Project Title	Narcissus: alternatives to the use of formaldehyde in hot water treatment tanks for the control of stem nematode and Fusarium basal rot
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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.

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Grower Summary

Headline

- There are a number of potential alternatives to the use of formaldehyde in hot-water treatment tanks, including use of some biocides, amendment of current HWT temperature regimes, incorporating pre-soaking and post warming stages or use of auxiliary devices.

Background and expected deliverables

Currently, stem nematode is controlled in the bulb industry by the use of regular hot-water treatment of narcissus stocks. In order to improve the control of stem nematode and to help suppress the transmission of basal rot in the hot-water treatment tank, formaldehyde is routinely added to the tank contents. However, this option is likely to cease after 2007 as no manufacturer or supplier of formaldehyde is willing to provide the data on its use that will facilitate its passage through an EC review of pesticides.

This project forms a preliminary study to a larger piece of work that is intended to provide growers of narcissus with information on how to maintain control of stem nematode and basal rot in the absence of formaldehyde.

Summary of the project and main conclusions

The project is intended to review the use of formaldehyde in hot-water treatment and to consider alternatives. Options considered included:

- Alternative biocides to directly replace formaldehyde
- Alternative fungicides to improve basal rot control in the HWT tank
- Amendments to the HWT regimes currently in use
- Pre-soaking bulbs before treatment
- Warm storage of bulbs before treatment
- Warm storage of bulbs after treatment
- Auxiliary devices that would improve the efficiency of HWT
- Alternative methods of using heat
- The potential in plant breeding for resistance to stem nematode in narcissus.

The findings of the review were as follows:

- Some biocides (chlorine dioxide, citric acid, iodophor disinfectants) and a fungicide (chorothalonil) were identified as having potential as direct replacements for formaldehyde in HWT and should be investigated further.
- There is considerable scope to amend HWT regimes by raising the temperature of HWT and/or increasing its duration. This should improve the kill of nematodes, but it will be necessary to consider the possible effects on the bulbs themselves.
- Pre-soaking bulbs should improve the control of stem nematodes by reactivating individuals that have entered the 'wool' stage, as well as aiding bulb wetting which improves conductivity of heat into the bulbs. Warm storage may be necessary to reduce HWT damage to the bulbs themselves if HWT regimes are changed but if 'wool' forms as a result of warm storage then greater nematode damage could result. This will need consideration when new HWT regimes are being designed.
- Post-treatment warm storage can greatly speed up the kill of stem nematodes compared to storage at ambient temperatures or immediate planting, which has the effect of cooling bulbs. The practical application of warm storage should be examined in more detail.
- The auxiliary devices looked at in the review (ozonation of HWT water, UV treatment of HWT water, flash heat treatment of diverted HWT water, filtration) all showed some promise technically, especially in the control of basal rot spores. However, all would need considerable development before they could be relied upon to give adequate control of stem nematodes and basal rot and the capital investment necessary to install appropriate machinery would be considerable. Further work on these devices is not recommended at this stage.
- Although the alternative methods of applying heat to bulbs (microwaves, radio waves, hot air) all had the advantage that bulbs could be treated dry, thus reducing disease transmission between bulbs, none were thought to be capable of heating a bulk of bulbs evenly. The fine level of control of final temperature necessary for the safe heat treatment of bulbs (+ or – 0.5°C) may also be beyond the described methods. Further investigation is not recommended.
- There are no nematode-resistant cultivars of narcissus known at present. Though there is some potential for breeding such varieties the finished product would be at least 15 years away. The large costs involved in such a breeding project, the uncertainty of the outcome and the time delay that would occur before any practical result might be available commercially rule out any work on breeding at this stage.

Financial benefits

It is unlikely that this project will produce any financial benefit for growers. The required outcome is that growers of narcissus will still be able to control stem nematode using hot-water treatment, even after the loss of formaldehyde for pesticide purposes. The new methods that evolve as a result of this project may exceed the cost of using formaldehyde.

Action points for growers

- Consider the alternatives to formaldehyde discussed in the report.
- Feed back comments to the grower co-ordinators of the project.

Science Section

Introduction

Hot water treatment (HWT) is the UK industry standard for controlling stem nematode *(Ditylenchus dipsaci,* 'bulb eelworm') in narcissus bulbs.

Since the 1930's, formaldehyde (formalin) has been included routinely in the HWT tank when treating narcissus bulbs, principally to speed the kill of free-swimming stem nematodes during treatment. Without it, any nematodes that enter the tank in the 'wool' stage (a resting stage of the nematode that is resistant to heat and nematicides) may not be killed by the standard exposure to hot water (3 hours at 44.4°C) used in HWT.

HWT with formaldehyde has also been shown to help control the spread of *Fusarium oxysporum* f. sp. *narcissi,* the cause of basal rot. It probably also gives incidental control of *Botrytis narcissicola*, the cause of smoulder.

