Project Title:	Hardy nursery stock: Evaluation of alternatives to aldicarb (Temik) for the control and management of leaf and bud nematodes
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Project Leader:	Jude Bennison, ADAS Boxworth Tel: 01954 268225 Fax: 01954 267659 Email: jude.bennison@adas.co.uk
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Key workers:	Jude Bennison, ADAS Boxworth (Project Leader) Heather Maher, ADAS Boxworth (Site Manager)
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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and 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.

PRINCIPAL WORKERS

J A Bennison, Senior Research Entomologist, ADAS Boxworth (Project Leader and author of report)

H M Maher, Senior Scientific Officer, ADAS Boxworth

AUTHENTICATION

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

..... JUDE BENNISON
Project Leader

Date.....

Report authorised by: .....

DR W E PARKER
Crop Protection Research Manager

Date.....

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GROWER SUMMARY

Headline

- Temik (aldicarb) gave excellent and persistent control of a heavy infestation of leaf and bud nematodes on Japanese anemones in a replicated pot experiment.
- Dynamec, Savona, Agri-50 or garlic did not control leaf and bud nematodes.
- No suitable alternative treatments to Temik were identified to take forward into a trial on a commercial nursery.
- The pot experiment will be repeated in year 2 of the project, using Japanese anemones at the start of nematode infestation and including some alternative potential treatments.

Background and expected deliverables

Leaf and bud nematodes, *Aphelenchoides* sp., are common, persistent and damaging pests of a range of economically significant nursery stock plants including *Anemone japonica*, *Buddleia*, *Viburnum* and *Weigelia*. Control currently depends on the use of Temik 10G (aldicarb) nematicide granules, which will be withdrawn from use in the UK in December 2007. An alternative control measure is urgently needed and this has been identified as a critical gap in the HDC Pesticide Gap Analysis (CP 17). A recent HDC-funded ADAS project (HNS 86) demonstrated that abamectin (Dynamec) can give useful suppression of the pest, but control is not as persistent or as robust as that given by Temik. The current project aims to build on knowledge and experience gained in project HNS 86, to evaluate alternatives to Temik for control of leaf and bud nematodes. The treatments to be tested in the project were selected by the HNS Panel in February 2004, from a list of potential treatments given in the proposal.

Expected deliverables include:

- Evaluation of alternative control measures to Temik, in a replicated pot experiment and a trial on a commercial nursery.
- Practical guidelines for growers on control and management of leaf and bud nematodes in HNS before the withdrawal of Temik in 2007.

Summary of the project and main conclusions

Pot experiment with Japanese anemones

- Temik gave excellent and persistent control of leaf and bud nematodes on heavily infested Japanese anemones, significantly reducing numbers of nematodes 21, 38 and 55 days after treatment (Figure 1).
- Dynamec as a single application, or a two-spray programme at either 14 or 28 day intervals, or as a three-spray programme at 14 day intervals did not give significant control of the pest.
- Foliar sprays of garlic, Savona or Agri-50 as a three-spray programme at 14 day intervals did not give significant control of the pest.

