

DEVELOPMENT OF A FIELD SAMPLING PLAN FOR GLASSY-WINGED SHARPSHOOTER VECTORED PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted July 1, 2004 to September 30, 2005.

ABSTRACT

Determining the location of grapevines infected with Pierce's disease (PD) in vineyards is a major objective of growers and researchers. Currently, there are no sampling protocols available except for field surveys based on PD symptoms. The work reported here was conducted in Kern County and the Coachella Valley towards developing a sampling plan to detect the locations of diseased grapevines within vineyards. Spatial distribution patterns of PD were characterized with spatial statistics. Results from Kern County sampling suggest that knowing the percentage of PD infection and the location of vineyards relative to citrus can predict the distribution pattern of PD in the vineyard. Research in the Coachella Valley suggests that PD distribution is highly localized within vineyards, and diseased grapevines are associated with two or more dead, replanted, or missing adjacent grapevines. These results bring us closer to developing reliable sampling protocols for PD in vineyards.

INTRODUCTION

A common sampling technique to detect the presence of PD in vineyards is to visually examine vines, collect symptomatic leaves from potentially infected vines, and confirm the presence of PD with enzyme-linked immunosorbent assay (ELISA). Locating vines infected with PD in a vineyard is required for current PD management, and the only reliable method for finding PD-infected vines is to examine every vine in the vineyard. A PD census was used in Kern County and this provided a cost-effective method (< \$5 per acre) for identifying infected vines in vineyards when PD infection was very low (Hashim and Hill 2003). As the infection level in a vineyard exceeded 1%, and more vines showed symptoms, it became increasingly difficult to observe and sample every symptomatic vine. It was especially difficult to distinguish PD symptoms when other stress factors, such as drought and salt damage, existed in vineyards. Such difficulties increase the sampling costs because many samples must be taken and confirmed with ELISA. Thus, the development of a cost-effective sampling program appropriate for the needs of growers and researchers is critical for PD monitoring and management.

The sampling plan we propose is a multi-step or sequential procedure using a series of grids with different spatial resolutions. For the first step we use a coarse grid to determine the overall proportion of infected vines, the spatial distribution patterns, and the spatial structure. This coarse grid also can locate patch areas if PD is aggregated in the vineyard. The information from the coarse grid is used to determine the next step in the sampling program. In step 2, we create intensive sampling grids ("fine" grids) around PD-infected vines determined in step 1. For every plant in the fine grid, we collect tissue for ELISA determination of *Xylella fastidiosa* (Xf) infection. It is essential that we make a correct assessment of PD infection for each vine, thus we do not depend on symptom expression that can be unreliable (Krell et al. 2005). The number of fine grids is determined by the distribution of infected vines determined in step 1, coupled with the size of the vineyard. Sampling within the fine grid reveals detailed structures and patterns of PD distribution, and identifies patch areas where PD is aggregated, the size of patch, and the direction of trends, if they exist. The fine grid also provides information to generate probability maps of PD incidence in the vineyard. Such maps guide where, and how intensively, we need to sample to find individual vines infected with Xf in the vineyard.

To develop our sequential grid-sampling programs, the construction and placement of coarse and fine grids is essential. We have been evaluating various sizes and patterns of sample grids based on the categorization of the spatial structure of PD distribution. Grids with different spatial resolutions have been superimposed on the census data to test the efficiency of

grids. This efficiency can be calculated by quantifying how well the grids match the PD incidence from the census data. These grids then are incorporated into the sequential grid-sampling program. Grids have been validated in Kern County and the Coachella Valley. Type I error (i.e., a vine is not infected but sampled with grids) and type II error (i.e., a vine is infected but not sampled with grids) will be calculated to evaluate the precision and accuracy of the sequential grid-sampling program. This procedure will allow us to choose the best series of grids to be used for the sampling program. Sensitivity analysis and cost analysis also will be used to optimize the sequential grid-sampling program. Sensitivity analysis identifies the effect of the grid size on the precision and accuracy of the sampling program, while cost analysis evaluates the economy of the sampling program by considering sampling costs, and accuracy and precision of the sampling program.

OBJECTIVES

The goal of this project is to develop a grid-sampling program for PD that can characterize the spatial distribution and determine the location of grapevines with PD based on the spatial structures and patterns of PD distribution in the vineyard. The objectives include:

1. Characterization of the spatial distribution of PD in vineyards.
2. Development of a sequential grid-sampling program.
3. Validation and optimization of the sampling program with cost analysis and sensitivity analysis.

