Project title:	**NARCISSUS BULB SCALE MITE: CONTROL USING HOT WATER AND DRY HEAT TREATMENTS**
Project number:	**BOF 25a**
Project leader:	Mr. Jozef Starzewski
Report:	Final Report, Year 2 (May 2000)
Key Workers:	Mr. Jozef Starzewski - Project leader Mr. Gordon Hanks*
Location of Project:	Central Science Laboratory, Sand Hutton, York, North Yorkshire. YO41 1LZ *Horticultural Research International, Kirton, Boston, Lincolnshire. PE20 1NN
Project Coordinators:	Dr. Gordon Flint, Winchester Bulbs Ltd., Varfell Farm, Long rock, Penzance, Cornwall TR20 8AQ Mr. Brian Taylor, O A Taylor & Sons Ltd., Washway House Farm, Holbeach, Spalding, Linconshire PE12 7PP
Date project commenced:	1 July 1998
Date completion due:	31 May 2000
Key words:	Bulb scale mite, dry heat treatments, formaldehyde, hot water treatments, HWT, Narcissus, *Steneotarsonemus laticeps* (Halbert).

CONTENTS

PRACTICAL SECTION FOR GROWERS

Background and objectives

The bulb scale mite (BSM) *Steneotarsonemus laticeps* (Halbert), is an increasingly important pest of narcissus, the damage being most obvious in forced crops, resulting in reductions of both yield and flower quality. In addition, narcissus bulbs destined for export to the USA must be free from bulb scale mite and pass Plant Health Inspections.

Narcissus stocks are routinely given a hot water treatment (HWT) containing 0.2% formaldehyde. This control method was first developed for use against the large narcissus fly *Merodon equestris* (Fabricius) and later adapted for the control of the stem nematode *Ditylenchus dipsaci* (Kühn). In recent years growers have noted increasing incidences of bulb scale mite. The high incidence of rejections by the Plant Health and Seeds Inspectorate (PHSI) of bulbs for export, during the 1989/90 season and subsequently, and the associated financial loss lead to the commissioning of this project.

The objective in year 1 of the project was to assess the effectiveness of the two different regimes of HWT employed by growers in Eastern England and South West England for the control of bulb scale mite on stocks for replanting. Following a successful outcome, the work in year 2 focused on :-

(i) the effectiveness of shorter periods of HWT for the control of bulb scale mite in bulbs for forcing and

(ii) the effectiveness of dry heat treatments as an alternative to methyl bromide fumigation for the control of bulb scale mite in bulbs destined for the export market.

Summary of results

Year 1 (1998/99)

The work conducted in the first year of this project confirmed that the standard hot water treatments as used in the main bulb growing regions of the UK on stocks for replanting (see Table below), are effective methods for the control of all developmental stages of the bulb scale mite (Starzewski, 1999).

Commercial hot water treatments for narcissus bulbs

Eastern Counties – standard HWT	**South West Counties – standard HWT**
Bulbs stored at ambient temperature prior to HWT	Bulbs stored at 30-35ºC for 7 days
	Bulbs soaked for 3 hours in 0.2% formaldehyde solution and wetting agent at ambient temperature
Bulbs subjected to 0.2% formaldehyde solution and wetting agent for 3 hours at 44.4ºC	Bulbs subjected to 0.2% formaldehyde solution and wetting agent for 3 hours at 46.7ºC

Year 2 (1999/2000)

i) ***HWT treatments for shorter time periods for the control of bulb scale mite in bulbs for forcing***

Narcissus bulbs of cv 'White Lion' severely infested with bulb scale mite were subjected to different durations of HWT using the Eastern Counties standard HWT regime. Hot water treatment at 44.4ºC (~112ºF) for a period of 30 minutes was fully effective in the control of bulb scale mite eggs and provided almost complete control of the adults (99.8% kill). Hot water treatment at 44.4ºC for periods of 60 minutes to 3 hours were fully effective for the control of eggs and adults.

As the cultivar 'White Lion' is not suitable for forcing, none of the bulbs were forced after HWT to assess for flower damage. Further assessment trials need to be conducted on a semi-commercial basis using suitable cultivars.

ii) Dry heat treatments for the control of bulb scale mite in bulbs for export

The temperature/time combinations 42 ºC for 3 hour, 46 ºC for 2 hours and 46 ºC for 3 hours were fully effective for the control of bulb scale mite eggs. The temperature/time combinations of 42 ºC for 3 hours, 44 ºC for 2 hours, 44 ºC for 3 hours, 46 ºC for 2 hours and 46 ºC for 3 hours were fully effective against BSM adults. Thus the minimum temperature/time combination found to be fully effective against both adults and eggs of the bulb scale mite was 42ºC for a 3 hour period.

All of the dry heat treatments employed in this study were found to have no significant effect on bulb vigour or the quality of the flowers that were produced, as assessed when the bulbs were grown on.

