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Testing antifeedants against the tomato potato psyllid (TPP) using EPG technique

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New Zealand Tamarillo Growers Association Incorporated

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Executive summary

Testing antifeedants against the tomato potato psyllid (TPP) using EPG technique

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Background

The tomato potato psyllid (TPP) (*Bactericera cockerelli*) is native to North America and was first detected in New Zealand in 2006. The pest primarily attacks plants in the Solanaceae (potato and tomato family) but can also be found feeding on some species of the Convolvulaceae (kumara and bindweed family). Both the adult and nymphal life stages of TPP cause damage to the host plants by feeding on the leaves, which results in 'psyllid yellows'. TPP transmits the bacterial pathogen *Candidatus* Liberibacter solanacearum, which is thought to be the causative agent of 'zebra chip' in potato tubers and stunted growth in fruit and leaves in tomatoes, capsicums and tamarillos. Liberibacter infection not only reduces crop yield and impacts the quality of the fruit, but ultimately also leads to the decline and death of the infected plant.

While past research has investigated the use of broad-spectrum insecticides against TPP, there was a need to complement this work by investigating other approaches which may be used as part of an effective integrated pest management program. The use of antifeedants provides an opportunity to potentially interrupt the successful transmission of Liberibacter by TPP.

The objective of this trial was to investigate possible antifeedants against TPP using the Electrical Penetration Graph (EPG) technique.

Methods

This trial was conducted in the laboratory at Plant & Food Research (Mt Albert) from Tuesday 11 April 2012 to Thursday 17 May 2012.

Experimental plants were grown under glasshouse conditions in an insect-free room. Due to short germination time and fast growth, tomato plants (cv. 'Money maker') were utilised for this trial. Plants were sprayed with one of four treatments: Neemazal (Neem Oil), Surround (Kaolin Clay), DC-Tron (Mineral Oil) or Tap Water (Control). A total of 16 replicates of each treatment were completed during the trial.

On each day of the experiment, four tomato plants were sprayed, each with one of the four treatments described above. Plants were allowed to dry for approximately 6 hours before being transferred to the Electrical Penetration Graph (EPG) laboratory. The treated plants were assigned to four channels of the EPG Giga 4 monitor, respectively. Insects were prepared for the EPG experiment by attaching an 18 µm diameter gold wire to the thorax of the insect using conductive silver paint. The other end of the gold wire was attached to a 4 cm long copper wire, which was connected to a copper nail inserted into the amplifier of the EPG Giga 4 monitor. One female adult TPP with gold wire attached was placed on each of the four plants and all four insects were monitored simultaneously using the four channels of the EPG monitor for 15 hours under lights at 23 ± 1°C.

The stylet penetration behaviour of the insects was recorded using WinDaq Pro+ software (DATAQ instruments, Ohio, USA) and the data were saved as WinDaq files for waveform measurements and analysis. The main EPG waveform parameters described below were measured during the EPG tests:

(np) non penetration (stylet has not penetrated into plant tissue)

(C) intercellular stylet penetration

(D) initial contact with phloem

(E1) salivation into phloem sieve tubes

(E2) phloem sap ingestion.

Key Findings

- There was no statistical difference in the mean percentage duration of salivation in phloem sieve tubes + phloem sap ingestion (E1 + E2) events or the mean number of sustained phloem feeding events for TPP on Surround-, Neemazal- and DC-Tron-treated plants 6–21 h after application when compared with individuals on control plants.
- Therefore, results of the study suggest that the treatment of tomato plants with Neemazal, Surround or DC-Tron does not deter TPP feeding enough to prevent phloem feeding and therefore Liberibacter transmission.
- The effect of these products on the 'feed or flight' activity of TPP was not tested in this trial.

