DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS **CSG 15**

Research and Development

Final Project Report

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Project title	To determine the longevity of adult bulb scale mites outside Narcissus bulbs, and to investigate soil as a potential source of infestation
DEFRA project code	**PHO186**
Contractor organisation and location	CENTRAL SCIENCE LABORATORY, SAND HUTTON, YORK, YO41 1LZ
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Executive summary (maximum 2 sides A4)

The Bulb Scale Mite (BSM) *Steneotarsonemus laticeps* Halbert is a serious and recurrent pest problem encountered in the commercial production of *Narcissus* bulbs in the United Kingdom. The current method for controlling this pest is by one of two standard methods of hot water treatments applied to dormant bulbs. Work done at CSL between 1998 and 2000 (Starzewski, 1999; 2000) provided conclusive experimental data that validated both standard hot water treatments as fully effective against both the eggs and motile stages of the BSM. Despite the routine use of hot water treatments stocks continue to become infested with BSM, potentially jeopardising exports of this crop to the United States. The work carried out during this project has provided new and valuable information, and is a further step to solving the problem of infestation and thereby safeguarding this important export sector.

A number of potential routes by which BSM infestations of *Narcissus* stocks could begin and spread, both in the field and in storage have been identified. Data collected by an earlier worker (Hodson, 1934) indicates that BSM will perish in the absence of a host but no experimental data was available. It has however been shown that the eggs of BSM could remain viable depending upon environmental conditions for up to 49 days before hatching (Lynch & Bedi, 1994). In earlier

experiments eggs removed from infested bulbs and held at 15°c and 90% Atmospheric Relative Humidity (RH) remained viable for up to 3 weeks (Starzewski, 1999). Spread from infested plants and bulbs through soil is reported to occur only over a limited distance (Doucette, 1936) and it has been stated that, 'treated stocks should be planted as soon as possible into land which has not recently grown a narcissus crop' (Anon, 1984), a statement which suggests that it is thought that there is some risk of re-infestation, again no data is available.

The work carried out for this project addressed three specific gaps in our understanding of the biology of BSM i.e. can BSM be detected in the soil surrounding an infested crop, how long will adult BSM survive in soil and will bulbs become infested if planted into soil collected from around a BSM infested crop.

The data obtained during this project has shown that:

1. Adult BSM can survive for between 5-6 day outside the Narcissus bulb.

2. Only dead adult BSM could be detected in the soil surrounding a BSM infested crop of *Narcissus* when sampled in August.

3. Non-infested *Narcissus* bulbs planted and grown on in soil collected from around a previously infested crop became infested with BSM.

Scientific report (maximum 20 sides A4)

Materials and Methods

Despite BSM being a common and widespread problem, locating an infested stock of *Narcissus* proved difficult. Fortunately on 31st July 2002 a sample of infested *Narcissus* bulbs var. 'Magnet' was received via the (Plant Health and Seeds Inspectorate) PHSI. The origin of this stock was quickly traced to a farm in Bedfordshire, from where soil samples and *Narcissus* bulbs were collected on the 6th August 2002. A non-infested stock of ~250 bulbs of *Narcissus* var. 'California' was supplied by a separate commercial supplier.

EXPERIMENT 1. To determine if BSM can be detected in the soil surrounding the infested plants, and then to compare this to the average number found on the plants.

50g soil samples were collected and bagged for laboratory analysis from 100 evenly spaced sampling points spread over the entire 14 x 250 m plot. The sampling points were paced out following a basic 'zig-zag' pattern. Ten of the bags of soil were randomly selected and all the invertebrates present were extracted using a standard floatation technique devised at CSL (Thind, 2000). The remaining 90 samples were examined visually for the presence of BSM under a dissecting microscope at X 8 magnification.

The second part of the experiment i.e. to assess of mite numbers on the growing crop was not possible. Because of the unavoidable delay in locating an infested stock, growing plants were no longer available. Indeed the bulbs from the infested crop had been harvested some five weeks earlier and the plot levelled. Despite this set back numerous loose bulbs were scattered across the plot. It was therefore decided that 100 of these bulbs would be collected at random and taken back to the laboratory for dissection as an alternative means of assessing the level of BSM infestation in the crop.

