SCEPTREPLUS

Final Trial Report

Trial code:	SP 11 Part 2
Title:	Egg laying deterrents for spotted wing drosophila, *Drosophila suzukii* – Part II
Crop	Blueberry (relevant to several other fruit crops)
Target	Spotted Wing Drosophila (SWD)
Lead researcher:	Michelle Fountain
Organisation:	NIAB EMR
Period:	Jan 2019 – Jul 2019
Report date:	01 June 2019
Report author:	Michelle Fountain
ORETO Number: **(certificate should be attached)**	Certificate number 321

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

15th April 2020 ……

Date Authors signature

Trial Summary

Introduction

Currently spotted wing drosophila (SWD) control is reliant on chemical insecticides and other products are needed in integrated pest management programs to protect fruit whilst extending the intervals between approved insecticides. In a previously reported study (SP 11 Egg laying deterrents for the spotted wing drosophila (SWD)) seven chemical treatments (calcium hydroxide, AHDB9919, calcium hydroxide plus AHDB9919, sodium hydrogen carbonate, AHDB9967, Urtica (fresh juice from nettle (*Urtica dioica*) and AHDB9931) were laboratory tested to determine if they reduced egg laying and adult emergence through an insecticidal, repellent or oviposition deterrent effect on SWD.

Blueberry and blackberry fruits were dipped in an aqueous solution of each chemical at the standard recommended rate, either 48 hours before or 48 hours after the fruit were exposed to SWD adults. Fruits were dipped 48 hours before being infested to determine if the treatments had insecticidal, repellent or oviposition deterrent effects. Fruits were dipped 48 hours after being infested with SWD to determine whether the treatments had curative insecticidal effects. The number of eggs and the number of adult SWD emerging after two weeks was recorded.

Treatment with Urtica gave reduced (~50%) numbers of emerging SWD adults on blueberry; demonstrating insecticidal effects, probably of short persistence. Urtica is a promising treatment worthy of further investigation. AHDB9931 reduced numbers of SWD emerging in tests where it was applied both before and after infestation of blackberry fruits. Calcium and AHDB9919 gave some reduction in numbers of emerging SWD, but the effect was not statistically significant. Of all the treatments tested, AHDB9931, Urtica, and calcium showed the greatest potential for reducing SWD in fruit.

In the study reported here, further experiments were done with the three most promising treatments, with increased numbers of fruits and SWD adults, using choice vs. no-choice test protocols. Field-testing would be the next step in confirming the efficacy of treatments.

Methods

Three treatments were tested; Calcium hydroxide $Ca(OH)_2$, Urtica and AHDB9931, in comparison to a water control. In the first no-choice experiment, SWD were allowed to lay eggs in fruit either before or after dipping in the treatments. In a second experiment SWD females were given a choice of laying eggs on treated or untreated fruits.

Ten previously-mated female SWD were added to each arena. There were ten replicates of each treatment and the numbers of eggs laid and the numbers of adults which emerged were recorded after incubation of fruit for 2 weeks.

Results

When SWD adults were exposed to the fruit before treatment (pre-dipping, no choice), there was no difference in the numbers of eggs laid or the numbers of adults that emerged from treated fruits compared to the untreated control. When fruit were exposed to SWD adults after treatment (post-dipping, no choice), the numbers of eggs were significantly higher in the fruits treated with Calcium hydroxide compared to the control and fruits treated with Urtica. Fewer adult SWD emerged from the fruits treated with Urtica compared to the fruits treated with Calcium hydroxide or AHDB9931, but not compared with the water-only control. It is not known why egg laying on, and subsequent SWD emergence from, the water control was lower.

In the choice test, where SWD were given a choice of treated or untreated fruit, there was no difference between treatments in numbers of eggs laid nor numbers of adult SWD that emerged subsequently.

Conclusions

Although this study set out to confirm findings of the previous study and determine the mechanism of mode of action of the treatments results were inconclusive.

Take home message:

- Previously (SP 11), numbers of SWD emerging from blueberry fruits treated with Urtica were reduced by 50%.
- SWD laid similar number of eggs in fruit pre-dipped in Urtica and the control, in the current study.
- This suggests that Urtica is more likely to affect eggs laid in fruit rather than deter egg laying.

- As the trials have not shown a convincing effect the results from these studies need interpreting with caution because of the failed control in the post-dipping test.

Objectives

Determine if three products previously tested in the preliminary experiments (SP 11) had toxic or repellent effects on SWD egg laying.

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 1/281	*Drosophila suzukii* – Efficacy evaluation of insecticides Bulletin OEPP/EPPO Bulletin (2013) 43(3). 386-388.	The experiment broadly followed the methods set out in the EPPO guideline
PP 1/225	Standard Minimum effective dose	
PM 7/115	*Drosophila suzukii* - identification	

There were deviations from the protocol because the EPPO PP 1/281 is for field-testing. We had a higher number of replicates (x10) but we had 10 fruits instead of 50. In addition, we used emergence rather than flotation testing to assess SWD survival to adult. As per the guideline we made 3 assessments – egg laying stage, then 1 and 2 weeks after incubation.

For detailed methodology see project report SP 11.

As requested by the protocol referees the numbers of females per plot was increased from 5 to 10 to reduce variability due to fecundity of individual females. The replicate number was increased to 10 and the fruit number per plot was to 10.

Experiments were done on blueberry so that we could validation previous results and count eggs in the fruit. We also compared a choice and no-choice test to determine the mechanism of action; repellent, deterrent, toxic etc.

Methodology

Fruits were washed and dried before use and spread onto absorbent laboratory roll to dry for 30 minutes. Fruits were dipped in treatments prepared at a single rate (Table 1, to represent the field rate) of each product for 5 seconds by placing into

mesh bags (Nylon 10 cm x 15 cm) and air-drying in a fume cupboard before placing into ventilated SWD rearing boxes (10 x 8 x 10 cm, Transpack UK Ltd) at 25^{o}C. Two tests were done;

NO CHOICE TEST: Each plot had 10 blueberries. Ten previously mated female SWD were housed with the fruit for 48 hours pre or post dipping (Table 1); **1) Pre-dipping:** Fruit was dipped and dried prior to SWD inoculation, **2) Post-dipping:** Fruit was exposed to SWD for 48 hours prior to dipping. There were 4 treatments x 2 (pre and post dip) x 10 replicate boxes = 80 boxes (total of 800 fruits).

CHOICE TEST: Female SWD were applied to fruits for 48 hours post dipping (Table 1). Each plot (SWD rearing box) had 10 treated fruits grouped at one side of the box and 10 distilled water treated fruits (20 fruits per box). There were 4 treatments x 10 replicate boxes = 40 boxes (800 fruits, half untreated).

