SFF11/058: IPM tools for psyllid management – Soft chemical options

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Executive summary

SFF11/058: IPM tools for psyllid management – Soft chemical options

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Since its discovery in 2006, the tomato/potato psyllid, *Bactericera cockerelli* (Sulc), has been considered a significant pest of potatoes and other solanaceous crops in New Zealand, resulting in an intensification of insect pest control measures, often with the use of broad-spectrum insecticides. In the search for more sustainable control methods in outdoor potato crops, a selection of softer chemicals was tested in laboratory bioassays to assess their effect on TPP behaviour and mortality. In the mortality study, the treatments were applied either once or twice. The products tested in both studies were Organic JMS Stylet Oil®, Excel Oil®, Eco-Oil®, Neem 600 WP and Sap Sucker Plus.

The 15 min behavioural studies of adult female TPP on treated potato leaves showed:

- A high repellence by Sap Sucker Plus followed by Organic JMS Stylet Oil® and Excel Oil® (measured by time spent off leaf and time spent feeding/probing).
- The mortality study on whole treated potato plants showed that: Numbers of nymphs were lower for all soft chemicals compared with water (control) 13 days after the first spray, with lowest numbers found on Organic JMS Stylet Oil® and Neem 600 WP
- 23 days after the first spray, Eco Oil® and Neem 600 WP seemed to have lost their effectiveness, as these treatments had a larger number of nymphs compared with the other soft chemicals
- On average, numbers of nymphs were reduced by the second spray to only ¼ of those found with one spray; however, the reduction in total nymphs compared to water was significant only for Organic JMS Stylet Oil® and Excel Oil®
- Numbers of large nymphs were reduced by more than half with the application of the second spray and numbers of small nymphs by almost 90%
- For all soft chemicals, significantly fewer adults had emerged compared with water for both 1 and 2 sprays
- 23 days after the first spray no nymphs or emerging adults were found on Organic JMS Stylet Oil® for either 1 or 2 sprays.

Given the efficacy of JMS Stylet Oil® and Excel Oil® at reducing TPP numbers and their probing/feeding deterrent qualities, these two products could warrant further testing in field trials. The impact of the tested soft chemicals on beneficial insects should also be assessed in future trials.

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1 Introduction

Since its discovery in New Zealand in 2006 the tomato/potato psyllid (TPP), *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae), has been regarded as a significant pest on both outdoor and greenhouse solanaceous crops, such as potatoes, capsicums and tomatoes. TPP has led to a considerable increase of insecticide applications in the horticultural industry and thus presents a serious challenge to the implementation of Integrated Pest Management (IPM) strategies (Teulon et al. 2009). TPP vectors the bacterial pathogen *Candidatus* Liberibacter solanacearum which has been identified as the cause of the disease 'zebra chip' in potato tubers (Munyaneza et al. 2007; Liefting et al. 2009). The economic impact of TPP and *Ca*. L. solanacearum in New Zealand has been in millions of dollars in terms of increased management costs, crop losses and loss of export markets (Teulon et al. 2009; Kale 2011)

The current TPP pest management practices in New Zealand potato crops rely on very regular applications of often broad-spectrum insecticides. These practices are not only costly but are likely to have a negative impact on the environment and non-target organisms, while increasing the potential for insecticide resistance in pest populations. As part of a sustainable IPM approach, the use of selective, cost-effective and environmentally sensitive products is therefore preferred.

'Softer' chemicals are described as compounds that are either more targeted in their specificity and/or have a reduced environmental impact. These products include; minerals, mineral oils, horticultural/vegetable oils, botanicals (extracts/oils, derivatives), insecticidal soaps and plant essential oils (Berry & Bourhill 2012). A number of softer chemical options are already on the market. However, to date they have only been considered for use in control of TPP on greenhouse crops (Walker et al. 2010; Walker et al. 2011). Softer chemical options may affect TPP life stages by direct mortality (Berry et al. 2009; Marcic et al. 2009) or may have a repellent effect on TPP adults, deterring them from settling, feeding and ovipositing (Al-Jabr 1999; Yang et al. 2010).

Berry & Bourhill's (2012) review on soft chemical options identified a range of products with potential for control of TPP in New Zealand potato crops. Based on this review and industry consultation (Fenton Hazelwood, pers. comm.), five soft chemicals were chosen for the study outlined in this report.

1.1 Objective

The objective of this study was to carry out bioassays on potato plants to determine the effects of a selection of softer chemicals on:

1. TPP adult behaviour responses

2. TPP adult, egg and early-instar mortality and oviposition.

2 Materials and methods

2.1 Insect source and rearing

TPP adults for testing were obtained from a laboratory colony at Plant & Food Research, Lincoln, Canterbury. This colony was originally established from adult TPP collected in greenhouse tomatoes in Auckland. TPP were reared on tomato plants (cv. 'Money Maker') in a controlled temperature growth room at 25°C, 40% humidity, 16:8 light:dark photoperiod. Only female TPP were used for the behaviour bioassay (sexed under a microscope). A mixture of male and female TPP were used for the mortality bioassay.

2.2 Plant material and application of soft chemicals

In vitro tissue culture potato plantlets (cv. 'Russet Burbank') were transferred into individual pots with potting mix and left to grow to a vegetative growth stage (8–12 leaves) in a controlled temperature growth room at 22°C, app. 50% humidity, 16:8 light:dark photoperiod. Based on the soft chemical literature review by Berry and Bourhill (2012) and industry consultation, five soft chemicals were chosen for the bioassays: Organic JMS Stylet Oil®, Excel Oil®, Eco-Oil®, Neem 600 WP and Sap Sucker Plus. A water control was also included, comprising a total of six treatments (Table 1). All five soft chemicals tested can be applied with a commercial spray boom; these products should not clog nozzles or require additional tank-cleaning. For this experiment the soft chemicals were applied with a spray bottle at the rate specified by the manufacturer for use on other Hemiptera.

Ten potato plants were used for each treatment. Leaves from four of the ten plants were used for the behaviour bioassay following spray application of all treatments to the selected leaves 24 h prior to the behaviour assessment. Six of the ten plants were used for the mortality bioassay with all treatments applied as direct sprays to the whole plants at the vegetative growth stage (8–12 leaves). In the mortality bioassay, a second application of all treatments was applied to three of the six plants, 10 days after the first spray application.

Table 1: Treatments used in the bioassays.

Trade name	Active ingredient	Mode of action	Formulation	Field rate
Organic JMS Stylet Oil®	Mineral Oil + adjuvant	Inhibits insect respiration	971 ml/litre EC	1.5 litre/100 litres
Excel Oil®	Mineral Oil	Inhibits insect respiration	843 g/litre EC	1 litre/100 litres
Eco-Oil®	Canola Oil	Inhibits insect respiration	851.5 g/litre EC	7.5 ml/litre
Neem 600 WP	Neem seed kernel extract	Insect growth regulator and antifeedant	600 g/kg neem seed kernel extract	1 kg/100 litres
Sap sucker Plus	Oxygenated monoterpenes neem oil, dispersants and adjuvants	Antifeedant and insect growth regulator	Information not accessible	240g in 12 litres water
Water	-	Control	-	-

2.3 Bioassay 1: TPP adult behaviour responses

2.3.1 Materials and methods

Bioassays based on the protocols of Liu and Trumble (2004) were carried out to assess adult TPP behaviour responses to excised treated leaves. The soft chemicals and the water control were applied to individual leaves still attached to the potato plant 24 hours prior to the experiment. When sprayed, the individual potato leaves were separated from the other leaves by a filter paper (Figure 1). We modified the arena described by Liu and Trumble (2004) using a 9 cm plastic Petri dish lined with a moistened filter paper (Figure 2). Immediately prior to the behaviour assessment, the excised treated potato leaf was cut off the plant and placed on top of the filter paper with the underside of the leaf facing up. The female TPP was taken from the holding Petri dish, placed on the leaf in the arena and immediately covered by a 4 cm glass Petri dish (Figure 2). The Petri dish was then placed under a binocular microscope with cold light for 5 min to allow the female to settle. All behaviour responses as described by Liu and Trumble (2004) (resting, off leaf, walking, feeding/probing, cleaning, jumping, oviposition) were then recorded into a digital voice recorder for 15 min. After each assay, the glass Petri dish was rinsed with water, followed by ethanol and dried with a paper towel. Assays always commenced at 10:30 am and a full replicate of six treatments was completed by 2:15 pm.