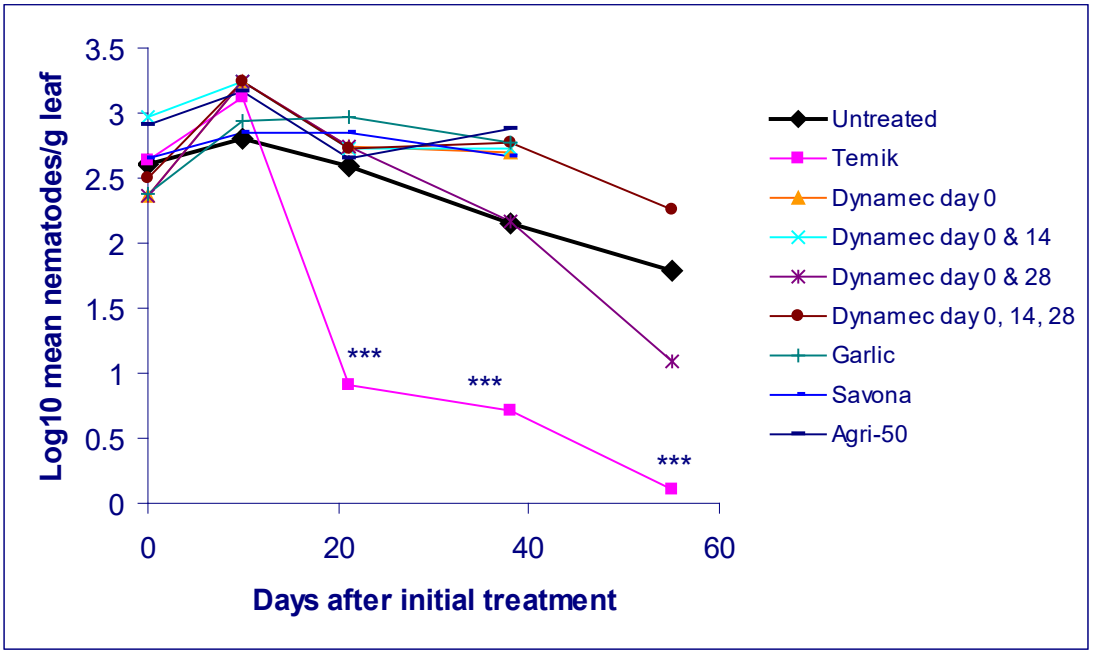

Figure 1. Log_{10} mean numbers of leaf and bud nematodes per gram of leaf tissue at day 0 (pre-treatment) and 10, 21, 38 and 55 days after first treatment
(*** = significantly different from the untreated controls, $P<0.001$).

- No suitable alternative treatment to Temik was identified to take forward to a trial on a commercial nursery in year 2 of the project.
- The pot experiment will be repeated in year 2, using Japanese anemones at the start of infestation with leaf and bud nematodes. Treatments will include the same Dynamec spray programmes as in the year 1 experiment and two additional candidates: Vydate (oxamyl) and a coded experimental product.

Financial benefits

- The project so far has shown that of the treatments included in the replicated pot test, only Temik gave significant reduction of leaf and bud nematodes on heavily infested Japanese anemones.
- Until further research is done in the project, no alternative treatments to Temik can be recommended.

Action points for growers

- Temik remains the most effective treatment for controlling leaf and bud nematodes on hardy nursery stock. Treatment should be applied in accordance with Specific Off-Label Approval 1932/2000 and is at grower's own risk.
- There is no label recommendation for use of Dynamec for leaf and bud nematodes and this pesticide has not controlled leaf and bud nematodes in this current project to date. Any use of Dynamec against this pest is at grower's own risk.
- Cultural control methods remain an important component of the management of leaf and bud nematodes:
  - ⇒ As the pest is commonly spread during plant propagation, cultural methods include avoiding using infested stock plants, hot water treatment of stock plants, or root propagation where appropriate.
  - ⇒ The pest can spread on infested plants and from plant to plant in films of water on the plant surface or in water splash, therefore spacing plants out and use of capillary irrigation rather than overhead watering can minimise spread.
  - ⇒ The pest can survive in a desiccated state in dry plant debris for several years, therefore strict nursery hygiene is an important cultural control component.
  - ⇒ The pest is unlikely to survive for more than four months in soil, in the absence of a host plant. However, many common weed species, e.g. chickweed and groundsel can be alternative hosts, so weed control should be maintained on previously infested ground.

SCIENCE SECTION

Introduction

HDC project HNS 60 confirmed that leaf and bud nematodes, *Aphelenchoides* spp. are common, persistent and damaging pests of a range of economically significant nursery stock plants including *Anenome japonica* (Japanese anemone), *Buddleia*, *Viburnum* and *Weigela* on many UK nurseries (Young, 1996). Control currently depends heavily on the use of aldicarb (Temik 10G) nematicide granules. In the current EU Review of pesticides, aldicarb has been excluded from Annex 1 of Directive 91/414, due to its risk to birds and earthworms. Consequently, all approvals for aldicarb will be withdrawn. However, aldicarb has been granted essential use status against nematodes in ornamentals in the UK until 2007, providing that alternative methods of control are developed. The current specific off-label approval (SOLA) for this use on ornamentals (1932/2000) will not be revoked until 31 December 2007.

