SCEPTREPLUS

Final Trial Report

Trial code:	SP 39
Title:	Capsids (efficacy trials)
Crop	Strawberry as a model crop. *Lygus rugulipennis* is also a pest of other fruit crops (e.g. raspberry), potato, brassicas, beet, legumes and protected cucumber and the results are therefore also expected to be applicable to these crops
Target	European tarnished plant bug (*Lygus rugulipennis*)
Lead researcher:	Dr Glen Powell
Organisation:	NIAB EMR
Period:	1st June 2019 – 29th February 2020
Report date:	5th May 2020
Report author:	Dr Glen Powell
ORETO Number: (certificate should be attached)	Certificate No. 411

I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained

…05/05/2020… ………Glen Powell………………………………
Date Author’s signature

Trial Summary

Introduction

The European tarnished plant bug (*Lygus rugulipennis*) is a capsid pest of multiple crops, affecting the soft fruit, field vegetable and protected edible sectors. The pest is sporadic but has a particularly large impact on strawberries (both outdoor and protected), where even low pest numbers in the crop may cause extensive fruit distortion ("cat-facing" damage) through feeding on flowers and developing fruits. Growers have few and reducing options for control of this pest: for example thiacloprid (e.g. Calypso) is currently relied upon extensively for control, but is unlikely to be available after 2020 (currently, there is a final use date of February 2021). In addition, application of insecticides for control of capsids often leads to collapse of the IPM system and resurgence of damage by other pests, particularly thrips and mites. The aim of this trial was to test three coded products with relatively specific action against sap-feeding pests such as aphids and whiteflies, to evaluate their potential to reduce capsid numbers and fruit damage.

Methods

"BugDorm"-caged plots enclosing strawberry plants were set out in a grid arrangement comprising five blocks inside a polytunnel at NIAB EMR. Plants were artificially infested with adult capsids (20 per cage). Three treatments (AHDB 9943, AHDB 9951 and AHDB 9966) were applied to separate plots, and all were re-applied 14 days later. Efficacy was determined by assessing capsid numbers and fruit damage, and making comparisons with untreated control plants. Assessments of capsid numbers and life stages via tap sampling were made before application and on 6 occasions after treatment. Fruit damage was scored on 5 occasions after treatment.

Results

All three treatments were associated with significant reductions in 1) the number of capsid nymphs within the cages (treatment effect $P<0.001$), and 2) the extent of capsid damage to fruit (treatment effect $P<0.005$). Treatments coded AHDB 9943 and AHDB 9966 had particularly clear impacts on numbers of the pest, with at least 74% fewer capsid nymphs recovered from plants treated with these products by the end of the trial.

Take home messages:

- All 3 products tested significantly reduced numbers of capsids, and subsequent feeding damage to fruit
- AHDB 9966 was the product associated with the greatest reductions in both capsid numbers and fruit damage.

Science section

Objectives

The aim of this study was to evaluate the efficacy of promising products identified in previous trials with *Lygus* species (as highlighted in the SP 39 Capsids Review and previous SCEPTRE trials) for control of adults and nymphs of the European tarnished plant bug (*Lygus rugulipennis*) feeding on the flowers and developing fruit of everbearer strawberries.

Trial conduct

UK regulatory guidelines were followed but EPPO guidelines took precedence. The following EPPO guidelines were followed:

Relevant EPPO guideline(s)		**Variation from EPPO**
PP1/152(4)	Design and analysis of efficacy evaluation trials	None
PP1/181(4)	Conduct and reporting of efficacy evaluation trials including good experimental practice	None
PP1/239(2)	Dose expression for plant protection products (PPPs)	None
PP1/135(4)	Phytotoxicity assessment	None

Test site

Item	Details
Location address	NIAB EMR, East Malling, West Malling, ME19 6BJ
Crop	Strawberry (*Fragaria × ananassa*)
Cultivar	Amesti
Soil or substrate type	Coir
Agronomic practice	Plants were transplanted to coir grow bags in the tunnel and put on drip fertigation on 7 May, initially using fertilizer for vegetative growth (Universol, Green; https://icl-sf.com/ie-en/products/ornamental_horticulture/2037-universol-green/). On 1 August plants were set up in the BugDorm cages (full dimensions?) and put on fertigation for flower development and fruiting (Universol, Violet; https://icl-sf.com/ie-en/products/ornamental_horticulture/2039-universol-violet/).
Prior history of site	N/A

Trial design

Item	Details
Trial design:	Randomized block
Number of replicates:	5
Row spacing:	N/A
Plot size: (w x l)	0.5 x 1.0 m (cage base dimensions)
Plot size: (m^2)	0.5 m^2 (cage base area)
Number of plants per plot:	8
Leaf Wall Area calculations	N/A

Treatment details

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
N/A	Untreated	N/A	N/A	N/A	N/A	N/A
AHDB 9966	N/A	N/A	N/A	21.80%	liquid	No
AHDB 9951	N/A	N/A	N/A	17.09%	liquid	No
AHDB 9943	N/A	N/A	N/A	500g/Kg	Water dispersible granules	No

Application schedule

Treatment number	Treatment: product name or AHDB code	Rate of active substance (ml or g a.s./ha)	Rate of product (l or kg/ha)	Application code
1	Untreated	N/A	N/A	N/A
2	AHDB 9966	43.60 ml	200 ml	A,B
3	AHDB 9951	85.45 ml	500 ml	A,B
4	AHDB 9943	80 g	0.16 Kg	A,B

Application details

Treatments were applied on 14th August, with cages allocated to treatments in a randomized block design. All treatments were re-applied once, on 28th August.

	Application A	Application B
Application date	14 Aug 2019	28 Aug 2019
Time of day	11:00	10:37
Crop growth stage (Max, min average BBCH)	71	81
Crop height (cm)	30	30
Crop coverage (%)	N/A	N/A
Application Method	Spray	Spray
Application Placement	Foliar	Foliar
Application equipment	CP2 2000 Series Knapsack Sprayer 20L	CP2 2000 Series Knapsack Sprayer 20L
Nozzle pressure	3 bar	3 bar
Nozzle type	Lurmark 30FCX04	Lurmark 30FCX04
Nozzle size	04	04
Application water volume/ha	1000 L	1000 L
Temperature of air - shade (°C)	21.5	24.0
Relative humidity (%)	75.0	72.5
Wind speed range (m/s)	N/A	N/A
Dew presence (Y/N)	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	N/A
Wetness of soil - 2-5 cm	N/A	N/A
Cloud cover (%)	N/A	N/A

Assessment details

During the week immediately before first treatments were applied, adult *L. rugulipennis* (18 females and 2 males) were released into each cage (see Appendix f for details). One day before treatments were first applied (on 13th August), tap sampling was carried out to obtain pre-treatment capsid numbers. Capsids were assessed by briefly unzipping the cage to open up the top, and holding a grey plastic tray (internal dimensions = 39 cm long x 29 cm wide x 9 cm deep) inside the cage, below the foliage level. The tray was moved along as the foliage immediately above was struck with palm of a gloved hand. When all plants had been sampled in this way (taking approximately 5 seconds per cage), the tray was removed briefly from the cage to allow counting of capsids. Female and male adults were recorded separately, and numbers and instars of any nymphs were also recorded. All capsids were returned to the cage and the zip re-closed.

