HDC Research Project Final Report

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Project leader:	**Mr John Young, ADAS Boxworth**
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I. Practical Section for Growers

Background

The increasing prevalence and awareness of bud and leaf nematode (*Aphelenchoides* spp.) attacking hardy ornamental nursery stock has caused concern amongst growers and was the subject of a recent HDC review (Young, 1996). Chemical control gives only short-term suppression of this widespread and insidious pest. The nematodes often go undetected in the propagation cycle as unhealthy stock plants do not always exhibit obvious external symptoms of attack.

Aldicarb (Temik) is the only chemical treatment currently available against bud and leaf nematode in hardy ornamentals. The avermectins, of which abamectin (Dynamec) is one, are known to possess nematicidal activity. Dynamec is approved and marketed in the UK as an insecticide and acaricide for use in protected and outdoor ornamentals against two-spotted spider mite and leaf miners. The use of Dynamec as a foliar spray against bud and leaf nematode could provide growers with an alternative to Temik, which may be of use in suppressing outbreaks of this pest.

This final report covers the results of the two-year project, HNS 86. The objective of the work was to investigate the basic toxicity of Dynamec against bud and leaf nematode in laboratory tests and to assess the activity of Dynamec applied as a foliar spray against the pest on a range of hardy ornamental hosts, in comparison with the standard treatment of Temik granules.

In the first year of the project, the *in vitro* toxicity of Dynamec was assessed against bud and leaf nematode. Plant trials were also done on two varieties of the woody host *Weigela* and one variety of the herbaceous host, Japanese *Anemone*. In the second year, further trials were done on *Saxifraga* and *Cistus* together with further *in vitro* toxicity tests to investigate the effect of temperature on the toxicity of Dynamec against the nematode.

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Key findings

- Dynamec (abamectin) was proved in laboratory tests to have nematicidal activity against the bud and leaf nematode (*Aphelenchoides ritzemabosi*).

- Dynamec acts by causing an apparently irreversible paralysis of the nematodes.

- An estimated dose of 0.03% Dynamec was required to immobilise 90% of the test nematodes after 24 hours of exposure to the chemical in laboratory tests at room temperature (*c*. 20°C).

- The toxicity and the speed of action of Dynamec against bud and leaf nematodes was reduced at low temperatures (3°C), compared with higher temperatures (25°C), in laboratory tests.

- Dynamec, applied as one or two-spray treatments, was shown to suppress populations of bud and leaf nematodes attacking *Weigela* (cvs Looymansii Aurea and Bristol Ruby), Japanese *Anemone, Saxifraga,* and *Cistus*, up to 9 weeks after treatment.

- The level of control obtained from Dynamec was, on occasions, similar to that achieved with the standard nematicide, Temik (aldicarb).

- Dynamec was not as persistent or reliable as Temik in controlling bud and leaf nematode or in suppressing their symptoms of attack.

- A second spray of Dynamec, applied one or four weeks after the first spray, did not confer any advantage over a single spray of Dynamec in terms of nematode control.

- Dynamec applied at twice the maximum label-recommended rate did not significantly improve the standard of bud and leaf nematode control, compared with the maximum label-recommended rate.

- Dynamec shows potential as a short-term suppressant treatment against bud and leaf nematode. In practice, periodic follow-up treatments are likely to be required to maintain the suppression of nematode populations.

- Dynamec is inherently less toxic and potentially less hazardous to humans than Temik. However, to safeguard human health, wildlife and the environment, certain safety precautions (as stated on the product label) must be adhered to when using Dynamec.

- Neither Dynamec nor the standard nematicide,Temik, should be viewed as eradicant treatments as small numbers of nematodes can survive treatment to give rise to later attacks.

Benefits and action points for growers

- The study has indicated that Dynamec is a potentially useful treatment in the short-term suppression of bud and leaf nematodes in hardy ornamentals.

- Results showed that, in some instances, Dynamec can give levels of nematode suppression often equivalent to the current standard, Temik, up to 9 weeks after treatment. It is likely that follow-up sprays of Dynamec will be required at two to three month intervals to maintain suppression of nematode infestations.

- There were indications that Dynamec will not offer the same long-term control of bud and leaf nematodes as that obtainable with Temik. Therefore, in the control of bud and leaf nematodes, Dynamec should be viewed as a useful alternative to Temik, rather than as a replacement chemical.

- The activity of Dynamec against bud and leaf nematodes was reduced by low temperatures (3°C), suggesting that sprays applied in cold winter conditions (e.g. less than 10°C) may be less effective.

- Dynamec currently holds Approval and label recommendations for use in outdoor or protected ornamentals for the control of spider mites or leaf miner. Any use of Dynamec to control bud and leaf nematodes in ornamentals would be at the grower's own risk.

- At the dose rates applied in this study (Dynamec applied at 0.05% in 3000 litres water/ha and Temik at 80 kg/ha), a single application of Dynamec cost £416/ha, whilst Temik was more expensive at £1127/ha.

- Dynamec is applied as a conventional high volume spray, which growers may find quicker and more convenient to use than Temik granules. The application of Temik calls for greater attention to detail in applying the correct dosage to individual plant containers.

- Unlike Temik, Dynamec is not an anticholinesterase pesticide and is not subject to the Poisons Rules 1982. Nevertheless, Dynamec is harmful to bees and aquatic life and a range of safety precautions (as stated on the product label) must be observed with Dynamec at all times to safeguard the environment, wildlife and human health.

- It is possible to use Dynamec in conjunction with integrated pest management (IPM) programmes. Dynamec is toxic on contact to some biocontrol agents (e.g. *Phytoseiulus persimilis* and *Encarsia formosa*). Therefore, a safe interval (2–3 weeks) must elapse before the re-introduction of biocontrol agents following Dynamec application.

II. Science Section

Introduction

The increasing prevalence of bud and leaf nematode (*Aphelenchoides* spp.) attacking hardy ornamental nursery stock has given rise to concern amongst growers and was recently the subject of an HDC review (Young, 1996). Chemical control only gives short-term suppression of this widespread and insidious pest. The nematodes often go undetected in the propagation cycle owing to infested stock plants which may not always exhibit obvious symptoms of attack. This project aims to investigate the feasibility of using abamectin (as Dynamec) as a foliar spray to suppress infestations of bud and leaf nematode.

Aldicarb (Temik) is the only chemical treatment currently available against bud and leaf nematode in hardy ornamentals. The avermectins, of which abamectin (Dynamec) is one, are known to possess nematicidal activity. The use of Dynamec as a foliar spray against bud and leaf nematode would provide growers with an alternative to aldicarb, which would be of use in suppressing outbreaks of the pest.

Abamectin is the name given to avermectin B_1, part of a larger group of naturally derived compounds known as avermectins. The avermectins are macrocyclic lactones which are produced by the soil micro-organism *Streptomyces avermitilis*, an actinomycete, the specific name of which implies averminous or 'no worms'. These compounds were originally discovered as anthelmintic (anti-worm) agents with activity against gastrointestinal worm parasites of domestic animals (Hotson, 1982). Subsequent work demonstrated avermectin activity against various insect and mite plant pests as well as the root-knot nematode *Meloidogyne incognita* (Stretton *et al.*, 1987).

Abamectin is approved and marketed in the UK as Dynamec, an insecticide and acaricide for use in protected and outdoor ornamentals. Dynamec is targeted mainly at two-spotted spider mite and leaf miners and gives control of these pests owing to its translaminar activity. The active ingredient penetrates leaf tissue and accumulates within the leaf structure. Although Dynamec is toxic on contact to some biocontrol agents (e.g. *Phytoseiulus persimilis* and *Encarsia formosa*), it has minimal impact on integrated pest management (IPM) programmes. A safe interval of 2–3 weeks is normally allowed to elapse before re-introducing foliar-active biocontrol agents following the application of Dynamec.

This final report covers work done during the course of a two-year HDC research project, HNS 86. During the first year of the project, experimental work was undertaken to investigate the *in vitro* toxicity of Dynamec against the bud and leaf nematode (*Aphelenchoides ritzemabosi*). Trials were also carried out against the nematode in two varieties of the woody host *Weigela* and one variety of the herbaceous host, Japanese *Anemone*. The objective of these trials was to evaluate the activity of Dynamec against that of the standard nematicide, Temik, for control of established infestations of bud and leaf nematode in commonly infested hardy ornamental hosts.

