SCEPTREPLUS

Final Trial Report

Trial code:	SP09 - W2020.507
Title:	Control of *Tuta absoluta* on tomatoes with novel insecticide sprays
Crop	Group: Protected crops - tomato
Target	*Tuta absoluta* - GNORAB
Lead researcher:	Professor Rosemary Collier
Organisation:	University of Warwick, School of Life Sciences, Wellesbourne, Warwick CV35 9EF
Period:	August 2019 – December 2019
Report date:	28/1/20
Report author:	Andrew Jukes and Rosemary Collier
ORETO Number: (certificate should be attached)	381

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.

Rosemary Collier

30 January 2020

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Date Authors signature

Trial Summary

Introduction

Currently most UK grown tomato crops are protected from damage due to larvae of *Tuta absoluta* by the use of a mating disruption pheromone. However, this treatment can be ineffective under certain circumstances, in which case a "knock down" insecticide treatment is required. The timing of this treatment is likely to be after feeding panes are observed (i.e. after the larvae have entered the leaf). Standard insecticidal treatments (spinosad and rynaxypyr) are becoming increasingly ineffective due to insect resistance. The aim of this trial was to build on the results obtained in SP09 – 2018 and evaluate some novel bio-insecticide treatments for control of *T. absoluta*.

Methods

Tomato seed (cv Espero F1) was sown into rockwool cells on 1 August 2019. After germination the cubes were placed on rock wool blocks (120 x 15 x 7 cm) to give a between-plant spacing of 30 cm (4 plants per block). The trial was arranged as a 4 x 4 Latin square with 4 rows of 4 blocks. Each plot consisted of 1 block and each plant was split into 2 heads to give 8 heads per plot. Irrigation was supplied through a drip feed attached to each plant. Nutrient feed was supplied daily.

A culture of *T. absoluta* provided by Rob Jacobson in 2018 was maintained on tomato plants in the Insect Rearing Unit at Warwick Crop Centre. Adult moths were collected in three cages. On 7 October the cages were transferred to the trial glasshouse compartment and left open, one per space, in the inter-row spaces. The moths were allowed to escape and lay eggs. Initially mines were seen to develop predominantly in the lower leaves of the plants immediately adjacent to the release sites. On 21 October a further cage of moths was collected from the laboratory culture and released at the front of the glasshouse compartment, ensuring an even distribution by shaking moths from the cage, and the existing infestation was allowed to go through a generation. Subsequently egg laying and infestation were much more even throughout the crop.

Spray treatments were applied in 1000 l/ha water using a knapsack sprayer with the boom held vertically. The drip treatment was applied in 10 ml using a laboratory pipette into the hole left when removing the drip irrigation spike. Treatments were applied on 6 and 13 November after mines had been observed in leaves throughout the whole crop.

Visual assessments of phytotoxicity were made 7 days after treatment. Two leaves per plant on 4 plants per plot were marked and damage was assessed by counting numbers of small mines (<15 mm) and large mines (>15 mm) which represent "new" mines and "established" mines respectively. Counts were done pre-treatment on 6 November and on 13 November, 1 week after the first treatment. The marked leaves were detached on 20 November (2 weeks after the first treatment and 1 week after the second treatment). The damage was re-assessed and the numbers of larvae were counted. A fresh set of leaves (2 leaves per plant, 4 plants per plot) were detached at a height of about 1.5 m on 4 December (4 weeks after first treatment). Numbers of small mines, large mines and larvae were counted.

Results

First assessments - 1 and 2 weeks after first spray

The change in numbers of large ("established") and small ("new") mines compared with pre-spray counts are displayed in Table A together with the numbers of live *T. absoluta*. None of the analyses were significant at the 5% level and when comparing changes in numbers of mines there is no evidence of control from any of the treatments. Small numbers of larvae and pupae were found and there is some evidence of a reduction in numbers in all treatments compared with the untreated control.

Table A. First assessments - 1 and 2 weeks after first spray. The change in numbers of large ("established") and small ("new") mines compared with pre-spray counts together with the numbers of live *Tuta absoluta*.

	Week 1			**Week 2**			**Live insects**		
	Large	**Small**	**Total**	**Large**	**Small**	**Total**	**Larvae**	**Pupae**	**Total**
Untreated	1.813	-1.250	0.563	3.781	0.656	2.625	0.875	0.063	0.938
AHDB9971 Drip	1.656	-1.125	0.531	2.250	1.344	1.938	0.500	0.031	0.531
AHDB9971 Spray	2.156	-1.313	0.844	3.000	1.438	2.281	0.688	0.031	0.719
AHDB9971 + AHDB9828 Spray	2.844	-0.906	1.938	3.750	0.656	1.563	0.594	0.063	0.656
F	0.577	0.201	1.444	0.393	0.654	0.224	0.284	0.286	0.291
p	0.641	0.894	0.279	0.760	0.596	0.878	0.836	0.835	0.831
df	12	12	12	12	12	12	12	12	12
SED	0.982	0.566	0.778	1.638	0.744	1.363	0.425	0.048	0.446
LSD (5%) (two-sided)	2.140	1.233	1.695	3.570	1.622	2.969	0.927	0.104	0.971
LSD (5%) (one-sided)	1.750	1.008	1.386	2.920	1.327	2.429	0.758	0.085	0.795

Second assessment - 4 weeks after first spray

The numbers of large ("established") and small ("new") mines are displayed in Table B along with numbers of live *T. absoluta* and the percentage of mines which contained larvae. The analyses of numbers of large mines, numbers of larvae and percentage of mines with larvae were significant at the 5% level. All treatments significantly reduced numbers of large mines, numbers of larvae and percentage of mines with larvae compared with the untreated control. Although the difference between treatments is not significant for large mines or numbers of larvae, a significant reduction of percentage mines with larvae in the AHDB9971 spray treatment compared with the drip treatment would suggest that the spray is the more effective.

Table B. Second assessment - 4 weeks after first spray. The numbers of large ("established") and small ("new") mines are displayed in the table below along with numbers of live *Tuta absoluta* and the percentage of mines which contained larvae.

	Number of mines			Number of larvae	Percentage of mines with larvae	
	Large	Small	Total		Ang	Back trans
Untreated	4.34	39.13	43.47	20.47	44.02	48.29
AHDB9971 Drip	1.03	40.38	41.41	13.06	34.20	31.59
AHDB9971 Spray	1.19	41.38	42.56	8.38	27.95	21.97
AHDB9971 + AHDB9828 Spray	0.97	32.22	33.19	8.94	31.54	27.36
F	27.35	0.247	0.310	5.092	25.30	
p	<0.001	0.862	0.818	0.017	<0.001	
df	12	12	12	12	12	
SED	0.444	11.772	11.991	3.496	1.936	
LSD (5%) (two-sided)	0.968	25.648	26.126	7.617	4.219	
LSD (5%) (one-sided)	0.792	20.981	21.371	6.231	3.451	

Conclusions

A population of *T. absoluta* rapidly established in the trial glasshouse compartment after laboratory-reared adults had been released. Although all life stages of the moth would have been present throughout the assessment period, results suggest that during the first assessments (1 and 2 weeks after the first spray) there were relatively small numbers of larvae established within the leaves and there was little evidence of control of these larvae. However, all of the treatments reduced subsequent re-infestation by the following cohort of larvae as witnessed by the reduction in large mines and larval numbers when the plants were re-assessed 4 weeks after the first spray. As all treatments (all containing AHDB9971) performed similarly there is no evidence of an additive effect due to AHDB9828.

Take home message:

AHDB9971 could provide sufficient control of *T. absoluta* larvae if applied immediately upon sight of fresh mines. Control of embedded larvae may be limited but higher levels of control of larvae hatching from freshly-laid eggs is likely. A number of spray or drip applications may be necessary to reduce *T. absoluta* numbers to acceptable levels.