Figure 1: Set up for application of treatments to individual potato leaves.

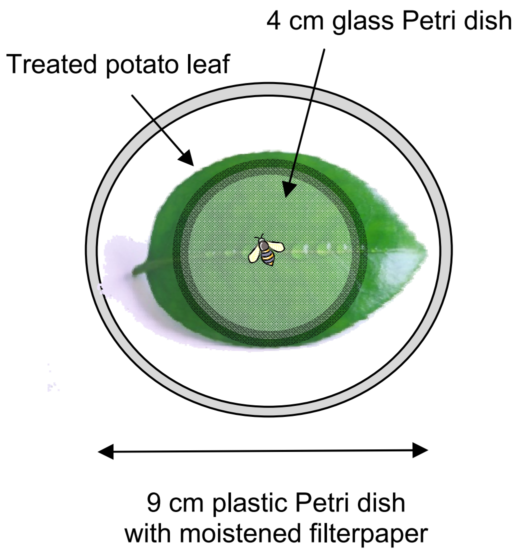

Figure 2: Arena set-up for TPP behaviour bioassay.

2.3.2 Trial design

All six treatments were assessed daily: Organic JMS Stylet Oil® (J), Excel Oil® (X), Eco-Oil® (C), Neem 600WP (N), Sap-sucker plus (S) and Water control (W). Twelve replicates of each treatment were assessed, with the order determined by a Latin rectangle (Figure 3), constructed with CycDesign (CycSoftware 2009). Assays were assessed in sequence, with a complete replicate assessed per assessor per day, and replicates assessed by each assessor in alternating order. Two replicates for each treatment were assessed daily, except the last two treatments.

		Insect Order					
		1st	*2nd*	*3rd*	*4th*	*5th*	*6th*
Rep (person/ day)	**1**	1 **X**	2 **N**	3 **W**	4 **C**	5 **S**	6 **J**
	2	7 **W**	8 **S**	9 **C**	10 **X**	11 **J**	12 **N**
	3	13 **S**	14 **W**	15 **J**	16 **N**	17 **C**	18 **X**
	4	19 **C**	20 **J**	21 **X**	22 **W**	23 **N**	24 **S**
	5	25 **N**	26 **X**	27 **S**	28 **J**	29 **W**	30 **C**
	6	31 **C**	32 **N**	33 **W**	34 **S**	35 **J**	36 **X**
	7	37 **J**	38 **C**	39 **N**	40 **S**	41 **X**	42 **W**
	8	43 **S**	44 **C**	45 **N**	46 **X**	47 **W**	48 **J**
	9	49 **W**	50 **J**	51 **X**	52 **N**	53 **S**	54 **C**
	10	55 **J**	56 **X**	57 **S**	58 **W**	59 **C**	60 **N**
	11	61 **N**	62 **W**	63 **J**	64 **C**	65 **X**	66 **S**
	12	67 **X**	68 **S**	69 **C**	70 **J**	71 **N**	72 **W**

Figure 3: Order of treatments for each replicate. Please refer to Section 2.3.2 for clarification of treatment codes.

2.3.3 Statistical analysis

Each behaviour was assigned a code: R resting, O off leaf, W walking, F feeding/probing, C cleaning, J jumping, E oviposition. Data were converted into the durations for each behaviour. The number of behaviour phases for each TPP was calculated (e.g. C, O, J, R is 4 phases), as was the total number of each behaviour type for each TPP. In addition, for jumps, the total number of jumps in the 15 min period was calculated.

Data for one TPP (number 13, S, Rep 3), which got its leg stuck to a droplet on its antennae and remained like that for the whole period, was excluded from the analysis as this is not normal behaviour.

Some initial analyses (using hierarchical generalized linear modelling, (Lee et al. 2006) details not presented) were carried out to assess whether there were substantial differences relating to Days, Replicates or Person. There were no strong differences relating to Days or Person. However, for one or two of the variables analysed, there were some strong replicate to replicate differences, but the analyses adjusting for these differences gave essentially the same conclusions, so for simplicity, the results presented here are from the unadjusted analyses.

Counts (number of phases, total jumps, mean number of jumps per jump phase, number of R, O, W, F, C, J, E phases) were analysed with Poisson generalized linear model (McCullagh & Nelder 1989), with a log. link. The analysis included an F-test for the Oil main effect (overall test for differences), and a contrast to compare each oil with water.

The percentage time (out of 15 min) spent on each phase (excluding jumping) was analysed with a Poisson log-linear model approach for multinomial data (McCullagh & Nelder 1989), adjusting for the continuous rather than discrete nature of the data by using the Pearson dispersion to assess treatments and for the calculation of confidence limits. Each behaviour was then analysed individually, as a binomial GLM with a logit link (using the Pearson dispersion from the multinomial analysis), to provide an assessment of differences between oils for each behaviour.

For the Poisson and Binomial analyses, 95% confidence limits for the means were obtained as part of the analysis on the transformed (link) scale, and back-transformed.

2.4 Bioassay 2: TPP adult, egg and early-instar mortality and oviposition

2.4.1 Materials and methods

A bioassay with whole potato plants was carried out to assess the effects of soft chemicals on TPP mortality and oviposition. Six whole potato plants in pots were used per treatment, consisting of Organic JMS Stylet Oil® (J), Excel Oil® (X), Eco-Oil® (C), Neem 600 WP (N), Sap-sucker plus (S) and Water control (W). Twenty-four hours after the plants had been sprayed with the different treatments they were covered with fine mesh cages (Figure 4) and 30 TPP adults from the laboratory colonies were added to each plant inside the cages. The cages were secured with tape around the base of the pots to prevent TPP from escaping. Each pot was placed in a white plastic container for watering.

Figure 4: Caged potato plant used in mortality bioassay.

Mortality assessments were carried out at three different time points over a 20-day period (4 May, 14 May and 24 May 2012). Three days after adding the adult TPP (4 May 2012) the total numbers of eggs and live and dead TPP in each cage were counted. The TPP adults were removed and the sex identified under a binocular microscope. After a further 10 days (14 May 2012), the number of remaining eggs and number of live nymphs hatched from the eggs were counted. On the same day, a second application of all six treatments was applied to three of the six potato plants per treatment, following removal of the cages from the plants. The cages were repositioned and the TPP nymphs were left to feed on the treated plants. After a further 10 days (24 May 2012), the numbers of live and dead nymphs and the number of live emerged adults were recorded. Nymphal stages were divided into Large, Medium and Small.

2.4.2 Trial Design

As mentioned above, each of the six treatments was applied either once (1) or twice (2), giving 12 treatment combinations in total. Three replicates of these treatments were used, each applied to a single potato plant in a pot (36 pots). The pots were laid out on benches in a controlled environment room at 22°C, app. 50% humidity, 16:8 light:dark photoperiod, using a Latinized resolvable row-column design (Figure 5), constructed with CycDesign (CycSoftware 2009).

