Project title:	Narcissus: Alternatives to the use of formaldehyde in HWT tanks for the control of stem nematode and *Fusarium* basal rot.
Project number:	BOF 61a
Project leader:	Mike Lole, ADAS.
Report:	Final Report, December 2010
Previous reports:	Third annual report, December 2009 Second annual report, December 2008 First annual report, December 2007
Key staff:	Mike Lole, ADAS Rosemaund Dr Erika Wedgwood, ADAS Boxworth Gordon Hanks, Warwick HRI
Location of project:	ADAS Arthur Rickwood; Warwick HRI, KRC & Wellesbourne
Industry Representative:	Mr Mark Clark, Grampian Growers
Date project commenced:	1st January 2007
Date project completed:	31st December 2010

The results and conclusions in this report are based on an investigation conducted over a four-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.

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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CONTENTS

GROWER SUMMARY

Headline

Fam 30 (an iodophore biocide) and Bravo 500 (chlorothalonil) each showed activity against spores of the basal rot fungus *Fusarium oxysporum* f.sp. *narcissi* when used as additives during the hot-water treatment (HWT) of narcissus.

Phytotoxic effects were minimal, but refinement of dose rates and top-up regimes is necessary before either material can be used with confidence in HWT.

Background and expected deliverables

Since at least the 1930's formaldehyde has been added to the contents of tanks used for the hot-water treatment (HWT) of narcissus bulbs, in order to speed up the kill of stem nematodes (*Ditylenchus dipsaci*) that entered the tank as 'wool' and to minimise the cross-contamination of bulbs with the spores of *Fusarium* basal rot (*Fusarium oxysporum* f.sp. *narcissi*). However, this use of formaldehyde was not supported through the EC review of pesticides by any manufacturer and as a result it has not remained legal to use formaldehyde in HWT beyond the end of 2008.

The loss of formaldehyde has prompted a search for an alternative method of dealing with stem nematodes and *Fusarium* basal rot during HWT. A preliminary review (BOF 61) showed that several biocides were suitable candidate replacements for formaldehyde, and the fungicide Bravo 500 also appeared to be suitable for use in HWT to control basal rot spores. Changes to the standard HWT regime (increased temperature, increased duration of exposure) also appeared to offer some promise.

The purpose of the work described in this report was to test the candidate alternative treatments to the use of formaldehyde in HWT so that the best treatment could be adopted by the bulbs industry when it was no longer permissible to use formaldehyde in HWT.

The preliminary part of the experimental work consisted of investigating the relative efficacy of the candidate alternatives, compared to the use of formaldehyde, by conducting tests *in vitro*. The most promising alternatives were then selected for further work utilising large-scale HWT and field trials.

The effects of a range of alternative biocides and HWT regimes on the 'wool' form of stem nematode, *Ditylenchus dipsaci*, and the chlamydospore stage of the basal rot organism, *Fusarium oxysporum* f.sp. *narcissi* were reported in the first annual report on this project. Before large-scale trials with the most promising candidates were undertaken the potential for the treatments to cause phytotoxic effects on treated bulbs was investigated. The results of this work were recorded in the second annual report. The third annual report contained details of the field trials that were set up in August 2008, and this report contains all of the above, plus the final results of the field trials.

Summary of the project and main conclusions

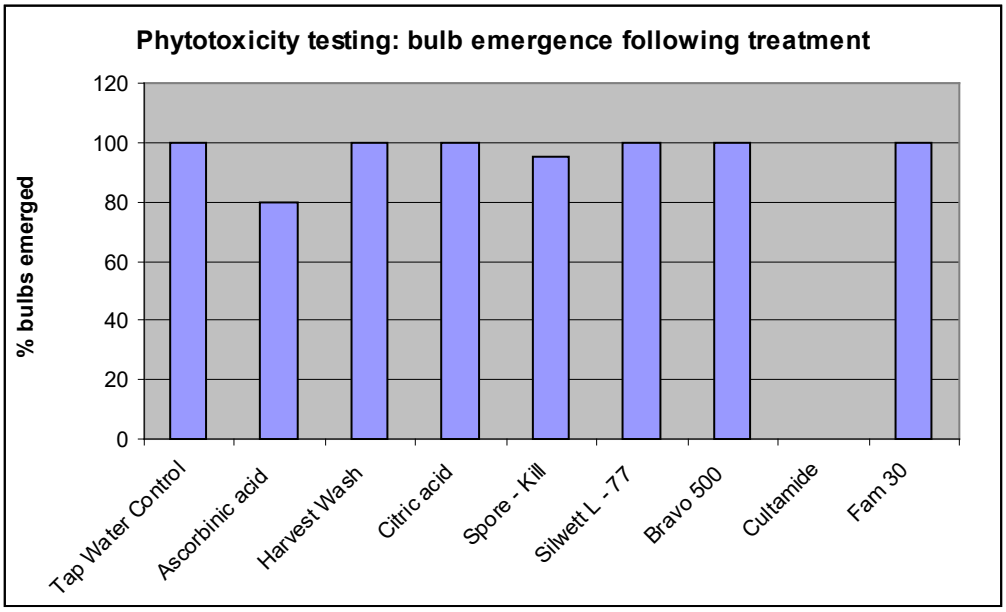
<u>Laboratory tests</u>

The first part of this project consisted of *in vitro* tests that compared the efficacy of a range of different chemicals and HWT regimes in controlling both stem nematode in the 'wool' form and the spores of basal rot. These chemicals/regimes had previously been shown to have potential either as direct replacements for formaldehyde in HWT, or as more efficient methods of HWT, in a previous project on this subject (BOF 61).

The candidate products were: ascorbinic acid, Harvest Wash (chlorine dioxide), citric acid, Spore-kill (natural product), Silwett L-77 (silicone wetter), Bravo 500 (chlorothalonil), Cultamide (hydrogen cyanamide) and FAM 30 (iodophor disinfectant). The most effective of these were FAM 30 (which killed both stem nematodes and basal rot spores) and Bravo 500 (which killed basal rot spores only). The HWT regimes tested included a range of temperatures between 44.4°C and 48°C, and durations of exposure of either 3 or 4 hours. Although these amended HWT regimes proved effective in controlling stem nematode, none gave any control of *Fusarium* chlamydospores, so no further work on the modification of HWT regimes was done.

Phytotoxicity tests

Phytotoxicity tests on the same candidate products and regimes were done. Bulbs were treated in autumn 2007 and then planted. Assessment of emerged growth in spring 2008 showed that FAM 30 and Bravo 500 did not produce any phytotoxic effects on treated bulbs sufficient to preclude their use in hot-water treatment. In contrast, when Cultamide (hydrogen cyanamide) was included in HWT no bulbs emerged the following spring. Cultamide had otherwise seemed to be a promising additive for use during HWT as it controlled both stem nematode and *Fusarium* chlamydospores. When compared to the effects of standard HWT (3 hours at 44.4°C), none of the alternative temperature/duration regimes appeared to produce severe phytotoxic effects.



Field growing-on tests

Treatments identified as effective in controlling stem nematode and/or *Fusarium* basal rot, and which did not appear to have severe phytotoxic effects, were tested on a larger scale. The treatments tested were:

- Bravo 500 at 1 litre of product /1000 litres of dip in HWT for 3 hours at 44.4°C
- Bravo 500 at 0.5 litres of product/1000 litres of dip in HWT for 3 hours at 44.4°C
- FAM 30 at 8 litres/1000 litres of dip in HWT for 3 hours at 44.4°C
- FAM 30 at 4 litres/1000 litres of dip in HWT for 3 hours at 44.4°C
- Standard commercial formaldehyde treatment at 5 litres formalin /1000 litres of dip in HWT for 3 hours at 44.4°C
- Control, HWT for 3 hours at 44.4°C, no additives.

The higher rates chosen of each product were the maximum rates recommended by the manufacturer for the nearest equivalent use.

Bulbs were treated and planted in autumn 2008 and assessed at emergence in spring 2009 and 2010. In spring 2009, bulbs given HWT without any disinfectant additive (i.e. without formaldehyde or any of the candidate replacement treatments) did not emerge as well as treated bulbs, which mostly produced good growth with acceptable flower numbers and quality. There were some minor phytotoxic treatment effects with the high rate of Bravo 500, but not with any of the other treatments. It is thought likely that the affected bulbs would have recovered by the second year after treatment. However, this cannot be confirmed because *Fusarium* basal rot virtually destroyed the whole of the trial prior to emergence in spring 2010, a result of having to select infected, sensitive varieties for the purpose of the trial.

