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AUTHENTICATION

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

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Practical Section for Growers

Background and objectives

The addition of the fungicide thiabendazole (as Storite Clear Liquid) to hot-water treatment (HWT) tanks is one of the main ways of managing basal rot of narcissus bulbs, the most serious fungal disease of narcissus crops in the UK. Bulb growers have been advised to add an acidifier, sodium bisulphate, to HWT tanks, to improve the effectiveness of the thiabendazole, but this practice had not been properly tested, nor are full recommendations available in print.

In an earlier HDC-funded project (BOF 43), the use of thiabendazole, with or without various additions of sodium bisulphate, was evaluated. Adding sodium bisulphate, to give a dip pH of 2.5-3.0, was shown to maintain a higher concentration of thiabendazole in the HWT tank, compared with using regular, non-acidified treatments. These rates of acidifier did not adversely affect crop growth.

The present extension of the project was set up to determine whether, when using an acidifier, reduced rates of thiabendazole would be effective in controlling basal rot. If so, this could result in significant cost reductions for the UK bulbs industry.

Summary of results

Can acidifier added to HWT improve the control of basal rot with Storite ?

Bulbs of narcissus cultivars Carlton and Golden Harvest were given standard HWT with full-, half- and quarter-rate Storite Clear Liquid, to which sodium bisulphate (sodium hydrogen sulphate) had been added to give a dip pH of 2.5-3.0. These treatments were compared with HWT using full-rate Storite but no acidifier, and acidifier but no Storite. Formalin, wetter and anti-foam preparation were added to all treatments.

The five treatments were:
1. Full-rate thiabendazole with no acidifier
2. Full-rate thiabendazole + acidifier
3. Half-rate thiabendazole + acidifier
4. Quarter-rate thiabendazole + acidifier
5. No thiabendazole + acidifier

Without added acidifier, the standard Storite dip had a pH value of 3.7, but when sodium bisulphate had been added the pH values were 2.5-2.8. Over the course of a 3-hour HWT, dip pH rose by about 0.2-0.4 pH units in all treatments. The target formaldehyde concentration was maintained with and without added acidifier. Using ‘dipsticks’ to measure pH was less accurate than using a simple pH meter. The treated bulbs have been planted in the field, and growth and disease control will be followed over a two-year-down growing period.

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Effect of acidifier in HWT on the levels on thiabendazole in dips

The concentrations of thiabendazole in dips and in bulbs were determined using a cup-plate diffusion assay. The target concentration of thiabendazole in full-rate dips was 1100 ppm, using 5 litres Storite Clear Liquid per 1000 litres dip. Where no acidifier had been added, the concentration of thiabendazole in solution in the full-rate dip fell rapidly, within a few minutes, to about 290 ppm, falling further to 260 ppm by the end of the 3-hour dip. Where acidifier had been added, the 'initial' concentration of thiabendazole in the full-rate treatment was 1102 ppm, falling to 963 over 3 hours. With half- and quarter-rate fungicide, the 'initial' values were 341 and 314 ppm, respectively, and the values after 3 hours were 392 and 289 ppm. It was confirmed that, in the control dip with no fungicide added, any trace of thiabendazole was below the limit of detection (1 ppm). It appeared that, when no acidifier was added, a large proportion of the thiabendazole rapidly precipitated out in the HWT equipment.

Effect of acidifier in HWT on the levels on thiabendazole in bulbs

The concentrations of thiabendazole in the outer bulb tissues (dry skin, two outer white scales and basal plate) and in the remaining inner bulb tissues were measured for the five treatments after HWT and 24 hours of drying. Although the lowest thiabendazole concentration was recorded in the non-acidified Storite dip, the highest concentration in bulbs was found when this treatment had been used. In these bulbs (non-acidified Storite dip) a white deposit was clearly visible after treatment, probably resulting from thiabendazole being deposited on the bulb surface during dipping. For the two cultivars, concentrations in this treatment were 546 and 364 ppm for the outer tissues, and 37 and 34 for the inner tissues. Where acidifier was used, the thiabendazole concentrations for the outer tissues were 350 and 196 ppm (full-rate), 106 and 156 ppm (half-rate) and 110 and 125 ppm (quarter-rate); for the inner tissues the concentrations varied only from 3 to 8 ppm. No traces of thiabendazole were found in control bulbs not treated with Storite.

Practical methods for maintaining a target pH level in HWT

In a supplementary observation, the changes in HWT dip pH were recorded over a 5-day working period using half-rate Storite and standard acidifier addition [2.5 litres of Storite Clear Liquid (½ rate) and 1.38 kg sodium bisulphate per 1000 litres of water]. At the start of the second and subsequent days the water level was topped-up to the original mark, noting the volume used (*ca.* 300 litres daily), and the dip chemicals were added as follows:

- Sodium bisulphate: at the original rate (0.138 kg per 100 litres of top-up) *plus* an amount estimated to regain the target pH (for the four successive days, 0.11, 0.29, 0.36 and 0.45 kg per 1000 litres dip),
- Formalin, wetter and anti-foam: at the original rate,
- Storite: at the same (half-rate) concentration as before (0.25 litres per 100 litres of top-up) *plus* 0.75 litres per 1 tonne of bulbs treated the previous day (the '½-tonne bins' held *ca.* 0.4 t bulbs each).

