Project title:	Narcissus: disinfectants for the control of stem nematode on bulb handling hardware and the fabric of buildings.
Project Number:	BOF 49
Report:	Final Report, December 2001
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Date Project Commenced:	April 2001
Date Completion due:	December 2001
Key Words:	narcissus, stem nematode, *Ditylenchus dipsaci,* disinfectant, iodophor/acid disinfectant, Antec Virudine, Fam 30

The results and conclusions in this report are based on a series of experiments 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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Commercial benefits of the project

This project has identified iodophor/acid disinfectants as the most appropriate type to use for controlling stem nematode on bulb handling machinery and the fabric of buildings. Products that are iodophor/acid based disinfectants include Fam 30 and Antec Virudine.

It has also been demonstrated that whilst it is possible to kill **active** stem nematodes with disinfectants, the dry 'wool' stage is tolerant of exposure to disinfectants for periods of at least ten minutes. This suggests that when machinery and buildings used for handling bulbs are being cleaned, they should be thoroughly wetted to re-activate nematode 'wool' and render the nematodes susceptible to disinfectants. This should be done 24 hours before the disinfectant is applied.

The corrosion risk posed by diluted solutions of the disinfectants FAM 30 and Antec Virudine is minimal and should not cause problems at the dilution rates recommended on the product labels.

Finally, the experiment confirms the adverse effect of soil contamination on the efficacy of disinfectants, and therefore reinforces the message that improved general cleanliness in bulb handling and storage is important.

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Background & Objectives

Stem nematode, *Ditylenchus dipsaci*, is a major threat to the UK narcissus industry. Bulbs infested by stem nematode decay within a few months of initial attack and the nematode can spread rapidly in the field. Control relies on hot-water treatment of dormant bulbs after harvesting prior to grading and subsequent sale or replanting. This process requires handling the bulbs on a large scale, which in turn involves the use of equipment and buildings. There is potential for stem nematode to spread from bulb stock to bulb stock during the handling phase, and growers need to maintain high

standards of hygiene in order to prevent this. In particular, stem nematodes have the capability to survive hostile conditions by forming what is known as 'wool'. This consists of a mass of dehydrated juvenile nematodes tangled together, in which form they may survive for many years in suspended animation. During this time they are very resistant to heat, chemicals etc.

Hygiene is obviously important in reducing cross-infestation and the use of disinfectants is one facet of this. However, not all disinfectants are effective against stem nematode and some are unsuitable for other reasons (e.g. potentially hazardous to humans or unpleasant to handle).

Previous work (Lole, 1990) showed that an iodophor/acid disinfectant designed for dairy use (product name Iosan, Ciba-Geigy) was the most effective replacement for traditional phenolic disinfectants such as Bray's Emulsion. Iosan is however no longer available. Consequently, re-evaluation of disinfectants for the control of stem nematodes is required. The aim of this project was to satisfy that need.

Summary of results and conclusions

Tests on active, free-swimming stem nematodes clearly demonstrated that there were differences in the efficacy of the different types of disinfectants (Table 1). FAM 30 and Antec Virudine were the most effective and rapid-acting products, providing 100% mortality in less than 5 minutes. H410 (which is now known as Agrichem Menno Florades) and Farm Fluid S were the next most effective products.

Where the disinfectant solutions were deliberately contaminated with soil, the efficacy of some disinfectants was significantly reduced, including those that were most effective as clean solutions. The iodophor/acid disinfectants remained the most effective of the products tested.

Checks with the product manufacturers of Antec Virudine and Fam 30 indicated that the corrosion posed at the recommended dilution rates of these products is minimal and should not cause problems for users.

The tests on stem nematode 'wool' demonstrated that this stage of the nematode could tolerate exposure to the disinfectants. Pieces of 'wool' steeped in disinfectant for 10 minutes retained the same viability as undipped 'wool when subsequently wetted and revived. Tests on the efficacy of disinfectants against previously wetted nematode 'wool' were not conducted but experience suggests that prior wetting of nematode 'wool' for at least 24 hours before treatment should activate the nematodes into the free living stage and hence improve the efficacy of the disinfectants.

Table 1. Effect of disinfectants on the survival of free-living stem nematodes

All products were tested at the recommended dilution rate on the product label.

