Project title:	Novel approaches for the management of leaf and bud nematodes (*Aphelenchoides* spp.) in hardy nursery stock
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DISCLAIMER

AUTHENTICATION

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

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GROWER SUMMARY

Headline

Several products, including insecticides and an elicitor of plant defences, reduced foliar nematode multiplication on a range of ornamental plants evaluated using a curative (on plants showing nematode symptoms) and preventative (on plants initially free from nematode infection) approach. These treatments led to improved post-treatment appearance for the plants compared with untreated plants. Treatments applied to the soil were also effective at reducing this route of nematode infection into plants.

Background

Foliar nematodes, also called leaf and bud nematodes (LBN), *Aphelenchoides* spp. cause serious damage to many ornamental plants grown in greenhouses, nurseries and field throughout the United States, Canada, and Europe. They are a significant foliar pest of hardy nursery stock plants (over 700 host species), whose feeding results in angular-shaped blotches on the leaves which are defined by the veins and often accompanied by leaf distortion. In the UK, *Aphelenchoides ritzemabosi* and *A. fragariae* are the two main foliar nematode species of economic importance.

The infestation usually starts at the base of the lower leaves where humidity is highest, and spreads upwards. LBN cause chlorotic lesions that can become necrotic. The lesions eventually turn blackish-brown and affected parts may shrivel. If buds or young leaves are infested, they may not develop properly and could become deformed. Flower development may also be affected. As ornamentals are sold for their aesthetic value, these plants are often unsaleable, making foliar nematode damage very costly for ornamental growers.

LBN problems have become important because of the withdrawal and subsequent loss of systemic nematicides, increased nursery production of vegetatively-propagated plants, and long distance movement of plants. A range of alternatives for the control and management of LBN have been evaluated previously (HNS 131 Final Report. Horticultural Development Company), the results suggesting that Dynamec (abamectin) is ineffective against LBN and that Vydate 10 G (oxamyl) was probably the most effective available product (Bennison, 2007)

Vydate 10 G currently has an extension of authorisation for minor use (EAMU) on protected ornamental plants. However, Vydate 10 G is not compatible with IPM programmes. Its use also requires precautions for theoperator and environmental protection, with a re-entry time to treated glasshouses and a harvest interval. In addition, the EAMU is uncertain after December 2017, hence the need for alternatives to Vydate 10 G to manage leaf and bud nematodes.

This project therefore developed new approaches for the management of these nematodes in hardy nursery stock by evaluating individually, and in combination, the efficacy of products derived from plant extracts and currently approved pesticides to reduce nematode infestation on plants.

The project has also evaluated the application of products that act as elicitors of plant defences to determine whether they can confer levels of resistance to nematodes. Elicitors are natural and synthetic compounds that induce defence responses in plants triggered by pathogen infection. These studies were carried out in the laboratory, glasshouse and at outdoor conditions in grower's nurseries.

Summary

After a series of tests conducted in the laboratory and with glasshouse bioassays earlier in the project, which had identified several potential products to be used for foliar nematode management, we proceeded further with field evaluation of foliar treatments and soil application to confirm product's efficacy for foliar nematode management on a range of plants in both glasshouse and outdoor conditions.

These potential products which include HDC 069 (plant extract), Movento (spirotetramat), Dynamec (abamectin) and HDC 071 (plant defence elicitor) were evaluated using robust nematode inoculation and product application methods to develop a novel nematode treatment programme. Products were tested individually and in combination as an integrated management approach to assess their efficacy using curative and preventative approaches.

The curative approach was carried out on a range of naturally infested ornamental plants which include *Gunnera mannicata*, *Cistus*, *Bergenia*, *Brunnera macrophylla*, *Dryopteris affinis*, *Astrantia major,* Japanese anemone and *Budlleja daviidi*, while the preventative approach programme was used to evaluate treatment efficacy on nematode-free plants of Japanese anemone and *Buddleja daviidi*. Furthermore, a soil treatment programme was evaluated to prevent nematode movement from soil to clean plant using Vydate 10 G (standard), HDC 070 (a plant extract), HDC 088 (bionematicide), HDC 084 (biopesticide) and HDC 101 (nematicide) in a glasshouse study.

The field results show that the foliar application programme of Dynamec, Movento, and HDC 071 as single treatments or in combination with HDC 071 as a curative treatment to already infested plants significantly reduced nematode populations by 61–97% when compared with an untreated control. Results on preventative treatments (with nematode-free plants inoculated with nematodes after the first treatment) showed a significant reduction in nematode multiplication on treated plants compared with untreated control. Nematode populations on Japanese anemone range between 157-609 / 5 g leaf from treated plants and 2570-5005 / 5 g leaf from untreated control. Buddleja plants gave 72-540 / 5 g leaf from treated plants while untreated control had 2454 / 5 g leaf.

The overall results show that Movento (spirotetramat), Dynamec (abamectin), HDC 069 (plant extract), HDC 071 (elicitor) in a foliar application programme resulted in effective management of foliar nematodes on a range of ornamental plants. HDC 071 in combination with Spirotetramat, Dynamec and HDC 069 also enhanced the management of foliar nematodes on a range of ornamental plants. The products above can limit nematode multiplication in already infected plants, and on asymptomatic plants. Results from applyin g treatments to the soil to target nematode infection via this route showed that SC 400, HDC 070, HDC 088 and HDC 084 alon g with Vydate 10 G (as the standard) significantly reduced nematode movement from infected soil to the plants and subsequent nematode infection compared with the untreated control.

Financial Benefits

Even though we cannot give an accurate financial benefits, plants treated with the products Movento (spirotetramat), Dynamec (abamectin), HDC 069 (plant extract) and HDC 071 (elicitor) were seen to be effective for foliar nematode management. Depending on the plants and size of the nursery, our previous discussions with growers suggest that an average size nursery could save between £2500 to £15,000 per annum, despite the additional cost of using the products mentioned above.

Action Points

Cultural control methods are an important component of the management of LBN within integrated pest management (IPM) programmes. The most effective of these is a programme of high crop hygiene as foliar nematodes can survive for several years in infested dried leaf debris.

Cultural control programmes should include:

- the removal and destruction of infested plants and debris
- avoiding replanting in contaminated soil

- sterilisation of pots and equipment
- if possible avoid the use of overhead irrigation and misting systems which create ideal conditions for nematode infection
- the use of Vydate 10 G (oxamyl) or the adoption of the products and programmes outlined in this report where necessary

SCIENCE SECTION

Introduction

Foliar nematodes (*Aphelenchoides* spp.), also called leaf and bud nematodes (LBN), are microscopic roundworms that live in leaf tissue and cause significant injury to many ornamental plants (Winslow, 1960). Foliar nematodes overwinter in the soil or in plant parts including rhizomes, bulbs, and buds, but generally not in the roots (Jagdale and Grewal, 2006). They are a significant foliar pest of hardy nursery stock plants (over 700 host species) whose feeding results in angular-shaped blotches on the leaves (Figure 1) which are delineated by the veins and often accompanied by leaf distortion (Kohl, 2010 *et al*; Kohl, 2011).

Figure 1. Symptoms of leaf and bud nematode infestation in Japanese anemone.

While foliar nematodes account for economic losses in many plant species, they are particularly detrimental to the floriculture industry, as the symptoms of nematode infection can directly decrease the market value of ornamental plants (An *et al*., 2017). In the UK, *Aphelenchoides fragariae and A. ritzemabosi* are the two main foliar nematode species of economic importance.

In the past, chemical treatments such as aldicarb, diazinon, parathion and oxamyl have been used for effective management of foliar nematodes (Johnson and Grill, 1975). However, due to regulatory issues and environmental concerns, most of these chemicals are no longer available to growers today. Modern chemical control methods have variable results, depending on the plant being treated (Bennison, 2007; Young, 2000). Chemical treatments tested may be successful at killing nematodes in a water suspension, but then fail to control nematodes when applied to infected leaves (Jagdale and Grewal, 2002; Jagdale and Grewal, 2004).

After an initial bioassay test on some products to determine whether they had contact mortality against nematodes, experiments were conducted to: (1) investigate the effect of two elicitor products HDC 071 and HDC 072 for their potential to induce plant defences, thereby conferrin g levels of resistance to multiplication of the foliar nematode (*A. fragariae*) on Weigela and Japanese anemone plants in glasshouses; (2) study the application of the elicitor – HDC 071 – with three treatment programmes to manage foliar nematode (*A. fragariae*) on Japanese anemone in a glasshouse; (3) investigate curative and preventative treatment programmes for the management of foliar nematode on nematode-free and naturally infested plants in growers nurseries using HDC071, Dynamec (abamectin), Movento (spirotetramat) and HDC 069 alone and in combination with HDC 071; (4) investigate the relationship between leaf symptoms and nematode population in the leaf; and (5) novel soil treatments to reduce A. *fragariae* infestation via the soil in Japanese anemone plants in a glasshouse study.

Materials and methods

Experiment 1

Contact mortality test of pesticide products for the management of *A*. *fragariae* in a laboratory bioassay.

The objective of this experiment was to investigate the contact effect of available products on *A. fragariae* in water

1.1 Materials

Nematodes: The nematodes (*Aphelenchoides* spp) were isolated from infected evergreen fern (*Woodwardia fimbrata*). *A. fragariae* was identified by morphology features and confirmed by molecular identification through nematode DNA extraction, using PCR techniques with Primers and Gel electrophoresis by Gel-Imager.

Nematodes were extracted from infested leaf tissues using the following method: - The leaves were cut into 1cm² pieces and soaked in tap water for 24h at room temperature. The nematodes that emerged from the leaf pieces were recovered using nested sieves of 20 mesh (850 µm) and 500 mesh (25 µm) and collected in a beaker. The suspension was left for 2 hours, and excess water was reduced on top of the suspension. Nematode populations were adjusted to 200 mixed stage individuals per ml by counting with a microscope. The nematodes were used within 2-3 days for laboratory experiments.

Pesticides: A range of biological and chemical pesticides were collected from agrochemical Companies in the UK (see Table 1). No recommendation specific to *Aphelenchoides* is available for these pesticides (except oxamyl); therefore the products were prepared based on the recommendations made by the companies for the management of insects, mites and other pests.

1.2 Method

An aliquot (4 ml) of a solution of each treatment (in distilled water) was transferred into each petri dish and 4 ml of a suspension containing 800 living nematodes was added to each dish to achieve a desired percentage active ingredient level for each treatment. The control was set up with nematode water suspension used as above while ordinary distilled water was used instead of concentrations of chemical and biological products. The percentage nematode mortality was recorded at 24, 48 and 72 h after exposure. At each observation, a thoroughly mixed 2 ml sub-sample from each dish was transferred into a 5cm diameter dish containing 10 ml of water and held at room temperature for 72 h for the recovery of nematodes.

Numbers of live and dead nematodes were counted after concentrating the suspension to 3ml. Death was defined by the complete lack of movement even after prodding with the tip of a micropipette.

Data analysis: Arcsine-transformed values of mean mortality data from this study were subjected to analysis of variance (ANOVA) using a General Linear Models Procedure (Minitab 17). Significant differences between treatments were determined with Tukey’s multiple range test at $P < 0.05$.

Table 1. Trade and chemical names, formulations and sources of pesticides used in this experiment

			Concentration level		
Product name	*Active ingredients*	*Manufacturers*	1*	2	3
Movento	*Spirotetramat*	Bayer Cropscience	0.5L/600L/ha	0.4L/600L/ha	0.3L/600L/ha
HDC 068	*biopesticide*	n/a	50L/500L/ha	25L/500L/ha	12L/500L/ha
Jet 5	*Peroxyacetic acid*	Certis	800ml/100L	650ml/100L	570ml/100L
HDC 069	*biopesticide*	n/a	743mg/L	550mg/L	350mg/L
Vydate10G	*Oxamyl*	DuPont	55kg/ha	40kg/ha	10kg/ha
HDC 070	*biopesticide*	n/a	4ml:96ml	4ml:120ml	4ml:144ml
Cercobin	*Thiocyanate*	Certis	1.1kg/500L/ha	900g/500L/ha	750g/500L/ha
Dynamec	*Abamectin*	Syngenta	50ml/100L	25ml/100L	10ml/100L

*Note that level 1 is the manufacturers recommended dosage for use against nematodes / other pests while 2 and 3 are reduced doses.

Experiment 2: Investigate two elicitor products HDC 071 and HDC 072 for their potential to induce plant defence against the multiplication of foliar nematode (A. fragariae) in Weigela and Japanese anemone plants under glasshouse conditions.

An experiment was conducted to investigate the efficacy of two elicitor products HDC 071 and HDC 072 for inducing plant defences against the foliar nematode *A. fragariae*. It was hypothesised that the elicitor products could trigger plant defences, increase plant resistance and reduce the multiplication of *A*. *fragariae* in leaves inoculated with nematode.

2.1 Materials

Nematodes: The nematode (*A. fragariae*) used in this test were extracted as described in section 1.1 above.

Elicitors: HDC 071 and HDC 072 were supplied by Syngenta Crop Protection UK Ltd.

Plants: Nematode-free plants (Weigela and Japanese anemone) were used in this test. The plants were grown in a glasshouse in individual two litre pots until they had at least six leaves. Fifteen plants were used for each species with 5 replicates per treatment.

2.2 Method

Glasshouse trials were conducted to evaluate the efficacy of two elicitor products on foliar nematode. The three treatments were: (i) HDC 071 + nematode (ii) HDC 072 + nematode, and (iii) Nematode only (control). All of the treatments were arranged in a randomised design with five replicates per treatment. Based on manufacturer's instructions, both elicitor products were dissolved in 1 litre of water. An adjuvant (Tween 20) was added at a dose of 100 µl per 100 ml of water to both elicitor and control treatments. Elicitor products were sprayed as a foliar application on plants until run off. Control treatments were sprayed onto plants until run off. Plants were left for 48 h before the direct inoculation of nematodes (*A. fragariae*) onto leaves. Using the method described by Zhen *et al*, (2012), three randomly selected leaves per plant were used. Leaves were injured by making 10 perforations of the leaf surface with a sharp needle scattered between veins at the upper side of the leaf. Leaves were wrapped with wet tissue paper (Kimpwipes, 11 by 21 cm; Kimberly–Clark). An aliquot (3 ml) suspension containing 200 live nematodes was dispensed onto the wet tissue paper. The plants were covered with black plastic bags after inoculation in order to maintain moist conditions. The bags and tissue paper were removed after 72 hours. All plants were completely randomised and kept in glasshouse condition of 25±2°C. Nematode multiplication was observed at 3, 5 and 8 weeks after inoculation by leaf extraction using the extraction method outlined above in Experiment 1, Section 1.1 to assess nematode multiplication.

