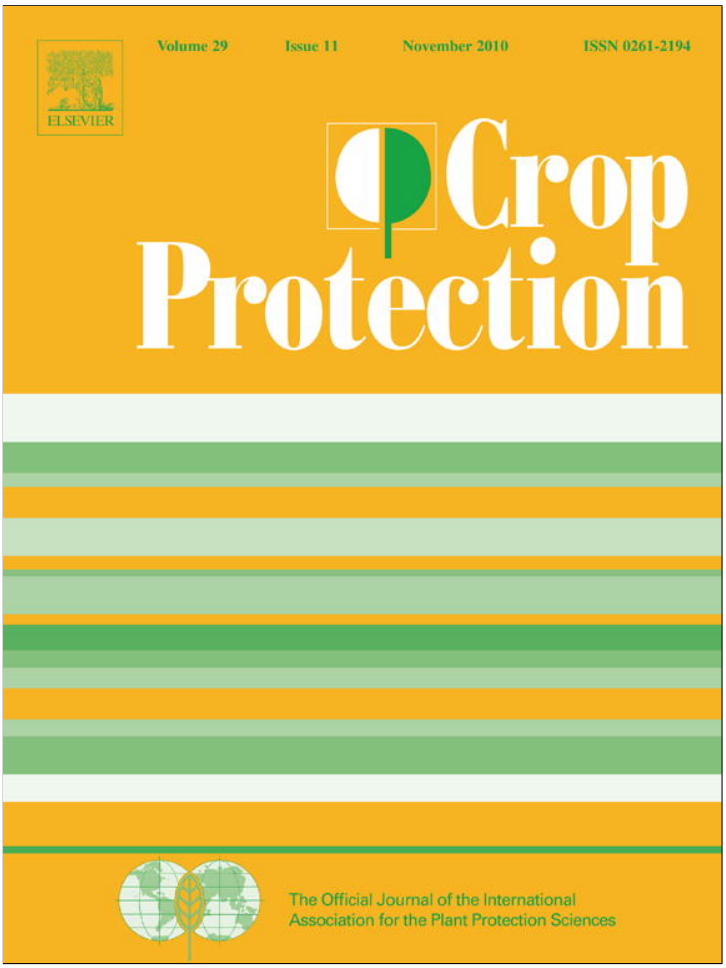

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Sequential sampling plans for estimating density of glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae) on citrus

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ABSTRACT

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar), is a serious pest of grapes and other crop and ornamental plants mainly through its role as a vector of the bacterium *Xylella fastidiosa* Wells. Citrus harbors large populations of this insect throughout much of the year in areas where the pest is problematic and improved understanding of the population dynamics and management of *H. vitripennis* on citrus may be key to its management in the broader agricultural landscape. In turn, the study of population dynamics and the development of management strategies require effective and efficient sampling methods. Within-tree sampling distribution studies revealed that adults and nymphs were more abundant and less variable in the upper strata of citrus trees (>1.5 m). They occurred in greater numbers on the southern quadrants of trees but relative variability did not differ due to cardinal direction. We developed and validated several fixed-precision sequential sampling plans for estimating the density of nymphs and adults of *H. vitripennis* using a pole bucket sampling method. Based on validation from resampling of independent data sets, Green's sequential sampling model, based on the Taylor's power law, provided the best overall performance in terms of providing mean density estimates with levels of precision equal to or better than the desired precision over a range of possible insect densities. Average sampling costs varied from about 21 to 189 min for a desired precision of 0.25 depending on insect density and whether the goal is to sample nymphs, adults or both stages combined. Further, the sampling plans developed on orange trees were robust, being equally effective on orange and lemon trees and on trees treated or not with insecticides.

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1. Introduction

The development of effective and sustainable management strategies for an invasive agricultural pest requires, at a minimum, a basic understanding of its ecology and implementation of control tactics that are least disruptive to its natural enemies. Indispensable to this goal are effective sampling tools for obtaining information on the distribution and abundance of the pest in its new environment, and their application in the development of management options. More often than not, management information, including sampling methods and plans for their implementation, may be lacking entirely for newly invasive pests in novel geographical regions, thus precluding species and crop-specific decisions that are the hallmark of knowledge-based pest management. While the lack of specific pest management information for an invasive species does not necessarily prevent effective control solutions from being implemented, sustainable pest management is better served by well-informed control practices including development of sampling plans (e.g., Naranjo and Ellsworth, 2009).

In the case of the entry of *Homalodisca vitripennis* (Germar), the glassy-winged sharpshooter, into California and its subsequent expansion through much of the state, prior knowledge of its field ecology and management was limited. Formal identification of *H. vitripennis* in California occurred in 1995 (Gill, 1995; Sorensen and Gill, 1996), but its presence in many parts of southern California was recognized well before this time, suggesting an introduction prior to 1990 (Blua et al., 1999). *H. vitripennis* is native to the southeastern United States and is a long known vector of the bacterium *Xylella fastidiosa* Wells, causal agent of phony peach disease and Pierce's disease of peach and grapes, respectively. Early efforts to control damage associated with the vectoring activity of *H. vitripennis* were focused largely on removing infected plants to reduce inoculum sources (Turner, 1933). Systemic insecticides for protection against phony peach disease were investigated decades ago (Kaloostian and Pollard, 1962) and again more recently (Dutcher et al., 2005), but concerted efforts to directly control *H. vitripennis* populations in its native territories have been sporadic.

