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Evaluation of selected essential oils as potential repellents for tomato/potato psyllid control

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June 2011

A report prepared for:

Horticulture New Zealand

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Plant & Food Research, Lincoln

SPTS No. 5617

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Executive summary

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Before the arrival of the tomato/potato psyllid (*Bactericera cockerelli* (Sulc), Hemiptera, Triozidae) (TPP), solanaceous crops in New Zealand greenhouses used Integrated Pest Management (IPM) practices based on biological control and pollination requirements. However, the advent of the TPP has increased the use of broad spectrum insecticides in greenhouses. The incorporation of IPM compatible control options such as soft chemical products into pest control strategies can enhance resistance management, reduce residue withholding periods and reduce toxicity to the environment and non-target species. Soft chemicals such as essential oils have previously been considered as alternative control measures for a number of insect pests. Studies have demonstrated that these oils can reduce insect pest populations via a number of mechanisms such as: their deterrent effect on oviposition, their capacity to delay development and their repellent and antifeeding effects.

The repellency and oviposition deterrent effects of 10 essential oils (Cedarwood. Lemongrass, Cinnamon, Wintergreen, Rosemary, Peppermint, Clove, Patchouli, D-limolene and Neem), to female TPP adults were tested on capsicum leaves in a no-choice laboratory bioassay. One hour after treatment, Neem and Cedarwood had significantly repelled female TPP adults compared with the untreated controls. Twenty-four hours after treatment, Peppermint, Clove, Patchouli, Wintergreen and Neem showed some repellency to female TPP adults compared with the untreated controls (but this was not significant). By 48 h, Neem and Patchouli had shown significant repellency to female TPP adults and all oils except Peppermint, Clove and Lemongrass had significantly deterred oviposition by female TPP adults as compared with the untreated controls. Further tests of these oils, including their antifeedant properties, are required to determine their effectiveness in reducing crop damage caused by TPP/*Ca*. L. solanacearum and their suitability for incorporation into an IPM programme.

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1 Introduction

The tomato/potato psyllid (*Bactericera cockerelli* (Sulc), Hemiptera, Triozidae) (TPP), first recorded in New Zealand in 2006, is a significant pest of solanaceous crops in both greenhouses and fields. The arrival of TPP in New Zealand and the recent identification of its role as a vector of the bacterial pathogen *Candidatus* Liberibacter solanacearum (Munyaneza et al. 2007) has presented a considerable challenge to the New Zealand greenhouse vegetable, tomato and potato industries. Plant damage symptoms associated with TPP are believed to cause 'psyllid yellows' (Teulon et al. 2009), and the bacterial pathogen *Ca*. L. solanacearum (vectored by TPP) has been identified as the cause of the disease 'zebra chip' in potato tubers (Liefting et al. 2008).

Before the arrival of TPP, New Zealand greenhouses used Integrated Pest Management (IPM) practices based on biological control and pollination requirements (Martin 2008; Zonda Resources Ltd 2009). However, the arrival and impact of TPP/*Ca*. L. solanacearum and an associated increase in the use of insecticides have threatened previously established IPM practices, including resistance management. The increased use of insecticides is also financially and environmentally unsustainable. Given the impact of TPP/*Ca*. L. solanacearum on IPM practices in these crops, IPM-compatible control options are preferred.

'Soft' pest control products are considered ecologically sustainable with low toxicity towards humans (workers), other mammals and non-target species, have no or very short withholding periods, and could be incorporated into IPM programmes (Smith 2009). Soft chemical options including horticultural soaps (e.g., PS1 and PS2), spraying oils (e.g., minerals oils and plant-based oils), botanical insect growth regulators (e.g., Neem products), Agri-50NF, Sucrose octanoate, kaolin (aluminium silicate), AkseBio2 and organosilicone surfactants have been used to control numerous species of insect pests (Ascher 1993; Cating et al. 2010; Martin 2001; Schmutterer 1990; Shaw et al. 2000; Srinivasan et al. 2008). In New Zealand to date, four soft chemicals have been evaluated as potential control options for TPP (Walker et al. 2010).

