Project title:	Managing ornamental plants sustainably (MOPS)
Project number:	CP 124
Work package title:	To investigate potential novel control methods for leaf and bud nematode
Work package leader:	Jude Bennison, ADAS
Report:	Annual report, February 2016
Previous report:	None
Key staff:	Jude Bennison, ADAS Kerry Maulden, ADAS
Location of work:	ADAS Boxworth, Cambs
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Date work completed (or expected completion date):	January 2016

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

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

Jude Bennison
Senior Research Entomologist
ADAS

Signature .....Date 9 February 2016

Report authorised by:

John Atwood
Project Leader
ADAS

Signature Date 9 February 2016

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GROWERS SUMMARY

Headline

- Of seven disinfectants tested for control of leaf and bud nematodes in infested leaves on damp sand, only Anigene (medical disinfectant) increased the percentage of dead nematodes after treatment but still killed only 9%. Menno Florades, Hortisept Pro, Unifect G and Anigene reduced nematode survival in the sand but did not eradicate them.

Background and expected deliverables

Leaf and bud nematodes, *Aphelenchoides* spp. are common, persistent and damaging pests of a range of economically important HNS plants. In the UK, both *A. ritzemabosi* and *A. fragariae* can occur. The nematodes enter the leaves through the stomata and also infest buds. Movement between plants, leaves and stems is facilitated by the presence of a film of water, provided either by rainfall or overhead irrigation. Feeding damage results in angular-shaped dark patches on the leaves, delineated by leaf veins and leaf distortion also often occurs. These damage symptoms can make infested plants unmarketable. HDC-funded project HNS 131 showed that of the conventional pesticides and biopesticides tested, the systemic pesticide, oxamyl (Vydate 10G) which had a SOLA for use on both protected and outdoor ornamentals, was the only effective alternative to aldicarb (Temik 10G) when Temik 10G was withdrawn in 2007. However, the SOLA (now EAMU) for Vydate 10G now only allows the use on outdoor ornamentals when applied by a mechanical granule applicator (not by hand-held equipment) followed by soil incorporation just before drilling or planting, thus growers of containerised and protected ornamentals can no longer use Vydate. Therefore cultural controls such as not taking cuttings from infested mother plants, using stringent nursery hygiene methods and sub-irrigation are the only current options for control of leaf and bud nematodes. However, these methods are not always fully effective and an effective alternative nematicide is urgently needed for control of this pest. Work in the current AHDB-funded PhD studentship project CP 104 'Novel approaches for the management of leaf and bud nematodes in HNS' aims to identify novel nematicides for control of the pest, therefore work in this project aimed to evaluate the potential role of disinfectants as part of a cultural control strategy.

In HDC-funded project HNS 147, disinfectants were tested in the laboratory for control of leaf and bud nematodes remaining in containers before re-use. When the nematodes were immersed in disinfectant solutions for one hour, two disinfectants used in animal health, FAM 30 and Trigene Advance (the latter is no longer available) reduced survival to 65% and 34% respectively.

The purpose of this experiment was to evaluate further disinfectants with potential for control of leaf and bud nematodes in infested leaf debris on sand beds as a clean-up procedure following an infested containerised HNS crop.

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Materials and methods

Seven disinfectants (Table 1) were tested as drenches to Japanese anemone leaves infested with leaf and bud nematodes (*Aphelenchoides fragariae*) placed onto damp sand in pots in a poly tunnel in December 2015. The treatments included four horticultural disinfectants, two used in animal health and one medical disinfectant. Currently, there is no register of authorized disinfectants, therefore use of those recommended for animal health or medical use would be at growers' own risk, see HDC Factsheet 03/14 'Use of chemical disinfectants in protected ornamental plant production' for further details. There were ten replicate pots per treatment (eight treatments including an untreated control) and each replicate was an individual 1.5 litre pot. Each pot was filled with one litre of a mixture of 90% sand and 10% peat-based herbaceous mix substrate to represent a commercial sand bed. Japanese anemone leaves infested with leaf and bud nematodes were collected from a commercial nursery and the species was confirmed as *A. fragariae* after extracting the nematodes and using a diagnostic key and microscopic examination. In order to infest the pots of sand, 80 leaves of similar size and with similar visual symptoms of nematode infestation were selected. Each leaf was cut in half along the mid-rib. Half of each leaf was used to estimate numbers of leaf and bud nematodes per g leaf tissue before treatment and the other half of each leaf was placed onto the sand surface in the 80 experimental pots so that numbers 'before' and 'after' treatment could be calculated. However, as different leaf halves were used for the 'before' and 'after' nematode counts these can only be used as a guide of any reductions in numbers given by the treatments.

The 80 experimental pots were set up and treated in two batches of 40, replicates 1-5 were set up on 8 December and replicates 6-10 on 15 December, in order to allow sufficient time to extract and count all the nematodes in treated leaves three days after treatment on 11 and 18 December respectively. Before treatments were applied, leaf and bud nematodes were extracted from the untreated halves of the leaves using a standard laboratory procedure and numbers of live and dead nematodes per gram of leaf tissue calculated. The products were applied to the sand using a small watering can with a rose. The label rate was used wherever a specific recommendation for treatment of sand beds was given. In other cases, the minimum dilution (maximum concentration) stated on the label was used. The application volume was 5 L per m^2 (equivalent to 88 ml per pot), as recommended on the Jet 5 label for use on sand beds (recommendation is 3-5 L per m^2 and growers usually use the maximum volume). The treated leaves were left for 72 hours before removal from the pots, followed by laboratory extraction leaf and bud nematodes using the same method as used for the pre-treatment leaf halves. In addition to extracting nematodes from treated leaves, leaf and bud nematodes were also extracted from a 50 ml sample of sand taken directly beneath each leaf, 72 hours after treatment.

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Table 1. Products tested (all treatments are commercially available as disinfectants for horticultural, animal health or medical use).