In the second and final year of the project, further *in vitro* toxicity testing was done to investigate the effect of temperature on the toxicity of Dynamec against the nematode. Further plant trials were also completed in the final year using Dynamec (including some higher dose-rates than those used in the first year) and Temik against bud and leaf nematode in *Saxifraga,* a herbaceous host, and Cistus, a woody host.

Materials and Methods

1) Dynamec **in vitro** *toxicity tests, Year 1.*

An initial exploratory test was carried out in order to prove and develop the methodology and to ascertain a suitable test concentration range for the Dynamec. Following the exploratory test, a total of three standardised toxicity tests were done at room temperature (*c.* 20°C).

The bud and leaf nematode species *A. ritzemabosi* was extracted from naturally infested lavender plants by placing chopped leaves into a nylon mesh bag and leaving them for 48 hours in a beaker of aerated tap water. The nematodes, which subsequently migrated from the leaf tissue into the surrounding water, were collected on a 53 μ sieve and placed in a small volume of water immediately prior to use.

The following concentrations of Dynamec were used in each of three tests:

1. Untreated
2. Dynamec @ 0.002% (0.36 ppm abamectin)
3. Dynamec @ 0.01% (2.8 ppm abamectin)
4. Dynamec @ 0.05% (9 ppm abamectin)
5. Dynamec @ 0.25% (45 ppm abamectin)
6. Dynamec @ 0.5% (90 ppm abamectin)

Each test solution was made up to a total volume of 10 ml with de-ionised water and placed in a nematode counting dish (Doncaster dish).

Tests were done on the following dates, with the following number of nematodes in each test solution:

Test identification	Test date	Number of nematodes per test concentration
Test 1	16 June 1998	30
Test 2	14 October 1998	60
Test 3	4 November 1998	50

Freshly extracted and visibly mobile nematodes were transferred to the test solutions by hand using a mounted needle or eyelash, with the aid of a low-power microscope. The nematodes were examined and counted under a low-power microscope at intervals of 1 hour, 4 hours and 24 hours after being placed in each test solution. The nematodes were counted on each occasion and classified as either mobile (visible swimming movements) or non-mobile (no visible signs of body movement).

Nematode recovery checks were made in Tests 1 and 2 to ascertain if any of the immobilised nematodes were capable of recovery. This was done immediately after the completion of the 24-hour post-treatment counts by removing up to 20 immobilised nematodes from each treatment and placing them in de-ionised water. However, in the case of the untreated controls, the majority of nematodes removed remained mobile throughout the observation. The nematodes were then examined after 3 hours and 24 hours for signs of renewed movement.

The toxicity test data were analysed using probit models fitted to each of the exposure times tested, using the Generalised Linear Model part of GENSTAT. The effective doses for LD50 and LD90 were calculated together with their 95% confidence limits. The LD90 for the one hour and four hour exposure times were outside of the range of results recorded, so only the 24 hour LD90 figure was included in the results.

2) Dynamec **in vitro** *toxicity/temperature tests, Year 2.*

Further toxicity tests were carried out in year two to investigate the effect of temperature on the toxicity of Dynamec against bud and leaf nematode. The temperatures chosen for these tests were intended to mimic the extremes of temperature encountered in the field at various times of the year. The tests were done at temperatures of 3°C ('low') and 25°C ('high') in a constant temperature incubator (± 1°C). The doses of Dynamec and the basic methodology of these tests were otherwise the same as detailed above for the first year of work. The test nematodes were, in this instance, extracted from infested *Cistus* plants. In the low temperature toxicity tests, each counting dish was left at laboratory temperature (*c*. 20°C) for 15 minutes prior to each assessment to allow the nematodes to regain movement.

The toxicity/temperature tests were done on the following dates, with the following number of nematodes in each test solution:

Test identification	Test date	Number of nematodes per test concentration
'Low' temperature (3°C)	8 September 1999	60
'High' temperature (25°C)	28 September 1999	50

As in the first year of testing, following 24 hours of exposure to the Dynamec treatments, the nematodes were checked for possible recovery by removing sub-samples of individuals from each treatment (30 and 20 nematodes for the low and high temperature tests, respectively) and transferring them to water for observation of renewed movement.

The toxicity/temperature test data were analysed using probit models fitted to each of the exposure times tested, as detailed for the first year of work above. As with the results of the first year's work, some of the calculated LD50 and LD90 values lay outside of the dose-range tested and were, therefore, excluded from the results.

3) Plant trials: Year 1

Dynamec was compared with Temik for the control of *A. ritzemabosi* infestations in *Weigela* cv. Bristol Ruby; *Weigela* cv. Looymansii Aurea and Japanese *Anemone* cv. Lady Gilmour. The *Weigela* infestations were of natural origin and the Japanese *Anemone* infestation was obtained through artificially inoculating healthy plants with nematodes extracted from the same plant variety. All results from the plant trials were subjected to analysis of variance (ANOVA) for each individual assessment date.

Anemone *inoculation technique*

Infested leaves of *Anemone* cv. Lady Gilmour were cut up and placed in a nylon mesh bags which were then immersed in beakers of tap water. The contents of each beaker were then constantly and vigorously aerated for 48 hours using an aquarium airpump connected to a diffuser (airstone). The nematodes were then collected on a 53 micron sieve and re-suspended in tap water. The nematode suspension was then used to inoculate the test plants by applying the suspension to run-off with a hand-held sprayer. The inoculated plants were placed on damp capillary matting, misted with water, covered with clear polythene and placed in a small perspex tunnel to maintain the free moisture and high humidity required to facilitate nematode movement and leaf invasion.

Treatments, plant trials, Year 1

The following treatments were applied in each plant trial conducted in Year 1:

1. Untreated. Plants sprayed with water only.
2. Temik 10G (10% w/w aldicarb) @ 80kg/ha. Applied to pot soil surface.
3. Dynamec (abamectin 1.8% w/v). One spray applied at a concentration of 0.05% in 3000 litres water/ha.
4. Dynamec (abamectin 1.8% w/v). Two sprays. Dose rates as in Treatment 3. First spray applied at the same time as Treatment 3; second spray applied 14 days after the first application.

The Dynamec dose rates reflected the maximum approved dose rates recommended on the current product label for leaf miner control in outdoor and protected ornamentals. The Temik was applied by hand to individual pots. The Dynamec was applied using a hand-held sprayer.

Experimental design, plant trials, Year 1

There were four replicates for each treatment. Each replicate consisted of five plants showing symptoms of *Aphelenchoides* infestation. Each batch of plants, comprising of the four replicates of each treatment, were labelled and placed in separate chambers. Each chamber measured approximately 2 m × 1 m × 1 m with a solid fibre-glass base on which was mounted a tubular frame covered with clear polythene. The plants were watered and misted regularly to maintain damp and humid conditions within each chamber.

Assessments, plant trials Year 1

Nematode infestations were assessed immediately prior to treatment and then 7 and 28 days after treatment (DAT). Final assessments were also done, the timing of which varied between 45 and 68 DAT. For the *Weigela* cv. Bristol Ruby, the final assessment was 68 DAT; for *Weigela* cv. Looymansii Aurea, 45 DAT (due to leaf senescence/drop); and for Japanese *Anemone* cv. Lady Gilmour, 62 DAT. In each assessment, five leaves showing nematode symptoms were taken per replicate (one from each plant). In the final assessment of *Weigela* cv. Bristol Ruby a mixture of leaves with and without symptoms were taken as there were too few leaves left with symptoms at that time.

To extract the nematodes, the leaf samples were weighed, chopped, and placed in a nylon mesh bag, the mesh being wide enough to allow the nematodes to swim through but keep plant material separate. The prepared leaf samples were then placed into beakers containing fresh tap water which was then aerated vigorously for 48 or 72 hours using air stones connected to aquarium air pumps. The nematodes were then collected, using a 53 micron sieve, and counted within a Doncaster Dish under a low-power binocular microscope. Where the numbers of nematodes were too great to count accurately the nematode solution was sub-sampled and 3 × 1ml aliquots were counted using a Hauxley counting slide.

