SAMPLING, SEASONAL ABUNDANCE, AND COMPARATIVE DISPERSAL OF GLASSY-WINGED SHARPSHOOTERS IN CITRUS AND GRAPES: SAMPLING PROGRESS REPORT

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ABSTRACT

The spatial distribution of nymphal and adult glassy-winged sharpshooter (GWSS) was studied in citrus orchards in Riverside, CA using a bucket sampling method. On average, about 2.4 times as many GWSS were collected in the upper half of the tree canopy compared with the lower half and about 1.6 times as many were collected on the south side of trees compared with the north side. The coefficient of variation (CV=SD/mean) was nearly 2 times lower in samples taken from the upper half of the canopy compared with the lower half, but there were no differences in the CVs among different compass directions. These findings were used to refine the sample unit for sampling GWSS in citrus. Sticky trap catches of GWSS adults were highly correlated with on-plant GWSS populations within a given year but the relationship was variable between years. Based on the bucket sampling method we present density-dependent sample size and sample cost estimates and a preliminary sequential sampling plan for estimating relative population density of GWSS in citrus. We have applied this sampling program towards estimating the incidence of *Xylella fastidiosa* in GWSS adults. A progressive increase in the proportion of adults positive for *X. fastidiosa* occurred from the time of adult emergence in late June, 2002 through April, 2003. The mean titer of *X. fastidiosa* in heads and thoraxes also increased progressively through this period, suggesting that the potential for vectoring *X. fastidiosa* may rise as the spring generation of adults ages.

INTRODUCTION

Decision-making in knowledge-based pest management depends upon sampling methods that provide reliable information on pest densities and distributions. Practical sampling methodology must balance sampling precision with simple and cost-effective collection techniques. In 2001 and 2002 four glassy-winged sharpshooter (GWSS) sampling methods were evaluated in citrus orchards. These included hand (bucket and beat net) and gasoline-powered (D-Vac and A-Vac) samplers. The bucket sampler was the most versatile and easiest to use with its extendable pole allowing access to foliage 4-6 m above ground. Samples obtained with the bucket sampler were also cleaner than those obtained with other methods and therefore required less handling during sample processing. All methods showed similar patterns of population change over time, but based on quantitative analyses the bucket sampler and the beat net were generally the least costly over the largest range of densities of both nymphal and adult stages. Studies in 2003 focused on quantifying the spatial distribution of GWSS nymphs and adults within citrus tree using the bucket sampler. These studies will help refine the sample unit and further reduce the cost of sampling. A preliminary sequential sampling plan is presented for the precise estimation of relative population density of GWSS. We also continued to examine the relationship between the abundance of GWSS on plants to adult GWSS capture on yellow sticky traps placed within orchards. Such studies will help define the utility of commonly used sticky traps as a method for monitoring GWSS abundance.

It is well recognized that the major threat of GWSS populations is the potential for vectoring *X. fastidosa* to uninfected grapevines in commercial vineyards. One practical application of a sampling plan would be to precisely estimate densities of GWSS within an orchard or vineyard and then determine what proportion are positive for *X. fastidiosa*. Accurate identification of individuals positive for *X. fastidiosa* is an essential part of an overall appraisal of the risk posed by a particular population. Work began in April, 2002 exploring ELISA, PCR, and culturing techniques for the detection of *X. fastidiosa* in GWSS. Sampling and evaluation of the proportion-positive among southern California populations of GWSS has been concentrated thus far in Riverside, owing to resident populations that remain sufficiently large year-round for continuous sampling. A temporal profile of the incidence of *X. fastidosa* in GWSS populations will provide information on when titers of *X. fastidiosa* are highest, and perhaps when dispersing adults are most inoculative. This approach, when integrated into an efficient sampling program providing reliable estimates of GWSS densities, may help to protect grape vineyards and other susceptible crops by recognizing when they are most vulnerable to transmission of *X. fastidiosa* by GWSS.