Product	Active ingredient(s)	Recommended use
Water control	-	-
Jet 5 (positive control)	peroxyacetic acid, hydrogen peroxide, acetic acid, ethoxylate	Horticultural & agricultural use including disinfection of sand beds and capillary matting
FAM 30	Iodophor (blend of iodine, sulphuric acid and phosphoric acid)	Animal health use
Menno Florades	benzoic acid	Horticultural use including disinfection of hard surfaces and capillary matting
Hortisept Pro	Dimethyl benzyl ammonium chloride, citric acid monohydrate and alcohols C9-C11- ethoxylated	Horticultural use including disinfection of hard surfaces, glasshouses, poly tunnels, matting
Rely+on Virkon	Potassium monopersulphate	Medical use
Unifect G	Gluteraldehyde and quaternary ammonium compounds	Horticultural use in protected crop structures
Anigene HLD_4V	Oxirane, 2-methyl-polymer with oxirane, mono(2-propylheptyl) ether, quaternary ammonium compounds, benzyl-C 12-16-alkyldimethyl, chlorides, N-(3-aminopropyl-N-dodecylpropane-1,3-diamine, didecyldimethylammonium chloride	Animal health use

Results and Conclusions

Numbers of live and dead leaf and bud nematodes per g leaf tissue before and after treatment

Mean numbers of live leaf and bud nematodes per g of leaf tissue were very high before treatment, with an overall mean across the treatments of 3,476 per g. Three days after treatment of the different leaf halves, none of the treatments had significantly reduced the number of live nematodes, with an overall mean across the disinfectant treatments of 2,935 per g. Numbers of dead nematodes per g of leaf tissue were very low before treatment, with an overall mean of 26.3 per g of leaf tissue. However, numbers of dead nematodes were significantly increased by Jet 5, FAM 30, Hortisept Pro, Unifect G and Anigene compared with the water controls (Table 2). In the water controls there was a mean of 34.6 dead nematodes per g of leaf whereas the effective treatments (all equally effective) increased mean numbers of dead nematodes to 123.2-171.6 per g

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of leaf. However, as mean numbers of live nematodes were so high, the percentage dead nematodes in the effective treatments were still low, ranging from 3.2% to 9.2%, compared with 1.2% in the water controls. Only Anigene significantly increased the percentage of dead nematodes, to 9.2% (Table 2 and Figure 1). These results indicate that none of the treatments are likely to be fully effective in controlling leaf and bud nematodes in heavily infested leaf debris on sand beds. Leaf and bud nematodes can survive for long periods in dry leaf tissue e.g. *A. ritzemabosi* can survive in dry infested leaves for up to three years but do not survive the winter in leaves or soil in normal outdoor conditions (Young, 1996). *Aphelenchoides fragariae* does not survive long in the soil in the absence of plants; research has shown that when infested plant tissue was buried 15 cm deep in soil, they only survived for up to three months, although this work did not include *A. ritzemabosi* (Young, 1996). These results indicate that if infested leaf debris remains in or on a sand bed following an infested crop, even if a disinfectant is used an interval of at least four months should be left before using it for fresh susceptible plants.

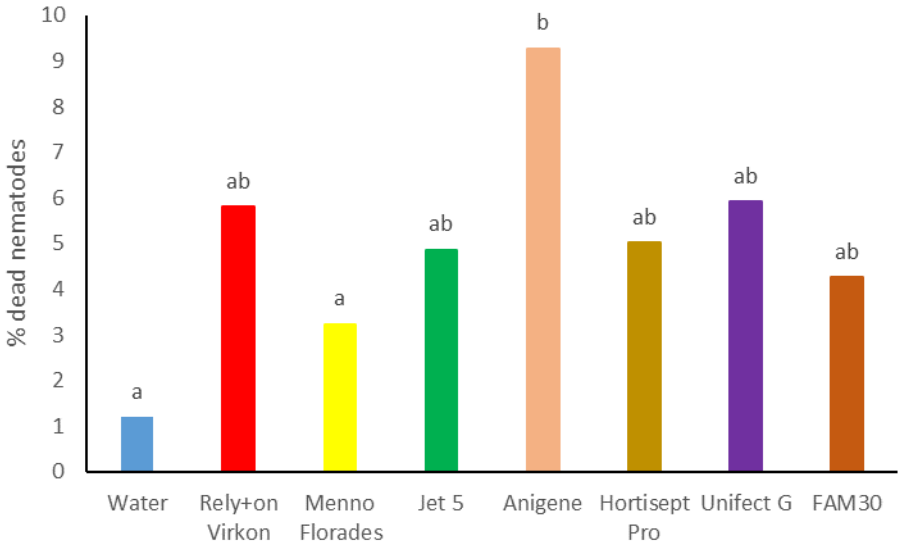

Figure 1. Percentage dead leaf and bud nematodes in infested leaf tissue 3 days after treatment. Bars sharing the same letters are not significantly different (P<0.05).

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Table 2. Mean numbers of live and dead nematodes per g leaf tissue before and 3 days after treatment and % dead nematodes after treatment.

Product name	Live before treatment	Live after treatment	Dead before treatment	Dead after treatment	% dead after treatment
1. Water control	4379	3616	28.1	34.6 a	1.2 a
2. Jet 5	3975	3214	27.0	123.2 b	4.9 ab
3. FAM 30	3222	3415	28.3	171.6 b	4.3 ab
4. Menno Florades	3009	2803	35.0	109.4 ab	3.2 a
5. Hortisept Pro	3746	2654	24.2	138.5 b	5.0 ab
6. Rely+on Virkon	2509	2510	23.2	105.2 ab	5.8 ab
7. Unifect G	3145	2711	23.9	138.9 b	5.9 ab
8. Anigene	3826	2560	20.9	133.7 b	9.3 b
	N.S.	N.S.	N.S.	P<0.05	P<0.05

NS = no significant differences between treatments. Numbers in a column sharing the same letters are not significantly different at P<0.05 based on least significant differences (LSD).

Numbers of live leaf and bud nematodes per 50 ml sand beneath infested leaves 3 days after treatment

Low numbers of live leaf and bud nematodes were found in the sand directly under the infested leaves three days after treatment, with a mean of 48.9 per 50 ml sand in water-treated controls. Menno Florades, Hortisept Pro, Unifect G and Anigene significantly reduced the numbers of live nematodes to 12.2, 12.3, 3.5 and 14.9 per 50 ml sand respectively (Figure 2). These four treatments were equally effective and Jet 5, FAM 30 and Rely+on Virkon were ineffective. Although the effective treatments gave a significant reduction in numbers of live nematodes in the sand beneath the leaves, none of them eradicated the nematodes. Previous research has shown that if *A. fragariae* were added to soil in a water suspension, they lived for only four weeks (Young, 1996), however this work did not include *A. ritzemabosi*. These results indicate that if sand beds are thoroughly cleared of all infested leaf debris following an infested crop, one of the disinfectants shown to have a significant effect could reduce the numbers of nematodes surviving in the sand but an interval of at least four weeks should be left before using the beds to grow any fresh plants susceptible to leaf and bud nematodes