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Emphasis on controlling *H. vitripennis* took on new urgency once the threat of expansion within California was comprehended. The point of reckoning came in early 2000 when federal, state and local representatives met in Temecula, CA, a relatively small wine-grape producing region in southern California where *H. vitripennis* had been established for years. The Temecula region is characterized by a mixture of citrus and grapes, an adjacent urban interface replete with landscape ornamentals and mild climate, all of which provided an ideal habitat for *H. vitripennis.* By early 2000, losses due to Pierce's disease were apparent with conspicuous gaps in vineyards where grape plants infected by *X. fastidiosa* had been pulled out to reduce in-field sources of inoculum. The epidemic of Pierce's disease had tracked the rising incidence of *H. vitripennis* in Temecula vineyards and become a textbook example of the consequences of an alien vector entering into a new region and exacerbating the Pierce's disease problem that had existed in California vineyards since the 19th century (Pierce, 1882; Hopkins and Purcell, 2002). With the recovery of *H. vitripennis* in Kern County in early 1998, the impact on vineyards in Temecula focused attention on the likelihood of similar scenarios playing out in the remaining 350,000 ha of grapes in California, many of which are cultivated in the San Joaquin Valley hundreds of kilometers to the north.

The crisis in Temecula also precipitated an urgent call for research to develop management approaches and improve understanding of *H. vitripennis* ecology and *X. fastidiosa* epidemiology (Meadows, 2001). As with any problem-solving effort, there was a need for quality tools to enable investigators to understand the fundamental nature of the problem and to evaluate potential solutions. Quantitative methods were required to facilitate objective comparison of various control approaches towards development of a successful management program. Not only was it important to have sampling tools that could provide accurate data for research, a long term goal was to provide a complete sampling program that could be incorporated into an IPM program for effectively managing *H. vitripennis*. The Pierce's disease problem in Temecula and other parts of California has been driven largely by high population densities of *H. vitripennis* that originate in citrus (Perring et al., 2001), not unlike the key role of citrus in Florida's *H. vitripennis* populations (Turner and Pollard, 1959; Adlerz, 1980). This presents a dilemma in carrying out IPM programs in citrus when the problem associated with *H. vitripennis*, i.e. the transmission of *X. fastidiosa* to grapes and other vulnerable crops, is of little concern to most citrus growers. Nevertheless, control in citrus is key to managing *H. vitripennis* populations, an observation substantiated by the resounding successes of regional control programs in California (Wendel et al., 2002; Hix et al., 2003; Stone-Smith et al., 2005).

Despite these regional successes, the multi-crop nature of the *H. vitripennis* and disease transmission problem will require the development of sampling plans and other components of an IPM program beyond grapes. Moreover, the role of *H. vitripennis* as a vector of the strain of *X. fastidiosa* that is the causal agent of citrus variegated chlorosis (CVC) disease of citrus in Brazil (Redak et al., 2004) points to the ongoing need to improve detection capabilities and evaluation of *H. vitripennis* population densities in several crops in California and elsewhere. Recently, we quantitatively compared four sampling methods including two vacuum devices, a beat net device, and a pole bucket device for estimating relative population density of *H. vitripennis* in citrus (Castle and Naranjo, 2008). The pole bucket sampler was found to be the most efficient method based on precision and cost, and had the added advantage of being most sensitive to detecting low infestations. As a step towards sustainable IPM for *H. vitripennis*, here we develop and validate sequential sampling plans based on the pole bucket method.

2. Materials and methods

2.1. Study site

We conducted all sampling and validation studies at the Agricultural Operations Farm, University of California, Riverside, CA between 2001 and 2003. The university farm contained a wide variety of citrus of varying ages that collectively harbored variable population of *H. vitripennis* allowing us to sample from a range of densities. Within-tree spatial distribution studies were conducted in a total of six orchards within one year. Trees in this study varied from 17 year old Washington navel oranges (grafted on Troyer citrange) ca. 3 m in height to >30 year old Valencia oranges (grafted on Troyer citrange) ca. 9 m in height. We conducted sampling for sample plan development primarily in two sections of a 5.2 ha Valencia orange grove (var. Frost Valencia grafted on Troyer citrange). Two samples were taken in a third orchard of Valencia oranges on the south side of the farm where *H. vitripennis* infestations were considerably lower. We collected additional samples for sample plan validation purposes on multiple dates over a two year period from three blocks of >30 year old oranges (var Frost Valencia grafted on Troyer citrange) and one block of lemon (var Lupe grafted on Cook) situated in the center of a 12-ha orchard. This orchard was part of a controlled study to examine the efficacy and residual activity of imidacloprid and thiamethoxam on *H. vitripennis* (Castle et al., 2005). We drew data sets from both treated and untreated plots within these orchards for validation purposes.