Following treatment fruit was stored in the ventilated boxes at 25^{o}C for two weeks. Twenty of the original fruits were also incubated to check for SWD already present in the tested fruit.

Test site

Item	Details
Location address	NIAB EMR
Crop	Blueberry fruits
Cultivar	Biloxi/Camposol
Soil or substrate type	NA
Agronomic practice	NA
Prior history of site	NA

Trial design

Item	Details
Trial design:	Randomized block
Number of replicates:	10
Row spacing:	NA
Plot size: (w x l)	NA
Plot size: (m^2)	NA
Number of plants per plot:	10 fruits per plot
Leaf Wall Area calculations	NA

Treatment details

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
NA	Calcium hydroxide Ca(OH)2	Calcium hydroxide E526 food grade / Mineral S- Water	NA	Calcium hydroxide Ca(OH)2	powder	No

NA	Urtica (100 % pure fresh plant juice)	Stinging nettle / Salus (UK) Ltd, Warrington, Cheshire	NA	*Urtica dioica*	liquid	No
AHDB9931	confidential	confidential	confidential	confidential	liquid	No
NA	Water (negative control)	NA	NA	NA	liquid	No

Table 1. Egg laying deterrent treatments

Trt	Active substance	Potential mode of action	Basic substance	Conc. (/l)	Rate of use (product)	Reason for inclusion
1	Calcium hydroxide Ca(OH)2	Change fruit surface pH, barrier i.e. physical	Yes	2 g	2 g/1	Calcium hydroxide (lime) registered as a basic substance in the EU. Used food grade basic substance.
2	Urtica	Change fruit surface pH	Yes	100 ml	1 in 10 dilution	Urtica is a basic substance and could potentially change the surface pH of the fruit. Pressed juice with 1.52 g of fresh stinging nettle per ml of product.
3	AHDB9931	-	No	16 ml	8 l per ha, so based on 500 l per ha	Potential repellent effects
4	Water (negative control)	-	-	-	-	Negative control.

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	Calcium hydroxide Ca(OH)2	2 g	1g/50ml	
2	Urtica	100 ml	1 in 10 dilution	
3	AHDB9931	16 ml	8 l per ha, applied at 500 l per ha	
4	Water (negative control)	-	-	-

Application details

	Application A No choice	Application B Choice	Application C	Application D
Application date	29 Jan 2019	20 Feb 2019		
Time of day	10:45	10:00		
Crop growth stage (Max, min average BBCH)	NA	NA		
Crop height (cm)	NA	NA		
Crop coverage (%)	NA	NA		
Application Method	Dipping	Dipping		
Application Placement	NA	NA		
Application equipment	NA	NA		
Nozzle pressure	NA	NA		
Nozzle type	NA	NA		
Nozzle size	NA	NA		
Application water volume/ha	NA	NA		
Temperature of air - shade	NA	NA		

(°C)				
Relative humidity (%)	NA	NA		
Wind speed range (m/s)	NA	NA		
Dew presence (Y/N)	NA	NA		
Temperature of soil - 2-5 cm (°C)	NA	NA		
Wetness of soil - 2-5 cm	NA	NA		
Cloud cover (%)	NA	NA		

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
SWD	*Drosophila suzukii*	DROSSU	Zero	Zero	High 15-20 eggs per fruit

[1] Mean percentage surface area damaged on youngest four leaves

[2] Non target pest. Not assessed pre-spraying

[3] percentage plants with caterpillar feeding holes

Assessment details

Assessments were; **Egg laying assessment:** Numbers of eggs in each of 5 blueberry fruit from each plot (both sides in the choice test) were counted under a dissecting microscope after 48 hours in the presence of SWD, **Adult emergence:** counts of emerging adults after incubation for 2 weeks.

	Evaluation Timing (DA)*				
Evaluation date	**After conventional insecticides**	**After Bio-insecticides**	**Crop Growth Stage**	**Evaluation type (efficacy,**	**Assessment**

			(BBCH)	phytotox)	
31 Jan 19		x	NA	efficacy	Egg count in fruit
15 Jan 19		x	NA	efficacy	Adult emergence from fruit
22 Feb 19		x	NA	efficacy	Egg count in fruit
08 Mar 19		x	NA	efficacy	Adult emergence from fruit

* DA – days after application

Statistical analysis

Significance level of $p<0.05$ used to compare all treatments with the untreated control and, in general, make no comparisons between treatments. Statistics were done in R package.

NO CHOICE TEST: Eggs and adult data: 1-way ANOVA

CHOICE TEST Eggs and adult data: 2-way ANOVA

Treatment means were compared using Tukey multiple comparisons of means 95% family-wise confidence level.

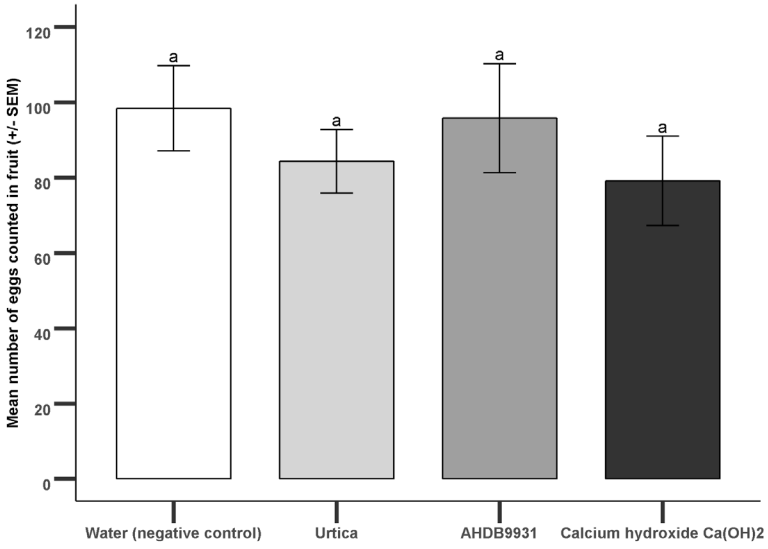
Results and Discussion

NO CHOICE TESTS: In the **pre-dipping** the no-choice test where 10 female SWD were applied to the fruit after treatment there was no significant difference in the numbers of eggs in the treatments compared to the water control or, indeed, between treatments. The numbers of eggs in a subsample of fruits were very high (Table 2: Fig. 1, Grand mean = 89.4). In addition there was no difference in the subsequent numbers of adults that emerged from 10 fruits (Fig. 1, Grand mean = 35.7). Low numbers of adults emerged compared to eggs laid – possibly because of competition/predation between larvae.