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The starting pH was 2.5, rising to about 3.1 over the course of three successive HWT periods on the first day of the test. Further sodium bisulphate was added at the start of each day's HWT, which brought the dip pH down to 2.7 – 2.8, rising by the end of each day to 3.0 - 3.2.

Action points for growers

- Full recommendations cannot be made until the completion of the project in December 2003. However, it does appear that adding an acidifer as sodium bisulphate (at a rate of about 1.38 kg per 1000 litres, giving a pH of 2.5 - 3.0), is a safe procedure for narcissus bulbs and may allow for more cost-efficient use of Storite fungicide. Any advice on fungicide rates should await the full results being available.

- As a general precaution, growers and contractors should check that the circulation in their HWT tanks minimises 'dead spots' where precipitated fungicide might accumulate.

Practical and financial benefits from the study

At present prices it costs about £50 to treat one tonne of narcissus bulbs with Storite used at a rate of 5 litres of Storite Clear Liquid per 1000 litres of dip. It costs less than 50p per tonne for the sodium bisulphate (acidifier) required to maintain high solubility of the added fungicide. The results from this project will show whether the rate of Storite used can be safely reduced.

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EXPERIMENTAL SECTION

Introduction

Basal or base rot caused by the fungal pathogen *Fusarium oxysporum* f.sp. *narcissi*, is the most important fungal disease affecting narcissus crops grown in the UK, and one major element in the management of the disease is the application of the fungicide thiabendazole (as Storite Clear Liquid) to the bulbs, usually by its inclusion in the hot-water treatment (HWT) tank. Despite the general effectiveness of this fungicide as demonstrated in trials and in practical use, in reality in many cases the control of basal rot is disappointingly poor. Added to this, this fungicide is expensive, costing about £50 to treat each tonne of bulbs at the recommended rate. In an earlier HDC-funded project, BOF 43, it was shown that the concentration of thiabendazole fell rapidly in HWT tanks, and that adding an acidifier, sodium bisulphate (sodium hydrogen sulphate), to the tank greatly improved the maintenance of thiabendazole concentrations during bulb treatment. The solubility of thiabendazole in water decreases with increasing pH. The maximum achievable concentration at pH 2.2 is 38.4 mg/ml (38,400 ppm), but this drops to 0.05 mg/ml (50 ppm) at pH 5.0 (Budvari *et al*., 1996; Spencer, 1981). The target thiabendazole concentration for narcissus dipping is 1100 ppm (1.1 mg/ml), using 5 litres of Storite Clear Liquid per 1000 litres of dip. The acidification of bulb dips to a pH of 2.5 – 3.0 resulted in a higher circulating concentration of thiabendazole being maintained (in project BOF 43, 0.79 mg/ml or 790 ppm after 5 days). This compared with the concentrations observed under normal use without acidifier (pH about 4.0, thiabendazole concentration 0.26 mg/ml or 260 ppm after 5 days). At these pH levels, there appeared to be no detrimental effects on the narcissus crop of acidic dip treatments. For the small additional cost of adding sodium bisulphate to tanks, the effectiveness of Storite should be enhanced. However, major financial benefits would follow if it were possible to use a reduced rate of Storite where an acidification treatment is applied.

The present project was set up to:

- Determine the effectiveness of acidified, reduced-rate thiabendazole HWT for the control of base rot in narcissus;
- Determine practical methods for maintaining a target pH in HWT tanks;
- Determine the persistence of thiabendazole in treated bulbs; and
- Produce recommendations for growers on using thiabendazole and sodium bisulphate in narcissus growing.

This first interim report describes work carried out up to the end of 2001, including the setting up of a field trial to determine the effectiveness of acidified, reduced-rate thiabendazole treatments, the maintenance of pH levels in bulb dips, and the concentrations of thiabendazole in bulb dips and treated bulbs.

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Materials and methods

Plant material

Bulbs of narcissus cultivars Carlton and Golden Harvest, taken from stocks grown at HRI Kirton, Lincolnshire for many years, were used for the project. These stocks had been grown using the usual 'two-year-down' growing system and typical husbandry methods for narcissus in the east of England (e.g., see ADAS, 1985). Bulb stocks were lifted in July 2001 and dried for 3 days at 35°C in ½-tonne bulk bins on a 'letter box' drying wall, before being moved to a controlled temperature store where 'second stage' drying and storage was continued at 17°C under fans. In previous years the stocks had received routine post-lifting dip and HWT treatments with thiabendazole, except that in 1999 only the HWT thiabendazole treatment had been given, therefore a moderate incidence of base rot was to be expected in these bulbs.

Bulbs were withdrawn from 17°C storage and passed down a cleaning, inspection and grading line. For each cultivar, *ca.* 300 kg of sound, undamaged bulbs of grade 10-12 cm circumference were allocated. From these, 20 10-kg and 20 5-kg lots of each cultivar were weighed out into net bags for use in experimental plots. As the design of the main experiment called for the HWT of full bulk bins of bulbs (to simulate commercial treatments), further bulbs (cv Golden Harvest) were allocated to provide a supply of full bins in which the small experimental lots were placed for treatment. Twenty, ½-tonne bins of bulbs were allocated for this purpose and for supplementary work. Bulb storage was continued at 17°C under fans until bulbs were required for HWT.

