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Source: Journal of Economic Entomology, 107(2):838-845. 2014.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/EC13328

SAMPLING AND BIOSTATISTICS

A Binomial Sequential Sampling Plan for *Bactericera cockerelli* (Hemiptera: Triozidae) in *Solanum lycopersicum* (Solanales: Solanacea)

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J. Econ. Entomol. 107(2): 838–845 (2014); DOI: http://dx.doi.org/10.1603/EC13328

ABSTRACT The tomato–potato psyllid *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) is a pest of many solanaceous plants, including tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.). In tomato, feeding by nymphs is associated with "psyllid yellows." *B. cockerelli* also vectors "*Candidatus* Liberibacter psyllaurous," an infectious bacterium that causes "vein greening" disease. Decisions about management action are much more effective when guided by robust sampling. However, there are few previous studies of potato psyllid spatial distribution in tomato fields, and no published sequential sampling plans for the pest in tomato. We studied *B. cockerelli* in various tomato fields in California and used these data to generate a sequential sampling plan. We found that juvenile *B. cockerelli* in tomato fields exhibit an edge effect, an aggregated distribution, and individuals are primarily located on the bottom of leaves. Psyllids were concentrated in the upper segments of plants, but this changed over time. Finally, we present three binominal sequential sampling plans for managing tomato psyllids in tomato fields. These plans differed from both those for bell pepper (*Capsicum annum* L.) and potato, indicating that *B. cockerelli* needs to be sampled using crop-specific sampling plans.

KEY WORDS tomato psyllid, zebra chip, *Candidatus* Liberibacter psyllaurous, *Bactericera cockerelli*

The potato–tomato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a phloem-feeding insect with a large geographic distribution that encompasses most of western North America, New Zealand, and parts of Central America (Butler and Trumble 2012a). *B. cockerelli* is a pest of many solanaceous crops, and uses host plants in >20 plant families (Pletsch 1947, Wallis 1955, Butler and Trumble 2012a). Damage from *B. cockerelli* occurs via three mechanisms. First, in some crops, such as bell pepper (*Capsicum annum* L.), damage is primarily due to sooty mold growth on honeydew or the direct result of feeding. Second, *B. cockerelli* vectors "*Candidatus* Liberibacter solanacearum" (syn. "Ca. L. psyllaurous") (CLP) (Hansen et al. 2008). CLP is the pathogenic cause of "zebra chip" (ZC) disease in potato (*Solanum tuberosum* L.) (Hansen et al. 2008, Liefting et al. 2008, Munyaneza et al. 2012) and "vein greening" disease in tomato (*Solanum lycopersicum* L.). Vein greening disease results in spiky and chlorotic apical growth; leaf mottling; midvein curling; shortened internodes; and small, deformed, poor-quality fruit (Liefting et al. 2008, McKenzie and Shatters 2009). Finally, feeding by *B. cockerelli* nymphs on tomato and potato can result in "psyllid yellows" (PY), an "infection" of unknown cause with symptoms that include reduction in growth, chlorosis or reddening of leaves, damaged internodes, and, importantly, reduced fruit size and quality in tomato (Pletsch 1947, Cranshaw 1994, Butler and Trumble 2012a). PY can result in plant death and yield reductions (Cranshaw 1994). Although little data are available to determine current losses in tomato from either PY or CLP, Liu and Trumble (2004) reported 85% losses in Mexico in 2001 because of *B. cockerelli.* Substantial loses have been observed in Arizona greenhouses, and Australia has quarantined New Zealand tomatoes (Crosslin et al. 2010). Collectively, this suggests considerable risk to California tomato agriculture.

The relation between the number of psyllids infesting a plant and severity of PY symptoms varies by tomato variety, but Liu and Trumble (2006) have demonstrated that as few as eight nymphs feeding on 2-wk-old plants can cause PY symptoms. Similarly, it has been demonstrated that feeding by a single adult psyllid can transmit CLP to potatoes (Buchman et al. 2011), and that the number of psyllids feeding influences bacterial titer, inoculation success, and inoculation time (Rashed et al. 2013). Such a comprehensive study has not been conducted in tomatoes but CLP translocation is known to vary among plants species (Levy et al. 2011), but 3 d of exposure to two psyllids is sufficient to transmit CLP to plants (S.M.P.,

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0022-0493/14/0838–0845$04.00/0

unpublished data). These values would indicate that, contrary to the more liberal approach suggested by Prager et al. (2013) for peppers, a conservative approach is needed in tomatoes.

