Project title:	The Role of Chemicals in the Location of Host Plants by Midge Pests of UK Fruit Crops.
Project number:	CP 73
Project leader:	Prof. David R Hall (NRI) Jerry Cross (EMR)
Report:	Annual, August 2011
Previous report:	None
Key staff:	Helen Thomas (PhD Student)
Location of project:	Natural Resources Institute, Chatham Maritime, Kent. East Malling Research, Kent.
Industry Representative:	[Name, Organisation, Address]
Date project commenced:	February 2011
Date project completed (or expected completion date):	January 2014

DISCLAIMER

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.

Prof David R Hall]
Professor of Chemical Ecology
Natural Resources Institute, University of Greenwich

Signature .... Date ....

Prof Jerry Cross
Science Programme Leader
East Malling Research

Signature .... Date ....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature .... Date ....

[Name]
[Position]
[Organisation]

Signature .... Date ....

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GROWER SUMMARY

Headline

Experiments have started to identify attractants for female raspberry cane midge.

Background and expected deliverables

Species of gall midge (Diptera: Cecidomyiidae) are important pests of many horticultural crops and are often very difficult to control by conventional means. NRI and EMR have made considerable progress in identification of female sex pheromones in this group of insects, and some are now in use for monitoring populations of several pest species. However, the female-produced sex pheromones attract only males. Attractants for the females, particularly mated females, would potentially be far more valuable for both monitoring and control of the pests. There is good evidence in several species of midge that mated females are attracted to their host plants for oviposition by specific odours from the plants. Although this has been known for over 40 years in some cases, the chemicals responsible for this attraction have not yet been identified.

This project will aim to identify the chemicals responsible for attraction of mated female midges to oviposition sites on their host crop for up to three species which are important pests of soft fruit and tree crops in the UK and where such attraction has been demonstrated previously. These are the raspberry cane midge, *Resseliella theobaldii*, the blackcurrant leaf midge, *Dasineura tetensii*, and the apple leaf midge, *D. mali*. Once the chemicals responsible have been identified and synthesised it may be possible to use synthetic lures for monitoring and controlling pest species.

Summary of the project and main conclusions

Work has begun on attractants for female raspberry cane midge. Observations have been made in growers' fields confirming attraction of female midges to split raspberry canes. Trapping experiments have been carried out with four different designs of trap baited with synthetic and natural lures. Horizontal or vertical sticky card traps caught more midges than delta traps, and water traps performed poorly. Catches of female midges were very low. Catches of male midges were higher, but there were no significant differences in numbers attracted to the unbaited traps and to those baited with either the synthetic or natural lures.

Midge larvae and pupae have been collected for further work during the winter using laboratory bioassays and electroantennography.

Financial benefits

- There are none to date

Action points for growers

- None have yet been identified. .

SCIENCE SECTION

Introduction

The overall aim of the project is to identify chemicals responsible for attraction of mated females of up to three species of midge to their host plants:

- The raspberry cane midge, *Resseliella theobaldii;*
- The blackcurrant leaf midge, *Dasineura tetensii*;
- The apple leaf midge, *Dasineura. mali*.
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The three target species are important pests of horticultural crops and identification of these attractants would provide a basis for development of new approaches to monitoring and control of these pests that would be compatible with both conventional IPM and organic strategies. The results will also advance our knowledge of the remarkable ability of insects to find their host plants in terms of whether a few key chemicals are involved or whether they use specific blends of several more ubiquitous chemicals.

The project began in February 2011 and practical work is only just starting. The first two months where spend reviewing existing literature to establish what work had already been done and to develop a research plan.

The first midge species to be focused on is the raspberry cane midge, *Resseliella theobaldi*. After mating females lay eggs in splits in the primocane of raspberry plants. Once hatched, the larvae feed on the tissue beneath the outer cortex, weakening the canes and reducing their productivity (Alford, 2007). An effective pheromone blend has been identified which attracts male midges (Hall et al. 2009) and this is now used commercially to monitor midge populations. Nijveldt (1963) found that females were rapidly attracted to freshly made splits and suggested that olfactory cues may play a part in split location. Hall et al. (2010) identified some of the chemicals released when a cane splits, using solid phase micro-extraction coupled to gas chromatography and mass spectrometry. There have been some field trials although the results were inconclusive.