Main experiment

There were five experimental HWT treatments, for each of which one ½-tonne bin of stock bulbs, containing four 10-kg and four 5-kg bags of each cultivar, was used. The weighed lots were 'buried' in each bin prior to treatment.

The five treatments were:

6. Full-rate thiabendazole with no acidifier
7. Full-rate thiabendazole + acidifier
8. Half-rate thiabendazole + acidifier
9. Quarter-rate thiabendazole + acidifier
10. No thiabendazole + acidifier

HWT was carried out in two front-loading tanks each capable of treating 2 x ½-tonne bulk bins using 5000 litres of dip. The tanks are also designed to work with a half load, and for the experiment each tank was loaded with 1 x ½-tonne bin using 3000 litres of dip. The treatment order was semi-randomised (such that full-rate Storite dips were available for other use at the end of the experimental treatments). Using tanks A and B respectively, treatments 3 and 5 were carried out on day 1, treatments 4 and 1 on day 2, and treatment 2 on day 3 (15-17 August 2001). Thus the treatment order in tank A was 3, 4 then 2, and in tank B treatment 5 then 1. Each HWT consisted of a 3 hour period at 44.4ºC, plus an initial period (of about 15 to 20 minutes) to regain the target temperature following loading the tank with bulbs.

Thiabendazole treatments were based on a full-rate of 5 litres Storite Clear Liquid (Banks Agriculture Ltd; 220 g a.i. per litre) per 1000 litres water. The acidifier treatment consisted of

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1.38 kg sodium bisulphate ($NaHSO_4$, sodium hydrogen sulphate) (technical grade powder; Banks Agriculture Ltd) per 1000 litres water. All treatments contained the following dip additives per 1000 litres:

- 5 litres commercial formalin (containing 38-40% formaldehyde)
- 300 ml non-ionic wetter (Activator 90)
- 40 ml anti-foam preparation (Croptex No Foam)

Before each treatment, the HWT tanks were cleaned and flushed thoroughly with mains water, filled with clean mains water to the 3000 litres mark, and brought to the required temperature overnight before chemicals were added, the acidifier (where appropriate) being added first, and the thiabendazole last.

After treatment the bins of bulbs were allowed to drain, dried under fans at ambient temperatures for 24 hours, then stored at ambient temperatures under fans until planting. During this storage period the weighed lots were recovered from the bins ready for planting. The 10-kg lots (to be used for crop records) were placed in 7.5 m-long lengths of tubular nylon netting (Netlon 'Oriented 1'), distributing the bulbs evenly by using plastic clips at intervals. The 5-kg lots (to be available for sampling at intervals) were not placed in netting.

Samples and records during HWT

The pH of mains water and (after all chemicals had been added) of the dip were recorded before each HWT, and dip pH was recorded 1 and 2 hours into the treatment time and at the end of the treatment. The pH was recorded using a simple portable, temperature-compensated pH meter (pH-temperature meter PHT3140, ebro Electronic GmbH) calibrated with fresh buffer solutions. Additional pH checks were made using pH indicator dipsticks that allowed pH to be discriminated to within 0.3 pH units (Pehanon pH 1.0 – 2.8 and pH 1.8 – 3.8; Macherey-Nagel).

Dip samples (*ca.* 100 ml) were taken from each tank for the determination of thiabendazole concentration, after the chemical additions had been made and allowing for thorough mixing, immediately before bulbs were added. Further samples were taken at the end of the treatment period. Samples were stored in polypropylene bottles and were immediately frozen (-18°C).

Further samples (*ca.* 100 ml) of dip were collected at the start and end of each HWT and refrigerated (4°C) for determining the concentration of formaldehyde using a test kit (Quantofix formaldehyde 10-200 mg/litre; Macherey-Nagel).

All samples were taken from a sampling port installed in the circulation of the HWT tanks. Dip temperature was monitored continuously and remained within acceptable limits.

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Planting and cultural practices

Bulbs were planted in the field on 13 September 2001. Following the usual procedures for planting field trials at Kirton, the trial area was ridged out and the position of plots marked in the furrows using fibreglass canes. The bulbs were placed evenly in the plots by hand. Each plot consisted of a single length of ridge (11.0 m long), into which were placed the 10-kg lots (in their 7.5 m-long nets) and the 5-kg lots (loose, in the remaining 3.5 m of the plot), the order of the two lots in the plots being randomised. The bulbs were then covered by splitting-back the ridges. This gave a planting rate of about 20 t/ha with ridges at 0.76 m centres.

The husbandry of the bulbs is following standard two-year-down commercial practices for the area (e.g., see ADAS, 1985). Fertilisers were applied according to analysis and MAFF recommendations (potash in the base pre-planting, nitrogen as a top-dressing pre-emergence). Weed control is by dormant season diquat + paraquat, pre-emergence cyanazine and post-emergence chlorpropham + linuron. Crops will receive a fungicide spray programme, with five sprays in the first year (iprodione, chlorothalonil, vinclozolin, mancozeb + benomyl, chlorothalonil) and three in the second year (iprodione, chlorothalonil, vinclozolin). Herbicides and fungicides are all used according to standard recommendations. Flowers will not be cropped. Crop records will be maintained and bulb yields will be determined at the conclusion of the experiment.

Field trial design and statistical analysis

In the field a randomised block design was used, with 10 treatments (2 cultivars x 5 treatments) and four blocks. Two metre gaps were left between plots in the same ridge. Guard bulbs were planted round the edges of the trial. The data will be subjected to the analysis of variance as appropriate, using the initial planting weight as a co-variate.