These results indicate either that 1) the treatments were not effective in deterring adult SWD from egg laying, 2) were not toxic to eggs laid after treatment application or 3) that the density of female SWD was too high, forcing female flies to lay eggs in the available fruits.

Table 2 **NO CHOICE TEST Pre-dipping -** Mean numbers of eggs laid in 5 fruits and adult SWD that emerged from 10 fruits in the pre-dipping test where SWD females were introduced to fruits post treatment.

Treatment	Eggs	Adults
Calcium hydroxide Ca(OH)2	79.1	37
Urtica	84.3	32.1
AHDB9931	95.8	28.8
Water (negative control)	98.4	44.7



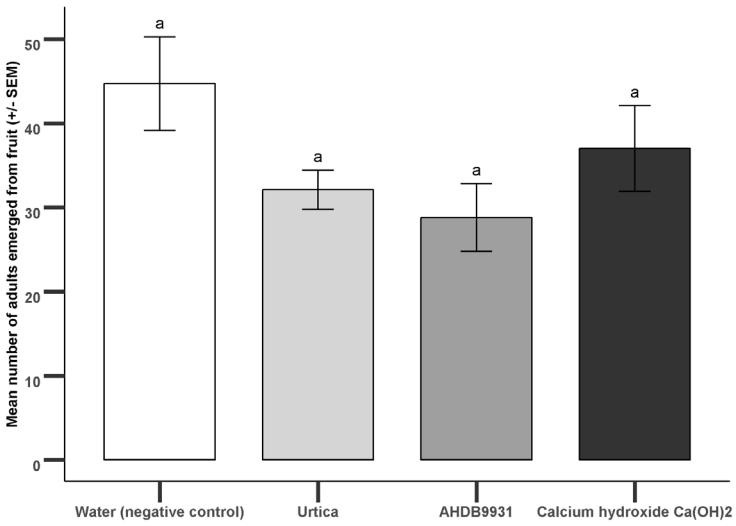
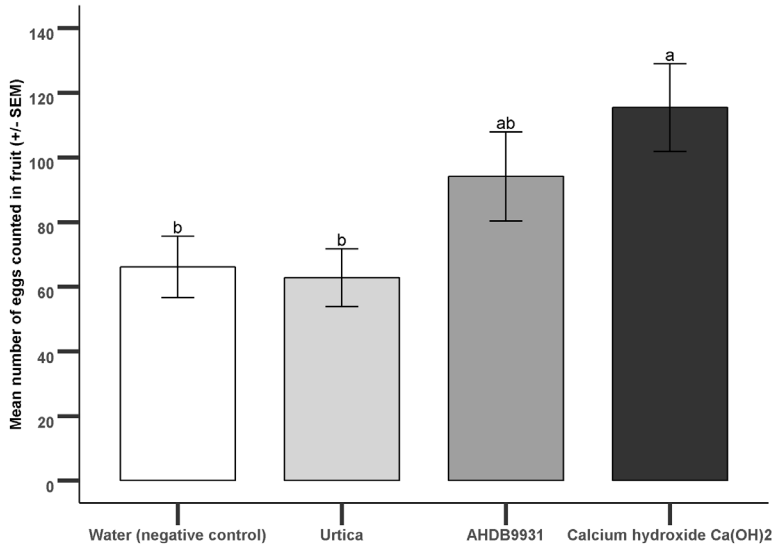


Figure 1. Mean numbers of eggs laid in 5 fruits and adult SWD that emerged from 10 fruits in the pre-dipping test where SWD females were introduced to fruits post treatment. Columns labelled with the same letter do not differ significantly.

In the **post-dipping**, no-choice, test where fruits were exposed to SWD for 48 hours before being dipped in the treatments, the numbers of eggs was significantly higher in the fruits treated with Calcium hydroxide compared to the control and Urtica treatments (Table 3; Fig. 2, P = 0.008). AHDB9931 was not different to the other treatments, giving intermediate results. As before, this was reflected in the numbers of adults emerging from the fruits. There were fewer SWD emerging from the Urtica treatment compared to the Calcium hydroxide and AHDB9931 treatments (Table 3: Fig. 2, P = 0.003). It is not known why the egg laying and subsequent SWD emergence from the water control was lower. If the treatments are compared, ignoring the control, numbers of eggs laid and numbers of SWD emerged from the Urtica treatment were almost half those from the other 2 treatments (Calcium hydroxide and AHDB9931), suggesting that Urtica may have some toxic effects on SWD eggs which have been laid already. These results need to be interpreted with caution.

Table 3. NO CHOICE TEST Post-dipping - Mean numbers of eggs laid in 5 fruits and adult SWD that emerged from 10 fruits in the post-dipping test where SWD females were introduced to fruits before treatment.

Treatment	Eggs	Adults
Urtica	62.8	19.1
Water (negative control)	66.1	23.5
AHDB9931	94.1	31.3
Calcium hydroxide Ca(OH)2	115.4	34.1



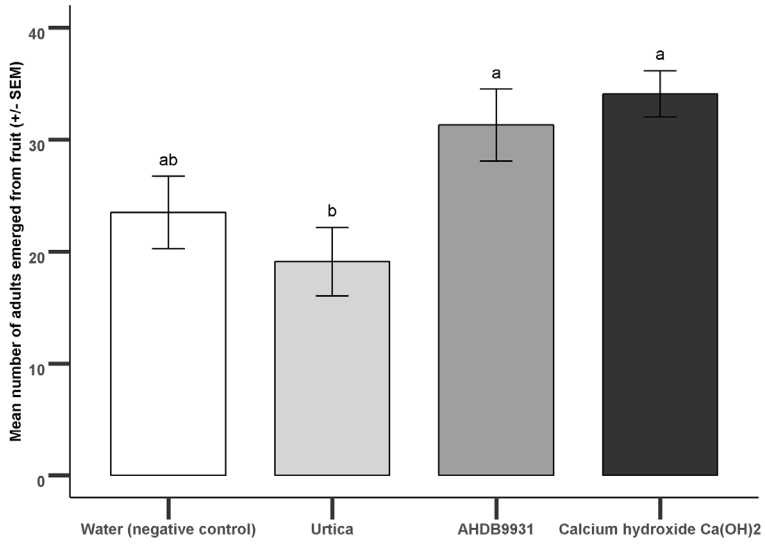


Figure 2. Mean numbers of eggs laid in 5 fruits and adult SWD that emerged from 10 fruits in the post-dipping test where SWD females were introduced to fruits before treatment. Columns labelled with the same letter do not differ significantly.

