Project title:	Combining biopesticides and other treatments to increase pest control
Project number:	FV 389
Project leader:	Rosemary Collier, University of Warwick
Report:	Annual, April 2012
Previous report:	First year of project
Key staff:	Andrew Jukes Marian Elliott
Location of project:	Warwick Crop Centre, Wellesbourne
Industry Representative:	
Date project commenced:	1 April 2011
Date project completed (or expected completion date):	31 March 2013

DISCLAIMER:

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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.

[Name]
[Position]
[Organisation]

Signature .... Date ....

[Name]
[Position]
[Organisation]

Signature .... Date ....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature .... Date ....

[Name]
[Position]
[Organisation]

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headline

Biopesticides, pesticides and behaviour modifiers have been identified that can be tested in combination to control important vegetable pests. Detailed laboratory tests are underway.

Background

There is much interest in identifying effective treatments for pests whilst reducing reliance on synthetic pesticides. One way to achieve this may be by 'combining' treatments to improve efficacy. Whilst this is done routinely with pesticide mixtures (e.g. Dovetail) and with pesticide/adjuvant combinations, there may be other improvements that could be achieved through, for example, combining insecticides or biopesticides with a treatment that modifies pest activity (and thereby pesticide uptake) or pest susceptibility. Such treatments could be applied at the same time or sequentially.

The aim of this project is to undertake a series of small-scale laboratory tests with pest insects that can be obtained easily from cultures to evaluate the potential of a range of treatments by comparing their activity separately and in combination. The term 'biopesticide' is used in the broadest sense, so could include biocontrol agents, botanicals or semio-chemicals. The results of this project will indicate which combinations of treatments may be worth exploring in more detail in future in trials on specific crop/pest combinations.

Summary

A literature review was undertaken to summarise the combinations of biopesticides, conventional pesticides and 'potentiators' that have been evaluated in previous studies and to understand the mechanisms involved in achieving improvements in pest insect control. A potentiator is a compound that is not pesticidal but which causes an increase in pest mortality when used with a pesticidal agent. Improvements in control as a result of treatment combinations can occur for a number of reasons associated with changes in the susceptibility or behaviour of the target insects.

The approaches to combining treatments vary considerably and may, for example, involve combining two microbial biopesticides (e.g. a fungal pathogen with *Bt*), a microbial biopesticide with a reduced dose of a chemical insecticide or a biopesticide based on a plant extract with a microbial biopesticide. The two main 'mechanisms' by which control is improved are, simplistically, where application of one treatment increases an insect's susceptibility to another, or where the application of one treatment increases the uptake of the second treatment and therefore the effective dose.

Biopesticides, pesticides and potentiators have been identified that can be tested in combination against a number of important pest insects of vegetables that are maintained in culture at Warwick Crop Centre, Wellesbourne. The test species and types of potential control agent/potentiator are shown in Table 1. Because many of these materials are being used in the SCEPTRE project a number of the individual materials must also be coded in this project.

Table 1. Potential control agents and potentiators for pest insects in current project.

	Insect cultures	**Potential control agents/potentiators**
Flies	*Cabbage root fly (Delia radicum), onion fly (Delia antiqua)*	Insecticide e.g. spinosad (drench or foliar application) Plant extract (drench, phytodrip or foliar application) Microbial Sugar/protein bait
Aphids	Cabbage aphid (*Brevicoryne brassicae*), peach-potato aphid (*Myzus persicae*), currant-lettuce aphid (*Nasonovia ribisnigri*)	Insecticide e.g. imidacloprid (phytodrip or drench application) Plant extract (drench, phytodrip or foliar application) Microbial
Caterpillars	Diamond-back moth (*Plutella xylostella*), large white butterfly (*Pieris brassicae*)	Insecticide e.g. spinosad (drench or foliar application) Sugar Plant extract (drench, phytodrip or foliar application) Microbial
Thrips	*Thrips nigripilosus, possibly* onion thrips (*Thrips tabaci*)	Insecticide (foliar application) Sugar Plant extract (foliar application) Microbial

The data obtained from preliminary tests on cabbage root fly, cabbage aphid, peach-potato aphid and diamond-back moth indicated considerable variability in the performance of biopesticide treatments between replicates. One of the biopesticides tested alone appeared to reduce egg-laying by female cabbage root flies (but did not increase mortality) and the same treatment also reduced aphid numbers. None of the biopesticides tested alone increased mortality of adult diamond-back moths compared with the untreated control.

Further tests are underway. Because of variability between replicates, it will be necessary to replicate tests in both space and time. It will also be important to make observations on insect behaviour during certain tests to determine the effects of different individual treatments and treatment combinations.

Financial Benefits

The results of this project will be relevant to the pests that infest a wide range of field vegetable crops (and protected and ornamental crops) and will indicate which combinations of treatments may be worth exploring in more detail in trials on specific crop/pest combinations in future. The proposed project is complementary to the LINK project submitted by a consortium led by HDC: Sustainable Crop and Environment Protection - Targeted Research for Edibles (SCEPTRE).

Action Points

- The results of this project are preliminary at present and there are no action points for growers.

SCIENCE SECTION

Introduction

There is much interest in identifying effective treatments for pests whilst reducing reliance on synthetic pesticides. One way to achieve this may be by 'combining' treatments to improve efficacy. Whilst this is done routinely with pesticide mixtures (e.g. Dovetail) and with pesticide/adjuvant combinations, there may be other improvements that could be achieved through, for example, combining insecticides or biopesticides with a treatment that modifies pest activity (and thereby pesticide uptake) or pest susceptibility. Such treatments could be applied at the same time or sequentially.

The aim of this project is to undertake a series of small-scale laboratory tests with pest insects that can be obtained easily from cultures to evaluate the potential of a range of treatments by comparing their activity separately and in combination. The term 'biopesticide' is used in the broadest sense, so could include biocontrol agents, botanicals or semio-chemicals. The results of this project will indicate which combinations of treatments may be worth exploring in more detail in future in trials on specific crop/pest combinations.

Materials and methods

Identify combinations of biopesticides/pesticides/behaviour modifiers that can be tested in combination.

Biopesticides and other materials available for pest control

The materials available for pest control fall into several categories and these are summarised below. Some materials may also act as 'potentiators'. A potentiator is a compound that is not pesticidal but which causes an increase in pest mortality when used with a pesticidal agent. Addition of sugar to an insecticidal treatment is an example of the use of a potentiator, in that it can increase probing by insects on leaf surfaces and thereby increase uptake of the insecticide.

Microbial pesticides

The main groups of entomopathogenic microbes that are formulated for pest control are the bacterium *Bacillus thuringiensis* (Bt), fungi and baculoviruses. Nematodes are also included in this category. The target pest groups, the species of entomopathogen and some examples of their horticultural targets are shown in Table 1. Further details about some of the products available are given in Appendix 1.

Table 1. Main groups of entomopathogenic organisms and horticultural targets.