Subsequent post-treatment assessments of capsid numbers were carried out on six occasions (dates given in the table below). In addition, starting at the third post-treatment assessment (on 23rd August) and continuing until the seventh and last post-treatment assessment (on 25th September), any ripe fruit was removed and scored according to the 4-level capsid damage scale applied in previous trials. Examples of damage matching each category on the scale are shown below (Figure A2), with 0 = no visible capsid damage; 1 = some slight damage visible, not acceptable as 1st class fruit; 2 = moderate damage overall but with some cat-facing distinctly visible on some of the fruit surface; 3 = severe damage with cat-facing damage affecting most of the fruit surface and entire shape of fruit distorted. At each of the seven post-treatment assessments, all plants were examined for any visual signs of phytotoxicity to foliage, flowers or fruit.

	Evaluation Timing (DA)*				
Evaluation date	**After conventional insecticides****	**After Bio-insecticides**	**Crop Growth Stage (BBCH)**	**Evaluation type (efficacy, phytotox)**	**Assessment number and description**
13 Aug 19	-1 (pre-assessment)	N/A	71	efficacy	0: Tap sampling of capsids
15 Aug 19	1	N/A	71	efficacy	1: Tap sampling of capsids
19 Aug 19	5	N/A	73	efficacy	2: Tap sampling of capsids
23 Aug 19	9	N/A	73	efficacy	3: Tap sampling of capsids, and damage score of fruit
29 Aug 19	15	N/A	81	efficacy	4: Tap sampling of capsids, and damage score of fruit
4 Sept 10	21	N/A	81	efficacy	5: Tap sampling of capsids, and damage score of fruit
11 Sept 19	28	N/A	85	efficacy	6: Tap sampling of capsids, and damage score of fruit
25 Sept 19	42	N/A	85	efficacy	7: Damage score of fruit

* DA – days after application; N/A – not applicable

** Note that evaluation timings (DA) are given relative to the first application of treatments on 14 Aug. All treatments were re-applied on 28th Aug (14 days after the first application) so the last 4 assessments (at 15, 21, 28 and 42 DA) were also 1, 7, 14 and 28 DA relative to the second application.

Statistical analysis

All statistical analyses were carried out using R 3.5. Generalized linear models were fitted to the data to establish whether treatment, assessment date or their interaction had an effect on (1) adult and nymph capsid numbers using Poisson regression with a log link function and (2) fruit damage score using ordinal regression.

Results

Mean numbers of observed capsids (adults and nymphs) and mean fruit damage scores are presented in the following table.

Assessment	Treatment	Mean adults	Mean nymphs	Mean fruit damage score
0	AHDB9943	1.32	0.10	N/A
0	AHDB9951	1.73	0.10	N/A
0	AHDB9966	2.14	0.10	N/A
0	Untreated	2.14	0.10	N/A
1	AHDB9943	0.31	0.10	N/A
1	AHDB9951	0.31	0.10	N/A
1	AHDB9966	0.92	0.10	N/A
1	Untreated	0.71	0.29	N/A
2	AHDB9943	0.31	0.10	N/A
2	AHDB9951	0.31	0.10	N/A
2	AHDB9966	0.10	0.10	N/A
2	Untreated	0.71	0.10	N/A
3	AHDB9943	0.51	2.59	0.20
3	AHDB9951	0.10	5.85	0.50
3	AHDB9966	0.10	2.97	0.19
3	Untreated	0.10	5.85	0.34
4	AHDB9943	0.31	1.06	0.42
4	AHDB9951	0.10	3.17	0.31
4	AHDB9966	0.10	0.67	0.63
4	Untreated	0.10	9.11	0.44
5	AHDB9943	0.10	2.01	0.81
5	AHDB9951	0.10	4.13	0.82
5	AHDB9966	0.10	0.48	0.48
5	Untreated	0.10	5.66	1.19
6	AHDB9943	0.10	1.63	1.34
6	AHDB9951	0.10	4.89	1.19
6	AHDB9966	0.10	0.67	1.04
6	Untreated	0.51	6.24	1.56
7	AHDB9943	N/A	N/A	1.76
7	AHDB9951	N/A	N/A	1.78
7	AHDB9966	N/A	N/A	1.56
7	Untreated	N/A	N/A	2.14

A summary of the statistical analyses, showing effects of treatment, assessment date and assessment x date interaction for each of the variables measured (numbers of adults observed, numbers of nymphs observed and fruit damage score) is shown in the following table.

		Adults		**Nymphs**		**Fruit score**	
	df	χ^2 value	P	χ^2 value	P	χ^2 value	P
Treatment	3	2.53	0.470	34.87	<0.001	13.05	<0.005
Assessment	6 (4)†	84.71	<0.001	319.03	<0.001	114.52	<0.001
Interaction	18 (12) †	5.56	0.998	27.57	0.069	20.61	0.056

† Numbers in brackets are the degrees of freedom (df) for the fruit damage score.

Adult capsids remained present and detectable in the cages at the pre-treatment (assessment 0), but tap-sampled numbers declined rapidly and very few adults were found by the third post-treatment assessment (on 23rd August). The analysis revealed a significant effect of assessment date (P<0.001) but no significant treatment, or treatment x assessment interaction effects. The output from the analysis, showing mean number of adults per cage, is plotted (Figure 1). Adult survival times within the experimental cages were therefore limited to just a few days and were not affected by treatment.

