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DISCLAIMER

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

- A Natural Product Matrix formulation containing a synthetic pyrethroid product and incorporating raspberry cane midge or blackberry leaf midge sex pheromone is promising for use for attract and kill of the target pests.

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. They are a significant cause of the use of traditional crop protection products. Raspberry cane midge is an important pest of raspberry, an important and valuable crop in the UK, and it can only be controlled currently with chlorpyrifos which has recently been withdrawn from use. 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 Defra Horticulture LINK 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 was 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 pest. 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 Defra Horticulture LINK project HL0175 (Cross et al., 2011) and a studentship funded by AHDB Horticulture, 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). There is clearly great potential to exploit host plant volatiles for control of gall midge pests.

The overall aim of this project was 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. It was decided that parallel work would be done on the blackberry leaf midge, which is also a serious pest of raspberry, to increase the productivity of the work and the chances of success.

Summary of the project and main conclusions

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, were developed, in collaboration with Bayer CropScience. We targeted 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 male midges of the respective species and kill in seconds, a big improvement on wax emulsion formulations (e.g. SPLAT) which were too slow acting. Disappointingly, we were not able to demonstrate their efficacy for control of their target pests on raspberry in replicated experiments in large field cages in the final year of the work, possibly because dose rates and/or density of deployment were too low. However, the formulations are promising, and further work is needed to explore rates of use and method of deployment.

Artificial sachet dispensers of the volatiles produced by splits in raspberry canes were produced during the project. However a reduction in egg laying by females was not proven where sachets were deployed in the field.

The Natural Product Matrix formulation has great potential for attract and kill of numerous other pests internationally.

Financial benefits

Raspberry cane midge is an important pest of raspberry, an important and valuable crop in the UK, and it can only be controlled currently with chlorpyrifos, which has been withdrawn from use. 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 A&K approach also has the advantages that comparatively small amounts of pheromone and crop protection product are likely to be required and that the pheromone is regarded as a co-formulant of the control product employed, considerably simplifying and reducing the cost of registration procedures.

Action points for growers

- 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 injury 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 cause of pesticide usage.

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.

In HortLINK project HL0175 (Raspberry Integrated Pest and Disease Management) preliminary work was done 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 was the most promising approach. 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 (Samietz *et al*., 2012). 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 LINK project HL0175 (Cross *et al*., 2011) and a studentship funded by the HDC 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). 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 unsplit 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, which repels females (van Tol *et al*., 2007).

Raspberry cane midge is an ideal choice of model species for 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 uninfested 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. It was decided that parallel work would be done on the blackberry leaf midge, which is also a serious pest of raspberry, to increase the productivity of the work and the chances of success. 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, Yr 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, Yr 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, Yrs 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, Yrs 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, Yrs 2-3)

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

As the work builds on the results from consecutive seasons and has incorporated two main pest species, the work packages have developed as below.

Detail of work objectives across the project.

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, Yr 1)
This work was carried out across all three years as different formulation types were tested.
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, Yr 1)
Work on this objective continued for years 1-3, working on insecticide dipped rubber septa, wax emulsion (SPLAT) and natural products matrix (NPM) formulations. This included laboratory bioassays and digital recordings of behaviour.
3. Determine the optimum number and release rate of MD or A&K sources/ha and how efficacy is affected by population size (EMR, Yrs 1-3)
In Yr 1 a field planting was inoculated with RCM larvae to provide a source of infestation for Yrs 2-3. Work was done in 12 field cages at EMR to compare MD and A&K formulations. Different formulations were tested in each year (Year 2, pheromone lures were coated with Decis 25 % WG; Year 3, a NPM was used). The experiment was widened to also include BLM as well as RCM. In both years, larvae of RCM and BLM were introduced. No work was done on population densities due to the progression in formulation types.
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, Yrs 1-2)
In Yr 1 Volatiles were optimised and a single sachet system was produced. In Yr 2 a field experiment was done on natural populations of RCM.
5. Determine the optimum number and release rate of host volatile sources/ha for disrupting and how efficacy is affected by population size (EMR, Yrs 2-3)
In Yr 3 the field experiment was repeated with a higher sachet density. A cage experiment was also done. The release rate did not need to be changed. As established populations were used, the population size was not altered.
6. Develop a host volatile dispensing formulation for practical use (NRI, Yr 3)
The host volatile sachets proved easy to use in the field, therefore no further work was done on this objective.
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, Yr 3)
Final year work concentrated on further results for Objective 2 with the new formulation.