OBJECTIVES

The overall objective of the research project is to develop statistically-sound sampling methods and plans for estimating density and inoculum potential of GWSS for research and management applications.

RESULTS

Spatial distribution and sampling. To better define the spatial distribution of GWSS nymphs and adults in citrus trees we used the bucket sampler to draw sample units from 8 distinct locations on the tree corresponding to upper and lower halves of the canopy at each of four compass directions (NE, NW, SE, SW). The sample unit consisted of 10 thrusts of a bucket sampler (a 5 gallon plastic bucket attached to an extendable pole and fitted with a funnel at the bottom that directed insects into a collection cup) into the tree canopy at the specified location. Samples were collected from 14 sites in 2003 that consisted of 6 orchards, each sampled on several dates between early June and late August. Within each orchard sample units from each of the 8 locations on the tree were collected at 20 sites. To minimize disruption, we collected only 2 sample units on opposite sides of any single tree (e.g. SE-upper and NW-lower). All samples were collected at UC-Agricultural Operations, Riverside, California. Analyses of each site individually and all sites pooled indicated clear directional and canopy height patterns in the distribution of both nymphal and adult GWSS (Figure 1). On average about 2.4 times as many GWSS were collected in the upper half of the tree canopy compared with the lower half and about 1.6 times as many were collected on the south side of trees compared with the north side. These patterns held when samples were collected in the afternoon. More importantly from a sampling perspective, the relative variation in counts also showed clear patterns (Figure 2). The coefficient of variation (CV=SD/mean) was nearly 2 times lower in samples taken from the upper half of the canopy compared with the lower half. There were no significant differences in the CVs among the different compass directions. These overall results suggest that samples should be taken from the upper half of the canopy. Based on coefficients variation, there is no particular advantage relative to compass direction; however, population densities would be expected to be different between the northern and southern portions of the tree and one or the other position should be used to standardize sample collection.

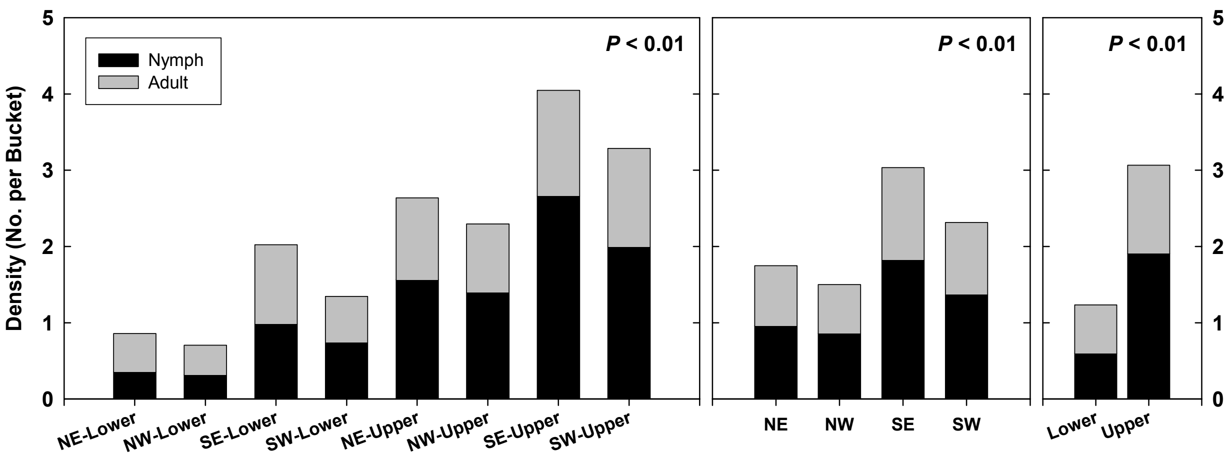

Figure 1. Spatial distribution of nymphal and adult GWSS on citrus trees based on pooled data from 14 sites during 2003. *P*-values denote the results of mixed-model ANOVA.