To date, *B. cockerelli* has been primarily considered a pest of potato. This is largely because of losses incurred in Texas due to ZC. In California, ≈5,600 ha of potatoes were planted in 2012 (U.S. Department of Agriculture–National Agricultural Statistics Service [USDA–NASS], 2012) and these are clearly at risk from *B. cockerelli* infestations. However, this area pales in comparison to the ≈90,000 ha of tomatoes grown in California in 2012 (USDA–NASS, 2012) that are susceptible to both PY and CLP. Currently, there are binomial sampling plans available for *B. cockerelli* in both bell peppers (Prager et al. 2013) and potatoes (Butler and Trumble 2012b). In addition, studies have examined sampling methods for *B. cockerelli* in potatoes (Martini et al. 2012, Yen et al. 2012). To date, there are no published sampling plans or methods for *B. cockerelli* in tomatoes. Acceptable infestation levels and pest distributions frequently vary among crops. Consequently, sampling plans often cannot be used across crops (Trumble et al. 1989), requiring sampling plans that are specific to a given crop and pest combination. This article addresses the deficiency of sampling recommendations for tomatoes by developing and presenting a binomial sequential sampling plan for *B. cockerelli* on tomato.

Materials and Methods

Sampling and Study Locations. In 2009 and 2010, biweekly sampling of entire tomato plants was conducted at two sites. The first site was a 24-ha commercial field in Oxnard, Ventura County, CA, planted with a "Roma" variety. The second field was located in Irvine, Orange County, CA, at the University of California's South Coast Research and Extension Center (SCREC). The Orange County field was 0.2 ha, planted with the "Bobcat" variety of tomatoes. In addition, this field was adjacent to a 0.2-ha field of bell peppers of variety 'Baron," and a similarly sized field of "Atlantic" variety potatoes. The fields at Orange County have been previously used in developing binomial sampling plans for psyllids in potato (Butler and Trumble 2012b) and bell pepper (Prager et al. 2013). In 2009, biweekly sampling was initiated with the appearance of psyllids, beginning in July and continued until November. In 2010, sampling began in May and was also conducted biweekly until November. Sampling was destructive and consisted of haphazardly selecting entire plants that were transported to the laboratory. Psyllids were tallied as eggs, nymphs, and adults, each of which was morphologically distinct. The use of both commercial and experimental fields for sampling gives a range of conditions, including pesticide-treated and untreated plants, varying weather conditions, different host plant varieties, and differing cultural practices. Consequently, the plans and conclusions derived from these samples should be robust under various conditions.

The Orange and Ventura County fields differed substantially in size, with larger rows in the Ventura fields. Consequently, cultural practices (including spacing) also varied between sites, and different sampling methods were required in Orange and Ventura counties. Fields in Ventura County were sampled using a systematic sampling design, while sampling in Orange County was conducted using a stratified random design. The number of plants sampled varied with the number of plants in a row, but in all sites a minimum of 8 and a maximum of 18 entire plants (all leaves, stems, and fruit) were sampled on each sampling date.

Psyllid Distributions. To effectively sample insects within fields, it is necessary to know the patterns of spatial distribution of the insect of interest. Without such information, one may unknowingly over (or under) collect from infested or uninfested areas. Because the use of a single dispersion index is often misleading (Myers 1978, Trumble et al. 1995), we calculated three different dispersion indices (Green's index, Taylor's power law, and Iwao's mean crowding). First, we calculated Green's index (C_x; Green 1966, Fortin 1999) 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 insects in I sample units. Next, we calculated Taylor's power law (Taylor 1961, 1965)

$$(s^2 = am^b),$$

which relates variance to abundance on a $\log_{10}$ scale, such that a population increase of 1 U on a $\log_{10}$ scale has the associated variance expected to increase by 2 U on a $\log_{10}$ scale. Thus, the term log a functions as a scaling factor and b (the slope) measures aggregation. Taylor's power law was calculated by regressing the $\log_{10}$ of variance (s^2) against the $\log_{10}$ mean psyllids. To determine if insect stage (egg, nymph, and adult), year, or sampling location influenced spatial distribution, we performed analysis of covariance (ANCOVA) using Taylor's power law but incorporated a term for life stage, year, or field. Each factor was examined in a separate model. Groups were pooled when nonsignificant ($P > 0.05$). After regressions, student's *t*-tests were used to determine whether the slopes of regression lines were different from 1.0.

