Project title:	Managing Spotted Wing Drosophila (SWD) in the UK: Determining its distribution and seasonal population dynamics
Project number:	SF 145
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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Report authorised by:

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CONTENTS

GROWER SUMMARY

Headline

- Monitoring for adult and larval spotted wing drosophila (SWD) has been optimised and contributions have been made to understanding the biology of the pest in UK habitats, and in managing fruit waste and pest control.

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 SWD within the UK, and to develop measures for its control. To this end five objectives have been set for the project;

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

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 SWD infestation and attraction on fruit farms.

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

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

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

Summary of the project and main conclusions

Objective 1 – Population dynamics

This was subdivided into two tasks. The first involved establishing a monitoring network across the UK, with fourteen sites; five in Kent (including East Malling Research), one in Surrey, two in the west Midlands, two in eastern England and four in Scotland (including the James Hutton Institute). This network has been successfully established, with 130 traps operating, and plans to add another site in the West Midlands in 2014. In addition, the design of trap and bait used, has been optimised to produce a considerable advance in efficiency and ease of use since the start of the project.

The first record of SWD in 2013 was made at East Malling Research in August. Since then, SWD has been captured in crops at each of the six national monitoring sites in Southern England, though at very low numbers. In East Anglia, the first SWD was recorded in December 2013. No SWD were found in the national monitoring traps in West Midlands or Scotland, though SWD is known to occur in the West Midlands on other farms. The numbers caught in crops have generally been very low and those caught were at the end of the year, so crop damage has been minimal. This is probably due to the exceptionally cold winter and spring in early 2013.

The second task was to study the distribution of SWD on farms in more detail. Therefore two farms (including EMR) were sampled with over 50 traps each, in a range of crops and in neighbouring wild areas and woodlands. SWD were detected throughout these farms, but were especially associated with particular woodlands and hedgerows. November and December saw a fall in catches in crops, but there was a steep increase in catches in wild areas during these months. This presumably reflected pest migration to more sheltered areas for the winter. SWD can remain active throughout the winter if the weather is mild.

To determine if SWD can utilise native plant species as hosts, fruit were collected from a range of species. SWD was found in berries collected from Elder and Blackberry, and it is known that they are also capable of using Dogwood, Sloe, Snowberry, Red Bryony and Nightshade.

Additional research is tracking the development of the ovaries in the female SWD flies. This work will allow scientists to predict when egg laying will start in wild hosts in the spring.

Objective 2 – Waste disposal

One conclusion from research in SWD infested countries is that crop hygiene is an important component of SWD control. However, consultations with UK soft fruit growers indicated that about 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.

As there is no published information on the conditions needed to kill SWD in fruit waste, this was investigated. It was found that fruit fermentation in bins was effective, but only in sealed containers which become anaerobic for up to 13 days. Further research will reveal if shorter containment periods are also successful at killing Drosophila larvae. Another consideration is that this fermented waste, although devoid of SWD, is highly attractive to SWD when opened to the environment. Therefore it cannot be simply spread on land, but has to be ploughed into the soil.

Objective 3 – Sampling and extraction methods

It is very difficult to discern SWD larvae in fruit by simple visual examination of the fruit and so various methods used around the world were assessed to quantify larval SWD infestation in blueberries, cherries and raspberries. All methods rely on stresses to induce the larvae to leave the fruit, sometimes encouraged by gently crushing the fruit.

Immersion of fruit in either a salt or sugar solution was trialled with some success, though 100% recovery was not achieved, even though the experiment was timed to assess the largest, 3rd, instars. Freezing overnight was found to generally produce lower counts, as well as taking longer to assess.

Preliminary trials on strawberries (not presented here) suggested that a different method may be required for this fruit.

Objective 4 – Lures/attract and kill technology

A wide variety of traps and baits have been developed around the world for SWD recording. Work at EMR compared the most promising of these traps for efficiency and ease of use. In collaboration with NRI a new synthetic attractant bait combination was developed and was shown to be attractive to SWD and more importantly more selective for this species. This new synthetic attractant is not yet commercially available to growers as it is still in trial.

Objective 5 – Crop protection

Insufficient SWD were present in the UK to undertake field trials in 2013. However, strawberry fruit with field doses of insecticide residues were assessed using a laboratory culture to determine the effectiveness of seven insecticides, including spinosad (Tracer), chlorpyrifos (Equity), lambda-cyhalothrin (Hallmark) chlorantraniliprole (Coragen), deltamethrin (Decis) pyrethrins (Spruzit) and a coded product. These were compared to an untreated control. Harvest interval was up to two weeks post insecticide application, to determine any effect of residue decay.

Spinosad, the coded product and chlorpyrifos gave control of SWD for up to two weeks after spraying (no adult SWD emerged from fruits exposed to SWD post spraying). Lamda-cyhalothrin and pyrethrins gave very short and variable control of SWD – up to two days. None of the other products were effective at reducing SWD in this strawberry trial. This trial will be repeated in 2014 on raspberry fruits. SWD develops insecticide resistance easily, and good crop hygiene and other non-chemical controls should be combined with rotations of modes of action of insecticides to prevent insecticide resistance.

Financial benefits

SWD poses a clear threat to the fruit industry, particularly soft fruit and cherries. Experience in other countries indicates that it has the capacity to spread rapidly, cause devastating damage to the industry and have an impact on the wider environment. There are clear, significant differences between the UK and other regions of the world where the pest has been found, in terms of climate, crops, varieties, growing systems and approved pesticides. However, damage has caused significant financial losses to many of the fruit growing countries and individual growers.

Action points for growers

- Growers should monitor susceptible crops and wild areas around crops so that they can predict the onset of egg laying by SWD.
- Growers should use the modified Biobest trap with the Dros-attract bait to monitor for SWD during the 2014 season.
- Growers should consult their BASIS qualified advisors before making a final choice of crop protection product.
- Crop hygiene needs to be maintained and waste fruit should be treated by containing in sealed vessels and then disposed of in a way which renders the waste unattractive to further SWD egg laying.
- Immersion of fruit in sugar or salt solution is recommended for detection of larval infestation in the crop.

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SCIENCE SECTION

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

Task 1.1 Population dynamics of adult SWD in vulnerable polytunnel and outdoor grown fruit crops at 10 sites in England (EMR + 9 farms) and 4 sites in Scotland (JHI+3 farms)

Materials and methods

Sites

Fourteen typical fruit farms, five in Kent (including East Malling Research), one in Surrey, two in the west Midlands, two in eastern England and four in Scotland (including the James Hutton Institute) were selected for the study. 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.

Table 1.1 Summary of fruit farms involves in the national monitoring survey

	Region and crops
	South East England (44 traps)
Farm 1	Raspberry, strawberry
Farm 2	Blackberry, blackcurrant, raspberry, strawberry
Farm 3	Cherry
Farm 4	Raspberry, strawberry
Farm 5	Blackcurrant, cherry, raspberry, strawberry
Farm 6	Blueberry, strawberry
	Eastern England (20 traps)
Farm 7	Blueberry, raspberry, strawberry
Farm 8	Blackberry, raspberry, strawberry
	West Midlands (22 traps)
Farm 9	Blackberry, blackcurrant, blueberry, raspberry, strawberry
Farm 10	Blueberry, cherry, raspberry, strawberry
	Scotland (44 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

SWD traps

SWD 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.

The design of trap and bait used evolved throughout 2013. Bottle traps with yeast/sugar water bait and a sticky collecting card were used initially, but although they were successful, the bait was messy and the trap difficult to use. A decision was taken in July (before any SWD had been caught) to switch to a synthetic bait (based on newly published work from the Yakima Agricultural Research Laboratory, Washington State which several labs in the US were starting to clearly show was much more effective and easier to use. It was also decided to switch to a modified version of the Biobest Droso trap which was more robust, though the sticky card was maintained. In a further step in September, it was decided to stop using the sticky collecting card and collect the SWD from the liquid bait because there was evidence that the SWD adults were escaping in large numbers from the sticky cards.

A full discussion of the evolution of SWD trapping is given under Objective 4.

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 is still continuing.

Results

The first SWD adult was captured at East Malling Research in August. Since then, SWD have been captured in crops at each of the six national monitoring sites in Southern England, though at very low numbers. The first SWD was recorded in East Anglia in December 2013. No SWD were found in the national monitoring traps in West Midlands or Scotland, though SWD is known to have been found in the West Midlands on other farms.

Total SWD catches rose slightly during September before rising more steeply in October. SWD catches in crops fell during late November, but there was a steady increase in woodland catches in December and January, before these too fell away. This is illustrated by the catches for the two farm case studies, (Figure 1.1).

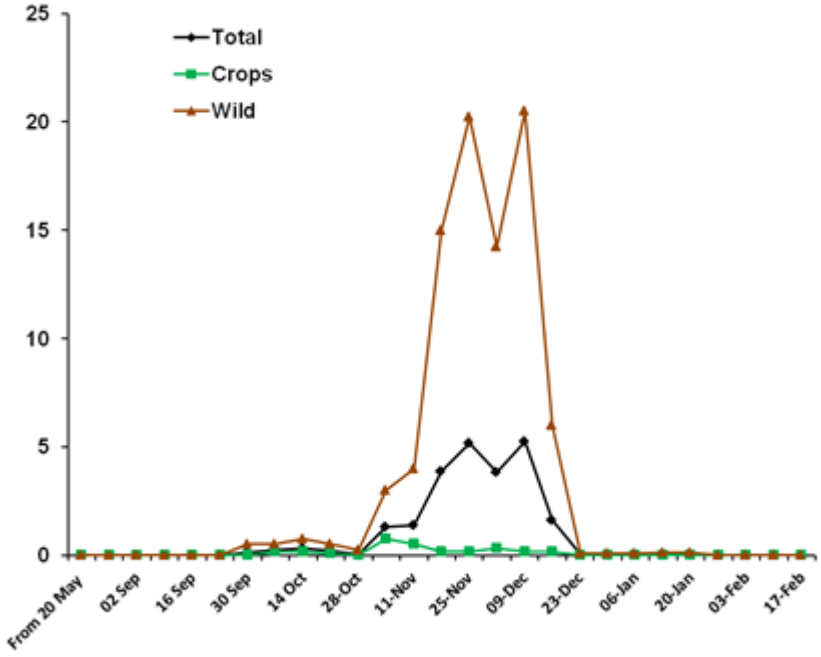

Figure 1.1. Mean numbers of SWD adults in main habitat types during 2013-14

Discussion

SWD numbers in the UK were very low throughout most of 2013, causing minimal crop damage. There is a danger however that this may lead to complacency.

SWD, like most insect pests, is known to respond to changes in climate. The UK had an unusually cold winter in early 2013, followed by a colder than average spring and early summer. Of the first nine months, only August was warmer than average. This presumably delayed the appearance and spread of SWD, so that for example the cherry crop was unaffected. However, in contrast the autumn and early winter was exceptionally mild. This presumably allowed the increase seem in October and November. It was clear too that SWD was found in most areas of middle and southern England. Thus there was a considerable reservoir of SWD available to re-emerge in the spring since the winter of 2013/4 continued to be mild.

One clear result of the monitoring survey was a sharp increase in numbers recorded in woodland in November and December, matched by a decrease elsewhere. This presumably reflects a migration to more sheltered areas, a phenomenon noted in other temperate countries at the northern limit of the SWD range. Whether it also is associated with continued reproduction is unclear, but a subject of research at EMR.

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 2014 inclusive.

Materials and methods

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 in each of Farms 1 and 2 (Table 1.2.1). Adults were trapped in yeast and sugar water bait with a sticky card trapping method until 30 September (Fig. 1.2.1) and afterwards with synthetic chemical based traps with a liquid trapping method (Fig. 1.2.2). See Objective 4 for details of baits. Traps within a pair were spaced 10 m apart.

Figure 1.2.1. Yeast and sugar water bait trap with sticky insert used initially in the trapping trial

Figure 1.2.2. Synthetic bait trap with yellow sticky and liquid bait trapping media – used from September onwards

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

Farm 1		Farm 2	
Trap pair no.	**Habitat**	**Trap pair no.**	**Habitat**
1	Cherry orchard	101	Strawberry
2	Cherry orchard	102	Strawberry
3	Hedgerow	103	Hedgerow
4	Woodland	104	*
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	Soft Fruit	119	Raspberry
20	Hedgerow	120	Hedgerow
21	Compost heap	121	Hedgerow
22	Hedgerow	122	Woodland
23	Hedgerow	125	Raspberry
24	Compost heap	126	Raspberry
		127	Raspberry
		128	Raspberry

* traps removed

Assessments

Adults: Weekly trap catch records for *D. suzukii* males and females, and other flies (total numbers). Numbers were initially counted in the field and then bought back to the lab for confirmation under a microscope. Later on in the trial *D. suzukii* were counted in the laboratory only.

Habitat: Monthly habitat assessments recorded the presence of fruiting plants.