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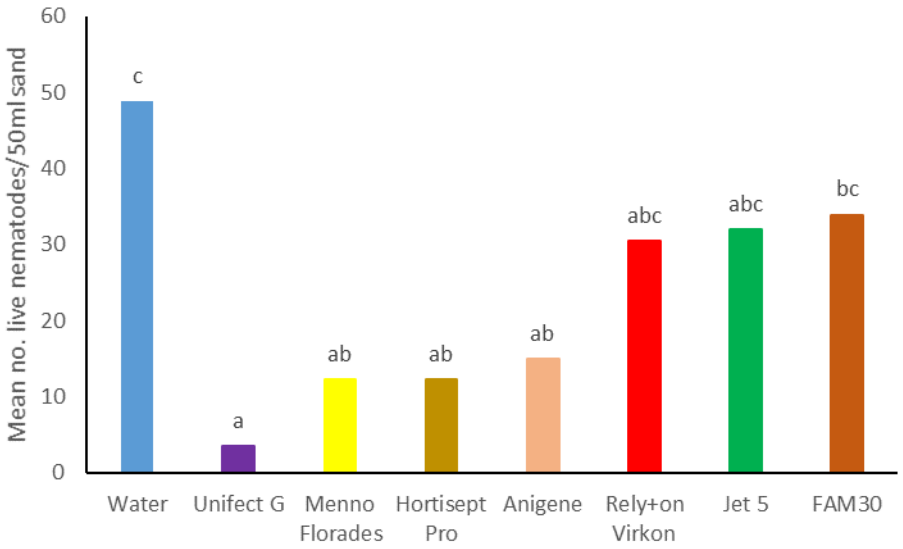

Figure 2. Mean numbers of live leaf and bud nematodes per 50 ml sand beneath infested leaf 3 days after treatment. Bars sharing the same letters are not significantly different (P<0.05).

Action points

- Thoroughly dispose of any plant debris from any areas including sand beds following plants infested with leaf and bud nematodes.
- Following physical removal of plant debris, a drench of Menno Florades, Hortisept Pro or Unifect G may reduce survival of leaf and bud nematodes that have entered the sand but is unlikely to eradicate them.
- If possible, leave any cleaned sand beds following plants infested with leaf and bud nematodes free from plants susceptible to the pest for at least four weeks to minimize nematode survival in damp sand. If any infested leaf debris remains, a period of at least four months should be left before using the sand bed for plants susceptible to the pest.
- Minimise the spread of leaf and bud nematodes on sand beds following infested plants by reducing or stopping overhead irrigation and by spacing plants widely to avoid foliar contact.

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SCIENCE SECTION

Introduction

Leaf and bud nematodes, *Aphelenchoides* spp. are common, persistent and damaging pests of a range of economically important HNS plants. In the UK, *A. ritzemabosi* is the main species found on HNS but *A. fragariae* can also occur. The nematodes enter the leaves through the stomata and also infest buds. Movement between plants, leaves and stems is facilitated by the presence of a film of water, provided either by rainfall or overhead irrigation. Feeding damage results in angular-shaped dark patches on the leaves, delineated by leaf veins and leaf distortion also often occurs. These damage symptoms can make infested plants unmarketable. Until its withdrawal in 2007, aldicarb (Temik 10G) was the most effective nematicide for control of leaf and bud nematode. HDC-funded project HNS 131 showed that the systemic pesticide, oxamyl (Vydate 10G) which had a SOLA for use on both protected and outdoor ornamentals, was the only effective treatment of a range of conventional pesticides and biopesticides tested (Bennison, 2007). However, this SOLA (now EAMU) now only allows the use of Vydate on outdoor ornamentals if it is applied by a conventional mechanical granule applicator (not by hand-held equipment) followed by soil incorporation just before drilling or planting, therefore growers of containerised and protected ornamentals can no longer use Vydate. Therefore the only current option for control of leaf and bud nematodes is cultural control such as avoiding taking cuttings from infested mother plants and using stringent nursery hygiene methods and sub-irrigation but these methods are not always fully effective. An effective alternative nematicide is needed for control of this pest. Work in the current AHDB-funded PhD studentship project CP 104 'Novel approaches for the management of leaf and bud nematodes in HNS' aims to identify novel nematicides for control of the pest. Therefore work in this project aimed to evaluate the potential role of disinfectants as part of a cultural control strategy.

Peroxyacetic acid (Jet 5) is widely used as a disinfectant in UK protected horticultural crops to clean floors and benches etc. between crops for control of disease pathogens. The same disinfectant (as ZeroTol) has been shown in the USA to have good activity against the leaf nematode *Aphelenchoides fragariae* when applied as a foliar spray (Jagdale & Grewal, 2002). Jet 5 was also tested by ADAS against the stem nematode, *Ditylenchus dipsaci* for the narcissus industry, in HDC project BOF 49 (Lole, 2011). This laboratory work showed that 75% of the nematodes were killed within one hour of immersion. Iodophor/acid disinfectants (FAM 30/Antec Virudine) were also tested against stem nematode in BOF 49 and killed 100% of the nematodes within five minutes of immersion. In HDC-funded project HNS 147, disinfectants were evaluated for control of leaf and bud nematodes remaining in containers before re-use. In laboratory tests where

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the nematodes were immersed in disinfectant solutions for one hour, two disinfectants used in animal health, FAM 30 and a halogentated tertiary amine (Trigene Advance, no longer available) reduced survival to 65% and 34% respectively (Lole, 2007).

The purpose of this proof of principle experiment was to evaluate potential disinfectants for control of leaf and bud nematodes in infested leaf debris on sand beds as a clean-up procedure following an infested containerised HNS crop.

Materials and methods

Site and crop details

Table 1. Test site and plot design information

Test location:	ADAS Boxworth
County	Cambridgeshire
Postcode	CB23 4NN
Soil type/growing medium	Sand and herbaceous mix sub substrate in a 90:10 ratio
Crop	Japanese anemone (detached leaves infested with leaf and bud nematodes)
Glasshouse or Field	Poly tunnel
Date of planting/potting	Potted on 8 December 2015
Pot size	1.5 litre pots
Number of plants per plot	1 pot for each of the 8 treatments
Trial design (layout in Appendix C)	Randomised block
Number of replicates	10
Plot size w (m), l (m), total area (m²)	One 1.5 L pot (surface area of sand in pot 176.7 cm^2)
Method of statistical analysis	Analysis of variance (ANOVA)

Seven disinfectants were tested as drenches to Japanese anemone leaves infested with leaf and bud nematodes (*Aphelenchoides fragariae*) placed onto damp sand in pots in a poly tunnel in December 2015. The treatments included four horticultural disinfectants, two used in animal health and one medical disinfectant. Currently, there is no register of authorized disinfectants, therefore

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use of those recommended for animal health or medical use would be at growers' own risk, see HDC Factsheet 03/14 'Use of chemical disinfectants in protected ornamental plant production' for further details.

Treatment details

Table 2. Detail of products tested (all treatments are commercially available as disinfectants for horticultural, animal health or medical use).

