SCEPTREPLUS

Year 1 Trial Report

Trial code:	SP 34
Title:	**Integrated Control of Tomato Russet Mite- Laboratory Efficacy Trial**
Crop	Tomato
Target	Tomato Russet Mite (TRM), *Aculops lycopersici* (Eriophyidae)
Lead researcher:	Dr Bethan Shaw
Organisation:	NIAB EMR (East Malling Research)
Period:	1st May 2020- 31st March 2022
Report date:	10th November 2020
Report author:	Dr Bethan Shaw
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.

10th November 2020	Bethan Shaw
Date	Authors signature

Trial Summary

Introduction
The Tomato Russet Mite *Aculops lycopersici* (Eriophyidae) (TRM) is a common and significant pest of tomato crops around the world and has become an increasing problem in UK tomato production. Unlike other Eriophyid mites, the TRM is oligophagous, reported to survive on a range of solanaceous plants (e.g. nightshades) and plants in other families, e.g. wild blackcurrant, wild gooseberry and blackberry. The visible symptoms of TRM infestation are discolouration of the stems to a brown/golden colour, shrivelling and browning of leaves, flower drop, and fruits exhibiting russeting. Severe infestations can lead to death of the plant. Even minor infestations can cause flower-drop, reduced fruit size, and fruits with visible TRM damage (russeting) which are unsaleable, causing financial losses to growers.

Current control options are limited, and growers mainly rely on sulphur-based products or conventional acaricides, which can upset the biocontrol options for other pests. The aim of this trial was to test conventional and novel chemistry and other control strategies (bioprotectants), which could be compatible with an IPM programme and used in the UK to manage this pest. Products were chosen after consultation with growers, agronomists, agro-chemical companies, other industry stakeholders and SCEPTREPlus consortium members.

Methods
Cultures of TRM were established from infested material collected by tomato growers and agronomists. Healthy tomato plants were housed in a quarantine facility at 26°C and infested from the donated tomato leaf material. Once populations built to a sufficient size, leaf samples were collected for dipping trials. The numbers of adult mites, which are distinguishable by their orange colour, were counted prior to being submerged in the treatment solutions for 5 seconds. At 4 and 20 hours post dipping, the numbers of live and dead adults were counted. The trial was repeated three times. Three products were tested in all three experiments, one product was tested in two experiments and one product was tested in one experiment only.

Results
Products AHDB9813 and AHDB9970 resulted in significantly higher mortality compared to the water-only control in Experiments 1 and 2 at the 20h assessment. AHDB9944 resulted in significantly higher mortality than the water-only control in Experiment 1 at the 20h assessment only. There was no statistically significant difference between the water-only control and any other treatment in Experiment 3 and there was no significant difference between the water-only control and any other treatment at the 4-hour assessment in Experiments 1 and 2.

Conclusions
Due to the issues associated with working on Eriophyid mites, there were concerns that the methodology used to assess these products for control of TRM would not be appropriate. However, an effective protocol was developed within this project to screen treatments prior to a larger, replicated, semi-field trial. We have identified three products (AHDB9944, AHDB9813 and AHDB9970), which show promise of providing control of TRM in glasshouse tomato. These will be included in the larger trial in the second year. It is possible that the products that did not result in TRM mortality, were not given enough time to take effect. However, there were concerns that by performing the bioassays on leaf disks, the desiccation of the plant material would result in high mortality in the water-only control, which would obscure any effect of the products. For this reason, no assessments were made >20 hours post dipping.

Take home message:

- This laboratory screening trial has identified three promising products that will be used in the larger, semi-field trial in the second year of this project.
- The methodology used, and the timing of assessments may not be appropriate for all plant protection products.

Objectives

The aim of this study was to evaluate the efficacy of promising products to control tomato russet mite (TRM). The products were identified in an earlier review (SP 34 Control of Tomato Russet mite – review of control measures) by Charles Whitfield, NIAB EMR.

