Horticultural Fellowship Awards

Final Report Form

Project title:	Maintaining the expertise for developing and communicating practical Integrated Pest Management (IPM) solutions for Horticulture
Project number:	CP 89
Project leader:	Jude Bennison, ADAS
Report:	Final Report , 18 April 2016
Previous reports:	Interim reports 2012, 2013, 2014 & 2015
Fellowship staff:	Jude Bennison, Senior Entomologist, ADAS Boxworth (lead Fellowship mentor) Mike Lole, Senior Entomologist, ADAS (mentor) Steve Ellis, Senior Entomologist, ADAS High Mowthorpe (mentor) Chris Dyer, Statistician, ADAS (mentor) Heather Maher, Senior Research Manager, ADAS Boxworth (mentor until August 2012, ad hoc training after this date) Kerry Boardman, Senior Research Manager, ADAS Boxworth (mentor on some projects, trainee on others)

("Trainees")	Gemma Hough, Entomologist, ADAS Boxworth (Fellowship trainee Entomologist until October 2015) Sacha White, Entomologist, ADAS Boxworth (Fellowship trainee Entomologist from May 2013) Chloe Whiteside, Research Technician, ADAS Boxworth (Fellowship trainee scientific support staff until October 2013, now an ADAS Horticultural Consultant) Robert Drummond, Technician, ADAS Boxworth (Fellowship trainee scientific support staff until October 2014) Abby Wood, Technician, ADAS Boxworth (Fellowship trainee scientific support staff until January 2014) Steven Richardson, Technician, ADAS Boxworth (Fellowship trainee scientific support staff)
Location of project:	ADAS Boxworth and commercial farms and nurseries
Industry Representative:	-
Date project commenced:	01 April 2011
Date project completed (or expected completion date):	18 April 2016

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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.

Jude Bennison
Senior Research Entomologist
ADAS

Signature ……..Date .....18 April 2016.....

Report authorised by:

ADAS
Barry Mulholland

Signature .....Date .....18 April 2016.....

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Progress Against Objectives

Objectives

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
1. Provide mentoring of two next generation ADAS research entomologists to equip them with the knowledge, skills, competencies and flexibility required to develop IPM strategies on horticultural crops.	31/03/2016	18/04/2016	18/04/2016
2. Deliver practical solutions to selected current and emerging pest management problems through specific applied research projects.	31/03/2016	18/04/2016	18/04/2016
3. Transfer knowledge and new IPM developments to the industry through a range of communication media.	31/03/2016	18/04/2016	18/04/2016

Summary of Progress

Objective 1: Mentor two 'next generation' IPM research Entomologists

Tom Pope was already in post at ADAS Boxworth at the start of the Fellowship. He joined ADAS in 2009 and worked with Jude Bennison and colleagues on a range of projects investigating the biology and control of various horticultural pests including aphids, cabbage root fly and vine weevil. As part of the Fellowship Tom led work on overwintering predatory mites in soft fruit, biological control of vine weevil, incidence of aphid hyperparasitoids and biological control of aphids on outdoor lettuce. In August 2012, Tom left ADAS to join Harper Adams University as a lecturer in entomology and applied pest management research, where he is now training future entomologists. Tom is now a valued research collaborator with ADAS, who has worked with Jude Bennison and her team in two Defra-funded IPM projects, the AHDB Horticulture vine weevil review, the current AHDB Horticulture project HNS 195 'Improving vine weevil control in HNS' and the current AHDB Horticulture project CP 124 'Managing Ornamental Plants Sustainably' (MOPS).

Gemma Hough joined ADAS Boxworth and replaced Tom Pope as a research entomologist in December 2012 after completing a AHDB Horticulture-funded PhD studentship on the biology and control of currant lettuce aphid at Warwick University. As part of the Fellowship Gemma took over work on biological control of vine weevil, biological control of aphids on lettuce and monitoring hyperparasitism in HNS. Gemma was involved in a range of AHDB

Horticulture projects which included the review of vine weevil control and MOPS (in which Gemma led the vine weevil work). Gemma also led AHDB Horticulture projects on *Scaptomyza flava* on baby-leaf salads (FV 408a) and evaluating aphid control strategies (FV 435). Gemma left ADAS in October 2015 to join Syngenta as Insecticide Development Manager.

Gemma Gillies joined ADAS Boxworth in October 2011 and assisted on Fellowship projects taking over work on biological control of vine weevil in August 2012. Gemma left ADAS to return to teaching in December 2012 and ADAS recruited Sacha White to replace her in its pest management team.

Sacha White joined ADAS in May 2013. Sacha completed his PhD at the University of Warwick, looking at the implications of new sustainable greenhouse systems for pests, diseases and biological control. He also completed the Integrated Pest Management MSc at Imperial College London and has previous experience in various aspects of entomological research. As part of the Fellowship Sacha has worked on the biological control of aphids in field-grown lettuce, the identification of thrips species on strawberry during 2014, controlling vine weevil larvae with the predatory beetle *Atheta coriaria* during 2015 and on using *Neoseiulus cucumeris* as a vector for entomopathogenic fungi for thrips control in 2016. Sacha has also worked on a projects investigating improved control of the invasive Oak Processionary Moth (Defra funded) and contributed toward the AHDB "Encyclopedia of pests and natural enemies in field crops" (AHDB cross-sector funded). He has been project leader for projects investigating insecticide resistance in the UK (part-AHDB Horticulture funded) and control of cabbage root fly (commercial), mangold fly in sugar beet (BBRO), cabbage stem flea beetle in oilseed rape (OSR) (AHDB Cereal and Oilseeds and commercial), slugs in wheat (commercial), wireworm in potatoes (commercial) and peach-potato aphid in OSR (commercial).

Mentoring activities during the fifth year of the Fellowship included:

Visits to commercial nurseries and farms

During 2015-2016 visits were made as follows:

Hardy nursery stock: Gemma Hough visited HNS growers while collecting plants infested with leaf and bud nematodes for the MOPS project, CP 124.

Soft fruit: Gemma Hough visited raspberry growers while monitoring the effects of pesticides applied for the control of spotted wing drosophila on predatory mites and spider mite control, in this Fellowship project and in AHDB Horticulture project SF 158 'Integrated Management of cane fruit pests and diseases'.

Field vegetables: Gemma Hough visited rocket growers in East Anglia while collecting *Scaptomyza flava* for project FV 408a.

Protected edibles: Kerry Boardman and Steven Richardson visited a protected edibles propagator whilst working on a CRD-funded experiment in project P2154 on using combinations of biopesticides and pesticides for control of cabbage root fly.

Pest and biocontrol agent identification

Laboratory training on identification of key horticultural pests was completed by Gemma Hough and Sacha White as well as key members of the scientific support team at ADAS Boxworth during 2015. Training courses included:

- Predatory mite identification (training given by Mike Lole)
- Thrips identification refresher course (given by Jude Bennison and Mike Lole)
- Extracting entomopathogenic nematodes from soil samples using a modified Baermann funnel (training given by biopesticides consultant Roma Gwynn and Jude Bennison)
- Extracting and identifying leaf and bud nematodes (training given by Heather Maher)
- Extracting DNA from nematodes and running a Loop mediated isothermal amplification (LAMP) method (training given by ADAS biotechnology consultant Ben Maddison)
- Training on mangold fly identification (training given by Mike Lole)

Scientific conferences attended:

- Gemma Hough gave a paper on currant-lettuce aphid at the EUCARPIA Leafy Vegetable congress, Murcia, Spain 14-17th April 2015
- Sacha White gave a paper on work completed in Defra-funded project TH0102 on Improving control of Oak Processionary Moth at the IOBC meeting Microbial and Nematode Control of Invertebrate Pests, Riga, Latvia 7-11th June 2015.

Objective 2: Deliver practical solutions to selected current and emerging pest management problems through specific applied research projects

2012 projects

● Contribution of overwintered predatory mites to pest mite control on strawberry – Tom Pope

● Aphid hyperparasitoids on protected edibles, soft fruit and ornamentals – Tom Pope

● Biological control of aphids on lettuce – Tom Pope

● Efficacy of entomopathogenic nematodes against vine weevil – Gemma Gillies

2013 projects

● Efficacy of entomopathogenic nematodes against vine weevil – Gemma Hough

● Aphid hyperparasitoids on protected ornamentals – Gemma Hough

● Biological control of aphids on lettuce – Gemma Hough and Sacha White

● Review of the control of leaf and bud nematodes – Gemma Hough

2014 projects

● Monitoring rose thrips (*Thrips fuscipennis*) at commercial strawberry sites – Gemma Hough, Sacha White and Steven Richardson

● Comparing damage by *T. fuscipennis* with *Frankliniella occidentalis* (western flower thrips) – Gemma Hough

● Literature review on current knowledge of *T. fuscipennis* biology, overwintering sites and natural enemies – Gemma Hough

● Potential of the predatory beetle, *Atheta coriaria*, for biological control of vine weevil – Sacha White

2015 projects

● Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil – Gemma Hough and Steven Richardson

● Determining the speed of kill of adult vine weevil when using E-nema weevil-stop traps – Gemma Hough and Steven Richardson

- Monitoring the effects of insecticide applications for spotted wing drosophila on predatory mites and two spotted spider mites in raspberries – Gemma Hough and Steven Richardson
- Using *Neoseiulus cucumeris* to deliver an entomopathogenic fungus to flowers for the control of thrips – Sacha White and Kerry Boardman

Objective 3: Transfer knowledge of new IPM developments to the industry

Knowledge transfer activities delivered by Gemma Hough and Sacha White in year five of this project related both to this project, and also to other horticultural projects, included:

Publications:

- Gemma co-authored an article with Jude Bennison in AHDB Horticulture News on the MOPS project Managing Ornamental Plants Sustainably (CP 124) April 2015 edition.
- Gemma co-authored an updated AHDB Horticulture Factsheet 10/12: Control of whitefly in protected ornamental crops with Jude Bennison and David Talbot.

Gemma authored the Fellowship report on monitoring the impact of pesticides applied for the control of spotted wing drosophila on raspberries which was then incorporated into the annual report for project SF 158 'Integrated Management of cane fruit pests and diseases'.

Presentations to industry:

- On behalf of Gemma Hough, Jude Bennison presented the results of the experiment on using the e-Nema weevil traps for the control of adult vine weevils with entomopathogenic nematodes at the AHDB Horticulture ornamentals conference in February 2016.
- Sacha and Kerry demonstrated the experiment assessing the use of *Neoseiulus cucumeris* as a vector of an entomopathogenic fungus for thrips control to AHDB Horticulture staff and members of the PE Panel in 2016.

Presentations at scientific conferences:

- EUCARPIA Leafy Vegetable congress – "Screening for host-plant resistance against Nr:0 and Nr:1 biotypes of *Nasonovia ribisnigri*" (Gemma)
- IOBC meeting Microbial and Nematode Control of Invertebrate Pests – "Entomopathogenic nematodes for the control of oak processionary moth in the UK" (Sacha)

- On behalf of Gemma Hough, Jude Bennison presented the results of the experiment on using the e-Nema weevil traps for the control of adult vine weevils with entomopathogenic nematodes at the AAB conference 'IPM – the 10 year Plan' in November 2015

Milestones not being reached

None

Do remaining milestones look realistic?

