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For accurate reporting, materials may be referred to by the name of the commercial product. No endorsement is intended of products mentioned, nor criticism of those not mentioned.

Grower Summary

Headline

- When using the fungicide thiabendazole, as Storite Clear Liquid, in the hot-water treatment (HWT) of narcissus bulbs, acidifying the dip maintains higher concentrations of dissolved thiabendazole, the active ingredient, in the tank.
- Using standard Storite/HWT treatments, effective concentrations of thiabendazole remain in the bulb for about 4 months, protecting the bulb during the critical autumn infection period.

Background and expected deliverables

The addition of the fungicide thiabendazole (as Storite Clear Liquid) to 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 recommendations available in print.

In an earlier phase of this project (BOF 43 Phase 1), 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 dissolved thiabendazole in the HWT tank, compared with using the standard, 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.

The expected deliverables from this project include the following:

- The avoidance of waste, and more cost-effective use, of Storite fungicide for managing basal rot in narcissus stocks.
- Understanding the dynamics of thiabendazole concentrations in narcissus bulbs, leading to the more rational design of measures to control basal rot.
- More attention to detail in the practice of HWT, perhaps the most critical treatment in the handling of narcissus bulbs.

Summary of the project and main conclusions

The effect of adding acidifiers in HWT on the concentration of thiabendazole in the dip
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.

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 0.2-0.4 pH units in all treatments. Using 'dipsticks' to measure pH was less reliable than using a simple pH meter. The target formaldehyde concentration was maintained irrespective of weather acidifier was added or not.

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. 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.

The concentrations of thiabendazole in dips were determined using a cup-plate diffusion assay and the results are shown in Table A below. 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 ppm 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. Thus, when no acidifier was added, a large proportion of the thiabendazole rapidly precipitated out in the HWT equipment.

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

Treatment (Storite rate and acidifier)	*Fungicide concentration (ppm)*		
	Expected	*Actual, at start of dip*	*Actual, at end of dip*
Full, no acidifier	1100	290	260
Full, + acidifier	1100	1102	963
Half, + acidifier	550	341	392
Quarter, + acidifier	275	314	289
None, + acidifier	0	0	0

Thiabendazole concentrations in bulbs following HWT and the effects of adding acidifier

The concentrations of thiabendazole were also determined in bulbs after treatment and planting. Thiabendazole levels in the outer bulb tissues and in the remaining inner bulb tissues were measured for the five treatments after HWT and 24 hours of drying. Although the lowest dissolved thiabendazole concentration had been recorded in the non-acidified Storite dip,

the highest concentration in bulbs was found with this treatment. This probably resulted from undissolved thiabendazole being deposited on the bulb surface during dipping. For cultivars Carlton and Golden Harvest, the concentrations in the non-acidified, full-rate dips 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, concentrations varied only from 3 to 8 ppm. No traces of thiabendazole were found in control bulbs not treated with Storite.

Some 90% of the thiabendazole in the bulbs was lost within 4 months of HWT, with little further loss over the next 3 months. During this 4 month period, concentrations of the fungicide in the outer bulb parts remained sufficient to control *Fusarium oxysporum* f.sp. *narcissi*, the basal rot fungus. Over the next 3 months, thiabendazole concentrations fell below effective levels (< 4 ppm).

Bulb and flower yields and levels of basal rot will be determined in 2003, after the normal two-year growing cycle, following which a full report and recommendations will be delivered.

Financial benefits

The full determination of financial benefits must await the conclusion of the project in 2003. If, as is likely, this shows that Storite rates can be halved by the addition of a cheap acidifier (less than £0.50 per tonne of bulbs treated), this would represent a saving of £25 per tonne of bulbs treated.

Action points for growers

- Adding sodium bisulphate to HWT (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. However, any firm 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 or undissolved fungicide might accumulate.
- The results confirmed that using Storite in HWT should protect bulbs from attack by basal rot over the critical post-planting autumn period, so the treatment is well worth using.

Full recommendations will be made after the completion of the project.

