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#### BOF 43A FINAL REPORT 2003

CONTENTS	Page no.
GROWER SUMMARY	1
SCIENCE SECTION	5
Introduction	5
Materials and methods	6
Results	12
Discussion	21
Acknowledgements	30
References	30
Figures	33

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# **GROWER SUMMARY**

## Headline

- When using Storite Clear Liquid ('Storite') in hot-water treatment (HWT), acidifying the dip to pH 2.5 3.0 helps maintain the concentration of dissolved thiabendazole in the tank without any adverse effects on growth.
- Acidification is done by adding an appropriate amount of sodium bisulphate (SBS).
- Acidification enables the rate of 'Storite' used to be reduced to quarter- or half-rate, reducing costs by up to 75%.

## Background and expected deliverables

The addition of the fungicide 'Storite' 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 (SBS, also known as sodium hydrogen sulphate), to HWT tanks, to improve the effectiveness of the thiabendazole, but this practice had not been properly tested, nor were recommendations available in print. In an earlier HDC-funded project, BOF 43, the use of thiabendazole, with or without various additions of SBS, was evaluated. Adding SBS, 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 treatment. The lower pH 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.

## Summary of the project and main conclusions

- Bulbs of narcissus cultivars 'Carlton' and 'Golden Harvest' were given five treatments:
  - 1. Full-rate 'Storite' with no SBS
  - 2. Full-rate 'Storite' + SBS
  - 3. Half-rate 'Storite' + SBS
  - 4. Quarter-rate 'Storite' + SBS
  - 5. SBS but no 'Storite'

Standard rates of formalin, non-ionic 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 SBS 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 (equivalent to 5 litres of commercial formalin per 1000 litres dip) was maintained irrespective of whether

SBS 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 SBS 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 SBS 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 total concentration of thiabendazole in dips was determined using a cup-plate diffusion assay. The target concentration of thiabendazole in full-rate dips was 1100 ppm (equivalent to 5 litres 'Storite' 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 SBS 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 plus SBS, the 'initial' values were 341 and 314 ppm, respectively, and the values after 3 hours were 392 and 289 ppm, respectively. Thus, when no SBS was added, a large proportion of the thiabendazole rapidly precipitated out in the HWT equipment.
- The concentrations of thiabendazole were also determined in bulbs after treatment and planting. Thiabendazole levels in the outer bulb tissues (i.e., the outer dry skin plus the two outermost white scales) and in the remaining inner bulb tissues were measured for the five treatments after HWT and drying for 24 hours. Although the lowest 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 suspended 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, respectively. Where SBS 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 relatively little, between 3 and 8 ppm. No trace of thiabendazole was found in control bulbs not treated with 'Storite'.
- Some 90% of the thiabendazole found in the bulbs from all treatments 24 hours after HWT was lost within 4 months of HWT. Little further loss occurred over the next 3 months. During this 7-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.
- In the first crop year there were conspicuously fewer flowers, in both cultivars, in the 'no SBS' and 'no Storite' treatments than in treatments which included 'Storite' (full, half or quarter rate) *and* SBS. In the case of the 'no Storite' treatment, this was probably due to extensive and rapid bulb rotting in the crucial weeks after planting, leading to extensive flower loss. In the 'no SBS' treatment, losses were presumably due to the effects of an ineffective fungicide treatment being expressed in the field. Otherwise, crops appeared normal.
- In the second crop year the effect of HWT treatments was different in the two cultivars. In 'Carlton', flower yield was reduced only in the 'full-rate Storite, no SBS' treatment, presumably as a result of continuing bulb losses. In 'Golden Harvest', flower yields were significantly lower in both 'no SBS' and 'no Storite' treatments than in the three treatments with 'Storite' *and* SBS. HWT treatment had no significant effect on either stem or foliage length in either cultivar. In other respects the crops appeared normal in all treatments.

- When 'Storite' was applied in the field as a foliar spray towards the end of the growing season, or as a shoot/soil drench after the senescent foliage had been flailed off, it was not possible to detect thiabendazole in the bulb tissues sampled between 1 day and 3 weeks after application.
- In 'Carlton', the effects of these treatments on bulb yields were relatively small; this was a healthy stock in which only a moderate amount of bulb rot would normally be expected, and only 2% of lifted bulbs were rejected at grading due to rotting. There was a significant effect of HWT treatment on the weight of sound (marketable) bulbs, with half- and quarter-rate 'Storite' treatments, with SBS, giving the highest yields, and the 'no Storite' treatment the lowest. HWT treatment did not have significant effects on the total *numbers* of sound or rotted bulbs, but there were clear trends: the numbers of marketable bulbs were highest where a half- or quarter-rate of 'Storite' was used with SBS, and the numbers of rotted bulbs were lowest where a full- or half-rate of 'Storite' was used with SBS. There were significantly higher yields in the larger (14-18 cm) grades of bulbs when a half-rate of 'Storite', plus SBS, had been used.
- The bulb yields for 'Golden Harvest', a basal rot-susceptible cultivar, showed much larger treatment effects. Overall, 14% of bulbs were rejected at grading owing to rots. There were highly significant effects of treatments on the total numbers of sound bulbs obtained, ranging from 132 per plot for the 'no Storite' treatment, to 397 per plot for the 'full-rate Storite, no SBS' treatment. The low numbers of bulbs obtained from the 'no Storite' treatment was linked to a large number of rotted bulbs (64 per plot) and a reduced yield across all bulb grades. The high numerical yield of the 'full-rate Storite, no SBS' treatment was associated with markedly higher bulb numbers in the small (<12 cm) grades of bulbs.
- After grading, bulbs were stored for further assessment 3 months later. In 'Carlton', as expected from the previous results, there were very few rotted bulbs, and no treatment effects could be discerned. In 'Golden Harvest' there were significant numbers of bulbs with basal rot: 9% in the 'no Storite' treatment, and 1-3% in other treatments. Combining all types of rots for 'Golden Harvest', 12% of bulbs were affected in the 'no Storite' treatment, and 4-6% in the other treatments. Only small numbers of 'Golden Harvest' bulbs were affected by neck or whole-bulb rots.
- It was noted that a significant number of these bulbs was infested by larvae of the large narcissus fly. Overall, 4 and 5% of 'Carlton' and 'Golden Harvest' bulbs, respectively, were affected.

# **Financial benefits**

By using a quarter rate of 'Storite' with SBS, fungicide costs can be reduced by 75% with no loss of effect. This reduces the cost of 'Storite' used from about £50 per tonne of bulbs to about £13 per tonne, allowing for the small cost (about £0.50) of the SBS required.

## Action points for growers

- 'Storite' should be used at a rate of 1.250 litres per 1000 litres of dip when making up tanks. After each dip, 0.375 litres of 'Storite' should be added to tanks for every 1 tonne of bulbs dipped. When the water level in the tank is topped-up, 0.125 litres of 'Storite' should be added for each 100 litres of water added.
- SBS should be added to the tank at a rate of 1.380 kg per 1000 litres of dip. When the

water level in the tank is topped-up, 0.138 kg of SBS should be added for each 100 litres of water added. At the start of each day's dipping, an additional 0.250 kg of SBS should be added per 1000 litres of dip, though this quantity should be adjusted according to the actual dip pH. A small portable pH meter should be used to check dip pH, perhaps at the start and end of each dip. The aim is to maintain a dip pH between 2.5 and 3.0, though the exact pH value is not critical. Lower pH values may be harmful to bulbs, and higher pH values will be ineffective in optimising the effect of 'Storite'.

- In all cases, the SBS should be added to the tank water first, and time for dissolving and mixing should be allowed, before adding 'Storite' and other ingredients.
- Other additives including formalin, non-ionic wetter and anti-foam compound and HWT conditions temperatures, durations, timing, etc. should continue to follow standard advice.
- Spot sampling of HWT dips for measurement of fungicide concentrations is recommended, to enable growers to become familiar with the thiabendazole dynamics of their tanks.
- A COSHH assessment should be carried out if the grower's HWT procedures have changed as a consequence of these findings.

