HDC Project BOF 64
Final Report (2007)

Narcissus: Determining the thiabendazole residues of bulbs following treatment with Storite Clear Liquid in hot-water treatment

Gordon R Hanks & Leanne Cozens
Warwick HRI, The Kirton Research Centre, University of Warwick

Project title:	Narcissus: Determining the thiabendazole residues of bulbs following treatment with Storite Clear Liquid in hot-water treatment
HDC project number:	BOF 64
Project leader(s):	Gordon Hanks Warwick HRI The Kirton Research Centre University of Warwick Kirton Boston Lincolnshire PE20 1NN T: 01205 725139 F: 01205 724957 E: Gordon.Hanks@warwick.ac.uk
Report:	BOF 64 Final Report (2007)
Previous reports:	None
Key worker(s):	Gordon R Hanks BSc, MPhil, FIHort, MIBiol, CBiol (Warwick HRI) Leanne Cozens BSc, DipBiol, DipChem, MIBiol, CBiol, MRSC (Warwick HRI)
Location:	Warwick HRI, Kirton, Lincolnshire (project management and HWT) CSL, York (chemical analysis)
Project co-ordinator(s):	Roy Willingham Wapentake Court Wood Lane Moulton Spalding Lincolnshire PE12 6QU
Date commenced:	1 September 2006
Date completion due:	31 January 2007
Keywords:	Basal rot, daffodil, disease control, fungicide, fungicide residue, *Fusarium oxysporum f.sp. narcissi,* hot-water treatment, *Narcissus*, thiabendazole.

CONTENTS Page no.

Narcissus: Determining the thiabendazole residues of bulbs following treatment with Storite Clear Liquid in hot-water treatment

Headline

This project determined the levels of thiabendazole residues in narcissus bulbs following treatment with quarter-rate Storite Clear Liquid in hot-water treatment for basal rot management. The data generated are being submitted to PSD to enable the environmental safety of the method to be evaluated.

Background and expected deliverables

For many years narcissus bulbs have been treated with the fungicide Storite Clear Liquid in order to manage basal rot caused by *Fusarium oxysporum f.sp. narcissi*. Most usefully, the fungicide is applied by adding it to the hot-water treatment tank. The HDC BOF Panel consider this use to be essential for maintaining the success of the UK bulb industry.

Recently, the environmental safety of this treatment has been questioned. However, the assessment that led to this was made without taking account of recent HDC findings that the use of Storite Clear Liquid could be reduced to a quarter-rate by the simple method of adding an acidifier to maintain the solubility of the active ingredient, thiabendazole (TBZ), during the treatment process. This project therefore provided samples of the treated bulbs that could be analysed to provide the relevant residue levels for submission to PSD.

Summary of the project and main conclusions

Bulbs of narcissus cultivars ‘Salome’ and ‘Carlton’, and pre-soaked bulbs of ‘Carlton’, were given standard hot-water treatment (HWT) with quarter-rate Storite Clear Liquid (1.25L formulation per 1000L water) to which an acidifier (sodium hydrogen sulphate, 12.42kg/1000L) had been added. Bulbs were sampled (1) before HWT, (2) at the end of HWT, (3) after HWT and a 24h period of cooling, ventilation and drying, and (4) after washing vigorously under running water for 30min. Samples were frozen and the concentration of TBZ determined by linked liquid chromatography-mass spectrometry.

The following average concentrations of TBZ (mg/kg FW) in the bulbs were found:

Stage of sampling	*Bulb stock*		
	‘Salome’	*‘Carlton’*	*Pre-soaked ‘Carlton’*
Before HWT	<1	<1	<1
After HWT	98	69	41
After drying	74	51	27
After washing	60	27	19

These data are being submitted to PSD for consideration.

Actions points for growers

- Following the outcome of PSD’s evaluation, a SOLA (0924/2007) has been issued for the use of Storite Clear Liquid (thiabendazole) in HWT of narcissus bulbs for basal rot control. Growers should ensure they have access to this SOLA Notice of Approval before using Storite Clear Liquid in narcissus HWT.

