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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with 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.]

AUTHENTICATION

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

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Signature ..... Date ...06 April 2018...

Report authorised by:

Louise Sutherland,

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GROWER SUMMARY

This project addresses the main pest problems reported by the UK strawberry industry, except for spotted wing drosophila (SWD), which is covered in other projects. Within this project, it is planned to work on five objectives over the five year duration:

1. Develop effective biological methods for managing western flower thrips, *Frankliniella occidentalis* (WFT), compatible with pesticide use against SWD, improve the reliability of biocontrol of WFT with predatory mites, and develop effective approaches to the use of entomopathogenic fungi (EPF) for control of WFT.
2. Refine pest control programmes on strawberry, integrating pesticides with phytoseiid mites.
3. Develop IPM compatible controls for European tarnished plant bug (*Lygus rugulipennis*), common green capsid (*Lygocoris pabulinus*), and strawberry blossom weevil (*Anthonomus rubi*).
4. Improve insecticide control of the potato aphid, *Macrosiphum euphorbiae,* so as to be more compatible with IPM programmes.
5. Improve the control of aphids through the growing season.

For ease of reading, this Grower Summary report is split into sections for each of the objectives being worked upon. In Year 3 of the project, Objectives 1, 2, 3, 4 and 5 were worked on and are reported here.

Western flower thrips

Objective 1 - Develop effective biological methods for managing western flower thrips, *Frankliniella occidentalis* (WFT), compatible with pesticide use against SWD, improve the reliability of biocontrol of WFT with predatory mites, and develop effective approaches to the use of entomopathogenic fungi (EPF) for control of WFT.

In Year 3 of the project, the work on WFT was broken into Tasks 1.1 and 1.2

Task 1.1. Develop and determine the efficacy and ease of use of the prototype extraction device for WFT and the predatory mite Neoseiulus cucumeris in commercial strawberry crops, by agronomist and growers

Task 1.2. Determine the distribution of Neoseiulus cucumeris on commercial strawberry plants after their introduction for WFT management

Headlines

- An extraction device has been developed to improve the level of detection of both WFT and predator numbers in strawberry plants.
- The presence of WFT as prey in strawberry plants increases the number *of N. cucumeris* on flowers and button fruits.

Background and expected deliverables

Task 1.1.

In 2015, methyl isobutyl ketone (MIK) was shown to be effective as a fumigant to extract arthropods from button fruit, with higher numbers recorded by extraction compared to 'by eye' assessments of flowers or fruits (see 2016 Annual Report). Three prototype monitoring devices, making use of this fumigant extraction method, were constructed. Based on grower/advisor feedback on the different designs and prototypes, a 'Tupperware' type device was chosen for further development based on its robustness, ease of use, and transparency. A few modifications were required, and to increase the ease of counting, a segmented counting surface was included.

Following initial laboratory studies to assess the efficacy of the device in extracting thrips and mites from flowers and fruit, further laboratory experiments were carried out in the summer and autumn of 2017 to achieve a more thorough calibration of the device with *N. cucumeris*. Field studies were also carried out during the summer by agronomists and growers to explore the efficacy and ease of use of the extraction device in commercial strawberry crops.

Task 1.2.

In 2016, when multiple releases of high numbers of *N. cucumeris* were made in small field plots, very few predators were recovered from flowers or button fruit after release. Some commercial growers have also reported finding very few or no predators in flowers or on fruit after multiple releases. In order to make rational decisions on release and sampling strategies for *N. cucumeris*, it is important to determine whether the mites are present on other parts of the plant, or if they are not surviving in the crop. In the first year of the project, the scientists recorded numbers of thrips and *N. cucumeris* on different aged flowers and fruits but did not record numbers on other plant parts. It is important to understand mite distribution on the plants as results will guide more effective sampling strategies, including the effective use of the prototype extraction device. Two experiments were set up to address the questions: Where do the mites disperse to when released onto the plant? What is the best plant part to sample to assess populations? Does the presence of WFT on the plants affect distribution of *N. cucumeris*? Is there a diurnal pattern of movement of *N. cucumeris* on strawberry button fruits and flowers?

Summary of the project and main conclusions

Task 1.1.

In laboratory experiments, single or groups of 10 button fruits were inoculated with known numbers of *N. cucumeris*. Mites were then extracted using the device containing MIK for 20 minutes and then fruits were washed further with ethanol to remove any remaining mites.

In addition, field assays tested the efficacy of the MIK and extraction device. Fruits were initially inspected using a hand lens, then arthropods extracted with the MIK in the extraction device before washing the fruit back in the laboratory with ethanol to remove further arthropods.

In the laboratory, from individual fruits placed in the extraction there was a close correlation between the numbers of *N. cucumeris* released and the numbers recovered (R^2=0.987) which indicated that around 57% of the mites that are actually present on the fruit were recovered.

When groups of 10 fruit were inoculated, the same calibration revealed that the device extracted about 60% of mites present on the fruit (R^2=0.993).

In the field test, no *N. cucumeris* could be seen on the fruit using a hand lens. However, the device recovered 27% of mites from button fruit and 5% from flowers. It was also possible to assess the presence of other arthropods on button fruit and flowers using the device. 68% and 81% of WFT were extracted using the device from button fruit and flowers, respectively.

The extraction device also increased detection of *Orius* on both button fruit (direct observation 26%; extraction device 85%) and flowers (direct observation 55%; extraction device 94%).

Hence the device can be used to make estimates of *N. cucumeris* in the field giving approximately 30% and 5% of the actual numbers present on fruit and flowers, respectively.

Task 1.2.

In a glasshouse experiment, to assess the distribution of *N. cucumeris* on strawberry plants after release, eighteen plants were placed in each of two glasshouse compartments at NIAB EMR. WFT from laboratory cultures were released onto plants in one compartment at approximately 20 mixed stages per plant; the second compartment had no WFT released. Five days after WFT release *N. cucumeris,* from a commercial supplier, was released onto each plant in both compartments at a rate of ~200 mites per plant. One, four and seven days after release, six plants were randomly selected from each treatment. Numbers of each plant part at the time of sampling were recorded and the plants were destructively sampled in the glasshouse; all plant parts were separated into closed containers. Plant parts assessed were: old leaves, recently expanded leaves, folded leaves, flowers, button fruit, remaining fruit, developing fruit clusters and the crown. In addition, a sample of the *N. cucumeris* carrier material from the leaf surfaces of each plant was taken. Numbers of *N. cucumeris* and WFT were counted from the different plant parts to assess distribution over the plant after release and the data analysed to determine if there were differences in *N. cucumeris* distribution when prey was present.

Results showed that, as in earlier studies, most WFT were found on the strawberry flowers and fruits. Most *N. cucumeris* had dispersed from the carrier material within one day of release, but around 50% of the total numbers of mites released were not recorded on the plants. *N. cucumeris* were recorded on all assessed plant parts but there were low numbers on the leaf samples. In the overall analyses of the results the presence of prey affected the distribution of *N. cucumeris* on the plants; there were significantly higher numbers of *N. cucumeris* on both flowers and fruits in the treatment where WFT had been released. These results confirmed earlier work that button fruit were the most effective plant parts to assess populations of *N. cucumeris* in the crop and highlights that the presence of prey (WFT) has a significant effect on the distribution of the predator.

In a following field experiment on a commercial crop to determine if there is a diurnal pattern of movement of *N. cucumeris* over the plant, several introductions of *N. cucumeris* were made. Data loggers were used to record temperature and humidity throughout the experimental period, and the photosynthetically active light levels (400-700 nm) were also

monitored. Button fruit and flower samples were taken five times during the day; 09.00; 12.00; 15.00; 18.00 and 21.00. Sampling was repeated on three days, with a one day gap between the first two samples and a four day gap between the second and third sample to allow the plants to recover and produce more open flowers and button fruits. Each assessment unit consisted of 10 flowers or 10 button fruit. These bulk samples were collected into ethanol and arthropods were extracted using our standard laboratory washing technique. Numbers of *N. cucumeris,* thrips adults and larvae and *Orius* adults and nymphs were counted. Arthropods recorded on the sample units in relation to sampling time and date, position within the tunnel, and environmental conditions (mean temperature and mean light intensity for the 60 mins before each sample) were analysed.

There was a diurnal pattern of movement of arthropods on strawberry. In the overall statistical analyses of the data, the mean temperature in the hour prior to sampling significantly affected the number of arthropods recorded in samples of flowers and button fruits. No other variable tested had any effect on arthropod distribution. Numbers of *N. cucumeris* declined by around 3% for every 1°C increase in mean temperature calculated per hour, over the range recorded in the experiment (18-33°C). Predatory *Orius* adults and WFT adults were recorded in higher numbers as the mean temperature increased whereas WFT larvae decreased in abundance. Numbers of *N. cucumeris* are likely to be lower in flowers and button fruit at higher temperatures. Therefore if very low numbers are recorded in samples it would be worth revisiting the plantation when temperatures have decreased to confirm establishment of the predator.

Financial benefits

Western flower thrips (*Frankliniella occidentalis*) causes bronzing of fruit. It has become difficult to control because of resistance to crop protection products and a lack of effective alternative biological controls. Financial losses can be high, exceeding £15m to the UK industry alone in 2013. This project is testing new approaches to monitoring and control of WFT whilst maintaining control of other pests, particularly by conserving and improving efficacy of introduced arthropod biocontrol agents and entomopathogenic fungi in the crop.

Action points for growers

- Sample button fruit to determine establishment of *N. cucumeris* in the crop.

- If temperatures are high, it is likely that fewer *N. cucumeris* will be found in the fruitlets and flowers and re-sampling to ascertain establishment may be needed.
- Avoid sampling for *N. cucumeris* in the mid-day heat.
- Sample mid-aged flowers to determine thrips numbers in the crop.
- Consider reducing the number of repeated applications of tank mixes of plant protection products as these may be harmful to introduced *N. cucumeris*.
- Careful thought needs to be given to the tank mixes used, ensuring that thrips and tarsonemid control is achieved early before SWD enters the crop and requires treatment.
- Reduce use of crop protection products where possible to ensure that *N. cucumeris* gains control of WFT before SWD control is needed.

Integrating pesticides with phytoseiid mites

Objective 2 - Refine pest control programmes on strawberry, integrating pesticides with phytoseiid mites.

In Year 3 of the project, the work on potato aphid concentrated on Task 2.2.

Task 2.2. In field, effect of insecticides commonly used to target spring aphids on the establishment of N. cucumeris, aphids and parasitoids

Headline

- Repeated applications of some fungicides can cause reductions of *N. cucumeris* numbers in the crop. This can be alleviated by further applications of *N. cucumeris.*

Background and expected deliverables

Predatory mites such as *Neoseiulus cucumeris* can form a very successful part of Integrated Pest Management (IPM). However, they can be vulnerable to plant protection products, including, potentially, fungicides. In addition, increased use of plant protection products against other pests, such as SWD, can potentially interfere with IPM. Although some plant protection products have been shown to be safe or only slightly harmful to *N. cucumeris* in single applications, in the field, products are applied multiple times, and in tank mixes. In Year 1 of the project, the scientists demonstrated that tank mixes of Nimrod/Teldor and Signum/Systhane and Aphox/Rovral had a detrimental effect on *N. cucumeris* numbers in strawberry. However, adverse effects were only statistically significant after the third spray application, suggesting that previous studies in the literature might have underestimated the toxicity of these products to *N. cucumeris* under normal commercial usage.

In Year 2, the science team tested Calypso (thiacloprid) and potassium bicarbonate+Activator90, products that the industry had suggested could be harmful to *N. cucumeris* over multiple applications or in tank mixes. These were compared to Nimrod+Teldor applications, a treatment tested in the previous year. We also tested whether a secondary addition of *N. cucumeris* could mitigate any effects of these spray treatments.

N. cucumeris were released onto strawberry plants before the trial began and three applications of plant protection products were applied, with assessment of adult and immature *N. cucumeris* numbers on button fruit made after each application. No evidence was found that Calypso, potassium bicarbonate+Activator90 or Nimrod+Teldor had a detrimental effect on *N. cucumeris* populations. An additional release of *N. cucumeris* after the second spray treatment led to an increase in adult *N. cucumeris* in the crop.

Neither Calypso nor the secondary addition of *N. cucumeris* had a significant effect on thrips numbers. However, there were significantly lower numbers of thrips in the potassium bicarbonate+Activator90 treated plots compared to the water controls. The reason for this was not clear.

Data on the introduction of *N. cucumeris* following a pesticide application is generally based on laboratory side-effects tests and does not consider timing, temperature or leaf expansion. A study began in March 2018 to test, in-field, effects of insecticides commonly used to target spring aphids on the establishment of *N. cucumeris* and other potential predators in the crop.

Summary of the project and main conclusions

Results will be reported at the field meeting in 2018 and reported in full in the 2019 annual report.

Financial benefits

From a pest like western flower thrips (WFT), strawberry growers can typically lose 20% or more of their fruit. For a crop yielding 30 tonnes/ha, this equates to 6 tonnes/ha and at a value of £2,400 per tonne, losses of £14,400 per hectare.

Frequent introductions of high numbers of predatory mites such as *Neoseiulus cucumeris* are not only expensive to purchase, but costly to introduce by hand. Potential damage or disruption to the mites caused by the use of harmful fungicide mixes or other crop protection products will lead to reduced efficacy of control and hasten the onset of WFT induced damage, resulting in further financial losses.

It is therefore vital that growers are better informed of those fungicide mixes or other products which may have an adverse effect on the expensive predatory mites which have been introduced.

Action points for growers

- Consider reducing the number of repeated applications of tank mixes of plant protection products as these may be harmful to introduced *N. cucumeris*.
- Careful thought needs to be given to the tank mixes used, ensuring that thrips and tarsonemid mite control is achieved early before SWD enters the crop and requires treatment.

IPM controls for capsids and blossom weevil

Objective 3. Develop IPM compatible controls for European tarnished plant bug (*Lygus rugulipennis*), common green capsid (*Lygocoris pabulinus*) and strawberry blossom weevil (*Anthonomus rubi*)

In Year 3 of the project, the work on capsids was broken into Tasks 3.1 and 3.2

Task 3.1. To investigate the potential of a multi-pheromone blue sticky trapping system for Lygus rugulipennis, Lygocoris pabulinus and Frankliniella occidentalis

Task 3.2. To investigate the potential of a push-pull system for control of capsids in strawberry

Headline

- Some early success has been gained in reducing capsid numbers in strawberry crops using a novel 'push-pull system' of control.

Background and expected deliverables

Task 3.1.

In strawberry, western flower thrips, *Frankliniella occidentalis* (WFT), causes bronzing of the fruit. It has become difficult to control because of resistance to crop protection products and lack of effective alternative biological controls. Financial losses can be high, exceeding £15m to the UK industry alone in 2013. From June onwards European tarnished plant bug, *Lygus rugulipennis*, becomes a damaging pest of strawberry requiring routine control. Feeding in flowers and on green fruits can cause up to 80% crop loss, rendering production uneconomical. Traditional crop protection products used for control can disrupt biological control agents and increase residues in fruits. *Lygocoris pabulinus* (common green capsid) is also a damaging pest, which tends to be sporadic in appearance and locally distributed within the crop.

Blue sticky traps are currently employed for WFT control. These can be enhanced with a WFT aggregation pheromone, which can typically double the catch. If these could also be used in conjunction with capsid pheromones this would potentially provide in-crop control of potentially three pest species. *L. rugulipennis* is currently trapped using a *Lygus* sex pheromone lure within a green bucket trap and cover; catches, including of females, can be increased with the addition of the plant volatile phenylacetaldehyde (PAA). The trapping

system for *L. pabulinus* uses the same pheromone lure, but attached to a blue sticky trap placed vertically in the crop.

Task 3.2.

Push-pull strategies have both an element which repels insect pests (the push), and an attractant source to draw the pest away from the crop (the pull). In addition the pull can be combined with a killing agent to prevent the pest re-entering the crop and to reduce population growth. Using synthetic semiochemicals, a push-pull system could be deployed to enable medium-term control of capsids. This study investigated whether; 1) the capsids, *L. rugulipennis* and *L. pabulinus*, could be repelled from a strawberry crop using hexyl butyrate (push system), 2) perimeter pheromone trapping system (pull system) could be used in conjunction with the repellent system for improved efficacy and 3) whether *Lygus* damage (i.e. cat-facing of the fruit), was reduced where treatments were applied.

Summary of the project and main conclusions

Task 3.1.

We investigated whether *L. rugulipennis* and *L. pabulinus* can be attracted to blue sticky traps with the addition of a *Lygus* sex pheromone lure + PAA only or whether the *Lygus* pheromone + PAA can be used in conjunction with the WFT pheromone, and, finally, if beneficial arthropods are also attracted to the trapping system.

Experiments were set up in multiple strawberry crops in mid to late June and covered a two-month period within 2017. Treatments included: 1) Blue dry sticky trap board - 25 cm x 10 cm, 2) blue dry sticky trap board + WFT pheromone lure, 3) blue dry sticky trap board + *Lygus* sex pheromone lure + PAA or 4) blue dry sticky trap board + WFT pheromone lure + *Lygus* sex pheromone lure + PAA. Traps were placed 10 m apart in a randomised block design.

As expected, *L. rugulipennis* and *L. pabulinus* were attracted to a blue sticky trap with *Lygus* sex pheromone + PAA. However, 20% of capsids could detach themselves from the blue sticky traps. The *Lygus* sex pheromone lure + PAA was compatible with the WFT pheromone and thrips catches were always higher when a WFT lure was present.

The PAA lure also appeared to attract lacewings and syrphids. PAA is essential to increase catches of the female *L. rugulipennis* however; the floral component may be detrimental to some beneficial species.

Task 3.2.

A field experiment was set up as a randomised block design, with four tunnelled strawberry crops as replicates. Each treated area was a 25 m x 25 m plot. Treatments included:1) Push - Hexyl butyrate (HB) sachets every 2 m, 2) Pull - Lygus sex pheromone + PAA in green "bucket traps" every 8 m around the perimeter of the plot, 3) Push–Pull – treatment 1 and 2 combined or 4) control plot with no traps or repellents. The experiment ran for two months from 4 July and the effect on capsid numbers throughout the season and resultant fruit damage was monitored.

There were significantly fewer adult and nymph *L. rugulipennis* where the 'push' was applied compared to where the 'push' was not applied. Differences were not statistically significant for *L. pabulinus* adults and nymphs, although overall numbers were lower where a treatment was applied. There was no significant effect of 'pull' only treatment when used alone.

There was also significantly less fruit damage where there was a 'push' treatment and a 'pull' treatment were combined compared to no treatment. To our knowledge this is the first study to show that a push-pull strategy could give significant control of capsids.

Financial benefits

Lygus rugulipennis (European tarnished plant bug) and *Lygocoris pabulinus* (common green capsid) are serious pests on everbearer strawberries causing crop losses by feeding on developing fruits which become deformed and unmarketable. Over 50% of fruit may be downgraded as a result of capsid feeding in unsprayed crops. The development of improved trap and monitoring systems for capsids will help growers to identify the exact time of their appearance in the crop, allowing control measures to be implemented at the optimum time. Should traditional spray control products be employed, the numbers required can be reduced by applying at the optimum time, saving money on unnecessary sprays. Novel control methods such as the 'push-pull system' will help to reduce reliance on traditional control products, which will further reduce crop protection costs for growers. Such a system will also enhance biological control methods employed for other pests, increasing their efficacy and reducing the need to introduce additional numbers of predatory mites, further reducing costs.

Action points for growers

- It is too early to identify any positive action points from the work on this objective so far.

Potato aphid

Objective 4. Improve insecticide control of the potato aphid, *Macrosiphum euphorbiae,* so as to be more compatible with IPM programmes.

In Year 3 of the project, the work on potato aphid concentrated on Task 4.2.

Task 4.2. Determine the effect of low and fluctuating temperatures on the ability of aphid parasitoids to parasitise the potato aphid, Macrosiphum euphorbiae.

Headline

- The parasitoids *Aphidius ervi* and *Praon volucre* require minimum temperatures of 8°C and 12°C respectively to effectively parasitise the potato aphid.

Background and expected deliverables

Several species of aphid are regularly found infesting strawberry crops. Five of the most frequently found and most damaging are the strawberry aphid (*Chaetosiphon fragaefolii*), the melon and cotton aphid (*Aphis gossypii*), the shallot aphid (*Myzus ascalonicus*), the glasshouse-potato aphid (*Aulacorthum solani*) and the potato aphid (*Macrosiphum euphorbiae*).

In recent years the control of early season aphids such as the potato aphid has become more problematic due to the withdrawal of commonly used insecticides. The remaining chemical options often have limited efficacy (AHDB Projects SF 140 and 156) and there is little evidence that biological controls are effective at the low temperatures experienced in early spring. The potato aphid causes damage to the crop through the production of honeydew and cast skins which result in sooty moulds and make the fruit unmarketable. Feeding action of these aphids can also result in distortion of the leaves and fruit. The species may breed all year round on strawberry crops if conditions allow and populations can build up rapidly in the spring.

Two aphid parasitoid species (*Aphidius ervi* and *Praon volucre*) commonly found in strawberry crops are known to readily parasitise potato aphid and may contribute to control. Both species occur naturally in the environment but can be introduced as biological control products as either a single species in the case of *A. ervi* or as part of a mix of six parasitoid

species (*Aphidius colemani, A. ervi, A. matricariae, Praon volucre, Ephedrus cerasicola* and *Aphelinus abdominalis*).

Temperature is a key factor in determining the developmental time of insect species. Current knowledge suggests that the lower developmental threshold of *P. volucre* from the egg to mummy stage is 3.8°C and for mummy to adult development is 5.5°C. In comparison, the lower developmental thresholds for egg to mummy development and mummy to adult development of *A. ervi* in *Sitobion avenae* are 2.2°C and 6.6°C respectively. Although parasitoid development at low temperatures is extremely slow, *A. ervi* has been found to have a negative effect on pea aphid reproductive capacity following oviposition. This suggests that even if the parasitoid larvae do not kill the adult aphids as quickly early in the season, they may still be effective at reducing aphid populations.

Temperature can also affect the ability of the parasitoid to successfully locate and parasitise the aphid. Previous work has shown that oviposition by *A. ervi* and *P. volucre* on the grain aphid remained low below 10°C in both species. Flight and walking activity both increased with temperature, with *A. ervi* being consistently more active than *P. volcure*. The lower flight threshold was 10°C for both species and walking activity continued down to 8°C. This suggests that these parasitoid species would still be capable of locating aphids at low temperatures early in the season.

The aim of this work was to determine the effect of low and fluctuating temperatures on the ability of *A. ervi* and *P. volucre* to parasitise the potato aphid.

Summary of the project and main conclusions

Air temperatures recorded in a polytunnel and an unheated glasshouse located in West Sussex confirmed that from early in the year, temperatures were above minimum thresholds for parasitoid activity. In the studied polytunnel, air temperatures rose above 12°C for at least 18% of the time in the month of February 2014, increasing to 33% in March and 52% in April. In the studied unheated glasshouse, air temperatures rose above 12°C for at least 11% of the time in the month of February 2015, increasing to 33% in March and 82% in April.

A series of experiments were completed under controlled temperature conditions. Each experiment used an unfurled strawberry leaf placed in a glass Petri dish with the stem immersed in 2.5 ml of water. The leaf was infested with 10 potato aphid nymphs and conditioned at the treatment temperature for 24 hours prior to the start of the experiment. Mated female parasitoids were separated out into a different glass Petri dish with access to

a 20% sugar solution and conditioned similarly. Two female parasitoids were introduced to each dish of aphids and left for 24 hours at the treatment temperature. The parasitoids were then removed and the aphids were maintained on the strawberry leaf at 20°C for a further seven days before they were dissected to determine if parasitism had occurred. To confirm parasitoid larval development at low temperatures, additional replicates of parasitised aphid treatments and 20 mummies of each species were maintained at the lowest constant temperature at which parasitism was previously observed.

The minimum temperature at which parasitism of potato aphid by *A. ervi* occurred under constant conditions was 8°C. The minimum temperature at which parasitism of the same aphid species by *P. volucre* occurred under constant conditions was 12°C. There were a greater number of dishes with parasitism occurring in *A. ervi* compared to *P. volucre* as a result of the lower temperature threshold. Development of parasitoid larvae inside the aphid host was confirmed for both species of parasitoid in aphids maintained at constant low temperatures for two weeks. Similarly, adult emergence from aphid mummies was also confirmed at these constant low temperatures for both species.

Where temperatures fluctuated between 2°C and then eight hours at 8, 13 or 18°C, the minimum temperature at which parasitism by *A. ervi* occurred was 8°C. The minimum temperature at which parasitism by *P. volucre* occurred under fluctuating conditions was 13°C.

Both parasitoid species responded to higher temperature fluctuations (8°C for *A. ervi* and 13°C for *P. volucre*) and parasitised aphids in less than two hours when switched from 2°C.

Financial benefits

Potentially, if not controlled, aphid infestations can lead to complete crop loss. No quantitative data on industry average losses resulting from aphid infestation is available but conservatively assuming that 1% of the crop is lost, this is equivalent to 507 tonnes of strawberries; worth £2.1 million per annum. Improved control as a result of this work would reduce the scale of these losses considerably.

Action points for growers

- Consider autumn applications (post-harvest) of insecticides for aphid control as these have been shown to reduce populations of aphids found in crops the following year.
- Carefully monitor both aphid numbers and their associated natural enemies within crops in order to determine the need for insecticide sprays. Do not treat all fields the

same. Consider the species of aphid prevalent and the damage it may cause, including plant virus spread.

- Where spring applications of insecticides are considered necessary, growers should ensure that there is good spray coverage, in particular the undersides of leaves and the crown of the plant. Consider the use of water sensitive papers to visualise how effectively spray applications achieve this.
- Some populations of aphid pests e.g. the melon and cotton aphid (*Aphis gossypii*) have developed insecticide resistance. Growers should ensure that they follow insecticide resistance management guidelines on the product label and rotate between insecticides with different modes of action.
- It is important to carefully consider the compatibility of the available insecticide options with aphid natural enemies as well as the biological control programmes used to control other pests of strawberry crops.
- Consider early season releases of *Aphidius ervi* to control potato aphid when daytime temperature exceed 8°C regularly for at least part of the day. *Praon volucre* is currently only available as part of a mix of parasitoid species (including also *A. ervi*) and may also be considered for releases when daytime temperatures exceed 12°C regularly for at least part of the day.
- Although aphid parasitism may occur at low temperatures, the development of the aphid parasitoid will be very slow at these temperatures and may take several weeks to complete. The absence of mummified aphids does not, therefore, reliably indicate lack of parasitoid activity. Carefully monitor aphid populations within crops for presence of adult parasitoids. If possible, move some aphid infested plants to a warmer environment for 7-10 days, checking regularly for presence of mummified aphids.

Aphid control

Objective 5. Improve control of aphids through the growing season.

In Year 3 of the project, the work on potato aphid concentrated on Task 5.1.

Task 5.1. Thresholds for aphids and natural enemies; assessments to demonstrate confidence in control strategies.

Headline

- Before June, there are very few natural enemies in strawberry crops and therefore other control measures should be employed to supress aphid populations until natural numbers build.

Background and expected deliverables

Strawberry crops are affected by a range of aphid pests. The most difficult to control is the potato aphid, as populations often resurge after spray application, probably due to incomplete control as shown in AHDB Project SF 140. In this project, it was also found that aphid numbers in the untreated plots had a tendency to decline rapidly by the end of the experiments because of the increases in natural enemies.

Crop protection sprays can be harmful to natural enemies which might otherwise be controlling pests in the crop. Often there is a lag between the build-up of the pest and the immigration and build-up of the predators and parasitoids. This lag period is often a critical time for the build-up of the natural enemies, but a time when sprays for aphids are more likely to be applied.

The aim of this study was to monitor and demonstrate the importance of naturally occurring aphid enemies in everbearer and June bearer strawberry crops. We compared three crops in both Junebearer and everbearer fields for aphid build-up in the crop, in relation to natural enemy appearance. We also aimed to demonstrate the effects of pest spray programmes on potato aphid and natural enemies and show the relationship between population ‘peaks and toughs’ of pest and natural enemies. Studies were made on two farms with historically different degrees of aphid and natural enemy numbers. On each farm, three Junebearer and three everbearer fields were selected. To obtain an overall picture of the changes in natural enemy populations throughout the year, fields were chosen within the same or as similar a landscape as possible on the farms. Hence they had the same potential pool of pests and natural enemies.

Summary of the project and main conclusions

Both farms were visited each week from 5 April until 30 August. At each visit, 25 plants were thoroughly searched in a different central row of the cropping area and the numbers and species of aphids and natural enemies were counted and plotted.

There was a high variability in aphid species and numbers between farms and between crops in the same landscape. The main pest was potato aphid although other pests (*Aphis gossypii*, thrips, two-spotted spider mites and glasshouse whitefly) were present. Winged aphids peaked on 9 June. The main aphid predators recorded were the green lacewing and hoverfly larvae. Hoverfly larvae were present in low numbers across the two farms through the season and green lacewing larvae became more prevalent from 4 July. It is known that a single larva of the marmalade hoverfly (*Episyrphus balteatus*) can consume 660-1,140 aphids during development and a single green lacewing larva 566-789 aphids before pupating. Other predators, such as spiders, ladybirds and *Orius* were also observed in low numbers.

The parasitoids *Praon* sp. and *Aphidius* sp. were the main species parasitising aphids. *Aphelinus* sp. parasitism was also present but at a lower incidence.

The pest and natural enemy fauna was more diverse in the ever-bearers than in the June bearers. In both crop types, there were delays in the natural enemy's population growth compared to the pest population growth. However, with the increase of natural enemies, the number of aphids declined. It is evident from this study, so far, that before June there are very few natural enemies in strawberry crops and therefore other control measures should be employed to supress aphid populations until natural numbers build. Controls introduced by growers should be sensitive to the natural enemies likely to enter the crop later in the season.

Financial benefits

Potentially, if not controlled, aphid infestations can lead to complete crop loss. No quantitative data on industry average losses resulting from aphid infestation is available but conservatively assuming that 1% of the crop is lost, this is equivalent to 507 tonnes of strawberries; worth £2.1 million per annum. Improved control as a result of this work would reduce the scale of these losses considerably.

Action points for growers

- Consider carefully early season applications of pesticides and wherever possible select products that are likely to be less harmful to aphid parasitoids and *N. cucumeris* that may or may not be obvious within the crop. Use either

https://www.koppert.com/side-effects/ or http://www.biobestgroup.com/en/side-effect-manual to help inform product selection.

SCIENCE SECTION

Objective 1. Develop effective biological methods for managing western flower thrips, Frankliniella occidentalis (WFT), compatible with pesticide use for control of spotted wing drosophila, Drosophila suzukii (SWD)

1.1 Develop and determine the efficacy and ease of use of the prototype extraction device for WFT and the predatory mite N. cucumeris in commercial strawberry crops, by agronomist and growers

Introduction

In 2015, methyl isobutyl ketone (MIK) was shown to be effective as a fumigant to extract arthropods from button fruit, with higher numbers recorded by extraction compared to 'by eye' assessments of flowers or fruits (see 2016 Annual Report). Three prototype monitoring devices, making use of this fumigant extraction method, were constructed (Fig. 1.1.1). Based on grower/advisor feedback on the different designs and prototypes, a "Tupperware" type device (Prototype 2 in Fig. 1.1.1) was chosen for further development based on its robustness, ease of use, and transparency. A few modifications were required, and to increase the ease of counting a segmented counting surface has been included.

1. Tin extraction device (10 x 10 cm)

2. Tupperware extraction device (10 x 10 cm)

3. Tiffin tin stainless steel extraction device (10.5 cm dia. X 9 cm height)

Figure 1.1.1. Prototype extraction devices sent to advisors for field testing. Prototype 2 was the preferred device based on the feedback received.

Following initial laboratory studies to assess the efficacy of the device in extracting thrips and mites from flowers and fruit (see 2017 Annual Report), further laboratory experiments were carried out in the summer and autumn of 2017 to achieve a more thorough calibration of the device with *N. cucumeris*. Field studies were also done during the summer in order to explore the efficacy and ease of use of the Prototype 2 extraction device in commercial strawberry crops, by agronomists and growers.

Methods

Laboratory experiments: Two laboratory trials were carried out testing different densities of *N. cucumeris* on individual and groups of button fruit.

Trial 1 - Inoculation of N. cucumeris *on individual button fruit.*

Button fruit (variety “Finesse”) were inoculated with 5 densities of mites, each with 15 replicates. An individual button fruit (Fig. 1.1.2) was placed in a clear 70 ml container and inoculated with either 0, 1, 3, 5 or 10 *N. cucumeris* mites. Individual adult female mites were transferred directly to the button fruit surface using a fine sable haired paint brush under a dissecting stereomicroscope (X60 magnification), and sealed in the container using stretched Parafilm. All containers were incubated overnight at ~20^{o}C before extraction sampling.

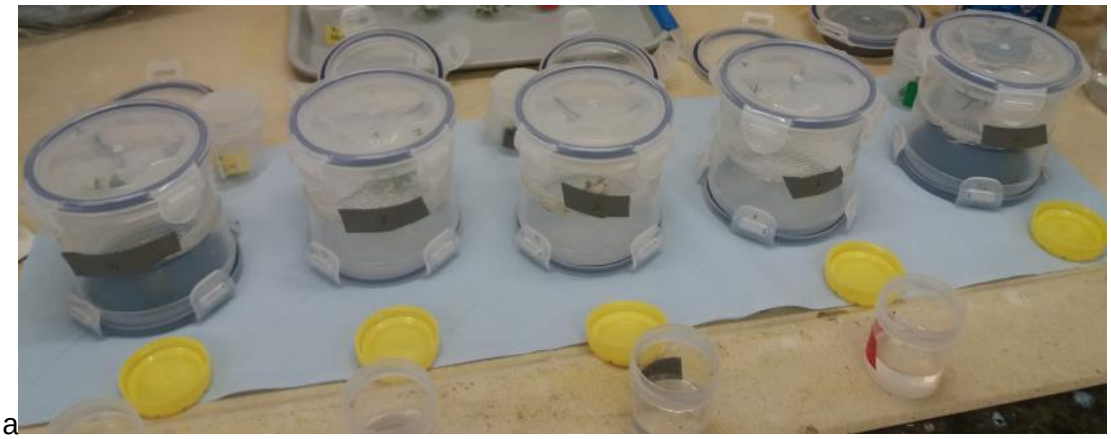
Trial 2 - Inoculation of N. cucumeris on 10 button fruits.

Groups of 10 button fruits (“Zara”) were inoculated with either 0, 10, 20 or 50 adult female *N. cucumeris* mites. Fifteen replicates of each inoculation density were prepared. Mites were collected using a filter pipette tip connected via tubing to a vacuum pump (Fig. 1.1.2), and transferred to a clear 315 ml plastic container holding 10 button fruits. Containers were sealed with plastic lids. All containers were incubated overnight at room temperature before extraction sampling.

Extraction was done in a fume hood to avoid the inhalation of the fumigant. One fumigant dispenser vial, containing 1000 mg of methyl isobutyl ketone (MIK) (Fig. 1.1.2), was opened and placed in the centre of the top compartment of each extraction device together with the fruit. When working with groups of 10 fruit, these were arranged in a single layer within the device. The device lid was then sealed and the fumigant left to act for 20 minutes. During this

time the empty overnight containers were checked under a microscope for the presence of *N. cucumeris*.

After the 20 minutes each device was tapped against the worktop twice before opening to release any insects trapped in the mesh. The button fruits were removed and placed in 70% ethanol back in the overnight container and later assessed using 70% ethanol wash mite extraction procedure (SOP 780). The bottom compartment was removed and specimens were identified and counted by examining the bottom lid of the device under a dissecting stereomicroscope, at X60 magnification. Bottom lids were cleaned with dry tissue to remove any remaining fruit debris or arthropods between uses.

a 

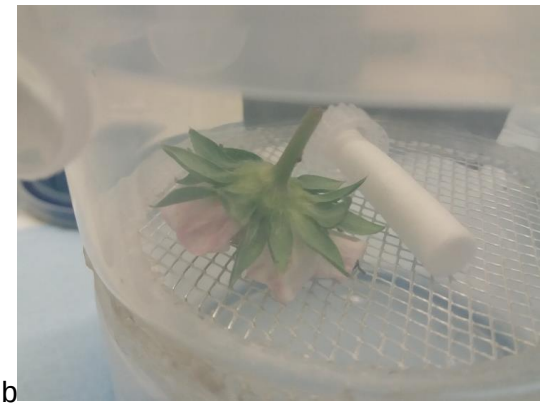
b 

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Figure 1.1.2. a) Prototype 2 extraction devices set up for experiment 1, b) single flower with MIK dispenser, c), d) 10 button fruit in device e) pooting *N. cucumeris*

Field experiments: A field site was selected on table top strawberries, variety Amesti. The crop was 2 years old with a history of WFT and TSSM (*Tetranychus urticae*) pest problems. Medium-sized button fruit and flowers were collected in the field, within 5 days of an application of *N. cucumeris* to the crop (Bioline Amblyline loose product) which was applied either by the grower and/or topped up by NIAB EMR staff. Each fruit was initially inspected using a hand lens (X20 magnification) and the numbers of thrips, mites, pest and other predators visible on each fruit recorded. Twenty fruit were placed into the Prototype 2 device (Fig. 1.1.1), arranged in a single layer, the lid replaced and left for 20 minutes within the cropping area for the MIK fumigant (dispensers initially contained 1000 mg of MIK, as for laboratory studies) to extract any arthropods present. The numbers of mites, WFT and other arthropods of note (e.g. *Orius*, lacewing larvae, etc.) were recorded, using a hand lens to examine the upper surface of the removable bottom lid of the device. Following extraction sampling, the fruit from the device was transferred to tubes of 70% ethanol and returned to the laboratory for washing and counting, following SOP 780. This methodology was repeated for samples of 20 strawberry flowers. Experiments with both flowers and fruit were repeated on 3 occasions (24 Jul, 8 Aug and 17 Aug), with 6 replicates of each plant structure collected on each occasion (placing 20 individual fruit or flowers in the device each time).

Numbers of arthropods observed in each set of fruit or flowers were used to calculate percentage detection, as a proportion of the total numbers present (total present = numbers extracted using the device plus numbers recovered using the ethanol wash technique).

Results

Trial 1 - Inoculation of N. cucumeris *on individual button fruit*

Across the inoculation densities; 1, 3, 5 and 10 mites, 43% of the released *N. cucumeris* were recovered in the extraction device. A further 3% of the released mites were recovered in the overnight incubation containers, and 35% of mites were later recovered from fruit via the 70% ethanol wash method. A total of 81% of the released mites were therefore accounted for through later recovery during this trial. The button fruit were collected from protected commercial production, and it is possible that some of them already harboured mites before the start of the experiment, meaning that some of the *N. cucumeris* that were recovered and counted may have been external contaminants. However, only two *N. cucumeris* were found using the 70% ethanol wash method (and none using the extraction device) in the “zero mite” control treatment, suggesting that contaminating mites were present but at very low numbers and would have had very little impact on the results of the experiment.

