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Project Leader:	Rosemary Collier Warwick HRI**,** The University of Warwick, Wellesbourne, Warwick CV35 9EF
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Location of project:	Warwick HRI**,** Wellesbourne, Warwick CV35 9EF
Project co-ordinators:	Adrian Jansen
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The results and conclusions in this report are based on an investigation conducted over a limited number of years. The conditions under which the experiment was carried out and the results obtained were reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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Signature:…… ***Date***: …….
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Director and Head of Department

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HortLINK HL0178 / HDC BOF 63 (incl. CP 36): Integrated control of bulb-scale mite in narcissus

GROWER SUMMARY

Headline

- Research on the biology and control of bulb-scale mite on narcissus is increasing our understanding of how mites are distributed in field crops and how they move between bulbs. The evidence to date suggests that mites disperse over short distances in the field, between bulbs and/or leaves that are in close contact.

- A number of physical control methods, such as exposure of bulbs to high temperatures (either in hot water or as dry bulbs) or low temperatures ('frosting' treatments) are being investigated, as well as a number of acaricide spray treatments.

Background and expected deliverables

The UK is the world-leader in production of narcissus. Some 4,300 ha of narcissus are field-grown, producing an annual saleable output of about 30,000 tonnes of bulbs and 600 million cut-flowers, of which perhaps 30% of bulbs and 40% of flowers are exported. To be cost-effective, narcissus production has become very intensive, increasing problems with pest and disease. Of the three major narcissus pests; large narcissus fly, stem nematode and bulb-scale mite, bulb-scale mite has received least attention.

Bulb-scale mite was once regarded as a sporadic pest, but for the last 10 years there has been a need for more effective control measures. While bulb-scale mite symptoms rarely cause concern in field-grown bulbs or in storage, temperatures in glasshouses, where bulbs are forced for cut-flowers and grown as pot-plants (an increasingly popular product), favour rapid multiplication of the pest, resulting in seriously damaged, distorted leaves and stems, with rejections and serious losses to producers.

With the loss of endosulfan there is no approved acaricide to control bulb-scale mite in glasshouse grown narcissus and a suitable acaricide for field application has yet to be identified. Other than hot-water treatment (HWT), there is no non-chemical means of control. However, standard HWT cannot be used to treat bulbs intended for sale or forcing, since it causes leaf and flower distortions in the year after treatment and despite the rigorous use of HWT on replanting stocks, bulb-scale mite problems continue to increase. The aim of this project is to develop an integrated control strategy for bulb-scale mite based on an understanding of its biology and ecology.

Summary of the project and main conclusions

Objective 1: Define the relationship between temperature and bulb-scale mite development

The work in Objective 1 was re-aligned at the end of 2008 because the development of populations under natural conditions was not fully understood. Some effort had already been devoted to monitoring populations in potted bulbs maintained outdoors and this effort was transferred to monitoring a stock of 'Dutch Master' infested with bulb scale mite that was planted at Wellesbourne. This is giving a clearer impression of how mite numbers change during the year and where the mites are found.

In field-grown bulbs, adult and egg numbers decreased during late winter 2008-9. A lower proportion of mites was present on the shoot tips during January and February, possibly an indication that the cold weather during this period had affected survival. Mite numbers continued to decline during the spring and then increased by a small amount in May-June, before decreasing again in late summer.

Objective 2: Discover when, where and how bulb-scale mite originates and spreads in field crops and in bulb storage

The use of planting troughs to examine the spread of bulb-scale mites between foliage over distance has indicated that mites may move between bulbs where their leaves touch or where the separation distance is 0.5m or less.

All of the commercial narcissus fields sampled in the South-West and the East in 2007 and 2008 and in the South-West in 2009 showed damage due to the presence of bulb-scale

mites, although some were at low levels of infestation. In general, infestations were greater in crops from the South-West. There was no evidence that damage was greater or less at the edges of the fields than towards the centre, but there were patches of infestation in the field. At all sites, *along* rows, high numbers in one sample were correlated with high numbers in adjacent samples (1 m apart) and vice versa. For greater than 1m separations, results were more variable. Results were less clear *between* rows, although there was some evidence of correlation between adjacent rows. This may be a reflection of the way that the bulbs are stored and then planted, or it may indicate the distance over which mites normally disperse from an infested bulb. Leaf 'bridges' may be effective mite routes, but the mites may also move between adjacent bulbs. There is no evidence to date that weeds are a source of bulb scale mites, but in the South-West naturalised narcissus (e.g. in field margins or dumps) are a potential source of bulb scale mite and bulb mite. No bulb scale mites were found on the naturalised narcissus sampled in the East.

Objective 3: Design optimal high or low temperature and/or chemical treatments to control bulb-scale mite in bulbs for replanting and for forcing, and ensure all stages in its life-history are killed and that crop quality is unaffected

In experimental situations, hot water treatment at all tested temperatures and durations killed bulb-scale mites, but warm-storage treatments were ineffective, as were frosting treatments. None of the acaricides tested were very effective when applied to narcissus foliage.

Objective 4 Design novel biological control strategies and test these as part of an integrated management strategy in commercial crops.

Potential control agents are to be tested in summer 2010.

Objective 5 Examine the link between bulb scale mite and smoulder disease.

Symptoms have been too slight to draw conclusions but further development of plants from an experiment is being followed.

Financial benefits

Effective control of bulb-scale mite in forced narcissus would reduce losses by an estimated 15 to 20%. It is expected that the recommendations would be taken up by the bulk of the industry, with a value of £2.9m to £3.9m annually. Bulb-scale mite is a prohibited pest for bulbs exported to the USA, with a zero tolerance. Effective control should increase the volume of bulbs eligible for the US market by 7.5 to 10% as the findings are taken up by all growers exporting to the US (these represent about 30% of the UK production area) – a potential increase of sales by up to £0.5m. However, increasing awareness of bulb-scale mite may lead to stricter tolerances for exports to other countries.

Action points for growers

- Growers should be aware that the debris accumulating in bulb handling and storage facilities may provide a source of mite infestation and that they should implement appropriate hygiene measures, by keeping these areas as free of dust and debris as possible.
- HWT procedures should be carefully maintained and treatment monitored.
- Any mite-controlling effects of a dry heat or frosting treatment are unreliable.
- In the South-West, growers should remove volunteer narcissus growing in field margins or close to newly planted crops.
- Further recommendations must await the completion of the project.

SCIENCE SECTION

Introduction

Steneotarsonemus laticeps (Halbert), the bulb-scale mite, is a pest of many economically important bulb species. In narcissus, feeding mites produce leaves with serrated margins and yellow flecking, and severe infestations can result in significant loss of the foliage and damaged flowers, or no flowers at all. Additionally, feeding damage promotes secondary infections, such as attack by the fungal pathogen smoulder (*Botrytis narcissicola* Kleb.), leading to further crop loss. Furthermore, for bulbs for export there may be little to no tolerance of bulb-scale mite infestations. There have been relatively few studies on the bulb-scale mite, as until recently it was considered to be only a sporadic pest. However, it has become increasingly common, although there is little understanding of the reasons for this increase.

The aim of this project is to develop an integrated control strategy for bulb-scale mite based on an improved understanding of its biology and ecology.

The project objectives are as follows:

1. Define the relationship between temperature and bulb-scale mite development.
2. Discover when, where and how bulb-scale mite originates and spreads in field crops and in bulb storage.
3. Design optimal high or low temperature and/or chemical treatments to control bulb-scale mite in bulbs for replanting and for forcing, and ensure all stages in its life-history are killed and that crop quality is unaffected.
4. Design novel biological control strategies and test these as part of an integrated management strategy in commercial crops.
5. Examine the link between bulb-scale mite and smoulder disease.
6. Deliver a prototype, improved programme for bulb-scale mite control.
7. Communicate with the industry.

The project milestones are as follows:

Year 1			
1.1	1 Apr 07	6	Initial literature review and grower consultation completed.
1.2	1 Apr 07	6	Field cultures of bulb-scale mite established.
2.1	1 Oct 07	12	Trapping and direct sampling methods developed and review of methods with HortLink PMC
4.1	1 Oct 07	12	Biological and microbial control methods reviewed
Year 2			
3.1	1 Apr 08	18	Effect of low temperatures determined
1.3	1 Oct 08	24	Relationships between environmental conditions and development defined.
2.2	1 Oct 08	24	Bulb distribution and movement in stores investigated
Year 3			
4.2	1 Oct 09	36	Small scale trials to evaluate biological control methods
2.3	1 Oct 09	36	Experiments on dispersal in field completed
4.3	1 Oct 09	36	Effects of acaricides determined
6.1	1 Oct 09	36	Initial commercial evaluation of alternative control techniques
Year 4			
3.2	1 Apr 10	42	HWT and (or) warm storage treatments defined
5.1	1 Jul 10	45	Link with smoulder disease investigated
7.1	1 Jul 10	45	Draft Fact sheet prepared
6.2	1 Oct 10	48	Integrated control strategies tested

Experimental

Preliminary work

Grower members of the consortium from Cornwall and Lincolnshire were interviewed by the PhD student working on the project for her to gain further insight into husbandry practices, current control strategies and theories on the origins of bulb-scale mite infestations. Reports documenting the discussions were compiled for her personal use.

Techniques for dissecting bulbs to assess mite numbers and damage, and for identifying infested bulb stocks were developed. These involve either making one or more cuts across the bulb to count the number of brown feeding marks or dissecting the whole bulb, or part of the bulb, scale by scale and examining the scales under the microscope to record feeding

marks and the numbers of mites and mite eggs. Historically, feeding marks have been used to assess the presence of mite infestations in bulb stocks. However, the data indicate that feeding marks in the bulb scales are not always correlated with the presence of mites. It would appear that darker feeding marks are associated with old infestations and that mites are usually found on scales with only pale marks. Assessing mite infestations by feeding marks therefore may be an inaccurate measure of the size of current infestations.

Objective 1: Define the relationship between temperature and bulb-scale mite development

Greenhouse and field cultures of bulb-scale mite were established to act as a source of experimental material. Figure 1.1 shows some of the mite-infested bulbs in a glasshouse at the Kirton Research Centre.

Figure 1.1: Mite-infested bulbs in a glasshouse at the Kirton Research Centre in February 2008

Laboratory rearing technique for bulb-scale mite

A key early objective was to design and test equipment to contain individuals or populations of bulb-scale mite for *in vitro* rearing purposes. Previous work by the Central Science Laboratory (CSL) developed a preliminary *in vitro* rearing cell, which incorporated live *Narcissus* bulb-scale (Lynch & Bedi, 1994). This rearing cell consisted of two glass microscope slides sandwiching an identically sized, but thicker, black Perspex slide, with a hole drilled in the middle centred over a piece of bulb-scale, the edges of which were sealed

with paraffin wax and sealed to the Perspex. This cell was replicated and tested, but was found to be unsuccessful, with mites escaping from the cell. Therefore the development of a modified form of the CSL apparatus was attempted.

A variety of materials were used to create small cells, with circular holes, creating a sealed arena around a piece of fresh *Narcissus* bulb-scale, which forms the base of the cell. Glass, Perspex and Darvic™ PVC sheet (Weston Hyde Products Ltd., UK) were used in the trials, with sealants for the bulb-scale of paraffin wax (M&B Laboratory Chemicals Ltd., Dagenham, UK), Fluoropolymer (Whitford Plastics Ltd., UK), Blu-tack™ (Bostick Ltd., UK) and rubber O-rings (RS, UK). Fine mesh floored cells with circular pieces of bulb-scale placed inside were also tested, but the bulb-scale dried within a few days, even with wax sealing the cut edges, so this was dismissed. Different diameter holes, as well as varying depths to the cell, were also tested. The most success has been obtained with 2mm-thick black Darvic™, with a 7mm-diameter hole for optimal viewing of the entire arena under a binocular microscope. The edge between the bulb and Darvic™ has been most effectively sealed with moulded Blu-tack™ around the rim of the arena, or with angled sides carved in the Darvic™ so that a 9mm O-ring could be placed beneath the bulb-scale to press up into the arena to create a seal (Figure 1.2)

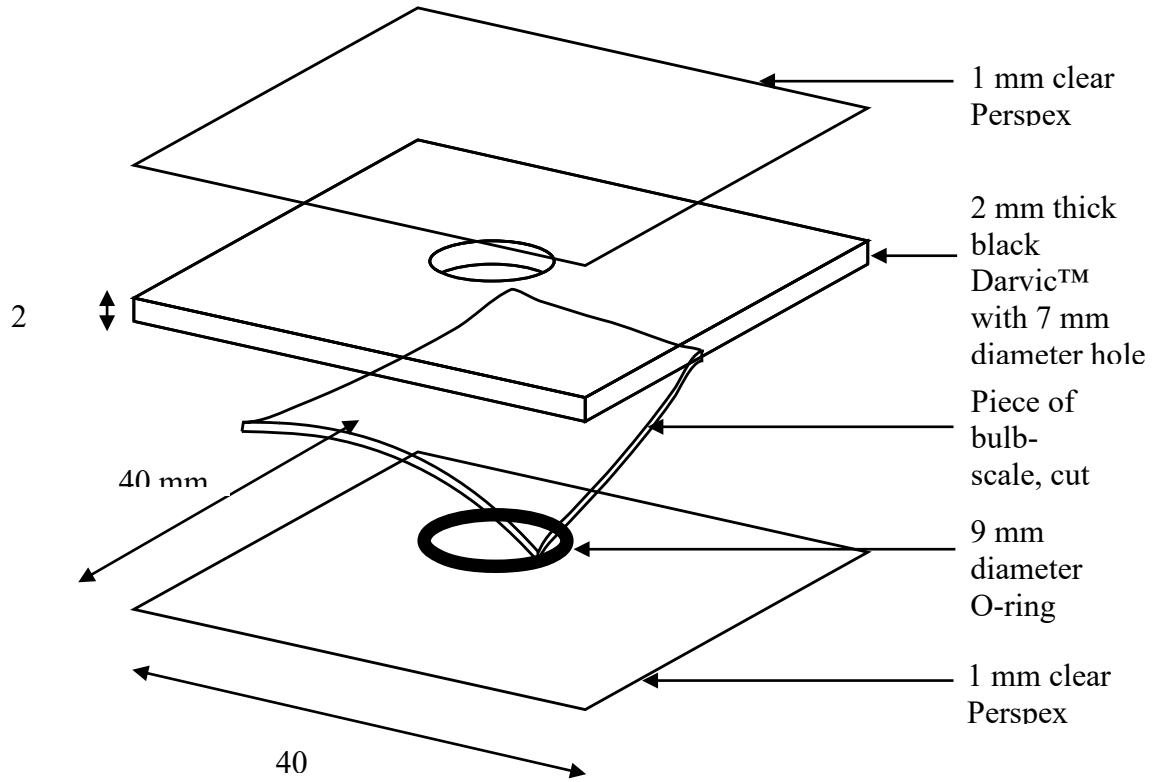

Figure 1.2: Expanded diagram of a cell design used to contain bulb-scale mite for *in vitro* work

For optimal survival of excised bulb-scales, high humidity is necessary to replicate conditions within the bulb. Previous work showed that a relative humidity of ~85% was most effective (Lynch & Bedi, 1994). Cells were therefore placed on metal-grid shelves over aqueous potassium chloride (KCl) solution (160g KCl made up to 400 ml) in sealed Perspex containers 155mm x 270mm x 100mm high (Stuart Plastics Ltd., UK), a method developed by De Courcy Williams *et al.* (2004). Humidity and temperature were monitored using a Squirrel data logger (Grant Instruments, UK). The bulb-scale remained fresh for the longest time when the convex side of the scale formed the base of the cell. Problems were encountered with loss of cells due to fungal growth on the bulb material. Bulb material was therefore surface sterilised before use. However, most success was obtained simply by careful selection of healthy material, and rinsing it with sterile water prior to use. Mould growth also occurred on the Blu-tack™, meaning that cells could only be used for one week to ten days before risk of fungal contamination. Using O-ring cells resulted in samples of bulb-scale remaining healthy for the longest periods (average of 20 days), although a ledge created by the O-ring hampered observation of the mites. It is therefore anticipated that the O-ring cell (Figure 1.1) will be used for rearing mites and the Blu-tack™ cell for life-studies.

A considerable amount of time was spent developing this technique but it was never considered robust enough to collect reliable data on mite development at different temperatures and because understanding of mite population development under natural conditions was limited the project was re-focussed to consider this (see below).

Additional studies

Images of all life-stages were taken using a Scanning Electron Microscope (SEM) to aid identification. Male and female adults, and large and small larvae, were critical point dried for examination. Images revealed the presence of folds in the integument of the smaller larvae, the pattern of which could be observed in the larger larvae and adult integument (Figure 1.3). This would suggest small larvae enlarge to stretch out the integument before pupating and moulting to an adult, implying that there is only one larval stage in development. Studies on other Tarsonemid mites have found male larvae to be smaller than female larvae, with a more prominent opisthosoma (Jeppson, Keifer & Baker, 1975). The different sized bulb-scale mite larvae could therefore be different sexes, ranging in size due to age.

In an attempt to increase numbers of mites available for future work, contact was made with a Dutch group working on *Hippeastrum*, to find out how to rear bulb-scale mite on *Hippeastrum*. Some *Hippeastrum* bulbs with a suspected infestation were potted up and

infested with bulb-scale mites. These are being maintained in a greenhouse at Wellesbourne.

Figure 1.3a.: Dorsal view of an adult female, showing patterning in the integument similar to the folds seen in smaller larvae and a much reduced opisthosoma (the hind part of the body behind the legs)

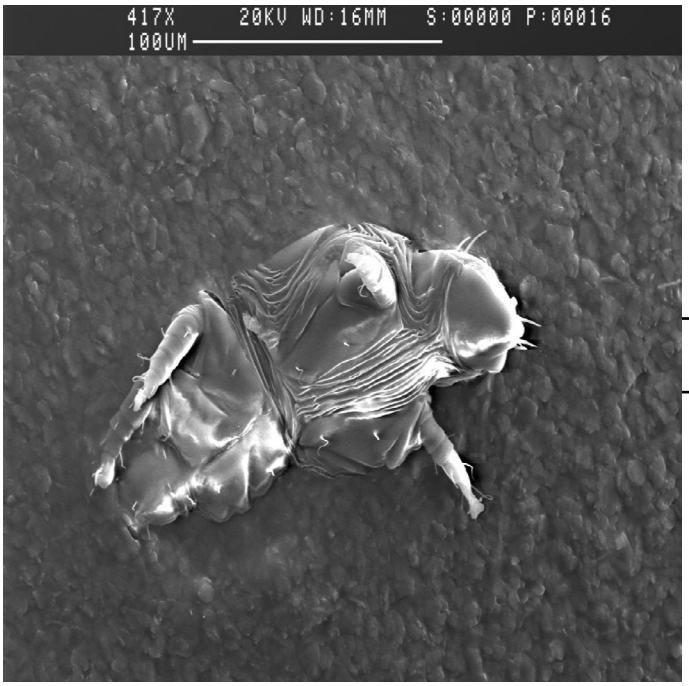

Figure 1.3b. Ventral view of a small larva, showing folds in the integument, particularly between the third leg pairs leading to the pronounced opisthosoma, possibly indicating this is a male (Jeppson *et al.*, 1975)

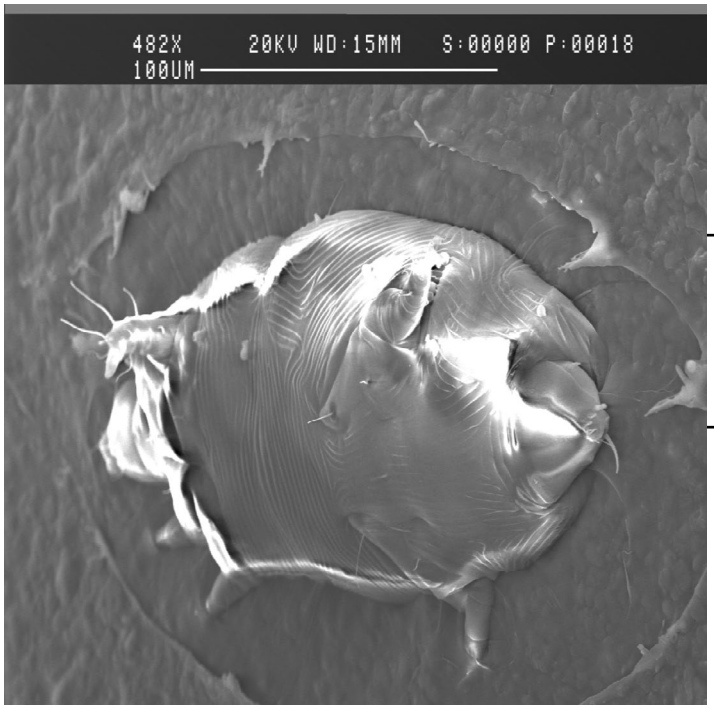

Figure 1.3c. Ventral view of a larger larva, showing patterning on the integument similar to the folds seen in smaller larvae. The less pronounced opisthosoma suggests this is a female (Jeppson *et al.*, 1975)

Development of bulb scale mite infestations under 'natural' conditions

As understanding of the development of bulb scale mite infestations under natural conditions was limited, the project was re-focused (see above) and experiments were set up to sample bulbs at regular intervals and determine the number and location of the different stages within the bulbs.

