Project title:	Semiochemical control of raspberry cane midge
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Project leader:	Prof. Jerry Cross, East Malling Research
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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.

Dr Chantelle Jay

Research Entomologist

East Malling Research

Signature C N Jay   Date April 2015

Report authorised by:

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GROWER SUMMARY

Headline

- Raspberry cane midge or blackberry leaf midge pheromone incorporated with a Natural Product Matrix plus pesticide look promising in the laboratory for use as an attract and kill formulation.

Background and expected deliverables

Plant feeding gall midges (Cecidomyiidae) are important pests of agricultural and horticultural crops in the UK and worldwide, often causing injury and serious crop losses. Their presence incurs significant use of crop protection products. Raspberry cane midge is an important pest of raspberry, an important and valuable crop in the UK, and at present, it can only be satisfactorily controlled with chlorpyrifos. Developing an alternative control method is therefore important.

Gall midges have powerful female-produced sex pheromones. The chemical structures of 17 species have been identified to date, including six of the most important pests of fruit crops in the UK identified by the EMR/NRI team (Hall et al., 2012). Many of these sex pheromones are successfully exploited for pest monitoring in commercial practice. However, there is also great potential to exploit them for control. We have identified the raspberry cane midge's sex pheromone (Hall et al., 2009), determined the optimum release rate for competitive attraction and have already demonstrated that it is possible to use it for control of the midge. In HortLINK project HL0175 (SF 74 – *Integrated pest and disease management for high quality raspberry production*) preliminary work was done to develop methods for controlling the raspberry cane midge using its sex pheromone (Cross et al., 2011). These trials indicated that Mating Disruption (MD) or Attract and Kill (A&K) with a high density of low dose sources was the most promising approach. Further work is needed to develop a suitable formulation for economic and practical use.

Female-produced sex pheromones attract only conspecific males. Attractants for the females, particularly mated females, would potentially be valuable for both monitoring and control of the pests. Traps baited with the attractants would give a better prediction of the laying of eggs and the appearance of larvae. There is good evidence for attraction of females of at least four species of midge – apple leaf midge, blackcurrant leaf midge, raspberry cane midge and wheat blossom midge – to volatiles from their host plants for oviposition. In previous work, in LINK project HL0175 (Cross et al., 2011) and a studentship funded by AHDB Horticulture, significant progress has been made in identification of chemicals released when raspberry canes split and become attractive to females of raspberry cane midge (Hall et al., 2011). There is clearly great potential to exploit host plant volatiles for control of gall midge pests.

The overall aim of this project is to develop an effective semiochemical-based control method for raspberry cane midge utilising the midge's sex pheromone and/or the host volatiles from cane splits.

Summary of the project and main conclusions

We have developed two promising new Natural Product Matrix amorphous flowable Attract and Kill (A&K) formulations containing the insecticide deltamethrin, which dispense the pheromones of the raspberry cane midge and the blackberry leaf midge, respectively, at suitable release rates. We have decided to work on the two midge species, which are both important pests of raspberry, to improve the chances of getting good results in efficacy trials. The formulations are highly attractive to midge males of the respective species and kill in seconds, a big improvement on wax emulsion formulations which were too slow. The next step of the project in the final year (2015-16) is to test their efficacy for control of their target pests on raspberry in replicated experiments in large field cages. We have also developed artificial sachet dispensers of the volatiles produced by splits in raspberry canes. The splits are attractive to raspberry cane midge females which use them to locate oviposition sites. Their efficacy for control of the midge by disruption of host finding is also being evaluated.

Financial benefits

Raspberry cane midge is an important pest of raspberry, an important and valuable crop in the UK, and at present, it can only be satisfactorily controlled with chlorpyrifos. Developing an alternative control method is therefore important. We have identified the midge's sex pheromone (Hall *et al*., 2009), determined the optimum release rate for competitive attraction, and have already demonstrated that it is possible to use it for control of the midge. Some midge sex pheromones have relatively complicated chemical structures and would be difficult and very costly to produce, but those of the raspberry cane midge could conceivably be produced on a large scale at comparatively low cost. The Attract & Kill approach also has the advantages that comparatively small amounts of pheromone and pesticide are likely to be required and that the pheromone is regarded as a co-formulant of the pesticide employed, considerably simplifying registration procedures.

