

Project Title: Optimising tarsonemid control on strawberry using predatory mites

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GROWER SUMMARY

Headline

- Predatory mite species shown to reduce tarsonemid mites in heavily infested glasshouse and polytunnel strawberry crops

Background and expected deliverables

The strawberry tarsonemid mite, *Phytonemus (Tarsonemus) pallidus* ssp. *fragariae*, sometimes called the strawberry mite, is a serious pest of strawberry. It feeds mainly on the upper surfaces of the young folded leaves of strawberry, making their surfaces rough and crinkled as they expand. Sometimes the leaves turn brown and die and the whole plant usually becomes stunted. Mites also feed in the flowers and fruits, seriously affecting yield and quality, which can halt berry production.

There has been a significant and threatening increase in the frequency and severity of attacks in UK strawberry production in the last few years, the pest was particularly bad in 2010 and 2011 and continues to be a problem in some crops. Strawberry tarsonemid mite can be particularly difficult to control with conventional crop protection products, because most acaricides are contact acting with no or, at best, limited translaminar activity. The mites are readily controlled when directly intercepted by an acaricide, but penetration into the young folded leaves, where the tarsonemid mites live and breed, is limited; spray penetration being the chief factor limiting efficacy. Furthermore, strawberry leaves are waxy and covered in hairs, and many products are not specifically formulated for the crop and have insufficient wetting properties.

The overall aim of this project is to identify effective predatory mites for prevention and control of strawberry tarsonemid mite in outdoor and glasshouse crops and improve application timing and treatment methods.

Summary of the project and main conclusions

The project objectives for 2012 were to evaluate six species of predatory phytoseiid mite for their effectiveness at controlling strawberry tarsonemid mite at low and high temperatures, for use in polytunnel and glasshouse conditions. We aimed to find the most effective predatory mite species in polytunnel and glasshouse crops, the most effective temperature for each predatory species to operate and the optimum distribution of the predatory mites on the strawberry plants.

Potted strawberry plants were inoculated with tarsonemid mites and placed in fleece open top cages with a barrier of grease to prevent the escape of predatory mites. There were four treatments for the glasshouse and polytunnel trial (including an untreated control) and six replicates of each treatment in a randomised block design. Three species of predatory mite were tested for the glasshouse and three for the polytunnel in both the summer and the autumn. Populations of tarsonemid and predatory mite (including motiles and eggs), were assessed on young folded, unfurled and old leaves on each plant after treatment of 30 predatory mites per plant.

The summer glasshouse trial was hampered by low numbers of tarsonemid mites in the untreated control compared to the predatory mite treated plots, even after repeated introductions of the pest. There were also significant differences between the treatments before the predatory mites were applied. An assessment was made of the numbers of aphids on each treatment to see if there was an interaction between the numbers of aphids and the numbers of tarsonemid mites found on the strawberry plants. Although higher in number on the untreated control, this was not significant. Significantly more tarsonemid mite eggs were found in the *A. swirskii* and *A. montdorensis* treatments compared to the *N. californicus* and untreated control. Indeed the untreated control had fewer eggs than the plants treated with predatory mites. There were more motiles in the plants treated with *A. swirskii* and *A. montdorensis* than either *N. californicus* or the untreated control. More predatory mites were found on the plants treated with *A. swirskii* and *A. montdorensis* compared to *N. californicus* and the untreated control.

The summer polytunnel experiment gave more promising results with fewer tarsonemid mites in the plots treated with *A. barkeri* and *N. cucumeris* compared to *A. andersonni* and the untreated control.

Identification of predatory mites from the cages of both the glasshouse and polytunnel trials showed virtually no cross contamination of predatory mite species between treatments. Only *N. cucumeris* was found across all treatments, but at low levels, but more *N. cucumeris* were recovered from the *N. cucumeris* treated plots. Very few *N. californicus* were recovered from the *N. californicus* treated plots, despite the lower numbers of tarsonemid mites compared to the other predatory mite treatments in the summer glasshouse trial. Encouragingly, all of the predatory mite species identified had individuals which contained eggs and eggs were laid on strawberry leaves showing that the mites could reproduce on strawberry plants. Predatory mites, where found, were distributed over the whole plant

compared to tarsonemid mites which were found predominantly in the young folded leaves. In both the autumn trials (glasshouse and polytunnel), numbers of tarsonemids had dropped and remaining predatory mites may have been entering diapause.

Financial benefits

Strawberry tarsonemid mite can cause devastating crop losses in highly valuable protected strawberry crops, with losses exceeding £10,000 per ha per annum in some instances. New effective predatory mite species, and more accurate timing of predators using the most effective species for the time of year, will reduce populations of tarsonemid mites in strawberry crops, reducing the need for chemical applications.

Action points for growers

- Results from this study suggest that *Neoseiulus californicus* is to be recommended as an effective treatment for tarsonemid mites in glasshouse strawberry and *Amblyseius barkeri* and *Neosiulus cucumeris* in polytunnel crops.
- For preventive treatments, it is essential that predatory mites are applied early in the season before tarsonemid mite populations can build up.

SCIENCE SECTION

Introduction

The strawberry tarsonemid mite, *Phytonemus (Tarsonemus) pallidus* ssp. *Fragariae*, is a serious pest of strawberry. It feeds mainly on the upper surfaces of the young folded leaves of strawberry, along the main vein, making leaf surfaces rough and crinkled as they expand (Cross, 2003). Sometimes the leaves turn brown and die and the whole plant usually becomes stunted. Mites also feed in the flowers and fruits, seriously affecting yield and quality, which can halt berry production. Damage is most severe in everbearing varieties and on plants grown under protection. June bearers can also be severely attacked.

Populations build up rapidly in warm conditions, the generation time being nine days at 25 °C (Smith & Goldsmith, 1936; Wisemann, 1941; Easterbrook *et al.*, 2003). The optimum temperature for development is between 22-28 °C (Wisemann, 1941). Female mites overwinter as adults in the crowns of the plants (Dustan & Matthewman, 1931; Harmsen, 1934; Alford, 1972; Jeppson *et al.*, 1975). Oviposition begins at 8 °C (Wisemann, 1941) with each female capable of laying 30-40 eggs during her lifetime (Smith & Goldsmith, 1936). In addition, reproduction is facultatively parthenogenetic (Masse, 1928-30).

There has been a significant increase in the frequency and severity of attacks in UK strawberry production in the last few years, mostly due to pesticide withdrawals, and the problem was particularly acute in 2010-11. Currently, UK growers use a combination of approaches to control the pest (Table 1).

Table 1. Current approaches to tarsonemid control

| Control/Prevention | Problem |
|---|---|
| Source clean certified planting material | Often low levels of infestation present |
| Inspect plantations frequently in spring and early summer for signs of damage and destroy infested plants | As % of infested plants rises, destruction of plants and loss of yield becomes costly and uneconomic |
| Apply predatory mites | Only partially effective because mites are not suitable for all conditions, timings, and application rates need to be optimised |
| Spray abamectin (Dynamec) or tebufenpyrad (Masai) when damaging infestations start to develop. Spirodiclofen (Envidor) has a SOLA for protected and outdoor strawberry for spider mite control (20093371, until 31/07/2013) | Partial control, delaying the spread or infestation and damage (see below) |

Difficulty of chemical control

Currently, UK approved chemical options for tarsonemid control on outdoor and protected strawberry are abamectin (Dynamec), fenpyroximate (Sequel) and tebufenpyrad (Masai):

- Tebufenpyrad (Masai) and abamectin (Dynamec) are only partially effective against *P. pallidus*
- The number of applications of abamectin (Dynamec) and tebufenpyrad (Masai) are limited to three and one respectively, but sprays used during flowering and fruiting on everbearers are undesirable
- Most acaricides are contact acting with no, or at best limited, translaminar activity. Lack of penetration into the young folded leaves is the chief factor limiting efficacy
- Furthermore, strawberry leaves are waxy and covered in hairs, and many products are not specifically formulated for the crop and have insufficient wetting properties
- Work by EMR in HDC project SF 79 (Fountain *et al.*, 2010) clearly demonstrated substantive improvements in the efficacy of abamectin (Dynamec) when admixed with a silicone wetter. Nevertheless a very high degree of efficacy is only likely to be achieved with a systemic acaricide

Predatory mites tested

The introduction of predatory mites for control of tarsonemids on strawberry is a recommended practice for control of tarsonemid and other pests in strawberry. Early research in the US identified *Typhlodromus* sp. as a controlling predatory mite of tarsonemids on strawberry (Huffaker & Spitzer, 1951; Huffaker & Kennet, 1953). Today, *Neoseiulus cucumeris* is used most commonly for biocontrol of strawberry tarsonemid mite in the UK, but other species may be more efficacious and cost effective. Larger predators such as anthocorids and *Orius* spp. are not effective because they cannot access the pest.

Biological control, although effective if applied when populations are low to moderate (Croft *et al.*, 1998), is slow acting and does not eliminate the pest on whole plants. This is probably because the position of *P. pallidus* in the fold of young strawberry leaves (Easterbrook *et al.*, 2001; 2003; Fitzgerald *et al.*, 2007; 2008) makes them inaccessible to natural enemies. Repeated and increasing introductions of predatory mites may need to be made until the predator has established (Petrova *et al.*, 2002). The most effective species may be temperature dependant, e.g. *A. andersoni* is active from <8 °C and *A.*

swirskii from 12 °C. This will have implications for applications of use. A more voracious predator may be needed if tarsonemid populations peak in high summer.

Neoseiulus barkeri is sold as a preventative treatment for tarsonemid mites. Some other commercially available species are reported to give some reductions in tarsonemid populations, although they are not specifically recommended for control of this pest. In laboratory tests on US species, predation on *P. pallidus* was highest by *Typhlodromus pyri* > *Neoseiulus fallacies* > *Neoseiulus californicus* > *Amblyseius andersoni* > *Galendromus occidentalis* (Croft *et al.*, 1998). Other workers found *N. californicus* and *N. cucumeris* to be more effective than *T. pyri* as predators of *P. pallidus* (Fitzgerald *et al.*, 2007). In UK crops *Phytoseiulus persimilis* used to control *Tetranychus urticae* was also found to keep *P. pallidus* in check (Simmonds, 1970). However, this species does not persist on strawberry plants.

