CDFA PIERCE'S DISEASE & GLASSY-WINGED SHARPSHOOER BOARD PROGRESS REPORT (JULY 2007 – FEBRUARY 2008)

I. PROJECT TITLE: Seasonal Transmission of *Xylella fastidiosa* by GWSS from Grapevines Infected for Various Lengths of Time

II. PRINCIPAL INVESTIGATOR AND COOPERATORS:

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III. LIST OF OBJECTIVES AND DESCRIPTION OF ACTIVITIES CONDUCTED TO ACCOMPLISH EACH OBJECTIVE:

Our goal is to elucidate details of vine-to-vine spread by GWSS. Vine to vine spread is affected by several factors such as when and where a vine is inoculated initially. Little information exists with regard to GWSS landing site selection and feeding behaviors on grapevine. Consequently, these factors affect location and time of inoculation and acquisition of *X. fastidiosa*. While this progress report addresses only the first objective, the objectives of the project are:

1. Document GWSS feeding preference, through the growing season, on established Cabernet Sauvignon and Chardonnay grapevines that either are healthy or have been infected with *X. fastidiosa* for 2, 3, or 4 years.
2. Evaluate the acquisition by GWSS, through the growing season, from established Cabernet Sauvignon and Chardonnay grapevines that either are healthy or have been infected with *X. fastidiosa* for 2, 3, or 4 years and determine the subsequent transmission from these acquisitions.
3. Determine the relationship between *X. fastidiosa* inoculation by GWSS at different times of the year and the development of the vine as a source for further acquisition by GWSS.

IV. SUMMARY OF MAJOR RESEARCH ACCOMPLISHMENTS AND RESULTS FOR EACH OBJECTIVE

We conducted experiments with Cabernet Sauvignon or Chardonnay grapevines from a mixed field-grown vineyard at the University of California in Riverside that was covered with 60% shade-cloth to protect them from PD. We placed GWSS adults individually in observation cages fabricated from acetate cylinders (25cm x 17cm diameter) with organdy sleeves attached to the ends. The cage was place over the base of a grape vine cane with the cane terminal looped back into it. The ends of the observation cage were sealed giving each GWSS access to immature and mature stems, petioles, and leaves inside during the summer months, or just immature and mature stems during the winter months. We made hourly observations from 700 to 1800 hours (Summer dates) or 900 to 1600 hours (Winter dates) over three days and noted which tissue each GWSS was on. Experiments were conducted on 29-31 August, 12-14 September 2007, 16-18 January, and 6-8 February 2008.

In experiments conducted in late August and mid September, GWSS were found on the cage in 12 to 16% of our observations (Figure 1). When on the cane, GWSS were observed on all tissue types, and although significant differences in tissues used occurred among varietals and genders in both experiments, GWSS did not show a substantial preference for a specific tissue consistently. However, they were found more frequently on immature stems, petioles, and leaves (46-67% of our observations) than mature tissue (21-37%) weather they were enclosed on Cabernet Sauvignon or Chardonnay vines, or were males or females (Figure1). In general, GWSS were most likely to be found on immature stems and leaves, and least likely to be found on immature petioles and mature stems and petioles (Figure 1). Importantly, averages of 34% and 36% of experimental GWSS across gender in experiments 1 and 2, respectively, switched from one tissue to another each hour on Cabernet Sauvignon. Hourly averages of 30% and 37% of GWSS on Chardonnay switched tissues in experiments 1 and 2, respectively. Averages of 34% and 39% of females switched tissues each hour, and averages of 30% and 34% of males switched tissues each hour in experiments 1 and 2, respectively.

In the January experiment, 13 of 20 GWSS died without having been observed on plant tissue. Two others were observed on tissue but died without feeding. The five remaining were observed to produce watery excreta, an indication of xylem feeding, and survived through the 3-day experiment. Of the five GWSS that fed, 4 fed from immature stems, and 1 from an old stem. Because only 5 GWSS survived the experiment, we did not further analyze feeding choice data.

In the February experiment, 6 of 20 GWSS died without having been observed on plant tissue. Three others were observed on tissue but died without feeding. The 11 remaining GWSS were observed to produce watery excreta and survived through the 3-day experiment. GWSS that died were replaced through the first two days of the experiment. GWSS on Cabernet Sauvignon or Chardonnay were found most frequently on the cage and immature stems in our February experiment (Figure 2). Significantly fewer GWSS were found on mature stems. When data were split by gender, similar numbers of female and male GWSS were found on the cage or immature stems. Significantly fewer females and males were found on mature stems (Figure 2). Analysis of the February experiment is on-going.