To calculate Iwao's mean crowding (Iwao 1968), we first calculated mean crowding using the equation:

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

where s^2 is the variance from sample counts and m is the mean of the sample counts. Mean crowding (m^*) was then regressed on the mean to generate the intercept (a) and slope (b) that were subsequently used to estimate α and β for solving the equation

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

Location of Psyllids Within Plants. We examined the distribution of juvenile (eggs and nymphs) tomato psyllids within plant strata (top, middle, or bottom third of the plant) using a generalized linear mixed model with a negative binomial distribution (R package MASS, R Development Core Team, Vienna, Austria; [Venables and Ripley 2002]). The negative binomial model accounts for overdispersion commonly found in count data. In the model, the dependent variable was number of psyllids and the fixed effect variables were strata, sampling week nested within year, and location. Initially, all interactions were included, but only the location × strata interaction was significant. Thus, a final model retaining the fixed variables: strata, location, and sample week nested within year, and the location × strata interaction was examined.

Development of Binomial Sequential Sampling Plan. We included all data sets that met the assumptions of the Resampling for Validation of Sample Plans (RVSP) Microsoft Excel plug-in, which was used for evaluating sampling plans (RVSP can be downloaded from: http://www.ars.usda.gov/pandp/docs.htm?docid = 10750). Overall, we sampled fields on 28 different occasions for the purpose of developing a binomial sequential sampling plan. There were no psyllids on one sampling date, so that event was eliminated from subsequent analysis resulting in 27 data sets. Of these sets, 13 were collected at the Orange County site and 15 at the Ventura County site. These data were collected in 2 yr, with 12 sampling events in 2009 and 16 in 2010.

The remaining suitable data sets were used to develop sampling plans following the methods described by Butler and Trumble (2012b) and Prager et al. (2013). This stepwise approach began with determining the empirical relationship between the proportion of plants infested with a specified number of psyllids (P_T) and mean psyllid density (m) using the equation:

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

where T is the tally threshold, which is held at one for binomial sampling plans, establishing one psyllid as the minimum for being considered infested. The remaining parameters are determined by regressing $\ln(m)$ on $-\ln(1 - P_T)$, with the slope of the regression line providing α and the intercept providing β.

The second step in developing our sampling plan involved generating stop lines using Wald's sequential probability ratio test, which is defined as:

$$X_{n(T)} \geq R_x + Q \text{ and } X_{n(T)} \leq R_x - S,$$

where the term $T_{n(t)}$ is the cumulative number of samples infested with at least T psyllids. The terms Q, R_x, and S are functions of the type I (α) and type II (β) error rates. A type I error is unnecessary treatment, while a type II error is failure to apply a necessary treatment. As is common when developing binomial sampling plans, we set our error rates to 0.10 for both α and β (Naranjo and Hutchison 1997, Hodgson et al. 2004, Galvan et al. 2007, Prager et al. 2013). We set the upper and lower boundaries of the action threshold at 0.1. The sampling plan was developed and validated using RVSP, performed with replacement, and 500 simulations. The final step of plan development consisted of evaluating the plan(s) based on mean sampling numbers and operating characteristic (OC) functions.

Table 1. Indices of spatial aggregation for juvenile tomato psyllids in tomato fields

Range of means	Green's index	Iwao's mean crowding regression			Taylor's power law		
		a	b	r^2	a	b	r^2
0.4–503.7	0.23	−67.2	98	0.75	1.95	1.80	0.95

All statistical analyses, except resampling, were performed using the R 2.15.0 (R Development Core Team, Vienna, Austria) statistical package.

Results

Selection of Sampling Unit. Prior sampling plans for *B. cockerelli* focused on all life-stages when sampling potato fields (Butler and Trumble 2012b) and only nymphs when sampling peppers (Prager et al. 2013). We performed ANCOVA using Taylor's power law regression including a term for life-stage. A nonsignificant effect of life-stage would indicate that all psyllid life-stages are distributed similarly, and thus all stages could be pooled. Conversely, a significant effect would suggest that life stages should be examined individually. Overall, Taylor's power law analyses revealed that there is a significant difference in spatial aggregation among life stages ($F = 9.7$; df = 2, 43; $P < 0.001$).