Earlier experiments at EMR (Fitzgerald, 2004) showed that *N. californicus* consumed similar numbers of tarsonemids to *N. cucumeris* when they were presented on a leaf arena, but this species was not tested on plants. However, it was found on the old rather than folded leaves. Currently, *N. californicus* may only be used in UK protected crops that are sealed throughout their life. However this species occurs in outdoor crops and efforts are being made to register the mite for use outdoors. Increasingly *Amblyseius swirskii* and *Amblyseius montdorensis* are being used to control a suite of pests in protected crops. *A. swirskii* has been shown to give good control of broad mite on azalea (Gobin *et al.*, 2011) and *Tarsonemus violae* on gerbera (Pijnakker & Leman, 2011).

The way forward

- The potential to exploit new species of predatory mite for the control of tarsonemid mite in strawberry needs to be explored
- In addition, the timing and methods of application are very important for the predator to be able to work effectively.

Objectives

The overall aim of the project is to identify effective predatory mites for prevention and control of strawberry tarsonemid mite in outdoor and glasshouse crops and improve application timing and treatment methods.

Project objectives for 2012:

1. To evaluate six species of predatory phytoseiid mites for their effectiveness at controlling strawberry tarsonemid mite at both low and high temperatures, for use in polytunnel and glasshouse conditions:
 - a. The most effective predatory mite species in polytunnel and glasshouse crops
 - b. The most effective temperature for each predatory species to operate
 - c. The distribution of the predatory mites on the strawberry plants

Materials and methods

Experimental design

A small plot replicated experiment comparing applications of 30 predatory mites (Table 2.1) per plant was carried out on caged tarsonemid mite infested everbearer strawberry plants (cv. Finesse) in a polytunnel (Rocks Farm) and a glasshouse (T) at East Malling Research (EMR) between March 2012 and March 2013.

Tarsonemid culture

Infested control plants from the previous years' experiment were kept in two glasshouses at EMR in order to culture the tarsonemid mites. Approximately 100 elite Finesse cold-stored strawberry runner plants were planted into individual pots and placed amongst the infested plants (Appendix 1) to increase the number of inoculation plants available for the trial. The mite populations were very slow to increase.

Plot infestation

To inoculate the trial plot with tarsonemid infected leaves from the glasshouse culture, young tarsonemid infested trifoliolate leaves were collected and on 8 May, 6 June and 2 July and placed between the folded leaves of the newly potted plants. Young leaves from the strawberry plants in the polytunnel were checked for tarsonemid mites on 10 July. The numbers of mites present were high but patchy across the plots, so a decision was made to apply the treatments.

Experimental design and layout

The experimental design consisted of 24 plots. A randomised block experiment with six replicates of four treatments was used (Table 2). For the polytunnel experiment a 22 x 6 m Spanish polythene tunnel (EMR plot code WF211, Rocks Farm) remote from other strawberry plantations was used. Plots consisted of cages made of horticultural fleece, the

cages were double skinned and the open top surface had a coating of fruit tree grease (Vitax Ltd.) to prevent mites from escaping. For the polytunnel experiment each cage was 50 cm x 50 cm x 100 cm and held eight plants which were drip irrigated (Appendix 1 photo 5). In the glasshouse experiment each replicate was in a separate glass house chamber, in order to maintain a minimum distance between the cages of 100 cm. The cages were 50 cm x 50 cm x 50 cm with four plants per cage fitted with drip irrigation (Appendix 1, Photo 6).

Treatments

Treatments were a single introduction of 30 mites per plant (Table 2). This was based on the Syngenta Bioline recommendation of 400 mites per m² curative (100 mites per plant) and 20 mites per m² preventative (five mites per plant) based on a standard strawberry planting density of 40,000 plants/ha. Normal mite applications involve shaking the mites out over the crop where not all of the mites reach the target. It was decided under consultation that as our application was made directly to the crown of the plant (Appendix 1, Photo 7) that 30 mites was a realistic number to apply.

Table 2. Six species of predatory mite were tested for efficacy for control of tarsonemid mite in strawberry in 2012

| Species | Commercially available | Native to UK | Use | Notes |
|--------------------------------|-------------------------------|---------------------|------------|---|
| <i>Amblyseius andersoni</i> | Yes | Yes | Polytunnel | May be effective at low temperature |
| <i>Amblyseius barkeri</i> | Yes | Yes | Polytunnel | Small species may be able to enter folded leaf more effectively |
| <i>Neoseiulus cucumeris</i> | Yes | Yes | Polytunnel | Commercial standard |
| <i>Amblyseius swirskii</i> | Yes | No | Glasshouse | Currently permit is for glasshouse use only |
| <i>Neoseiulus californicus</i> | Yes | No | Glasshouse | Occurs widely in commercial strawberry, but only licenced for release in glasshouse crops |
| <i>Amblyseius montdorensis</i> | Yes | No | Glasshouse | Currently permit is for glasshouse use only |

Treatment application

Treatments were applied as single point inoculations of 30 mites per plant, applied directly to the crown of each plant. One ml filtered pipette tips were cut down to make collection chambers, the mites were then individually sucked in to these chambers using a high volume air pump. Thirty mites were collected into each chamber and the chambers were capped with para-film. Plants were inoculated simultaneously (*i.e.* four or eight collection chambers were required). Each mite species was set up as an isolated collection station to prevent cross contamination between species. Pipette tips were checked under a microscope and the mites were moving freely inside prior to introduction to the plants. The treatments were applied in the summer and the experiment was set up again in the autumn with newly infested strawberry plants.

Assessments

A pre-treatment assessment was made of the degree of tarsonemid mite infestation in the glasshouse (9 July 2012) and the polytunnel (10 July 2012). For the summer 'high temperature' experiments two young trifoliolate leaves from the glasshouse plots and five young leaves from each of the polytunnel plots were collected and examined using a microscope and the numbers of tarsonemid mites and eggs were recorded. A note was made of any predatory mites.

The predatory mite treatments were introduced three days later. Populations of tarsonemid mite and the number and location of predators within the crop were assessed on 17 July, 1 August and 14 August in the glasshouse experiment and 26 July and 6 August in the polytunnel experiment. The three-day assessment was not included, based on the information gathered from the first assessment of the glasshouse experiment, since three days was not long enough for the predators to have an assessable effect. Five young (folded), five medium (unfurling) and five old leaves were collected from each plot and placed in separate plastic bags to keep the three ages of leaves separate. The upper and lower surface of each leaf was examined under a microscope in the laboratory. The numbers of tarsonemid and predatory mite motiles and eggs were recorded. Thrips and spider mite numbers were also noted. Any predatory mites found were mounted onto microscope slides with Hoyer's medium for later identification to species.

In the autumn for the 'low temperature' trials no pre-assessment was conducted as the plants were growing very slowly and any pre-assessment would have removed significant plant material required for a post-inoculation assessment. The glasshouse experiment was inoculated on 26 September and assessed on 3, 9 and 23 October, whilst the polytunnel

experiment was inoculated on 27 September and assessed on 4, 10 and 24 October (Table 3).

Table 3. Dates of activities conducted in each of the four experiments

| | Glasshouse six reps (four plants) | | Polytunnel six reps (eight plants) | |
|------------------------------|--|---------------|---|---------------|
| | Summer | Autumn | Summer | Autumn |
| Pre-assessment | 9 July | none | 18 July | none |
| Predator introduction | 12 July | 26 September | 21 July | 27 September |
| First assessment | 17 July | 3 October | 26 July | 4 October |
| Second assessment | 1 August | 9 October | 06 August | 10 October |
| Third assessment | 14 August | 23 October | - | 24 October |

Plot maintenance

All plants were supplied with drip irrigation. The plantation was inspected weekly to check for pests, disease and any other problems. Plants were de-blossomed and de-fruited before the trials were started and on each inspection to encourage new leaf growth favoured by tarsonemid mites.

Meteorological records

Half-hourly temperature and humidity records were taken using two Lascar USB-502 loggers in the glasshouse, the polytunnel and within the cages (see Appendix 2-5). For comparison external weather data was collected from the EMR weather station (Appendices 6, 7).

Statistical analysis

Repeated measures ANOVA, covariance adjusted for pre-treatment was done where applicable. Analyses was conducted on $\text{Log}_{10}(\text{mean}+1)$ transformed data.

Results

Summer glasshouse experiment

The summer glasshouse experiment was pre-assessed on 9 July (two young tri-foliate leaves per plot, Appendix 1 photos 1-3). There were no significant differences in the numbers of tarsonemid mite eggs between any of the treatments ($P = 0.258$, sed 0.2774, lsd 0.5913) however the number of motile mites between the treatments was significantly different ($P = 0.028$, sed 0.1831, lsd 0.3902) (Figure 1). There were significantly more tarsonemids in the *A. swirskii* (to be treated) plants than the *A. montdorensis* plants.