RESULTS

We have conducted landscape-scale censuses and vineyard-scale sampling in Bakersfield (Kern County) and in the Coachella Valley (Riverside County) for the past four growing seasons (2001-2004) to identify vineyards with PD. Data from this year (2005) are still being collected and analyzed.

Kern County sampling

Census data from 215 vineyard blocks in Kern County showed a total of 52 blocks with PD. Most of the infected blocks (82%) were within ¼ mile of citrus, suggesting that proximity to citrus is an important criterion to consider when sampling for PD in this area. Of 10 cultivars that we sampled, we found that “Flame” had the highest number of vineyards with a PD incidence greater than 1% (Table 1). Spatial analyses with geostatistics and spatial analysis with distance indices (SADIE) found that the distribution of diseased grapevines was dependent on the overall PD incidence in the vineyard. When the incidence was < 0.1%, there was no spatial structure to the infection. Vineyards that had 0.1 - 1% incidence showed a “trend” distribution pattern, with areas of low to high infection. When the PD incidence was between 1% and 5%, the pattern of disease was random, and a clumped distribution existed when disease incidence was > 5%. A couple of vineyards showed enough PD every year to examine year-by-year PD distributions. In these vineyards, we found that the PD distribution patterns were consistently PD-incidence dependent. For example, disease distribution in a vineyard was random when the disease incidence was 0.8% in 2001. In 2002 disease incidence exceeded 5% and distribution was clumped. In 2003 and 2004, disease incidence was 1.3% and 0.8%, respectively, and distributions were random. Further investigation of vineyards with > 5% PD incidence revealed that the diseased grapevines were aggregated and they were spatially correlated within ca. 23-28 m (the “range” in Table 2). This suggests an appropriate size for coarse and fine grids for grid sampling plans to find diseased grapevines. We are continuing our work of constructing and testing coarse (ca. 21 m sampling distance) and fine grids (sample every vine within 25 m from a known diseased vine) in Kern County vineyards, and we will begin sampling in the second week of October.

Coachella Valley sampling

Each year from 2001-2004, we have surveyed all vineyards in the Coachella Valley. Consistent with our work in Kern County, we found that “Flame” vineyards had the highest number of PD-infected sites with an incidence greater than 1% (Table 1). One vineyard had a higher disease incidence (3.8%) than the other 6 vineyards (<0.01%), and in this field, the diseased grapevines were spatially aggregated, forming a patch. Further investigation of this vineyard at the interplant scale (using fine grids) revealed that PD within the patch was aggregated, and diseased grapevines were spatially correlated within 26 m (“range” in Table 2). This result is consistent with the aggregation size of the vineyards in Kern County. All vineyards with PD in the Coachella Valley were located adjacent to citrus groves indicating that citrus affects the incidence and severity of PD in nearby grapes. However, proximity to citrus did not affect PD distribution in all vineyards, similar to the findings in the Temecula vineyards (Perring et al. 2001) and Kern County vineyards. Coarse grid sampling detected spatial aggregation of PD in the one vineyard that had sufficient PD incidence. Fine grid sampling showed that 82% of the infected vines in the Coachella Valley were adjacent to two to six consecutive missing, dead, or replanted grapevines in a row (Figure 1). This potential signature of PD symptomatic areas can be used to locate where to examine plants for disease symptoms, or where to take samples to test with ELISA. We hypothesized that such areas might be detectable with remote sensing and in 2005, we tested this hypothesis in the Coachella Valley. We used three aerial images (1-m resolution natural color image taken in August 2000, 1-m resolution IR natural color image taken in spring 2002, and 2-foot resolution natural color image taken in August 2004). From these images we identified 122 signature areas with inconsistent canopies that contained potential missing, dead, or replanted grapevines. We referred to these areas as “holes”, and we visited each hole identified by the images. This sampling revealed that 57 of the holes still existed; some had been replanted, some were holes created by other factors in the field (like power poles), and others were in vineyards that had been removed since the images were taken.

Sampling these 57 holes, we confirmed the presence of PD-infected vines in 14% of them. Preliminary studies in Kern County indicate that remote sensing of holes can be used to identify PD-sampling areas.