Data analysis was carried out using arcsine-transformed values of percentage nematode reproduction and subjected to analysis of variance (ANOVA) using a General Linear Models Procedure (Minitab 17). Significant differences between treatments were determined with Tukey's multiple range test at $P < 0.05$. Variables considered include treatments, time (week) and nematode count.

Experiment 3: Application of the elicitor HDC 071 within different treatment programmes to manage foliar nematode (A. fragariae) on Japanese anemone in a glasshouse test

Objective of this experiment was to evaluate the effect of different treatment programmes against single dose of elicitor on multiplication of nematodes in Japanese anemone

3.1 Materials

Plants and nematodes used were from the same source as described from the 2 previous experiments (Sections 1 and 2). HDC 071 – a synthetic elicitor, supplied by Syngenta Crop Protection UK Ltd.

3.2 Methods

This experiment had five treatments, with each replicated five times. Plants were arranged in a randomised design. The two factors considered were nematode and HDC 071. Treatments (Trt) were:

- Trt 1 = HDC 071 (+ nematode) at week 1 (x 1 application)
- Trt 2 = HDC 071 (+ nematode) at week 1 and 3 (x 2 applications)
- Trt 3 = HDC 071 (+ nematode) at week 1 and 5 (x 2 applications)
- Trt 4 = HDC 071 (+ nematode) at week 1, 3 and 5 (x 3 applications)
- Trt 5 = Nematode only - control

Treatments with HDC 071 and control had an adjuvant (Tween 20) at a dose of 100 µl per 100 ml of water. All treatments were applied as a foliar spray on plants to run off. Two days after the first HDC 071 application in Week 1, 200 live nematodes were inoculated directly onto three randomly selected leaves per plant (as described in Experiment 2, Section 2.2). Plant maintenance and duration of sampling were the same as described in Experiment 2, Section 2.2.

Data analysis: as in the previous experiments, arcsine-transformed values of percentage nematode reproduction from this study were subjected to analysis of variance (ANOVA) using a General Linear Models Procedure (Minitab 17). Significant differences between treatments were determined with Tukey's multiple range test at $P < 0.05$.

Experiment 4: To investigate the curative approaches for the management of foliar nematode on naturally infested plants in grower's nurseries

The objective of this study was to determine the curative potential and efficacy of selected novel products as post-infection control measures for foliar nematodes (*A. fragariae*) on naturally infested hardy nursery plants.

4.1 Materials

Plants: Seven naturally infected plants species - *Astrantia major*, *Gunnera mannicata*, *Bergenia crassifolia*, *Brunnera macrophylla*, *Astilboides tabularis*, *Dryopteris filix-mas* and *Anemone hupehensis* were isolated in a nursery in Cuxham, Oxfordshire, while *Buddleja daviidi* and Cistus were isolated at a nursery in Hereford. These infected plants were isolated based on visual symptoms of nematode infection shown on the leaves. The symptoms observed on the leaves include blotches, discolouration, angular leaf spots and brown lesions characteristically contained in the patterns of the leaf veins. Leaf samples randomly taken from a selection of plants confirmed infestation by *A. fragariae*. Plants were kept in a quarantine area isolated from other 'clean' plants.

Pesticides: Products used were the same for both locations. These include Movento (spirotetramat) - a systemic insecticide supplied by Bayer CropScience Limited UK; Dynamec (abamectin) – a contact and translaminar insecticide supplied by Syngenta Crop Protection UK Ltd; and HDC 071 – a synthetic elicitor, supplied by Syngenta Crop Protection UK Ltd. These products were used as individual treatments and also in combination with HDC 071.

Method

The field trials were conducted in two locations to evaluate the efficacy of different products as individual treatments and in combination with HDC 071 on foliar nematode infected plants.

Field study 1 with seven infected plant species had six treatments with four replicates per plant species (Figure 2).

Field study 2 with Infested *Buddleja daviidi* and *Cistus corbariensis* had the same six treatments as in Field Study 1, but with four replicates for *Buddleja* (Figure 3) and ten replicates for *Cistus* (Figure 4).

Figure 2. Layout of the experimental trial with randomised block design at Babylon Nursery, Cuxham – Oxfordshire – Field Study 1

Figure 3. Layout of *Buddleja* experimental plants at Wyevale Nurseries - Field Study 2

Plants in the two locations were arranged in 2 litre and 4 litre pots in a randomised block design. The same number of treatments (six) was used in both Field Study 1 and 2.

Figure 4. Layout of Cistus experimental plants at Wyevale Nurseries - Field Study 2

The six treatments were (i) Movento, (ii) Dynamec, (iii) HDC 071, (iv) Movento + HDC 071, (v) Dynamec + HDC 071, and (vi) control (Water). Based on manufacturer's instructions, all products were prepared in 1 litre of water with doses of Movento (spirotetramat) at 1.67 ml / 1 litre of water (equivalent to 0.5 L/ha/300 L water); Dynamec (containing 18 g/l abamectin) at 500 µl / 1 litre water (equivalent to 50 ml/ha/100 L water) and HDC 071 at 0.175 g / 1 litre water (equivalent to 35 g/ha/200 L water). An adjuvant (0.01% Tween 20) was added at the rate of 100 µl per 1 litre of water to all treatments including the water control. A hand-held pressurised sprayer (1 litre) supplied by Scientific Laboratory Supplies (SLS) UK, was used to spray products as a foliar application on each plant until all parts of the plant were well covered (to run off). control treatments had only water + Tween 20 sprayed on the plants.

Movento and Dynamec were applied two times while HDC 071 had three applications. All the treatments were applied together on the same day at the start of the trial. Movento and HDC 071 treatments had a 14 day interval between each application while Dynamec had a 7 day interval between applications. Plants were left in the nursery under protected glasshouse conditions throughout the duration of study. Plants were watered daily, and assessed for nematode population by leaf sampling before the first treatment application and at eight weeks after the final treatment application. Leaf sampling was carried out on plants to determine their initial (referred to as p_i) and final (referred to as p_f) nematode population. Leaf samples (including both symptomatic and asymptomatic) were randomly taken per infested plant during both (p_i and p_f) sampling periods. Leaves were cut into 1 cm² sections, and 5 g fresh weight of leaf sample was used for nematode extraction. Nematodes were extracted from leaves of plants using the method outlined in previous Experiment 2 in Section 2.

Visual scoring of nematode symptoms on leaves was carried out on each plant at the beginning and end of the trial, with a value of '0' used for clean (no symptoms) and '10' as maximum symptoms score per leaf.

Data analysis

Data of nematode population and leaf symptom score at pre-treatment (p_i) and post-treatment (p_f) were analysed by analysis of variance (ANOVA) using a General Linear Models Procedure (Minitab 17). Variables include treatment, plant species, leaf symptom score and nematode population count (initial and final). Significant differences between treatments were determined with Fisher's multiple range test at $P < 0.05$.

Experiment 5: To determine the efficacy of Movento and Dynamec (selected foliar insecticides), HDC 069 (plant extract) and HDC 071 (synthetic chemical elicitor), as individual treatments, and in combination for preventative management of A. fragariae on nematode-free plant

The objective of this study was to determine the preventative potential and efficacy of selected products as pre-infection control measures for foliar nematodes (*A. fragariae)* on clean 'nematode-free' hardy nursery plants.

The trials were conducted in three locations (2 grower nurseries at Cuxham and Hereford), and the Plant Growth Unit at SRUC Edinburgh, United Kingdom.

5.1 Materials

*Nematode***.** The nematodes (*Aphelenchoides fragariae*) were sourced and extracted from infected Japanese anemone plants (Honorine Jobert) maintained at SRUC as outlined in Experiment 2. *A. fragariae* was identified based on morphological and morphometric features (Siddiqi, 1975). Nematodes for the study were extracted from the infected anemone leaves according to the technique described by Zhen *et al* (2012) as explained in Experiment 2 – Section 2. The nematodes were used for the studies within 2-3 days after extraction.

*Plants***:** Certified nematode-free Japanese anemone were used in two locations (Cuxham and Edinburgh) while *Buddleja daviidi* was used in Hereford. Leaf samples taken at random from these plants confirmed that they were nematode free. These plants were kept in an isolated area from other nursery plants to avoid any potential nematode infestation.

Pesticides: Products used were the same for the three locations: Movento (spirotetramat) - a systemic insecticide supplied by Bayer CropScience Limited UK; Dynamec (abamectin) – a contact and translaminar insecticide supplied by Syngenta Crop Protection UK Ltd; HDC 069 **-** a plant extract with anti-feedant and repellents properties known to have toxic effects on insects; and HDC 071 – a synthetic elicitor, supplied by Syngenta Crop Protection UK Ltd. These products were used as individual treatments and also in combination with HDC 071.

5.2 Method

The three locations had the same number of treatments (eight) with five replicates each (Figures 5, 6 and 7).

Figure 5. Layout (left) of the foliar nematode (preventative) experimental trial and treatment application (right) on Japanese anemone plants at Babylon Nursery, Cuxham, UK – location one

Figure 6. Pictures of layout on *Buddleja* experimental plants at Wyevale Nurseries Hereford, UK - location two

Figure 7. Layout of nematode preventative study on Japanese anemone at SRUC Plant Growth Unit, Edinburgh, UK – Location three.

Plants in 2 litre pots of the three locations were arranged in a randomised block design (Figures 5, 6 and 7) with same 8 treatments and 5 replicates per trial.

The eight treatments were (i) Movento, (ii) Dynamec, (iii) HDC 071 (elicitor), (iv) HDC 069 (v) Movento + HDC 071, (vi) Dynamec + HDC 071, (vii) HDC 069 + HDC071 and (viii) control (Water). Based on manufacturer's instructions, all products were prepared in 1 litre of water with doses of Movento (spirotetramat) 1.67 ml / 1 litre of water (equivalent to 0.5 L/ha/300 L water); Dynamec (containing 18 g/l abamectin) – 500 µl / 1 litre water (equivalent to 50 ml/ha/100 L water); HDC 069 - 2.5ml / 1 litre water (equivalent to 3 L/ha/1200 L water) and HDC 071 - 0.05 g / 1 litre water (equivalent to 50 g/ha/1000 L water). In addition, an adjuvant (0.01% Tween 20) was added at the rate of 100 µl per 1 litre of water to all treatments including the control (water).

Apart from an additional treatment of HDC 069, which had three applications and an interval of 7 days between applications in this study, the method and timing of treatment application were the same as carried out in experiment 4 Section 4). Control treatments had only water + Tween 20 sprayed on the plants. Nematode inoculation was carried out 4 days after the first treatment application on all the plants as suggested by Cole (1999). Inoculation was carried out directly on three randomly selected leaves (Zhen *et al,* 2012). This method was successfully used in previous studies of experiment Sections 2 and 3.

Plants were left in the nurseries and glasshouse under ambient conditions throughout the duration of the study. Daily watering was carried out on the plants.

Leaf sampling was carried out on all the plants eight weeks after nematode inoculation to determine the nematode population in the leaves. Nematode extraction was carried out according to the technique described (Section 2) (Zhen *et al.,* 2012).

Visual scoring of nematode symptoms on the three inoculated leaves was carried out on each plant at the end of the trial. A value of '0' was used for clean (no visual symptoms) and '10' as maximum symptom score per leaf.

Statistical analyses

Data of nematode population and leaf symptom at sampling were analysed by analysis of variance (ANOVA) using a General Linear Models Procedure (Minitab 17). Variables include treatment, plant species, leaf symptom score and nematode population count. Significant differences between treatments were determined with Fisher's multiple range test at P <0.05.

Experiment 6: To investigate the relationship between the visual symptoms of Aphelenchoides fragariae leaf infection and corresponding nematode population on leaves of Weigela and Japanese anemone in a glasshouse study

6.1 Materials

Nematode: The nematodes (*Aphelenchoides fragariae*) were isolated from infected evergreen fern (*Woodwardia fimbrata)* and multiplied on the leaves of clean Japanese anemone. Nematodes species used were from same source as Section 2.

Plants: Certified nematode-free *Weigela* 'Bristol Ruby' and Japanese anemone were used in this test. The plants were grown individually in 2 L pots until they had at least six leaves.

6.2 Method

This study had 12 plants as replicate for each plant species including the control. Nine plants were inoculated with nematodes while three plants were used as non-inoculated controls per species. All plants were completely randomised and kept in the glasshouse for 10 weeks at 22 ± 2°C - 25 ± 2° C. Three leaves were randomly selected per plant. Direct nematode inoculation of 50, 100 and 200 nematodes per leaf was carried out according to the method described by Zhen *et al*, (2012), as used in the previous Section 2. The leaves of three control plants were not inoculated. Leaf sampling for nematode population was undertaken from 3 weeks after inoculation when the symptoms were noticeable.

During observations, different degrees of nematode symptoms were observed on leaves of both plant species. Leaves showing symptoms of various degrees of foliar nematode infection were carefully selected, scored and categorised into 6 groups (%) according to their degree of infection based on total leaf area with the aid of Computer Software (Image J-win64). The 6 categories include (1) = 0% - (clean), (2) = 1-10%, (3) = 10-15%, (4) = 25-50%, (5) = 50-75% and (6) = 75 -100%. Five leaves were randomly selected from each of the 6 categories for nematode extraction to obtain the nematode population in relation to their degree of symptoms. Nematode extraction was carried out according to the technique described (Section 2) Zhen *et al.,* (2012).

Experiment 7: To investigate novel soil treatment for the management of Aphelenchoides fragariae on Japanese anemone in a glasshouse study

The objective of this study was to determine the potential of several products as control measures for limiting foliar nematode infection of plants from nematode-infected soil on clean 'nematode-free' plants.

7.1 Materials

Nematode: The nematodes (*Aphelenchoides fragariae*) were isolated from infected evergreen fern (*Woodwardia fimbrata)* and multiplied on the leaves of clean Japanese anemone. Nematodes used were from same source as used in the above Section 2.