2.2. General sampling method

Prior analyses of four sampling devices (Castle and Naranjo, 2008) indicated that a pole bucket sampling device was the most efficient and flexible sampling tool for assessing the relative density of *H. vitripennis* in citrus. The device consisted of a lightweight 19 l plastic paint bucket firmly attached to a 3.7 m extension pole made from rigid, but lightweight electrical conduit (2.2 cm dia.). The original bottom of the bucket was cut away and replaced by a large plastic funnel riveted to the outer sides of the bucket that directed dislodged adults and nymphs into a plastic container screwed into a lid fastened to the bottom of the funnel. The basic design was adapted from the beat bucket device used to sample arthropods in cotton (Knutson et al., 2008) and similar to the funnel collection method used to sample arthropods from deciduous fruit trees (Bostanian and Herne, 1980). The attachment of a rigid pole to the bucket sampler provided extended reach and permitted access to both lower and upper sections of the trees. For sample plan development and validation, a sample unit consisted of thrusting the device into the foliage five times at five different sites (25 total thrusts) around the circumference of an individual tree at ca. 1.5 m and above in height. For the five thrusts at a given site, the bucket was moved slightly so that different portions of the general site were sampled.

2.3. Within-tree distribution

To examine the spatial distribution of *H. vitripennis* nymphs and adults in citrus trees we drew sample units from 8 distinct locations on each tree corresponding to upper (approximately >1.5 m) and lower halves of the canopy at each of four compass directions (NE, NW, SE, SW).The sample unit consisted of five thrusts into the tree canopy at the specified location. As noted above, the bucket was moved slightly with each thrust so that different portions of the site were sampled. To minimize disruption, we collected only two sample units on diagonally opposite sides of any single tree (e.g. SE – upper and NW – lower) and thus one sample consisted of four

consecutive trees (two unique sample units per tree). We collected samples from six orchards over five sampling dates for a total of 14 orchard-date samples between early June and late August. The sample size for any given orchard-date was 20 (80 total trees). We conducted all sampling from 0900 to 1200 h. All samples were frozen and stored until they could be processed in the laboratory. Counts of nymphs and adults were recorded separately.

We used a two-way, mixed-model ANOVA in SAS (Littell et al., 1996) to test for differences due to cardinal direction and height. Means and SD were calculated across the 20 samples for each site (orchard/date combination) and used as response variables in further analyses. Site (orchard/date combinations) and associated interaction terms were entered as random effects and the Kenward–Roger option was used to estimate degrees of freedom for *F*-tests. We tested for differences in mean density and variability (coefficient of variance = SD/mean) of nymphs, adults or both combined relative to locations within trees. Data in all cases were transformed as $\ln(x+1)$ to achieve normality and homogeneity of variance. If significant differences among cardinal directions were indicated we used an adjusted Tukey test to separate means while controlling the experiment-wise error rate at 0.05 (Littell et al., 1996).

2.4. Sample plan development

We collected a total of 25 samples on 14 dates over a two-year period with each sample consisting of 20 sample units. All sampling was conducted from 0900 to 1200 h. As before, we froze samples for later processing in the laboratory and recorded counts of nymphs and adults separately. We modeled the sampling distributions for nymphs, adults or both combined using both Taylor's (1961) power law, $S^2 = am^b$ and Lloyd's (1967) mean crowding index, $m^* = m + (S^2/m - 1)$ where m = mean, S^2 = variance and a and b are parameters. For the power law we regressed $\ln(S^2)$ on $\ln(m)$ to derive estimates of $\ln[a]$ and b, and for mean crowding we regressed m^* on m to derive estimates of α and $ß$ from $m^* = \alpha + ßm$ (Iwao, 1968, 1977). Fixed-precision sequential sampling models were developed using Green's (1970) method to calculate sampling stop lines from the Taylor's model as:

$$T_n \geq \left(an^{1-b}/D^2\right)^{1/(2-b)} \quad (1)$$

where T_n is the critical cumulative count over n samples, and D is precision, measured as SEM/m. Similarly, we used Kuno's (1969) method to develop alternate fixed-precision sequential sampling stop lines from Lloyd's model:

$$T_n \geq (a+1)/\left(D^2 - (\beta - 1)/n\right) \quad (2)$$

Kuno's stop line is subject to the sample size restriction $n > (\beta - 1)/D^2$.

2.5. Sample plan validation

We tested the performance of the six sequential sampling plans developed using a resampling approach and software developed by Naranjo and Hutchison (1997). After entering sample plan parameters in the software, each independent data set was randomly sampled (with replacement) until the sequential stop line limits terminated sampling. The mean and variance of the sample were then calculated and used to estimate precision (D). This process was repeated for a total of 500 times to arrive at an average performance and its associated variability for each independent data set. We were able to extract a total of 61 data sets from the experiment described above with mean densities of 0.1–76 for nymphs, 0.1–34 for adults, and 0.1–79 for both combined with sample sizes of 14–48. We retained information on the sources of the data sets (orange, lemons, treated with insecticides or untreated) in order to examine sample plan robustness relative to these factors for a plan developed entirely in orange orchards. To further characterize sample plan performance we calculated average expected precisions, sample sizes and sampling costs as a function of insect density categories. Here we used the relationships developed by Castle and Naranjo (2008) to estimate density and stage-specific sampling costs (time).

