CENTRAL SCIENCE LABORATORY

Report to: Dr E Moorhouse
Horticulture Development Council
18 Lavant Street
Petersfield
Hants GU32 3EW

CSL Contract Manager: Miss S M Lynch
Central Science Laboratory
London Road
Slough
Berks SL3 7HJ

Date of issue of report: 20 May 1994

No. of pages in report: 28 including 1 table & 9 Figures

This is CSL copy no. 2 Issued to:

Report for CSL
Contract No. CC06077

The development of an *in vitro* method for culturing the bulb scale mite (*Steneotarsonemus laticeps* Halbert) and its use for life history studies.

PRINCIPAL WORKERS

Sophie M Lynch, Senior Acarologist

Aju Bedi, BSc MSc MPhil

AUTHENTICATION

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

..... Contract Manager

Signature

Date.....20/5/94.....

Report authorised by: .....

Date: .....24/5/94.....

CONTENTS

THE DEVELOPMENT OF AN *IN VITRO* METHOD FOR CULTURING THE BULB SCALE MITE (*STENEOTARSONEMUS LATICEPS* HALBERT) AND ITS USE FOR LIFE HISTORY STUDIES

S M T Lynch and A Bedi

Introduction

The Bulb Scale Mite (*Steneotarsonemus laticeps* (Halbert 1923)) has long been recognised as a potentially serious pest of the bulb industry. The first record of *S. laticeps* probably dates back to 1892 (Michael, 1892) although it was not actually described and named until 1923. Heavy infestations were observed in North America from 1925 onwards and were associated with Narcissus bulbs imported from Holland (Doucette, 1929). Subsequently it appears to have become a major problem in cultivated Narcissus because the conditions under which the bulbs are grown, in order to achieve the maximum output of flowers and bulbs, would appear to favour the rapid multiplication of the mite population within the bulb. Infested bulbs which are forced exhibit the most serious and obvious signs of damage, with a considerable reduction in yield and quality of flowers. Immediately bulbs infested with *S. laticeps* are subjected to the abnormally high temperatures used during forcing, a rapid increase in the mite populations occurs. Infested bulbs grown without forcing do not usually display a high level of infestation or damage. For bulbs grown in the open there may, however, be a long term cumulative effect. Hodson (1934) reported that cold, wet weather during May and June hampers the increase of *S. laticeps*, but warm, dry weather during this period results in an extremely rapid increase in the populations.

In the United Kingdom during 1989 and 1990 there was a greater incidence of rejections, by the PHSI, of bulbs for export because of *S. laticeps* infestation. In

1990 2.6% of stocks were rejected for this reason (PHSI figures) at an estimated cost of £75,000. Presumably the infested stocks had to be replaced and the infested crop itself had to be discarded or suffered a considerable drop in value. The actual problem during these years may have been greater than this, in that stocks known by the grower to be infested would presumably not have been presented for export. The reason for the sudden upsurge of infestations during this period is not known but a combination of factors may have been involved. These might be, for example, a failure to detect initial low levels of infestation combined with the temperature and humidity conditions needed for the rapid increase of the mite. There is very little known about the effects of physical conditions such as temperature and humidity on *S. laticeps* or its population growth. Beer (1954) suggested that optimum conditions for the various species of Tarsonemid mite studied involved a combination of warm temperature, high humidity and low light intensity.

The most detailed observations on the life history were made by Blattney (1933) and Hodson (1934) and these have been summarised in a review by Lynch (1993). The studies made by Hodson (1934) were the more detailed, but were hampered by the lack of a suitable culturing method and controlled conditions under which to make observations. Hodson's initial attempts to monitor the life history were by using either bulb scales isolated in glass cells or by applying glass cells containing mites to the outside of skinned bulbs or bulb foliage. These initial methods were unsuccessful, and for his most successful observations he isolated single female mites on very small, skinned, dormant bulbs. Even with this latter method the data obtained were minimal, and the observations made under uncontrolled and fluctuating conditions.

There appear to have been no subsequent published observations on the biology and physical requirements of *S. laticeps* and so very little is known about the biology of this important pest. In order to facilitate control of *S. laticeps* it is evident that

further information is required on the factors that control its survival and breeding rates. In particular, information is needed on the effects of low temperatures in depressing reproduction and on the determination of the maximum reproductive potential at higher temperatures. Such information would be essential when considering the development of physical control methods during storage and might explain why *S. laticeps* is a problem in certain years on Narcissus grown in the open. The establishment of an upper and lower lethal temperature would also be of benefit. Fox Wilson (1939) quoted an upper thermal death point of between 38°C and 49°C but did not say how this was obtained; it is possible that he was referring to the temperatures used during the hot water treatment of bulbs. There would, in any case, appear to be no substantial evidence in the literature for the choice of the temperature used in the hot water method of treating bulbs to control *S. laticeps* infestation.

The reason that there is such limited specific information for *S. laticeps*, is because of the special difficulties involved in making observations on mites which live principally between the fleshy scales within the bulb, and would, therefore, not normally be visible during their reproductive cycles.

In order to make accurate observations on the biology and effects of various physical conditions it is necessary to develop an *in vitro* method of culturing *S. laticeps*. Such a method would need to yield reliable supplies of mites of known ages and stages which could be used in experimental work. Unfortunately the living bulb does not make an easy or consistent medium on which to culture mites for laboratory investigation. A further complication is that any food medium used needs to account of the fact that the mites have piercing mouthparts which are used to feed through the epidermal layer. Other bulb mite pests (e.g. *Rhizoglyphus robini* Claparède) and other species of this genus have more adaptable mouthparts and are much easier to culture on a different range of substrates or food media away from

the actual bulb. Although, during the growing and flowering season, *S laticeps* spends time outside the bulb on the aerial part of the plant it would be reasonable to presume that because of its adaptation to living a large amount of its life history within the bulb, it probably has a much lower tolerance to fluctuations in humidity. This again would make it more sensitive to experimental procedures which do not take this into account. A successful culturing technique would therefore need to provide a high humidity, irrespective of temperature, and to address the problem of the specialised feeding requirements. Following on from the development of a successful culturing method it would be necessary to find a method of isolating individuals at all stages in the life cycle so that observations on development time and longevity could be made at various temperatures and humidities, and in the presence or absence of biocides. The present study was undertaken to overcome these difficulties and to develop an *in vitro* culturing method representing, as nearly as possible, conditions within the normal habitat of the mite (i.e. the bulb) and which could be further developed so that observations on the effect of physical conditions, and especially temperature, on the development of *S. laticeps* could be made.