SCIENCE SECTION

INTRODUCTION

The background to this project was fully described in the previous Annual Report (December 2001). Briefly, basal rot (caused by *Fusarium oxysporum* f.sp. *narcissi*), the most important fungal disease affecting narcissus crops in the UK, is largely managed by treating the bulbs after lifting or before planting with the MBC fungicide thiabendazole (as Storite Clear Liquid). Probably the most usual method of application is to add the fungicide to the tank in which the bulbs receive their routine hot-water treatment (HWT), an expensive method since Storite costs about £50 per tonne of bulbs treated. In an earlier part of this project (see Final Report BOF 43 Phase1, December 2000), it was established that the rapid loss of dissolved thiabendazole that occurs in HWT tanks during this process could largely be prevented by acidifying the dip solution to a pH of 2.5 to 3.0 using sodium bisulphate.

This project extension was set up in 2001 to investigate this procedure further. In particular, the project sought to:

- Discover whether the concentration of Storite (and thereby the cost of treatment) could be reduced if its active ingredient were maintained in solution using acidification;
- Determine post-treatment concentrations of thiabendazole in the outer and inner tissues of bulbs over a two-year growing cycle;
- Produce definite recommendations for growers on using Storite and acidifier in HWT.

The previous Annual Report (2001) described the dynamics of thiabendazole concentration in the HWT tank during bulb treatment, and its immediate post-treatment concentrations in treated bulbs. The current report updates the earlier results, describing bulb thiabendazole concentrations in the first year after treatment. A Final Report will be issued in December 2003, following bulb yield and basal rot assessments of the two-year-down crop.

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 basal rot was to be expected in these bulbs.

Bulbs were withdrawn from 17°C storage in July-August 2001, 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:
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

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 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 HWT 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.

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 each plot being randomised. The bulbs were covered with soil by splitting-back the ridges. This gave a planting rate of 20 t/ha with ridges at 0.76 m centres.

The husbandry of the bulbs followed 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 was by dormant season diquat + paraquat, pre-emergence cyanazine and post-emergence chlorpropham + linuron. Crops received 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 were used according to standard recommendations. Flowers were not cropped. Crop records were maintained during the growing season, and bulb yields and level of bulb rots will be determined at the conclusion of the experiment.

Field trial design and statistical analysis

For the field trial an incomplete Trojan design was used, a 'row and column' layout that ensures an even distribution of treatments across the trial. There were four replicates. Gaps 2m-long were left between plots in the same ridge, and guard bulbs were planted round the edges of the trial. These 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 of each cultivar 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 outside layer of 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, 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 the other treatments (all of which contained acidifier).

For each cultivar, further sets of bulbs were lifted from the field from each of the four replicate plots of treatments 1 (full-rate Storite with no acidifier) and 2 (full-rate Storite + acidifier) in December 2001, March 2002 and June 2002 for thiabendazole analysis. Bulbs were quickly washed free of soil in running cold water and allowed to drain. Three bulbs per plot were divided as described above, but in addition to separating each bulb into outer and inner parts, the roots and the growing shoots (all material above the bulb 'neck') were also removed for separate analysis.

Determination of thiabendazole concentrations in dips and bulbs

Thiabendazole concentrations in HWT tank solutions and bulbs were determined using a 'cup-plate' diffusion bioassay (Carder, 1986; Yarden *et al*., 1985). Samples of HWT tank fluids were taken from the beginning and end 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 48h. Each dilution of every sample was placed in two wells on each of two diffusion plates.

Bulb samples were kept frozen until assessments of thiabendazole content were made. Each sample was weighed and placed in 15ml water (outer samples) or a volume equivalent to 1.5 times the weight of bulb tissue (inner samples). All samples in water were agitated gently for 6h to allow thiabendazole on and in the tissues to diffuse into the water. All outer diffusates were assayed directly by diffusion plate bioassay (as above) using 40µl volumes of an appropriate dilution. All inner diffusates were concentrated ten-fold before thiabendazole assay. Each dilution of every sample was placed in one well on each of two diffusion plates. The bulb parts subsequently collected from samples taken from field plots during the first growing season were subjected to a similar procedure, except that they were eluted in a volume of water equivalent to 1.5 times the weight of bulb tissue and all were concentrated ten-fold before thiabendazole assay.

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. Thiabendazole concentrations were calculated from a standard curve constructed by using a set of fungicide dilutions ranging in concentration from 10 to 100ppm. The limit of detection was 1ppm. 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 (Tables 2-5) 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:

- 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.

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[1]. 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. 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, thereby disturbing undissolved material.