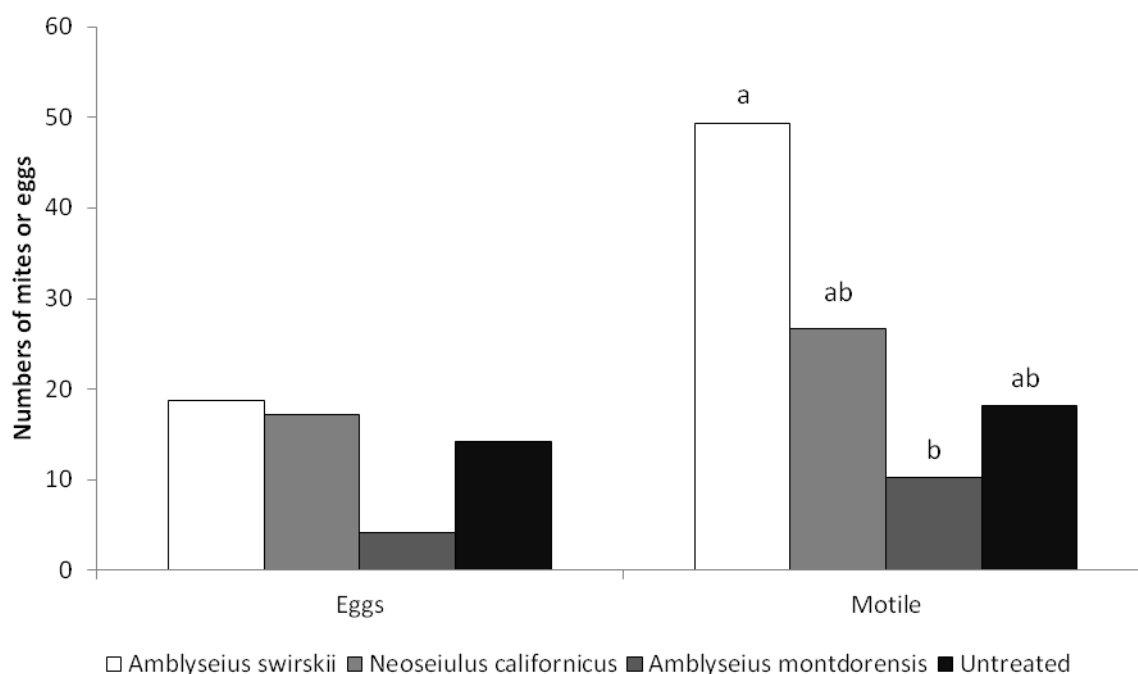


Figure 1. Mean numbers of tarsonemid mites and eggs per leaf per plot in the pre-assessment of the glasshouse experiment in the summer

The summer glasshouse experiment was assessed on 17 July, 1 August and 14 August. Two young, two medium and two old tri-foliate leaves per plot were assessed for the total numbers of motile mites, eggs, predatory mites and predatory mite eggs (Table 4, Figure 2). There were significant differences in the numbers of tarsonemid mite eggs between the treatments and the control ($P = 0.003$, sed 0.053, lsd 0.113), the number of motile mites between the treatments was also significantly different ($P = 0.006$, sed 0.050, lsd 0.106).

The numbers of predators recorded, whilst low, were consistent between treatments and significant ($P = 0.024$, sed 0.015, lsd 0.031). Only the numbers of predatory mite eggs had no significant relationship to treatment ($P = 0.125$, sed 0.006, lsd 0.012) (Table 4).

Table 4. ANOVA table of actual and \log_{10} ($n=1$) transformed data on the numbers of tarsonemid (Tar) and predatory (Pred) mite motiles and eggs in the summer glasshouse trial

| | Tar Eggs | Tar Eggs \log_{10} | Tar Motile | Tar Motile \log_{10} | Pred | Pred \log_{10} | Pred Egg | Pred Egg \log_{10} |
|----------------------------|-------------------------------|----------------------|-------------------------------|------------------------|-------------------------------|------------------|-------------------------------|----------------------|
| <i>A. swirskii</i> | 8.8 | 0.34 | 4.52 | 0.28 | 0.23 | 0.06 | 0.02 | 0.01 |
| <i>N. californicus</i> | 3.86 | 0.23 | 1.41 | 0.15 | 0.08 | 0.02 | 0.05 | 0.01 |
| <i>A. montdorensis</i> | 8.86 | 0.35 | 6.66 | 0.32 | 0.22 | 0.06 | 0.06 | 0.02 |
| Untreated | 1.4 | 0.14 | 1.04 | 0.15 | 0.06 | 0.02 | 0.01 | 0 |
| Young | 13.25 | 0.63 | 7.15 | 0.55 | 0.15 | 0.04 | 0.06 | 0.02 |
| Medium | 3.2 | 0.15 | 2.29 | 0.12 | 0.15 | 0.04 | 0.03 | 0.01 |
| Old | 0.73 | 0.02 | 0.78 | 0.02 | 0.14 | 0.04 | 0.02 | 0.01 |
| 1 st assessment | 5.63 | 0.27 | 4.76 | 0.28 | 0.07 | 0.02 | 0 | 0 |
| 2 nd assessment | 5.83 | 0.26 | 2.06 | 0.17 | 0.22 | 0.06 | 0.07 | 0.02 |
| Treat | | | | | | | | |
| F pr. | | 0.003 | | 0.006 | | 0.024 | | 0.125 |
| s.e.d. | | 0.053 | | 0.05 | | 0.015 | | 0.006 |
| l.s.d. | | 0.113 | | 0.106 | | 0.031 | | 0.012 |
| Leaf age | | | | | | | | |
| F pr. | | <.001 | | <.001 | | 0.907 | | 0.144 |
| s.e.d. | | 0.055 | | 0.045 | | 0.009 | | 0.005 |
| l.s.d. | | 0.112 | | 0.092 | | 0.018 | | 0.01 |
| Time | | | | | | | | |
| F pr. | | 0.692 | | <.001 | | <.001 | | <.001 |
| s.e.d. | | 0.031 | | 0.027 | | 0.009 | | 0.004 |
| l.s.d. | | 0.06 | | 0.052 | | 0.017 | | 0.008 |
| | No sig treat/leaf interaction | | No sig treat/leaf interaction | | No sig treat/leaf interaction | | No sig treat/leaf interaction | |

There were significantly more tarsonemid eggs in the *A. swirskii* and *A. montdorensis* treatments compared to the *N. californicus* and untreated control. Indeed the untreated control had fewer eggs than the plants treated with predatory mites. This was reflected in the numbers of motile tarsonemid mites, where there were more motiles in the plants treated

with *A. swirskii* and *A. montdorensis* than in either those treated with *N. californicus* or the untreated control (Fig. 1).

More predatory mites were found on the plants treated with *A. swirskii* and *A. montdorensis* compared to *N. californicus* and the untreated control. This was obviously partly due to the higher numbers colonising plants before the treatments were applied. In addition it was suggested that the untreated plants had more aphids on them and this may have impeded the tarsonemid mite population growth on the plants.

We analysed the numbers of aphids on four young trifoliolate leaves per plot. No significant differences between the treatments was found (ANOVA on Log¹⁰ transformed data, P = 0.864, sed 0.360, lsd 0.768, Fig. 3). However, the numbers of aphids was relatively high and may have contributed to the unusual result. Unfortunately, applications of mixed aphid parasitoids did not reduce numbers of aphids, even though parasitoids could be seen searching plants. The parasitoids were probably added too late to give significant control.

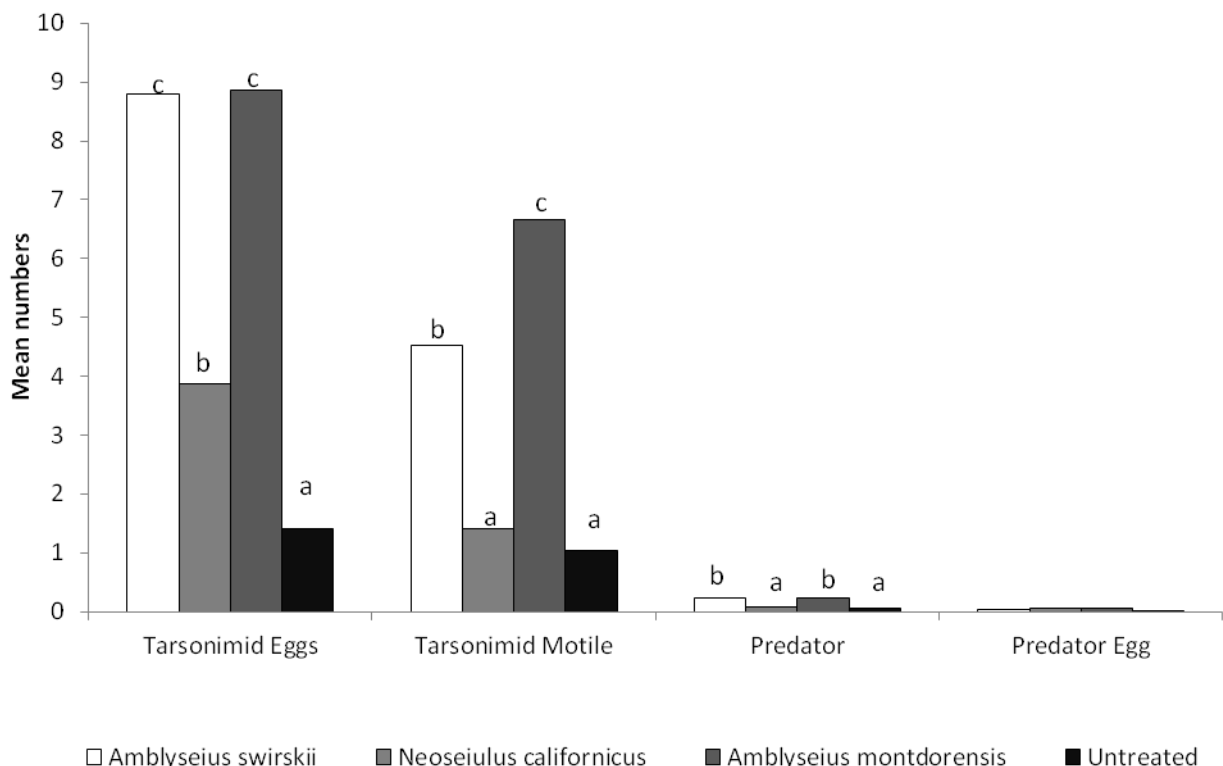


Figure 2. Numbers of tarsomemid and predaoty mite motiles and eggs in the summer glasshouse experiment after application of predatory mite treatments