Action points for growers

- At this stage of the project, no changes to growing practice are being advised.

SCIENCE SECTION

Introduction

Plant feeding gall midges (Cecidomyiidae) are important pests of agricultural and horticultural crops in the UK and worldwide, often causing damage and serious crop losses. In the UK wheat blossom midges, swede midge, brassica pod midge, pea midge, raspberry cane midge, pear midge and the leaf curling midges that attack apple, blackberry, blueberry, cherry, raspberry and pear are all serious pests which growers attempt to control with pesticides, with varying degrees of success. They are a significant reason for pesticide usage.

Gall midges have powerful female-produced sex pheromones. The chemical structures of 17 species have been identified to date, including of six of the most important pests of fruit crops in the UK identified by us (Hall *et al*., 2012). Many of these sex pheromones are successfully exploited for pest monitoring in commercial practice. However, there is also great potential to exploit them for control.

In HortLINK project HL0175 (HDC project SF 74, Integrated Pest and Disease Management for Raspberry Production) preliminary work was done by us to develop methods for controlling the raspberry cane midge using its sex pheromone (Cross *et al*., 2011). The efficacies of several Mating Disruption (MD), Attract and Kill (A&K) and a Mass Trapping (MT) treatment, comprising a wide range of dispenser/device types and dose rates of pheromone, were evaluated. These trials indicated that MD or A&K with a high density of low dose sources were the most promising approaches. One of the main problems encountered was sustaining an adequate release of pheromone through the season. A proprietary wax emulsion formulation (SPLAT) was the best for ease of application and steady release rate, and the most promising for further development.

Recently, work in Sweden and Switzerland has demonstrated sex pheromone mating disruption control of the swede midge (Ylva Hilbur and Jorg Samietz, pers. comm.). However, the high doses of pheromone were released from impractical dispensers and further work is needed to develop a suitable formulation for economic and practical use.

Female-produced sex pheromones attract only conspecific males. Attractants for the females, particularly mated females, would potentially be far more valuable for both monitoring and control of the pests. Traps baited with the attractants would give a better prediction of the laying of eggs and the appearance of larvae. Traps or other devices could be used to lure mated females away from the host crop or the attractant chemicals could be used to disrupt the ability of the females to find the host plant and lay their eggs. There is good evidence for attraction of females of at least four species of midge – apple leaf midge, blackcurrant leaf midge, raspberry cane midge and wheat blossom midge – to volatiles from their host plants for oviposition. Unlike many other plant-based attractants for insects, the attraction seems to be remarkably specific.

In previous work in SF 74, LINK project HL0175 (Cross *et al*., 2011), and a studentship funded by the HDC (CP 73) significant progress has been made in the identification of chemicals released when raspberry canes split and become attractive to females of raspberry cane midge (Hall *et al*., 2011). This work has involved the use of novel techniques for trapping volatile compounds, analysis by gas chromatography (GC) linked to electroantennography to detect biologically-active compounds and by GC-mass spectrometry (MS) to identify them. Eleven compounds [(Z)-3-hexenol, 6-methyl-5-hepten-2-ol, linalool, myrtenal, geranial, citronellol, methyl salicylate, myrtenol, nerol, geraniol, benzyl alcohol] were identified as being produced in larger amounts from splits in raspberry canes in comparison with un-split canes. Three of the compounds [6-methyl-5-hepten-2-ol, myrtenal and myrtenol] gave strong electroantennogram responses. However, as yet we have been unable to develop a synthetic lure attractive to females. We believe the blend and release rate need careful adjustment. In addition, a suitable trap design for trapping females has yet to be identified.

As far as we are aware, there have been no successful attempts to exploit host plant volatiles for control of gall midge pests, but there is clearly great potential. In the Netherlands, non-host volatiles are used commercially to protect grafting wounds from attack by the red bud borer (*Resseliella oculiperda*) on newly grafted apple rootstocks by incorporating them into the grafting tape used to secure and protect the graft wood, and repelling females (van Tol *et al*., 2007).