Objectives

1. To evaluate novel insecticides and bioinsecticides as foliar sprays or drip irrigation treatments for the control of *T. absoluta* on tomato
2. To assess the toxicity of treated leaves to commonly used bio-control insects
3. To monitor the treated crop for phytotoxicity

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**
PP 1/152 (3)	Design and analysis of efficacy evaluation trials	None
PP 1/135 (3)	Phytotoxicity assessment	None
PP 1/181 (3)	Conduct and reporting of efficacy evaluation trials including GEP	None
PP 1/275 (1)	Tuta absoluta	Plot size 8 heads instead of 10

Test site

Item	**Details**
Location address	University of Warwick Wellesbourne Campus Wellesbourne Warwick CV35 9EF
Crop	Tomato
Cultivar	Espero F1
Soil or substrate type	Rockwool
Agronomic practice	See Appendix A
Prior history of site	n/a

Trial design

Item	**Details**
Trial design:	4 x 4 Latin square
Number of replicates:	4
Row spacing:	100 cm
Plot size: (w x l)	0.5 x 1.2m
Plot size: (m^2)	0.6
Number of plants per plot:	4 (8 heads)
Leaf Wall Area calculations	

Treatment details

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
Untreated						
AHDB9971	N/D	N/D	N/D	N/D	N/D	N/D
AHDB9828	N/D	N/D	N/D	N/D	N/D	N/D

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	Control			
2	AHDB9971	36.4 g	1.4 l	A C
3	AHDB9971	36.4 g	1.4 l	B D
4	AHDB9971	36.4 g	1.4 l	A C
3	AHDB9828		0.2 l	A
4	AHDB9828		0.1 l	C

Application details

	Application A	Application B	Application B	Application B
Application date	6/11/19	6/11/19	13/11/19	13/11/19
Time of day	14.00	14.00	14.00	14.00
Crop growth stage (Max, min average BBCH)	702	702	702	702
Crop height (cm)	170	170	170	170
Crop coverage (%)	N/A	N/A	N/A	N/A
Application Method	Spray	Irrigation drip	Spray	Irrigation drip
Application Placement	Foliar	Rockwool block	Foliar	Rockwool block
Application equipment	Berthoud Vermorel 2000HP	Pipette	Berthoud Vermorel 2000HP	Pipette
Nozzle pressure	2 bar	N/A	2 bar	N/A
Nozzle type	05F110	N/A	05F110	N/A
Nozzle size	05	N/A	05	N/A
Application water volume/ha	1000	10 ml/block	1000	10 ml/block
Temperature of air - shade (°C)	19	19	19	19
Relative humidity (%)	N/A	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	N/A	N/A	N/A
Wetness of soil - 2-5 cm	N/A	N/A	N/A	N/A
Cloud cover (%)	N/A	N/A	N/A	N/A

Untreated levels of pests/pathogens at application and through the assessment period

Common name	Scientific Name	EPPO Code	Infestation level pre-application	Infestation level at start of assessment period	Infestation level at end of assessment period
	T. absoluta	GNORAB	3.5 large mines and 2.8 small mines per leaf	5.3 large mines and 1.7 small mines per leaf	4.3 large mines and 39.1 small mines per leaf

Method

Glasshouse Trial

Plant production and design

Tomato seed (cv Espero F1) was sown into rockwool cells on 1 August 2019. After germination the cubes were placed on rock wool blocks (120 x 15 x 7 cm) to give a between-plant spacing of 30 cm (4 plants per block). The trial was arranged as a 4 x 4 Latin square with 4 rows of 4 blocks. Each plot consisted of 1 block and each plant was split into 2 heads to give 8 heads per plot. Irrigation was supplied through a drip feed attached to each plant. Nutrient feed was supplied daily.

Insect production and trial inoculation

A culture of *T. absoluta* provided by Rob Jacobson in 2018 was maintained on tomato plants in the Insect Rearing Unit at Warwick Crop Centre. Adult moths were collected in three cages. On 7 October the cages were transferred to the trial glasshouse compartment and left open, one per space, in the inter-row spaces. The moths were allowed to escape and lay eggs. Initially mines were seen to develop predominantly in the lower leaves of the plants immediately adjacent to the release sites. On 21 October a further cage of moths was collected from the laboratory culture and released at the front of the glasshouse compartment, ensuring an even distribution by shaking moths from the cage, and the existing infestation was allowed to go through a generation. Subsequently egg laying and infestation were much more even throughout the crop.

Treatments

Spray treatments were applied in 1000 l/ha water using a knapsack sprayer with the boom held vertically. The drip treatment was applied in 10 ml using a laboratory pipette into the hole left when removing the drip irrigation spike. Treatments were applied on 6 and 13 November after mines had been observed in leaves throughout the whole crop.

Assessments

Visual assessments of phytotoxicity were made 7 days after treatment. Two leaves per plant on 4 plants per plot were marked and damage was assessed by counting numbers of small mines (<15 mm) and large mines (>15 mm) which represent “new” mines and “established” mines respectively. Counts were done pre-treatment on 6 November and on 13 November, 1 week after the first treatment. The marked leaves were detached on 20 November (2 weeks after the first treatment and 1 week after

the second treatment). The damage was re-assessed and the numbers of larvae were counted. A fresh set of leaves (2 leaves per plant, 4 plants per plot) were detached at a height of about 1.5 m on 4 December (4 weeks after first treatment). Numbers of small mines, large mines and larvae were counted.

Effect on bio-control agents

Bio-control agents typically used in tomato production were supplied by Fargro. Leaves (1 per plot per species of bio-control agent) were removed from the glasshouse trial the day after the first treatment (7 November) and placed in plastic cups (7.5 cm diameter, 10.5 cm height) with a small piece of dampened filter paper to help keep the leaves fresh. There were 4 replicates for each treatment. Bio-control agents were added to the pots (1 species per pot) and the pots were sealed with parafilm. Numbers of live and dead insects/mites were counted 1 day later with the exception of *Encarsia formosa* which was slow to emerge, so counts were performed 6 days after introduction.

Phytoseilus persimillis (Phytoline P - predatory mite) was supplied as live adults in vermiculite. 0.75 g of vermiculite was weighed into each pot.

Diglyphus isaea (Digline I – parasitic wasp) was supplied as live adults. Approximately 15 adults were added to each pot (3 replicates per treatment due to low numbers supplied).

Macrolophus pygmaeus (Macroline P – predatory bug) was supplied as live adults. Approximately 15 adults were added to each pot.

Encarsia formosa (Encarline F – parasitic wasp) was supplied as pupae on cards (60 per card). One card was added to each pot.

Assessment details

Evaluation date	Evaluation Timing (DA)*	Crop Growth Stage (BBCH)	Evaluation type (efficacy, phytotox)	Assessment
5/11/19	-1	702	Pre-spray	Leaf damage
13/11/19	7 (1st spray)	702	Phytotoxicity	Leaf damage
13/11/19	7 (1st spray)	702	Efficacy	Leaf damage
20/11/19	14 (1st spray), 7 (2nd spray)	702	Efficacy	Larvae/pupae numbers and leaf damage
4/12/19	28 (1st spray), 21 (2nd spray)	703	Efficacy	Larvae/pupae numbers and leaf damage

* DA – days after application

Statistical analysis

The data were analysed by ANOVA using the Excel data package. Data was transformed before analysis where required. In all cases plot means were used

Results

Phytotoxicity

No phytotoxic effects were observed with any treatment

Glasshouse Trial

First assessments - 1 and 2 weeks after first spray

The numbers of large ("established") and small ("new") mines pre-spray, 1 week after 1st spray and 2 weeks after 1st spray (1 week after second spray) are presented in Table 1 and Figure 1. None of the analyses were significant at the 5% level using an F-test and there is no evidence of treatment differences. The change in numbers of large and small mines compared with pre-spray counts (Figure 2) are displayed in Table 2 together with numbers of live *Tuta absoluta* (Figure 3). A small number of dead larvae were counted (see appendix). None of the analyses are significant at the 5% level using an F-test and when comparing changes in numbers of mines there is no evidence of control from any of the treatments. Small numbers of larvae and pupae were found and there is some evidence of a reduction in numbers in all treatments compared with the untreated control.

Table 1 Mean numbers of large, small and total mines per leaf in marked tomato leaves pre-spray, 7 days after spraying and 14 days after the first spray.