3. Results

3.1. Within-tree distribution

Nymphal stages of *H. vitripennis* were more abundant on the south side of trees than the north ($F = 13.3_{3,50.7}$, $P < 0.01$); coefficients of variation were highest on samples collected from the NW quadrant of the tree and lowest from samples collected on the two southern quadrants ($F = 8.3_{3,49}$, $P < 0.01$) (Fig. 1A). More nymphs were sampled from the top portion of the tree (>1.5 m) than lower ($F = 10.3_{1,23.6}$, $P < 0.01$), but coefficients of variation did not differ relative to vertical strata of the tree ($P > 0.05$). Patterns were a little different for the adult stage (Fig. 1B). More adult *H. vitripennis* were sampled from southern quadrants than northern quadrants on trees ($F = 9.9_{3,77.9}$, $P < 0.01$), but there was no difference relative to

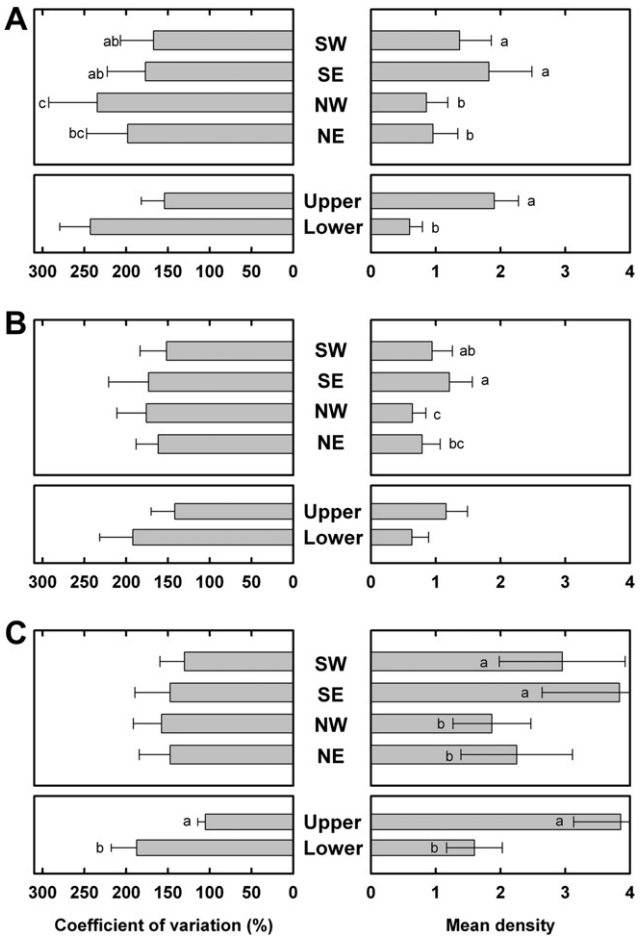

Fig. 1. Within-tree distributions of *H. vitripennis* nymphs (A), adults (B) and both stages combined (C) on citrus trees using a pole bucket sampling device, Riverside, CA. Bars accompanied by different letters are significantly different ($P < 0.05$) based on a two-way, mixed-model ANOVA with mean separation for compass direction by adjusted Tukey tests.

vertical strata in abundance ($P > 0.05$) and no differences in coefficients of variation relative to cardinal direction or vertical strata ($P > 0.05$). Combining both adult and nymphal stages, the patterns were distinct with more insects being sampled from southern quadrants ($F = 17.8_{3,50.9}$, $P < 0.01$) and higher vertical strata of trees ($F = 10.2_{1,19.9}$, $P < 0.01$) (Fig. 1C). There were no differences in coefficients of variation relative to cardinal direction ($P > 0.05$), but the relative variation of samples from the upper strata of the tree was much lower than those collected from the lower strata ($F = 12.2_{1,25.2}$, $P < 0.01$). There were no significant interactions between strata and quadrant for abundance or coefficients of variation for any insect stage ($P > 0.05$).

3.2. Sample plan

Patterns of abundance and relative variation for within-tree distributions showed that the greatest abundance and lowest variability were achieved when insects (both nymphs and adults) were collected in the upper portions (>1.5 m in height) of the tree. Although insects were generally more abundant on southern exposures, the variation in counts did not differ relative to cardinal direction. Thus, our sampling unit for further sample plan development consisted of thrusting the pole bucket device into the foliage five times at five different sites (25 thrusts per tree) around the circumference of an individual tree at ca. 1.5 m and above in height. This provided good coverage of the upper perimeter of the tree. Based on a representative range of densities, Taylor's power law provided a good fit to the sampling distribution data for nymphs, adults and both stages combined (Table 1). Lloyds mean crowding model also fit the sampling distribution data for nymphs and adults well, but less so for adults and nymphs combined. Using these model parameters, sequential sampling stop lines for two levels of desired sampling precision (0.15 and 0.25) are provided for both Green's and Kuno's method in Fig. 2. In general, Green's method required fewer samples to estimate mean abundance (cumulative count divided by sample size) with the same level of precision compared with Kuno's method. Kuno's method was more efficient at very low nymphal densities, but less so at moderate and high densities for this stage. This is largely due to the sample size restriction inherent in the Kuno sequential sampling model (see Section 2.4).

3.3. Sample plan validation

Validation is an important process in developing a useful and generalized sample plan. A resampling approach using independent field data was employed here to gauge the performance of various sample plans for nymphs, adults and both stages combined. In general, Green's sequential sampling plan was conservative for nymphs, arriving at estimates of mean density with better than desired precision over a broad range of insect densities (Fig. 3A, Table 2). As expected, there was considerable variation in precision from one resampling bout to the next. Kuno's plan produced slightly poorer levels of precision than desired at very low mean densities but much more conservative (and higher) levels of precision at moderate to high mean densities (Fig. 3B, Table 2). As with Green's method there was considerable resampling variation. Mean sample sizes were very close to those predicted by the sampling model for both Green's and Kuno's method (Fig. 3C,D). Estimated sampling costs declined with increasing density to a point and then increased again due to the time required to count insects (Table 2). Because of the minimum sample size restriction in the Kuno's model the cost of sampling increased dramatically as density increased. The validation process also showed that the sampling plans developed may have broad applicability in the citrus system. The sampling plans developed from orange trees appear to work equally well for oranges, lemons and orchards that have been treated or not with insecticides (Fig. 3).