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1 Introduction

The tomato/potato psyllid (TPP) (*Bactericera cockerelli:*) is native to North America and was first detected in New Zealand in 2006. The pest primarily attacks plants in the Solanaceae (potato and tomato family) but can also be found feeding on some species of the Convolvulaceae (kumara and bindweed family) (Liefting et al. 2009; MPI 2012). Both the adult and nymphal life stages of TPP (Figures 1A & 1B) cause damage to the host plants by feeding on the leaves, which results in 'psyllid yellows' (Sengoda et al. 2010; Brown et al. 2010). TPP transmits the bacterial pathogen *Candidatus* Liberibacter solanacearum, which is thought to be the causative agent of 'zebra chip' in potato tubers (Sengoda et al. 2010) and stunted growth in fruit and leaves in tomatoes, capsicums and tamarillos (Brown et al. 2010). Liberibacter infection not only reduces crop yield and impacts on the quality of the fruit but ultimately also leads to the decline and death of the infected plant (Sengoda et al. 2010).

A B

Figure 1. (A) TPP adults and eggs; (B) TPP nymphs.

While past research has investigated the use of broad-spectrum insecticides against TPP (Page et al. 2011), there was a need to complement this work by investigating other approaches which may be used as part of an effective integrated pest management programme. The use of antifeedants provides an opportunity to potentially interrupt the successful acquisition and/ or transmission of Liberibacter by TPP. Little research has been published concerning the efficacy of antifeedants against TPP. A recent study investigated the residual effects of insecticides on TPP behaviour (Butler et al. 2011). Insecticides tested included imidacloprid, kaolin particle film, horticultural spray oil, abamectin, and pymetrozine. All insecticides significantly reduced probing durations and increased the amount of time adult psyllids spent off the leaflets, suggesting that these chemicals may be deterrents to feeding as well as repellents. Several overseas studies have also been conducted on antifeedants against the Asian citrus psyllid (*Diaphorina citri*). Pymetrozine had moderate antifeedant effect but produced this by modifying the behaviour of the psyllids due to the neurotoxic affect of the insecticide (i.e. uncoordinated leg movements and wing stretching (Boina et al. 2010)). Imidacloprid was also found to reduce feeding of Asian citrus psyllid when ingested at sublethal concentrations (Boina et al. 2009) although initial feeding on plants still occurred.

The objective of this trial was to investigate possible antifeedants against TPP using the Electrical Penetration Graph (EPG) technique. The EPG technique allows the quantification of stylet penetration activities and real feeding (ingestion) of an insect (Sandanayaka et al. 2007). It is a system consisting of an electrical circuit which is completed when an insect penetrates the plant with its stylet. This completed circuit is amplified and displayed on a computer screen as a graph with different waveforms indicating different insect activities. Previous investigations have indicated that adult TPP produce five distinct EPG waveform types (Sandanayaka et al. 2011). The waveforms representing salivary sheath secretion and other stylet pathway activities (C), first contact with phloem (D), salivation in phloem sieve tubes (E1) and phloem sap ingestion (E2) were similar to the Asian citrus psyllid, *Diaphorina citri*, which has had the relationship between wavelength and stylet position confirmed by histological studies (Sandanayaka et al. 2011).

Products for this trial were chosen in discussion with the New Zealand Tamarillo Growers Association (NZTGA). The products are discussed briefly below:

Surround (Distributed by Elliott Chemicals Ltd)

Surround is a natural mineral-based product used for the control of sunburn and heat stress on apples and in vineyards. The active ingredient in Surround is kaolin clay. Surround was chosen for this trial as it is a product which could potentially create a physical barrier to TPP feeding.

Neemazal (Distributed by Sustain-Ability / EcoGrape Ltd)

Neemazal is a broad-spectrum insecticide derived from the Neem tree seed kernel. The active ingredient in Neemazal is azadirachtin. It is a slow acting insecticide which can inhibit feeding and moulting of larvae and also inhibit feeding in adults. Neemazal was chosen for this trial as overseas research has suggested that Neem products have antifeedant properties against aphids (Nisbet et al 1993).

DC- Tron (Distributed by Fruitfed Supplies)

DC- Tron is a highly paraffinic mineral oil. It is currently used in orchards but usually with an insecticide as a surfactant.

2 Methods

This trial was conducted in the laboratory at Plant & Food Research (Mt Albert) from Tuesday 11 April 2012 to Thursday 17 May 2012.

2.1 Insects

Adult female TPP were obtained from a laboratory colony reared on a mixture of tomato and capsicum plants.