#### SCIENCE SECTION

#### **INTRODUCTION**

By common agreement amongst most UK bulb growers, basal rot (base rot) caused by the fungal pathogen Fusarium oxysporum f.sp. narcissi is the most important fungal disease affecting narcissus crops grown in this country. The main element in management of the disease is the use of the fungicide thiabendazole (as Storite Clear Liquid, 'Storite'<sup>1</sup>), which is usually applied by including the product in the hot-water treatment (HWT) tank (ADAS, 1985, 1988). Despite the general effectiveness of this fungicide, as demonstrated over many years in trials and in practical usage, in some instances the control of basal rot achieved is disappointing. Bulb growers have long been concerned about the white, scummy deposits that appear on bulbs taken from the first few dips in the HWT tank, suspecting that this contains active ingredient that has come out of solution and has been deposited on bulbs and other surfaces (including the bottom of the tank), thereby seriously depleting the amount of thiabendazole remaining in solution in the tank. Also, there have been reports that analyses of tank dips had indicated lower-than-expected concentrations of thiabendazole (various growers, personal communications). Two random samples of tank bottom sludge were found to contain 4405 and 563 ppm thiabendazole, very high, but rather different, amounts (A. Jukes, personal communication). Despite these concerns, apparently no advice addressing the problem could be elicited from representatives of the pesticide suppliers; one responded, without addressing the solubility issue, that "only 5% [of thiabendazole] degraded at 43°C after 3 weeks, and 10% at 54°C after 1 week" (AgrEvo UK Ltd., personal communication). Similar concerns about the loss of benomyl (as Benlate Fungicide, another benzimidazole compound) in HWT tanks had been demonstrated earlier (Hanks, 1990b). A further concern with 'Storite' is that this formulation is expensive. It costs about £50 to treat a tonne of bulbs in HWT at the full, recommended rate, which becomes prohibitive when bulb prices, notoriously cyclical, drop as low as about £200 per tonne (for commonplace cultivars).

Anecdotal recommendations to add an acidifier, SBS (sodium bisulphate or sodium hydrogen sulphate, NaHSO<sub>4</sub>) to 'Storite' dips have been circulating for several years in the UK bulb community, apparently originating from agrochemical salesmen (various bulb growers, personal communications). This recommendation relates to the decreasing solubility of thiabendazole in water as its pH increases. The maximum achievable thiabendazole concentration in water at pH 2.2 is 38.4 mg/ml (38,400 ppm), but it falls to 0.05 mg/ml (50 ppm) at pH 5.0 (Spencer, 1981; Budvari et al., 1996). The target thiabendazole concentration for narcissus dipping is 1.1 mg/ml (1100 ppm), based on using 5 litres of 'Storite' per 1000 litres of dip initially, topping up tanks with 'Storite' at the same rate, and adding 1.5 litres 'Storite' for every tonne of bulbs that had been treated in the tank. The unwritten advice for acidifying bulb dips appears to have originated in other research to improve the solubility, and therefore the effectiveness, of benzimidazole fungicides. This work involved the control of Verticillium wilt of cotton (Buchenauer & Erwin, 1972) and of Dutch elm disease (Prasad, 1972; Clifford et al., 1976, 1977). These researchers used mineral acids to acidify fungicide solutions. The advice for bulb dipping was to use SBS, which dissociates in aqueous solution to give the ions Na<sup>+</sup>, H<sup>+</sup> and SO4<sup>2-</sup>, therefore being acidic and in fact behaving like a solution

<sup>&</sup>lt;sup>1</sup>For convenience, and where the context is clear, Storite Clear Liquid is referred to simply as 'Storite' for the rest of the report.

of dilute sulphuric acid (Wood & Holliday, 1963). Rates of SBS used in HWT by bulb growers ranged from 0.9 to 1.2 kg per 1000 litres, reportedly giving dip pH values of 4.2 to 5.2 in commercial use (R. Willingham, personal communication).

In a one-off trial at Kirton in 1992-1994, part of a MAFF-funded project to reduce pesticide usage in bulb growing, narcissus bulbs received HWT with full-rate 'Storite' (no acidifier) or half-rate 'Storite' with acidifier (1.4 kg SBS per 1000 litres dip, a 0.01N solution with a theoretical pH of 2.5). This acidification appeared to improve the control of basal rot in a heavily diseased stock of 'Golden Harvest', and was without adverse side-effects in a healthy 'Carlton' stock (G.R. Hanks, unpublished data). Acidifying 'Storite' HWT dips was also tested in an earlier HDC-funded project (BOF 43; Hanks, 2000). It was shown that the concentration of thiabendazole fell rapidly in HWT tanks, and that adding SBS to the tank greatly improved the maintenance of the fungicide concentration during treatment. The acidification of bulb dips to pH 2.5 - 3.0 resulted in a circulating concentration of thiabendazole of 0.79 mg/ml (790 ppm) after 5 days, compared with 0.26 mg/ml (260 ppm) under normal use without acidifier. At the pH stated, acidification did not appear to result in phytotoxic damage to the narcissus bulbs or plants. For the small additional cost of adding SBS to HWT tanks, i.e. about £0.50 per tonne of bulbs treated, the effectiveness of 'Storite' in basal rot management will be enhanced. Further, assuming that the label recommendation for using 'Storite' on bulbs was calculated to take account of the normal loss of active ingredient in aqueous solution, significant financial benefits should follow if acidification enabled a reduced rate of fungicide to be used with confidence.

The current project (BOF 43a) was set up in 2001 to investigate this practice further. In particular, the project sought to:

- Discover whether the concentration of 'Storite' (and thereby the cost of treatment) could be reduced, without loss of disease control, if its active ingredient were maintained in solution by acidification with SBS;
- 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 SBS in HWT.

## **MATERIALS AND METHODS**

#### Plant material

Bulbs of narcissus cultivars 'Carlton' and 'Golden Harvest', taken from stocks grown at Horticulture Research International (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; Hanks, 2002). Bulb stocks were lifted in July 2001 and dried for 3 days at 35°C ('high temperature drying') 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. Prior to 2001 the stocks had received routine post-lifting dip *and* HWT treatments with thiabendazole and formalin, except that in 1999 thiabendazole and formalin had only been given in HWT; therefore, as these cultivars are susceptible to basal rot, a moderate incidence of the disease was to be expected in controls and ineffective treatments.

Bulbs were withdrawn from  $17^{\circ}$ 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 nylon net bags for use in experimental plots. Bulb storage was continued at  $17^{\circ}$ C under fans until bulbs were required for HWT. As the design of the main experiment called for the HWT of full bulk bins of bulbs (to simulate commercial practice), further bulbs (cv 'Golden Harvest') were allocated to provide a supply of full bins in which the small experimental lots were buried amongst the loose bulbs for treatment. Twenty,  $\frac{1}{2}$ -tonne bins of bulbs were allocated for this purpose and for supplementary work.

## 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 bulbs of each cultivar, was used. The weighed lots were placed in each bin prior to treatment. The five treatments were:

- 6. Full-rate 'Storite' with no SBS
- 7. Full-rate 'Storite' + SBS
- 8. Half-rate 'Storite' + SBS
- 9. Quarter-rate 'Storite' + SBS
- 10. SBS but no 'Storite'

HWT was carried out in two front-loading tanks each capable of treating 2 x  $\frac{1}{2}$ -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  $\frac{1}{2}$ -tonne bin using 3000 litres of dip. The treatment order was semi-randomised (such that full-rate 'Storite' dips could be put to further use at the conclusion 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 in tank A 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. Although tanks were cleaned between treatments (see below), this order of dips served as a precaution minimising the contamination of later treatments by thiabendazole residues from previous treatments.

Each HWT consisted of a 3-hour period at 44.4°C, plus an initial period (of about 15 to 20 minutes as required) 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 Cargill Agriculture Ltd; 220 g a.i. per litre) per 1000 litres water. The acidification treatment consisted of adding 1.38 kg SBS (sodium bisulphate technical grade powder, Banks Cargill Agriculture Ltd) per 1000 litres water. All treatments contained the following dip additives per 1000 litres:

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

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, when the SBS (where appropriate) was added first and the 'Storite' 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 ('Oriented 1'; Netlon Ltd), distributing the bulbs evenly by using plastic clips at intervals. Planting bulbs in netting enables a near-100% recovery of the bulbs at the end of the experiment, and does not significantly affect growth (G.R.Hanks, unpublished data). The 5-kg lots (to be available for sampling at intervals) were not placed in netting for planting.

#### 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 HWT 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 during HWT and remained within acceptable limits throughout.

## Planting and cultural practices

Bulbs were planted in the field on 13 September 2001. After ploughing and cultivation, and following the usual procedures for planting bulb 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 (this order of herbicides has been found to work well on

narcissus crops at Kirton, though it differs from commercial practice in which chlorpropham + linuron are applied pre-emergence and cyanazine post-emergence). 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

The numbers of flowers per plot was recorded in both crop years, where necessary distinguishing between normal (marketable quality) flowers, damaged flowers (usually due to HWT) and dead ('blasted') buds. In the second crop year, stem and foliage lengths were recorded for ten central stems or plants in each plot.

Bulb were lifted from the field on 21 July 2003, placed in bulb trays and dried at ambient temperatures by fans for two weeks. Bulbs were then cleaned, clusters split by hand, and graded, recording the numbers and weights of sound (marketable) bulbs in each grade (<8, 8-10, 10-12... and >18 cm circumference) and the number of rotted bulbs (which were then discarded).