Sample plan validation for adults and for adults and nymphs combined showed that sampling plans for these stages are less conservative compared with those for nymphs, resulting in closer to desired precision across most mean densities (Figs. 4 and 5, Table 2). Again, there was considerable variation between resampling bouts for any independent data set and broad generality relative to oranges, lemons and the use of insecticides. The performance of Green's and Kuno's plans in terms of sampling costs was more comparable for adults and for nymphs and adults combined than for nymphs alone (Table 2). Still, Green's plan resulted in generally lower sampling costs with equal precision compared with Kuno's plan, especially at very low densities.

4. Discussion

A sampling plan is a structured set of rules that guide sampling activities that, at a minimum, includes determination of the nature and size of the sample unit as well as the number of sample units that need to be collected to meet a predetermined criterion. In a companion study (Castle and Naranjo, 2008) we quantitatively evaluated four sampling methods and determined that a pole bucket method provided the most consistent precision for estimating relative densities of all stages of *H. vitripennis* with the lowest cost. In the present study, we refined the sample unit to collecting insects from the upper portion of citrus trees based on comparisons of relative variation among tree strata and cardinal direction, and then devised and validated sequential sampling plans. Sequential sampling offers a simple means of estimating population density by automatically minimizing the number of sample units needed to satisfy a predetermined level of precision. Sequential sampling plans have been developed for estimating insect density and as aids for pest management in many systems (Pieters, 1978; Hutchison, 1994). Surprisingly, no formal sampling plan of any kind has previously been developed for *H. vitripennis* in any cropping system. Given the importance of the citrus system in driving local and regional *H. vitripennis* population dynamics (Perring et al., 2001; Sisterson et al., 2008), our proposed sampling plans should be useful in future studies of the population ecology and management of this pest.

Both nymphs and adults of *H. vitripennis* were more abundant on the southern exposures of citrus trees, but only for the nymphs was the relative variation of this abundance also lower on the

Table 1
Sequential sample plan parameters for estimating the relative density of *H. vitripennis* in citrus with a pole bucket sampler, Riverside, CA.

	Density range	*N*	Green's plan			Kuno's plan		
			Taylor ln[*a*](SE)	Taylor *b*(SE)	r^2	Iwao α(SE)	Iwao β(SE)	r^2
Nymphs	0.1–8.7	17	1.285(0.140)	1.583(0.077)	0.97	−0.760(1.160)	3.386(0.289)	0.90
Adults	0.1–16.1	25	0.679(0.098)	1.282(0.049)	0.97	0.276(0.464)	1.309(0.059)	0.95
Both	0.1–21.6	25	0.717(0.230)	1.425(0.115)	0.87	1.873(1.811)	1.409(0.190)	0.70