An effective control measure for the pest has been identified as a critical gap (***) in HDC Project CP 17 (Pesticide Gap Analysis for Ornamental Crops 2003), and therefore it is essential that work is done to find an effective replacement for Temik.

The main aim of the project is to evaluate existing and novel alternatives to Temik for the control and management of leaf and bud nematodes on hardy nursery stock. The specific objectives are:

1. Test candidate treatments in a small-scale experiment with infested plants.
2. Test candidate treatments showing promise in a larger-scale trial on a commercial nursery.
3. Produce a factsheet to communicate the results of the project to growers and to recommend alternative treatments to Temik for the control and management of leaf and bud nematodes.

The work in year 1 of the project was designed to meet Objective 1.

Materials and methods

Experiment location and plant material

The work was done in a shade tunnel at ADAS Boxworth, Cambridge. The plants used were Japanese anemones. These were naturally infested with leaf and bud nematodes and were obtained from a commercial nursery.

Candidate treatment selection

Various candidate treatments were included in the original project proposal made to the HDC HNS Panel in February 2004. The Panel selected the following treatments to be included in this project:

Dynamec

Dynamec (abamectin) is approved for the control of two-spotted spider mite, western flower thrips and leaf miners on protected and outdoor ornamentals. ADAS research in HNS 86 demonstrated that sprays of this translaminar pesticide gave useful suppression of leaf and bud nematode, but control was not as persistent or as robust as that given by Temik (Young, 2000). The results indicated that a spray of Dynamec at the maximum label rate (as recommended for leaf miner control) can suppress the pest for up to nine weeks after treatment, but that repeated treatments would be needed at two to three month intervals to maintain this suppression. As a result of this research, some growers who do not wish to continue using Temik are now using Dynamec on HNS for partial control of leaf and bud nematodes.

Savona

Savona (fatty acids) are approved in the UK as a foliar spray for control of various pests including aphids, whiteflies and spider mites on both outdoor and protected ornamentals. Certain ornamental species are susceptible to damage by Savona and details are given on the label. Fatty acids (trade name Insecticidal Soap) have been shown in the USA to give 72% control of leaf nematodes 48 days after treatment when used as a foliar spray on *Hosta*, although it gave no control in previous *in vitro* laboratory tests when tested as a water suspension (Jagdale & Grewal, 2002).

Agri-50E

Agri-50E (alginate polysaccharide) has recently become available in the UK for the control of various pests including whiteflies and aphids. Like the plant extract products Majestik and Eradicoat, Agri-50 does not need to be registered as a pesticide as it acts by mechanical action, thus it can be used on any crop, at grower's own risk. Agri-50 was developed in the USA and current research there is focussing on its potential as a nematicidal soil drench.

Garlic

Garlic-based products are available in the UK and are sold as plant bio-stimulants e.g. Garlic Barrier. There is increasing interest in the potential repellent or toxic effects of garlic on various pests. One supplier is currently seeking approval for the use of a garlic product as pesticide. According to one supplier, garlic extracts have some

activity on nematodes in the soil, and there may be some systemic activity when applied to the soil, or contact activity when applied to the foliage.

Experiment design

The work was done as a randomised complete block design with five replicates of each of the nine treatments. There were eight plants in each plot, all plants having similar numbers of leaves with symptoms of leaf and bud nematode infestation. The eight plants in each plot were contained in a plastic pallet with raised feet, so that any run-off of Temik from treated compost during irrigation periods did not contaminate any pots in adjacent plots. The other treatments were applied as foliar sprays and a plastic spray shield was used in the paths between the pallets to prevent the risk of cross-contamination between plots during application. The pallets were stood on woven groundcover matting on the floor of the shade tunnel. Treatment dose rates and application timings are given in Table 1.

Table 1. Experimental treatments, product dose rates and dates of application.