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

[1] Figures start on page 16.

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 before dilution, the target concentration. A 'blank' test (water only) did not elicit 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 (Storite rate and acidifier)	*Fungicide concentration (ppm)*		
	Expected	*Actual, at start of dip*[1]	*Actual, at end of dip*[1]
Full, no acidifier	1100	290 ± 41	260 ± 48
Full, + acidifier	1100	1102 ± 218	963 ± 102
Half, + acidifier	550	341 ± 45	392 ± 42
Quarter, + acidifier	275	314±41	289 ± 48
None, + acidifier	0	0	0

[1] Mean value and standard deviation for four replicates

Thiabendazole concentrations in bulbs

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

- Using Storite at full rate, much higher thiabendazole concentrations were found in bulbs from treatment 1, where no acidifier was used, than in treatment 2, despite the much lower than expected concentration of thiabendazole found in the non-acidified dip solution compared with the acidified dip (Table 1). This was true for both inner and outer bulb tissue samples, with inner samples retaining between 15% and 50% of the total amount of fungicide taken up by a bulb. The larger numerical differences in concentrations shown in Table 2 between inner and outer samples reflected the much greater weight of the inner portions, which often represented over 80% of bulb mass.

- As expected, thiabendazole concentrations in bulbs treated with half-rate Storite (treatment 3) were proportionally lower. The concentrations in the quarter-rate treatment (treatment 4) were similar (see above).
- In control bulbs (no Storite in HWT) thiabendazole could not be detected. Therefore, untreated bulb samples taken before HWT were not analysed for thiabendazole.
- 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 the low-rate treatments.

Table 2. Thiabendazole concentrations in outer and inner samples of bulbs from five HWT treatments, sampled after HWT and 24 hours drying (August 2001).

Treatment (Storite rate and acidifier	*Thiabendazole concentrations (ppm)*			
	Outer samples		*Inner samples*	
	Carlton	*Golden Harvest*	*Carlton*	*Golden Harvest*
Full, no acidifier	546 ± 162	364 ± 62	37 ± 5	34 ± 12
Full, + acidifier	350 ± 96	196 ± 59	8 ± 3	6 ± 6
Half, + acidifier	106 ± 33	156 ± 37	6 ± 6	3 ± 3
Quarter, + acidifier	110 ± 21	125 ± 36	7 ± 1	5 ± 2
None, + acidifier	0	0	0	0

The data above showed clear differences between treatments with respect to the quantities of thiabendazole acquired by bulbs during HWT with Storite present. Subsequently, samples of bulbs were lifted at three dates during the first growing season from each of the two full-rate Storite treatments. The data in Table 3 showed that bulbs lifted during December 2001 (when green shoots were emerging above ground) displayed a dramatic reduction in fungicide concentration in outer samples. Between ten- and sixty-fold lower values were recorded compared to pre-planting concentrations obtained three months' earlier. Inner samples also demonstrated a decline, but only of the order of three- to ten-fold reductions. The levels of thiabendazole in shoot samples were below the limit of detection, and in further samples shoots were not analysed. However, roots showed slightly higher concentrations of fungicide than inner samples, indicating either uptake by roots of chemical from the soil environment or translocation from other regions of the growing bulb into the roots.

Table 3. Thiabendazole concentrations in bulb parts sampled December 2001.

Treatment (Storite rate and acidifier)	*Thiabendazole concentrations (ppm)*[1]							
	Outer samples		*Inner samples*		*Roots*		*Shoots*	
	Carlton	*GH*	*Carlton*	*GH*	*Carlton*	*GH*	*Carlton*	*GH*
Full, no acidifier	48 ± 14	22 ± 16	14 ± 8	3 ± 3	22 ± 17	13 ± 16	0	0
Full, + acidifier	17 ± 9	3 ± 4	3 ± 3	1 ± 2	13 ± 10	2 ± 3	0	0

Table 4 shows fungicide levels from samples taken in March 2002, about 6 months after planting. In general, concentrations are similar to those seen in December. The relatively unchanged levels of fungicide observed may be due to low soil temperature during this period and a corresponding reduction in microbial activity, since the latter is likely to be the major cause of fungicide degradation.

Table 4. Thiabendazole concentrations in bulb parts sampled March 2002.