Treatment	Active ingredient(s)	Manufacturer	Formulation type
1. Water (-ve control)	-	-	-
2. Jet 5 (+ve control)	peroxyacetic acid, hydrogen peroxide, acetic acid, ethoxylate	Certis	Aqueous solution
3. FAM 30	Iodophor (blend of iodine, sulphuric acid and phosphoric acid)	Evans Vanodine International PLC	Liquid
4. Menno Florades	benzoic acid	Fargro Ltd	Soluble concentrate
5. Hortisept Pro	Dimethyl benzyl ammonium chloride, citric acid monohydrate and alcohols C9-C11- ethoxylated	LQ Solutions	Liquid
6. Rely+on Virkon	Potassium monopersulphate	Du Pont	Powder
7. Unifect G	Gluteraldehyde and quaternary ammonium compounds	Aromany	Liquid
8. Anigene HLD_4V	Oxirane, 2-methyl-polymer with oxirane, mono(2-propylheptyl) ether, quaternary ammonium compounds, benzyl-C 12-16-alkyldimethyl, chlorides, N-(3-aminopropyl-N-dodecylpropane-1,3-diamine, didecyldimethylammonium chloride	Medimark Scientific	Liquid

There were ten replicate pots per treatment (eight treatments including an untreated control) and each replicate was an individual 1.5 litre pot. The tunnel sides were rolled down and the doors were closed throughout the experiment. The pots were stood on plant pot saucers on woven ground-cover matting and a mixture of 90% sand and 10% peat-based herbaceous mix substrate was used to represent a typical commercial sand bed slightly contaminated with substrate. Japanese anemone leaves infested with leaf and bud nematodes were collected from a commercial nursery and 80 infested leaves of similar size and with similar visual symptoms of nematode infestation were selected. Each leaf was cut in half along the mid-rib. Half of each leaf was used to estimate

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numbers of leaf and bud nematodes per g leaf tissue before treatment and to identify the nematode species using diagnostic keys and microscopy: the species was confirmed as *Aphelenchoides fragariae*. The other half of each leaf was placed onto the sand surface in the 80 experimental pots so that numbers 'before' and 'after' treatment could be calculated. However, as different leaf halves were used for the 'before' and 'after' nematode counts these can only be used as a guide of any reductions in numbers given by the treatments.

The 80 experimental pots were treated in two batches of 40, on 8 and 15 December, in order to allow sufficient time to extract and count all the nematodes in treated leaves three days after treatment on 11 and 18 December (this procedure for 40 pots took a whole working day).

Table 3. Treatments

Product name or MOPS code number	**Application timing**	**Product rate**	**Spray volume (L/m^2)**
1. Water (-ve control)	-	-	5
2. Jet 5	Once	8 ml in 1 L water (1:125)	5
3. FAM30	Once	10 ml in 1 L water (1:100)	5
4. Menno Florades	Once	40 ml in 1 L water (1:25)	5
5. Hortisept Pro	Once	10 ml in 1 L water (1:100)	5
6. Rely+on Virkon	Once	10g in 1 Litre water (1:100)	5
7. Unifect G	Once	40 ml in 1 L water (1:25)	5
8. Anigene HLD_4V	Once	20 ml in 1 L water (1:50)	5
Application timing			
A1 (replicates 1-5)	8 December 2015		
A2 (replicates 6-10)	15 December 2015		

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All treatments were applied once. Label rates were used wherever a specific recommendation for treatment of sand beds was given. In other cases, the minimum dilution (maximum concentration) stated on the label was used. The application volume used was 5 L per m^2, as recommended on the Jet 5 label for use on sand beds (recommendation is 3-5 L per m^2 and growers usually use the maximum volume). This volume was equivalent to 88 ml per pot which was applied with a small watering can fitted with a rose.

Target pest(s)

Table 4. Target pest(s)

Common name	Scientific Name	Infestation level pre-application
Leaf and bud nematode	*Aphelenchoides* sp.	Half an infested leaf per pot

Assessments

Numbers of live and dead leaf and bud nematodes per g leaf tissue before and after treatment

Before treatments were applied, leaf and bud nematodes were extracted from the untreated halves of the leaves using a standard laboratory procedure. The leaf halves were weighed and then cut into small pieces (5mm x 5mm) and placed in individual one litre beakers containing 600 ml of tap water. The water in each beaker was aerated using an aquarium pump and air stone for 72 hours to encourage the nematodes to leave the leaf tissue. After 72 hours, the contents of each beaker was sieved and any nematodes collected on a 45 micron sieve. The nematodes were then washed in to a Doncaster dish (counting dish) and the number of live/mobile nematodes (visible swimming) and dead nematodes (non-mobile) were counted and numbers per gram of leaf tissue were calculated.

The same procedure was used to assess numbers of live and dead leaf and bud nematodes per g of treated leaf tissue three days after treatment.

Numbers of live leaf and bud nematodes per 50 ml sand after treatment

Three days after treatment, 50 ml sand from directly beneath each treated leaf was taken on 11 and 18 December respectively. The nematodes were extracted using a modified Baermann funnel method in which each sand sample was immersed in a pot of water for 72 hours during which time any live nematodes swam through a milk filter for collection and counting under a binocular microscope.

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Temperatures and relative humidity in the poly tunnel

Dataloggers were used to record ambient temperatures and relative humidities in the poly tunnel every 30 minutes during the experiment.

Statistical analysis

The data on numbers of nematodes per g leaf tissue and per 50 ml sand were analysed using analysis of variance (ANOVA) to calculate means, variance and LSDs ($P<0.05$).

Results

Control of leaf and bud nematodes

Numbers of live and dead leaf and bud nematodes per g leaf tissue before and after treatment

Mean numbers of live leaf and bud nematodes per g of leaf tissue were very high before treatment, with an overall mean across the treatments of 3,476 per g. Three days after treatment, none of the treatments had significantly reduced the number of live nematodes, with an overall mean across all disinfectant treatments of 2,935 per g. Numbers of dead nematodes per g of leaf tissue were very low before treatment, with an overall mean of 26.3 per g of leaf tissue. However, numbers of dead nematodes were significantly increased by Jet 5, FAM 30, Hortisept Pro, Unifect G and Anigene compared with the water controls (Table 5 and Figure 4). In the water controls there was a mean of 34.6 dead nematodes per g of leaf whereas the effective treatments increased mean numbers of dead nematodes to 123.2-171.6 per g of leaf. Each of the five treatments that significantly increased numbers of dead nematodes were equally effective. However, as mean numbers of live nematodes were so high, the percentage dead nematodes in the effective treatments were low and only Anigene gave a significant increase, from 1.2% dead in the water controls to 9.3% dead ($P<0.05$, Table 5 and Figure 5).

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Table 5. Mean numbers of live and dead nematodes per g leaf tissue before and 3 days after treatment and % dead nematodes after treatment.