Code	Treatment	Product dose rate	Application timing
1	Untreated	-	-
2	Temik 10G	10% w/w granules at 80 kg/ha	28/9/04
3	Dynamec	1.8% e.c. at 50 ml/100 l water	28/9/04
4	Dynamec	1.8% e.c. at 50 ml/100 l water	28/9/04, 12/10/04
5	Dynamec	1.8% e.c. at 50 ml/100 l water	28/9/04, 26/10/04
6	Dynamec	1.8% e.c. at 50 ml/100 l water	28/9/04, 12/10/04, 26/10/04
7	Garlic (coded product 0042)	99.9% e.c. at 0.25% v/v	28/9/04, 12/10/04, 26/10/04
8	Savona	50% e.c. at 2% v/v	28/9/04, 12/10/04, 26/10/04
9	Agri-50E	30% e.c. at 300 ml/100l	28/9/04, 12/10/04, 26/10/04

Treatment application methods

All treatments except Temik were applied with a knapsack sprayer as foliar sprays to just before run-off in 1,000 litres water per ha. Temik was applied in accordance with Specific Off-Label Approval 1932/2000, by sprinkling the granules onto the compost followed by watering in to before run-off.

Treatment timings

Dynamec was applied as a single treatment (day 0) or as a 2-spray programme (days 0 and 14 or days 0 and 28) or as a 3-spray programme (days 0, 14 and 28). The other foliar spray treatments were applied as a 3-spray programme (days 0, 14 and 28).

Irrigation

Plants were watered overhead twice per day, in the morning and early evening, using an automatic sprinkler. The plants were not irrigated for 24 hours after the foliar treatment sprays had been applied.

Assessments

Nematode infestations were assessed on five occasions:

1. Pre-treatment (day 0, 28 September 2004)
2. 10 days after first treatment date (8 October 2004)
3. 20 days after first treatment date (18 October 2004)
4. 38 days after first treatment date (5 November 2004)
5. 55 days after first treatment date (Treatments 1, 2, 5, 6 only, 22 November 2004)

Assessment methods

At each assessment date, one leaf was sampled from each of the eight plants per plot. Leaves of similar size and showing similar damage symptoms were selected from the same location on each plant. The eight leaves from each plot were combined, weighed and chopped into evenly sized pieces. The chopped leaves from each plot were placed into beakers containing 600 ml of fresh tap water. The water in each beaker was aerated for 72 hours using air stones connected to aquarium pumps. The nematodes were then collected by pouring the suspension through a 53 micron sieve. Numbers of live nematodes were counted in a Doncaster dish under a low-power microscope. If nematode numbers were too high to count accurately the suspension was sub-sampled and diluted as appropriate.

Temperature records

Temperatures inside the shade tunnel were recorded for the duration of the experiment using a Tinytalk datalogger, placed inside an empty plant pot.

Statistical analysis

Mean numbers of nematodes per plot were converted to mean numbers per gram of leaf tissue before analysis. The raw data were subjected to a $\log_{10}$ transformation prior to doing an Analysis of Variance. The data from the four Dynamec treatments at the assessment 10 days after the first treatment were combined before analysis, as on this date only one application of Dynamec had been made to all treatments. Similarly, at the assessment 21 days after the first treatment, data from Dynamec treatments 3 and 5 were combined as on this date only one application had been made, and data from treatments 4 and 6 were combined as on this date two applications had been made to both, on days 0 and 14.

Results

Control of leaf and bud nematodes

The transformed (log_{10}) and back-transformed (i.e. transformed back to the original scale) mean numbers of leaf and bud nematodes per gram of leaf tissue at each assessment date are given in Table 2 and shown in Figures 1 and 2 respectively. Untransformed means are given in Table 3 in Appendix I.

Table 2. Transformed (log_{10}) mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 10, 21, 38 and 55 days after treatment (back-transformed means are shown in brackets below the transformed means).