Treatment (Storite rate and acidifier)	*Thiabendazole concentrations (ppm)*					
	Outer samples		*Inner samples*		*Roots*	
	Carlton	*GH*	*Carlton*	*GH*	*Carlton*	*GH*
Full, no acidifier	65 ± 37	47 ± 41	12 ± 12	4 ± 4	19 ± 32	14 ± 25
Full, + acidifier	8 ± 8	2 ± 3	1 ± 1	0	4 ± 5	1 ± 1

Three months later, when natural leaf senescence was very advanced, the thiabendazole concentrations in all samples were very low or below the limit of detection (Table 5). Soil temperatures would have risen over this period. The decreases in fungicide concentrations observed between March and June 2002 were of the same order as those noted between August and December 2001. All parts of all bulbs, except the outer portions of Carlton bulbs from both treatments and Carlton roots from treatment 1, now displayed concentrations of thiabendazole below those required to inhibit the growth of *Fusarium oxysporum* f.sp. *narcissi* (<4ppm).

Table 5. Thiabendazole concentrations in bulb parts sampled June 2002.

Treatment (Storite rate and acidifier)	*Thiabendazole concentrations (ppm)*					
	Outer samples		*Inner samples*		*Roots*	
	Carlton	*GH*	*Carlton*	*GH*	*Carlton*	*GH*
Full, no acidifier	4 ± 7	1 ± 2	1 ± 1	0	4 ± 8	0
Full, + acidifier	4 ± 12	0	0	0	0	0

Figure 3 presents the thiabendazole concentrations for all sample dates. In view of the very low or undetectable levels of thiabendazole in the June samples, and the likelihood of further decreases throughout the summer and autumn months, it was decided not to continue routine sampling to measure further fungicide concentrations.

Crop growth

Crop growth in 2002 appeared normal. There were fewer flowers in treatments 1 and 5 than in other treatments. This is likely to have been due to increased bulb rotting as a consequence of having no Storite (treatment 5) or a low, ineffective concentration of Storite (treatment 1) in HWT. Flower and bulb yields and bulb rots will be assessed in 2003, after the two-year-down growing cycle.

DISCUSSION

In this project, 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. The concentrations of thiabendazole in dips and in bulbs were determined using a cup-plate diffusion assay. Full rate Storite corresponded to a target thiabendazole concentration of 1100 ppm. The initial results were summarised in the previous Annual Report:

- 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. In a 5-day HWT run, 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.
- 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. Thus, when no acidifier had been added, a large proportion of the thiabendazole rapidly precipitated out in the HWT equipment.
- After HWT and drying, the highest thiabendazole concentration in bulbs was found when the non-acidified Storite dip treatment had been used, although this treatment gave the lowest concentration in the dip. A white deposit was clearly visible on these bulbs after this treatment, probably resulting from undissolved thiabendazole being deposited on the bulb surface.
- For the two cultivars Carlton and Golden Harvest, concentrations in the non-acidified treatment were 546 and 364 ppm for the outer tissues, and 37 and 34 for the inner tissues, respectively. Where acidifier was used with a full Storite rate, the thiabendazole concentrations for the outer tissues were 350 and 196 ppm, and for the inner tissues the concentrations varied only from 3 to 8 ppm.

During 2001-2002, the thiabendazole concentrations of bulbs were determined three, six and nine months after planting. Most thiabendazole was found in the outer parts of the bulb, and here some 90% of the thiabendazole was lost between August and December. There was little further loss of thiabendazole over the following three months, perhaps due to low temperatures, but by June 2002 concentrations had fallen to levels that would no longer control the basal rot pathogen. These findings indicate that using Storite in HWT would not protect bulbs from attack in the summer one year after planting, irrespective of whether full or reduced rates were used, nor whether an acidifier was used or not. Nevertheless, for the protection of bulbs in the late-summer and autumn after planting, a critical infection period when soil temperatures are still high and the roots are emerging from the basal plate (creating points of entry for pathogens), significant savings might be achieved through using acidified half- or quarter-rate Storite in HWT.

The completion of the project awaits the determination of flower and bulb yields and bulb rot assessments in the second year of the crop, 2003. This will determine, on a practical level, whether reduced-rate Storite treatments control basal rot.