Despite the disappointing loss of the field trial in its second year, this project has identified two materials that could function as replacements for formaldehyde in HWT to help prevent the spread of *Fusarium* basal rot between bulbs in the treatment tank. However, work elsewhere in the meantime has highlighted the need for further evaluation of the optimum rates of inclusion of Bravo 500 or FAM 30 in HWT tanks, together with refinement of top-up regimes, before growers can ensure that they are using these materials in the most efficient and cost-effective way. HDC-funded projects BOF 61b, 61c, 70, 71 and 71a have been commissioned to provide this supplementary information.

Financial benefits

Financial benefits to growers resulting from this work are very difficult to quantify. Using an additive in the HWT tank in order to minimise disease transmission during the process has a cost – FAM 30 at 8 litres/1000 litres of dip solution costs approximately £43.00 for each 1000 litres treated and Bravo 500 at 1 litre/1000 litres of dip solution costs approximately £5.80 for each 1000 litres treated. However, giving HWT to an infected bulb crop without any additive could result in the cross-infection of much of the crop with basal rot or stem nematodes, with the consequent risk of loss, and not giving HWT at all risks serious losses due to stem nematode attack if the stock is infested.

Action points for growers

- Treatment of an infected stock within the HWT tank is essential to prevent disease and nematodes cross-contaminating healthy bulbs (the cost of this is far outweighed by the potential losses that are likely to occur if treatment is omitted).
- To minimise the spread of *Fusarium* within the HWT tank, FAM 30* or Bravo 500* are the most suitable products, with a low risk of phytotoxicity to bulbs post planting.
- To minimise the spread of stem nematode within an infested stock FAM 30 is the most suitable product.

(* A SOLA for the use of Bravo 500 (MAPP number 14548) as a dip to control basal rot in narcissus is currently available, SOLA number 0943/2011, expiry date 3 March 2015. The use of FAM 30 is permissible within current regulations).

SCIENCE SECTION

Introduction

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In this report each set of data has been reported in chronological order.

Year 1 - in vitro testing

Materials and Methods

Test organisms

Taking into account the purpose of the inclusion of formaldehyde in HWT tanks, it was necessary to assess the effect of the candidate replacement treatments on the appropriate stages of the target organisms. These are the 'wool' stage of stem nematode, *Ditylenchus dipsaci*, and the chlamydospore stage of the basal rot organism, *Fusarium oxysporum* f.sp. *narcissi*.

Stem nematode 'wool' consists of a tangled mass of dehydrated final-stage juveniles. 'Wool' is formed when conditions for the nematodes become unsuitable because of a combination of overcrowding and deterioration/desiccation of the host plant tissue in which they have developed. The nematodes leave the host bulb and form a dry mass externally, often close to the basal plate. Clumps of 'wool' so formed can remain viable, if kept in dry ambient conditions, for many years and will then revive if re-wetted. For the purposes of the experimental work, however, it was necessary to use 'wool' that was similar to that which might be found in a commercial bulb stock, that would have been formed in the time between harvesting this infested crop and subjecting it to HWT, a matter of weeks. 'Wool' for use in these tests was therefore produced by collecting infested bulb material from widely-separated sources (in Cornwall, Lincolnshire and Bedfordshire) and allowing it to air-dry in nets in an unheated building before picking off any nematode masses that had formed on the surface of dry bulb scale material. This 'wool' was kept in Petri dishes in a refrigerator at 5°C until it was required for experimental purposes.

Fusarium oxysporum chlamydospores were obtained by firstly culturing material from infected bulbs onto plates containing modified Nash medium (Nash & Snyder, 1962). Colonies were then transferred to potato dextrose agar (PDA) and grown on for 10 days. It was found that these cultures naturally produced chlamydospores within the medium. The plates were scraped to collect the chlamydospores and the 'mycelial slurry' was added to dry talc to form a 'dry paste'. This was then air-dried for 3 weeks before use under sterile conditions. When required for experimental purposes a stock suspension of the fungus was made up in sterile distilled water immediately prior to use.

Treatments

a) Alternative chemicals.
Eight different biocide/fungicide products were tested for their efficacy in controlling stem nematode 'wool' and basal rot chlamydospores *in vitro*. These are listed in Table 1.

Tests on 'wool' were done in square plastic Petri dishes ('multicells') comprising 25 compartments, each measuring 20 mm x 20 mm x 17 mm deep. Five replications of each test were done, so that each complete set of replicates occupied 1 row of 5 compartments in a multicell and 2 complete multicells were required to house the 8 candidate treatments plus 2 controls.

Table 1. List of biocide/fungicide treatments used in *in vitro* tests – 2007

Code	Active ingredient	Product	Required rate of active	Necessary rate of product
T1	N/a	Tap water control	N/a	N/a
T2	Ascorbinic acid	99% l-ascorbinic acid	0.006%	0.06 g / litre water
T3	Chlorine dioxide	Harvest Wash	50 ppm	0.25ml / 100 ml water + 0.025g activator
T4	Citric acid	Commodity product (100%)	1%	1g / 100 ml water
T5	'Natural product'	Spore-kill	0.75%	0.75ml / 100 ml water
T6	Silicone wetter	Silwett L-77	0.08% /0.02%	0.1ml / 100 ml water
T7	Chlorothalonil	Bravo 500 (500 g/l)	0.05%	0.1ml / 100 ml water
T8	Hydrogen cyanamide	Cultamide	6%	24 ml / 76 ml water
T9	Iodophor disinfectant	FAM 30	0.8%	0.8ml / 100 ml water
T10	N/a	Tap water control	N/a	N/a

Before testing began, small fragments of 'wool' were excised from larger pieces and were soaked in tapwater overnight to check for viability. Once the excised fragment was shown to be viable the parent pieces of 'wool' were divided into fragments comprising approximately 50-100 nematodes, for eventual use in the experiment. Stock solutions of the candidate materials were made up at the dilutions indicated in Table 1. Two ml of the first solution was added to each of the first 5 compartments of a multicell, providing 5 replicates of this treatment. This was repeated for each of the candidate materials and for both of the control treatments, providing 5 replicates of each treatment and occupying 2 multicells in total. Treatments were arranged so that there was one set of control replicates in each multicell. The loaded multicells were then placed in a water bath at 44.4°C so that they were partially submerged and were allowed to reach equilibrium with the temperature of the bath. A single fragment of 'wool' was then put into each compartment of both multicells, which were then maintained at 44.4°C for 3 hours. After this time the multicells were removed from the water bath and the nematodes in the compartments were directly observed for signs of activity. The contents of each compartment were then separately transferred to a boiling tube, 1 tube per replicate. The boiling tubes were each filled with tap water at ambient temperature (approx 40 ml) to dilute the test solution. After 1 hour, to allow the nematodes to settle, most of the supernatant liquid was removed and the tubes were refilled with tap water to further dilute the residue. The nematodes were then left in the boiling tubes overnight to allow time for the revival of active nematodes from the 'wool'. The following day the supernatant liquid was removed from the boiling tubes and 3 ml of the liquid, taken from the bottom of the boiling tube and therefore containing the nematodes, was transferred to a clean compartment in a new multicell, where the nematodes could be examined for signs of activity.

This test confirmed that all of the nematodes in all of the treatments had failed to survive. The experiments with HWT showed that this was likely to have been largely due to the effects of the heat treatment used and the result therefore showed no indication of which were likely to be the most effective chemical treatments. The experiment was therefore repeated, almost

identically except that the water bath was kept at ambient temperature (approx 18°C) rather than 44.4°C.