	Pre-spray			**7 days post spray**			**14 days post spray**		
	Large	**Small**	**Total**	**Large**	**Small**	**Total**	**Large**	**Small**	**Total**
Untreated	3.531	2.750	6.281	5.344	1.500	6.844	7.313	2.156	9.469
AHDB9971 Drip	3.781	2.781	6.563	5.438	1.656	7.094	6.031	3.000	9.031
AHDB9971 Spray	3.344	2.313	5.656	5.500	1.000	6.500	6.344	2.438	8.781
AHDB9971 + AHDB9828 Spray	3.781	2.719	6.500	6.625	1.813	8.438	7.531	2.469	10.000
F	0.115	0.156	0.194	0.330	1.141	0.429	0.233	0.281	0.083
p	0.950	0.924	0.899	0.804	0.372	0.736	0.871	0.838	0.968
df	12	12	12	12	12	12	12	12	12
SED	0.886	0.787	1.330	1.483	0.466	1.832	2.135	0.940	2.626
LSD (5%) (two-sided)	1.931	1.716	2.898	3.232	1.016	3.991	4.652	2.048	5.721
LSD (5%) (one-sided)	1.580	1.404	2.370	2.644	0.831	3.264	3.806	1.675	4.679

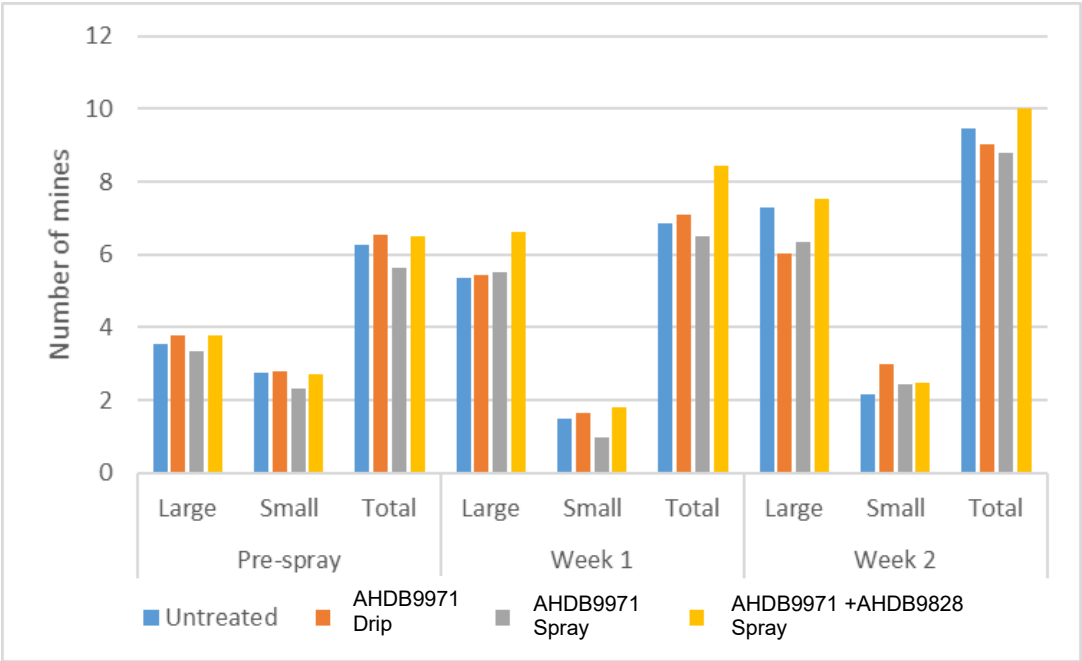

Figure 1 Mean numbers of large, small and total mines per leaf in marked tomato leaves pre-spray, 1 week after spraying and 2 weeks after first spray.

Table 2 Mean change in numbers of mines per leaf from pre-spray numbers 1 and 2 weeks after the first spray and the numbers of live larvae and pupae.

	Week 1			**Week 2**			**Live insects**		
	Large	**Small**	**Total**	**Large**	**Small**	**Total**	**Larvae**	**Pupae**	**Total**
Untreated	1.813	-1.250	0.563	3.781	0.656	2.625	0.875	0.063	0.938
AHDB9971 Drip	1.656	-1.125	0.531	2.250	1.344	1.938	0.500	0.031	0.531
AHDB9971 Spray	2.156	-1.313	0.844	3.000	1.438	2.281	0.688	0.031	0.719
AHDB9971 + AHDB9828 Spray	2.844	-0.906	1.938	3.750	0.656	1.563	0.594	0.063	0.656
F	0.577	0.201	1.444	0.393	0.654	0.224	0.284	0.286	0.291
p	0.641	0.894	0.279	0.760	0.596	0.878	0.836	0.835	0.831
df	12	12	12	12	12	12	12	12	12
SED	0.982	0.566	0.778	1.638	0.744	1.363	0.425	0.048	0.446
LSD (5%) (two-sided)	2.140	1.233	1.695	3.570	1.622	2.969	0.927	0.104	0.971
LSD (5%) (one-sided)	1.750	1.008	1.386	2.920	1.327	2.429	0.758	0.085	0.795

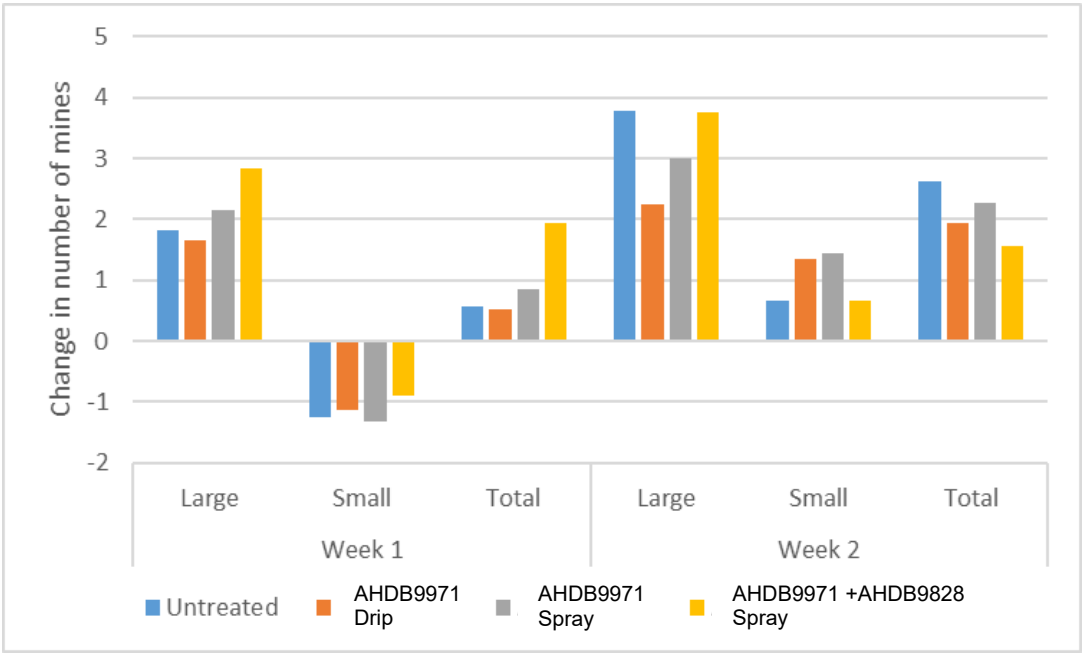

Figure 2 Mean change in numbers of mines per leaf from pre-spray numbers 1 and 2 weeks after first spray

