Horticultural Fellowship Awards

Interim 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:	Interim, 31 March 2014
Previous reports:	Interim reports 2012 and 2013
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 Maulden, Senior Research Manager, ADAS Boxworth (mentor)

("Trainees")	Gemma Hough, Entomologist, ADAS Boxworth (Fellowship trainee Entomologist and Project Manager from Dec 2012) 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 a Trainee Horticultural Consultant) Robert Drummond, Technician, ADAS Boxworth (Fellowship trainee scientific support staff) 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):	31 March 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 .....

Report authorised by:

Dr Tim O'Neill

Horticulture Research Manager

ADAS

Signature ..... Date .....

CONTENTS

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	ongoing	-
2. Deliver practical solutions to selected current and emerging pest management problems through specific applied research projects.	31/03/2016	ongoing	-
3. Transfer knowledge and new IPM developments to the industry through a range of communication media.	31/03/2016	ongoing	-

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 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, already working with Jude Bennison and her team in two Defra-funded IPM projects and the HDC Vine weevil review.

Gemma Hough joined ADAS Boxworth and replaced Tom Pope as a research entomologist in December 2012 after completing a HDC-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. She is already involved in the delivery of two HDC-funded projects on improving biological control of aphids on protected herbs (PE 006a),

and a review of vine weevil control. Gemma is also project leader for *Scaptomyza flava* on baby-leaf salads (FV 408a) and Evaluating aphid control strategies (FV 435).

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 has now 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 and on the identification of thrips species on strawberry. Sacha is also involved in the delivery of projects investigating improved biological control of the invasive oak processionary moth (Defra), slug control in wheat (commercial), insecticide resistance in the UK (part HDC funded) and contributing toward a pest and beneficial encyclopedia (AHDB-HGCA funded).

Mentoring activities during the second year of the Fellowship included:

Visits to commercial nurseries and farms

Visits were made by Gemma Hough and Sacha White with Senior ADAS entomologists, Jude Bennison, Steve Ellis and ADAS Horticultural Consultant Harriet Roberts. Nurseries and farms visited included:

Hardy nursery stock: Gemma Hough and Sacha White participated in the delivery of Integrated Pest Management workshops with Jude Bennison for HNS growers. Gemma Hough also visited growers to monitor aphids and hyperparastism.

Soft fruit: Gemma Hough and Sacha White visited a soft fruit nursery with fruit consultant Harriet Roberts to monitor for vine weevil and identify symptoms of other pests. Sacha also visited a strawberry crops to monitor for thrips species and damage.

Field vegetables: Gemma Hough and Sacha White visited lettuce growers to identify pests and monitor aphids and parasitism. They also participated in the delivery of an IPM workshops on reducing pesticide residues in leafy salads.

Protected edibles: Gemma Hough and Sacha White visited various sweet pepper growers to collect aphid samples for a project on aphid resistance. Gemma also visited a herb

grower and participated in the delivery of an IPM workshop with Jude Bennison for protected and outdoor herb growers.

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. Training courses included:

- Aphid parasitoid, hyperparasitoid spp. and *Scaptomyza* spp. identification (training given by Tracie Evans and Heather Maher to Gemma Hough and ADAS scientific support staff. Following this training given by Gemma Hough to Sacha White).
- Microphotography and video stills (training given by Tom Pope, Harper Adams University).
- Nematode extraction and identifying leaf and bud nematodes (training given by Jude Bennison and Kerry Maulden).
- Identifying the cause of pest damage on horticultural crops (training given by Gemma Hough, Sacha White and Jude Bennison to ADAS scientific support staff).
- Thrips species identification (training given by Jude Bennison to Gemma Hough, Sacha White, Kerry Maulden and Steven Richardson).

Technical updates on biocontrol agents, biopesticides, pesticides and horticultural research

Technical meetings with ADAS horticultural colleagues, suppliers of pesticides, biopesticides and biocontrol agents were attended throughout the year. These meetings provided updates on new products under development or those recently available for use by UK growers e.g. Bayer crop science, BASF, Sygenta, Landseer Ltd. Industry commodity group meetings and HDC research update meetings were also attended e.g. HDC Herbaceous perennial technical discussion group.

Scientific conference attended by Gemma Hough and Sacha White include:

- AAB Pushing Back the Frontiers
- Aphid Special Interest Group. Royal Entomological Society meeting.
- IOBC/wprs Working Group Insect Pathogens and Entomoparasitic Nematodes. Biological Control- its unique role in organic and integrated production.

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

Efficacy of entomopathogenic nematodes against vine weevil

The aim of this project was to assess the efficacies of four commercially available nematode products Nemasys L® (*Steinernema kraussei*), Nemasys H® (*Heterorhabditis bacteriophora),* Nematop® (*Heterorhabditis bacteriophora)* and Larvanem® (*Heterorhabditis bacteriophora)* and the entomopathogenic fungus, Met52® (*Metarhizium anisopliae),* for the control of vine weevil larvae. Efficacy of Met52 combined with each of the nematode products was also determined.

The experiment was done in a poly tunnel at ADAS Boxworth. On 20 June 2013, ten bare-rooted everbearer strawberry plants were planted per standard one metre-long grow-bag of either 80% peat and 20% wood fibre or coir. Met52 had been incorporated into the growing medium in some of the bags by the supplier. Vine weevil eggs were added on 23 August (15 eggs per plant) and curative applications of the nematode products were made on 16 September. In early November, plants were destructively sampled and the numbers of live larvae in each grow-bag were recorded.

Aphid hyperparasitoids on hardy nursery stock

Aphid hyperparasitoids were collected from a hardy nursery stock (HNS) site in Norfolk where the grower used regular releases of the aphid parasitoid mix which includes the six parasitoid species *Aphidius colemani*, *Aphidius ervi* and *Aphelinus abdominalis*, *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola*.

The site was sampled on three occasions and parasitised (mummified) aphids were collected. Where possible, the aphid species and primary parasitoid genus were identified from the appearance of the 'mummy'. Evidence of primary parasitoid emergence (indicated by a neat circular exit hole) or hyperparasitoid emergence (indicated by a ragged emergence hole) was also recorded. Where there was no emergence hole, the mummified aphids were kept in the laboratory until either a primary or a hyperparasitoid emerged and this was then identified.

Biological control of aphids on lettuce

The population dynamics of aphids in response to the release of the parasitoid *Aphidius colemani* in two organic lettuce crop was monitored. Aphid numbers, mummies and

predator numbers were recorded on sequential dates. Mummies were collected and where possible, the aphid species, primary parasitoid and any hyperparasitoid genus were identified.

The release strategy used by the grower was also evaluated. Currently the grower walks through the field regularly distributing mummies onto the crop. A dispersal experiment was carried out to determine whether parasitoids released from one location would spread throughout the rest of the field. Releasing parasitoids from one location would reduce the labour costs compared to the current release strategy.

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

Knowledge transfer activities delivered by Gemma Hough and Sacha White in year 3 of this project related both to this project, and also to other horticultural projects, and included:

Publications (with input from experienced ADAS colleagues):

Gemma Hough:

● HDC News articles on the entomology fellowship (CP 89), the leaf miner *Scaptomyza flava* (FV 408) and the biological control of herb aphids (PE 006a).

● Update of HDC Factsheet 10/12 Whitefly (in progress).

● Pope, T., Gundalai, E., Hough, G., Wood, A., Bennison, J., Prince, G., and Chandler, D. (2013) How far does a weevil walk? *Aspects of Applied Biology,* 119, 97-104.

● Update- HDC Herb Best Practice Guide: http://herbs.hdc.org.uk/

Sacha White:

● Poster on DEFRA project on the improved control of oak processionary moth (TH0102) for Defra Plant Health Summit, January 2014.

● AHDB- funded encyclopaedia on pest and beneficials (in progress).

Presentations to industry:

● Maintaining the Expertise for Developing & Communicating Practical IPM Solutions for Horticulture (2011-2016) – HDC Studentship Conference (Gemma Hough).

● RPDE Event- Leafy Salads: Pesticide residue reduction strategies and current research (Gemma Hough and Sacha White).

● RPDE Event- Integrated Pest Management on protected and outdoor herbs (Gemma Hough and Jude Bennison).

● RPDE Event - Integrated pest and disease management workshop for hardy nursery stock (Sacha White and Jude Bennison).

● IRAC meeting - Combating insecticide resistance in major UK pests (Sacha White).

● HGCA monitoring meeting - Combating insecticide resistance in major UK pests (Sacha White).

● HGCA agronomy workshop - Pests: managing resistance with less chemistry (Sacha White).

● MSc lecture at Harper Adams University College on IPM (Jude Bennison and Gemma Hough)

Presentations at scientific conferences:

● AAB Pushing Back the Frontiers - Biological control of vine weevil larvae on protected strawberry (Gemma Hough).

● The tip of the iceberg: Biological control of aphids on organic field grown lettuce. Aphid Special Interest Group. Royal Entomological Society meeting (Sacha White).

● Improved biological control of herb aphids. Aphid Special Interest Group. Royal Entomological Society meeting (Gemma Hough).

Milestones not being reached

None

Do remaining milestones look realistic?

Yes

Other achievements in the last year not originally in the objectives

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

- HDC-funded project PE 006a - Protected herbs: improved biological control of aphids.
- CRD-funded project PS2134 - Use of refuge traps to disseminate entomopathogenic fungi for the control of adult vine weevil.
- HortLINK project HL001107 - Biological, semiochemical and selective chemical management methods for insecticide resistant western flower thrips on protected strawberry.
- DEFRA-funded project FFG 1146 – Tree health: review and analysis of control strategies for established pests and pathogens of trees to inform current and future management.
- AHDB-HGCA funded project – Pests and Beneficials Encyclopaedia for Arable and Field Crops.
- DEFRA-funded (CRD) – PS2722 Combating insecticide resistance in major UK pests
- DEFRA-funded – TH0102 Improved Control Methods for Oak Processionary Moth

Gemma is also project leader for the HDC project FV408a Baby-leaf Cruciferae: Improved control of *Scaptomyza flava,* and will be working closely with Jude Bennison on the management of experiments within the Managing Ornamental Plants Sustainably (MOPS) project. In addition to the technical skills learnt through involvement on these projects, this work has provided several knowledge transfer opportunities as previously discussed. These activities were delivered by Gemma Hough and 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.

None

GROWER SUMMARY

Headline

- All the entomopathogenic nematode products and Met52 in a coir substrate significantly reduced the numbers of live vine weevil larvae in substrate-grown strawberry when compared with untreated controls.
- Aphid hyperparasitism shows annual and seasonal variation. Percentage aphid hyperparasitism was between 0 and 95% on a HNS nursery during 2013. Compared to 2012 percentage hyperparasitism was similar in May but higher in August following a warm summer.
- Monitoring of parasitised potato aphid (*Macrosiphum euphorbiae*) mummies on an outdoor organic lettuce crop showed that naturally occurring parasitoids such as *Praon volucre* and *Aphidius ervi* were responsible for most of the parasitism rather than *A. colemani* which was released by the grower. Predators and entomopathogenic fungi were also observed. The control of aphids was likely to be due to the natural enemy community rather than one individual species.

Background

Efficacy of entomopathogenic nematodes against vine weevil

Vine weevil (*Otiorhynchus sulcatus*) remains one of the most serious problems in both soft fruit and nursery stock industries. In order to reduce damage caused by this pest, controls can be targeted against both the larvae in the soil and the adult weevils within the crop. Biological control of vine weevil is preferable to the use of insecticides in Integrated Pest Management (IPM) programmes. Current options for biological control of vine weevil larvae are entomopathogenic nematodes (various species and products) and the entomopathogenic fungus *Metarhizium anisopliae* (Met52).