The use of formaldehyde in hot water dips is covered under Plant Protection Products Regulations (PPPR). Formaldehyde is therefore required to be notified as a pesticide under list 4 of the EC 91/414 directive. However, no manufacturer is willing to provide the necessary data to support its continued use as a PPPR product, which is likely to result in the rapid withdrawal (perhaps in 2-4 years from the time of writing) of formaldehyde as a horticultural disinfectant or commodity substance.

Only very sparse information is available on the suitability of alternative chemicals for use in HWT. At present, there are no viable alternatives – chemical, biological, cultural or other – to using HWT with formaldehyde for stem nematode control.

It is likely that the current approval for the use of the fungicide thiabendazole (as Storite Clear Liquid) as an HWT additive will also be withdrawn or curtailed within the next 1-2 years, as the manufacturer is reluctant to support the dip recommendation, due to environmental concerns. Therefore the loss of not only formaldehyde, but also thiabendazole, must be considered.

An alternative way of achieving complete control of stem nematode and suppression of fungal pathogens during HWT must be developed as a matter of urgency. The aim of this project is to examine possible alternatives to the use of formaldehyde in HWT for the control of stem nematodes and basal rot and to make recommendations as to future research and development that may be required to identify a suitable substitute.

Section 1. Pesticides and disinfectants as potential alternatives to the use of formaldehyde in HWT for control of stem nematodes and fusarium basal rot.

Biocides

Chlorine dioxide

This product is available in the UK and is used especially in the food industry. Good efficacy was demonstrated in the USA against *Fusarium oxysporum* f. sp. *narcissi* when used in the HWT tank (Chastagner & Riley, 2002). Information is available on the influence of pH, metal ion concentration and water hardness on the effectiveness of chlorine dioxide against Fusarium conidia (Copes *et al,* 2004). There is no information on the use of this material to control stem nematodes.

Hydrogen peroxide/peracetic acid

Various formulations are available in the UK (e.g. Jet 5, Sanprox P). Efficacy against *F. oxysporum* f. sp. *narcissi* chlamydospores when used in HWT at 0.5% has been demonstrated (Hanks & Linfield, 1999). In laboratory studies it was also effective against free-swimming stem nematodes, and stem nematodes in the wool stage. The disadvantages of these materials are i) the concentration in a HWT tank rapidly decreases due to reaction with organic matter, and ii) they are expensive. The Dutch consider the action of Jet 5 against nematodes in HWT is poor. Disposal of waste solution should not be a problem with this disinfectant.

Glutaraldehyde

This disinfectant is widely used in hospitals and to a limited extent in horticulture. There is evidence of efficacy against *F. oxysporum* (HNS 63) and nematodes (?). Organic matter has little effect on antimicrobial activity, and it is non-corrosive to metals. However, it is a hazardous chemical (hazardous to breathing; skin and eye irritant), and its activity is markedly affected by pH, being most active in alkaline conditions (whereas HWT tanks are normally acidified to pH 2.5 if thiabendazole is being used). It is also relatively expensive. It is available in the UK in mixture with QAC disinfectants (e.g. Unifect G, Vitafect PepMV)
[Van Aartrijk commented that the Dutch were not looking at glutaraldehyde as a replacement due to the risk of ‘chloride damage’].

Citric acid

Citric acid is commonly used as a disinfectant in animal health and human medicine at dilutions of 0.2–0.5%, giving good control of bacteria and viruses. No information on its efficacy against Fusarium has been found. When tested as a disinfectant against

Verticillium albo-atrum conidia it proved ineffective (PC 186A). The Dutch report efficacy against stem nematodes, but less than that of formalin (Van Aartrijk).

Sodium hypochlorite

Widely used for treatment of drinking and swimming pool water, sodium hypochlorite is a cheap disinfectant, which has activity against *F. oxysporum*. Treatment of fresh fruit and vegetables with chlorinated water reduces post - harvest rots. Though there does not seem to be any information on the susceptibility of stem nematode, various other nematode species may be controlled by immersion in solutions containing between 0.1% and 4% hypochlorite. Use of chlorine in water is currently under scrutiny due to the production of trihalomethanes (THMs) as a by-product of chlorinating water that contains organic materials. The occurrence of THMs in drinking water is considered a health risk. The antimicrobial activity of hypochlorite is reduced by organic matter, light, and heavy metal ions. Hypochlorite is toxic to plant growth so if used to treat bulbs in HWT the effect on subsequent growth would need to be assessed. It is likely that a continuous monitoring and dosing system would be required to maintain a target dose in HWT water, due to the rapid inactivation of hypochlorite.

