LINKING WITHIN-VINEYARD SHARPSHOOTER MANAGEMENT TO PIERCE'S DISEASE SPREAD

Principal Investigator:
Matt Daugherty
Department of Entomology
UC Riverside
Riverside, CA 92521
matt.daugherty@ucr.edu

Researchers:
Adam Zeilinger
Department of Entomology
UC Riverside
Riverside, CA 92521
adamz@ucr.edu

Tracy Pinckard
Department of Entomology
UC Riverside
Riverside, CA 92521
tracy.pinckard@ucr.edu

Cooperators:
Frank Byrne
Department of Entomology
UC Riverside
Riverside, CA 92521
frank.byrne@ucr.edu

Objectives and activities to accomplish objectives

In Southern California vineyards chemical control, at both the area-wide and local scales, constitutes one of the primary methods of limiting the density or activity of the invasive vector, the glassy-winged sharpshooter (*Homalodisca vitripennis*; Castle et al. 2005). Yet the extent to which these treatments, particularly at the field-level scale, actually suppress Pierce's disease spread is not well documented.

The bacterial pathogen *Xylella fastidiosa* is endemic to the Americas, and is widespread throughout the western and southeastern U.S. This xylem-limited bacterium is pathogenic to a wide variety of plants, including several important crop, native, ornamental, and weedy species (Purcell 1997). In the Western U.S. the most economically significant host is grapevine, in which *X. fastidiosa* causes Pierce's disease. Multiplication of the bacterium in vines plugs xylem vessels, which precipitates leaf scorch symptoms and typically kills susceptible vines within a few years (Purcell 1997).

X. fastidiosa can be spread by several species of xylem sap-feeding insects, the most important being the sharpshooter leafhoppers (Severin 1949). Historically Pierce's disease prevalence has been moderate, with a pattern that is consistent with primary spread into vineyards from adjacent riparian habitats by the native blue-green sharpshooter (*Graphocephala atropunctata*). However, beginning in the late 1990s severe outbreaks occurred in southern California and the southern San Joaquin Valley that are attributable to the recent establishment of the glassy-winged sharpshooter. This invasive sharpshooter is not inherently more efficient at transmitting the pathogen than are native sharpshooters (Almeida and Purcell 2003). Instead its threat as a vector appears to stem from a combination of ability to achieve extremely high densities (Blua et al. 1999) and promote vine-to-vine (i.e. secondary) disease spread (Almeida et al. 2005).

Citrus trees themselves are not susceptible to the strains of *X. fastidiosa* found in the U.S. (though strains found in Brazil have caused significant economic losses to their citrus industry – Purcell 1997). None-the-less citrus plantings figure prominently in the epidemiology of *Xylella* diseases in California. Many portions of southern California and the southern San Joaquin Valley have vineyards in close proximity to citrus groves (Sisterson et al. 2008). This is important because citrus is a preferred habitat for the glassy-winged sharpshooter at key times of the year, allowing this vector to achieve very high densities (Blua et al. 2001). High vector populations then disperse seasonally out of citrus into nearby vineyards, resulting in clear gradients of Pierce's disease prevalence (i.e. proportion of infected plants) as a function of proximity to citrus (Perring et al. 2001).

Given the importance of citrus in Pierce's disease epidemiology, citrus groves have been the focus of area-wide chemical control programs, initiated in the Temecula and Coachella Valleys in the early 2000s and shortly afterward in Kern and Tulare Counties (Sisterson et al. 2008). The southern California programs use targeted application of systemic insecticides, such as imidacloprid, to limit *H. vitripennis* populations residing within citrus. Census data in citrus show substantial year to year variation in sharpshooter abundance that may stem from incomplete application, the use of less effect organically-derived insecticides, or inadequate irrigation to facilitate uptake - which makes the consistent management of sharpshooter populations a challenge (Toscano and Gispert 2009). None-the-less trap counts have been, overall, much reduced compared to pre-area-wide counts. The effect of chemical control can be seen clearly in early insect surveys which found significantly fewer sharpshooters in treated relative to untreated citrus and in vineyards bordering treated versus untreated groves (R. Redak and N. Toscano, unpublished data). Thus, these area-wide control programs have been considered successful in southern California (Toscano and Gispert 2009), and the swift implementation of an area-wide management program in Kern County has been credited with limiting the severity of Pierce's disease outbreaks (Sisterson et al. 2008).