Insecticidal oils, including those of botanical or mineral origin, are a favourable pest control option for management of numerous pest insects, especially soft-bodied insects (Yang et al. 2010). In addition to controlling pest insects through direct mortality, insecticidal oils can also act as insect repellents (Butler et al. 1989, 1993; Larew & Locke 1990; Liang & Liu 2002; Schmutterer 1990; Stansly & Liu 1994; Weathersbee & McKenzie 2005; Yang et al. 2010; Zaka et al. 2010). Repelling TPP adults from landing and settling on the leaves of host plants, and deterring them from feeding and oviposition could help eliminate or reduce the transmission of the bacterial pathogen *Ca*. L. solanacearum (Yang et al. 2010).

The aim of this study was to determine the repellency and deterrent effects of 10 essential oils on settling behaviour and oviposition of female TPP on capsicum leaves, in a no-choice laboratory bioassay.

2 Methods

2.1 Insect source and rearing

TPP adult females used for testing were obtained from a laboratory colony at Plant & Food Research, Lincoln, Canterbury. This colony was originally established from adult TPP collected from greenhouse tomatoes in Auckland. TPP were reared on tomato plants ('Moneymaker') in a controlled temperature growth room at 25°C, 60% humidity, 16:8 light:dark photoperiod.

2.2 Trial design

2.2.1 Plant material and application of essential oils

Potted capsicum plants ('Giant Bell') were grown from seed in a controlled temperature growth room at 25°C, 60% humidity, 16:8 light:dark photoperiod (Figure 1). New leaves of capsicum, with petioles attached, were taken from the potted plants and dipped in one of 10 essential oils (Figure 2). For the control treatment the leaves were dipped in a surfactant mix. An untreated control was also used. The treated and control leaves were dipped for a period of 10 seconds and then inverted on to a drain tray to simulate spray run-off (Figure 3). All leaves (including untreated) were then left in the controlled temperature growth room (25°C, 60% humidity, 16:8 light:dark photoperiod) to dry for a period of 20 minutes. Four replicates of each treatment, control and untreated leaf were tested.

Figure 1. Potted capsicum plants grown from seed in the controlled temperature growth room.

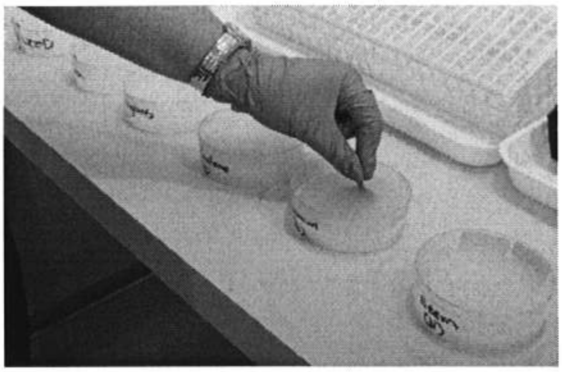

Figure 2. Capsicum leaves were dipped in one of 10 essential oils or surfactant mix (control) for a period of 10 seconds.

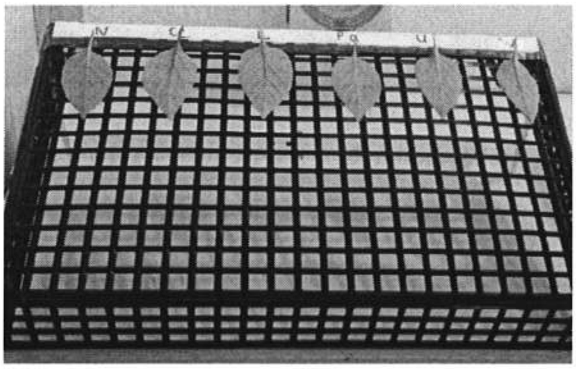

Figure 3. After dipping, capsicum leaves were inverted onto a drain tray to simulate spray run-off, and were then left to dry for a period of 20 minutes.