Plants: Certified nematode free plants of Japanese anemone were used for this glasshouse study. Japanese anemone was chosen since it exhibits moderate susceptibility to *Aphelenchoides fragariae*. Plants were grown in 2 litre pots containing sterilised compost (Fisons Levington) and maintained in a glasshouse condition of 22 ± 2°C - 25 ± 2°C.

Pesticides: Based on manufacturer's instructions, all products were prepared in 1 litre of water and applied to 2 L pot with doses as:

- Vydate (Oxamyl) 0.11 g / pot (11 g/1 L water) - equivalent to 55kg/ha
- HDC 068 (660 µl in 100 ml water), 5 ml / pot - equivalent to 50 L/ha/500 L water
- HDC 070 (1:24), 5 ml / pot- equivalent to 4 ml/96 ml product/water
- HDC 088 (0.16 g/1 ml/2 L pot) 16 g / 1 L water - equivalent to 80 kg/ha/500 L water
- HDC 084 (76 µl/2 L pot), 0.76 ml / 1 L water - equivalent to 37.9 L/ha/500 L water
- HDC 101 (2.1 ml / 1L) - equivalent to 625 ml/ha/300 ml water; and
- Water (control) in 1 litre.

7.2 Method

This experiment was conducted to study the effect of drenching soil with a range of products to limit foliar nematode (*A. fragariae*) infection of plants via the soil infection route. Plants were laid out in a randomised block design with seven treatments and six replicates (Figure 8).

Figure 8. Layout of the soil treatment trial with randomised block design at SRUC Glasshouse, Edinburgh, UK.

The required dose rates of the products or water (as the control) were carefully drenched around the plant in the sterilised moist soil in each 2 litre pot. Twenty-four hours after the application of the soil treatments, 1000 mixed stages of nematodes (*A*. *fragariae*) in 3ml of water were carefully dispensed using a glass pipette round the surface of the soil in each pot, including the control (water) treatment. The plants were left in the glasshouse condition for 10 weeks at 22 ± 2°C - 25 ± 2° C. Observations were made at 5 and 8 weeks after nematode inoculation to assess nematode populations in leaves. At weeks 5 and 8, three leaf samples per plant were randomly collected at the base, middle and growin g point of the plant for nematode extraction. Nematode extraction was carried out according to the technique described (Section 2) by Zhen *et al* (2012). Nematode population from leaves is expressed as number per 5 g leaf. Soil sampling was carried out at week 8 after nematode inoculation to determine the population of *A*. *fragariae* in soil, with 30 g of soil collected from round the base of the plant per pot up to root zone and mixed thoroughly. Three 10 g sub-samples were collected from the total 30 g soil sample per pot. The 10 g sub-samples of soil were used for nematode extraction using Baermann funnel technique. Nematode numbers were expressed per 10 g soil (Jagdale and Grewal, 2002).

Data analysis**:** Nematode population values from both soil and leaf extraction were subjected to an analysis of variance (ANOVA) using a General Linear Models Procedure (Minitab 15). Significant differences between categories were determined with Fishers multiple range test at *P* <*0*.05.

Results

Experiment 1: Contact mortality tests of pesticide products for the management of A. fragariae in a laboratory bioassay

Results (Table 2 and Figure 9) showed that at 72h, HDC 068, HDC 070 and Dynamec had significant high mortality rates (>75%) in direct contact with the nematodes compared to the other products tested including the current EAMU for Vydate 10G. Some of the other products like HDCI 69, Movento and Vydate 10 G which had average contact mortality (Table 2), are known to have a systemic action within plants, therefore mortality could be gradual rather than immediate.

Table 2. Effect of biological and chemical pesticides at three different levels (doses) of concentrations in water on *Aphelenchoides fragariae* after 72 hours exposure

	Mortality (%) at 72h for 3 levels of Concentration		
Products	level 1*	level 2	level 3
Movento	8.8d	8.3de	7.4d
HDC 068	95.8a	83a	28.3b
HDC 069	33.8c	17.6cd	13.1cd
Jet5	40.5c	32.6b	11.4cd
Dynamec	94.4ab	78.8a	59.8a
Cercobin	10d	6.9de	7.1d
Vydate	32.6c	29.8bc	20.6bc
HDC 070	86.4b	74.1a	68a
Water	4d	4.5e	3.9d

Data are percentage mortality means of four replicates, and values in the same column followed by the same letter are not significantly different (Tukey's multiple range test, $P < 0.05$).

*Note that level 1 is the manufacturers recommended dosage for use against nematodes/other pests while levels 2 and 3 are reduced doses.

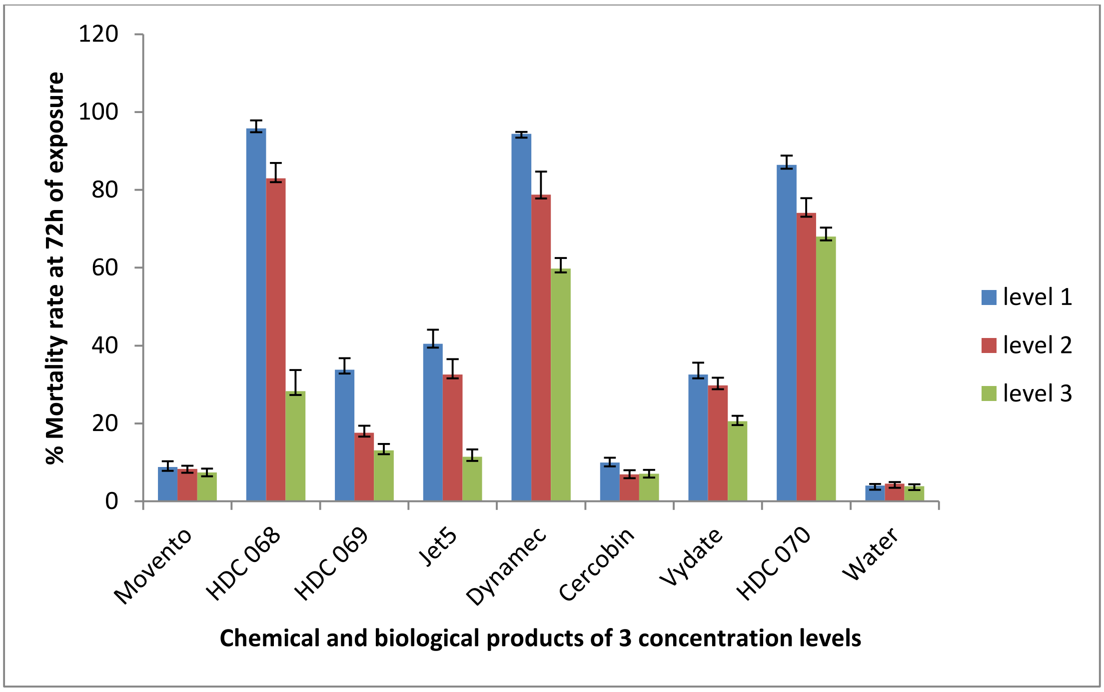

Figure 9. Contact mortality test of chemical and biological pesticides with three different levels of concentration on *Aphelenchoides fragariae* at 72h exposure in water. Error bars are standard error of the mean (±SE) of four replicates. Level 1 is the manufacturers recommended dosage for use against nematodes/other pests while levels 2 and 3 are reduced doses.

Experiment 2: Investigate two elicitor products HDC 071 and HDC 072 for their potential to induce plant defence against the multiplication of foliar nematode (A. fragariae) in Weigela and Japanese anemone plants under glasshouse conditions.

The elicitor treatment HDC 072 had a highest mean percentage reproduction rate (2706%) at week 3 on Japanese anemone (Figure 10), but dropped to the lowest value (343.5%) at week 5 between the three treatments (Table 3.). The elicitor treatment HDC 071 had a small increase in nematode multiplication on Japanese anemone (Figure 10) and had the lowest overall nematode population (324%) compared with HDC 072 (343.5%) during the study (Figure 10). The nematode reproduction in the control treatment was significantly higher ($P < 0.05$) compared to HDC 071 and HDC 072, despite the variability in the data. The control was also observed to have a steady percentage increase in nematode multiplication throughout the duration of the study in both plant species. Although the control had a low reproduction rate on Weigela, ranging from 25%, 63.4% and 72.1% at week 3, 5 and 8 respectively (Figure 10), the highest values of 540.5%, 1992% and 2082% were obtained on the control treatment during the same duration on Japanese anemone (Figure 10, Table 3).

Table 3. Test of two elicitor products on the reproduction of *A. fragariae* in a glasshouse when inoculated with 200 nematodes / leaf on Weigela and Japanese anemone. Data are percentage mean values of nematode reproduction of five replicates when inoculated with 200 nematodes / leaf (N = nematodes)

Duration	Treatment	Mean Nematode Reproduction (%) at 3, 5 and 8 week after inoculation	
		Weigela	Anemone
3 week	HDC 072+N	7.5	2706
	HDC 071 +N	24.8	324
	N	25.2	540.5
5 week	HDC 072+N	78.1	343.5
	HDC 071 +N	57.6	550
	N	63.4	2082
8 week	HDC 072+N	59.3	876
	HDC 071+N	30.8	751
	N	72.1	1992.5

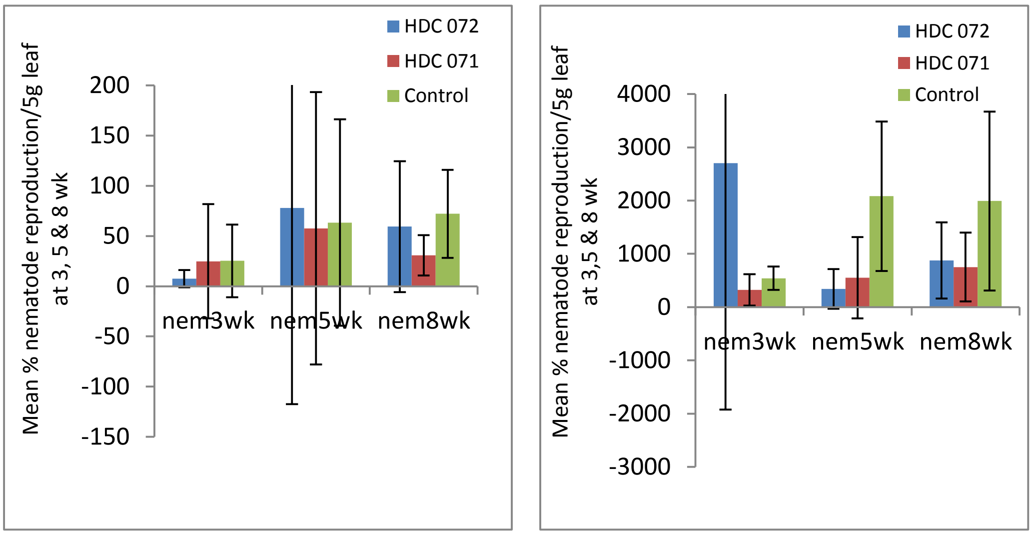

Figure 10 showing effect of two elicitor products on mean% nematode reproduction (± SEM) on Weigela (left) and Japanese anemone (right) plants at 3, 5 and 8 weeks when inoculated with 200 nematodes / leaf in glasshouse. Data are percentage mean values of five replicates.

Experiment 3: Application of the elicitor HDC 071 within different treatment programmes to manage foliar nematode (A. fragariae) on Japanese anemone in a glasshouse test

Figure 11 shows that three application doses of HDC 071 significantly reduced nematode multiplication (treatment 4, P <0.05), compared with other treatments, especially the control. Counts obtained at week 5 from all HDC 071 treatments showed a significantly lower nematode population during the study compared with the control (P <0.05); treatment 4 had the least mean value (49) at week 5, while treatment 1 had the highest mean value (591) at week 8 among other HDC 071 treatments. The highest nematode population mean (2580.3) during the study was recorded in the control treatment at week 8 (Figure 11).

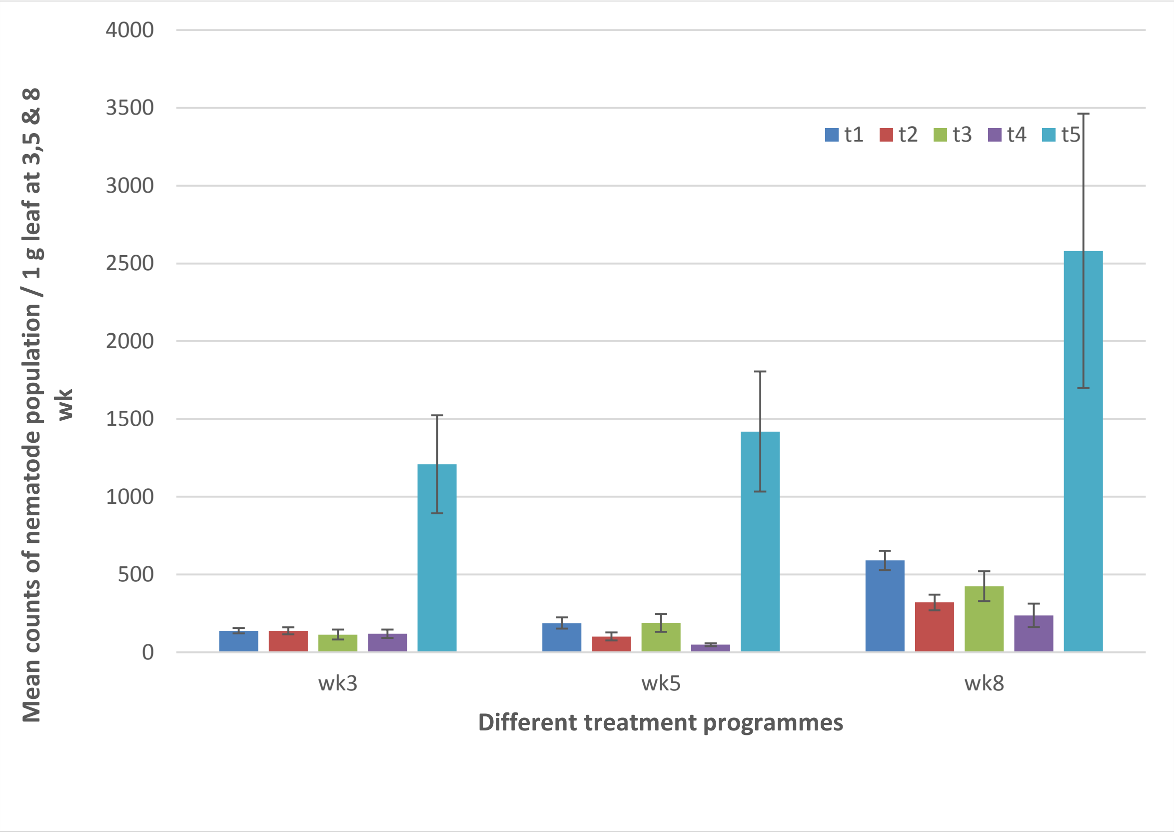

Figure 11. Mean counts of nematode population after an initial inoculation of 200 nematodes per leaf, on Japanese anemone at 3, 5 and 8 weeks with differing HDC 071 application programmes: t1 = HDC 071 (+ nematode) at week 1; t2 = HDC 071 (+ nematode) at week 1 and 3; t3 = HDC 071 (+ nematode) at week 1 and 5; t4 = HDC 071 (+ nematode) at week 1, 3 and 5; t5 = Nematode only. Error bars are standard error of the mean (±SE) of five replicates. Values are means of 5 replicates per treatment.