Bacteria		
Flies	*Bacillus thuringiensis*	Fungus gnats (e.g. Gnatrol http://www.entomology.umn.edu/cues/mnla/gnatrol.pdf)
Caterpillars	*Bacillus thuringiensis*	Range of pests e.g. diamond-back moth, small white butterfly (UK – Dipel)
Beetles	*Bacillus thuringiensis*	Colorado potato beetle (e.g. Novodor http://www.valentbiosciences.com/agricultural_products/agricultural_products_8.asp)
Viruses		
Caterpillars	Granulosis virus (codling moth)	Codling moth (UK – CyD-XTM granulosis virus http://www.certiseurope.co.uk/fileadmin/downloads_uk/products/insecticides/Cydx_granulovirus_for_codling_moth_in_apples_and_pears.pdf)
Fungi		
Flies	*Metarhizium anisopliae, Beauveria bassiana*	Cabbage root fly (Bruck *et al*., 2005; Meadow *et al*., 2000)
Aphids	*Beauveria bassiana*	*Myzus persicae, Brevicoryne brassicae, Nasonovia ribisnigri* in Defra project HH3117TFV (Defra, 2006) with Botanigard (not available in UK http://www.bioworksinc.com/products/botanigard-22wp.php
Caterpillars	*Beauveria bassiana* and others	Diamond-back moth (Ali *et al*, 2010; Vickers *et al*., 2004; Wraight *et al*., 2010)
Thrips	*Beauveria bassiana, Lecanicillium muscarium*	Naturalis- l (available in UK) Mycotal (available in UK)
Whitefly	*Beauveria bassiana, Lecanicillium muscarium*	Naturalis- l (available in UK) Mycotal (available in UK) *Bemisia tabaci* (Islam *et al*., 2010; 2011)
Beetles	*Metarhizium anisopliae*	Vine weevil (UK – Met 52) Pollen beetle (Butt *et al*., 1998)
Nematodes		
Flies	*Steinernema feltiae*	Sciarid fly larvae, leatherjackets (UK - Nemasys Leatherjacket Killer)
Caterpillars	*Steinernema carpocapsae*	UK - Nemasys Caterpillar and Codling Moth Killer
Thrips	*Steinernema feltiae*	Western flower thrips (Nemasys)
Beetles	*Steinernema kraussei*	Vine weevil (UK - Nemasys Vine Weevil Killer**)**

Plant extracts

There are a number of pesticides which are derived from plants (also included in the term biopesticide). These include garlic, chilli extract, pyrethrum, neem, limonene and relatively new products such as BugOil and Requiem (not available in the UK). Table 1.2 summarises the targets for some of these substances and products.

Table 1.2. Targets for biopesticides based on plant extracts

Garlic extract	
Flies	Cabbage root fly (HDC project FV 242a; http://www.ecospray.com/graph.html)
Aphids	Cabbage aphid (http://www.ecospray.com/graph.html)
Caterpillars	Weak evidence (e.g. Sewak *et al*. 2008)
Thrips	No strong evidence
Beetles	No strong evidence
Chilli extract (capsaicin)	
Various species?	Dayan *et al* (2009); Oparaeke *et al*. (2005) e.g. Hot pepper wax (http://www.hotpepperwax.com/)
Food sources (baits)	
Flies	Addition of sugar/yeast baits increased control of cabbage root fly and large narcissus fly with insecticides (HDC projects FV 242a, BOF 55). DOW product (GF-120® NF NATURALYTE® FRUIT FLY BAIT)
Neem	
Flies	Turnip fly (Meadow *et al*.,) Onion fly (Tanzubil *et al*., 2004) Cabbage root fly – no effect (Pats & Isman, 1998)
Aphids	*Brevicoryne brassicae* (Zaki, 2008) Currant-lettuce aphid (Neemazal)
Caterpillars	Cabbage moth (Meadow *et al*., 2012)
Thrips	*Frankliniella occidentalis* (Thoeming *et al*., 2003) *Thrips tabaci* (Al-mazra'awi *et al*., 2009)
Beetles	Pollen beetle, weevils (Neemazal label)
Whitefly	*Bemisia tabaci* (Islam *et al*., 2010; 2011)
Pyrethrum	
Flies	Blueberry maggot (Barry *et al*., 2005).
Aphids	UK - Pyrethrum 5 EC, Spruzit
Caterpillars	UK - Pyrethrum 5 EC, Spruzit
Thrips	UK - *Thrips tabaci* populations are resistant to synthetic pyrethroids
Beetles	UK - Pyrethrum 5 EC, Spruzit
Other plant extracts	
Aphids	Requiem (AgraQuest, 2011) BugOil (Yang *et al*. 2010) Limonene (Hollingsworth, 2005)
Thrips	Requiem (AgraQuest, 2011)
Whitefly	Requiem (AgraQuest, 2011) BugOil (Yang *et al*. 2010) Limonene (Hollingsworth, 2005)

Pheromones

Pheromones are also classified as biopesticides. Pheromones are compounds that are secreted by animals that influence the behaviour or development of other members of the same species. They may be sex attractants, or cause insects to aggregate (aggregation pheromones) or disperse (e.g. aphid alarm pheromone). They may be used independently to control pest species e.g. confusion technique where large amounts of pheromone are released into the environment (Wu *et al.*, 2012) or as in the approach being researched currently at Rothamsted Research using genetic modification to allow wheat to release aphid alarm pheromone (Rothamsted Research, 2012). They might also be used in combination with other control methods e.g. using attraction to sex pheromone to increase uptake of insecticide (Mitchell, 2002), using aphid alarm pheromone to increase acquisition of fungal conidia by enhancing target insect movement (Roditakis *et al*., 2000) or the attraction of male moths into chambers where they become contaminated with infective fungal conidia and then return to the crop, disseminating the pathogen amongst their own population (Furlong *et al*., 1995; Vickers *et al*., 2004).

Examples of the effects of combining biopesticide treatments with insecticides and other biopesticides

Microbial and microbial

The speed of kill and overall efficacy of microbial biopesticides are usually less than that of many chemical pesticides. Generally the main aim of combining treatments is to identify synergistic interactions that give greater pest mortality, faster speed of kill or which enable a reduction in application rates to save money (Chandler, 2011).

As an example, Wraight and Ramos (2005) investigated the effect of combining *Beauveria bassiana* and *Bt* against field populations of Colorado potato beetle (*Leptinotarsa decemlineata*). *Beauveria bassiana* was very infectious to beetle larvae in laboratory experiments but gave slow and inadequate control in the field whilst *Bt* could give some control in the field but was more expensive than chemical pesticides. Previous research showed that development of the beetle was retarded when treated with sub-lethal doses of *Bt*. This suggested that *Bt* would prolong the interval between larval moults and thus enhance the activity of *B. bassiana* (i.e. there would be more time for the fungus to penetrate the insect cuticle before being lost through moulting). In addition, starvation induced by *Bt* could affect the susceptibility of larvae to *B. bassiana. Bt* and *B. bassiana* were applied to field plots. The *B. bassiana* product gave no, or a very low-level, of control in the field while *Bt* gave between 40 – 50% control depending on dose. However, when *B. bassiana* was combined with *Bt,* the level of control increased to 80 – 85%.

Microbial and pheromone

Roditakis *et al*. (2000) showed that the application of the aphid alarm pheromone, E β farnesene, increased the movement of *Myzus persicae* on leaf discs of pepper in a laboratory test, which caused them to pick up more spores of the entomopathogenic fungus *Lecanicillium longisporum* (=*Verticillium lecanii*), leading to an increase in fungus-induced mortality. Unfortunately biological and chemical constraints including problems in handling, storing and applying such a volatile and unstable compound have prevented its practical use and combining E β farnesene and fungus is probably not a practical option (Roditakis *et al*., 2000).

Microbial and insecticide

The effect on pest control of simultaneous applications of microbial biopesticides and chemical pesticides has been investigated in a range of studies seeking higher pest mortality and improved speed of kill. Research has also been done to investigate the role of microbial biopesticides in preventing or delaying the development of chemical pesticide resistance, and also to look at the effect of sub-lethal quantities of chemical pesticide on the performance of microbial biopesticides. The majority of studies have concerned entomopathogenic fungi (Chandler, 2011).

For example, Kpindou *et al.* (2001) combined *Metarhizium anisopliae* with lambda-cyhalothrin in order to improve speed of kill of grasshoppers. Here the chemical pesticide gave rapid knockdown with mortality due to the *M. anisopliae* beginning two days after application. Cuthbertson *et al.* (2008a, b; 2010) investigated the potential of combining the entomopathogenic fungus *Lecanicillium muscarium* with chemical pesticides in an IPM programme for tobacco whitefly (*Bemisia tabaci*). The fungus was applied to plants 24 hours after the chemical insecticides. Although spore germination was affected by some of the chemical insecticides or physically-acting products such as fatty acids, mortality of second instar whitefly larvae was higher in combinations than when the fungus or the pesticides were applied on their own. Cuthbertson *et al*. (2008b) also investigated the compatibility of the entomopathogenic nematode *Steinernema carpocapsae* with a range of synthetic insecticides. Here the use of nematodes in combination with thiacloprid resulted in higher levels of mortality in *B. tabaci* than when the chemical was used on its own.