On 6 sampling dates in 2002 and 8 dates in 2003 we placed yellow sticky traps (Pherocon AM, 22.8 x 27.9 cm) within orchards to capture GWSS adults. Traps were attached to 1 m wooden stakes, placed between rows, and left exposed for 72 hours. We estimated on-plant densities of GWSS (mainly adults) with the bucket method in these same orchards at the time of trap placement. On-plant counts were highly correlated with trap catches in each year; however, regression analyses indicated that the relationships between plant densities and trap counts were very different between years. Samples in both years were collected between late June and early October in the same orchards. These preliminary findings suggest that the prediction of field populations of GWSS from trap catches may be problematic. A similar problem was demonstrated by Naranjo et al. (1995) using sticky traps to predict population densities of sweetpotato whitefly in the cotton system.

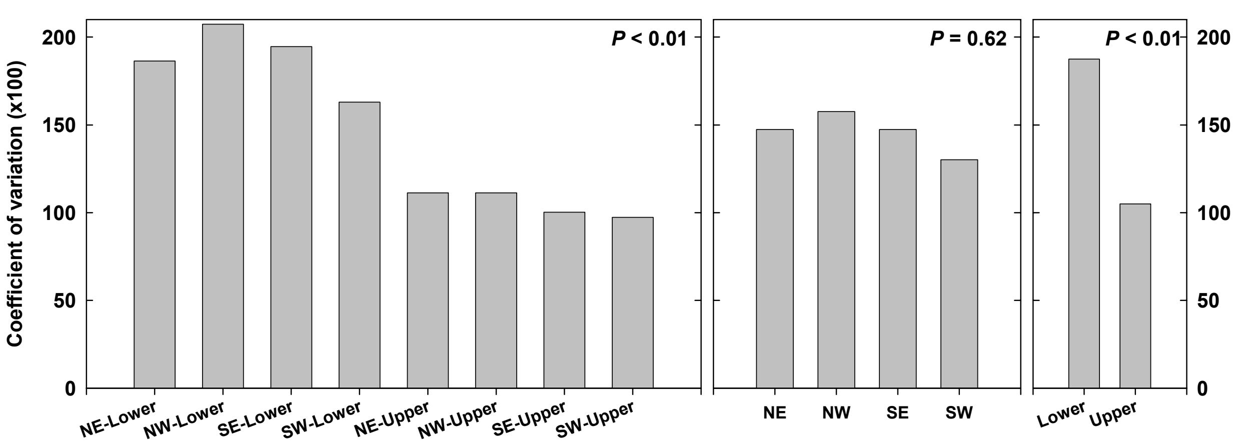

Figure 2. Relative variation (CV = SD/mean) of GWSS counts (nymph + adult) from samples on different portions of citrus trees based on pooled data from 14 sites during 2003. *P*-values denote the results of mixed-model ANOVA.

Based on bucket sampling data collected from 2001 to 2003 we calculated density-dependent sample size and sampling cost curves for two levels of statistical precision (Figure 3). The sample size curve is given by $n = am^{b-2}/D^2$, where n is sample size, D is statistical precision (SE/mean), m is mean density, and a and b are parameters from the Taylor power law (Taylor 1961) relating the sample variance to the sample mean. Sample cost was estimated as the product of the total time need to collect and process a single sample unit and sample size. Sample size and sample cost increase with higher levels of precision and decline as density increases. For example, at a precision of 0.25 less than 10 sample units would be needed to estimate relative densities over 10 GWSS per bucket. Sample costs plateau regardless of precision because of the increased time required to count increasing numbers of GWSS. Because population density is unknown at the time of sampling, we formulated a preliminary sequential sampling plan (Figure 3) using the method of Green (1970). In a sequential plan the need for additional sample information is assessed following the collection of each sample unit so that no more sample units than necessary are collected.

