Project title:	Combining biopesticides and other treatments to increase pest control
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Project leader:	Rosemary Collier, University of Warwick
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Industry Representative:	Matthew Rawson
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The results and conclusions in this report are based on an investigation conducted over a two-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

Headline

Several biopesticide products were effective against important vegetable pests (cabbage root fly, aphids and diamond-back moth) when applied alone and there was evidence of additive and synergistic effects between biopesticides.

Background

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

Summary of the project and main conclusions

The aim of this project was to undertake a series of small-scale laboratory tests with pest insects that can be obtained easily from cultures (cabbage root fly [*Delia radicum*], cabbage aphid [*Brevicoryne brassicae*], peach-potato aphid [*Myzus persicae*], currant-lettuce aphid [*Nasonovia ribisnigri*] and diamond-back moth [*Plutella xylostella*]) to evaluate the potential of a range of treatments by comparing their activity separately and in combination. The term 'biopesticide' used here includes biocontrol agents, botanicals or semio-chemicals.

A literature review was first 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. These improvements 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 *Bacillus thuringiensis*), a microbial biopesticide with a reduced dose of a chemical insecticide, or a biopesticide based on a plant extract with a microbial biopesticide. Simplistically, the two main mechanisms by which control is improved are 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 were identified that can be tested in combination against a number of vegetable pests. Because most of these materials are being used in the SCEPTRE (Sustainable Crop and Environment Protection – Targeted Research for Edibles) project, the individual products were coded in this project.

The biopesticides were tested in a laboratory situation using potted plants and by infesting them with insects from cultures. For the cabbage root fly, two types of test were undertaken. Firstly, adult flies were exposed to plants whose foliage had been treated with foliar sprays of the test biopesticides. This was either in a 'choice' (flies confined with several treatments) or 'no-choice' (flies confined with one treatment) situation, to investigate effects on fly survival and egg-laying by female flies. Secondly, biopesticides were applied to the compost surrounding the potted plants and the plants were then inoculated with cabbage root fly eggs. In this case, some of the biopesticides were applied with a reduced dose of Tracer (spinosad) (5% or 10% of recommended rate) to investigate whether reduced doses of insecticide and biopesticides might act additively or even synergistically. For aphids, foliar sprays of the biopesticides were applied to infested plants and for diamond-back moth, adult moths were confined with plants whose foliage had been treated with foliar sprays of the test biopesticides.

Cabbage root fly

Most of the biopesticides applied as foliar sprays did not increase cabbage fly mortality. However, HDCI020, or treatments including HDCI020, did increase fly mortality on several occasions in no-choice tests, particularly during the first few days when residues were fresh. Numbers of cabbage root fly larvae/pupae were reduced by several treatments applied to the module compost (Figure A). Of the treatments applied alone, HDCI019 and HDCI021 were most effective and Tracer was also surprisingly effective at a reduced dose. HDCI049 was effective in combination with a reduced dose of Tracer. Treatments that reduced the number of cabbage root fly larvae/pupae also reduced root damage (scored on a 0–5 scale), and some of them increased root weight, compared with the untreated control. There was some evidence – requiring confirmation – that HDCI049 and a reduced dose of Tracer worked synergistically.

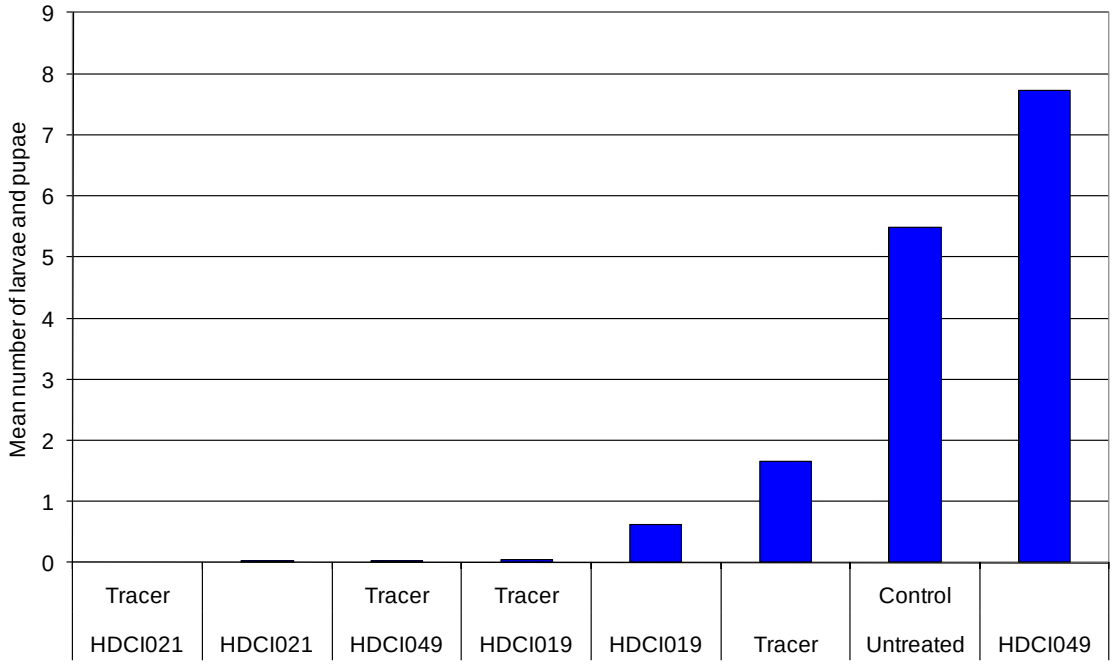

Figure A. Cabbage root fly – biopesticides applied to module compost –mean number of larvae + pupae recovered per plant (back-transformed data). Tracer was applied at 5% of the recommended rate.

Aphids

The levels of control achieved with single products in all the experiments on aphid control (three species) were summarised. Whilst there is a considerable amount of variation, this provides an overview of the performance of the different products when applied alone. The treatments that reduced aphid numbers were HDCI024 (as a spray and a drench), HDCI025 and HDCI026, and of these, foliar sprays of HDCI024 and HDCI025 appeared to be the most effective.

In an experiment on peach-potato aphid there was no evidence that addition of HDCI023, HDCI025 or HDCI026 improved the already good control by HDCI024. In another experiment on peach-potato aphid, addition of either HDCI023 or HDCI026 appeared to improve control by HDCI025, but these were not statistically significant differences. In a third experiment, addition of HDCI023 to HDCI025 did not improve the already good control.

In an experiment on currant-lettuce aphid, all combinations of four products were examined (Figure B). There was an indication that a combination of HDCI026 with another product improved control more than might be expected from an additive effect alone, but this effect requires confirmation.

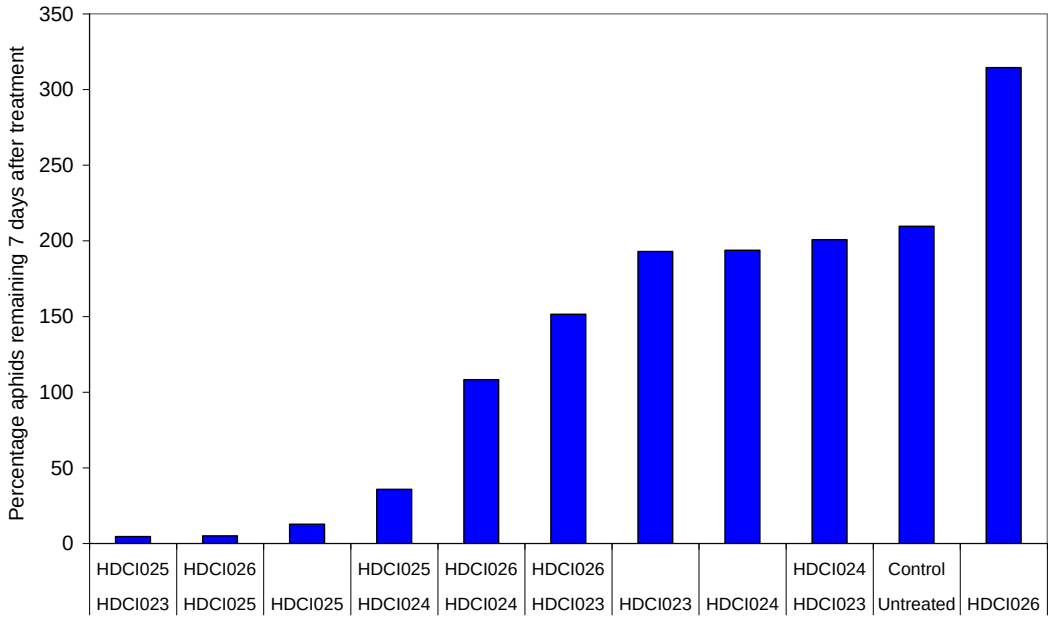

Figure B. Currant-lettuce aphid – mean percentage aphids remaining seven days after treatment. Back-transformed means.

Diamond-back moth

The results of the early experiments on diamond-back moth were hard to explain; this pest requires further experimental work to look at single treatments and interactions. In the later experiments, two of the biopesticides gave statistically significant control of diamond-back moth. The observed control from a combination of these two products was better than predicted from their use alone but this effect requires confirmation (Figure C).

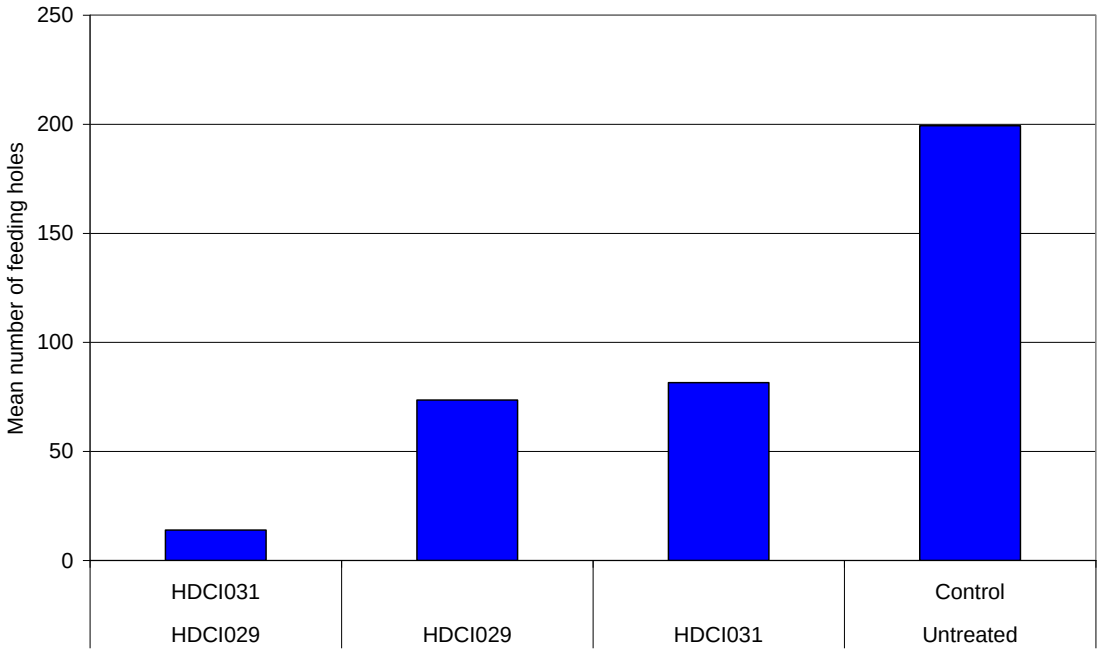

Figure C. Diamond-back moth – mean number of feeding holes per plant. Back transformed data.

General conclusions

The consistency of results was varied, being greatest in the experiments on the use of treatments applied to the compost to control cabbage root fly, and least in experiments with diamond-back moth. With the exception of the former experiments, further repeats would be desirable to increase confidence in the findings.

Time had to be spent evaluating individual treatments in preliminary experiments, which whilst not directly related to biopesticide combinations, has increased understanding of the products and provided information that is complementary to the data collected in the SCEPTRE project.

The study has shown that improved control was achieved with some simultaneous applications of two biopesticides and that this effect may be additive or, in some cases, synergistic. Experiments with reduced doses of Tracer in compost treatments were undertaken to determine whether there were possible synergistic or additive effects of insecticides and biopesticides, and this appears to be the case for this pest and method of application. Further work is needed to explore other pest x biopesticide combinations and to determine how these might be used effectively in the field, particularly in terms of methods and timings of application and the persistence of such biopesticide treatments.

Financial benefits

This project, which is complementary to the HortLINK SCEPTRE project, is relevant to pests that infest a wide range of field vegetable, protected and ornamental crops. The results have indicated which treatments and combinations of treatments may be worth exploring in more detail in future trials.

Action points for growers

- No change of practice is recommended at this stage, since further work is required before most of the coded products will be available for growers to use. However, several of the products are being evaluated in the SCEPTRE project, which may facilitate their availability.

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 was 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.

Most of the biopesticides tested are part of the SCEPTRE (Sustainable Crop and Environment Protection – Targeted Research for Edibles) project and thus they have to be presented as coded products.

Materials and methods

The project had two objectives:

1. Identify combinations of biopesticides/pesticides/behaviour modifiers that can be tested in combination
2. Determine the effect of combinations of treatments identified in 1) on control of key groups of pest insect

Objective 1. Identify combinations of biopesticides/pesticides/behaviour modifiers that can be tested in combination

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.

Objective 2. Determine the effect of combinations of treatments identified in 1) on control of key groups of pest insect

The experimental part of the project involved a number of laboratory tests using pest insect species reared in the Insect Rearing Unit at Warwick Crop Centre. Because of time limitations, the work was done on cabbage root fly, three species of pest aphid and diamond-back moth.

The test plants were grown in a greenhouse or in a constant environment room in the Insect Rearing Unit.

Cabbage root fly

Treatments to control cabbage root fly (*Delia radicum*) were applied in two ways:

- Spray treatments to the foliage of cauliflower plants
- Drench treatments to the compost in modules containing cauliflower plants

The cabbage root flies were reared in the Insect Rearing Unit at Warwick Crop Centre and the test plants, which were cauliflower cv. Skywalker, were grown in a greenhouse.

Experiments using treatments applied to plant foliage

The treatments and rates used to apply them as foliar sprays are shown in Table 2a.

Table 2a. Treatments and rates used in experiments on cabbage root fly control where treatments were applied to plant foliage.

Product code	Rate applied (ml/ha)
HDCI016	4000
HDCI017	4000
HDCI018	3000
HDCI019	4000
HDCI020	2400
HDCI021	3000

The experiments consisted mainly of ‘no-choice’ tests (Experiments 2.1.1–2.1.7) where treated potted plants (9 cm square pots) were placed in individual Perspex cages (38 x 38 x 38 cm), fixed numbers of cabbage root fly adults were released and then assessments of fly

mortality and numbers of eggs laid were made at intervals. There was one ‘choice’ test (Experiment 2.1.8) where four plants (each treated with a different substance) were placed in each of two compartments in a large rotating cage. The rotating cage was a wooden-framed test chamber with two equal sized compartments (160 x 160 x 63 cm high) arranged one above the other. Each compartment contained a 145 cm diameter turntable, which rotated once every four minutes. As the adult cabbage root fly is positively phototactic, the rotation ensured that everything placed on the turntables was exposed equally to the insects, which tended to aggregate near the fluorescent lights used to illuminate the test chamber.

The protocol for undertaking ‘no-choice’ tests to evaluate the performance of biopesticides applied as foliar sprays or drenches to cauliflower plants to assess the impact on fly mortality and egg-laying was as follows:

- Spray foliage of potted cauliflower plants with treatments using Knapsack sprayer, with exception of untreated control plants.
- Cover surface of compost with a layer of silver sand.
- Place each plant in a separate cage.
- Introduce ten female and ten male (6–8 days old) cabbage root flies.
- Assess fly mortality at intervals.
- Count numbers of eggs laid after a specified number of days.

To extract and count the eggs, the plants were removed from the cages one by one and the silver sand covering the potting compost was rinsed into a container using a wash bottle containing tap water. More water was added to the container plus ‘anti-foam’ (Foam-fighter — Sangosse Limited). The water was stirred and the cabbage root fly eggs floated to the surface, where they were counted. If the experiment was continuing, the surface of the soil surrounding each plant was covered with fresh silver sand and the plants were replaced in the appropriate cages.

For the ‘choice’ test in the rotating cage, treated plants were obtained as described above. Each turntable had space for four custom-made trays. Each of these was segment-shaped and covered one quarter of the turntable. For these experiments the trays were filled to the top with sieved soil and a single empty plant pot (9 x 9 cm) was inserted into the centre of each tray to hold a pot containing a cauliflower plant. Fixed numbers of cabbage root fly

adults were released into the cage and then the numbers of eggs laid on each plant were assessed.

Experiments using treatments applied to module compost

In these experiments (2.1.9–2.1.11), the biopesticides were applied to the compost in which the test plants were grown to determine effects on the survival of cabbage root fly larvae and the consequent effects on the plant roots. Plants (cauliflower cv. Skywalker) were generally 6–8 weeks old, but in Experiment 2.1.11, older plants were used. Some of the biopesticide treatments were applied with a reduced dose of Tracer (spinosad) to look for evidence of additive or synergistic effects between reduced doses of insecticide and biopesticides.

Experiment 2.1.9 used potted cauliflower plants (cv. Skywalker) grown in 9 cm square pots and with 7–9 leaves. There were five replicates of each treatment in each test and there were three tests in total. The plants were treated with the biopesticides as shown in Table 2b. Tracer (spinosad) was applied at 10% of the standard dose. Twenty newly laid cabbage root fly eggs were inoculated onto each plant and the plants were placed in a greenhouse. Then after approximately four weeks, the plants were destructively sampled, the roots washed and weighed and the larvae and pupae were removed from the compost by flotation and counted.

Table 2b. Treatments applied to control cabbage root fly larvae from eggs inoculated onto cauliflower plants (Experiment 2.1.9).

Treatment	Application method	Dose/plant
HDCI019	Drench	0.163 ml
HDCI021	Drench	0.12 ml
Tracer[1] + HDCI019	Drench	0.0012 ml + 0.163 ml
Tracer[1] + HDCI021	Drench	0.0012 ml + 0.12 ml
Tracer[1]	Drench	0.0012 ml
Untreated control		

[1] 10% of commercial rate (12 ml/1000 plants)

Further experiments were undertaken using the same approach (Experiments 2.1.10 and 2.1.11) and in this case an even lower dose of Tracer was applied (Table 2c).

Table 2c. Treatments applied to control cabbage root fly larvae from eggs inoculated onto cauliflower plants (Experiments 2.1.10 and 2.1.11).

Treatment	Application method	Dose/plant
HDCI019	Drench	0.163 ml
HDCI021	Drench	0.12 ml
HDCI049[3]	Soil surface	0.5 g
Tracer[2] + HDCI019	Drench	0.0006 ml + 0.163 ml
Tracer[2] + HDCI021	Drench	0.0006 ml + 0.12 ml
Tracer[2] + HDCI049[3]	Drench + Soil surface	0.0006 ml + 0.5 g
Tracer	Drench	0.0012 ml
Untreated control		

[2] 5% of commercial rate (12 ml/1000 plants)

[3] Based on commercial rate of 0.5 kg/m^3 and assuming volume of soil in pot = 1litre.

Aphids

The aphids tested (cabbage aphid [*Brevicoryne brassicae*], peach-potato aphid [*Myzus* persicae] and currant-lettuce aphid [*Nasonovia ribisnigri*]) were all reared in the Insect Rearing Unit at Warwick Crop Centre. The test plants were grown in pots in a greenhouse or in the Insect Rearing Unit and were either cauliflower (cv. Skywalker) or lettuce (cv. Saladin). The plants were generally 4–6 weeks old when infested with aphids. The general protocol for testing aphids was as follows:

- Infest cauliflower or lettuce plants with aphids
- If infestation not uniform, count aphids on plants prior to treatment
- Spray plants with treatments using Knapsack sprayer, with exception of untreated control plants
- Place each plant in a cage (38 x 38 x 38 cm)
- Count aphids remaining after a specified number of days

Treatments were applied at the rates shown in Table 2d.

