Project Title:	Improving integrated pest management in strawberry
Project number:	SF 156
Project leader:	Michelle Fountain, NIAB-EMR, New Road, East Malling, Kent ME19 6BJ
Report:	Annual report, March 2016
Previous report:	None
Key staff:	Jerry Cross, Jean Fitzgerald, Chantelle Jay, Phil Brain, David Buss, Adrian Harris (NIAB-EMR) Bryony Taylor, David Moore, Emma Thompson (CABI) William Kirk, Clare Sampson (Keele University) David Hall, Dudley Farman (NRI) Tom Pope (Harper Adams University) Robert Irving (ADAS), Neil Audsley (Fera)
Location of project:	East Malling Research, CABI and grower sites
Industry Representative:	Marion Regan, Hugh Lowe Farms
Date project commenced:	01 April 2015
Date project completed (or expected completion date):	31 March 2020

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

[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.

Michelle Fountain

Research Leader in Entomology

East Malling Research, New Road, East Malling, Kent ME19 6BJ

Signature ..... Date ...01/03/16...

Report authorised by:

Jerry Cross

PPESCM Programme Leader

East Malling Research, New Road, East Malling, Kent ME19 6BJ

Signature ..... Date ..03/03/16

[Name]

[Position]

[Organisation]

Signature ..... Date .....

Contents

GROWER SUMMARY

Headline

The overall aim of this project is to increase the efficacy of existing IPM measures and explore new and emerging controls for the most damaging strawberry pests whilst maintaining control of spotted wing drosophila, *Drosophila suzukii* (SWD), thus enhancing the growth and profitability of the UK strawberry industry.

Within this project, it is planned to work on four differing 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.
    1.1. Improve the reliability of biocontrol of WFT with predatory mites, particularly *Neoseiulus cucumeris.*
    1.2. Develop effective approaches to the use of entomopathogenic fungi (EPF) for control of WFT.
    1.3. Investigate more effective predators for 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. The first year's work concentrated solely on Objectives 1 and 2, so only these are reported on in this first annual report.

Objective 1 - Develop effective biological methods for managing western flower thrips, Frankliniella occidentalis (WFT), compatible with pesticide use against SWD

Headline

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

Background and expected deliverables

In the first year of the project the major target was western flower thrips (WFT). Except for SWD, WFT is currently the most serious pest of UK strawberry and financial losses can be high. WFT is difficult to control because it is resistant to all crop protection products currently available to UK strawberry growers.

At present, growers rely on introductions of the predatory mite *Neoseiulus cucumeris* (formerly called *Amblyseius cucumeris*) to control WFT. It is relatively inexpensive to mass produce and can be introduced in large numbers but only predates first-instar WFT larvae. However, biocontrol with *Neoseiulus cucumeris* sometimes fails. This is usually due to 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*. Growers find it difficult to assess whether *N. cucumeris* populations have established adequately and whether they are in balance with 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 ratio thresholds for interpreting relative populations.

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. There are three existing EPF products currently available in the UK:

1) Naturalis L (Belchim): sprayable formulation containing *Beauveria bassiana* approved for control of aphids, beetles and whitefly in protected edibles and ornamentals.

2) Met52 granular biopesticide (Fargro); granular formulation containing *Metarhizium anisopliae* incorporated into growing media for control of a wide range of pests including thrips

and vine weevil in a wide range of crops, including strawberries. A liquid (sprayable) formulation of this product is under development by the parent company, Novozymes.

3) Mycotal (Koppert); sprayable formulation containing *Lecanicillium lecanii* approved for control of whitefly in a wide range of protected crops, including strawberries.

Unfortunately, grower experience with spraying these products for controlling thrips in strawberries has been disappointing. Another approach for exploiting EPFs is to apply them to the growing media.

Expected deliverables in Year 1:

- Development of a rapid and easy method of assessing populations of western flower thrips and *N. cucumeris* and their distribution on flowers and fruits suitable for use by agronomists.
- Determine whether the addition of adjuvants significantly improves spore distribution, adhesion and biological efficacy of EPFs against WFT.

Summary

To gain the background information needed to develop effective sampling strategies and treatment thresholds for WFT, samples of individual flowers and 'button' fruit were collected from two commercial crops, where *N. cucumeris* were being released, every two weeks from April to September. In addition, replicate samples of different plant parts, from unopened flowers to ripe fruit, were collected twice from each of two plantings to determine the distribution of pest and predator over the plant. One-off collections of flowers and fruit were also made from 12 crops that had different levels of WFT on the plants. Numbers of WFT and *N. cucumeris* were extracted and recorded in the lab and the data used to determine the most effective sampling strategy for *N. cucumeris* and to model the interaction between pest and predator.

In experiments to develop a field-based extraction/monitoring system, three fumigants were tested in replicated experiments for their efficacy in extracting WFT and *N. cucumeris* from flowers and fruit. The most effective fumigant was used successfully in a prototype extraction/monitoring device in the field. Further development of this system will be done in 2016.

To determine if a method could be developed to enable *N. cucumeris* to be more easily visualised on plants, lab experiments were undertaken to assess the efficacy of staining the mites with CalcoRed, but this proved to be ineffective.

In 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 Q1 2016 and will be 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. Samples were also collected for testing by a molecular technique (QPCR); this method requires further development to give reliable results and CFU counts should be used for future assessments. Results from these experiments have been used to refine protocols for use in laboratory and field experiments in 2016.

The main findings of the research from year 1 are:

- Potentially the most appropriate plant part to sample to assess numbers of *N. cucumeris* in strawberry crops is young button fruit.
- Mid aged/old flowers are the most appropriate stages to sample to assess numbers of adult WFT present.
- There was no consistent pattern of distribution for thrips larvae, with larvae being found in flowers and on fruit
- Thrips and *N. cucumeris* were extracted easily from plant samples in the field using the fumigant methyl isobutyl ketone (MIK).
- A prototype monitoring device making use of the MIK fumigant extraction method was constructed and will be field tested in 2016.
- A preliminary analysis of distribution of *N. cucumeris* and WFT was completed. The maximum mean number of WFT in a sample of a given size to ensure the probability of less than 5% fruit damage with different damage thresholds has been calculated.
- Improved bioassay protocols have been developed for assessing the efficacy of entomopathogenic fungi against WFT in 2016.

Financial benefits

Western flower thrips, *Frankliniella occidentalis* (WFT), causes bronzing of the 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

- Inspect 'button' fruit to determine if *N. cucumeris* have established and assess numbers of thrips larvae in the crop.
- Continue to monitor thrips adults in mid-aged flowers.
- Monitor and make repeated releases of *N. cucumeris* as necessary.

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

Headline

- Growers should be aware that repeated applications of some fungicides can cause significant reductions in predatory mite populations

Background and expected deliverables

The efficient and successful use of predatory mites relies on careful coordination of crop protection product spray strategies to maintain predators in the crop. This coordination forms part of Integrated Pest Management (IPM) and can work very successfully. However, strawberries are vulnerable to attack by other pests, including SWD, leading to an increase in use of crop protection products which are not always compatible with IPM programmes. Information is needed on how control products used for SWD and other pests and diseases can be used without disrupting interactions between WFT and *N. cucumeris*. Studies also suggest that tank mixtures of fungicides and insecticides can have synergistic effects on mite toxicity whilst spray timing in relation to mite release may also be crucial.

Expected deliverables in year 1:

- Demonstration of which tank mixes are harmful to *N. cucumeris* on strawberry.

Summary

To determine if the reduction in *N. cucumeris* numbers often seen in commercial crops is due in part to applications of various crop protection products, the effect of repeated applications of three commonly used tank mixes of fungicides that are classed as harmless to predatory mites were compared to spinosad and assessed in a replicated field experiment. The fungicide mixes included Amistar (azoxystrobin) & Rovral (iprodione), Nimrod (bupirimate) & Teldor (fenhexamid) and Signum (boscalid + pyraclostrobin) & Systhane (myclobutanil). The aphicide/fungicide mix of Aphox (pirimicarb) & Rovral (iprodione) was also included. These

mixes were chosen in consultation with growers and assessed in a replicated field experiment. It should be noted that products already known to be toxic to predatory mites were excluded. *N. cucumeris* was released onto the plants before the trial began and three applications of the fungicide mixes were applied, with assessment of predator numbers made after each application.

No significant treatment effects were found for the pre-assessment and first two assessments when analysed separately. However the third assessment showed a significant reduction in *N. cucumeris* numbers exposed to repeated applications of Nimrod/Teldor and Signum/Systhane. Tracer and Aphox/Rovral did not affect numbers of *N. cucumeris* on the strawberry leaves. A repeated measures analysis of the entire sample set showed a reduction in phytoseiid mite numbers through the course of the trial for Nimrod/Teldor and Signum/Systhane, as well as Aphox/ Rovral.

A widely used website associated with a biocontrol company defines Nimrod (bupirimate) and Signum (boscalid + pyraclostrobin) as safe to *N. cucumeris* and Teldor (fenhexamid) as only slightly harmful. Systhane (myclobutanil), was described as safe to the related species *Amblyseius californicus*. Growers could therefore assume that application of these compounds would be fully compliant with an IPM programme, when in fact these results suggest that this might not be the case where repeated applications are made.

Aphox was already reported as harmful to *N. cucumeris* (Koppert side effects guide, 2016). Although Aphox use on strawberries will be restricted from 2016, applications of Aphox could explain past biological control failures.

Each treatment was applied three times and it was only after the third application that significant differences in phytoseiid mite numbers were found. Hence, small cumulative impacts on *N. cucumeris* populations might be significant over time. A similar pattern has been reported for the use of phosalone on apple trees, where a single application had no effect on predatory mite numbers, but two applications reduced the population (Raudonis et al, 2004).

It is also possible that the compounds in tank mixes combine additively or even synergistically, so that the effect is greater than that of each individually, but this needs to be confirmed.

In summary, these results suggest that repeated applications of fungicides can have a detrimental effect on predatory mite populations.

Financial benefits

The two most damaging pests to strawberry at present are the spotted wing drosophila and western flower thrips. If left uncontrolled, both can cause total loss of crop.

If certain crop protection products are used to control spotted wing drosophila, they can disrupt the biological control programme employed for western flower thrips and other pests of strawberry, thereby resulting in serious or total crop loss caused by WFT and other pests. For this reason, it is important to research and develop improved integrated pest management programmes which avoid such an imbalance in the pest/predator ratio.

Similarly, if the typical crop protection spray programme used for other pests or fungal diseases is not sympathetic to the predatory mites being employed to control WFT and other strawberry pests, then IPM programmes will break down and lead to major crop losses.

Assuming a typical return for strawberries of £2.30/kg to growers (Defra Basic Horticultural Statistics 2014) and a yield of 20 tonnes/ha, then total crop loss would lead to a financial loss of £46,000/ha. The development of a reliable integrated pest management programme which does not fail, can therefore save growers from making such heavy losses.

Action points for growers

- Consider reducing the application of tank mixes of fungicides as these may be harmful to introduced predatory mites.

SCIENCE SECTION

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

Introduction

We hypothesise that *Neoseiulus cucumeris* can control WFT effectively and that control failures are likely to be due to inadequate numbers being released and the distribution of predators within the crop and on the plants. Currently growers find it difficult to assess whether *N. cucumeris* populations have established in the crop and whether they are in balance with 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 for interpreting relative populations.

Task 1.1.1. Develop practical methods for assessment of populations of WFT and N. cucumeris suitable for use by agronomists and growers (EMR, Keele Year 1)

1.1.1.1: To determine the optimum age of everbearer strawberry flowers/fruitlets to sample for quantifying N. cucumeris and WFT populations and how this is affected by WFT population density.

Materials and methods

Two commercial everbearer crops in Kent were selected for study based on the population density of WFT present. One planting had very low numbers of WFT (Site 1). This site had a first year crop of the variety Jubilee, planted in coir in pots on tabletops. The second was a first year planting of the variety Triumph, planted into raised beds that had been used in 2014 (Site 2). This planting had higher numbers of WFT, the planting had had 5-6 adult WFT per flower in 2014. On both plantings *N. cucumeris* had been introduced in spring. The *N. cucumeris* release programmes for each site are shown in Table 1. At Site 1, in addition to *N. cucumeris*, *Hypoaspis miles* was released on 16 March (88 / m^2), and *Orius* on 4 and 18 May and 1 June (3 / m^2). At Site 2, *Orius* (3 / m length of bed) were released on 1 July. During 2015 at Site 1, in addition to fungicides, applications of Aphox were made on 10 and 22 April, Floramite on 22 April, Apollo on 22 April and Calypso on 31 May. At Site 2 applications of Calypso were made on 15 & 22 May. See Appendix 1 for full spray programmes; Site 1 below is shown in the Appendix as Site 8 and Site 2 as Site 7 (i.e. the one-off samples from Sites 7 and 8 are from the same sites sampled every fortnight throughout the season.

Table 1. *N. cucumeris* release programmes at Sites 1 & 2 in Kent

Site 1			Site 2		
Date	Predator	Release rate	Date	Predator	Release rate
30 March	*N. cucumeris*	1 sachet / 2m*‡	18 April	*N. cucumeris*	1 sachet / 2m
6 April	*N. cucumeris*	1 sachet / 2m	29 May	*N. cucumeris*	50 / plant
13 April	*N. cucumeris*	1 sachet / 2m	04 June	*N. cucumeris*	50 / plant
8 June	*N. cucumeris*	10 / plant	01 July	*N. cucumeris*	50 / plant
13 June	*N. cucumeris*	10 / plant			
27 June	*N. cucumeris*	10 / plant			
6 July	*N. cucumeris*	10 / plant			

* length of bed

‡ *N. cucumeris* sachets from Bioline are reported to contain c. 1000 mites when they are sealed. The mites reproduce within the sachets and at 24ºC emerge at around 450 per week over six weeks or longer. One sachet per 2m length of bed is the recommended commercial rate of use.

When all flower and fruit development stages were present 4 replicate bulk samples of 20 sampling units (buds/flowers/fruitlets) were collected directly into 70% alcohol at each site. Sample units assessed were: 1) unopened flowers; 2) newly opened flowers; 3) middle aged flowers; 4) oldest flowers (with petals still attached); 5) young 'button' green fruits with calyx attached; 6) mature green fruits with calyx attached; 7) young white fruits with calyx attached; 8) ripe fruits with calyx attached. Samples were taken to the lab and *N. cucumeris* and WFT extracted by washing the samples in alcohol. Total numbers of each life stage of *N. cucumeris* and WFT present in each sample were recorded under a stereo microscope. This sampling protocol was repeated three times at Site 2 (12, 28 May and 18 August) and twice at Site 1 (28 May and 18 August).

Counts were square root transformed and means were compared by ANOVA.

Results

Individual sample data: Mean number (with standard error of the mean) of thrips and mites per 20 sample units from Site 1 are shown in Figs 1-4. There were very low numbers of thrips at Site 1 in May (Fig. 1) with less than 1 adult per 20 old flowers. Numbers increased during the season but in August there was a mean of just over 2 adults per 20 mid-aged flowers (Fig. 3). Conversely there were high numbers of *N. cucumeris* present in May (Fig. 2), with highest

numbers recorded on the old flowers (mean of 70 per 20 units) and button fruit (Photograph 1, below) (mean of 62 per 20 units). By 18 August numbers of *N. cucumeris* had dropped, with highest numbers (mean of 11 per 20 units) on the ripe fruit (Fig. 4). The unopened and just opened flowers had less than 1 mite per 20 units.

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

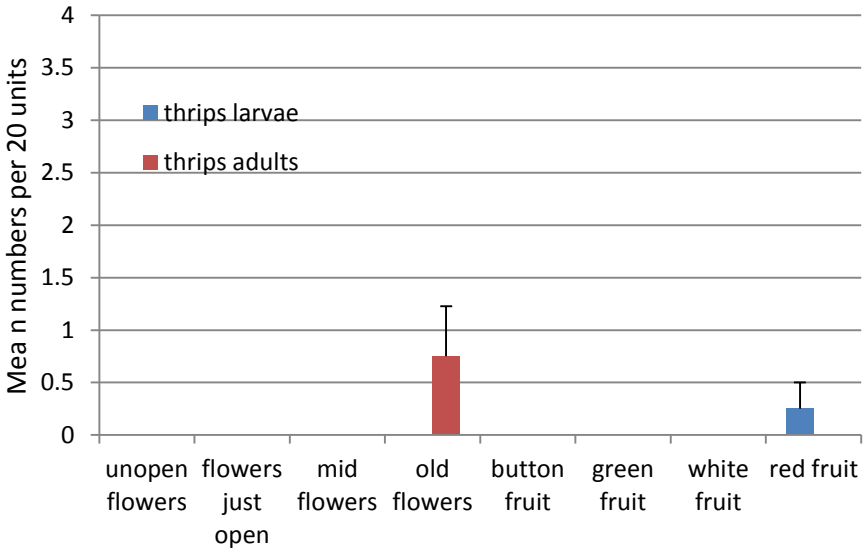

Figure 1. Mean numbers (± SE) of thrips per 20 sample units at Site 1 on 28.05.15

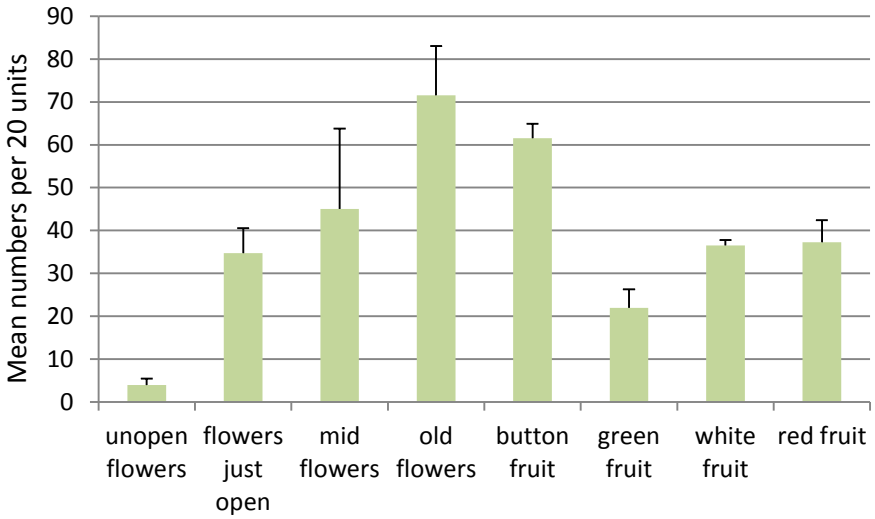

Figure 2. Mean numbers (± SE) of *N. cucumeris* per 20 sample units at Site 1 on 28.05.15

Analysis of the counts of thrips and N. cucumeris at Site 1 on 28 May are shown in Table 2. Significantly higher numbers of *N. cucumeris* were recorded on old flowers and button fruit.

Table 2. Means of sqrt(counts) and back-transformed means for thrips and *N. cucumeris* at Site 1 on 28.05.15. Numbers on plant parts with the same letter in the column are not significantly different

	Thrips adults		Thrips larvae		*N. cucumeris*	
Stage	Mean	Backtrans	Mean	Backtrans	Mean	Backtrans
Unopen flowers	0.000b	0.000	0.000a	0.000	1.720d	2.960
Flowers just open	0.000b	0.000	0.000a	0.000	5.830c	33.985
Mid aged flowers	0.000b	0.000	0.000a	0.000	6.293bc	39.606
Old flowers	0.604a	0.364	0.000a	0.000	8.371a	70.080
Button fruit	0.000b	0.000	0.000a	0.000	7.833ab	61.362
Green fruit	0.000b	0.000	0.000a	0.000	4.629c	21.431
White fruit	0.000b	0.000	0.000a	0.000	6.038bc	36.463
Red fruit	0.000b	0.000	0.250a	0.063	6.062bc	36.744
Sig of Stage	0.027		0.455		<0.001	
SED	0.179		0.125		0.907	
LSD	0.370		0.258		1.872	

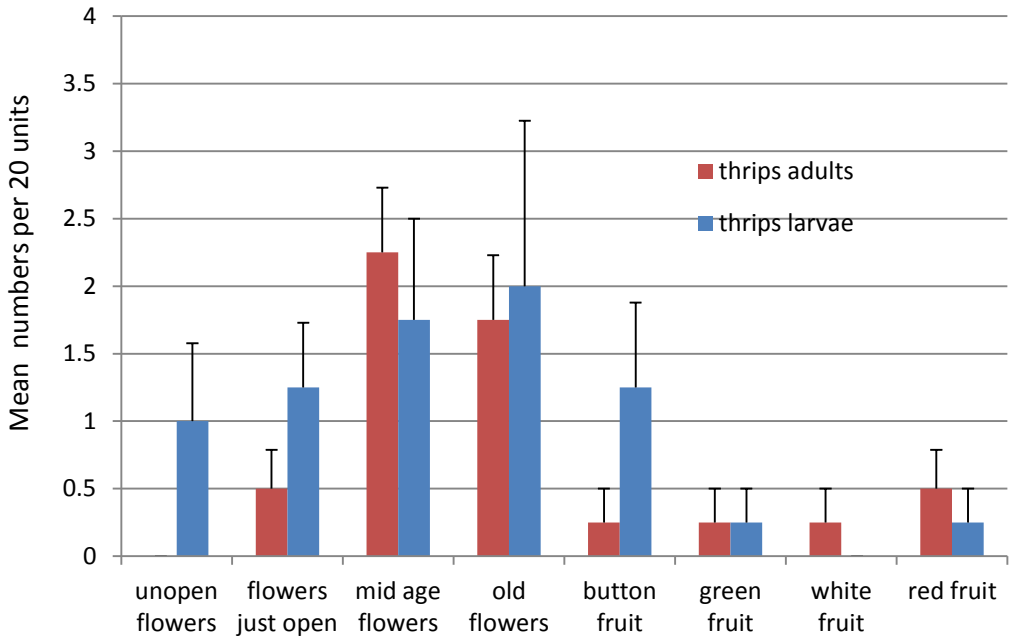

Figure 3. Mean numbers (± SE) of thrips per 20 sample units at Site 1 on 18.08.15

Analysis of numbers of thrips and N. cucumeris at Site 1 on 18 August are shown in Table 3. Significantly higher numbers of thrips adults were recorded on mid aged and old flowers and higher numbers of larvae were found on mid aged flowers, however there was no significant difference among all flower stages and button fruit. Highest numbers of N. cucumeris were recorded on old flowers and green, white and red fruit.