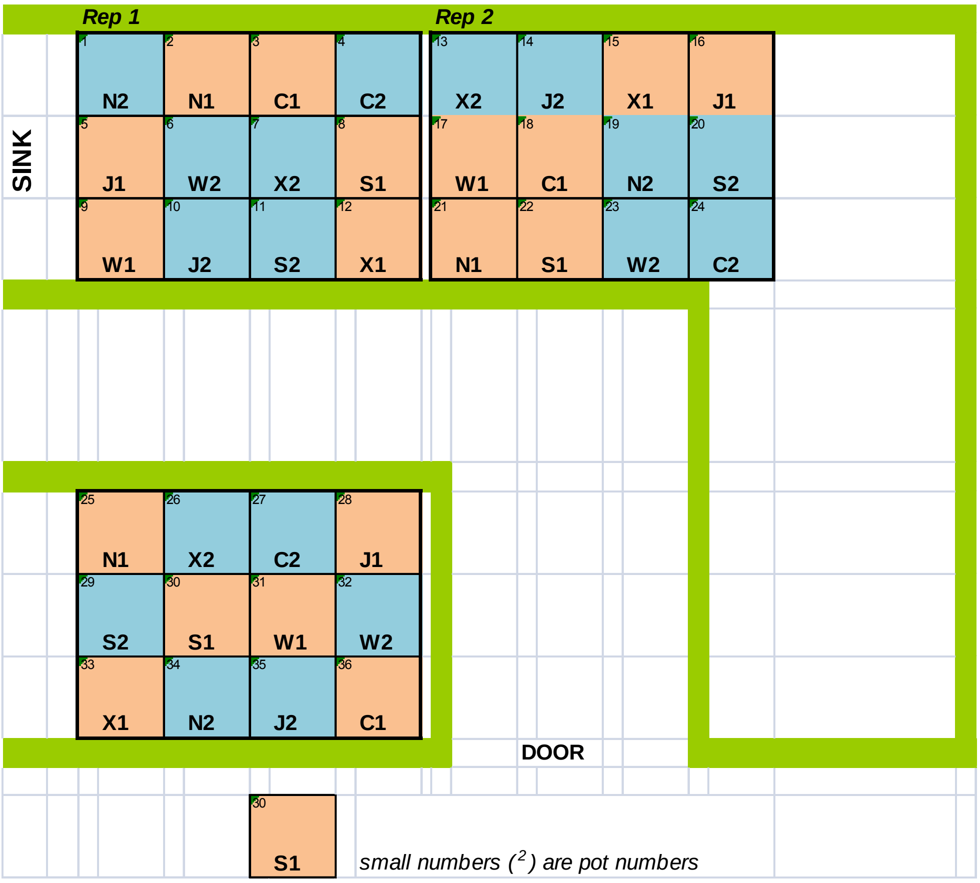

Figure 5: Layout of pots in the controlled environment room. Green lines indicate bench edges. Please refer to 2.4.1 for clarification of treatment codes. The number 1 or 2 following the code indicates number of soft chemical sprays.

2.4.3 Statistical analysis

Some initial analyses were done to assess whether there was any spatial patterning in the data. These were done using the hierarchical generalized linear model approach (Lee et al. 2006); however, since spatial effects, including those associated with replicates, were largely very small, such effects were ignored in the final analyses.

Counts were analysed using Poisson generalized linear model with a log. link (GLM, McCullagh & Nelder 1989). This included contrast to assess differences between oils (including water), number of sprays, and the interaction between these. These were assessed with F-tests done in the analysis of deviance carried out as part of the analysis. Estimated mean counts and 95% confidence limits associated with the means were obtained on the link (log) scale, and back-transformed.

For the analysis of eggs per female, the egg counts were also analysed with a Poisson GLM, with a modification. The log(number of females) was included as an 'offset' (parameter-less explanatory variable), and the estimated eggs/ female obtained by predicting for numbers of females= 1 (i.e. log(females)=0). The analysis was otherwise as above.

Numbers of remaining eggs for two cages on the second assessment date (24 May) had been clearly affected by female TPP that had not been removed on the first assessment date, so these were deleted before analysis and data summary

The percentages of nymphs in each size category were also analysed together to assess whether the proportions of nymphs by size varied between the treatments. This was done using the Poisson log-linear model for multinomial data (McCullagh & Nelder 1989). As for the analyses of counts, treatment contrasts were assessed using F-tests, and means and confidence limits obtained.

All analyses were carried out with GenStat (GenStat Committee 2011).

3 Results

3.1 Bioassay 1: TPP adult behaviour responses

3.1.1 Counts of TPP behaviour phases

The total number of phases (e.g. Cleaning, Off leaf, Jumping, Resting is 4 phases) (Table 2, Figure 6) varied between the treatments, with the highest number found in the water treatment (mean of 7.6 per TPP) followed by Neem (7.0). Sap Sucker Plus had significantly fewer phases (1.6; *P* = 0.016) than the water control.

Table 1: Mean number of each behaviour type per TPP for each Oil (95% Confidence limits)[†]

Oil	Phases	C	F	J	O	R	W
W	7.6 (4.6,12.5)	0.7 (0.4,1.5)	0.8 (0.4,1.4)	0.8 (0.3,2.0)	0.6 (0.3,1.2)	2.6 (1.4,4.6)	2.2 (1.1,4.4)
J	5.4 (3.0,9.8)	0.0 (0.0,*)	0.3 (0.1,0.8)	1.4 (0.7,2.9)	1.3 (0.8,2.2)	0.9 (0.3,2.4)	1.5 (0.6,3.5)
X	5.8 (3.3,10.3)	0.4 (0.2,1.1)	0.2 (0.1,0.8)	1.3 (0.6,2.8)	0.9 (0.5,1.7)	1.8 (0.9,3.6)	1.2 (0.4,3.1)
C	3.3 (1.5,7.0)	0.2 (0.0,0.7)	0.5 (0.2,1.1)	0.5 (0.2,1.7)	0.6 (0.3,1.2)	0.8 (0.3,2.2)	0.8 (0.2,2.5)
N	7.0 (4.2,11.8)	0.7 (0.3,1.4)	0.6 (0.3,1.2)	0.5 (0.2,1.7)	1.1 (0.6,1.9)	1.9 (1.0,3.8)	2.2 (1.1,4.4)
S	1.6 (0.5,4.7)	0.0 (0.0,*)	0.0 (0.0,*)	0.2 (0.0,1.3)	1.0 (0.6,1.8)	0.3 (0.0,1.6)	0.2 (0.0,2.1)

* upper Confidence Limit for 0 cannot easily be obtained. [†] E omitted. 0 for all but N which had 0.1 (0,0.6)

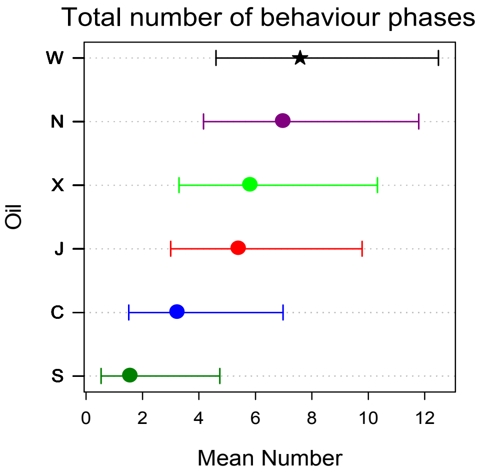

Figure 6: Mean number of behaviour phases per TPP, ordered by mean number. Error bars show 95% confidence limits for the means.