Quaternary Ammonium Compounds (QACs)

These disinfectants tend to be expensive, are fungistatic rather than fungicidal, are less active in the presence of organic matter, and tend to foam. They are therefore unlikely to be suitable for use in a HWT.

Phenolics

Although these disinfectants have broad-spectrum activity and are effective in the presence of organic matter, they can be corrosive and are persistent in the environment. Difficulty with environmentally safe disposal of waste solution probably precludes use of this type of disinfectant in HWT.

Iodine/Iodophors

HDC-sponsored work in both 1991 and 2001 (BOF 46) showed that the iodophor disinfectants were the most effective disinfectants for the control of stem nematodes when applied as cold solutions *in vitro*. This work was done to identify a replacement for phenolics for use on bulb handling equipment and the fabric of buildings and was completed at ambient temperatures. The effect of iodophor disinfectants on stem nematodes at HWT temperatures has not been studied, nor has the effect on *Fusarium* or the narcissus bulbs themselves, but the products may have potential for this use.

Fungicides

Benzimidazole fungicides (Carbendazim, thiophanate methyl, thiabendazole)
All three fungicides were shown to give good control of fusarium basal rot when used as a post-lifting dip combined with HWT (Hanks, 1996). One potential problem with reliance on MBC fungicides is the risk of resistance development. There are reports of resistance to MBC fungicides in some Fusarium species (e.g. *F. graminearum*).

Prochloraz (e.g. Sportak)
This fungicide has activity against *F. oxysporum* species, and its use in HWT has been shown to reduce fusarium basal rot (e.g. Hanks, 1996). Although stable in water at 20ºC, it was reported that prochloraz is unstable at "high" temperatures (Van Aartrijk).

Chlorothalonil
A protectant fungicide with some activity against Fusarium species, when used both post-lifting and in HWT, this fungicide was found to give good increases in bulb yields. Chlorothalonil is relatively inexpensive and there is considered to be a reduced risk of resistance development.

Strobilurin fungicides
Some fungicides in this group (e.g. azoxystrobin) have been shown to have activity against *F. oxysporum* when used as a soil drench treatment on growing plants. Several fungi have developed resistance to this group of fungicides.

Conclusions

Some of the materials discussed above have potential for the control of *Fusarium oxysporum*, stem nematode, or both in HWT. In some cases, e.g. hydrogen peroxide/peracetic acid and the benzimidazole fungicides on *Fusarium*, the potential is probably already proven. In other cases investigation will be required.

Recommendations

It is recommended that the candidate materials listed below are assessed for their effectiveness in controlling important pathogens of narcissus at HWT temperatures. Initial tests should be done in vitro: -
Chlorine dioxide on stem nematode
Citric acid on stem nematode and *Fusarium*
Iodophor disinfectants on stem nematode and *Fusarium*

Chorothalonil on *Fusarium*

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Section 2. Evaluation of the potential for changes to accepted HWT practice

Hot-water treatment, that is the immersion of bulbs in a tank at a pre-determined temperature for a defined period, was pioneered in Britain by Ramsbottom in 1919 (Tompsett, 2006) and has been used for the control of stem nematode (*Ditylenchus dipsaci*) in narcissus since at least the 1920's (Staniland, 1933). The treatment first applied widely in the UK, 3 hours immersion at 43.3°C, was based on Ramsbottom's and Staniland's experiments and gave partial control of the nematode with little damage to the bulbs (Green, 1964). However, in the 1950's it became apparent that this standard treatment was not controlling stem nematode adequately (Winfield, 1970) and further work was done in the 1960's and 1970's into improving HWT practice in order to enhance control of the pest.

Raising the temperature of HWT was favoured, but damage to the bulbs, particularly the flower initials, often resulted. It was discovered that this 'HWT damage' could be prevented by storing the bulbs at elevated temperatures before HWT – typically 30°C for 7 days or 35°C for 5 days. However, this also resulted in some acclimatisation of the stem nematodes, (requiring the HWT temperature to be raised further), and encouraged the production of nematode 'wool', a resting stage resistant to heat and pesticides (Woodville & Morgan, 1961). Pre-soaking the bulbs before HWT, for a minimum of 3 hours, was found to improve the kill of nematodes after warm storage (Winfield, 1970), probably because this reactivated nematodes that were in the 'wool' stage.