The first experiment used potted bulbs that had been used previously for an HDC-funded trial on 'narcissus physiological rust' (BOF 62) and which were known to be infested with bulb-scale mite. These potted bulbs were of mixed varieties with a single variety per pot, and were maintained outside on a gravel standing ground at Wellesbourne. A sample of 30 bulbs (randomised over varieties) per month was examined in detail over a period of several days and the data are summarised by month in Figures 1.4, 1.5 and 1.6. The largest numbers of eggs were found on the foliage in January – March, and thereafter egg numbers remained relatively low (less than 0.5 per bulb) (Figures 1.4 and 1.6). The largest numbers of adults were found in June and all of these were inside the bulbs as the foliage had died back by that time (Figure 1.5 and 1.6).

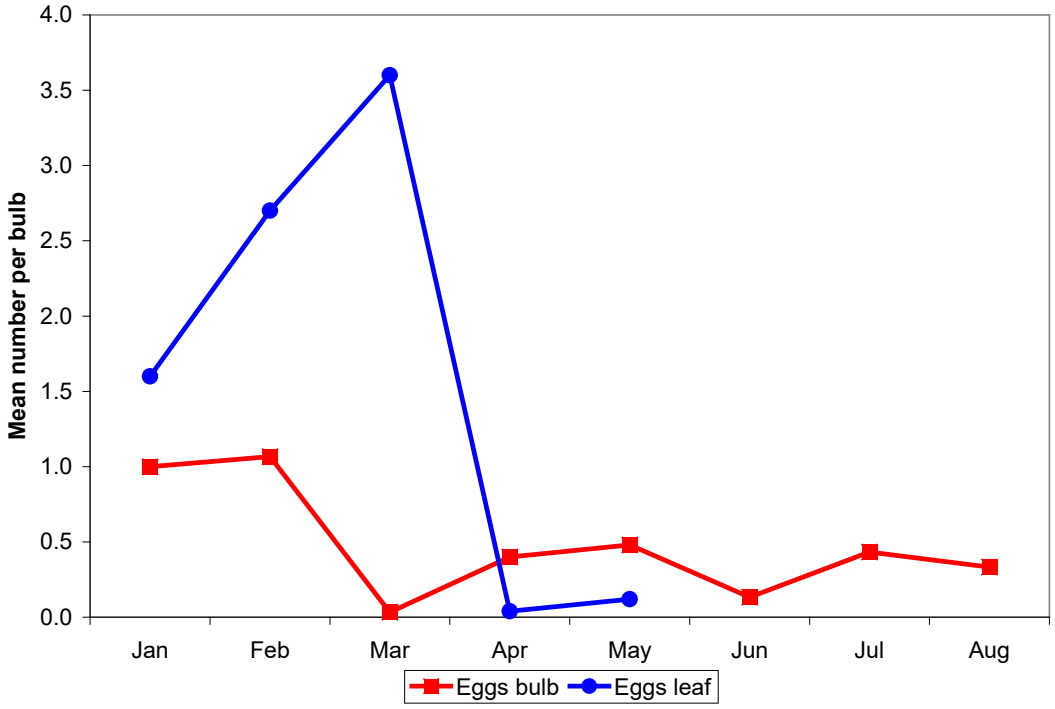

Figure 1.4.: Mean number of mite eggs per bulb or leaf in samples taken from potted bulbs stood outdoors at Wellesbourne in 2008

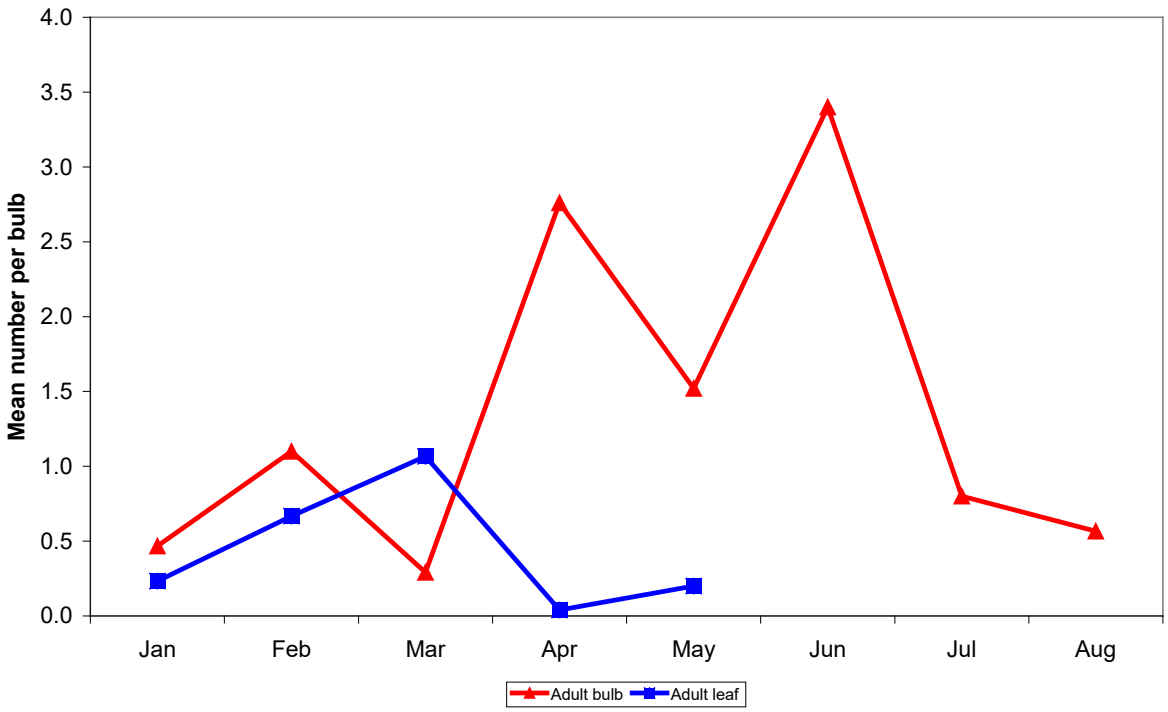

Figure 1.5: Mean number of mite adults per bulb or leaf in samples taken from potted bulbs stood outdoors at Wellesbourne in 2008

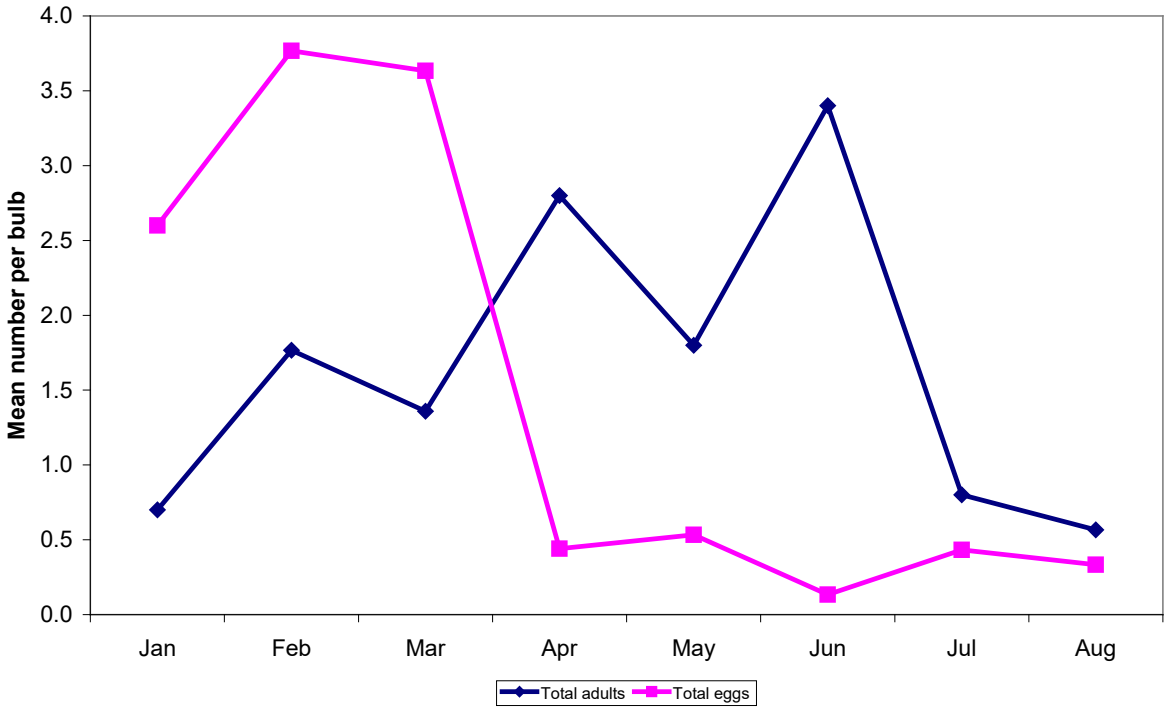

Figure 1.6.: Mean number of mite eggs and adults per bulb plus leaf in samples taken from potted bulbs stood outdoors at Wellesbourne in 2008

A stock of more heavily infested bulbs (cv Dutch Master) was obtained in summer 2008, and the focus of regular sampling was moved to these from November 2008, once they had been planted in the field.

In this instance, a sample of 10 bulbs was removed from the field plot every month and the bulbs were assessed in detail. Figures 1.7 – 1.10 summarise the numbers of mites recovered during November 2008 – February 2009. Adult and egg numbers decreased during late winter. Of particular interest is the lower proportion of mites that were present on the shoot tips during January and February, possibly an indication that the cold weather during this period had affected survival in this more exposed location.

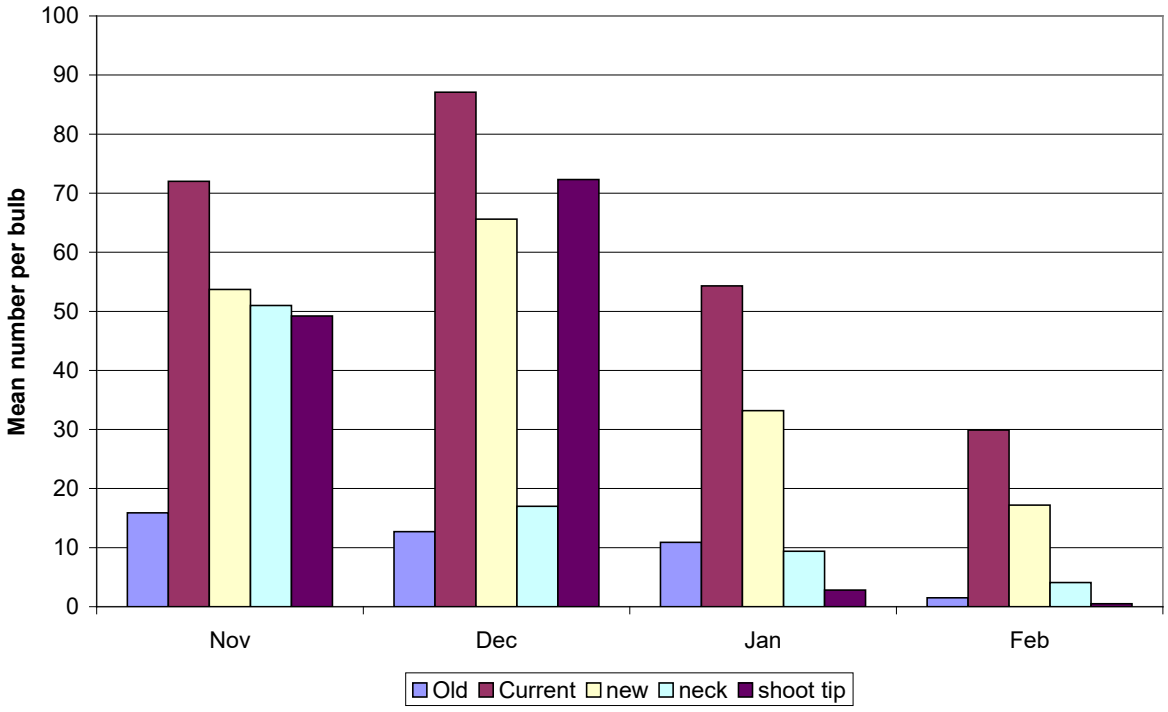

Figure 1.7: Mean number and distribution of mite adults per bulb in samples taken from cv Dutch Master grown in the field at Wellesbourne in 2008-9. 'Old', 'current' and 'new' refer to the bulb scales from bulb units flowering in 2008, 2009 and 2010, respectively.

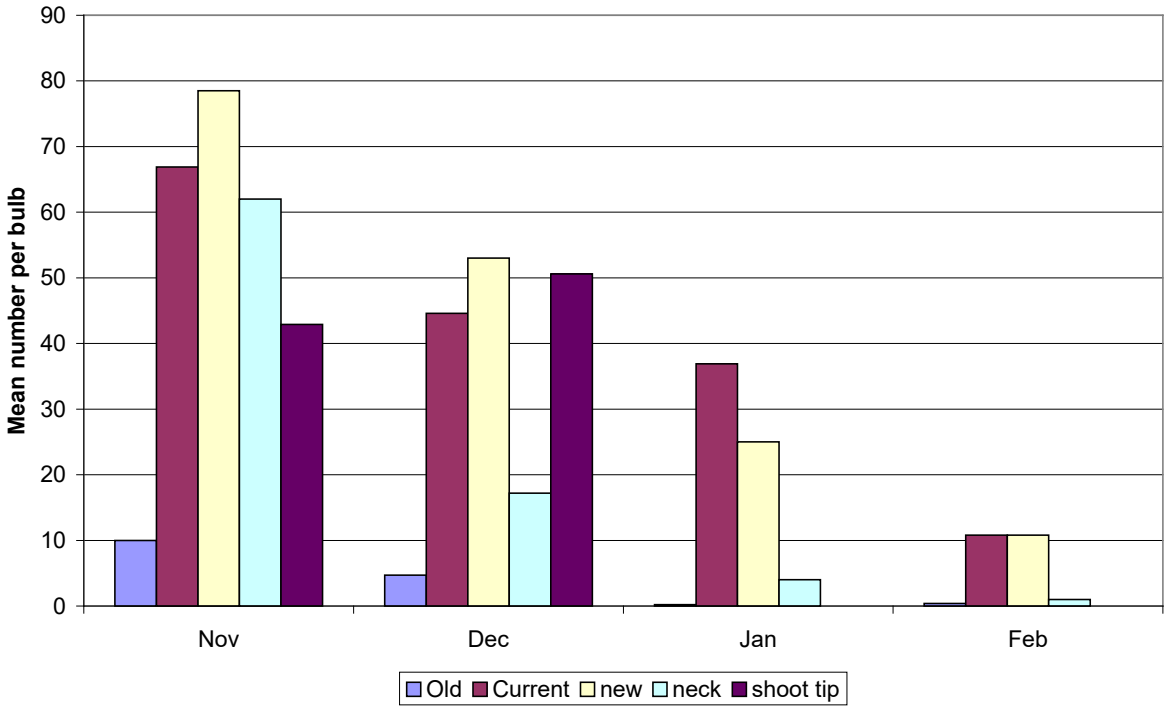

Figure 1.8: Mean number and distribution of mite eggs per bulb in samples taken from cv Dutch Master grown in the field at Wellesbourne in 2008-9. 'Old', 'current' and 'new' refer to the bulb scales from bulb units flowering in 2008, 2009 and 2010, respectively.

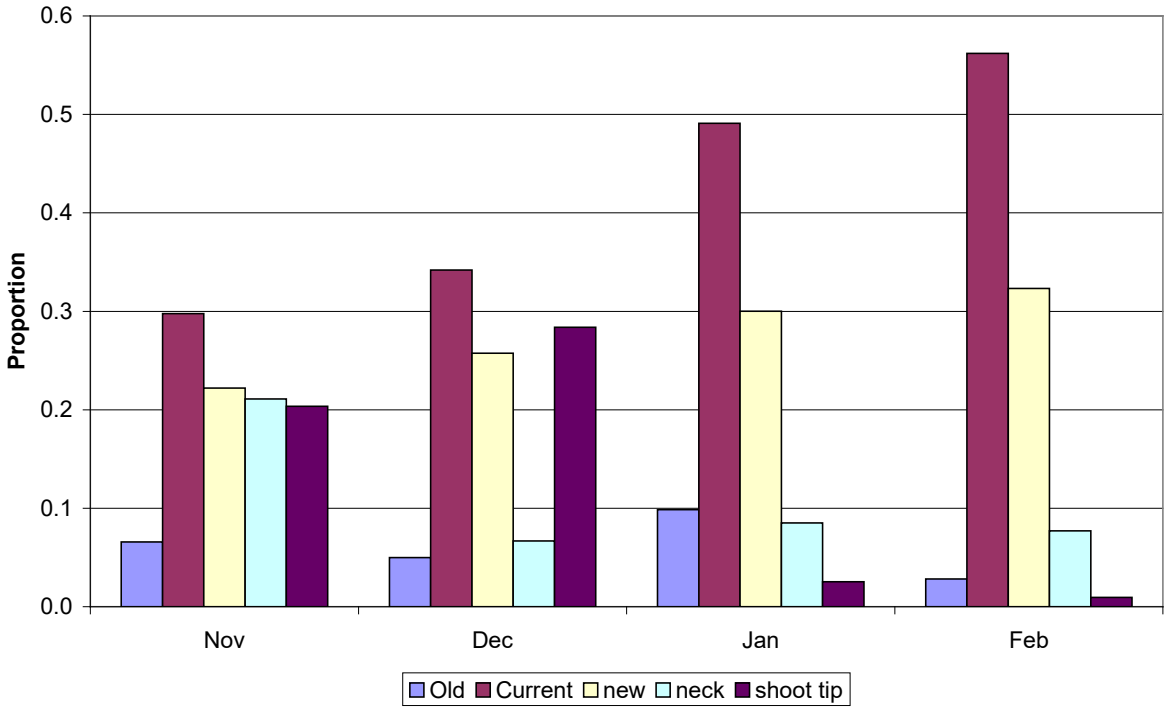

Figure 1.9: Proportion (of total mites found) of mite adults found in each part of the bulb in samples taken from cv Dutch Master grown in the field at Wellesbourne in 2008-9 Old', 'current' and 'new' refer to the bulb scales from bulb units flowering in 2008, 2009 and 2010, respectively.