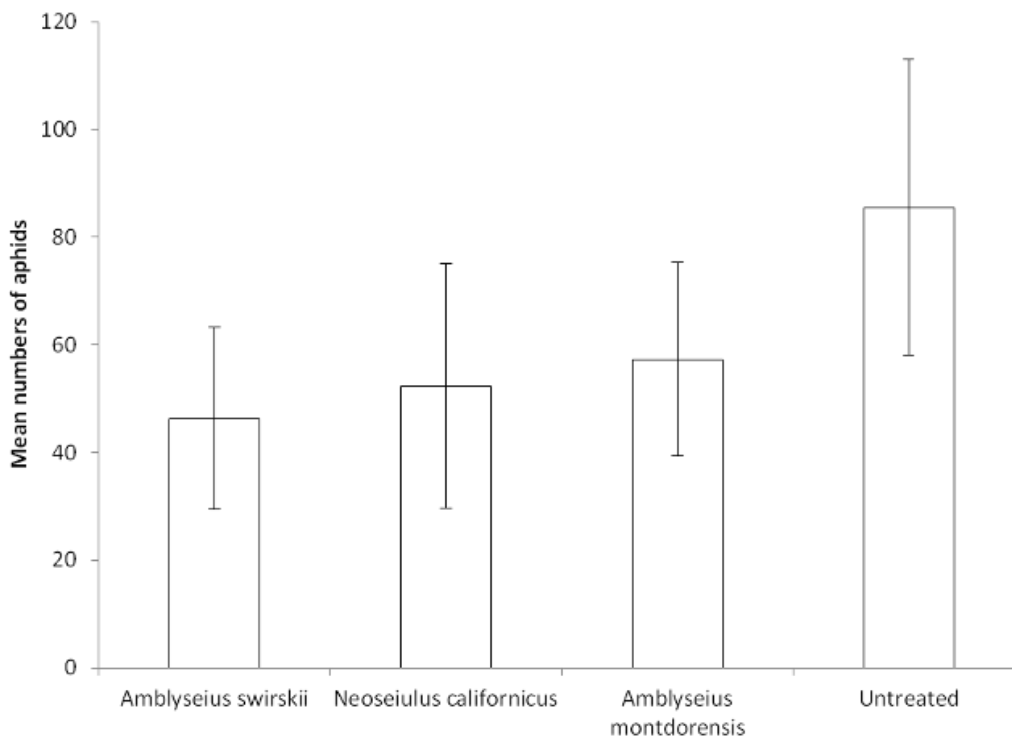


Figure 3. Numbers of aphids in the summer glasshouse experiment after application of predatory mite treatments

The predatory mites collected from the leaves were identified to species, and an interaction matrix constructed to look for cross contamination between treatments. Very few non-applied species were found in other cages. Only *N. cucumeris* was found across all the treatments, but at low levels (Table 5). Very few *N. californicus* were recovered from the *N. californicus* treated plots, despite the lower numbers of tarsonemid mites compared to the other predatory mite treatments.

Table 5. Numbers of predatory mite species recovered from each leaf of each treatment in the summer glasshouse experiment. NB: juveniles could not identified to species

| Leaf Age | Treatment | <i>A. swirskii</i> | <i>A. swirskii</i> with egg | <i>N. californicus</i> | <i>N. californicus</i> with egg | <i>A. montdorensis</i> | <i>A. montdorensis</i> with egg | <i>N. cucumeris</i> | <i>N. cucumeris</i> with egg |
|----------|------------------------|--------------------|--------------------------------|------------------------|------------------------------------|------------------------|------------------------------------|---------------------|---------------------------------|
| Young | <i>A. swirskii</i> | 5 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Medium | <i>A. swirskii</i> | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Old | <i>A. swirskii</i> | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Young | <i>N. californicus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Medium | <i>N. californicus</i> | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Old | <i>N. californicus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Young | <i>A. montdorensis</i> | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 1 |
| Medium | <i>A. montdorensis</i> | 0 | 0 | 0 | 1 | 5 | 4 | 0 | 0 |
| Old | <i>A. montdorensis</i> | 0 | 0 | 0 | 0 | 6 | 1 | 0 | 0 |
| Young | Untreated | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Medium | Untreated | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Old | Untreated | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Autumn glasshouse experiment

The autumn glasshouse experiment was assessed on 3, 9 and 23 October. Two young, medium and old tri-foolate leaves per plot were assessed for the total numbers of tarsonemid and predatory motile mites and eggs.

There were no significant differences in the numbers of tarsonemid mite eggs between any of the treatments ($P = 0.490$, sed 0.023, lsd 0.049), and neither was there any significant difference between the numbers of motile tarsonemid mites ($P = 0.550$, sed 0.037, lsd 0.078).

The numbers of predators recorded were very small and not significant ($P = 0.490$, sed 0.023, lsd 0.049). The numbers of predatory mite eggs were also low ($P = 0.625$, sed 0.006, lsd 0.014, Table 6). It was noted that some predatory mites could be found aggregating in

folded leaves and many had changed to a redder colour. Numbers of all mites significantly reduced over the course of this experiment.

Table 6. ANOVA table of actual and \log_{10} ($n=1$) transformed data on the numbers of tarsonemid (Tar) and predatory (Pred) mite motiles and eggs in the autumn glasshouse trial

| | Tar Eggs | Tar Eggs \log_{10} | Tar Motile | Tar Motile \log_{10} | Pred | Pred \log_{10} | Pred Egg | Pred Egg \log_{10} |
|----------------------------|----------|----------------------|------------|------------------------|------|------------------|----------|----------------------|
| <i>A. swirskii</i> | 0.74 | 0.07 | 0.92 | 0.11 | 0.36 | 0.07 | 0.03 | 0.01 |
| <i>N. californicus</i> | 0.22 | 0.04 | 0.31 | 0.05 | 0.22 | 0.04 | 0.05 | 0.01 |
| <i>A. montdorensis</i> | 0.27 | 0.04 | 0.45 | 0.08 | 0.27 | 0.04 | 0.01 | 0 |
| Untreated | 0.14 | 0.04 | 0.41 | 0.07 | 0.26 | 0.04 | 0.03 | 0.01 |
| Medium | 0.22 | 0.02 | 0.33 | 0.04 | 0.27 | 0.02 | 0.01 | 0 |
| Old | 0.14 | 0.03 | 0.13 | 0.03 | 0.32 | 0.03 | 0.03 | 0.01 |
| Young | 0.66 | 0.09 | 1.1 | 0.16 | 0.25 | 0.09 | 0.04 | 0.01 |
| 1 st assessment | 0.69 | 0.08 | 0.74 | 0.1 | 0.4 | 0.08 | 0 | 0 |
| 2 nd assessment | 0.36 | 0.06 | 0.81 | 0.12 | 0.32 | 0.06 | 0.08 | 0.02 |
| 3 rd assessment | 0.02 | 0 | 0.01 | 0.01 | 0.11 | 0 | 0 | 0 |
| Treat | | | | | | | | |
| F pr. | | 0.49 | | 0.55 | | 0.49 | | 0.625 |
| s.e.d. | | 0.023 | | 0.037 | | 0.023 | | 0.006 |
| l.s.d. | | 0.049 | | 0.078 | | 0.049 | | 0.014 |
| Leaf age | | | | | | | | |
| F pr. | | <.001 | | <.001 | | <.001 | | 0.412 |
| s.e.d. | | 0.012 | | 0.018 | | 0.012 | | 0.005 |
| l.s.d. | | 0.023 | | 0.037 | | 0.023 | | 0.01 |
| Time | | | | | | | | |
| F pr. | | <.001 | | <.001 | | <.001 | | <.001 |
| s.e.d. | | 0.015 | | 0.017 | | 0.015 | | 0.005 |
| l.s.d. | | 0.03 | | 0.036 | | 0.03 | | 0.01 |

Summer polytunnel experiment

The summer polytunnel experiment was pre-assessed on 10 July. Five young tri-foliate leaves per plot were assessed for the total number of tarsonemid and predatory motile mites and eggs (Appendix 1 photos 1-3). There were no significant differences in the numbers of tarsonemid mite eggs ($P = 0.287$, sed 0.161, lsd 0.319) or numbers of motile mites between the treatments ($P = 0.317$, sed 0.122, lsd 0.243) (Figure 4).

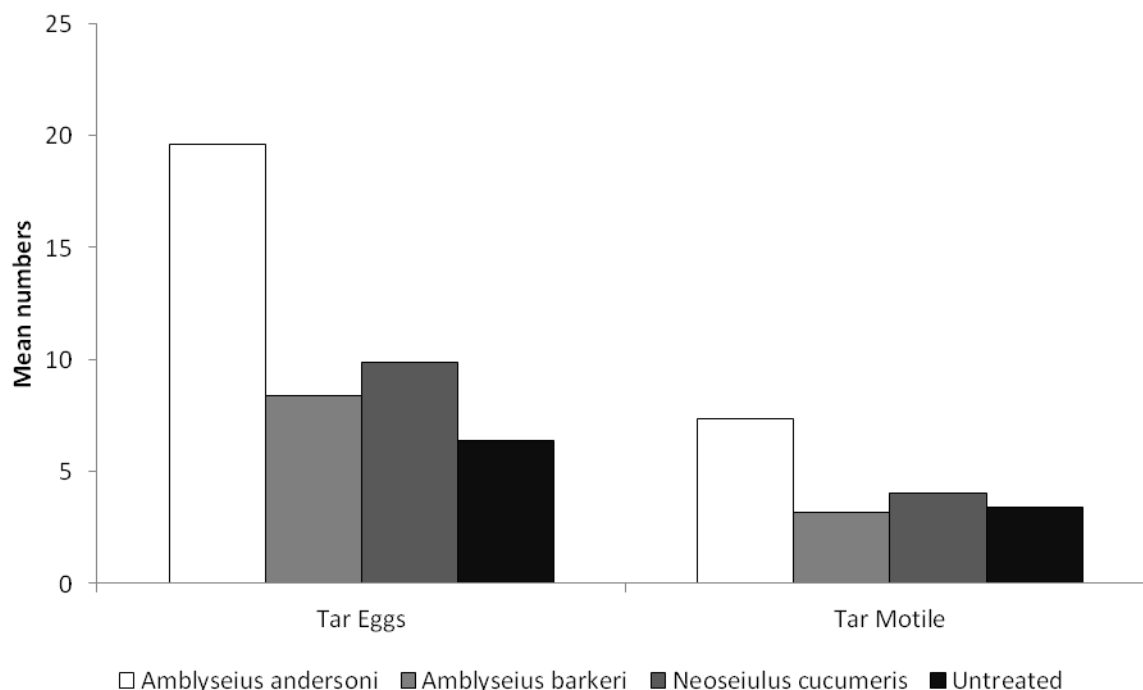


Figure 4. Mean numbers of tarsonemid mites and eggs per leaf per plot in the pre-assessment of the polytunnel experiment in the summer

The summer polytunnel experiment was assessed on 26 July and 06 August. Five young, medium and old tri-foliate leaves per plot were assessed for the total numbers of motile tarsonemid and predatory mites and eggs. The values were low, so data from the two assessment dates were pooled for analysis.