Experiment 4 - To investigate the curative approaches for the management of foliar nematode on naturally infested plants in grower's nurseries

Field Study 1.

Gunnera manicata: There was a significant (*P* <0.05) reduction in reproduction factor (RF) between all the treatments compared to the control (Table 4, Figure 12).

Table 4. Mean (± SE) Reproduction Factor (RF – p_i /p_f) from 5 g of leaf for *Aphelenchoides* infected *Gunnera manicata* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Nematode per 5 g leaf				
Gunnera manicata				
Treatments	***pi***	***pf***	***RF***	***ROC%***
Movento	740.1	557.5	0.78 b	89.1
Dynamec	1656.5	922.6	0.53 bc	92.6
HDC 071	923.9	575.7	0.59 bc	91.8
Movento+HDC071	828.9	140.9	0.20 c	97.2
Dynamec+HDC071	540.5	331.9	0.60 bc	91.7
Control	449.1	3254.2	7.16 a	

Values obtained as the reproduction factor (RF) range between 0.20 and 7.16. (Table 4) Movento + HDC 071 had the lowest RF (0.20) while the untreated control gave the highest RF of 7.16 (Table 4). Significant (P <0.05) reduction of the RF of nematodes was observed in all the treatments compared to the control (Figure 12).

Reduction of nematode population over the control (ROC) expressed as a percentage (Jagdale and Grewal, 2002), had the highest value with Movento + HDC 071 (97.2%) followed by 92.6% with Dynamec. The lowest value (89.1%) was obtained with Movento (Table 4).

In general, there were significant differences in RF (*P* <0.05) between all treatments and the control, and all the treatments caused a >80% reduction in nematode population in leaves compared with the control in this plant species (Table 4).

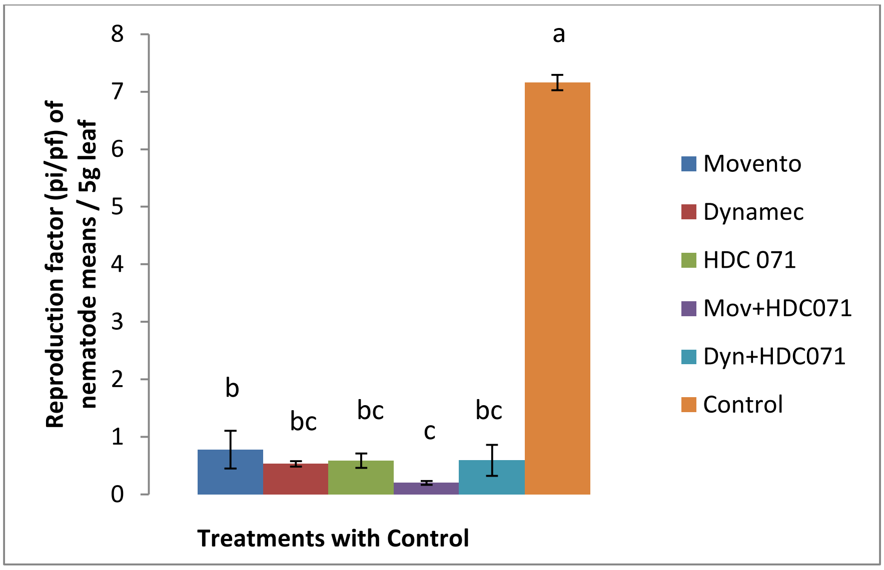

Figure 12. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected *Gunnera manicata* plants with the untreated 'control'. Bars with the same letter are not significantly different (Fisher's multiple range test, *P* <*0*.05).

Dryopteris filix-mas: There was a significant (*P* <0.05) reduction in reproduction factor (RF) between all the treatments and the untreated control (Table 5, Figure 13). An illustration of the leaf symptoms in the control and the Movento + HDC071 treatment is shown in Figure 13.

Table 5. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected *Dryopteris filix-mas* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Nematode per 5 g leaf				
Dryopteris filix-mas				
Treatments	$\boldsymbol{p_i}$	$\boldsymbol{p_f}$	**RF**	**ROC(%)**
Movento	2334.5	354.7	0.16 b	92.04
Dynamec	3931.5	503.4	0.14 b	93.33
HDC 071	4456.6	562.6	0.12 b	94.04
Movento+HDC071	2890.4	234.2	0.09 b	95.84
Dynamec+HDC071	5138.8	427.7	0.10 b	95.36
Control	5581.1	14785.2	2.07 a	

Values obtained as the reproduction factor (RF) range between 0.09 and 2.07 (Table 5). Movento + HDC 071 had the lowest RF (0.09) while the untreated control gave the highest RF value of 2.07 (Table 5). Significant reduction (*P* <0.05) of the RF of nematodes was observed in the leaves in all the treatments compared to the untreated control (Figure 13).

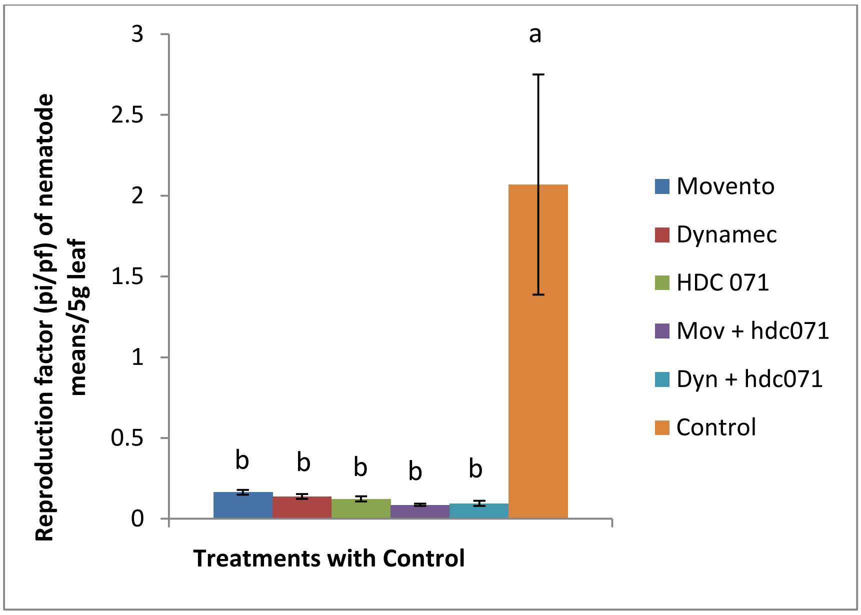

Figure 14. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected *Dryopteris filix-mas* plants with the untreated 'control'. Bars with the same letter are not significantly different (Fisher's multiple range test, *P* <*0*.05).

Reduction of nematode population over the untreated control (ROC) expressed as a percentage (%) indicate a range of values between 92.04 - 95.84% (Table 6). Movento + HDC 071 had the highest (ROC) value (95.84%) closely followed by 95.36% with Dynamec + HDC 071. The lowest value (92.04%) was obtained with Movento (Table 5).

In general, there were significant differences in RF (*P* <0.05) between all treatments and the control, and all the treatments caused a >90% reduction in nematode population in leaves compared with the control in this plant species (Figure 14; Table 5).

Bergenia cordifolia: Results of reproduction factor (RF) on nematode population showed a significant (*P* <0.05) reduction in all the treatments compared with the control (Figure 15; Table 6). Values obtained as the RF range between 0.30 with HDC 071 and 1.43 in control. The four treatments (Movento, HDC 071, Dynamec + HDC 071 and Movento + HDC 071) had no difference (*P* >0.05) between them. Significant reduction of the RF of nematodes (*P* <0.05) was observed in the leaves in all the treatments compared to the control (Figure15).

Figure 13. Untreated 'control' plant (left) of *Dryopteris filix-mas* before the application of treatment and treated plant (foliar applied Movento + HDC 071 - elicitor (right) after eight weeks of treatment application in the grower's nursery.

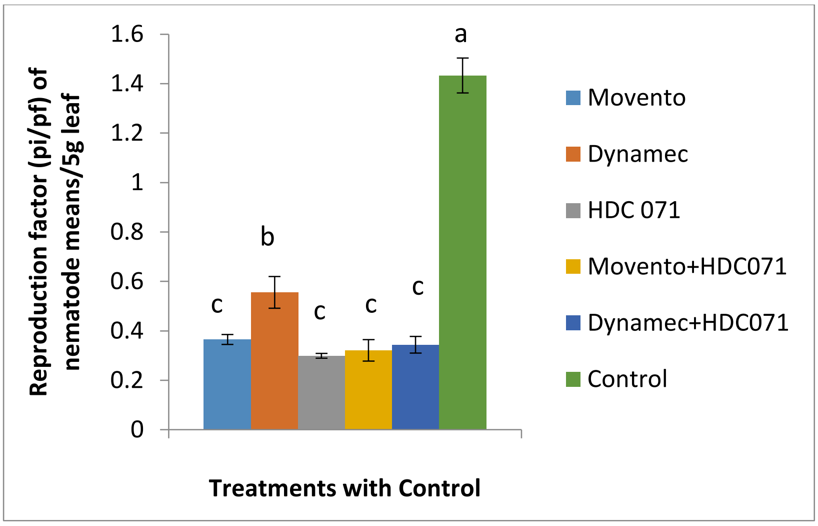

Figure 15. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected *Bergenia cordifolia* plants with the untreated 'control'. Bars with the same letter are not significantly different (Fisher's multiple range test, $P < 0.05$).

Reduction of nematode population over control (ROC) expressed as a percentage show that HDC 071 obtained the highest value of 79.1% followed by 77.6% in Movento + HDC 071. The lowest value (61.2%) was obtained with Dynamec (Table 6).

Table 6. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected *Bergenia cordifolia* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, P <0.05).

	Nematode per 5 g leaf			
	Bergenia cordifolia			
Treatments	p_i	p_f	***RF***	***ROC(%)***
Movento	2529.4	900.6	0.36 c	74.5
Dynamec	1529.9	994.2	0.56 b	61.2
HDC 071	2080.9	632.2	0.30 c	79.1
Movento+HDC071	1100.8	383.7	0.32 c	77.6
Dynamec+HDC071	1689.0	512.1	0.34 c	76.0
Control	1127.7	1595.5	1.43 a	

Generally, there was only a significant difference in RF results (P <0.05) between Dynamec and the other treatments excludin g control (Figure 15), while all the treatments caused a >60% reduction in nematode population in leaves compared with the control.

Astrantia major**:** There was a significant reduction (P <0.05) in reproduction factor (RF) between all the treatments and the untreated control (Figure 16; Table 7).

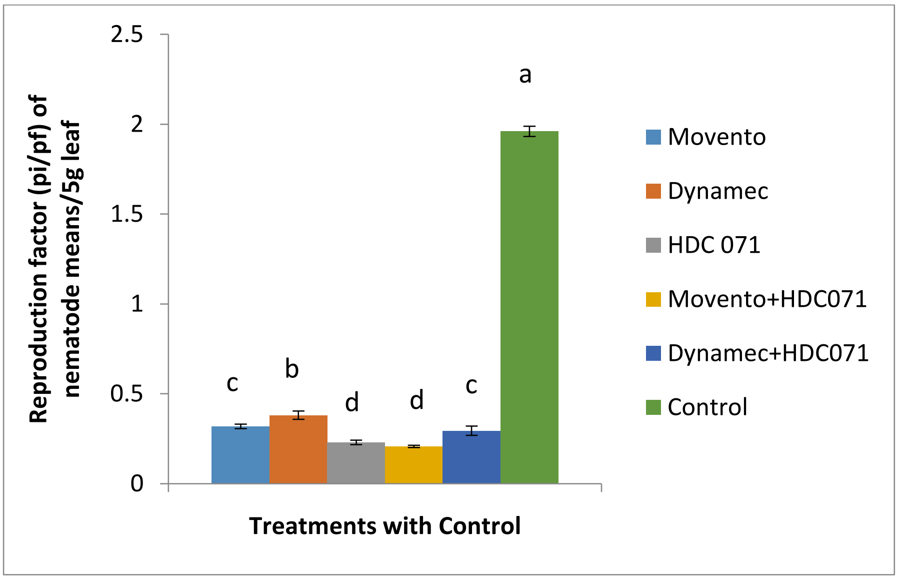

Figure 16. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected *Astrantia major* plants with the untreated 'control'. Bars with the same letter are not significantly different (Fisher's multiple range test, P <0.05).

The reproduction factor (RF) results range between 0.21 – 1.96. The untreated control had the highest RF value of 1.96 while Movento + HDC 071 had the lowest RF of 0.21. A significant (P <0.05) reduction of the RF of nematodes was observed in the leaves of all the treatments compared to the control (Figure 16).

Table 7. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected *Astrantia major* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <*0*.05).

Nematode per 5 g leaf				
Astrantia major				
Treatments	***pi***	***pf***	***RF***	***ROC(%)***
Movento	3988.9	1217.8	0.32 c	83.7
Dynamec	3656.9	1510.4	0.38 b	80.5
HDC 071	4972.8	1121.6	0.23 d	88.3
Movento+HDC071	3989.2	853.0	0.21 d	89.4
Dynamec+HDC071	4599.4	1382.8	0.29 c	85.0
Control	3654.2	7242.1	1.96 a	

Reduction of nematode population over the control (ROC) expressed as a percentage had the highest value with Movento + HDC 071 (89.4%) followed by 88.3% with HDC 071. The lowest value (80.5%) was obtained with Dynamec (Table 7).