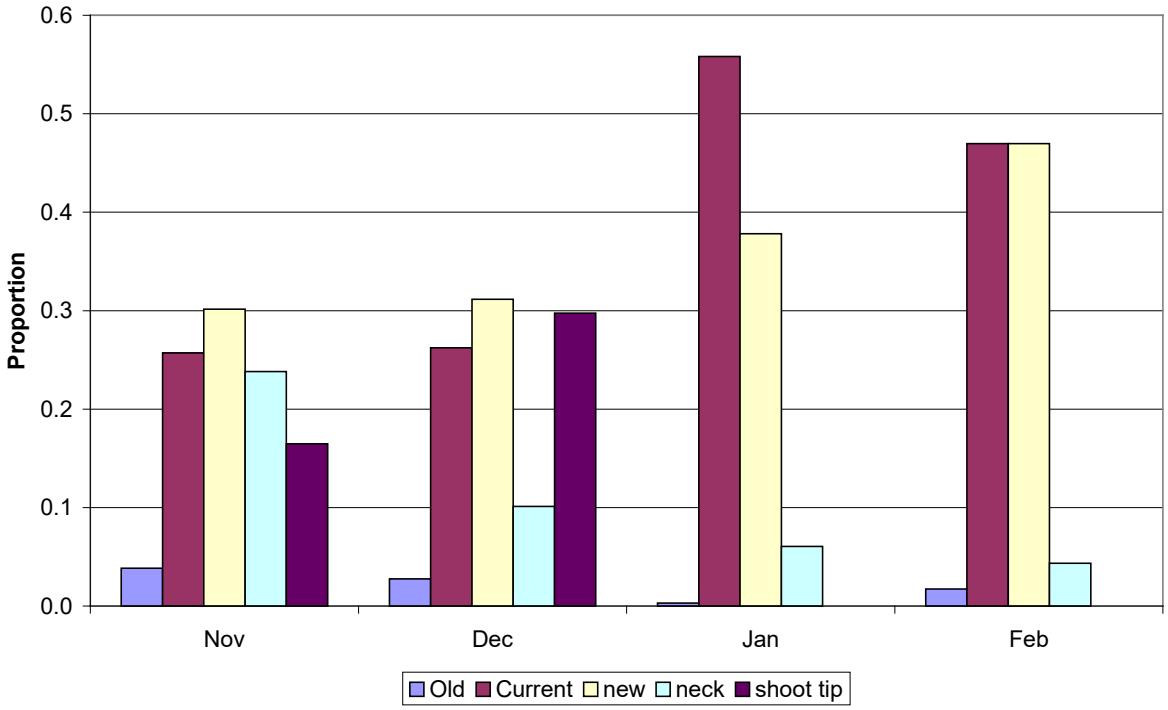

Figure 1.10: Proportion (of total eggs found) of mite eggs found in each part of the bulb in samples taken from cv Dutch Master grown in the field at Wellesbourne in 2008-9. 'Old', 'current' and 'new' refer to the bulb scales from bulb units flowering in 2008, 2009 and 2010, respectively.

Figures 1.11 and 1.12 show the numbers of mite eggs and adult and immature mites found in different locations in the bulbs up to October 2009. Sampling is continuing into 2010.

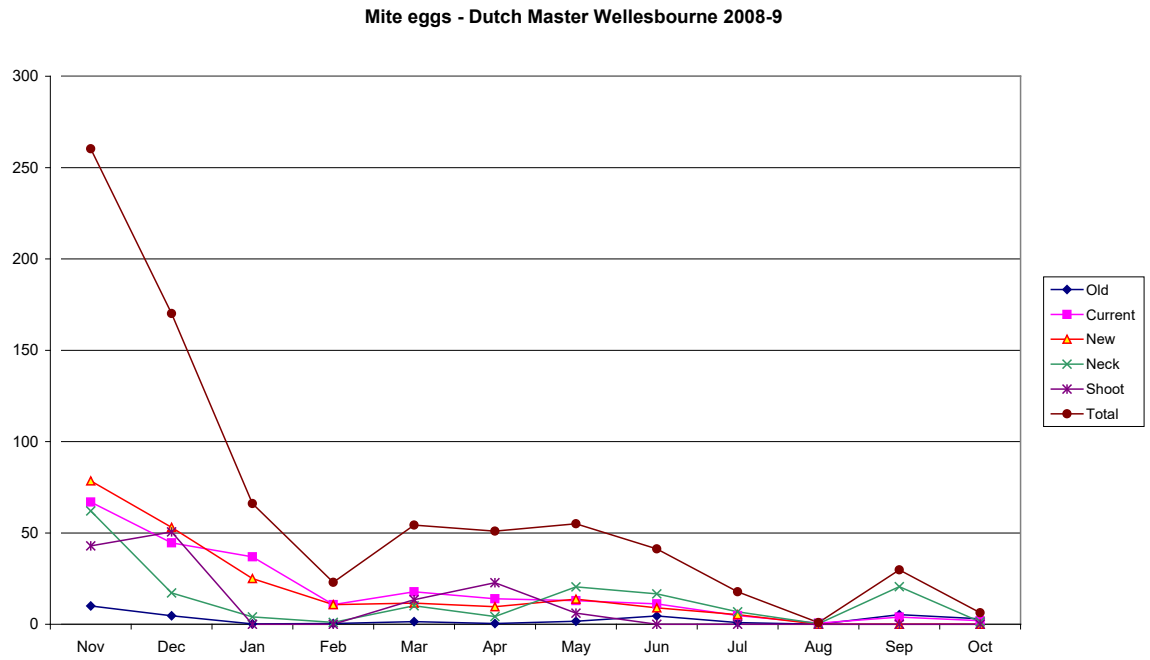

Figure 1.11: Numbers of mite eggs found in each part of the bulb in samples taken from cv Dutch Master grown in the field at Wellesbourne in 2008-9. 'Old', 'current' and 'new' refer to the bulb scales from bulb units flowering in 2008, 2009 and 2010, respectively.

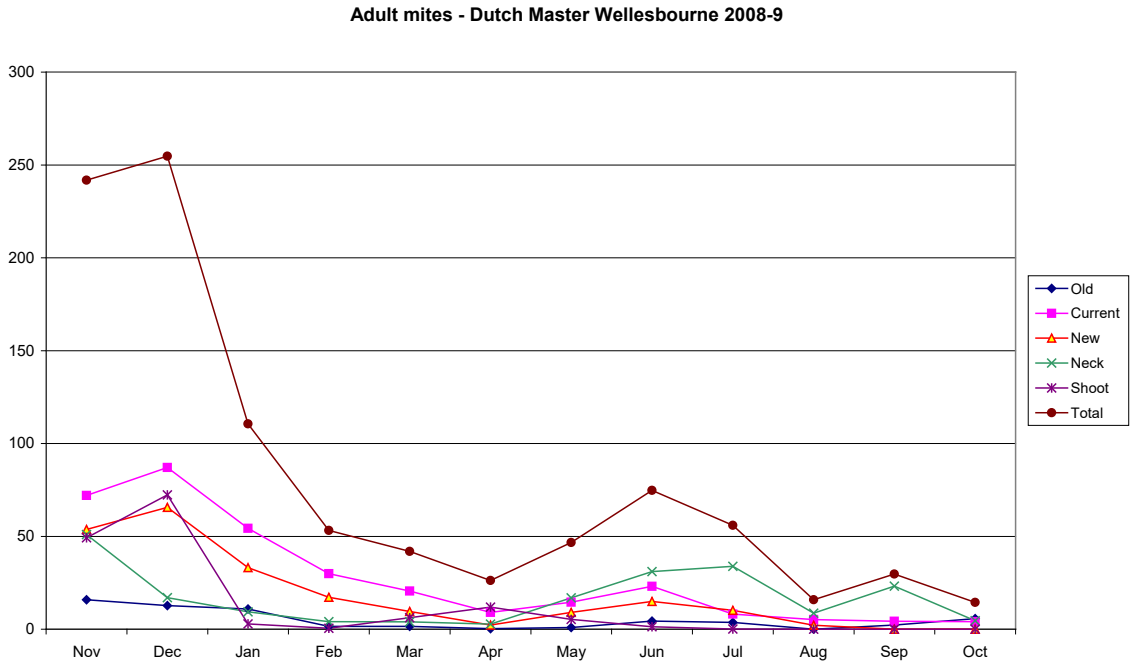

Figure 1.12: Numbers of mite eggs found in each part of the bulb in samples taken from cv Dutch Master grown in the field at Wellesbourne in 2008-9. 'Old', 'current' and 'new' refer to the bulb scales from bulb units flowering in 2008, 2009 and 2010, respectively.

Objective 2: Discover when, where and how bulb-scale mite originates and spreads in field crops and in bulb storage

Determine the spatial distribution of BSM in the field by taking samples from commercial crops

2007

Between 5 July and 9 August 2007 five narcissus fields were selected for sampling in each of Cornwall and eastern England (Table 2.1). The fields sampled were typical of the region, had a large block of a single cultivar, and included crops of various ages (crop-years). Ten, 10-bulb samples were dug from each field in the pattern shown in Figure 2.2, which included samples from both sides of the field and from edge, central and intermediate ridges. When digging bulbs, a check was made for bulb-scale mite symptoms (e.g. serrated edges of leaves and stems, curly leaves or bright green foliage) in the immediate vicinity of the sample. Each 10-bulb sample was placed in a labelled nylon net bag and transported to the Kirton Research Centre, where samples were stored in a ventilated location at room temperature and assessed within 7 days.

Table 2.1: Narcissus crops sampled for bulb-scale mite spatial distribution in 2007

Crop number	Region	Location	Cultivar	Crop-year
1	Eastern England	1	Golden Harvest	2
2	Eastern England	2	Carlton	1
3	Eastern England	3	Fortune	2
4	Eastern England	4	Kerensa	2
5	Eastern England	5	Standard Value	1
6	Cornwall	1	Ice Follies	2
7	Cornwall	2	Golden Harvest	3
8	Cornwall	3	Golden Harvest	7
9	Cornwall	4	Golden Harvest	2
10	Cornwall	5	Ice Follies	2

To estimate the amount of bulb-scale mite damage, each bulb was cut transversely 2/3rds of the way up. The presence or absence of brown feeding mark symptoms was recorded using

a 0 to 5 scoring system (0, none; 1, up to 2 small marks; 2, up to two conspicuous marks; 3, up to 5 marks; 4, ≥5 marks; 5, larger areas of damage), and the mean score was calculated for each group of 10 bulbs.

No foliar symptoms were seen. A summary of feeding mark scores is shown in Figure 2.2. There was mite damage in all 10 crops, although the overall level of damage was low (all mean scores were <1). There was no evidence that damage was consistently greater or less at the edges of the fields than towards the centre. Most of the crops sampled were either in their first or second crop-year, and there was no obvious correlation between the age of crops and the incidence of bulb-scale mite symptoms. Even in the one long-term (7-year) crop, the scores were not consistently high. The main finding was that crops in Cornwall generally showed more damage than those from eastern England, though it should be noted that the average age of the crops in Cornwall (2.3 years, if the long-term crop was excluded) was slightly greater than that in the east (1.6 years), this representing the tendency for using longer-term crops in the South-West.

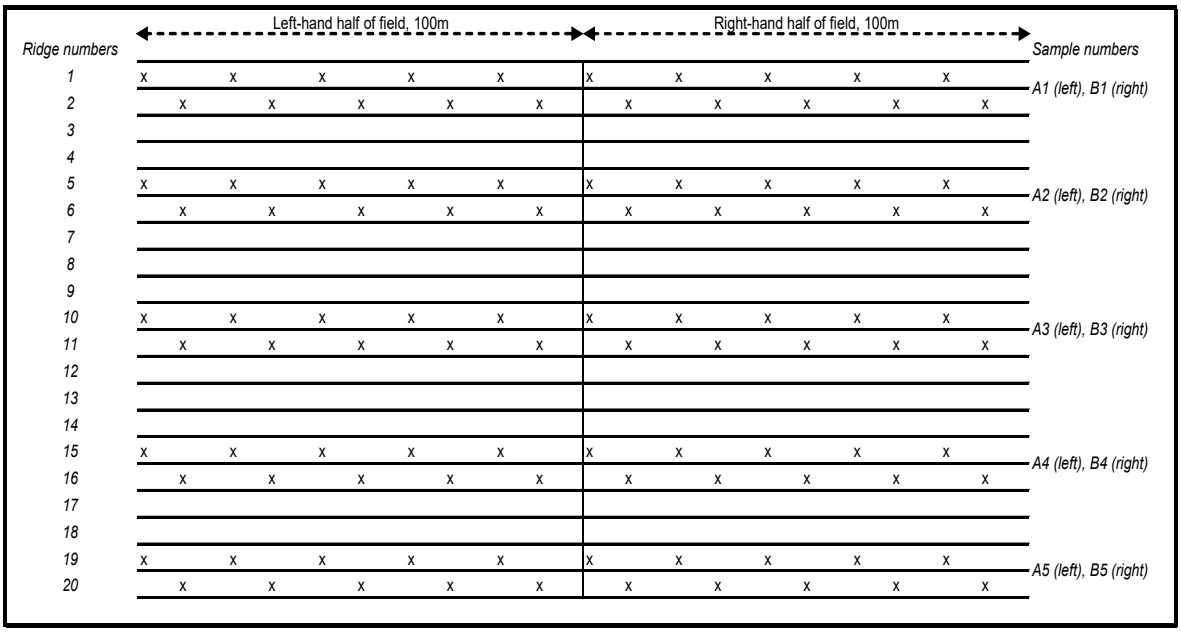

Figure 2.1: Plan used to sample 100 bulbs per field to determine the spatial distribution of mite-infested bulbs. 10 bulb-samples were taken from each of the 10 areas indicated. This illustration shows a field of 20, 200m-long ridges, the plan being adapted to suit actual field dimensions

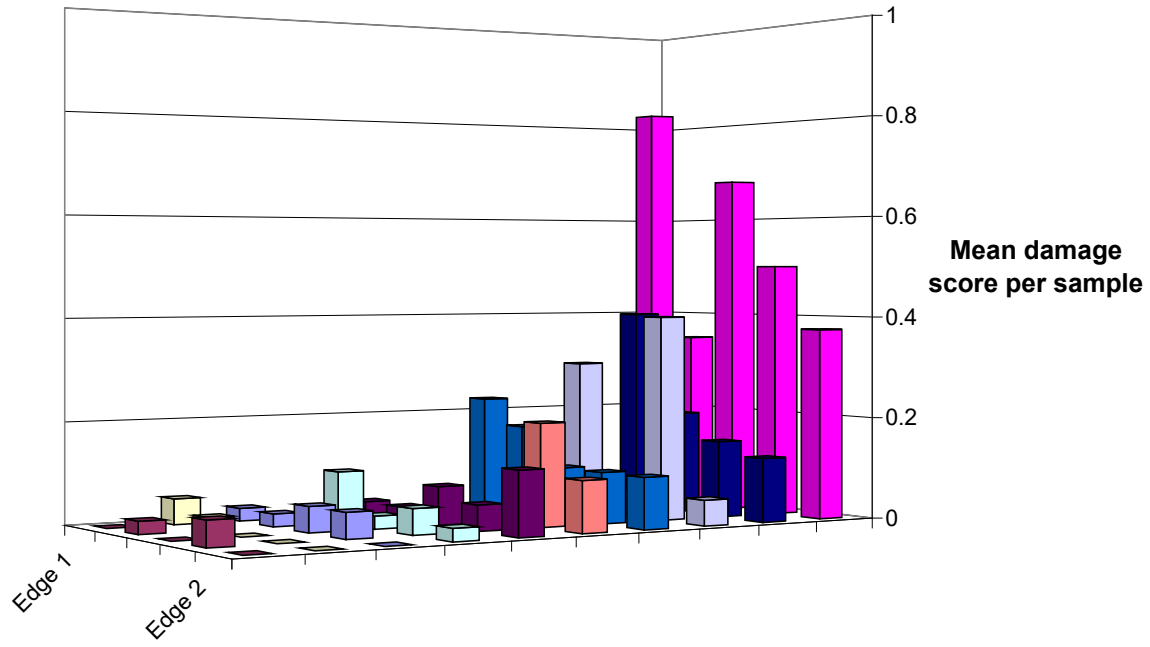

Figure 2.2: The distribution of mite-infested bulbs in ten commercial crops sampled in 2007. X-axis: crops 1 to 10 (left to right) (presented in the order used in Table 2.1 so the first five crops from the left are from Eastern England and the second five crops are from Cornwall); y-axis: ridge position, from edge 1 to edge 2. The figures are means of the left- and right-hand replicates

On 5 November 2007 additional samples were taken from one of the more heavily-damaged Cornish crops, the long-term Golden Harvest crop (No. 8 in Table 2.1), to obtain further information about the distribution of mite-damaged bulbs. A 'clump' of ten bulbs was taken from each of 25 locations on a 5 x 5 grid (approximately 5 rows x 5 metres), as shown in Figure 2.3. This sampling was repeated in three locations across the field. The samples were transported to Kirton for storage and assessed as previously described. The incidence of mite damage was measured as the percentage of bulbs in each 10-bulb sample showing feeding marks and the severity of damage (mean damage score). The data were analysed to determine whether there was any evidence of association between the incidence of mite damage in clumps of bulbs at different distances from one another.

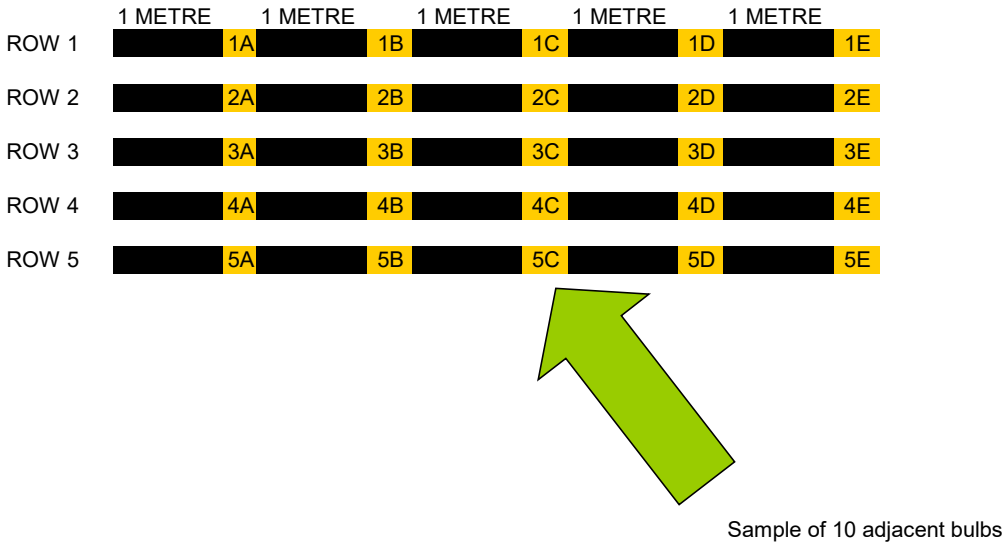

Figure 2.3: Sampling grid used in 2007 to collect a second set of bulb samples to assess spatial distribution of bulb-scale mites

Figure 2.4 shows the percentage of damaged bulbs in one of the 5 x 5 grids, and Figure 2.5 shows the mean damage score for the same grid. These data indicated that infestation occurred in patches in the field. The data were first analysed using a Generalised Linear Model Analysis of the proportion of undamaged bulbs, assessing for differences between the blocks, between ridges within blocks, and between samples along ridges. There was evidence for differences between blocks, but no strong evidence for systematic differences between ridges or between samples.