For the tests on *Fusarium oxysporum* chlamydospores, a stock suspension was made up by mixing some of the 'dry paste' containing spores (see above) with sterile distilled water. All work with open tubes of spore suspension was carried out under sterile conditions. Solutions of the test chemicals were also made up, at double the concentrations required for the tests. For each test chemical, 5 ml of the double-strength solution was put into each of 5 lidded, sterile, plastic universal tubes of 20 ml capacity. Five ml of sterile tap water (STW) were also added to each of a further 5 tubes, to act as untreated controls. This gave a total of 45 tubes, which were placed in racks in a water bath at 44.4°C until the temperatures had equalised. The temperature within the boiling tubes was measured by installing a thermocouple sensor in STW in an extra tube that was placed in the water bath alongside the other tubes, and taking readings from this thermocouple. When the temperature within the tubes had equalised with that of the water bath, a 5ml aliquot of the stock *Fusarium* spore suspension was added to each boiling tube, diluting the chemical already in the tube to the required concentration. When the temperature in each tube had returned to 44.4°C timing was started. The spores were exposed to the chemicals at 44.4°C for 3 hours, after which the tubes were removed to an ambient water bath to cool. The tubes were then centrifuged for 3 minutes to concentrate the spores in the bottom of the tubes. The supernatant was removed, the tubes were refilled with STW and they were agitated. They were centrifuged for a second time, the supernatant was again removed and the tubes re-filled with STW for the second time. This centrifugation and re-suspension was done in order to remove most of the chemical from the spores. The supernatant was removed for a final time to leave 3 ml containing the 'plug' of spores and talc. Two ml of 0.1% w/v cold tapwater agar was then added to each tube and agitated. One ml of this dilute agar suspension was then taken from each tube and was plated-out over the surface of Nash medium in a 9cm round Petri dish. One dish was used for each replicate of each chemical, giving a total of 45 dishes. These dishes were incubated at 20°C for 5 days, or until growth was visible, whichever was the sooner. *Fusarium* mycelial coverage of the plates was recorded.

b) Alternative temperature regimes

The susceptibility of both the nematodes and the fungal spores to a range of different temperature treatment regimes was tested in a series of laboratory experiments. It was originally intended to use the same range of regimes on both the nematodes and the fungal spores, but the first results indicated that there were grounds for modifying the range of tests to be used on each. The range of temperature regimes tested is listed in Table 2 below.

The effects of the temperature treatment regimes on stem nematode 'wool' were tested using boiling tubes and a water bath capable of maintaining the chosen temperature +/- 0.1°C. Ten ml of tap water was put into each of 5 boiling tubes, capacity 40 ml, for each treatment to be tested. The boiling tubes were placed, part submerged, in a rack in the water bath and the temperatures of the contents were allowed to acclimatise with that of the bath. Temperatures of the contents of the boiling tubes were measured using a thermocouple immersed in the contents of a separate, but identical, 40 ml boiling tube that was held in the water bath for that purpose. When the temperatures in the boiling tubes had stabilised at the chosen temperature a fragment of nematode 'wool' that had previously been confirmed to contain viable individuals was dropped into each tube. The tubes were maintained in the water bath until the required exposure time had elapsed. Simultaneously, control treatments were run using 'wool' in boiling tubes containing 5 ml of water in a second water bath at ambient temperature (nominally 18°C). When the heat treatment was complete the boiling tubes in the 44.4°C water bath were transferred to the 18°C water bath and allowed to cool. When cooling was complete the bottom 3 ml of the contents of each boiling tube were extracted using a pipette and transferred to a single cell in a multicell Petri dish. The control tubes were similarly treated. Multicells provide a convenient means of containing and examining a

number of nematode samples simultaneously. The contents of the multicells were observed *in situ* using a low-power binocular microscope. Nematodes were considered to be alive if they showed signs of movement 12-96 hours after treatment. Doubtful specimens were manipulated with a hair mounted on a dissecting needle until their status could be clarified.

Table 2. The temperature treatment regimes used in tests on stem nematode 'wool' and *Fusarium* basal rot chlamydospores

Code	Pre-warming temperature	HWT Regime	Used on:
H1	Ambient	3hrs 18°C	Nematodes + fungi
H2	Ambient	3hrs 44.4°C	Nematodes + fungi
H3	Ambient	4hrs 44.4°C	Nematodes + fungi
H4	30°C	3hrs 18°C	Nematodes + fungi
H5	30°C	3hrs 46°C	Nematodes + fungi
H6	30°C	3hrs 47°C	Fungi only
H7	30°C	3hrs 47.5°C	Fungi only
H8	30°C	3hrs 48°C	Fungi only
H9	Ambient	2hrs 44.4°C + 1hr 47°C	Fungi only
H10	Ambient	1hr 18°C	Nematodes only
H11	Ambient	1hr 44.4°C	Nematodes only
H12	Ambient	2hrs 18°C	Nematodes only
H13	Ambient	2hrs 44.4°C	Nematodes only
H14	Ambient	4hrs 18°C	Nematodes only

The effects of the temperature regimes on the *Fusarium* chlamydospores were also tested using the system of tubes and a water bath, but using sterile plastic universal tubes with lids. For those treatments carried out at 44°C a stock suspension of spores was produced immediately prior to use, from spore-containing talc and sterile distilled water maintained at 30°C for 7 days before use ('pre-warmed'). For each treatment, 5 tubes were allocated and were filled with 5 ml of STW. These tubes were placed in a water bath set to the chosen temperature and were allowed to acclimatise. Temperatures of the contents of the universal tubes were measured using a thermocouple immersed in the contents of a separate, but identical, universal tube that was held in the water bath for that purpose. Simultaneously, 5 more tubes were filled with 5 ml of STW and were placed in the second water bath, at ambient temperature, to act as controls. When the temperature in the tubes had reached the chosen temperature 5 ml of a stock chlamydospore suspension was transferred to each. At the same time, 5 ml of stock suspension was transferred to each of the control tubes. The chlamydospores were exposed to the chosen temperature for the required time, when the tubes from the heated water bath were transferred to the ambient water bath and allowed to cool. Both sets of tubes were then centrifuged for 3 minutes, and the supernatant was removed to leave 3 ml containing the spores and talc. Two ml of 0.1% w/v water agar was added to each tube and agitated. One ml of this dilute agar suspension was then taken from each tube in turn and plated-out over the surface of Nash medium in a 9 cm round Petri dish. This was repeated for all tubes, each time using a different Petri dish for the culture. The dishes were then incubated for 5 days at 20°C and any *Fusarium* mycelial growth was recorded.

Results and discussion

The results of the testing of the effects of the various chemical treatments on stem nematode 'wool' are given in Table 3.

Table 3. The effects on survival of stem nematodes of the inclusion of chemical treatments in immersion water at two different temperatures (3 hour exposures) - 2007

Treatment	Mean % nematode survival

Code	Active	Product	At 44.4°C	At 18°C
T1	N/a	Tap water	0	43
T2	Ascorbinic acid	l-ascorbinic acid	0	49
T3	Chlorine dioxide	Harvest Wash	0	27
T4	Citric acid	Citric acid	0	23
T5	'Natural product'	Spore-kill	0	23
T6	Silicone wetter	Silwett L-77	0	21
T7	Chlorothalonil	Bravo 500	0	15
T8	Hydrogen cyanamide	Cultamide	0	0
T9	Iodophor disinfectant	FAM 30	0	0
T10	N/a	Tap water	0	26

The initial experiment was done using an immersion-water temperature of 44.4°C. No surviving nematodes were detected 24 hours after the treatments were completed, including the untreated controls. It was probable, therefore, that the lethal effects of the treatments were due to the elevated temperature that was used (in order to replicate the standard HWT temperature) rather than to the chemicals. The experiment was therefore repeated using the lower immersion temperature of 18°C in order that the potential lethal effects of the chemicals would not be masked by the lethal effect of temperature. In this second experiment two chemicals gave outstanding control of the nematodes. These were Cultamide and the FAM 30, both of which appeared to cause 100% mortality in the nematodes after 3 hours exposure at the rates employed. This result with the FAM 30 is in accord with previous tests done in 1990 (Lole, 1990) and, for the HDC, in 2002 (BOF 49).

The results of the testing of the effects of the chemicals on the viability of *Fusarium oxysporum* f.sp*. narcissi* chlamydospores are given in Table 4.

The germination and growth of viable *Fusarium* chlamydospores on the plates in the incubator was generally rapid, so that the majority of plates were completely covered by mycelial growth within 2 days of plating out. Growth on the plates seeded with spores from the ascorbinic acid, chlorine dioxide, citric acid and silicone wetter treatments was indistinguishable from the growth seen on the plates that were seeded with spores from the water treatments, so it must be concluded that these treatments had little effect. Growth on the plates seeded with spores that had received the Spore-kill natural product treatment had only reached 3% cover after 2 days, so this treatment either suppressed the germination of the spores or killed a large proportion of them outright – it is not possible to say which. After 6 days the growth on the 'Spore-kill plates' was similar to that on the 'water-treated plates', covering 100% of the plate surface. There was, however, no growth at all on any of the plates seeded with spores that had been immersed in solutions of Bravo 500, Cultamide or FAM 30, even after 8 days incubation. It may be concluded, therefore, that each of these products caused 100% mortality of *Fusarium* chlamydospores when they were included in solutions in which the spores were suspended.