Because adult *B. cockerelli* are typically difficult to see and sample, and there were significant differences among stages, we chose to exclude adults from most analyses and in development of our sampling plan. When ANCOVA was repeated after removing adults, the distribution of eggs and nymphs was not significantly different (ANCOVA: $F = 0.28$; df = 1, 28; $P = 0.3$). Therefore, we focused our sampling plan on the sum of eggs and nymphs, which are both easier to locate within plants and less mobile.

Within Field Distributions. To examine the spatial distribution of psyllids within fields, we calculated three indices of spatial aggregation (Table 1). To different extents, all three suggested aggregated distributions of juvenile psyllids within fields. Specifically, Greens's index was above zero, although only moderately. To test for potential effects of sampling and site, we initially performed Taylor's power law regression as ANCOVA including these terms. There was no significant effect of site (ANCOVA: $F = 3.8$; df = 1, 17; $P = 0.07$), or of year (ANCOVA: $F = 4.04$; df = 1, 15; $P = 0.63$). Consequently, we conducted a single Taylor's regression analysis using all data pooled. The resulting model was a strong fit and had a slope that differed significantly from 1 (TTEST: $T = 13.17$; df = 1; $P < 0.001$). Finally, Iwao's mean crowding regression resulted in a slope of 0.75, which was significantly different from 1 (TTEST: $T = 5.7$; df = 1; $P < 0.001$).

To test for a potential "edge effect," we examined the number of plants infested with juvenile psyllids using a combined data set from the two sites in 2009 and 2010. There were significantly more infested plants on the edge of the field (101) versus within the field (55; Chi-square: $\chi^2 = 7.94$; df = 1; $P < 0.01$). Although the minimum number of juvenile psyllids found was zero on both the edge and within the field, the maximum on the edge of the field was 8,576 versus only 430 within the field, and the population means differed significantly (Wilcoxon rank sum test: W = 26670, $P < 0.001$).

Within Plant Distribution. Juvenile tomato psyllids showed a significantly nonrandom distribution among types of plant tissue (Fishers exact test: $P < 0.001$), with an extreme preference for leaves (640 observations) relative to stems (40 observations) or flowers (2 observations). In addition, juvenile psyllids demonstrated a significant preference for the abaxial (bottom) of leaves (median = 38.5) versus the adaxial (top) of leaves (23.0) (Wilcoxon rank sum test: W = 13985; $P < 0.001$). This trend also exists when eggs (top = 27.5, bottom = 48.5; W = 3024.5; $P < 0.05$) and nymphs are examined individually (top = 13.5, bottom = 30.5; W = 4084.5; $P < 0.05$).

There is a significant difference with respect to plant strata (top, middle, and bottom) ($\chi^2 = 17.009$; df = 2; $P < 0.001$), of location ($\chi^2 = 9.9$; df = 1; $P < 0.001$), and of time nested within year ($\chi^2 = 17.009$; df = 2; $P < 0.001$), in addition to a strata × location interaction ($\chi^2 = 8.275$; df = 2; $P < 0.05$; Fig. 1).

Binomial Sequential Sampling Plan. We initially developed multiple binomial sequential sampling plans for tomatoes based on rates of infestation from 8% (0.5 psyllids per plant) to 89% infestation (10 psyllids per plant). After eliminating those plans that were clearly unsuitable (OC slope of zero, too many samples required, etc.) we settled on three plans for further evaluation. These plans were based on 27, 57, and 70% infestation (equivalent to 1, 3, and 5 juvenile psyllids per plant; Fig. 2). The quality of the sampling plants can be evaluated based on the OC curves, in which a "steeper" curve indicates lower error probabilities relative to the action threshold (Naranjo and Hutchison 1997). Evaluating OC revealed a sharper decline in the 57% infestation plan (Fig. 3), the 27% plan was less optimal (Fig. 3), and the plan based on 70% infestation was both least optimal and least conservative (Fig. 3).

Stop lines for each of the three sequential sampling plans are presented in Fig. 4. To apply the plan, one collects samples until the cumulative number of infested plants falls above the upper stop line, which indicates treatment is necessary, or below the lower line, which indicates no treatment is required. When the cumulative number of infested plants is between the two lines, more samples need to be taken. The 57% infestation sample plan requires an average of 18 samples; however, as many as 100 samples may be required to make a decision. The 27% plan requires an average of eight samples, which was also the maximum number required to reach a decision in our resampling. The 70% plan requires an average of 13, but up to 100 samples were required in resampling to reach a decision.

