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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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CONTENTS

Contents

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 four 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.

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

Headline

- Advances in monitoring western flower thrips and *Neoseiulus cucumeris* in strawberry crops have been made.

Background and expected deliverables

At present, growers rely on introductions of the predatory mite *Neoseiulus cucumeris* (formerly called *Amblyseius cucumeris*) to control WFT. It is relatively inexpensive to release and can be introduced in large numbers. However, *N. cucumeris* only predates first-instar WFT larvae. Biocontrol with *Neoseiulus cucumeris* sometimes fails. The reasons for failure are not well understood but are thought to be caused by insufficiently early or frequent introductions, poor predator viability and/or adverse effects of crop protection programmes. For effective biocontrol, a high proportion of flowers must contain *N. cucumeris*. It is difficult to assess whether *N. cucumeris* populations have established adequately and whether they are in balance with their prey and so developing grower-friendly methods for estimating WFT and *N. cucumeris* predator-prey ratio thresholds in relation to fruit damage would be very useful.

In the first year of the project, different flower and fruit stages were assessed to determine which plant parts should be used to improve sampling strategies. Fruit consistently had higher numbers of *N. cucumeris* than any flower stages, so button fruit were chosen as the stage to be used for assessing numbers of *N. cucumeris*. Methyl isobutyl ketone 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. A prototype monitoring device making use of this fumigant extraction method was constructed and initial experiments showed promise, but further testing is necessary.

Data were collected from commercial crops where *N. cucumeris* had been released by the growers. Results of the occurrence of *N. cucumeris* and WFT in individual flowers and button fruit indicated aggregation for both species. Results from this analysis have been used to develop a prototype model to estimate the maximum mean number of WFT in a sample of a given size to ensure the probability of low levels of fruit damage. However, these data did not

cover a sufficiently large range of WFT and *N. cucumeris* densities and more data (with a wider range of thrips and predatory mite numbers) are needed to develop a more reliable model.

Strawberry crops need a second line of defence against WFT, such as curative spray treatments of entomopathogenic fungi (EPF). For effective control of a target pest, spores of an EPF strain have to adhere to the pest's cuticle, then germinate and penetrate the cuticle to cause mycosis. Efficacy requires an adequate number of spores to adhere in vulnerable parts of the body, then adequate high humidity and temperature for a sufficient period for spore germination and infection. Mortality occurs after a few days, but insects stop feeding, moving and reproducing well before death. Unfortunately, grower experience with spraying EPFs for controlling thrips in strawberries has been disappointing. In the first year, results from bioassays showed some promise for the use of EPF for WFT control within an IPM system, if application and spore retention are good. In the first year experiments to determine if the efficacy of entomopathogenic fungi to control WFT can be improved, three adjuvants were tested in conjunction with the EPF Naturalis L in laboratory bioassays and replicated field experiments. Effects on WFT mortality and on spore deposition, both on the treated surface and on treated thrips, were assessed. WFT mortality was low in these experiments. However improvements to bioassay techniques have been made during 2016 which were used in future assays. No significant difference in deposition/retention of spores could be identified between adjuvants following spraying. However, significantly higher deposition/retention was observed on flowers compared to leaves in all treatments.

Summary of the project and main conclusions

Extraction device for determining predator-prey ratios

An experiment was set up to derive predator and prey data for inclusion in the model to predict likely damage scenarios with particular ratios of *N. cucumeris* to WFT. However, despite three releases of 100 *N. cucumeris* per plant very few mites were found on the plants after release. The very low numbers of *N. cucumeris* recorded after high rates of application were unexpected and this requires further investigation to understand the dispersion of predators on plants after predator release. Since very few *N. cucumeris* were recorded on the button fruit or flowers, it was not possible to develop the modelling aspect of the project further in 2016.

However, the use of methyl isobutyl ketone in a prototype extraction device was effective at removing *N. cucumeris* and thrips adults and larvae from plant material. Field assessment of the device is now needed.

Control of WFT using entomopathogenic fungi

The new EPF formulation of Met52 OD (Fargro), which is recommended for use as a foliar spray, was tested in a laboratory bioassay against adult female WFT using a direct dosing method. The concentration 1.25L in 300L water equates to the aim of depositing 250 spores per mm^2 in the field for an effective dose to be applied. Results from bioassays show some promise for the use of EPF for WFT control within an IPM system, if application and spore retention are good. There were two experiments, similar in their methodology: in the first experiment there was 44% higher WFT mortality after 6 days at the highest label dose compared to the untreated control. In the second experiment there was over 40% WFT mortality after 6 days and nearly 70% mortality after 8 days, at the highest label dose, compared to the untreated control. However, it should be noted that there was around 40% WFT mortality in a blank oil control.

Financial benefits

Western flower thrips, *Frankliniella occidentalis* (WFT), causes bronzing of fruit and 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. 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.
- Sample mid-aged flowers to determine thrips numbers in the crop.
- The new EPF formulation, Met52 OD (Fargro) is available in 2017 for use as a foliar spray against WFT in strawberry.
- Consider reducing the number of repeated applications of tank mixes of plant protection products as these may be harmful to introduced *N. cucumeris*.

Integrating pesticides with phytoseiid mites

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

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. Also, increased use of plant protection products against other pests, such as SWD, can potentially interfere with IPM. In addition, 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 we 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.

Summary of the project and main conclusions

For effective biocontrol of WFT with *N. cucumeris*, crop protection products safe to the predator need to be integrated into the overall management programme. Some compounds that are regarded as relatively safe for predatory mites, may be applied multiple times, and combined in tank mixes, where they may act additively or synergistically against the predator. In Year 2 we 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 is not clear.

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 control is achieved early before SWD enters the crop and requires insecticide treatments.

Potato aphid

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

Headline

- Good spray coverage of strawberry crops is required in order to achieve effective control of potato aphid, *Macrosiphum euphorbiae*, in spring when plants are relatively compact.

Background and expected deliverables

Several species of aphid are regularly found affecting 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 which may distort plants, contaminate fruits with honeydew and sooty moulds (e.g. *Aphis gossypii* and *Macrosiphum euphorbiae*) and vector viruses, such as mottle virus (e.g. *C. fragaefolii* and *A. gossypii*). Aphicide 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 products.

The Defra HortLINK project HL0191 (SF 94) demonstrated that product applications in the autumn may effectively reduce numbers of potato aphid on the crop the following spring. It is, however, not always possible to time applications in the autumn and so product applications in the spring may be required. There is a need to identify which products would be more effective under cooler spring temperatures before crops have begun to grow and when the canopy is still relatively compact.

In recent years growers have reported increasing problems in controlling the potato aphid, *M. euphorbiae*. Difficulty in controlling this aphid pest appears to be linked to the need for good spray coverage (AHDB Horticulture project SF 140). This problem is being exacerbated by the strawberry growing season being bought forward and extended by protected cropping with crops under fleece and tunnels and a reducing range of products available, with recent withdrawals of chlorpyrifos and pirimicarb.

Summary of the project and main conclusions

Two experiments to investigate the improvement of potato aphid control were completed; Experiment 1 was done in a ventilated research polytunnel. The experiment was a randomised block design with 5 replicates of each treatment (Table 1) including an adjuvant only and an untreated control. Each replicate consisted of a single potted strawberry (*Fragaria*

x *ananassa*) plant (cv. Driscolls Diamond). All plants used were infested with potato aphids. Products were applied using an air assisted knapsack sprayer using a water volume of 1,000 l/ha.

Table 1. Treatments

Treat No.	Product	Active ingredient	Product dose (/ha)	HI	EAMU Approval
1	Hallmark with zeon technology 100g/l CS	lambda-cyhalothrin	0.075 l	3 d	1705/11
2	Hallmark with zeon technology 100g/l CS + Silwet L-77	lambda-cyhalothrin + trisiloxane ethoxylate	0.075 l 0.25 l		*
3	Calypso	thiaclorprid	0.250 l	3 d	2132/14
4	Calypso + Silwet L-77	thiacloprid + trisiloxane ethoxylate	0.250 l 0.25 ml		*
5	Chess 50% w/w WG	pymetrozine	0.400 kg	3 d	0504/07
6	Chess 50% w/w WG + Silwet L-77	pymetrozine + trisiloxane ethoxylate	0.400 kg 0.25 ml		*
7	Silwet L-77	trisiloxane ethoxylate	0.25 ml	-	-
8	Water control	-		-	-

*Note that strawberry crops are not permitted to be sprayed at full label rates when applied together with Silwet L-77 and should instead be sprayed at 50% of the full label rate.

Analysis of data for all assessments and additional comparisons between the separate treatments indicated that the treatments could be separated into three groups based on product efficacy. ‘Group A’ gave 100% control (Hallmark and Hallmark + Silwet), ‘Group B’ (Calypso and Calypso + Silwet) gave moderate control initially (approx. 75% reduction in aphids numbers three days after spray application) but aphid numbers started to increase again eight days after spray application and ‘Group C’ (Chess, Chess + Silwet, Silwet and the water control) gave no control . No significant difference was found between Chess and Chess + Silwet when compared with Silwet or the water control. Where complete control was not achieved there was evidence that a greater proportion of aphids in the crown of the plant survived the spray application than aphids on other parts of the plant.

Experiment 2 was done in controlled environment rooms and was a fully randomized experiment with 5 replicates of each treatment (Table 1) including a Silwet applied on its own, a water control and an untreated control. Each replicate consisted of a single aphid infested strawberry leaf (cv. Elsanta). In order to validate results from Experiment 1 and to determine the importance of spray coverage this experiment was divided into two bioassays. In the first bioassay, uninfested fully expanded strawberry leaves were sprayed on both surfaces to run-off and allowed to dry by placing the leaves on several layers of tissue paper before infesting each leaf with 20 potato aphid nymphs (1-3 instar). The second bioassay was prepared in the same way; however, leaves were infested with 20 potato aphid nymphs before spraying to run-off and allowing to dry. After spraying, the petioles of the leaves were wrapped in damp tissue paper and leaves were placed separately in filter paper lined Petri dishes (90 mm diameter). Each leaf was maintained in a Petri dish in a controlled environment room set to 20^{o}C and 60% RH.

The treatments Calypso, Calypso + Silwet, Hallmark and Hallmark + Silwet killed all aphids regardless of whether the aphids were directly sprayed or placed onto a leaf that had already been sprayed. Hallmark and Hallmark + Silwet gave 100% kill within 24 hours in both cases whereas Calypso and Calypso + Silwet gave 100% kill within 24 hours only when aphids were directly sprayed. Chess + Silwet and Silwet applied on its own killed all aphids but only when aphids were directly sprayed. Chess applied without Silwet did not kill all aphids when aphids were directly sprayed or placed onto a leaf that had already been sprayed. Aphid mortality on leaves sprayed with water or left untreated was low.

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 p.a.. 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 aphicides 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 to determine the need for aphicide sprays.
- Where spring applications 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 such as the melon and cotton aphid (*Aphis gossypii*) have developed aphicide resistance. Growers should ensure that they follow resistance management guidelines on the product label and rotate between products with different modes of action.
- It is important to carefully consider the compatibility of the available product options with aphid natural enemies as well as the biological control programmes used to control other pests of strawberry crops.

SCIENCE SECTION

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

Task 1.1.2. Development of N. cucumeris:WFT predator:prey ratios required for effective biocontrol at different temperatures to avoid fruit damage, for use by growers/agronomists

Introduction

Earlier projects have shown that *Neoseiulus cucumeris* can control WFT effectively. The reasons for control failures are not fully understood but are thought to be often due to inadequate numbers being released early in the season, infrequent releases, poor establishment due to use of incompatible pesticides and an uneven distribution of predators on the plants. Currently it is difficult to assess whether *N. cucumeris* populations have established in the crop and whether they are effectively regulating their prey. It is crucial to develop grower-friendly methods for estimating WFT and *N. cucumeris* populations in relation to fruit damage, and to develop attendant predator-prey thresholds to ensure satisfactory biocontrol and to avoid fruit damage.

Effect of predator: prey ratios on subsequent fruit damage

A preliminary analysis of *N. cucumeris* and WFT distribution was completed in 2015. Data were collected from commercial crops where *N. cucumeris* had been released by the growers. For nearly all samples with mean counts of WFT (adults or larvae) and *N. cucumeris* >0.5 per flower or button fruit, results indicated aggregation for both WFT and *N. cucumeris*. Results from this analysis have been used to develop a prototype model to estimate the maximum mean number of WFT in a sample of a given size to ensure the probability of only 5% fruit having numbers that could cause damage. However, these data did not cover a sufficiently large range of WFT and *N. cucumeris* densities and most flowers or button fruit did not have thrips or mites present. More data (with a wider range of thrips and predatory mite numbers) were needed to develop a more reliable model. The aim of the research in 2016 was to determine the effects of different predator:pest ratios to avoid high populations of WFT developing that might pose a risk of fruit damage, and to use these data to develop the model further.

Methods

Experimental design: An experiment was set up at Rocks Farm, East Malling in a purpose planted plot. Six plots, measuring 6 m wide and 8 m in length, each with three raised beds covered with polythene mulch, were planted with bare rooted strawberries (var. Capri) on 5 May 2016. Polytunnels were erected over these plots. Strawberries were planted 0.5 m apart in the row with two staggered rows per bed (100 plants per plot). Each tunnel was separated from the others by at least 6 m and was provided with automatic irrigation and fertigation. Weed cover on the ground between the tunnels was minimised by mowing. Plants were de-blossomed on 27 May and tunnels erected on 6 June. The East Malling standard commercial fungicide programme was applied to all plants throughout the season, using only products recorded as safe for predatory mites (see Appendix 1 for details). No insecticides were applied except where mentioned below. The experiment was designed to obtain distribution data for *N. cucumeris* and WFT to add to data collected in 2015 for the modelling work.

WFT release: Initial samples from the planting showed that there were no, or very low numbers of, WFT present on 27 June; but very low numbers of other thrips species were recorded. An application of Tracer (spinosad) was made to remove these other species WFT were then released onto each plant. To ensure sufficient WFT were released, a sample was taken on 28 June from a commercial site that was having problems with WFT control, to assess WFT numbers. Subsequently this commercial planting was used as a source of WFT for the experiment; this population of WFT was resistant to Tracer. Flowers were collected and used to infest the experimental plants; a replicated bulk sample of flowers was assessed to estimate the total number of WFT released per plant. Mean numbers of WFT per flower recorded from these bulk samples was 8 larvae and 11 adults. One flower was pushed into the crown of each plant in all plots on 1 July; thus an average of c. 20 WFT were released per plant. Subsequent bulk samples taken from the experimental plot before *N. cucumeris* were released showed that WFT had established; on 5 July there was a mean of 1 larva and 5 adults per flower and on 11 July a mean of 4 larvae and 1 adult per flower. WFT were also present on the button fruit; on 5 July there was a mean of 0.7 larvae and adults and on 11 July 3 larvae and 0.3 adults per button fruit. Replicate data loggers, recording temperature and humidity, were placed in tunnels at the time of thrips release. After sampling (Table 1.1) it was observed that several species of thrips were present on the plants. An application of Tracer (spinosad) was applied on 16 August to reduce numbers of non-WFT present (WFT are resistant to Tracer and so survived the application). Although Tracer can be harmful to *N. cucumeris* for short periods of time (<1 week) it can be successfully integrated into thrips management strategies; results with multiple applications in the first year of this project showed no reduction in populations compared with control treatments.

N. cucumeris release: *N. cucumeris* were released from loose commercial product provided in bran with *Tyrophagous putrescentiae* prey mites as food. The volume of bran carrier required to obtain the required dose was estimated in the lab by counting mites in replicate 1 ml sub samples of the product. The required volume of carrier for each estimated release rate was then calculated. This volume was sprinkled over each plant in the relevant plot; the product was well mixed by rotating the containers before dispensing. Release rates of 4, 25 and 100 mites per plant were agreed. Each rate was applied to two plots on 20 July. This range was chosen to enable us to obtain a range of predator:prey ratios over time. After assessment of intermediate bulk samples it was clear that *N. cucumeris* were present at very low numbers in the flowers and button fruit even in the highest initial release rate. It was decided to release *N. cucumeris,* again, on 19 August, and subsequently for a third time on 24 August (Table 1.1). In the second and third releases the low rate of release was increased to 10 per plant.

Assessments: The main samples consisted initially of 20 individual 'button' fruit and 20 mid aged flowers from the same plant placed into 70% alcohol from each tunnel (i.e. 40 flowers and fruits from each release rate of *N. cucumeris*). After initial assessments of sub samples from these collections, due to the low numbers of *N. cucumeris* recorded in the crop, the number of units assessed was reduced to 10 flowers and 10 fruit from the same plant in each sample (i.e. 20 flowers and fruits from each release rate of *N. cucumeris*).

Photograph 1. Pictures showing typical button fruits. Some senescing petals may be visible on some fruits

Numbers of *N. cucumeris* and WFT in each sampling unit were counted in the laboratory using our standard ethanol plant washing protocol. Several 'bulk' samples were also taken to determine establishment of WFT and *N. cucumeris*. Details are given in Table 1.1. At the time the main samples were taken, 20 just-open flowers per tunnel were tagged with the date. The aim was that these flowers should be, where possible, from the same plants that the flower and button fruit samples were taken from. These fruit were inspected at the white fruit stage

to assess any thrips damage to the developing fruits; the number of seeds surrounded by bronzing on each tagged fruit was recorded. On each main sampling occasion the number of flowers, button fruit, green fruit, white fruit and red fruit were recorded on 10 plants in each plot.

Table 1.1. Timetable of experiment

Date	**Activity**
27 June	Bulk samples of 2 x 10 flowers and 2 x 10 button fruit assessed before WFT release
30 June	Tracer (spinosad) applied to all plots to reduce numbers of all thrips species except WFT
1 July	WFT released in flowers from commercial site
5 July	Bulk sample of flowers taken to assess WFT population establishment
11 July	Bulk sample of flowers taken to assess WFT population establishment
20 July	Release of *N. cucumeris*. Rates of 4, 25 or 100 per plant; each rate in 2 tunnels
28 July	First sampling of individual flowers and individual button fruits from each tunnel
4 August	Second sampling of individual flowers and individual button fruits from each tunnel
16 August	Tracer (spinosad) applied to all plots to reduce numbers of all thrips species except WFT
19 August	Bulk sample of 10 flowers taken from each treatment to determine if WFT is main species present
19 August	Second release of *N. cucumeris*. Released at 10 (increased from 4 for first release), 25 and 100 per plant
22 August	Bulk sample of 10 button fruit from each treatment to assess success of second *N. cucumeris* release
24 August	Third *N. cucumeris* release at 10, 25 and 100 per plant as for second release
25 August	Third full sample taken 1 day after *N. cucumeris* release
31 August	Fourth full sample taken

Analysis: This experiment was set up to obtain data for the model to establish the predator prey ratios required to minimise fruit damage, therefore no statistical analysis was possible of the effects of different release rates of *N. cucumeris* on thrips numbers or fruit damage. Mean numbers and the standard error of the mean of *N. cucumeris* and thrips from 20 flowers or button fruit were calculated and are shown in Figures 1-5 to give an overview of the results.