In general, there were significant differences in RF (*P* <0.05) between all treatments and the control.

Brunnera macrophylla: There was a significant reduction (*P* <0.05) in reproduction factor between all the five treatments compared with untreated control (Figure 17; Table 8).

Values obtained as the reproduction factor (RF) range between 0.15 and 2.04. The control treatment had the highest RF (2.04) with the lowest value (0.15) obtained with Dynamec + HDC 071. A significant (*P* <0.05) reduction of the RF of nematodes was observed in the leaves in all the treatments compared to the control (Figure17).

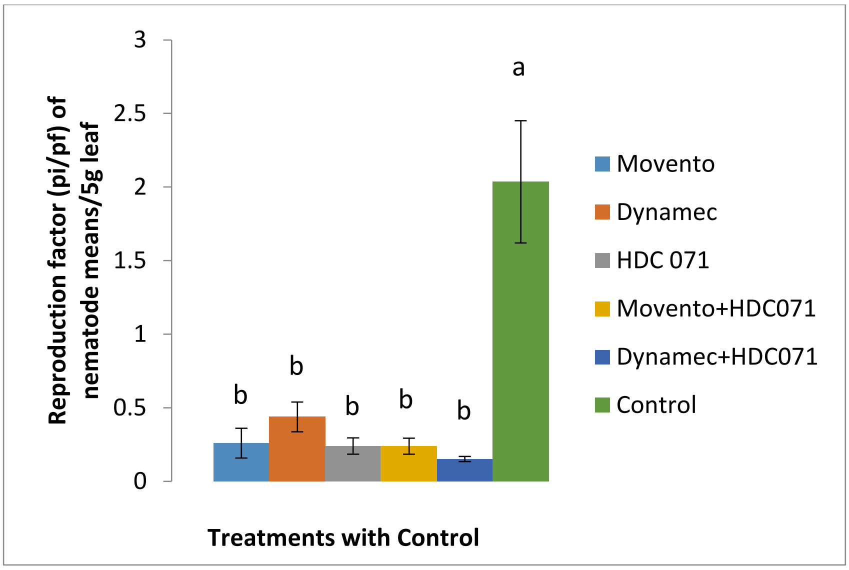

Figure 17. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected *Brunnera macrophylla* plants with the untreated 'control'. Bards with the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Reduction of nematode population over control (ROC) expressed as a percentage had the highest value of 92.5% with Dynamec + HDC 071 (Table 8). The lowest value (78.5%) was obtained with Dynamec. In general, there were significant differences in RF (*P* <0.05) between all the treatments and the control (Figure 17).

Table 8. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected *Brunnera macrophylla* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Nematode per 5 g leaf				
Brunnera macrophylla				
Treatments	p_i	p_f	***RF***	***ROC(%)***
Movento	8753.1	1131.8	0.26 b	87.2
Dynamec	2629.3	1519.3	0.44 b	78.5
HDC 071	6051.6	1077.1	0.24 b	88.2
Movento+HDC071	4221.0	821.8	0.24 b	88.2
Dynamec+HDC071	2892.3	484.8	0.15 b	92.5
Control	2454.0	3537.2	2.04 a	

Astilboides tabularis: there was a significant (*P* <0.05) reduction in reproduction factor (RF) between all the treatments and the control (Figure 18; Table 9).

The values obtained as the reproduction factor (RF) range between 0.17 and 2.92. Movento + HDC 071 had the lowest RF value (0.17) while the untreated control gave the highest RF of 2.92. There was a significant (*P* <0.05) reduction of the RF of nematodes observed in all the treatments compared with the untreated control (Figure 18).

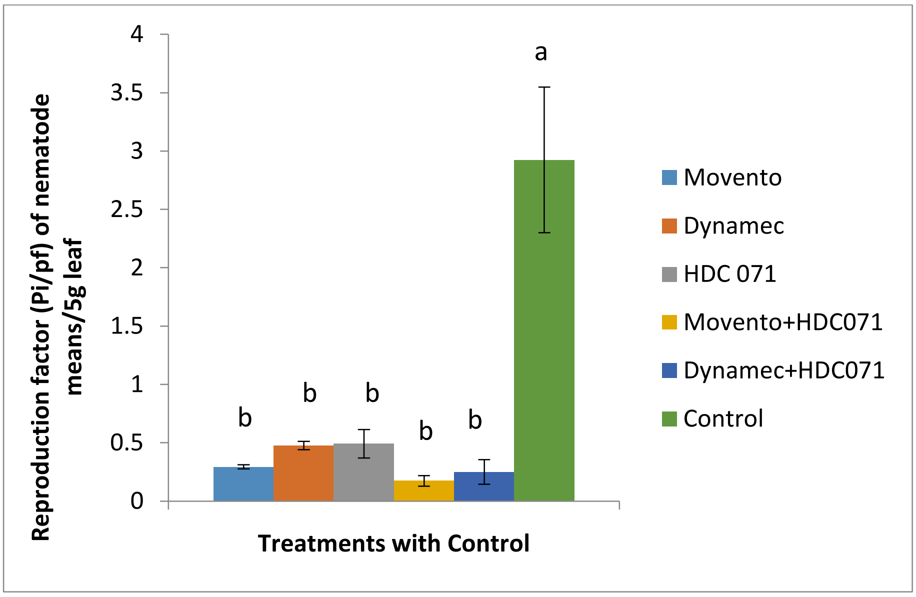

Figure 18. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected *Astilboides tabularis* plants with the untreated 'control'. Bars with the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Reduction of nematode population over the control (ROC) had 94.06% as the highest value with Movento + HDC 071 followed by 91.45% with Dynamec + HDC 071. The lowest value (83.19%) was obtained with HDC 071 (Table 9).

Table 9. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected *Astilboides tabularis* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Nematode per 5 g leaf				
Astilboides tabularis				
Treatments	***pi***	***pf***	***RF***	***ROC(%)***
Movento	8122.194	2363.154	0.29 b	89.96
Dynamec	9566.865	4720.268	0.48 b	83.74
HDC 071	1606.25	1045.151	0.49 b	83.19
Movento+HDC071	5798.776	628.7594	0.17 b	94.06
Dynamec+HDC071	7731.625	1129.324	0.25 b	91.45
Control	9046.452	33198.62	2.92 a	

In general, there were significant differences in RF (*P* <0.05) between all the treatments and the control.

Japanese anemone: There was a significant (*P* <0.05) reduction in reproduction factor (RF) between all the treatments and the untreated control (Figure 19; Table 10).
Values obtained as the reproduction factor (RF) range between 0.04 and 2.22. Movento + HDC 071 had the lowest RF (0.04) while the untreated control gave the highest RF of 2.22. Significant (*P* <0.05) reduction of the RF of nematodes was observed in the leaves of all the treatments compared to the control (Figure 19).

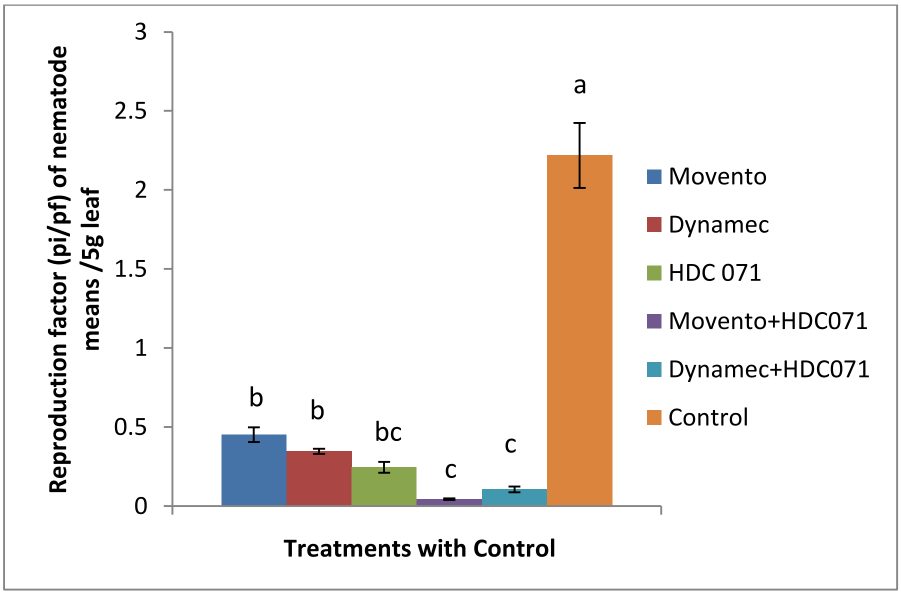

Figure 19. Mean (± SE) Reproduction Factor (RF – p_i /p_f) from 5 g of leaf for *Aphelenchoides* infected Japanese anemone plants with the untreated 'control'. Bars with the same letter are not significantly different (Fisher's multiple range test, P <0.05).

Reduction of nematode population over the control (ROC) expressed as a percentage had the highest value with Movento + HDC 071 (98.04%) followed by 95.23% with Dynamec + HDC 071. The lowest value (79.63%) was obtained with Movento (Table 10).

Table 10. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected *Japanese anemone* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, P <0.05).

	Nematode per 5 g leaf			
	Japanese anemone			
Treatments	***pi***	***pf***	***RF***	***ROC (%)***
Movento	2732.536	1218.378	0.45 b	79.63672
Dynamec	6636.642	2293.788	0.35 bc	84.4143
HDC 071	2877.411	697.789	0.25 bc	88.92595
Movento+HDC071	6812.223	291.8795	0.04 c	98.04841
Dynamec+HDC071	4292.742	445.187	0.11 c	95.23595
Control	4573.576	10086.45	2.22 a	

In general, there were significant differences in RF (P <0.05) between all treatments and the control, and all the treatments caused a >75% reduction in nematode population in the leaves of Japanese anemone compared with the control.

A summary of the comparison of the Movento and Dynamec treatments ± HDC 071 is presented in Table 11 and summarised below.
Japanese anemone: Results of RF indicate a significant difference between Movento + HDC 071 (0.04) and Movento alone (0.45) at P <0.05 (Table 11). Similarly, addition of HDC 071 gave a significant reduction in RF (0.11) with Dynamec + HDC 071 compared to an RF of 0.35 with Dynamec alone (P <0.05; Table 11).

Dryopteris affinis: There was no difference (*P* >0.05) in RF between Dynamec + HDC 071 (0.09) and Dynamec (0.13). However, addition of HDC 071 gave a significant reduction in RF (*P* <0.05) with Movento + HDC 071 (0.09) and Movento alone (0.16, Table 11).

Astilboildes tabularis: There was a significant (*P* <0.05) reduction of RF with Movento + HDC 071 (0.17) compared to Movento alone (0.29; Table 11). However, addition of HDC 071 gave no difference (*P* >0.05) between Dynamec + HDC 071 (0.25) and Dynamec alone (0.47, Table 11).

Brunnera macrophylla: Results of RF obtained with Movento + HDC 071 (0.23) and Movento alone (0.26) gave no significant (*P* >0.05) difference (Table 11). In contrast, there was a significant reduction in RF with Dynamec + HDC 071 (0.15) compared to Dynamec alone (0.43) (*P* <0.05; Table 11).

Table 11. Plant species response to the addition of elicitor – HDC 071 (‘with’ and 'without') on the novel management programme of foliar nematode in grower’s nursery

Plants	Trmt	RF	Plants	Trmt	RF
J.anemone	Movento vs Mov+HDC 071	0.45184a 0.04332b	**Astilboides**	Movento vs Mov+HDC 071	0.29366a 0.17365b
J.anemone	Dynamec vs Dyna+HDC 071	0.34579a 0.10571b	**Astilboides**	Dynamec vs Dyna+HDC 071	0.4753a 0.2501a
Dryopteris affinis	Movento vs Mov+HDC071	0.16455a 0.08614b	**Brunnera**	Movento vs Mov+HDC071	0.2601a 0.2394a
Dryopteris affinis	Dynamec vs Dyna+HDC071	0.13794a 0.09587a	**Brunnera**	Dynamec vs Dyna+HDC071	0.4383a 0.1518b

Data are mean values of four replicates and separated by Fisher’s multiple range test. RF – reproduction factor (pi/pf).

Field 2 Study:

Experiment 4: To investigate the curative approaches for the management of foliar nematode on naturally infested plants in grower's nurseries

Buddleja *daviidi*: There was a significant (P <0.05) reduction in reproduction factor (RF) between all the treatments and control (Table 12; Fi g 20). Values obtained as the RF range between 0.07 and 2.91 (Table 12). Movento + HDC 071 had the lowest RF (0.07) while the untreated control had the highest RF value of 2.91. A significant reduction of the RF of nematodes (P <0.05)was observed in the leaves in al the treatments compared to the control.

Table 12. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected Buddleja *daviidi* plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, P <0.05).