In order to calibrate the fumigation technique in terms of its success in extracting mites that are present on the fruit, the mean number of extracted mites was calculated for each original inoculation density, and plotted against the mean number of mites actually present on the fruit at the time of extraction (i.e. numbers extracted using the device plus the numbers recovered via the 70% ethanol wash method). Linear regression revealed a close correlation between these variables (R^2=0.987), and the trend line (y=0.57x + 0.07) indicates that the extraction device recovers approximately 57% of the mites that are actually present on the fruit (Fig. 1.1.3).

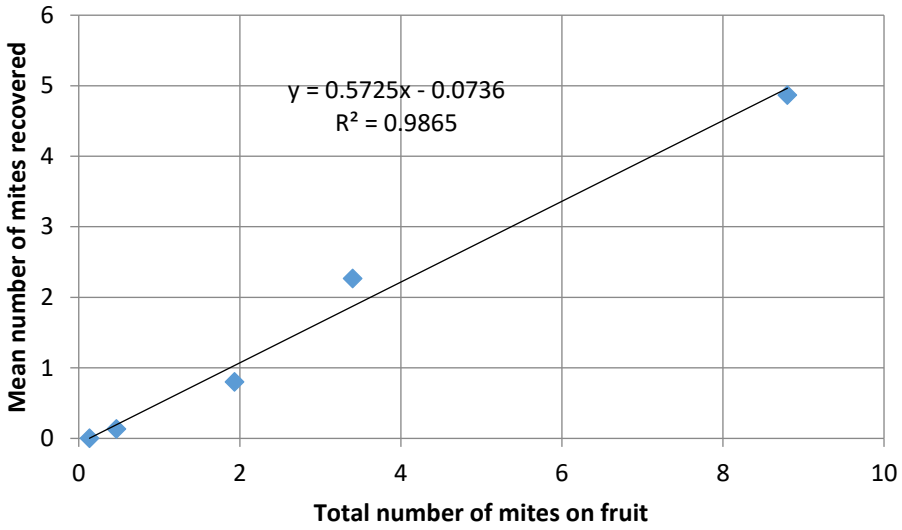


Figure 1.1.3. Linear regression showing the relationship between mean number of mites recovered using the extraction device and the number known to be present on the fruit at the time of extraction (number recovered using device + number subsequently recovered from fruit using the ethanol wash technique), based on the experiment where individual button fruit were inoculated with mites.

Trial 2 - Inoculation of N. cucumeris *on 10 button fruits*

When groups of 10 fruit were inoculated with different numbers of mites (0, 10, 20 or 50 individuals), 30% of the released *N. cucumeris* were recovered in the extraction device overall. A further 20% of the mites were recovered in the overnight incubation containers, and 24% of mites were later recovered from fruit via the 70% ethanol wash method. A total of 74% of the released mites were therefore recovered via one of the three modes of detection during this trial, a lower proportion than the previous trial where mites were transferred directly to fruit using a brush. Only two *N. cucumeris* were detected in the “zero mite” control treatment, despite the use of 150 fruits in total, suggesting that background levels of natural mite infestation were very low on the fruit used in this experiment.

The same calibration approach previously applied to the individual fruit extraction trial was repeated for this second experiment with larger numbers of released mites. The mean number of mites extracted using the device was again calculated for each original inoculation

density, and plotted against the mean number of mites actually present on the fruit at the time of extraction (i.e. numbers extracted using the device plus the numbers recovered via the 70% ethanol wash method), as an estimate of extraction success. Linear regression revealed a close correlation between these variables (R^2=0.993), and the trend line (y=0.60x - 0.49) indicates that the extraction device recovers approximately 60% of mites present on the fruit (Fig. 1.1.4).

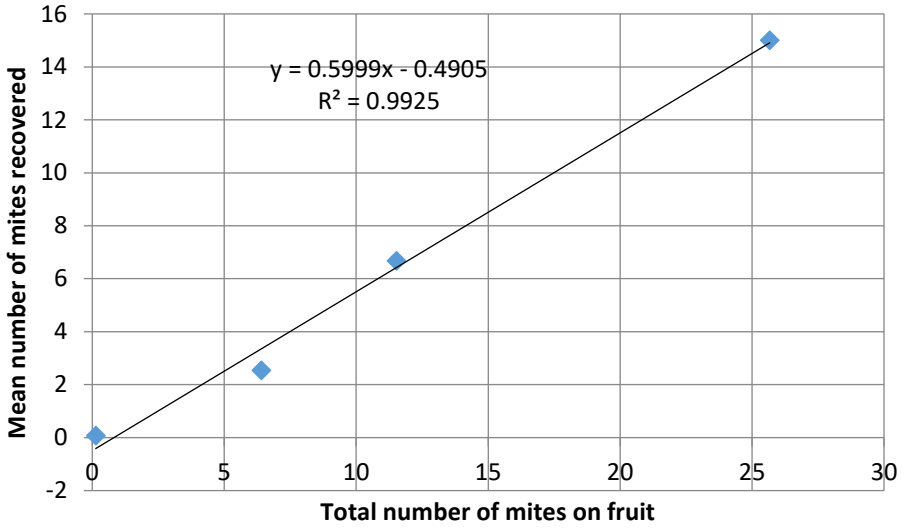


Figure 1.1.4. Linear regression showing the relationship between mean number of mites recovered using the extraction device and the number proven to be present on the fruit at the time of extraction (number recovered using device + number subsequently recovered from fruit using the ethanol wash technique), based on the experiment where groups of 10 button fruit were inoculated with mites.

No *N. cucumeris* were observed on the plant surfaces using a hand lens, but the extraction device revealed the presence of *N. cucumeris* on button fruit and flowers in the field. The device recovered 27% of mites on button fruit, but only 5% of mites present on flowers were extracted from flowers (Fig. 1.1.5).

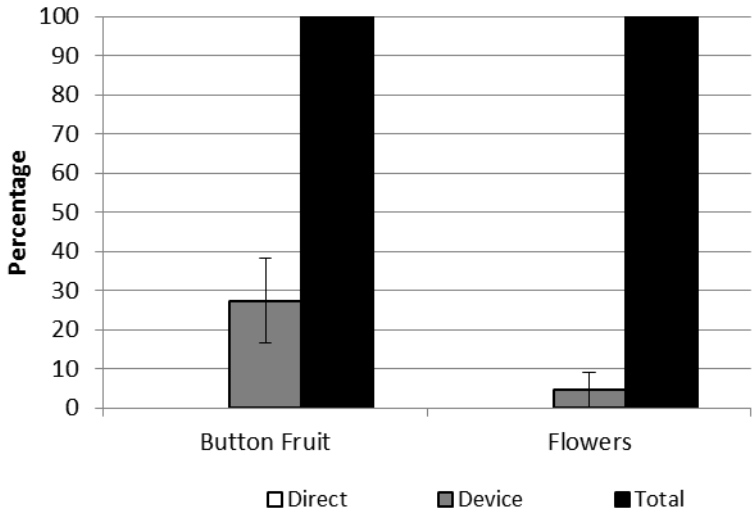


Figure 1.1.5. Mean percentage detection (± standard errors) of mites (*N. cucumeris*) on plant surfaces by direct observation of the fruit or flower surface, or by using the extraction device. Percentage calculations are based on the total numbers of mites detected (numbers detected using the device plus numbers later counted using the ethanol wash technique) on each set of twenty fruit or flowers

Under field conditions, it was also possible to assess the presence of other arthropods on button fruit and flowers using the device. The numbers of WFT and anthocorids (*Orius* species) detected directly and using the device were also expressed as percentages of total numbers and are summarised in Figures 1.1.6 (WFT) and 1.1.7 (*Orius*). Although a relatively small mean proportion (12%) of WFT were observed directly on button fruit surfaces, this increased to 68% using the device. Similarly, a higher proportion of WFT were fumigation-extracted from flowers (81%) than could be seen on the flower surface using a hand lens (24%). The extraction device also increased detection of *Orius* species on both button fruit (direct observation 26%; extraction device 85%) and flowers (direct observation 55%; extraction device 94%).

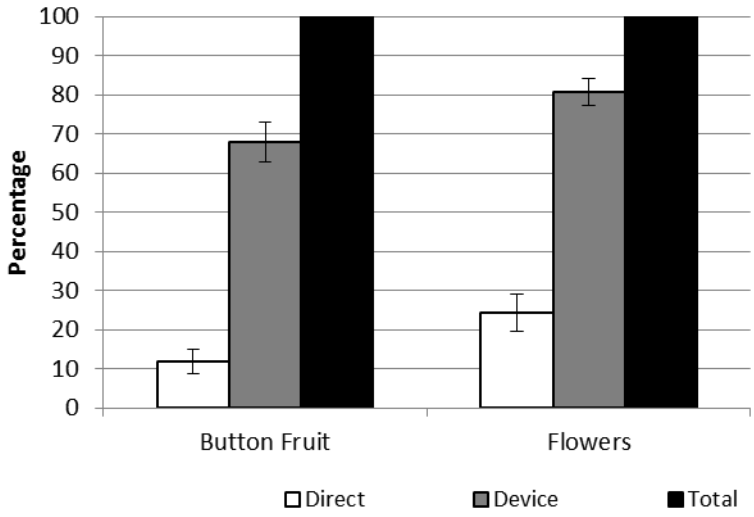


Figure 1.1.6. Mean percentage detection (± standard errors) of thrips (WFT) on plant surfaces by direct observation of the fruit or flower surface, or by using the extraction device. Percentage calculations are based on the total numbers of mites detected (numbers detected using the device plus numbers later counted using the ethanol wash technique) on each set of twenty fruit or flowers

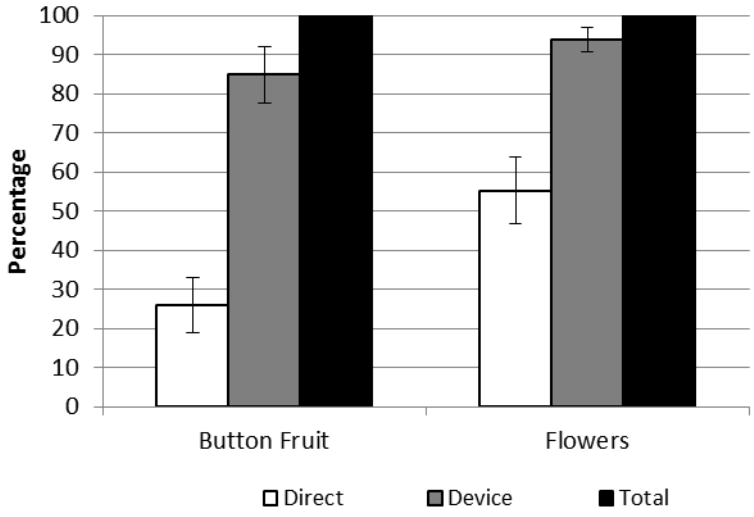


Figure 1.1.7. Mean percentage detection (± standard errors) of anthocorids (*Orius*) on plant surfaces by direct observation of the fruit or flower surface, or by using the extraction device. Percentage calculations are based on the total numbers of mites detected (numbers detected using the device plus numbers later counted using the ethanol wash technique) on each set of twenty fruit or flowers

Discussion

Laboratory inoculation of individual fruit, by direct transfer using a paint brush, showed that the extraction device gives a highly reliable estimate of the numbers of mites present. Although a substantial portion (35%) of mites in the first inoculation experiment remained on the fruit surface after the attempt to extract them via the fumigation method, the numbers of mites recovered using the device remained consistent and therefore were a predictable portion (approximately 57%) of the total numbers of mites that were present on the fruit.

When groups of 10 fruit were inoculated with larger numbers of mites in the controlled laboratory-based experiments, 74% of mites were recovered by one of the three methods (in the extraction device, remaining in the overnight container, or subsequently washed from fruit in a solution of ethanol). This was a lower proportion of total mite recovery than in the previous experiment (81%), when mites were transferred to and contained with individual fruit. This difference could be caused by the different transfer methods that were used: in the second experiment, mites were not placed on fruit but released into the container in the pipette tip and were required to locate fruit during the overnight incubation. This difference in transfer methods accounts for the much higher proportion of released mites that were recovered from the container after pipette transfer (20%) compared to direct transfer via brush (3%).

The second experiment, sampling groups of 10 fruit, achieved a consistent proportional recovery of mites using the extraction device. Based on the linear regression analysis (R^2=0.987; y=0.60x - 0.49), the extraction device recovered approximately 60% of the mites that were present on the fruit (Fig. 1.1.4). Based on this proportion, and the 57% figure obtained in the previous experiment, it would be reasonable to multiply numbers of mites counted in the extraction device by a fixed average correction factor (1.70) to obtain a reliable estimate of the numbers of mites present on the sampled fruit when the device was operated under standardised conditions in the laboratory.

However, under field operation with more variable conditions and using a hand lens rather than a microscope, the recovery of mites from button fruit using the extraction device represented a much lower proportion (27%) of those present on the plant surface. It would therefore be advisable for growers and agronomists to multiply field-extracted counts by a higher correction factor (3.5) in order to estimate numbers of *N. cucumeris* present on fruit sampled in the field. While this is a reasonable multiplier for field-sampled fruit, based on the data presented here, it would not apply to strawberry flowers, which have a more complex microtopography and therefore provide mites with a greater variety of folded and recessed

refuges. A greater proportion of mites therefore remained secreted in flowers, even when they were killed using MIK, and only 5% were counted having fallen to the bottom of the extraction device.

The extraction device was more effective as a method for recovering numbers of larger arthropods, and facilitated detection of a high proportion (>85%) of the total *Orius* that were present on both fruit and flowers. The device also substantially improved recovery of WFT, increasing detection of this pest on both fruit and flowers to a much higher level than was achieved via direct field inspection of plant surfaces. The relatively low proportional extraction of *N. cucumeris*, compared with WFT and *Orius*, is an inevitable consequence of the smaller body size and positive thigmotactic behaviour of these predatory mites. Despite these constraints, the laboratory experiments show that the device can be operated to provide a reliable estimate of the numbers of mites present on plant material. The device can also be used to provide estimates of mite numbers under field conditions, where numbers of extracted *N. cucumeris* are likely to represent approximately 30% and 5% of the actual numbers present on fruit and flowers, respectively.

Future Work

- Improve MIK dispenser release
- Determine minimum time interval required for maximum *N. cucumeris* extraction (currently using 20 minutes)
- Determine the maximum number of uses of the MIK dispenser
- Investigate temperature effects on *N. cucumeris* extraction

Objective 1.2. Determine the distribution of Neoseiulus cucumeris on commercial strawberry plants after their introduction for WFT management

Introduction

In 2016, in experiments at NIAB EMR where multiple releases of high numbers of *N. cucumeris* were made, very few predators were recovered from flowers or button fruit after release. Some commercial growers have also reported finding very few or no predators in flowers or on fruit after multiple releases. In order to make rational decisions on release and sampling strategies for this predator it is important to determine whether the mites are present on other parts of the plant, or if they are not surviving in the crop for some reason. In the first year of the project we recorded numbers of thrips and *N. cucumeris* on different aged flowers and fruits but did not record numbers on other plant parts. It is important to understand mite distribution on the plants as results will guide more effective sampling strategies, including the effective use of the prototype extraction device. Two experiments were done. The first experiment was a small scale glasshouse experiment to address the questions:

- Where do the mites disperse to when released onto the plant?
- What is the best plant part to sample to assess populations?
- Does the presence of WFT on the plants affect distribution of *N. cucumeris*?

The second experiment was a field scale investigation to address the question:

- Is there a diurnal pattern of movement of *N. cucumeris* on strawberry button fruits and flowers?

Methods

Experiment 1

There were two treatments; strawberry plants with WFT populations present and plants with no WFT. Eighteen potted Flamenco plants were placed in each of 2 glasshouse compartments at NIAB EMR. Initial replicate samples showed that there were no *N. cucumeris* or WFT on these plants before the start of the experiment. WFT from laboratory cultures were released onto plants in one compartment at approximately 20 mixed stages per plant; the second compartment had no WFT release. Five days after WFT release (when young larvae were present on the infested plants), *N. cucumeris* from a commercial supplier were released onto each plant in both compartments. Numbers of *N. cucumeris* in 10

replicates of a set volume (1 ml) of the carrier in which the mites are supplied were counted, and this information was used as the basis for calculating the volume of carrier to release on the plants to obtain the required release rate of approx. 200 *N. cucumeris* per plant. The mean number of *N. cucumeris* per 1 ml carrier in these samples was 22 (adults + immatures). Thus 10 ml of carrier containing *N. cucumeris* were released onto each plant. Releases were made by NIAB EMR staff by gently sprinkling the required volume onto each plant in both compartments. Data loggers were used to record temperature throughout the experimental period.

All samples were taken at the same time of day (early afternoon), 1, 4 and 7 days after release (DAR) of *N. cucumeris*. On each sample date 6 plants were randomly selected from each treatment. Numbers of each plant part present at the time of sampling were recorded from each sampled plant. The plants were destructively sampled in the glasshouse; all plant parts were separated into closed containers. Plant parts assessed were: old leaves (10 leaves taken at random from the total leaves collected per plant), recently expanded leaves (all present), folded leaves (all present), flowers (all stages present), button fruit (all present) (Fig. 1.2.1), remaining fruit (all stages present), developing fruit clusters, crown (cut off at soil surface with short pieces of stem remaining). In addition, a sample of the carrier material from the leaf surfaces of each plant was taken. All samples were held in a cold store until assessed.

Fig. 1.2.1. Typical button fruits. Some senescing petals may be visible on some fruits

Numbers of *N. cucumeris*, WFT (if present) and *Tyrophagous putrescentiae* (the prey mites that are supplied by the biocontrol company with the *N. cucumeris*) were counted from the different plant parts to assess distribution over the plant after release. A weighed volume of carrier was examined directly under a microscope, as were leaf samples, since earlier samplings had shown that leaf hairs and surface debris washed from the leaves made

counting of arthropods very difficult. All remaining stages were washed in bulk in 70% alcohol in the laboratory, using our standard washing method; there was thus one composite sample for each stage per plant per sampling occasion.

To obtain an estimate of the total of *N. cucumeris* per plant (since only 10 mature leaves were assessed per plant), the mean number per leaf (calculated from the 10 leaves assessed) was multiplied by the number of leaves present at the time of sampling. Numbers of *N. cucumeris* from a bulk sample include mites from all the individual sample units within that bulk.

A GLM with the Poisson distribution and a log link was used to compare the total number of *N. cucumeris* per plant part per replicate plant. The average numbers of *N. cucumeris* per plant part were analysed, where a plant part was defined as, for example, all the mature leaves. All three sampling dates were combined in a single analysis. Since only 10 mature leaves were sampled, but the total number of mature leaves per plant was counted, an offset of ln(#Mature leaves per plant/10) was used for the mature leaf counts, and 0 for all other plant parts to produce corrected means. Comparisons of the percentages recorded on individual plant parts between treatments (i.e. with and without WFT release) were carried out using likelihood ratio tests. For comparisons of the mean counts on each plant part, t-tests on the log-link scale were used.

Results and Discussion

Temperature records: Temperatures recorded in the two glasshouse compartments during the experiment are shown in Figs. 1.2.2 and 1.2.3.

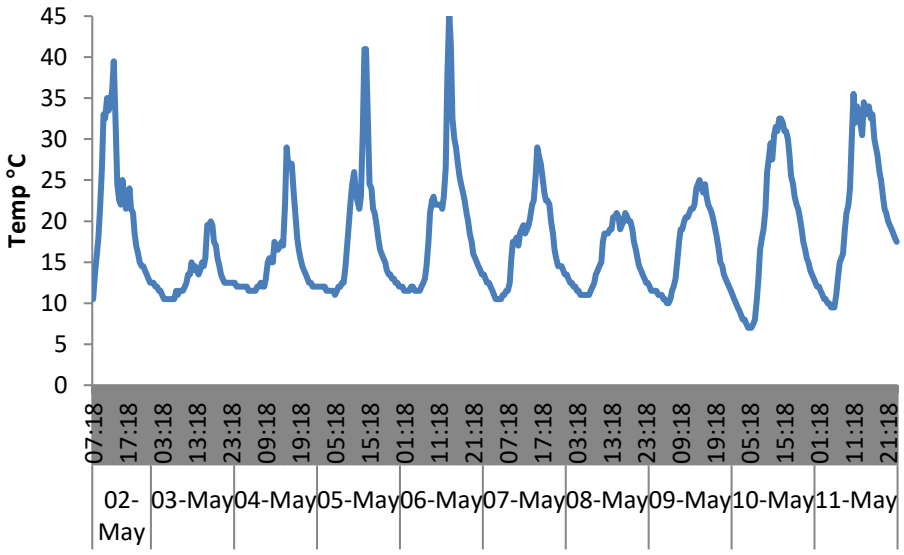


Figure 1.2.2. Temperatures recorded in the compartment where both WFT and *N. cucumeris* were released

Samples from both compartments were collected between 13.00 and 15.00 hrs. Mean temperatures recorded in the compartments at 13.00 hrs on the sample days were 30.5°C on 5 May, 19°C on 8 May and 32.5°C on 11 May.

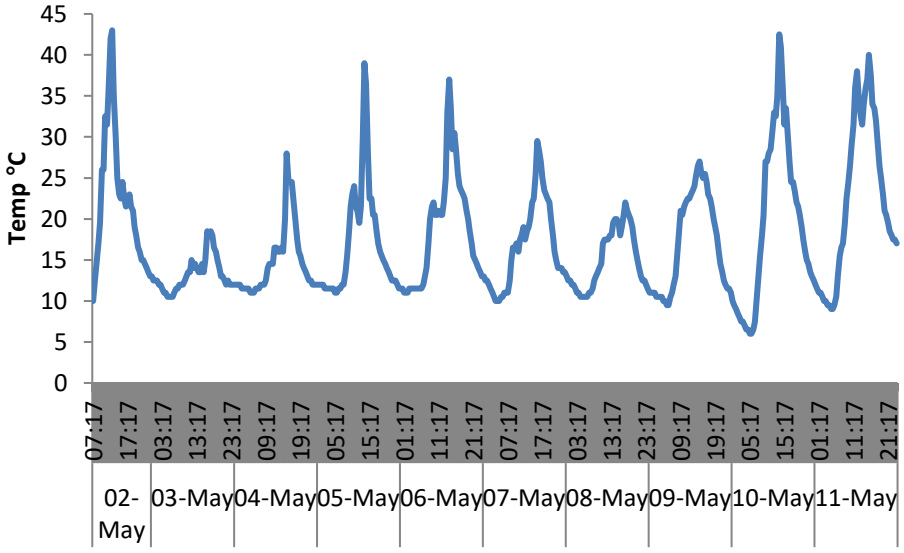


Figure 1.2.3. Temperatures recorded in the compartment where only *N. cucumeris* were released

Plant parts: The mean number of number of plant parts present on each sampling occasion for the two treatments are shown in Figs. 1.2.4 and 1.2.5. Plants in both treatments were flowering and fruiting throughout the sampling period.

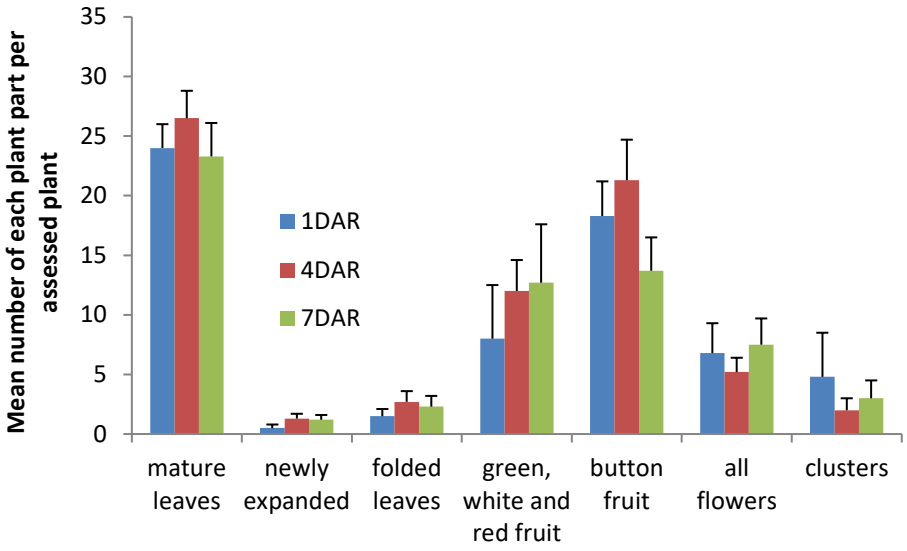


Figure 1.2.4. Mean number of parts on 6 plants 1, 4 and 7 days after release (DAR) of *N. cucumeris* at 200 per plant. No WFT were released on these plants

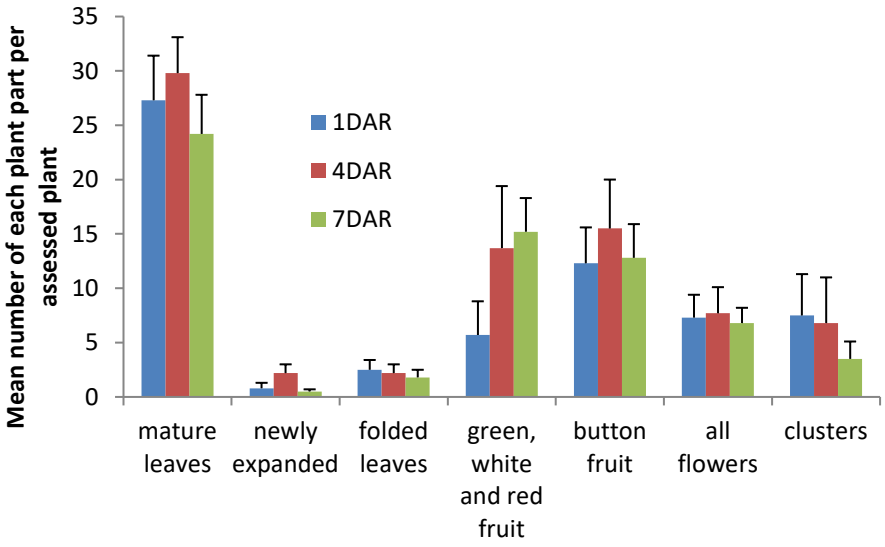


Figure 1.2.5. Mean number of parts on 6 plants 1, 4 and 7 days after release (DAR) of *N. cucumeris* at 200 per plant. WFT were released on these plants

Carrier: At the time of release, 1 ml of carrier material weighing 0.17 g (mean of 5 replicates) contained on average 22 *N. cucumeris*. Thus in 1 g of carrier there was an estimated 130 mites at the time of release.

Estimated numbers of *N. cucumeris* remaining in the carrier on the leaf surface after release is shown in Fig. 1.2.6. Means are for 12 samples (one from each plant assessed). Mean weight of carrier sampled at 1 and 4 days after release (DAR) was 0.38 g and 7 DAR was 0.23 g. Thus an average of 85% of the released mites had moved from the carrier 1 DAR.

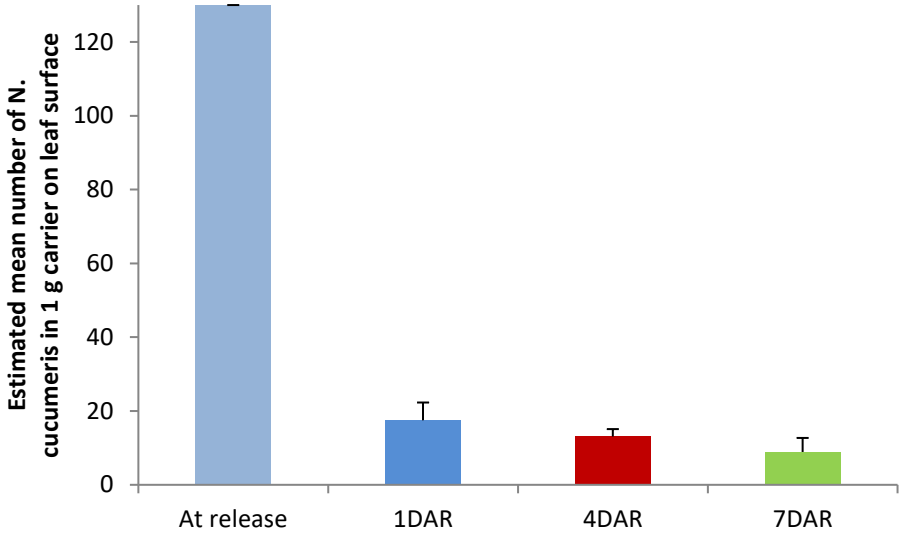


Figure 1.2.6. Estimated mean numbers of *N. cucumeris* remaining in 1 g of carrier material on the leaf surface 1, 4 and 7 days after release (DAR)

Distribution of N. cucumeris: Graphical illustrations of the percentages of *N. cucumeris* recorded on different plant parts in the two treatments are shown in Figs 1.2.7-1.2.14. Statistical analysis of the data is given after the graphs.

Distribution of N. cucumeris *where no WFT released*: The percentage of the estimated total per plant recorded on the different plant parts is shown in Fig. 1.2.7 for the no WFT release treatment. Estimated total number of *N. cucumeris* per plant, obtained by totalling numbers recorded from each plant part assessed, in the treatment where WFT were not released ranged from 55-78 (mean 66.8) 1 DAR, from 49-71 (mean 59.7) 4 DAR and from 40-103 (mean 66.1) 7 DAR; the estimated total number of *N. cucumeris* released per plant was 200. Using these minimum and maximum mean estimates of number released and recorded on the plants 58-76% of mites released were not subsequently recorded on the plants. There was a trend for the percentage of *N. cucumeris* on leaves declining with time (Fig. 1.2.7); at

release most *N. cucumeris* would have been in the carrier or moving from this onto the leaf surface, with numbers declining as the mites moved to other plant parts. Very few *N. cucumeris* were recorded on young and folded leaves and on developing clusters.

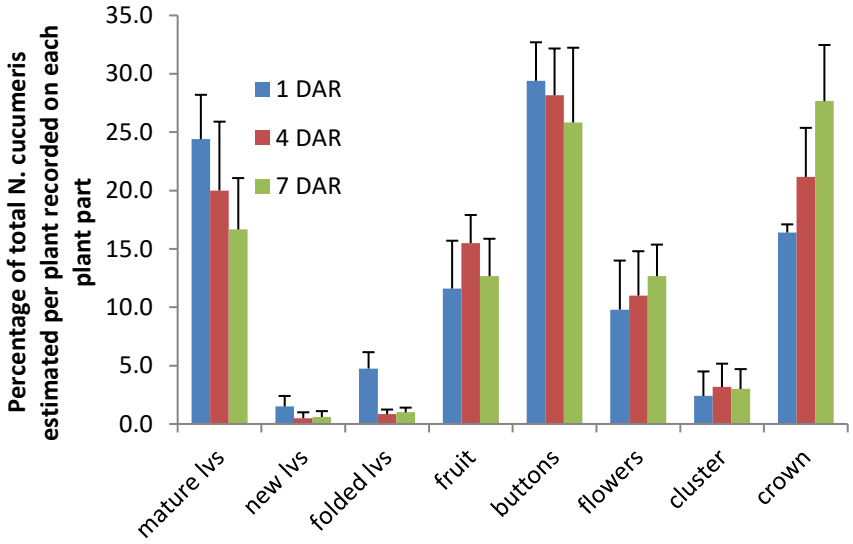


Figure 1.2.7. Percentage of the total number of *N. cucumeris* recorded on each plant part from 6 plants 1, 4 and 7 days after release (DAR). No WFT were released on these plants. Data from the bulk samples of fruit and flowers include numbers from all the units in each bulk sample

Mean numbers of *N. cucumeris* on individual plant parts calculated from the bulk samples with reference to numbers of plant parts present in the bulks are shown in Figs. 1.2.8 and 1.2.9. Leaves of all ages were combined. Taking into account the range of surface areas of the different plant parts it appears that higher numbers per unit area were present on the flowers and button fruit (Fig. 1.2.9). Higher numbers were present on the leaves 1 DAR, and there was a trend of a declining numbers on leaves after this. Numbers were higher on the flowers and fruits and were high in the crown.

Figure 1.2.8. Mean numbers *of N. cucumeris* 1, 4 and 7 days after release (DAR) on individual plant parts within bulk samples where no WFT were released on the plants

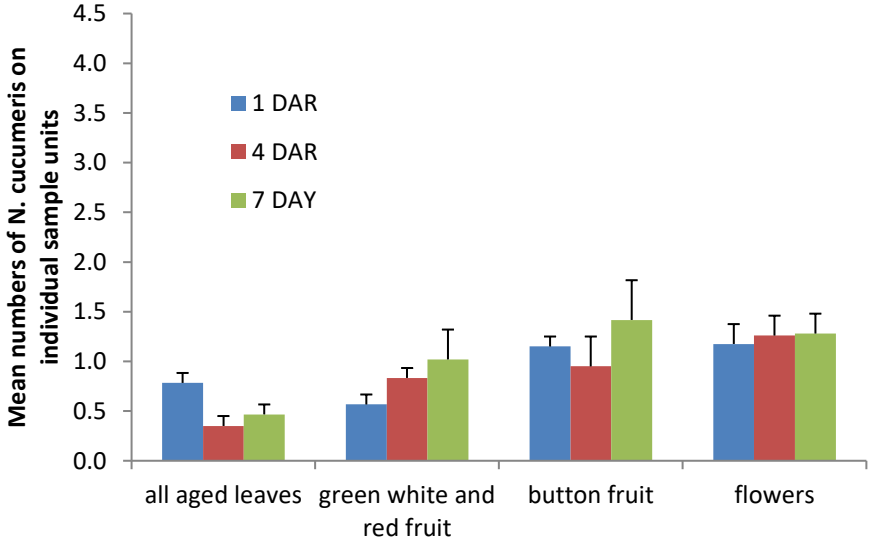


Figure 1.2.9. Mean numbers *of N. cucumeris* 1, 4 and 7 days after release (DAR) on individual plant parts within bulk samples where no WFT were released on the plants (same data as Fig. 1.2.8 but at clearer scale with crown omitted)

Distribution of N. cucumeris *where WFT were released*: The percentage of the estimated total per plant recorded on the different plant parts are shown in Fig. 1.2.10 for the WFT release treatment. Estimated total number of *N. cucumeris* per plant, obtained by totalling numbers recorded from each plant part, in the treatment where WFT were released ranged from 41-101 (mean 80.5) 1 DAR, from 58-88 (mean 74.5) 4 DAR and from 62-129 (mean 91) 7 DAR; the estimated total number of *N. cucumeris* released per plant was 200. Using these minimum and maximum mean estimates of number released and recorded on the plants 47-73% of mites released were not subsequently recorded on the plants.

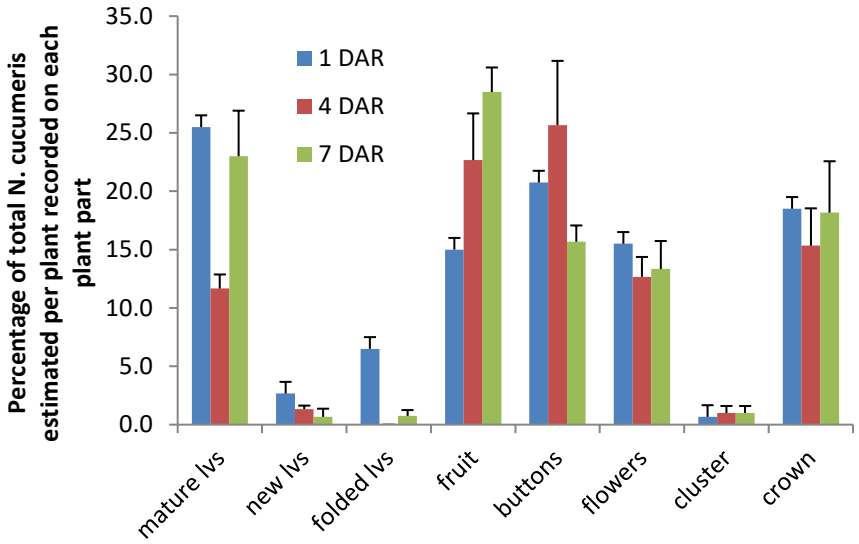


Figure 1.2.10. Percentage of the total number of *N. cucumeris* recorded on each plant part from 6 plants 1, 4 and 7 days after release (DAR) where WFT were released on the plants. Data from the bulk samples of fruit and flowers include numbers from all the units in each bulk sample

Mean numbers of *N. cucumeris* on individual plant parts calculated from the bulk samples with reference to numbers of plant parts present in the bulks are shown in Figs. 1.2.11 and 1.2.12. Leaves of all ages were combined. At 7 DAR higher numbers of *N. cucumeris* were recorded on the mixed age fruit samples (excluding button fruit) (Fig. 1.2.12).

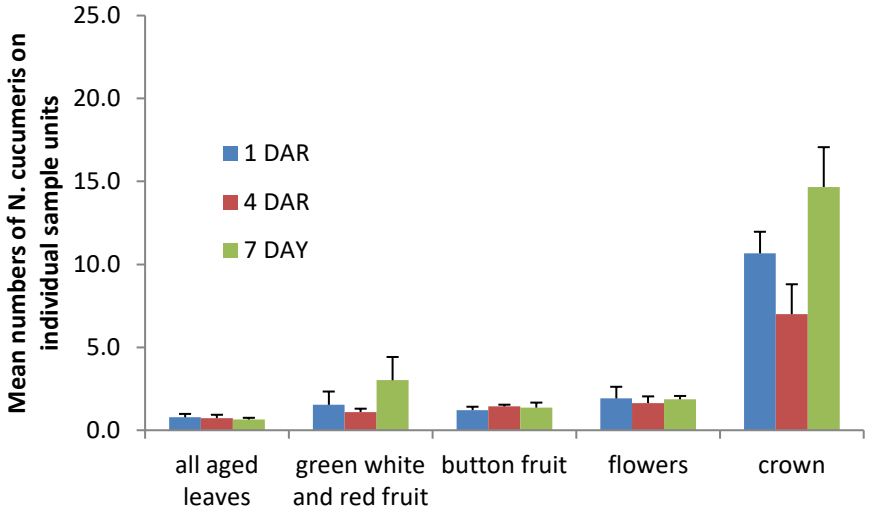


Figure 1.2.11. Mean numbers *of N. cucumeris* on individual plant parts within bulk samples 1, 4 and 7 days after release (DAR) where WFT were released on the plants

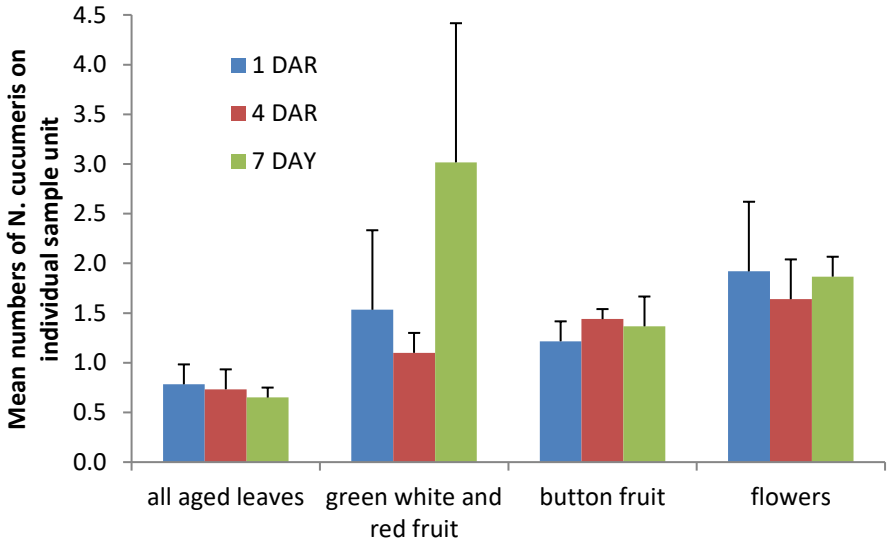


Figure 1.2.12. Mean numbers *of N. cucumeris* on individual plant parts within bulk samples 1, 4 and 7 days after release (DAR) where WFT were released on the plants (same data as Fig. 1.2.11 but at clearer scale with crown omitted)

Distribution of WFT on plants: Mean numbers of WFT adults on the different plant parts sampled in the treatment where WFT were released are shown in Fig. 1.2.13. Numbers were highest 1 DAR and declined over time. Numbers were highest on the flower samples. As expected very few WFT adults were recorded on leaves or in the crown.