Results

Despite three releases of *N. cucumeris* over the course of a month (20 July-24 August) very few *N. cucumeris* were recorded in the button fruits assessed (Figure 1.1). Eight days after the first release there was a mean of 0.7 mites per button fruit at the high release rate (100 per plant), and lower numbers at the other two rates. Before the second release, numbers had declined to a mean of 0.3 per fruit in the high rate release. The subsequent two releases in August did not result in higher numbers of *N. cucumeris* on the fruit. *N. cucumeris* numbers in flowers were lower than in button fruit; there were a total of 11, 2, 11 and 2 mites in all samples (80 flowers) on the four assessment dates.

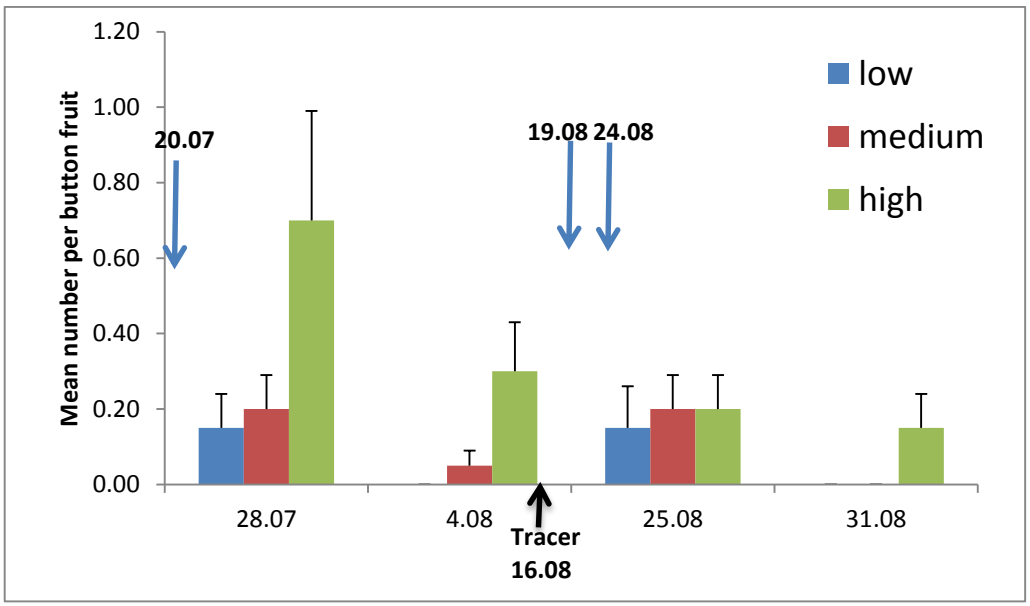


Figure 1.1. Mean number of *N. cucumeris* on individual button fruit after releases at three rates onto the plants. Release dates are shown with arrows. (*N. cucumeris* release rate: low=4 on first release and 10 on subsequent releases; medium=25 and high=100 per plant)

As was seen in Year 1 sampling programmes, thrips larvae were present on the button fruit (Figure 2), so prey was available for *N. cucumeris.* Numbers of thrips larvae were higher in the flowers than the button fruit at the end of August (Figures 1.2 & 1.3). Numbers of adult

thrips were higher in flowers than button fruit (Figures 1.4 & 1.5) with numbers in flowers ranging from a mean of 3 to 12 per flower (Figure 1.5).

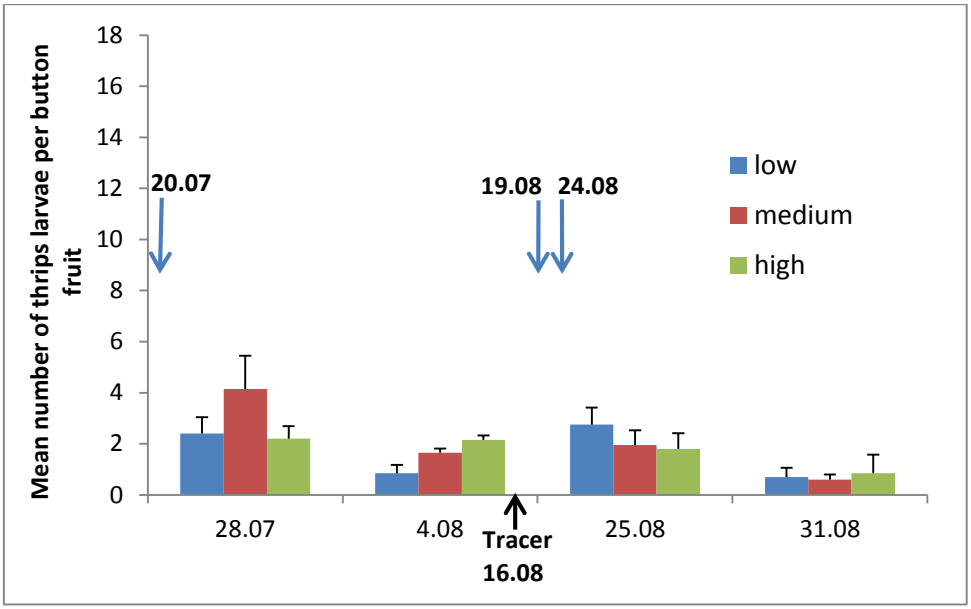


Figure 1.2. Mean number of thrips larvae on button fruit during the experiment in treatments where *N. cucumeris* were released at different rates (low=4 on first release and 10 on subsequent releases; medium=25 and high=100 per plant). *N. cucumeris* (above) and Tracer (below) application dates shown with arrows

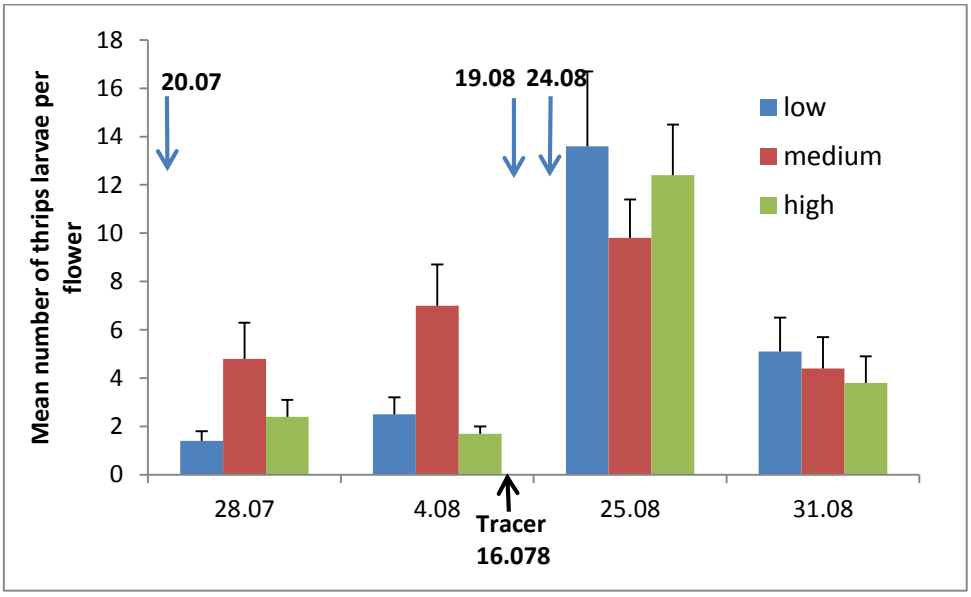


Figure 1.3. Mean number of thrips larvae on flowers during the experiment in treatments where *N. cucumeris* were released at different rates (low=4 on first release and 10 on subsequent releases; medium=25 and high=100 per plant. *N. cucumeris* (above) and Tracer (below) application dates shown with arrows

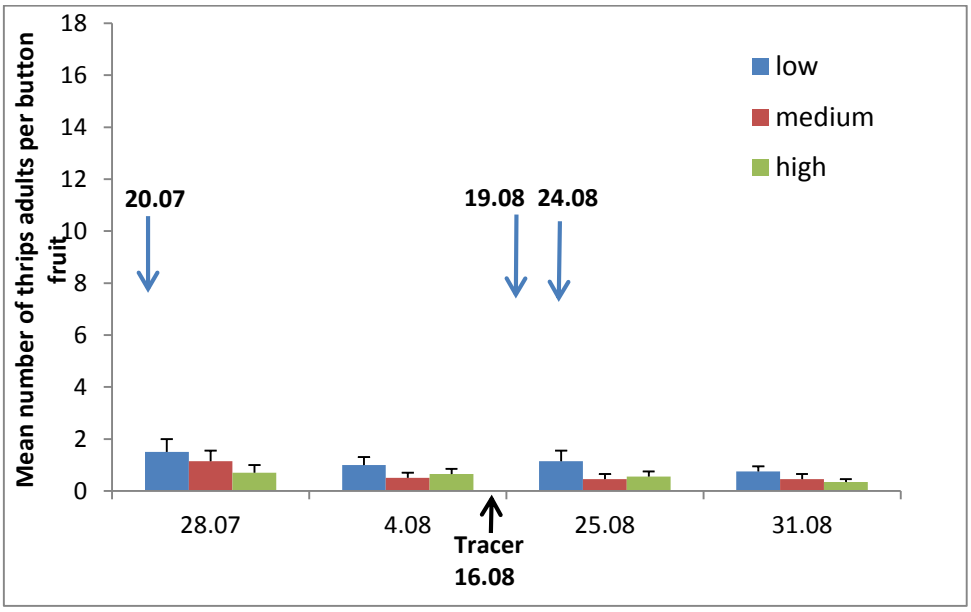


Figure 1.4. Mean number of thrips adults per button fruit during the experiment in treatments where *N. cucumeris* were released at different rates (low=4 on first release and 10 on subsequent releases; medium=25 and high=100 per plant). *N. cucumeris* (above) and Tracer (below) application dates shown with arrows

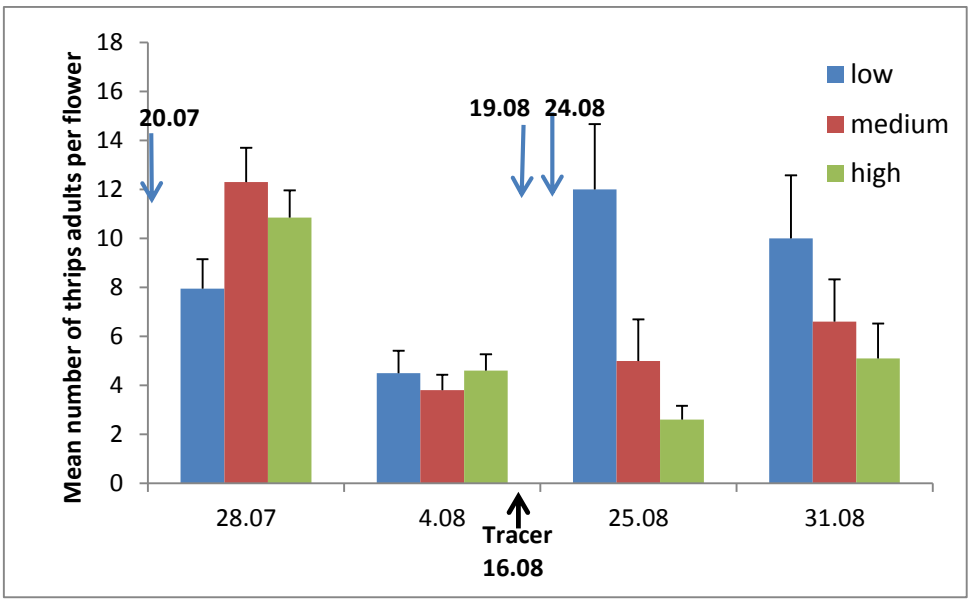


Figure 1.5. Mean number of thrips adults per flower during the experiment in treatments where *N. cucumeris* were released at different rates (low=4 on first release and 10 on subsequent releases; medium=25 and high=100 per plant). *N. cucumeris* (above) and Tracer (below) application dates shown with arrows

The very low numbers of *N. cucumeris* recorded after high rates of application were unexpected and this requires further investigation. Three applications of 100 *N. cucumeris* per plant were applied at the highest rate; current commercial recommendations vary but range from 50-250 per m^2. If strawberries are planted in double rows at 0.3 m spacing this is equivalent to around 50 mites per plant so the highest rate used was twice that recommended. In addition, growers in the West Midlands are advised to use rates of 25 per plant every 2 weeks if they are in a low risk area and 50 per plant in high risk situations (Robert Irving pers comm.). The aim of this experiment was not to obtain good biocontrol of WFT, but to obtain plants with different ratios of pest to predator to develop the model to predict population development and potential crop damage, so the releases were not made as early or as frequently as would be done to control the pest.

Fungicides were applied routinely to the planting and it is possible that these may have had some effect on *N. cucumeris* numbers. Most of the fungicides were applied before the beginning of the experiment so are unlikely to have affected mite numbers (Appendix 1 and Figure 1.1). In the first post-release sample, although mite numbers were low there were more recorded in the plots where the high release had been made (Figure 1.1). Fungicides applied after this sample and before the second sample (where mite numbers had decreased further) were Amistar, Systhane and Teldor. Work in Year 1 of this project suggested that tank mixes of Signum and Systhane or Nimrod and Teldor (fungicides that are considered safe to predatory mites when applied separately) were damaging to *N. cucumeris*, although results reported in the current report found no deleterious effects of 3 applications of tank mixes of Nimrod and Teldor. The Tracer application made on 16 August to remove any other species of thrips may have affected *N. cucumeris* populations present at the time of application but two releases of the predator were made after this, the second application was made eight days after the insecticide was applied. Studies by Rahman *et al* (2011) suggest that the threshold for residual activity of Tracer (time at which 25% of mites tested on the residues would die) for *N. cucumeris* is three days and that the residues would be safe for releases of mites after six days. Other workers (such as Van Driesche et al 2006) suggest a shorter persistence for Tracer; in this case residues only two hours old were not toxic to *N. cucumeris*. In the current report, no significant effect of Tracer on *N. cucumeris* was recorded in field experiments. It is possible that there may be other more long term effects e.g. on reproduction.

There was very little fruit bronzing recorded on the tagged fruit in any treatment in this experiment. Maximum damage was recorded on 19 September, where four out of 35 white fruits assessed had bronzing on half the surface. Fifty nine out of 120 fruits tagged on 30

August and recorded on 22 September had no visible thrips damage. Thirty five tagged flowers did not develop into fruits. Damage on remaining fruits was not high; maximum damage recorded was bronzing around 20 seeds. Only 11 fruits had bronzing around more than five seeds. This damage would not have caused downgrading of the fruit to Class 2.

The numbers of flowers and fruits on plants during the experiment are shown in Figure 1.6 and temperature data from the tunnels in Figure 1.7. Flower numbers were low on the plants in samples assessed on 25 August onwards (Figure 1.6); this may have resulted in higher numbers of thrips being recorded on the flowers that were present.

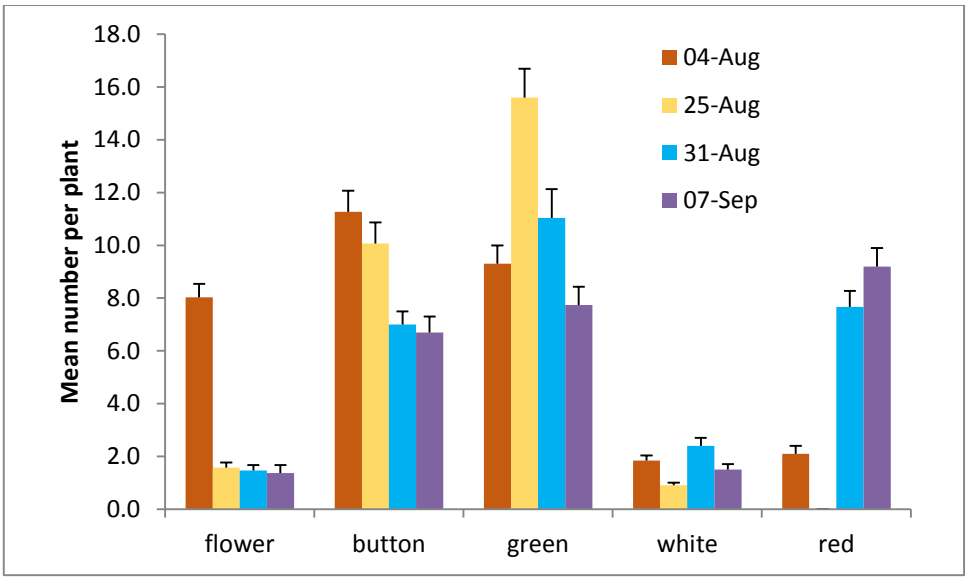


Figure 1.6. Mean number of flowers or different stage fruits on plants on four sampling dates during the experiment

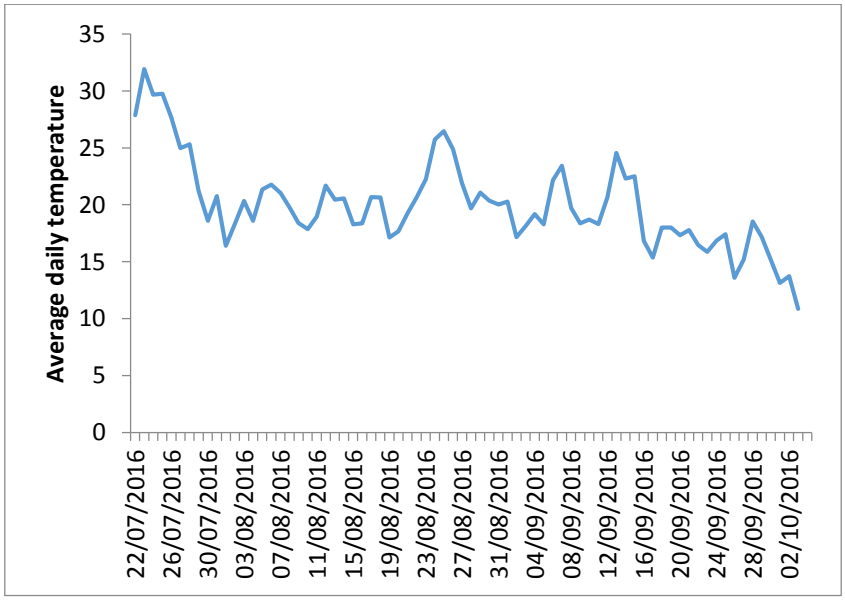


Figure 1.7. Average daily temperature (°C) in the experimental tunnels in 2016

References

Rahman, T., Spafford, H., Broughton, S. (2011). Compatibility of spinosad with predaceous mites (Acari) used to control *Frankliniella occidentalis* (Pergande) (Tysanoptera: Thripidae). Pest Management Science 67 (8) 993-1003.

Van Driesche, R.G., Lyon, S., Nunn, C. (2006). Compatibility of spinosad with predacious mites (Acari: Phytoseiidae) used to control western flower thrips (Thysanoptera: Thripidae) in greenhouse crops. Florida Entomologist 89 (3) 396-401.