To assess the potential for storage rots, bulbs from the middle grades (10-12 and 12-14 cm) were replaced in trays and stored at ambient temperatures in a shed for three months. One hundred bulbs per tray were taken at random and cut in half lengthways; the presence or absence of bulb rots (basal rot, neck rot, whole-bulb rot and mummified (dried) bulbs), and of the number of bulbs with large narcissus fly larvae, were recorded.

## Field trial design and statistical analysis

For the main field trial an incomplete Trojan design was used, a 'row and column' layout that ensured 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. The data were subjected to the analysis of variance as appropriate. When initial planting weight was included as a co-variate in the analysis, the co-variate effect was not significant, therefore un-adjusted data have been quoted throughout.

## 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, the outermost two white scales, and the outside layer of the base plate were removed ('outer tissues'). The outer tissues and the remaining parts ('inner tissues') 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 the 'full-rate Storite, no SBS' treatment, but were not seen on bulbs from the other treatments, all of which

### contained SBS.

For each cultivar, further sets of bulbs were lifted from the field from each of the four replicate plots of the 'full-rate Storite, no SBS' and the 'full-rate Storite + SBS' treatments 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 tissues, the roots and the growing shoots (all material above the bulb 'neck') were also removed for separate analysis.

Following analysis of the June 2002 bulb samples, since only very low or non-detectable thiabendazole concentrations had been found in that sample, no subsequent bulb samples were tested.

### Pre-lifting 'Storite' application

The application of 'Storite' as a spray in the field, shortly before lifting bulbs, could be a further means of controlling bulb rots. Since no bulb sampling was deemed necessary in the second crop year (see above), control plots (which had received no 'Storite' treatment) were utilised to examine whether thiabendazole was taken up by the plants when applied as a prelifting spray.

On 3 June 2003, when the crop foliage was starting to become yellow, control (untreated) bulbs were recovered and sampled, and one part of the plots was sprayed to run-off with thiabendazole (as 25ml 'Storite' in 5 litres water), using a precision sprayer and a single medium-quality nozzle. These bulbs were sampled 1 and 3 weeks later. The same spray application was applied to other ends of the plots on 23 June 2003, when the foliage was becoming senescent, and these bulbs were also sampled 1 and 3 weeks later. On 7 July 2003, the remaining foliage on another part of the plots was flailed off to ground level, following which 'Storite' (same concentration and sprayer as above) was applied as a directed spray to the cut ends of the bulb shoots; bulbs were sampled 1 day and 1 week later. These bulbs were sampled, extracted and tested for thiabendazole concentration as described above.

#### 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 dips 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 1.8 mm-layer of potato dextrose agar was poured into a shallow glass tray and allowed to set. Then 0.75 ml of a spore suspension of *Fusarium oxysporum* f. sp. *narcissi* (HRI isolate LVB Na2), containing 1 x 10<sup>6</sup> spores/ml, was spread evenly over the agar surface. Using a cork borer 7 mm in diameter, discs were removed from the agar layer at regular spacing (centres 40 mm apart horizontally and vertically). Aliquots of test samples (40  $\mu$ l) were placed in these wells, the plate covered and incubated at 25°C for 48 h, when measurements were made. Each dilution of every sample

was placed in two wells on each of two diffusion plates. This procedure quantified the concentration of dissolved thiabendazole circulating in the dip, excluding any suspended material that settled out during the 30 minutes' standing and any insoluble fungicide that would not be able to diffuse outwards from the wells. An earlier study had shown that about 90% of the circulating thiabendazole in the tank is expected to be in the dissolved form (BOF 43; Hanks, 2000).

Bulb samples were kept frozen until assessments of thiabendazole content were made. Each sample was weighed and placed in 15 ml 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 6 h 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 40  $\mu$ l 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 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 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 (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 extractable thiabendazole per gram of bulb tissue ( $\mu g/g$ , or ppm).

## Supplementary observation

To determine the practicalities of HWT with acidification, 15 further  $\frac{1}{2}$ -tonne bins of bulbs were dipped in sequence, three bins per day for 5 days (3 September to 7 September 2001). The same HWT set-up as above was employed (one  $\frac{1}{2}$ -tonne bin with 3000 litres of dip per load). HWT duration and temperature were as described for the main experiment and the dip consisted of half-rate 'Storite' along with SBS, 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:

- SBS: 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, respectively)
- 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 topup) *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 SBS (see above) to be added to maintain the target pH (2.5 - 3.0).

## 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
  - SBS 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 SBS; 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<sup>2</sup>. 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 the meter readings. Determinations using dipsticks appeared consistently to over-estimate pH by about 0.4, compared with meter readings:

pH by dipstick	3.5	3.5	3.2	3.5	3.5	3.2	3.2	3.5
pH by meter	3.1	3.1	2.9	3.0	3.0	2.9	3.0	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 SBS 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 precipitated 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 concentration on-scale. All ten samples (i.e., taken at the start and end of the five HWT

<sup>&</sup>lt;sup>2</sup> Figures start on page 33.

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treatments) resulted in readings corresponding most closely to 100 mg/litre, equivalent to 2000 mg/litre before dilution, which was 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 SBS 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 <sup>1</sup>/<sub>4</sub>-rate treatment was higher than expected. One possibility is that, because the <sup>1</sup>/<sub>4</sub>-rate dip was done in tank A after this tank had been used the previous day for the <sup>1</sup>/<sub>2</sub>-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, despite the cleaning procedures. No thiabendazole was detected in bulbs from the 'no Storite' treatment, indicating that there was no contamination of the bulbs from earlier applications.

Treatment		Fungicide concentration	<i>(ppm)</i>
('Storite' rate and	Expected	Actual, at	Actual, at
SBS)		start of dip <sup>1</sup>	end of dip <sup>1</sup>
Full, no SBS	1100	$290\pm41$	$260 \pm 48$
Full, + SBS	1100	$1102 \pm 218$	$963\pm102$
Half, + SBS	550	$341 \pm 45$	$392\pm42$
Quarter, + SBS	275	314±41	$289\pm48$
None, + SBS	0	0	0

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

<sup>1</sup> Mean value and standard deviation for four samples

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 the treatment where no SBS was used, than in the full-rate 'Storite' treatment with SBS, 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' were proportionally lower. The concentrations in the quarter-rate treatment were similar (see

above).

- In control bulbs (no 'Storite' in HWT) thiabendazole could not be detected. Therefore, untreated (control) 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 the two 'full-rate Storite' treatments, but this trend was reversed in the low-rate treatments.

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

Treatment	Thiabendazole concentrations (ppm) <sup>1</sup>								
('Storite' rate and	Oute	er samples	Inn	er samples					
SBS	'Carlton'	'Golden Harvest'	'Carlton'	'Golden Harvest'					
Full, no SBS	$546\pm162$	$364\pm62$	$37 \pm 5$	$34 \pm 12$					
Full, + SBS	$350\pm96$	$196\pm59$	$8\pm3$	$6 \pm 6$					
Half, + SBS	$106\pm33$	$156\pm37$	$6\pm 6$	$3\pm3$					
Quarter, + SBS	$110 \pm 21$	$125 \pm 36$	$7 \pm 1$	$5\pm 2$					
None, + SBS	0	0	0	0					

<sup>1</sup> Mean value and standard deviation for 16 samples (see text).

The data above showed clear differences between treatments with respect to the quantities of thiabendazole acquired by bulbs during HWT with 'Storite'. 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, compared with previous samples, 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 the chemical from the soil environment, or translocation from other regions of the growing bulb into the roots.

Treatment		Thiabendazole concentrations (ppm) <sup>1</sup>											
('Storite' rate	Outer se	amples	Inner samples		Roots		Shoots						
and SBS)	'Carlton'	'GH'	'Carlton'	'GH'	'Carlton'	'GH'	'Carlton'	'GH'					
Full, no SBS	$48\pm14$	$22 \pm 16$	$14 \pm 8$	$3\pm3$	$22 \pm 17$	$13\pm16$	0	0					
Full, + SBS	$17\pm9$	$3\pm4$	$3\pm3$	$1\pm 2$	$13 \pm 10$	$2\pm3$	0	0					

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

<sup>1</sup> Mean value and standard deviation for 16 samples (see text).

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.

Treatment	Thiabendazole concentrations (ppm) <sup>1</sup>										
('Storite' rate	Outer samples		Inner sa	mples	Roots						
and SBS)	'Carlton'	'GH'	'Carlton'	'GH'	'Carlton'	'GH'					
Full, no SBS	$65\pm37$	$47\pm41$	$12 \pm 12$	$4\pm4$	$19\pm32$	$14\pm25$					
Full, + SBS	$8\pm8$	$2\pm3$	$1 \pm 1$	0	$4 \pm 5$	$1 \pm 1$					

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

<sup>1</sup> Mean value and standard deviation for 16 samples (see text).

