

A sequential binomial sampling plan for potato psyllid (Hemiptera: Triozidae) on bell pepper (*Capsicum annuum*)

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Abstract

BACKGROUND: Potato psyllids (*Bactericera cockerelli* Sulc) are a pest on solanaceous crop plants, including bell peppers. Potato psyllids vector *Candidatus Liberibacter psyllaeus*, but bell peppers (*Capsicum annuum* L.) do not exhibit symptoms from infection. Potato psyllids show variation in spatial patterns and host choice with cultivar and plant species. Consequently, a study of spatial distribution and sampling plan specific to bell peppers is necessary for management of this insect pest, as those developed for other crops are unlikely to transfer among crops.

RESULTS: Potato psyllids were evenly distributed on both sides of leaves but prefer the top two-thirds of pepper plants. Within fields, psyllids demonstrated an aggregated spatial distribution, but the edge effect observed in other crop plants was absent. Eggs and nymphs had similar spatial distributions that differed from adults. A series of nymph-based sampling plans were examined. Sampling plans based on an infestation of less than 41% of plants infested (5 nymphs plant⁻¹) were statistically unacceptable, while little difference was found between the 41% infestation plan and 56% (20 nymphs plant⁻¹) infestation plan. At 41%, an average of 11 and maximum of 49 samples would be necessary to make a treatment decision.

CONCLUSION: The binomial sequential sampling plan presented here offers an important yet simple tool for managing potato psyllids in bell pepper.

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Keywords: *Bactericera cockerelli*; RVSP; peppers; zebra chip

1 INTRODUCTION

The potato/tomato psyllid (*Bactericera cockerelli* Sulc) is a pest that can reportedly live on species in over 20 host plant families.^{1,2} This includes commercial crop species: potato, tomato, eggplant and bell pepper. These psyllids are associated with 'psyllid yellows' in tomato and 'zebra chip disease' (ZC) in potatoes. ZC is caused by the bacterial pathogen *Candidatus Liberibacter psyllaeus* (CLP) (also known as *Ca. L. solanacearum*), which is vectored by *B. cockerelli* and can be transmitted with very limited feeding.³ To control ZC, economic thresholds for potato must be set extremely low, at levels that growers treat as a near-zero tolerance. Bell peppers can host CLP, and it can be transmitted from infected plants to psyllids.⁴ However, they do not appear to exhibit CLP symptoms, although Camacho-Tapia *et al.*⁵ have found CLP symptoms in chili peppers. Problems caused by *B. cockerelli* in bell peppers include wilting due to feeding, and 'honeydew' accumulation on the fruit serves as a medium for sooty mold fungus growth.⁶ Economic thresholds for potato psyllids in commercial bell peppers can therefore be set substantially higher than for either tomato or potato. In potatoes, the near-zero-tolerance sampling schemes required to prevent losses due to pathogen effects necessarily focus on all life stages that can acquire or transmit the pathogen.⁴ This is not a consideration in bell peppers, where CLP does not result in discoloration or taste effects. Consequently, an approach and plan for managing psyllid damage to peppers can be less conservative than one for tomato or potato. This includes focusing

on a single life stage and possibly allowing for greater levels of infestation. Although, in instances where a pepper field is being managed because of its potential as an inoculum, this may be less applicable.

A binomial sampling plan has recently been published for *B. cockerelli* on potatoes in California.⁷ This is the only sampling plan published for *B. cockerelli* on any plant. However, it is known that both host plant and plant variety influence life history parameters of *B. cockerelli*.^{8–11} Consequently, crop-specific sampling plans and spatial analysis are necessary for optimal management of these pests.

Current recommendations for scouting potato psyllids in pepper involve the use of sticky cards to monitor adults. However, when sampling potato psyllids on plants in the field, adults are difficult to count as they quickly move mid-count (personal observation). Eggs are abundant and sessile, but they are difficult to see and hence count. Nymphs reflect a compromise as they exhibit limited mobility, are more visible than eggs and reflect

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the resident population rather than transients. Further, methods of nymph sampling have already been examined for *B. cockerelli* in potato,¹⁰ although a nymph-specific sequential sampling plan does not yet exist. Here, a sampling plan for *B. cockerelli* nymphs in peppers is presented. This plan should be of primary assistance to management of potato psyllids in peppers, but will also aid in monitoring populations that may move to neighboring fields with ZC-susceptible crops.