Bulb sampling for thiabendazole determination

Bulbs were sampled on 15 August 2001, prior to HWT, taking five lots of five bulbs each at random from the 5-kg lots of each cultivar. Individual bulbs were cut lengthwise into quarters, following which the outer brown bulb scales and first two white bulb scales were removed, together with the base plate. These outer tissues and the remaining inner parts of each bulb were placed in separate polythene bags and frozen (-18°C).

After the treated bulbs had been dried for 24 hours, on the same day, further bulbs (five from each 5-kg lot) were taken, divided into outer and inner tissues as before, and deep-frozen. At this stage white deposits, presumed to be fungicide, were obvious on bulbs sampled from treatment 1 (full-rate Storite, no acidifier), but were not evident on bulbs from any other treatment.

It is planned to recover further bulb samples from the field for analysis at 3-monthly intervals (or until two successive samples have non-detectable concentrations of thiabendazole).

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Determination of thiabendazole concentrations in dips and bulbs

Thiabendazole concentrations in HWT tank solutions and bulbs were determined using a 'cup-plate' diffusion bioassay (Yarden *et al*., 1985; Carder, 1986).

Samples of HWT tank fluids were taken from the beginning ('B') and end ('E') of bulb-dipping cycles (see above) and were kept in a frozen state until assessments of thiabendazole content were made. Each sample was thawed, shaken, allowed to stand for 30 minutes and diluted in water (the dilutions tested ranged from 1:2 to 1:40). A 1.8mm-layer of potato dextrose agar was poured into a shallow glass tray and allowed to set. Then 0.75ml of a spore suspension of *Fusarium oxysporum* f. sp. *narcissi* (isolate LVB Na2), containing 1 x 10^6 spores/ml, was spread evenly over the agar surface. Using a cork borer 7mm in diameter, discs were removed from the agar layer at regular spacing (centres 40mm apart horizontally and vertically). Aliquots of test samples (40µl) were placed in these wells, the plate covered and incubated at 25ºC for 48 hours. Each dilution of every sample was placed in two wells on each of two diffusion plates.

Bulb samples from each of the five treatments were kept frozen until assessments of thiabendazole content were made. Samples of outer tissues ('O', outer scales plus base plates) and of the remaining inner tissues ('I') were analysed separately (see above). Two bulbs from each of four replicates of all five treatments were tested. Each sample was weighed and placed in 15ml water (all 'O' samples) or a volume equivalent to 1.5 times the weight of bulb tissue (all 'I' samples). All samples in water were agitated gently for 6 hours to allow thiabendazole on and in the tissues to diffuse into the water. All 'O' diffusates were assayed directly by diffusion plate bioassay (as above) using 40µl volumes of an appropriate dilution. All 'I' diffusates were concentrated ten-fold before thiabendazole assay. Each dilution of every sample was placed in one well on each of two diffusion plates.

In the diffusion bioassay, circular zones of agar with no visible fungal growth were seen where the fungicide had diffused outwards from the wells and inhibited fungal growth. The diameters of these zones were recorded after incubation at 25°C for 48 hours. Thiabendazole concentrations were calculated from a standard curve constructed by using a set of fungicide dilutions ranging in concentration from 10 to 100 ppm. The limit of detection was 1 ppm. For tank dip samples, the means and standard deviation values for the four replicates are presented (Table 1). For bulb samples, the figures for means and standard deviations shown (Table 2) are for 16 values, i.e., two diffusion plate values for each of two bulbs from each of four replicates. These values were adjusted to take account of the weight of bulb tissue in each sample, and are presented as micrograms of thiabendazole per gram of bulb tissue (µg/g or ppm).

Supplementary observation

To determine the practicalities of HWT with an acidifier, 15 further ½-tonne bins of bulbs were dipped in sequence, three bins per day for 5 days (3 September to 7 September 2001), using the same HWT set-up as above (one ½-tonne bin with 3000 litres of dip per load). The HWT duration and temperature were as described for the main experiment, and the dip consisted of half-rate Storite and full-rate acidifier, formalin, non-ionic wetter and anti-foam material, used as described above. At the start of the second and subsequent days the water level was topped-up to the original mark, noting the volume used (*ca.* 300 litres daily), and the dip chemicals were added as follows:

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- Sodium bisulphate: at the original rate (0.138 kg per 100 litres of top-up) *plus* an amount estimated to regain the target pH (for the four successive days, 0.11, 0.29, 0.36 and 0.45 kg per 1000 litres dip)
- Formalin, wetter and anti-foam: at the original rate
- Storite: at the same (half-rate) concentration as before (0.25 litres per 100 litres of top-up) *plus* 0.75 litres per 1 tonne of bulbs treated the previous day (the '½-tonne bins' held *ca.* 0.4 t bulbs each)

For each treatment, the pH of the dip was recorded (using a pH meter) before the addition of bulbs, after 1 and 2 hours and at the end of HWT. Spot checks of pH were also made at random intervals using indicator dipsticks (as above). The records were used to estimate a daily additional amount of sodium bisulphate (see above) to be added to maintain the target pH (2.5 – 3.0). A sample of the dip was taken for thiabendazole determination at the end of the final treatment, using the procedures given above.