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.

Thiabendazole concentrations (ppm) <sup>1</sup>									
Outer samples		Inner samples		Roots					
'Carlton'	'GH'	'Carlton'	'GH'	'Carlton'	'GH'				
$4\pm7$	$1\pm 2$	$1 \pm 1$	0	$4\pm 8$	0				
$4 \pm 12$	0	0	0	0	0				
	Carlton' $4 \pm 7$	Outer samples'Carlton''GH' $4 \pm 7$ $1 \pm 2$	Outer samplesInner sa'Carlton''GH' $4 \pm 7$ $1 \pm 2$ $1 \pm 1$	Outer samplesInner samples'Carlton''GH''Carlton' $4 \pm 7$ $1 \pm 2$ $1 \pm 1$ 0	Outer samplesInner samplesRoot'Carlton''GH''Carlton''GH' $4 \pm 7$ $1 \pm 2$ $1 \pm 1$ 0 $4 \pm 8$				

<sup>1</sup> Mean value and standard deviation for 16 samples (see text).

Figure 3 presents the thiabendazole concentrations for all sample dates. In view of the very low or undetectable levels of thiabendazole in the June 2002 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.

### Pre-lifting 'Storite' application

Detectable levels of thiabendazole were not found in any of these samples.

### Crop growth and flower production

Flower numbers and stem and foliage lengths are shown in Tables 6 ('Carlton') and 7 ('Golden Harvest'). In the first crop year (2001 - 2002) in both cultivars, there were conspicuously fewer flowers in the 'no SBS' and 'no Storite' treatments than in treatments which included 'Storite' (full, half or quarter rate) *and* SBS. In the case of the 'no Storite' treatment, this was probably due to extensive and rapid bulb rotting in the crucial weeks after planting, leading to extensive flower loss. It is possible that all the acidified fungicide treatments had a better curative effect than the non-acidified treatment, and that was why there were more flowers in the first year from those bulbs. The effect on flower numbers persisted into the second year for both cultivars, even though the full, no SBS treatment gave very similar percentage weight increases to the acidified treatments. The presence of the larger numbers of small sized bulbs in the 'full-rate, no SBS' treatment for both cultivars probably explains this. Poor productivity of first-year-down narcissus crops is often compensated by increased growth in the second year, as a result of decreased inter-plant competition following the earlier losses.

In the second crop year (2002 - 2003) the effect of HWT treatments was different in the two cultivars. In 'Carlton', flower yield was reduced only in the 'full-rate Storite, no SBS' treatment, possibly as a result of continued bulb losses, while there were only non-significant differences between the four SBS-containing treatments. In 'Golden Harvest', flower yields were significantly lower in both 'no SBS' and 'no Storite' treatments, than in the three treatments with 'Storite' *and* SBS. Between-plot variations in flower numbers were high in the case of 'Golden Harvest' (see the large standard errors in Table 7), so it was not possible to discern the optimum 'Storite' rate in this regard. HWT treatment had no significant effect on either stem or foliage length in either cultivar. In other respects the crops appeared normal in all treatments.

'Carlton'.							
HWT ('Storite'		Flowers per	plot (2002)		Flowers per	Stem length	Foliage length
rate and SBS)	Normal	Damaged	Dead buds	Total	plot (2003) <sup>a</sup>	(2003) (mm)	(2003) (mm)
Full (no SBS)	119	21	4	143	402	694	605
Full (+ SBS)	422	18	1	440	474	684	580
Half (+ SBS)	456	33	0	488	532	671	608

516

191

\*\*\*

21.9

494

486

35.2

691

697

ns

12.7

Table 6. Effect of 'Storite' and SBS treatments in HWT on flower production for a two-year crop of narcissus 'Carlton'.

<sup>a</sup> All flowers were normal in the second crop year.

13

27

ns

11.3

1

6

1.5

503

158

25.8

\*\*\*

Quarter (+ SBS)

None (+ SBS)

SED (19 d.f.)

*Significance<sup>b</sup>* 

<sup>b</sup>ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

627

632

ns

20.8

HWT ('Storite'		Flowers per	plot (2002)		Flowers per	Stem length	Foliage length	
rate and SBS)	Normal	Damaged	Dead buds	Total	plot (2003) <sup>a</sup>	(2003) (mm)	(2003) (mm)	
Full (no SBS)	46	8	32	86	313	689	618	
Full (+ SBS)	155	11	13	178	370	715	659	
Half (+ SBS)	204	6	8	217	367	697	642	
Quarter (+ SBS)	194	8	20	221	419	733	663	
None (+ SBS)	89	11	23	123	314	715	624	
SED (19 d.f.)	9.1	1.5	2.4	10.7	51.5	25.5	20.1	
Significance <sup>b</sup>	***	*	***	***	ns	ns	ns	

**Table 7.** Effect of 'Storite' and SBS treatments in HWT on flower production for a two-year crop of narcissus 'Golden Harvest'.

<sup>a</sup> All flowers were normal in the second crop year.

<sup>b</sup> ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

#### Bulb yield

In 'Carlton', the effects of treatments on bulb yields were relatively small (Tables 8 and 9). This was a reasonably healthy stock, in which only a moderate amount of bulb rot would normally be expected, and, overall, only 2.2% of lifted bulbs were rejected due to rotting at grading. There was a significant effect of HWT treatment on the weight of sound (marketable) bulbs, with half-and quarter-rate 'Storite' treatments (with SBS) giving the highest yields, and the treatment with no 'Storite' the lowest. Expressed as the percentage weight increase in bulbs from planting, the increases varied from 150% (with no 'Storite') to 198% (with half-rate 'Storite' and SBS). Overall, HWT treatment did not have significant effects on the total *numbers* of sound or rotted bulbs, but there were clear trends. Marketable yields were highest where a half- or quarter-rate of 'Storite' was used with SBS, and numbers of rotted bulbs were lowest where a full- or half-rate of 'Storite' was used with SBS, than in other treatments. Except in the full- and half-rate treatments with SBS, there was considerable variation in the number of rotted bulbs between replicates, probably representing a rapid local spread from the infected bulbs occurring sporadically under these conditions. These facts seemed connected with significantly higher yields in the larger (14-18 cm) grades when a half-rate of 'Storite', plus SBS, had been used.

The results for 'Golden Harvest', a basal rot-susceptible cultivar, showed much larger treatment effects (Tables 10 and 11). Overall, this variety had 14.2% of bulbs rejected through rots at grading. There were significant (P<0.05) effects of treatments on the total numbers of sound bulbs obtained, ranging from 132 per plot for the 'no Storite' treatment, to 397 per plot for the 'full-rate Storite, no SBS' treatment, other treatments giving intermediate yields. The low numbers of bulbs obtained from the 'no Storite' treatment was linked to a large number of rotted bulbs (64 per plot) and a reduced yield across all bulb grades. The high numerical yield of the 'full-rate Storite, no SBS', treatment was associated with markedly higher bulb numbers in the small (<12 cm) grades of bulbs. These results were largely matched by the yields expressed as weights lifted. The highest percentage weight increases were obtained with the quarter-rate 'Storite' treatment (99%) and 'full-rate Storite' treatment (-24%). Even the highest increases here were very poor, but not, however, atypical of a poor 'Golden Harvest' stock.

HWT ('Storite'	Number of sound bulbs in grades and in total, and of rotted bulbs									
rate and SBS)	<i>&lt;8cm</i>	8-10cm	10-12cm	12-14cm	14-16cm	16-18cm	>18cm	Total sound	Rotted	
Full (no SBS)	67	136	159	116	74	17	1.3	570	17	
Full (+ SBS)	56	102	156	118	74	18	2.8	526	7	
Half (+ SBS)	53	119	162	130	89	28	2.3	584	2	
Quarter (+ SBS)	50	123	154	150	80	24	2.3	582	19	
None (+ SBS)	48	100	138	129	70	20	2.8	508	20	
SED (19 d.f.)	6.7	15.9	15.0	16.8	5.5	3.09	1.02	38.4	11.7	
<i>Significance<sup>a</sup></i>	ns	ns	ns	ns	*	*	ns	ns	ns	

Table 8. Effect of 'Storite' and SBS treatments in HWT on bulb yield (numbers) for a two-year crop of narcissus 'Carlton'.

<sup>a</sup>ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

Table 9. Effect of 'Storite' and SBS treatments in HWT on bulb yield (weights) for a two-year crop of narcissus 'Carlton'.