Of the individual behaviours (Table 2, Figure 7), the mean number per TPP varied between treatments for Cleaning (*P* < 0.001), Probing/Feeding (*P* = 0.009) and Resting (*P* = 0.018). However, numbers of phases off-leaf did not vary substantially between treatments (*P* = 0.334). No TPP Cleaned for either JMS Stylet Oil or Sap Sucker Plus, this was significantly less than with Water (*P* < 0.001). No feeding was done by TPP on Sap Sucker Plus, which was significantly lower than for Water (*P* < 0.001). Resting phases were highest with Water (2.6), significantly more than for either Sap Sucker Plus (0.25, *P* = 0.02) and Eco-Oil® (0.75; *P* = 0.049), and slightly higher than for JMS Stylet Oil (0.92; *P* = 0.07). The highest numbers of Jump phases were recorded for JMS Stylet Oil and Excel Oil® (mean 1.4, 1.3 respectively) and the lowest for Sap Sucker Plus (0.2). The number of Jump phases for Water was mid-range (0.75 per TPP).

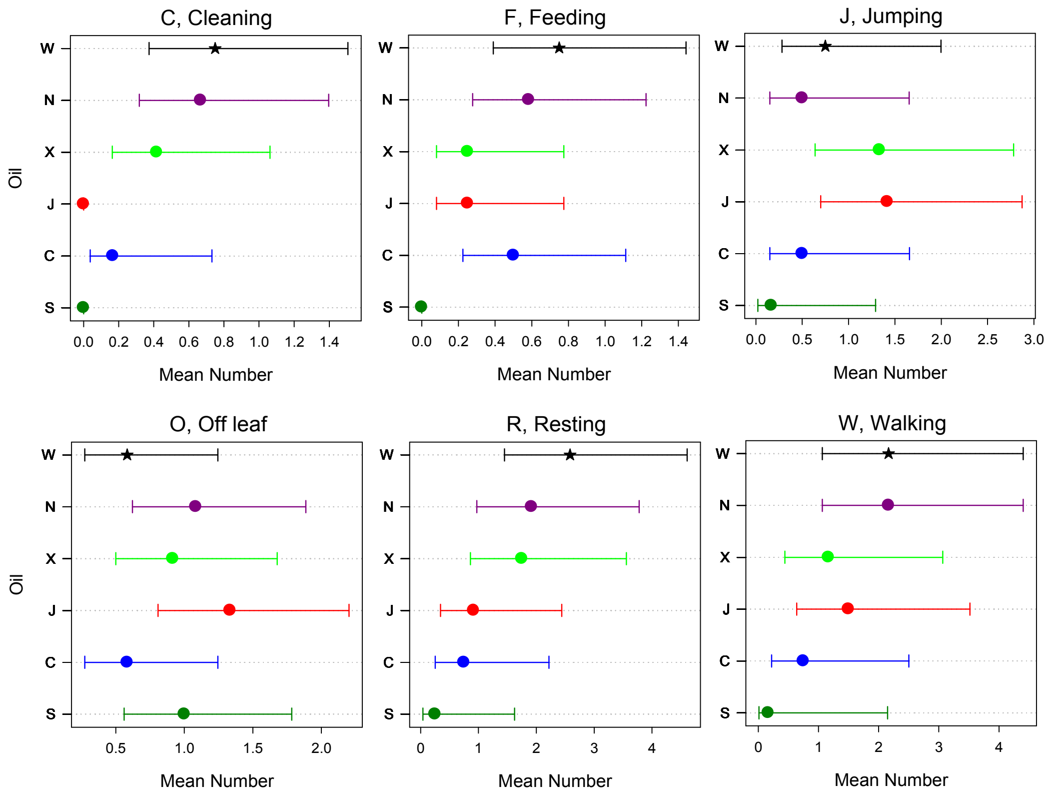

Figure 7: Mean number of each behaviour type per TPP, ordered by mean total number of phases. Error bars show 95% confidence limits for the means (omitted when number=0).

The initial behaviour for each TPP is summarised in Table 3. Of the 71 TPP analysed, 42 began the 15 min period off the leaf and 29 stayed off leaf for 15 min, 15 started resting and 2 rested for 15 min, 11 started probing/feeding and 7 probed/fed for 15 min and 3 started walking. No TPP started the experiment with cleaning, oviposition or jumping.

Table 3: Number of TPP initially performing each behaviour for each treatment (number that did not change from that behaviour).

Oil	F	O	R	W	Total;
W	4 (3)	3 (2)	4 (1)	1 (0)	12 (6)
J	1 (0)	9 (7)	1 (0)	1 (0)	12 (7)
X	1 (1)	8 (5)	3 (0)	0	12 (6)
C	2 (1)	6 (5)	3 (1)	1 (0)	12 (7)
N	3 (2)	5 (1)	4 (0)	0	12 (3)
S	0	11 (9)	0	0	11 (9)
Total	11 (7)	42 (29)	15 (2)	3 (0)	71 (38)

3.1.2 Percentage time spent on each behaviour

The pattern of percentage time spent on each behaviour varied substantially between the treatments ($P < 0.001$) (Table 4, Figures 8 & 9). The percentage of time spent off-leaf increased from around 35% for Water to 77% for JMS Stylet Oil and almost 90% for Sap Sucker Plus ($P < 0.001$ for the overall difference between treatments). This seems largely to be at the expense of the percentage time spent probing/feeding or cleaning ($P < 0.001$ and $P = 0.014$ respectively for overall difference between treatments).

Table 4: Percentage time spent doing each behaviour type for each oil.

Oil	Phase	%C	%F	%O	%R	%W
W	7.6 (4.6,12.5)	11.1 (4.4,25.1)	30.5 (18.3,46.2)	35.4 (22.3,51.2)	19.2 (9.8,34.3)	3.8 (0.8,16.7)
J	5.4 (3.0,9.8)	0.0 (0.0,1.7)	7.2 (2.3,20.5)	77.2 (61.8,87.7)	13.3 (5.8,27.6)	2.3 (0.3,15.7)
X	5.8 (3.3,10.3)	2.2 (0.3,15.7)	10.0 (3.8,23.8)	60.3 (44.7,74.2)	25.6 (14.5,41.0)	1.9 (0.2,15.8)
C	3.3 (1.5,7.0)	1.0 (0.1,18.2)	28.2 (16.4,43.9)	53.4 (38.1,68.1)	16.3 (7.7,31.0)	1.1 (0.1,17.6)
N	7.0 (4.2,11.8)	9.7 (3.6,23.5)	33.0 (20.3,48.8)	33.8 (21.0,49.6)	18.4 (9.2,33.4)	4.9 (1.2,17.8)
S	1.6 (0.5,4.7)	0.0 (0.0,1.7)	0.0 (0.0,1.7)	88.2 (74.1,95.1)	11.4 (4.6,25.4)	0.4 (0.0,33.9)