Research into imidacloprid uptake by grape also has been initiated, and target concentrations high enough to suppress glassy-winged sharpshooter activity (approx. 10 μg/L of xylem sap) can be achieved and will endure for several weeks in mature vines (Byrne and Toscano 2006). This information coupled with the success of area-wide programs in citrus appears to have led to relatively widespread adoption by grape growers of imidacloprid application in vineyards to reduce further exposure to *X. fastidiosa*. In Temecula Valley, for example, it is estimated that 70% of vineyards use imidacloprid, at an approximate cost of $150-200 per acre (N. Toscano, personal communication). Yet consistent treatment of vineyards with systemic insecticides is neither universal, nor have there been any measures of how effective these costly treatments are at reducing Pierce's disease incidence.

We are studying the epidemiological significance of chemical control in vineyards, via a multi-year series of field surveys in Temecula Valley. This work will address gaps in empirically-derived observations regarding the cascading effects of vineyard imidacloprid applications on glassy-winged sharpshooter abundance and, ultimately, Pierce's disease severity.

The overall goal of this project is to understand **does within-vineyard sharpshooter chemical control reduce vector pressure and Pierce's disease spread?** This goal includes the following two specific objectives:

1. *Link within-vineyard sharpshooter management to Pierce's disease prevalence*.
2. *Quantify the form of disease progression – primary versus secondary spread*

In 2011, we continued to survey Pierce's disease prevalence, imidacloprid concentrations *in planta*, and arthropod densities in the 34 sites we had identified and surveyed in previous years. To survey disease prevalence, we conducted visual surveys of vine symptoms in the Fall for all vines in each site. These visual surveys were adjusted for false positives using plate culturing of bacteria and for false negatives using ELISA. For false positive testing, we collected petioles from 50 randomly selected vines that were visually categorized as symptomatic for Pierce's disease. We then plate cultured isolates from these petioles for detection of *X. fastidiosa*. For false negative testing, we collected petioles from 100 randomly selected asymptomatic vines. We then conducted ELISA tests for the presence of *X. fastidiosa*. We estimated a false positive and a false negative rate and used these to adjust the disease prevalence based on the visual surveys.

To survey insecticide use, in late summer 2011 we collected leaf samples from 10 vines at each site. For each site 2 vines were sampled in 5 different rows spread throughout the vineyard block. For each vine sampled we collected 2 healthy, fully expanded leaves from mid-cane. These samples were then subjected to an ELISA analysis to calculate imidacloprid concentration, using a slight modification to established methods (Castle et al. 2005, Byrne and Toscano 2006). Briefly, from each leaf we used a #6 cork borer to punch a disc of leaf tissue, weighed that disc, and then ground it in 1% methanol. After incubation dilutions were made and this material was added to Envirologix Imidacloprid Quantiplate Kit.

Finally, we surveyed population densities of *H. vitripennis*, the native smoke-tree sharpshooter, *Homalodisca littura*, and generalist natural enemies. We placed 4-8 yellow sticky-card traps at 0.5-1 m above the vines at each site. The number of traps depended on the area of vines planted. Traps were replaced monthly. For each trap, we counted the number of *H. vitripennis, H. littura*, and four groups of generalist predators: minute pirate bugs (*Orius* spp.), assassin bugs (Reduviidae), big-eyed bugs (*Geocoris* spp.), and spiders (Aranea). These four groups appear to be the most important generalist predators of *H. vitripennis* (Fournier et al. 2008). The total seasonal density of *Homalodisca* spp. and predators was calculated for each site between February and September.