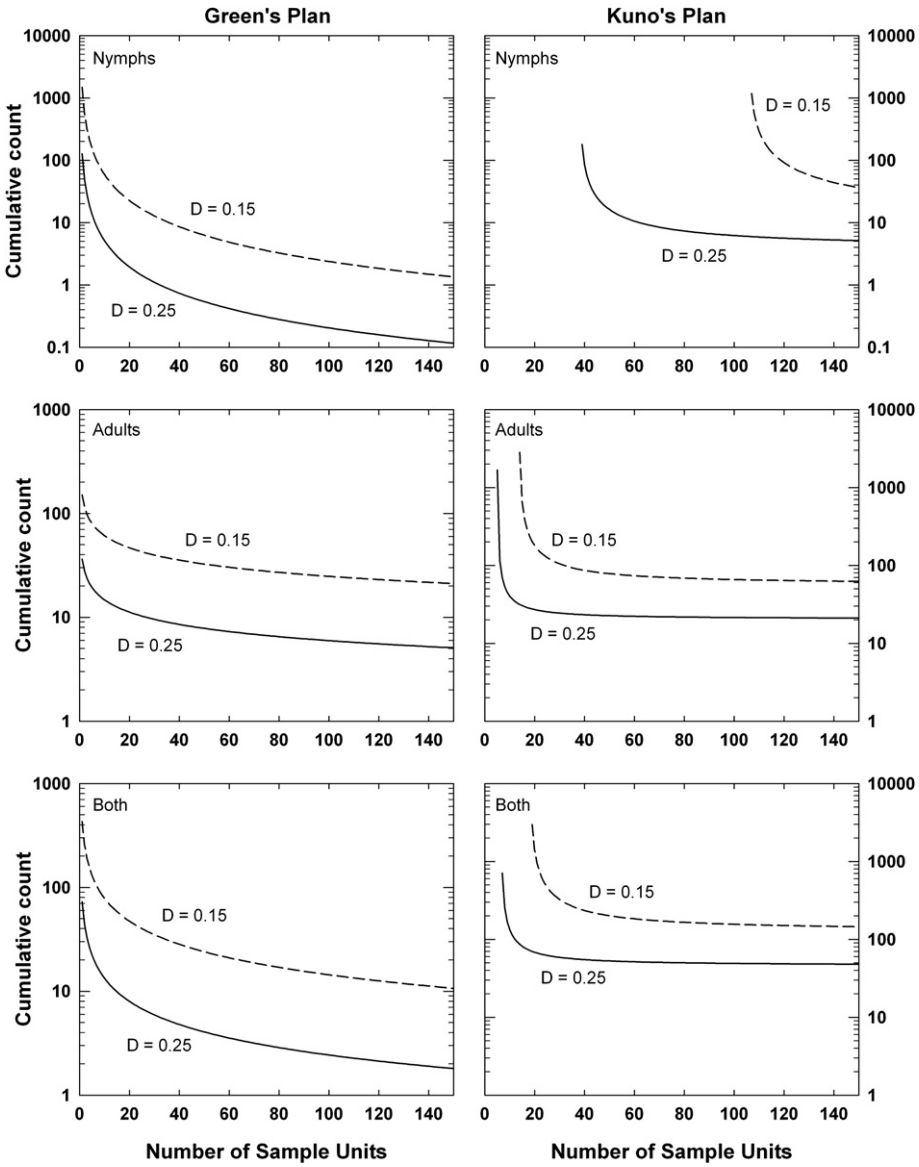

Fig. 2. Fixed-precision (D) sequential sampling plans based on Green's (1970) and Kuno's (1969) methods for estimating the relative density of *H. vitripennis* nymphs, adults and both stages combined on citrus trees using a pole bucket sampling device. Note differences in the y-axis scale.

southern portion of trees. We speculate that perhaps *H. vitripennis* are using more southern exposures as a means of thermoregulation or maybe there are differences in plant quality relative to the inclination of the sun's rays. In support of the former, it is worth noting that all our sampling activities were confined to morning hours when the accumulation of additional heat may be most beneficial. We also observed a strong pattern of higher abundance of *H. vitripennis* on the upper strata of citrus trees, especially for nymphs, and for nymphs and adults combined. This could once again be related to thermoregulation given the shading of the lower canopy by adjacent trees, but may also reflect plant quality. We often noticed that productive branches were sparser on the lower portions of the trees. There also was a strong pattern of lower variability in the upper canopy of citrus trees when counts of adults and nymphs were combined. This likely represents a more consistent preference for the upper canopy, and by targeting the upper canopy for sampling, fewer sample units would be needed to achieve the same sampling precision compared with collecting samples from the lower canopy or the whole tree.

The Kuno (1969) and Green (1970) fixed-precision, sequential approaches are commonly used for developing insect sampling plans. We found that Green's plan was most efficient over the wide range of potential densities where the plan would be used. The Kuno method resulted in lower sampling costs for nymphs at very low densities, and marginally lower costs for adults at slightly higher densities (see Table 2). The most significant drawback to the Kuno plan is the high minimum sample imposed by the approach under certain circumstances. For example, a minimum sample size of 39 was required for nymphs above a density of 5 per pole bucket. As is typical of aggregated populations described with the Taylor (1961) or Lloyd (1967) mean–variance models, sample size declined with increasing density for most of the plans. Overall sampling costs also declined with density up to a point, but then increased as it took longer to process samples with high densities of insects. Using the Green sampling plan for detection of any mobile stage, we estimate that it would require, on average, 54 sample units and a total sampling time of 169 min to achieve a sampling precision of 25%. Many fewer samples (5) and much

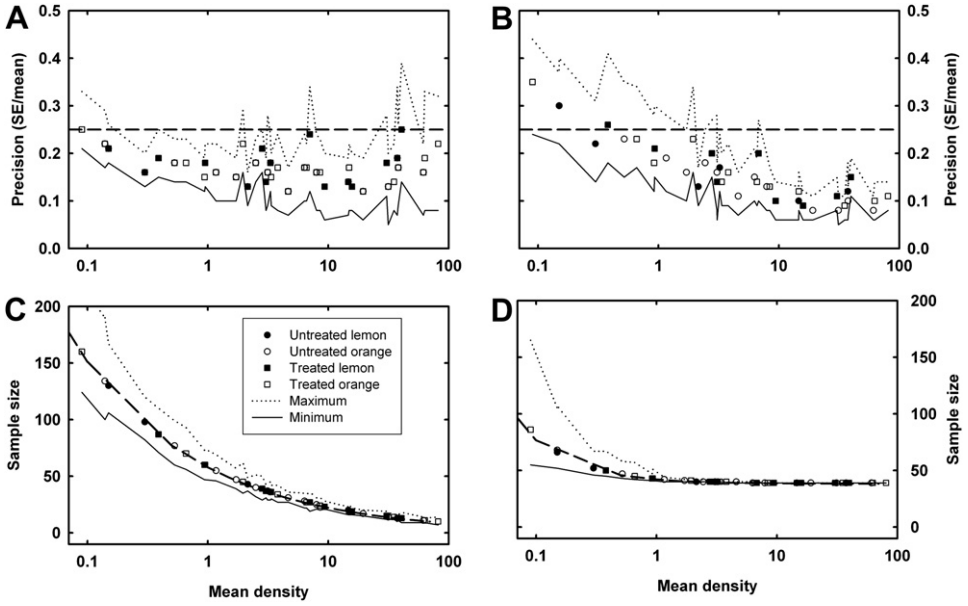

Fig. 3. Validation of fixed-precision sequential sampling plans based on Green's (1970) (A and C) and Kuno's (1969) (B and D) methods for estimating the relative density of *H. vitripennis* nymphs on citrus trees based on resampling of multiple independent data sets from untreated and insecticide-treated orange and lemon orchards. The dashed line at $Y = 0.25$ in A and B denotes the desired precision level; dashed lines in C and D denote the desired sample size curves for each method at a precision of 0.25.

less time (ca. 30 min.) would be required to estimate densities that would most likely warrant some kind of control action should, for example, the protection of an adjacent vineyard be the goal.