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
PP1/223(2)	Introduction to the efficacy evaluation of plant protection products	None
PP1/213(4)	Resistance risk analysis	None
PP1/315(1)	*Aculops lycopersici* on tomato	None

Mite culturing

Tomato seedlings (v. Alicante) were purchased from 'Spadework', Ofham, West Malling on 28th May 2020. The plants were maintained in an insect excluded room within the quarantine facility at NIAB EMR at 20°C under a 16:8 light dark cycle to promote growth, and then transferred to 1L pots after 2 weeks. Any flowers were removed to encourage foliage growth. I plants were transferred to culture cages within the quarantine facility at 26°C under a 16:8 light dark cycle to promote the pest population's growth once TRM infested material began to arrive from growers and agronomists. Tomato foliage infested with TRM was collected by growers and agronomists from commercial crops and sent to NIAB EMR to establish a culture. The infested material was checked under a microscope and any predators and pests other than TRM were physically removed. Samples that contained TRM were transferred to the culture cages, ensuring that there was contact between the infested material and a healthy plant (Appendix Figure 2). A healthy plant was added to each cage each week, ensuring there was contact with the existing plant to enable mites to colonise the new plant. Leaf samples were taken once a week to assess pest establishment. Several infestations of mites were required to establish a stable population. Towards the end of the experiment, plants of cv.. Moneymaker were introduced to prolong the life of the culture; however, foliage from this variety was not used for the dipping bioassay.

Test site

Item	Details
Location address	Quarantine facility, NIAB EMR, New Road, East Malling, Kent, ME19 6BJ
Crop	Tomato
Cultivar	Alicante and Moneymaker
Soil or substrate type	M52 compost in 1L pots

Trial design

Item	Details
Trial design:	Randomised block design
Number of replicates:	5/6 replicates depending on experiment

Treatment details

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
Water-only control	NA (deionised water)					
AHDB9970	N/D	N/D	N/D	N/D	N/D	Transact (0.5 L in 100 L)
AHDB9944	N/D	N/D	N/D	N/D	N/D	NA
AHDB9813	N/D	N/D	N/D	N/D	N/D	NA
AHDB9812	N/D	N/D	N/D	N/D	N/D	NA
AHDB9818	N/D	N/D	N/D	N/D	N/D	NA

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)	Recommended rate of formulated product per 250ml, as used for dipping solutions (rates per L)	Application code
1	Water-only control (deionised water)	NA	NA	NA	A,B,C
2	AHDB9970	766 g/ha	16 L/ha	4 ml/ 250ml (plus 1.25 ml/250ml Transact) (16 ml/L and 5ml/L)	A,B,C
3	AHDB9944	147.6 g/ha	0.9 L/ha	0.23 ml/250ml (0.9 ml/L)	A,B,C
4	AHDB9813	160 g/ha	200 g/ha	0.5 g/250ml (2 g/L)	A,B,C
5	AHDB9812	1.2 L/ha	25 L/ha	6.25 ml/250ml (25 ml/L)	A
6	AHDB9818	2.9 L/ha	7 L/ha	1.75 ml/250ml (7 ml/L)	B,C

Application details

Leaves were removed from the infested tomato plants on the morning dipping was to be performed and mites were counted under a microscope. Only the adults (orange in colour) were assessed (see comment in 'Assessment methodology'). Leaves were organised into blocks with similar numbers of mites on each leaf, this ensured an even distribution of mites within every treatment. Then, leaves were separately assigned a random treatment code, within each block (see Appendix b Figure 3). A single leaf was placed in a 90 mm Petri dish lined with filter paper, with the top side of the leaf facing upwards. In cases where the number of mites on a single leaf were very high, the leaves were cut into sections. Solutions were produced using recommended rates from manufacturers in one litre of deionised water (see 'Application schedule' table above for measurements). The recommended weight/volume was transferred to a 1L Pyrex measuring beaker and 250ml water added. The solution was mixed thoroughly until combined. For AHDB9970, the adjuvant 'Transact' was also used as required in hard water areas (as recommended by the product label). Treatment solutions were made 10 minutes prior to the first dipping. Prior to dipping the solutions were stirred. Leaves were dipped into the solution for 5 seconds using forceps, ensuring that the whole leaf was completely submerged for the 5-second duration. After dipping, the leaf was returned to the Petri dish (topside facing upwards), the lid was replaced, and then left to dry. The same process was performed for the untreated control using deionised water. A minimum of 15 minutes was left between dipping each block. These waiting periods were used to ensure there was a sufficient time interval between the treatment of blocks, to count the mites for the assessments at 4 and 20 h post dipping. In the preliminary trial where these waiting periods were not introduced, we found the assessments of the final block occurred after 6 and 22 hours post dipping.

AHDB9812 performed poorly in Experiment 1 and so was removed from the trial (see discussion). SCEPTREPlus advisors were keen to investigate AHDB9818, so it replaced AHDB9812 in Experiments 2 and 3. Five blocks were treated in Experiment 1 and 2, and 6 blocks in Experiment 3.