	Nematodes per 5 g leaf			
Treatments	pi	pf	RF	ROC(%)
Movento	2092	356	0.17b	94.1
Dynamec	2901.9	499	0.17b	94.1
HDC 071	2476	446.4	0.18b	93.8
Mov+HDC071	2700.5	178.2	0.07b	97.7
Dyn+HDC071	1765	283	0.17b	94.1
Control	1841	5065	2.91a	

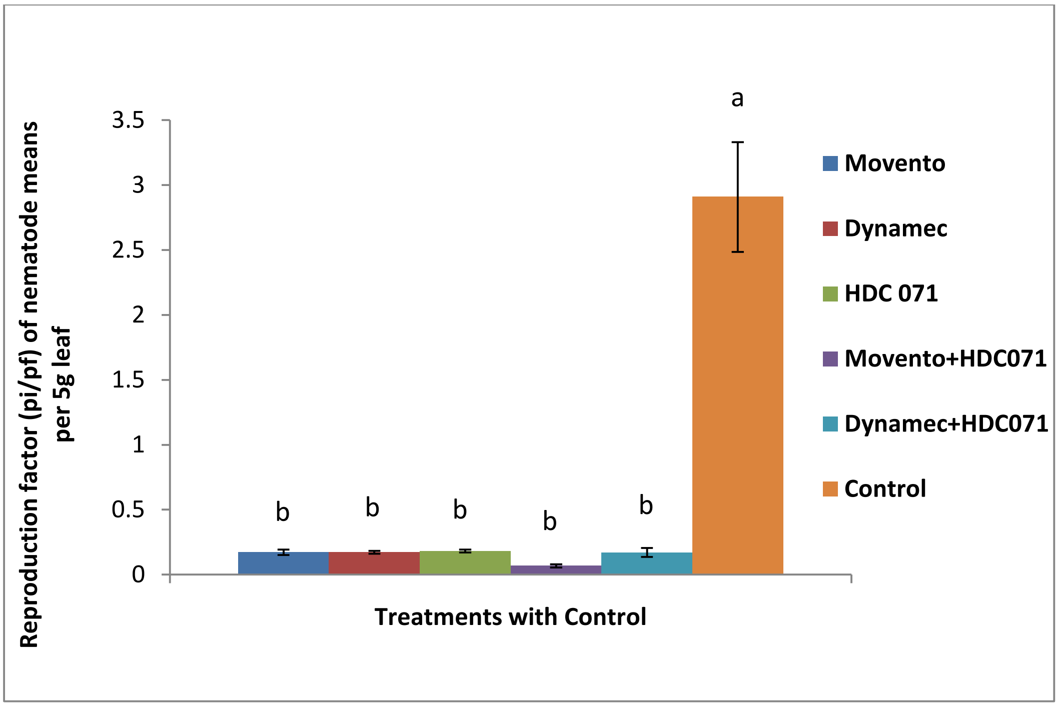

Figure 20. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected *Buddleja daviidi* plants with the untreated 'control'. Bars with the same letter are not significantly different (Fisher's multiple range test, P <0.05).

Reduction of nematode population over the control (ROC) expressed as a percentage had the highest value with Movento + HDC071 (97.7%), while the lowest value (93.8%) was obtained with HDC 071 (Table 12). In general, there were significant differences in RF (P <0.05) between all treatments and the control, and all the treatments caused a >90% reduction in nematode population in leaves compared with the untreated control in this (*Buddleja daviidi)* plant species (Table 12).

Cistus: There was a significant (P <0.05) reduction in RF between all the treatments and the control (Table 13; Figure 21).

The RF values obtained from Cistus range between 0.13 and 1.58 (Table 13). Movento + HDC 071 had the lowest RF (0.13) closely followed by Dynamec + HDC 071 (0.14) while the untreated control gave the highest RF of 1.58 (Table 13). There was a significant reduction of the RF of nematodes as observed in all the treatments compared to the untreated control (Figure 21).

Reduction of nematode population over the control (ROC) expressed as a percentage, had the highest value with Movento + HDC 071 (91.5) followed by 90.9% with Dynamec + HDC 071. The lowest value (78%) was obtained with Movento (Table 13).

In general, there were significant differences in RF (*P* <0.05) between all treatments and the control, and all the treatments caused a >75% reduction in nematode population in leaves compared with the control (Table 13).

Table 13. Mean (± SE) Reproduction Factor (RF – p_i / p_f) from 5 g of leaf for *Aphelenchoides* infected Cistus plants. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

	Nematodes / 5 g leaf			
Trmts	p_i	p_f	RF	ROC(%)
Movento	3130	1020	0.35 b	78.0
Dynamec	2331	629	0.30 bc	80.8
HDC 071	2717	771	0.28 bc	82.0
Movento+HDC071	3283	409	0.13 c	91.5
Dynamec+HDC071	2331	330	0.14 c	90.9
Control (water)	2544	3802	1.58 a	

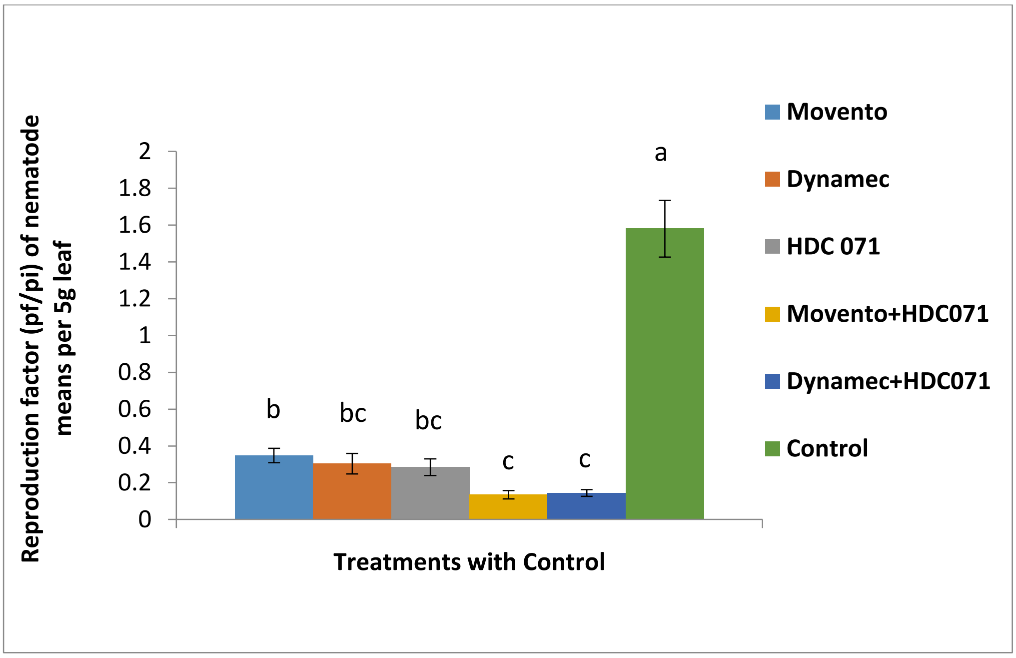

Figure 21. Mean (± SE) Reproduction Factor (RF – p_i/p_f) from 5 g of leaf for *Aphelenchoides* infected Cistus plants with the untreated 'control'. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Table 14. Reproduction Factor (RF) values in response to the addition of elicitor – HDC 071 ('with' and 'without') to insecticides on the novel management programme of foliar nematode at Field site 2

Plants	Treatment	RF	Plants	Treatment	RF
	Movento	0.1726a		Movento	0.3475a
Buddleja	vs		***Cistus***	vs	
	Mov+HDC071	0.0673b		Mov+HDC071	0.1349b
	Dynamec	0.1728a		Dynamec	0.3035a
Buddleja	Vs		***Cistus***	vs	
	Dyna+HDC071	0.1709a		Dyna+HDC071	0.1437b

Data are means of four replicates (Buddleja) and ten replicates (Cistus) plants. Values not sharin g a common letter indicate a significant difference (*P* <0.05) according to Fisher individual test.

RF - Reproduction factor (p_i / p_f) per 5 g leaf

Results from Buddleja and Cistus indicate that an addition of HDC 071 with Movento had a significant ($P < 0.05$) reduction in reproduction factor (RF) compared with Movento alone - Table 14. Similarly there was a significant reduction ($P < 0.05$) with reduced RF values in Dynamec + HDC 071 when compared with Dynamec alone in both Buddleja and Cistus plants (Table 14)

Experiment 5: To determine the efficacy of Movento and Dynamec (selected foliar insecticides), HDC 069 (plant extract) and HDC 071 (synthetic chemical elicitor), as individual treatments, and in combination for preventative management of *A. fragariae* on nematode-free plant.

Field location 1 - Babylon Nursery

Japanese anemone: There was a significant ($P < 0.05$) difference in final nematode population between all the treatments and the untreated control (Table 15; Figure 22). Mean values obtained for nematode population in the leaves range between 157 and 2570 (Table 15). Movento + HDC 069 had the lowest mean population (157) while the untreated control gave the highest mean population of 2570 (Table 15). A significantly lower nematode population ($P < 0.05$) was observed in the leaves in all the treatments compared to the untreated control (Figure 22; Table 15).

Table 15. Mean (± SE) nematode population from 5 g of leaf eight weeks after inoculation with 200 *A. fragariae* on Japanese anemone. Columns followed by the same letter are not significantly different (Fisher's multiple range test, $P < 0.05$)

Treatments	**Mean Nematode population/5 g leaf**
Movento	381.4 bc
Dynamec	408.9 bc
HDC 069	503.5 b
HDC 071	476 b
Dyna+HDC 071	196.5 de
Movento+HDC071	157.8 e
Control(Water)	2570.2 a

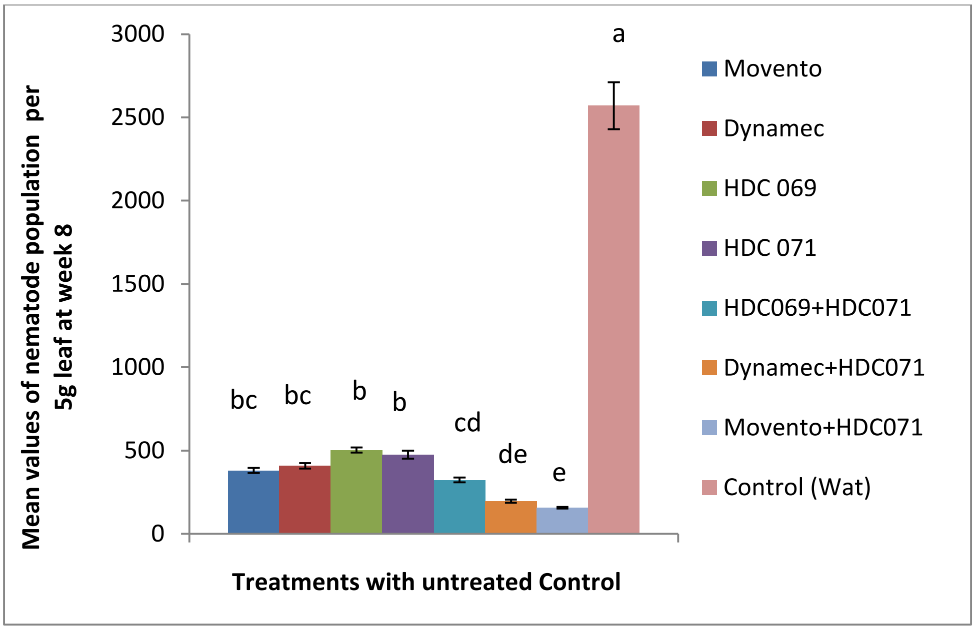

Figure 22. Mean (± SE) nematode population from 5 g of leaf eight weeks after inoculation with 200 *A. fragariae* on Japanese anemone. Bars with the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

All the treatments mixed with HDC 071 had a significantly lower nematode population than the solo treatments (Figure 22; Table 16).

Table 16. Response to HDC 071 ± pesticides on the management of *A. fragariae* inoculated on leaves of Japanese anemone. Columns followed by the same letter are not significantly different (Fisher's multiple range test, P <0.05).

Treatments	**Mean nematode population 5 g of leaf**
Movento	381.45 a
vs	
Mov+HDC 071	157.76 b
Dynamec	408.95 a
vs	
Dyna+HDC 071	196.49 b
HDC 069	503.53 a
vs	
HDC 069+HDC 071	323.77 b

Data are mean values of five replicates, separated by Fisher's multiple range test, *P* <0.05.

Experiment 5: To determine the efficacy of Movento and Dynamec (selected foliar insecticides), HDC 069 (plant extract) and HDC 071 (synthetic chemical elicitor), as individual treatments, and in combination for preventative management of *A. fragariae* on nematode-free plant.

Field location 2 - Wyevale Nurseries

Buddleja daviidi: There was a significant (*P* <0.05) difference in final nematode population between all the treatments and the untreated control (Table 17). Mean values obtained for nematode population in the leaves range between 72 and 2454 (Table 17). Movento + HDC 071 had the lowest mean value (72.9) while the untreated control gave the highest mean value of 2454 (Table 17). Significant (*P* <0.05) reduction of the nematode population was observed in the leaves in all the treatments compared to the untreated control (Figure 23; Table 17).

Table 17. Mean (± SE) nematode population from 5 g of leaf eight weeks after inoculation with 200 *A. fragariae* on *Buddleja daviidi*. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05)

Treatments	**Mean nematode population 5 g of leaf**
Movento	362.2 c
Dynamec	475.4 b
HDC 069	540.7 b
HDC 071	531.7 b
HDC069 + HDC071	141.2 d
Dynamec +HDC 071	158.6 d
Movento+HDC 071	72.9 d
Control (Water)	2454 a

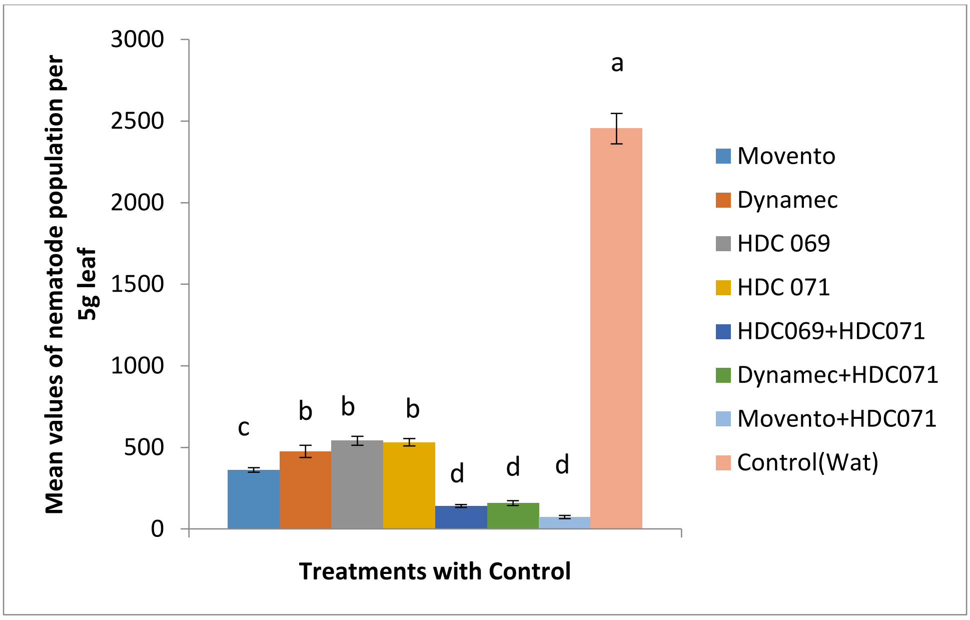

Figure 23. Mean (± SE) nematode population from 5 g of leaf eight weeks after inoculation with 200 *A. fragariae* on *Buddleja daviidi*. Bars with the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

All the treatments mixed with HDC 071 had a significantly lower nematode population than the solo treatments (Figure 23; Table 18).