Table 4. The effects of the inclusion of chemicals in immersion water (3 hours exposure, 44.4°C) on survival and development of *Fusarium* chlamydospores

Treatment			Mean % cover by *Fusarium* after:		
Code	Active	Product	2 days	6 days	8 days
T1	N/a Tap water	Sterile tap water	100	100	100
T2	Ascorbinic acid	l-ascorbinic acid	100	100	100
T3	Chlorine dioxide	Harvest Wash	100	100	100
T4	Citric acid	Citric acid	100	100	100
T5	'Natural product'	Spore-kill	3	100	100
T6	Silicone wetter	Silwett L-77	100	100	100
T7	Chlorothalonil	Bravo 500	0	0	0
T8	Hydrogen cyanamide	Cultamide	0	0	0
T9	Iodophor disinfectant	FAM 30	0	0	0
T10	N/a Tap water	Sterile tap water	100	100	100
T11	N/a Tap water	Sterile tap water	100	100	100

It is notable that the Cultamide and the FAM 30 both apparently gave 100% mortality of both stem nematodes AND *Fusarium* chlamydospores.

The results of the experiments assessing the effects of different HWT regimes on nematode survival are given in Table 5. It should be noted that these experiments were not all completed at the same time – some experiments were repeated, wholly or partially, in order to confirm some of the results obtained in the original experiment.

Table 5. Survival of stem nematodes after hot-water treatment of 'wool' fragments

Treatment				Nematode score, days after treatment (DAT)		
Code	Temp. °C	Duration (hours)	Date applied	1 DAT	4 DAT	21 DAT
H10	18	1	30/10	XX		XX
H10	18	1	13/12	XXX	XXX	
H12	18	2	30/10	XXX		XX
H12	18	2	13/12	XXX	XXX	
H1	18	3	25/10	XXX		
H4	18	3	30/10	XXX		XX
H1	18	3	13/12	XXX	XXX	
H14	18	4	13/12	XXX	XXX	
H11	44.4	1	30/10	0		XX
H11	44.4	1	13/12	0	X	
H13	44.4	2	30/10	0		XX
H13	44.4	2	13/12	0	X	
H2	44.4	3	25/10	0		
H2	44.4	3	30/10	0		0
H2	44.4	3	13/12	0	0	
H3	44.4	4	13/12	0	0	
H5	46	3	30/10	0		0

Key to nematodes score:
0 = no live nematodes detected
X = 1 live nematode detected per replicate (mean)
XX = 2 -9 live nematodes detected per replicate (mean)
XXX = 10+ live nematodes detected per replicate (mean)

When stem nematodes that have been treated in the 'wool' form are examined immediately after treatment it is very easy to get a false impression of their viability, as they often appear immobile and apparently lifeless. In fact, nematodes that are gradually resuscitating from the 'wool' stage may take several hours to become rehydrated to the point where they can make voluntary movements. It is also the case that nematodes that have been subjected to a sub-lethal heat shock take on the appearance of nematodes that have been killed – such nematodes lie very straight and stiff compared to viable nematodes. However, rehydrating nematodes can 'come round' after quite long intervals, and nematodes apparently dead after heat treatment may also recover after several hours or days. It is necessary therefore to examine specimens at least 24 hours after treatment and, on at least one occasion, more than 2 days after treatment. Table 5 contains the results of such examinations. The results show that 'wool' immersed in water at 18°C had revived to a considerable extent 24 hours later, whereas that immersed in water at 44.4°C showed no sign of activity after 24 hours had elapsed. However, when the latter was examined after longer recovery periods (4 days or 21 days), a few individuals in the batches exposed for either one or two hours had recovered and were active. No survival was noted, however, in nematodes exposed as 'wool' to 44.4°C for three hours, no matter how long after treatment observations were made.

Table 6 summarises the results of the experiments that assessed the effects of different hot-water treatment regimes on the survival and growth of *Fusarium oxysporum* f.sp*. narcissi* chlamydospores. The results of these tests were fairly clear-cut. None of the treatments applied, not even the most extreme, that is exposure to 48°C for 3 hours, appeared to have any detrimental effect on the viability of the spores.

Table 6. The effects of different hot-water treatment regimes on the germination and growth of chlamydospores of *Fusarium oxysporum* f.sp. *narcissi*

Treatment				*Fusarium* growth	
Code	Pre-warming temp (°C)	Treatment temp (°C)	Duration (hours)	No. of plates	Mean % plate coverage
H1	18	18	3	5	100
H2	18	44.4	3	5	100
H3	18	44.4	4	5	100
H4	30	18	3	10	100
H5	30	46	3	5	100
H6	30	47	3	5	100
H7	30	47.5	3	5	100
H8	30	48	3	5	100
H9	18	44.4 then 47	2 then 1	5	100
H4 extra	30	18	3	2	100

Conclusions

- Hot-water treatment applied at a temperature of 44.4°C for 3 hours is sufficient to give 100% kill of stem nematodes that are exposed in the 'wool' stage.
- Some nematodes will survive exposure to 44.4°C for 1 or 2 hours.
- Hot-water treatment, using exposures to temperatures up to 48°C for 3 hours, or 44.4°C for 4 hours, does not give satisfactory control of *Fusarium oxysporum* f.sp. *narcissi* chlamydospores.
- Cultamide and FAM 30 at the rates used will give 100% control of stem nematodes when nematode 'wool' is exposed to solutions of either for 3 hours at 18°C.
- Bravo 500, Cultamide and FAM 30 at the rates used will each give 100% control of *Fusarium oxysporum* chlamydospores when these are exposed to solutions for 3 hours at 44°C.

Years 1-2 – Phytotoxicity testing

Materials and Methods

The effects of a range of alternative biocides and HWT regimes on the 'wool' form of stem nematode, *Ditylenchus dipsaci*, and the chlamydospore stage of the basal rot organism, *Fusarium oxysporum* f.sp. *narcissi* were reported in the first annual report on this project. Before large-scale trials with the most promising candidates were undertaken the potential for the treatments to cause phytotoxic effects on treated bulbs was investigated.

Methodology

The effects of biocides and HWT regimes on the survival and growth of narcissus were tested in a small-scale experiment at ADAS Arthur Rickwood (Cambs.) between September 2007 and March 2008. There were eighteen treatments (Table 7). Standard, commercial, single-nosed bulbs, cv Carlton, were used.

Table 7. List of treatments used in phytotoxicity experiments. Treatments prefixed 'H' were hot-water treatments. Those prefixed 'T' were biocide treatments

Treatment Ref.	Treatment	Product	Rate of use
H1	Control – ambient temp	-	
H2	3hrs @ 44.4 ºC	-	
H3	4hrs @ 44.4 ºC	-	
H4	Control 2 – ambient temp	-	
H5	3hrs @ 46.0 ºC	-	
H6	3hrs @ 47.0 ºC	-	
H7	3hrs @ 47.5 ºC	-	
H8	3hrs @ 48.0 ºC	-	
H9	2hrs @ 44.4 ºC + 1hr @ 47.0 ºC	-	
T1	Tap water control	-	
T2	Ascorbinic acid	99% l-ascorbinic acid	0.06 g/litre water
T3	Chlorine dioxide	Harvest Wash	0.25 ml/100 ml water + 0.025 g activator
T4	Citric acid	Commodity product	1 g/100 ml water
T5	Natural product	Spore-kill	0.75 ml/100 ml water
T6	Silicone wetter	Silweet L-77	0.1 ml/100 ml water
T7	Chlorothalonil	Bravo 500	0.1 ml/100 ml water
T8	Hydrogen cyanamide	Cultamide	24 ml/76 ml water
T9	Iodophor disinfectant	FAM 30	0.8 ml/100 ml water

The bulbs used for treatments H4 to H9 were warm-stored at 30°C for 1 week before treatment (warm-storage is known to alleviate the damaging effects of the higher HWT temperatures on flower bud development). All other treatments were applied without prior warm storage. Biocide treatments were applied by dipping bulbs in solutions of the biocides in tap water at a temperature of 44.4°C for three hours.

