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

A. Project title: Seasonal Transmission of *Xylella fastidiosa* by GWSS from Grapevines Infected for Various Lengths of Time.

B. CDFA contract number: 07-0300

C. Time period covered by the progress report: July 2007 – June 2008

D. Principal Investigator and Cooperators:

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E. 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. 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.

F. Research accomplishments and results for each objective:

Choice Tests for Grapevine Tissue Selection

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 placed over the base of a Cabernet Sauvignon or Chardonnay grape vine cane with the cane terminal looped back into it. The ends of the observation cage were sealed giving a single GWSS in each cage access to old and young stems, petioles, and leaves inside. The grapevines were 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 made hourly observations during daylight hours over three days to determine the location of each GWSS. This experiment was conducted twice in the winter 2008 and once in summer 2008.

Results of the two winter trials were pooled for purposes of reporting here. GWSS were found on the cage walls in 49% of our observations in winter trials. In the winter, neither leaves nor petioles were available to the sharpshooters, and GWSS were found on old stems and young stems in 11% and 40% of the observations, respectively. Those tissue preferences differed somewhat among the two varietals and the two sexes (Figure 1). The major departure from these numbers was the preference for the old stem among sexes; females and males were on the old stem in 20% and 1% of the observations, respectively. The general preference for the young stem over the old stem was consistent among varietals and among sexes. Changes in GWSS position occurred in 14% of the observations, considerably less than the 35% exhibited in the trials of August and September 2007. There was little difference in the tendency of GWSS to change positions among varietals nor among sexes.

The trial in July 2008 offered GWSS young and old leaf and petiole tissue in addition to young and old stems. GWSS were found on the cage wall 12% of the time (Figure 1). The general preference for young tissue that was found in the winter occurred in July. GWSS chose young leaves, petioles, or stems in 67% of the observations compared to 21% for the older tissues. The young stem was the preferred tissue, both among varietals and among sexes. However, there were some differences in tissue selection among varietals and among sexes. The old stem was selected 24% of the time on Cabernet Sauvignon but only 5% of the time on Chardonnay. The young leaf and young petiole each were selected in 1% of the observations on Cabernet Sauvignon, while they were selected 8% and 7% of the time on Chardonnay. Among sexes, females chose the old stem in 22% of the observations, but males chose that tissue in only 7% of the observations. Among tissue types of any age, leaves, petioles, and stems were chosen in 12%, 5%, and 83% of the observations, respectively. Changes in GWSS position occurred in 21% of the observations, and that rate of change was consistent among the varietals and among the sexes.

No-choice Tests Quantifying Feeding on Grapevine Tissues

No-choice feeding trials were conducted on the same mixed field-grown vineyard at the University of California in Riverside. Individual GWSS were caged on selected grapevine tissue in 50 ml polypropylene centrifuge tubes (Thermo Fisher Scientific Inc., Waltham, MA) by one of two methods. The first method, modified from Andersen et al. (1992), was for use on cordons, stems, and petioles. The cages were made by melting a transverse hole in the side of the tube using hot metal cylinders of diameters similar to the grape tissues. The tube was pressed onto the plant tissue, so the GWSS had access to about 2.5 cm length of the plant through the hole. The cage was affixed and sealed to the tissue by wrapping the tube and tissue with ca. 2 cm wide strips of Parafilm (Pechiney Plastic Packaging, Menasha, WI). The screw cap was tightened, and the cage rested vertically so that excreta collected in the bottom of the tube. The second cage design was for use on leaf tissue. The mouth of an intact 50 ml tube was pressed to the abaxial leaf surface with a piece of coiled spring steel in a clothes-pin like fashion (Blua and Perring 1992). One end of the spring held the 50 ml tube. The other end of the spring had a plastic ring on which was glued a foam pad 1 cm thick by 3 cm in diameter which gently held the leaf against the polypropylene tube, giving the insect access to leaf tissue of ca. 5.7 cm^2. This cage, too, was oriented vertically, so excreta drained to the bottom of the cage. Each cage type was loosely covered with aluminum foil in order to shade it from direct sunlight.