	Application A	**Application B**	**Application C**
Application date	13 August 2020	20 August 2020	1 October 2020
Time of day (start of dipping)	Block 1 12:50 Block 2 13:12 Block 3 13:33 Block 4 13:52 Block 5 14:12	Block 1 12:16 Block 2 12:36 Block 3 12:56 Block 4 13:10 Block 5 13:30	Block 1 11:45 Block 2 12:00 Block 3 12:15 Block 4 13:30 Block 5 13:45 Block 6 13:00
Application Method	Dipped into solution for 5 seconds	Dipped into solution for 5 seconds	Dipped into solution for 5 seconds

Assessment methodology

It is well-known that eriophyid mites are difficult to work with experimentally. Consequently, a preliminary trial was undertaken to validate the methodology to be used in the replicated trials (results not shown). During this investigation it was found that the orange adult TRM were much more visible compared to the other life stages, and therefore easier to assess. In addition, the numbers of juveniles in the preliminary trial were so high that the mortality assessment took a significant period of time, resulting in a delay in assessments (i.e., block 2 should have been assessed at 16:15, but the assessment actually started at 17:00). Also, within this trial, it was concluded that the mortality assessment itself (probing the mites with a paintbrush bristle) could be causing the death of the mites. Therefore, a decision was made to perform the 4-hour assessment on the topside of the leaf and the 20-hour assessment on the

underside of the leaf, this was to prevent a destructive assessment method from interfering with the results.

Assessment details

Assessments were made 4 and 20 hours post dipping to assess for rapid and slower effects of the treatments. Information provided for product AHDB9812 stated that mortality would occur within 2-4 hours of application. Mortality was assessed by checking for movement in response to physical contact. This was done by using a single bristle of a fine paintbrush to gently touch each mite. If no movement occurred, it was assumed that the mite was dead. As the assessment method could be harmful to the mites, the 4-hour assessment was performed on the top of the leaf and the 20-hour assessment performed on the underside of the leaf.

	Evaluation Timing		
Evaluation date	**After conventional insecticides**	**After Bio-insecticides**	**Assessment**
13 August 2020	4 hours	4 hours	Mortality on topside of leaf
14 August 2020	20 hours	20 hours	Mortality on underside of leaf
20 August 2020	4 hours	4 hours	Mortality on topside of leaf
21 August 2020	20 hours	20 hours	Mortality on underside of leaf
1 October 2020	4 hours	4 hours	Mortality on topside of leaf
2 October 2020	20 hours	20 hours	Mortality on underside of leaf

Statistical analysis

Statistical analysis was performed using R in R-Studio. The proportion of dead to live mites at each time-point was modelled using logistic regression. If the data was over-dispersed, the model was refitted using the quasi-binomial family with logit link function. Analysis of deviance was used to check for overall treatment effect. Post-hoc means and contrasts were estimated using the R package ‘emmeans’. Contrasts between the water-only control and treated samples were controlled for by family-wise error using Dunnett's test.

Results

Raw data can be seen in Appendix 3. In the replicated laboratory trial, there was no statistically significant difference between the water-only control and any of the other treatments in any of the experiments at the 4-hour assessment (Figure 1). There was also no significant difference between the water-only control and any of the treatments in Experiment 3 at the 20-hour assessment. This outcome was attributed to very low mite numbers on the leaves (average 9 mites in Experiment 3 compared to 26.7 and 17.5 in Experiment 1 and 2 respectively), resulting in no significant differences between treatments in Experiment 3.

AHDB9970 significantly reduced survival in comparison with the water-only control 20 hours post dipping in Experiments 1 and 2 ($p=0.0003$ and $p=0.0001$ respectively) (Table 1). AHDB9813 also significantly reduced survival in comparison with the water-only control 20 hours post dipping in Experiments 1 and 2 ($p<0.0001$ and $p=0.0001$

respectively). AHDB9944 significantly reduced survival in comparison with the water-only control in Experiment 1 (p = 0.009) but not in Experiment 2.

AHDB9812 was replaced by AHDB9818 after it had shown poor efficacy in Experiment 1. However, AHDB9818 did not significantly reduce survival in comparison with the water-only control in Experiment 2.