HWT ('Storite'	Weight of sound bulbs in grades and in total (kg/plot)							% weight	
rate and SBS)	<i>&lt;8cm</i>	8-10cm	10-12cm	12-14cm	14 <b>-</b> 16cm	16-18cm	>18cm	Total	<i>increase</i> <sup>a</sup>
Full (no SBS)	0.89	3.49	6.14	6.87	6.18	2.03	0.17	25.8	158
Full (+ SBS)	0.83	3.40	6.26	7.60	6.17	2.01	0.41	26.7	167
Half (+ SBS)	0.81	3.24	6.91	7.70	7.53	3.24	0.34	29.8	198
Quarter (+ SBS)	0.74	3.40	6.30	8.85	6.69	2.65	0.34	29.0	190
None (+ SBS)	0.71	2.55	5.51	7.50	5.90	2.32	0.41	24.9	150
SED (19 d.f.)	0.087	0.316	0.551	0.854	0.403	0.404	0.171	1.57	-
Significance <sup>b</sup>	ns	(*)	ns	ns	*	(*)	ns	*	-

<sup>a</sup>% weight increase = ((lifted weight – planed weight) x 100) / planted weight.

<sup>b</sup> ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

	Number of sound bulbs in grades and in total, and of rotted bulbs								
	<i>&lt;8cm</i>	8-10cm	10-12cm	12 <b>-</b> 14cm	14 <b>-</b> 16cm	16-18cm	>18cm	Total sound	Rotted
Full (no SBS)	69	85	99	68	56	18	3.0	397	31
Full (+ SBS)	47	72	83	57	50	22	2.5	334	31
Half (+ SBS)	32	40	64	45	39	23	4.5	247	78
Quarter (+ SBS)	39	58	80	65	53	31	6.0	332	34
None (+ SBS)	12	25	33	32	19	10	1.3	132	64
SED (19 d.f.)	15.8	13.3	15.0	11.6	10.3	5.5	1.60	59.2	16.8
Significance <sup>a</sup>	*	**	**	*	*	*	(*)	**	*

Table 10. Effect of 'Storite' and SBS treatments in HWT on bulb yield (numbers) for a two-year crop of narcissus 'Golden Harvest'.

<sup>a</sup> ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

Table 11. Effect of 'Storite' and SBS treatments in HWT on bulb yield (weights) for a two-year crop of narcissus 'Golden Harvest'.

HWT ('Storite'	Weight of sound bulbs in grades and in total (kg/plot)							% weight	
rate and SBS)	<8cm	8-10cm	10-12cm	12 <b>-</b> 14cm	14 <b>-</b> 16cm	16-18cm	>18cm	Total	<i>increase</i> <sup>b</sup>
Full (no SBS)	1.00	2.22	4.10	4.28	5.06	2.33	0.46	19.5	95
Full (+ SBS)	0.63	2.01	3.67	3.63	4.57	2.72	0.38	17.6	76
Half (+ SBS)	0.51	1.14	2.89	2.94	3.67	2.85	0.73	14.7	47
Quarter (+ SBS)	0.52	1.58	3.56	4.30	4.82	4.13	1.00	19.9	99
None (+ SBS)	0.20	0.68	1.42	2.04	1.79	1.29	0.20	7.6	-24
SED (19 d.f.)	0.188	0.390	0.656	0.783	0.949	0.784	0.285	3.17	-
Significance <sup>b</sup>	*	*	*	(*)	*	*	ns	*	-

<sup>a</sup> % weight increase = ((lifted weight – planted weight) x 100) / planted weight. <sup>b</sup> ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

#### Storage rot assessment

The results of storage assessments are given in Tables 12 ('Carlton') and 13 ('Golden Harvest'). In 'Carlton', as expected from the previous results, there were very few rotted bulbs, and no treatment effects could be discerned. In 'Golden Harvest' there were significant numbers of bulbs with basal rot: 9% in the 'no Storite' treatment, and 1-3% in other treatments. Combining all types of rots for 'Golden Harvest', 12% of bulbs were affected in the 'no Storite' treatment, and 4-6% in the other treatments. Only small numbers of 'Golden Harvest' bulbs were affected by neck or whole-bulb rots. Storage rots and most rotted bulbs found immediately after lifting will largely be the result of infections occurring during the second season and the treatments applied during 2001 will not have any persistent fungicidal effects beyond spring 2002. However, treatments that reduced the number of bulb rots during the first season may reduce the number of rots found in the second season by limiting the number of diseased bulbs acting as inoculum sources.

It was noted that significant numbers of these bulbs were found to be infested by larvae of the large narcissus fly. Overall, 3.9 and 4.8% of 'Carlton' and 'Golden Harvest' bulbs, respectively, were affected.

Carnon .							
HWT ('Storite'	% of bulbs in categories						
rate and SBS)	Basal rot	Neck rot	Whole-bulb rot	All categories			
Full (no SBS)	0.25	0	0	0.25			
Full (+ SBS)	0	0.25	0	0.25			
Half (+ SBS)	0	0.25	0	0.25			
Quarter (+ SBS)	0.25	0.50	0.75	1.50			
None (+ SBS)	0.25	0.50	0	0.75			
SED (19 d.f.)	-	-	-	0.619			
Significance <sup>a</sup>	-	-	-	ns			

Table 12. Effect of 'Storite' and SBS treatments in HWT on storage rot assessments for 'Carlton'.

<sup>a</sup> ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

Harvest . HWT ('Storite'	% of bulbs in categories							
rate and SBS)	Basal rot	Neck rot	Whole-bulb	Mummified	All			
			rot	bulbs	categories			
Full (no SBS)	2.25	1.25	1.75	0.25	5.50			
Full (+ SBS)	3.00	1.75	1.00	0	5.75			
Half (+ SBS)	1.75	0.79	1.79	0	4.34			
Quarter (+ SBS)	1.25	1.25	1.25	0.25	4.00			
None (+ SBS)	8.95	1.20	2.34	0	12.49			
SED (19 d.f.)	2.441	-	-	-	2.869			
Significance <sup>a</sup>	*	-	-	-	(*)			

Table 13. Effect of 'Storite' and SBS treatments in HWT on storage rot assessments for 'Golden Harvest'.

<sup>a</sup> ns, not significant; (\*), \*, \*\* and \*\*\*, significant at P<0.1, 0.05, 0.01 and 0.001 levels of probability, respectively.

### DISCUSSION

#### Basal rot and the UK bulb industry

In the history of bulb growing in the UK, basal rot has not always been a major problem. In the 1930s, for example, narcissus stocks with basal rot could not always be found for experiments at Kirton! Then, bulb losses were more likely to be due to stem nematode, *Ditylenchus dipsaci* (Wallace, 1937). It was reported in 1969 that, in the Rosewarne variety trials, basal rot was not being observed even in cv 'Golden Harvest' (Wallis, 1969). Starting in the early 1970s, however, basal rot became more troublesome (Tompsett, 1972), and this initiated a major R&D effort. While climatic change may have had a role in these events, it is certain that cultural practices in UK narcissus growing had begun to change significantly from around this time, for example with two-year-down growing, higher planting rates, and less of labour-intensive inputs such as bulb inspection or rogueing (Rees, 1972; Hanks, 2002a). Bulb pathologist David Price pointed out in 1977 that "recent changes in almost every aspect of narcissus husbandry favour the spread of disease" (Price, 1977).

#### Advice on basal rot control

Basal rot control has long been practised using a combination of chemical treatments and bulb husbandry practices (see reviews of Melville (1980) and Linfield & Hanks (1994a), and the standard advisory texts Moore et al. (1979) and ADAS (1985, 1988)). Much of the advice given has emphasised the importance of cumulative good practice, maintained over several years, in reducing basal rot infestations (as demonstrated by, e.g., Hanks, 1996). Inevitably, disease control by fungicides has tended to receive more attention in commercial growing than the more labour-intensive or inconvenient use of crop growing and husbandry practices (such as site selection, or maintaining good farm hygiene). Applied research at Kirton and Rosewarne over many years, mainly MAFF-funded, led to the formulation of sets of recommendations that, applied together and consistently, were considered a practical way of managing basal rot. These so-called 'commandments' were widely available, for example, in poster format (ADAS & GCRI, undated [ca. 1980]) and as HDC 'fact-sheets' (Linfield & Hanks, 1994b; Hanks, 2002b). Despite this, experience and anecdotal evidence showed that following these rules did not always guarantee a low level of basal rot! The reverse could also be true – in some cases good control was achieved despite not all the recommendations being followed.

#### Development of current fungicide usage

Basal rot control was originally based on using cold dips or HWT with formalin (Hawker, 1935, 1940). Later, mercury fungicides were used, but these caused some damage to the crops (Miller & Gould, 1967), and in the 1970s the value of the benzimidazole (MBC) fungicides benomyl and, especially, thiabendazole was established (Gould & Miller, 1970, 1971a, 1971b). These fungicides were most effective when applied as post-lifting, 30 minute immersions in aqueous fungicides (1000 or 2000 ppm a.i.) at 25°C, within 1 day of lifting. The fungicides were also successful when applied as a heavy dusting onto bulbs, a practice unacceptable today.