2.2 Plants

Experimental plants were grown under glasshouse conditions in a room without TPP. Due to short germination time and fast growth, tomato plants (cv. 'Money maker') were utilised for this trial. Plants of approximately 20–30 cm in height were used in the experiments.

2.3 Treatments

Spray treatments are given in Table 1. A total of 16 replicates of each treatment were completed during the trial. On each experimental day, four tomato plants were sprayed, each with one of the four treatments described below. Plants were sprayed using a 1-L hand trigger sprayer, ensuring spray coverage on both the top and underside of leaves. Plants were allowed to dry for approximately 6 h before being transferred to the Electrical Penetration Graph (EPG) laboratory.

Table 1. List of treatments used in the trial.

Product	Rate per 100 L	Rate per 500 ml
Neemazal (Neem oil)	500 ml	2.5 ml
Surround (Kaolin clay)	2.5 kg	12.5 g
DC-Tron (Mineral oil)	1 L	5 ml
Control	Tap water	

2.4 Electrical Penetration Graph (EPG) readings

The treated plants were assigned to four channels of the EPG Giga 4 monitor, respectively (Figure 2). One insect was placed on each of the four plants and all four insects were monitored simultaneously using the four channels of the EPG monitor for 15 h.

All insects were prepared for the EPG tests as follows. Females were collected from the colony and starved for 6 h prior to EPG tests. The insects were immobilised under CO_2 for 2–3 s to attach an 18 µm diameter gold wire to the thorax of the insect using conductive silver paint (Figure 3). The other end of the gold wire was attached to a 4-cm-long copper wire, which was connected to a copper nail inserted into the amplifier of the EPG Giga 4 monitor. A second electrode was placed into the damp soil around the plant. Wired insects were held for a recovery period of 10–15 min and then placed one by one on the leaves of the treated plants, according to the numerical order of the channels The stylet penetration behaviours of the insects were recorded for 15 h using WinDaq Pro+ software (DATAQ instruments, Ohio, USA) and the data were saved as WinDaq files for waveform measurements and analysis. Sixteen

replicates (i.e. separate insects) of each treatment were carried out but insects that escaped during recordings or failed to settle on the plants were excluded from analysis.

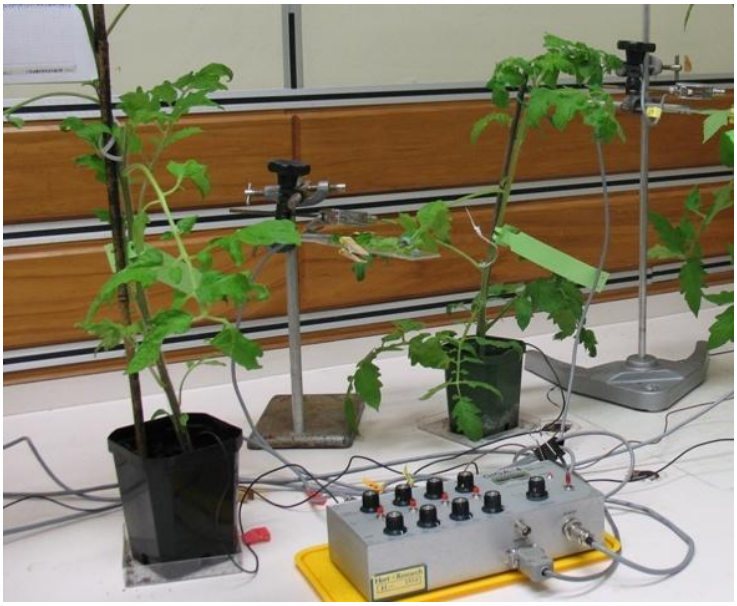


Figure 2. Treated plants connected to the EPG Giga 4 monitor.

Figure 3. An adult psyllid attached to 10 µm diameter gold wire using conductive silver paint for EPG recording.

2.5 Data analysis

2.5.1 Wave forms

The main EPG waveform parameters (Figure 4) described below were measured during the EPG tests:

(np) non penetration (stylet has not penetrated into plant tissue)

(C) intercellular stylet penetration

(D) initial contact with phloem

(E1) salivation into phloem sieve tubes

(E2) phloem sap ingestion.