Moran's I statistic was then calculated for pairs of samples at different distances from one another, to identify any patterns of spatial association between levels of damage. Separate analyses were done based on the mean damage score and the proportion of undamaged bulbs (Table 2.2). The cells shaded in yellow indicate significant levels of association at the indicated distances. Values in the row labelled 0 are for different separation distances along the bulb rows (1, 2 and 3m), while values in the other rows also include the separation between ridges (Row 1 indicates one bulb-row apart, Row 2 means two bulb-rows apart, etc). For example, Row 1, Column1 (with a value of 3.778 for Moran's I statistic) represents the samples are one ridge apart and are also separated by 1m along a bulb row. Overall, there was evidence for association both between samples up to 2m apart along a row, and between samples in adjacent ridges.

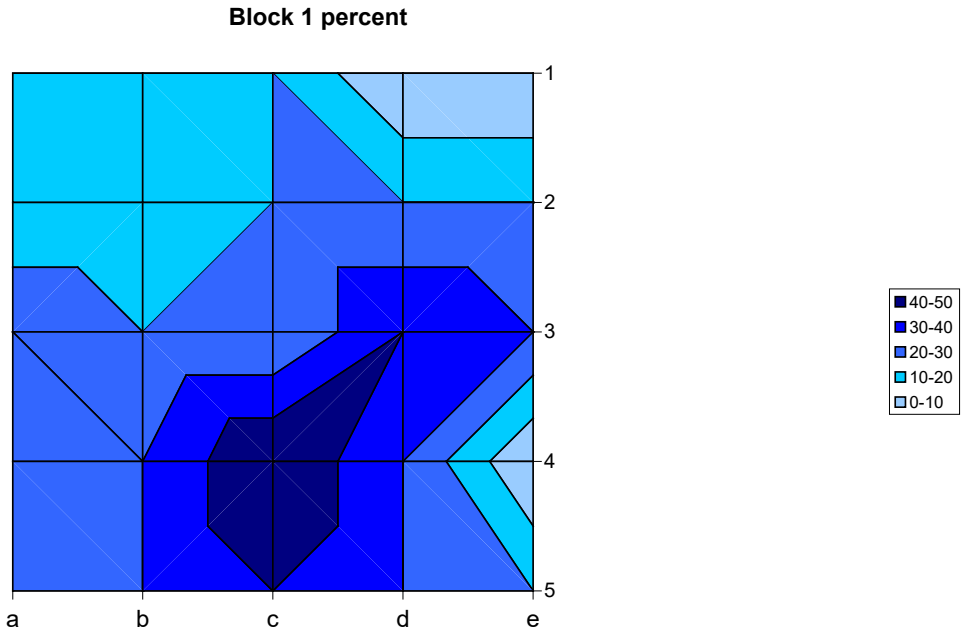

Figure 2.4: The percentage of damaged bulbs in one of the 5 x 5 grids in 2007

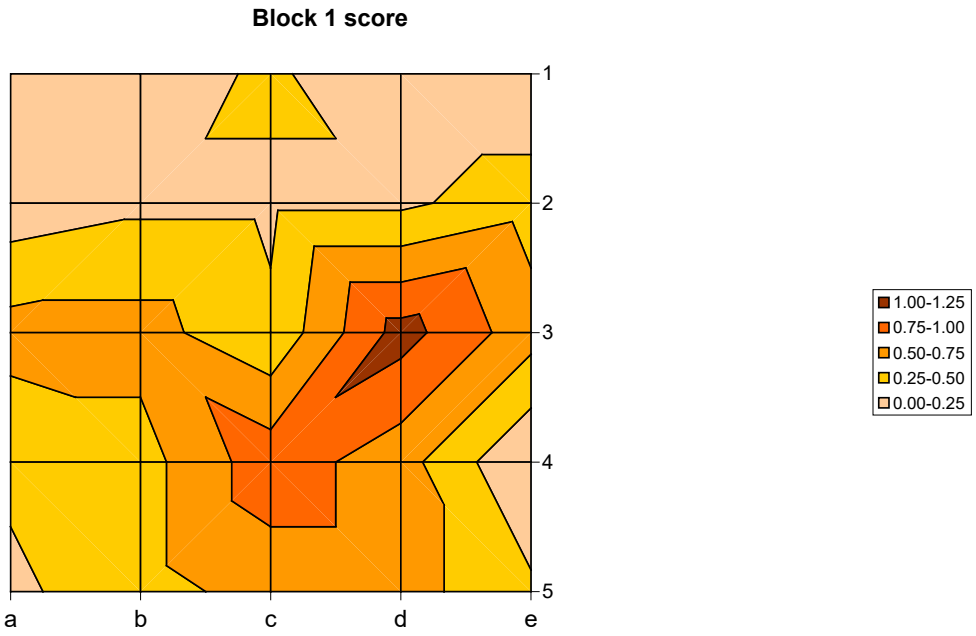

Figure 2.5: The mean damage score for the bulbs in the same grid as Figure 2.4.

Table 2.2: Summary of analysis of spatial distribution data using Moran's I statistic (see text for explanation)

a) Mean damage score

Number of ridges apart	Distance between samples (m)			
	0	1	2	3
0	*	4.565	2.165	2.065
1	2.643	3.778	2.866	0.744
2	-0.172	1.386	1.909	0.301
3	-0.321	-0.195	-0.368	0.439

b) Proportion of undamaged bulbs

Number of ridges apart	Distance between samples (m)			
	0	1	2	3
0	*	4.902	1.904	0.403
1	2.544	3.2	2.817	0.998
2	-0.121	0.824	1.96	1.318
3	-0.16	-0.094	-0.378	0.747

2008

Between 17 July and 21 August 2008 further bulb samples were taken from five Cornish and five eastern stocks, where available using the same bulb stocks and fields as in 2007 (Table 2.3). The sampling procedures described in Figure 2.3 was used, except that the sampling of the 5 x 5 grids was replicated only twice at each site, and the bulb unit (i.e. in the old, current or new bulb unit) in which feeding marks were located was noted.

The summary results for 2008 are given in Table 2.4. Overall the percentage of bulbs with feeding marks, and the feeding mark score, were markedly higher in the Cornish samples than in those from eastern England. From the different stocks, the percentage of bulbs with feeding marks varied between 0.2 and 2.5 (mean, 1.4%) in eastern samples (data presented in Table 2.4 as numbers of bulbs with feeding marks out of a total of 500) and from 0.5 to 25.8 (mean, 11.6%) in Cornish samples (data presented in Table 2.4 as numbers of bulbs with feeding

marks out of a total of 500). The average feeding mark scores were 0.13 for eastern samples and 2.21 for Cornish samples. In four out of five samples from the east, the distribution of feeding marks was about equally split between scales of the current and old bulb units, while in four out of five Cornish samples more feeding marks were found in the scales of the old bulb units, implying there had been different infestation rates each year in each region. The average age of crops in Cornwall and the East (excluding the one long-term crop) was similar to that in 2007, 2.5 and 1.4 years, respectively. As previously found, there was no clear correlation between the incidence of feeding marks and the age of the crop, though in 2008 the 8-year-down crop did have the highest feeding mark score. In the three cases where crops were examined in two successive years, there was no obvious increase in bulb-scale mite infestation year-on-year, except in the long-term crop.

The examples in Figure 2.6 show similar distributions to those found in 2007. Several samples evidenced the patch-wise spread of infestations, and, overall, infestations appeared to spread either from the edges or from within crops.

Table 2.3: Narcissus crops sampled for bulb-scale mite spatial distribution in 2008. Where the crop used was the same as in 2007, this is indicated by *

County	Location	Cultivar	Crop-year
Eastern England	1	Spellbinder	1
Eastern England	2	Tamara	1
Eastern England	3	Carlton	1
Eastern England	4	Carlton	2
Eastern England	5	Standard Value	2*
Cornwall	1	Standard Value	3
Cornwall	2	Standard Value	3
Cornwall	3	Golden Harvest	8*
Cornwall	4	Golden Harvest	3*
Cornwall	5	Ice Follies	1

Table 2.4: Summary of bulb-scale mite feeding mark (FM) data for samples of ten narcissus crops in 2008

Region	***Farm***	***No. of bulbs with FM (per 500 bulbs)***	***For bulbs with FM, percentage in different bulb units***			***FM score (average of 50, 10-bulb samples)***
			New	*Current*	*Old*	
East	1	5	0	83	17	0.12
	2	12	0	75	25	0.28
	3	11	9	45	45	0.22
	4	1	0	0	100	0.01
	5	4	25	50	25	0.04
	Mean	**7**	**7**	**51**	**42**	**0.13**
Cornwall	1	75	0	0	100	3.07
	2	31	3	52	45	0.96
	3	54	0	0	100	2.08
	4	129	0	4	96	4.90
	5	2	0	0	100	0.04
	Mean	**58**	**1**	**11**	**88**	**2.21**

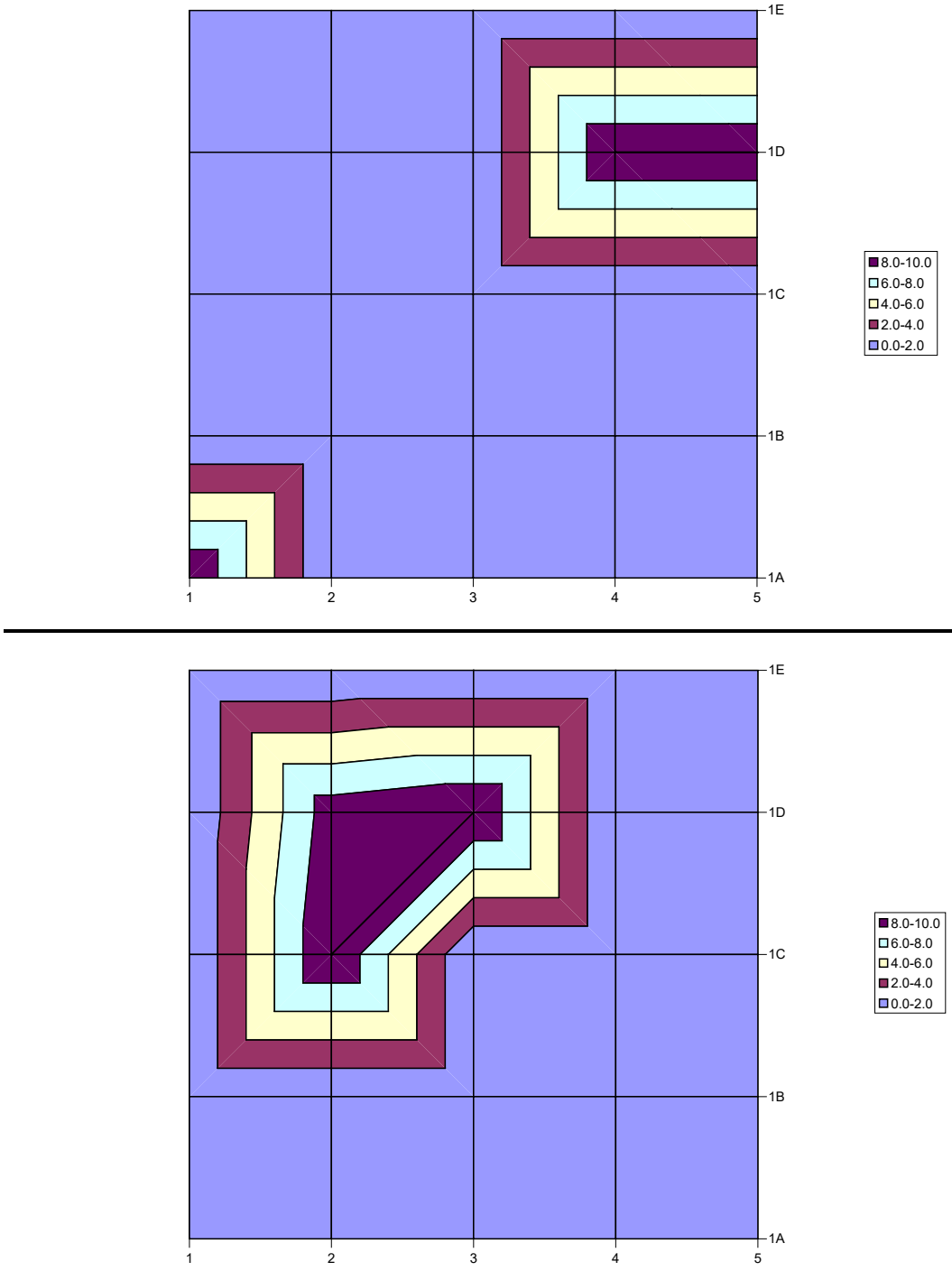

Figure 2.6: Two examples of the distribution of feeding marks (as the percentage of damaged bulbs in 5 x 5 grids) from samples taken in 2008.

The data from 2008 were subjected to statistical analysis as described above. All eastern sites and 1 south-western site had damage levels that were too low for analysis. The data from the 4 remaining locations in south-west showed the following:

Along rows

- At all sites high numbers in one sample were correlated with high numbers in adjacent sample (1 m apart) or vice versa
- For >1m separations the results were more variable
- For 3 m separations there were mainly negative correlations suggesting that if damage was high in one spot, then bulbs 3 m away would have lower damage (the reasons for this are unclear)

Between rows

- The results were less clear – there was some evidence of correlation between adjacent rows.

2009

In 2009, five Cornish crops were sampled using a 10 x 10 grid i.e. 10 rows x 10 metres long. A sample of 10 bulbs was taking from each sampling point (at metre intervals along each row). These bulbs were assessed for the presence of feeding marks.

Examples of the distribution of damaged bulbs in these 10 x 10 grids are shown in Figure 2.7. The data are being subjected to further analysis.

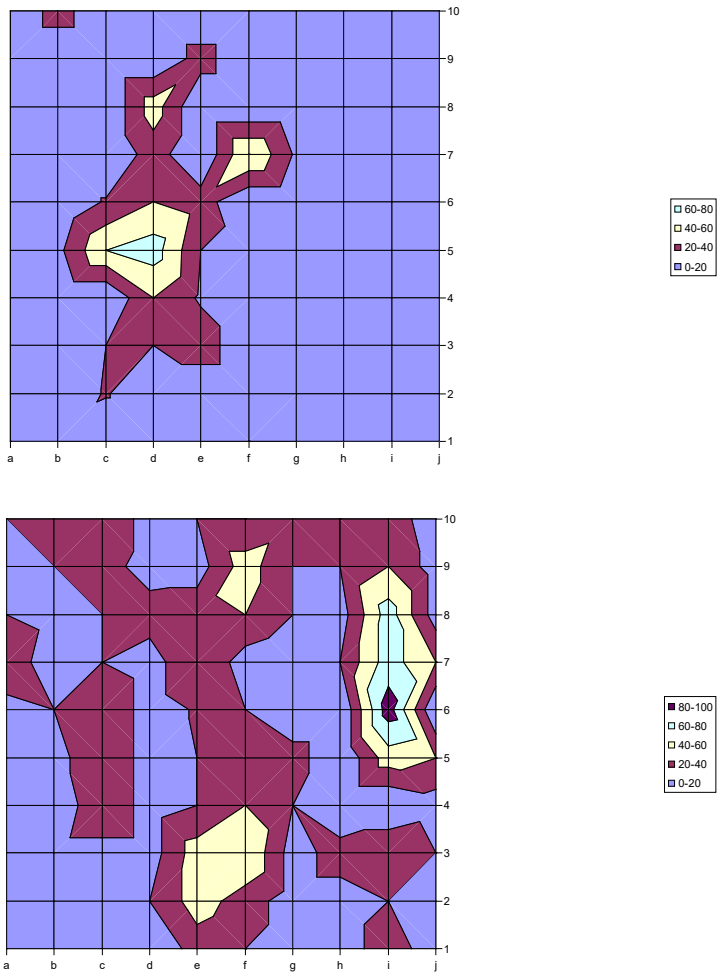

Figure 2.7: Two examples of the distribution of feeding marks (as the percentage of damaged bulbs in 10 x 10 grids) from samples taken in 2009.

Can you find bulb-scale mites on common bulb-field weeds?

A preliminary evaluation was carried out on 27 May 2008 on a weedy commercial bulb-field in Lincolnshire. Five plants each of the predominant weeds at the site, rose-bay willow-herb (*Chamaenerion angustifolium*), annual sow-thistle (*Sonchus oleraceus*), groundsel (*Senecio vulgaris*) and potato ground-keepers (*Solanum tuberosum*) were collected (by cutting off at ground level) at random from each section of a 3 x 3 grid across a narcissus cv Golden Ducat crop. The leaf blades and stems, and especially in leaf axils and around leaf bases, were examined under a low-power binocular microscope. No mites were found.

On 28 May 2008 further checks of weeds were carried out at Kirton. Individual weeds, typically groundsel and knotgrass (*Polygonum aviculare* agg.), growing immediately adjacent

to narcissus cv Carlton plants that had distinct bulb-scale mite symptoms, were examined. Again, no mites were found.

The approach was tested again at in 2009 at sites in Cornwall and in the large plot of cv Dutch Master at Wellesbourne. No live bulb scale mites were found on any of the weeds examined.

Are bulb scale mites present on naturalised bulbs?

Samples of naturalised bulbs were taken in the east and the south-west in 2009. The bulbs (10 per clump) were sent to Wellesbourne where the shoots were examined for mites. Figure 2.8 shows the mean numbers of bulb scale mites and bulb mites per bulb shoot in each location. No bulb scale mites were found in the samples from Lincolnshire. Bulb mites were found in both regions.

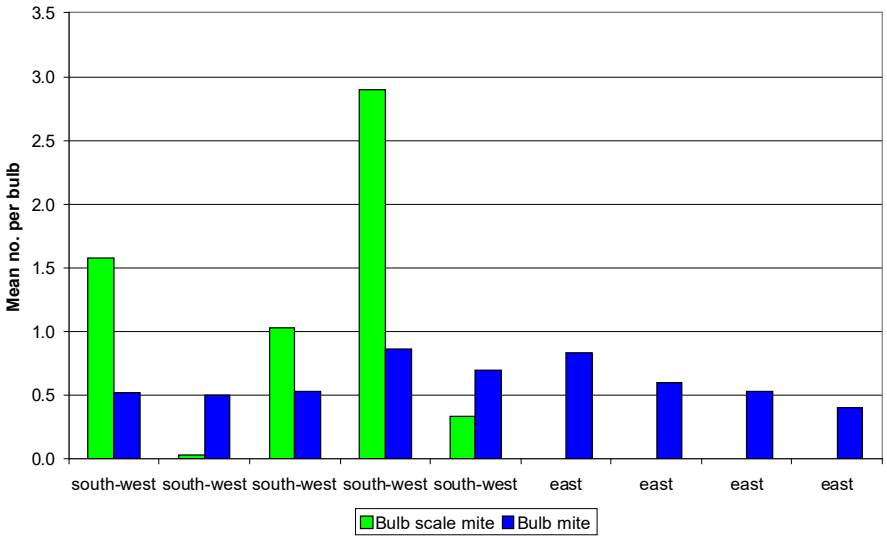

Figure 2.8: The mean numbers of mites per bulb shoot in the south-west and the east in 2009.