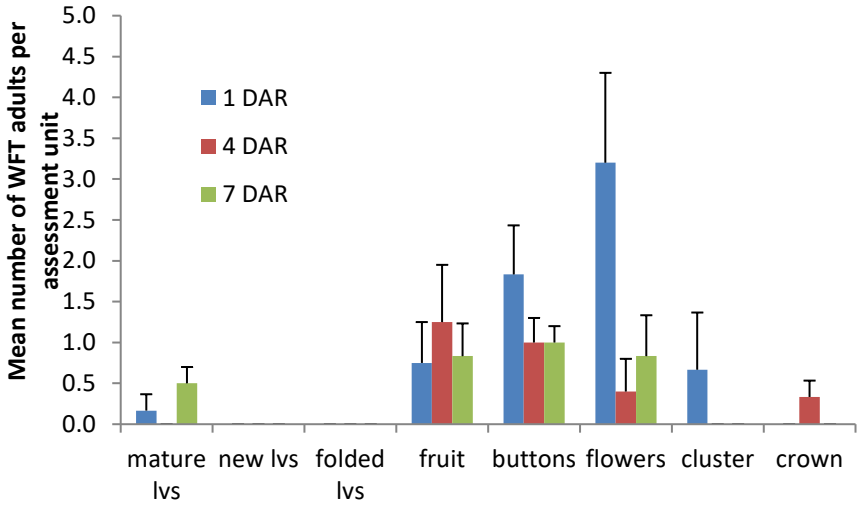


Figure 1.2.13. Mean number of WFT adults on sampled plant parts 1, 4 and 7 days after release (DAR) of *N. cucumeris*

Mean numbers of WFT larvae on the sampled plant parts are shown in Fig. 1.2.14. Numbers were low 1 DAR of *N. cucumeris* due to the phenology of the pest; numbers of WFT adults declined over time and numbers of larvae increased.

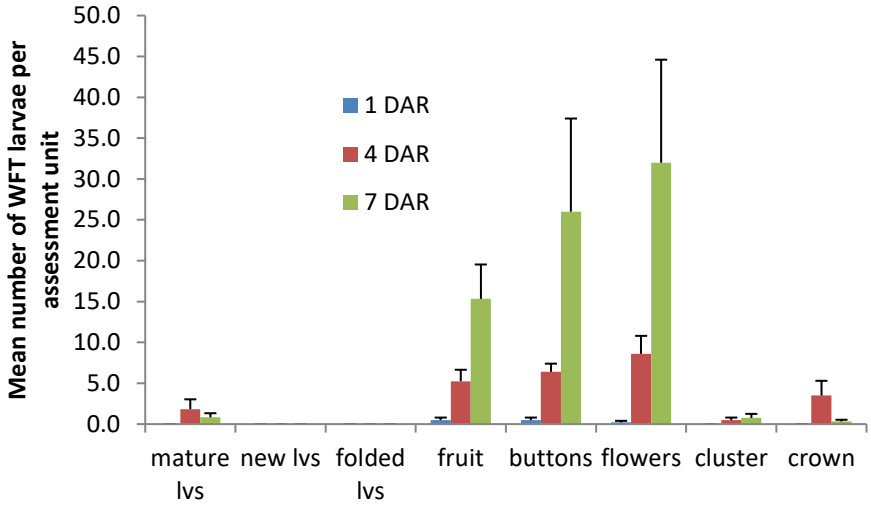


Figure 1.2.14. Mean number of WFT larvae on sampled plant parts 1, 4 and 7 days after release (DAR) of *N. cucumeris*

No WFT were recorded on the plants in the treatment where WFT were not released. Very low numbers of *Tyrophagous putrescentiae* (the prey supplied commercially with *N. cucumeris*) were recorded in the initial counts of mites in the carrier, and on the sampled plants. In other work at NIAB EMR we have observed that numbers of the prey mites vary widely from batch to batch of *N. cucumeris* (as do numbers of *N. cucumeris* per 1 ml of carrier). The condition of the product on delivery may well affect distribution of *N. cucumeris* on plants and their ability to establish in the crop, as will availability of food for the predators; *N. cucumeris* will feed on pollen as well as WFT and *Tetranychus urticae* (spider mite) on plants.

Analyses of N. cucumeris *distribution*: Numbers of *N. cucumeris* recorded on plants increased over time (p=0.05); this may in part be due to the mites leaving the carrier and moving out onto the plants. There was a significant treatment effect (p=0.05) with overall numbers of adults higher in the treatment where WFT had been released. It is not clear why this is the case as the same estimated numbers were released on all plants. However, it is possible that with WFT present more mites were arrested on the plant. There was also a significant effect of treatment (WFT release or not) on numbers recorded on the different plant parts (Tables 1.2.1-1.2.3). Where WFT had been released there were higher numbers of *N. cucumeris* adults on the flowers (P=0.03) and older fruit (P=0.003) compared with where WFT were not present (Table

1.2.1) with similar numbers on the button fruits. This suggests that when prey (WFT) is present on the plants adult mites are likely to be found in the flowers and fruits where the prey is located and when WFT are absent mites will be found elsewhere, presumably as they search for alternative food. Numbers of *N. cucumeris* immatures were significantly higher on button fruit when there were no WFT present (P=0.02) and on the older fruit (P=0.008) when WFT were present (Table 1.2.2). There was no significant effect of treatment on *N. cucumeris* egg distribution (Table 1.2.3).

When mean numbers of *N. cucumeris* per plant part were compared between the two treatments there were significantly higher numbers of mites on both flowers (p=0.024) and fruits (p=0.037) in the treatment where WFT had been released, again suggesting that the mites were found in highest numbers at locations where their prey were present.

Table 1.2.1. Tables of overall mean numbers (taken from GLM Analysis) showing distribution of adult *N. cucumeris* on different plant parts in treatments where WFT were present or absent from the plants. Significant differences between treatments are shown in red. 240 df

	folded leaves	new leaves	mature leaves	cluster	flower	button fruit	all other fruit	crown
No WFT	0.78	0.17	7.99	1.06	**4.94**	12.28	**6.39**	9.94
+ WFT	0.78	0.28	12.44	0.33	**8.28**	12.67	**12.06**	8.28
SED (of ln ratio)	0.99	5.73	0.29	0.87	**0.23**	0.16	**0.21**	0.20
ln ratio	0.0	-0.51	-0.44	1.15	**-0.52**	-0.03	**-0.63**	0.18
Sig (P)	1.000	0.929	0.129	0.186	**0.025**	0.847	**0.003**	0.348

Table 1.2.2. Tables of overall mean numbers (taken from GLM Analysis) showing distribution of immature *N. cucumeris* on different plant parts in treatments where WFT were present or absent from the plants. Significant differences between treatments are shown in red. 240 df

	folded leaves	new leaves	mature leaves	cluster	flower	button fruit	all other fruit	crown
No WFT	0.28	0.17	4.47	0.50	2.50	**5.61**	**1.44**	3.94
+ WFT	0.72	0.33	5.49	0.22	2.89	**3.39**	**3.33**	2.50
SED (of ln ratio)	4.82	4.85	0.29	4.83	0.26	**0.22**	**0.31**	0.25
ln ratio	-0.95	-0.69	-0.21	0.81	-0.14	**0.50**	**-0.84**	0.46
Sig (P)	0.843	0.887	0.477	0.867	0.575	**0.020**	**0.008**	0.072

Table 1.2.3. Tables of overall mean numbers (taken from GLM Analysis) showing distribution of *N. cucumeris* eggs on different plant parts in treatments where WFT were present or absent from the plants. There were no significant differences between treatments. 240 df

	folded leaves	new leaves	mature leaves	cluster	flower	button fruit	all other fruit	crown
No WFT	0.06	0.00	3.08	0.00	0.44	1.39	0.28	1.28
+ WFT	0.06	0.00	3.47	0.06	0.56	0.44	0.39	0.78
SED (of ln ratio)	22.35	27.37	0.42	24.99	0.62	11.18	11.19	0.36
ln ratio	0.00	0.00	-0.12	-6.80	-0.22	1.14	-0.34	0.50
Sig (P)	1.000	1.000	0.772	0.786	0.720	0.919	0.976	0.175

Conclusions

- As in earlier studies, most WFT were found on the flowers and fruits
- Most *N. cucumeris* had dispersed from the carrier material within one day of release
- It was estimated that around 50% of the total estimated number of mites released were not subsequently recorded on the plants; they were possibly lost to the soil or ground surface at the time of application
- *N. cucumeris* were recorded on all the assessed plant parts
- When comparing numbers on individual units within the bulk samples, lower numbers of *N. cucumeris* were recorded on leaves (any age); similar numbers were recorded on fruit and flowers
- The presence of prey affected the distribution of *N. cucumeris* on the plants
    - There were significantly higher numbers of *N. cucumeris* immatures on older fruits in the treatment where WFT had been released and on button fruits when no WFT were present
    - There were significantly higher numbers of *N. cucumeris* adults on the flowers and older fruit where WFT had been released
    - There was no effect of prey presence on distribution of *N. cucumeris* eggs
- When designing an optimised sampling strategy for *N. cucumeris* it is important to take into account the relationship between numbers recorded and surface area of the different plant parts sampled. Numbers were generally higher or similar in button fruit compared with other fruit stages sampled (as determined in earlier work funded by AHDB) and the surface area is smaller potentially increasing the efficacy of extraction of the mites using the field extraction device.

Experiment 2: Is there a diurnal pattern of movement of *Neoseiulus cucumeris* on strawberry button fruits and flowers?

Introduction

In experiment one undertaken in May and June, the distribution of *N. cucumeris* on different parts of potted strawberry plants was assessed. All samples were taken at the same time of day from leaves, fruits, flowers and crowns. As in earlier experiments the highest percentage of mites recovered were recorded on the fruits. A field experiment was set up to determine if the distribution of *N. cucumeris* on button fruits and flowers changes at different times of day. This work is important as it has been suggested, in other research on different crops that predatory mites move to different plant parts depending on humidity (Ferrero et al. 2010). This has not been assessed on strawberry. Understanding any changes in potential distribution of the predator on the conventional sampling units at different times of day would enable more effective sampling strategies to be developed.

Methods

A commercial table top strawberry crop in Kent was chosen for the experiment. Amesti were planted at 6 plants per bag in double staggered rows. There were 5 table tops per tunnel. Two table top beds in one tunnel were used for the experiment. The crop had received several introductions of *N. cucumeris* during the growing season. Numbers of *N. cucumeris* and WFT were assessed in samples taken from flowers and button fruits before the start of the experiment, and based on the results of these assessments it was decided to do another release to increase the numbers of predators in the experimental area. Numbers of *N. cucumeris* in a set volume of carrier from a commercial supplier were counted and used as the basis to calculate the volume of carrier to release on the plant to obtain the required release rate. The release rate needed was an estimated 200 per plant; this is the rate used in the glasshouse experiment described under experiment 1. The volume required was released onto two beds of plants, an outer bed and the central bed, in one tunnel. Since these beds may experience different temperatures samples from these beds may enable us to obtain more information on any mite movement that is related to temperature.

Easy Log data loggers were used to record temperature and humidity throughout the experimental period and were set to record every 5 mins. Three loggers were placed along the outer bed and three along the central bed where mites had been released.

The photosynthetically active light levels (400-700 nm) on the inner bed were also monitored during the experiment using a Data Hog2 quantum sensor (Skye Instruments); this instrument averages 5 readings over 5 mins to give a single reading.

Samples were taken at five times during the day; 09.00; 12.00; 15.00; 18.00; 21.00. Sampling was repeated on three days, with a one day gap between the first two samples and a 4 day gap between the second and third sample to allow the plants to recover and produce more open flowers and button fruits. Each sample consisted of 10 flowers or 10 button fruits. The aim was to take 10 replicate samples (5 x flowers and 5 x button fruit) at each assessment time on both inner and outer beds. These bulk samples were collected into alcohol and taken to the lab where arthropods were extracted using our standard alcohol washing technique. Numbers of *N. cucumeris* were counted from the samples to determine distribution over time. Thrips adults and larvae and *Orius* adults and nymphs were also recorded from the samples.

Mean and standard error (SE) of numbers of *N. cucumeris* on each plant part at the different sampling times were calculated. Numbers of *N. cucumeris* (all active stages), WFT larvae and adults and *Orius* adults and nymphs on these plant parts in relation to sampling time and date, position (inner vs outer bed), and environmental conditions (mean temperature for the 15 and 60 mins before each sample and mean light intensity for the 60 mins before the sample) were analysed using forward step-wise regression to find the best model for each variate. The analyses were all carried out using a GLM with the Poisson distribution and a log-link. Where the slope of the relationship with the environmental variate (average temperature, etc.) is not affected by any of the treatment factors the analysis is a covariate analysis, so presented means are adjusted to the average environmental variate. This was so for all variates except *Orius*.

Results

Mean temperature records from inner and outer beds are shown below (Figs. 1.2.15 and 1.2.16) for the days on which samples were taken. Temperature rose earlier in the day on the inner beds compared to the outer beds, but overall, differences were relatively small. Maximum temperature was higher on 29 August than on the previous sample days.

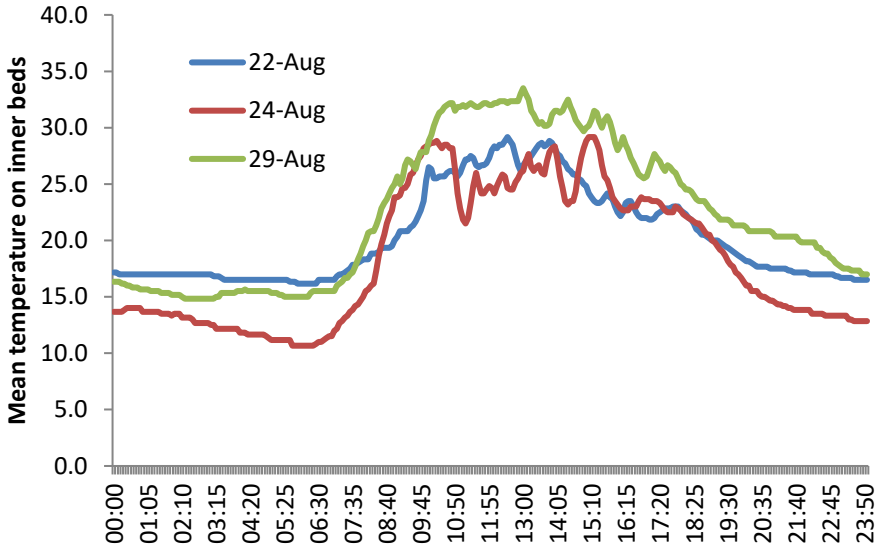


Figure 1.2.15. Mean temperature recorded on inner bed on the sample days

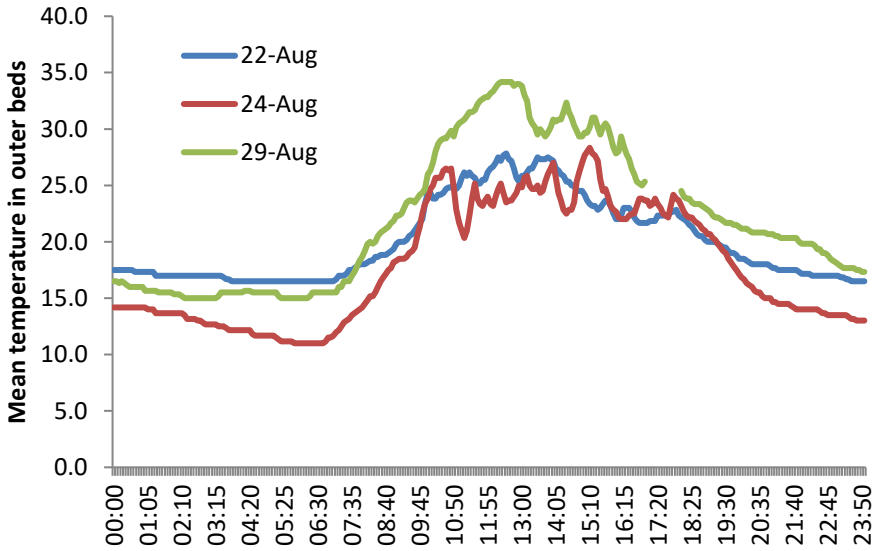


Figure 1.2.16. Mean temperature recorded on outer bed on the sample days

The photosynthetically active light levels (400-700 nm) recorded on the centre bed during the days the samples were taken are shown in Fig. 1.2.17. Light intensity was very low on 22 August; during this day the Kent area was overcast with only 1 hour of sunshine recorded at the nearby Met Office weather station at Manston.

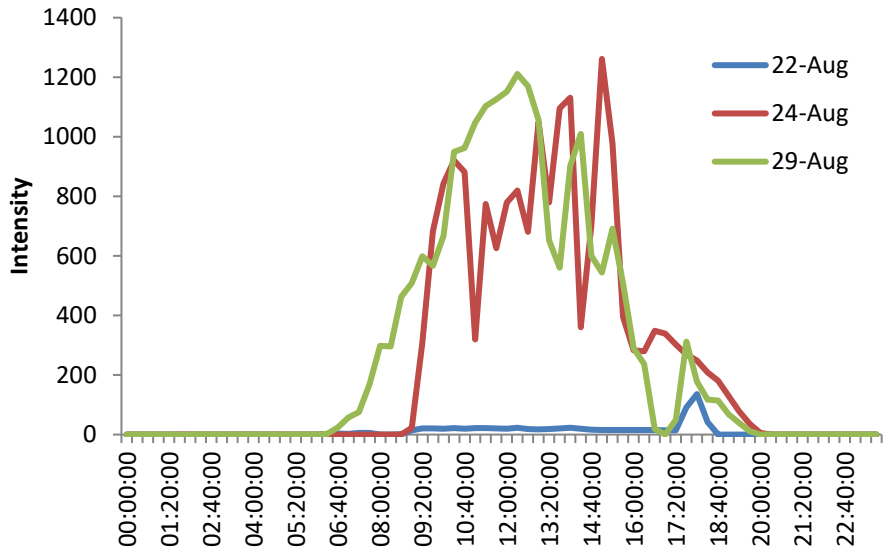


Figure 1.2.17. Light intensity recorded on centre bed on the three sampling days; sunshire hours recorded at Manston were 1, 4, 10 and at Heathrow were 0, 6, 4 on the three days respectively

The mean number of *N. cucumeris* per 10 flowers or button fruits are shown in Figs 1.2.18 and 1.2.19. There was a high level of variability between numbers recorded in replicate samples on each sampling occasssion.

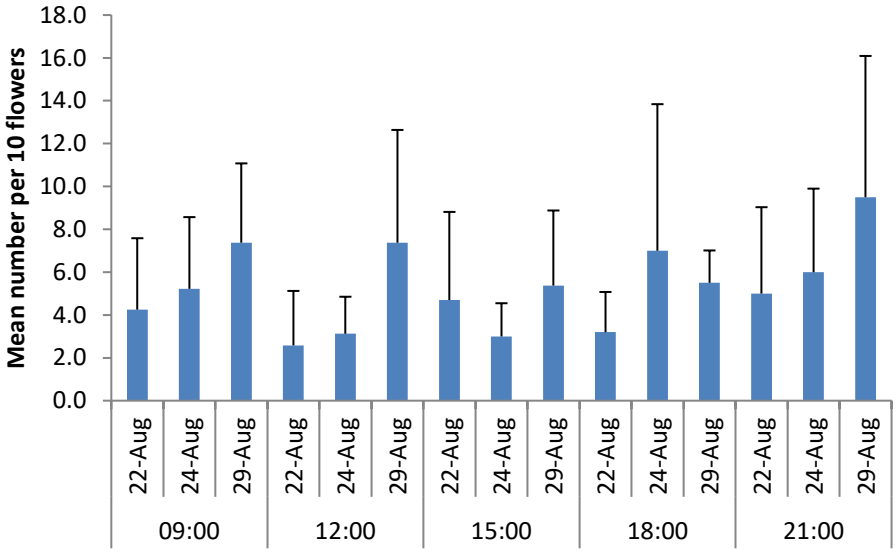


Figure 1.2.18. Mean numbers of *N. cucumeris* (all stages) per 10 flowers

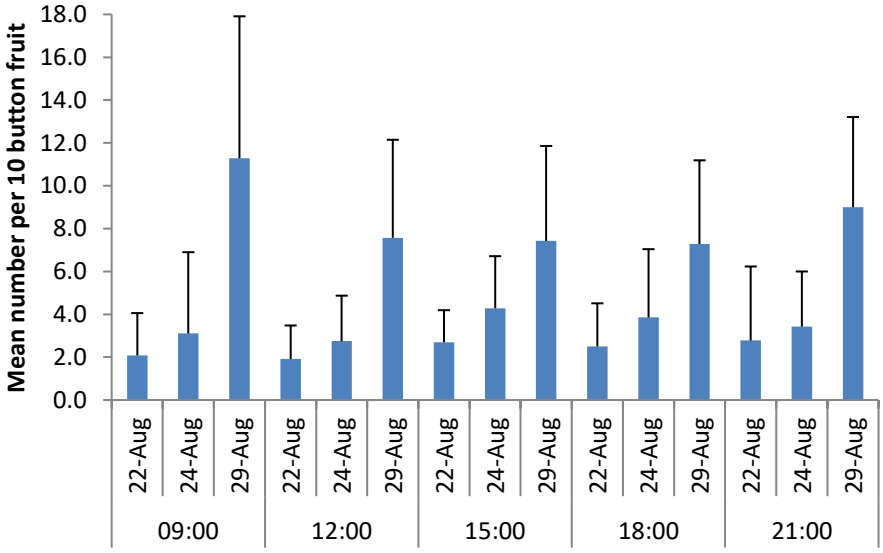


Figure 1.2.19. Mean numbers of *N. cucumeris* (all stages) per 10 button fruits

In the statistical analyses the final models for each variate showed significant effects of temperature on numbers of WFT larvae, *N. cucumeris* and *Orius* adults (Table 1.2.4) in flowers and button fruit; for WFT adults there was an effect of temperature on distribution on flowers only. For *Orius* adults the effect on distribution was significant only on the first sample date.

Table 1.2.4. Effect of temperature on distribution of arthropods recorded in strawberry flowers and button fruits

	Slope of regression line	P value	Estimated % increase/decrease in counts per degree rise in average temperature over the range recorded in the expt
WFT larvae	-0.0305	0.048	-3.0
WFT adults	0.0274	0.047	2.8
N. cucumeris [+]	-0.0224	0.033	-2.5
Orius adults	0.074	0.011	7.7

[+] all active stages

Mean temperatures in the hour before samples were taken are shown in Fig. 1.2.20. The analysis allowed an estimate to be made of the impact changes in temperature might have on numbers of arthropods recorded in sample units (Table 1.2.4); over the range of temperatures recorded during the experiment a 1°C increase in temperature could result in around a 3% reduction in numbers of *N. cucumeris* in sample units (Table 1.2.4). There was no effect of recorded light intensity on distribution of any of the arthropods recorded. There was no evidence of any environmental effects on distribution of *Orius* nymphs.

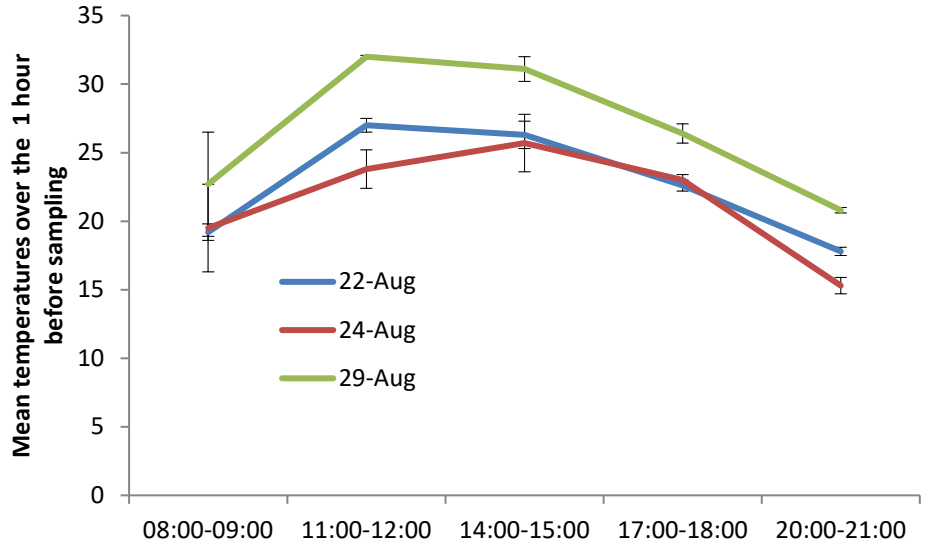


Figure 1.2.20. Mean temperatures recorded in the hour before sampling

Conclusions

- The mean temperature in the hour prior to sampling affected the number of arthropods recorded in samples of flowers and button fruits
    - Numbers of *N. cucumeris* declined by 2.5% for every 1°C increase in mean temperature calculated per hour, over the range recorded in the experiment (18-33°C)
    - Numbers of *N. cucumeris* are likely to be lower in flowers and button fruit at higher temperatures. Therefore if low numbers are recorded in samples it would be worthwhile to revisit the planting when temperatures have decreased to confirm establishment of the predator
- Predatory *Orius* adults and WFT adults were recorded in higher numbers as the mean temperature increased
- WFT larvae decreased in abundance as the mean temperature increased

1.2. Making applications of entomopathogenic fungi (EPF) effective for control of WFT

This work was suspended until 2018 due to delays in Met52 OD (Fargro) availability. Potential work in 2018 could include grower field testing of Met 52 OD with assessments for mycosis in aphids, thrips (including rose thrips) and *N. cucumeris* and other natural enemies.

Objective 2. Refine pest control programmes on strawberry, integrating pesticides with phytoseiid mites.

Task 2.1. In field, effect of insecticides commonly used to target spring aphids on the establishment of *N. cucumeris*, aphids and parasitoids

Introduction

This work will be done in spring 2018 and will be reported upon completion. Below is an outline of the proposed study. Data on the introduction of *N. cucumeris* and residual time of pesticides is laboratory generated. This field study will look at the effect of insecticides commonly used to target spring aphids on the establishment of *N. cucumeris* and other predators.

Table 2.1.1. Treatments applied to control aphids. *A = adult, N = nymph, E = eggs of *N. cucumeris*. 1 = harmless, 2 = slightly harmful, 3 = moderately harmful, 4 = harmful (Koppert and Biobest side-effects websites). !R= red, Y = yellow, G = green

Product!	Harm*	persistence	MAPP No:	Active(s)	Target
R. Hallmark	4A N4 E4	8-12 W	12629	lambda-cyhalothrin	Range of pests
Y. Calypso	A1	0	11257	Thiacloprid	capsids
G. Untreated control	-	-	-	-	-

The experiment will be a randomised block experiment with 6 replicates of each treatment including an untreated control. Plots will be whole tunnels.

Plots will be sprayed by the grower using standard spray apparatus on table top strawberry. Sprays to be applied at 500-1000 l/ha (depending on growers recommendation). *N. cucumeris,* at 200 mites per plant (recommended release is 200 m^2), will be added to the centre 20 m of each row following the spray application. Spray application will be supervised by a NIAB EMR PA1, PA6 and PA9 qualified staff at the volume rate specified in the protocol.

The growers standard spray programme for non-aphid pests and disease control will be applied across the entire site. All spray records will be documented. Because the grower will treat the whole plantation in a uniform way this will reduce variation between plots. All grower spray programmes including fungicides will be requested at the end of the trial. We will also request records of *Phytoseiulus* and *N. cucumeris* releases and gain understanding with the grower that any treatments applied need to be applied equally to the whole area. Using a commercial plantation with commercial spraying apparatus will allow us to test a grower relevant situation.

2 lascar EL-USB-2 data loggers will be deployed in a Stevenson screen in the middle of the target area to collect hourly temperature and humidity levels. In addition two further data loggers will be placed at either end of the plantation where temperature is more likely to be slightly different (=6 data loggers). Wet and dry bulb temperature with aspirated psychrometer, wind speed and direction before and after spraying will be measured.

At each assessment the numbers of ***N. cucumeris*** on either leaves or flowers or button fruit (depending on availability) will be recorded by sampling into polythene bags and doing direct counts in the laboratory. Assessments will continue up to 84 days depending on results (see timeline below). The sample size will be adjusted so that enough *N. cucumeris* can be recorded for statistical analyses. We will begin by sampling 20 units from the control to assess the numbers of mites and then adjust the sample size depending on the numbers of mites recovered. It is possible that if *N. cucumeris* numbers remain low in the control plots we will make additional releases. This will be closely monitored with each assessment. Predatory mite adults, nymphs and eggs will be counted on each sampling unit. A small sample of adults from each treatment will be mounted on microscope slides for identification to species on each occasion. We will subsample predators and i.d. to species.

Aphids numbers will be counted on each of 20 plants in each of the 18 plots. Aphid colonies will be collected and incubated in the laboratory at NIAB EMR to assess for emerging 'wild' populations of **parasitoids** (known to take longer in the spring). See timeline below.

Thrips numbers will be noted as part of this study but are likely to be in very low numbers at this time of year and are not the focus of this study.

In addition, on each occasion, samples of leaves will be collected and sent to BGG who will coordinate leaf residue testing.

A note will be made of any phytotoxic effects but this is not expected as these are approved products.

Timeline;

Day	**Action**
0	Pre assessment and leaf sample
1	Apply sprays
7	Introduce *N. cucumeris* to centre 20 m of each plot
14	Assessment, parasitoid and leaf sample
21	Assessment, parasitoid and leaf sample
28	Assessment, parasitoid and leaf sample
35	Assessment, parasitoid and leaf sample
42	Assessment, parasitoid and leaf sample
84	Assessment, parasitoid and leaf sample

NB: during the trial we will expect the grower to introduce *N. cucumeris* at their standard programme equally to the whole trial area.

Objective 3. Develop IPM compatible controls for European tarnished plant bug, *Lygus rugulipennis*, common green capsid, *Lygocoris pabulinus*, and strawberry blossom weevil, *Anthonomus rubi*.

Task 3.1. To investigate the potential of a multi-pheromone blue sticky trapping system for *Lygus rugulipennis*, *Lygocoris pabulinus* and *Frankliniella occidentalis*

Introduction

In strawberry the western flower thrips, *Frankliniella occidentalis* (WFT), causes bronzing of the fruit and has become difficult to control because of resistance to insecticides and lack of effective alternative biological controls. Financial losses can be high, exceeding £15m to the UK industry alone in 2013. From June onwards European tarnished plant bug, *Lygus rugulipennis*, becomes a damaging pest of strawberry requiring routine treatment with insecticides. Feeding in flowers and on green fruits can cause up to 80% crop loss, rendering production uneconomic and insecticidal products used for control can disrupt biological control agents and increase pesticide residues in fruits. *Lygocoris pabulinus* (common green capsid), is also a damaging pest, which tends to be sporadic in appearance and locally distributed within the crop.

Growers need practical solutions which ideally target multiple pests. Currently blue sticky traps are employed for WFT control. These can be enhanced with a WFT aggregation pheromone, which can typically double the catch (Sampson, 2014). If these could also be used in conjunction with capsid pheromones this would potentially provide in-crop control of three pest species. Currently *L. rugulipennis* is trapped using a *Lygus* sex pheromone lure within a green bucket trap and cover; catches, including of females, can be increased with the addition of the plant volatile phenylacetaldehyde (PAA). The trapping system for *L. pabulinus* uses the same pheromone lure, but attached to a blue sticky trap placed vertically in the crop.

Objectives

To investigate whether:

- *L. rugulipennis* and *L. pabulinus* can be attracted to a blue sticky trap with the addition of a *Lygus* sex pheromone lure + phenylacetaldehyde (PAA)
- The *Lygus* pheromone + PAA can be used in conjunction with the WFT pheromone

- Beneficial arthropods are also attracted to the trapping system

Methods

The experiments were set up on multiple sites in mid to late June and covered a 2 month period within 2017 (running continuously). Sites (Fig. 3.1.1. and Table 3.1.1.) were:

1. Langdon Manor Farm, Goodnestone, Faversham, Kent ME13 9DA. By kind agreement of Alastair Brooks.
2. Ewell Farm, Graveney Rd, Faversham ME13 8UP, Edward Vinson. By kind agreement of Sean Figgis.
3. NIAB EMR, New Road, East Malling, ME19 6BJ.

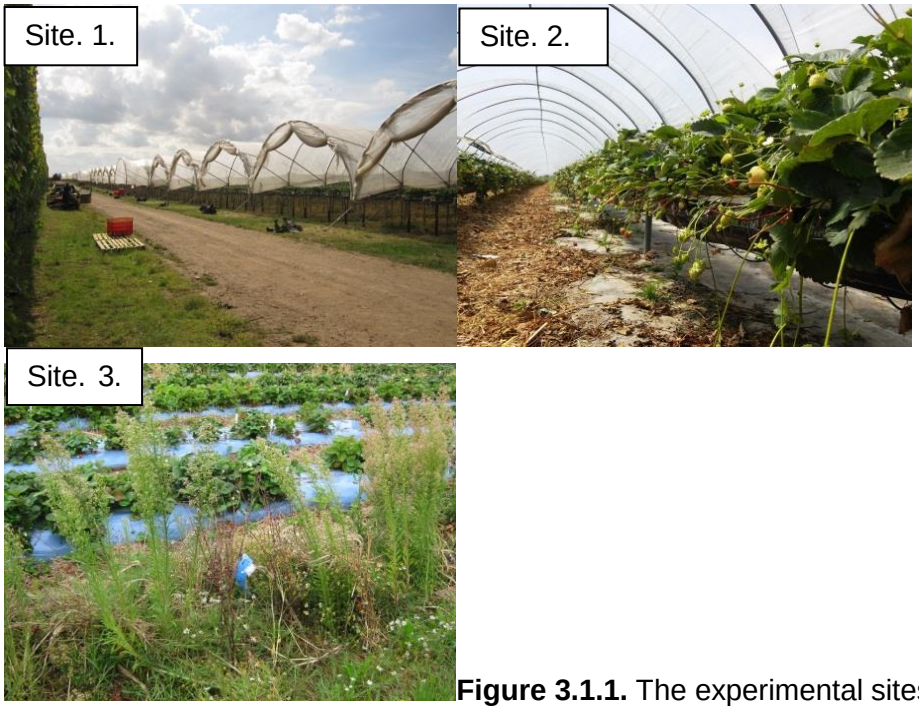


Figure 3.1.1. The experimental sites in 2017

Table 3.1.1. Site details and set up and assessment dates

Site	Growing method	Plantation	Variety	Number of replicates	Set up	Assessments
1	Standard table-top	Trackside	Amesti	20	13 Jun	27 Jun, 13, 25 Jul, 8 Aug
2	Low table-top	Sandbanks, Sandyfield	Eve's delight	15	22 Jun	6, 20, 31 Jul
3	Weeds	Surrounding strawberry field	Multiple varieties	6	29 Jun	12, 26 Jul, 9, 21 Aug

The strawberry sites were chosen to maximise the likelihood of catching WFT, but also with the possibility of trapping the capsid species. The standard height and low height table-top systems both used commercial coir grow bags with staggered planting holes. Both of the varieties were everbearers, with Amesti at Site 1 and Eve's delight at Site 2. An experiment was also set up at NIAB EMR in a naturally occurring weed strip surrounding a mixed strawberry variety planting, in raised beds with blue polythene mulch. This was chosen as capsid numbers are generally high in weed plots which contain *Matricaria* and *Chenopodium* (fat-hen), and this would maximise catches of capsid species.

Treatments were:

1. Blue dry sticky trap board 25 cm x 10 cm, as advised by Russell IPM, as the control
2. Blue dry sticky trap board + WFT pheromone lure
3. Blue dry sticky trap board + *Lygus* sex pheromone lure + phenylacetaldehyde (PAA)
4. Blue dry sticky trap board + WFT pheromone lure + *Lygus* sex pheromone lure + PAA

Replicates were placed at least 10 m apart and organised as a randomised block design. Pheromone lures were attached onto dry blue sticky traps (provided by Russell IPM, UK) (Fig. 3.1.2.). The phenylacetaldehyde and *Lygus* sex pheromone lures were prepared at NRI, University of Greenwich.

- Phenylacetaldehyde (PAA) was formulated in polyethylene sachets (1 ml on dental roll in polyethylene sachet 50 mm x 50 mm x 250 µm thick), release rate 6.7 mg/d at 22°C
- *Lygus* sex pheromone was formulated in 1 ml disposable pipettes (10 mg hexyl butyrate + 0.3 mg (E)-2-hexenyl butyrate + 2 mg (E)-4-oxo-2-hexenal + 1 mg Waxoline Black in 100 µl sunflower oil on cigarette filter), release rate of hexyl butyrate 0.93 ± 0.05 (S.E.) µg/hr at 27°C
- The WFT lure was provided by Bioline AgroSciences UK as the product Thripline. This product is an aggregation pheromone that attracts both males and females. The pheromone is encapsulated in rubber lures (septa) and is released gradually over several weeks

The *L. rugulipennis* pheromone lures were hooked onto the blue sticky trap using a modified paper clip. The PAA sachet was attached where necessary using a paper clip/bulldog clip to the side. The WFT lure was inserted into a hole punched into the sticky trap using a single hole punch. The blue traps and lures were renewed monthly. New lures did not need to be added on every occasion. The blue traps were placed horizontally in the strawberry sites and were attached to the metal hoops of the growing system structures. The traps were placed vertically in the weed experiment and were held 15 cm above ground level, supported on a white fibreglass cane. The orientation was determined by the support structures and practicality in the different situations.