Table 2d. Treatments and rates used in experiments on aphid control where treatments were applied to plant foliage.

Product code	Rate applied (ml/ha)
HDCI022	4000
HDCI023	4000
HDCI024	3000
HDCI025	2400
HDCI026	4000
HDCI027	3000

Diamond-back moth

The diamond-back moths (*Plutella xylostella*) tested were reared in the Insect Rearing Unit at Warwick Crop Centre. The test plants were grown in pots in a greenhouse and were cauliflower (cv. Skywalker). The plants were generally 4–6 weeks old when used in experiments.

The general protocol for testing diamond-back moth was as follows:

- Spray potted test plants with treatments using Knapsack sprayer, with exception of untreated control plants
- Place each plant in a cage
- Release fixed numbers of young moths
- As appropriate, count dead moths, eggs and/or larval feeding holes after defined periods

Treatments were applied at the rates shown in Table 2e.

Table 2e. Treatments and rates used in experiments on control of diamond-back moth where treatments were applied to plant foliage.

Product code	Rate applied (ml/ha)
HDCI032	4000
HDCI030	4000
HDCI033	3000
HDCI028	4000
HDCI029	2400ml/ha
HDCI031	3000

Analysis

The data were summarised in Excel and subjected to Analysis of Variance (ANOVA). Some data sets were transformed prior to analysis to normalise the variance.

Results

Objective 1. Identify combinations of biopesticides/pesticides/behaviour modifiers that can be tested in combination

1.1 Biopesticides and other materials available of 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 1.1. Further details about some of the products available are given in the Appendix.

Table 1.1. 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*	Vine weevil (UK — Met 52)

	anisopliae	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 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, as in, for example, the 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).

1.2 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, 1% 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 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 and 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 24 hours before the fungus, but not when the insecticide was applied 24 hours 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 anti-feedant leading to increased acquisition of fungal spores. The apparent anti-feedant 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 mechanised 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.

Objective 2. Determine the effect of combinations of treatments identified in 1) on control of key groups of pest insect.

Experiment 2.1.1 'No-choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment, following treatment of the plants and the release of the flies, eggs were counted after two days and seven days. The numbers of dead flies were recorded. Fly mortality was very low and was not analysed. The egg count data were square root-transformed prior to analysis. Only the HDCI020 treatment reduced egg-laying compared with the untreated control (total number of eggs); none of the other treatments were significantly different from the control (Table 2.1.1 and Figure 2.1.1).

Table 2.1.1. Cabbage root fly – no choice test – mean number of eggs per cage after two days, from 2–7 days and over the whole period (0–7 days). A square root transformation was used prior to analysis. Treatments that were significantly different from the control are in bold and underlined.

	Eggs 0–2 days		**Eggs 2–7 days**		**Total number eggs (0–7 days)**	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI020	4.15	17.20	2.82	7.95	**5.06**	25.64
Untreated control	6.00	36.04	6.41	41.11	8.86	78.41
HDCI019	6.01	36.06	6.75	45.55	9.22	85.06
HDCI018	7.38	54.41	6.49	42.10	10.20	103.94
HDCI016	8.27	68.41	5.87	34.40	10.21	104.32
HDCI017	7.51	56.35	7.07	49.93	10.49	110.09
F	3.14		1.60		3.38	
df	18		18		18	
p	0.03		0.21		0.03	
LSD	2.48		3.67		3.29	

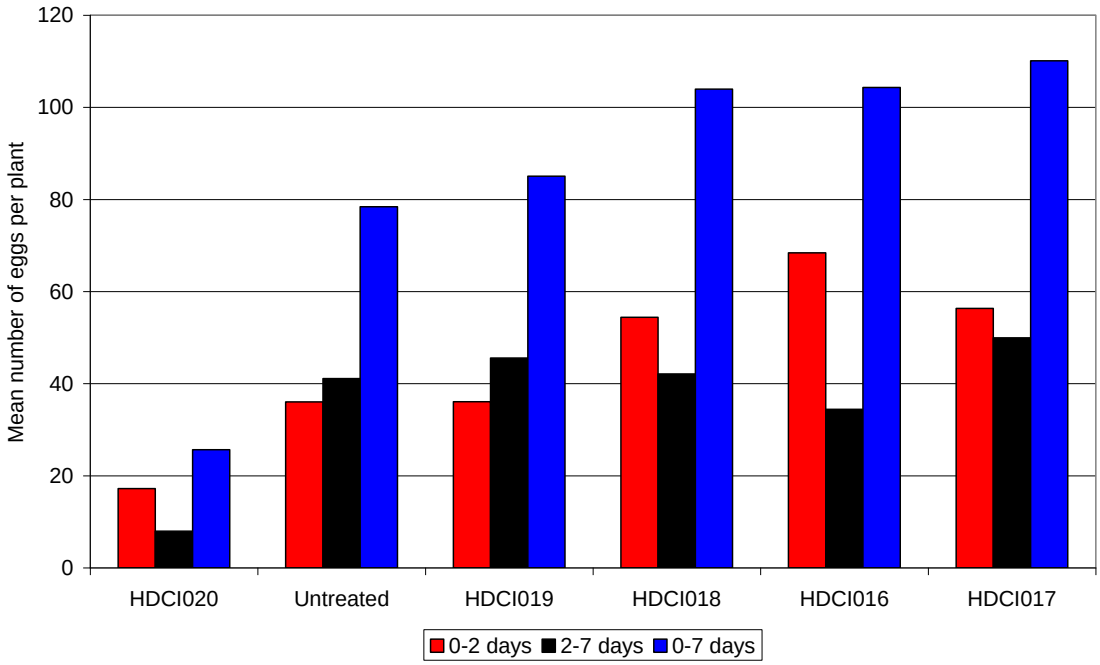

Figure 2.1.1. Mean number of eggs per cage after two days, from 2–7 days and over the whole period (0–7 days). Back-transformed data.

Experiment 2.1.2 'No-choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment, following treatment of the plants and the release of the flies, eggs were counted after two days and seven days. The numbers of dead flies were recorded. The dead fly and egg count data were square root-transformed prior to analysis.

Table 2.1.2a and Figure 2.1.2a show the mean number of dead flies per cage after two days, from 2–7 days and over the whole period (0–7 days). The analyses showed a statistically significant difference in the numbers of dead flies between days 0–2, but there was little difference between treatments subsequently. Between days 0–2, all treatments using HDCI020 killed more flies compared with the untreated control. The effect was not so apparent between days 2–7.

Table 2.1.2a. Mean number of dead flies per cage after two days, from 2–7 days and over the whole period (0–7 days). A square root transformation was used prior to analysis. Treatments that were significantly different from the control are in bold and underlined.

	Dead flies 0–2 days		**Dead flies 2–7 days**		**Total number dead flies**	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
Untreated control	0.17	0.03	0.90	0.81	0.97	0.94
HDCI021	0.86	0.74	0.57	0.32	1.43	2.04
HDCI020 + HDCI019	**1.21**	1.46	0.67	0.44	1.44	2.06
HDCI021 drench	0.83	0.69	1.00	1.00	1.54	2.37
HDCI020	**0.93**	0.86	1.28	1.63	1.71	2.92
HDCI020 + HDCI016	**1.33**	1.76	1.11	1.23	**1.93**	3.72
F	2.74		0.60		1.37	
df	30		30		30	
p	0.04		0.70		0.26	
LSD	0.71		0.99		0.80	

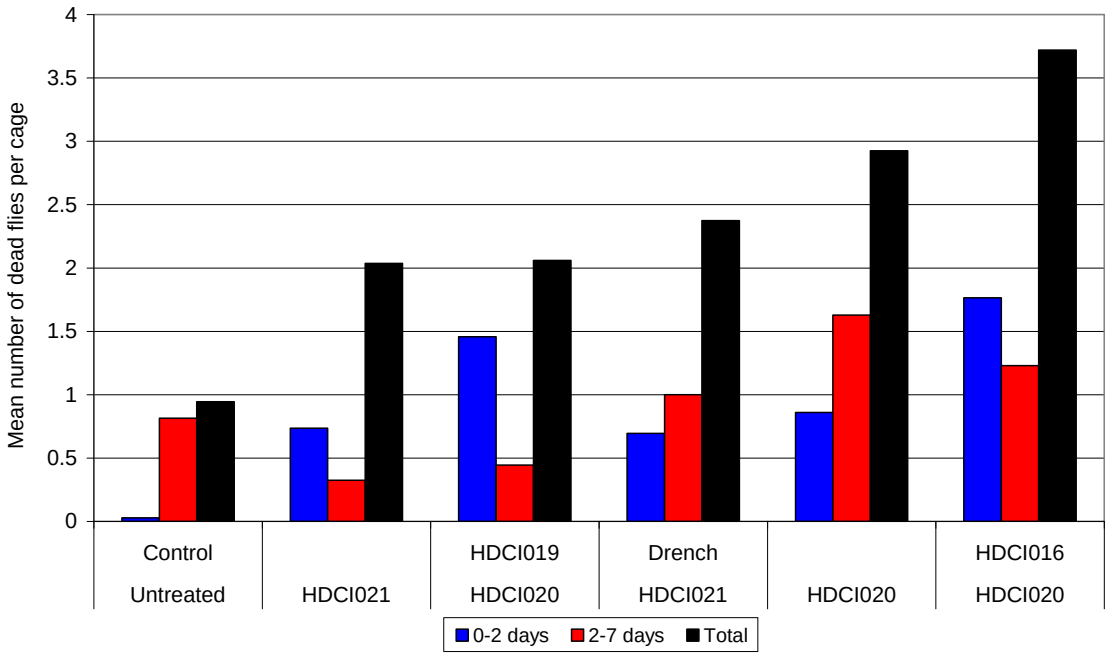

Figure 2.1.2a. Mean number of dead flies per cage after two days, from 2–7 days and over the whole period (0–7 days). Back-transformed data.

Table 2.1.2b shows the mean number of eggs per plant after two days, from 2–7 days and over the whole period (0–7 days). The analyses show a statistically significant difference in the numbers of eggs laid between days 2–7 and in the total number of eggs. Surprisingly, the treatments HDCI021 (applied as a drench) and HDCI020 + HDCI019 increased the total numbers of eggs laid compared with the untreated control.

Table 2.1.2b. Mean number of eggs per plant after two days, from 2–7 days and over the whole period (0–7 days). A square root transformation was used prior to analysis. Treatments that were significantly different from the control are in bold and underlined.

	Eggs 0–2 days		**Eggs 2–7 days**		**Total number of eggs**	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI021	3.07	9.43	1.57	2.48	3.99	15.89
Untreated control	3.89	15.10	2.77	7.65	4.81	23.14
HDCI020	4.30	18.47	2.54	6.43	5.22	27.23
HDCI020 + HDCI016	3.23	10.44	4.34	18.80	5.52	30.45
HDCI020 + HDCI019	5.52	30.51	**4.89**	23.96	7.44	55.30
HDCI021 drench	5.86	34.39	**5.63**	31.75	**8.16**	66.66
F	2.08		4.83		3.05	
df	30		30		30	
p	0.095		0.002		0.024	
LSD	2.33		2.06		2.66	

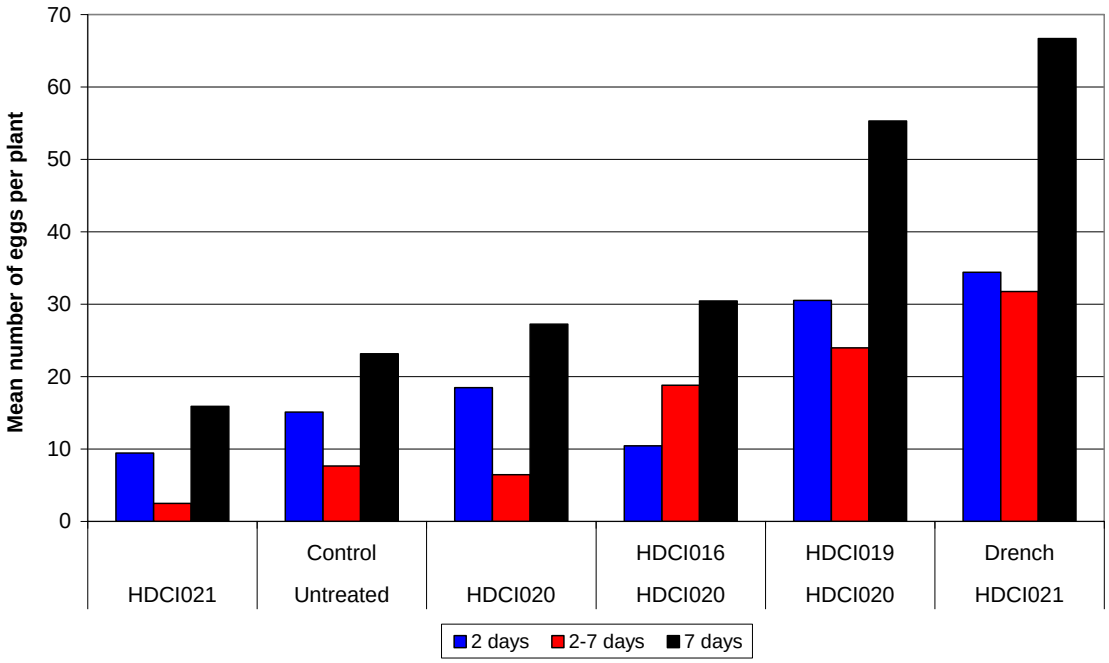

Figure 2.1.2b. Mean number of eggs per plant after two days, from 2-7 days and over the whole period (0-7 days). Back-transformed data.

Experiment 2.1.3 'No-choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment, following treatment of the plants and the release of the flies, eggs were counted after two days and six days. The numbers of dead flies were recorded. The dead fly and egg count data were square root-transformed prior to analysis.

There were no statistically significant differences in fly mortality ($p>0.05$) (Table 2.1.3a and Figure 2.1.3a). Overall, there were no statistically significant differences between treatments. However, when comparing means using the LSDs, the combined biopesticide treatment (HDCI020 + HDCI021) reduced the number of eggs laid between days 0–2 and the total numbers of eggs laid compared with the untreated control (Table 2.1.3b and Figure 2.1.3b). The difference between treatments was greatest within the first two days, when the treatments were fresh.

Table 2.1.3a. Cabbage root fly – no choice test – numbers of dead flies (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Dead flies days 0–2		Total dead flies	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
Untreated	0.35	0.13	2.17	4.72
HDCI021	0	0	2.58	6.64
HDCI020	0.43	0.19	2.80	7.83
HDCI020 + HDCI021	1.10	1.22	2.85	8.11
F	2.63		0.52	
df	12		12	
p	0.10		0.68	
LSD	0.88		1.32	

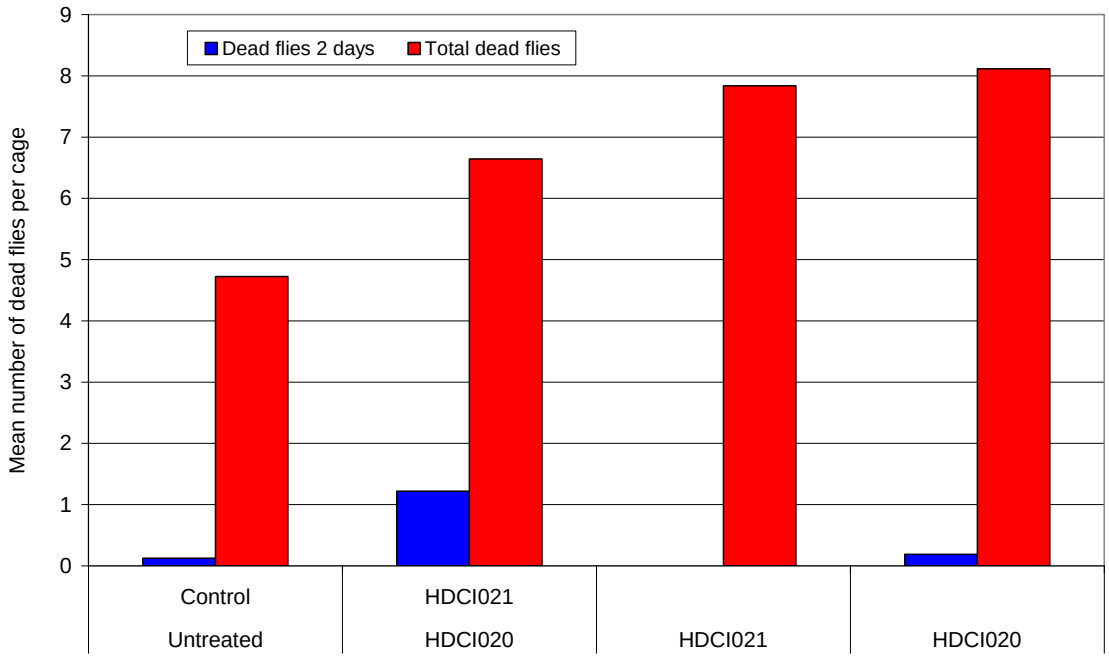

Figure 2.1.3a. Cabbage root fly – no choice test – mean number of dead flies per cage after two days and the total number of dead flies. Back-transformed data.

Table 2.1.3b. Cabbage root fly – no choice test – numbers of eggs laid (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Eggs days 0–2		Eggs days 2–6		Total eggs	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI020 + HDCI021	**4.12**	16.99	6.16	37.90	**7.46**	55.63
HDCI020	7.57	57.31	6.16	38.00	9.95	98.91
HDCI021	8.41	70.81	4.81	23.15	10.10	101.92
Untreated	9.89	97.74	7.79	60.71	12.75	162.46
F	3.24		0.95		2.28	
df	12		12		12	
p	0.06		0.45		0.13	
LSD	4.19		3.85		4.41	

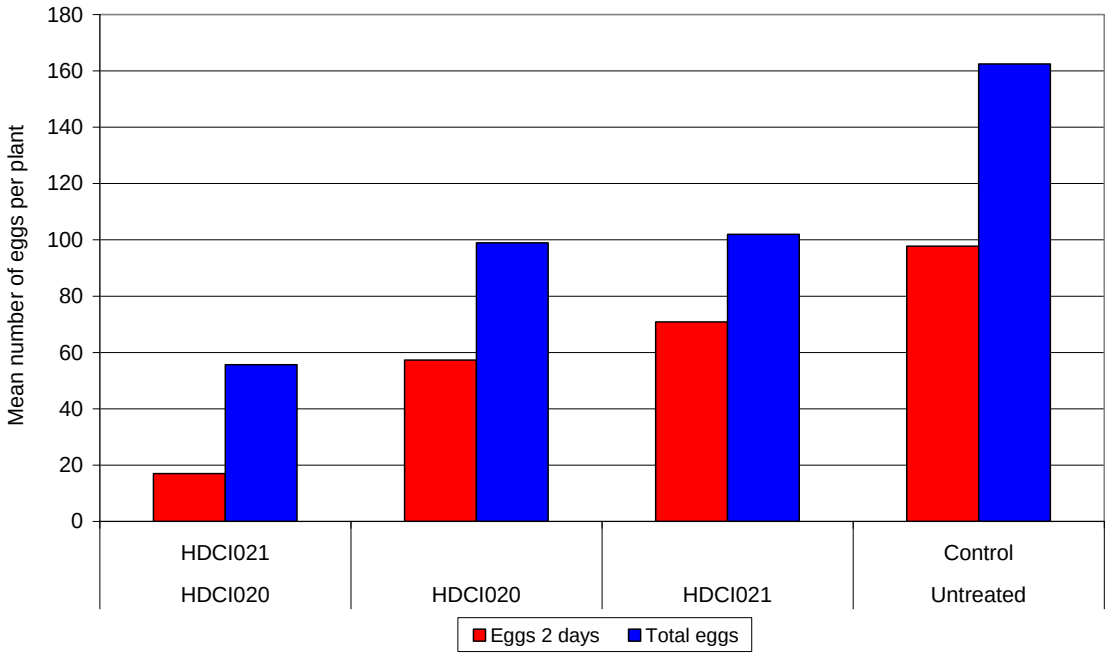

Figure 2.1.3b. Cabbage root fly – no choice test – mean number of eggs per plant. Back-transformed data.