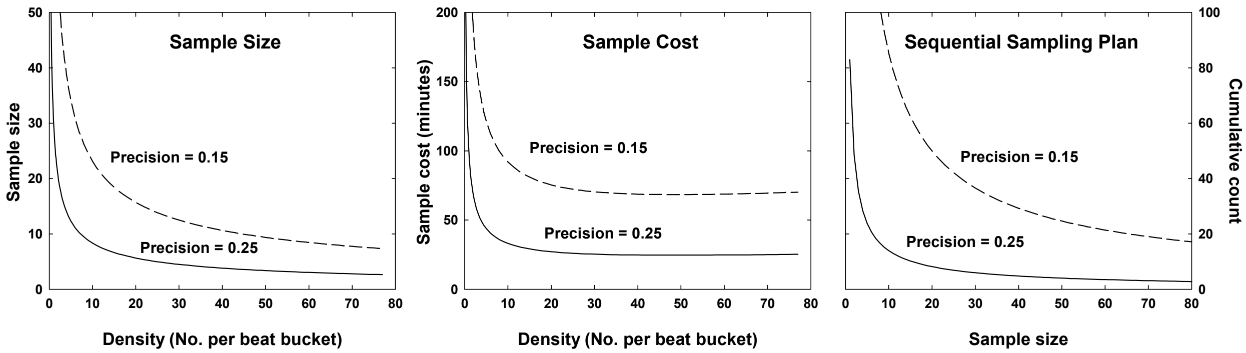

Figure 3. Sample size, sample cost, and a preliminary sequential sampling plan for the bucket method at two levels of statistical precision (SE/mean).

Incidence of *X. fastidiosa* in GWSS populations. Primary spread of *X. fastidiosa* in grapevines occurs when vectors such as GWSS move into vineyards and transmit *X. fastidiosa* at some unknown level. To estimate what proportion are positive for *X. fastidiosa* and possibly inoculative, GWSS adults were collected periodically from the sampling orchards and frozen for detection of *X. fastidiosa*. PCR, ELISA and media culturing techniques were employed, but quantitative ELISA conducted with negative controls and GWSS-based standards provided the most informative results. A greenhouse colony of GWSS started from eggs and maintained on *X. fastidiosa*-free plants served as the source of negative controls (Figure 4a) and "clean" adults that, when homogenized in known concentrations of *X. fastidiosa*, provided a standard curve (Figure 4b) for estimating *X. fastidiosa* titers in field-collected GWSS (Figure 5).