EXPERIMENT 2. To determine under controlled conditions, the longevity of adult BSM when separated from the host plant

Ten replicates of ten adult BSM were incubated under optimum conditions i.e. 15ºC and 90% relative humidity in separate closed cells on a soil substrate. Two sets of controls each comprising ten replicates of ten mites were set up at the same time as the experimental treatment and incubated in parallel. One set of controls were on plain filter paper and the other on detached bulb scales as per the methodology of Lynch & Bedi (1994). Both treatments and controls were observed on a daily basis until the adult mites in the soil cells had all died. This observation was extended to a point when any eggs laid in the soil cells during this period had either perished or hatched and the larvae produced had also died.

EXPERIMENT 3. To determine under controlled condition, if a clean *Narcissus* stock can become infested when planted in soil taken from around a BSM infested narcissus stock.

From 100 sampling points marked on the field plot, 20 points were randomly selected. From each of the 20 points sufficient soil was collected in order to fill one standard 30 cm diameter plant pot (approx 10 cubic litres). The soil was then brought back to CSL and the 20 samples (approx 200 cubic litres) were combined and mixed. The bulk of soil was then divided into two equal portions. One portion was heat-sterilised in an autoclave at 128ºC for 1.5 hours and the other left in its original state.

From a stock of Narcissus bulbs var. 'California', supplied as BSM free, a random sample of 50 bulbs were removed, dissected and examined for the presence of BSM at X8 magnification under a dissecting microscope. On the 13/08/02 twenty replicates of ten bulbs were taken from the remaining stock at random. Ten replicates were planted into separate 30 cm plant pots containing heat-sterilized soil and ten replicates were planted into separate 30 cm plant pots of soil that had not been sterilised (Control). Each set of replicates was then set into a field plot with the pots buried up to rim level. The two sets of replicates were separated from each other by a gap of 8 meters and both sets of replicates were then left to grow-on until early March.

Between the 11-13/03/03 the treated and untreated replicates were harvested separately, the foliage was examined and the individual bulbs dissected and assessed for the presence of BSM under a dissecting microscope at X 8 magnification.

Results

Experiment 1.

Visual examination of the 90 x 50 g soil samples taken randomly from the plot in which the infested crop had been grown found no evidence of BSM adults or eggs. Live examples of other mite species were present in particular *Siteroptes* sp. (Protigmata; Pygmephoridae). Of the ten samples extracted using the standard CSL floatation technique one was found to contain two adult female specimens of BSM, these were slide mounted and when examined were found to be heavily infiltrated by fungal hyphae, indicating that they had been dead for some time. The floatation extraction also detected a range of other mites (**Table 1.)**, once again including large numbers of *Siteroptes* sp. Some mite eggs were also successfully extracted from the soil but these were clearly too large to be those of BSM, being more typical of the eggs the Astigmatid species *Rhizoglyphus robini* Claparède also commonly found in the samples.

Table 1. Mites extracted from soil samples and numbers found

Sample	1	2	3	4	5	6	7	8	9	10
Parasitus fimetorum		1					1			
Rhizoglyphus robini		5+e	1	3	3+e		4		4	1
Siteroptes sp.	15		1	5	25	39		16	46	4
Steneotarsonemus laticeps							2			
Tarsonemus sp.		1					1			3
Tyrophagus similes							3			2
Oribatida			4		1				1	
Uropodina						1	2	1		

+e =eggs found

In the absence of whole plants 100 bulbs were collected from the plot, dissected and examined in the laboratory under a dissecting microscope at X 8 magnification. It was found that 23 (23%) of the bulbs showed evidence of BSM infestation i.e. typical feeding damage and the presence of dead adult mites, of these 19 (19%) of the bulbs had live mites or eggs present. The level of infestation in the bulbs varied from one or two to in excess of 60 live adults and eggs per bulb (**Table 2.).**

As with the soil samples the bulbs also had appreciable numbers of other mite species present, again numerous specimens of *Siteroptes* sp. and also *Rhizoglyphus robini*.