Materials and Methods

Four different methods were tried and these included (Methods 1 and 2) variations of the methods used by Hodson (1934). The details of each method are given below. In all the experiments the food medium was obtained from non-infested bulbs ('Golden Harvest' cultivar of Narcissus, supplied by HRI Kirton). To ensure that the bulbs were not infested a random selection were cut open, the scales separated and checked under a light microscope. Although in some instances other mites, *Rhizoglyphus robini* and *Tyrophagus putrescentiae* (Schrank), were found on the outside of some of the bulbs, no *S. laticeps* were found either on the outside or within these non-infested bulbs. The mites used for the first two methods were

obtained from infested stock of the Narcissus cultivar 'Texas' supplied by HRI Kirton. The mites were either picked out from between dissected bulb scales by hand using mounted needles or a single haired artists' paint brush, or were washed out of the bulbs with distilled water and filtered on to black filter paper (Schleicher & Schull nr 551, 100 mm) using a Buchner Funnel. Approximately twenty mites, from each bulb used, were mounted on microscope slides and their identity confirmed as *S. laticeps*. In the course of the study it became increasingly difficult to obtain sufficient mites from the 'Texas' bulbs and an alternative supply was sought. The mites used for methods three and four were obtained from infested Narcissus bulbs of the cultivars 'Ice King' and 'St Patricks Day' purchased from a local garden centre. These bulbs were heavily infested and displayed all the classic symptoms of an *S. laticeps* infestation. When viewed under a microscope large populations of mites in all stages were found and could be picked-off easily for the experimental work. The identity of these mites was checked as before.

Method 1

Bulbs were separated into scales and individual scales were isolated in 50mm diameter perspex cells cut into 70mm perspex squares. The cells were 3 mm deep. Black filter paper (Schleicher & Schull nr 551) formed the floor of the cell which had gently sloping sides. The cut ends of the scales were sealed with wax (BDH Paraffin wax pellets pastillated, congealing point 57-60°C) using an artists` paint brush and the whole scale was held in position in the centre of the floor of the cell by wax painted right round the edges of the scale in contact with the cell floor (see Figure 1). Twenty to thirty mites were placed on to each scale and the cell covered with a glass square held in place by "Bulldog" clips at each corner. Twenty cells were set-up, ten with mites and ten without. Five cells with mites and five without were placed in a desiccator on a platform over distilled water. The remaining cells were placed in a desiccator on a platform over a potassium hydroxide solution of a

density of 1.108 g/cm2 giving an equilibrium relative humidity of 90%. The desiccators were kept in the dark in a constant temperature and humidity room maintained at 20°C. They were removed, and the cells observed under a light microscope, on a daily basis.

Results

Within two days the mites had abandoned the scales and were all observed shrivelled and dead around the perimeter of the cell. No eggs had been laid. The cells were retained for a further two weeks, in order to monitor the condition of the scales and any signs of deterioration (i.e. drying out, fungal growth). At the end of this time the cells containing the dead mites had fungal growth, but those without mites showed no apparent evidence of fungal growth or deterioration.

Method 2

Small, shallow perspex cells 20 mm in diameter and 8 mm deep were attached to the sides of skinned bulbs by means of a wax seal around the base of the cell. The cell was sealed by a perspex cover with a tiny, 2 mm, central hole. Using a single haired artists' brush, twenty mites were introduced into each cell through the hole in the cover. A coverslip was then placed over the hole and held in place with vacuum grease (Apiezon AP101 grease). Two cells were placed on each of four bulbs. The bulbs were then stood upright on damp filter paper in crystallising dishes. The bulbs and dishes were placed in a propagator and kept in the dark in a CTH room at 20°C and 60% RH for two weeks during which time they were removed for daily observations.

Results

Most of the cells contained eggs between the second and sixth day and there was evidence of feeding damage. However, as in Hodson's experiments (Hodson, 1934) there were considerable problems with condensation inside the cells and this trapped many of the mites and eggs in water droplets, causing high mortality and fungal growth. Mites on the sides of the cells were also difficult to see and count. The bulbs themselves showed evidence of sprouting and in some cases the outer skinned surfaces became discoloured, brown and oxidised. The method was, therefore, abandoned.

Method 3

Bulbs were separated into individual scales and the cut ends were sealed with wax. Each scale was gently flattened and sandwiched between a glass microscope slide and a black perspex strip 50 mm long and 25 mm wide. Each perspex strip contained, offset to one side, a hole 18 mm in diameter and 3 mm deep, with gently sloping slides. This hole formed a cell, the floor of which was made by the bulb scale (Figure 2). The join between the base of the perspex cell walls and the floor was sealed with wax to prevent the mites escaping, leaving a central area of bulb scale on which the mites could live and feed. The edges of the perspex and glass 'sandwich' were also sealed with wax (Figure 3). Approximately twenty mites were placed in each cell and the cells were sealed using a glass microscope slide held at each corner with a "Bulldog " clip (Figure 4). Ten cells containing mites were placed in a desiccator containing potassium hydroxide solution giving an equilibrium relative humidity of 90%. Ten cells without mites were placed under identical conditions in another desiccator. The desiccators were kept in the dark in a CTH room maintained at 20°C and removed daily for observations.

Results

The cells containing no mites were examined for signs of bulb scale deterioration. The scales remained in good condition, with no apparent signs of deterioration, for six to eight weeks.

Of the cells containing mites, four showed signs of fungal growth and were discarded. In the remaining six cells there was evidence of feeding lesions (Figure 5) and the mites appeared to be healthy. After nine days there were 50+ eggs laid per cell and at this time the original females were removed. Numerous larvae appeared from day 11 onwards and by day 25 most had become adults. These F1 adults were allowed to lay eggs before they, in turn, were removed and development of the eggs followed through to the F2 generation. At this time (55 days) the F2 females were moved into new cells as the original scales were showing multiple feeding lesions and signs of deterioration. The observations were carried on until the F4 generation appeared, after which time the cultures were allowed to continue without removing the new females. The entire cultures were transferred to new cells as the bulb scales deteriorated, approximately every 6-8 weeks.

Method 4

For this method the mites were placed on agar to which macerated or whole bulb scales were added.

20 g of no. 3 agar (Unipath) was dissolved in 1 litre of sterile distilled water (distilled water autoclaved at 121°C for 15 minutes) and placed in a 650 watt microwave oven set at high for 15 minutes. The agar solution was then divided into two separate 500 ml portions.

One ampoule of the antibiotic chlorophenicol was mixed with 3 ml of acetone and added to one of the portions of agar. This portion was then divided into five bottles each containing 100 ml of agar + chlorophenicol solution. The second portion of agar was also divided equally between five 100 ml bottles. All ten bottles were then autoclaved at 121°C for one hour. Narcissus bulbs were placed in beakers which were then covered with greaseproof paper and were also autoclaved at 121°C for one hour.

250 g of the anti-fungal agent "Nystatin" was mixed with 100 ml of sterile distilled water. This "Nystatin" solution, the bottles of agar solution and the bulbs were then refrigerated until needed for the experimental work.

Six agar plates were prepared, two using the agar only solution and four using the agar + chlorophenicol solution. To prepare the plates the 100 ml bottles of agar solution were placed in a boiling water bath for 20 minutes, allowed to cool to between 40-45°C and then placed in a water bath at 50°C before pouring the agar onto individual, sterile, lidded 90 mm diameter petri dishes to a depth of between 5-8 mm. At the same time 28 g of the autoclaved bulbs was macerated in a 'stomacher' for 2 minutes with 28 ml of sterile distilled water to form a thick paste. A further 28 ml of sterile distilled water was then mixed-in to produce a thick liquid. The six plates were then prepared as follows:

Plate 1, 4 ml of the macerated bulb liquid was pipetted onto the plate, followed quickly by 100 ml of warm agar. The agar and liquid were stirred rapidly, the lid was added to the plate which was then allowed to cool.

Plate 2, The same procedure was followed using 100 ml of the agar + chlorophenicol solution.