The long-established HWT regime has therefore been gradually replaced by new regimes that come in a variety of forms, with and without pre-warming and pre-soaking. Adding to the confusion, different narcissus-growing countries have adopted different regimes, as illustrated in the table below (taken from various sources):

Authority	Temp °C	Duration (hours)	Comments
Australia	43.3	4	
	44.4	3	
EPPO*	45.0	4	Pre-warm @ 25-30°C for 10 days; pre-soak 24hrs @ ambient
	47.0	4	Pre-warm @ 25-30°C for 10 days.
India	45.0	4	
Netherlands	43.0	2	Standard regime – annual treatment
	46.0	4	Regime for suspected nematode-infested bulbs
New Zealand	43.0	4	
UK	44.4	3	

	44.4	3	Partial pre-warming: 18°C for 14 days before HWT
	46.0	3	Pre-warm @ 30°C for 7 days or 35°C for 5 days, pre-soak for 3 hours
US	43.8	4	Pre-soak for 2 hours @ 24°C

* EPPO = European Plant Protection Organisation

Few of the HWT regimes above are identical. The recommended temperatures range between 43.0 and 47.0°C, and the duration of treatments is either 2, 3 or 4 hours. There is no real consistency between the temperature and duration of the treatment. One would perhaps expect it to be possible to apply a higher temperature for a shorter time, but the regimes above do not conform to this. There is however often a requirement for pre-warming and pre-soak treatments where the higher temperatures (46.0 or 47.0°C) are used.

Tompsett (1975) confirmed that pre-warming of narcissus bulbs before HWT produced a reduction in all kinds of plant damage but necessitated both pre-soaking and a higher HWT temperature to maintain control of the nematode. He also considered that 'omitting formaldehyde from HWT significantly increased the eelworm attack, thereby reducing the yield of flowers and bulbs'. His recommendation was to warm-store bulbs for 7 days at 30°C or for 5 days at 35°C, followed by a pre-soak for at least 3 hours and then HWT at 46.1°C for 3 hours with formaldehyde added to the HWT tank. This became one of the UK's standard recommendations. However, Tompsett also mentioned in his paper that 'HWT at 47.8°C without pre-soaking appears satisfactory in experiments'. Whilst no duration of treatment at this temperature was given, nor any description of the degree of stem nematode control achieved or the level of plant damage sustained, there nevertheless seems to be some possibility that treatment at this very high temperature could succeed in giving satisfactory nematode control. There is a note of caution. In some early work in Ireland Hewitt (1914) immersed infested narcissus bulbs in water heated to 49°C for one, three or six hours, which killed all of the nematodes present but unfortunately also killed the bulbs. It is likely that Hewitt did not acclimatise his bulbs by pre-warming, however.

Green (1964), in his *in vitro* experiments on aqueous suspensions of *Ditylenchus dipsaci*, also looked at the effects of higher temperatures. He came to the conclusion that heat may have a different effect when applied above 47°C than when it is applied below 46°C. Below 46°C a linear relationship exists between duration of exposure and mortality, but above 47°C the rate of achieving 50% kill of nematodes increases suddenly and disproportionately. He proposed that this was accounted for by the existence of two thermal death mechanisms, one of which operated only above 47°C. If this is the case, then it is possible that utilising a HWT temperature in excess of 47°C may give enhanced control of the nematode. However, there is a risk that such a high temperature applied during HWT may be particularly

hazardous to the bulbs themselves. Acclimatisation to such temperatures by pre-warming the bulbs in store may be effective, but this would need testing.
Green also studied the effect of warm-storage after HWT on nematode survival. He concluded that storage at 25°C or 30°C not only increased the level of mortality of stem nematode but also increased the rate at which mortality occurred. For instance, he gave HWT (3 hours at 44°C) to stem nematodes *in vitro*, and found that when subsequently stored at 30°C mortality had reached 80% in 15 days, whereas for those stem nematodes that were stored at 15°C after treatment mortality was still less than 50% after 40 days. A period of warm storage for narcissus bulbs after HWT may therefore enhance the effects of the treatment on stem nematode. However, it must be borne in mind that warm storage of bulbs after treatment can provide ideal conditions for basal rot or *Rhizopus* soft rot to thrive. The high-risk storage temperatures for basal rot lie between 21°C and 30°C, so post-treatment storage temperatures must lie outside this range. Post-treatment bulbs must also be dried immediately, to prevent the development of *Rhizopus*.

In California, where the standard HWT regime was reported as 4 hours at 44.4°C, Qiu *et al*. (1993) showed that this treatment was sufficient to control stem nematode without adding formalin. 2.5 hours at this temperature was sufficient when formalin was added and caused no crop damage. With formalin, control was achieved by shorter times at higher temperatures, e.g. 45 minutes at 48°C or 30 minutes at 50°C, though all caused crop damage.

Standard practice in the UK is to carry out HWT once all the flower initials have been formed and before the root initials have developed too far. This means mid- to late-July in the south-west and late-July to early August in the east. However, whenever stocks are infested (or suspected of being infested) with stem nematode it is recommended that they are lifted early (June) and receive HWT promptly, avoiding warm storage that will lead to the production of nematode ‘wool’ (Hastings and Newton, 1934; Winfield and Hesling, 1966). In this case crop damage has to be accepted to save the stock. Hence there is scope for using early HWT as a way of improving nematode control, especially where the first-year flower crop is not important.