The day before the start of the test, GWSS adults were collected from citrus at Agricultural Operations, UCR, and placed in a cage with a potted rough lemon plant. The following morning, adults were isolated and sexed and then placed individually into the tube cages. Cages were inspected daily and the presence of excreta noted. Cages with dead GWSS were removed, and the amount of excreta was weighed. Up to 1.5 ml of excreta from each cage was frozen for future analysis of chemical content. At the end of the trial, all remaining cages were collected, GWSS mortality was noted, and excreta was weighed.

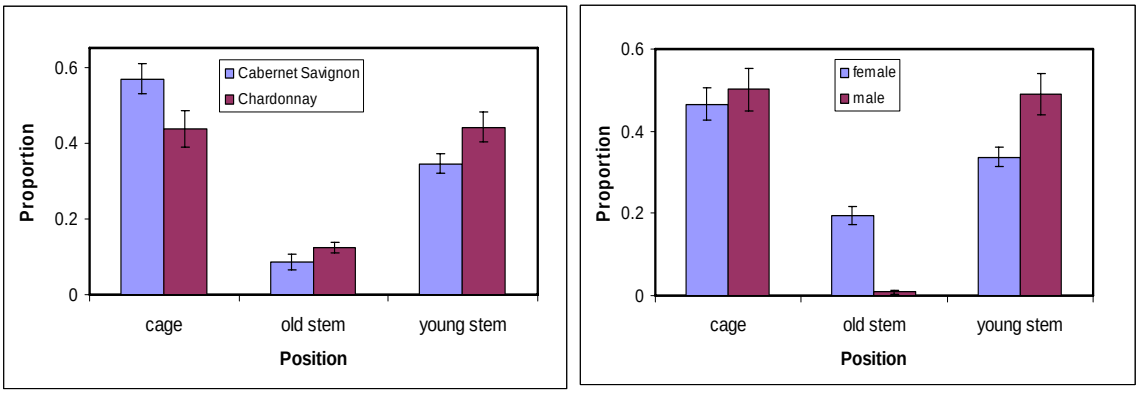
During the winter trials, GWSS were placed on cordons, old stems, and young stems; leaves and petioles were not available. The overall GWSS feeding rate was 0.37 g of excreta per day, but there was considerable variation among sharpshooters (Figure 2). In no case did discernible feeding occur on cordons, tissue several years old with thick dry bark. The old stems were covered with dry, but much thinner bark. Feeding on the old stem averaged 0.92 g of excreta per day and on the young stem, 0.57 g, however those amounts were not significantly different at $p=0.05$. There were no significant differences in feeding among varietals nor among the sexes. Survivorship in the winter trials averaged 2.04 days, and there were no significant differences in survivorship among varietals nor sexes. There were significant differences in survivorship among GWSS on different tissues (Figure 2). Of 29 GWSS on cordons, only 6 lived into the second day for an average survivorship of 1.2 days, significantly less than on the other tissues. Among all insects, only one insect that produced no excreta survived as long as 3 days, and only one insect that produced excreta died before the end of the trial.

Sharpshooters fed on all tissues except cordons in the July trial (Figure 2), averaging 0.51 g of excreta per day. Feeding on non-cordon tissues was highly variable, but there were some significant differences. Excreta produced on young stems was significantly more than excreta from young petioles, old leaves, and cordons. There were no significant differences among varietals, sexes, nor tissue age (i.e. old leaves, petioles, and stems vrs. young leaves, petioles, and

stems). Among tissue types there were significant differences in feeding. Significantly more excreta was produced on stems (0.92 g) than on petioles (0.45 g), leaves (0.41 g), and cordons (0 g), and excreta from the petioles and leaves was significantly greater than from the cordons. Average GWSS survivorship in the July trial was 4.06 days. As in the winter, only survivorship on cordons was significantly less than that on other tissues (Figure 2). Other than cordons, there were no significant differences among leaves, petioles, and stems. In addition, survivorship among varietals and among sexes was not significantly different. In this and the winter trial, observations more frequent than once per day might have revealed more differences in survivorship, however that would have come at the risk of disturbing GWSS feeding.