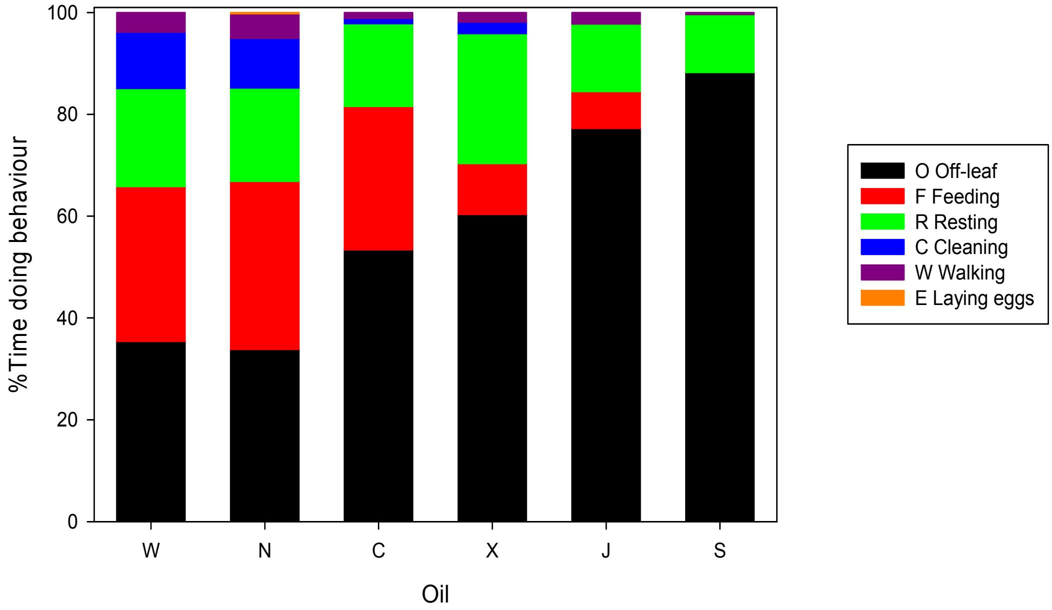

Figure 8: Percentage time spent on each activity for TPP on each oil. Note treatments other than water are ordered by the percentage time for Off-leaf, and activities are ordered by the percentage time over all oils for each activity.

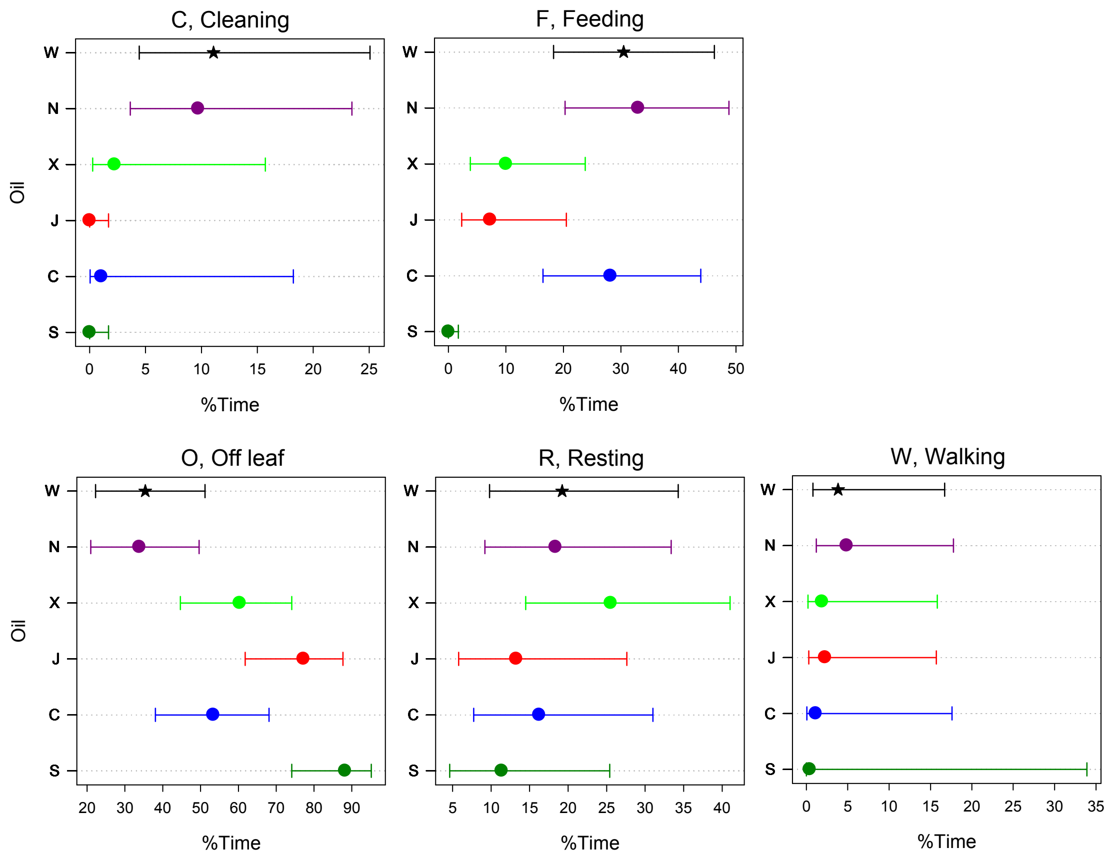

Figure 9: Percentage time spent doing each behaviour type for each oil. Error bars are 95% confidence limits.

3.2 Bioassay 2: TPP adult, egg and young-instar mortality and oviposition

3.2.1 First assessment: TPP adult mortality and oviposition

On the first assessment day (4 May 2012) (1 spray applied), neither total numbers of TPP, numbers of eggs or numbers of eggs laid per female varied significantly with soft chemical ($P > 0.28$) (Table 5,

Figure 0).

Table 5: 4th May, Mean total TPP and eggs per cage and eggs per female (95% confidence limits).

	Total TPP*		No Eggs		Eggs/Female	
Oil	1	2**	1	2**	1	2**
W	28.7 (22.9,35.8)	28.3 (22.7,35.4)	27.7 (11.7,65.5)	24.7 (9.9,61.4)	1.9 (0.7,4.7)	1.7 (0.6,4.5)
J	25.0 (19.7,31.7)	21.0 (16.2,27.2)	9.0 (2.0,40.8)	19.3 (6.9,54.1)	0.9 (0.2,3.4)	2.3 (1.0,5.3)
X	28.0 (22.4,35.1)	28.0 (22.4,35.1)	11.3 (3.0,43.5)	20.7 (7.6,55.9)	0.8 (0.2,3.3)	1.4 (0.5,4.1)
C	24.0 (18.8,30.6)	26.7 (21.2,33.6)	2.3 (0.1,45.3)	21.0 (7.8,56.4)	0.2 (0.0,3.7)	1.5 (0.5,4.2)
N	25.3 (20.0,32.1)	26.0 (20.6,32.8)	6.7 (1.2,37.3)	5.0 (0.7,37.9)	0.4 (0.1,2.9)	0.5 (0.1,3.0)
S	29.3 (23.5,36.6)	24.7 (19.4,31.4)	20.7 (7.6,56.0)	7.7 (1.5,39.3)	1.4 (0.5,4.1)	0.5 (0.1,3.0)

*Note: Mean numbers of adult TPP per treatment does not add up to 30, as not all released adults were refound on the assessment day.

**Note: Only 1 spray applied for all treatments at this point in time. Plants assigned to this column are the plants that were to receive a second spray on 24 May.