Conclusions

Currently-accepted HWT regimes are not likely to give complete control of stem nematode without the addition of formaldehyde or an equivalent disinfectant or pesticide to the HWT tank.
Treatment of bulbs at very high temperatures (47°C plus) may be possible, and may exploit a thermal death mechanism that temperatures below 46°C do not utilise.

Extending the duration of treatment from three hours to four is technically feasible and may maintain the level of nematode control without formalin being added.

The effects of HWT may be better regulated if the duration of the treatment is related to the grade of bulbs being treated.

Raising HWT temperatures and extending the duration of treatment is likely to have a damaging effect on treated bulbs. Acclimatisation by pre-warming and pre-soaking ought to minimise this but may not always be possible.

Routine pre-soaking may improve nematode control.

Post-treatment storage at temperatures between 30°C and 35°C may enhance the lethal effect of HWT on stem nematode without increasing the risk of disease.

Early lifting and early HWT (without any warm storage) could be used for better nematode control where the first-year flower crop is not important.

Economic effects of changing the HWT regime.

The current UK 'standard' HWT regime consists of 3 hours exposure to 44.4°C, with formaldehyde included in the HWT tank. If formaldehyde has to be omitted, as seems likely in the future, then a replacement regime may have to be more complex. Such a regime could consist of a period of pre-warming, perhaps a week, followed by a pre-soak for 3 hours, then HWT at say 47.5°C, for 4 hours, and finally (after surface-drying the bulbs) warm storage at 30°C or more for 14 days.

A grower who was to adopt the new regime would require the following:

A pre-warming facility. This would need to be capable of storing that proportion of the farm's production that could be given HWT within a period identical to the intended duration of the pre-warming period. So, for example, if it takes 4 weeks to give HWT to the entire production of a farm and the pre-warming duration is 7 days, then the pre-warming facility would need to be capable of storing a quarter of the farm's total production. If the pre-warming period were only 5 days then the facility would need to store a smaller proportion of the total production, in this case approximately one sixth. This has long been standard practice in the south-west and presumably could be used more widely.

A pre-soak facility. In previous experience a pre-soak period of 3 hours has been sufficient. If this were the case, then the pre-soak facility would need to have the same capacity as the HWT tanks in order not to introduce a delay into the procedure.

HWT tanks capable of sustaining the higher water temperature reliably for up to four hours. If the HWT period dictated by the new regime is 4 hours rather than the standard 3 hours of current regimes then this effectively reduces the capacity of the system to three-quarters of its previous level. This will result in a requirement to increase the HWT capacity, which could be done by beginning HWT earlier in the season, (not desirable because of enhanced

damage), or finishing it later, (inconvenient from the management point of view, but management may have to change; late HWT can be ameliorated by 2 weeks storage at 18°C, see above) or by increasing the number of HWT tanks.
A post-treatment warm-storage facility capable of drying the bulbs immediately after HWT and then storing them at 30°C minimum for 2 weeks. The capacity necessary would depend upon the throughput of the HWT tanks.

The capital investment necessary to achieve the above would on some farms be considerable.

Recommendations

Experimentally determine the effect of giving infested bulbs HWT @ 47.0°C, 47.5°C or 48.0°C for 3 hours or 4 hours, compared with 44.4°C for 3 and 4 hours, on the survival of stem nematodes and on bulb/flower damage.
Experimentally determine the influence of pre-warming on the survival rate of stem nematodes and on bulb/flower damage.
Experimentally determine the influence of pre-soaking on the survival rate of stem nematodes and on bulb/flower damage.
Experimentally determine the effects of post-treatment warm storage on the survival rate of stem nematodes and on bulb/flower damage.
Conduct field tests to determine how the modified treatments affect flower damage, the rate of foliar dieback and the levels of Fusarium basal rot.

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Section 3. Potential for auxiliary devices during HWT and alternative devices that might replace HWT

Auxiliary devices to HWT

Ozone generator (ozonation)

Ozone is a powerful oxidising agent and systems have been developed to treat water (e.g. drinking water, swimming pool water, recycled irrigation solution from hydroponic crops), bubbling the gas through an enclosed column. Excess ozone is absorbed to prevent escape to the atmosphere. Treatment with ozone was effective against *F. oxysporum* after 10 - 20 min (van Aachter, 1988; Runia, 1990) in small-scale tests, but was not fully effective on a semi-commercial scale; a high concentration is required to kill fungal spores. Although ozone treatment was initially a leading system in the Netherlands for treatment of recycled nutrient solution, it has been superseded by other systems. It has the disadvantages of a high capital cost (£13,000 - £20,000 for 2-5 m^3/hr), a relatively high running cost (production of ozone by UV lamp or electrical discharge) and the need constantly to monitor ozone in the treatment room (risk to human health).