Table 3. Means of sqrt(counts) and back-transformed means for thrips and *N. cucumeris* at Site 1 on 18.08.15. Numbers on plant parts with the same letter in the column are not significantly different

	Thrips adults		Thrips larvae		*N. cucumeris*	
Stage	Mean	Backtrans	Mean	Backtrans	Mean	Backtrans
Unopen flowers	0.000b	0.000	0.707ab	0.500	0.433d	0.188
Flowers just open	0.500b	0.250	0.957ab	0.916	0.604d	0.364
Mid aged flowers	1.470a	2.160	1.250a	1.563	2.977bc	8.863
Old flowers	1.287a	1.655	0.992ab	0.984	3.243ab	10.517
Button fruit	0.250b	0.063	0.933ab	0.871	1.877c	3.524
Green fruit	0.250b	0.063	0.250b	0.063	3.258ab	10.613
White fruit	0.250b	0.063	0.000b	0.000	3.146ab	9.898
Red fruit	0.500b	0.250	0.250b	0.063	4.344a	18.870
Sig of Stage	0.001		0.153		<0.001	
SED	0.322		0.482		0.606	
LSD	0.665		0.995		1.251	

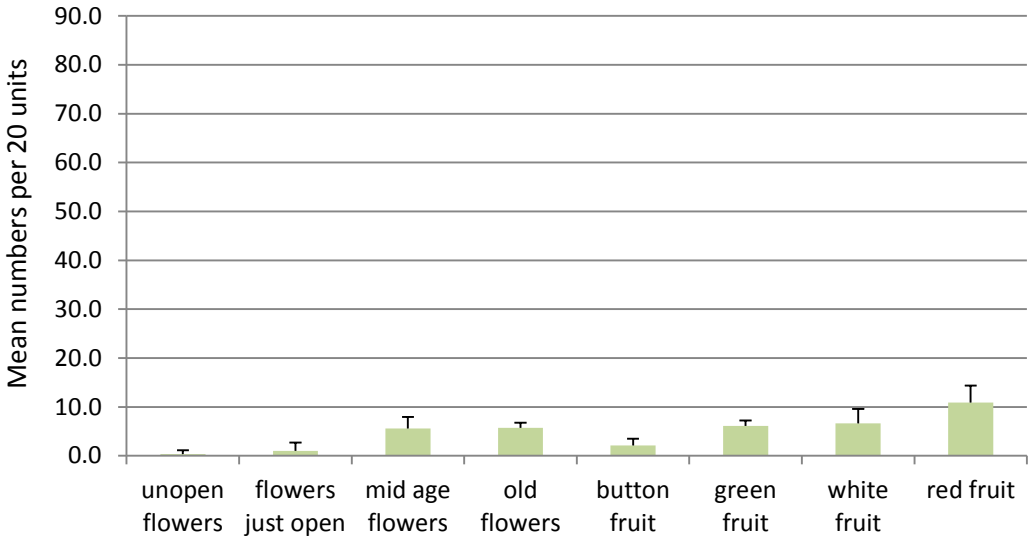

Figure 4. Mean numbers (± SE) of *N. cucumeris* per 20 sample units at Site 1 on 18.08.15

Mean number of thrips and mites per 20 sample units from Site 2 are shown in Figs 5-10. At the first sample on 12 May there were no red fruit present in the crop. Thrips numbers were higher at this site than Site 1. Numbers of thrips adults were highest on the mid-aged flowers (Fig. 5) with a mean of nearly 12 per 20 flowers. Thrips larvae were highest on the button fruit, at a mean of 7 per 20 fruits. Highest numbers of *N. cucumeris* were recorded on the green fruit (mean of 24 per 20 fruits) (Fig. 6). By 28 May numbers of thrips had increased with highest numbers of adults on the mid age (mean of 26 per 20 units) and old flowers (mean of 31 per 20 units) (Fig. 7). Highest numbers of thrips larvae were recorded on the old flowers (mean of 30 per 20 units) and button fruit (mean of 24 per 20 units). Numbers of *N. cucumeris* were lower than at Site 1 and were highest on the button, green and white fruit, with a mean of 27 per 20 units on the button fruit (Fig. 8). By 18 Aug thrips numbers had increased further with a mean of 95 adults per 20 mid aged flowers (i.e. around 5 per flower) and 30-42 larvae per 20 open flowers of different ages (Fig. 9). Number of *N. cucumeris* had declined with highest numbers (mean of around 3 per 20 units) recorded on the red fruit (Fig. 10).

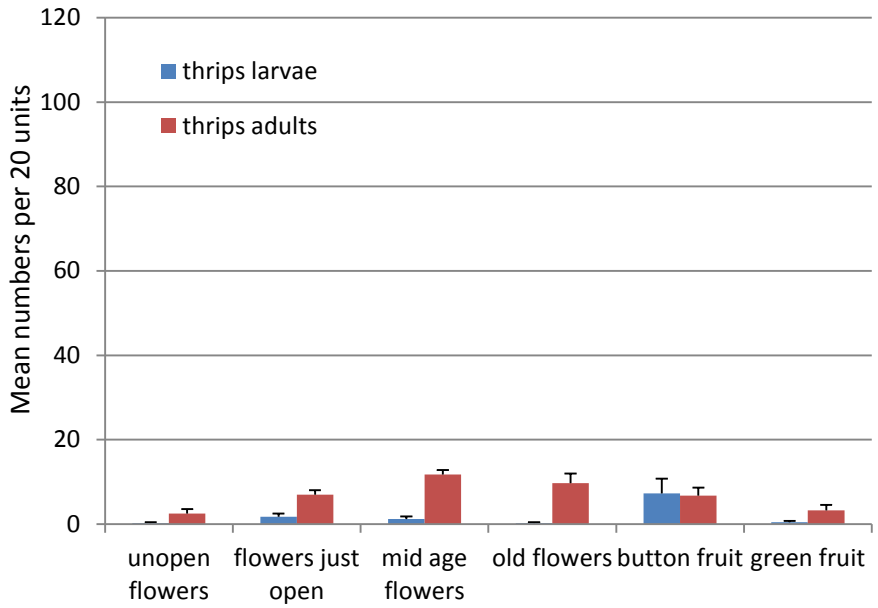

Figure 5. Mean numbers (± SE) of thrips per 20 sample units at Site 2 on 12.05.15

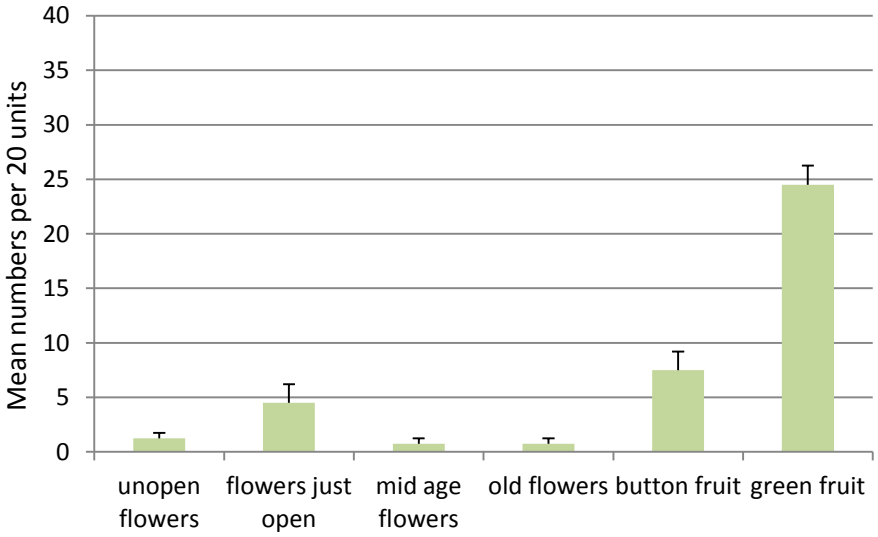

Figure 6. Mean numbers (± SE) of *N. cucumeris* per 20 sample units at Site 2 on 12.05.15

Since all fruit stages were not present on 12 May, no analysis of the data was done.

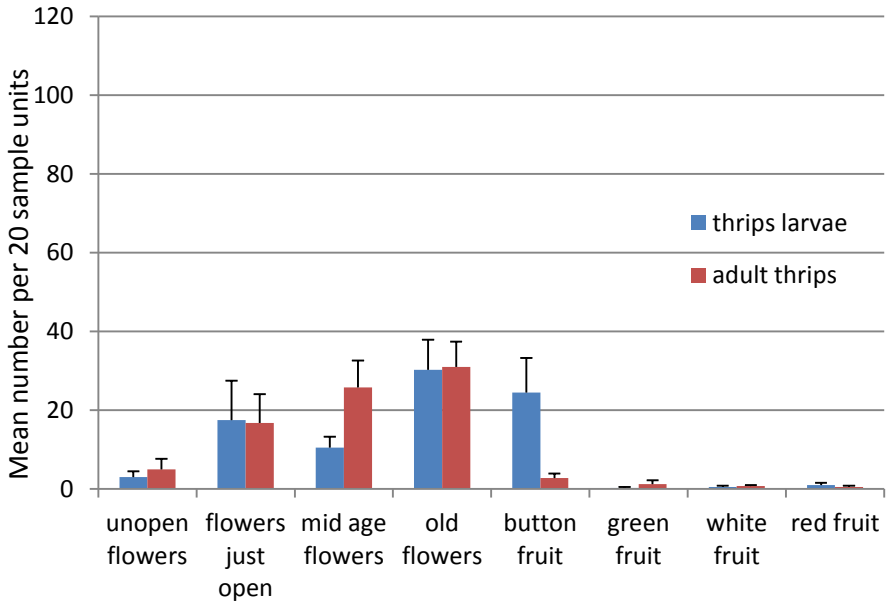

Figure 7. Mean numbers (± SE) of thrips per 20 sample units at Site 2 on 28.05.15

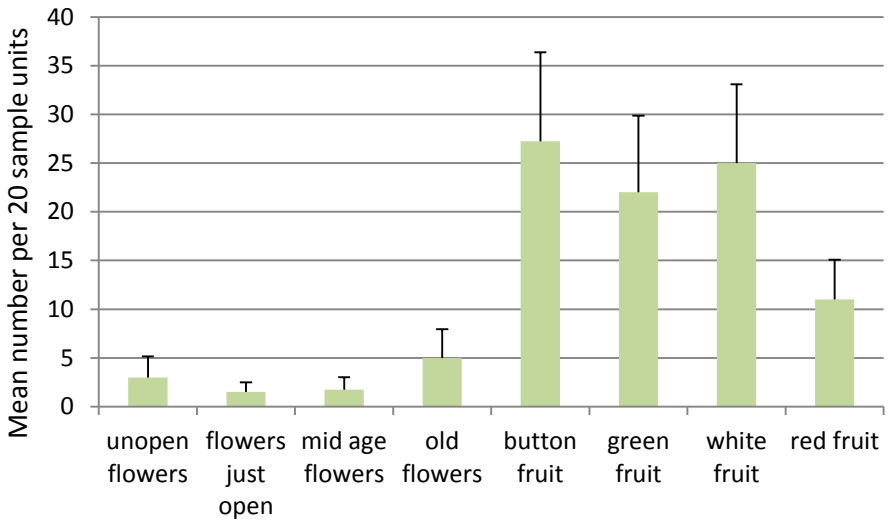

Figure 8. Mean numbers (± SE) of *N. cucumeris* per 20 sample units at Site 2 on 28.05.15

Analysis of counts of thrips and N. cucumeris at Site 2 on 28 May are shown in Table 4. Significantly higher numbers of thrips adults were recorded on mid aged and old flowers and of larvae on old flowers and button fruit. Significantly higher numbers of N. cucumeris were recorded on the button, green and white fruit.

Table 4. Means of square route (counts) and back-transformed means for thrips and *N. cucumeris* at Site 2 on 28.05.15. Numbers on plant parts with the same letter in the column are not significantly different

	Thrips adults		Thrips larvae		*N. cucumeris*	
Stage	Mean	Backtrans	Mean	Backtrans	Mean	Backtrans
Unopen flowers	1.978c	3.914	1.448cd	2.097	1.649c	2.719
Flowers just open	3.746b	14.033	3.414abc	11.654	1.061c	1.125
Mid aged flowers	4.948ab	24.482	3.143bc	9.875	1.140c	1.300
Old flowers	5.476a	29.985	5.359a	28.721	2.155c	4.645
Button fruit	1.545c	2.388	4.621ab	21.358	5.158a	26.606
Green fruit	0.750c	0.563	0.250d	0.063	4.633a	21.464
White fruit	0.750c	0.563	0.500d	0.250	4.953a	24.537
Red fruit	0.500c	0.250	0.707d	0.500	3.272b	10.707
Sig of Stage	<0.001		<0.001		<0.001	
SED	0.794		1.040		0.532	
LSD	1.640		2.147		1.098	

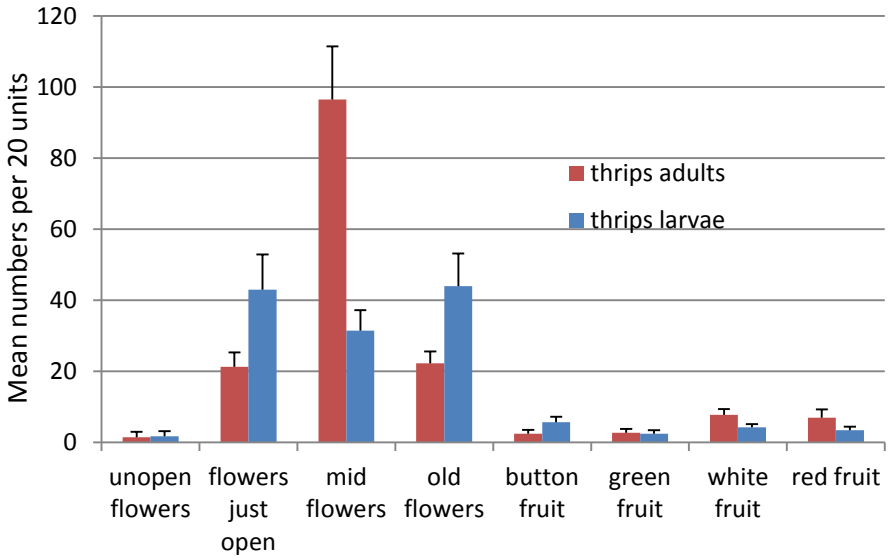

Figure 9. Mean numbers (± SE) of thrips per 20 sample units at Site 2 on 15.08.15

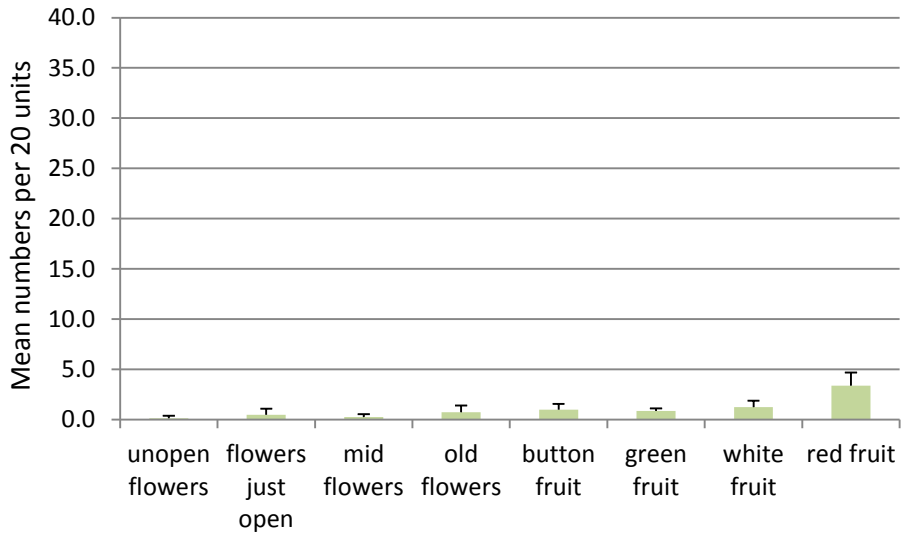

Figure 10. Mean numbers (± SE) of *N. cucumeris* per 20 sample units at Site 2 on 15.08.15

Analysis of number of thrips and N. cucumeris at Site 2 on 15 August are shown in table 5. Significantly higher numbers of thrips adults were recorded on old flowers and thrips larvae on all open flowers. Significantly higher numbers of N. cucumeris were recorded on red fruit on this date.

Table 5. Means of square route (counts) and back-transformed means for thrips and *N. cucumeris* at Site 2 on 18.08.15. Numbers on plant parts with the same letter in the column are not significantly different

	Thrips adults		Thrips larvae		*N. cucumeris*	
Stage	Mean	Back	Mean	Back	Mean	Back
Unopen flowers	0.612d	0.375	0.862c	0.744	0.250e	0.063
Flowers just open	4.550b	20.703	6.413a	41.132	0.707cde	0.500
Mid aged flowers	9.733a	94.722	5.536a	30.650	0.500de	0.250
Old flowers	4.677b	21.870	6.526a	42.588	1.037bcde	1.074
Button fruit	1.346cd	1.811	2.342b	5.483	1.366bc	1.866
Green fruit	1.413cd	1.995	1.492bc	2.226	1.311bcd	1.718
White fruit	2.719c	7.394	2.027bc	4.111	1.537b	2.361
Red fruit	2.553c	6.520	1.801bc	3.244	2.559a	6.549
Sig of Stage	<0.001		<0.001		<0.001	
SED	0.714		0.711		0.395	
LSD	1.473		1.468		0.815	

The percentage of each stage on the different plant parts over all sample occasions at Sites 1 & 2 are shown in Figs 11-13.

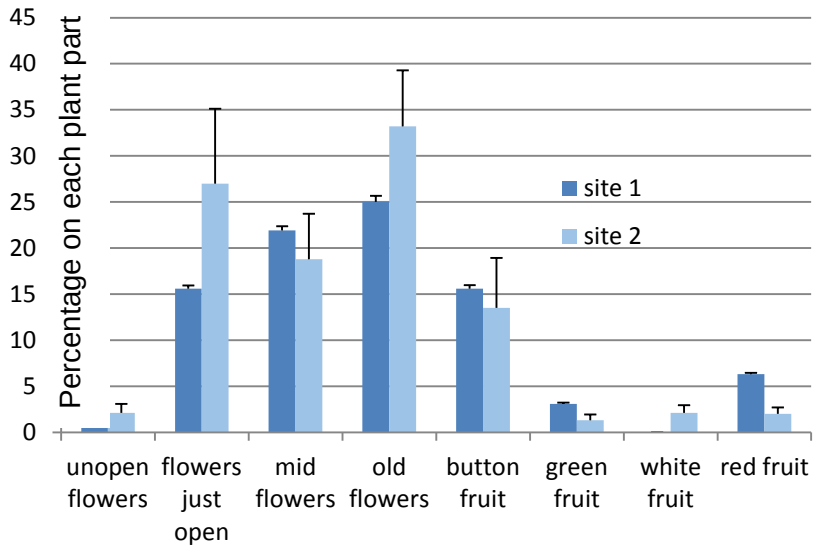

Figure 11. Percentage (± SE) of larval thrips on each plant part over all sample occasions

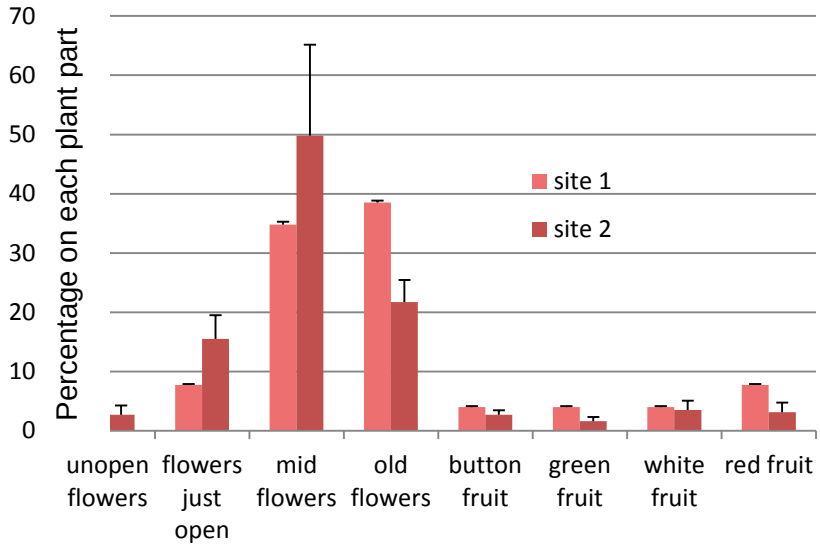

Figure 12. Percentage (± SE) of adult thrips on each plant part over all sample occasions

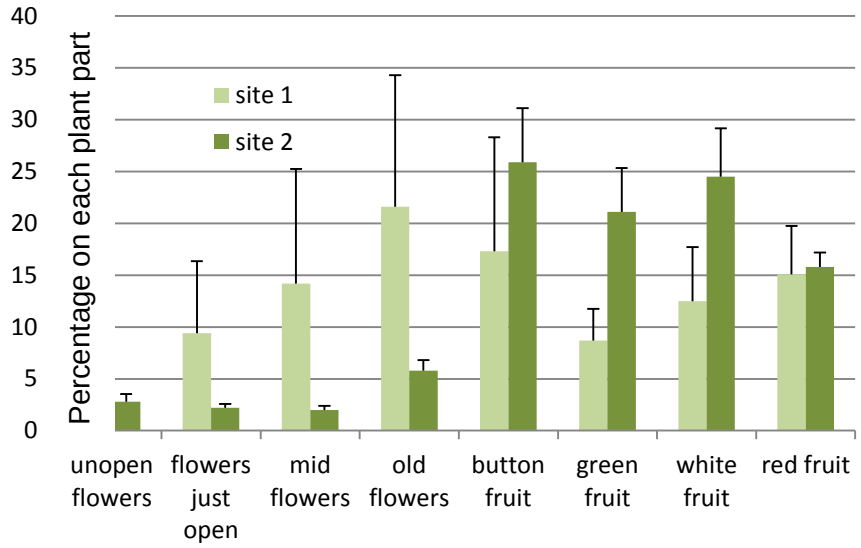

Figure 13. Percentage (± SE) of *N. cucumeris* on each plant part over all sample occasions

In the analysis of percentage of *N. cucumeris* on all flower vs all fruit stages on the two dates at each site, apart from site 1 on 28 May, there were always significantly higher numbers of mites on the fruit compared with the flowers (Table 6) (see also results in section 1.1.1.2).