Raspberry cane midge is an ideal choice of model species for the development of semiochemical based control methods, for the following reasons:

- It is an important pest of raspberry, an important and valuable crop in the UK, and it can only be controlled currently with chlorpyrifos. Developing an alternative control method is therefore important;
- We have identified the midge's sex pheromone (Hall *et al*., 2009), determined the optimum release rate for competitive attraction, and have already demonstrated that it is possible to use it for control of the midge, though we have not yet perfected a formulation and method of application. This was the first time control of a midge pest

with a sex pheromone was demonstrated and is a very significant scientific breakthrough;

- Some midge sex pheromones have relatively complicated chemical structures and would be difficult and very costly to produce but those of the raspberry cane midge could conceivably be produced on a large scale at comparatively low cost. The A&K approach also has the advantages that comparatively small amounts of pheromone and pesticide are likely to be required and that the pheromone is regarded as a co-formulant of the pesticide employed, considerably simplifying registration procedures;
- We have identified the key host plant volatile compounds produced by cane splits that are used for female attraction and these are not produced continuously by the plant in large amounts, only when and where cane splits occur.

Raspberry cane midge is thus a good model midge species and the results will be more generally applicable to other species. In this project, we are using large field cages and potted un-infested plants for development work so that we can make replicated comparisons using known artificially introduced populations of midges.

The overall aim of this project is to develop an effective semiochemical-based control method for raspberry cane midge utilising the midge's sex pheromone and/or the host volatiles from cane splits.

Component objectives are as follows:

1. Develop a suitable formulation for sustained and adequate release of the raspberry cane midge pheromone for competitive mating disruption (MD) or attract and kill (A&K) (NRI, Year 1);

2. Investigate inclusion of an insecticide for enhancing efficacy through kill of male midges when they contact the dispensing formulation or a target device, i.e. determine whether an A&K formulation can be developed which is likely to give better results than MD (EMR, Year 1);

3. Determine the optimum number and release rate of MD or A&K sources/ha and how efficacy is affected by population size (EMR, Years 1-3);

4. Determine whether cane split finding by female raspberry cane midge can be disrupted by artificially provided host volatiles and optimise the blend for doing so (EMR, NRI, Years 1-2);

5. Determine the optimum number and release rate of host volatile sources/ha for disrupting and how efficacy is affected by population size (EMR, Years 2-3);

6. Develop a host volatile dispensing formulation for practical use (NRI, Year 3);

7. Evaluate the efficacy of the sex pheromone and/or host volatile formulations in the field, alone versus in combination, in comparison with untreated and standard grower insecticide controls (EMR, NRI, Year 3).

Materials and methods

Objective 1. Develop a suitable formulation for sustained and adequate release of the raspberry cane midge pheromone for competitive mating disruption (MD) or attract and kill (A&K) (NRI, Year 1)

In 2013 the release of the raspberry cane midge sex phermomone 2-acetoxy-5-undecanone from a wax emulsion formulation was measured. In 2014 this was compared with the release from a natural products matrix formulation (NPM) formulation (0.5% loading; 1.8 g) as measured by trapping of volatiles on Porapak (mean of two reps; dollops maintained and collections carried out in wind tunnel at 27°C and 8 km/hr wind-speed).

Objective 2. Investigate inclusion of an insecticide for enhancing efficacy through kill of male midges when they contact the dispensing formulation or a target device, i.e. determine whether an A&K formulation can be developed which is likely to give better results than MD (EMR, Year 1)