Improved knowledge of the spatial distribution of *H. vitripennis* within trees coupled with sequential sampling plans provides a foundation for more selective and efficient pest management. Although the regional control programs conducted in California have been highly effective at reducing *H. vitripennis* populations, they were carried out at great expense due to large acreages of citrus that were treated with imidacloprid (Wendel et al., 2002; Hix et al., 2003; Stone-Smith et al., 2005). The capacity of *H. vitripennis* populations to rebound following area-wide applications of imidacloprid has already been demonstrated in Temecula. High captures of insects on traps reinforced with visual inspections in late summer/early fall of 2008 prompted repeat treatments with imidacloprid on approximately 1000 acres of citrus in Temecula during spring 2009 (Toscano et al., 2009). This example portends the possibility of additional follow up treatments in the problem regions of California that were previously subjected to similar regional control programs. The ability to delimit areas experiencing threatening densities of *H. vitripennis* will become increasingly important given the possibility of diminishing federal funds in support of regional control programs in the future. The sampling plans we have developed will provide valuable tools for identifying problem areas on a local scale and encourage more focused abatement efforts at a reduced cost.

Table 2
Summary of resampling validation of sequential sampling plans for *H. vitripennis*, Riverside, CA.

Density (per sample unit)	Green's plan			Kuno's plan		
	Mean precision	Mean sample size	Mean cost (min)[a]	Mean precision	Mean sample size	Mean cost (min)
Nymphs						
<1	0.191	97	189.2	0.253	56	108.3
1–5	0.164	40	91.6	0.164	40	92.9
5–10	0.172	26	77.7	0.142	39	117.9
10–20	0.140	19	80.8	0.098	39	167.7
20–40	0.162	14	97.4	0.100	39	274.9
>40	0.205	11	123.4	0.110	39	430.3
Adults						
<1	0.218	91	180.7	0.218	101	199.5
1–5	0.230	18	40.7	0.249	15	33.5
5–10	0.211	9	24.8	0.210	9	25.1
10–20	0.246	6	21.1	0.225	7	26.2
>20	0.206	5	25.9	0.186	6	31.2
Both						
<1	0.215	54	168.9	0.148	116	364.4
1–5	0.213	20	68.1	0.188	27	90.3
5–10	0.210	11	42.3	0.193	14	51.4
10–20	0.207	8	33.4	0.179	11	44.8
20–40	0.211	5	29.9	0.169	9	47.8
>40	0.286	5	39.7	0.232	8	63.8

Density ranges based on 4–15 individual validation data sets that were each resampled 500 times; desired precision = 0.25, minimum sample size = 5.

[a] Density-dependent and stage-specific costs estimated from the sample cost equations in Castle and Naranjo (2008).

A sampling plan is generally developed with a restricted set of data but is then implemented over a broader range of novel areas where environmental, agronomic and other factors may vary. Validation is a means of testing the expected performance of a sample plan under novel conditions and has become more common over the past decade due to the availability of easy to use software (Nyrop and Binns, 1991; Naranjo and Hutchison, 1997; Binns et al., 2000). We employed an approach based on the resampling of independent field data sets of *H. vitripennis* populations in citrus to validate our sampling plans. In general, we found that both Green's and Kuno's plans were somewhat conservative over a broad range of insect densities, providing better than expected precision while calling for sample sizes very close to those specified. The plans were more conservative for nymphs alone than for adults, or nymphs and adults combined. In all cases there was considerable variability about the mean response emphasizing the stochastic nature of the sampling process (Hutchison et al., 1988). In addition, our validation studies also pointed to the robust nature of the sampling plans that were developed solely on untreated orange trees, but performed equally well for untreated lemons and both orange and lemons treated with insecticides. Insecticides have often been shown to alter insect sampling distributions, mostly by decreasing