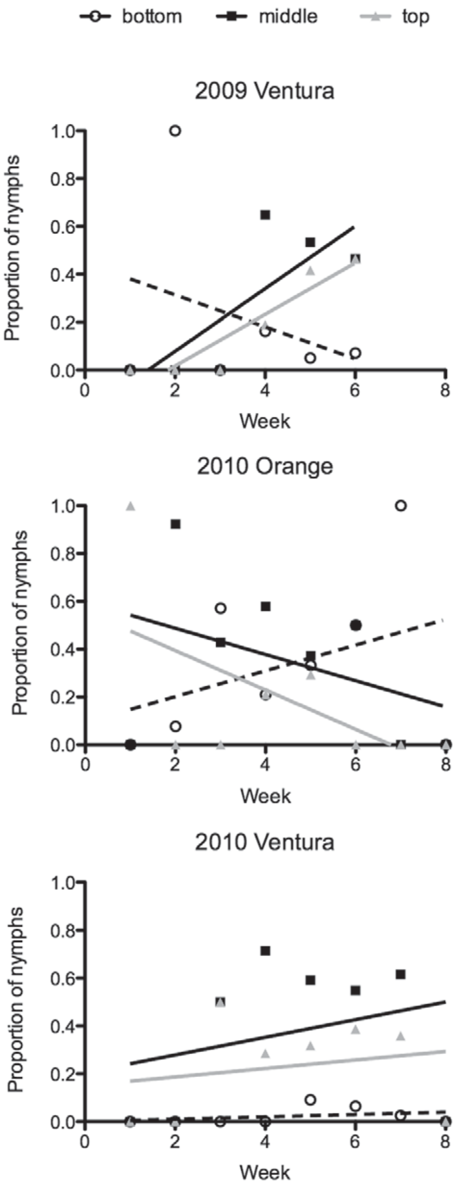

Fig. 1. The proportion of psyllid nymphs in the top (gray triangles), middle (squares), and bottom (circles) thirds of plants over time in 2009 and 2010 at the Ventura County and Orange County sampling sites. No data are shown for Orange County in 2009 as nymphs were only detected on 2 d.

Discussion

A major concern when developing a pest management sampling plan is that it will result in a failure to take necessary action. One way to reduce this risk is to sample in those areas where the pest is more likely to be located. To that end, it is important to determine where psyllids are located within fields and within plants.

Previous studies of within-field *B. cockerelli* distribution in the fields have suggested an "edge effect" with more psyllids of all stages in border areas than

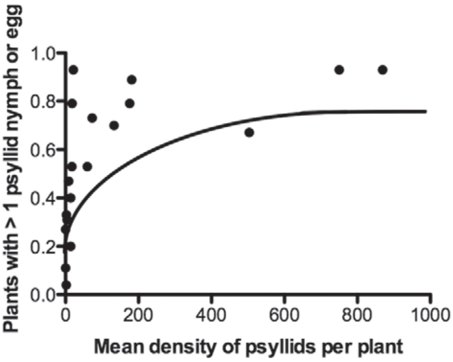

Fig. 2. Relationship between the proportion of tomato plants infested with one or more *B. cockerelli* nymph or egg and the mean number of psyllid nymphs per plant, $R^2 = 0.55$.

within the field (Butler and Trumble 2012b, Martini et al. 2012), and a similar effect is found with ZC infected potato plants on edges (Workneh et al. 2012). In our study, we found a greater number of infested plants and a greater number of psyllid eggs and nymphs on the edges of tomato fields. Interestingly, bell peppers are the only closely examined crop where an affinity for the edges of fields was not observed. Our findings suggest that sampling efforts in tomato fields should concentrate on the edges of fields, although collecting some samples within fields is still prudent as psyllids are occasionally found within fields. Similar to pepper (Prager et al. 2013) and potato (Butler and Trumble 2012b), we found that psyllid eggs and nymphs are aggregated within fields. Such aggregation suggests that sampling must be spaced throughout the edges of fields because one can easily find a densely (un)infested field if only sampling one location. The tomato psyllid aggregation is similar to findings for other psyllid species including *Diaphorina citri* Kuwayama (Tsai et al. 2000) and *Trioza ertreae* (Del Guercio) (Samways and Manicom 1983). Of interest, these psyllids failed to aggregate regardless of sampling location (Orange County or Ventura County). This is interesting because the various sampling sites were subjected to different management schemes and pesticide applications, which are known to cause a shift from aggregated to random distributions (Trumble 1985). We found that patterns for spatial aggregation of eggs and nymphs were not significantly different, while they did differ from distributions of adults. This may result from the relative mobility of adults relative to other life stages, or may instead be an artifact of the relative difficulty of locating adults versus eggs and nymphs.