2.2.2 Essential Oil treatments

Ten essential oils were selected for testing and formulated by John Thompson Bioforce Ltd. Each formulation consisted of an oil and surfactant mix. The following is a list of the 10 oils that were selected for testing:

1. Cedarwood
2. Lemongrass
3. Cinnamon
4. Wintergreen
5. Rosemary
6. Peppermint
7. Clove
8. Patchouli
9. D-limolene
10. Neem

2.2.3 Essential Oil application rates

In greenhouse vegetable crops, oils are commonly applied at a rate of 250-500 mL/100 L. Application rates higher than this have been found to cause plant phytotoxicity. All essential oils and the surfactant mix (control) were applied at a rate of 250 ml/100 L (advised by John Thompson Bioforce Ltd).

2.2.4 Psyllid introduction

After the treated capsicum leaves had dried, each leaf (treated, control and untreated) was inserted into a round block of moistened Sunshine™ Floral Foam and placed into a 120 mL (92 mm high x 46 mm wide) vented plastic screw-top container, each containing 10 gravid female psyllids (Figure 4). Containers were then laid out on a bench within the controlled temperature growth room (25°C, 60% humidity, 16:8 light:dark photoperiod) in a 4 x 12 array, using a layout determined by a randomized Latinized row-column design constructed with CycDesign (CycSoftware 2009) (Figures 5 and 6). Psyllids were then left to settle on the leaf or inner surfaces of each container for a period of 1 h.

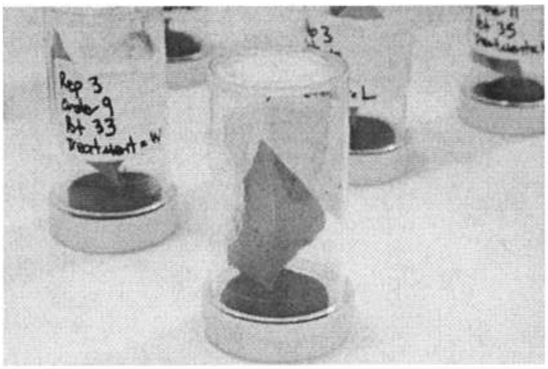

Figure 4. Each capsicum leaf was inserted into a round block of moistened Sunshine™ Floral Foam and placed into a 120 ml vented plastic screw-top container, each containing 10 gravid female psyllids.

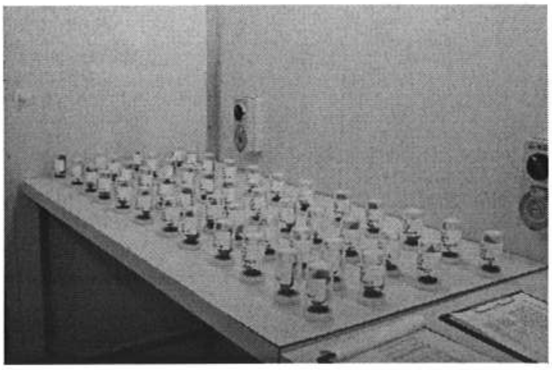

Figure 5. Containers were laid out on a bench within the controlled temperature growth room in a 4 x 12 array.

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
Replicate 1	1 N	2 Ce	3 L	4 Pa	5 U	6 W	7 Cl	8 S	9 D	10 Pe	11 Ci	12 R
2	13 S	14 Pe	15 W	16 Ci	17 D	18 L	19 R	20 N	21 U	22 Ce	23 Pa	24 Cl
3	25 D	26 R	27 Pa	28 Pe	29 Cl	30 N	31 Ci	32 Ce	33 W	34 L	35 U	36 S
4	37 Ci	38 U	39 Cl	40 S	41 Ce	42 R	43 L	44 Pa	45 Pe	46 D	47 N	48 W

18 L — Plastic container, with capsicum leaf and 10 gravid female psyllids

Treatments

Ce	Cedarwood	**Pe**	Peppermint	**S**	Surfactant mix only
L	Lemongrass	**Cl**	Clove	**U**	Untreated
Ci	Cinnamon	**Pa**	Patchouli		
W	Wintergreen	**D**	D-limolene		
R	Rosemary	**N**	Neem		

Figure 6. Layout of containers in controlled temperature growth room: 4 x 12 array using a Latinized row-column design.