Narcissus: Determining the thiabendazole residues of bulbs following treatment with Storite Clear Liquid in hot-water treatment

Introduction

The fungicide formulation Storite Clear Liquid (hereafter referred to as 'Storite'), of which the active ingredient (a.i.) is the benzimidazole fungicide thiabendazole (TBZ), is the material of choice for treating narcissus bulbs to manage basal rot caused by *Fusarium oxysporum f.sp. narcissi*. This fungicide has been used, often (but not always) successfully, for many years by the UK bulb sector. Storite currently has on-label approval for use on narcissus bulbs, either as a post-lifting bulb spray, or by inclusion in a bulb dip (such as a cold dip given after lifting or in the hot-water treatment (HWT) which all narcissus bulbs should receive before re-planting). Both methods have their advantages and disadvantages, but many growers prefer the HWT option since (1) prompt drying, a major consideration at bulb lifting time, is slowed by spraying bulbs, and (2) post-lifting treatment would treat all bulbs (whether for sale or re-planting), whereas it is necessary to treat only bulbs for re-planting. A more general concern amongst narcissus producers was the relatively high cost of the Storite product, even though application as a bulb spray used about half the amount of product compared with bulb dipping.

Storite's licence holder, Syngenta Crop Protection UK Ltd., recently (2005-2006) reviewed its label recommendation for the product. By summer 2006 it was evident that Syngenta were unable to support the use as a bulb dip (Syngenta and the HDC, corporate communications). For the reasons given above, many bulb growers wished to retain the HWT application, the more so because recent HDC-funded research (Projects BOF 43 and 43a) had shown that a half- or quarter-rate Storite application in HWT was effective against basal rot if an acidifier (sodium hydrogen sulphate (SHS), also called sodium bisulphate) was added to the tank to maintain the a.i. in solution (benzimidazole fungicides such as TBZ are strongly soluble in water only at low pH values). The cost of adding SHS is very small, so using quarter-rate Storite would considerably mitigate against the high cost of full-rate Storite treatment, without loss of effectiveness.

In considering the proposal for treating bulbs in quarter-rate Storite in HWT, PSD apparently concluded that there were insufficient data available from the earlier HDC projects to permit approval for bulb dip use even using a much reduced rate (Syngenta, HDC and PSD, corporate communications). It was pointed out by PSD that Project BOF 43a was based on one trial, one cultivar, a half load in the HWT tank and using a bioassay for determining in-bulb levels of TBZ, and that the analytical work had not been done under compliance with Good Laboratory Practice (GLP).

In order to maximise the likelihood that PSD might reconsider their decision relating to the quarter-rate HWT use of Storite, further data (complying with the conditions listed above) were urgently needed. This situation became evident only in August 2006, towards the end of the bulb dipping season; realistic tests therefore needed to be carried out as soon as possible. These would involve the HWT of bulbs of two different cultivars with quarter-rate Storite plus acidifier, and determining TBZ concentrations in bulbs at the start and end of the 3-hour HWT period and at a pre-planting stage (i.e. after the ventilation, cooling and surface-drying that normally follows HWT). Because many bulb stocks are routinely 'pre-soaked' in water and formalin before HWT – a process that would reduce subsequent dip uptake in HWT itself – a set of pre-soaked bulbs was also included in the trial. To obtain additional information, one set of bulbs was washed prior to planting, to determine the amount of a.i. liable to be leeched from the bulbs into the soil once planted; from previous investigations (BOF 43a) the remainder of the TBZ, left within the bulb, would be expected to break-down *in situ* over the next about six months. These data would indicate the amount of TBZ liable to enter the environment following commercial-scale HWT and the planting of treated bulbs, allowing PSD to make a judgement on the suitability of continued Storite use in HWT. *It is important to note that no other as-effective fungicide, or alternative (e.g.*

biological) method of basal rot management, is available at this time; the use of Storite in HWT (at a reduced rate) was considered essential, by the HDC Bulb & Outdoor Flower Panel and others, for continued success of UK narcissus production.