Modelling the distribution of predator and prey on plants to develop damage thresholds

The data collected above was used to further develop the model. Since very few *N. cucumeris* were recorded on the button fruit or flowers it was not possible to develop the predator distribution modelling further.

There were higher numbers of total thrips (adults plus larvae) on flowers than on fruit and the number of thrips on individual flowers or button fruit was highly skewed (Figure 1.8), with high numbers of WFT (>60) on a few flowers, while most flowers had less than 20. The distribution of thrips followed a negative binomial model as was seen from the 2015 data; the estimated aggregation (dispersion) parameter of the negative binomial distribution was similar between the two years. This supports the use of a negative binomial distribution for thrips in designing sampling schemes for the pest.

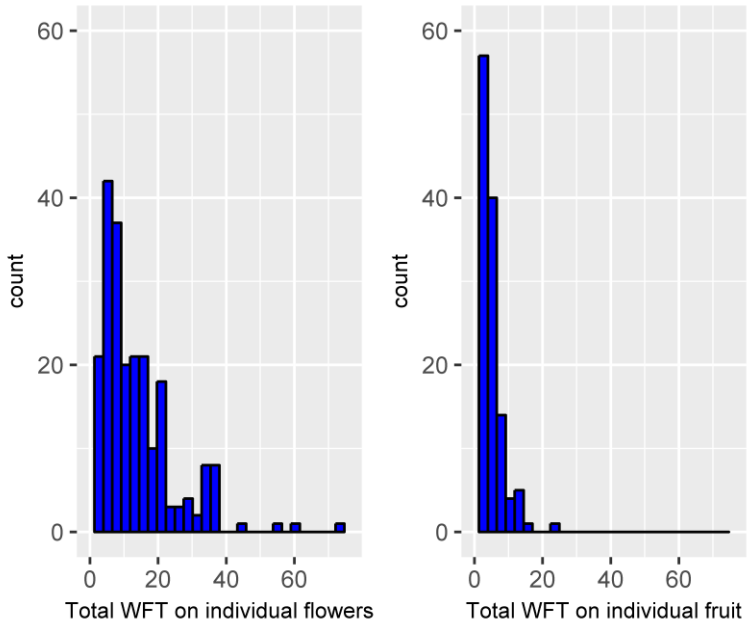


Figure 1.8. Distribution of WFT (larvae and adults) on individual flowers and button fruit in the experimental plot in 2016.

Development of field extraction device for thrips and N. cucumeris

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 2015 Annual Report). A prototype monitoring device making use of this fumigant extraction method was constructed and initial experiments showed promise. In 2016, lab studies assessed the efficacy of the device in extracting thrips and *N. cucumeris* from flowers and fruit.

Methods

The efficacy of the method was assessed using individual chrysanthemum and strawberry flowers and strawberry button fruit (10 replicates of each species/stage). Ten *N. cucumeris* adults, WFT larvae or adults were transferred onto the plant material in the extraction device. The fumigant, adsorbed on a cigarette filter, was then placed in the device and the device closed. After 10 minutes numbers of dead arthropods seen with a hand lens on the bottom plate of the extractor were recorded. The plant material was then washed using the NIAB EMR standard ethanol washing technique (NIAB EMR SOP 780), to ensure all arthropods had been recovered.

Analysis: Mean numbers of *N. cucumeris* extracted from chrysanthemum flowers, strawberry flowers and button fruit and of thrips adults and larvae extracted from chrysanthemum flowers were compared using ANOVA.

Results

Mean efficacy for *N. cucumeris* extraction using MIK was consistently 50-60% and for thrips was around 80%. There was no significant difference in efficacy of extraction from the three flower/fruits tested for *N. cucumeris* ($P>0.05$) or between thrips adults and larvae extracted from chrysanthemum flowers ($P>0.05$). Results for *N. cucumeris* are shown graphically in Figure 1.9.

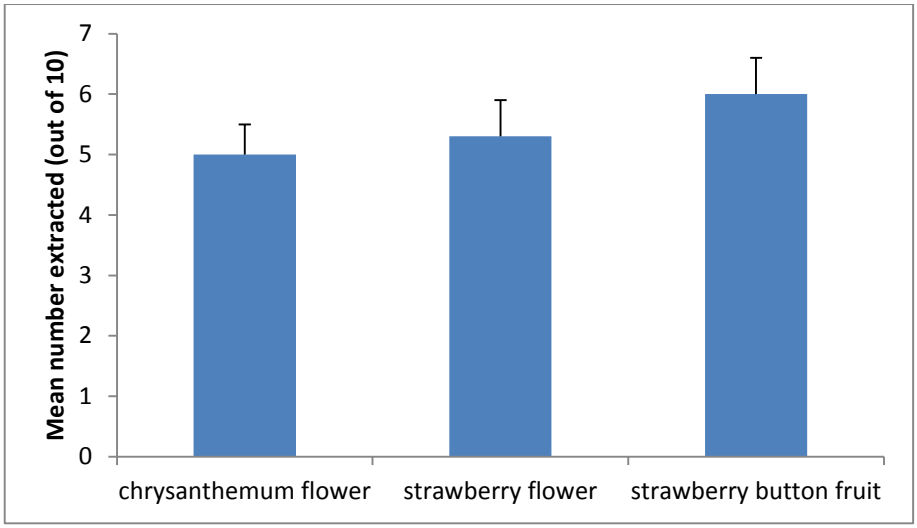


Figure 1.9. Efficacy of extraction of *N. cucumeris* from flowers and fruit using MIK

Discussion

The extraction device was effective at removing *N. cucumeris* and thrips adults and larvae from plant material.

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

Introduction

Predators and/or crop protection products are currently the major methods employed for the control of *Frankliniella occidentalis* (Western Flower Thrips, WFT) in the UK. However, the use of entomopathogenic fungi to control WFT is low. There are three existing EPF products available currently in the UK which list WFT as a target on their label: Naturalis L (Belchim), Met52 G (Fargro) and Met52 OD (Fargro). Naturalis L is a sprayable formulation containing *Beauveria bassiana* approved for control of whitefly, thrips, spider mites and capsid. Met52 G is a granular biopesticide containing *Metarhizium anisopliae* which is incorporated into growing media for control of a range of pests including thrips and vine weevil in a wide range of crops, including strawberries. Met52 OD (Fargro) is a new oil dispersion formulation containing *M. anisopliae* which is now available for use on strawberry and recommended as a foliar spray for control of adult and larval stages of WFT.

The lifecycle of the thrips means that the insect may be found in different parts of the strawberry. The adults primarily feed on flower pollen, thus are found within the flower heads. Females lay eggs on leaves underneath the epidermis or within flower tissues. The first and second instars develop on the plant then migrate, or drop, to the base of the plant, where they pupate and go through a pre-pupal and pupal stage. Adults emerge and are attracted to flowering plants, specific colours and volatiles (Cloyd, 2009). Cloyd (2009) suggested that the key to efficacy of EPFs on strawberries is to target adults which have been shown to be more susceptible to *B. bassiana* than nymphs due to different cuticular thicknesses and, as they reside in the flower heads, which tend to have a higher humidity than other plant parts, conditions are more favourable for fungal infection. The author also outlines that efficacy is related to the numbers of spores contacted by the thrips; higher spore contact/pick up leads to higher mortality. Factors which may influence the efficacy of EPFs may be temperature and humidity.

Naturalis L has had variable results in polytunnel grown strawberries and the use of additional adjuvants with this formulation was the focus of work in Year 1. In Year 2 the focus was on a sprayable *M. anisopliae* formulation. As many strawberries are grown in coir which is pre-bagged and imported from Sri Lanka it is difficult to incorporate Met52 G into the system. This would be easier with pot and tray grown strawberries; however, currently only a small percentage of strawberries are tray grown. The new Met52 OD formulation which will be

available to growers in the 2017 season is intended for use as a repeated foliar spray only. This Task will therefore focus on evaluating the efficacy of Met52 OD against WFT.

Objective: Determine an LD 50 for Met52 with Western Flower Thrips (WFT) adults.

Methods

Experiment 1

Product quality control check: A sample of Met52 was sent to CABI for QC testing on 29 June and refrigerated on arrival (CABI no. 183/16 allocated).

On 1 July 2016, a germination test was set up using 183/16 by removing a small amount of suspension in a Pasteur pipette and adding to 9 ml Shellsol T oil. The suspension was sonicated for 3 min then a small amount was spread onto 3 x 55 mm Sabouraud Dextrose Agar + Distilled Water (SDA+DW; prepared 27 June 2016) plates using a micro-spatula and incubated for 24h at 25°C. Plates were removed from the incubator and assessed for germination. A minimum of 300 conidia were counted and classed as germinated or non-germinated. Germinating conidia were counted as conidia where the germ tubes were longer than the length of the conidium itself. Percentage viability was calculated.

For colony forming unit (CFU) assessments, sample 183/16 was shaken well and three x 1 ml were removed and added to three x 9 ml sterile distilled water and mixed. However, these samples were too concentrated to form a uniform suspension, so 100 µl of formulation were added to 9 ml of sterile water and mixed (x3). Samples were sonicated for 3 min to break up any chains of conidia. From this, 1 ml of suspension was added to 9 ml of sterile water and mixed thoroughly. This was repeated to form three dilution series of suspensions down to 10^{-8}. From these vials, 200 µl was removed and spread across 2 x 90 mm Sabouraud Dextrose Agar + Tap Water (SDA+TW) plates and then incubated at 25°C for three days. After three days, the numbers of CFUs were assessed for each dilution and the CFU/ml were calculated.

Dose response assay

Treatments and application: The field rate for Met52 OD was given as 1.25 L/300-1600 L water per ha. A dilution series was prepared using the highest field concentration (1.25 L Met 52 /300 L water) then 3 x 1:1 dilutions were prepared to create a dose response curve (Table 1.2.1). Diluted Codacide oil (Microcode Ltd, Bury St Edmunds, Suffolk, UK) (emulsifiable oil) was applied at a rate to represent the quantity of oil in the strongest formulation (formulation control). A completely untreated control was also included. The experiment used a

randomised block design with 5 replicates of 6 treatments; with each block being on the same shelf of an incubator.

Where a treatment was applied, a well was made by pushing a piece of fine lens tissue (Fisher Scientific) into a plastic sample tube (2 cm diam, 6.5 cm height) into which 10 thrips females were placed, adult females are larger than males and it is therefore important to standardise by size. These were then briefly cooled, to prevent the thrips from flying on opening the tube, by placing at 4°C prior to dosing. To dose the thrips, 1 ml of formulation was applied topically using a P1000 Gilson Pipetman to the 10 WFT adults (Table 1.2.1). Due to the nature of the tissue this allowed the solution to move through the tissue to allow coverage of the thrips but prevented drowning. The thrips were then collected using a fine sable haired paintbrush (using a new brush for each treatment) and placed into a glass honey jar container (7 cm diam, 9.5 cm height) containing a moisture wick, damp filter paper and a green bean pod. The jar was closed with a plastic lid which had a 2.8 cm diam. ventilation hole covered with a thrips proof mesh. To ensure that the seal was tight and to prevent the thrips from escaping, the outer rim of the glass jar was wrapped with white PTFE tape. Bioassay containers were held in an incubator set at 25 °C and 70% RH with a 14:10 day length. Assessments of mortality were made on day 4, 6, 8 and 11.

Table 1.2.1. Treatments and amounts required for the stock suspension.

Treatment	**Met 52**	**Distilled Water**	**Codacide**
1.25 l in 300 l water/ ha (1)	417 µl	99.58 ml	0
1.25 l in 600 l water/ha (2)	50 ml Trt1 (i.e. 208.5 µl product)	50 ml	0
1.25 l in 1200 l water/ha (3)	50 ml Trt2 (i.e. 104 µl product)	50 ml	0
1.25 l in 2400 l water/ha (4)	50 ml Trt3 (i.e. 52 µl product)	50 ml	0
Diluted Codacide oil as a formulation control (5)	0	99.58 ml	417 µl
Untreated control	0	0	0

Thrips washings: An extra application was set up to assess the numbers of spores picked up by the treated thrips per treatment. Five adult female thrips were dosed as described above and transferred individually using a clean paintbrush into separate 500 µl sterile PCR tubes. Thrips were placed on an ice block and transported back to CABI where they were refrigerated until the next day. Thrips were washed using 200 µl of sterile 0.05% Tween 80.

Tubes were vortexed then sonicated for 3 min to loosen spores from the thrips cuticle. Suspensions were plated onto 90 mm ¼ potato dextrose agar+rose bengal+chloramphenicol media (PDA+RB+C) (selective media to minimise contaminant overgrowth) and incubated at 25°C for three days, after which CFUs of *M. anisopliae* were counted.

Thrips mycosis assessment: Dead thrips from assay dates 11, 13, 15 and 18 July 2016, days 4, 6, 8 and 11, were sent to CABI for surface sterilisation to check for signs of mycosis. The surface sterilisation method was as per Grundschober et al. (1998); thrips were dipped in 74% ethanol for 1 min, then 3% NaOCI (sodium hypochlorite) for 3 min and twice in sterile distilled water for 3 min each. Surface sterilised thrips were plated onto 55 mm SDA+DW plates and incubated at 20°C for 4 days. After 4 days thrips were checked for signs of *M. anisopliae* growth.

Experiment 2

Treatments and application: A new unopened bottle of MET52 OD was used for experiment 2 and the product was QC'd. As for Experiment 1, Met52 OD was used at specified rates (Table 1.2.2.). Codacide was again used as a formulation control, based on the amount of EPF product at the highest recommended field rate, i.e. 1.25 l in 300 l water per ha (rather than the highest concentration of product used in the experiment, 1.25 l in 150 l water per ha), and a completely untreated control was included. A separate bean dip treatment was also added, where two green beans per replicate were dipped into a solution at 1.25 l in 300 l water per ha and allowed to dry before placing into the bioassay containers. A randomised block design with six replicates of eight treatments was used, with each block being on the same shelf of an incubator (Table 1.2.3)

Table 1.2.2. Treatments and amounts required for the stock suspension in Experiment 2.

Treatment	Met 52 OD	Dist. Water	Codacide	Method of application
1.25 l in 150 l water per ha (1)	834 µl	99.17 ml	0	Direct
1.25 l in 300 l water per ha (2)	Serial dilution, 50 ml from above Trt (i.e. 417 µl product)	50 ml	0	Direct
1.25 l in 600 l water per ha (3)	Serial dilution, 50 ml from above Trt (i.e. 208.5 µl product)	50 ml	0	Direct
1.25 l in 1200 l water per ha (4)	Serial dilution, 50 ml from above Trt (i.e. 104 µl product)	50 ml	0	Direct
1.25 l in 2400 l water (5)	Serial dilution, 50 ml from above Trt (i.e. 52 µl product)	50 ml	0	Direct
Codacide (6)	0	99.58 ml	417 ul	Direct
Untreated (7)	0	0	0	Direct
1.25 l in 300 l water per ha (8)	417 µl	99.58 ml	0	Bean dipped and allowed to dry

The methodology was as in Experiment 1, although on dosing, 0.5 ml not 1 ml was topically applied to the 10 female WFT adults in a lens tissue (Fisher Scientific) well held in a plastic sample tube. In Experiment 2, the methodology was changed as all thrips were fully covered on application of the first 0.5 ml of treatment. The bioassay containers were as in Experiment 1, although two green beans and a drop of distilled water on parafilm were added to each container to increase the humidity and allow additional water for the insects. Thrips for the untreated and bean dip treatments were added directly to the bioassay containers. The bioassay containers were held in an incubator set at 25° C, 70% RH, with a 14:10 day length. Assessments of mortality were done on Day 4, 6, 8 and 11. Any signs of mycosis were recorded. Thrips that died were held at 4° C and sent to CABI each week, on a next day delivery.

Table 1.2.3. Randomisation plan, where each block is a separate incubator shelf.

Block	Bioassay Unit	Treatment		Block	Bioassay Unit	Treatment
1	1	5		2	1	2
1	2	8		2	2	7
1	3	3		2	3	5
1	4	1		2	4	4
1	5	4		2	5	1
1	6	6		2	6	8
1	7	7		2	7	6
1	8	2		2	8	3
3	1	5		4	1	8
3	2	6		4	2	2
3	3	8		4	3	3
3	4	7		4	4	7
3	5	3		4	5	4
3	6	4		4	6	5
3	7	1		4	7	1
3	8	2		4	8	6
5	1	1		6	1	4
5	2	5		6	2	7
5	3	7		6	3	5
5	4	4		6	4	2
5	5	3		6	5	1
5	6	8		6	6	3
5	7	2		6	7	6
5	8	6		6	8	8

Thrips washings: These were done as in Experiment 1.

Thrips mycosis: This was done as in Experiment 1; however, the sterilisation process was slightly modified as the thrips were not dipped in ethanol prior to their wash in NaOCI. This was done as the previous methodology may have been overly harsh, leading to less thrips showing positive for mycosis due to the EPF.

Analysis: Results of Experiment 1 were analysed by ANOVA. For Experiment 2, the number dead at each date was analysed using a GLM (Generalised Linear Model) with the binomial distribution and a probit link. Treatments were compared using means on the probit scale.

Dose response curves versus log(Dose) were fitted to the mortalities for each day using all treatments except Codacide and Dipped. These were fitted using maximum likelihood assuming that the mortalities were binomially distributed, using the procedure PROBITANALYSIS in Genstat.

Results

Experiment 1

Product quality control check: Results showed that CFU's per ml for Met 52 OD were 3.12 x 10^{12} ± 5.21 x 10^{11} per litre of product, compared to a label stated CFU of 2 x 10^{12}. Product viability was 74% after 24h at 25°C.

Dose response assay: The LT_{50} (time to kill 50% of thrips) was quickest for 1.25 L in 300L (between D4-6), then 1.25 L in 600L (D6), then 1.25 L in 1200 L (between D8-11) and 1.25 L in 2400 L (D11) (Figure 1.2.1). The untreated control also reached LT_{50} at D11, however was not reached with the Codacide control. LD50 at approximately five days was reached by formulation strength 1.25 L in 300 L water and six days for 1.25 L in 300 L water. LD90 was only reached by 1.25 L in 300 L. ANOVA for Day 4, 6 and 8 (including both the untreated and Codacide controls) were significant at P <0.001 (20 d.f.), with s.e.d. of 7.63, 5.89 and 7.84 respectively.

