SFF09-143

Sustainable Tomato/Potato Psyllid Management

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Contents

Milestones 1, 2 and 3 - Tomato-Potato Psyllid Insecticide Trials (2009–12)

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Executive summary

Three main-crop potato insecticide trials were undertaken at Pukekohe Research Station to test different spray regimes for their efficacy in controlling the tomato-potato psyllid (TPP) and symptoms of zebra chip (ZC). Three to five treatments were used: insecticide drench and weekly foliar sprays (i.e. weekly 'standard blocks' spray programme); insecticide drench and different threshold-based foliar sprays (i.e. reduced spray programmes); and no insecticides (i.e. untreated). Reduced spray programmes were based on nominal action thresholds utilising either the number of TPP nymphs per middle leaf or the mean number of TPP adults per yellow sticky trap. Thresholds based on infestations of TPP nymphs on plants led to a range of 5–8 applications of insecticides, approximately half the number applied in the weekly spray programme. The incidence of ZC damage in treatments triggered by nymph thresholds were unacceptable, ranging from 5 to 27% ZC. However, thresholds based on sticky trap catches led to a 50% reduction in sprays with <1% ZC when the 'standard blocks' sequence of insecticides was applied. The sticky trap action threshold gave promising results that deserve further investigation to assess if this method may be used as an economic action threshold, being an economic injury level (EIL) 'trigger'. Also, the 'standard blocks' sequence of Avid®, Movento® and Sparta® gave the best results of all the insecticide rotation strategies tested in these 'insecticide' trials.

Naturally occurring predators responded to TPP plant infestations, with brown lacewing and small hover fly being the most important foliage-dwelling predators, with their populations increasing with increasing infestations of TPP to peak at more than 200 predators per plant. However, from January onwards even these large populations of predators were not able to keep TPP numbers below an economic injury level. Overall, a sound insecticide resistance management strategy is urgently required along with proven economic action thresholds to reduce insecticide use in potatoes.

Introduction

Milestones 1, 2 and 3 of this SFF project were to undertake 3 years of main crop insecticide trials at Pukekohe Research Station to test different insecticides and action thresholds and their impact on TPP populations and damage to potato crops. The type and number of treatments to be compared were discussed with the project team and included an untreated control treatment, plus a weekly spray programme to try and ensure we had "good control" and "poor control" of TPP infestations for meaningful comparisons with treatments with reduced numbers of foliar-applied insecticides. The 'standard blocks' calendar treatment was based on weekly applications of blocks of insecticides of different mode of action (MoA) as part of an insecticide resistance management (IRM) strategy. This spray programme was also designed to use broad-spectrum insecticides later in the crop cycle to 1) minimise impacts on natural enemies, 2) rely less on insecticides that may cease to be available over the medium term (e.g. the organo-phosphate insecticides), and 3) for control of late season infestations of potato tuber moth. The treatments also took into account the registration or imminent registration of new insecticides of different MoA and these were incorporated into the trials as appropriate, in discussion with the project team. The weekly 'standard' spray programme used in all trials was based on blocks of Avid®, Movento®, Sparta® (when it gained registration), Karate® and Tamaron® (see Tables 1, 2 and 3 and Appendix for more details).

Objective

To complete 3 years of main crop potato insecticide trials at Pukekohe Research Station to test different spray regimes for their efficacy in controlling TPP and zebra chip (ZC).

Methods

Three to five treatments were used: insecticide drench and weekly foliar sprays (i.e. weekly 'standard blocks' spray programme); insecticide drench and threshold-based foliar sprays (i.e. reduced spray programmes); and no insecticides (i.e. untreated control plots). Tables 1, 2 and 3 list the treatments and insecticides used. Actara® was normally used to minimise damage from other vectors (aphids) that might mask the impacts of TPP and ZC. TPP and associated insects in the trial were monitored weekly using four yellow sticky traps and sampling plants from late November to late March. Initially eight plants were assessed weekly (non-destructively) from each of the four plots in each treatment (32 plants per treatment), by counting and recording all TPP life stages on the whole plant. As plants grew larger, only single stems were assessed. After 3 weeks, partly because of time constraints, the method was changed to assessing 100 middle leaves, comprising 25 middle leaves per plot (2 middle leaves from separate stems from 12 plants, plus 1 more middle leaf per plot). Previous research had focussed on determining a practical, cost-efficient method of assessing potato plants, and it was shown that assessing a middle leaf is a reliable indicator of the infestations on a whole plant (Walker et al. 2012 (in press); Walker, unpublished data). Middle leaf counts of TPP nymphs and the mean number of TPP per trap per week were used for the action thresholds. In year 3 of the trials the trap threshold was defined by a 'high risk increase in trap catch' as determined by the senior author.

Assessments at harvest

The trials were machine harvested and tuber yields and size-grades were assessed. For tuber yield and quality assessments, the two middle beds from each plot were dug and a 5 m length defined in the middle of each plot (these middle beds were not sampled during the monitoring period of the trial). All tubers within this area were graded visually into 'marketable' (undamaged and > 60 g) or 'reject' (< 60 g, green, diseased or damaged by PTM) categories. The weights of the two grades were recorded. A representative sub-sample of ≥30 tubers (approximately one tuber chosen from each plant in the middle beds) was also picked from this area in each plot for assessment of specific gravity and ZC. This method of sub-sampling was used in year 3 to minimise variations within a plot, because plants within a plot may exhibit different symptoms of TPP damage (G.P. Walker, personal observation). Tuber specific gravity was determined by calculating the difference in weight of these ≥ 30 marketable tubers in air and in water.

Cooking tests for 'zebra chip'

Thirty of the representative sample of marketable tubers were selected for cooking tests ('crisping') to determine the incidence of ZC. A single slice of approximately 2 mm was cut from the centre of each tuber with a stainless steel mandoline slicer and fried in canola oil in batches of 10 for 3–3.5 min at 180ºC. Each individual crisp was then assessed on a 0–9 scale for symptoms of ZC where 0 = no browning and 9 = dark brown (Walker et al. 2012). The percentage of crisps in each category was recorded for each treatment. A sub-sample of tubers from all treatments was also frozen for future assessment of infection by *Candidatus* Liberibacter solanacearum.

Table 1. Trial treatments for the main crop potato trial at Pukekohe Research Station, 2009/10 (includes list of insecticides applied).

Treatment name	Treatment
Threshold (0.1)	Actara® & action (foliar spray) threshold of 0.1 TPP nymph per middle leaf (or equivalent infestation for stem or plant). Spray programme: blocks of three Avid®, two Movento®, one Oberon®
Weekly	Actara & weekly foliar applications from 100% emergence. Blocks of two Avid, three Karate, three Avid, four Tamaron, two Karate.
Untreated	Untreated

Table 2. Trial treatments for the main crop potato trial at Pukekohe Research Station, 2010/11(includes list of insecticides applied).

Treatment name	Treatment
Threshold (start)	Actara® & action (foliar spray) threshold of 0.1 TPP nymph per middle leaf. Spray programme: from start of 'standard blocks' sequence of three Avid®, three Movento®, one Tamaron®.
Threshold (same)	Actara & action (foliar spray) threshold of 0.1 TPP nymph per middle leaf. Spray programme: apply same product as that used in the same week in the 'standard' weekly spray programme. Applications of three Sparta®, two Karate®, three Tamaron.
Weekly	Actara & weekly foliar applications from 100% emergence. Blocks of three Avid, three Movento, three Sparta, three Karate and three Tamaron.
Untreated	Untreated

Table 3. Trial treatments for the main crop potato trial at Pukekohe Research Station, 2011/12 (includes list of insecticides applied).

Treatment name	Treatment
Trap threshold ('knockdown' inssecticides)	Actara® & action (foliar spray) threshold at increase in trap catches (above 3 per trap). Spray programme: blocks of three Avid®, two Proteus®, three Tamaron®.
Trap threshold ('standard' sequence)	Actara & action (foliar spray) threshold at increase in trap catches (above 3 per trap). Spray programme: three Avid, two Movento®, three Sparta®.
Threshold (nymphs)	Actara & action (foliar spray) threshold of 0.1 TPP nymph per middle leaf. Spray programme: three Avid, two Movento.
Weekly	Actara & weekly foliar applications from 60% emergence. Blocks of three Avid, two Movento, four Sparta, three Karate®, four Tamaron.
Untreated	Untreated.

More details on the trial protocols and parameters, crop management, method of insecticide application and rates of insecticide, plus the assessments made are presented in Appendix 1, a 'Working Sheet' of the trial in 2011/12 following this MS report.

Results

Figures 1, 2 and 3 present data showing the trends in infestations of TPP nymphs, mean trap catches of TPP adults, timing of spray applications and also trends of the common foliage-dwelling predators (Figure 1). Table 4 lists the first observations and peaks of TPP

nymphs in the crops and first catches and the peak mean trap catch per week of adult TPP. Tables 5, 6 and 7 summarise results from the three trials of the marketable weight, reject weight, mean tuber weight, specific gravity (with standard errors) and ZC symptoms for all treatments for the three trials.

In year 1, ZC damage was unacceptable in all treatments, ranging from 10% in the weekly ('standard blocks') sprayed plots to 64% ZC in the remotely located untreated plots (Table 5). In year 2, ZC damage once again was unacceptable in all treatments, with the best result in the reduced spray treatments (5% ZC) where we followed the sequence of insecticide applications as in the weekly 'standard blocks' treatment ('start' threshold) (Table 6). In year three, two treatments resulted in an acceptably low incidence of ZC, the weekly 'standard blocks' treatment (0%) and one of the reduced spray treatments (<1%). The reduced spray treatment that controlled ZC ("trap threshold standard") used the trap threshold and followed the same sequence of insecticides as the 'standard blocks' weekly treatment (Table 7).

Table 4. Timing of first observations of tomato-potato psyllid (TPP) nymphs on plants, TPP adults on sticky traps and peak numbers for main crop trials over 3 years.

Trial	First TPP nymphs	Peak of TPP nymphs	First trap catch	Peak of trap catches
2009/10	15 December	259 (8 Feb.)	1 December	121 (22 Feb.)
2012/11	30 December	1121 (15 Mar.)	7 December	286 (8 Mar.)
2011/12	22 December	185 (29 Feb.)	6 December	54.5 (21 Feb.)

Table 5. Summary of average weights (kg/10 m^2), specific gravity (with standard errors) and zebra chip score of harvested potatoes following 'weekly', 'reduced' and 'untreated' treatments in the main crop trial at Pukekohe Research Station, 2009/10.

Test	Weekly	Reduced	Untreated
Marketable wt.	35.62 (1.11)	25.29 (3.85)	19.88 (0.50)
Reject wt.	2.66 (0.46)	5.32 (1.55)	5.74 (0.54)
Specific gravity	1.083 (0.0016)	1.075 (0.0007)	1.066 (0.0019)
ZC symptoms*	10%	27%	64%

*120 tubers (30 from each replicate) (ZC score >3)

Table 6. Summary of average weights (kg/10 m^2), specific gravity (with standard errors) and zebra chip score of harvested potatoes following 'weekly', 'reduced' and 'untreated' treatments in an early trial at Pukekohe Research Station, 2010/11.

Test	Weekly	Threshold (start)	Threshold (same)	Untreated
Marketable wt.	46.5 (0.85)	37.53 (2.85)	36.39 (2.71)	22.31 (3.44)
Reject wt.	3.32	3.24	3.20	3.30
Mean tuber wt.	0.222 (0.014)	0.172 (0.015)	0.185 (0.015)	0.114 (0.010)
Specific gravity	1.071 (0.0014)	1.066 (0.0016)	1.068 (0.0016)	1.055 (0.0018)
ZC symptoms*	12.4%	5%	9%	22.3%

*120 tubers (30 from each replicate), (ZC score >3)

Table 7. Summary of average weights (kg/10 m^2), specific gravity (with standard errors) and zebra chip score of harvested potatoes following different spray regimes in a main crop trial at Pukekohe Research Station, 2011/12.

Test	Weekly	Trap threshold (knockdown)	Trap threshold (standard)	0.1 leaf nymph threshold	Untreated
Marketable wt.	59.2 (.51)	59.2 (4.18)	60.7 (3.03)	53.8 (2.16)	34.1 (1.52)
Reject wt.[#]	0.40	0.39	0.50	0.64	0.48
Specific gravity	1.0774 (0.001)	1.0745 (0.002)	1.0769 (0.0005)	1.0736 (0.002)	1.0592 (0.001)
ZC symptoms*	0%	5%	1%	7%	32%

*120 tubers (30 from each replicate) (ZC score >2)
[#]only includes undersize tubers

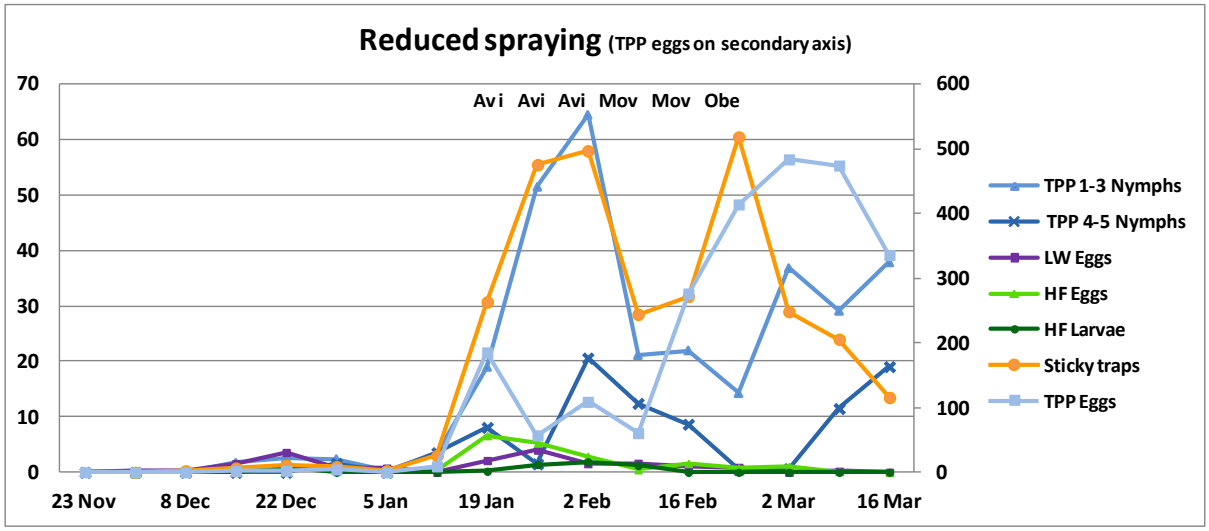


Figure 1. Mean number of brown lacewing (LW) eggs, eggs and larvae of small hover fly (HF), tomato-potato psyllid (TPP) eggs (shown on right axis), small (1st to 3rd instar) and large (4th to 5th instar) TPP nymphs per plant, and mean number of adult TPP captured per sticky trap per week on traps (left axis), reduced spray treatment, main crop trial 2009–10, Pukekohe Research Station. Timing of foliar-applied insecticides presented: Avi = Avid®, Mov = Movento®, Obe = Oberon®.

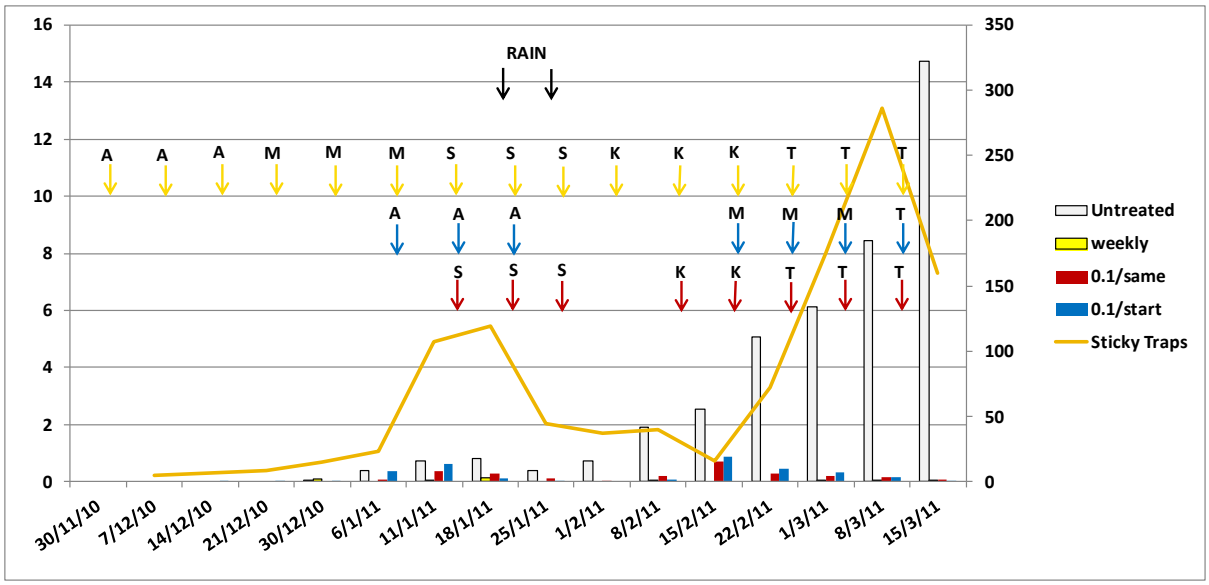


Figure 2. Average number of tomato-potato psyllid (TPP) nymphs per middle leaf (bars and left axis), and mean number of adult TPP captured per sticky trap per week (line and right axis), 2010–11 main crop trial, Pukekohe Research Station. Arrows indicate heavy rainfall events and timing of foliar applied insecticides (A = Avid®, M = Movento®, S = Sparta®, K = Karate®, T = Tamaron®).

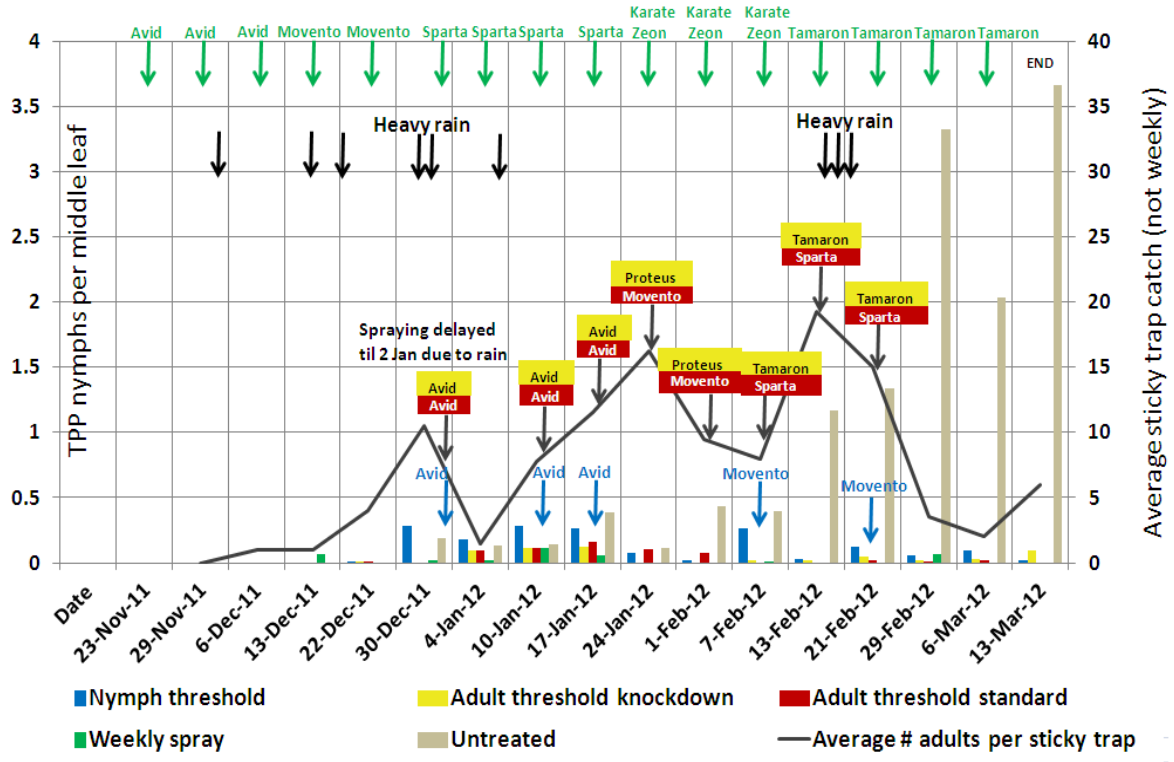


Figure 3. Average number of tomato-potato psyllid (TPP) nymphs per middle leaf (bars and left axis), and mean number of adult TPP captured per sticky trap (black line and right axis), 2011–12 main crop trial, Pukekohe Research Station. Arrows indicate heavy rainfall events and timing of foliar applied insecticides.

Other results including trends in populations of foliage dwelling predators are available in the PowerPoint slideshows presented at the SFF team meetings, plus in Walker et al. (2011) and Walker et al. (2012, in press). In summary, naturally occurring predators responded well to the first TPP infestations in main crop potatoes, with brown lacewing (*Micromus tasmaniae*) and small hover fly (*Melanostoma fasciatum*) being the most common species. Lacewings became abundant in December and there was normally another peak in lacewing activity about mid-February. Hover fly was common in mid-December, and became the dominant predator found on foliage by late December. Hover fly responded to the increases in TPP infestations in December; populations increased markedly and peaked at about 200 eggs and larvae per plant. However, from mid-February onwards, predator populations declined while TPP nymphal populations remained relatively high (Walker et al. 2011; Walker et al. 2012, unpublished data). It appears that from about mid-January onwards, predators are not able to keep TPP numbers below an economic injury level (EIL).

Discussion

Action (spray) thresholds based on plant infestations by TPP nymphs were not useful, resulting in excess ZC damage in all treatments tested over the 3 years of main crop trials. However, the trap catch threshold gave promising results that deserve further investigation. In year 1, plants were deliberately placed under stress (minimal irrigation) to test their ability to produce healthy tubers in the presence of the new pest. However, the dry season, along with reduced irrigation, led to excessive damage in all treatments (Table 5). In year 3, heavy rainfall in December and cooler conditions over January and February appeared to affect all insect populations, with infestations remaining low until later than in the previous 2 years (Figure 3). The TPP generations were not delayed but the first damaging flight (recorded in the previous 2 years) was greatly reduced, mainly caused by very wet weather (and heavy rainfall) in late December 2011 (Figures 3).

Reliable action (spray) thresholds rely on background knowledge of the economic injury levels (EILs) caused by a pest. In our trials, including 4 years of early crop trials, we have noted that trap catches have been no higher than 3 per trap and there was little or no ZC damage to early crops (Walker et al. 2012, in press). In the year three trial we used this background information and did not intervene with an insecticide spray until the trap catches increased markedly above 3 per trap. Our results show that trap catches were above 8 per trap before an insecticide was applied (see Figure 3). More research is required to better define the economic action threshold based on sticky trap catches. Using this threshold, the number of insecticide applications was reduced by 50% with <1% (1 tuber with ZC from 120 tested) and 5% ZC, when using the 'standard blocks' sequence of insecticides compared with using knockdown insecticides, respectively. These trials indicated that the 'standard' sequence of blocks of Avid, Movento and then Sparta resulted in the least ZC damage to tubers. As well as a reduced spray programme based on EILs, an insecticide resistance management strategy for potatoes is urgently required, not only to minimise any development of resistance in TPP, but also to mitigate the resistance already documented for potato tuber moth and green peach aphid in the north of the North Island.

Extension information

- Results from 3 years of main crop potato trials at Pukekohe Research Station indicate that damaging populations of TPP may appear in December and main crops require protection with well-timed, effective insecticides from about early January through to late March.
- The 'standard blocks' sequence of foliar applied insecticides using blocks of Avid, Movento and Sparta resulted in less ZC damage than blocks of insecticides in different sequences.
- The sticky trap action threshold tested in year 3 (2011/12) gave promising results that deserve further investigation. This threshold used a baseline of about 3 per trap per

week, following results from early crop trials undertaken over 4 years that show that this number in traps results in little or no ZC damage to the crop (Walker et al. 2012, in press).

- Naturally occurring predators respond well to TPP plant infestations with brown lacewing and small hover fly being the most important foliage-dwelling predators. However, from January onwards they are not able to keep TPP below an economic injury level (EIL).
- It is important that the economic injury levels (EILs) for TPP are identified so that validated economic action thresholds can be used to reduce the reliance on calendar spraying of insecticides.
- A robust insecticide resistance management (IRM) strategy is urgently required, not only to minimise any development of resistance in TPP, but also to mitigate the resistance to these same insecticides already documented for potato tuber moth and green peach aphid in the north of the North Island.

References

Walker GP, MacDonald FH, Larsen NJ, Wallace AR 2011. Monitoring of tomato-potato psyllid and associated insects in unsprayed potatoes in New Zealand. New Zealand Plant Protection 64: 269–275.

Walker GP, MacDonald FH, Puketapu A, Fergusson H, Wright P, Anderson JAD 2012. A field trial to assess damage by *Bactericera cockerelli* to early potatoes at Pukekohe. New Zealand Plant Protection 65:148-154.

Output: please refer to Appendix MS1, which is a separate document to this report.

Appendix 1

SFF TPpsyllid (main crop) insecticide trial, Pukekohe RS

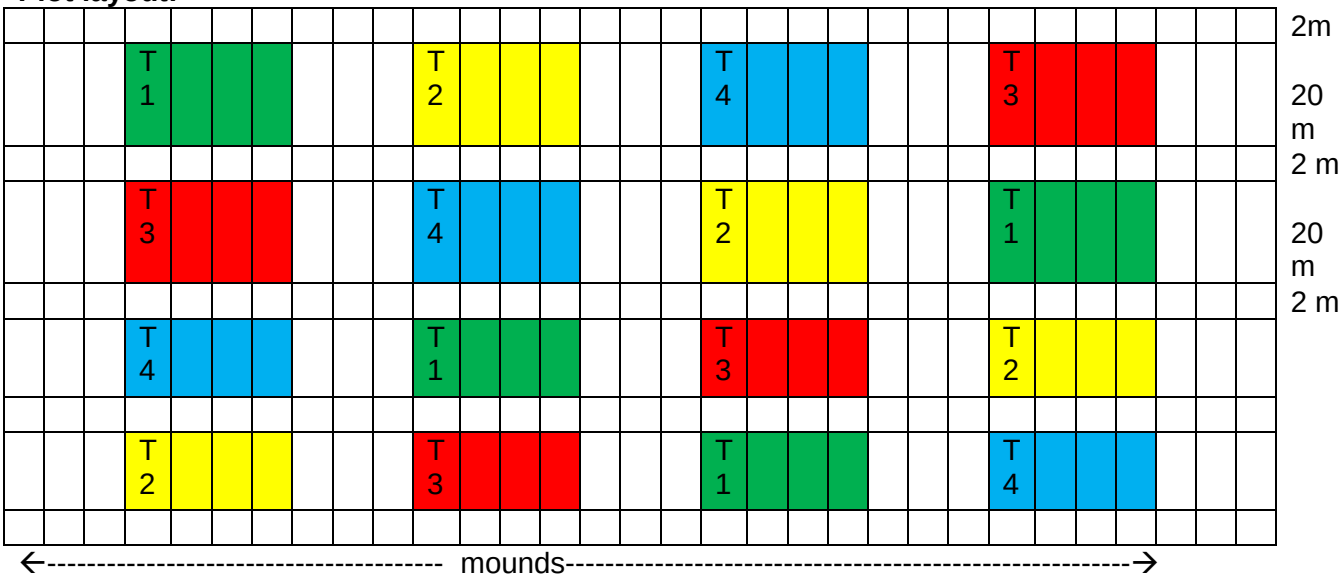
Graham Walker and Peter Wright (Plant & Food Research)

Variety: **Moonlight** (susceptible) **Plant (seed tuber) spacing**: 30 cm
Planted: range 1: 31 Oct.; remote: 1 Nov. **Harvested:**

Treatments: 5
Number of replications: 4 plots per treatment
Number of plots: 16

Plot Size: eight mounds **(6 m) x 20** metres long

Plot layout:



Tractor rows consist of 3 rows and are not planted (guards).
Treatment plots are 8 rows wide.

<u>TRIAL PROTOCOLS:</u>

4 insecticide treatments versus untreated: the insecticide trial will be a fully replicated 4 treatment trial. At a nearby locality (on the research station) a large (30 x 30 m) planting of untreated tubers will be used as an untreated remote control. This trial layout will be used to minimise the pressures exerted when putting untreated control plots within a 'small' plot trial, being:

1) Treated plots are put under extreme (and artificially-high) pest pressure by having untreated (heavily infested with TPP) plots immediately adjacent to treated plots
2) Mobile insects (in particular, predators) are subjected to insecticidal effects when untreated controls are placed immediately adjacent to treated plots. Therefore the 'real' impacts of natural enemies of TPP are 'masked'.