The chief commercial objective of the project was therefore to maintain, for UK bulb producers, a safe, effective, economical and environmentally acceptable means of managing basal rot disease. This would reduce the sometimes considerable losses due to the disease, increasing the supply of quality bulbs available for the multiple retailer and export markets. The UK exports a large proportion of its narcissus bulb and cut-flower production, an outstanding example of a horticultural success which should be encouraged.

Materials and methods

Material

In August 2006 bulbs (grade 12-14cm circumference) of narcissus cultivar 'Carlton' were taken from stocks grown at The Kirton Research Centre (KRC), and 'Salome' bulbs were obtained from a commercial source. At the time of use, the bulbs were free of soil and were surface-dry. Six, *ca*. 25kg-nets of 'Carlton' bulbs and three, *ca*. 25kg-nets of 'Salome' were allocated for the project. Each net of bulbs was weighed just prior to pre-soaking or HWT (see below). One tonne of further bulbs from the 'Carlton' stock was allocated for use as 'fillers' (see below). Between operations, bulbs were stored at 17°C.

Bulb treatment plant

The KRC HWT tanks (Secker Welding, Holbeach, UK) used were based on a standard design with front-loading dipping tanks and overhead holding (slave) tanks. Purpose-built for commercial-scale practice under experimental conditions, the facility consists of four independent tanks each holding up to two half-tonne bulk bins. Prior to use, the tanks, associated pipe-work, etc., was thoroughly washed out followed by rinsing with plain water.

Pre-soaking treatment (see below) was applied in a *ca.* 0.5t-capacity general purpose dipping tank equipped with suitable dip circulation but without heating.

Pre-soaking treatment

On 27 September 2006 three nets of 'Carlton' bulbs were soaked overnight for 14h in a general purpose tank containing commercial formalin (containing 38-40% formaldehyde) (5L per 1000L) and non-ionic wetter (0.3L per 1000L) at ambient temperature. After removal from the tank the nets were allowed to drain (*ca*. 10min) before continuing to HWT. These bulbs are referred to below as 'pre-soaked bulbs'. The other six nets of bulbs were not pre-soaked.

HWT treatments

On 27 September 2006, the day before HWT treatments were carried out, an HWT tank was filled with 3000L of mains water and heated to and maintained at 44.4°C. On the next morning the pH of the plain water, measured using a digital pH meter, was 8.2, typical of the mildly alkaline mains water of the site.

The following chemicals were then added to the 3000L of circulating water, and allowed to dissolve and disperse, in order:

- Technical grade sodium hydrogen sulphate (4.14kg)
- Storite Clear Liquid (3.75L)
- Commercial formalin (15L)
- Non-ionic wetter (900ml)
- Anti-foam product (120ml)

After the addition of all the chemicals the pH of the dip solution was 2.6, as found earlier (BOF 43a).

After taking out sample bulbs (see below), the three nets each of pre-soaked 'Carlton' bulbs and of non-pre-soaked 'Carlton' and 'Salome' bulbs were randomly placed into two clean, heat-sterilised ½-tonne, wooden bulk bins previously part-filled with further loose 'Carlton' bulbs acting as 'fillers'. There were four nets in one bin and five nets in the other. The bins were then topped-up with further filler bulbs. The empty weight of each bin and the final total weight of each bin were recorded using a 1t weigh-bridge. The two bins were loaded into the HWT tank which was then run for 3h at 44.4°C once the required temperature had been regained following the loading of the bins (*ca*. 15min).

After the 3h-HWT the pH of the dip solution was measured and was 3.3. After a suitable drain-down period (*ca*. 10min) the two bins were removed from the tank and the volume of dip left in the tank noted (2900L).

Post-HWT drying and washing

Immediately after HWT the bins of bulbs were ventilated, cooled and dried for 24h at ambient temperature using high-capacity fans mounted in covers that fitted on the tops of the bulk bins.

Following removal of sample bulbs, the nets were re-tied and washed vigorously under running tap-water for 30min and then placed on a clean surface and allowed to drain for *ca*. 30min before further bulb samples were taken.