Action points for growers

- The standard UK HWT are fully effective in controlling bulb scale mite in bulbs for replanting. Thus, all planting stocks of narcissus should be free from infestation if treated and managed correctly.
- To ensure that HWT treatments are fully effective certain basic principles should always be applied:
  - Good hygiene measures before and after HWT
  - Ensure that treated and untreated bulbs are always handled and stored separately
  - Clean equipment between stocks to prevent cross contamination
  - Ensure that treated bulbs are stored in a clean area away from the untreated bulbs to minimise the risk of re-infestation

- Ensure that the necessary health and safety measures are in place, that COSHH regulations are observed and that the treatment tank is working properly and is well maintained.
- Ensure that the formaldehyde and wetting agent is used at the recommended rate.
- Ensure that the treatments are timed from when the tank temperature returns to the treatment temperature, after the addition of the bulbs.

- The work has shown that 1 hour HWT can be effective in controlling both the egg and adult stages of bulb scale mite. There are some indications from work published in 1970's (Winfield, 1971)[1] that HWT for 1 hour prior to forcing should not lead to any damage of forced bulbs. However, robust and detailed information is sparse and further trials are needed on a range of suitable forcing cultivars before any recommendations can be made.
- Dry heat treatments have potential for the control of bulb scale mite in bulbs for export as an alternative to methyl bromide fumigation. No robust grower recommendations can be made at this stage.

Anticipated financial benefits

In 1990, 2.6% of narcissus stocks destined for export were rejected due to the presence of live bulb scale mite. At the time this amounted to an estimated loss of £75,000, and prompted the commissioning of project BOF25a. The most recent figures quoted by the MAFF Basic Horticultural Statistics for 1998 indicate that the value of narcissus bulbs exported was £9.45 million. Therefore improved measures for bulb scale mite control will allow UK growers to produce quality crops of forced flowers and retain or perhaps increase the valuable export market.

[1] Winfield, A.L. (1971). Control of bulb scale mite and stem nematode of narcissus, and reclaiming forced bulbs. Plant Pathology 20: 10-13.

SCIENCE SECTION

Introduction

Historically, investigation of the control of BSM has been directed towards identifying the most effective temperature/time combination for use in hot water treatments. Different authors have proposed various combinations; 30 minutes at 40ºC (Blattny, 1933); one hour at 43.3ºC (Hodson, 1934); one hour 30 minutes at 43.4ºC (Doucette, 1936); three hours at 44.4ºC and four hours at 43.3ºC (Winfield, 1958).

Water vapour heat treatments, as a method of controlling BSM have also been investigated; the method entails circulating super-saturated, warmed air through bulb stocks. It is reported that using this method, complete BSM mortality resulted after two hours at temperatures of between 43.3ºC and 43.9ºC (Doucette, 1936). Spruijt and Blanken (1933), using a two hour treatment at 48.9ºC, found that vapour-treated Narcissus bulbs produced flowers equal to those of untreated controls and the foliage was not injured by this treatment.

Neither the hot water nor the water vapour heat treatments reported above identified the upper lethal temperature (ULT) for any of the life stages of the BSM, although they do give some indication of the likely temperature. Fox-Wilson (1939) states that the upper thermal death point (equivalent to the ULT) lies between 38ºC and 49ºC. The accurate identification of this point is key to the design of any effective heat treatment for the control of BSM.

Studies conducted in year 1 of the project (1998/99) confirmed that the standard HWT as used in the main bulb growing regions of the UK, are effective methods for the control of all the developmental stages of the BSM. Work in year 2 will evaluate reduced time exposures to HWT for the control of BSM in bulbs for forcing. It was

agreed at the HDC project review meeting in June 1999 that dry heat treatments should be examined as a means of controlling BSM infestations in Narcissus bulbs destined for forcing or export. It would be inadvisable to subject such stocks to HWTs, because of the risk of flower damage and bulb discolouration. The use of methyl bromide fumigation, although effective for the control of BSM in dry bulbs, is to be phased out in the foreseeable future, so an effective alternative needs to be sought.

Materials and Methods

A BSM infested stock of the Narcissus cultivar 'White Lion', that had been grown at CSL Sand Hutton since 1997, was used for the work. The bulbs were harvested and then stored at 15°C prior to use.

HWT for reduced exposure periods - Preliminary experiment.

Prior to carrying out the planned HWT, a brief study was made of the rate at which the core temperature of Narcissus bulbs increase once immersed in HWT at 44.4 °C. Taking a single replicate of 25 bulbs, a thermocouple was inserted into the centre of one of the bulbs via the root plate. The bulbs were then given a standard HWT in a laboratory water bath, during which the temperature of the monitored bulb was recorded at regular intervals. The temperature of the water bath was monitored at regular intervals throughout the treatment with a 0-100°C (1/10th°C incremental) mercury/glass thermometer. After treatment the bulbs were removed and allowed to return to ambient temperature, the rate of cooling was also monitored at regular intervals.