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Results

pH values of dips

The following pH values were recorded prior to starting HWT:

- Tanks filled with plain mains water, pH 7.3 – 7.4
- Sodium bisulphate added, pH 2.5
- Formalin, wetter and anti-foam added, pH 2.5 – 2.6
- Storite added, pH 2.5 (full-rate), 2.8 (½-rate), 2.6 (¼-rate)
- No sodium bisulphate; formalin, wetter, anti-foam and Storite (full-rate) added, pH 3.7

Typically, the pH of the dip rose by 0.1 units once the bulbs had been loaded.

The changes in dip pH values over the course of HWT are shown for the five treatments in Figure 1 (page 14). In all treatments, the pH of the dip drifted steadily upwards over the course of the *ca.* 3 hour treatment. The pH of the non-acidified dip was about 1.0 pH units higher, overall, than that of acidified dips (whether or not these contained Storite). The three Storite rates all gave pH values falling within acceptable limits.

The pH of dips was also checked at random intervals using indicator dipsticks, and compared with meter readings. Determinations using dipsticks appeared consistently to over-estimate pH by about 0.4, compared with meter readings:

pH by dipstick	*pH by meter*
3.5	3.1
3.5	3.1
3.2	2.9
3.5	3.0
3.5	3.0
3.2	2.9
3.2	3.0
3.5	3.0

Supplementary observation on pH values of dips

The changes in dip pH values over the 5-day period are shown in Figure 2 (page 15). The figure shows a starting pH of 2.5, which rises to about 3.1 over the course of three successive HWT periods on the first day of the test. With the addition of further sodium bisulphate before the start of dipping on subsequent days, the pH was reduced to 2.7 – 2.8, never regaining the original level of 2.5. By the end of each day's HWT, dip pH had risen to 3.0 - 3.2. Inflexions can be seen in the graphs of the pH records, corresponding to the unloading and re-loading of bulbs from the tanks, presumably a result of pumping the dip to and back from the holding tank.

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Formaldehyde determination

The test kit used for the determination of formaldehyde in dips produced a colour change allowing the discrimination of formaldehyde concentrations of <10, 20, 40, 60, 100 and >200 mg/litre. Dip samples were first diluted 20-fold with tap water to bring the formaldehyde concentration on-scale. All ten samples (i.e., taken at the start and end of the five HWT treatments) resulted in readings corresponding most closely to 100 mg/litre, equivalent to 2000 mg/litre in the non-diluted dip, the target concentration. A 'blank' test (water only) did not produce a colour change.

Thiabendazole concentrations in dips

Table 1 shows the thiabendazole concentrations at the start and end of the five HWT treatments. The main findings were:

- The concentrations of thiabendazole at the start and the end of bulb dipping varied by no more than 15% within any of the treatments.
- Only 26% of the fungicide that was added to the HWT tank in the absence of any acidifier and before any bulbs had been added was detected in the assay.
- Between 62 and 114% of the amounts of fungicide expected to be present in the three acidified treatments was detected.

The thiabendazole concentration determined for the ¼-rate treatment was higher than expected. One possibility is that, because the ¼-rate dip was done in tank A after this tank had been used the previous day for the ½-rate dip, this could have resulted in some 'stuck' precipitated fungicide from the previous treatment being solubilised by the more acidic solution of the fresh dip.

Table 1. Thiabendazole concentrations expected and observed in five HWT treatments.

Treatment	Fungicide concentration (ppm)		
	Expected	Actual, at start of dip[1]	Actual, at end of dip[1]
1 (1xTBZ – A)	1100	290±41	260±48
2 (1xTBZ + A)	1100	1102±218	963±102
3 (½TBZ + A)	550	341±45	392±42
4 (¼ TBZ + A)	275	314±41	289±48
5 (A only)	0	0	0

[1] mean value and standard deviation for four replicates

TBZ = thiabendazole (ai of Storite Clear Liquid)
A = acidifier as Sodium bisulphate

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Thiabendazole concentrations in bulbs

Table 2 shows the thiabendazole concentrations recorded for bulb samples taken after HWT and 24 hours drying.

- The concentrations of thiabendazole in bulb tissues of both cultivars were up to fifteen times higher in outer samples than in inner samples.
- Uptake of fungicide by Carlton bulbs was slightly greater than Golden Harvest in treatments 1 and 2 but this trend was reversed in treatments 3 and 4.
- Despite the much lower than expected concentration of fungicide found in the non-acidified dip solution (treatment 1, Table 1), this treatment resulted in the highest concentrations of thiabendazole recorded in and (or) on the bulbs.

Table 2. Thiabendazole concentrations in outer and inner samples of bulbs from five HWT treatments.

Treatment	Thiabendazole concentrations (ppm)[1]			
	Outer samples		Inner samples	
	Carlton	Golden Harvest	Carlton	Golden Harvest
1 (1xTBZ – A)	546±162	364±62	37±5	34±12
2 (1xTBZ + A)	350±96	196±59	8±3	6±6
3 (½TBZ + A)	106±33	156±37	6±6	3±3
4 (¼ TBZ + A)	110±21	125±36	7±1	5±2
5 (A only)	0	0	0	0

[1] mean value and standard deviation for eight bulbs (two bulbs from each of four replicates, assayed twice, i.e. sixteen values)

TBZ = thiabendazole (ai of Storite Clear Liquid)
A = acidifier as Sodium bisulphate

Bulbs will be lifted from the replicate plots of treatments 1 and 2 (both full-rate thiabendazole treatments) of both varieties 3, 6, 12 and 18 months after planting. These bulbs will be divided into outer and inner samples as before (except that roots will be removed and assayed separately), and the concentrations of thiabendazole determined. This will allow the anticipated bio-degradation of fungicide to be followed over two growing seasons.