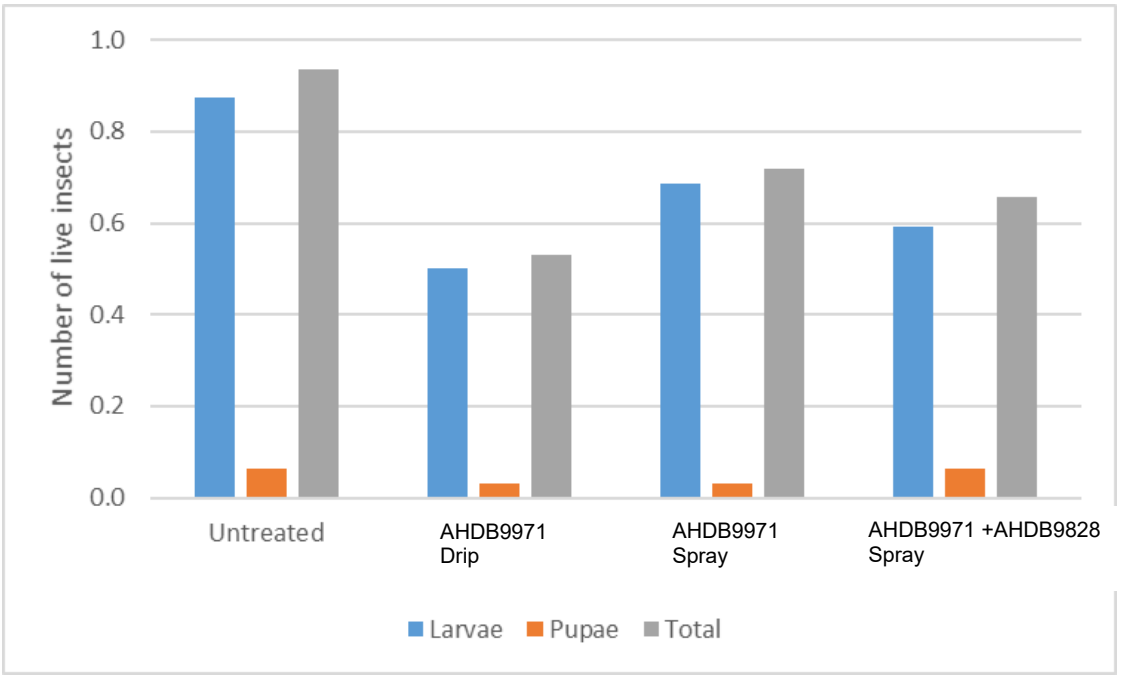

Figure 3 Mean numbers of live larvae and pupae per leaf 2 weeks after first spray

Second assessment - 4 weeks after first spray

The numbers of large (Figure 4) and small mines are displayed in Table 3 together with the numbers of live *T. absoluta* (Figure 5) and the percentage of mines which contained larvae (Figure 6). The data on percentage of mines with larvae were Angular transformed before analysis. The analyses of the numbers of large mines, numbers of larvae and percentage of mines with larvae were significant at the 5% level using an F-test. All treatments significantly reduced numbers of large mines, numbers of larvae and percentage of mines with larvae compared with the untreated control. Although the difference between treatments is not significant for large mines or numbers of larvae, there was a significant reduction in percentage mines with larvae in the Azatin spray treatment compared with the drip treatment.

Table 3 Mean numbers of mines, mean number of larvae per leaf and percentage of mines with larvae in leaves assessed 4 weeks after the first spray.

	Number of mines			**Number of larvae**	**Percentage of mines with larvae**	
	Large	**Small**	**Total**		**Ang**	**Back trans**
Untreated	4.34	39.13	43.47	20.47	44.02	48.29
AHDB9971 Drip	1.03	40.38	41.41	13.06	34.20	31.59
AHDB9971 Spray	1.19	41.38	42.56	8.38	27.95	21.97
AHDB9971 + AHDB9828 Spray	0.97	32.22	33.19	8.94	31.54	27.36
F	27.35	0.247	0.310	5.092	25.30	
p	<0.001	0.862	0.818	0.017	<0.001	
df	12	12	12	12	12	
SED	0.444	11.772	11.991	3.496	1.936	
LSD (5%) (two-sided)	0.968	25.648	26.126	7.617	4.219	
LSD (5%) (one-sided)	0.792	20.981	21.371	6.231	3.451	