Investigation of movement of bulb-scale mite between growing bulbs

On 6 February 2007, groups of infested bulbs (cv Carlton; 8-10 cm grade) and hot-water-treated narcissus bulbs (cv. Golden Harvest; 12-14cm grade) were planted in 1 m-long plastic planting troughs. Two replicates of the following treatments were set up:

- Control 8 hot-water-treated bulbs only

- Bulbs touching — 8 hot-water-treated bulbs and 8 infested bulbs
- Foliage touching — 8 hot-water-treated bulbs and 8 infested bulbs – two sets of bulbs separated by a 0.5 m gap with foliage tied together in the middle
- 0.5 m apart — 8 hot-water-treated bulbs and 8 infested bulbs – foliage of two sets not touching
- 1 m apart — 8 hot-water-treated bulbs and 8 infested bulbs – foliage of two sets not touching

The troughs were stood initially in a polytunnel at Warwick HRI, Wellesbourne but when the weather became warmer they were moved outside to the Dutch Lights area (23 April 2007). A further two replicates were set up on 13 May 2007 and these were also stood outside in the Dutch Lights area. They were again provided with shelter in a polytunnel during the coldest part of winter 2008-2009 (moved in on 8 January 2009) in case severe frosting might kill the mites. The troughs were then sampled destructively during February and March 2009 (one replicate at a time) and the numbers of mite adults and eggs were recorded. Figure 2.9 shows the percentage of 'clean' bulbs that became infested with mites. This suggests that mites move between bulbs most readily when the bulbs are close to one another.

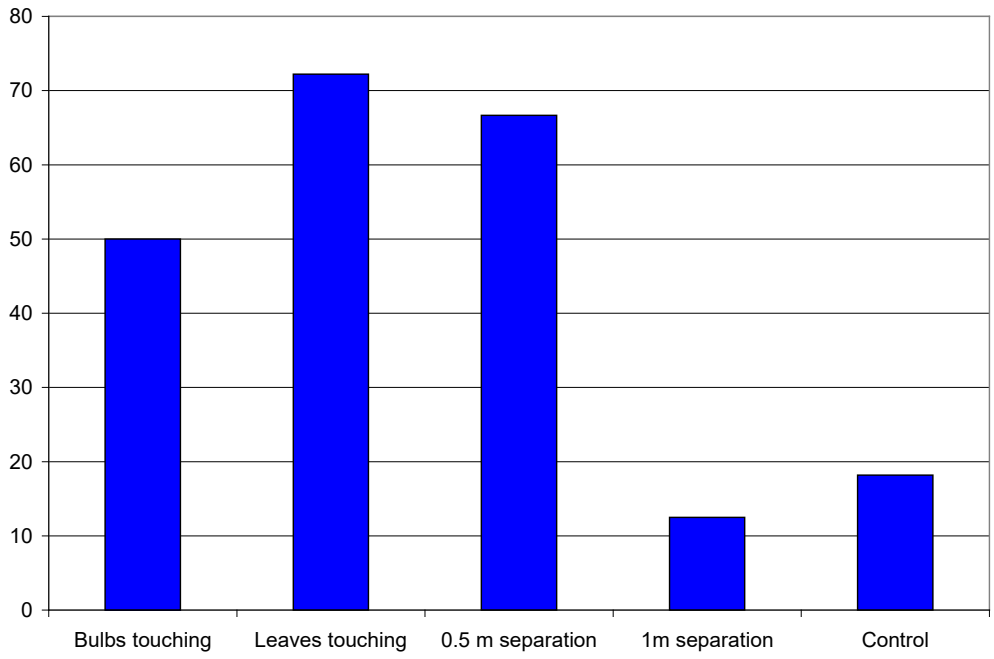

Figure 2.9: The percentage of 'clean' bulbs that became infested in the trough trial in 2007-8.

A similar experiment was set up on 5 March 2009 (four replicates), using a heavily-infested stock of cv Dutch Master and hot-water-treated bulbs (cv Golden Harvest), all of which were dug from field plots established at Wellesbourne in autumn 2008. All troughs were stood outside in the Dutch lights area at Wellesbourne. These will be sampled destructively in 2010.

A field trial was planted at Wellesbourne in late summer 2009 to extend studies on mite dispersal. Replicated plots of bulbs were planted with un-infested (cv Golden Harvest) and infested bulbs (cv Dutch Master) separated by:

- 0 m
- 0.5 m
- 1 m
- 2 m
- 4 m

There was also an un-infested control treatment. These bulbs will be assessed in late summer 2010.

Investigate extent of movement of bulb-scale mite between bulbs and around bulb stores and bulb-handling facility

2007

To determine whether mite infestations can arise from the exposure of bulbs to dust and debris during the handling and storage of dry bulbs, dust/debris samples were collected from the premises of seven bulb growers and merchants in August 2007. At each site, samples were taken from up to six locations, representing the whole bulb storage and handling process from the initial bulb cleaning lines and drying walls (or drying floors) through to hot-water treatment, grading and pre-planting or pre-sales storage areas (see Table 2.5 for details of locations).

The dust/debris samples were taken to Kirton and, after thoroughly mixing each sample, 50ml samples were mixed with sets of nine test bulbs in paper bags and stored at 20°C for 4 weeks (representing the storage phase when infestation could take place). The test bulbs were cv Golden Harvest that had previously received HWT at the elevated temperature of 46.4°C to ensure they carried no live bulb-scale mites. For each dust sample eight replicate

bags of bulbs were set up. As controls, 24 additional bags of nine bulbs each were set up with no added dust.

After 4 weeks the bulbs were planted in 20 cm-diameter pots by tipping the contents of the bag (including the dust/debris) onto a peat growing medium, spacing the nine bulbs evenly and topping up the pot with growing medium. The pots were watered, placed outdoors on a standing ground, covered with fleece and kept watered as needed. When the bulbs were ready to force (judged by the development of the bud in the neck of the bulb) they were moved to a glasshouse (heated to 17°C, vented at 20°C).

After 4 weeks in the glasshouse the incidence and severity of bulb-scale mite symptoms (serrated leaf/stem margins, curled leaves and feeding marks on leaves/stems) were recorded (Table 2.6 and Figure 2.10). In the control, there was a low incidence of symptoms of bulb-scale mite damage (about 0.7 per pot of nine bulbs), and this would have been caused by mite activity prior to HWT in 2007. With few exceptions, an elevated incidence of bulb-scale mite symptoms was seen in bulbs that had been mixed with dust samples. Dust from all farms resulted in an increased incidence of symptoms with dust from at least some locations on the farm, but no one location was consistently infective across all farms. Further, the overall incidence of damage varied somewhat between farms. In many instances the incidence of damage rose from the about 0.7 per pot of the controls to 2.0 to 3.0 per pot. The highest incidence of symptoms was seen using dust from the grading line and pre-planting holding areas of one farm, the drying wall and the main bulb storage area of another farm, and the pre-planting storage area of a third. In general, the highest scores were seen in dust samples from drying, grading and storage areas (Tables 2.5 and 2.6).

Table 2.5: Summary of farm locations used for dust samples in 2007, with incidence of bulb-scale mite symptoms. The symptoms seen in the control treatment may reflect a low level of infestation in this 'clean' hot-water-treated stock or may be due to causes other than bulb-scale mite

Farm no.	Location ref.	Location description	Symptoms per pot
Control			0.7
1	B	Cleaning line	1.7
	C	Drying wall	2.0
	D	Grading line	2.2
	E	HWT shed	1.8
	F	Storage area	0.9
2	B	Cleaning line	0.6
	C	Drying wall	0.7
	D	Grading line	0.5
	E	HWT shed	1.8
	F	Storage area	1.5
3	B	Cleaning line	0.5
	C	Drying wall	1.7
	D	Grading line	2.6
	F	Storage area	3.0
4	A	Cleaning line	1.2
	B	Grading line	1.2
	C	Cold store	1.8
	D	Drying wall	2.7
	E	Storage area	2.5
	F	Storage area	0.8
	G	Drying wall	1.7
5	B	Cleaning line	0.8
	C	Drying wall	0.4
	D	Grading line	1.7
	E	HWT shed	1.0
	F	Storage area	3.0
6	A	Grading line	2.3
	B	Grading line	1.4
	C	Drying wall	1.2
	D	Drying wall	1.2
	E	Drying wall	1.2
	F	Heat store	1.3
7	A	Cleaning line	1.3
	B	Drying wall	1.3
	C	Drying wall	2.3
	D	Grading line	1.9
	E	HWT shed	1.6
	F	Storage area	1.7

Table 2.6: Summary of bulb-scale mite symptoms from dust samples taken at different types of locations in 2007

Location	Number of samples	Minimum score	Maximum score
Control		0.7	
Cleaning line	6	0.5	1.7
Drying wall	11	0.4	2.7
Grading line	8	0.5	2.6
Heat store	1	1.3	1.3
HWT shed	4	1.0	1.8
Storage area	7	0.8	3.0
Cold store	1	1.8	1.8

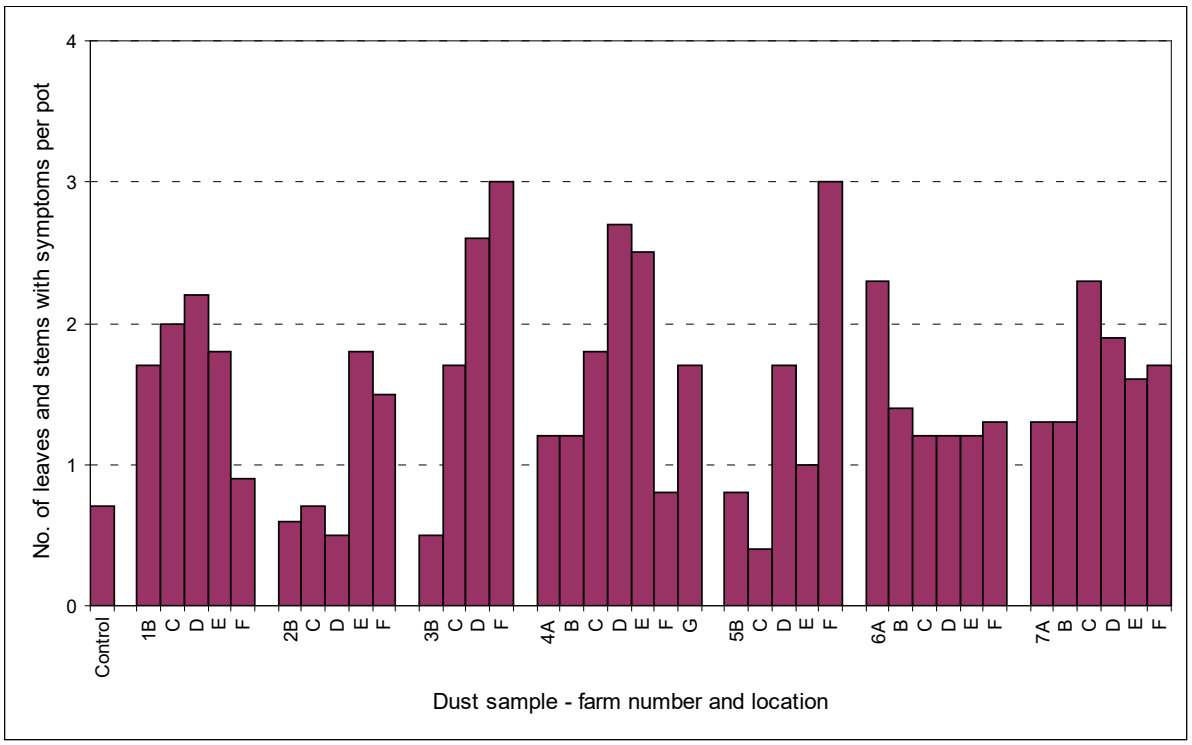

Figure 2.10: The number of leaves and stems with symptoms of bulb-scale mite infestation from dust samples taken in different locations at seven farms (see Table 2.5 for location codes). Data from the 2007-2008 experiment assessed in spring 2008

After foliar senescence the pots were moved to a ventilated, non-heated mesh tunnel and grown-on. Over several days in December 2008 bulbs were recovered from their pots, cut transversely and scored for the presence and severity of feeding marks on the current bulb units (Figure 2.11). While several of the dust treatments produced higher scores than in the controls, there was, however, no correlation between the spring and autumn assessments

(Figure 2.12), and the reasons for this need to be investigated. It may be that assessments made in the following autumn are not valid because of the dispersal of mites over the previous spring and summer.

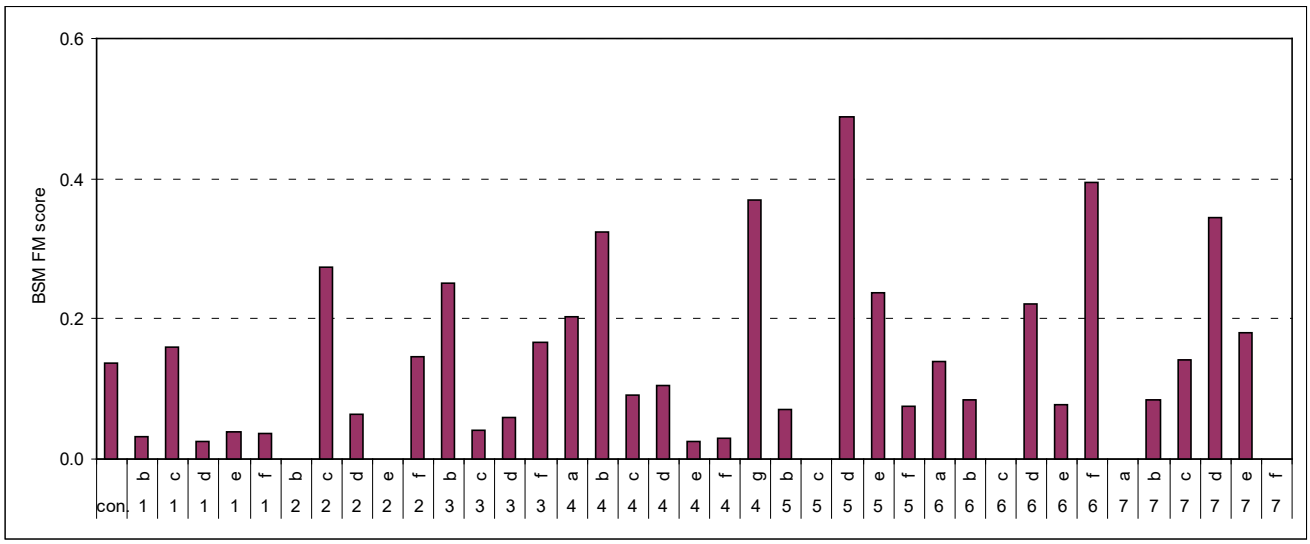

Figure 2.11: Feeding mark scores in bulbs previously treated with dust samples taken in different locations at seven farms. Data from the 2007-2008 experiment assessed in autumn 2008

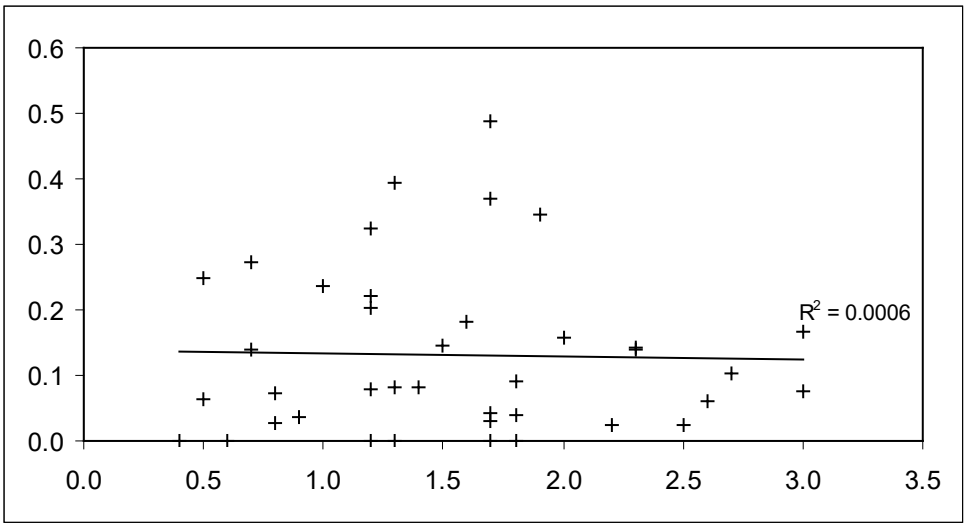

Figure 2.12: Correlation plot of foliage symptoms (in spring 2008) against feeding mark scores (in autumn 2008) in bulbs previously treated with dust samples taken in different locations at seven farms. Data from Figures 2.7 and 2.8

In addition to the main series of dust samples, further samples were collected in late-May 2007 from one Cornish farm. One sample was collected from each of 10 separate drying floors before the company's usual pre-season cleaning of facilities. The volumes collected were variable and the samples contained a proportion of parts of bulbs as well as dust and general debris. After transport to Kirton the samples were placed in calico bags and stored at room temperature until required. These 'pre-season samples' were otherwise treated as described above. The results (Figure 2.13) showed that several samples had increased incidence of bulb-scale mite symptoms above control levels, though to a variable extent. There were also a few leaves and stems with symptoms in the control treatment.

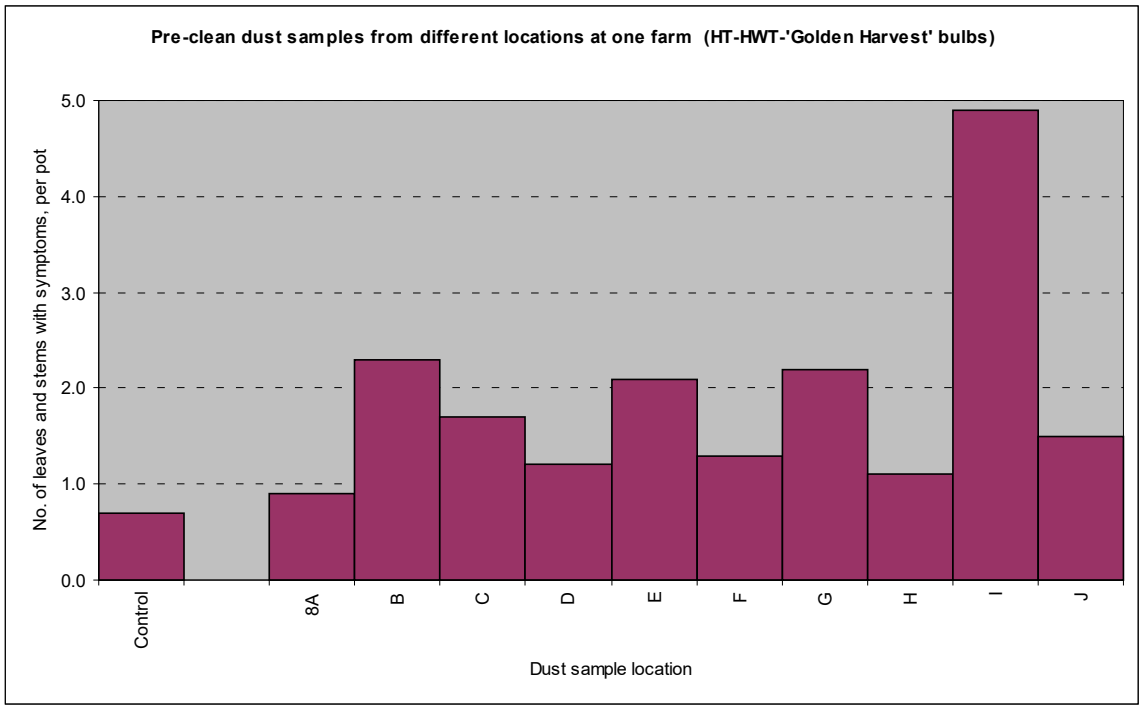

Figure 2.13: The number of leaves and stems with symptoms of bulb-scale mite infestation from dust samples taken from different drying floors prior to seasonal cleaning

2008

The transmission of infestations via dust and debris was further investigated in 2008. In August, dust samples were collected from two Lincolnshire and two Cornish sites that had produced infective samples in 2008. At each site samples were collected from four locations at each site, the drying wall, the grading (or mixing) line, the storage area for bulbs awaiting despatch, and the storage area for bulbs for replanting. Each of these locations was divided into three sections, and samples were collected from each section, making 48 samples in all

(four farms x four locations x three sub-locations). The samples were tested using similar procedures to those of 2007. The bulbs were planted in pots on 14 October 2008, grown-on in an unheated mesh tunnel, and transported to Wellesbourne in February 2009. The pots were kept in a glasshouse at Wellesbourne and shoots were sampled for the presence of live mites. No mites were found.