No further milestones to complete

Training undertaken

At ADAS Boxworth, Gemma Hough (until October 2015) and Sacha White worked alongside experienced horticultural scientists, including pathologists and weed scientists, as well as other entomologists. In addition, there was the opportunity to work on a wide range of research and technology transfer projects both within and outside of the Fellowship. Through this work, training on the design, completion analysis and communication of horticultural experiments was given by senior ADAS entomologists, Jude Bennison and Steve Ellis, the ADAS statistician, Chris Dyer, and other experienced members of staff.

See Appendices for full list of training undertaken.

Expertise gained by trainees

- Thrips species identification – Gemma Hough and Sacha White and key scientific support staff are now able to identify key horticultural thrips pests after in-house specialist training. This experience was used within the HortLINK project HL001107 (Biological, semiochemical and selective chemical management methods for insecticide resistant western flower thrips on protected strawberry), in the ADAS Thrips Identification Service for soft fruit growers and at the AHDB Horticulture soft fruit agronomists thrips training session on 12 Feb 2015.
- Predatory mite identification - Gemma Hough and Sacha White and key scientific support staff are now able to identify predatory mites after in-house specialist training. This experience was used within the HortLINK project HL001107 (Biological, semiochemical and selective chemical management methods for insecticide resistant western flower thrips on protected strawberry) and in AHDB Horticulture-funded project SF 158 'Integrated Management of cane fruit pests and diseases'.
- Aphid, parasitoid and hyperparasitoid identification – following the resignation of Tom Pope and Tracie Evans (research technician who had developed specialist skills in aphid

parasitoid and hyperparasitoid identification), Tom and Tracie returned to ADAS to give training in these key skills to Gemma Hough and scientific support staff in March 2013. Gemma then provided this training to Sacha White. This has enabled the continuity of the skills in the IPM team at ADAS Boxworth, who are now able to identify all commercially available aphid parasitoid species and hyperparasitoid genera.

- Gemma Hough and scientific support staff at ADAS Boxworth are now proficient in identifying the leaf miner *Scaptomyza flava* and this has enabled them to complete AHDB Horticulture-funded project FV 408 and continue with FV408a, on improving control of this pest on baby-leaf Cruciferae.
- Microphotography and videos (training given by Tom Pope, Harper Adams University). Gemma Hough and scientific support staff are now proficient in taking photographs and videos using the microscope which have been used in IPM presentations to the industry and at scientific conferences.
- Free living nematode extraction and identifying leaf and bud nematodes (training given by Jude Bennison and Heather Maher). This enabled Gemma Hough and Kerry Boardman to identify leaf and bud nematode species for use in the MOPS project CP 124.
- Entomopathogenic nematode extraction from soil, infectivity bioassays and lipid content analysis using image analysis software.
- Extracting DNA and using the loop mediated isothermal amplification (LAMP) technique (training given by ADAS biotechnology consultant Ben Maddison). This enabled Gemma Hough to complete work on developing a PCR method for identifying *Aphelenchoides* species in the MOPS project CP 124.
- Knowledge transfer skills – Gemma Hough and Sacha White have spoken at several industry conferences/technical meetings and written various project reports and technology transfer publications.
- Gemma Hough attended the AHDB Horticulture Oomycete workshop (2014) to develop a broader understanding of issues the industry faces and to support her BASIS commercial horticulture training.

Other achievements in the last year not originally in the objectives

Trainees have worked with experienced ADAS entomologists and collaborating scientists on a wide range of horticultural projects over the last year. These included:

- CRD-funded project PS2134 - Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil. Site managed by Gemma Hough.
- AHDB Horticulture project CP 124 Managing Ornamental Plants Sustainable (MOPS). Gemma led the vine weevil work during 2014 and leaf and bud nematode LAMP work.
- AHDB Horticulture project FV408a Baby-leaf Cruciferae: Improved control of *Scaptomyza flava* – Led by Gemma Hough
- DEFRA-funded (CRD) PS2722 - Combating insecticide resistance in major UK pests. Led by Sacha White.
- AHDB Cereal and Oilseeds funded Project RD-2140025 - Cabbage stem flea beetle larval survey (2015/16 extension). Led by Sacha White.
- BBRO-funded Project 15/10 – Monitoring and control of mangold fly. Led by Sacha White.

Changes to Project

Are the current objectives still appropriate for the Fellowship?

Indicate any changes to the ordinal objectives that you would like to make and provide any information that you can to support this decision.

No further Objectives remaining

Grower Summary

Headline

- Three young entomologists and key ADAS scientific support staff have been mentored in order to equip them to develop and communicate IPM strategies on horticultural crops, thus maintaining this expertise in the industry. Novel strategies investigated in the final year of the project paved the way for further research on control of vine weevil, western flower thrips and two-spotted spider mite within IPM programmes.

Background

Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil

Vine weevil is one of the most problematic pests in soft fruit crops and hardy nursery stock. Larval feeding in the roots causes plant stunting, wilting and death while adult feeding on foliage renders ornamentals unmarketable. Although biological control methods are available for vine weevil larvae, control of adults currently relies on chemical insecticides, which provide unreliable control and interfere with Integrated Pest Management (IPM) programmes. Novel IPM methods of controlling adult vine weevils is therefore a priority.

Vine weevils are known to aggregate during the day in sheltered locations and this behaviour has been exploited to design artificial refuges for use in lure-and-kill control methods. Previous Defra-funded work has shown that adult vine weevils pick up fluorescent powder or powdered formulations of entomopathogenic fungi (EPF) from within artificial refuges and then spread it to other weevils either when visiting refuges or whilst feeding at night. The most effective EPF for use in such refuges was confirmed as *Metarhizium brunneum*. When Defra-funded work assessed the control efficacy of artificial refuges containing *M. brunneum* in semi-field conditions the results were unclear due to a natural fungal infection in the vine weevil culture and so the experiment was repeated in this Fellowship project.

Using *Neoseiulus cucumeris* to deliver an entomopathogenic fungus to strawberry flowers for the control of thrips

Western flower thrips (WFT) is serious pest of strawberry, causing fruit damage that can make the crop unmarketable. On most fruit farms WFT is resistant to all available pesticides, thus control relies on the use of predators. Biological control on soft fruit crops is now threatened by use of pesticides for control of spotted wing drosophila (SWD). Foliar sprays of entomopathogenic fungi (EPF) can kill WFT, are not affected by insecticides used against

SWD and are compatible with IPM programmes. However, targeting WFT in the flowers with foliar sprays is difficult. Work in this project investigated whether predatory mites can be used to carry spores of the EPF, *Beauveria bassiana*, to flowers to infect and kill WFT.

Determining the speed of kill of adult vine weevil when using E-nema Nematop® Käfer-Stopp traps

Entomopathogenic nematodes (EPN) are known to provide effective control of vine weevil larvae. E-nema in Germany have recently developed a product for the home garden market using nematodes to control adult vine weevils. The Nematop® Käfer-Stopp (weevil-stop) trap is a wooden refuge trap filled with a gel containing the EPN, *Steinernema carpocapsae*. The weevils shelter in the trap and become infected by the EPN. The speed of weevil kill was unknown therefore work in this project investigated this.

Monitoring the effects of insecticide applications for spotted wing drosophila on predatory mites and two spotted spider mites in raspberries

The invasive pest SWD is currently controlled with pesticides and these are likely to have an impact on IPM programmes. Control of the two-spotted spider mite (TSSM, *Tetranychus urticae*) is an example of an important raspberry pest for which an IPM programme has been developed. Growers release the predatory mite *Phytoseiulus persimilis* and the predatory midge *Feltiella acarisuga* for control of TSSM and also integrate acaricides. *Amblyseius andersoni* and *Neoseiulus) californicus* are naturally-occurring predatory mites that also help regulate TSSM populations. Work in this project investigated the impacts of SWD control on predatory mites and control of TSSM on raspberry crops on two commercial farms.

Summary

Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil

Roguard® traps (used for trapping cockroaches) containing talc and fluorescent powder mixed with *M. brunneum* or just talc and fluorescent powder (untreated control) were used as artificial refuges. Six traps of either treatment were placed in an insect-proof mesh 'tent' cage with potted strawberry plants. Five replicate cages of each treatment were assessed. Forty marked weevils were released into each cage and after approximately five weeks the number of dead and live adult weevils in each cage was assessed. The presence on the bodies of fluorescent powder (indicating that they had entered a refuge trap or had come into contact with another weevil which had), white or grey-green hyphae/spores (indicating that it had

been infected with fungus) and their location was also recorded. All weevils were subsequently incubated and observed for six weeks to check for evidence of fungal infection.

In total 95.5% and 94.5% of weevils were recovered at the end of the experiment from the untreated and treated cages (traps containing *M. brunneum*) respectively. In the treated cages, 37.5% were found dead, 57% were found alive, 5.5% were missing and 3% had obvious signs of fungal infection. In the untreated cages, 24.5 % were found dead, 71% were found alive, 4.5% were missing and none had signs of infection. Analysis showed that there were significantly more vine weevil adults found dead in the treated cages compared to in the untreated cages. Following incubation, significantly more were found to be infected with *M. brunneum* in the treated cages (41.7%) than the untreated cages (0%).

This work showed that the traps were effective in intiating a fungal epidemic amongst the vine weevils. However, as few weevils were observed with infection until after incubation at optimum temperature and humidity, it is likely that the success of this method is highly dependent on suitable environmental conditions. Further work on effective EPF dose and formulation would be needed to further develop this method if approval could be gained for using an EPF in this way.

Using *Neoseiulus cucumeris* to deliver an entomopathogenic fungus to strawberry flowers for the control of thrips

Laboratory tests were set up in plastic boxes to investigate the ability of *N. cucumeris* to carry powdered *Beauveria bassiana* (Botanigard®) spores to flowers. Chrysanthemum flowers were used as strawberry flowers were unavailable. Three treatments were investigated; (1) Botanigard® only, (2) *N. cucumeris* only and (3) Botanigard® mixed with *N. cucumeris*. The treatment was placed at one end of the box on a chrysanthemum leaf. At the other end of the box was placed either a chrysanthemum flower (treatments 2 and 3) or commercial pollen (Nutrimite™) on a chrysanthemum leaf (treatment 1). Fifteen adult WFT were added to each box. The boxes were then placed in an incubator set at 23°C and after one week the numbers of live and dead adult WFT were counted. The WFT were then incubated on damp filter paper for a further week and assessed for *B. bassiana* sporulation. Petals from the flowers were also incubated on selective agar and assessed for the presence of *B. bassiana* spores.

Significant differences in WFT mortality were found at the end of the bioassay (100% in the Botanigard® only treatment, 8% in the Botanigard® and *N. cucumeris*, and 0% in the *N. cucumeris* only treatment). Following incubation, 76% of WFT in the Botanigard® only treatment were found to have *B. bassiana* infection compared with 33% in the Botanigard® and *N. cucumeris* treatment but these differences were not statistically significant. No WFT infection occurred in the *N. cucumeris* only treatment. No petals in the *N. cucumeris* only

treatment were found to have *B. bassiana* spores on them compared to 67% in the Botanigard® and *N. cucumeris* treatment.