There were no significant differences between the treatments in the numbers of tarsonemid mite eggs ($P = 0.763$, sed 0.042, lsd 0.090). However there were significant differences in the numbers of motile tarsonemid mites treated with different predatory mite species ($P = 0.062$, sed 0.028, lsd 0.060). Fewer tarsonemid mites were found in the plots treated with *A. barkeri* and *N. cucumeris* compared to *A. andersoni* and the untreated control (Fig. 5). The

numbers of predatory motiles and eggs recorded were small and neither showed any significant differences ($P = 0.365$, sed 0.015, lsd 0.032 and $P = 0.324$, sed 0.002, lsd 0.005 respectively) (Table 7).

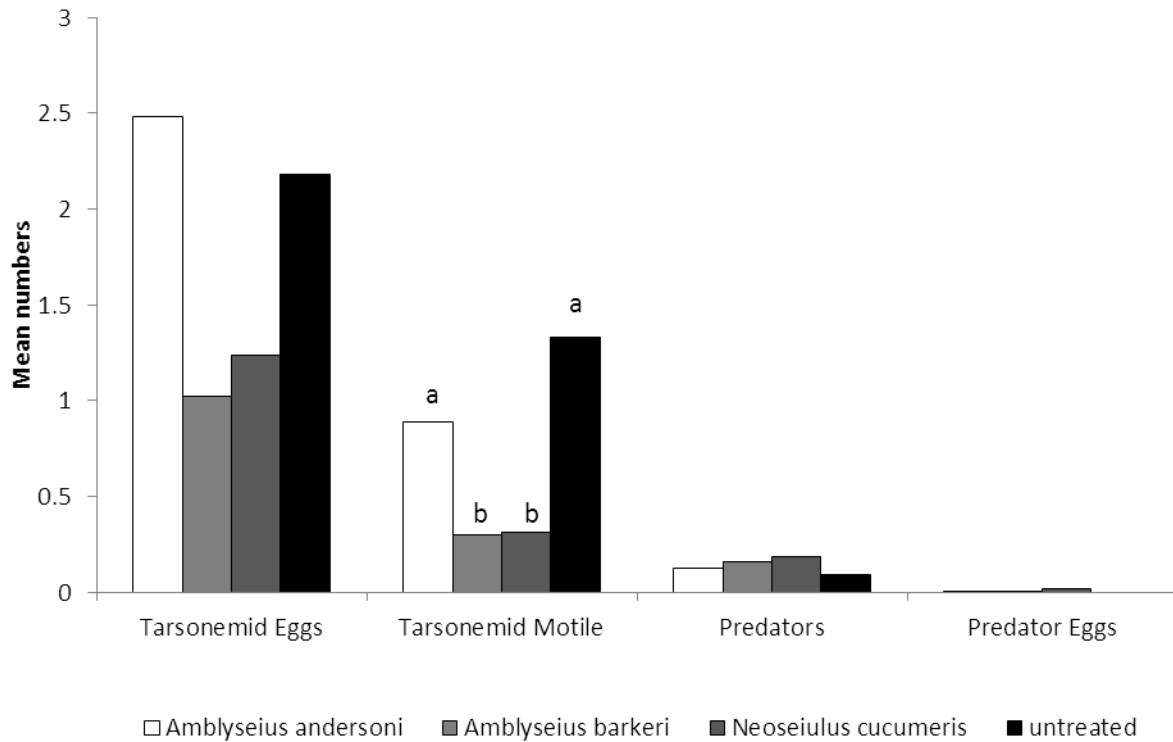


Figure 5. Numbers of tarsonemid and predatory mite motiles and eggs in the summer polytunnel experiment after application of predatory mite treatments

The predators collected were identified, and an interaction matrix constructed to look for cross contamination between treatments. Results show (Table 8) that the mite species applied to the plots were in the correct cages (plots). Only *N. cucumeris* was ubiquitous across all of the treatments including the untreated control, but more *N. cucumeris* were recovered from the *N. cucumeris* treated plots (Table 8).

Table 7. ANOVA table of actual and log₁₀ (n=1) transformed data on the numbers of tarsonemid and predatory mite motiles and eggs in the summer polytunnel trial

| | Tar Eggs | Tar Eggs log ₁₀ | Tar Motile | Tar Motile log ₁₀ | Pred | Pred log ₁₀ | Pred Egg | Pred Egg log ₁₀ |
|----------------------------|-------------------------------|----------------------------|-------------------------------|------------------------------|-------------------------------|------------------------|-------------------------------|----------------------------|
| <i>A. andersoni</i> | 2.48 | 0.13 | 0.89 | 0.08 | 0.13 | 0.03 | 0.01 | 0 |
| <i>A. barkeri</i> | 1.02 | 0.11 | 0.3 | 0.06 | 0.16 | 0.05 | 0.01 | 0 |
| <i>N. cucumeris</i> | 1.24 | 0.11 | 0.31 | 0.06 | 0.19 | 0.05 | 0.02 | 0 |
| Untreated | 2.18 | 0.15 | 1.33 | 0.13 | 0.09 | 0.03 | 0 | 0 |
| Medium | 0.95 | 0.08 | 0.16 | 0.03 | 0.19 | 0.05 | 0 | 0 |
| Old | 0.05 | 0.01 | 0.01 | 0 | 0.05 | 0.01 | 0 | 0 |
| Young | 4.19 | 0.3 | 1.96 | 0.22 | 0.19 | 0.05 | 0.02 | 0.01 |
| 1 st assessment | 2.50 | 0.16 | 0.94 | 0.1 | 0.23 | 0.06 | 0.01 | 0 |
| 2 nd assessment | 0.96 | 0.09 | 0.48 | 0.07 | 0.06 | 0.02 | 0.01 | 0 |
| Treat | | | | | | | | |
| F pr. | | 0.763 | | 0.062 | | 0.365 | | 0.324 |
| s.e.d. | | 0.042 | | 0.028 | | 0.015 | | 0.002 |
| l.s.d. | | 0.09 | | 0.06 | | 0.032 | | 0.005 |
| Leaf age | | | | | | | | |
| F pr. | | <.001 | | <.001 | | 0.001 | | 0.02 |
| s.e.d. | | 0.034 | | 0.024 | | 0.011 | | 0.002 |
| l.s.d. | | 0.068 | | 0.049 | | 0.023 | | 0.005 |
| Time | | | | | | | | |
| F pr. | | 0.003 | | 0.151 | | <.001 | | 0.805 |
| s.e.d. | | 0.024 | | 0.017 | | 0.008 | | 0.002 |
| l.s.d. | | 0.047 | | 0.034 | | 0.016 | | 0.004 |
| | No sig treat.leaf interaction | | No sig treat.leaf interaction | | No sig treat.leaf interaction | | No sig treat.leaf interaction | |

Table 8. Numbers of specific predatory mite species recovered from each leaf of each treatment in the summer polytunnel experiment. NB: juveniles could not be identified to species

| Leaf Age | Treatment | <i>A. andersoni</i> | <i>A. andersoni</i> with egg | <i>A. barkeri</i> | <i>A. barkeri</i> with egg | <i>N. cucumeris</i> | <i>N. cucumeris</i> with egg | <i>A. californicus</i> | <i>A. californicus</i> with egg |
|----------|---------------------|---------------------|---------------------------------|-------------------|-------------------------------|---------------------|---------------------------------|------------------------|------------------------------------|
| Young | <i>A. andersoni</i> | 1 | 1 | 0 | 0 | 2 | 2 | 0 | 0 |
| Medium | <i>A. andersoni</i> | 1 | 0 | 0 | 0 | 2 | 2 | 0 | 0 |
| Old | <i>A. andersoni</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Young | <i>A. barkeri</i> | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| Medium | <i>A. barkeri</i> | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 0 |
| Old | <i>A. barkeri</i> | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Young | <i>N. cucumeris</i> | 0 | 0 | 0 | 0 | 4 | 5 | 0 | 0 |
| Medium | <i>N. cucumeris</i> | 0 | 0 | 0 | 0 | 2 | 8 | 1 | 0 |
| Old | <i>N. cucumeris</i> | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Young | Untreated | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Medium | Untreated | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Old | Untreated | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Autumn polytunnel experiment

The autumn polytunnel experiment was assessed on 4, 10 and 24 October. Five young, medium and old tri-foolate leaves per plot were assessed for the numbers of tarsonemid and predatory mite motiles and eggs. There were almost significant differences in the numbers of tarsonemid mite eggs between the treatments ($P = 0.079$, sed 0.009, lsd 0.020). However, there were no significant differences in the numbers of motile mites between the treatments ($P = 0.591$, sed 0.018, lsd 0.028).

The numbers of predatory mites and predatory mite eggs recorded were small and neither showed any significant differences between treatments ($P = 0.172$, sed 0.013, lsd 0.028 and $P = 0.937$, sed 0.005, lsd 0.0 respectively) (Table 9). As with the glasshouse trial, it was noticed that predatory mites were aggregating in folded leaves (up to seven per leaf) and

were a darker red colour, which indicated that they may have been entering a winter diapause.

Table 9. ANOVA table of actual and \log_{10} (n=1) transformed data on the numbers of tarsonemid (Tar) and predatory (Pred) mite motiles and eggs in the autumn polytunnel trial

| | Tar Eggs | Tar Eggs \log_{10} | Tar Motile | Tar Motile \log_{10} | Pred | Pred \log_{10} | Pred Egg | Pred Egg \log_{10} |
|----------------------------|----------|----------------------|------------|------------------------|------|------------------|----------|----------------------|
| <i>A. andersoni</i> | 0.29 | 0.03 | 0.17 | 0.04 | 0.24 | 0.06 | 0.07 | 0.01 |
| <i>A. barkeri</i> | 0.10 | 0.02 | 0.23 | 0.04 | 0.19 | 0.05 | 0.04 | 0.01 |
| <i>N. cucumeris</i> | 0.07 | 0.02 | 0.23 | 0.04 | 0.12 | 0.03 | 0.02 | 0 |
| Untreated | 0.41 | 0.04 | 0.32 | 0.05 | 0.14 | 0.04 | 0.01 | 0 |
| Medium | 0.21 | 0.02 | 0.08 | 0.01 | 0.12 | 0.03 | 0.01 | 0 |
| Old | 0.05 | 0.01 | 0.07 | 0.02 | 0.19 | 0.05 | 0.08 | 0.01 |
| Young | 0.4 | 0.05 | 0.56 | 0.1 | 0.2 | 0.05 | 0.01 | 0 |
| 1 st assessment | 0.56 | 0.06 | 0.53 | 0.08 | 0.21 | 0.06 | 0.01 | 0 |
| 2 nd assessment | 0.09 | 0.02 | 0.16 | 0.03 | 0.11 | 0.03 | 0.1 | 0.01 |
| 3 rd assessment | 0 | 0 | 0.03 | 0.01 | 0.2 | 0.05 | 0 | 0 |
| Treat | | | | | | | | |
| F pr. | | 0.079 | | 0.591 | | 0.172 | | 0.937 |
| s.e.d. | | 0.009 | | 0.013 | | 0.013 | | 0.005 |
| l.s.d. | | 0.02 | | 0.028 | | 0.028 | | 0.011 |
| Leaf age | | | | | | | | |
| F pr. | | <.001 | | <.001 | | 0.061 | | 0.25 |
| s.e.d. | | 0.009 | | 0.008 | | 0.009 | | 0.004 |
| l.s.d. | | 0.018 | | 0.017 | | 0.018 | | 0.009 |
| Time | | | | | | | | |
| F pr. | | <.001 | | <.001 | | 0.004 | | 0.014 |
| s.e.d. | | 0.009 | | 0.01 | | 0.008 | | 0.004 |
| l.s.d. | | 0.019 | | 0.021 | | 0.016 | | 0.008 |

Higher temperatures were reached in the cages in the summer in the glasshouse and polytunnel. The humidity was higher in the autumn (Table 10).