Work to date has involved field studies to take advantage of natural populations of *R. theobaldii*. Initial observational work was done to confirm that female midges are attracted to split canes and this led on to a trapping trial to investigate lures and trap design.

Materials and Methods

Initial field observations

Once the midges began to emerge in April preliminary observations were carried out in plantations at Mockbeggar Farm, Higham, and East Malling Research Station, both in Kent. Red delta traps baited with synthetic sex pheromone lures were hung in an open ended polytunnel at Mockbeggar (cv. Tulameen) and in plots WE195 (cv. Glen Ample), CT2, DF174, CW 132 - 133 and CW 136-137. The sticky bases were monitored and once the numbers in the field were more than 10 midges on the sticky paper, artificial split observations were begun.

Splits (approx 10 cm long) were made with a mounted needle in growing primocane and observed to see if female midges were attracted. In some of the later observations a pooter was used to collect the midges visiting the splits so that their sex could be confirmed in the lab. Once the midges arrived they walked the length of the split before inserting their ovipositors under the primocane skin, presumably to lay eggs, before flying off.

Trapping experiment

Location

The experimental work was carried out in cv. Maravilla potted primocane raspberries at Court Lodge Farm, Kenward Road, Yalding, Kent. The site is farmed by Clockhouse Farms Ltd, Linton, owner Robert Pascall. The total study area measures 85 m x 228.8m and includes a total of 30 rows of raspberry cane (Figure 1).

Figure 1. Arial photograph of Court Lodge Farm with study area marked in red.

Treatments

Four different trap designs (Figure 2) were compared.

1. White delta trap (20 cm x 27 cm x 11 cm high) with a sticky base (18.5 cm x 18.5 cm).
2. Horizontal white water trap constructed from a plant saucer (24 cm dia x 4 cm deep) with an upright section of plastic pipe (2 cm dia, 5 cm high) glued in the centre to support the cane sections.
3. Horizontal sticky trap consisting of a piece of red corrugated plastic (20 cm x 20 cm) with a sticky base (18.5 cm x18.5 cm) clipped to the upper surface using bulldog clips. A nail was pushed through from the underside for mounting the cane.
4. Vertical sticky trap made from a piece of corrugated plastic (19 cm x 11 cm) with an aperture (2.5 cm x 12 cm) cut into the centre. A sticky base was cut into four strips and one strip attached with bulldog clips to either side of the aperture on both the front and back of the trap. A loop of wire was pushed down through the top of the trap to make legs which could be pushed into the soil to support the trap. Nails were pushed up through the bottom and down through the top of the trap to hold the cane sections.

The size of the catching surface was the same in the vertical, horizontal and delta traps (18.5 cm x 18.5 cm sticky base) and the water trap size was chosen to be as close to these dimensions as possible.

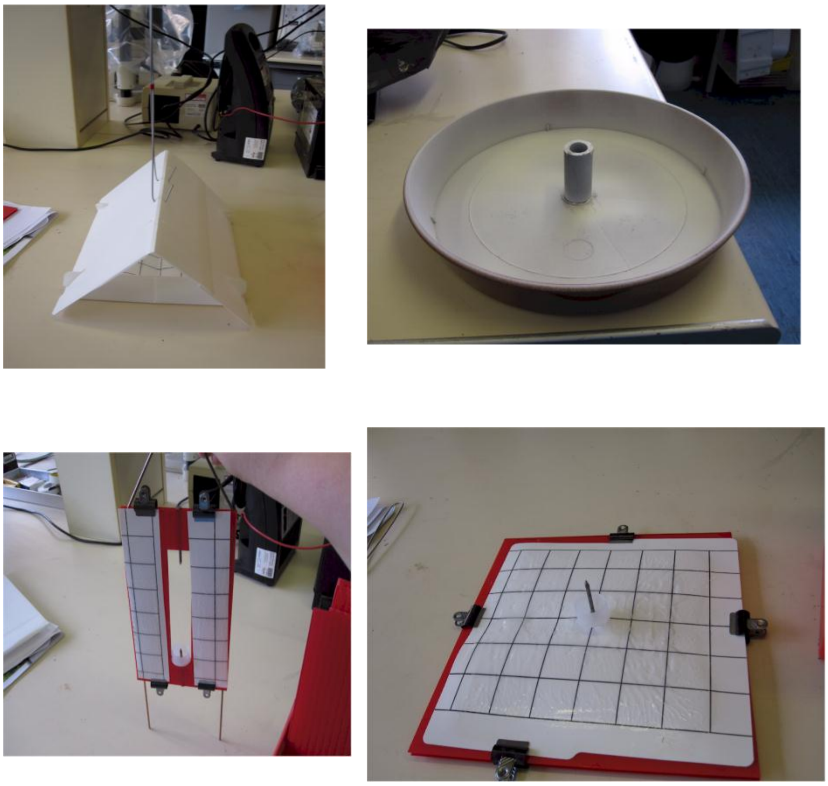

Figure 2. The trap designs (clockwise from top): white delta trap, water trap, vertical sticky trap, horizontal sticky trap.