Deltamethrin, a synthetic pyrethroid (SP), was chosen as the ‘attract and kill’ insecticide because SPs have rapid knockdown and the parent company, Bayer Crop Science, agreed to support the project. Their Decis formulation, containing deltamethrin as an active ingredient (25 g/l EC), is approved and used in raspberry in the UK. Decis is also used as a coating inside the lid of a trap (the Decis trap) for the Mediterranean fruit fly (*Ceratitis capitata)* in S. Europe. Laboratory bioassays in 2013 showed that a long exposure (5 minutes) to 1 % deltamethrin in the wax emulsion formulation significantly increased the percentage of the predatory midge *Aphidoletes aphidimyza* affected (with twitching or mortality) reaching 100 % after 1 hr, however shorter exposure times (60 secs or 5 secs) typical of those that may be seen in a field situation, affected less than 50 % of individuals. SPLAT technology was therefore found to be not suitable for A&K with 1 % deltamethrin, though the technology may be suitable for mating disruption. Further bioassays showed that the deltamethrin formulation as used in the Decis traps affected 100% of the midges within one hr of a five second exposure, with 50 % of those individuals developing symptoms 20 mins after exposure. This could have a good potential for A&K for midges.

Work has continued on this objective in 2014 using *A. aphidimyza*, which is readily available year round from biocontrol suppliers. A range of laboratory pesticide bioassays were completed to identify suitable formulations for A&K. A bioassay with *A. aphidomyza* (males and females), on Decis 25 % WG treated 12.22 kg/ha Petri dishes was carried out with a 10 second exposure. Bioassays were also carried out on male midges, using rubber lures treated with two concentrations of Decis 25 % WG with only a brief five second exposure. Standard and waterproof formulations of Decis 25 % WG were also tested on a rubber lure.

To ensure that there was no repellent effect of formulations, in 2013 digital videos were taken of the behaviour of midges near a wax emulsion formulation, with specific pheromones and with and without a pesticide incorporated. This was done for the blackberry leaf midge, the raspberry cane midge and the apple leaf midge. Midges were seen to walk on and around the wax emulsion when it had dried (one+ days), in some cases for at least a minute. In 2014, the pheromone of the apple leaf midge was incorporated into the NPM with two concentrations of the pesticide, 1.25 and 3.75 %. The behaviour of the apple leaf midges near the NPM was recorded on two dates after the NPM (approx. 1 cm in diameter) was applied to the plastic dish.

Objective 3. Determine the optimum number and release rate of MD or A&K sources/ha and how efficacy is affected by population size (EMR, Years 1-3)

An experiment was done in the large cage facility at EMR to look at control of blackberry leaf curling midge and raspberry cane midge by A & K on raspberries. Twelve cages, each 12 m x 1.5 m x 2 m (L x W x H) in size, were used (Figure 1). These were separated by 24 m. Each cage contained 18 potted raspberries, of three cultivars, Glen Moy, Autumn Bliss and Glen Clova (Figure 2), placed in a zig-zag linear formation and supplied with drip irrigation. Treatments were pheromone lures coated with Decis 25 % WG, the formulation of Decis as used in Decis traps, pheromone lure alone and untreated control, with four replicates of each treatment in a randomised block design. Each pesticide treated pheromone lure was dipped in 2.86 g of Decis 25 % WG dissolved in 100 ml of water and allowed to dry. Lures were dipped on 30 April, and again on 1 July 2014. The rubber septa were attached inside the lip of each plant pot, with blackberry leaf midge on one side and raspberry cane midge on the other side. The cultivars were set up in groups, to give a randomised split-plot design. The plots were artificially infested with midge larvae collected from infested commercial plantings. Assessments for the blackberry leaf curling midge were frequent (five in total) so as not to miss generations.

For the raspberry cane midge experiment, artificial splits were made in the canes post-inoculation and these were assessed after two weeks by cutting the canes and looking for the presence of cane midge under a binocular microscope. Two main assessments were carried out on 1 and 20 August 2014.

Figure 1. The field cages

Figure 2. Three cultivars of raspberry plants in the cages.

