

Sub-sampling plants to monitor tomato-potato psyllid (*Bactericera cockerelli*) and associated insect predators in potato crops

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Abstract Sub-sampling of potato plants was assessed as a practical tool for monitoring *Bactericera cockerelli*, tomato-potato psyllid (TPP) and other key pests and their predators at Pukekohe. The total numbers of the key insect species and all their life stages on: (1) the bottom leaf; (2) a middle leaf (the bottom leaf on the top half of the stem); (3) the bottom half of the stem; and (4) the top half of the stem, were compared with numbers on the whole stem. Assessing the top half of the stem was the most reliable sub-sampling method for the three main insects sampled, while assessing a middle leaf also gave a reliable estimate for two out of the three insects. A crop scouting protocol for monitoring TPP infestations based on sampling 100 middle leaves off 50 randomly selected plants in a crop is now recommended by the potato industry in New Zealand.

Keywords tomato-potato psyllid, *Bactericera cockerelli*, insect predators, plant sub-sampling, potatoes.

INTRODUCTION

Bactericera cockerelli (Sulc) (Hemiptera: Triozidae), commonly known as tomato-potato psyllid (TPP), is a serious pest of potatoes in Central and North America and New Zealand. The psyllid and its associated disease, zebra chip, is estimated to have cost the New Zealand potato industry around \$60 million since it was first found in New Zealand in 2006 (Anonymous 2013). Most of this cost is due to crop impacts (yield and quality) and control costs (additional insecticides and spray applications). In developing a robust IPM programme for potatoes, cost-effective methods are required so that crop scouts can monitor these crops to

make any control decisions in a timely manner. Therefore, sampling of leaves and plants as monitoring tools for TPP are being tested in replicated insecticide field trials at the Pukekohe Research Station (Walker et al. 2013).

Although TPP monitoring systems have been developed in some states in the United States of America, the TPP phenology and density on crops can vary considerably between states and between countries. Hence there is a need to develop and validate a monitoring system under New Zealand conditions. Leaf sampling methods and economic thresholds are still being developed for TPP (Butler & Trumble 2012a). At present in

the USA yellow sticky traps are recommended for detecting the first influx of TPP (Anonymous 2012) and to initiate leaf sampling (UC IPM Online 2008). Goolsby et al. (2007) report that this combination (yellow sticky traps and leaf samples) appears to provide a complete picture of the life cycle (of TPP) and the impact of an IPM programme in potatoes. Plant sampling also allows scouts to assess the efficacy of insecticide treatment, the infestations of other pests, such as aphids and potato tuber moth (*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)), and the populations of foliage-dwelling predators, all of which may impact on any control/spray decision.

Examinations of leaf samples have been described as tedious and time-consuming (Butler & Trumble 2012a) and sampling whole potato plants is even more time-consuming and less cost-effective. Thus, this paper reports on a field trial comparing insect infestations on different parts of potato plants for validation of a reliable plant sub-sampling method under a range of crop management practices, and also makes recommendations for sampling TPP and key predators in potato crops in New Zealand.

MATERIALS AND METHODS

Trial layout and crop management

The trial was conducted in the summer of 2010 within a potato growing region at the Plant & Food Research Pukekohe Research Station (174°55'E, 37°10'S). The trial utilised a randomised block design of two insecticide treatments using four replicates plus a remotely located untreated crop. Seed tubers of 'Moonlight' (certified PT 2nd generation 50–100 g seed) were planted on 30 October 2009. Plots were 20 m long and 3 m wide with four rows of potatoes spaced 750 mm apart, with plants 250 mm apart within rows and planted to a depth of ca 250 mm. Each plot comprised about 320 tubers and plots were separated by non-planted buffers, 2 m wide. The untreated control treatment was located remotely to reduce the high pest pressure of untreated plots immediately adjacent to treated plots. This untreated planting was 0.3 ha, located

about 200 m north of the replicated plot trial site and divided visually into four quadrants for monitoring. The soil type at Pukekohe Research Station is described as a Patumahoe mottled clay loam and has a pH of 6.1. Soil fertility was amended with an application of 2.5 t/ha "Potato Mix" (N:P:K = 6:6:6) at planting. Metribuzin and linuron were applied prior to crop emergence for weed control. Mancozeb was applied for blight control at recommended rates at intervals of 7–10 days throughout the growing season and the crop was irrigated twice. Treatments were (1) (calendar) 14 weekly applications of foliar-applied insecticides, (2) (reduced) six applications using a nominal action threshold of 10 TPP nymphs per 100 middle leaves and (3) (unsprayed) untreated remote control. Full details of materials and methods for insecticide treatments are reported in Anderson et al. (2013).