2 MATERIALS AND METHODS

2.1 Psyllid sampling

Sampling was conducted biweekly in 2009 and 2010. In 2009, samples were initiated with the appearance of psyllids in July, while 2010 sampling began in May; final samples were collected in November of both years. A total of three field sites were sampled. In both 2009 and 2010, 0.2 ha plots of variety 'Baron' at the University of California's South Coast Research and Extension Center in Orange County (Irvine, CA) were sampled using a stratified random design (area divided into portions which are sampled).¹² Additionally, commercial fields were sampled in Ventura County, California, each year, and a different field was used in each year. In 2009, 0.02 ha plots of organically grown bell peppers (variety 'Islander') were sampled. In 2010, a 60 ha commercial bell pepper field (variety 'Crusader') was sampled. Fields in Ventura County were sampled using a systematic sampling design (samples collected at regular intervals).¹² Different sampling methods, stratified versus systematic, were necessitated by particulars of the cultural practices in the different sites. These included row length and plant spacing. In all sites, a minimum of eight and a maximum of 18 entire plants (all leaves, stems and fruit) were sampled on each sampling date. In total, 26 field sets were collected from fields representing varying size, varieties and pest management schemes. Thus, plans reflect the range of field conditions that may be encountered when sampling.

2.2 Psyllid distribution

As an initial step in developing a sampling plan, it is necessary to determine the spatial distribution of the target insect within the field. As the use of a single index of dispersion can be misleading,^{13,14} conclusions were based on the consensus of three different indices: Iwao's mean crowding, Green's index and Taylor's power law. To obtain Iwao's mean crowding, mean crowding (m^*) was first determined from the equation

$$m^* = m + (s^2/m) - 1$$

where m is the mean and s^2 is the variance from the sample counts. Mean crowding (m^*) was then regressed on the mean, resulting in the intercept (a) and slope (b), which were used to estimate α and β in solving the equation

$$m^* = \alpha + \beta m$$

Green's index (C_x) was calculated from the equation

$$C_x = (s^2/m) - 1/(n - 1)$$

where s^2 is the variance, m is the mean number of insects in i sample units and n is the total number of insects in i sample units.

Taylor's power law ($s^2 = am^b$),^{15,16} which quantifies the relationship of variance (s^2) to the mean density (m), was calculated

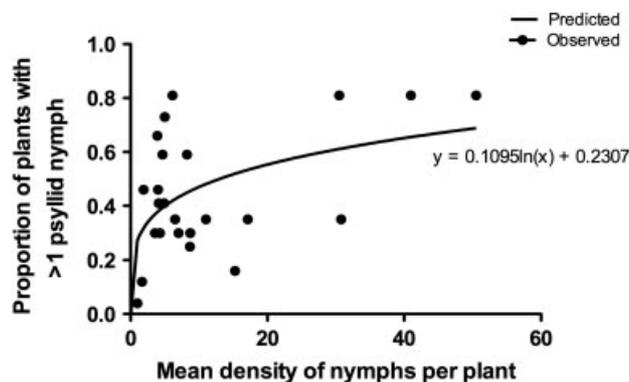


Figure 1. Relationship between the proportion of potato plants infested with one or more *B. cockerelli* nymphs and the mean number of psyllid nymphs per plant.

by regressing the log of variance on the log mean. The intercept and slope provided a and b respectively. Log a is a scaling factor, and the slope (b) measures aggregation. Student's t -tests were used to determine whether the slopes of regression lines were different from 1.0. Analysis of covariance (ANCOVA) was used to compare the slopes and intercepts among life stages, years and fields. When results were insignificant ($P > 0.05$), groups were pooled. All linear regressions and t -tests were performed using the R statistical package, v.2.150.