Plate 3, As plate 2, but 1 ml of the "Nystatin" solution was added to the agar + chlorophenicol solution just before pouring.

Plate 4, 100 ml of warmed agar only solution was poured onto a plate and about six individual unsterilised bulb scales (convex side up and with cut ends unwaxed) were positioned at random on the surface of the agar. The agar was then allowed to cool and set.

Plate 5, As for plate 4, but using agar + chlorophenicol solution to which 1 ml of "Nystatin" solution had been added .

Plate 6, As for plate 5, but in this case the cut ends of the bulb scales were sealed with wax and the scales placed touching each other on the surface of the agar. Some scales were placed convex side up and others concave side up (Figure 6). Six 9 mm diameter holes were drilled in the lid of this plate. The holes were sealed with circles of filter paper (Whatman No. 1) held down with micropore tape (Boots) (Figure 7).

All six plates were then refrigerated.

The following day 20-30 mites were placed in each plate and the sides of the plate resealed with micropore tape in order to prevent the mites escaping. The mites placed in plate 6 were first washed in a 50% ethanol/sterile distilled water solution. All six plates were kept in the dark in an incubator at 20°C and removed daily for observations.

Results

Plates 1 - 4 had considerable amounts of condensation and soon became completely covered with fungal growth. All the mites died and the plates were discarded after 12 days.

Plate 5, again there were problems with condensation, although not as much as in plates 1 - 4. Fungal growth was most prevalent on the agar between the scales but not on the scales themselves. By day 14 some of the scales had started to oxidise quite badly. By day 16, however, eggs, larvae and resting larvae or "pupae" (see Lynch, 1993) were all present together with the original adults. The resting larvae showed signs, however, of fungal attack and the plates were finally discarded on day 20.

Plate 6 had no condensation and there was very little fungus present. However, by day 10 the bulb scales had begun to oxidise; those which were placed concave side up deteriorating much more than those placed convex side up. 40+ eggs were present on day 10, together with many of the original females and 34 larvae. By day 17 resting larvae were present, but by day 22 the scales were very badly oxidised and the plate was discarded.

In all of the plates the mites appeared to have some difficulty in walking on the agar, which was perhaps too soft.

Further development of Method 3

Because the third method appeared to be successful in maintaining several generations of *S. laticeps*, an attempt was made to develop the method further for some preliminary life history observations. The same experimental procedure was

followed using identical perspex strips, but this time containing two equidistant cells 10 mm in diameter and 1.5 mm deep (Figures 8 & 9). All the mites for this experiment were taken from the infested "St Patricks Day" cultivar bulbs. The non-infested bulb scales were again taken from the "Golden Harvest" cultivar supplied by Kirton HRI.

Five female mites of unknown age were placed in each of one of the cells in each strip and allowed to lay a "countable" number of eggs (maximum 50) before being moved to the second cell in the strip where they could lay further eggs. The cells were placed in desiccators in CTH rooms maintained at five temperatures. All were kept at 90% relative humidity. The temperatures used were 10°C, 15°C, 20°C, 25°C and 30°C. Initially seven cells were set up at each temperature, but because of high mortality at the two highest temperatures, a further five cells were set up at 25°C and ten cells at 30°C. Cells without mites were also set-up in order to observe any deterioration of the bulb scales in the absence of mites. Observations were made on a daily basis.

Results

Table 1 shows the mean number of eggs laid per cell at each temperature, the maximum observed percentage hatch, the minimum time taken for the first adult progeny to appear from the time the first egg was observed, and the maximum observed longevity of the female parents.

No adult progeny were produced at the two highest temperatures, 25°C and 30°C. At 30°C a total of six eggs was produced in seventeen cells and none hatched. At 25°C eggs were laid (see Table 1) but few (7%) hatched, and none reached the resting larva stage. There was also a problem with wax lifting away from the bulb

at 25°C and 30°C, possibly because of the temperature. The bulb scales at these temperatures appeared enlarged and dry in both the cells with and without mites.

At 10°C the bulb scale in one of the cells became badly oxidised at an early stage and the cell was discarded. In four of the remaining six cells a total of 17 eggs were produced but only four (24%) hatched and no adult progeny were produced. In the two remaining cells 76 eggs were produced and 55 (72%) hatched. Adult progeny were produced in both of these cells.

At both 15°C and 20°C eggs were produced in all the cells, and in sufficient numbers that, for each, a duplicate cell had to be set-up. Five of the seven cells kept at 15°C produced adults, as did four of the seven cells kept at 20°C.

At 10°C, 15°C and 20°C the bulb scale in cells containing mites again showed evidence of feeding lesions. Those without mites remained healthy for 6-8 weeks at 20°C and 8-10 weeks at 10°C and 15°C.

Discussion

1. Culturing

A simple method (method 3) of culturing *S. laticeps* has been developed. The method allows the production of large numbers of progeny, and cultures have been maintained for in excess of four generations. These cultures are still alive at the time of writing. It has also been shown that the method 3 can be further developed for life history studies under varying physical conditions and some preliminary results have been obtained. A second method (method 4, Plate 6) may also be feasible if problems with fungal contamination and oxidation of the bulb scales can be overcome.

Culture method 3 allows populations of *S. laticeps* to be reared on a continuous basis using their natural food medium and, to some extent, mimics their natural habitat within the bulb scales. It provides them with their natural egg laying and breeding surface, although the air space above the mites in the culture cells would probably not be so large as between scales in the bulb. The glass cover, *in lieu* of an opposing scale, would also not allow the retention or availability of such a high relative humidity as living bulb material. Despite this the method has been proven successful over several generations, although it could, no doubt, be further improved. It does allow all of the mites present in the culture to be observed at one time and cultures can be reared in the same cells for 6-8 weeks at 20°C, and for 2-3 weeks longer at 15°C, before being transferred to new cells. Further development of the method would involve keeping cultures at various combinations of temperature, humidity and light intensity to find which would give the best productivity.

Hodson`s (1934) attempts were inconsistent probably because of the fluctuating and uncontrolled conditions under which they were carried out. The use of whole bulbs, albeit ones that were small, must have made accurate observations extremely difficult, if not impossible, because of the large and varied surface over which the mites could spread and remain concealed.

2. Life History

The results shown in Table 1 suggest that, taking into account the mean number of eggs laid, the percentage hatch and the adult longevity, the optimum temperature for development of these mites is around 20°C, and that at 25°C and above no development takes place. The results at 10°C suggest some heterogeneity in response, with some females being able to reproduce and others not. At 15°C and 20°C the life history was completed more quickly than the 49 days recorded by

Hodson (1934). At all temperatures the maximum observed adult longevity was equal to or longer than that found by Hodson (1934). As the adults were of unknown age at the beginning of the experiments the actual longevity will be longer than that observed. The same argument can be applied to Hodson's observations. Hodson (1934) did not observe males until at least the third generation. In these experiments numerous males were observed from the first generation onwards.

Although these results are interesting and confirm what Hodson (1934) suspected, that the life history could be considerably shortened at higher temperatures, and therefore during the forcing process, they should not be used as a definitive set of values for *S. laticeps*. The present work should be considered a feasibility study. There were insufficient replicates for a complete life history study and further work needs to be carried out in order to confirm and improve on the results obtained to date.