In laboratory experiments, Ye *et al*. (2005) modelled the mortality of chrysanthemum aphid, *Macrosiphoniella sanborni*, in response to time and dose of *B. bassiana* applied alone or with sub-lethal concentrations of imidacloprid. These experiments provided very strong evidence of a potentiating effect of imidacloprid on fungal virulence. In a different study, one percent of the recommended dose of imidacloprid, applied systemically, dramatically increased movement of *M. persicae* (Roditakis *et al*., 2000). This resulted in greater mortality from infection by *Verticillium lecanii* in experiments where aphids were exposed to insecticide-treated leaf discs that had been sprayed with fungal conidia. A comparison with results from an experiment where conidia were sprayed directly onto aphids which were feeding on insecticide-infused pepper discs established that synergy was due to an indirect effect of the insecticide, i.e. through increased movement, rather than a direct effect through insecticide-weakened insects becoming more susceptible to disease.

Furlong & Groden (2001) found that applying sub-lethal concentrations of imidacloprid together with spores of *B. bassiana* resulted in increased mortality of larvae of Colorado potato beetle in laboratory tests. This occurred when imidacloprid was applied at the same time as the fungus or when it was applied 24h before the fungus, but not when the insecticide was applied 24h after the fungus. The imidacloprid inhibited larval feeding and it was suggested that stress due to starvation made the larvae more susceptible to the fungus. The possibility that fungal infection made the insect susceptible to normally sub-lethal concentrations of imidacloprid was ruled out, as there was no increase in mortality when the insecticide was applied after the fungus.

Microbial and plant extract

Shah *et al.* (2008) showed that both *M. anisopliae* and neem cake (a by-product of neem oil production) were effective against early instar larvae of the black vine weevil when incorporated into compost and that the addition of neem cake enhanced the efficacy of *M. anisopliae*. They suggested that the neem cake caused greater movement of the larvae by acting as a repellent or antifeedant leading to increased acquisition of fungal spores. The apparent antifeedant properties of the neem cake also resulted in reduced larval growth which may have weakened the larvae, making them more susceptible to the fungus.

Similarly, Mohan *et al.* (2007) found most isolates of *B. bassiana* tested to be compatible with neem oil and that a combination was more effective against tobacco budworm. This improved efficacy was seen both by increased mortality and faster speed of kill. Barčić *et al.* (2006) investigated the efficacy of *Bt*, neem and pyrethrins for the control of the Colorado potato beetle. Here the combinations were found to have greater efficacy and persistence compared to the individual components. James (2003) combined azadirachtin (from neem) with the entomopathogenic fungus *Paecilomyces fumosoroseus* to control *Bemisia argentifolii*. Higher levels of mortality were recorded when the azardirachtin and the entomopathogenic fungus were combined in sequential sprays separated either by two hours or three days.

Plant extract and insecticide

A laboratory experiment on diamond back moth, *Plutella xylostella*, showed that extracts of chilli 3% or garlic 2% in combination with half doses of dichlorvos and endosulfan proved to be as effective as that of the chemical insecticides alone (Sewak *et al*., 2008).

Plant extract and plant extract

A study was undertaken to determine the efficacy of seven natural compounds compared with piperonyl butoxide (PBO) in synergising pyrethrum, with the intention of formulating an effective natural synergist with pyrethrum for use in the organic crop market. They were tested on houseflies. Dillapiole oil and parsley seed oil showed the greatest potential as pyrethrum synergists. Piperonyl butoxide remained the most effective synergist, possibly owing to its surfactant properties, enhancing penetration of pyrethrins (Joffe *et al*., 2011).

Insecticide and pheromone

Insecticide based 'lure and kill' uses a combination of a pheromone (or other attractant) and an insecticide to kill the target pest. The insects responding to the pheromone attractant are lured into direct physical contact with the insecticide. Cook *et al*. (2002) showed that there is potential for addition of dodecyl acetate component of the alarm pheromone of the western flower thrips (*Frankliniella occidentalis* Pergande) to enhance insecticidal control of this species on strawberry by including it in the spray solution. Russell IPM has developed an Attract and Kill system Tac-37 for tomato moth (*Tuta absoluta*) (Russell IPM, 2012). TAC-37 can be applied using a hand dispensing gun. Application can be mechanized for large scale field application.

Insecticide and bait

Feeding stimulants can be used to increase the ingestion of insecticides that might not be so effective through direct contact with sprays or with residues. HDC-funded research showed in laboratory tests and a field cage test that the efficacy of Tracer (spinosad) as a foliar spray to control cabbage root fly could be improved considerably by the addition of a feeding stimulant (sugar or sugar + yeast) (FV 242a). Similar studies showed this was also the case for large narcissus fly adults (BOF 55). Such treatments were not effective in the open field and studies on the cabbage root fly indicated this was likely to be due to the short persistence of the insecticide on the foliage combined with the continued immigration of insects over a period of several weeks.

Determine the effect of combinations of treatments identified in 1) on control of key groups of pest insect.

Test species and potential control agents/potentiators

The test species and potential control agents/potentiators (e.g. sugar) for this project are shown in Table 2.1. Because many of these materials are part of the SCEPTRE project a number of the individual materials must be coded.

Table 2.1. Potential control agents and potentiators for pest insects in current project.

	Insect cultures	**Potential control agents/potentiators**
Flies	*Cabbage root fly (Delia radicum), onion fly (Delia antiqua)*	Insecticide e.g. spinosad (drench or foliar application) Plant extract (drench, phytodrip or foliar application) Microbial Sugar/protein bait
Aphids	Cabbage aphid (*Brevicoryne brassicae*), peach-potato aphid (*Myzus persicae*), currant-lettuce aphid (*Nasonovia ribisnigri*)	Insecticide e.g. imidacloprid (phytodrip or drench application) Plant extract (drench, phytodrip or foliar application) Microbial
Caterpillars	Diamond-back moth (*Plutella xylostella*), large white butterfly (*Pieris brassicae*)	Insecticide e.g. spinosad (drench or foliar application) Sugar Plant extract (drench, phytodrip or foliar application) Microbial
Thrips	*Thrips nigripilosus, possibly* onion thrips (*Thrips tabaci*)	Insecticide (foliar application) Sugar Plant extract (foliar application) Microbial

Research questions for each group of test species

Flies

The model system is cabbage root fly on brassica. Test substances can be applied to brassica transplants as drench or phytodrip treatments. They can be applied at any stage as foliar sprays.

Questions to be addressed are:

- How do plant extracts or microbial biopesticides affect fly mortality and behaviour (particularly egg-laying)?
- How do plant extracts or mcrobials affect larval mortality?

- Do any of the plant extracts have systemic activity?
- Does a combination of plant extracts and/or microbials have a greater effect on fly and larval mortality and behaviour?
- Do plant extracts increase the uptake/performance of insecticide sprays?
- Does a sub-lethal dose of insecticide enhance the performance of microbial insecticides or plant extracts?

Aphids

The model system is aphids – *Myzus persicae* and *Brevicoryne brassicae* on brassica and *Nasonovia ribisnigri* on lettuce. Test substances can be applied to transplants as drench or phytodrip treatments. They can be applied at any stage as foliar sprays.

Questions are:

- How do plant extracts or microbials affect aphid mortality and behaviour?
- Does a combination of plant extracts/microbials have a greater effect on aphid mortality and behavior?
- Do plant extracts increase the uptake/performance of insecticide?
- Does a sub-lethal dose of insecticide enhance the performance of microbial insecticides or plant extracts?
- Do any of the plant extracts have systemic activity?