HWT for reduced exposure periods

Following the methodology used in the first year of this project (Starzewski, 1999), all the treatments were carried out under laboratory conditions in a 100-GS Safelabs System Ltd. externally-vented fume cabinet. The experimental treatments were done in a Bayer SE20, 20 litre, 0-80°C circulating water bath. Prior to each treatment replicate the water bath was filled with a fresh solution of 0.2% formaldehyde, to which the non-ionic wetting agent 'Enhance' (87% w/w Alkyl phenol ethyl oxide) was added, at a rate of 0.64ml/litre (John Fisher, Winchester Growers Limited, pers. comm.). Temperature was monitored by a thermocouple probe placed in the centre of the water bath, attached to a Dimplex electronic thermometer (+/- 1°C) and by a 0-100°C (1/10th°C incremental) mercury/glass thermometer. This was inserted into the end of the water bath furthest from the heating element. Before each treatment the water bath was calibrated to run at 44.4°C as indicated by the mercury/glass thermometer. The temperature of the water bath was monitored prior to each treatment over a one hour period to ensure a stable temperature had been reached. Four replicates of 150 infested bulbs were placed separately and sequentially into a stainless steel mesh cage and immersed into the formaldehyde and wetting agent solution in the water bath, running at a temperature of 44.4°C, ensuring a 1:3 bulb to solution ratio. The temperature of the water bath was monitored as described previously. The treatment was considered to have begun once the water bath had returned to the treatment temperature (44.4°C) after the addition of the bulbs. The temperature of the water bath was monitored at 30 minute intervals throughout the treatment. At intervals of ½, 1, 1½, 2, 2½ and 3 hours, 25 bulbs were removed from the water bath, allowed to return to ambient temperature, at which time 5 bulbs per time treatment were selected at random for dissection. A count was made of the live

and dead adult mites, and 25 eggs were gently removed with a single hair brush and placed into a cell, labelled and incubated at 15°C and 90% relative humidity (Lynch & Bedi, 1994). The cells were observed daily for egg hatch, over a 30 day period. Controls consisted of four replicates of 25 untreated bulbs. Five bulbs were selected at random from each replicate, dissected and a count made of the live and dead mites. Two cells of 25 eggs were labelled, set up alongside those of the experimental treatments and also incubated at 15°C and 90% relative humidity. The cells were monitored for egg hatch over a 30 day period.

Dry heat treatments - Two preliminary experiments

The first experiment was a brief study to monitor the rate at which the core temperature of the Narcissus bulbs increased once exposed to a dry heat treatment. Taking a single replicate of 25 bulbs, a thermocouple was inserted into the centre of one of the bulbs via the root plate. The bulb was then placed amongst the other bulbs in the replicate, in a laboratory incubator running at 30°C, during which the temperature of the monitored bulb was recorded at regular intervals. The temperature of the incubator was also monitored throughout with a glycerol buffered 0-100°C (1/10th°C incremental) mercury/glass thermometer. After treatment the bulbs were removed and allowed to return to ambient temperature, cooling was monitored at regular intervals. This procedure was repeated at 40, 45 and 50°C on fresh replicates of bulbs.

The second preliminary experiment was undertaken to identify the most appropriate range of dry heat treatments to be investigated for the construction of thermal death curves for the adult stages of the BSM.

The experiment consisted of 12 replicates of 25 Narcissus bulbs. Two replicates were separately subjected to one of six dry heat treatments; 30, 35, 40, 45, and 50 for a

period of three hours (i.e. the same exposure period as used in a standard HWT). After treatment the replicates were allowed to return to ambient temperature before three bulbs were selected randomly from each for dissection. A count of the numbers of live and dead adult mites was made. Controls consisted of two replicates of five untreated bulbs per temperature treatment, taken at random from the main stock. These were dissected and the number of live and dead adults were counted. The resulting data was plotted as a graph and regression analysis carried out in order to identify the likely value for the ULT for this exposure period. This data was used to select the most suitable range of temperatures for investigation at exposure periods of ½, 1, 2 and 3 hours. The Effective Dose (ED) of the various combinations of temperature and exposure period necessary to produce 50%, 99% and 99.9% mortality, are hereafter denoted as ED50, ED99 and ED99.9 respectively.

Dry heat treatments - for the construction of thermal death curves of both the adult and egg stages of the BSM

The experiment consisted of 80 replicates of 25 Narcissus bulbs. Four replicates were subjected separately to one of 20 dry heat treatments (comprising four exposure periods, ½, 1, 2 and 3 hours and five temperatures 36, 38, 42, 44 and 46°C).

Four replicates (one per shelf) were loaded into a LMS 0-50°C fan-assisted cooled incubator set at 5°C above that of the required treatment temperature. The shelves were labelled positions 1 to 4 from top to bottom.

The temperature of the incubator was monitored by a glycerol buffered 0-100°C (1/10th°C incremental) mercury/glass thermometer. Each treatment was considered to have begun once the interior temperature of the bulbs had reached the required treatment temperature,and at that point the incubator temperature was reduced by 5°C. The internal bulb temperature was monitored by an internal thermocouple

thermometer inserted into the centre of one bulb, via the root plate. The time taken to reach treatment temperature was also recorded.

At ½, 1, 2 and 3 hour intervals a single replicate was removed from the incubator and allowed to return to ambient temperature. Five bulbs were selected at random from each replicate for dissection. A count was made of the numbers of live and dead adults. Twenty-five eggs were gently removed using a single hair brush and incubated at 15°C and 90% relative humidity over a 30 day period, and observed for egg hatch.

The procedure was repeated four times for each temperature on fresh sets of replicates. To counter any variability of temperature within the incubator the four replicates for each exposure period were taken from a different shelf, sequentially from shelves 1 to 4 on each experiment run. The thermocouple-monitored bulb was also placed sequentially on shelves 1 to 4.