Weigela cv. Bristol Ruby, Year 1

All treatments were applied on 30 July 1998, with a second Dynamec application 14 days later on Treatment 2. Plants were selected for inclusion in the trial only if at least eight leaves per plant were showing visible symptoms of nematode attack. Only the lower leaves were displaying substantial symptoms at the time of treatment. The pots were watered prior to the application of the treatments. The plant chambers (see above) were initially placed in a glasshouse and placed under shade netting. However, owing to hot weather which was in danger of damaging the plants, the chambers were transferred to a cooler, shaded, outdoor area on the 7 August. Final assessments were done on 8 October 1998.

Temperatures for the trial period under glass (28 July–7 August): maximum 43.7°C, minimum 21.1°C, mean 27.9°C. Outdoor shade period (8 August–8 October): maximum 31.0° C, minimum 4.8°C, mean 14.5°C.

Japanese anemone cv. Lady Gilmour, Year 1

All treatments were applied on 20 October 1998, with the second application of Dynamec on Treatment 2 applied 14 days later. The plant chambers were placed in a shade tunnel (47% shade). The final assessment was on the 21 December 1998. Temperatures over trial period: maximum 16.5°C, minimum -3.5°C, overall mean 5.1°C. Maximum relative humidity (RH) 97.9%, minimum RH 72.6%.

Weigela cv. Looymansii Aurea, Year 1

All treatments were applied on the 3 November 1998, with the second application of Dynamec on Treatment 2 applied 14 days later. The plant chambers were set up in a shade tunnel (47% shade.). The final assessment was made on 18 December 1998. Temperatures over trial period: Maximum 16.5°C, Minimum -3.3 °C, overall mean 5.0°C. Maximum RH 97.9%, minimum RH 72.6%.

4) Plant trials: Year 2

In the second year of the project, Dynamec was compared with Temik for the control of bud and leaf nematode in *Saxifraga* cv. James Bremner and *Cistus* cv. Corbariensis, both with natural infestations.

Treatments, plant trials, Year 2

The following treatments were applied in each plant trial conducted in Year 2:

1. Untreated. Plants with water only.
2. Temik 10G (10%w/w aldicarb) @ 80kg/ha. Applied to compost surface.
3. Dynamec (abamectin 1.8%w/v). One spray applied at a concentration of 0.05% in 3000 litres of water.
4. Dynamec (abamectin 1.8%w/v). Two sprays at a concentration of 0.05%, the first applied as in Treatment 3, followed by a second application 35 days later.
5. Dynamec (abamectin 1.8%w/v). One spray applied at a concentration of 0.1% in 3000 litres of water.
6. Dynamec (abamectin 1.8%w/v). Two sprays applied at a concentration of 0.1%. The first applied as in Treatment 5, followed by a second application 35 days later.

The Dynamec rate in Treatments 3 and 4 was at the maximum Approved dose-rate, as recommended on the product label. Treatments 5 and 6 were included as additional treatments in Year 2. In these treatments, Dynamec was applied at double the maximum label-recommended rate as single- and two-spray treatments respectively. The high-rate treatments of Dynamec (5&6) were included in the final year of work as the findings of the first year suggested that higher rates merited investigation. All of the Dynamec treatments were applied as high volume sprays using an Oxford Precision CO_2-powered sprayer. As in the first year of work, the Temik was applied by hand to individual pots.

Experimental design, plant trials, Year 2

The same basic design was employed as in Year 1. Each replicate consisted of four plants for the *Saxifraga* plants (3 litre pots) and five plants for the *Cistus* plants (1 litre pots).

Assessments, plant trials, Year 2

Plants were assessed for nematode infestation immediately prior to treatment and then at 7, 28, 35 and 63 days after treatment (DAT). For each assessment of the *Saxifraga* plants four points were sampled within the foliar 'cushion' of each plant. Although the symptoms of nematode attack were not readily visible within the *Saxifraga* foliage, foliage suspected of harbouring nematodes was selected for these assessments whenever possible. For the *Cistus* plants, five leaves exhibiting nematode symptoms were taken (one from each plant) on each assessment date.
The same methodology for extracting nematodes from plant material was used as detailed above for Year 1

Saxifraga *cv. James Bremner*

All treatments were applied on the 13 April 1999. The second applications of Treatments four and six were applied 35 days later. The plant chambers were kept in a shade tunnel (47% shade). The trial period was from mid-April to mid-June. Temperatures over the trial period were: maximum 27.7°C, minimum -2.0°C, overall mean 12.7° C. The maximum relative humidity (RH) was 98.8%; minimum RH, 37.4%.

Cistus *cv. Corbariensis*

All treatments were applied on 10 August 1999. The second applications of Treatments four and six, were applied 35 days later. The plants were kept in a shade tunnel (47% shade). The trial period was from mid-August to mid-October. Temperatures over the trial period were: maximum 29.0°C, minimum 1.8°C, overall mean 14.4°C. The maximum RH was 104.1%, minimum RH 40.1%.

Results

Table 1. The effect of Dynamec on the mobility of *A. ritzemabosi* in Toxicity Test 1 (16/06/98). 30 nematodes were tested per treatment at room temperature.

Treatment & nematode category		Exposure time		
		1 hour	4 hours	24 hours
Untreated	Mobile	30	30	29
	Non-mobile	0	0	1
	% non-mobile	0	0	3
Dynamec 0.002%	Mobile	29	28	7
	Non-mobile	1	2	22
	% non-mobile	3	7	76
Dynamec 0.01%	Mobile	23	15	0
	Non-mobile	5	16	31
	% non-mobile	18	52	100
Dynamec 0.05%	Mobile	25	16	0
	Non-mobile	5	13	29
	% non-mobile	17	45	100
Dynamec 0.25%	Mobile	23	13	0
	Non-mobile	7	15	30
	% non-mobile	23	54	100
Dynamec 0.50%	Mobile	2	14	0
	Non-mobile	27	16	30
	% non-mobile	93	53	100

Table 2. The effect of Dynamec on the mobility of *A. ritzemabosi* in Toxicity Test 2 (14/10/98). 60 nematodes were tested per treatment at room temperature.

Treatment & nematode category		Exposure time		
		1 hour	4 hours	24 hours
Untreated	Mobile	60	59	56
	Non-mobile	0	1	4
	% non-mobile	0	2	7
Dynamec 0.002%	Mobile	58	58	24
	Non-mobile	2	2	36
	% non-mobile	3	3	60
Dynamec 0.01%	Mobile	58	14	4
	Non-mobile	2	46	56
	% non-mobile	3	77	93
Dynamec 0.05%	Mobile	50	23	2
	Non-mobile	7	34	55
	% non-mobile	12	60	96
Dynamec 0.25%	Mobile	54	29	3
	Non-mobile	6	31	57
	% non-mobile	10	52	95
Dynamec 0.50%	Mobile	11	13	3
	Non-mobile	50	47	57
	% non-mobile	82	78	95

Table 3. The effect of Dynamec on the mobility of *A. ritzemabosi* in Toxicity Test 3 (4/11/98). 50 nematodes were tested per treatment at room temperature.

Treatment & nematode category		Exposure time		
		1 hour	4 hours	24 hours
Untreated	Mobile	50	48	46
	Non-mobile	0	2	4
	% non-mobile	0	4	8
Dynamec 0.002%	Mobile	50	46	11
	Non-mobile	0	4	39
	% non-mobile	0	8	78
Dynamec 0.01%	Mobile	43	32	9
	Non-mobile	7	18	41
	% non-mobile	14	36	82
Dynamec 0.05%	Mobile	35	29	1
	Non-mobile	15	21	49
	% non-mobile	30	42	98
Dynamec 0.25%	Mobile	46	35	7
	Non-mobile	4	15	43
	% non-mobile	8	30	86
Dynamec 0.50%	Mobile	1	39	4
	Non-mobile	49	11	46
	% non-mobile	98	22	92

Table 4. Calculated Lethal Dose 50 (LD50) and Lethal Dose 90 (LD90) values from *in vitro* tests of Dynamec against *A. ritzemabosi* at room temperatures in 1998, (% concentration of Dynamec).

Exposure time		Lethal dose (% Dynamec)	95% confidence range: Lower limit	Upper limit
1 hour	LD50	0.2875	0.2201	0.3940
	LD90	NA	NA	NA
4 hours	LD50	0.1421	0.0878	0.2539
	LD90	NA	NA	NA
24 hours	LD50	0.0011	0.0008	0.0015
	LD90	0.0315	0.0217	0.0488

Table 5. A summary of the effect of Dynamec on the mobility of *A.ritzemabosi* in three *in vitro* toxicity tests conducted at room temperatures in 1998, (% non-mobile nematodes).