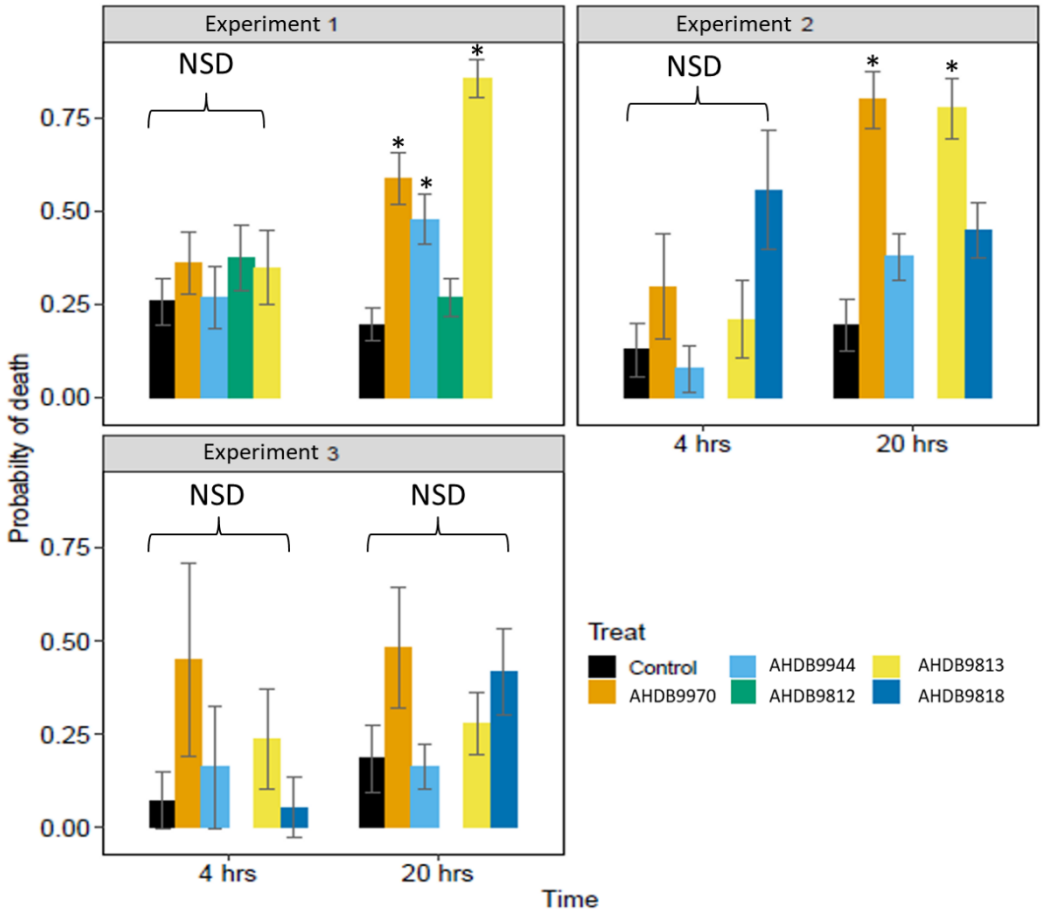

Figure 1. Probability of death of TRM from Experiments 1 (top left), 2 (top right) and 3 (bottom left) at 4 and 20 hours post dipping with treatments. * indicates significant difference from the water-only control within each time point and each experiment. NSD indicates no significant difference between the water-only control and each treatment. Note (water-only) Control, AHDB9970, AHDB9944 and AHDB9813 were applied in all three experiments. AHDB9812 was applied only in Experiment 1. AHDB9818 was applied in Experiments 2 and 3.

Table 1. Table of p-value results for treatments in comparison with the water-only control in all experiments at the 20-hour assessment. The 4-hour assessment is not included as there were no significant differences for any experiment. The Z score produced in

the Dunnett's test indicates how removed the treatment is from the median of the control. Those in bold indicate significance *p*<0.05.

	Experiment 1		Experiment 2		Experiment 3	
Treatment	**Z score**	**Z score**	**p. value**	**p. value**	**Z score**	**p. value**
AHDB9970	3.955	**0.0003**	4.333	**0.0001**	1.734	0.2492
AHDB9944	3.03	**0.0092**	1.847	0.2015	-0.158	0.9955
AHDB9813	5.598	**<0.0001**	4.126	**0.0001**	0.845	0.7761
AHDB9812	1.014	0.67	NA	NA	NA	NA
AHDB9818	NA	NA	2.271	0.08	1.662	0.2833

Discussion

Within the first year of this project, we have successfully developed a laboratory-based bioassay to screen products for the control of TRM. There had been concerns about the methodology as Eriophyid mites are prone to desiccation in laboratory trials. After discussions with M. Easterbrook, the decision was made to keep the mites on tomato leaves to delay desiccation, and to perform a dipping bioassay. A decision was made to only assess adult mites in this laboratory-based screening, but in the semi-field trials planned for year 2, it would be beneficial to determine the impact of the products on the juvenile stages of the mite. Smaller areas of leaf may have to be sampled to do this; this would ensure that assessments are not overly prolonged that they interfere with the assessment timing. In addition, the 4 and 20-hour assessments were performed on different sides of the leaves, as the assessment process appeared to cause mortality. This was not ideal, as there was some variation in mite numbers between the tops and undersides of the leaves, with more found on the undersides (see Appendix c).