Sampling bulbs and TBZ determination

Before HWT a sample (sample 1) of bulbs (≥ 1.0kg and containing at least 10 bulbs) was extracted from each of the nine, 25kg-nets. Each sample taken was placed in a clean, labelled polyethylene (PE) bag and placed in a freezer until despatch for analysis. The temperature of the freezer was logged at daily intervals and varied between -17°C and -26°C with a mean temperature over the relevant period of -24.3°C.

Further, similar bulb samples were extracted from each 25kg-net at the end of the 3h-HWT (sample 2), after ventilating and drying for 24h (sample 3) and after washing for 30min (sample 4).

Bulb samples were kept frozen and despatched to CSL, York, on 2 November 2006. Full details of the methods used at CSL, and the full results, are reproduced in Appendix C. Briefly, frozen bulb samples were homogenised in the presence of solid carbon dioxide and samples extracted by homogenisation with ethyl acetate/sodium sulphate and filtered. An aliquot of filtrate was evaporated to dryness under a stream of nitrogen and the residue dissolved in methanol with ultrasonication. A sample was then separated by liquid chromatography and quantified by linked mass spectrometry (LC-MS). The method gives the total TBZ content of the sample, i.e. including both dissolved and suspended TBZ. When validated these methods showed mean recoveries, from narcissus bulb fortified with 3 or 100mg TBZ per kg, of 117.6% (relative standard deviation (RSD), 6.5%) and 85.3% (RSD, 2.5%), respectively. The work at CSL was carried out in compliance with GLP standards.

The results are presented in Table 1 as the 36 individual values and as means of the three replicates of each cultivar x treatment x sample time combination; all measurements are expressed in mg of TBZ per kg fresh weight of bulbs (mg/kg FW), i.e. parts per million (ppm). These data were subjected to the analysis of variance, and standard errors of the difference of means (SEDs) and probability levels (P) at the appropriate degrees of freedom (df) are also given in Table 1.

Sampling dip and TBZ and formaldehyde determination

Following the addition of all chemicals to the HWT tank, but before the bulbs were loaded, a sample of dip solution (250ml) was taken in a clean, labelled PE bottle and placed in a freezer (as above) until despatch for TBZ determination. A further sample (100ml) was taken in a clean, labelled PE bottle and refrigerated (4°C) until formaldehyde determination which was carried out at KRC on the following day.

At the conclusion of the 3h-HWT period a further dip sample was taken as at the beginning.

Dip samples for TBZ determination were sent to and analysed by CSL. After shaking, sub-samples were made up in methanol, diluted as appropriate, and analysed by LC-MS (see above and Appendix C). Three sub-samples were analysed for each of the two samples. All measurements are expressed in mg of TBZ per L of dip (mg/L).

Dip samples for formaldehyde determination were analysed by a standard titration method (Appendix A). Samples taken at the start and end of HWT both gave formaldehyde concentrations of 0.18%, compared with a target concentration of 0.20% (based on using 5L of commercial formalin (38-40%) per 1000L of dip. The figure of 0.18% is typical of formaldehyde concentrations found over a series of HWT runs in an earlier study (BOF 43); it indicated that the dip components or conditions used did not degrade formaldehyde to any substantial degree.

Results

General comments

- The weights of bulb samples, of '25kg-nets' of bulbs, and of bulk bins are given in Appendix B. Although TBZ concentrations in bulbs given below were expressed in mg/kg FW, the information in the Appendix would allow other calculation to be made, if required.
- As expected from earlier studies, the Kirton mains water had a slightly alkaline pH value, and this was reduced to 2.6 following chemical additions and rose slightly (to about 3.3) by the end of HWT.
- As mentioned under Materials and Methods, formaldehyde concentrations were as expected at both the start and the end of HWT, indicating no interference from the other chemicals in the dip.
- Bulbs sampled before HWT showed no detectable level of TBZ, in line with the earlier study.

TBZ concentrations

As determined using LC-MS, TBZ concentrations in the 36 bulb samples examined varied from <1 to 101.0mg/kg FW (Table 1, Figure 1 and Appendix C). Analysis of variance for concentrations in the bulbs showed that both factors – bulb cultivar/treatment and sampling stage - and the interaction between them, were statistically significant at the 0.1% level, meaning that the differences between the means were highly unlikely to have arisen by chance (Table 1).