UV light treatment

UV light in the range 200-280 nm can be used to kill microorganisms in water. The lethal dose for *Fusarium* conidia is relatively low (c. 100 mJ/cm^2), but that for chlamydospores is likely to be significantly greater. In the Netherlands, a low capacity (9-18 L/h) flat-film lamp with a high intensity (430-800 mJ/cm^2) eliminated *Fusarium* (Runia & Klomp, 1990), whereas a high capacity lamp (200-400 L/h) applying a moderate intensity (100-200 mJ/cm^2) resulted in <20% kill of *F. oxysporum*. An UV system with a low capacity (2m^3/h) has a relatively low capital cost and may be suitable for use with high quality water (i.e. with good light transmission). Solids and dissolved organic matter cause a rapid decline in efficacy. A filtration system is therefore likely to be essential if treating water from a HWT tank. The running cost of UV treatment is relatively high (electricity and UV lamps).

Flash heat treatment of HWT water

Systems are available to treat re-cycled irrigation water from hydroponic crops by a short-duration high temperature treatment. *Fusarium oxysporum* f. sp. *melongea* conidia and chlamydospores were killed by treatment at 90ºC for 4 minutes. The capital cost of a commercial system to treat 5m^3/h is around £20,000; running costs are relatively high (1-4 m^3 gas/m^3 of water). Water is diverted into a heat exchanger, where it is rapidly heated to 90ºC, through a second heat exchanger to 95ºC for 30 seconds, and then passed through the first heat exchanger again where heat is transferred to the incoming water. Heat treatment will control many organisms and it will be possible to define conditions that kill nematodes as well as fungal spores. It is less dependent on water quality than UV, ozone or chlorine treatment.

Chlorination

See under ‘Biocides; sodium hypochlorite’, above.

Filtration

Potentially, pathogenic organisms (fungal spores, nematodes) could be extracted from HWT water by running it through a filter system. However, the small size of the fungal spores would necessitate a fine filter and the large quantities of bulb debris and soil that contaminate the HWT water as soon as bulbs are introduced would make a filter system, whether a slow sand filter, a fast sand filter or a mechanical filter, difficult to operate effectively and economically.

Conclusions

Each of the auxiliary methods of treatment discussed above would require considerable capital investment if it were to be implemented on a commercial scale. In the absence of proven efficacy, with some potential technical difficulties to be overcome and with the existence of some other potentially viable alternatives it is difficult to justify pursuing the auxiliary methods at this juncture.

Alternatives to HWT – heat application

Heat treatment - microwave, radiowave

A system is under development for direct heating of soil using electromagnetic radiation (‘Agratron’), and prototype equipment has been designed to heat wet substrates (e.g. mushroom compost) using radiowave radiation. Both systems require a large electricity supply and are likely to be expensive. Potentially, microwaves and radiowaves could be used to heat bulbs and have the advantage that the bulbs would be treated in the ‘dry’ state, minimising the transfer of pathogenic organisms from one bulb to another. Reassurance would be required on the ability to heat bulbs uniformly and to control heating so as not to exceed a set temperature (which would cause damage to bulbs). The cost of heating via these methods relative to that of heating bulbs in water would also need establishing.

Heat treatment – hot air

Heating bulbs using hot air rather than hot water also has the theoretical advantage that the bulbs could be treated in the dry state, minimising transfer of pathogens. However, the controlled, consistent transfer of heat from hot air to each bulb in a bulk of narcissus bulbs so that each more-or-less simultaneously reaches the specified core temperature and remains at that temperature (± 0.5°) is likely to prove to be very difficult in practice.

Conclusions

The application of heat to control pests in living bulbs must be finely controlled, in order to kill the pests without causing phytotoxic effects. It seems unlikely that any of the alternative methods of applying heat considered above would prove technically adequate.

Alternatives to HWT – Field treatments

In theory, if narcissus bulbs free of stem nematode are planted into soil that is also free of the pest, and appropriate hygiene measures are taken for however long the crop is *in situ*, then there should be no problem with stem nematode in that crop. However, in practice it is very difficult to be completely confident that a field is free of stem nematode infestation and it is almost equally difficult to ensure that a bulb stock is completely free of the pest. The biology of stem nematode is such that it can reproduce very rapidly, so that even a very low initial infestation, in either the field or the bulbs, can result in significant damage in the timescale of a typical narcissus crop.

For field treatments to be effective in preventing damage to narcissus by stem nematode they would need to be able to kill all of the individuals present in the soil prior to planting and/or they would need to be able to kill nematodes within the bulb, or at least prevent them from travelling from an infested bulb to an uninfested one once the bulbs were in the ground.

The biology of stem nematode and the potential of different field treatments to control the pest are examined below.