Plant sampling

Weekly sampling commenced when approximately 60% of the plants had emerged. Eight plants were randomly selected (a representative plant chosen every 4–5 m into the plot and also choosing plants in alternating rows) from each of the four plots or quadrants for each treatment and whole plants were examined. When the whole plant became too big to be quickly assessed (after 3 weeks), the number of stems per plant were counted and one representative stem was sub-sampled, with all insect species and all their life stages recorded on (1) the bottom leaf, (2) a middle leaf (the bottom leaf on the top half of the stem), (3) the bottom half of the stem including leaves and (4) the top half of the stem including leaves, flowers and terminal leaflets. The four sub-samples were added together to give the number of insects per stem and multiplied by the number of stems on the particular plant to give estimated numbers of insects per plant. It was likely that the destructive sampling method used dislodged adult TPP, so the numbers recorded on plants would be an under-estimate of the infestation. Thus TPP adult infestations were not assessed in this sub-sampling investigation.

Analysis

The experimental unit used for evaluating the different sub-sampling units (listed above) for use as crop scouting tools was the whole stem; whole plant estimates are multiples of the stem counts. For the untreated plot, weighted linear regressions on the replicate mean counts (of eight sampled plants and plant components) for each week, for species' life stages commonly present, were used to estimate mean numbers of insects and life stage per stem from the counts on the upper or lower stem or middle or bottom leaf (from weeks when plant components were first sampled). Weights used were the reciprocal of mean stem counts because the variance of individual counts was proportional to the observed mean. Intercepts were consistently not significantly different from zero, so regression lines were fitted through the origin, giving simple expressions for estimated stem means (slope of the line multiplied by the relevant sub-sample mean).

For the principal objective of estimating whole plant means of TPP nymphs from leaf samples, for early detection of infestations on plants, similar weighted regression was repeated on stem data where stem means were less than five (relevant for detecting low infestations of TPP on potato plants), using data for all three treatments. For this regression, data were further aggregated over the four replicates each week to weekly sample means (of $4 \times 8 = 32$ plants) in order to achieve sufficient stability for useful regression estimates. Similar regression analyses were done for two predators of TPP, eggs and nymphs of hoverfly (*Melanostoma fasciatum* (Macquart) (Diptera: Syrphidae) (commonly known as small hoverfly)) and lacewing (*Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) (brown lacewing) (data not shown, but estimators from the stem components were not as precise as for TPP). Approximate confidence limits for these regression slopes were obtained as the 10% and 90% quantiles (lines below and above which 10% of the observed stem means lie) of the linear regressions. Estimation of insect numbers per plant would be a simple extension, using easily counted stems per plant. Analyses were performed with GenStat Release 12.2 and 14.1.

RESULTS

Plant sampling

The trends in infestations of TPP (including adult TPP captured in yellow sticky traps placed in the crop), other pests and foliage-dwelling predators during this trial in the untreated planting are reported in Walker et al. (2011).

The relationships in the untreated crop between components of a sampled stem and the total stem count, for means of eight stems sampled per quadrant, for each of the four quadrants are shown in Figures 1 to 3. Data plotted are replicate means for each week. The middle leaf counts have been added to the upper stem counts, and the bottom leaf counts to the lower stem counts, so the sum of the two half stem counts is the total stem count. The regression line through the origin is drawn where the adjusted R^2 value for the regression (i.e. percentage of the variance accounted for) is greater than 80%. These graphs all examine relationships for the sum of eggs and nymphs or eggs and larvae only, and data are only presented for TPP, hoverfly and lacewing. Numbers on the graphs identify the week of sampling, from 4 to 12. Data are not presented after week 12 because the large numbers of TPP would be unacceptable to any grower, so the data are not relevant for this study. These results show that the upper stem is the most reliable sub-sampling unit for all three main insects sampled, but that sampling a middle leaf is also a reasonably reliable indicator of infestations of TPP eggs and nymphs, and hoverfly eggs and larvae on the whole stem and plant.