Crop growth

Crop growth, bulb yields and disease control will be reported later.

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Discussion

Storite Clear Liquid is an acidic formulation of the fungicide thiabendazole. When used for narcissus HWT with mains water of pH 7.0-7.5 and other standard HWT additives (formalin, wetter and anti-foam preparation), it gives a dip pH of about 4.0. The pH of mains water in important bulb-growing areas of the UK is often close to neutrality or slightly alkaline (various Personal Communications), so the conditions in which the project was carried out should be close to practice on many bulb-growing farms. Thiabendazole is reported to be stable in acid and alkaline aqueous solution (Spencer, 1981), but the pH achieved will not be sufficiently low to achieve maximum solubility (see Introduction). It appears that this factor led to 'informal' recommendations, presumably originating with representatives of the agrochemical industry, for acidifying HWT dips containing Storite with sodium bisulphate (sodium hydrogen sulphate). Rates quoted at the time were 0.5 to 1.2 kg sodium bisulphate per 1000 litres. A 'standard' rate of 1.38 kg sodium bisulphate per 1000 litres was used in project BOF 43, equivalent to a 0.01M solution which is expected to give a pH of 2.18 (in practice about 2.5, see below). This rate was shown to be effective in maintaining near-maximal thiabendazole concentrations without any adverse effects on the narcissus crop. In that study, a lower pH level (pH 1.9) resulted in some crop damage, particularly to flower yields. In the present tests, the resultant dip pH was similar whether a full-, half- or quarter-rate of Storite was used. Over the course of a day's HWT, the pH of the acidified dip consistently rose by about 0.5 pH units, so further sodium bisulphate was added daily. In this case, some 0.3 kg sodium bisulphate per 1000 litres was needed daily, and a simple guide to calculate the required amount to add will be produced. This requirement to add more acidifier is probably because alkaline compounds present in soils and debris or possibly released from bulbs neutralise some of the acidifying potential of the sodium bisulphate. In practice, in farm-scale studies at Kirton, the pH values determined tended to be slightly higher (by 0.2 – 0.4 pH units) than the expected values or the values measured under laboratory conditions at Wellesbourne, due to some undetermined factor. However, the pH needed to maintain high thiabendazole solubility for bulb dipping purposes is unlikely to be this critical. It has also been reported that a dip pH of 4.9-5.3 was obtained in one instance of commercial bulb treatment despite using 1.17 kg sodium bisulphate per 1000 litres when treating reclaimed (ex-forced) bulbs, possibly because of a neutralising effect of adhering growing medium. Formalin concentrations seemed to be unaffected by the pH of the dips. It was found that an inexpensive temperature-compensating pH meter appeared adequate for monitoring pH in the HWT tank, while colour-change pH dipsticks appeared less reliable. pH meters should be calibrated regularly against reference buffer solutions, and, in an on-farm situation, the fragile electrode would have to be handled carefully to avoid damage.

Only 26% of the fungicide that was added to the HWT tank in the absence of any acidifier, and before any bulbs had been added, was detected in solution. This implies that it was lost rapidly from the circulating fluid, before the test sample was taken. A similar rapid reduction in circulating levels of thiabendazole was communicated in the final report of BOF 43. It was believed then that fungicide was lost from the tank fluid by absorption by or adsorption to bulbs, wooden bins and soil or other debris. This time the reduction occurred before any bulbs or bins had been place in the tank. It is known that the solubility of thiabendazole in aqueous solutions decreases with increasing pH with a maximum solubility of only 50ppm (0.05mg/ml) at pH 5. It is likely that at a pH near 4.0, as has been shown to occur in non-acidified dip solutions when bulbs are treated (see BOF 43 Final Report), the solubility will drop below the 1100mg/litre target concentration, causing some of the fungicide to precipitate. It is suspected that much of this precipitate will become deposited on surfaces within the HWT equipment, and hence removed from circulation. This quantity of chemical

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(up to 74%) will be unavailable for treating the bulbs. Some will stay in suspension, where it can, presumably, adhere to the outer surface of the bulbs. It is not known whether thiabendazole deposited from suspension on the bulb surface, or dissolved thiabendazole which may permeate the bulb's outer layers to some extent, is the more effective in treating basal rot. The location of the 'missing' fungicide requires further investigation, but good circulation with the elimination of dead-spots is obviously a pre-requisite for minimising losses of thiabendazole by deposition. Fluctuations in thiabendazole concentrations during dipping operations were observed in BOF 43 and can be linked, to some extent, to occasions when bins are added or removed or dip is pumped to and from the treatment tank. In Figure 2 inflexions in the pH graph are also seen at about 3-hourly intervals, as bins are removed and replaced.