The experiment resulted in 16 replicates per temperature, four at each temperature and exposure period combination, giving a total of 80 treated replicates. Controls consisted of 20 replicates of 25 bulbs, one per treatment, taken at random from the main stock at the time each treatment was running. Ten bulbs from each replicate were dissected and a count was made of the numbers of live and dead adults. One cell of 25 untreated eggs were gently removed from each control replicate with a single hair brush and incubated at 15°C and 90% relative humidity over a 30 day period.

The data produced was plotted and linear regression analysis was carried out. The lethal exposure period for both the adult and egg stages at each temperature was determined at the 50%, 99% and where possible the 99.9% confidence intervals as appropriate.

Growing-on trial

The Narcissus variety used ('White Lion', the only BSM-infested stock of bulbs available) is not suitable for forcing. It was therefore decided that a growing-on trial instead of a forcing trial should be undertaken in order to determine if the dry heat treatments had any effect upon the bulbs and the flowers.

Bulbs were delivered to HRI Kirton on 23 September 1999 and were planted without receiving any further treatments.

Following the usual procedures for planting field trials at Kirton, the trials area was ridged out, the position of plots was marked in the furrows using fibre-glass canes, the bulbs were placed evenly in the plots by hand, and the bulbs were covered by splitting-back the ridges. Each plot consisted of a 1m length of ridge. Plots were separated by non-planted 1m gaps within the ridges and by non-planted ridges between each planted ridge, to minimise the spread of pathogens between plots. There were four replicate plots of each of the 21 treatments, except that there were five replicate plots of the control (treatment 21). The trial was arranged in a randomised block design, with four replicate blocks to accommodate the extra replicate of the control, this was regarded as forming a 22nd treatment with three missing plots. Each plot consisted of 20 bulbs.

Husbandry procedures followed those usually used at HRI Kirton. Potash fertiliser was applied pre-planting and cultivated in as usual, according to soil analysis and standard MAFF fertiliser recommendations (no phosphate fertiliser was required). Nitrogen fertiliser was applied in winter as a pre-emergence top-dressing. Weed control was by applying diquat + paraquat in autumn, cyanazine in winter pre-emergence, and chlorpropham + linuron post-emergence. From emergence, the crop received a regular

fungicide spray programme (involving iprodione, chlorothalonil, vinclozolin, mancozeb and benomyl), with three sprays before flowering and two sprays after flowering.

All flowers were recorded, although the 'rogue' flowers can be separated in the raw data file if required (they are labelled 'Y'). Flowers were picked (at a commercial cropping stage, when the spathe was just splitting) three times a week over the main flowering period. Flowers were graded by length (overall length of the stem plus flower bud, in 5cm-wide bands) after cropping and were placed in vases of plain tap-water in a vase-life room. When individual flowers were fully open, the overall diameter of the flower (across the widest dimension) was recorded, and flowers were examined and classified as either undamaged or according to abnormalities present. Plants were examined in the field for symptoms of pest and HWT damage on two occasions, 17 March and 26 April 2000. As an additional measure of crop vigour, the length of ten central leaves in each plot were recorded on 26 April 2000, when leaves were fully grown.

Photographs of typical plots and of damage symptoms in the field and in the vase-life room, were taken. **(Plates 1-10)**

Results

Hot water treatments for reduced exposure periods - Preliminary experiment

The preliminary experiment revealed that the bulb core temperature reached treatment temperature from ambient (22.4°C) in approximately 45 minutes. The temperature of the water bath fell on addition of a replicate of Narcissus bulbs, but returned to the pre-set temperature after approximately 15 minutes. The core temperature of the bulbs 15 minutes after immersion was approximately 39.5°C. After treatment and removal from the water bath the bulbs returned to ambient temperature in approximately 15 minutes.

Hot water treatments for reduced exposure periods

Complete dissection of the five randomly selected bulbs from each treatment replicate revealed the presence of adult mites of both sexes, larvae and numerous eggs. All were to be found in the top third of the bulbs. The number of both live and dead adult mites were counted from each dissected bulb, including the controls. The total number of adults ranged from 0-247, the average number per bulb being 74. Very large numbers of larvae and eggs were also present which could not be counted.

The shortest exposure period of 30 minutes was not sufficient to kill all adult mites, with approximately 0.2 % surviving this treatment. Treatments of 1 hour or longer were fully effective. In the controls, 54.5% of the total number of the adult mites found were alive.

None of the eggs removed from any of the HWT replicates hatched over the 30 day observation period, whereas egg hatch in the controls reached 84% over the 30 day observation period, with no further egg hatch after day 21 of the experiment.

Dry heat treatments - Two preliminary experiments

In the first experiment to monitor the heating of a Narcissus bulb subjected to a dry heat treatment, the core temperature of the bulb reached treatment temperature from ambient (22.4°C) after approximately 140 minutes following the introduction of the Narcissus bulbs to the incubator. To raise the core temperature of a bulb to the required treatment temperature more quickly it was necessary to set the incubator at 5°C above that temperature. By doing so the core temperature would be achieved after 24 to 28 minutes. At this point the incubator was adjusted to the required temperature. The core temperature would then remain stable throughout the rest of the exposure period. After treatment and removal from the incubator, the bulbs returned to ambient temperature after approximately 30 minutes. A slight drop in incubator temperature was observed when bulbs were first put in, but treatment temperature was rapidly regained.