Product name or MOPS code	Live before treatment	Live after treatment	Dead before treatment	Dead after treatment	% dead after treatment
1. Water control	4379	3616	28.1	34.6 a	1.2 a
2. Jet 5	3975	3214	27.0	123.2 b	4.9 ab
3. FAM 30	3222	3415	28.3	171.6 b	4.3 ab
4. Menno Florades	3009	2803	35.0	109.4 ab	3.2 a
5. Hortisept Pro	3746	2654	24.2	138.5 b	5.0 ab
6. Rely+on Virkon	2509	2510	23.2	105.2 ab	5.8 ab
7. Unifect G	3145	2711	23.9	138.9 b	5.9 ab
8. Anigene	3826	2560	20.9	133.7 b	9.3 b
	N.S.	N.S.	N.S.	P<0.05	P<0.05

NS = no significant differences between treatments. Numbers in a column sharing the same letters are not significantly different (P<0.05).

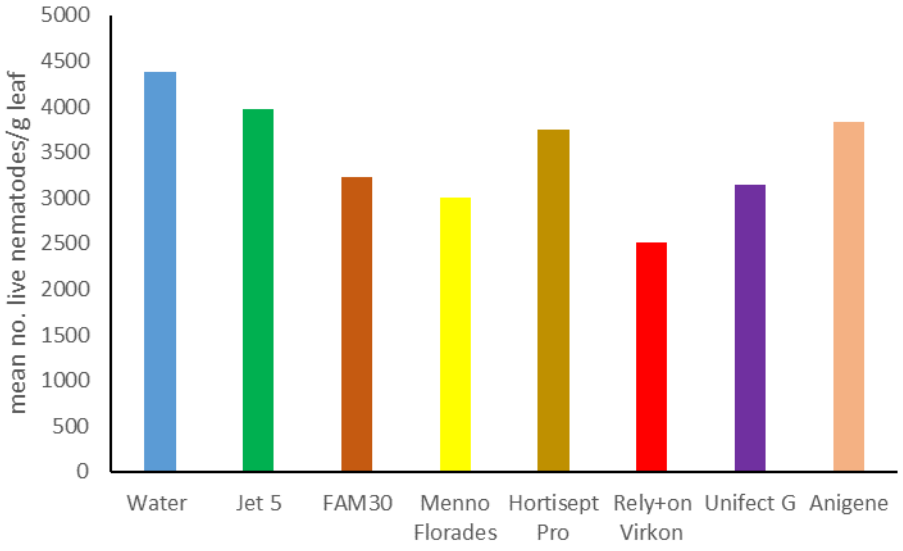

Figure 1. Mean numbers of live leaf and bud nematodes per g leaf tissue before treatment

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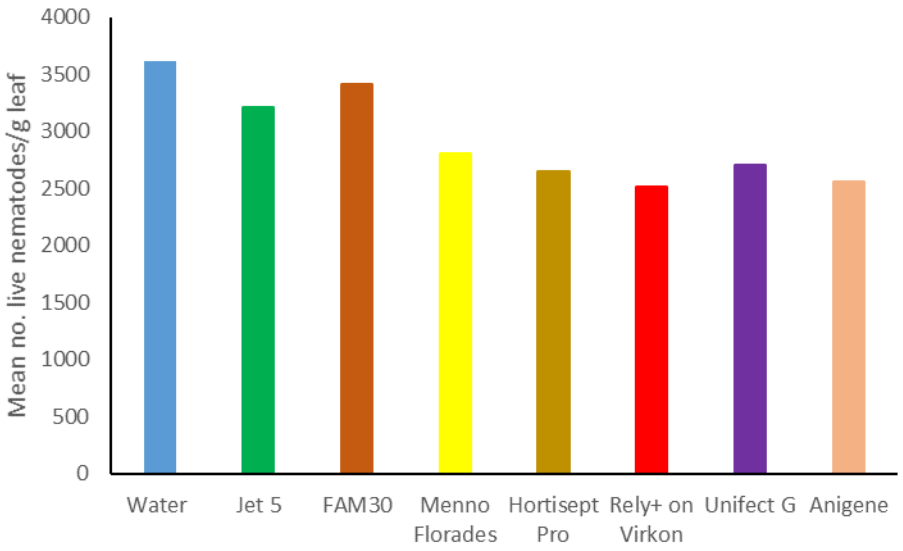

Figure 2. Mean numbers of live leaf and bud nematodes per g leaf tissue 3 days after treatment

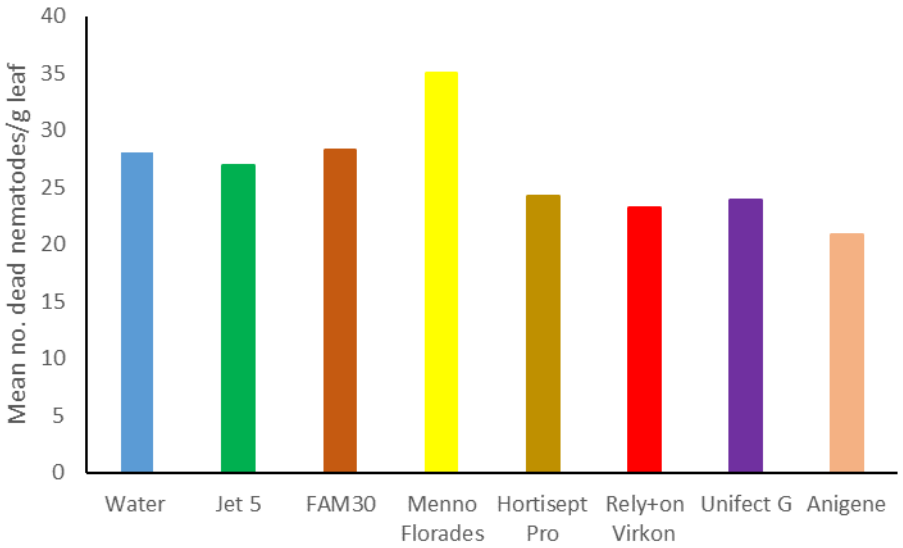

Figure 3. Mean numbers of dead leaf and bud nematodes per g leaf tissue before treatment