Table 6. Percentage of *N. cucumeris* recorded on all flower stages compared to all fruit stages

	Date	All flower stages	All fruit stages	P value
Site 1	28.05.15	49.68	50.32	ns
	18.08.15	31.60	68.40	<0.01
Site 2	28.05.15	11.66	88.34	<0.001
	15.08.15	20.00	80.00	<0.001

Discussion

These results, from two sites and five sampling occasions over one season suggest that mid aged/old flowers are the most effective plant stages to sample to get accurate assessments of numbers of adult WFT present. Thrips larvae were present in the flowers but also on the young button fruit. However to get a reasonable estimate of number of *N. cucumeris* present the results suggest that it is more effective to take samples of fruit. For practical reasons, within this project, it was decided to focus on young 'button' fruit, where both *N. cucumeris* and thrips larvae were generally present. Growers and advisors currently assess flowers for

presence of thrips and *N. cucumeris*. This research suggests that counts from flowers, although giving a good assessment of thrips adults present, underestimates the number of *N. cucumeris* present. To get a clear picture of thrips population development in crops growers should still select mid-aged flowers where possible. However, the aim of this research was to investigate the distribution of thrips and *N. cucumeris* on different plant parts and to select an appropriate stage from which predator prey ratios can be estimated to enable predictions of potential damage to be made, and from that, to enable release strategies for *N. cucumeris* to be clarified.

1.1.1.2: To determine the best sampling strategy for N. cucumeris and WFT for management of the biocontrol process: Comparison of predator and prey numbers in flowers and button fruits

Materials and methods

At the same 2 sites used for the identification of the most effective sample units for determining the presence of *N. cucumeris* in 1.1.1.1 above, samples of 50 individual mid aged flowers and 50 button fruit were collected every 2 weeks from May/June to August. These data are being used in the statistical modelling of the distribution of the predator and prey (see below). They are also used here to confirm the results of the sampling described in 1.1.1.1. The mean numbers of thrips and *N. cucumeris* in each of the 50 sample units was square root transformed and subjected to ANOVA.

Results

At Site 1 where numbers of thrips remained low throughout the season, in the first two samples much higher numbers of *N. cucumeris* were recorded per button fruit than per flower (Fig. 14 and Table 7) and numbers were still significantly higher in button fruit than flowers on 9 July (Table 7). In the remaining samples there were similar numbers of *N. cucumeris* on flowers and fruits, with numbers decreasing through the season.

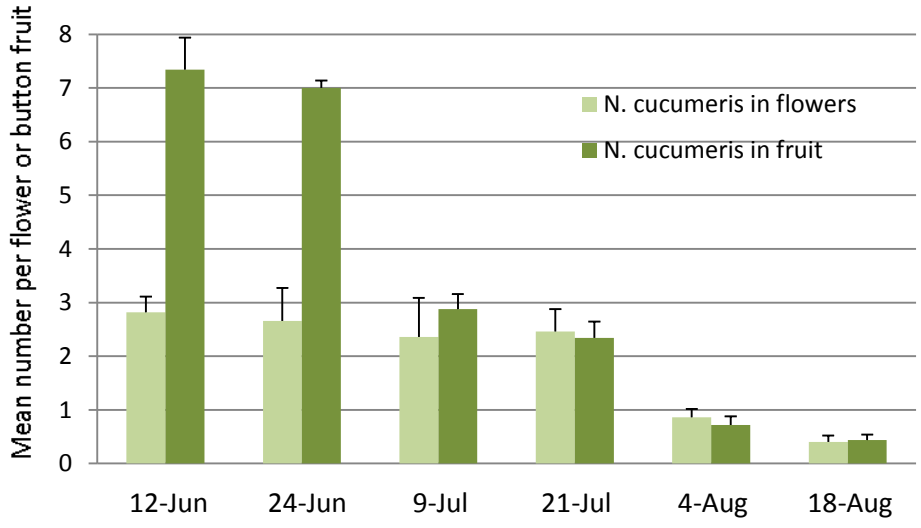

Figure 14. Comparison of numbers (± SE) of *N. cucumeris* in flowers and button fruit fortnightly from 12 Jun at Site 1

Figure 14 shows mean numbers of *N. cucumeris* from 50 individual flowers or individual button fruit through the season. Figures 2 and 4 show means of replicates of 20 plant parts. Comparing the results of the two sampling programmes, on 28 May (Figure 2) there was a mean of 45 *N. cucumeris* per 20 flowers and 62 per 20 button fruit (these are the plant parts collected and assessed in Figure 14). This approximates to 2.3 mites per flower and 3.1 per button fruit. By 12 June (15 days later) when the first individual flowers and button fruits were assessed, there was a mean of 2.8 mites per flower and 7.3 per button fruit. Between the two sample dates there had been a release of *N. cucumeris* at approx. 10 per plant on 8 June (Table 1). There were also releases of predators on 13 and 27 June and on 6 July. *N. cucumeris* numbers were significantly higher in button fruit than flowers on 12 and 24 June and 9 July after these releases (Table 7). Numbers decreased in samples taken later in July and August, perhaps because no more releases were made and mites were being lost to the crop on harvested fruit (see Figures 2, 4, 8 and 10).

Thrips numbers were low throughout the sampling period (Table 7). There were higher numbers of thrips larvae on flowers than button fruit on 21 July and 18 August and of thrips adults on flowers on 18 August.

Table 7. Comparison of mean number of thrips and *N. cucumeris* recorded fortnightly in samples of 50 flowers or 50 button fruit from Site 1. Numbers are significant at the <0.001 (red), <0.01 (yellow) and <0.05 (green) levels.

square root (thrips larvae)

Source \ Date	12.06.15	24.06.15	09.07.15	21.07.15	04.08.15	18.08.15
Flowers	0.000	0.000	0.040	0.120	0.120	0.220
Button fruit	0.020	0.000	0.048	0.020	0.040	0.040
Sig of difference	0.666	1.000	0.858	0.032	0.085	<0.001
SED	0.046					
d.f.	294					
LSD	0.091					

square root (thrips adults)

Source \ Date	12.06.15	24.06.15	09.07.15	21.07.15	04.08.15	18.08.15
Flowers	0.080	0.080	0.020	0.068	0.020	0.117
Button fruit	0.028	0.000	0.020	0.040	0.080	0.000
Sig of difference	0.226	0.061	1.000	0.507	0.160	0.007
SED	0.043					
d.f.	294					
LSD	0.084					

square root (*N. cucumeris*)

Source \ Date	12.06.15	24.06.15	09.07.15	21.07.15	04.08.15	18.08.15
Flowers	1.534	1.047	0.954	1.314	0.622	0.289
Button fruit	2.601	2.452	1.561	1.270	0.517	0.379
Sig of difference	<0.001	<0.001	<0.001	0.797	0.539	0.594
SED	0.170					
d.f.	294					
LSD	0.335					

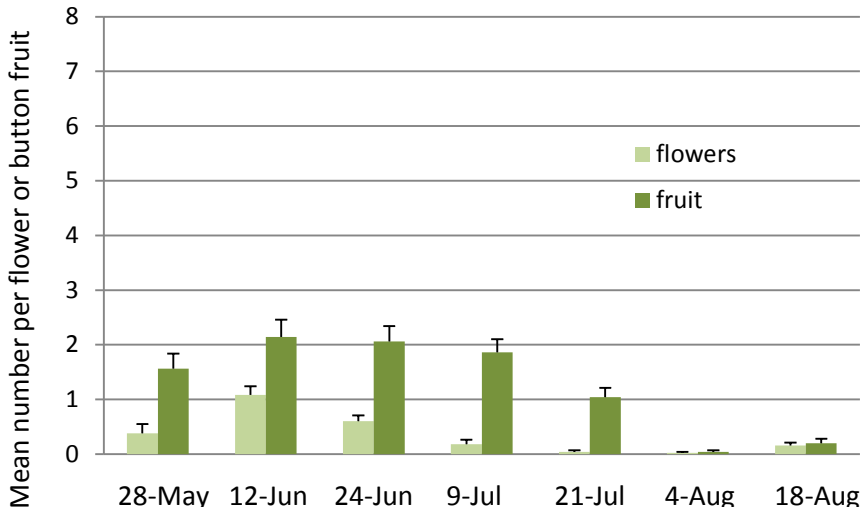

Figure 15. Comparison of numbers (± SE) of *N. cucumeris* in flowers and button fruit fortnightly from 28 May at Site 2

At Site 2 numbers of *N. cucumeris* were generally lower than at Site 1 (Fig. 15 and Table 8). There were always higher numbers of predators on the button fruit than in the flowers. This difference was significantly different on all sampling dates from 28 May to 21 July (Table 8). Figure 15 shows mean numbers of *N. cucumeris* from 50 individual flowers or individual button fruit through the season. Figures 6 and 8 show means of replicates of 20 plant parts. Comparing the results of the two sampling programmes, mean numbers of *N. cucumeris* per mid aged flower on 12 May was 0.1 and on button fruit was 0.4 (Figure 6). Numbers on 28 May were 0.1 per flower and 1.4 per button fruit (Figure 8). This compares well with the individual plant part samples shown in Figure 15 for 28 May. Releases of *N. cucumeris* were made on 29 May, 4 June and 1 July at 50 per plant. Numbers of *N. cucumeris* were significantly higher on button fruit than on mid-aged flowers until 21 July (Table 8). As for Site

1, numbers of mites decreased in August, perhaps as a result of removal on ripe fruit at harvest.

Numbers of thrips adults were similar or higher on the flowers than the fruit; the differences were significant for 28 May, 9 July and 4, 18 August (Table 8). Numbers of thrips larvae were not consistently different in the button fruit or flowers (Table 8).

Table 8. Comparison of mean number of thrips and *N. cucumeris* recorded fortnightly in samples of 50 flowers or 50 button fruit from Site 2. Numbers are significant at the <0.001 (red), <0.01 (yellow) and <0.05 (green) levels.

square root (thrips adults)

Source \ Date	28.05.15	12.06.15	24.06.15	09.07.15	21.07.15	04.08.15	18.08.15
Flowers	1.173	0.773	0.341	1.725	1.926	2.169	1.953
Button fruit	0.040	0.838	0.157	0.191	2.030	0.982	0.323
Sig of difference	<0.001	0.656	0.205	<0.001	0.475	<0.001	<0.001
SED	0.146						
d.f.	343						
LSD	0.146						

square root (thrips larvae)

Source \ Date	28.05.15	12.06.15	24.06.15	09.07.15	21.07.15	04.08.15	18.08.15
Flowers	0.200	0.068	0.108	0.776	2.761	3.261	1.148
Button fruit	0.020	0.513	0.367	1.713	0.356	1.618	0.653
Sig of difference	0.198	<0.01	0.065	<0.001	<0.001	<0.001	<0.001
SED	0.140						
d.f.	343						
LSD	0.275						

square root (*N. cucumeris*)

Source \ Date	28.05.15	12.06.15	24.06.15	09.07.15	21.07.15	04.08.15	18.08.15
Flowers	0.216	0.779	0.516	0.143	0.040	0.020	0.160
Button fruit	0.937	1.159	1.166	1.113	0.739	0.040	0.165
Sig of difference	<0.001	<0.01	<0.001	<0.001	<0.001	0.871	0.969
SED	0.123						
d.f.	343						
LSD	0.242						

Discussion

Comparison of numbers of arthropods in mid-aged flowers and button fruit throughout the growing season on two sites showed that *N. cucumeris* numbers were always higher in the button fruit than in flower samples from May to end of July. Numbers then declined, possibly because mites on fruit were being removed during harvest and not replaced by additional releases. Results from sampling in 1.1.1.1 and 1.1.1.2 indicate that growers may be underestimating numbers of *N. cucumeris* in their crops by assessing numbers present only in flower samples. Results also showed that thrips adults were in general present in greater numbers in the flowers, whereas, although there was no consistent pattern of distribution of thrips larvae, they were present in both button fruit and flowers.

1.1.1.3: Development of methods to accurately quantify predator and prey numbers in the field

Materials and methods: fumigant extraction

This work aimed to compare fumigation methods for extracting predatory mites and WFT from plant samples that could be developed into a field-based monitoring system. Three volatile organic compounds (methyl isobutyl ketone (information supplied by Berry Gardens Growers), amyl acetate and ethyl acetate) used as fumigants were assessed and compared with the standard ethanol washing technique used in previous projects. Six replicate samples of 10 button fruit were picked into glass jars containing a grid to support the fruit above the bottom of the jar. Each fumigant was adsorbed onto a small cigarette filter which was laid on top of the grid. Mites and WFT extracted and killed by each fumigant were washed from the jars and recorded under a stereomicroscope. Samples collected directly into ethanol were used as the control ethanol washing treatment; arthropods were washed off the plant material in the laboratory and counted under a stereomicroscope.

Results

For thrips adults and larvae significantly lower numbers were extracted using ethyl acetate ($P=0.05$); there was no difference between the other treatments (Fig. 16 and Table 9). For *N. cucumeris* significantly higher numbers were extracted in the standard ethanol washing technique ($P<0.001$). Differences between the other treatments were also significant with higher numbers extracted in the methyl isobutyl ketone treatment (Fig. 16 and Table 9).

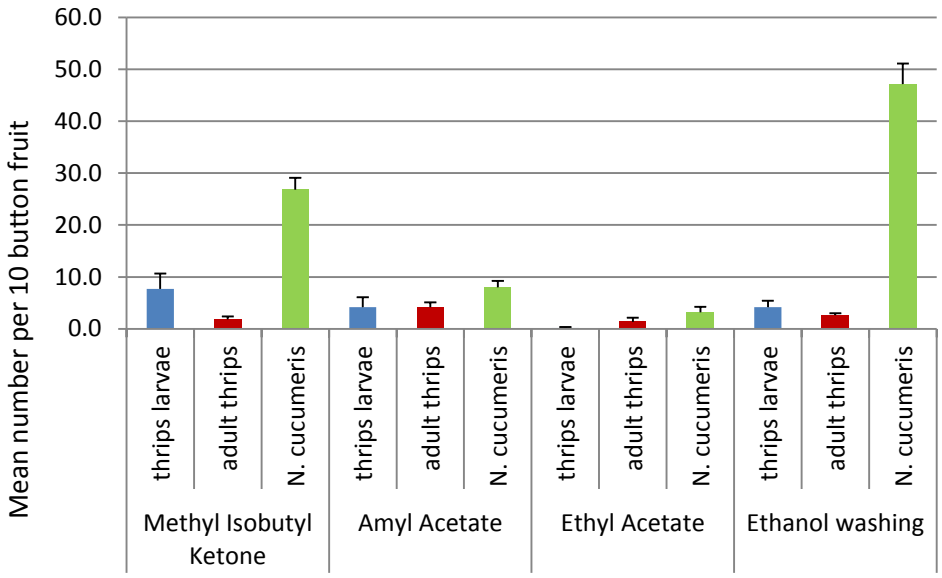

Figure 16. Mean numbers of invertebrates (± SE) extracted using fumigant extraction or the ethanol washing technique

Table 9. Square root transformed numbers of thrips and *N. cucumeris* recorded after extraction by organic fumigants compared with the ethanol washing technique

	Ethanol washing	Methyl isobutyl ketone	Amyl acetate	Ethyl acetate	lsd p=0.05
Thrips larvae	1.91a	2.39a	1.55a	0.17b	1.381
Thrips adults	1.62a	1.20ab	1.97a	0.78b	0.802
N. cucumeris	6.84a	5.16b	2.79c	1.67d	0.744

Techniques within a row followed by the same letter are not significantly different (p=0.05)

Discussion

This study, using naturally infested button fruit, suggested that methyl isobutyl ketone was the most effective fumigant at extracting arthropods from button fruit. The experiment used six replicates for each treatments, each containing 10 button fruit and the results showed little variability within treatments (see Figure 16). However, it should be borne in mind that the actual number of WFT or *N. cucumeris* in each sample was not known. To confirm these results it would be useful to set up a laboratory experiment where known numbers of arthropods were introduced onto the plant material and the percentage extracted assessed.

Materials and methods: Comparison of extraction techniques

Since methyl isobutyl ketone was the most effective fumigant at extracting *N. cucumeris* from fruit this was used in a comparison of different arthropod extraction methods. There were 6 replicates of each method. These methods were:

a) direct visual examination and counting in the field using a hand lens.
b) fumigant extraction; this was done with an improved monitoring device shown in Fig. 17. Thrips and *N. cucumeris* were counted in the field using a hand lens held above/below the extracted arthropods. The extracted arthropods were then washed out of the monitoring device with ethanol and re-counted in the lab under a stereomicroscope.
c) heat / light extraction in the lab using Tullgren funnels. Arthropods were captured in ethanol and counted as above
d) extraction by ethanol washing (standard method used as a control)

Figure 17. Prototype monitoring device

Results

As in earlier studies it is clear that assessing fruits (or flowers) with a hand lens in the field underestimated the numbers of thrips larvae and *N. cucumeris* present in the crop when compared with the standard ethanol washing technique (Fig. 18 and Table 6). Assessing arthropods in the prototype monitoring device in the field was difficult and resulted in very low counts. This was because the depth of the sampling pot made it impossible to clearly see the predatory mites from above, and the fact that the container was not of a clear plastic material made it difficult to count through the base of the container. However, when the containers were washed in the lab and arthropods recounted it was clear that the extraction method had

been successful, with numbers of thrips larvae and *N. cucumeris* similar to those extracted by the ethanol washing technique (Fig. 18 and Table 10). Further work is needed to develop a more usable monitoring device, preferably with a counting dish that can be removed from the container for assessing numbers of arthropods under a hand lens in the field if necessary. This work is ongoing at NIAB EMR. Numbers of adult thrips were very low in the Tullgren extractions as there was no barrier to adult flight away from the heat and light.

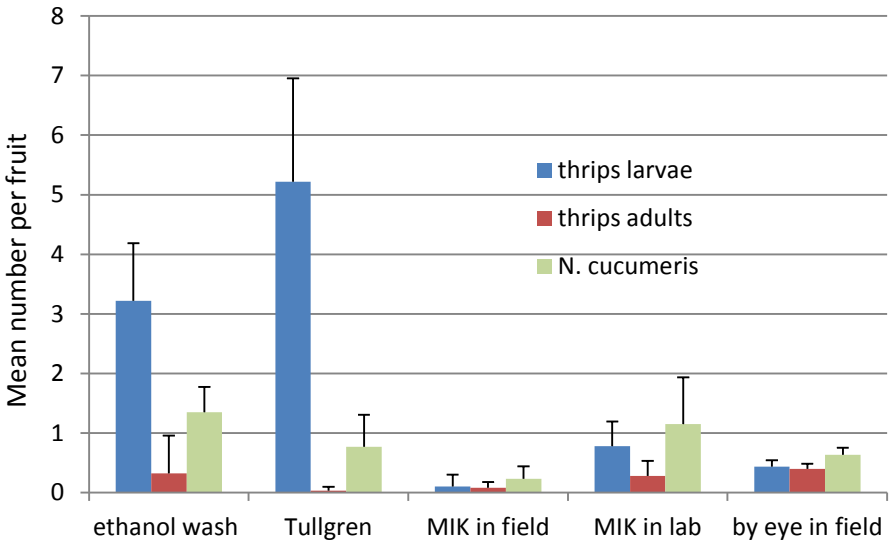

Figure 18. Mean numbers of invertebrates (± SE) using different extraction methods

Table 10. square root transformed counts per button fruit recorded in the different extraction methods

	Thrips adults		Thrips larvae		*N. cucumeris*	
Treatment	Mean	Backtrans	Mean	Backtrans	Mean	Backtrans
Ethanol washing	0.439	0.193	1.781	3.173	1.155	1.334
tullgren	0.105	0.011	2.267	5.140	0.845	0.714
MIK in field	0.233	0.054	0.211	0.044	0.429	0.184
MIK in lab	0.506	0.256	0.863	0.744	1.039	1.080
eye	0.626	0.392	0.633	0.401	0.795	0.632
Significance	0.003		<0.001		<0.001	
SED	0.130		0.141		0.123	
d.f	25		25		25	
LSD	0.267		0.290		0.253	
LSD - Lower	0.000		0.000		0.500	
LSD - Upper	0.267		0.290		0.753	

Discussion

These results show that thrips and *N. cucumeris* can be successfully extracted from plant samples in the field using the fumigant methyl isobutyl ketone (MIK). Extracted arthropods can be collected directly on a flat surface for counting and this could be developed as a rapid monitoring technique. Prototype monitoring devices were constructed and further work on this is ongoing. The standard lab ethanol washing technique, as shown in earlier work, was very effective at extracting arthropods but this technique could not so easily be used as the basis of a field monitoring system since it relies on a two stage process; washing the plant parts with a liquid and then filtering any extracted arthropods in the liquid out onto a collecting device for counting.