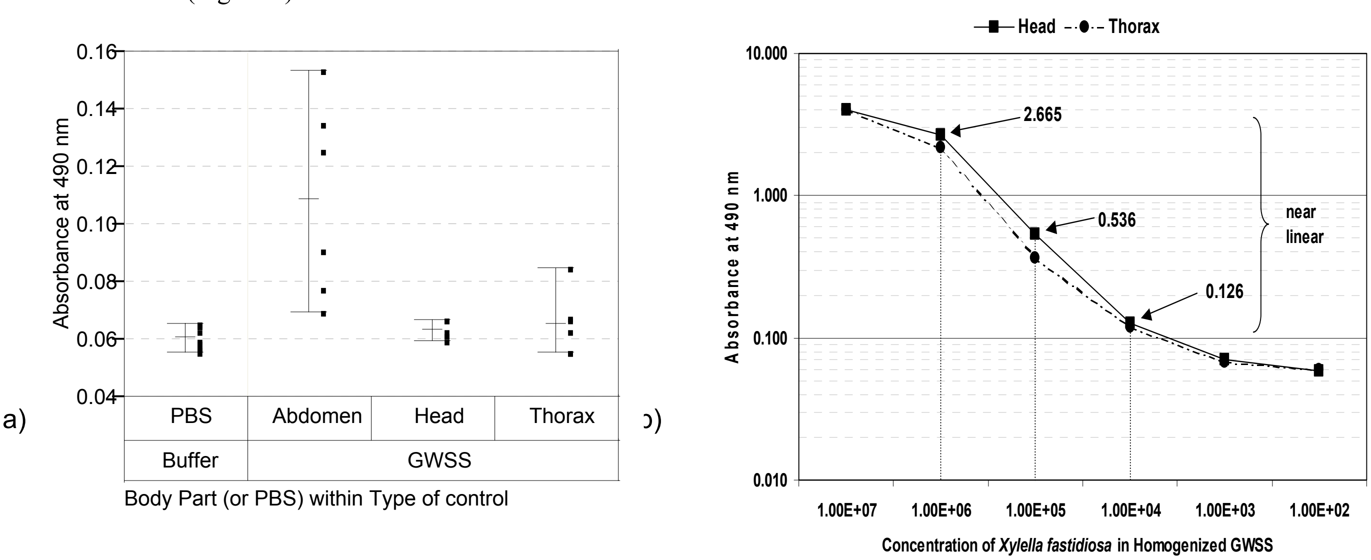

Figure 4. ELISA results for (a) GWSS adults (n=6) reared from eggs on *X. fastidiosa*-free plants grown in a greenhouse. The mean response for each set of points is indicated by the horizontal dash near the midpoints of the vertical lines (range). $Absorbance_{490}$ values for the head and thorax only were not significantly different from the PBS buffer control. A standard curve using *X. fastidiosa*-free GWSS adults was produced (b) and used to estimate the concentration of *X. fastidiosa* in field-collected GWSS adults represented in Figure 5.

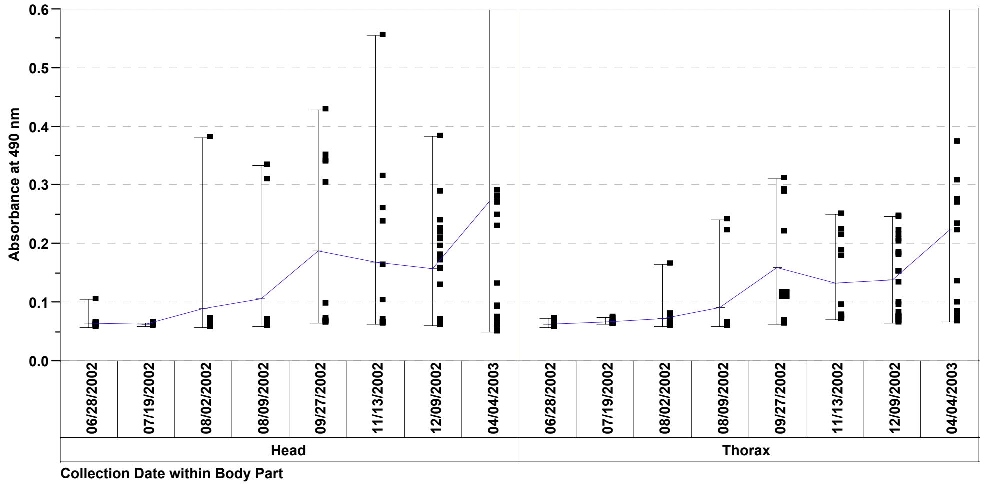

Figure 5. Progressive incidence of *X. fastidiosa* in GWSS adults collected in Riverside, California between June '02 and April '03. An identical pattern of increasing mean titers (represented by the traversing line) of *X. fastidiosa* in both head and thorax segments can be observed, but with consistently higher mean levels in the head.

CONCLUSIONS

Sampling is a fundamental component of the study of population dynamics and central to the development of robust strategies for pest management. The results of our research have focused on the development of an efficient method for estimating densities of GWSS in citrus. Based on considerations of precision and cost we have identified a bucket sampler as an efficient sampling method. Further study of the spatial distribution of GWSS within citrus trees has helped to refine the sample unit and further reduce sampling costs. We present a preliminary sequential sampling plan that will enable researchers and pest managers to precisely estimate the relative density of GWSS at a minimal cost. Further work will be needed to independently test the validity of this sampling plan. For pest management, additional research will be needed to define problematic population densities requiring control. Coupling information on the incidence of *X. fastidiosa* in GWSS will provide better definition of threshold densities.

FUNDING AGENCIES

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