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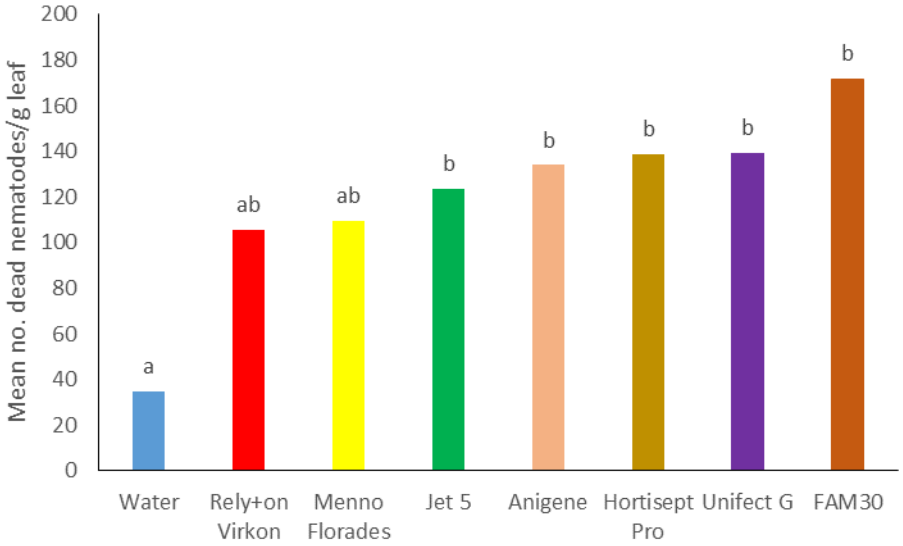

Figure 4. Mean numbers of dead leaf and bud nematodes per g leaf tissue 3 days after treatment. Bars sharing the same letters are not significantly different (P<0.05).

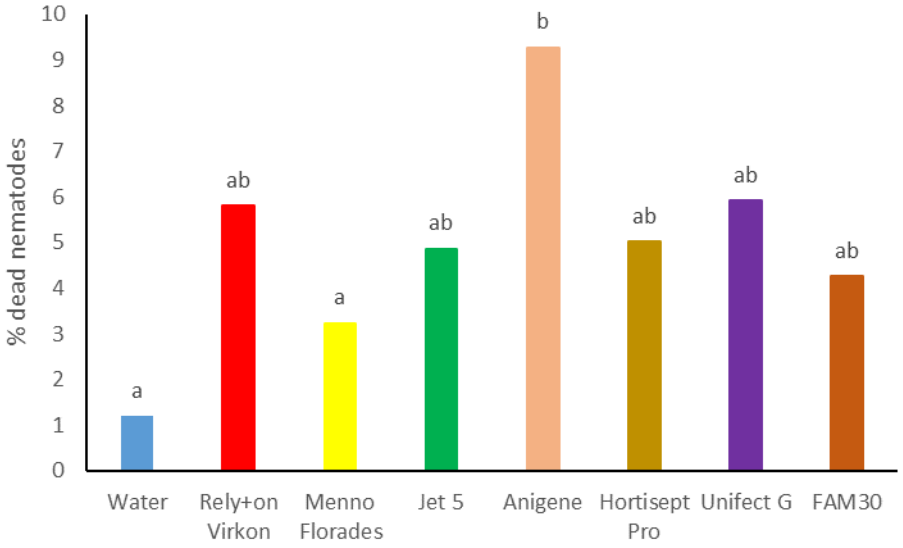

Figure 5. Percentage dead leaf and bud nematodes in infested leaf tissue 3 days after treatment. Bars sharing the same letters are not significantly different (P<0.05).

Numbers of live leaf and bud nematodes per 50 ml sand beneath infested leaves 3 days after treatment

Low numbers of live leaf and bud nematodes were found in the sand directly under the infested leaves three days after treatment, with a mean of 48.9 per 50 ml sand in water-treated controls. Menno Florades, Hortisept Pro, Unifect G and Anigene significantly reduced the numbers of live nematodes to between 3.5 and 14.9 per 50 ml sand (Table 6 and Figure 6). These four treatments were equally effective and Jet 5, FAM 30 and Rely+on Virkon were ineffective.

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Table 6. Mean numbers of live nematodes per 50 ml sand beneath infested leaves 3 days after treatment. Numbers in a column sharing the same letters are not significantly different (P<0.05).

Product name or MOPS code	Mean nos live nematodes 3 days after treatment
1. Water control	48.9 c
2. Jet 5	32.0 abc
3. FAM 30	33.9 bc
4. Menno Florades	12.2 ab
5. Hortisept Pro	12.3 ab
6. Rely+on Virkon	30.4 abc
7. Unifect G	3.5 a
8. Anigene	14.9 ab
	P<0.05

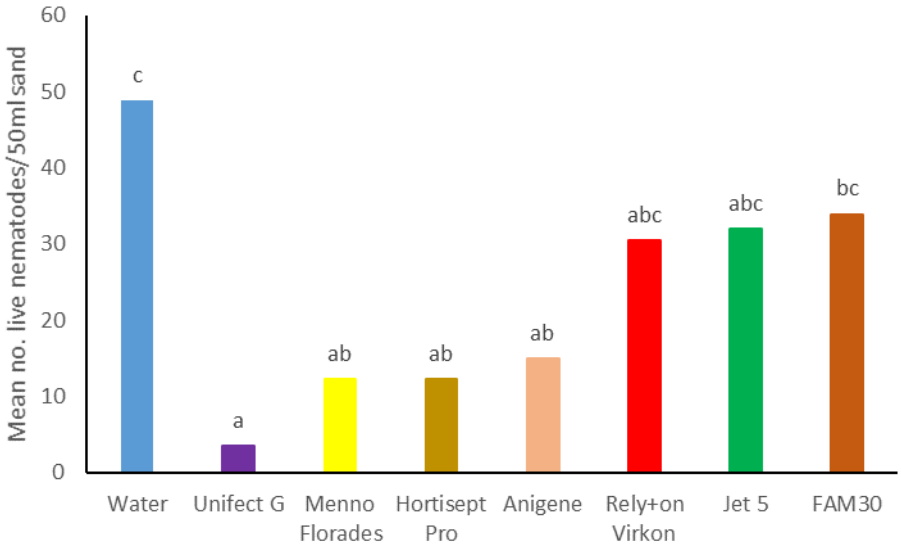

Figure 6. Mean numbers of live leaf and bud nematodes per 50 ml sand beneath infested leaf 3 days after treatment. Bars sharing the same letters are not significantly different (P<0.05).