Further samples were taken in 2009. Dust samples were taken from four farms x four locations x three sub-locations, and un-infested bulbs (cv Kerensa) were incubated with the dust as described above. These were potted up and the bulbs will be assessed for live mites in 2010.

Are growers re-planting infested bulbs?

Overall, the evidence suggests that growers may be re-planting infested bulbs. In the summer of 2009, growers sent samples of bulbs to Wellesbourne so that they could be sampled destructively to search for mites. Each stock of bulbs was sampled twice: 1) immediately after hot-water treatment and 2) immediately before planting. Over 80 samples were received. Sub-samples of these bulbs are being sampled destructively to search for mites. The remaining bulbs have been potted up for examination later on.

Determine whether cultivars are differently susceptible to bulb-scale mite and whether bulb-scale mite show cultivar preferences

2007-8

'Golden Harvest' bulbs (from Kirton stocks) and bulbs of cultivars 'Counsellor', 'Dutch Master', 'California', 'St Keverne', 'Golden Ducat', 'Ice Follies' and 'Carlton' (supplied by a Consortium member) were used as test bulbs. This was a mix of popular cultivars including various possible susceptibilities and what was available at the time from the industry partners.

The supplied bulbs were not specially treated, and therefore variable degrees of infestation with bulb-scale mites would be expected. In August 2007, groups of nine test bulbs were placed into brown paper bags, and five mite-infested bulbs were added to half of the bags. The bags were stored at 17°C for 4 weeks (representing the storage phase when infestation could take place). For each cultivar there were eight pots with mite-infested bulbs and eight without infested bulbs. The bulbs were planted in pots of a peat growing medium by tipping

out the contents of the bag onto growing medium in a 20 cm-diameter plant-pot, arranging the bulbs evenly, with the mite-infested bulbs in the centre of the pot, and topping-up with growing medium in a standard fashion. The pots were watered well and placed in a cold store at 9°C. When the bulbs of each cultivar had received a sufficiently long cold treatment they were moved to a glasshouse (details as above) for growing-on.

After 4 weeks in the glasshouse the incidence and severity of bulb-scale mite symptoms were recorded, as previously described. Only a low incidence of bulb-scale mite symptoms was seen on any of these plants, with no indication of differences between cultivars.

After foliar senescence the pots were moved to a ventilated, non-heated mesh tunnel and grown-on. In November 2008 bulbs were recovered from their pots, cut transversely and scored for the presence and severity of feeding marks in the current bulb units (Figure 2.10). As there were no clear differences in the amount of symptoms for each variety planted with inoculator bulbs and those planted alone, it appeared that most feeding marks were the result of prior infestations, which naturally varied between varieties and stocks. It was seen that the feeding marks were almost always in the scales of the current bulb unit, which indicated that the addition of inoculator bulbs had been totally ineffective.

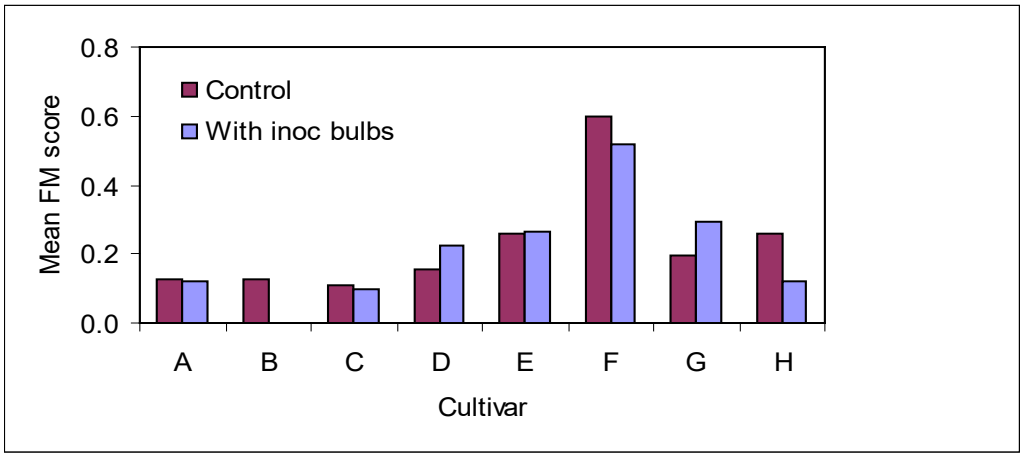

Figure 2.12: The number of leaves and stems per pot with symptoms of bulb-scale mite infestation in eight narcissus varieties grown alone ('control') or with added inoculator bulbs ('with inoc bulbs'). Cultivars: A, Carlton; B, Golden Ducat; C, Dutch Master; D, Ice Follies; E, Counsellor; F, California; G, Golden Harvest and H, St Keverne

2008-9

In order to collect further data on varietal differences, and hopefully to explain the result obtained in 2007-8, this experiment was repeated in 2009. The same bulb stocks as before were used, with the addition of cv. Hollywood, but the bulbs of all stocks received standard

HWT prior to set-up; in addition, Golden Harvest bulbs were tested following both standard HWT and HT-HWT. The UK standard is 3 hours x 44.4°C and the 'extra hot' treatment was 3 hours x 46.4C (originated from the earlier HWT experiment where a range of temperatures from 44.4 to 48.4°C were tested). The bulbs were planted in pots on 21 October 2008, placed in a 9°C cold store, moved to Wellesbourne in February 2009, and are currently being grown-on for examination.

Objective 3: Design optimal high or low temperature and/or chemical treatments to control bulb-scale mite in bulbs for replanting and for forcing, and ensure all stages in its life-history are killed and that crop quality is unaffected

Determine what hot-water treatment (HWT) regimes are effective in controlling all stages of the bulb-scale mite life-cycle

2007

In 2007 mite-infested bulbs (cv. 'Carlton', 8-10 cm grade) were used to test the effects of HWT regimes. On 3 September netted groups of nine bulbs each were treated for 2, 3 or 4 hours at 42.4, 44.4 or 46.4°C, with a further group of bulbs remaining untreated as controls. This was repeated with fresh sets of bulbs on 4 and 5 September, giving three 'replicates' of each of the ten treatments. Following standard practice, the HWT dip contained formaldehyde (as commercial formalin), a prochloraz fungicide (as Mirage 40EC), non-ionic wetter and anti-foam preparation. To simulate commercial HWT conditions, the netted groups of bulbs were placed in HWT tanks already fully loaded with stock bulbs (cv 'Carlton'), and treatments were timed from when the netted bulbs were added to the tank, assuming these bulbs would warm up rapidly. After HWT the bulbs were removed from the tank, cooled and surface-dried by standing under strong ventilation, and stored at 17°C. The bulbs were planted on 17 September in 20 cm-diameter plant-pots and placed in a 9°C store until judged ready for forcing (11 January 2008), when the pots were moved to a heated glasshouse.

After 4 weeks in the glasshouse the incidence and severity of BSM symptoms on leaves and stems were recorded. Very few symptoms were present, and only on control (non-HWT) bulbs (Table 3.1).

After a further 1 week in the glasshouse, two random shoots from each pot were excised and all leaf and stem surfaces were examined under a low-powered (LP) microscope for bulb-scale mites and eggs. There were numerous mites and eggs on all control plants, and a

few on one only of the treated plants. After a further 4 weeks additional shoot samples, from the controls and the 3h x 44.4°C treatment only, were examined. The earlier result was confirmed (Table 3.1).

Table 3.1: BSM assessments of forced plants in the glasshouse, following different HWT regimes, 2007-2008 experiment (assessed spring 2008)

HWT	BSM symptoms (mean no. of leaves and stems per pot with symptoms, at week 4)	BSM/eggs on leaves (mean incidence score)	
		Week 5	Week 9
None	0.7	3.7	6.0
2h 42.4°C	0	0	-
3h 42.4°C	0	0.3	-
4h 42.4°C	0	0	-
2h 44.4°C	0	0	-
3h 44.4°C	0	0	0.3
4h 44.4°C	0	0	-
2h 46.4°C	0	0	-
3h 46.4°C	0	0	-
4h 46.4°C	0	0	-

-, not assessed

After foliar senescence the pots were moved to a ventilated, non-heated mesh tunnel and grown-on. Over several days in late-October 2008 bulbs were recovered from their pots. All bulbs were cut transversely and then scored for the presence and severity of feeding marks on the scales, also recording whether feeding marks were on the current, previous or new bulb units. Numerous feeding marks occurred on scales of both the previous and current bulb units (Figure 3.1). As expected, feeding marks were found on previous bulb units in both control and treated bulbs (since HWT cannot eliminate feeding marks made prior to treatment, but should control current mite activity). In the current bulb units the highest feeding mark score occurred on control bulbs, but bulbs from various hot-water treatments also showed some evidence of mite activity, indicating only a partial control of bulb-scale mite by the treatments had been achieved.

In addition, bulbs from three key treatments (the control, the standard HWT of 3h x 44.4°C, and the extreme HWT of 4h x 26.4°C) were fully dissected into individual scale pieces and all pieces were examined under a LP microscope for the presence and numbers of bulb-

scale mites and eggs. Table 3.2 shows the distribution of feeding marks and bulb-scale mites to the generations of bulb units. The data confirmed that both current and previous bulb units contained feeding marks, that those in the current scales were fewer where HWT had been given, and that no feeding marks occurred in the new bulb units at this stage. However, in new bulb units bulb-scale mites were sometimes found without accompanying feeding marks; to a lesser extent this effect was also seen in current bulb units. Table 3.3 shows the numbers of scale pieces with feeding marks and mites, and the total numbers of mites. It confirms that there were more feeding marks in control bulbs than in treated bulbs. However, one of the two HWT treatments examined contained active and inactive mites and mite eggs, like the control, suggesting that new infestations had taking place by the autumn of the year following HWT. In this particular case, the number of mites and eggs was skewed by very high numbers of mites and eggs in one of the replicates.

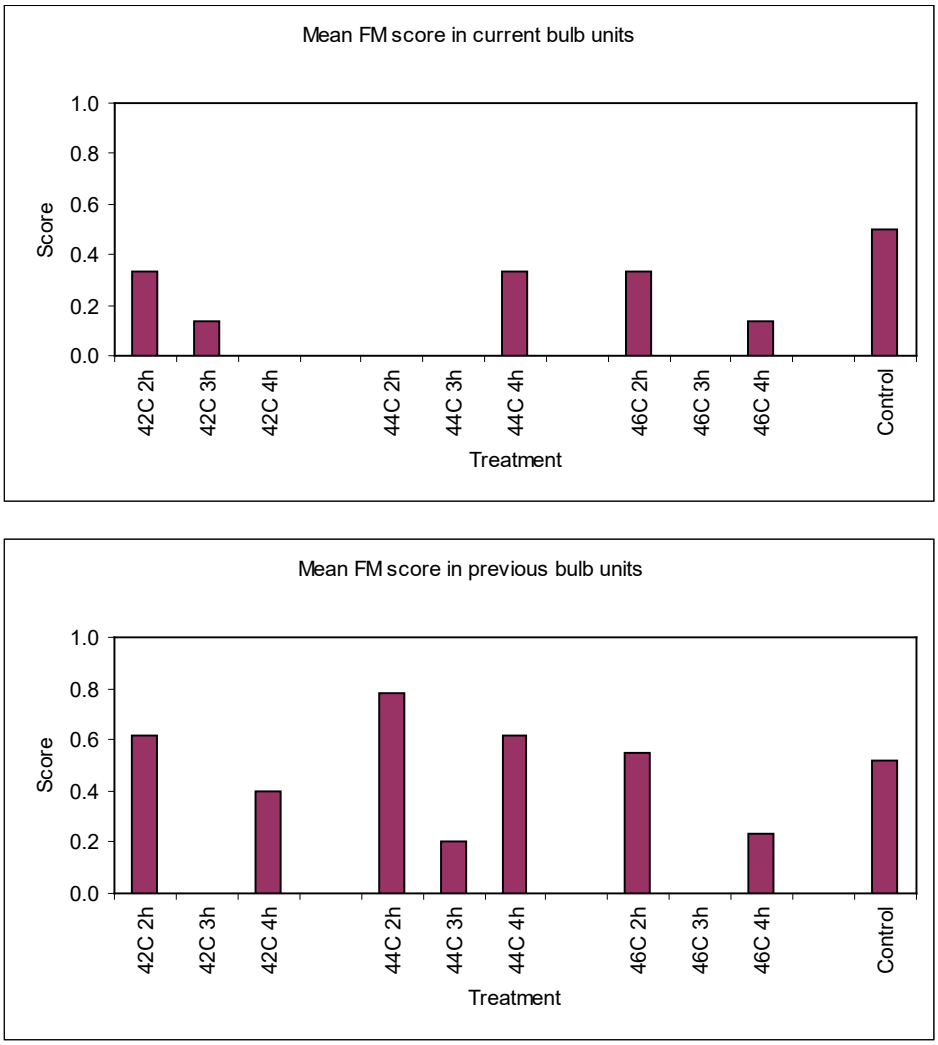

Figure 3.1: Feeding mark scores in current (top) and old bulb units (bottom) of bulbs previously given hot-water treatment at the temperatures and durations stated. Data from the 2007-2008 experiment assessed in autumn 2008

Table 3.2: Bulb-scale mites assessments of plants grown-on after glasshouse forcing following key HWT regimes, 2007-2008 experiment (assessed autumn 2008)

No. of scales per bulb with:	Bulb unit	44C 3h	46C 4h	Control
Feeding marks	Previous	0.27	0.73	2.18
	Current	0	0.27	2.62
	New	0	0	0
BSM but no feeding marks	Previous	0	0	0
	Current	0	0.17	0
	New	0	1.33	0.83

Table 3.3: BSM assessments of plants grown-on after glasshouse forcing, following different HWT regimes, 2007-2008 experiment (autumn 2008)

HWT	Number of scale pieces with:				Number of mites		
	Feeding marks	Active BSM	BSM eggs	Inactive BSM	Active BSM	BSM eggs	Inactive BSM
44C 3h	0.27	0.00	0.00	0.00	0.0	0.0	0.0
46C 4h	0.93	2.00	0.83	0.17	57.2	11.0	0.2
Control	4.80	1.67	0.83	0.67	16.2	9.0	2.0

2008

The HWT experiment was repeated in 2008, using infested bulbs of cv. 'Dutch Master'. As in 2007, netted groups of bulbs (ten each) were treated for 2, 3 or 4 hours at 42.4, 44.4 or 46.4°C or remained untreated as controls; the three replicates were treated on 28 August, 29 August and 1 September 2008. The bulbs were planted on 12 September, methods as before, placed in a 9°C store until judged ready for forcing (29 December 2008) and then moved to a heated glasshouse.

After 4 weeks in the glasshouse the incidence and severity of bulb-scale mites symptoms on leaves and stems were recorded. Mite symptoms were common on control plants but occurred only rarely on treated bulbs (Table 3.4). These bulbs were re-located to a glasshouse at Wellesbourne on 3 February 2009 and then shoots were removed from each plant (by severing them at the top of the bulb) and examined for mites. The results are summarised in Figure 3.2. There were no live mites in shoots from hot-water-treated bulbs, although there were live mites in the shoots taken from the untreated bulbs.

Table 3.4. Bulb scale mite damage symptom assessments of forced plants in the glasshouse, following different HWT regimes, 2008-2009 experiment (assessed spring 2009)

HWT	BSM symptoms (mean no. of leaves and stems per pot with symptoms)
None	6.0
2h 42.4°C	0.0
3h 42.4°C	0.0
4h 42.4°C	0.0
2h 44.4°C	0.0
3h 44.4°C	0.7
4h 44.4°C	0.3
2h 46.4°C	0.0
3h 46.4°C	0.7
4h 46.4°C	0.0

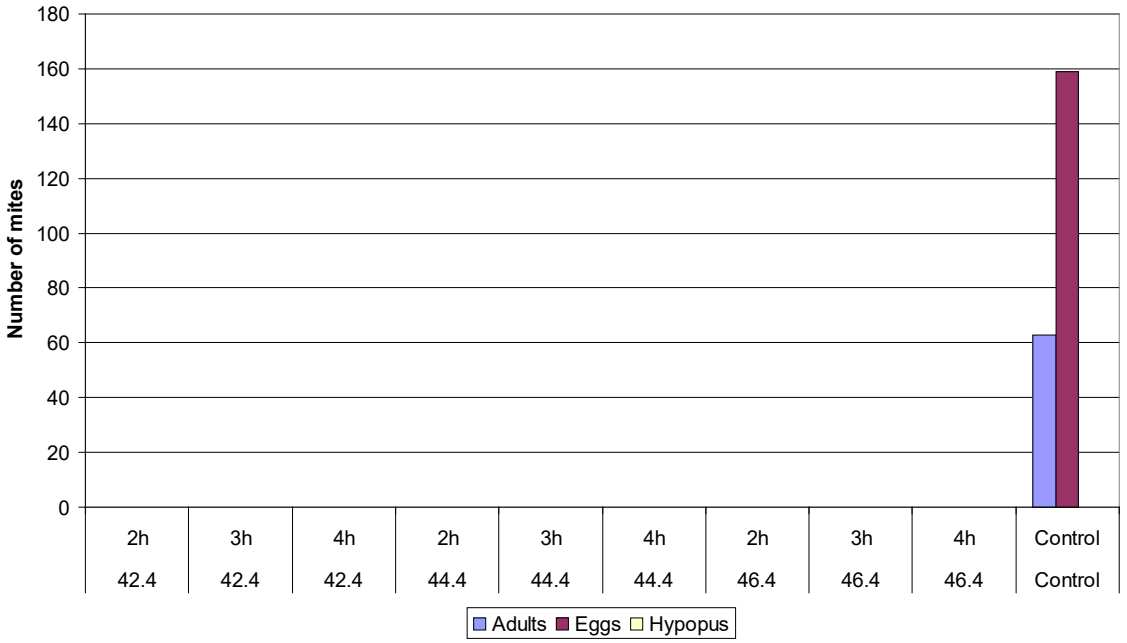

Figure 3.2: Number of live mites per shoot from bulbs previously given hot-water treatment in 2008 at the temperatures and durations stated.

2009

A further hot water treatment trial was undertaken in 2009. Mite-infested Dutch Master bulbs from the plot at Wellesbourne were treated by a consortium member.