In current usage, i.e. without using an acidifier, Storite seems to provide effective control of basal rot despite losses of active ingredient during HWT. Even the 'quarter-rate + acidifier' treatment maintained a higher circulating level of thiabendazole than the full-rate without acidifier suggesting that it may be possible to use reduced rates of Storite where these can be maintained in solution by acidification. The only in-bulb concentrations of thiabendazole available at present are those measured 24 hours after HWT and drying, although further samples will be examined at 3-month intervals. Quite large variations in fungicide content were observed between individual bulbs of both varieties tested in all treatments, possibly reflecting the variety in condition and shape of bulbs used, but nevertheless there were very clear differences between treatments. In all treatments where fungicide was present in the dip the concentrations of thiabendazole in the outer parts of bulbs (dry skins, two outer white scales and base plate), 106-546 ppm, were much higher than for the remaining inner parts of the bulbs (3-37 ppm). The greater concentration found in the outer samples indicates a poor penetration by the tank solution to the inner portions of the bulbs, or that most of the fungicide is deposited on the outer surface. Reductions of between 20-70% in concentrations of the fungicide in the outer samples were recorded when half-rate Storite was used in place of full-rate. The corresponding reductions between half-rate and quarter-rate were much less apparent and reflect the smaller than expected differences in actual fungicide concentrations determined in dip solutions of these two treatments. Unexpectedly, the highest concentrations of thiabendazole were found in bulbs treated with Storite at the full rate but with no acidifier (364-546 ppm in outer samples, 34-37 ppm in the inner samples). It is possible that the higher pH, although leading to lower solubility of thiabendazole, facilitates the deposition of the fungicide on the bulbs. However, thiabendazole concentrations of only 3 ppm are adequate to prevent the growth of *Fusarium oxysporum* f.sp. *narcissi* in culture, so even the lowest concentrations recorded in inner bulb samples in the quarter-rate treatment should be adequate to prevent tissue rot by *Fusarium*. Determination of the rate of loss of thiabendazole from bulb tissues will allow us to predict the likely duration of effectiveness of the fungicide in inhibiting growth of the basal rot pathogen.

So far, the acidification of Storite bulb dips has been evaluated only at HWT temperatures, and its effect in cold dips is not known. When bulbs are sprayed with Storite post-lifting, further acidification in that case was found (in project BOF 43) to be unnecessary. This is probably because the higher thiabendazole concentration used gives a lower pH in the spray tank, keeping the chemical in solution at least until the spray hits the bulbs. The concentration of fungicide at different positions in spray-treated bulbs is unknown at present. For use in HWT, sodium bisulphate is a cheap and convenient material to use for acidification, readily soluble and dissociating to hydrogen and sulphate ions in water and generating a low pH at a relatively low concentration (pH 2.18 at 1.38 kg per 1000 litres). Automated pH control of HWT tanks could be envisaged, with solutions metered to tanks as required.

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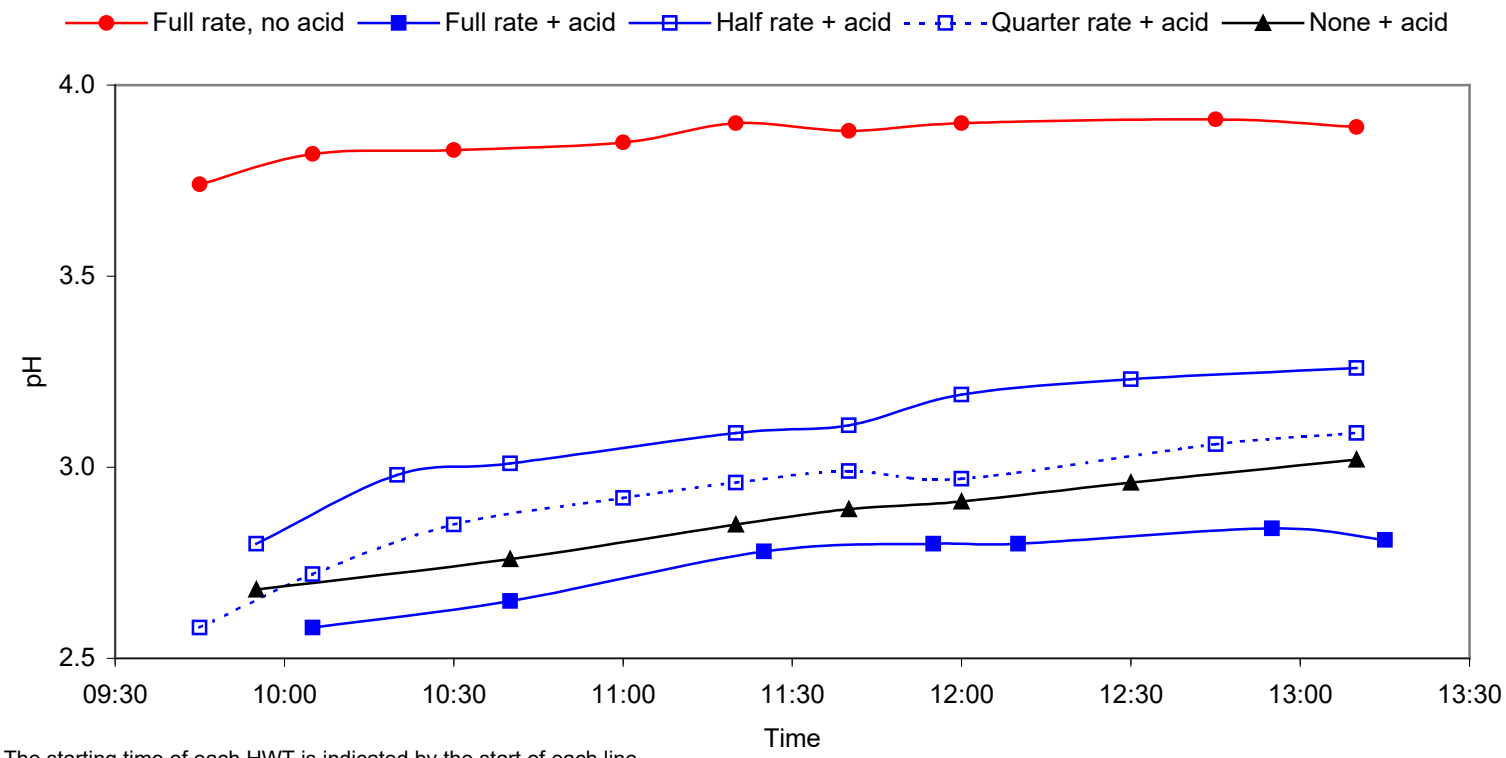
Figure 1. Dip pH for five HWT treatments at different rates of thiabendazole +/- acidifier (changes in pH shown over 3 hour HWT)