4 sticky traps (located N, E, S, W) will be placed out at both planting locations, monitored weekly, psyllid species identified and counted (1 set of traps for the 4 treated plots and 1 set of traps at the untreated planting).

Treatment 1:
Monitor treatment weekly (traps and plant sampling). Calendar (weekly) spray programme:

Actara in furrow at planting. Start foliar applications at 60% plant emergence, being:
3 Avid
2 Movento
4 Sparta
3 Karate Zeon (100ml rate)
3 methamidophos

Treatment 2:
Monitor treatment weekly (traps and plant sampling)

Actara in furrow at planting. Start foliar applications when trapping indicates 1st damaging summer generation/flight of TPP (flying into crop).
***When plant sampling action threshold is exceeded, apply knockdown insecticides (for adults and nymphs):
3 Avid
2 Proteus
3 Tamaron
3 Karate
Repeat blocks of Tamaron & Karate as required
*****But recent manuscript (Buchman, Sendoga & Munyaneza, 2011) reports that adults more efficient transmitters of Lib. Then nymphs. So, we should respond to adult increase as well, or as a priority. Numbers of both adults and nymphs were low in December and early January; so decided after monitoring each week (or twice weekly) whether to spray (main focus on trap catches).**

Treatment 3:
Monitor treatment weekly (traps and plant sampling). Start foliar applications when trapping indicates 1st damaging summer generation/flight of TPP. After that, action at 0.1 TPP nymphs per middle leaf (or equivalent infestation threshold for stem/plant).

Actara in furrow at planting.
When plant sampling action threshold is exceeded, apply same a.i. used that week in treatment 1
*****But recent manuscript (Buchman, Sendoga & Munyaneza, 2011) reports that adults more efficient transmitters of Lib. Then nymphs. So, we should respond to adult increase as well, or as a priority. Numbers of both adults and nymphs were low in December and early January; so decided after monitoring each week (or twice weekly) whether to spray (main focus on trap catches). Difference from T2 is using non-knockdown as well as knock-down (= last years order of blocks, being Avid, Movento, Sparta, etc.**

Treatment 4:
Monitor treatment weekly (traps and plant sampling). Action at 0.1 TPP nymphs per middle leaf (or equivalent infestation threshold for stem/plant), apply same a.i. used that week in treatment 1, unless action threshold reached same week as for treatment 3. If so, to make Treatments 3 and 4 different, follow MoA order from start as in treatment 1.

Actara in furrow at planting
When action threshold exceeded, apply same a.i. used that week in treatment 1 (unless threshold reached same week as for T3, then a.i.'s in same order as for treatment 1)

Nymph infestation not same timing as adult increase, and used same order as T1 to maximise predators (pest infestations low and predator numbers high)

Treatment 5:
Untreated control. No Actara
Monitor treatment weekly (traps and plant sampling)

Insecticide applications:

Actara will be applied by knapsack sprayer (single nozzle) at planting as an in-furrow spray over the tubers at 4 g/100m row applied in 50-100L of water per ha. A Jacto Condor 800 AM-12 tractor-mounted spray rig will be used to spray the potato plants. Nozzles used will be ceramic hollow cone Jacto JA-4 nozzles spaced 50 cm apart. Spraying volume will be 400 L /ha at 120 psi.

Insecticide treatments:

Treatment	Rate (ml/ha) + adjuvant
Untreated	-
Actara	4 g/100m row
Avid	600 ml + Eco oil(@ 1ml/L tank mix)
Movento	350 ml of Movento 240SC + 2 L PARTNER (note Movento product brief (Novachem 2012) says 1 litre esterified vegetable oil)
Oberon 240SC	600 ml
Karate Zeon	100 ml + non-ionic wetting agent
methamidophos	1000 ml
Sparta	500 ml + wetter spreader e.g. Bond Extra as per label
Proteus	650 ml/ha + wetter spreader

Week (date sprayed)	1: GREEN T1 - Actara & weekly (blocks of 3)	2: YELLOW T2 – treat at trap catch increase indicating 1st summer generation, then knockdowns at 0.1 threshold *** (changed to adult catches and nymph counts)	3: RED T3 – Treat at trap catch increase indicating 1st summer generation, then use same a.i. as used that week in T1 *** (changed to adult catches and nymph counts)	4: BLUE T4 – threshold of 0.1 nymph per middle leaf (then use spray order from START to maximise predators)	5: WHITE (untreated) T5 – no insecticides
Planting	Actara	Actara	Actara	Actara	
wk 1- Emergence 23 Nov	Avid	Sticky trap catches	Sticky trap catches	Nymph threshold (0.1)	
wk 2 – 30 Nov	Avid	0.0	0.0	0	
wk 3 – 7 Dec	Avid	1.0	1.0	0	
wk 4 – 14 Dec	Movento	1.0	1.0	0	
wk 5 – 22 Dec	Movento	1.5	1.5	0	
wk 6 – 28 Dec	Sparta	9.75 - Avid	9.75 - Avid	-	
Wk 6 – 2 Jan				0.28 – spray needed, but heavy RAIN! (not sprayed until 2 Jan) - Avid	
wk 7 – 5 Jan	Sparta	3.5	3.5	0.18 – no spray although over threshold (sampled on 4 Jan. Sprayed 2 days earlier – delay due to rain)	
wk 8 – 11 Jan	Sparta	9.0 - Avid	9.0 - Avid	0.28 – Avid	
12 Jan		9.0 – no spray because sprayed day before	9.0 - no spray because sprayed day before		
wk 9 – 18 Jan	Sparta	11.2 - Avid	11.2 - Avid	0.26 – Avid	
20 Jan		10	10		
wk 10 – 25 Jan	Karate Zeon	10+ 6.25 = 16.25 - Proteus	16.25 - Movento	0.08 – no spray	
wk 11 – 1 Feb	Karate Zeon	9.5 - Proteus	9.5 - Movento	0.02 – no spray	
wk 12 – 8 Feb	Karate Zeon	9.1 – Tamaron	9.1 - Sparta	0.26 – Movento	
wk 13 – 15 Feb	Tamaron	19 - Tamaron	19 - Sparta	0.03 – no spray	
wk 14 – 21 Feb	Tamaron	13 - Tamaron	13 - Sparta	0.12 - Movento	
wk 15 – 29 Feb	Tamaron	3.5 – no spray	3.5 – no spray	0.06 – no spray	
wk 16 – 7 Mar	Tamaron	2.3 – no spray	2.3 – no spray	0.07 – no spray	
wk 17 – 13 Mar	FINAL SAMPLE	FINAL SAMPLE	FINAL SAMPLE	FINAL SAMPLE	
wk 18					
wk 19					
wk 20					

Crop management:
All other crop production inputs e.g. ground base fertilisers (2 t/ha 8:8:8), herbicides, irrigation and fungicide programs to be the same for all treatments.

Land area required:
Crops will be grown on 0.75 m wide mounds. Plots will be 20 m (length) x 6 m (width) (8 mounds wide). There will be a 2 m unplanted buffer area between plots.

Seed tubers:
Plots will have 8 mounds of potatoes. Rows will be 75 cm apart with 30 cm between plants in the row. Number of plants per plot is 5528 x 16 plots = 8448. Number of seed tubers required for the replicated trial is 8448. Seed tubers are c. 100 g each = 845 kg seed tubers required.

Plus remote (untreated) planting: 16 mounds (12 m) x 20 m = 16 x 66 = 1056 = 105 kg

845 + 105 = 950 kg = approx. 1,000 kg = 1 Tonne.

SAMPLING METHOD:

Weekly sampling (15-16 weeks):

- At emergence, sample 8 whole plants per plot x 4 replicates = 32 plants
- When too labour-intensive, sample 8 stems per plot (1 stem from different, representative plants) x 4 reps. = 32 stems
- When TPP nymphs are found, change to sampling 25 middle leaves per plot x 4 plots = 100 middle leaves per treatment (1 leaf off each of 2 stems per plant = 12.5 plants per plot)

Action thresholds: economic injury level (EIL) or economic threshold (ET) not known. Treatment 1 spray programme will be weekly applications after 60% emergence. For treatments 2 and 3, apply foliar applications when trap catches indicate damaging flights of TPP, then the 0.1 threshold. For treatment 2, use knockdown insecticides for control of adults and nymphs. For treatment 4, insecticide applications only when crop sampling counts reach 0.1 nymphs per middle leaf (from 100 leaves). Also assess infestations of other pests and natural enemies.

Assessments:
A sub-sample of tubers used in the trial and produced from all treatments will be weighed and assessed for dry matter and ZC and a sub-sample frozen for future assessment of Liberibacter infection (however Liberibacter assessment is not in the budget).

Harvest assessments

1. 5 m x 2 mounds in centre of plot
   - Total weight
   - Marketable tubers
   - Reject tubers
2. Cooking test for zebra chip – 30 random marketable tubers.

3. Specific gravity vs zebra chip test
   - 100 random marketable tubers – specific gravity – range of salt solutions – all treatments

Milestone 4 - Psyllid National Monitoring

Jessica Dohmen-Vereijssen, Natasha Taylor, Nina Jorgensen

Executive summary

The seasonal abundance and distribution of the tomato/potato psyllid, *Bactericera cockerelli* (Sulc.) (TPP), was assessed using weekly sticky trap monitoring in commercial potato, tomato and tamarillo crops during the 2009-10, 2010–11 and 2011-12 growing seasons. At an additional two sites, weekly sticky trap and plant assessments were performed.

The most important findings and conclusions are:

- TPP numbers were highly variable between sites and regions. Generally, the North Island had higher TPP numbers than the South Island.
- In general, for both the North and South Islands, the abundance of TPP appeared to peak between early February and late March, with declining numbers towards mid-April. This means that early potatoes in the North Island may escape psyllid/*Candidatus* Liberibacter solanacearum (Lso) damage and could be grown without chemical psyllid control.
- The monitoring (both sticky trap and plant assessment) was influenced by crop management and local climate.
- Greater TPP numbers were generally found on the edge of a crop rather than in the middle. This is a similar observation to that reported from TPP monitoring programmes in the USA.
- The sticky trap monitoring programme is most useful at the beginning of the cropping season, providing an indication of when TPP could be expected to arrive in the crop. Sticky trap monitoring should be accompanied by actual plant assessments throughout the growing season to give a true indication of pest infestation in the crop.
- Sticky traps recorded psyllids 1-4 weeks earlier than plant assessments did.

Further studies should include trapping and plant assessments in unsprayed crops and development of a less labour-intensive weather-based supervised control system for TPP. In addition, this study has shown that in many cases, the first one or two insecticide applications can be omitted, as they were applied too early in the season. Education of growers regarding this would increase profits and decrease TPP insecticide resistance.

Introduction

Since 2006, solanaceous crops grown in New Zealand have been affected by an exotic insect pest, the tomato/potato psyllid (*Bactericera cockerelli* (Sulc), (Hemiptera, Triozidae) (TPP). The arrival of TPP in New Zealand and the identification of its role as a vector of the bacterial pathogen *Candidatus* Liberibacter solanacearum (Lso) have presented a considerable challenge to the New Zealand greenhouse vegetable, tamarillo, tomato and potato industries. Seasonal monitoring in New Zealand's main crop growing areas for these crops was needed to understand the population dynamics of this pest.

Insect monitoring is an important Integrated Pest Management (IPM) tool and is commonly carried out to determine presence or absence, seasonal migration and phenology of insect pests. Additionally, insect monitoring can provide important information to aid pest management decisions, such as estimating damaging population densities and evaluating the efficacy of control measures, and monitoring contributes to the development of action thresholds for insecticide applications and sustainable management.

Objectives

The aims of this project were (1) to develop simple and effective monitoring tools for the potato psyllid by comparing actual numbers of psyllids in crops (foliage samples) with less intensive sampling methods such as sticky traps and (2) using these data to understand the population dynamics (timing and magnitude of psyllid infestations) of the potato psyllid in different locations and crops (seed, processing, table) under New Zealand conditions, potentially to predict psyllid outbreaks.

Outline of the methodology

- Yellow sticky trap monitoring was conducted in commercial potato, tomato and tamarillo crops in the North and/or South Islands over three growing seasons (2009-10, 2010-11 and 2011-12).
- In the third year, sampling sites throughout the country from Northland to Southland comprised a total of 41 monitoring sites (1 tomato, 6 tamarillo and 34 potato crops). Not all sites were funded through SFF; the majority were funded through commercial, Horticulture Australia Limited or Plant & Food Research internal funding.
- All sampling was influenced by normal commercial practices (e.g., spraying) and localised environmental effects (e.g., rainfall).
- As for the previous year, two additional sites in Canterbury and in Hawke's Bay were selected for weekly trap and plant assessment (see also Berry et al. 2011, Appendix MS4, separate from this report). These assessments were made in the same field to correlate the two trapping techniques, which could simplify monitoring in the future.
- Yellow sticky traps: Double sided yellow sticky traps (BugScan®, 25 cm x 10 cm) were mounted on wire frames or stakes just above plant height. Traps were placed 5 m from the field edge and where possible at the north, south, east and west corners of a site. Traps were collected and replaced weekly.
- Plant assessment: Fifty plants per crop were assessed weekly. At first emergence, whole plants were sampled until this became too labour-intensive; then it was switched to sampling whole stems. Numbers of all TPP life stages on two whole stems (all leaves attached) per plant (each stem facing other rows) were recorded.
- Weekly monitoring data were collated by researchers at Plant & Food Research Lincoln & Hawke's Bay and submitted to Stephen Ogden/Sally Anderson for the weekly national psyllid monitoring update.

Year 3 results

The following observations were made:

- The first TPP flights were recorded early November 2011 in the North Island and late November 2011 in the South Island (Figure 1C). For both regions, TPP numbers on sticky traps were most abundant from mid-January to mid-April.
- Higher TPP numbers were recorded in the North Island than in the South Island (Figure 1C).
- Although TPP numbers were high on average, the TPP numbers in Hawke's Bay and South Auckland regions were considerably lower for the 2011-2012 season than they had been for the two previous seasons (Figure 1).
- The Northland sticky traps in tamarillo orchards recorded lower numbers of TPP than in the previous season.
- The 2011-2012 TPP numbers in Canterbury were lower than in the 2010-2011 season, but higher than in the 2009-2010 season (Figure 1).
- Given previous years' indication of TPP in the Southland region, a regular monitoring site was set up for the third year of this SFF. TPP were recorded in low numbers from early January to mid-March 2012 when the last set of sticky traps was removed (Figure 1C).

- There was no correlation between number of adults on sticky traps and any of the life stages of the whole-plant assessment. However, sticky traps recorded psyllids 1-4 weeks earlier than plant assessments did.

Combined results over the 3 years of the study

- TPP numbers were highly variable between sites and regions. Generally, the North Island had higher TPP numbers than the South Island (Figure 2).
- In general, for both the North and South Islands, the abundance of TPP appeared to peak between early February and late March, with declining numbers towards mid-April (Figure 2). This means that early potatoes in the North Island may escape psyllid/Lso damage and could be grown without chemical psyllid control.
- The monitoring (both sticky trap and plant assessment) was influenced by crop management and local climate.
- Greater TPP numbers were generally found on the edge of a crop rather than in the middle. This is a similar observation to that reported from TPP monitoring programmes in the USA.
- The sticky trap monitoring programme was most useful at the beginning of the cropping season, providing an indication of when TPP could be expected to arrive in the crop. Sticky trap monitoring should be accompanied by actual plant assessments throughout the growing season to give a true indication of pest infestation in the crop.
- Sticky traps recorded psyllids 1-4 weeks earlier than plant assessments did.

Discussion & Extension information

This work has provided insight into the population dynamics of TPP in New Zealand. It is now generally known when TPP may arrive in the crop in a specific area. This will aid growers in controlling TPP more sustainably; for example, by being able to omit the first insecticide sprays. Besides increasing profits, this will also decrease insecticide resistance development in TPP.

There were differences in psyllid numbers between the North and the South Islands, which can be explained by climate differences. However, zebra chip disease, caused by Lso, has been observed in South Island potatoes; thus control of the psyllid population is also necessary there. An important question that arises from this is how prevalent Lso is within the psyllid population that arrives in a crop. Plant & Food Research this year may obtain funding to research this.

Yellow sticky traps indicated the presence of psyllids 1-4 weeks earlier in the season than plant assessments did; this has also been shown by Cameron et al. (2009). This makes these traps a good monitoring option early in the season.

There was no correlation between the numbers of adults on the sticky traps and any of the psyllids' life stages on the plants. This can, however, be explained by the insecticide applications, which firstly disturb adult psyllids and subsequently lead to increased number of adults in sticky traps, and secondly cause mortality of part of the population. For future research, we suggest repeating these two monitoring techniques on unsprayed potato crops. However, the early-season monitoring data can also be used to look for correlations between weather parameters and first psyllid flight. If a correlation can be found, a region-specific weather-based supervised control system could be developed, which would omit the sometimes unreliable and always labour-intensive psyllid monitoring.

List of Outputs:

- Presentation given at SFF meeting 28 February 2012
- Presentation will be given by Natasha Taylor at the Psyllid Conference 26 & 27 July 2012, Ellerslie Events Centre, Auckland

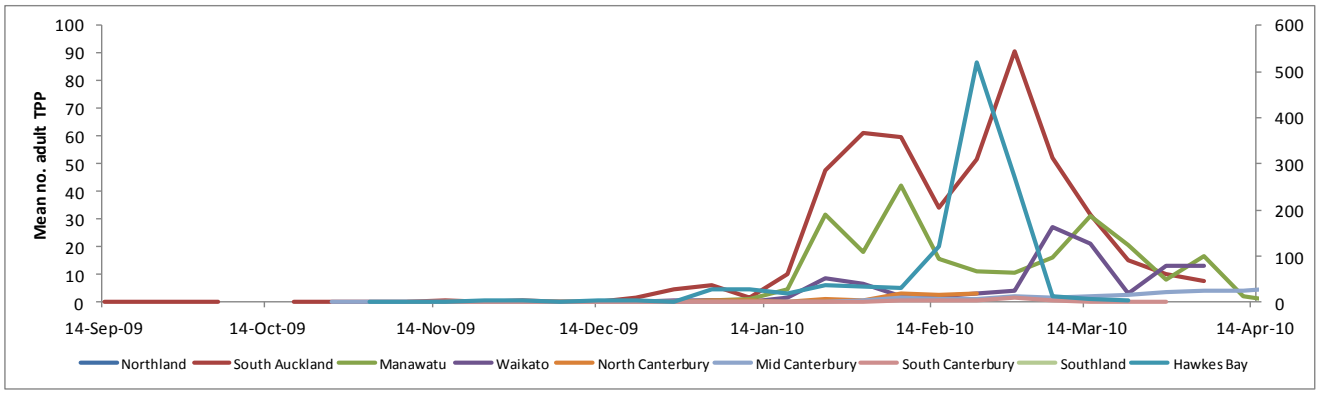
References

Berry NA, Jorgensen N, Taylor N 2011. Tomato Potato Psyllid trap and plant monitoring – SFF Support. A Plant & Food Research report prepared for Potatoes NZ. SPTS No. 6189. 20p.

Cameron PJ, Surrey MR, Wigley PJ, Anderson JAD, Hartnett DE, Wallace AR 2009. Seasonality of *Bactericera cockerelli* in potatoes (*Solanum tuberosum*) in South Auckland, New Zealand. New Zealand Journal of Crop and Horticultural Science 37: 295-301.

This milestone report has been published as:
Dohmen-Vereijssen J, Taylor N, Jorgensen N 2012. MS 4 Psyllid National Monitoring - Year 3 activity and final report. A Plant and Food Research report prepared for Market Access Solutionz Limited. SPTS No. 7265. 9p.

A



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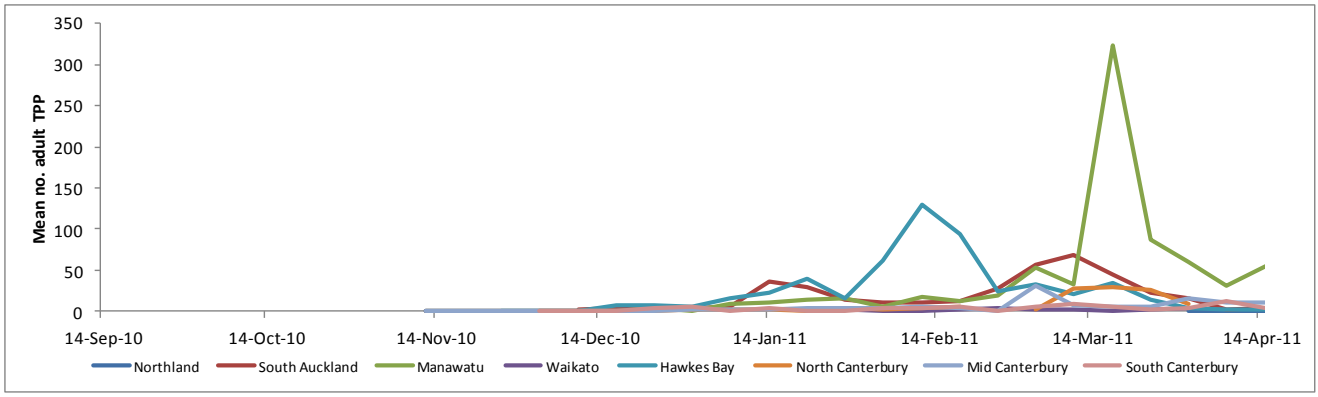

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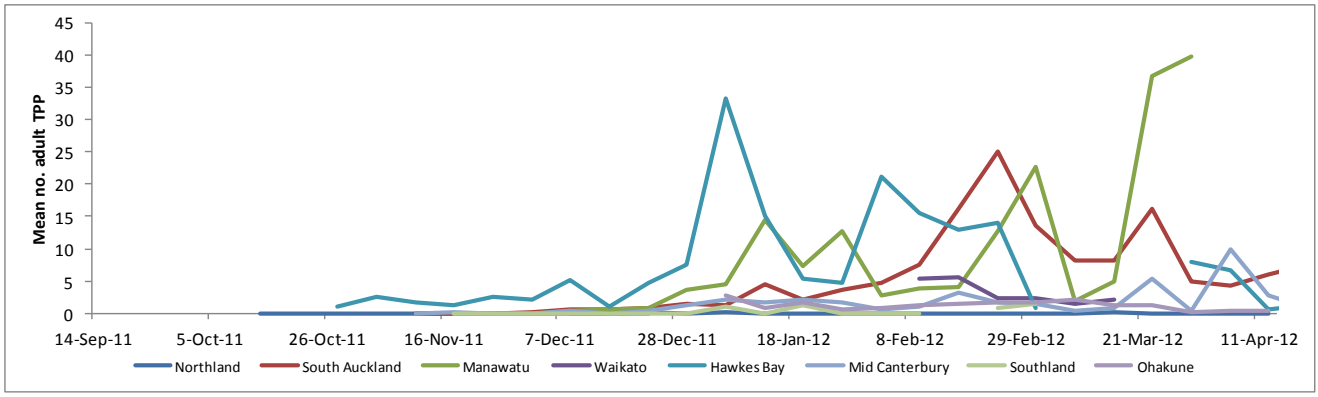
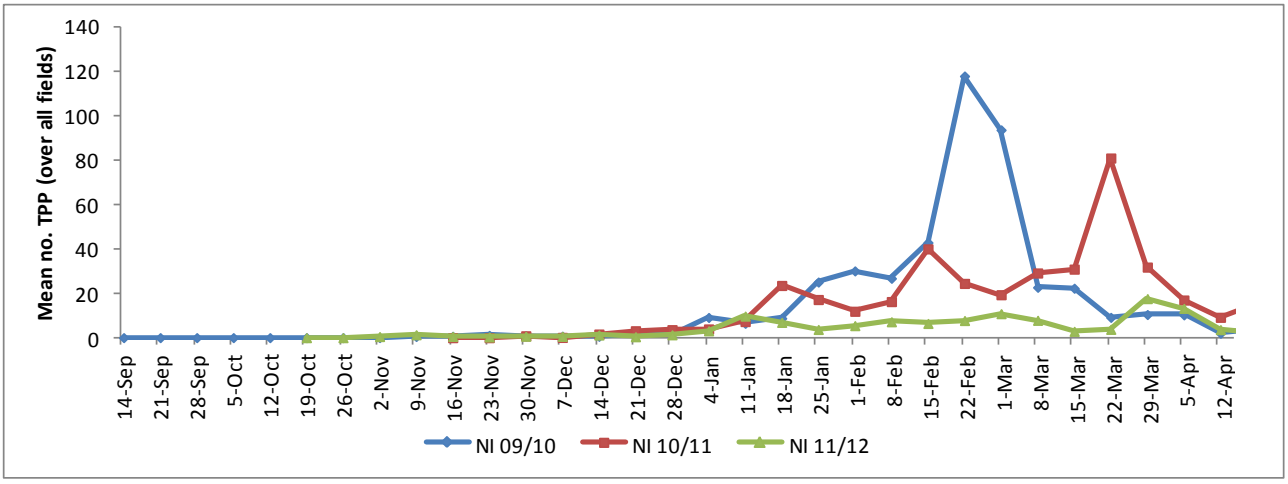


Figure 1. Adult potato/tomato psyllid yellow sticky trap catches for different sites for the (A) 2009-10, (B) 2010-11 and (C) 2011-12 growing seasons. Please note that in (A) the secondary right-hand y-axis is for Hawke's Bay only; for other regions, the primary left-hand y-axis applies.

A



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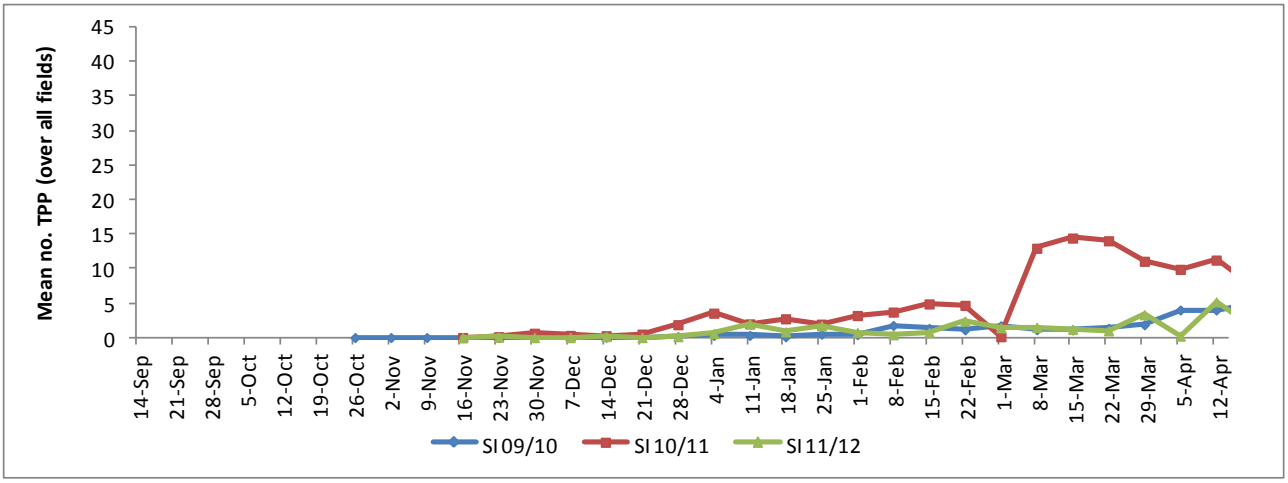

Figure 2. Mean number of adult tomato/potato psyllids trapped on yellow sticky traps averaged over all sites for each of the three monitoring seasons in the (A) North Island and (B) South Island.

Milestone 5 - Improved Liberibacter Diagnostics

Sam Beard, Farhat Shah, Ian Scott

Executive summary

- Potato plants were infected with '*Candidatus* Liberibacter solanacearum' (Lso) via Lso-positive tomato and potato psyllids (TPP) and the translocation of Lso throughout the plant over time was measured. The distribution of Lso within plants was uneven, with spatial and temporal differences. Titre was also variable between plant tissues.
- Lso could not be detected until 2-3 weeks post-infection in stolons and in the middle point of the stem. Detection did not occur until 5 weeks post-infection in petioles and in the upper portion of stems.
- These results lead to recommendations for field sampling of Lso in potato crops. First, samples should be collected from mid-point stem or stolon tissue, not foliar material or upper stems. Second, testing should be delayed until 2-3 weeks after recognised exposure to TPP. These guidelines are designed to avoid the generation of false-negative test results.
- PCR testing of TPP collected from crops may provide a more practical approach than testing plant material. TPP can be processed immediately after collection and testing TPP for Lso could provide information about the infective potential of the TPP population.