Objective 4. Determine whether cane split finding by female raspberry cane midge can be disrupted by artificially provided host volatiles and optimise the blend for doing so (EMR, NRI, Years 1-2)

Fourteen compounds have been shown to be released by raspberry canes on splitting and are anticipated to be involved in attracting the female raspberry cane midge to suitable sites for oviposition. These have been identified and slow-release formulations have been developed for all or sub-sets of these compounds to test in the field. To determine the effect of additional host plant volatiles on female egg laying in fresh splits, an experiment was carried out in polytunnel grown raspberries at Langdon Manor Farm, courtesy of Alastair Brooks. The addition of dispensers containing cane split volatiles was compared with untreated control plots, with eight replicates in a randomised block design. The volatile dispensers were a complex blend of synthetic chemicals, some added into a slow release vial and some added to a dental roll, so that all chemicals would have a similar release rate, both placed inside a single sachet. There was 100 µl total in the vial and 100 µl total on the dental roll. The vials contained Z3-hexenyl acetate, 6-methyl-5-hepten2-one, Z3-hexenol and benzyl alcohol. The dental rolls contained 6-methyl-5-hepten2-ol, decanal, linalool, myrtenal, citral, geranial, citronellol, myrtenol, geraniol and geranyl acetone. On 8 September 2014 volatiles were put in a 3 x 3 square grid with total square size of 2.5 m x 2.5 m, i.e. three dispenser sachets on each of three plants in each of three rows. Artificial splits were made on eight separate canes in the middle row, four canes close to the central dispenser, two up the row and two down the row, and four canes in between the lures, again both up and down the row. The canes were collected two weeks later on 25-26 September 2014 and the splits were assessed for midge larvae under a binocular microscope.

Objective 5. Determine the optimum number and release rate of host volatile sources/ha for disrupting and how efficacy is affected by population size (EMR, Years 2-3)

To be completed in 2015-2016.

Results

Objective 1. Develop a suitable formulation for sustained and adequate release of the raspberry cane midge pheromone for competitive mating disruption (MD) or attract and kill (A&K) (NRI, Year 1)

Volatile collections in the wind tunnel showed a more sustained, albeit lower release rate, with values of 0.06 to 0.03 ug/h/g over 14 days with the NPM formulation (Fig. 3), as opposed to 8.0 to 2.4 ug/h/g over 14 days with the wax emulsion formulation (Fig. 4).

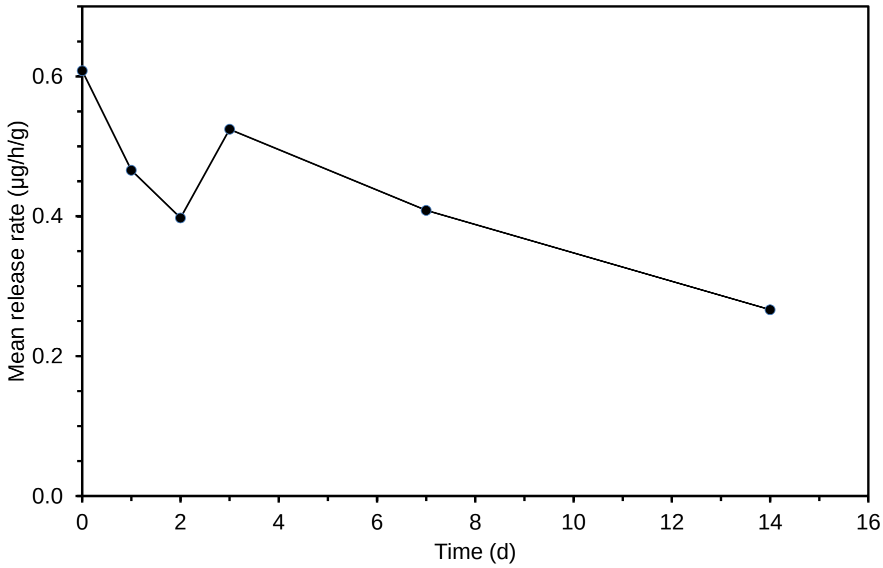

Fig. 3. Release of 2-acetoxy-5-undecanone from Natural Product Matrix dollops (0.5% loading; 1.8 g) as measured by trapping of volatiles on Porapak (mean of two reps; dollops maintained and collections carried out in wind tunnel at 27°C and 8 km/hr wind-speed).