Figure 1. GWSS preference on field-grown Cabernet Sauvignon and Chardonnay grapevines in choice experiments conducted in winter 2008 (16 January, 6 February) and summer 2008 (1 July). Bars represent average proportions of GWSS (±SE) observed on various tissue types.

Winter 2008



Summer 2008

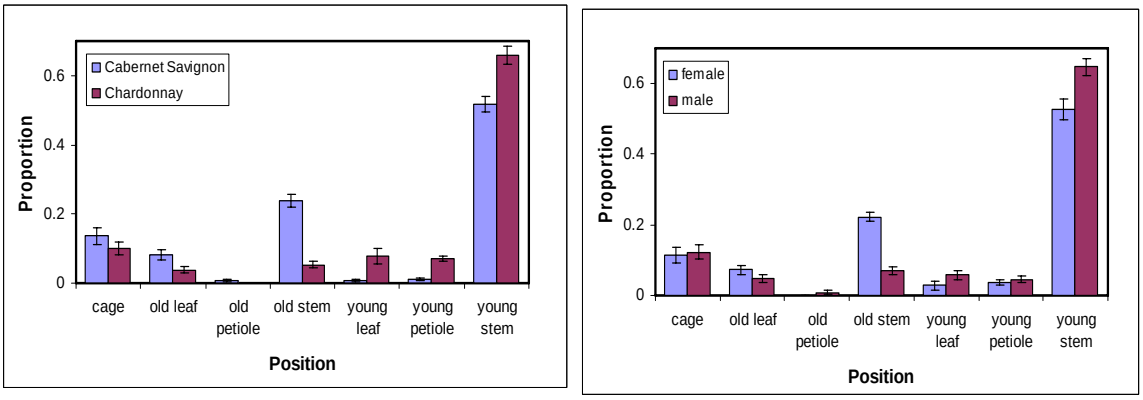
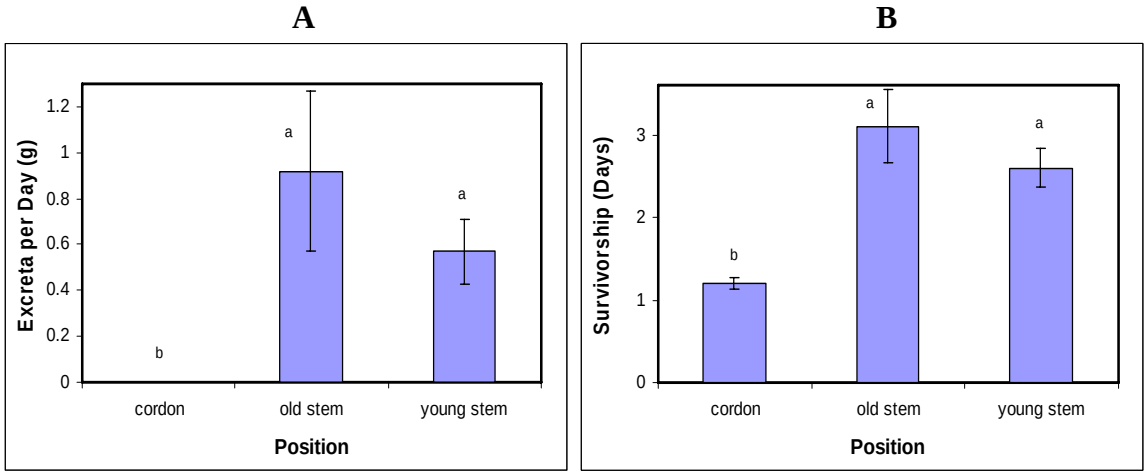
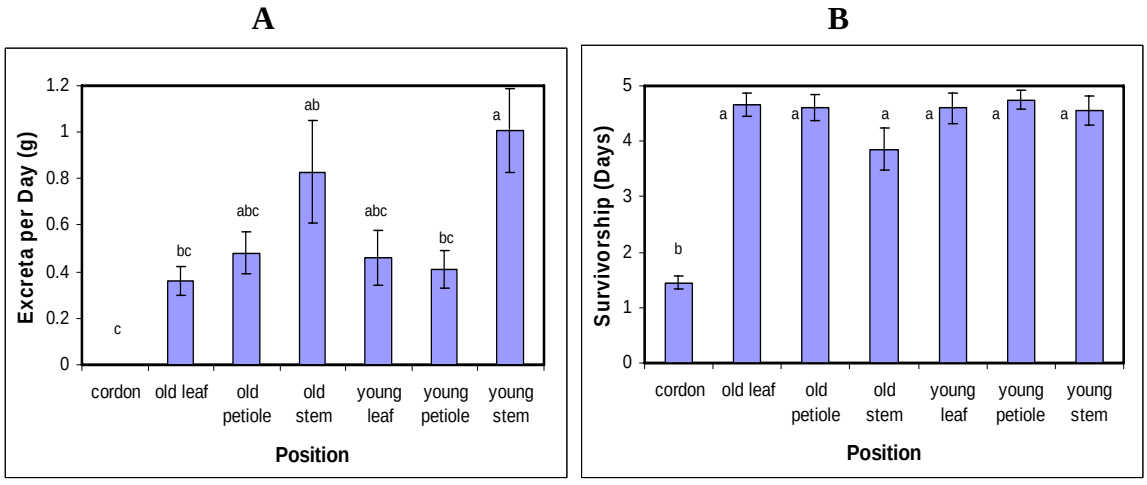