1.1.1.4: To determine if the use of Calco Red to stain Neoseiulus cucumeris in culture makes them more easily visualised in the field.

Materials and methods

Growers had remarked that it was difficult to see predatory mites in strawberry flowers. This work was done to determine if colouring the mites would make them easier to count in the field. In addition several species of predatory mites may be present in strawberry fields, and not all of these will predate thrips. Thus if a successful method to stain *N. cucumeris* could be developed and used commercially it would be possible for growers to be sure that any mites they can see are *N. cucumeris* (predatory mites cannot be identified to species except under a high power microscope).

The initial work on this was done by SyngentaBioline. Calco Red (Hagler and Jackson, 2001) was added to cultures of *Thyreophagus entomophagus*, which are used as prey for several predatory mite species. The dye was either added in powder form or dissolved in oil prior to mixing with the diet. The expectation was that the colour from the dye would be absorbed by the fatty components of the prey mites and that, when fed to predatory mites, the colour would be transferred to the predator.

Results

When the dye was added as powder to the prey diet no real colour transfer to the mites was seen. When the dye was dissolved in oil prior to mixing, a more intense colour was seen in the culture diet, and the mites developed a pink colour, but were not particularly dark. When the stained prey mites were fed to predatory mites, in this case *N. californicus*, the red colour was taken into the gut of the predators and was quite visible (Fig. 18); the gut of phytoseiid

mites is H shaped and this can be clearly seen in Fig. 18. However, since the colour is in the gut rather than the whole body we would expect the colour to be transient. The *N. californicus* were also exposed to the Calco Red in the rearing medium, but there was no evidence that the cuticle had been stained (Figure 18).

In a repeat of this experiment at EMR pollen was stained for 10 min in a solution of 0.15g Calco Red/10ml ethanol and allowed to dry. Replicate samples of adult *N. cucumeris* were added to a glass petri dish with the stained pollen (10 individuals) and 10 more adults were added to a petri dish with normal pollen as controls. The petri dishes were observed every day and the pollen was changed every 3 days.

Two days after the experiment was set up, mites that had been feeding on stained pollen were taking up the dye, but this was mainly in the gut (Figure 19). Five days after exposure the mites had become quite red. On this day the mites from one of the replicates feeding on stained pollen were transferred to feed on normal pollen. Every day after that, the red colour diminished from the gut. There was no evidence that the mite body had been permanently stained.

Figure 18. Calco Red stain seen in gut of *N. californicus* fed on stained prey mites

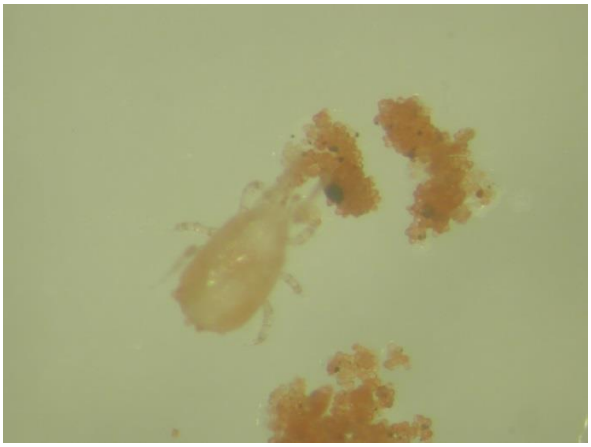

Figure 19. Calco Red stain seen in the gut of *N. cucumeris* fed on stained pollen

Discussion

It proved possible to detect Calco Red in the gut of predatory mites, both by feeding them on stained prey mites or on stained pollen. However, the dye did not appear to permanently stain the gut or be taken up into the body of the mites. Because of this it was agreed not to continue with this strand of research.

Reference

Hagler JR and Jackson CG (2001) Methods for marking insects: Current techniques and future prospects. *Annu Rev Entomol*, 46, 511-543.

1.1.1.5. To determine thresholds and sampling strategies required for successful predator-prey-damage relationships in commercial crops.

Materials and methods

In summer 2015, 12 commercial strawberry plantings were identified where *N. cucumeris* was being introduced for WFT biocontrol, including sites where WFT biocontrol had been successful, partially successful and unsuccessful. Samples of 50 individual button fruit were collected into alcohol from each planting. Numbers of *N. cucumeris* and WFT on individual fruit from each sample were counted in the laboratory. Damage on 20 white fruit was recorded at each site as numbers of seeds surrounded by bronzing. A damage score was attributed to the crop at each site based on the total number of bronzed seeds recorded on 20 fruits: 0-10 seeds = 0; 10-100 seeds = 1; 100-1000 seeds = 2. The bronzing damage recorded was very low and would not have caused downgrading of fruit. The number of flowers and different fruit stages were also recorded on 15-30 plants per site close to the areas sampled for thrips and *N. cucumeris*. Data from this sampling programme, together with that from sampling undertaken in 1.1.1.2 and previous studies are being analysed to determine predator prey ratios for successful and unsuccessful biocontrol.

Results

Predator and prey occurrence in different crops: Table 11 shows the mean number of thrips and *N. cucumeris* per button fruit at the 12 plantings sampled, together with the damage assessment made in these plantings. There was no clear correlation between predator:prey ratios and fruit damage, with highest damage seen at ratios of 1:3 and 1:83. However, at 5 out of 6 sites where mean numbers of *N. cucumeris* were higher than adult thrips numbers no fruit damage was seen, whereas, when numbers of thrips adults were higher than *N. cucumeris* low levels of fruit damage were recorded (Table 11). The actual highest damage recorded, in total seeds bronzed, was at site 6 (535), followed by site 4 (410) and site 10 (285). However, it is likely that damage to the fruit would have been caused by populations of thrips feeding on the developing fruits several weeks before the actual assessment. It is also possible that the varieties planted may have different susceptibilities to thrips feeding. The crop with the highest numbers of WFT present was site 6; this site had received a Tracer application to attempt to reduce thrips populations and this may have affected the number of predators present after the application. Fruit at this site was not bronzed but was small and misshaped.

Table 12 shows the mean number of different plant developmental stages at each site at the time of the pest and predator assessments. At site 11 there was insufficient white fruit to make

a damage assessment. At site 6, which had the highest pest:predator ratio and high numbers of thrips larvae, the remaining fruits were small and misshaped and there was also visible thrips damage to the flowers. Table 13 shows the timing and rates of *N. cucumeris* released at these sites. All these data were analysed for distribution characteristics of thrips and predatory mites in individual flowers or button fruit (see below).

Table 11. Mean numbers of thrips and *N. cucumeris* per button fruit in 10 different crops

Site and variety	Date collected	Thrips		*N. cucumeris*		Pred:prey ratio	Damage score
		Larvae	Adults	Immatures	Adults		
1: Red glory	July	6.6	0.6	0.02	0.2	1:33	1
2: Jubilee	July	2.5	0.8	0.2	0.06	1:13	1
3: Amesti	July	6.2	1.8	0.1	0.5	1:13	1
4: Jubilee	July	3.4	0.5	0.2	0.2	1:10	2
5: Amesti	July	6.7	1.4	0.3	0.3	1:14	2
6: Amesti	July	8.5	1.5	0.02	0.1	1:83	2
7. Triumph	July	4.7	0.4	0.02	1.0	1:5	0
8. Jubilee	July	0.04	0.02	0.4	1.9	1:0.3	0
9: Dream	Early Sept	1.8	0.2	0.4	0.2	1:3	0
10: Dream	Early Sept	2.7	0.2	0.4	0.6	1:3	2
11: Elsanta	Early Sept	1.1	0.1	0.4	0.4	1:1.5	-
12: Elsanta	Early Sept	0.04	0.02	0.4	0.3	1:0.9	0

Damage was not assessed at Site 11

Table 12. Mean number of different plant stages per plant in assessed crops

		Mean number per plant of each stage				
Site and variety		Flowers	Button fruit	Green fruit	White fruit	Red fruit
1: Red glory	July	2	2	1	2	0.6
2: Jubilee	July	3	6	4	3	2
3: Amesti	July	2	2	3	3	2
4: Jubilee	July	3	5	3	3	1*
5: Amesti	July	2	3	3	2	0.5
6: Amesti	July	0.3	5	2	2	3**
7. Triumph	July	N/A	N/A	N/A	N/A	N/A
8. Jubilee	July	N/A	N/A	N/A	N/A	N/A
9: Dream	Early Sept	0.2	2	0.6	1	1
10: Dream	Early Sept	1	4	2	4	2
11: Elsanta	Early Sept	3	5	3	0.3+	0
12: Elsanta	Early Sept	3	5	3	2	0*

+insufficient fruit to assess damage

*some fruit very small and misshapen

** some fruit very small and misshapen, also damage to flowers

Data not available for sites 7 and 8

Table 13. Timing and approx. rate of release of *N. cucumeris* per plant on the 10 sampled plantings 2015.

Site	Week number 2015																												
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1								25	50		25		25		25		25		25			55	25		25				
2	S		S	60	60	60	60	60	30	30	90	180	180	30			30												
3															25		25		25										
4					S+125	125		125	125		375		500		S+750			500	750	875	750	150	S+150		150				
5					S+125	125		125	125		375		500		S+750			500	750	875	750	150	S+150		150				
6					S+125	125		125	125		375		500		S+750			500	750	875	750	150	S+150		150				
7							S						50	50				50											
8					S	S	S								10	10		10	10										
9																					S+18	116	140	75	40		150	25	
10																					S+18		40	25	40		50	25	
11																					S+18		40	25	40		50	25	
12*																													

Sites 9-12 were under glass
S=1 sachet / 2m length of bed; $S^{+}1$=sachet / 1m length of bed
Other biocontrol agents also released at some sites. Highlighted weeks (yellow) are when samples were taken
*=information not provided by host grower

Analysis of distribution of predators and prey:

A preliminary analysis of the distribution of WFT and *N. cucumeris* in individual flowers and button fruits has been completed. The data used was from the one-off samples from 12 crops shown in Table 11 and from the season long assessments shown in Figures 14 and 15, together with data collected in the HortLink Project HL01107 completed in 2014. WFT and mite counts were very low on many sampling occasions and do not contribute much information to characterising distribution properties. For WFT, eight larval and seven adult counts out of the total 17 flower samples cannot be fitted to a distribution because of the low numbers; four of 17 mite samples on flowers failed the fitting for the same reason. On button fruit, eight out 23 samples failed to fit a distribution because of low numbers of both WFT larvae and adults; only three out of the 23 samples failed for the predatory mites. For nearly all samples with mean counts of WFT (adults or larvae) and mites > 0.5, a negative binomial distribution fitted the counts data better ($P < 0.01$) than a Poisson distribution (as expected), indicating aggregation for WFT and mites.

The extent of aggregation varied with mean counts but such a relationship does not exhibit any regular pattern and hence is practically impossible to model. Aggregation is greater for WFT larvae than WFT adults and is greater on flowers than on button fruit for both WFT and *N. cucumeris*, particularly as there were very low number of mites/WFT on many flowers (Figure 20).

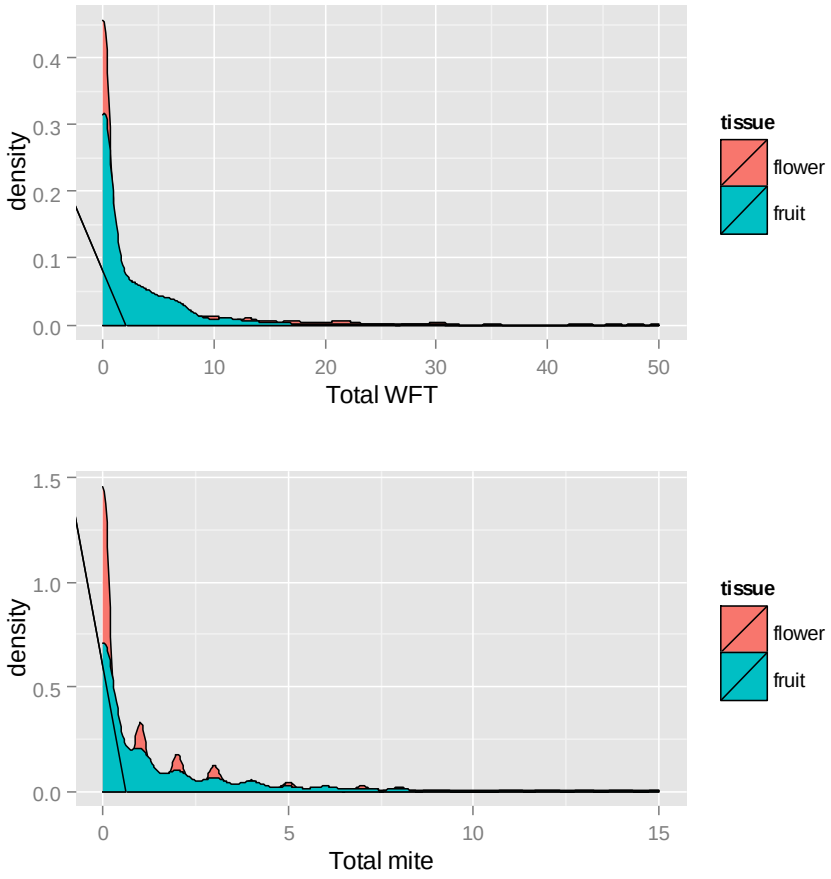

Figure 20. Density plot of total WFT and *N. cucumeris* on individual flowers and button fruit.

As expected the number of *N. cucumeris* is inversely related to total WFT on the same flowers or fruit (Fig. 21). If the WFT damage threshold is assumed to be in the range of 4-8 WFT (including adults and larva) per button fruit/flower (as indicated by the results obtained in the previous Hort LINK project), it appears that you need to have at least five mites per flower or button fruit (Figure 21) to ensure that WFT numbers are below this threshold; however, from the sampling programme there were very few flowers or button fruit on which there were at least five mites present (Figure 20).

GLM modelling (hurdle models) was used to assess whether the presence and number of predatory mites significantly reduced the incidence of flowers/button fruit with WFT and the number of WFT in flowers/button fruit. The GLM analysis indicated that both the incidence of flowers/button fruit with WFT and the number of WFT were significantly ($P < 0.01$) reduced by the presence of predatory mites.

(a) On flowers, increasing the number of *N. cucumeris* by one will increase the odds ratio of a flower being healthy (i.e. free of WFT) by ca. 20%

(b) On button fruit, increasing the number of *N. cucumeris* by one will increase the odds ratio of a fruit being free of WFT by ca. 30%

(c) On those flowers with WFT present, the relationship between WFT and mite numbers is: ln(WFT) = -0.1605 * mite + intercept (where intercept depends on site specific factors), namely the relationship of the number of WFT with mite numbers is of a negative exponential type

(d) On those button fruit with WFT present, the relationship between WFT and mite numbers is: ln(WFT) = -0.2006 * mite + intercept (where intercept depends on site specific factors).

(e) These are results from preliminary data analysis since we cannot easily associate the presence and number of predatory mites or WFT (hence their ratio) with the likelihood of flower/fruit damage. Experiments will focus on this aspect in 2016.

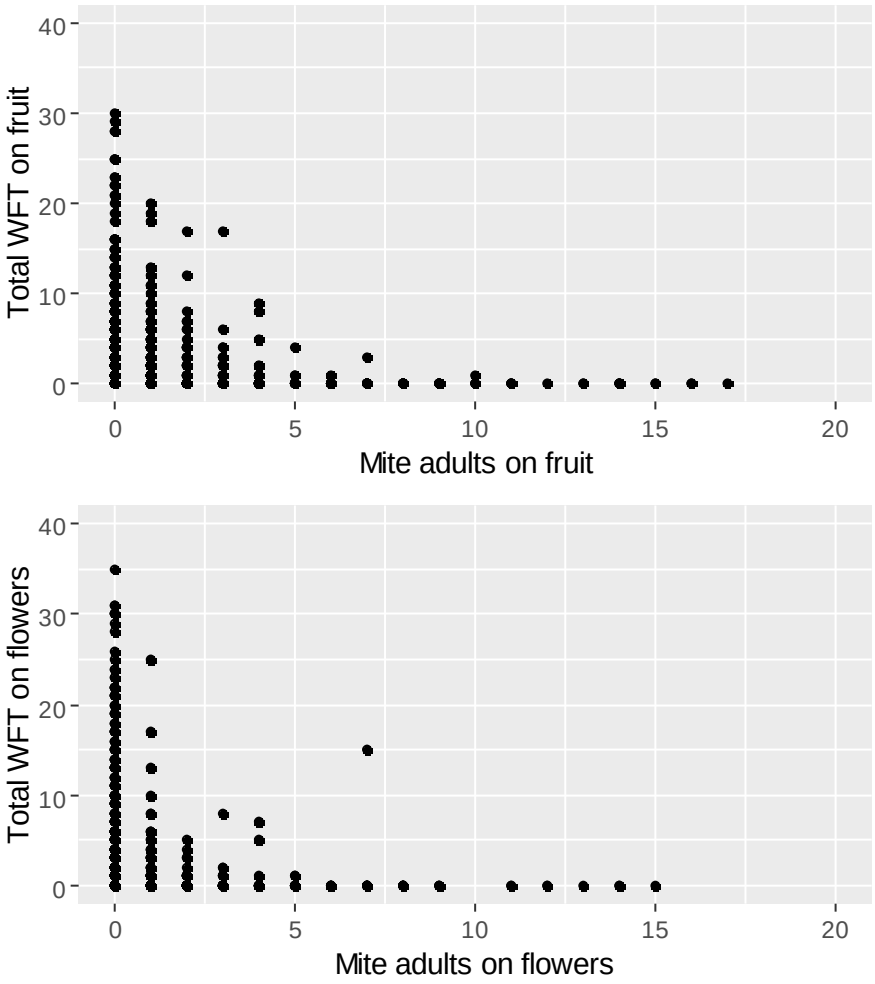

Figure 21. The relationship between total number of WFT and *N. cucumeris* on the same flowers or button fruit

Assuming that numbers of WFT per fruit follows a negative binomial distribution with a common theta parameter (the dispersion parameter of negative binomial distribution – linking the distribution mean to variance), we conducted preliminary studies to determine a sampling

strategy. To develop a sampling protocol for WFT on button fruit, we have to know the following information: WFT damage threshold and the maximum tolerance level with WFT exceeding the damage threshold. Based on the results obtained in the previous Hort LINK project, we assumed that the WFT damage threshold is between 4-8 individuals and that growers can tolerate a maximum of 5% of individual fruit with the number of WFT exceeding the damage threshold (4-8). Based on these assumptions, we derived the maximum mean WFT values from a sample of a given size to ensure that there are 5% of fruit with WFT exceeding the corresponding threshold for a maximum of 5% times (Table 14). For example, for a sample size of 20 and WFT damage threshold of 5, if the mean number of WFT in the sample of 20 fruit is 0.7 per fruit, then we can predict that only in 5% of cases will there be more than 5% of individual flowers with numbers of WFT exceeding 5.

Table 14. The estimated maximum mean numbers of WFT per flower/fruit from a bulk sample (10, 15, 20, 25 and 30) of flowers or fruits. Any value over the means will indicate more than a 5% chance that 5% of individual fruit/flowers will have thrips numbers exceeding the damage threshold (DT, as calculated in Clare Sampson's PhD). For example, at >0.94 for a bulk sample size of 20 you will have at least a 5% chance that 5% of fruit will have thrips number of more than 6.

Numbers of WFT per flower or fruit (assumed DT)	Bulk sample size				
	10	15	20	25	30
4	0.40	0.42	0.44	0.44	0.46
5	0.64	0.68	**0.70**	0.70	0.72
6	0.88	0.92	0.94	0.96	0.98
7	1.12	1.18	1.20	1.22	1.24
8	1.38	1.44	1.48	1.50	1.52

Discussion

Further work is needed to develop relationships between *N. cucumeris* and WFT for satisfactory biocontrol. The final output from this work is a quantitative relationship of the damage to individual flowers/fruit with the number of thrips as well as predatory mites present in the same flower/fruit. The data from 2015 samples did indicate the expected relationship of this ratio (thrips/mites) with the likelihood of damage. However, this ratio did not cover a sufficiently large range and most flowers or button fruit did not have thrips or mites present. Hence, more data (with a wider range of thrips and predatory mites) are needed to develop a more reliable model.

Conclusions

- Potentially the most appropriate plant part to sample to assess numbers of *N. cucumeris* in strawberry crops is young button fruit
- Mid aged/old flowers are the most effective plant stages to sample to get accurate assessments of numbers of adult WFT present
- There was no consistent pattern of distribution for thrips larvae, with larvae being found in flowers and on fruit
- Although no definitive experiment with set numbers of arthropods in each sample was undertaken, thrips and *N. cucumeris* were successfully extracted from plant samples collected directly from the field using the fumigant methyl isobutyl ketone (MIK)
- A prototype monitoring device making use of this solvent extraction method was constructed. Further work on this is ongoing
- Calco Red stained the gut of predatory mites, but the dye did not appear to be taken up into the body of the mite.
- A preliminary analysis of distribution of *N. cucumeris* and WFT has been completed. For nearly all samples with mean counts of WFT (adults or larvae) and mites > 0.5, results indicated aggregation for both WFT and *N. cucumeris*.
- Results of the analysis have been used to estimate the maximum mean number of WFT in a sample of a given size to ensure the probability of only 5% fruit damage. Further work is required to validate these results.