Following this work in the USA, and with the increasing concern over basal rot in the UK, many fungicide trials were carried out at Kirton and Rosewarne (see, e.g., Tompsett, 1976, 1980, 1984), and the information was incorporated into the advisory literature already referenced. The work was summarised in detail in an earlier HDC-funded report (BOF 31; Linfield & Hanks, 1994a). The trials concentrated on testing formulations of benomyl, thiabendazole and another benzimidazole fungicide, carbendazim (methyl 2benzimidazolecarbamate, MBC), in post-lifting, ambient temperature dips, and in HWT. In the case of thiabendazole especially, various formulations were tested, such as Storite Flowable, thiabendazole dust, Tecto WP and a hypophosphorous acid formulation. As a group, benzimidazole fungicides were more effective than other fungicide types, though many of those tested gave some degree of control. Amongst the benzimidazoles, thiabendazole gave the most consistent results, especially in the acidic thiabendazole hypophosphite formulation Storite Clear Liquid ('Storite').

#### Further developments in fungicide practices

Later developments saw 'Storite' tested as a post-lifting, on-line spray application, as well as in a post-lifting dip or in the HWT tank. Trials also included a 'double treatment' with 'Storite' applied both as a post-lifting application and in HWT, a method useful in the case of severely infested bulb stocks (Hanks, 1996; Hanks & Linfield, 1996). For post-lifting sprays and dips and HWT applications, fungicides other than thiabendazole were effective to some extent, though they were not as consistently beneficial as thiabendazole (Hanks, 1996; Hanks & Linfield, 1996). The double treatment allowed thiabendazole to be applied post-lifting, with a non-benzimidazole fungicide in HWT, conferring the benefits of using fungicides of more than one mode-of-action group. In one series of treatments, various formulations of thiabendazole, imazalil and thiabendazole + imazilil were applied as post-lifting dips or sprays, or as double treatments (post-lifting spray plus HWT). For controlling basal rot, the double treatments were better than post-lifting sprays alone, which were in turn more effective than post-lifting dips, though all treatments were effective to a useful extent. When using a post-lifting spray, conventional spray nozzles, ultrasonic sprayers and electrostatic sprayers were all effective, preferably when operated over bulbs moving on a roller table, though the best treatment was 'Storite' applied through ultrasonic nozzles (G.R.Hanks, unpublished data).

As an alternative to applying HWT at the usual time, usually late-July or early-August, HWT may be applied earlier, even post-lifting. This is more effective in controlling basal rot (and stem nematode), and therefore useful for treating badly infested stocks, although damage to the bulbs is greater (Linfield & Hanks, 1994a).

Other methods of application are feasible. Fungicide sprays may be applied to bulbs at lifting or at planting, with spray equipment mounted on the lifting or planting machines, or a separate bulb dip can be used at some point between lifting and re-planting. These alternatives may have been used occasionally in commerce, but have not been the subject of controlled trials. All these methods involve application between lifting and planting, and a further possibility is to apply the fungicide as a foliar spray in the field, prior to lifting. Few trials appear to have been carried out to test this procedure. However, it was reported that fortnightly benomyl sprays did not prevent basal rot when bulbs were subsequently lifted, dipped in inoculum and stored (Tompsett, 1972; ADAS, 1983), while a benomyl drench 3 weeks before lifting failed to control rots that were controlled by post-lifting and HWT treatments (Briggs, 1973). In the present project no detectable amounts of thiabendazole were found in or on bulbs that had received a spray application of 'Storite' at the end of the growing season and had been extracted and analysed between 1 day and 3 weeks later.

### Application rates and costs of 'Storite'

In the UK, 'Storite' is used on potato tubers as well as narcissus bulbs, but the application rate for bulbs is five times that used on potatoes. Trials confirmed the need to use the higher rate with narcissus (G.R.Hanks, unpublished data). Perhaps this was related to differences in shape and surface texture that affect the amount of coating achieved, but, whatever the reason, the high 'Storite' application rate recommended for treating bulbs is regarded by the industry as a major expense.

Bulb prices are notoriously cyclical. In times of high bulb prices (say, £500 per tonne for commonplace cultivars), the cost of 'Storite' – about £50 to treat each tonne of bulbs by dipping or HWT, or £25 per tonne as a post-lifting spray - may be acceptable to growers. In many cases, these recommended rates have probably been reduced by growers for reasons of economy. When prices fall, say to around £200 per tonne, using 'Storite' is considered by many growers to be prohibitively expensive. Hence there is considerable pressure to consider the adoption of cheaper fungicides, and to ensure that 'Storite' – if used – is employed in the most cost-effective way. The results of the present project showed that effective basal rot control could be achieved with half- or quarter-rate 'Storite', if the dip is acidified to pH 2.5 to 3.0. Thus, 'Storite' costs could be reduced by 50 - 75%; the cost of the SBS used is very low, about £0.50 per tonne of bulbs treated.

## Solubility of benzimidazoles

A general problem with benzimidazole pesticides is their low water solubility, which, in the anthelminthics field, has led to a search for more soluble analogues (Nielsen *et al.*, 1992). The introduction and proven effectiveness of Storite Clear Liquid ('Storite') made it the material of choice for advisors and growers. However, as the present project showed, the low solubility of thiabendazole, other than at low pH values, still poses problems with its use as a bulb dip. As shown in an earlier, HDC-funded project (BOF 43; Hanks, 2000), the concentration of thiabendazole circulating in HWT tanks falls rapidly from the start of treatment due to precipitation linked to rising pH. Similar effects had been demonstrated earlier with benomyl (Hanks, 1990b). As mentioned in the Introduction, there were anecdotal recommendations that an acidifier, SBS, should be added to 'Storite' dips, and these arose from research addressing similar problems in the control of Dutch elm disease (*Ceratocystis ulmi*) and of *Verticillium* wilt of cotton.

Prasad (1972) studied the effects of pH on the penetration and translocation of benomyl in young elm trees (*Ulmus americana*). Benomyl (1500 ppm) attained maximum systemic action at pH 3.2; at higher pH values translocation was reduced, ceasing at a pH of 9.2. Acidic preparations of benomyl penetrated and accumulated more rapidly at disease sites, enabling lower doses to be used. Working on the control of *C. ulmi* in *Ulmus* spp., Clifford *et al.* (1976, 1977) found that carbendazim dissolved in dilute hydrochloric, orthophosphoric or hypophosphorous acids, and aqueous solutions of thiabendazole hypophosphite, were most

active in controlling the disease. Formulations containing thiabendazole hypophosphite were more readily translocated, and remained longer in the twigs, than other formulations. The protectant effects of carbendazim increased with increasing concentrations of acid (hydrochloric or orthophosphoric) up to a base/acid molar ratio of 1:3. Studying *Verticillium* wilt in cotton, Buchenauer & Erwin (1972) used benomyl and thiabendazole dissolved in dilute hydrochloric acid (HCl). Pre-infection sprays of thiabendazole-HCl (2500 ppm) thiabendazole) reduced the symptoms of *V. albo-atrum*, and post-infection sprays (5000 ppm) inhibited further symptom development. Similar results were obtained when the fungicides were dissolved in dilute nitric or sulphuric acids. Thiabendazole-HCl penetrated leaf tissue faster than thiabendazole suspension. Working with isolated cuticle from apple leaves, Solel & Edgington (1973) studied the transcuticular movement of fungicides. For benzimidazoles, the rate of movement was thiophanate-methyl > thiophanate > benomyl > carbendazim > thiabendazole. When the solubility of benomyl and thiabendazole was increased using acidified water, the rate of movement increased four-fold.

In the present study, the maintenance of a near-target concentration of dissolved thiabendazole, by lowering the pH, was clearly demonstrated. Because of the existing anecdotal advice to use SBS in bulb dips, this material was used throughout the present study. The authors are not aware of other acidifiers being used in bulb dipping.

#### Uptake of thiabendazole

Benzimidazole fungicides are systemic in action. The route of entry of benzimidazoles into bulbs has not been specifically determined, though it was previously assumed that the effectiveness of prompt, post-lifting spray or dip treatments was due to uptake by the roots which are still present (although their natural senescence will be quite advanced at this time). However, basal rot control did not appear to differ when various parts of the freshly lifted bulbs were immersed in 'Storite' solution before storage (G.R.Hanks, unpublished data). 'Storite' dips controlled basal rot using de-rooted bulbs as well as using intact bulbs with roots, and treatments in which bulbs with the dry outer scales removed were stood upsidedown in a shallow container of 'Storite' solution resulted in better control than in the case of intact, fully immersed bulbs. This suggests that uptake over the whole surface is effective, but better uptake may be obtained through living tissues rather than through the dead outer scales.

