Project title:	Novel approaches for the management of leaf and bud nematodes (*Aphelenchoides* spp.) in hardy nursery stock
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[The results and conclusions in this report are based on an investigation conducted over a one-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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GROWER SUMMARY

Headline

Research is on-going to identify novel and suitable approaches to manage leaf and bud nematodes (eelworms) in hardy nursery stock. These novel methods will potentially serve as alternatives to Vydate 10G.

Background

Foliar nematodes, also called leaf and bud nematodes (LBN), *Aphelenchoides* spp. cause serious damage to many ornamental plants grown in greenhouses, nurseries, and in the landscape settings 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 delineated 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 upward. LBN cause brown to black, or chlorotic, vein-delineated angular lesions that can become necrotic. The lesion eventually turns blackish-brown and affected parts may shrivel. If buds or young leaves are infested, they may not develop properly and may 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.

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 land, sterilisation of pots and equipment, and if possible avoiding the use of overhead irrigation and misting systems which create ideal conditions for nematode infection.

LBN problems have become important because of the revocation and subsequent loss of systemic nematicides, increased nursery production of these vegetatively propagated plants and long distance movement of plants. A range of alternatives for the control and management of LBN have been evaluated previously, the results suggesting that Dynamec (abamectin) was found to be ineffective against LBN and it was proposed that Vydate 10G (oxamyl) was the most effective available product.

Vydate 10G has an extension of authorisation for minor use (EAMU) for use on protected ornamental plants. However, oxamyl is not compatible with IPM programmes. Its use also requires precautions for operator and environmental protection, with a re-entry time to treated glasshouses and a harvest interval. In addition, its continued availability is uncertain.

This project therefore, aims to develop 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 will also evaluate the application of products that act as elicitors of plant defences to determine whether they can confer levels of resistance to nematodes. These studies are being carried out in the laboratory, glasshouse and thereafter at grower's nurseries. For this project - (guided by advice from UK industry), the focus will be on Japanese anemone, Hosta and Weigela plants.

The overall aim of this study will be to develop new guidelines for the integrated management of foliar nematodes in hardy nursery stock utilising existing and novel methods.

Summary

This research project aims to target stages of the routes of nematode invasion into plants, investigate the efficacy of some potential plant protection products and characterise the defence mechanisms induced by elicitors against nematodes.

Laboratory bioassays were conducted to evaluate contact mortality of *Aphelenchoides fragariae* using biological and chemical products including Movento, Jet 5, Vydate 10G, Cercobin and Dynamec and three experimental products. Results after 72 hours of exposure showed that HDCI 68, HDCI 70 and Dynamec had high significant mortality rates of foliar nematodes. This was followed by Movento, HDCI 69 and Vydate 10G with average mortality while Cercobin, Jet 5 and Control (Water) had less contact effect. This project therefore aims to utilise the results of the above laboratory bioassay in a glasshouse study in order to evaluate protection against LBN movement from the soil into plants.

The promising and potential products will be used either as contact or systemic form on plants to prevent nematode infection. This will be evaluated as soil and foliar treatments to assess efficacy.

In addition, the results from on-going tests of 'elicitors' will be incorporated as part of the integrated pest management approaches on foliar nematode control.

Financial Benefits

At this stage in the project we are not yet at a stage to be able to give an accurate estimate of financial benefit to growers. However, the financial benefits will become clearer once data from glasshouse and nursery trials in Years 2 & 3 have been obtained.

Action Points

It is too early to offer growers specific action points to achieve significant benefits for leaf and bud nematode management. The current best practice of crop hygiene, destruction of infested plants, sterilising pots and containers and minimising overhead irrigation and misting should be maintained by growers, with use of oxamyl 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). 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 delineated by the veins and often accompanied by leaf distortion (Kohl, 2010 *et al*; Kohl, 2011). In the UK, *Aphelenchoides ritzemabosi* and *A. fragariae* 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 & 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 & Grewal, 2002; Jagdale & Grewal, 2004).

Initially, an experiment was conducted to investigate the contact mortality of some currently available chemical and bio pesticide products on foliar nematodes, *Aphelenchoides* spp.

Materials and methods

A bioassay experiment was conducted in 108 (including control) 5cm plastic petri dishes to test the contact mortality of chemical and biological products (Table 1) on *Aphelenchoides* spp. at room temperature of 25°C. The experiment evaluated 8 products at 3 dose rates: full recommended dose (Level 1) and two lower dose rates (Level 2 and Level 3) depending on the product, with each level replicated 4 times. Water was used as the control with the same number of replicates (n=4).

2.1 *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. Nematodes were extracted from infested leaf tissues. 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 (850µm) and 500 mesh (25µm) and collected in a beaker. The suspension was left for 2 hours, and excess water was reduced on top of the suspension. Nematode concentration was adjusted to 200 mixed stage individuals per millilitre by counting with a microscope. The nematodes were used within 2-3 days for laboratory experiments.

- *Pesticides*: A list of biological and chemical pesticides was 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.

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
HDCI 69	*biopesticide*	n/a	50L/500L/ha	25L/500L/ha	12L/500L/ha
Jet 5	*Peroxyacetic acid*	Certis	800ml/100L	650ml/100L	570ml/100L
HDCI 69	*biopesticide*	n/a	743mg/L	550mg/L	350mg/L
Vydate10G	*Oxamyl*	DuPont	55kg/ha	40kg/ha	10kg/ha
HDCI 70	*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

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

2.2 *Methods*

An aliquot (4ml) of a solution of each treatment (in distilled water) was transferred into each petri dish and 4ml of a suspension containing 800 living nematodes was added to each dish to achieve a desired percentage active ingredient (a.i) 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 72h after exposure. At each observation, a thoroughly mixed 2ml sub-sample from each dish was transferred into a 5cm diameter dish containing 10ml of water and held at room temperature for 72h 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 15). Significant differences between treatments were determined with Tukey's multiple range test at $P<0.05$.