Lack of water sources in the Botanigard® only treatment are likely to have contributed to the high WFT mortality observed. The presence of *B. bassiana* spores on the petals in the Botanigard® and *N. cucumeris* treatment shows that the Botanigard® was taken to the flowers, although both WFT and *N. cucumeris* may have carried them there. *N. cucumeris* were observed moving freely with spores on their bodies. Furher work would be needed using whole plants in a more realistic environment to furher investigae this potential novel method for using entomopathogenic fungi for WFT control, if approval could be gained for using EPF in this way.

Determining the speed of kill of adult vine weevil when using E-nema Nematop® Käfer-Stopp traps

The experiment assessed two treatments; Nematop® Käfer-Stopp (weevil-stop) traps with the *S. carpocapsae* nematode gel (treated) or traps without the gel (untreated control). Each treatment had five replicates with each replicate consisting of an insect cage containing either treated or untreated traps. Each cage contained of a seed tray filled with a coir substrate with a trap and a sprig of yew (as a food source for the weevils) placed on top. Five adult vine weevils were released into each cage. The cages were kept in a glasshouse maintained at conditions optimal to *S. carpocapsae* activity. The numbers of live and dead vine weevils were assessed regularly for thirty days. Dead vine weevils were removed and dissected to determine whether nematode infections were evident.

At the end of the experiment 92% and 8% of the weevils had died in the treated and untreated cages respectively. Of the weevils that had died on the treated cages, 83% were confirmed to contain nematodes (Figure 1). The first dead weevils were observed after nine days and 50% died after approximately 15 days. Although the traps were effective they are currently too expensive for commercial use. The traps will be further investigated together with a vine weevil lure in the current AHDB Horticulture project HNS 195 ‘Improving vine weevil control in HNS’.

Figure 1. Entomopathogenic nematodes *Steinernema carpocapsae* inside a dissected vine weevil adult body

Monitoring the effects of insecticide applications for spotted wing drosophila on predatory mites and two spotted spider mite control in raspberries

Raspberry crops on two commercial farms that had SWD on site were monitored. Site 1 used IPM for TSSM control and the varieties monitored were Tulameen and Maravilla. Site 2 relied on naturally occurring predators and acaricides for TSSM control and the variety monitored was Kweli. The sites were monitored on several occasions during the summer, before and after pesticides were used for SWD control. On each visit assessments were made of TSSM incidence and severity and relevant predator establishment.

At Site 1 on cv. Tulameen, low numbers of *P. persimilis* (released by the grower) and *A. andersoni* and *N. californicus* (naturally-occurring) survived an application of thiacloprid (Calypso) and TSSM numbers remained stable during May and June. On cv. Maravilla, low numbers of *P. persimilis* (released by the grower), *A. andersoni* and *N. californicus* (both naturally-occurring) survived three applications of spinosad (Tracer) and one application of chlorpyrifos (Equity, no longer available). Predatory mites were considered responsible for reducing TSSM numbers to negligible levels between 11 August and 2 September.

At Site 2 on cv. Kweli, the acaricides clofentezine (Apollo) and abamectin (Dynamec) together with naturally-occurring *A. andersoni* and *N. californicus* maintained TSSM populations at low levels. A proportion of the *A. andersoni* and *N. californicus* populations survived acaricides applied for TSSM control, Tracer and pyrethrum applied for SWD control, and Equity and Calypso applied for control of other pests. These results highlight the importance of naturally-occurring predatory mites in maintaining spider mite control when applying pesticide programmes for control of SWD and other pests.

Financial Benefits

- Growers of soft fruit crops and agronomists will benefit from being aware that naturally-occurring predatory mites can play an important role in maintaining spider mite control when applying pesticide programmes for control of spotted wing drosophila and other pests.
- Growers of HNS and soft fruit crops will benefit from being aware that the e-nema vine weevil traps can lead to high mortalities of adult vine weevils within 30 days. The traps are currently too expensive for commercial use but further development of the traps together with the identification of a vine weevil lure is being done in the current AHDB Horticulture project HNS 195 'Improving vine weevil control in HNS'.
- Results of this project have demonstrated that powdered formulations of entomopathogenic fungi have potential for use in a novel lure and kill approach for adult vine weevils and in a novel approach for using predatory mites to carry the fungal spores to flowers. Further development of these novel approaches is dependent on the potential approval of the use of entomopathogenic fungi in these methods.
- The horticultural industry will benefit from the mentoring of three young entomologists and key ADAS scientific support staff in developing and communicating new IPM strategies for horticultural crops, thus maintaining this UK expertise.

Action Points

- Growers of soft fruit susceptible to spotted wing drosophila (SWD) should be aware that naturally-occurring predatory mites can play an important role in maintaining two-spotted spider mite control when using pesticides for control of SWD. Identification of these mites can be done by ADAS on request, via ADAS soft fruit consultants or direct to jude.bennison@adas.co.uk
- Growers of HNS and soft fruit should keep up to date on further developments of the e-nema vine weevil trap for control of adult vine weevils via the results of current project HNS 195 'Improving vine weevil control on HNS', which will be available on the AHDB Horticulture website and reported in AHDB Horticulture Grower.

Science Section

Introduction

Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil

Vine weevil is a serious pest of soft fruit and nursery stock crops. Adult weevils feed on the leaves, rendering ornamental plants unmarketable, and the larvae feed on the roots, causing plant stunting, wilting and death. Although non-chemical control methods are available for vine weevil larvae (e.g., entomopathogenic nematodes (EPN) and the entomopathogenic fungus (EPF), *Metarhizium brunneum*) control of adult weevils is currently reliant on the use of chemical pesticides which give unreliable control and are often incompatible with Integrated Pest Management (IPM) programmes. This project is building on the Defra-funded projects PS2134 and PS2140 carried out by ADAS, Harper Adams University and Warwick University, which showed that adult vine weevils enter artificial refuges containing fluorescent powder/EPFs, pick up the powder and spread it to other weevils when foraging or aggregating together within other refuges. The projects also confirmed that the EPFs effectively killed adult vine weevils within refuges in laboratory tests. When the best performing EPF was confirmed (*Metarhizium brunneum*) and tested in semi-field conditions the results were unclear due to a natural infection in the culture and as a result this experiment was repeated in the Fellowship project.

Using *Neoseiulus cucumeris* to deliver an entomopathogenic fungus to strawberry flowers for the control of thrips

When this experiment was planned, entomopathogenic fungi (EPF) were not used for the control of WFT on strawberry but there is now an EAMU for the use of Botanigard (*Beauveria bassiana*) as a foliar spray for western flower thrips (WFT) control on protected strawberry. This will be of interest to growers as EPF can be safely integrated into an IPM programme and are unaffected by insecticides used for spotted wing drosophila control or the control of other 'IPM disruptors' invading the crop. However, WFT are difficult to target with foliar sprays as they hide within the flowers. This experiment aimed to evaluate a novel approach where *N. cucumeris* is used to deliver EPF spores to the flowers and subsequently provide control of WFT. Use of EPF in this way is not currently approved in the UK, but the approach has been developed by Biobest using bumble bees to vector both EPF and additional pollen (www.biobestgroup.com/en.

Determining the speed of kill of adult vine weevil when using E-nema Nematop® Käfer-Stopp traps

Growers currently have limited IPM-compatible options with which to control vine weevil adults and there is an urgent need to develop effective alternatives to the use of broad spectrum insecticides. In this respect two CRD-funded projects (PS2134 and PS2140) investigating the potential of refuge traps for infecting adult weevils with an entomopathogenic fungus have recently been completed which demonstrated the potential of a lure and infect or lure and kill approach based on the use of artificial refuges. However, further development of this approach would require the approval of a fungal formulation for use in the refuges.

E-nema have recently developed the Nematop® Käfer-Stopp trap, which is a modification of the grooved wooden boards sometimes used for monitoring adult vine weevils. The grooves are filled with a gel containing the entomopathogenic nematode (EPN), *Steinernema carpocapsae* (Figure 1) so that adult weevils seeking refuge in the grooves on the underside of the boards become infected with the nematodes. Currently the Nematop® Käfer-Stopp traps are sold for home garden use in both Germany and the UK but are likely to be too expensive to be used under most commercial situations. The development of this product provides a model on which a cost-effective lure and kill approach could be developed for commercial use by growers of hardy nursery stock or soft fruit. In the recently funded AHDB Horticulture project HNS 195 (Improving vine weevil control in HNS), the project team led by ADAS will investigate identifying effective vine weevil lures in order to enhance the efficacy of this potential 'lure and kill' control method and will discuss with e-nema the possible development of an alternative trap design for cost-effective commercial use. The use of EPNs as the killing agents has the advantage that any product developed would not require pesticide registration thus there would be no regulatory hurdles.

The Nematop® Käfer-Stopp traps have been shown by e-nema to be effective for the control of adult vine weevil but the speed of kill was unknown therefore this experiment aimed to fill this gap in knowledge.

Figure 1. E-Nema Nematop® Käfer-Stopp trap with gel containing nematodes in grooves.

Monitoring the effects of insecticide applications for spotted wing drosophila on predatory mites and two spotted spider mites in raspberries.

To date, all UK work on spotted wing drosophila (SWD) has focused on damage limitation. As a result no work has looked at the wider implications of spraying for SWD on other pest targets or on maintaining the successful IPM approaches developed over the past 10 years while controlling SWD. Two-spotted spider mite (TSSM) can be a devastating pest of raspberries, especially on crops grown under glass or in polytunnels and in hot weather. Phytoseiid predatory mites are the main natural enemies of TSSM. The two main naturally occurring (overwintering) species in raspberry are *Amblyseius andersoni* (predominantly) and *Amblyseius (= Neoseiulus) californicus* (also common). These regulate TSSM populations to a greater or lesser extent but not reliably. In recent years, growers have also successfully used introductions of *Phytoseiulus persimilis* (a predatory mite) and *Feltiella acarisuga* (a predatory gall midge) and/or acaricides for control of TSSM in outdoor/protected raspberry and blackberry crops. However, it is known that some of the pesticides used for the control of SWD are harmful to biological control and in other countries this has led to serious outbreaks of TSSM on SWD-infested crops.

Outbreaks of TSSM and other mites, as a result of disruption of biocontrol by naturally occurring and introduced predatory mites through sprays of insecticides for SWD and/or capsid bugs, is an immediate and serious challenge facing the UK cane fruit industry. The Integrated Pest Management (IPM) of Cane Fruit Pests and Diseases project (SF 158) aimed to address this gap in knowledge during 2015 with the support from the trainees on this Fellowship. The collaborative project aimed to assess the extent to which programmes of sprays for SWD or capsid bugs disrupt the control offered by naturally occurring and introduced predatory mites.

Materials and methods

Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil

Large gauze ‘tent’ cages (1.45 x 1.45 m) were prepared to simulate a soft fruit crop. Each cage contained twelve 1.5 L pots each containing a strawberry plant (cv. Malling Centenary). The pots were arranged in the centre of a cage in a 4x3 rectangle with each pot touching.

The experiment had an untreated control (fluorescent powder and talc) and a *M. brunneum* + talc + fluorescent powder (isolate 275.86) fungal treatment (Table 1).