The second preliminary experiment was to establish a suitable range of temperatures for use in the main experiment. The proportion of dead adult mites recorded from three hour treatments at 30°C and 35°C was 18.3% and 66.7% respectively. No adult mites survived a three hour treatment at 40, 45 or 50°C. The data obtained was plotted and a regression line produced **(Fig 1).** From this line the temperature that would be expected to result in 99.9% mortality (ED99.9) of adult BSM was estimated to be 38.77°C (upper 95% confidence interval 39.70°C, lower 95% confidence interval 38.02°C). Based on this result and the observations of earlier workers the temperatures selected for the main experiment were 36, 38, 42, 44 and 46°C.

Dry heat treatments

The numbers of live and dead adult mites found in each treated replicate and control were counted. The proportion of dead adults vs. exposure period for each temperature were plotted **(Figs 2-6).** The data produced for each temperature were transformed by Logistic regression **(Figs 7-12).** The transformed data for 36, 42, 44, and 46°C behaved in a linear fashion. The data obtained for the 38°C treatment showed a decrease in the proportion of dead adults at the 60 minute exposure period, before a general increase at 2 and 3 hours. As a result of this it was necessary to fit a quadratic function to the 38°C treatment data **(Fig 8)**. A linear function could be fitted to the data by omitting the 30 minute data points. **(Fig 9)**. From the regression lines produced for each of the five selected temperatures, the exposure periods that would be expected to result in the mortality of 50% (ED50), 99% (ED99) and where possible 99.9% (ED 99.9) of the adult BSM were estimated **(Table 1)**. The proportion of dead adult mites in the treatments and controls expressed as a mean percentage are presented **(Table 2)**. The proportion of dead adult mites generally increased with both increasing treatment temperature and exposure period.

The controls for the 44°C treatment exhibited significantly lower background mortality ($\chi^2 = 40.8$ $p < 0.001$).

The egg mortality vs. the exposure period was plotted for each of the experimental temperatures on one graph **(Fig 13).** All the treatments showed higher egg mortalities than the controls (control mortality 11.8%).

Table 1.

Temperature (°C)		Estimated (mins)	Upper 95% CI(mins)	Lower 95% CI(mins)
36	ED 50 ED 99	43.5 19674	48.0 45381	38.3 10317
38*	ED 50 ED 99	37.2 4969	45.0 11867	28.5 2730
42	ED 50 ED 99	26.5 151	27.7 172	25.3 136
44	ED 50 ED 99 ED 99.9	24.8 60.2 94.2	25.9 70.6 122.3	23.3 54.0 78.7
46	ED 50 ED 99 ED 99.9	11.5 41.4 78.8	5.8 50.2 140.4	15.7 37.1 60.7
Treatment 36° 38° 42° 44° 46°	Slope 1.73 2.16 6.07 11.91 8.27	SE 0.114 0.205 0.247 1.15 1.66	χ^2 239.9 110.6 1270.5 686.4 66.1	p <0.001 <0.001 <0.001 <0.001 <0.001

*Results are for a linear model with the 30 minute treatment omitted, note that this model is also non linear

Table 2.

Proportion of dead adult mites as a mean percentage of 4 replicates for each exposure period and the controls.

Temperature (°C)	30 min %	60mins %	120mins %	180 mins %	Controls %
36	42.2	56.7	65.5	74.8	61.9
38	78.1	67.1	65.2	87.2	61.0
42	60.3	81.8	99.3	100.0	60.2
44	72.9	98.8	100.0	100.0	54.4
46	96.9	99.7	100.0	100.0	60.4

The 36°C plot showed decreasing egg mortality with increasing exposure period for the 1 and 2 hour treatments, and then stabilised. Similarly the 38 °C plot showed decreasing egg mortality with increasing exposure period for the 1 and 2 hour treatments, but then egg mortality increased sharply for the 3 hour exposure period.

The lines plotted for the 42, 44 and 46°C treatment showed a progressive increase in egg mortality with increasing temperature. The egg mortality in the treatments and controls expressed as a mean percentage are presented **(Table 3.)**.

Table 3.

Egg mortality as a mean percentage of 4 replicates for each exposure period and the controls.

Temperature (°C)	30 min %	60mins %	120mins %	180 mins %	Controls %
36	59.8	55.4	22.0	29.7	13.7
38	60.3	27.1	18.0	97.0	9.3
42	59.8	60.7	87.3	100.0	11.2
44	92.0	93.0	95.0	94.0*	9.6
46	83.5	87.5	100.0	100.0	15.1

* considered to be an anomolous result- see conclusion.

Growing-on trial

A large amount of data was collected in the course of the field trial and tabulated. Statistical analysis of the data revealed no significant differences between the treated and untreated groups of bulbs **(Appendix)**.