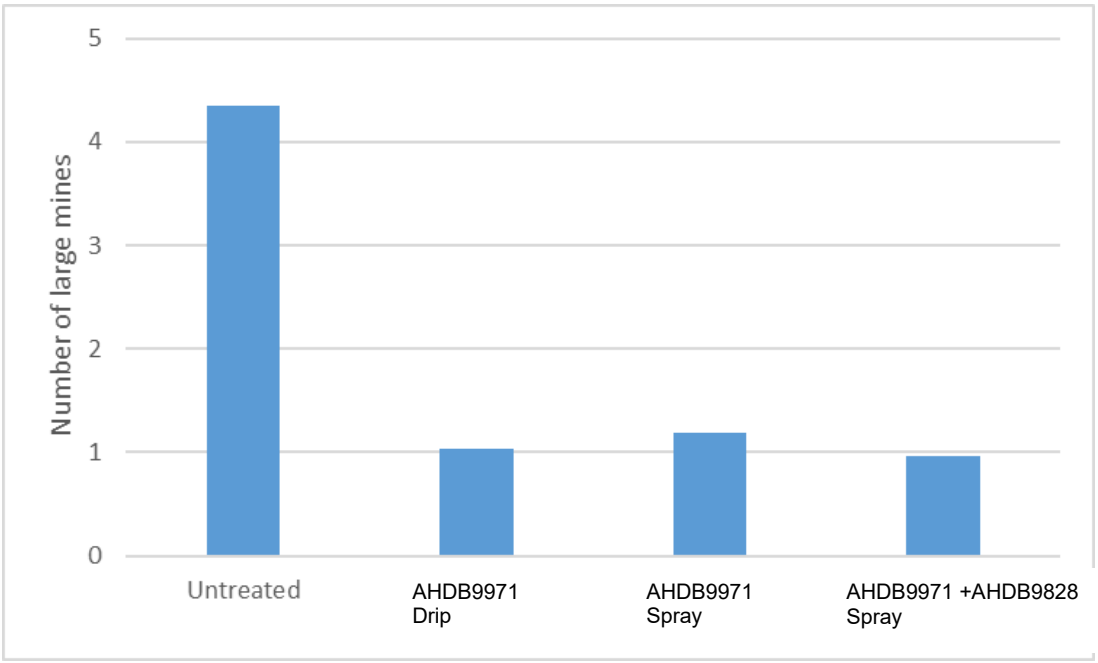

Figure 4 Mean number of large mines per leaf 4 weeks after the first spray

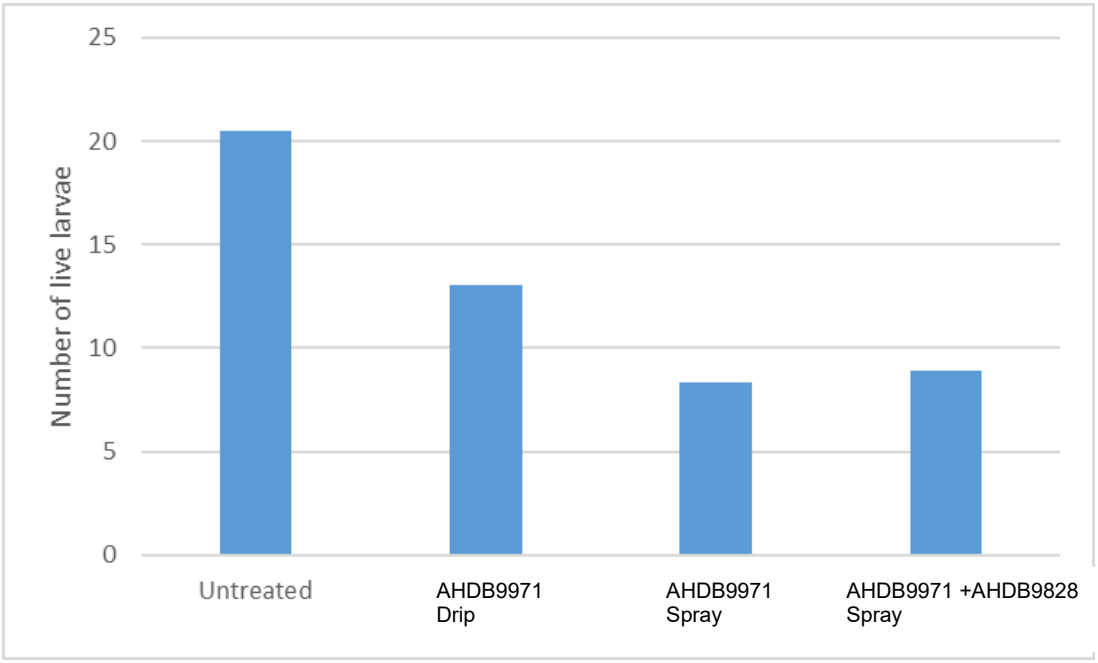

Figure 5 Mean number of larvae per leaf 4 weeks after the first spray

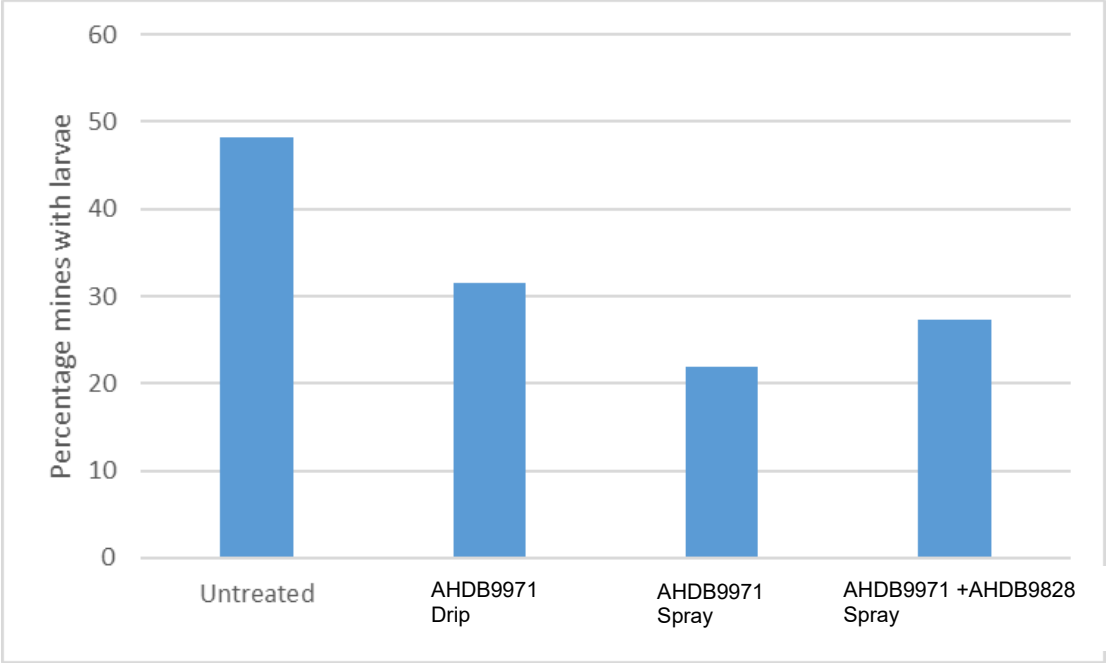

Figure 6 Percentage mines with larvae 4 weeks after the first spray

Effect on bio-control agents

The numbers of *Phytoseilus persimillis* and *Diglyphus isaea* 1 day after introduction, the percentage live *Macrolophus pygmaeus* 1 day after introduction and the number of live *Encarsia formosa* 6 days after introduction are presented in Table 4. Data on percentage live *M. pygmaeus* were Angular transformed before analysis. None of the analyses were significant at the 5% level using an F-test and there is no evidence of treatment differences. Very few dead insects were observed for all species except *M. pygmaeus* (where the percentage live insects was analysed) but even with this species there was no evidence of increased mortality due to any of the treatments compared with the untreated control.

Table 4 Mean numbers of bio-control insects after exposure to treated leaves

	Number live			**Percentage live *M. pygmaeus***	
	P. persimillis	***D. isaea***	***E. formosa***	**Ang**	**Back trans**
Untreated	32.5	14.0	10.8	76.4	94.5
AHDB9971 Drip	19.0	15.0	10.3	76.9	94.9
AHDB9971 Spray	26.5	13.3	10.3	90.0	100
AHDB9971 + AHDB9828 Spray	30.5	14.3	10.5	82.2	98.2
F	1.308	0.423	0.023	0.966	
p	0.317	0.742	0.995	0.440	
df	12	12	12	8	
SED	7.374	1.509	2.229	9.077	
LSD (5%) (two-sided)	16.066	3.480	4.857	19.777	

LSD (5%) (one-sided)	13.142	2.806	3.973	16.178	

Discussion

The structure, size and rate of growth of the tomato crop presents problems for undertaking replicated trials on a range of insecticide/bio-insecticide products. Due to financial and other constraints, experimental approaches have to be at a scale smaller than for commercial production, but this trial was conducted as close as possible to commercial conditions.

In this trial a population of *T. absoluta* rapidly established in the trial glasshouse compartment after laboratory-reared adults had been released. Although all life stages of the moth would have been present throughout the assessment period results suggest that during the first assessments (1 and 2 weeks after the first spray) there were relatively small numbers of larvae established within the leaves and there was little evidence of control of these larvae. However, all of the treatments reduced subsequent re-infestation by the following cohort of larvae as witnessed by the reduction in large mines and larval numbers when the plants were re-assessed 4 weeks after the first spray. The fact that there was no difference in the numbers of small mines between treated and untreated leaves would suggest that some larval feeding occurs (to create the mine) before larvae are killed or deterred from feeding.

All of the treatments tested here were based on AHDB9971 and have been shown to be at least partially effective when applied either as a drip or a spray. There is some evidence that two sprays are more effective than two applications by drip but the differences are small. The final treatment tested AHDB9828 added to AHDB9971. The rationale was that the AHDB9971 could irritate the larvae and draw them out of the leaves thus making them more susceptible to infection with AHDB9828, however there was no obvious additive control effect from this treatment.

In laboratory trials on detached leaves from the glasshouse trial none of the bio-control agents assessed (*P. persimillis, D. isaea, E. formosa* and *M. pygmaeus*) exhibited any increased mortality on treated leaves compared with the untreated control. However, in a glasshouse situation where direct contact from the spray could occur, mortality might increase with the sprays but is likely to be unaffected by the drip application.

Conclusions

- Two applications of AHDB9971 were partially effective as either a spray or a drip in reducing damage and numbers of *T. absoluta* larvae.
- Treatments are more effective in the control of newly-emerged larvae than larvae established in leaves.
- Treatment with AHDB9971 at the first sign of mines, and weekly applications thereafter, may ultimately give sufficient control of *T. absoluta*.
- In this trial, AHDB9828 gave no additional control
- Neither fresh residues of AHDB9971 or AHDB9828 increase mortality of commonly used bio-control agents.

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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. We would also like to thank G's for providing the lettuce seed and for their technical advice.

Appendix

a. Crop diary – events related to growing crop

Crop	Cultivar	Planting/sowing date	Row width (m)
Tomato	Espero	1/8/19	0.3

Active ingredient(s) / fertiliser(s) applied to the trial area

Date	Product	Rate	Unit
All	Solufeed	3.5	E.C.

Details of irrigation regime

Date	Type, rate and duration	Amount applied (mm)
All	As required to maintain Rockwool slabs at 70% water content	

b. Trial diary

Date	Event
01-Aug	150 tomato seed 'Espero' sown into rockwool cells
20-Aug	Tomatoes transplanted from cell into rockwool cubes
28-Aug	Pinch-out tomatoes to produce two healthy side shoots
16-Sep	Tomatoes transplanted into slabs
05-Nov	Pre spray assessment of mines on marked leaves
06-Nov	First sprays and drip applied
13-Nov	Marked leaves re-assessed
13-Nov	Second sprays and drips applied
20-Nov	Marked leaves detached, mines re-assessed and larvae counted
04-Dec	New set of leaves @ approx 1.5m assessed for mines and larvae

c. Raw data from assessments

Glasshouse trial – Pre-spray assessment

				Number of mines		
Plot	Treatment	Plant	Leaf	Large	Small	Total
1	3	1	1	0	3	3
1	3		2	5	6	11
1	3	2	3	0	3	3
1	3		4	4	5	9
1	3	3	5	6	1	7
1	3		6	5	2	7
1	3	4	7	2	3	5
1	3		8	2	4	6
2	4	1	9	0	1	1
2	4		10	2	5	7
2	4	2	11	2	3	5
2	4		12	3	2	5
2	4	3	13	2	2	4
2	4		14	9	10	19
2	4	4	15	3	1	4
2	4		16	3	5	8
3	1	1	17	4	5	9
3	1		18	3	4	7
3	1	2	19	2	3	5
3	1		20	1	6	7
3	1	3	21	1	2	3
3	1		22	2	2	4
3	1	4	23	2	6	8
3	1		24	3	2	5
4	2	1	25	15	6	21
4	2		26	1	7	8
4	2	2	27	4	2	6
4	2		28	5	2	7
4	2	3	29	2	1	3
4	2		30	4	2	6
4	2	4	31	0	2	2
4	2		32	1	1	2
5	1	1	33	1	1	2
5	1		34	1	4	5
5	1	2	35	1	1	2
5	1		36	5	2	7
5	1	3	37	9	3	12
5	1		38	11	3	14
5	1	4	39	0	10	10
5	1		40	15	5	20