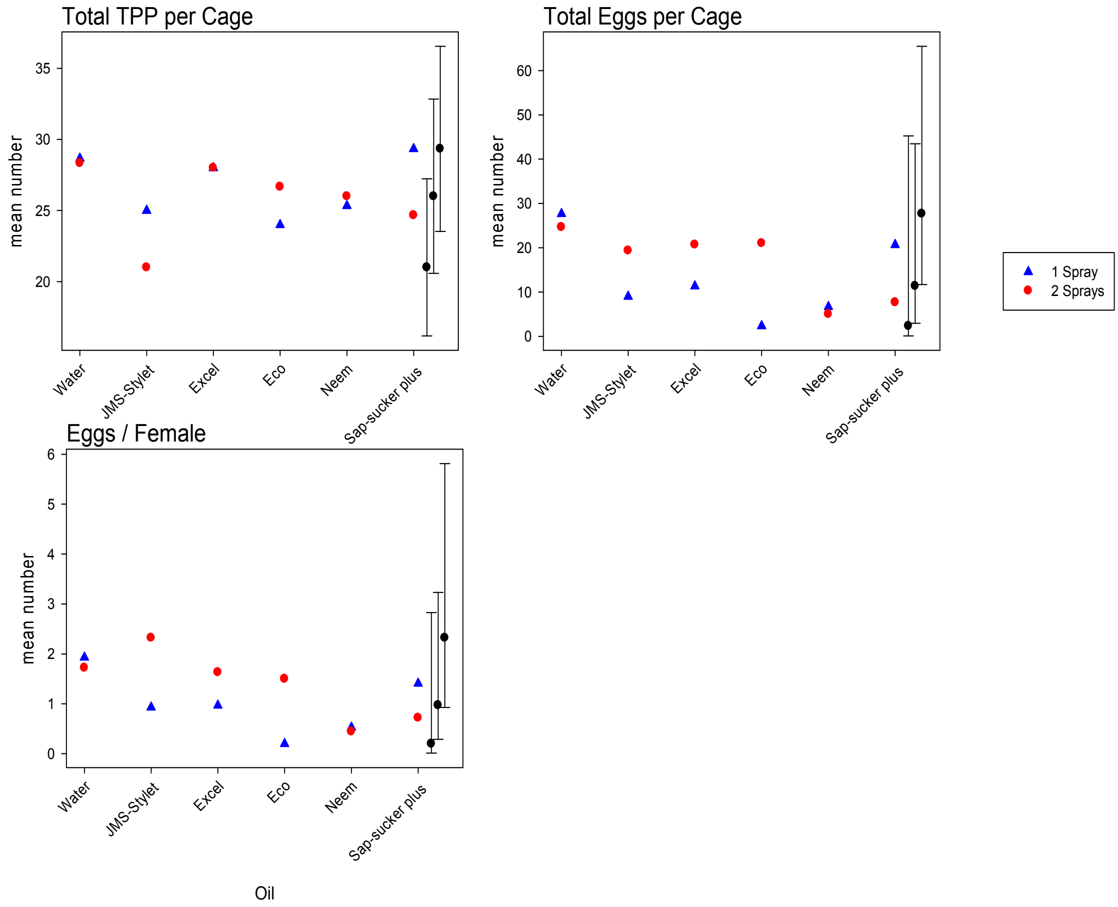

Figure 10: 4th May, Mean total TPP and eggs per cage and eggs per female. Error bars show 95% confidence limits for the highest, a mid-range, and a small mean.

Note: Only 1 spray applied for all treatments at this point in time. “2 sprays” indicates the plants that were to receive a second spray on 24 May.

3.2.2 Second assessment: TPP egg and nymph mortality

On the second assessment day (14 May) (1 spray applied), there was no substantial variation between the treatments in the number of eggs remaining ($P > 0.28$ for all effects). Variation in the number of nymphs on each plant ranged from 0 to 50 per cage and varied strongly with soft chemical ($P = 0.003$ for the main effect). Nymph numbers were highest for water (mean = 36.5/cage) and lowest for JMS Stylet (mean = 2.0/cage; $P = 0.006$). Numbers for the other soft chemicals were also lower than for water, varying from 4.3/ cage for Neem 600 WP ($P = 0.004$) to 17.8 for Eco-Oil® ($P = 0.09$) (Table 6, Figure 11).

Table 6: 14th May, Mean eggs remaining, and live nymphs per cage (95% confidence limits).

	Eggs Remaining		Nymphs	
Oil	1	2**	1	2**
W	1.9 (0.9,4.2)	1.7 (0.8,3.9)	41.0 (21.0,79.9)	32.0 (15.0,68.1)
J	0.9 (0.2,3.6)	2.3 (0.9,5.8)	4.0 (0.5,33.9)	0.0 (0.0,*)
X	1.0 (0.3,3.2)	1.6 (0.7,4.0)	9.0 (2.2,37.4)	14.0 (4.5,43.9)
C	0.2 (0.0,2.8)	1.5 (0.6,3.6)	16.7 (5.8,47.5)	19.0 (7.1,50.7)
N	0.5 (0.1,2.5)	0.4 (0.1,2.7)	5.0 (0.7,33.8)	3.7 (0.4,34.2)
S	1.4 (0.6,3.4)	0.7 (0.2,3.1)	17.3 (6.2,48.4)	1.3 (0.0,54.1)

*Cannot be estimated

**Note: Only 1 spray applied for all treatments at this point in time. Plants assigned to this column are the plants that were to receive a second spray on 24 May.

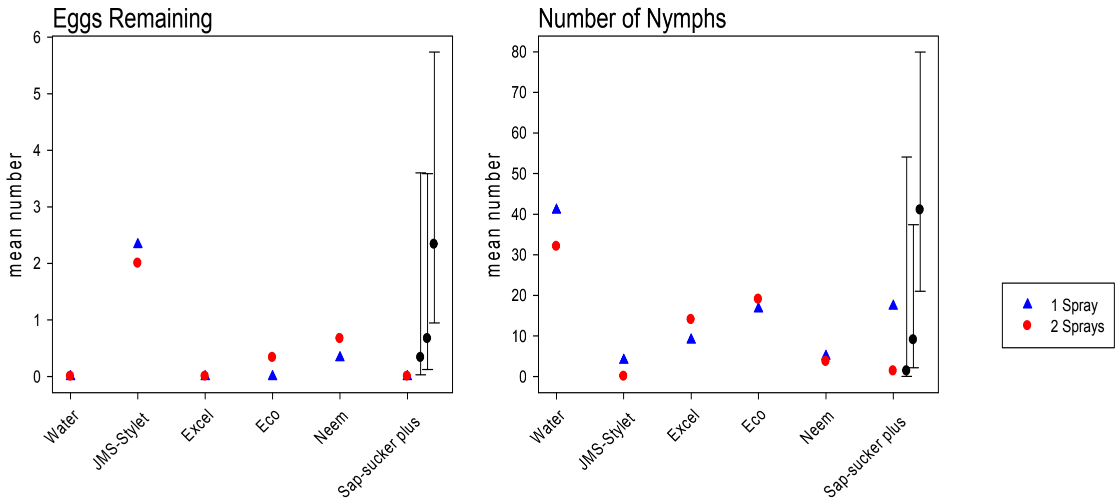

Figure 11: 14th May, Mean eggs remaining, and live nymphs per cage. Error bars show 95% confidence limits for the highest, a mid-range, and a small mean.

Note: Only 1 spray applied for all treatments at this point in time. “2 sprays” indicates the plants that were to receive a second spray on 24 May.

3.2.3 Third assessment: TPP nymph mortality, nymph size and adult emergence

On the third assessment day (24 May) (2 sprays applied to half of the plants), numbers of live nymphs varied with soft chemical (P = 0.008 for the main effect) and number of sprays (P = 0.004). However, the difference between 1 and 2 sprays was relatively similar for all soft chemicals (P = 0.580). This non-significant interaction means it is not entirely legitimate to make the comparisons between 1 and 2 sprays, and therefore further analyses used averages for 1 and 2 sprays combined. On average, numbers of nymphs were reduced by the second spray to only ¼ of those found with 1 spray (Table 7, Figure 12). However, the reduction in total nymphs compared with water was only significant for Organic JMS Stylet Oil® and Excel Oil® (P < 0.001; P = 0.035). Numbers of emerged adults varied between all treatments (P < 0.001), but was relatively unaffected by the number of sprays (P = 0.517 for the main effect). Adult numbers were lower for all soft chemicals than for water (P < 0.001 for all) (Table 7, Figure 12).

Table 7: 24th May, Mean total Nymphs and Adults emerged per cage (95% confidence limits).