Treatment	**Day 0 (pre-treatment)**	**Day 10**	**Day 21**	**Day 38**	**Day 55**
Untreated	2.61 (405.4)	2.8 (630.0)	2.59 (384.5)	2.15 (141.6)	1.79 (60.9)
Temik	2.63 (428.5)	3.12 (1314.2)	0.92*** (7.2)	0.71*** (4.1)	0.10*** (0.3)
Dynamec at day 0	2.37 (232.9)	3.24 (1716.9)	2.74 (548.5)	2.70 (504.8)	-
Dynamec at days 0 & 14	2.97 (932.3)	3.24 (1716.9)	2.73 (531.1)	2.73 (536.0)	-
Dynamec at days 0 & 28	2.3 (229.1)	3.24 (1716.9)	2.74 (548.5)	2.16 (143.9)	1.10 (11.5)
Dynamec at Days 0,14 & 28	2.49 (310.2)	3.24 (1716.9)	2.73 (531.1)	2.72 (597.4)	2.26 (179.3)
Garlic at days 0,14 and 28	2.38 (241.1)	2.94 (866.0)	2.97 (923.7)	2.77 (591.9)	-
Savona at days 0,14 & 28	2.66 (450.9)	2.85 (710.2)	2.85 (706.9)	2.67 (468.9)	-
Agri-50 at days 0,14 and 28	2.91 (810.0)	3.16 (1444.4)	2.66 (451.9)	2.87 (743.7)	-
SED (df) for transformed data	0.41 (36 df)	0.32 min.rep 0.25 max-min 0.16 max.rep (39 df)	0.36 min.rep 0.31 max-min 0.25 max.rep (38 df)	0.36 (36 df)	0.36 (16 df)

- no assessment on that date

NB on day 7 all Dynamec treatments had only received one application at day 0, thus the mean value is given for all four Dynamec treatments. Similarly, on day 21, treatment 3 (Dynamec at day 0) and treatment 5 (Dynamec at days 0 and 28) had both received only one application, thus a mean value of these two treatments is given. Similarly, on day 21, treatment 4 (Dynamec at days 0 and 14) and treatment 6 (Dynamec at days 0, 14 and 28) had both received two applications, at day 0 and 14, thus a mean value of these two treatments is given.

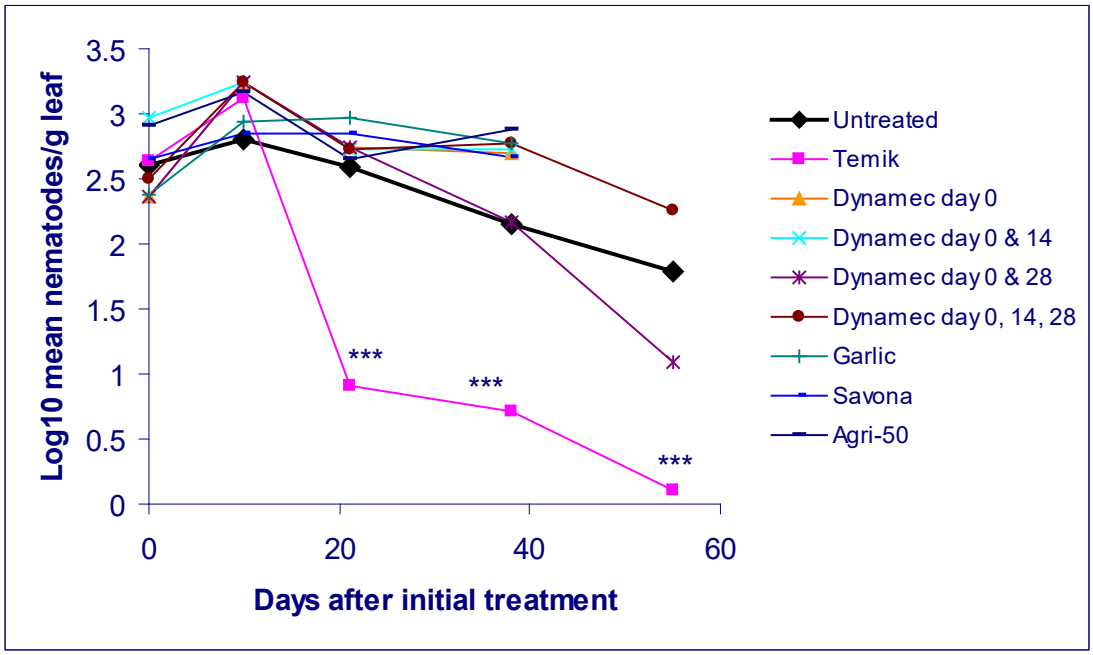

Figure 1. Log_{10} mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 10, 21, 38 and 55 days after first treatment.