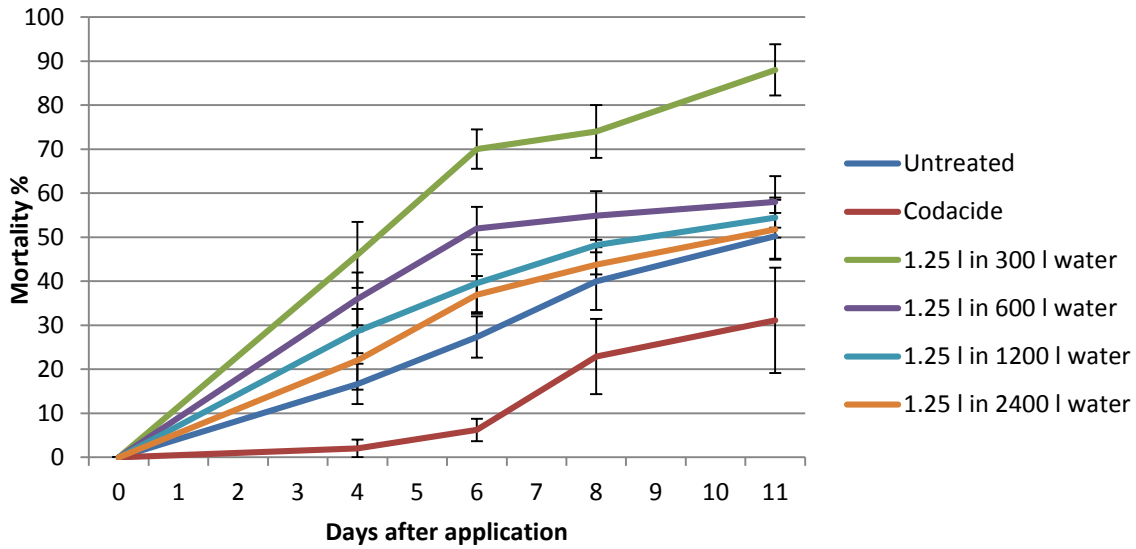


Figure 1.2.1. The effect of Met52 OD on WFT adult females (standard error of the means shown).

Thrips washings: Thrips washings showed that treatments applied different levels of spores (Figure 1.2.2), increasing with concentration; however washings per treatment had variable results.

The highest mortality was observed in the most concentrated treatment; 1.25 L in 300 L of water. According to washings of thrips this treatment deposited approximately 120 ± 61.4 spores per insect; however it should be noted that the range in CFU deposition was relatively wide for this treatment, i.e. 7 - 303 CFU per insect.

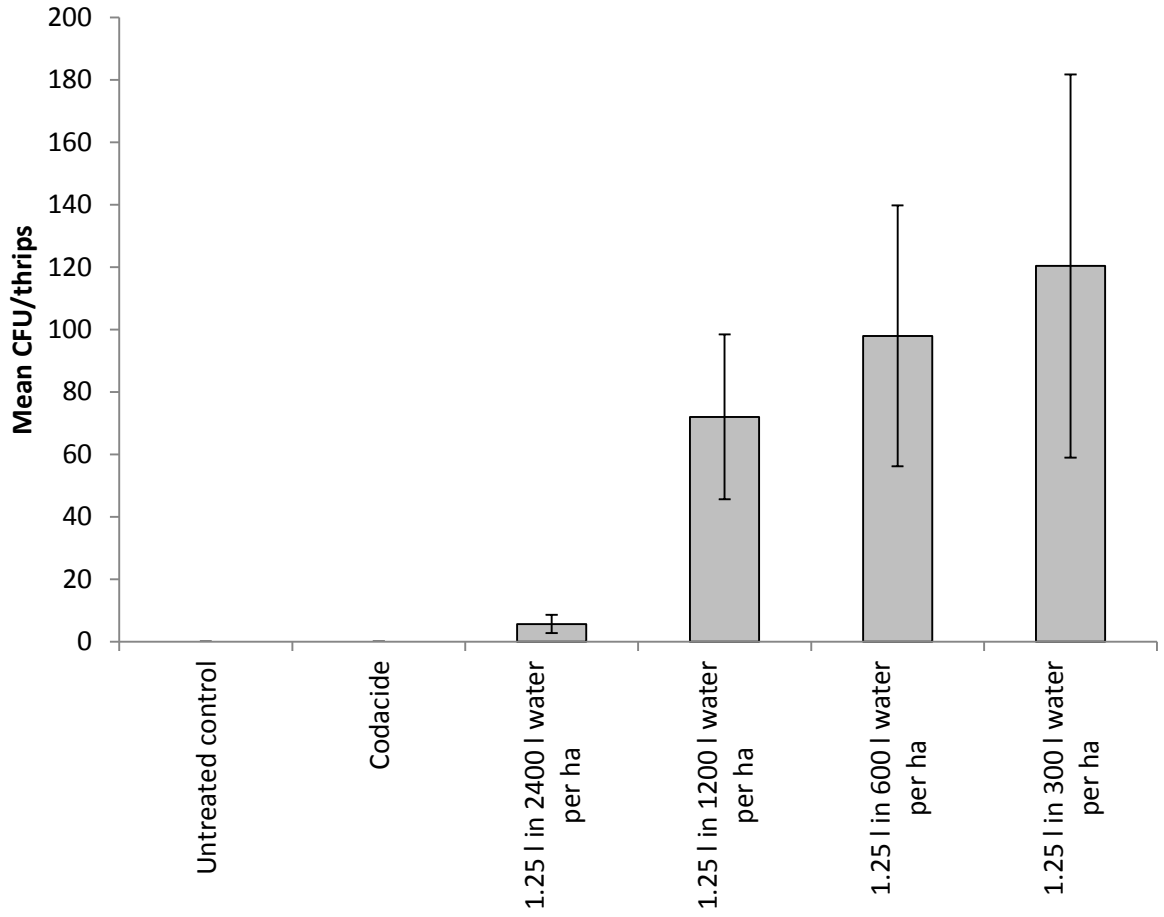


Figure 1.2.2. Mean numbers of colony forming units (CFU) washed from thrips from each treatment (n=5, ±1SE) Thrips mycosis assessment: Following surface sterilisation 16-25% of thrips treated with Met52 OD showed signs of mycosis (Figure 1.2.3.). It is not known for certain if dead thrips showing no mycosis following surface sterilisation had been killed by Met52 OD but as controls showed no mycosis it is likely that they had been. Surface sterilisation followed a published methodology. However, as thrips are so small, there is a

chance that both internal and external microorganisms were sterilised during the process leading to false negatives, therefore real mycosis levels may be higher than those observed.

For all Met52 OD treatments, CFU recovery plateaued at Day 6 with the exception of the weakest treatment (1.25 L in 2400 L) for which mycosis increased from Day 6 to Day 11; indicating, perhaps, a slow action of the fungus at the lower dose.

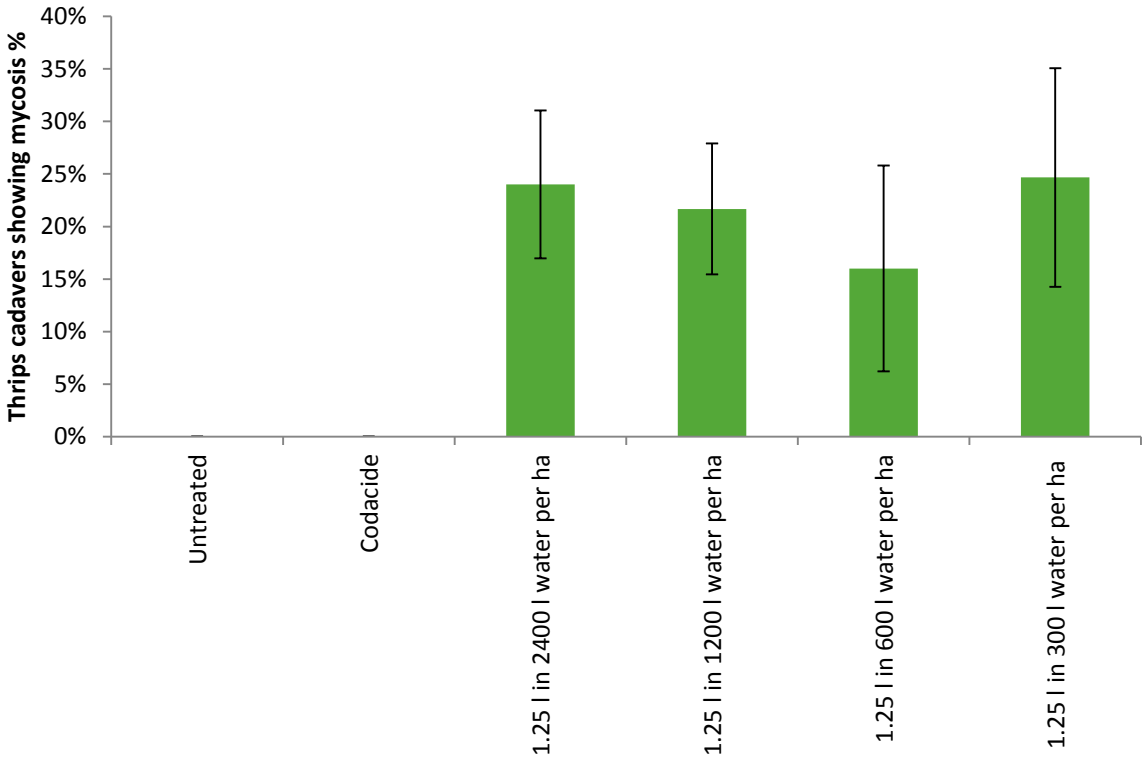


Figure 1.2.3. Percentage mycosis per treatment, as a total of numbers of dead thrips per treatment over 11 days

Experiment 2

Product quality control check: Results showed that CFU's per ml for the second bottle of Met 52 OD were $1.22 \times 10^{12} \pm 4.41 \times 10^{11}$ per litre of product, compared to a label stated CFU of 2×10^{12}. Product viability was 74% (as per Experiment 1) after 24 h at 25° C.

Dose response: The results of the dose response bioassay were analysed by probit analysis for Treatments 1-5 & 7. Codacide and the bean dip treatments are included in the mean percentage mortality (Fig. 1.2.4), this is linked to the probit analysis and therefore it is not appropriate to present SE. The significance of treatment comparisons is shown in Table 1.2.4. The control mortality increased significantly on Day 11, so mortality from Day 4 – 8 was used when assessing product efficacy. At the highest field rate concentration (Trt 2) 50 % mortality was reached by Day 6 compared to an untreated control mortality of 6 %.

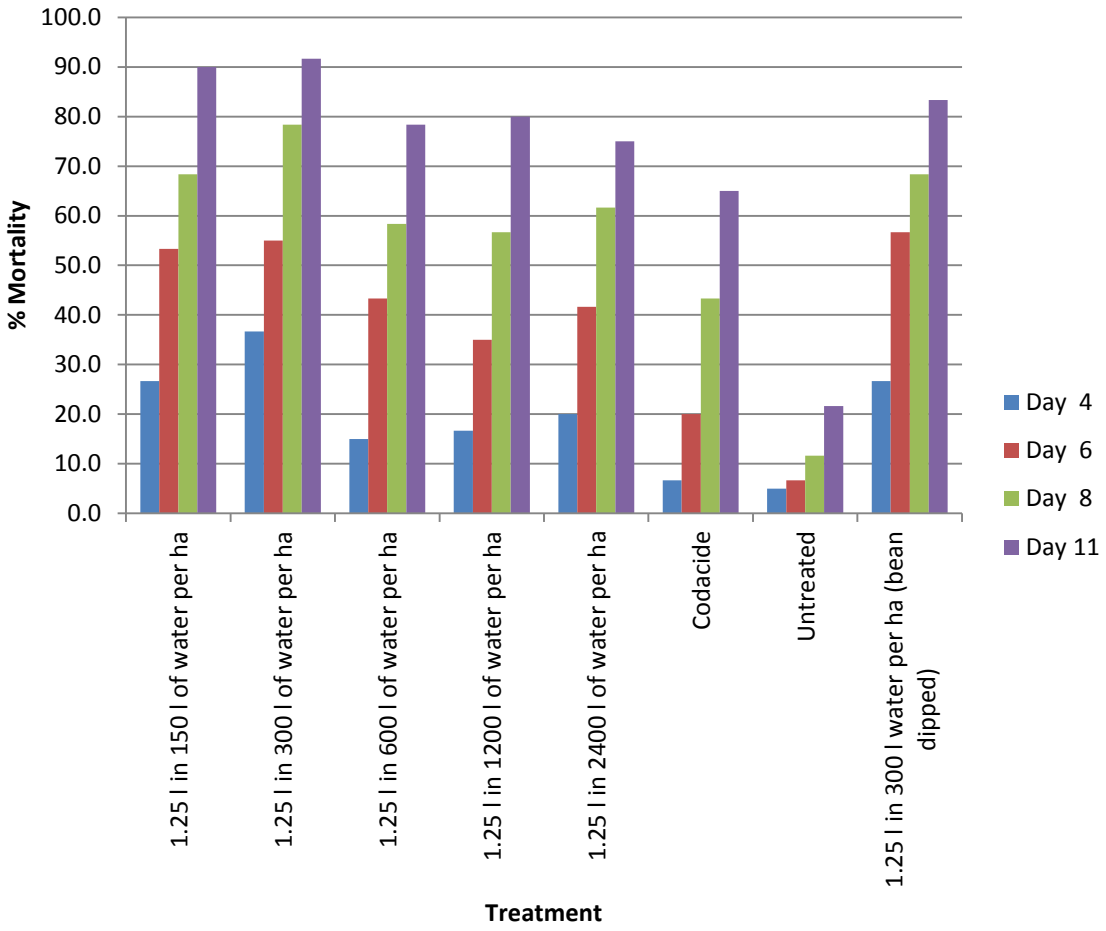


Figure 1.2.4. The mean % mortality of adult female WFT. Treatment values are amounts of Met52 OD, or Codacide where stated. Method of application was by direct application of 0.5 ml of treatment to 10 WFT females, or by introduction of 10 WFT females to dipped French beans.

Table 1.2.4. Significance of treatment comparisons, where green, orange and red shading indicates significance at the P = 0.05, 0.01 and 0.001 levels respectively. Where the amounts of Met52 OD are: Trt 1 = 1.25 l in 150 l water per ha, Trt 2 = 1.25 l in 300 l water per ha, Trt 3 = 1.25 l in 600 l water per ha, Trt 4 =1.25 l in 1200 l water per ha, Trt 5 = 1.25 l in 2400 l water per ha, Trt 6 = Codacide, Trt 7 = untreated, Trt 8 = 1.25 l in 300 l water per ha (bean dipped) . Trts 1-6 are direct application and Trt 8 is a dipping method.

Day	Means	Trt								
4	26.7	1								
	36.7	2	0.228							
	15.0	3	0.112	0.007						
	16.7	4	0.178	0.013	0.799					
	20.0	5	0.379	0.040	0.464	0.631				
	6.7	6	0.005	0.000	0.147	0.094	0.037			
	5.0	7	0.003	0.000	0.078	0.049	0.019	0.696		
	26.7	8	0.992	0.228	0.112	0.178	0.379	0.005	0.003	
			1	2	3	4	5	6	7	8
6	53.3	1								
	55.0	2	0.854							
	43.3	3	0.271	0.200						
	35.0	4	0.043	0.028	0.348					
	41.7	5	0.199	0.143	0.853	0.451				
	20.0	6	0.000	0.000	0.007	0.067	0.011			
	6.7	7	0.000	0.000	0.000	0.001	0.000	0.039		
	56.7	8	0.712	0.853	0.143	0.018	0.100	0.000	0.000	
			1	2	3	4	5	6	7	8
8	68.3	1								
	78.3	2	0.216							
	58.3	3	0.255	0.020						
	56.7	4	0.186	0.012	0.853					
	61.7	5	0.442	0.048	0.708	0.576				
	43.3	6	0.006	0.000	0.100	0.143	0.045			
	11.7	7	0.000	0.000	0.000	0.000	0.000	0.000		
	68.3	8	0.992	0.216	0.255	0.186	0.442	0.006	0.000	
			1	2	3	4	5	6	7	8
11	90.0	1								
	91.7	2	0.751							
	78.3	3	0.086	0.047						
	80.0	4	0.130	0.074	0.821					
	75.0	5	0.035	0.019	0.665	0.511				
	65.0	6	0.002	0.001	0.106	0.067	0.231			
	21.7	7	0.000	0.000	0.000	0.000	0.000	0.000		
	83.3	8	0.286	0.174	0.486	0.636	0.262	0.024	0.000	
			1	2	3	4	5	6	7	8

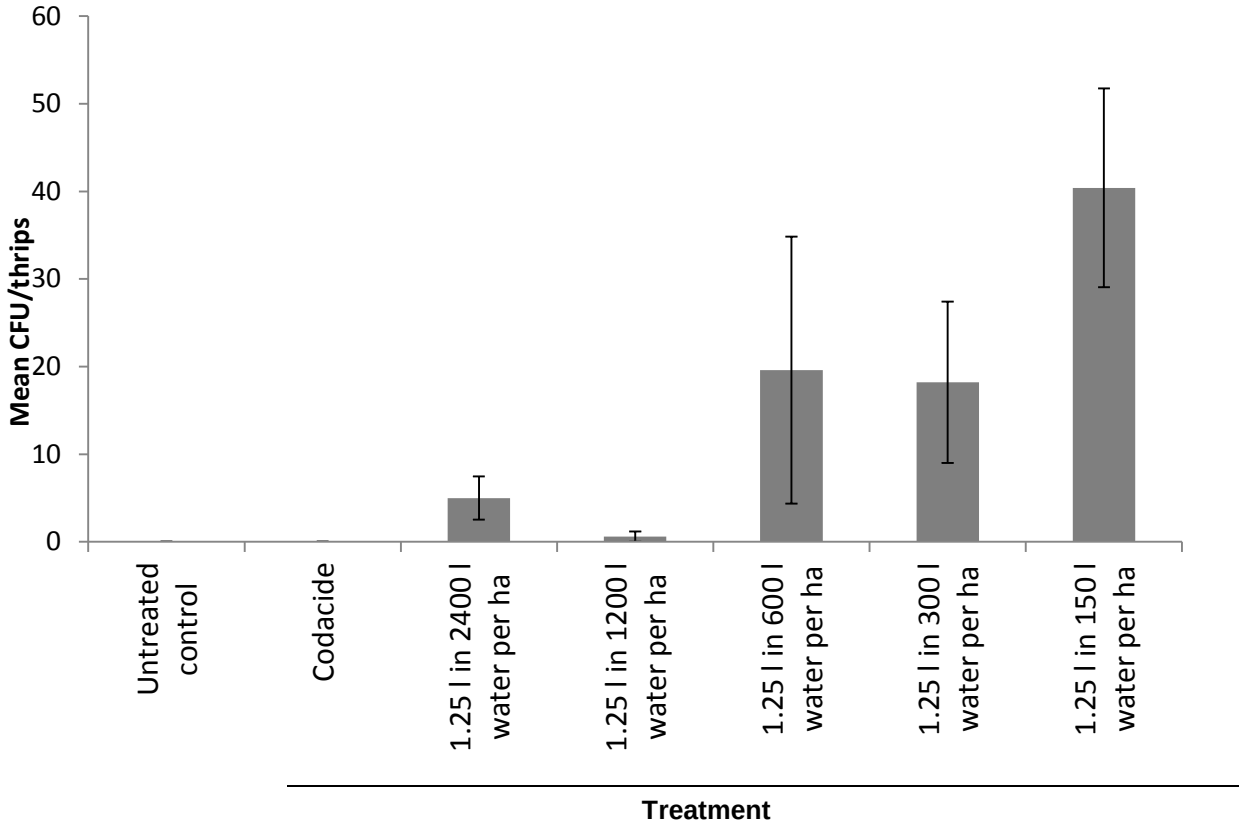


Figure 1.2.5. Mean numbers of colony forming units (CFU) washed from thrips from each treatment (n=5, ±1SE)

Thrips washings: There were indications that the highest spore concentration applied the highest level of spores per thrips (Figure 1.2.5.). However, as with Experiment 1 there was variability within treatments. The most concentrated treatment deposited on average 40 spores per insect, however the range was 11 to 81 per insect. The LD50 of Met52 OD on Day 6 of the experiment was 703 µl of product in a total of 100 µl water. The LD50 of Met52 OD on Day 8 of Experiment 2 was 37.7 µl of product in a total of 100 µl water. The recommended field rate equates to 417 µl of product in a total of 100 µl water.