Table 2. Numbers of live and dead BSM and eggs found by dissection of 100 randomly selected bulbs from the infested site.

Bulb	Adults		Eggs	Bulb	Adults		Eggs
No	Live	Dead	Number	No	Live	Dead	Number
3		10+		**40**	1		1
4	20+	10+	2	**42**	1	2	
5		10+	3	**46**		10+	
6	1			**49**	30+	10+	7
11	40+	100+	20+	**57**	20+	10+	
13		5	40+	**62**	5	20+	2
19	1	3	5	**71**	10+	20+	10+
22	50+		10+	**76**	20+	5	7
25	8	5		**87**	1	10+	
34		20+	10+	**95**	10+	20+	10+
36	20+			**97**		20+	
38		2					

Experiment 2.

Daily observations made of all the mite cells revealed that in both the soil cells and the plain filter paper cells the adult mites all died by day 6 and day 7 respectively, and produced very few eggs (12 in total). The observation period was extended to day 9 by which time all the eggs produced had either collapsed or had hatched and the larvae produced had also perished. In the cells made using detached bulb scales 89% of the adult mites were still alive on day 9 and throughout this period continued to lay eggs (123 in total), 57 of which hatched to produce larvae that remained alive up to the termination of the experiment **Table 3.**

Table 3.

	Numbers of surviving life stages present (sum of 10 replicates)								
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Soil									
Adults	103	76	51	34	16	0	0	0	0
Eggs	0	4	5	5	1	1	1	1	0
Larvae	0	0	0	0	3	0	0	0	0
Filter paper									
Adults	103	63	32	23	2	1	0	0	0
Eggs	7	7	6	5	4	4	4	3	1
Larvae	0	0	0	1	2	0	0	0	0
Control – On detached bulb scales									
Adults	103	99	99	98	98	96	93	93	93
Eggs	0	15	26	33	47	51	57	67	66
Larvae	0	0	0	0	5	13	27	41	57

Experiment 3.

Prior to planting 50 of the bulbs from the non-infested stock of *Narcissus* var. 'California' were selected at random and dissected to check for the presence of BSM. No live BSM or eggs were detected, but one bulb did have some evidence of a previous infestation with about ten dead adult mites being found.

The replicates of *Narcissus* planted in the heat-sterilised soil were harvested on the 11/03/02. No mites were observed on the foliage and no live BSM adults or eggs were found when the bulbs were dissected, but there was some evidence old BSM infestations in 5 (5%) of the 100 bulbs, with up to 10 dead mites found in each of these bulbs. Comparison using Fischer's exact test at Bayesian 95% confidence intervals (with uniform prior distribution) found there to be no significant difference between the number of dead mite populations observed in bulbs sampled prior to planting or between the bulbs planted in the heat-sterilised soil and those planted into non-sterilized soil (p 0.44) . The *Narcissus*

replicates planted in the untreated soil were harvested on the 13/03/02 . No mites were observed on the foliage. When the bulbs were dissected it was found that 21(21%) showed evidence of BSM infestation and of these 19 (19%) were found to have live adults and eggs, clearly the numbers of live mite and eggs found were significant different to the heat sterilized replicates ($p < 0.0001$).

Bulbs grown in heat sterilised soil

Replicate	Bulb 1	2	3	4	5	6	7	8	9	10
1										
2										
3			**D1**							
4										
5							**D1**			
6										
7				**D1**						
8										
9						**D1**				
10			**D1**							

Bulbs grown non-sterilised soil

Replicate	Bulb 1	2	3	4	5	6	7	8	9	10
1	**L1**									
2		**L2e**						**L1**		
3				**D1**	**L1e**				**L1**	
4		**L2e**					**L1**			
5	**L1**				**L1**					
6	**L2**	**L2e**	**L1**			**L3e**		**L3e**		
7							**L3e**			
8						**L2**				
9	**L2e**	**L1**								
10				**L2**	**D1**					