For treatment, batches of five bulbs were placed in a bag of coarse-mesh netting, which kept the sample together but allowed the free circulation of water among the bulbs. Treatments were applied in a laboratory water-bath maintained at the required temperature. After treatment, the nets of bulbs were air-dried and then stored in an un-lit incubator at 30°C until planting.

The treated bulbs were planted on 11 September 2007 in a peaty loam field soil at ADAS Arthur Rickwood. Because the application of the treatments had to be staggered over several days, the time between treatment and planting varied from treatment to treatment, between 4 and 13 days.

Experiment design

The hot-water treated bulbs were planted out in a randomised complete block design of nine treatments replicated four times. The biocide-treated bulbs were planted out similarly in a randomised complete block design with four replicates.

Assessments

In spring 2008, after emergence and during flowering, observations were made of the number of bulbs emerging in each plot and the mean foliage height, flower stem length and flower diameter.

Any visible signs of phytotoxic effects, such as discolouration or distortion of the foliage or flowers were also recorded, on a subjective scale of 0 to 10 (0 = no visible phytotoxic effects, 10 = all foliage with severe symptoms of distortion and/or discolouration, or no plant survival).

Results and discussion

Assessments of the various phytotoxicity parameters were made on the following dates: bulb survival - 10th January 2008; foliage and flower stem heights - 22nd February 2008; flower number and size - 26th March 2008; phytotoxicity index - 4th April 2008. The results of these assessments are presented in Table 8.

Table 8. Results of phytotoxicity assessments, January – April 2008

Code	Treatment	Mean no. surviving plants per plot	Mean no. of open flowers per plot	Mean flower stem length (mm)	Mean foliage height (mm)	Mean flower diam. (mm)	Phyto-toxicity index (0=nil, 10=severe)
H1	Control 1– ambient	5.0	5.0	425	416	96	0.0
H2	3hrs @ 44.4 °C	5.3	3.5	403	407	87	3.3
H3	4hrs @ 44.4 °C	5.0	4.0	402	395	94	6.3
H4	Control 2 – ambient	5.0	4.8	409	420	97	0.8
H5	3hrs @ 46.0 °C	5.0	5.0	419	399	92	5.8
H6	3hrs @ 47.0 °C	5.0	4.8	432	445	98	3.8
H7	3hrs @ 47.5 °C	4.8	5.0	395	419	93	1.8
H8	3hrs @ 48.0 °C	5.0	4.5	417	436	94	3.3
H9	2hrs @ 44.4 °C + 1hr @ 47.0 °C	5.0	3.8	375	367	90	6.8
T1	Tap water control	5.0	4.5	369	371	89	3.8
T2	Ascorbinic acid	4.0	3.5	360	350	95	3.8
T3	Harvest Wash	5.0	4.8	412	395	96	3.3
T4	Citric acid	5.0	4.3	398	392	93	3.3
T5	Spore-Kill	4.8	5.3	391	400	97	8.8
T6	Silwett L-77	5.0	2.5	342	336	90	7.0
T7	Bravo 500	5.0	4.5	403	387	97	3.5
T8	Cultamide	0.0	0.0	-	-	-	10.0
T9	FAM 30	5.0	4.5	381	399	96	3.3
	Mean	**4.65**	**4.19**	**371.9**	**372.6**	**88.3**	**4.33**
	SED	0.28	0.64	21.5	23.0	3.9	1.62
	LSD (*P*=0.05)	0.56	1.28	43.1	46.3	7.9	3.25

Most treatments had no significant effect on the number of surviving plants, but the Cultamide treatment significantly reduced the number of surviving bulbs, to zero. Treatment T2, ascorbinic acid, also produced significantly fewer plants, 4 per plot vs. 5 in the controls ($p = 0.05$). The number of flowers produced was more variable than the number of plants surviving. The most damaging treatment (apart from Cultamide) was Silwett L-77, which resulted in only 10 flowers from 20 surviving plants. This was a statistically-significant difference from the control treatments ($p = 0.05$), which produced between 18 and 20 flowers from 20 plants. The apparent reduction in foliage height and flower-stem length recorded for the Silwett L-77 treatment was not statistically significant when compared to other treatments.

In general, the hot-water treatments produced similar, relatively minor symptoms of phytotoxicity, which would be familiar to many growers. Amongst the biocide treatments, the citric acid, Harvest Wash, Bravo 500 and FAM 30 disinfectant did not produce statistically-significant effects on the survival, growth and flowering of narcissus. As already reported, ascorbinic acid significantly reduced survival of the bulbs by 20% ($p = 0.05$). Silwett L-77 reduced flower numbers from a mean of 4.2 flowers/5 bulbs to 2.5 (significant at $p = 0.05$) and also caused more discolouration and distortion of the foliage (phytotoxicity index 7.0, significantly different from the control treatments at $p = 0.05$). The Spore-Kill did not significantly affect survival, growth or flower production, but the symptoms of discolouration and distortion it produced were the most severe of any of the treatments in which bulbs survived. The phytotoxicity index for the Spore-Kill was 8.75, compared with the overall mean of 4.3. Treatment with Cultamide appeared to be lethal to the bulbs.

Conclusions

The following conclusions were drawn from the *in vitro* testing of candidate treatments reported in the first annual report on this project, plus the phytotoxicity investigations reported above.

- HWT applied at a temperature of 44.4°C for 3 hours is sufficient to give 100% kill of stem nematodes that are exposed in the 'wool' stage.
- HWT, using exposures to temperatures up to 48°C for 3 hours, or 44.4°C for 4 hours, does not give satisfactory control of *Fusarium oxysporum* f.sp. *narcissi* chlamydospores.
- Cultamide and FAM 30 disinfectants at the rates used will give 100% kill of stem nematodes when nematode 'wool' is exposed to solutions of either for 3 hours at 18°C
- Bravo 500, Cultamide and FAM 30 at the rates used will each give 100% kill of *Fusarium oxysporum* chlamydospores when these are exposed to solutions for 3 hours at 44.4°C.
- Cultamide is lethal to narcissus bulbs when used as a constituent of the HWT dip at the experimental rate. Using some other biocides – ascorbinic acid, Silwett L-77 and Spore-Kill – had undesirable phytotoxic effects.

Years 2-4 – Field trial 2008-10

Materials and methods

A stock of *Narcissus* cv 'Golden Harvest' (a basal rot-susceptible cultivar) known to carry a significant level of basal rot due to *Fusarium oxysporum* f. sp. *narcissi*, was lifted from the field at the Kirton Research Centre (KRC) of Warwick HRI, University of Warwick, Kirton, Lincolnshire in July 2008. The bulbs were placed in standard bulb trays and promptly surface-dried under fans blowing at ambient temperatures. Once surface-dried (*ca.* 5 days) the bulbs were subjected to the normal on-line manual cleaning, inspection and grading. The bulbs did not receive any fungicide, washing or dipping treatments. Five-hundred kg of bulbs of grade 12-14 cm (circumference) were allocated for the field trial and stored at ambient temperatures in a large agricultural shed with good air movement. From the remainder of the stock, similarly treated but un-graded, 12 'half-tonne' bulk bins of bulbs were allocated to provide bulk loads for HWT (see below) and were stored in the same conditions as the smaller quantity.

A stock of narcissus cv 'Carlton' bulbs known to be heavily infested with stem nematode (*Ditylenchus dipsaci*) was lifted from a quarantined field at KRC in July 2008 and placed in bulb trays. These bulbs were kept in isolation from other bulb stocks and were allowed to air-dry at ambient temperatures, and were then cleaned by hand. A random sample of 50 bulbs was taken and cut transversely to check for stem nematode symptoms: most bulbs had several 'brown rings' and a few were fully rotted or had only one or two 'brown rings', and a few also showed nematode 'wool' on the outside near the base plate. Representative tissue samples were examined under a x40 microscope, and stem nematodes were easily found in all cases; it was also confirmed that the 'wool' was viable. The remaining bulbs were well mixed to facilitate an even distribution of infestation, and *ca.* 1200 bulbs were allocated for use as 'infector bulbs' for the field trial. They were stored in isolation at ambient temperatures until required.

In August 2007 24 lots of bulbs were made up, each consisting of 13.3kg of the 'Golden Harvest' bulbs well-mixed with 50 'Carlton' infector bulbs. Four replicate lots of bulbs were allocated for each of six treatments. Each lot of bulbs was split equally between two, 5 m-long lengths of extruded nylon tubular netting ('Netlon Oriented 1', Netlon Ltd), spread evenly along each length of netting and secured at intervals with plastic clips. Pairs of nets were wired together with a label and stored in a 17°C store until HWT. The bulbs were planted in netting because this ensures full recovery when subsequently harvested.