Introduction

Lso is a phloem-limited bacterium that causes economically important disease in several solanaceous crops. It is vectored by the tomato and potato psyllid (TPP). Lso cannot be cultured and therefore PCR-based methods are required for Lso diagnostics. Work completed in years 1 and 2 of milestone 5 of the SFF 09 143 project led to the development of a highly sensitive quantitative PCR (qPCR) system for the specific detection and quantitation of Lso in infected plants. However, Lso is known to be unevenly distributed in the host plant (Ian Scott, pers comm.) [1-3]. To establish a robust sampling strategy for Lso detection in field material, an understanding of the spatial and temporal distribution of Lso within the host plant is required in order to select the most appropriate sample at the correct time.

Following infection via TPP, Lso causes systemic infection throughout the host plant, spreading via the phloem [4]. However, bacterial titre varies significantly among different areas and tissues of the plant, and some sub-samples of the same plant may test positive while others test negative for the presence of Lso (Pitman, A. pers comm.) [3]. Some researchers have noted the uneven distribution of Lso within the potato plant, reporting the highest bacterial titres in root and stolon tissues (up to 100-fold higher than other tissues), with lower titres in tubers and above-ground tissues such as leaves, petioles and stem [1, 2]. In addition, Lso titre may remain below the level of detection for some weeks once infection has occurred, and the temporal distribution of Lso following infection may influence Lso titre in specific tissues. It has been suggested that Lso distribution may follow the source-to-sink movement of photosynthates [3], therefore spread to certain tissues may be influenced by the developmental stage of the plant.

No systematic studies have addressed the localisation and timing of Lso infection in multiple tissues in the host plant. A study by Levy, et al. [3] investigated the translocation of Lso in tomato and potato plants following infection via TPP caged on to a single leaf. Samples were collected weekly from each plant over an eight week period from upper-, middle- or lower-tier petioles and tested via PCR and qPCR. In tomato, Lso was detected earlier and more reliably in upper-tier petioles, with detection first occurring three weeks post-infection. Detection in middle- and lower-tier petiole occurred later and was sporadic. In contrast, no clear patterns were observed in potato among the four cultivars tested [3]. Lso

was detected in three out of four cultivars after three weeks, however there was no clear difference in detection rates in upper-, middle- and lower-tier petioles. The PCR and qPCR methods used in the study have been shown to be ~100-fold less sensitive than those developed in the first phase of milestone 5 (Levy, J., pers comm.), suggesting that a more sensitive diagnostic tool might produce different results.

In order to understand the translocation of Lso in potato a shadehouse trial was performed at PFR, Lincoln. Detection and titre of Lso was assessed using the Lso-specific qPCR tool developed in years 1 and 2 of milestone 5.

Objective

The objective of this study to was to characterise the distribution of Lso in infected potato plants over time. Plants were inoculated with Lso via infective TPP and then destructively sampled at weekly intervals. Stratified samples were taken and assessed by qPCR for the presence and titre of Lso. Plants were inoculated at either of two different time points corresponding to different growth stages of the plant in order to test the effect of growth phase and the source-to-sink movement of photosynthates on Lso translocation. The outcome of this study has informed sampling strategies for field testing of potatoes for Lso.

Outline of the methodology

Experimental design

Tubers (cv. 'Moonlight') were obtained from a 2010-2011 field trial in Pukekohe and tested via qPCR for the presence of Lso. 120 Lso-negative tubers were selected for planting. Forty-eight tubers were planted in four plots of twelve, each surrounded by a row of buffer plants (Figure 1). Twenty-four tubers were inoculated with Lso during flowering (A), and the remainder inoculated after flower drop (B). Plants were harvested at 1, 2, 3, 4, 5 and 7 weeks post-inoculation, corresponding to conditions A1-5 + A7 and B1-5 + B7. There were four replicates plants per condition (Figure 2). Two stems were allowed to develop per plant, one of which was inoculated (infected stem) and the other was not (uninfected stem).

For each plant, samples were taken from the upper petiole, upper stem, middle petiole, middle stem and stolon of each stem – 10 samples per plant. Upper petiole and stem samples were taken from the uppermost fully unfurled branch. Middle petiole and stem samples were taken from a branch at the midpoint of the stem. For each stem, all stolons were recovered and combined into a single sample. In total, 480 samples were processed.

Inoculation

Two adult TPP from an Lso-positive Lincoln colony were placed into clip cages and clipped onto a single leaf positioned 3/4 of the way up the stem. Insects were given a 72 h inoculation access period (IAC) before the clip cages were removed. Immediately after removal, plants were sprayed with Tamaron (0.83 ml/L) to remove any eggs or escaped TPP. TPP were applied to inoculation A 6 weeks after planting (4 weeks after full emergence) while plants were flowering. Inoculation B plants were infected three weeks later, after flowers had dropped (Figure 2).

DNA extraction

Plant DNA was extracted using a Cetyltrimethylammonium bromide (CTAB) protocol. Approximately 100 mg tissue was ground in a 1.7 mL centrifuge tube with a micropestle, then 550 µl 2x CTAB buffer (2% CTAB, 50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1.4 M NaCl) was added and the sample incubated overnight at 50°C. 550 µl chloroform:IAA (24:1) was added and samples agitated for 2-5 min prior to centrifugation at 8,000 rpm for 15 min. 400 µl supernatant was removed to a fresh 1.7 mL centrifuge tube containing 80 µl PEG solution (4% v/v Polyethelene glycol, 2 M NaCl). Samples were mixed by inversion, incubated at 4°C for 2 h and then centrifuged at 13,000 rpm for 10 min. The supernatant was removed to a fresh 1.7 mL centrifuge tube containing 400 µl isopropanol, inverted to mix and subsequently incubated at -20°C for 2 h. Samples were centrifuged at 13,000 rpm for 10 min, the supernatant was discarded and the pellet washed with 70% ethanol. The pellet was

air-dried and resuspended in 70 µl EB (10 mM Tris-HCL pH8.5). DNA was quantified using a Nanodrop 100 spectrophotometer (Thermo Fisher). All samples contained between 50 – 200 ng/µl gDNA.

TPP DNA was extracted using a Zygem reagents (Zygem, NZ). TPP were ground in a 1.7 mL centrifuge tube with a micropestle, then 50 µl of a reagent mix containing 1 µl *prepGEM*^{TM} enzyme, 5 µl Buffer Green and 44 µl water was added (*prepGEM*^{TM} Bacteria Kit, ZyGEM). Samples were incubated at 75°C for 60 min then at 95°C for 10 min. Samples were allowed to cool to room temperature, then centrifuged at 13,000 rpm for 2 min to pellet debris.

Quantitative PCR

Single-step seminested SYBR Green qPCR targeting the Lso 16S rRNA gene was performed in 10 µl reactions containing 1x iTaq SYBR Green Mastermix with ROX (Biorad), 1 µl template, 50 nM primer LsoF (3'-GTCGAGCGCTTATTTTTAATAGGA-5'), 300 nM of primer Lso16SF (3'-ATACCGTATACGCCCTGAGAAG-5') and 300 nM of primer Lso16SRI (5'-TCGTAGCCTTGGTAGGCATT-3'). TPP ITS2 SYBR Green qPCR was performed in 10 µl reactions containing 1x iTaq SYBR Green Mastermix with ROX (Biorad), 1 µl template, 300 nM of primer TPP ITS2F (AAAGCGACGTGTGGAAGAACC) and 300 nM of primer TPP ITS2R (GGTTGTGTGTGTCCGGGGAAG). The amplification parameters for both assays were: 95°C for 3 min, followed by 40 cycles of 95°C for 20 s, 60°C for 20 s, and 72°C for 20 s, with data capture during the 72°C step, followed by a melt curve analysis (65°C to 90°C, 0.3°C s^{-1}). The absolute quantification standard curve method was used to quantify Lso and TPP ITS2 target gene copy number.

A plasmid, pZCn, containing one copy each of the Lso 16S rRNA and TPP ITS2 target sequences was linearised with the restriction enzyme ScaI. Plasmid serial dilutions were prepared in water to specific plasmid copy numbers according to the formula: number of copies = (6.02 x 10^{23}/MW) x Mass (MW = molecular weight of linearised pZCn plasmid; Mass = mass of linearised pZCn in grams). Quantity values were calculated from the standard curve using Stepone software (v2.2.2, Applied Biosystems). All qPCR reactions were performed in triplicate – Lso qPCR samples were considered positive for Lso if amplification was observed in at least two of the triplicate wells for each sample. Averages were calculated for Cycle threshold (Ct) and Quantity (copy number) values. For potato samples, Lso titre was normalised and expressed as genome units per microgram of gDNA. Three copies of the 16S rRNA gene are present in Lso, GU/µg was calculated according to the formula: (mean of Lso quantity 16S rRNA/3) / µg plant gDNA per qPCR reaction. For TPP samples, Lso titre was normalised and expressed as Lso ratio according to the formula: (mean of Lso quantity) / (mean of TPP ITS2 quantity).

Results

Lso transmission efficiency

The efficiency of Lso transmission was measured by calculating the percentage of each replicate set of four plants with at least one sample from any tissue testing positive for Lso. 100% transmission efficiency was observed only for one condition, B3 (Figure 5). Transmission efficiencies for all other conditions ranged between 25 – 75%, even up to 7 weeks post-infection. This indicates that the inoculation protocol used was insufficient for full Lso transmission, or that a proportion of TPP within the populations used did not contain a high enough Lso titre for transmission.

At each inoculation, surplus individuals from the TPP population used for inoculation were tested for Lso by qPCR; 12 individuals were tested at inoculation A, and 5 at inoculation B (Figure 4). Substantial variation was observed in the Lso titre between TPP individuals, with up to ~1000-fold (A) and ~100-fold (B) differences in titre observed.

Visible symptoms in Lso-infected plants

Formal symptomology was not scored, however no noticeable symptoms such as leaf cupping or rolling, purpling, chlorosis or aerial tuber formation were observed. Instead, at

around 4-5 weeks post-inoculation, infected stems began to rapidly decline, showing yellowing then browning and stem death over 2-3 weeks (Figure 3).

Lso detection in infected stems

DNA was extracted from 5 different sites in the infected stem of each plant and tested by qPCR for Lso. Clear differences in the presence and titre of Lso were observed between the tissues and locations sampled. For both inoculation conditions, Lso was detected as early as 1-2 weeks post-infection in the middle stem or stolon tissues (Figure 5A, C). By three weeks post-infection (conditions A3 and B3), Lso was detected in 25 – 100% of the four replicates in both middle stem and stolon samples. By contrast, Lso was not detected in any upper petiole, upper stem or middle petiole samples until 5 weeks post infection. At 5 weeks post-infection, Lso was detected in 25 – 75% of samples in all tissues.

Lso was detected in the various tissues at comparable time points for both A and B inoculations. For middle stem and stolon samples the rate of Lso detection was 0 – 25% in conditions A1-A4, and 0 – 100% for conditions B1-B4. This suggests there may be a higher rate of early Lso detection in inoculation B.

For each replicate set of four plants, the Lso titre was averaged for each sample site (Figure 5B, D). For both A and B inoculations, the highest titre was observed in stolon tissue, with titre generally increasing with the number of weeks post-infection. The middle stem provided the next highest titre in both A and B. The lowest titres were observed in upper petiole, upper stem, and middle petiole samples with titres between ~8-300-fold lower than those observed in the stolon. All samples from condition B7 were degraded and thus titre values are not accurate.

Lso detection in uninfected stems

Lso was detected in the uninfected stems of some plants, demonstrating the translocation of Lso from infected to uninfected stem via the mother tuber. As anticipated, the rate of Lso detection in the uninfected stem was lower than for infected stems (Figure 6). An anomaly was observed for one plant in condition A1 where 4/5 samples in the uninfected stem tested positive for Lso one week post infection, while the corresponding infected stem tested negative. Excluding condition A1, at 5 weeks post-infection Lso had been detected in single samples from 3 plants (A5, B2 and B4). At 7 weeks post-infection Lso was detected in the majority of plants in both A and B (67% and 100% of plants, respectively). Lso titre was lower in uninfected stems than in infected stems, and again showed the highest titre in the stolon.

Discussion

The results of this study aid in the development of a sampling strategy for the detection of Lso in potato crops infested by TPP. The data showed that the choice of sampling site and tissue affected the likelihood of detecting Lso in infected plants. This has clear implications for field sampling of crops.

Lso could be detected as soon as 1 week post-infection, however Lso was more reliably detected 2-3 weeks post-inoculation in both inoculation conditions, consistent with the findings of Levy et al. Detection was first observed in middle stem and stolon tissue. Lso was not detected in petioles or in the upper portion of stems until 5 weeks post-infection. These results suggest guidelines for field sampling of potato crops for Lso infection in order to avoid generation of false-negative results.

- Samples should be collected from either stolon or the middle stem; petioles and upper portions of the plant should be avoided.
- Samples collected in the first three weeks following the first sighting of TPP on plants may be infected with Lso yet remain below the levels of detection by qPCR.

Given the delay before plant testing is advisable, a more practical approach might be to collect TPP on crops and determine the Lso status of the TPP population. TPP can be

processed immediately after collection and would provide information about the infective capacity of a population. Lso appears to have a low incidence in TPP populations in NZ, and often those individuals that are infected have a very low Lso titre (Pitman, A., pers comm.). It is important to note, however, that the relationship between Lso titre in TPP and disease transmission has not been fully investigated. Experimental evidence would be required to determine whether low-titre TPP may present a disease risk to crops.

No clear differences were observed between the two inoculation time points used in this study. It has been hypothesised that Lso translocation in plants may follow the source-to-sink movement of photosynthates, however no evidence to support this was observed. The first inoculation was during flowering, timed to occur during movement of photosynthates towards the growing tips of the plant. The second inoculation was after flower drop, during the tuber bulking phase where photosynthates are transported below ground. The two inoculation time points were only 3 weeks apart – the first time point may have been too late for Lso infection to coincide with significant movement of photosynthates to upper portions of the plant.

The transmission efficiency of Lso via TPP to the plant was less than 100% in all conditions with the exception of B3. This complicated the analysis, as it was not possible to distinguish between a plant that was uninfected, or infected yet below the limit of detection. The transmission efficiency may have been affected by the protocol used for TPP inoculations and/or by the variable titre of Lso in the TPP populations. Clip cages contained 2 adults TPP which were given a 72 hr IAC. A refined TPP inoculation protocol including increased number of TPP per cage could increase the transmission efficiency.

Milestone 5 three-year summary

- A cost-effective DNA extraction protocol was developed and optimised for potato material, including tubers. The protocol provided increased DNA yields and significantly reduced costs over previous commercial kit-based methods.
- A new semi-nested PCR assay for the detection of Lso in plant and TPP samples was developed. This method was approximately 10-fold more sensitive than the previously used assay and could be completed in half of the time.
- A new quantitative PCR (qPCR) assay was developed for the specific detection and quantitation of Lso. The qPCR assay showed a further 10-fold increase in sensitivity over the seminested PCR assay and allows for the determination of Lso titre in infected plant and TPP samples. This tool has important applications as the most sensitive diagnostic available for field testing, and as a research tool to further the understanding of Lso biology.
- A shadehouse trial investigating the timing and distribution of Lso within infected potato plants provided valuable information to inform strategies for field sampling of infected crops. To avoid generation of false-negative results, samples should be collected from mid-stems or stolons approximately 3 weeks after TPP are first sighted. Sampling of petiole or foliar tissue, or at earlier time points, reduces the accuracy of the test result.

Extension information – to assist with SFF reporting

An initial experimental design was proposed in which Lso-positive tubers would be planted, and then harvested at various time points and tested by qPCR for the distribution of Lso throughout the plant. Following consultation at the SFF meeting in October 2011, a modified plan was proposed in which Lso-negative tubers would be panted and then infected with Lso by TPP. Plants would then be sampled over a time course and tested by qPCR for the distribution of Lso throughout the plant.

Future experiments could address the source-to-sink hypothesis in more detail. It is possible that the inoculation time points used in this study were too late to observe preferential movement of Lso towards the growing tips of the plant. This could be addressed with earlier inoculations. The TPP inoculation procedure could be revised to improve Lso

transmission efficiency, which would give more power to the experiment and simplify interpretation. A simplified sampling strategy in which a second uninfected stem was not included would provide scope for multiple infection conditions and the inclusion of more sampling locations and tissues.

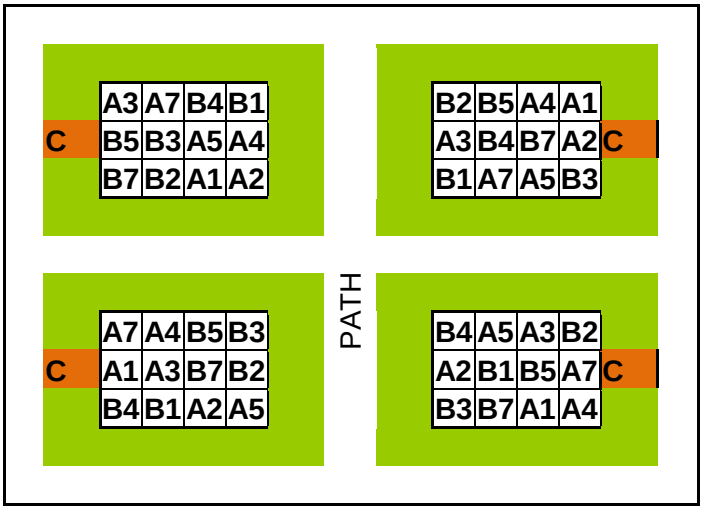

Figure 1. Plot layout. Green: buffer plants; orange: control uninfected plants.

Nov 24th	Dec 7th	Dec-Jan	Jan 12th	Jan 19th	Jan 26th	Feb 2nd	Feb 9th	Feb 16th	Feb 23rd	Mar 1st	Mar 8th	Mar 15th	Mar 22nd
Planted	Full emergence	Vegetative growth /Flowering							Flowers drop, tuber bulking, maturation				
			end IAP	A1	A2	A3	A4	A5	-	A7			
						end IAP	B1	B2	B3	B4	B5	-	B7

Figure 2. Experimental timeline.

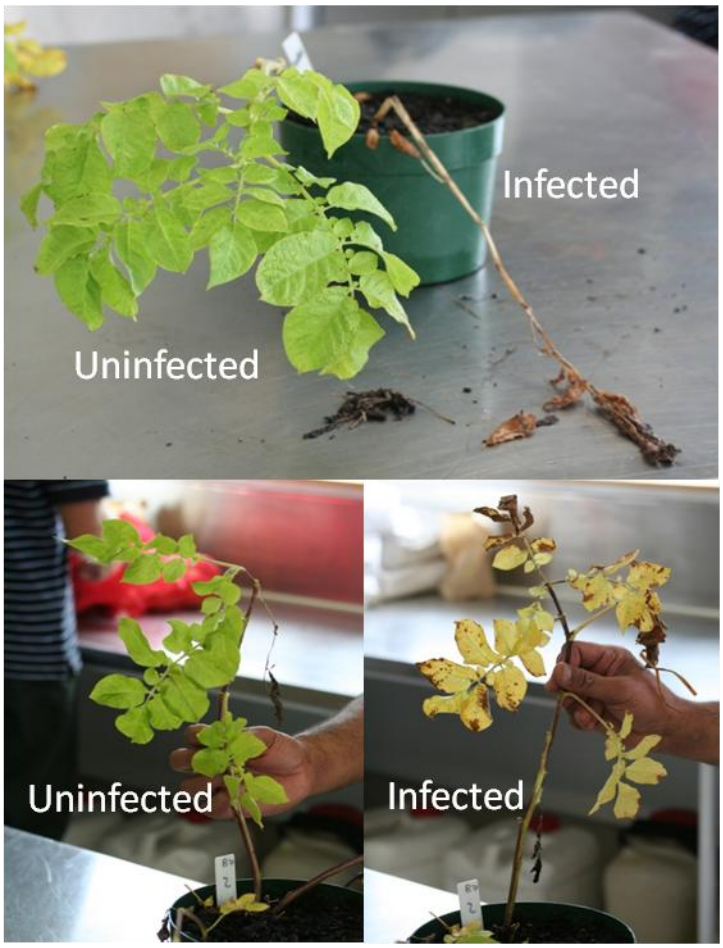

Figure 3. Visible symptoms observed in Lso-infected stems 4-6 weeks post-inoculation. Upper panel – infected and uninfected stems of plant A5-2; lower panel – infected and uninfected stems of plant B7-2.

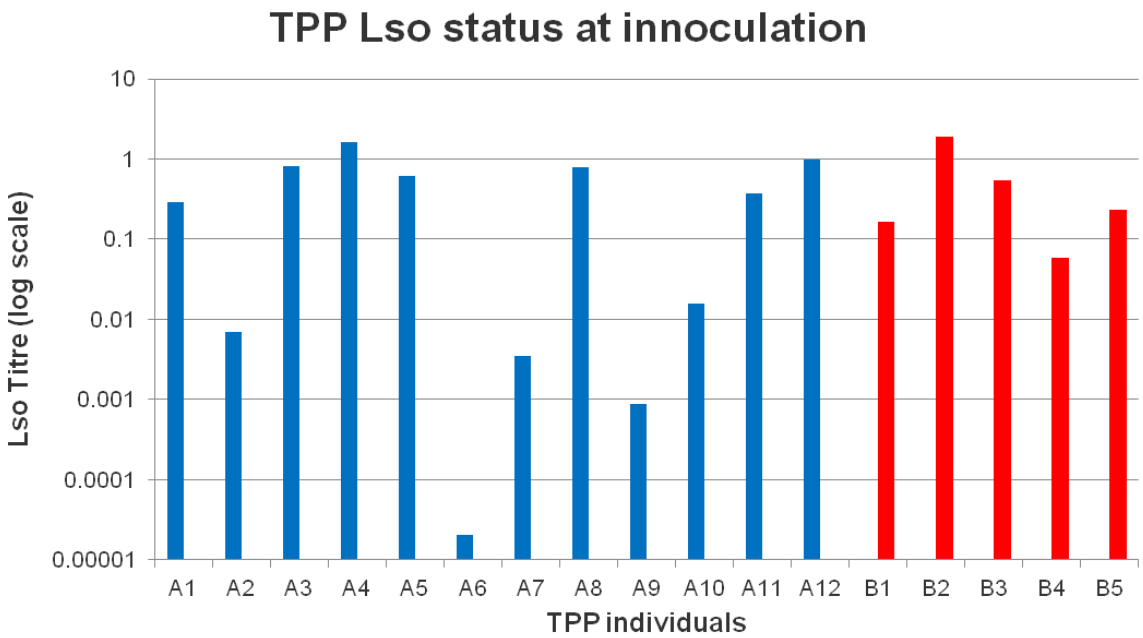

Figure 4. Lso status of TPP individuals. 12 individuals tested at time of inoculation A, 5 individuals tested at time of inoculation B.

A: percent positive Lso

	A1	A2	A3	A4	A5	A7
Infected Stem, Inoc. A						
Upper Petiole	0%	0%	0%	0%	75%	67%
Upper Stem	0%	0%	0%	0%	50%	67%
Mid Petiole	0%	0%	0%	0%	67%	0%
Mid Stem	0%	25%	25%	25%	75%	67%
Stolon	25%	0%	25%	25%	75%	67%

B: average Lso Titre (GU/µg)

	A1	A2	A3	A4	A5	A7
Infected Stem, Inoc. A						
Upper Petiole	--	--	--	--	10	32
Upper Stem	--	--	--	--	14	121
Mid Petiole	--	--	--	--	128	--
Mid Stem	--	6	271	7	30	285
Stolon	99	--	35	2	729	1052

C: percent positive Lso

	B1	B2	B3	B4	B5	B7
Infected Stem, Inoc. B						
Upper Petiole	0%	0%	0%	0%	25%	50%
Upper Stem	0%	0%	0%	0%	50%	50%
Mid Petiole	0%	0%	0%	No sample	33%	No sample
Mid Stem	0%	50%	50%	75%	75%	50%
Stolon	0%	0%	100%	75%	75%	50%

D: average Lso Titre (GU/µg)

	B1	B2	B3	B4	B5	B7
Infected Stem, Inoc. B						
Upper Petiole	--	--	--	--	8	3*
Upper Stem	--	--	--	--	7	2*
Mid Petiole	--	--	--	--	2	--
Mid Stem	--	31	277	56	24	1*
Stolon	--	--	27	168	576	11*

Figure 5. Lso qPCR results for all infected-stem samples. A, the percentage of positive samples out of the four replicate plants for each sample location and time point for the infected stem of inoculation condition A; B, the average Lso titre of the four replicate plants for each sample location and time point for inoculation A; C, the percentage of positive samples out of the four replicate plants for each sample location and time point for the infected stem of inoculation condition B; D, the average Lso titre of the four replicate plants for each sample location and time point for inoculation B.
*Sample degraded

A: percent positive Lso	A1	A2	A3	A4	A5	A7
Uninfected Stem, Inoc. A						
Upper Petiole	25%	0%	0%	0%	25%	25%
Upper Stem	33%	0%	0%	0%	0%	25%
Mid Petiole	25%	0%	0%	0%	0%	0%
Mid Stem	25%	0%	0%	0%	0%	67%
Stolon	0%	0%	0%	0%	0%	50%

B: Average of GU/µg	A1	A2	A3	A4	A5	A7
Uninfected Stem, Inoc. A						
Upper Petiole	4	--	--	--	18	14
Upper Stem	16	--	--	--	--	25
Mid Petiole	5	--	--	--	--	--
Mid Stem	89	--	--	--	--	120
Stolon	--	--	--	--	--	228

C: percent positive Lso	B1	B2	B3	B4	B5	B7
Uninfected Stem, Inoc. B						
Upper Petiole	0%	0%	0%	0%	0%	0%
Upper Stem	0%	0%	0%	0%	0%	50%
Mid Petiole	0%	0%	0%	0%	0%	50%
Mid Stem	0%	0%	0%	0%	0%	50%
Stolon	0%	25%	0%	25%	0%	100%

D: Average of GU/µg	B1	B2	B3	B4	B5	B7
Uninfected Stem, Inoc. B						
Upper Petiole	--	--	--	--	--	--
Upper Stem	--	--	--	--	--	0.7*
Mid Petiole	--	--	--	--	--	0.6*
Mid Stem	--	--	--	--	--	0.7*
Stolon	--	2.4	--	7.5	--	8.8*

Figure 6. Lso qPCR results for all uninfected-stem samples. A, the percentage of positive samples out of the four replicate plants for each sample location and time point for the uninfected stem of inoculation condition A; B, the average Lso titre of the four replicate plants for each sample location and time point for inoculation A; C, the percentage of positive samples out of the four replicate plants for each sample location and time point for the infected stem of inoculation condition B; D, the average Lso titre of the four replicate plants for each sample location and time point for inoculation B.
*Sample degraded

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2. Li, W.B., et al., *Multiplex real-time PCR for detection, identification and quantification of 'Candidatus Liberibacter solanacearum' in potato plants with zebra chip.* Journal of Microbiological Methods, 2009. 78(1): p. 59-65.
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Milestone 6 - Soft Chemicals in the Greenhouse Industry

Melanie Walker and Nadine Berry

The work described in this milestone has been published in two reports:

Walker MK, Butler RC, Berry NA 2010. Evaluation of selected soft chemicals as potential control options for tomato/potato psyllid. A Plant & Food Research report prepared for Horticulture New Zealand. SPTS No. 3937. 12p.

Berry NA, Bourhill A 2012. Review of soft chemical options and research for insect pest control. A Plant & Food Research report prepared for Potatoes New Zealand. SPTS No. 6065. 18.

Please refer to Appendix MS6-1 and 6-2 for the full reports, which are separate files to this document.

Change of milestone, October 2011:
A review of the Soft Chemical milestone (Milestone 6) by the tomato and capsicum industry prior to the October 2011 project planning meeting identified that a continuation of the soft chemical research would be of little commercial benefit. As a result, the project team agreed to terminate the soft chemical research milestone and redirect the remaining budget to support the Entomopathogen research (Milestone 8).

