

Project title: Increasing hoverfly populations in apple orchards for control of apple aphids

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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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GROWER SUMMARY

Headline

- Dispensers of the plant volatiles methyl salicylate and phenyl ethanol, with or without (*E*)- β -farnesene or a mix of farnesene isomers, increased the number of hoverflies caught in baited traps indicating adult attraction.

Background and expected deliverables

Apple aphids are ongoing pest problems and biological control can help to reduce the severity of attack or eliminate the pest altogether. Hoverfly larvae are voracious predators of aphids and if adults can be attracted into the orchard early in the season, and/or encouraged to overwinter in or close to orchards (hoverflies overwinter either as adults or pupae depending on the species), this increase in predators would be an important component of an IPM strategy. Biocontrol is particularly effective where ants are discouraged from protecting the aphids. Hoverfly adults respond to plant produced volatiles and to components specific to aphid feeding.

This project aimed to determine whether volatiles can be used to attract hoverflies into orchards and whether they then act as effective predators of aphids, reducing aphid populations in the orchard.

Summary of the project and main conclusions

Replicated experiments were carried out over three years to determine whether volatile compounds, either applied individually or as blends of volatiles, were attractive to hoverfly adults in apple orchards. The volatiles were soaked onto dental roll held inside a polyethylene sachet. These were hung inside delta traps, with sticky bases, which were placed in apple orchards. The species and numbers of hoverflies in the trap catches were recorded.

In 2016, twenty nine different species of hoverflies were found in the orchards, with 1,700 individuals identified. Species found in the spring included *Episyrphus balteus*, *Eupoedes spp.* and *Platycheirus spp.*. Some species, such as certain *Platycheirus spp.* are thought to be able to overcome ant defence which is important when considering effective aphid control. There were clear indications that synthetic volatiles acted as attractants for hoverflies found naturally in apple orchards.

These results showed that a blend of volatiles was consistently attractive to hoverflies, significantly increasing the number of hoverflies in trap catches. In particular a dispenser with the two volatile chemicals methyl salicylate and phenyl ethanol, or a dispenser with the three volatiles methyl salicylate, phenyl ethanol and (*E*)- β -farnesene are promising. Sachets

containing methyl salicylate are already marketed in the US as PredaLure™ to attract beneficial insects. In addition to the hoverfly attraction, a combined dispenser may also attract other orchard beneficials such as lacewings, as shown in our 2015 results. The use of beneficial species is compatible with IPM and organic control programmes in orchards. Whilst there are no recommendations for growers as yet, the results from the work will be taken forward in another AHDB project, TF 223, where the dispensers will be assessed in large plots in newly planted orchards.

Financial benefits

Apple trees are subject to a number of aphid pests including the rosy apple aphid (*Dysaphis plantaginea*), the rosy leaf curling aphid (*Dysaphis devectora*), the green apple aphid, (*Aphis pomi*), the woolly apple aphid (*Erisoma lanigerum*) and the apple grass aphid (*Rhopalosiphum insertum*). When conditions are favourable pest numbers can increase rapidly. The rosy apple aphid is the most damaging of these and high numbers result in curled leaves and misshapen fruits, which can lead to economic losses. The Assured Produce threshold for RAA suggests that crop protection product application is justified if one aphid is found in the orchard pre-blossom. Some organic orchards suffer 100% crop loss from rosy apple aphid.

In this project hoverflies were attracted to traps baited with volatile lures. Now that suitable volatile blends have been demonstrated we may be able to manipulate beneficial species numbers in orchards in the future, with the aim of ultimately reducing pest numbers. The use of plant volatiles to attract beneficial species is compatible with Integrated Pest Management (IPM) and organic control programmes in apple orchards. IPM strategies reduce product inputs, residues on the fruit and the risk of development of pest resistance to products. Attraction of hoverflies into orchards would also be economically favourable as the adults are important pollinators; they are reported to be the most important pollinator group after wild or managed bees.

Action points for growers

- No specific recommendations for growers have resulted from this work, but it will be taken forward in Project TF 223.

SCIENCE SECTION

Introduction

Aphids can be serious pests of apple and pear trees. Many naturally occurring predators attack aphids and other pests in apple orchards, but often migrate into orchards as pest populations increase, and thus too late in the season to prevent damaging populations of the pest from occurring. Hoverflies (Family: Syrphidae) are important predators of aphids early in the season; adults have a high fecundity and the larvae are voracious predators. One of the most common early season species is *Episyrphus balteatus* which is a migratory hoverfly with larvae that feed on a range of aphid species, but which can overwinter as adults in UK orchards. Therefore a possible control strategy would be to encourage more hoverflies into the orchards before pest numbers increase on the trees.

Predation by hoverflies and other beneficial species is more effective when the ants that have a mutualistic relationship with the aphids are excluded from the system. Tree-banding and supplementary sugar feeding have been shown to reduce ant attendance which has led to an increase in control by beneficial insects and a reduction in aphid numbers (Stewart-Jones et al., 2008; Nagy et al., 2013; Nagy et al., 2015). Some of the main hoverfly species are vulnerable to ant attack in the early larval stages, early in the season, although the later stages, especially the third instar larvae, may have some defence strategies against ant attack (Nagy, pers comm.).