Traps were unbaited or baited with one of two different lures. The “natural” lure was an excised cane section (12 cm) with a split (10 cm long) made in the epidermis using a mounted needle. The “synthetic” lure was that developed by Hall et al. (2010) containing a blend of nine compounds in a sealed polyethylene sachet (5 cm x 5 cm x 120 μ thick) and four compounds in a polyethylene vial (22 mm x 8 mm x 1 mm thick). The vial was placed inside the sachet.

The synthetic lures were suspended in the delta and vertical traps using a paperclip. A section of old dried out cane was used as a support for the synthetic sachets in the horizontal sticky and water traps. The synthetic volatile sachet was attached to the top of the dry cane using a nail. Sections of primocane with the base cut at an angle of 45° were wedged into the white delta traps and secured with a nail (Figure 3).

Figure 3. Sachet suspended on dry cane in water trap (left) and primocane wedged in delta trap (right)

Experimental design

A randomised complete block design was used with four blocks. The plots were a single trap and each block ran the full length of the study area (228.8 m or 104 metal poly tunnel hoops). Block one was located in the third row, block two in row eleven, block three in row nineteen and block four in row twenty seven. The traps were laid out along the row at a distance of eight hoops (18 m) apart.

In addition two red delta traps baited with pheromone lures and located near to but not in the study area were checked daily to gauge the male raspberry cane midge population. Also splits were made in 10 canes close to each of the pheromone traps. These splits were marked with coloured tape and checked for eggs the day after being made, although this was abandoned after two days as no eggs were found.

Each day the excised cane sections where changed and water in the water traps topped up. Counts of the numbers of raspberry cane midge males and females were made every three days. Sticky card, inserts and trap water were changed after each count had been made. Sticky card inserts were stored in small pizza boxes until the number of midge and other insects had been counted.

The insects caught in the traps were sorted into three groups: male *R. theobaldii*, female *R. theobaldii* and by-catch, which included all insects which are not raspberry cane midge, including midges of other species.

The data were analyzed using ANOVA in Genstat with trap design and +/- lure or cane section as treatment factors.

Results

Initial field observations

During the field observations at the farm and research station in Kent it was seen that midges where attracted to the splits within 20 min (Table 1). Once the midges arrived they walked the length of the split before inserting their ovipositors under the primocane skin, presumably to lay eggs, before flying off.

Table 1. Summary of data collected from observing arrival of *R. theobaldii* at artificial splits

Location	Observations	Midges Seen	Females	Males
Mockbeggar Farm	8	6	0	1
EMR – Plot WE195	18	8	3	0

The observational work led to the baiting of a red delta trap in the Mockbeggar Farm polytunnel with a section of excised split cane. This was left for seven days after which time 94 midges had been caught, all of which were male. This finding suggests that males are also attracted to splits in primocane, possibly looking to find females.

These initial observations also led to investigation into different types of trap which could catch insects visiting a split living cane. A delta trap with an aperture cut in the top and bottom to allow the cane to pass through was tried, but it was found that the delta trap was too heavy for the cane to support. A trap was also created using the central section of a 2-litre drinks bottle with sticky paper attached to the inside surface. This trap did not catch many insects at all and so was abandoned.

Trapping experiment

Table 2 shows the mean number of males, females and by-catch caught in each of the two weeks of the experiment. Combined mean catches of males and by-catch for the two weeks are shown in Figures 4 and 5.

Table 2. Mean numbers of male and female *R. theobaldii* and by-catch insects caught in four trap designs (D delta trap; W water trap; H horizontal sticky trap; V vertical sticky trap) with three lures over two successive weeks.