In particular the results at higher temperatures need further investigation. The higher mortalities and failure to complete the life cycle may be the result of failure to maintain a sufficiently high relative humidity or they may be a function of the effect of the higher temperatures on the bulb scale tissue. Although large numbers of eggs can be laid by some females and the life cycle completed at 10°C, further data is needed at this and at lower temperatures which would be relevant to the prevention of a build up of infestation during storage.

The development of a successful culturing method opens up the possibility of looking at the effects of insecticides, fumigants and hot water treatment on *S. laticeps*. Mackie *et al* (1942) claimed that methyl bromide treatment would kill *S. laticeps* at temperatures between 15°C and 21°C. They found that the treatment must be repeated after 10-14 days because the eggs survived. Gurney and Gandy (1974) in their fumigation trials state that no eggs hatched during the fourteen day

period in which the bulbs were dissected after treatment. They found that the mites and eggs killed by the methyl bromide remained plump and healthy looking although microscopic examination showed them to be dead.

Our experiments have shown that the duration of the egg phase can vary between six and forty-nine days. This would mean that the fourteen day criterion for hatching after fumigant treatment is not an appropriate measure of egg viability and subsequent hatching success. The culturing method described here could be used for isolating individuals and eggs after treatment and monitoring their subsequent survival. The same approach could be adopted for the hot water treatment as there appears to be no confirmed evidence proving the efficacy of this method in killing all stages of *S. laticeps*.

Conclusions

1. A technique has been developed which allows *S. laticeps* (the bulb scale mite) to be cultured and observed for a number of generations on their natural food medium, bulb scales. This provides the potential for producing large numbers of known age mites for studies on physical, chemical and biological control.

2. The culturing technique has been modified to allow detailed studies of the life history of the bulb scale mite at different temperatures.

3. Preliminary results indicate an optimum temperature for development from egg to adult of around 20°C. Following harvesting in July, narcissus bulbs are frequently stored outdoors for a considerable period (2-3 months). External ambient temperatures during this period, can in some years be quite high (18°C). The results demonstrate that these storage temperatures are approaching ideal conditions for the rapid build up of *S. laticeps* populations.

4. The results indicate that even at 10°C, large numbers of eggs can be laid and the life cycle completed. Bulbs for forcing which may have already been stored outside at near optimum conditions are "vernalised" by placing them at less than 9°C for 9 weeks prior to forcing. The results indicate that not only is there the potential for a large build up of the infestation during the outside storage period but also for a continued slow but steady increase and development of this population during "vernalisation".

Depending on the market, forcing occurs over a range of times (2-6 weeks) and temperatures which can be as high as 21°C. Our work demonstrates that this could result in a further large increase in an already rapidly increasing infestation.

5. Our results show a very high mortality and cessation of development at around 25°C. Bulbs which are simply to be replanted outside are usually hot water treated at temperatures between 43-44°C. This often results in damage to the bulbs. It would appear that such high temperatures may not be necessary to control *S. laticeps* although the hot water treatment is also used to control other organisms.

6 Preliminary results also indicate a considerable variation in the duration of the egg period prior to hatching, the highest being 49 days.

In previous fumigation experiments, bulbs have been examined for egg hatch 10-14 days after treatment and the treatment considered successful if none was found. It has also been suggested that treatments are repeated after 14 days to ensure complete control of bulb scale mite, this being considered the limit of duration of the egg stage. Our results show clearly that this is not necessarily true, and that there may still be the potential for build up of infestation long after this period.

In conclusion the development of the culturing technique has made the investigation of a number of uncertain areas possible and could provide important information on the apparent failure of control measures in the past.

Future Research Areas

1. Establishment of more accurate data on the effects of temperature on the life history, particularly at the lower and higher temperatures, would provide essential information which could be applied to prevent and possibly even eradicate build up of infestations during storage, forcing and the growing season.

Additional investigations into the effect of light and humidity as a contributory factor to build up of infestations would be of further benefit.

2. Investigations into the potential of *S. laticeps* to survive fumigation and hot water treatments. It is now possible to remove and incubate eggs after such treatments in order to assess their success or failure. Previous problems of "reinfestation" may in fact have been simply due to failure or possibly incomplete success of these treatments and the consequent build up of the resulting residual infestation. This work now makes it possible to test the success of such treatments and could provide important information on the apparent failure of control measures in the past.

3. Observations during these trials suggest that *S. laticeps* can move rapidly over a considerable distance. This would appear to contradict some of the previous observations and has implications for the spread of the mite. Little is known about this subject although it has been conjectured that the mites spread from plant to plant over the foliage during the growing season and over the mat of wilting foliage at the end of the season. The reasons for such migrations are obscure as also are

the ways in which apparently "clean" bulbs become infested. There is also no information on frequency of occurrence of infestation on different cultivars or whether indeed some cultivars are more susceptible to infestation than others. Although *S. laticeps* is known to infest Amaryllis bulbs and other members of the Amaryllidacidae, it does appear to have a preference for narcissus. Feeding trials on, different cultivars, other bulbs and investigations into the effect of relevant plant volatiles on the mite would add considerably to our knowledge in this area and have possible implications for control. It is also very clear that further trials on method of spread are necessary.

4. Development of a method for detection and quantitative estimation of infestation levels in bulbs. Present methods of detection are dependant on the visual recognition of infestation symptoms. During storage this can be extremely difficult and often symptoms are not recognised until considerable damage has been caused i.e. during the forcing process.

During the course of these experiments preliminary investigations with a flotation technique used for the assessment of mite populations in stored food products, demonstrated the presence of *S. laticeps* in bulbs which appeared to be uninfested. There is a possibility that the method could be modified, not only to detect the presence of bulb scale mite, but to make a quantitative assessment of the infestation. Many of the existing cases of "new" infestations or even in some cases "reinfestation" may in fact be simply a failure to detect small populations of bulb scale mite initially. This may result in lightly infested stocks (presumed mite free) being subjected to conditions, such as forcing, which result in a rapid increase in the infestation level and the development of more obvious symptoms.

Development of an accurate detection and quantitative assessment method, would not only be of benefit in establishing "clean" or mite free bulbs, but would enable

light infestations, which might otherwise go unnoticed, to be detected at an early stage. Used in conjunction with improved knowledge of the effects of physical conditions, such as temperature, on the life history, this would allow the grower to make a more informed choice as to the ultimate use of the bulbs. It would ensure that only non-infested bulbs were used for forcing and that lightly infested bulbs could perhaps be used for planting outside. Bulbs known to be only lightly infested and destined for this purpose could be stored until needed, at a temperature or under conditions which would prevent an increase in the level of infestation. It would also allow the grower to make an early decision on whether bulbs, depending on the degree of infestation, were worth keeping or should be discarded. Such a technique would enable infestations to be monitored during storage, and the growing season. It would also allow for the screening of imported bulbs and indeed those for export. This would save a considerable amount of time and money involved in the process of returning bulbs later found to be infested and therefore unacceptable.

5. At present only endosulfan is available for chemical control of *S. laticeps*. The efficacy of other insecticides/acaracicides should be considered for treatment of both bulbs, foliage and storage areas.

Acknowledgements

We would like to thank Mr B B Thind (CSL) for his help when designing the cells, Mr J Banks (CSL) for his advice on microbiological techniques and Mr G Kelly (3rd year BSc Joint Honours student at Roehampton Institute of Higher Education) for his assistance with setting up the cells and making experimental observations.