The starting time of each HWT is indicated by the start of each line

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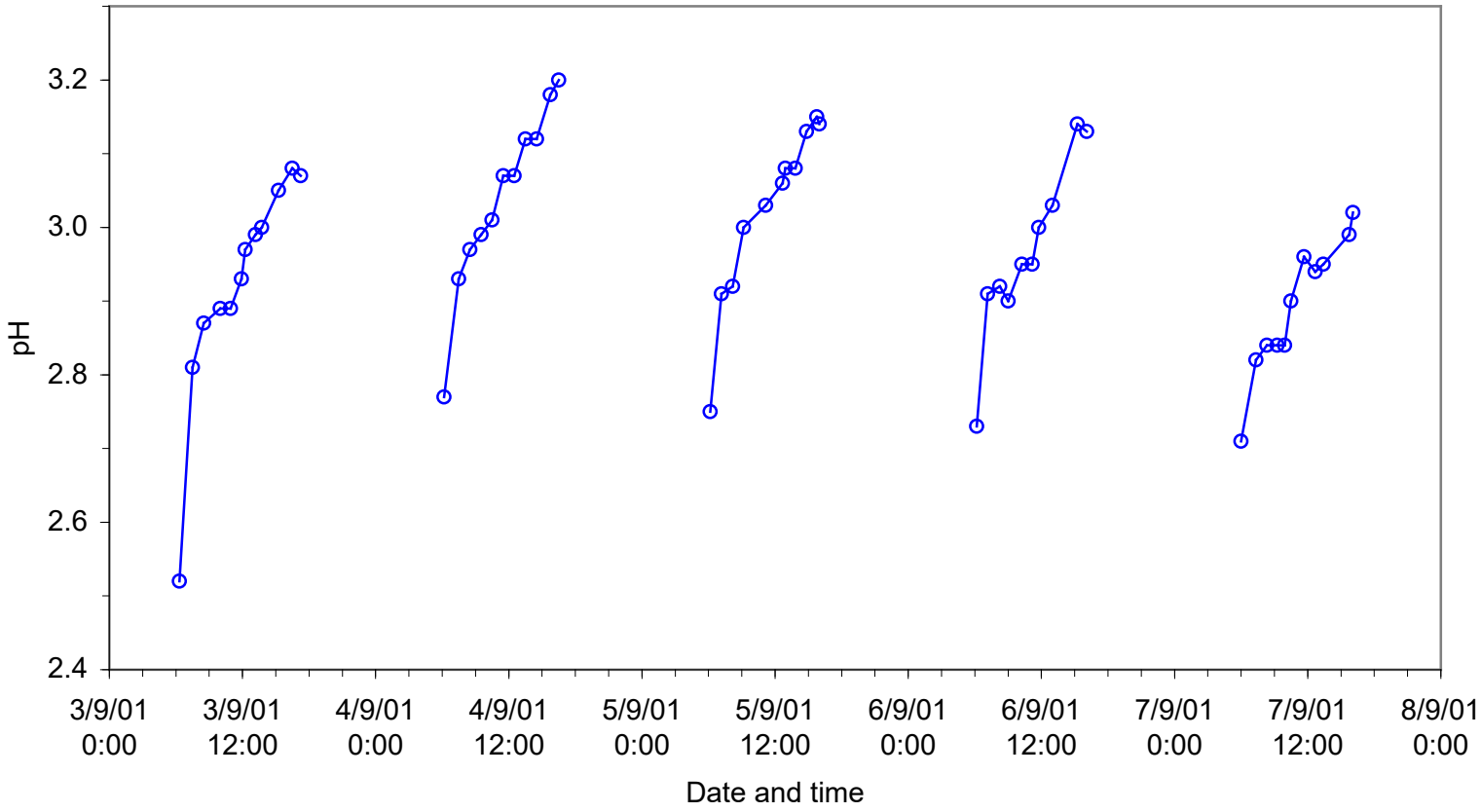
Figure 2. Changes in Dip pH over 5 days of HWT; 3 bins dipped per day; pH adjusted at the start of each day by the addition of more acidifier (sodium bisulphate)



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