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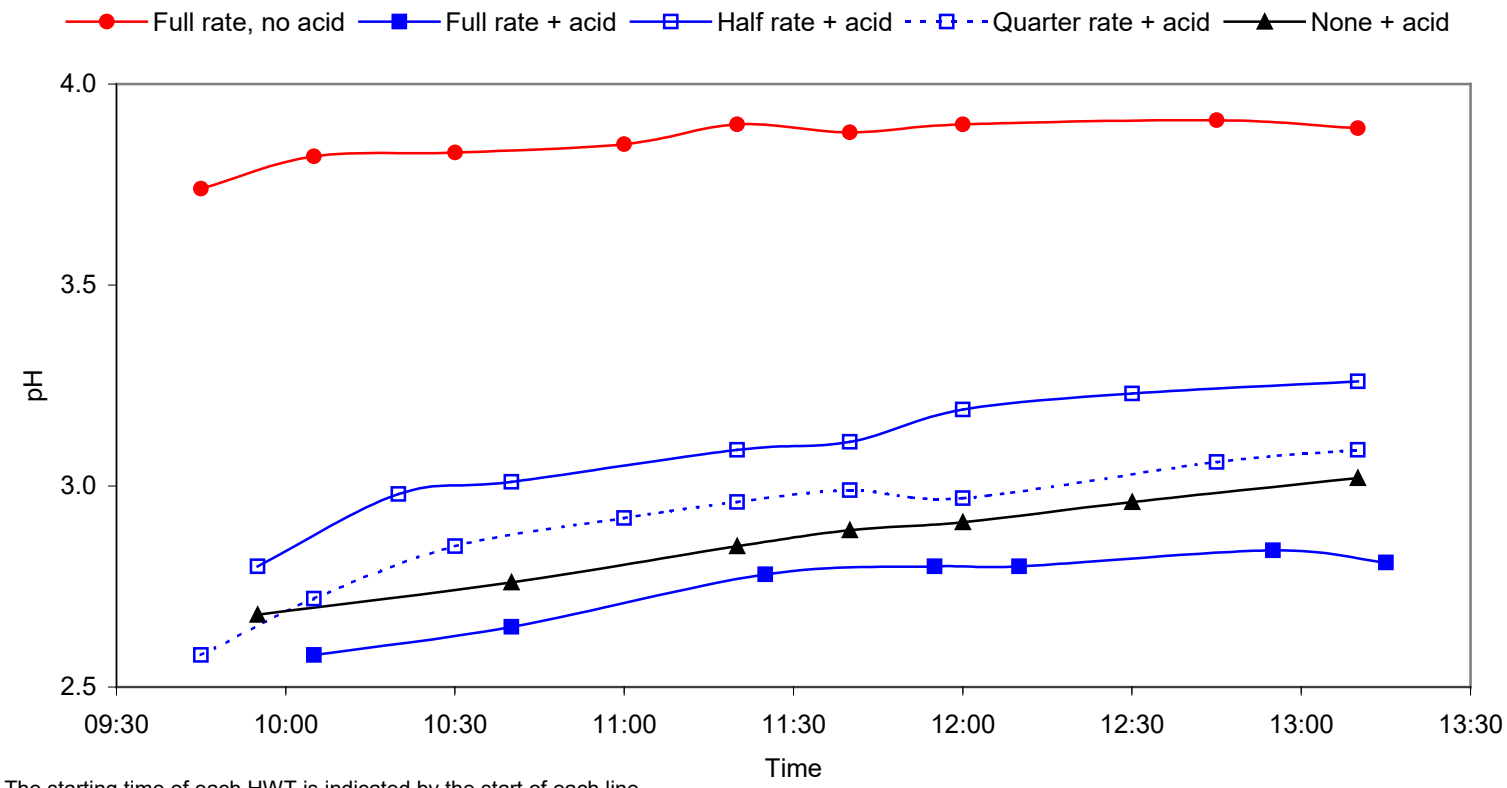
Figure 1. Dip pH for five HWT treatments