Fruit samples: Samples of fruits were taken from different locations (edges, centre) in each focus crop, each week from the green fruit stage until the end of harvest. Within each soft fruit crop 50 fruits from between each paired 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 fruit were sampled from each crop and measurements of colour (comparison with Ctfil colour chart), hardness/softness (measurements with penetrometer/durometer) and sugar content (refractometer measurements of °Brix) made weekly until the end of harvest.

Reproductive stage of females: Collected males and females were measured and dissected under a microscope to determine sexual maturity and gravidness through the season.

Results

Trapping: Trapping began on 23 April 2013 and the first *D. suzukii* male was captured in a hedgerow adjacent to a soft fruit farm on 12 Aug (Fig. 1.2.3). The first individuals trapped were males. There was a lag period of at least two weeks and then occasional individual *D. suzukii* continued to be caught in crops and wild areas throughout August and September, after the cherry harvest. *D. suzukii* numbers caught in crops began to increase in October and mid-November before falling. From November onwards the majority of the *D. suzukii* were trapped in neighbouring woodlands (Figs. 1.2.4, 1.2.5), but adults were still widely distributed across both farms. *D. suzukii* were still being trapped in December 2013. There were particular woodlands or hedgerows on both farms where *D. suzukii* were caught almost every week. Adult *D. suzukii*

tended to be trapped in areas that contained blackberry and elderberry and became more abundant in crops once the wild fruits were diminishing around the end of September.

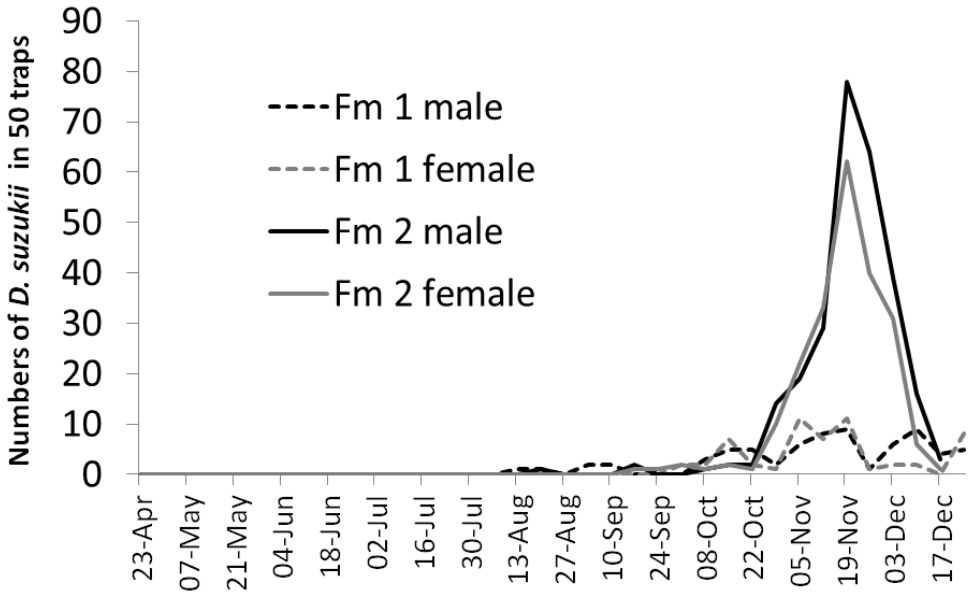

Figure 1.2.3. Adult SWD trap catches at Farms 1 and 2

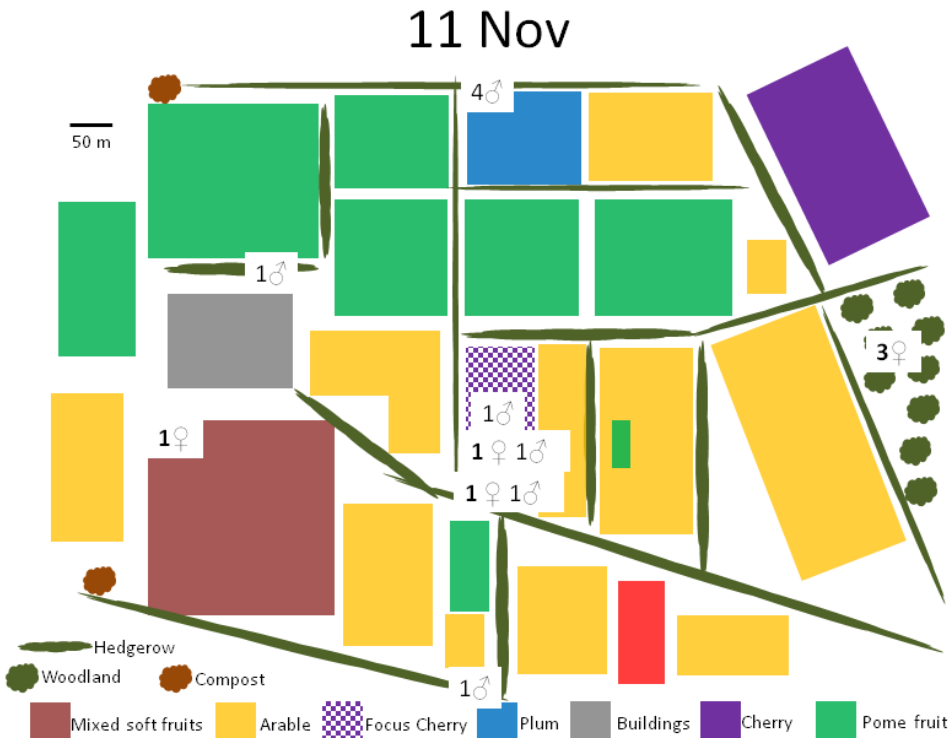

Figure 1.2.4. Numbers and locations of male and female *D. suzukii* trapped on 11 November on Farm 1

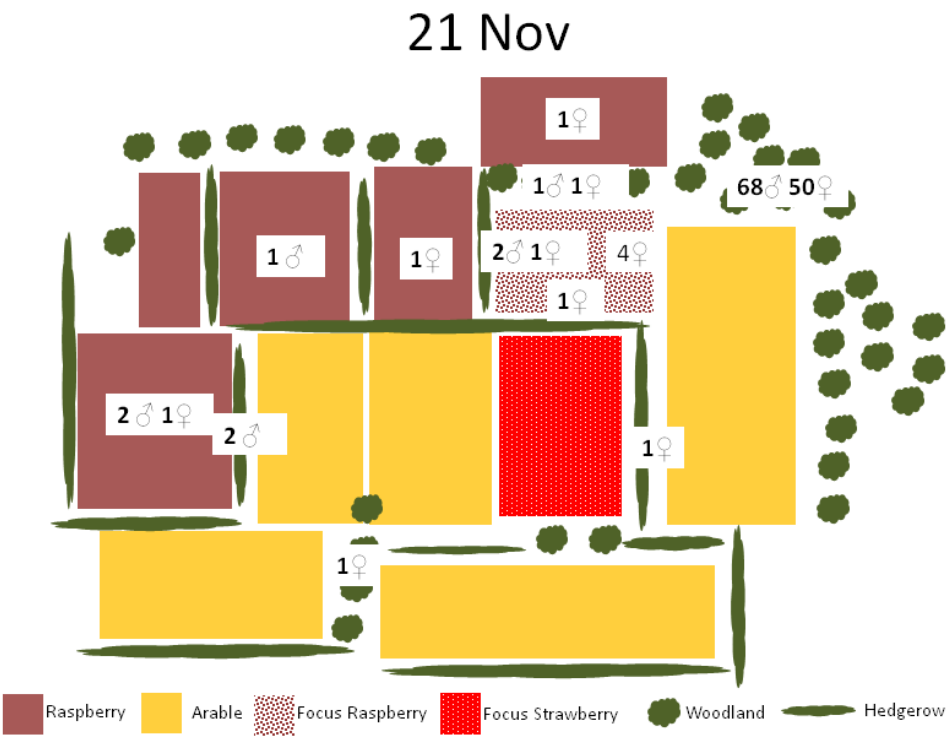

Figure 1.2.5. Numbers and locations of male and female *D. suzukii* trapped on 21 November on Farm 2

Habitat: In general *D. suzukii* numbers were higher in woodlands than crops throughout the year (Figs. 1.2.6, 1.3.7). A peak in December around a compost heap at Farm 1 was shown to be the result of open disposal of waste fruit.

Wild blackberry came into flower on 25 June and there were green-red fruits by 5 August. Blackberries had ripened by 10 September and by 8 October there were no fruit remaining. This reduction in available ripe fruits seemed to coincide with an increase in *D. suzukii* adults found in the crops and woodlands.

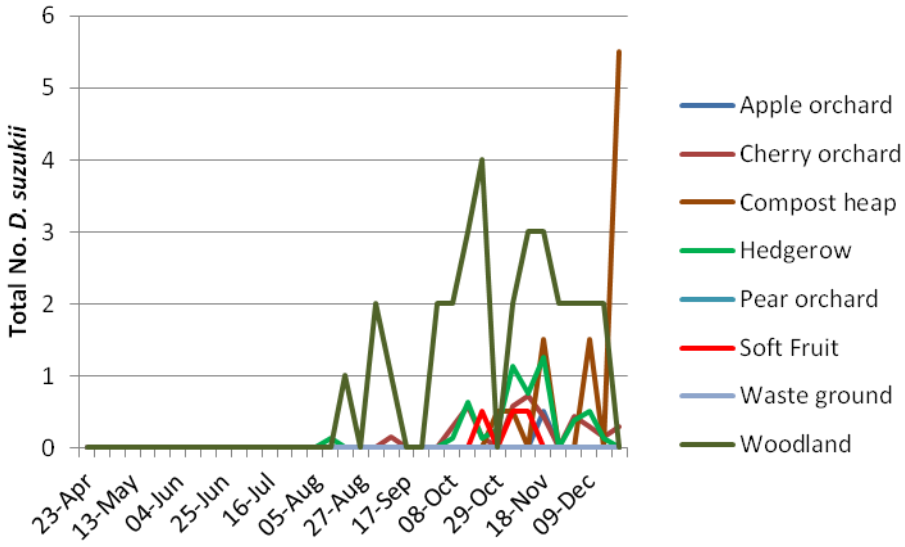

Figure 1.2.6. Mean numbers of *D. suzukii* captured per habitat type at Farm 1

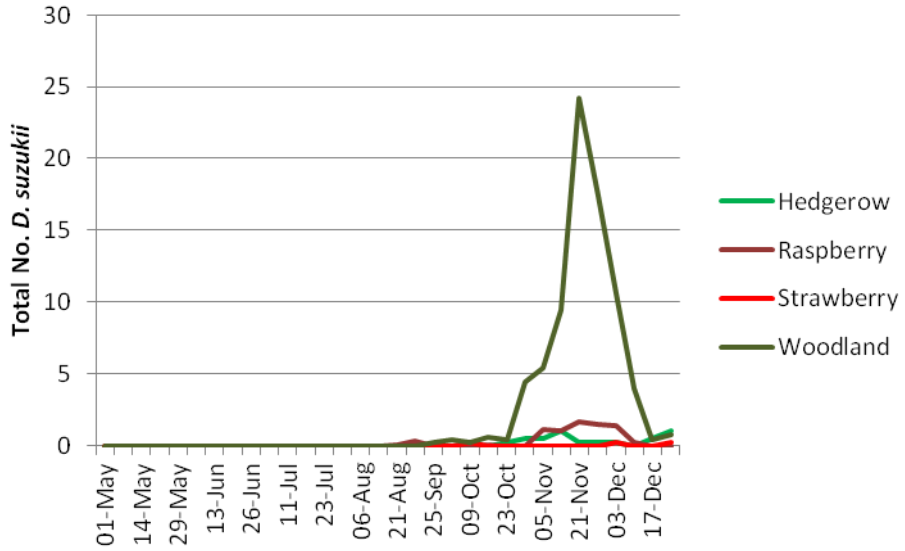

Figure 1.2.7. Mean numbers of *D. suzukii* captured per habitat type at Farm 2

Fruit samples: Records were made of fruit quality, but are not reported here. One male *D. suzukii* emerged from raspberry fruit picked on 5 November and one male and two females from the strawberry fruit picked on 1 October. At this time, all fruits were ripe and the crops were not being treated as the harvest had finished. Because the numbers of emerging *D. suzukii* from the fruit were so low it was not possible to correlate these with the growth stage and quality attributes of the fruits.

Reproductive stage of females: This work is beginning from samples of male and female *D. suzukii* collected weekly and stored in 70% alcohol.

Spray programmes in crops: The insecticides applied to the cherry crop at Farm 1 included Dipel on 17 May and 5 June, Aphox on 27 June and 17 July and a pyrethrum on 23 July. These

applications were made before any *D. suzukii* was detected on the farm.

Farm 2 applied Apollo on 9 April, Chlorpyrifos and Decis on 11 May, Calypso on 7 June, Dynamc on 9 April, Naturalis on 11 September, and Tracer on 20 September.