Thrips mycosis assessments: Following surface sterilisation 9-45% of thrips treated with Met52 OD showed signs of mycosis (Figure 1.2.6.) with no clear trend across treatments. As in Experiment 1 it is not known for certain if dead thrips showing no mycosis following surface sterilisation had been killed by Met52 OD but as controls showed no mycosis it is likely that they had been.

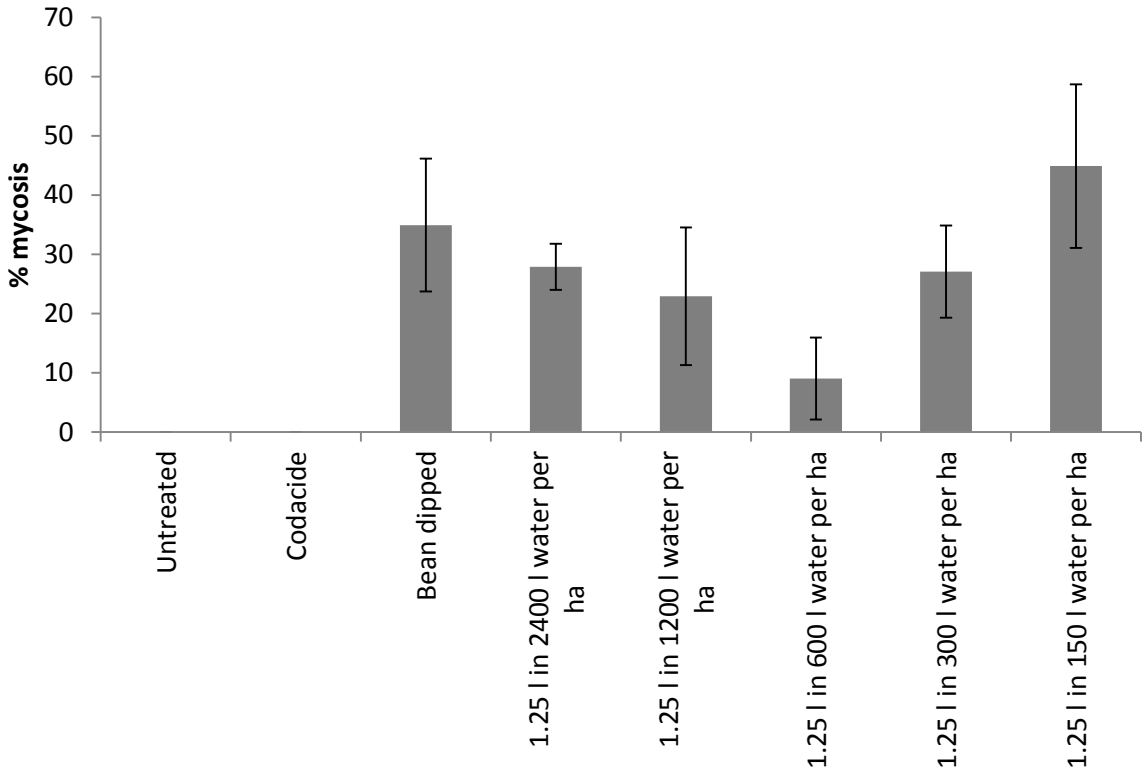


Figure 1.2.6. Percentage mycosis per treatment, as a total of numbers of dead thrips per treatment over 11 days (±1SE)

Comparison to other work using Met52 OD against Frankliniella occidentalis

Metarhizium anisopliae F52 (the same isolate as Met 52 OD) was tested against *Frankliniella occidentalis* in the USA (Wraight et al., 2016). Their laboratory bioassay used laboratory-produced fungal material which was formulated using water and a surfactant (0.01% Silwet) and applied to a bean leaf surface, not topically applied to the insect. Results showed a viability level of 66% of *M. anisopliae* F52, but no correlation between viability and LC50. LC50 was found to be 161 viable spores per mm^2 on average for *M. anisopliae* after 5 days. Our highest concentration of 1.25 L Met52 OD in 300 L in Experiment 2, if applied evenly to a surface, would equate to 250 spores per mm^2 and for the 1.25 L in 600 L treatment approximately 125 spores per mm^2, thus our results are broadly comparable to this study based on laboratory results.

Conclusions and further work

Results from bioassays show some promise for the use of EPF for WFT control within an IPM system. In Experiment 1 there was 70% mortality after six days using the highest label dose compared to <10% mortality with the Codacide control (although the untreated control mortality was higher). In Experiment 2 there was over 50% mortality after six days and nearly 80% mortality by eight days, with untreated control mortality at 11%. However, in this experiment the codacide mortality was high, half of that of the Met52 OD.

Wraight et al. (2016) tested the efficacy of F52 against WFT under variable moisture conditions in a greenhouse (via mist-spraying of the floor for various times post EPF application). They used a laboratory mass produced isolate of F52 (same strain as Met52 OD but not commercially produced) which was suspended in Silwet® surfactant. Spores applied to plants against WFT were sensitive to RH conditions post-application. A satisfactory level (>50%) of WFT mortality in flowers was achieved when greenhouse RH was maintained above 70% for at least 28 hours out of 40, post-application.

Whilst the results of Wraight et al. (2016) showed that the isolate was sensitive to moisture conditions in the greenhouse and responded well to extended conditions of high RH (≥70% RH), their fungus was not formulated in an oil, in contrast to Met52 OD. Formulating fungal spores in an oil may improve their tolerance to lower moisture conditions. Chapple *et al*., (2000) stated that the requirement for an elevated level of RH for an extended period of time or reliance on 'dew point' may be completely negated through formulation in oil.

Other work on fungi for WFT control, using *Beauveria bassiana* (Ugine et al., 2007), showed that high volume application rates led to significantly more conidia deposited per mm^2 than "low" volume application rates (935 l/ha compared to 3,740 l/ha at 2×10^{14} conidia per ha).

Their conclusions were that high volume applications of high EPF rates, repeated every five days, were the only method found to reduce thrips populations. They suggested spraying to run off to deliver more conidia to thrips which were residing in cryptic habitats. However, spraying at such high volume application rates may be too costly for growers and may also lead to run off and loss of active ingredient into the soil. Of note, their formulation was a wettable powder, thus again may not confer the advantages that the Met52 OD oil formulation could give in terms of negating the effects of suboptimal moisture conditions.

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

Task 2.1. Investigate how to minimise the adverse effects of pesticides used for control of other pests (SWD, capsid bugs) on biocontrol of WFT by N. cucumeris (NIAB EMR Yr 1,2)

Introduction

The use of phytoseiid mites to control phytophagous thrips, mites, and whitefly on crops has become increasingly important in recent years in the UK. For example, of the 3,981 ha of strawberries grown in the UK in 2014, 975 ha were treated with *N. cucumeris* (Garthwaite et al., 2015). Biocontrol in this way can be very effective, but phytoseiid mites are vulnerable to some plant protection products and their use requires careful coordination of crop protection spray as part of an Integrated Pest Management (IPM) strategy. This in turn requires data on the toxicity of products to the predatory mites.

Some products are known to be harmful to phytoseiid mites, for example pyrethroids (see HDC review SF133; Fountain and Medd, 2015), and this knowledge can be incorporated into planning an IPM strategy. Other compounds are regarded as relatively safe for predatory mites, but recent studies (Sampson, 2014) have suggested repeated applications could lead to unexpected pest problems. In the field compounds are applied multiple times, and combined in tank mixes, where they may act additively or synergistically; in 2014 strawberries in the UK received, on average, 13 fungicides, five sulphur, four insecticides, three herbicides, three biological control agents, two physical control agents, two acaricides and two molluscicides (Garthwaite et al., 2015).

In Year 1 of this project, we tested common tank mixes over multiple applications. Nimrod/Teldor, Signum/Systhane and Aphox/ Rovral. Reduced numbers of *N. cucumeris* were observed, but only after the third spray application. Five of these six products were fungicides.

For Year 2 we tested two more products that the industry had suggested could be harmful to *N. cucumeris* over multiple applications. These were compared to the Nimrod/Teldor treatment from the previous year. We tested whether a secondary addition of *N. cucumeris* could mitigate the effects of spray treatments.

Materials and methods

Choice of treatments: Calypso (thiacloprid) and potassium bicarbonate+Activator90 were chosen following a survey of 11 growers and agronomists in the industry and feedback from the steering committee on 9 June 2016. Compounds already known to be toxic to predatory mites were excluded. Nimrod + Teldor were included (Table 2.1).

Treatment programme: The trial took place between 1 July and 1 August 2016 in tunnels of strawberries (var. Triumph) planted (> 2 yo) in beds (Figure 2.1) on a commercial plantation. Each tunnel consisted of four beds; the 2nd row of which (reading left to right) was used. A randomised block design was utilised with five replicates of eight treatments, the plots were arranged end to end in a bed. Each plot was 6 m long and separated by an untreated area of 4 m to reduce mite migration between plots.

Figure 2.1. Photograph of trial site

N. cucumeris were released as a loose product within the experimental plots. Plots were 4.5 m^2 (10 plants). Assessment of the Bioline and Koppert *N. cucumeris* supplied gave 4.4 and 7.5 mites per ml of the product carrier, respectively. Products were mixed and the volume of carrier applied to each plant was calculated based on these means so that approx. 460 mites per m^2 were applied (46 *N. cucumeris* per plant) for each release.

Three days later a pre assessment was made of the numbers of *N. cucumeris* in each plot and the first treatments were applied with a Birchmieier air assisted motorised knapsack sprayer at 1,000 l/ha.

Spray treatments were applied 10 days apart (max. intervals for Nimrod) with the exception of Calypso which had approval for a maximum of two applications at the maximum dose (no crop destruct had been approved for this trial). All plots had received an initial introduction of *N. cucumeris*, with half the plots for each spray programme reapplied with an additional application of *N. cucumeris* after the 2nd spray application (Table 2.2). Temperature and humidity were monitored over the course of the trial (Figure 2.2).

Assessments: Pre and post spray assessments were made by visual counts of the numbers of phytoseiids and thrips on 20 button fruit. Button fruit were then washed using the NIAB EMR standard ethanol washing technique (NIAB EMR SOP 780). A number of specimens were mounted on microscope slides to confirm the identity of the phytoseiids as *N. cucumeris*.

Statistics: Following consultation with the NIAB EMR statistician, the data for each sampling date was analysed by one way ANOVA following square root transformation and then the entire data set was analysed by Repeated Measures ANOVA with the pre-treatment assessment as a covariate.

Table 2.1. Plant protection product treatments applied.

Treatment	Products	Second release of cucumeris	Active ingredients	Use	Application rate (/ ha in 1000 l/ha)
1	Calypso	No	Thiacloprid	Insecticide	0.25 l
2	Calypso	Yes	Thiacloprid	Insecticide	0.25 l
3	Potassium bicarbonate*	No	Potassium bicarbonate	Fungicide	7.0 kg
4	Potassium bicarbonate*	Yes	Potassium bicarbonate	Fungicide	7.0 kg
5	Nimrod and Teldor	No	Bupirimate (Nimrod) and Fenhexamid (Teldor)	Fungicide	Nimrod, 1.4 l/ha; Teldor, 1.5 kg/ha
6	Nimrod and Teldor	Yes	Bupirimate (Nimrod) and Fenhexamid (Teldor)	Fungicide	Nimrod, 1.4 l/ha; Teldor, 1.5 kg/ha
7	Untreated	No	Water	-	
8	Untreated	Yes	Water	-	

* with Activator90 Non-Ionic Wetting Agent (1l/ ha in 1000 l/ha)

Table 2.2. Programme of treatments and assessments

Date	Day	Plots with single *N. cucumeris* application	Plots with 2 *N. cucumeris* applications
1 July	0	Inoculate plots with *N. cucumeris*	Inoculate plots with *N. cucumeris*
4 July	3	Pre assess	Pre assess
5 July	4	1st spray	1st spray
11 July	10	1st assessment	1st assessment
15 July	14	Spray 2 all plots	Spray 2 all plots
19 July	18	-	Add *N. cucumeris*
21 July	20	2nd assessment	2nd assessment
25 July	24	3rd spray (except Calypso)	3rd spray (except Calypso)
1 Aug	31	3rd assessment	3rd assessment

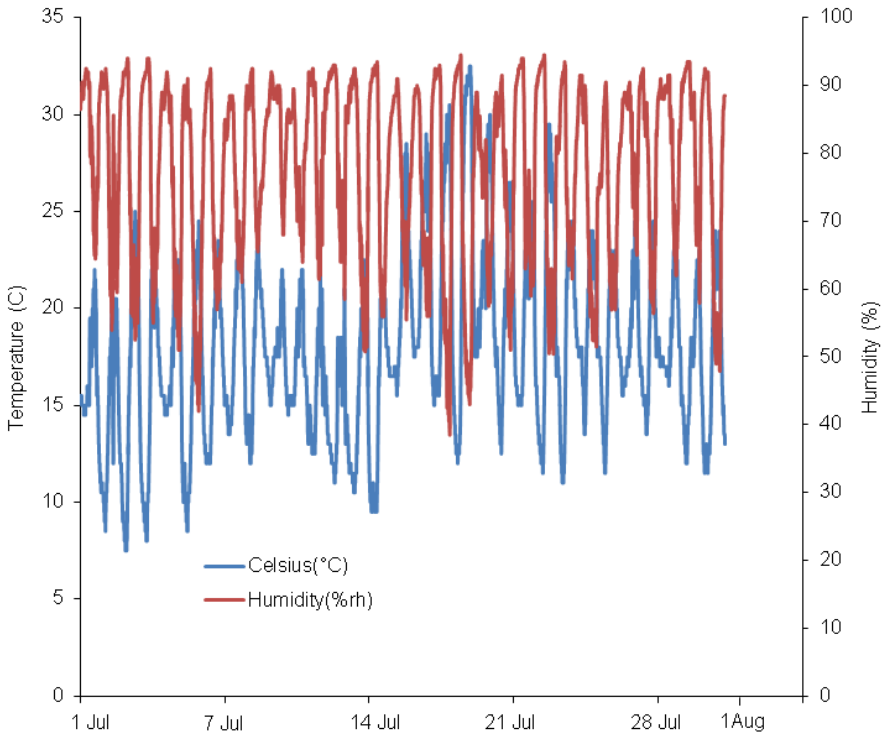


Figure 2.2. Climatic conditions over the course of the trial

Results

Effect of treatments on N. cucumeris numbers: Numbers of *N. cucumeris* (adults and immatures combined) were not significantly affected by spray treatment at any time point compared to the water control; mean numbers of *N. cucumeris* per 20 button fruit in the different treatment are shown in Table 2.3.

Table 2.3. Mean numbers of *N. cucumeris* per 20 button fruit before and after three spray applications. Treatments followed with + had 2nd release of *N. cucumeris* after the second spray application. SE of means shown in parentheses.

	Assessment			
	Pretrt	1	2	3
Water	10.0 (1.4)	7.2 (0.9)	7.0 (1.3)	6.0 (0.4)
Water+	5.8 (1.0)	9.8 (1.5)	11.6 (1.7)	5.8 (1.7)
Calypso	9.8 (3.8)	7.4 (1.3)	7.8 (2.3)	6.8 (1.0)
Calypso+	5.0 (2.0)	8.8 (2.1)	8.8 (3.5)	8.4 (2.0)
P. bicarb	9.2 (1.7)	10.0 (2.9)	4.4 (1.3)	6.6 (1.6)
P. bicarb+	8.6 (2.5)	11.2 (3.0)	9.8 (3.1)	6.8 (1.6)
Nimrod+Teldor	6.6 (1.9)	8.6 (2.7)	6.6 (1.9)	5.6 (0.9)
Nimrod+Teldor+	6.6 (1.2)	9.8 (3.0)	5.0 (0.6)	7.6 (2.3)

However, the second addition of *N. cucumeris* to half of the plots after the second spray application, led to an overall significant increase in adult *N. cucumeris* compared to plots where no second release of *N. cucumeris* was done ($F=4.99$; df 1,15; $p<0.05$). At the third assessment there was no significant effect of any of the spray applications on *N. cucumeris* numbers.

Effect of treatments on thrips numbers: Overall, there was a significant decrease in thrips numbers over time ($F=3.66$; df 2,56; $p<0.05$), but there was no significant difference in thrips numbers caused by the second addition of *N. cucumeris*. Mean numbers of thrips before and after treatment per 20 button fruit are shown in Table 2.4.

Crop damage: The crop suffered heavy thrips damage, so that a projected harvest of 25 t/ha was 9 t/ha by the end of the season.

Figure 2.4. Mean numbers of thrips per 20 button fruit before and after three spray applications. Treatments followed with + had 2nd release of *N. cucumeris* after the second spray application. SE of means shown in parentheses.

	Assessment			
	Pretrt	1	2	3
Water	96.0 (19.5)	81.2 (23.4)	74.0 (12.3)	38.2 (3.0)
Water+	99.6 (16.5)	129.0 (29.1)	56.0 (2.6)	55.6 (17.9)
Calypso	103.6 (14.3)	88.2 (14.5)	86.0 (6.0)	29.4 (4.8)
Calypso+	117.6 (35.2)	97.2 (15.5)	56.6 (4.8)	48.0 (9.9)
P. bicarb	110.4 (19.9)	67.2 (3.5)	39.2 (5.4)	37.4 (12.6)
P. bicarb+	76.2 (9.6)	81.2 (18.4)	48.4 (6.7)	41.6 (7.4)
Nimrod+Teldor	111.4 (19.0)	100.4 (13.6)	64.4 (6.6)	30.6 (8.1)
Nimrod+Teldor+	64.2 (17.7)	74.6 (10.7)	60.2 (7.0)	44.8 (15.9)

Discussion (see Table 2.1 for active ingredient of products)

There was no evidence from this trial that Calypso or Potassium bicarbonate+Activator90 had a detrimental effect on *N. cucumeris* numbers after three applications (two applications of Claypso). This is encouraging for the industry as Calypso (thiacloprid) is one of the options available for capsid and aphid control, and Potassium bicarbonate is a commonly applied fungicide whose use increased by 103% between 2012 and 2014 (Garthwaite et al., 2015). Thiacloprid had been previously reported not to be harmful to *N. cucumeris* (Cuthbertson et al., 2012), whilst potassium bicarbonate had also been reported in the literature to be harmless to the related species *Amblyseius swirskii* (Gradish et al., 2011).