Experiment 2.1.4 'No-choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment, following treatment of the plants and the release of the flies, eggs were counted after one day and six days. The numbers of dead flies were recorded. The dead fly and egg count data were square root-transformed prior to analysis.

There were statistically significant differences in fly mortality (Table 2.1.4a and Figure 2.1.4a). In particular, HDCI020 and HDCI020 + HDCI016 increased fly mortality (0–1 days and total respectively) compared with the untreated control. There were no statistically significant differences in the numbers of eggs laid ($p>0.05$) (Table 2.1.4b and Figure 2.1.4b).

Table 2.1.4a. Cabbage root fly – no choice test – numbers of dead flies (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Dead flies days 0–1		Total dead flies	
Treatment	Square root transform	Back transform	Square root transform	Back transform
HDCI016	0.50	0.25	1.46	2.12
Untreated	0.00	0.00	1.64	2.69
HDCI020	**1.04**	1.07	1.78	3.16
HDCI020 + HDCI016	0.85	0.73	**2.45**	5.99
F	2.64		4.18	
df	12		12	
p	0.10		0.03	
LSD	0.87		0.65	

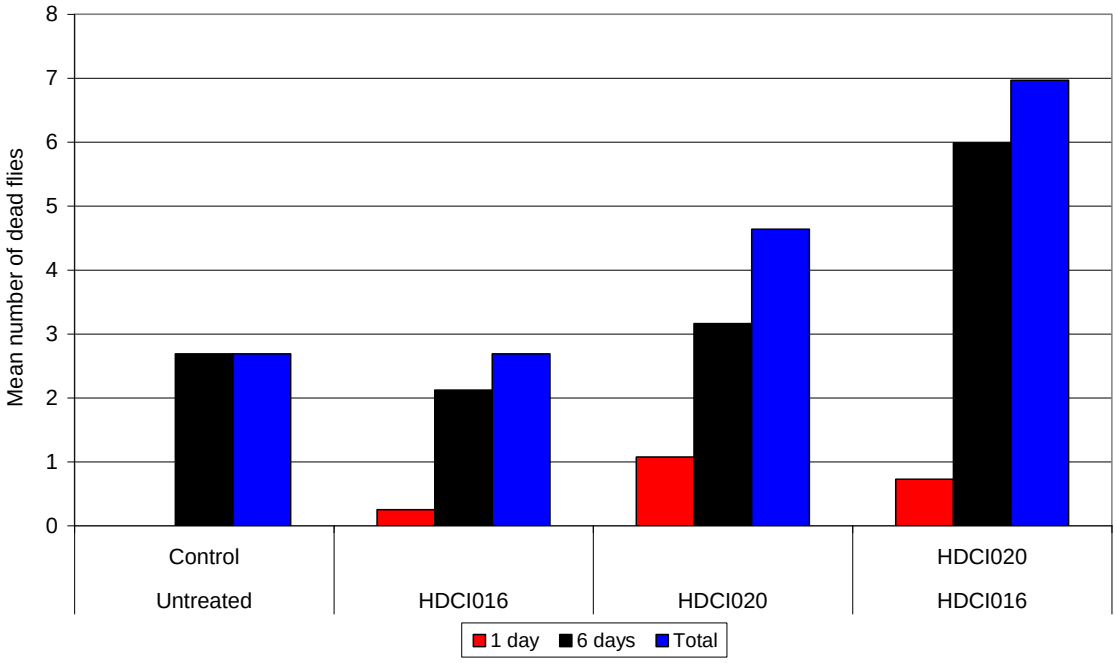

Figure 2.1.4a. Cabbage root fly – no choice test – mean number of dead flies per cage after one day, 1–6 days and the total number of dead flies. Back-transformed data.

Table 2.1.4b. Cabbage root fly – no choice test – numbers of eggs laid (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Eggs days 0–1		Eggs days 1–6		Total eggs	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI020	3.12	9.73	4.59	21.07	5.63	31.64
HDCI020 + HDCI016	2.68	7.19	5.20	27.08	5.99	35.86
Untreated	5.80	33.59	5.08	25.76	7.72	59.65
HDCI016	5.71	32.60	6.33	40.03	9.23	85.11
F	1.39		0.27		1.03	
df	12		12		12	
p	0.29		0.84		0.41	
LSD	4.33		4.34		5.05	

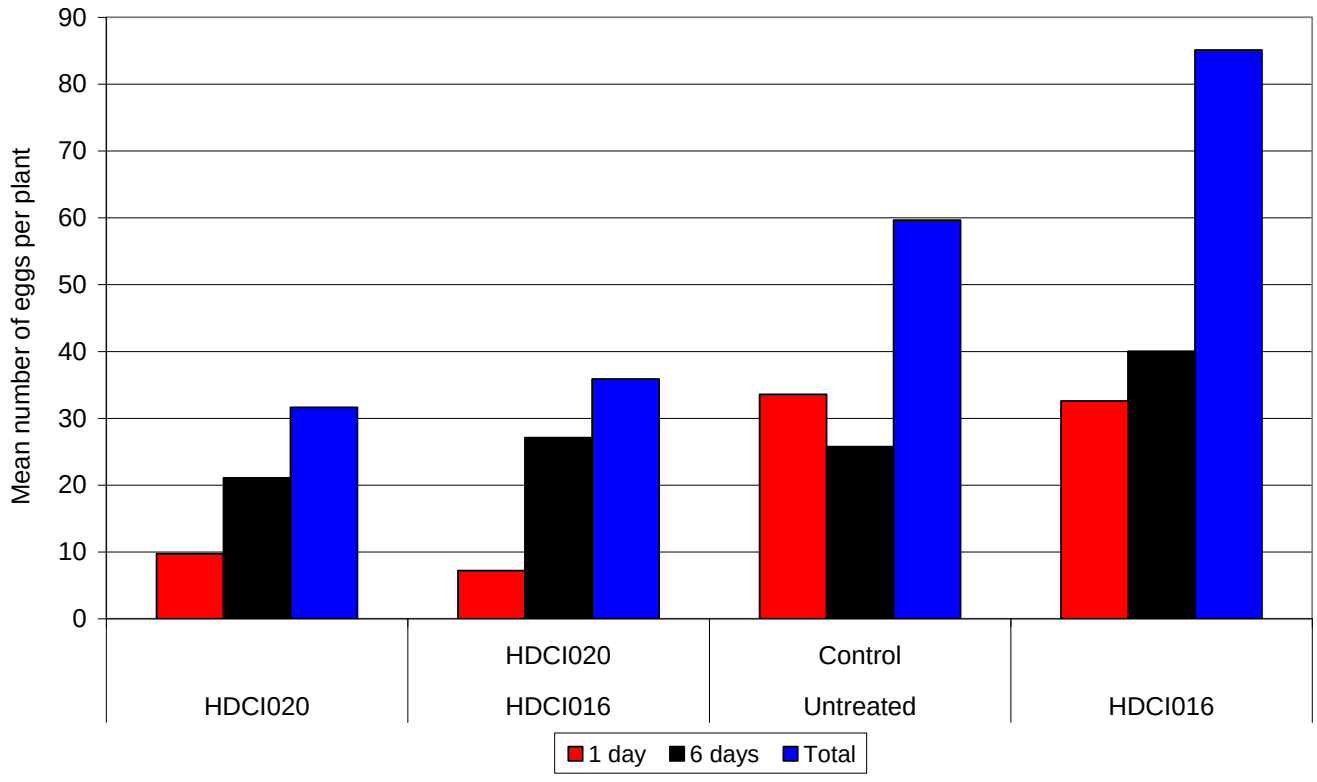

Figure 2.1.4b. Cabbage root fly – no choice test – mean number of eggs per plant. Back-transformed data.

Experiment 2.1.5 'No-choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment, following treatment of the plants and the release of the flies, eggs were counted after one day and six days. The numbers of dead flies were recorded. The dead fly and egg count data were square root-transformed prior to analysis.

There were statistically significant differences in fly mortality (Table 2.1.5a and Figure 2.1.5a). In particular, HDCI021 + HDCI016 increased total fly mortality compared with the untreated control. There were no statistically significant differences in the numbers of eggs laid ($p>0.05$) (Table 2.1.5b and Figure 2.1.5b).

Table 2.1.5a. Cabbage root fly – no choice test – numbers of dead flies (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Dead flies days 0–1		Total dead flies	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI016	0.50	0.25	1.46	2.12
HDCI021	0.00	0.00	1.52	2.30
Untreated	0.00	0.00	1.64	2.69
HDCI021 + HDCI016	0.50	0.25	**2.41**	5.79
F	n/a		3.90	
df			12	
p			0.04	
LSD			0.69	

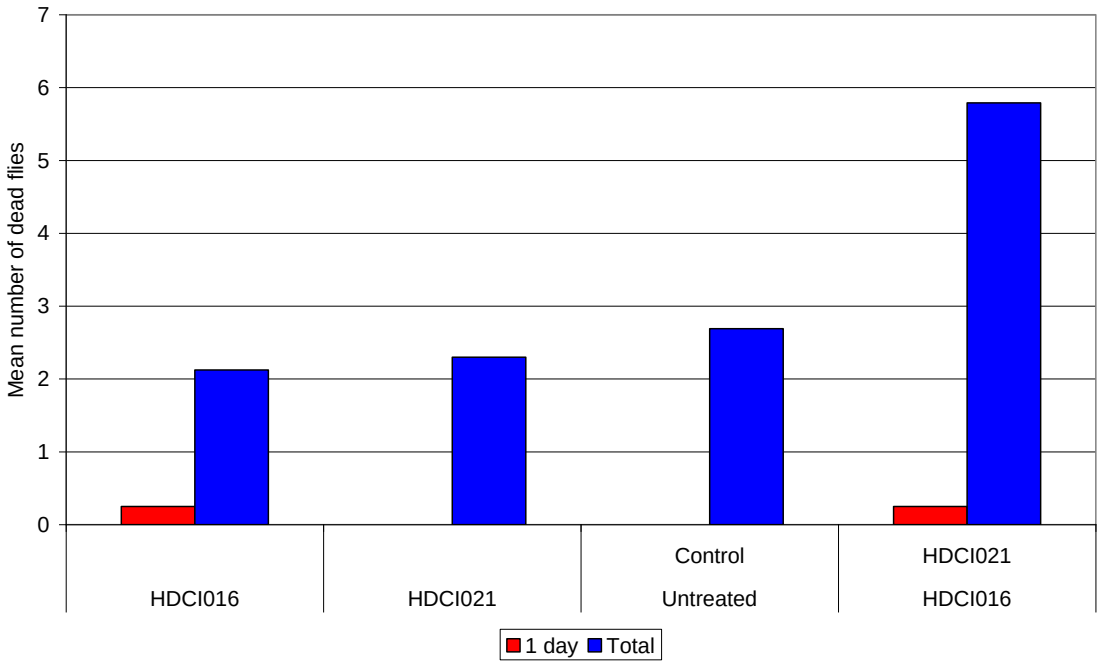

Figure 2.1.5a. Cabbage root fly – no choice test – mean number of dead flies per cage after one day and the total number of dead flies. Back-transformed data.

Table 2.1.5b. Cabbage root fly – no choice test – numbers of eggs laid (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Eggs days 0–1		Eggs days 1–6		Total eggs	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI021	5.05	25.51	4.91	24.11	7.58	57.47
Untreated	5.80	33.59	5.08	25.76	7.72	59.65
HDCI016 + HDCI021	6.30	39.67	4.47	20.01	7.79	60.67
HDCI016	5.71	32.60	6.33	40.03	9.23	85.11
F	0.10		0.31		0.20	
df	12		12		12	
p	0.96		0.82		0.89	
LSD	5.00		4.43		5.25	

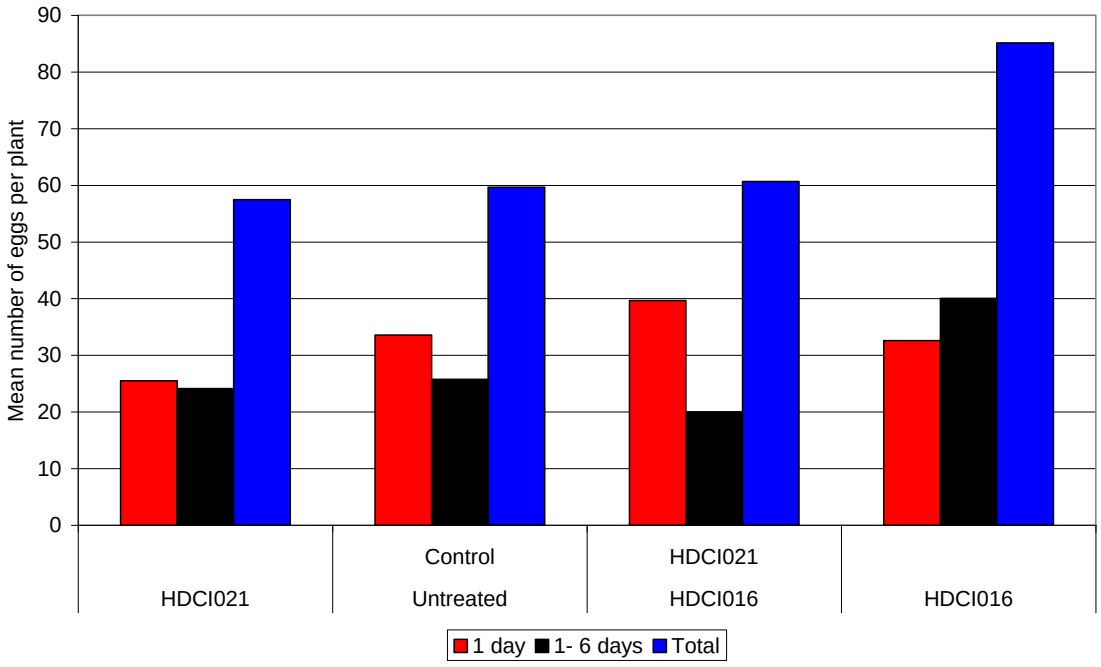

Figure 2.1.5b. Cabbage root fly – no choice test – mean number of eggs per plant. Back-transformed data.

Experiment 2.1.6 'No-choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment, following treatment of the plants and the release of the flies, eggs were counted after one day and six days. The numbers of dead flies were recorded. The dead fly and egg count data were square root-transformed prior to analysis.

There were no statistically significant differences in fly mortality (Table 2.1.6a and Figure 2.1.6a) or in the numbers of eggs laid ($p>0.05$) (Table 2.1.6b and Figure 2.1.6b).

Table 2.1.6a. Cabbage root fly – no choice test – numbers of dead flies (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Dead flies days 0–1		**Total dead flies**	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI019	0.25	0.06	2.12	4.48
HDCI021 + HDCI019	0.00	0.00	2.34	5.48
Untreated	0.50	0.25	2.47	6.12
HDCI021	0.00	0.00	2.61	6.80
F			0.28	
df			12	
p			0.84	
LSD			1.22	

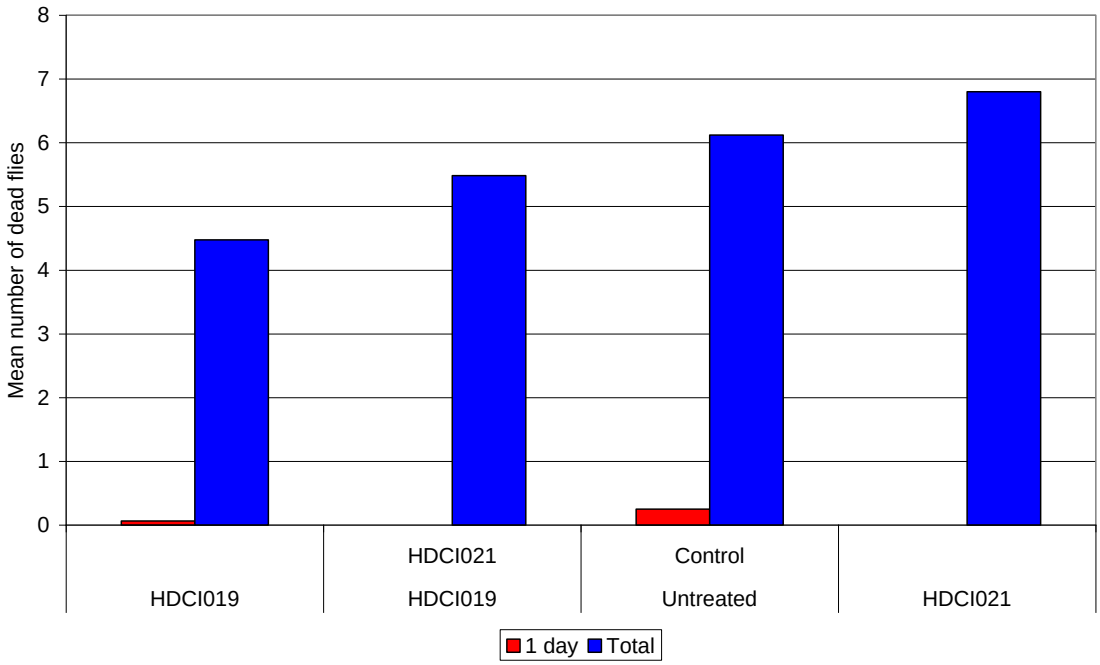

Figure 2.1.6a. Cabbage root fly – no choice test – mean number of dead flies per cage after one day and the total number of dead flies. Back-transformed data.

Table 2.1.6b. Cabbage root fly – no choice test – numbers of eggs laid (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Eggs days 0–1		**Eggs days 1–6**		**Total eggs**	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI021 + HDCI019	4.80	23.08	4.21	17.71	6.46	41.72
HDCI019	5.27	27.79	5.61	31.45	7.74	59.89
HDCI021	5.65	31.87	6.31	39.76	8.61	74.13
Untreated	7.37	54.27	5.52	30.46	9.34	87.19
F	1.25		0.77		1.06	
df	12		12		12	
p	0.34		0.53		0.40	
LSD	3.08		3.06		3.70	

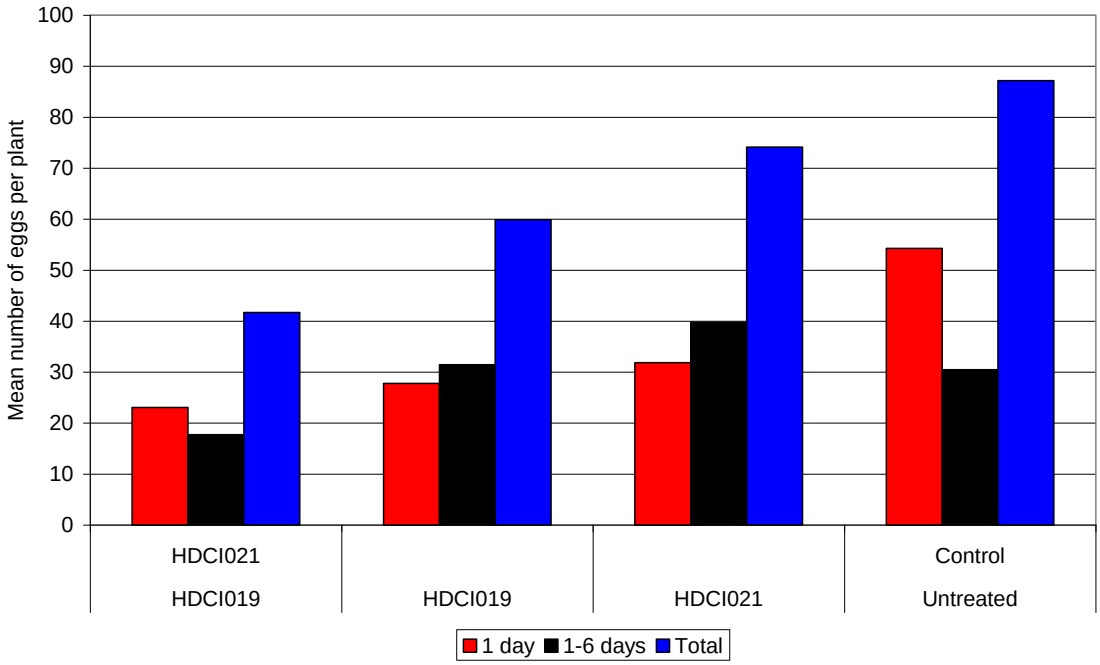

Figure 2.1.6b. Cabbage root fly – no choice test – mean number of eggs per plant. Back-transformed data.