Many plants respond to herbivore feeding by producing volatiles that act to reduce herbivore colonisation. These herbivore induced plant volatiles (HIPVs) have been shown to be attractive to some beneficial insects (Scutareanu et al., 1997; James, 2005). One volatile, methyl salicylate, has been used to encourage beneficial insects into grapes and hops (James and Price, 2004), and following this James (2005) tested a range of HIPVs in grassland and hop gardens as attractants for beneficial insects. Mallinger et al. (2011) also used methyl salicylate as an attractant in soybean. Hesler (2016) studied green lacewing and syrphid volatile attractants in corn and soybean. Methyl salicylate dispensers are commercially available in the US as PredaLure™ sachets. As part of a Defra HortLINK project (TF 181, HL 0194) which aimed to develop novel control strategies for pear psylla, in experiments monitoring beneficials by using delta traps holding dispensers containing plant volatiles, we found that methyl salicylate and phenyl ethanol were more effective at trapping hoverflies than control traps with no volatiles.

Volatile components of aphid honeydew and aphid pheromones may also be attractive to hoverflies (Stökl et al., 2011). The aphid alarm pheromone (*E*)- β -farnesene (trans- β -farnesene) has been shown to repel aphids, whilst attracting beneficials such as parasitoids

and predators. The use of (*E*)- β -farnesene in a push-pull strategy has also been explored using wheat, genetically modified to produce the volatile (Bruce, 2015). Farnesene, an analogue of farnesol, is a collective name that refers to a number of stereoisomers of alpha-farnesene and beta-farnesene. It can be purchased relatively inexpensively as a mix of the stereoisomers, or at a higher cost for the (*E*)- β -farnesene single isomer. However, due to the versatile industrial applications of the chemical, yeast strains have been adapted for the biosynthesis of isoprenoids such as β -farnesene (C₁₅H₂₄) (McPhee, 2013).

Hoverflies are thought to use host and plant volatiles in the penultimate stage of short range prey detection (Almohamad et al., 2009), although these volatiles may not have an effect on the final searching behaviour (Joachim and Weisser, 2015). If an appropriate species can be attracted into the orchard when pest numbers are still low in spring this could offset the use of an early season insecticide application.

Summary of volatile work in 2014 and 2015

In this project, replicated experiments were carried out to assess the effects of the use of volatiles alone and in combination to encourage hoverflies into orchards either early in the season or in the autumn.

In 2014, both a phenyl ethanol and a combined phenyl ethanol + methyl salicylate dispenser caught significantly higher numbers of hoverflies in the early spring (April and May) than the untreated control, and the combined dispenser had higher catches across the season (April to July). In the autumn, a combined phenyl ethanol + methyl salicylate + (*E*)- β -farnesene dispenser caught significantly higher numbers of hoverflies than either of the chemicals individually. Due to orchard space constraints the phenyl ethanol + methyl salicylate dispenser was not included in this experiment.

In 2015, to determine whether the effect in the autumn 2014 was due to the addition of the (*E*)- β -farnesene, an experiment was set up to compare the triple dispenser, the phenyl ethanol + methyl salicylate and a phenylacetaldehyde and methyl salicylaldehyde dispenser, compared to an untreated control. The combined phenyl ethanol + methyl salicylate + (*E*)- β -farnesene dispenser caught significantly higher numbers of hoverflies across the spring season (12 April to 22 June) than the phenyl ethanol + methyl salicylate, the phenylacetaldehyde and methyl salicylaldehyde or the untreated control. The phenylacetaldehyde and methyl salicylaldehyde dispenser captured significantly more hoverflies than the control for the June data alone, but not when all data was combined. The phenyl ethanol and methyl salicylate dispenser hoverfly capture did not differ significantly from the control, or from the phenylacetaldehyde and methyl salicylaldehyde dispenser. In a summer trapping experiment, there were few hoverflies, however there was a significant

increase in the numbers of green lacewing, *Chrysoperla carnea*, adults in the combined phenyl ethanol plus methyl salicylate traps compared to single compound traps or the control. This experiment also tested the source of the methyl salicylate i.e. the NRI produced sachet compared with the commercially available Predalure™ sachet, and found no significant difference between the two sources.

As increased numbers of hoverflies are found in the orchards in autumn, and we have found an attraction to volatile compounds at this time, we explored the possibility of attracting species that overwinter, leading to populations of adults in the spring and an earlier production of the larvae, however this was done in small plots and low numbers of hoverflies were caught in the early spring, which meant that an effect could not be determined.

The aim of the experiments in 2016 was to determine the efficacy of single, or blends of, compounds to attract hoverfly adults into orchards.

Materials and methods

The experiment was set up in the spring/summer of 2016 across three sites in Kent:

Site 1. Location – Coxheath, Kent, Variety Envy, tree spacing – 3.6 x 1.4 m

Site 2. Location – Chart Sutton, Kent, Varieties Topaz, Red Windsor, Cox Royale, Crimson Crisp, tree spacing – 3.3 x 1.2 m

Site 3. Location – West Malling, Kent, Variety Gala, tree spacing – 4.27 x 2.13 m

The experiment was a randomised block design, with blocks running down the tree rows (or down paired rows). There were 5 blocks with 7 treatments plus an untreated control. All traps were at least 15 m apart both between and within rows.

Treatments were dispensers containing either single volatiles of methyl salicylate (Sigma-Aldrich, UK), phenyl ethanol (Sigma-Aldrich, UK), mixed farnesene isomers (Sigma-Aldrich, UK) or (*E*)- β -farnesene (Bedoukian research, Danbury, CT 06810, US) or these chemicals in different combinations (Table 1). Each dispenser had a 0.5 ml loading of each volatile soaked on a dental roll in a polyethylene sachet 5 x 5 cm (Figure 1). Dispensers were produced by the Natural Resources Institute, University of Greenwich, Chatham Maritime, UK. The dispensers were hung inside a delta trap with a sticky insert (size 15 cm x 15 cm) (Figure 2).

