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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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

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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.

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Project Leader

Date.............................

Report authorised by: ....

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Principal Research Entomologist

Date....................……….

CONTENTS

GROWER SUMMARY

Headline

- With the imminent withdrawal of Temik 10G from use in December 2007, Vydate 10G (oxamyl) has been identified as a potential replacement.

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 *Weigela*. Control currently depends on the use of Temik 10G (aldicarb) nematicide granules, which will be withdrawn from use in the UK in December 2007.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 previous HDC-funded project conducted by ADAS (HNS 86) demonstrated that abamectin (Dynamec) could give useful suppression of the pest, but control was 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 Hardy Nursery Stock before the withdrawal of Temik in 2007.

Summary of the project and main conclusions

Pot experiment with Japanese anemones

- As in the year 1 experiment, Temik 10G gave excellent and persistent control of leaf and bud nematodes on Japanese anemones, significantly reducing numbers of nematodes 21, 34 and 48 days after treatment (Figure 1).
- Vydate 10G also gave significant control of the pest, although control was not as effective as that given by Temik 10G on the final two assessment dates, 34 and 48 days after treatment.

- 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 control the pest.
- Foliar sprays of a coded experimental treatment, applied as a three-spray programme at 14 day intervals did not control the pest.

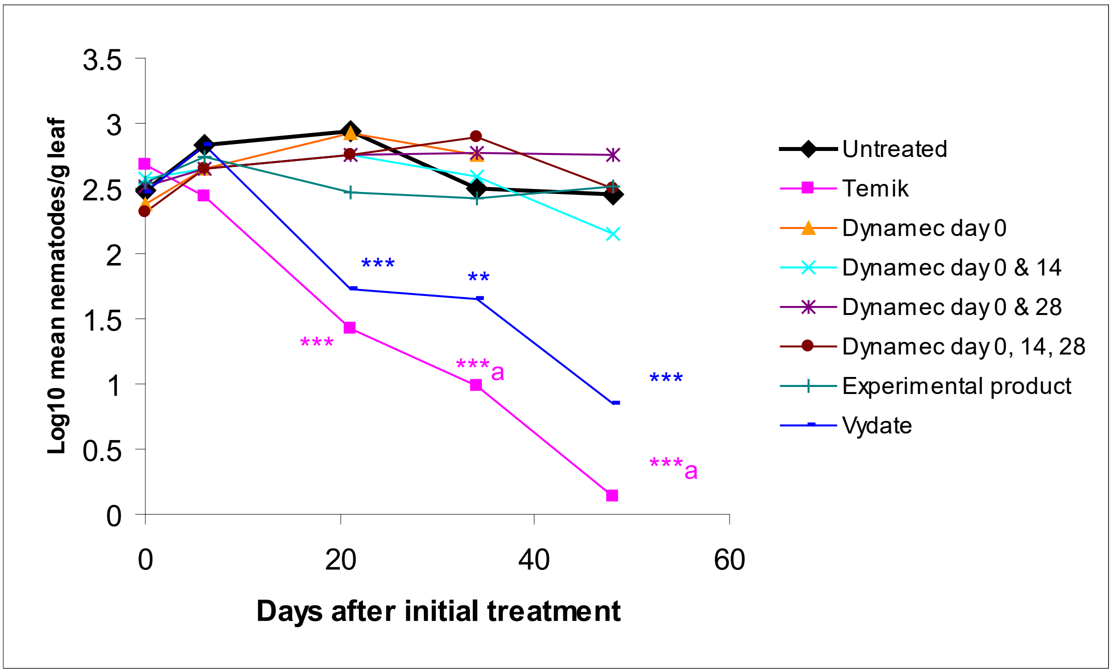

Figure 1. Log_{10} mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 6, 21, 34 and 48 days after first treatment.
** or *** significantly lower than in untreated controls.
a Temik significantly lower than Vydate.

- The experiment will be repeated in year 3, using a woody host e.g. *Weigela*. Treatments will include Temik 10G, Vydate 10G, a biological control agent and a novel plant extracts product if available.