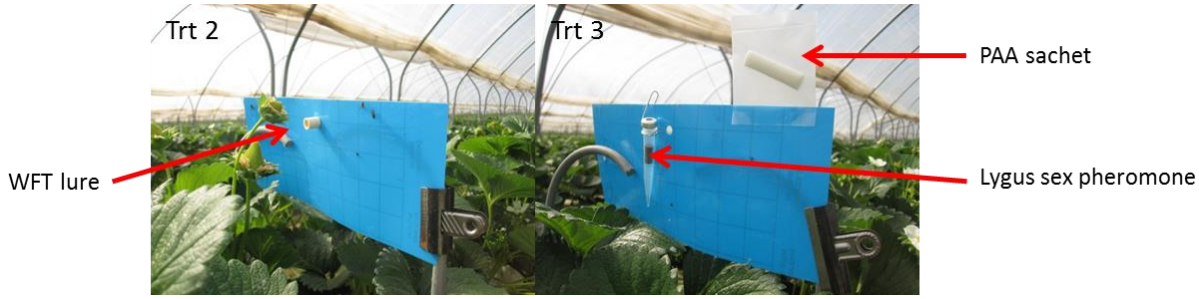


Figure 3.1.2. The blue sticky trap attached to the support poles of the strawberry growing system, showing Trt 2, the WFT pheromone lure and Trt 3, the *Lygus* pheromone lure and PAA sachet

Capsid assessments: The numbers of *Lygus rugulipennis* and *Lygocoris pabulinus* caught on the traps were counted on both sides of the blue sticky traps every 2 weeks when the traps were changed. In addition any *Liocoris tripustulatus* and *Lygus pratensis* were also recorded.

Thrips: The number of thrips on the traps was assessed at each trap change date for one side of the trap, under a binocular microscope. This was the same side as the pheromone and volatile dispensers, and the reverse side to the black line markings on the blue trap. It was only possible to accurately count thrips on sticky traps in the laboratory. The proportion of thrips that were WFT in the crop was assessed by collecting a twenty flower sample directly into ethanol and identifying from slide preparations all adult thrips found.

Predators: Natural enemies on the traps were recorded on one side of the blue sticky trap (as for the thrips assessments) at each trap change date, including Coccinelidae (ladybirds), Syrphidae (hoverflies), Neuroptera (lacewings), *Orius*, other Anthocoridae and other notable predatory species such as soldier beetles. Other beneficial species noted were bees, spiders and butterflies.

Data loggers were used to record temperature and humidity throughout the experimental period in each crop.

Data was analysed using square root transformed data and REML variance components analysis (linear mix model) for Site 1 due to an imbalance in the data, and with ANOVA for a complete randomised block design for Sites 2 & 3. To determine whether there was an interaction between the treatments, the analyses were structured to firstly compare any effect of the individual components. Therefore to determine the effect of the WFT lure, any of the treatments containing the WFT lure i.e. Trts 2 and 4 were compared with any of the treatments without the WFT lure i.e. Trts 1 and 3. Similarly to determine the effect of the *Lygus* sex pheromone lure + PAA sachet, any of the treatments containing the *Lygus* sex pheromone lure + PAA sachet i.e. Trts 3 and 4 were compared with any of the treatments without the *Lygus* sex pheromone lure + PAA sachet i.e. Trts 1 and 2. Finally the interaction between the treatments was determined. Additional snapshot ANOVAs with a focus on capsid species were also done to determine the effect of treatments on individual dates where required.

A small experiment was also set up to determine if the capsid species could be lost from the traps or indeed moved position within the dry sticky glue. Eight blue sticky traps were set up, as for the previous weed experiment, on 14 August, at the edge of a weed plot at NIAB EMR. Repeated monitoring of the traps was done, on 17, 21, 23 & 25 August, with any insect catches marked on the traps by circling with permanent ink around the insects.

Results

There were a number of significant results across the sites with a consistent increase in total thrips wherever the WFT lure was present and an increase in lacewings wherever the *Lygus* sex pheromone lure and PAA were present. Results which were significant at two or more sites were an increase in syrphids and bees, and a decrease in *Orius* sp., wherever the *Lygus* sex pheromone lure + PAA treatment was present. Interactions with an effect on capsids or thrips were considered if significant.

Site 1: There were few capsids at site 1 and a mixed thrips population, which included WFT. The results of the effect of the WFT lure on the arthropod species is shown in Table 3.1.2. and the effect of the *Lygus* sex pheromone lure + PAA is shown in Table 3.1.3.
There was no effect of either the WFT lure or the *Lygus* sex pheromone lure + PAA and no interaction between these two lure combinations for the total number of capsids either with REML or with snapshot analysis. Although there was a significant decrease of *L. tripustulatus*, numbers of this capsid were extremely low, therefore it is difficult to know how valid this result is.

Table 3.1.2. The effect of the Western Flower Thrips (WFT) lure (Thripline) on the square-root numbers of arthropods caught on blue sticky traps at Site 1. REML variance components analysis compared any of the treatments containing the WFT lure with any of the treatments without the WFT lure. ***, ** and * denote a significance at the *P*<0.001, 0.01 and 0.05 level, respectively d.f. 1, 42+.

Arthropod	**Sqrt No. Without WFT lure**	**Sqrt No. With WFT lure**	**s.e.d.**	***P***
Lygus rugulipennis	0.113	0.130	0.0540	0.956
Lygocoris pabulinus	0.103	0.100	0.0434	0.972
Liocoris tripustulatus	0.0294	0.00	0.0155	↓0.039 *
Lygus pratensis	0.0303	0.0270	0.0274	0.780
Total Capsids	0.249	0.223	0.0649	0.584
Thrips (total catch, incl. WFT)	6.35	8.17	0.1555	↑<0.001 ***
Syrphidae	2.97	2.67	0.1550	↓0.019 *
Bees	0.854	0.745	0.0883	0.153
Ladybirds	0.101	0.107	0.0387	0.855
Solider Beetles	0.308	0.332	0.0502	0.612
Lacewings	0.677	0.582	0.0766	0.138
Orius sp.	1.121	1.342	0.1060	0.064
Spiders	0.264	0.373	0.0761	0.124
Butterflies	0.322	0.368	0.0667	0.702

Table 3.1.3. The effect of the *Lygus* sex pheromone lure (*Lygus* lure) + phenylacetaldehyde (PAA) sachet on the square-root numbers of arthropods caught on blue sticky traps at Site 1. REML variance components analysis compared any of the treatments containing the *Lygus* lure + PAA with any of the treatments without the *Lygus* lure + PAA. ***, ** and * denote a significance at the *P*<0.001, 0.01 and 0.05 level respectively, d.f. 1, 42+.

Arthropod	**Sqrt No. Without *Lygus* lure + PAA**	**Sqrt No. With *Lygus* lure + PAA**	**s.e.d.**	***P***
Lygus rugulipennis	0.118	0.126	0.0540	0.948
Lygocoris pabulinus	0.0876	0.1159	0.0434	0.510
Liocoris tripustulatus	0.0021	0.0229	0.0155	0.296
Lygus pratensis	0.0125	0.0448	0.0274	0.346
Total Capsids	0.190	0.282	0.0649	0.200
Thrips (total catch incl. WFT)	7.413	7.106	0.1555	0.069
Syrphidae	2.552	3.089	0.1550	↑<0.001 ***
Bees	0.692	0.907	0.0883	↑0.010 **
Ladybirds	0.105	0.103	0.0387	0.972
Solider Beetles	0.351	0.289	0.0502	0.119
Lacewings	0.397	0.863	0.0766	↑<0.001 ***
Orius sp.	1.400	1.063	0.1060	↓0.004 **
Spiders	0.309	0.328	0.0761	0.816
Butterflies	0.290	0.400	0.0667	0.108

The overall total thrips numbers were doubled wherever the WFT lure was present, consistent with a previous study (Sampson, 2014) (sqrt count without a WFT lure = 6.35, with a WFT lure = 8.17, s.e.d. = 0.1555, *P* <0.001, d.f. = 1, 51). There was a significant effect of date, with the difference between the traps with and without a WFT lure decreasing with time (Fig.3.1.3, *P* <0.001, d.f.= 3, 178). There was also an interaction between the treatments, with fewer thrips where the *Lygus* sex pheromone + PAA trt was also present with the WFT lure (s.e.d. = 0.220, *P* = 0.019, d.f. = 1, 51, Fig. 3.1.4).

The numbers of syrphids were highest at the first sample date with a mean of 49 per trap across treatments, falling to approximately 2 per trap at the later dates. Therefore the effect of treatment is presented from a snapshot ANOVA from the first sample date (Fig. 3.1.5). If the treatments are examined across the season there were fewer syrphids wherever a WFT lure was present compared to wherever a WFT lure was not present (sqrt numbers: without a WFT lure in the trts = 2.968, with a WFT lure in the trts = 2.673, s.e.d. = 0.1550, *P* = 0.019, d.f. = 1, 56). There were more syrphids wherever a *Lygus* sex pheromone lure + PAA sachet were present than wherever a *Lygus* sex pheromone lure + PAA sachet were not present (sqrt numbers: without a *Lygus* sex pheromone lure + PAA in the trts = 2.552, with a *Lygus* sex pheromone + PAA in the trts = 3.089, s.e.d. = 0.1550, *P* = <0.001, d.f. = 1, 56). There was no interaction between the WFT lure and the *Lygus* sex pheromone + PAA sachet. If the data from the first assessment only is analysed using ANOVA (for 12 reps) then the increase in syrphid catch is only seen in the *Lygus* sex pheromone + PAA treatment (Fig. 3.1.5).

Although there was a significant increase in the numbers of bees where there was a *Lygus* sex pheromone lure + PAA in the trts, there were fewer than 1 bee per trap across the season (sqrt numbers: without a *Lygus* sex pheromone lure + PAA in the trts = 0.692, with a *Lygus* sex pheromone lure + PAA in the trts = 0.907, s.e.d. = 0.0883 , *P* = 0.01, d.f. = 1, 52). Lacewing numbers increased as the season progressed to 1 lacewing per trap in August. There was a significant increase in lacewing catches where there was a *Lygus* sex pheromone lure + PAA in the trts (sqrt numbers: without a *Lygus* sex pheromone lure + PAA in the trts = 0.397, with a *Lygus* sex pheromone lure + PAA in the trts = 0.863, s.e.d. = 0.0766 , *P* < 0.001, d.f. = 1, 52). There was also a significant interaction between the two treatments (s.e.d. 0.1084, *P* = 0.049, d.f. 1, 52, **Fig. 3.1.6**). Although there was a decrease in numbers of *Orius* spp. with a *Lygus* sex pheromone lure + PAA in the trts, compared to without, there were low numbers of *Orius* (sqrt numbers: without a *Lygus* sex pheromone lure + PAA in the trts = 1.4, with a *Lygus* sex pheromone lure + PAA in the trts = 1.06, s.e.d. = 0.106, *P* = 0.004, d.f. = 1, 54).

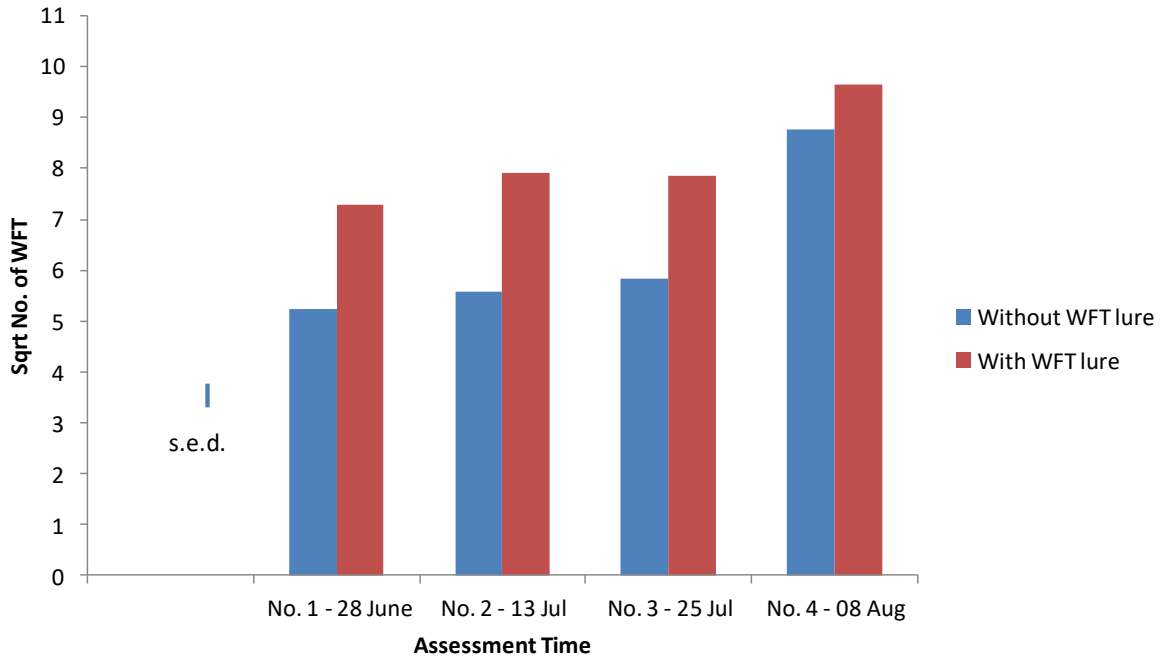


Figure 3.1.3. The effect of date on mean square-root total thrips numbers caught on dry glue blue sticky traps per trap, comparing any treatments without a WFT pheromone lure and treatments with a WFT pheromone lure

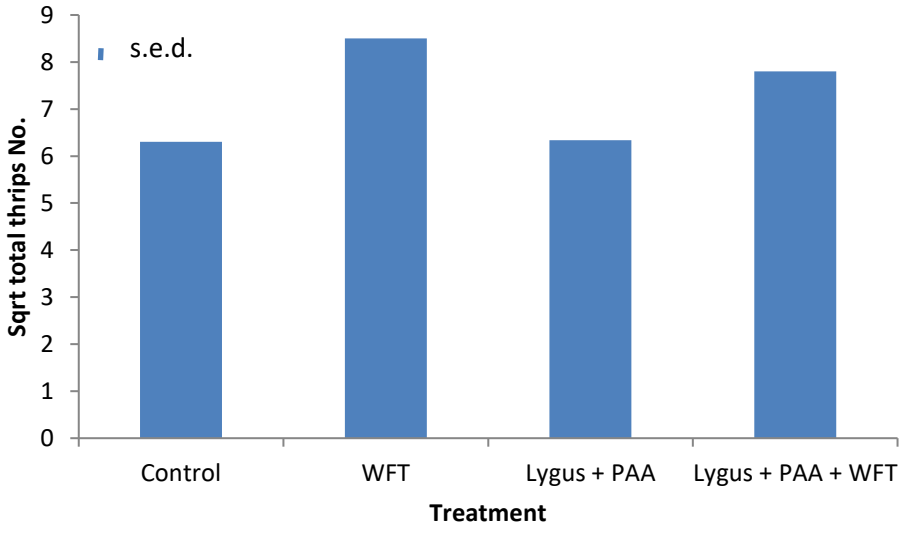


Figure 3.1.4. The effect of treatment on mean square-root total thrips numbers caught on dry glue blue sticky traps per trap, with 4 trap assessments across the season at site 1. Treatments were WFT pheromone lure (WFT), *Lygus* sex pheromone lure + PAA (*Lygus* +

PAA), a combination of the two (*Lygus* + PAA + WFT) and an untreated control (blue sticky trap alone).

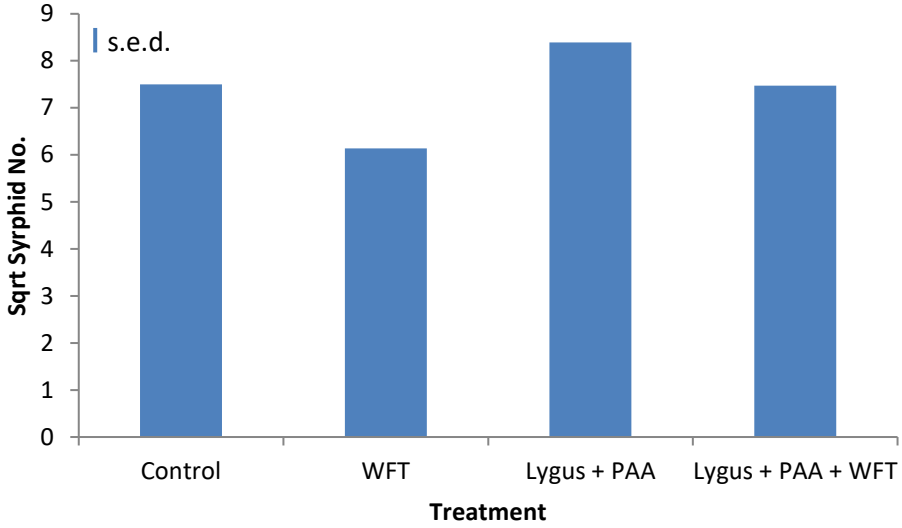


Figure 3.1.5. The effect of treatment on mean square-root total syrphid numbers caught on dry glue blue sticky traps per trap, at Site 1 for assessment 1. Treatments were WFT pheromone lure (WFT), *Lygus* sex pheromone lure + PAA (*Lygus* + PAA), a combination of the two (*Lygus* + PAA + WFT) and an untreated control (blue sticky trap alone) (s.e.d. = 0.598, *P* = 0.007, d.f. = 3, 33).

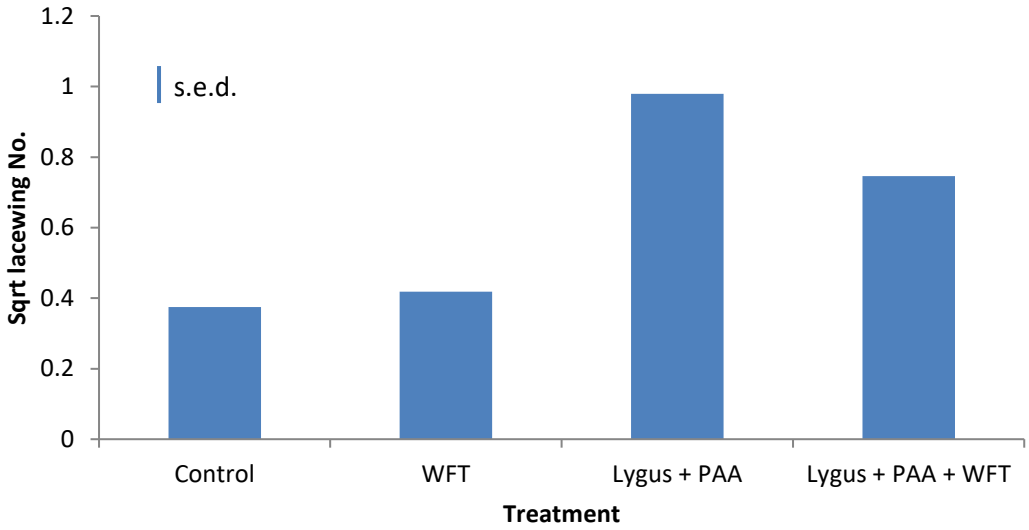


Figure 3.1.6. The effect of treatment on mean square-root total lacewing numbers caught on dry glue blue sticky traps per trap, with 4 trap assessments across the season at site 1. Treatments were WFT pheromone lure (WFT), *Lygus* sex pheromone lure + PAA (*Lygus* +

PAA), a combination of the two (*Lygus* + PAA + WFT) and an untreated control (blue sticky trap alone).

Site 2: As with site 1 there were few capsids at site 2 and a mixed thrips population, which included WFT. Overall ANOVA analyses across the season were done to look at the effects firstly of the WFT lure, comparing traps with and without the WFT lure (Table 3.1.4), then of the *Lygus* sex pheromone lure + PAA, again comparing traps with and without these volatiles (Table 3.1.5), then to look at interactions between the WFT lure and the *Lygus* sex pheromone lure + PAA.

As expected there was an overall increase in thrips numbers where the WFT pheromone lure was present (sqrt numbers: without the WFT pheromone lure = 5.99, with the WFT pheromone lure = 7.76, s.e.d. = 0.1642, $P < 0.001$, d.f. = 1, 42), but no interaction between the treatments.

There was also an increase in the number of butterflies (sqrt numbers: without the WFT pheromone lure = 0.109, with the WFT pheromone lure = 0.238, s.e.d. = 0.0508, $P = 0.028$, d.f. = 1, 42).

Table 3.1.4. The effect of the Western Flower Thrips (WFT) lure (Thripline) on the square-root numbers of arthropods caught on blue sticky traps at Site 2. ANOVA compared any of the treatments containing the WFT lure with any of the treatments without the WFT lure. ***, ** and * denote a significance at the P<0.001, 0.01 and 0.05 level, respectively d.f.1, 42.

Arthropod	**Sqrt No. Without WFT lure**	**Sqrt No. With WFT lure**	**s.e.d.**	***P***
Lygus rugulipennis	0.113	0.095	0.0549	0.744
Lygocoris pabulinus	0.124	0.097	0.0485	0.582
Liocoris tripustulatus	0.016	0.015	0.0182	0.976
Total Capsids	0.251	0.177	0.0717	0.307
Thrips (total catch, incl. WFT)	5.986	7.762	0.1642	↑<0.001 ***
Syrphidae	0.902	0.725	0.1092	0.111
Bees	1.009	0.894	0.1146	0.323
Ladybirds	0.056	0.089	0.0545	0.392
Solider Beetles	0.060	0.034	0.0322	0.423
Lacewings	0.497	0.511	0.0878	0.881
Orius sp.	1.221	1.097	0.1324	0.355
Spiders	0.432	0.476	0.0742	0.554
Butterflies	0.109	0.238	0.0568	↑0.028 *

Table 3.1.5. The effect of the *Lygus* sex pheromone lure (*Lygus* lure) and phenylacetaldehyde (PAA) sachet on the square-root numbers of arthropods caught on blue sticky traps at Site 2. ANOVA compared any of the treatments containing the *Lygus* lure + PAA with any of the treatments without the *Lygus* lure + PAA. ***, ** and * denote a significance at the P<0.001, 0.01 and 0.05 level, respectively, d.f. 1, 42.

Arthropod	**Sqrt No. Without *Lygus* lure + PAA**	**Sqrt No. With *Lygus* lure + PAA**	**s.e.d.**	***P***
Lygus rugulipennis	0.081	0.127	0.0549	0.404
Lygocoris pabulinus	0.053	0.168	0.0485	↑0.022 *
Liocoris tripustulatus	0.015	0.016	0.0182	0.976
Total Capsids	0.127	0.300	0.0717	↑0.021 *
Thrips (total catch incl. WFT)	6.860	6.888	0.1642	0.868
Syrphidae	0.627	1.000	0.1092	↑0.001 **
Bees	0.796	1.107	0.1460	↑0.009 **
Ladybirds	0.022	0.122	0.0545	↑0.013 *
Solider Beetles	0.039	0.056	0.0322	0.604
Lacewings	0.200	0.808	0.0878	↑<0.001 ***
Orius sp.	1.425	0.892	0.1324	↓<0.001 ***
Spiders	0.550	0.358	0.0742	↓0.013 *
Butterflies	0.172	0.176	0.0568	0.944

Wherever the *Lygus* sex pheromone lure + PAA was present, compared to wherever the *Lygus* sex pheromone lure + PAA was absent, there were significant increases in capsid numbers (for the total number of capsids, driven by the catches of *L. pabulinus*, common green capsid, as a category) (Table 3.1.5). Capsid numbers increased over time, with the most capsids caught by the last assessment, between 21 and 31 July. Wherever the *Lygus* sex pheromone lure + PAA was present there was also an increase in the numbers of syrphids, bees, ladybirds and lacewings, but decreases in the numbers of *Orius* spp. and spiders (Table 3.1.5).

Site 3: There were generally less effects of the treatments at site 3, and the significant results are described below.

Capsids were present at the weed site 3; however neither the WFT pheromone lure, nor the *Lygus* sex pheromone lure + PAA had an effect on catches. *Lygus rugulipennis* was the most prevalent capsid species, an average of 1 per trap were caught when analysed across the season.

Thrips were also present at site 3; however the thrips complex did not include WFT. As was found at the other sites, there was still an increase in thrips numbers on the blue traps where a WFT pheromone lure was present compared to where it was absent (sqrt numbers: without a WFT pheromone = 7.454, with a WFT pheromone lure = 9.04, s.e.d. = 0.342, P = <0.001, d.f. = 1, 15). There was no effect of the *Lygus* sex pheromone lure + PAA on trap catches of thrips.

There was an increase in the lacewing trap catch wherever the *Lygus* sex pheromone lure + PAA was present, although numbers of lacewings were low (sqrt numbers: without *Lygus* sex pheromone lure + PAA = 0.11, with *Lygus* sex pheromone lure + PAA = 0.33, s.e.d. = 0.079, P = 0.012, d.f. = 1, 15). Butterfly numbers were reduced wherever the WFT lure was present, the opposite result from site 2. However, numbers of butterflies were low (sqrt numbers: without a WFT pheromone lure = 0.213, with a WFT pheromone lure = 0.083, s.e.d. = 0.044, P = 0.01, d.f. = 1, 15).

The capsid movement experiment was set up to determine if the larger capsid species could walk free from the dry sticky glue on the blue traps. When blue sticky traps were monitored daily it was clear that there was some movement of capsids (which were mainly *L. rugulipennis*) and some losses from the traps, however the majority of capsids remained on the traps. By the 25 August, 11 days after the traps were set up, 20% of the capsids had been

lost and 10% had moved but remained on the traps (total of 148 capsids, Fig. 3.1.7). It should be noted that these traps were outside, not under polytunnels. These sticky traps were dry glue type, which has fewer losses than the wet glue type (Russell IPM, pers. comm.).

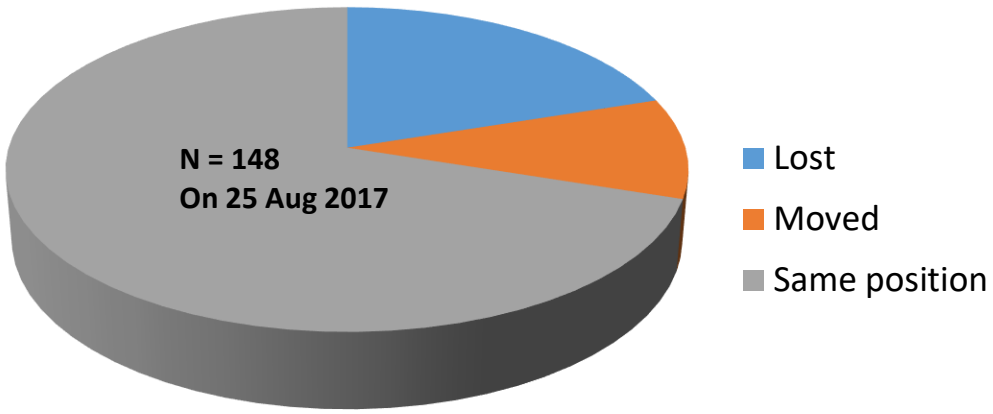


Figure 3.1.7. The number of capsids (*Lygus rugulipennis*) that were lost, moved, or remained in the same position following initial trapping on a dry blue sticky trap (Russell IPM, UK) over an 11 day period (traps checked on four occasions).

Discussion

The experiments have shown that *L. rugulipennis* and *L. pabulinus* can be attracted to a blue sticky trap with the addition of a *Lygus* sex pheromone + phenylacetaldehyde (PAA). However, as the standard green bucket traps were not included as a control, we have not determined whether this would be a more effective method of trapping. The capsid detachment experiment showed that 20% of the trap catches were being lost from the blue sticky traps; therefore it is not 100% effective as a trapping method. It was not possible to determine whether the escaped adults died or, in the case of the females, could continue to lay eggs.

The *Lygus* sex pheromone lure + PAA can be used in conjunction with the WFT pheromone lure. The thrips catches are always higher when a WFT lure is present. The catches are also

still higher than the control when the *Lygus* sex pheromone lure + PAA is used in conjunction with the WFT pheromone. However, in one experiment there is evidence of an interaction between the two treatments, and the WFT catches for the combined treatment were less than the WFT pheromone alone.

The addition of the PAA improves *L. rugulipennis* female trap catches (Koczor et al., 2012). This plant volatile has also been known to attract noctuid moths and has been shown to be a generic attractant (El-Sayed et al., 2008), including for beneficials, such as green lacewings (Toth et al., 2009) and syrphids (Hesler, 2016). In this study lacewings and syrphids were trapped in higher numbers where the *Lygus* pheromone lure and the PAA were present. It is essential to increase the trap catches of the female *L. rugulipennis* if this system is to be used as a trapping, rather than a monitoring, system. However, the floral component may be detrimental to some beneficial species.

On balance, to preserve the natural enemies in the crop and to control the pest, improving the floral attractants in the green bucket trap design may be an alternative route for *L. rugulipennis* control that would be of value.

Future work

- To optimise the volatile blend for the female attractant sachets (currently PAA) which accompany the *Lygus* sex pheromone lure, but for use in the green bucket traps.

Task 3.2. To investigate the potential of a push-pull system for control of capsids in strawberry.

Introduction

The European tarnished plant bug, *Lygus rugulipennis*, becomes a damaging pest of strawberry, requiring routine treatment with insecticides, usually from June onwards in everbearer crops. Feeding in flowers and on green fruits can cause up to 80% crop loss, rendering production uneconomic and products used can disrupt biological control agents and increase pesticide residues in fruits. *Lygocoris pabulinus* (the common green capsid) may also be a damaging pest, and its appearance within crops tends to be sporadic and locally distributed. A push-pull system could be deployed to enable medium-term control, which could be integrated into an IPM system. Push-pull strategies are designed to have an element which is unattractive to insect pests (such as repellence or masking), the push, combined with an attractant source to draw the pest away from the crop, the pull. The pull can be combined with a killing agent to prevent the pest re-entering the crop and to reduce population growth. Commonly these strategies are employed in developing countries using plants as both trap crop and repellent (Cook *et al*, 2007). This study investigated whether;

- Capsids, *L. rugulipennis* and *L. pabulinus*, could be repelled from a strawberry crop using hexyl butyrate (push system)
- A perimeter pheromone trapping system (pull system) could be used in conjunction with the repellent system for improved efficacy
- *Lygus* damage i.e. cat-facing of the fruit, was reduced where treatments were applied.

Methods

The experiment was set up as a randomised block design, with four tunnelled strawberry crops acting as replicates (and as blocks). These were on different farms (sites), with one crop at each site (and the crops at sites 3 and 4 situated close to each other; see Appendix 3.2.1):

Site 1. Hugh Lowe Farms, Mereworth, Kent. ME18 5NF by kind agreement of Tom Pearson.

Site 2. Edward Vinson Farms, Faversham, Kent. ME13 8UP by kind agreement of Sean Figgis.

Sites 3 & 4. Quaives Farm, part of Kelsey Farms group, Grove Road, Wickhambreaux, Canterbury, Kent. CT3 1RY by kind permission of John Ricks.

All of the sites were tunnel grown strawberries, using standard height systems, and using grow-bags with staggered planting holes. Varieties differed between the sites, with Amesti grown at sites 1, 3 and 4, and Sweet Eve 2 at site 2.

Each treated area was a 25 m x 25 m plot. These were 3 or 4 tunnels wide depending on the tunnel span at each site (i.e. 8 or 6 m tunnel spans). Plots were set up either at the corners of the crop as in Fig. 3.2.1, or along the edge of the crop, depending on pest pressure. Plots were greater than 60 m apart to avoid interaction between the treatments.

Lures were prepared at NRI.

- Hexyl butyrate (HB) was formulated in polyethylene sachets (1 ml on a dental roll in polyethylene sachet 50 mm x 50 mm x 120 µm thick) with release rate of 18 mg/d at 22°C.
- Phenylacetaldehyde (PAA) was formulated in polyethylene sachets (1 ml on dental roll in polyethylene sachet 50 mm x 50 mm x 250 µm thick), release rate 6.7 mg/d at 22°C
- *Lygus* sex pheromone was formulated in 1 ml disposable pipettes (10 mg hexyl butyrate + 0.3 mg (*E*)-2-hexenyl butyrate + 2 mg (*E*)-4-oxo-2-hexenal + 1 mg Waxoline Black in 100 µl sunflower oil on cigarette filter), release rate of hexyl butyrate 0.93 ± 0.05 (S.E.) µg/hr at 27°C.

Treatments were:

1. Push - Hexyl butyrate (HB) in polyethylene sachets stapled to the polythene of the strawberry bags within the rows, 1 every 2 m, with a central block of 8 x 8 HB sachets at 2 m spacing
2. Pull - *Lygus* sex pheromone + female *Lygus* attractant PAA in green “bucket traps” (Agralan UK, *Lygus rugulipennis* trap system) every 8 m around the perimeter of the plot with 12 traps in total
3. Push–Pull - Hexyl butyrate sachets applied to strawberry bags as above + *Lygus* sex pheromone + female *Lygus* attractant PAA, perimeter traps. Note that the hexyl butyrate block was 5 m away from the pull traps to prevent interference with the pheromone as hexyl butyrate is a component of the *Lygus* sex pheromone
4. Control plot with no traps or repellents

Treatments were randomised.

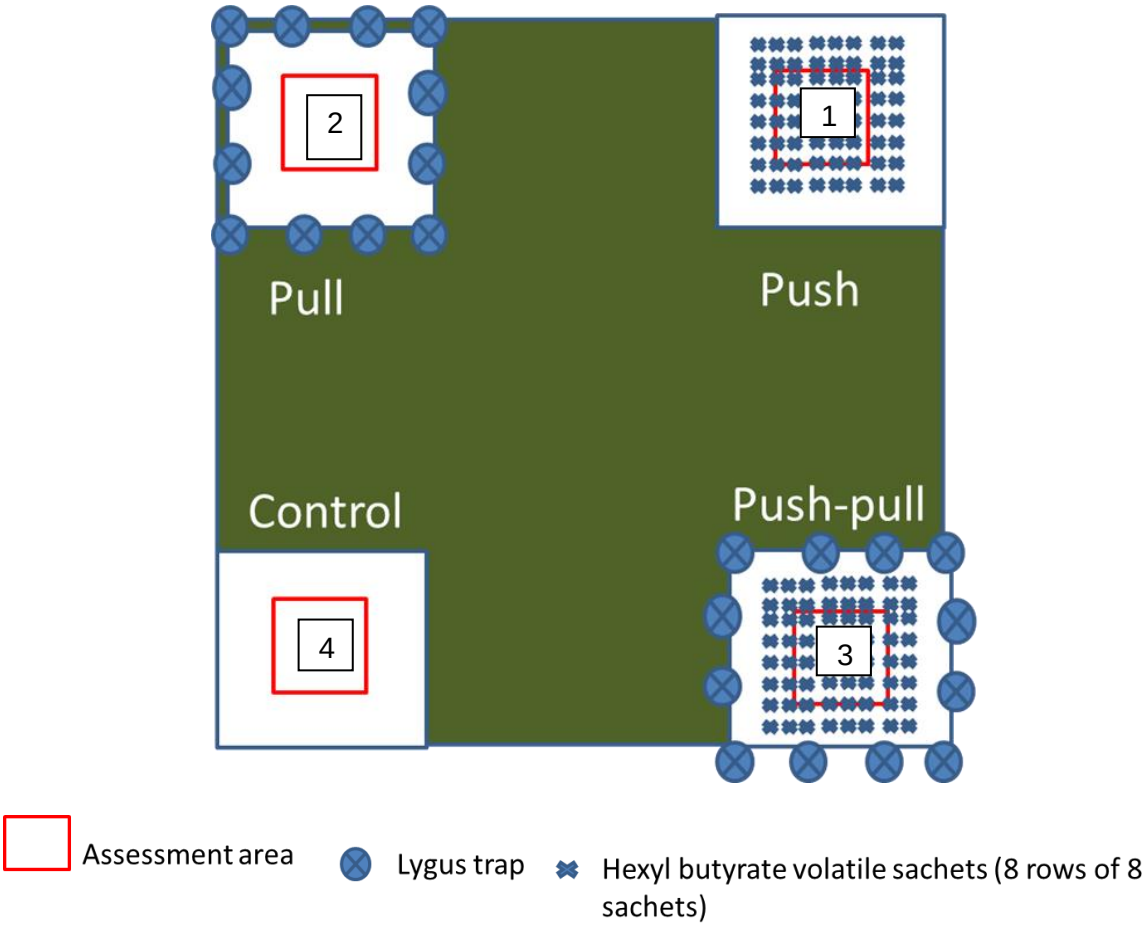


Figure 3.2.1: Diagrammatic representation of an experimental block of the push-pull experiment, showing: **1. Push** Hexyl butyrate sachets within the rows, 1 every 2 m, **2. Pull** *Lygus* sex pheromone + female *Lygus* attractant (PAA) traps every 8 m around the perimeter of the plot**, 3. Push-Pull** Hexyl butyrate sachets + *Lygus* sex pheromone + female *Lygus* attractant (PAA) perimeter traps and **4. Control plot** with no traps or repellents

The 'pull' perimeter traps were placed in-between two grow bags or at the end of the row in between the metal support and the first grow bag (Figs. 3.2.2 a & b). The 'push' hexyl butyrate sachets were stapled to the grow bag (Fig. 3.2.3) in a situation where they would not touch developing fruit. The semiochemical release units were renewed after 1 month.

Figures 3.2.2. a) 'Pull' perimeter trap showing placement in the crop; **b)** positioning of the *Lygus* sex pheromone lure and female *Lygus* attractant PAA sachet within the trap

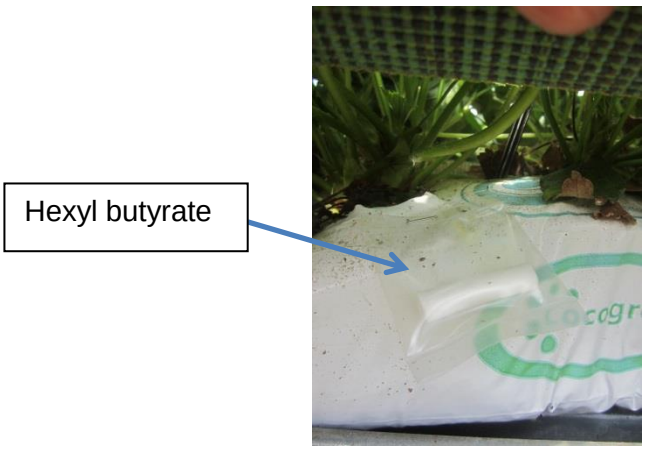


Figure 3.2.3. 'Push' Hexyl butyrate sachet stapled to the grow bag

The experiment was run for two months in 2017 and was set up on 4 July at Site 1, 5 July at Site 2 and 11 July at Sites 3 & 4. A grower spray programme was used, which differed at each site (Appendix 3.2.2). Growers were advised that non-essential insecticide sprays should be avoided to prevent target pests being killed. Data loggers recorded temperature and humidity throughout the experimental period in each crop (Appendix 3.2.3.).