The aim of this project was to assess the efficacies of four commercially available nematode products Nemasys L® (*Steinernema kraussei*), Nemasys H®, Nematop® and Larvanem® (all *Heterorhabditis bacteriophora)* and the entomopathogenic fungus, Met52® (*Metarhizium anisopliae),* for the control of vine weevil larvae. Efficacy of Met52 combined with each of the nematode products was also determined.

Aphid hyperparasitoids on protected ornamentals

Aphid parasitoids are widely used for biological control of aphids within IPM programmes on many protected crops. Until recently, biological control of aphids on protected crops relied mainly on three aphid parasitoid species:

- *Aphidius colemani* for control of e.g. the peach-potato aphid, *Myzus persicae* and the melon-cotton aphid, *Aphis gossypii*.
- *Aphidius ervi* and *Aphelinus abdominalis* for control of e.g. the potato aphid, *Macrosiphum euphorbiae* and the glasshouse-potato aphid, *Aulacorthum solani*.

Use of aphid parasitoids on some crops has increased recently, due to the availability of a new mix of six parasitoid species. The new mix contains the above three parasitoid species plus an additional three species (*Aphidius matricariae*, *Ephedrus cerasicola* and *Praon volucre*) which has extended the range of aphid species that can be parasitised, and have thus led to further uptake of aphid parasitoids on a range of crops. In 2005, in a MAFF (now Defra)-funded project on developing IPM in outdoor HNS, ADAS confirmed that hyperparasitoids (secondary parasitoids which parasitise the primary aphid parasitoids) were a potential problem in naturally- parasitised aphids in outdoor HNS (Buxton *et al.* 2005). More recent investigations by Rob Jacobson in HDC-funded project PC 295, 295a and 295b have shown that breakdown in aphid control by parasitoids in mid-summer on some sweet pepper nurseries were predominantly due to the presence of hyperparasitoids (Jacobson 2010, 2011).

During 2011 in this Fellowship project, the presence of hyperparasitism was monitored and confirmed in sweet pepper, protected strawberry and hardy nursery stock crops. A range of aphid species were parasitised by both *Aphidius* spp. and *Praon* spp. The hyperparasitoid species identified were similar to those recorded in PC 295 and 295a and b, including *Asaphes suspensus*, *Asaphes vulgaris*, *Dendrocerus carpenteri*, *Dendrocerus laticeps* and *Pachyneuron* sp. On protected strawberry, hardy nursery stock (HNS) and sweet pepper hyperparasitism reached 5, 32 and 25% respectively in 2011. During 2012 on a HNS site hyperparasitism reached 50% on 18 May and 70% on 1 August. The aim during 2013 was to continue monitoring hyperparasitism at the same HNS site.

Biological control of aphids on lettuce

Control of aphids on lettuce with pesticides is becoming increasingly difficult due to the limited number of pesticides available, pressures to reduce pesticide use and the increasing aphid resistance issues relating to both insecticides and to resistant cultivars which have been observed on lettuce for the peach-potato aphid, *Myzus persicae* and to currant-lettuce

aphid, *Nasonovia ribisnigri* respectively. A major grower has reported achieving successful control of aphids in organic outdoor lettuce through the release of parasitoids. The use of biological control in field-grown lettuce, particularly for organic growers, could be an important component of an Integrated Pest Management (IPM) programme.

During 2012 in this Fellowship project, the effect of releasing parasitoids in an outdoor organic lettuce crop was monitored but only low levels of parasitism were observed. Low parasitism was likely to have been due to the presence of the entomopathogenic fungi which killed most of the aphids that had infested the plants after planting. The aim during 2013 was to continue monitoring parasitism following the release of parasitoid and evaluate the grower's current release strategy.

Summary

Efficacy of entomopathogenic nematodes against vine weevil

The aim of this project was to assess the efficacies of four commercially available nematode products Nemasys L® (*Steinernema kraussei*), Nemasys H® (*Heterorhabditis bacteriophora),* Nematop® (*Heterorhabditis bacteriophora)* and Larvanem® (*Heterorhabditis bacteriophora)* and the entomopathogenic fungus, Met52® (*Metarhizium anisopliae),* for the control of VW larvae. Efficacy of the Met52 combined with the Nemasys L, Nematop Larvanem and Nemasys H was also determined.

The experiment was done in a poly tunnel at ADAS Boxworth. On 20 June, ten bare-rooted everbearer strawberry plants were planted per standard one metre-long grow-bag (either coir or 80% peat and 20% wood fibre). Vine weevil eggs were added on 23 August (15 eggs per plant) and curative applications of the nematode products were made on 16 September. In early November, plants were destructively sampled and the numbers of live larvae in each grow-bag were recorded.

The results were as followed:

● All the nematode products and Met52 in a coir substrate significantly reduced the numbers of live vine weevil larvae in substrate-grown strawberry when compared with untreated controls.

● Met52 in coir was as effective as Larvanem, Nematop and Nemasys H but less effective than Nemasys L. Met52 in a peat substrate was ineffective.

● Nemasys L (*Steinernema kraussei)* and Larvanem (*Heterorhabditis bacteriophora)* were the best performing products and were not significantly different in their reduction of mean numbers of live vine weevil larvae. Nematop and Nemasys H (both *Heterorhabditis bacteriophora)* were not significantly different than Larvanem but did not reduce the mean number of vine weevil larvae as effectively as Nemasys L.

● Combining nematodes with Met52 did not significantly improve the control of vine weevil larvae compared to when using nematodes alone.

Aphid hyperparasitoids on protected ornamentals

Aphid hyperparasitoids were collected from a hardy nursery stock (HNS) site in Norfolk where the grower used regular releases of a new aphid parasitoid mix which included the six parasitoid species *Aphidius colemani*, *Aphidius ervi* and *Aphelinus abdominalis*, *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola*.

The site was sampled on 23 May, 16 July and 13 August and hyperparasitism ranged between 0-44, 0-90 and 13-95% at each date respectively with the highest parasitism on *Solanum* sp., Cosmos Chocamocha and Cistus x purpureus. The main aphid species was the potato aphid, *Macrosiphum euphorbiae.* The hyperparasitoid species identified were *Dendrocerus* sp. *Asaphes* sp. and *Alloxysta brevis*.

During 2012, hyperparasitism on 18 May was between 0-50% which was similar to the 0-44% observed this year on 23 May 2013. However, by 13 August 2013, following a prolonged July heat wave, hyperparasitism increased and was higher (13-95%) compared with 1 August 2012 (17-70%).

Biological control of aphids on lettuce

Following reports that a major lettuce grower had been achieving successful control of aphids in organic outdoor lettuce through the release of parasitoids (*Aphidius colemani*), it was decided to evaluate the population dynamics of aphids in response to the release of parasitoids in an organic lettuce crop. Between 4 June 2013 and 17 July 2013 two fields were monitored and the presence of aphids, mummies and natural enemies were recorded (Objective 1). In both monitored fields natural parasitism was occurring i.e. parasitoid species which had not been released by the grower. It was concluded that the release of *A. colemani* into the field is unlikely to have made a significant contribution to the control of the aphid populations. As *Macrosiphum euphorbiae* was the most common aphid recorded and it is not readily parasitised by *A. colemani*, it is likely that the control of aphids was due to

the natural enemy community rather than one individual species. Syrphid (hoverfly) larvae were observed in high numbers in Objective 1.

A second experiment was also carried out to evaluate the grower's parasitoid release strategy (Objective 2). The release strategy used by the grower involved walking through the field distributing mummies onto the crop at repeated locations. Determining whether the grower could release parasitoids from one location and achieve the same control as the currently used strategy would allow a less labour intensive method to be used.

Following the release of *A. colemani* into designated release areas, aphid numbers were observed to decrease two weeks later in both the release and non-release areas. The confirmation of *A. colemani* mummies 35 m into the non-release areas as early as three weeks into the experiment suggests that the parasitoids were able to move out of the release areas fairly quickly (assuming that they were not naturally occurring *A. colemani*). Overall few *A. colemani* were recorded in the crop and as in Objective 1, more natural parasitism (*Praon volucre* and *Aphidius ervi)* and predation (spiders) was observed. Spiders were observed in high numbers in Objective 2. This suggests that a range of parasitoids and predators contributed to the aphid control and it was not possible to confirm whether releasing parasitoids at fewer locations was as effective as the current grower release strategy.

Financial Benefits

- No clear financial benefits could be determined from this experiment
- Biocontrol of aphids usually requires regular releases of parasitoids. High proportions of aphid hyperparasitoids reduce the effectiveness of these parasitoids, resulting in increased losses caused by aphids. Growers will benefit from being aware of this risk on a range of horticultural crops so that they can adapt their IPM programmes if needed.
- Growers are not always confident of using entomopathogenic nematodes for control of vine weevil in strawberry, and are unsure of which product to buy. Growers will benefit from the results in this project which compared the efficacy of different products for the control of vine weevil larvae allowing them to make an informed choice.

Action Points

- Growers using aphid parasitoids in any crop should be aware that aphid hyperparasitism may occur. Look out for ragged emergence holes in aphid 'mummies' as an indicator that hyperparasitoids are present.
- Seek advice from your biocontrol supplier or IPM consultant if there are high levels of aphid hyperparasitism. It is likely that you will need to switch from using aphid parasitoids to aphid predators, and/or IPM-compatible pesticides.
- Growers should take care when using Met52 and nematodes which can be sensitive to temperature and moisture. Apply the products when conditions are suitable for optimum efficacy.
- Natural beneficial insects can help to control aphid populations. Use the parasitoid mix rather than a single species on crops that can be infested with a range of aphid species.

SCIENCE SECTION

Introduction

Efficacy of entomopathogenic nematodes against vine weevil

Vine weevil (*Otiorhynchus sulcatus*) remains one of the most serious problems in both soft fruit and nursery stock industries. In order to reduce damage caused by this pest, controls can be targeted against both the larvae in the soil and the adult weevils within the crop. Biological control of vine weevil is preferable to the use of insecticides in Integrated Pest Management (IPM) programmes. Current options for biological control of vine weevil larvae are entomopathogenic nematodes (various species and products) and the entomopathogenic fungus *Metarhizium anisopliae* (Met52).

The aim of this project was to assess the efficacies of four commercially available nematode products Nemasys L® (*Steinernema kraussei*), Nemasys H® (*Heterorhabditis bacteriophora),* Nematop® (*Heterorhabditis bacteriophora)* and Larvanem® (*Heterorhabditis bacteriophora)* and the entomopathogenic fungus, Met52® (*Metarhizium anisopliae),* for the control of vine weevil larvae. Efficacy of Met52 combined with the all of the nematode products was also determined.

Aphid hyperparasitoids on hardy nursery stock

Aphid parasitoids are widely used for biological control of aphids within IPM programmes on many protected crops. Until recently, biological control of aphids on protected crops relied mainly on three aphid parasitoid species:

- *Aphidius colemani* for control of e.g. the peach-potato aphid, *Myzus persicae* and the melon-cotton aphid, *Aphis gossypii*.
- *Aphidius ervi* and *Aphelinus abdominalis* for control of e.g. the potato aphid, *Macrosiphum euphorbiae* and the glasshouse-potato aphid, *Aulacorthum solani*.

Use of aphid parasitoids on some crops has increased recently, due to the availability of a new mix of six parasitoid species. The new mix contains the above three parasitoid species plus an additional three species (*Aphidius matricariae*, *Ephedrus cerasicola* and *Praon volucre*). The mix is produced by Viridaxis in Belgium and is available as various products, such as Aphidsure mix ® (for use on various crops) and Aphidsure fragaria ® (for strawberry) supplied by BCP Certis and FresaProtect ® (for strawberry) and OrnaProtect ® (for ornamentals) from various other suppliers. These products have extended the range of

aphid species that can be parasitised, and have thus led to further uptake of aphid parasitoids on a range of crops, particularly those such as HNS and soft fruit that can be attacked by a wide range of aphid species.