*** Significantly different from Untreated controls, $P<0.001$

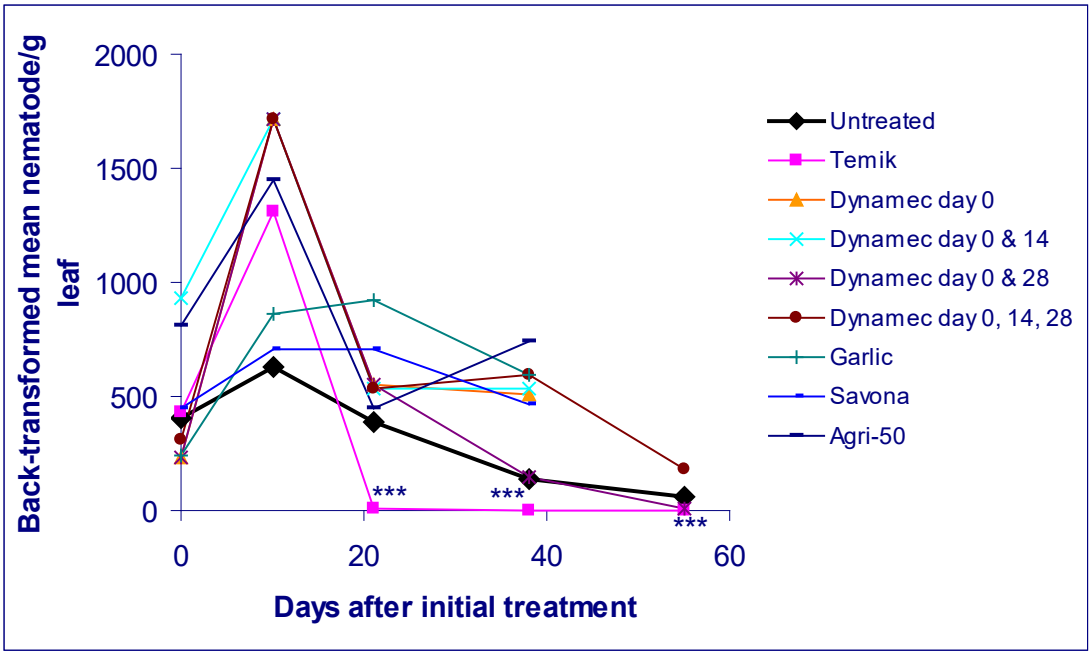

Figure 2. Back-transformed mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 10, 21, 38 and 55 days after first treatment.
*** Significantly different from Untreated controls, $P<0.001$

Temperature records

Temperatures during the experimental period are shown in Figure 3.

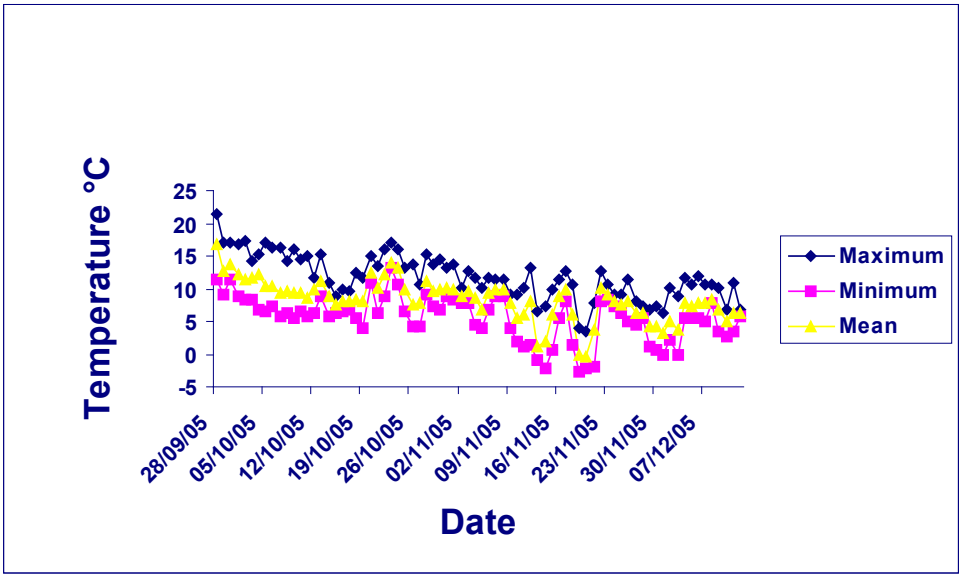

Figure 3. Mean, maximum and minimum temperatures in shade tunnel during experiment.