Aspects of stem nematode biology

Stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, is an endoparasite of largely the above-ground parts of growing plants. It is within plant tissue that the nematode spends most of its life cycle and reproduces, but the nematode is also capable of persisting outside the plant, mainly as active stages in the soil but also, in very dry conditions, in a state of suspended animation awaiting reactivation by moisture.

Persistence in the soil can vary from a few weeks to several years. Soil type plays an important part in influencing this, with persistence being least in sandy soils and greatest in clays. The nematode also has a wide host range including many common weeds (Goodey *et al.*, 1965) and can 'tick over' in the cotyledons and young stems of non-host plants, so it will readily persist between host crops if weeds are allowed to proliferate. Crop rotation is not therefore a reliable way of ensuring that narcissus remains free of stem nematode. (This is complicated by the fact that the nematode exists in a number of morphologically-identical races, each of which has a different host range, which sometimes overlap between races).

In the dry, inanimate phase (sometimes called 'wool' from the appearance of the tangled mass of desiccated juveniles) the nematodes can remain viable for many years. This is less likely to occur under field conditions as it is likely that the desiccated nematodes would be re-wetted quite rapidly, but 'wool' on pieces of dry bulb scale in a dry environment, like the inside of a bulb handling facility, could easily remain viable between successive seasons, and probably a lot longer, and are capable of re-infecting a 'clean' crop.

The population of stem nematodes in the soil is never very high, unlike some more typical soil-dwelling species of nematode, and in the absence of a host crop the population rapidly becomes so low that it is difficult to detect. Whilst the soil sampling and processing techniques available are adequate for the detection of significant free-living plant-pathogenic nematodes in the soil they are not capable of detecting the very low levels of stem nematode that are still able to lead to crop damage. Risk assessment by soil sampling is therefore impractical for sensitive crops (Whitehead, 1998). The reproductive capability of stem nematode in host plant tissues is very high. At the optimum temperature of approximately 15°C the whole life cycle takes about 3 weeks, and since each female is capable of laying 500 eggs populations can increase very rapidly. Indeed, Hesling (1970) reported that stem nematode in narcissus increased 18,000-fold in narcissus bulbs in a single growing season. Undetectable low soil populations of stem nematode are therefore capable of causing serious economic damage in a crop like narcissus which may be left down for two years or even longer.

Stem nematode is not very mobile in the soil. Unassisted, infestation may spread at about 1m per year. However, individuals may be carried longer distances in free water streams on the soil surface, such as might occur during heavy rain or floods, and further dissemination may occur in clumps of soil attached to farm machinery, boots etc. Accidental cross-contamination of a field of narcissus is therefore a constant risk for bulb growers.

Potential of Field Treatments

Field treatments to control stem nematodes have not in the past been commonplace. Firstly, without a reliable method of detecting the pest in the soil any treatment would have to be made on a prophylactic basis, which is unsatisfactory on grounds of expense and environmental damage. Secondly, for many years all bulb material has been hot-water treated before replanting, which has been considered adequate to produce a good crop of flowers and bulbs even if it does not eradicate the pest, so there has been no incentive to carry out additional field treatments. There is therefore no history of the usage of field treatments to control stem nematode in narcissus, though attempts have been made to do this experimentally.

The options for field treatments include the use of steam, soil fumigation and the application of granular nematicides.

Steam. Steam has been used to partially sterilise field soils prior to growing crops in Britain for some years. However, such treatment is only a viable option financially on very high value crops such as salads, for the control of soil-borne pathogens, and its use for the control of stem nematode has not been studied. It is unlikely to be financially viable on narcissus even if it is technically adequate.

Soil fumigants. Soil fumigants are pesticides that act in the soil in the gaseous phase. Methyl bromide is no longer permitted for use as a soil fumigant in Britain, but 1,3 dichloropropene, metam-sodium and dazomet are all still available. 1,3 dichloropropene is itself the active ingredient but metam-sodium and dazomet both produce the active principle, methyl isothiocyanate, on breakdown in the soil. All three can produce very high levels of kill of nematodes. For instance, 500 l/ha of 1,3 dichloropropene produced 98.9% kill of stem nematodes buried 12.5 cm deep in an organic field in New York State, USA (Lewis & Mai, 1958) and dazomet at 880kg/ha decreased the number of stem nematodes in a field soil after cropping from 1200/l soil to 0/l soil (Whitehead and Tite, 1972). In practice, however, nothing less than 100% control would be required in order to prevent stem nematode infection of narcissus and it is unlikely that this could be achieved on a consistent basis.