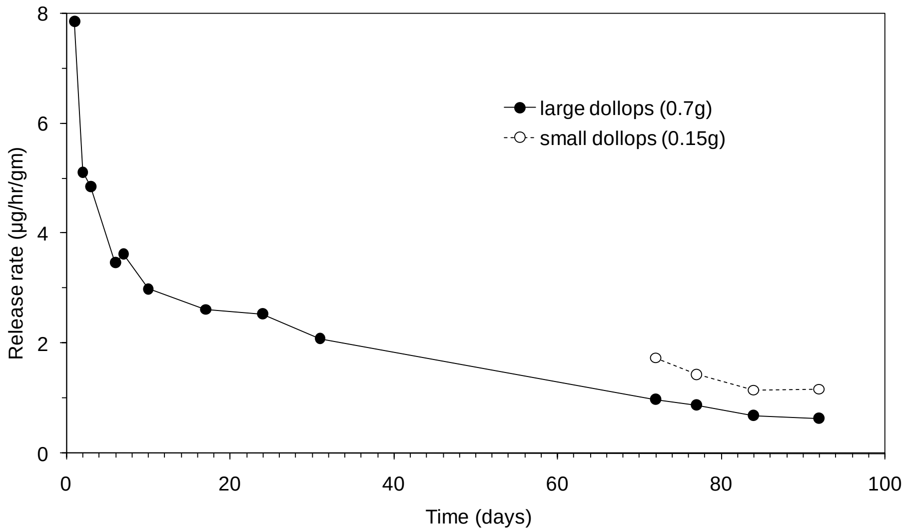

Fig. 4. Release of 2-acetoxy-5-undecanone from Wax Emulsion Formulation dollops (0.5% loading) as measured by trapping of volatiles on Porapak (mean of two reps; dollops maintained and collections carried out in wind tunnel at 27°C and 8 km/hr wind-speed).

Objective 2. Investigate inclusion of an insecticide for enhancing efficacy through kill of male midges when they contact the dispensing formulation or a target device, i.e. determine whether an A&K formulation can be developed which is likely to give better results than MD (EMR, Year 1)

Bioassays with *A. aphidomyza* (males and females), on Decis 25 % WG treated 12.22 kg/ha Petri dishes with a 10 second exposure showed that after 20 minutes, 86 % of midges were affected (twitching or mortality) in the Decis 25 % WG treatment compared to 20 % affected in the control treatment. The bioassays with the male midges, using rubber lures treated with two concentrations of Decis 25 % WG with only a brief five second exposure, found that the high rate was more effective than the low rate. In a further experiment the control treatment had no mortality at either 10 or 60 minutes, however in the high rate treatment 40 % of midges were affected after 10 minutes and 87 % were affected after 60 minutes. Standard and waterproof formulations of Decis 25 % WG on a rubber lure were similar in performance. Very few midges were dead after 10 minutes, the majority of effects were seen within an hour, although there was also high mortality in the control treatment. All midges were affected after an hour with the standard Decis 25 % WG formulation and 73 % were affected with the waterproof formulation compared to 45 % in the control.

The digital videos of the behaviour of midges near the pheromone of the apple leaf midge incorporated into the NPM with two concentrations of the pesticide, 1.25 and 3.75 %, showed that the formulation did not appear to be repellent, however it remained sticky and the midges were unable to walk from it once they had touched it.

Objective 3. Determine the optimum number and release rate of MD or A&K sources/ha and how efficacy is affected by population size (EMR, Years 1-3)

In the large cage experiment to look at control of blackberry leaf curling midge and raspberry cane midge by A&K on raspberries, two generations of blackberry leaf curling midge were seen, however larvae were most numerous for the second generation, assessed on 2 September. There was a significant reduction in the total number of larvae per plot for the pheromone alone treatment, with the control, pheromone plus pesticide and pheromone alone treatments having 46, 49 and 24 larvae respectively ($p = 0.005$, d.f. = 6, s.e.d. = 5.02), although there was no difference in the total number of shoots affected, having 19, 21 and 14 shoots affected per plot ($p = 0.228$, d.f. = 6, s.e.d. = 3.49).