Conclusions

The brief experimental study to monitor the core temperature of a single Narcissus bulb undergoing a hot water treatment found that the core temperature of a bulb did not reach treatment temperature from ambient (21.4°C) until approximately 45 minutes after the treatment had begun. This compares to a figure of 60 minutes as quoted by Woodville & Morgan (1961), and reflects the generally small size of the bulbs in the experimental stock. This information should be treated cautiously since

data was obtained from observation of a single bulb, without replication. The temperature of the water bath, although initially depressed on the addition of the bulbs, was regained after approximately 15 minutes. HWT are timed from the point at which the treatment bath returns to temperature following the addition of the bulbs. This is based on the assumption that the bulbs must have also reached treatment temperature. Clearly this result confirms this is not the case and that this assumption is wrong. It was also noted that after removal from the water bath the treated bulbs took approximately 15 minutes to return to ambient temperature. The lag in returning to ambient temperature after treatment, would partially compensate for the initial delay in reaching the required treatment temperature as previously noted.

The finding that a HWT of one hour at 44.4 °C is fully effective against the adults and eggs of the BSM, is close to that of Winfield (1958), who found that a HWT for one hour at 43.3°C (110°F) would kill most active stages and eggs of the BSM, but would not effect complete eradication. The slight difference of 1.1°C (2°F) in temperature between this experimental study and that of Winfield (1958) appears to be critical to the success or failure of a 60 minute treatment. It was not within the scope of the project to determine if the mites surviving the 30 minute treatment were still viable. This is perhaps a point that should be investigated further. Winfield (1958) also stated that a good crop of flowers can be taken from a stock, with least risk of loss of blooms, if the bulbs are given a one hour treatment at 43.3°C (110°F) in the season prior to forcing. Before this treatment could be recommended it would be advisable to assess the efficacy of the one hour HWT in commercial conditions and check the effects of this treatment on the quality of a crop from a range of Narcissus varieties used for forcing. In practice the use of a one hour HWT would be only used for the control of BSM as it would be inadequate as a means of controlling the other major

bulb pests, the stem nematode *Ditylenchus dipsaci* and the Narcissus fly *Merodon equestris.*

With regard to the dry heat treatments, this project has produced the first data on the efficacy of such treatments as a means of controlling the BSM. As with the HWT, two experiments were carried out prior to the dry heat treatments, to give a guide as to how quickly bulbs reached treatment temperature. Air is a poorer conductor of heat than water and it took more than three times as long for the core temperature of a dry heat-treated bulb to reach treatment temperature than it did for a comparable hot water-treated bulb. From a practical point of view this would not be acceptable both in terms of time and cost. Through experimentation it was found that the time taken to reach treatment temperature could be reduced considerably by the inclusion of a short period of preheating at a higher temperature, thus making the process more practical. After treatment and removal from the incubator, it was noted that the bulbs took approximately 30 minutes or twice as long as the hot water-treated bulb to return to ambient temperature. This can be explained in terms of the different ways in which heat would be lost from the bulbs in the two treatments. The hot water-treated bulbs would be losing heat by radiation, but also more rapidly through the evaporation of liquid residues. The dry heat-treated bulbs would be losing heat only through radiation, and would therefore cool down more slowly. The lag in cooling after dry heat treatment would partially compensate for the initial delay in reaching treatment temperature.

Having established an appropriate method, a suitable range of five treatment temperatures were selected. Reference to existing published data was of limited use since much of the information refers to HWT. A useful starting point was a paper by Fox-Wilson (1939) in which the upper thermal death point for the BSM is quoted as

between 38°C(~102°F) and 49°C(~120°F). A limited number of dry heat treatments was therefore carried out at five intervals, between 35 and 50°C for a standard three hour exposure period. For expediency the numbers of replicates at each temperature were limited. The experiment indicated that the critical temperature for a three hour exposure period was between 38°C and 40°C. The five temperatures selected for the main investigation were chosen to straddle these two temperatures, with a bias towards higher temperatures to take account of the expected reduction in efficacy at exposure periods of less than three hours.

Logistic regression analysis of the data relating the proportion of dead adults and the exposure period for each temperature (with the exception of the 38°C data) produced a series of linear response lines, the slopes of which increased with increasing temperature as would be expected.

The data for the 36°C treatment showed a lower proportion of dead adults present in the 30 minute treatment than were found in the controls. One possible explanation for this is that a 30 minute exposure period at this temperature resulted in the emergence of more adult mites, thus reducing the proportion of dead mites observed. Increasing exposure periods at this temperature had little effect on the adult mites. The proportion of dead mites increased slightly with lengthening exposure periods but this data was comparable to the controls.

The shape of the response curve for the 38°C data was not linear and had to be fitted to a quadratic function. A one hour exposure at 38°C resulted in a smaller proportion of dead adults being found. This result can be explained in the same way as the observation for the 36°C/30 minute data i.e. treatment at this temperature results in the emergence of more adult mites thus reducing the proportion of dead mites observed. As the exposure period increases to two and three hours the proportion of

dead adult mites starts to increase. The use of a 38°C dry heat treatment for the control of the adult BSM would in any case not be practical as the estimated ED99 from the regression model gave a treatment time of 82.8 hours.