References

BEER, R. E., 1954. A revision of the Tarsonemidae of the Western Hemisphere (Order Acarina). *Kansas Univ. Sci. Bull.*, **36**, 1091-1387.

BLATTNEY, C., 1933. On "Red Burn" disease of Amaryllis caused by a mite, *Tarsonemus hydrocephalus* Vitzhum. *Gartenbauwissenschaft*, **7**, 425-437. (seen in English translation).

DOUCETTE, C.F., 1929. A tarsonemid mite attacking Narcissus. *J. econ. Ent.*, **22**, 423-424.

FOX-WILSON, N.D.H., 1939. Contributions from the Wisley Laboratory LXXXVII. Amaryllis pests. *J. Roy. hort. Soc.*, **64**, 318-326.

GURNEY, B. and GANDY, D.G., 1974. Methyl bromide for control of the bulb scale mite, *Steneotarsonemus laticeps* (Halb.). *Pl. Path.*, **23**, 17-19.

HALBERT, J.N., 1923. Notes on Acari, with descriptions of new species. *J. Linn. Soc., Zool.*, **35**, 363-392.

HODSON, W.E.H., 1934. The bionomics of the bulb scale mite, *Tarsonemus approximatus* Banks var. *Narcissi* Ewing. *Bull ent. Res.*, **25**, 177-185.

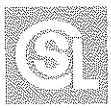
LYNCH, S.M.T., 1993. The bulb scale mite, *Steneotarsonemus laticeps* (Halbert) - a review. *Unpublished report for Horticultural Development Council.*

MACKIE, D.B., STEINWEDEN, J.B. and CARTER, W.B., 1942. Methyl bromide fumigation of Narcissus bulbs for the control bulbs and bulb mites. *Mimeographed Report California department of Agriculture, Sacremento.*

MICHAEL, A. D., 1892. Narcissus bulbs attacked by Acari . *Jl R. hort. Soc.*, **15**, xxvii-xxviii.

Table 1 Life history observations made using Method 3

	10°C	15°C	20°C	25°C	30°C
Mean eggs/cell	15.5	23.9	30.3	13.0	0.4
Maximum % hatch in one cell	82.6	92.8	81.8	27.2	0
Minimum egg to adult time (days)	51	22	15	-	-
Maximum adult longevity (days)	60+	60+	37	31	28



Appendix 1

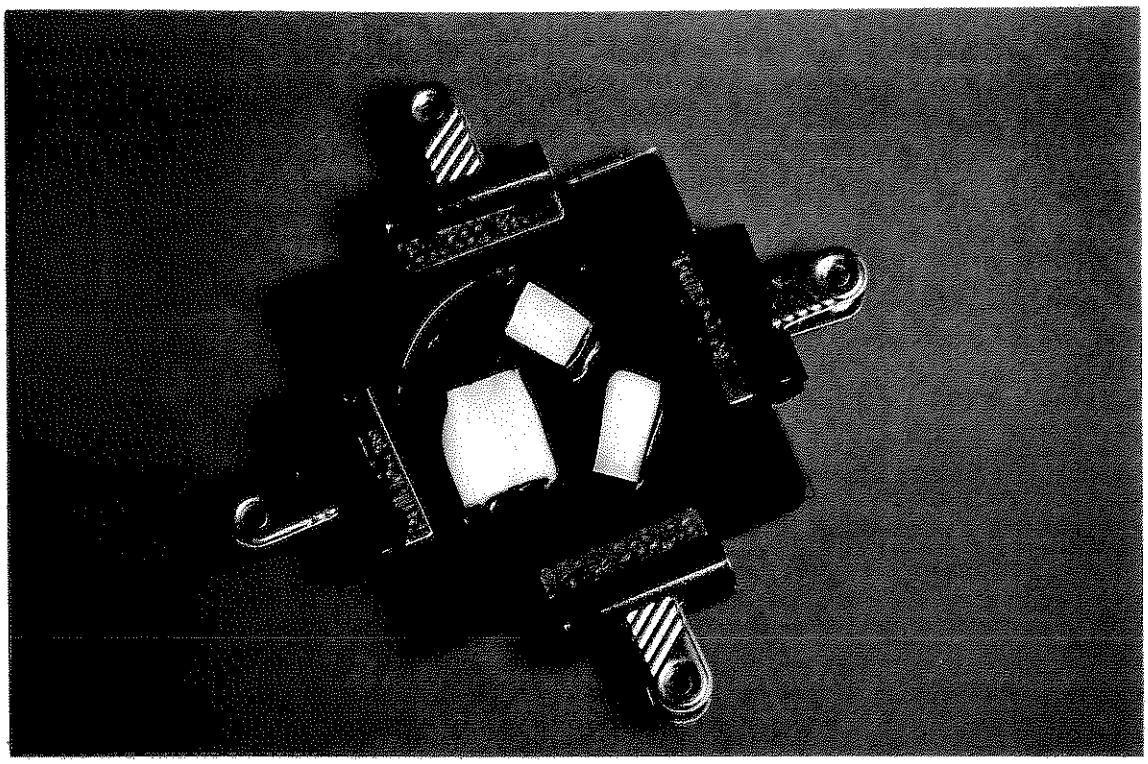


Figure 1. Assembled cell with bulb scale "islands" as used for culturing method 1.

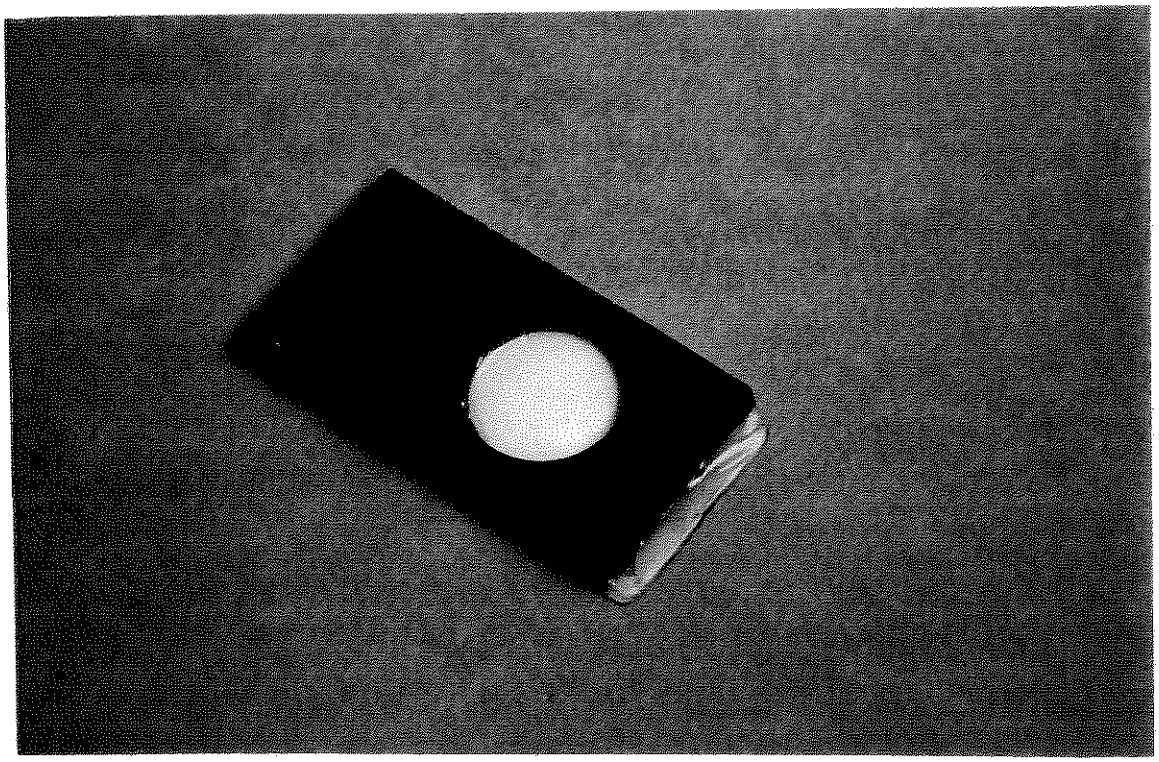


Figure 2. Cell, used for culturing method 3, opened to show bulb scale "floor".