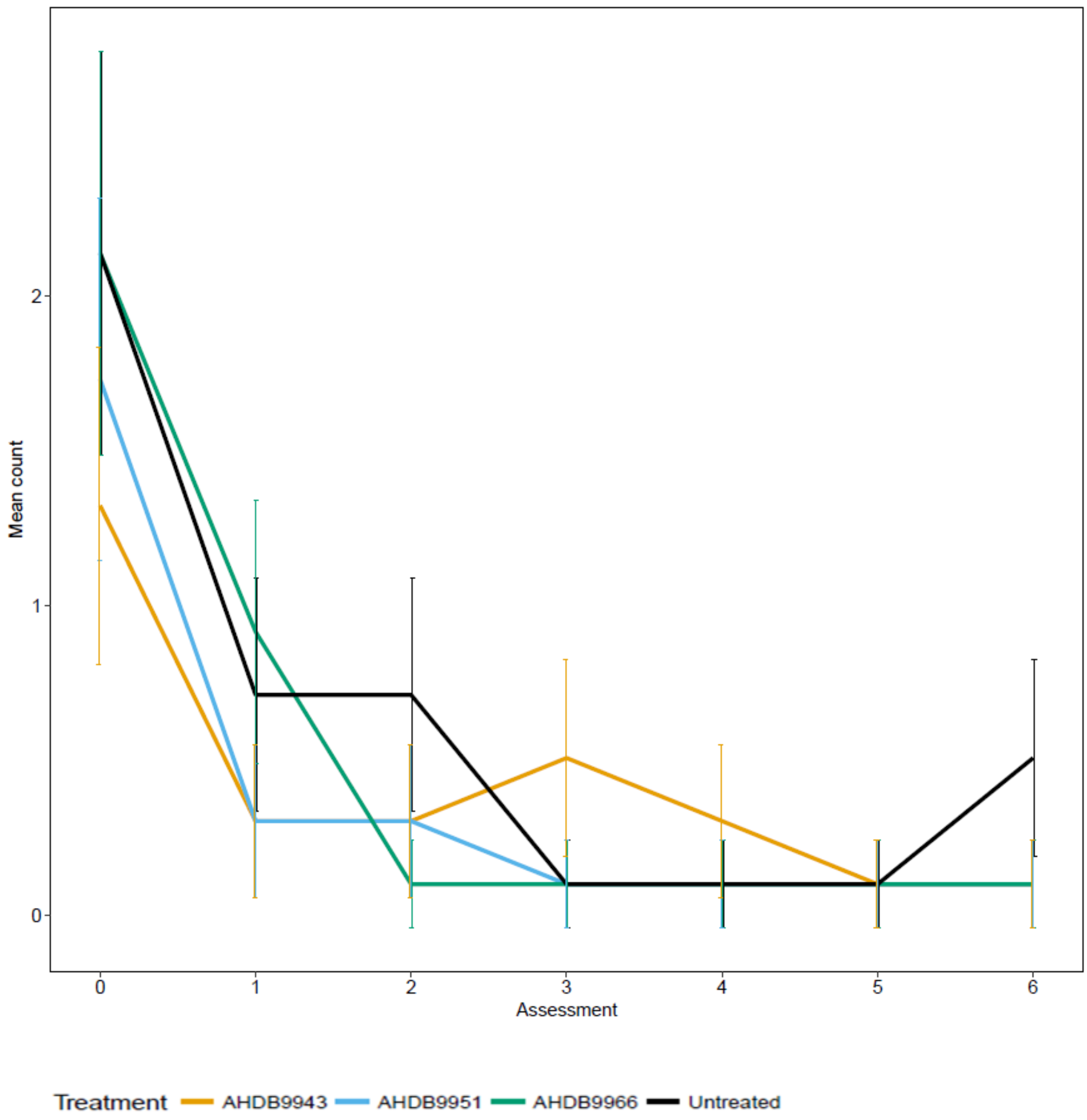

Figure 1. Mean numbers of adults observed per treatment (with standard errors). See Assessment Details section and table above for details of the sampling date corresponding to each assessment number.

Counts of tap-sampled capsid nymphs showed that, although adult capsids were short-lived within the cages, females were able to deposit viable eggs and these hatched and led to the establishment of a second generation. Nymphs had started to appear by the third post-treatment assessment (23rd August). The model fitted to these data revealed substantial effects of treatment on the numbers of nymphs. The output of the model, showing mean number of nymphs per cage, is plotted below (Figure 2).

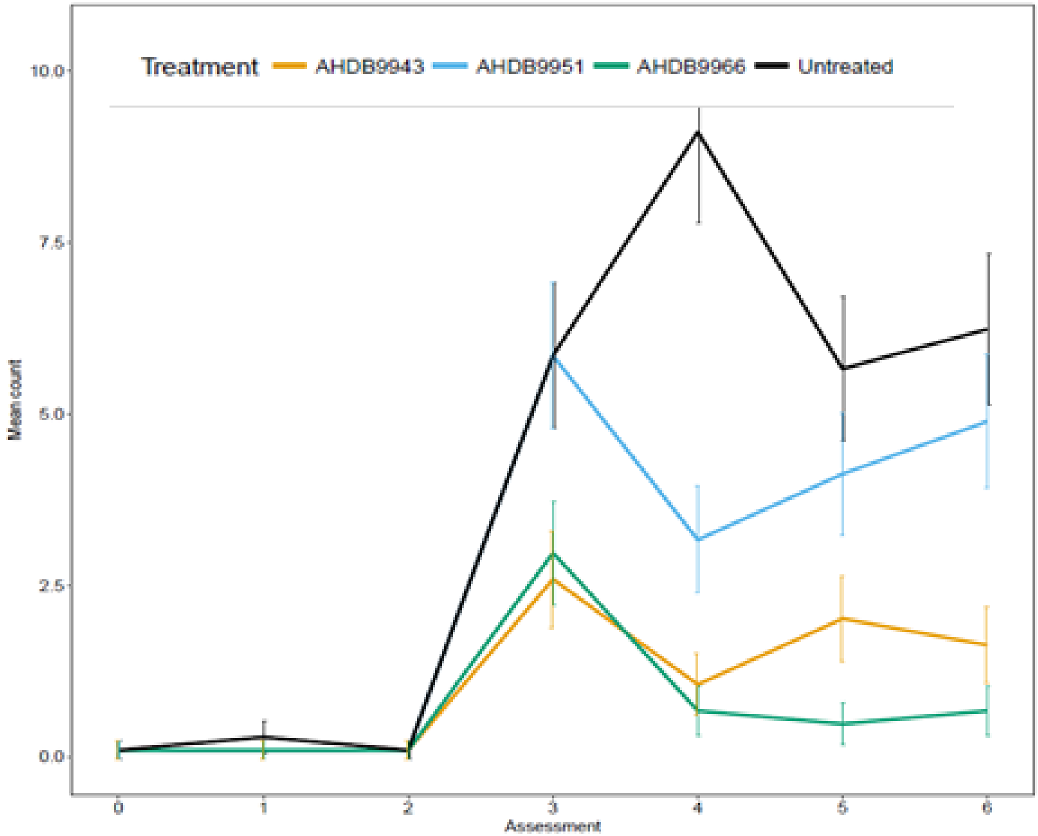

Figure 2. Mean numbers of nymphs sampled per treatment (with standard errors). See Assessment Details section and Table above for details of the sampling date corresponding to each assessment number.