The shape of the dose response lines for the egg mortality data (measured by the numbers of eggs that did not hatch) was much the same as that for the adults. At 36°C, egg mortality decreased with increasing exposure period up to 2 hours and then stabilised, suggesting that this temperature favours the development of the BSM eggs. At 38°C the egg response line is similar to that adult response line. Egg mortality decreases with lengthening exposure periods up to 1 hour and then increases for exposures of 2 and 3 hours. At 42, 44 and 46°C egg mortality simply increased with lengthening exposure period. The data collected for the 44°C 3 hour treatments (94% mortality) appears to be anomalous when compared to the result for the 42°C / 3 hour, 46°C / 2 hour and 46 °C / 3hour treatments, that all resulted in 100% egg mortality. Based on a calculation of degree minutes it would be expected that a 44°C / 3 hour treatment would achieve 100% egg mortality. Clearly there is a case for repeating the experiment to confirm this data.

The eggs of BSM were found to be as susceptible as the adults to dry heat treatments, whereas they were more susceptible to the HWT. The minimum dry heat treatment required to give 100% mortality of both the eggs and adult stages was found to be 42 ºC /3 hours, comparable to the conditions that apply in a standard hot water treatment. If it is assumed that the input of heat energy via either HWT or dry heat treatment has the same overall effect on BSM, some other factors are contributing to the greater effectiveness of the HWT, as compared to the dry heat treatment. The most obvious factors are the additives in the hot water treatment, formaldehyde and a non-ionic wetting agent which may have some toxic effects. If this is the case it would be

worth conducting a study of the toxicity of these additives, as a means of enhancing the efficacy of the hot water treatments against the BSM.

The possibility of damage to bulbs and subsequently the blooms that they produce, is the main reason why HWT are considered unsuitable as a control method for BSM in stocks that are destined for forcing. It was unfortunate that the only infested stock available at the outset of this project was of 'White Lion', a cultivar that is not suitable for forcing. The assessment of the effects of dry heat treatments on bulb vigour and flower production had therefore to be made through a growing-on trial. It was found that the dry heat treatment had no significant effect on any of the measured characters. The trial only suggests that dry heat treatments would be a suitable control method for BSM. As previously stated it has been found (for HWT at least) that the damage to a bulb and the flowers produced subsequently, is amplified when the bulb is forced. Further experimentation is needed to determine the effects that dry heat treatments have on forced stocks before such a treatment could be considered on a commercial scale.

The data obtained in the second year of this project has given the first indications of the efficacy of dry heat treatments as a means of controlling the BSM. The data should however be viewed with caution, as is it has been obtained with reference to one population of BSM and one cultivar of Narcissus. The stock of 'White Lion' used was atypical as it had been untreated for three seasons, and the bulbs were generally rather small. Additional data needs to be obtained by experimentation with fresh stocks of other cultivars (preferably those used for forcing) before a dry heat treatment could be selected for a trial on a commercial scale.

Future work

This study has identified specific areas that require further investigation which should be considered for funding in the future.

The chemical additives used in hot water treatments appear to have some toxicity to the BSM, and this should be investigated further.

The viability of adult mites that survive a 30 minute HWT needs to be examined.

More developmental work is required before a dry heat treatment could be recommended for use in field trials, specifically this is:

• The dry heat treatment experiments need to be repeated and with a range of different cultivars.

• The effects of dry heat treatments on forced stocks need to be determined.

• Other temperature/time combinations need to be examined to complete the study.

References

Briggs, (1999). *ADAS HORTICULTURE* BULB NOTES. N° 92. 12th July 1999.

Blatney, C. (1933). On a "Red Burn" disease of Amaryllis caused by a mite, *Tarsonemus hydrocephalus* Vitzthum. Gartenbauewissenschaft, **7:** 425-437.

Doucette, C.F. (1936). Observations on the bulb scale mite as a major pest of Narcissus. J. econ. Ent. **29:** 1103-1105.

Fox-Wilson, N.D.H. (1939). Contributions from the Wisley Laboratory LXXXVII. Amaryllis Pests. JI R. hort. Soc.**64:** 318-326.

Lynch, S. M. T and Bedi, A. (1994). The development of an *in vitro* method for culturing the Bulb Scale Mite (*Steneotarsonemus laticeps* (Halbert) and its use in life history studies. Horticultural Development Council report for project BOF25

Starzewski, J. C. (1999). Narcissus: bulb scale mite control using hot water treatments.Horticultural Development Council first annual report for project BOF25a

Hodson, W.E.H. (1934). The bionomics of the Bulb Scale Mite, *Tarsonemus approximatus* Banks var. *narcissi* Ewing. Bull. ent. Res.**25:** 177-185.

Spruijt, F.T & Blanken, F.S. (1933). Vapour heat treatments for the control of bulb pests and its effect on the growth of the Narcissus bulbs. J. econ. Ent. **26:** 613-620.

Woodville H.C. & Morgan, H.G. (1961). Lethal times and temperatures for bulb eelworm (*Dytlenchus dipsaci*) Exp. Hort. **5:** 19-22.

Appendix - Statistical analysis of the data obtained from the growing-on trial

Numbers of stems, leaves and flowers were analysed using general linear models with Poisson errors and a log link function. Where the data were over-dispersed the scale factor was estimated from the data and the necessary correction applied.

Results are presented as analysis of deviance tables. In each case the model:- **block +time +temperature +time.temperature** interaction was fitted to the data and the significance of a variable was estimated using backward elimination. Changes in deviance are tested against a chi^2 disrtribution to assess their significance.