Temperatures and relative humidity in the poly tunnel

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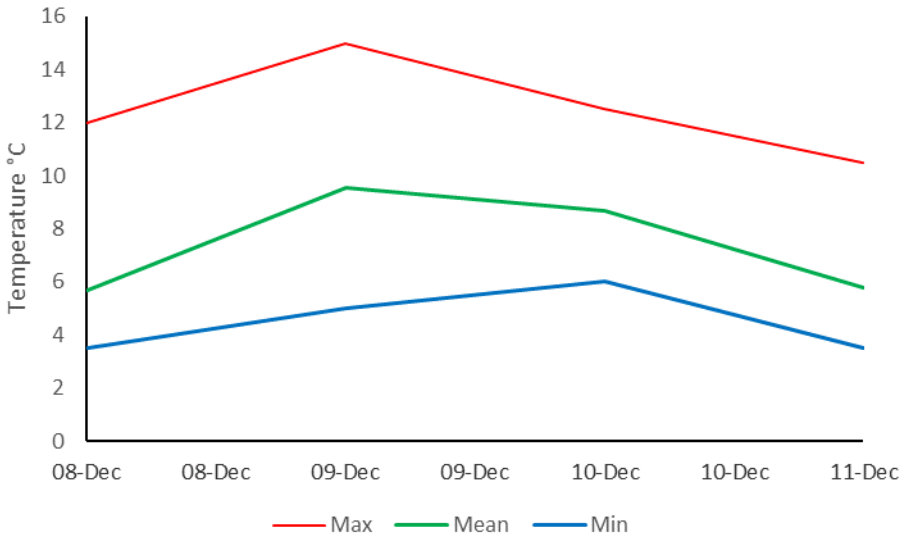

Figure 7. Mean, maximum and minimum temperatures in the poly tunnel during the experiment on first batch of 40 plants (replicates 1-5)

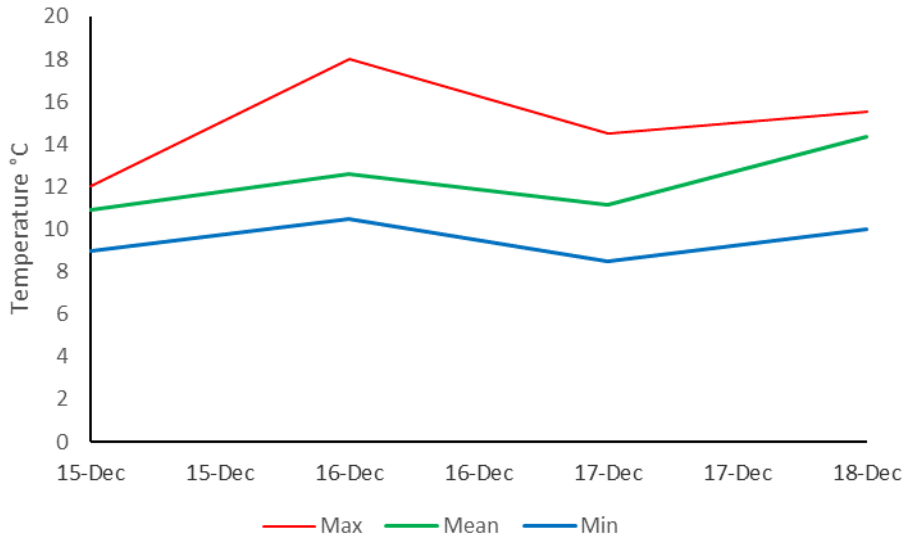

Figure 8. Mean, maximum and minimum temperatures in the poly tunnel during the experiment on the second batch of 40 plants (replicates 6-10)

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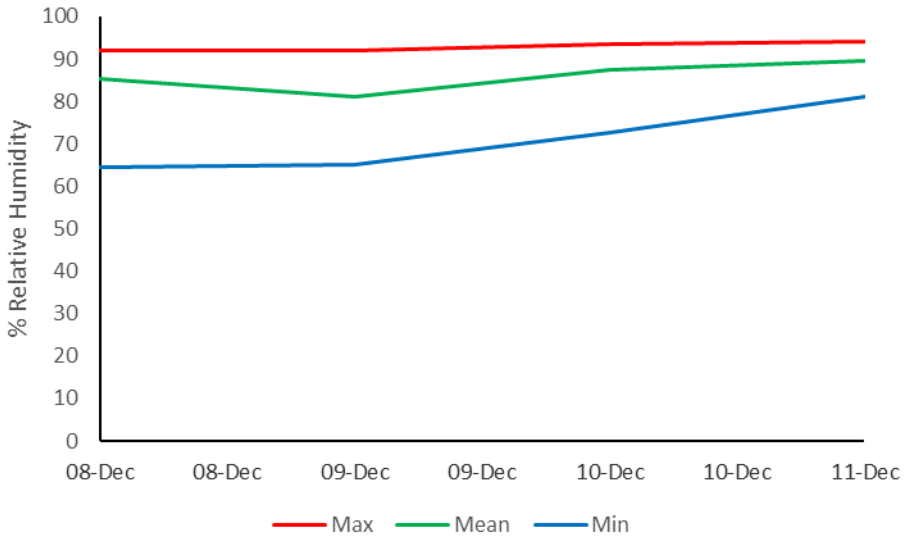

Figure 9. Mean, maximum and minimum relative humidities in the poly tunnel during the experiment on first batch of 40 plants (replicates 1-5)

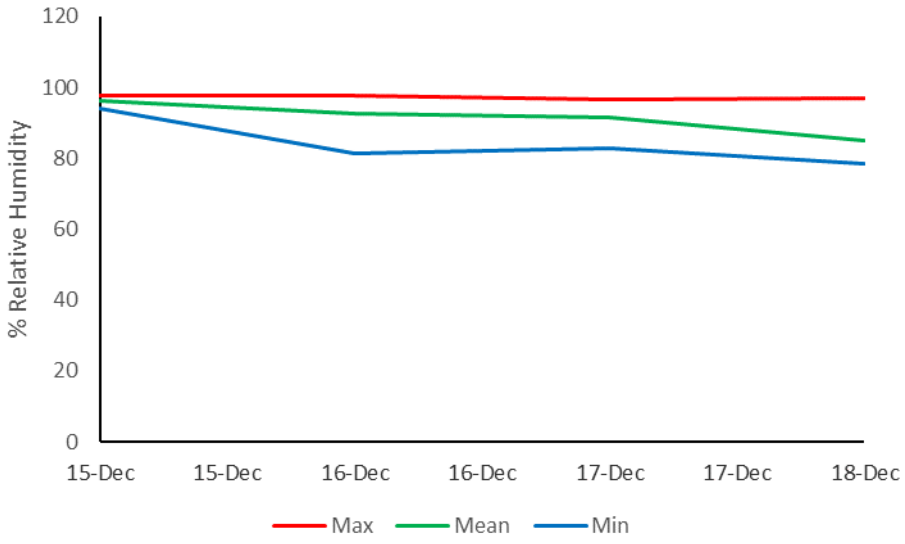

Figure 10. Mean, maximum and minimum relative humidities in the poly tunnel during the experiment on second batch of 40 plants (replicates 6-10)

Formulations

No problems were encountered during mixing or application of any of the product formulations under test.

Effect on non-targets

No other pests or invertebrates were noted during completion of this experiment.