The effect on capsid numbers throughout the season and resultant fruit damage was monitored. Tap samples within the assessment area of the crops were done every 2 weeks on 4 occasions (Table 3.2.1) to record the capsid species, sex and life-stage (nymphs and adults) (60 plants were tapped per plot). Insect numbers from the tap samples were analysed following a square root transformation over 4 assessment dates for *L. rugulipennis* adults and

nymphs, and *L. pabulinus* adults and nymphs. The numbers (and sex where possible) of adult *L. rugulipennis* and *L. pabulinus* in the perimeter traps of the pull and push-pull treatments were counted every 2 weeks following set-up (dates as for the tap samples in Table 3.2.1). The difference between these treatments on the different dates at the different sites was analysed using ANOVA.

Table 3.2.1. Dates for capsid tap samples within each crop assessment area, 2017

Location	**Date of experiment set-up**	**Tap sample 1**	**Tap sample 2**	**Tap sample 3**	**Tap sample 4**
Site 1	4 Jul	18 Jul	1 Aug	17 Aug	31 Aug
Site 2	5 Jul	18 Jul	2 Aug	15 Aug	4 Sep
Site 3	11 Jul	27 Jul	11 Aug	22 Aug	7 Sep
Site 4	11 Jul	27 Jul	11 Aug	22 Aug	7 Sep

Flowers were tagged at each visit to relate numbers of pest to subsequent damage. Crop damage was assessed for 100 fruits per plot on four occasions. These were categorised as zero, slight, moderate and severe capsid damage (Fig. 3.2.4). The timing of the first assessment was determined by following tagged flowers, and subsequent assessments were at two-week intervals (Table 3.2.2). All fruit at the same development stage on a plant was assessed to prevent bias. The area and length of the crop that was assessed was recorded. Damage assessments were started in August and were carried through until mid-September.

Table 3.2.2. Dates for strawberry damage assessments within each crop assessment area, 2017

Location	Date of experiment set-up	Damage assessment 1	Damage assessment 2	Damage assessment 3	Damage assessment 4
Site 1	4 Jul	1 Aug	17 Aug	31 Aug	15 Sep
Site 2	5 Jul	2 Aug	15 Aug	4 Sep	15 Sep
Site 3	11 Jul	11 Aug	22 Aug	7 Sep	21 Sep
Site 4	11 Jul	11 Aug	22 Aug	7 Sep	21 Sep

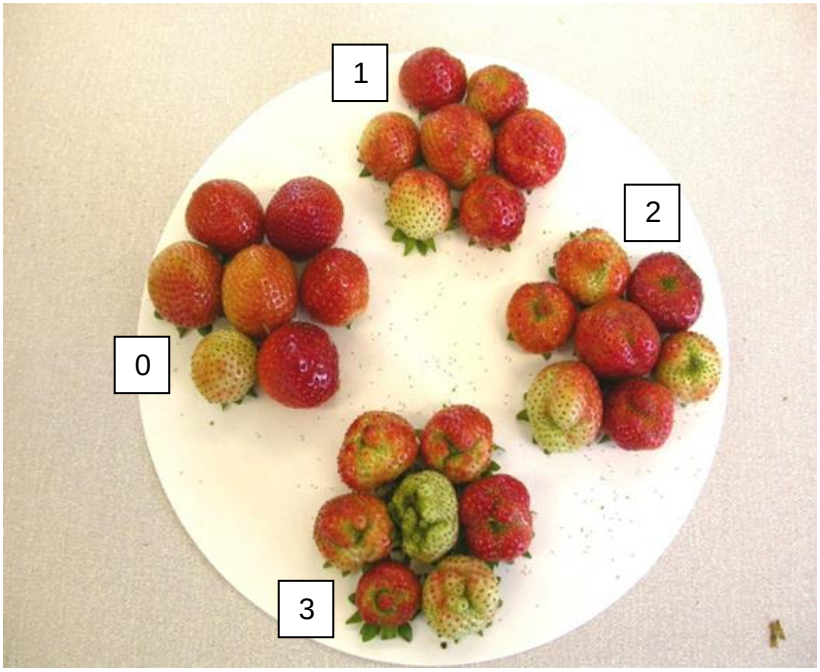


Figure 3.2.4. *Lygus* damage categories for strawberry fruits; from left working clockwise, 0 = no damage, 1 = low damage, 2 = moderate damage, 3 = high damage

Data for damage were analysed by firstly calculating a damage score. The damage score was determined for analysis using the formula (%0*0 + %1*1 + %2*2 + %3*3)/3. Values ranged from 0 if all of the fruits are in the '0' category, to 100 if all of the fruits are in the '3' category. Whilst this does not relate directly to the mean % damage, this allows data between

plots to be compared statistically and to be transformed for analysis; in this case an angular transformation was used prior to ANOVA. Overall effects of the 'push' treatment, the 'pull' treatment and any potential interaction between the treatments were examined. The percentage of fruits in each category were also analysed using ANOVA, comparing the effect of treatment on the % of fruit with low damage (in categories 0 + 1) and the percentage of fruit with zero damage (in category 0).

Results

There were generally low numbers of *L. rugulipennis* in the plots. However there were significantly fewer adults and nymphs where the 'push' was applied, i.e. if hexyl butyrate sachets were present (i.e. in the 'push' and the 'push-pull' treatments), compared to where the 'push' was not applied (i.e. in the 'pull' and the 'control' treatments). Overall numbers of *L. rugulipennis* adults per plot (per date) for 'no push' were 0.1 with 'no push' and 0.01 with 'push'. The data were analysed using square root transformed counts which is shown in Fig. 2.2.5 (P = 0.048, s.e.d. = 0.0865, l.s.d. = 0.1958, d.f. = 1,9).

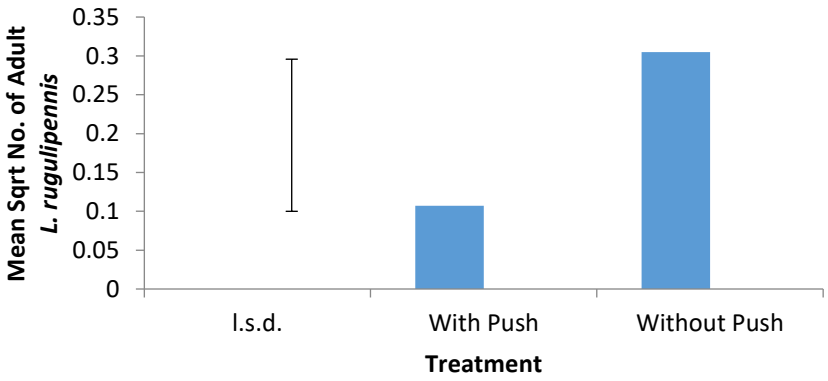


Figure 2.2.5. The effect of the 'push' treatment, hexyl butyrate sachets, on the mean square root number of *L. rugulipennis* adults per plot.

Overall mean numbers of *L. rugulipennis* nymphs per plot (per date) were 0.1 with 'no push' and 0.01 with 'push'. As above the data were analysed using square root transformed counts which is shown in **Fig. 2.2.6** (P = 0.033, s.e.d. = 0.0974, l.s.d. = 0.2204, d.f. = 1,9).

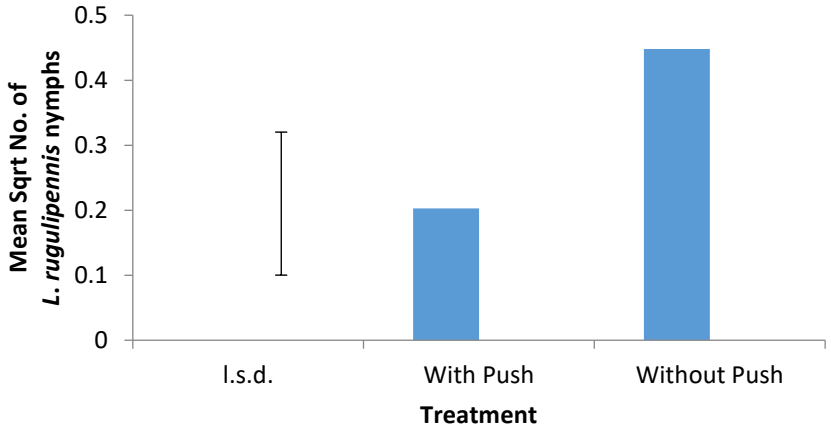


Figure 2.2.6. The effect of the 'push' treatment, hexyl butyrate sachets, on the mean square root number of *L. rugulipennis* nymphs per plot.

Differences were not statistically significant for the *L. pabulinus* adults and nymphs, although overall numbers were lower where a treatment was applied.

There were no significant effects where the 'pull' treatment (*Lygus* perimeter traps) was used i.e. in the 'pull' alone or in the 'push-pull', compared to where the 'pull' treatment was not present, i.e. in the 'control' or the 'push' treatment, on either *L. rugulipennis* or *L. pabulinus* adults or nymphs.

The numbers of capsid bugs, adults and nymphs for both *L. rugulipennis* and *L. pabulinus* were analysed to determine the effect of date using a square root transformation. Although, numbers of capsids in the crop increased over time, there was no significant effect of date in any case (Fig. 2.2.7).

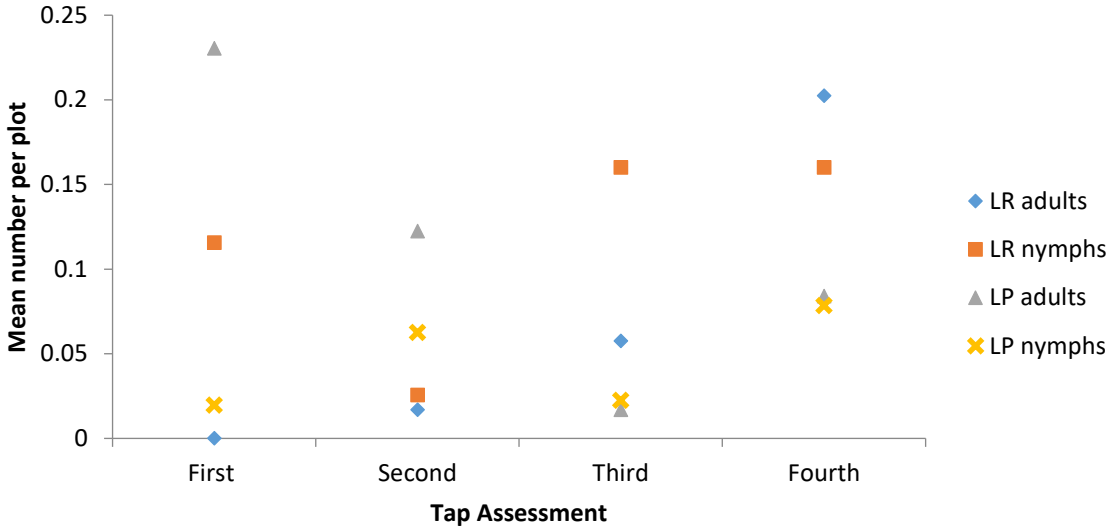


Figure 2.2.7. The back transformed numbers of *L. rugulipennis* (LR) and *L. pabulinus* (LP) adults and nymphs per plot, averaged across all treatments

There was no significant difference in the numbers of *L. rugulipennis* caught in the *Lygus* sex pheromone + PAA perimeter traps between the two treatments which contained a 'pull'. Across the two-month experimental period there was a mean of 12 *L. rugulipennis* caught per plot (total of 12 traps) in the 'pull' treatment and 8 per plot in the 'push-pull' treatment. Although there were some females, there were 11 times more males. There were only 5 *L. pabulinus* (both male and female) caught in the perimeter trap catches across both of the treatments.

Following angular transformation of the damage score there was significantly less fruit damage where there was a 'push' with the hexyl butyrate sachets when the treatments with the 'push' (i.e. Trt 1 'push' and Trt 3 'push-pull) were compared with the treatments without the 'push' (i.e. Trt 2 'pull' and Trt 4 'control' (Table 3.2.3).

Following angular transformation of the damage score, there was also significantly less fruit damage where there was a 'pull' when the treatments with the 'pull' (i.e. the 'pull' and the 'push-pull) were compared with the treatments without the 'pull' (i.e. the 'push' and the 'control') (Table 3.2.4).

Table 3.2.3. The effect of the 'push' component, hexyl butyrate sachets, on the mean Damage Score for strawberry fruits, following an angular transformation, with a lower score indicating less damage (*P* = 0.019, s.e.d.=1.307, l.s.d.=2.956, d.f.=1,9)

	Damage Score	
Treatment	**Angular transformed**	**Back transformed**
With 'push'	17.86	9.40
Without 'push'	21.60	13.55

Table 3.2.4. The effect of the 'pull' component, *Lygus* sex pheromone lures + female *Lygus* attractant phenyl acetaldehyde (PAA), in perimeter green bucket traps, on the mean Damage Score for strawberry fruits, following an angular transformation, with a lower score indicating less damage (*P* = 0.013, s.e.d. = 1.307, l.s.d. = 2.956, d.f .= 1,9)

	Damage Score	
Treatment	**Angular transformed**	**Back transformed**
With 'pull'	17.86	9.24
Without 'pull'	21.76	13.74

There was no evidence of a 'push' x 'pull' interaction (*P* = 0.653). As there is no interference between the treatments, it is possible to combine the treatments in the field.

When the damage score was analysed following an angular transformation, there was less fruit damage where a treatment was present (*P* = 0.016, s.e.d. = 1.848, l.s.d. = 4.181, d.f. = 3,9, Fig. 3.2.7). The least damage was seen in the combined attractant and repellent treatments; push-pull, although this was not statistically different to the other two treatments.

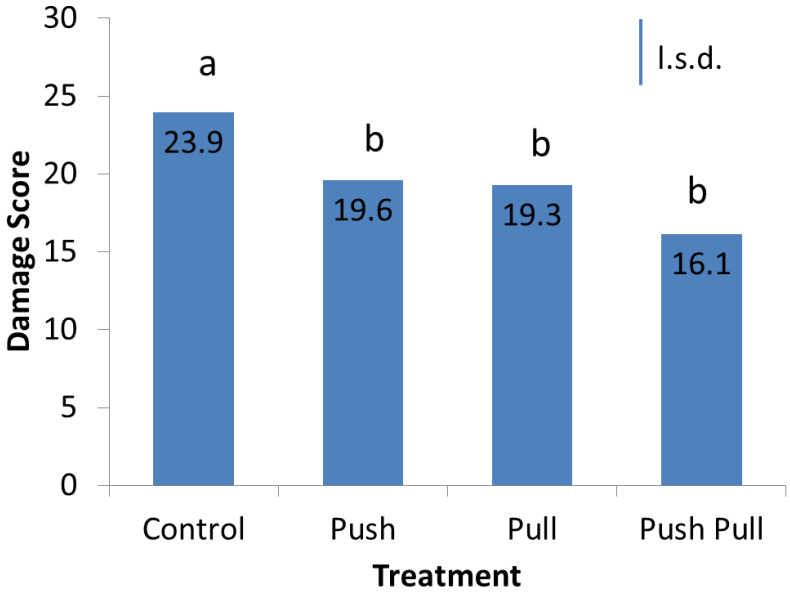


Figure 3.2.7. The effect of a push, pull or push-pull treatment on the strawberry fruit damage score, following angular transformation.

The angular transformed mean damage score is also shown for each of the four assessment dates in Table 3.2.5. There was a significant effect of date where the mean damage score, decreased across the four assessment dates ($P < 0.001$, s.e.d. = 1.909, l.s.d. = 3.871, d.f. = 3,36). Overall, the date by treatment effect was not significant at the 5% level ($P = 0.980$, s.e.d. = 3.787, l.s.d. = 7.63, d.f. = 9,45)

Table 3.2.5. Angular transformed mean damage score across four assessment dates.

	Damage Assessment			
Treatment	**1**	**2**	**3**	**4**
Control	26.67	24.23	20.98	23.84
Push	25.54	20.47	16.19	16.12
Pull	23.77	19.45	16.29	17.56
Push-Pull	22.80	16.38	12.67	12.68
Mean	23.93	19.58	19.27	16.13

We can also look at what this means for the grower by comparing the effects of the treatments on the % of fruit with low damage (in categories 0 + 1) and with zero damage (in category 0). The analysis was done on angular transformed data, and this data is presented in Table 3.2.6. The back transformed % means are also presented in Figure 3.2.8, to give understanding representation of the data in real terms. The percentages of fruit with low or zero damage were significantly higher with the push-pull treatment than in the untreated control.

Table 3.2.6. The effect of treatments on the mean percentage (following angular transformation) of strawberry fruits with low damage (category 0+1) or zero damage (category 0) due to cat-facing by *L. rugulipennis (*means followed by different letters are significantly different $P < 0.05$).

	Mean % with low damage		**Mean % with zero damage**	
Treatment	**Angular transformed**	**Back transformed**	**Angular transformed**	**Back transformed**
Control	70.36 a	88.70	54.77 a	66.73
Push	75.26 ab	93.52	60.85 b	76.28
Pull	75.32 ab	93.58	61.19 b	76.78
Push-Pull	78.06 b	95.72	65.55 b	82.87
P	0.043		0.006	
s.e.d.	2.236		2.198	
l.s.d.	5.059		4.972	
d.f.	3,9		3,9	

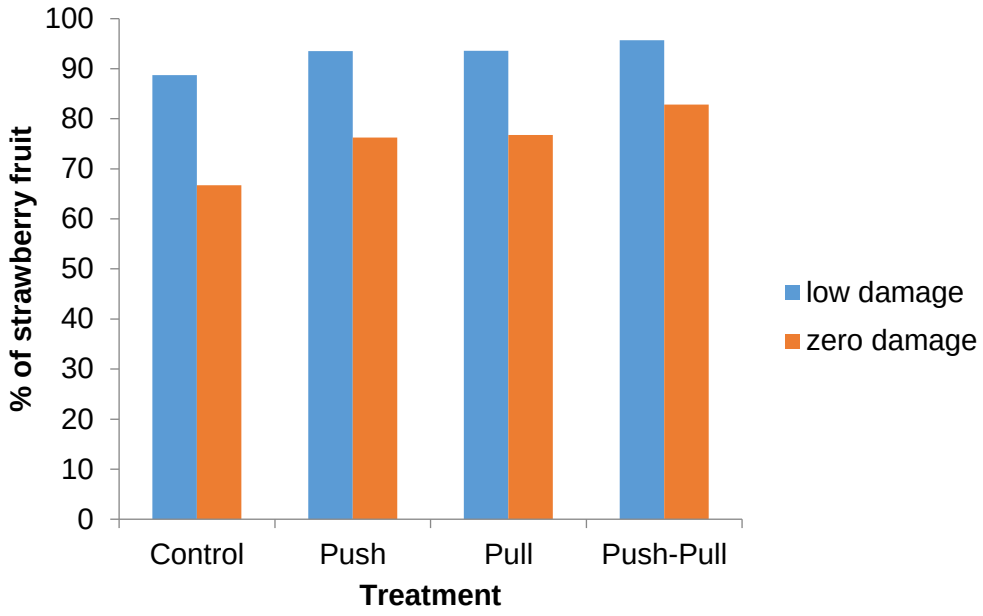


Figure 3.2.8. The effect of treatment: 'push' hexyl butyrate sachets within the crop, 'pull' *Lygus* sex pheromone + female *Lygus* attractant phenyl acetaldehyde (PAA) in perimeter traps, 'push-pull' a combination of the two treatments, compared to an untreated control, on the mean percentage of strawberry fruits with low damage (category 0+1) or zero damage (category 0) due to cat-facing by *Lygus rugulipennis*.

Conclusions

This study is the first time that a push-pull management programme giving significant control of capsids has been demonstrated and is a significant achievement. Although the separate components had some effects, the components of the system can be combined in the field to produce the most effective treatment. Although there are many proposed push-pull systems in agriculture these often could not be replicated, and the interactions within the system were not analysed (Eigenbrode *et al*., 2016), both of which are demonstrated in this work. This strategy will be a useful tool in an IPM system.

Future Work

- Confirm capsid Push-Pull system
- Test hexyl butyrate with another repellent compound (Russell IPM)

- Improve female capsid trapping with additional floral compounds

Objective 4 Improve insecticide and biological control of the potato aphid, *Macrosiphum euphorbiae*, so as to be more compatible with IPM programmes

Task 4.2. Determine the effect of low and fluctuating temperatures on the ability of aphid parasitoids to parasitise the potato aphid, *Macrosiphum euphorbiae*.

Introduction

Several species of aphid are regularly found infesting strawberry crops. Five of the most frequently found and most damaging are the strawberry aphid (*Chaetosiphon fragaefolii*), the melon and cotton aphid (*Aphis gossypii*), the shallot aphid (*Myzus ascalonicus*), the glasshouse-potato aphid (*Aulacorthum solani*) and the potato aphid (*Macrosiphum euphorbiae*). Damage is caused by direct feeding causing distortion and contamination of fruits and foliage with honeydew and sooty moulds (e.g. *Aphis gossypii* and *Macrosiphum euphorbiae*) and vectoring of viruses, such as mottle virus (e.g. *C. fragaefolii* and *A. gossypii*). Insecticide resistance further complicates management of these pests. Populations of the melon and cotton aphid are for example known to be resistant to pyrethroid and carbamate insecticides (Furk & Hines, 1993; Marshall et al., 2012).

Biological control of Macrosiphum euphorbiae

In recent years the control of early season aphids such as the potato aphid (*Macrosiphum euphorbiae*) has become more problematic due to the withdrawal of commonly used insecticides such as chlorpyrifos and pirimicarb. *Macrosiphum euphorbiae* causes damage to the crop through the production of honeydew and cast skins which result in sooty moulds and make the fruit unmarketable (Trumble et al., 1983). Feeding action of these aphids can also result in distortion of the leaves and fruit (Irving et al., 2012). The species may breed all year round on strawberry crops if conditions allow (Alford, 1984) and populations can build up rapidly in the spring. Currently available chemical control options may give variable levels of control of this pest and may not be compatible with biological control programmes (AHDB Horticulture project SF 140 and 156). For example, lambda-cyhalothrin is effective at controlling populations of *M. euphorbiae*, however this is not an IPM compatible product and early season applications may disrupt natural parasitoids populations moving into the crop.

Any introductions of aphid parasitoids should ideally be done in late winter or early spring before aphid populations become established (Dassonville et al., 2013), however, there are concerns over the effectiveness of biological controls at these low temperatures. There are currently no economic thresholds for *M. euphorbiae* in assurance schemes e.g. Red Tractor Assurance http://assurance.redtractor.org.uk/contentfiles/Farmers-6576.pdf

Figure 4.2. Potato aphid, *Macrosiphum euphorbiae*, on strawberry leaf petiole

Two aphid parasitoid species commonly found in strawberry crops are known to readily parasitise and may contribute to control of *M. euphorbiae*: *Aphidius ervi* (Sidney et al., 2010a) and *Praon volucre* (Di Conti et al., 2008). Both species occur naturally in the environment but can be introduced as biological control products as either a single species in the case of *A. ervi* or as part of a mix of six parasitoid species (*Aphidius colemani, A. ervi, A. matricariae, Praon volucre, Ephedrus cerasicola* and *Aphelinus abdominalis*). The outcome of larval competition inside aphid hosts parasitised by both species suggests that the activity of *A. ervi* may be reduced in the presence of *P. volucre* (Sidney et al., 2010b) however the mix of parasitoids species has the advantage of controlling multiple aphid species found in strawberry crops (Dassonville et al., 2013).

Effects of low and fluctuating temperatures on parasitoid development

Temperature is a key factor in determining the developmental time of insect species. Current knowledge suggests that the lower developmental threshold of *P. volucre* in *Sitobion avenae* from the egg to mummy stage is 3.8°C and for mummy to adult development is 5.5°C with a duration in degree days of 126°D and 150°D respectively (Sigsgaard, 2000). A similar study

of the parasitoid in *M. euphorbiae* found a similar developmental threshold and duration of 5.17°C and 243°D respectively (De Conti et al., 2011). In comparison, the lower developmental thresholds for egg to mummy development and mummy to adult development of *A. ervi* in *Sitobion avenae* are 2.2°C and 6.6°C respectively, with a duration of 159°D and 79°D respectively (Sigsgaard, 2000). Under experimental conditions, development of *A. ervi* in the mummy stage has been observed to continue at constant temperatures as low as 4°C in (Ismail et al., 2013). The estimated developmental threshold and duration of *M. euphorbiae* are 1°C and 145°D, which suggests that the aphid host is better adapted to low temperatures than the parasitoid species and that biological control, particularly by *P. volucre*, may not be as effective in colder conditions (De Conti et al., 2011). Although parasitoid development at low temperatures is extremely slow, *A. ervi* has been found to have a negative effect on pea aphid reproductive capacity following oviposition (Digilio et al., 2000). This suggests that even if the parasitoid larvae do not kill the adult aphids as quickly early in the season, they may still be effective at reducing aphid populations.

Typically, estimates of developmental time based on data collected at constant temperatures are longer in duration than those based on data collected under fluctuating temperatures within a non-injurious range (Hagstrum & Milliken, 1991; Colinet et al., 2015). Fluctuating temperature conditions have also been found to reduce the fitness costs associated with low temperatures of both *A. ervi* (Ismail et al., 2013, Colinet & Hance, 2010) and *P. volucre* (Colinet & Hance, 2010) compared to constant temperature conditions. Air temperatures recorded in polytunnels and glasshouses often show large fluctuations between daytime and night-time conditions meaning that for at least part of the day temperatures will exceed these developmental thresholds even early in the season. Preliminary work by Viridaxis has shown that parasitoid emergence from aphid mummies occurs during warmer days in polytunnels even when night time temperatures are at or close to 0°C (Dassonville et al., 2013). A study looking at the thermal range of *A. ervi* on *M. euphorbiae* noted the suitability of the parasitoid for early season aphid control in bell peppers at temperatures as low as 8°C (Flores-Mejia et al., 2016).

Effects of low and fluctuating temperatures on parasitoid activity

Temperature can also affect parasitoid-host dynamics through modifications in insect behaviour and activity, such as the ability of the parasitoid to successfully locate and parasitise the aphid. A study by Langer et al., (2004) tested the activity of *A. ervi* and *P. volucre* at low temperatures with the aphid host *S. avenae*. This study showed that oviposition

remained low below 10°C in both species. Flight and walking activity both increased with temperature, with *A. ervi* being consistently more active than *P. volcure*. The lower flight threshold was 10°C for both species and walking activity continued down to 8°C. This suggests that these parasitoid species would still be capable of locating aphids at low temperatures early in the season. In a separate study, defensive behaviours of *M. euphorbiae* in response to *A. ervi* were reduced at 12°C compared to 28°C which may lead to more frequent successful ovipoisition at low temperatures (Moiroux et al., 2016).

Aphidius ervi *and* Praon volucre *overwintering strategies*

Aphidius ervi overwinters in the larval stage and diapause appears to be primarily influenced by photoperiod and temperature with a minor effect of aphid morph (sexual or asexual) (Christiansen-Weniger & Hardie, 1997; Christiansen-Weniger & Hardie, 1999). *Praon volucre* diapause initiation however appears to be strongly influenced by aphid morph independently of environmental cues (Polgár et al., 1991). The appearance of sexual (oviparae) aphid individuals in autumn therefore acts as a cue for diapause induction and may have an impact on parasitoid populations in subsequent years. Both species are also capable of remaining active over winter in temperate climates if temperatures are suitable and anoholocyclic aphid hosts are available (Polgár et al. 1995; Langer & Hance, 2000). *Macrosiphum euphorbiae* is primarily holocyclic (Langer & Hance, 2000), however both parasitoid species will parasitise anholocyclic hosts such as the grain aphid, *S. avenae* (Langer et al., 2004). The mechanism of diapause termination in these species is unclear however it is likely to occur as a result of warmer temperatures and hormonal cues occurring in the spring. In aphid-parasitoid systems, the choice of aphid host can influence the fitness of the emerging parasitoid wasp. The thermal tolerance of *A. ervi* and *P. volucre* overwintering in *M. euphorbiae* has not yet been tested, however no effect of aphid host on thermal tolerance was recorded in other *Aphidius* species overwintering in grain aphids (Alford et al., 2017).

Aims & Objectives

The aim of this work was to determine the effect of low and fluctuating temperatures on the ability of *A. ervi* and *P. volucre* to parasitise the potato aphid, *M. euphorbiae*. Lower temperature thresholds for parasitism by these species have been observed in other aphid hosts, but the thresholds for *M. euphorbiae* have not yet been studied. The impact of fluctuating temperatures on the ability to parasitise has not yet been investigated in aphid-

parasitoid systems. In particular, the ability of *A. ervi* and *P. volucre* to respond to warmer 'daytime' temperatures following a period of low temperature is currently unknown. The objectives of this work are therefore as follows:

- To determine the lower temperature threshold for parasitism of *M. euphorbiae* by *A. ervi* and *P. volucre* under constant temperature conditions
- To determine the lower temperature threshold for parasitism of *M. euphorbiae* by *A. ervi* and *P. volucre* under fluctuating temperature conditions
- To determine the time taken for *A. ervi* and *P. volucre* to respond to higher temperatures under fluctuating conditions and successfully parasitise *M. euphorbiae*.

Materials and methods

All experiments were performed at Harper Adams University in Panasonic controlled environment cabinets (model no. MLR-352-PE) at 4,000 lux, 12:12 L:D, 90% RH, at the described temperatures.

Macrosiphum euphorbiae, Aphidius ervi and *Praon volucre* adults were obtained from laboratory cultures at Harper Adams University maintained at 20°C, 16:8 L:D, 60% RH. Prior to each experiment, adults of *M. euphoria* were separated into individual mesh-lidded rearing pots (10 cm diameter and 10 cm high) on fresh strawberry leaves and maintained under controlled conditions (20°C, 16:8 L:D, 60% RH) for 3-4 days for nymph production to occur. Aphid nymphs (2^{nd}-4^{th} instar) were used in all experiments. Both parasitoid cultures were reared on strawberry plants infested with *M. euphorbiae*.

Air temperature data were recorded inside a polytunnel located in Pulbourough, West Sussex, in 2014. Another set of air temperature data were recorded inside an unheated glasshouse located in Walburton, West Sussex in 2015. Data for the months February to April were summarised to represent typical early season conditions within these systems. Additional data of external air temperatures were obtained from a nearby meteorological station (MIDAS, 2017) in 2014 and from the same site as the glasshouse in 2015. Additional air temperature data from six polytunnels located in Kent for April 2017 were obtained and summarised to compared with the existing polytunnel data and assess the level of variation across one site.

1.1 Experiment 1 – Determine minimum temperature threshold for successful parasitism under constant conditions

An unfurled strawberry leaf was placed in a glass Petri dish with the stem immersed in 2.5ml of water. The leaf was infested with ten *M. euphorbiae* nymphs and conditioned at the treatment temperature for 24 hours prior to the start of the experiment. Mated female parasitoids were separated out into a different glass Petri dish with access to a 20% sugar solution and conditioned similarly. Two female parasitoids were then introduced to each dish of aphids and left for 24 hours at the treatment temperature. The parasitoids were then removed and the aphids were maintained on the strawberry leaf at 20°C for a further seven days before they were dissected to determine if parasitism had occurred. Dissection of the aphids was only possible when the aphid was still alive after seven days. If an aphid had been parasitised but subsequently died before the dissections were completed the aphid would have been scored as being dead and not parasitised. As such parasitism may in some cases have not been recorded when in fact it had occurred. Four treatment temperatures were tested: 8, 10, 12 and 20°C as well as two control treatments without parasitoids which were maintained at 8°C and 20°C. To confirm parasitoid larval development at low temperatures, three additional replicates of parasitised aphid treatments and 20 mummies of each species were maintained at the lowest constant temperature at which parasitism was previously observed: 8°C for *A. ervi* and 12°C for *P. volucre*. Aphids were maintained for two weeks prior to dissection to confirm larval development and aphid mummies were monitored for parasitoid emergence.

1.2 Experiment 2 – Determine minimum temperature threshold for successful parasitism under fluctuating conditions

Experiment 2 was set up as described in section 1.1, however the insects were conditioned at 2°C. Following the introduction of the parasitoids, insects were maintained at 2°C for 16 hours before being moved to a higher treatment temperature for 8 hours (typical day-length in February). The parasitoids were then removed and the aphids were maintained on the strawberry leaf at 20°C for a further seven days before they were dissected to determine if parasitism had occurred. Three treatment temperatures were tested: 8, 13 and 18°C.

1.3 Experiment 3 – Determine time taken for parasitoids to respond to higher temperatures under fluctuating conditions

Experiment 3 was set up described in section 1.2, however the insects remained at the treatment temperature for shorter periods of time, 2 hours and 4 hours, before the parasitoids were removed. The aphids were then maintained on the strawberry leaf at 20°C for a further seven days before they were dissected to determine if parasitism had occurred.

1.4 Experiment 4 – Determine the effect of aphid numbers on mortality

Experiment 4 was set up as described in section 1.1, however the number of insects was varied between treatments. One parasitoid was introduced to dishes containing either 5 or 30 aphids and held at the treatment temperature for 24 hours. One female aphid parasitoid was used to test whether the effect of superparasitism was influencing the results recorded. Superparasitism occurs where a parasitoid may parasitise an aphid host that has already been parasitised by a second parasitoid (e.g. van Alphen & Visser, 1990). Repeated parasitism of the aphid host may reduce the survival of the aphid and lead to high levels of mortality but lower levels of recorded parasitism. Two treatment temperatures were used: 20°C and either 8°C for *A. ervi* or 12°C for *P. volucre*, these being identified as the lowest constant temperatures at which parasitism was found to occur for each species in Experiment 1. An additional experiment with one parasitoid and 30 aphids was performed under fluctuating conditions where the insects were initially held at 2°C for 16 hours and then kept at 8°C for *A. ervi* or 13°C for *P. volucre*. These were identified as the lowest fluctuating temperatures at which parasitism was found to occur for each species in Experiment 2.

Successful parasitism was determined by presence or absence of at least one parasitoid larva within at least one aphid in a Petri dish. The effect of parasitoid species and temperature on the number of dishes where parasitism occurred was analysed using a generalized linear model with binomial errors. The effect of treatments on aphid mortality counts was assessed using generalized linear models with poisson errors with the exception of experiment 4 where count data were converted to proportions and binomial errors were used instead. Likelihood ratios were used to determine the significance of model parameters and multiple comparisons of means were done using the glht function in the ‘multcomp’ package.

Results

Meteorological data

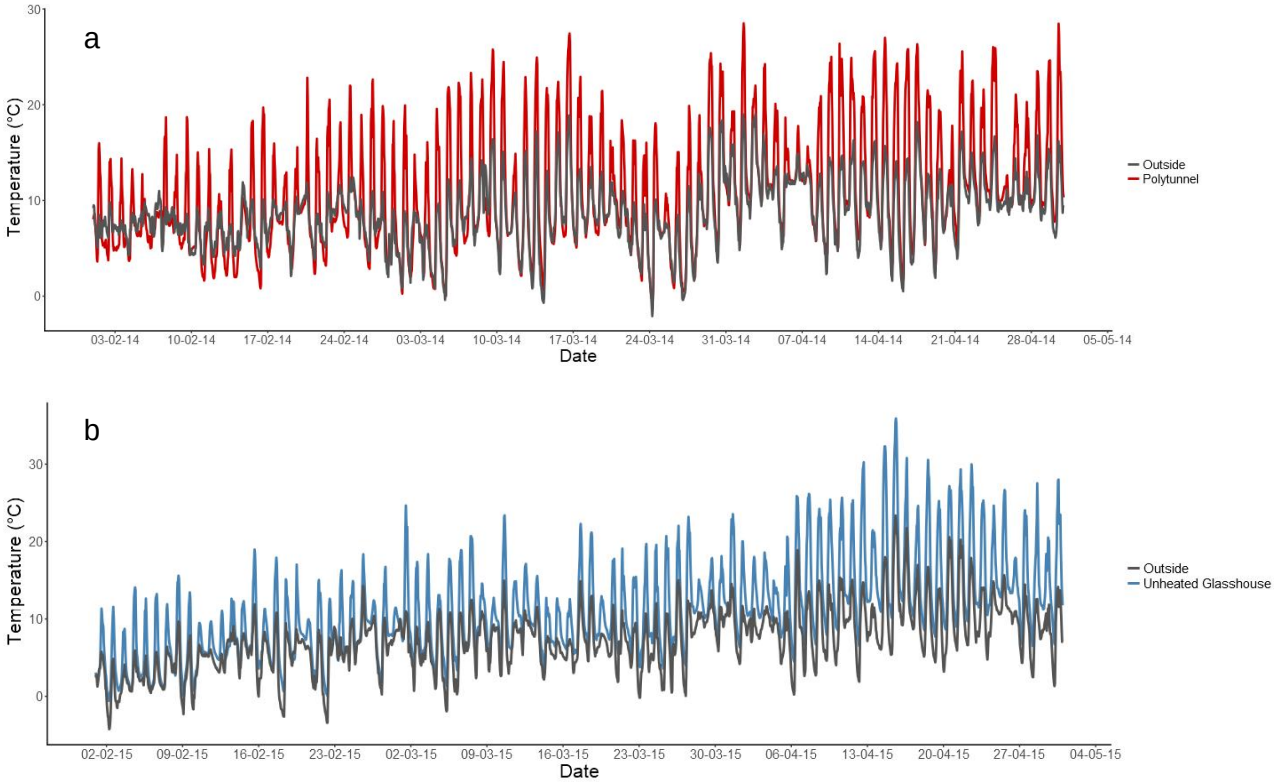


Figure 4.3. Air temperatures inside and outside of **a.** a polytunnel (Pulborough, West Sussex, 2014) and **b.** an unheated glasshouse (Walberton, West Sussex, 2015) recorded between February and April.