Number of stems

Source	df	Change in Dev (χ^2)	p
Block	3	4.97	0.17
time x temp	12	12.72	0.39
time	3	1.20	0.75
temp	4	0.31	0.99

No significant effects on the number of shoots of block, time or temperature were detected.

Number of leaves

Source	df	Change in Dev (χ^2)	p
Block	3	4.36	0.22
time x temp	12	19.69	0.073
time	3	2.02	0.57
temp	4	3.76	0.42

No significant effects on the number of leaves of block, time or temperature were detected.

Number of flowers

Source	df	Change in Dev (χ^2)	p
Block	3	12.90	0.005
time x temp	12	18.19	0.11
time	3	0.98	0.81
temp	4	13.02	0.011

Significant differences in the number of flowers between blocks were found. Time also showed significant differences in the number of flowers. Means for average number of flowers in each block are given in the worksheet “average flowers”. These data are represented in graphical form in the chart “Nat flowers ch”. There appear to be no systematic differences in the number of average number of flowers in each of the temperature treatments in each block.

Flower Size

Flower size was analysed using standard analysis of variance techniques.

There are no significant effects of temperature and time on the diameter of flowers. There is, however a significant effect of cropping date (see table below). Examination of the residuals (Cook's distance) however show that two points have an influence on the estimate of the effect of cropping date. Cases 38 and 60 appear to have very small average flower size. Case 58 also appears to be influential with a large positive residual.

ANOVA table for Diameter of flowers (with all points included)

Source	df	SS	MS	F	p
block	3	70.85	23.62	0.80	0.50
temp	4	139.42	34.86	1.18	0.33
time	3	131.41	43.8	1.48	0.23
time x temp	12	451.88	37.66	1.27	0.26
day	1	204.37	204.37	6.89	0.01
residual	56	1660.59	29.65		
total	79	2658.52			

Omitting these points results from the analysis results in the estimate for cropping day becoming non significant.

ANOVA table for Diameter of flowers (with cases 38 and 60 omitted)

Source	df	SS	MS	F	p
block	3	17.43	5.81	0.29	0.83
temp	4	124.9	31.23	1.54	0.20
time	3	50.37	16.79	0.82	0.49
time x temp	12	229.6	19.13	0.94	0.52
day	1	30.41	30.41	1.50	0.23
residual	54	1098.2	20.34		
total	77	1550.9			

DAMAGE ON FLOWERS

Proportion of undamaged, streaked and brown-patched flowers were analysed using GLMs with binomial errors and a correction factor for over dispersion in the data. The resulting statistics are then compared to an F-distribution. No significant effects (at the 5% level) of cropping day, treatment time or treatment tempreture were detected.

Undamaged flowers

Analysis of deviance for the effect of cropping date

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
Day	3.805	1	3.805	3.805	0.056
Full model	56	56	1.000		
bl +temp*time	59.085	57			

Analysis of deviance for the time temperature interaction

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
temp.time	12.65	12	1.054	1.017	0.37
bl +temp*time	59.085	57	1.037		
bl +temp +time	71.733	69			

Analysis of deviance for the time main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
time	3.916	3	1.305	1.255	0.30
bl +temp +time	71.733	69	1.040		
bl +temp	75.649	72			

Analysis of deviance for the temp main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
temp	3.924	4	0.981	0.933	0.45
bl +temp	75.649	72	1.051		
bl	79.573	76			

Analysis of deviance for the block main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
bl	3.792	3	1.264	1.027	0.39
mean	79.573	76	1.047		
total	83.365	79			

Streaked flowers

Analysis of deviance for the effect of cropping date

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
Day	3.072	1	3.027	3.027	0.087
Full model	56	56	1.000		
bl +temp*time	59.072	57			

Analysis of deviance for the time temperature interaction

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
temp.time	9.909	12	0.826	0.80	0.65
bl +temp*time	59.072	57	1.036		
bl +temp +time	68.981	69			

Analysis of deviance for the time main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
time	3.155	3	1.052	1.052	0.38
bl +temp +time	68.981	69	1.000		
bl +temp	72.136	72			

Analysis of deviance for the temp main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
temp	0.924	4	0.231	0.231	0.92
bl +temp	72.136	72	1.002		
bl	73.06	76			

Analysis of deviance for the block main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
bl	4.169	3	1.390	1.446	0.23
mean	73.06	76	0.96		
total	77.229	79			

Petals poorly coloured or brown

Analysis of deviance for the effect of cropping date

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
Day	0.199	1	0.199	0.199	0.66
Full model	56	56	1.000		
bl +temp*time	56.197	57			

Analysis of deviance for the time temperature interaction

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
temp.time	14.54	12	1.212	1.23	0.29
bl +temp*time	56.157	57	0.985		
bl +temp +time	70.738	69			

Analysis of deviance for the time main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
time	7.037	3	2.346	2.288	0.085
bl +temp +time	70.738	69	1.025		
bl +temp	77.775	72			

Analysis of deviance for the temp main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
temp	1.082	4	0.271	0.251	0.91
bl +temp	77.775	72	1.080		
bl	79.557	76			

Analysis of deviance for the block main effect

Source	Deviance	d.f.	Mean deviance	Ratio (F)	p
bl	2.424	3	0.808	0.772	0.52
mean	79.577	76	1.047		
total	82.001	79			