Conclusions

- *D. suzukii* was widespread on the farms, but trap catches were generally higher in woodlands and hedgerows where wild blackberry was growing
- There were particular woodlands or hedgerows on both farms where *D. suzukii* were caught almost every week
- *D. suzukii* numbers were too low in 2013 to cause damage to commercially cropped fruit
- The first *D. suzukii* male was captured in a hedgerow adjacent to a soft fruit farm on 12 August
- The first individuals trapped were males
- There was a lag period of at least two weeks
- Populations peaked in mid-November before falling
- *D. suzukii* were still being trapped in December 2013
- Open disposal of waste fruit was attractive to *D. suzukii*
- *D. suzukii* only emerged from cropped fruit after harvest was completed

Task 1.3. Identify the common wild host plants of SWD adults and larvae in the UK

Objective

By identifying the wild host plants that SWD can breed on it may be possible to highlight problem areas and possible 'hot spots' or reservoirs of SWD 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. Although this task was not due to be explored until years two and three, we began some preliminary tests with wild and commercial fruits and leaf litter. Ripening and ripe fruits were collected from the field and then tested for either natural emergence or whether flies would lay eggs and develop in fruits (no choice tests). No choice tests consisted of introducing males and females into a Perspex box along with a sample of fruit. This allowed us to determine whether the plant was a potential host for SWD, but not whether it was a preferred host. The natural emergence tests examined whether flies were utilising fruit and leaf litter in the wild for their development.

In April 2013 a paper was published by Bellamy *et al.* (2013) which gave a Host Potential Index (HPI) determined by reproductive success of SWD on fruit. From this paper it was concluded that raspberry had a HPI of 1 ranked out of 7 different fruits. The raspberry no choice emergence SWD numbers could then be used as the indicator for optimum result in the other fruits trialled.

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 20°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 3 weeks. At the end of this time the total numbers of male and female SWD 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 SWD populations. 10 cm x 5 cm x 3.5 cm Perspex boxes were filled with fruit and were then sealed inside a 20 cm^3 bug dorm cage along with one sheet of paper towel sprayed with distilled water. The bug dorm was stored within a large polythene bag to maintain humidity (Fig. 1.3.2) and maintained at 20°C for 4 weeks. The boxes were kept damp by spraying the paper towel with distilled water weekly. After this period of time the numbers of adult SWD were recorded. Keeping cultures for longer periods risks a second generation emergence.

Natural emergence from leaf litter

Five bags of approximately 5 kg of leaf litter was collected from the surrounding areas of SWD national monitoring traps in woodlands and orchard edges at one farm. These traps were catching high numbers of adults long after harvest and fruiting had ended. These were mixed samples of leaves which were sorted in 600 mm x 900 mm polythene bags hung from a steel frame. Within the bag a modified Droso trap containing 300 ml of Gasser liquid bait was hung to attract the flies out of the leaf litter. The bags were stored at 20°C for four weeks (Fig. 1.2.3).

Figure 1.3.2. Experimental set up of the natural emergence from leaf litter experiment

Results

No choice emergence

SWD introduced into boxes with fruits had a second generation emerge from dogwood (*Cornus sanguinea*), elderberry (*Sambucus* sp.), fig (*Ficus carica*, var. Brown Turkey), Guelder rose (*Viburnum opulus*), Japanese rose (*Rosa rugosa*), nightshade (*Solanum* sp.), raspberry (*Rubus*), red bryony (*Bryonia dioica*), rowan (*Sorbus* sp.), sloe (*Prunus spinosa*), spindle (*Euonymus europaeus*), snowberry (*Symphorcarpus* sp.) and wall cotoneaster (*Cotoneaster horizontalis*) (Table 1.3.1).

Species tested in which SWD did not appear to develop included damson (*Prunus domestica*), hawthorn (*Crataegus* sp.), holly (*Ilex aquifolium*), ivy (*Hedera helix*), pyracantha (*Pyracantha* sp.) and rose (*Rosa acicularis*).

Table 1.3.1. Field collected fruits exposed to adult male and female SWD and resulting emergent adult SWD (no choice)

Description	Total SWD (per gram)
Wall cotoneaster (*Cotoneaster horizontalis*)	2.9
Fig (*Ficus carica*)	2.3
Japanese rose (*Rosa rugosa*)	0.7
Rowan (*Sorbus* sp.),	0.3
Spindle (*Euonymus europaeus*)	0.2
Guelder rose (*Viburnum opulus*)	0.1
Hawthorn (*Crataegus* sp.)	0
Ivy (*Hedera helix*)	0
Holly (*Ilex aquifolium)*	0
Pyracantha (*Pyracantha* sp.)	0

Natural emergence

SWD naturally emerged from raspberry, elderberry and blackberry. It did not emerge from cotoneaster, snowberry, Guelder rose, dogwood, hawthorn, red bryony and rose. There was only one replication of each fruit and so these will be retested in 2014.

Table 1.3.2. Field collected fruits and the resulting naturally emerged adult SWD

Description	Total weight of fruit (g)	Total SWD
Raspberry	1300	24
Elder	1000	8
Blackberry	719	6
Cotoneaster	193	0
Snowberry	273	0
Guelder rose	450	0
Dogwood	193	0
Hawthorn	700	0
Red bryony	99	0
Rose	355	0

Natural emergence from leaf litter

No SWD emerged from the leaf litter. From the no choice trial, several potential wild SWD hosts have been identified. In the no-choice laboratory tests SWD was able to lay eggs and develop in dogwood, sloe, snowberry, red bryony, Guelder rose and nightshade although no SWD emerged from these fruits collected in the field.

By using the HPI (Host Potential Index) we can use the SWD adult emergence from raspberry in the no choice trial as the threshold. We can then use this as comparison for future no choice trials as an indication to its success as a host fruit.

Conclusion

Several wild, commercial and garden species were shown to be potential hosts for SWD. Conversely, the grape variety Red Globe did not support development, despite reports in the literature of grapes being hosts for SWD. Some of the potential hosts which SWD was not able to develop in were quite hard berries e.g., rose, hawthorn and ivy. Studies in the literature comparing cultivars of commercial fruit have found a strong negative correlation of vulnerability to SWD and fruit firmness (eg. Kinjo *et al.* 2012, Lee *et al.* 2011) and this presumably is one consideration of host preference for SWD

However, there was only one replicate of some of these fruit and so these will be retested in 2014.

No SWD emerged from the leaf litter samples taken from one site. This was tested as a possible resting place for SWD, given that adults were active at that time. This suggests they are elsewhere, perhaps in the plant canopy, though again this requires further testing.

References

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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 SWD infestation and attraction on fruit farms. (Yrs 1-4)

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

Consultations with soft fruit growers indicated that about 20% of the strawberry crop and 10-15% of the raspberry crop is waste. Until now, soft fruit waste is mainly disposed of in a 'compost heap' which gradually rots down over several months. Cherry and plum waste is not usually collected from under the trees. Cherries grown in polytunnels produce about 5% crop waste whereas cherries grown outside produce between 10 and 100% waste depending on the season and levels of splitting and subsequent fruit rot due to rainfall. Quantities of fruit waste produced by individual companies can range from <1 tonne to >100 tonnes per week during peak season.

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

There is no published information on the conditions needed for eradicating different life stages of SWD from fruit waste. A review of conditions needed to eradicate various life stages of other Diptera species showed that the eggs of some species could survive for several hours above 50˚C whereas larvae were more sensitive to temperature and were killed after 1 hour at 45˚C. However, these temperatures would be difficult to achieve in a composting system using a high moisture waste such as fruit, unless a large amount of dry organic material is added. *Drosophila melanogaster* is very tolerant of deprivation of oxygen supply (anoxia) and the larvae can survive for several hours in an atmosphere of pure nitrogen. However, killing *Drosophila* species by subjecting fruit waste to an anaerobic treatment was considered feasible.

Two types of sealed vessels were used for treating fruit waste: 500 litre plastic pallet boxes (Fig. 2.1) and 100 litre plastic barrels. The vessels were filled with waste fruit to within 10 cm of the lids. Three 500-litre and three 100-litre vessels were filled with strawberry waste and one 100-litre vessel was filled with plum waste. The lids of these vessels were then sealed. A further 500-litre vessel was filled with strawberry waste and a 100-litre vessel filled with plum waste; these vessels were covered but left unsealed as controls. Gas measurements of the headspace air in sealed vessels showed that the oxygen level became undetectable after 12 hours whereas the carbon dioxide level exceeded 21 % v/v. In the unsealed vessels, the oxygen level did not go below 8 %v/v and the carbon dioxide level remained at or below 8 %v/v. After two weeks in the sealed vessels, the strawberry or plum waste had separated into two layers: 90% consisting of underlying liquid and a 10% surface layer of partially degraded fruit and leaf waste.

Figure 2.1 Strawberry fruit waste in 500 litre plastic boxes

Two-litre samples of the surface fermented waste fruit from the above vessels was placed in 5 litre plastic containers and covered with a fine mesh to exclude any drosophila. After three weeks, the containers were checked for any drosophila that may have emerged from the waste. No drosophila emerged from the fruit waste treated in the sealed vessels but abundant drosophila adults were present in the fruit waste from the unsealed vessels (Fig. 2.2).

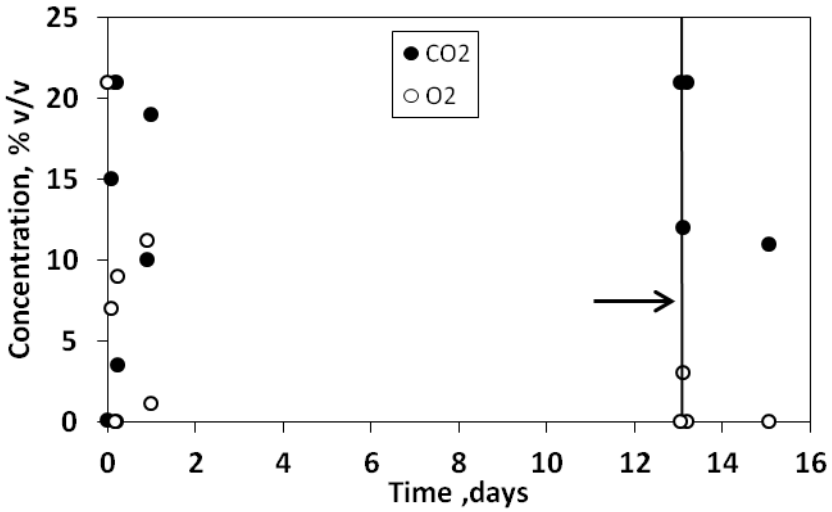

Figure 2.2 Oxygen and carbon dioxide concentrations in the headspace of 500 litre sealed vessels containing strawberry fruit waste

The attractiveness of the fermented strawberry waste from the above sealed vessels was tested by placing 10 g samples in Petri dishes in plastic cages with 4 female and 2 male SWD adults. Fresh strawberries were used as control. The adults were removed after 6 days and the cages tested for the presence of further adults after 3 weeks. Although the number of emerging adults was lower from the fermented waste than from the fresh fruit, the fermented waste was still attractive and retained its capacity to rear a complete life cycle of SWD.

Anaerobic treatment of strawberry and plum wastes in sealed vessels is therefore effective in eradicating any *Drosophila* spp. present in the waste but does not eliminate its attractiveness to SWD. A preliminary test using the above fermented waste has shown that it degrades rapidly if spread and mixed into the soil surface. Further tests are needed to determine whether soil-waste mixtures remain attractive to SWD.

Objective 3: To develop and evaluate sampling and extraction methods for quantifying SWD infestations in different soft and stone fruits. (Yrs 1-3)

Objectives

It is very difficult to discern SWD larvae in fruit by simple visual examination of the fruit. A variety of methods have been developed around the world to improve SWD detection by inducing the larvae to exit the fruit, either by immersion in a solution of by freezing the fruit. It is unclear which of these methods is the most efficient, and if indeed this varies for different fruit.

The objective therefore is to determine the efficacy of detection of different methods of quantifying SWD larval infestations in different fruits including flotation and freezing methods. These will be compared to direct examination of the fruit by dissection, and by counts of adult emergence.

Materials and methods

Infestation of fruit

Fruit (100g of blueberry or raspberry), was added to the base of a (228*121*66 mm) plastic box over a layer of paper towel to stabilise the fruit (Figure 3.1). The number of fruit was noted to form another variable. Only healthy, undamaged, fruit was used.

Figure 3.1 Fruit incubation box

SWD (10 females and 5 males per box) were added to infest the fruit, the boxes were sealed to prevent fly 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 7 days, this period of incubation having been shown previously to give late instar larvae.

Treatments

Three possible methods of larval assessment were assessed and compared to two controls, manual dismemberment of fruit and counting of larvae, and counts of adult emergence. Each was replicated six times.

The three methods of larval assessment were as follows:

Sugar

Fruit (100g) was placed in a clear plastic bag and gently crushed as this increases larval extraction by 50% compared to use of whole fruit (Dreves *et al.,* 2013). Fruit was then covered with a sugar solution (1 Kg sugar/ 5.5l water) and 1-2 drops spray tank de-foamer and observed for 20 mins, with gentle mixing at 10 mins.