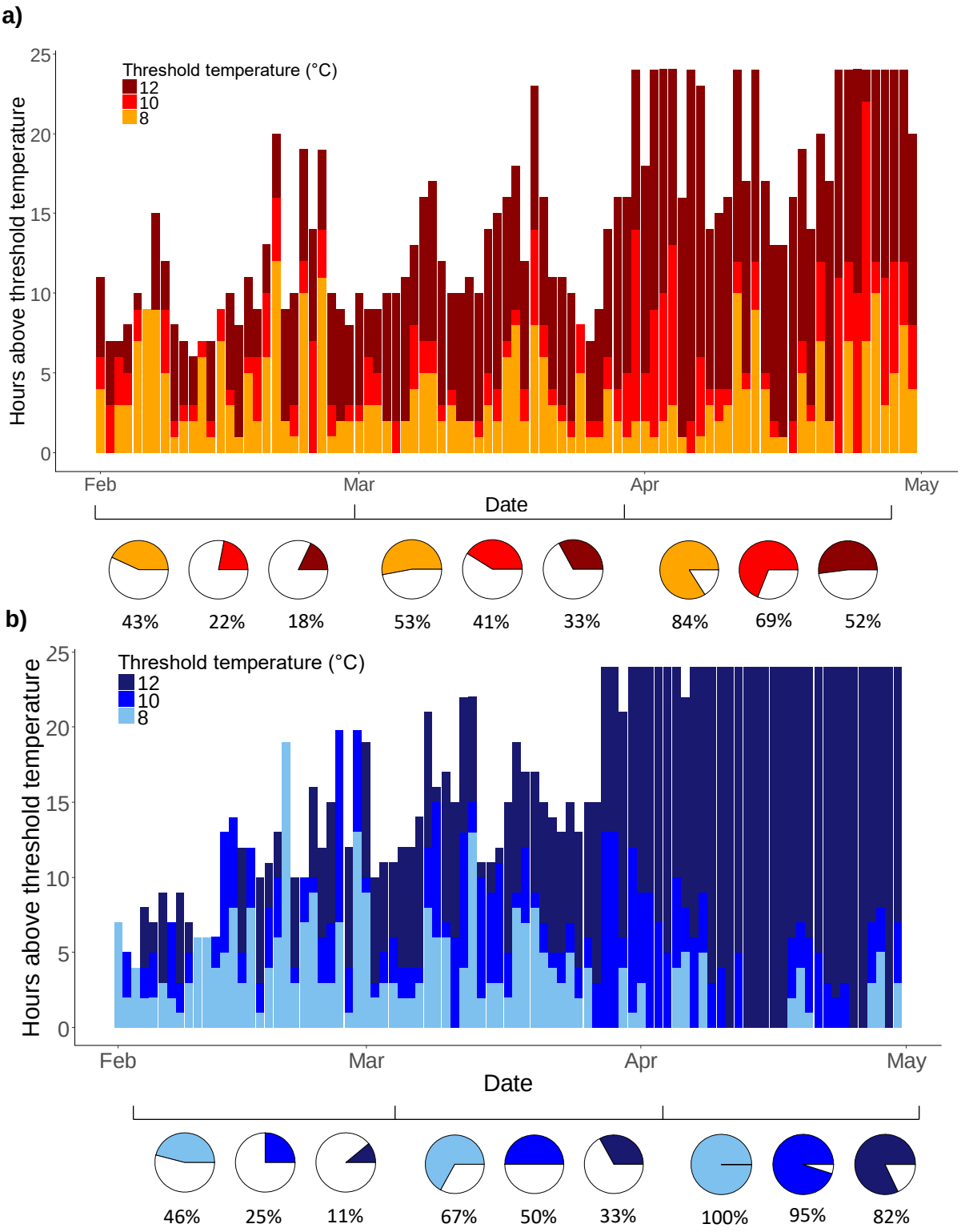


Figure 4.4. Number of hours per day air temperature exceeded threshold temperatures of 8, 10 and 12°C and the percentage hours per month air temperatures exceeded the same

thresholds for the months of February, March and April in **a.** a polytunnel (2014) and **b.** an unheated glasshouse (2015), both located in West Sussex.

Table 4.1. Summary data for April air temperatures recorded in polytunnels in Kent (2017) compared to the polytunnel (2014) and unheated glasshouse (2015) located in West Sussex.

	Mean Temp Apr (± SEM) (°C)	Min Temp Apr (°C)	Max Temp Apr (°C)
Polytunnels Kent (all) 2017	10.63 (± 0.07)	-1.5	26.5
Polytunnel 2014	13.74 (± 0.28)	1.6	28.5
Glasshouse 2015	19.4 (± 0.35)	8.35	42.4

1.1 Experiment 1 – Determine minimum temperature threshold for successful parasitism under constant conditions

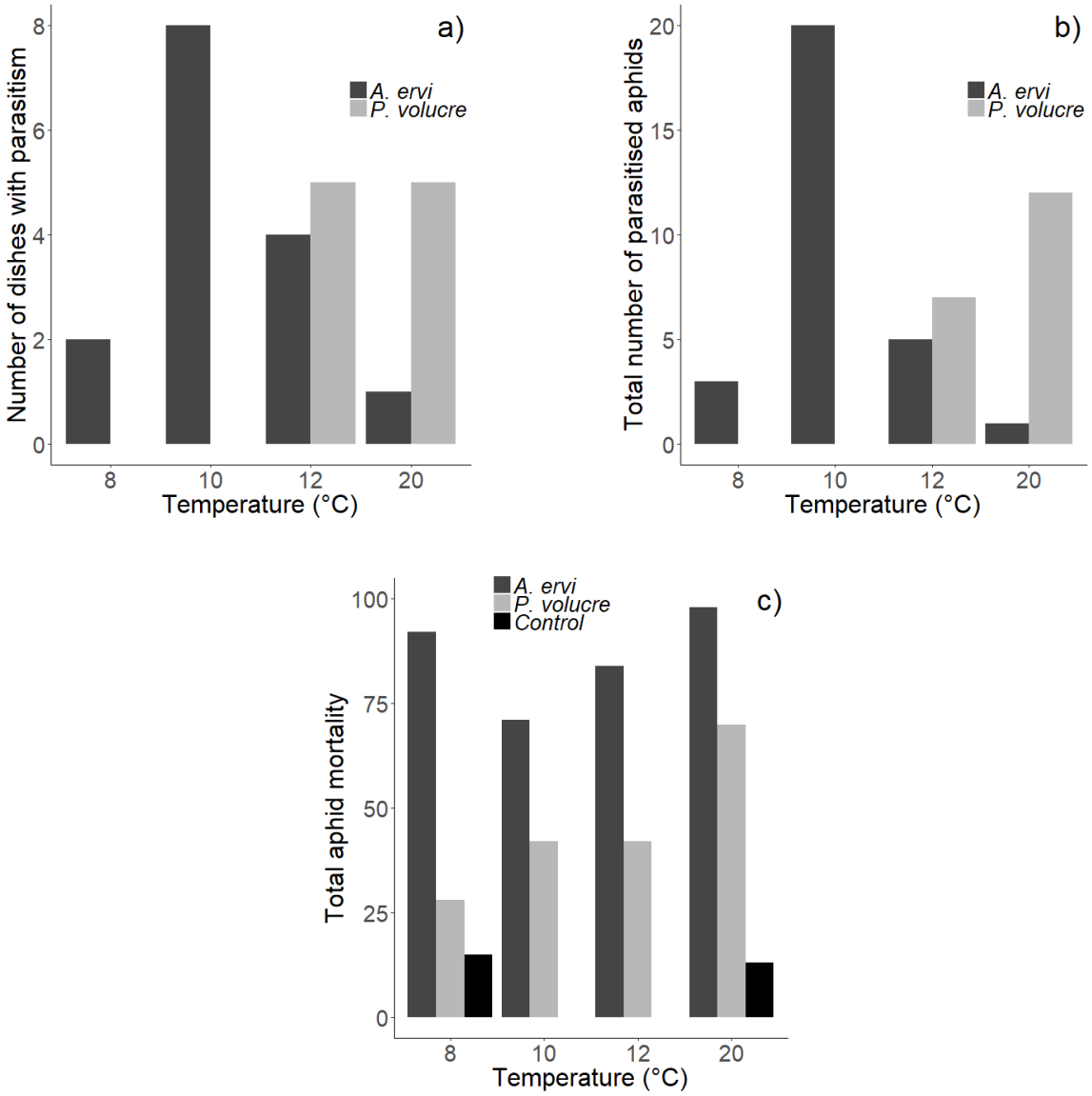


Figure 4.5. a. Number of Petri dishes (*n* = 10) with at least one parasitised aphid at each constant treatment temperature for each parasitoid species. **b.** Total number of parasitised aphids at each constant temperature for each parasitoid species (*n = 100*). **c.** Total aphid mortality in all Petri dishes at each constant treatment temperature and each parasitoid species. NB: Error bars are not suitable for this data as values are count data (as advised by statistician)

For the temperatures studied, the lowest temperature at which parasitism by *A. ervi* occurred under the constant conditions tested was 8°C and for *P. volucre* it was 12°C. There were a greater number of dishes with parasitism occurring in *A. ervi* compared to *P. volucre* as a result of the lower temperature threshold (X^2 = 11.651, df = 3,1, P < 0.001). Parasitism did not increase with temperature but a difference was observed between species at different temperatures (X^2 = 10.187, df = 3,1, P < 0.001) (Fig. 4.5a).

Aphidius ervi treatments had significantly higher aphid mortality than *P. volucre* treatments overall (X^2 = 24.702, df = 76,1, P < 0.001) and both treatments had significantly higher aphid mortality than the controls at 20°C (P < 0.001). At 8°C, *A. ervi* had higher aphid mortality than the control (P < 0.001) however *P. volucre* did not. Aphid mortality did not increase with increasing temperature with *A. ervi* but did with *P. volucre* (X^2 = 7.442, df = 76,1, P < 0.001) (Fig. 4.5c). In preliminary work, aphid mortality in the absence of parasitoids but otherwise following the described experimental design was low at just 15% at 8°C and 13% at 20°C after 8 days.

Larval development was confirmed for both species of parasitoid in aphids maintained at constant low temperatures for two weeks (Figure 4.6). Of the 20 aphid mummies of each species which were maintained at constant low temperatures, 15 *A. ervi* emerged and 14 *P. volucre* had emerged after 2 weeks.

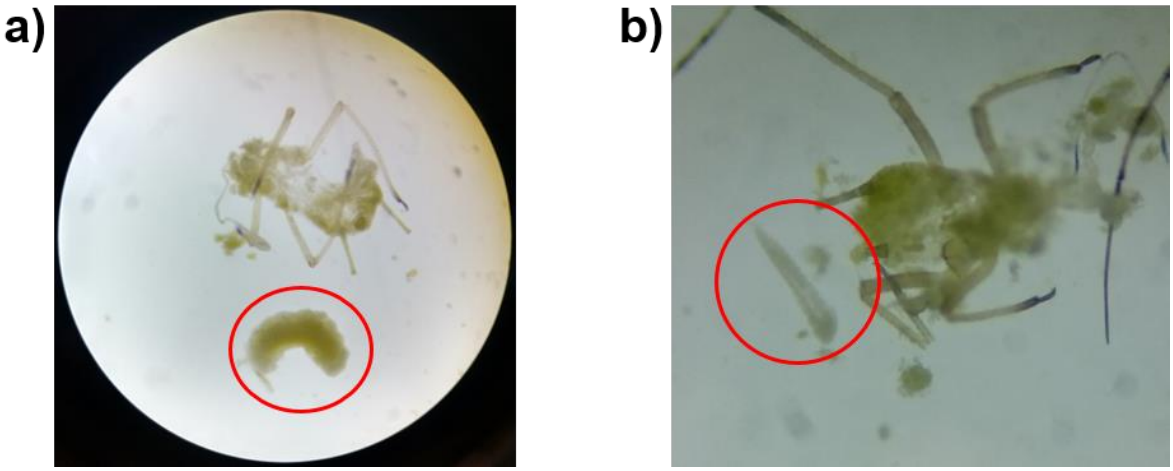


Figure 4.6. Microscope images of *Aphidius ervi* larva dissected from *Macrosiphum euphorbiae* after a) 7 days at 20°C and b) 14 days at 8°C

1.2 Experiment 2 – Determine minimum temperature threshold for successful parasitism under fluctuating conditions

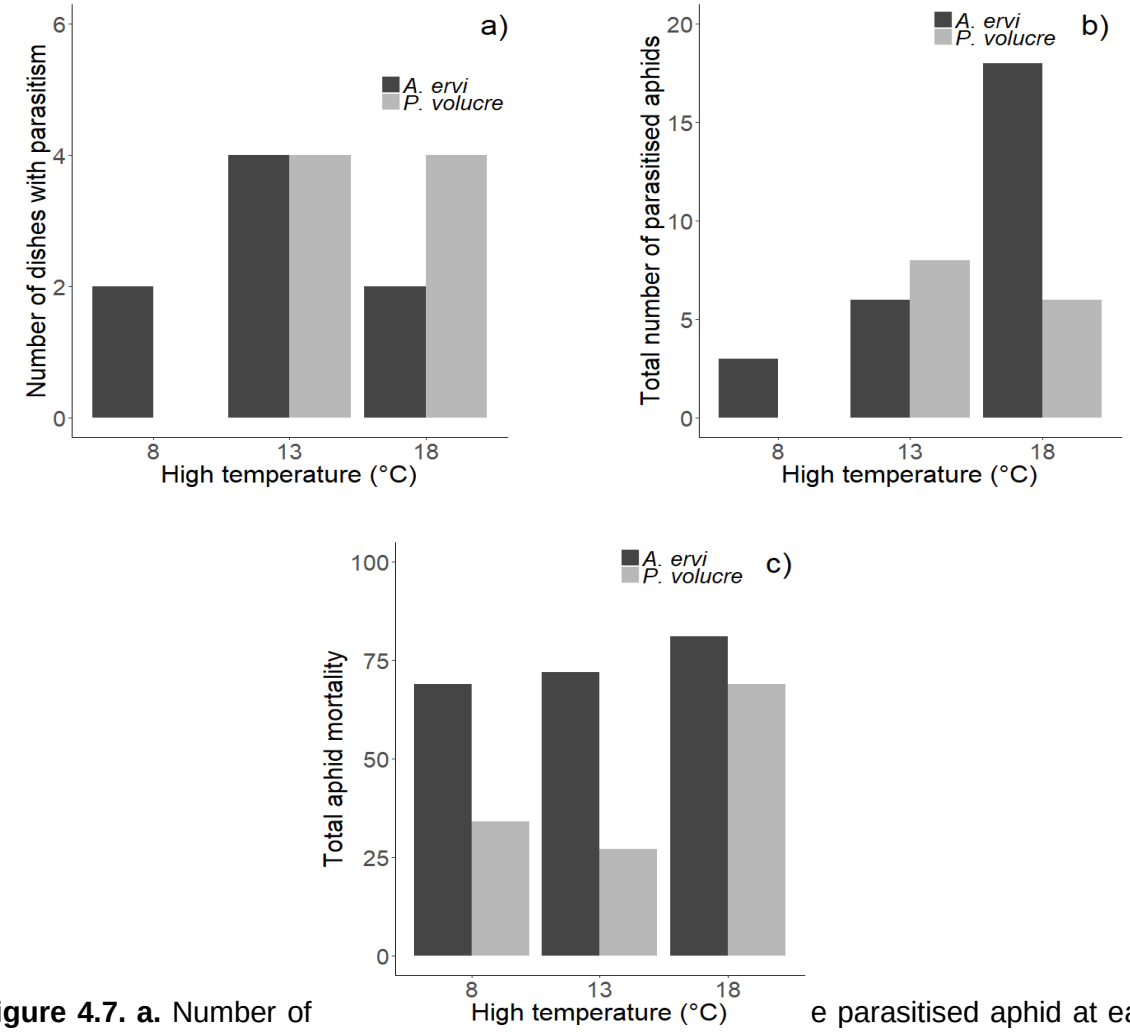


Figure 4.7. a. Number of e parasitised aphid at each fluctuating treatment temperature for each parasitoid species. Temperature shown represents the higher temperature fluctuation from a low temperature of 2°C, 16:8 L:D. **b.** Total number of parasitised aphids (*n = 100*) at each fluctuating temperature for each parasitoid species. **c.** Total aphid mortality at each fluctuating treatment temperature for each parasitoid species. NB: Error bars are not suitable for this data as values are count data (as advised by statistician)

The minimum temperature studied at which parasitism by *A. ervi* occurred under fluctuating conditions was 8 °C. The minimum temperature studied at which parasitism by *P. volucre* occurred under fluctuating conditions was 13 °C (Fig. 4.7a). There was no effect of temperature or species on the incidence of parasitism.

Aphidius ervi treatments had significantly higher aphid mortality than *P. volucre* treatments (X^2= 13.681, df = 56,1, P < 0.001). Aphid mortality did not increase with increasing temperature with *A. ervi* but did with *P. volucre* (X^2 = 5.86, df = 56,1, P = 0.01) (Fig. 4.7c).

1.3 Experiment 3 – Determine time taken for parasitoids to respond to higher temperatures under fluctuating conditions

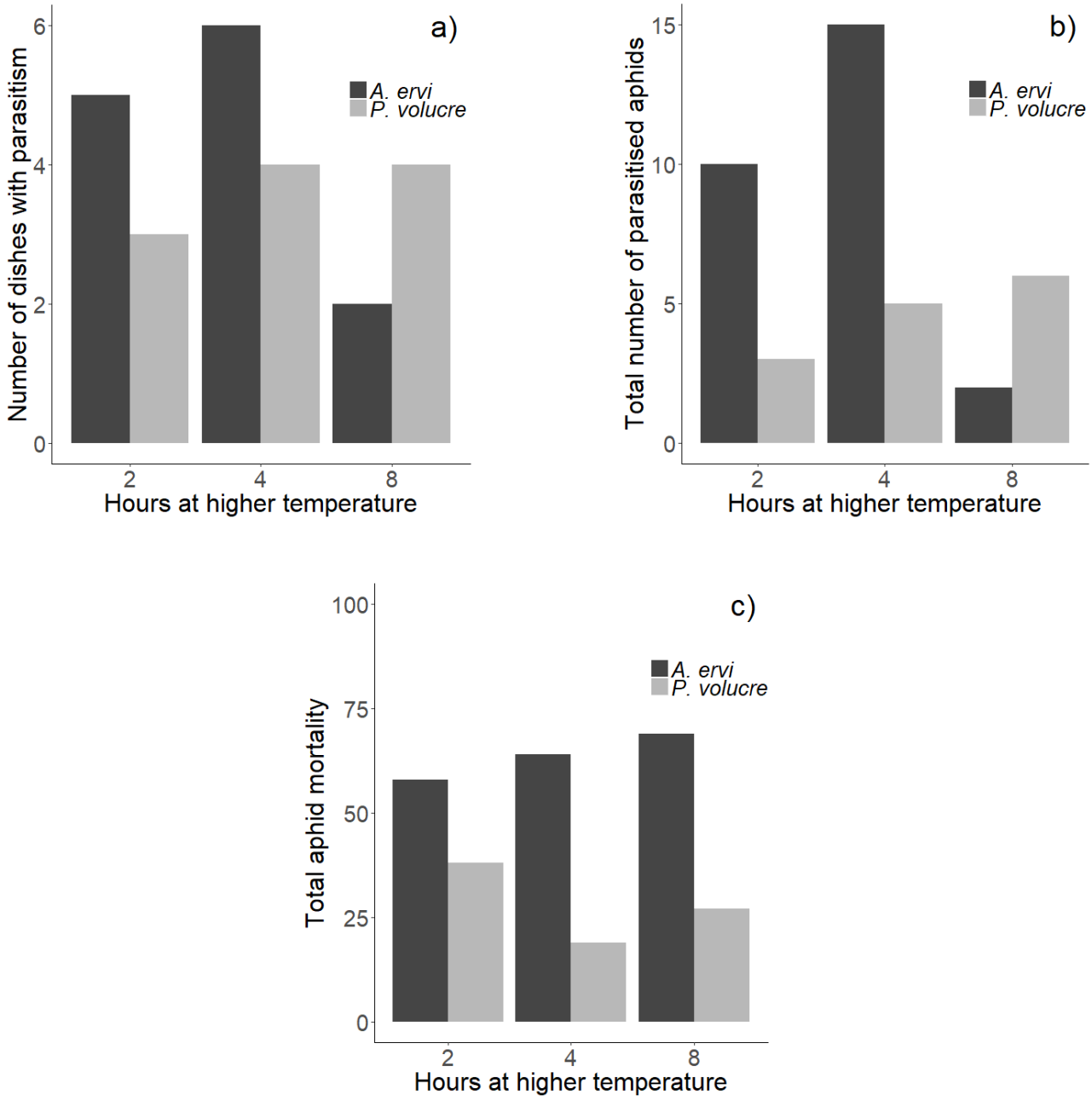


Figure 4.8. a. Number of Petri dishes (*n* = 10) with at least one parasitised aphid for each exposure time at the higher fluctuating temperature for each parasitoid species. **b.** Total number of parasitized aphids (*n = 100*) for each exposure time at the higher fluctuating temperature for each parasitoid species. **c.** Total aphid mortality for each exposure time at the higher fluctuating temperature for each parasitoid species. NB: Error bars are not suitable for this data as values are count data (as advised by statistician)

Both parasitoid species responded to higher temperature fluctuations and parasitised aphids in under two hours. Shorter exposure times increased the incidence of parasitism overall (X^2 = 15.528, df = 57,3, P = 0.001) which appeared to be largely as a result of low incidence of parasitism in the *A. ervi* treatment after 8 hours. There was no interaction between parasitoid species and time of exposure on parasitism (Fig. 4.8a).

Aphid mortality was higher in *A. ervi* treatments than *P. volucre* treatments overall (X^2 = 32.047, df = 56,1, P < 0.001). Aphid mortality did not increase with increasing time of exposure with *A. ervi* or *P. volucre* (Fig. 4.8c).

1.4 Experiment 4 – Determine the effect of aphid numbers on mortality

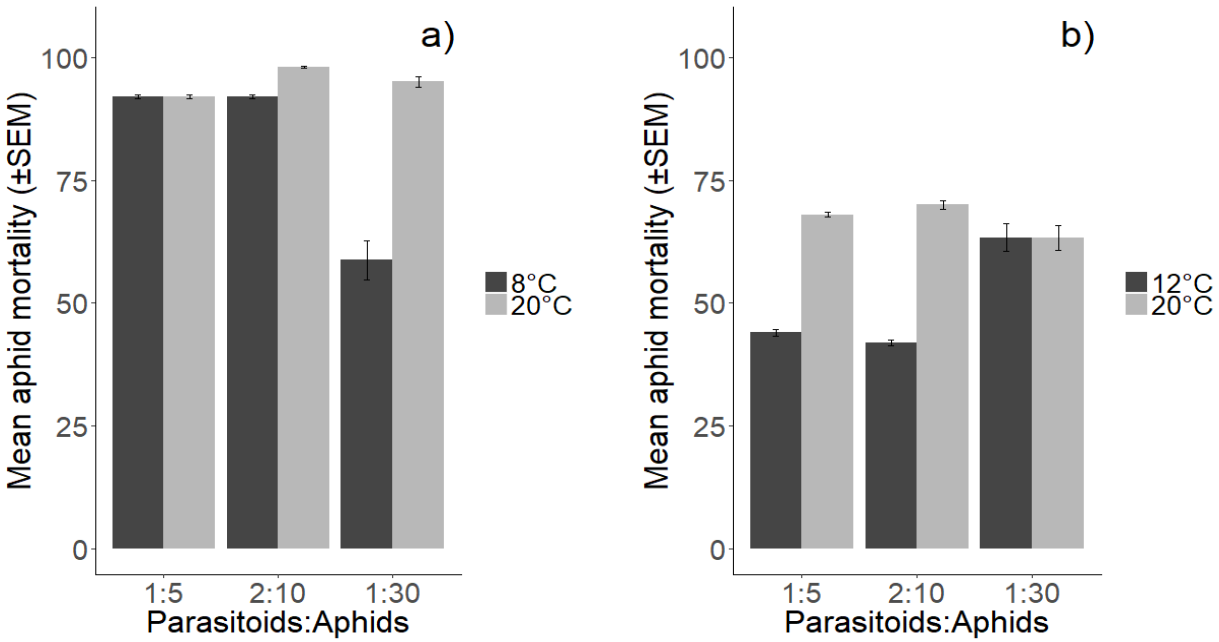


Figure 4.9. a. Mean aphid mortality (± SEM) for each treatment ratio of *Aphidius ervi* to *Macrosiphum euphorbiae* at 8 °C and 20 °C. **b.** Mean aphid mortality (± SEM) for each treatment ratio of *Praon volucre* to *Macrosiphum euphorbiae* at 12 °C and 20 °C.

Aphid mortality was lower in the *P. volucre* treatments (Fig. 4.9b) compared to the *A. ervi* treatments (Fig 4.9a) overall (X^2= 5.698 df = 99,3, P = 0.001). Aphid mortality did not change with temperature or with different numbers of parasitoids to aphids in either species (Fig. 4.9). Importantly, there was no evidence that aphid mortality was higher when two parasitoids were present than when one parasitoids was present. When two parasitoids are present it is possible superparasitism may occur and that this may in turn lead to increased aphid mortality as a result of repeated stings as each parasitoid lays an egg inside the aphid host. When multiple eggs are laid inside an aphid host the outcome is either that only one parasitoid survives to complete its development inside the aphid or that the aphid dies and no parasitism is apparent.

Discussion

- Daytime and nightime temperatures in polytunnels and unheated glasshouses varied considerably in the months of February, March and April.
- In the studied polytunnel, air temperatures rose above 12°C for at least 18% of the time in the month of February 2014, increasing to 33% in March and 52% in April.
- In the studied unheated glasshouse, air temperatures rose above 12°C for at least 11% of the time in the month of February 2015, increasing to 33% in March and 82% in April.
- In both systems, daytime temperatures consistently exceeded air temperatures recorded outside. This difference was amplified at higher temperatures, particularly in the unheated glasshouse.
- Polytunnel temperature data collected from a site near West Malling in Kent in 2017 for the month of April showed little variation between tunnels.
- The lowest temperature tested at which *M. euphorbiae* parasitism occurred with *A. ervi* under the constant temperature conditions tested was 8°C. The lowest temperature tested at which parasitism occurred with *P. volucre* under the constant temperature conditions tested was 12°C. This broadly agrees with earlier studies of the thermal range of *A. ervi* (Flores-Mejia et al., 2016) and the activity of both species at low temperatures (Langer et al., 2004).
- Parasitoid larval development was confirmed in both species at low temperatures under constant conditions (8°C for *A. ervi* and 12°C for *P. volucre*) which is consistent with previous estimates of lower thermal development for these stages of 2.2°C and 3.8°C respectively in *S. avenae* aphid hosts.

- Parasitism thresholds under fluctuating conditions were consistent with those under constant conditions, with *A. ervi* and *P. volucre* parasitising *M. euphorbiae* at higher temperature fluctuations of 8°C and 13°C respectively.
- Parasitism by both species occurred under fluctuating conditions within two hours of the parasitoids being moved to the higher temperature.
- Total aphid mortality increased in Petri dishes containing *A. ervi* as exposure to the higher temperature increased, while decreasing in Petri dishes containing *P. volucre*.
- Incidence of parasitism did not increase with increasing temperatures under constant or fluctuating conditions owing to high levels of aphid mortality. Control treatments without parasitoids kept under constant temperature conditions at 8°C and 20°C showed significantly lower mortality demonstrating that the aphids died as a result of the parasitoid presence rather than the experimental conditions.
- Total aphid mortality generally increased with temperature under both constant and fluctuating conditions. It is likely that as temperature increased, parasitoid activity increased leading to greater aphid disturbance or more frequent attacks. This may have resulted in aphids moving away from the food source and starving; repeated stings (oviposition attempts) triggering aphid defence mechanisms, which incur a fitness cost; or from parasitoids feeding on the aphid host. Both outcomes may have contributed to increased aphid mortality due to parasitoid presence. High aphid mortality was found in a project investigating aphid parasitoids in protected herbs which used a similar experimental set-up. Here, the presence of *Aphidius colemani* resulted in an average aphid mortality of 62.7% when introduced into to a Petri dish containing hawthorn-parsley aphid (HDC PE 006).
- In the *A. ervi* treatments, aphid mortality was high even at the lowest temperature of 8°C, and was higher, although not statistically significantly so, than at 10°C under constant conditions. It is possible that although parasitoid activity is reduced at lower temperatures, the costs incurred to the aphid as a result of defence mechanisms are higher due to the adverse conditions. This has been observed in a previous study of aphid defensive strategies involving *Aphidius matricariae* and *Myzus persicae* (Bannerman et al., 2011).
- Aphid parasitoids use host-marking behaviour to discriminate between parasitised and unparasitised hosts. Different individuals of the same species however, may not respond to the mark left on a parasitized aphid. To determine whether the two different parasitoids were continually attacking the same aphid, the number of parasitoids was reduced to one per dish, with five aphids to maintain the original ratio. Additional

replicates were also set up with 30 aphids to one parasitoid to determine whether increased aphid numbers would reduce the frequency of attacks and therefore overall mortality. In this experiment, neither increased aphid numbers nor reduced parasitoid numbers affected aphid mortality.

- From the data presented here showing early season polytunnel and glasshouse temperatures, and the activity of *A. ervi* and *P. volucre* at temperatures typically found in polytunnels at this time of the year, both species have the potential to be used as part of a biological control programme for early season *M. euphorbiae.* Both species have the ability to parasitise at low temperatures, however *A. ervi* has a lower temperature threshold and appears to be more active than *P. volucre* at low temperatures based on the levels of aphid mortality observed in these experiments.
- At low temperatures, development of the parasitoid larvae to the mummy stage will be extremely slow, meaning mummies will not be visible soon after application of biological controls in the early season. An absence of mummies does not mean that the biological control has been unsuccessful. Parasitised aphids have greatly reduced reproduction and mummification will progress over a longer time period.
- Although outside temperatures are generally lower than in polytunnel and glasshouse systems, the data presented here show that overwintering parasitoid populations are likely to become active before *M. euphorbiae* populations build up in the spring.

Conclusions

- *Aphidius ervi* is capable of parasitising *Macrosiphum euphorbiae* at temperatures as low as 8°C and *Praon volucre* at temperatures as low as 12°C.
- Fluctuating temperatures had no effect on the ability of the parasitoids to parasitise *M. euphorbiae* and both species were able to respond to short periods, as little as two hours, of higher temperatures.
- Daytime air temperatures in glasshouses and polytunnels frequently exceed temperature thresholds for parasitoid activity early in the season (February to April).
- *Aphidius ervi* was responsible for higher aphid mortality than *P. volucre*, possibly due to differences in aggression or activity levels. Regular disturbance or attack of aphids is likely to result in mortality. Both species were active at these low temperatures
- Aphid defence mechanisms may also be reduced or costlier at lower temperatures, making them more vulnerable to disturbance or attack.

- Increased numbers of aphids or reduced numbers of parasitoids had no effect on aphid mortality in these experiments.
- Both species have the potential to be used as early season biological control in polytunnels or glasshouses. *Aphidius ervi* is particularly suitable due to the lower temperature threshold for parasitism and high levels of activity at lower temperatures.
- The slow development of parasitoid larvae at low temperatures means that evidence of parasitism in the form of mummified aphids may not be apparent.
- Early season applications of insecticides may reduce the efficacy of natural and introduced biological control agents.

Future work

The high levels of aphid mortality in the laboratory system indicates that both parasitoid species are highly active at low temperatures. It would be beneficial for the next stage of this work to introduce parasitoids to aphid infested plants at low and fluctuating temperatures in a cage trial or semi-field setting. This would allow aphid and parasitoid behaviour to be observed in a more field-realistic setting to determine their ability to search for and locate aphid hosts, and the ability of the hosts to respond. It would also be beneficial to investigate the effects of low and fluctuating temperatures on *M. euphorbiae* fitness and defence responses, particularly in the presence of aphid parasitoids.

Objective 5 Improve control of aphids through the growing season

Task 5.1. Thresholds for aphids and natural enemies; assessments to demonstrate confidence in control strategies.

Introduction

Strawberry crops are affected by a range of aphid pests. The most difficult to control is the potato aphid, *Macrosiphum euphorbiae*, as infestations often resurge after pesticide application, probably due to incomplete control as shown in project SF 140. In this project it was also found that aphid numbers in the untreated plots had a tendency to decline rapidly by the end of the experiments because of the increases in natural enemies.

Insecticide sprays can be harmful to natural enemies which might otherwise be controlling pests in the crop. Often there is a lag between the build-up of the pest and the immigration and build-up of the predators and parasitoids. This lag period is often a critical time for the build-up of the natural enemies, but a time when sprays for aphids are more likely to be applied.

The aim was to monitor and demonstrate the importance of naturally occurring aphid enemies in strawberry crops under different spray programmes in relation to aphid populations and aphid damage. This study;

- Compared 3 crops on each of 2 sites, both in June bearer and ever-bearer fields for aphid build-up in the crop in relation to natural enemy appearance
- Demonstrated the effects of insecticide spray programmes on *M. euphorbiae* and natural enemies
- Showed the relationship between population ‘peaks and troughs’ of pest and natural enemies

Materials and methods

Studies were done on two farms with historically different degrees of aphid and natural enemy numbers. On each farm 3 June- and 3 ever-bearer fields were selected. To obtain an overall picture of the changes in natural enemy populations throughout the year, fields were within the same or similar landscape as possible on the farms. Hence they had the same potential pool of pests and natural enemies. Crop details including varieties were recorded for June- (Table 5.1.1a) and ever-bearer plantations (Table 5.1.1b).

Table 5.1.1a. Main characteristics of the June bearer fields used for the assessments

Farm	Plantation	No.tunnels	Variety	Date planted	Date protected	Planting	Crop habit
1	1	4	Olivia	2016	22 Feb	Staggered planting in grow bags	Tall, upright stems
1	2	17	Malling Centenary	17 Jan	Planted in covered tunnels	Trays coir bags on drainage sheet on the ground	As above
1	3	17	Flair	17 Jan	Planted in covered tunnels	Trays coir bags on drainage sheet on the ground	As above
2	1	22	Flare	28 Jan	Planted in covered tunnels	4 crowns in a pot (all examined)	Very tall upright stems
2	2	8	Malling Centenary	10 Jun 2016	18 Jan	4 crowns in a pot	Short stems, more leaves
2	3	14	Malling Centenary	10 Jun 2016	22 Jan	Staggered planting in grow bags – one plant examined	As above

Crop husbandry was the standard grower practice. The crop growth stage and understory management were recorded at each visit (APPENDIX. 5.1.1). There were notable differences between fields but as no numerical data was collected this could not be analysed. Most fields were mown approximately every 3rd week; either the alleyways only or the alleyways and under the tables. There were a variety of flowering weeds present at low density within the tunnels through the cropping season including red dead-nettle, chickweed, dandelion, groundsel, speedwell, shepherd's purse and bindweeds and some natural enemy beneficial

plants such as nettles at one site. In general the habitat within the tunnels was considered poor for pollinators and natural enemies as even when there were flowering weeds these were isolated individual plants.

Table 5.1.1b. Main characteristics of the everbearer fields used for the assessments

Farm	Plantation	No.tunnels	Variety	Date planted and protected	Planting	Crop habit
1	4	6	Amesti	24 Mar	Staggered planting in grow bags – one plant examined	Tall, upright stems
1	5	22	Amesti	4 Apr	As above	As above
1	6	22	Amesti	11 Apr	As above	As above
2	4	14	Katrina	27 Mar	As above	As above
2	5	10	Katrina	27 Mar	As above	As above
2	6	10	Zara	27 Mar	As above	As above

Around the perimeter of the fields Farm 2 had a more diverse flora with mixed hedgerows. The most commonly found plants were poplar, common hazel, common elder, blackthorn, blackberry, field maple, sweet chestnut and ivy. Farm 1 had poplar windbreaks. Records of the flowering plants around the tunnels were made at each visit (APPENDIX 5.1.2). Similarly to the habitat within the tunnels, most of flowering weeds (such as dandelion, clover and *Sonchus sp.)* were isolated individuals. Umbelliferous plants were present along the hedgerows in most of the fields throughout the cropping season. At fields 1.2 and 1.3 shepherd's purse was found all along the beginning and ending of the tunnels.

The growers standard spray programme was applied to all crops including biocontrol introductions in some crops (Table 5.1.2).

Table 5.1.2. Spray programme and biocontrol introduction in strawberry fields

Field	Product	Active ingredient	Units	Date	Applied		
					Area (ha)	Rate	Quantity
June bearer fields (farm.field)							
1.1	Hallmark With Zeon Technology	lambda-cyhalotrin	L	07 Mar	2.0	0.075	0.15
1.1	Chess WG (13310)	pymetrozine	Kg	21 Mar	2.0	0.4	0.8
1.1	Calypso	thiacloprid	L	21 Mar	2.0	0.25	0.5
1.1	Aphiscout	*Aphidius colemani, Aphidius ervi, Aphelinus abdominalis, Praon volucre,* and *Ephedrus cerasicola*	Pack	24 Apr	4.3	14.061	60 dispensers
1.1	Aphiscout	As above	Pack	8 May	4.3	14.061	60 dispensers
1.2	Aphox	pirimicarb	kg	06 Apr	1.5	0.56	0.846
1.3	Aphox	pirimicarb	kg	06 Apr	3.2	0.56	1.762
2.1	Calypso	thiacloprid	L	22 Mar	2.6	0.25	0.65
2.1	Masai (10223)	tebufenpyrad	kg	22 Mar	2.6	0.75	1.95
2.1-2.3	Phytoline	*P. persimilis*	Pack	04 Apr		8000 /ha	

Table 5.1.2 continued.. Spray programme and biocontrol introduction in strawberry fields

Ever-bearer fields (farm.field)							
1.4	Calypso	thiacloprid	L	20 Apr	3.4	0.250	0.848
1.4	Chess WG	pymetrozine	Kg	20 Apr	3.4	0.400	1.356
1.4	Spidex 10000	*P. persimilis*	Pack	26 Apr	3.4	11.796	40.0
1.4	Thripex bulk	*N. cucumeris*	Pack	04 May	3.4	0.885	3.001
1.4	Spidex	*P. persimilis*	Pack	11 May	3.4	11.796	40.0
1.4	Thripex bulk	*N. cucumeris*	Pack	17 May	3.4	0.885	3.001
1.4	Thripex bulk	*N. cucumeris*	Pack	01 Jun	3.4	0.590	2.001
1.4	Thripex bulk	*N. cucumeris*	Pack	07 Jun	3.4	0.590	2.001
1.4	Thripex bulk	*N. cucumeris*	Pack	14 Jun	3.4	0.885	3.001
1.4	Thripex bulk	*N. cucumeris*	Pack	30 Jun	3.4	0.885	3.001
1.4	Thripex bulk	*N. cucumeris*	Pack	13 Jul	3.4	0.885	3.001
1.5-1.6	Calypso	thiacloprid	L	25 Apr	1.6	0.250	0.411
1.5-1.6	Chess WG	pymetrozine	Kg	25 Apr	1.6	0.400	0.657
1.5-1.6	Masai (10223)	tebufenpyrad	Kg	25 Apr	1.6	0.750	1.232
1.5-1.6	Spidex	*P. persimilis*	Pack	26 Jun	1.6	12.180	20.0
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	04 May	1.6	0.782	1.284
1.5-1.6	Spidex	*P. persimilis*	Pack	10 May	1.6	12.180	20.0
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	17 May	1.6	0.782	1.284

1.5-1.6	Spidex	*P. persimilis*	Pack	01 Jun	1.6	9.965	16.363
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	01 Jun	1.6	0.586	0.962
1.5-1.6	Spidex	*P. persimilis*	Pack	15 Jun	1.6	9.965	16.363
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	15 Jun	1.6	0.782	1.284
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	22 Jun	1.6	0.782	1.284
1.5-1.6	*Orius laevigatus* Nymphs (2000)		Pack	22 Jun	1.6	1.6	24.062
1.5-1.6	*Orius laevigatus* Adults (1000)		Pack	22 Jun	1.6	1.6	48.124
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	30 Jun	1.6	0.782	1.284
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	13 Jul	1.6	0.782	1.284
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	03 Aug	1.6	0.782	1.284
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	18 Aug	1.6	0.782	1.284
1.5-1.6	Thripex bulk	*N. cucumeris*	Pack	02 Sep	1.6	0.782	1.284

Data loggers recorded temperature and humidity throughout the experimental period in each crop and data for the Case Studies (see below) are in APPENDIX 5.1.3.