The treatments were:

- Treatment 1 – no disinfectant
- Treatment 2 – half rate FAM30
- Treatment 3 - full rate FAM 30
- Treatment 6 - FAM 30 + Bravo 500 – both half rates
- Untreated

The bulbs were returned to Wellesbourne in the week beginning 21 September and then 10 pots x 8 bulbs from each treatment were potted up and stood outside in the Dutch Lights area. They were later moved to a polytunnel to protect them from extreme cold. They will be assessed in spring 2010.

Determine whether warm-storage treatments are effective in controlling all stages of the bulb-scale mite life-cycle without causing damage to glasshouse-forced bulbs

2007

Bulbs of a mite-infested stock (cv. 'Carlton', 8-10 cm grade) were used in this trial at Wellesbourne in 2007. Bulbs were treated in netted groups of nine bulbs each, with three replicates (occasions) of each treatment. The treatments were storage for 1, 2 or 3 hours at 42, 44 or 46°C, with further untreated bulbs serving as controls. The three replications were carried out on 2, 3 and 4 October 2007, respectively. Treatments were applied in a laboratory fan oven (Gallenkamp Size 2 'Hotbox', 0.125 m^3 capacity) calibrated using a separate thermometer. The bulbs, in nets, were put in the oven on a wooden board, and treatments were timed from when the oven re-gained the target temperature after closing the door. After treatment, the bulbs from each treatment and replicate were put into separate paper bags (to prevent possible transfer of mites between treatments) and they were then transported to the Kirton Research Centre where they were planted in 20 cm-diameter pots with the nine treated bulbs planted around the edge of the pot and five previously hot-water treated 'Golden Harvest' test bulbs were planted in the centre. The pots were cold-stored and moved to a heated glasshouse on 29 February 2008.

After 4 weeks in the glasshouse the incidence and severity of bulb-scale mite symptoms on leaves and stems were recorded (Table 3.5). There was a very low incidence of symptoms, and hence no evidence for a beneficial effect of warm storage.

After a further 1 week in the glasshouse, two random shoots from each pot were excised and all leaf and stem surfaces were examined under a low-powered (LP) microscope for bulb-scale mite and eggs (Table 3.5). There were numerous mites and eggs on control (untreated) plants, and fewer on the warm-stored bulbs, probably indicating a measure of control by these warm storage treatments.

Table 3.5: Bulb-scale mite assessments of forced plants in the glasshouse, following different warm storage treatments, 2007-2008 experiment (assessed spring 2008). The figures given are the marginal means for temperature treatments (i.e. means across all treatment durations)

Heat treatment	BSM symptoms on leaves (mean no. of leaves per pot with symptoms)	BSM/eggs on leaves (mean incidence score)
Control (none)	0	2.7
42.0°C	0.2	0.3
44.0°C	0.1	1.0
46.0°C	0.4	0.7

After foliar senescence the pots were moved to a ventilated, non-heated mesh tunnel and grown-on. In November 2008 the bulbs were recovered from their pots, cut transversely and scored for the presence and severity of feeding marks on the scales, also recording whether feeding marks were on the current, previous or new bulb units. However, only two bulbs were found with feeding marks in the current bulb units, and so the data are not presented.

2008

The warm-storage experiment was repeated in 2008, using infested bulbs of cv. 'Dutch Master'. As in 2007, netted groups of bulbs (10 each) were treated for 1, 2 or 3 hours at 42, 44 or 46°C or remained untreated as controls. There was a single treatment of 48 hours at -2°C. The three replicates were treated in the week beginning 6 October 2008. The bulbs were planted in the week beginning 13 October, kept in a glasshouse and shoots from each pot were sampled in April 2009. The numbers of mites per shoot are shown in Figure 3.3. None of the treatments appeared to have controlled the mites.

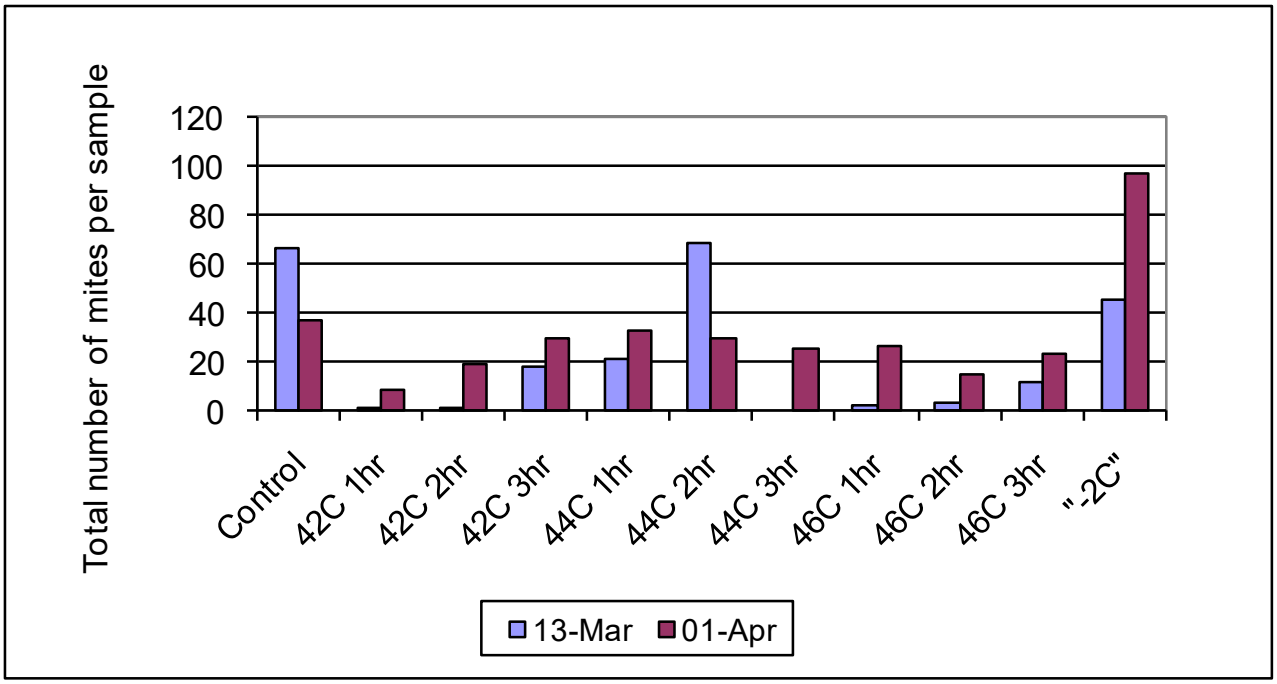

Figure 3.3: Number of live mites per shoot from bulbs previously given warm storage treatment in 2008 at the temperatures and durations stated.

2009

Further 'extreme' warm storage treatments have been applied to mite-infested bulbs cv Dutch Master in late summer 2009. Bulbs of a mite-infested stock (cv. Dutch Master) were used in this trial at Wellesbourne in 2009.

Bulbs were treated in netted groups of nine bulbs each, with three replicates (occasions) of each treatment. The treatments were storage for 2, 4 or 8 hours at 46, 50, 54°C, with further untreated bulbs (6 x 9 bulbs) serving as controls. The three replications were carried out in October 2009. Treatments were applied in a laboratory fan oven (Gallenkamp Size 2 'Hotbox', 0.125 m^3 capacity) calibrated using a separate thermometer.

The bulbs, in nets, were put in the oven on a wooden board, and treatments were timed from when the oven re-gained the target temperature after closing the door.

After treatment, the bulbs from each treatment and replicate were put into separate paper bags (to prevent possible transfer of mites between treatments) and they were then planted in 20 cm-diameter pots. These were kept outside and then moved to a polytunnel to protect them from extreme cold. They will be assessed in spring 2010.

Determine whether moving forced bulbs from the glasshouse to freezing temperatures overnight ('frosting') is effective in controlling bulb-scale mite without adversely affecting crop quality

2007

On 17 September 2007, groups of nine mite-infested bulbs (cv 'Carlton', grade 8-10 cm) were placed with five hot-water treated 'Golden Harvest' test bulbs in paper bags (317 x 305 mm). Twenty-four bags were set up, the top of each bag was folded over, and the bags were placed in a controlled-temperature store at 9°C for 5 weeks. On 22 October 2007, the bulbs from each bag were planted in pots, the infected bulbs being planted around the edge of the pot and the five treated 'Golden Harvest' test bulbs planted in the centre. The pots were returned to the 9°C cold store. On 8 January 2008, after a total of 15 weeks at 9°C, the pots were moved to a glasshouse. After 3 days in the glasshouse the pots were subjected to different 'frosting' treatments by placing them in a -1°C cold store for 12, 24 and 36 h before being returned to the glasshouse. One set was non-frosted as a control. There were six replicate pots for each of the treatments and control.

After 4 weeks (5 February 2008) the incidence and type of foliar symptoms of bulb-scale mite were recorded (Table 3.6). A relatively low incidence of symptoms was seen at this examination, in all treatments.

After a further week in the glasshouse, random shoots were excised from each pot and the leaf/stem surfaces were examined under a LP microscope (Table 3.6). All six replicates of the control (untreated) bulbs carried several to many live bulb-scale mites. Of the 12 lots of bulbs frosted for 12 or 24 hours, half carried one to many mites. Half of the plants frosted for 36 hours carried one to a few mites. Mite eggs were found on only five pots from among the control plants and plants frosted for 12 hours. This suggests that the 'frosting' treatment has potential and should be further investigated.

Table 3.6: Bulb-scale mite assessments of forced plants in the glasshouse, following different 'frosting' treatments, 2007-2008 experiment (assessed spring 2008)

Frosting treatment	BSM symptoms on leaves (mean no. of leaves per pot with symptoms)	BSM/eggs on leaves (mean incidence score)
Control (none)	0.7	4.3
12h	0.7	1.2
24h	0.2	1.8
36h	0.5	0.8

After foliar senescence the pots were moved to a ventilated, non-heated mesh tunnel and grown-on. In November 2008 bulbs were recovered from their pots, cut transversely and scored for the presence and severity of feeding marks on the scales, also recording whether feeding marks were on the current, previous or new bulb units. Feeding marks occurred frequently on scales of the current bulb units (Figure 3.7). The outstanding result is that much lower feeding mark scores occurred in the 12h-frosting treatment, with higher scores in the longer frosting treatments and in the controls, a difficult result to interpret.

Table 3.7: Bulb-scale mite assessments of plants grown-on after glasshouse forcing, following different frosting treatments, 2007-2008 experiment (assessed autumn 2008)

Frosting treatment	Feeding mark score in current bulb units
12h	0.27
24h	0.50
36h	0.49
None (control)	0.47

2008

A further experiment on 'frosting' was carried out in 2008, to develop these findings. 'Dutch Master' bulbs were potted-up on 24 September 2008, the bulbs for one treatment having been stored at -2°C for the previous 48 hours, after which all pots were placed in a 9°C cold store. On 16 January 2009, all pots were moved to a heated glasshouse. After three days, bulbs allocated to receive 24-, 48- and 72-hour 'frosting' treatments were moved to a cold store at -2°C and were returned to the glasshouse after the appropriate period. One batch of

pots received no 'frosting' treatment, as controls. These pots were transported to Wellesbourne in February 2009.

Shoots were examined on 10-11 March 2009 and the average counts of mites and eggs are presented in Figure 3.3. Only the dry, cold treatment appeared to reduce the numbers of eggs, compared with the control. No treatment appeared to reduce the number of adults.

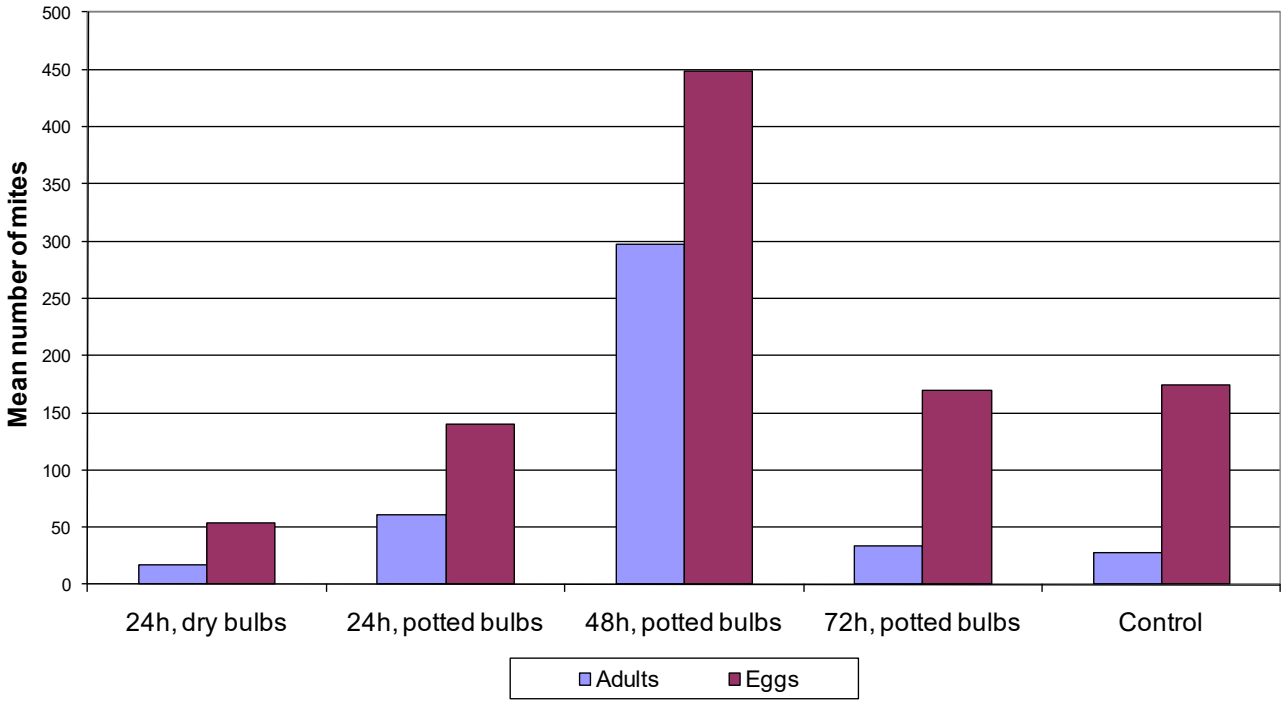

Figure 3.3: Numbers of bulb-scale mite adults and eggs in bulb shoots following 'frosting' treatments, 2008-2009 experiment

Evaluate fogging as a method of mite control in bulb stores

The possibility of using fogging to control bulb-scale mites on bulbs in-store was investigated during 2008. Formalin, 'Jet 5' and pybuthrin-based insecticides were considered. Despite assertions from some segments of the industry that they *will* be able to use formaldehyde after 2008, this seemed unlikely to be a realistic interpretation of the situation. 'Jet 5' is also likely to be withdrawn for such uses. Pybuthrin insecticides have apparently been used extensively for the fogging of produce stores, but discussions with the registration holder clarified that the material cannot be used to treat produce in stores. Consequently this approach has been aborted.

Effect of acaricides and disinfectants applied as sprays

The first trials focused on acaricides applied as sprays. Mite-infested bulbs (grade 8-10 cm) (9 per pot) were potted with un-infested bulbs (5 per pot) in 20 cm diameter pots of compost in September 2007. The pots were watered well and placed outside on 'standing ground' at Wellesbourne. In December 2007, the pots were placed inside a polytunnel at Wellesbourne to protect them from extreme weather. A range of acaricides were sourced from pesticide companies and these were applied to the bulbs as foliar sprays (5 replicate pots per treatment). The acaricides tested in this trial were: pirimiphos-methyl (Actellic), Fenpyroximate (Sequel), Bifenazate (Floramite) and two coded treatments (Exp C and Exp U2 (spirotetramat)). There was also an acaricide-free control treatment. There were two trials. Each trial was sprayed twice. The first trial was sprayed on 15 and 29 March the second on 16 and 30 May 2008. The bulbs were then grown on and samples of the foliage were assessed for the presence of live mites in April – June 2008 (one replicate at a time) by sampling 10 cm of the lower leaf taken from the bulb neck upwards. Figure 3.3 summarises the data from the two trials. The bulbs were later assessed (mid November – early December 2008) for the presence of feeding marks and these data are shown in Figures 3.4 and 3.5. The relatively large counts from the pots treated with Exp U2 suggests that there was considerable variation between infestation levels in individual pots prior to treatment. None of the treatments controlled mites completely.

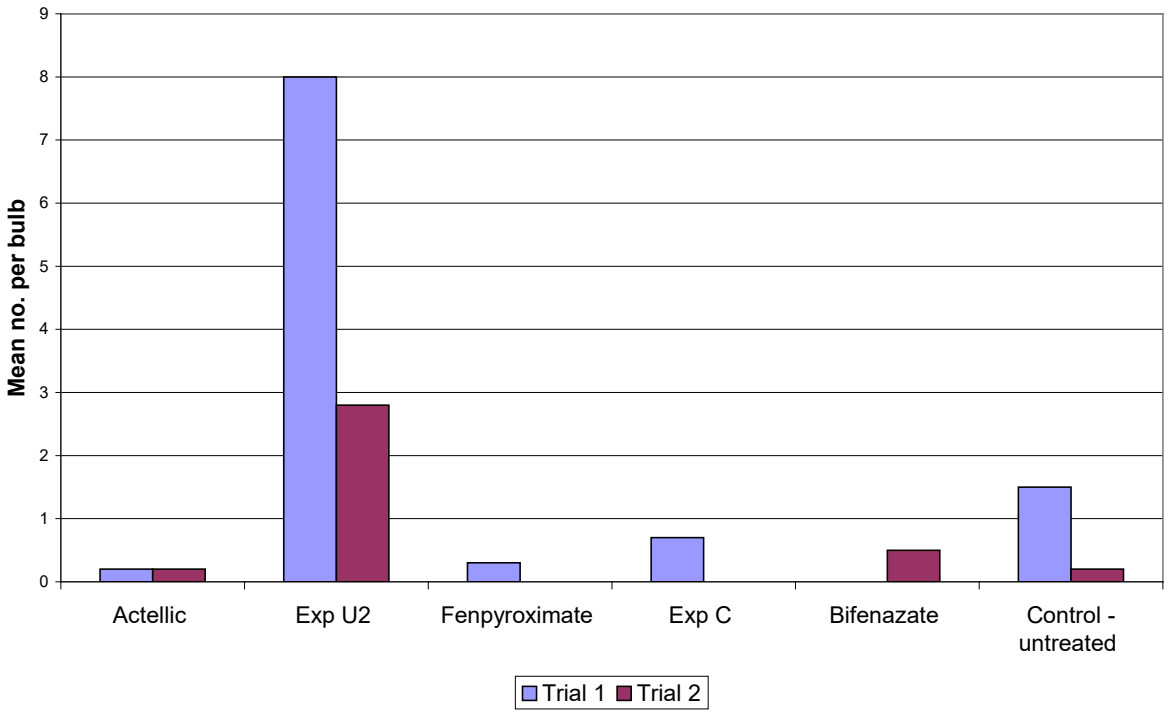

Figure 3.3. The mean number of bulb scale mites (all stages) recovered from the foliage of potted Narcissus treated with foliar sprays (2007-8).