Summary of Years 1 & 2

Objective 1. To 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), the release of the raspberry cane midge sex pheromone 2-acetoxy-5-undecanone in volatile collections in the windtunnel was measured from different formulations. An existing wax emulsion formulation (SPLAT) gave a release rate of 8.0 to 2.4 µg/h/g over 14 days. However, as the wax emulsion formulation locked up the insecticide, midges touching the surface died too slowly, and an alternative natural products matrix formulation (NPM) formulation was developed in conjunction with Bayer CropScience. This formulation resulted in rapid dose transfer and kill. It showed a more sustained, albeit lower release rate, with values of 0.6 to 0.3 µg/h/g over 14 days.

Objective 2. Deltamethrin, a synthetic pyrethroid (SP), was chosen as the attract and kill insecticide because SPs have rapid knockdown and the parent company, Bayer CropScience 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 in S. Europe.

Laboratory bioassays

Initial bioassays were done on the predatory midge *Aphidoletes aphidimyza* as a model species. This insect is available from commercial biocontrol companies all year round and is a similar size to the target species. 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 1 hr of a 5 second exposure, with 50 % of those individuals developing symptoms 20 mins after exposure. In 2014, 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 5 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.

Recording of insect behaviour

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 (BLM), the raspberry cane midge (RCM) and the apple leaf midge (ALM). Midges were seen to walk on and around the wax emulsion when it had dried (1+ days), in some cases for at least a minute. In 2014, 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. To determine the optimum number and release rate of MD or A&K sources/ha, in 2014 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, separated by 24 m. Each cage contained 18 potted raspberries, of 3 varieties, Glen Moy, Autumn Bliss and Glen Clova (Image 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 varieties 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, with each plot receiving the same number of larvae.

Assessments for the blackberry leaf curling midge were frequent (5 in total) so as not to miss generations. Two generations of BLM 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). To assess the effect on raspberry cane midge, artificial splits were made in the canes post-inoculation and these were assessed after 2 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. 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 ($p = 0.115$, 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 ($p = 0.145$, 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. 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. 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 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.

In 2014, 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, Langdon Manor Farm. The addition of dispensers containing cane split volatiles was compared with untreated control plots, with eight replicates in a randomised block design. Volatiles were put on 8 September 2014 in a 3 x 3 square grid with total square size of 2.5 m x 2.5 m, i.e. 3 dispenser sachets on each of 3 plants on each of 3 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. 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). This suggests that cane split finding by female raspberry cane midge may be difficult to disrupt by artificially provided host volatiles.

Materials and methods

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, Yr 1)*

In 2015, videoing and observations were done in the laboratory to record bioassays of commercially purchased *Aphidoletes aphidomyza* with different viscosities of the NPM both with and without pesticide incorporation. Whilst there was no pheromone component this gave an indication of the time to kill, the stickiness of the dollop, the coverage of the formulation on the insect, whether the insect would sink into the dollop, or whether the insect could 'touch and escape' form the dollop. Digital videos were also done in a field setting for with the standard formulation both for RCM and BLM: RCM were videoed both in a soil grown field plot at EMR and in the field cages at EMR, the BLM were recorded in a commercial field plot. Note that all recordings were done on a dollop dispensed onto either a natural substrate or onto a glass Petri dish. Plastic Petri dishes, whilst disposable, have issues with static that affect the behaviour of the insects.

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, Yrs 1-3)*

An experiment was done in the large cage facility at EMR (Image 1) to look at control of blackberry leaf curling midge and raspberry cane midge by A & K on raspberries. There were four replicates of three treatments 1) A&K, 2) MD, 3) Untreated control, in a randomised split-plot block design (Fig. 1). There were 9 pots with the variety Glen Ample and 9 pots with the variety Kweli per cage (Image 2). A treatment of 10,000 0.2 g dollops per ha was used i.e. 1 dollop per m^2 and a total of 3.6 g per cage (=14.4 g/treatment) for each species. Each plot was an 18 m^2 ground area cage. Therefore:

- Treatment 1 had 1 x 0.2 g dollop of NPM + deltamethrin + *R. theobaldi* pheromone and 1 x 0.2 g dollop of NPM + deltamethrin + *D. plicatrix* pheromone per pot.
- Treatment 2 had 1 x 0.2 g dollop of NPM + *R. theobaldi* pheromone and 1 x 0.2 g dollop of NPM + *D. plicatrix* pheromone.
- Treatment 3 was untreated.