The analysis revealed that treatment had a significant (P<0.001) overall effect on numbers of tap-sampled nymphs. *Post-hoc* pairwise comparisons confirmed that all three treatments were associated with significant reductions in numbers of nymphs (AHDB 9943 P<0.001; AHDB 9966 P<0.001; AHDB 9951 P<0.05) compared with numbers in untreated control cages. Assessment date also had a significant (P<0.001) effect on numbers of nymphs detected, but there was no significant interaction between treatment and assessment date.

Mean fruit damage scores were also affected by treatment (Figure 3). The analysis revealed significant overall effects of treatment (P<0.005) and assessment date (P<0.001) on fruit damage, but no significant treatment x assessment date interaction. *Post-hoc* pairwise comparisons confirmed that fruit damage scores were lower for all 3 treatments, compared with the controls. This was highly significant (P<0.001) for AHDB 9966, but was also significant (P<0.05) for the other two treatments. At the end of the trial (7th post-treatment assessment), the mean fruit damage scores for plots treated with AHDB 9966, 9951 and 9943 were 72.9, 82.8 and 81.9 % of control (untreated) values respectively. No signs of phytotoxicity were observed in any of the plants, at any stage of the trial.

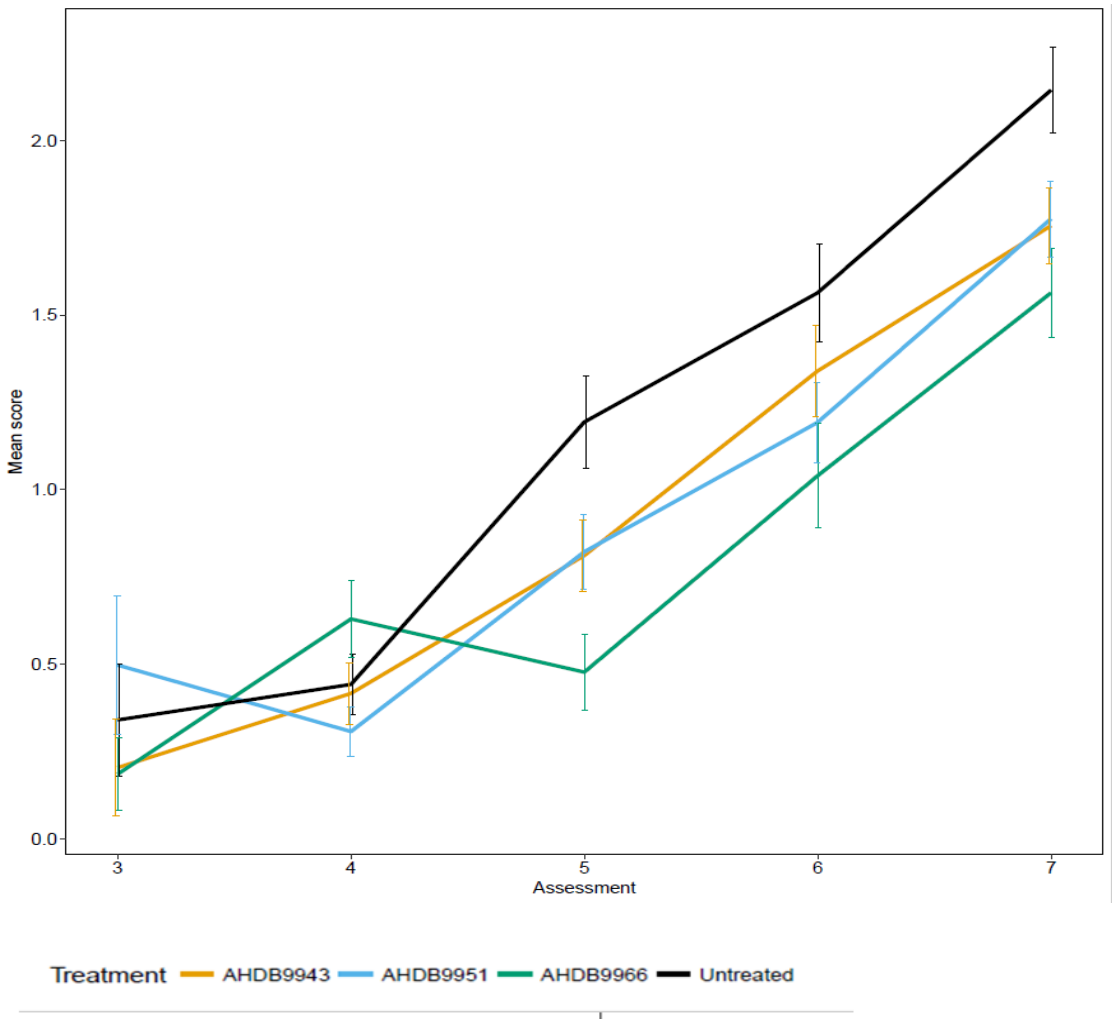

Figure 3. Mean fruit damage score per treatment (with standard errors). See Assessment Details section and Table above for details of the sampling date corresponding to each assessment number.

Discussion

Although adult capsids collected from the field and introduced into cages only survived for 2-3 days, this was sufficient time for them to lay eggs. The second generation of capsids developed successfully during the experiment (see untreated control). Feeding by the capsids led to significant damage to fruit with extensive 'cat facing' and distortion during the experiment.

All three treatments resulted in significant reductions in the numbers of capsid nymphs without showing phytotoxic effects. The applied treatments were all synthetic insecticides with proven efficacy against other sap-feeding Hemiptera such as aphids, whiteflies and planthoppers. Treatment AHDB 9966, was particularly promising when tested against *L. rugulipennis* in this work, reducing numbers of capsid nymphs to very low levels at the later assessments (mean numbers of nymphs per plot at the fifth and sixth post-treatment assessments were just 6.9 and 9.4 % of numbers on control plots, respectively). AHDB 9966 has been demonstrated to have very high efficacy against sap-feeding pests. The insecticide has been shown to be effective, even against biotypes of aphids (*Myzus persicae*), whiteflies (*Bemisia tabaci*) and planthoppers (*Nilaparvata lugens*) that are resistant to neonicotinoids and other classes of insecticides (Sparks et al. 2013). The active ingredient (AI) of AHDB 9966 acts as an agonist at insect nicotinic acetylcholine receptors (nAChRs), and therefore binds to these receptors in place of acetylcholine. The AI is also translocated systemically within plants (Cui et al. 2018), and is present in both phloem and xylem of foliar-sprayed crops. The presence of this insecticide within the vascular tissue helps to explain its rapid action against phloem feeders such as aphids and whiteflies. Capsids have a different feeding strategy, ingesting the sap released by mesophyll cells that have been damaged through the mechanical activity of their stylets and secretion of salivary enzymes (Powell, 2020). However, insecticides with systemic and translaminar movement, such as AHDB 9966, are likely to be present in mesophyll cells and therefore effective through feeding uptake by capsids.