For the raspberry cane midge experiment, on 1 August, there was no effect of treatment on either total larvae per plot with 41, 119 and 67 larvae for the control, pheromone plus pesticide and pheromone alone treatments respectively ($p = 0.115$, d.f. = 6, s.e.d. = 31.7), or number of canes affected with 7.5, 9 and 10 canes for the control, pheromone plus pesticide and pheromone alone treatments respectively ($p = 0.280$, d.f. = 6, s.e.d. = 1.414). There was also no effect on 20 August, with 182, 106 and 30 total larvae per plot for the control, pheromone plus pesticide and pheromone alone treatments respectively ($p = 0.145$, d.f. = 6, s.e.d. = 65) and 9, 8.5 and 5 total shoots per plot for the control, pheromone plus pesticide and pheromone alone treatments respectively ($p = 0.214$, d.f. = 6, s.e.d. =2.233).

Objective 4. Determine whether cane split finding by female raspberry cane midge can be disrupted by artificially provided host volatiles and optimise the blend for doing so (EMR, NRI, Years 1-2)

In the field experiment to determine the effect of additional host plant volatiles on female egg laying in fresh splits, both orange and white larvae were present in the samples, however there was no difference between the treated and control plots with a mean of 44 and 28 larvae per plot respectively ($p = 0.288$, d.f. = 7, s.e.d. = 14.12). It may be that the plots were not large enough to see an effect, although plots were well spaced out by at least 15 m in all directions.

Discussion

The results from the cage experiment in 2014 were disappointing as a high standard of control was not demonstrated, and with no benefit for the A&K treatment. It may be that dipping the lures in the Decis 25 WP every eight weeks was not sufficient given that the lures were not protected, although this is unlikely. However this still does not explain why fewer blackberry leaf midge larvae were found per plot in the pheromone treatment.

The NPM formulations are showing huge potential and assays in 2015 are looking at different viscosities of the NPM formulation, with and without the pesticide incorporated, in laboratory bioassays. The cage experiments will be repeated in 2015 using the NPM formulations.

Although the volatile experiment did not show the result as expected it was interesting to see that the female midges still found the artificial splits even with the high rate volatile lures in the vicinity. It may be that the plots were not far enough apart to see an effect between treatments, even though they were well spaced out by at least 15 m in all directions. Thilda Nilsson in her MSc thesis suggests distances of greater than 20 m for pheromone point sources.

Conclusions

We have developed two promising new Natural Product Matrix amorphous flowable Attract and Kill (A&K) formulations containing the insecticide deltamethrin which dispense the pheromones of the raspberry cane midge and the blackberry leaf midge, respectively, at suitable release rates. The formulations are highly attractive to midge males of the respective species and kill in seconds, a big improvement on wax emulsion formulations which were too slow. The next step of the project in the final year (2015-16) is to test their efficacy for control of their target pests on raspberry in replicated experiments in large field cages.

Knowledge and Technology Transfer

Presentation to the Leafy Salads Group, EMR, March 2015

- 2 April 14: J Cross gave a 45 minute seminar about the project to CRD staff
- 24 Oct 14: J Cross gave a 30 minute presentation about the project to Waitrose technical staff and management and their Berry Gardens Growers suppliers.
- 26 Nov 14: J Cross gave a 30 minute presentation about the project at the HDC/EMRA Soft Fruit day at EMR
- 11 Dec 14: J Cross gave a 1/2 day technical seminar to 15 Bayer Crop Science experts and specialists at Monheim, Germany, and where commercial development opportunities were discussed
- 19 Jan 2015: J Cross gave a brief overview of the project to senior staff from Rothamsted Research
- 28 Jan 2015: J Cross gave a 30 minute talk about the project to ASDA staff and their Berry Gardens Grower suppliers
- 19 Mar 15: J Cross included a description of the project and its results in the science presentation to the final meeting for HortLINK project HL01105 (blackcurrant IPDM)

Glossary

A&K - Attract and Kill

BLM - Blackberry Leaf Midge

NPM - Natural Product Matrix

RCM - Raspberry Cane Midge

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