6	2	1	41	5	4	9
6	2		42	10	5	15
6	2	2	43	0	3	3
6	2		44	3	1	4
6	2	3	45	1	7	8
6	2		46	17	8	25
6	2	4	47	1	3	4
6	2		48	4	3	7
7	3	1	49	4	4	8
7	3		50	3	2	5
7	3	2	51	4	1	5
7	3		52	7	1	8
7	3	3	53	4	1	5
7	3		54	3	4	7
7	3	4	55	2	1	3
7	3		56	7	6	13
8	4	1	57	4	0	4
8	4		58	7	3	10
8	4	2	59	0	2	2
8	4		60	2	2	4
8	4	3	61	8	2	10
8	4		62	15	0	15
8	4	4	63	1	1	2
8	4		64	4	1	5
9	2	1	65	4	2	6
9	2		66	4	3	7
9	2	2	67	0	1	1
9	2		68	6	2	8
9	2	3	69	1	2	3
9	2		70	4	11	15
9	2	4	71	1	0	1
9	2		72	2	2	4
10	1	1	73	0	2	2
10	1		74	6	3	9
10	1	2	75	0	3	3
10	1		76	5	1	6
10	1	3	77	1	3	4
10	1		78	4	1	5
10	1	4	79	3	1	4
10	1		80	6	1	7
11	4	1	81	0	1	1
11	4		82	1	10	11
11	4	2	83	2	2	4
11	4		84	13	5	18
11	4	3	85	0	1	1

11	4		86	15	5	20
11	4	4	87	1	2	3
11	4		88	10	5	15
12	3	1	89	6	4	10
12	3		90	9	3	12
12	3	2	91	1	1	2
12	3		92	4	0	4
12	3	3	93	3	1	4
12	3		94	4	3	7
12	3	4	95	1	1	2
12	3		96	1	0	1
13	4	1	97	0	3	3
13	4		98	10	2	12
13	4	2	99	0	1	1
13	4		100	0	4	4
13	4	3	101	0	3	3
13	4		102	3	2	5
13	4	4	103	1	0	1
13	4		104	0	1	1
14	3	1	105	0	1	1
14	3		106	0	2	2
14	3	2	107	1	1	2
14	3		108	6	5	11
14	3	3	109	2	1	3
14	3		110	8	3	11
14	3	4	111	2	1	3
14	3		112	1	0	1
15	2	1	113	1	2	3
15	2		114	7	0	7
15	2	2	115	2	1	3
15	2		116	3	1	4
15	2	3	117	2	1	3
15	2		118	5	0	5
15	2	4	119	1	3	4
15	2		120	5	1	6
16	1	1	121	8	2	10
16	1		122	2	0	2
16	1	2	123	4	3	7
16	1		124	4	1	5
16	1	3	125	0	2	2
16	1		126	2	1	3
16	1	4	127	5	2	7
16	1		128	2	3	5

Glasshouse trial – 1 week after 1st treatment assessment

				Number of mines		
Plot	Treatment	Plant	Leaf	Large	Small	Total
1	3	1	1	2	3	5
1	3		2	8	1	9
1	3	2	3	2	1	3
1	3		4	7	6	13
1	3	3	5	5	2	7
1	3		6	8	1	9
1	3	4	7	4	1	5
1	3		8	7	0	7
2	4	1	9	1	0	1
2	4		10	7	0	7
2	4	2	11	6	0	6
2	4		12	6	3	9
2	4	3	13	5	1	6
2	4		14	17	3	20
2	4	4	15	2	2	4
2	4		16	9	1	10
3	1	1	17	8	2	10
3	1		18	4	4	8
3	1	2	19	3	1	4
3	1		20	2	0	2
3	1	3	21	5	0	5
3	1		22	3	1	4
3	1	4	23	5	3	8
3	1		24	7	2	9
4	2	1	25	12	5	17
4	2		26	4	5	9
4	2	2	27	6	1	7
4	2		28	7	0	7
4	2	3	29	2	1	3
4	2		30	6	0	6
4	2	4	31	0	2	2
4	2		32	1	2	3
5	1	1	33	2	1	3
5	1		34	4	3	7
5	1	2	35	3	3	6
5	1		36	6	2	8
5	1	3	37	12	3	15
5	1		38	12	0	12
5	1	4	39	10	5	15
5	1		40	17	2	19

6	2	1	41	8	0	8
6	2		42	12	5	17
6	2	2	43	0	3	3
6	2		44	3	0	3
6	2	3	45	9	1	10
6	2		46	18	4	22
6	2	4	47	6	2	8
6	2		48	4	1	5
7	3	1	49	7	1	8
7	3		50	7	0	7
7	3	2	51	4	0	4
7	3		52	12	2	14
7	3	3	53	5	0	5
7	3		54	7	1	8
7	3	4	55	3	0	3
7	3		56	17	2	19
8	4	1	57	4	2	6
8	4		58	10	3	13
8	4	2	59	0	1	1
8	4		60	2	3	5
8	4	3	61	10	2	12
8	4		62	13	1	14
8	4	4	63	1	0	1
8	4		64	4	1	5
9	2	1	65	7	2	9
9	2		66	3	2	5
9	2	2	67	1	1	2
9	2		68	7	2	9
9	2	3	69	2	1	3
9	2		70	18	5	23
9	2	4	71	1	0	1
9	2		72	3	0	3
10	1	1	73	1	0	1
10	1		74	8	3	11
10	1	2	75	1	1	2
10	1		76	6	0	6
10	1	3	77	3	1	4
10	1		78	6	3	9
10	1	4	79	3	1	4
10	1		80	7	2	9
11	4	1	81	2	1	3
11	4		82	16	4	20
11	4	2	83	4	1	5
11	4		84	23	1	24
11	4	3	85	1	2	3

11	4		86	19	3	22
11	4	4	87	2	4	6
11	4		88	19	8	27
12	3	1	89	7	1	8
12	3		90	13	2	15
12	3	2	91	1	0	1
12	3		92	4	0	4
12	3	3	93	3	2	5
12	3		94	9	0	9
12	3	4	95	2	0	2
12	3		96	1	0	1
13	4	1	97	4	6	10
13	4		98	14	0	14
13	4	2	99	1	0	1
13	4		100	2	0	2
13	4	3	101	1	2	3
13	4		102	6	0	6
13	4	4	103	1	2	3
13	4		104	0	1	1
14	3	1	105	1	0	1
14	3		106	1	2	3
14	3	2	107	1	1	2
14	3		108	12	1	13
14	3	3	109	3	0	3
14	3		110	9	2	11
14	3	4	111	3	0	3
14	3		112	1	0	1
15	2	1	113	5	0	5
15	2		114	9	0	9
15	2	2	115	2	0	2
15	2		116	3	2	5
15	2	3	117	3	1	4
15	2		118	4	0	4
15	2	4	119	2	3	5
15	2		120	6	2	8
16	1	1	121	7	1	8
16	1		122	2	0	2
16	1	2	123	3	2	5
16	1		124	5	1	6
16	1	3	125	1	0	1
16	1		126	2	0	2
16	1	4	127	8	1	9
16	1		128	5	0	5