Financial benefits

- The results of this work to date suggest that Vydate 10G is an effective alternative to Temik.

- Its identification will ensure that Hardy Nursery Stock growers are able to continue controlling leaf and bud nematodes, thus saving commercial plant losses to this pest.

Action points for growers

- Temik 10G 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. This SOLA will be revoked on 31 December 2007.

- Vydate 10G may be the most effective alternative to Temik 10G after December 2007, but so far its efficacy has only been tested on a herbaceous host, Japanese anemone. Its efficacy on a woody HNS host e.g. *Weigela* should be tested in year 3 of the project.

- Vydate 10G (oxamyl) is a carbamate nematicide and like Temik it has systemic activity. It 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. Oxamyl has achieved Annex 1 listing in EU Directive 91/414, so the approval for use of Vydate should continue, subject to re-registration at member state level.

- 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 two consecutive experiments on Japanese anemone in this project. 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 December 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.

Summary of work to meet Objective 1 in year 1

In a replicated experiment on potted Japanese anemones in year 1, no suitable alternative treatments to Temik were identified to take forward into a trial on a commercial nursery. Temik gave excellent and persistent control of a heavy infestation of leaf and bud nematodes, but Dynamec, Savona, Agri-50 and garlic did not control the pest.

Work to meet Objective 1 in year 2

As no candidate treatments had been identified for testing in a trial on a commercial nursery, the pot experiment on Japanese anemones was repeated in year 2, using plants at an earlier stage of infestation and including some alternative treatments.

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 the same plants used in the Year 1 experiment. The plants which had been treated with Temik in Year 1 were not used in the Year 2 experiment.

Candidate treatment selection

Dynamec

Dynamec (abamectin) is approved for the control of two-spotted spider mite, western flower thrips and leaf miners on protected and outdoor ornamentals. Previous 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.

However, in year 1 of the current project, HNS 131, Dynamec did not control a heavy infestation of leaf and bud nematodes, when applied in the autumn as a single application, or as a two-spray programme at either 14 or 28 day intervals, or as a three-spray programme at 14 day intervals. It was agreed with the HDC to include the same Dynamec treatments in the Year 2 experiment, but to evaluate treatments applied at lower nematode infestation levels.

Vydate

Oxamyl (Vydate) is a carbamate nematicide with known efficacy against leaf and bud nematodes, and was relied on for control of the pests in the USA until 1995, when it was withdrawn from the market (Jagdale & Grewal, 2002). Like Temik it has systemic activity. It 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. Oxamyl has achieved Annex 1 listing in EU Directive 91/414, subject to re-registration at member state level.

Experimental coded product

An experimental product was included in the year 2 experiment, the identity of which is confidential.

Experiment design

The work was done as a randomised complete block design with five replicates of each of the eight treatments. There were seven plants in each plot, all plants having similar numbers of leaves with symptoms of leaf and bud nematode infestation. The seven plants in each plot were contained in a plastic pallet with raised feet, so that any run-off of Temik or Vydate from treated compost during irrigation periods did not contaminate any pots in adjacent plots. When applying treatments as foliar sprays, 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	13/9/05
3	Dynamec	1.8% e.c. at 50 ml/100 l water	13/9/05
4	Dynamec	1.8% e.c. at 50 ml/100 l water	13/9/05, 26/9/05
5	Dynamec	1.8% e.c. at 50 ml/100 l water	13/9/05, 10/10/05
6	Dynamec	1.8% e.c. at 50 ml/100 l water	13/9/05, 26/9/05, 10/10/05
7	Experimental product		13/9/05, 26/9/05, 10/10/05
8	Vydate 10G	10% w/w granules at 55 kg/ha	13/9/05

Treatment application methods

Dynamec and the experimental product 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 (SOLA) 1932/2000, by sprinkling the granules onto the compost followed by watering in to before run-off. Vydate was applied using the same method as used for Temik, at the 55 kg/ha rate given on SOLA 0020/93, which is the maximum rate for use on outdoor ornamentals. Vydate may be used at 110 kg/ha on protected ornamentals, using SOLA 0020/93, which is recommended for control of alien leaf miners. However, the suppliers of Vydate advised that 55 kg/ha should be sufficient for use as a nematicide (this is the label recommended rate for use against soil-dwelling nematodes on outdoor crops e.g. potato).