Research accomplishments and results

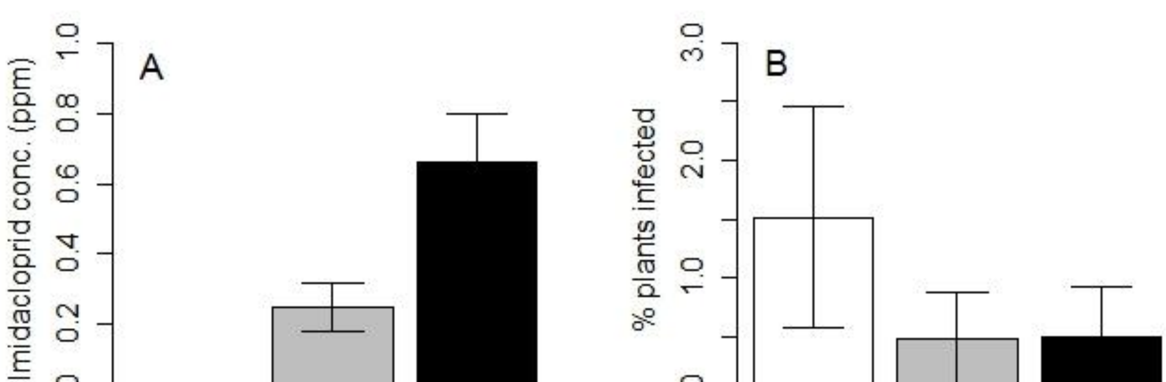
Our estimates of imidacloprid concentrations *in planta* match our classes for management practices: mean imidacloprid concentrations were 0.5 ± 0.5 (mean ± SE) ppb for untreated sites, 249 ± 68 ppb for mixed treated sites, and 662 ± 139 ppb for regularly treated sites (Fig. 1A).

Estimates of disease prevalence were generally quite low, but also highly variable, patterns that correspond roughly with imidacloprid treatment class (Fig. 1B). Disease prevalence tended to be higher in untreated sites, but there was no apparent difference between mixed and treated sites (Fig. 1B). In untreated sites, 1.5 ± 0.9% (mean ± SE) of vines were infected with Pierce's disease, whereas in mixed and treated, 0.4 ± 0.4% and 0.5 ± 0.4% of vines were infected, respectively.

We collected a total of 135 *H. vitripennis* and 56 *H. littura* in all sites from February through September, 2011. Untreated sites tended to have the highest seasonal densities of *Homalodisca* spp. with 0.05 ± 0.02 *H. vitripennis* and 0.02 ± 0.01 *H. littura* $trap^{-1}$ day^{-1} (Fig. 1C). Mixed treatment sites tended to have lower densities of *H. vitripennis* (0.004 ± 0.001 $trap^{-1}$ day^{-1}) than regularly treated sites (0.02 ± 0.01 $trap^{-1}$ day^{-1}). The densities of *H. littura* were similar in mixed and treated sites (mixed, 0.007 ± 0.002; treated, 0.005 ± 0.002 $trap^{-1}$ day^{-1}).

We collected a total of 446 minute pirate bugs, 29 assassin bugs, 42 big-eyed bugs, and 2460 spiders in all sites between February and September. We found little apparent difference in the total seasonal density of all predators collectively among management classes (Fig. 1D). The mean density was 0.60 ± 0.12 $trap^{-1}$ day^{-1} in untreated sites, 0.51 ± 0.05 $trap^{-1}$ day^{-1} in mixed treatment sites, and 0.57 ± 0.05 $trap^{-1}$ day^{-1} in regularly treated sites.