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

Task 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; Yr 1)

Introduction

Predators and/or crop protection products are the major methods employed for the control of *Frankliniella occidentalis* (Western Flower Thrips, WFT) in the UK currently, however the use of entomopathogenic fungi to control WFT is low. There are two existing EPF products available currently in the UK which list WFT as a target on their label: Naturalis L (Belchim) and Met52 (Fargro). Naturalis is a sprayable formulation containing *Beauveria bassiana* approved for control of whitefly, thrips, spider mites and capsid. Met52 is a granular biopesticide containing *Metarhizium anisopliae* incorporated into growing media for control of a wide range of pests including thrips and vine weevil in a wide range of crops, including strawberries. According to the Naturalis label, in trials, only 30-50% control was achieved against WFT on strawberries, compared to 'high levels' of control on cucumber. Jacobson et al. (2001) demonstrated high efficacy of Naturalis L (75% reduction in population) on cucumbers using a high volume spray against WFT, and stressed that the key to efficacy is good application/coverage by spores to ensure WFT come into contact with the target material. Met52 does not directly specify the use against WFT on the label in the UK (at the time of writing) although efficacy is claimed in the technical notes for the product. This Task will focus on improving the efficacy of Naturalis L against WFT on strawberry crops in the UK.

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 to the base of the plant where they pupate and go through a pre-pupa 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 EPF's 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. Other factors which may influence the efficacy of EPFs may be

temperature and humidity. Humidity can be artificially raised at the point of spraying through high volume applications such as those used by Naturalis L.

A biopesticide application needs to target mobile 'exposed' thrips or aim to get formulation to penetrate into the flower heads where adults are likely to reside. It is not known why the Naturalis formulation is less effective on strawberries; however it may be hypothesised that the problem may be due to ambient conditions, low spore contact by the thrips or low efficacy of the product against WFT. The literature states that high efficacy can be expected against WFT using Naturalis, therefore the problem is not due to the isolate (Jacobson et al., 2001). Ambient conditions may contribute to lower efficacy, but the main drivers affecting efficacy such as temperature and humidity may be difficult for a grower to control. The high volume application used by Naturalis L should artificially increase ambient humidity at the point of application to negate humidity problems. Contact with spores however, is a factor which may be influenced through experimentation with formulation and application methods.

One approach to increasing spore retention is to alter the formulation of the biopesticide. Under current regulatory guidelines, this would be expensive to achieve as a dossier would have to be put together for the EU for approval of a change in formulation. Another approach however, could be to investigate the use of adjuvants to increase the retention of spores on the leaf surface. Adjuvants are additives which may be added to the tank mix to improve the performance of a pesticide. In terms of Naturalis, the biopesticide is very effective against other insects or against WFT on other plant types, however the performance needs enhancing on strawberry. This piece of work examines how the control of WFT can be improved through the use of adjuvants added to the tank mix at the point of spraying. Future work will investigate application methodologies.

Materials and methods

Adjuvant selection: There are a variety of different types of adjuvant which may be used.

- Surfactants act to allow water to spread more evenly over the plant surface
- Penetrants work to allow more chemical to pass through the plant surface
- Stickers/spreaders act to spread formulation across the leaf surface and secure it to the leaf surface.

When selecting a suitable adjuvant to use in a tank mix a number of factors need to be considered

- The plant health following spray with an adjuvant

- The existing formulation (an oil dispersion in this case); what would be compatible with it and improve its pick up/penetration
- What is registered for use in the UK with horticultural crops
- The health of the spores in the formulation- will the adjuvant affect the viability of spores when mixed into a tank mix
- How would you mix the tank mix and oil dispersion to get effective use of adjuvant

Naturalis is an oil dispersion (the formulation is stored as a spore-oil concentrate). This is then mixed with a volume of water to create a tank mix in which oil droplets and spores are evenly dispersed. There would be two ways to try and increase the retention of spores on the strawberry. One would be to look at the addition of a sticker to ensure that the oil droplets contained in the tank mix are not washed straight from the plant during application. The other approach would be to reduce the amount of water applied with the Naturalis as this may be washing spores/oil from the leaf resulting in the majority of formulation in the soil. Different spray volumes are to be examined in a separate component of this project; therefore this report will concentrate on the addition of off the shelf adjuvants. A list of adjuvants categorised as 'stickers' which are able to be used on strawberries according to the UK pesticide guide are given in Appendix 2. Among these stickers are several shared chemistries and some distinct chemistries and Appendix 3 groups together those products of interest.

Many formulations contain 10-45 % styrene-butadeine co-polymers which are found in extenders/stickers. The formulations they are found in general are emulsifiable concentrate or soluble concentrates. They are derived from rubber/latex and some of the formulations have been reported to act as a UV screen which would have additional advantages for fungal spores. The mode of action is that the adjuvant "absorbs the impact of the droplet on the target and imparts a stickiness to the surface enabling increased deposition without run-off" (Bond- DeSangosse). The mode of action acts to increase deposition and reduce any run off of the tank mix.

Trisiloxane organosilicone copolymers are found in quantities of 10-41% in several of the adjuvants. These are surfactants which are likely to act to suspend either the styrene-butadeine in the concentrate or to allow the product to mix with oil. Possibilities are that a concentration which is too high will cause the formulation to spread too much across the leaf/ flower, however too low and there is a possibility that a stable mixed tank mix will not be formed. These surfactants are listed as non-ionic and these have a greater possibility of maintaining the viability of the spore, however the effect of their addition into the tank mix should be assessed in terms of spore viability. The have shown toxicity to insects and mites on their own previously (Cowles et al., 2000).

Of these chemistries a short list was drawn up which consisted of:

1) Codacide oil (95 % emulsifiable oil). The mode of action will be to increase the concentration of oil in the formulation to allow a higher chance that the oil droplets will bind with the leaf surface.
2) Bond: An emulsifiable concentrate formulation containing 45.0 % w/w styrene-butadiene copolymers (EAC 1) and 10.0 % w/w alkoxylated alcohols (EAC 2). The mode of action will be to increase retention of spores on the leaf/flower surface
3) Silwet – L77 – an organosilicone surfactant containing a minimum of 80 % w/w polyalkylene oxide modified heptamethyl trisiloxane and a maximum of 20 % w/w allyloxypolyethylene glycol methyl ether. This may help the formulation to penetrate the flower head.

Quality control of Naturalis L: On receipt of Naturalis L from Fargro, a CFU count was set up to ensure that the product was viable. The formulation was allocated a CABI no. 175/15. Using a sterile pipette tip, 1 ml of formulation was removed from the bottle and added to 9 ml of sterile tap water. Three separate vials were prepared. Vials were shaken to mix the formulation and then sonicated for 3 min to break up any chains of conidia. Following sonication three 1/10 dilution series were prepared down to 10^{-7}. Each dilution series was used to prepare 3 sets of CFU counts on SDA-TW 90mm agar plates. For each series, 200 µl of suspension was taken from each vial and spread across the plate using a sterile glass spreader. Plates were incubated at 25°C for 3 days and CFU's were counted.

Range finding trials: On 10/08/2015 assays were set up to determine the dose at which Naturalis will kill WFT. For the assay, 360 µl Naturalis was added to 30 ml tap water and the formulation was inverted 10 times, then 15 ml was pipetted into 15 ml tap water. This was inverted 10x and the same dilution was repeated a further 4 times. This gave a dilution series of equivalent to that shown in Table 1. The suspensions were prepared at 2x strength as the Burkard Sprayer delivered the equivalent of 474 L/ha (0.305 ml/plate at 10 bar pressure).

Table 1 Strengths of dilutions used in range finding experiment

Dilution	Strength
1	0.6 % v/v (2 x label strength)
2	0.3 % v/v (1 x label strength)
3	0.15 % v/v (0.5 x label strength)
4	0.075 % v/v (0.25 label strength)
5	0.0375 % v/v (0.125 label strength)

Formulations were placed in glass vials which were attached to the Burkard sprayer. Blank formulation (Codacide plus water) was sprayed initially as a control and then the weakest dilution (5) through to the strongest dilution (1) were applied to 5 x sterile 90 mm petri dishes (Bibby Sterilin) lined with sterile 90 mm filter papers. A sixth plate was prepared without filter paper so the spores from the petri dishes could be enumerated. Dishes were allowed to dry for approximately 30 min before 5 x adult WFT and small bean leaf as a food source were added to each and sealed using parafilm. Plates were stored at 20°C and adult thrips were assessed after 7 d for mortality (length of mortality assessment was based on similar assays from the literature). Extra plates were set up using dilution 2 to assess the number of spores picked up by the thrips. Three thrips were used for spore enumeration using CFU counts.

For enumeration of spores from thrips, thrips were kept in a dark box for 22 h and then placed in a freezer to kill them. Three thrips were removed using 1ml filter tip pipette tips attached to a VWR vacuum pump and added to 200 µl sterile tween (0.05%). The tubes were vortexted and then sonicated for 3 m to remove the spores from the thrips cuticle. The 200 µl of Tween 80 0.05% was then removed and spread across a 90mm SDA-TW-Ab (Sabouraud Dextrose Agar plus tap water and Chloramphenicol) plate. Plates were incubated for 3 d at 25°C. After 1d it was observed that many of the spores had been washed from the thrips cuticle thus 3 agar plugs (5 mm) were removed using a cork borer and placed inside 55 mm petri dishes for incubation (to prevent plates from becoming uncountable). These were incubated for 24 h at 25°C so spores could germinate and viability could be assessed.

Deposition of spores: 10 ml of Shellsol T were added to each of the spray deposition plates and agitated. Liquid was pumped and dispersed *in situ* using a 10 ml Gilson pipette. The washings were placed in glass vials and a 1/10 dilution series was prepared. To allow for differing concentrations of the formulation, different dilution strengths were tested. For formulation strengths 1 and 2 (the strongest dilutions) the dilutions 10^{-3} and 10^{-4} were used, for formulation strengths 3 and 4, dilutions 10^{-2} and 10^{-3} were used and for 5 (the weakest) 10^{-1} were plated out. To plate out, a 200 µl sub-sample was spread across 3 x SDA-DW-Ab plates and incubated for 3 d at 25°C.

Field trials: A trial was set up using existing formulations of EPF in combination with a range of off-the-shelf adjuvants to enhance the efficacy of products. Adjuvants were applied in admixture with Naturalis at field label rate and spore deposition in flowers and leaves, spore pick up by WFT and biological efficacy was assessed against controls where no adjuvants have been applied. Treatments are shown in Table 2. Sprays were applied to potted flowering strawberry plants (var. 'Flamenco') using a Birchmeier B245 air assisted knapsack sprayer using a micron restrictor nozzle. In total 30 plants were treated as 5 replicates of 6 plants. Temperature, humidity and wind-speed records were taken at the start and end of spraying.

Table 2. Products and rates used in the experiments				
Product	**Trt**	**Colour**	**Rate/ha %**	**Amount product in tank ml**
Silwet	1	R	0.05	0.75
Naturalis + Silwet	2	RR	0.3 + 0.05	4.5 + 0.75
Bond	3	Y	0.14	2.1
Naturalis + Bond	4	YY	0.3 + 0.14	4.5 + 2.1
Codacide	5	B	0.3	4.5
Naturalis + Codacide	6	BB	0.3 + 0.3	4.5 + 4.5
Naturalis	7	BLK	0.3	4.5
Water	8	G	-	-

Bioassay methods: Due to a lack of thrips in culture for this experiment, the approach was amended slightly so mortality could be assessed in vitro, rather than on the plant as planned. Following spraying, six flowers were taken from each treatment (1 per replicate) on Day 0. These were placed individually into Sterilin™ tubes and 10 WFT, which had been collected from culture into a filter tip using an air pump, were introduced to each tube; tubes were sealed with Parafilm™ and samples were held at 20 °C. A piece of bean pod 2 cm x 1 cm was added to each tube after 4 days as a food source as the flowers wilted. The bioassay samples were assessed 7 days after treatment and the numbers of live and dead/mycosed thrips counted. Samples were randomised within the cabinet.

The experiment was repeated with 5 replicates of each treatment. Sprays were applied to 6 plants on the 11 September. A 2 cm x 1 cm bean pod was introduced on Day 1 and with assessments on Day 4, Day 7, Day 10 and Day 17.

Testing tank mixes: Samples were taken of the pre-spray tank mix and the post spray tank mix in clean glass vials (20 ml) and were stored at 5°C overnight until processing. The following day, 1ml of stock was added to 9 ml of sterile Tween 80 0.05 % and mixed thoroughly and a 1 in 10 serial dilution was prepared down to 10^{-3} (based on expected values). From this, 200µl of suspension was plated onto 2 x 90mm SDA+Cu+Ab plates (selective media) and incubated for 3 d at 25°C.

Deposition of spores on leaf/flower/thrip samples: Eight different treatments were sprayed in total onto five sets of six strawberry plants (var. 'Flamenco') during the field trials (Table 2). From each of the five sets of six strawberry plants, the following samples were taken:

1) Strawberry flower for thrips spore pick up experiment
2) Strawberry flower for deposition CFU count
3) Strawberry flower for deposition QPCR
4) Leaf- (1 lobe of 3) for CFU deposition

5) Leaf - (1 lobe of 3) for QPCR deposition

Strawberry flowers for thrips-spore pick up experiment: Flowers were left with their stems on and six thrips were added per tube. Tubes were incubated for 12h at 25°C and then at ambient temp/daylight for 12h. Due to time restrictions tubes were then placed at 7-10°C for the weekend prior to processing to reduce their activity and stop fungal metabolism.

Samples for deposition assessment (2-5): Leaf samples were photographed on a black background card (during the field trial) with a measuring tape as a scale bar prior to being collected in glass vials (20 ml). Leaves were handled by the stem to minimise handling/spore transfer. The background card was changed with every treatment to avoid cross contamination. Flowers were too small to be dissected and photographed in the field, therefore they were collected whole and dissected and photographed in the lab using the same method as above. Flowers were dissected to allow more accurate surface area estimation. Those destined for QPCR were placed in cryovials (2 ml Nalgene) and those for CFU counts were placed in clean glass vials containing 10 ml 0.05% Tween 80 (sterile). Photographs of leaves/flowers were analysed using Image J software to give leaf / flower areas. Where a data point was missing, the average for that sample was used to give spore density.

Processing of CFU samples: Thrips were separated into separate vials and 200 µl of sterile 0.05 % Tween were added. Leaf samples were left in situ in their vials and 10 ml of 0.05 % Tween (sterile) were added. Flowers and leaves in the 10 ml of Tween / thrips in 200 µl of Tween were vortexed for 5 seconds then sonicated for 3 min to loosen any spores. Prior to plating samples were given a final shake to loosen spores. Using each sample, 200 µl of suspension were plated onto 90 mm SDA+Cu+Ab plates and incubated for 3 d at 25°C.

QPCR: Calibration curve preparation: To enable quantification of Naturalis, a calibration curve was prepared. Plates were prepared to harvest pure spores of *B. bassiana* by pipetting and spreading 200 µl of 1/10, 1/100 and 1/000 dilutions of Naturalis onto SDA + TW plates. Plates were allowed to grow until sporulation, at 25 °C, Plates were then stored at 5 °C until use. Spores were removed from the plates using a microspatula and collected in a sterile Eppendorf tube. Five calibration weights were prepared into separate sterile Eppendorf tubes. Weights were as follows: 1) 0.01160 g, 2) 0.00481 g, 3) 0.00250 g, 4) 0.00112 g, 5) 0.00017 g. DNA was extracted from these samples using DNeasy plant mini kit (Qiagen). To enable spore count quantification, three tubes were prepared using 1 ml of 0.05 % Tween 80, to which 0.00082 g, 0.00029 g and 0.00084 g of spores were added. A 1/10 dilution was prepared for each and then the suspension was enumerated using a haemocytometer.

QPCR: Preparation of leaf, flower and thrips samples

Due to different size of starting material (Leaves, Flowers and Thrips), different but comparable DNA extraction techniques were used.

Sample preparation and DNA extraction from leaves using DNeasy® Plant Maxi Kit: Leaves were removed from original sample tubes and transferred into 50ml Fisherbrand centrifuge tubes. Leaves were freeze dried overnight then stored in a -80 °C freezer until use. Freeze dried material was disrupted using sterile homogeniser, then 5 ml of AP1 buffer and 10 µl of RNase A were added to samples. Each sample was vortexed for 10 sec and incubated overnight at 55°C. After lysis was performed, the supernatant was transferred into a new sterile tube. Approximately 5 ml of supernatant was retrieved. From this stage, the DNeasy plant Maxi kit protocol was followed (https://www.qiagen.com/gb/resources/resourcedetail?id=6b9bcd96-d7d4-48a1-9838-58dbfb0e57d0&lang=en). At step 6, 2.5 ml of flow through was retrieved. DNA was eluted in 500 µl of AE buffer. All centrifugation steps were performed at 3000 x g.

Sample preparation and DNA extraction from flowers using DNeasy® Plant Mini Kit: Flower samples were exposed to liquid nitrogen for 2-3 minutes, then, under sterile conditions they were transferred into 2ml sterile Eppendorf tubes. The original tube was rinsed with 200 µl AP1 buffer and buffer was transferred in to the new tube with flower material. Flower material was disrupted by using a sterile micropestle (Fisherbrand). A further 500 µl of AP1 and 4 µl of RNase were added to the remaining buffer and flower sample. Each sample tube was vortexed and incubated at 55°C overnight. Following overnight incubation, tubes were centrifuged for 1 min at 14 000 rpm. Supernatant was transferred in to a new 1.5 ml sterile tube. Approximately 450 ml of supernatant was retrieved. Further steps were followed according the protocol. At step 11 DNA was eluted in 100 µl of AE buffer.

Sample preparation and DNA extraction from thrips using DNeasy® Plant Mini Kit: In total three thrips were combined in to one 1.5 ml sterile Eppendorf tube except: Control treatments “No adjuvant 1”, “No adjuvant 5”, “Bond only 2”, and “Silwet only 1” where only two thrips were combined. Thrips were disrupted in 100 µl of AP1 buffer by using a sterile micropestle. A further 300 µl of AP1 buffer was then added along with 4 µl of RNase A. Samples were then vortexed and incubated overnight at 55 °C. Following this, the DNeasy plant mini kit protocol was followed. DNA was eluted in 100 µl of AE buffer awaiting QPCR.

QPCR of samples

QPCR was undertaken using a Realplex® Mastercycler QPCR machine (Eppendorf, UK). PCR was undertaken according to the method described by Bell *et al*. (2009) using MGB Taqman® probes (Life Technologies, UK). Primers and probes are described in Table 1. The *Beauveria*-specific probe was labelled with VIC (Life Technologies, UK). The *Beauveria* probe was quoted to be specific to *B. bassiana* in the paper used; however personal communications with a primer/probe design company has suggested that it would be a genus-level probe, thus we will refer to it as a *Beauveria* spp. probe.

Table 1. List of primers and probes used in the current study

Primer name	Sequence	Reference
Fungal fwd	AGA TAC CGT CGT AGT CTT AAC CAT AAA CT;	Bell et al., 2009
Fungal rev	TTC AGC CTT GCG ACC ATA CT	Bell et al., 2009
Fungal probe	6-FAM-CGT TCG GCA CCT TAC	Bell et al., 2009
Bbass fwd	GCC GGC CCT GAA ATG G	Bell et al., 2009
Bbass rev	GAT TCG AGG TCA ACG TTC AGA AG	Bell et al., 2009
Bbass probe	VIC-ACA GCT CGC ACC GGA	Bell et al., 2009

Results

Quality control results: CFU results showed that the mean CFU/ml in Naturalis were 1.06 x 10^7 /ml ± 2.1 x 10^6. The label stated dose for Naturalis is 2.3 x 10^7 /ml, so slightly lower than quoted on the label.

Range finding protocol: Bioassay results showed poor thrips mortality in all treatments, with no difference between the highest dilution and the controls (Fig. 1). No CFU's were observed growing on plates from the thrips washing experiment. Results obtained from the depositions plates (Fig. 2) showed that the dilution series had been prepared well and there was evidence of a good serial dilution. The results obtained however were much lower than had been expected for this assay. Plates for dilution 2 did not have any CFU's therefore results are not quoted on the graph.