CONCLUSIONS

The results showed that patches of PD were detected with big grids and most diseased vines were located with small grids. Validation of sampling grids will be continued and sampling plans will be optimized with sensitivity and cost-benefit analyses. Our work from Kern County suggests that knowing the percentage of PD infection and the location of vineyards relative to citrus can predict the distribution pattern of PD in the vineyard. Coachella Valley data suggests PD distribution is highly localized within vineyards and diseased grapevines were associated with two or more dead, replanted, or missing adjacent grapevines. Such inferences can be used to develop a spatially-oriented sampling program with sampling grids. The development of this sequential grid-sampling program provides three fundamental roles in PD management and research. First, it enables growers to locate vines infected with PD in the vineyard when the high incidence of infected vines precludes a vineyard census. Second, growers will be able to identify problem areas in their vineyards. Third, the sampling program provides a method for standardizing PD sampling statewide. Progress in these areas, i.e. locating individual vines, identifying problem areas in a vineyard, and standardizing areawide monitoring, not only will help growers make informed decisions in their own vineyards, but will assist researchers trying to understand the epidemiology of glassy-winged sharpshooter (GWSS) *Xf* in California. The incidence-dependent spatial distribution of PD and signature areas (i.e. “holes”) found in the Coachella Valley are very important discoveries, because they imply that by knowing the percentage of PD incidence or signature areas for PD, we can predict the distribution pattern of PD in the vineyard. These patterns then become the foundation upon which a spatially-oriented sampling program with sampling grids can be developed. Ultimately, this program will reduce cost and increase efficiency of PD sampling.

Table 1. *Vitis vinifera* cultivar in vineyards with $\geq 1\%$ PD grapevine in Kern County and the Coachella Valley.

Location	Cultivar	Number of vineyards
Kern County	Flame	17
	Red Globe	14
	Thompson Seedless	8
	Crimson	3
	Perlette	3
	Jade	2
	Superior	2
	Autumn Royal and Princess	1
	Black Emerald	1
	French Colombard	1
Coachella Valley	Flame	3
	Perlette	2
	Superior Seedless	1
	Thompson Seedless	1

Table 2. Semivariograms for within-block spatial structure of high PD incidence distribution from Kern County and the Coachella Valley.

Vineyard	Model	Nugget	Sill	Range	R^2
Kern County A	Spherical	0.139	0.176	23.4 m	0.95
Kern County B	Exponential	0.020	0.188	27.5 m	0.87
Coachella Valley	Spherical	0.053	0.118	26.0 m	0.88

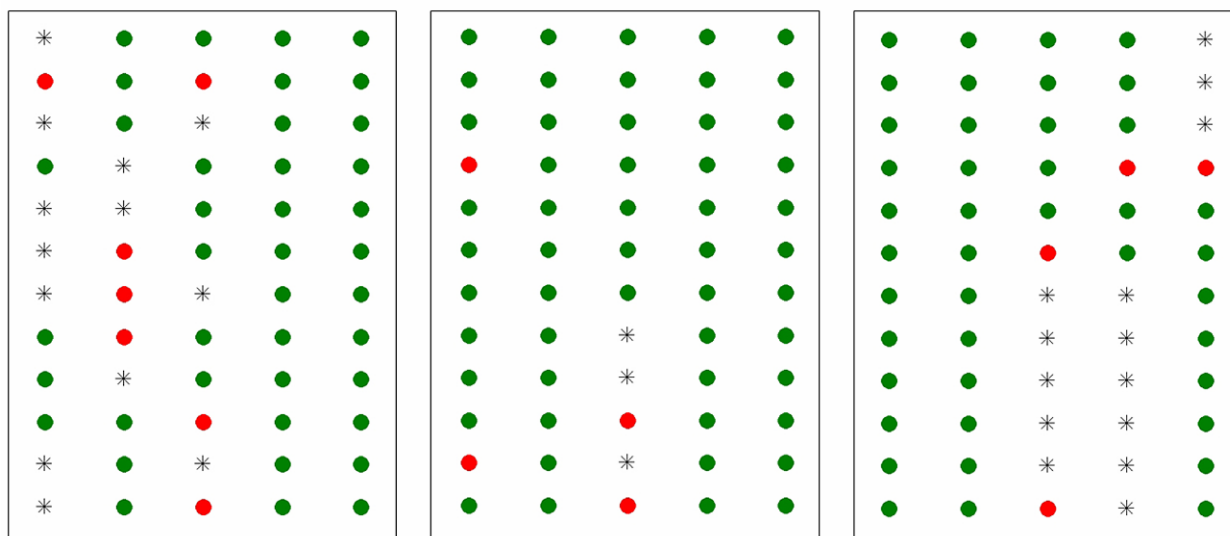


Figure 1. Examples of area symptoms of PD found by grid sampling. Red and green circles indicate diseased and healthy grapevines, respectively, and asterisks indicate missing, dead, and replanted grapevines.

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FUNDING AGENCIES

Funding for this project is being provided by the University of California Pierce’s Disease Grant Program.