Caterpillars

The model system is diamond-back moth on brassica. Test substances can be applied to transplants as drench or phytodrip treatments. They can be applied at any stage as foliar sprays.

Questions are:

- How do plant extracts/microbials affect adult and larval mortality and behaviour?
- Does a combination of plant extracts/microbials have a greater effect on mortality and behavior?
- Do plant extracts increase the uptake/performance of insecticides/microbials?
- Does a sub-lethal dose of insecticide enhance the performance of microbial insecticides or plant extracts?
- Do any of the plant extracts have systemic activity?

Thrips

The model system is *Thrips nigripilosus* on lettuce. *Thrips tabaci* will be tested if a culture can be established in summer 2012. Test substances can be applied as foliar sprays.

Questions are:

- How do plant extracts/microbials affect thrips mortality?
- Does a combination of plant extracts have a greater effect on mortality?
- Do plant extracts increase the uptake/performance of insecticide?
- Does a sub-lethal dose of insecticide enhance the performance of microbial insecticides or plant extracts?
- Do any of the plant extracts have systemic activity?

Materials and methods

Standard protocols have been developed and tested for flies, aphids and caterpillars, using substances applied alone as foliar treatments and are summarised below. Further protocols will be developed for thrips and to observe any other effects of the treatments (e.g. behavioural effects).

Test materials

The test materials are coded using the HDC system and for reasons of confidentiality it is not possible at this stage to provide detail about the mode of action of the individual compounds. Each test is based on up to 6 different substances/materials (Table 2.2).

Table 2.2 Codes for test materials by pest species

Pest species	Product code
Cabbage root fly (*Delia radicum*)	HDCI016
Cabbage root fly (*Delia radicum*)	HDCI017
Cabbage root fly (*Delia radicum*)	HDCI018
Cabbage root fly (*Delia radicum*)	HDCI019
Cabbage root fly (*Delia radicum*)	HDCI020
Cabbage root fly (*Delia radicum*)	HDCI021
Brevicoryne brassicae and *Myzus persicae*	HDCI022
Brevicoryne brassicae and *Myzus persicae*	HDCI023
Brevicoryne brassicae and *Myzus persicae*	HDCI024
Brevicoryne brassicae and *Myzus persicae*	HDCI025
Brevicoryne brassicae and *Myzus persicae*	HDCI026
Brevicoryne brassicae and *Myzus persicae*	HDCI027
Plutella xylostella	HDCI028
Plutella xylostella	HDCI029
Plutella xylostella	HDCI030
Plutella xylostella	HDCI031
Plutella xylostella	HDCI032
Plutella xylostella	HDCI033

Control of adult cabbage root fly and impact on egg-laying

1. Spray cauliflower plants with treatments using Knapsack sprayer
2. Cover surface of compost with a layer of sieved soil
3. Place each plant in a cage
4. Introduce 10 female and 10 male (6-8 days old) cabbage root flies
5. Assess fly mortality daily
6. Count numbers of eggs laid after a specified number of days

Mortality of *Brevicoryne brassicae* or *Myzus persicae*

1. Infest cauliflower plants with aphids
2. Count aphids on plants prior to treatment
3. Spray plants with treatments using Knapsack sprayer
4. Place each plant in a cage
5. Re-count aphids after a specified number of days

Effect on colonization of winged *Brevicoryne brassicae* or *Myzus persicae*

1. Spray cauliflower plants with treatments using Knapsack sprayer
2. Place each plant in a cage
3. Release known numbers of winged aphids
4. Assess aphid mortality daily
5. Monitor plants for build up of colonies
6. Count numbers of aphids if colonies develop

Control of adult diamond-back moth and impact on egg-laying

1. Spray cauliflower plants with treatments using Knapsack sprayer
2. Place each plant in a cage
3. Release diamond-back moths
4. Assess moth mortality daily
5. Count numbers of eggs laid after a specified number of days

Control of diamond-back moth caterpillars

1. Spray cauliflower plants with treatments using Knapsack sprayer
2. Place each plant in a cage
3. Infest cauliflower plants with a known number of diamond-back moth caterpillars
4. Assess caterpillar mortality daily

Results

The initial tests have shown that there is a need for considerable replication in time and space as responses are quite variable between replicates. Figures 2.1-2.5 summarise results from the initial tests. Test materials are identified by a code.

Cabbage root fly

In the tests with cabbage root fly, there was no significant mortality due to any of the treatments, just a low level of 'background' mortality in all cages including the control (data not shown). However, when adults were exposed to plants freshly-treated with T5, there was evidence of sub-lethal effects and some of the flies appeared to be knocked-down for a short period of time. These flies soon recovered. Despite this recovery, there was a noticeable effect on egg-laying (Figure 2.1), with fewer eggs laid on plants treated with T5.

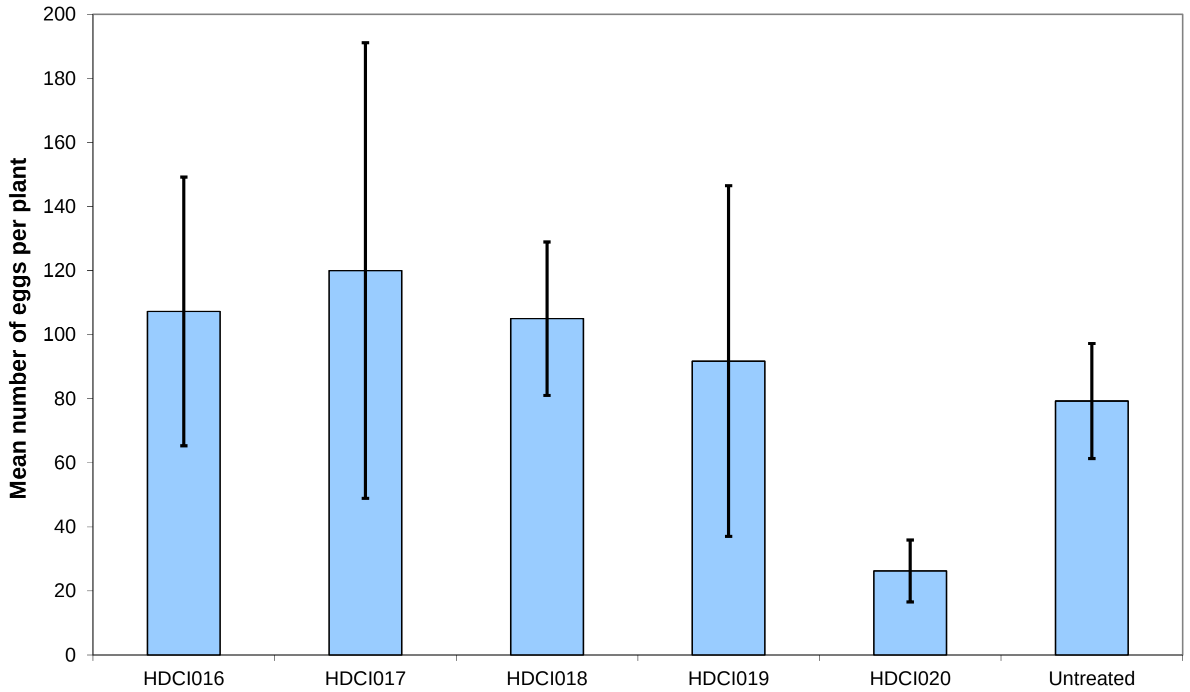

Figure 2.1. Mean number of cabbage root fly eggs per plant 6 days after flies were exposed to treated plants.

Brevicoryne brassicae

The test plants were infested with similar numbers of aphids prior to treatment. Aphid numbers were re-assessed 3 days (Figure 2.2) and 7 days (Figure 2.3) after spraying. Again T5 appeared to be the most effective treatment.