2.3 Development of sampling plan

After pooling of all field sites and collection dates, 28 field datasets were collected for the purpose of developing a binomial sampling plan. On three sampling dates, no psyllids were present, and one dataset contained eggs and a single adult but no nymphs. Eliminating these datasets resulted in 24 datasets used in calculating the empirical relationships between density and infestation. In addition, four sets contained too few samples or were otherwise inapplicable. These sets were also discarded, resulting in a total of 20 datasets for use in sampling plan development. Sampling plans were developed following the steps outlined in Galvan *et al.*¹⁷ and Butler and Trumble.⁷

In the initial step, the empirical relationship between the proportion of plants infested with a given number of psyllids (P_T) and mean psyllid density (m) (Fig. 1) was determined using the equation

$$\ln(m) = \alpha + \beta \ln[-\ln(1 - P_T)]$$

where T is the tally threshold and represents the requisite number of psyllids present on a plant to be classified as infested. For a binomial plan, this value is set at 1. The parameters α and β were estimated by linear regression of $\ln(m)$ on $-\ln(1 - P_T)$ and are the slope (α) and intercept (β) of the regression line. To determine the associations among density relationships in various life stages (adults, nymphs, eggs), the homogeneity of slopes among stages was examined. A significant difference in slopes ($p < 0.05$) indicated that life stage should be examined separately, while no difference would support pooling of stages. A similar approach was used in comparing years and fields.

In the second step of developing the sampling plan, stop lines were generated using Wald's sequential probability ratio test as calculated by the RVSP (Resampling for Validation of Sample Plans) Microsoft Excel plug-in (RVSP can be downloaded for free

at <http://www.ars.usda.gov/pandp/docs.htm?docid=10750>, along with accompanying literature and references). One important benefit of this resampling method is that it is independent of underlying statistical assumptions.¹⁸ SPRT calculations require the parameters θ_1 and θ_2 , the upper and lower boundaries for decision action thresholds, the type I error (α) and the type II error (β). A type I error occurs if treatment is applied when pest density is actually below the threshold, while a type II error would be non-treatment when the pest density is actually above the threshold. Resampling was conducted with replacement using values of 0.1 for α and β .

In the final step, operating characteristic functions and mean sample numbers for each threshold of interest were used to validate the plan.

3 RESULTS

3.1 Selection of sampling unit and spatial distribution

Comparison of Taylor's power law relationships among life stages revealed significantly different slopes (ANCOVA: $F = 4.90$; $df = 3, 17$; $P < 0.02$). Further analysis with Tukey's HSD *post hoc* test revealed that adults differed significantly from eggs and nymphs, but nymphs and eggs did not differ from each other. For the present analysis, it was therefore decided to sample nymphs on whole plants, as this is the most time-efficient and least visually taxing combination of factors.

Three indices of spatial aggregation were calculated (Table 1), which gave similar results. Iwao's mean crowding regression showed a very strong fit with slopes below, but not significantly different, from 1 (t -test: $t = 0.637$; $df = 1$; $P = 0.72$), an indication of aggregation. Green's index was substantially greater than 0, which also indicates aggregation. Finally, the spatial distribution of nymphs, as tested by Taylor's power law, did not differ among fields (ANCOVA: $F = 0.91$; $df = 2, 18$; $P = 0.4$), between years (ANCOVA: $F = 0.18$; $df = 1, 18$; $P = 0.67$) or among sampling dates within a year (ANCOVA: $F = 0.0010$; $df = 1, 18$; $P = 0.98$). Consequently, a single Taylor's power law regression was performed for all data on nymphs pooled. The pooled power regression yielded a slightly poorer fit than Iwao's mean crowding regression (Table 1), and the slope was marginally different from 1 (t -test: $t = 5.33$; $df = 1$; $P = 0.059$), suggesting a random or aggregated spatial aggregation.

While plot size considerations made it difficult to examine edge effects in Orange County fields and the 2009 Ventura County data, the 2010 data from Ventura County were suitable. For these data, edge is defined as any plant in the outer rows, or the first or final 20 m of 100 m plots. This resulted in a total of 90 plants. There was no significant difference in the distribution of infested plants on the edge and in the center of these fields. Thirty-five infested plants were located on the edge versus six within the field, and four uninfested plants were on the edge versus none in the center of the field (chi-square: $\chi^2 = 0.675$; $df = 1$; $P = 0.41$). There was also no difference in the mean number of nymphs collected on the edge of the field (124.6 ± 186.8) and within the field (205.2 ± 348.3) (t -test: $t = 0.561$; $df = 5.207$; $P = 0.60$).