Hot-water treatment (HWT)

For HWT the KRC suite of four independent, 1-tonne capacity HWT tanks of a standard 'front-loading' commercial design, with overhead holding tanks for the dip, were used. Two days before they were required, the cleaned HWT tanks were filled to the 5000 litre mark with plain tap-water and set to run at 44.4°C, after which the dip temperature was checked at intervals against a certified mercury-in-glass total immersion thermometer (CIS Calibration Laboratories). On the day HWT was to begin, for each of the six treatments the four replicate lots of mixed bulbs were 'buried' in the bulk loads of bulbs contained in two 'half-ton' bulk bins (see above). The experimental bulbs were treated in bulk in this way because it mimics a commercial HWT operation.

Having taken account of the efficacy and phytotoxicity findings from the earlier, laboratory-based tests (see above), HWT treatments were based around testing the iodophore biocide

(disinfectant) 'FAM 30' and the fungicide chlorothalonil (tested as 'Bravo 500').[1] Six hot-water treatments were tested:

1. Control – no biocide or fungicide added
2. Current standard treatment – commercial formalin (38-40% formaldehyde) added to tank at 5 litre per 1000 litre water
3. Bravo 500 at full-rate - 1 litre 'Bravo 500' (containing 500g chlorothalonil per litre) per 1000 litre water
4. Bravo 500 at ½-rate – 500ml 'Bravo 500' per 1000 litre water
5. Iodophore biocide at full-rate - 8 litre 'FAM 30' per 1000 litre water
6. Iodophore biocide at ½-rate - 4 litre 'FAM 30' per 1000 litre water

All six treatments also contained the following standard additives:

- 0.3 litre non-ionic wetter (as 'Fighter F Activator') per 1000 litre water
- 1 dose of anti-foam preparation (as 'No Foam') per 1000 litre water.

Replication of HWT tanks was impractical, so treatments 1, 2, 4 and 6 were carried out on 20 August 2008 and treatments 3 and 5 were carried out on the next day, full-rate treatments being done in the same tanks as used for the respective half-rate treatments in order to reduce waste and possible contamination. For all treatments the chemicals in the tanks were added well before the bulbs and the tank dip was circulated (*ca.* 1 hour) to ensure mixing of all components. Prior to carrying out treatments 3 and 5, the respective tanks were topped-up to the 5000 litre mark with tap-water, noting the volume needed and then adding the appropriate quantity of non-ionic wetter and anti-foam preparation to replace that 'lost' through bulb treatment.

Each HWT was run for 3 hours at 44.4°C, timing the 3-hour period from when the target temperature was regained following addition of the bulbs (requiring *ca.* 20 minutes). Throughout treatment, tank temperatures were logged electronically at 5-minute intervals, and a summary of the temperatures achieved is given in Table 9; this shows that the temperatures achieved were well within expected commercial tolerances. After the HWT period the bulb bins were removed from the tanks and cooled, ventilated and surface-dried under fans blowing at ambient temperatures for 24 hours. The 24 sets of netted bulbs were extracted from the bins and stored at 17°C until planting.

Table 9. Summary of dip temperatures (°C) for the six treatments

	1	*2*	*3*	*4*	*5*	*6*
Average	44.41	44.41	44.46	44.31	44.26	44.48
Maximum	44.65	44.65	44.70	44.55	44.58	44.71
Minimum	44.22	44.15	44.22	44.11	43.57	44.24

Planting and husbandry

The treated bulbs were planted on 1 October 2008 in sandy loam soil in 'Long Meadow Centre' field at Warwick HRI, Wellesbourne, Warwickshire for growing-on for two years. Before use, the trial site had been soil-sampled and fertiliser applied as needed, followed by ploughing, cultivation and forming into ridges. The position of the plots was marked in the furrows with canes, following which the nets of bulbs were lain evenly in the furrows and the ridges re-formed to cover the bulbs. The trial layout was a randomised block design with four blocks. Each plot was two ridges-wide and 5 m-long, giving a planting rate equivalent to 17.5 t/ha based on ridges at 0.76 m centres. Along the ridges, 5m-long un-planted sections were

[1] Discussions between HDC and Chemicals Regulations Directorate (CRD) staff had led to a specific off-label approval (1848/2009) being given for 'Bravo 500', and had established that approval from the CRD was not needed for the proposed use of 'FAM 30'.

left between adjoining plots to counter the spread of stem nematode which can occur along the ridge/furrow system.

The trial area received the following standard crop sprays, applied in accordance with their approval documents:

- Pre-crop-emergence contact herbicide: glyphosate (as 'Cleancrop Tungsten'), 5 December 2008
- Post-crop-emergence residual herbicide: linuron (as 'Alpha Linuron 50'), 23 February 2009
- Fungicide: tank-mix azoxystrobin (as 'Amistar') + tebuconazole (as 'Folicur'), 26 February 2009
- Fungicide: chlorothalonil (as 'Bravo 500'), 13 March 2009
- Fungicide: tank-mix azoxystrobin (as 'Amistar') + tebuconazole (as 'Folicur'), 30 March and 14 April 2009
- Post-flowering residual herbicide: tank-mix isoxaben (as 'Flexidor 125') + metazachlor (as 'Butisan S') + florasulam (as 'Boxer'), 14 May 2009
- Fungicide: mancozeb (as 'Dithane 945'), 10 June 2009
- Pre-crop-emergence contact herbicide: glyphosate (as 'Cleancrop Tungsten'), 9 November 2009
- Pre-crop-emergence residual herbicide: pendimethalin + linuron, 8 December 2009
- Fungicide: tank-mix azoxystrobin (as 'Amistar') + tebuconazole (as 'Folicur'), 11 March and 27 April 2010

Hand-weeding was carried out when needed. Between growing seasons, July to August 2009, the trials area was cultivated, hand-weeded and the ridges were re-formed.

Assessments

In the 2009 growing season the following assessments were made on each plot:

- Number of emerged shoots (checked at intervals and finally counted on 7 May)
- Incidence and severity of stem nematode and other pest and disease symptoms (inspected at about monthly intervals, counts and scores made if appropriate)
- Number of flowers and buds of marketable quality (counted on 19 March when about 2/3rds the flowers were fully open)
- Number of flowers with defects and nature of defects (19 March)
- Number of buds not opening (19 March)
- Stem length (mean of 10 plants taken at random in the centre of each plot, measured from soil level to base of spathe, 12 May)
- Foliage length (mean of the longest leaves of 10 plants taken at random in the centre of each plot, measured from soil level to leaf tip, 12 May)
- Time of foliage senescence (estimated percentage of green leaf area remaining in each plot at an appropriate date)

Where stem nematode infection (either in leaf spikkels or in bulb scales) was suspected, small pieces of tissue were excised, macerated in a drop of water on a microscope slide, and examined under x40 magnification. Stem nematode, if present, is usually easily seen by this procedure.

Similar assessments (and assessments of bulb yields) were planned for the 2010 growing season, but there was little crop emergence and the possibilities for carrying out normal crop assessments were very restricted. Towards the end of the growing season all bulbs that had produced any foliage were dug out and examined to determine the cause of bulb loss.

Data were examined using the analysis of variance where appropriate.

Results

First crop year (2009)

The survival of bulbs in the various treatments was assessed by recording the total number of shoots emerging in each plot. The means indicated that bulbs in the 'FAM 30' treatments (both rates), in the half-rate Bravo 500 treatment, and in the standard (formaldehyde) treatment contained similar numbers of viable bulbs (Figure 1, Table 10). By comparison, shoot numbers were significantly lower in the full-rate Bravo 500 treatment and further lowered in the control, which had received no biocide or fungicide in HWT (Figure 2).