Table 1. Treatments tested for hoverfly attraction, loaded onto a dental roll inside a polyethylene sachet, hung inside a delta trap

Compounds in the volatile dispenser	Trt
Blank control	1
Methyl salicylate	2
Phenyl ethanol	3
(<i>E</i>)- β -farnesene	4
Farnesene mix of isomers	5
Methyl salicylate + phenyl ethanol	6
Methyl salicylate + phenyl ethanol + (<i>E</i>)- β -farnesene	7
Methyl salicylate + phenyl ethanol + farnesene mix	8



Figure 1. Volatile dispenser as produced by NRI



Figure 2. Apple orchard with a white delta trap hanging mid-canopy

The experiment was set up on 3 May 2016 at Site 1, on 12 May 2016 at Site 2, and following low trap catches at Site 1, the experiment was set up at Site 3 on 16 June 2016. Traps were checked fortnightly and all hoverflies were identified to species where possible. Collections were taken on 19 May, 2 June and 15 June at Site 1, on 20 May, 2 June, 17 June, 6 July and 26 July at Site 2 and on 6 July and 25 July at Site 3. Lures were changed on 17 June at Site 2. Count data of numbers of hoverflies were square-root transformed,

and analysed using a Generalised Linear Model with a Poisson distribution and a log-link. A Fisher's Test was used to determine between treatment differences. Analysis of individual genera was also done with untransformed data.

Results

Only 4 female hoverflies were caught at Site 1 between 3 May and 17 June 2016, 1 *Episyrphus balteatus*, 2 *Meliscaeva auricollis* and 1 *Platycheirus* spp, therefore these data were not analysed. Sites 2 and 3 had higher numbers of hoverfly adults and in total 1700 hoverflies were caught and identified to species or group level. Twenty nine species were identified. The species, sex and date of catch are in Appendix 1 for Site 2 and in Appendix 2 for Site 3. For the analysis, as we were specifically interested in attracting hoverflies for aphid control, early in the season, the total numbers were grouped as 'May and June' and as 'May, June and July' for Site 2, with totals for July at Site 3 (Table 2).

Table 2. Mean square-root total numbers of adult hoverflies per trap

	Site 2		Site 3			
Volatile dispenser	May and June only	Fisher's test	May, June & July	Fisher's test	July	Fisher's test
1. Untreated control	1.64	ab	2.51	a	1.33	a
2. Methyl salicylate	2.99	c	4.91	b	2.67	b
3. Phenyl ethanol	2.51	bc	5.28	bc	4.04	c
4. (<i>E</i>)- β -farnesene	1.63	ab	5.23	bc	2.34	ab
5. Farnesene mix of isomers	1.14	a	2.90	a	1.41	a
6. Methyl salicylate + phenyl ethanol	2.80	c	6.12	cd	4.56	cd
7. Methyl salicylate + phenyl ethanol + (<i>E</i>)- β -farnesene	2.94	c	7.52	e	5.37	d
8. Methyl salicylate + phenyl ethanol + farnesene mix	2.50	bc	7.12	de	4.32	cd
p	0.005		<0.001		<0.001	
d.f.	28		28		28	
s.e.d.	0.507		0.553		0.575	
l.s.d.	1.038		1.132		1.179	

The results are presented graphically in Figures, 3, 4 and 5, showing the back-transformed mean totals per trap with the Fisher's test values shown above. For 'May and June' only at Site 2 (Figure 3) the methyl salicylate, phenyl ethanol, methyl salicylate + phenyl ethanol, methyl salicylate + phenyl ethanol + (*E*)- β -farnesene and the methyl salicylate + phenyl ethanol + farnesene mix treatments were all significantly different from the untreated

control. The (*E*)- β -farnesene and the farnesene mix of isomers did not significantly attract hoverflies as single dispensers. When examining the data from Site 2 across the whole season (Figure 4), all catches were significantly higher from the control apart from the farnesene mix of isomers. Methyl salicylate + phenyl ethanol + (*E*)- β -farnesene had the highest catches, albeit not significantly different from the methyl salicylate + phenyl ethanol + farnesene mix. There was no significant difference between the latter and the methyl salicylate + phenyl ethanol without a farnesene addition, however there were significantly more catches in the methyl salicylate + phenyl ethanol + (*E*)- β -farnesene treatment compared to the methyl salicylate + phenyl ethanol alone.