The insecticide AI in AHDB 9951 also acts on insect nAChRs (but via a different mode of action to AHDB 9966) to cause rapid effects on sap feeding pests (Nauen et al. 2015). AHDB 9951 is also translocated systemically within plants, moving mainly through the xylem (Nauen et al. 2015). This insecticide, applied to strawberry plants, caused significant reductions in numbers of capsid nymphs sampled from the plants. However, the impact was not as dramatic as for AHDB 9966. At the fourth post-treatment assessment, numbers of nymphs on AHDB 9951-treated plots represented

40.9% of numbers on untreated control plots. By the end of the experiment (at the fifth and sixth assessments respectively) mean numbers of capsid nymphs had increased on plots treated with AHDB 9951, to 72.4 and 78.1 % of control numbers.

The third product tested in this trial (AHDB 9943) was very effective, being linked with clear reductions in numbers of capsids, with just 25% of nymphs recorded at the end of the trial (sixth post-treatment assessment), compared with numbers on untreated plots. AHDB 9943 shares some features with the other two treatments tested: in particular, it is translocated systemically within plants and also has selective activity against sap feeding insects (Roditakis et al. 2014). However, the AI in AHDB 9943 acts via a different mechanism to the other two insecticides tested here, being a chordotonal organ modulator shown to target Kir channels. This results in disruption of normal salivary secretion and excretory functions, leading to lethal inhibition of feeding behavior (Ren et al. 2018). The resultant antifeedant effects lead to starvation-based mortality (Morita et al. 2007) and therefore a slow and progressive effect, rather than rapid knock-down of the pest.

The capsid densities in this trial, with the pest confined to strawberry plants within cages, were kept at particularly high levels. Even at very low pest densities (just one capsid detected per 40 sampled plants), capsid feeding can be linked with significant fruit distortion and losses in soft fruit crops (Cross, 2004). The artificially high and constant capsid density resulted in significant accumulated fruit damage in all treatments (Figure 3). However, the significant reductions in fruit damage levels associated with all three treatments are particularly encouraging. The caged approach was necessary to guarantee fruit damage during this experiment and maximize the chances of detecting significant effects with minimal replication, but it would certainly now be worthwhile testing the same treatments under more realistic, commercial field, conditions (with the pests at lower densities and able to move away from plants).

A recent review of capsid damage and control options (Powell, 2020) highlighted new efficacy testing as an important recommendation to provide growers of capsid-damaged crops with options for management, particularly given the situation with recent and imminent withdrawals of effective PPPs. Other recommended areas for capsid research included investigations to explore the potential advantages of salt additives to enhance the efficacy of insecticides. Including sodium chloride in tank mixes has enabled Australian cotton growers to achieve effective control of capsid pests at lower insecticide dose rates, with the added benefits of reduced impact on beneficial pollinators and natural enemies. The active ingredients tested in this trial could be combined with salt for further investigation. Additional possible avenues for research and exploitation include formulations of insecticide enclosed in feeding capsules (attract and kill microcapsules) that are currently in development for control of *L. rugulipennis* and other capsid pests. This would need to be tested for phytotoxic effects to strawberry plants.

Conclusions

- All three treatments were associated with significant reductions in the number capsid nymphs within the cages.
- The extent of capsid damage to fruit was significantly reduced following application of all treatments.
- Treatments AHDB 9943 and AHDB 9966 had particularly clear impacts on numbers of the pest, with at least 70% fewer capsids recovered from plants treated with these products, compared to the untreated control, by the end of the trial.
- AHDB 9966 was the product associated with the greatest reductions in capsid numbers and fruit damage (90.6 and 27.1 % reductions at the final assessments respectively, compared to untreated plots).

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Agrii, Alpha Biocontrol Ltd, Andermatt, Arysta Lifescience, BASF, Bayer, Belchim, Bionema Limited, Certis Europe, Dow, DuPont, Eden Research, Fargro Limited, FMC, Gowan, Interfarm, Lallemand Plant Care, Novozymes, Oro Agri, Russell IPM, Sumitomo Chemicals, Syngenta, UPL.

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Roditakis E, Fytrou N, Staurakaki M, Vontasb J & Tsagkarakoua A 2014. Activity of flonicamid on the sweet potato whitely *Bemisia tabaci* (Homoptera: Aleyrodidae) and its natural enemies. *Pest Management Science* 70: 1460-1467 (https://doi.org/10.1002/ps.3723)

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Appendix

a. Crop diary – events related to growing crop

Date	Event
07/05/2019	Plants from cold store transplanted into grow bags and moved to tunnel. Put on fertigation (3 min, once per day)
22/05/2019	Runners removed from this date, at weekly intervals
30/05/2019	Biological controls applied for spider mite and thrips: *P. persimilis* applied at 4 per plant, *N. cucumeris* applied at 1 sachet (250) every 1.4 m
31/05/2019	Fertigation increased to 7 min, twice per day
03/06/2019	Fertigation increased to 10 min, twice per day
04/07/2019	Ripe fruit removed
01/08/2019	Ripe fruit removed. Bags placed in BugDorm cages as needed for experiment (tops of cages left open). Fertigation changed to 6 times per day for 20 seconds to avoid puddling and consequent trapping of mobile capsids

b. Trial diary

Date	Event
06/08/2019	Ripe and green fruit removed. Alternative capsid food sources (frozen and defrosted fly larvae, green beans and bee pollen) placed into the base of each cage. Adult capsids (8 females and 2 males) released into each cage and the top opening immediately zipped closed
12/08/2019	A further 10 adult female capsids released into each cage. Fresh green beans and fly larvae provided
13/08/2019	Pre-assessment of capsid numbers
14/08/2019	Treatments applied
15/08/2019	Assessment 1. Capsid numbers
19/08/2019	Assessment 2. Capsid numbers
23/08/2019	Assessment 3. Capsid numbers and fruit damage
28/09/2019	Treatments re-applied
29/08/2019	Assessment 4. Capsid numbers and fruit damage
4/09/2019	Assessment 5. Capsid numbers and fruit damage
11/09/2019	Assessment 6. Capsid numbers and fruit damage
25/09/2019	Assessment 7. Fruit damage

c. Trial photos

Figure A1. Layout of BugDorm cages (a) and detail of closed cage showing entry of fertigation pipe (b). Stacked empty fruit crates were used to raise cages to 53 cm above ground level, facilitating access for sampling.