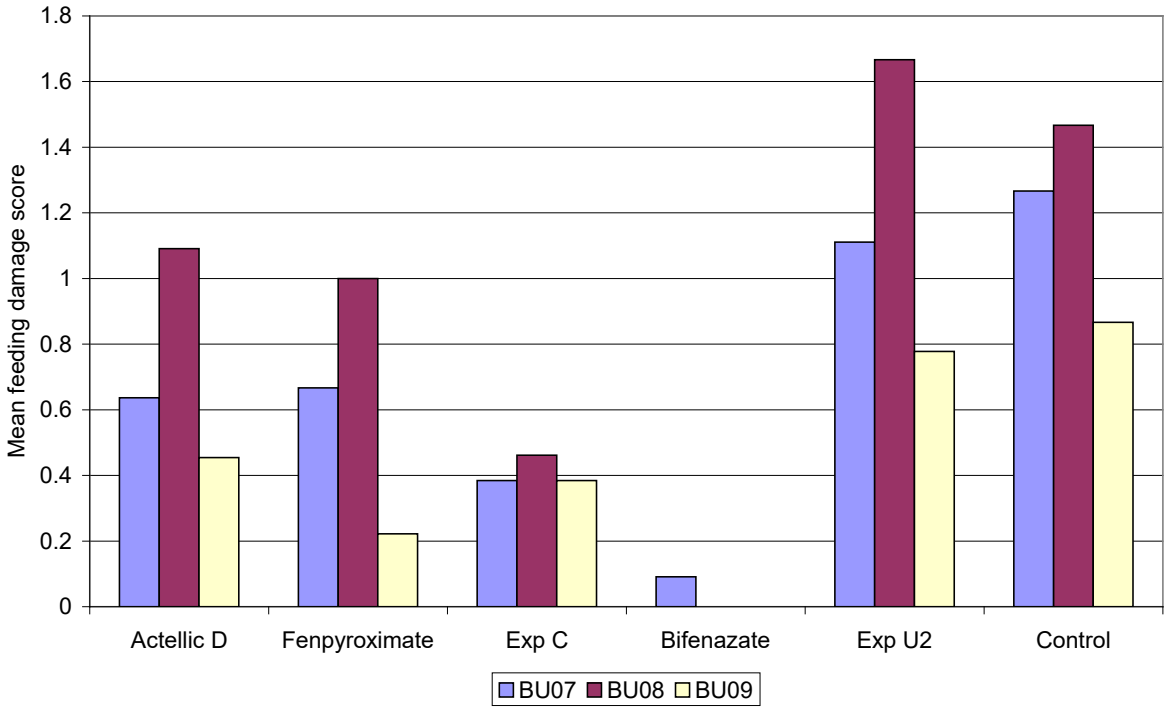

Figure 3.4. The mean feeding damage score of bulbs treated with foliar sprays (Trial 1, 2007-8)

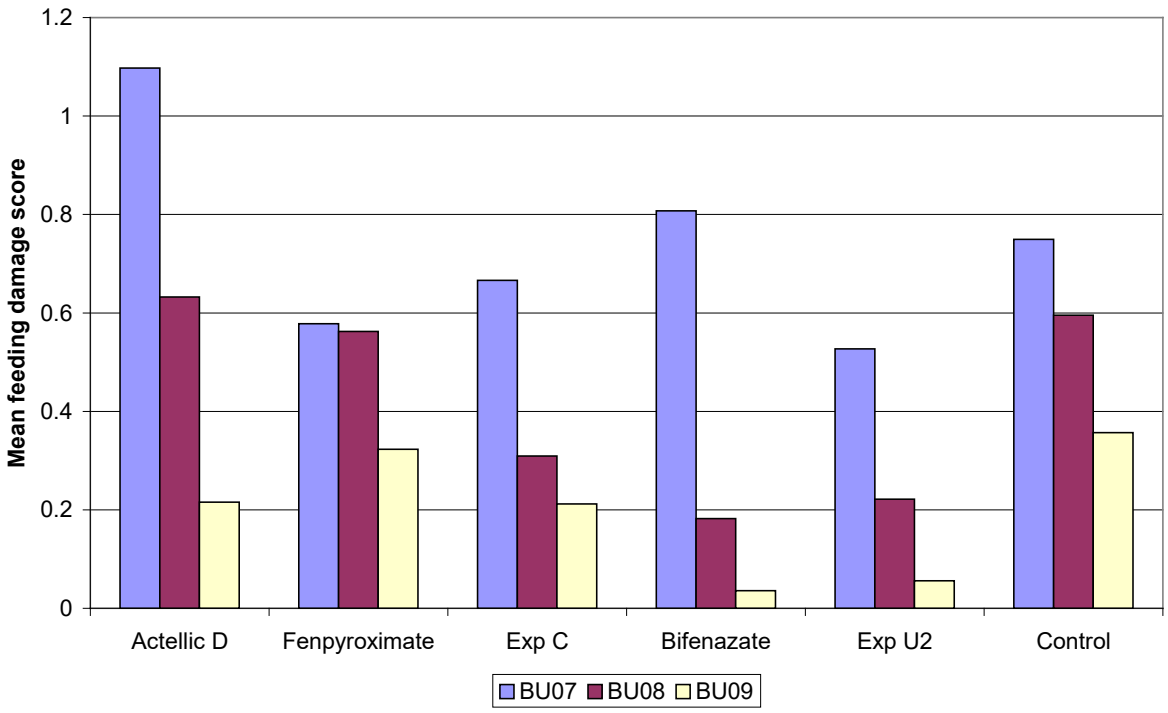

Figure 3.5. The mean feeding damage score of bulbs treated with foliar sprays (Trial 2, 2007-8)

Three similar trials were done in spring 2009 using infested bulbs cv Dutch Master (6 replicates per treatment). The treatments were: Actellic (pirimiphos methyl), Sequel (fenpyroximate), Exp C, Floramite (bifenazate) and Movento (spirotetramat) in Trials 1 & 2. Additional treatments were tested in Trial 3 (Dynamec (abamectin) & Masai (tebufenpyrad)). Samples of shoots were taken from each pot and examined for mites. The results are summarised in Figures 3.6 – 3.8. No treatment appeared to be consistently effective.

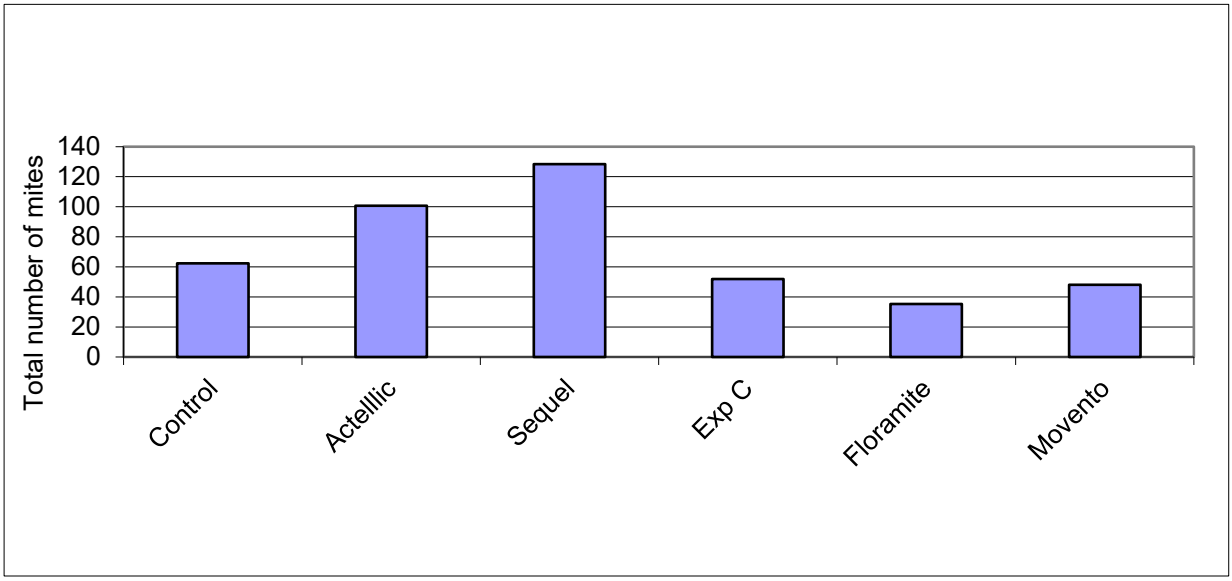

Figure 3.6. The number of mites per shoot from bulbs treated with foliar sprays (Trial 1, 2009).

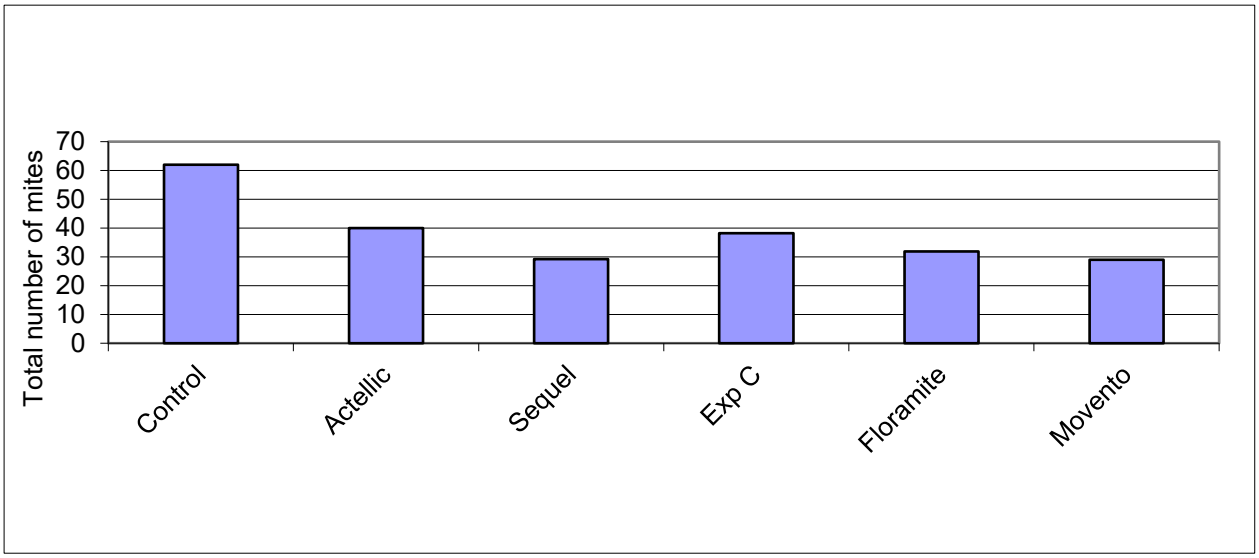

Figure 3.7. The number of mites per shoot from bulbs treated with foliar sprays (Trial 2, 2009).

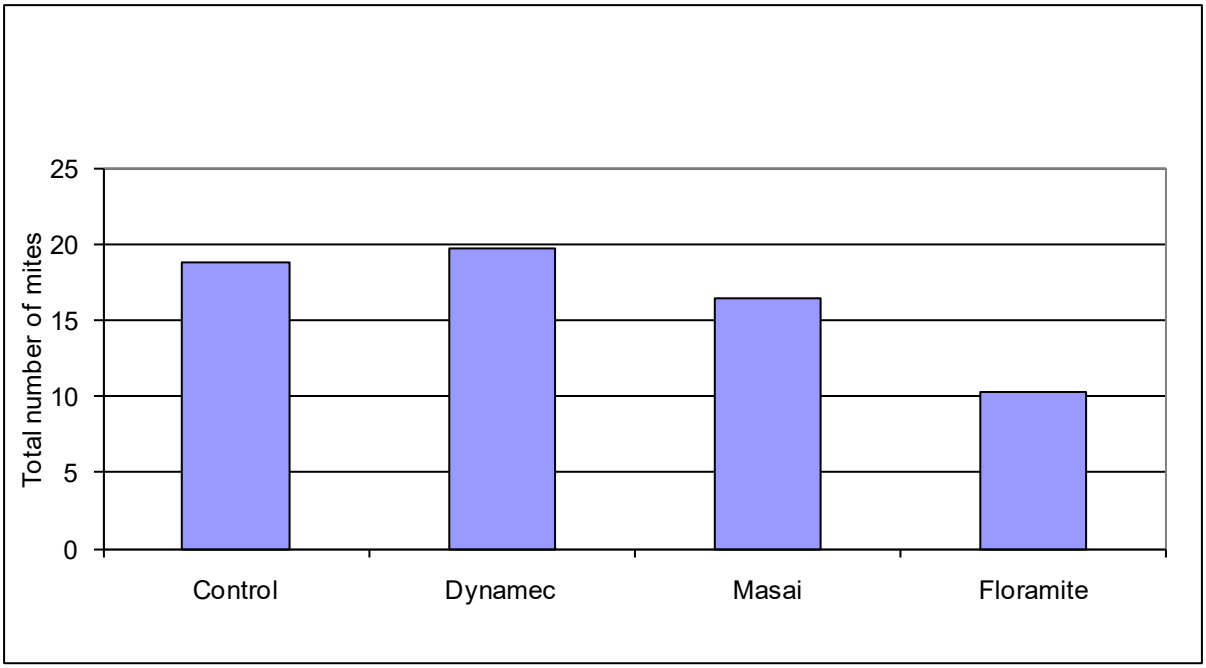

Figure 3.8. The total number of mites per shoot from bulbs treated with foliar sprays (Trial 3, 2009).

Objective 4: Design novel biological control strategies and test these as part of an integrated management strategy in commercial crops

Biological control could provide an alternative control strategy. An initial literature review was done to identify potential biological control agents. The predatory mite *Hypoaspis aculeifer* (Canestrini) has demonstrated an ability to suppress the bulb mite, *Rhizoglyphus robini* Claparède on lilies in laboratory trials (Lesna, Sabelis & Conijn, 1996). A limiting factor in the study was the ability of the predatory mite to penetrate between the bulb-scales, where *R. robini* sought refuge. Bulb-scale mite, *Steneotarsonemus laticeps,* is a substantially smaller mite, penetrating deep within bulbs, therefore the potential success of any predator will depend on its ability to reach them. A recent study to identify predators of bulb-scale mite on *Hippeastrum* identified a number of suitable candidates (Messelink & van Holstein-Saj, 2006). They found that predation efficacy was correlated with predator body size and concluded that the best candidate was the small mite *Neoseiulus barkeri* Hughes. This species would be a good candidate to test against bulb-scale mite in this project. Microbes, on the other hand, might provide a more feasible control strategy, since, for example, fungal-infected, but not yet dead mites, could transmit infections to the bulb-scale mite population in the scales, which might be unreachable by predators.

Objective 5: Examine the link between bulb-scale mite and smoulder disease

2006

It has been reported that bulb-scale mite infestations increase the incidence of smoulder, and this was investigated in a series of tests, starting with a field trial planted in 2006 at the Kirton Research Centre. In spring 2007 three replicate plots were (1) inoculated with smoulder debris, (2) inoculated with smoulder debris and bulb-scale mite debris/bulbs and (3) left untreated as controls. The trial was left down for two years and was assessed for the incidence and severity of smoulder and bulb-scale mite symptoms each year. Only very occasional symptoms were observed, and, because the inoculation methods appeared to be ineffective, it was decided to carry out further work on pot-grown bulbs.

2007-8

On 21 September 2007 groups of nine test bulbs of 'Golden Harvest' (previously treated with standard HWT) were placed alone or with five mite-infested bulbs (<8 cm grade) in food-quality brown paper bags (317 x 305mm). Twenty bags were set up with mite-infested bulbs and 20 with test bulbs only. The top of each bag was folded over and the bags were placed in a controlled-temperature store at 17°C for 4 weeks. On 19 October 2007 the bulbs from each bag were planted in pots, with nine test bulbs around the edge of the pot and (where appropriate) five mite-infested bulbs in the centre. As described above, the pots were placed in a cold store and on 22 February 2008, five smoulder-infected leaves were placed among the shoots in each pot of the appropriate treatments and all pots were lightly enclosed in a clear polythene bag. The pots were then moved to the glasshouse, after which the growing medium and shoot surfaces in each pot were sprayed with water and the polythene bags closed for 3 days before the bags were removed. The plants were assessed for foliar symptoms through the growing season, after which they were moved to an unheated mesh tunnel and bulbs bisected and examined in November 2008. In both cases no clear smoulder symptoms, and only a very low incidence of bulb-scale mite symptoms, were observed. Because of the ineffectiveness of the earlier treatments, a different approach was used in 2008-2009.

2008-9

Fifty pots each of the infested 'Dutch Master' stock and 50 of the HT-HWT 'Golden Harvest' stock, five bulbs per pot, were planted on 23 September 2008 and placed in a 9°C cold store. The plants were transferred to Wellesbourne in early February 2009, where they were grown-on in an unheated glasshouse. Examined in March 2009, few symptoms of bulb-scale

mite, and no symptoms of smoulder symptoms, were seen, and the plants will be assessed again later in the season. Bulb-scale mite symptoms and any interaction between symptom frequency will be examined.

Summary

Objective 1 Define the relationship between temperature and bulb scale mite development.

The work in Objective 1 was re-aligned at the end of 2008 because the way populations cycle under natural conditions was not fully understood. Some effort had already been devoted to monitoring populations in potted bulbs maintained outdoors and this effort was transferred to monitoring a stock of Dutch Master infested with bulb scale mite that was planted at Wellesbourne. This is giving a clearer impression of how mite numbers change during the year and where the mites are found.

Objective 2 Discover when, where and how bulb scale mite originates and spreads in field crops and in bulb storage.

The spatial distribution of mite-damaged bulbs in commercial crops has been investigated. All crops have shown some evidence of mite damage, although in some cases levels were very low. On average, there was more damage in the south-west than eastern England and in 2009, sampling was focused in the south-west. Mite damage appears to occur in 'patches' and the size of these patches suggests that mites could be moving out from a central infested bulb - usually the patches are 1-2 m wide. The 'trough' experiments with clean and infested bulbs planted at different distances also indicate that mite movement occurs most when the bulbs are close and the foliage touches.

Weeds and naturalized narcissus have been sampled to determine whether they could be sources of mites. Nothing was found on the weeds but naturalised bulbs do act as reservoirs for bulbs scale mite and bulb mite, although no bulb scale mites were found in Lincolnshire. Bulb handling units have been sampled to determine whether mites could be transferred to 'clean' bulbs on dust and waste material. In Year 1 (2007) there were indications that this could be happening, not confirmed in Year 2 (2008) and so this is being repeated in 2009. Overall, the evidence suggests that growers may be re-planting infested

bulbs. In 2009, growers provided samples of hot-water treated bulbs to determine whether this is the case.

Objective 3 Design optimal high or low temperature and/or chemical treatments to control bulb scale mite in bulbs for replanting and for forcing, and ensure all stages in its life-history are killed and that crop quality is unaffected.

The study has confirmed that 'optimal' hot water treatment kills bulb scale mite. Warm storage treatment has not been effective to date - but a wider temperature range has been explored in 2009-10 storage treatments. 'Frosting' has not controlled mites. Of the acaricides applied to narcissus foliage, the most promising appears to be Floramite (bifenazate) but it still does not provide a very good level of control. There appears to be no suitable chemical available to treat bulbs in store by fogging. Hot water treatment on an experimental scale appeared to be effective.

Objective 4 Design novel biological control strategies and test these as part of an integrated management strategy in commercial crops.

Potential control agents are to be tested in summer 2010.

Objective 5 Examine the link between bulb scale mite and smoulder disease.

Symptoms have been too slight to draw conclusions but further development of the plants from the experiment is being followed.

Objective 6 Deliver a prototype, improved programme for bulb scale mite control.

If infested bulbs are being planted then it appears that management and treatment between lifting and planting may be the key.

Technology transfer

An article about the project was published in HDC News in March 2009.

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