Table 10. Mean temperature and humidity inside the cages during the summer and autumn trials

| | Temperature °C | | Humidity | |
|------------|----------------|--------|----------|--------|
| | Summer | Autumn | Summer | Autumn |
| Polytunnel | 18.7 | 13.5 | 75% | 83% |
| Glasshouse | 21.9 | 15.2 | 73% | 80% |

Conclusions

- The summer glasshouse trial was hampered by low numbers of tarsonemid mites in the untreated control compared to the predatory mite treated plots even after repeated introductions of the pest
- There were also significant differences between the treatments before the predatory mites were applied. An assessment was done of the numbers of aphids on each treatment and although higher in number on the untreated control, this was not significant
- Significantly more tarsonemid eggs were found in the *A. swirskii* and *A. montdorensis* treatments compared to the *N. californicus* and untreated control. Indeed the untreated control had fewer eggs than the plants treated with predatory mites
- There were more motiles in the plants treated with *A. swirskii* and *A. montdorensis* than either *N. californicus* or the untreated control
- More predatory mites were found on the plants treated with *A. swirskii* and *A. montdorensis* compared to *N. californicus* and the untreated control
- The summer polytunnel experiment gave promising results, with fewer tarsonemid mites in the plots treated with *A. barkeri* and *N. cucumeris* compared to *A. andersonni* and the untreated control
- Identification of predatory mites from the cages of both the glasshouse and polytunnel trials showed virtually no cross-contamination of predatory mite species between treatments
- Only *N. cucumeris* was found across all treatments, but at low levels, but more *N. cucumeris* predatory mites were recovered from the *N. cucumeris* treated plots
- Very few *N. californicus* were recovered from the *N. californicus* treated plots, despite the lower numbers of tarsonemid mites compared to the other predatory mite treatments in the summer glasshouse trial

- Encouragingly, all of the predatory mite species identified had individuals which contained eggs, and eggs were laid on strawberry leaves - showing that the mites could reproduce on strawberry plants
- Predatory mites, where found, were distributed over the whole plant, compared to tarsonemid mites, which were found predominantly in the young folded leaves
- In both the autumn trials (glasshouse and polytunnel) the numbers of tarsonemids had dropped and remaining predatory mites may have been entering diapause

Future work

In the final two years of the project we will:

1. Review the data available on predatory mite compatible chemical treatments in strawberry
2. Determine the most effective timings and application rates of *A. barkeri* and *N. cucumeris* to control tarsonemid mites in polytunnels
3. Test the most successful predatory mite species and application strategies in a field trial in strawberry under polythene

Acknowledgements

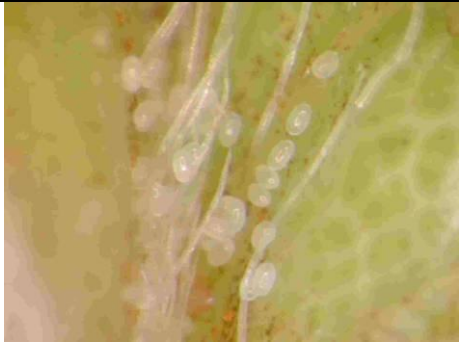







We are grateful to Graham Caspell and his team at EMR for the erection and maintenance of the polytunnel and husbandry of the plants. We would also like to thank Bethan Shaw, Judit Linka and Antonio Llorente of EMR, who assisted with the spraying, sampling and mite counts.

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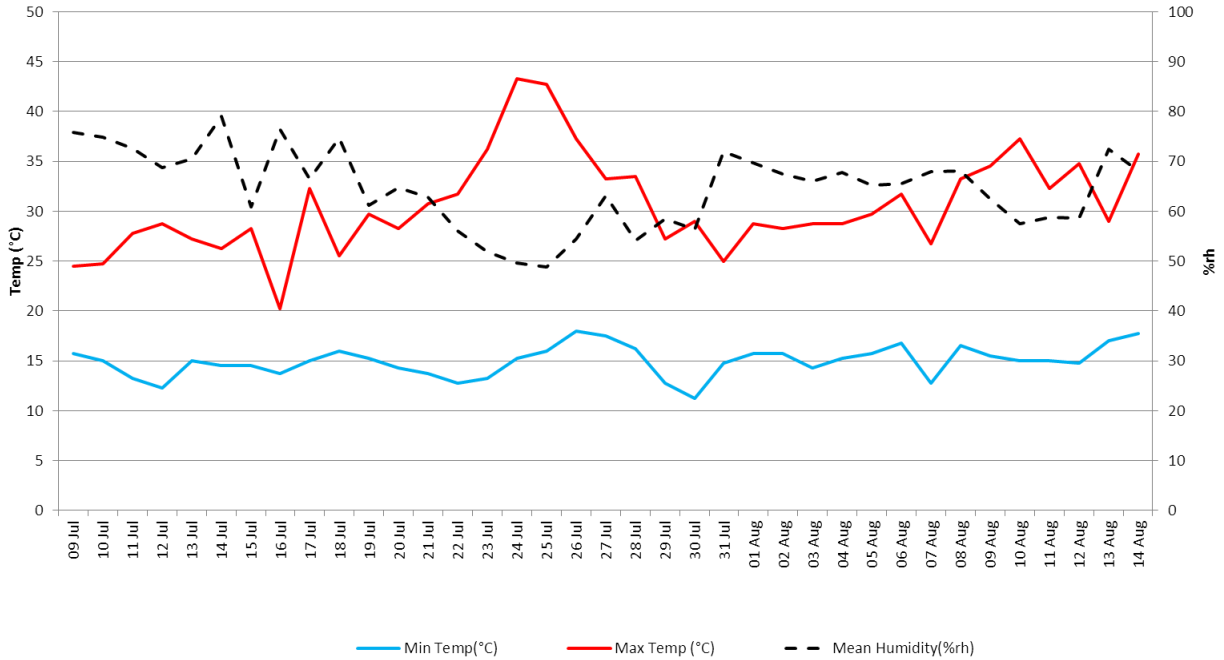
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Appendix 1. Photographs from HDC strawberry trial

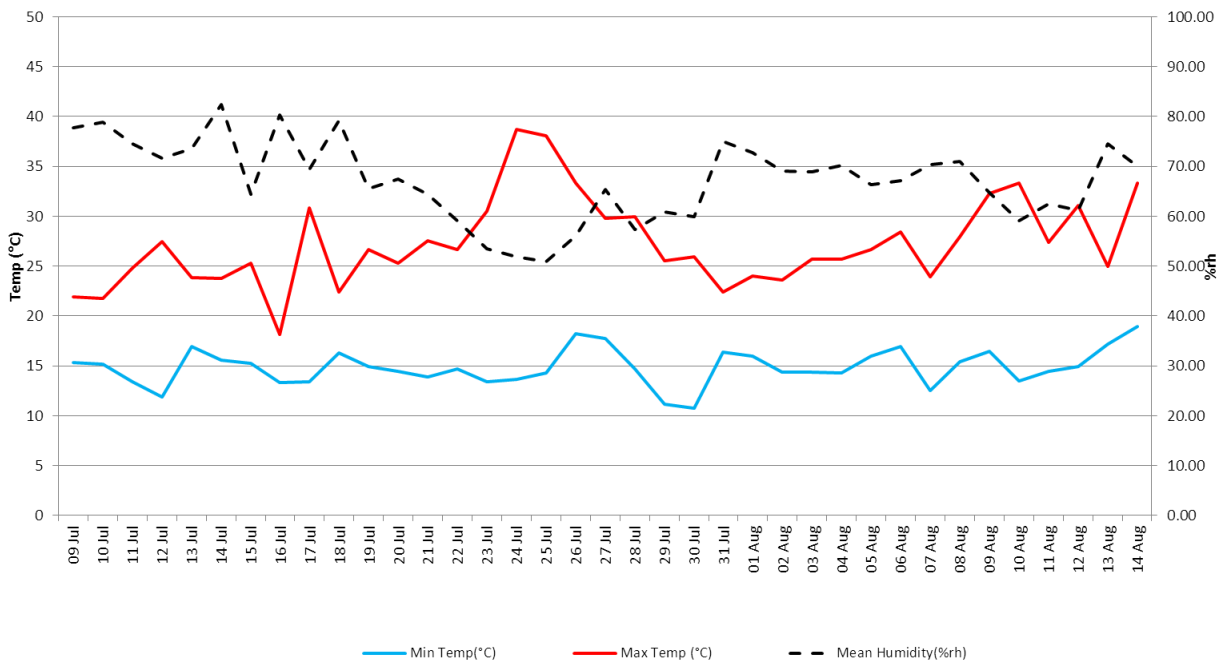
| | |
|---|--|
| <p>1</p>  <p>Tarsonemid mite eggs</p> | <p>2</p>  <p>Tarsonemid mite eggs and nymph</p> |
| <p>3</p>  <p>Tarsonemid mite adult</p> | <p>4</p>  <p>Tarsonemid damage to strawberry leaf</p> |
| <p>5</p>  <p>Polytunnel and cages used in trial, autumn 2012</p> | <p>6</p>  <p>Glasshouse cages, summer 2012</p> |
| <p>7</p>  <p>Potted strawberry plant showing mite release tube</p> | <p>8</p>  <p>Tarsonemid culture plants, October 2012</p> |