Disinfectant type	Product(s)	LE 95 (minutes) No soil	Mortality @ 60min. (%) No soil	LE 95 (minutes) Soil added	Mortality @ 60min. (%) Soil added
Polyethoxylated alcohol + iodine + inorganic acids*	FAM 30	3	100	16	100
	Antec Virudine	4	100	9	100
Benzoic acid	H 410	22	100	45	100
Tar acids	Farm Fluid S	19	100	-	93
Organic acids	Jeyes Fluid	-	96	53	100
Peroxygen compounds + organic acids	Antec Virkon S	-	60	-	89
Quaternary ammonium compounds	Antec Ambicide	-	70	-	77
Peroxyacetic acid	Jet 5	-	75	-	40
Tap water	_	-	0	-	0

* Otherwise known as iodophor/acid disinfectants

LE 95 = the time taken, in minutes, to achieve 95% mortality. Where 95% mortality was not achieved in 60 minutes the actual % mortality at 60 minutes has been recorded.

Action Points for Growers

- Clean machinery, pallets, containers, buildings, etc. before disinfecting. An industrial vacuum cleaner is preferable for this purpose.
- Thoroughly wet all surfaces with water at least 12 hours before treatment with disinfectant in order to revive nematode 'wool'. It may be desirable to re-wet at intervals to prevent drying out and ensure maximum revival of 'wool'.
- Choose an iodophor/inorganic acid disinfectant such as Fam 30 or Antec Virudine, for use on machinery and buildings used in bulb handling. Checks with the product manufacturers of Antec Virudine and Fam 30 indicated that the corrosion risk posed at the recommended dilution rates of these products is minimal and should not cause problems for users. Always check the product label prior to use.
- Avoid recontamination of cleaned areas and machinery.

Anticipated practical and financial benefits from the study

- More appropriate choice of disinfectant
- Better levels of control of stem nematode on machinery and buildings
- Reduced cross-infestation of stocks during bulb handling.

In the UK the total saleable output of narcissus bulbs is estimated as approximately 30,000 tonnes/year. If 60% (18,000 tonnes) is entered for PHSI Plant Passporting/Export Certification, of which 2% fails due to stem nematode infestation, 360 tonnes would be rejected. Costed at a nominal price of £50/tonne as against a farm-gate price of £350/tonne for healthy stock, this represents a cost to the industry of £108,000 per year in lost sales. If the outcome of this project reduced this problem by only 25%, then the annual saving to the industry would be £27,000.

Science Section

Introduction

Stem nematode (*Ditylenchus dipsaci* (Kuhn) Filipjev) is a major pest of cultivated narcissus in the UK. The nematode is a facultative endoparasite of stems and leaves (not roots), able to survive in the soil for a few weeks, which can allow it to spread from plant to plant in the field. Reproduction in the host plant is rapid, and numbers of the nematode can readily build up to the point where damage is so severe that secondary organisms (fungi, bacteria, mites) can invade and destroy the plant. (Whitehead, 1998)

Growers keep the pest in check by subjecting the bulbs to regular hot-water treatment. The standard treatment is to harvest, sort and grade the bulbs (normally every second year) and subsequently immerse in water at 44.4^{o}C for 3 hours in order to kill the nematode. During the handling of the bulbs it is possible for machinery and the fabric of buildings to become contaminated with stem nematodes, which may then transfer to other bulb stocks and infest them. A particular hazard is ‘wool’, a tangled mass of dehydrated juvenile stem nematodes that may be attached to pieces of bulb debris. The nematodes in ‘wool’ are in a form of suspended animation and are very difficult to kill. Stem nematodes in the ‘wool’ form have been known to survive in dry conditions for 25 years, becoming active again on rehydration.

To minimise the risk of cross-infestation of stocks of bulbs by stem nematodes during the handling stage of bulb cultivation, hygiene is of prime importance. Removal of soil, bulb debris etc. forms part of this process, but the industry also needs to use disinfectants in order to reduce the risk of nematode survival on machinery and buildings. Traditionally, phenolic disinfectants were used for this purpose but the number of products available began to diminish in the 1980’s. Work at this time (Lole, M.J. 1990) identified a limited number of potential replacements, but these in turn have become superseded. The aim of this work was to assess a range of currently available materials in order to identify the most suitable for use in the bulb industry.

Materials and Methods

The experiments were done in the laboratory at ADAS Wolverhampton. The active nematodes used in some of the tests were extracted from infested narcissus bulbs collected from a field near Spalding, Lincolnshire in April 2001. The nematode 'wool' used in the remaining tests was collected in 1999 from infested bulbs of Lincolnshire origin and was held in the laboratory for the intervening period.

The disinfectants tested are listed in Table 2. These were tested as solutions in clean water and also in the presence of soil contamination, to assess their effectiveness under conditions appropriate to the field. They were tested on the 'wool' stage of stem nematode as well as on active nematodes freshly extracted from bulb tissue.