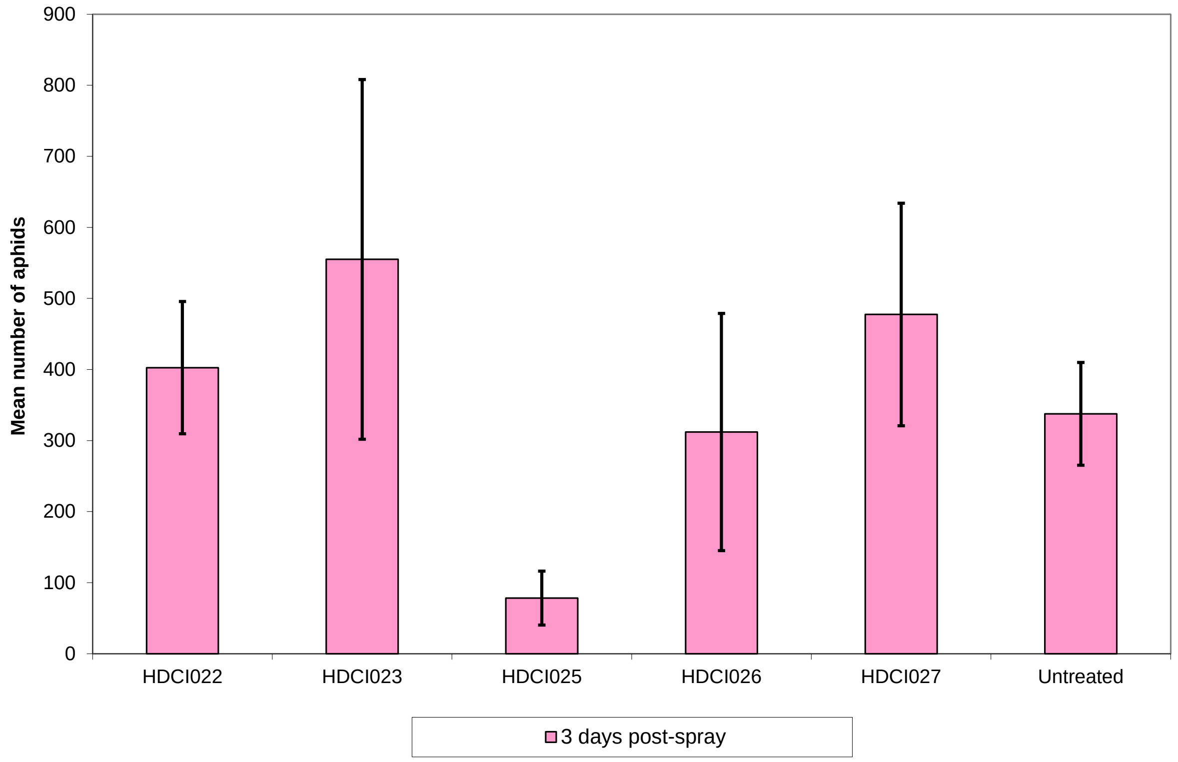

Figure 2.2. *Brevicoryne brassicae* - mean number of aphids per plant 3 days after treatment.

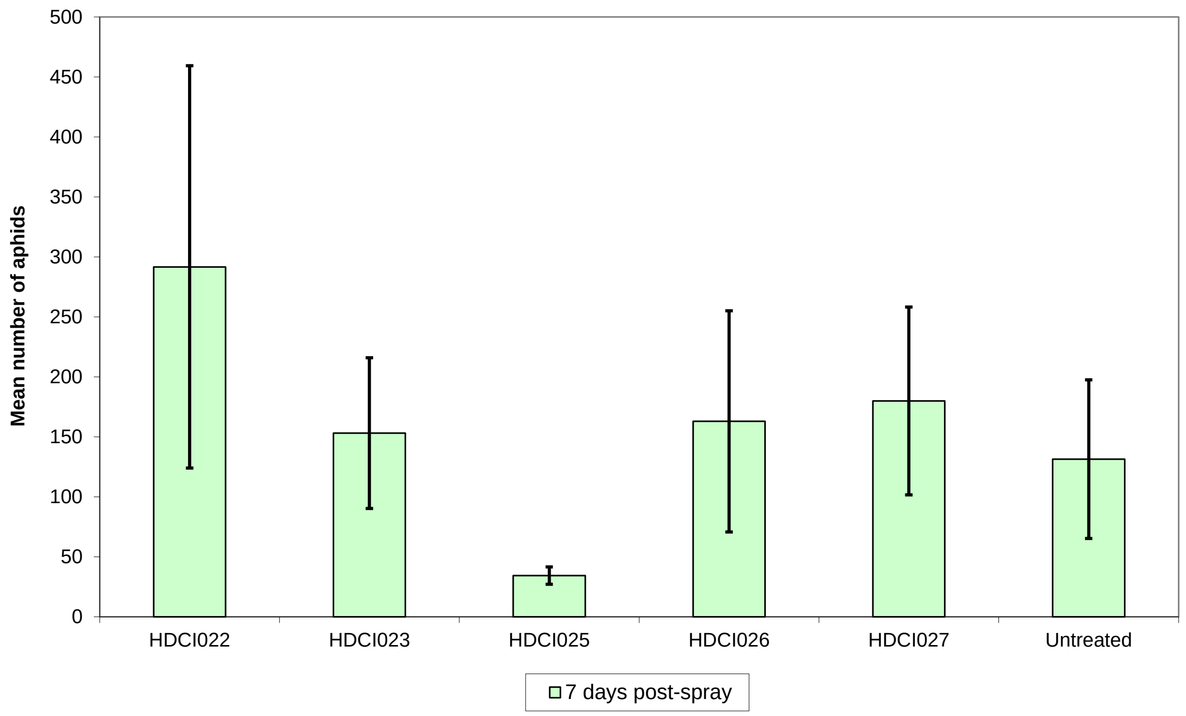

Figure 2.3. *Brevicoryne brassicae* - mean number of aphids per plant 6 days after treatment.

Myzus persicae

Aphid numbers were assessed 5 days after spraying (Figure 2.4). Again T5 appeared to be the most effective treatment.

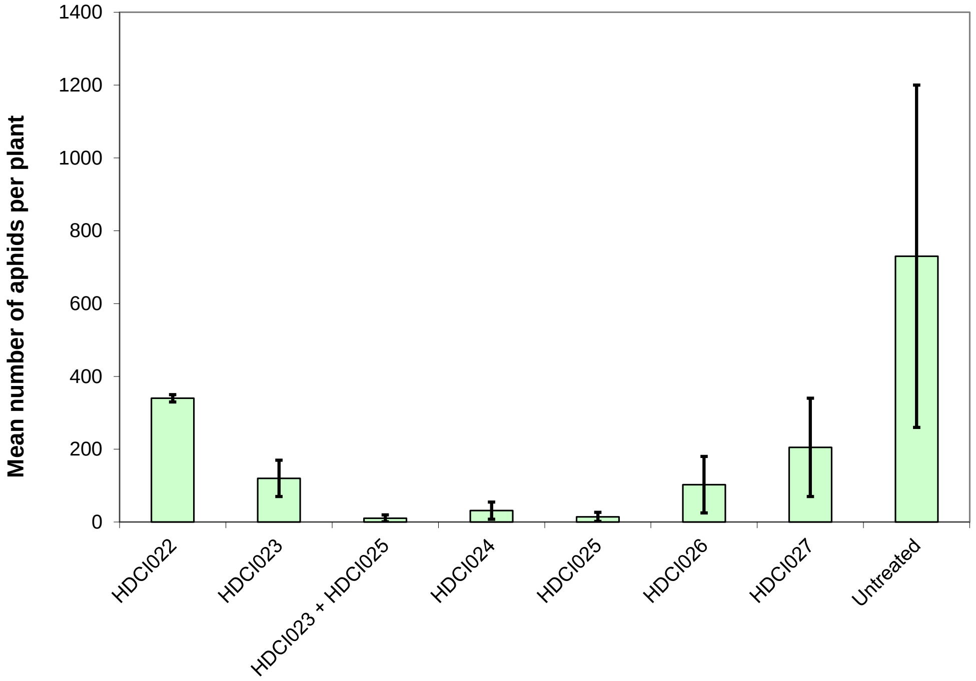

Figure 2.4. *Myzus persicae* - mean number of aphids per plant 5 days after treatment.