In 2005, in a MAFF (now Defra)-funded project on developing IPM in outdoor HNS, ADAS confirmed that hyperparasitoids (secondary parasitoids which parasitise the primary aphid parasitoids) were a potential problem in naturally- parasitised aphids in outdoor HNS (Buxton *et al.* 2005). Seven species of hyperparasitoids were confirmed in this project. More recent investigations by Rob Jacobson in HDC-funded project PC 295, 295a and 295b have shown that breakdown in aphid control by parasitoids in mid-summer on some sweet pepper nurseries were predominantly due to the presence of hyperparasitoids (Jacobson 2010, 2011).

During 2011 in this Fellowship project the presence of hyperparasitism was monitored and confirmed in sweet pepper, protected strawberry and hardy nursery stock crops. A range of aphid species were parasitised by both *Aphidius* spp. and *Praon* spp. The hyperparasitoid species identified were similar to those recorded in PC 295 and 295a and b, including *Asaphes suspensus*, *Asaphes vulgaris*, *Dendrocerus carpenteri*, *Dendrocerus laticeps* and *Pachyneuron* sp. On protected strawberry, hardy nursery stock (HNS) and sweet pepper hyperparasitism reached 5, 32 and 25%.

During 2012 the presence of hyperparasitism was monitored in hardy nursery stock crops.

The aim during 2013 was to continue monitoring hyperparasitism at a HNS site

Biological control of aphids on lettuce

Control of aphids on lettuce with pesticides is becoming increasingly difficult due to the limited number of pesticides available, pressures to reduce pesticide use and the increasing aphid resistance issues to both insecticides and to resistant cultivars which has been observed on lettuce for the peach-potato aphid, *Myzus persicae* and to currant-lettuce aphid, *Nasonovia ribisnigri* respectively. A major grower has reported achieving successful control of aphids in organic outdoor lettuce through the release of parasitoids. The use of biological control in field-grown lettuce, particularly for organic growers, could be an important component of an Integrated Pest Management (IPM) programme.

During 2012 in this Fellowship project, the effect of releasing parasitoids in an outdoor organic lettuce crop was monitored but only low levels of parasitism were observed. Low parasitism was likely to have been due to the presence of the entomopathogenic fungi which killed most of the aphids that had infested the plants after planting. The aim during

2013 was to continue monitoring parasitism following the release of parasitoids and to evaluate the grower's parasitoid release strategy.

Materials and methods

Efficacy of entomopathogenic nematodes against vine weevil

The experiment consisted of fourteen treatments (Table 1). There were two untreated coir treatments (treatments 1 and 2) and two Met52 coir treatments (treatments 8 and 9).

Table 1 Treatments, rates and methods of application

Trt. num	Product name	Active substance	Supplier	Substrate	Label recommended rate	Equivalent nematodes per litre of compost	Application method
1	Untreated	-	-	Coir	-	-	-
2	Untreated	-	-	Coir	-	-	-
3	Untreated	-	-	Peat	-	-	-
4	Nemasys L	*Steinernema kraussei*	BASF	Coir	25,000 per plant	10,000 per l	Drench
5	Nemasys H	*Heterorhabditis bacteriophora*	BASF	Coir	25,000 per plant	10,000 per l	Drench
6	Nematop	*H. bacteriophora*	e-Nema	Coir	25,000 per plant	10,000 per l	Drench
7	Larvanem	*H. bacteriophora*	Koppert	Coir	1 million per m^2	10,000 per l	Drench
8	Met52	*Metarhizium anisopliae* var. *anisopliae*	Novozymes	Coir	500 g/m^2	-	Substrate incorporation
9	Met52	*M. anisopliae* var. *anisopliae* F52	Novozymes	Coir	500 g/m^2	-	Substrate incorporation
10	Met52	*M. anisopliae* var. *anisopliae* F52	Novozymes	Peat	500 g/m^2	-	Substrate incorporation
11	Met52 + Nemasys L	*M. anisopliae* var. *anisopliae* F52 + *S. kraussei*	Novozymes + BASF	Coir	500 g/m^2 + 25,000 per plant	10,000 per l	Substrate incorporation + Drench
12	Met52 + Nemasys H	*M. anisopliae* var. *anisopliae* F52	Novozymes + BASF	Coir	500 g/m^2 + 25,000 per plant	10,000 per l	Substrate incorporation + Drench
13	Met52 + Nematop	*M. anisopliae* var. *anisopliae* F52 + *H. bacteriophora*	Novozymes + e-nema	Coir	500 g/m^2 + 25,000 per plant	10,000 per l	Substrate incorporation + Drench
14	Met52 + Larvanem	*M. anisopliae* var. *anisopliae* F52	Novozymes + Koppert	Coir	500 g/m^2 + 1,000,000 per m2	10,000 per l	Substrate incorporation + Drench

N.B. All nematode products were recommended on the product leaflet to be applied at 100 ml of water per plant except for Nematop which was recommended at 200ml per plant. Due to concern about the risk of water logging and potential nematode run-off, all treatments were applied at 100ml of water per plant.

Experimental plants and substrate:

Standard one metre-long grow-bags, each containing 25 litres of substrate were obtained from Bulrush Horticulture Ltd. Four grow-bags contained 80% peat and 20% wood fibre, 24 contained coir, four contained Met52 incorporated into 80% peat and 20% wood fibre and 24 contained Met52 incorporated into coir.

Bare-rooted everbearer strawberry plants (cv. Calypso) were purchased from Hargreaves Plants Ltd.

Mealworm test for Met52:

Grow-bags were tested for the presence of Met52 by carrying out a mealworm test (Figure 2).

On 4 July 2013, substrate samples were taken from each grow-bag and were placed in Petri dishes to which ten mealworms were added. The Petri dish was sealed and kept in an incubator at 25°C 16L:8D. After 7-10 days the presence of mealworms infected with *M. anisopliae* were recorded.

Figure 2 Mealworm test

Experiment design

Ten strawberry plants were planted per grow-bag on 20 June. This was later reduced to six plants per bag as explained below. Each grow-bag represented a treatment plot, and there were four replicates per treatment except for treatment 1 and 8 which had eight replicates each. Treatments were arranged in a randomised block design in a polytunnel at ADAS Boxworth, Cambridgeshire (Figure 3).

Figure 3 Strawberry experiment in grow-bags in a polytunnel at ADAS Boxworth

Irrigation, temperatures and reduction in numbers of plants per bag

Overhead irrigation was used to establish the plants between 20 June and 27 June; those plants which did not establish were replaced. Automatic drip irrigation was used thereafter. Due to a malfunction with the Dosatron between 27 June and 16 July, feed was not delivered correctly. Although this was rectified four plants furthest away from the drippers in each coir bag failed to recover (Figure 4). In order to standardize the number of healthy plants per grow-bag, on 23 August the four plants which failed to establish, and an equivalent four plants in each peat bag, were removed by cutting them just above the crown leaving six plants per bag.

Temperature of the substrate at root depth was measured throughout the experiment using four identical data loggers.

Figure 4 Four of the six strawberry plants failed to establish

Vine weevil egg infestation

On 23 August, 15 vine weevil eggs were washed onto the soil around the stem of each of the six plants (Figure 5). An additional 60 eggs were kept on a damp filter paper in the laboratory and their viability was assessed by recording egg hatch. Fifty-six of the eggs hatched (93%) and the larvae were recovered.

Figure 5 Infesting strawberry plants with vine weevil eggs

Nematode applications

On 5 September, curative applications of each nematode product were applied as per supplier's recommendations to all ten planting holes (Table 1). All ten planting holes were treated as the roots of the removed four plants remained in the substrate and could potentially be attacked by vine weevil larvae. Nematodes were applied with a syringe rather than a sprayer or through the irrigation lines, to ensure dose accuracy to each plant (Figure 6).

Figure 6 Nematode application using syringe

For each product, counts of active nematodes in six sub-samples of the nematode suspension were completed before application. Nematode suspensions were diluted where necessary to make sure all nematodes products were applied at 250 nematodes per ml of water (in the experiment Nemasys L and Nemasys H were diluted slightly to standardize the dose rates, see Table 17). The following method was used:

1) Packs of 50 million nematodes were examined for microbial spoilage. Packs were emptied into a 1L beaker then mixed thoroughly with 500ml of water. The beaker contents were then diluted to 2L in a measuring cylinder and aerated for five minutes.

3) The air supply was turned off and after a few seconds 80ml (representing the 2 million nematodes needed for the experiment) was transferred into a bucket containing 7,920ml of water.

4) The solution was aerated again and a 5ml pipette was used to take a sample which filled a single counting chamber of a haemocytometer. Using a binocular microscope, counts of live infective juvenile nematodes were then made under each 1ml grid which was repeated six times (a count of 250 nematodes per ml was expected). The numbers of infective juveniles in each pack were determined by calculating the mean of the six counts multiplied by the dilution factor (200,000).

Assessment of vine weevil larvae and plant vigour

Between 11-14 November, the grow-bags were destructively sampled and the numbers of live vine weevil larvae were recorded by carefully searching through the roots, substrate

and breaking open the crown of the plant (Figure 7). Vine weevil larvae were collected from each grow-bag and kept in the laboratory in a Petri dish on damp filter paper to see if further infection developed.

Figure 7 Vine weevil larvae in the crown of the plant and substrate surrounded by red frass produced by the larvae feeding on the roots.

Visual assessments were also made of plant vigour (plant size and foliage health) before destructive sampling using a scale of 1-5 (Figure 8) as follows:

5 - large and healthy

4 - small and healthy

3 - discolored leaves

2 - wilted

1 - dead

5)  **4)** 

Figure 8 Vigour score: 5) Large and healthy 4) small and healthy 3) discoloured leaves 2) wilted 1) dead

Control of other pests and diseases

Regular applications of biological control agents were applied to control other pests e.g. aphids, spider mites and thrips. The biological control agents used included the predatory mite *Neoseiulus (Amblyseius) cucumeris* for thrips control, a mix of six aphid parasitoid species for aphid control and the predatory mite *Phytoseiulus persimilis* for spider mite control.

Statistical analysis

Data on the numbers of live larvae and plant vigour for each treatment were subjected to analysis of variance (ANOVA).

Aphid hyperparasitoids on hardy nursery stock

Site selection

Parasitised aphids were collected from a protected HNS site in Norfolk where the grower used regular releases of the aphid parasitoid mix of six species; *Aphidius ervi, A. colemani, A. matricariae, Praon volucre*, *Ephedrus cerasicola* and *Aphelinus abdominalis.*

Sampling and identification

The site was visited on 23 May, 16 July and 13 August. Aphid mummies were collected from protected hardy nursery stock crops including: Coronilla, Pittosporum, Coprosmam, Fuchsia, Photinia, Solanum, Phormium, Hebe, Euphorbia, Euryops, Cistus, Akebia, Sallya, Cosmos, Escallonia and Lavandula. The aphid species present on each of the crops were noted. Aphid mummies were brought back to ADAS Boxworth. If the parasitoid had already emerged, a record was made where possible detailing the aphid species, the primary parasitoid species (based on aphid mummy colour and morphology) and whether it was characteristic of a primary parasitoid or hyperparasitoid (i.e. round or jagged emergence

hole respectively). Where no emergence hole was found, the mummies were placed in glass Petri dishes in the laboratory at approximately 20°C until either a primary parasitoid or a hyperparasitoid adult emerged. Emerging parasitoid species were identified to species and emerging hyperparasitoids were identified to genus.

Biological control of aphids on lettuce

First objective

The aim of the first part of this study was to record the numbers and species of aphids and the levels of parasitism on two outdoor organic lettuce crops where the grower was releasing *Aphidius colemani* for the control of aphids. The work was carried out on an organic lettuce crop in Cambridgeshire.

Assessments: Two new plantings were monitored through to harvest. The first field was planted week beginning 27 May and sampled on 4, 12, 19, 26 June and 2 and 10 July (Figure 9). The second field was planted week beginning 17 June and sampled on 19, 26 June and 2, 10 and 17 July. The two fields were separated by another field and hedgerows.