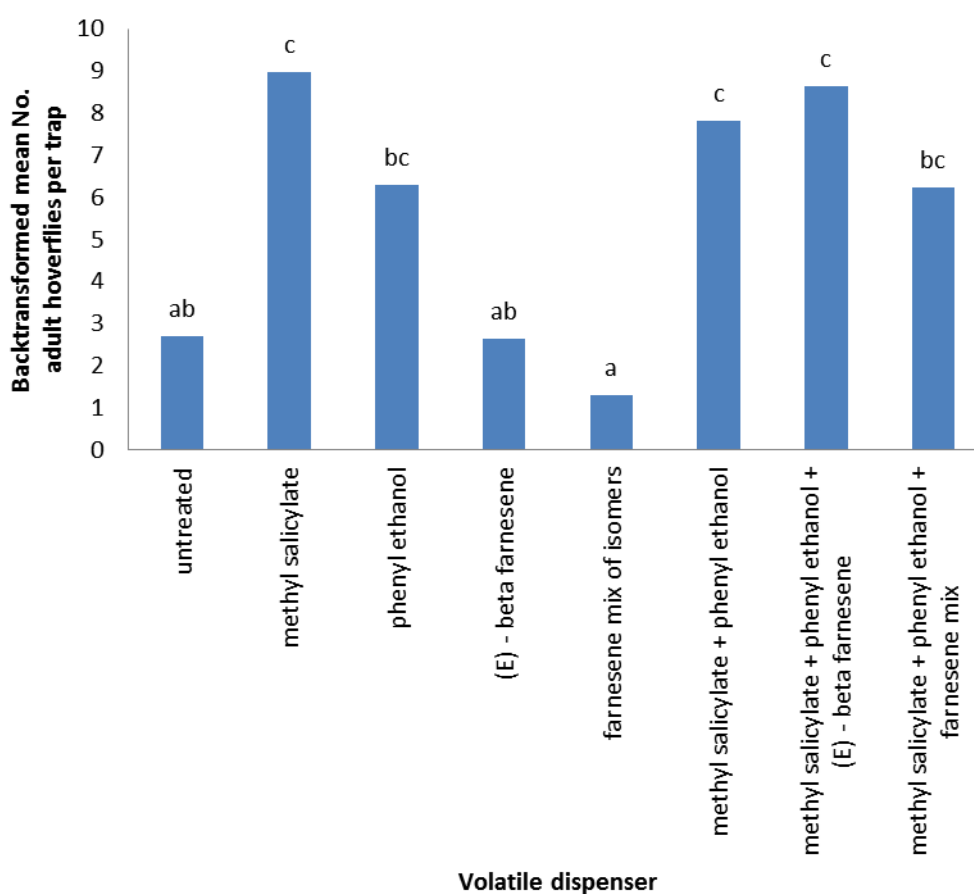


Figure 3. Back-transformed mean numbers of hoverflies per trap at Site 2 in May and June 2016. Bars with different letters are significantly different at $P < 0.05$.

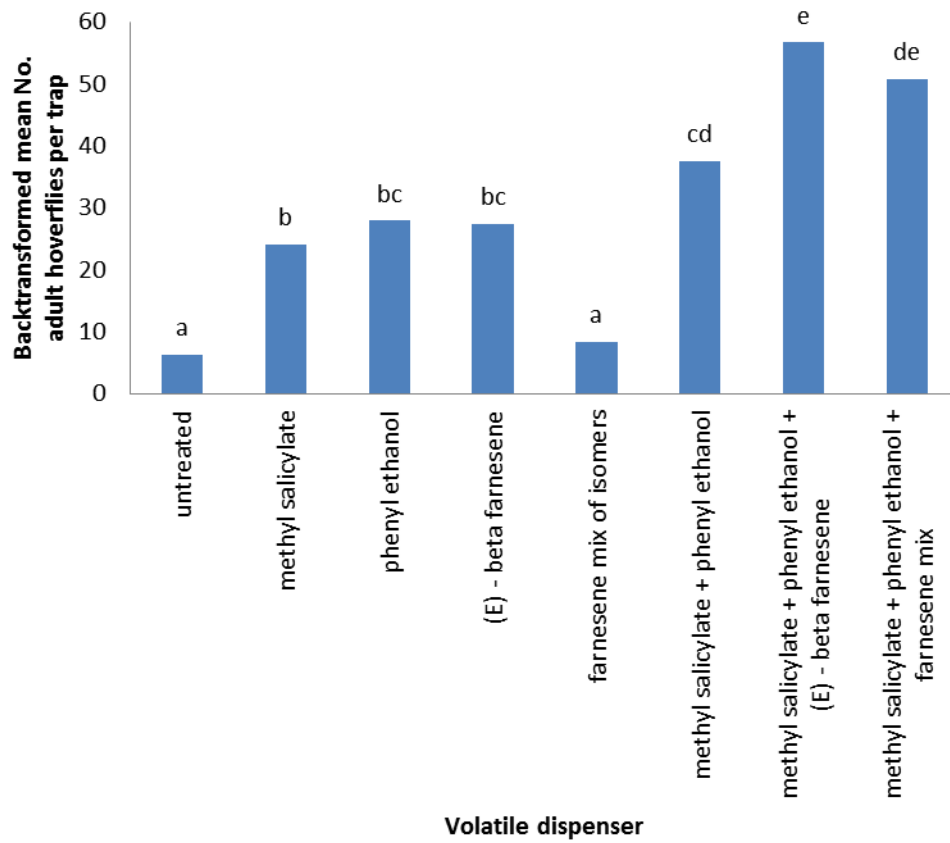


Figure 4. Back-transformed mean numbers of hoverflies per trap at Site 2 in May to July 2016. Bars with different letters are significantly different at $P < 0.05$.