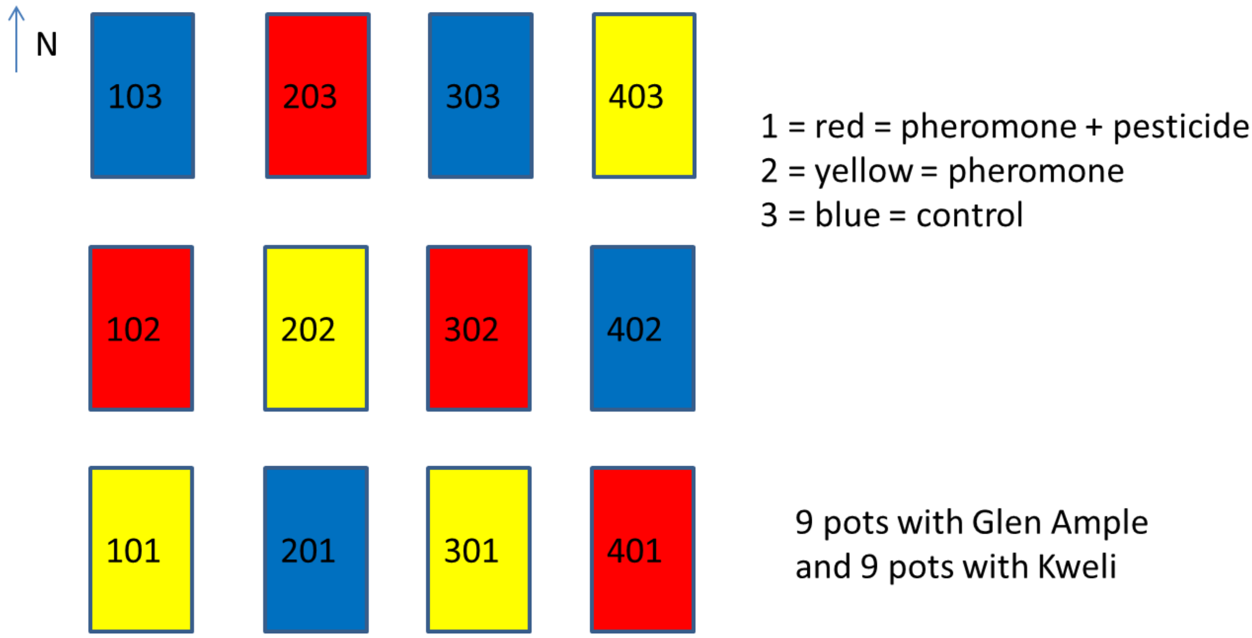

Figure 1. Experimental plan to determine the effect of NPM formulations on MD and A&K

Treatments were applied by adding a 0.2 g ‘dollop’ to a small piece of white card, which was then attached to the plant pot, so that it was on the surface of the soil, with a paper clip (Image 3). They were applied at the start of the experiment in the first week of July and a new ‘dollop’ was introduced on 7 August 2015.

The plots were artificially infested with late instar larvae that were ready to pupate. These were applied to the soil surface in the plant pots. The blackberry leaf midge larvae were collected from infested commercial plantings and were inoculated on 29 July (60 per cage) and 21 August (20 per cage). Raspberry cane midge were inoculated on 8 July (60 per cage) and 28 July (60 per cage). To assess the effect of the treatments on raspberry cane midge, artificial splits were made in the canes post-inoculation and these were assessed after a further 2 weeks by either direct observation in the field, or by cutting the canes and looking for the presence of cane midge under a binocular microscope. Assessments were made according to the schedule in Table 1. Assessments for the blackberry leaf curling midge were done on the 12 and 25 August by looking at the number of damaged tips, leaves and the number of larvae present.