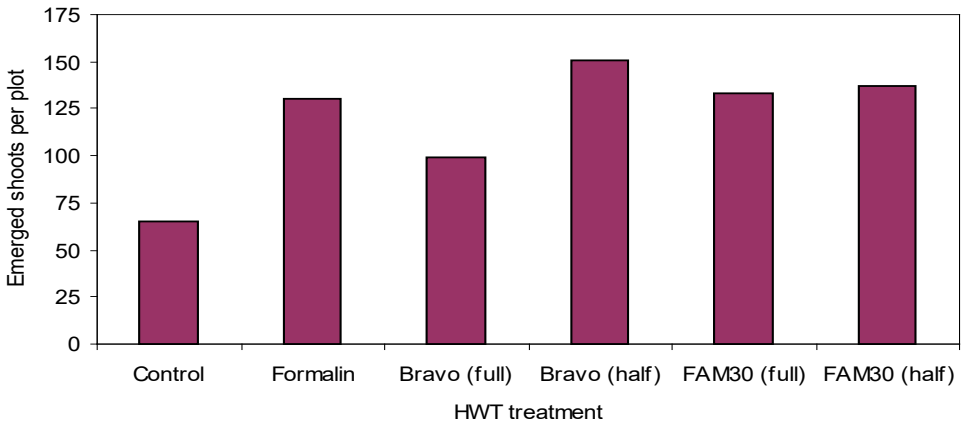

Figure 1. Number of shoots per plot in the year following hot-water treatment

Table 10 Plant performance in the year following HWT

HWT	Shoots (number/plot)	Flowers (number/plot)	Leaf length (mm)	Stem length (mm)
Control	65.0	33.0	45.5	48.7
Formalin	130.5	73.0	49.1	51.9
'Bravo' (full-rate)	99.5	55.5	51.7	55.5
'Bravo' (half-rate)	150.5	89.8	50.8	52.9
'FAM 30' (full-rate)	132.8	73.0	49.2	52.7
'FAM 30' (half-rate)	137.3	71.8	49.8	53.8
LSD(5%)	24.79	15.41	4.09	4.43
Significance	***	***	NS	NS
***, significant at the 0.1% level of probability; NS, not significant				

Left: The two rows with labels are the full-rate ‘FAM 30’ treatment
Right: The two rows with labels are the standard formalin treatment, with controls on their right

Figure 2. Views of plots in March 2009

Flower yield was assessed when the crop was nearly in full bloom. The flower count varied with HWT treatment in the same way as the number of emerging shoots (Figure 3, Table 10). Bulbs in ’FAM 30’ treatments (both rates), in the half-rate Bravo 500 treatment and in the standard (formaldehyde) treatment produced similar numbers of flowers, while numbers were significantly reduced in the full-rate Bravo 500 treatment and further reduced in the control which had received no biocide or fungicide in HWT.

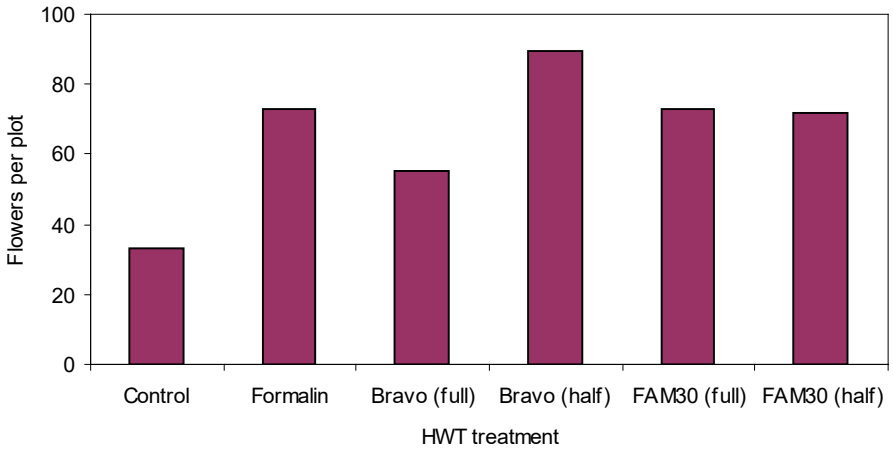

Figure 3. Number of flowers per plot in the year following HWT

Flower quality. Over the whole trial, just nine of the flowers counted showed defects, and this comprised bud-damage on short stems due to damage from slugs, and was therefore unrelated to HWT. There were also an additional eight dead flower buds (with the appearance of ‘drumsticks’), which could be ascribed to HWT damage, but the distribution of these did not appear to be correlated with any particular treatment or replicate block.

Stem and foliage length. While stems and leaves appeared shorter in the control plots, analysis of variance suggested there were no significant differences between these data (Figure 4, Table 10).

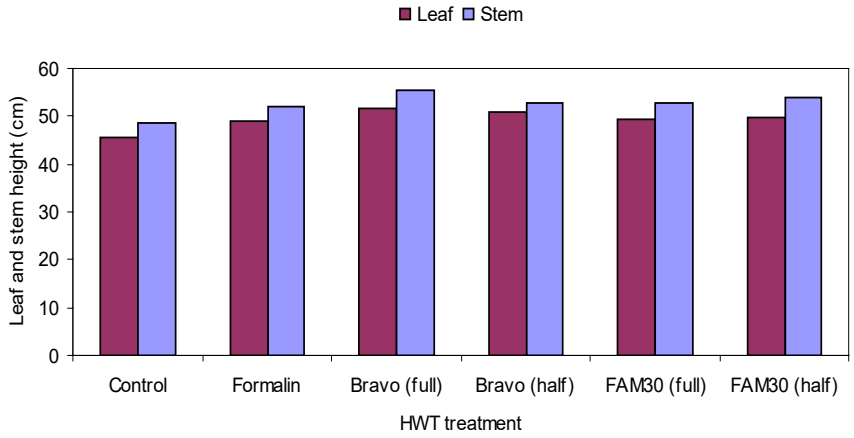

Figure 4 Leaf and stem lengths in the year following different HW treatments

Plant health. At no time were 'classic' or 'textbook' spikkels (leaf lesions characteristic of stem nematode infestations) seen anywhere in the trial. Tissues from any 'suspect' lesions were teased out in water under a low-power microscope, a process that will easily detect stem nematodes, if present, but none was found.

Exploratory digging in empty stretches of ridge in the control plots showed that bulb death was largely due to typical *Fusarium* basal rot. Across the trial 11 plants were noted with premature foliar die-down, characteristic of rotting due to basal rot, but these appeared to be randomly scattered around the trial.

Several plants were found with shoot damage characteristic of bulb scale mite (*Steneotarsonemus laticeps*) infestation, and/or with foliar virus symptoms, but these were spread generally across the trial and appeared to be stock problems.

As expected, occasional plants were found with smoulder (*Botrytis narcissicola*) primaries or leaf lesions. These were very few in number and appeared to be randomly distributed.

Foliar senescence. By mid-May *ca.* 5% of the leaf area was senescent (yellowing) and leaves were starting to fall into the furrows. No convincing differences in the progress of senescence were observed between any plots.

Second crop year (2010)

In early-2010 the bulbs in the trial were late to emerge, and not unexpectedly so, given the unusually cold weather experienced. However, other bulb stocks planted adjacent to the trial began to emerge normally and, when only a very few shoots had emerged in the trial plots by mid-February, it became apparent that most bulbs had died. The total number of emerging shoots was recorded on 6 April 2010 (Figure 5, Table 11). The two 'Bravo 500' treatments gave the largest number of shoots, followed by the half-rate 'FAM 30' treatment, while the other three treatments gave very few. However, the between-replicate variability of the data was high, and the analysis of variance showed that the effect of the treatments on shoot numbers was not statistically significant

In mid-March sample stretches of the formalin plots were dug up, avoiding any surviving shoots. It was found that most bulbs had rotted away, and of those that were recoverable, most had the typical appearance of basal rot when bisected.

In early April, further samples were dug from the full-rate 'FAM 30' and full-rate 'Bravo 500' plots, and again it was seen that most bulbs had rotted fully as a result of basal rot. In a few cases, some areas of healthy, white bulb scales remained: when examined, these showed no 'brown ring' symptoms typical of stem nematode. Several samples of such adjacent healthy bulb tissue were examined, and only a solitary nematode was found. Bulb mites and larvae of small narcissus fly, both secondary pests, were present.

During the March to May period the remaining foliage was checked regularly. Some 'suspect' (but not 'classic') stem nematode lesions (spikkels) were examined, but no nematodes were found.