Table 18. Response to HDC 071 ± pesticides on the management of *A. fragariae* inoculated on leaves of *Buddleja daviidi* - Field location 2

Treatments	**Mean nematode population / 5g of leaf**
Movento	362.2 a
vs	
Mov+HDC071	72.94 b
Dynamec	475.4 a
vs	
Dyna+HDC071	158.6 b
HDC 069	540.69 a
vs	
HDC069+HDC 071	141.18 b

Data are mean values of five replicates, and values are separated by Fisher's multiple range test, *P* <0.01.

Experiment 5: To determine the efficacy of Movento and Dynamec (selected foliar insecticides), HDC 069 (plant extract) and HDC 071 (synthetic chemical elicitor), as individual treatments, and in combination for preventative management of *A. fragariae* on nematode-free plant.

Field location 3 - SRUC Edinburgh

Japanese anemone*:* There was a significant (*P* <0.05) difference in final nematode population between all the treatments and the untreated control (Table 19). The mean values for nematode population in the leaves range from 206.5 to 5005 (Table 19). The lowest nematode population of 206.5 was obtained from Movento + HDC 071 while the untreated control gave the highest nematode population of 5005 (Figure24; Table 19). There was a significantly lower (*P* <0.01) nematode population observed in the leaves in all the treatments compared to the control (Figure 24; Table 19).

Table 19. Mean (±SE) nematode population from 5 g of leaf eight weeks after inoculation with 200 *A. fragariae* on Japanese anemone. Columns followed by the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

Treatments	***Mean nematode population / 5 g of leaf***
Movento	423 bc
Dynamec	584.7 b
HDC 069	609.2 b
HDC 071	576.8 b
HDC069+HDC071	314.2 c
Movento+HDC071	206.5 c
Dynamec+HDC071	292.4 c
Control (Water)	5005 a

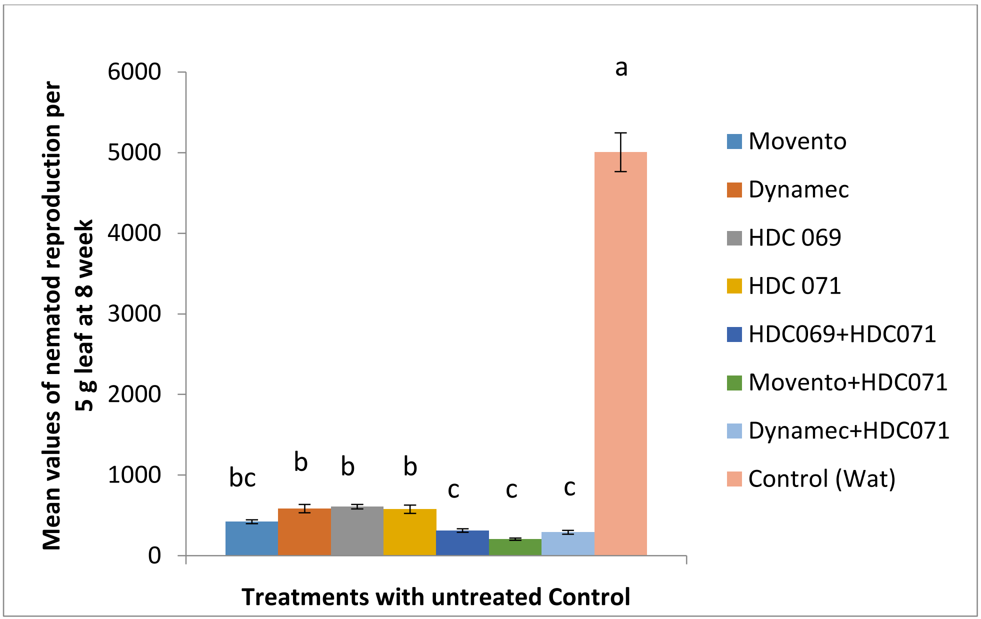

Figure 24. Mean (± SE) nematode population from 5 g of leaf eight weeks after inoculation with 200 *A. fragariae* on Japanese anemone. Bars with the same letter are not significantly different (Fisher's multiple range test, *P* <0.05).

All the treatments mixed with HDC 071 had a significantly lower nematode population than the solo treatments (Figure 24; Table 20).

Table 20. Response to HDC 071 ± pesticides on the management of *A. fragariae* inoculated on leaves of Japanese anemone. Columns followed by the same letter are not significantly different (Fisher's multiple range test, P <0.05)

Treatments	***Mean nematode population / 5 g of leaf***	
Movento vs Mov+HDC071	423.35 206.48	a b
Dynamec vs Dyna+HDC071	584.7 292.4	a b
HDC 069 vs HDC069+HDC071	609.21 314.18	a b

Data are mean values of five replicates, and values are separated by Fisher's multiple range test, *P* <0.01, *P* <0.05.

A summary of the comparison of the Movento, Dynamec and HDC 069 treatments ± HDC 071 is presented in Table 20. There were significant differences (*P* <0.05; *P* <0.01) in nematode population in the three treatments (Table 20). The results show that all the treatments mixed with HDC 071 had a significantly lower nematode population than the solo treatments (Table 20).

Experiment 6: To investigate the relationship between the visual symptoms of *Aphelenchoides fragariae* leaf infection and corresponding nematode population on leaves of Weigela and Japanese anemone in a glasshouse study

Japanese anemone: Results of nematode population per leaf category analysed from 5 replicates against the percentage of leaf area indicate that the higher the lesion percentage, the more nematode population was found per leaf category (Fi g 25; Table 20).

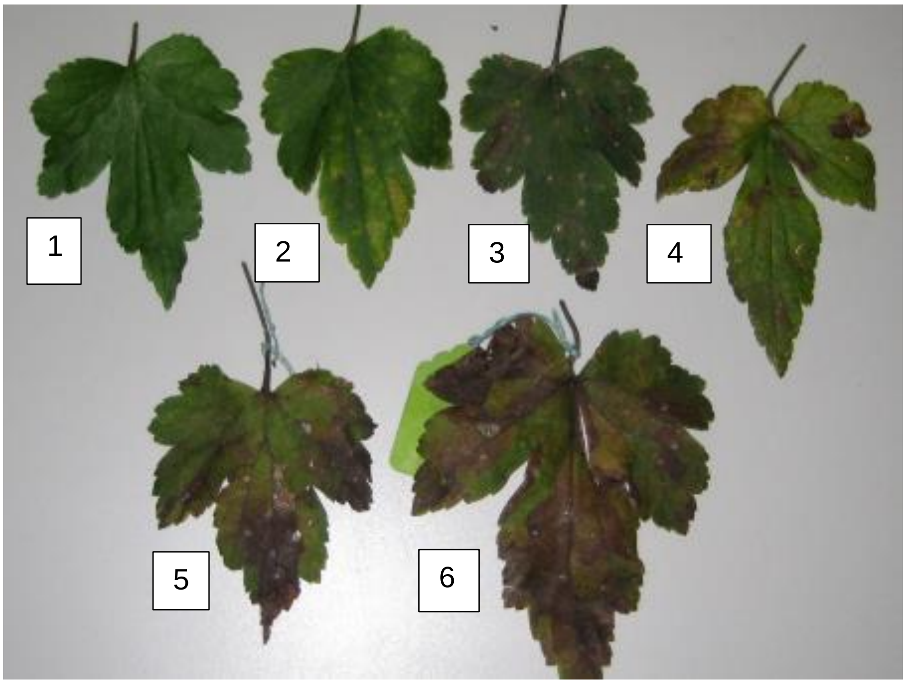

Figure 25. Picture of Japanese anemone leaves showing degrees of lesion infection by *Aphelenchoides fragariae* at 5 -10 weeks after inoculation with 50, 100 and 200 nematodes in glasshouse.

The results of leaf 'category 1' with 0% lesion indicate no nematode, while nematode populations found in category 2 to category 6 increased in response to increasing lesion percentage (1-100%; 867 -10,712) per 5 g leaf (Table 21). This indicates that the larger the lesion on leaf, the higher the nematode population (Table 21).

Table 21. Relationship between visual leaf lesion symptoms (%) and nematode population when inoculated with 50, 100 and 200 nematodes / leaf in a glasshouse study on Japanese anemone. Data are nematode means of 5 replicates. Means that do not share a letter are significantly different (Fisher's multiple range test P <0.05)

Leaf category	% lesion on total leaf area	Mean nematode count/5g leaf
1	0%	0
2	1 - 10%	867 d
3	10 - 15%	1832 d
4	25 - 50%	3955 c
5	50 - 75%	6053 b
6	75 - 100%	10,712 a

All the leaf categories (1-6) were significantly different (P <0.05) from each other in both lesion area and nematode population on leaf, with the exception of categories 2 and 3.

Weigela 'Bristol Ruby'

Results of leaf 'category 1' with 0% lesion indicate no nematodes present, while nematode Results of nematode population per leaf category analysed from 5 replicates against the percentage of leaf area indicate that the higher the lesion, the more nematode population was found per leaf category (Table 22; Figure 26)**.** Population found in category 2 to category 6 increased in response to increasing lesion percentage (1-100%; 728 - 8582) per 5 g leaf (Table 22). This indicates that the higher the lesion on leaf, the higher the nematode population (Table 22). All the leaf categories (1-6) were significantly different (*P* <0.05) from each other in both lesion area and nematode population on leaf.

Table 22. Relationship between leaf lesion symptoms (%) and nematode population when inoculated with 50, 100 and 200 nematodes / leaf in a glasshouse study on Weigela. Data are nematode means of five replicates. Means that do not share a letter are significantly different (Fisher's multiple range test P <0.05).

Leaf category	% lesion on total leaf area	Mean nematode count/5 g leaf
1	0	0 e
2	1 -10%	728 d
3	10 - 15%	1396 d
4	25 - 50%	3548 c
5	50 - 75%	4375 b
6	75 - 100%	8582 a

Figure 26. Picture of Weigela 'Bristol Ruby' showing degrees of lesion infection by *Aphelenchoides fragariae* at 5 -10 weeks after inoculation with 50, 100 and 200 nematodes

Experiment 7: To investigate novel soil treatment for the management of *Aphelenchoides fragariae* on Japanese anemone in a glasshouse study

Results obtained from the seven treatments namely Vydate (Oxamyl), HDC 068, HDC 070, HDC 088, HDC 084, HDC 101 and control (water) from soil and leaf samples indicate that there were significant differences (*P* <0.05) between all the treatments and untreated control (Figure 27).

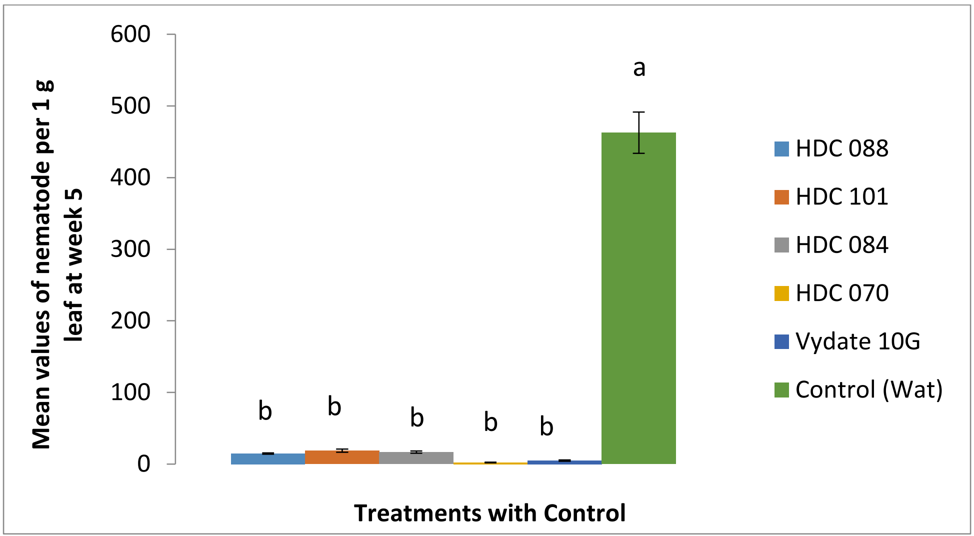

Figure 27. Showing nematode population per 1 g leaf at week 5 after nematode inoculation in soil of clean Japanese anemone plants.

The smallest nematode population at week 5 after nematode inoculation was 2 nematodes /1 g leaf from HDC 070, while 463 nematodes were observed in the untreated control (Figure 27). Results at week 8 after nematode inoculation gave the smallest number (8) from Vydate 10 G while 1382 (highest) nematodes were observed in the untreated control (Figure 28). In general, there were significantly lower nematode populations in all the treatments compared with the control (Figure 27 and 28). There was no result from the HDC 068 treatment as the product was phytotoxic to the plants**.**

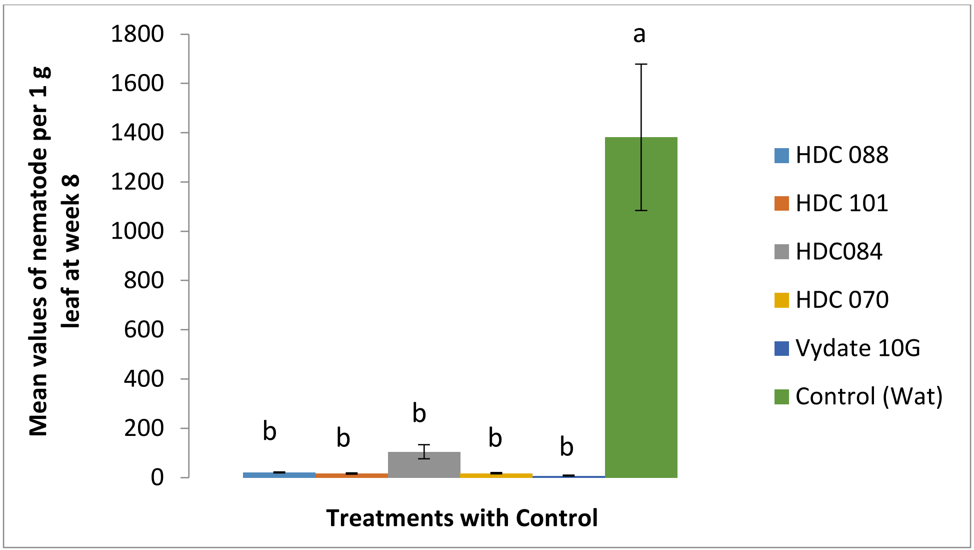

Figure 28. Nematode population per 1 g leaf at week 8 after 1000 nematode inoculation on soil of clean Japanese anemone

Results from soil treatments show that HDC 068 (which was phytotoxic) had the smallest number of 5 nematodes per 10 g soil samples (3). The highest nematode population (58) was found in the soil of the untreated control (Table 23).