2.2.5 Psyllid behavioural responses and mortality

The number of live psyllids that settled on each leaf or the inner surfaces of each container was recorded after 1, 24 and 48 h. After 48 h, psyllids were removed from each container and the total number of eggs deposited on each leaf was counted under a stereo microscope. The number of dead psyllids per container was also recorded after 1, 24 and 48 h.

2.3 Statistical Analysis

Some initial analyses (details not presented) were carried out to assess whether there were important trends in the data relating to the position of containers on the bench. As none were found, the analyses did not include any adjustments for spatial position.

For each assessment, the number of dead psyllids as a percentage of the total was analysed using a binomial generalized linear model with a logit link (McCullagh & Nelder 1989), with dispersion estimated. The analyses included an overall F-test for differences between the treatments (done within an analysis of deviance). Contrasts comparing each treatment with the untreated control and the surfactant only treatment were also assessed.

The numbers of eggs were analysed similarly, with a Poisson generalized linear model (McCullagh & Nelder 1989) with a log link. This analysis included log (Initial number of psyllids) as an offset (McCullagh & Nelder 1989), essentially to give an analysis of final egg numbers per initial psyllid applied.

The number of psyllids on the leaf as a percentage of all live psyllids (number on leaf + number on container) was analysed for each assessment, using a binomial generalized linear model, as for the percentage dead above. Note that an analysis of the number on the leaf as a percentage of the total psyllids (including dead) would produce different results.

In the results, the mean percentage or number of eggs/ psyllid are presented along with associated 95% confidence intervals. These were obtained on the transformed (logit or log scale) and back-transformed to percentages or counts. For the percentages, where the mean was 0%, the upper confidence limit is not available directly from the analysis, so was estimated using modified version of Blyth's formula (Blyth 1986) $100 \times \left(1 - 0.025^{\frac{\sqrt{d}}{n}}\right)$, where *d* is the estimated dispersion and *n* the total number of psyllids applied initially for a treatment. Similarly, the lower confidence limit where the percentage was 100 was estimated as $100 \times \left(0.025^{\frac{\sqrt{d}}{n}}\right)$.

3 Results

3.1 Psyllid settling

The percentage of live female TPP adults that settled on their leaves 1, 24 and 48 h after treatment is shown in Table 1. The percentage of psyllids on the leaf varied increasingly between the treatments from the 1 h to the 48 h assessment (p=0.320, 0.132, 0.026 for the 1h, 24h and 48 h assessments for overall differences between treatments). At 1 h, the percentage of psyllids on leaves was lower than the untreated control for all treatments except Cinnamon. However, this difference was significant only for Neem and Cedarwood (p=0.015 and p=0.037, with p>0.05 for the other treatments). No treatment had a significantly lower percentage of psyllids on the leaf than the surfactant mix (control) at this time (p>0.05).

By 24 h, the percentage of psyllids on leaves had increased for all treatments, with the untreated control falling mid-range at 87%. However, the lowest percentage was found for the surfactant mix (control), with 67% on the leaf. Peppermint, Clove, Patchouli, Wintergreen and Neem had lower percentages of settled psyllids compared with the untreated controls (but these differences were not significant).

By 48 h, the percentage of psyllids on leaves was above 90% for all but Neem, Patchouli, Cedarwood and Peppermint. Percentages for Neem and Patchouli were significantly lower than the untreated control (p=0.032 and p=0.036), but none varied significantly from the surfactant mix (control).

Table 1. Percentage of live female TPP adults settled on the leaf 1, 24 and 48 h after treatment (95% confidence limits in parentheses).