The screening process has identified two products that did not result in significant mortality within the 20-hour assessment period. In addition, there was no difference between treatments at the 4-hour assessment in any of the experiments. It may be beneficial to replace the 4-hour assessment with a 48+ hour assessment as some products may take longer to be effective. The justification for not performing an assessment >24 hours post dipping, was due to concerns that water-only control mortality would be high, as a result of the desiccation of leaves. As this screening used leaf disks, after 24 hours some of the leaves had started to curl and stiffen. A more appropriate methodology would be to treat leaves while they were still attached to the plant, which would prevent desiccation from occurring. We propose a modified methodology for Year 2 of this project; treating whole plants with products and removing leaves at defined time points (i.e., 24, 48 and 96 hours post treatment) to make assessments. In addition, the use of whole plants would increase the amount of material available, meaning that different leaves could be assessed after each time. It would have been beneficial to include an acaricide with known efficacy within this trial to confirm that 100% mortality is achievable with this method. However, we felt the resources would be better-used if untested products were screened.

From this dipping trial we have highlighted three products that should be taken forward into the larger, replicated, semi-field trial in Year 2. AHDB9970, AHDB9813 and AHDB9944 all reduced TRM adult survival in Experiment 1 and AHDB9970 and AHDB9813 in Experiment 2. Several other products that were identified by the SP34 review were not screened in Year 1 of this project. These products will be considered for evaluation in Year 2. Some of the un-tested products are bioprotectants, which require specific conditions to fully assess their efficacy. To accommodate this, we may run the trials differently using commercial glasshouses sites.

AHDB9812 was included in Experiment 1 but did not significantly reduce mortality in comparison with the water-only control. However, AHDB9812 is typically used in conjunction with sulphur products to enhance the effect of sulphur. It appears that when used alone, AHDB9812 has no impact on mortality but it should not be disregarded, as it could improve the efficacy of other products. This would need to be confirmed.

AHDB9818 was applied in Experiments 2 and 3 but did not reduce survival in comparison with the water-only control. As this is a product based on fatty acids, it may be that efficacy could be improved by combining it with an adjuvant, as was done for AHDB9970 (also a fatty acid). For AHDB9970 the use of an adjuvant is recommended in hard water areas, as is the case where these trials were executed, although a similar recommendation was not made for AHDB9818. The efficacy of the adjuvant alone was not tested.

Conclusions

Three products have been identified for evaluation in Year 2 of this project (AHDB9970, AHDB9813 and AHDB9944). An effective protocol has been developed to screen products against TRM, which can be used to detect efficacy within 20 hours of application. It would be beneficial to test this protocol with an acaricide with known efficacy, to confirm that 100% mortality is achievable.

Acknowledgements

Several acknowledgements are due in relation to the successful delivery of the first year of trials for this project. We would like to thank Phil Morley who provided vital advice and guidance on the proposed treatments and facilitated the collection of TRM to establish the cultures. The TGA and agronomists involved in sourcing TRM material and sending it to us for this work. Lore Vervaet for her advice on establishing the culture. Mike Easterbrook for advice on the trial methodology. Greg Deakin for statistical analysis and Zoe Clarke for technical support with the experiments and contributions to the report.

We would like to thank the AHDB for funding this work and the crop protection companies for their financial and in kind contributions. Thank you to Bayer, Certis, UPL and Fargro for their specific in-kind contributions to this trial.

Finally, thank you to the SCEPTREPlus team for their guidance and support with this project during a challenging year.