Table 1. Treatment list.

Treatment number	Treatment	Amount of talc and fluorescent powder mix (50:50) per trap (g)	Amount of fungus per trap (g)	Total powder per trap (g)
1	Untreated	0.4	0	0.4
2	*Metarhizium brunneum* (isolate 275.86) 1 x 10^8	0.1	0.3	0.4

Each treatment was replicated five times. The experiment was carried out in a polytunnel. Roguard® traps were used as artificial refuges and were filled with 0.4 g of the appropriate treatment on 17 July (Figure 2).

Figure 2. Refuge traps containing 0.4 g of fluorescent powder in each central well.

Fungal spores were cultured by Warwick Crop Centre and sent to ADAS where the appropriate amount of spores, fluorescent powder and talc were weighed, combined and shaken together to mix.

On 17 July, six refuge traps of the appropriate treatment were added to each cage. A refuge trap was placed between each plant pot (Figure 3). Adult weevils were taken from the ADAS culture and 40 weevils were placed into each cage on the foliage of the plants. The weevils were marked on their backs with bright nail varnish before release so that they were easier to find in subsequent assessments. A data logger was placed in each of two of the cages to monitor temperature.

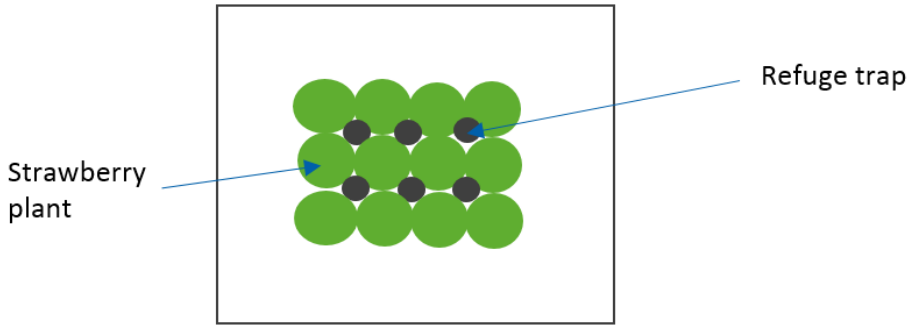

Figure 3. Layout out of strawberry plants and refuge traps in each cage.

After approximately five weeks, (24-27 August), the number of dead and live adult weevils in each cage was assessed. The cage floor, cage roof, plants, refuge traps, underside of pots, roots and surrounding substrate were searched for weevils. When a weevil was found a note was made of whether it had fluorescent powder on its body (indicating it had entered a refuge trap or had come into contact with another weevil which had), whether it was clearly infected with fungus (white or grey-green hyphae/spores), whether it was alive or dead and finally where it was found.

All the weevils recovered from the cages (alive, dead and dead + infected) were kept individually on damp filter paper in sealed Petri dishes containing strawberry leaves/yew as food and incubated for up to six weeks at 22°C to check for any additional mortality through development of a pathogen and evidence of sporulation. The infection status and mortality of the weevils were checked each week and any weevils that had developed a fungal infection were sent to Warwick Crop Centre for confirmation of the species responsible. The final assessment took place on 7 October.

Using *Neoseiulus cucumeris* to deliver an entomopathogenic fungus to strawberry flowers for the control of thrips

Neoseiulus cucumeris (provided in bran by Syngenta Bioline) were used to determine whether predatory mites could act as vectors to carry spores of the entomopathogenic fungus, *Beauveria bassiana*, to flowers to infect WFT. The experiment was originally planned to be done with strawberry flowers but as these were unavailable when the experiment was set up in March 2016, pot chrysanthemum flowers were used instead. Botanigard®, containing *B. bassiana* strain GHA as a powder was provided by Certis and was used in the experiments at 100 x the recommended rate to demonstrate a proof of concept. The experiment consisted of three treatments, a control without *N. cucumeris* i.e. Botanigard® and bran mix (Botanigard®

only), a control without Botanigard® i.e. *N. cucumeris* only, and Botanigard® and *N. cucumeris* (Table 2). Prior to the experiment, the average of the number of *N. cucumeris* in 0.1 g of the bran substrate was determined. On average 0.1 g of the substrate contained 13 predatory *N. cucumeris*. It was decided that 0.5 g *N. cucumeris*-bran mixture would be a suitable amount to use in each bioassay box. Five replicates were used for each of the three treatments.

Table 2. Treatment list.

Trt no.	Treatment	Rate
1	Bran only, x 100 Botanigard®	0.03g Botanigard® in 0.5g bran
2	*N. cucumeris*, no Botanigard®	0.5 g *N. cucumeris* mixture
3	*N. cucumeris*, x100 Botanigard®	0.03g Botanigard®in 0.5g *N. cucumeris* mixture

All experiments were set up in 16 x 22 cm ventilated plastic boxes. A chrysanthemum leaf was placed at one end of the box. The appropriate treatment (Table 1) was added to the leaf. At the other end of the boxes used for Treatment 1, a pinch of Nutrimite™ was added to an additional leaf as a food source for the WFT. A chrysanthemum flower was not added to this control treatment as it was discovered that the chrysanthemum flowers bought for the bioassay contained low numbers of *N. cucumeris.*

In treatments 2 and 3, a chrysanthemum flower was included, with its stem inserted into a damp cube of Oasis® that had been covered with cling film (Fig 4). The cling film prevented the Oasis® from desiccating and also prevented moisture leading to condensation in the boxes in order to prevent the WFT from drowning. This was because in a pilot bioassay, where it was aimed to manipulate the relative humidity in the boxes to remain above 70% (the minimum recommended RH for Botanigard optimum efficacy), most of the WFT drowned in droplets of condensation in the boxes. In addition to using cling film around the Oasis®, other modifications of the pilot bioassay method used in the final bioassays were not using damp dental roll inserted into holes in the base of the boxes and standing the boxes on a tray of damp capillary matting. The boxes had two ventilation holes in the lids that were screened with thrips-proof mesh.

Figure 4. Bioassay used for treatments 2 and 3.

Fifteen female WFT from the ADAS laboratory culture were added to each box by pooting them into a tube and placing the open end of the tube near the flower (for the *N. cucumeris* only and Botanigard® and *N. cucumeris* treatments) and near the leaf with Nutrimite™ on (Botanigard® only treatment). The lid was then placed firmly onto the box. All boxes were placed in an incubator set at 23°C with 16:8 hour light: dark cycle for one week. A data logger was placed in one of the boxes to monitor temperature and relative humidity.

After seven days the numbers of live and dead adult WFT were counted. These were then placed on damp filter paper in separate petri dishes, which were put inside sealed boxes to maintain humidity. The boxes were then placed in an incubator at 23°C for one week. This provided ideal humidity conditions to ensure that any spores picked up by the thrips could germinate. All thrips were spaced apart from each other to minimise contamination between them. After seven days the thrips were examined for *B. bassiana* sporulation, which would demonstrate whether they had been infected by Botanigard®. The petals of the flowers (*N. cucumeris* only and Botanigard® and *N. cucumeris* treatments) were also tested for the presence of *B. bassiana* spores by placing the petals in 9 cm petri dishes on selective modified PDA media (39 g of PDA, 1 g yeast extract, 0.5 g chloramphenicol, 0.25 g cyclohexamide, 0.004 g thiabendazole, 0.01 g rose Bengal and 1,000 ml of deionized water). Three petals were placed on each petri dish with two petri dishes being used for each bioassay. These were sealed and placed in an incubator at 23°C.

Determining the speed of kill of adult vine weevil when using E-nema weevil-stop traps

This experiment was set up on 14 October 2015 in a research glasshouse at ADAS Boxworth. There were two treatments: cages containing either Nematop® Käfer-Stopp traps with the nematode gel or traps without the gel (untreated control). Each treatment had five replicates with each replicate consisting of an insect cage (50 x 50 x 50 cm) containing a seed tray filled with damp coir substrate. The Nematop® Käfer-Stopp traps (either with or without nematodes) were then placed grooved side down on the surface of the substrate. The trap was then watered from above as recommended, using a hand-held plant mister. Five adult vine weevils were released into each cage onto the growing media. A sprig of yew was provided on each side of the trap as a source of food (Figure 5).

Figure 5. Nematop® Käfer-Stopp trap on coir substrate with yew as a food source for the weevils in each cage.

The weevils were marked with yellow nail varnish before they were released so that they could be found easily during assessments. The cages were arranged in a randomised design in a glasshouse compartment (Figure 6) set at a minimum temperature of 15°C, the lower threshold for optimum performance of *S. carpocapsae*. Air temperatures in one of the cages were monitored with a data logger. After four days the minimum temperature was increased to 20°C to ensure that night-time temperatures did not drop below 15°C. The traps and the growing media were watered regularly using a plant mister in order to keep the conditions damp for the nematodes.

Figure 6. Arrangement of treatment cages in the glasshouse.

The cages were monitored every two to three days for thirty days. On each occasion the numbers of live and dead vine weevils in the cages were assessed after searching inside the cages, in the substrate and under the yew and seed tray. Any dead vine weevils were removed and dissected in a dish of water in the laboratory to record any nematodes present in order to confirm whether their death was caused by nematode infection. When the weevils were dissected it was necessary to crush their heads and bodies as the nematodes are often found inside the heads in the early stages of infection.

Monitoring the effects of insecticide applications for spotted wing drosophila on predatory mites and two spotted spider mites in raspberries.

Raspberry crops on two farms which had SWD on site were selected for monitoring. Site one was using IPM for TSSM control and the varieties monitored were Tulameen and Maravilla. Site two relied on naturally occurring predators and insecticides and the variety monitored was Kweli. An initial visit to Site 1 was made on 12 May prior to any fruit ripening and therefore before the SWD control programmes had started. Return visits and assessments were made after applications of an insecticide, which was approved for use against SWD. Site one (IPM used) was visited on 17 June, 11 August and 2 September. Site 2 was initially visited on 10 June when flowers and fruit were present and then on 29 June, 30 July and 2 September. At

both sites the numbers of TSSM and predatory mites were recorded on each visit. Table 3 shows the assessment dates and dates of insecticide applications for Site 1 and Table 4 shows the dates for Site 2.

Table 3. Assessment dates and dates of insecticide applications at Site 1. TSSM = two-spotted spider mite, SWD = spotted wing drosophila.

Date	**Spray/Visit**
29 April	Apollo 50 SC (clofentezine) for TSSM
05 May	Apollo 50 SC (clofentezine) for TSSM
05 May	Aphox (pirimicarb) for aphids
12 May	1st assessment of Tulameen
08 June	Calypso (thiacloprid) for raspberry beetle (also affects SWD)
17 June	2nd assessment of Tulameen
11 August	1st assessment of Maravilla
15 August	Tracer (spinosad) for thrips and capsids
24 August	Equity (chlorpyrifos) for SWD
25 August	Tracer (spinosad) for SWD
29 August	Tracer (spinosad) for SWD
2 September	2nd assessment of Maravilla

Table 4. Assessment dates and dates of insecticide applications at Site 2. TSSM = two-spotted spider mite, SWD = spotted wing drosophila.