Glasshouse trial – 2 weeks after 1st treatment assessment

				Number of mines			Number of *T.absoluta*		
Plot	Treatment	Plant	Leaf	Large	Small	Total	Larvae	Pupae	Dead
1	3	1	1	4	8	12	4	0	0
1	3		2	12	2	14	2	0	0
1	3	2	3	3	12	15	3	0	0
1	3		4	11	10	21	1	0	1
1	3	3	5	7	2	9	3	0	0
1	3		6	10	2	12	0	0	0
1	3	4	7	6	4	10	2	1	0
1	3		8	8	2	10	2	0	0
2	4	1	9	1	2	3	1	0	0
2	4		10	7	2	9	1	0	0
2	4	2	11	6	3	9	1	0	0
2	4		12	8	4	12	1	0	0
2	4	3	13	7	1	8	1	0	0
2	4		14	19	5	24	0	0	0
2	4	4	15	4	2	6	0	0	0
2	4		16	10	0	10	0	0	0
3	1	1	17	10	5	15	3	0	0
3	1		18	7	1	8	0	0	0
3	1	2	19	2	9	11	6	0	0
3	1		20	3	1	4	0	0	0
3	1	3	21	5	1	6	0	0	0
3	1		22	3	1	4	1	0	0
3	1	4	23	5	3	8	0	0	0
3	1		24	11	1	12	0	0	0
4	2	1	25	15	4	19	0	0	0
4	2		26	3	5	8	0	0	0
4	2	2	27	6	3	9	1	0	0
4	2		28	7	1	8	0	0	0
4	2	3	29	2	3	5	0	0	0
4	2		30	7	0	7	0	0	0
4	2	4	31	0	2	2	0	0	0
4	2		32	1	3	4	2	0	0
5	1	1	33	7	0	7	1	0	0
5	1		34	9	3	12	2	1	0
5	1	2	35	9	1	10	3	0	0
5	1		36	15	1	16	1	0	0
5	1	3	37	14	6	20	2	0	0
5	1		38	16	3	19	1	0	0
5	1	4	39	23	7	30	2	0	0
5	1		40	18	5	23	1	0	0
6	2	1	41	10	2	12	1	0	0

6	2		42	12	7	19	0	1	0
6	2	2	43	0	3	3	0	0	0
6	2		44	3	2	5	0	0	0
6	2	3	45	10	0	10	0	0	0
6	2		46	20	20	40	2	0	0
6	2	4	47	5	6	11	2	0	0
6	2		48	6	0	6	0	0	0
7	3	1	49	4	1	5	0	0	0
7	3		50	11	1	12	0	0	0
7	3	2	51	4	0	4	0	0	0
7	3		52	15	0	15	0	0	0
7	3	3	53	5	0	5	1	0	0
7	3		54	7	2	9	1	0	0
7	3	4	55	4	0	4	0	0	0
7	3		56	17	5	22	1	0	0
8	4	1	57	4	5	9	0	0	0
8	4		58	11	6	17	1	0	0
8	4	2	59	0	2	2	1	0	0
8	4		60	2	3	5	0	0	0
8	4	3	61	12	2	14	2	0	0
8	4		62	12	1	13	0	0	0
8	4	4	63	1	0	1	0	0	0
8	4		64	4	1	5	1	0	0
9	2	1	65	4	6	10	0	0	0
9	2		66	8	4	12	0	0	0
9	2	2	67	3	2	5	0	0	1
9	2		68	6	2	8	0	0	1
9	2	3	69	2	1	3	1	0	0
9	2		70	21	2	23	2	0	1
9	2	4	71	1	1	2	1	0	0
9	2		72	3	2	5	0	0	0
10	1	1	73	1	0	1	0	0	0
10	1		74	11	3	14	1	0	0
10	1	2	75	2	4	6	0	0	0
10	1		76	6	1	7	0	0	0
10	1	3	77	3	2	5	1	0	0
10	1		78	7	2	9	0	0	0
10	1	4	79	4	2	6	1	0	0
10	1		80	7	3	10	1	0	0
11	4	1	81	2	1	3	0	0	0
11	4		82	19	5	24	1	0	0
11	4	2	83	4	2	6	0	0	0
11	4		84	23	1	24	0	1	0
11	4	3	85	2	1	3	0	0	0
11	4		86	19	4	23	0	0	0

11	4	4	87	5	1	6	2	0	0
11	4		88	22	2	24	0	0	0
12	3	1	89	7	3	10	0	0	0
12	3		90	17	3	20	0	0	0
12	3	2	91	1	1	2	0	0	0
12	3		92	4	0	4	0	0	0
12	3	3	93	4	3	7	0	0	0
12	3		94	7	1	8	0	0	0
12	3	4	95	2	0	2	0	0	0
12	3		96	1	0	1	0	0	0
13	4	1	97	11	4	15	3	0	0
13	4		98	14	4	18	0	1	0
13	4	2	99	1	0	1	0	0	0
13	4		100	2	3	5	1	0	0
13	4	3	101	3	2	5	0	0	0
13	4		102	5	1	6	1	0	0
13	4	4	103	1	4	5	1	0	0
13	4		104	0	5	5	0	0	0
14	3	1	105	1	0	1	0	0	0
14	3		106	1	2	3	0	0	0
14	3	2	107	1	1	2	1	0	0
14	3		108	13	2	15	0	0	0
14	3	3	109	3	4	7	0	0	1
14	3		110	9	2	11	0	0	0
14	3	4	111	3	4	7	1	0	0
14	3		112	1	1	2	0	0	0
15	2	1	113	5	0	5	0	0	0
15	2		114	10	1	11	1	0	0
15	2	2	115	3	2	5	2	0	0
15	2		116	3	2	5	1	0	0
15	2	3	117	5	3	8	0	0	0
15	2		118	4	1	5	0	0	0
15	2	4	119	2	3	5	0	0	0
15	2		120	6	3	9	0	0	0
16	1	1	121	8	0	8	1	0	0
16	1		122	2	1	3	0	0	0
16	1	2	123	4	1	5	0	0	0
16	1		124	5	0	5	0	0	0
16	1	3	125	1	1	2	0	0	0
16	1		126	2	1	3	0	0	0
16	1	4	127	9	0	9	0	0	0
16	1		128	5	0	5	0	1	0

Glasshouse trial – 4 weeks after 1st treatment assessment

				Number of mines			Number of *T.absoluta*	
Plot	Treatment	Plant	Leaf	Large	Small	Total	Larvae	Dead
1	3	1	1	3	112	115	15	0
1	3		2	0	82	82	21	0
1	3	2	3	1	84	85	6	0
1	3		4	1	85	86	20	0
1	3	3	5	0	52	52	13	0
1	3		6	1	56	57	14	0
1	3	4	7	0	84	84	5	0
1	3		8	4	55	59	6	0
2	4	1	1	1	56	57	8	0
2	4		2	1	40	41	7	0
2	4	2	3	2	24	26	6	0
2	4		4	5	40	45	10	0
2	4	3	5	1	54	55	17	0
2	4		6	0	22	22	4	0
2	4	4	7	0	28	28	6	0
2	4		8	0	26	26	9	0
3	1	1	1	2	46	48	29	0
3	1		2	3	39	42	23	0
3	1	2	3	4	45	49	31	0
3	1		4	8	29	37	14	0
3	1	3	5	4	49	53	17	0
3	1		6	3	20	23	9	0
3	1	4	7	2	46	48	17	0
3	1		8	5	36	41	15	0
4	2	1	1	0	19	19	7	0
4	2		2	1	20	21	7	0
4	2	2	3	1	46	47	19	0
4	2		4	0	44	44	12	0
4	2	3	5	2	25	27	9	0
4	2		6	1	34	35	15	0
4	2	4	7	0	20	20	5	0
4	2		8	0	22	22	7	0
5	1	1	1	4	70	74	29	0
5	1		2	3	49	52	18	0
5	1	2	3	1	60	61	29	0
5	1		4	3	76	79	40	0
5	1	3	5	10	77	87	36	0
5	1		6	6	60	66	45	0
5	1	4	7	7	63	70	37	0
5	1		8	11	30	41	25	0