Several of the studies already cited showed that the extent of movement of benzimidazole fungicides in plant tissues is low even when using an acidic formulation (Buchenauer & Erwin, 1972). In *U. americana*, benomyl penetration into, and movement from, the leaves is slow, although appreciable amounts were absorbed through the bark, were translocated and acted effectively against *C. ulmi* (Prasad & Travnick, 1973). Possibly, absorption of benzimidazoles through the rough outer surfaces of narcissus bulbs could be considered similar to that which occurred through the bark of elm twigs. However, Clifford *et al.* (1976, 1977) considered it necessary to develop a novel pressure-injection apparatus for applying carbendazim to elm bark, despite which the distribution of the fungicide in the trees remained non-uniform, requiring injection at multiple points around the trunk. These findings may partly explain some disappointing effects of thiabendazole in the control of basal rot of narcissus. In the present study, penetration of the compound to the inner bulb scales was low, and it is possible that most of the compound 'in the outer tissues' may be largely superficial.

### Fungicidal activity of thiabendazole

The benzimidazole fungicides are generally regarded as both protective and curative, though it is not definitively known whether thiabendazole has a fungistatic or curative action on the basal rot pathogen. In *Verticillium* wilt of cotton, benzimidazoles work by suppressing fungal spread (Buchenauer & Erwin, 1972), and benomyl is understood to have a protectant action on Dutch elm disease (Prasad & Travnick, 1973; Clifford *et al.*, 1977). Experience suggests that, in narcissus bulbs, control of basal rot is likely to be ineffective if any visible rot has already penetrated beyond the basal plate. Many trials have shown that, while effective fungicide treatments reduce subsequent yield loss and storage losses due to basal rot, even 'Storite' treatments are incapable of curtailing immediate losses (G.R.Hanks, unpublished data). In one study obviously rotted bulbs (determined by visual inspection and by feel) were rigorously removed at planting (Millar, 1977). When compared with controls, yield losses were still apparent two years later, suggesting that many bulbs carry incipient symptoms. Ineffective fungicide treatments may actually increase basal rot, presumably by providing water to encourage and spread fungal growth.

#### Persistence of thiabendazole

In Ulmus spp., Clifford et al. (1977) reported that internal levels of carbendazim decreased sharply 55 to 97 days after pressure-injection, while Coosemans & van Assche (1983) reported that the half-life of thiabendazole in apple peel was well over one month. In the present study, concentrations of thiabendazole in narcissus bulb tissues had fallen to ineffective levels by about 6 months after application. Little information is available about the concentrations of thiabendazole found in narcissus bulbs after treatment. However, in an earlier HDC-funded test (BOF 6a: Norris, 1992) bulbs were treated with 'Storite' in various ways and thiabendazole concentrations determined in all cases one week after HWT. Bulbs received a post-lifting 15-minute cold dip, were stored for 6 weeks, pre-soaked for 3 hours at ambient temperatures, and received HWT at 46°C. Control (untreated) bulbs showed a thiabendazole concentration of 1 ppm, and where 'Storite', at the same starting concentration had been applied in the post-lifting dip, in the pre-soak tank, or in HWT, concentrations in bulbs after HWT were 6, 13 and 63 ppm, respectively. In other bulbs, which were not presoaked but received HWT at 44.4C with 'Storite', the concentration was 98 ppm. These data illustrate how easily thiabendazole, acquired by bulbs in earlier treatments, can subsequently become depleted by the following treatments in which the fungicide is not added.

In this project, thiabendazole concentrations of bulbs were determined three, six and nine months after planting. Most thiabendazole was always found in the outer parts of the bulb, and here some 90% of the fungicide was lost between August and December. There was little further loss 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, or 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 could be achieved through using acidified half- or quarter-rate 'Storite' in

### HWT.

#### Corrosion

HWT tanks are usually constructed of mild steel, without permanent anti-corrosion treatment such as galvanising, although current advice is to apply a rust-preventing zinc- or aluminiumbased paint at the end of the treatment season, after cleaning (Gratwick & Southey, 1986). Although this aspect has not been studied specifically, one bulb grower, who has acidified cold and HWT dips using SBS for the last 15 years, aiming for a pH of 3.0, reported that some pipe-work and one pump needed replacing only in the 2002 - 2003 season, despite carrying out some 200 dips per annum (personal communication). This suggests that corrosion as a result of adding SBS is unlikely to be severe.

#### Determination of thiabendazole concentrations in dips

The analytical methods available for thiabendazole include spectrophotometry (Wood, 1976) chromatography and high-performance liquid (HPLC) (D.C.Brown, personal communication), as well as the biological assay used in the present study. Thiabendazole is very stable in aqueous suspensions and solutions regardless of pH, and is stable to heat and light (Wood, 1976; Tomlin, 1997), so no special precautions to minimise microbial degradation appear to be needed in handling samples up to analysis, other than storing samples frozen until used (D.C.Brown, personal communication; D.Stainton, personal communication). Unfortunately, none of these methods is suitable for giving instant results: spectrophotometric and chromatographic methods require apparatus to be set up for a batch run of samples, and the biological assay takes two days to produce a result. Hence, information on tank concentrations would have to be gained retrospectively. However, the current study has shown that pH measurement supplemented by maintenance of pH at a value below 3.2 should ensure that almost all of the added thiabendazole stays in solution. None of the available assay methods can be used to quantify thiabendazole whilst in suspension since they are all dependant on the chemical being in true solution for correct operation. However spectrophotometry and HPLC can use organic solvents to solubilise suspensions and the biological assay can handle samples with SBS added to dissolve particulate thiabendazole. In an earlier study the HPLC analysis revealed that over 90% of circulating thiabendazole was in the dissolved form even though over 70% of that added to non-acidified dip solutions was 'missing' even before bulbs were added. This suggests that this 'missing' chemical is trapped as deposits on the pipe-work, pumps or tanks of the HWT equipment.

### Recommendations

These findings indicate that, when using 'Storite' in HWT to control basal rot, a reduced rate of 'Storite' together with the addition of SBS should be adopted. The rates of SBS used did not have any detected adverse effects on the bulbs or plants, neither did there appear to be any adverse interaction with other dip components, such as formalin. It is likely that these recommendations could also be applied to cold dip treatments, but the concentrated 'Storite' solutions applied as bulb sprays are already relatively acidic and do not require further acidification (BOF 43; Hanks, 2000). Although other methods of application are possible, in commerce only post-lifting sprays or addition to HWT tanks appear to be practical; which one is adopted will depend on the preferences and practices of the individual grower.

- For a quarter-rate application, 'Storite' should be used at a rate of 1.250 litres per 1000 litres of dip when making up tanks. After each dip, 0.375 litres of 'Storite' should be added to tanks for every 1 tonne of bulbs dipped. When the water level in the tank is topped-up, 0.125 litres of 'Storite' should be added for each 100 litres of water added.
- SBS should be added to the tank at a rate of 1.380 kg per 1000 litres of dip. When the water level in the tank is topped-up, 0.138 kg of SBS should be added for each 100 litres of water added. At the start of each day's dipping, an additional 0.250 kg of SBS should be added per 1000 litres of dip, though this quantity should be adjusted according to the actual dip pH. A small portable pH meter should be used to check dip pH, perhaps at the start and end of each dip. The aim is to maintain a dip pH between 2.5 and 3.0, though the exact pH value is not critical; lower pH values may be harmful to bulbs, and higher pH values will be ineffective in optimising the 'Storite'. It is important to follow the instructions for maintaining the electrodes of pH meters in good condition. To some extent, dip pH will be influenced by the pH of the local water supply. However, water authorities aim to maintain a pH just above 7.0, and spot enquiries in the South Holland and Cornwall bulb-growing areas found the pH of mains water to range from "just below 7" to 7.4. In any case, the buffering capacity of tap water is generally quite low and would be easily overcome by relatively high concentrations of the added SBS.
- In all cases, the SBS should be added to the tank water first, and time to allow solution and mixing should be allowed, before adding 'Storite' and other ingredients.
- Other additives including formalin, wetter and anti-foam compound and HWT conditions temperatures, durations, timing, etc. should continue to follow the standard advice. Bulbs for HWT should be clean, to avoid the adsorption and inactivation of thiabendazole on soil particles.
- Spot sampling of HWT dips for measurement of thiabendazole concentrations is recommended, to enable growers to become familiar with the dynamics of their tanks.
- A COSHH assessment should be carried out if the grower's HWT procedures have changed.

How these recommendations are implemented should take account of the desirability (1) to reduce the likelihood of resistance to benzimidazole developing, (2) to plan for the contingency of thiabendazole or the specific 'Storite' formulation being lost, and (3) to develop 'integrated' systems of pest and disease management, less reliant on pesticides.