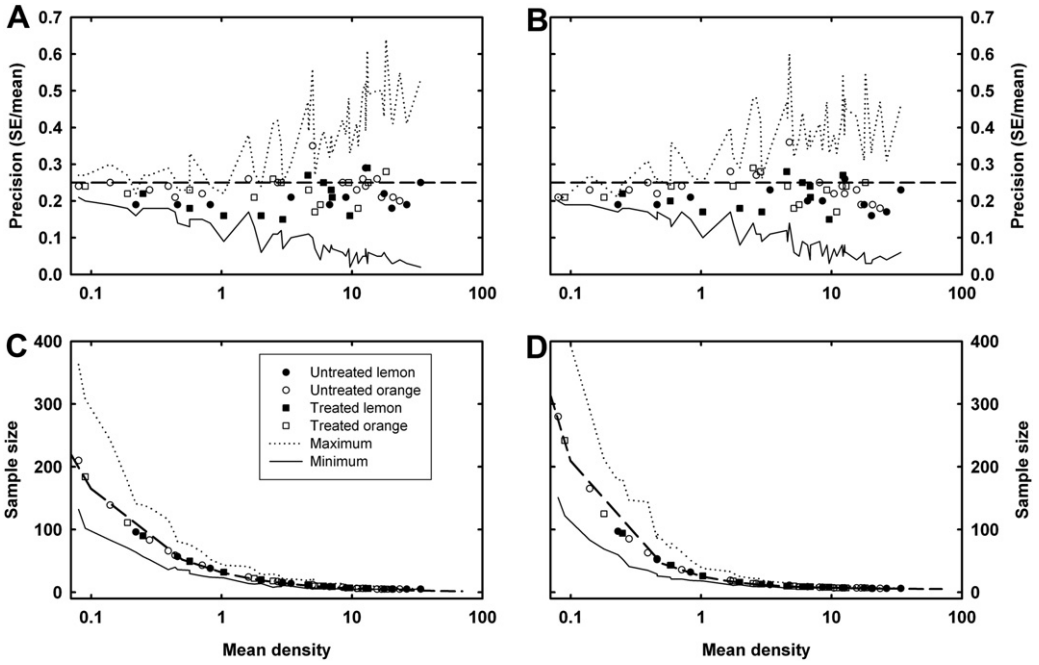

Fig. 4. Validation of fixed-precision sequential sampling plans based on Green's (1970) (A and C) and Kuno's (1969) (B and D) methods for estimating the relative density of *H. vitripennis* adults on citrus trees based on resampling of multiple independent data sets from untreated and insecticide-treated orange and lemon orchards. The dashed line at $Y = 0.25$ in A and B denotes the desired precision level; dashed lines in C and D denote the desired sample size curves for each method at a precision of 0.25.

aggregation (Trumble, 1985; Taylor, 1987; Liu et al., 1993; Polston et al., 1996; O'Rourke et al., 1998), but sometimes by increasing spatial clumping (Tonhasca et al., 1994). The sampling plans we present here have broad utility as they performed equally well whether or not insecticides were applied. This versatility allows the plans to be used as tools for basic study of population dynamics, for estimating insecticide efficacy, and for undertaking pest management programs that will likely be dealing with insect density estimation and decision-making in both sprayed and unsprayed citrus over the season. Further efficiencies in decision-making for pest management application could be achieved by using some of the knowledge gained here to develop sequential classification plans once action or economic thresholds are developed for *H. vitripennis*.

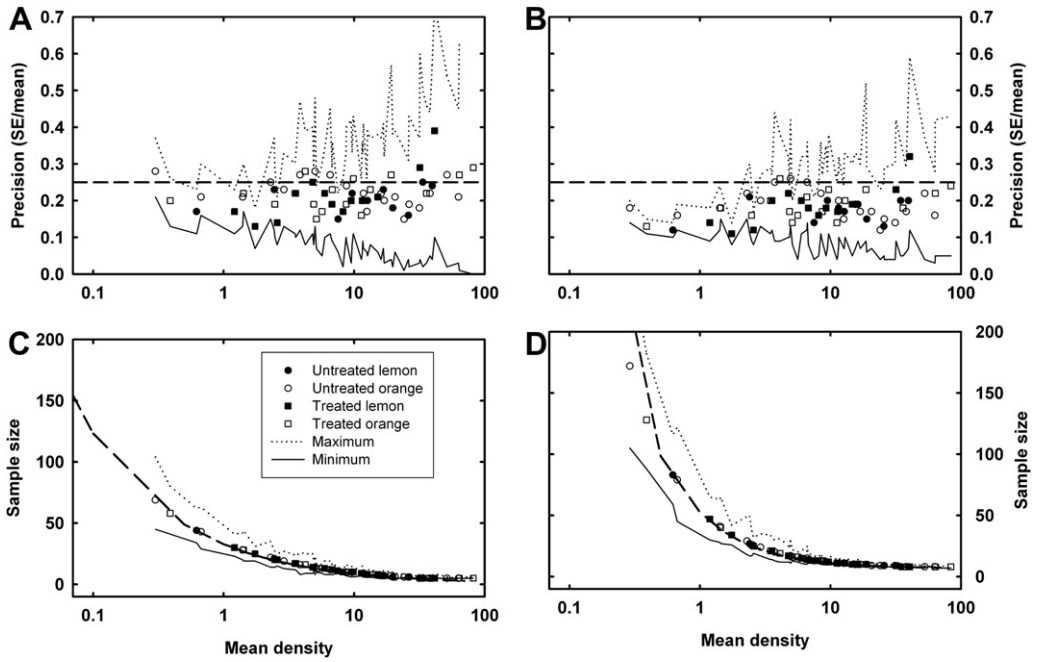

Fig. 5. Validation of fixed-precision sequential sampling plans based on Green's (1970) (A and C) and Kuno's (1969) (B and D) methods for estimating the relative density of *H. vitripennis* nymphs and adults combined on citrus trees based on resampling of multiple independent data sets from untreated and insecticide-treated orange and lemon orchards. The dashed line at $Y = 0.25$ in A and B denotes the desired precision level; dashed lines in C and D denote the desired sample size curves for each method at a precision of 0.25.

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