Figure 4 presents data comparing the mean number of TPP nymphs per whole stem and the mean number per middle leaf of the same stem for the three treatments, when the mean number of nymphs per stem is less than five, relevant for detecting low infestations of TPP on potato plants. Estimated numbers of nymphs per stem are 6.8 (SE 0.57) times the mean number on the 32 middle leaves sampled each week (adjusted $R^2=76\%$, $P<0.001$). The test for different slopes for the individual treatments was significant ($P<0.05$), but this was influenced by only three samples from treated plots, not enough to substantiate a need for separate multipliers for different treatments.

TPP eggs + nymphs

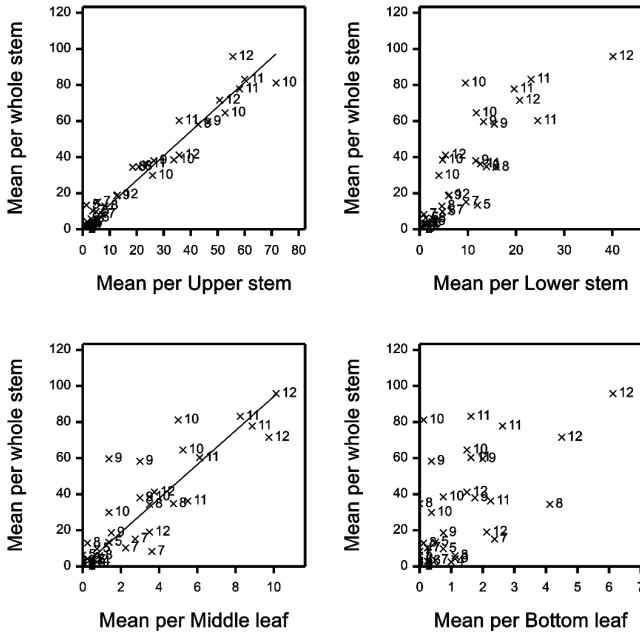


Figure 1 Mean numbers of tomato-potato psyllid (TPP; *Bactericera cockerelli*) eggs and nymphs per upper and lower stem, middle and bottom leaf compared with the whole potato stem (untreated plants only). Data plotted are replicate means for each week (n=8). The regression line through the origin is drawn where the adjusted R² value for the regression is greater than 80%. Regression slopes are all significantly greater than 0, P<0.001. Numbers on the graphs identify the week of sampling, from 4 to 12. Slope, SE and adjusted R² values are, clockwise from top left: 1.36, 0.031, 96.2; 3.07, 0.179, 77.7; 17.6, 1.99, 39.2; 9.40, 0.504, 80.7.

Hoverfly eggs + larvae

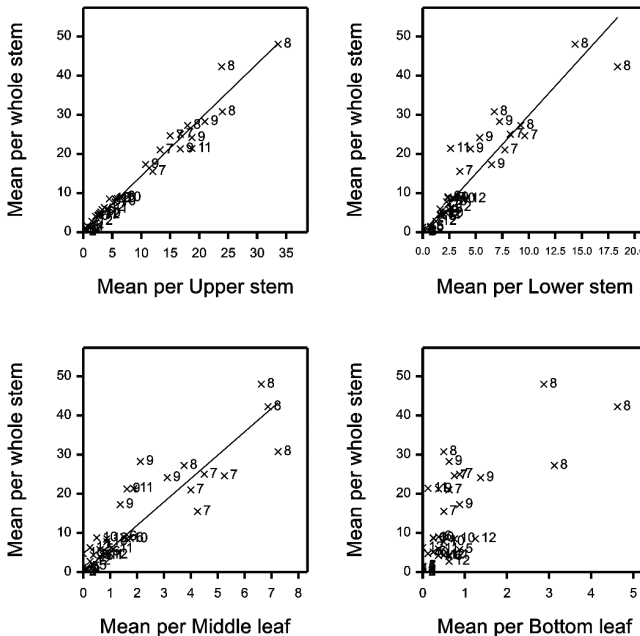


Figure 2 Mean numbers of hoverfly (*Melanostoma fasciatum*) eggs and larvae per upper and lower stem, middle and bottom leaf compared with the whole potato stem (untreated plants only). Data plotted are replicate means for each week (n=8). The regression line through the origin is drawn where the adjusted R² value for the regression is greater than 80%. Regression slopes are all significantly greater than 0, P<0.001. Numbers on the graphs identify the week of sampling, from 4 to 12. Slope, SE and adjusted R² values are, clockwise from top left: 1.43, 0.026, 97.6; 2.98, 0.117, 89.0; 12.40, 1.18, 51.3; 5.97, 0.293, 83.4.