CHOICE TESTS: When SWD were housed in a box and given a choice of fruit that had been treated with one of the three products or a water control, and untreated fruit there was no difference between treatments in the numbers of eggs laid (Table 4: Fig. 3, Grand mean = 37.6), nor the numbers of SWD adults that emerged subsequently (Fig. 3, Grand mean = 21.2). Hence, no deterrent effect of egg laying in treated blueberry fruit was detected. One possible explanation for lack of effect is that the density of female SWD in the boxes was too high. The numbers of eggs laid in this experiment were roughly half that of the previous experiment, but the data cannot be compared because the experiments were done at a different time and hence the fecundity of the SWD culture could have been different.

Table 4. **CHOICE TEST -** Mean numbers of eggs laid in 5 fruits and adult SWD that emerged from 10 fruits when SWD were given a choice of whether to lay eggs in the treated or untreated (control) fruits.

Treatment	Choice	Eggs	Adults
Calcium hydroxide Ca(OH)2	Control	42.2	18.7
Calcium hydroxide Ca(OH)2	Treated	25.9	17.7
AHDB9931	Control	39.8	18.1
AHDB9931	Treated	35.5	22.6
Urtica	Control	41.4	24.6
Urtica	Treated	41.3	23.6
Water (negative control)	Control	38.1	18.5
Water (negative control)	Treated	36.7	25.7

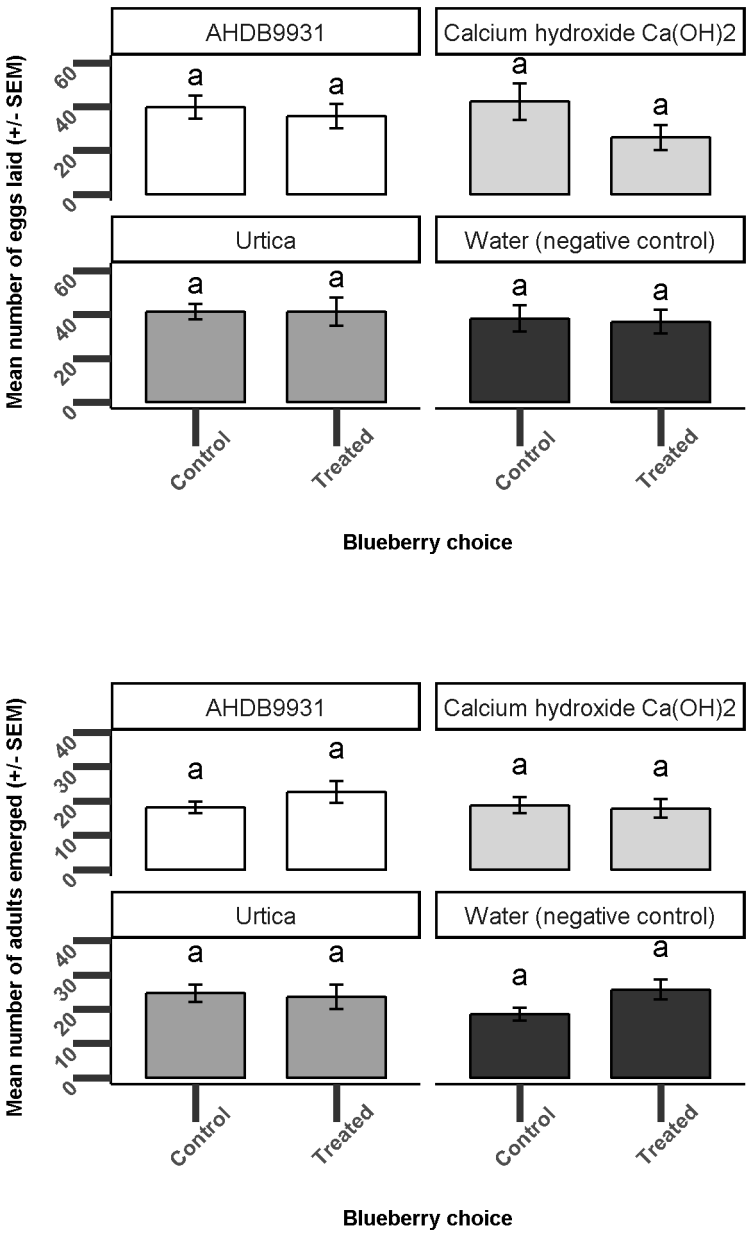


Figure 3. Mean numbers of eggs laid in 5 fruits and adult SWD that emerged from 10 fruits when SWD were given a choice of whether to lay eggs in the treated or untreated (control) fruits. Columns labelled with the same letter do not differ significantly.

Phytotoxicity

NA

Conclusions

Although this studied set out to confirm findings of the previous study and determine the mechanism of mode of action of the treatments the results were inconclusive.

Take home message:

- Previously (SP 11), numbers of SWD emerging from blueberry fruits treated with Urtica were reduced by 50% compared with the control.
- However, in the current study, SWD laid similar number of eggs in fruit pre-dipped in Urtica and the control.
- This suggests that Urtica is more likely to affect eggs laid in fruit rather than deter egg laying.
- As the trials have not shown a convincing effect the results from these studies need interpreting with caution because of the failed control in the post-dipping test.

Acknowledgements

We would like to thank the AHDB for funding this work, Adrian Harris for help with treatments and the staff at NIAB EMR who helped with the egg counts.

We are grateful for the financial and in kind contributions from the crop protection manufactures and distributors involved with the SCEPTREplus programme as listed below:
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.