Salt

Fruit (100g) was placed in a clear plastic bag and gently crushed as this increases larval extraction by 50% compared to use of whole fruit (Dreves *et al.,* 2013). Fruit was then covered with a salt solution (4 cups water to every 1/4 cup salt) and 1-2 drops spray tank de-foamer and observed for 20 mins, with gentle mixing at 10 mins.

Freezing

Fruit (100g) was placed in a clear plastic bag and frozen overnight. Reportedly, large larvae will exit the fruit and die on the surface. Fruit was examined visually next day.

The two control methods were as follows.

Direct observation: Fruit (100g) was dismembered under a binocular microscope and larvae observed directly.

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

Assessments

Visual counts of swimming larvae in the salt and sugar treatments. Visual counts under binocular microscope for the dissected and frozen fruit. Counts of emerged adults for emergence treatments.

Results

The results are presented in Figures 3.2, 3.3 and 3.4. Incubation was timed to give 3rd instar larvae, the largest and most visible. In all cases, there was no significant difference between sugar and salt emergence, and in all trials except one (the second blueberry trial) these were scored significantly lower than dissection, which was assumed to catch 100% of larvae.

Freezing generally gave a lower count than either sugar or salt immersion but the results were inconclusive.

Immersion in carbonated water was also investigated, but abandoned after one trial as there were major difficulties in seeing the larvae (data not shown). Strawberries were also investigated but it became clear that their greater size would require a different pre-treatment.

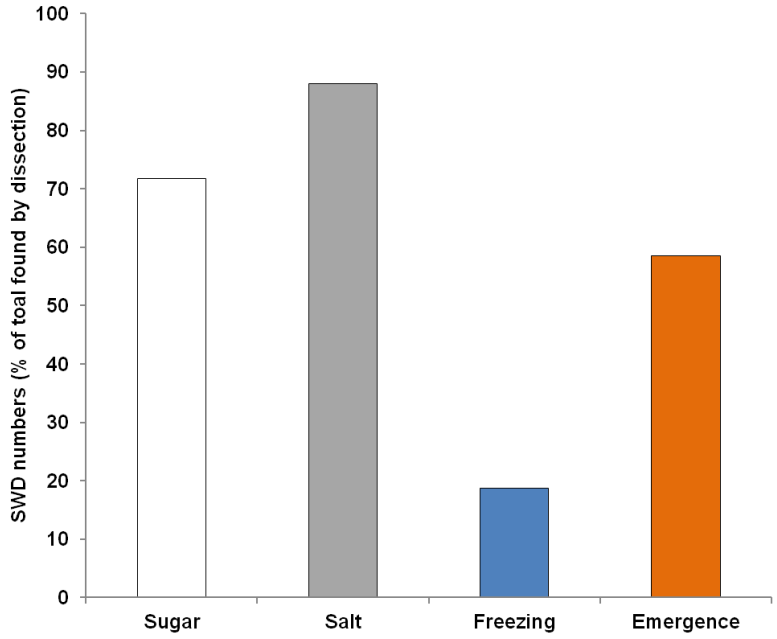

Figure 3.2. Counts of larval SWD obtained following various treatments of blueberries. Results given as percentage of larvae found by dissection of fruit. Summary of two experiments

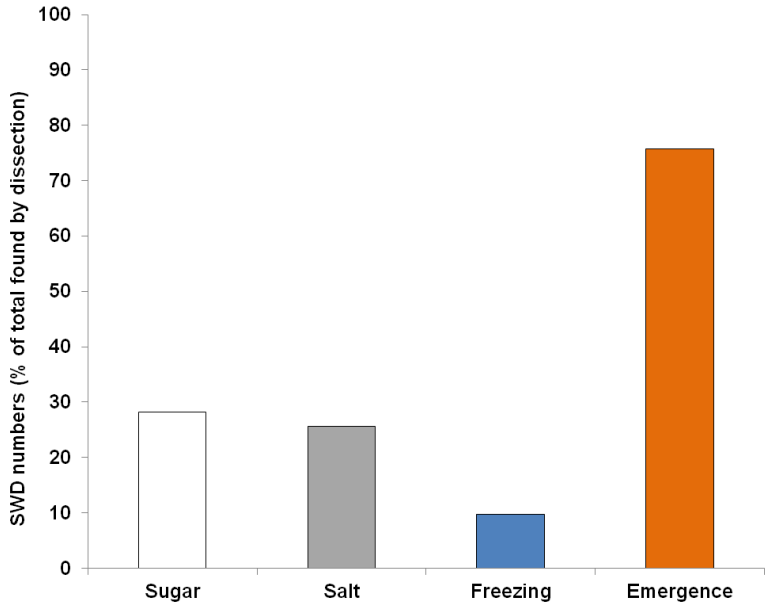

Figure 3.3. Counts of larval SWD obtained following various treatments of raspberries. Summary of two experiments

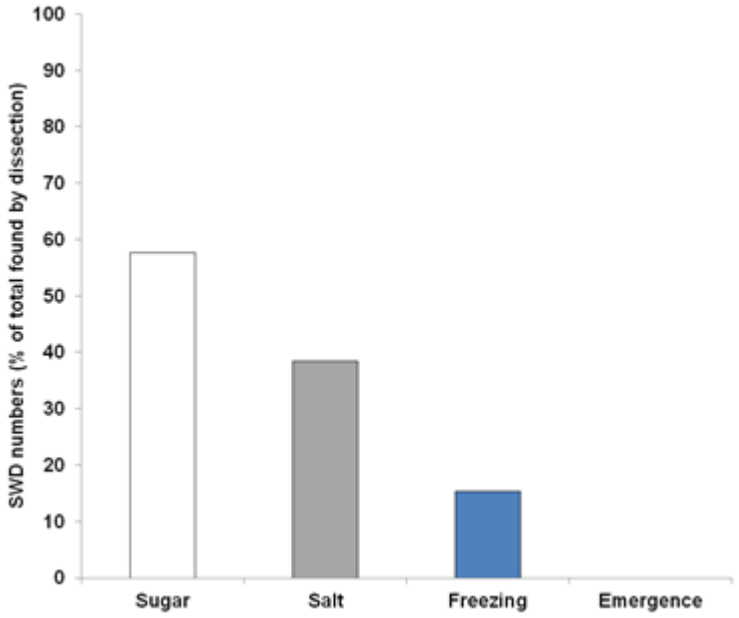

Figure 3.4. Counts of larval SWD obtained following various treatments of cherries. Single experiment

Conclusions

Sugar and salt immersion both appear to detect SWD larvae in blueberries, raspberries and cherries, with little difference between them. Freezing overnight generally gave a lower count of SWD, and together with the greater delay involved in determining a result, this method would appear to be less useful.

This is work in progress, with another trial on cherries required. Preliminary trials on strawberries (not presented here) suggested that a different method may be required for this fruit, involving cutting the fruit into smaller pieces. The single trial on carbonated water immersion suggested this method would be less useful than sugar or salt due to the reduced visibility of larvae in this solution.

It is interesting that in neither blueberries nor raspberries did adult emergence match the counts recorded by dissection, suggesting a level of mortality during pupation or adult emergence.

References

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

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

Introduction

A wide variety of traps and lures is available for monitoring for the presence of SWD. The aim of this work is to investigate whether mass trapping or lure-and-kill approaches can be used for controlling SWD as part of IPM programmes against the pest.

Most work to date has involved lures based on various natural fermentation products such as vinegar, apple cider and wine (e.g. Landolt *et al*., 2012a; 2012b). Recently a four-component blend of synthetic chemicals has been reported to be attractive to SWD (Cha *et al*., 2012, 2013a). This consists 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 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 mass trapping or lure-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.

Materials and methods

Dispensing systems

Ethanol and acetic acid were dispensed from an aqueous solution containing 7.2% ethanol and 1.6% acetic acid (v/v) with 1% boric acid to prevent microbial growth.

Vial dispensers were polypropylene vials (4 ml) with a hole (3 mm dia) in the lid. Methionol or a 1:1 mixture of acetoin and water (1 ml) was deposited on a cotton dental roll (40 mm x 8 mm) in the vial.

Sachet dispensers (50 mm x 50 mm x 120 µ thick) were made by heat sealing layflat LDPE tubing (Transatlantic Plastics, Southampton, UK). "Baggie" dispensers were also tested, made from resealable LDPE bags (57 mm x 76 mm x 50 µ thick). The test material (1 ml) was deposited on a cotton dental roll as above.

Also assessed were dispensers for acetoin and methionol from Trecé (sealed plastic capsules, approx. 15 mm x 7 mm) and International Pheromone Systems Ltd. (various combinations of wax, polyethylene vial and paper sachets).

Measurement of release rates

Release of ethanol and acetic acid from aqueous solution was measured by weighing and taking samples at intervals. The samples were diluted (50 µl in 5 ml of water), acetone added as internal standard (50 µl of 5% acetone) and analysed by GC on a Poraplot capillary column (10 m x 0.32 mm i.d.) with oven temperature programmed at 60°C for 2 min then at 10°C/min to 200°C.

Release rates from other dispensers were measured by weighing and/or by collection and analysis of volatiles. For the latter, dispensers were placed in a round-bottomed flask (1 l) and air drawn in through an activated charcoal dispenser (20 cm x 2 cm; 10-18 mesh) and out through a collection filter (4 mm i.d.) containing Porapak Q (50-80 mesh, 200 mg). Volatiles were eluted with dichloromethane (Pesticide Residue Grade, 1ml). Decyl acetate (5 µg) was added as internal standard and the solutions were analysed by GC using a capillary column (30

m x 0.32 mm i.d. x 0.25 µ film thickness) with helium carrier gas (2.4 ml/min) and oven temperature programmed at 50°C for 2 min then at 10°C/min to 250°C.

Release rate measurements were continued for at least 20 d and results are means of at least two replicates.

Traps

Traps were Droso traps (BioBest, Westerlo, Belgium) with 20 extra 4 mm holes drilled into the body of the trap to maximise catches of SWD (Figure 4.1). These had either a drowning solution or a folded sticky card to retain SWD entering (Figure 4.2).

Figure 4.1. Modified Droso-trap

Figure 4.2. Traps with either drowning solution (left) or yellow sticky card (right) as a trapping agent

Baits

Liquid baits tested in field tests were yeast/sugar, the Gasser wine/vinegar mixture, and the synthetic solution of ethanol and acetic acid (Table 4.1). They were changed weekly.

Table 4.1. Liquid bait recipes

Bait	Recipe (1 litre)
Yeast/sugar	4 Tbs yeast: 16 Tbs sugar: 1 L water
Gasser	1 l Gasser bait, 1 ml detergent, 0.05 g boric acid
Synthetic	912 ml H_2O: 24 ml ethanol: 5.3ml acetic acid: 3 g boric acid, 1 ml detergent

The synthetic liquid bait was combined with acetoin and methionol dispensed either from separate polypropylene vials (4 ml) with a hole (3 mm dia) in the lid as above, or from the Trece dispensers as above.

Field test 1

Two sites were selected; one in the South East of England (Site 1) and the second in the West Midlands (Site 2). Due to low adult numbers, Site 1 was removed from the trial after four weeks. Site 2 was regularly visited by agronomists from a leading marketing group. There were two replicates of the six treatments (Table 1, Treatments 1-6). There were three baits and two collection methods. The traps were set up in a randomised block design with 10 m between traps. One replicate was placed along the edge of woodland and the second in an adjacent raspberry crop. Traps were checked weekly from 24 September - 19 October 2013 and then checked every three weeks until 4 December 2013 when the trial ended.

Table 4.2. Treatments in field tests

Trt No.	Bait	Trapping method
1	Yeast/sugar	Card/Excluder Grid
2	Yeast/sugar	Drowning Solution/ No Grid
3	Gasser	Card/Excluder Grid
4	Gasser	Drowning Solution/ No Grid
5	Synthetic + acetoin, methionol vials	Card/Excluder Grid
6	Synthetic + acetoin, methionol vials	Drowning Solution/ No Grid
7	Synthetic + acetoin, methionol Trecé	Drowning Solution/ No Grid
8	Synthetic + acetoin, methionol Trecé	Drowning Solution/ No Grid

Field test 2

Two additional treatments using the Trecé dispensers (Table 2, Treatments 7 and 8) were added to the above experiment from 3 October 2013. For the last two assessment periods (29 October

– 4 December 2013) a third replicate of all eight treatments was installed, and results were analysed for these two collection periods only.