Table 1. Timetable of assessments for raspberry cane midge

Date splits were made in raspberry canes			Date of assessment	
Colour code	Variety	Date	Field	Lab
red	Glen Ample	08/07/2015	06/08/2015	-
red	Kweli	08/07/2015	13/08/2015	-
yellow	Glen Ample	24/07/2015	06/08/2015	13/08/2015
yellow	Kweli	28/07/2015	13/08/2015	-
blue	Glen Ample	07/08/2015	-	21/08/2015
blue	Kweli	12/08/2015	-	28/08/2015
white	Glen Ample	21/08/2015	-	08/09/2015
white	Kweli	28/08/2015	-	15/09/2015

Image 1. The field cages

Image 2. Raspberry varieties Glen Ample and Kweli in the cages

Image 3. The NPM dollops applied to cards and attached to the pots

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, Yrs 2-3)*

Experiment 1

Following the A&K cage experiment, a further experiment was set up in the cages late in the season to utilise any emerging midges from the pots. The Glen Ample plants were removed from the cages, and five new Glen Ample plants were added to the cages, spaced evenly along the cage. The Kweli plants were left in the cages, but cut back to ground level, as any pupae would already be in the soil. Only the cages which had previously been used as control and pheromone treatments in the previous experiment were used. These were randomised so that there was an equal split between the treatments in the second experiment. The treatments were ‘control’, with no host volatile source, and ‘treated’, with a host volatile dispenser attached to a tent peg which was stuck into the soil close to the plant. The volatile dispensers were as described in Objective 4. The experiment was in a randomised block design with 4 replicates of the two treatments. Two splits were made in each of two canes per plant on 29 September 2015, and the canes were cut and collected on 19 October and larvae were counted under a binocular microscope. The experiment was started later than anticipated due to the delay in the supply of one of the chemicals.

Experiment 2

In 2015 an experiment to determine the effect of high density host plant volatiles on female egg laying in fresh splits, was carried out in polytunnel grown raspberries, Langdon Manor Farm. The addition of dispensers containing cane split volatiles, as in objective 4, was compared with untreated control plots, with four replicates in a randomised block design. The volatile dispensers were as in experiment 1. The volatiles were put out on 2 October 2015, however the temperature was still warm in the tunnels at this time. A separate tunnel was used for each block, with three tunnels in between. The plots were widely spaced (25 m) and at either ends of the tunnel, but at least 10 m into the tunnel to minimise edge effects. There were three rows of raspberry per tunnel and volatiles were attached to the lower plant wires on every other plant (Image 4), with 5 per row to form to form a 5 x 3 square grid with total square size of approx. 3 m x 2.5 m, i.e. 15 dispenser sachets on each of 5 plants on each of 3 rows. Artificial splits were made on the 15 canes close to the dispensers. The canes were collected two weeks later on 20 October 2015 (Image 4). The position of each cane in the grid was marked. The splits were assessed for midge larvae under a binocular microscope.

Images 4a and b. The experiment in a commercial planting at Langdon Manor Farm: the plant volatile dispenser and cutting canes

Results

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, Yr 1)

In 2015, the digital videos and observations again focussed on the NPM (Image 5). The laboratory bioassays using commercially purchased *Aphidoletes aphidomyza* with different viscosities of the NPM both with and without pesticide incorporation showed that all viscosities were sticky to the insects and it was difficult for insects to escape from the dollop when they had flown in to it. As there was no viscosity level which was firm enough for an insect to walk over, in order to pick up a dose of pesticide and then to fly away, it was considered that the standard dose would be used, as insects could potentially sink into the dollop and allow further insects to contact the substance. The digital videoing in the field was not successful for RCM as only 3 midges were observed. Recordings in the commercial blackberry plot were successful and showed the BLM arriving at the 'dollops' within minutes. The majority of BLM became fully stuck on the dollop, although one individual that walked towards and touched the dollop with one leg was able to release from the dollop and fly away. The dollops were filmed at ground level or on the soil surface of the pots, depending on the planting.