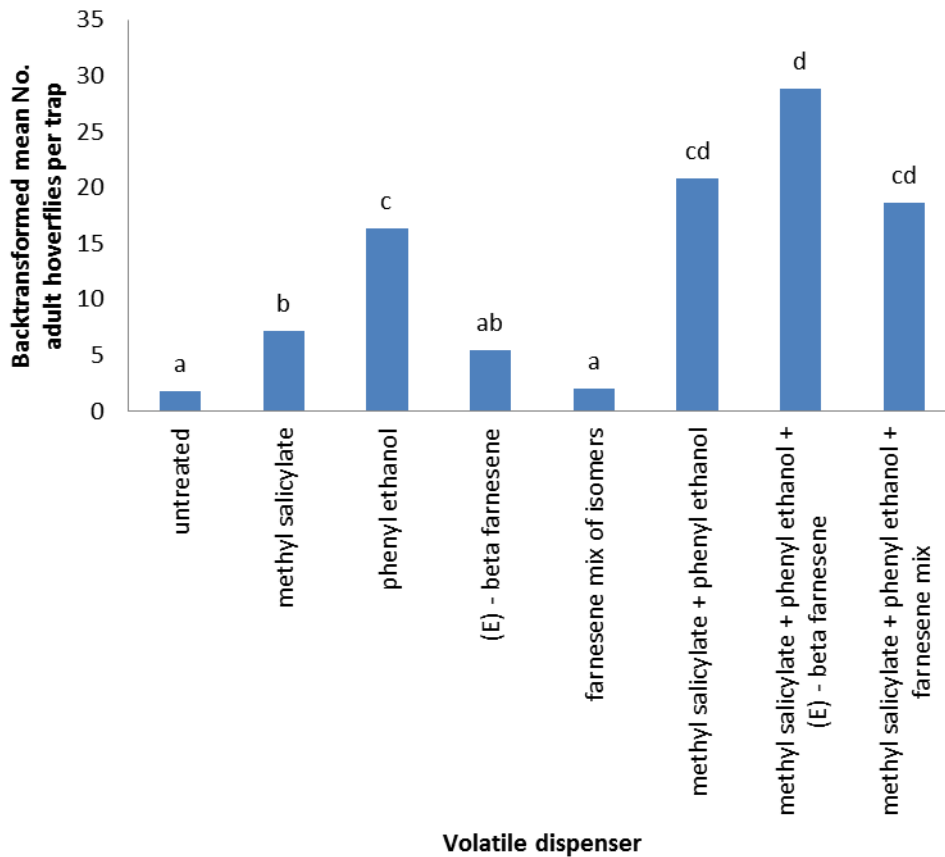


Figure 5. Back-transformed mean numbers of hoverflies per trap at Site 3 in July 2016. Bars with different letters are significantly different at $P < 0.05$.

The data for Site 3 (Figure 5), again, shows the highest catches for the methyl salicylate + phenyl ethanol + (*E*)- β -farnesene, although not significantly different from the methyl salicylate + phenyl ethanol or the methyl salicylate + phenyl ethanol + farnesene mix.

The data was also analysed according to three of the main hoverfly genera that are found early in the season. This included all data from Sites 2 & 3 and all the sample dates. For *Episyrphus* spp., the methyl salicylate, phenyl ethanol and (*E*)- β -farnesene treatment had the highest trap catches (Table 3); with the between treatment differences in Table 4. The *Episyrphus* spp. were mainly *Episyrphus balteatus* (Figure 6), with only 5 individuals that may have been another *Episyrphus* sp.

Table 3. The effect of volatile dispenser on mean delta trap catches for *Episyrphus* spp., per trap per date, sorted according to mean count.

Treatment	Mean Count	Group*
Untreated (U)	0.333	a
Farnesene mix of isomers (FM)	0.400	a
Methyl salicylate (MS)	0.633	ab
Methyl salicylate + phenyl ethanol (MS + PE)	1.000	bc
(E)- β -farnesene (EBF)	1.267	cd
Phenyl ethanol (PE)	1.333	cd
Methyl salicylate + phenyl ethanol + farnesene mix (MS + PE + FM)	1.800	d
Methyl salicylate + phenyl ethanol + (E)- β -farnesene (MS + PE + EBF)	2.700	e

*Groups with different letters are statistically significant from each other at $P < 0.05$.

Table 4. Compared significance table showing the comparisons between volatile treatments on mean delta trap catch, per trap and date, for *Episyrphus* spp.

Treatment	Values							
U	1.00	0.67	0.09	0.00	0.00	0.00	0.00	0.00
FM	0.67	1.00	0.21	0.00	0.00	0.00	0.00	0.00
MS	0.09	0.21	1.00	0.11	0.01	0.01	0.00	0.00
MS + PE	0.00	0.00	0.11	1.00	0.33	0.23	0.01	0.00
EBF	0.00	0.00	0.01	0.33	1.00	0.82	0.09	0.00
PE	0.00	0.00	0.01	0.23	0.82	1.00	0.15	0.00
MS + PE + FM	0.00	0.00	0.00	0.01	0.09	0.15	1.00	0.02
MS + PE + EBF	0.00	0.00	0.00	0.00	0.00	0.00	0.02	1.00
	U	FM	MS	MS + PE	EBF	PE	MS + PE + FM	MS + PE + EBF



Figure 6. Adult *Episyrphus balteatus*

Table 5. The effect of volatile dispenser on mean delta trap catches for *Eupoedes* spp., per trap per date, sorted according to mean count.

Treatment	Mean Count	Group*
Untreated (U)	0.050	a
Farnesene mix of isomers (FM)	0.100	a
(<i>E</i>)- β -farnesene (EBF)	0.300	b
Methyl salicylate (MS)	0.467	bc
Methyl salicylate + phenyl ethanol + farnesene mix (MS + PE + FM)	0.483	bc
Phenyl ethanol (PE)	0.650	c
Methyl salicylate + phenyl ethanol + (<i>E</i>)- β -farnesene (MS + PE + EBF)	0.717	cd
Methyl salicylate + phenyl ethanol (MS + PE)	0.867	d

***Groups with different letters are statistically significant from each other at $P < 0.05$.**

Table 6. Compared significance table showing the comparisons between volatile treatments on mean delta trap catch, per trap and date, for *Eupeodes* spp.