2.5.2 Comparison of treatments

Using the MASS (Venables & Ripley 2002) package with R (R Development Core Team, 2012), negative binomial generalized linear models were used to model the number and duration of phases. To compensate for the difference in recording times, predictions were done on a standard 15 h for all treatments.

The negative binomial model is one of the log family, a consequence of which is that the standard errors are on the log scale. Those standard errors have been added to and subtracted from the mean (also on the log scale) and the resulting three values back-transformed. Although the untransformed values are presented in the Results section, the transformed values are presented in Table 3 within the Appendices.

Analysis of percentage data used quasibinomials models which make an adjustment for the dispersion which is not identical to what is expected in a binomial distribution. Standard errors are indicated in Table 3 in a way similar to that used for negative binomial models.

Analysis of variables which measured durations were analysed by simple ANOVA.

The probabilities listed in the tables relate to the probability of obtaining the corresponding means and standard errors if there was no difference between the treated population and the control population.

3 Results

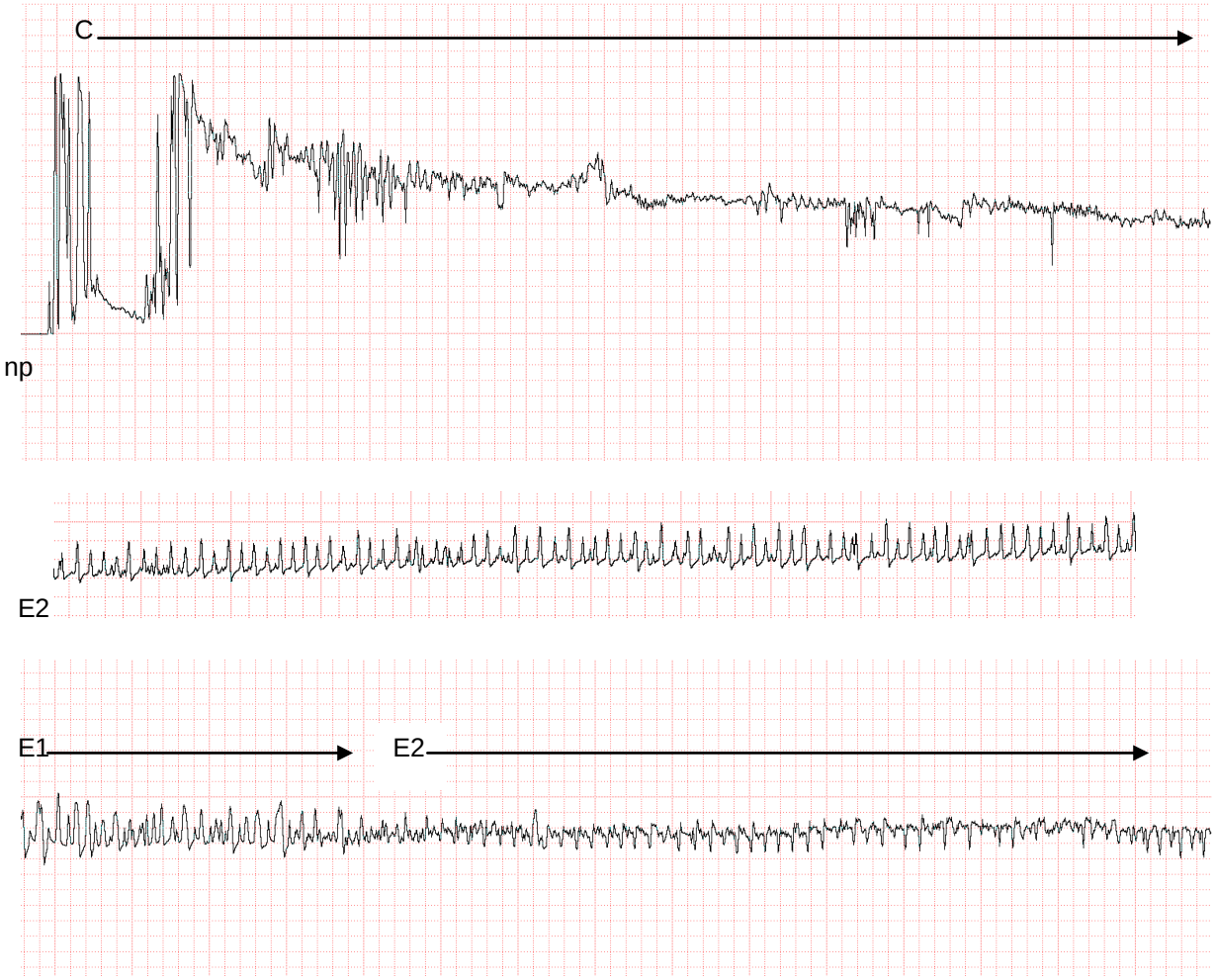


Figure 4. Main EPG waveforms representing probing and feeding of TPP on tomato leaves. Scale= 0.4 s/division.

3.1 EPG waveform analysis

Characteristics of the main waveforms produced by TPP feeding on tomato plants are summarised in Figure 4.

Waveform C indicates penetration and salivary sheath secretion (probing activities) in the epidermis, mesophyll or parenchyma cells.

The phloem phase (E) is represented by two waveforms (E1 and E2). Waveform E1 indicates salivation into the phloem sieve tubes.

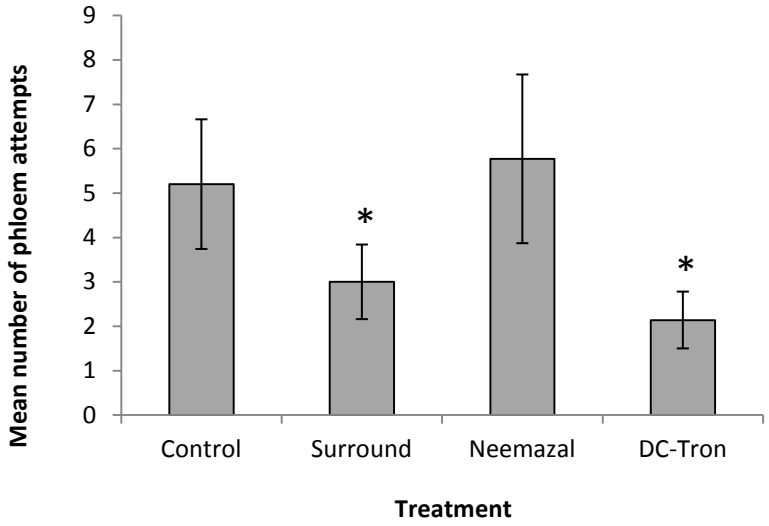
3.2 Comparison of treatments

The mean durations (expressed as a percentage) of various TPP feeding events observed on control and treated plants are shown in Table 2.

Table 2. Mean (± SEM) duration (expressed as a percentage) and the mean (± SEM) number of times that TPP adults spent performing various feeding behaviours on control plants or treated plants during the EPG recording period.