Treatment	**Test number**	**Exposure time**		
		1 hour	**4 hours**	**24 hours**
Untreated	1 (16/6/98)	0	0	3
	2 (14/10/98)	0	2	7
	3 (04/11/98)	0	4	8
	Mean	0	2	6
Dynamec 0.002%	1 (16/6/98)	3	7	76
	2 (14/10/98)	3	3	60
	3 (04/11/98)	0	8	78
	Mean	2	6	71
Dynamec 0.01%	1 (16/6/98)	18	52	100
	2 (14/10/98)	3	77	93
	3 (04/11/98)	14	36	82
	Mean	12	55	92
Dynamec 0.05%	1 (16/6/98)	17	45	100
	2 (14/10/98)	12	60	96
	3 (04/11/98)	30	42	98
	Mean	20	49	98
Dynamec 0.25%	1 (16/6/98)	23	54	100
	2 (14/10/98)	10	52	95
	3 (04/11/98)	8	30	86
	Mean	14	45	94
Dynamec 0.50%	1 (16/6/98)	93	53	100
	2 (14/10/98)	82	78	95
	3 (04/11/98)	98	22	92
	Mean	91	51	96

Table 6. The effect of placing *A. ritzemabosi* into untreated water to observe any recovery following 24 hour exposure to Dynamec test solutions at room temperatures in 1998. 20 nematodes were observed per treatment in each test, (% non-mobile nematodes).

Treatment	Test number	Time in water	
		3 hour	24 hours
Untreated	1 (16/06/98)	0	20
	2 (14/10/98)	na	na
	3 (04/11/98)	10	0
	Mean	5	10
Dynamec 0.002%	1 (16/06/98)	85	75
	2 (14/10/98)	na	na
	3 (04/11/98)	80	70
	Mean	83	73
Dynamec 0.01%	1 (16/06/98)	100	100
	2 (14/10/98)	na	na
	3 (04/11/98)	100	95
	Mean	100	98
Dynamec 0.05%	1 (16/06/98)	100	100
	2 (14/10/98)	na	na
	3 (04/11/98)	100	100
	Mean	100	100
Dynamec 0.25%	1 (16/06/98)	100	100
	2 (14/10/98)	na	na
	3 (04/11/98)	100	95
	Mean	100	98
Dynamec 0.50%	1 (16/06/98)	100	100
	2 (14/10/98)	na	na
	3 (04/11/98)	95	90
	Mean	98	95

Table 7. The effect of Dynamec on the mobility of *A. ritzemabosi* in the low temperature/toxicity test (8/9/99). 60 nematodes were tested per treatment at 3°C.

Treatment & nematode category		Exposure time		
		1 hour	4 hours	24 hours
Untreated	Mobile	59	58	59
	Non-mobile	1	2	1
	% non-mobile	2	3	2
Dynamec 0.002%	Mobile	59	51	22
	Non-mobile	1	9	38
	% non-mobile	2	15	63
Dynamec 0.01%	Mobile	55	53	33
	Non-mobile	5	7	27
	% non-mobile	8	12	45
Dynamec 0.05%	Mobile	56	25	15
	Non-mobile	4	35	45
	% non-mobile	7	58	75
Dynamec 0.25%	Mobile	46	21	4
	Non-mobile	14	39	55
	% non-mobile	23	65	92
Dynamec 0.50%	Mobile	41	15	1
	Non-mobile	19	44	59
	% non-mobile	32	73	98

Table 8. Calculated Lethal Dose 50 (LD50) and Lethal Dose 90 (LD90) values from *in vitro* low temperature/toxicity tests of Dynamec against *A. ritzemabosi* conducted at 3°C, 1999, (% concentration of Dynamec).

Exposure time		**Lethal dose (% Dynamec)**	**95% confidence range: Lower limit**	**Upper limit**
1 hour	LD50	NA	NA	NA
	LD90	NA	NA	NA
4 hours	LD50	0.0694	0.0435	0.1164
	LD90	NA	NA	NA
24 hours	LD50	0.0049	0.0030	0.0076
	LD90	0.1665	0.0933	0.3550

Table 9. The effect of Dynamec on the mobility of *A. ritzemabosi* in the high temperature/toxicity test (28/9/99). 50 nematodes were tested per treatment at 25°C.

Treatment & nematode category		Exposure time		
		1 hour	4 hours	24 hours
Untreated	Mobile	50	50	49
	Non-mobile	0	0	1
	% non-mobile	0	0	2
Dynamec 0.002%	Mobile	47	43	4
	Non-mobile	3	7	32
	% non-mobile	6	14	64
Dynamec 0.01%	Mobile	44	21	1
	Non-mobile	6	29	49
	% non-mobile	12	58	98
Dynamec 0.05%	Mobile	34	8	1
	Non-mobile	16	42	49
	% non-mobile	32	84	98
Dynamec 0.25%	Mobile	25	12	3
	Non-mobile	25	38	47
	% non-mobile	50	76	94
Dynamec 0.50%	Mobile	5	7	2
	Non-mobile	45	44	48
	% non-mobile	90	88	96

Table 10. Calculated Lethal Dose 50 (LD50) and Lethal Dose 90 (LD90) values from *in vitro* high temperature/toxicity tests of Dynamec against *A. ritzemabosi* conducted at 25°C, 1999, (% concentration of Dynamec).

Exposure time		Lethal dose (% Dynamec)	95% confidence range: Lower limit	Upper limit
1 hour	LD50	0.1125	0.0762	0.1728
	LD90	NA	NA	NA
4 hours	LD50	0.0146	0.0091	0.0227
	LD90	0.3887	0.2081	0.9132
24 hours	LD50	0.0013	0.0008	0.0021
	LD90	0.0228	0.0132	0.0464

Table 11. The effect of placing *A. ritzemabosi* into untreated water to observe any recovery following 24 hour exposure to the Dynamec test solutions, 1999. 30 nematodes were observed in the low temperature test and 20 in the high temperature test (% non-mobile nematodes).

Treatment	**Test identity**	**Time in water**	
		3 hour	**24 hours**
Untreated	Low temp.	17	17
	High temp.	0	0
Dynamec 0.002%	Low temp.	90	43
	High temp.	100	75
Dynamec 0.01%	Low temp.	90	63
	High temp.	100	100
Dynamec 0.05%	Low temp.	100	77
	High temp.	100	100
Dynamec 0.25%	Low temp.	100	97
	High temp.	90	100
Dynamec 0.50%	Low temp.	100	100
	High temp.	100	100

Table 12. The effect of Temik and Dynamec treatments on the numbers of *A. ritzemabosi* infesting *Weigela* cv. Bristol Ruby, (numbers/gram leaf tissue).

Treatment	Days after treatment (DAT)			
	0 (pre-treatment)	7	28	68
Untreated	705	27	136	17
Temik (Day 0)	319	5	2**	0
Dynamec (Day 0)	553	15	15**	4
Dynamec (Days 0 & 14)	217	30	18**	8
SED (12 d.f.)	177.4	11.8	35.8	8.2

** Significantly different from untreated ($P < 0.01$)

Table 13. The effect of Temik and Dynamec treatments on the numbers of *A. ritzemabosi* infesting Japanese *Anemone* cv. Lady Gilmour, (numbers/gram leaf tissue).

Treatment	Days after treatment (DAT)			
	0 (pre-treatment)	7	28	62
Untreated	411	790	1473	686
Temik (Day 0)	1156*	99	226	30
Dynamec (Day 0)	334	374	962	463
Dynamec (Days 0 & 14)	319	36	924	612
SED (12 d.f.)	253	390.9	621.1	378.6

* Significantly different from untreated ($P < 0.05$)

Table 14. The effect of Temik and Dynamec treatments on the numbers of *A. ritzemabosi* infesting *Weigela* cv. Looymansii Aurea, (numbers/gram leaf tissue).