Figure 2. GWSS feeding on field-grown Cabernet Sauvignon and Chardonnay grapevines in no-choice experiments conducted Winter 2008 (26 February, 4 March) and Summer 2008 (15 July). Bars represent A) average amount (g) of excreta per day (±SE) measured from various tissue types, B) average GWSS survivorship (days) (±SE) on the same tissues. Different letters above bars represent statistically significant differences among means at p = 0.05 (ANOVA followed by Tukey's studentized range test for mean separation).

Winter 2008



Summer 2008



G. Publications, reports, and presentations where the information generated from research was presented:

Publications

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.

Presentations

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.

H. Research relevance statement:

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. We are comparing GWSS feeding in choice and no-choice circumstances, and our future work will evaluate GWSS feeding behavior when confronted with PD-infected and healthy grapevines. We also plan to 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.

References Cited:

Andersen, P. C., B. V. Brodbeck, and R. F. Mizell III. 1992. Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. J. Insect Physiol. 38: 611-622.

Blua, M. J. and T. M. Perring. 1992. Alatae production and population increase of aphid vectors on virus-infected host plants. Oecologia. 92: 65-70.

Turner, W.F., and H.N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. U.S. Dept. Agric. Tech. Bull. 1188.

I. Summary in lay terms of the specific accomplishments of the research project:

This study aims to define specific orientation and feeding behaviors by the glassy-winged sharpshooter (GWSS) in on grapevines that may influence vine-to-vine spread of *Xylella fastidiosa*, the Pierce's disease bacterium. We have learned that GWSS adults males and females prefer immature tissues to mature tissues on Cabernet Sauvignon and Chardonnay grapevines, yet sharpshooters moved frequently throughout the day. Immature stems and leaves were consistently the most used tissue, while immature petioles and mature stems and petioles were the least used. While our studies have shown a preference by GWSS for young grapevine tissues, a substantial amount of their time is spent on older tissue. All of our studies indicate that GWSS does not feed on cordons. They do feed however on older tissues at the base of canes which could result in transmission of PD that leads to systemic infection. One practical suggestion to minimize systemic infection of PD in the winter would be to delay pruning as long as possible, thus offering sharpshooters young tissue that, if infected, would be pruned off.

J. Summary and status of intellectual property produced during this research project:

Aside from the published proceedings and the presentation at the CDFA PD conference, no intellectual property was produced as a result of this research project.