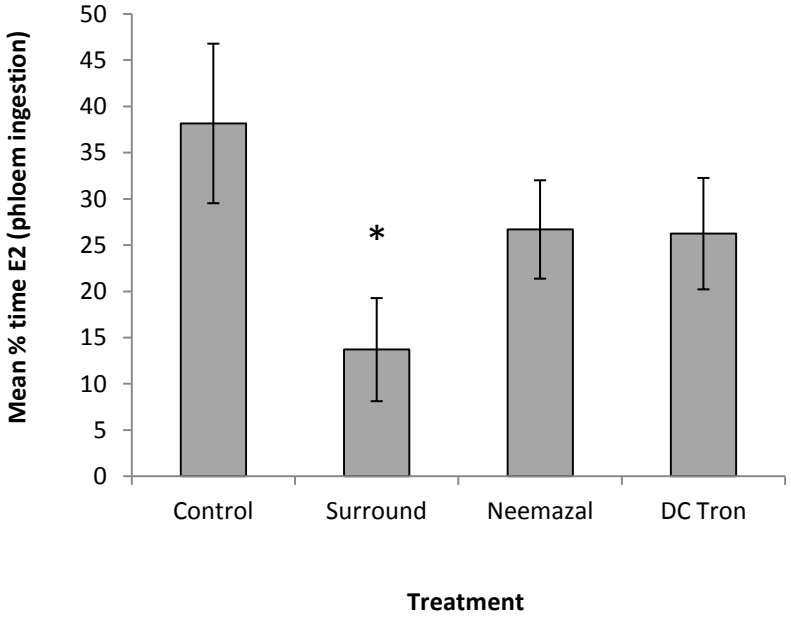
Feeding events	Control	Surround	Neemazal	DC-Tron
Mean time to 1st probe (minutes)	5.57 ± 1.80	6.84 ± 2.35	12.83 ± 10.56	2.39 ± 0.73
Mean % duration non-penetration	7.95 ± 2.90	16.84 ± 6.20	22.07 ± 8.55	19.80 ± 8.78
Mean number of non-penetration events (np)	12.07 ± 2.73	13.40 ± 2.77	14.69 ± 3.00	13.14 ± 2.55
Mean number of phloem attempts (D)	5.20 ± 1.46	3.00 ± 0.84	5.77 ± 1.90	2.14 ± 0.64
Mean % duration of E1 events	5.55 ± 2.01	12.70 ± 6.40	13.30 ± 4.18	6.90 ± 2.12
Mean number of E1 events	6.40 ±1.71	5.07 ±1.42	9.31 ± 3.21	4.57 ± 1.61
Mean % duration of E2 events	38.17 ± 8.62	13.71 ± 5.58	26.71 ± 5.32	26.25 ± 6.02
Mean number of E2 event	3.67 ± 0.81	2.93 ± 0.92	5.46 ± 2.12	3.36 ± 1.41
Mean number of sustained E2 (>10 min) events	1.53 ± 0.26	1.20 ± 0.44	1.38 ± 0.50	1.57 ± 0.60
% time of E (E1 + E2) events	42.47 ± 8.44	26.41 ± 8.20	41.19 ± 7.30	33.15 ± 6.34

[a] X ± X indicates treatments within the same row that are significantly different from the control ($\alpha < 0.05$).



.* indicates treatments that are significantly different from the untreated control (α < 0.05).

Figure 5. The mean (± SEM) number of phloem attempts by TPP on treated plants



* indicates treatments that are significantly different from the untreated control (α < 0.05).

Figure 6. The mean (± SEM) duration (expressed as a percentage) of phloem ingestion (E2) by TPP on treated plants.

DC-Tron

The mean time to first probe for TPP on DC-Tron treated plants was not significantly different to TPP on control plants. Although the mean percentage duration of non-penetration was higher for TPP on DC-Tron-treated plants when compared with individuals on control plants, this difference was not statistically significant (Table 2). The mean number of phloem attempts was significantly lower on DC-Tron-treated plants than on control plants (Table 2, Figure 5). While the mean number of E1 events (salivation in phloem sieve tubes), the mean number of E2 events (phloem sap ingestion) and the mean percentage duration of E2 events were all lower

on DC-Tron-treated plants than on the control plants, these differences were not statistically significant (Table 2, Figure 6). The mean number of sustained E2 activities between DC-Tron-treated plants and control plants was not significantly different.

Surround

The mean time to first probe for TPP on Surround-treated plants was not significantly different from TPP on control plants. The mean percentage duration of non-penetration was not significantly different for TPP on Surround-treated plants when compared with individuals on control plants (Table 2). The mean number of phloem attempts was also not significantly different between TPP on control plants and TPP on surround-treated plants (Table 2, Figure 5). Although the mean number of E1 events (salivation into phloem sieve tubes) and E2 events (phloem sap ingestion) were both lower on surround-treated plants than on control plants, these differences were not statistically significant (Table 2). Nonetheless, the mean percentage duration of E2 activities was significantly lower on surround-treated plants than on control plants (Table 2, Figure 6). The mean number of sustained E2 activities between Surround-treated plants and control plants was not significantly different.

Neemazal

The mean time to first probe for TPP on Neemazal-treated plants was not significantly different from TPP on control plants. Although the mean percentage duration of non-penetration was higher for TPP on Neemazal-treated plants than for TPP oncontrol plants, this difference was not statistically significant. The mean number of phloem attempts was not significantly different on Neemazal-treated plants compared with control plants (Table 2, Figure 5). While the mean number of E1 events (salivation in phloem sieve tubes), the mean number of E2 events (phloem sap ingestion) and the mean percentage duration of E1 activities were all higher on Neemazal-treated plants than on control plants, these differences were not statistically significant (Table 2, Figure 6). The mean number of sustained E2 activities between Neemazal-treated plants and control plants was not significantly different.