Figure 1. Differences among management classes in (A) mean imidacloprid concentrations (ppm); (B) mean percent of plants infected by *X. fastidiosa*; (C) mean seasonal densities of *H. vitripennis* (GWSS, white bars) and *H. littura* (STSS, grey bars); and (D) mean seasonal densities of predators. Observations are from Temecula Valley, 2011. Seasonal arthropod densities are from February through September.



Overall, we found clear differences in imidacloprid concentrations among our management classes. However, this pattern did not translate to clear differences in disease prevalence, densities of *Homalodisca* spp., or densities of predators. While untreated sites tended to have higher disease prevalence than mixed or regularly treated sites, we found no apparent difference between the two treatment classes. Our current results suggest that moderate rates of imidacloprid application may be sufficient to reduce disease prevalence.

We found that untreated sites tended to have the highest densities of *Homalodisca* spp. Surprisingly though, sites in the mixed treatment class tended to have the lowest densities of *Homalodisca* spp., especially for *H. vitripennis*, the primary vector of *X. fastidiosa*. One potential cause of increased *H. vitripennis* in regularly treated sites is the reduction of natural enemy populations due to excessive imidacloprid applications. However, our results indicate that release from predation is unlikely; we found little difference in the densities of predators among management classes.

Publications and reports

Gruber, B.R., and M.P. Daugherty 2012. Predicting the effects of seasonality on the risk of pathogen spread in vineyards: vector pressure, natural infectivity, and host recovery. Plant Pathology, *accepted*.

Daugherty, M.P. 2011. Linking within-vineyard sharpshooter management to Pierce's disease spread. California Department of Food and Agriculture. pp. 28-31. 2011 Pierce's Disease Research Symposium. Sacramento, CA.

Presentations on research

Daugherty, M.P. 2011. The effectiveness of within-vineyard sharpshooter control for limiting Pierce's disease spread. Temecula Valley Grape Day. Temecula, CA.

Daugherty, M.P. 2011. Regional epidemiology of Pierce's disease in California and the implications for management. Pierce's Disease Research Symposium. Sacramento, CA.

Research relevance

Pierce's disease management in southern California vineyards hinges on chemical control of populations of the vector, the invasive glassy-winged sharpshooter (*Homalodisca vitripennis*), residing in citrus. Yet the effectiveness of chemical control programs at reducing Pierce's disease spread has not been documented. We are conducting a series of surveys in treated and untreated vineyards in Temecula Valley to determine the relative economic value of within-vineyard chemical control for Pierces disease management. These surveys will be conducted over three consecutive years to evaluate the relative importance of vector pressure and chemical control for disease spread. Ultimately, survey data will be used to quantify rates of secondary spread and the spatial distribution of *Xylella fastidiosa* strains, which is needed for drawing inferences regarding sharpshooter movement and pathogen sources.

Lay summary

One of the main tools for dealing with the glassy-winged sharpshooter in southern California and the southern San Joaquin vineyards is the application of insecticides. Systemic insecticides (imidacloprid) are regularly applied to citrus, which is a preferred plant type for sharpshooter, to reduce insect abundance before they move into vineyards. These treatment programs have been successful, reducing sharpshooter populations to a fraction of what they once were. Grape growers frequently use systemic insecticides in vineyards as well to reduce further the threat of sharpshooters spreading Pierce's disease among vines. However, no measurements have been made about whether these costly insecticide treatments are effective at curbing disease spread. We are conducting a series of disease surveys in Temecula Valley to understand whether chemical control of glassy-winged sharpshooter in vineyards is justified. Results from the surveys thus far indicate that the frequency of disease in vineyards varies greatly, but generally fields that are treated regularly or even intermittently tend to have lower sharpshooter pressure and fewer diseased vines than those that are not treated with imidacloprid. We will continue these disease surveys for an additional year to more fully evaluate this question.

Status of funds

Currently, the progress we have made on the project matches up with the proposed timeline. Remaining funds are fully committed towards completing the required project tasks, therefore we expect to exhaust these funds in the same timeframe as originally budgeted.

Intellectual property

N/A

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