Appendix

a. Crop diary – events related to growing crop

NA

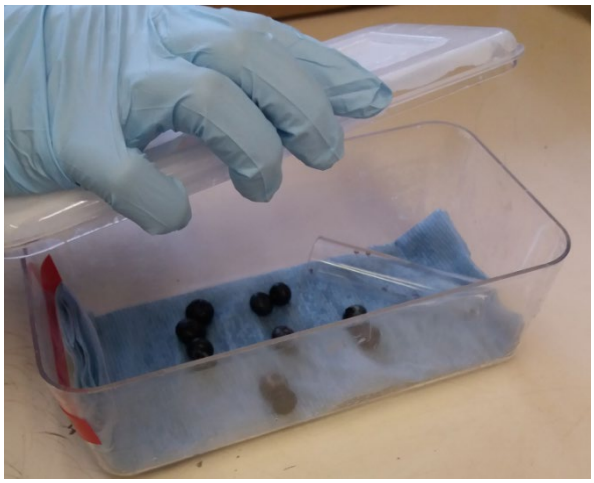
b. Trial diary

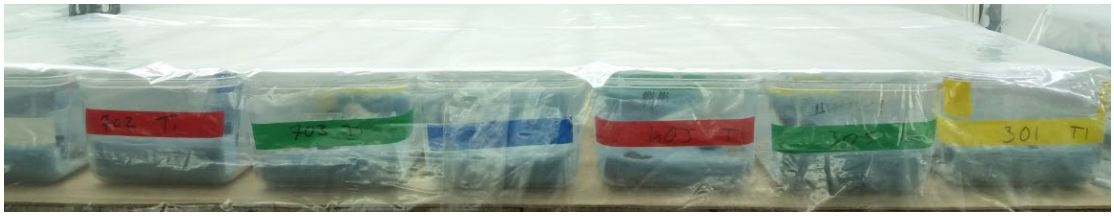
<table>
<tr><th>Date and name</th><th>Record of work done, observations made or reference to lab or field book entry (give book and page numbers)</th></tr>
<tr><td></td><td>NO CHOICE TESTS</td></tr>
<tr><td>17/01/2019</td><td>Protocol finalised. Equipment assembled.</td></tr>
<tr><td>28/01/2019</td><td>Prep boxes</td></tr>
<tr><td>29-31/01/2019</td><td>
<table>
<tr><th>Treatment I (pre-dipping)</th><th>Date</th><th>Time from</th><th>to</th></tr>
<tr><td>Dipping</td><td>29-Jan</td><td>10:45</td><td>11:20</td></tr>
<tr><td>Inoculation</td><td>29-Jan</td><td>14:30</td><td>15:00</td></tr>
<tr><td>SWD removed</td><td>31-Jan</td><td>15:30</td><td>15:45</td></tr>
<tr><td>Egg Assessment</td><td>31-Jan</td><td></td><td></td></tr>
<tr><td>Adult assessment</td><td>15-Feb</td><td></td><td></td></tr>
<tr><th>Treatment II (post-dipping)</th><th>Date</th><th>Time from</th><th>to</th></tr>
<tr><td>Dipping</td><td>31-Jan</td><td>15:30</td><td>16:00</td></tr>
<tr><td>Inoculation</td><td>29-Jan</td><td>12:45</td><td>13:15</td></tr>
<tr><td>SWD removed</td><td>31-Jan</td><td>12:30</td><td>12:45</td></tr>
</table>
</td></tr>
<tr><td>31/02/2019</td><td>Egg assessments</td></tr>
<tr><td>08/02/2019</td><td>Boxes checked for emergence. No adults found</td></tr>
<tr><td>15/02/2019</td><td>Adult emergence assessments</td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td>CHOICE TESTS</td></tr>
<tr><td></td><td>
<table>
<tr><th>Date</th><th>Time</th><th>Step</th></tr>
<tr><td>19-Feb</td><td>*</td><td>Preparation</td></tr>
<tr><td>20-Feb</td><td>09:00</td><td>Prepare treatments</td></tr>
</table>
</td></tr>
</table>

	09:00	Prepare 40 mesh bags with 10 fruits each
	10:00	Dipping fruit 5 seconds
	10:00	Collect flies 40 tubes with 10 females
	10:45	Prepare incubation boxes with control fruit
	12:30	Inoculation treatment boxes with flies
	13:30	Transfer boxes to CT
22/02/2019	Remove the flies + count eggs + split the 20 fruits in 2 boxes	
08/03/2019	Adult emergence assessments	

c. Any photographs.

NO CHOICE: Top left: treated fruits in boxes, Top right: inoculation with SWD, Bottom: incubation of fruit for SWD emergence testing





CHOICE TEST: Innoculation of treated and untreated blueberry fruits

d. Climatological data during study period

Incubated at 25^{o}C

e. Raw data from assessments

NO CHOICE Pre-dipping

Pre-dipping (dipping time 1)										Egg count (pairs of egg breathing tubes counted)										
										Berry										
Pot number	**Block**	**Trt!**	**Treat!**	**Label**	**Dipping time**	**Date inoculated**	**When inoculated**	**Date dipped**	**Flies removed**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
1	1	1	Calcium hydroxide Ca(OH)2	101	1	29/01/2019	14:30	29/01/2019	31/02/2019	10	11	13	18	24	9	11	6	9	8	109
3	1	3	AHDB9931	103	1	29/01/2019	14:30	29/01/2019	31/02/2019	12	3	11	2	8	19	5	7	1	9	77
4	1	2	Urtica	104	1	29/01/2019	14:30	29/01/2019	31/02/2019	4	16	6	4	17	5	9	9	17	18	105
5	1	4	Water (negative control)	105	1	29/01/2019	14:30	29/01/2019	31/02/2019	9	4	11	34	14	4	8	14	12	4	114
6	2	1	Calcium hydroxide Ca(OH)2	201	1	29/01/2019	14:30	29/01/2019	31/02/2019	13	8	13	4	3	14	6	9	12	3	85
7	2	4	Water (negative control)	202	1	29/01/2019	14:30	29/01/2019	31/02/2019	2	6	2	5	4	7	19	9	5	3	62
8	2	2	Urtica	203	1	29/01/2019	14:30	29/01/2019	31/02/2019	0	11	7	19	10	5	10	8	5	10	85
10	2	3	AHDB9931	205	1	29/01/2019	14:30	29/01/2019	31/02/2019	20	11	10	14	12	5	9	4	5	1	91
11	3	2	Urtica	301	1	29/01/2019	14:30	29/01/2019	31/02/2019	7	16	20	12	4	10	2	2	3	13	89
12	3	3	AHDB9931	302	1	29/01/2019	14:30	29/01/2019	31/02/2019	9	15	7	7	12	8	17	4	6	21	106
13	3	1	Calcium hydroxide Ca(OH)2	303	1	29/01/2019	14:30	29/01/2019	31/02/2019	12	5	9	20	9	7	6	12	16	8	104
15	3	4	Water (negative control)	305	1	29/01/2019	14:30	29/01/2019	31/02/2019	25	21	12	13	18	8	7	3	11	6	124
17	4	4	Water (negative control)	402	1	29/01/2019	14:30	29/01/2019	31/02/2019	15	0	2	13	0	3	3	2	2	2	42
18	4	3	AHDB9931	403	1	29/01/2019	14:30	29/01/20	31/02/201	4	1	0	2	6	4	3	0	3	3	26