Treatment	1 h	24 h	48 h
Cedarwood	40.0 (19.8,64.2)	89.5 (69.6,96.9)	83.8 (67.0,92.9)
Lemongrass	52.6 (29.1,75.1)	94.4 (73.8,99.0)	90.3 (72.4,97.1)
Cinnamon	75.0 (49.5,90.2)	92.5 (73.6,98.2)	100.0 (90.9,100.0)
Wintergreen	71.1 (45.0,88.0)	75.8 (53.3,89.5)	92.3 (72.0,98.2)
Rosemary	52.6 (29.1,75.1)	93.3 (69.6,98.8)	96.2 (74.8,99.5)
Peppermint	55.0 (31.6,76.4)	81.1 (60.2,92.4)	84.4 (66.1,93.7)
Clove	64.1 (39.1,83.3)	81.1 (60.2,92.4)	100.0 (90.7,100.0)
Patchouli	65.0 (40.2,83.7)	81.1 (60.2,92.4)	77.4 (58.3,89.4)
D-limolene	51.3 (28.3,73.8)	93.9 (71.8,99.0)	93.5 (75.9,98.5)
Neem	31.6 (13.7,57.4)	68.6 (46.9,84.3)	77.3 (54.0,90.8)
Surfactant mix	54.1 (30.0,76.4)	66.7 (45.4,82.8)	92.0 (71.1,98.2)
Untreated	71.8 (46.1,88.4)	87.2 (67.5,95.7)	96.8 (78.2,99.6)

3.2 Psyllid oviposition

The estimated number of eggs laid per female TPP adult 48 h after treatment is shown in Table 2. Numbers of eggs laid per psyllid varied between the treatments (p=0.013 for overall treatment differences). The number of eggs laid per psyllid was greatest in the untreated control, significantly so when compared to all but the Peppermint, Clove and Lemongrass treatments (p varying from <0.001 to 0.029). When compared to the surfactant mix (control), only the

Lemongrass treatment had significantly higher numbers of eggs (p=0.043). Only Neem had fewer eggs than the surfactant-only treatment.

Table 2. Estimated numbers of eggs laid per female TPP adult 48 h after treatment (95% confidence limits in parentheses).

Treatment	Number of eggs/psyllid
Cedarwood	8.1 (4.8,13.8)
Lemongrass	12.0 (7.6,18.8)
Cinnamon	9.4 (5.8,15.5)
Wintergreen	7.2 (4.1,12.9)
Rosemary	7.2 (4.0,12.8)
Peppermint	10.0 (5.8,17.5)
Clove	10.6 (6.6,17.0)
Patchouli	6.7 (3.7,12.0)
D-limolene	7.3 (4.2,12.9)
Neem	2.2 (0.8,6.3)
Surfactant mix	6.1 (3.2,11.5)
Untreated	17.2 (11.9,24.9)

3.3 Psyllid mortality

The percentage mortality of female TPP adults 24 and 48 h after treatment is shown in Table 3. No psyllids had died after 1 h, so these data are not presented. By 24 h, some psyllids had died in all treatments except Cinnamon and the untreated controls. However, by 48 h, there were some dead psyllids in all treatments. At 24 h, the highest percentage dead was in one replicate of the D-limonene treatment (40% dead). At 48 h, there was at least one replicate with no dead psyllids in all treatments except Wintergreen, Rosemary, Neem, surfactant mix (control) and the untreated control. The highest percentage dead was 80%, for one pot containing a leaf dipped in Rosemary oil. Overall, the percentage of psyllids that were dead did not vary significantly between the treatments at 24 or at 48 h (p=0.233, p=0.438 for overall treatment differences respectively).

Table 3. Percentage mortality of female TPP adults 24 and 48 h after treatment (95% confidence limits in parentheses).