Plutella xylostella

Adult and egg numbers were assessed 5 days after spraying. Adult mortality was very low in all cages. The numbers of eggs laid were variable but none of the treatments appeared to have a major effect (Figure 2.4). The numbers of feeding holes due to the eggs which had hatched are shown in Figure 2.5. This may be another useful parameter to measure in future tests.

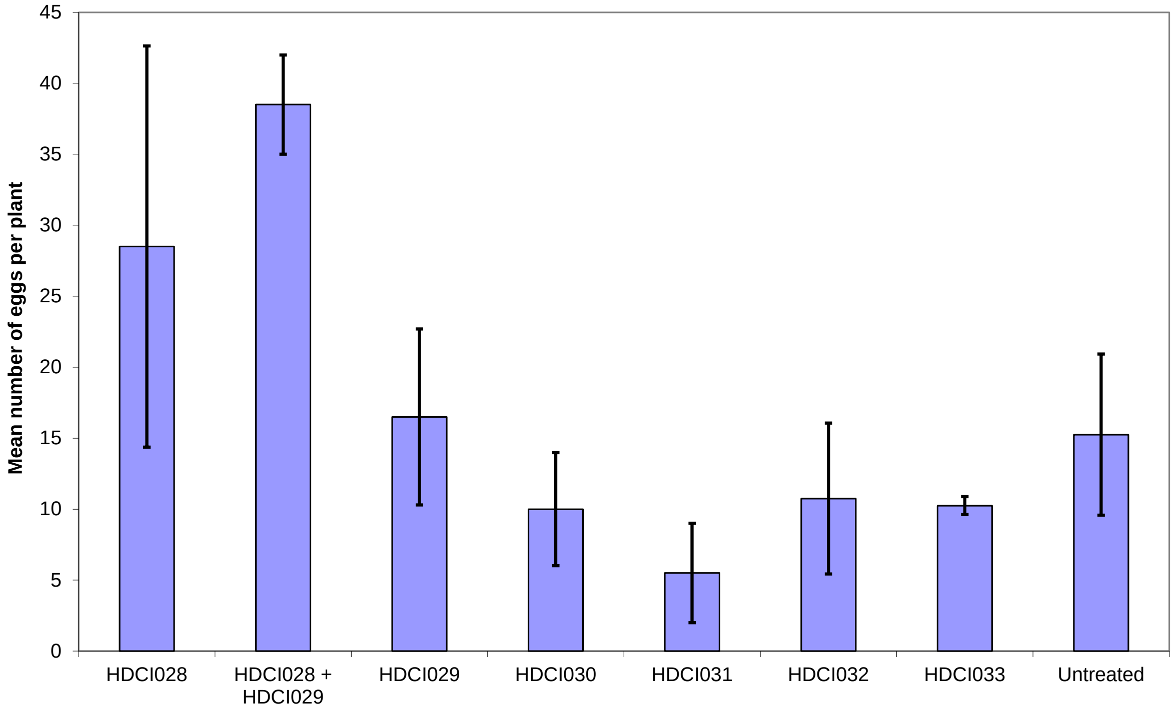

Figure 2.5. *Plutella xylostella* - mean number of eggs per plant 5 days after treatment.

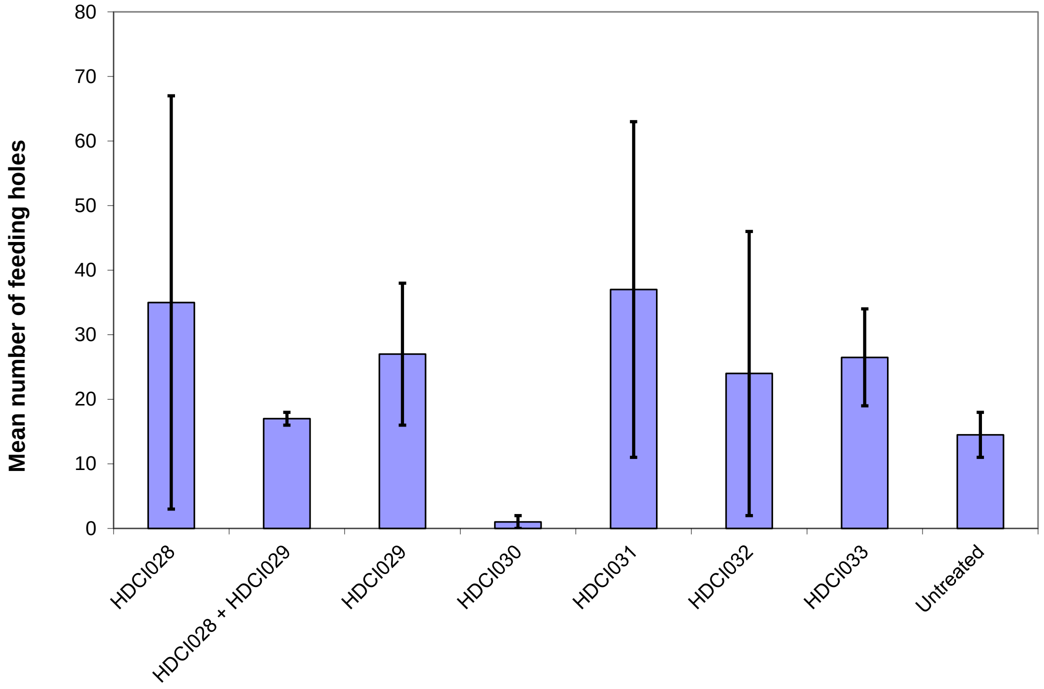

Figure 2.6. *Plutella xylostella* - mean number of feeding holes per plant 5 days after treatment.

Discussion

The review of biopesticides and other materials that might be used to improve pest control indicate a range of possible interactions based on changes in insect behaviour and/or insect susceptibility to treatments. Obviously, in terms of the test species, the range of substances that can be evaluated is more limited, based on the susceptibility of the test species to certain substances and the availability of substances to test. However, there is potential to test a number of interactions and to address the range of questions in Section 2.2.

The preliminary data shown in the Results section indicate that there is considerable variability within treatments and it will be necessary to replicate tests further in both space and time. It will also be important to make observations on insect behaviour during certain tests to determine the effects of different individual treatments and treatment combinations. Further tests are underway.

Conclusions

The results presented here represent the first part of the study and as such it is difficult to draw clear conclusions about the materials tested at this stage.

Knowledge and Technology Transfer

An article will be published in HDC News in summer 2012

References

AgraQuest (2012). http://requieminsecticide.com/

Ali, S., Huang, Z. & Ren, S. (2010). Production of cuticle degrading enzymes by *Isaria fumosorosea* and their evaluation as a biocontrol agent against diamondback moth. Journal of Pest Science 83: 361-370.

Al-mazra'awi, M. S.; Al-Abbadi, A.; Shatnawi, M. A.; Ateyyat, M. (2009). Effect of application method on the interaction between *Beauveria bassiana* and neem tree extract when combined for *Thrips tabaci* (Thysanoptera: Thripidae) control. Journal of Food, Agriculture & Environment 7: 869-873.

Arpaia, S. & van Loon, J.J.A. (1993). Effects of azadirachtin after systemic uptake into *Brassica oleracea* on larvae of *Pieris brassicae*. Entomol. Exp. Appl. 66: 39–45.

Barcic, J.I., Bazok, R., Bezjak, S., Culjak, T.G., and Barcic, J. (2006). Combinations of several insecticides used for integrated control of Colorado potato beetle (*Leptinotarsa decemlineata*, Say., Coleoptera : Chrysomelidae). Journal of Pest Science 79, 223-232.