Lacewing eggs + larvae

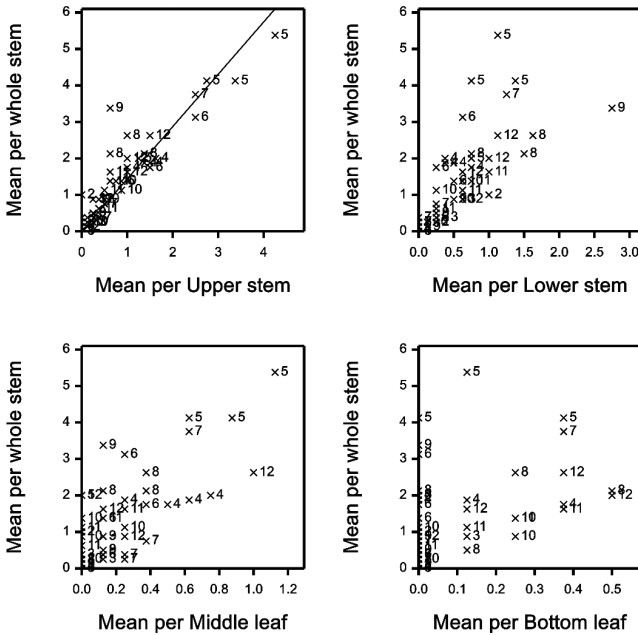


Figure 3 Mean numbers of lacewing (*Micromus tasmaniae*) eggs and larvae per upper and lower stem, middle and bottom leaf compared with the whole potato stem (untreated plants only). Data plotted are replicate means for each week (n=8). The regression line through the origin is drawn where the adjusted R² value for the regression is greater than 80%. Regression slopes are all significantly greater than 0, P<0.001. Numbers on the graphs identify the week of sampling, from 4 to 12. Slope, SE and adjusted R² values are, clockwise from top left: 1.44, 0.065, 80.7; 2.12, 0.168, 50.2; 6.63, 1.16, 0.0 (16.6 with intercept fitted); 4.43, 0.390, 41.7.

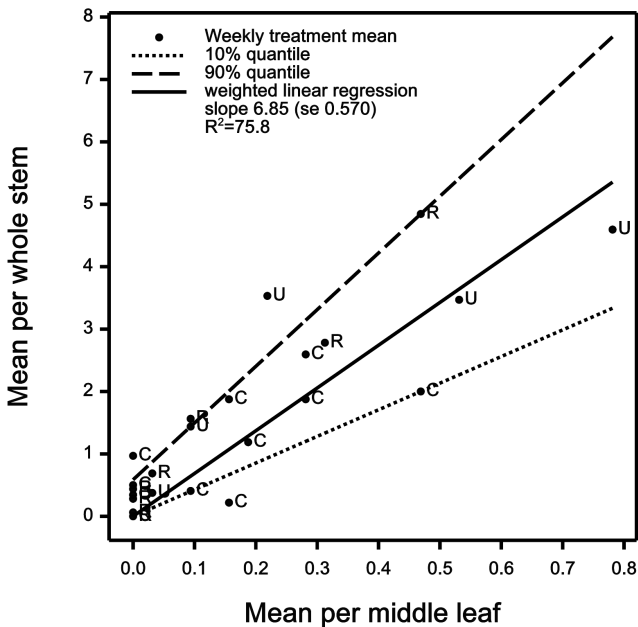


Figure 4 Weighted linear regression through the origin of the mean number of tomato potato psyllid (TPP; *Bactericera cockerelli*) nymphs per whole stem on the mean number per middle leaf on the same potato stem (n=32), for weeks when mean per whole stem was less than five (estimates shown on graph, R² value is adjusted R²). The regression slope is significantly greater than 0, P<0.001. Quantiles for the same regression are also shown. The 10% quantile has slope 4.27 (SE 1.26) and intercept 0.0 (SE 0.024), while the 90% quantile has slope 9.08 (SE 2.50) and intercept 0.59 (SE 0.232). Points are labelled by treatment (C= calendar spray, R= reduced spray, U= unsprayed).