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 experimental product was applied as a 3-spray programme (days 0, 14 and 28). Temik and Vydate were applied as a single treatment (day 0).

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, 13 September 2005)
2. Six days after first treatment date (19 September 2005)
3. 21 days after first treatment date (4 October 2005)
4. 34 days after first treatment date (17 October 2005)
5. 48 days after first treatment date (All treatments except treatment 3, 1 November 2005)

Assessment methods

At each assessment date, one leaf was sampled from each of the seven plants per plot. Leaves of similar size and showing similar damage symptoms were selected from the same location on each plant. The seven 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 six 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 6, 21, 34 and 48 days after treatment (back-transformed means are shown in brackets below the transformed means).

Treatment	Day 0 (pre-treatment)	Day 6	Day 21	Day 34	Day 48
Untreated	2.49 (308.0)	2.84 (684.5)	2.93 (858.0)	2.49 (310.9)	2.45 (279.5)
Temik	2.69 (487.7)	2.44 (273.2)	1.42*** (25.2)	0.99***a (8.7)	0.14***a (0.4)
Dynamec at	2.38	2.64	2.92	2.76	-

day 0	(240.5)	(439.6)	(838.5)	(569.2)	
Dynamec at days 0 & 14	2.58 (380.1)	2.64 (439.6)	2.75 (565.2)	2.59 (385.4)	2.15 (139.6)
Dynamec at days 0 & 28	2.52 (330.1)	2.64 (439.6)	2.92 (838.5)	2.77 (587.8)	2.76 (579.8)
Dynamec at Days 0,14 & 28	2.32 (207.9)	2.64 (439.6)	2.75 (565.2)	2.89 (775.2)	2.49 (310.9)
Experimental product at days 0,14 and 28	2.55 (353.0)	2.75 (560.0)	2.47 (294.1)	2.42 (260.8)	2.51 (325.6)
Vydate	2.48 (298.9)	2.84 (684.5)	1.73*** (52.1)	1.65** (43.6)	0.85*** (6.1)
SED (df) for transformed data	0.27 (32 df)	0.29 min.rep 0.23 max-min 0.14 max.rep (35 df)	0.27 min.rep 0.23 max-min 0.19 max.rep (34 df)	0.30 (32 df)	0.32 (28 df)

- no assessment on that date
** significantly different from untreated controls, *P*<0.01
*** significantly different from untreated controls, *P*<0.001
a Temik significantly different from Vydate, *P*<0.05

NB on day 6 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.