Appendix 2. Mean half hour weather data from two Lascar 502 temperature and humidity loggers for the duration of the summer glasshouse experiment, data for the glasshouse and the cages within the glasshouse

Temperature and humidity for glasshouse T Summer 2012

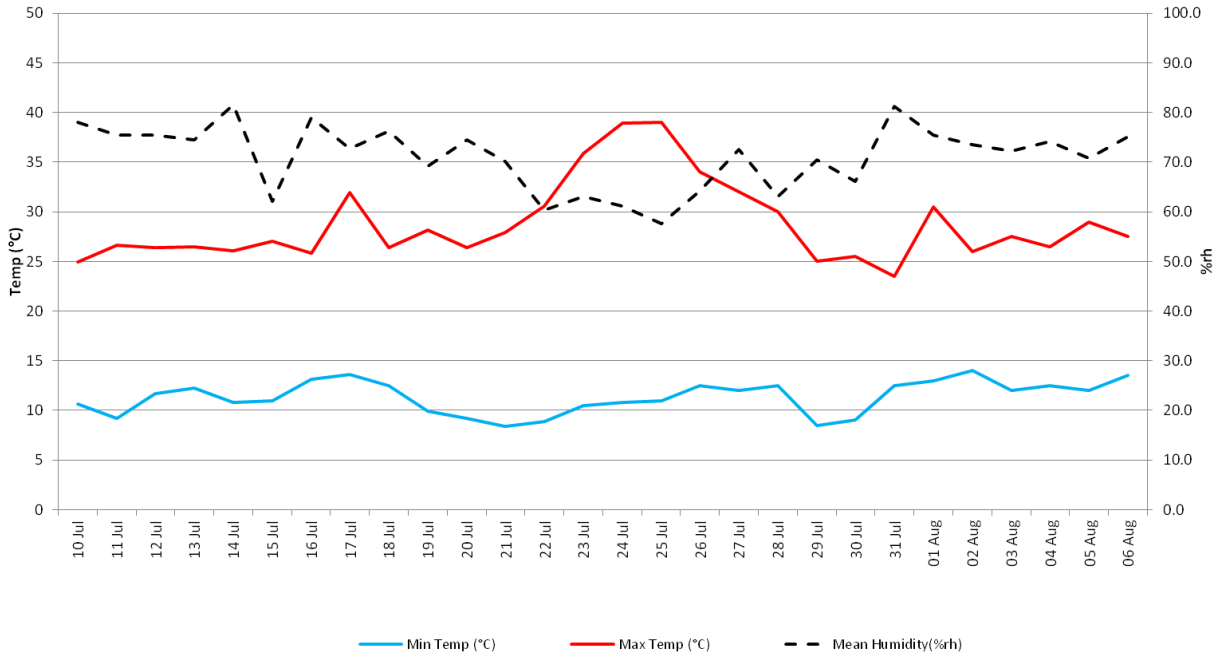


Temperature and Humidity for Cages in Glasshouse T Summer 2012

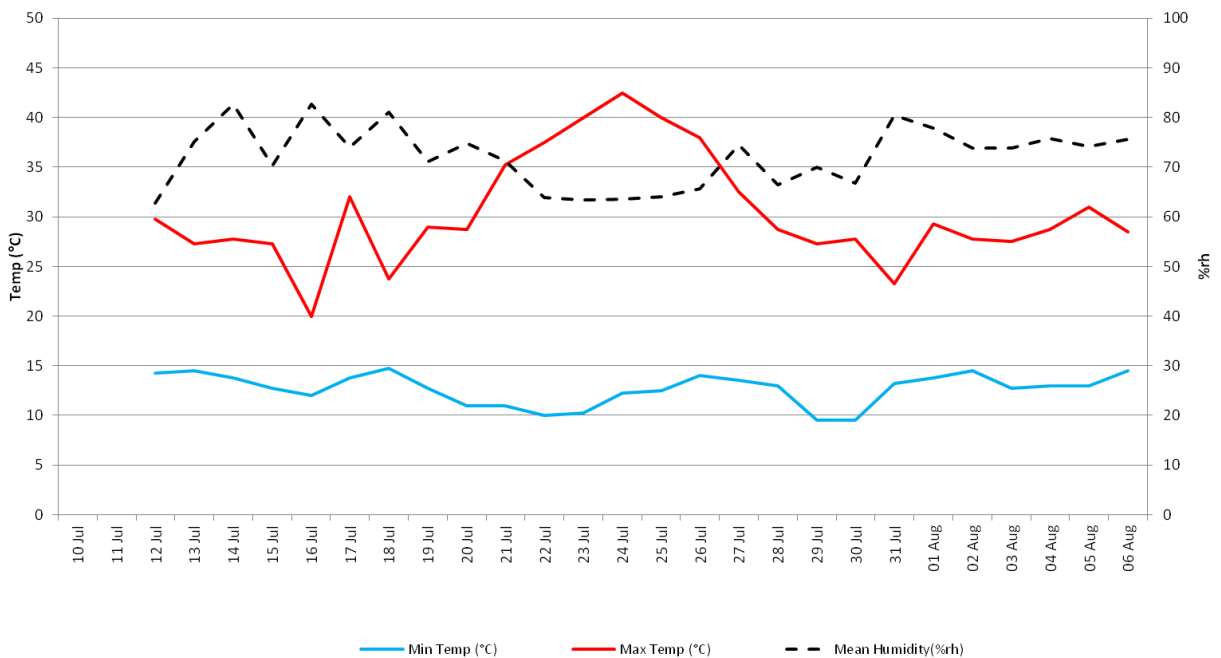


Appendix 3. Mean half hour weather data from two Lascar 502 temperature and humidity loggers for the duration of the summer polytunnel experiment, data for the polytunnel and the cages within the polytunnel

Temperature and Humidity for polytunnel Summer 2012

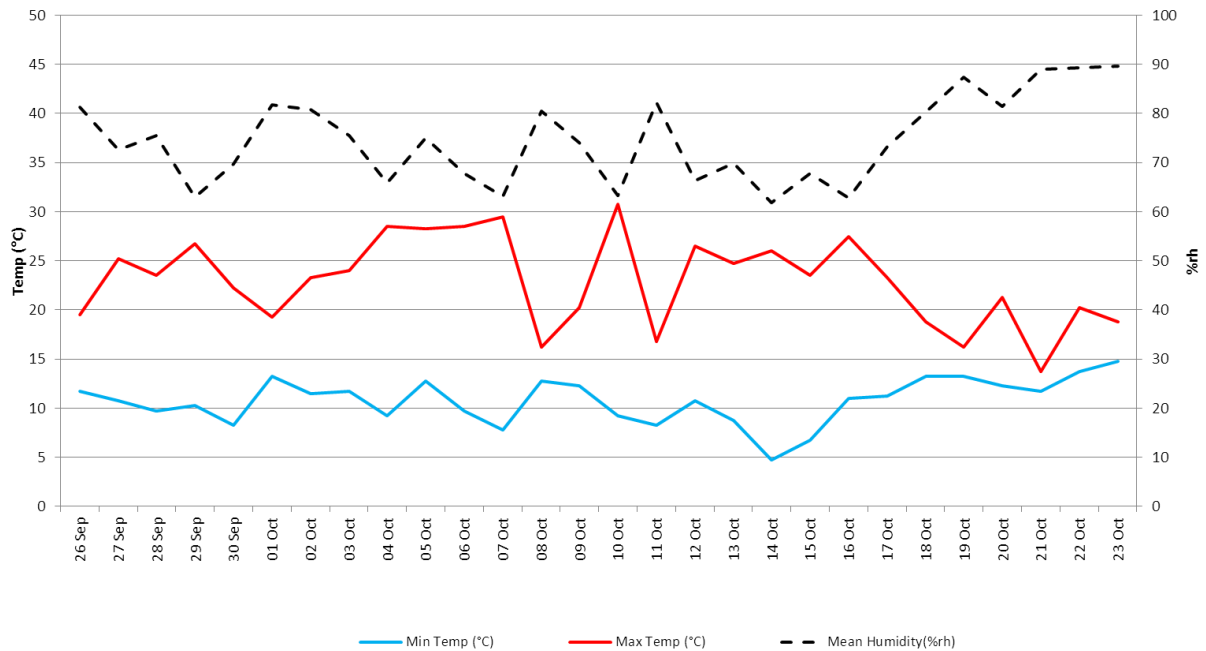


Temperature and Humidity for Cages in polytunnel Summer 2012

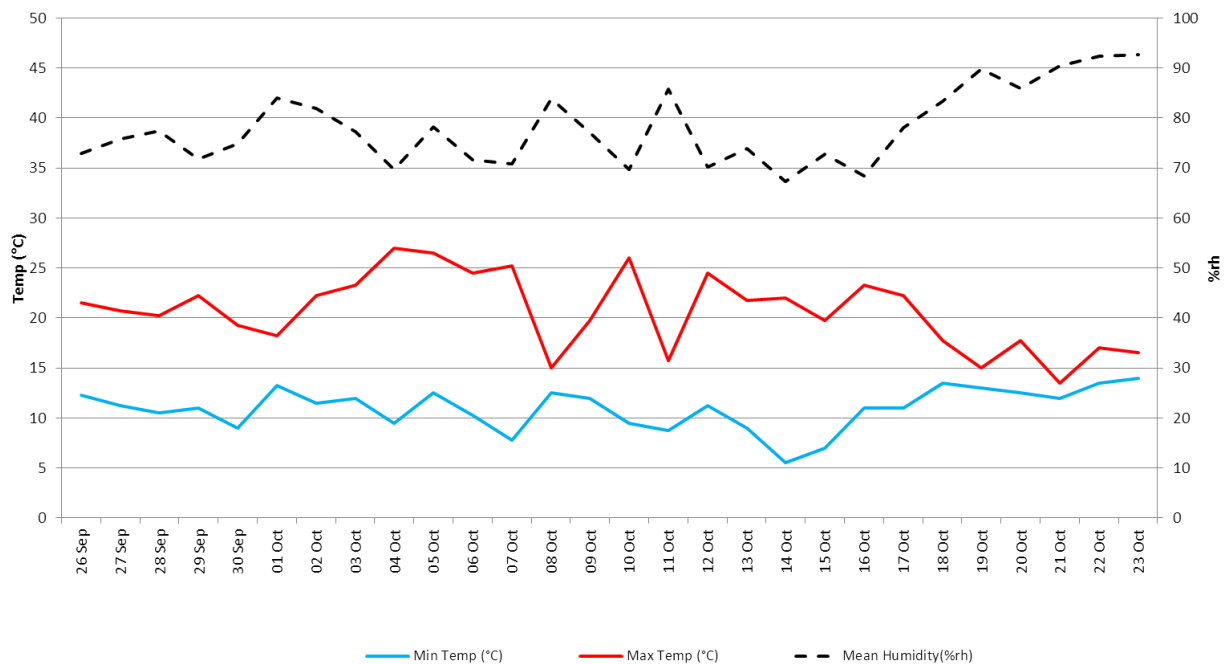


Appendix 4. Mean half hour weather data from two Lascar 502 temperature and humidity loggers for the duration of the autumn glasshouse experiment, data for the glasshouse and the cages within the glasshouse