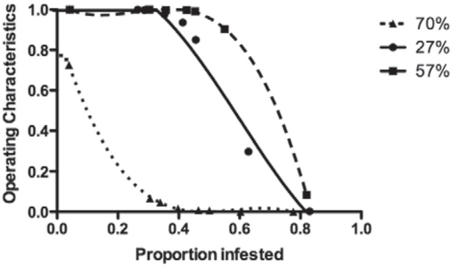

Fig. 3. OC curves for tomato psyllid binomial sequential sampling plans with action thresholds of 27 (one psyllid threshold, triangles), 57 (three psyllids, circles), and 70% (five psyllids, squares) of plants with at least one psyllid nymph or egg.

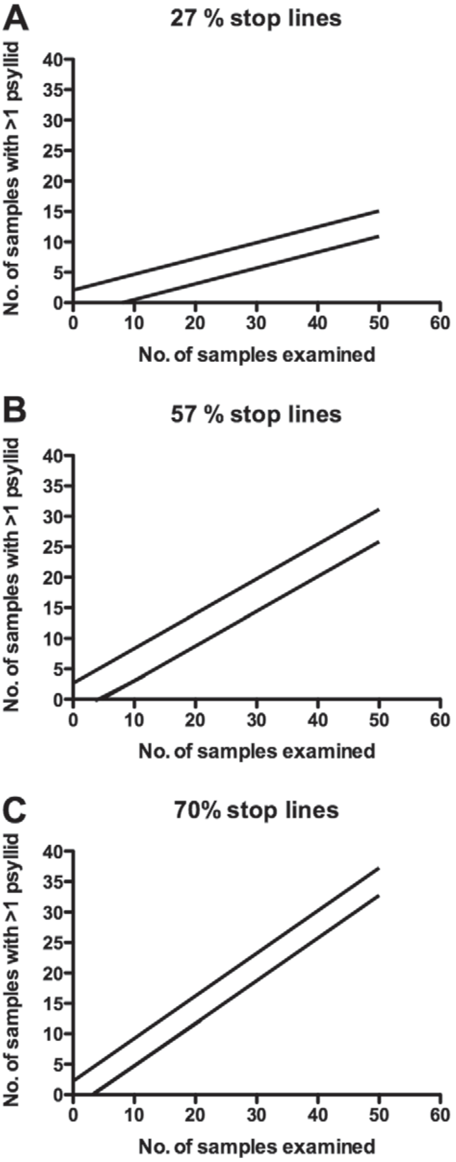

Fig. 4. Decision lines for the binomial sequential sampling plan based on a rates of 27 (A), 57 (B), and 70% (C) of tomato plants infested with at least one *B. cockerelli* egg or nymph.

In multiple studies of psyllids in potatoes (Butler and Trumble 2012b, Martini et al. 2012, Workneh et al. 2012) and in bell peppers (Prager et al. 2013), psyllids were reportedly concentrated in the upper two-thirds of plant material. Our data suggest that this is also the case in tomato, with some nuances. Analyses revealed effects of year, site, and time on the numbers of psyllids in different strata of plants. There are a few possible explanations for these differences. First, this observation may reflect differences among life-stages. Some previous studies excluded adults or eggs, and thrips have been shown to distribute differently in tomatoes based on life-stage (Reitz 2002). A second explanation is that the psyllid population density at the Ventura County study site was much higher than that at SCREC in both years, and this may have led to different distributions. Such a phenomenon is known to occur in aphids (Musser and Shelton 2003). We also found an effect of time, suggesting that psyllids may be either relocating over time or that the position of psyllid eggs and nymphs changes as stems elongate and new growth forms. Many psyllids prefer new growth (Liu and Trumble 2006, Munyaneza et al. 2007, Ikeda and Ashihara 2008), and it is possible that while eggs are laid in lower parts of plants, the nymphs relocate to newer growth over time. Finally, tomatoes in Ventura County commercial sites were staked while those in the Orange County site were not, and it is possible that stratification was more distinct in Ventura County than in Orange County, where plants tend to fall down into furrows. Whatever the explanation, we suggest that whole plants be inspected early in the season when they are smaller. After the first couple of weeks, sampling should focus on the top two-thirds of plants similar to potato (Butler and Trumble 2012b). We found that psyllids were primarily located on the abaxial side of leaves. This suggests that sampling for psyllids in tomato should focus on the undersides of leaves.