Barry, J.D., Sciarappa, W.J., Teixeira, L.A.F., & Polavarapu, S. (2005). Comparative effectiveness of different insecticides for organic management of blueberry maggot (Diptera: Tephritidae). Journal of Economic Entomology 98:1236-1241.

BOF 55 (2006). Novel insecticide treatments to control large narcissus fly. HDC Final Report.

Bruck, D.J., Snelling, J.E., Dreves, A.J. & Jaronski, S.T. (2011). Laboratory bioassays of entomopathogenic fungi for control of *Delia radicum* (L.) larvae. Journal of Invertebrate Pathology 89 (2005) 179–183.

Butt, T.M., Carreck, N.L., Ibrahim, L. & Williams, I.H. (1998) Honey-bee-mediated infection of pollen beetle (*Meligethes aeneus* Fab.) by the insect-pathogenic fungus, *Metarhizium anisopliae*. Biocontrol Science and Technology 8: 533-538.

Chandler, D. (2011). A desk study of current knowledge on the combined use of microbial biopesticides and chemical pesticides in Integrated Pest Management. Unpublished.

Cook, D.F., Dadour, I.R., Bailey, W.J. (2002). Addition of alarm pheromone to insecticides and the possible improvement of the control of the western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). International Journal of Pest Management 48: 287-290.

Cuthbertson, A.G.S., Blackburn, L.F., Northing, P., Luo, W.Q., Cannon, R.J.C., and Walters, K.F.A. (2008a). Further compatibility tests of the entomopathogenic fungus *Lecanicillium muscarium* with conventional insecticide products for control of sweetpotato whitefly, *Bemisia tabaci* on poinsettia plants. Insect Science 15, 355-360.

Cuthbertson, A.G.S., Mathers, J.J., Northing, P., Prickett, A.J., and Walters, K.F.A. (2008b). The integrated use of chemical insecticides and the entomopathogenic nematode, *Steinernema carpocapsae* (Nematoda: Steinernematidae), for the control of sweetpotato whitefly, *Bemisia tabaci* (Hemiptera : Aleyrodidae). Insect Science 15, 447-453.

Cuthbertson, A.G.S., Blackburn, L.F., Northing, P., Luo, W., Cannon, R.J.C., and Walters, K.F.A. (2010). Chemical compatibility testing of the entomopathogenic fungus *Lecanicillium muscarium* to control *Bemisia tabaci* in glasshouse environment. International Journal of Environmental Science and Technology 7, 405-409.

Defra (2006). Aphid control in Lettuce and Brassica crops - HH3117TFV. http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=12163

Dayan, F.E. , Cantrell, C.L., Duke, S.O. (2009). Natural products in crop protection. Bioorganic and Medicinal Chemistry 17: 4022-4034

DOW (accessed 22 April 2012). GF-120® NF NATURALYTE® FRUIT FLY BAIT. http://msdssearch.dow.com/PublishedLiteratureDAS/dh_0057/0901b80380057a6f.pdf?filepath=ca/pdfs/noreg/010-20585.pdf&fromPage=GetDoc

Furlong, M.J., and Groden, E. (2001). Evaluation of synergistic interactions between the Colorado potato beetle (Coleoptera : Chrysomelidae) pathogen *Beauveria bassiana* and the insecticides, imidacloprid, and cyromazine. Journal of Economic Entomology 94, 344-356.

Furlong, M.J., Pell, J.K., Choo, O.P. & Rahman, S.A. (1995). Field and laboratory evaluation of a sex pheromone trap for the autodissemination of the fungal entomopathogen *Zoophthora radicans* (Entomophthorales) by the diamond-back moth, *Plutella xylostella* (Lepidoptera: Ypnomeutidae). Bulletin of Entomological Research 85: 331-337.

FV 242a (2004). Brassica crops: evaluation of non-OP insecticides for controlling the cabbage root fly. HDC Final Report.

Hollingsworth, R.G. (2005). Limonene, a citrus extract, for control of mealybugs and scale insects. Journal of Economic Entomology 98, 772-779.

Islam, M.T., Castle, S.J., & Ren, S. (2010). Compatibility of the insect pathogenic fungus *Beauveria bassiana* with neem against sweetpotato whitefly, *Bemisia tabaci*, on eggplant. Entomologia Experimentalis et Applicata 134: 28-34.

Islam, M.T., Omar, D., Latif, M.A., & Morshed, M.M. (2011). The integrated use of entomopathogenic fungus, Beauveria bassiana with botanical insecticide, neem against *Bemisia tabaci* on eggplant. African Journal of Microbiology Research 5: 3409-341.

James, R.R. (2003). Combining azadirachtin and Paecilomyces fumosoroseus (Deuteromycotina : Hyphomycetes) to control *Bemisia argentifolii* (Homoptera : Aleyrodidae). Journal of Economic Entomology 96, 25-30.

Joffe, T., Gunning, R.V., Allen, G.R., Kristensen, M., Alptekin, S., Field, L.M., & Moores, G.D. (2011). Investigating the potential of selected natural compounds to increase the potency of pyrethrum against houseflies *Musca domestica* (Diptera: Muscidae). Pest Management Science 68:178-184.

Kpindou, O.K.D., Lomer, C.J., Langewald, J., Togo, T., and Sagara, D. (2001). Effect of a mixture of lambda-cyhalothrin and spores of *Metarhizium anisopliae* (flavoviride) var. acridum Driver and Milner (biopesticide) applied to grasshopper larvae in Mali. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 125, 249-253.

Kumar, P., Poehling, H.-M., & Borgemeister, C. (2005). Effects of different application methods of azadirachtin against sweetpotato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants. Journal of Applied Entomology 129, 489-497.

Marks, D. (2006). The regulation of BugOil: Experiences from North America and the EU. Outlooks on Pest Management 17: 15-17.

Meadow, R., Seljåsen, R. & Brynildsen, P. (2012). The effect of neem extracts on the turnip root fly and the cabbage moth. http://www.neemazal.de/deu/intland/Files/WS9%20gemuesebau%203.pdf

Meadow, R., Vandberg, J.D., Shelton, A.M. (2000). Exchange of inoculum of *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes) between adult flies of the cabbage maggot *Delia radicum* L. (Diptera: Anthomyiidae). Biocontrol Sci Technol 10: 479-485.

Meadow, R., Vandenberg, J.D. & Shelton, A.M. (2000). Exchange of inoculum of *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes) between adult flies of the cabbage maggot *Delia radicum* L. (Diptera: Anthomyiidae). Biocontrol Science and Technology 10: 479-485

Mitchell, E.R. (2002). Promising new technology for managing diamondback moth (Lepidoptera: Plutellidae) in cabbage with pheromone. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes 37: 277-290.

Mohan, M.C., Reddy, N.P., Devi, U.K., Kongara, R., and Sharma, H.C. (2007). Growth and insect assays of *Beauveria bassiana* with neem to test their compatibility and synergism. Biocontrol Science and Technology 17, 1059-1069.

Neemazal http://www.ecogrape.com/neemazal

Oparaeke, A.M. , Dike, M.C., Amatobi, C.I. (2005). Evaluation of botanical mixtures for insect pests management on cowpea plants. Journal of Agriculture and Rural Development in the Tropics and Subtropics. 106: 41-48.

Osman, M.Z. & Port, G.R. (1990). Systemic action of neem seed substances against *Pieris brassicae*. Entomol. Exp. Appl. 54: 297–300.

Pats, P. & Isman, M.B. (1998). Effect of neem on adult longevity, oviposition and larval development of the cabbage fly, *Delia radicum* (L.) (Dipt., Anthomyidae). Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie 122: 125-127

Roditakis, E., Couzin, I. D, Balrow, K., Franks, N. R. & Charnley, A. K. (2000). Improving secondary pick up of insect fungal pathogen conidia by manipulating host behaviour. Ann. appl. Biol. (2000), 137:329–335.

Rothamsted Research (2012). A new generation of insect resistant GM crops: Transgenic wheat synthesising the aphid alarm signal. http://www.rothamsted.ac.uk/ProjectDetails.php?ID=5010.