4 Conclusions

The objective of this trial was to investigate possible antifeedants against TPP using The Electrical Penetration Graph (EPG) technique. The numbers of TPP in tamarillo orchards are much lower than in potato or tomato crops (Page-Weir NEM et al. 2010, 2012; Sally Anderson pers. comm.). However, the impact of Liberibacter on tamarillo plants is severe, with complete dieback with attempted regrowth or death of the plant occurring within 2–3 months of first symptoms. Reducing the transmission of Liberibacter by TPP by deterring the feeding of immigrating infected adults using antifeedants has the potential to reduce transmission of an already small population without frequent application of toxic pesticides, which may then only be needed for treating immature resident TPP populations when detected.

The results of the study suggest that the treatment of tomato plants with Neemazal, Surround or DC-Tron does not deter TPP feeding enough to prevent phoem feeding, and therefore Liberibacter transmission, 6–21 h after application. Although the mean percentage duration of non-penetration was higher for TPP on Neemazal- and DC-Tron-treated plants than for TPP on control plants, this difference was not statistically significant. Also, there was no statistical difference in the mean percentage duration of salivation in phloem sieve tubes + phloem sap ingestion (E1 + E2) events or the mean number of sustained phloem feeding events for TPP on Surround-, Neemazal- and DC-Tron-treated plants when compared with TPP on control plants.

The mean time to first probe for TPP on DC-Tron-, Surround- and Neemazal-treated plants was not significantly different from TPP on control plants. The time to first probe provides information on the settlement of the insect on each treatment plant. In this experiment insects were attached to a wire and were possibly forced to probe a treated plant unlike insects in the field which have a choice to fly away if they prefer not to settle on a particular plant.

Results collected in this trial suggest that the products tested do not provide enough antifeedant activity to prevent transmission of Liberibacter. The effect of these products on the ‘feed or flight’ activity of TPP was not tested in this trial.

5 Acknowledgements

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6 References

Boina D, Onagbola EO, Salyani M, Stelinski LL 2009. Antifeedant and sublethal effects of imidacloprid on Asian citrus psyllid, *Diaphorina citri*. Pest Management Science 65: 870–877.

Boina D, Youn Y, Folimonova S, Stelinski LL 2010. Effects of pymetrozine, an antifeedant of Hemiptera, on Asian citrus psyllid, *Diaphornia citri*, feeding bahavior, survival and transmission of *Candidatus* Liberibacter asiaticus. Pest Management Science 67: 146–155.

Brown JK, Rehman M, Rogan D, Martin RR, Idris AM 2010. First report of "*Candidatus* Liberibacter psyllaurous" (synonym "Ca. L. solanacearum") associated with 'tomato vein-greening' and 'tomato Psyllid yellows' diseases in commercial greenhouses in Arizona. Plant Disease 94(3): 376.

Butler CD, Byrne CD, Byrne FJ, Keremane ML, Lee RF, Trumble JT 2011. Effects of insecticides on behavior of adult *Bactericera cockerelli* (Hemiptera: Triozidae) and transmission of *Candidatus* Liberibacter psyllaurous. Journal of Economic Entomology 104(2): 586–594.

Liefting LW, Weir BS, Pennycook SR, Clover GRG 2009. "*Candidatus* Liberibacter solanacearum", associated with plants in the family Solanaceae. International Journal of Systematic and Evolutionary Microbiology 59: 2274–2276.

MPI Biosecurity 2012. Potato/Tomato Psyllid. http://www.biosecurity.govt.nz/pests/potato-tomato-psyllid [Accessed 12 June 2010].

Nisbet AJ, Woodford JAT, Strang RHC, Connolly JD 1993. Systemic antifeedant effects of azadirachtin on the peach-potato aphid *Myzus persicae*. Entomologia Experimentalis et Applicata 68: 87–98.