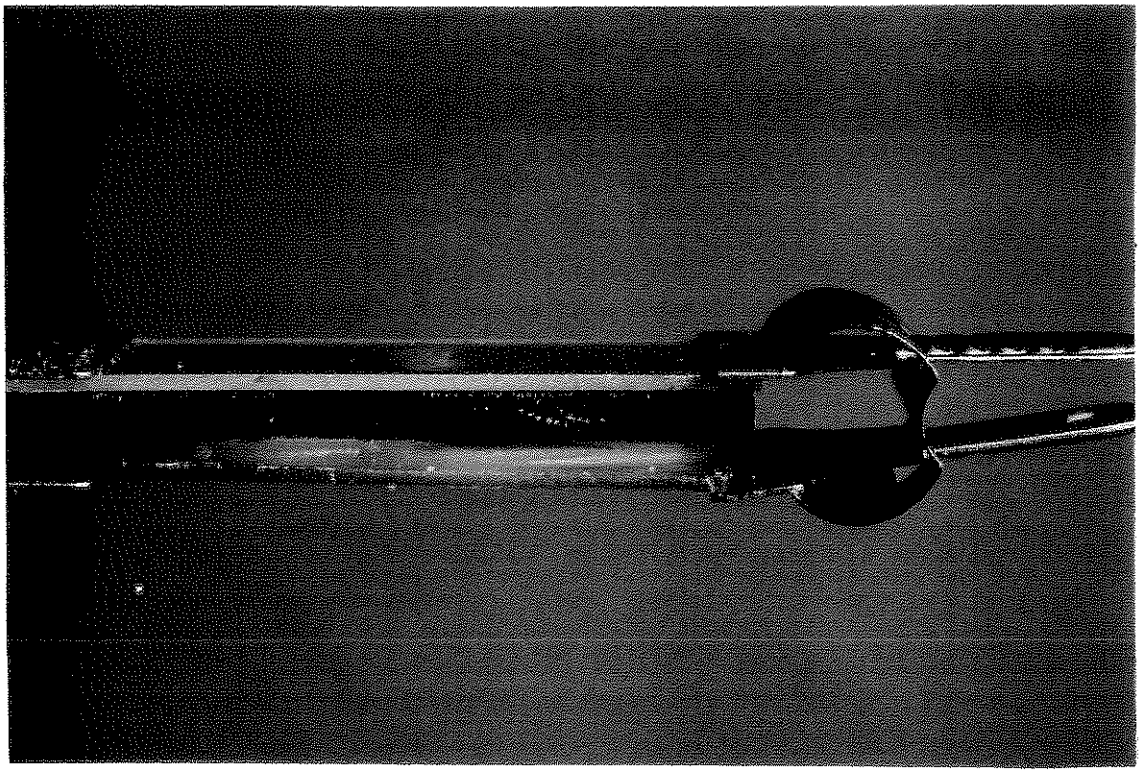


Figure 3. Side view of assembled cell, used for culturing method 3, showing cell edges sealed with wax.

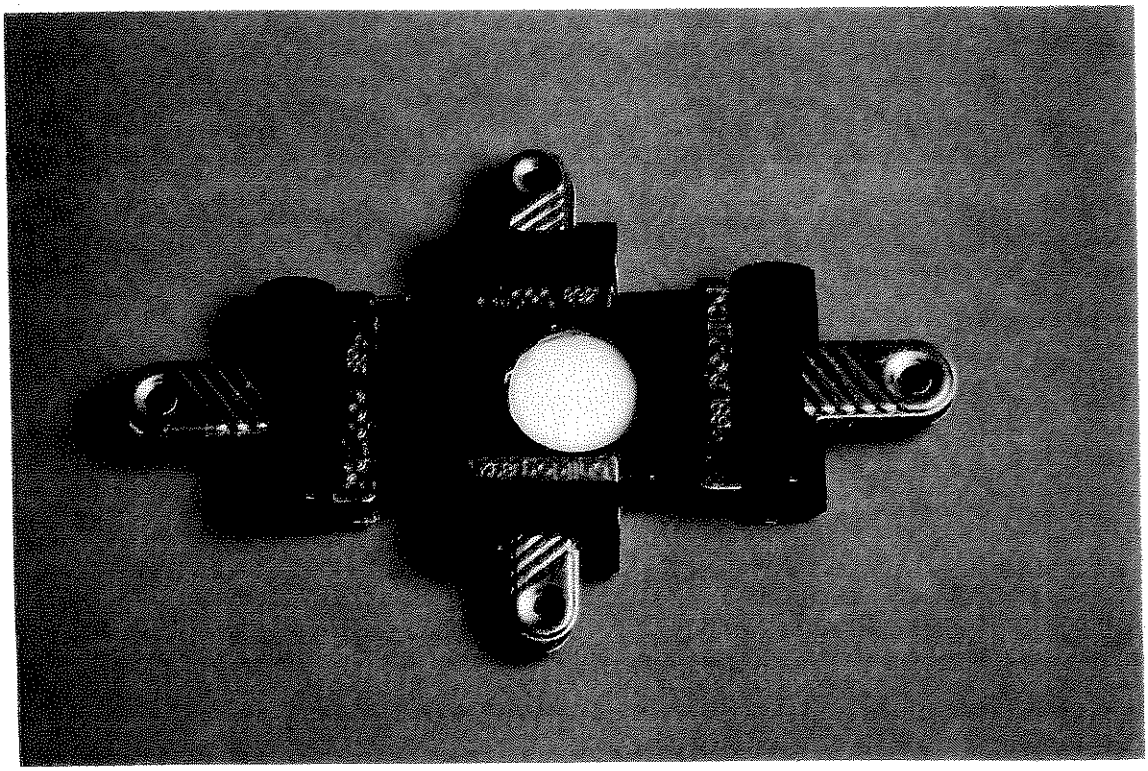


Figure 4. Assembled cell used for culturing method 3.