Experiment 2.1.7 'No-choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment, following treatment of the plants and the release of the flies, eggs were counted after one day and six days. The numbers of dead flies were recorded. The dead fly and egg count data were square root-transformed prior to analysis.

There were no statistically significant effects on early fly mortality (Table 2.1.7a and Figure 2.1.7a). There was a statistically significant difference in the numbers of eggs laid in the first day. The HDCI020 treatment decreased early egg numbers compared with the untreated control (Table 2.1.7b and Figure 2.1.7b).

Table 2.1.7a. Cabbage root fly – no choice test – numbers of dead flies (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Dead flies days 0–1		Total dead flies	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI019	0.25	0.06	2.12	4.48
HDCI020	0.75	0.56	2.30	5.29
Untreated	0.50	0.25	2.47	6.12
HDCI020 + HDCI019	0.50	0.25	3.02	9.15
F	0.57		0.57	
df	12		12	
p	0.83		0.64	
LSD	1.25		1.60	

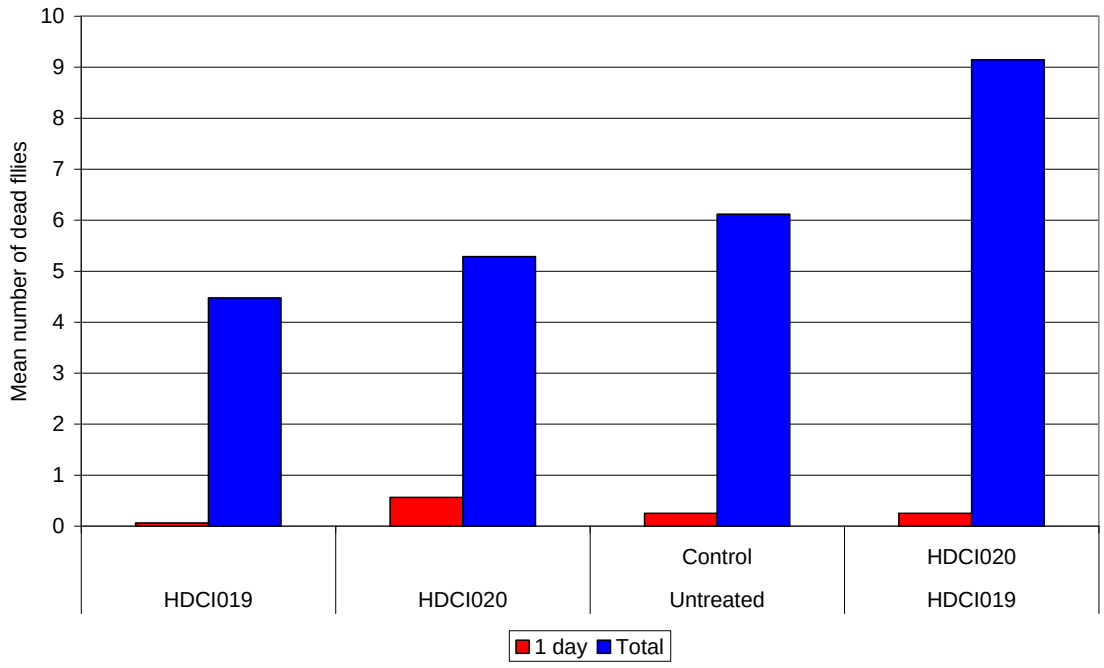

Figure 2.1.7a. Cabbage root fly – no choice test – mean number of dead flies per cage after one day and the total number of dead flies. Back-transformed data.

Table 2.1.7b. Cabbage root fly – no choice test – numbers of eggs laid (data square root-transformed prior to analysis). Treatments that were significantly different from the control are in bold and underlined.

	Eggs days 0–1		Eggs days 1–6		Total eggs	
Treatment	Square root transform	Back transform	Square root transform	Back transform	Square root transform	Back transform
HDCI020	**<u>3.09</u>**	9.54	5.83	33.98	6.67	44.53
HDCI019	5.27	27.79	5.61	31.45	7.74	59.89
HDCI020 + HDCI019	4.91	24.13	6.09	37.05	8.06	65.00
Untreated	7.37	54.27	5.52	30.46	9.34	87.19
F	4.09		0.07		1.27	
df	12		12		12	
p	0.03		0.97		0.33	
LSD	2.67		2.86		3.00	

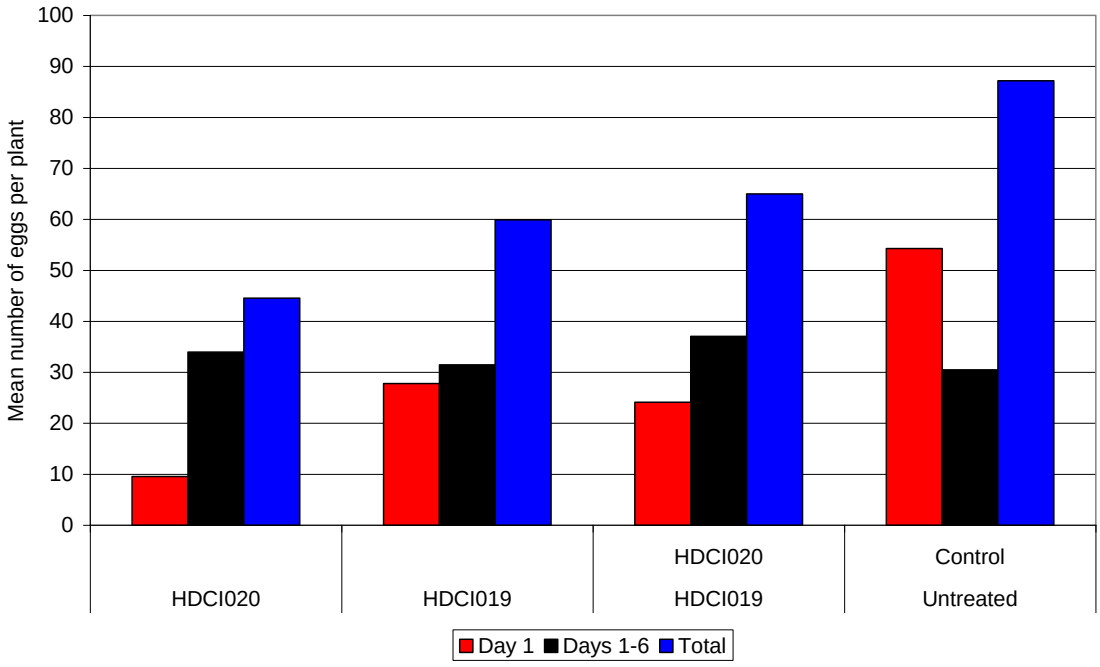

Figure 2.1.7b. Cabbage root fly – no choice test – mean number of eggs per plant. Back-transformed data.

Experiment 2.1.8 'Choice' test to evaluate the performance of biopesticides applied as foliar sprays to cauliflower plants

In this experiment the potted plants were treated with the biopesticides and then the pots were placed in a rotating cage, which contained one plant of each of the four different treatments. A total of three male and 20 female flies were added to each cage. The female flies were 5–6 days old and ready to lay eggs. The flies were left for 2–3 days and then the plants were removed and the eggs were counted. New sand was placed around each plant and the plants were then put back in the rotating cage and left for a further five days when the plants were removed and the eggs were counted.

There were no statistically significant differences between treatments ($p>0.05$) (Table 2.1.8). The results are summarised in Figures 2.1.8a and 2.1.8b.

Table 2.1.8a. Cabbage root fly – choice test – mean number of eggs per plant. Data square root-transformed.

	Mean number of eggs per plant			
	After 2–3 days		After 7–8 days	
Treatment	Square root transform	Back transform	Square root transform	Back transform
HDCI020	5.25	27.60	6.87	47.19
Untreated	7.58	57.53	7.75	60.04
HDCI019	7.27	52.87	7.89	62.18
HDCI021	8.49	72.08	9.05	81.84
F	2.05		1.34	
df	12		20	
p	0.16		0.29	
LSD	2.94		2.91	

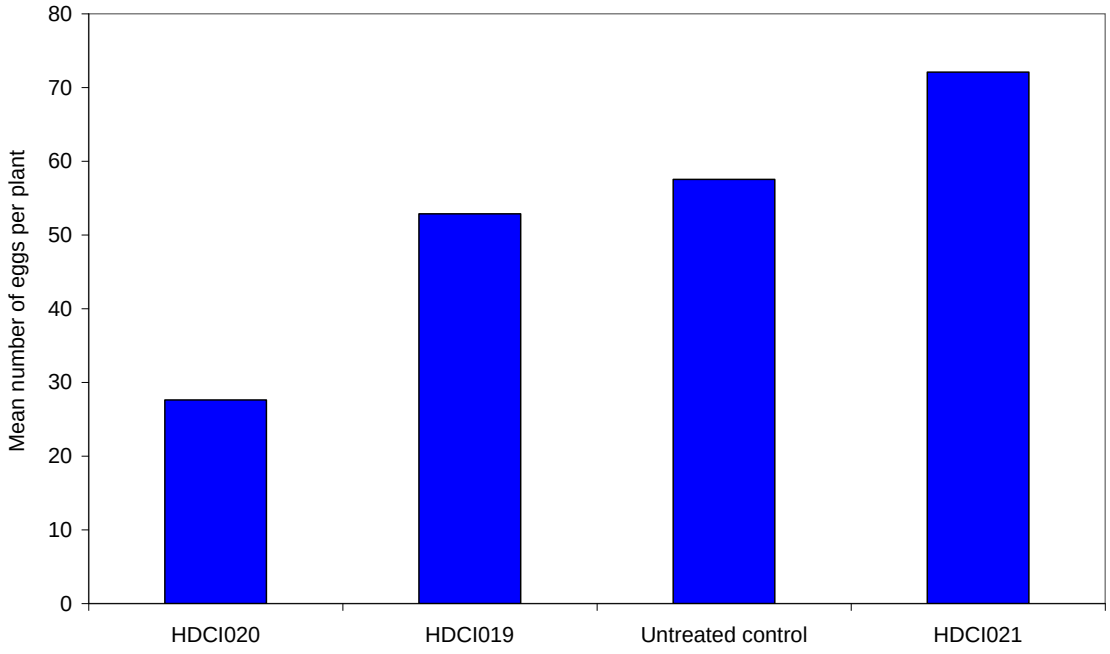

Figure 2.1.8a. Cabbage root fly – choice test – mean number of eggs per plant after 2–3 days. Back-transformed data.

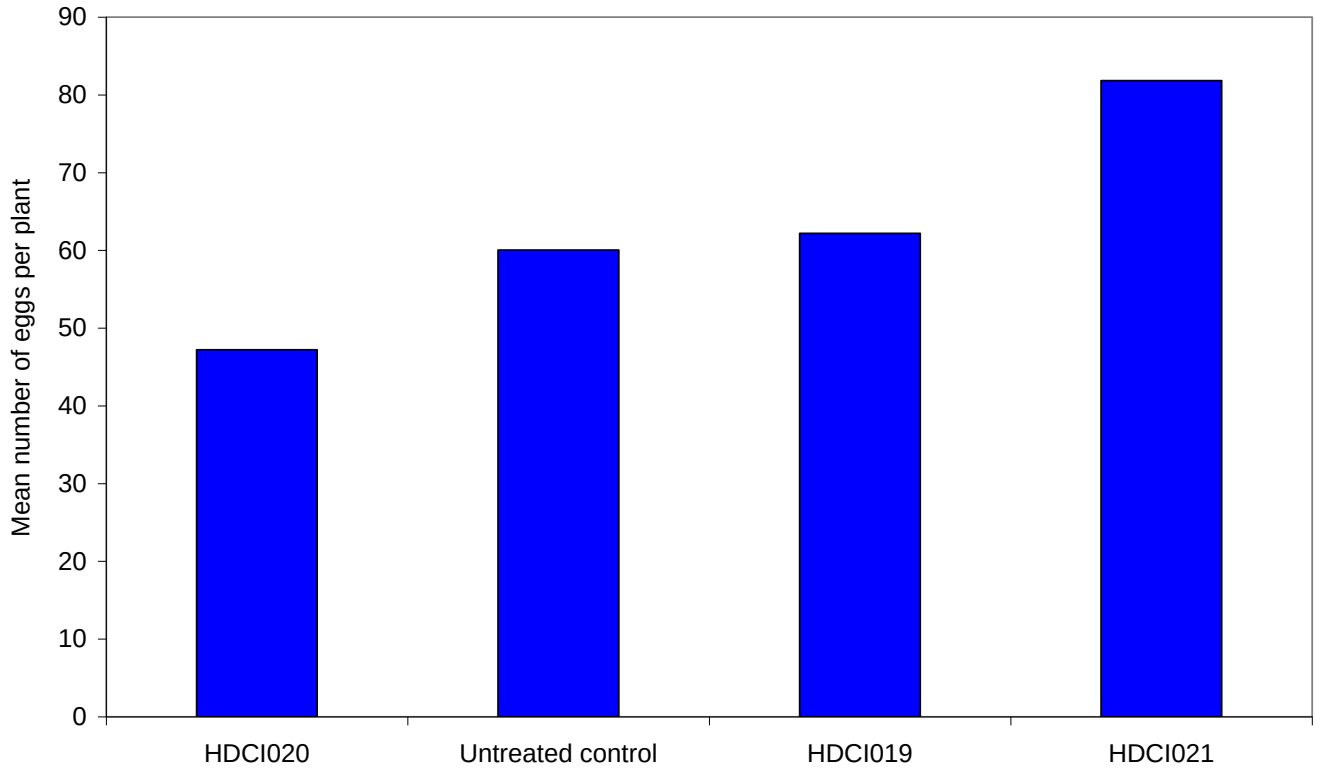

Figure 2.1.8b. Cabbage root fly – choice test – mean number of eggs per plant after 7–8 days. Back-transformed data.

Effect of biopesticides applied to the module compost

In these experiments, the biopesticides were applied to the compost in which the test plants were grown to determine effects on the survival of cabbage root fly larvae and on the plant. Plants (cauliflower cv. Skywalker) were generally 6–8 weeks old, but in Experiment 2.1.11, older plants were used.

Experiment 2.1.9 Effect of biopesticides applied to the module compost

All of the plants treated with biopesticides were heavier than those from the untreated control pots (Table 2.1.9b and Figure 2.1.9a) and with the exception of Tracer alone (at 10% approved dose); this was a statistically significant difference. There were no statistically significant differences between the biopesticide treatments. All of the biopesticide treatments (including Tracer at 10% of recommended dose) resulted in very low numbers of larvae and pupae (<1 per plant) and all of the treatments reduced the numbers of larvae and pupae compared with the untreated control (Table 2.1.9b and Figure 2.1.9b).

Table 2.1.9b. Cabbage root fly – biopesticides applied to module compost – summary of data analysis using ANOVA. Treatments that were significantly different from the control are in bold and underlined. Insect count data were square root transformed prior to ANOVA.

		Number of larvae and pupae per plant	
Treatments	**Plant weight (g)**	**Square root transform**	**Back transform**
Tracer + HDCI019	**9.94**	**0.23**	0.05
Tracer + HDCI021	**8.33**	**0.38**	0.14
Tracer	7.42	**0.45**	0.21
HDCI019	**9.20**	**0.66**	0.43
HDCI021	**8.41**	**0.73**	0.53
Untreated	3.93	2.82	7.96
F	8.22	23.14	
df	84	84	
p	<0.001	<0.001	
LSD	4.01	1.10	

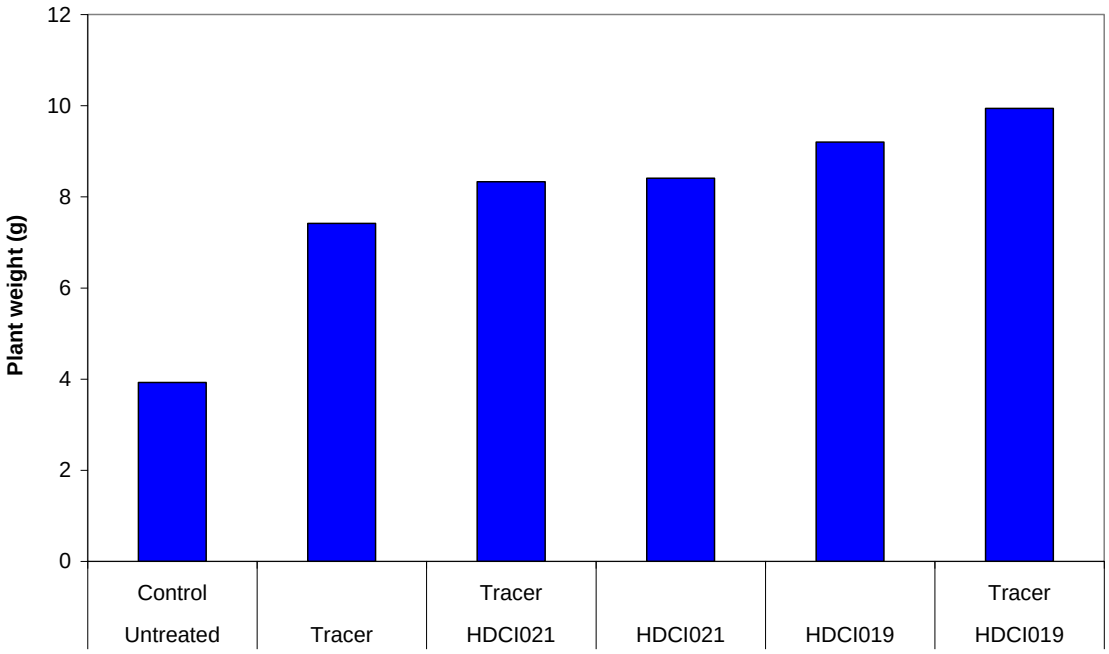

Figure 2.1.9a. Cabbage root fly – biopesticides applied to module compost – mean plant weight (g).

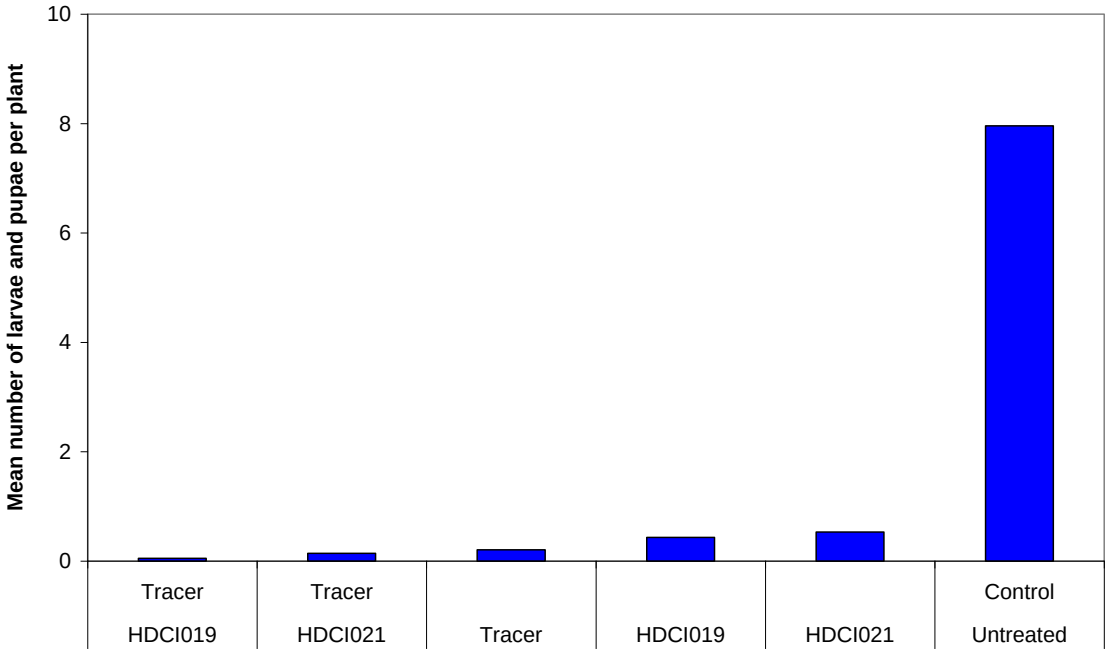

Figure 2.1.9b. Cabbage root fly – biopesticides applied to module compost – mean number of larvae + pupae recovered per plant (back-transformed data).