Discussion

Mean numbers of nematodes per gram of leaf tissue were high in all plots at the start of the experiment, ranging from 464 to 1010 per g leaf (untransformed data, Table 3, Appendix I). However, there was no significant difference between pre-treatment infestation levels. Mean numbers of nematodes per g leaf were also variable between the five replicate plots per treatment, despite leaves of similar size, position and symptom expression being selected. For example, in the five untreated plots, numbers of nematodes per g leaf varied from 18 to 1556 (untransformed data, Table 3, Appendix I). This variation is typical of leaf and bud nematode infestations and was also experienced in the previous project, HNS 86. Due to this previous experience, additional numbers of plants per plot and additional replicate plots per treatment were used in the current project compared with HNS 86.

Ten days after the first treatment had been applied, none of the treatments had reduced numbers of nematodes. At 21, 38 and 55 days after the first treatments had been applied, only Temik had significantly reduced numbers of nematodes (Table 2). Temik gave better control in this experiment than on Japanese anemones in HNS 86, on which Temik did not give significant control during the experimental period, although control was given on *Weigela*, *Saxifrage* and *Cistus* (Young, 2000).

None of the Dynamec treatments gave effective control of nematodes at any assessment date. This was a similar result to that obtained on Japanese anemones in HNS 86. However, in HNS 86, as with the Temik treatment, Dynamec applied as either a one or two-spray programme gave significant reduction of nematodes on *Weigela*, *Saxifrage* and *Cistus* (Young, 2000), although nematode infestations were generally lower than in the current project.

In HNS 86, laboratory results indicated that Dynamec might be less effective against leaf and bud nematodes over a 24-hour period at 3°C than at 25°C (Young, 2000). In the current project, Dynamec was applied on 28 September, 12 October and 26 October. Mean temperatures remained at approximately 10°C between 28 September and early November and did not fall to 3°C or below until the second and third weeks in November (Figure 3). Minimum temperatures fell to 3°C or below during November. In HNS 86, Dynamec was ineffective against the pest on Japanese anemones in an experiment done at a similar time of year to that in the current project, although some efficacy was shown on *Weigela* during a similar time period.

Although insufficient data are available to draw firm conclusions on the effect of temperature on the efficacy of Dynamec for nematode control, the product is known to degrade faster at high temperatures and is more persistent at low temperatures (Sopp, personal communication). In year 2 of the project, the experiment will be

done on Japanese anemones at the start of an infestation during the summer rather than in the autumn, to evaluate the treatments at warmer temperatures and at lower nematode levels. This will be more more representative of how and when the plants are treated against the pest in commercial practice.

Garlic, Savona and Agri-50E applied at 0, 14 and 28 days did not reduce numbers of nematodes. This is the first time garlic and Agri-50 have been rigorously tested against leaf and bud nematodes, although the suppliers of both treatments report some activity against soil-dwelling nematodes (personal communications). Although Savona has not previously been tested against leaf and bud nematodes, a very similar American fatty acids product, Insecticidal Soap, gave some control of the pest when used as a foliar spray on Hosta (Jagdale & Grewal, 2002).

Conclusions

- Temik gave excellent and persistent control of leaf and bud nematodes on heavily infested Japanese anemones, significantly reducing numbers of nematodes 21, 38 and 55 days after treatment.
- Dynamec as a single application, a two-spray programme at either 14 or 28 day intervals, or a three-spray programme at 14 day intervals did not give significant control of the pest.
- Foliar sprays of garlic, Savona or Agri-50 as a three-spray programme at 14 day intervals did not give significant control of the pest.
- No suitable alternative treatment to Temik was selected to take forward to a trial on a commercial nursery, to meet Objective 2 in the project.