	Nymphs Alive		Adults Emerged	
Oil	1	2	1	2
W	19.3 (8.3,44.9)	10.3 (3.3,32.7)	16.0 (10.0,25.7)	14.3 (8.7,23.6)
J	0.0 (0.0,*)	0.0 (0.0,*)	0.0 (0.0,*)	0.0 (0.0,*)
X	2.7 (0.3,25.8)	0.3 (0.0,203.8)	0.0 (0.0,*)	0.0 (0.0,*)
C	23.7 (11.1,50.7)	1.3 (0.1,33.0)	0.7 (0.1,6.8)	2.0 (0.5,7.6)
N	15.3 (6.0,39.5)	5.3 (1.1,26.5)	5.3 (2.3,12.1)	2.7 (0.8,8.5)
S	8.7 (2.5,30.5)	0.0 (0.0,*)	2.0 (0.5,7.6)	1.0 (0.2,6.6)

* Cannot be estimated

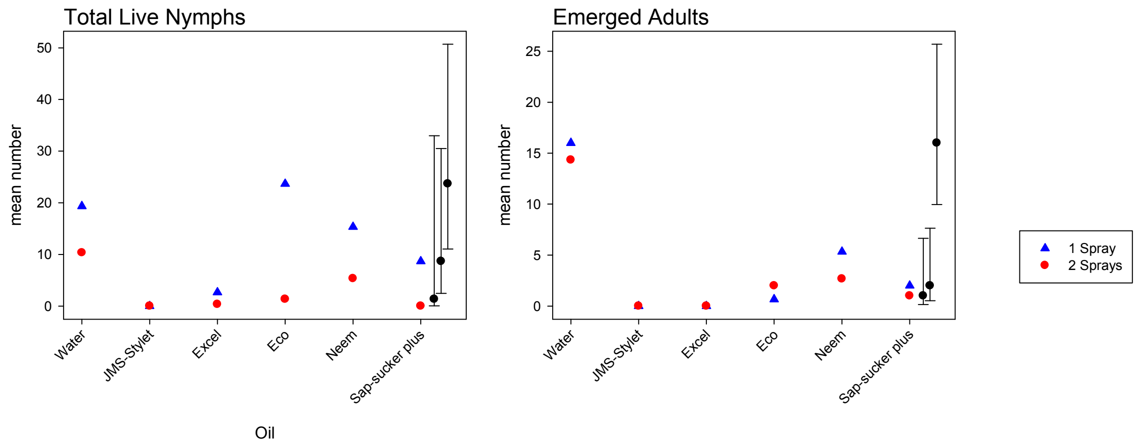

Figure 12: 24th May, Mean total Nymphs and Adults emerged per cage. Error bars show 95% confidence limits for the highest, a mid-range, and a small mean.

Numbers of large, medium and small nymphs varied between soft chemicals (*P* = 0.004, 0.028, 0.008 respectively) and with the number of sprays (*P* = 0.014, 0.001, 0.007 respectively). Numbers of large nymphs were reduced by more than half with the application of the second spray, and numbers of small nymphs by almost 90%. Large nymphs were most numerous with water for both 1 and 2 sprays followed by Neem 600 WP and Eco-Oil®. No large nymphs were found with Organic JMS Stylet Oil®. Mean small nymphs were below 1 per cage for all soft chemicals except Eco-Oil® and Neem 600 WP at 1 spray only. No small nymphs were found with JMS Stylet. For medium nymphs, the spray effect varied noticeably between the soft chemicals (*P* = 0.046 for the soft chemical by Number of sprays interaction). This is primarily because for almost all soft chemicals, numbers were higher with one spray than with two, but for Neem 600 WP, the numbers were slightly higher with two sprays (2.3 v. 1 per cage). The largest numbers of medium nymphs were for Eco-Oil®, one spray (6.7), followed by Sap Sucker Plus, one spray (3.0). Once again, no medium nymphs were found with JMS Stylet Oil® (Tables 8 & 9; Figures 13 & 14).

Table 8: 24th May, Mean number of large, medium and small nymphs per cage (95% confidence limits).

	Large		Medium		Small	
Oil	1	2	1	2	1	2
W	17.0 (7.8,37.2)	9.7 (3.4,27.3)	2.3 (0.8,7.0)	0.3 (0.0,6.0)	0.0 (0.0,*)	0.3 (0.0,12.2)
J	0.0 (0.0,*)	0.0 (0.0,*)	0.0 (0.0,*)	0.0 (0.0,*)	0.0 (0.0,*)	0.0 (0.0,*)
X	0.7 (0.0,34.9)	0.0 (0.0,*)	1.7 (0.5,6.1)	0.0 (0.0,*)	0.3 (0.0,12.2)	0.3 (0.0,12.2)
C	9.0 (3.1,26.4)	1.0 (0.0,25.3)	6.7 (3.5,12.7)	0.0 (0.0,*)	8.0 (3.8,16.7)	0.3 (0.0,12.2)
N	12.0 (4.7,30.5)	2.7 (0.4,19.3)	1.0 (0.2,5.3)	2.3 (0.8,7.0)	2.3 (0.6,9.1)	0.3 (0.0,12.2)
S	5.3 (1.3,21.6)	0.0 (0.0,*)	3.0 (1.1,7.9)	0.0 (0.0,*)	0.3 (0.0,12.2)	0.0 (0.0,*)

* Cannot be estimated.

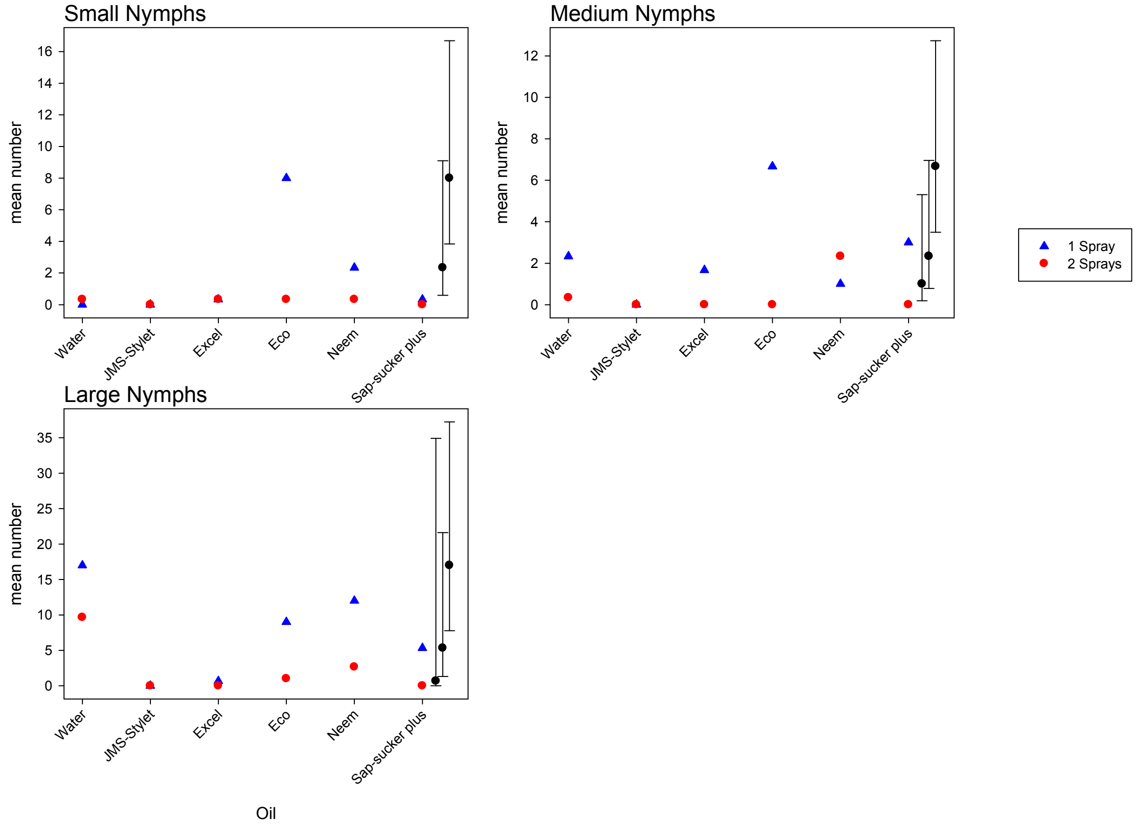

Figure 13: 24th May, Mean number of large, medium and small nymphs per cage. Error bars show 95% confidence limits for the highest, a mid-range, and a small mean.