Experiment 2.1.10 Effect of biopesticides applied to the module compost

The roots of all of the plants treated with HDCI021 or HDCI019 were heavier than those from the untreated control pots (Table 2.1.10a and Figure 2.1.10a). Those treated with Tracer (5% of recommended dose) and/or HDCI049 were not.

With the exception of HDCI049 alone, all of the biopesticide treatments (including Tracer at 5% of recommended dose) resulted in a lower damage score than the untreated control (Table 2.1.10a, Figure 2.1.10b).

With the exception of HDCI049 alone, all of the biopesticide treatments (including Tracer at 5% of recommended dose) resulted in lower numbers of larvae and pupae per plant than the untreated control (Table 2.1.10a and Figure 2.1.10c).

Table 2.1.10a. Cabbage root fly – biopesticides applied to module compost – summary of data analysis using ANOVA. Treatments that were significantly different from the control are in bold and underlined. Insect count data were square root transformed prior to ANOVA.

Treatments	Plant weight (g)	Mean damage score (0–5)	Number of larvae and pupae per plant	
			Square root transform	Back transform
Tracer + HDCI019	**2.75**	**0.2**	**0.10**	0.01
Tracer + HDCI021	**4.00**	**0.1**	**0.10**	0.01
HDCI019	**3.28**	**0.6**	**0.37**	0.14
HDCI021	**1.98**	**0.8**	**0.54**	0.29
Tracer + HDCI049	0.91	**3.1**	**1.46**	2.13
Tracer	0.88	**3.6**	**1.95**	3.81
HDCI049	0.25	5	2.54	6.45
Untreated	0.25	5	2.79	7.80
F	19.63	44.84	21.53	
df	72	72	72	
p	<0.001	<0.001	<0.001	
LSD	0.91	0.89	0.67	

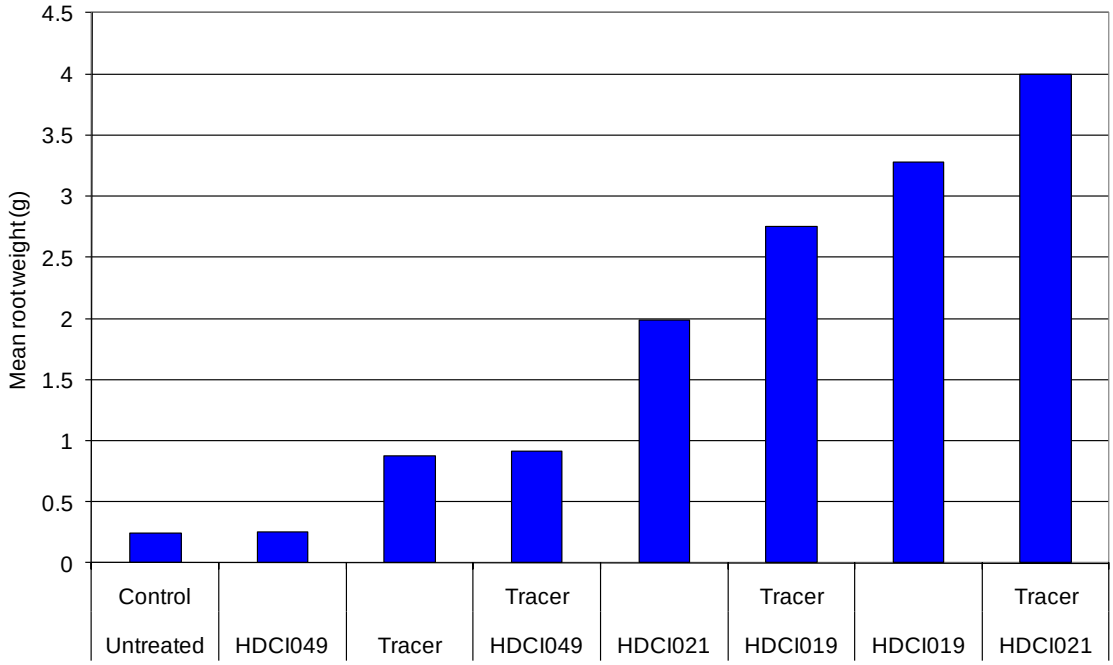

Figure 2.1.10a. Cabbage root fly – biopesticides applied to module compost – mean weight of plant roots.

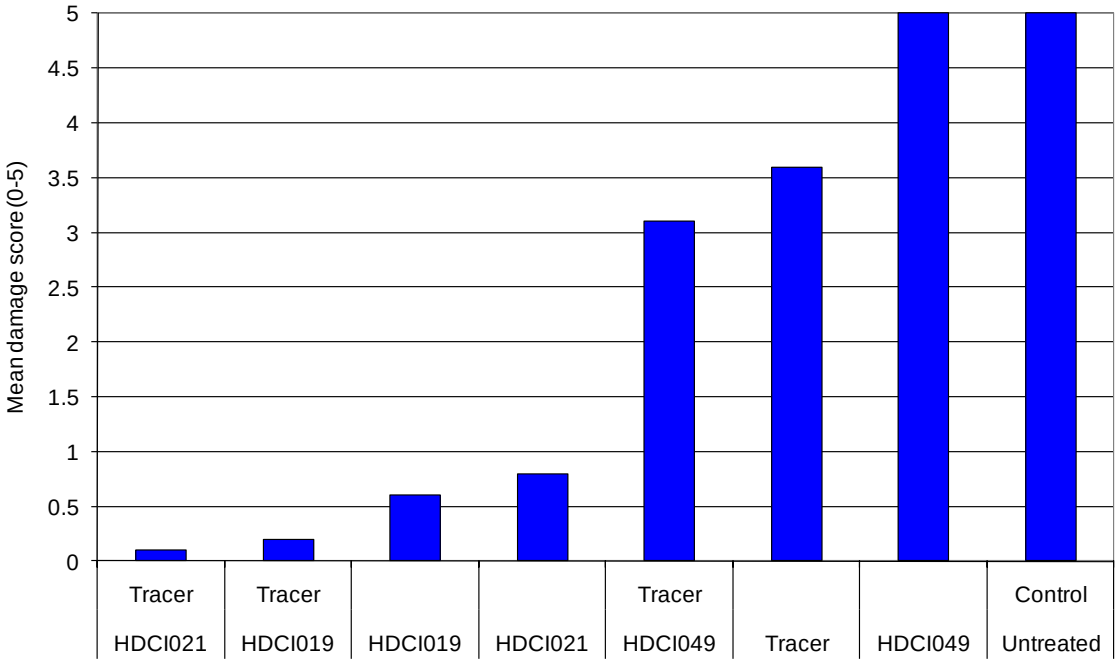

Figure 2.1.10b. Cabbage root fly – biopesticides applied to module compost – mean root damage score.

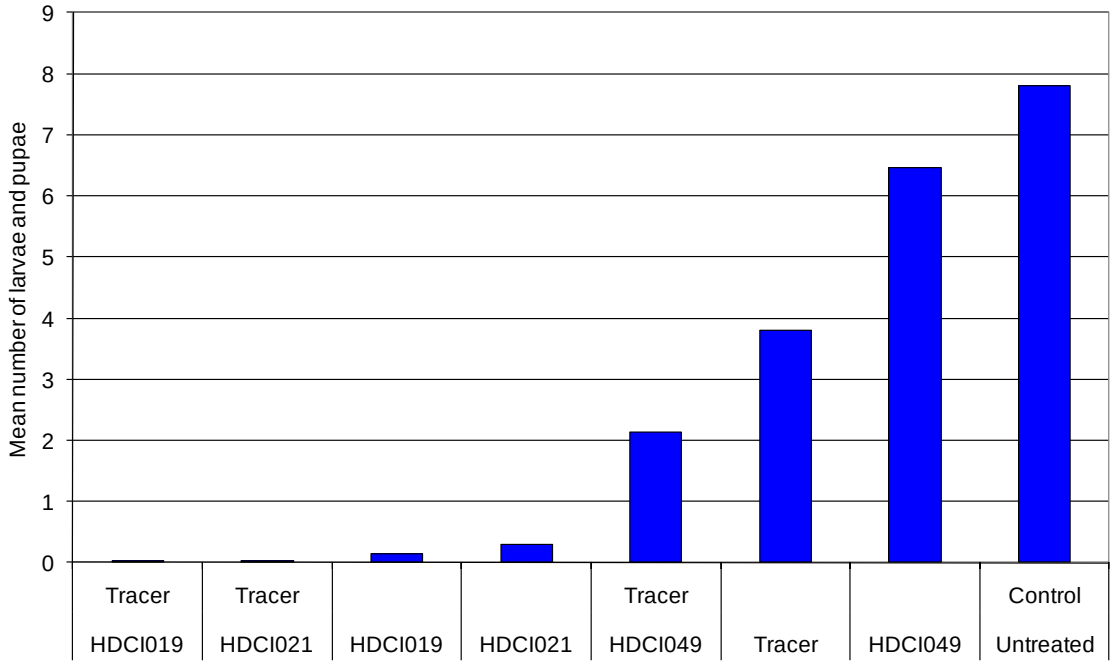

Figure 2.1.10c. Cabbage root fly – biopesticides applied to module compost – mean number of larvae + pupae recovered per plant (back-transformed data).

Experiment 2.1.11 Effect of biopesticides applied to the module compost

Older plants were used in this experiment and root weight was not measured. With the exception of HDCI049 alone, all of the biopesticide treatments (including Tracer at 5% of recommended dose) resulted in a lower damage score than the untreated control (Table 2.1.11a, Figure 2.1.11a).

With the exception of HDCI049 alone, all of the biopesticide treatments (including Tracer at 5% of recommended dose) resulted in lower numbers of larvae and pupae per plant than the untreated control (Table 2.1.11a and Figure 2.1.11b).

Table 2.1.11a. Cabbage root fly – biopesticides applied to module compost – summary of data analysis using ANOVA. Treatments that were significantly different from the control are in bold and underlined. Insect count data were square root transformed prior to ANOVA.

	Number of larvae and pupae per plant		
Treatments	**Mean damage score (0–5)**	**Square root transform**	**Back transform**
Tracer + HDCI021	**0.00**	**0.00**	0.00
HDCI021	**0.67**	**0.17**	0.03
Tracer + HDCI049	**0.50**	**0.17**	0.03
Tracer + HDCI019	**0.17**	**0.24**	0.06
HDCI019	**1.17**	**0.79**	0.62
Tracer	**1.67**	**1.29**	1.66
Untreated	3.00	2.34	5.49
HDCI049	3.17	2.78	7.73
F	21.29	13.00	
df	40	40	
p	<0.001	<0.001	
LSD	0.75	0.85	

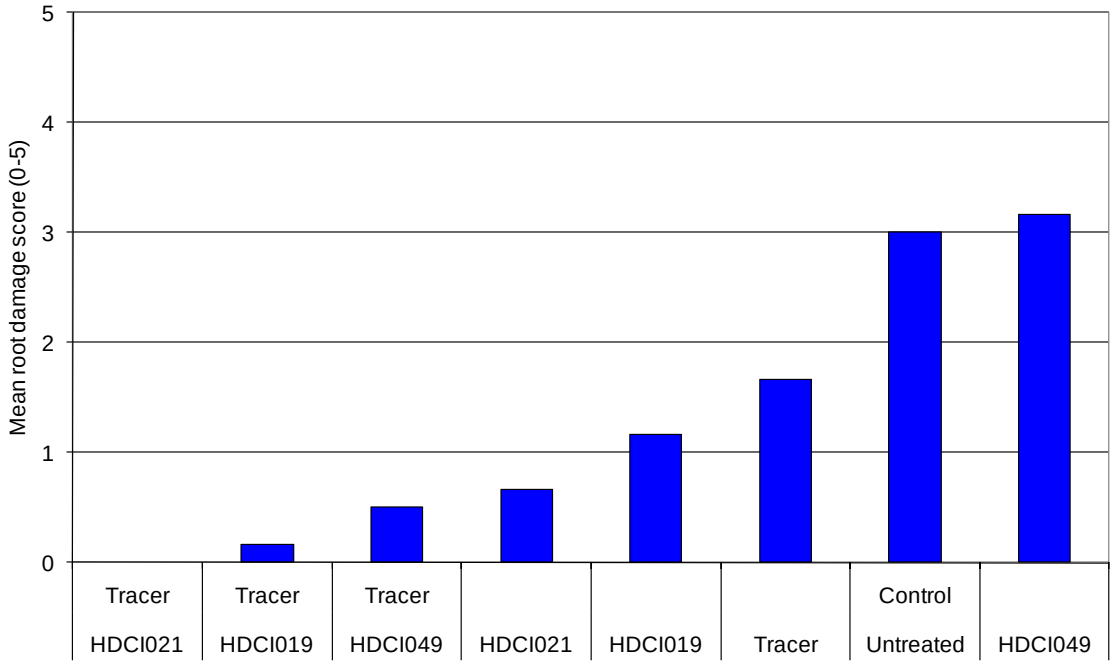

Figure 2.1.11a. Cabbage root fly – biopesticides applied to module compost – mean root damage score.

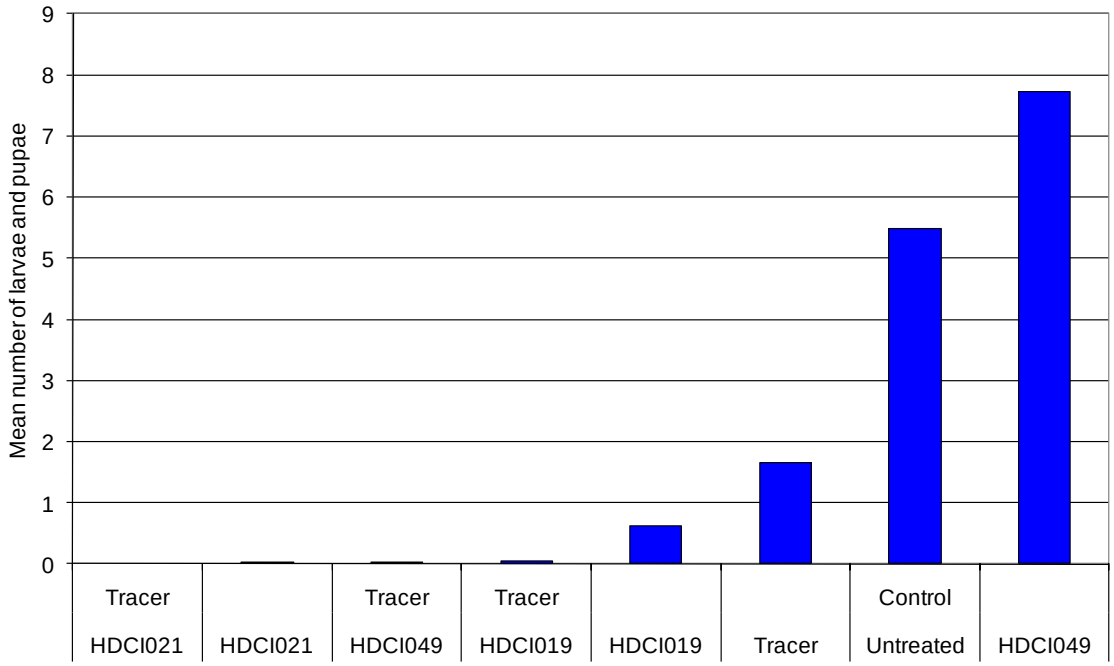

Figure 2.1.11b. Cabbage root fly – biopesticides applied to module compost – mean number of larvae + pupae recovered per plant (back-transformed data).

2.2 Aphids

Experiment 2.2.1 Peach-potato aphid (Myzus persicae) – no choice test

In this experiment, the distribution of aphids was relatively uniform and no pre-spray aphid counts were made. The infested plants were sprayed and the numbers of aphids remaining were recorded after five days. The data were square root-transformed prior to ANOVA.

Compared with the untreated control there were statistically significant reductions in aphid numbers for HDCI023, HDCI024, HDCI025, HDCI026 and HDCI023 + HDCI025 (Table 2.2.1 and Figure 2.2.1).

Table 2.2.1. Peach-potato aphid – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatments	Mean number of aphids remaining five days after treatment (square root-transformed)	Back-transformed mean number of aphids remaining five days after treatment
HDCI023 + HDCI025	**2.74**	7.49
HDCI025	**3.31**	10.92
HDCI024	**5.12**	26.24
HDCI026	**9.21**	84.79
HDCI023	**10.70**	114.54
HDCI027	13.40	179.64
HDCI022	18.44	339.93
Untreated control	25.38	644.28
F	3.39	
df	8	
p	0.054	
LSD	13.94	

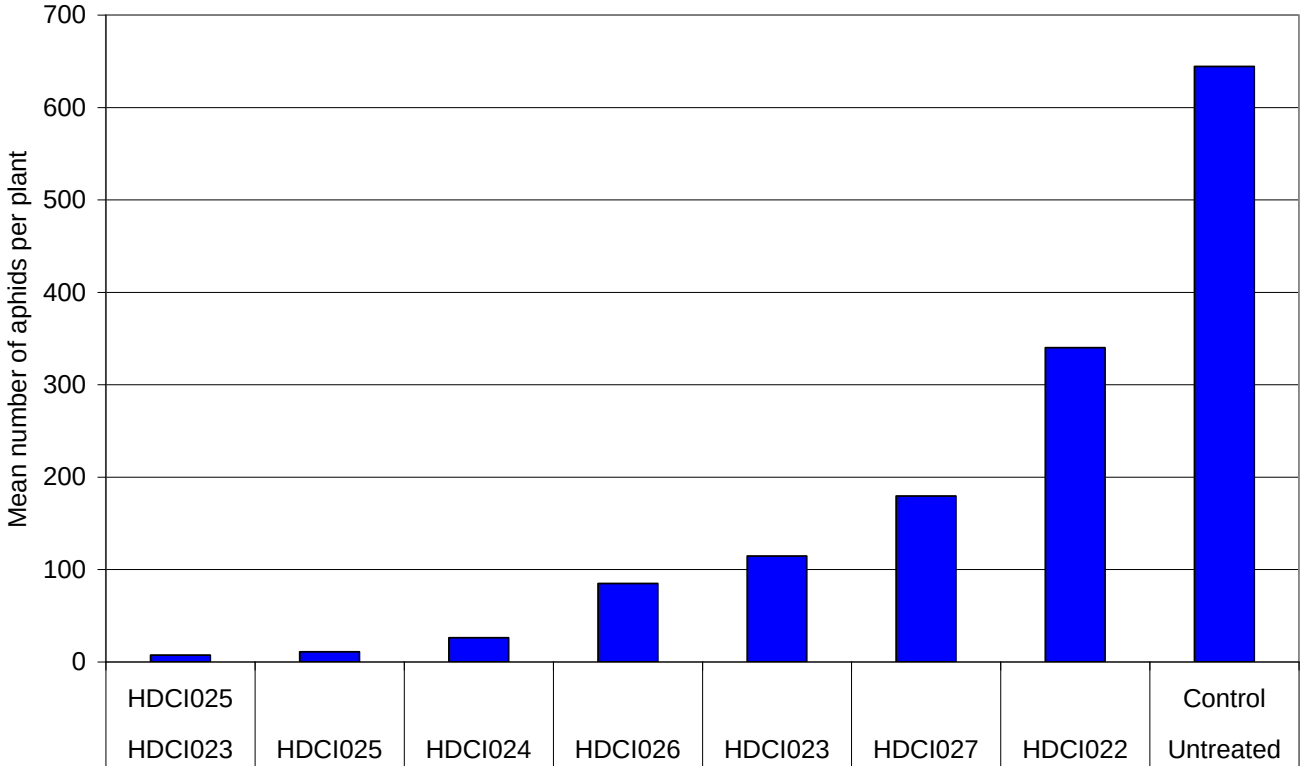

Figure 2.2.1. Peach-potato aphid – mean number of aphids remaining five days after treatment – no choice test. Back-transformed means.