Date	Spray
16 April	Equity (chlorpyrifos)
6 May	Apollo (clofentezine) and abamectin with wetter for TSSM
14 May	Calypso (thiacloprid) for aphids
2 June	Pyrethrum + Codacide for cane midge
10 June	1st assessment of Kweli
22 June	Pyrethrum for cane midge
29 June	2nd assessment of Kweli
3 July	Pyrethrum for cane midge
14 July	Apollo (clofentezine) for TSSM and chlorpyrifos for caterpillars
25 July	Abamectin for TSSM
29 July	Pyrethrum + Codacide for cane midge
30 July	3rd assessment of Kweli
5 August	Pyrethrum + Codacide for cane midge
17 August	Tracer (spinosad) for SWD and caterpillars
22 August	Pyrethrum with SPO 58 and Codacide for cane midge
28 August	Pyrethrum + Codacide for SWD
2 September	Pyrethrum + Codacide for SWD
2 September	4th assessment of Kweli

At each site 10 plots were selected within the crop which were 1 m long. On each visit assessment records were made of TSSM incidence and severity and relevant predator establishment within each plot.

TSSM assessment

Two spotted spider mite damage was quantified by assessing the incidence of leaf speckling and webbing on 15 representative leaves at each plot. Five fully expanded leaves in each of the bottom, middle and top thirds of floricane and primocane canopy were examined (15 leaves examined in total per plot). Leaves were selected that had signs of TSSM damage but where no visible damage was observed leaves were chosen at random. Where TSSM were present, the number of mites was estimated using a hand lens according to the following scale;

0 – No mites

1 – 1-5 mites and eggs/minor speckling

2 – 6-10 mites and eggs /minor speckling

3 – 11-20 mites and eggs /moderate speckling

4 – 21- 40 mites and eggs /moderate speckling

5 – 41 + mites and eggs /Severe speckling webbing visible

Predatory mites and eggs

A record was made of the presence of predatory mites, and predatory mite eggs if visible, on the same 15 leaves that were examined per plot for TSSM. The same scoring system was used as for TSSM. *Phytoseiulus persimilis* were identified in the field and any other predatory mites were collected for examination in the laboratory (e.g. *A. andersoni* or naturally occurring *N. californicus*) in order to confirm the effects of SWD insecticides on the different species.

Other pests

During the assessments records were made of the presence (live and dead) of other pests if these occurred in high numbers, such as aphids, blackberry leaf midge, cane midge, etc.

Results and Discussion

Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil

Of the 40 weevils released into each cage between 34 and 40 adult weevils were recovered per cage. A total of 200 weevils (40 x 5 cages) were released for each treatment and a total of 191 (95.5%) and 189 (94.5%) of these weevils were recovered in the untreated and *M. brunneum* (isolate 275.86) cages respectively. In the untreated cages, 49 (24.5 %) were found dead, 142 (71%) were found alive and nine (4.5%) were missing. None of the weevils found had obvious signs of mycosis (infection by a fungus) when found in the cages (prior to incubation).

In the *M. brunneum* treatment, 75 (37.5%) were found dead, 114 (57%) were found alive and 11 (5.5%) were missing. Six weevils were found with obvious signs of mycosis when collected from the cages (prior to being incubated) and the fungus was confirmed to be *M. brunneum* (Figure 7).

Figure 7. Vine weevil adult infected with *Metarhizium brunneum*

The weevils which were not recovered could have been missed during the assessment or could have died and disintegrated. Weevils were found on the floor of the cage, under the pots, within the substrate, on the surface of substrate, on the strawberry plant, under the capillary matting in the refuge traps and around the roots of the plants.

A logistic regression analysis was performed on the proportion of vine weevils found dead in the cages (prior to incubation) which showed that there were significantly more vine weevil adults found dead in the treated cages compared with the untreated cages (df(1,8), deviance ratio= 8.77, chi p= 0.003). In the untreated cages a proportion of 0.26 (26%, s.e = 0.032) of the vine weevil adults in the untreated cages were found dead compared to a proportion of 0.40 (40%, s.e= 0.036) in the treated cages.

Daily average temperatures and maximum daily temperatures in the polytunnel remained above 15°C (the minimum temperature for *M. brunneum* development) during the experiment, however daily minimum temperatures fell below 15°C on 33 of the 40 days (Figure 8). As infection is dose-dependent in addition to temperature-dependent it is also possible that some weevils only picked up a small number of spores, which would increase the time needed for infection to develop.

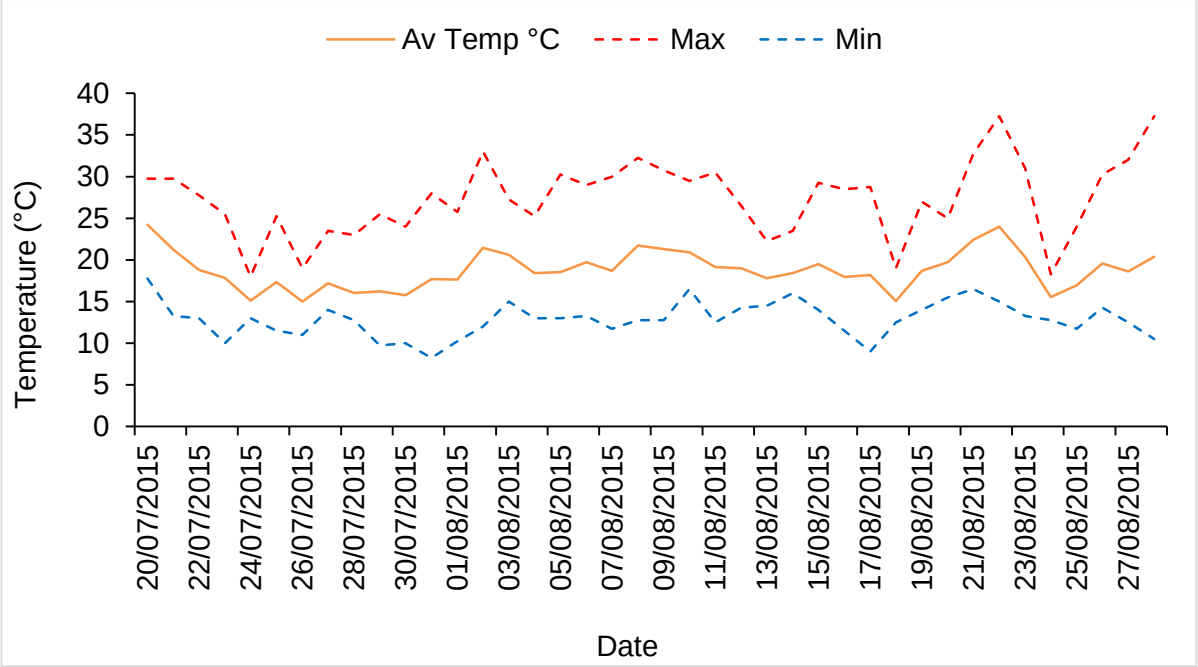

Figure 8. Average, maximum and minimum temperatures in the polytunnel

Untreated cages

In the untreated cages, 137 (71.7%) of the 191 weevils found had fluorescent powder on their body indicating they had entered a refuge trap or had come into contact with another weevil which had. During the assessment, 24 (12.6%) adult weevils were found inside the refuge traps.

Following incubation of the 191 weevils found from the untreated cages, 48 (25.13%) were still alive and 143 (74.9%) were dead. None of the weevils died from *M. brunneum* infection. The majority of these weevils were colonised by a secondary saprophytic fungus which developed during incubation, the remaining weevils showed no signs of mycosis. One weevil died from a natural infection with *Beauvaria bassiana*. The data is summarised in Table 5.

Cages treated with M. brunneum

In the *M. brunneum* (isolate 275.86) treatment, 145 (77.1%) of the weevils found had orange fluorescent powder on their bodies indicating they had entered a refuge trap or come into contact with another weevil which had. 13 (6.9%) adult weevils were found in the refuge traps during the assessment.

Following incubation of the 188 weevils found in the *M. brunneum* (isolate 275.86) treatment; eight (4.3%) were still alive and healthy and 180 (95.7%) were dead. Of the 180 dead weevils,

75 (41.7%) died from infection with *M. brunneum*. As with the untreated weevils, the remaining 105 dead weevils (58.3%) were either colonised by a secondary saprophytic fungus which developed during incubation or alternatively showed no signs of mycosis. The data is summarised in Table 5.

Table 5. Number and percentage of live, dead and *M. brunneum* infected weevils following incubation

		Total weevils found and incubated out of 200	Weevils alive after incubation	Weevils dead after incubation*	Of those dead- infected with *M. brunneum*	Of those dead- death by other causes*
Untreated	**Num**	191	48	143	0	143
	%	95.5	25.1	74.9	0	100
***M. brunneum* (isolate 275.86)**	**Num**	188	8	180	75	105
	%	94	4.3	95.7	41.7	58.3

* Weevils which did not die from *M. brunneum* infection either showed no signs of mycosis or were infected by secondary saprophytic fungi which developed as a result of incubation. One weevil in the untreated treatment died from a naturally occurring *B. bassiana* infection.

A logistic regression analysis was performed on the proportion of vine weevils found dead after incubation which showed that there was significantly more vine weevil adults found dead in the treated cages (traps containing *M. brunneum*) compared to the untreated cages (traps containing no *M. brunneum*) (df(1,8), deviance ratio= 35.92, chi *p*= <0.001). In the untreated cages a proportion of 0.75 (75%, s.e = 0.031) of the vine weevil adults in the untreated cages were found dead compared to 0.96 (96%, s.e= 0.015) in the treated cages.

A logistic regression analysis was also performed on the proportion of vine weevils infected with *M. brunneum* after incubation which showed that there were significantly more infected vine weevil adults in the treated cages compared with in the untreated cages (df(1,8), deviance ratio= 105.57, chi *p*= <0.001). In the untreated cages none (0%, s.e = 0) of the vine weevil adults in the untreated cages were infected compared with a proportion of 0.42 (42%, s.e= 0.037) in the treated cages (Fig 9).

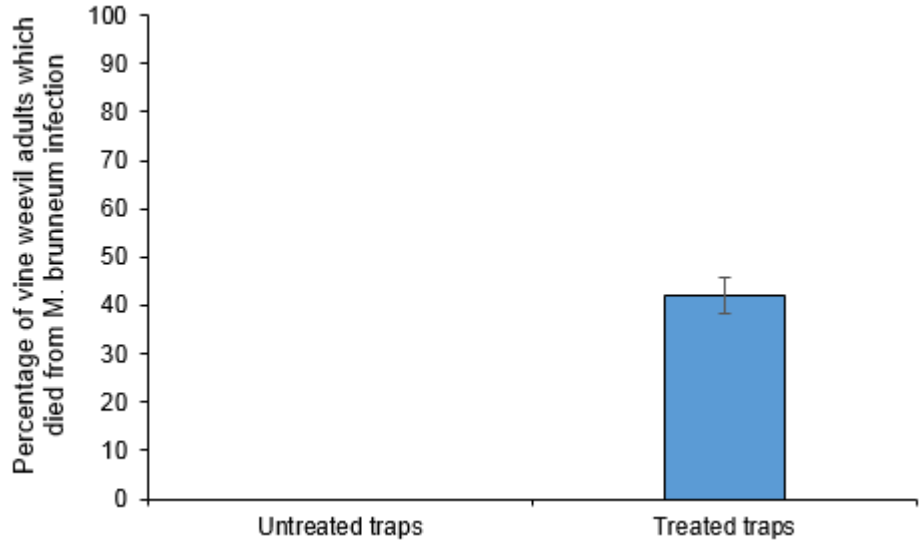

Figure 9. Percentage of adult vine weevils which died from *M. brunneum* infection in the untreated cages (traps with no *M. brunneum*) and the treated cages (traps with *M. brunneum*).