Results

Release of ethanol and acetic acid

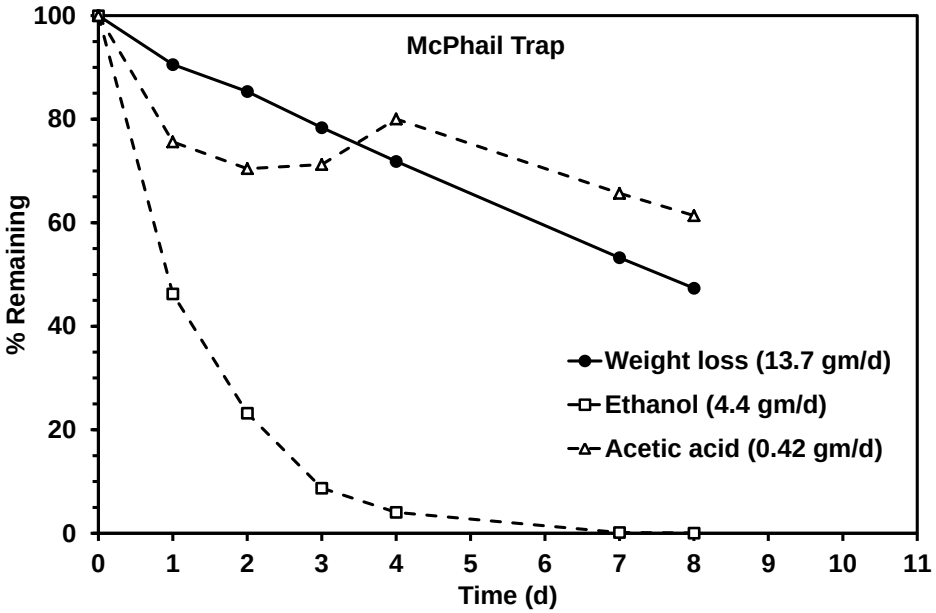

Figure 4.3. Release of ethanol and acetic acid from 300 ml aqueous solution of 7.2% ethanol and 1.6% acetic acid (v/v) from Agrisense Dome (McPhail) trap at 22°C in laboratory

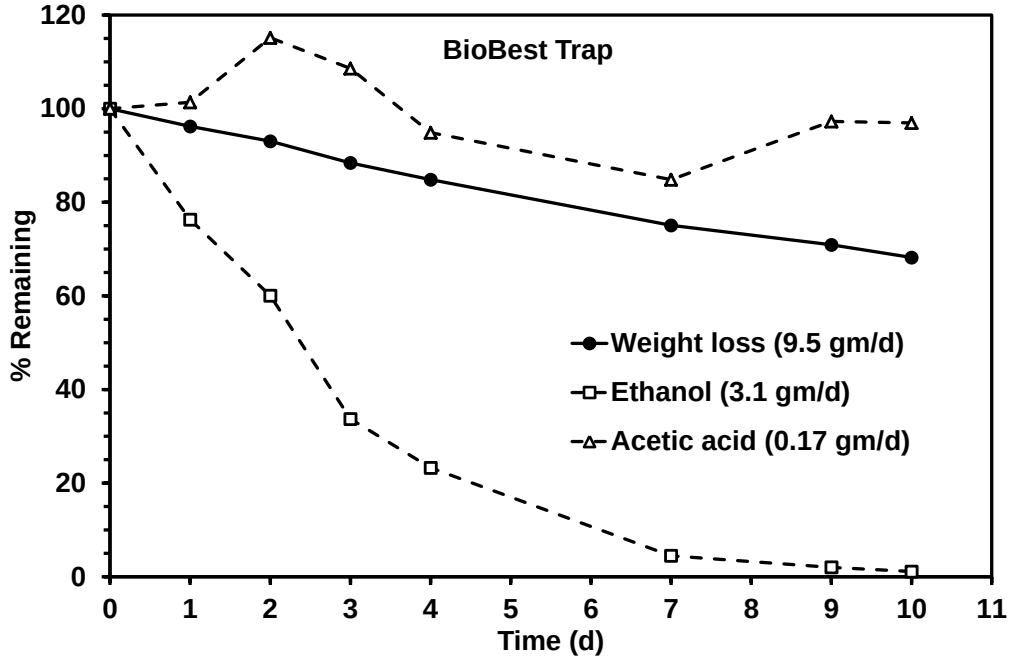

Figure 4.4. Release of ethanol and acetic acid from 300 ml aqueous solution of 7.2% ethanol and 1.6% acetic acid (v/v) from BioBest trap at 22°C in laboratory

Measurements of release of ethanol and acetic acid from the synthetic drowning solution were made in the laboratory at 22°C from both Agrisense Dome (McPhail) and BioBest traps. Results showed that essentially all the ethanol was released within 7 days in both traps but release of acetic acid was much slower (Figures 4.3 and 4.4).

Ethanol is released at 3-4 gm/d from the aqueous solution. Release of ethanol from vial and sachet dispensers was much slower (Table 3). Release rates of 170 mg/d or 100 mg/d could be achieved from a 4 ml vial or double-size Baggy sachet, where 3 ml would last approx. 20 d or 30 d respectively.

Acetic acid is released at 170-420 mg/d from the aqueous solution. It should be possible to design vial or sachet dispensers releasing at approx. 100 mg/d such that 3 ml would last approx. 30 d.

Table 4.3. Release rates of ethanol and acetic acid from aqueous solution (300 ml aqueous solution of 7.2% ethanol and 1.6% acetic acid (v/v)) and other dispensers at 22°C in laboratory

	Weight Loss (mg/d)			Analysis (mg/d)	
	3mm Vial[1]	**Sachet[2]**	**Baggy3**	**Dome**	**BioBest**
Ethanol	170.2	7.0	47.3	4,400	3,100
Acetic acid	53.2	36.5		420	170

[1] 4 ml polypropylene vial with 3 mm diameter hole
[2] 50 mm x 50 mm x 120 µ thick LDPE sachet, 1 ml
[3] 57 mm x 76 mm x 50 µ thick LDPE resealable sachet, 1 ml

Release of acetoin and methionol

Release of acetoin from the 4 ml vials with 3 mm hole, as used by Cha *et al*. (2012, 2013a, 2013b) lasted for approx. 40 d under laboratory conditions at 22°C (Figure 4.5). This was confirmed when vials exposed in the field for one month were subsequently maintained in the laboratory and they ran out after another approximately 2 weeks (Figure 4.6).

Release of methionol was much slower and the vials were still releasing after 157 d under laboratory conditions (Figures 4.5 and 4.6).

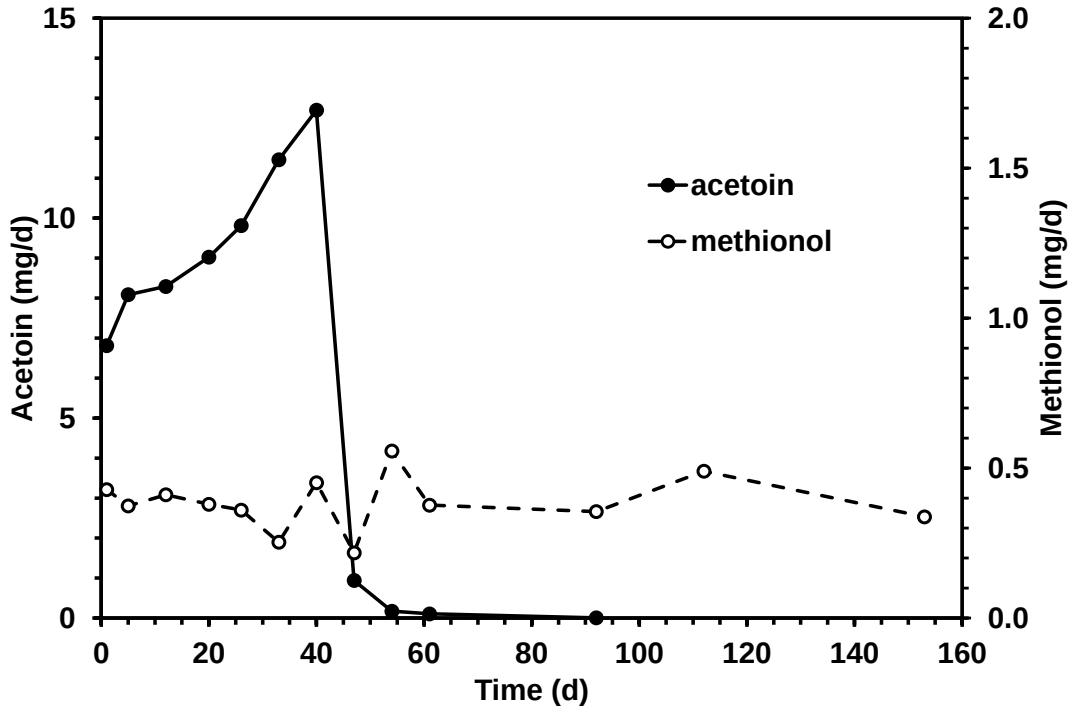

Figure 4.5. Release of acetoin and methionol from 4 ml polypropylene vials with 3 mm dia hole in laboratory at 22°C as measured by collection and analysis of volatiles

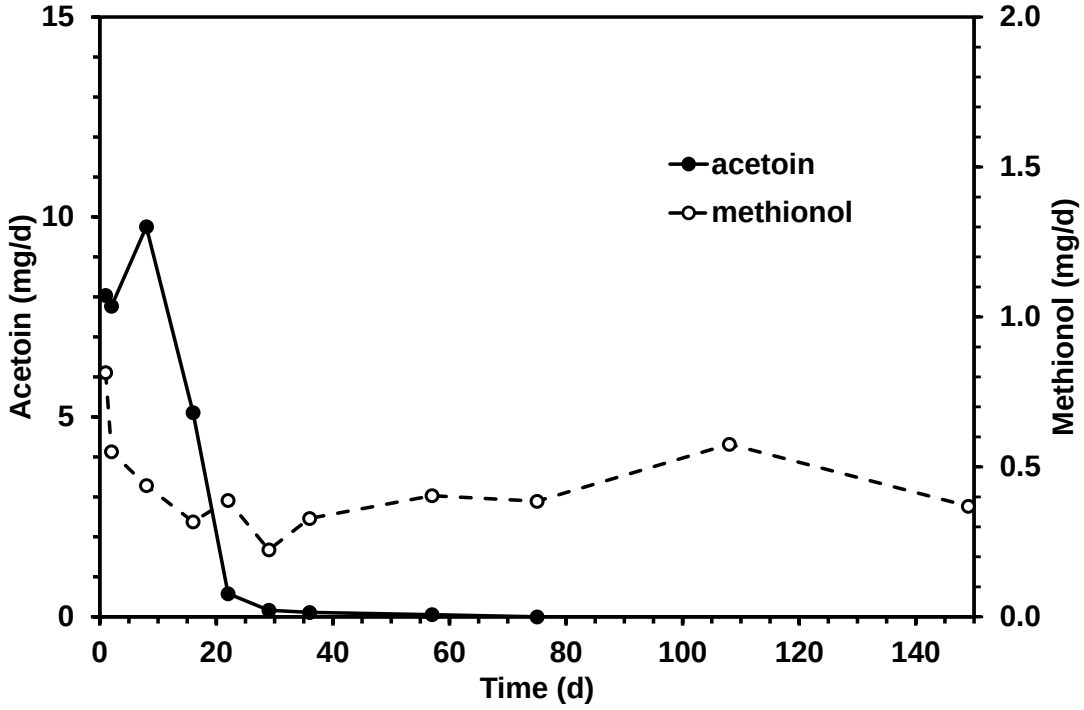

Figure 4.6. Release of acetoin and methionol from 4 ml polypropylene vials with 3 mm dia hole returned from field after 4 wk and then maintained in laboratory at 22°C, as measured by collection and analysis of volatiles

Table 4.4. Release rates of methionol and acetoin from vials and sachets as measured by weight loss and collection and analysis of volatiles

	Weight Loss (mg/d)		Entrainment (mg/d)	
	3mm Vial[1]	Sachet[2]	3mm Vial[1]	Sachet[2]
Methionol		1.4	0.37	2.58
Acetoin/water 1:1	34.7	3.1	8.09	2.15
Acetoin	17.1	5.0	3.20	
Water	52.9	3.6		

[1] 4 ml polypropylene vial with 3 mm diameter hole
[2] 50 mm x 50 mm x 120 µ thick LDPE sachet, 1 ml

Release of acetoin from sachet dispensers was much slower than from the vials and that of methionol was much faster (Table 4.4). It should be possible to make a sachet releasing acetoin at approx. 10 mg/d for which 1 gm would last 100 d. A sachet of methionol containing 1 ml would last approx. 400 d.

Release of acetoin from the Trecé dispensers was 1/5 that of the rate from the vial, but continued for at least 100 d under laboratory conditions (Figure 4.7, Table 4.5). The release rate of methionol from the Trecé dispensers was similar to that from the vial and the dispenser similarly continued releasing for at least 100 d (Figure 4.7, Table 4.5).