Temperature and humidity for glasshouse T Autumn 2012

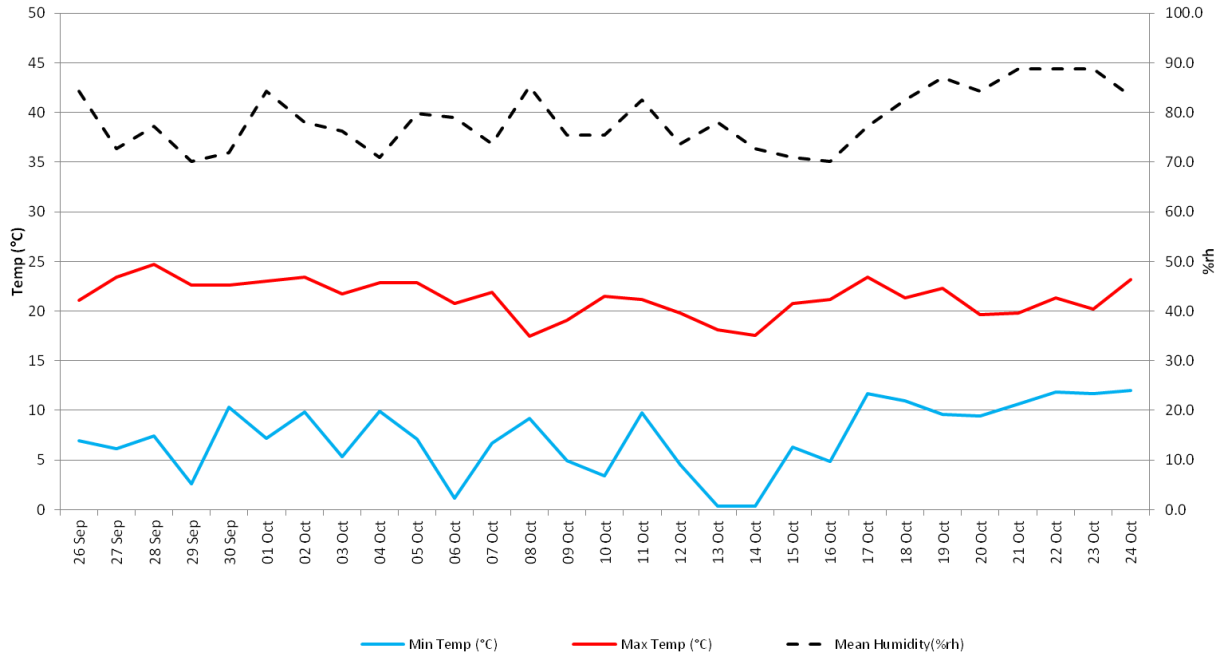


Temperature and Humidity for Cages in Glasshouse T Autumn 2012

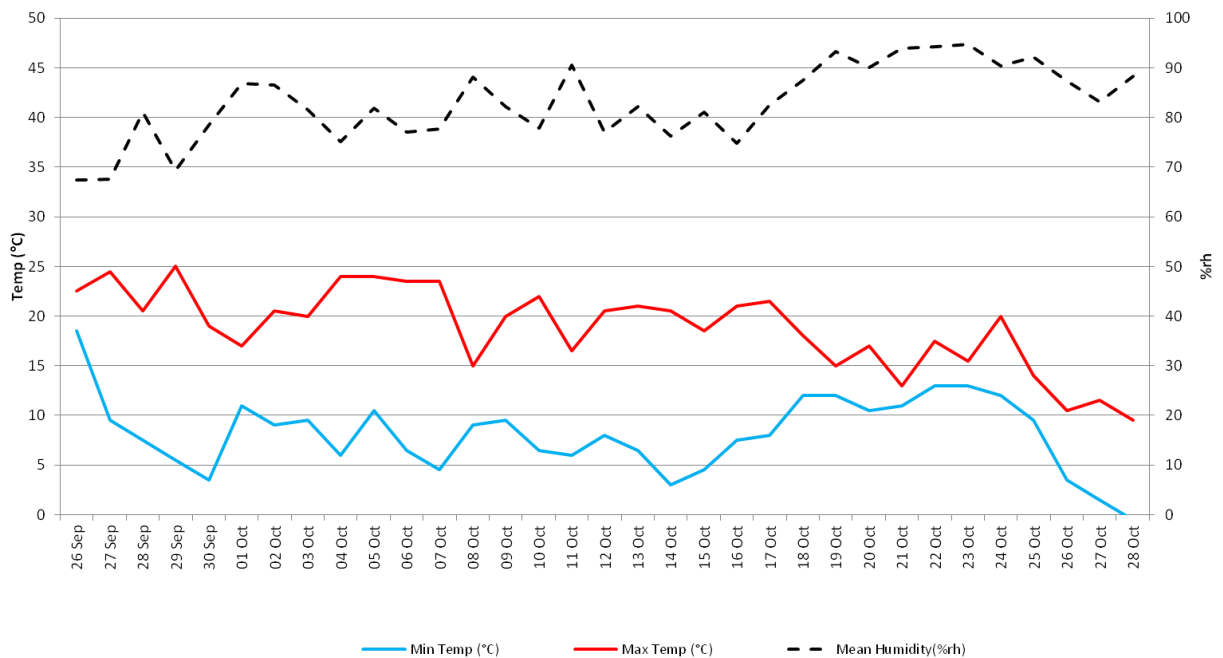


Appendix 5. Mean half hour weather data from two Lascar 502 temperature and humidity loggers for the duration of the autumn polytunnel experiment, data for the polytunnel and the cages within the polytunnel

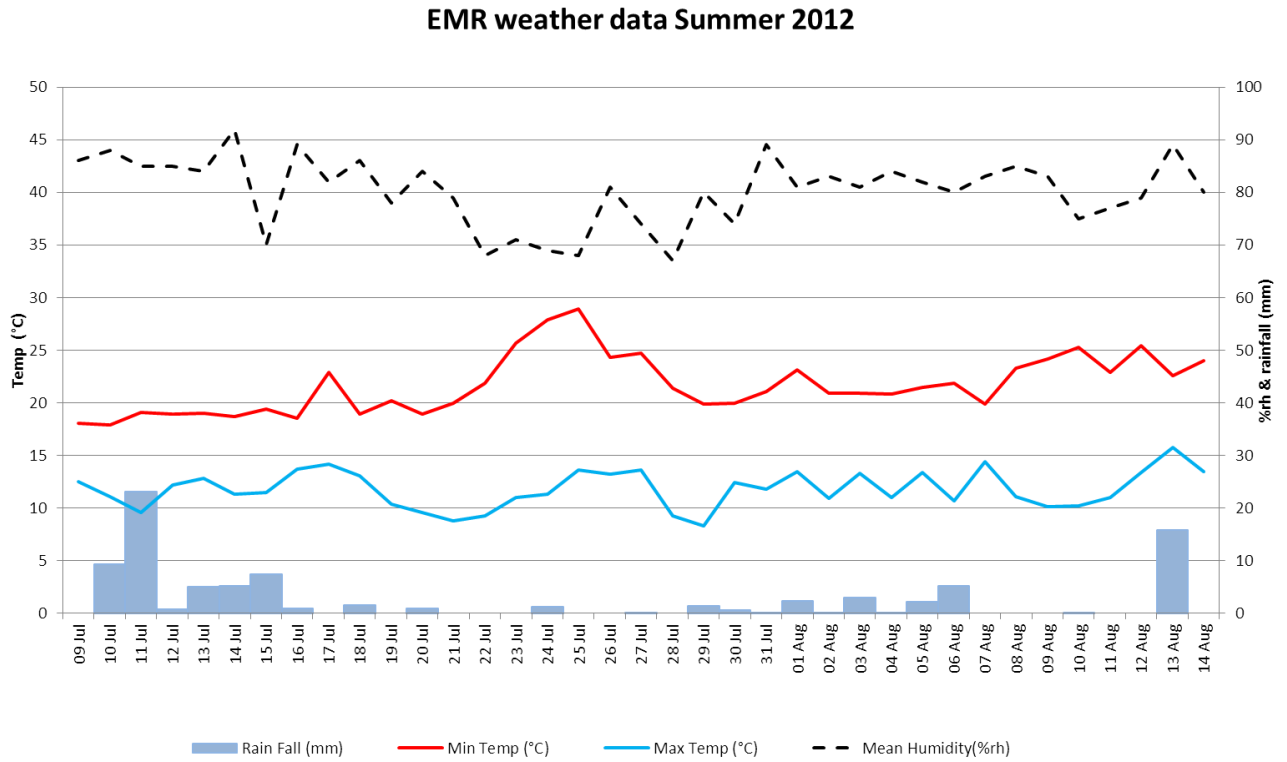
Temperature and Humidity for polytunnel Autumn 2012



Temperature and Humidity for Cages in polytunnel Autumn 2012



Appendix 6. Mean daily weather data from the EMR weather station for the duration of the summer experiments



Appendix 7. Mean daily weather data from the EMR weather station for the duration of the autumn experiments