	Week 1				Week 2			
Lure	**D**	**W**	**H**	**V**	**D**	**W**	**H**	**V**
Females								
Synthetic	0.0	0.0	0.0	0.0	0.75	0.00	0.00	0.00
Cane	0.0	0.0	0.0	0.0	1.00	0.00	0.00	0.25
No Bait	0.0	0.0	0.0	0.0	0.75	0.25	0.50	0.50
Males								
Synthetic	45.3	0.0	32.2	41.5	30.0	0.2	18.7	77.8
Cane	13.3	0.0	35.3	57.5	14.2	0.5	12.7	87.8
No Bait	18.5	0.0	78.4	46.8	32.5	0.0	16.2	62.8
By-catch								
Synthetic	68.5	5.0	105.2	48.8	275.0	35.5	164.8	168.5
Cane	22.5	5.7	72.1	70.0	22.0	84.8	161.5	173.5
No Bait	16.2	3.7	73.1	70.8	31.0	48.8	158.2	155.2

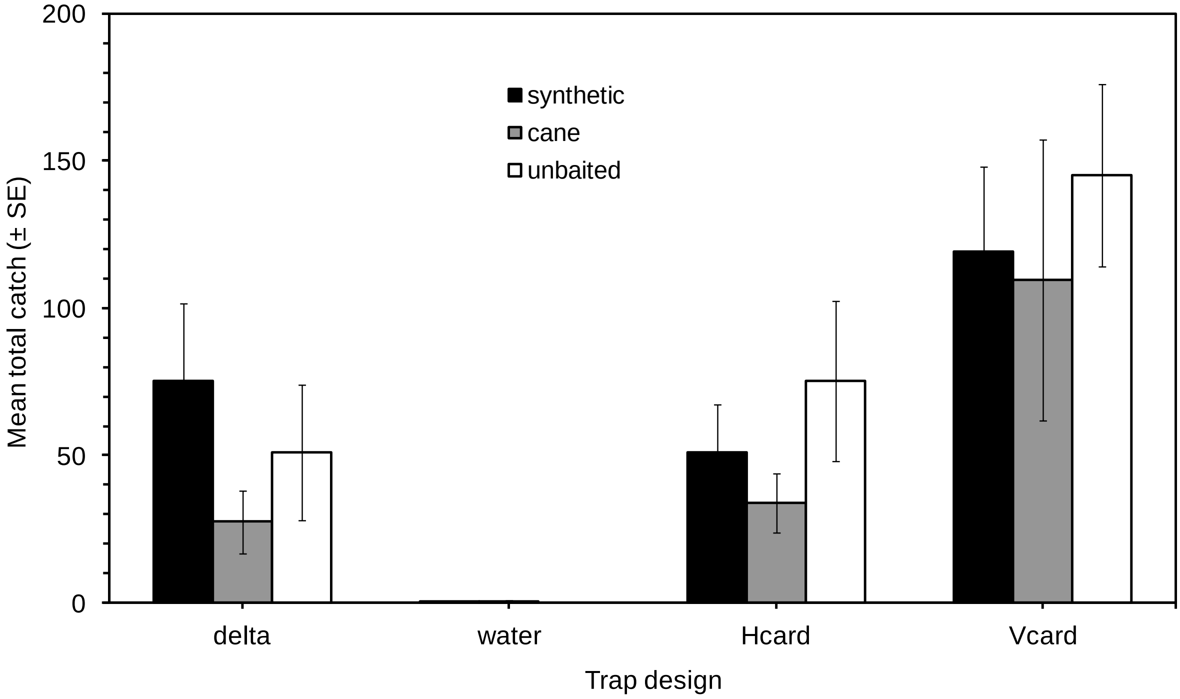

Figure 4. Mean catches of male *R. theobaldii* in different trap designs baited with synthetic lures or a split raspberry cane (8-22 July 2011).