Recommendations for research in Year 2

- In consultation with the HDC, the project co-ordinator and the HNS Panel, it was decided to repeat the small-scale pot experiment in the summer of year 2 of the project, using the same Japanese anemones as used in the year 1 experiment. The plants treated with Temik will be discarded and the treatments will be applied at an earlier stage of infestation than in year 1.
- As it is possible that Dynamec will give better control of a lighter infestation of the pest, the Dynamec treatments will be repeated in year 2.
- Vydate 10G (oxamyl) will be included in the experiment, as although this treatment was not selected by the Panel for the experiment in year 1, UK approval for this systemic carbamate nematicide is likely to continue after 2007, when Temik will be withdrawn (Powell, personal communication). Vydate has known efficacy against leaf and bud nematodes, and was relied on for control of the pest

in the USA until 1995, when it was withdrawn from the market (Jagdale & Grewal, 2002). Vydate 10G is currently approved in the UK for control of soil and root-dwelling nematodes on outdoor field crops, and it also has specific off-label approval (SOLA 0020/93) for control of alien leaf miner species on both outdoor and protected ornamentals.

- A coded pesticide will be included in the experiment, the identity of which cannot be revealed at present due to a confidentiality agreement with the supplier.
- Other potential treatments identified in the original project proposal were Jet 5 (peroxyacetic acid) and *Xenorhabdus*, the symbiotic bacteria extracted from entomopathogenic nematodes. The same disinfectant as Jet 5 (trade name ZeroTol) has been shown in the USA to have good activity against leaf nematodes when applied as a foliar spray (Jagdale & Grewal, 2002). However, it is unlikely that Jet 5 will be registered as a pesticide in the UK, so in agreement with the HDC, this will not be included in the year 2 experiment. Although *Xenorhabdus* has been shown in the USA to be highly toxic to leaf and bud nematodes, use of the bacteria is not currently approved in the UK for use as a biopesticide and there are no current commercial plans to pursue approval. Therefore this treatment will not be included in the year 2 experiment.

Technology transfer

- Jude Bennison has discussed the results of the project to date with HNS growers during consultancy work and with suppliers of the experimental treatments.
- Jude Bennison discussed the aims of the project in a presentation on IPM on UK hardy nursery stock, at the IOBC conference 'IPM in glasshouse crops' in Finland, April 2005.
- An article in HDC News is scheduled for the November 2005 issue.

Acknowledgements

Thanks to the following for supplying materials free of charge for the experiment:

- Allensmore Nurseries for supplying the Japanese anemones.
- Bayer Crop Science for supplying the Temik.
- Fargro for supplying the Dynamec and the Agri-50E.
- Koppert UK Ltd for supplying the Savona.

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Appendix 1

Figure 4. Symptoms of leaf and bud nematode infestation on Japanese anemone (angular leaf blotches limited by leaf veins).

Table 3. Untransformed mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 10, 21, 38 and 55 days after treatment

Treatment	Day 0 (pre-treatment)	Day 10	Day 21	Day 38	Day 55
Untreated	795	791	701	615	142
Temik	1010	1335	29	6	0.5
Dynamec at day 0	464	2448	823	733	-
Dynamec at days 0 & 14	967	2448	773	639	-
Dynamec at days 0 & 28	556	2448	823	338	28.6
Dynamec at Days 0,14 & 28	830	2448	773	787	445
Garlic at days 0,14 and 28	511	1340	1315	974	-
Savona at days 0,14 & 28	732	1155	967	1023	-
Agri-50 at days 0,14 and 28	1001	2322	895	861	-

- no assessment on that date

NB on day 7 all Dynamec treatments had only received one application at day 0, thus the mean value is given for all four Dynamec treatments. Similarly, on day 21, treatment 3 (Dynamec at day 0) and treatment 5 (Dynamec at days 0 and 28) had both received only one application, thus a mean value of these two treatments is given. Similarly, on day 21, treatment 4 (Dynamec at days 0 and 14) and treatment 6 (Dynamec at days 0, 14 and 28) had both received two applications, at day 0 and 14, thus a mean value of these two treatments is given.