Treatment	**Days after treatment (DAT)**			
	0 (pre-treatment)	**7**	**28**	**45**
Untreated	55	59	216	180
Temik (Day 0)	209	103	240	35**
Dynamec (Day 0)	179	38	65	51**
Dynamec (Days 0 & 14)	82	108	180	105
SED (12 d.f.)	67.7	66.1	159.7	34.0

** Significantly different from untreated ($P < 0.01$)

Table 15. The effect of Temik and Dynamec treatments on the numbers of *A. ritzemabosi* infesting *Saxifraga* cv. James Bremner, (numbers/gram leaf tissue).

Treatment	**Days after treatment (DAT)**				
	0 (pre- treatment)	**7**	**28**	**35**	**63**
Untreated	35	17	8	5	7
Temik	14	23	0.1*	0.04**	0.1***
Dyn. label rate x1	14	13	2*	1**	1***
Dyn. label rate x2	11	4*	0.4*	0.3**	0.04***
Dyn. double rate x1	15	33	1*	0.4**	1***
Dyn. double rate x2	15	16	0.4*	0.03**	0.04***
SED (18 d.f.)	11.2	12.4	2.4	1.0	1.0

* Significantly different from untreated ($P < 0.05$)
** Significantly different from untreated ($P < 0.01$)
*** Significantly different from untreated ($P < 0.001$)

Table 16. The effect of Temik and Dynamec treatments on the numbers of *A. ritzemabosi* infesting *Cistus* cv. Corbariensis, (numbers/gram leaf tissue).

Treatment	**Days after treatment (DAT)**				
	0 (pre- treatment)	**7**	**28**	**35**	**63**
Untreated	160	68	455	106	97
Temik	209	132	9***	3***	14
Dyn. label rate x1	118	97	139***	6***	80
Dyn. label rate x2	115	91	43***	14***	62
Dyn. double rate x1	188	113	148***	14***	15
Dyn. double rate x2	163	168	80***	3***	4
SED (18 d.f.)	49.0	60.2	60.5	22.1	37.8

*** Significantly different from untreated ($P < 0.001$)

Table 17. Visual assessment of bud and leaf nematode leaf symptoms on *Weigela* cv. Looymansii Aurea and Japanese *Anemone* cv. Lady Gilmour, assessed on 26 July 1999, nine months after initial treatments were applied, (data are symptom assessment categories as detailed in table footnote).

Treatment	***Weigela* cv.Looymansii Aurea**	***Anemone* cv. Lady Gilmour**
Untreated	4	4
Temik (Day 0)	1	1
Dynamec (Day 0)	4	4
Dynamec (Days 0 & 14)	4	4

Symptom assessment categories:

1 No symptoms, plants marketable
2 Lower leaves with slight damage, plants marketable
3 Moderate & severe symptoms on lower and middle. Plants unmarketable
4 Moderate & severe symptoms on lower, middle and top leaves. Plants unmarketable

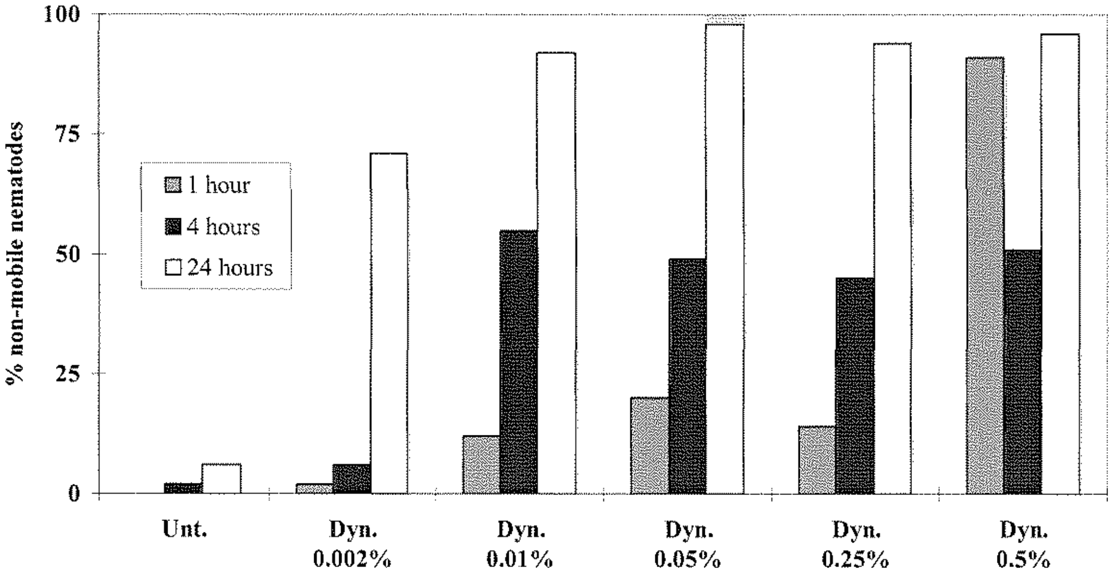

Figure 1. The mobility of *A. ritzemabosi* exposed to a range of Dynamec concentrations for up to 24 hours in *in vitro* toxicity tests, (% non-mobile nematodes as means of three tests).

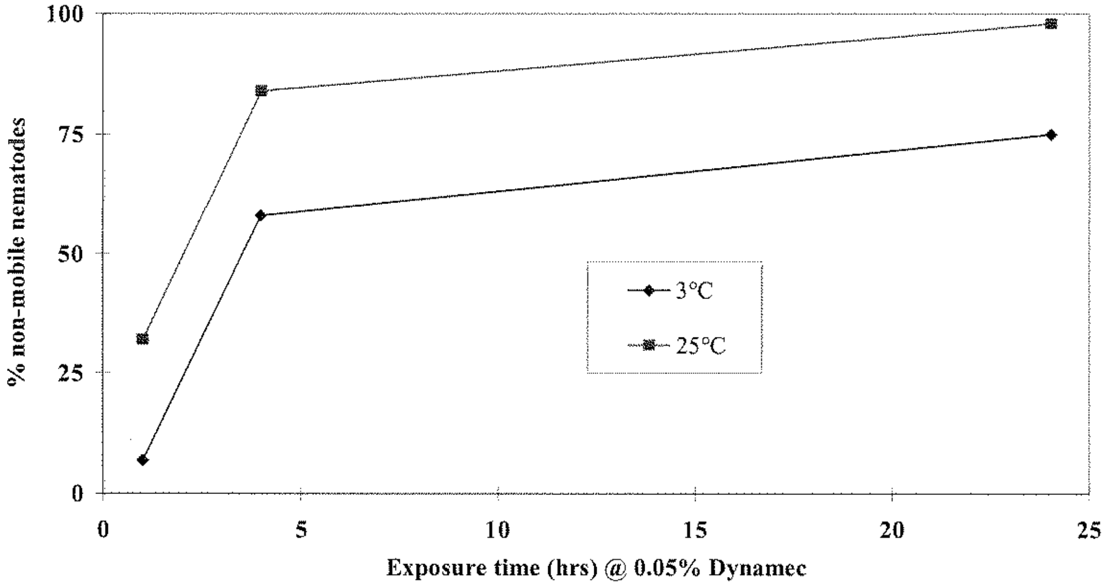

Figure 2. The mobility of *A. ritzemabosi* exposed to 0.05% Dynamec at two contrasting temperatures for up to 24 hours (% non-mobile nematodes).

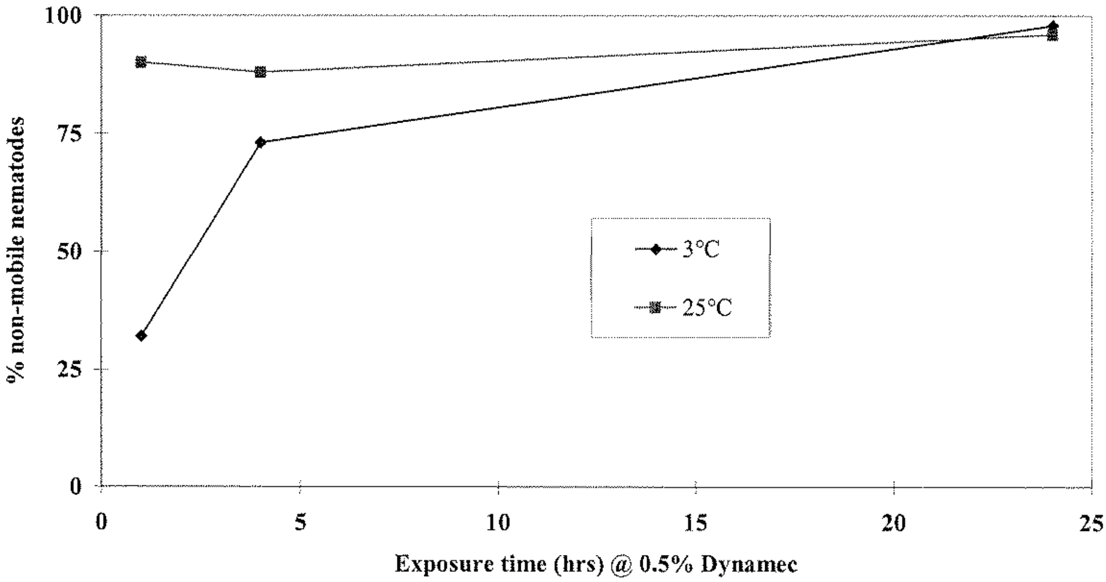

Figure 3. The mobility of *A. ritzemabosi* exposed to 0.5% Dynamec at two contrasting temperatures for up to 24 hours (% non-mobile nematodes).