Treatment	Values							
U	1.000	0.313	0.001	0.000	0.000	0.000	0.000	0.000
FM	0.313	1.000	0.012	0.000	0.000	0.000	0.000	0.000
EBF	0.001	0.012	1.000	0.139	0.107	0.005	0.001	0.000
MS	0.000	0.000	0.139	1.000	0.895	0.178	0.074	0.007
MS + PE + FM	0.000	0.000	0.107	0.895	1.000	0.224	0.098	0.010
PE	0.000	0.000	0.005	0.178	0.224	1.000	0.659	0.172
MS + PE + EBF	0.000	0.000	0.001	0.074	0.098	0.659	1.000	0.355
MS + PE	0.000	0.000	0.000	0.007	0.010	0.172	0.355	1.000
	U	FM	EBF	MS	MS + PE + FM	PE	MS + PE + EBF	MS + PE

For *Eupeodes* spp., the methyl salicylate and phenyl ethanol, and the methyl salicylate, phenyl ethanol and (*E*)- β -farnesene treatment had the highest trap catches (Table 5), with the compared significances between the treatments shown in Table 6. The *Eupoedes* spp. were either *E. luniger* or *E. corollae*.

Table 7. The effect of volatile dispenser on mean delta trap catches for *Platycheirus* spp., per trap per date, sorted according to mean count.

Treatment	Mean Count	Group*
Untreated (U)	0.016	a
Farnesene mix of isomers (FM)	0.020	ab
(<i>E</i>)- β -farnesene (EBF)	0.042	bc
Methyl salicylate (MS)	0.047	c
Phenyl ethanol (PE)	0.082	d
Methyl salicylate + phenyl ethanol + farnesene mix (MS + PE + FM)	0.116	de
Methyl salicylate + phenyl ethanol + (<i>E</i>)- β -farnesene (MS + PE + EBF)	0.129	e
Methyl salicylate + phenyl ethanol (MS + PE)	0.137	e

***Groups with different letters are statistically significant from each other at $P < 0.05$.**

Table 8. Compared significance table showing the comparisons between volatile treatments on mean delta trap catch, per trap and date, for *Platycheirus* spp.

Treatment	Values							
U	1.000	0.617	0.025	0.007	0.000	0.000	0.000	0.000
FM	0.617	1.000	0.080	0.026	0.000	0.000	0.000	0.000
EBF	0.025	0.080	1.000	0.631	0.010	0.000	0.000	0.000
MS	0.007	0.026	0.631	1.000	0.034	0.000	0.000	0.000
PE	0.000	0.000	0.010	0.034	1.000	0.111	0.031	0.013
MS + PE + FM	0.000	0.000	0.000	0.000	0.111	1.000	0.567	0.372
MS + PE + EBF	0.000	0.000	0.000	0.000	0.031	0.567	1.000	0.749
MS + PE	0.000	0.000	0.000	0.000	0.013	0.372	0.749	1.000
	U	FM	EBF	MS	PE	MS + PE + FM	MS + PE + EBF	MS + PE

For *Platycheirus* spp., the methyl salicylate and phenyl ethanol, the methyl salicylate, phenyl ethanol and (*E*)- β -farnesene, and the methyl salicylate, phenyl ethanol and farnesene isomer mix treatments all fell within the highest trap catch group (Table 7), with the compared significances between the treatments shown in Table 8. The *Platycheirus* spp. included *P. albimanus*, *P. fulviventris*, *P. peltatus* and *P. punctulatus*.

Discussion

The results clearly show that hoverflies can be attracted to specific volatiles. This is seen from examining the total hoverfly catch and also catches of species and genera found early in the season. This was important in order to develop a dispenser that will be effective early season and to encourage species that may add to an effective control programme. Different species may be important at different timings in the season, and may have different defence strategies against ant attack. In the early period (April) the first and second instar larvae of some of the main species such as *Episyrphus balteatus* can be vulnerable to ant attack (Nagy et al., 2015). Later in the season (mid-May onwards) as the aphid colonies are larger this effect is not seen as strongly and all main species can successfully develop. Some species such as some *Playcheirus* and *Epistrophe* species are still typically present in the ant-protected aphid colonies. During the observations that led to the aforementioned paper, Csaba Nagy had not seen an ant attack these species (pers. comm). When examining the trap catches of some of the early species, they followed a similar response pattern to the volatiles as the data for the overall total hoverfly catch.

To decide which volatile blend to use on a commercial scale, cost of purchase of a dispenser and deployment are major considerations. An approximate cost of spraying an orchard with an insecticide such as Calypso may be in the region of £65 for 1 hectare, including tractor costs, operator costs and the chemical at recommended rate (depending on the distance between orchards and staff salary). In field studies in the US, the preliminary work in hop gardens used methyl salicylate at a rate of 448 dispensers/ha (James and Price 2004). Other studies (Khan et al. 2008) suggested that lower deployment rates (180 sachets/ha) were more attractive to all predator groups. A recent study in Oregon strawberry fields using 30-day 2 g PredaLure™ dispensers documented attraction of some natural enemies, particularly green lacewings (Chrysopidae) and *Orius tristicolor* at a range of approximately 10 m or less from the point source (Lee 2010). Woods et al. (2011) deployed commercial PredaLure™ dispensers at 185/ha and at 123 dispensers/ha, the latter rate determined by the cost of an acaricide that the dispensers were effectively replacing. Therefore if like Woods et al. (2011) we deployed the dispensers at a rate to obtain comparable costs for the product they are intended to replace and at a deployment rate between 185 or 123 dispensers per hectare then the purchase cost of each dispenser would need to be between 35 and 53 pence (which is not taking into account costs of deployment).