Both farms were visited each week from 5 Apr (1 day per farm). The last assessments in June bearer crops were on 13 Jun. From 20 Jun to 30 Aug the assessments were made in ever-bearer crops. Each time, in each crop, 25 plants were thoroughly searched in a different central row. A standard crop walking procedure was followed. The assessment was started 10 plants in from the edge and the evaluated plants were at least 10 plants apart to avoid assessing many infested plants in one hotspot of aphids. A plant was focused on from a distance and then walked towards and counts of pests and natural enemies recorded.

Aphids: Numbers of aphids were counted, 1. In the canopy and 2. In the crown (Fig. 5.1.1). Aphid species were identified on site and samples bought back to the laboratory for identification where necessary. A note was made when winged forms appear and the weeks they were present.

Table 5.1.2 continued.. Spray programme and biocontrol introduction in strawberry fields

Ever-bearer fields (farm.field)							
2.4-2.6		*Hyposapis miles*	Pack	20 Mar	1.17	88.2 mites / m^2	
2.4-2.6		*N. cucumeris*	Pack	03 Apr	1.17	290 mites / m^2	
2.4-2.6		*N. cucumeris*	Pack	10 Apr	1.17	290 mites / m^2	
2.4-2.6	Spidex	*P. persimilis*	Pack	10 Apr	3.4	50000	
2.4-2.6	Calypso Masai	thiacloprid tebufenpyrad	L kg	12 Apr	1.17	0.25 0.75	0.2925 0.8775
2.4-2.6		*N. cucumeris*	Pack	17 Apr	1.17	290 mites / m^2	
2.4-2.6	Dynamec	abamectin	L	21 Apr	1.17	0.05 /100 l water	
2.4-2.6		*Orius sp.*	Pack	08 May	1.17	3 / m^2	
2.4-2.6	Spidex	*P. persimilis*	Pack	08 May	3.4	50000	
2.4-2.6		*Orius sp.*	Pack	22 May	1.17	3 / m^2	
2.4-2.6	Spidex	*P. persimilis*	Pack	29 May	3.4	50000	
2.4-2.6		*Orius sp.*	Pack	05 Jun	1.17	3 / m^2	
2.4-2.6		Loose *Amblyseius*	Pack	12 Jun	1.17	50 mites / m^2	
2.4-2.6	Spidex	*P. persimilis*	Pack	19 Jun	3.4	50000	

2.4-2.6		Loose *Amblyseius*	Pack	19 Jun	1.17	50 mites / m^2	
2.4-2.6		Loose *Amblyseius*	Pack	03 Jul	1.17	50 mites / m^2	
2.4-2.6		Loose *Amblyseius*	Pack	10 Jul	1.17	50 mites / m^2	
2.4-2.6	Kumulus	sulphur	kg	26 Jul	1.17	0.2 /100 l water	
2.4-2.6	Kumulus	sulphur	kg	22 Aug	1.17	0.2 /100 l water	
2.4-2.6	Tracer	spinosad	L	05 Sep	1.17	0.15	0.1755
2.4-2.6	Benevia	cyantraniliprole	L	12 Oct	1.17	0.75	0.8775

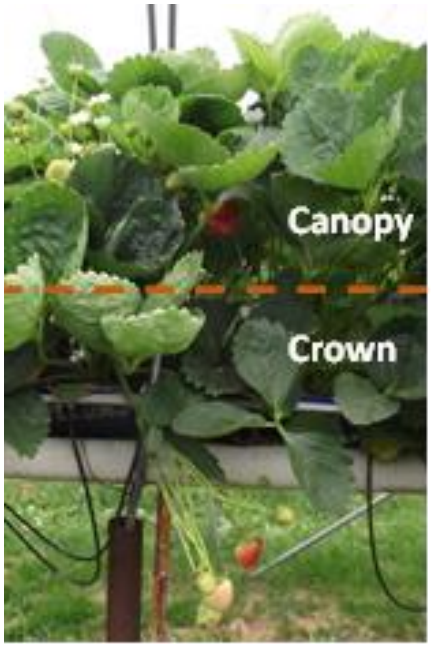


Figure 5.1.1. Canopy and crown parts of the assessed plants

Parasitoids: The numbers of parasitized aphids were counted and mummies were collected on leaves and bought back to the laboratory for identification (Table 5.1.3, Fig. 5.1.2).

Table 5.1.3. Reported efficiency of parasitic wasps against common aphids in strawberry (Viridaxis)

Aphid/Parasitoid	*Aphidius colemani*	*Aphidius ervi*	*Aphelinus abdominalis*	*Ephedrus cerasicola* (cryptic species)	*Praon volucre*
Aphis gossypii	+++		X	X	+
Aulacorthum solani	X	++	++	+++	++
Macrosiphum euphorbiae	X	+++	+++		+++
Myzus ascalonicus			X	X	X
Myzus persicae	+++	+	++	++	++

Figure 5.1.2. Aphids parasitized by different types of parasitic wasps (from the left: *Aphidius* sp., *Praon volucre, Aphelinus abdominalis*)

Predators: Immature and, where was possible, adult stages of natural enemies on the plants were recorded including Coccinelidae, Spiders, Syrphidae, Neuroptera, Orius, Anthocoridae, and other notable predatory species, including Soldier beetles.

Pest damage: An overall score of aphid damage (skins, honeydew and fungi) was done each week for each assessed plant in every field. The damage values were; 0 – *none*, 1 – *slight* – some aphid skins, 2 - *moderate* – some aphid skins and honeydew but confined to leaves and 3 – *severe* – fruit/flowers affected, possible sooty moulds.

Results

Differences between farms and crops

In June bearers *M. euphorbiae* was the dominant pest (Fig. 5.1.3a); however, other aphid species were also present in low numbers, such as glasshouse-potato aphids, strawberry- and shallot- aphids. Hoverflies and lacewings were present in low numbers, but the main recognisable biocontrol agents were parasitic wasps. In June bearer crops there were differences between the numbers of aphids and natural enemies in the different fields at each farm (Fig. 5.1.3a). Everbearers plantations had more diverse pest and natural enemy populations (Fig. 5.1.3b).

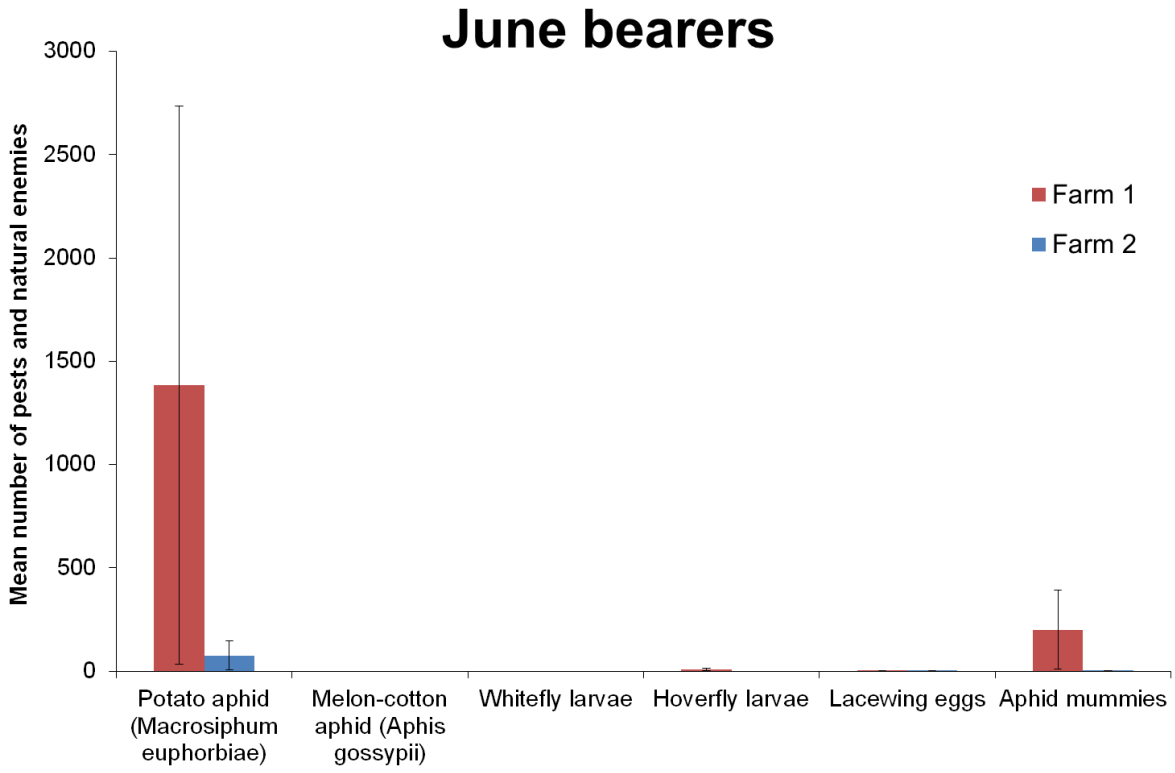


Figure 5.1.3a. Mean number (+/- SE) of aphids, whitefly and natural enemies in 25 plants in June bearer strawberry crops on 2 farms

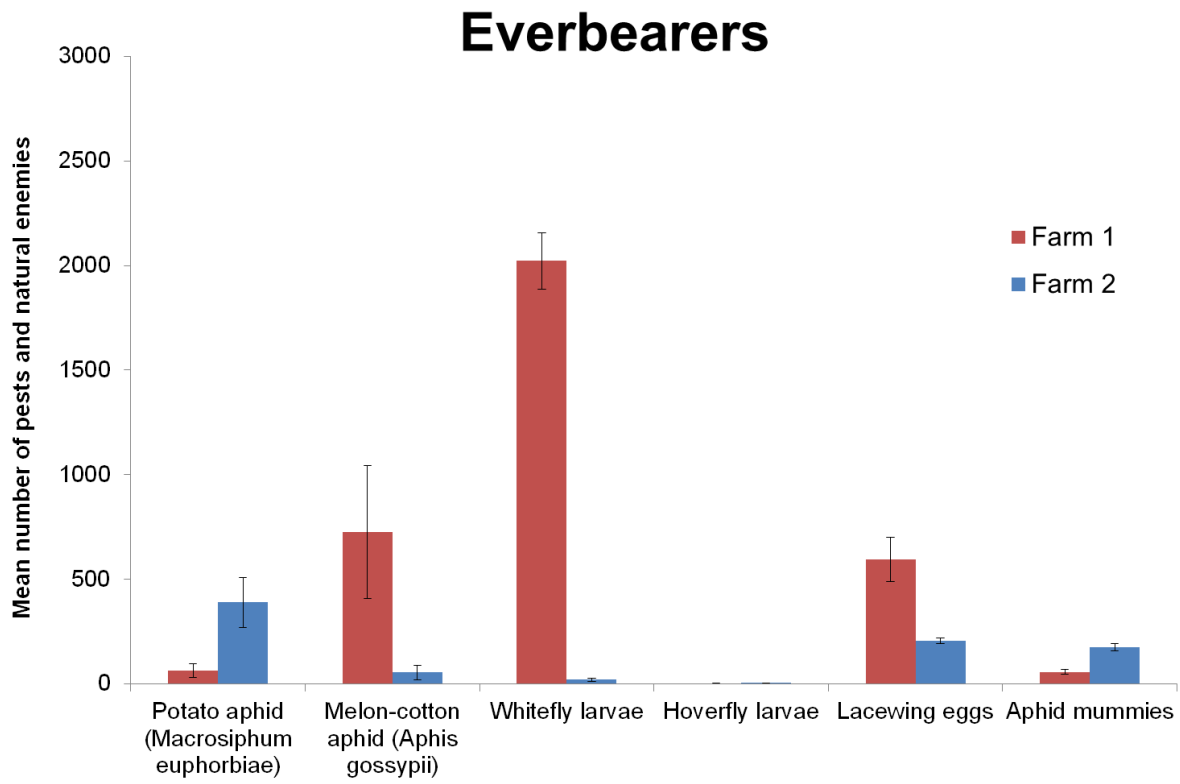


Figure 5.1.3b. Mean number (+/- SE) of aphids, whitefly and natural enemies in 25 plants in ever-bearer strawberry crops on 2 farms

Main pests and parasitic wasps: In June bearers the main pest was *M. euphorbiae* (Fig. 5.1.4a). From end of June to end of May winged aphids within the *M. euphorbiae* colonies were recorded, with a peak on 09 Jun (Fig. 5.1.4a). The number of parasitized aphids increased approximately 4 weeks after the increase in aphid numbers. In both June- and ever-bearers *Aphidius* sp. and *Praon volucre* were the main parasitoids observed (Fig. 5.1.5a,b). There were lower numbers in everbearers which may be a consequence of declining aphid numbers (Fig. 5.1.5b). The everbearer pest population was more diverse than June bearers (Fig. 5.1.4b). Apart from *M. euphorbiae*, Melon-cotton- (*Aphis gossypii*) and peach-potato aphids (*Myzus persicae*) were also present in considerable numbers, as were *Trialeurodes vaporariorum*, the glasshouse whitefly (Fig. 5.1.4a,b).

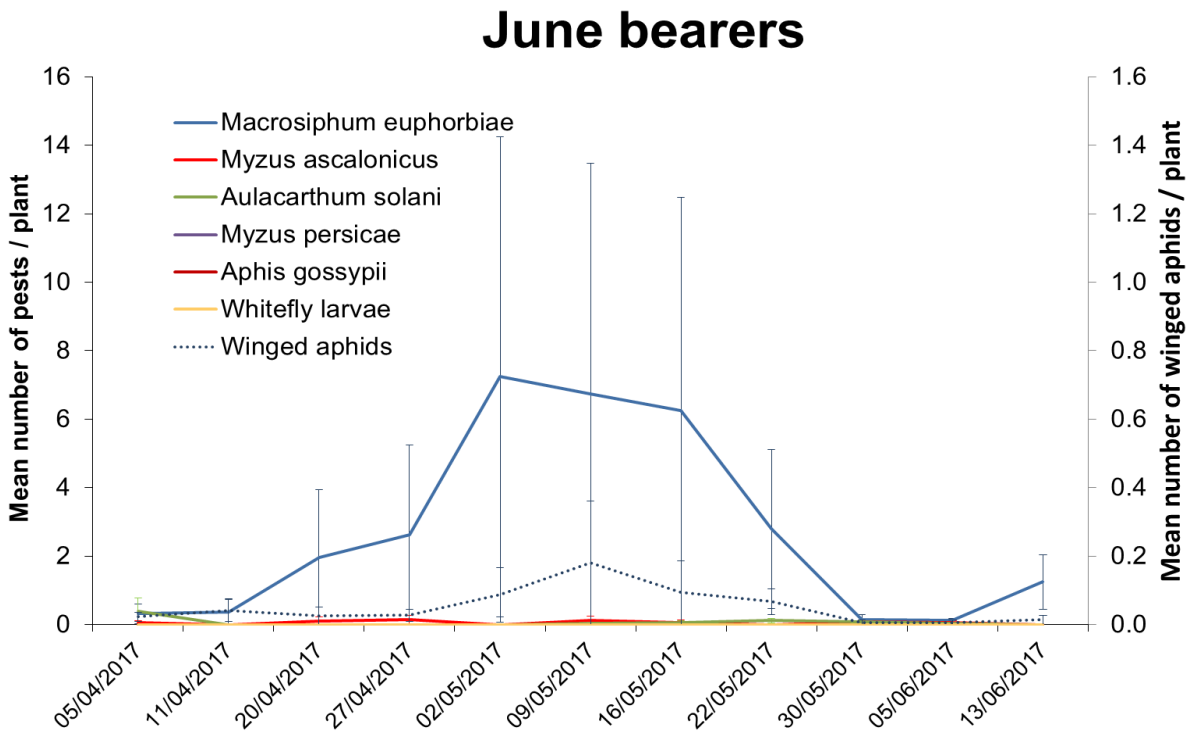


Figure 5.1.4a. Mean number (+/- SE) of aphids and whitefly per plants in June bearer strawberry crops on 2 farms

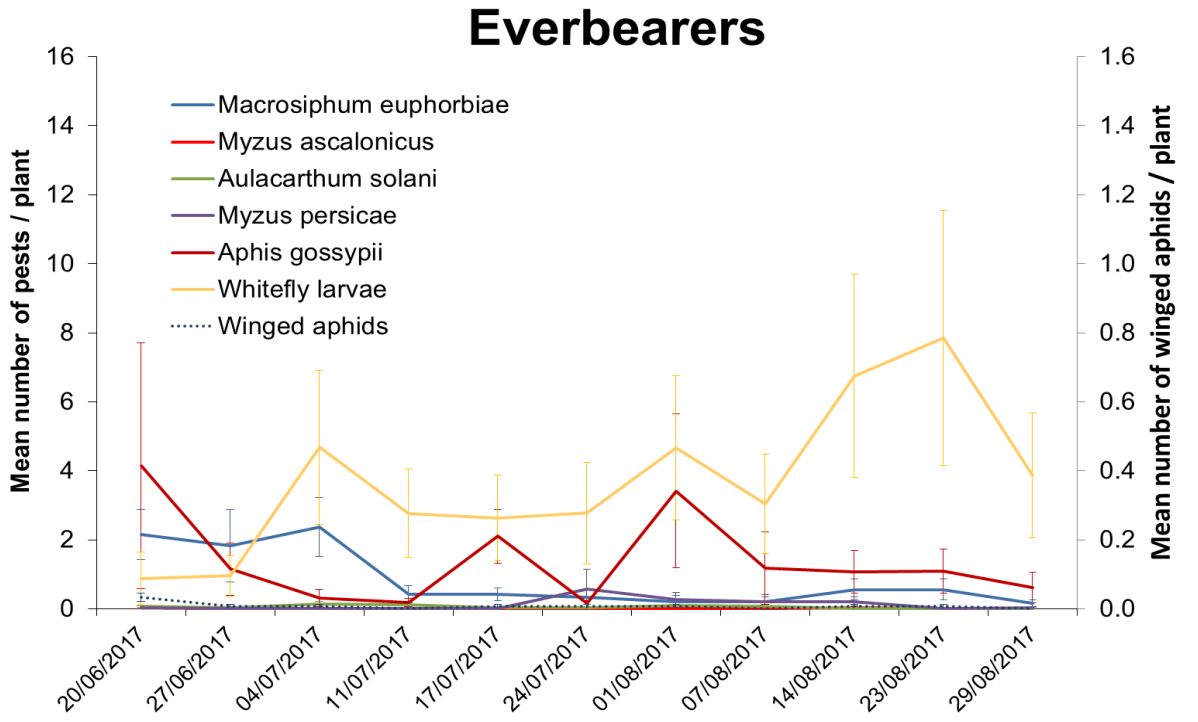


Figure 5.1.4b. Mean number (+/- SE) of aphids and whitefly per plants in ever-bearer strawberry crops on 2 farms

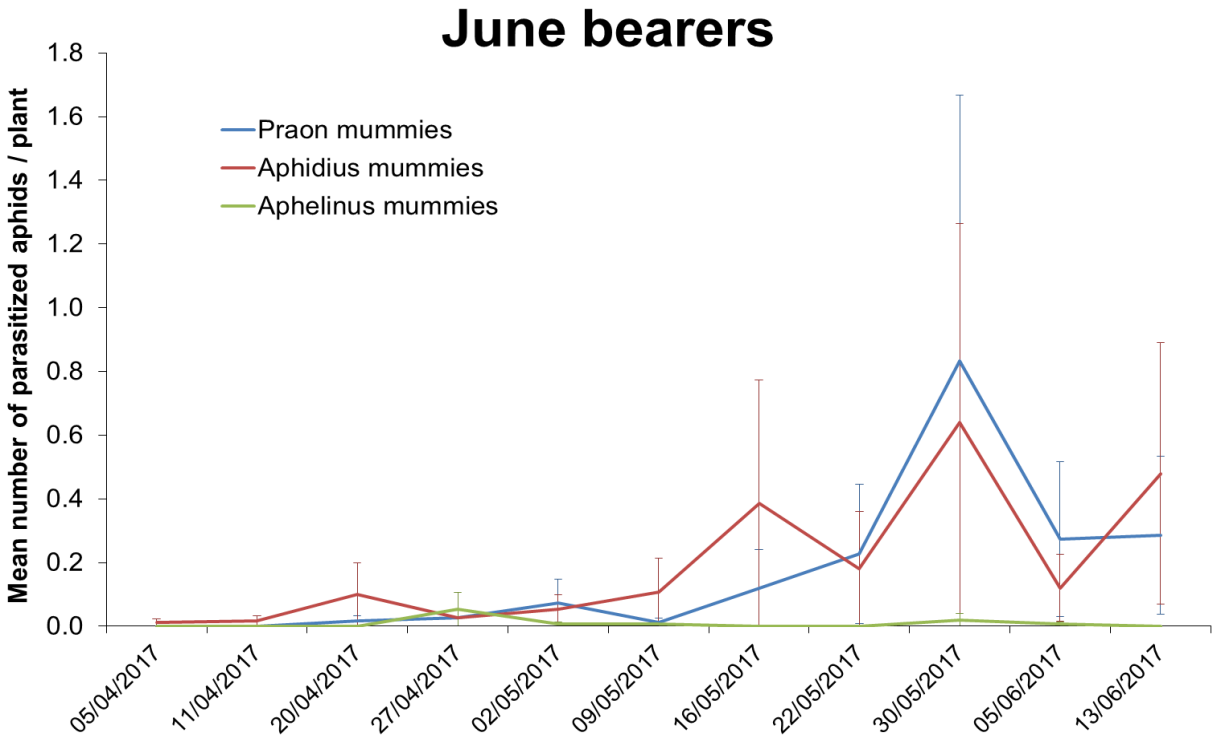


Figure 5.1.5a. Mean number (+/- SE) of parasitized aphids per plant in June bearer strawberry crops on 2 farms

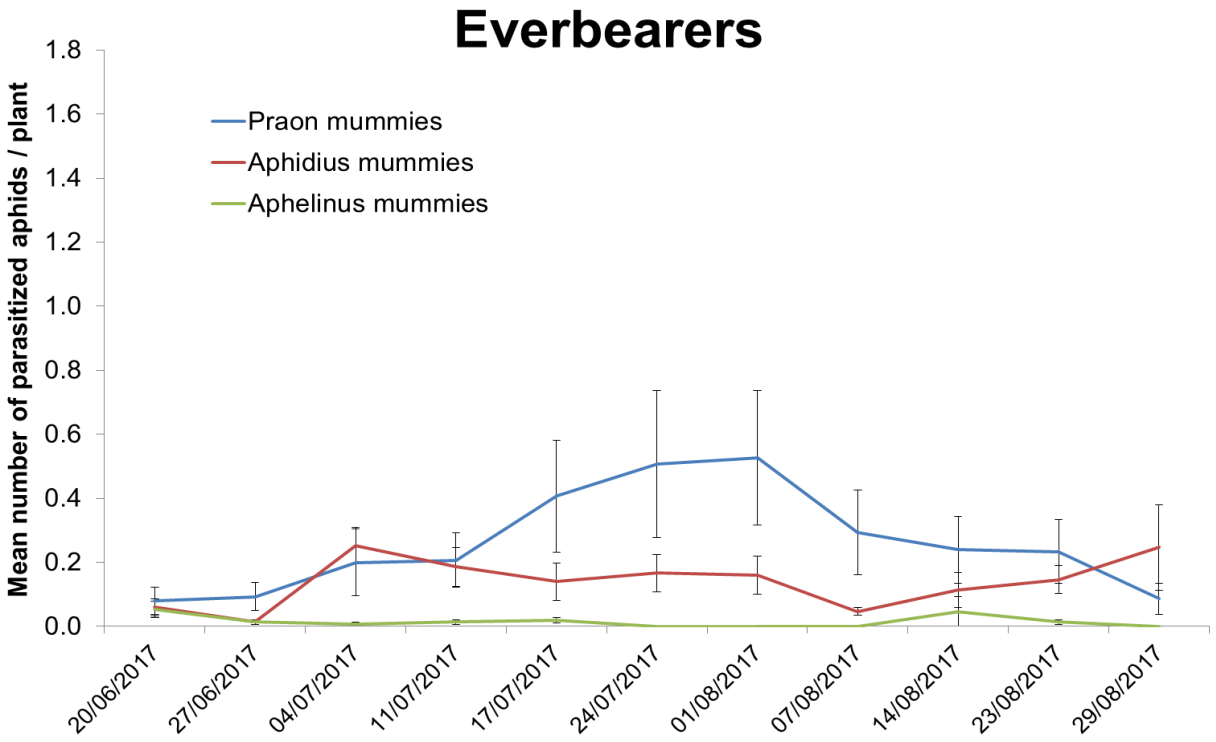
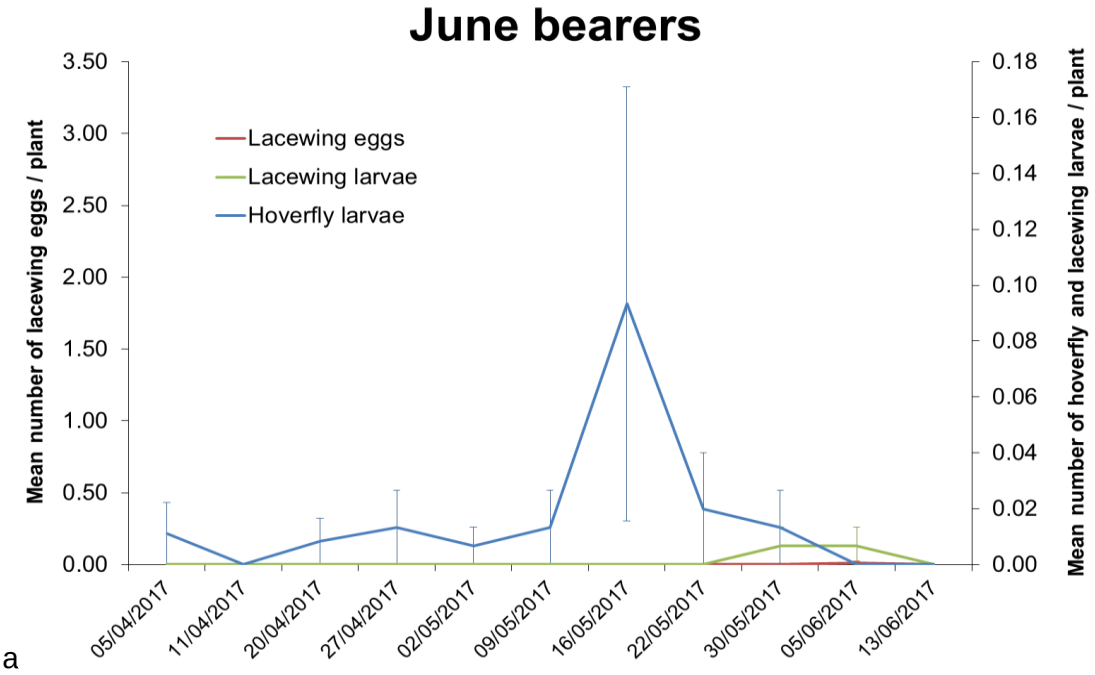
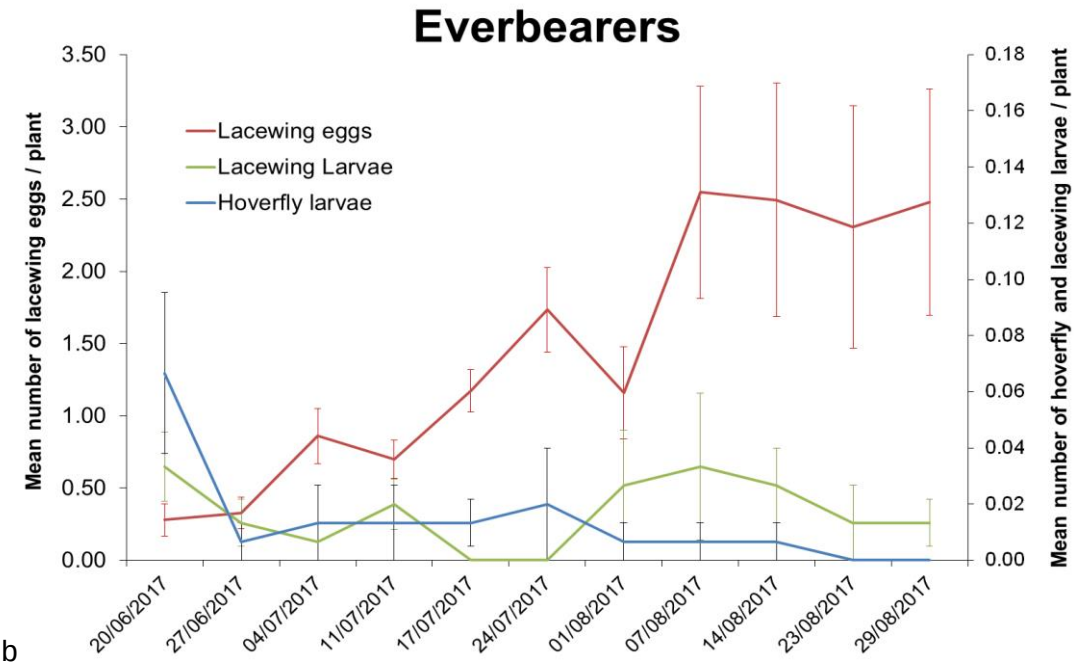


Figure 5.1.5b. Mean number (+/- SE) of parasitized aphids per plant in ever-bearer strawberry crops on 2 farms

Predators: Hoverfly larvae were present throughout the season (Fig. 5.1.6a,b), although in low numbers, a maximum of 0.48 per plant was found at any one visit. Green lacewing (*Chrysoperla sp.*) larvae became more prevalent from 4 Jul (Fig. 5.1.6b). Other predators, such as spiders, ladybirds were also observed, but only in low numbers (less than 2 per week in all of the plants).



a



b

Figure 5.1.6. Mean number (+/- SE) of lacewing eggs, larvae and hoverfly larvae per plants in a) June bearer strawberry crops and b) ever-bearer strawberry crops on 2 farms

Case studies

In the majority of the June bearer fields aphids were not in significant numbers. However the data collected in one of the fields allowed us to make a phenological plot of the key peaks and troughs in aphid and natural enemy numbers (Fig. 5.1.7).

The mean numbers of aphids began to increase from the end of Apr. In this field a mixture of parasitic wasps was introduced on 24 Apr. As the mean numbers of parasitoid mummies increased the numbers of aphids in the plants decreased, with a steep decline by the end of May. From the date of the first introduction it took almost a month for the increase in mummified aphids to be apparent.

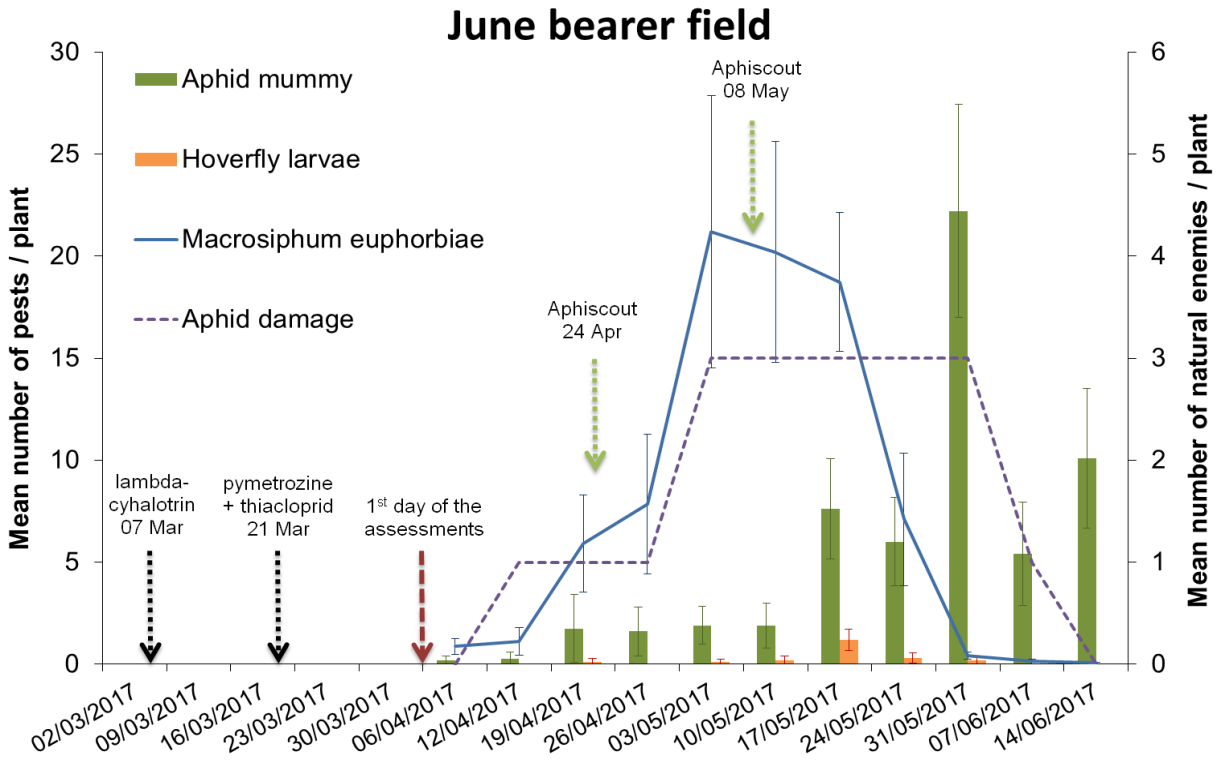


Figure 5.1.7. Mean number (+/- SE) of aphids, parasitized aphids (mummies), lacewing eggs and hoverfly larvae per plant in a June bearer field. The second axis also shows the maximum aphid damage is given; 0 – *none*, 1 – *slight* – some aphid skins, 2 - *moderate* – some aphid skins and honeydew but confined to leaves and 3 – *severe* – fruit/flowers affected, possible sooty moulds

A maximum score of aphid damage was made for each plant, where the values varied from 0 (no damage) to 3 (severe damage) (Fig. 5.1.7 and 5.1.8). Table 5.1.5 summarize how many plants from 25 assessed had severe damage (3 – *severe* – fruit/flowers affected, possible sooty moulds). There was a peak of damage to plants, but only a maximum of 3 of 25 plants, in May. This coincided with a peak in aphid numbers. However, aphid populations and damage declined following the parasitoid introduction. Ever-bearer plants did not have a high incidence of aphid damage (Table 5.1.4).

Table 5.1.4. Number of plants with maximum aphid damage per week in the case study plantations (June- and ever-bearer fields)**;** 0 – *none*, 1 – *slight* – some aphid skins, 2 - *moderate* – some aphid skins and honeydew but confined to leaves and 3 – *severe* – fruit/flowers affected, possible sooty moulds.

June bearers		**Everbearers**	
Date	**Number of plants with severe damage caused by aphids/25 assessed plants**	**Date**	**Number of plants with severe damage caused by aphids/25 assessed plants**
06 Apr	0	20 Jun	0
12 Apr	0	27 Jun	2
19 Apr	0	04 Jul	0
26 Apr	0	11 Jul	0
03 May	3	17 Jul	0
10 May	3	24 Jul	0
17 May	2	01 Aug	0
24 May	1	07 Aug	0
31 May	1	14 Aug	0
07 Jun	0	24 Aug	0
14 Jun	0	29 Aug	0

In comparison to the June bearer fields the pest and natural enemy fauna was more diverse in the ever-bearer crops probably reflecting the emergence of a more diverse species assemblage as the season progressed (Fig. 5.1.8). The main pest in this field was *M. euphorbiae,* although other pests e.g. *Aphis gossypii*, thrips and glasshouse whitefly were present in considerable numbers in some fields. Similarly to the June bearer field, there was a delay in the increase of natural enemies present in comparison to the aphid population. As the mean numbers of parasitoid mummies and lacewing eggs (which are good representatives of the larvae present) increased, the numbers of aphids in the plants decreased. Towards the end of the study (from 25 Jul) small numbers of *Encarsia formosa* parasitoids were recognised in the whitefly larvae. It is not certain what caused the decline in whitefly at the end of Jul, however it has been demonstrated (Koppert website https://www.koppert.com/products/products-pests-diseases/chrysopa/) that lacewing larvae can feed on whitefly larvae.

Against mites and thrips several biocontrol agents were released throughout the season (Table 5.1.2).