Russell IPM (2012). http://www.russellipm-agriculture.com/solutions.php?id_ctg=1&lang=en

Seljåsen, R., Meadow, R. (2006). Effects of neem on oviposition and egg and larval development of *Mamestra brassicae* L: Dose response, residual activity, repellent effect and systemic activity in cabbage plants. Crop Protection 25: 338-345

Sewak, N. , Ahmad, S., Mishra, M.S. (2008). Bioefficacy of indigenous products and their combinations against diamond back moth, *Plutella xylostella* (Linnaeus). Pestology 32: 23-27.

Shah, F. A., Gaffney, M., Ansari, M. A., Prasad, M. & Butt, T. M. (2008) Neem seed cake enhances the efficacy of the insect pathogenic fungus *Metarhizium anisopliae* for the control of black vine weevil, Otiorhynchus sulcatus (Coleoptera: Curculionidae). Biological Control. 44, 111-115.

Tanzubil, P.B.,Terbobri, P. & Ansoba, E. (2004). Incidence and control of onion fly, *Delia antiqua*, at some irrigation sites in the Upper East Region of Ghana. Ghana Journal of Agricultural Science 32: 205-211.

Thoeming, G., Borgemeister, C., Sétamou, M. and Poehling, H-M. (2003). Systemic Effects of Neem on Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Journal of Economic Entomology 96:817-825.

Vickers, R.A., Furlong, M.J., White, A. & Pell, J.K. (2004). Initiation of fungal epizootics in diamondback moth populations within a large field cage: Proof of concept for auto-dissemination. Entomologia Experimentalis et Applicata 111: 7-17.

Wraight, S.P., and Ramos, M.E. (2005). Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. Journal of Invertebrate Pathology 90, 139-150.

Wraight, S.P., Ramos, M.E., Avery, P.B., Jaronski, S.T., Vandenberg, J.D. (2010). Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops Journal of Invertebrate Pathology 103: 186-199.

Wu, Q.-J., Zhang, S.-F., Yao, J.-L., Xu, B.-Y., & Zhang, Y.-J. (2012). Management of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) by mating disruption. Insect Science (Article in press) DOI: 10.1111/j.1744-7917.2011.01497.x

Yang, X., Zhang, Y., Hua, L., Peng, L., Munyaneza, J.E., Trumble, J.T. & Liu,T. (2010). Repellency of selected biorational insecticides to potato psyllid, *Bactericera cockerelli* (Hemiptera: Psyllidae). Crop Protection 29: 1320-1324.

Ye, S.D., Dun, Y.H., and Feng, M.G. (2005). Time and concentration dependent interactions of Beauveria bassiana with sublethal rates of imidacloprid against the aphid pests Macrosiphoniella sanborni and Myzus persicae. Annals of Applied Biology 146, 459-468.

Zaki, F.N. (2008). Field application of plant extracts against the aphid, *B. brassicae* and the whitefly, *B. tabaci* and their side effects on their predators and parasites. Archives of Phytopathology and Plant Protection 41: 462-466

Appendix

Further details on some of the biopesticide products described in the section 'Biopesticides and other materials available for pest control' and some additional materials that have activity against pest insects. Sources of information are indicated.

Requiem Agraquest	Active Ingredient: 25% of essential oil extract of *Chenopodium ambrosioides* nr. Ambrosiodes. Control: whiteflies, aphids, mites, thrips and other sucking pests in high-value fruits and vegetables. Active against all lifecycle stages – eggs to adults. Attacks the exoskeleton of targeted pests, punches holes in their fatty tissues. This degradation of the exoskeleton causes a loss of fluid that kills the insects. Clogs trachea - respiration in insects is dependent on a network of tubes, called trachea, to exchange gases. Without the ability to pass air through the openings in the tracheal system, the insect suffocates and dies. Disrupts Feeding - confuses insects' chemoreceptors, discouraging their ability to locate food sources. Without the ability to feed on protected crops, virus transmission is reduced and pests die. http://requieminsecticide.com/
Bugoil Plant Impact	Active ingredient: 94% canola oil, 0.6% thyme oil (*Thymus vulgaris*), 0.6% tagetes oil (Tagetes erecta) and 0.001% wintergreen oil (Gaultheria procumbens) (Yang *et al*. 2010)
Majestik Certis	Active Ingredient: 49% w/w maltodextrin Control: A contact insecticide that works by physical means. Spider mites, whitefly, aphid. Crops: Outdoor and protected crops. http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
Met52 Granular Bioinsecticide	Active Ingredient: 2%w/w *Metarhizium anisopliae* var. *anisopliae* strain F52. Control: Black vine weevil (*Otiorhynchus* spp.) larvae. Crops: Ornamental plant production and named soft fruit. Protected and outdoor, container and open ground. Application Method: Pre-planting granule incorporation. http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
Pyrethrum 5 Ec	Active Ingredient: 20% w/v pyrethrum extract (5% w/v pyrethrins). Control: Chewing and sucking pests including aphids, caterpillars, whitefly and red spider mite. Crops: All edible and non-edible crops. Application Method: Foliar spray. http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
Mycotal	Active Ingredient: *Verticillium lecanii* 16.1%w/w. Control: Whitefly. Crops: Protected crops of: tomato, cucumber, runner bean, broad bean, French bean, aubergine, lettuce, pepper and ornamental plant production. Application Method: Foliar spray. Comment: Requires minimum of 80% relative humidity and 18^{o}C http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
Naturalis-L Belchim	Active Ingredient: 7.16%w/w *Beauveria bassiana* ATCC 74040 Control: Whitefly and thrips with activity on a range of other pests ncluding spider mites. Crops: All edible crops (protected) and ornamental plant production (protected).

	Application Method: Spray. http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
Savona Koppert	Active Ingredient: Potassium salts of fatty acids 49% w/w Control: Whitefly, mealybugs, scale insects, aphids and spider mites. Crops: Tomato, cucumber, pepper, pumpkin, brussels sprout, cabbage, lettuce, peas, beans, fruit trees, ornamental shrubs and trees. Application Method: Foliar spray. http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
Sb Plant Invigorator Fargro	Ingredients: Foliar lattice, linear sulphanate, 0.37% w/w Iron chelate (Fe), 9.57% w/w Nitrogen (N) and natural products. Control: A wide range of pests including whitefly, aphid, spider mite, mealy bug, hard and soft scale insects, and bay sucker psyllids. Controls by physical means and therefore exempt from registration as a pesticide. Crops: Protected and outdoor edible and ornamental crops. Application Method: Foliar spray http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
Spruzit Certis	Active Ingredient: 4.59 g/l pyrethrins. Contains a naturally derived oil. Control: A broad spectrum contact insecticide for use against biting and sucking insects. Controls insect adults and larvae and some stages egg stages of bugs. Crops: All edible and non-edible crops. Application Method: Foliar spray. http://www.fargro.co.uk/catalogue-pesticide/catalogueinsecticide.pdf
NeemAzal-T/S	Active Ingredient: Broad spectrum botanical Insecticide derived from the Neem tree seed kernel. The formulation is naturally based neem extract, sesame oil and a surfactant from a renewable resource. Control: Slow acting naturally based anti-feeding insecticide. When used early, or prior to an increase in pest numbers, it leads to feeding inhibition and moulting, also to a reduction in fecundity and breeding ability. The formulation of the product greatly assists the transport of the active ingredient into the leaf. thrips, white fly, aphid (also *Nasonovia ribisnigri*), caterpillar, scale insects, mealy bug. aphids, bronze beetle, erinose mite, whitefly, leaf-mining flies, mealy bug , scale insects, potato tuber moth, brown beetle (grass grub), cicada, weevils and midges. It should be combined with *Bacillus thuringiensis* (Bt) insecticides to provide more complete protection against caterpillar where they are a problem with multiple generations. Application Method: Spray. http://www.ecogrape.com/neemazal