Fifty plants were assessed on each sampling date by walking from the edge of the planting to the centre in a 'W' pattern. Plants were sampled at random and for each plant the number and species of aphid, the number of mummies (noting whether they were *Aphidius*, *Praon* or *Ephedrus*/*Aphelinus* species by observing the mummy colour as pale brown, beige or black respectively) and any aphid predators or pathogens were recorded.

Mummies were collected from each plant. Where a parasitoid had already emerged from a mummy it was recorded whether the emergence hole was characteristic of a primary parasitoid or hyperparasitoid (i.e. round or jagged respectively). The aphid species parasitised was also used to determine the parasitoid species e.g. when a *M. euphorbiae* mummy was found that had been parasitised by an *Aphidius* spp. it was assumed that this was *A. ervi* as it has a very high efficacy against this bigger aphid species. *Aphidius colemani* does not readily parasitise *M. euphorbiae* and has a preference for smaller hosts such as *M. persicae,* the peach-potato aphid. For some mummies the parasitoid never emerged and were too damaged for the host aphid species to be identified.

If no emergence hole was present, the mummies were placed in a petri dish and brought back to ADAS Boxworth where the adult parasitoids were allowed to emerge and the species identified.

Figure 9 Lettuce assessments being carried out in an organic lettuce crop

Parasitoid release: In both fields, starting when aphid numbers were low a parasitoid species mix of *A. colemani* and *A. ervi* were released weekly at a very low rate of 0.01 per m2. When aphid numbers began to increase at the end of June and the beginning of July the grower decided to release *A. colemani* instead of the mix as it was a lower cost option. The release rate was between 0.1 – 0.3 per m² each week. This release strategy was decided by the grower.

Second objective

The second part of this study aimed to determine whether parasitoids released from one area of the field could disperse to the rest of the field. Currently the grower releases parasitoids by walking over the entire field distributing mummies from the container onto the ground and plants at regular intervals. Determining whether parasitoids could be released from designated areas in the field and disperse throughout the rest of the field could improve the release strategy and reduce labour costs involved with their release.

Experiment layout: A 300m by 60m area of a lettuce field was divided into five areas (Figure 10). Two of these areas measured 130 x 50m and were designated non-release areas (NRAs). The three remaining areas were designated release areas (RAs), two of which measured 10x50m and one was 20 x 50m.

Parasitoid release: Parasitoids (*Aphidius colemani*) were released weekly at a rate of 0.28-0.4 per m² in the release areas.

Assessments: In both the RAs and NRAs the number and species of aphids, the number of mummies and any aphid predators or pathogens were recorded in the same way as in the first objective. In the release areas two lettuces were sampled at each of five, 15, 25 and 35m across the 50m width of the field (10 lettuces sampled per release area).

In the NRAs two lettuces were sampled at each of five, 15, 25 and 35m across the 50m width of the field and at 10, 35, 65, 95 and 120m along the 130m length of the field (40 lettuces sampled per non-release area).

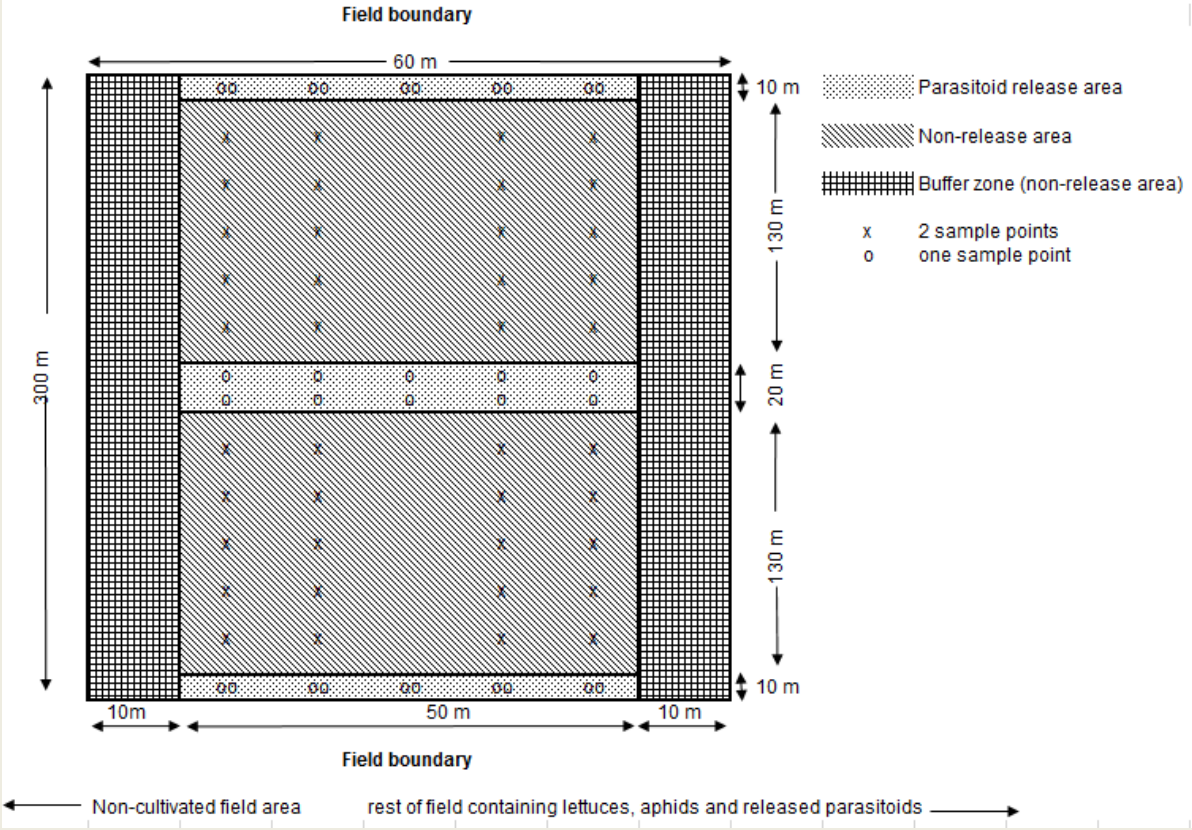


Figure 10 Experiment layout used to measure parasitoid dispersal in Objective 2.

Results and Discussion

Efficacy of entomopathogenic nematodes against vine weevil

Effects of treatment on the number of live larvae

● Analysis of the mean number of live larvae per grow-bag showed a highly significant effect ($F(13,39)$ 15.67, $P < 0.001$) of treatment (Figure 11). As expected the highest numbers of live larvae were recorded in the untreated grow-bags containing either peat (44.3 larvae per bag equivalent to a mean of 7.4 per plant) or coir substrate (39.5 (untreated coir 1) and 44 (untreated coir 2) larvae per bag equivalent to 6.6 and 7.3 larvae per plant respectively). High numbers of larvae (statistically similar numbers to the untreated bags) were also observed in grow-bags containing Met52 in a peat substrate, indicating that this treatment was not effective.

● When considering the effect of nematode treatments alone in coir (without Met52), Nemasys L and Larvanem were the most effective nematode products, reducing the mean number of larvae per bag to 1.5 and 7.5 respectively (equivalent to means of 0.3 and 1.3 per plant respectively). These two treatments were not significantly different from each other.

● Nematop and Nemasys H reduced the numbers of live larvae per bag to 16 and 20.5 respectively (equivalent to means of 2.7 and 3.4 per plant respectively) and were not significantly different from Larvanem. However, these two treatments did not reduce vine weevil larvae as well as Nemasys L.

● When used alone, Met52 in a coir substrate significantly reduced the mean number of larvae per bag when compared with numbers in untreated bags, to 23.5 (Met52 coir 1) and 19.5 (Met52 coir 2) (equivalent to means of 3.9 and 3.3 per plant respectively). However, Met52 in a coir substrate was not as effective as Nemasys L in coir but was not significantly different than Larvanem, Nematop and Nemasys H in coir.

● The difference in the performance between Met52 in peat and coir substrates could possibly have been due to a combination of differences in substrate moisture and nutrition. Between 27 June and 16 July the Dosatron was not delivering feed correctly. As the peat substrate had more nutrients naturally available the plants established better than the plants in the coir substrate. This led to a discrepancy in watering where providing sufficient water for the coir plants resulted in the peat substrates being drier as the larger established plants took up more water. These differences in substrate moisture could have affected the performance of Met52. However, this irrigation discrepancy was rectified quickly and evidence of some control by Met52 in peat would have been expected.

● When each nematode product was combined with Met52 the numbers of live larvae per grow-bag were not significantly lower compared with treatments where the nematode products were used alone. Therefore combining Met52 with nematodes did not result in improved control.

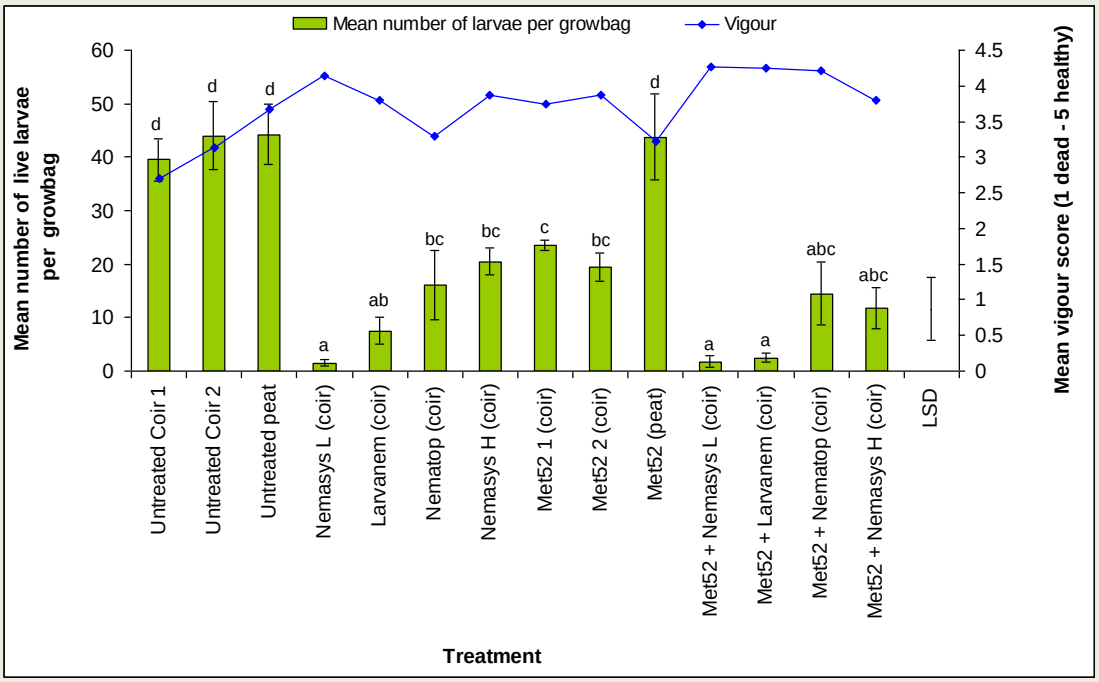


Figure 11 Mean numbers of live vine weevil larvae per grow-bag with standard error of the mean. The least significant difference (LSD) was used to determine any significant differences. Different letters above bars indicate a significant difference. The mean vigour score per grow-bag is also shown.

Effects of treatment on plant vigour

Analysis of the average plant vigour score per plot showed that despite some visual difference in plant vigour (Figure 8) there was no significant effect of treatment on plant vigour observed during the experiment (Figure 12).