Image 5. A Natural Product Matrix ‘dollop’ with some attracted adult midges stuck to the surface. Other midges which escape died very rapidly from insecticide exposure

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, Yrs 1-3)

In 2015 the large cage experiment again looked at control of BLM and RCM by A&K on raspberries, this time using the NPM. There were low numbers of BLM found on the Kweli plants, and statistical analysis was not possible. This may have been due to the stage of the plants as previous damage was recorded on the same plants in a pre-treatment assessment showing that this variety is susceptible to BLM. The inoculations of raspberry cane midge experiment worked well this season. However, the mean total number of RCM collected per cage (from all assessments and both species of raspberry) were not significantly different, with 1166, 1268 and 1243 RCM in the cut splits in the A&K, MD and untreated control treatments respectively ($P = 0.946$, d.f. 6, s.e.d. 318.4, l.s.d. 779.2), full data and analyses can be seen in Table 2. The temperatures in the cages can be seen in Appendix 1.

Table 2. The effect of attract and kill (pheromone + pesticide) and mating disruption (pheromone only) treatments on the number of raspberry cane midge collected from man-made splits in two varieties of raspberry, Glen Ample and Kweli across 9 assessment dates in August and September.

		Assessment								
	Variety	**GA**	**GA field***	**GA lab***	**K**	**K**	**GA**	**K**	**GA**	**K**
	No. RCM Larvae	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
pheromone + pesticide	**total**	**1.25**	**81**	**45.5**	**269**	**204**	**106**	**151.5**	**64**	**245**
	white	0	7.5	22.5	73.8	48.2	16.2	20.8	32	102
	orange	1.25	73	23	196	156	89	130.8	31.3	142
pheromone	**total**	**1.5**	**70**	**42**	**233**	**196**	**83**	**146.5**	**60**	**436**
	white	0	10.2	16.5	60	49.2	19.2	24	36	172
	orange	1.5	60	25.5	173	147	63	122.5	24.5	264
control	**total**	**1.75**	**56**	**61.2**	**207**	**204**	**94**	**198.2**	**104**	**318**
	white	0	7.2	42.5	49	46	21.8	26.2	68	104
	orange	1.75	49	18.8	158	158	72	172	36.2	214
Statistics	s.e.d. total	0.825	41.1	19.65	50.1	43.2	28.8	20.35	41.4	183.1
	l.s.d. total	2.019	100.6	48.09	122.7	105.8	70.5	49.79	101.4	448
	d.f.	6	6	6	6	6	6	6	6	6
	p	0.837	0.838	0.606	0.498	0.981	0.744	0.081	0.54	0.6

Variety = Glen Ample (GA) and Kweli (K)

*These were the same canes, but given the longer development time of an extra 7 days between the field and the lab assessment, it can be seen that there are fewer orange pupae, as these have dropped to the ground to pupate. Larvae change from white to orange as they develop.

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, Yrs 2-3)*

Experiment 1

This experiment to look at the effect of plant volatiles on female egg laying was carried out in field cages. Few larvae were found, perhaps due to the delayed start, and some of the orange larvae were already dead, perhaps due to fungal pathogens. Although low numbers of larvae were found (Table 3), the larvae were only found in 1 of the 4 blocks when a host plant volatile dispenser was next to the raspberry plant.

Table 3. The effect of host volatile dispensers on the numbers of RCM larvae found in cut splits.

	Total number of larvae from 10 splits (2 canes per pot)			
	Control		Treated	
Block	White	Orange	White	Orange
1	0	1a 7d	0	8d
2	13	2a 1d	0	0
3	2	5	0	0
4	5	1	0	0

Where a = alive d = dead, larvae change from white to orange as they develop.

Experiment 2

This experiment to determine the effect of high density host plant volatiles on female egg laying in fresh splits, was carried out in polytunnel grown raspberries. Again, few larvae were found, and they were mainly present in two blocks (Table 4). The larvae were mainly orange, with one white larva found. Whilst the data are not able to be analysed statistically, this does suggest that the female midges are still able to find splits and lay even when a plant volatile dispenser is nearby.

Table 4. Total number of raspberry cane midge larvae found in the high density plant volatile experiment, with number of canes with damage shown in parenthesis.

Block	Treated	Control
1	21 (5)	1 (1)
2	8 (2)	6 (1)
3	0	6 (2)
4	0	0

The temperatures in the tunnels were still warm, reaching 20.5 °C as seen in Appendix 2.