Points (1) and (2) require the use of other fungicides or formulations to be considered. Storite Clear Liquid is manufactured only for the UK, and is mainly used on narcissus bulbs, since

other formulations are preferred for use on potatoes. Where reasonable management of basal rot has already been achieved with 'Storite', alternate HWT treatments could substitute a fungicide of another mode-of-action group for the benzimidazole. Several have been shown to be active, though not as effective as 'Storite', but at present only carbendazim (another benzimidazole) and prochloraz are approved for this purpose in the UK. Other candidates, such as captan and chlorothalonil fungicides (Hanks & Linfield, 1996), would require off-label approval for this usage. A similar, but more restrictive, situation exists for post-lifting spray applications: only 'Storite' is approved for such use, though the other fungicides mentioned are known from experimental work to have some effect when applied in this way (Hanks & Linfield, 1996).

Point (3) requires the industry to consider the physical (handling) and cultural (growing) methods that can augment basal rot management by chemicals. Physical methods include the use of high air-flow rates for rapid drying, preferably below 20°C or at 35°C, bulb storage at 17°C, careful bulb handling throughout, careful inspection and rejection of diseased or damaged bulbs, and the cleaning and disinfecting of bulb stores and other facilities. Cultural methods include early lifting and late re-planting. New approaches to basal rot control also include biological, plant breeding and pathogen screening approaches, which have recently been considered elsewhere (Hanks & Carder, 2004).

It should be recorded that advice has been given (1) against reducing the rate of 'Storite' used to treat basal rot-susceptible cultivars, and (2) that there is a possibility of the corrosion of tanks and equipment when using acidic solutions (D.Turner, Banks Cargill Agriculture Ltd., personal communication).

## Additional recommendations

The results of the present study highlight the problems that may arise when pesticide treatments are adopted without having determined the fate of the pesticide. Uptake and persistence should be determined as a matter of course.

In this study many bulbs were found infected with large narcissus fly larvae. This should draw attention to the often unappreciated occurrence of this pest in the eastern counties.

#### Legal aspects of using SBS

The legal position on using SBS in bulb dips has been considered, and current information is summarised below:

- In response to an enquiry from the HDC, the Pesticides Safety Directorate (PSD) wrote (16 September 1998) "... the use of such acidifiers does not fall to be regulated as either a pesticide or adjuvant under current UK pesticide legislation. Use of acidifiers as described in your letter is legal as long as all the statutory conditions of use of the appropriate pesticide product are not infringed. However, it may be advisable for the HDC to check their liability should they subsequently recommend such practices". This view was confirmed orally by PSD in October 2000 to Tim Briercliffe, ADAS, enquiring on behalf of the HDC.
- Further enquiries were made in October 2000 by Tim Briercliffe in relation to the Groundwater Regulations 1998 and the Health and Safety Executive (HSE). On the former, the Environment Agency wrote: "... I have considered SBS with respect to classification

under the Groundwater Directive. It does not appear to fall within the categories identified under either List 1 or List 2. The provisional classification is therefore that it does not fall within List 1 or List 2 under this Directive. This is however a provisional classification. All classifications made here at the [EA Exotoxicology] National Centre are circulated to a steering group for comment and in addition there is also an external consultation process. All classifications remain as provisional until these steps have been undertaken." SBS is therefore not covered at present under this Directive.

• Finally, HSE stated that SBS is not listed as a pesticide, adjuvant or commodity substance. SBS is classified as 'harmful' on the CHIP Approved Suppliers List, and the applicable risk and safety phrases are shown on the product safety sheet. "HSE do not see a problem with using the acid as long as an appropriate COSHH assessment has been conducted".

A further suggestion is that spent dip should be neutralised before disposal. It has been suggested that sodium hydroxide solution could be used to adjust the solution to pH 7 (G. Barrett, personal communication). Arguably, the pH need only be raised to the pH of the dip before SBS were added (about pH 4), since dips of this pH are already disposed of without problems. Handling solid or dissolved sodium hydroxide in a farm situation is considered by the authors to be inappropriate, though the amount required could easily be calculated. Alternatively, ground chalk or sodium bicarbonate could be used, but this would produce copious carbon dioxide production and sludge formation. The authors recommend that the simplest approach would be simply to allow the pH of the last one or two dips to rise naturally by adding no further SBS at this point. In any case, growers should be reducing top-up at the end of a dip's working life in order to minimise wastage and disposal problems.

## Further R&D

The above recommendations and requirements point at several areas for further R&D, both near-market and more strategic, including the following:

- The possibility of using other (cheaper) benzimidazole formulations (e.g., Storite Excel<sup>3</sup>) for bulb HWT, in association with SBS. The work should include studies to improve the uptake and distribution of thiabendazole by the bulb.
- Testing and developing recommendations for fungicides that could be alternatives to thiabendazole, including prochloraz. Possibly, this should include testing low-rate fungicide 'cocktails' as used in the Netherlands (such as prochloraz, a benzimidazole, captan and formaldehyde; Vreeburg *et al.*, 1993), which proved damaging to narcissus yields in an earlier HDC study (Hanks & Linfield, 1996). Any studies should include work on the persistence and stability of the materials, and of any adverse phytotoxic effects such as lower bulb and flower yields. This should go hand-in-hand with monitoring the status of fungicide tolerance in UK *Fusarium oxysporum* isolates.
- Developing an automatic dosing system for adjusting the pH of HWT tanks, as used in metering and adjusting nutrient solutions.
- Investigating novel methods of fungicide application, such as incorporation of fungicides into wax sprays as used with fruits (e.g., El-Refai & Mahmoud, 1982), fog or smoke application of thiabendazole in bulk stores as used in potatoes (Cayley *et al.*,

<sup>&</sup>lt;sup>3</sup> This replaced Storite Flowable.

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1979), and using drenching equipment as designed for treating fruit (Gratwick & Southey, 1986, p.20).

• Improving physical and cultural means of managing basal rot, including the development of automated, non-destructive detection methods to enable bulbs with low levels of internal rots to be removed, so avoiding the re-planting of a large amount of inoculum.

#### ACKNOWLEDGEMENTS

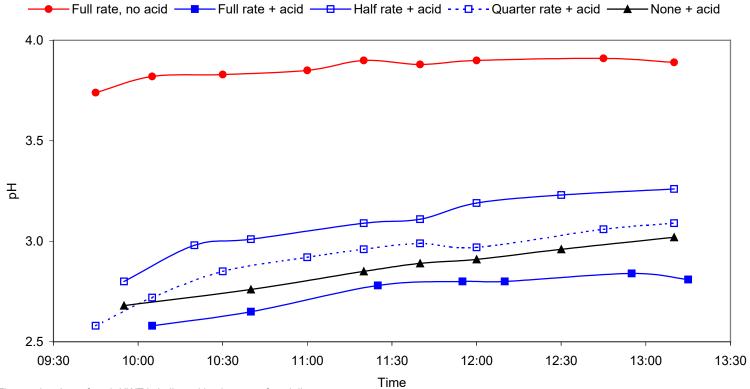
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#### Figure 1. Dip pH for five HWT treatments

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

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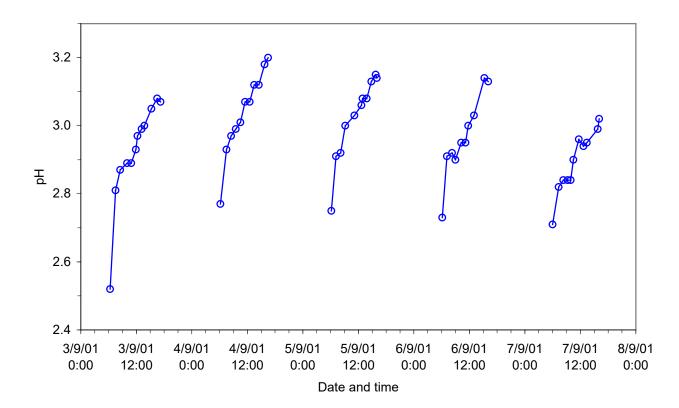
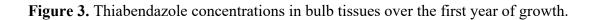
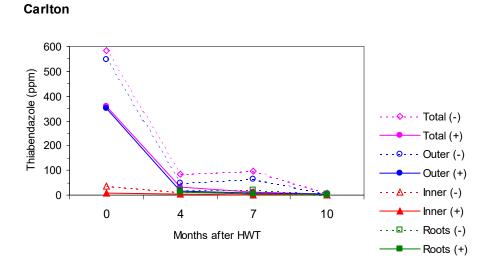


Figure 2. Dip pH over 5 days of HWT

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**Golden Harvest** 