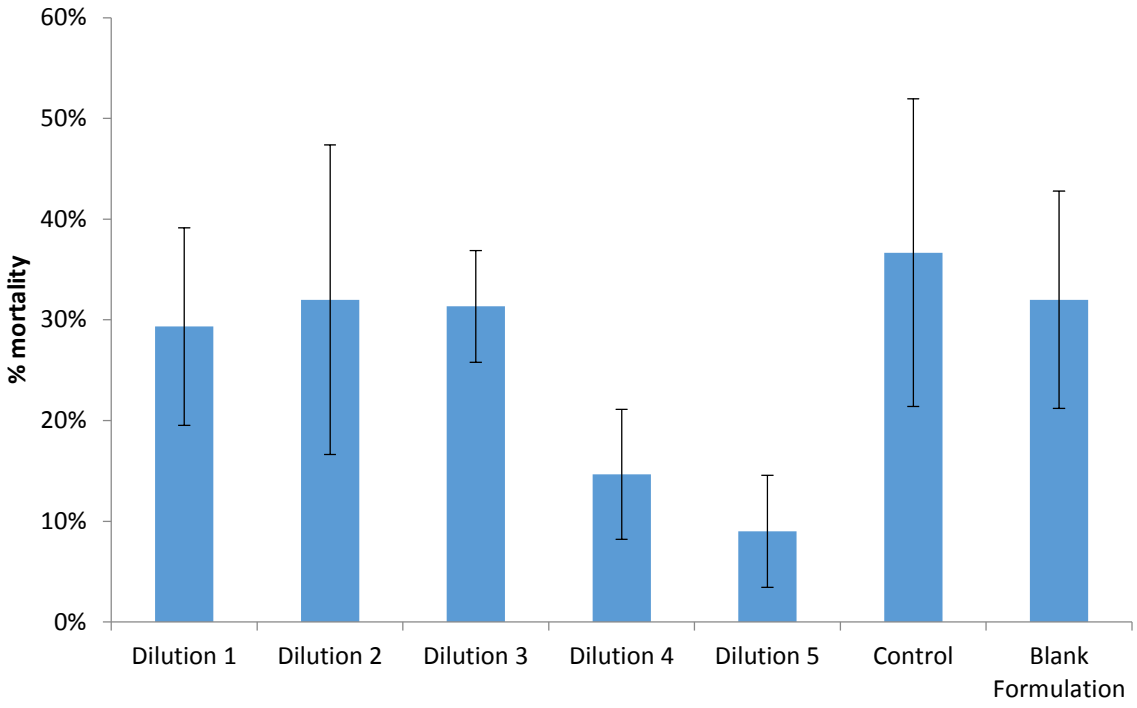

Figure 1. Adult thrips mortality results from the range finding protocol after 7d

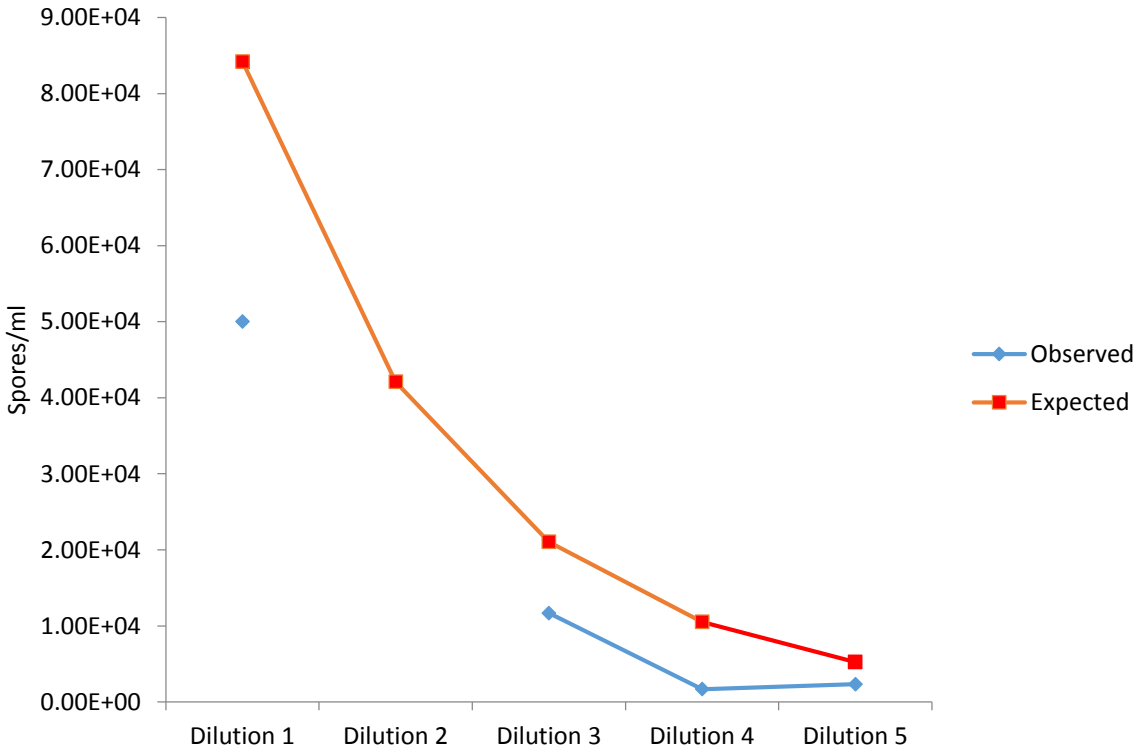

Figure 2. Results from the spray deposition plates in range finding bioassay. Only one plate was sampled per dilution (given sprayer space restrictions) therefore no SE can be applied

Field trial results: Figure 3 gives the results of the CFU counts obtained from the tank mixes. The observed values of the tank mixes prior to spraying were, in general, much higher than the label recommended amount and always lower in the post spray sample. This is common as spores can stick to the inside of the tank and reduce the deposited value. The CFU given by the manufacturer accounts for this.

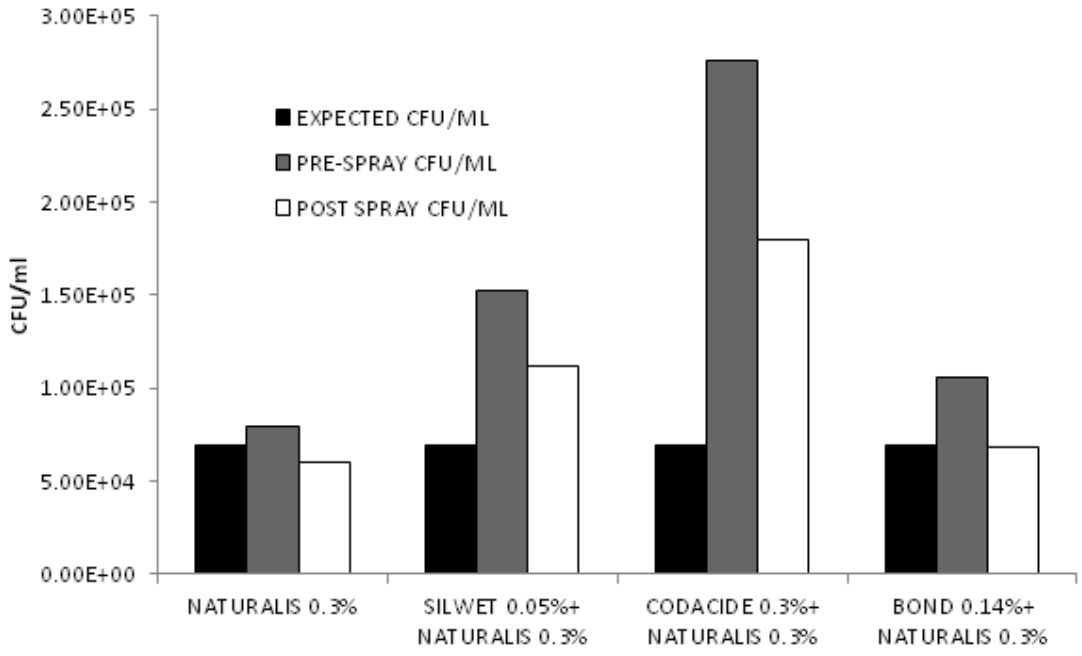

Figure 3. CFU counts taken from pre and post spray tank mixes/ml. One formulation per tank mix was prepared with 3 pseudo-replicates

The average CFU count per cm^2 from both the flower and leaf samples is shown in Figure 4. A two-way ANOVA showed that there was no significant interaction between treatment and leaves/flowers, therefore this term was removed from the model ($F=0.556$, $p= 0.64809$). Subsequent analysis showed there to be no significant difference between the recovery of spores between treatments (from both leaves and flowers) ($F=2.027$, $p= 0.12978$). There was however a significant difference in density of spores recovered between leaves and flowers, with flowers retaining significantly more spores than leaves in all treatments ($F=11.346$, $p=0.00198$). When normalised in relation to tank mix spores/ml, deposition as an amount of the total sprayed is highest in the Naturalis treatment (Figure 5). QPCR results returned on leaves and flowers were negative for *Beauveria bassiana* DNA. This is likely to be due to DNA extraction methodology than a lack of *Beauveria bassiana* sprayed.

Figure 6 shows the number of spores of *Beauveria bassiana* recovered from each thrips during the bioassay. The figures show that thrips pick up was low, although the highest level of pick-up was observed using the Bond adjuvant.

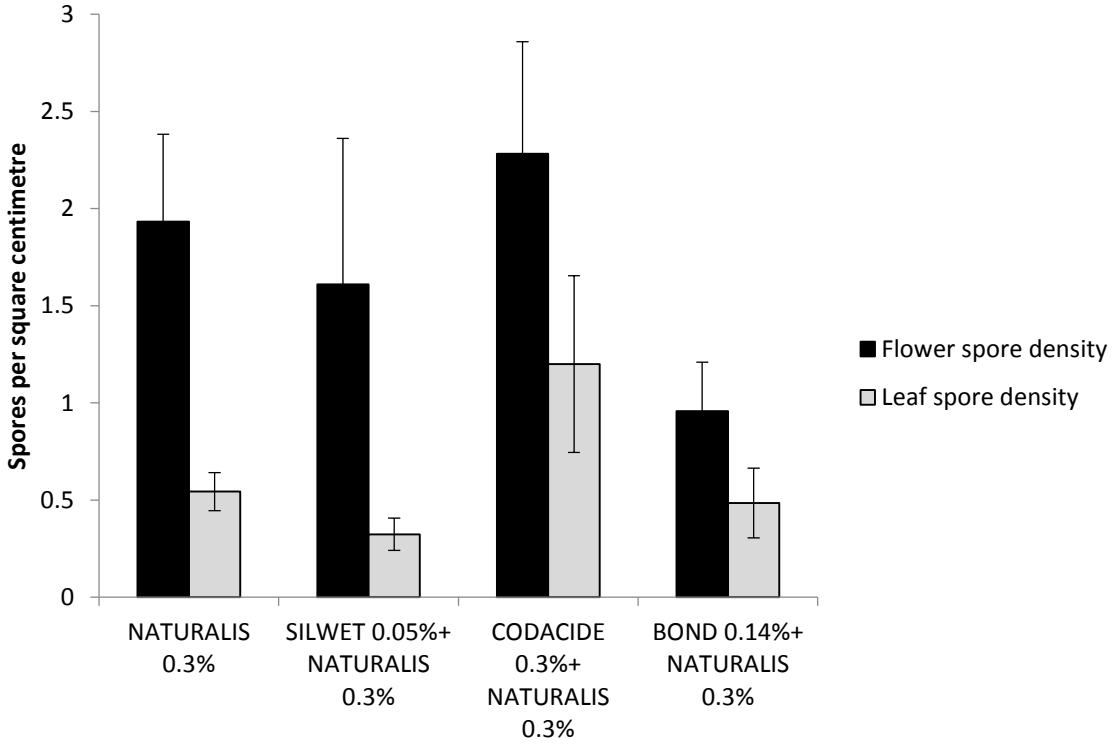

Figure 4. Summary of CFU densities per leaf and per flower from different treatments during August field trial. No significant difference was shown between treatments (F=2.027 p=0.12978), however significantly more spores were retained on flower than leaves (F=11.346,p= 0.00198).

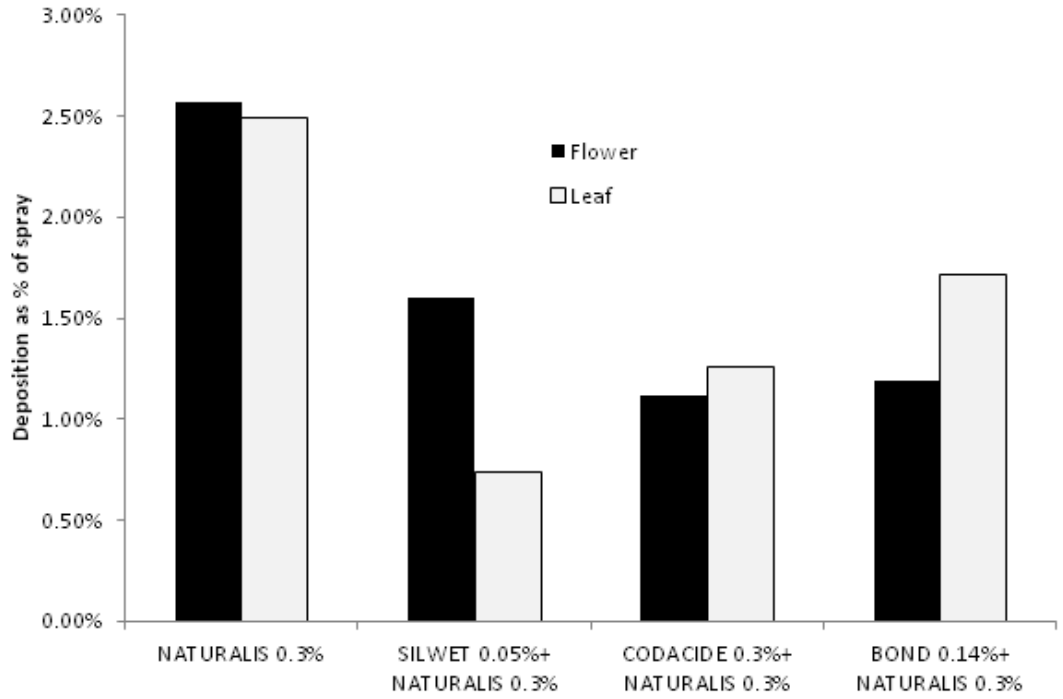

Figure 5. Graph showing the proportion (hence no error bars) of CFU deposition per leaf/flower as a % of the amount sprayed

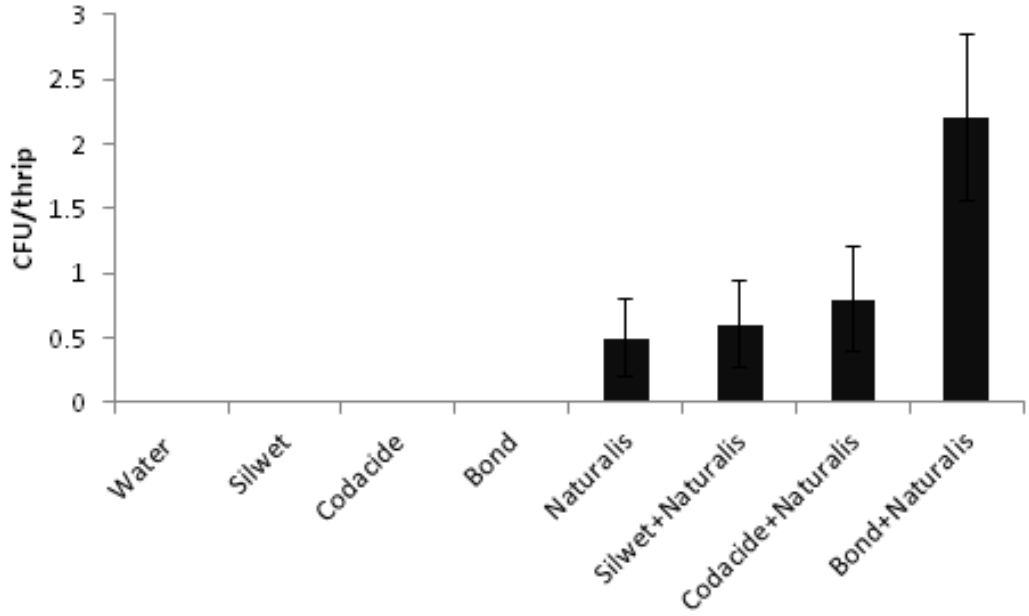

Figure 6. CFU's per thrips following fungal ID/plate checking (Note on chart axis, CFU/thrips)

QPCR Results

Fluorescence profiles returned for the flower and flower samples showed that only the calibration curves were *Beauveria bassiana* positive for both assays therefore no further analysis could be carried out. The thrips assay did show some replication of *Beauveria bassiana* DNA among the samples indicating that DNA was extracted and amplified during the experiment. The majority of C_t values returned however, were $>C_t$ 30, meaning it took over 30 cycles to reach a threshold value. Figure 7 shows the calibration curve for the thrips assay plotted with a best fit line. This shows that once sample are over C_t 30, the accuracy of prediction breaks down i.e. replicates of calibration sample 5 should predict the same number of spores in each replicate, however the three replicates predicted between 382-1844 spores. Figure 8 shows the numbers of spores predicted using this calibration curve, however, the results predicted positive values for known negative samples at this high C_t level, meaning an accurate prediction cannot be achieved at this stage.

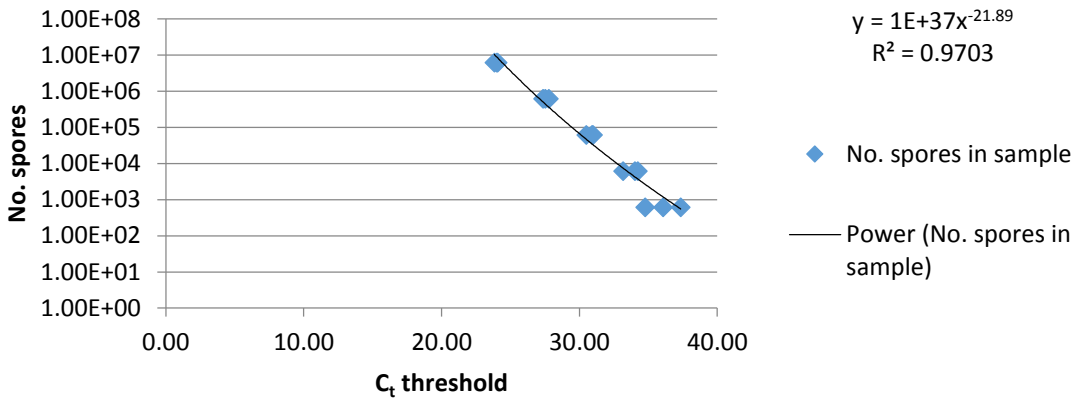

Figure 7. Calibration curve from thrips QPCR assay.

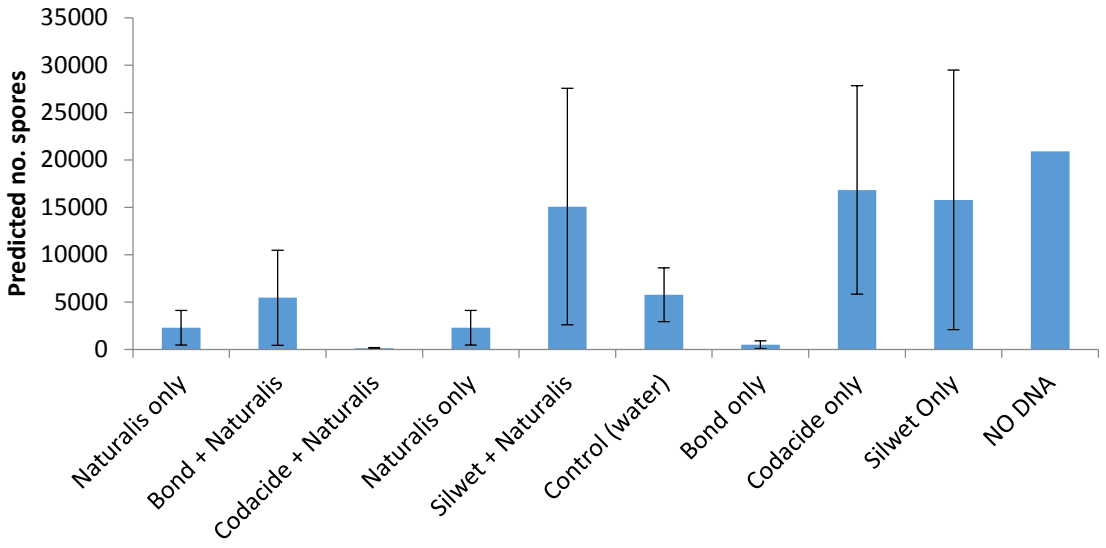

Figure 8. Predicted numbers of spores per 3 thrips derived for calibration curve results

Spore Efficacy Results: The mortality results for the first experiment were not statistically significant with over 80% mortality in all treatments including the control, the reasons for this are not known and results are not presented. The second experiment had a low mortality; this was not statistically significant (Table 3). It was important to note that 1st instar larvae were found on the bean pods at the Day 11 assessment.

Table 3. Mean % mortality of female WFT (based on 5 replicates of 5 adults per unit) at 4, 7 and 11 days after product application.

Product	Day 4	Day 7	Day 11
Silwet	0	12	16
Naturalis + Silwet	16	52	60
Bond	10	44	52
Naturalis + Bond	0	8	36
Codacide	12	20	24
Naturalis + Codacide	0	8	16
Naturalis	0	20	28
Water	10.7	25.3	29.3
F. pr.	0.42	0.263	0.229
rep.	5	5	5
d.f.	32	32	32
s.e.d.	9.32	19.96	18.97
l.s.d.	18.97	40.66	38.64

Discussion

Range finding trials: Given time constraints, range finding trials were conducted one week prior to the field trials therefore results from this did not directly feed into the field trial experimental set up. The QC results showed that the formulation was viable on spraying; however the spray deposition results were lower than expected when CFU's were checked. This may have been due to spore viability being affected during spraying, or poor recovery from deposition plates. No viable spores were removed from the thrips in this experiment; therefore it is unclear whether thrips picked up any spores using this bioassay technique. The bioassays did not show any difference in mortality of thrips compared to the control; therefore the bioassay technique needs to be improved. One theory could be that thrips did not pick up enough spores from the filter paper either due to the texture of the substrate or because they spent the majority of time residing on the bean pod which was untreated. Amendments to the bioassay technique have been made during Q4 of y1 in order to improve on these results and in addition, reduce control mortality. Improvements include using a glass jar system for the bioassay to contain thrips and using a whole bean pod dipped in formulation instead of spraying filter paper substrate. This improves on the first assay technique as the body of the jars are glass can be cleaned easily and WFT were unlikely to escape. Also, thrips are likely to remain in contact with the surface which has been sprayed rather than reside on a non-sprayed surface as with the original design. This method shall be employed in future work.