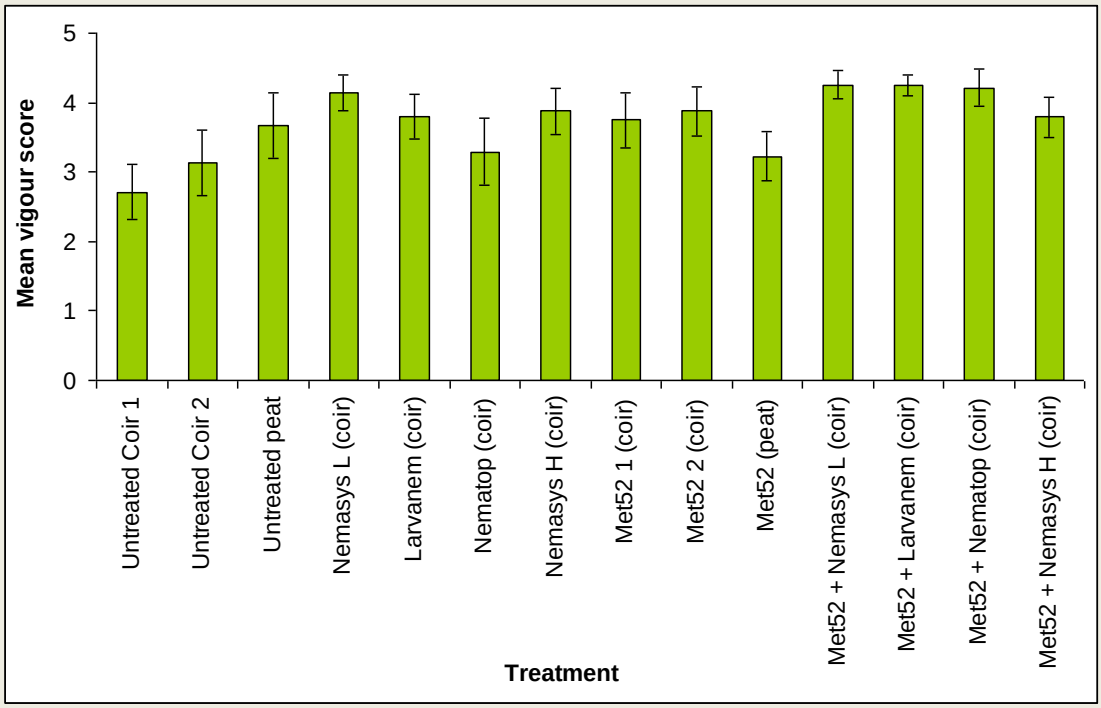


Figure 12 Mean foliage vigour score per plot for all treatments (5 very healthy, 1 dead).

When removing the peat treatments from the analysis to compare the treatments in a coir substrate only, a significant effect ($F(11,33)= 2.11$, $p= 0.048$) of treatment on the vigour scores was observed (Figure 13). Only the combined Met52 and Nemasys L treatment had significantly better vigour than the untreated coir controls. This suggests that the plant vigour scores were similar across all other treatments.

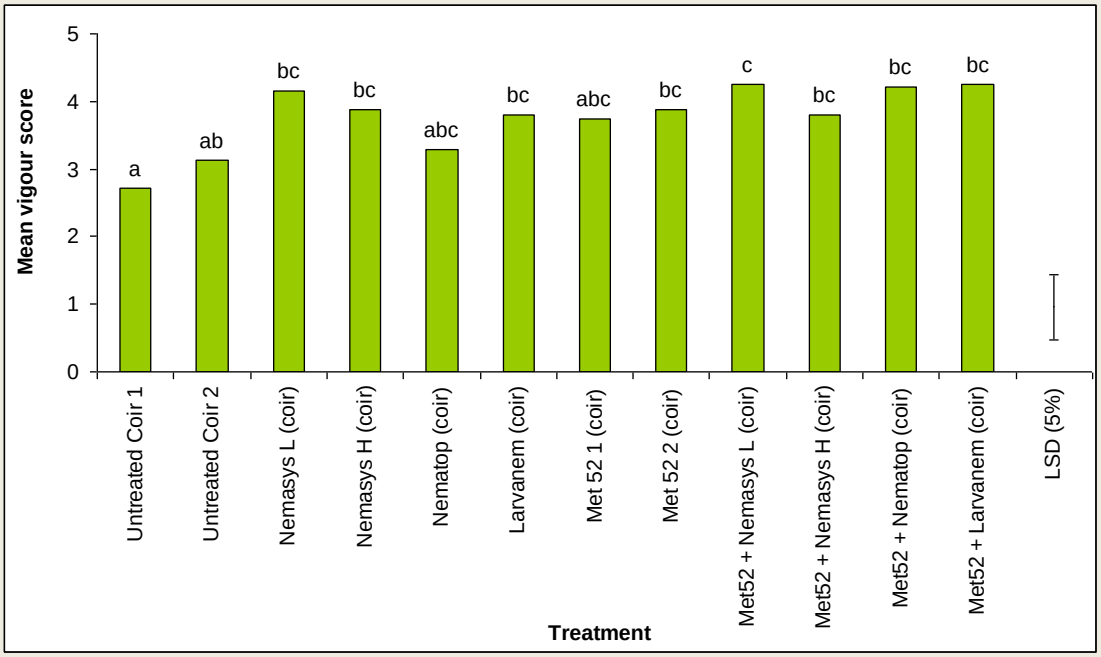


Figure 13 Mean foliage vigour score per plot for coir only treatments (5 very healthy, 1 dead). The least significant difference (LSD) was used to determine any significant differences. Different letters above bars indicate a significant difference.

In the 2012 experiment, no effect on vigour was observed between treatments and untreated controls, indicating that more than a mean of 60 live larvae per grow-bag (equivalent to six larvae per untreated control plant) were required before immediate visible crop damage occurs. It is thought that the damage observed during the 2013 experiment was due to the later planting date meaning the plants were not as well established prior to being infested with vine weevil eggs. Furthermore, Dosatron problems with delivering feed and irrigation early on in the experiment is likely to have also effected establishment. These factors may have made these plants more susceptible to vine weevil feeding damage than those in the 2012 experiment.

Substrate temperatures

The critical period for substrate temperatures for nematode activity was between the date of nematode application (5 September) and the date assessments were done on surviving vine weevil larvae (11-14 November). During this period, temperatures remained within the activity range of Nemasys L (5-30°C). Minimum substrate temperatures were dropping below 14°C (lower limit of Larvanem) prior to the start of the experiment and average temperatures began to drop below 14°C by 9 September (Figure 14 and Table 15). However, substrate temperature did not appear to adversely affect the level of control provided by Larvanem which gave as effective control as Nemasys L. Nematop and Nemasys H are reported to have a lower minimum temperature (>12°C) than Larvanem (14°C), however these two treatments were less effective than Nemasys L.

The critical period for substrate temperatures for Met52 was between vine weevil egg hatching and the date assessments were done on surviving vine weevil larvae (11-14 November). The activity range of Met52 is reported on the technical leaflet to be between 15-30°C. During the experimental period average temperatures remained above 15°C until 8 September. Therefore, following egg infestation on 23 August, the larvae (after taking a few days to hatch) would only be exposed to Met52 for up to two weeks before temperatures fell and the activity of Met52 was reduced. Furthermore, minimum temperatures were already falling below 15°C at the beginning of the experiment on 22

June meaning the quality of newly formed spores may have been reduced (see Met52 product leaflet). Lower than optimum temperatures could explain the performance of Met52 in the experiment. As previously discussed, Dosatron problems may also have affected the performance of Met52 in the peat substrate as it was drier than the coir substrate.

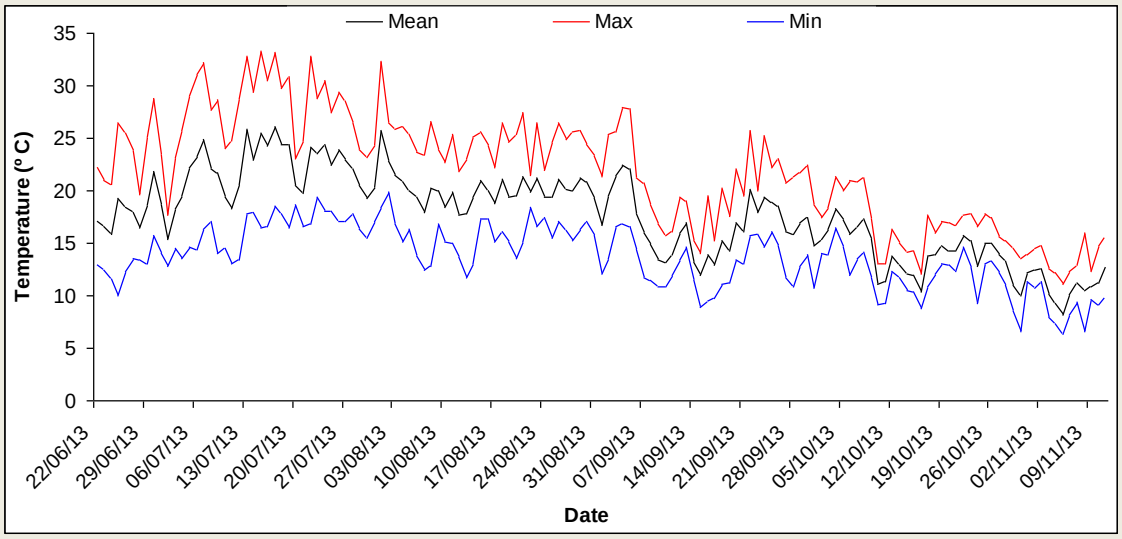


Figure 14 Mean average, maximum and minimum substrate temperatures recorded by four data loggers at root level throughout the experimental period.

Table 15 The optimum temperature range for the nematode products used as per the manufacturer's instructions supplied with the products.

Product	Effective temperature range (°C)
Nemasys L	5-30
Larvanem	14-33
Nematop	>12
Nemasys H	12-30
Met52	15-25

Substrate mealworm test

All the samples taken from the Met52-treated grow-bags had almost 100% of the mealworms infected by *M. anisopliae* after seven days (Table 14). However, of the 28 untreated grow-bags which should not have contained Met52, seven of the grow-bags contained a few infected mealworms after seven days. By day 11, samples from a further five untreated grow-bags contained low numbers of infected mealworms. This result indicated that the 'untreated' grow-bags contained a low level of Met52, however this did not seem to affect the experimental results. It is thought that mealworms are more susceptible to infection by Met52 than vine weevil larvae.

Table 16 Results from the substrate mealworm test to determine the presence or absence of Met52 in each grow-bag used in the experiment

Treatment	Infection after 7 days	Infection after 11 days	Treatment	Infection after 7 days	Infection after 11 days
Met52 + Larvanem (coir)	Yes		Nemasys H (coir)	No	
Met52 + Nematop (coir)	Yes		Untreated (coir)	No	Yes
Met52 + Nemasys H (coir)	Yes		Met52 (Peat)	Yes	
Nemasys H (coir)	No	Yes	Nemasys L (coir)	No	
Untreated (coir)	No		Larvanem (coir)	Yes	
Met52 (coir)	Yes		Met52 (coir)	Yes	
Met52 (peat)	Yes		Met52 + Larvanem (coir)	Yes	
Nemasys L (coir)	No	Yes	Nematop (coir)	No	
Untreated (coir)	No		Met52 (coir)	Yes	
Met52 (coir)	Yes		Untreated (coir)	No	
Met52 + Nemasys L (coir)	Yes		Met52 + Nemasys L (coir)	Yes	
Nematop (coir)	No		Met52 + Nemasys H (coir)	Yes	
Untreated (peat)	No		Untreated (peat)	No	
Larvanem (coir)	Yes		Met52+ Nematop (coir)	Yes	
Untreated (peat)	Yes		Met52 + Nemasys L (coir)	Yes	
Met52 + Nemasys L (coir)	Yes		Met52 + Nemasys H (coir)	Yes	
Nematop (coir)	Yes		Met52 (peat)	Yes	
Met52 + Nematop (coir)	Yes		Nemasys H (coir)	Yes	
Met52 + Larvanem (coir)	Yes		Met52 + Larvanem (coir)	Yes	
Met52 (peat)	Yes		Met52 + Nematop (coir)	Yes	
Met52 +Nemasys H (coir)	Yes		Met52 (coir)	Yes	
Nemasys H (coir)	No		Met52 (coir)	Yes	
Met52 (coir)	Yes		Nemasys L (coir)	No	
Nemasys L (coir)	No		Nematop (coir)	No	Yes
Untreated (coir)	Yes		Larvanem (coir)	No	Yes
Untreated (coir)	No		Untreated (peat)	Yes	
Larvanem (coir)	No		Untreated (coir)	No	
Met52 (coir)	Yes		Untreated (coir)	No	

Counts of nematodes

Active counts of the nematodes within each product were estimated (Table 15). All packs of nematodes claimed to contain 50 million active juveniles per pack. Nemasys L contained the most nematodes per pack, followed by Nemasys H, with both above the claimed 50 million content, while Nematop and Larvanem contained slightly less. Means calculated were based on 6x 1 ml sub-samples.