DISCUSSION

It was important to compare the data from sub-sampling untreated potatoes with results from insecticide-treated plants (Figure 4) to ensure this method was relevant for growers because most main crop potatoes are treated with insecticides for control of TPP and zebra chip. Sub-sampling methods in potatoes have been assessed in North America, and Martini et al. (2012) reported that plant stem mid-portion TPP counts showed the strongest correlation with total counts of TPP, while Butler & Trumble (2010) recommended sampling the underside of leaves in the middle or top of a plant. Butler & Trumble (2012b) also described a binomial sequential sampling system for TPP in potatoes based on presence/absence of TPP on whole plants. It is concluded from the present trial that sub-sampling of plants would be more cost-effective than sampling whole plants, and is therefore a quicker (more practical) monitoring tool for scouting crops. For TPP it was found that 32 middle leaves gave a reasonably reliable estimate of the numbers of TPP eggs and nymphs per stem, with a multiplier for nymphs, for the present trial, of 7 times the number per middle leaf. Because 10% and 90% quantiles for the estimated multiplier were 4.3 and 9.1 (Figure 4), it is recommended that the sample size for routine scouting is increased to 100 middle leaves, to achieve greater precision for the sample means (standard errors will be approximately halved). It is further suggested that estimating TPP numbers could be done more cost-effectively by sampling the middle leaf of each of two stems on 50 plants chosen at random within the crop. This untested protocol should be satisfactory even in the presence of moderate clustering because a minimum of 50 independent observations is assured.

In the present study the concentrations of TPP and other insects along the edges of trial plots were not assessed because the plots were small compared to commercial crops. In the USA, Butler & Trumble (2012b) reported that more plants were infested on the edges of the field. In New Zealand it has also been observed that insect infestations, pests and natural enemies, are often more concentrated at crop margins and that

observed symptoms of TPP damage are normally greater on the outer-most bed of potatoes (G.P. Walker, personal observation). Therefore, crop management decisions need to take this situation into consideration, potentially by including crop margins in the sampling plan. Further research will be required to test this observation and develop a more robust sampling plan.

It has previously been reported that hoverfly and lacewing are likely to be important predators of TPP and aphids in potatoes (MacDonald et al. 2010; Walker et al. 2011). Almohamad et al. (2010) reported that predatory hoverfly females avoid ovipositing in aphid colonies in which con-specific larvae or their tracks are already present. This helps explain the even distribution of eggs and larvae of hoverfly found in field studies in New Zealand (G.P. Walker, personal observation). The present results (Figure 2) show that the middle leaf sub-sampling should also be a useful tool for monitoring hover fly, which is the most common foliage-dwelling predator in potatoes in summer crops (Walker et al. 2011). The results were also assessed for lacewing eggs and larvae (and adults) but the reliability was not as good as for hoverfly (Figure 3 and other results not shown). Brown lacewing is more readily observed at night (Leathwick & Winterbourn 1984) so large numbers of adults would not be expected in the crop during daylight sampling. However, lacewing is present on potato plants all year, is the most common insect located on potatoes in spring and early summer (Walker et al. 2011), and is likely to be an important predator of aphids and TPP (MacDonald et al. 2011).

The 'middle leaf' sub-sampling system has been used in IPM development trials (Walker et al. 2013) and, along with monitoring of TPP adults using yellow sticky trap catches, these studies have led to recommendations that early-crop potatoes do not normally require insecticide applications (Walker et al. 2012). However, leaf sampling and sticky traps are limited in their value until they can be developed to make informed control decisions for main crop (processing) potatoes. There is no known publication reporting a predictive relationship between the number of adults on traps and the numbers of nymphs in the foliage.

Also, Buchman et al. (2011) reported that adult TPP are highly efficient vectors of *Candidatus Liberibacter solanacearum* (the causal agent of zebra chip), and nymphs are less efficient than adults at transmitting this organism. Therefore, IPM decision tools, such as economic action thresholds for spray decisions, may need to be based on monitoring of sticky trap catches of adult TPP. However, monitoring of plants is also important to confirm efficacy of controls, trends in infestations of different TPP life stages and population dynamics of other pests and natural enemies.

Knowledge of the levels of *Candidatus Liberibacter solanacearum* infection in invading TPP populations, combined with economic action thresholds, will help determine whether populations of TPP warrant control or not (Butler & Trumble 2012b). The sub-sampling plan presented below should also help contribute to the development of a comprehensive IPM programme for potatoes in New Zealand.

Recommendations for sub-sampling potato crops

Based on information gained from this research and similar research undertaken overseas, and practicality in the real world, the recommendation for crop sampling is to assess 50 randomly selected whole plants, until they become too large to be cost-effective to sample. Subsequently, the sampling recommendation is to assess two middle leaves off different representative stems from 50 randomly selected plants, including plants near the margins of the crop. This middle leaf crop scouting method is now recommended by Potatoes NZ (Anonymous 2012).

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