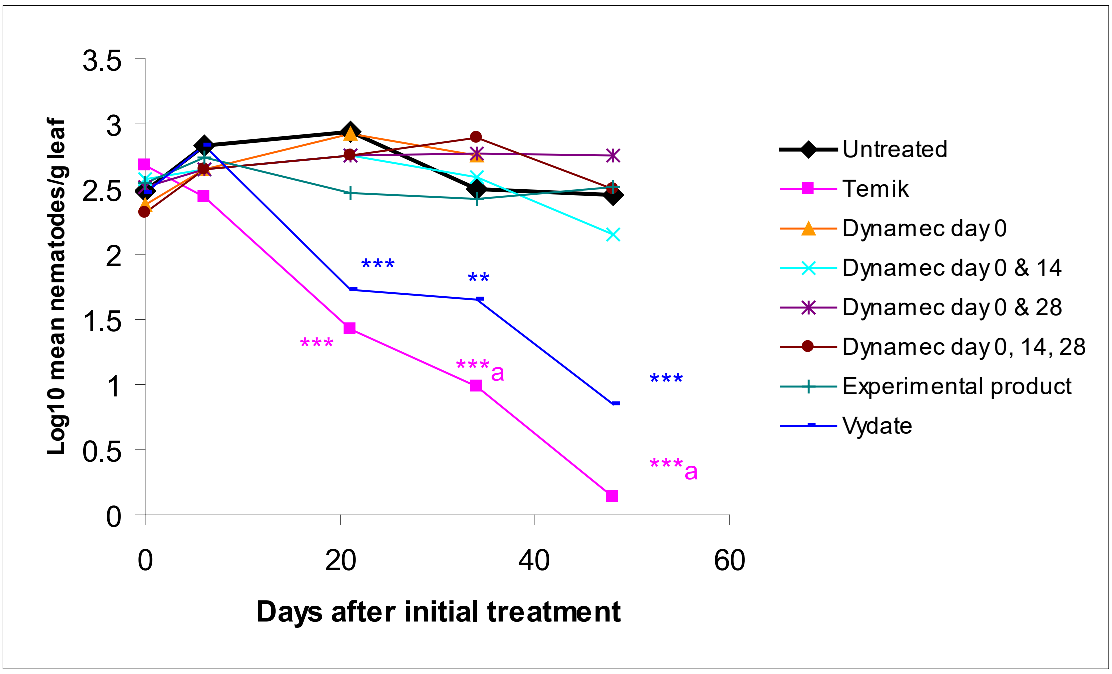

Figure 1. Log_{10} mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 6, 21, 34 and 48 days after first treatment.

** significantly different from untreated controls, $P<0.01$

*** significantly different from untreated controls, $P<0.001$

a Temik significantly different from Vydate, $P<0.05$

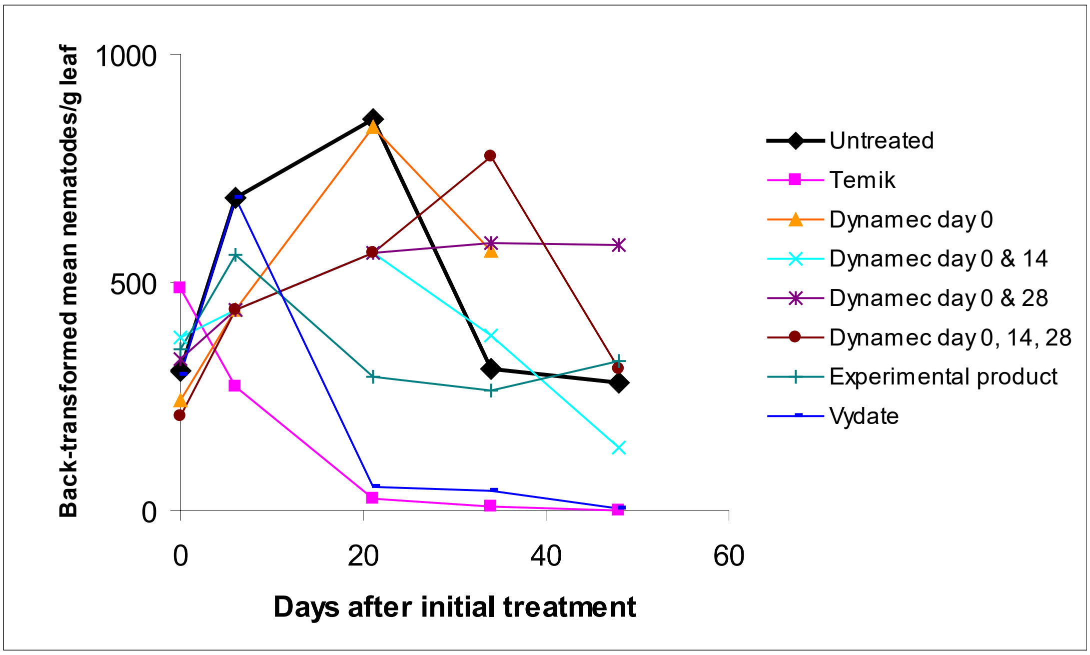

Figure 2. Back-transformed mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 6, 21, 34 and 48 days after first treatment.

Temperature records

Temperatures during the experimental period are shown in Figure 3.