An analysis of variance based on the six sub-samples showed that Nemasys L and Nemasys H had similar numbers of nematodes per ml of water but had significantly more than Larvanem and Nematop. Larvanem and Nematop had similar numbers of nematodes per ml of water. Nemasys L and Nemasys H numbers were adjusted to standardise numbers of nematodes delivered per plant to 250 per ml (see methods). Despite having slightly lower numbers of nematodes per ml than Nemasys L (Table 15), Larvanem was not significantly different compared with Nemasys L in reducing the number of vine weevil larvae.

Table 17 Mean nematodes counts per 1ml sub-sample for each product. The least significant difference (LSD) was used to determine any significant differences. Different letters next to the mean indicate a significant difference.

Products	Mean numbers per 1ml sub-sample	Estimated numbers per pack
Nemasys L	304.8 a	60,960,000
Nemasys H	286.3 a	57,260,000
Larvanem	235.7 b	47,140,000
Nematop	234 b	46,800,000

Aphid hyperparasitoids on hardy nursery stock

Mummified aphids were collected from a range of hardy nursery stock crops including: Coronilla, Pittosporum, Coprosmam, Fuchsia, Photinia, Solanum, Phormium, Hebe, Euphorbia, Euryops, Cistus, Akebia, Sallya, Cosmos, Escallonia and Lavandula.

On 23 May the parasitised aphids were almost all *Macrosiphum euphorbiae* except for *Myzus ornatus* on Coronilla and a *Myzus* sp. on Photinia (Table 18). Estimated parasitism in the crops ranged between 0-25% with an average of 5.5%. Sixty one mummies were collected. The aphids were mainly parasitised by *Aphidius* sp. (36 mummies) followed by *Praon volucre* (12 mummies), *Ephedrus cerasicola* (five mummies) and *Aphelinus abdominalis* (four mummies). Four mummies were too damaged to be identified.

Only *Aphidius ervi* and *Aphidius matricariae* were observed to emerge from *Aphidius* mummies. *Aphidius ervi* was the most common of the two species. No parasitoids emerged from 11% of the mummies collected. Hyperparasitism ranged between 0-44% and three species were present (*Alloxysta brevis*, *Asaphes* sp. and *Dendrocerus* sp.). The most hyperparasitism occurred on *Solanum* sp.

Table 18 Numbers of parasitised aphids and percentage hyperparasitised collected from a range of HNS crops sampled on 23 May 2013.

Plant	Estimated % parasitism in the crop	No. mummified aphids collected	Aphid species (identified on the crop and as mummies)	Primary parasitoid	Hyperparasitoid	%parasitoid emergence	% hyper parasitism
Sollya	None	0	None	-	-	0	0
Coprosma 'Pacific night'	<5	7	*M. euphorbiae*	*A. ervi* and *Aphidius* sp.		71	40
Pittosporum	0	0	*M. euphorbiae*			0	0
Coronilla	5-10	12	*M. ornatus* and *M. euphorbiae*	*A. ervi*, *Aphidius* sp., *A. abdominalis* and *P. volucre*	*Asaphes* sp.	100	17
Pittosporum	0	0	*M. euphorbiae*	-	-	0	0
Coronilla	25	7	*M. ornatus*	*Aphidius* sp., *A. matricariae*, *A. ervi* and *A. adominalis*		57	0
Fuchsia	0	0	*M. euphorbiae*	-	-	0	0
Photinia	<5	11	*M. euphorbiae*, *Myzus sp.*	*A. ervi*, *A. matricariae*, *A. abdominalis* and *E. cerasicola.*	*A. brevis*	100	27
Phormium	10	14	*M. euphorbiae*	*Aphidius*, *A. matricariae P. volucre* and *E. cerasicola.*	*Dendrocerus* sp. and *A. brevis*	93	15
Solanum	5	10	*M. euphorbiae*	*A. ervi* and *Aphidius* sp.	*Asaphes* sp. and *Dendrocerus* sp.	90	44
Photinia	0	0	*M. euphorbiae*	-	-	0	0

On 16 July the parasitised aphids were mainly *M. euphorbiae* but *M. ornatus, Aulacorthum circumflexum*, *Aphis* sp. and *Aphis gossypii* were also present (Table 19). Estimated parasitism in the crops ranged between 1-25% with an average of 11.6%. One hundred and forty seven mummies were collected. The aphids were mainly parasitised by *Aphidius* sp. (82 mummies) followed by *P. volucre* (60 mummies), *A. abdominalis* (three mummies) and *E. cerasicola* (one mummy). One mummy was too damaged to be identified.

Only *A. ervi* and *A. colemani* were observed to emerge from *Aphidius* mummies. *Aphidius ervi* was the most common of the two species. No parasitoids emerged from 16% of the mummies collected. Hyperparasitism ranged between 0-90% and three species were present (*Alloxysta brevis*, *Asaphes* sp. and *Dendrocerus* sp.). The most hyperparasitism occurred on Cosmos Chocamocha.

Table 19 Numbers of parasitised aphids and percentage hyperparasitised collected from a range of HNS crops sampled on 16 July 2013.

Plant	Estimated % parasitism in the crop	No. mummified aphids collected	Aphid species	Primary parasitoid	Hyperparasitoid	%parasitoid emergence	% hyper parasitism
Cistus Maculata	<1	3	*M. euphorbiae*	*A. ervi* and *A. abdominalis*		100	33
Hebes mergret	5	10	*M. euphorbiae, M. ornatus* and *A. circumflexum*	*Aphidius* sp. and *P. volucre*		80	75
Escallonia Red dream	15	22	*M. euphorbiae*	*A. ervi, Aphidius* sp., *A. abdominalis* and, *P. volucre*	*Dendrocerus* sp.	82	58
Cistus 'Maculatus'	<1	2	*M. euphorbiae*	*A. ervi*		100	0
Euryops pectinatus	20	19	*M. euphorbiae*	*A. ervi, Aphidius* sp. and *P. volucre*	*Dendrocerus* sp.	84	25
Solanum laxum 'album'	10	22	*M. euphorbiae*	*A. ervi, Aphidius* sp. *A. abdominalis,* and *P. volucre*	*Asaphes* sp., *A. brevis, Dendrocerus* sp.	86	63
Akebia Quanta	25	22	*M. euphorbiae*	*Aphidius* sp. and *P. volucre*	*Asaphes* sp.	100	68
Sallya heterophyll	<1	1	*M. euphorbiae* and *A. gossypii*	*A. ervi*		100	0
Hebe Margret	20	12	*M. ornatus* and *Aphis* sp.	*A. colemani* and *Aphidius* sp.	*Dendrocerus* sp.	92	64
Cosmos chocamocha	25	16	*M. euphorbiae*	*A. ervi, Aphidius* sp. and *P. volucre.*	*Asaphes* sp., *Dendrocerus* sp.	63	90
Euphorbia amygdaloides 'Purpurea'	15	17	*M. euphorbiae*	*P. volucre*	*Dendrocerus* sp., *Asaphes* sp.	71	75
Photinia	<1	0	*M. euphorbiae*	-	-	-	-

On 13 August the parasitised aphids were mainly *M. euphorbiae* but *M. ornatus, Myzus persicae, Aphis gossypii* and *Aphis* sp. were also present (Table 20). Estimated parasitism in the crops ranged between 0-100% with an average of 68.8%. One hundred and thirty two mummies were collected. The aphids were mainly parasitised by *Aphidius* sp. (69 mummies) followed by *P. volucre* (59 mummies) and *A. abdominalis* (four mummies). The majority of the parasitoids had already emerged from the mummies prior to collection so the most numerous *Aphidius* sp. could not be determined. No parasitoids emerged from 31% of the mummies collected. Hyperparasitism ranged between 13-95% and three species were present (*A. brevis*, *Asaphes* sp. and *Dendrocerus* sp.). The most hyperparasitism occurred on Cistus x purpureus.

Table 20 Numbers of parasitised aphids and percentage hyperparasitised collected from a range of HNS crops sampled on 13 August 2013.

Plant	Estimated % parasitism in the crop	No. mummified aphids collected	Aphid species (identified on crop and as mummies)	Primary parasitoid	Hyperparasitoid	%parasitoid emergence	% hyper parasitism
Akebia Quinata	85	7	*M. euphorbiae*	*Aphidius* sp. and *P. volucre*		86	33
Corinilla and Solanum laxum 'album'	100	33	*M. euphorbiae* and *M. ornatus*	*A. colemani, Aphidius* sp. and *P. volucre*	*Asaphes* sp.	58	37
Cistus x purpureus	100	10	*M. euphorbiae*	*Aphidius* sp.	*Dendrocerus* sp.	40	75
Euryops	80	24	*M. euphorbiae, M. ornatus* and *Aphis* sp.	*Aphidius* sp. and *P. volucre*	*Asaphes* sp.	63	13
Euryops	Collected from a bag of cuttings	7	*Aphis* sp. and *M. euphorbiae*	*Aphidius* sp. and *P. volucre*	*Asaphes* sp.	86	17
Iris	15	22	*M. euphorbiae* and *M. persicae*	*Aphidius* sp., *A. abdominalis* and *P. volucre*	*A. brevis* and *Asaphes* sp.	86	58
Cistus x purpureus	90	26	*M. euphorbiae*	*Aphidius* sp. and *P. volucre*	*Asaphes* sp. and *Dendrocerus* sp.	73	95
Coronilla glauca citrina	80	3	*M. euphorbiae* and *A. gossypii*	*P. volucre*		100	33
Cosmos chocamocha	0	0	None	-	-	-	-

The data confirms that hyperparasitism was again widespread in a range of HNS crops during 2013. There was no indication that the threat of hyperparasitism had decreased between 2012 and 2013. During 2012, hyperparasitism on 13 August 2013 was higher (between 13 and 95%) compared with 1 August 2012 (between 0 and 70%) which was likely due to the prolonged July heat wave.

Biological control of aphids on lettuce

First objective

In the first field which was monitored *M. euphorbiae* was the most common aphid species. Sampling began on 4 June and aphid numbers peaked at 23.8 per plant on 2 July (Figure 21). On the next sampling date on 10 July, aphid numbers had reduced to 5.5 per plant. On 10 July when aphid numbers had decreased, the number of mummies per plant was 0.1 (equivalent to one mummy every 10 plants) and the number of syrphid (hoverfly) larvae was 0.7 per plant (equivalent to one syrphid larvae every 1.4 plants). In Field 1 the release of *A. colemani* could not be confirmed as the main contributing factor to the decline in aphid numbers as the main aphid species present was *M. euphorbiae* which is not readily parasitised by *A. colemani.* Control of *M. euphorbiae* would have been more effective using *A. ervi.* It is likely that the syrphid larvae were the main contributors to the decline in aphid numbers. This has been observed in other trials where syrphid larvae composed more than

85% or more of the predators in organic lettuce and were thought to be primarily responsible for reducing the number of aphids (Smith et al., 2008).