Figure 1. GWSS preference on field-grown Cabernet Sauvignon and Chardonnay grapevines in choice experiments initiated on 29 August 2007 (Experiment 1) and 11 September 2007 (Experiment 2). Bars represent average proportions of GWSS (±SE) observed on various tissue types. Different letters above bars represent statistically significant differences among means at p = 0.05 (ANOVA followed by t-tests for mean separation).

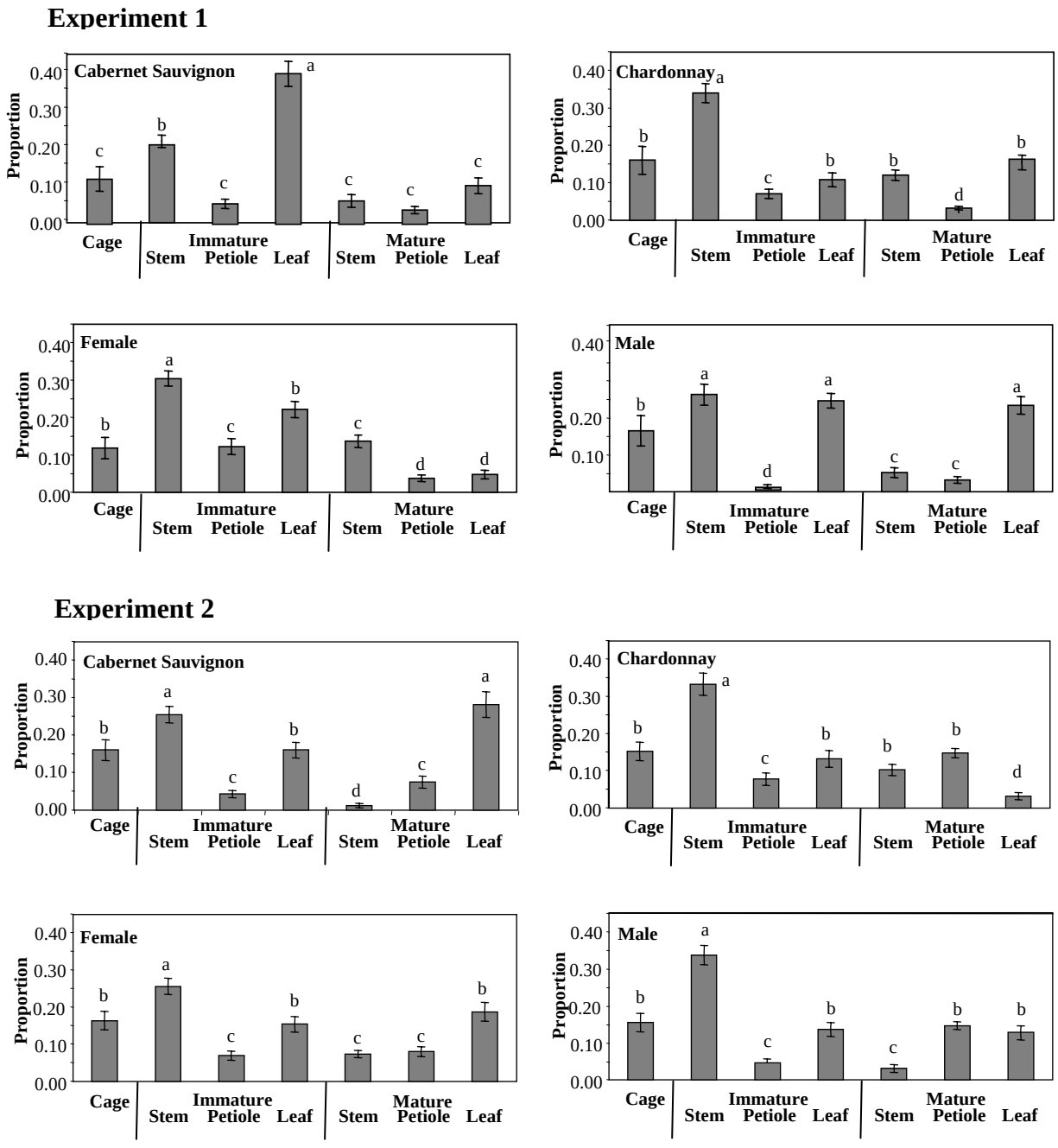
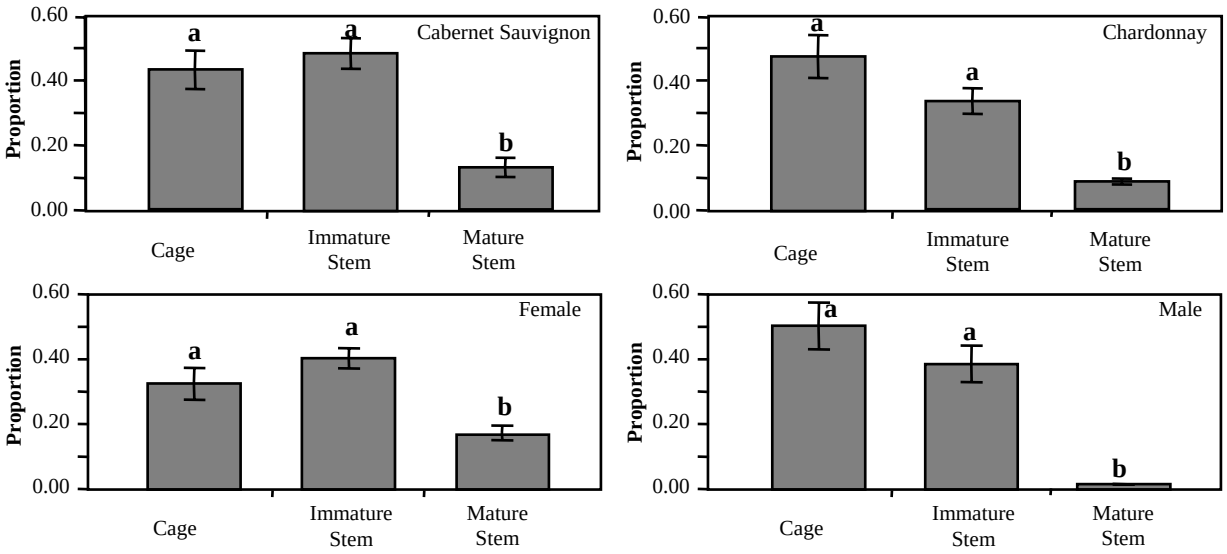