Treatment	24 h	48 h
Cedarwood	5.0 (0.7,26.9)	7.5 (1.1,36.7)
Lemongrass	5.3 (0.8,28.1)	18.4 (5.4,47.1)
Cinnamon	0.0 (0.0,11.5)	5.0 (0.5,36.2)
Wintergreen	13.2 (4.0,35.4)	31.6 (12.8,59.1)
Rosemary	21.1 (8.4,43.7)	31.6 (12.8,59.1)
Peppermint	7.5 (1.6,28.9)	20.0 (6.4,47.7)
Clove	5.1 (0.8,27.5)	28.2 (10.9,55.8)
Patchouli	7.5 (1.6,28.9)	22.5 (7.7,50.1)
D-limolene	15.4 (5.2,37.4)	20.5 (6.6,48.6)
Neem	7.9 (1.7,30.1)	42.1 (19.9,68.1)
Surfactant mix	2.7 (0.2,29.7)	32.4 (13.2,60.3)
Untreated	0.0 (0.0,11.8)	20.5 (6.6,48.6)

4 Discussion/Conclusions

Softer chemicals are preferable greenhouse pest control options due to their low toxicity to the environment, beneficial insects and pollinators, and their nil or short withholding periods. Softer chemicals, such as horticultural oils and soaps, have broad applications against insect and mite pests (Baxendale & Johnson 1990; Larew & Locke 1990; Saxena 1995). For example, Baxendale & Johnson (1990) found that the horticultural oil SUNSPRAY® was highly effective against homopterous insects, such as boxwood psyllid *Psylla buxi* (L.) and calico scale *Eulecanium cerasorum* (Cockerell). Liu & Stansly (1995) also found that SUNSPRAY used as a dip was at least as effective as a pyrethroid for the control of *Bemisia argentifolii* on tomatoes under greenhouse and laboratory conditions.

In the present study, TPP mortality was not considered an objective in the selection of essential oils for testing. The principal aim was to assess the repellency and deterrent effects of various essential oils on adult female TPP. The essential oils Neem and Cedarwood had significant repellency to female TPP adults 1 h after treatment compared with the untreated controls. Twenty-four hours after treatment, Peppermint, Clove, Patchouli, Wintergreen and Neem showed some repellency to female TPP adults compared with the untreated controls (but this was not significant). By 48 h, Neem and Patchouli had significant repellency to female TPP adults. Forty-eight hours after treatment, all the oils except the Peppermint, Clove and Lemongrass treatments had significantly deterred oviposition by female TPP adults as compared with the untreated controls. Therefore, 48 h after treatment, Neem and Patchouli oils reduced oviposition and significantly repelled adult female TPP.

There are few published studies on the repellent effects of horticultural or essential oils on TPP; however, their repellent effects on other insect pests have been reported in various studies (Larew & Locke 1990; Liu & Stansly 1995). For example, Saxena & Basit (1982) found that the volatiles of *Eucalyptus globulus* (Myrtaceae) and *Coriandrum sativum* (Umbelliferae) inhibited oviposition by the leafhopper *Amrasca devastans.* Saxena (1995) reported the repellent effect of a spray application of 1.4% emulsified neem oil on the Asiatic citrus psyllid on citrus under laboratory and field trials.

Many essential oils are also reported to have insect antifeedant properties (Huang et al.1997; Carpinella et al. 2003; Yang et al. 2010). Identification of those oils with settling, oviposition and feeding deterrent properties may be important in reducing the rate of *Ca.* L. solanacearum transmission by TPP (Yang et al. 2010). Use of an effective oil/s could be a complementary or alternative method to the use of traditional insecticides in greenhouse crops. Of the essential oils tested in this study, Neem and Patchouli reduced oviposition and significantly repelled adult female TPP. Laboratory studies on the antifeedent properties of Neem and Patchouli oils on TPP are necessary to determine their effectiveness in reducing crop damage caused by TPP/*Ca.* L. solanacearum. Further tests under greenhouse conditions would then be required to determine efficacy, appropriate method of application and suitability for inclusion in an IPM programme.

Potential options for Year 3 soft chemical trials will be discussed with the Sustainable TPP Management SFF Project Team at the meeting planned for 21st June 2011.

5 Acknowledgements

Thanks to Nina Jorgensen Plant & Food Research Lincoln and Natasha Taylor Plant & Food Research Hawke's Bay, for technical assistance. Thanks to John Thompson Bioforce Ltd for formulating and supplying samples of the 10 essential oils for testing. This research was funded by the Sustainable TPP Management SFF.

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