The chemicals used in the dispensers are available commercially and current costs are shown in Table 9.

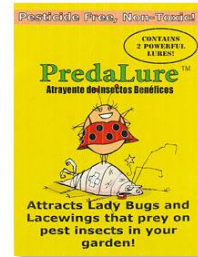
Table 9. Cost of the chemicals used in the dispensers, prices correct as of May 2017

Chemical	Supplier	Cost	Amount
Methyl salicylate	Sigma-Aldrich Ltd, UK	£49	1 l
Phenyl ethanol	Sigma-Aldrich Ltd, UK	£88	1 kg
Mixed farnesene isomers	Sigma-Aldrich Ltd, UK	£209.50	1 kg
(<i>E</i>)- β -farnesene	Bedoukian research, Danbury, CT 06810, US	£300	100 ml
(<i>E</i>)- β -farnesene (as analytical standard)	Sigma-Aldrich UK	£335	1 ml
(<i>E</i>)- β -farnesene	Amyris Inc, USA	\$100 plus import tax	1 kg

The chemicals methyl salicylate and phenyl ethanol are relatively inexpensive to purchase. In addition to this the polyethylene dispenser and material onto which the volatile is loaded, plus technician time needs to be considered. However a combined methyl salicylate and phenyl ethanol dispenser would be feasible. The mixed farnesene isomers are also feasible to include, although it is uncertain whether the extra costs would be warranted given that there is no significant improvement in trap catches over and above the methyl salicylate and phenyl ethanol dispenser. The (*E*)- β -farnesene used in these experiments was purchased from Bedoukian research, Danbury, CT 06810, US. This product is expensive (although less so than the analytical standard from Sigma-Aldrich UK), however the product is not currently on their website for purchase. The chemical company Amyris Inc, USA, is now using the new yeast production method to produce (*E*)- β -farnesene, which could be a more cost effective solution. This could make a triple lure with methyl salicylate, phenyl ethanol and (*E*)- β -farnesene of interest. As it is still arguable as to whether this would be cost effective if targeting hoverflies alone, it should be borne in mind that a combined dispenser may have a use in attracting other orchard beneficials such as lacewings (as shown from work in 2015), ladybirds, heteroptera, parasitoids etc which may also target other pests. Therefore it would be reasonable to conclude that a combined dispenser containing the two components methyl salicylate and phenyl ethanol, or a triple dispenser with the addition of (*E*)- β -farnesene (should this become cost effective) would be the way forward, and indeed will be used in TF 223. Sources of hoverfly adults could also be provided, e.g. flowering herbs, in orchard surrounds. With the withdrawal of products such as chlorpyrifos and pirimicarb in recent years, alternative control options are valuable in an IPM system.

Conclusions

- Work to date has shown that the combination dispensers are consistently attractive to hoverfly species
- The work will be carried forward to TF 223 where large plot studies will be done. The work in TF 223 will be able to assess the effects on attraction of hoverflies into orchards and the subsequent effects on aphid control
- The use of plant volatiles to attract beneficial species is compatible with IPM and organic control programmes in orchards
- Producing a double (methyl salicylate and phenyl ethanol) or triple (methyl salicylate, phenyl ethanol and (*E*)- β -farnesene, depending on supplier) volatile dispenser could be cost effective. Production of methyl salicylate sachets as PredaLureTM in the US has shown that a commercial market could be possible
- This would not need pesticide approval as it is attracting a beneficial insect



Knowledge and Technology Transfer

28 Feb 2017 EMRA AHDB Tree Fruit Day.

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Glossary

Plant volatiles – these are chemicals that are produced by the plant. These can be referred to as herbivore induced plant volatiles (HIPVs) if the chemicals are upregulated following feeding by a pest.

References

- Almohamad R., Verheggen F.J., Haubruge E. 2009. Searching and oviposition behaviour of aphidophagous hoverflies (Diptera: Syrphidae): a review. *Biotechnology, Agronomy, Society and Environment* 13 467-481.
- Bruce, T.J.A., Aradottir G. I., Smart, L.E., Martin J.L., Caulfield J.C., Doherty A., Sparks C.A., Woodcock C.M., Birkett M.A., Napier J.A., Jones H.D., Pickett J.A. 2015. The first crop plant genetically engineered to release an insect pheromone for defence. *Scientific Reports* 5, 11183; doi: 10.1038/srep11183
- Hesler, L.S. 2016. Volatile semiochemicals increase trap catch of green lacewings (Neuroptera: Chrysopidae) and flower flies (Diptera: Syrphidae) in corn and soybean plots. *Journal of Insect Science* 16 77: 1-8.
- James D.G., Price T.S. 2004. Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *Journal of Chemical Ecology* 30 1595-1610.
- James D.G. 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Journal of Chemical Ecology* 31 481-495.
- Joachim C., Weisser W. W. 2015. Does the aphid alarm pheromone (E)- β -farnesene act as a kairomone under field conditions. *Journal of Chemical Ecology* 41 267-75.
- Khan, Z. R., D. G. James, C. A. O. Midega, J. A. Pickett. 2008. Chemical ecology and conservation biological control. *Biological Control* 45: 210–224.
- Lee. J.C. 2010. Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. *Environmental Entomology* 39: 653-660
- Mallinger R.E., Hogg D.B., Gratton C. 2011. Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. *Journal of Economic Entomology* 104 115-124.
- McPhee, D. 2013. in *Catalytic Process Development for Renewable Materials* (eds Imhof, P. & van der Waal, J. C.) Ch. 3, 51–79 (Wiley-VCH)
- Nagy C., Cross J.V., Markó V. 2013. Sugar feeding of the common black ant, *Lasius niger* (L.), as a possible indirect method for reducing aphid populations on apple by disturbing ant-aphid mutualism. *Biological Control* 65 24-35.
- Nagy C., Cross J.V., Markó V. 2015. Can artificial nectaries outcompete aphids in ant-aphid mutualism? Applying artificial sugar sources for abts to support better biological control of rosy apple aphid, *Dysaphis plantaginea* Passerini in apple orchards. *Crop Protection* 77 127-138.