Figure 2. GWSS preference on field-grown Cabernet Sauvignon and Chardonnay grapevines in a choice experiment initiated on 6 February 2008. Bars represent average proportions of GWSS (±SE) observed on various tissue types. Different letters above bars represent statistically significant differences among means at p = 0.05 (ANOVA followed by t-tests for mean separation).



V. PUBLICATIONS OR REPORTS RESULTING FROM THE PROJECT

Perring, T. M., C.A. Farrar, and M.J. Blua. 2007. Seasonal transmission of *Xylella fastidiosa* by the glassy-winged sharpshooter from Grapevines Infected for various lengths of time. Pp. 54-57 *In* T. Esser (ed.) Proceedings, 2007 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

VI. PRESENTATIONS ON RESEARCH

Perring, T. M., C. A. Farrar, M. J. Blua, T. R. Pinckard. 2007. Seasonal transmission of *Xylella fastidiosa* by the glassy-winged sharpshooter from Grapevines Infected for various lengths of time. Poster presentation. 2007 Pierce's Disease Research Symposium, San Diego, CA.

VII. RESEARCH RELEVANCE STATEMENT

Pierce's disease (PD), a disease of grapes caused by the bacteria, *Xylella fastidiosa* Wells et al., was described in California in the 1880s during an epidemic in Orange County (Pierce 1882). A second epidemic occurred in Tulare County in the 1930s (Hewitt et al. 1949), and until the mid-1990s, it was considered only a minor problem in vineyards close to riparian areas. In the early 1990s a new vector, the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (formerly *Homalodisca coagulata* Say), was introduced into the state (Sorenson and Gill 1996), and became associated with a devastating epidemic of PD in the Temecula Valley. Since 1994, at least 1,500 acres of vineyards have been lost to the disease in California; in the Temecula Valley alone, losses have been estimated at $13 million (Wine Institute 2002). The GWSS has

