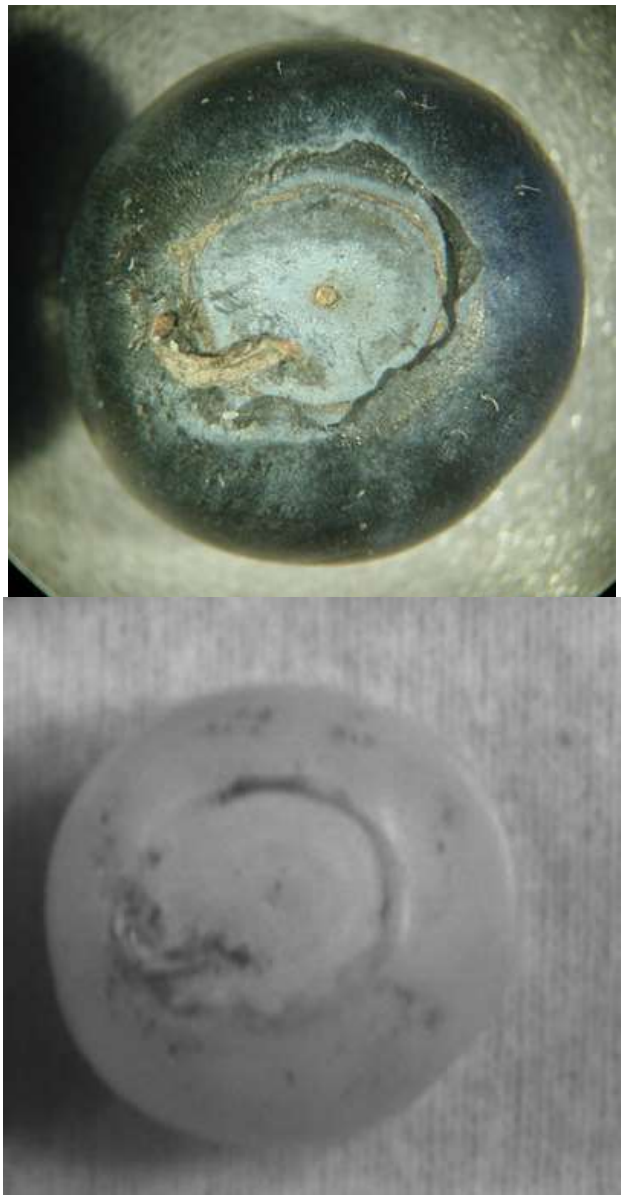


Figure 6.2.1 Image produced from the compound microscope (left) and the hyperspectral image (right)

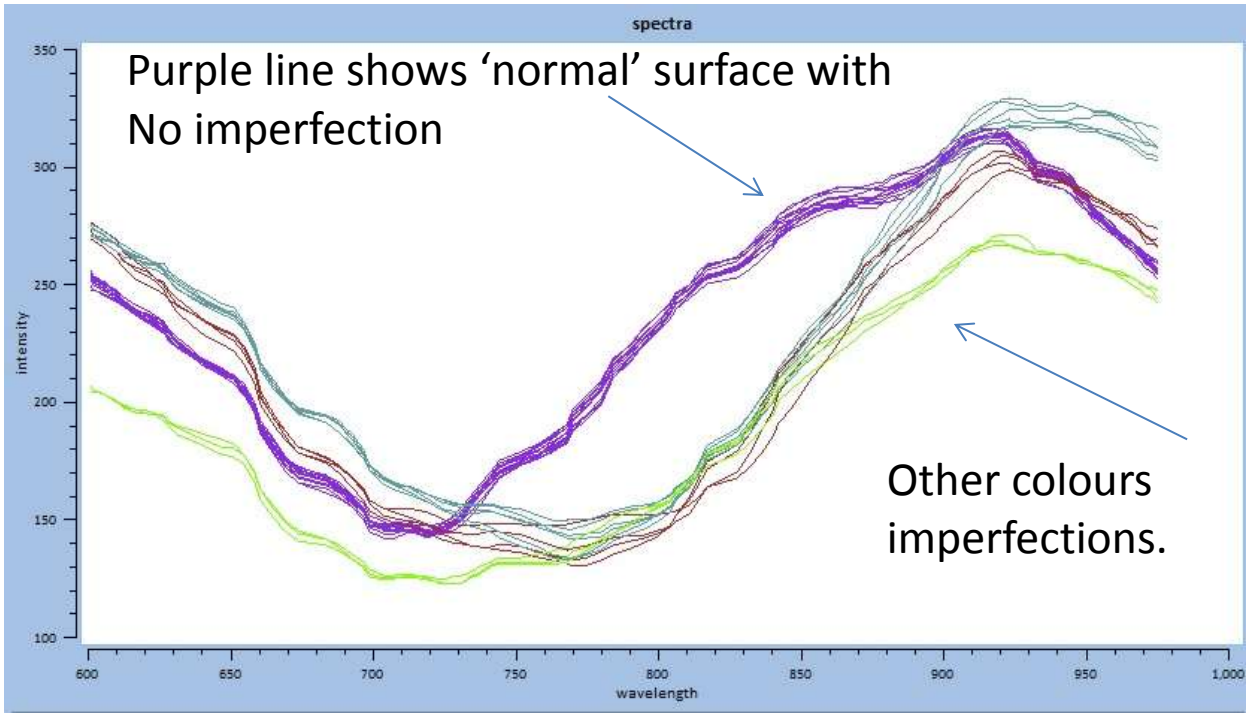


Figure 6.2.2 Image showing different wavelengths and intensities which means an imperfection on the fruits' surface

Discussion

It is possible to highlight the impure surface areas of the blueberries using hyperspectral imaging although not all impurities that were highlighted were due to *D. suzukii* eggs and damage. Validation with a compound microscope would be needed to predict the accuracy of the technique.

Future work

Objective 1

- Continue the National Monitoring Programme;
- Scale down the habitat assessments to focus crops and surrounding area and do more intense studies on the ripening and vulnerability of cherry varieties.

Objective 2

- Repeat infested bin test with *D. suzukii;*
- Fine tune and investigate further composting options;
- Carry out a similar study on cherry waste – not yet done;
- Look at the efficacy of the bin method at low temperatures (beginning and end of season).

Objective 3

- Extraction method efficacy on younger larvae.

Objective 4

- More focus on dry baits;
- More focus on attract and kill methodologies.

Objective 5

- Spray trial on cherry;
- Resistance testing.

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

Target date (31/03/2014)		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	

Target date (31/03/2014		No. of months from start date	Description of milestone	Progress
17	31/03/2017	48	Economics of treatment options for different types of fruit waste and scales of production quantified	
18	31/03/2017	48	Standard Operating Procedure and final report submitted	
19	31/03/14	12	Efficacy of detection and economic costs of different methods of quantifying larval infestations in different fruits	Ongoing
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	Ongoing
22	31/03/16	36	Target device and identify suitable insecticide(s) for attract and kill formulation developed	
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	* See note
26	31/03/17	48	Study on variation in susceptibility of *D. suzukii* populations to 3 insecticides in 3 successive years completed	

*A method has been established, however, there were insufficient *D. suzukii* in culture for bioassays. Cultures of laboratory and UK strains of *D. suzukii* are currently being expanded and it is expected that the tests will be done in early 2015