In conclusion, the traps were effective in intiating a fungal epidemic amongst the adult vine weevils. However, little infection was observed at the time the weevils were collected from the cages. Development of *M. brunneum* infection required incubation at optimum conditions suggesting that the sucess of this method is very temperature and humidity dependent. Futhermore, an infection rate of 40% was achieved when six traps were used in a 2.1 m² area. Therefore, using the ,methods tested in this experiment, the number of traps required in a commercial situation to achieve good control is unlikely to be cost effective or practical. However, as the dose rate of *M. brunneum* used in the traps was based on that recommended for control of vine weevil larvae, it is possible that further work on the effective dose rate required to control adult weevils, together with improved formulation could improve the infection rate. This, together with the development of an effective lure for vine weevil adults (being developed in current AHDB project HNS 195) could lead to a cost-effective strategy for commercial use, if approval could be gained for using *M. brunneum* in this way.

Using *Neoseiulus cucumeris* to deliver an entomopathogenic fungus to strawberry flowers for the control of thrips

The treatment had a clear effect on percentage mortality of the WFT with significantly greater mortality ($P < 0.001$, $F = 1488.9$, $df = 14$) in treatment 1 (100% mortality, Botanigard® only) than treatment 3 (8% mortality, Botanigard® and *N. cucumeris*), which in turn had significantly greater mortality than treatment 2 (0% mortality, *N. cucumeris* only) (Fig 10).

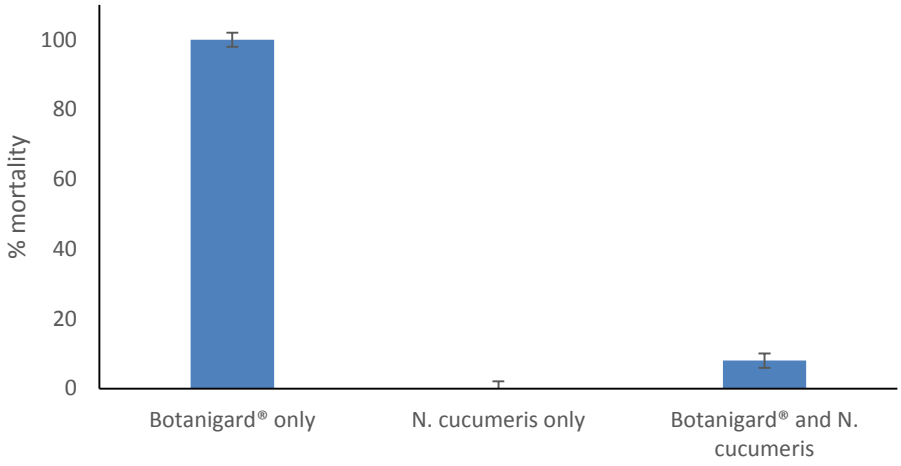

Figure 10. Percentage mortality of WFT after seven days in bioassay boxes containing either Botanigard® and *N. cucumeris*, only Botanigard® or only *N. cucumeris*.

Following incubation, the percentage of thrips that exhibited *B. bassiana* sporulation was 76% in the Botanigard® only treatment, 0.2% in the *N. cucumeris* only treatment and 33.3% in the Botanigard® and *N. cucumeris* treatment, however these differences were not statistically significant (Fig. 11).

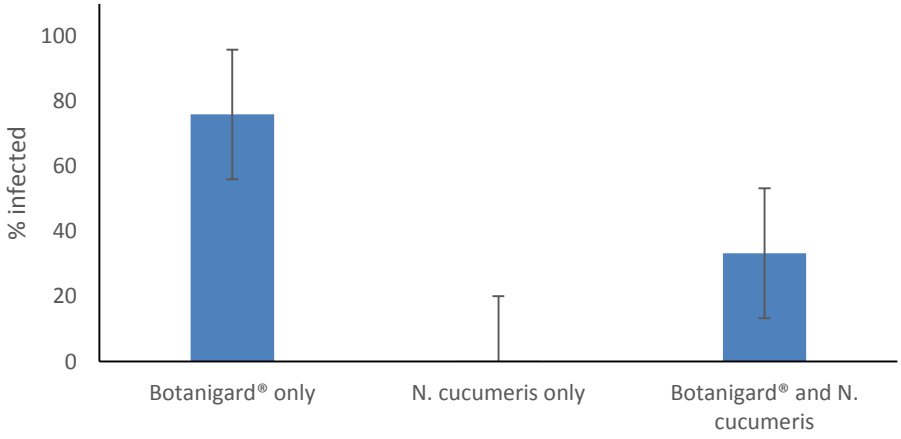

Figure 11. Percentage of WFT infected with Botanigard® after seven days in an incubator follwing the bioassay.

Culturing of petals on selective media (Fig 12) showed that 67% of petals in the Botanigard® and *N. cucumeris* treatment and 0% of petals in the *N. cucumeris* only treatment had *B. bassiana* spores on them at the end of the bioassay.

Figure 12. *B. bassiana* sporulating on selective media, demonstrating the presence of *B. bassiana* spores on petals.

Temperature data from the bioassays (Fig 13) show that conditions throughout the experiment were within the optimum temperature range (20-30^{0}C) for Botanigard® and *N. cucumeris* (18.9-26.7^{0}C).

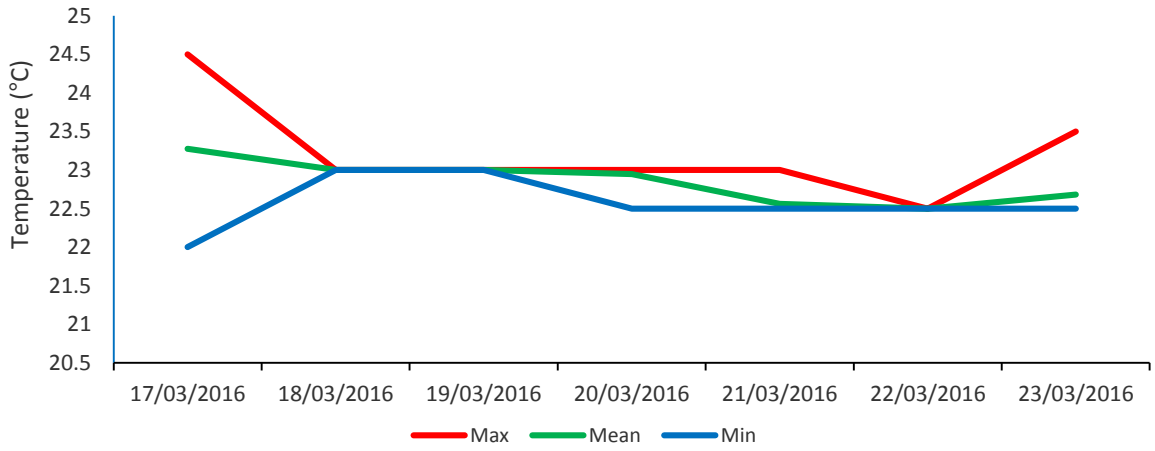

Figure 13. Mean, maximum and minimum temperatures within the bioassay boxes during the experiment.

Humidity data from the bioassays (Fig 14) show that humidity in the boxes was generally lower than the optimum range for Botanigard® (>70% RH) and *N. cucumeris* (65-75% RH). However, the optimum RH for *N. cucumeris* is for successful egg-laying rather than activity.

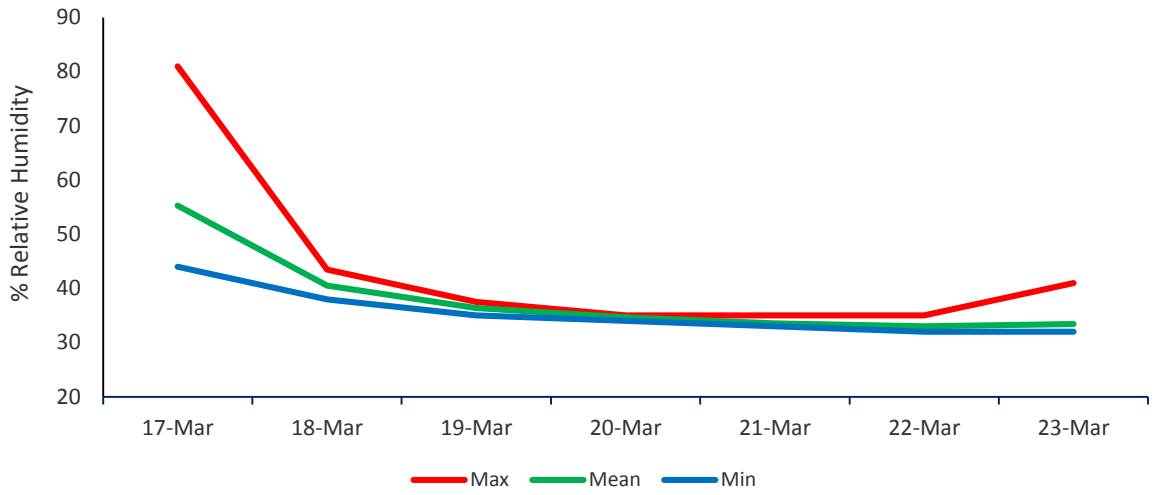

Figure 14. Mean, maximum and minimum relative humidities within the bioassay boxes during the experiment.

The results showed that Botanigard® alone (treatment 1) provided higher levels of WFT mortality than the Botanigard® and *N. cucumeris* treatment (treatment 3). However, as Nutrimite™ was used as a food source for the WFT in the Botanigard® only treatment rather than a flower, the high mortality in this treatment may have been due to a lack of water sources for the WFT.

Of the WFT recovered from the bioassays, 76% in the Botanigard® only treatment were subsequently found to have *B. bassiana* infection compared with 33% in the Botanigard® and *N. cucumeris* treatment (Fig 15). Although not significantly higher, the high infection rate in the Botanigard® only treatment may have been due to WFT moving around the bioassay box. WFT are very active at 23°C and it is likely that some of them picked up *B. bassiana* spores by visiting the chrysanthemum leaf on which the Botanigard® was placed. Although not significantly lower, it is possible that infection rates in the Botanigard® and *N. cucumeris* treatment may have been lower due to WFT adults actively avoiding the chrysanthemum leaf containing the Botanigard® in this treatment due to the presence of *N. cucumeris*, thus reducing their likelihood of picking spores. Research in the United States has indicated that the presence of *N. cucumeris* (which only predates WFT first instar larvae) can reduce the abundance of WFT second instar larvae and subsequently WFT adults WFT due to non-predatory effects (Jandricic & Frank, 2014).