6	2	1	1	1	30	31	10	0
6	2		2	0	64	64	23	0
6	2	2	3	3	52	55	25	0
6	2		4	2	68	70	30	0
6	2	3	5	3	39	42	11	0
6	2		6	1	23	24	9	0
6	2	4	7	0	29	29	8	0
6	2		8	2	50	52	22	0
7	3	1	1	7	38	45	7	0
7	3		2	5	23	28	5	1
7	3	2	3	2	31	33	5	0
7	3		4	3	33	36	12	0
7	3	3	5	0	14	14	6	0
7	3		6	0	22	22	2	0
7	3	4	7	0	27	27	7	0
7	3		8	0	37	37	9	0
8	4	1	1	0	27	27	8	0
8	4		2	0	10	10	4	0
8	4	2	3	1	34	35	13	0
8	4		4	1	46	47	20	0
8	4	3	5	0	28	28	13	0
8	4		6	1	19	20	3	0
8	4	4	7	0	15	15	3	0
8	4		8	3	32	35	7	0
9	2	1	1	0	72	72	15	0
9	2		2	2	60	62	12	0
9	2	2	3	2	52	54	15	0
9	2		4	2	75	77	8	0
9	2	3	5	1	88	89	28	0
9	2		6	5	76	81	36	0
9	2	4	7	0	18	18	5	0
9	2		8	0	17	17	7	0
10	1	1	1	8	47	55	21	0
10	1		2	5	35	40	14	0
10	1	2	3	1	36	37	15	0
10	1		4	5	27	32	12	0
10	1	3	5	6	25	31	16	0
10	1		6	6	27	33	19	0
10	1	4	7	1	60	61	21	0
10	1		8	0	34	34	20	0
11	4	1	1	1	9	10	3	0
11	4		2	1	41	42	11	0
11	4	2	3	0	15	15	6	0
11	4		4	0	23	23	5	0
11	4	3	5	0	18	18	5	0

11	4		6	0	18	18	7	0
11	4	4	7	7	34	41	6	0
11	4		8	0	41	41	16	0
12	3	1	1	2	4	6	1	1
12	3		2	1	25	26	8	0
12	3	2	3	1	27	28	10	0
12	3		4	0	24	24	8	0
12	3	3	5	0	19	19	6	0
12	3		6	0	41	41	6	0
12	3	4	7	0	14	14	4	0
12	3		8	0	14	14	4	0
13	4	1	1	1	50	51	16	0
13	4		2	1	54	55	21	0
13	4	2	3	1	27	28	6	0
13	4		4	1	47	48	7	0
13	4	3	5	0	37	37	9	0
13	4		6	1	64	65	16	1
13	4	4	7	1	26	27	8	0
13	4		8	0	26	26	6	0
14	3	1	1	0	27	27	4	0
14	3		2	2	45	47	10	0
14	3	2	3	1	50	51	12	0
14	3		4	1	37	38	10	0
14	3	3	5	1	49	50	6	0
14	3		6	0	69	69	14	0
14	3	4	7	2	20	22	2	0
14	3		8	0	24	24	9	0
15	2	1	1	1	28	29	8	0
15	2		2	1	38	39	10	0
15	2	2	3	1	43	44	11	0
15	2		4	0	43	43	9	0
15	2	3	5	0	17	17	5	0
15	2		6	0	14	14	3	0
15	2	4	7	1	27	28	9	0
15	2		8	0	39	39	18	0
16	1	1	1	1	13	14	8	0
16	1		2	3	25	28	18	0
16	1	2	3	3	27	30	15	0
16	1		4	3	20	23	16	0
16	1	3	5	2	16	18	12	0
16	1		6	3	21	24	12	0
16	1	4	7	11	30	41	13	0
16	1		8	5	14	19	9	0

Bio-control insects

			Number	
Species	Plot	Treatment	Live	Dead
P. persimillis	1	3	28	0
	2	4	41	0
	3	1	29	0
	4	2	12	0
	5	1	30	0
	6	2	16	0
	7	3	35	0
	8	4	27	0
	9	2	22	0
	10	1	20	0
	11	4	39	0
	12	3	14	0
	13	4	15	0
	14	3	29	0
	15	2	26	0
	16	1	51	0
D. isaea	1	3		
	2	4	13	0
	3	1	16	0
	4	2	15	0
	5	1	13	0
	6	2	15	0
	7	3	16	0
	8	4	15	0
	9	2		
	10	1	13	0
	11	4	15	0
	12	3	14	0
	13	4		
	14	3	10	1
	15	2	15	0
	16	1		
M. pygmaeus	1	3	13	0
	2	4	13	1
	3	1	15	0
	4	2	16	0
	5	1	15	0
	6	2	12	7

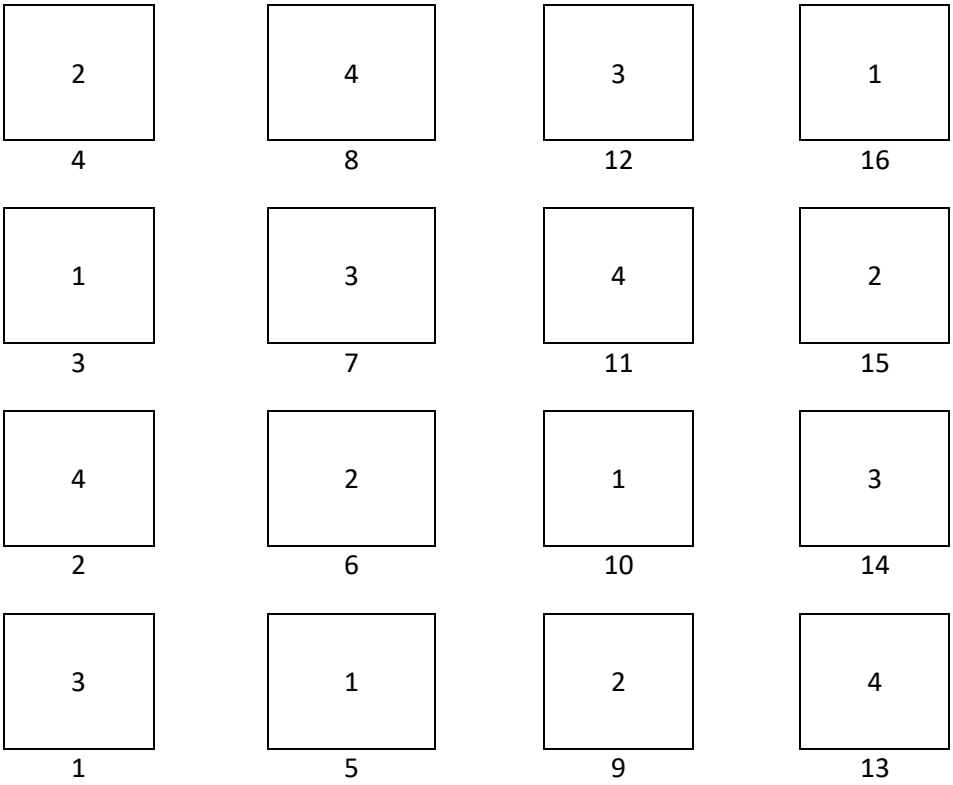
	7	3	13	0
	8	4	13	1
	9	2	11	0
	10	1	12	5
	11	4	15	0
	12	3	12	0
	13	4	15	0
	14	3	15	0
	15	2	14	1
	16	1	13	2
E. Formosa	1	4	10	0
	3	2	11	0
	4	1	12	0
	5	2	12	0
	5	3	9	0
	6	1	7	0
	7	4	7	0
	8	3	8	0
	9	1	17	0
	10	2	8	0
	11	3	10	0
	12	4	13	0
	13	3	14	0
	14	4	12	0
	15	1	7	0
	16	2	10	0

d. Photographs of the trial

Photo 1. The trial on 21 October 2019.

Photo 2. Foliage damaged by *Tuta absoluta* larvae.

Trial number	W2020.507
Sponsor	SceptrePlus
Crop	Tomato
Location	GH E



1	Untreated	
2	AHDB9971	Drip
3	AHDB9971	Spray
4	AHDB9971 + AHDB9828	Spray

Certificate of

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

This certifies that

Warwick Crop Centre, School of Life Sciences

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

Date of issue: **6 October 2017**
Effective date: **20 March 2017**
Expiry date: **19 March 2022**

Signature Alison Richardson
Authorised signatory

Certification Number
ORETO 381

HSE
Chemicals Regulation Division

Department of Agriculture and Rural Development