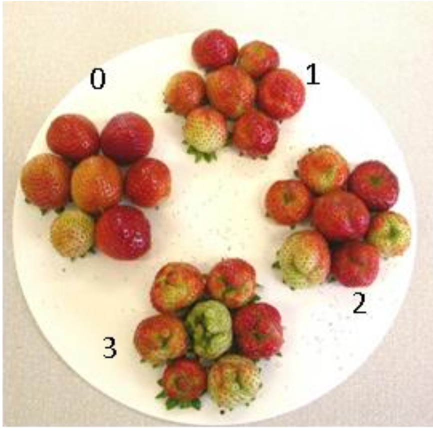


Figure A2. Fruit damage scale. 0: no damage; 1: minor distortion but acceptable for sale as fresh fruit; 2: clear distortion, unacceptable for fresh sale; 3: extreme cat-facing.

d. Climatological data

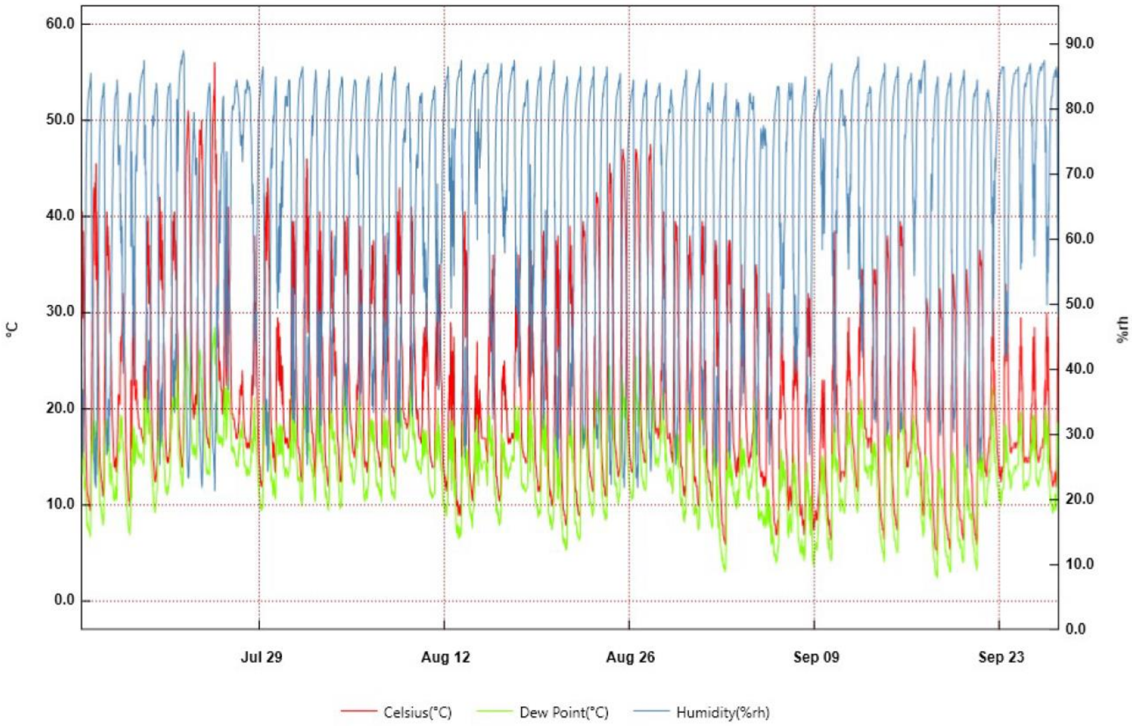


Figure A3. Temperature (°C) and humidity (%rh) data recorded during the trial period.

e. Raw data

Date	Assess-ment number*	Plot number**	Tap-sampled live capsid numbers:			Numbers of fruits in each damage category:			
			Male adults	Female adults	Numbers and instars of nymphs	0	1	2	3
13/08/2019	0	101	0	1	0	N/A	N/A	N/A	N/A
13/08/2019	0	102	0	1	0	N/A	N/A	N/A	N/A
13/08/2019	0	103	1	1	0	N/A	N/A	N/A	N/A
13/08/2019	0	104	1	0	0	N/A	N/A	N/A	N/A
13/08/2019	0	201	0	0	0	N/A	N/A	N/A	N/A
13/08/2019	0	202	0	2	0	N/A	N/A	N/A	N/A
13/08/2019	0	203	0	4	0	N/A	N/A	N/A	N/A
13/08/2019	0	204	0	2	0	N/A	N/A	N/A	N/A
13/08/2019	0	301	1	1	0	N/A	N/A	N/A	N/A
13/08/2019	0	302	0	0	0	N/A	N/A	N/A	N/A
13/08/2019	0	303	0	1	0	N/A	N/A	N/A	N/A
13/08/2019	0	304	1	0	0	N/A	N/A	N/A	N/A
13/08/2019	0	401	2	2	0	N/A	N/A	N/A	N/A
13/08/2019	0	402	1	2	0	N/A	N/A	N/A	N/A
13/08/2019	0	403	1	1	0	N/A	N/A	N/A	N/A
13/08/2019	0	404	0	0	0	N/A	N/A	N/A	N/A
13/08/2019	0	501	1	2	0	N/A	N/A	N/A	N/A
13/08/2019	0	502	0	2	0	N/A	N/A	N/A	N/A
13/08/2019	0	503	0	0	0	N/A	N/A	N/A	N/A
13/08/2019	0	504	0	3	0	N/A	N/A	N/A	N/A
15/08/2019	1	101	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	102	0	1	1 x Instar 5	N/A	N/A	N/A	N/A
15/08/2019	1	103	1	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	104	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	201	0	1	0	N/A	N/A	N/A	N/A
15/08/2019	1	202	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	203	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	204	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	301	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	302	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	303	0	1	0	N/A	N/A	N/A	N/A
15/08/2019	1	304	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	401	1	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	402	0	2	0	N/A	N/A	N/A	N/A
15/08/2019	1	403	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	404	0	1	0	N/A	N/A	N/A	N/A