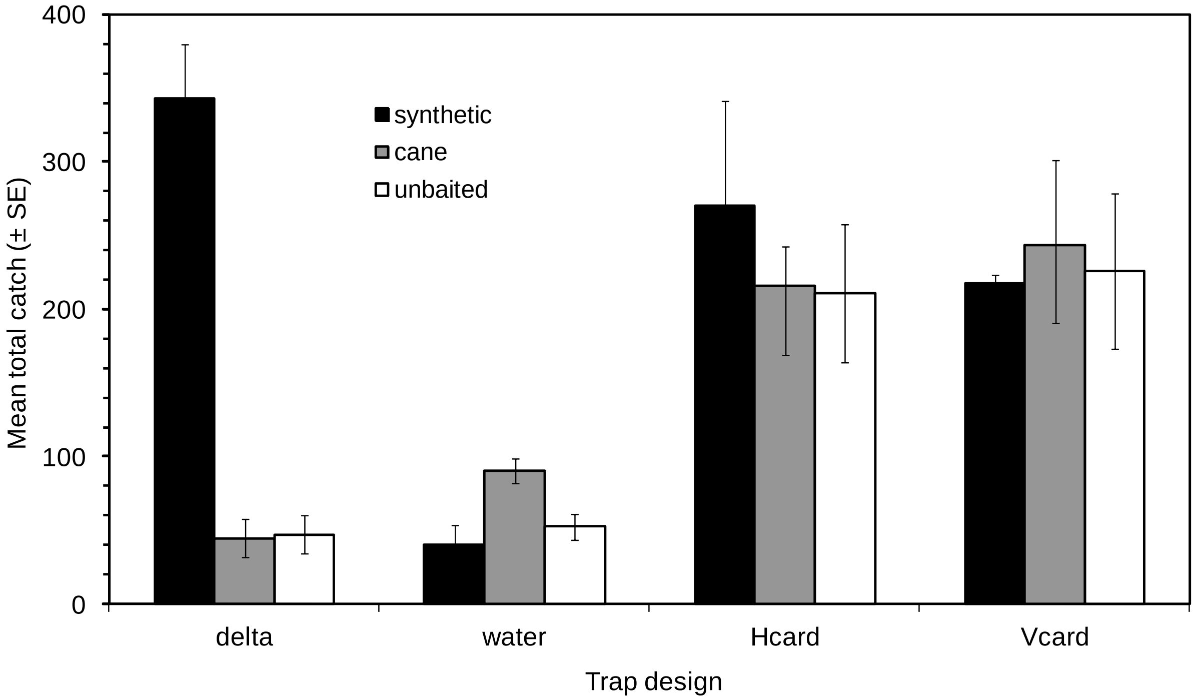

Figure 5. Mean by-catches in different trap designs baited with synthetic lures or a split raspberry cane (8-22 July 2011).

The Genstat ANOVA yielded the following F-Values (Table 3) indicating the significance of trap and lure type on the number of males, females and by-catch each week:

Table 2. F-Values after ANOVA on trap catch data over two successive weeks (*** very highly significant; no females were caught in Week 1).

Variable	Week 1			Week 2		
	Males	Females	By-catch	Males	Females	Bycatch
Lure	0.564		0.097	0.921	0.462	0.004
Trap	**<0.001*** **		**<0.001*** **	**<0.001*** **	0.057	**<0.001*** **
Lure and trap	0.097		0.027	0.852	0.962	**<0.001*** **

From Table 2 it can be seen that the numbers of female *R. theobaldii* caught by all traps and lure combinations were low.

In both weeks the numbers of males caught were lower in the water traps than in all the other traps (Table 2), giving a significant trap effect in the ANOVA (Table 3). The numbers of males in the other traps varied with bait and week, but generally the vertical traps seemed to catch the most male raspberry cane midges (Figure 4).

In the case of the by-catch, the water traps again had lower catch numbers (Table 2), giving a significant effect in the ANOVA in both weeks (Table 3). The catches with the other trap types were extremely variable. The effect of trap and lure combined was significant for the by-catch in the second week. In fact for both weeks, the by-catch in the delta traps baited with the synthetic lure was much higher than with the other lures (Figure 5).

Discussion and conclusions

The initial observations suggested that females are attracted to split primocanes. However, this was not evident in the trapping experiment. It is possible that excising the cane from the plant reduced its attractiveness or that females are simply put off by the appearance of the traps. Similarly the synthetic blend did not attract midges of either sex. Hall et al. (2010) reported some attraction, but results were very variable and inconsistent.

In conclusion more work is needed to develop a bioassay to show that females are attracted to the split canes and to further test the synthetic blend and improve its formulation. Large numbers of late larvae and pupae of *R. theobaldii* have been collected. It is hoped these will emerge over the winter so that adults can be used for bioassay and electroantennogram work. Further chemical work will be carried out on volatiles from raspberry canes.

Knowledge and Technology Transfer

A poster was presented at the HDC studentship conference at East Malling in June. The poster outlined the aims of the project and initial work.

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