Table 9: 24th May, Mean percentage of large, medium and small nymphs per cage (95% confidence limits).

	Large		Medium		Small	
Oil	1	2	1	2	1	2
W	87.9 (73.8,95.0)	93.5 (72.1,98.8)	12.1 (5.0,26.2)	3.2 (0.3,26.8)	0.0 (0.0,*)	3.2 (0.3,26.8)
J	-	-	-	-	- -	
X	25.0 (4.6,69.6)	0.1 (0.0,*)	62.5 (22.9,90.3)	0.0 (0.0,*)	12.5 (1.1,64.0)	100 (*,100.0)
C	38.0 (25.6,52.2)	75.0 (16.4,97.9)	28.2 (17.4,42.2)	0.0 (0.0,*)	33.8 (22.0,48.0)	25.0 (2.1,83.6)
N	78.3 (60.8,89.3)	50.0 (23.5,76.5)	6.5 (1.7,22.2)	43.8 (19.1,71.9)	15.2 (6.4,32.1)	6.2 (0.6,43.3)
S	61.5 (38.2,80.6)	-	34.6 (16.7,58.3)	-	3.8 (0.4,30.7)	-

* technically hard to estimate; - No Nymphs present.

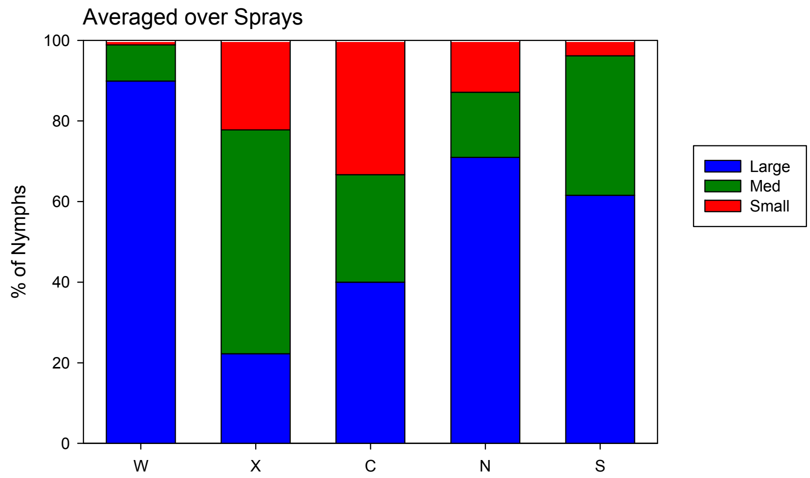

Figure 14: Percentage of nymphs that were of each size, averaged over sprays. Note: there were no nymphs for J, and no nymphs for S with 2 Sprays.

4 Discussion & conclusions

Based on the 15 min behaviour bioassay, Sap Sucker Plus and JMS Stylet Oil® produced the strongest repellent effect on adult female TPP, followed by Excel Oil® and Eco Oil®, with 88%, 77%, 60% and 53% of the time spent off the leaf respectively. However, on Eco-Oil® a relatively large amount of the time was spent feeding/probing (28%) compared with Sap Sucker Plus (0%), JMS Stylet Oil® (7%) and Excel Oil® (10%). An initial repellent and probing/feeding deterrent effect is important when considering the potential risk of adult TPP settling in a potato crop and transmitting disease. Buchman et al. (2011) reported that a single adult TPP could transmit *Ca*. L. solanacearum to a potato plant in as a little as 6 h, resulting in the development of zebra chip symptoms. Neem 600 WP did not induce a difference in behaviour when compared with water. Oviposition was even observed on one occasion on this product. This result differs from that found by Walker et al (2011) who showed significant repellence to female adult TPP after 1 h. However, a different Neem formulation was used in these trials.

In the TPP mortality study, there was no obvious adult mortality or oviposition-deterrent effect for any of the soft chemicals over a 3-day period after the spray application date. However, 13 days after the first spray (14 May); nymph numbers were lower for all soft chemicals when compared with water. The lowest number of nymphs was found on Organic JMS Stylet Oil® followed by Neem 600 WP, Sap Sucker Plus, Excel Oil®, and Eco Oil®, respectively. Results were not significant for Eco-Oil®. The reduction in nymph numbers could suggest that the products have some residual effect on egg hatching rates and/or young instar mortality. Organic JMS Stylet Oil® and Neem 600 WP showed good potential for early control of TPP.

Twenty-three days after the first spray (24 May), Eco Oil® and Neem 600 WP seemed to have lost their effect, as these treatments had a larger number of nymphs compared with the other soft chemicals. However, the number of emerged adults was only slightly higher than for the other soft chemicals. Ten days after the second spray (24 May), nymph numbers were lower for all soft chemicals compared with water. However, the reduction in total nymphs compared with water was only significant for Organic JMS Stylet Oi® and Excel Oil®. Walker et al. (2010) reported 48% TPP nymphal mortality with Excel Oil® in a potted plant bioassay on capsicum and even higher mortality for Eco-Oil® (58%). For all soft chemicals, significantly fewer adults had emerged compared with the water control for both 1 and 2 sprays. Numbers of large nymphs were reduced by more than half with the application of the second spray, and numbers of small nymphs by almost 90%. This is not surprising as many of the soft chemicals kill by direct contact. No nymphs or emerging adults were found on Organic JMS Stylet Oil® for either 1 or 2 sprays. John Trumble (University of California, Riverside, USA) reported very good repellence of up to 4 weeks with Organic JMS Stylet Oil® (Brian Smith, pers. comm.).

Although soft chemicals have the potential to control TPP through repellency and mortality, there is also a need to consider potential adverse effects of using these products in the cropping system. One potential risk with soft chemicals is phytotoxicity. In this experiment we noticed some phytotoxicity to leaves sprayed with Neem 600 WP, Excel Oil® and Eco-Oil®. The phytotoxicity appeared most severe on leaves sprayed with Neem 600 WP. Phytotoxicity from other Neem products, Excel Oil® and Eco-Oil® has also been reported in the industry (Stuart Attwood, pers. comm.). The use of soft chemicals also creates the potential risk of adversely affecting beneficial insects in the cropping system. Soft chemical oils could be defined as broad spectrum insecticides, as they are not systemic in the plant, but work via direct contact, e.g. by inhibiting insect respiration (Berry & Bourhill, 2012). The detrimental effects of soft chemicals (or organic pesticides) have been reported before (Johnson & Krugner 2004; Bahlai et al. 2010).

5 Overall recommendations

Given the efficacy of JMS Stylet Oil® and Excel Oil® at reducing TPP numbers and their probing/feeding deterrent qualities, these two products could warrant further testing in field trials. Sap Sucker Plus was also notable for its high repellence effect in the 15 min behavioural studies of adult female TPP, but left noticeable residue on the leaves. The impact of the tested soft chemicals on beneficial insects should also be assessed in future trials.

6 Acknowledgements

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