Table 2 List of Disinfectants Tested

Disinfectant Type	Product	Recommended label dilution rates for general disinfection
Polyethoxylated alcohol + iodine + inorganic acids	FAM 30	1:400
Polyethoxylated alcohol + iodine + inorganic acids	Antec Virudine	1:400
Benzoic acid	H 410	1:33
Tar acids	Farm Fluid S	1:400
Organic acids	Jeyes Fluid	1:143
Peroxygen compounds + organic acids	Antec Virkon S	1:100
Quaternary ammonium compounds	Antec Ambicide	1:100
Peroxyacetic acid	Jet 5	1:125

The tests on the active nematodes were done in embryo dishes - solid, rectangular glass vessels (40 mm x 40 mm x 15 mm) with a hemispherical recess of 30 mm diameter in the upper surface. 1 ml of tap water was measured into each dish. 10 stem nematodes freshly extracted from infested bulb tissue were then transferred into each dish using an eyelash mounted on a dissecting needle. Adults or final stage juveniles were chosen at random. Disinfectant solutions were made up using clean tap water at

double the strength recommended by the manufacturer for general farm use. At time zero, 1 ml of one of these disinfectant solutions was added to a dish of nematodes in tap water, producing a final disinfectant solution at the manufacturer's recommended concentration. The nematodes in the dish were then observed under the binocular microscope, using under-stage lighting. The number of nematodes showing signs of activity 1, 5, 10, 20, 30 and 60 minutes after the disinfectant solution was added was recorded. Where necessary, nematodes were manipulated with the mounted needle in order to test activity. The experiment was repeated twice (ie replicated three times) with each disinfectant.

Further double-strength solutions of the disinfectants were then made up, but this time 10% by weight of a Lincolnshire silt soil was added and the resulting suspension was agitated for a few seconds before being allowed to settle for one hour. The experiment above was then repeated for each disinfectant using the soil-amended solutions. Nematode viability was recorded in the way described above.

Further tests were conducted, on nematode 'wool'. A fragment of nematode 'wool' (approx. 2 mm^3) was placed on a Whatman cellulose nitrate sterile membrane filter (47 mm diameter, 0.45 μm pore size) held on a Buchner funnel and was inundated with a droplet of disinfectant solution. After 10 minutes the disinfectant solution was removed by suction through the membrane filter using the Buchner funnel. The 'wool' fragment was then rinsed with clean tap water to remove traces of disinfectant before transferring it to one cell of a multicell petri dish (25 cell, each 20 mm^3, Bibby Sterilin Ltd), containing 4 ml clean tap water. This was repeated for each of the disinfectants. Pieces of untreated 'wool' were simultaneously added to clean tap water in other cells of the dish. As it can take 12 hours for nematodes to revive from the wool stage, the contents of the cells were left for 18 hours before checking for relative viability under the binocular microscope. Comparisons were made between the proportion of viable nematodes present in fragments of disinfectant-treated wool and that in untreated fragments from the same origin. The results were recorded.

Results and Discussion

The results of the tests on active nematodes are presented in Table 3.

Table 3: Effect of disinfectants on survival of free-living stem nematodes.

Disinfectant type	Product(s)	LE 95 (minutes) No soil	Mortality @ 60min. (%) No soil	LE 95 (minutes) Soil added	Mortality @ 60min. (%) Soil added
Polyethoxylated alcohol + iodine + inorganic acids*	FAM 30	3	100	16	100
	Antec Virudine	4	100	9	100
Benzoic acid	H 410	22	100	45	100
Tar acids	Farm Fluid S	19	100	-	93
Organic acids	Jeyes Fluid	-	96	53	100
Peroxygen compounds + organic acids	Antec Virkon S	-	60	-	89
Quaternary ammonium compounds	Antec Ambicide	-	70	-	77
Peroxyacetic acid	Jet 5	-	75	-	40
Tap water	_	-	0	-	0

* Otherwise known as iodophor/acid disinfectants

LE 95: the time taken, in minutes, to achieve 95% mortality. Where 95% mortality was not achieved in 60 minutes the actual % mortality at 60 minutes has been recorded

The most rapid kill of free-living stem nematodes was given by the two polyethoxylated alcohol + iodine + inorganic acid (iodophor/acid) products. When used in clean water each product gave 100% mortality in less than 5 minutes, and though the addition of soil contamination increased the time taken to achieve the same effect by between 2- and 5-fold, these products still out-performed the remainder when soil contamination was present. Benzoic acid and tar acids both gave 100% mortality in around 20 minutes; of these, the benzoic acid was much less adversely affected by the addition of soil contamination than was the tar acids, which then failed to give complete control within the hour. None of the other materials tested gave complete kill of the nematodes within one hour.