Table 23. Mean (± SE) value from 10 g of soil 8 weeks post-infection with *Aphelenchoides fragariae* in pots of Japanese anemone plants. Data are nematode means of 6 replicates. Columns followed by the same letter are not significantly different (Fisher's multiple range test, P <0.05).

Treatments	Mean nematode population /10 g soil	
HDC 088	14.33	cd
HDC 101	22.83	c
HDC 084	38	b
HDC 070	21	c
Vydate 10G	4.667	de
HDC 068	2.833	e
Control (water)	57.5	a

In general, there was significantly lower nematode population in all the treatments compared with the control (P <0.05).

Discussion

The key outcomes from this research are the identification of commercially available products that have a negative effect against leaf and bud nematodes. These products have the potential to be utilised in an integrated programme of nematode management to reduce nematode symptoms, by reducing nematode multiplication in a range of ornamental plants.

Products derived from bioassay studies were identified that could be used to manage leaf and bud nematodes, which provided an indication of products to further investigate as alternatives to the current 'standard' nematicide - Vydate 10 G (oxamyl). Products that demonstrated contact mortality to leaf and bud nematodes included Dynamec (abamectin), HDC 068 (plant extract), HDC 069 (plant extract) and HDC 070 (plant extract). The insecticide Movento (spirotetramat) did not exhibit high contact mortality against leaf and bud nematodes in this bioassay. However, spirotetramat was retained for further study due to it reducing reproduction of cereal cyst nematode (*Heterodera avenae)* when applied to the foliage of sprin g wheat on two nematode infested fields in the United States (Smiley *et al*, 2011).

Past research work has reported that repeated applications of abamectin reduced both *A. fragariae* and *Ditylenchus dipsaci* populations in Lamium and Phlox in the USA (LaMondia 1999). Youn g and Maher (2000), demonstrated the activity of abamectin against leaf and bud nematodes (*Aphelenchoides ritzemabosi*) *in vitro* and *in vivo*. Abamectin was also reported to have potential to control foliar nematodes on hardy nursery stock in the UK (Young, 2000). However, abamectin was found to be ineffective among a range of alternatives evaluated for the control and management of leaf and bud nematodes (Bennison, 2007). Programmes using HDC 069 and spirotetramat have not been fully evaluated for their capacity to suppress foliar nematodes in hardy nursery plants. These products were therefore chosen to be used in field trials in a range of hardy ornamentals which had been naturally infested with leaf and bud nematodes, or were artificially inoculated with nematodes.

In addition to the products that had contact mortality against leaf and bud nematodes, the use of the plant defence elicitors HDC 071 and HDC 072 both reduced nematode multiplication in infected leaves compared to untreated leaves in a pilot study on Japanese anemone and Weigela.

Although there are no previous reports on the use of HDC 071 or HDC 072 to control *A. fragariae*, HDC 071 has been reported to induce resistance to pathogens when applied to plants (Kessman *et al*., 1994). Plant defence elicitors including HDC 071 and HDC 072 are not directly antimicrobial (Vallad and Goodman, 2004). Other authors recommended the use of HDC 071 in combinations with fungicides and bactericides in tomato spray programs in North Carolina, USA for increased plant resistance and reduction of early blight (*Alternaria solani*) inoculum levels (Ivors and Louws, 2007). HDC 071 has showed significant reduction (up to 74%) in root-knot nematode (*Meloidogyne incognita*) reproduction in tomato (Molinari and Baser, 2010). The use of elicitor + pesticide combinations could be valuable in reducing pesticide use, and could delay pesticide-resistance development and increased longevity in their use (Ivors and Louws, 2007).

The elicitor HDC 071 was further tested in a glasshouse study as a foliar application on inoculated Japanese anemone leaves to determine the number of treatments necessary to obtain the most reduction in nematode multiplication. A programme of three applications at 2 week intervals between applications proved to be the most effective programme. Consequently field trials were carried out in nurseries using HDC 071 alone and in combination with pesticides identified from the contact mortality bioassays: HDC 069, spirotetramat and abamectin. Application of HDC 069, Movento and Dynamec with or without the elicitor HDC 071 significantly reduced the reproductive capacity and symptoms of leaf and bud nematodes on plants compared to untreated plants. The elicitor HDC 071 alone in a 3-spray programme also significantly reduced nematode symptoms and multiplication.

The activity of abamectin could be influenced by the use of adjuvants and manipulation of product application as carried out in this study. Early application of treatment before sunshine was carried out on pre-irrigated plants about 1 hour before product application. The results of this study is at odds with the report of HDC project HNS 131 (Bennison, 2007), which evaluated a range of alternatives for the control and management of LBN, and found Dynamec to be ineffective in controlling leaf and bud nematodes.

Foliar application of Movento (spirotetramat) caused a significant reduction in the reproduction factor (RF) of the nematode population. This suggests that continuous multiplication of nematode populations in the leaves was altered through foliar application. Spirotetramat is an insecticide with both phloem and xylem mobility (ambimobile) in many plant species. Movento is a registered product for controlling many suckin g insect pests including aphids, with an extension of authorisation for minor use (EAMU) in the UK. The results from trials in this study indicate significant potential for foliar nematode control on ornamental plants using spirotetramat. There was no observed phytotoxicity on any plant species from Movento. Spirotetramat consistently reduced *Heterodera glycines* and *Meloidogyne incognita* development to reproductive maturity with a single application to foliage at 1-2 weeks after inoculation, with nematodes on soybean plants

(*Glycine max* cv. Hutcheson) grown in a glasshouse (Van g *et al*., 2013), so a similar effect was seen in this current study.

The plant extract HDC 069 has insecticidal properties, based on multi-action pathways which include anti-mitotic effects, anti-feedant activity, insect growth regulator effects, fecundity suppression, sterilization, oviposition repellency, and harmful effects on the endocrine system and damage to the cuticle of larvae, preventin g them from moultin g (Mulla and Su, 1999; Howard, *et al.*, 2009). This study shed light on the potential of HDC 069 as a nematicide against leaf and bud nematode. The results in this study are in agreement with a review by Khalil (2013), who reported HDC 069 as a promisin g tool in integrated nematode management programs. Foliar application of HDC 069 at the manufacturer's recommended rate in this study reduced nematode nematode multiplication.

HDC 071 is registered in the UK as a plant protection product, and approved for use on Barley and protected Chrysanthemum against fungal diseases. Foliar application of HDC 071 on naturally infected plants caused a significant reduction of nematode population over the control (ROC%) which ranged between 79% (*Bergenia*) to 91% (*Gunnera manicata)*. The results from this study are similar to those seen with foliar fungal pathogens, where HDC 071 protected tobacco plants from angular leaf spot compared with controls (Cole, 1999), and caused a 70% reduction in *Rhynchosporium commune* infection on sprin g barley in a glasshouse experiment (Walters *et al.,* 2014).

There was similarity between results obtained from the 2 year nurseries in term of efficacy due to the addition of HDC 071 to HDC 069, abamectin and spirotetramat in most of the plant species tested. Results also confirmed that addition of HDC 071 to the three pesticides above as a curative treatment or preventative approach had a significantly lower reproduction factor (RF) and suppressive effect on nematode populations compared to use of the insecticides alone. Spirotetramat + HDC 071 gave a consistent nematode population reduction in most of the plant species investigated.

Similar observations have been seen in field experiments where HDC 071 plus fungicide combinations provided the most consistent disease control of *R*. *commune* on barley crops (Walters *et al*, 2014). Other authors recommended the use of HDC 071 in combinations with fungicides and bactericides in tomato spray programs in North Carolina, USA for increased plant resistance and reduction of early blight (*Alternaria solani*) (Ivors and Louws, 2007).

The adjuvant Tween 20 used in this study had a good compatibility with all the products tested and may well contribute to the efficacy of these products.

Results obtained from the leaf visual symptoms and corresponding nematode population on leaves of Weigela and Japanese anemone plants demonstrated a direct relationship between the degree of lesion symptoms on leaves and the leaf nematode population. This observation is key for growers to determine the extent of damage caused to the plant, and to help in takin g a decision of whether it is worth treatin g plants to make them viable for sale, or if the plants are beyond saving.

Results on both Weigela and Japanese anemone were similar in terms of corresponding levels of nematode population with symptoms/lesion percentage. The lower the lesion score, the lower the nematode population recorded in both Weigela and Japanese anemone. Similar observations were obtained from degrees of symptom severity and percentages of lesions caused by foliar nematode (*Aphelenchoides fragariae)* on leaves of 'Guacamole' Hosta plants (Zhen *et al*., 2012).

The results from seven products evaluated as novel soil treatments demonstrated that all the treatments significantly reduced nematode population at week 5 and 8 when compared with the untreated control (in both soil and leaf samples). HDC 068 showed a phytotoxic effect on plants. Future work using HDC 068 could consider the use of reduced dose rates and application of treatment to soil before plants are introduced. Results obtained on 'standard' nematicide - Vydate 10 G during the soil treatment study confirmed the past report of HDC project HNS 131 that oxamyl (Vydate 10G) was effective against leaf and bud nematodes (Bennison, 2007).

However, all the treatments had a significantly reduced nematode population in both soil and leaf samples when compared with the control in this study, and could be utilised as soil treatments to prevent nematode infection via this route.

As a key components of an Integrated Pest Management (IPM) programme for leaf and bud nematode, we would recommend a high level of hygiene (sanitation, increase plant spacing, reduced overhead irrigation, isolation of suspected nematode infected plants and avoiding the use of potential nematode infected soil to reduce the risk of infection by foliar nematodes in both open and protected nurseries. Use of a pesticide-based programme (with or without elicitors) should only be adopted in high risk situations (e.g. previous history of nematode infection or nematode presence in soil/compost) or at the earliest signs of nematode infection.

Conclusions

The key outcomes from this research on foliar nematode management are:

- Products that have contact mortality against leaf and bud nematodes have been identified.
- The insecticides HDC 069, spirotetramat (Movento) and abamectin (Dynamec) demonstrate effective management of foliar nematodes on a range of ornamental plants in a foliar application programme.
- The elicitor HDC 071 demonstrated effective management of foliar nematodes on a range of ornamental plants in a foliar application programme.
- HDC 071 in combination with HDC 069, spirotetramat or abamectin enhanced the management of foliar nematodes on a range of ornamental plants.
- The above products have an effect on plants by limiting nematode reproduction. Consequently they can limit nematode multiplication in already infected plants, and limit reproduction in asymptomatic plants.
- Development of a severity ratin g demonstrated that leaf symptoms are an accurate indication of the population of leaf nematodes within symptomatic leaves.
- Soil treatments with various products significantly reduced the infestation of Japanese anemone via the soil route of nematode infection. HDC 088, HDC 101, Vydate, HDC 070 and HDC 084 were all effective and can play a role in nematode management should they be available for use in ornamentals.

Knowledge and Technology Transfer

Description	***Date***
Presentation: 2014 Postgraduate Conference at SRUC	19-20/03/2014
Poster Presentation at HDC Annual Studentships Conference	16-17/09/14
Bio-fumigation International Conference – Newport UK	09-12/09/14
Presentation: 2014 at Advances in Nematology Annual Conference, London	16/12/2014
Presentation on Managing Leaf and Bud Nematode at AHDB HPTDG Technical Meeting, London	11/2/2015
Poster Presentation at Postgraduate Conference at SRUC, Edinburgh	19-20/3/15

Presentation at Agri-Science Young Researchers Conference by Syngenta Agrochemical Company, Bracknell UK	8/7/2015
Poster at AHDB - Horticulture Annual Studentships Conference	16-17/9/2015
Presentation at Society of Chemistry and Industry (SCI), David Miller Awards and Horticulture Group AGM, University of Reading, UK	18/9/2015
Poster at School of Biology Graduate Students Poster's Day at the University of Edinburgh, Edinburgh.	23/9/2015
Presentation at Herbaceous Perennial Technical Discussion Group (Winter Meeting) at RHS Wisley (Woking) Surrey	16/2/2016
Poster Presentation at first AHDB Ornamental Pests, Diseases and Weed Control Conference at Stoneleigh Park, Kenilworth	23/02/16
Presentation: 2016 Postgraduate Conference at SRUC, Edinburgh	22-23/03/2016
Presentation of Project Findings at Panel Meeting Hardy Nursery Stock - AHDB Stoneleigh, Kenilworth	03/11/2016
Platform Presentation at AHDB Annual Studentships Conference	16-17/11/2016
Platform Presentation at Association of Applied Biologists (AAB) Nematology Group Annual Conference, London	13/12/2016
Presentation at Herbaceous Perennial Technical Discussion Group (Winter Meeting) at RHS Wisley (Woking) Surrey	08/2/2017

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Appendices

Appendix 1 - Future work recommended

- HDC 071 is no longer supported in the UK by Syngenta Crop Protection, so will not be available for much longer. However, limited results with another elicitor product HDC 072 suggest similar activity to HDC 071 in terms of limiting nematode reproduction in infected foliage. There are other elicitors available that warrant evaluation such as laminarin (Vacciplant), Sitko SA, Softguard (Chitosan)
- Investigate combinations of elicitors, and pesticides like HDC 069, Spirotetramat and Dynamec for the management of foliar nematode: look at varyin g dose and timings in developin g an effective programme of treatments
- Field trials of the soil treatments HDC 088, HDC 101, HDC 070 and HDC 084 are required to demonstrate prevention of infection via nematode infested soil in a range of ornamental plants
- Look at the combined approach of soil treatments with foliar treatments as an overall management approach for foliar nematodes
- Work needed on more plants species on nematode symptom severity for leaf ratin g assessment