Field trial results: Bioassay results from the first field trial commenced in August 2015 had high mortality under all treatments therefore results were discarded. Results from the deposition work showed that initial tank mix CFU's were much higher than expected for all formulation prepared and especially so for the Codacide+Naturalis formulation. Post spray samples were lower in all cases and still higher than expected for both Silwet - L77 + Naturalis and Codacide + Naturalis. This could be due to the way tank mixes were prepared i.e. there could have been residual spores in the tank leading to an additive effect in samples, although this is unlikely as the tank was triple rinsed. The drop in levels from pre-spray tank mixes and post-spray levels are to be expected given the materials from which spray tanks are made. Spores of *Beauveria bassiana* are hydrophobic and may stick to materials such as plastic. This will have been accounted for in product development and CFU label values should account for this drop is spore level during the spray process.

Deposition figures from the CFU counts showed that there was no significant difference in spore recovery between adjuvant treatments, however significantly more spores were deposited on flowers. It may be hypothesised that the complex structure of the flower may contribute to higher spore retention and the flat leaf surface contribution to lower spore retention. This is a promising result as the target stages of WFT will be found within the flower. Further work to ascertain which parts of the flower are retaining the most spores could provide further information as to whether spores are penetrating the areas where the thrips reside. Even though no significant difference was found between treatments, the highest spore deposition was observed in the Codacide + Naturalis treatment, however as the tank mix was shown to have a higher number of spores initially, this is likely to be due to the spores/ml in the starting formulation rather than any deposition difference. A comparison of CFU numbers deposited per treatment (not density) with tank mix concentration showed that Naturalis retained the most spores compared to the number sprayed. This experiment would need to be repeated to confirm these results. A comparison with QPCR results was not possible for leaves and flowers, as the assay did not return any *Beauveria bassiana* positive samples. The aim of the QPCR assay was to avoid the washing step as there may have been differences in spore retention between treatments. Given the current results, the protocol may need to be modified, as we hypothesise spores may need to be separated from the flower/plant material prior to DNA extraction to obtain optimal results, as there may have been an overload of non-fungal DNA in sample columns during DNA extraction. Further refinements to assay procedure may yield positive results in the future such as improving specificity of the probes or using a wash step to remove spores from the plant material prior to QPCR analysis.

Washing spores from the thrips showed that most spores were picked up from the Bond + Naturalis treatments; however the numbers recovered were very low (1-2 per thrip) and further work to confirm this would need to be carried out. This would link in with work to see if spore deposition is occurring in areas where the thrips reside. QPCR results for thrips were shown to be inconclusive. The C_t (cycle threshold) value given in the assay informs of how many PCR cycles it takes for DNA to reach a threshold level, this was then compared to a calibration curve prepared from known numbers of Naturalis spores. The C_t values for samples returned were in general >30, and the plot of the calibration curve showed that calibration sample replication at this level broke down meaning readings could not be accurate (variable readings were given to samples of the same concentration). Future work for both CFU counts and QPCR analysis should examine pooling more thrips together to ensure that a readable number of spores are returned in experiments, this would improve the accuracy in spore pick up estimation.

The bioassay results (from the repeated assay) gave more promising results with highest mortalities seen in the Naturalis + Silwet treatment, compared to the Silwet only control; however there is a still a problem with high control mortality in other treatments i.e. Bond only. Further protocol improvements have been underway in Q1 of 2016 and this will aim to reduce control mortality so the effect of Naturalis can be confirmed.

Dues to the late start to the project in 2015, the numbers of thrips in culture were lower than required to achieve the original planned size of trial, however for 2016 work, this shall be undertaken earlier in the season when thrips numbers in culture are higher.

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 (EMR Yr 1)

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 3,849 ha of strawberries grown in the UK in 2012, 2,417 ha were treated with *Phytoseiulus persimilis* and 2,567 ha with *N. cucumeris*. Together with other predatory mites this represents a 20 fold increase since 2001 (Garthwaite et al., 2013).

The efficient and successful use of predatory mites relies on careful coordination of crop protection spray strategies to maintain predators in the crop. This coordination forms part of IPM and can work very successfully. However, strawberries are vulnerable to attack by capsid bugs and SWD leading to an increase in crop protection product usage. The products generally recommended for SWD control are spinosad and lambda-cyhalothrin, and the latter is also used to control capsids (organophosphates are not normally used on strawberries during the growing season because of the long harvest intervals). Pyrethroids are highly toxic to phytoseiids (see HDC review SF133) and spinosad is generally regarded as harmful to *N. cucumeris* (for example, IPM impact; Koppert; Biobest websites). Therefore one of the reasons for failure of *N. cucumeris* establishment in crops, and subsequent failure of thrips control, is likely to be the detrimental effect of control products on these predators.

Furthermore, predatory mites within a crop are exposed to a range of other compounds, many of which are potentially toxic. However, there are gaps in our knowledge of the compatibility of some of these products with *N. cucumeris*. In addition, whilst some compounds are regarded as relatively safe for predatory mites, recent studies (Sampson, 2014) have suggested that this might not be the case. Crop protection products such as fungicides can also be toxic, and tank mixes can have unexpected synergistic effects. In addition, laboratory toxicity trials may use only one application, whilst in a farm situation sprays might be applied multiple times.

Materials and methods

Choice of treatments: Compounds already known to be toxic to predatory mites were excluded. Following analysis of 226 tank mixes from a recent survey of spray programmes and WFT control (Sampson, 2014), and in consultation with growers, the following common pesticide tank mixes were chosen for assessment (Table 1).

Table 1. Pesticides used in the trial.

Pesticides	Application rate (/ ha in 1000 l/ha)	Notes
Amistar (azoxystrobin) and Rovral (iprodione)	Amistar, 1 l/ha; Rovral, 1kg/ha	Two widely applied fungicides often used in combination.
Nimrod (bupirimate) and Teldor (fenhexamid)	Nimrod, 1.4 l/ha; Teldor, 1.5 kg/ha	Two widely applied fungicides often used in combination.
Signum (boscalid + pyraclostrobin) and Systhane (myclobutanil)	Signum, 1.8 kg/ha; Systhane, 0.23 l /ha	Two widely applied fungicides often used in combination.
Aphox (pirimicarb) and Rovral (iprodione)	Aphox, 560 g/ha; Rovral, 1 kg/ha	An insecticide and fungicide. Aphox is reported to be harmful to *N. cucumeris*, but this combination was requested by growers as it is a common early tank mix.
Tracer (spinosad)	150 ml / ha	Although spinosad is already known to be harmful, data on multiple doses is limited. Tracer was found to be very widely used, in 16/20 farms studied, with an average of 2.4 applications per season. Also functioned as a positive control.
Untreated	-	-

Treatment programme: The trial took place in tunnels of strawberries (variety Scarlet) planted in beds (Fig. 1) between 23 June and 9 July 2015 (Fig. 2). Each tunnel consisted of five beds, the middle row of which was used. A randomised block design was utilised with 6 replicates of 6 treatments, the plots arranged end to end in a bed, each plot was 8 m long. Each plot was separated by an untreated area of 8 m to reduce mite migration between plots.

N. cucumeris were released as a loose product within the experimental plots following manufacturer's instructions at a rate of 500 per plant. Three days later, a pre assessment was made of the numbers of *N. cucumeris* in each plot and the first treatments were applied (Table 1). Each treatment was applied three times, seven days apart, using a motorised knapsack sprayer at 1000 l/ha.

Figure 1. Photograph of trial site

Pre and post spray assessments were made by visual counts of the numbers of mites on 20 furled leaves (NB: there were no flowers at the beginning of the trial and experience shows that *N. cucumeris* can be found on this strawberry leaf stage). Following consultation with a 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 following square root transformation.

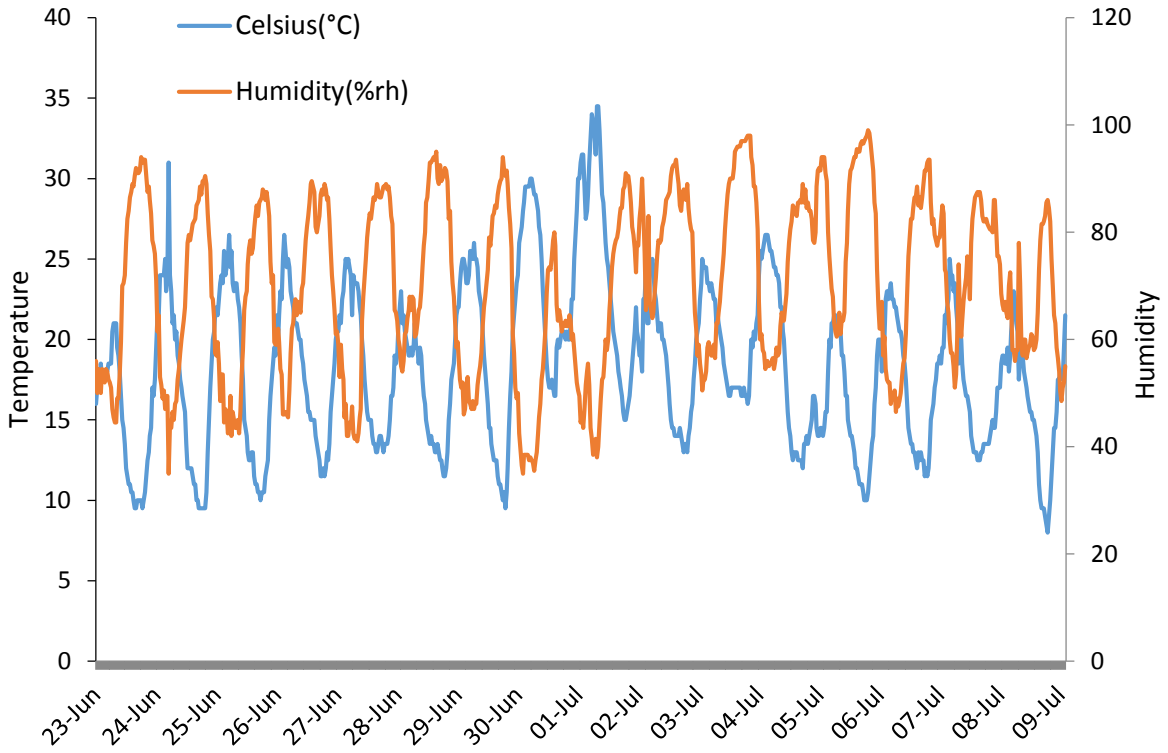

Figure 2. Climatic conditions over the course of the trial

Results

N. cucumeris numbers declined for all the populations from the 1st to 2nd assessments, which could be associated with unknown environmental factors, or dispersal in search of further prey. However, it was noticeable that numbers declined more rapidly in some of the treatments and numbers in the untreated controls were increasing at the third assessment, suggesting that the treatments themselves were also having a detrimental effect on the populations. This is considered in the analysis as all treatments are compared to the untreated control and the statistical methods used were selected to take account of these factors.

No significant ($P<0.05$) treatment effects were found for the pre-assessment and first two assessments when analysed separately, however the third assessment showed a significant reduction in *N. cucumeris* numbers exposed to repeated applications of Nimrod/Teldor and Signum/Systhane (Fig. 3). Tracer and Aphox/Rovral did not affect numbers of *N. cucumeris* on the strawberry leaves. A repeated measures analysis of the entire sample set showed a significant ($p<0.05$) reduction in phytoseiid mite numbers through the course of the trial for Nimrod/Teldor and Signum/Systhane and also Aphox/ Rovral.

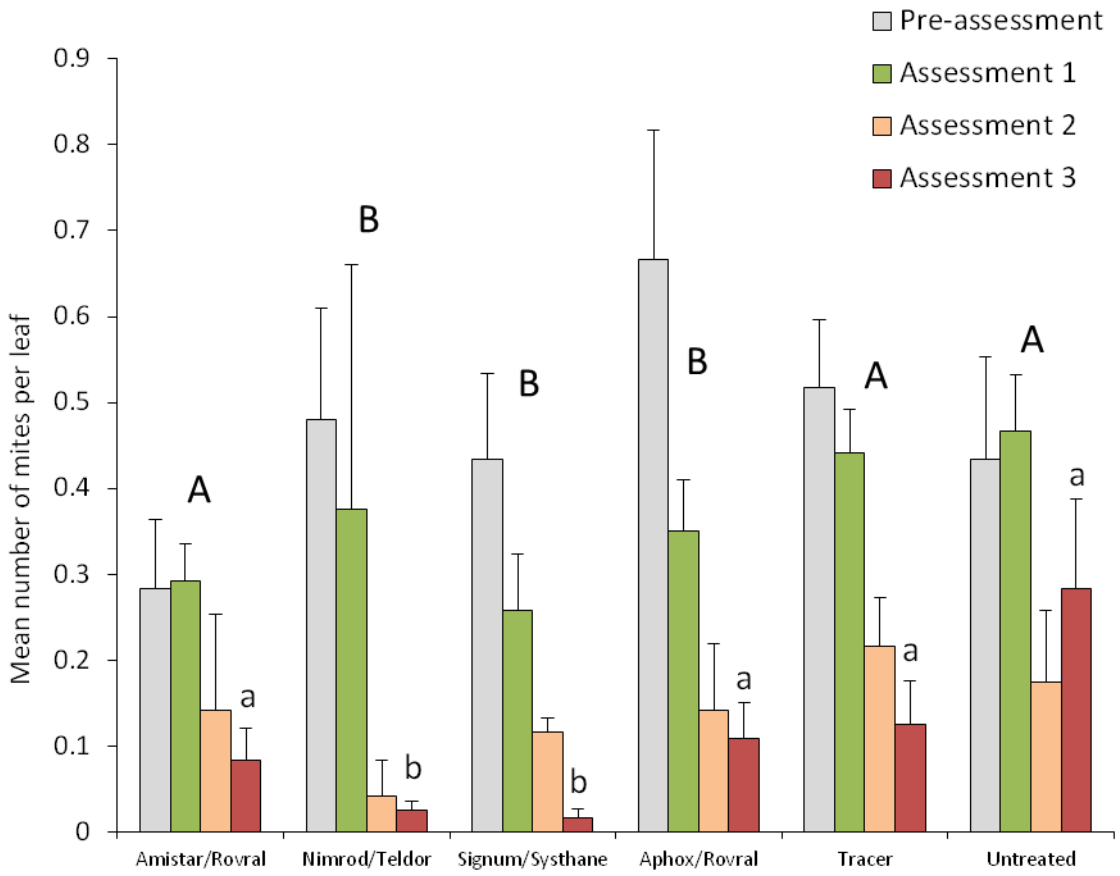

Figure 3. Effect of various pesticides in combination or alone over three applications. Groups of columns with different upper case letters above are significantly different over the course of the trial ($p<0.05$). Columns with different lower case letters above are third assessments with significantly different counts ($p<0.05$)

Discussion

A widely used website associated with a biocontrol company defines Nimrod (bupirimate) and Signum (boscalid + pyraclostrobin) as safe to *N. cucumeris* and Teldor (fenhexamid) as only slightly harmful. Systhane (myclobutanil), was described as safe to the related species *Amblyseius californicus*. Growers could therefore assume that application of these compounds would be fully compliant with an IPM programme, when in fact these results suggest that this might not be the case where repeated applications are made.

Aphox was already reported as harmful to *N. cucumeris* (Koppert side effects guide, 2016). Although Aphox use on strawberries will be restricted from 2016 onwards applications of Aphox could explain past biological control failures.

Each treatment was applied three times and it was only after the third application that significant differences in phytoseiid mite numbers were found. Hence, small cumulative impacts on *N. cucumeris* populations might be significant over time. A similar pattern has been reported for the use of phosalone on apple trees, where a single application had no effect on predatory mite numbers, but two applications reduced the population (Raudonis et al, 2004).

It is also possible that the compounds in tank mixes combine additively or even synergistically, so that the effect is greater than that of each individually, but this needs to be confirmed.

In summary, these results suggest that repeated applications of fungicides can have a detrimental effect on predatory mite populations.

Knowledge and Technology Transfer

23 July 2015 SF 156 PROGRAMME MANAGEMENT GROUP (PMG) MEETING at Hugh Lowe Farms, Park Field, Comps Lane

November 2015: Presentation by Dr Jean Fitzgerald 'Improving integrated pest management in strawberry' at AHDB Soft Fruit Day

11 February 2016 Presentation by Dr Jean Fitzgerald and Dr Chantelle Jay 'Control of Western Flower Thrips in tunnel grown strawberries at AHDB Horticulture Agronomists Day

14 January 2016 SF 156 PROGRAMME MANAGEMENT GROUP (PMG) MEETING at EMR

January 2016: Presentation by Dr Jean Fitzgerald 'Control of Western Flower Thrips in tunnel grown strawberries' at Syngenta Bioline Technical Day

References

Abdullah Z, Greenfield B, Ficken K, Taylor J, Wood M & Butt T (2015) A new attractant for monitoring western flower thrips, Frankliniella occidentalis in protected crops. Springer Plus 4: 1-9.

Cloyd R (2009) Western Flower Thrips (Frankliniella occidentalis) Management on Ornamental Crops Grown in Greenhouses: Have We Reached an Impasse? Pest Techology 3: 1-9.

Cowles R, Cowles E, McDermott A & Ramoutar D (2000) "Inert" Formulation Ingredients with Activity: Toxicity of Trisiloxane Surfactant Solutions to Twospotted Spider Mites (Acari: Tetranychidae). Journal of Economic Entomology 93: 180-188.

Garthwaite, D.G., Hudson, S. Barker, I., Parrish, G., Smith, L. & Pietravalle, S. (2013). Pesticide Usage Survey report 251. Soft fruit in the United Kingdom. London. Defra.

Imai T, Maekawa M & Murai T (2001) Attractiveness of methyl anthranilate and its related compounds to the flower thrips, Thrips hawaiiensis (Morgan), T. coloratus Schmutz, T. flavus Schrank and Megalurothrips distalis (Karny) (Thysanoptera: Thripidae). Applied Entomology and Zoology 36: 475-478.

Koppert side effects guide, (2016).http://www.koppert.com/side-effects/#

Murai T, Imai T & Maekawa M (2000) Methyl Anthranilate as an attractant for two thrips species and the thrips parasitoid Ceranisus menes Journal of Chemical Ecology 26: 2257-.

Raudonis,L., Surviliene, E., Valiuskaite, A. (2004). Toxicity of pesticides to predatory mites and insects in apple-tree site under field conditions. Environ Toxicol., 19, 291-5.

Sampson, C. (2014). Management of pesticide resistant western flower thrips on tunnel grown strawberry: a study of the reasons for successes and failures on commercial production sites. HDC Report.