We found no evidence this year that Nimrod and Teldor applied together were harmful to *N. cucumeris*; this is contradictory to the results in 2015. It is unclear why there is this discrepancy as the same concentrations and spray equipment were used, but sampling time, differences in environmental conditions, spray coverage etc. could be contributing factors. However, it should be noted that different sampling methods in each year were used; in 2015 young folded leaves were sampled and in 2016 button fruit were sampled. It could be that the pesticides are not as harmful in the button fruit as *N. cucumeris* is better protected under the calyx – however this has not been tested.

Numbers of *N. cucumeris* reduced over the course of the trial in all plots in 2016 regardless of the plant protection products applied. The addition of further *N. cucumeris* on Day 18 (after the 2nd spray application) was reflected in increased numbers of adults two days later, but not in the assessment on Day 31, after a third spray treatment. The number of thrips was high on all plots, causing significant crop loss.

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

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).

Insecticide applications in the autumn (e.g. thiacloprid) effectively reduce numbers of *M. euphorbiae* on the crop the following spring compared to untreated controls (less than 50 aphids/100 leaves compared to more than 400 aphids/100 leaves) (HortLINK HL0191/HDC SF 94). It is, however, not always possible to time insecticide applications in the autumn and so insecticide applications in the spring may be required. There is a need to identify which insecticide products would be more effective under cooler spring temperatures before crops have begun to grow and the canopy remains relatively compact.

In recent years growers have reported problems in controlling aphids in early spring, in particular *M. euphorbiae*. Difficulty in controlling this aphid pest appears to be linked to the need for good spray coverage (AHDB Horticulture project SF 140). This problem is exacerbated by the strawberry growing season being bought forward through use of protected cropping e.g. increasing use of fleece and tunnels, and insecticide products based on a reducing range of active ingredients; most notably, the recent withdrawals of chlorpyrifos and pirimicarb, two widely used insecticides in strawberry crops.

Macrosiphum euphorbiae damages crops primarily through the production of copious amounts of honeydew, which may result in the growth of sooty moulds. This together with cast skins may make the fruits unmarketable (Trumble et al., 1983). *Macrosiphum euphorbiae* feeding may also distort the leaves and berries (Irving et al., 2012).

M. euphorbiae is a medium to large species of aphid (wingless adults are 1.7-3.4 mm long) with a spindle shaped body that is yellowish green or pinkish in colour and has very long siphunculi (Figure 4.1). Under suitable conditions this species is capable of breeding throughout the year on strawberry crops. Reproduction is entirely asexual and populations can build up rapidly in the spring. Populations often build throughout April with winged aphids being produced by the end of the month (Alford, 2007). 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 4.1. Potato aphid, *Macrosiphum euphorbiae*, on strawberry leaf petiole

Biological Control

Aphid pests may be controlled by predators, parasites and pathogens (Hagen & van den Bosch 1968). In fruit crops aphid parasitoids should be considered the first line of defence and it is possible to release the appropriate parasitoid species for the aphid pest or a mix of six parasitoid species (*Aphidius colemani*, *A. ervi*, *A. matricariae*, *Praon volucre*, *Ephedrus cerasicola* and *Aphelinus abdominalis*) to cover a range of potential aphid pest species attacking strawberry crops (Irving et al., 2012). HortLINK Project HL0191, SF 94, investigated the aphid parasitoid *Aphidius eglanteriae* as a control of *Chaetosiphon fragaefolii*. *Aphidius eglanteriae* proved difficult to mass produce and so an alternative species, *E. cerasicola,* was assessed for its effectiveness in reducing *Chaetosiphon fragaefolii* populations. A mix of the six parasitoid species was also used and compared with *E. cerasicola* alone and an untreated control. Results showed that releasing parasitoids onto aphid-infested plants significantly reduced the populations of both *C. fragaefolii* and *M. euphorbiae*. Distributors of the six species mix of aphid parasitoids of aphids recommend that parasitoids are introduced to crops before aphids are seen in order to increase the potential to control populations effectively. There is a perception, however, that aphid parasitoids will not emerge at the low

temperatures experienced in polytunnels in winter and early spring. Despite this, it has been demonstrated that, under common winter conditions for a polytunnel (temperatures around 0°C at night and between 5°C and 20°C during the day), no mortality was recorded amongst aphid parasitoids introduced into crops as aphid mummies and the expected number of adult parasitoids emerged from these mummies (Dassonville et al., 2013).

Conventional crop protection products

Conventional insecticides are typically used to control aphid pests on strawberry, however, the range of active ingredients available has reduced with the withdrawal of chlorpyrifos (see withdrawal notice 20160814) and pirimicarb (see withdrawal notice 20160148). The withdrawal of chlorpyrifos is likely to be particularly important as this insecticide was used by many growers post-harvest to reduce aphid numbers in crops to be overwintered. The range of effective insecticide options is further limited by development of insecticide resistance in species such as *A. gossypii*. Available insecticides fall into the following groups based on mode of action (see IRAC website: http://www.irac-online.org/modes-of-action).

Pyrethroids/Pyrethrins (IRAC group 3A) – lambda-cyhalothrin (e.g. Hallmark with Zeon Technology) has an EAMU for use on strawberry crops while pyrethrins (e.g. Pyrethrum 5 EC) has on-label approval. Populations of *A. gossypii* have developed resistance to these insecticides. Where effective, these insecticides provide control through direct contact with the pest through disruption of the sodium channels in the insect nervous system. Both lambda-cyhalothrin and pyrethrins are harmful to biocontrol agents but while lambda-cyhalothrin has a persistence of more than eight weeks, pyrethrins have persistence of just one day to a week and so are more compatible within an IPM programme.

Neonicotinoids (IRAC group 4A) - thiacloprid (e.g. Calypso) has an EAMU for use on strawberry crops. There are no reported cases of resistance to thiacloprid amongst species of aphid found on strawberry. This insecticide provides control through contact and systemic activity as nicotinic acetylcholine receptor agonists. It is moderately harmful to predatory mites used as biocontrol agents and *Orius* predatory bugs (Irving et al., 2012). A second neonicotinoid, acetamiprid, only approval for non-harvested strawberry, e.g., nursery plants and the harvest interval is 365 days.

Pymetrozine (IRAC group 9B) - pymetrozine (e.g. Chess WG) has an EMAU for use on strawberry crops. There are no reported cases of resistance to pymetrozine amongst species of aphid found on strawberry. This insecticide works primarily through systemic activity as a

homopteran feeding blocker. It is safe or only slightly harmful to natural enemies and biocontrol agents, including aphid parasitoids (Irving et al., 2012).

Fatty acids – fatty acids C7-20 (e.g. Flipper) has on-label approval for use on strawberry crops. There are no reported cases of resistance to fatty acids. This insecticide provides control through contact, typically dissolving the waxy coating of the insect cuticle and disruption of the insect tracheal system. Fatty acids are typically harmful to active stages of natural enemies and biocontrols but only when they come into direct contact and there is residual toxicity.

Maltodextrins – maltodextrins (e.g. Majestik) has on-label approval for use on strawberry crops. There are no reported cases of resistance to maltodextrins. This insecticide provides control through contact, typically coating and drying on the insect, ultimately killing the insect through suffocation. Maltodextrins are typically harmful to natural enemies and biocontrols but only when they come into direct contact and there is residual toxicity.

The aim of this work was to improve insecticide control of the potato aphid, *Macrosiphum euphorbiae*, so as to be more compatible with IPM programmes.

Materials and methods

Site: Experiment 1 was done in a ventilated research polytunnel at Harper Adams University (Crop and Environment Research Centre). Experiment 2 was done in controlled environment rooms at the Jean Jackson Entomology Laboratory at Harper Adams University.

Experimental Design: Experiment 1, was a randomised block experiment with five replicates of each of eight treatments (including a water control). Each replicate consisted of a single potted strawberry (*Fragaria* x *ananassa*) plant (cv. Driscoll's® Diamond™). Potted strawberry plants were separated using horticultural fleece plot dividers (Figure 4.2). The experiment largely followed the EPPO PP1/252 protocol for efficacy evaluation of insecticides against aphids on strawberry. The only difference being the use of single potted plants in each plot to allow for more detailed assessments.

Figure 4.2. Experimental set-up used in Experiment 1 with potted strawberry plants separated by horticultural fleece dividers.

Experiment 2, was a fully randomized experiment with five replicates of each of nine treatments (including a water control and an unsprayed control). Each replicate consisted of a single aphid infested strawberry (*Fragaria* x *ananassa*) leaf (cv. Elsanta) (Figure 4.3). Leaves were sprayed before or after being experimentally infested with aphids. After spraying, each leaf was maintained in a ventilated plastic Petri dish placed in a controlled environment room set to 20 ^{o}C and 60% RH at the Jean Jackson Entomology Laboratory. The experiment largely followed a standard testing protocol developed by the Insecticide Resistance Action Committee (IRAC). The only difference being that leaves were sprayed to 'run-off' rather than being dipped into each test solution.

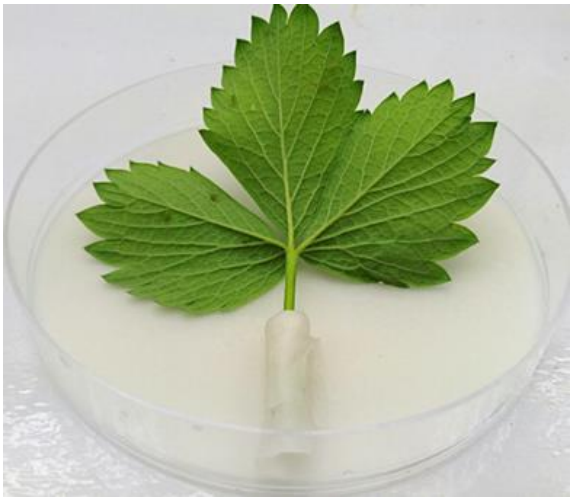


Figure 4.3. Experimental set-up used in Experiment 2 with a single aphid infested strawberry leaf in a ventilated Petri dish.

Treatments: Experiment 1 – treatments listed in Table 4.1 were applied using an air assisted knapsack sprayer. Products applied as per label recommendations and at the same rate with the addition of Silwet L-77. Overwintered soil grown strawberry plants (commercial fruit crop grown in Staffordshire), which were naturally infested with *M. euphorbiae*, were carefully dug up and taken back to Harper Adams University in late March 2016. Plants were grown on in 5 l pots in a ventilated polytunnel during the experiment. The numbers of aphids on these plants were initially low so they were supplemented with additional aphids collected, also from commercial fruit crops grown in Staffordshire in the weeks before the experiment began. Aphid numbers were supplemented by placing infested leaves onto each potted strawberry plant. The experiment was started once aphid populations reached approximately 20 aphids/half plant.

Experiment 2 – in order to validate results from Experiment 1 and to determine the importance of spray coverage, two leaf bioassays were completed using each of the treatments listed in Table 2 as well as an unsprayed control. This experiment was divided into two bioassays. In the first bioassay, leaves were sprayed before being infested with aphids and in the second bioassay leaves were sprayed after being infested with aphids. In both cases strawberry leaves were sprayed on both surfaces to run-off and allowed to dry at room temperature by placing the leaves on several layers of tissue paper. Leaves were infested with 20 *M. euphorbiae* nymphs (1-3 instar). After spraying, the petioles of the leaves were wrapped in damp tissue paper and leaves were placed separately in Petri dishes lined with damp filter paper (90 mm diameter).

Table 4.1. Treatments

Treat No.	Product	Active ingredient	Product dose (/ha)	HI	Approval
1	Hallmark with Zeon technology 100 g/l CS	lambda-cyhalothrin	0.075 l	3 d	1705/11
2	Hallmark with Zeon technology 100 g/l CS + Silwet L-77	lambda-cyhalothrin + trisiloxane ethoxylate	0.075 l 0.25 l		*
3	Calypso	thiaclorprid	0.250 l	3 d	2132/14
4	Calypso + Silwet L-77	thiacloprid + trisiloxane ethoxylate	0.250 l 0.25 l		*
5	Chess 50% w/w WG	pymetrozine	0.400 kg	3 d	0504/07
6	Chess 50% w/w WG + Silwet L-77	pymetrozine + trisiloxane ethoxylate	0.400 kg 0.25 l		*
7	Silwet L-77	trisiloxane ethoxylate	0.25 l	-	-
8	Water control	-		-	-

*Note that strawberry crops are not permitted to be sprayed at full label rates when applied together with Silwet L-77 and should instead be sprayed at 50% of the full label rate.

Treatment Application: Experiment 1 - treatments were applied using a water volume of 1000 l/ha using an air assisted knapsack sprayer (Stihl, model SR340) by PA1, PA6 and PA9 qualified Crop and Environmental Research Centre member of staff. Water sensitive papers were attached to outer, middle and inner leaves using paper clips before spraying one plot with the water control in order to determine spray coverage throughout the plant canopy.

Experiment 2 - treatments were applied at a rate equivalent to 1000 l/ha using a handheld atomiser by PA1 and PA6 qualified Crop and Environmental Research Centre member of staff.

Assessments: Experiment 1 – a detailed pre-treatment assessment of the numbers and positions on each strawberry plant of aphids was recorded on the day before the treatments were applied. One half of the plant was used for these assessments and the area of the plant to be assessed was marked and kept the same throughout the experiment. Plots were

randomly allocated to treatments, ensuring that all treatments had a range of densities of aphids and that the relative positions of the aphids were similar. Analysis of the aphid counts completed before treatment application confirmed that there was no significant difference in aphid numbers at the start of the experiment. Similar post treatment application assessments were done 1, 3, 8 and 15 days after the spray application, counting the total numbers and positions of live aphids on each plant. At each count the aphid species was confirmed. The crop was also checked for the presence of natural enemies, recording the numbers of each species. See following table for summary of experiment activities:

Table 4.2. Timetable of experiment

Date	**Activity**
21 March	Overwintered soil grown strawberry plants collected from commercial fruit crop. Plants were all naturally infested with potato aphids.
22 March	Plants potted into 5 l pots and placed into ventilated polytunnel at Harper Adams University
15 April	Each plant infested with approximately 10 M. euphorbiae collected from a commercial fruit crop
2 May	Pre-treatment assessment of aphid numbers on each strawberry plant
3 May	Insecticide applications and water control applied
4 May	1 day post-treatment assessment of aphid numbers on each strawberry plant
6 May	3 day post-treatment assessment of aphid numbers on each strawberry plant
11 May	8 day post-treatment assessment of aphid numbers on each strawberry plant
18 May	15 day post-treatment assessment of aphid numbers on each strawberry plant

Experiment 2 – a pre-treatment assessment of the numbers of aphids on each leaf was completed to ensure there were 20 aphids present at the start of the experiment. Post treatment assessments were done 1, 3 and 6 days after spray application and aphids were scored as alive, moribund or dead.

Husbandry: Experiment 1 – plants were checked regularly for evidence of diseases and other pests. No diseases or other pests were recorded during the experiment and so sprays or biocontrol releases for diseases and other pests were not required.

Experiment 2 – the damp tissue paper used to wrap around the petiole of each leaf was checked at each assessment and re-wetted as required.

Meteorological records: Experiment 1 – air temperature, cloud cover and relative humidity (RH) were recorded when the spray applications were made (Table 4.3). Additional weather data was available from a Met Office weather station (location 52.783, -2.433), which was approximately 400 m from the polytunnel in which the experiment was completed. Additionally, temperature and humidity data was collected inside the polytunnel using Tinytag data loggers (model TGP-4500).

Experiment 2 – leaf bioassays were completed in a controlled environment room, set to a constant 20 ^{o}C and 60% RH.

Table 4.3. Meteorological conditions when spray applications were made.

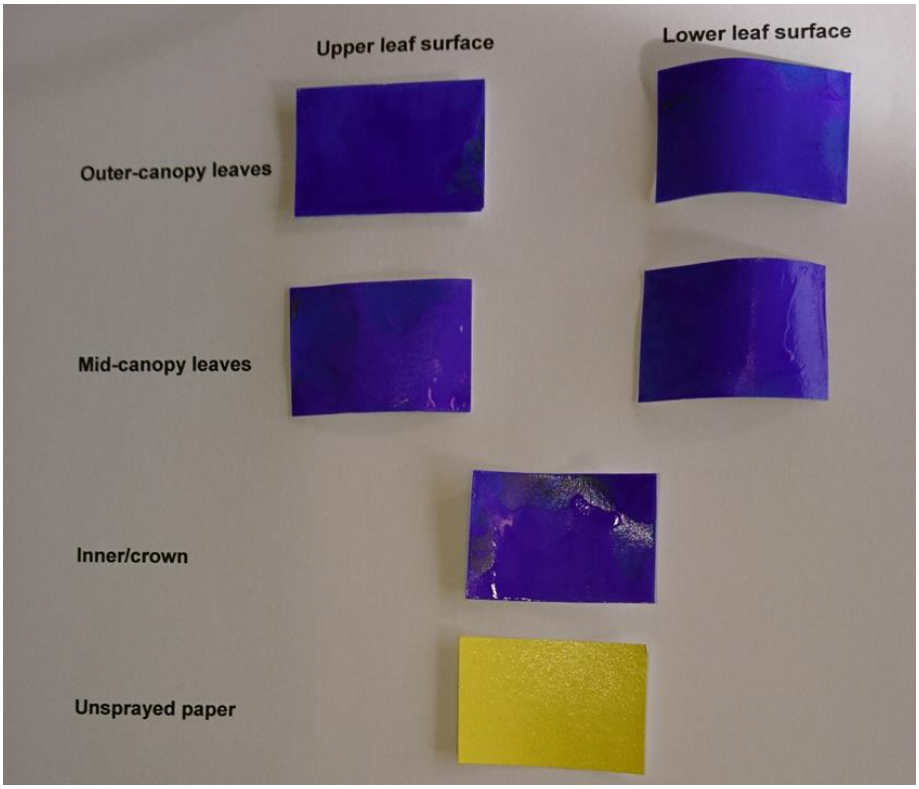
Date	Time of spray application	Temperature (ºC)		Relative humidity (%)	Cloud cover
3rd May 2016	5-6pm	13.3		53.5	25%

Statistical analysis: Experiments 1 and 2 - data were tested for normality and found to have a Poisson distribution. As a result a square root transformation was used to normalise the data. A repeated measures ANOVA was then completed, with treatment as the explanatory variable and days post spray as the error variable. *Post-hoc* Bonferroni adjusted pairwise comparisons were undertaken with the significance level set at $P = 0.05$. A further ANOVA analysis and LSDs ($P<0.05$) were used to determine significance between treatments within the same dates.

Compliance: The study was conducted to ORETO standards (Harper Adams University ORETO Certification Number 343). EPPO guideline EPPO PP1/252 was followed as closely as possible for the treatment and assessment of the polytunnel trial and standard IRAC testing protocol for the leaf bioassays.

Results

Experiment 1 – meteorological data both inside and outside the polytunnel during the experimental period is presented in Figure 4.5. Inspection of the water sensitive papers confirmed that good spray coverage was achieved regardless of the position of the papers on the plant (complete colour change of papers placed throughout the strawberry plant canopy, Figure 4.4).