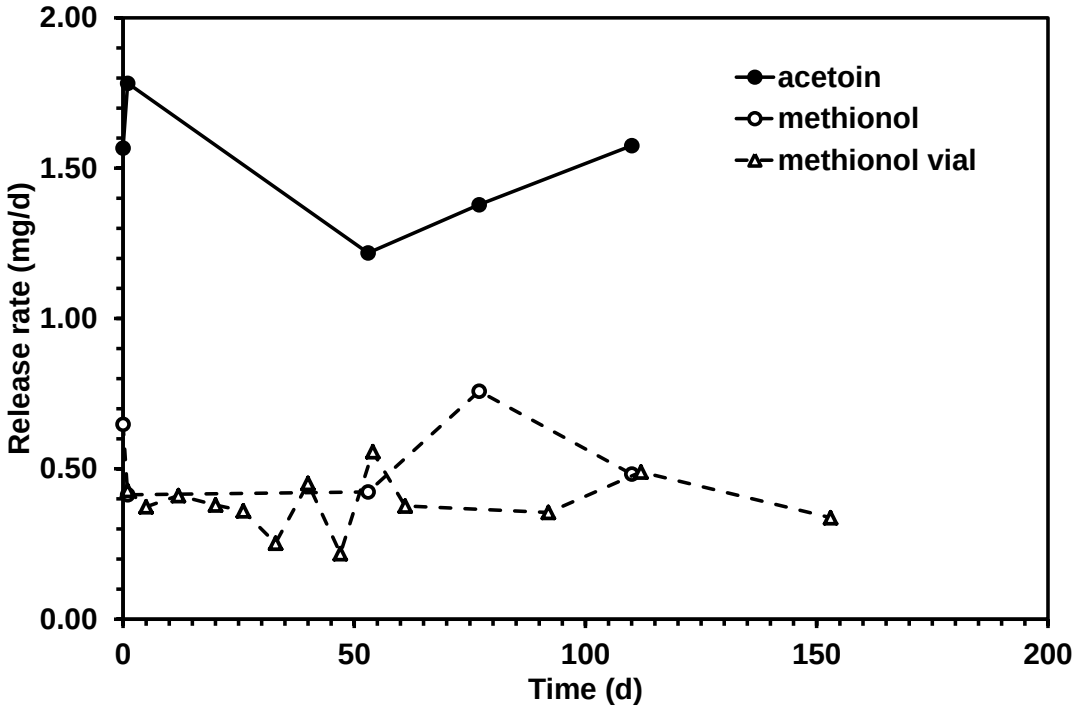

Figure 4.7. Release of acetoin and methionol from Trece dispensers compared with methionol from 4 ml polypropylene vials with 3 mm dia hole maintained in laboratory at 22°C, as measured by collection and analysis of volatiles

Table 4.3. Release rates of methionol and acetoin from Trece dispensers in laboratory at 22°C

	Weight Loss (mg/d)	Entrainment (mg/d)
Methionol	1.6	0.55
Acetoin	4.9	1.50

Various release rates were shown by dispensers provided by IPS and most of these were long-lived (Table 4.4).

Table 4.4. Release rates of methionol and acetoin from dispensers provided by IPS/Chun in laboratory at 22°C as measured by weight loss

	Weight Loss (mg/d)	
	Acetoin	**Methionol**
Wax Squat	19.3	25.2
Wax Tube	1.4	0.7
Wax Squat in Paper Sachet	6.1	2.0
Bottle	4.6	

Field test 1

Due to the small replication (*N* = 2), data transformation was not necessary for the statistical analysis (Table 4.5). There was a significant difference between the method of trapping adult male and female SWD (sticky card and drowning solution), but there is no significant difference between the two methods for the total *Drosophila* numbers. Numbers of SWD trapped in the drowning solution were higher than those caught on the sticky card. Although the raw data showed that Gasser bait had higher catches of SWD, this was not statistically significant, probably due to the low number of replicates (Table 4.5; Figure 4.8) and one very high reading (Figure 4.10) on 12 November 2013.

The selectivity towards SWD relative to other *Drosophila* species was similar for all baits and collection methods (Table 4.6).

Field test 2

For this trial trapping method was not a factor. There were three replicates, but only two data collections were made before the end of this trial and values were missing, resulting in using predicted means for analysis (Table 4.7). Significant differences were found between the baits for both male and female (Figure 4.9) SWD numbers with the Gasser bait catching more than the

others, although this was only on one occasion (Figures 9, 10). There was no significant difference between total drosophila numbers captured with the different baits.

In this experiment, the selectivity towards SWD relative to other *Drosophila* species was apparently much higher for the Gasser bait with drowning solution (Table 4.8), but this was not seen in Field Test 1 and is probably due to the low number of replications.

Table 4.5. Mean catches of *Drosophila suzukii* in Field Test 1 (24 September – 4 December 2-13; *N* = 2)

Bait	**Collection Method**	**Males**	**Females**	**Total drosophila**
Yeast	Card	19.5	8.0	332
Yeast	Drown	36.0	20.0	304
Gasser	Card	9.0	3.0	208
Gasser	Drown	67.5	42.5	498
Synthetic	Card	14.5	5.5	216
Synthetic	Drown	16.5	11.5	292
Bait	F pr.	NSD	NSD	NSD
	s.e.d.	9.12	7.05	83.6
	d.f.	5	5	5
	l.s.d.	23.44	18.13	215.0
Collection method	F pr.	0.022	0.021	NSD
	s.e.d.	7.44	5.76	68.3
	d.f.	5	5	5
	l.s.d.	19.14	14.80	175.6
Bait + collection	F pr.	NSD	NSD	NSD
	s.e.d.	12.90	9.97	118.3
	d.f.	5	5	5
	l.s.d.	33.15	25.64	304.1

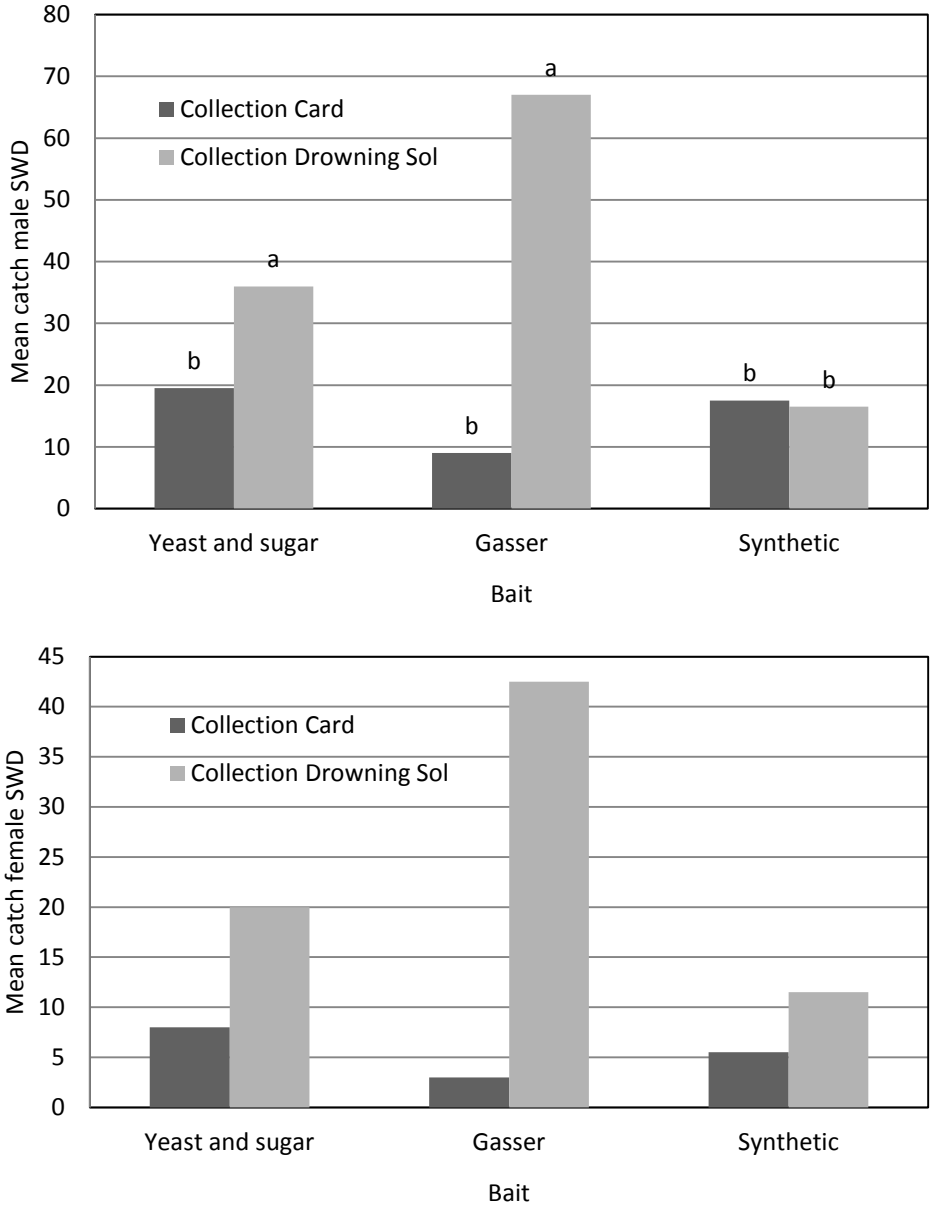

Figure 4.8. Mean catches of male (upper) and female (lower) SWD in Field Test 1 (24 September – 4 December 2013; *N* = 2; means for the same bait with different letters are significantly different $P < 0.05$)

Table 4.6. Percentage SWD in total drosophila catch in Field Test 1 (*N* = 2)

Bait	Collection	Total drosophila	SWD	% SWD	
Yeast/sugar	Card	856	55	6%	10%
Yeast/sugar	Solution	897	112	12%	
Gasser	Card	806	24	3%	12%
Gasser	Solution	1199	219	18%	
Synthetic	Card	545	46	8%	8%
Synthetic	Solution	730	56	8%	

Table 4.7. Mean numbers of *Drosophila suzukii* over two collection dates in Field Test 2

Bait	Males		Females		Total drosophila	
	12/11/13	04/12/13	12/11/13	04/12/13	12/11/13	04/12/13
Yeast and sugar	3.0	4.7	2.7	5.3	22.3	77.7
Gasser	13.7	4.0	15.3	7.0	36.0	38.7
Synthetic	2.3	1.3	2.0	2.3	19.7	55.7
Trecé	1.8	1.8	0.8	0.6	3.6	17.6

			Male	Female	Total drosophila
	Bait	F pr.	<0.001	0.007	NSD
	Date	F pr.	0.040	NSD	NSD
	Bait + Date	F pr.	0.002	NSD	NSD
		s.e.d min	1.462	1.462	14.52
		s.e.d max	1.887	1.887	15.53
		d.f.	18	18	18
		l.s.d.	3.758	8.268	52.801

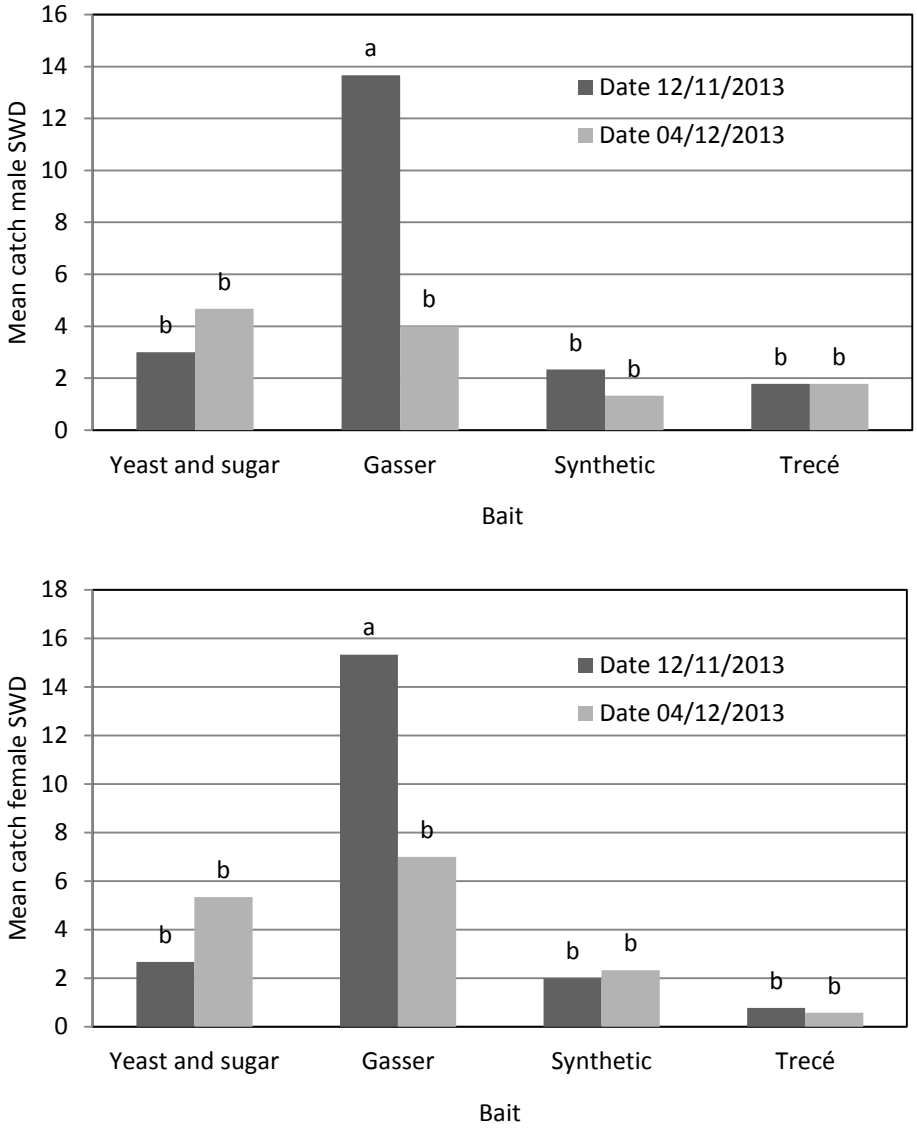

Figure 4.9. Mean catches of male (upper) and female (lower) SWD in Field Test 2 (29 October – 4 December 2013; *N* = 3; means with different letters are significantly different $P < 0.05$)