Appendix 1: Husbandry records from sites sampled for N. cucumeris numbers

Site 1: Husbandry records. Taylors Red Glory. 2015

Heading	Product	Date
Fungicides	Systhane 20 EW	12/06/2015
Fertiliser	Calmax	05/06/2015
Fungicides	Amistar	
Fungicides	Systhane 20 EW	28/05/2015
Fungicides	Switch	
Fungicides	Amistar	17/05/2015
Fungicides	Stroby WG	08/05/2015
Fungicides	Scala	
Fungicides	Rovral WG	28/04/2015
Fungicides	Fortress	
Fungicides	Amistar	21/04/2015
Fertiliser	Maxicrop	
Insecticides	Aphox	
Insecticides	Pyrethrum 5 EC	14/03/2015
Fungicides	Signum	

Heading	Product	Date
Fungicides	Serenade ASO	
Fungicides	Rovral WG	
Fungicides	Rovral WG	
Fungicides	Kindred	21/09/2015
Fungicides	Topas	
Fungicides	Signum	12/09/2015
Fungicides	Rovral WG	
Fungicides	Amistar	03/09/2015
Fertiliser	Potassium Bicarbonate	
Adjuvants	Codacide	26/08/2015
Fungicides	Switch	
Fungicides	Systhane 20 EW	24/08/2015
Fungicides	Teldor	
Fungicides	Nimrod	17/08/2015
Fertiliser	Potassium Bicarbonate	
Fertiliser	Maxicrop	15/08/2015
Fertiliser	Potassium Bicarbonate	
Fertiliser	Maxicrop	11/08/2015
Insecticides	Chess WG	
Fungicides	Systhane 20 EW	08/08/2015
Fungicides	Frupica SC	
Fertiliser	Potassium Bicarbonate	
Adjuvants	Codacide	01/08/2015
Fungicides	Serenade ASO	28/07/2015
Fungicides	Teldor	
Fungicides	Nimrod	18/07/2015
Fertiliser	Potassium Bicarbonate	09/07/2015
Fertiliser	Maxicrop	
Fungicides	Nimrod	04/07/2015
Fungicides	Teldor	
Insecticides	Tracer	
Fungicides	Switch	29/06/2015
Fungicides	Topas	
Fungicides	Frupica SC	12/06/2015

Site 2 Husbandry Records.
Jubilee. 2015

Date	Spray Name	Spray Type	S	Reason
29/08/2015	AQ10WG	Miscellaneous		mildew
29/08/2015	Maxicrop Triple	Trace Elemen		
29/08/2015	Inca	Trace Elemen		
25/08/2015	Teldor	Fungicide		botrytis
25/08/2015	Maxicrop Triple	Trace Elemen		
25/08/2015	Inca	Trace Elemen		
25/08/2015	hortiboost	Growth Regul		
17/08/2015	Potassium Bicarbonate	Trace Elemen		plant health
30/07/2015	vegro			mildew bortrytis
30/07/2015	serenade	Fungicide		
30/07/2015	AQ10WG	Miscellaneous		
18/07/2015	Systhane 20 EW	Fungicide		mildew bortrytis
18/07/2015	Inca	Trace Elemen		
18/07/2015	Maxicrop Triple	Trace Elemen		
18/07/2015	Amistar	Fungicide		
18/07/2015	hortiboost	Growth Regul		
26/06/2015	Inca	Trace Elemen		mildew, plant health
26/06/2015	hortiboost	Growth Regul		
26/06/2015	AQ10WG	Miscellaneous		
19/06/2015	serenade	Fungicide		mildew bortrytis
19/06/2015	potizon	Trace Elemen		
19/06/2015	Maxicrop Triple	Trace Elemen		
19/06/2015	AQ10WG	Miscellaneous		
05/06/2015	serenade	Fungicide		mildew bortrytis
05/06/2015	Maxicrop Triple	Trace Elemen		
05/06/2015	AQ10WG	Miscellaneous		
05/06/2015	kelpack	Trace Elemen		
05/06/2015	cal max	Trace Elemen		
16/04/2015	Maxicrop Triple	Trace Elemen		botrytis, mildew
16/04/2015	Frupica SC	Fungicide		
16/04/2015	Fortress	Fungicide		
07/04/2015	Teldor	Fungicide		botrytis, mildew
07/04/2015	Systhane 20 EW	Fungicide		
07/04/2015	Maxicrop Triple	Trace Elemen		
07/04/2015	Aphox	Insecticide		
25/03/2015	Teldor	Fungicide		botrytis
25/03/2015	Maxicrop Triple	Trace Elemen		
16/02/2015	Teldor	Fungicide		mildew bortrytis
16/02/2015	Scala	Fungicide		
16/02/2015	Maxicrop Triple	Trace Elemen		
28/01/2015	Switch	Fungicide		aphid, botrytis, mildew
28/01/2015	Signum	Fungicide		
28/01/2015	Maxicrop Triple	Trace Elemen		
13/01/2015	Switch	Fungicide		botrytis
13/01/2015	Scala	Fungicide		
13/01/2015	Rovral WG	Fungicide		
13/01/2015	Maxicrop Triple	Trace Elemen		
14/10/2014	Equity	Insecticide		insect pests
14/10/2014	Signum	Fungicide		mildew bortrytis aphids
14/10/2014	Hallmark With Zeon Te	Insecticide		
14/10/2014	Fortress	Fungicide		

Site 3. Husbandry Records. Amesti. 2015			
DATE	**PRODUCT**	**PRODUCT USE**	**Reasons for use**
05-May	pyrethrum	insecticide	aphids
05-May	chess	insecticide	aphids
05-May	urea	nutrient	feed
22-May	fortress	fungicide	mildew
22-May	Chess	insecticide	aphids
22-May	Pyrethrum	insecticide	aphids
22-May	urea	nutrient	feed
22-May	Calcium Flo	nutrient	feed
22-May	Maxicrop	nutrient	feed
04-Jun	Pot Bicarb	nutrient	mildew
04-Jun	Sulphur flo	nutrient	feed
04-Jun	codacide oil	adjuvant	adjuvant
08-Jun	fortress	fungicide	mildew
08-Jun	Calcium Flo	nutrient	feed
08-Jun	Maxicrop	nutrient	feed
12-Jun	calypso	insecticide	Aphids, vine weevil
12-Jun	systhane	fungicide	mildew
12-Jun	Maxicrop	nutrient	feed
16-Jun	Pot Bicarb	nutrient	mildew
16-Jun	Sulphur flo	nutrient	feed
16-Jun	codacide oil	adjuvant	adjuvant
23-Jun	dynamec	insecticide	red spider
23-Jun	apollo	insecticide	red spider
23-Jun	slither	wetter	adjuvant
23-Jun	Maxicrop	nutrient	feed
23-Jun	nimrod	fungicide	mildew
23-Jun	Calcium Flo	nutrient	feed
26-Jun	switch	fungicide	mildew, botrytis
26-Jun	Calcium Flo	nutrient	feed
26-Jun	Maxicrop	nutrient	feed
30-Jun	Pot Bicarb	nutrient	mildew
30-Jun	Sulphur flo	nutrient	feed
30-Jun	codacide oil	adjuvant	adjuvant
11-Jul	nimrod	fungicide	mildew
11-Jul	tracer	insecticide	thrips
11-Jul	aphox	insecticide	aphids
11-Jul	Calcium Flo	nutrient	feed
16-Jul	Pot Bicarb	nutrient	mildew
16-Jul	codacide oil	adjuvant	adjuvant
22-Jul	Pot Bicarb	nutrient	mildew
22-Jul	codacide oil	adjuvant	adjuvant
22-Jul	systhane	fungicide	mildew
22-Jul	Calcium Flo	nutrient	feed

22-Jul	X Change	adjuvant	0
27-Jul	Pot Bicarb	nutrient	mildew
27-Jul	Sulphur flo	nutrient	feed
27-Jul	codacide oil	adjuvant	adjuvant
07-Aug	Pot Bicarb	nutrient	mildew
07-Aug	Sulphur flo	nutrient	feed
07-Aug	codacide oil	adjuvant	adjuvant
19-Aug	Pot Bicarb	nutrient	mildew
19-Aug	Sulphur flo	nutrient	feed
19-Aug	Maxicrop	nutrient	feed
19-Aug	slither	wetter	adjuvant
22-Aug	nimrod	fungicide	mildew
22-Aug	Calcium Flo	nutrient	feed
22-Aug	urea	nutrient	feed
22-Aug	Maxicrop	nutrient	feed
29-Aug	Rovral WG	fungicide	botrytis
29-Aug	systhane	fungicide	mildew
29-Aug	urea	nutrient	feed
29-Aug	Calcium Flo	nutrient	feed
29-Aug	Maxicrop	nutrient	feed
03-Sep	systhane	fungicide	mildew
03-Sep	urea	nutrient	feed
03-Sep	Rovral WG	fungicide	botrytis
03-Sep	Calcium Flo	nutrient	feed
03-Sep	Maxicrop	nutrient	feed
11-Sep	Pot Bicarb	nutrient	mildew
11-Sep	Sulphur flo	nutrient	feed
11-Sep	codacide oil	adjuvant	adjuvant
17-Sep	teldor	fungicide	botrytis
17-Sep	nimrod	fungicide	mildew
17-Sep	urea	nutrient	feed
17-Sep	Calcium Flo	nutrient	feed
17-Sep	Maxicrop	nutrient	feed
25-Sep	Rovral WG	fungicide	botrytis
25-Sep	Amistar	fungicide	mildew
05-Oct	signum	fungicide	mildew, botrytis
15-Oct	scala	fungicide	botrytis

Sites 4, 5 and 6. Husbandry records. 2015.

Site 4=Jubilee

Sites 5 & 6=Amesti

Product		Date	Comment
Topas	Fungicides	27/04/2015	Mildew
CalMax	Fertiliser	04/05/2015	
Maxicrop Concentrate	Fertiliser		Feed
Frupica SC	Fungicides		Spider
Systhane 20 EW	Fungicides		Mildew
Maxicrop Triple	Fertiliser	11/05/2015	Feed
CalMax	Fertiliser		
Nimrod	Fungicides		Botrytis
Teldor	Fungicides		Botrytis
Maxicrop Triple	Fertiliser	19/05/2015	Feed
Kumulus Df	Fungicides		Mildew
Serenade ASO	Fungicides		Botrytis
CalMax	Fertiliser	26/05/2015	
Maxicrop Triple	Fertiliser		Feed
Scala	Fungicides		Botrytis
Topas	Fungicides		Mildew
Maxicrop Triple	Fertiliser	05/06/2015	Feed
CalMax	Fertiliser		
Calypso	Insecticides		Aphids
Systhane 20 EW	Fungicides		Mildew
Nimrod	Fungicides		Botrytis
Maxicrop Triple	Fertiliser	10/06/2015	Feed
Sulphur Flowable	Fertiliser		1.5ml per litre of spray. Feed
Potassium Bicarbonate	Fertiliser		Feed
Maxicrop Triple	Fertiliser	20/06/2015	Feed
Topas	Fungicides		Mildew
Maxicrop Triple	Fertiliser	24/06/2015	Feed
Sulphur Flowable	Fertiliser		1.5ml per litre of spray. Feed
Potassium Bicarbonate	Fertiliser		Feed
Maxicrop Triple	Fertiliser	27/06/2015	Feed
Systhane 20 EW	Fungicides		Mildew
Maxicrop Triple	Fertiliser	02/07/2015	Feed
Potassium Bicarbonate	Fertiliser		Feed
Maxicrop Triple	Fertiliser	06/07/2015	Feed
CalMax	Fertiliser		
Clayton Concorde	Fungicides		Mildew
Maxicrop Triple	Fertiliser	01/08/2015	Feed
Potassium Bicarbonate	Fertiliser		Feed
CalMax	Fertiliser	07/08/2015	
Maxicrop Triple	Fertiliser		Feed
Urea	Trace Elements		Feed

Site 7 Husbandry. Triumph. 2015				
Pesticide applications				
31-Mar	Paraat			
05-Apr	Equity	Fortress		
17-Apr	Systhane			
20-Apr	Challenge		(interrow)	
24-Apr	Fortress	FAST Boron		
30-Apr	Stroby	FAST Boron		
08-May	Topas	FAST Boron		
15-May	Systhane	Calypso		
22-May	Amistar	Calypso		
29-May	Topas			
09-Jun	Systhane			
16-Jun	Amistar			
23-Jun	Topas			
29-Jun	Pot bicarb			
03-Jul	Systhane			
10-Jul	Pot bicarb			
15-Jul	Pot bicarb	Sulphur		
20-Jul	Amistar			
24-Jul	Pot bicarb			
29-Jul	Nimrod			
04-Aug	Pot bicarb			
12-Aug	Nimrod			
15-Aug	Pot bicarb	SP058	(silicon wetter)	
20-Aug	Scala	Systhane		
25-Aug	Pot bicarb	SP058		
27-Aug	Topas	Signum		
04-Sep	Systhane	Teldor		
09-Sep	Nimrod	Switch		
17-Sep	Amistar	Rovral WG	Hallmark	
22-Sep	Kindred	Switch		
30-Sep	Rovral WG	Sulphur		
09-Oct	Scala			
29-Oct	Fenomenal			

Appendix 2. Adjuvants

Addit	An emulsifiable concentrate formulation containing 780.2 g/l oil (rapeseed triglycerides) (EAC 1);	All Edible Crops, All Non Edible Crops	0.125%
BioSyl	!% w/w polyoxyehtylen-alpha-methyl-omega[3-(1,3,3,3-tetramethyl-3-trimethylsiloxy)-disiloxanyl]propylether and 32.67% ethylene oixide condensate An emulsion, oil in water formulation containing 32.67 % w/w alkoxylated alcohols (EAC 1) and 1.0 % w/w trisiloxane organosilicone copolymers (EAC 2); (detailed formulation as specified in Appendix 2 of the application form submitted 13 March 2013 submitted in support of this List Entry and as clarified in CRD's document dated 16 May 2013)	pesticides on range of fruit and veg crops; up to and including first fruit development of strawberries	max 1%
Bond	An emulsifiable concentrate formulation containing 45.0 % w/w styrene-butadiene copolymers (EAC 1) and 10.0 % w/w alkoxylated alcohols (EAC 2);	up to and including first fruit development of strawberries	max 0.14%
Designer	An emulsifiable concentrate formulation containing 25.0 % w/w styrene-butadiene copolymers (EAC 1) and 7.1 % w/w trisiloxane organosilicone copolymers (EAC 2)	up to and including first fruit development of strawberries	0.13%
Elan Xtra	A soluble concentrate formulation containing 530 g/l ethylene oxide-propylene oxide copolymers (EAC 1) and 415 g/l trisiloxane organosilicone copolymers (EAC 2)	up to and including first fruit development of strawberries	0.2%
Grenadier	An emulsifiable concentrate formulation containing 700 g/l alkoxylated alcohols (EAC 1) and 150 g/l rapeseed fatty acids (EAC 2);	up to and including first fruit development of strawberries	0.75%
Headland Guard	A soluble concentrate formulation containing 10.0 % w/w styrene-butadiene copolymers (EAC 1);	up to and including first fruit development of strawberries	0.1%
Impala	An emulsifiable concentrate formulation containing 700 g/l	up to and including first fruit	0.75%

	alkoxylated alcohols (EAC 1) and 150 g/l rapeseed fatty acids (EAC 2);	development of strawberries	
Intracrop Agwet Gtx	A soluble concentrate formulation containing 500 g/l alkoxylated alcohols (EAC 1);	up to and including first fruit development of strawberries	0.1%
Intracrop Bla	A liquid concentrate formulation containing 22.0 % w/w styrene-butadiene copolymers (EAC 1)	up to and including first fruit development of strawberries	0.14%
Intracrop Bla-Tex	A liquid concentrate formulation containing 22.0 % w/w styrene-butadiene copolymers (EAC 1);	up to and including first fruit development of strawberries	0.14%
Intracrop Boost	An emulsion, oil in water formulation containing 32.67 % w/w alkoxylated alcohols (EAC 1) and 1.0 % w/w trisiloxane organosilicone copolymers (EAC 2);	up to and including first fruit development of strawberries	1%
Intracrop Cogent	An emulsion, oil in water formulation containing 32.67 % w/w alkoxylated alcohols (EAC 1) and 1.0 % w/w trisiloxane organosilicone copolymers (EAC 2);	up to and including first fruit development of strawberries	1%
Intracrop Mica AF	An emulsifiable concentrate formulation containing 250 g/l ethylene oxide-propylene oxide copolymers (EAC 1), 70.2 g/l styrene-butadiene copolymers (EAC 2) and 41.5 g/l trisiloxane organosilicone copolymers (EAC 3);	up to and including first fruit development of strawberries	0.25%
Intracrop Neotex	A liquid concentrate formulation containing 22.0 % w/w styrene-butadiene copolymers (EAC 1);	up to and including first fruit development of strawberries	0.14%
Intracrop Novatex	A liquid concentrate formulation containing 22.0 % w/w styrene-butadiene copolymers (EAC 1);	up to and including first fruit development of strawberries	0.14%
Intracrop salute	An emulsion, oil in water formulation containing 18.9 % w/w trisiloxane organosilicone copolymers (EAC 1);	up to and including first fruit development of strawberries	0.75%
Intracrop salute AF	An emulsifiable concentrate formulation containing 250 g/l ethylene oxide-propylene oxide copolymers (EAC 1), 70.2 g/l styrene-butadiene copolymers (EAC 2) and 41.5 g/l trisiloxane	up to and including first fruit development of strawberries	0.25%

	organosilicone copolymers (EAC 3);		
Intracrop Sapper	An emulsion, oil in water formulation containing 18.9 % w/w trisiloxane organosilicone copolymers (EAC 1);	up to and including first fruit development of strawberries	0.25%
Intracrop Sapper AF	An emulsifiable concentrate formulation containing 250 g/l ethylene oxide-propylene oxide copolymers (EAC 1), 70.2 g/l styrene-butadiene copolymers (EAC 2) and 41.5 g/l trisiloxane organosilicone copolymers (EAC 3);	up to and including first fruit development of strawberries	0.25%
Intracrop Signal XL	A liquid concentrate formulation containing 22.0 % w/w styrene-butadiene copolymers (EAC 1);	up to and including first fruit development of strawberries	0.14%
Intracrop Tonto	An emulsion, oil in water formulation containing 32.67 % w/w alkoxylated alcohols (EAC 1) and 1.0 % w/w trisiloxane organosilicone copolymers (EAC 2);	up to and including first fruit development of strawberries	1%
Intracrop Zenith AF	An emulsifiable concentrate formulation containing 250 g/l ethylene oxide-propylene oxide copolymers (EAC 1), 70.2 g/l styrene-butadiene copolymers (EAC 2) and 41.5 g/l trisiloxane organosilicone copolymers (EAC 3);	up to and including first fruit development of strawberries	0.25%
Level	A soluble concentrate formulation containing 10.0 % w/w styrene-butadiene copolymers (EAC 1);	up to and including first fruit development of strawberries	0.1%
Profit oil	An emulsifiable concentrate formulation containing 95.0 % w/w oil (rapeseed triglycerides) (EAC 1);	All Edible Crops (aerial application), All Non Edible Crops (aerial application) with all pesiticdes	12.5%
Reward oil	An emulsifiable concentrate formulation containing 95.0 % w/w oil (rapeseed triglycerides) (EAC 1);	All Edible Crops (aerial application), All Non Edible Crops (aerial application) with all pesiticdes	12.5%
Siltex	An emulsion, oil in water formulation containing 18.9 % w/w trisiloxane organosilicone copolymers (EAC 1);	up to and including first fruit development of strawberries	0.25%
Siltex AF	An emulsifiable concentrate formulation	up to and including first fruit	0.25%

	containing 250 g/l ethylene oxide-propylene oxide copolymers (EAC 1), 70.2 g/l styrene-butadiene copolymers (EAC 2) and 41.5 g/l trisiloxane organosilicone copolymers (EAC 3);	development of strawberries	
Spray Fix	An emulsifiable concentrate formulation containing 45.0 % w/w styrene-butadiene copolymers (EAC 1) and 10.0 % w/w alkoxylated alcohols (EAC 2);	up to and including first fruit development of strawberries	0.14%
Stika	An emulsifiable concentrate formulation containing 22.5 % w/w styrene-butadiene copolymers (EAC 1) and 10.0 % w/w alkoxylated alcohols (EAC 2);	up to and including first fruit development of strawberries	0.14%
Zig Zag	A soluble concentrate formulation containing 36.5 % w/v styrene-butadiene copolymers (EAC 1) and 5.6 % w/v trisiloxane organosilicone copolymers (EAC 2);	up to and including first fruit development of strawberries	0.125%

Appendix 3: Compounds of interest

Soluble concentrate	A liquid concentrate formulation containing 22.0 % w/w styrene-butadiene copolymers (EAC 1)	• Intracrop Bla • Intracrop Bla-Tex • Intracrop Neotex • Intracrop Novatex
Soluble concentrate	A soluble concentrate formulation containing 10.0 % w/w styrene-butadiene copolymers (EAC 1)	• Headland Guard • Level
Soluble concentrate	A soluble concentrate formulation containing 36.5 % w/v styrene-butadiene copolymers (EAC 1) and 5.6 % w/v trisiloxane organosilicone copolymers (EAC 2)	• Zig zag
Soluble concentrate	A soluble concentrate formulation containing 500 g/l alkoxylated alcohols (EAC 1)	• Intracrop Agwet GTX
Soluble concentrate	A soluble concentrate formulation containing 530 g/l ethylene oxide-propylene oxide copolymers (EAC 1) and 415 g/l trisiloxane organosilicone copolymers (EAC 2)	• Elan Xtra
Emulsifiable concentrate	An emulsifiable concentrate formulation containing 22.5 % w/w styrene-butadiene copolymers (EAC 1) and 10.0 % w/w alkoxylated alcohols (EAC 2)	• Stika
Emulsifiable concentrate	An emulsifiable concentrate formulation containing 25.0 % w/w styrene-butadiene copolymers (EAC 1) and 7.1 % w/w trisiloxane organosilicone copolymers (EAC 2)	• Designer
Emulsifiable concentrate	An emulsifiable concentrate formulation containing 250 g/l ethylene oxide-propylene oxide copolymers (EAC 1), 70.2 g/l styrene-butadiene copolymers (EAC 2) and 41.5 g/l trisiloxane organosilicone copolymers (EAC 3);	• Intracrop Mica AF • Intracrop Salute AF • Intracrop Sapper AF • Intracrop Zenith AF
Emulsifiable concentrate	An emulsifiable concentrate formulation containing 45.0 % w/w styrene-butadiene copolymers (EAC 1) and 10.0 % w/w alkoxylated alcohols (EAC 2);	• Bond • Spray Fix
Emulsifiable concentrate	An emulsifiable concentrate formulation containing 700 g/l alkoxylated alcohols (EAC 1) and 150 g/l rapeseed fatty acids (EAC 2);	• Grenadier • Impala
Emulsifiable concentrate	An emulsifiable concentrate formulation containing 780.2 g/l oil (rapeseed triglycerides)	• Addit

Emulsifiable concentrate	An emulsifiable concentrate formulation containing 95.0 % w/w oil (rapeseed triglycerides) (EAC 1);	• Profit oil • Reward oil
Emulsion	An emulsion, oil in water formulation containing 18.9 % w/w trisiloxane organosilicone copolymers (EAC 1);	• Intracrop salute • Intracrop Sapper • Siltex
Emulsion	An emulsion, oil in water formulation containing 32.67 % w/w alkoxylated alcohols (EAC 1) and 1.0 % w/w trisiloxane organosilicone copolymers (EAC 2);	• Bio Syl • Intracrop Boost • Intracrop Cogent • Intracrop Tonto

Appendix 4: Activities and Milestones schedule

			YEAR 1				YEAR 2				Milestone
ID	**Description**	**Organisation Responsible**	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	**Date mm/dd/yyyy**
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. cucumerissuitable 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

Cloyd R (2009) Western Flower Thrips (*Frankliniella occidentalis*) Management onOrnamental Crops Grown in Greenhouses: Have We Reached an Impasse? Pest Techology 3: 1-9.

Jacobson RJ, Chandler D, Fenlon J & Russell KM (2001) Compatibility of Beauveria bassiana (Balsamo) Vuillemin with Amblyseius cucumeris Oudemans (Acarina: Phytoseiidae) to control Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) on cucumber

plants. Biocontrol Science and Technology 11: 391-400. doi:10.1080/09583150120055808.