								19	9												
19	4	2	Urtica	404	1	29/01/2019	14:30	29/01/2019	31/02/2019	6	4	3	9	10	7	15	10	5	4	73
20	4	1	Calcium hydroxide Ca(OH)2	405	1	29/01/2019	14:30	29/01/2019	31/02/2019	11	7	0	11	14	18	30	27	9	15	142
22	5	2	Urtica	502	1	29/01/2019	14:30	29/01/2019	31/02/2019	8	3	0	0	0	6	3	8	7	6	41
23	5	3	AHDB9931	503	1	29/01/2019	14:30	29/01/2019	31/02/2019	22	7	14	10	22	27	3	26	7	34	172
24	5	4	Water (negative control)	504	1	29/01/2019	14:30	29/01/2019	31/02/2019	15	17	7	16	3	7	3	5	6	15	94
25	5	1	Calcium hydroxide Ca(OH)2	505	1	29/01/2019	14:30	29/01/2019	31/02/2019	0	2	0	1	5	2	6	0	3	13	32
26	6	2	Urtica	601	1	29/01/2019	14:30	29/01/2019	31/02/2019	4	6	0	12	9	16	7	10	17	5	86
27	6	3	AHDB9931	602	1	29/01/2019	14:30	29/01/2019	31/02/2019	22	1	20	21	9	6	15	49	5	4	152
28	6	4	Water (negative control)	603	1	29/01/2019	14:30	29/01/2019	31/02/2019	14	20	17	14	3	3	28	12	19	12	142
30	6	1	Calcium hydroxide Ca(OH)2	605	1	29/01/2019	14:30	29/01/2019	31/02/2019	1	5	12	3	12	1	9	22	5	4	74
31	7	2	Urtica	701	1	29/01/2019	14:30	29/01/2019	31/02/2019	2	19	10	17	15	2	11	10	0	14	100
32	7	3	AHDB9931	702	1	29/01/2019	14:30	29/01/2019	31/02/2019	0	3	3	4	17	17	8	7	10	18	87
33	7	4	Water (negative control)	703	1	29/01/2019	14:30	29/01/2019	31/02/2019	0	8	25	6	21	3	3	13	1	23	103
34	7	1	Calcium hydroxide Ca(OH)2	704	1	29/01/2019	14:30	29/01/2019	31/02/2019	2	1	0	0	3	3	7	0	3	1	20
36	8	3	AHDB9931	801	1	29/01/2019	14:30	29/01/2019	31/02/2019	7	14	9	3	1	3	14	0	8	3	62
37	8	2	Urtica	802	1	29/01/2019	14:30	29/01/2019	31/02/2019	12	10	9	0	15	6	2	0	7	14	75
38	8	4	Water (negative control)	803	1	29/01/2019	14:30	29/01/2019	31/02/2019	15	9	12	11	2	10	18	10	3	3	93
39	8	1	Calcium hydroxide	804	1	29/01/2019	14:30	29/01/20	31/02/201	2	3	1	9	5	2	6	6	1	0	89

			Ca(OH)2					19	9	3					1			5		
41	9	2	Urtica	901	1	29/01/2019	14:30	29/01/2019	31/02/2019	7	9	4	9	1	4	5	4	3	7	53
43	9	4	Water (negative control)	903	1	29/01/2019	14:30	29/01/2019	31/02/2019	21	1	6	3	5	9	2	2	5	6	60
44	9	1	Calcium hydroxide Ca(OH)2	904	1	29/01/2019	14:30	29/01/2019	31/02/2019	10	3	5	2	12	3	0	4	4	2	45
45	9	3	AHDB9931	905	1	29/01/2019	14:30	29/01/2019	31/02/2019	16	2	6	24	5	32	7	2	31	8	133
46	10	1	Calcium hydroxide Ca(OH)2	1001	1	29/01/2019	14:30	29/01/2019	31/02/2019	6	9	3	6	38	6	5	0	13	5	91
47	10	4	Water (negative control)	1002	1	29/01/2019	14:30	29/01/2019	31/02/2019	14	12	11	2	15	16	13	13	37	17	150
48	10	2	Urtica	1003	1	29/01/2019	14:30	29/01/2019	31/02/2019	13	3	0	12	20	29	9	23	22	5	136
49	10	3	AHDB9931	1004	1	29/01/2019	14:30	29/01/2019	31/02/2019	3	2	7	12	6	0	1	8	6	7	52

NO CHOICE Post-dipping

Post-dipping (dipping time 2)										Egg count (pairs of egg breathing tubes counted)										
										Berry										
Pot number	**Block**	**Trt!**	**Treat!**	**Label**	**Dipping time**	**Date inoculated**	**When inoculated**	**Date dipped**	**Flies removed**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
1	11	1	Calcium hydroxide Ca(OH)2	1101	2	29/01/2019	12:45	31/01/2019	31/02/2019	6	7	20	8	3	10	4	27	5	0	90
3	11	3	AHDB9931	1103	2	29/01/2019	12:45	31/01/2019	31/02/2019	5	2	3	1	5	14	15	15	4	2	66
4	11	2	Urtica	1104	2	29/01/2019	12:45	31/01/2019	31/02/2019	7	17	10	8	11	0	3	2	6	16	80
5	11	4	Water (negative control)	1105	2	29/01/2019	12:45	31/01/2019	31/02/2019	8	2	10	3	6	3	1	0	4	*	37
6	12	1	Calcium hydroxide Ca(OH)2	1201	2	29/01/2019	12:45	31/01/2019	31/02/2019	21	11	13	19	29	8	2	0	8	1	112
7	12	4	Water (negative control)	1202	2	29/01/2019	12:45	31/01/2019	31/02/2019	4	12	2	8	15	5	0	8	3	7	64
8	12	2	Urtica	1203	2	29/01/2019	12:45	31/01/2019	31/02/2019	2	11	5	6	2	2	9	7	0	1	45
10	12	3	AHDB9931	1205	2	29/01/2019	12:45	31/01/2019	31/02/2019	5	4	15	3	2	8	11	12	8	9	77
11	13	2	Urtica	1301	2	29/01/2019	12:45	31/01/2019	31/02/2019	20	8	10	18	3	14	5	9	6	2	95
12	13	3	AHDB9931	1302	2	29/01/2019	12:45	31/01/2019	31/02/2019	13	28	7	5	21	18	37	6	30	15	180
13	13	1	Calcium hydroxide	1303	2	29/01/2019	12:45	31/01/2019	31/02/2019	2	16	41	24	13	25	18	21	4	38	202