Before HWT, bulbs had TBZ concentrations below the limit of detection (<1mg/kg FW); after HWT, concentrations had risen to an average of 69mg/kg FW, and subsequently fell to averages of 50 and 38mg/kg FW after drying and washing, respectively. Overall, 'Salome' had the greatest concentration (58mg/kg), while this was lower in 'Carlton', and particularly in pre-soaked 'Carlton', at 38 and 22mg/kg FW, respectively.

The concentrations of the two dip samples averaged 86.8 and 90.1mg/L, not significantly different from one another taking into account the standard deviations (SD) (Table 2 and Appendix C).

Figure 1. Summary of TBZ concentrations in three stocks of bulbs at three stages of treatment.

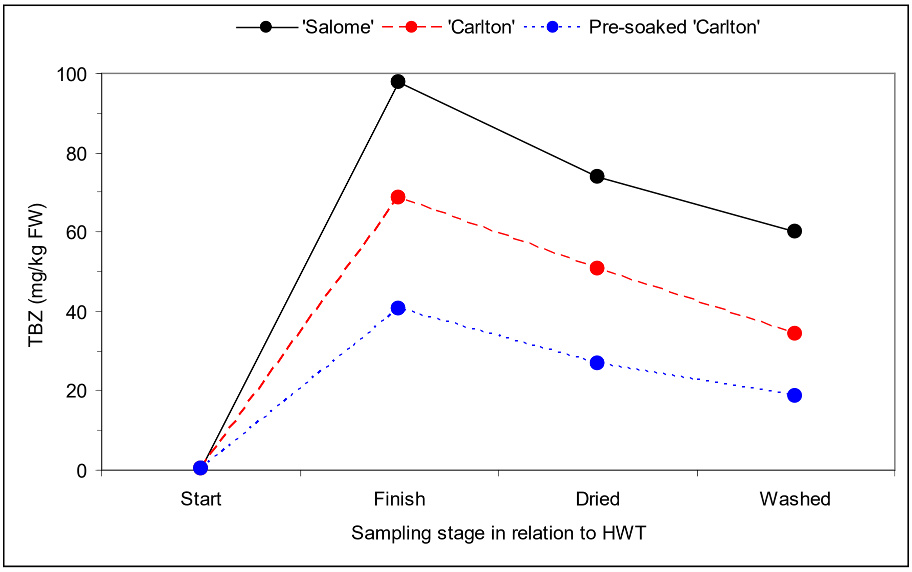

Table 1. TBZ residues in narcissus bulbs determined using LC-MS following various bulb treatments, with summary of analysis of variance. The means for the treatment combinations are shown in the upper part of the table, with the marginal means for the main factors below.

Bulb cultivar and treatment	*Sample stage*	*Replicate number*	*TBZ residue (mg/kg FW)*	
			Replicates	*Mean*
'Salome'	Before HWT	1	<1	
		2	<1	<1
		3	<1	
	After HWT	1	101.0	
		2	99.6	97.6
		3	92.3	
	After drying	1	73.4	
		2	65.3	74.0
		3	83.4	
	After washing	1	60.9	
		2	61.1	60.0
		3	58.1	
'Carlton'	Before HWT	1	<1	
		2	<1	<1
		3	<1	
	After HWT	1	64.9	
		2	75.1	68.8
		3	66.3	
	After drying	1	43.6	
		2	54.3	50.8
		3	54.4	
	After washing	1	30.1	
		2	35.5	34.5
		3	38.0	
'Carlton' (pre-soaked)	Before HWT	1	1.6	
		2	<1	<1
‘		3	<1	
	After HWT	1	41.8	
		2	45.1	40.6
		3	35.0	
	After drying	1	24.0	
		2	33.6	26.8
		3	22.8	
	After washing	1	19.0	
		2	16.5	18.7
		3	20.6	
SED (22df)				3.85
Significance (P)				<0.001***
Marginal means for bulb cultivar and treatment				
'Salome'				58.1
'Carlton'				38.4
'Carlton' (pre-soaked)				21.8
SED (22df)				1.92
Significance (P)				<0.001***
Marginal means for bulb cultivar and treatment				

Before HWT	0.6
After HWT	69.0
After drying	50.3
After washing	37.8
SED (22df)	2.22
Significance (P)	<0.001***

Table 2. TBZ residues in bulb dips determined using LC-MS.