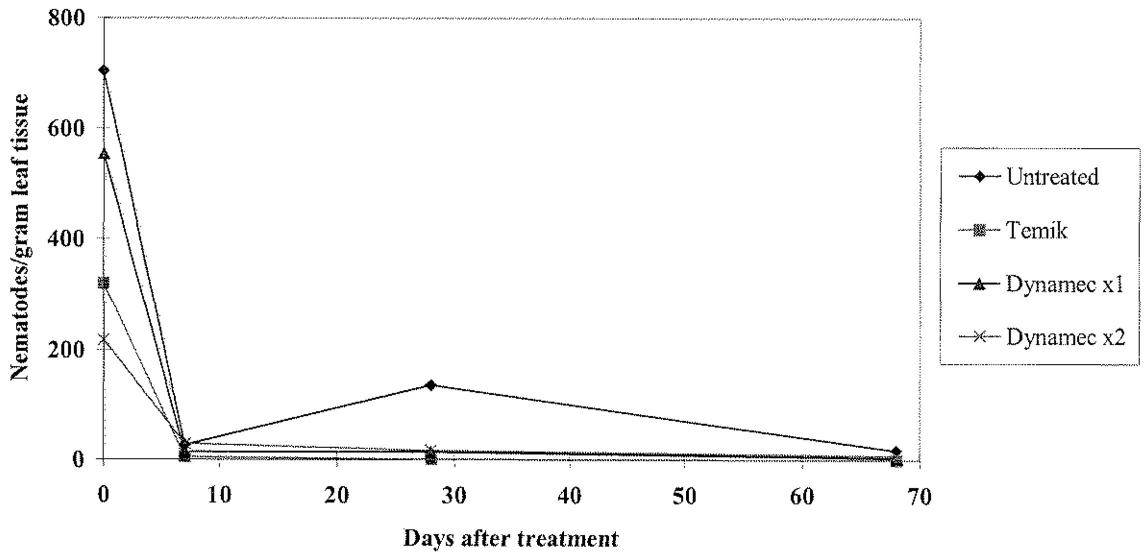

Figure 4. The effect of Dynamec and Temik treatments on *A. ritzemabosi* infestation of *Weigela* cv. Bristol Ruby.

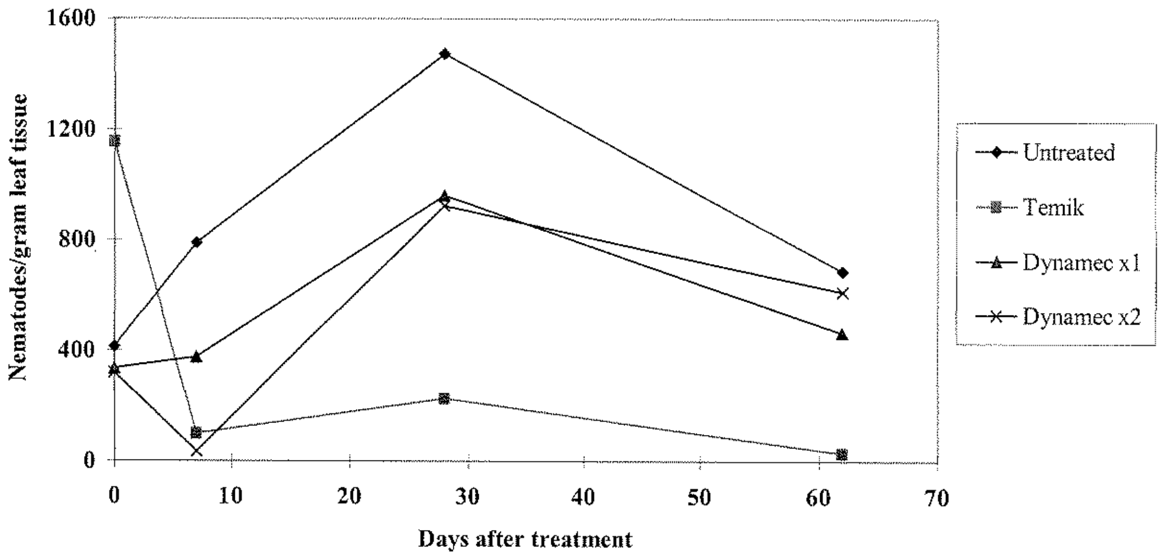

Figure 5. The effect of Dynamec and Temik treatments on *A. ritzemabosi* infestation of Japanese *Anemone* cv. Lady Gilmour.

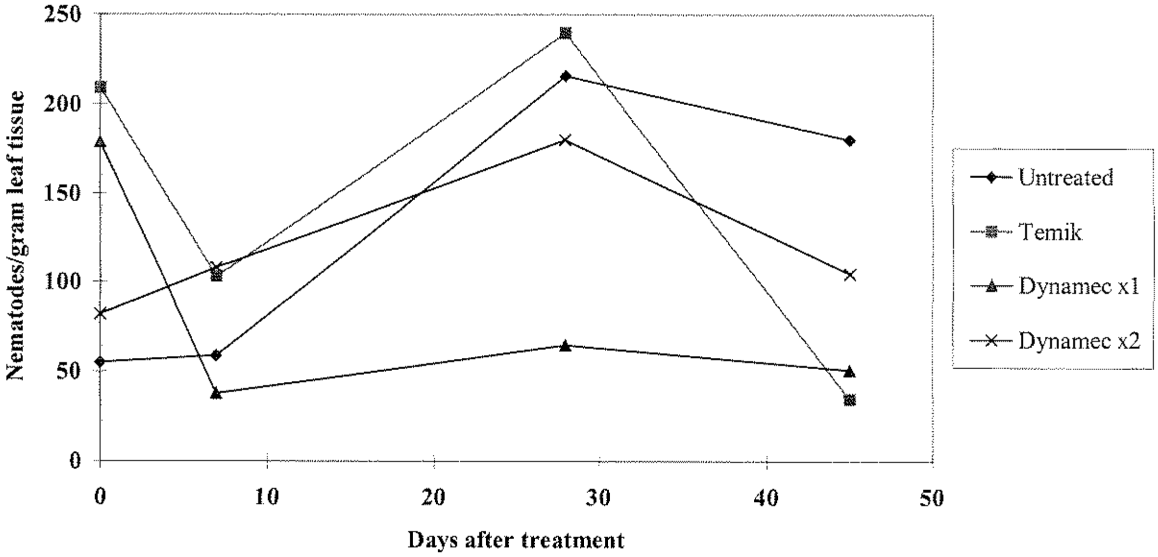

Figure 6. The effect of Dynamec and Temik treatments on *A. ritzemabosi* infestation of *Weigela* cv. Looymansii Aurea.