			Ca(OH)2																	
15	13	4	Water (negative control)	1305	2	29/01/2019	12:45	31/01/2019	31/02/2019	29	14	6	26	11	7	1	5	3	3	105
17	14	4	Water (negative control)	1402	2	29/01/2019	12:45	31/01/2019	31/02/2019	18	3	15	13	28	6	2	3	14	11	113
18	14	3	AHDB9931	1403	2	29/01/2019	12:45	31/01/2019	31/02/2019	23	11	14	3	14	4	10	16	24	0	119
19	14	2	Urtica	1404	2	29/01/2019	12:45	31/01/2019	31/02/2019	2	3	3	1	8	7	18	16	3	1	62
20	14	1	Calcium hydroxide Ca(OH)2	1405	2	29/01/2019	12:45	31/01/2019	31/02/2019	21	17	24	12	17	5	6	8	11	9	130
22	15	2	Urtica	1502	2	29/01/2019	12:45	31/01/2019	31/02/2019	3	1	3	3	4	4	1	0	0	0	19
23	15	3	AHDB9931	1503	2	29/01/2019	12:45	31/01/2019	31/02/2019	3	8	1	11	15	4	15	5	16	5	83
24	15	4	Water (negative control)	1504	2	29/01/2019	12:45	31/01/2019	31/02/2019	10	4	13	7	21	17	6	9	5	11	103
25	15	1	Calcium hydroxide Ca(OH)2	1505	2	29/01/2019	12:45	31/01/2019	31/02/2019	18	5	1	5	9	7	5	4	2	3	59
26	16	2	Urtica	1601	2	29/01/2019	12:45	31/01/2019	31/02/2019	2	2	3	7	11	8	1	3	17	8	62
27	16	3	AHDB9931	1602	2	29/01/2019	12:45	31/01/2019	31/02/2019	3	6	4	14	1	15	13	36	17	15	124
28	16	4	Water (negative control)	1603	2	29/01/2019	12:45	31/01/2019	31/02/2019	10	4	1	1	3	7	1	2	19	5	53
30	16	1	Calcium hydroxide Ca(OH)2	1605	2	29/01/2019	12:45	31/01/2019	31/02/2019	22	2	2	15	13	2	17	8	9	10	100
31	17	2	Urtica	1701	2	29/01/2019	12:45	31/01/2019	31/02/2019	7	4	2	9	1	0	2	6	0	5	36

32	17	3	AHDB9931	1702	2	29/01/2019	12:45	31/01/2019	31/02/2019	15	15	4	5	6	11	10	9	20	2	97
33	17	4	Water (negative control)	1703	2	29/01/2019	12:45	31/01/2019	31/02/2019	2	1	2	5	1	14	10	9	2	1	47
34	17	1	Calcium hydroxide Ca(OH)2	1704	2	29/01/2019	12:45	31/01/2019	31/02/2019	17	11	7	9	4	22	1	0	10	1	82
36	18	3	AHDB9931	1801	2	29/01/2019	12:45	31/01/2019	31/02/2019	0	0	3	4	7	1	2	3	4	2	26
37	18	2	Urtica	1802	2	29/01/2019	12:45	31/01/2019	31/02/2019	8	4	0	16	8	17	3	7	4	14	81
38	18	4	Water (negative control)	1803	2	29/01/2019	12:45	31/01/2019	31/02/2019	6	9	5	2	3	0	4	15	8	4	56
39	18	1	Calcium hydroxide Ca(OH)2	1804	2	29/01/2019	12:45	31/01/2019	31/02/2019	0	26	2	10	3	4	12	7	11	5	80
41	19	2	Urtica	1901	2	29/01/2019	12:45	31/01/2019	31/02/2019	10	1	10	2	0	3	4	0	1	9	40
43	19	4	Water (negative control)	1903	2	29/01/2019	12:45	31/01/2019	31/02/2019	0	0	0	1	12	2	7	2	4	*	28
44	19	1	Calcium hydroxide Ca(OH)2	1904	2	29/01/2019	12:45	31/01/2019	31/02/2019	24	19	25	11	3	25	12	7	3	22	151
45	19	3	AHDB9931	1905	2	29/01/2019	12:45	31/01/2019	31/02/2019	4	6	2	4	7	8	6	7	7	0	51
46	20	1	Calcium hydroxide Ca(OH)2	2001	2	29/01/2019	12:45	31/01/2019	31/02/2019	11	16	12	23	7	26	6	15	22	10	148
47	20	4	Water (negative control)	2002	2	29/01/2019	12:45	31/01/2019	31/02/2019	5	6	7	1	0	2	18	12	4	*	55
48	20	2	Urtica	2003	2	29/01/2019	12:45	31/01/2019	31/02/2019	2	14	11	18	6	10	4	11	8	24	108

49	20	3	AHDB9931	2004	2	29/01/2019	12:45	31/01/2019	31/02/2019	5	13	4	6	5	8	17	10	26	24	118

CHOICE TEST

						Egg count (pairs of egg breathing tubes counted)										
						Berry										
Pot number	**Block**	**Trt!**	**Treat!**	**Label**		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
1	1	4	Water (negative control)	101	C	0	0	9	3	0	6	3	0	0	1	22
2	1	4	Water (negative control)	101	T	8	13	1	2	4	0	0	0	3	0	31
3	1	2	Urtica	102	C	5	6	1	9	0	4	1	1	0	5	32
4	1	2	Urtica	102	T	1	3	1	3	4	9	1	4	0	0	26
5	1	3	AHDB9931	103	C	8	13	11	6	2	1	0	0	7	2	50
6	1	3	AHDB9931	103	T	3	4	4	4	0	0	9	2	7	2	35
7	1	1	Calcium hydroxide Ca(OH)2	104	C	0	5	7	6	0	1	0	0	0	4	23
8	1	1	Calcium hydroxide Ca(OH)2	104	T	0	1	1	1	0	2	0	0	1	0	6
9	2	2	Urtica	201	C	8	2	1	6	2	1	7	10	0	0	37
10	2	2	Urtica	201	T	6	3	1	3	4	1	6	3	2	3	32
11	2	3	AHDB9931	202	C	4	3	2	0	3	4	2	1	0	3	22
12	2	3	AHDB9931	202	T	1	0	2	2	1	1	0	2	0	0	9
13	2	4	Water (negative control)	203	C	3	4	7	1	1	6	4	0	0	0	26
14	2	4	Water (negative control)	203	T	1	0	0	1	4	1	2	5	2	0	16
15	2	1	Calcium hydroxide Ca(OH)2	204	C	1	2	10	0	2	1	1	0	0	3	20
16	2	1	Calcium hydroxide Ca(OH)2	204	T	2	4	3	2	0	1	1	0	0	0	13
17	3	1	Calcium hydroxide Ca(OH)2	301	C	4	6	2	5	2	0	3	0	3	6	31
18	3	1	Calcium hydroxide Ca(OH)2	301	T	2	0	0	5	0	3	4	0	1	1	16
19	3	2	Urtica	302	C	13	1	2	2	7	9	1	4	3	4	46
20	3	2	Urtica	302	T	2	2	2	5	3	4	6	1	1	0	26
21	3	3	AHDB9931	303	C	0	0	2	1	2	1	1	11	3	0	21
22	3	3	AHDB9931	303	T	0	4	1	8	1	0	5	2	0	14	35