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Discussion

The results indicate that although Anigene, a medical disinfectant, increased the percentage kill of leaf and bud nematodes in heavily infested leaf tissue compared with water-treated controls, only 9% kill was achieved. Therefore none of the disinfectant treatments tested are likely to be fully effective in eradicating leaf and bud nematodes in heavily infested leaf debris on sand beds. Leaf and bud nematodes can survive for long periods in dry leaf tissue e.g. *A. ritzemabosi* can survive in dry infested leaves for up to three years but do not survive the winter in leaves or soil in normal outdoor conditions (Young, 1996). *Aphelenchoides fragariae* does not survive long in the soil in the absence of plants; research has shown that when infested plant tissue was buried 15 cm deep in soil, they only survived for up to three months, although this work did not include *A. ritzemabosi* (Young, 1996). These results indicate that if infested leaf debris remains in or on a sand bed following an infested crop, even if a disinfectant is used an interval of at least four months should be left before using it for fresh susceptible plants.

Although three of the horticultural disinfectants (Menno Florades, Hortisept Pro and Unifect G) and the medical disinfectant Anigene gave significant reductions in numbers of live nematodes in the sand beneath the leaves three days after treatment compared with water-treated controls, none of them are likely to eradicate nematodes moving into the sand from heavily infested leaves. Previous research has shown that if *A. fragariae* were added to soil in a water suspension, they lived for only four weeks (Young, 1996), however this work did not include *A. ritzemabosi*. This result indicates that if sand beds are thoroughly cleared of all infested leaf debris following an infested crop, an interval of at least four weeks should be left before using the beds to grow any fresh plants susceptible to leaf and bud nematodes.

Conclusions

- A drench of Anigene, a medical disinfectant, killed 9% *Aphelenchoides fragariae* in heavily infested Japanese anemone leaves placed onto damp sand to represent sand beds. The other six disinfectants did not kill significantly more than the water-treated controls. None of the disinfectants tested are likely to be effective in controlling leaf and bud nematodes in heavily infested leaf debris on sand beds.
- Three horticultural disinfectants; Menno Florades, Hortisept pro and Unifect G and the medical disinfectant Anigene gave significant reductions in numbers of live nematodes in the sand beneath the leaves compared with water-treated controls However, none of them are likely to eradicate nematodes that have moved into the sand from heavily infested leaves.

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Jagdale, G.B. and Grewal, S. (2002). Identification of alternatives for the management of foliar nematodes in floriculture. Pest Management Science 58:451-458.

Lole, M. (2007). Ornamentals: control of pests, pathogens and weed seeds on re-used plant containers. Final report to HDC on project HNS 147.

Young, J.E.B. (1996). A review of the biology and control of leaf and bud nematodes in outdoor ornamentals. Report to HDC on project HNS 60.

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Appendix A – Study conduct

ADAS is officially recognised by United Kingdom Chemical Regulations Directorate as competent to carry out efficacy testing. The experiments reported were carried out according the internal ADAS operating procedures

GLP compliance will not be claimed in respect of this study.

Relevant EPPO/CEB guideline(s)		**Variation from EPPO**
PP 1/152(3)	Design and analysis of efficacy evaluation trials	none
PP 1/181(3)	Conduct and reporting of efficacy evaluation trials including GEP	none
PP 1/261 (1)	Disinfection in plant production	none

There were no significant deviations from the EPPO and national guidelines

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Appendix B – Meteorological data

Location of the weather station	On site (ADAS Boxworth)
Distance to the trial site	0 m
Origin of the weather data	Weather station for long term average Data logger for average conditions during the trial

Long-term averages from *location* Boxworth 30 year mean (outdoors)				
Month/period	**Av temp (ºC)**	**Min temp (ºC)**	**Max temp (ºC)**	**Rainfall (mm)**
May	11.9	7.0	16.8	43.7
June	14.9	9.7	20.0	48.6
July	17.4	12.0	22.9	48.6
August	17.4	12.4	22.5	56.3
September	14.5	10.1	19.0	52.8

Average conditions during the trial:

Month/period	**Av temp (ºC)**	**Min temp (ºC)**	**Max temp (ºC)**	**Av RH (%)***	**Rainfall (mm)**
Poly tunnel (reps 1-5)	7.4	3.5	15	85.9	n/a
Poly tunnel (reps 6-10)	12.3	8.5	18	91.4	n/a

***protected crops only**

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Appendix C – Agronomic details

Other pesticides - active ingredient(s) / fertiliser(s) applied to the trial area

Date	Product	Rate	Unit
-	-	-	-

Details of irrigation regime (pot-grown crops)

Type of irrigation system employed (e.g. overhead sprinkler, hand watering, drip, ebb and flow, capillary sandbed or capillary matting)
No irrigation used after treatments were applied to damp sand in pots stood on plant pot saucers on woven ground cover matting

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Appendix D – Trial layout in poly tunnel

PLOT	1	2	3	4	5	6	7	8
BLOCK	1	1	1	1	1	1	1	1
TREATMENT	2	6	1	3	5	7	8	4
PLOT	11	12	13	14	15	16	17	18
BLOCK	2	2	2	2	2	2	2	2
TREATMENT	3	5	2	8	4	1	7	6
PLOT	21	22	23	24	25	26	27	28
BLOCK	3	3	3	3	3	3	3	3
TREATMENT	8	1	4	7	6	3	5	2
PLOT	11	12	13	14	15	16	17	18
BLOCK	2	2	2	2	2	2	2	2
TREATMENT	4		3	5	8	2	1	7
PLOT	21	22	23	24	25	26	27	28
BLOCK	3	3	3	3	3	3	3	3
TREATMENT	5	2	7	1	6	4	8	3

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Appendix E – Copy of the Certificate of Official Recognition of Efficacy Testing Facility or Organisation

Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

ADAS UK Limited

complies with the minimum standards laid down in Regulation (EC) 1107/2009 for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Agriculture/Horticulture
Stored Crops
Biologicals and Semiochemicals

Date of issue: 10 May 2013
Effective date: 18 March 2013
Expiry date: 17 March 2018

Signature *Authorised signatory*

Certification Number
ORETO 339

HSE Chemicals Regulation Directorate

Department of Agriculture and Rural Development

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Appendix F – *Photographs*

Figure 1. Japanese anemone leaf infested with *A. fragariae*

Figure 2. Infested leaf cut in half for estimation of pre-treatment numbers of nematodes and for use in disinfectant experiment

Figure 3. Experiment layout in poly tunnel

Figure 4. Infested leaf half on damp sand after treatment with disinfectant

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Figure 5. Laboratory extraction of nematodes from sand under infested leaf

Figure 6. Laboratory extraction of nematodes from infested leaf tissue

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