A rapid decline in aphid populations, as observed in this experiment, also occurred during the 2012 monitoring trial (Bennison, 2013). The timing of the crashes in both years coincided with the aphid 'mid-summer crash; which commonly occurs during July and results in aphid numbers remaining low for up to eight weeks (Karley *et al*., 2004).

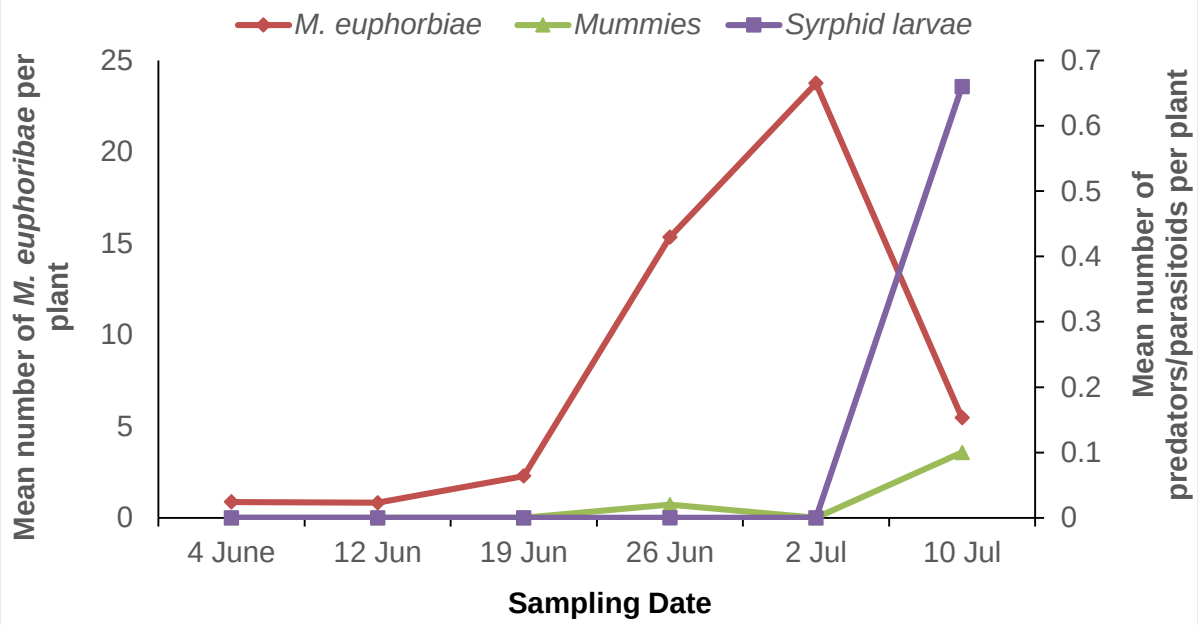


Figure 21 Mean number of *M. euphorbiae*, mummies and syrphid larvae per plant recorded in Field 1 on each sampling date.

In the second field which was monitored, *M. euphorbiae* was again the most common aphid species but *M. persicae* was also observed in low numbers. Sampling began on 19 June and *M. euphorbiae* numbers peaked at 16.4 per plant on 17 July (Figure 22). Unfortunately this was the last sampling date before the field was harvested and it is unknown whether aphid's numbers would have decreased. On the17 July the numbers of mummies also peaked at 1.23 per plant. No predators were observed. As the number of aphids did not decline during the monitoring period the control provided by the release of *A. colemani* could not be determined.

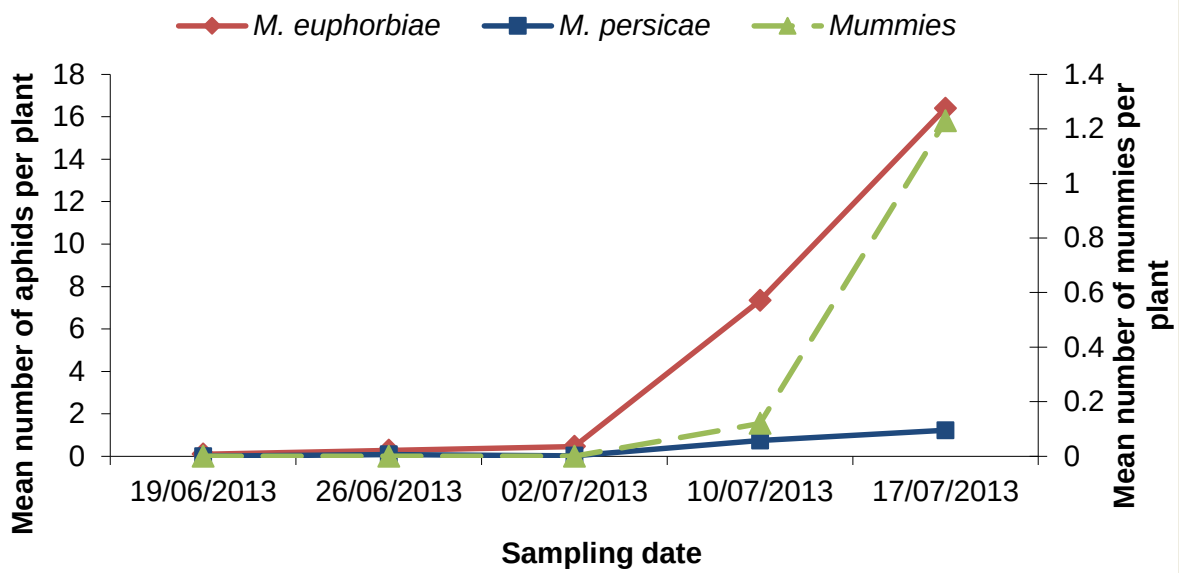


Figure 22 Mean number of *M. euphorbiae*, *M. persicae* and mummies per plant recorded in Taylor's house ground on each sampling date.

From both fields a total of 37 mummies were collected. Parasitoids were identified when they emerged from the mummies. Where a parasitoid had already emerged from a mummy the aphid species was used to determine the parasitoid species e.g. when a *Macrosiphum euphorbiae* mummy was found which had been parasitised by an *Aphidius* spp. it was assumed that this was *A. ervi* as it has a very high efficacy against this bigger aphid species. *Aphidius colemani* is not recommended for the control of *M. euphorbiae* and is regarded not to parasitise (or give low percentage parasitism) of *M. euphorbiae*. Viridaxis report observing parasitism under laboratory and semi field conditions. *Aphidius colemani* is generally more effective against smaller aphids such as *M. persicae*. Some *Aphidius* mummies could not be identified to species as the mummy was too damaged to identify the aphid species and the parasitoid never emerged.

Of the mummies collected 67.6% were *A. ervi* and only 5.4% were identified as *A. colemani* which was the parasitoid being released (Figure 23). The high number of *A. ervi* was due to the aphids mainly being *M. euphorbiae*. This suggested that a lot of natural parasitism was occurring in the field. Other studies on lettuce crops have also identified a range of braconid wasp species parasitising aphids where no parasitoids had been released (Nebreda *et al*., 2005).

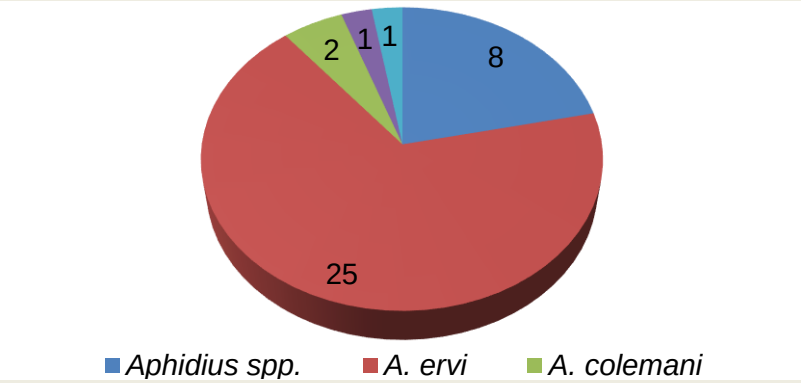


Figure 23 Proportion of the mummies collected which had been parasitised by *Aphidius* spp, *A. ervi, A. colemani, A. matricariae* and *P. volucre.*

Second objective

The number of aphids (including *M. persicae, M. euphorbiae, Myzus ascalonicus, Aphis spp and Aulacorthum solani*) in areas where parasitoids were released (RAs) and where they were not released (NRAs) followed a similar trend (Figure 24). Aphid numbers per plant peaked in week two with 4.6 and 3.4 per plant in RAs and NRAs respectively. Following this date the number of aphids decreased to 0.7 per plant in both RAs and NRAs and remained low for the rest of the experiment. In both the RAs and NRAs the main aphid species recorded during the first four weeks was *M. persicae* and in weeks five and six it was *M. euphorbiae*.

The number of mummies in the RAs varied between 0.05 and 0.18 per plant (equivalent to 1 every 20 plants and 1 every 5.6 plants respectively). In the NRAs the number of mummies gradually increased at each sampling week until week six where they reached 0.18 per plant (Figure 25). This suggests that over time parasitoids released in the RAs gradually moved into the NRAs or natural parasitoids moved into the NRA. In the first two weeks of the experiment the lower numbers of mummies is likely to be due to the time required for a parasitised aphid to develop into a mummy. Each week more mummies developed and by week three, the number of aphids was observed to decrease (Figure 24 and 25).

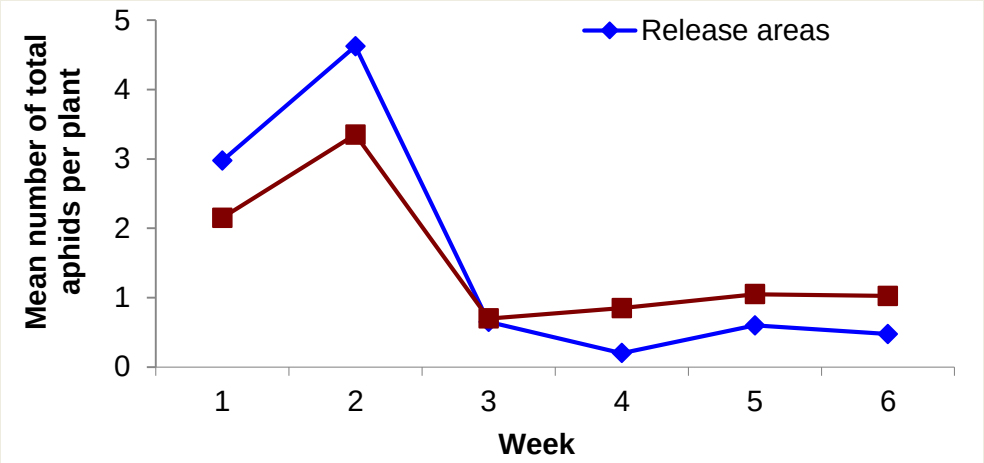


Figure 24 Mean number of total aphids per plant in parasitoid release and non-release areas in the dispersal experiment