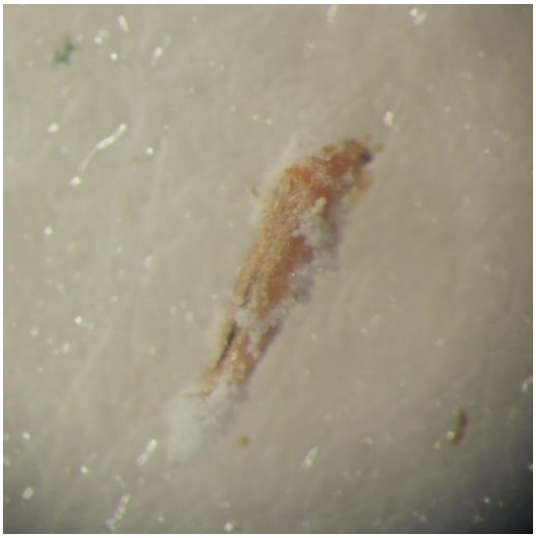

Figure 15. *B. bassiana* sporulating on a WFT adult.

The presence of *B. bassiana* spores on the petals in the Botanigard® and *N. cucumeris* treatment (as confirmed by the selective media results) shows that the Botanigard® was taken to the flower. It is not possible to determine whether WFT or *N. cucumeris* took the spores to the flower and as flowers were not used in the Botanigard® only treatment (treatment 1) the likelihood of WFT being the vector could not be assessed. However, inspection of the *N. cucumeris* using a microscope showed that the mites were able to move freely while carrying the spores (Fig 16).

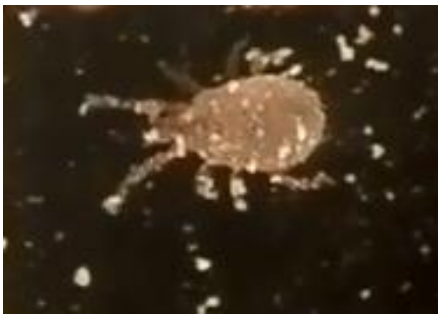

Figure 16. *N. cucumeris* with *B. bassiana* spores on its cuticle.

This work was not able to demonstrate whether using predatory mites as a vector of the spores of entomopathogenic fungi would improve control efficacy of entomopathogenic fungi against WFT. This was partially due to sub-optimal humidity conditions in the bioassay boxes due to the problem of WFT drowning in condensation when optimum humidities were maintained. The use of beneficial arthropods to vector entomopathogenic fungi remains a

potentially useful technique, both by better targeting biopesticides and by taking them to habitats within the crop where the microclimate is more conducive to the activity of the pathogen. Further work would be justified on this approach using whole plants in a semi-field experiment, if use of *Beauveria bassiana* or other species of entomopathogenic fungi in this way had a likely route to approval.

Determining the speed of kill of adult vine weevil when using E-nema weevil-stop traps

The first dead vine weevils were recorded in the treated cages nine days after set-up, when 4% of the weevils had died. Numbers of dead weevils increased in treated cages over the following weeks. Dead weevils were recorded on the floor of the cage, in the coir substrate, under the traps and under the yew. There was a clear relationship between the treatment and weevil mortality, with 92% of the weevils in the cages with the Nematop® Käfer-Stopp traps containing the nematode gel dying after thirty days (Fig 18). All but four of these weevils (83%) were confirmed to contain nematodes (Fig 17).

Figure 17. Nematodes (*Steinernema carpocapsae*) within the body of a vine weevil adult

Two of the four weevils that did not contain nematodes may have been assessed too soon (one day) after death for the nematodes to be detectable. In the control cages with the blank traps without nematodes, dead weevils were found on only two dates, 17 and 23 days after set-up, with only 8% of the weevils dying by day 23 (Fig 18). These weevils were confirmed to have died due to natural causes as no nematodes were detected when they were dissected. In the control cages with the blank traps, live vine weevils were often found taking

refuge in the empty grooves (Fig 20) but were also found under the seed trays and in the corners of the cages.

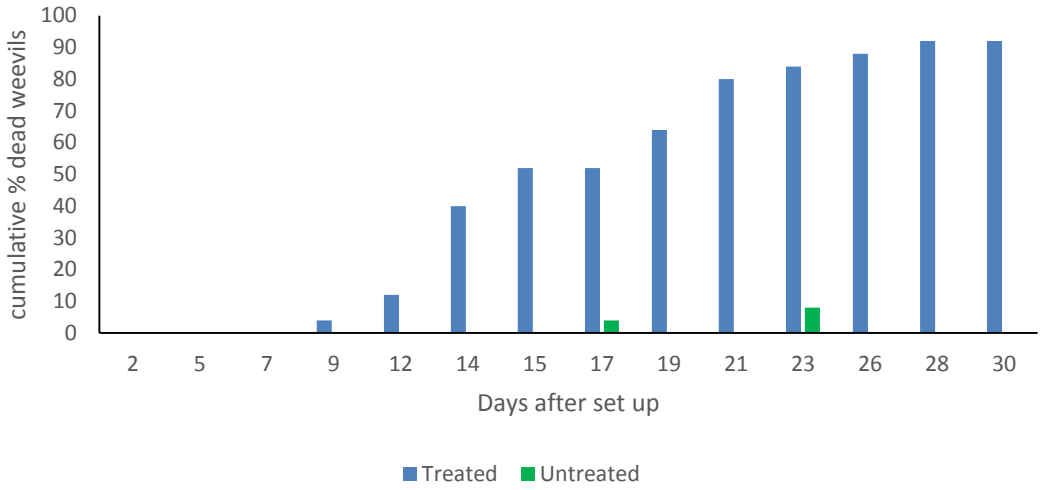

Figure 18. Cumulative percent vine weevil mortality over time in Nematop® Käfer-Stopp traps with (treated) and without (untreated) nematode gel.

Non-linear regression analysis of the cumulative mortality over time was well described by a logistic curve ($P < 0.001$, variance accounted for = 97.8%) (Fig 19). This is useful as it means reliable predictions can be made of the time until kill, e.g. the time until 50% mortality is predicted to be 15.5 days after set-up.

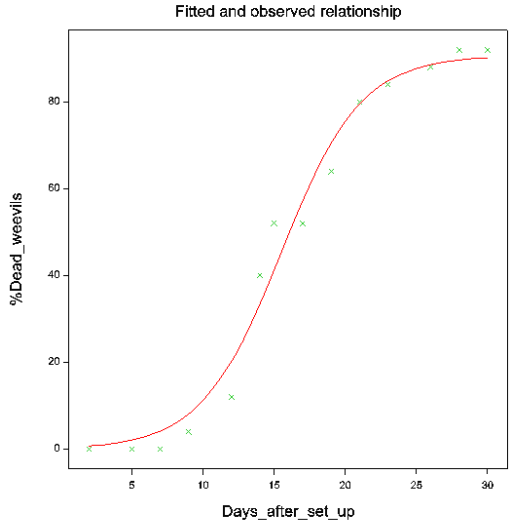

Figure 19. Logistic curve (red line) fitted to the observed cumulative mortality (green crosses) in the Nematop® Käfer-Stopp traps with nematode gel treatment.

Figure 20. Live vine weevils marked with yellow nail varnish in empty grooves on the underside of a blank Nematop® Käfer-Stopp trap.

Mean daily air temperatures in the cages were within the optimum temperatures for *S. carpocapsae* (15-30°C) throughout the experiment period (Fig 21). Minimum night temperatures dropped below 15°C (to 14.5°C) on only one date on 18 October. Maximum daytime temperatures exceeded 30°C (31°C) on only one date on 20 October.

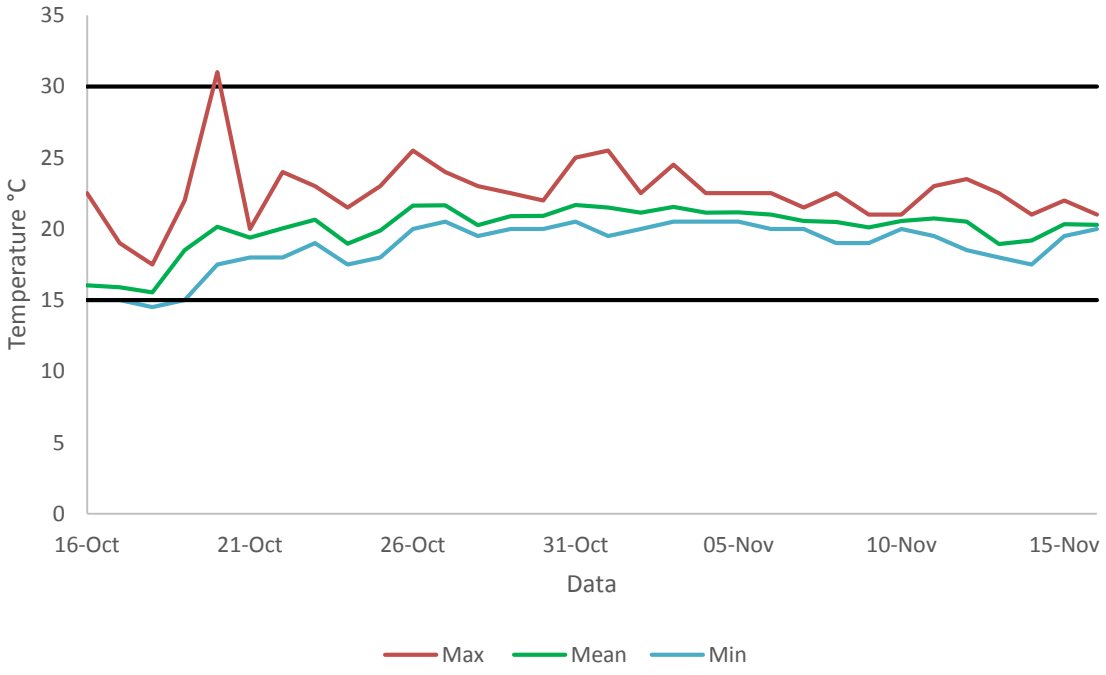

Figure 21. Mean maximum, minimum and mean air temperatures in one of the cages during the experiment. The black horizontal lines indicate the optimum minimum and maximum temperatures for *Steinernema carpocapsae* activity (15 and 30°C respectively).

In conclusion the Nematop® Käfer-Stopp traps treated with *S. carpocapsae* killed 92% of the released vine weevil adults within 30 days. There is considerable interest in the traps by UK commercial growers of hardy nursery stock, however the traps would currently be too expensive for commercial use. Further development of the traps together with a vine weevil lure is being done by ADAS, Harper Adams University and the Natural Resources Institute at the University of Greenwich in the current AHDB Horticulture-funded project HNS 195 (Improving vine weevil control in HNS). This research should help to develop a more cost-effective commercial strategy for using nematodes for control of adult vine weevils.

Monitoring the effects of insecticide applications for spotted wing drosophila on predatory mites and two spotted spider mites in raspberries.

The results presented are for TSSM and predators found on the lower leaves in the canopy as incidence of the pest was much lower in the middle and upper canopy areas.