Results

Results below in (Table 2 & Figure 1) showed that at 72h, HDCI 68, HDCI 70 and Dynamec had high significant mortality rates (>75%) in direct contact with the nematodes above the other products tested.

Some of the other products like HDCI 69, Movento and Vydate 10G which had average contact mortality (Table 3) 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 3 different levels (doses) of concentrations in water on *Aphelenchoides fragariae* after 72 hours exposure

Products	Mortality (%) at 72h for 3 levels of Concentration		
	level 1	level 2	level 3
Movento	8.8d	8.3de	7.4d
HDCI 68	95.8a	83a	28.3b
HDCI 69	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
HDCI 70	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, $P<0.05$).

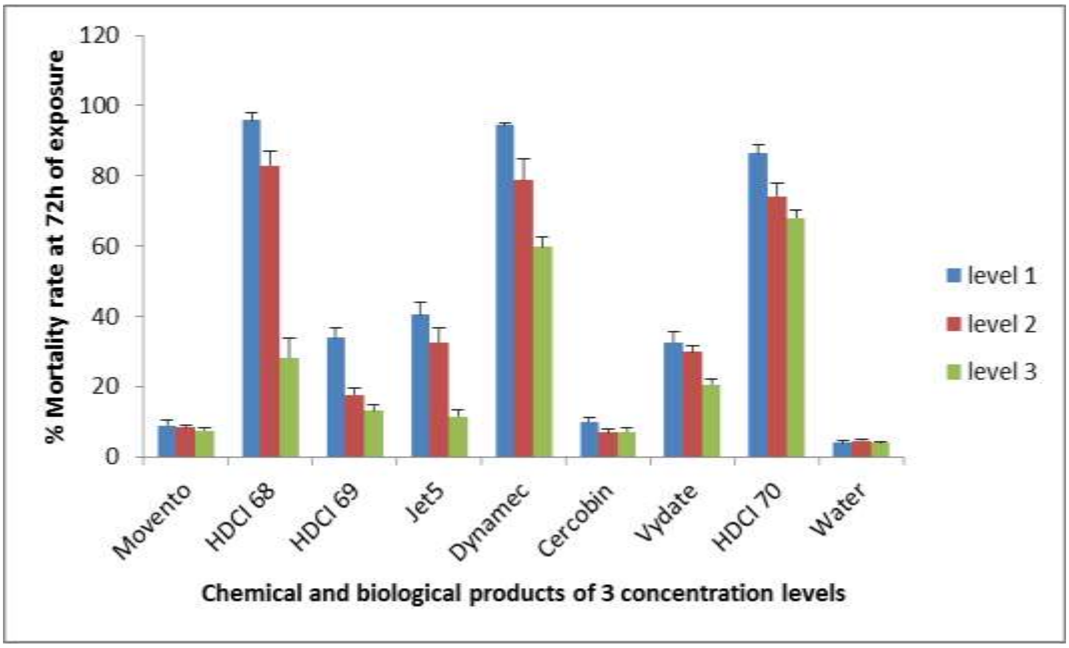

***Figure 4**. Contact mortality test of chemical and biological pesticides with three different levels of concentration on Aphelenchoides fragariae at 72h exposure in water. Error bars are standard error of the mean (±SE) of four replicates.*

Table 3. Mortality classification (%) for chemical and biological pesticides of 3 different levels of concentration on *Aphelenchoides fragariae* in water after 72h exposure

Percentage Mortality Classification at 72hours after exposure						
	Below average (<50%)		Average (50%)		Above average (>50%)	
	Products	*means*	*Products*	*means*	*Products*	*means*
Level 1	Movento	8.8d	HDCI 69	33.8c	HDCI 68	95.8a
	Cercobin	10d	Jet5	40.5c	Dynamec	94.4ab
	Control(Wt)	4d	Vydate10G	32.6c	HDCI 70	86.4b
Level 2	Cercobin	6.9de	HDCI 069	17.6cd	HDCI 68	83a
	Movento	8.3de	Jet5	32.6b	Dynamec	78.8a
	Control(Wt)	4.5e	Vydate10G	29.8bc	HDCI 70	74.1a
Level 3	Movento	7.4d	Vydate10G	20.6bc	Dynamec	59.8a
	Cercobin	7.1d	HDCI 68	28.3b	HDCI 70	68a
	Jet5	11.4cd				
	HDCI 69	13.1cd				
	Control(Wt)	3.9d				

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

Discussion

The bioassay study results provide an indication of products which warrant further investigation as alternatives to Vydate 10G. From the results, some products which are currently used as disinfectants, bio-fumigants and insecticides showed considerable contact mortality rates on foliar nematodes.

There is a need to evaluate these products along with products known to act systemically like Vydate 10G, Movento and HDCI 69 in glasshouse studies on plants.

This preliminary test shows that the objective of this project is progressing, although the positive results on plants will finally determine their effectiveness.

Conclusions

- Promising products that have contact mortality against leaf and bud nematodes have been obtained through this bioassay experiment.
- Evaluation of elicitor products on Hosta, Weigela and Japanese anemone plants in the glasshouse are on-going and will be reported in the next annual report.
- The next study will evaluate the efficacy of contact materials from the above contact bioassay results in the glasshouse. This is to test their ability in preventing leaf nematode infection from soil.
- Soil and foliar treatments of systemic products and elicitors to start December 2014
- See Appendix 1 for future work plan and milestones.

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	9-12/09/14

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