Granular nematicides. There are six granular nematicides currently registered in Britain. These comprise the oxime carbamates aldicarb and oxamyl, the carbamates benfuracarb and carbosulfan and the organophosphates ethoprophos and fosthiazate. They are in commercial use for the control of pests such as potato cyst nematode on potato, free-living nematodes on sugar beet, carrot and parsnip, and stem nematode on onion. They are normally applied to the soil at drilling/planting and may protect the host crop from attack for up to 12 weeks, depending on temperature, soil moisture etc. Though this is an adequate performance as far as controlling the pests on the product labels is concerned, it is unlikely that any of these products would persist for long enough to prevent infection of a narcissus crop, which may remain in the same field for 2 or more years.

Conclusions

The persistence of stem nematode in the absence of a host crop, its reproductive capability, the difficulty of detecting the low levels of nematode population that can result in severe crop damage and the properties of the available field treatments conspire to make it very unlikely that field treatment, rather than hot-water treatment of dormant bulbs, would give acceptable control of stem nematode in narcissus.

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Section 4. A plant breeding solution?

Standard texts (e.g. Bergman *et al*., 1978; Lane, 1984; MLNV/CADB, 1990; Byther and Chastagner, 1993; Hanks, 1993; Le Nard and De Hertogh, 1993a) do not mention any differential sensitivity (i.e. resistance (or tolerance) and susceptibility) of narcissus cultivars or species to attack by the narcissus race of *D. dipsaci*. Further, a search of the world scientific literature (concluded September 2006) revealed no reports of differential sensitivity between narcissus cultivars or species to the pest. Therefore it should be assumed that all narcissus might be attacked, so at present there are no prospects for breeding a solution through the production of stem nematode-resistant new cultivars. However, the existence of many host-specific races of *D. dipsaci*, and the differential susceptibility of various hosts to different *D. dipsaci* races (e.g. narcissus are attacked vigorously by the narcissus and tulip races but only weakly by the hyacinth race), might imply there is potential for genetic manipulation. Besides narcissus, a breeding approach to the management of pest nematodes does not appear to have been considered in the case of other major bulbous ornamentals (De Hertogh and Le Nard, 1993; Le Nard and De Hertogh, 1993b).

The preceding comments relate to commercial narcissus growing, and do not mean that there is no experience-derived or anecdotal information on the issue deriving from the considerable body of narcissus enthusiasts. In a review targeted at this audience, Beaumont (1950) listed numerous examples of resistance/susceptibility in different narcissus groups and cultivars, but the bulk of his comments refer to fungal and viral diseases and large narcissus fly. However, it is stated that the various groupings within the yellow trumpet cultivars are about equally susceptible to the different insect [*sic*] pests (his list includes *D. dipsaci*), and that *Poeticus* cultivars behave differently to other divisions, with "large areas of bulbs [rotting] away with eelworm (stem nematode] in the field almost before the pest has been diagnosed in the foliage." Some specialist monographs consulted (e.g. Wells, 1989) do not appear to give any useful examples of different reactions to *D. dipsaci*.

The bulk of narcissus breeding is carried out, using conventional plant breeding methods, by specialist growers and enthusiasts. These breeders have the show-bench in mind, so the emphasis has been on achieving perfect form and colour and novel colour combinations and flower forms. Cultivars with commercial potential (e.g. having good flowering season, vigour and shape/colour attributes) may be spotted in shows, purchased and bulked up for commercial exploitation. Some commercially-orientated narcissus breeding programmes have been carried out, however, notably those at Rosewarne (especially for early-flowering, outdoor, large yellow trumpet and long-cup cultivars; Tompsett, 1984) and at Littlehampton/Wellesbourne (for base-rot resistant 'Carlton'- or 'Golden Harvest'-like types;

Bowes *et al*., 1996). These programmes have almost exclusively used conventional breeding methods, although mutation techniques were used in some cases at Littlehampton and in a few other cases with no spectacular outcomes (Misra, 1990; Rahi *et al*., 1998).

At Wellesbourne a genetic transformation system for narcissus has been developed (Sage, 2001, 2002). In other Defra-funded research, Robinson *et al*. (2006) are carrying out a project to establish the technologies necessary for future varietal improvements in commercial narcissus crops. This involves developing rapid clonal propagation using liquid culture in 'Bioreactors', establishing molecular marker and genetic fingerprinting protocols to aid the identification of marker genes for base rot resistance, and securing a core collection of narcissus diversity for future breeding. Resistance to basal rot is the widely agreed top priority for future commercial narcissus breeding research, with resistance to potyviruses (or their aphid vectors) also considered important.

Conclusions

Given the lack of information on the interaction between *D. dipsaci* and narcissus genotypes, it would seem that a plant breeding solution for stem nematode is many years away at the present time. The current management techniques for stem nematode, probably based round an upgraded HWT protocol, will be needed for probably 10-15 years at least. Using genetic transformation, excellent receptor cultivars are available to host pest- and disease-resistant genes and so provide the main types required by the industry, such as 'Carlton' (large-cup type), 'Golden Harvest' (trumpet type) and 'Tête-à-Tête' (miniature).

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