Appendix

a. Trial diary

Date	Event
28/05/20	Collected 10 Alicante tomato plants from Spadework, West Malling. Moved into CT1
17/06/20	Infested tomato material arrived from a grower. The material was collected on the 16th June prior to the grower applying a spray to target the pest. Have also spoken to an agronomist who is sending some infested material also.
18/06/20	Re-potted tomato plants into compost that contains Met52 - this is to control vine weevil. Hopefully will not impact the plants above ground. Added infested material from the Isle of White samples into the cage.
22/06/20	Added new plants to cages.
29/07/20	Added new plants to cages
8/07/20	New infested material collected from a grower. This has been added to a new cage in CT1 with new plants.
21/07/20	Have taken a sample of leaves today and only found 1 live mite out of 10 leaves. Concerned the cultures are not establishing. Will contact growers to see if we can get anymore plant material.
22/07/20	More infested material collected from a grower. Checked the leaves but could only find a few mites. Spoke to Joe Martin at AHDB and expressed concerns that the culture is unstable and will crash before products come to test. Will aim to do a leaf dipping experiment next week with one product to gather data before culture dies out completely and test methodology.
27/07/20	Material collected from a grower has been transferred onto new plants but moved into CT3 (within the quarantine facility). Advice was provided by a PhD student who suggested increasing the temperature and trying to keep the humidity as low as possible- in addition, watering the plants less frequently as they prefer stressed toms. Plant material was taken from the original plants to be used in a dipping experiment, but not enough mites could be found (only two leaves out of twenty with 6/7mites on each). Decided to put them back into the culture cages and hope the increase in temp will stimulate population growth.
30/07/20	Products have started to arrive. Have received 3 products so far.
04/08/20	4th product has arrived.
05/08/20	Checked plants today and watered tomatoes. Looking stressed due to lack of water but otherwise ok. Have received emails today to confirm that more infested material is being sent from a grower. Also, material from a second grower has arrived today
07/08/20	New material arrived and has been transferred to cages in quarantine and a cage in my office which is exposed to natural light and high temperatures. Quarantine room set to 26 degrees C still and from samples taken to check the mites, it seems they are bulking up nicely. Will do first trial as soon as the other products arrive.
12/08/20	5th product arrived. 6th will be delivered at a later date as being sent from Europe.
13/08/2020	1st dip. Have set up a dipping trial today with 4 treatments and a control and 5 reps. Have used the suggested rates from manufactures when no label rate is available. Aiming to repeat this experiment with the same products once more. If mites are still available, it will be repeated twice more with the other products. All TRM came from CT3 culture cage 3
20/08/2020	2nd dip Have set up the second repeat of the tipping trial but have replaced one of the products. Chose to keep the dipping time frequency the same (5 seconds submerged in the solutions, 1 minute between treatments, 15 minutes between blocks). Hoping that with two people performing the assessment it should leave enough time between samples. I.e., block 1 assessed at 16:15 by person A. Block 2 assessed at 16:30 by person B. Block 3 assessed by person A etc. Have done 6 reps this time. All TRM came from CT3 culture cage 2.
24/09/20	New plants added to culture cages in quarantine. All fruit stripped from plants. Seem to have spider mite outbreaks in cage 1 and 3 but cage 2 is looking good. The leaves from these new plants will be used for the dipping trial next week- it seems that a week is enough time for the mites to transfer onto them. Data sent to Greg Deakin for analysis from the first two trials

1/10/20	3rd dip Have set up the third repeat of the tipping trial. Chose to keep the dipping time frequency the same (5 seconds submerged in the solutions, 1 minute between treatments, 15 minutes between blocks). All TRM came from CT3 culture cage 2.
2/10/20	At the final assessment there were much lower numbers of mites than in 1 and 2. Do not think there is time/money to try again this year. Still happy with results from Experiments 1 and 2.
9/10/20	Plants all transferred to freezer and cultures dismantled.

b. Photos from trial

Figure 2. Healthy tomato plants (Alicante) standing tall in an insect cage with grower-collected infested material surrounding the bases of the pots (in contact with the stems to allow mites to transfer onto healthy plants).