15/08/2019	1	501	0	1	0	N/A	N/A	N/A	N/A
15/08/2019	1	502	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	503	0	0	0	N/A	N/A	N/A	N/A
15/08/2019	1	504	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	101	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	102	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	103	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	104	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	201	0	1	0	N/A	N/A	N/A	N/A
19/08/2019	2	202	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	203	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	204	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	301	0	2	0	N/A	N/A	N/A	N/A
19/08/2019	2	302	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	303	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	304	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	401	0	1	0	N/A	N/A	N/A	N/A
19/08/2019	2	402	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	403	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	404	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	501	0	1	0	N/A	N/A	N/A	N/A
19/08/2019	2	502	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	503	0	0	0	N/A	N/A	N/A	N/A
19/08/2019	2	504	0	0	0	N/A	N/A	N/A	N/A
23/08/2019	3	101	0	0	4 x Instar 1	1	0	0	0
23/08/2019	3	102	0	0	2 x Instar 1	3	1	0	0
23/08/2019	3	103	0	0	4 x Instar 1	1	0	0	0
23/08/2019	3	104	0	0	1 x Instar 1	4	0	0	0
23/08/2019	3	201	0	0	5 x Instar 1	3	0	1	0
23/08/2019	3	202	0	0	4 x Instar 1	5	1	0	0
23/08/2019	3	203	0	0	4 x Instar 1	1	0	0	0
23/08/2019	3	204	0	0	4 x Instar 1	1	1	0	0
23/08/2019	3	301	0	0	5 x Instar 1, 1x Instar 2	2	0	0	0
23/08/2019	3	302	1	1	4 x Instar 1	3	0	0	1
23/08/2019	3	303	0	0	2 x Instar 1	1	0	0	0
23/08/2019	3	304	0	0	7 x Instar 1	3	0	1	0
23/08/2019	3	401	0	0	6 x Instar 1	1	1	1	1
23/08/2019	3	402	0	0	3 x Instar 1	1	0	0	0
23/08/2019	3	403	0	0	0	1	0	0	0
23/08/2019	3	404	0	0	8 x Instar 1	6	2	0	0
23/08/2019	3	501	0	0	10 x Instar 1	0	1	0	0
23/08/2019	3	502	0	0	2 x Instar 1	17	1	1	0
23/08/2019	3	503	0	0	9 x Instar 1	6	0	0	0
23/08/2019	3	504	0	0	3 x Instar 1	3	0	0	0
29/08/2019	4	101	0	0	2 x Instar 2	17	3	0	0

29/08/2019	4	102	0	0	18x Instar 1	7	2	0	2
29/08/2019	4	103	0	0	2 x Instar 1	7	2	0	0
29/08/2019	4	104	1	0	0	1	1	1	1
29/08/2019	4	201	0	0	2 x Instar 1	6	1	0	2
29/08/2019	4	202	0	0	0	1	3	2	0
29/08/2019	4	203	0	0	11 x Instar 2, 1 x Instar 4	6	1	1	0
29/08/2019	4	204	0	0	4x Instar 1, 3 x Instar 3	7	1	1	0
29/08/2019	4	301	0	0	5 x Instar 1, 1x Instar 2	7	7	1	0
29/08/2019	4	302	0	0	0	13	1	2	0
29/08/2019	4	303	0	0	1 x Instar 1	4	4	1	2
29/08/2019	4	304	0	0	2 x Instar 1, 5 x Instar 2, 1 x Instar 3	13	3	2	0
29/08/2019	4	401	0	0	1 x Instar 1	18	4	0	0
29/08/2019	4	402	0	0	0	3	3	0	0
29/08/2019	4	403	0	0	1 x Instar 1	15	4	2	1
29/08/2019	4	404	0	0	1 x Instar 1	14	1	2	0
29/08/2019	4	501	0	0	3 x Instar 1, 4 x Instar 2	17	3	1	0
29/08/2019	4	502	0	0	0	21	5	2	0
29/08/2019	4	503	0	0	0	6	2	1	0
29/08/2019	4	504	0	0	1 x Instar 1, 1 x Instar 2	20	2	1	0
04/09/2019	5	101	0	0	1 x Instar 1, 1 x Instar 2, 1 x Instar 4	4	3	2	1
04/09/2019	5	102	0	0	1 x Instar 1, 1 x Instar 2, 3 x Instar 3, 4 x Instar 4	8	2	7	6
04/09/2019	5	103	0	0	0	7	1	0	0
04/09/2019	5	104	0	0	1 x Instar 3	9	2	3	2
04/09/2019	5	201	0	0	2 x Instar 1	7	6	1	1
04/09/2019	5	202	0	0	0	14	2	1	1

04/09/2019	5	203	0	0	4 x Instar 2, 4 x Instar 3, 1 x Instar 4	7	2	0	7
04/09/2019	5	204	0	0	3 x Instar 1, 2 x Instar 2, 3 x Instar 3, 3 x Instar 4	17	3	3	0
04/09/2019	5	301	0	0	1 x Instar 1, 3 x Instar 2, 1 x Instar 3, 2 x Instar 4	8	0	3	1
04/09/2019	5	302	0	0	3 x Instar 1, 1 x Instar 3, 1 x Instar 4	10	8	3	7
04/09/2019	5	303	0	0	1 x Instar 2, 1 x Instar 4	6	2	3	2
04/09/2019	5	304	0	0	1 x Instar 1, 1 x Instar 3, 1 x Instar 4	13	8	5	4
04/09/2019	5	401	0	0	3 x Instar 2, 1 x Instar 3	6	3	2	4
04/09/2019	5	402	0	0	0	8	1	0	1
04/09/2019	5	403	0	0	0	13	4	2	1
04/09/2019	5	404	0	0	3 x Instar 2	6	3	3	5
04/09/2019	5	501	0	0	1 x Instar 3	4	4	0	0
04/09/2019	5	502	0	0	0	4	1	0	1
04/09/2019	5	503	0	0	0	5	1	1	2
04/09/2019	5	504	0	0	1 x Instar 1, 1 x Instar 2	9	1	1	2
11/09/2019	6	101	0	0	2 x Instar 3	5	2	8	3
11/09/2019	6	102	0	2	2 x Instar 1, 3 x Instar 2, 1 x Instar 4	9	5	3	12
11/09/2019	6	103	0	0	0	5	0	4	1
11/09/2019	6	104	0	0	1 x Instar 4	6	3	2	3
11/09/2019	6	201	0	0	1 x Instar 1, 2 x Instar 2	4	1	2	0
11/09/2019	6	202	0	0	0	5	5	1	1
11/09/2019	6	203	0	0	1 x Instar 3, 2 x	0	2	1	8