Milestone 7 - Host Range Testing of BCA (*Tamarixia triozae*)

Robin Gardner-Gee

Executive summary

Bactericera cockerelli is a North American pest species known in New Zealand as the tomato/potato psyllid (TPP). First reported in New Zealand in 2006, it has now become a major pest on both greenhouse and outdoor solanaceous crops in New Zealand. Searches conducted between 2006 and 2008 failed to identify any natural enemies within New Zealand that were likely to control TPP on tomatoes, so in 2009 a North American parasitoid, *Tamarixia triozae,* was imported into quarantine facilities at the Mt Albert Research Centre, Auckland, for assessment as a biological control agent. Host-range testing has been carried out to evaluate the potential for *T. triozae* to impact negatively on non-target psyllid species in New Zealand. *Tamarixia triozae* did not oviposit on six of the eight non-target psyllid species it was exposed to in no-choice screening tests. *Tamarixia triozae* did oviposit on two native psyllid species, *Trioza curta* and *Trioza panacis*. However, the oviposition rate on both was lower than the oviposition rate on the target pest TPP. In addition, no *T. triozae* adults emerged from parasitized *T. curta*, suggesting that *T. triozae* would not be able to maintain itself over time in situations where *T. curta* was the only host available. *Tamarixia triozae* did emerge from parasitized *T. panacis* nymphs but the first generation female parasitoids from *T. panacis* had reduced ability to produce further offspring compared with parasitoids that emerged from their usual host (TPP). Testing indicates that *T. triozae* will attempt to use novel species as hosts, and that *T. triozae* is capable of developing in at least some of these novel species. Consequently species such as *T. panacis* (and possibly other native psyllid species) could act as alternative hosts to TPP for *T. triozae.* The impacts of this non-target parasitism are unlikely to be severe for widespread and abundant psyllid species, but rare species may be vulnerable.

Introduction

Description of the target pest and potential biological control agents

The tomato/potato psyllid (*Bactericera cockerelli* (Sulk) Hemiptera: Triozidae) is a North American pest that was first reported in New Zealand in 2006 (Teulon et al. 2009). This psyllid has been found to vector the bacterial pathogen *Candidatus* Liberibacter solanacearum (Liefting et al. 2009) and has now become a major pest on both greenhouse and outdoor solanaceous crops in New Zealand (Teulon et al. 2009). The presence of the tomato/potato psyllid (TPP) has disrupted existing integrated pest management (IPM) programmes, and effective biological control agents (BCAs) are urgently required to restore IPM in solanaceous crops. Since 2006 Crop & Food Research (now Plant & Food Research) has searched intensively for potential BCAs, both within New Zealand and overseas. This research effort is summarised below.

In December and January 2006–07 Crop & Food Research conducted searches around Auckland for natural enemies of the common native psyllid *Trioza vitreoradiata* which feeds on a widespread native tree (karo; *Pittosporum crassifolium)* (Workman et al. 2006; Pedley & Workman 2007). Seven natural enemies (six predators and one parasitoid) of *T. vitreoradiata* were found to be common at the 10 sites surveyed in Auckland. Laboratory trials indicated that all the predators readily fed on TPP but the parasitoid did not attack TPP. The three most promising predators (two lady bird beetle species and a lacewing species) were then used in a small-scale glasshouse trial to investigate their ability to control TPP infestations on capsicum and tomato plants. All three predators were effective at reducing the number of psyllids on capsicum but only the lacewing (*Drepanacra binocular*) appeared to have potential as a BCA on tomato, as the two ladybird species trialled avoided going onto the tomato plants.

In 2007 Crop & Food Research undertook a literature review (for Horticulture New Zealand) evaluating natural enemies already occurring in New Zealand and those present overseas that could be useful for covered crops in New Zealand (Workman & Davidson 2007). The review found that, in addition to the natural enemies already in New Zealand, four overseas species had potential to provide effective biological control of TPP. These were a predatory mirid (*Dicyphus hesperus*), two predatory green lacewings (*Chrysoperla carnea* and *C. rufilabris*) and a parasitic wasp (*Tamarixia triozae*).

Following this review, Crop & Food Research undertook a glasshouse trial to determine the ability of five natural enemies (that already occur in New Zealand) to adapt to greenhouse conditions and control TPP in covered crops of capsicums and tomatoes (Workman 2008). The greenhouse trial results were similar to the earlier small-scale trials, as once again the natural enemies reduced TPP on capsicums, but failed to achieve any control of TPP on tomatoes. Consequently, research effort focused on overseas species that have potential to control TPP on tomatoes. In 2008 a Horticulture NZ application was approved to import 10 arthropod species (*Eretmocerus mundus, Delphastus catalinae, Dicyphus hesperus, Macrolophus caliginosus, Amblyseius swirskii, Amblyseius degenerans, Typhlodromips montdorensis, Orius laevigatus, Tamarixia triozae,* and *Chrysoperla carnea*) into containment. These species are used against a range of greenhouse pests overseas and it was intended that at least some of the species would be imported into containment for host specificity tests and evaluation as biological control agents for the New Zealand greenhouse industry. Of these species, *Tamarixia triozae* was selected as the most promising BCA for TPP on tomatoes and was imported into quarantine on 27 February 2009.

Description of the proposed biological control agent Tamarixia triozae (Burks 1943) (Hymenoptera: Eulophidae)

In 1939 parasitism was observed on TPP on uncultivated hosts in North America and attributed to a hymenopteran parasitoid in the *Tetrastichus* genus (Eulophidae) (Romney 1939). The species was described 1943 by Burks as *T. triozae* and has subsequently transferred to *Tamarixia* by Boucek (1988). *Tamarixia* species are primary parasitoids of Psylloidea (La Salle 1994), although reports of non-psyllid hosts do exist (Zuparko et al. 2011). *Tamaraxia triozae* is a small wasp (0.7–1.05 mm in length) that is an ectoparasitoid of psyllids. Adult female *T. triozae* typically lay a single egg on the ventral surface of the host and the larva develops as an external parasitoid beneath the body of the host. It is widespread in North America (Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, New Mexico, Texas, Washington) and more recently has been recorded from Mexico (Lomeli-Flores & Bueno 2002; Zuparko et al. 2011).

Efficacy of agent

Early field observations in the USA suggested *T. triozae* was unlikely to achieve control of TPP in outdoor crops because of poor synchronization between the psyllid and the parasitoid, high parasitoid pupal mortality and patchy establishment within crops (Pletsch 1947; Johnson 1971). Parasitism rates of TPP by *T. triozae* were below 20% in southern California fields (Butler & Trumble 2012). However, surveys of unsprayed pepper crops in Oaxaca, Mexico, have found that *T. triozae* can achieve over 80% parasitism of TPP (Bravo & Lopez 2007; cited in Luna Cruz 2010). In addition, laboratory studies point to the potential of *T. triozae* as a BCA in some crops, as these studies indicate that the life cycle of *T. triozae* is almost half the time of its host on peppers (P. Workman unpublished data; Rojas et al. 2009; Rojas 2010) and that *T. triozae* causes host death through feeding as well as through parasitism (P. Workman unpublished data; Rojas et al. 2009; Vega 2010). Liu et al. (2012) suggest that releases of commercially reared *T. triozae* into outdoor crops late in the growing season could be feasible, providing some level of non-chemical control at a time when transmission of Liberibacter is less damaging to crop yields.

In New Zealand most fresh pepper and tomato crops are grown under cover, rather than outdoors, hence the potential use of *T. triozae* within glasshouses is of interest. Overseas, inundative releases of *T. triozae* have been undertaken within glasshouses, with anecdotal reports of successful control, and *T. triozae* is sporadically available from commercial insectaries. For example, *T. triozae* was commercially produced in Canada, until demand for it lessened due to a decline in psyllid populations, at which time the parasitoid became uneconomic to produce (D. Gillespie, pers. comm.). In Canada, *T. triozae* reportedly provided effective control of psyllids on capsicum greenhouse crops, provided the pest was detected early and sufficient numbers of the parasitic wasp were released (Elmhirst 2005). *Tamarixia triozae* is the only biological control agent that has been commercially reared specifically for use against TPP in glasshouses.

Source of agent

Tamarixia triozae was imported by Plant & Food Research into containment at the quarantine facility at Mt Albert Research Centre (MARC), Auckland, on 27 February 2009. Approval to import *T. triozae* was obtained under the HSNO Act, 1996 and HSNO Order, 1998 (ERMA Approval Code: NOC002530-39) and the Biosecurity Act, 1993 (MAF Biosecurity, Permit to Import Live Animals: 2008035896). Specimens were imported from Koppert Mexico (S.A.de C.V. Av. Del marquee # 38-1, Parque Industrial Bernardo Quintana (3rd Etapa), Municipico El Marques, 76246 Queretaro, Mexico). On the arrival of *T. triozae* into the Plant & Food Research quarantine facility, samples of 10 females and 10 males were placed in 95% ethanol and deposited with the New Zealand Arthropod Collection (NZAC), Landcare Research, Auckland, New Zealand. Further samples of 10 females and 10 males were sent to Dr Ian Scott, Plant & Food Research, Lincoln, for molecular analysis. A sample of 100 *T. triozae* (bred from the initial imported stock) was supplied to Dr Louise Malone, Plant & Food Research, Auckland, in April 2009 for standard examination for internal pathogens. There were no signs of any viruses, fungi, or other pathogens in any of the smears that were made from the insects. Since importation, *T. triozae* have been maintained in containment on TPP nymphs reared in non-quarantine glasshouse colonies at MARC.

Hosts in the native range of agent

Tamarixia triozae is a widespread parasitoid, occurring in many arid or semi-arid regions through North America and also in Mexico (La Salle 1994; Lomeli-Flores & Bueno 2002). It attacks psyllids from a number of families (Zuparko et al. 2011). At the species level, psyllids typically exhibit very narrow host-plant ranges (Burckhardt 1994). However, some hosts used by *T. triozae* are notable exceptions; TPP, for example, can complete development on at least 40 host-plant species (Wallis 1955). In addition, the range of psyllid hosts used by *T. triozae* means that the parasitoid is found in association with a wide range of plants with varying growth forms (Table 1).

TPP is the most well-studied of the species parasitized by *T. triozae*. TPP nymphs typically feed on the undersides of leaves of their host-plant and seldom move. Other psyllid species attacked by *T. triozae* feed on flower buds (e.g. *Calophya californica*), or on woody branches (e.g. *Calophya nigrella*) (Jensen 1957). Many of the species parasitized by *T. triozae* have free-living nymphs but *Euphalerus vermiculosus* nymphs form a waxy cell that completely surrounds them (Jensen 1957).

Table 1: Psyllid hosts of the parasitoid *Tamarixia triozae* and the host-plants used by the psyllid species (data from Wallis 1955; Jensen 1957; Zuparko et al. 2011; Ouvrard 2012).

Psyllid family	Psyllid species	Host-plant family	Host-plant species	Host-plant details
Calophyidae	*Calophya californica*	Anacardiaceae	*Rhus integrifolia* *Rhus ovata*	Genus members are typically shrubs and small trees growing 1–10 m tall
	Calophya nigrella	Anacardiaceae	*Rhus trilobata*	
	Calophya nigripennis	Anacardiaceae	*Rhus capallina*	
	Calophya triozomima	Anacardiaceae	*Rhus trilobata*	
Psyllidae	*Ceanothia ceanothi*	Rhamnaceae	*Ceanothus tomentosus*	Genus members are typically shrubs growing 0.5–3 m tall
	Euglyptoneura minuta	Rhamnaceae	*Ceanothus crassifolius*	
	Euphalerus vermiculosus	Rhamnaceae	*Ceanothus leucodermis*	
	Pexopsylla cercocarpi	Rosaceae	*Cercocarpus betuloides* *Cercocarpus ledifolius*	Genus members are deciduous shrubs or small trees, growing 3–6 m tall
Triozidae	*Bactericera cockerelli*	Solanaceae Convolvulaceae Lamiaceae	40 + host species including many solanaceous crop species such as potato, tomato and eggplant	Hosts include vines, shrubs and herbaceous plants
	Bactericera minuta	Salicaceae	*Salix exigua* *Salix lasiandra* *Salix lasiolepsis* *Salix longifolia*	Genus members are deciduous trees and shrubs, normally growing in moist soils
	Bactericera nigricornis	Asteraceae, Apiaceae Brassicaceae, Liliaceae, Solanaceae	Various including onion, potato and carrot	Various growth habits including herbaceous plants
	Trioza albifrons	Urticaceae	*Urtica* sp.	Genus members are typically annuals or perennial herbaceous plants
	Trioza beameri	Rhamnaceae	*Rhamnus californica*	Evergreen shrub growing to 2–5 m tall

Description of psyllid fauna in New Zealand

Psyllids belong to the superfamily Psylloidea, which is relatively well documented in New Zealand (Tuthill 1952; Dale 1985). Of the six families of Psylloidea, two families (Phacopteronidae and Carsidaridae) are not found in New Zealand and another two (Calophyidae and Homotomidae) are each represented by only a single adventive species (P. Dale, pers. comm.). Of the two remaining families, Psyllidae is dominated by adventives

(34 species of which 23 are adventives) whereas Triozidae has 51 species, 50 of which are endemic and only one of which is adventive (P. Dale, pers. comm.). New Zealand psyllids feed on many native plant genera, including *Alseuosmia, Carmichaelia, Dacrydium, Discaria, Dodonaea, Fuchsia, Pseudopanax,* and *Schefflera.* They are not associated with host deaths but some disfigure their hosts, causing pitting and yellow streaks to appear on distorted leaves. More than half of the New Zealand psyllids occur on small trees and shrubs of open country, with 17 of these open country species occurring in lowland-subalpine areas and another 25 more occurring from lowland to alpine areas (Dale 1985). Many are found on seral-stage plant hosts (e.g. *Kunzea ericoides*) (Dale 1985). Very few are found on large trees or in mature forests (Dale 1985). Most (57%) are widespread, occurring in all three main islands. Diversity increases towards the south, and one third of species do not occur in the North Island (Dale 1985). Overall the New Zealand psyllids are predominantly a cold-adapted shrub-land fauna, with a relatively small northern sub-tropical element (Dale 1985). Correspondingly, most are not active all year round. However, a few will oviposit all year round if suitable foliage is available (e.g. *Trioza curta, T. vitreoradiata, T. panacis* and *Ctenarytaina* species).

Psyllid species in New Zealand of value as biological control agents or of cultural or conservation value

No psyllid species are recorded as having specific cultural value in New Zealand, but there is one beneficial exotic psyllid species. The broom psyllid *Arytainilla spartiophila* (Psyllidae) has been introduced into New Zealand as a biological control agent for Scotch broom (*Cytisus scoparius*). The broom psyllid was imported from England by the DSIR in 1992, and released throughout New Zealand in the mid-1990s (Hayes 2005). It is now widespread and common through both the North and South Islands (Hayes 2005).

The 2001 publication “Conservation requirements of New Zealand’s nationally threatened invertebrates” did not list any psyllids as threatened or in need of conservation attention (McGuinness 2001). However, in a more recent revision of the conservation status of New Zealand Hemiptera two psyllid species have been designated “Threatened : Nationally Critical”, the highest category in current threat classification system (Stringer et al. 2012). The first of these is an undescribed species of *Anomalopsylla* (Psyllidae) that has only been collected from a single plant of *Olearia solandri* at Port Underwood, east of Picton, northern South Island. This single plant was last searched for psyllids in 1982 and at that time the plant was at risk from gorse encroachment (P. Dale, pers. comm.). It is possible that the plant and the associated psyllid population have been lost in the subsequent three decades (P. Dale, pers. comm.). However, the host-plant species *O. solandri* is widespread and it is possible the *Anomalopsylla* species may be present on other *O. solandri* plants in the area (P. Dale pers. comm.).

The second Nationally Critical psyllid species, *Psylla* aff. *carmichaeliae* (Psyllidae), is also undescribed and is also known from only a single location, Woodside Creek, Marlborough, northern South Island. The psyllid population was examined most recently in 2008 (P. Dale, pers. comm.). The host-plant is *Carmichaelia torulosa*, and the conservation status of this plant species is “Threatened: Nationally Endangered” (Stringer et al. 2012). A third psyllid species, *Gyropsylla zealandica* (Psyllidae), has been placed in the “At Risk: Naturally Uncommon (Sparse)” category (Stringer et al. 2012). This is the largest psyllid in New Zealand but only a few specimens (15) have ever been collected (Dale, 1985). All records are from southern New Zealand, with locations ranging from 1220 m asl in the western South Island to near sea level on Stewart Island (Dale 1985). In addition, many New Zealand psyllid species are endemic to New Zealand and therefore have scientific value as local products of evolution.

Aims of the study

In New Zealand the importation and release of new organisms (including biological control agents) is regulated by the Hazardous Substances and New Organisms (HSNO) act (1998), administered by the Environmental Protection Authority (EPA). The purpose of the HSNO act is to "protect the environment, and the health and safety of people and communities, by preventing or managing adverse effects of.... new organisms". The EPA is required to consider the effects that a new organism is likely to have on native species (and on valued introduced species), and is directed to decline any application for the release of a new organism if significant displacement of a native species will occur as a result of the release. Given this regulatory framework, assessment of potential non-target impacts is an important part of any application for release of a new organism in New Zealand. *Tamarixia triozae* has not been released as a classical BCA outside its native range before and little is known about its response to novel psyllid species. Given the wide distribution of *T. triozae* in its home range, it was assumed from the outset that *T. triozae* would be able to establish outdoors in at least some areas of New Zealand and hence the parasitoid could encounter non-target psyllid species outside the glasshouse or crop environment. The aims of the present study were to expose *T. triozae* to a range of psyllid species that occur in New Zealand and determine what, if any, non-target impacts *T. triozae* was likely to have if released into the New Zealand environment.

Methods

Development of species list for host range testing

Within weed biocontrol the centrifugal-phylogenetic approach to host range testing is well established and research has shown that the more closely related a non-target plant species is to the target weed species, the more likely it is to be attacked by exotic insects introduced to control the weed (Pemberton 2000). This approach is of less value, however, when assessing the risk of entomophagous insects introduced to control other insects, as insect phylogenies are often poorly understood and host utilisation by entomophagous insects is often not determined, or is poorly predicted, by taxonomic affinities (Hoddle 2004). Kuhlmann et al. (2006) analysed a number of host-range testing programmes for entomophagous insects, and concluded that although phylogeny was a valuable starting point for assessing host range, other criteria were as important. Kuhlmann et al. proposed that species lists for host testing of entomophagous insects should be assembled by considering species that fall into the following three categories: 1) ecologically similar species (i.e. species that live in the same the habitat as the target species, or in habitats immediately adjacent to the agricultural system used by the target species, species that share the same host-plants as the target species); 2) phylogenetically related species; 3) safeguard species (i.e. species that are beneficial, rare native species especially if related to the target species and/or species that are attacked by congeners of the entomophagous insect under consideration). The initial list that results may then need to be reduced using practical considerations (e.g. accessibility of species, ease of rearing) and further ecological filters (e.g. phenological asyncronization) (Kuhlmann et al. 2006).

Following this approach, an initial species list was established that contained all native members of Triozidae and Psyllidae, as *T. triozae* attacks a range of psyllid species within these families in its home range and the target pest (TPP) is a member of Triozidae. One exotic psyllid species, *Arytainilla spartiophila* (Psyllidae), was added to the list as this species has been introduced into New Zealand as a weed biological control agent. The resulting list of 68 species was then reduced by focusing on lowland native psyllid species (from both Psyllidae and Triozidae), as these species could occur in habitats adjacent to agricultural crops attacked by TPP (e.g. forest reserves, roadside margins, amenity plantings, abandoned agricultural land). The final list consisted of seven native species (Table 2) plus *A. spartiophila.* The seven native species represent the major taxonomic groups within the native psyllid fauna and all were easily collected within the Auckland

region, an area where TPP is a widespread pest. Three species were of particular interest (*Trioza curta, Trioza panacis* and *Trioza vitreoradiata*) as they are parasitized in the wild by another *Tamarixia* species already present in New Zealand (R. Gardner-Gee, unpublished data).

Table 2. Seven native psyllid species and their common hosts. Combinations marked with * were used in host specificity tests.

Psyllid family	Psyllid species	Host-plant family	Host-plant species	Host-plant details
Psyllidae	*Acizzia dodonaeae*	Sapindaceae	*Dodonaea viscosa**	Evergreen shrub growing to 1–3 m
	Ctenarytaina clavata	Myrtaceae	*Leptospermum scoparium**	Evergreen shrub and trees growing to 2–5 m tall
			Kunzea ericoides	
	Psylla apicalis	Fabaceae	*Sophora chathamica*	Genus members are small trees and shrubs
			Sophora fulvida	
			Sophora tetraptera	
			Sophora sp.*	
Triozidae	*Trioza curta*	Myrtaceae	*Syzygium maire*	Trees, up to 25 m
			*Metrosideros excelsa**	
			Metrosideros robusta	
			Metrosideros umbellata	
	Trioza "ohumata" (an undescribed species)	Asteraceae	*Brachyglottis kirkii**	A forest epiphyte or ground shrub to 3 m tall
	Trioza panacis	Araliaceae	*Pseudopanax crassifolius*	Shrubs and small trees, occurring in forest or scrub environments
			Psuedopanax discolor	
			Pseudopanax ferox	
			*Pseudopanax lessonii**	
	Trioza vitreoradiata	Pittosporaceae	*Pittosporum colensoi*	Genus members are trees and shrubs growing to 2–30 m tall
			*Pittosporum crassifolium**	
			Pittosporum eugenioides	
			Pittosporum tenuifolium	

The native psyllid species selected utilise native host plants from six families, two of which (Asteraceae and Fabaceae) contain species that are recorded as host plants of TPP in the USA (Wallis 1955). However, there are no confirmed reports of TPP breeding on plants within these families and it is likely that the records refer to adult feeding rather than breeding (Wallis 1955; Martin 2008). Within the USA, TPP breeds almost exclusively on plants in the family Solanaceae; the only other plants it is known to breed on in the wild are within the Convolvulaceae family (Wallis 1955). Laboratory studies indicate that TPP can also complete development on *Micromeria chamissonis* (Lamiaceae) (Wallis 1955; Martin 2008). Within New Zealand, TPP has been recorded breeding only on plants in the Solanaceae and Convolvulaceae families (Martin 2008). No native psyllids utilise these two families in New Zealand (Dale 1985).

Source of psyllids used in host range tests

Nymphs from multivoltine species (*Acizzia dodonaeae, Trioza curta, T. panacis, T. vitreoradiata*) were collected from the field on host-plant foliage and placed into holding containers for 1–3 weeks. Foliage stems were placed into water to maintain freshness. Adult psyllids that emerged during this time were transferred to large mesh cages (700 x 700 x 700 mm) containing clean potted specimens of their host plant and reared through multiple generations in unheated glasshouse units. Nymphs from these colonies were used as required for host-range testing. Univoltine species and some multivoltine species could not be effectively reared in this manner (*Arytainilla spartiophila, Ctenarytaina clavata, Psylla apicalis, Trioza* "ohumata"). For these species the nymphs used in testing were collected directly from the field. When direct use of field-collected nymphs was necessary, a sub-sample of each collection was placed into holding containers and monitored for the emergence of native parasitoids. This precaution was necessary due to the presence in New Zealand of at least one *Tamarixia* species, the eggs of which are indistinguishable from *Tamarixia triozae* eggs. *Tamarixia* adults did not emerge from any of the sub-samples from collections used for testing.

Identification of psyllids in the field was based on host-plant association and general morphology. However, nymphs and/or adults were collected from all the colonies and collections used in the testing programme and stored in alcohol for further examination. All specimens were examined by a psyllid taxonomist, Pam Dale, and all were found to be correctly identified.

General description and justification of host range tests

Oviposition tests

A range of choice and no-choice oviposition tests were carried out in small cages to gain information about the host range of *T. triozae* (Figure 1). Tests of this type are routinely used in host specificity testing, especially in containment situations where space limitations preclude large-scale tests (Van Driesche & Murray 2004). All tests were undertaken in containment conditions at 22ºC, with a 16:8 h photoperiod.

Preliminary no-choice tests were carried out to determine appropriate test conditions. Eight cages (vented "cookie jars") were established, each with three female *T. triozae* (3–10 days old, probably mated). Each cage contained either approximately 50 non-target psyllid nymphs (4^{th} and 5^{th} instars) on shoots of their host plant or 50 TPP nymphs (4^{th} and 5^{th} instar) on capsicum leaves. Foliage stems were placed in small vials of water and the vial necks were stopped with cotton wads to prevent the parasitoids drowning in the water. Psyllid nymphs used in these tests were sourced from laboratory colonies that were free of any parasitoids. Each cage was maintained for at least 7 days and every 2–3 days the old foliage was removed and fresh foliage (with psyllid nymphs) was added. The number of parasitoids was counted at each foliage change to ensure no mortality had occurred. Once removed from the cage, foliage was examined under binocular microscope and each psyllid nymph was inverted and examined for the presence of parasitoid eggs. In these eight cages, the initial 48-h results were the same as the 7-day results (i.e. if oviposition occurred at all, it was detected after 48 h). In all eight tests with TPP, *T. triozae* oviposited on TPP within 48 h. In the eight cages with non-target psyllids, oviposition was only detected in one of the cages, and in this case the oviposition occurred within 48 h. On the basis of these results the cage set-up described above was used in all subsequent screening tests and oviposition was checked after 48 h.

The screening no-choice tests were undertaken to determine if *T. triozae* would oviposit on non-target species in the absence of the target species (TPP). Eight psyllid species were tested (Table 2). For each psyllid species, a minimum of 15 cages with non-target psyllids were set up, along with 15 cages with TPP nymphs only (control cages). When possible, six cages were set up simultaneously (three with non-target psyllids, three with TPP) using the

same cohort of *T. triozae*. If oviposition failed to occur in a control cage, the results from all cages using that cohort were discarded. To investigate *T. triozae* responses to non-target psyllids in more detail, one sequential no-choice test was also carried out using *T. curta* as the non-target species. Three female parasitoids were placed in a cage as per usual, and were then offered either TPP nymphs or *T. curta* nymphs over a 15-day period.

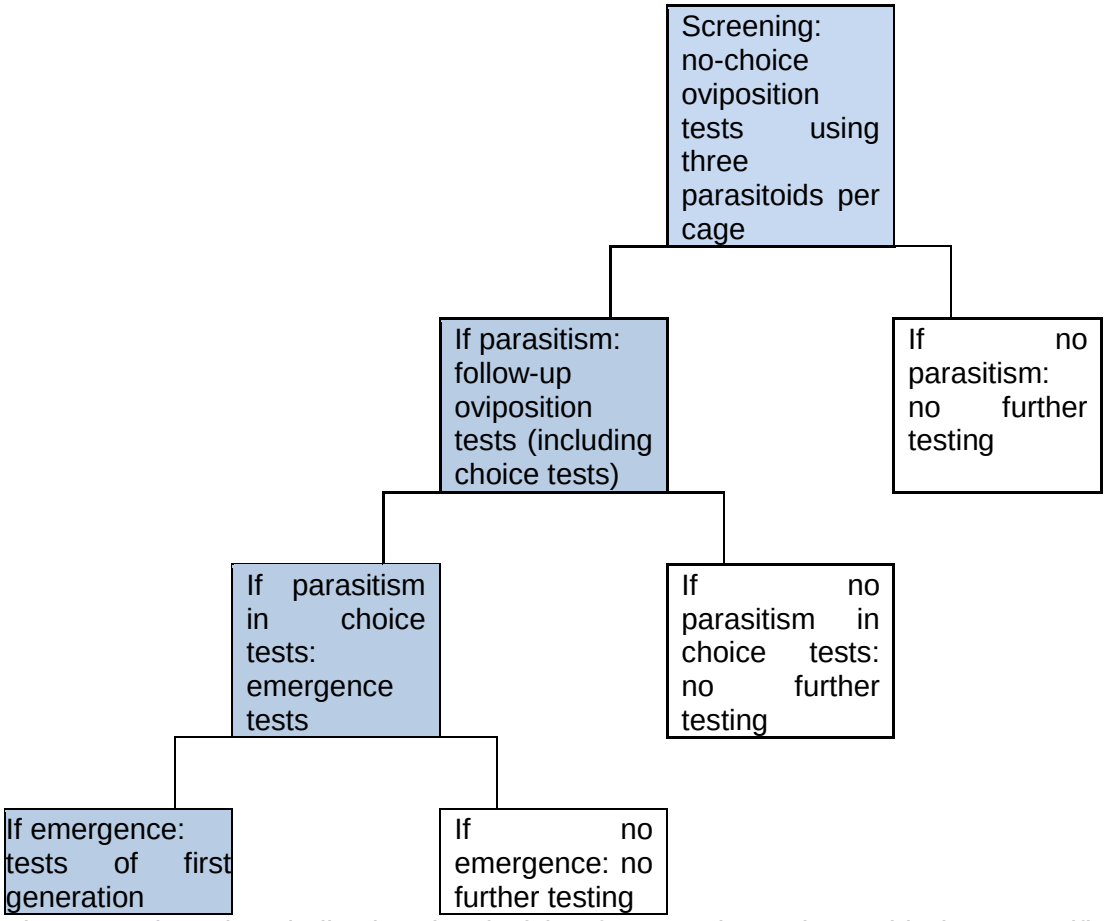

Figure 1. Flow chart indicating the decision framework used to guide host specificity tests with *Tamarixia triozae.*

Follow-up tests
If oviposition was detected in the screening tests, then follow-up tests were undertaken to determine parasitoid responses in choice situations and to gather further data on no-choice responses. Smaller cages were set up as per Table 3 and psyllid nymphs were examined for parasitoid eggs after 48 h. For these tests 20 cages were set up simultaneously (five of each treatment) using the same cohort of *T. triozae*. If oviposition failed to occur in the positive control cages, the results from all cages using that cohort were discarded.