Sample stage	*Sub-sample number*	*TBZ residue (mg/L)*	
		Sub-samples	*Mean (SD)*
Start of HWT	1	91.7	85.8 (5.1)
	2	82.8	
	3	82.9	
End of HWT	1	89.2	90.1 (1.0)
	2	91.2	
	3	89.8	

Discussion

Taking account of the different methods used in the present study and in the earlier ones previously referred to (BOF 43 and 43a), the findings on TBZ concentrations in narcissus bulbs following application of quarter-rate Storite with acidifier in HWT are considered to be in broad agreement. In BOF 43a, the mean TBZ concentrations in bulbs sampled after HWT and re-drying bulbs, were 110 and 125mg/kg FW in 'Carlton' and 'Golden Harvest', respectively. These figures were derived from samples of the outer parts of the bulb only (i.e. of the dry brown scales plus the outer two white bulb scales), where *ca*. 95% of the residue was found. In the present study, bulbs sampled at the same stage of the treatment process gave concentrations of 74, 51 and 27mg/kg FW, respectively, for bulbs of 'Salome', 'Carlton' and pre-soaked 'Carlton'. When sampled at the end of the HWT period, before bulbs were ventilated, cooled and surface-dried for a period of 24h, the corresponding concentrations of TBZ were 98, 69 and 41mg/kg FW, respectively.

From the above findings, and since bulb handling regimes will vary considerably between bulb producers and particular circumstances, it seems reasonable to expect the concentration of TBZ in bulbs following quarter-rate Storite treatment to lie in the broad range of 25 to 100mg/kg FW. This is a wide range, and one major source of this variability would be the water content of the bulbs at the start of HWT, as illustrated by the differences between pre-soaked and non-pre-soaked 'Carlton' bulbs. Many bulb stocks are pre-soaked before HWT, usually as a consequence of using a warm storage treatment (used to reduce subsequent HWT-induced bulb damage) – warm storage dehydrates bulbs, which causes any stem nematodes (*Ditylenchus dipsaci*) present to adopt the water-repellent, temperature-resistant 'wool stage' that is so effective in spreading infestation. The prior soaking of the bulbs will reduce considerably their capacity for water uptake during a subsequent immersion. In this study, 'Salome' bulbs contained more TBZ than 'Carlton' bulbs. While there may be differences in TBZ uptake or in the amounts of water that can be taken up by different cultivars of bulbs due to differences in internal structure, it seems more likely that simple differences in bulb handling and amount of drying could account for these differences. Thus, it appeared that the 'Salome' bulbs used here were relatively dehydrated by the time of receipt or use, a '24kg-net' of bulbs of 'Salome' having only 93% of the weight of a net of 'Carlton', despite all nets having held, initially, the same approximate weight and volume of bulbs.

What estimate of TBZ concentration should be used in calculating likely residues from the fungicide application? Previous studies (BOF 43a) showed that, following Storite treatment in HWT, the bulk (>90%) of TBZ present was in the outer layers of the bulb, and it is likely that much of this was deposited on, rather than absorbed by, the bulb. The decrease in TBZ concentrations in bulbs between the end of HWT and the end of a 24h cooling, ventilating and drying period, shows that marked losses occur during this process: for the three stocks, 24, 26 and 34% of the TBZ content present at the end of HWT had been lost by the end of the 'drying' period. On average, this indicates that some 51mg of TBZ would be included per kg of bulbs planted; at an average bulb planting density of 17.5t/ha, this would equate to <0.9kg TBZ per hectare. Of this TBZ, it is likely that a relatively limited amount would enter the soil, the bulk remaining *in situ* were it would be degraded over a period of about 6 months (BOF 43a). In the present study, HWT-treated, dried bulbs were subjected to simulated leeching by vigorous washing before planting; for the three stocks, 14, 13 and 8 mg of TBZ per kg FW of bulbs was lost in this way, which would represent, on average, a TBZ load into the soil of <0.25kg/ha.