4.4. Water sensitive papers after spray application.

The repeated measures ANOVA found a significant difference between the treatments (F = 17.4, d.f. = 7, 191 *P*<0.0001). Post-hoc pairwise comparisons (Bonferroni adjusted) further put the treatments into three groups, A B and C as illustrated in Figure 4.6, based on overall treatment effect. Significance 'Group A' included Hallmark and Hallmark + Silwet, 'Group B' included Calypso and Calypso + Silwet, and 'Group C' included Chess, Chess + Silwet, Silwet and the water control. No significant difference was found between Chess and Chess + Silwet when compared with Silwet or the water control.

Additional analysis further assessed at which date significant differences between treatments could be observed. Hallmark and Hallmark + Silwet had reduced aphid numbers significantly more than any other treatment by the time assessments were completed eight days post spray application. Calypso and Calypso + Silwet were differentiated from other treatments in terms of efficacy by the time assessments were completed 15 days post spray application. Chess and Chess + Silwet did not differentiate from Silwet applied on its own or the water control on any assessment date (Table 4.4).

Distribution of the aphids throughout the plant changed during the experiment. For treatments Hallmark and Hallmark + Silwet, no aphids were recorded on the last assessment, and very few aphids were present on plants at earlier assessments and so any distribution data for

these treatments was highly variable and should be treated with caution. For all the other treatments, aphid numbers increased overall and increases in numbers were seen in the crown and leaves as well as on the flower petals, bracts, stem and petioles as these positions on the plant became available as the plants grew. When the aphids were found on the leaves, they were found on the under leaf surface in much greater numbers than on the upper leaf surface (Figure 4.7). The relative proportion of aphids found in the crown increased immediately after spray application regardless of the treatment applied.

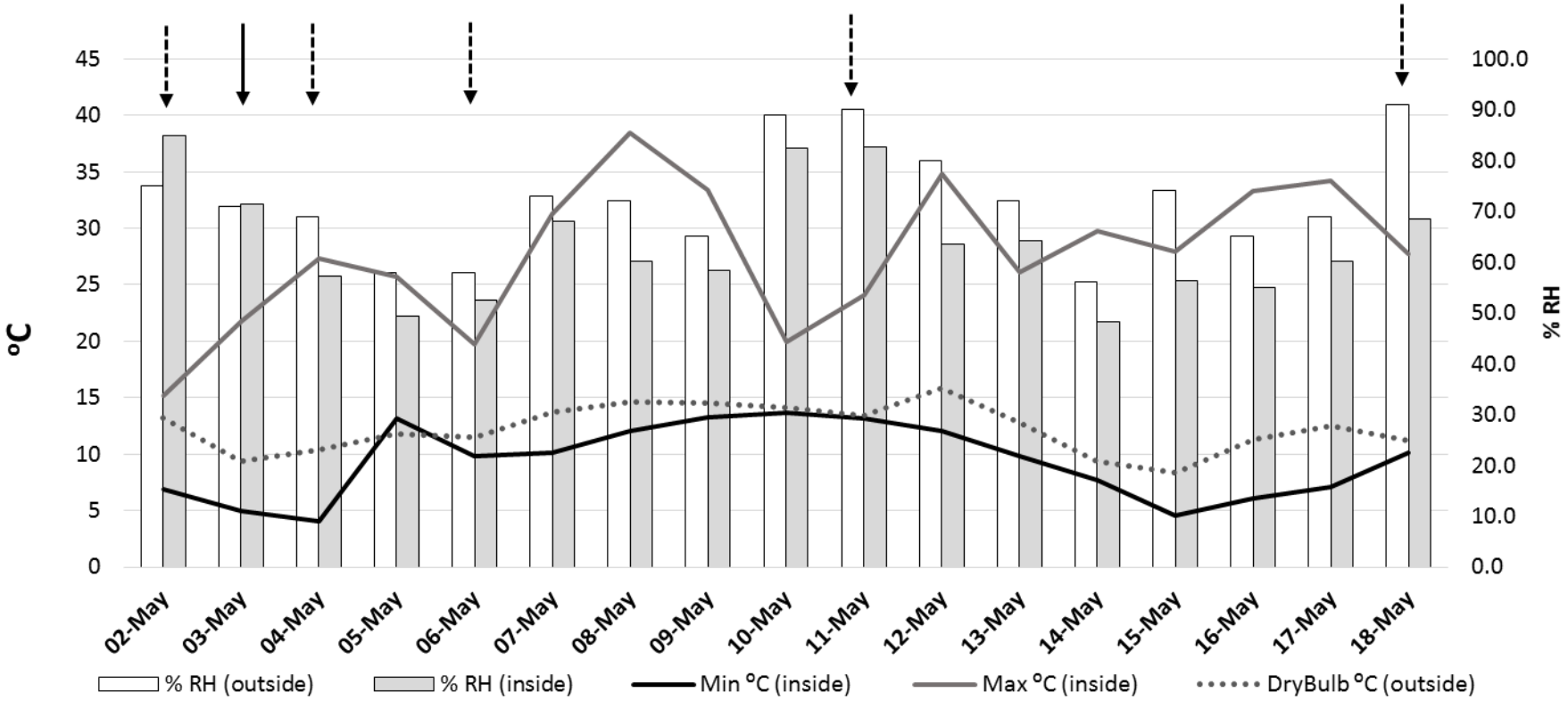


Figure 4.5. Weather data for the duration of Experiment 1. Percent relative humidity (%RH) outside and Drybulb represent measurements taken outside of the polytunnel. Mean percent relative humidity inside, minimum and maximum temperatures represent measurements taken inside the polytunnel using Tiny Tags (model no. TGP-4500). Solid arrow = spray application date, hatched arrows = assessment dates

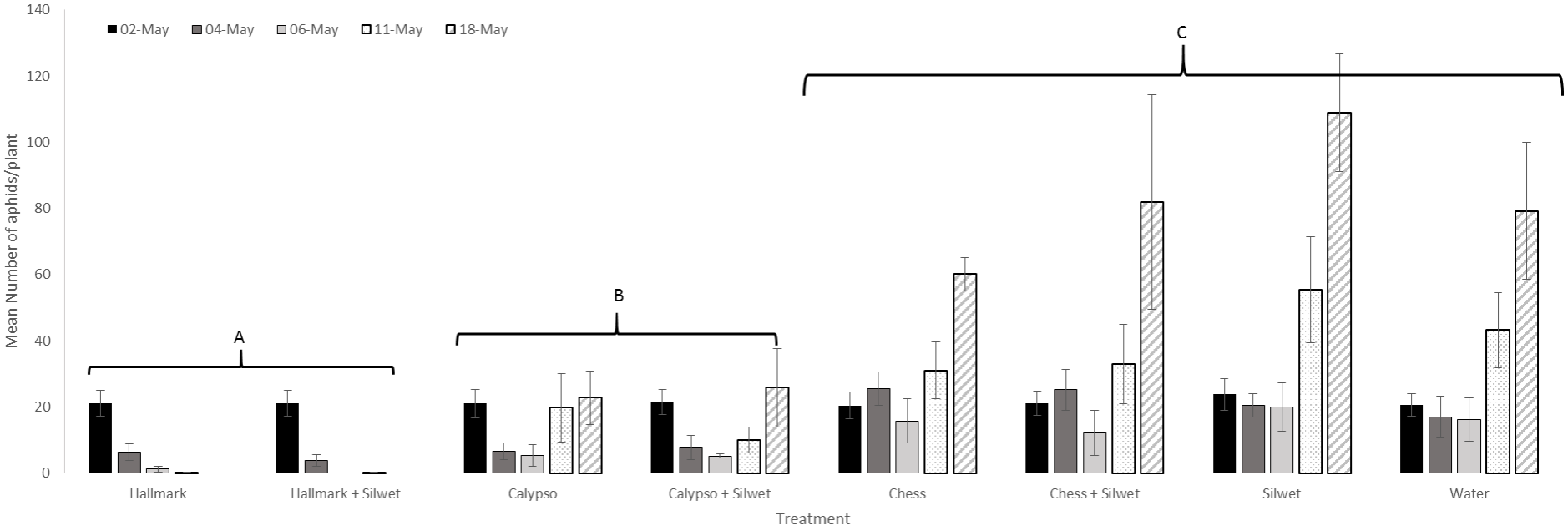


Figure 4.6. Mean numbers of *Macrosiphum euphorbiae* per half plant in Experiment 1. The letter groupings indicate significantly different groupings as found in the Bonferroni post hoc analysis. Error bars indicate +/- SEM. Pre-treatment aphid counts completed on 2 May and aphid counts completed 1, 3, 8 and 15 days after treatment application completed on 4, 6, 11 and 18 May, respectively.

Table 4.4. Mean and square root transformation of mean numbers of *Macrosiphum euphorbiae* per half plant in Experiment 1. The same lower case letter denotes not significantly different from each other between treatments within the same dates. The upper case letters denote overall significance of each treatment. Pre-treatment aphid counts completed on 2 May and aphid counts completed 1, 3, 8 and 15 days after treatment application completed on 4, 6, 11 and 18 May, respectively.

Treatment/Date		Actual mean				Square root of mean					
	02-May	04-May	06-May	11-May	18-May	02-May	04-May	06-May	11-May	18-May	
1 Hallmark with Zeon technology 100 g/l CS	21.2	6.4	1.2	0.2	0	4.52a	2.12ab	0.68ab	0.20a	0.000a	A
2 Hallmark with Zeon technology 100 g/l CS+ Silwet L-77	21.2	3.8	0	0.2	0	4.53a	1.48a	0.000a	0.20a	0.000a	
3 Calypso	21	6.6	5.4	19.8	22.8	4.49a	2.37ab	1.88bc	3.60bc	4.44b	B
4 Calypso + Silwet L-77	21.6	7.8	5.2	10	25.8	4.57a	2.31ab	2.26bcd	2.97b	4.66b	
5 Chess 50% w/w WG	20.4	25.6	15.8	31	60.2	4.44a	4.96c	3.71cd	5.37bcd	7.73c	C
6 Chess 50% w/w WG + Silwet L-77	21.2	25.2	12.2	33	82	4.53a	4.84c	2.84cd	5.41cd	8.27c	
7 Silwet L-77	23.8	20.4	20	55.4	108.8	4.79a	4.45c	4.16d	7.07d	10.30c	
8 Water control	20.6	17	16.2	43.2	79.2	4.47a	3.83bc	3.72cd	6.37d	7.94c	
						P	<0.0001				
						F	17.4				
						d.f.	7, 191				

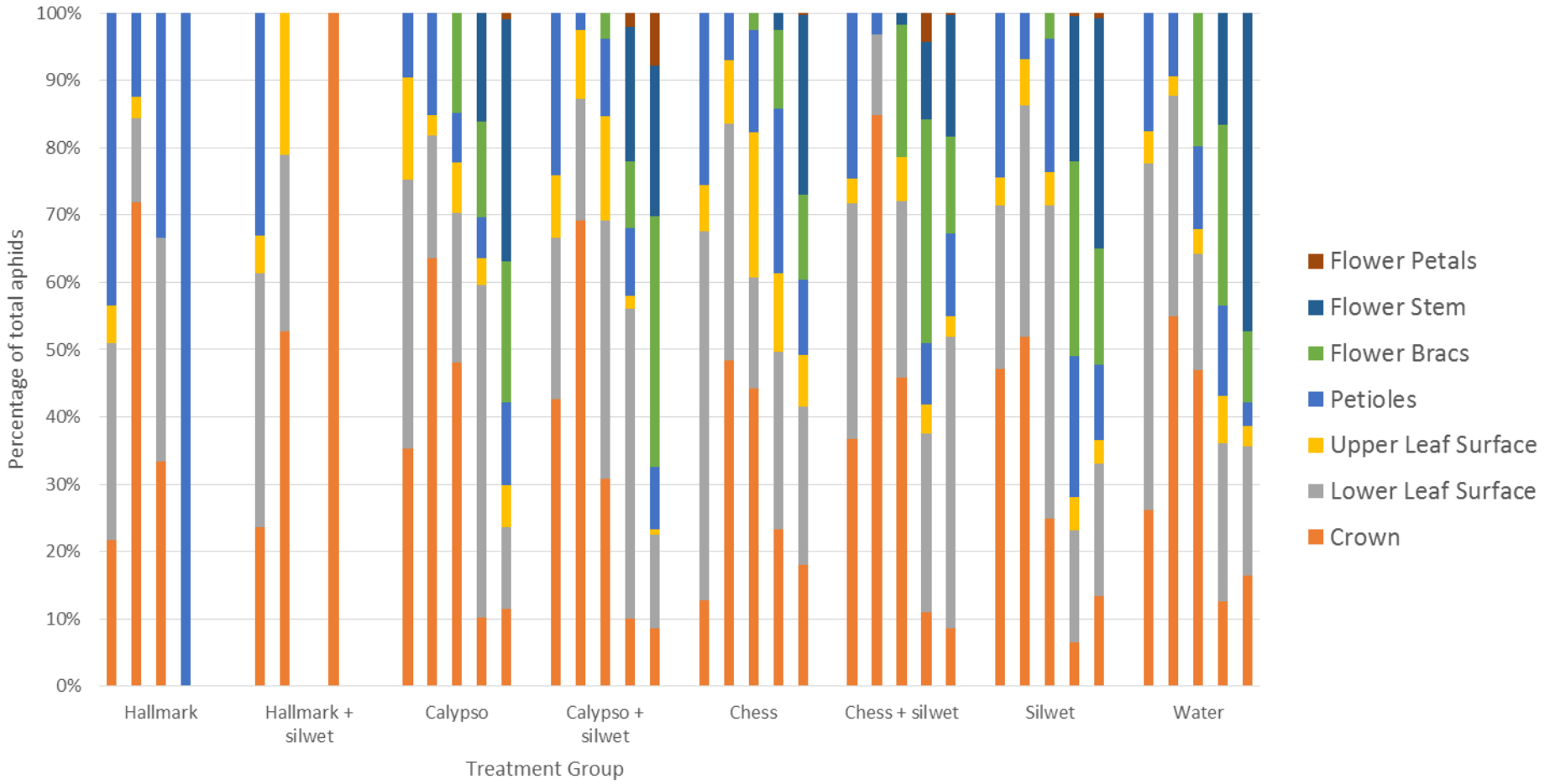


Figure 4.7. Proportions of aphids found on different parts of the strawberry plants throughout the experimental period.

Experiment 2 – in the bioassay where the leaves were infested with aphids before treatment application, there was a significant difference between the treatments (F = 17.7, d.f. = 8, 170 *P*<0.0001). *Post-hoc* analysis indicates that the untreated and water controls were not significantly different from each other. Chess when applied without the wetter killed significantly more aphids than the controls but was significantly less effective than the other treatments tested. The remaining treatments, Chess + Silwet, Calypso, Calypso + Silwet, Hallmark and Hallmark + Silwet and Silwet alone, killed all of the aphids in the experiment and were not statsically different in their performance (Figure 4.8). For each of these treatments 100% mortality was observed by the assessment completed three days after treatment application (Table 4.5), except for Silwet applied on its own where 100% mortality was recorded six days after treatment application.

In the bioassay where leaves were infested with aphids after treatment application, there was also a significant difference between the treatments (F = 26.7, d.f. = 8, 170 *P*<0.0001). Post-hoc analysis found that the water and untreated controls as well as Silwet applied on its own were not statistically different from each other. Chess and Chess + Silwet killed significantly more aphids than either of the controls, however, these two treatments were significantly less effective compared to the other treatments tested. The remaining treatments, Calypso, Calypso + Silwet, Hallmark and Hallmark + Silwet, killed all the aphids in the experiment and were not statistically different in their performance (Figure 4.9). In both Hallmark and Hallmark + Silwet, all aphids were either dead or moribund by the assessment completed one day after treatment application. By the time of the assessment completed six days after treatment application there was 100% mortality in these two treatments. Calypso and Calypso + Silwet killed nearly all of the aphids six days after treatment application, only two aphids remained in a moribund state. In the remaining treatments, Chess, Chess + Silwet, Silwet and the water and untreated controls there living aphids on the leaves at the end of the experiment (Table 4.6).

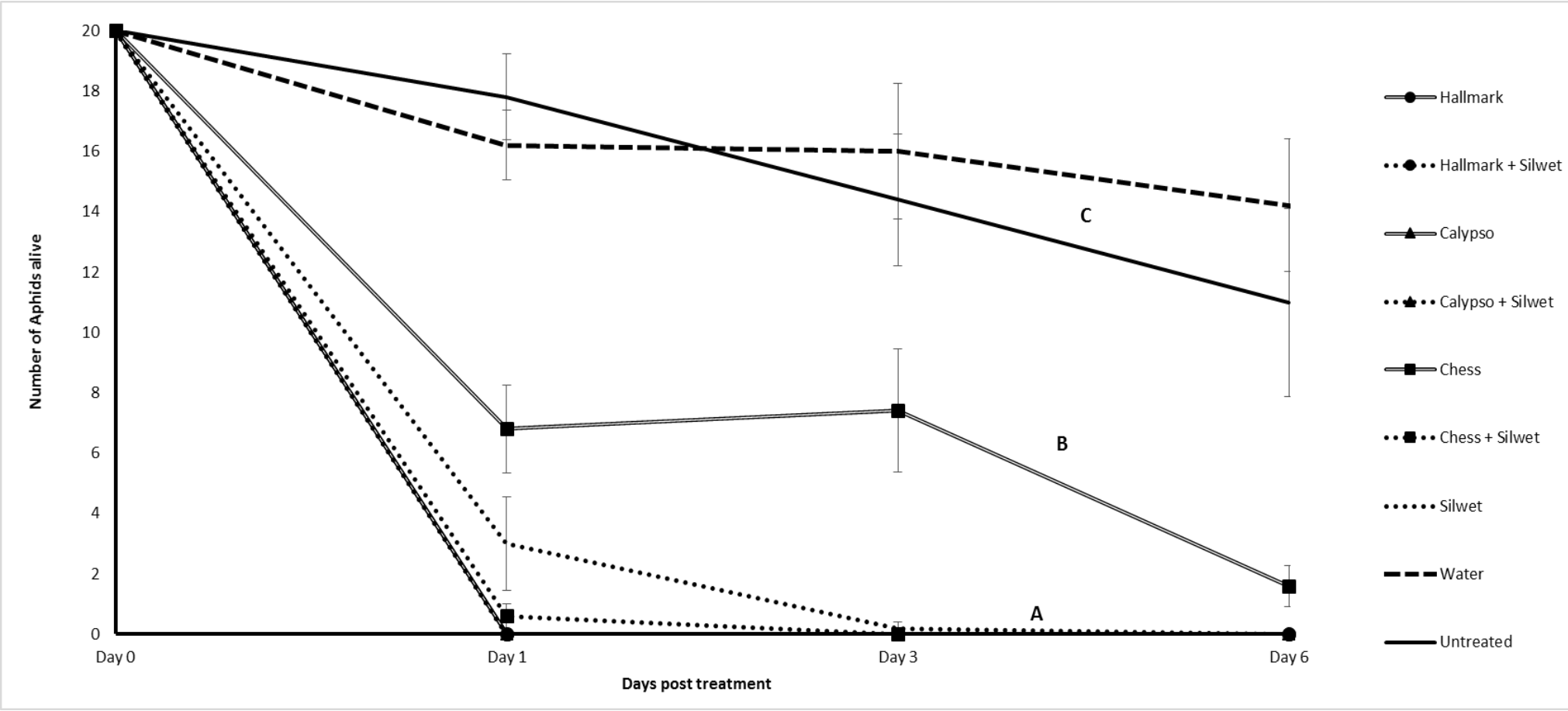


Figure 4.8. *Macrosiphum euphorbiae* survival in the leaf bioassay when leaves were sprayed after being infested with aphids. Each uppercase letter denotes a group of treatments which are not significantly different from one another at the *P* = 0.05 level. 'Group A' includes Hallmark, Hallmark + Silwet, Calypso, Calypso + Silwet, Silwet, Chess + Silwet; 'Group B' includes Chess; 'Group C' includes Water and Untreated. Error bars represent +/- SEM