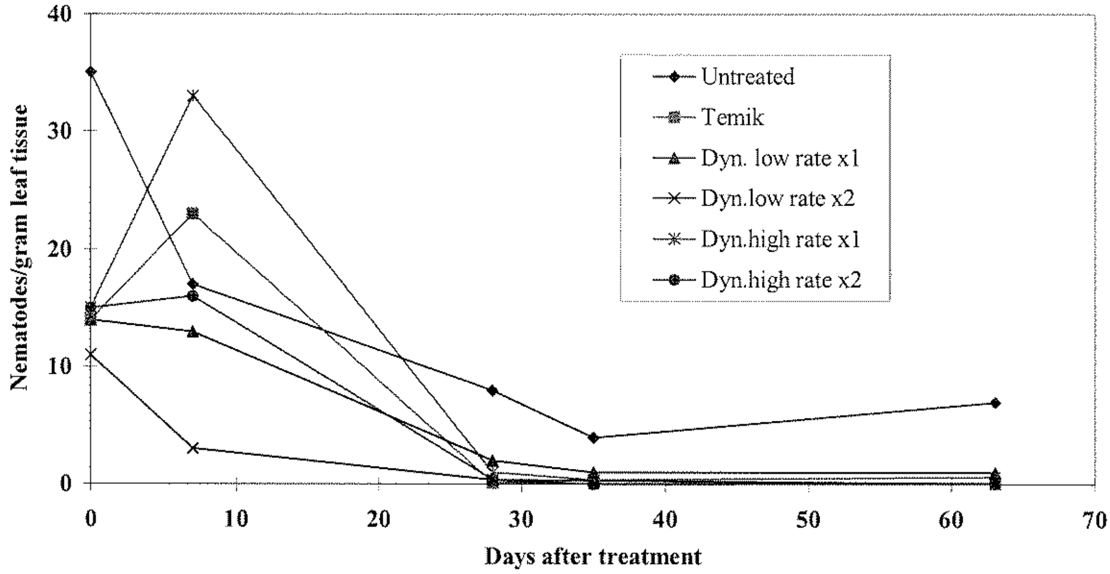

Figure 7. The effect of Dynamec and Temik treatments on *A. ritzemabosi* infestation of *Saxifraga* cv. James Bremner.

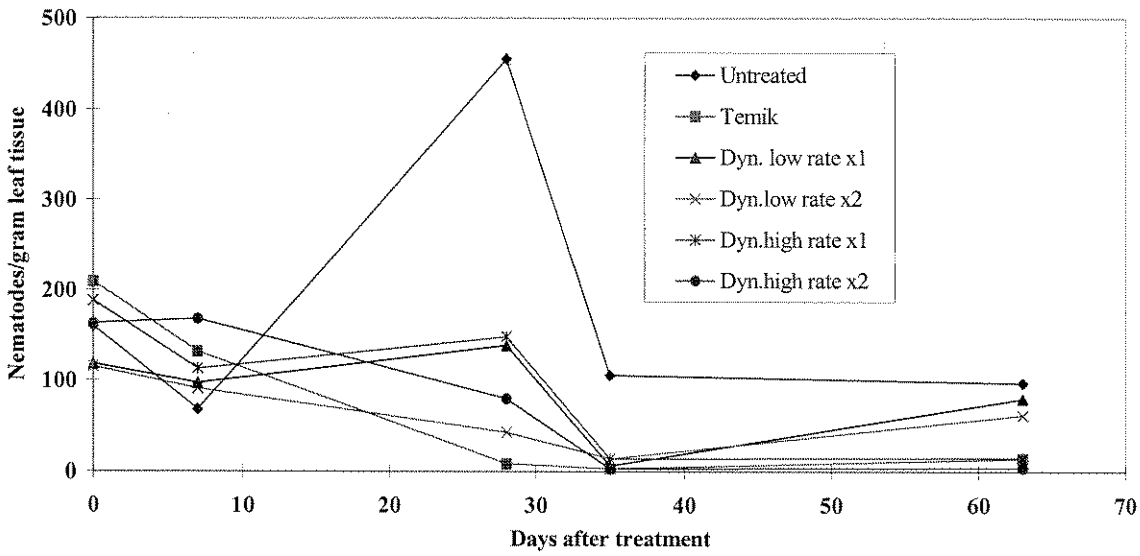

Figure 8. The effect of Dynamec and Temik treatments on *A. ritzemabosi* infestation of *Cistus* cv. Corbariensis.

Discussion

Toxicity tests

The toxicity tests confirmed that Dynamec (abamectin) possesses activity against bud and leaf nematodes (*Aphelenchoides* spp.). The avermectins inhibit nerve transmission in motor neurons mediated by the neurotransmitter GABA (gamma-aminobutyric acid). They enhance GABA binding to the nerve receptor, which results in increased chloride ion flow into the nerve cell (Quarles, 1991). When chloride ion flow becomes large enough, nerve transmission ceases. Paralysis is the primary symptom in target animals. The avermectins have a high degree of specificity against invertebrate targets as, in humans and other mammals, the GABA neurons are all in the brain and are not directly concerned with muscle activation. At the doses used for crop protection purposes, negligible amounts of avermectins cross the blood-brain barrier in mammals.

The paralysing action of abamectin was observed in the toxicity tests. Nematode movement ceased fairly rapidly following exposure to abamectin. However, there was some doubt as to whether or not the paralysed nematodes were dead. Therefore, the results were expressed in terms of mobile or non-mobile nematodes (Tables 1,2,3&5). The recovery tests were done to observe if any of the paralysed nematodes were capable of recovery (Tables 6&11). The results indicated that nematode paralysis would appear to lead to eventual death; there was very little evidence of nematode recovery following exposure to abamectin for 24 hours. The majority of the nematodes observed appeared to be permanently disabled by the Dynamec, particularly at doses greater than 0.002% Dynamec.

Although some immediate effects of treatment were visible within one hour of exposing the nematodes to Dynamec, the chemical appeared to be relatively slow acting, as the largest effects, in terms of the number of immobilised nematodes, were not visible until after 24 hours of exposure (Figure 1). The LD50 and LD90 doses give the concentrations of Dynamec required to attain 50% or 90% mortality of the test nematodes (Table 4). The 24 hour LD90 was estimated as 0.03% Dynamec (room temperature tests). This value corresponds closely to the label recommended dose rates, which vary from a spray concentration of 0.025% Dynamec for spider mite control, to 0.05% Dynamec for use against leaf miners.

In the tests done to evaluate the effect of temperature extremes on the toxicity of Dynamec against bud and leaf nematode, the results indicated that the toxic action of Dynamec was slowed down at the low temperature of 3°C, compared with 25°C (Tables 7&9). The 24 hour LD 90 for Dynamec 3°C was 0.17%, whilst the equivalent value for the 25°C test was 0.03% (Tables 8&10). This observation confirms that the toxicity of Dynamec against bud and leaf nematode tends to be reduced at low temperatures, compared with high temperatures. The 24 hour LD 90s for the 25°C and room temperature tests (*c.* 20°C) corresponded with each other very well (Tables 4&10). At doses equivalent to, or less than, 0.05% Dynamec, the proportion of nematodes immobilised by Dynamec after 24 hours at 3°C was consistently less than that at 25°C (Figure 2). However, at doses greater than 0.05% Dynamec, although the proportion of immobilised nematodes was initially lower at 3°C than at 25°C, by the time of the 24 hour assessment the numbers of nematodes immobilised by Dynamec was ultimately similar at both temperatures (Figure 3). Clearly, these observations have practical implications, as the activity of Dynamec applied to outdoor or unheated nursery stock in winter may well be reduced.

Plant trials

In the *Weigela* cv. Bristol Ruby trial, Temik and both of the Dynamec treatments significantly reduced nematode numbers at 28 days after treatment (DAT) ($P < 0.01$). However, by the end of this study, nematode numbers had also undergone a substantial decline in the untreated control. At 68 DAT, no nematodes were detected in the Temik treatment but small numbers remained in all other treatments, including the untreated control (Table 12 & Figure 4).

The variability observed in the numbers of nematodes is typical of that often found in the assessment of bud and leaf nematode populations. The nematode populations tended to be inherently variable between plants and between leaves on the same plants, despite the fact that the leaves were showing similar symptoms. Bud and leaf nematode populations were also shown to be highly variable over time, with populations reaching well defined peaks, followed by rapid declines. This relationship is poorly understood, but is thought to be related to environmental conditions as well as the the physiological state of the host plants. The analysis of the nematode populations took account of this inherent variation in order to detect real differences between treatments.

In the *Anemone* trial, the Temik pre-treatment nematode numbers were significantly greater than the other treatments ($P < 0.05$). However, in all subsequent post-treatment assessments the numbers of nematodes in the Temik treatment were much lower than the other treatments, although this was not statistically significant (Table 13 & Figure 5). The Dynamec sprays appeared relatively ineffective on *Anemone*, although a low nematode count was observed 7 DAT, compared with the untreated (non-significant).