The set up differed from the screening tests in that a single parasitoid was used per cage. The use of three parasitoids per cage in the initial screening tests can be regarded as a "worst case scenario" and these tests provide information about the likely behavior of a small group of parasitoids confined with a non-target host. A more realistic scenario, however, is that a solitary parasitoid encounters a non-target host, and testing with a single parasitoid per cage more closely simulates this scenario.

The follow-up tests also examined parasitoid-induced mortality as well as oviposition, by counting the number of psyllid nymphs alive and dead after the 48-h test period. To be able to determine the extent to which mortality was due to the parasitoid, the follow up tests

included “negative control” cages that contained psyllid nymphs (target and non-target) but not the parasitoid; data from these cages indicated the background level of mortality, which can be high in host-specificity testing (Berndt et al. 2009).

Table 3. Summary of follow-up test protocols. These tests were carried out if the initial screening no-choice tests detected parasitism of a non-target psyllid species.

	Negative control	Choice test	No-choice test	Positive control
Psyllids:	Non-target psyllid on naturally infested leaf with at least 20 late instars AND Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)	Non-target psyllid on naturally infested leaf with at least 20 late instars AND Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)
Tamarixia triozae:	None	1 female	1 female	1 female
Cages:	Vented plastic pottles (100 mm diameter)			
Duration:	48 h			
Replicates:	15 per treatment			
Variables:	Egg numbers per psyllid nymph, numbers of live and dead nymphs			

Emergence tests

If the follow-up tests detected parasitism of a non-target psyllid species in a choice situation, emergence tests were then conducted to determine whether *T. triozae* was capable of completing its development on non-target species. The set-up for the emergence tests was similar to the follow-up tests (Table 4), but instead of assessment at 48 h, the parasitoids were removed at 48 h and the leaves were held within the cages for 3 weeks (usual emergence time for *T. triozae* is 14–16 days under quarantine conditions). At the end of this 3-week period cages were examined and the number of emerged *T. triozae* adults was recorded. The nymphs were not checked for parasitism or removed from the host leaf until the end of the 3-week period, as there is evidence that some psyllid species are intolerant of dislodgement. For example the lilly pilly psyllid (*Trioza eugeniae*) is gall forming, like *Trioza curta*. If lilly pilly psyllid nymphs are dislodged from their galls, they do not re-establish in their galls, or settle elsewhere, and eventually die of desiccation (Young 2003). For these tests 10 cages were set up simultaneously (five of each treatment) using the same cohort of *T. triozae*. If oviposition failed to occur in the positive control cages, the results from all cages using that cohort were discarded.

Table 4. Summary of emergence test protocols. These tests were carried out if the follow-up choice tests detected parasitism of a non-target psyllid species.

	No-choice	Positive control
Psyllid/s	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)
Tamarixia triozae	1 female (removed after 48 h)	1 female (removed after 48 h)
Cages:	Vented plastic pottles (100 mm diameter)	
Duration:	3 weeks	
Replicates:	10 per treatment	
Variable:	Numbers of adult parasitoids emerged	

First-generation performance tests

If emergence of parasitoid adults from a non-target host was detected, further tests were undertaken to assess the oviposition rates of parasitoids derived from non-target hosts compared with parasitoids derived from TPP (protocols given in Table 5). The ten replicates (cages) for each treatment were all set up at the same time. The parasitoids used for the 20 tests with *T. panacis*-derived *T. triozae* were from the same cohort. Similarly the parasitoids used for the 20 tests with TPP-derived *T. triozae* were from the same cohort. To assess emergence rates, a separate set of cages was set up following the same protocol, but parasitoids were removed after 48 h and the cages were incubated for 3 weeks. At the end of this 3-week period cages were examined and the number of emerged *T. triozae* adults was recorded.

Table 5. Summary of next-generation test protocols. These tests were carried out if emergence of *Tamarixia triozae* occurred from a non-target host.

	No-choice test 1	Positive control 1	No-choice test 2	Positive control 2
Psyllid/s:	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)
Tamarixia triozae:	1 female from non-target host	1 female from non-target host	1 female from TPP	1 female from TPP
Cages:	Vented plastic pottles (100 mm diameter)			
Duration:	48 h			
Replicates:	10 of each treatment			
Variable:	Egg numbers per psyllid nymph			

Data analysis

Using the MASS (Venables & Ripley 2002) package with R (R Development Core Team 2012), negative binomial generalized linear models were used to model the numbers of parasitized psyllids under the various conditions. Emergence of *Tamaraxia triozae* in subsequent generations was analysed similarly. Mortality data were examined in a similar way with the number dead offset by the number of psyllids exposed to the test conditions. The model was used to predict the percentage mortality.

In some analyses, there was evidence of a significant effect of the cohort on the numbers observed. Calculating separate means for each cohort and averaging the result gave an almost identical result to the one obtained by simply removing cohort from the model. In those cases, the probability associated with the response under consideration was calculated with cohort as a blocking factor.

Analysis using a more straightforward Poisson model could not be used because of the degree of overdispersion which is typical of this kind of data. A negative binomial model estimates the overdispersion and gives more realistic estimates of relevant probabilities. 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 to give the values presented in the results Tables 6 and 7.

Results

Oviposition tests

Tamarixia triozae oviposited on TPP nymphs in all positive control cages, but did not oviposit on six of the eight non-target psyllid species tested (Figure 2). The parasitoid did lay eggs on two native non-target species, *Trioza curta* and *Trioza panacis*. In the sequential no-choice test *T. triozae* consistently oviposited on *T. curta*, even after repeated exposure to its target host TPP (Figure 3).

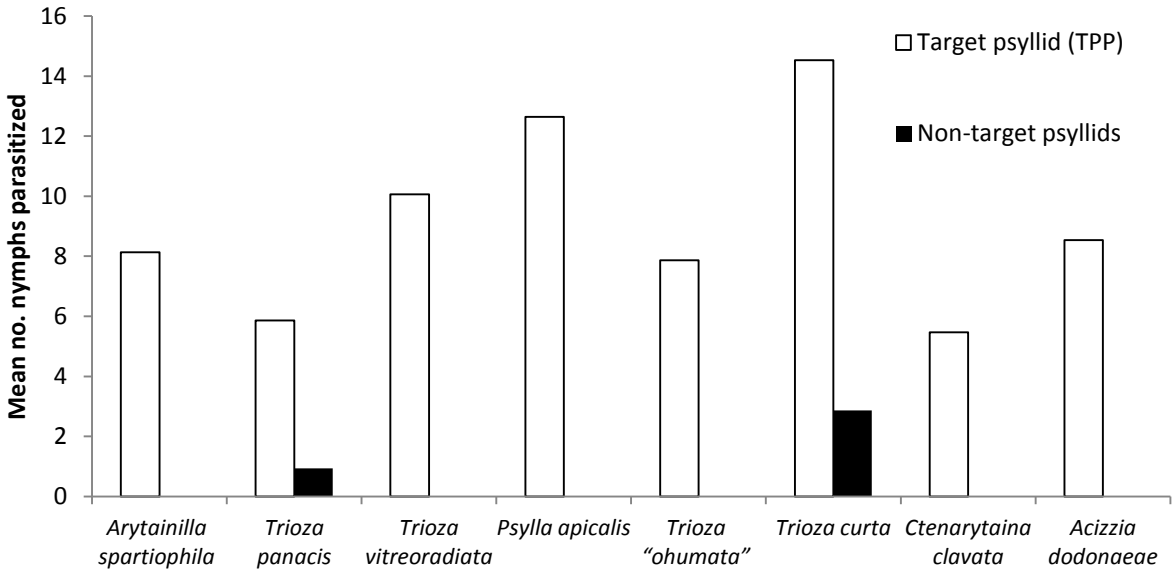

Figure 2. Mean number of nymphs with *Tamarixia triozae* eggs (parasitized nymphs) after 48 h in no-choice screening tests. In these tests *T. triozae* females (three per cage) were offered nymphs of either the non-target species (species given in x-axis) or the target species (TPP).

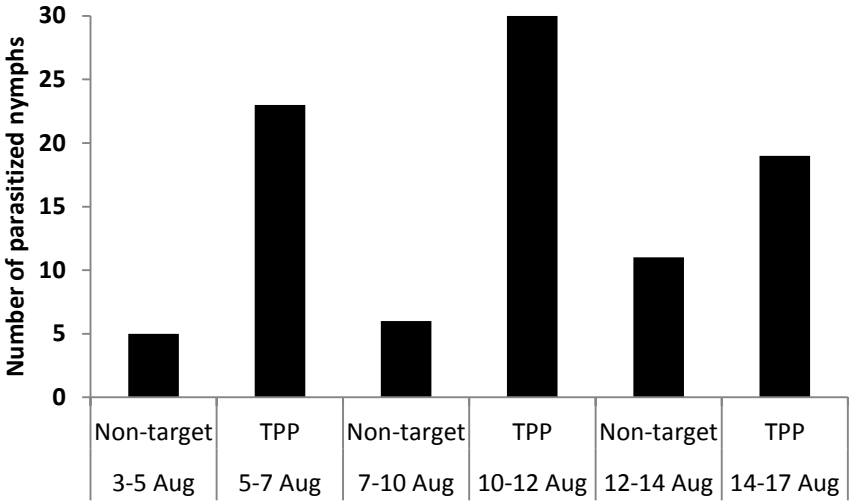

Figure 3. Number of nymphs with *Tamarixia triozae* eggs (parasitized nymphs) in a sequential no-choice test. In this test a single cage of *T. triozae* females (three per cage) were offered nymphs of either the non-target species (*Trioza curta*) or the target species (TPP) over a 14-day period.

Follow-up tests

Follow-up tests were conducted for two non-target psyllid species, *Trioza curta* and *Trioza panacis*. *Tamarixia triozae* parasitized both non-target species in choice and no-choice cages but generally laid fewer eggs on the non-target species than on the target TPP (Table 6). The parasitoid had no significant effect on the mortality of *T. panacis* (Table 7). However, there is some evidence that the parasitoid did affect the mortality of *T. curta*, as there was a significant increase in mortality in the choice cages (21% of *T. curta* nymphs died) compared with the negative control cages without the parasitoid (12% of *T. curta* nymphs died) (Table 7). Under the same test conditions TPP mortality in cages with the parasitoid was 25–35%, whereas in negative control cages without the parasitoid TPP mortality was 4–7% (Table 7).

Table 6: Predicted number of nymphs parasitized by a single *Tamarixia triozae* female in 48 h (prediction -1 SE, prediction +1 SE). Differences between pairs of non-target versus target results are statistically significant if results within the pair have different letters following them.

Test type	Tests with *T. curta* as non-target		Tests with *T. panacis* as non-target	
	Non-target	Target (TPP)	Non-target	Target (TPP)
Choice	0.8 (0.6, 1.1) [a]	4.7 (4.0, 5.5) [b]	0.2 (0.1, 0.4) [a]	4.4 (3.5, 5.5) [b]
No-choice	3.0 (2.3, 3.9) [a]	5.6 (4.4, 7.1) [a]	0.2 (0.1, 0.4) [a]	6.1 (5.5, 6.7) [b]

Table 7: Predicted percentage of nymphs dead in 48 h (prediction -1 SE, prediction +1 SE). Choice and no-choice results with an * are significantly higher than the background level of mortality (indicated by the negative control results) for the species.

Test type	Tests with *T. curta* as non-target		Tests with *T. panacis* as non-target	
	Non-target	Target (TPP)	Non-target	Target (TPP)
Negative control	12.4 (10.4,14.8)	6.6 (5.3, 8.3)	7.7 (5.5, 10.8)	3.5 (2.6,4.9)
Choice	20.9 (17.8, 24.5)*	25.8 (23.1, 28.9)*	5.2 (3.7, 7.4)	32.1 (28.8, 36.0)*
No-choice	18.1 (15.4, 21.3)	34.5 (21.2, 38.1)*	12.8 (9.4, 17.5)	30.4 (27.2, 34.0)*

Emergence tests

Emergence tests were conducted for the same two species, *Trioza curta* and *Trioza panacis*. *Tamarixia triozae* did not complete development on *Trioza curta* nymphs: in the emergence tests no parasitoids emerged from *T. curta* after 3 weeks whereas *T. trioza* consistently emerged from TPP nymphs under the same conditions. To confirm this result, several additional investigations were undertaken. On one occasion, parasitized nymphs from the no-choice screening tests (26 *T. curta* and 237 TPP) were transferred to fresh leaf material and held for 21 days. No parasitized *T. curta* nymphs survived beyond 14 days and no *T. triozae* adults emerged from these nymphs, whereas *T. triozae* adults emerged from 44% of the parasitized TPP nymphs. In another investigation *T. triozae* adults were released into three large mesh cages each containing a host plant with 200+ late instar *T. curta* nymphs (10 female *T. triozae* and 4 male *T. triozae* per cage). Cages were examined regularly over a 4-week period but no additional adult parasitoids were seen within these cages.

Tamarixia triozae was able to complete development on *Trioza panacis* nymphs. No emergence was detected in the emergence tests (probably due to poor leaf condition), but further investigations were then undertaken, similar to those described above. *Tamarixia triozae* adults were released into three large mesh cages each containing a host plant with 200+ late instar *T. panacis* nymphs (10 female *T. triozae* and 4 male *T. triozae* per cage). Cages were examined regularly over a 4-week period. By the end of the 4-week period the cages contained between 40 and 160 adult *T. triozae,* clearly indicating that the female *T. triozae* originally released into the cages had laid eggs onto *T. panacis* and that a least a portion of those eggs had successfully hatched and completed development on *T. panacis*.

First-generation performance tests

The egg-laying performance of the first generation of *T. triozae* that had developed on *T. panacis* was compared with the performance of *T. triozae* that had emerged from TPP. Egg-laying was significantly affected by both the source of the parasitoids used in the test (P = 0.004), and the host offered to the parasitoids (P = 0.00001). There was no significant interaction between the terms. Parasitoids that had developed on the non-target psyllid laid significantly more eggs on average, on both hosts, than parasitoids that had developed on TPP (Figure 4a). Emergence was also significantly affected by both the source of the parasitoids used in the test (P = 0.00009), and the host offered to the parasitoids (P = 0.01). There was no significant interaction between the terms. Parasitoids that had developed on the non-target psyllid produced significantly fewer adult parasitoids on average, on both hosts, than parasitoids that had developed on TPP (Figure 4b).

Discussion

Interpretation of host range tests

The host range of a parasitoid species can be defined as the set of species that can support development of the parasitoid. A distinction is usually made between the physiological (or fundamental) host range and the ecological host range. The physiological host range consists of the species that can support parasitoid development under laboratory conditions, whereas the ecological host range consists of the species actually used by the parasitoid in the field for successful reproduction (Haye et al. 2005). The host range tests reported here provide the first information available on the physiological host range of the potential biological control agent, *Tamarixia triozae*. The parasitoid *T. triozae* did not lay eggs on six psyllid species it was offered in a series of small-scale no-choice tests. For each psyllid species, a total of 45 adult female *T. triozae* were confined with nymphs of the species, in 15 separate cages. Under the same test conditions, *T. triozae* females laid eggs on its usual host, indicating that the parasitoids were in a physiological state that allowed them to readily attack an acceptable host (i.e. the parasitoids were competent). Given these results, these six psyllid species can be regarded as lying outside the host range of *T. triozae* and are unlikely to be parasitized by *T. triozae* in the wild. Five of the species are native (*Acizzia*

dodonaeae, *Ctenarytaina clavata, Psylla apicalis, Trioza* "ohumata" and *Trioza vitreoradiata*) while one is an exotic beneficial (*Arytainilla spartiophila*).

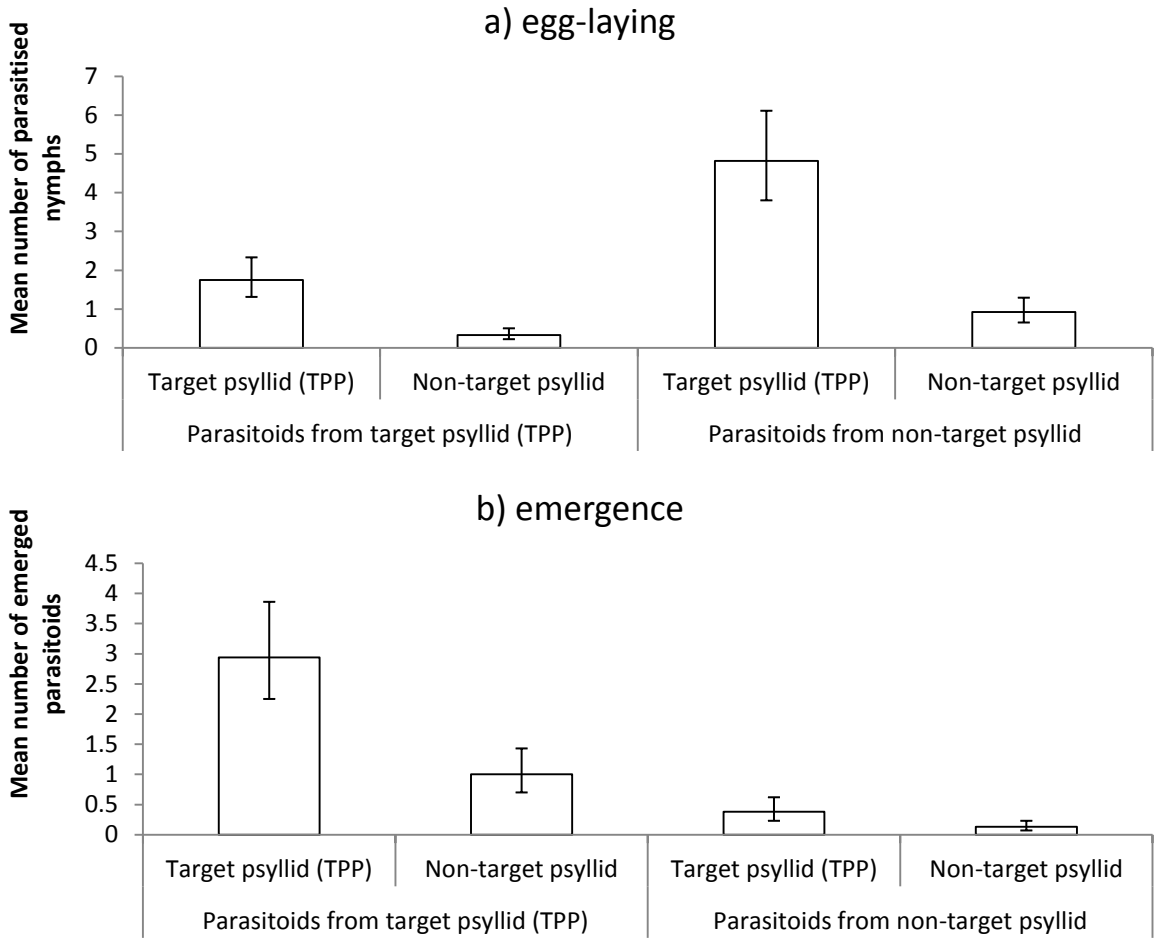

Figure 4. Performance of *Tamarixia triozae* adult females from different hosts, when offered the target psyllid (TPP) or a non-target psyllid (*Trioza panacis*), as measured by a) egglaying and b) emergence of adult parasitoids. The female parasitoids used in these tests had developed on either the target psyllid (TPP) or the non-target psyllid *T. panacis*.

Tamarixia triozae did oviposit on two native psyllid species (*Trioza curta* and *Trioza panacis*) in both choice and no-choice tests, but was unable to complete development on one of these species (*T. curta*). These results suggest that *T. curta* also lies outside the physiological host range of *T. triozae.* Successful development did occur on *T. panacis*, but the rate of egg-laying on *T. panacis* was low and the parasitoids that emerged from *T. panacis* had reduced ability to produce further offspring compared with parasitoids that emerged from their usual host (TPP). Hence, while *T. panacis* lies within the physiological range of *T. triozae,* it does not appear to be an optimal host for the parasitoid.

Extrapolation of test results to impacts in the field

Studies have shown that the physiological host range is often greater than the ecological host range of a species, a discrepancy that arises because laboratory tests cannot predict parasitoid host searching and other behaviours that occur in complex open environments (Froud & Stevens 2003; Haye et al. 2005). Furthermore, even if a non-target species does lie within the ecological host range of a parasitoid and parasitism occurs in the field as well

as the laboratory, this may not significantly affect the non-target species. A major analysis of over 5000 insect introductions for classical biological control of insects found that only 87 (1.7%) of the introductions had any non-target effects recorded (Lynch et al. 2001). In the majority of these 87 cases, the data available indicated that the introduced agents utilised non-target hosts at a low level and did not generate sufficient mortality to cause population-level effects (Lynch et al. 2001). Quantitative evidence of severe impacts (e.g. > 40% long-term population suppression of either global or local populations) existed for at least ten introductions. However, when patterns of data recording and research effort are taken into account, the authors conclude that it is possible as many as 10% of all introductions have had serious population level effects (Lynch et al. 2001). Unfortunately, predicting detrimental non-target effects continues to be difficult (Parry 2009; Barratt et al. 2010). For example, even when levels of parasitism of a non-target host equals the parasitism rates on the target species, the effect on the two populations may be different (Barratt et al. 2010). Understanding the population level impacts of non-specific agents remains one of the major challenges in biocontrol research.

With *Tamarixia triozae*, the low level of parasitism observed on *T. panacis*, together with the evidence that *T. panacis* may not be an optimal host, suggests that *T. triozae* is not likely to cause high mortality in local populations if field parasitism did occur following release. In addition, both *T. panacis* and its hosts are widespread, occurring from Auckland in the north to Manapouri in the south, and from sea level to subalpine (Dale 1985). Given this distribution pattern, it is unlikely that *T. triozae* would locate and deplete all *T. panacis* populations, as several authors have noted that *T. triozae* has patchy distribution within its home range, occurring in abundance at one site but not at other nearby sites with abundant hosts present (Pletsch 1947; Johnson 1971). There is also some evidence that *T. triozae* has limited ability to locate hosts over distance (Johnson 1971). This may also allow some *T. panacis* populations to remain undetected by *T. triozae*.

Other *Tamarixia* species have been successfuly used in a number of biocontrol programmmes (Table 8). The *Tamarixia* species used appear to be monophagous within their home range (Zuparko et al. 2011). Despite this high level of host specificity, non-target effects have been reported for one species, *Tamarixia dryi,* introduced onto Reunion Island in 1974, to control the citrus psyllid *Trioza erytreae* (Aubert & Quilici 1983). On Reunion Island, *T. dryi* rapidly reduced its host numbers, eventually completely expirpating *T. erytreae*. Prior to the release of *T. dryi,* another psyllid*, Trioza litseae (eastopi),* was widespread and abundant, especially on its main host, a common Reunion Island shrub, *Litsea chinensis*. Parasitism of *T. litseae* by *T. dryi* was first detected in 1978, and heavy levels of parasitism were observed in 1980. By this stage the target pest *T. erytreae* was considered to be locally extinct and by 1981–82 *T. litseae* had also dropped to extremely low abundance on its main host (Aubert & Quilici 1983). Although *T. litseae* was first described from Reunion Island, some authors do not appear to regard it as native there, and the successful control of *T. erytreae* on Reunion Island has been attributed to the ability of the parasitoid to maintain high numbers by using *T. litseae* as an alternative host (Aubert & Quilici 1983; Halbert & Manjunath 2004). Other authors have cited the host switching of *T. dryi* on Reunion Island as an example of unexpected severe non-target effects that can arise from classical biological control (Samways 2005; Parry 2009). Host-range testing was apparently not conducted prior to the release of *Tamarixia dryi* on Reunion Island. If tests had been done, then the non-target attack on *T. litseae* could have been predicted and accounted for in the decision making process.

Table 8. *Tamarixia* species that have been introduced into countries outside of their home range as biological control agents (based on Zuparko et al. 2011). *Tamarixia triozae* has been included for comparative purposes. *Two other hosts have been recorded for *Tamarixia radiata*, but are thought to be mistaken identifications (Wager-Page 2010).

Parasitoid	Introduced against	Number of parasitoid hosts recorded in home range	Areas where introduced	Non-target effects reported?	Provides some control in area of introduction?
Tamarixia dahlsteni	*Trioza eugeniae*	1	North America	No	Yes
Tamarixia dryi	*Trioza erytreae*	1	Reunion and Mauritius	Yes	Yes
Tamarixia leucaenae	*Heteropsylla cubana*	1	Asia and Africa	No	No
Tamarixia radiata	*Diaphorina citri*	1*	North America, South Americ,Asia, plus islands in the Pacific and Indian Oceans	No	Variable
Tamarixia schina	*Calophya schini*	1	North America	No	Yes
Tamarixia triozae	-	13	-	-	-

Extrapolation of results to psyllid species not tested

The native psyllids selected for testing were representative of the taxonomic diversity of the New Zealand native psyllid fauna; hence the results suggest that most native psyllid species are likely to lie outside the physiological host range of the parasitoid. However, the results also indicate that *T. triozae* may attempt to use novel species as hosts, and that *T. triozae* is capable of successfully developing in at least some of these novel species. Changes in parasitoid host use post-introduction are referred to as "parasitoid drift" and appear to be reasonably common (Follett et al. 2000). For example, 16% (51/313) of the exotic parasitoids introduced into North America for classical biological control have been recorded using novel native hosts (Hawkins & Marino 1997). Drift can arise through host switching, host range expansion or host shifting and may involve genetic change in the parasitoid population as it adapts to its new environment, or a parasitoid may be pre-adapted to use a non-target species (e.g. if the parasitoid responds to a chemical signal released by both the original and novel host) (Follett et al. 2000). *Tamarixia triozae* utilises a diverse range of psyllid hosts within its home range, suggesting some level of behavioural or developmental flexibility that may enable it to expand or change its host range in a new environment. If the laboratory results reported here are typical of *T. triozae*'s response to novel hosts, then 15–30% of native psyllid species in New Zealand (i.e. 10–20 species) could potentially be parasitized by *T. triozae* in the field. As noted above, not all parasitism will lead to successful development, or result in population level effects. Nonetheless, these figures do raise concerns, especially for rare psyllid species.

Rare psyllid species

Two threatened endemic psyllid species occur in lowland habitats that *T. triozae* could potentially invade. Both species are in the Psyllidae family, a family *T. triozae* is known to attack in its home range (Zuparko et al. 2011). Unfortunately, very little is known about the biology of either species and population numbers are thought to be too low to allow collection for host range testing (P. Dale, pers. comm.). It is possible that rare species may avoid attack if they are remote from agricultural areas where exotic parasitoids are likely to be concentrated, because their low numbers may not attract the exotic parasitoids. However,

exotic parasitoids can invade natural habitats over time (Henneman & Memmott 2001; Barratt et al. 2007), hence such a refuge may be temporary rather than permanent. Louda et al. (2003) reviewed 10 projects that had quantitative data on non-target effects and concluded that non-target attack on rare species could accelerate their decline and increase the risk of extinction. Such impacts are considered more likely if a natural enemy is able to maintain high population levels on another host (Louda et al. 2003).

Recommendations for future work

Concerns regarding non-target effects of biological control agents have increased markedly in the past three decades and there is wide agreement that agents with narrow host ranges are likely to be lower risk, more environmentally benign, and more cost effective to research than generalists. Despite this agreement, releases of agents with broad host ranges continue to occur, often because of extreme pest pressure that requires urgent action, or because more host-specific agents are not known for the target pest (Jenner & Kuhlmann 2009). Both these factors influenced the decision to import *Tamarixia triozae* into containment in New Zealand and begin host range testing. Very little published information on *T. triozae* was available at the outset of this project, and this report represents the first attempt to consider the safety of *T. triozae* as a biological control agent outside its region of origin. The host testing has proceeded with the working assumption that *T. triozae* was likely to establish in at least some areas of New Zealand. Test results indicate that some non-target parasitism is likely if *T. triozae* encounters novel psyllids in its new environment, so it is now important to gain a better understanding of the potential distribution of *T. triozae* in New Zealand. This is a cost-effective research option that could significantly improve our understanding of the risks *T. triozae* poses to the New Zealand psyllid fauna. In particular, climate modelling may be able to determine if the New Zealand alpine environment could create a significant refuge for native psyllid species.