In adjacent fields sampled with identical methods and on the same days, we found that psyllids are not evenly distributed. Although there were no strong patterns, in 2009 adult psyllids appeared far more commonly on pepper than on tomato, a trend which was less distinct but also apparent in 2010. Conversely, juvenile stages were more common in tomato fields than in pepper, even on those dates where adults were prevalent in peppers. Although we cannot completely rule out a difference in the efficacy of sampling in the different crops, these findings seem to indicate that psyllids may not prefer, or use, crops equally. Another possible explanation of this pattern is response to insecticides. Different crops at SCREC are managed using a series of regimes specific to crops and to life-stages, and it is known that some insecticides are repellent to *B. cockerelli* (Butler et al. 2011). Consequently, these findings may indicate that psyllid adults are settling onto peppers, which are managed with less repellent compounds, or may indicate differences in the efficacy of psyllid treatment regimes in the pepper fields relative to tomato fields. Unfortunately, our data do not allow for a specific test of this hypothesis. However, it has been demonstrated that immature life stages vary between treated and untreated potato fields in Texas (Goolsby et al. 2012). Regardless, these results strongly suggest that sampling must be conducted using methods specific to a given crop, a finding also supported by the differences in within-plant distributions among crops, and the lack of edge effects in peppers relative to tomatoes and potatoes.

Sampling and action plans for insect pests are typically presented in one of two forms. Enumerative plans use exact counts to generate more precision (Pedigo 1994), but this necessitates more counting with an increase in associated costs. Binomial sampling plans are less precise but require less time to implement. These time savings are important as they make it more practical to survey multiple fields and to survey more often.

Currently, there are no economic thresholds for tomato psyllids in any crop (Butler and Trumble 2012b, Prager et al. 2013). However, because in potato ZC disease is an extreme concern and the primary target of management, a near zero-tolerance approach is typically taken. This is in contrast to recommendations for bell pepper, where CLP infection is not a concern and thresholds are assumed to be higher (Prager et al. 2013). Because tomatoes are subject to infection with CLP but are also subject to many other diseases and pests (Kennedy 2003), we assume that they require a more conservative management approach than peppers.

We present plans based on three different levels of infestation, the most conservative plan is based on an average of one psyllid per plant, which is the equivalent of 27% of an infected field. This 27% plan requires an average and a maximum of eight samples. Based on OC curves, we determined that a moderate plan based on an average of three eggs or nymphs per plant (57% infested field) was optimal. This plan requires an average of 18 samples, but has a very large maximum of >100 samples. Consequently, we recommend no >50 samples be taken before postponing to a future date or deferring to a more conservative plan. We also present a plan based on five psyllid eggs or nymphs (70% infestation) that requires an average of 13 samples and a maximum of >100. The 70% plan is least optimal based on OC curves, and thus should only be used if one is looking to specifically minimize treatment action.

Our data strongly suggest that fields of different crops must be sampled individually, using a plan specific to that crop. To facilitate this, we present binomial sequential sampling plans for tomato psyllid nymphs and eggs in tomato fields at three different levels of infestation. In combination with previously published sampling plans for peppers and potatoes, this should substantially improve psyllid management and lead to the development of area wide pest management schemes.

Acknowledgments

We thank W. Carson, B. Vindiola, J. Butler, and G. Kund for assistance in the field. C. Mogren, K. Hladun, B. Vindiola, D. De La Riva, W. Carson, and G. Kund provided comments that improved an earlier version of this manuscript. Funding for the work was provided in part by U.S. Department of Agriculture–The Specialty Crop Research Initiative (USDA–SCRI) grant (2009-34381-20036) and U.S. Department of Agriculture–Risk Avoidance and Mitigation Program (USDA–RAMP) grant (2009-51101-05892).

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Received 16 July 2013; accepted 7 January 2014.