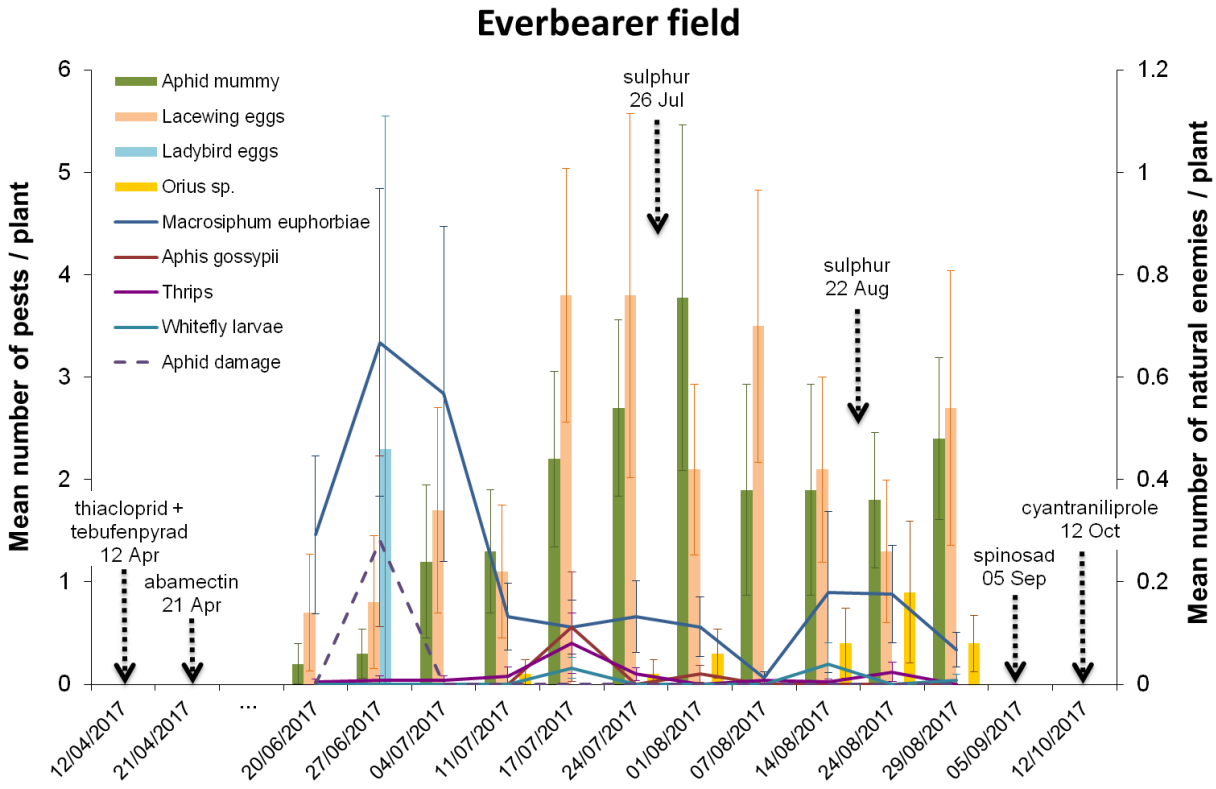


Figure 5.1.8. Mean number (+/- SE) of aphids, parasitized aphids (mummies), lacewing eggs and hoverfly larvae per plants in an ever-bearer field. On the secondary axis the maximum aphid damage value is given; 0 – *none*, 1 – *slight* – some aphid skins, 2 - *moderate* – some aphid skins and honeydew but confined to leaves and 3 – *severe* – fruit/flowers affected, possible sooty moulds

Discussion

- There is a high variability in aphid species and numbers not only between farms, or between the different fields in the same farm, but between plants in the same field.
- In both farms the main pest was *M. euphorbiae* though other pests such as *Aphis gossypii,* thrips, two-spotted spider mites and glasshouse whitefly were present in considerable numbers.
- From the end of May to the end of June winged aphids were presented with a peak on 09 Jun.

- In both farms the main aphid predators were the green lacewing and hoverfly larvae. Hoverfly larvae were present in low numbers through the two crops through the season and green lacewing larvae became more prevalent from 04 Jul.
    - A single larva of the widespread marmalade hoverfly (*Episyrphus balteatus*) consumes between 660 and 1,140 aphids during development.
    - A single green lacewing larva consumes between 566 and 789 aphids before pupating.
- Other predators such as spiders, ladybirds and *Orius* sp. were also observed, but only in low numbers.
- *Praon* sp. or *Aphidius* sp. were the main parasitoid found parasitizing aphids in strawberry. *Aphelinus* sp. parasitism was also present but at a lower incidence.
- In the assessed fields the pest and natural enemy fauna was more diverse in the ever-bearers than in the June bearers.
- In the ever-bearer crops at Farm 1 apart from aphids, glasshouse whitefly was present during the time of the assessments.
- Towards the end of the study (from 25 Jul) small numbers of *Encarsia formosa* parasitoids were recognised in the whitefly larvae.
- In both crops there were delays in the natural enemy's population growth comparing to the pest population growth. However, with the increase of natural enemies, the number of aphids declined.
- It is evident from this study, so far, that before June there are very few natural enemies in strawberry crops and therefore other control measures should be employed to supress aphid populations until natural numbers build.
- Controls introduced by growers should be sensitive to the natural enemies likely to enter the crop later in the season.
- Future considerations should be focused on how SWD exclusion mesh will effect the influx of natural enemies into strawberry crops?

Future Work

Follow the success of spot treating individual colonies of different species of aphid with soaps (Majestic or Savona) in comparison to insecticides.

Acknowledgements

We would like to thank the funders of the research AHDB Horticulture for their support. We would also like to thank all growers for the use of their plants and crops and Berry Gardens for continued support in sourcing sites and growers. We also thanks Charlotte Rowley and the technicians at NIAB EMR for help with treatment application and Dr Rob Graham and Phil Brain for their advice on the statistics used.

Knowledge and Technology Transfer

18-20 Apr 2017. Fountain. 2017 International Heteroptera Symposium, Pests for the Next Decade: Lygus, Plant and Stink Bug, Monterey Bay, CA. *Controlling Lygus in strawberry with semiochemical traps*

4-5 September 2017 – Charlotte Rowley and Tom Pope – AAB – *Advances in IPM*.

21 November 2017 EMR Association/AHDB Soft Fruit Day *New predators of WFT* (Chantelle Jay, NIAB EMR)

21 November 2017 EMR Association/AHDB Soft Fruit Day *The latest research into WFT control and a device to extract pest and predators* (Jean Fitzgerald and Adrian Harris, NIAB EMR)

21 November 2017 EMR Association/AHDB Soft Fruit Day *The benefits of hoverflies in strawberry crops* (Dylan Hodgkiss, NIAB EMR)

21 November 2017 EMR Association/AHDB Soft Fruit Day *The latest research into SWD control* (Madeleine Cannon and Michelle Fountain, NIAB EMR)

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APPENDIX 3.2.1.

Experimental Sites

Site 1. Hugh Lowe Farms, Mereworth, Kent, Variety Amesti. Note the four treatments were set up in a line at the southern edge of the crop adjacent to a hedgerow/windbreak with a cereal crop behind. The tunnels were staggered and therefore in all cases the outer start of the plots were at least 8 m into the crop.

Site 2. Edward Vinson Farms, Faversham, Kent. Variety Sweet Eve 2. The four treatments were set up in a grid, although as the planting was in blocks, each treatment was placed 8 m into the edge of the crop.

Sites 3 & 4. Quaives Farm, Grove Road, Wickhambreaux, Canterbury, Kent. Variety Amesti. Site 3 in shown in blue, and site 4 in red. Each treatment started at the edge of the crop.

APPENDIX 3.2.2. Spray records provided for each site.

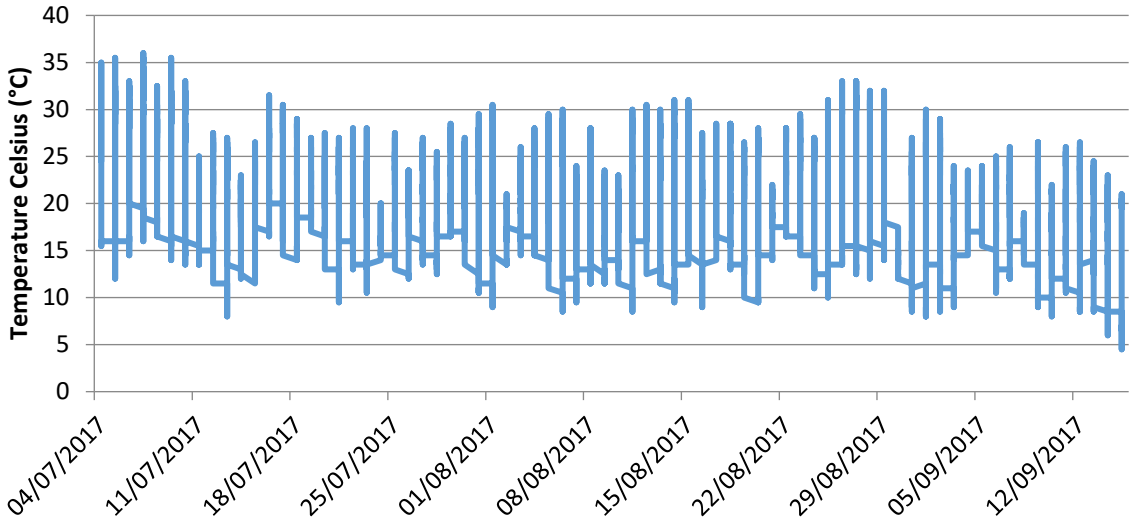
Site	Product	Application date 2017	Rate	A.I.	Notes
Site 1	Nimrod	05/07	As label	Bupirimate	
Site 1	Tracer	05/07	As label	Spinosad	
Site 1	Kumulus	25/07	As label	Sulphur	
Site 1	K50	25/07	As label		
Site 1	SPO 58	25/07	As label		
Site 1	Luna sensation	30/07	As label	Fluopyram Trifloxystrobin	
Site 1	Maxicrop	30/07	As label		
Site 1	Hortiphyte	30/07	As label		
Site 1	Nimrod	11/08	As label	Bupirimate	
Site 1	Hortiphyte	11/08	As label		
Site 1	K50	23/08	As label		
Site 1	Kumulus	23/08	As label	Sulphur	
Site 1	Serenade	23/08	As label		
Site 2	Fast Manganese	08/07	3.250 l/ha		
Site 2	Systhane 20 EW	08/07	0.450 l/ha	Myclobutanil	
Site 2	Fast Iron	12/07	6.000 l/ha		
Site 2	Fast trac	18/07	1.000 l/ha		
Site 2	Sinpro	18/07	1.500 l/ha	Iprodione	
Site 2	Tracer	18/07	0.150 l/ha	Spinosad	EAMU 1238/17
Site 2	Topas	18/07	0.500 l/ha	Penconazole	

Site 2	Hallmark with Zeon Technology	28/07	0.075 l/ha	Lambda-cyhalothrin	EAMU 1705/11
Site 2	Systhane 20 EW	28/07	0.450 l/ha	Myclobutanil	
Site 2	Fast trac	28/07	2.000 l/ha		
Site 2	Potassium bicarbonate	04/08	5.000 kg/ha		
Site 2	Wetcit	04/08	0.5000 l/ha		
Site 2	Pyrethrum 5 EC	08/08	1.100 l/ha	Pyrethrins	
Site 2	Sinpro	08/08	1.500 l/ha	Iprodione	
Site 2	Talius	08/08	0.190 l/ha	Proquinazid	
Site 2	Fast formula 1	08/08	3.000 l/ha		
Site 2	Amistar	17/08	1.000 l/ha	Azoxystrobin	
Site 2	Fast formula 1	17/08	3.000 l/ha		
Site 2	Potassium bicarbonate	24/08	10.000 kg/ha		
Site 2	Wetcit	24/08	1.000 l/ha		
Site 2	Luna sensation	31/08	0.800 l/ha	Fluopyram Trifloxystrobin	
Site 2	Systhane 20 EW	09/09	0.450 l/ha	Mycobutanil	
Site 2	Decis	09/09	0.500 l/ha	Deltamethrin	EAMU 1643/13
Site 2	Benevia 10 OD	14/09	0.750 l/ha	Cyantraniliprole	EAMU 1559/17
Site 2	Nimrod	14/09	1.400 l/ha	Bupirimate	
Site 2	Teldor	14/09	1.500 kg/ha	Fenhexamid	
Site 2	Fast formula 1	14/09	3.000 l/ha		

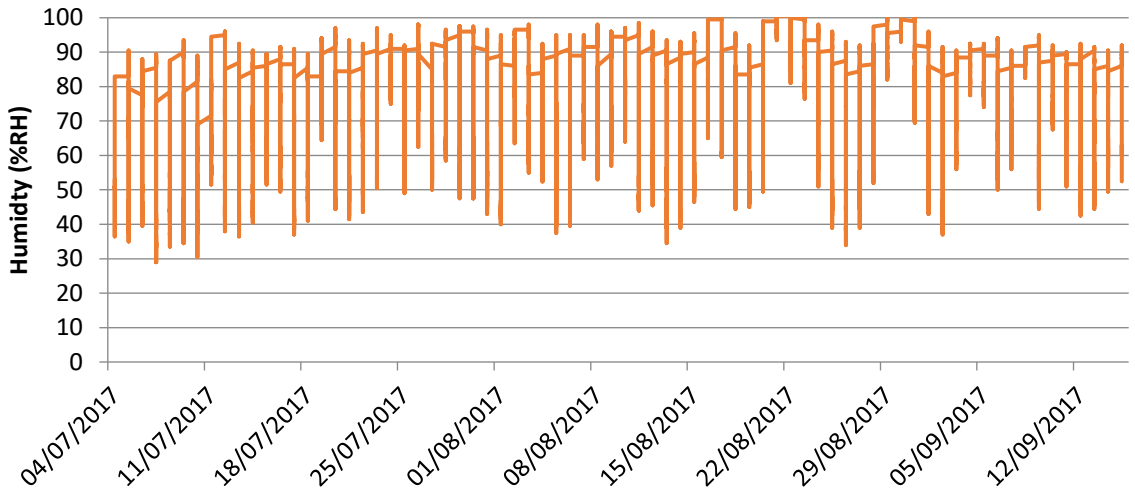
NB: Sites 3&4 – spray programmes not provided by growers

APPENDIX 3.2.3. Air temperature and humidity records within the polytunnels at the push-pull experimental sites

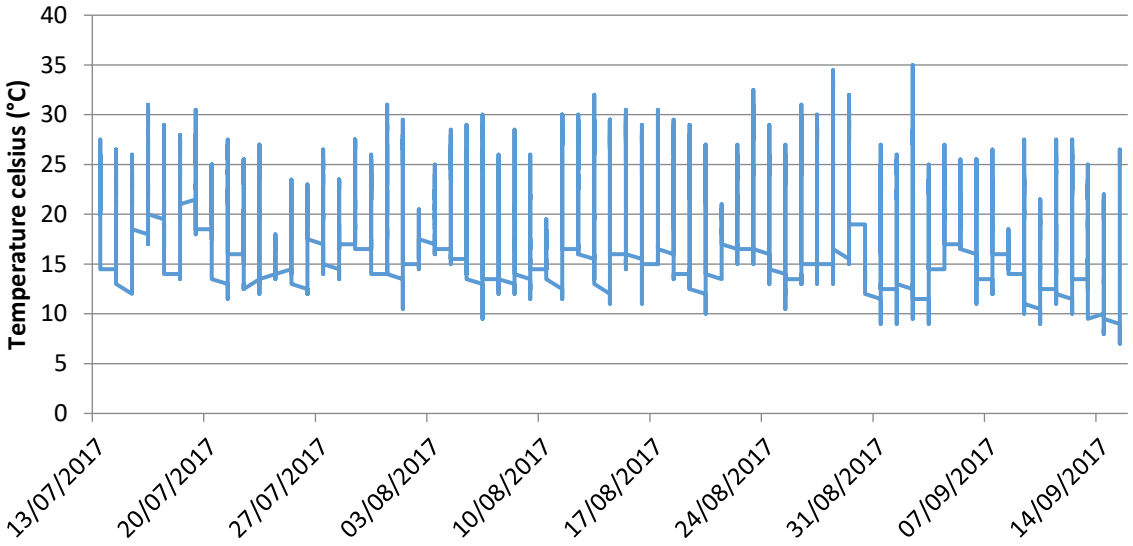
a) Temperature records at Site 1 between 4 Jul and 15 Sep 2017



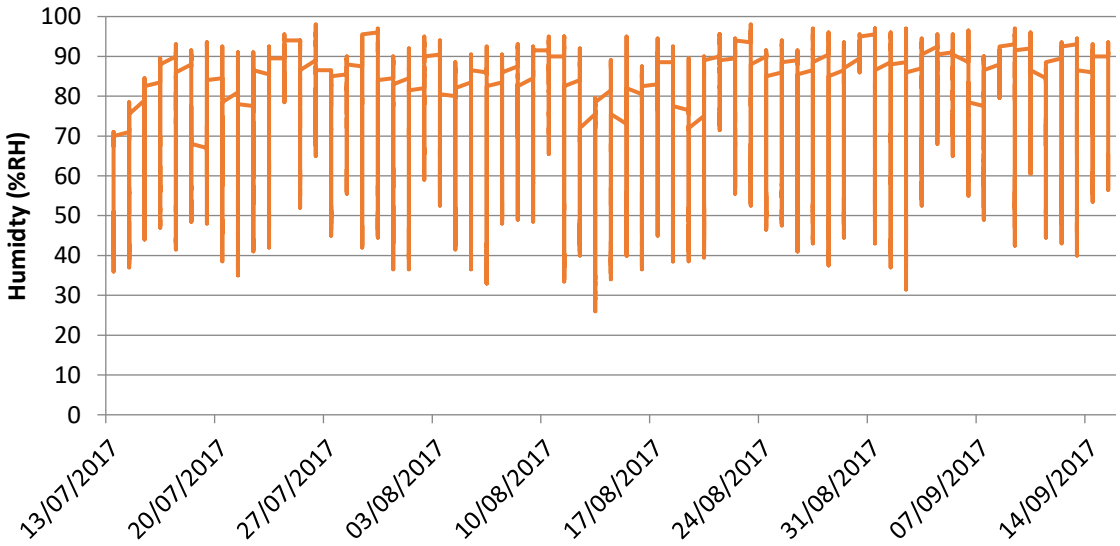
b) Humidity records at Site 1 between 4 Jul and 15 Sep 2017



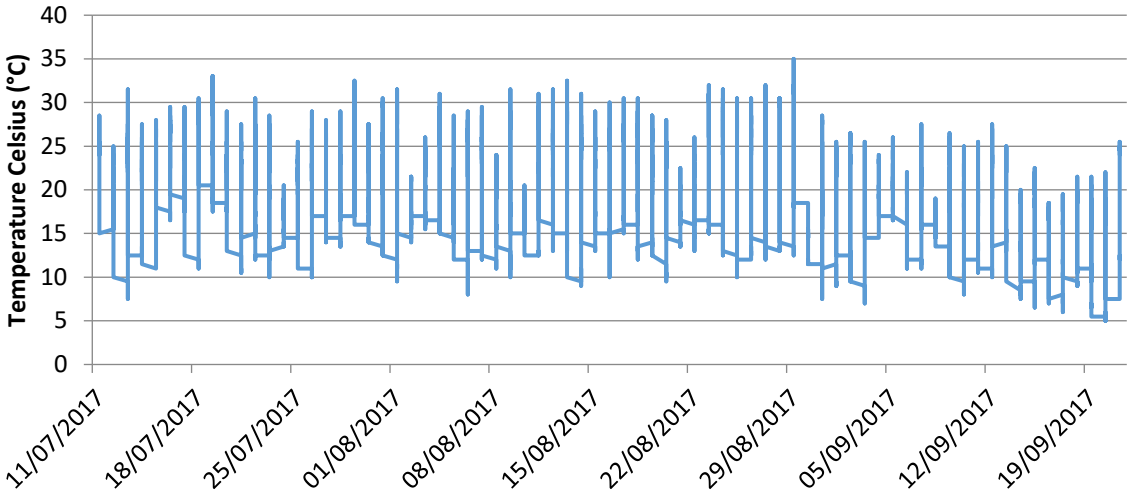
c) Temperature records at Site 2 between 13 Jul and 15 Sep 2017



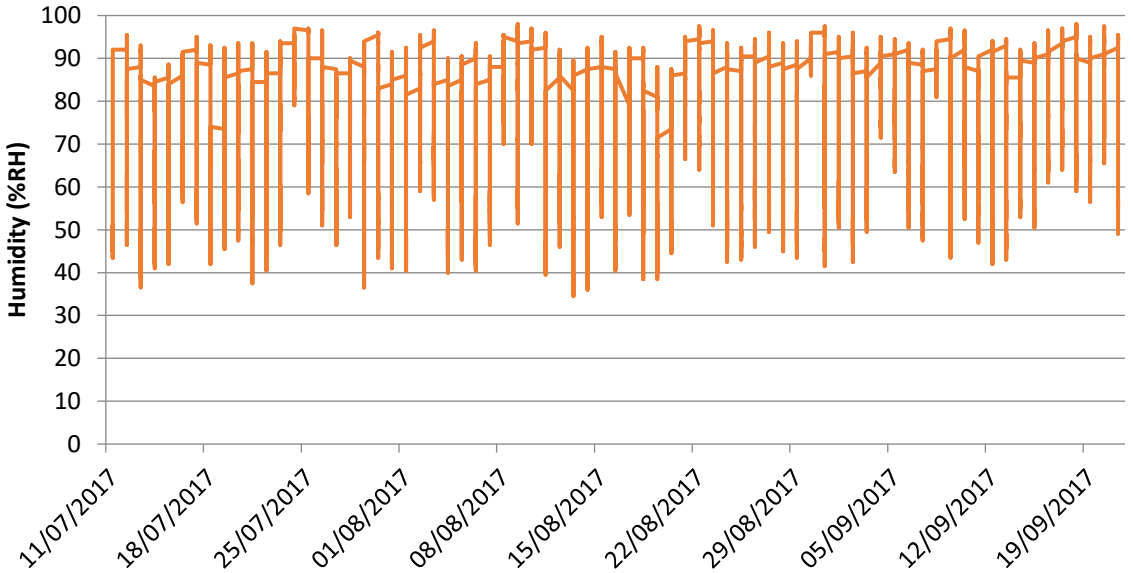
d) Humidity records at Site 2 between 13 Jul and 15 Sep 2017



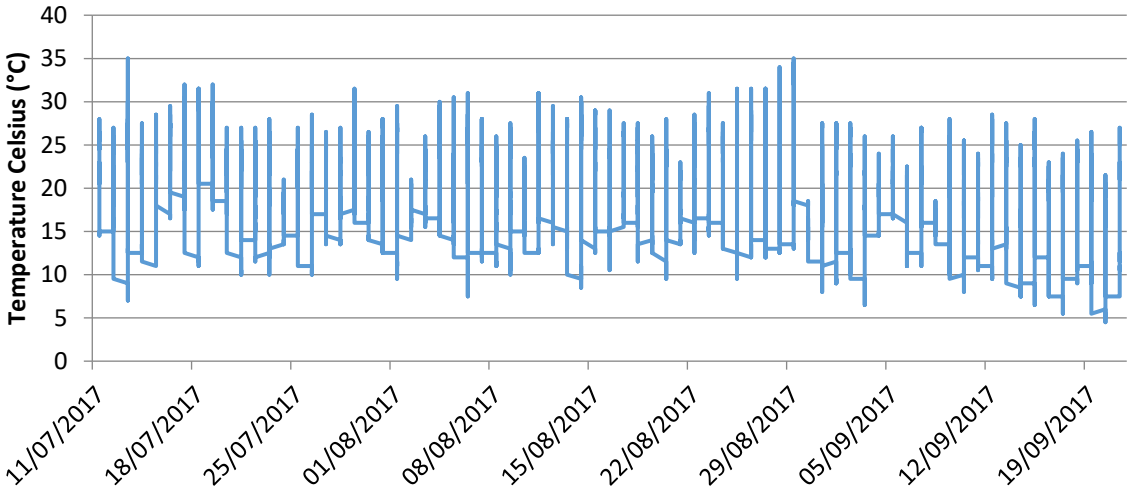
e) Temperature records from Site 3 between 11 Jul and 21 Sep 2017



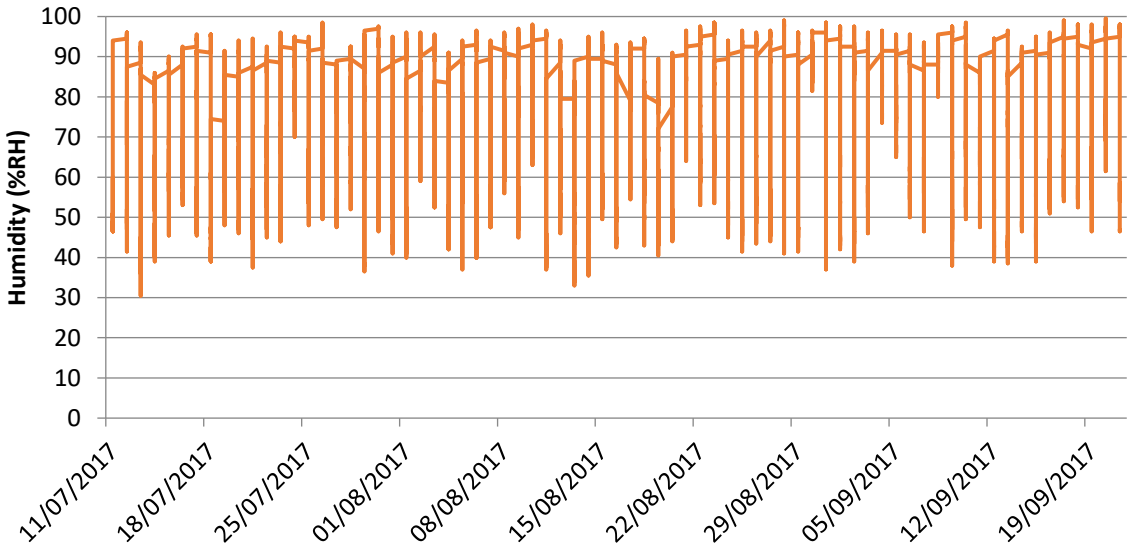
f) Humidity records from Site 3 between 11 Jul and 21 Sep 2017



g) Temperature records from Site 4 between 11 Jul and 21 Sep 2017



h) Humidity records from Site 4 between 11 Jul and 21 Sep 2017



APPENDIX 5.1.1. Summary table of the understory management of the fields used for the assessments

Farm	Plantation	Understory management
1	1	Mown grass between tables, herbicide use under the tables.
1	2	Bare ground with patches of speedwell, grass, shepherd's purse, groundsel, dock, etc.
1	3	Bare ground with patches of speedwell, grass, shepherd's purse, nettles, fumitory, groundsel.
2	1	Until 20 Apr unmown grass with red dead-nettle, chickweed, dandelion, groundsel.
		From 27 Apr mown grass.
2	2	Mown grass between tables, unmown under, with groundsel, knotgrass.
2	3	Until 20 Apr unmown grass with red dead-nettle, chickweed, dandelion, groundsel.
		From 27 Apr to 30 May unmown grass sprayed with herbicide.
		From 05 Jun herbicide use between table, unmown grass under table, with field bindweed, chickweed.
1	4	Mown grass between tables, herbicide use under the tables.
1	5	Mown grass between tables, unmown under tables.
1	6	Mown grass.
2	4	Mown grass between tables, unmown under tables with bindweeds (*Convolvulus arvensis, Calystegia sepium*).
2	5	Unmown grass with bindweeds (*Convolvulus arvensis, Calystegia sepium*).
2	6	Unmown grass with bindweeds (*Convolvulus arvensis, Calystegia sepium*), groundsel, chickweed, cleavers, nettles.

APPENDIX. 5.1.2a. Summary table of flowering plants at each June bearer field on given date

date	Field					
	2.1	**2.2**	**2.3**	**1.1**	**1.2**	**1.3**
05-06 Apr	*Taraxacum officinale, Stellaria media*	*Taraxacum officinale, Stellaria media*	*Taraxacum officinale, Stellaria media, Veronica persica*	-	*Veronica sp., Taraxacum officinale*	*Capsella bursa-pastoris*
11-12 Apr	*Taraxacum officinale, Stellaria media*	*Taraxacum officinale, Stellaria media*	*Taraxacum officinale, Stellaria media, Veronica persica, Pentaglottis sempervirens, Laurus cerasus, Symphytum officinale, Sonchus arvensis*	-	*Veronica sp., Taraxacum officinale*	*Capsella bursa-pastoris, Senecio vulgaris*
20-19 Apr	-	-	*Sinapis arvensis, Sonchus asper*	*Veronica sp., Taraxacum officinale*	*Veronica sp., Taraxacum officinale*	*Capsella bursa-pastoris, Senecio vulgaris*
24-26 Apr	-	-	Umbelliferous plants (Parsleys)	*Veronica sp., Taraxacum officinale*	*Matricaria recutita*	*Capsella bursa-pastoris*
02-03 May	Umbelliferous plants (Parsleys)	Umbelliferous plants (Parsleys), *Taraxacum officinale*	Umbelliferous plants (Parsleys), *Crataegus monogyna, Taraxacum officinale, Pentaglottis sempervirens*	*Veronica sp., Taraxacum officinale*	*Capsella bursa-pastoris*	*Capsella bursa-pastoris*
09-10 May	Umbelliferous plants (Parsleys)	Umbelliferous plants (Parsleys)	Umbelliferous plants (Parsleys)	*Veronica sp., Taraxacum officinale*	*Capsella bursa-pastoris*	*Capsella bursa-pastoris*

17 May	Umbelliferous plants (Parsleys)	*Taraxacum officinale, Sonchus sp., Anthemis sp., Senecio vulgaris*	*Sambucus nigra, Pentaglottis sempervirens*	*Ranunculus sp.*	*Capsella bursa-pastoris*	*Matricaria sp.*
22-24 May	Umbelliferous plants (Parsleys)	Umbelliferous plants (Parsleys), *Senecio vulgaris*	Umbelliferous plants (Parsleys), *Sambucus nigra, Pentaglottis sempervirens*	*Ranunculus sp.*	*Cardamine hirsute, Sinapis arvensis, Senecio vulgaris*	*Capsella bursa-pastoris, Matricaria sp.*
30-31 May	Umbelliferous plants (Parsleys)	Umbelliferous plants (Parsleys)	Umbelliferous plants (Parsleys), *Sambucus nigra*	*Ranunculus sp., Anthemis sp., Trifolium repens*	*Taraxacum officinale, Veronica sp., Cardamine hirsute*	*Capsella bursa-pastoris, Matricaria sp., Taraxacum officinale, Sonchus sp., Sambucus nigra, Papaver rhoeas,* Umbelliferous plants (Parsleys)
05-07 Jun	*Sonchus sp., Matricaria sp.*	*Trifolium repens, Ranunculus sp., Epilobium sp.*	*Trifolium repens, Ranunculus sp., Sambucus nigra, Rubus sp., Rosa sp., Anthemis sp., Sonchus sp.*	*Ranunculus sp., Anthemis sp., Trifolium repens*	*Taraxacum officinale, Veronica sp., Cardamine hirsute, Capsella bursa-pastoris*	*Capsella bursa-pastoris, Matricaria sp., Taraxacum officinale, Sonchus sp., Sambucus nigra, Papaver rhoeas,* Umbelliferous plants (Parsleys), *Convolvulus sp*.

4 Jun			*Trifolium repens, Papaver rhoeas*	*Veronica sp.,* *Cardamine hirsute, Capsella bursa-pastoris, Senecio vulgaris, Rumex sp.*		*sp., Taraxacum officinale, Sonchus sp., Sambucus nigra, Papaver rhoeas,* Umbelliferous plants (Parsleys), *Convolvulus sp.*

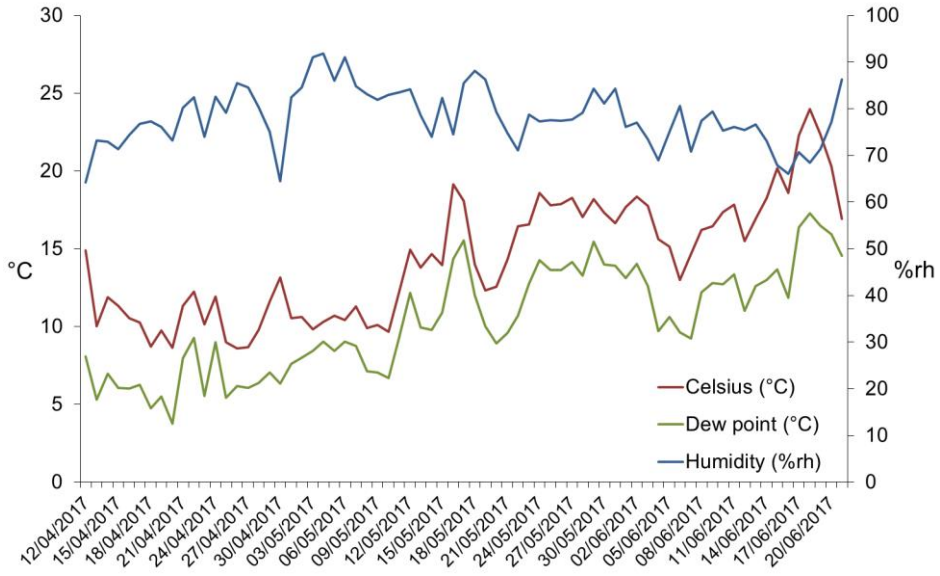
APPENDIX. 5.1.2b. Summary table of flowering plants at each ever-bearer field on given date

date	**Field**					
	2.4	**2.5**	**2.6**	**1.4**	**1.5**	**1.6**
20-21 Jun	*Trifolium repens, Sonchus sp.*	*Trifolium repens, Sonchus sp.,* Umbelliferous plants (Parsleys), *Ranunculus sp.*	*Trifolium repens, Sonchus sp.,* Umbelliferous plants (Parsleys), *Ranunculus sp.*	*Papaver rhoeas, Ranunculus sp., Convolvulus sp.*	-	Umbelliferous plants (Parsleys), *Sonchus sp*.
27-28 Jun	*Trifolium repens*	*Trifolium repens,* Umbelliferous plants (Parsleys)	*Trifolium repens, Taraxacum officinale,* Umbelliferous plants (Parsleys)	*Papaver rhoeas, Ranunculus sp.*	-	Umbelliferous plants (Parsleys*), Sonchus sp.*
04-05 Jul	*Trifolium repens, Taraxacum officinale, Umbelliferous plants (Parsleys), Convolvolus arvensis, Sonchus sp., Anthemis sp.*	*Trifolium repens,* Umbelliferous plants (Parsleys), *Cirsium arvense, Anthemis sp., Clematis vitalba*	*Trifolium repens,* Umbelliferous plants (Parsleys), *Cirsium arvense, Urtica dioica*	*Ranunculus sp., Sonchus sp.*	-	Umbelliferous plants (Parsleys), *Sonchus sp.*

11-12 Jul	*Trifolium repens,* *Umbelliferous plants (Parsleys),* *Clematis vitalba*	*Trifolium repens,* Umbelliferous plants (Parsleys), *Cirsium arvense, Taraxacum officinale, Anthemis sp., Clematis vitalba*	*Trifolium repens,* Umbelliferous plants (Parsleys), *Cirsium arvense, Urtica dioica*	*Ranunculus sp., Sonchus sp., Taraxacum officinale, Anthemis sp.*		Umbelliferous plants (Parsleys), *Sonchus sp.*
17-18 Jul	*Trifolium repens,* Umbelliferous plants (Parsleys), *Anthemis sp., Clematis vitalba*	*Trifolium repens,* Umbelliferous plants (Parsleys), *Cirsium arvense, Taraxacum officinale, Anthemis sp.*	*Trifolium repens,* Umbelliferous plants (Parsleys), *Urtica dioica*	*Ranunculus sp., Sonchus sp., Taraxacum officinale, Anthemis sp., Trifolium repens*	*Trifolium repens*	Umbelliferous plants (Parsleys), *Sonchus sp.*
24-25 Jul	*Trifolium repens, Taraxacum officinale,* Umbelliferous plants (Parsleys), *Anthemis sp., Convolvulus arvensis, Calystegia sepium*	*Trifolium repens, Taraxacum officinale,* Umbelliferous plants (Parsleys), *Anthemis sp., Convolvulus arvensis, Calystegia sepium*	*Trifolium repens, Taraxacum officinale,* Umbelliferous plants (Parsleys), *Anthemis sp., Convolvulus arvensis, Calystegia sepium, Senecio vulgaris, Stellaria media, Galium aparine, Urtica dioica*	*Ranunculus sp., Sonchus sp., Taraxacum officinale, Anthemis sp., Trifolium repens, Veronica sp.*	*Trifolium repens, Veronica sp., Chenopodium sp., Sonchus sp.*	Umbelliferous plants (Parsleys), *Sonchus sp.*
01-02 Aug	Umbelliferous plants (Parsleys), *Anthemis sp., Sonchus sp.*	*Trifolium repens, Taraxacum officinale,* Umbelliferous plants (Parsleys), *Anthemis sp., Convolvulus arvensis, Calystegia sepium*	*Trifolium repens, Taraxacum officinale, Anthemis sp., Senecio vulgaris, Galium aparine, Urtica dioica,*	*Sonchus sp., Taraxacum officinale, Anthemis sp., Trifolium repens, Veronica sp.*	*Trifolium repens, Veronica sp., Chenopodium sp., Sonchus sp., Rumex sp.*	*Veronica sp., Chamomilla sp., Anagallis arvensis, Sonchus sp., Trifolium repens*

07-09 Aug	*Anthemis sp., Sonchus sp., Taraxacum officinale*	*Trifolium repens, Taraxacum officinale, Anthemis sp.*	*Trifolium repens, Taraxacum officinale, Urtica dioica*	*Sonchus sp., Taraxacum officinale, Anthemis sp., Trifolium repens, Veronica sp.*	*Trifolium repens, Veronica sp., Chenopodium sp., Sonchus sp., Rumex sp.*	*Veronica sp., Chamomilla sp., Anagallis arvensis, Sonchus sp., Trifolium repens*
14-16 Aug	*Anthemis sp., Sonchus sp., Taraxacum officinale, Trifolium repens*	*Trifolium repens, Taraxacum officinale*	*Trifolium repens, Taraxacum officinale, Urtica dioica*	*Sonchus sp., Taraxacum officinale, Anthemis sp., Trifolium repens, Veronica sp.*	*Trifolium repens, Veronica sp., Chenopodium sp., Sonchus sp., Rumex sp.*	*Veronica sp., Chamomilla sp., Anagallis arvensis, Sonchus sp., Trifolium repens*
24-23 Aug	*Taraxacum officinale,* Umbelliferous plants (Parsleys), *Ranunculus repens*	*Trifolium repens, Taraxacum officinale, Rumex sp., Polygonum aviculare, Stellaria media,* Umbelliferous plants (Parsleys)	*Taraxacum officinale, Senecio vulgaris, Chenopodium album*	*Sonchus sp., Taraxacum officinale, Anthemis sp., Trifolium repens, Veronica sp.*	*Trifolium repens, Veronica sp., Chenopodium sp., Sonchus sp., Rumex sp.*	*Veronica sp., Chamomilla sp., Anagallis arvensis, Sonchus sp., Trifolium repens*
29-30 Aug	*Taraxacum officinale,* Umbelliferous plants (Parsleys), *Clematis vitalba, Trifolium repens, Cirsium arvense, Hedera helix*	*Trifolium repens, Taraxacum officinale, Rumex sp., Senecio vulgaris, Malva neglecta, Polygonum aviculare, Stellaria media,* Umbelliferous plants (Parsleys)	*Trifolium repens, Taraxacum officinale, Senecio vulgaris, Rumex sp., Chenopodium album, Urtica dioica,* Umbelliferous plants (Parsleys)	*Sonchus sp., Taraxacum officinale, Anthemis sp., Trifolium repens, Veronica sp.*	*Trifolium repens, Veronica sp., Chenopodium sp., Sonchus sp., Rumex sp., Polygonum aviculare, Senecio vulgaris*	*Veronica sp., Chamomilla sp., Anagallis arvensis, Sonchus sp., Trifolium repens, Polygonum aviculare*

APPENDIX. 5.1.3a. Meteorological records of the June bearer case study plantation.



APPENDIX. 5.3b. Meteorological records of the ever-bearer case study plantation.