Experiment 2.2.2 Cabbage aphid (Brevicoryne brassicae) – no choice test

In this experiment, following infestation of the plants, the distribution of aphids was not uniform and pre-spray counts were made. The infested plants were sprayed and the numbers of aphids remaining were recorded after three and seven days. The data were expressed as a percentage of the pre-spray count and were $\log_{10}$ transformed prior to ANOVA.

After three days, and compared with the untreated control, there was a statistically significant reduction in aphid numbers for HDCI025 only (Table 2.2.2 and Figure 2.2.2). After seven days, although numbers on the plants treated with HDCI025 were still low there were no statistically significant differences between treatments as the numbers of aphids had declined on most of the other treatments; the reason for which is not known.

Table 2.2.2. Cabbage aphid – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatments	Mean percentage aphids remaining three days after treatment (square root-transformed)	Back-transformed mean percentage aphids remaining three days after treatment	Mean percentage aphids remaining seven days after treatment (square root-transformed)	Back-transformed mean percentage aphids remaining seven days after treatment
HDCI025	**0.94**	7.78	0.73	4.32
HDCI026	1.44	26.41	0.99	8.68
Untreated control	1.72	51.14	1.13	12.57
HDCI022	1.82	64.40	1.16	13.59
HDCI023	1.87	72.34	1.34	20.76
HDCI027	1.85	69.42	1.35	21.58
F	3.65		0.35	
df	18		18	
p	0.02		0.88	
LSD	0.56		1.18	

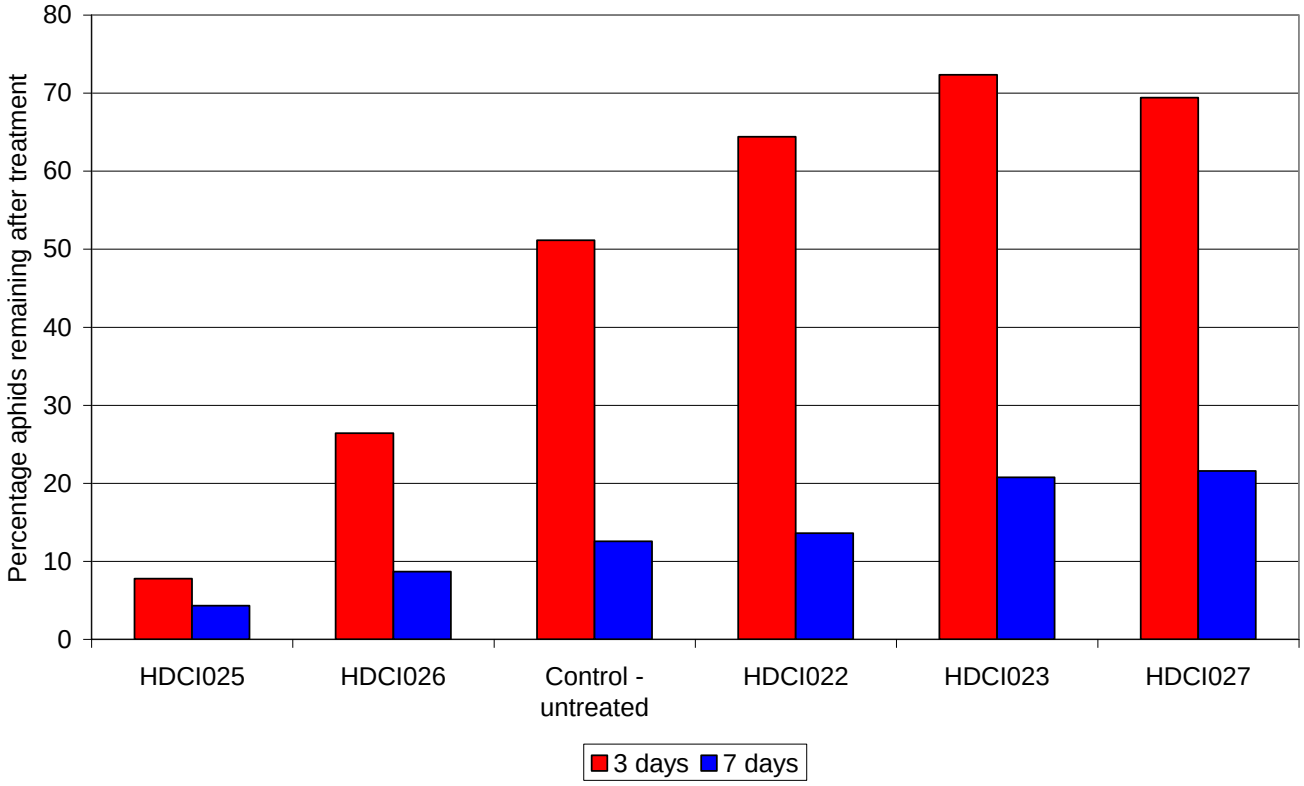

Figure 2.2.2. Cabbage aphid – mean percentage aphids remaining after treatment – no choice test. Back- transformed data.

Experiment 2.2.3 Peach-potato aphid (Myzus persicae) – no choice test

In this experiment, following infestation of the plants, the distribution of aphids was not uniform and pre-spray aphid counts were made. The infested plants were sprayed and the numbers of aphids remaining were recorded after seven days. The data were expressed as a percentage of the pre-spray count and were $\log_{10}$ transformed prior to ANOVA.

After seven days and compared with the untreated control there was a statistically significant reduction in aphid numbers for three of the test treatments (Table 2.2.3 and Figure 2.2.3). The control with the HDCI024 spray was very good and there appeared to be no improvement from combining it with another treatment.

Table 2.2.3. Peach-potato aphid – no choice test – data log_{10} transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatments	Mean percentage aphids remaining seven days after treatment (log_{10} transformed)	Back-transformed mean percentage aphids remaining seven days after treatment
HDCI024	**0.16**	2.45
HDCI024 + HDCI025	**0.22**	2.65
HDCI023 + HDCI024	**0.73**	6.34
HDCI024 + HDCI026	1.10	13.58
HDCI024 drench	1.42	27.48
Untreated control	2.41	257.20
F	4.77	
df	6	
p	0.042	
LSD	1.34	

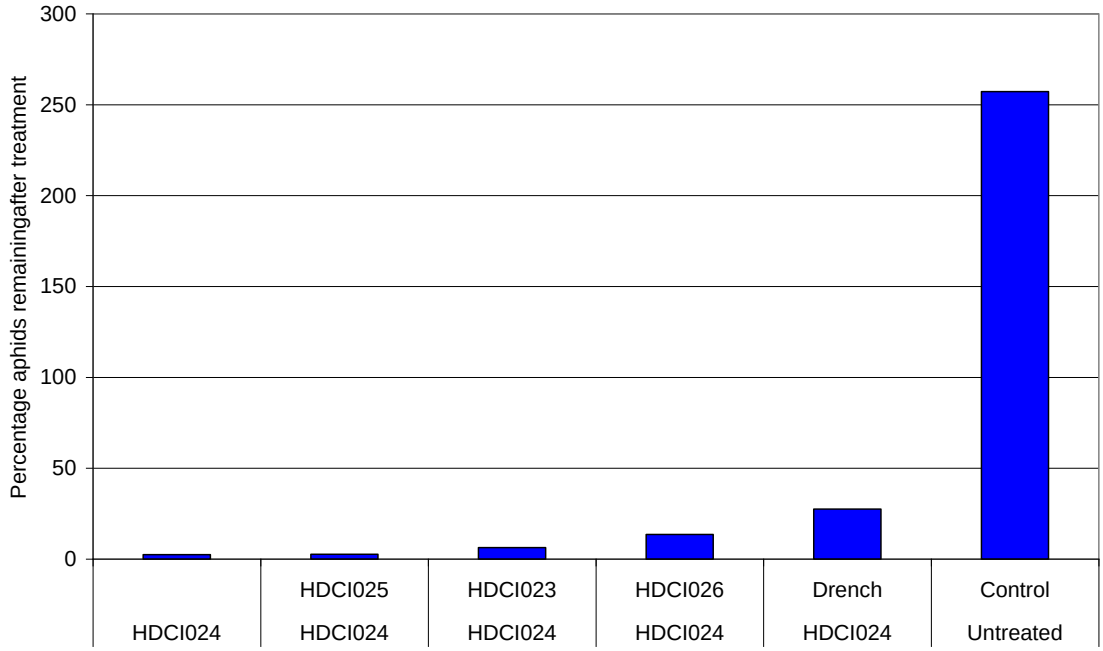

Figure 2.2.3. Peach-potato aphid – mean percentage aphids remaining seven days after treatment no choice test. Back-transformed means.

Experiment 2.2.4 Peach-potato aphid (Myzus persicae) – no choice test

In this experiment, following infestation of the plants, the distribution of aphids was not uniform and pre-spray aphid counts were made. The infested plants were sprayed and the numbers of aphids remaining were recorded after seven days. The data were expressed as a percentage of the pre-spray count and were $\log_{10}$ transformed prior to ANOVA.

After seven days and compared with the untreated control there was a statistically significant reduction in aphid numbers for HDCI024 and HDCI025 + HDCI026 (Table 2.2.4 and Figure 2.2.4). Although the HDCI025 treatment alone did not produce a statistically significant reduction in aphid numbers compared with the untreated control, there was no statistically significant difference in the levels of control provided by HDCI025 alone or by HDCI025 + HDCI026. In this case the HDCI024 drench treatment was ineffective.

Table 2.2.4. Peach-potato aphid – no choice test – data log_{10} transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatments	Mean percentage aphids remaining seven days after treatment (log_{10} transformed)	Back-transformed mean percentage aphids remaining seven days after treatment
HDCI024	**1.63**	42.50
HDCI025 + HDCI026	**1.78**	59.58
HDCI023 + HDCI023	2.11	127.56
HDCI025	2.21	163.66
Untreated control	2.39	247.09
HDCI024 drench	2.45	281.03
F	3.88	
df	18	
p	0.015	
LSD	0.5	

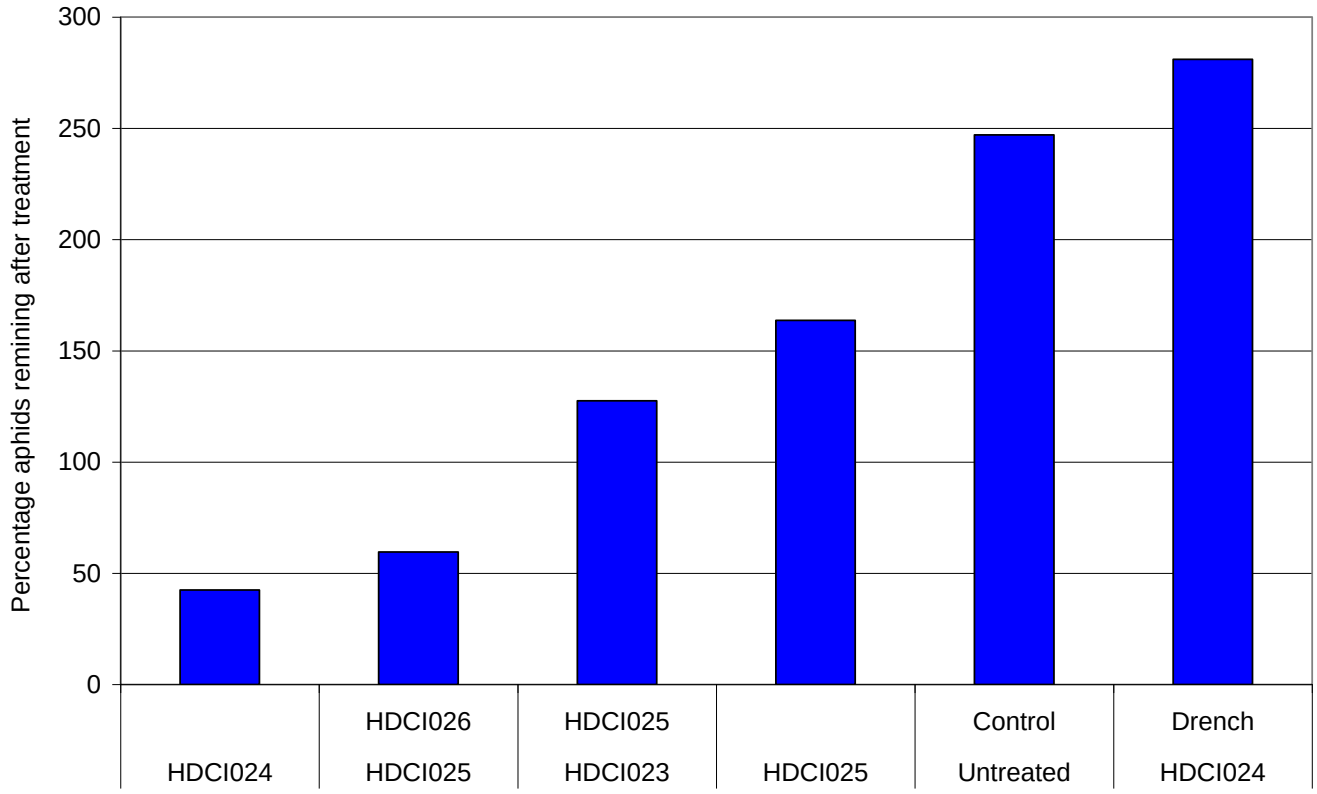

Figure 2.2.4. Peach-potato aphid – mean percentage aphids remaining seven days after treatment no choice test. Back-transformed means.

Experiment 2.2.5 Currant-lettuce aphid (Nasonovia ribisnigri) – no choice test

The test plants were infested with aphids and the infestation was allowed to develop. When sufficient numbers of aphids were present, the numbers of aphids on each plant were recorded and then the plants were treated and placed in individual cages. Seven days after treatment, the plants were removed from the cages and the numbers of aphids on each plant were recorded. The numbers of aphids remaining were expressed as a percentage of those recorded prior to treatment. The data were $\log_{10}$ transformed prior to ANOVA.

Compared with the untreated control there were statistically significant reductions in aphid numbers for HDCI025, HDCI023 + HDCI025, HDCI024 + HDCI025 and HDCI025 + HDCI026 (Table 2.2.5 and Figure 2.2.5).

Table 2.2.5. Currant-lettuce aphid – no choice test – data log_{10} transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatments	Mean percentage aphids remaining seven days after treatment (log_{10} transformed)	Back-transformed mean percentage aphids remaining seven days after treatment
HDCI023 + HDCI025	**0.66**	4.57
HDCI025 + HDCI026	**0.70**	4.99
HDCI025	**1.10**	12.69
HDCI024 + HDCI025	**1.55**	35.72
HDCI024 + HDCI026	2.03	108.19
HDCI023 + HDCI026	2.18	151.48
HDCI023	2.29	192.94
HDCI024	2.29	193.74
HDCI023 + HDCI024	2.30	200.79
Untreated control	2.32	209.56
HDCI026	2.50	314.49
F	6.67	
df	33	
p	<0.001	
LSD	0.77	

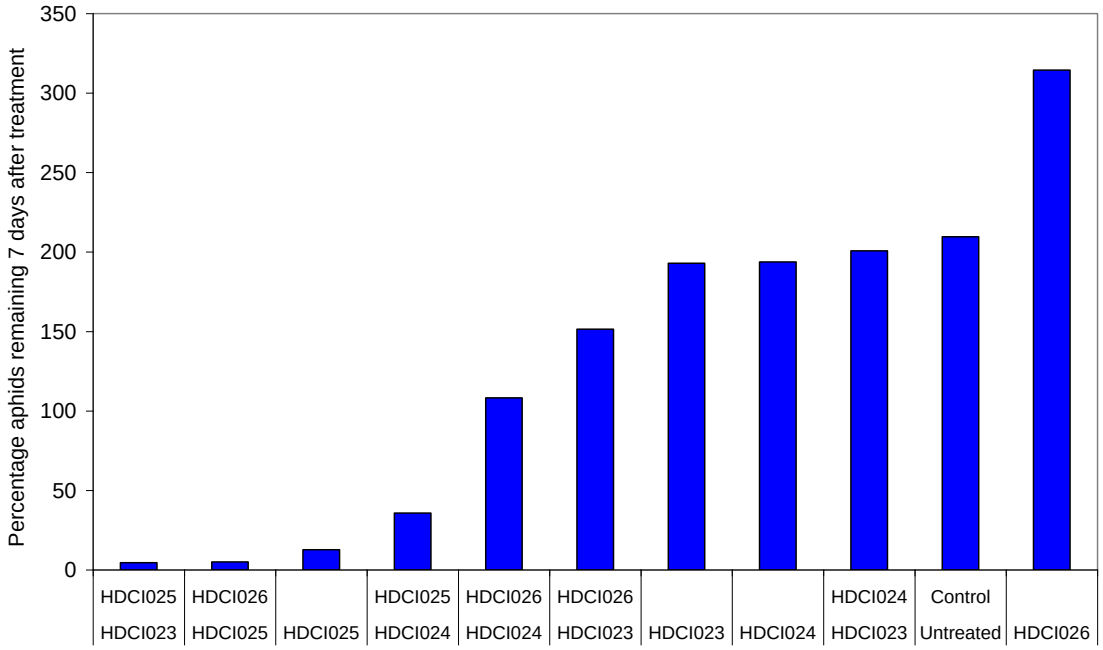

Figure 2.2.5. Currant-lettuce aphid – mean percentage aphids remaining seven days after treatment no choice test. Back-transformed means.

2.3 Diamond-back moth (*Plutella xylostella*)

Experiment 2.3.1 Foliar spray treatments to control diamond-back moth

In this experiment, most of the products available were tested alone for activity against diamond-back moth. There were no statistically significant differences between treatments in either the number of eggs laid or in larval feeding holes (Tables 2.3.1a and b: Figures 2.3.1 a and b).

Table 2.3.1a. Diamond-back moth – no choice test – data square root-transformed.

	Eggs 0–2 days		Eggs 2–7 days		Total number eggs (0–7 days)	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI033	0.00	0.00	2.21	4.87	2.21	4.87
HDCI032	1.22	1.50	1.87	3.48	2.45	5.99
HDCI029	1.50	2.25	2.72	7.42	3.16	10.00
Untreated	1.82	3.32	2.37	5.60	3.23	10.45
HDCI030	3.00	8.99	1.83	3.33	3.55	12.60
HDCI028	2.66	7.07	2.58	6.66	3.71	13.73
F	1.29		0.38		0.49	
df	6		6		6	
p	0.38		0.85		0.77	
LSD	3.27		2.06		2.95	

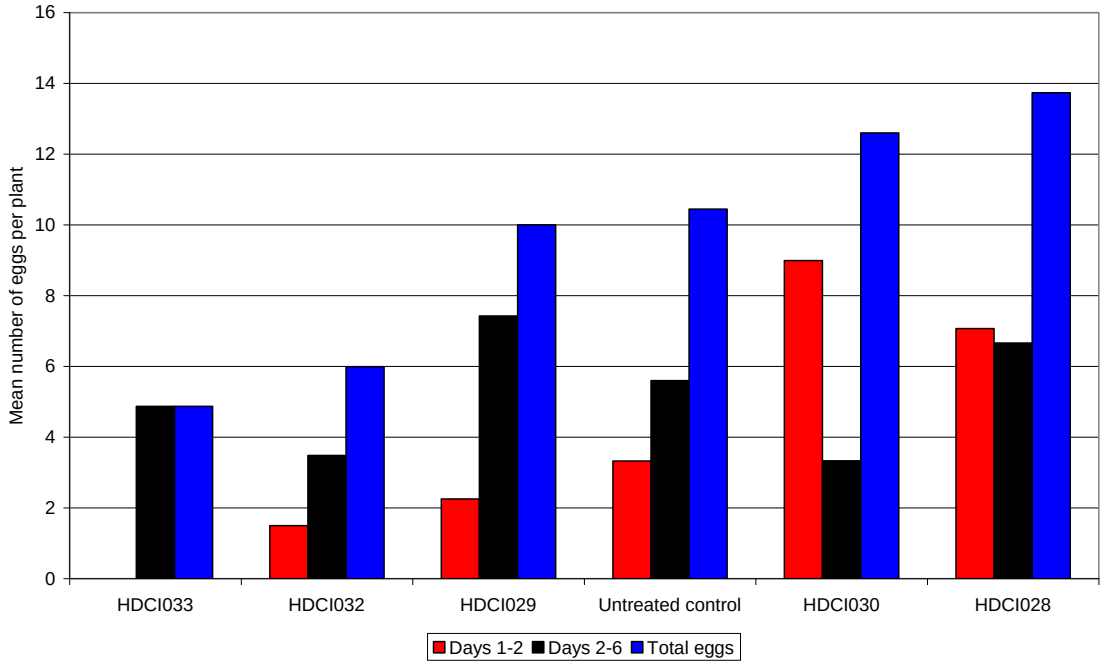

Figure 2.3.1a. Diamond-back moth – mean number of eggs per plant – no choice test. Back-transformed data.