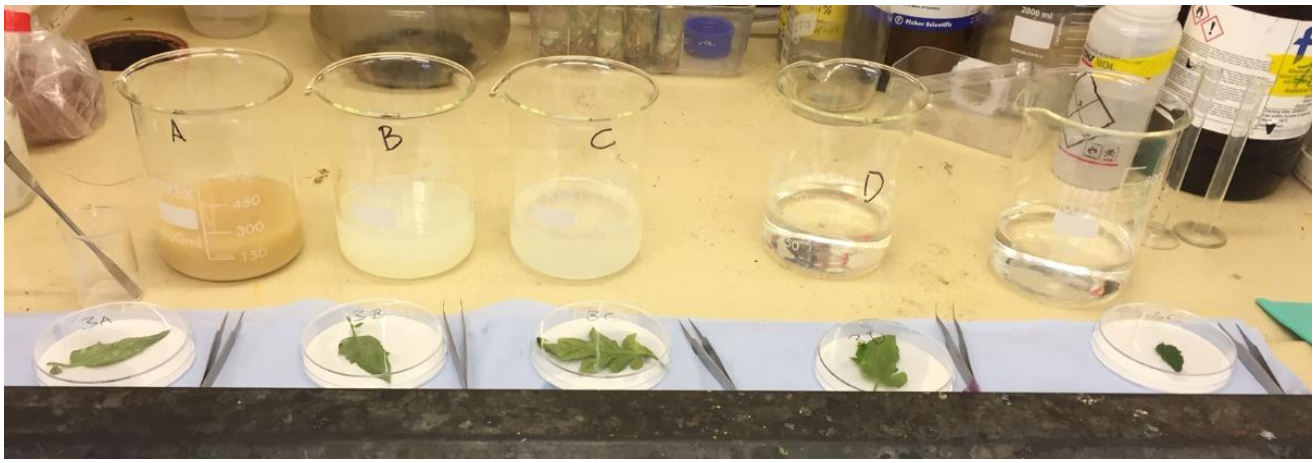

Figure 3. Solutions in a fume hood with leaves after dipping. Each solution had specific forceps for dipping to prevent cross-contamination of products.

c. Raw data from assessments.

Experiment 1. a. Average counts for live, dead, and total mites at 4-hour and 20-hour assessments along with standard error of the mean. b. Actual counts for live, dead, and total mites at 4-hour and 20-hour assessments for each treatment. Note that 4-hour assessments were performed on the topside of the leaf and 20-hour assessments on the underside due to the destructive assessment process.

a.

Treatment	Average Live 4h	Average Dead 4h	Average total 4h	Average Live 20h	Average Dead 20h	Average total 20h
Water-only control	15.4±3	5.4±4.6	20.8±3.6	28.2±1.8	7±2.4	35.2±6.2
AHDB9970	9.4±4.8	5.6±3.1	15±6.7	9±2.1	12.8±3.5	21.8±5.9
AHDB9944	9±1.8	3.4±3.6	12.4±1.9	12.6±1.1	11.8±2.7	24.4±4.6
AHDB9813	6.2 ±1.9	3.6±1	9.8±1.9	2.8±1.7	17.2±2.5	20±2.5
AHDB9812	9.6±4.9	5.2±4.8	14.8±6.1	23.4±1.7	8.6±1.2	32±4.4

b.

Block	Treatment	4 hour- top of leaf			20 hour- bottom of leaf		
		Live	Dead	Total	Live	Dead	Total
1	AHDB9813	7	4	11	2	16	18
	AHDB9944	5	6	11	8	3	11
	AHDB9970	6	4	10	2	13	15
	AHDB9812	28	10	38	38	8	46
	Water-only Control	23	5	28	37	8	45
2	AHDB9813	4	0	4	2	9	11
	AHDB9944	10	0	10	11	11	22
	AHDB9970	11	3	14	17	12	29
	AHDB9812	11	4	15	21	5	26
	Water-only Control	11	11	22	30	3	33
3	AHDB9813	5	2	7	0	24	24
	AHDB9944	15	4	19	25	15	40
	AHDB9970	0	3	3	4	3	7
	AHDB9812	4	7	11	24	8	32
	Water-only Control	7	0	7	11	4	15
4	AHDB9813	13	2	15	6	17	23
	AHDB9944	9	5	14	4	19	23
	AHDB9970	3	4	7	6	11	17
	AHDB9812	2	0	2	8	12	20
	Water-only Control	15	7	22	28	4	32
5	AHDB9813	2	10	12	4	20	24
	AHDB9944	6	2	8	15	11	26

	AHDB9970	27	14	41	16	25	41
	AHDB9812	3	5	8	26	10	36
	Water-only Control	21	4	25	35	16	51

Experiment 2. a. Average counts for live, dead, and total mites at 4-hour and 20-hour assessments along with standard error of the mean. b. Actual counts for live, dead, and total mites at 4-hour and 20-hour assessments for each treatment. Note that 4-hour assessments were performed on the topside of the leaf and 20-hour assessments on the underside due to the destructive assessment process.

a.

Treatment	Average Live 4h	Average Dead 4h	Average total 4h	Average Live 20h	Average Dead 20h	Average total 20h
Water-only control	7.5±3.8	1±0.6	8.5±3.7	10.7±4.0	2.3±1.5	13±5.4
AHDB9970	2.8±1.1	1.2±0.7	4±1.0	2.2±0.9	7.8±2.7	10±3.5
AHDB9944	6±0.9	0.5±0.2	6.5±1.0	13.3±2.4	8±1.2	21.3±3.3
AHDB9813	4.5±0.4	1.2±0.5	5.7±0.7	2.3±2.3	7.7±1.2	10±2.8
AHDB9818	1.7±1.5	2±1.3	3.7±2.0	10.7±3.9	7.8±4.3	18.5±7.7

b.