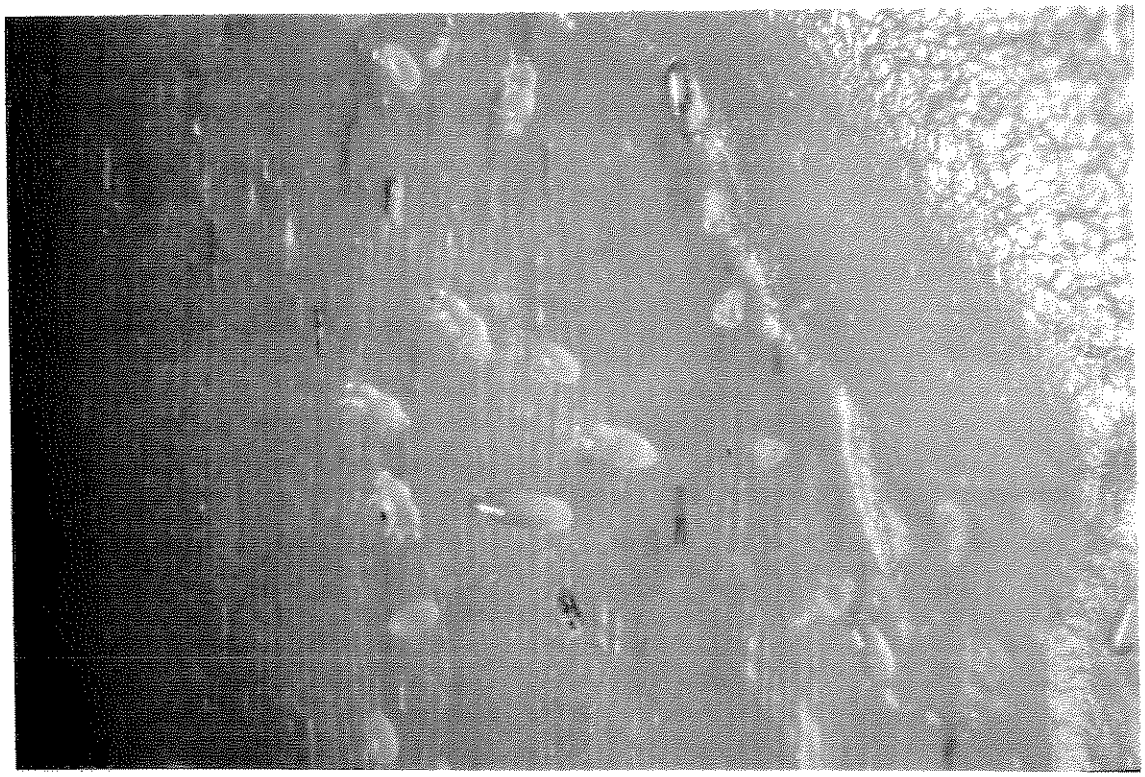


Figure 5. *Steneotarsonemus laticeps* (eggs, larvae and females) on a bulb scale from culturing method 3 - the brownish marks are feeding lesions.

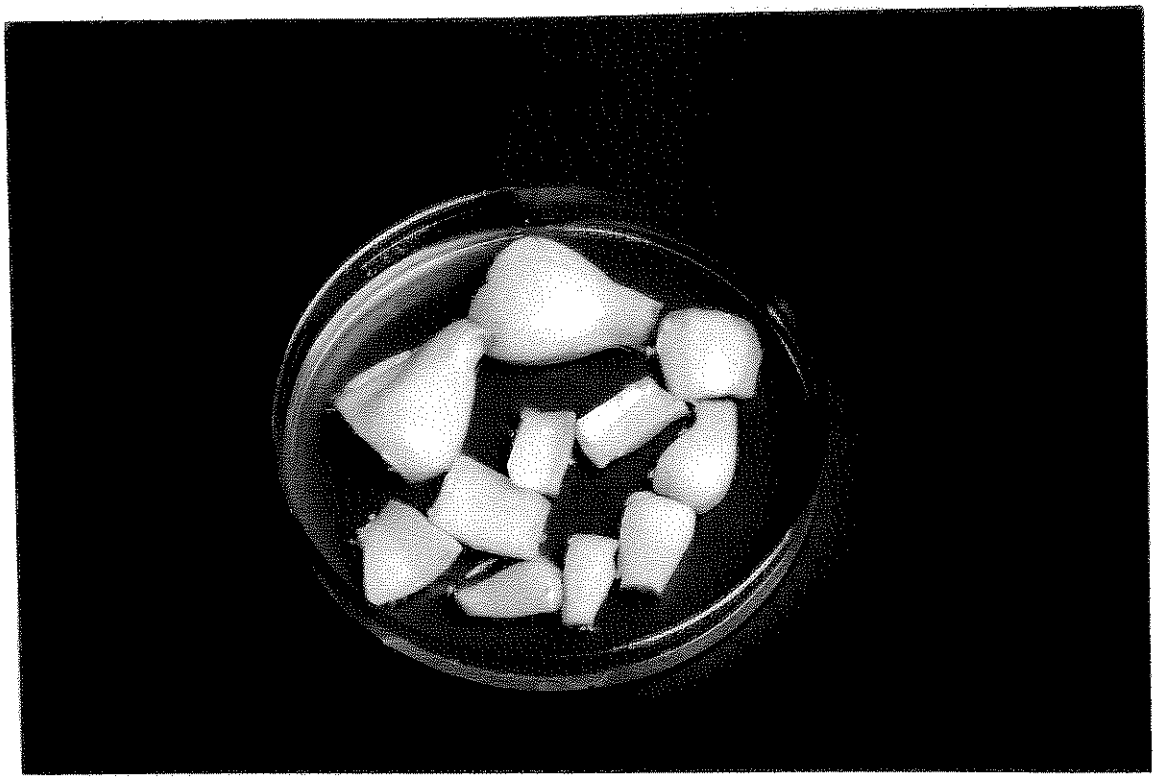


Figure 6. Culturing method 4 - an agar plate of type 6, opened to show bulb scales sealed onto agar with wax.