Table 2.3.1b. Diamond-back moth – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatments	Mean number of feeding holes (square root transformation)	Mean number of feeding holes (back-transformed)
Untreated	4.18	17.50
HDCI032	5.22	27.25
HDCI030	5.77	33.27
HDCI029	6.78	45.95
HDCI033	8.89	78.97
HDCI028	9.62	92.46
F	0.58	
df	6	
p	0.72	
LSD	9.70	

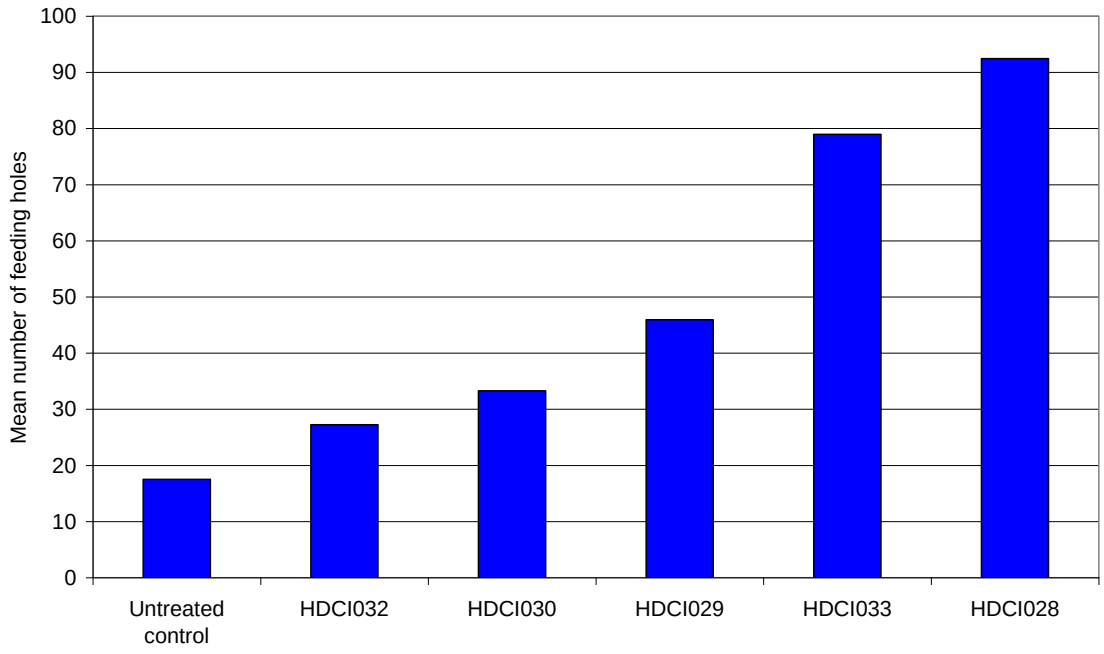

Figure 2.3.1b. Diamond-back moth – mean number of feeding holes – no choice test. Back-transformed data.

Experiment 2.3.2 Foliar spray treatments to control diamond-back moth

In this experiment some of the treatments used in Experiment 2.3.1 were re-tested and some additional treatments were added. There were no statistically significant differences between treatments in either the number of eggs laid or in larval feeding holes (Tables 2.3.2a and b: Figures 2.3.2 a and b).

Table 2.3.2a. Diamond-back moth – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

	Eggs 0–2 days		**Eggs 2–7 days**		**Total number eggs (0–7 days)**	
Treatment	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**	**Square root transform**	**Back transform**
HDCI031	2.12	4.50	0.50	0.25	2.21	4.87
Untreated	1.91	3.66	1.21	1.46	2.29	5.24
HDCI030	2.87	8.24	1.00	1.00	3.29	10.79
HDCI033	2.96	8.74	1.32	1.75	3.40	11.59
HDCI028	3.96	15.71	1.32	1.75	4.29	18.37
HDCI029	3.07	9.44	3.87	14.98	4.94	24.44
HDCI028 + HDCI029	3.79	14.37	4.74	22.50	6.20	38.42
HDCI032	5.82	33.87	2.93	8.57	6.53	42.62
F	1.89		2.62		2.67	
df	8		8		8	
p	0.20		0.10		0.10	
LSD	2.93		3.10		3.29	

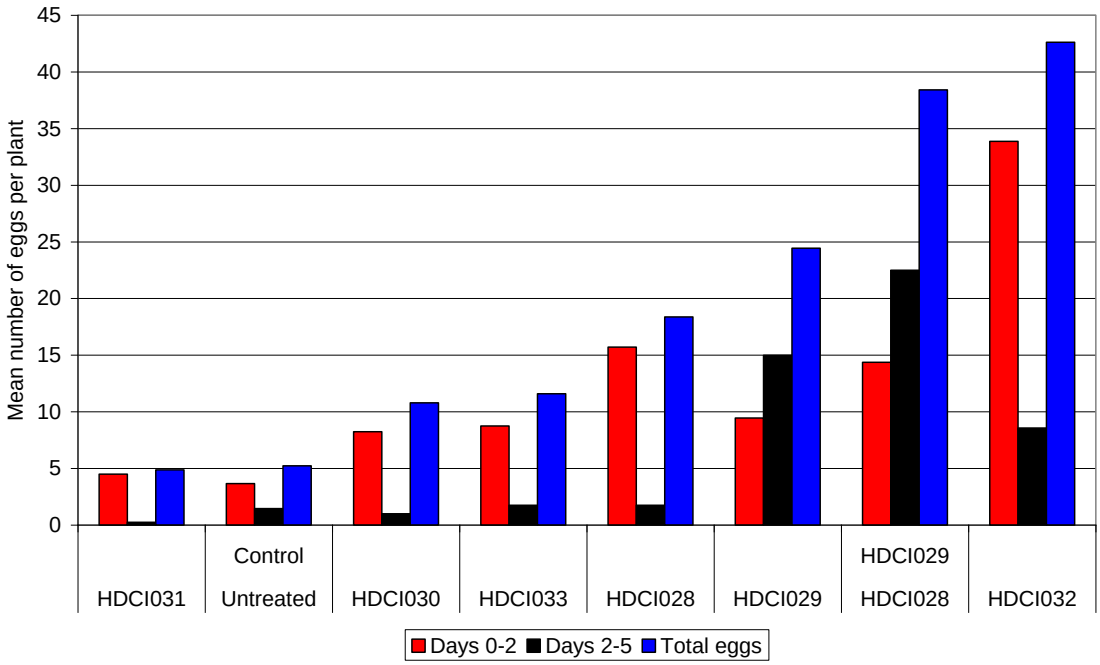

Figure 2.3.2a. Diamond-back moth – mean numbers of eggs per plant – no choice test. Back-transformed data.

Table 2.3.2b. Diamond-back moth – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatment	Mean number of feeding holes (square root transformation)	Mean number of feeding holes (back-transformed)
HDCI032	4.10	16.80
HDCI030	0.71	0.50
HDCI033	5.09	25.96
HDCI028	4.96	24.59
HDCI029	5.08	25.83
Untreated	3.78	14.29
HDCI031	5.63	31.66
HDCI028 + HDCI029	4.12	16.99
F	0.75	
df	8	
p	0.64	
LSD	5.81	

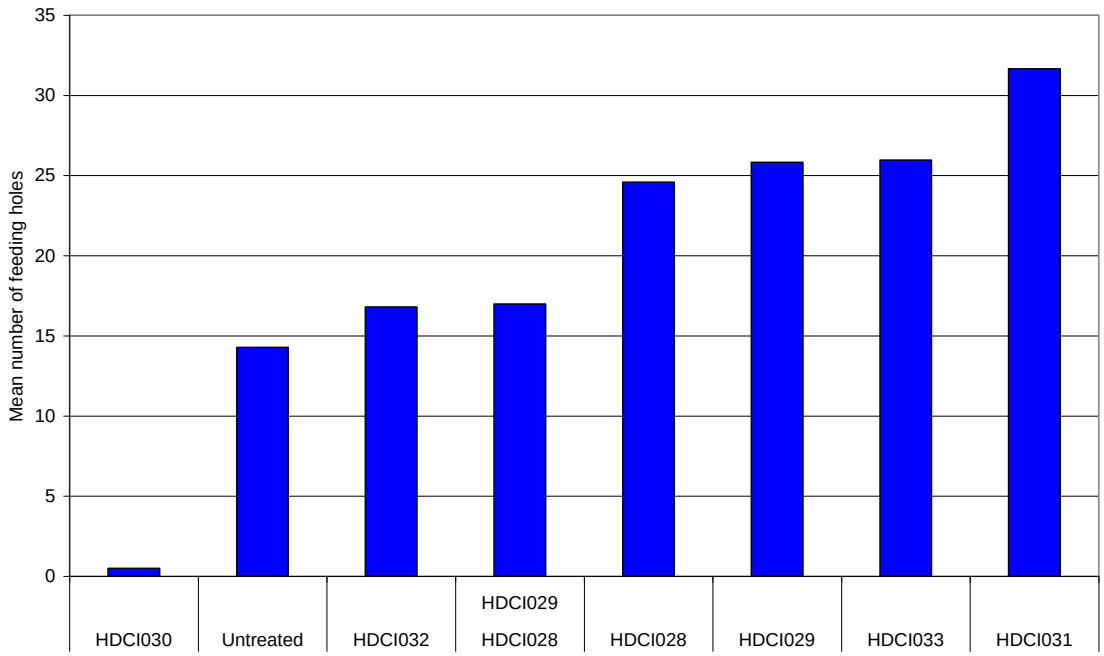

Figure 2.3.2b. Diamond-back moth – mean number of feeding holes – no choice test. Back-transformed data.

Experiment 2.3.3 Foliar spray treatments to control diamond-back moth

Published evidence indicated that treatments HDCI031 and HDCI029 should be effective against diamond-back moth. This experiment focused on HDCI031 applied as a foliar spray, a drench treatment or in combination with other biopesticides as foliar sprays. There were statistically significant effects of treatment on the number of feeding holes after six days compared with the untreated control (with the exception of plants treated with HDCI031 + HDCI028) and all treatments had reduced the number of feeding holes compared with the untreated control by ten days. Addition of HDCI028, HDCI029 or HDCI032 did not improve control by HDCI031.

Table 2.3.3. Diamond-back moth – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

	Six days		Ten days	
Treatment	**Mean number of feeding holes (square root transformation)**	**Mean number of feeding holes (back-transformed)**	**Mean number of feeding holes (square root transformation)**	**Mean number of feeding holes (back-transformed)**
HDCI031	**4.72**	22.28	**9.80**	96.00
HDCI031 + HDCI029	**5.24**	27.42	**10.82**	117.17
HDCI031 Drench	**4.72**	22.28	**18.56**	344.55
HDCI031 + HDCI032	**10.74**	115.34	**20.84**	434.29
HDCI031 + HDCI028	11.50	132.36	**21.33**	454.99
Control	12.35	152.58	25.10	629.84
F	16.92		135.46	
df	18		6	
p	0.042		P<0.001	
LSD	0.95		1.29	

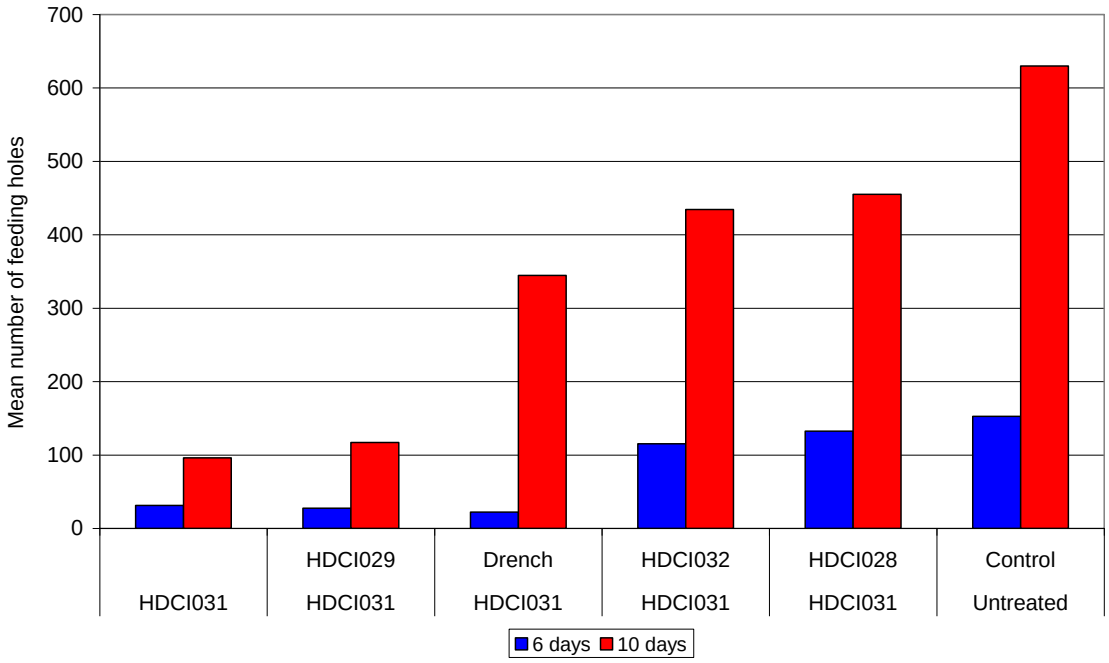

Figure 2.3.3. Diamond-back moth – mean number of feeding holes – no choice test. Back-transformed data.

Experiment 2.3.4 Foliar spray treatments to control diamond-back moth

This experiment focused on HDCI029 applied as a foliar spray or in combination with other biopesticides as foliar sprays. HDCI031 (spray and drench treatments) was included as a positive control. All treatments reduced the number of feeding holes compared with the untreated control. A combination of HDCI029 + HDCI032 was more effective than HDCI029 alone or HDCI029 + HDCI028.

Table 2.3.4. Diamond-back moth – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatment	Mean number of feeding holes (square root transformation)	Mean number of feeding holes (back-transformed)
HDCI029 + HDCI032	**8.47**	71.69
HDCI031	**8.96**	80.31
HDCI029 + HDCI028	**11.78**	138.70
HDCI029	**12.54**	157.35
HDCI031 Drench	**14.28**	203.85
Untreated	17.77	315.82
F	16.92	
df	18	
p	<0.001	
LSD	2.50	

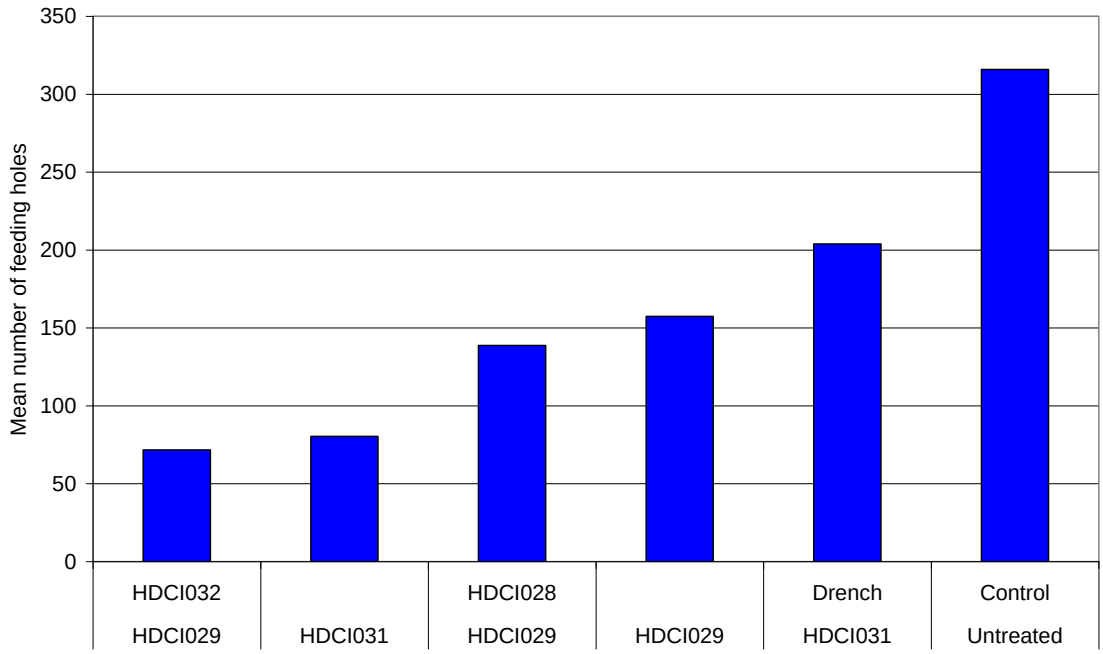

Figure 2.3.4. Diamond-back moth – mean number of feeding holes – no choice test. Back-transformed data.

Experiment 2.3.5 Foliar spray treatments to control diamond-back moth

This experiment focused again on HDCI029, HDCI031 and the two treatments combined, all as foliar sprays. HDCI029 + HDCI031 and HDCI029 alone reduced the numbers of feeding holes compared with the untreated control.

Table 2.3.5. Diamond-back moth – no choice test – data square root-transformed. Treatments that were significantly different from the control are in bold and underlined.

Treatment	Mean number of feeding holes (square root transformation)	Mean number of feeding holes (back-transformed)	Mean number of dead moths
HDCI029 + HDCI031	**3.74**	13.95	1.60
HDCI029	**8.57**	73.51	1.29
HDCI031	9.03	81.52	1.49
Untreated	14.12	199.33	1.31
F	6.46		0.14
df	12		12
p	0.008		0.93
LSD	5.14		1.21

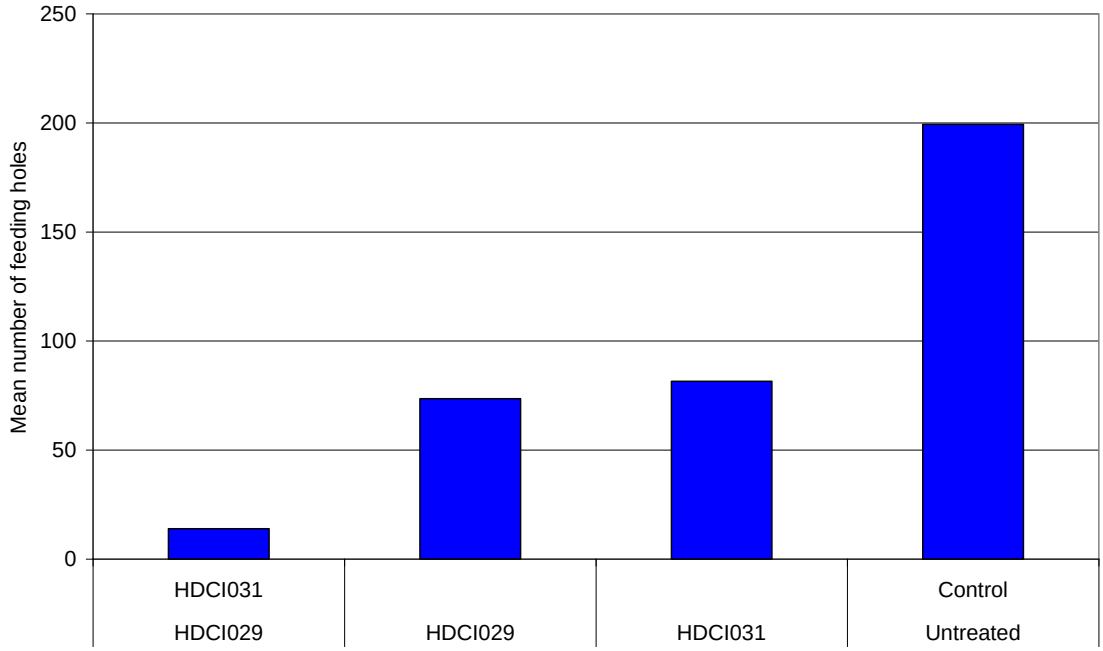

Figure 2.3.5a. Diamond-back moth – mean number of feeding holes per plant – no choice test. Back-transformed data.