Block	Treatment	4 hour- top of leaf			20 hour- bottom of leaf		
		Live	Dead	Total	Live	Dead	Total
1	AHDB9813	4	0	4	0	11	11
	AHDB9944	5	0	5	10	10	20
	AHDB9970	6	0	6	1	8	9
	AHDB9818	0	7	7	11	6	17
	Water-only Control	3	3	6	9	0	9
2	AHDB9813	5	3	8	2	8	10
	AHDB9944	9	0	9	14	7	21
	AHDB9970	3	2	5	5	20	25
	AHDB9818	1	0	1	20	9	29
	Water-only Control	11	0	11	12	3	15
3	AHDB9813	6	1	7	0	12	12
	AHDB9944	8	1	9	14	7	21
	AHDB9970	1	0	1	0	4	4
	AHDB9818	0	0	0	2	5	7
	Water-only Control	3	0	3	5	0	5
4	AHDB9813	6	2	8	12	10	22
	AHDB9944	9	1	10	18	11	29
	AHDB9970	6	1	7	3	7	10
	AHDB9818	8	3	11	23	26	49

	Water-only Control	23	1	24	28	8	36
5	AHDB9813	6	1	7	0	5	5
	AHDB9944	5	1	6	24	13	37
	AHDB9970	1	4	5	4	8	12
	AHDB9818	1	2	3	8	1	9
	Water-only Control	5	2	7	10	3	13

Experiment 3. a. Average counts for live, dead, and total mites at 4-hour and 20-hour assessments along with standard error of the mean. b. Actual counts for live, dead, and total mites at 4-hour and 20-hour assessments for each treatment. Note that 4-hour assessments were performed on the topside of the leaf and 20-hour assessments on the underside due to the destructive assessment process.

a.

Treatment	Average Live 4h	Average Dead 4h	Average total 4h	Average Live 20h	Average Dead 20h	Average total 20h
Water-only control	3.3±2.0	0.3±0.2	3.7±2.1	5.2±0.7	1.3±0.3	6.5±0.6
AHDB9970	0.8±0.2	0.7±0.2	1.5±0.2	2±0.7	2±0.7	4±0.9
AHDB9944	1.3±0.6	0.3±0.3	1.7±0.6	11.3±3.4	2.7±1.1	14±3.5
AHDB9813	2.7±0.7	1±0.6	3.7±0.8	8.8±4.1	4.2±1.2	13±5.2
AHDB9818	2.7±0.8	0.2±0.2	2.8±0.8	4.2±1.5	3.7±1.1	7.8±2.2

b.

Block	Treatment	4 hour- top of leaf			20 hour- bottom of leaf		
		Live	Dead	Total	Live	Dead	Total
1	AHDB9813	4	1	5	2	5	7
	AHDB9944	3	0	3	5	5	10
	AHDB9970	0	1	1	3	5	8
	AHDB9818	3	1	4	10	8	18
	Water-only Control	2	1	3	3	2	5
2	AHDB9813	4	1	5	14	6	20
	AHDB9944	0	0	0	24	1	25
	AHDB9970	1	0	1	2	1	3
	AHDB9818	4	0	4	4	4	8
	Water-only Control	0	0	0	4	2	6
3	AHDB9813	4	0	4	5	5	10
	AHDB9944	3	0	3	6	1	7
	AHDB9970	1	1	2	0	3	3
	AHDB9818	3	0	3	1	4	5
	Water-only Control	1	0	1	7	0	7

4	AHDB9813	1	4	5	5	1	6
	AHDB9944	0	0	0	18	2	20
	AHDB9970	1	1	2	0	2	2
	AHDB9818	1	0	1	2	4	6
	Water-only Control	2	0	2	5	1	6
5	AHDB9813	0	0	0	27	8	35
	AHDB9944	0	2	2	12	7	19
	AHDB9970	1	0	1	3	0	3
	AHDB9818	0	0	0	1	2	3
	Water-only Control	13	1	14	7	2	9
6	AHDB9813	3	0	3	0	0	0
	AHDB9944	2	0	2	3	0	3
	AHDB9970	1	1	2	4	1	5
	AHDB9818	5	0	5	7	0	7
	Water-only Control	2	0	2	5	1	6

d. 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