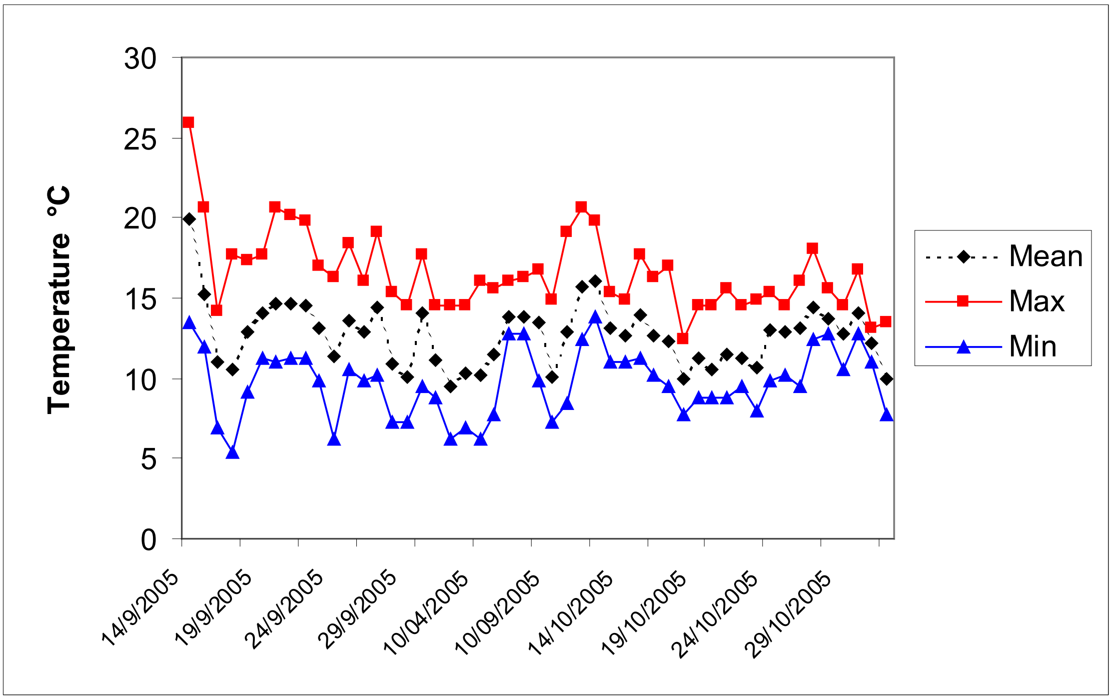

Figure 3. Mean, maximum and minimum temperatures in shade tunnel during experiment.

Discussion

Mean numbers of nematodes per gram of leaf tissue were high in all plots at the start of the experiment, ranging from 296 to 661 per g of leaf (untransformed data, Table 3, Appendix I). However, these initial numbers were lower than in the year 1 experiment (which ranged from 464 to 1010 per g of leaf), as intended, to test the alternative treatments to Temik at lower nematode infestations.

There was no significant difference between pre-treatment infestation levels. Six days after the first treatment had been applied, none of the treatments had reduced numbers of nematodes, and this was similar to the results in the year 1 experiment 10 days after the first treatment date. At 21, 34 and 48 days after the first treatments had been applied, both Temik and Vydate had significantly reduced numbers of nematodes (Table 2 and Figures 1 and 2). At 34 and 48 days after treatment, Temik gave significantly better reduction of nematodes than Vydate (Table 2 and Figures 1 and 2). At the final assessment 48 days after treatment, Temik and Vydate had reduced numbers of nematodes to a mean of one and 15 per g of leaf respectively (untransformed data, Table 3, Appendix I).

None of the Dynamec treatments gave effective control of nematodes at any assessment date. This was a similar result to that given in the Year 1 experiment in the current project and to that given 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 year 1 experiment in the current project, temperatures fell to 3°C or below towards the end of the experimental period. However, in the year 2 experiment, which was done earlier in the year than in year 1, overall temperatures were higher, with mean temperatures ranging from 10-15°C (Figure 3). Minimum temperatures in the year 2 experiment did not fall below 5°C. The results of the year 2 experiment indicate that Dynamec is ineffective against leaf and bud nematodes even at moderate temperatures between 5°C (minimum) and 20°C (maximum).