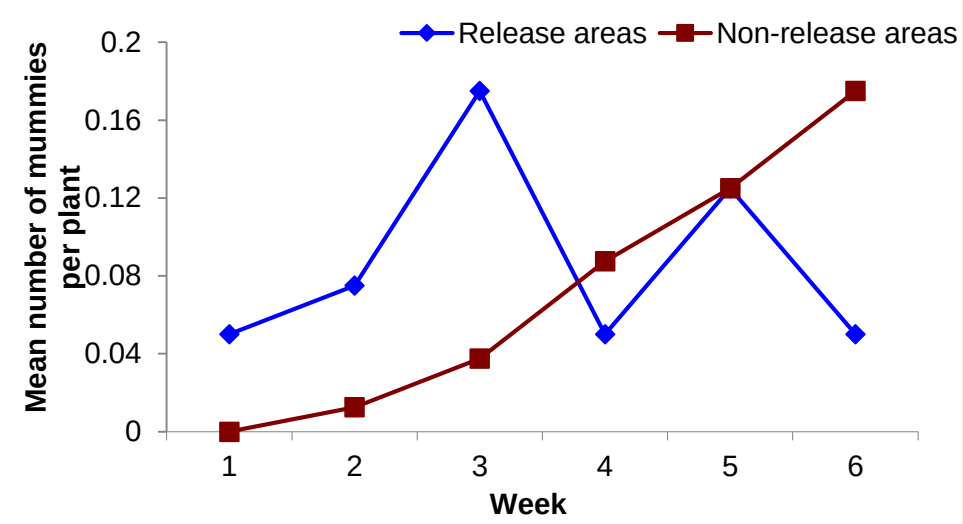
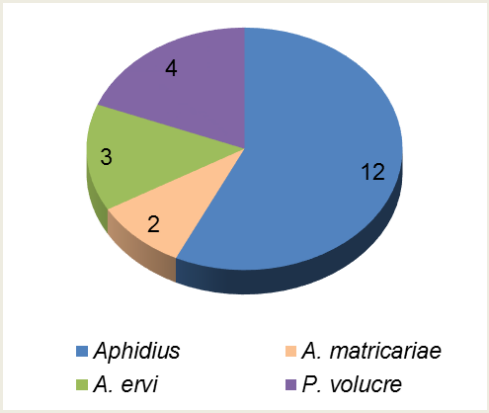


Figure 25 Mean number of mummies per plant in parasitoid release and non-release areas in the dispersal experiment

A total of 21 and 35 mummies were collected from the RAs and NRAs respectively (Figure 26). The majority of mummies were unidentified *Aphidius* spp. This was because either the parasitoid had already emerged when the mummy was collected or no parasitoid emerged following collection (mummies were kept and monitored for emergence for two months). Of the mummies collected, 43% in the RAs and 40% in the NRAs were definitely parasitised by naturally occurring parasitoids, i.e., by parasitoids positivley identified as other than *A. colemani*, which was released by the grower. *A. colemani* was positively identified as the parasitising species in only two cases. The two *A. colemani* mummies were found 35 m into the non-release areas three and five weeks into the experiment respectively and, as

mummies can take two weeks to form after oviposition by the adult parasitoid, this suggests that the parasitoids are capable of quickly dispersing from designated release points (assuming these are not naturally occuring *A. colemani*). Hyperparasitism was observed in 12.5% of the mummies collected (7 out of 63, Figure 27).

a)



b)

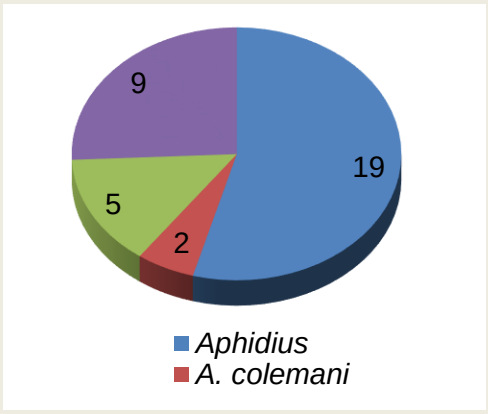


Figure 26 The number and species of mummies in release (a) and non-release (b) areas in the dispersal trial

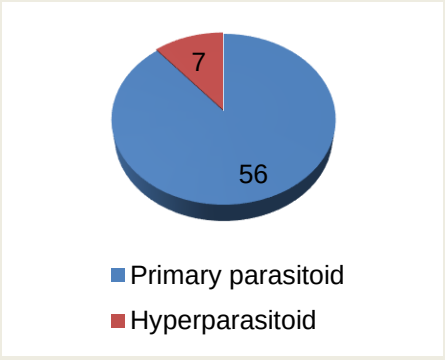


Figure 27 12.5 % of mummies which were parasitised by primary parasitoids or hyperparasitoids

When looking at the number of aphids infected by an entomopathogenic fungus, numbers increased prior to the decline of the aphids in week three (Figure 28) which would have contributed to the decline in aphid numbers. In the NRAs the numbers of aphids infected peaked at 0.2 per plant (equivalent to one per 5 plants) in week two and in the RAs they peaked at 0.12 per plant in week three (one every 8.3 plants). Numbers decreased after this date. Aphid population crashes have been observed in other studies in response to a fungal epizootic (Nielsen and Hajek, 2005).

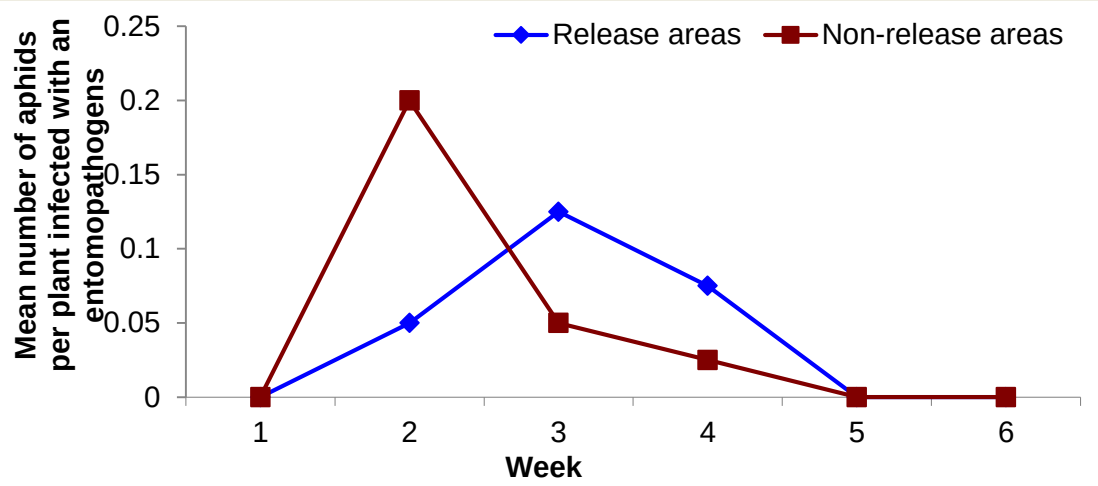


Figure 28 Mean number of aphids infected with entomopathogenic fungus per plant in parasitoid release and non-release areas in the dispersal experiment

Of the predators recorded spiders were the most common and present throughout the dispersal experiment (Figure 29). Spider numbers peaked in week five with a mean number of 0.8 spiders per plant (one spider per 1.25 plants). When aphid numbers declined in week three there was 0.3 spiders per plant in both RAs and NRAs (one spider per 3.3 plants). Various studies have indicated that spiders are important predators of aphids in a range of crops and should be considered in conservation biological control (e.g. Kuusk et al., 2008, Gontijo, et al., 2013).

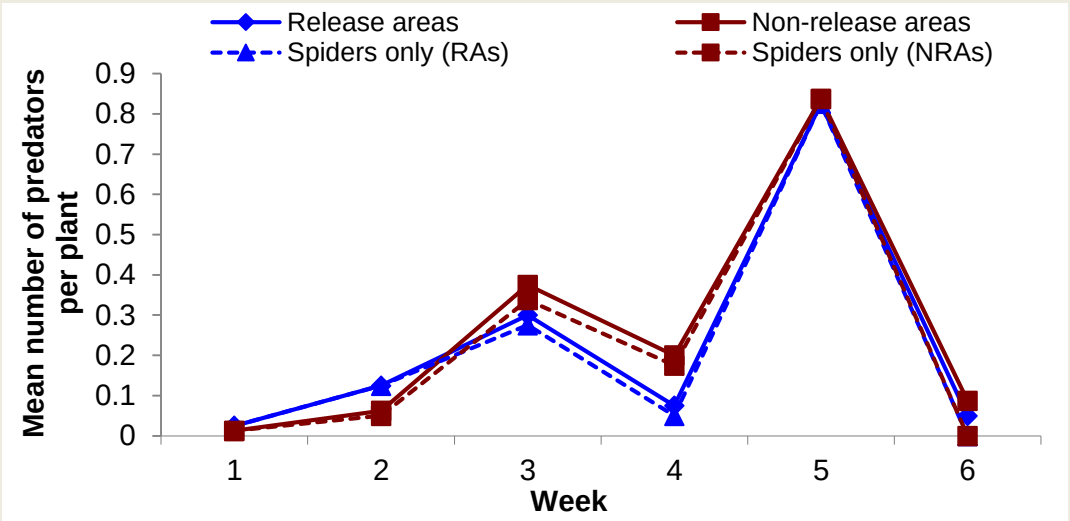


Figure 29 Mean number of predators per plant in parasitoid release and non-release areas in the dispersal experiment

Conclusions

Efficacy of entomopathogenic nematodes against vine weevil

● All the nematode products and Met52 in a coir substrate significantly reduced the numbers of live vine weevil larvae in substrate-grown strawberry when compared with untreated controls.

● Met52 in coir was as effective as Larvanem, Nematop and Nemasys H but less effective than Nemasys L.

● Met52 in a peat substrate was ineffective.

● Nemasys L (*Steinernema kraussei)* and Larvanem (*Heterorhabditis bacteriophora)* were the best performing products and were not significantly different in their reduction of mean numbers of live vine weevil larvae. Nematop and Nemasys H (both *Heterorhabditis bacteriophora)* were not significantly different than Larvanem but did not reduce the mean number of vine weevil larvae as well as Nemasys L.

●Combining nematodes with Met52 did not significantly improve the control of vine weevil larvae compared to when using nematodes alone.

● Vine weevil larvae feeding damage and plant vigour was similar across all treatments when analysing peat and coir substrates together. When analysing coir treatments alone the combined Met52 and Nemasys L treatment had significantly better vigour than the untreated coir controls.

Aphid hyperparasitoids on hardy nursery stock

● Percentage aphid hyperparasitism was between 0 and 95% on a HNS nursery during 2013.

● In August hyperparasitism was higher than in 2013 following a warm summer.

● The hyperparasitoid species *Dendrocerus* sp. *Asaphes* sp. and *Alloystra brevis* were identified.

Biological control of aphids on lettuce

● Natural parasitism by species which had not been released by the grower was observed in all of the trials. *A. colemani* was identified from mummies in very few cases.

● The release of *A. colemani* into the field may have contributed to the control of the aphid populations but it could not be confirmed as the main contrib uting factor, particularly in Objective 1 where *Macrosiphum euphorbiae* was the most common aphid which is not well parasitised by *A. colemani.*

● It is likely that the control of aphids was due to the natural enemy community rather than one individual species.

● Predators observed in high numbers included syrphid (hoverfly) larvae (Objective 1), spiders (Objective 2) and entomopathogenic fungi (Objective 2). These are likely to have made a significant contribution to aphid control.

● In Objective 2, the confirmation of *A. colemani* mummies 35m into the non-release areas as early as three weeks into the experiment suggests that the parasitoids dispersed out from the release areas fairly quickly (assuming they were not naturally-occurring *A. colemani*). Further work is needed to determine whether releasing parasitoids from fewer locations would provide similar levels of control compared to releasing them throughout the field.

● In conclusion, the contribution of the released parasitoids to aphid control is likely to have been relatively minor in comparison to naturally-occuring parasitoids and predators. However, it is difficult to quantify their contribution without a control field in which no parasitoids were released.

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):

● HDC News articles on the entomology fellowship (CP 89)

Presentations:

● Maintaining the Expertise for Developing & Communicating Practical IPM Solutions for Horticulture (2011-2016) – HDC Studentship Conference (Gemma Hough).

Scientific conferences:

● AAB Pushing Back the Frontier - Biological control of vine weevil larvae on protected strawberry (Gemma Hough).

Glossary

Hyperparasitism – when a primary parasitoid developing within its host is attacked by a secondary parasitoid. Here, this refers to naturally-occurring secondary parasitoids which attack the aphid parasitoids being used as biological control agents to control aphid pests.

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