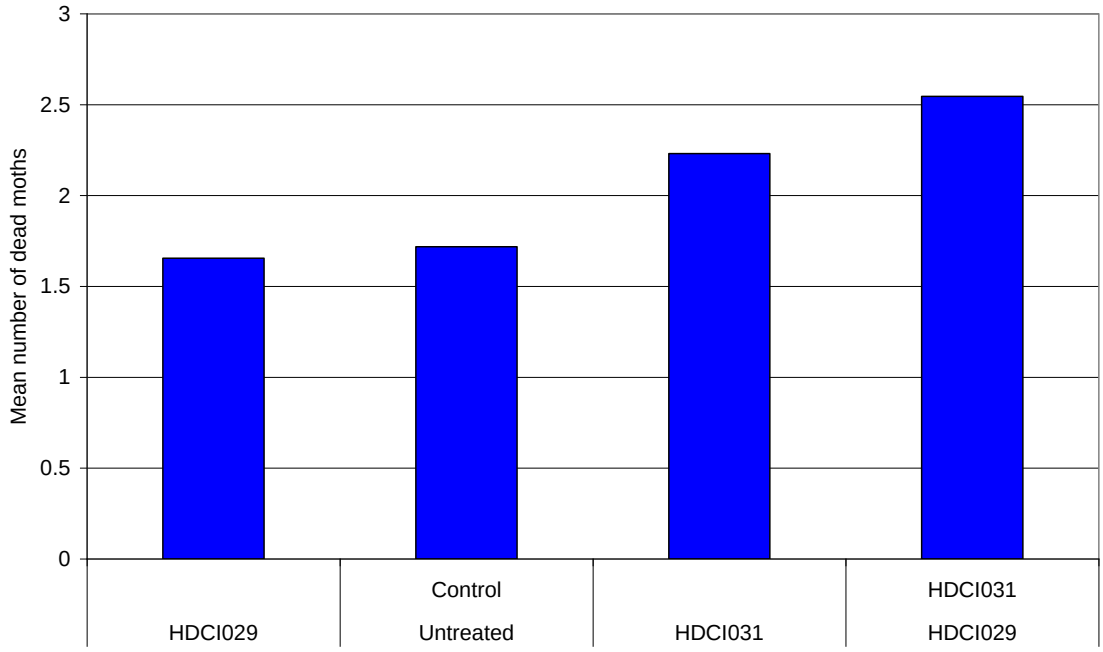

Figure 2.3.5b. Diamond-back moth – mean number of dead moths – no choice test. Back-transformed data.

Discussion

3.1 Cabbage root fly

Effect of biopesticides applied as foliar sprays on adult cabbage root fly mortality (Experiments 2.1.1–2.1.7)

Most of the biopesticides did not increase mortality of adult cabbage root flies. However, HDCI020, or treatments including HDCI020, did increase fly mortality on several occasions in the no-choice tests and this was particularly for fly mortality during the first few days when biopesticide residues were fresh (Table 3.1.1).

Table 3.1.1. Treatments producing statistically significant increases in cabbage root fly mortality in no-choice tests with foliar treatments.

Expt	Early flies	Total flies
2.1.2	HDCI020 HDCI020 + HDCI016 HDCI020 + HDCI019	HDCI020
2.1.3		
2.1.4	HDCI020 HDCI020 + HDCI016	
2.1.5		HDCI021 + HDCI016
2.1.6		
2.1.7		

Effect of biopesticides applied as foliar sprays on egg-laying by female cabbage root fly (Experiments 2.1.1–2.1.8)

Most of the biopesticides did not decrease egg-laying when applied as foliar sprays to plants. However, HDCI020 did decrease egg laying on two occasions (Table 3.1.2).

Table 3.1.2. Treatments producing statistically significant decreases in egg-laying in no-choice or choice tests with foliar treatments.

Expt	Type of test	Early eggs	Later eggs	Total eggs
2.1.1	No choice			HDCI020
2.1.2	No choice			
2.1.3	No choice			
2.1.4	No choice			
2.1.5	No choice			
2.1.6	No choice			
2.1.7	No choice	HDCI020		
2.1.8	Choice			

Effect of biopesticides applied to the module compost

Numbers of cabbage root fly larvae/pupae were reduced by a number of treatments applied to the module compost. Of the treatments applied alone, HDCI019 and HDCI021 were most effective and Tracer was also surprisingly effective at a reduced dose. HDCI049 was effective in combination with reduced dose of Tracer. Treatments that reduced the number of cabbage root fly larvae/pupae also reduced root damage (score 0–5), and some of them also increased root weight, compared with the untreated control.

If the effects of combining two pesticides are additive then it may be possible to predict the outcome of the combination of treatments as follows:

Expected outcome = no. in untreated control x proportion controlled by A x proportion controlled by B.

This can then be compared with the observed outcome as in Table 3.1.3 and Figure 3.1.1.

Table 3.1.3. Treatments producing statistically significant decreases in numbers of cabbage root fly larvae.

Experiment	Reduction in numbers of larvae/pupae	Reduction in root damage (score 0-5)	Increase in root weight
2.1.9	HDCI019 HDCI021 Tracer Tracer + HDCI019 Tracer + HDCI021	n/a	HDCI019 HDCI021 Tracer + HDCI019 Tracer + HDCI021
2.1.10	HDCI019 HDCI021 Tracer Tracer + HDCI049 Tracer + HDCI019 Tracer + HDCI021	HDCI019 HDCI021 Tracer Tracer + HDCI049 Tracer + HDCI019 Tracer + HDCI021	HDCI019 HDCI021 Tracer + HDCI019 Tracer + HDCI021
2.1.11	HDCI019 HDCI021 Tracer Tracer + HDCI049 Tracer + HDCI019 Tracer + HDCI021	HDCI019 HDCI021 Tracer Tracer + HDCI049 Tracer + HDCI019 Tracer + HDCI021	n/a

Table 3.1.4. Observed and expected outcomes of pesticide combinations from Experiments 2.1.9–2.1.11.

Expt	Tracer plus:	'Untreated' number of pupae	Proportion remaining after Tracer alone	Proportion remaining after biopesticide alone	Numbers predicted from combination	Observed numbers
1.9	HDCI019	7.96	0.21	0.05	0.08	0.05
1.10	HDCI019	7.8	0.49	0.02	0.08	0.01
1.11	HDCI019	5.49	0.3	0.11	0.18	0.01
1.10	HDCI049	7.8	0.49	0.83	3.17	2.13
1.11	HDCI049	5.49	0.3	1.41	2.32	0.01
1.9	HDCI021	7.96	0.21	0.07	0.12	0.14
1.10	HDCI021	7.8	0.49	0.04	0.15	0.01
1.11	HDCI021	5.49	0.3	0.01	0.02	0.00

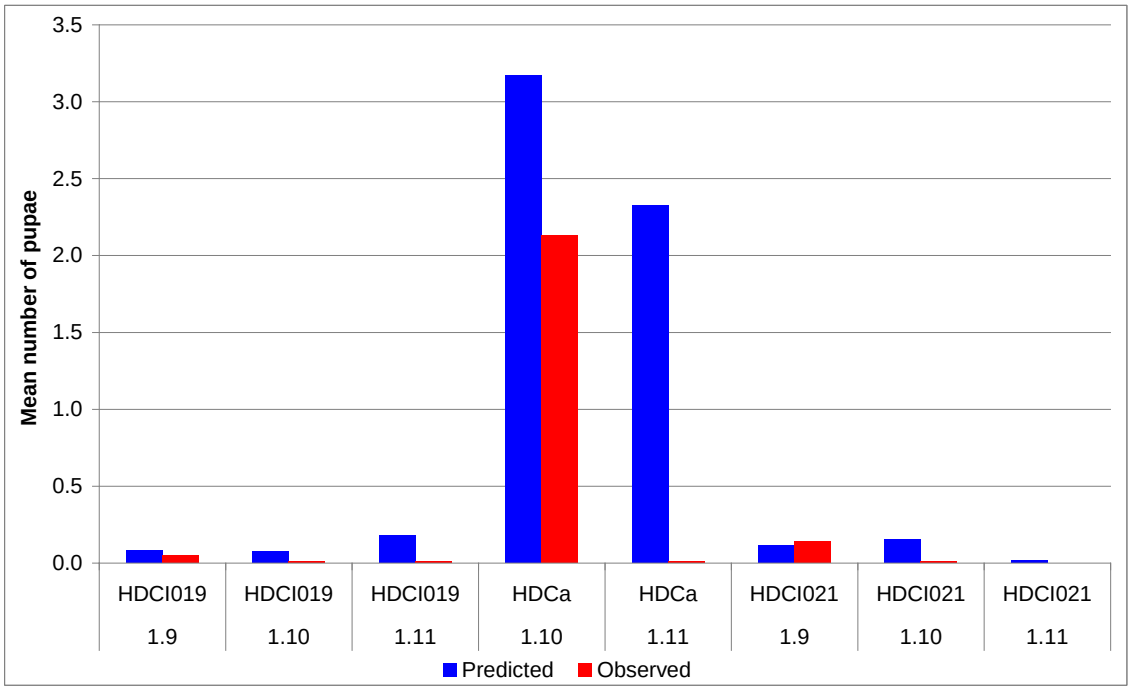

Figure 3.1.1. Observed and expected outcomes of pesticide combinations from Experiments 2.1.9–2.1.11.

In all but one case, the observed control was better than predicted and this difference was relatively large in the instance of Tracer and HDCI049, suggesting synergistic activity.

3.2 Aphids

Table 3.2.1 summarises the levels of control achieved with single products in all the experiments on aphid control (three species). Whilst there is a considerable amount of variation, this provides an overview of the performance of the different products when applied alone; it is not a definitive analysis. The treatments that reduced aphid numbers were HDCI024 (as a spray and a drench), HDCI025 and HDCI026, and of these, HDCI024 and HDCI025 sprays appeared to be the most effective.

Table 3.2.1. Summary of levels of control achieved with single products in different experiments (bb = cabbage aphid, mp = peach-potato aphid, nr = currant-lettuce aphid; 2.2.2a = first assessment; 2.2.2b = second assessment in experiment 2.2.2). Treatments applied as foliar sprays unless indicated otherwise.

Product	Expt	Species	Back-transformed mean number of aphids remaining	Aphids remaining as a percentage of control treatment	Mean percentage remaining for each treatment
HDCI022	2.2.2a	bb	64	126	
HDCI022	2.2.2b	bb	14	108	
HDCI022	2.2.1	mp	340	53	96
HDCI023	2.2.2a	bb	72	141	
HDCI023	2.2.2b	bb	21	165	
HDCI023	2.2.1	mp	115	18	
HDCI023	2.2.5	nr	193	92	104
HDCI024	2.2.1	mp	26	4	
HDCI024	2.2.3	mp	2	1	
HDCI024	2.2.4	mp	43	17	
HDCI024	2.2.5	nr	194	92	29
HDCI024 drench	2.2.3	mp	27	11	
HDCI024 drench	2.2.4	mp	281	114	62

Product	Expt	Species	Back-transformed mean number of aphids remaining	Aphids remaining as a percentage of control treatment	Mean percentage remaining for each treatment
HDCI025	2.2.2a	bb	8	15	
HDCI025	2.2.2b	bb	4	34	
HDCI025	2.2.1	mp	11	2	
HDCI025	2.2.4	mp	164	66	
HDCI025	2.2.5	nr	13	6	25
HDCI026	2.2.2a	bb	26	52	
HDCI026	2.2.2b	bb	9	69	
HDCI026	2.2.1	mp	85	13	
HDCI026	2.2.5	nr	314	150	71
HDCI027	2.2.2a	bb	69	136	
HDCI027	2.2.2b	bb	22	172	
HDCI027	2.2.1	mp	180	28	112
Untreated	2.2.2a	bb	51	100	
Untreated	2.2.2b	bb	13	100	
Untreated	2.2.1	mp	644	100	
Untreated	2.2.3	mp	257	100	
Untreated	2.2.4	mp	247	100	
Untreated	2.2.5	nr	210	100	100

Combining treatments

In Experiment 2.2.3 there was no evidence that addition of HDCI023, HDCI025 or HDCI026 improved the already good control by HDCI024. In Experiment 2.2.4, addition of either HDCI023 or HDCI026 appeared to improve control by HDCI025 but these were not statistically significant differences. In Experiment 2.2.1, addition of HDCI023 to HDCI025 did not improve the already good control.

In Experiment 2.2.5, all combinations of four products were examined and the results are summarised in Table 3.2.2 using the approach described in Section 3.1 for cabbage root fly. In general, differences in observed and predicted control were relatively small. However, there was an indication that a combination of HDCI026 with another product improved control more than might be expected from an additive effect alone.

Table 3.2.2. Analysis of data from Experiment 2.5.

Treatments	**Percentage remaining after treatment**	**'Control' as a proportion of untreated**
HDCI023 + HDCI025	0	0
HDCI025 + HDCI026	5	0.02
HDCI025	13	0.06
HDCI024 + HDCI025	36	0.17
HDCI024 + HDCI026	108	0.52
HDCI023 + HDCI026	151	0.72
HDCI023	193	0.92
HDCI024	194	0.92
HDCI023 + HDCI024	201	0.96
Untreated	210	1.00
HDCI026	314	1.50

Table 3.2.3. Observed and expected outcomes of pesticide combinations from Experiment 2.2.5. Mean number of aphids on untreated plants = 210.

Product 1	Product 2	Proportion remaining after Product 1 alone	Proportion remaining after Product 2 alone	Numbers predicted from combination	Observed numbers
HDCI023	HDCI024	0.92	0.92	178	201
HDCI023	HDCI025	0.06	0.92	12	0
HDCI023	HDCI026	1.50	0.92	290	151
HDCI024	HDCI025	0.06	0.92	12	36
HDCI024	HDCI026	0.92	1.5	290	108
HDCI025	HDCI026	0.06	1.5	19	5

3.3 Diamond-back moth

The results of the early experiments on diamond-back moth were hard to explain. In the later experiments, two of the biopesticides gave statistically significant control of diamond-back moth. From Experiment 2.3.5 it is possible to undertake the same analysis as for cabbage root fly (Table 3.1.4) and currant-lettuce aphid (Table 3.2.4), for a combination of these two products. Once again, the observed control from a combination of two products was better than predicted from their use alone (Table 3.3.1). This species requires further experimental work to investigate interactions.

Table 3.3.1. Analysis of data from Experiment 2.3.5.

Treatments	Observed feeding holes	Observed 'Control' as a proportion of untreated	Numbers of feeding holes predicted from combination
Untreated	199	1.00	
HDCI029	74	0.37	
HDCI031	82	0.41	
HDCI029 + HDCI031	14	0.07	30

General discussion

This study covers 21 different experiments on the control of pest insects by biopesticides. The consistency of results produced by the different experimental set-ups varies, with consistency being greatest in the experiments on the use of treatments applied to the compost to control cabbage root fly and least in experiments with diamond-back moth. This may be associated also with the amount of replication, as it is far easier to manage large replications of cabbage root fly inoculation experiments. With the exception of the cabbage root fly inoculation experiments, further repeats would be desirable to increase confidence in the findings. In addition, some time had to be spent evaluating individual treatments in preliminary experiments, which whilst not directly related to biopesticide combinations, has increased understanding of the products and provided information that is complementary to the data collected in the SCEPTRE project.

The study has shown that there are some combinations where control is improved by the application of two biopesticides simultaneously and that this effect may be additive or, in some cases, synergistic. The experiments with reduced doses of Tracer in compost treatments were undertaken to determine whether there were possible synergistic or additive effects of insecticides and biopesticides and this appears to be the case for this pest and method of application.

There is a need for further studies to explore more pest x biopesticide combinations and to determine how these might be used effectively in the field. This is particularly related to application methods and timings, and the persistence of such biopesticide treatments, which has not been tested at all in this project.

Conclusions

Cabbage root fly

- Of the four biopesticides evaluated as foliar sprays, only one biopesticide increased fly mortality, generally when residues were fresh.
- The same biopesticide was the only one to reduce egg-laying.
- There was little evidence of synergistic effects on fly mortality or egg-laying when two biopesticides were combined.
- Of the three biopesticides evaluated for control of cabbage root fly larvae, two produced significant reductions when applied alone and also increased root weight and reduced cabbage root fly damage.

- All three biopesticides evaluated for control of cabbage root fly larvae were applied with reduced doses of Tracer and with all three there was evidence of additive and sometimes synergistic effects. The evidence for synergistic effects was particularly strong for Tracer and HDCI049.
- Future studies should further investigate synergism for control of cabbage root fly larvae.
- For all of the biopesticides tested, persistence may be a problem in a field situation.

Aphids

- Two of the biopesticides (HDCI024 and HDCI024) appeared to provide relatively effective aphid control when applied on their own.
- A third biopesticide (HDCI026) appeared to have some efficacy on its own and there is also some evidence that it improved the control of other biopesticides when applied in combination. This requires further investigation.
- There is potential to investigate further combinations with all of the species.
- For all of the biopesticides tested, persistence may be limited in a field situation.

Diamond-back moth

- Two of the biopesticides evaluated gave statistically significant control of diamond-back moth.
- The observed control from a combination of these two products appeared to be additive and possibly synergistic.
- More studies on this pest are required.

General

- The consistency of results produced by the different experimental set-ups varies, with consistency being greatest in the experiments on the use of treatments applied to the compost to control cabbage root fly and least in experiments with diamond-back moth.
- With the exception of treatments applied to the compost to control cabbage root fly, further repeats would be desirable to increase confidence in the findings.
- Time had to be spent evaluating individual treatments in preliminary experiments, which whilst not directly related to biopesticide combinations, has increased understanding of the products and provided information that is complementary to the data collected in the SCEPTRE project.

- The study has shown that there are some combinations where control is improved by the simultaneous application of two biopesticides and that this effect may be additive or, in some cases, synergistic.
- The experiments with reduced doses of Tracer in compost treatments to control cabbage root fly were undertaken to determine whether there were possible synergistic or additive effects of insecticides and biopesticides and this appears to be the case for this pest and method of application.
- There is a need for further studies to explore more pest x biopesticide combinations and to determine how these might be used effectively in the field. This is particularly related to the persistence of such biopesticide treatments which has not been tested at all in this project.

Knowledge and technology transfer

An article was written for HDC News in 2012.

Glossary

Synergism: the interaction or cooperation of two or more organizations, substances, or other agents to produce a combined effect greater than the sum of their separate effects.

Additive: characterized by, relating to, or produced by addition: *the combination of these factors has an additive effect.*

Source: Oxford Dictionaries on-line.

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Appendix

Further details on some of the biopesticide products described in Section 1 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 including 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 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.ecograpе.com/neemazal