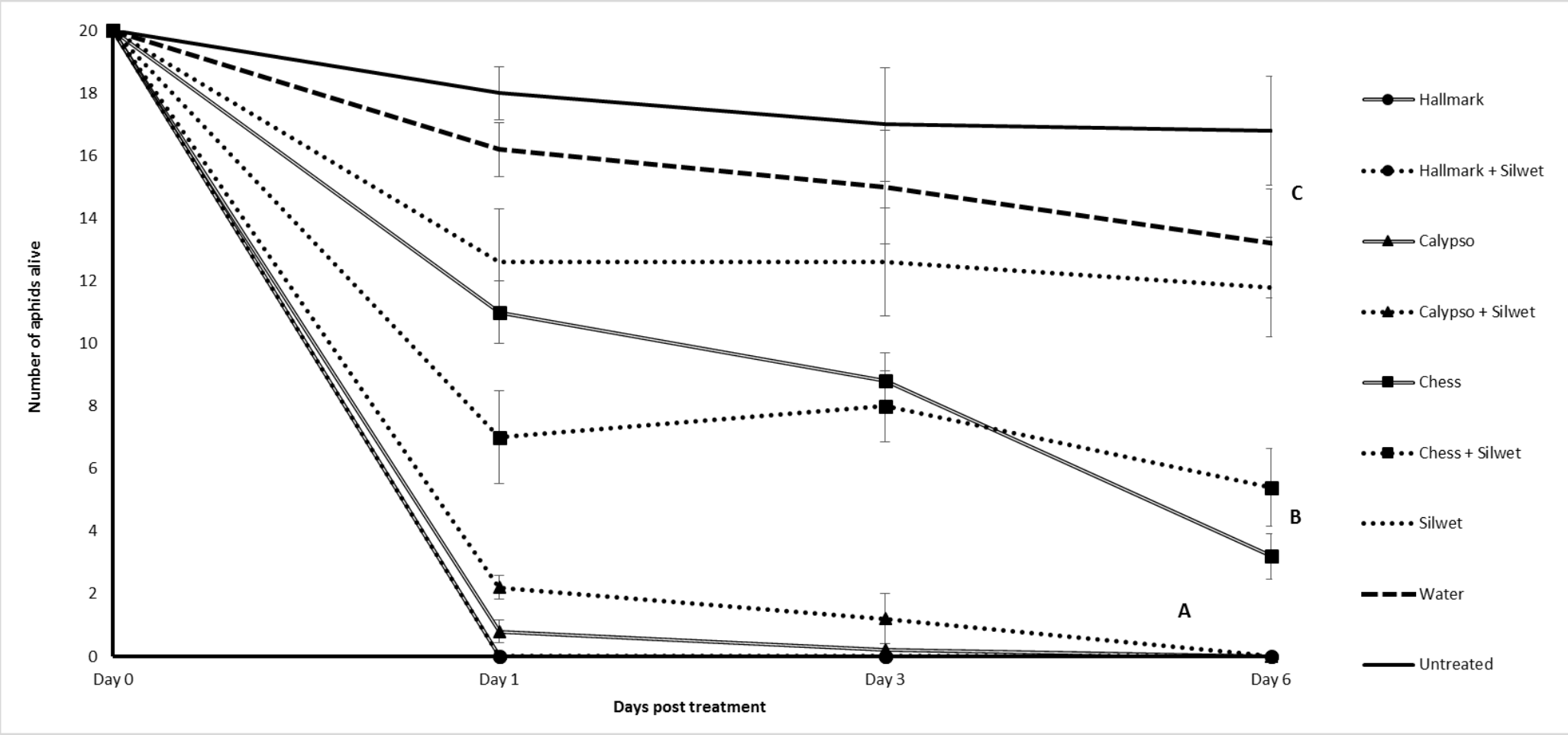


Figure 4.9. *Macrosiphum euphorbiae* survival in the leaf bioassay when leaves were sprayed before being infested with aphids. Each uppercase letter denotes a group of treatments which are not significantly different from one another at the *P* = 0.05 level. 'Group A' includes Hallmark, Hallmark + Silwet, Calypso, Calypso + Silwet; 'Group B' includes Chess and Chess + Silwet; 'Group C' includes Water, Untreated and Silwet. Error bars represent +/- SEM.

Table 4.5. Mean numbers of *Macrosiphum euphorbiae* per leaf when leaves were sprayed after being infested with aphids. The same lower case letter denotes that treatments were not significantly different from each other (analysis completed on transformed data) within the same dates. The upper case letters denote overall significance of each treatment

Treatment/Day	**Mean numbers of aphids**										
	Day 0	Day 1			Day 3			Day 6			
	Alive	Alive	Moribund	Dead	Alive	Moribund	Dead	Alive	Moribund	Dead	
Hallmark	20a	0d	0.6	19.4	0d	0	20	0c	0	20	A
Hallmark + Silwet	20a	0d	0.8	19.2	0d	0	20	0c	0	20	
Calypso	20a	0d	1.6	18.4	0d	0	20	0c	0	20	
Calypso + Silwet	20a	0d	1.6	18.4	0d	0	20	0c	0	20	
Chess	20a	6.8b	1	12.2	7.4b	0	12.6	1.6b	1	17.4	B
Chess + Silwet	20a	0.6d	0.2	19.2	0d	0	20	0c	0	20	A
Silwet	20a	3c	0.2	16.8	0.2c	0	19.8	0c	0	20	
Water	20a	16.2a	0	3.8	16a	0	4	14.2a	0	5.8	C
Untreated	20a	17.8a	0	2.2	14.4a	0.2	5.4	11a	0.2	8.8	

Table 4.6. Mean numbers of *Macrosiphum euphorbiae* per leaf when leaves were sprayed before being infested with aphids. The same lower case letter denotes treatments were not significantly different from each other (analysis completed on transformed data) within the same dates. The upper case letters denote overall significance of each treatment

Treatment/Day	**Mean numbers of aphids**										
	Day 0	Day 1			Day 3			Day 6			
	Alive	Alive	Moribund	Dead	Alive	Moribund	Dead	Alive	Moribund	Dead	
Hallmark	20a	0g	0.2	19.8	0e	0	20	0c	0	20	A
Hallmark + Silwet	20a	0g	0.8	19.2	0e	0.2	19.8	0c	0	20	
Calypso	20a	0.8f	5.6	13.6	0.2de	0.2	19.6	0c	0.2	19.8	
Calypso + Silwet	20a	2.2e	4.6	13.2	1.2d	0.2	18.6	0c	0.2	19.8	
Chess	20a	11c	0.4	8.6	8.8c	0.4	10.8	3.2b	0.4	16.4	B
Chess + Silwet	20a	7d	2	11	8bc	1	11	5.4b	0.4	14.2	
Silwet	20a	12.6bc	1.6	5.8	12.6ab	0	7.4	11.8a	0	8.2	C
Water	20a	16.2ab	0.2	3.6	15a	0	5	13.2a	0	6.8	
Untreated	20a	18a	0	2	17a	0	3	16.8a	0	3.2	

Discussion

- Note that strawberry crops are not permitted to be sprayed at full label rates when applied together with Silwet L-77 and should instead be sprayed at 50% of the full label rate.
- Strawberry plants (cv. Driscoll's® Diamond™) collected from a commercial strawberry crop in Staffordshire in late March were infested with *M. euphorbiae.* Plants were infested with additional *M. euphorbiae* collected from commercial strawberry crops also grown in Staffordshire.
- In the polytunnel experiment aphid numbers on the water control were approximately 20/half plant at the start of the experiment but had increased to approximately 80/half plant by the end of the experiment.
- Results for lambda-cyhalothrin (e.g. Hallmark) in the polytunnel experiment were broadly similar to those recorded in AHDB Horticulture project SF 140. When this insecticide was applied on its own it had reduced aphid numbers by 70% just 24 hours after spray application and by 80% when applied with the wetter Silwet L-77 over the same period. Lambda-cyhalothrin with or without the addition of Silwet L-77 has 100% control of *M. euphorbiae* by the time of the final aphid assessment completed 15 days after spray application. The rapid control of aphid populations reflects the mode of action of this insecticide and the fact that unlike for *A. gossypii*, resistance to pyrethroid insecticides has not been reported for *M. euphorbiae* (Foster & Blackshaw, 2012; Foster pers. comm.).
- Previous work in AHDB Horticulture project SF 140 and results presented here indicate that pyrethroids such as lambda-cyhalothrin effectively control overwintered *M. euphorbiae*. Although not IPM compatible, these insecticides, as well as natural pyrethrins, may be useful as an autumn insecticide application to reduce overwintering aphid populations.
- The insecticide thiacloprid (e.g. Biscaya), with or without the addition of Silwet L-77, had reduced aphid numbers by over 60% just 24 hours after spray application but aphid populations had recovered to more than 20 aphids/half plant by the end of the polytunnel experiment. This result suggests that the activity of thiacloprid on overwintered strawberry plants may be more reliant on contact activity than systemic activity. Indeed, for another neonicotinoid insecticide, imidacloprid, foliar sprays to control aphids on banana have been shown be effective on young or old leaves but less effective on leaves emerging after spray application (Robson et al., 2007). It is not known whether this observation applies equally to thiacloprid on strawberry but would provide an explanation for the results observed.
- The insecticide pymetrozine, with or without Silwet L-77, had only slightly reduced aphid numbers (20-40% reduction) three days after spray application but after 15 days numbers

had recovered and were similar to Silwet L-77 applied on its own or the water control in the polytunnel experiment. As a selective feeding blocker pymetrozine was expected to be slower acting than the other insecticides tested but the recovery of aphid populations suggests that the slight control seen was through contact (pymetrozine may work through direct contact with the insect e.g. Fuog et al., 1998) as well as through systemic activity. It is not known, however, if there was poor uptake of pymetrozine or if the poor control was due to aphids surviving on new growth not protected by the insecticide. A sample of aphids was sent to Rothamsted Research and was confirmed to be susceptible to this insecticide, although a slight tolerance was noted (Foster pers. comm.).

- Pymetrozine (e.g. Plenum) was also found to be less effective than lambda-cyhalothrin, for example, in project SF 140. Interestingly, pymetrozine was also found to be less effective than another selective feeding blocker, flonicamid, in SF 140. Flonicamid is not currently permitted for use in strawberry crops but would provide a useful IPM compatible alternative to pymetrozine.
- It has been suggested that the stability of pymetrozine is affected by water pH. Syngenta do not consider water pH to be a factor in determining the efficacy of pymetrozine unless this is unusually alkaline or acidic or the spray was prepared more than 24 hours in advance of application. The water pH at Harper Adams University is only slightly alkaline (pH 7.5) and the spray was prepared on the day of use and so this is unlikely to an explanation for the results seen.
- For the use of pymetrozine (e.g. Plenum) of Brassica crops with waxy leaves it is recommended to use an approved seed oil or methylated seed oil derivative adjuvant (minimum content 90% w/w) in order to improve penetration and uptake by the plant. It is likely that overwintered strawberry leaves present a similar problem in achieving good uptake of pymetrozine. Further work could investigate the potential of oil base adjuvants to improve early season efficacy of this insecticide.
- There is little evidence from the polytunnel experiment that the addition of a wetter, in this case Silwet L-77, was important in improving the efficacy of the insecticides tested. In addition, Silwet L-77 did not itself reduce aphid numbers in this experiment. It is, however, worth noting that in SF 140 that lambda-cyhalothrin and pyrethrum significantly reduced numbers of *M. euphorbiae* for at least one week when applied at 50% of the full label rate.
- Based on the positions of the aphids on the plants it is noticeable that the proportion of aphids recorded on the crowns increased following the spray application. This suggests that aphid mortality was higher on more exposed parts of the plant than in the crown. The proportion of aphids recorded in the crown then declined for each treatment (excluding lambda-cyhalothrin with or without Silwet L-77, where aphid numbers were low) as aphid

numbers on the underside of leaves and flower stalks increased in real terms and relative to the crown.

- Results from the leaf bioassays largely confirmed those from the polytunnel experiment. Lambda-cyhalothrin, with or without Silwet L-77, gave 100% control regardless of whether aphids were directly sprayed or came into contact with spray residue on the leaf surface. Speed of kill was, however, improved where aphids were directly sprayed (100% mortality less than three days after spray application). Where aphids only came into contact with spray residue 100% mortality was only achieved after six days. Results were similar for thiacloprid with evidence of an improved speed of kill where aphids were directly sprayed. Pymetrozine gave similar levels of control when aphids were directly sprayed (87% control) or were placed onto treated leaves (82% control) after six days.
- Silwet L-77 was effective against *M. euphorbiae* when aphids were directly sprayed. This was apparent when Silwet was applied on its own and may explain why pymetrozine gave 100% control within three days when it was applied with this wetter. This result is perhaps not surprising as active ingredient, trisiloxane ethoxylate, is known for example to be toxic to immature whitefly (Mascarin et al., 2013).

Conclusions

- Spray coverage using an air assisted knapsack sprayer was good on upper leaf surfaces but relatively poor on lower leaf surfaces, where the highest numbers of aphids were recorded.
- Lambda-cyhalothrin effectively controls the potato aphid, *M. euphorbiae*, on overwintered strawberry plants for at least two weeks under polytunnel conditions.
- Thiacloprid was not effective against *M. euphorbiae* on overwintered strawberry plants but did show good efficacy in leaf bioassays.
- Pymetrozine was not effective against *M. euphorbiae* on overwintered strawberry plants and was not 100% effective in leaf bioassays.
- Silwet L-77 did not improve the efficacy of any of the insecticides tested in the polytunnel experiment but had insecticidal properties when it directly contacted the aphids in the laboratory bioassay.
- Plant protection products applied with Silwet L-77 should only be used at 50% of the full label rate on strawberry. Results presented here used full label rates of each insecticide.
- Growers require a wider range of effective IPM compatible controls of *M. euphorbiae* in spring.

Future work

A slight tolerance to pymetrozine was reported for aphids used in this study and so an investigation of the clonal variation of *M. euphoribiae* to survive periods of starvation and susceptibility to this insecticide and flonicamid, an insecticide with a very similar mode of action, would be useful. Aphid pests of strawberry are often effectively controlled by naturally occurring predators and parasitoids as well as through releases of biocontrols. Improved understanding of aphid numbers and associated natural enemies early in the season would enable growers to minimise the use of early season insecticide applications targeted against *M. euphorbiae.* Where early season releases of aphid biological controls, both predators e.g. *Aphidoletes aphidimyza*, and parasitoids e.g. *Praon volucre* are made there is currently little understanding of efficacy when temperatures fluctuate between those that permit or preclude foraging activity. Knowledge of the efficacy of biological controls under fluctuating temperatures that mimic early spring conditions would, therefore, be of use to growers before releasing predators and parasitoids into crops.

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Knowledge and Technology Transfer

23 November 2016 - Tom Pope, David Buss, Chantelle Jay, Jean Fitzgerald - EMRA/AHDB Horticulture Soft Fruit Day

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Appendix 1: Pesticides applied to experimental planting in 2016

Date	All in 1000 l / ha	
27.05.16	Parat	3 kg
10.06.16	Frupica	0.6 l
	Teldor	1.5 kg
17.06.16	Kindred	0.6 l
	Pek Acid	5 kg
24.06.16	Scala	2 l
	Teldor	1.5 kg
30.06.16	Nimrod	1.4 l
	Teldor	1.5 kg
	Tracer	0.25 l *
08.07.16	Scala	2 l
	Teldor	1.5 kg
	Pek Acid	5 kg
28.07.16	Amistar	1 l
	Systhane	0.45 l
	Teldor	1.5 kg
12.08.16	Topenco	0.5 l
16.08.16	Tracer	0.25 l *
19.08.16	Frupica	0.6 l
26.08.16	Systhane	0.45 l
02.09.16	Topenco	0.5 l
	Pek Acid	5 kg
09.09.16	Scala	2 l
16.09.16	Systhane	0.45 l
23.09.16	Signum	1.8 kg
	Systhane	0.45 l
30.09.16	Switch	1 kg
07.10.16	Scala	2 l
14.10.16	Nimrod	1.7 l

*Insecticide requested to reduce numbers of thrips other than WFT

APPENDIX 2: Activities and Milestones schedule

ID	Description	Organisation Responsible	YEAR 1				YEAR 2				Milestone Date mm/dd/yyyy
			Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
1	**Develop effective biological methods for managing western flower thrips, Frankliniella occidentalis (WFT), compatible with pesticide use against SWD.**										
1.1.1	Develop practical methods for assessment of populations of WFT and N. cucumeris suitable for use by agronomists and growers	EMR, Keele									31 Mar 2016
1.1.2	Development of attendant dynamic WFT-A. cucumeris-temperature-damage thresholds for use by growers/agronomists	EMR, Keele									31 Mar 2017
1.2.1	To investigate the use of adjuvants to increase the number of spores adhering to the cuticle of WFT so increasing mycosis and biological efficacy	EMR, CABI									31 Mar 2016
1.2.2	To investigate whether application of semiochemicals improves spore uptake by WFT and efficacy of EPF's	EMR, CABI, NRI									31 Mar 2017

2	**Refine pest control programmes on strawberry, integrating pesticides with phytoseiid mites.**											
2.1	Investigate how to minimise the adverse effects of pesticides used for control of other pests (SWD, capsid bugs) on biocontrol of WFT by A. cucumeris	EMR										31 Mar 2016
3	**Develop IPM compatible controls for European tarnished plant bug, Lygus rugulipennis, common green capsid, Lygocoris pabulinus, and strawberry blossom weevil, Anthonomus rubi.**											
3.1	Develop practical pesticide management programme for capsid bugs, in field, that does not interfere with predatory mite releases											31 Mar 2017
4	**Improve insecticide control of the potato aphid, Macrosiphum euphorbiae, so as to be more compatible with IPM programmes.**											
4.1	Determine the efficacy of insecticides used to control M. euphorbiae when diluted by use of higher water volumes, persistence of these applications and efficacy at lower temperatures	Harper Adams										31 Mar 2017