					Instar 4, 2 x Instar 5				
11/09/2019	6	204	0	0	1 x Instar 2, 1 x Instar 3, 3 x Instar 4, 3 x Instar 5	14	5	1	4
11/09/2019	6	301	0	0	5 x Instar 2, 2 x Instar 3, 1 x Instar 4	0	2	2	1
11/09/2019	6	302	0	0	2 x Instar 1, 1 x Instar 3	7	4	7	7
11/09/2019	6	303	0	0	3 x Instar 2	2	3	3	2
11/09/2019	6	304	0	0	4 x Instar 1, 2 x Instar 2, 5 x Instar 4, 1 x Instar 5	10	10	6	6
11/09/2019	6	401	0	0	1 x Instar 1, 1 x Instar 2	0	3	2	0
11/09/2019	6	402	0	0	0	4	2	1	1
11/09/2019	6	403	0	0	0	3	4	2	4
11/09/2019	6	404	0	0	1 x Instar 2, 4 x Instar 3	4	4	1	3
11/09/2019	6	501	0	0	2 x Instar 2, 4 x Instar 3, 2 x Instar 4	8	4	1	2
11/09/2019	6	502	0	0	0	3	3	0	1
11/09/2019	6	503	0	0	1 x Instar 2	2	3	3	3
11/09/2019	6	504	0	0	1 x Instar 4	5	3	4	4
25/09/2019	7	101	N/A	N/A	N/A	1	3	5	3
25/09/2019	7	102	N/A	N/A	N/A	0	1	5	3
25/09/2019	7	103	N/A	N/A	N/A	4	0	3	2
25/09/2019	7	104	N/A	N/A	N/A	4	4	4	5
25/09/2019	7	201	N/A	N/A	N/A	1	2	4	4
25/09/2019	7	202	N/A	N/A	N/A	0	2	1	1
25/09/2019	7	203	N/A	N/A	N/A	0	7	8	8
25/09/2019	7	204	N/A	N/A	N/A	2	12	19	8
25/09/2019	7	301	N/A	N/A	N/A	0	0	2	0
25/09/2019	7	302	N/A	N/A	N/A	1	3	3	2
25/09/2019	7	303	N/A	N/A	N/A	4	5	5	7
25/09/2019	7	304	N/A	N/A	N/A	2	1	10	1
25/09/2019	7	401	N/A	N/A	N/A	0	1	4	1

25/09/2019	7	402	N/A	N/A	N/A	5	9	11	5
25/09/2019	7	403	N/A	N/A	N/A	3	3	10	5
25/09/2019	7	404	N/A	N/A	N/A	0	0	3	3
25/09/2019	7	501	N/A	N/A	N/A	0	1	6	6
25/09/2019	7	502	N/A	N/A	N/A	0	2	4	1
25/09/2019	7	503	N/A	N/A	N/A	2	1	7	3
25/09/2019	7	504	N/A	N/A	N/A	5	8	15	7

*0=pre-assessment. See Assessment Details Table above for full descriptions of assessments

**see Figure A4 for plot layouts

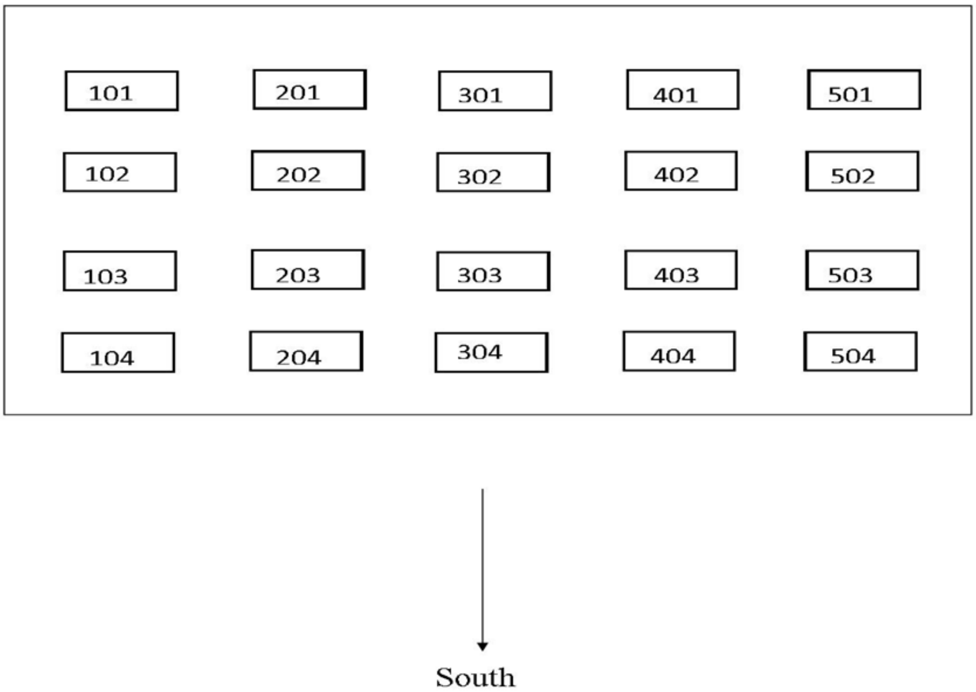
N/A: not applicable (not scored at this assessment)

f. Trial design

The tests were done on artificially infested pot-grown flowering everbearer strawberry plants in cages within a polytunnel. This ensured that testing was done against known life stages without emigration or immigration, so that the test insects were fully exposed to the treatments.

The trial layout is shown below (Figure A4), A randomised complete block design with 5 replicates of 4 treatments (= 20 plots) was used. Each plot consisted of 8 flowering everbearer strawberry plants (cv. Amesti) rooted in a single bag of coir-based substrate and contained in a 0.5 x 0.5 x 1.0 m BugDorm cage.

Figure A4. Layout of BugDorm cages showing plot numbers within the polytunnel.



Treatments were evaluated in comparison with an untreated control. The randomisation of treatments to plots is given in Table A1 below.

Table A1. Randomisation of treatments

Block 1		Block 2		Block 3		Block 4		Block 5	
Plot	Treatment	Plot	Treatment	Plot	Treatment	Plot	Treatment	Plot	Treatment
101	9951	201	9943	301	untreated	401	9951	501	untreated
102	untreated	202	9966	302	9943	402	9966	502	9966
103	9966	203	untreated	303	9966	403	9943	503	9951
104	9943	204	9951	304	9951	404	untreated	504	9943

Fertigation pipes were fixed to the corners of the BugDorm frames (Figure 2b), holding the pipes in place while allowing the cage zips to be fully closed to contain insects during the trial. Any runners and ripe or developing fruit were removed so that only flowers and button fruit were available to capsids at the start of the trial.

Between 6th and 12th August, wild adult *L. rugulipennis* were caught in sweep nets from arable areas of the NIAB EMR estate (where large populations were feeding on weeds such as fat hen - *Chenopodium album* - and shepherd's purse – *Capsella bursa-pastoris*) and released into the cages (totals of 18 females and 2 males per cage). Some of the sweep-netted adult capsids were brought into the laboratory and examined using a binocular dissecting microscope to confirm that the captured capsids were *L. rugulipennis* (identified based on use of the density of hairs covering the corium; Nau 2004). Fresh organic green beans, bee pollen and dead (frozen for storage and defrosted) blowfly larvae were also placed in each cage (and refreshed at least once per week) as supplementary food.

g. ORETO certificate

Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

NIAB EMR

complies with the minimum standards laid down in Regulation (EC) 1107/2009 for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Agriculture/Horticulture
Biologicals and Semiochemicals
Stored Crops

Date of issue:	12 July 2018
Effective date:	1 January 2018
Expiry date:	31 December 2022

Signature

Authorised signatory

Certification Number
ORETO 411

HSE
Chemicals Regulation Division

Department of
Agriculture and
Rural Development