Site 1 cv. Tulameen

On the initial assessment on 12 May, following two applications of Apollo (clofentezine) on 29 April and 5 May, the numbers of TSSM recorded had an average score of 2.4 (6-10 mites per leaf). Numbers of TSSM were similar on the second assessment on 17 June. *Phytoseiulus persimilis* were recorded on both dates (average score 0.1 i.e. less than one per leaf). Calypso (thiacloprid) was applied on 8 June for raspberry beetle control. This insecticide is known to be 'moderately harmful' (kills 50-75%) for up to two weeks after application (www.biobestgroup.com and www.koppert.com.) Calypso may have prevented *P. persimilis* numbers from increasing, however not all were killed. Low numbers of other predatory mite species were recorded (average score of 0.3 on 12 May, increasing to 0.5 on 17 June i.e. less than one per leaf). On 12 May, all these predatory mites were confirmed as *Amblyseius andersoni* and on 17 June, 73% were *A. andersoni* and 27% were *Neoseiulus californicus*). On 17 June, the numbers of predator eggs, mainly *Amblyseius* spp., were given an average score of 0.5 eggs per leaf. These results indicated that some of these predatory mite species survived the application of Calypso on 8 June and were continuing to lay eggs.

Site 1 cv. Maravilla

On the first assessment date on 11 August spider mite numbers were given a mean score of 3.2 (10-20 mites per leaf). However, most of the spider mites were dead. On the same date, low numbers of *P. persimilis* and other species of predatory mites were recorded (mean score of 0.2 and 0.5 respectively i.e. less than one per leaf). On the second assessment date on 2

September the numbers of TSSM had dropped with an average score of 0.02 per leaf. No acaricides had been applied during the monitoring period therefore it is likely that spider mite control was due to predatory mites, particularly *Amblyseius andersoni* which were present in similar numbers on 2 September to those recorded on 11 August. This was despite applications of Tracer (spinosad) on 15, 25 and 29 August and Equity (chlorpyrifos, no longer available) on 24 August (Table 3). On 2 September, the 'other' species of predatory mites were confirmed as 90% *A. andersoni* and 10% *N. californicus*. No information is available on the Biobest or Koppert side effects lists on the impact of pesticides on *A. andersoni* although both websites report that Tracer is 'safe' to *N. californicus* (kills less than 25%) and that chlorpyrifos (e.g. Equity) is 'moderately harmful' (kills 50-75%) to *N. californicus*. The monitoring results in this project indicated enough naturally-occurring predatory mites and *P. persimilis* survived applications of pesticides applied for control of SWD and other pests to maintain control of the spider mite population.

Site 2 cv. Kweli

Figure 22 shows the average score per lower leaflet for two spotted spider mites, *Amblyseius / Neoseiulus* spp., *P. persimilis* and their eggs at Site 2. Very few spider mites were observed during the season, with an average score of less than one (i.e. less than five mites per leaflet) at each sampling date. On 10 June, 29 June, 30 July and 2 September the average score for TSSM was 0.15, 0, 0.007, and 0.1 per leaflet respectively.

No predators were being introduced by the grower at this site but acaricides were applied for spider mite control. Apollo (clofentezine) was applied with Dynamec (abamectin) on 6 May, Apollo was applied again on 14 July and Dynamec was applied on 25 July (Table 4).

Natural populations of predatory mites were observed during the monitoring period. On the first assessment on 10 June, the average score for predatory mites was 0.6 per leaflet, when 82% were confirmed as *N. californicus* and 18% confirmed as *A. andersoni*. On the assessments on 29 June, 30 July and 2 September the average scores for predatory mites were 0.2, 0.3 and 0.1 per leaflet respectively. On the final assessment on 2 September, 90% were confirmed as *A. andersoni* and 10% as *N. californicus*. Numbers of naturally-occurring *P. persimilis* were negligible. It was concluded that acaricides, together with naturally-occurring predatory mites maintained spider mite populations at low levels at this site and that some *A. andersoni* and *N. californicus* survived both acaricides used for spider mite control and insecticides applied for SWD and other pests.

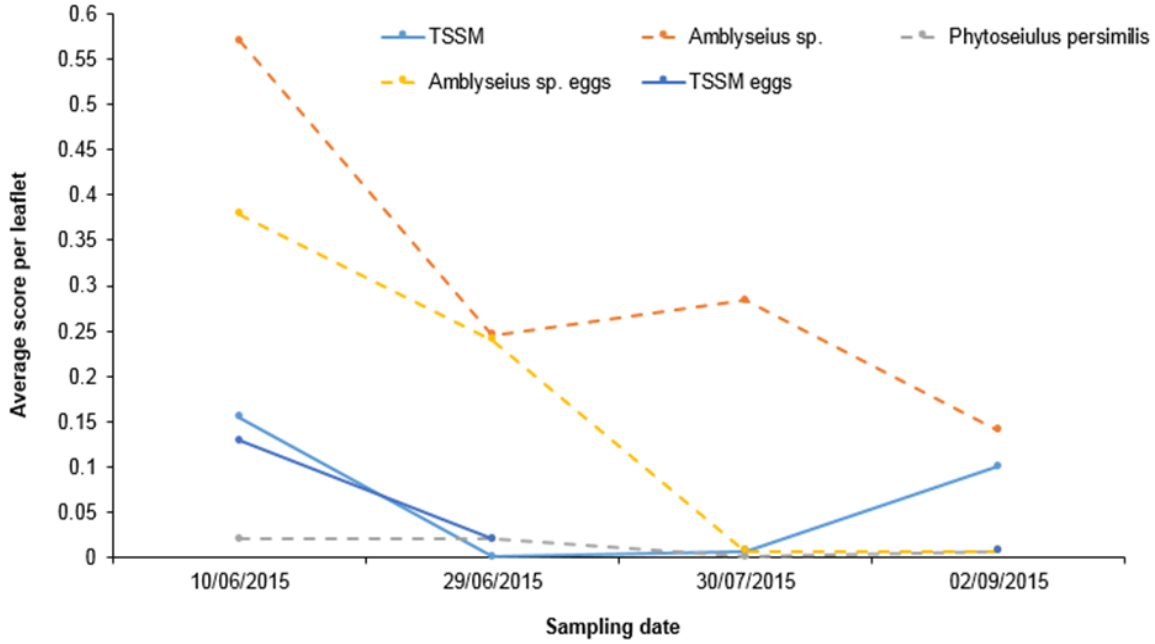

Figure 22. The average score per lower leaflet for two spotted spider mites, *Amblyseius/Neoseiulus* spp. *P. persimilis* and their eggs at Site 2. Score below 1 indicates less than one mite or egg per leaflet.

Conclusions

Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil

- Refuge traps can be used to infect adult vine weevils with *Metarhizium brunneum*.
- Further work on effective dose rate, formulation and finding an effective vine weevil lure could help to make the method cost-effective for commercial use. This would require approval for use of the EPF in the traps.

Using *Neoseiulus cucumeris* to deliver an entomopathogenic fungus to strawberry flowers for the control of thrips

- *N. cucumeris* were seen to carry *Beauveria bassiana* spores.
- Spores were carried to flowers in the bioassays either by *N. cucumeris* or WFT adults and this led to infection of WFT adults following incubation at optimum humidities for fungal development.
- Further work on whole plants in a semi-field experiment under more realistic conditions would be needed to further evaluate this novel approach to using entomopathogenic fungi. This work would be justified if there is a clear route to approval for using a fungus in this way.

Determining the speed of kill of adult vine weevil when using E-nema weevil-stop traps.

- Nematop® Käfer-Stopp traps killed 92% of weevils in 30 days and 50% of weevils in 15.5 days. The traps are being further investigated together with vine weevil lures in the current AHDB Horticulture project HNS 195 'Improving vine weevil control in HNS'.

Monitoring the effects of insecticide applications for spotted wing drosophila on predatory mites and two spotted spider mites in raspberries.

- At Site 1 on cv. Tulameen, low numbers of *P. persimilis* (released by the grower), *A. andersoni* and *N. californicus* (naturally-occurring) survived an application of Calypso on 8 June for raspberry beetle control and numbers of spider mite remained stable between 12 May and 17 June. Calypso is also one of the insecticides recommended for SWD control.
- At Site 1 on cv. Maravilla, low numbers of *P. persimilis* (released by the grower), *A. andersoni* and *N. californicus* (naturally-occurring) survived applications of Tracer on 15, 25 and 29 August and Equity on 24 August and were concluded to have been responsible for the decrease in numbers of spider mites to negligible numbers between 11 August and 2 September.
- At Site 2 on cv. Kweli, the acaricides Apollo and Dynamec, together with naturally-occurring predatory *A. andersoni* and *N. californicus* maintained spider mite populations at low levels. A proportion of the *A. andersoni* and *N. californicus* populations survived both the acaricides used for spider mite control, Tracer and pyrethrum applied for SWD control and Equity and Calypso for control of other pests.
- The monitoring results from both sites highlight the important role of naturally-occurring predatory mites in maintaining spider mite control when applying pesticide programmes for control of SWD and other pests.

Knowledge and Technology Transfer

The results of each research project were discussed informally with the growers hosting the trial.

Publications (with input from experienced ADAS colleagues):

- Gemma Hough co-authored an article on results of the MOPS project (CP 124) with Jude Bennison in an AHDB Horticulture News article, April 2015 edition.

Sacha White and Jude Bennison are co-authoring an article on the final results of the Fellowship project in AHDB Horticulture News, due in the June 2016 edition.

Gemma Hough co-authored an updated AHDB Horticulture Factsheet 10/12: Control of whitefly in protected ornamental crops with Jude Bennison and David Talbot.

Gemma Hough authored the Fellowship report on monitoring the impact of pesticides applied for the control of spotted wing drosophila on raspberries which was then incorporated into the annual report for project SF 158 'Integrated Management of cane fruit pests and diseases'

Presentations to industry:

On behalf of Gemma Hough, Jude Bennison presented the results of the experiment on using the e-nema weevil traps for the control of adult vine weevils with entomopathogenic nematodes at the AHDB Horticulture ornamentals conference in February 2016.

- Sacha and Kerry demonstrated the experiment assessing the use of *Neoseiulus cucumeris* as a vector of an entomopathogenic fungus for thrips control to AHDB Horticulture staff and members of the PE Panel in March 2016.

Presentations at scientific conferences:

- EUCARPIA Leafy Vegetable congress – "Screening for host-plant resistance against Nr:0 and Nr:1 biotypes of *Nasonovia ribisnigri*" (Gemma Hough)
- IOBC meeting Microbial and Nematode Control of Invertebrate Pests – "Entomopathogenic nematodes for the control of oak processionary moth in the UK" (Sacha White)
- On behalf of Gemma Hough, Jude Bennison presented the results of the experiment on using the e-Nema weevil traps for the control of adult vine weevils with entomopathogenic nematodes at the AAB conference 'IPM – the 10 year Plan' in November 2015

Glossary

Mycosis - the presence of parasitic fungi in or on any part of the body.

References

Jandricic, S. & Frank, S.D. (2014). Too scared to eat: Non-consumptive effects of predatory mites reduce plant damage by western flower thrips larvae. *IOBC wprs Bulletin* **102**, 111-115.