Scutareanu P., Drukker B., Bruin J., Posthumus M.A., Sabelis M.W. 1997. Volatiles from Psylla-infested pear trees and their possible involvement in attraction of anthocorid predators. *Journal of Chemical Ecology* 23 2241-2260.

Stewart-Jones A., Pope T.W., Fitzgerald J.D., Poppy G.M. 2008. The effects of ant attendance on the success of rosy apple aphid populations, natural enemy abundance and apple damage in orchards. *Agricultural and Forest Entomology* 10 37–43.

Stökl J., Brodmann J., Dafni A., Ayasse M., Hansson B.S. 2011. Smells like aphids: orchid flowers mimic aphid alarm pheromones to attract hoverflies for pollination. *Proceedings of the Royal Society of Biology* 278 1216–1222.

Woods, J.L., James, D.G., Lee, J.C. 2011. Evaluation of airborne methyl salicylate for improved conservation biological control of two-spotted spider mite and hop aphid in Oregon hop yards. *Experimental and Applied Acarology* 55: 401.

APPENDIX 1 The species of hoverflies caught in delta traps in an apple orchard at Site 2, from 12 May to 26 July in spring/summer 2016

Site 2	20/05			02/06			17/06			05/07			26/07			Grand Total
	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	
Species	10	1	9	156	27	129	76	18	58	267	79	189	666	108	558	1175
<i>Cheilosia</i> spp.				1	0	1				6	3	3	18	0	18	25
<i>Episyrphus balteatus</i>				5	2	3	14	8	6	37	12	25	77	33	44	133
<i>Eristalis nemorum</i>				2	0	2				8	1	7	8	0	8	18
<i>Eristalis pertinax</i>	1	0	1	5	0	5	1	0	1	2	0	2				9
<i>Eristalis tenax</i>										1	0	1	1	0	1	2
<i>Eupeodes corollae</i>				5	0	5	14	2	12	43	16	27	5	0	5	67
<i>Eupeodes luniger</i>				2	0	2	10	3	7	8	2	6	28	3	25	48
<i>Helophilus pendulus</i>	1	0	1	2	0	2	5	1	4							8
<i>Melanostoma millenium</i>										15	9	7	78	24	54	93
<i>Melanostoma scalare</i>				1	0	1	1	0	1	7	0	7	152	8	144	161
<i>Meliscaeva auricollis</i>				1	0	1				82	27	55	146	31	115	229
<i>Myathropa florea</i>							6	0	6	1	0	1	1	0	1	8
<i>Platycheirus albimanus</i>				1	0	1	1	0	1				5	0	5	7
<i>Platycheirus peltatus</i>							1	0	1							1
<i>Platycheirus punctulatus</i>													1	0	1	1
<i>Platycheirus</i> spp.				2	0	2	6	0	6	39	2	37	120	3	117	167
<i>Rhingia campestris</i>	8	1	7	128	25	103	16	4	12	5	3	2	1	0	1	158
<i>Sericomyia silentis</i>										1	0	1				1
<i>Sphaerophoria scripta</i>													1	0	1	1
<i>Syrirta pipiens</i>				1	0	1										1
<i>Syrphus ribesii</i>										10	4	6	12	4	8	22
<i>Volucella bombylans</i>							1	0	1							1
<i>Volucella inflata</i>										1	0	1	2	1	1	3
<i>Xanthogramma citrofasciatum</i>													5	1	4	5
<i>Xanthogramma pedissequum</i>										1	0	1	5	0	5	6

APPENDIX 2 The species of hoverflies caught in delta traps in an apple orchard at Site 3, from 16 June to 25 July, in spring/summer 2016

Site 3	06/07			25/07			Grand total
	Total	♂	♀	Total	♂	♀	
Species list	224	40	187	297	71	226	521
<i>Cheilosia</i> spp.	7	0	7	18	2	16	25
<i>Chrysotoxum verralli</i>	2	1	1				2
<i>Episyrphus balteatus</i>	41	7	35	105	41	64	146
<i>Episyrphus</i> spp.				5	1	4	5
<i>Eristalis nemorum</i>	2	0	2				2
<i>Eupeodes corollae</i>	19	5	14	20	1	19	39
<i>Eupeodes luniger</i>	43	3	40	21	6	15	64
<i>Melanostoma millenium</i>				40	4	36	40
<i>Melanostoma scalare</i>	9	1	8	33	1	32	42
<i>Meliscaeva auricollis</i>	22	6	16	16	5	11	38
<i>Platycheirus albimanus</i>	22	2	20	7	0	7	29
<i>Platycheirus fulviventris</i>	3	2	1				3
<i>Platycheirus</i> spp.	34	4	32	24	4	20	58
<i>Sericomyia silentis</i>	1	0	1				1
<i>Sphaerophoria scripta</i>	1	0	1	4	3	1	5
<i>Syrphus ribesii</i>	14	9	5				14
<i>Xanthandrus comtus</i>				4	3	1	4
<i>Xanthogramma pedissequum</i>	1	0	1	3	0	3	4