Page-Weir NEM, Chhagan A, Jamieson LE, Kean AM, Poulton J, Davis VA, Griffin M, Connolly PG 2012. The phenology of, and efficacy of insecticides against, the tomato/potato psyllid (TPP) in tamarillos. Plant & Food Client Report (No. yet to be allocated).

Page-Weir NEM, Jamieson LE, Chhagan A, Curtis C 2010. The phenology of, and efficacy of insecticides against, the tomato/potato psyllid (*Bactericera cockerelli*) in tamarillos. Plant & Food Client Report No. 39778.

Page-Weir NEM, Jameison LE, Chhagan A, Connolly PG, Curtis C 2011. Efficacy of insecticides against the tomato/ potato psyllid (*Bactericera cockerelli*). New Zealand Plant Protection 64: 276–281.

R Development Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Sandanayaka WRM, Chhagan A, Ramankutty P 2007. Host plant testing of the spittle bug *Carystoterpa fingens* by stylet penetration behaviour. New Zealand Plant protection 60: 78–84.

Sandanayaka WRM, Page-Weir NEM, Fereres A 2011. Real time EPG recordings of tomato/potato psyllid (*Bactericera cockerelli*) feeding on tomato. New Zealand Plant Protection 64: 294.

Sengoda VG, Munyaneza JE, Crosslin JM, Buchman JL, Pappu HR 2010. Phenotypic and etiological differences between Psyllid yellows and zebra chip diseases of potato. American Journal of Potato Research 87(1): 41–49.

Venables WN, Ripley BD 2002. Modern applied statistics with S. Fourth Edition. New York, Springer. ISBN 0-387-95457-0

Appendix

Table 3. Mean (± 1 SEM) duration (expressed as a percentage) and the mean (± 1 SEM) number of times that that TPP adults spent performing various feeding behaviours on control plants or treated plants during the EPG recording period.

Feeding events	Control	Surround	Neemazal	DC-Tron
Mean time (minutes) to 1st probe	5.48 (0.57, 10.4)	6.84 (2.05, 10.40) 0.595	12.83 (7.02, 17.33) 0.595	2.39 (1.85, 7.48) 0.595
Mean % duration of non-penetration (np)	7.95 (4.40, 14.00)	16.84 (11.30, 24.30) 0.283	22.07 (15.30, 30.70) 0.133	19.80 (13.6, 27.90) 0.183
Mean number of non-penetrations (np)	12.07 (10.20, 15.10)	13.40 (10.9, 16.10) 0.819	14.69 (11.8, 18.90) 0.587	13.14 (10.90, 16.10) 0.496
Mean number of phloem attempts (D)	5.20 (4.20, 7.50)	3.00 (2.20, 4.00) 0.121	5.77 (4.20, 7.70) 0.981	2.14 (1.60, 3.10) 0.034
Mean % duration of E1 events	5.55 (3.40, 9.00)	12.70 (8.80, 18.00) 0.179	13.30 (8.90, 19.40) 0.169	6.90 (3.80, 12.30) 0.778
Mean number of E1 activities	6.40 (45.0, 89.60)	5.07 (3.60, 7.00) 0.490	9.31 (6.50, 13.00) 0.551	4.57 (3.30, 6.60) 0.409
Mean % duration of E2 events	38.17 (31.20, 45.60)	13.71 (8.90, 20.60) 0.024	26.71 (18.90, 36.30) 0.336	26.25 (18.50, 435.80) 0.3174
Mean number of E2 activities	3.67 (2.80, 5.40)	2.93 (2.10, 4.10) 0.543	5.46 (3.80, 7.70) 0.494	3.36 (2.40, 4.80) 0.791
Mean number of sustained E2 activities	1.53 (1.20, 2.20)	1.20 (0.90, 1.60) 0.513	1.38 (1.00, 1.90) 0.746	1.57 (1.20, 2.20) 0.956
Mean % duration of E (E1 + E2) events	42.47 (34.90, 50.40)	26.41 (19.30, 35.00) 0.169	41.19 (31.40, 51.70) 0.921	33.15 (24.10, 43.60) 0.471

[a] X ± X indicates treatments within the same row that are significantly different from the control (α < 0.05)

[b] (MeanLse, MeanPse)

[c] P value