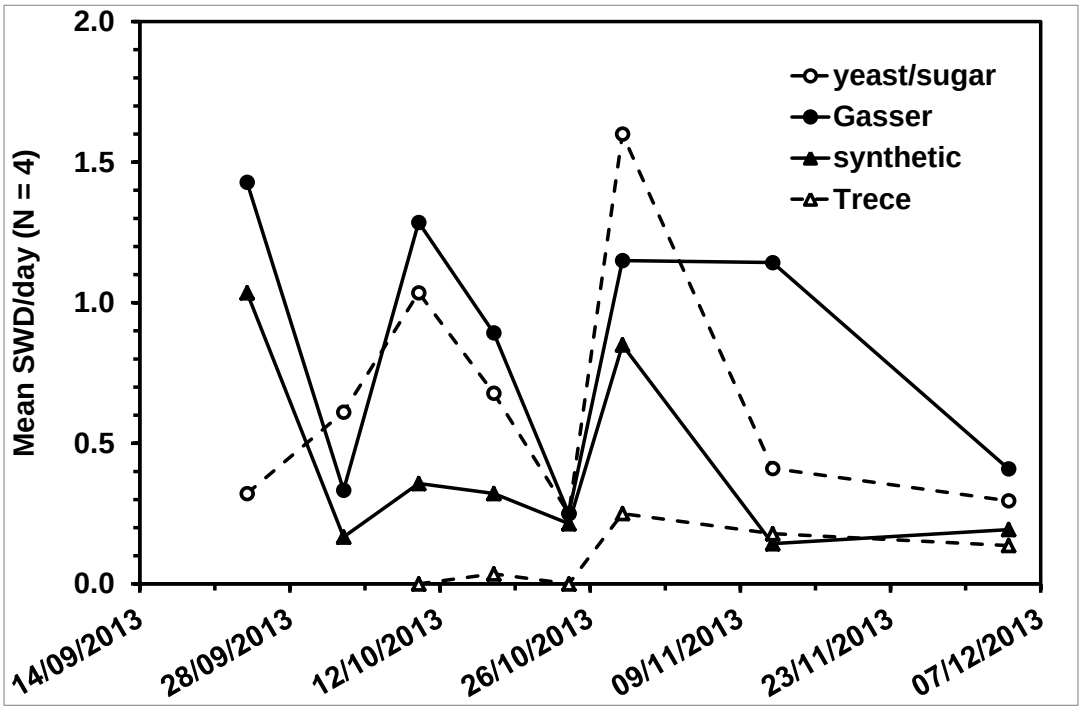

Figure 4.10. Mean catches per day of SWD in the two field tests (*N* = 4 including both types of collection method)

Table 4.8. Percentage SWD in total drosophila catch in Field Test 2 (*N* = 2)

Bait	Collection	Total drosophila	SWD	%SWD	
Yeast/sugar	Card	195	24	12%	14%
Yeast/sugar	Solution	300	47	16%	
Gasser	Card	115	13	11%	39%
Gasser	Solution	224	120	54%	
Synthetic	Card	154	25	16%	13%
Synthetic	Solution	226	24	11%	
Trece_1	Solution	74	15	20%	19%
Trece_2	Solution	68	12	18%	

Discussion

Dispensing systems

The Cha synthetic lure for SWD (Cha *et al*., 2013a; 2013b) consists of ethanol, acetic acid, acetoin and methionol.

The ethanol and acetic acid are dispensed from the aqueous “drowning” solution used as capture medium in standard traps. The studies above showed that the ethanol in this is exhausted within 7 d of exposure in standard SWD traps at 22°C, although there is still 60-80% of the acetic acid remaining after this time. Thus the drowning solution must be replaced at least

every 7 d, and this possibly applies to other baits such as apple cider and wine which are essentially aqueous solutions of ethanol.

The rate of release of ethanol from the aqueous solution was measured at 3-4 gm/d. To obtain this release rate from a specific dispenser would require one containing at least 30 ml to last even the 7 d, and this would be prohibitively large to fit into a trap in addition to the drowning solution. It is not known whether this high release rate is essential. Release of ethanol from vial and sachet dispensers was much slower. Release rates of 170 mg/d or 100 mg/d could be achieved from a 4 ml vial or double-size Baggy sachet, where 3 ml would last approx. 20 d or 30 d respectively.

Acetic acid is released at 170-420 mg/d from the aqueous solution. It should be possible to design vial or sachet dispensers releasing at approx. 100 mg/d such that 3 ml would last approx. 30 d.

In the Cha lure the acetoin and methionol are dispensed from cotton pads in 4 ml vials with a 3 mm diameter hole in the lid. The release rate of acetoin is 34 mg/d by weight loss. The release rate of methionol cannot be measured by weight loss as it is hygroscopic, absorbing water from the atmosphere, and the release rate measured by entrainment was 0.4 mg/d.

The acetoin dispenser containing 1 ml of a 1:1 aqueous solution of acetoin in water (i.e. 500 mg acetoin) lasted for approx. 40 d at 22°C, and thus dispensers should be replaced or refreshed after 1 month in the field. Using solid acetoin in the vial halved the release rate, but this would presumably double the lifetime. This could be increased further, at least x 5, as more solid acetoin could be loaded into the vial.

Release of acetoin from sachet dispensers was much slower than from the vials and that of methionol was much faster. It should be possible to make a sachet releasing acetoin at approx. 10 mg/d for which 1 gm would last 100 d. A sachet of methionol containing 1 ml would last approx. 400 d.

Release of acetoin from the Trecé dispensers was 1/5 that of the rate from the vial, but continued for at least 100 d under laboratory conditions. The release rate of methionol from the Trecé dispensers was similar to that from the vial and the dispenser similarly continued releasing for at least 100 d.

Trapping results

In almost all instances more SWD were caught in the traps with the drowning solution than in traps with the sticky card as trapping device. This result is in line with previous observations that SWD can escape from the adhesive (Figure 4.10).

Figure 4.10. Sticky card showing where SWD landed and escaped (dotted circles)

There were few statistically significant differences between catches with the different baits, probably because only two replicates were run. Overall catches were higher in traps with the Gasser > yeast/sugar > synthetic > Trecé baits, but this was largely due to a particularly high catch with the Gasser bait on one occasion (Figure 10). Catches were generally lower with the synthetic baits than with the Gasser wine/vinegar mix or the yeast/sugar. This is in contrast to the results of Cha *et al*. (2013b) who found that dome and cup traps baited with the synthetic bait caught at least as many SWD as traps baited with apple cider vinegar in the US and a wine/vinegar mixture in Germany.

Trap catches with the Trecé dispensers for acetoin and methionol tended to be lower than those with the synthetic bait where these two compounds were dispensed from vials. Results on release rates above indicated that the only difference between these two baits would have been the lower release rate of acetoin from the Trecé dispensers, indicating that this compound is indeed important for attraction of SWD.

Advantages and disadvantages of baits

As collection devices the sticky cards were much simpler to transport and could be posted. The drowning solution was filtered through a paint filter which could be folded and posted in a sealable plastic wallet and then disposed of after inspection.

The yeast and sugar bait, whilst very attractive to SWD, made the traps sticky and disposal of the solution posed problems when in the field. The drowning solution and decaying insects were

attractive to other insects, such as earwigs, bees and large Diptera, making searching and identification of SWD difficult. The liquid is also cloudy and thick and it was difficult to identify the catch contents without washing the sample several times. The fermentation of the yeast and sugar caused foaming and expansion so the liquid could not be stored in airtight bottles.

The Gasser bait was a blend of red wine and vinegar and appeared to increase specificity to SWD. The Gasser liquid required no preparation before use in the field. The liquid was transparent with a watery consistency, making identification of SWD in the trap easy. However, in warmer weather the liquid congealed slightly which impeded identification. Adding 0.05g of boric acid prevented this. According to an agronomist Gasser is also attractive to wasps. However, this only seemed to be a problem for one of the agronomists for around 6 weeks. This attraction was also observed with the yeast sugar solution.

The synthetic bait vials had a long life expectancy, with the acetoin in the white vial maintaining the correct release rate for over a month and the methionol in the black vial lasting at least 100 days at 22°C. The drowning solution was changed weekly. The synthetic bait did not appear to attract wasps. Furthermore it appeared to positively select for *Drosophila* species, greatly reducing the amount of other insects attracted to and caught in the trap. The undiluted chemicals needed to be mixed in a fume cupboard with personal protective equipment and the process of producing the liquid bait was time consuming. The boric acid is soluble in water but can crystallise at the bottom of the containers before dilution. The boric acid also crystallised in colder weather, making the drowning solution difficult to filter. The vials were re-usable, topping the acetoin up every month and the methionol every three months. This required a full face guard, spray suit and nitrile gloves.

The two sachets produced by Trecé were deployed in the traps and required no preparation. The Trecé lures are clean to use in the field and simple to dispose of.

Conclusions

These studies were carried out to develop an optimal trapping system in terms of attractiveness to SWD and ease of operation on a large scale, for use in mass trapping of SWD. Progress was also made in developing synthetic lures for use in a lure-and-kill approach. The key conclusions were as follows:

- The synthetic mixture of ethanol and acetic acid must be replaced every week, and this probably applies to drowning solutions based on fermentation products.
- The current dispenser for acetoin must be replaced every month, although longer-lived dispensers are available.

- The methionol dispenser lasts for at least 120 d at 22°C.
- In trapping tests, more SWD were caught in traps with the drowning solution as the collection medium compared with the sticky card.
- There were no significant differences in catches with the different baits, although the trend was for the Gasser > yeast/sugar > synthetic > Trecé baits.
- There were no significant differences in the selectivity of the different baits for SWD relative to other *Drosophila* species.

References

Cha, D.H., Adams, T., Rogg, H., and Landolt, P.J. (2012) Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing Drosophila, Drosophila suzuki. *Journal of Chemical Ecology*, 38:1419-31.

Cha, D.H., Adams, T., Werle, C.T., Adamczyk, J.J. , Rogg, H. and Landolt, P.J. (2013a). A four-component blend of fermented bait volatiles is attractive to spotted wing Drosophila, *Drosophila suzuki* (Diptera: Drosophilidae). *Pest Management Science*

Cha, D.H., Hesler, S.P., Cowles, R.S., Vogt, H., Loeb, G.M. and Landolt, P.J. (2013b). Comparison of a synthetic chemical lure and standard fermented baits for trapping *Drosophila suzuki* (Diptera: Drosohilidae). *Environmental Entomology*, 42:1052-1060.

Landolt, P.J., Adams, T., and Rogg, H. (2012a) Trapping spotted wing Drosophila, *Drosophila suzukii* (Matsumara) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. *Journal of Applied Entomology*, 136:148-154.

Landolt, P.J., Adams, T., Davis, T., and Rogg, H. (2012b) Spotted wing Drosophila, *Drosophila suzukii* (Matsumara) (Diptera: Drosophilidae), trapped with combinations of wines and vinegars. *Florida Entomologist*, 95: 326-332.

Objective 5. (Yrs 1-4) To obtain evidence for the effectiveness of different plant protection products including biopesticides and for developing an insecticide resistance management strategy for SWD. (Yrs 1-4)

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

Introduction

It is important to establish chemical control levels for SWD. Whilst bioassays are important in this regard, they are only part of the picture. In a farm situation, SWD will encounter plants treated with insecticide, which could either give direct mortality, act as deterrent (especially in the case of pyrethroids) or impact on larval development. Each of these factors could be important commercially. The ideal would be to run field trials but there were insufficient SWD in the summer of 2013 in the UK for this to be viable. Consequently, the selected crop (strawberries) was sprayed with a field dose, and then harvested fruit was infested with flies in the laboratory after an interval of time. Harvest interval was up to two weeks post insecticide application, to determine any effect of residue decay.

Materials and methods

Plants and site

Strawberry plants (cv. Finesse, everbearer) were grown in a 5 bed row polytunnel at East Malling Research. The rows were 1 m wide, 1.1m apart, with two plants per row and five rows per tunnel (Figure 5.1). Plants were provided with tape fertigation and an overall spray programme of mildew fungicides was applied to prevent mildew establishing.