These findings proved useful in assessing the environmental impact of using reduced-rate Storite in HWT to control basal rot in narcissus bulbs and following the outcome of PSD's evaluation, a SOLA (0924/2007) has been issued for this use. Growers should therefore ensure they have access to this SOLA Notice of Approval before using Storite Clear Liquid in narcissus HWT.

The findings also indicate how the environmental impact of this essential use of TBZ might be reduced further, for example (1) by pre-soaking bulbs, (2) by using carefully observed drain-down procedures for bulbs exiting HWT tanks, and (3) by improved containment of dust and bulb skins in the period between the end of HWT and planting.

Acknowledgements

We thank the staff of Warwick HRI, KRC, especially Rodney Asher, for their skilled assistance in carrying out this work.

Appendix A: Method for determining formaldehyde concentrations in dips.

ADAS

The determination of formaldehyde content in HWT tanks

Ministry of Agriculture, Fisheries and Food

THE DETERMINATION OF FORMALDEHYDE CONTENT IN HWT TANKS

Formaldehyde is measured titrimetrically employing the iodine method as devised by Romijn.

A known volume of a standard iodine solution is added to the formaldehyde/water mixture. The solution is made alkaline with sodium hydroxide when the resulting hypoiodite oxidises the formaldehyde to formic acid. Excess hypoiodite forms into the iodate salt of sodium and on the addition of hydrochloric acid elemental iodine is released. The iodine is titrated with sodium thiosulphate employing the normal starch end point thus ascertaining the amount of iodine used in the reaction

Sampling

An initial sample should be taken from the HWT tank after the formaldehyde has been added and adequate mixing taken place Variations have been found between different sides of the tank on the first sampling due to inadequate mixing. Samples should be taken at half-hourly intervals whilst the bulbs are being treated to assess the formaldehyde content. With experience it should be possible to reduce the number of samplings per day.

Apparatus		Minimum quantity
250 cm^3	stoppered Erlenmeyer flasks (conical)	3
150 cm^3	beakers	3
25 cm^3	measuring cylinders	3
10 cm^3	measuring cylinders	2
25 cm^3	burette	1
Filter funnels	polythene	3
1000 cm^3	volumetric flasks	2
Washbottle · polythene		1
Filter papers · 15 cm diam. Whatman No. 2		
Burette stand		

Reagents

1. Iodine solution 0.1N (0.05M)
2. Sodium Thiosulphate solution 0.1N (0.1M)
3. Sodium Hydroxide solution, 2N
4. Hydrochloric acid, 2N
5. Starch solution, 1% $^{M}/_{v}$

Reagents 1 and 2 can be prepared from CVS solutions (concentrated volumetric solutions)
Reagents 3 and 4 can be prepared from CVS solutions by purchasing 1N CVS solutions and diluting to half the stated volume.
Reagent 5 can be prepared by adding 1g soluble starch to 100 cm^3 boiling water.

Distilled water should be used for the preparation of the reagents and also to rinse glassware after washing. Instructions for the preparation of the reagents are given on the container.

Filter the soil/water suspension through a Whatman No 2 filter paper discarding the first few cc's. Measure 10 cm^3 of the filtrate in a measuring cylinder ensuring a correct meniscus reading. Transfer to the 250 cm^3 Erlenmeyer flask, and add 5 cm^3 distilled water. Measure 25 cm^3 iodine solution 0.1N in a measuring cylinder and add to the filtrate in the flask. Add 4 cm^3 sodium hydroxide solution 2N, mix, stopper and allow to stand for 10 minutes. Add 6 cm^3 hydrochloric acid 2N and titrate the liberated iodine with sodium thiosulphate solution 0.1N until a pale straw colour is obtained. Add a few drops of starch solution 1% $^M/v$ and continue the titration until the colour changes from blue to colourless. Record the titre. The end-point is rapid and sharp and the sodium thiosulphate solution should be added dropwise after the addition of the starch solution. A blank determination must be carried out daily using 10 cm^3 distilled water instead of the filtrate.