Tables 4 and 5 express the data in a different format, which has been statistically analysed.

Table 4 Mean number of nematodes surviving at indicated elapsed time after initial exposure to disinfectant: no added soil

Product	Time 0	1 min	5 min	10 min	20 min	30 min	60 min
Fam 30	10	2.33	0	0	0	0	0
Antec Virudine	10	9.67	0	0	0	0	0
H410	10	10	6.33	4	1.33	0.33	0
Farm Fluid S	10	10	6.0	4.67	0.67	0	0
Jeyes Fluid	10	10	10	10	7	2	0.33
Antec Virkon S	10	10	8.67	8.33	7.67	7	4
Ambicide	10	10	9.33	9.33	9	8	3
Jet 5	10	10	6.33	4.67	4	2.67	2.67
Tap water	10	10	10	10	10	10	10
S.E.D		0.720	0.869	1.276	1.346	1.478	1.434

Table 5 Mean number of nematodes surviving at indicated elapsed time after initial exposure to disinfectant: soil added

Product	Time 0	1 min	5 min	10 min	20 min	30 min	60 min
FAM 30	10	6	2.83	1.5	0	0	0
Antec Virudine	10	9.67	3.33	0	0	0	0
H410	10	9.33	6.5	3.83	1.83	1	0
Farm Fluid S	10	10	8	7.33	4.5	3.33	0.33
Jeyes Fluid	10	10	10	10	6.5	3.16	0.16
Antec Virkon S	10	10	8.67	8.33	6.83	4.83	2.5
Ambicide	10	10	9.67	9.67	9.5	9	2.67
Jet 5	10	10	8	7	6.67	5.67	4.33
Tap water	10	10	10	10	10	10	10
S.E.D		1.220	1.746	1.502	1.738	1.937	1.363

The results of the tests on nematode 'wool' are included in Table 6.

Table 6. Effect of disinfectants on the viability of nematode 'wool'

Disinfectant	Mean score*, treated replicates	Mean score*, untreated controls
FAM 30	3	2.5
Antec Virudine	2.6	3
H 410	1.7	2
Farm Fluid S	2	2
Jeyes Fluid	2	2
Antec Virkon S	2.3	2
Antec Ambicide	2	2
Jet 5	2	2

***** Score system: No viable nematodes present = 0
1 - 10 viable nematodes present = 1
11 - 100 viable nematodes present = 2
101+ viable nematodes present = 3

Replication of the work on 'wool' was necessary because of the inherent variability in viability of nematodes within and between clumps of 'wool' (a 'clump' is an aggregated mass of nematodes from a single source). To eliminate as much of this variability as possible, all replicates per disinfectant, three treated and two untreated, used fragments of 'wool' taken from the same clump. Taking the inherent variability into account, the results above do not indicate that any of the disinfectants used had any effect on the viability of nematode 'wool'.

Conclusions

1) Iodophor/acid disinfectants (which contain polyethoxylated alcohol, iodine and inorganic acids) were the most effective and rapid-acting against free living stem nematodes and are recommended for use in disinfecting machinery and premises that may be contaminated with stem nematode (*Ditylenchus dipsaci*). Such disinfectants include the commercial products FAM 30 and Antec Virudine.

2) The presence of soil contamination reduced the effectiveness of some disinfectants, but the iodophor/acid disinfectants remained the most effective of the products tested.

3) None of the disinfectants gave control of nematodes in the 'wool' stage of suspended animation. It is therefore desirable to wet, and thereby reactivate, nematode 'wool' before instigating the disinfection procedure.

Corrosion Risk

The iodophor/acid disinfectants identified by this project as being the most effective for the control of free living stem nematode, contain significant amounts of inorganic acids (5-20% sulphuric and phosphoric acids) and in their undiluted state are corrosive, as warned on the packaging. This raises concerns that the disinfectants might damage bulb-handling machinery and buildings.

Information on the corrosion risks has been provided by the manufacturers of Fam 30 and Antec Virudine. In summary, the corrosion risk posed by the diluted products is minimal and should not cause a problem for users of the products at the dilution rates recommended. Further information is included in Appendix 2 of the report.

Technology Transfer

Preliminary results have been published in HDC Project News (No 76, September 2001).

The results were presented at a seminar organised by the HDC for bulb growers, held at Duchy College, Camborne, Cornwall on 15th November 2001.

References

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