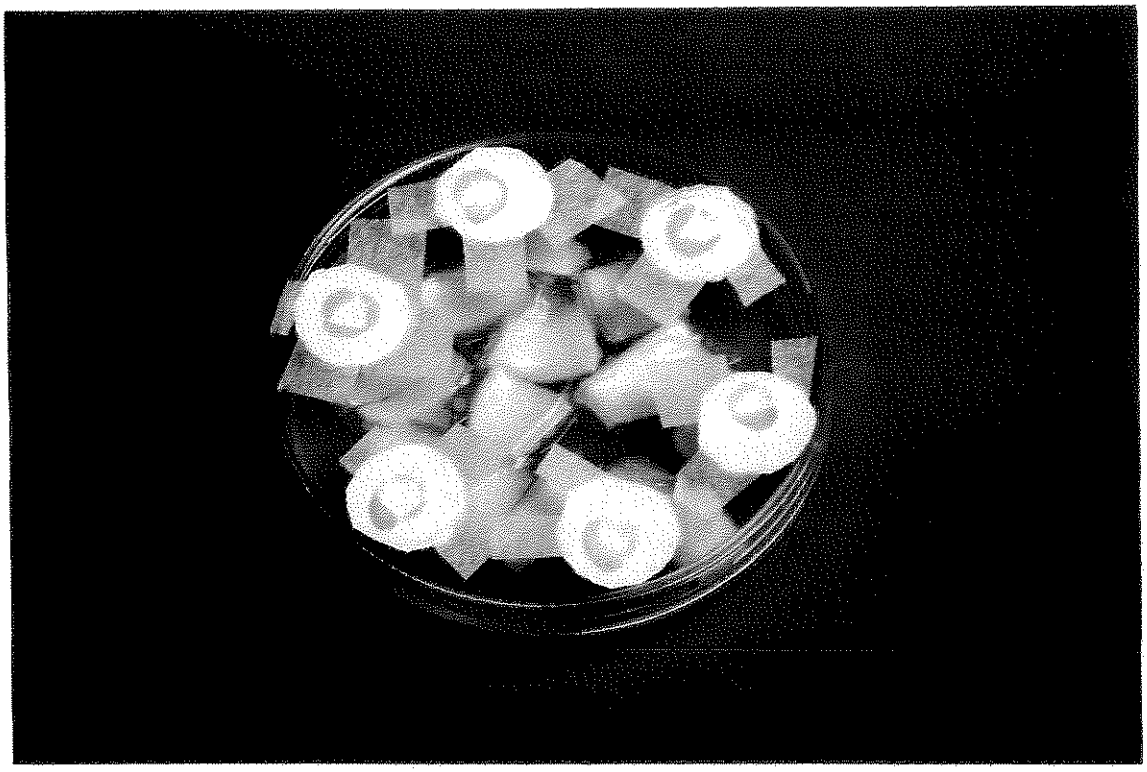


Figure 7. Culturing method 4 - an agar plate of type 6 showing aeration holes in petri dish lid.

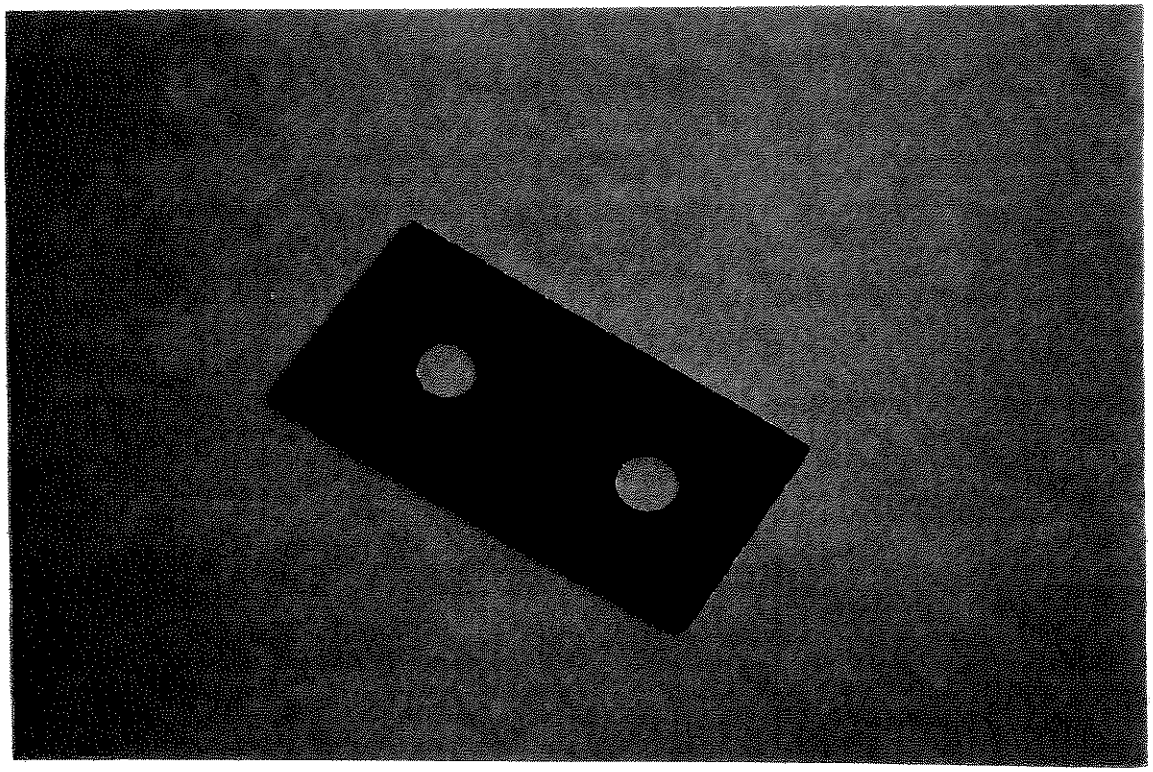


Figure 8. The perspex strip with two equidistant holes used to construct cells for life history studies.

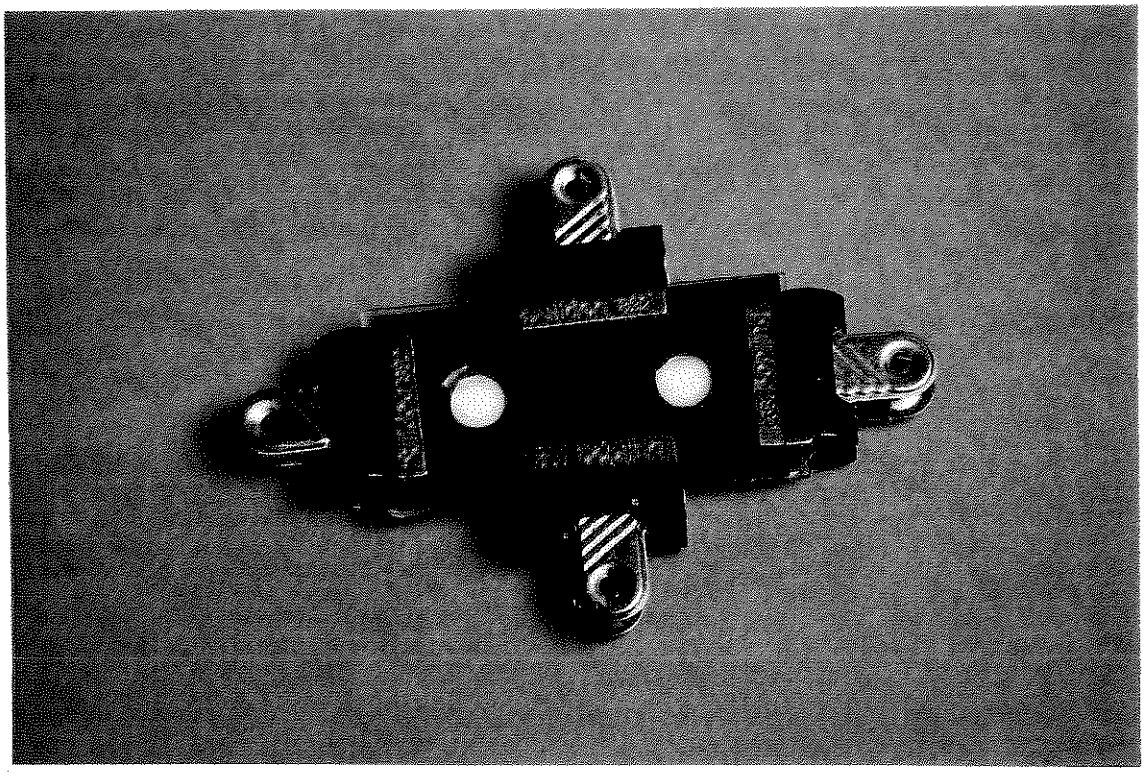


Figure 9. Assembled cell as used for life history studies.