Alpine refuges

Parasitism of the native fauna can only occur if *T. triozae* actually encounters an acceptable native psyllid host. Spatial refuges for psyllid species have been mentioned above, but climate refuges may also be important. *Tamarixia triozae* is recorded mainly from warm dry / arid areas in its home range, and New Zealand's alpine and sub-alpine areas may be climatically unsuitable for the parasitoid (D. Logan, unpublished data). This is an area that deserves further research. Although only five native psyllid species are exclusively alpine (i.e. only occur in alpine herbfields), another 25 species are found from lowland to alpine situations and another 17 species occur from lowland to sub-alpine situations (Dale 1985). Hence almost 70% of the native psyllid fauna occur in alpine to subalpine situations. One other species occurs only in the subantarctic islands (Dale 1985). Climate may protect these species from potential *T. triozae* parasitism in all or part of their ranges.

Summary

The aim of this study was to evaluate the potential for deleterious effects by *T. triozae* on native or valued psyllid species in New Zealand. *Tamarixia triozae* was known to parasitize a number of psyllid species in its home range, and as no information was available about the response of the parasitoid to novel hosts, host range testing was considered necessary prior to any application for release in New Zealand. Testing was carried out using seven common native psyllid species and one beneficial (an exotic psyllid species introduced for weed biocontrol). *Tamarixia triozae* did not oviposit on the beneficial psyllid species or on five of the seven native psyllid species it was exposed in no-choice tests. *Tamarixia triozae* did oviposit on two native psyllid species in both choice and no-choice tests, but was only able to complete development on one of these species (*Trioza panacis*). The rate of egglaying on *T. panacis* was low and the parasitoids that emerged from *T. panacis* had reduced ability to produce further offspring compared with parasitoids that emerged from their usual host (TPP). The native psyllids selected for testing were representative of the taxonomic diversity of the New Zealand native psyllid fauna; hence the results suggest that most native psyllid

species are likely to lie outside the physiological host range of the parasitoid. Nonetheless, a significant portion of the native psyllid fauna could be attacked by *T. triozae* in the field, if it is assumed that *T. triozae* will overlap with most native psyllids in space and time. Climate modelling will be an important focus for future work.

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Milestone 8 - Entomopathogens

Nicola Mauchline

Milestone 8 commenced on 1 September 2011 and was completed by 30 June 2012, and was comprised of six components as outlined in Table 1.

Table 1. Breakdown of the components comprising Milestone 8 within project SFF 09/143.

	Description	Due Date
1	Obtain entomopathogenic products and fungal isolates	1 Dec 2011
2	Develop a suitable bioassay	20 Dec 2011
3	Rearing and culturing	20 Dec 2011
4	Laboratory Screening	31 May 2012
5	Greenhouse Screening	31 May 2012
6	Final Report	30 June 2012

Executive Summary

Bactericera cockerelli is an important pest of including greenhouse capsicum and tomato crops, outdoor tomatoes, potatoes and tamarillos in New Zealand. Entomopathogenic fungi could provide feasible integrated pest management options for control of this pest. Seven entomopathogen products and four fungal isolates were sourced from overseas and within New Zealand. These were evaluated in the laboratory and greenhouse using adult (immersion) and nymph (detached leaf) bioassays as a means of selecting suitable candidates for larger scale greenhouse trials.

Screening of entomopathogens using bioassays

Bioassay results showed that *B. cockerelli* adults and nymphs are susceptible to a number of entomopathogens. Under laboratory conditions (25°C ± 1.1°C), BotaniGard® ES and K4B3 resulted in significantly greater mortality of adults than the conventional standard, Oberon®, or the entomopathogen standard, eNtocide L™ ($P \leq 0.07$). K4B3, BotaniGard® ES, BotaniGard® 22WP, Mycotrol® O and Met52® EC, and three *Lecanicillium muscarium* isolates resulted in adult mortality above 90% within 72 h of application. Mortality of adults was more rapid under greenhouse than laboratory conditions. K4B3 resulted in 100% mortality of nymphs, while BotaniGard® ES, BotaniGard® 22WP and Met52® EC, and *L. muscarium* isolates gave greater than 55% nymph mortality 5 days after application. Younger nymphs succumb more quickly to the treatments than older nymphs. *Isaria fumosorosea*-based products and isolates were less successful at killing both adult and nymph *B. cockerelli*, with the application of NoFly™ WP, PreFeRal® 20WG and isolate F129 resulting in less than 90% mortality of adults and less than 60% mortality of nymphs.

K4B3, BotaniGard® ES and Met52® EC were selected as the best potential candidates for subsequent greenhouse and host plant trials based on overall efficacy and obtaining a rapid solution for industry.

Impact of environmental conditions on isolate growth and germination

All isolates (*Lecanicillium* and *Isaria*) had high rates of germination at 20°C and 25°C and showed consistent growth at 15°C, 20°C and 25°C. In contrast, all isolates had limited or no germination or growth at 30°C or within the greenhouse in February. Light had no affect on germination. These results are consistent with most fungal entomopathogens.

Effect of host plant on the susceptibility to entomopathogens
Reduction of *B. cockerelli* numbers was comparable between host plants for each insect life-stage and treatment. That is, host plant had no effect on pathogen efficacy.

Caged greenhouse trials
The greenhouse trial using *B. cockerelli*-infested capsicums showed mortality of *B. cockerelli* was significantly greater with BotaniGard® ES (overall reduction of 88%) than with Met52® EC (66%) or Oberon® (49%) ($P < 0.0001$). BotaniGard® ES resulted in the greatest reduction in adults (75%).
The greenhouse trial using infested tomatoes showed mortality of *B. cockerelli* was greatest with BotaniGard® ES (overall reduction of 83%), followed by eNtocide L™ (63%), Oberon® (40%) and Met52® EC (33%), ($P \leq 0.0001$). The application of K4B3 resulted in severe phytotoxicty and consequently was eliminated from further evaluation.

Environmental stability
Variable differences in mortality between entomopathogenic treatments at different times of the year were evident. The mortality of *B. cockerelli* when treated with BotaniGard® ES and Met52® EC was 88% and 66%, respectively, in March to April (average temperature 19.4°C and relative humidity 80%), and 82% and 33% respectively, in April to March (average temperature 14.3°C and RH 83%). The performance of BotaniGard® ES was not influenced by changing environmental conditions unlike Met52® EC.

Introduction
The incursion and subsequent spread of *Bactericera cockerelli* in New Zealand has had a serious impact on many primary industries, including greenhouse capsicum and tomato crops, outdoor tomatoes, potatoes and tamarillos. In addition to the economic impact, the presence of *B. cockerelli* has and will continue to put any Integrated Pest Management (IPM) programmes within these industries at risk. In some crops control of *B. cockerelli* is currently achieved by weekly applications of insecticides. This control strategy is unsustainable in the longer term, with insecticide resistance, outbreaks of secondary pests, safety risks to humans and other mammals, and a decrease in biodiversity all very real concerns for industry. Biopesticides could provide feasible options for inclusion in IPM programs and reduce the current reliance on chemical insecticides.

Records of the association between insect and pathogen can be traced back hundreds of years, with fungi being the first micro-organism to be associated with insects. Over 170 years ago Agostino Bassi demonstrated that a fungus (*Beauveria bassiana*) caused disease in silkworm (Steinhaus 1956). By 1874 the first attempts to use fungi to control insects were made by Pasteur and Le Conte (Steinhaus 1956). Significant headway was made by Elie Metschinikoff who attempted to use *Metarhizium anisopliae* against the wheat chafer (*Anisoplia austriaca*) (Lacey et al. 2001).

Over 700 species of entomopathogenic fungi have now been described, of which Samson et al. (1988) list 51 genera of fungi with entomopathogenic species. Of these, several have been associated with Hemipteran species including psyllids, scale insects, aphids and whitefly (Lacey et al. 2009; Mauchline et al. 2011; Hall 1981; Goettel et al. 2010). The mouthparts (piercing and sucking) of Hemiptera are not designed to ingest contact pathogens, therefore the most common pathogen of Hemipterans are entomopathogenic fungi. These infect through penetration of the insect's integument. Entomopathogenic fungi have been observed regulating and effectively controlling populations of aphid and whitefly (Lacey et al. 2009; Goettel et al. 2010). Jaques & Patterson (1962), Villacarlos & Robin (1989) and Wraight et al. (2009) have reported epizootics of fungi (Entomophthorales and Hypocreales) naturally regulating some psyllid species.

Today many species of entomopathogenic fungi are formulated as effective biopesticides, many of which are based on the fungi *Lecanillicum lecanii, Beauveria bassiana* and *Metarhizium* spp. Evaluations of fungi against *B. cockerelli* are limited to trials performed on potatoes in Central America. These evaluations include inundative introductions of *Metarhizium anisopliae, B. bassiana* and *Isaria fumosorosea.* These introductions significantly reduced the number of psyllid eggs and nymphs compared to the controls, and decreased plant damage, and increased tuber yield (Lacey et al. 2009; Lacey et al. 2011). Evaluations to date and herein only include pathogens isolated from other psyllid species or other Hemipterans, and commercially formulated fungal biopesticides.

NB: Refer to final report for references (Appendix MS8).

Objectives

The overall objective of this project was to identify, source and evaluate a range of fungal entomopathogens, including commercial biopesticide formulations and isolates, against *B. cockerelli* under laboratory and greenhouse conditions. The objectives below were established:

1. Obtain commercial products and isolates
2. Passage and re-isolate fungal isolates
3. Develop suitable bioassays for laboratory and greenhouse screening
4. Assess efficacy of products and isolates against *B. cockerelli* under laboratory conditions
5. Assess growth and germination of isolates in response to variation in temperature and light
6. Assess efficacy of selected products and/or isolates when *B. cockerelli was* reared on different host plants, including tomato, potato and capsicum
7. Assess efficacy of 2–3 products and/or isolates under greenhouse conditions at different times of the year.

This project forms part of the Sustainable Farming Fund project "Sustainable Psyllid Management" (SFF 09.143).

Outline of Methodology

- Entomopathogen products and fungal isolates were received into the Plant & Food Research (PFR) insect pathology laboratory in Te Puke between November 2011 and January 2012. Fungal isolates were passaged through adult *B.cockerelli* and subsequent culturing was performed using the newly passaged isolates.

- Tomato and capsicum plants were grown at PFR Te Puke or by Oakdale Organics (Pukekohe). A regular supply of B*. cockerelli* was provided by R. Gardener-Gee and A. Puketapu (PFR, Mt. Albert).

- Seven entomopathogen products, four fungal isolates, a microbial standard, a conventional standard Oberon® and a water control were applied to adult *B. cockerelli* using an immersion vacuum assay and to nymphs using a detached lead assay. Daily and cumulative mortality data was obtained from both assays under laboratory (25°C ± 1.1°C) and greenhouse conditions.

- The growth and germination of four fungal isolates (3 x *Lecanicillium muscarium,* 1 x *Isaria fumosorosea)* were assessed when held at 15°C, 20°C, 25°C, 30°C and in the greenhouse, under ambient light and no light.

- Selected entomopathogen products were applied to tomato, potato and capsicum infested with *B. cockerelli* that had been reared for at least two generations on the

respective host plants. Data was corrected against the water control and presented as percent reduction in *B. cockerelli*.

- Caged greenhouse trials were performed in March and April 2012 using *B. cockerelli* infested capsicum and tomato, respectively. Candidate treatments, identified from the adult and nymph bioassays, were applied alongside microbial and conventional standards. Both trials received two applications and mortality assessments were completed two weeks after the first application. Data was corrected against the water control and presented as percent reduction in *B. cockerelli*.

- The stability of entomopathogens with changes in temperature and humidity is discussed in reference to the results obtained within this project and the recommendations for specific entomopathogens.

Outline of Results (numbers below are aligned to objective numbers)

1. Seventeen commercial entomopathogen products and isolates were identified as candidates for screening against *B. cockerelli* in New Zealand. Due to importation, legal and time constraints, 12 of the 17 candidates were successfully sourced, and 11 were evaluated, including seven entomopathogen products and four fungal isolates.

2. All fungal isolates were passaged through adult B. cockerelli and successfully cultured for the duration of the project.

3. Bioassays were developed that enabled the screening of a range of fungal entomopathogens against *B. cockerelli* adults and nymphs under laboratory and greenhouse conditions.

4. All entomopathogens screened showed varying degrees of activity against nymphs and adults. K4B3, BotaniGard® ES and Met52® EC outperformed the conventional insecticide standard, Oberon®, and all other entomopathogens screened, and were therefore selected as the best potential candidates for subsequent trials.

5. Temperature and light influenced the growth and germination of fungal isolates.

6. Host plant (capsicum, tomato and potato) had no effect on the observed efficacy of selected entomopathogens against *B. cockerelli.*

7. Caged greenhouse trials performed on capsicum and tomato confirmed the efficacy of BotaniGard® ES and Met52® EC. BotaniGard® ES demonstrated more consistent efficacy in response to changes in environmental factors.

Discussion, recommendations and extension information

Fungal entomopathogens have demonstrated efficacy against *B. cockerelli* and should be considered for inclusion in future IPM programs.

The results presented provide an indication of product and isolate efficacy under specific environmental conditions. Both BotaniGard® ES and Met52® EC are suited to greenhouse temperatures, but the activity of these fungi will be dependent on the amount of time conditions are optimal, that is 23–25°C and 25–30°C, respectively. As such, timing spray applications to correspond to periods in the growing season when higher greenhouse temperatures are maintained is likely to maximise the effectiveness of the spray. In greenhouse environments where environmental conditions are not controlled, the use of these entomopathogens would be best suited to early spring, early and late summer and autumn.

As a continuation from the research presented here, it is recommended that candidate entomopathogens are tested under a broader range of environmental conditions. Such work could be performed within a controlled environment facility, with results validated within commercial greenhouses.

Overall it is strongly recommended that further trials are completed to compare potential candidate entomopathogens on targeted crops throughout a growing season. It is also recommended that the effects of candidate entomopathogens on non-target organisms, such as predators and parasitoids of *B. cockerelli*, are examined prior to the inclusion within an IPM programme.

List of Outputs:

- Presentation given at SFF meeting 28 February 2012.
- Final report 30 June 2012.
- Paper to be presented at Psyllid 2012 conference (26-27 July 2012) entitled 'Evaluation of entomopathogens for the control of tomato potato psyllid'.

This milestone report has been published as:
Mauchline N 2012. Evaluation of entomopathogens for the control of tomato potato psyllid, Bactericera cockerelli. A Plant & Food Research reportfor the Sustainable Farming Fund. SPTS no. 7186. 43p.

Milestone 9 & 10 - Monitoring of Psyllids in a Tamarillo Orchard / Tamarillo Insecticide Trials

Lisa Jamieson, Natalie Page, Asha Chhagan, Craig Watson, Robin Nitschke

Executive summary The phenology of tomato/potato psyllid (TPP) in tamarillos and efficacy of insecticides against TPP

The tomato/potato psyllid (*Bactericera cockerelli:* TPP) 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, resulting in a condition known as '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 affects the quality of the fruit but ultimately also leads to the decline and death of the infected plant (Sengoda et al. 2010). TPP has also been found carrying the phytoplasma *Ca.* Phytoplasma australiense; however, transmission of this phytoplasma by TPP has not yet been confirmed.

Investigations are underway to understand the phenology of the TPP in various regions of New Zealand, its host range and transmission biology. This information, in conjunction with the development of spray programmes targeted to the susceptible life stages of psyllids, will assist growers in making informed decisions about when to spray to more effectively control TPP in their crops.

This report outlines the progress and results of the following investigations:

- Phenology (seasonal abundance) of TPP in tamarillo orchards
- Susceptibility of different life stages of TPP to insecticides
- Efficacy of insecticide residues against TPP.

Phenology of TPP in Tamarillo orchards

TPP on leaves of 10 branches and 3–5 yellow sticky traps were monitored fortnightly in each of three blocks within each of two tamarillo orchards in Whangarei. Monitoring began in October 2009 in the first orchard and in February 2010 in the second orchard and finished in June 2012. Liberibacter disease symptoms were also monitoring on 10 trees in each block.

Key findings:

- TPP in Northland have multiple overlapping generations
- TPP appears to overwinter as late instar nymphs in tamarillos
- There is low survivorship from young nymphs to old nymph in tamarillos
- There appears to be a constant migration of adult TPP into tamarillo orchards (this is based on monitoring and on grower observation)
- Development of a more effective spray programme from 2009 to 2012 has likely made an impact on the number of TPP found in the tamarillo orchards that were monitored
- Disease symptoms progressed quickly over the summer months, with some trees showing severe symptoms within 6 weeks.

Testing the efficacy of insecticides against TPP nymphs and adults
Potted capsicum plants were infested with TPP eggs and nymphs and sprayed with one of the following 11 insecticides to determine the direct toxicity to these life stages: Avid® + mineral oil, Calypso®, Confidor®, Delegate®, NeemAzal – T/S™, Ovation™50WDG + mineral oil, Talstar®, Pyradym® + mineral oil, Oberon®, DC-Tron®, Movento® + Partner®. Results were compared to TPP survival on untreated control plants.

The numbers of TPP eggs, nymphs and adults on each plant were assessed 1, 2, 4, 6, 8, 10 and 12 weeks after treatment. To determine the efficacy of insecticide residues against adults landing on treated plants, adults were bagged on to leaves once sprays had dried and mortality assessed 3 days later.

Key findings:

- Avid + oil, Talstar, Oberon and Movento gave effective control of TPP nymphs over a 6-week period
- Avid + oil and Talstar had good knockdown effect against TPP nymphs, while Oberon and Movento took a couple of weeks to become effective
- Ovation + oil and the mineral oil treatment controlled TPP nymphs for up to 2 weeks after treatment
- Treatment with Neemazal reduced abundance of TPP nymphs on plants 1, 2 and 6 weeks after application compared with on unsprayed plants
- Confidor treatment did not result in a significant reduction in numbers of TPP nymphs, indicating that the rate applied and therefore taken up by the plant may not have been high enough.

Testing the efficacy of spray residues against TPP nymphs and adults
Potted capsicum plants were treated with one of five insecticides: Avid® Sparta™. NeemAzal – T/S™, Oberon®, Movento® + Partner®. Early instar (1st, 2nd or 3rd) and late instar (4–5th) TPP nymphs were placed on leaves and adult TPP were bagged on treated plants on days 1, 3, 7, 17, 21 and 28 after treatment. Early and late instar nymphal mortality and TPP life stage were assessed 7 days after first exposure to treated plants. Also, adult mortality and the number of eggs laid on the leaf in the enclosed bag were assessed 3 days after adults had been placed on the plants.

Key findings:

- On plants where nymphs were exposed to 1-, 3-, 7-, 17- and 21-day-old residues of Avid or Movento there were significantly fewer TPP than on untreated plants.
- Plants with 3-, 7-, 17- and 21-day-old Oberon residues had significantly fewer TPP than the untreated control plants.
- The numbers of TPP on Sparta-treated plants were variable, with significantly fewer TPP on plants with nymphs that had been exposed to 3-, 17- and 21-day-old residues than TPP on untreated plants.
- There was no significant reduction in the number of TPP on plants where nymphs had been exposed to residues of Neemazal compared with the untreated plants.
- There was no statistically significant reduction in the number of TPP on plants where nymphs had been exposed to 28-day-old residues of any of the products tested.
- The percent mortality of adults exposed to 1-, 3-, 17-, 21- and 28-day-old residues of Sparta on plants was significantly higher than that of adults on untreated control plants.
- Adults exposed to 3-, 7- and17-day-old Avid residues also had significantly higher mortality than adults on untreated control plants. However, adults exposed to 21- and 28-day-old Avid residues showed a similar level of mortality to those on untreated plants.

- There was a higher level of mortality for adults exposed to 1- and 3-day-old residues of Movento, Oberon and Neemazal (41–62% mortality) than adult mortality on untreated control plants (17–21%). However, there was no significant difference in adult mortality when exposed to 7-, 21- and 28-day-old residues of Movento, Oberon and Neemazal compared with adults on untreated plants.
- Fewer eggs were laid by adults exposed to 1- and 3-day-old residues of each of the insecticide treatments than those on unsprayed leaves. This reduction in egg laying persisted for longer with Sparta, with adults exposed to 7-, 17- and 21-day-old Sparta residues laying significantly fewer eggs than those on the other treatments. This was probably due to the high level of adult mortality caused by this product. A reduction in egg laying was not observed for adults exposed to the other insecticide residues older than 7 days.

Introduction

The tomato/potato psyllid (*Bactericera cockerelli:* TPP) was first identified in New Zealand in 2006. Since its discovery, TPP has spread throughout many regions of New Zealand, infesting plants in the Solanaceae and some species of Convolvulaceae. Examples of plants attacked include tomato, potato, capsicum, tamarillo, egg plant, kumara, cape gooseberry and chilli (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 can result in 'psyllid yellows' as seen in tomatoes and potatoes (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), stunted growth in fruit and leaves in tomatoes (Brown et al. 2010), and leaf curling and yellowing in capsicums (MAF 2008b). Tamarillos have tested positive for *Ca*. L. solanacearum (MAF 2008a) with plants exhibiting similar symptoms of yellowing, leaf curling and stunted growth.

A B

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

Liberibacter infection not only reduces crop yield and affects the quality of the fruit but ultimately also leads to the decline and death of the infected plant (Sengoda et al. 2010). TPP has also been found carrying the phytoplasma *Ca.* Phytoplasma australiense; however, transmission of this phytoplasma by TPP has not yet been confirmed. Investigations are underway to understand the phenology of the TPP in various regions of New Zealand, its

host range and transmission biology. This information, in conjunction with the development of spray programmes targeted to the susceptible life stages of psyllids, will assist growers in making informed decisions about when to spray timings to more effectively control TPP in their crops.

This report outlines the progress and results of the following investigations:

- Phenology (seasonal abundance) of TPP in tamarillo orchards
- Susceptibility of different life stages of TPP to insecticides
- Efficacy of insecticide residues against TPP.

Phenology of TPP in tamarillo orchards

Aim

The aim of this project was to determine the seasonal abundance of the different life stages of TPP on tamarillo trees through field monitoring, and to relate this information to results from potted plant insecticide trials to determine the optimum timing of insecticide applications to control TPP.

Methods

Monitoring nymphs

Four blocks within two orchards in Whangarei with moderate to high numbers of TPP were located. Within each block, 10 trees infested with TPP were tagged (40 trees in total). The numbers of TPP eggs, early nymphs (first, second and third instars), late nymphs (fourth and fifth instars), and adults on five young and five mature leaves on each of the tagged trees, were examined and recorded fortnightly from October 2009 (orchard 1) and February 2010 (orchard 2) until June 2012. These trees were monitored by two tamarillo growers. Insecticides were applied to these blocks and are summarised in Appendix 1.

Monitoring seasonal flight activity of adults

Within each block, five yellow sticky traps (19 x 18 cm) were also hung in the tamarillo trees and replaced fortnightly. At the time of collection, the traps were covered with a single layer of clear plastic wrap and sent to Plant & Food Research, Auckland, where TPP adults on each sticky board were identified and counted using a microscope. Period of trapping was as above.

Disease monitoring

A rating scale from 1 to 5 was created in conjunction with tamarillo growers to monitor disease symptom development (Figures 1 & 2) on the 40 tagged tamarillo trees, whereby:

1 = Juvenile leaf pinking and cupping. Tree otherwise normal (Figure 2A).
2 = More pronounced pinking and cupping. Change in tree colour – paling.
3 = Juvenile leaves no longer pink – yellow and cupped. Scorching of leaf margins/leaf spotting (Figure 2B).
4 = Juvenile leaves dropped/branch tips scorched (Figure 3A).
5 = Attempted re-growth or total defoliation (Figure 3B).

Once a tree reached a disease score of 5, any further observations were made on a ‘replacement tree’ close by. Period of disease monitoring was as above.

A B

Figure 2. (A) Early leaf pinking and cupping = score 1. (B) Yellowing and cupping of tamarillo leaves = score 3.

A B

Figure 3. (A) Tamarillo branch tip scorching = score 4. (B) Attempted re-growth, small leaves = score 5.

Data analysis

All data recording sheets and sticky traps were sent to Plant & Food Research, Auckland. Data management and calculations were conducted in Microsoft® Office Excel 2007.

Graphs were produced using Origin 7.5 [(PC/Windows XP) Copyright 2004, OriginLab Corporation].

Results

Monitoring nymphs

The mean number of tomato/potato psyllid eggs, nymphs and adults found per leaf on tamarillo plants each fortnight is shown in Figure 4.

Eggs were predominantly found over the summer months. Early and late instar nymphs were found throughout the year in low numbers apart from a peak in numbers at the start of

monitoring when branches already infested with TPP were selected for monitoring. Eggs and nymphs were rarely found during the 2011–12 season.

Adults were intermittently found on monitored branches during the summer months of 2009–10 and 2010–11 but remained at low numbers. Adults were not found on monitored branches over the summer of 2011–12.

Differences in weather patterns may account for the changes in TPP population numbers between the seasons. However, since monitoring began, new sprays that are known to have greater efficacy against TPP have been used, as well as more frequent spray applications. This could explain the ongoing lower numbers of TPP found since the 2010 season.

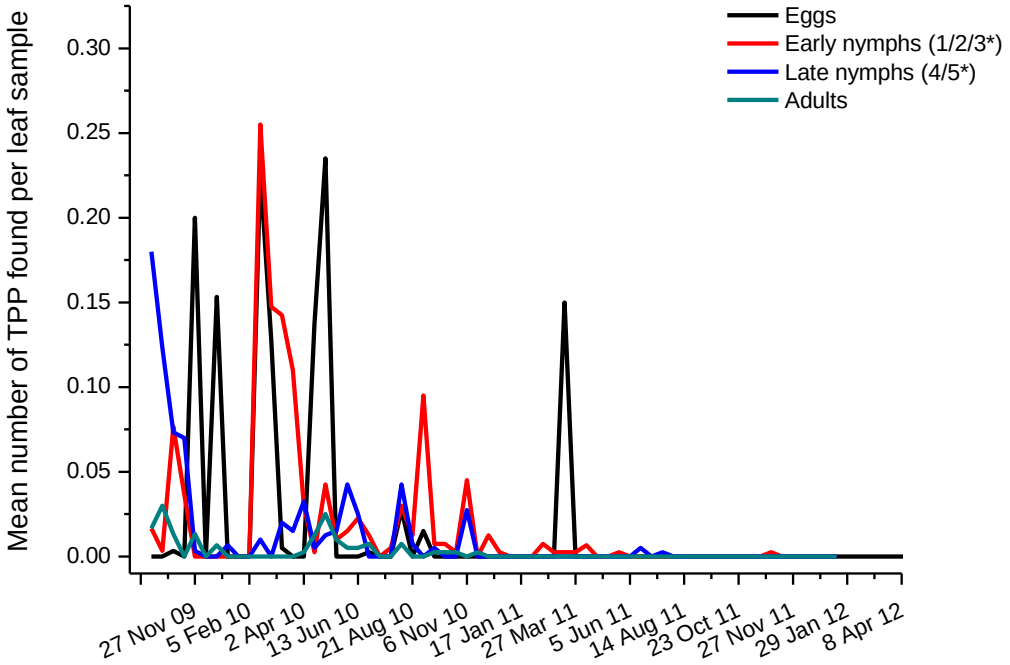

Figure 4. The mean number of tomato/potato psyllid eggs, nymphs and adults found per leaf on tamarillo plants each fortnight.

Monitoring adult flight activity
The mean number of tomato/potato psyllid adults found per sticky trap, per fortnight in tamarillo orchards is shown in Figure 5.

The highest number of adult TPP caught on sticky traps was a mean of six adults per trap, per fortnight, in March 2010. As with the number of nymphs that were found, the number of adults found has also declined. This may also be due to the new spray regime.

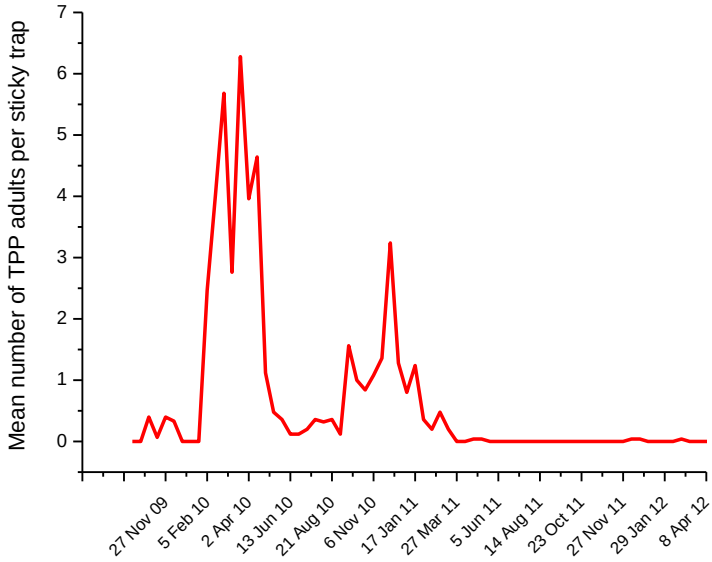

Figure 5. The mean number of tomato/potato psyllid adults found per sticky trap, per fortnight in tamarillo orchards.