23	3	4	Water (negative control)	304	C	6	3	4	7	5	8	2	5	3	4	47
24	3	4	Water (negative control)	304	T	0	3	3	2	4	5	11	4	3	6	41
25	4	3	AHDB9931	401	C	3	9	7	3	4	9	2	2	15	8	62
26	4	3	AHDB9931	401	T	0	2	4	5	3	0	1	3	2	6	26
27	4	1	Calcium hydroxide Ca(OH)2	402	C	4	11	9	7	17	5	11	13	5	3	85
28	4	1	Calcium hydroxide Ca(OH)2	402	T	2	7	4	2	5	7	3	5	2	4	41
29	4	2	Urtica	403	C	9	4	4	13	2	16	3	4	2	6	63
30	4	2	Urtica	403	T	3	2	5	6	8	0	0	12	3	8	47
31	4	4	Water (negative control)	404	C	0	2	13	9	3	4	6	3	5	4	49
32	4	4	Water (negative control)	404	T	0	3	2	5	8	3	4	5	1	2	33
33	5	4	Water (negative control)	501	C	0	2	4	2	2	1	1	2	0	0	14
34	5	4	Water (negative control)	501	T	0	1	2	1	4	4	0	0	0	0	12
35	5	3	AHDB9931	502	C	2	0	1	0	4	4	5	4	1	3	24
36	5	3	AHDB9931	502	T	2	1	1	6	0	6	0	3	3	0	22
37	5	1	Calcium hydroxide Ca(OH)2	503	C	3	0	1	0	0	5	2	0	3	3	17
38	5	1	Calcium hydroxide Ca(OH)2	503	T	2	2	1	0	2	2	2	1	3	0	15
39	5	2	Urtica	504	C	5	3	4	5	3	6	11	1	0	3	41
40	5	2	Urtica	504	T	9	13	16	3	6	4	14	8	4	0	77
41	6	2	Urtica	601	C	4	2	10	4	7	5	9	4	1	8	54
42	6	2	Urtica	601	T	6	2	3	5	6	5	5	4	2	11	49
43	6	3	AHDB9931	602	C	9	3	9	3	5	5	3	5	6	8	56
44	6	3	AHDB9931	602	T	5	5	4	9	8	4	9	13	4	3	64
45	6	1	Calcium hydroxide Ca(OH)2	603	C	7	2	4	6	8	3	2	4	7	5	48
46	6	1	Calcium hydroxide Ca(OH)2	603	T	4	6	7	9	2	3	6	7	8	4	56
47	6	4	Water (negative control)	604	C	6	4	8	2	1	4	5	6	3	7	46
48	6	4	Water (negative control)	604	T	3	0	6	3	6	8	7	2	3	2	40

49	7	1	Calcium hydroxide Ca(OH)2	701	C	0	0	4	4	1	2	2	4	0	2	19
50	7	1	Calcium hydroxide Ca(OH)2	701	T	0	0	0	0	0	0	0	3	2	2	7
51	7	2	Urtica	702	C	12	1	8	2	2	1	2	2	3	0	33
52	7	2	Urtica	702	T	0	0	6	10	11	4	4	0	0	9	44
53	7	3	AHDB9931	703	C	4	11	5	8	6	7	2	3	8	9	63
54	7	3	AHDB9931	703	T	2	9	7	10	1	2	16	3	4	3	57
55	7	4	Water (negative control)	704	C	2	0	0	2	0	2	3	1	0	0	10
56	7	4	Water (negative control)	704	T	5	3	4	3	7	1	0	3	1	7	34
57	8	1	Calcium hydroxide Ca(OH)2	801	C	6	12	2	3	9	13	7	6	9	11	78
58	8	1	Calcium hydroxide Ca(OH)2	801	T	6	4	5	2	11	3	4	7	3	4	49
59	8	2	Urtica	802	C	2	2	9	5	3	5	8	6	3	3	46
60	8	2	Urtica	802	T	2	3	2	1	4	3	5	6	2	0	28
61	8	4	Water (negative control)	803	C	4	8	3	2	5	2	8	4	7	3	46
62	8	4	Water (negative control)	803	T	2	4	6	8	5	10	17	9	6	5	72
63	8	3	AHDB9931	804	C	4	7	0	2	3	3	8	4	6	2	39
64	8	3	AHDB9931	804	T	4	5	1	12	4	5	3	7	6	8	55
65	9	1	Calcium hydroxide Ca(OH)2	901	C	0	8	7	25	3	12	6	5	0	3	69
66	9	1	Calcium hydroxide Ca(OH)2	901	T	5	3	2	2	9	3	4	0	2	6	36
67	9	4	Water (negative control)	902	C	12	7	3	3	7	10	0	2	14	10	68
68	9	4	Water (negative control)	902	T	0	4	4	1	2	16	2	3	1	3	36
69	9	3	AHDB9931	903	C	1	4	5	9	1	0	0	5	0	4	29
70	9	3	AHDB9931	903	T	0	1	0	4	8	5	0	3	0	2	23
71	9	2	Urtica	904	C	0	2	1	3	8	8	3	4	0	7	36
72	9	2	Urtica	904	T	8	12	2	8	2	7	9	7	8	8	71
73	10	2	Urtica	1001	C	1	0	6	0	3	1	3	3	3	6	26
74	10	2	Urtica	1001	T	1	1	0	5	2	4	0	0	0	0	13

75	10	3	AHDB9931	1002	C	8	4	3	1	1	0	3	1	8	3	32
76	10	3	AHDB9931	1002	T	0	2	2	4	0	2	2	0	8	9	29
77	10	1	Calcium hydroxide Ca(OH)2	1003	C	0	3	0	0	9	4	5	4	5	2	32
78	10	1	Calcium hydroxide Ca(OH)2	1003	T	9	0	1	0	4	0	3	0	1	2	20
79	10	4	Water (negative control)	1004	C	1	3	10	3	4	1	5	7	7	12	53
80	10	4	Water (negative control)	1004	T	0	3	7	9	9	6	7	0	2	9	52

f. Trial design

All trials had 4 treatments (including a water treated control) and 10 replicates.

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