L = Live adult mites present **D**= Dead adult mites present
Numbers present **1**= 1-10, **2**= 10-20, **3** = 20+ **e** = eggs

Discussion

None of the life stages of the BSM could be detected visually in the soil that surrounded the previously infested *Narcissus* crop at the time of sampling, but two dead adult mites were found in one of the ten samples examined using the CSL standard floatation method (Thind, 2000). The specimens had obviously been dead for some time as the body contents were heavily infiltrated by fungal hyphae. Hodson (1934) in describing the seasonal biology of this pest observes that the adult mites disperse freely over the foliage from early June and can be found until the foliage starts to wither. As no fresh BSM specimens were found (although other live mite species including another tarsonemid species were detected) it is likely that the individuals found here had died and were part of the detritus falling from the infested crop. BSM eggs can also be found on foliage and flower stems (Anon, 1992), and one would expect that a proportion of these would

become detached and fall to the ground. Although the eggs of another mite species were found, no BSM eggs were detected. BSM eggs are quite fragile and are easily damaged and it is possible that any present in the soil may have been destroyed by mechanical action in the handling of the soil. Hodson (1934) also states that BSM 'appear only able to exist for a very short time when removed from the host plant ', but this was not quantified. The experiment to determine the longevity of adult BSM when removed from the bulb found that the adult mites all died after five or six days on the filter paper or soil substrate and produced very few eggs (six and five respectively) none of which survived beyond day nine. This compares to the adult mortality of ~10% (10 of 104) in the control cells i.e. on bulb scales over the same period, with 123 eggs having being laid by day nine. These results clearly show that neither live adults or eggs of the bulb scale mite are likely to be found in the soil surrounding an infested crop and that the adults will only survive for a maximum of 6 days in the absence of the host.

Although it was not possible to sample whole plants from the infested *Narcissus* crop it was possible to collect a random sample of bulbs left over from the harvest. Despite many of these being damaged or even fragmentary, healthy colonies of BSM including both adults and/or eggs were still present in 19% of these bulbs.

All the replicates planted in the heat sterilised soil remained free of live BSM infestations, although there was evidence of some old infestation in 5 (5%) of the bulbs as compared to 1: 50 (2%) of the bulbs examined at the start of the experiment. The most striking finding was that 19% of the bulbs planted in non-sterilised soil were found to contain live BSM and eggs, with each replicate containing up to five infested bulbs, a completely unexpected result. The lack of any live infestations in the *Narcissus* stock when sampled at the outset of the experiment and in the replicates planted in the sterilised soil rules out any intrinsic level of infestation as being the source of this result. Both sets of replicates were planted in the same plot at CSL that had no previous history of *Narcissus* cultivation and under the same cultural conditions. The bulbs planted in the sterilized soil did not appear to perform as well this perhaps indicating that the sterilization process may have had an affect on soil fertility. Since neither live adults or eggs were to be found in the soil sampled from the infested site, the infestation of the bulbs planted at CSL can only be explained in two ways. Firstly the eggs and or adults of BSM are to be found in the soil from around an infested crop, but could not be detected using the methods employed. This appears to be the least likely alternative as other species of mites and mite eggs of comparable dimensions were successfully detected. Secondly and perhaps the most likely answer is that some other source of infestation was present in the untreated soil such fragments of infested bulb material. It has already been clearly shown that such material will maintain residual populations of BSM.

Conclusions

Based on the data presented here it appears that soil in itself does not harbour BSM infestations, at least during August. Bulbs planted into soil taken from a field in which an infested crop have been grown can become infested, probably through contact with infested bulbs and bulb fragments carried over from the previous crop that act as a reservoir of infestation.

The standard recommendation that bulbs should be planted 'into land which has not recently grown a *Narcissus* crop' (Anon, 1984) is somewhat vague, however the data provided here provides evidence to support this advice. At the present time there is no data to indicate how persistent BSM infestations are, but it would be reasonable to assume that that an infestation will remain provided suitably infested host material is present. The complete removal of all crop residues post harvest would therefore seem to be a sensible precautionary measure to prevent of reduce infestation by BSM.

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