Disease symptoms

Eighteen of the initial 40 trees (45%) monitored have since developed disease symptoms and died. Another seven trees which were replacements for dead trees have also died. This gives a total of 25 trees out of 47 (53%) which have died due to Liberibacter infection since October 2009. From the time that infection was first noticed to trees reaching a score of 5 (most severe symptoms) was approximately 2 months, although in some trees disease progression was much faster (4–6 weeks until score of 5).

Early disease symptoms are very similar to drought symptoms and are easily confused. During the summer months, there were several trees that were scored as showing early symptoms (score 1) that did not develop further symptoms, and eased once there was rain.

Discussion

The main findings of the phenology work are as follows:

- TPP appears to overwinter as nymphs in tamarillo orchards.
- There is low survivorship of TPP from young nymph to older nymph in tamarillos.
- There appears to be a constant migration of adult TPP into the orchards (this is based on monitoring and on grower observation).
- The new spray programme has likely made an impact on the number of TPP found in the tamarillo orchards that were monitored.

These results may be influenced by seasonal difference (e.g. dry summer (drought) vs. wet summer), and as unmanaged solananaceous crops die from TPP burden, there may be less sources of TPP to infest tamarillo orchards.

Disease symptoms progressed quickly over the summer months, with some trees showing severe symptoms within 6 weeks. Early results have indicated that TPP were not found on plants with disease symptoms, indicating a reduced attraction of these plants for feeding or laying eggs. There is significant crop loss due to Liberibacter infection which has a detrimental effect on orchard production.

Testing the efficacy of insecticides against TPP nymphs and adults

Aim

The aim of this project was to determine the efficacy of various insecticides against TPP nymphs and adults in a potted plant trial.

Methods

Plants & Insects

For insecticide trials whereby large numbers of TPP need testing capsicum plants were used because earlier preliminary trials showed that there was insufficient egg laying, low nymph establishment and low survival of nymphs on tamarillo plants. Adult TPP of mixed sex were released into a glasshouse unit with capsicum plants (McGregors 'Californian Wonder') to allow egg laying. These eggs were left to hatch so that after 2 weeks there was a mixture of eggs and early instar nymphs on the capsicum plants. This was done in preference to manually infesting plants with eggs and young nymphs as previous work had revealed that these life stages could be damaged when handling. Late instar nymphs, from a laboratory colony, were transferred onto plants using a fine tipped paintbrush so that a minimum of 15 late instar nymphs were on each plant.

Treatments

Plants were assigned treatments (Table 1) and moved to an outdoor spray area to ensure no spray drift between treatments. Treatment rates were based on label rates or those agreed on in consultation with growers.

Table 1. The active ingredient, trade name and rate of treatments.

Treatment (a.i.)	Trade name	Rate (a.i./100 litres)
untreated control		
abamectin+oil	Avid® + mineral oil	70 ml + 500 ml
thiacloprid	Calypso®	60 ml
Imidacloprid[a]	Confidor®	0.1 ml in 100 ml [a]
spinetoram	Delegate®	10 g
azadirachtin	NeemAzal – T/S™	500 ml
buprofezin+oil	Ovation™50WDG + mineral oil	25 g + 500 ml
bifenthrin	Talstar®	40 ml
pyrethrin + oil	Pyradym® + mineral oil	50 ml + 25 ml
spiromesifen	Oberon®	60 ml
mineral oil	DC-Tron®	1000 ml
spirotetramat + oil polymer	Movento® + Partner®	40 ml + 50ml

[a] Applied as a soil drench.

Four litres of each treatment was mixed and applied using a 5 L hand sprayer. On 3 June 2010, treatments were applied starting at the uppermost leaves and working towards the base of the plant, ensuring that the top of each leaf was sprayed but that there was minimal spray run-off. Four replicate plants were treated with each treatment. The control plants received no treatment.

Plants were left outside for approximately 3 h to dry before being moved back into a glasshouse where the temperature was held between 25 and 30°C. Plants were placed into treatment groups and enclosed within a mesh cage. At this time an additional 40 adult TPP of mixed sex were enclosed in a small mesh bag over a leaf on each plant to test residue activity. The numbers of live and dead adults in these bags were counted 3 days after treatment. The numbers of live or dead TPP nymphs on each plant were assessed 1, 2, 4, 6, 8, 10 and 12 weeks after treatment.

On 30 July 2010, spirotetramat treatments were similarly carried out (delayed due to incorrect rate applied on 3 June) and compared with separate controls (control 2) set up at that time. All plants were sprayed and assessed using the methods described above.

By 8 weeks after treatment untreated control plants had significantly deteriorated due to high TPP infestation. Numbers of TPP on controls began to decline as the plants died. All control plants were dead at 12 weeks. Due to this, only data from the first 6 weeks of monitoring are presented when the plants were seen to be healthy.

Statistical analysis

Percentage mortalities of adults exposed to residues were angular transformed and then compared among treatments using analysis of variance (ANOVA). Least significant differences (LSDs) were calculated to separate treatments where the ANOVA demonstrated significant differences ($P < 0.05$). Transformed percentage mortalities of adults exposed to spirotetramat residues were analysed separately and compared to transformed mortalities of adults in untreated plants set up at the same time (control 2). The analysis was performed using GenStat (version 10) ((PC/Windows XP) Copyright 2006, Lawes Agricultural Trust (Rothamsted Experimental Station)).

Differences in populations of early and late nymphs were assumed to be influenced only by the treatment and the number at day zero. It was assumed that differences between the quality of the plant material in the various treatments was negligible during the 6-week period investigated. Numbers of nymphs on the days in question, relative to the number on day zero, were analysed to compare the treatments with the control group. Two proportions were compared:

T_t/T_0 and C_t/C_0 where:

T_t = number of nymphs on treated plants at time t, T_0 = number of nymphs on treated at time 0, C_t = number of nymphs on untreated control plants at time t, and C_0 = number of nymphs on untreated control plants at time 0.

The R version 2.12.1 (R Development Core Team) generalized linear model used the negative binomial model to adjust for the high levels of overdispersion. Because of the necessary approximations made in the assumptions (number of TPP not influenced by plant quality), the Type I error rate was set at 0.01 to lessen the possibility of spurious differences. For the spirotetramat experiment, the numbers of nymphs were compared with those on control plants by a pairwise t-test.

Results

Adult mortality

Residues of Avid + oil and Talstar resulted in significantly ($P < 0.001$) higher adult mortality than residues from other treatments and controls, with 85−93% of adults dead 3 days after treatment (Figure 6). Adult TPP mortality on leaves with residues of Pyradym + oil, mineral oil, Ovation + oil or Movento was not significantly different ($P \geq 0.05$) from that on untreated leaves (Figure 6).

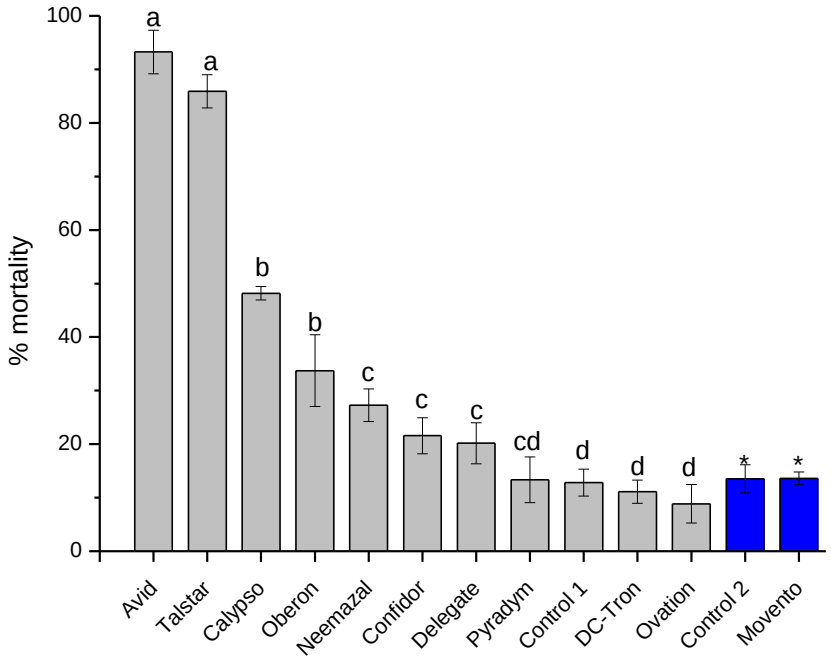

Figure 6. The percentage mortality of tomato/potato psyllid (*Bactericera cockerelli*) adults 3 days after treatment on capsicums. Vertical lines represent the standard errors of the means. Means followed by the same letter are not significantly different at *P* = 0.05. The bars indicated by * are from a later spirotetramat experiment where the control and treatment were not significantly different from each other.

Nymphal mortality

Table 2, Figure 7 and Figure 8 summarise the mean number of nymphs on treated and untreated plants. There were differences in nymph numbers among plants allocated to treatments prior to spray application, therefore the proportion of nymphs on plants compared with day zero is presented in Table 3.

At 1 and 2 weeks after treatment, Ovation+ oil-, mineral oil-, Neemazal-, Talstar-, Avid + oil- and Calypso-treated plants had a lower proportion of live TPP than the untreated control. However, by 4 weeks after treatment the proportion of TPP on Ovation+ oil- and mineral oil-treated plants had begun to increase and there was no longer a significant difference when compared with the controls (Table 3). The Neemazal treated plants still had a significantly lower proportion of TPP than the control at week 6, largely due to the increase in numbers in the control at this time compared with day 0. Calypso had a significantly lower proportion of TPP for up to 4 weeks after treatment.

Table 2. The mean number (± SEM) of live tomato/potato psyllid nymphs before and after treatment.

Treatment	Post treatment assessments (weeks)				
	0	1	2	4	6
Control	215.25 ± 66.24	265 ± 45.08	384.75 ± 54.25	210.25 ± 39.74	343.5 ± 60.11
Ovation	127.75 ± 26.46	68.25 ±5.41	37 ± 4.30	122.25 ± 22.61	216.5 ± 68.33
DC- Tron	94.25 ± 39.78	60.25 ± 17.78	31.25 ± 7.09	55.25 ± 15.55	149.5 ± 25.14
Oberon	93.50 ± 9.72	121.25 ±26.79	15 ± 4.67	3.25 ± 1.97	4.5 ± 4.5
Neemazal	102.25 ± 14.01	54.5 ± 11.76	52.75 ± 10.97	67.75 ± 15.73	67 ± 20.19
Confidor	73.25 ± 15.10	174 ± 32.05	91.5 ± 20.40	46.75 ± 6.47	71.25 ± 20.67
Delegate	93.75 ± 11.24	169.75 ± 28.78	84.5 ± 14.62	46.25 ± 6.71	122 ± 15.04
Talstar	73.75 ± 10.68	7 ± 1.47	4.75 ± 2.13	3.5 ± 2.22	6.25 ± 3.06
Pyradym + oil	99.50 ± 10.74	92.5 ± 14.23	74.25 ± 17.93	42.25 ± 9.63	202.75 ±30.84
Calypso	161.75 ± 25.05	85.5 ± 16.66	57.25 ± 7.88	56.5 ± 3.40	249.75 ± 47.22
Avid + oil	205.75 ± 27.49	17.25 ± 5.22	0.75 ± 0.75	5 ± 4.36	0 ± 0
Control 2	113.75 ± 24.76	149 ± 13.68	358 ± 60.64	122.5 ± 11.82	161.25 ± 52.96
Movento + oil	120 ± 22.15	60.75 ± 18.13	14.25 ± 11.74	25.5 ± 11.75	0 ± 0

Table 3. The relative difference in numbers of live tomato/potato psyllid nymphs on each treatment compared with time 0. Values >1 indicate an increase in number of TPP, while values < 1 indicate a decrease in number of TPP from the start of the experiments.

Treatment	Post treatment assessments (weeks)			
	1	2	4	6
Control	1.23	1.79	0.98	1.60
Ovation	0.53*	0.29*	0.96	1.69
DC-Tron	0.64*	0.33*	0.59	1.59
Oberon	1.30	0.16*	0.03*	0.05*
Neemazal	0.53*	0.52*	0.66	0.66*
Confidor	2.38	1.25	0.64	0.97
Delegate	1.81	0.90*	0.49	1.30
Talstar	0.09*	0.06*	0.05*	0.08*
Pyradym + oil	0.93	0.75*	0.42*	2.04
Calypso	0.53*	0.35*	0.35*	1.54
Avid + oil	0.08*	0.00*	0.02*	0.00[1]
Control 2	1.31	3.15	1.08	1.42
Movento + oil	0.51	0.12*	0.21*	0.00[1]

[a] * indicates a significant difference ($P < 0.01$) between a treatment and the corresponding control.

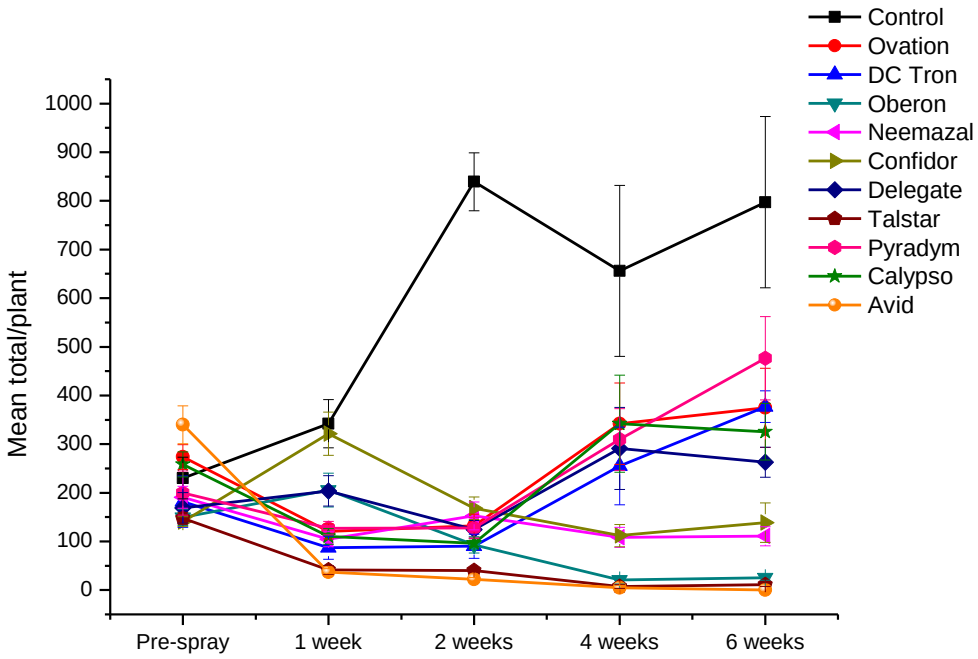

Figure 7. The mean total number (± SEM) of tomato/potato psyllids per plant on each assessment date up to 6 weeks after spraying.

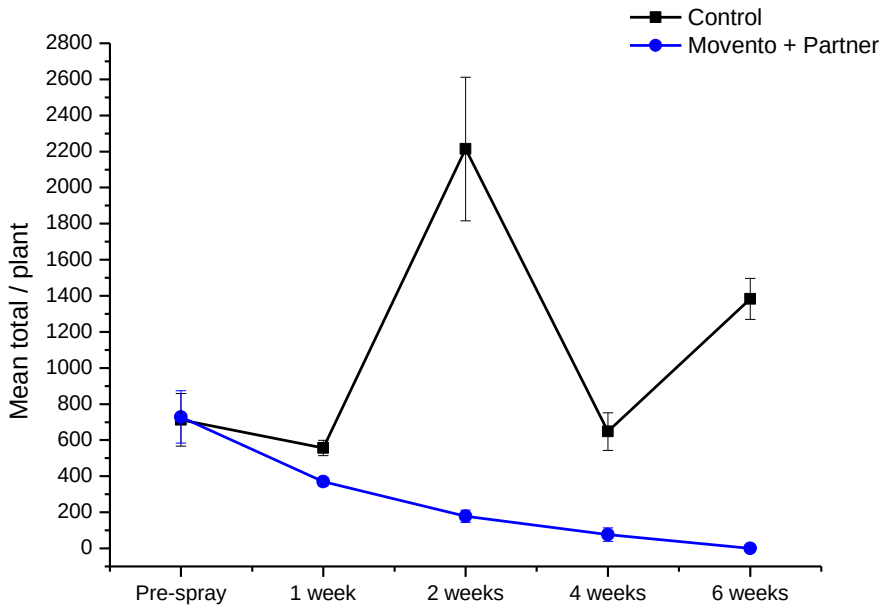

Figure 8. The mean total number (± SEM) of tomato/potato psyllids per plant up to 6 weeks after spraying.

Delegate treatment resulted in a significantly lower proportion of nymphs at week two only. Two weeks after treatment numbers of nymphs on Oberon- and Movento + oil-treated plants had significantly reduced and remained lower than on untreated plants for up to 6 weeks. Pyradym + oil-treated plants showed a significant difference in TPP infestation when compared with untreated controls at 2 and 4 weeks, but by 6 weeks there was no longer a difference between treatments and untreated controls.

After 6 weeks all TPP nymphs were dead on Avid + oil- and Movento+ oil-treated plants and numbers of nymphs on Overon-, Neemazal- and Talstar-treated plants remained significantly lower than on untreated plants.

Numbers of TPP nymphs on Confidor-treated plants were not significantly different from those on untreated controls at any of the assessment times.

Discussion

The residual activity of insecticides is important against highly mobile pest life stages such as TPP adults which can avoid direct exposure and re-infest plants soon after application. Avid + oil and Talstar residues resulted in high mortality (>80%) of TPP adults. Although the mineral oil treatment in the present study had no significant affect on adult mortality, some mineral oils have been shown to have repellent effect on TPP and reduced oviposition on tomato for up to 3 days after treatment (Yang et al. 2010). The persistence of insecticidal residues against TPP adults and nymphs and their impact on TPP egg laying, feeding and transmission of *Candidatus* Liberibacter solanacearum is currently being investigated (N.E.M. Page-Weir, Plant & Food Research, pers. comm.).

Of the 11 products tested, Avid + oil, Talstar, Oberon, and Movento gave effective control of TPP nymphs over a 6-week period. Avid + oil and Talstar had good knockdown effect against TPP nymphs, while Oberon and Movento took a couple of weeks to become effective. These results support those reported in previous bioassays on TPP nymphs where Avid (Vega-Gutierrez et al. 2008; Berry et al. 2009; Walker & Berry 2009) and Movento (Berry et al. 2009) treatment resulted in effective control of TPP. Effective control of pear psyllids (*Psylla pyri*) using Movento has also been reported (Brück et al. 2009). Previous studies have also shown Oberon to give good control of TPP (Berry et al. 2009; Walker & Berry 2009; Tucuch-Haas et al. 2010).

Ovation + oil and the mineral oil treatment controlled TPP nymphs for up to 2 weeks after treatment. These results support those reported in Berry et al. (2009), where 44% nymphal mortality was recorded at 7 days after treatment with Oberon. Treatment with Neemazal reduced abundance of TPP nymphs as was shown in a laboratory bioassay (Berry et al. 2009). The use of low mammalian toxicity products, such as mineral oil and Neemazal, may be effective when incorporated with other control strategies (e.g. biocontrol, plant resistance, early season harvest window) as part of an IPM programme.

Soil application of Confidor has been found to have a significant impact on immature stages of the Asian citrus psyllid (*Diaphorina citri*) on citrus trees (Sétamou et al. 2010) and has resulted in 53% mortality of TPP nymphs on capsicum seedlings after 7 days. In this study Confidor treatment did not result in a significant reduction in numbers of TPP nymphs indicating that the rate applied and therefore taken up by the plant may not have been high enough.

Very little peer reviewed material on the efficacy of insecticides against psyllids, in particular TPP in New Zealand, is available. Most published research has been undertaken in the United States, Mexico and Central America where growing conditions are different. Therefore, trials testing the efficacy of insecticides used in New Zealand against TPP are an

important step towards the establishment of an IPM programme for affected industries and ongoing research is required.

Testing the efficacy of spray residues against TPP nymphs and adults

Aim

The aim of this investigation was to determine the efficacy of various insecticide residues against TPP nymphs and adults in a potted plant trial.

Methods

Treatments

On 20 June 2011, individual potted capsicum plants (McGregor's 'Californian Wonder') were assigned treatments (Table 4) and moved to an outdoor spray area to ensure no spray drift between treatments. Treatment rates were based on label rates or those agreed on in consultation with growers.

Four litres of each treatment was mixed and applied using a 5-L hand sprayer. Treatments were applied starting at the uppermost leaves and working towards the base of the plant, ensuring that the top of each leaf was sprayed but that there was minimal spray run-off. Four replicate plants were treated with each treatment giving a total of 24 plants per treatment. The control plants were sprayed with tap water only.

Plants were left outside for approximately 3 h to dry before being moved back into a glasshouse where the temperature was held between 25 and 30°C.

Table 4. The active ingredient, trade name and rate of treatments.

Treatment (a.i.)	Trade name	Rate (a.i./100 litres)
Untreated control		Tap water
abamectin	Avid®	70 ml
spinetoram	Sparta™	50 ml
azadirachtin	NeemAzal – T/S™	500 ml
spiromesifen	Oberon®	60 ml
spirotetramat + oil polymer	Movento® + Partner®	40 ml + 100 ml

Insects

On days 1, 3, 7, 17, 21 and 28 after treatment (DAT), four plants from each treatment group were moved to a separate glasshouse cubicle for infestation with TPP. TPP were collected from a glasshouse colony at Plant & Food Research, Mt Albert. Three leaves on each of the four capsicum plants in each treatment group were used – one leaf for early instar (1st, 2nd or 3rd) TPP, one for late instar (4–5th) TPP, and another enclosed in a mesh bag with TPP adults.

To transfer TPP onto treated plants, leaves infested with 30 early instar were collected from the colony. Each leaf was then attached to a leaf on the treated capsicum plant using a small amount of Blu-tak. This was done as the early instar nymphs were too delicate to be transferred by paintbrush and left on their original capsicum leaf could move freely to the new leaf without being injured. Thirty late-instar TPP were collected using a fine paint brush and were carefully transferred onto a leaf of the treated capsicum plant. Adult TPP were collected into individual tubes using a mechanical aspirator; each tube contained 30 adults. These were then transferred into small mesh bags, which were subsequently enclosed over a leaf of the capsicum plant using a twist tie.

Treatments were kept on separate trays and covered by large fine mesh bags, to prevent interference by other insects present in the glasshouse. This experiment was conducted in a glasshouse unit where the temperature was held between 25 and 30°C.

Adult mortality and the number of eggs laid on the leaf in the enclosed bag were assessed 3 days after setup. Early and late instar nymphal mortality, and TPP life stage were assessed 7 days after setup.

Statistical analysis

Utilising the MASS (Venables et al. 2002) package with R (R Development Core Team), negative binomial generalized linear models were used to model the numbers of insects and eggs. Analysis of mortality data used a similar model but with an offset to allow for the differences between the number of adults in each treatment. Analysis using a more straightforward Poisson model could not be used because of the degree of overdispersion which is typical of this kind of data. A negative binomial model estimates the overdispersion and gives more realistic estimates of relevant probabilities.

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 Tables 9, 10, 11 and 12 within the Appendices. 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 for the same number of days after treatment.

Results

Efficacy of residues against nymphs

The mean number of TPP (nymphs + those that survived to adult stage) 7 days after exposure to aged residues is presented in Table 5 and Figure 9. On plants where nymphs were exposed to 1-, 3-, 7-, 17- and 21–day-old residues of Avid or Movento there were significantly fewer TPP than on untreated plants. However, the number of TPP on plants with 28-day-old Avid residues increased substantially. Plants with 3-, 7-, 17- and 21-day-old Oberon residues had significantly fewer TPP than the untreated control plants. However, there was no statistical difference in the number of TPP on plants where nymphs had been exposed to 28-day-old Oberon residues. The number of TPP on Sparta-treated plants was variable, with significantly fewer TPP on plants with nymphs that had been exposed to 3-, 17- and 21-day-old residues compared with numbers of TPP on untreated plants. There was no significant reduction in the number of TPP on plants where nymphs had been exposed to residues of Neemazal compared with the untreated plants.

Efficacy residues against adults

The mean percentage mortality of TPP adults exposed to insecticide residues of different ages is presented in Table 6 and Figure 10. The percent mortality of adults exposed to 1-, 3-, 17-, 21- and 28-day-old residues of Sparta-treated plants was significantly higher (31–72% mortality) than mortality of adults on untreated control plants (12–34% mortality). Adults exposed to 3-, 7- and17-day-old Avid residues also had significantly higher mortality than adults on untreated control plants. However, adults exposed to 21- and 28-day-old Avid residues showed a similar level of mortality as those on untreated plants. There was a higher level of mortality for adults exposed to 1- and 3-day-old residues of Movento, Oberon and Neemazal (41–62% mortality) compared with adult mortality on untreated control plants (17–21%). However, there was no significant difference in adult mortality when exposed to 7-, 21- and 28-day-old residues of Movento, Oberon and Neemazal compared with adults on untreated plants.

The mean number of eggs laid by TPP adults exposed to insecticide residues of various ages is presented in Tables 7 and 8. Overall, egg laying on the untreated plants was variable and ranged between 7.5 and 528 eggs laid over the 3 days that the adults were caged on to the leaf at the different times. This variability was probably due to several factors including

the differences in glasshouse temperature over each 3-day period and the variation in age of adults collected. Due to the low numbers of eggs laid on the control and treated leaves for exposures to 1-day-old residues, the data for 1-day-old residues is not presented. Fewer eggs were laid by adults exposed to 1- and 3-day-old residues of all of the insecticide treatments than those on unsprayed leaves. This reduction in egg laying continued for adults exposed to 7-, 17- and 21-day-old Sparta residues, probably due to the high level of adult mortality caused by this product. Reduction in egg laying was not observed for adults exposed to the other insecticide residues older than 7 days.

Discussion

Plants used in this research were kept in a glasshouse for the entirety of the trial; therefore, residues were not exposed to the natural elements of rainfall and UV light. As a result products can only be compared with each other and extrapolating length of effective residue information out to the field is not recommended.

In this trial, residues of Avid and Movento remained effective against TPP nymphs for up to 21 days. Residues of Oberon and Sparta were also effective against nymphs for this period, although not consistently at all times. Residues of Neemazal were not effective against nymphs.

Residues of Sparta were most effective against TPP adults, causing between 31 and 71% mortality for the 28-day period tested. This was a slightly higher level of adult mortality then reported by Gardener-Gee et al. (2012) where residues of Cyazypyr, sulfoxaflor and Sparta increased adult mortality, whereby at 1 DAT, mortality was 19-30% in these treatments (compared with 9% in the control), while at 14 DAT mortality was 17-29% (compared with 8% in the control).

Residues of Avid were effective against adults for up to 17 days. Residues of Oberon and Neemazal caused between 42 and 50% mortality of TPP adults for up to 3 days and were more effective than untreated adults. Trials conducted by Dohmen-Vereijssen et al. (2012) suggest that a related Neem product, Neem 600 WP, showed some residual effect on egg hatching rates and/or young instar mortality.

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The authors would like to thank NZ Tamarillo Growers and Sustainable Farming Fund for funding this research.

Table 5. Mean number (± SEM) of tomato/ potato psyllids (nymphs and adults) after 7 days' exposure to aged residues.

	Age of residues (days)											
	1		3		7		17		21		28	
Control	24.50	5.17	46.75	16.52	46.50	12.32	68.50	12.56	42.25	5.39	43.75	4.87
Avid	2.25	1.31*	7.00	6.01*	5.50	2.63*	4.75	2.46*	6.25	3.28*	103.00	33.52*
Movento	4.25	1.60*	9.25	3.64*	16.00	2.12*	5.25	1.60*	9.25	2.46*	30.75	8.13
Oberon	13.50	13.50	8.00	1.96*	15.50	4.41*	26.50	9.13*	12.50	3.01*	38.75	11.01
Neemazal	16.50	14.75	32.00	17.02	39.00	10.06	54.25	4.71	39.75	5.94	82.25	14.82*
Sparta	14.75	6.73	3.25	1.65*	27.25	6.54	8.75	2.46*	6.25	2.87*	27.75	6.75

* indicates treatments within the same column that are significantly different from the control ($\alpha < 0.05$).

Table 6. Mean percentage (± SEM) mortality of adult tomato/ potato psyllids per treatment.