Calculation

1 cm^3 0.1N Iodine = 0.0015015g Formaldehyde
Iodine used = Blanktitre - sample titre = a

$$a \times 0.0015015 \times \frac{100}{10} = {}^M/v \text{ formaldehyde}$$

SAFETY PRECAUTIONS

1. Safety spectacles or goggles must be worn whilst sampling, and throughout the analytical procedure.
2. Any spillages of reagents on skin, eyes or clothing should be removed immediately by flushing under cold water for at least 5 minutes. Medical attention should be sought immediately should any of the reagents come into contact with the eyes.
3. Wash hands and exposed skin before eating, drinking or smoking and after the job is completed.
4. The method should be carried out by a competent trained person. It is assumed that the user is familiar with the requirements of the Health and Safety at Work Act 1974.

ADAS Analytical Chemistry Department
Cambridge RO
December 1980

Table of volumes (Formaldehyde solution) to correct to 0.2% $^M/v$ Formaldehyde

% $^M/v$ Formaldehyde found (by titration) in HWT solution	gals Formaldehyde solution/1000 gals water - to correct to 0.2% $^M/v$
0.20	0.00
0.19	0.25
0.18	0.50
0.17	0.75
0.16	1.00
0.15	1.25
0.14	1.50
0.13	1.75
0.12	2.00
0.11	2.25
0.10	2.50
0.09	2.75
0.08	3.00
0.07	3.25
0.06	3.50
0.05	3.75
0.04	4.00
0.03	4.25
0.02	4.50
0.01	4.75

Appendix B: Bulb, net and bin weights

Table B1. Weights of '25kg-nets' of bulbs used as replicates.

Bulb variety and treatment	*Replicate number*	*Weight (kg)*
'Carlton'	1	24.96
	2	25.07
	3	25.00
'Carlton' (pre-soaked)	1	25.02
	2	24.97
	3	25.01
'Salome'	1	23.40
	2	23.26
	3	23.38

Table B2. Whole bin weights as used in HWT tests.

Bin and position	*Weight empty (kg)*	*Weight full (kg)*	*Weight dried (kg)*
1 (upper)*	73.0	471.5	472.0
2 (lower)**	69.0	467.5	465.0

*Containing 'Carlton' 1, 'Carlton' pre-soaked 1 and 2 and 'Salome' 3.
**Containing 'Carlton' 2 and 3, 'Carlton' pre-soaked 3 and 'Salome' 1 and 2.

Table B3. Weights of '10 bulb' samples used for analysis.

Bulb variety and treatment	*Sample stage*	*Replicate number*	*Weight (g)*
'Carlton'	Before HWT	1	1065.4
	Before HWT	2	1048.8
	Before HWT	3	1020.3
'Carlton' (pre-soaked)	Before HWT	1	1071.8
	Before HWT	2	1050.0
	Before HWT	3	1145.9
'Salome'	Before HWT	1	1027.6
	Before HWT	2	1056.3
	Before HWT	3	1034.3
'Carlton'	After HWT	1	1356.4
	After HWT	2	1389.1
	After HWT	3	1160.0
'Carlton' (pre-soaked)	After HWT	1	1258.0
	After HWT	2	1395.8
	After HWT	3	1131.7
'Salome'	After HWT	1	1559.2
	After HWT	2	1301.4
	After HWT	3	1549.8
'Carlton'	After drying	1	1242.8
	After drying	2	1056.1
	After drying	3	1246.9
'Carlton' (pre-soaked)	After drying	1	1375.2
	After drying	2	1498.9
	After drying	3	1328.6
'Salome'	After drying	1	1573.6
	After drying	2	1495.9
'	After drying	3	1553.8

‘Carlton’	After washing	1	1269.6
	After washing	2	1089.8
	After washing	3	1134.7
‘Carlton’ (pre-soaked)	After washing	1	1355.7
	After washing	2	1262.1
	After washing	3	1062.9
‘Salome’	After washing	1	1556.7
	After washing	2	1428.2
	After washing	3	1375.0

Appendix 3: TBZ determinations: report from CSL