To determine whether nymphs were distributed evenly among vertical sections of plants, plants were measured and separated into thirds (top, middle, bottom) at the time of sampling. Thus, each sample represented the spatial distribution at a given time, rather than at an absolute height. There was a significant difference in the number of nymphs within vertical sections of plants (ANOVA: $F = 9.33$; $df = 2, 492$; $P < 0.001$), with more nymphs in the top and middle sections of the plants. Also, infested leaves (those with one or more nymphs) were more common in the top and middle of

Table 1. Indices of spatial dispersion

Range of means	Green's Index	Iwao's mean crowding regression			Taylor's power law		
		<i>a</i>	<i>b</i>	<i>r</i> ²	<i>a</i>	<i>b</i>	<i>r</i> ²
2.5–50.5	38.27	0.49	1.57	0.99	1.9	1.63	0.89

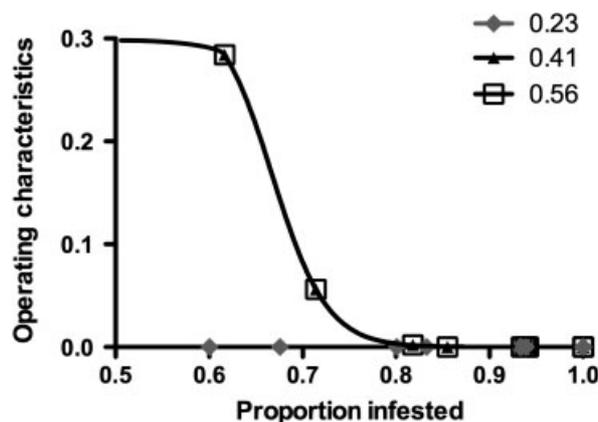


Figure 2. Operating characteristic curves for potato psyllid binomial sequential sampling plans with action thresholds of 23% (gray diamonds), 41% (triangles) and 56% (squares) of plants with at least one psyllid nymph.

plants than the bottom (chi-square: $\chi^2 = 80.65$; $df = 1$; $P < 0.001$). Psyllid nymphs showed a preference for leaves to all other types of plant tissue; only four infested plants contained nymphs on tissue other than leaves (three on stems, one on fruit). Nymphs did not show a preference for the side of leaves, with an average of 14.8 on the bottom of leaves and 13.5 on the top (t -test: $t = 0.595$; $df = 1109$; $P = 0.55$).

3.2 Binomial sequential sampling plan

Binomial sampling plans, based on the presence/absence of psyllids, were initially developed on the basis of action thresholds ranging from 15% infestation (mean density of 0.5 nymphs plant⁻¹) to 60% infestation (mean density of 30 nymphs plant⁻¹) (Fig. 1). Each plan was evaluated on the basis of steepness of operating characteristic curves. Action thresholds below 30% generated slopes of 0 (Fig. 2) and were rejected. Among the remaining plans, the plan based on 41% infestation (mean density of 5 nymphs plant⁻¹) was nearly identical to that of 56% infestation (Fig. 2). Thus, the 41% infestation plan was optimal, and is presented in Fig. 3. Under the plan, if during sampling the cumulative number of infested plants is above the upper threshold line, management action should be taken. Conversely, if the cumulative number of infested plants falls below the lower decision line, no action is necessary. When the cumulative number of infested plants is between the two decision lines, additional sampling is necessary. The average number of samples for this plan is 11 plants, with an absolute maximum of 49 plants. Therefore, if no decision is reached after sampling 50 plants, sampling should be postponed to a future date.