Discussion

In the 2015 season it was difficult to show effective control of RCM with the different A&K treatments in the large cage experiment. This may have been because the inoculations with larvae were too effective, producing too many adult midges, with a total of over 1100 larvae found per treatment. Ideally, additional experiments would also be done with differing population densities, however this was not possible as the formulation type changed in each year. This gave a single season to work with each formulation on the main objective i.e. to determine if an A&K formulation could be developed. Although our experiments were inconclusive, this does not necessarily mean that this approach would not be effective in the field, as video studies in commercial blackberry plantings showed that the BLM was attracted within minutes to the NPM dollop, with the majority of the midges getting stuck immediately. This work is still promising, but needs to be further explored.

The raspberry volatile work did not achieve high RCM numbers in 2015, however, cane midge larvae were present in some canes adjacent to plant volatile dispensers, as also seen in 2014. Therefore it seems unlikely that this would be a promising treatment to prevent egg laying, in the current format, especially given the number of chemicals required in these dispensers. However, as the two experiments in 2015 gave different results it would be worth progressing this research.

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. It has been difficult to test their efficacy for control of their target pests on raspberry in replicated experiments in large field cages, however videos of blackberry midge behaviour around NPM dollops in a commercial blackberry planting showed that the midges could be attracted in the field within minutes, therefore the concept could potentially work in commercial plantings. This formulation could have significant advantages over other pheromone based control strategies including greater activity,

speed of action/dose transfer and lower costs. The formulation has potential for A&K of numerous pests of crops worldwide, which will be explored in future research.

Knowledge and Technology Transfer

2 Apr 2014: J Cross gave a 45 minute seminar about the project to CRD staff.

24 Oct 2014: J Cross gave a 30 minute presentation about the project to Waitrose technical staff and management and their Berry Gardens Growers suppliers.

26 Nov 2014: J Cross gave a 30 minute presentation about the project at the HDC/EMRA soft fruit day at EMR.

11 Dec 2014: J Cross gave a 1/2 day technical seminar to 15 Bayer 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.

18 Mar 2015: Presentation to the Leafy Salads Group.

19 Mar 2015: J Cross included a description of the project its results in the science presentation to the final meeting for HortLINK project HL01105 (blackcurrant IPDM).

22 Jul 2015: C Jay included a description of the project as part of a walking tour at Fruit Focus, with approx. 60 visitors to the stand.

25 Nov 2015: J Cross presented the results of the work as an oral presentation at the AHDB Soft Fruit Day.

Glossary

A&K - Attract and Kill

BLM - Blackberry Leaf Midge

NPM - Natural Product Matrix

RCM - Raspberry Cane Midge

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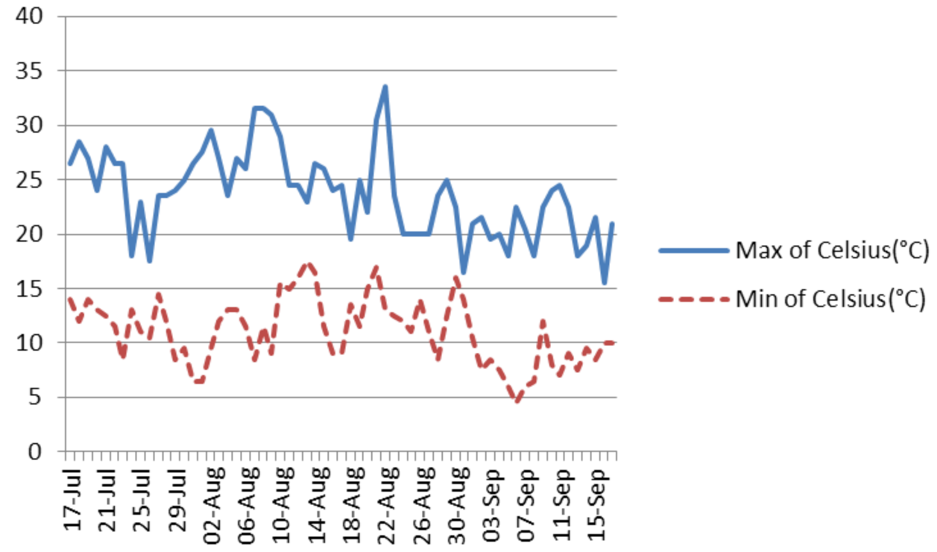
Appendices

Appendix 1

The temperatures in the field cages



Appendix 2

The temperatures in the polytunnels