different feeding and dispersal capabilities than native insect sharpshooter vectors and these attributes are thought to have contributed to the increased number of PD-infected grapevines in California (Almeida et al. 2005, Blua et al. 1999, Redak et al. 2004). Like other insect-borne plant pathogen systems, there are two potential types of pathogen spread: primary or secondary spread. Primary spread occurs when the pathogen is obtained by the vector from sources outside the crop and transported and inoculated into the crop. Secondary spread occurs when the vector acquires the pathogen from infected vines in the vineyard, and subsequently inoculates healthy vines within the same vineyard (i.e. vine to vine spread). It is thought that *X. fastidiosa* spread with native California vectors was the result of primary spread, but that rapid spread by GWSS may be the consequence of primary and secondary spread (Almeida et al. 2005, Hill 2006). Consequently, the best strategy for managing PD spread by GWSS is to control the vector. This strategy, which is simple in concept, yet difficult and costly to implement over large geographic areas, is exactly the approach of the areawide management program being implemented by USDA, CDFA, County Agricultural Commissioner's Offices, and the University of California (for example Toscano and Gispert 2005, Stone-Smith et al. 2005). This strategy has been so effective at slowing the spread of PD throughout the state that the "crisis" phase of the PD-GWSS problem appears to have passed. Unknown, of course, is what will happen should the areawide management program be reduced. Due to this uncertainty, researchers continue to search for alternative solutions, both short and long term. Understanding details of primary and secondary spread of *X. fastidiosa* by GWSS can assist in the development of alternatives to the areawide management program. For example, to reduce primary spread, efforts must focus on reducing bacteria-carrying GWSS from entering healthy vineyards, through continued areawide or local treatment programs outside the vineyard, barriers, trap crops, and/or removal of pathogen sources outsides the vineyard. Reduction of secondary spread can be accomplished by in-field control of GWSS, finding and roguing infected vines in the vineyard (Varela et al. 2001), and/or minimizing acquisition from infected vines and transmission to healthy vines.

The relationship among time of inoculation, location of inoculation, and disease progression in the vine likely plays a role in determining whether disease becomes chronic and when a vine becomes a source plant for additional spread. When another PD vector, the blue-green sharpshooter, *Graphocephala atropunctata*, infected grapevines early in the season, more persistent infections resulted than from later season infection (Purcell 1981). A potential difference between blue-green sharpshooter transmission and GWSS transmission is that the former is known to prefer feeding at the tips of canes (Purcell 1976), whereas the latter has been reported to feed on older plant parts. Almeida et al. (2005) demonstrated that GWSS could even transmit *X. fastidiosa* to dormant vines in the field, and Almeida et al. (2005) found that GWSS also could acquire *X. fastidiosa* from dormant vines in the greenhouse. However acquisitions from dormant vines in the field were negative. Whether these transmissions and acquisitions are important to disease spread depends on GWSS feeding preferences during the winter months when the vines are dormant. Similarly, it is possible that infection at certain times of the season may not become systemic because infection is pruned out at end of year, or environmental conditions limit bacterial spread (Feil and Purcell 2001, Fiel et al. 2003, Hill 2006).

Vine to vine spread of *X. fastidiosa* by GWSS has been hypothesized as a critical component of devastating PD epidemics that occurred in Temecula and in the General Beale area of Kern

County. A fundamental understanding of this type of spread can lead to strategies insuring that epidemics of these proportions do not occur elsewhere. GWSS landing and feeding behavior, and tissue feeding capacity combine with grapevine phenology, and *X. fastidiosa* phenology to make vine to vine spread possible. Particularly important is the tendency for GWSS to move frequently in grapevines, as shown in this study, and their characteristic short hopping flights (Turner and Pollard 1959) that would maximize within-vineyard spread of *X. fastidiosa*. Increased movement by GWSS in search for optimal host tissue would increase the chance of contact with infected and healthy grapevines alike.

Our future studies will compare GWSS feeding in no-choice circumstances, and GWSS flight orientation and behavior when confronted with PD-infected and healthy grapevines. We will also examine the relationship between *X. fastidiosa* inoculation by GWSS at different times of the year and the development of the vine as a source for further acquisition by GWSS.

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VIII. LAY SUMMARY OF RESULTS

This study was designed to define specific orientation and feeding behaviors by the glassy-winged sharpshooter (GWSS) in association with grapevines that influence vine-to-vine spread of *Xylella fastidiosa*, the Pierce's disease bacterium. Thus far, we have conducted experiments in summer and winter in which we made hourly observations on the location of individual GWSS adults given access to mature tissue and a terminal tip on the same cane. Although GWSS were observed on all tissues, both males and females preferred immature tissues to mature tissues on Cabernet Sauvignon and Chardonnay grapevines, yet sharpshooters moved frequently throughout the day. In the summer, immature stems and leaves were consistently the most used tissue, while immature petioles and mature stems and petioles were the least used. In the winter, immature stems were used more than mature stems. Winter survivorship of GWSS was associated with xylem feeding.

IX. STATUS OF FUNDS

According to the most recent records the PI has (through December 2007), the project had utilized $52,698 or 35% of the 2 year allocation for this project. Given that just 25% of the project time frame was completed, the PI will make adjustments to the spending on this project to insure financial health through the 2 year time frame.

X. SUMMARY AND STATUS OF INTELLECTUAL PROPERTY PRODUCED DURING THIS RESEARCH PROJECT

No intellectual property was produced as a result of this research project.