In the *Weigela* cv. Looymansii Aurea trial, Temik and the single spray of Dynamec significantly reduced nematode numbers 45 DAT ($P < 0.01$). The two-spray Dynamec treatment did not appear to confer any advantages over the single spray treatment (Table 14 & Figure 6).

In the trial using *Saxifraga* cv. James Bremner, the untreated nematode population declined naturally during the course of the study. However, all of the chemical treatments significantly reduced the numbers of nematodes in the assessments made 28, 35 and 63 days after initial treatment, compared with the untreated ($P < 0.05$). The numbers surviving were very low by the time of the final assessment (63 DAT) and there were no significant differences between any of the chemical treatments. The symptoms of attack in the Saxifraga were not very distinctive. Classical angular leaf-blotching was visible in all of the test plant species except the *Saxifraga*. The indication of nematode symptoms in the Saxifraga was the presence of a mild distortion and stunting of the leaf-whorls. It is possible that, in commercial practice, these type of mild symptoms could go unnoticed in propagation lines for a long period of time.

In the *Cistus* trial, the untreated nematode population peaked at 28 days after initial treatment and then subsequently declined. All of the chemical treatments significantly reduced nematode numbers in the 28 and 35 DAT assessments (Table 16). By the time of the final 63 DAT assessment, none of the nematode counts of the chemical treatments were significantly lower than untreated but numbers were lowest in association with the Temik and double-rate Dynamec treatments (Table 16 and Figure 8).

In general, Temik, the standard nematicide, was shown to be a very robust and persistent treatment. In comparison, Dynamec, applied as a single or two-spray treatment, appeared in some of the tests (e.g. *Weigela* cv. Looymansii Aurea, *Saxifraga* and *Cistus*) to give levels of control similar to Temik. However there was a trend in the results to suggest that the persistence of Dynamec was not as great as that of Temik. In the final assessments, which ranged between 45 and 68 days after initial treatment, the lowest numbers of nematodes were, with the exception of *Cistus*, consistently associated with the Temik treatment. This observation was verified by the reappearance of nematode symptoms in *Weigela* cv. Looymansii Aurea and Japanese *Anemone*, approximately nine months after these plants were originally treated. The visual assessments of symptoms made at that time clearly indicated that the plants treated with Temik remained free of symptoms. In contrast, nematode symptoms had resurged in the Dynamec treatments, which, by that time, were no different in appearance to the untreated plants (Table 17).

The two-spray Dynamec treatments did not appear to confer any advantage over the application of a single spray. Furthermore, the double-rate Dynamec used on the *Saxifraga* and *Cistus* did not significantly reduce nematode numbers to any greater extent than that of the standard label-rate applications of Dynamec. However, in the *Cistus*, by the time of the final assessment 63 days after the initial treatments were applied, there was a trend for lower numbers of nematodes in the double-rate Dynamec than in the standard label-rate Dynamec treatments (Table 16).

The results confirm that although both Temik and Dynamec are capable of suppressing bud and leaf nematodes attacks, neither are capable of eradicating or permanently suppressing the pest. Small numbers of surviving nematodes invariably always remained at the end of each trial period. These survivors would clearly be capable of giving rise to renewed attack when the residual activity of these chemicals diminishes after a period of time.

Dynamec shows potential as an alternative nematicidal agent against bud and leaf nematodes. The results suggest that Dynamec will be of use in the short-term suppression of bud and leaf nematode populations. However, the standard of nematode control obtained with Dynamec is, on balance, likely to be inferior in terms of persistence and reliability, than that obtainable with Temik. The secondary sprays of Dynamec used in this work were applied two or four weeks after the first treatments, but did not appear to confer any additional advantages in terms of nematode control when assessed approximately two months after the initial sprays. However, it is likely that in practice, repeat sprays of Dynamec may be required at approximately three monthly intervals in order to maintain suppression of bud and leaf nematode populations.

There have been conflicting reports from American workers studying the use of abamectin against *Aphelenchoides* spp. in ornamentals. Walker *et al.* (1997) found that a single application of abamectin to control *Aphelenchoides fragariae* attacking *Begonia* was relatively ineffective. However, LaMondia (1996) found encouraging levels of control from abamectin used against *A. fragariae* attacking *Lamium maculatum*. Furthermore, two applications of abamectin (one week apart) were no better than a single application. LaMondia concluded that abamectin may be of use in the management of foliar nematodes and his findings support the results presented in this report.

The maximum approved label recommended dose rate of Dynamec was used in the plant studies reported here. No phytotoxic effects were observed following the use Dynamec on any of the species of plants tested. On the basis of the work reported here, higher rates of Dynamec than those currently recommended on the product label would not appear to be justified for bud and leaf nematode control. Dynamec is currently Approved and recommended for the control of spider mites and leaf miners in protected and outdoor flower crops and ornamentals. Any application of Dynamec to non-edible ornamental crops (outdoor or protected) for the control of bud and leaf nematodes would, therefore, be done entirely at the grower's own risk.

Conclusions

- Dynamec (abamectin) was shown in *in vitro* laboratory tests to have nematicidal activity against the bud and leaf nematode (*Aphelenchoides ritzemabosi*). Dynamec acts by causing an apparently irreversible paralysis of the nematodes.

- An estimated dose of 0.03% Dynamec was required to immobilise 90% of nematodes after 24 hours (24 hour LD 90) *in vitro* exposure at room temperature (*c.* 20°C). The maximum label-recommended rate of Dynamec corresponds to a spray concentration of 0.05% Dynamec.

- Dynamec, applied as one or two-spray treatments, was shown to suppress populations of bud and leaf nematodes attacking *Weigela, Anemone, Saxifraga and Cistus*, up to nine weeks after treatment.

- The level of control obtained from Dynamec was, on occasions, similar to that achieved with the standard nematicide, Temik (aldicarb). No phytotoxic effects were observed with Temik or Dynamec in any of the plant species tested .

- A second spray of Dynamec, applied one or four weeks after the first spray, did not confer any advantage over a single spray of Dynamec.

- Dynamec applied at twice the maximum label-recommended rate did not significantly improve the standard of bud and leaf nematode control, compared with the maximum label-recommended rate.

- Dynamec did not appear to be as persistent or reliable as Temik in controlling bud and leaf nematode or in suppressing their symptoms of attack.

- The efficacy of Dynamec against bud and leaf nematodes may be diminished in low temperatures (3°C). *In vitro* tests showed that toxicity and speed of action of Dynamec against bud and leaf nematode was reduced at low temperatures (3°C), compared with high temperatures (25°C).

- Neither Dynamec nor the standard nematicide,Temik, should be viewed as eradicant treatments as small numbers of nematodes can survive treatment to give rise to later attacks.

- Dynamec shows potential as a short-term suppressant treatment against bud and leaf nematode. Periodic follow-up treatments, possibly at two to three month intervals, may be required to maintain the suppression of nematode populations.

- Dynamec currently holds Approval and label recommendations for use in outdoor or protected ornamentals for the control of spider mites or leaf miner. Any use of Dynamec to control bud and leaf nematodes in ornamentals would be at the grower's own risk.

References

Hotson I K. 1982. The avermectins: a new family of antiparasitic agents. *Journal of the South African Veterinary Association* **53** (2): 87–90.

LaMondia J A. 1996. Efficacy of Avid (abamectin) against the foliar nematode *Aphelenchoides fragariae*, 1995. *Fungicide and Nematicide* Tests **51**:185.

Stretton A O W. 1987. Biological activity and mode of action of avermectins. In: *Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists,* eds. J A Veech & D W Dickson, pp 136–146. Society of Nematologists: Hyattsville, MD.

Quarles W. 1991. Avermectins: Successful Biopesticides. *IPM Practitioner* **XIII** (5/6), 6–15.

Walker J T, Oetting R D, Johnston-Clark M, Melin J B. 1997. Evaluation of newer chemicals for control of foliar nematode on Begonia. *Journal of Environmental Horticulture* **15** (1): 16–18.

Young J E B. 1996. A review of the biology and control of leaf and bud nematodes in outdoor ornamentals. *HDC Project Report HNS 60.* Horticultural Development Council: East Malling.