The experimental product did not give control of nematodes at any assessment date.

Conclusions

- Temik gave excellent and persistent control of leaf and bud nematodes on infested Japanese anemones, significantly reducing numbers of nematodes 21, 34 and 48 days after treatment.
- Vydate also gave significant control of nematodes 21, 34 and 48 days after treatment, although control was not as effective as Temik 34 and 48 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.
- An experimental product applied as a three-spray programme at 14 day intervals did not give significant control of the pest.
- Vydate is the only effective alternative to Temik that has been identified so far in this project.

Recommendations for research in Year 3

- Test Vydate (compared with Temik) on a woody plant host e.g. *Weigela*, to determine whether its systemic activity is as effective as on a herbaceous host, Japanese anemone.
- Test 'Nemagold' (*Tagetes* and seaweed extract, currently unavailable in the UK) if a sample can be procured from the Spanish supplier. This product is recommended as a soil drench against soil-dwelling nematodes and claims to have a repellent effect on nematodes, and to stop nematode growth and movement in the soil, causing death. The product has not been tested against leaf and bud nematodes but is worth trying as a foliar spray (personal communication with product supplier).
- Test entomopathogenic nematodes (epns) as a foliar spray, as suggested in the original project proposal in February 2004. Epns, currently available either as compost drenches, e.g. for the control of vine weevil and sciarid flies, or as foliar sprays against thrips, contain a symbiotic bacteria. The bacteria, *Xenorhabdus* spp. is released inside the insect gut once the nematodes have entered the insect host's body. It is the bacteria that kills the host, rather than the nematodes themselves. Although epns are unlikely to be able to penetrate other nematodes as they do with larger pests, the bacteria itself has been shown to act as a biological nematicide if released in water or soil containing nematodes. Research has been done in the UK on the use of bacteria from epns for the control of various pests including root-knot nematodes (Tabin *et al*, 2003) and this research is still in progress at Reading University and at other research institutes (Gowen, personal communication). Recent work at Ohio State University has shown that bacteria from the same epns is highly toxic to leaf and bud nematodes. Although various species of epns are available in the UK, use of the symbiotic *Xenorhabdus* sp. bacteria itself is not currently approved for use as a biopesticide. However, work in the proposed project using the nematodes as a foliar spray is justified, as sprays of the nematodes may lead to release of the bacteria on the leaf or bud surface when the epns die. (Other research in the USA has shown that soil applications of either live or dead EPNs can lead to suppression of root lesion nematode populations, La Mondia *et al, 2000*).

- In the original project proposal in February 04, it was suggested that the possible potential for other novel controls should be investigated, such as plant extracts, including *Tagetes*, mustard, Sudan grass, celery seed oil and other potential essential oils. It was suggested that a literature review should be done and other scientists contacted to identify any promising treatments. At the end of the current project in December 06, it is recommended that this suggestion is discussed with the Panel for their consideration of a future project.

Technology transfer

- HDC News article November 2005 issue.

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- Allensmore Nurseries for supplying the Japanese anemones.
- Bayer Crop Science for supplying the Temik.
- DuPont (UK) Ltd for supplying the Vydate.
- Fargro for supplying the Dynamec.
- The supplier of the experimental product.

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Table 3. Untransformed mean numbers of leaf and bud nematodes per g leaf tissue at day 0 (pre-treatment) and 6, 21, 34 and 48 days after treatment

Treatment	Day 0 (pre-treatment)	Day 6	Day 21	Day 34	Day 48
Untreated	535	750	951	648	479
Temik	585	474	29	9	1
Dynamec at day 0	412	445	938	875	-
Dynamec at days 0 & 14	451	797	943	742	438
Dynamec at days 0 & 28	388	981	1037	703	987
Dynamec at Days 0,14 & 28	296	551	657	965	383
Experimental product	384	931	833	371	447
Vydate	661	845	91	59	15

- no assessment on that date

NB on day 6 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.