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

Figure 2. Dip pH over 5 days of HWT

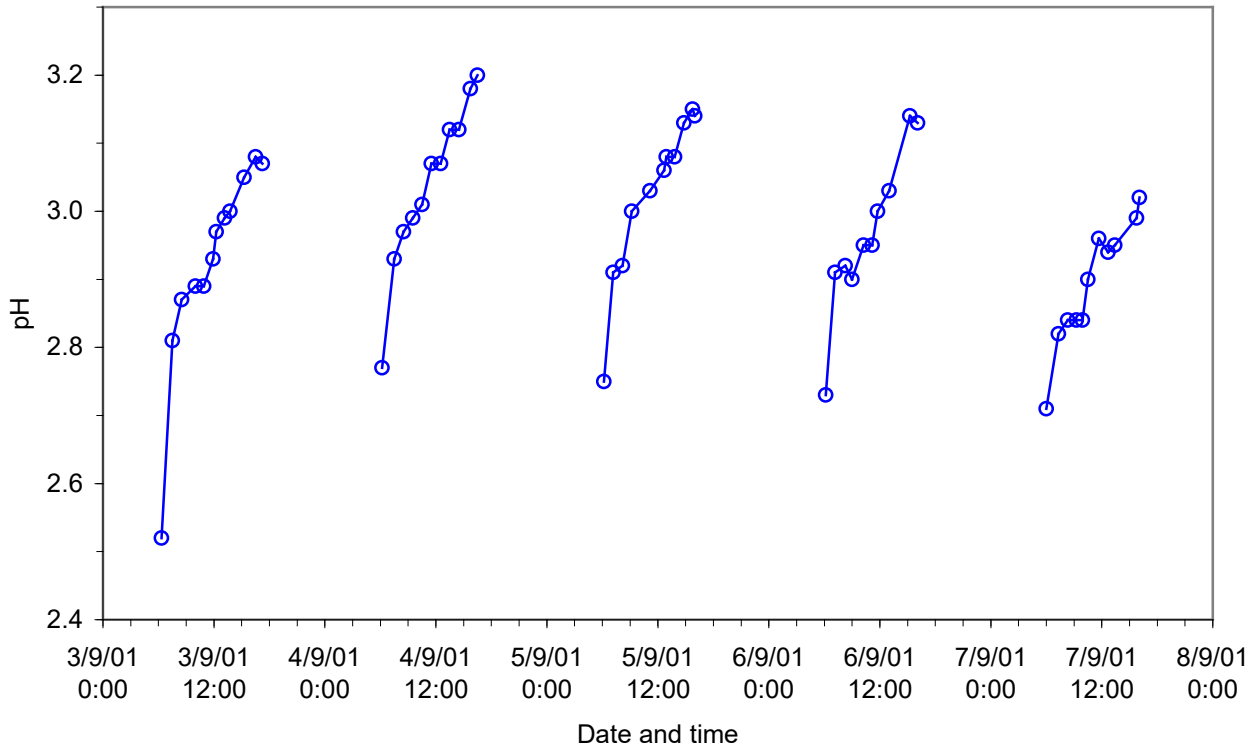
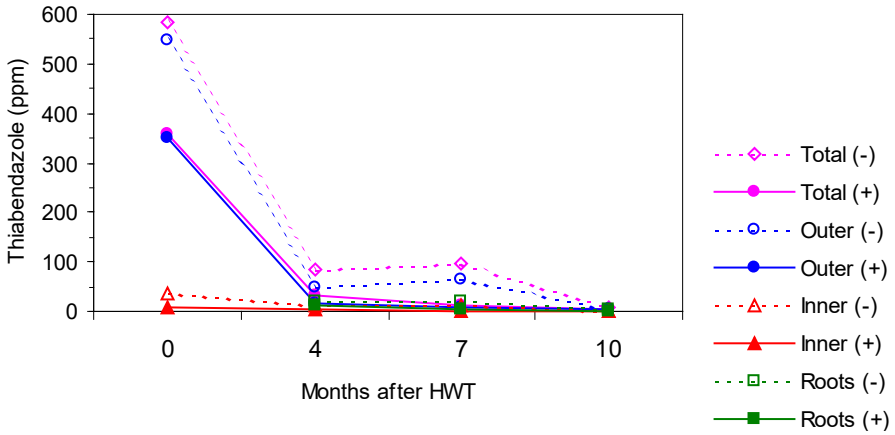


Figure 3. Thiabendazole concentrations in bulb tissues over the first year of growth

(+) = plus acidifier
(-) = no acidifier

Carlton



Golden Harvest