On 14-15 July 2010 all bulbs associated with foliage were dug up. They were roughly cleaned and the number and weight of these relatively sound bulbs in each plot were recorded (Figure 5). The highest bulb recovery was found in the half-rate 'Bravo 500' treatment, while the poorest results were found in the control, formalin and full-rate 'FAM 30' treatments; in the full-rate 'Bravo 500' treatment, bulb weight particularly was reduced. However, with 10 out of the 24 plots having no bulbs recovered, it was not surprising that the between-replicate variability of the data was high, with analysis of variance showing that the effect of the treatments on bulb numbers and weights was not statistically significant (Table 11).

The recovered bulbs were bisected, the numbers of healthy bulbs and mummified (completely dried out) bulbs noted, and the incidence of pest and disease symptoms (basal rot, 'brown rings', bulb scale mite feeding marks and damage due to large narcissus fly) recorded. The percentages of bulbs healthy, mummified or with symptoms were:

Mummified bulbs	52%
Healthy bulbs	25%
Large narcissus fly damage	12%
Basal rot damage	6%
Bulb scale mite damage	2%
'Brown ring' symptoms	2%

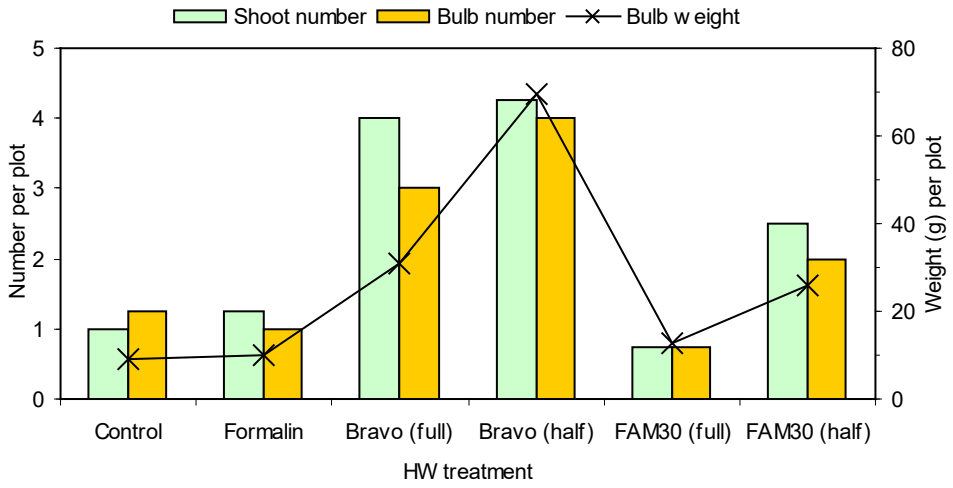

Figure 5 Shoot number (in April), and number and weight of bulbs recovered (in July) for the second crop year following HWT. Numbers and weights were treatment means.

Table 11. Number of shoots and yields of bulbs in the second crop year

HWT	Shoots/plot	Bulb number/plot	Bulb weight (g)/plot
Control	1.0	1.3	9.0
Formalin	1.3	1.0	10.0
'Bravo' (full-rate)	4.0	3.0	31.0
'Bravo' (half-rate)	4.3	4.0	69.5
'FAM 30' (full-rate)	0.8	0.8	12.5
'FAM 30' (half-rate)	2.5	2.0	26.0
LSD(5%)	4.74	4.02	66.55
Significance	NS	NS	NS

Discussion

While it is not uncommon for trials on pest and disease control in daffodils to show no or only trace incidence of target pathogen symptoms, it is unusual (but not unprecedented) to experience the near-complete loss of all plants in daffodil trial plots under extreme conditions. In this trial it was important to provide for pest (stem nematode) and disease (basal rot) pressure so that the experimental HWT with 'FAM 30' and 'Bravo 500' could be thoroughly tested; unfortunately, in this case, this pressure proved too great, and while gaps were evident in the plots in the year after HWT, by the second growing season very few shoots emerged. Because other, adjacent, daffodil stocks on the same site grew normally in 2009 and 2010, a more general cause of this loss, say due to adverse weather or soil conditions, can be ruled out. This left the obvious cause of plant loss as the basal rot and/or stem nematode present in the bulbs. Pest and disease symptoms were noted on shoots throughout the trial. No undisputed ('text-book') nematode-induced leaf or stem spikkels (lesions) were seen, neither (with a single exception) were stem nematodes found in the tissues of suspect spikkels or in 'healthy adjacent' bulb tissue. On the other hand, most recovered bulbs and bulb debris were either mummified (rotted and dried out following total rotting of the bulb) or rotted with a wet, dark brown rot typical of basal rot. Other pests were found, namely bulb mite and small narcissus fly, but these are not primary pests of daffodils, while this level of bulb loss is not known to result from large narcissus fly or bulb scale mite infestations. The loss of bulbs was likely due to a catastrophic level of basal rot.

Despite this situation, it does appear that the trial provided useful information about the crop safety of the tested HWT additives, 'FAM 30' and 'Bravo 500', which was the main objective of the project. In the first crop year, among the plots treated with formalin, 'Bravo 500' or 'FAM 30', shoot emergence, flower yields and crop quality were similar, except that in the plants treated with full-rate 'Bravo 500' there was some reduction in shoot and flower numbers. Similar, mildly growth-depressing effects have been seen on daffodils in the year following treatment in HW with another fungicide, thiabendazole (as 'Storite Clear Liquid'), from which the crop fully recovered after a further year's growth (Hanks, 1992). This effect of full-rate versus half-rate 'Bravo 500' treatments was seen even in the few plants that survived to the second year. The effect of full-rate 'FAM 30' may also have been mildly detrimental, a similar effect to that observed in a later field trial (BOF 61b).

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Overall Summary

Discussion

Each separate element of this project has been discussed in the relevant sections above, and the reader is referred to those sections as appropriate.

Conclusions

The overall conclusions of the project are as follows:

- Of the eight materials identified as candidates with the potential to act as replacements for formaldehyde in HWT, only two (Cultamide and FAM 30) demonstrated activity against stem nematode during *in-vitro* tests. Both of these materials and the fungicide Bravo 500 were also active against the chlamydospores of *Fusarium oxysporum* f.sp. *narcissi*, the causal organism of basal rot.
- When the phytotoxic effects of Cultamide, FAM 30 and Bravo 500 were assessed, Cultamide proved lethal to narcissus bulbs when used during the HWT dipping process. The phytotoxic effects of the FAM 30 and Bravo 500 during the same tests were minor.
- In the first year of a 2-year experiment, all treatments (except full-rate Bravo 500) were as effective as formaldehyde in facilitating the production of shoots and flowers by treated bulbs when compared to bulbs that had been given HWT without additives.
- The full rate of cholorothalonil resulted in fewer shoots and flowers being produced by treated bulbs compared with the other additive treatments, presumably due to sublethal phytotoxic effects.
- In the second year, the challenge provided by the basal rot infection was so severe that the majority of bulbs were killed outright by the disease, whether treated with formaldehyde, treated with either of the experimental materials, or given no additive treatment.
- The addition of materials with fungicidal activity during HWT may reduce cross-contamination of bulbs during the process and give some beneficial effects during the first year after planting, but it is unlikely to have any long-term effects on basal rot in a diseased stock.

Recommendations

This work has shown that both FAM 30s and Bravo 500 have the potential to replace formaldehyde as the standard additive in bulb-dipping tanks during HWT. However, it is evident that more development work could usefully be done on these materials.

The dose rates used in the experiments described above were chosen as the maximum likely to be allowable in practice, since if they were not efficacious at such rates they would be of no practical use. Having proved to be effective at these high rates, it would now be useful to examine the effects of lower rates of these materials, to determine the minimum effective rates. This could result in cost savings and reductions in pesticide residues.

During the HWT process it is necessary to ‘top-up’ the bulb dipping tanks at intervals, to maintain both water volumes and the levels of additives, which tend to evaporate, be adsorbed onto soil and debris, react with HWT-tank structural materials and/or be removed on treated bulbs. This current work has not examined the longevity of FAM 30 or Bravo 500 during HWT treatment and so top-up rates have not been established. If these treatments are to be commercially practical then such information will be necessary to allow their efficient use.

Knowledge and Technology Transfer

Presentations of the interim results of this project were made by Mike Lole to HDC BOF levy payers on 29 April 2009 at the Penventon Hotel, Redruth, Cornwall and on 26 February 2009 at the ADAS National Bulb Consultancy Centre meeting, Holbeach, Lincolnshire.

The preliminary findings also appeared in an article by Mike Lole in *HDC News*, March 2009, p.17.

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