Figure 5.1.1. Experimental site

Experimental design

A randomised block system with five replicates of nine treatments was utilised, the plots in each block arranged end to end in a row, each plot 2.2 m long.

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, assuming a plant density of 40,000 plants/ha, i.e. each plant received 25 ml of sprayate or until runoff for maximum coverage (Table 5.1.1).

Table 5.1.1. Insecticide treatments

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	Decis	0.2 ml / l	200 ml
7	Pyrethroids	Spruzit	4.59 g/l EC	20 l
8	Untreated			
9	Untreated			

† SOLA 1291, ‡ SOLA 1705

Assessments

Five fruit were removed from each plot on each sample day (0, 1, 2, 3, 4, 8 and 14 post application), leaving 2 plants at each end of each plot as spray guards.

Assessments were performed in ventilated Perspex plastic boxes (228*121*66 mm) covered with fine mesh (300mm * 400 mm) held tightly in place with electrical tape (Figure 5.1.2). Fruit was placed on paper towel to absorb leakage. Flies (four female, two male) were introduced by pooter, with the collection bottle removed and outlet blocked. Boxes were kept for at least 3 weeks at 20 ^{o}C in the EMR quarantine facility and assessed weekly for adult emergence.

Following consultation with a statistician, results were analysed in Genstat by General Analysis of Variance with the two control groups combined (if testing for significant difference between the two groups shows no difference).

Figure 5.1.2. Fruit incubation box

Results

Fruit was harvested immediately after spray application and at 1, 2, 3, 4, 8 and 14 days post application. The results are presented in Fig. 5.1.3, 5.1.4 and 5.1.5.

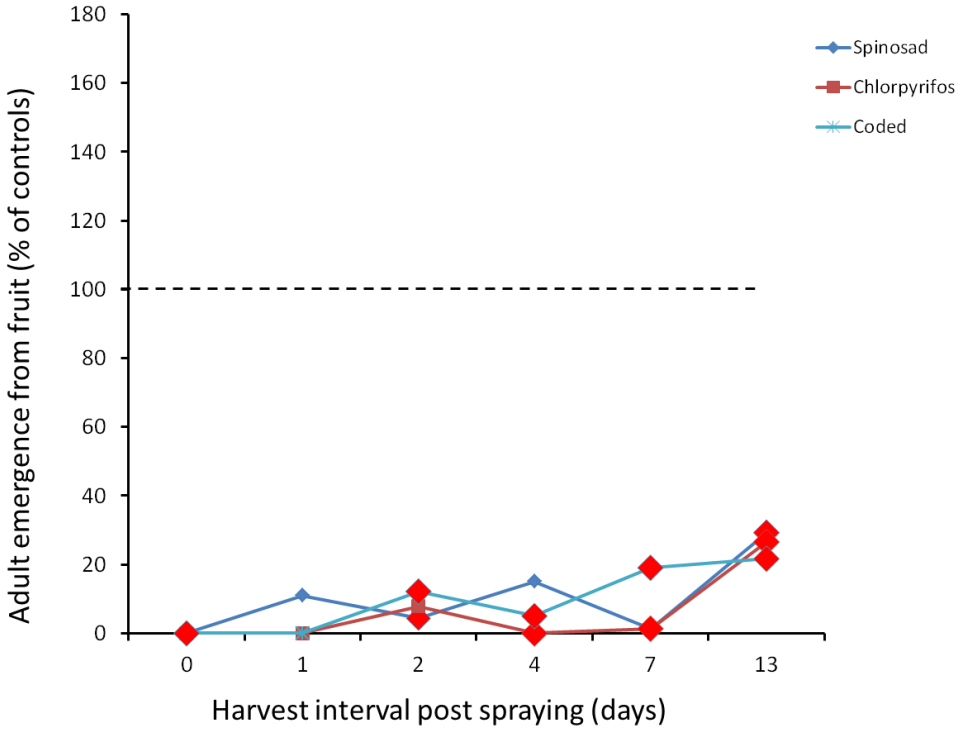

Figure 5.1.3. Adult emergence from fruit exposed to flies various days after spraying, spinosad, chlorpyrifos and coded product. Red marks indicate significant difference to control (P<0.05)

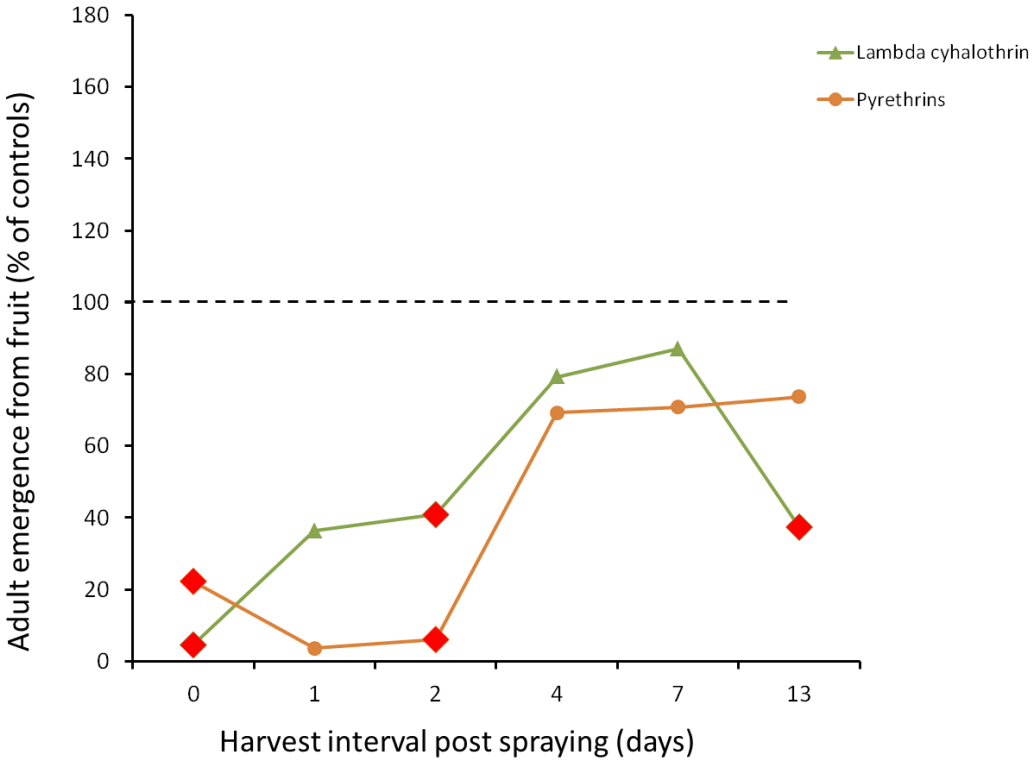

Figure 5.1.4. Adult emergence from fruit exposed to flies various days after spraying, lambda cyhalothrin and pyrethrins. Red marks indicate significant difference to control (P<0.05)

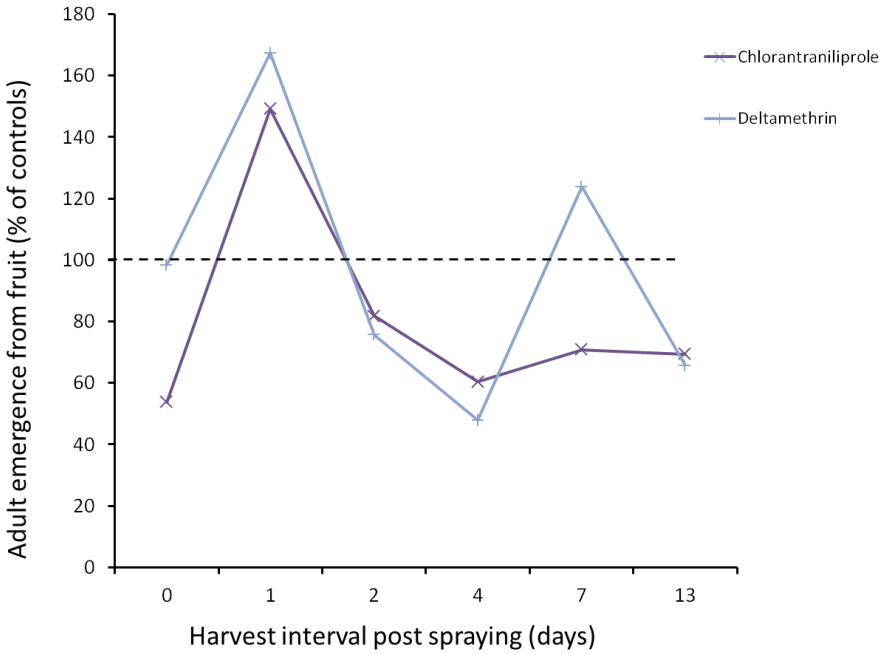

Figure 5.1.5. Adult emergence from fruit exposed to flies various days after spraying, chlorantraniprole and deltamethrin

The effectiveness of SWD chemical control varied with insecticide and time post spraying. The most effective at time 0 were spinosad, chlorpyrifos, the coded product, the pyrethrin mixture and lambda cyhalothrin. Over time the effectiveness of the pyrethrin mixture and lambda cyhalothrin gradually deteriorated.

Conclusions

Insufficient SWD were present in the UK to undertake field trials. However, strawberry fruit with field doses of insecticide residues were assessed using a laboratory culture to determine effectiveness of seven insecticides: spinosad (Tracer), chlorpyrifos (Equity), lambda-cyhalothrin (Hallmark) chlorantraniliprole (Coragen), deltamethrin (Decis) pyrethroid (Spruzit) and a coded product. These were compared to an untreated control. Harvest interval was up to two weeks post insecticide application, to determine any effect of residue decay.

Spinosad, the coded product and chlorpyrifos gave control of SWD for up to 2 weeks after spraying (no adult SWD emerged from fruits exposed to SWD post spraying). Lamda-cyhalothrin and pyrethrin gave very short and variable control of SWD – up to 2 days. None of the other products were effective at reducing SWD in this strawberry trial. This trial will be repeated in 2014 on raspberry fruits. SWD develops insecticide resistance easily and so good crop hygiene and other non-chemical controls should be combined with rotations of modes of action of insecticides to prevent insecticide resistance.

Task 5.2. Monitor the susceptibility (LC_{50} values) of SWD populations in the UK to the three insecticide groups used to control SWD (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)

Insufficient UK SWD populations have been available to perform bioassays. However, optimisation of technique has already been performed on laboratory cultures of SWD. Males and females will be assessed separately as they have different insecticide tolerances (P. Shearer, personal communication). In addition, care will be taken to apply insecticides at a similar time of day, as circadian variation has been shown to have a marked effect on SWD insecticide tolerance (Hamby *et al*., 2013 and J. Chiu, personal communication)

Proposed protocol

Bioassay apparatus

The bioassay apparatus is a plastic Petri dish (9cm diameter) with a filter paper disk placed in the bottom and a gridded lid. Eight flies are added by pooter and the Petri dish halves joined and sealed with parafilm and the dish is stored in a refrigerator for 3 hours.

Insecticide application

Insecticide to be applied using a Burkhard sprayer. Insecticide diluted so that 0.4ml is applied per dish to give the field rate, assuming a uniform flat surface. For example, pyrethrins (Spruzit) 10 ml/l, maltodextrin (Majestik) 25 ml/l, spinosad (Tracer) 25 ml/l.

After spraying a small piece of cotton wool soaked in 30% sucrose is placed on the lid of each dish for nutrition and then each dish is placed in an individual “bakers” bag on a tray and incubated at 20 ^{o}C.

Mortality to be assessed after 24 hours.

Doses will be adjusted to give a range of five mortality values, to calculate LD_{50}. This will require an initial “ranging” series of doses to determine the approximate resistance status of the population, which would be expected to vary considerably depending on part insecticide treatment.

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.

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 SWD and their changing spatial distributions determined for 2014	
7	31/03/2016	36	Phenology and population dynamics of each life stage of SWD and their changing spatial distributions determined for 2015	
8	31/03/2017	48	Phenology and population dynamics of each life stage of SWD and their changing spatial distributions determined for 2016	
9	31/03/2015	24	Common wild host plants of SWD adults and larvae in the UK identified	
10	31/03/2016	48	SWD overwintering sites investigated and whether SWD 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 SWD 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 SWD and indicator *Drosophila* species tested	
16	31/03/2017	48	Collection and disposal optimised for different types and scales of fruit waste; sanitization and loss of attractiveness confirmed	
17	31/03/2017	48	Economics of treatment options for different types of fruit waste and scales of production quantified	
18	31/03/2017	48	Standard Operating Procedure and final report submitted	

Target date (31/03/2014)		No. of months from start date	Description of milestone	Progress
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 SWD larvae in field crops and harvested fruit determined and protocols produced	
21	31/03/15	24	Synthetic lure for SWD developed	
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	
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 SWD populations to 3 insecticides in 3 successive years completed	

*A method has been established, however, there were insufficient SWD in culture for bioassays. Cultures of laboratory and UK strains of SWD currently being expanded and it expected that the tests will be done in early 2014