	Age of residues (days)											
	1		3		7		17		21		28	
Control	17.04	2.35	20.00	4.76	33.57	13.47	31.71	2.66	12.28	2.26	14.04	1.18
Avid	51.69	10.39*	53.96	3.56*	64.18	7.36*	50.00	5.49*	25.96	10.93	10.77	1.35
Movento	43.22	6.08*	44.09	6.65*	15.79	5.83	15.45	2.08*	12.39	3.15	10.65	4.31
Oberon	49.61	4.05*	42.31	7.43*	22.90	7.83	13.04	1.10*	18.69	4.21	14.68	6.13
Neemazal	51.26	1.04*	61.94	3.09*	23.53	9.23	18.13	2.04*	21.49	5.50	5.04	2.12
Sparta	46.34	4.52*	50.74	10.68*	52.73	8.15	71.54	9.48*	59.48	15.42*	31.01	9.47*

* indicates treatments within the same column that are significantly different from the control ($\alpha < 0.05$).

Table 7. Mean number (± SEM) of tomato/ potato psyllid eggs laid by adults (3 days after placement of adults on treated plants) *(Mean number of adults recovered).*

	Age of residues (days)											
	1		3		7		17		21		28	
Control	7.50	3.92 (*33.75)*	353.75	29.69 (*30.00)*	161.25 *(35.75)*	49.91	528.00	83.78 (*30.75)*	189.75 *(28.50)*	33.04	36.25 *(28.50)*	14.48
Avid	0.50	0.50* *(29.50)*	20.00	5.67* *(34.75)*	56.50 *(33.50)*	19.29*	430.25	38.14 *(30.00)*	221.50 *(26.00)*	54.70	34.25 *(32.50)*	13.83
Movento	2.25	1.44 *(29.50)*	6.75	3.90* *(31.75)*	59.0	17.01* *(33.25)*	486.75 *(30.75)*	114.31	169.00 *(28.25)*	17.17	69.75 *(30.50)*	18.01
Oberon	0.00	0.00 *(32.25)*	2.75	2.13* (39.00)	84.50	23.60 *(32.75)*	568.25	87.99 *(28.75)*	148.00 *(26.75)*	43.30	52.50 *(27.25)*	14.51
Neemazal	5.25	1.89 *(29.75)*	10.25	9.59* *(38.75)*	57.75 *(29.75)*	20.73*	486.00	60.30 *(40.00)*	245.00 *(26.75)*	28.90	43.50 *(29.75)*	16.54
Sparta	12.0	7.47 *(30.75)*	74.25	47.84 *(34.00)*	58.75 *(27.50)*	18.40*	217.50 *(32.50)*	63.11*	83.50	19.46* *(29.00)*	26.75	6.56 *(32.25)*

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

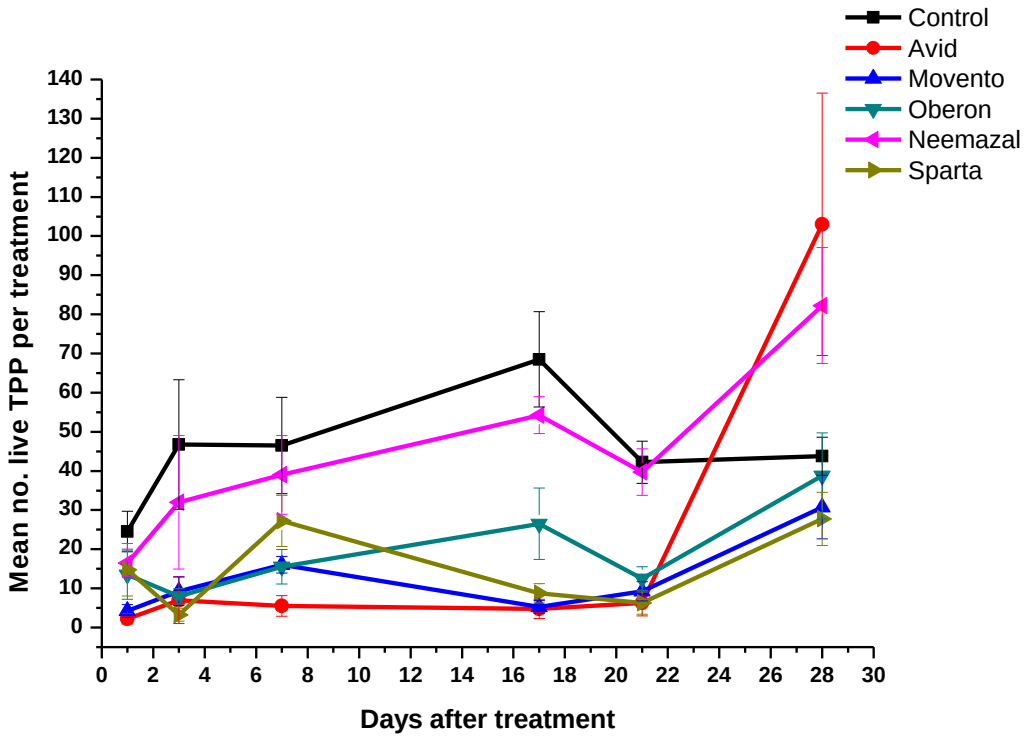

Figure 9. Mean number (± SEM) of tomato/potato psyllids (nymphs and adults) per treatment.

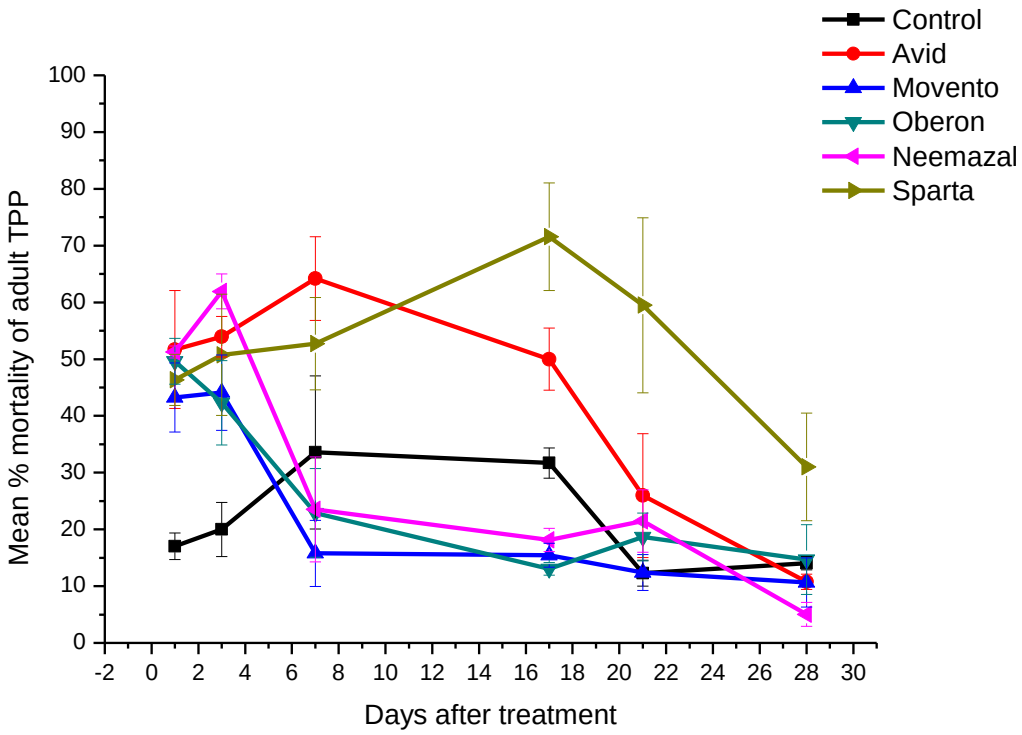

Figure 10. Mean percentage (± SEM) mortality of adult tomato/potato psyllids per treatment.

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Appendices

Appendix 1. Insecticides applied in the monitoring blocks.

Grower 1			Grower 2		
Date	Insecticide	Active ingredient	Date	Insecticide	Active ingredient
7/1/2009	Avid	Abamectin			
20/1/2009	Avid	Abamectin			
16/2/2009	Decis	Deltamethrin			
2/3/2009	Decis	Deltamethrin			
18/3/2009	Nuvos	Dichlorvos			
	Chess	Pymetrozine			
31/3/2009	Nuvos	Dichlorvos			
28/8/2009	Decis	Deltamethrin			
27/9/2009	Chess	Pymetrozine			
	Decis	Deltamethrin			
21/10/2009	Decis	Deltamethrin			
28/10/2009	Decis	Deltamethrin			
6/11/2009	Decis	Deltamethrin	4/11/2009	Calypso	Thiacloprid
				Nuvos	Dichlorvos
16/11/2009	Decis	Deltamethrin			
24/11/2009	Tamaron	Methamidaphos	30/11/2009	Calypso	Thiacloprid
				Talstar	Bifenthrin
9/12/2009	Tamaron	Methamidaphos			
21/12/2009	Movento	Spirotetramat			
27/12/2009	Movento	Spirotetramat			
13/1/2010	Tamaron	Methamidaphos	6/1/2010	Chess	Pymetrozine
				Talstar	Bifenthrin
				Avid	Abamectin
25/1/2010	Talstar	Bifenthrin			
8/2/2010	Decis	Deltamethrin	12/2/2010	Calypso	Thiacloprid
				Nuvos	Dichlorvos
17/2/2010	Tamaron	Methamidaphos			
26/2/2010	Avid	Abamectin			
11/3/2010	Avid	Abamectin			
22/3/2010	Avid	Abamectin			
8/4/2010	Tamaron	Methamidaphos			
25/6/2010	Nuvos	Dichlorvos	10/6/2010	Nuvos	Dichlorvos
9/7/2010	Nuvos	Dichlorvos			
12/8/2010	Nuvos	Dichlorvos			
24/8/2010	Nuvos	Dichlorvos			
10/9/2010	Nuvos	Dichlorvos	10/9/2010	Avid	Abamectin
24/9/2010	Nuvos	Dichlorvos			
28/9/2010	Nuvos	Dichlorvos			
7/10/2010	Decis	Deltamethrin	2/10/2010	Avid	Abamectin
15/10/2010	Talstar	Bifenthrin			
	Calypso	Thiacloprid			

26/10/2010	Talstar	Bifenthrin			
3/11/2010	Venom	Bifenthrin			
17/11/2010	Venom	Bifenthrin	2/12/2010	Movento Calypso Talstar	Spirotetramat Thiacloprid Bifenthrin
1/12/2010	Movento Tamaron	Spirotetramat Methamidaphos	5/12/2010	Movento Talstar	Spirotetramat Bifenthrin
15/12/2010	Movento Nuvos	Spirotetramat Dichlorvos	22/12/2010	Movento Talstar	Spirotetramat Bifenthrin
5/1/2011	Lorsban Calypso	Chlorpyifos Thiacloprid			
11/1/2011	Tamaron	Methamidaphos	10/1/2011	Movento Talstar	Spirotetramat Bifenthrin
17/1/2011	Lorsban	Chlorpyifos			
26/1/2011	Tamaron Calypso	Methamidaphos Thiacloprid			
2/2/2011	Lorsban	Chlorpyifos	1/2/2011	Tamaron Calypso	Methamidaphos Thiacloprid
24/2/2011	Calypso	Thiacloprid	28/2/2011	Tamaron	Methamidaphos
28/2/2011					
11/3/2011	Verdex	Abamectin	17/3/2011	Avid	Abamectin
17/3/2011					
29/3/2011	Diazanon Verdex	Diazanon Abamectin			
17/4/2011	Verdex	Abamectin	13/4/2011	Nuvos	Dichlorvos
21/4/2011	Nuvos	Dichlorvos			
21/5/2011	Nuvos	Dichlorvos			
23/5/2011	Verdex	Abamectin			
9/6/2011	Verdex	Abamectin			
5/7/2011	Verdex	Abamectin			
4/8/2011	Verdex	Abamectin			
5/9/2011	Diazanon Verdex	Diazanon Abamectin			
7/10/2011	Verdex	Abamectin	9/10/2011	Avid Calypso	Abamectin Thiacloprid
28/10/2011	Verdex	Abamectin			
17/11/2011	Verdex	Abamectin	3/11/2011	Avid Calypso	Abamectin Thiacloprid
20/11/2011			20/11/2011	Movento	Spirotetramat
8/12/2011	Movento Verdex	Spirotetramat Abamectin			
21/12/2011	Movento Verdex	Spirotetramat Abamectin	21/12/2011	Sparta	Spinetoram
5/1/2012	Sparta	Spinetoram	10/1/2012	Sparta	Spinetoram
19/1/2012	Sparta	Spinetoram	19/1/2012		
2/2/2012	Sparta	Spinetoram	1/2/2012	Tamaron	Methamidaphos

13/2/2012	Diazanon	Diazanon	13/2/2012		
20/2/2012	Sparta	Spinetoram	17/2/2012	Tamaron Chess	Methamidaphos Pymetrozine
2/3/2012	Verdex Chess	Abamectin Pymetrozine	6/3/2012	Avid Chess	Abamectin Pymetrozine
15/3/2012	Verdex Chess	Abamectin Pymetrozine	22/3/2012	Avid	Abamectin
7/4/2012	Verdex	Abamectin			
4/5/2012	Diazanon Nuvos	Diazanon Dichlorvos			
15/5/2012	Diazanon Verdex	Diazanon Abamectin			
29/5/2012	Diazanon Verdex	Diazanon Abamectin			
22/6/2012	Verdex	Abamectin			

Appendix 2. Mean number (± 1 SEM) of tomato/ potato psyllid per treatment.

	1 DAT	3 DAT	7 DAT	14 DAT	21 DAT	28 DAT
Control	24.50 (17.30, 34.70)	46.75 (31.60, 69.10)	46.50 (37.40, 57.80)	68.50 (54.60, 85.90)	42.25 (34.50, 51.70)	43.75 (35.10, 54.50)
Avid	2.25* (1.40, 3.60) 0.000	7.00* (4.60, 10.70)	5.50* (4.10, 7.40) 0.000	4.75* (3.50, 6.50) 0.000	6.25* (4.80, 8.20) 0.000	103.00* (83.30, 127.30)
Movento	4.25* (2.80, 6.40) 0.001	9.25* (6.10, 14.0) 0.005	16.00* (12.60, 20.40)	5.25* (3.90, 7.10) 0.000	9.25* (7.20, 11.90)	30.75 (24.60, 38.50)
Oberon	13.50 (9.40, 19.30)	8.00* (5.20, 12.20)	15.50* (12.20, 19.70)	26.50* (20.90, 33.70)	12.50* (9.90, 15.80)	38.75* (31.10, 48.30)
Neemazal	16.50 (11.60, 23.50)	32.00 (21.60, 47.40)	39.00 (31.30, 48.60)	54.25 (43.20, 68.20)	39.75 (32.40, 48.70)	82.25* (66.50, 101.80)
Sparta	14.75* (10.30, 21.10)	3.25* (2.0, 5.20) 0.000	27.25 (21.70, 34.20)	8.75* (6.60, 11.50)	6.25* (4.80, 8.20) 0.000	27.75 (22.10, 34.80)

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

[b] (MeanLse, Mean Pse)

[c] *P* value

Table 10. Mean percentage (± 1 SEM) mortality of adult tomato/ potato psyllid per treatment.

	1 DAT	3 DAT	7 DAT	17 DAT	21 DAT	28 DAT
Control	17.04 (13.80, 21.00)	20.00 (16.30, 24.50)	33.57 (23.10, 39.60)	31.71 (27.00, 37.10)	12.28 (9.00, 16.70)	14.04 (10.40, 18.80)
Avid	51.69* (45.50, 58.80)	53.96* (48.00, 60.60)	64.18* (51.00, 83.50)	50.00* (43.90, 56.90)	25.96 (20.50, 33.50)	10.77 (8.00, 14.80)
Movento	43.22* (37.60, 49.70)	44.09* (38.60, 50.40)	15.79 (11.50, 21.40)	15.45* (12.30, 19.40)	12.39 (9.10, 16.90)	10.65 (7.80, 14.80)
Oberon	49.61* (43.80, 56.20)	42.31* (37.40, 47.90)	22.90 (17.20, 30.50)	13.04* (10.10, 16.90)	18.69 (14.50, 24.90)	14.68 (11.10, 20.00)
Neemazal	51.26* (45.10, 58.30)	61.94* (55.90, 68.60)	23.53 (18.00, 32.20)	18.13* (15.10, 21.80)	21.49 (16.60, 27.80)	5.04 (3.20, 7.80) 0.052
Sparta	46.34* (40.60, 52.90)	50.74 * (44.90, 57.20)	52.73 (40.90, 68.60)	71.54* (64.50, 79.40)	59.48* (48.60, 71.80)	31.01* (24.70, 38.70)

[a] * indicates treatments within the same column that are significantly different from the control (α < 0.05**).**

[b] (MeanLse, Mean Pse)

[c] *P* value

Table 11. Mean number (± 1 SEM) of tomato/ potato psyllid eggs laid by adults (3 days after placement on plants).

	1 DAT	3 DAT	7 DAT	17 DAT	21 DAT	28 DAT
Control	7.50 (4.50, 12.50)	353.75 (201.10, 622.30)	161.25 (117.40, 221.50)	528.00 (446.90, 623.80)	189.75 (155.60, 231.40)	36.25 (24.90, 52.90)
Avid	0.50* (0.20, 1.20)	20.00* (11.30, 35.50)	56.50* (41.00, 77.90)	430.25 (364.00, 508.50)	221.50 (181.70, 270.00)	34.25 (23.50, 50.00)
Movento	2.25 (1.30, 4.00)	6.75* (3.70, 12.30)	59.00* (42.80, 81.40)	486.75 (411.90, 575.20)	169.00 (138.50, 206.20)	69.75 (48.00, 101.30)
Oberon	0.00 (0.00, Inf) 0.996	2.75* (1.50, 5.20) 0.000	84.50 (61.40, 116.30)	568.25 (481.00, 671.30)	148.00 (121.20, 180.70)	52.50 (36.10, 76.30)
Neemazal	5.25 (31.0, 8.90)	10.25* (5.70, 18.40)	57.75* (41.90, 79.70)	486.00 (411.30, 574.30)	245.00 (201.00, 298.60)	43.50 (29.90, 63.30)
Sparta	12.0 (7.30, 19.70)	74.25 (42.10, 130.90)	58.75* (42.60, 81.00)	217.50* (183.70, 257.50)	83.50* (68.20, 102.30)	26.75 (18.30, 39.10)

[a] * indicates treatments within the same column that are significantly different from the control (α < 0.05**).**

[b] (MeanLse, Mean Pse)

[c] *P* value

Table 12. Mean number (± 1 SEM) of tomato/ potato psyllid eggs laid by adults (7 days after placement on plants).

	1 DAT	3 DAT	7 DAT	17 DAT	21 DAT	28 DAT
Control	30.00 (15.60, 57.80)	115.75 (81.10, 165.30)	51.25 (34.50, 76.20)	91.75 (48.50, 173.50)	74.25 (48.10, 114.60)	16.25 (7.70, 34.10)
Avid	2.00* (1.00, 4.20) 0.006	1.00* (0.50, 1.80) 0.000	19.25 (12.80, 28.90)	17.50 (9.20, 33.40)	22.50 (14.40 35.00)	13.50 (6.40, 28.40)
Movento	2.00* (1.00, 4.20) 0.006	6.75* (4.50, 10.10)	20.25 (13.50, 30.40)	27.25 (14.30, 51.80)	86.75 (56.20, 133.90)	0.00 (0.00, Inf) 0.996
Oberon	0.00 (0.00, Inf) 0.998	27.75* (19.30, 40.00)	24.25 (16.20, 36.30)	77.75 (41.10, 147.10)	55.00 (35.60, 85.00)	13.75 (6.50, 28.90)
Neemazal	2.75 (6.60, 24.80)	29.25* (20.30, 42.10)	53.25 (35.80, 79.20)	42.25 (22.30, 80.10)	53.75 (34.80, 83.10)	16.75 (8.00, 35.10)
Sparta	0.00 (0.00, Inf) 0.998	3.50* (2.20, 5.40) 0.000	12.50* (8.30, 18.90)	12.75* (6.70, 24.40)	9.50* (6.00, 15.00)	2.75 (1.20, 6.10) 0.101

[a] * indicates treatments within the same column that are significantly different from the control (α < 0.05**).**

[b] (MeanLse, Mean Pse)

[c] *P* value

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

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.

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.

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

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

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.

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	

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.

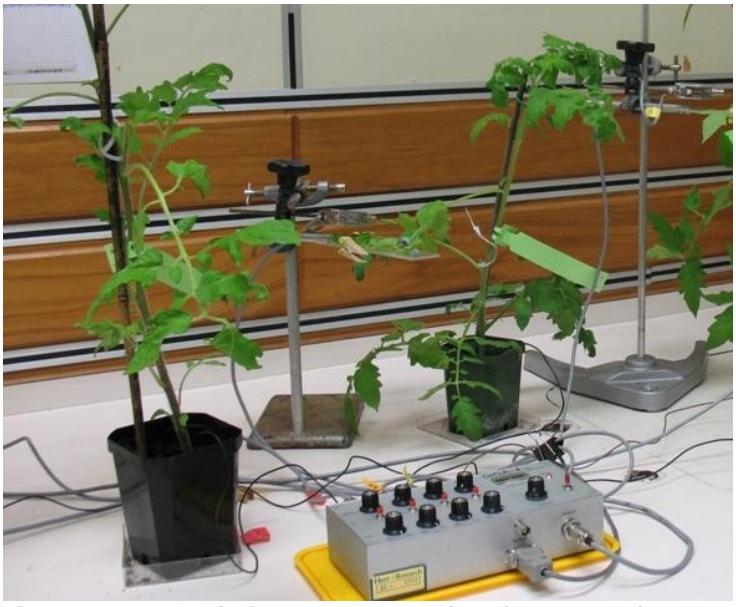


Figure 2. Treated plants connected to the EPG Giga 4 monitor.

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

Data analysis

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.

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.

Results

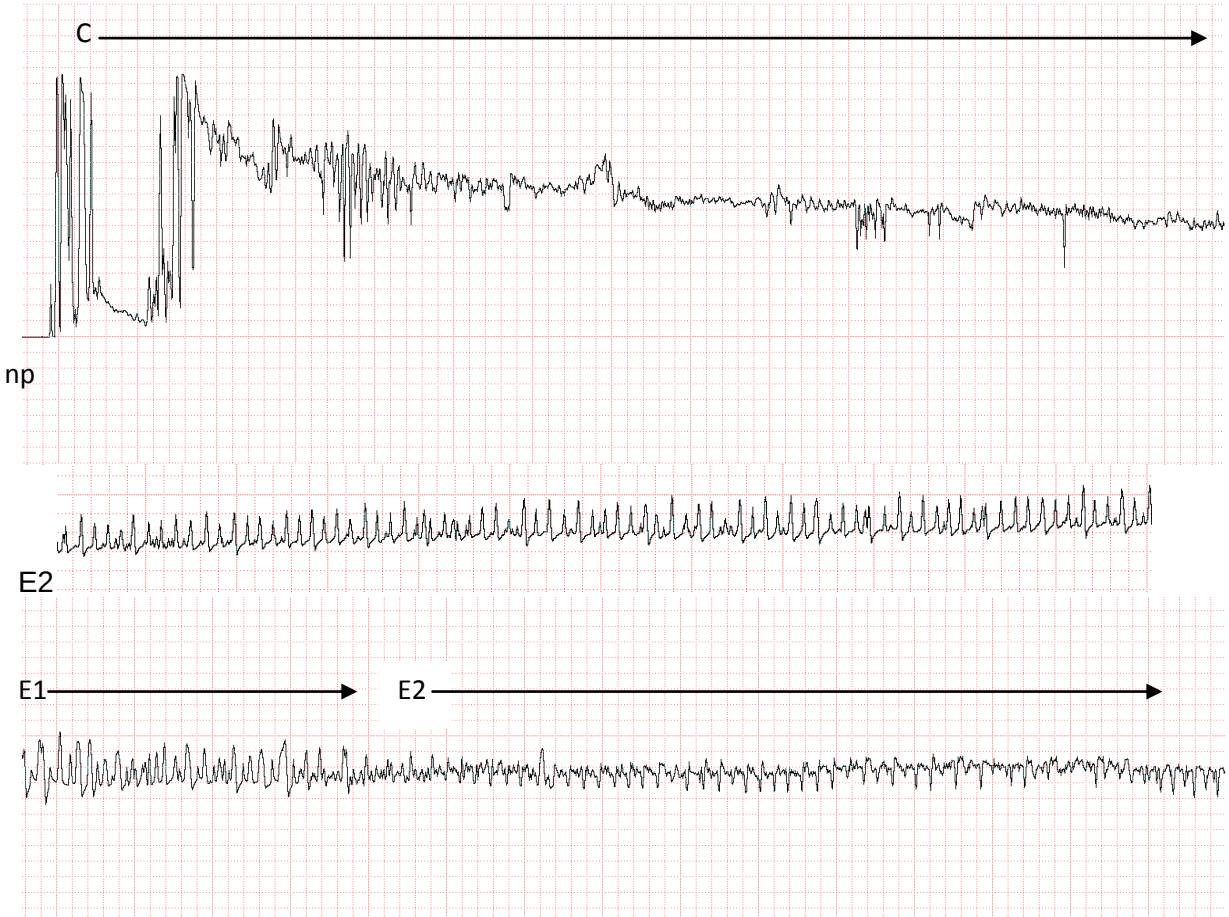


Figure 4. Main EPG waveforms representing probing and feeding of TPP on tomato leaves. Scale= 0.4 s/division.

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.

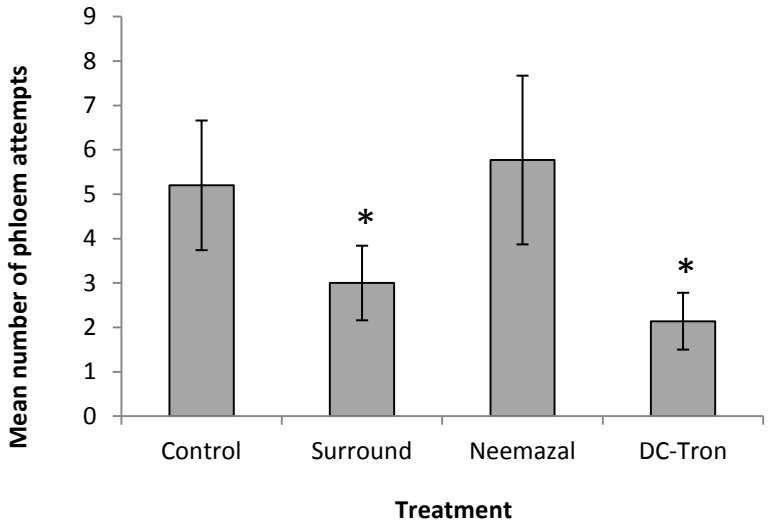
Comparison of treatments

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

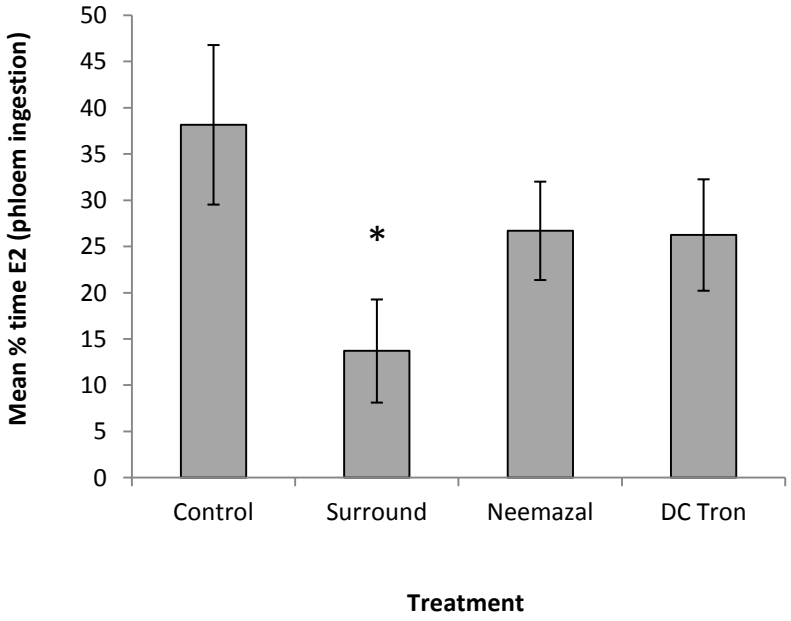
Table 2. Mean (± SEM) duration (expressed as a percentage) and the mean (± SEM) number of times that TPP adults spent performing various feeding behaviours on control plants or treated plants during the EPG recording period.

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 on control 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.

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.

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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 ($\alpha < 0.05$)

[b] (MeanLse, MeanPse)

[c] P value