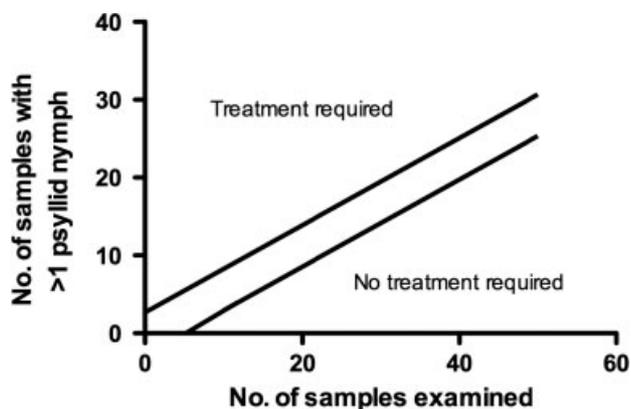


Figure 3. Decision lines for the binomial sequential sampling plan based on a rate of 41% infested with at least one potato psyllid nymph.

4 DISCUSSION

An overall sampling plan must consider both spatial and practical elements. Spatially, it is necessary to know where to search for the pest in question, or there is a risk of falsely assuming a negative. In peppers, as in potato, psyllid nymphs were located primarily on leaves in the top two-thirds of plants. Unlike potato, there was no preference for a particular side of a leaf. Practical considerations of sampling include the ability to see the insect, accessibility within the field, counting accuracy, precision, and considerations of cost and time. In this regard, nymphs are an ideal unit for sampling potato psyllids in peppers as they are more sessile and abundant than adults, but easier to see than eggs.

In the present sample set, psyllid nymphs were distributed at similar frequencies on the edge and interior of fields. This contradicts findings for potato psyllids in potato fields.^{7,10} Possibly, this lack of edge effect resulted from the severe level of infestation in the field sampled. It is also possible that the pattern stems from the different crops. Martini *et al.*¹⁰ have shown that spatial distribution of potato psyllids varies with potato variety, and there are known preferences with tomato variety as well.¹⁹ A similar pattern may apply to edge effects. Three dispersion indices were used to evaluate the spatial distribution of nymphs throughout the sampled fields, and all were in agreement that nymphs were aggregated. These results match those for psyllids in California potato fields⁷ and for psyllid nymphs in Texas potato fields.¹⁰ Other psyllid species known to aggregate include *Diaphorina citri* Kuwayama²⁰ and *Trioza ertreae* (Del Guccio).²¹ Taylor's power law demonstrated that spatial aggregation was consistent among a series of factors including year and field. This suggests that aggregation of potato psyllid nymphs in crops is common regardless of insecticide treatment or environmental variation. This was somewhat surprising, as pesticide applications in other crops have changed populations from aggregated to random distributions.²²

Sampling plans can be either enumerative, in which case all individuals are counted, or binomial, which is based on presence/absence. Enumerative plans offer greater precision,¹² but at a cost of efficiency and time. When a sampling plan is intended to inform management decisions, as in IPM, efficiency is of primacy because of the time involved with sampling multiple fields and implementing treatment. This makes a binomial sampling scheme preferential, as it is faster and less costly. Here, a sampling plan for psyllid nymphs in bell pepper fields is presented. The plan can be carried out quickly and inexpensively because

it will require, on average, only 11 presence/absence samples to make a decision about insecticide application. To date, no economic threshold exists for potato psyllids on any crop, so Butler and Trumble⁷ presented sampling plans for potato psyllids on potato that were based on multiple thresholds. The plan here is based on a single threshold, an average density of 5 nymphs plant⁻¹ (41% infestation). This exceeds the highest rate for potato, but 'zebra chip' symptoms are not a problem for peppers, and pepper fields have both a greater range of overall and per-plant psyllid densities. In addition, the damage from potato psyllids on peppers is visibly detectable, while ZC symptoms are often delayed until it is too late for action, because early exposure leads to the most extreme symptoms and damage.⁴ Thus, growers can be more conservative in their pesticide applications to peppers than would be required for tomato or potato. The exception is a 'good neighbor' policy, where applications are made to bell peppers in order to reduce risk to nearby fields of CLP-susceptible crops. This plan should provide an important contribution to management of potato psyllids in bell pepper and, by extension, in neighboring fields of other solanaceous crops.

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