CDFA contract number: 07-0300

CDFA PIERCE'S DISEASE & GLASSY-WINGED SHARPSHOOER BOARD PROGRESS REPORT (JULY 2007 – FEBRUARY 2009)

A. Project title:

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

B. Principal Investigator and Cooperators:

Principal Investigator:
Thomas M. Perring, Professor, Department of Entomology, University of California, Riverside, CA 92521, phone: (951) 827-4562, e-mail: thomas.perring@ucr.edu

Cooperators:
Russell L. Groves, Assistant Professor and Extension Specialist, Department of Entomology, University of Wisconsin, 537 Russell Laboratories, 1630 Linden Drive, Madison, WI 53706, phone (608) 2623229, e-mail: groves@entomology.wisc.edu

Mark Sisterson, Research Entomologist, USDA-ARS, San Joaquin Valley Agricultural Sciences Station, 9611 S. Riverbend Ave., Parlier, CA 93648, phone (559) 596-2840, e-mail: msisterson@fresno.ars.usda.gov

Charles A. Farrar, SRA, Department of Entomology, University of California, Riverside, CA 92521, phone: (951) 827-4518, e-mail: charles.farrar@ucr.edu

Tracy Pinckard, SRA, Department of Entomology, University of California, Riverside, CA 92521, phone: (951) 827-4518, e-mail: tracy.pinckard@ucr.edu

C. Time period covered by the progress report: July 2007 – February 2009

D. Objectives, Activities, Progress and Findings:

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.

We have been forced to modify the original objectives due to the fact that suspected infections of our grapevines have not proven to be consistent. At the time we started in July 2007, selected vines in our field cages had been needle-inoculated in May 2003, May 2004, and May 2005 by cooperator Groves. According to Dr. Groves, the vines tested positive in 2004 (for 2003 inoculations) and 2005 (for 2004 inoculations), but due to his departure in 2006, the 2005 inoculations were not verified. Interestingly our first evaluation of all the vines on August 28, 2007 showed almost no infection with *X. fastidiosa*. It is unclear why the infections did not become systemic, but the fact that we had no multi-year infections dictated a revision of our original plans. On November 5, 2007, we needle inoculated the set of vines that had been inoculated in 2003 by scraping the bark on the cordons to expose green tissue for inoculation (Figure 1).

Figure 1. Scraping away bark on the grapevine cordon (left) to expose green tissue for needle inoculation (right).

While waiting for these infections, we proceeded with experiments to document GWSS feeding biology through the season on the two varieties of grapes in our field cages, Cabernet Sauvignon and Chardonnay. Detailed results from these studies have been presented in previous reports; here we provide a summary.

GWSS feeding biology was studied in three seasons using choice and no-choice studies. Choice studies were conducted in the Fall 2007 (29 August and 11 September 2007), Winter 2008 (16 January and 6 February 2008) and Summer 2008 (1 July 2008). For this research, 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 grapevine cane with the cane terminal looped back into the cage. 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 cage. We made hourly observations during daylight hours over three consecutive days to determine the location of each GWSS.

When given a choice, GWSS males and females chose to feed on young leaf, petiole, and stem tissue compared to the same tissues on older parts of the cane. However, there was substantial time spent feeding on old stem tissue, a phenomenon that would result in more rapid chronic infection than feeding on young tissue. We also learned that throughout the day, GWSS adults change position frequently between the various tissues, a characteristic that would support the rapid spread of *X. fastidiosa* that has been associated with GWSS.

No-choice studies were conducted in the Winter 2008 (26 February, 4 March), Summer 2008 (15 July), and Fall 2008 (19 September). Individual GWSS were caged on selected grapevine tissue in 50 ml polypropylene centrifuge tubes 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. 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 each 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.

In these studies, we found that GWSS adults were not able to feed on cordon tissue, regardless of the time of year. They were able to feed on old and young grapevine tissue throughout the year, but the relative amount of feeding on this tissue varied with the season.

Returning to the problems associated with obtaining infected vines, we assayed every vine in our field cages on August 22, 2008 and this evaluation yielded one strong positive and 2 weak positive vines. We are not sure why our inoculations in the field cages do not take but we again inoculated the vines that were inoculated in 2003 (and 2007) in September 2008. Similar to the 2007 inoculation, we removed bark from the cordon wood and needle inoculated into green tissue. Another sampling of all vines occurred on December 4, 2008 and 27 vines were confirmed to by *X. fastidiosa* positive by ELISA.

GWSS preference for infected/non-infected grapevine tissue

Integrating all of the information from ELISA positive vines, symptomatic vines, and vines we infected twice, we selected canes from putative infected and non-infected Cabernet Sauvignon or Chardonnay grapevines to study GWSS choice for infected or non-infected tissue. Two trials were conducted on 19-21 February and 25-27 February 2009. Because of the time of year, there were no leaves or petioles on the canes. All tissue had a woody outward appearance, but we confirmed that the internal tissue was green, so GWSS would be able to feed. We placed GWSS adults individually in observation cages. The cage was placed over a section of cane from an infected vine and a section of cane from a non-infected vine (Figure 2). The infected cane was marked with a small wire label. The ends of the observation cage were sealed giving a single GWSS in each cage access to infected or non-infected cane tissue. We made hourly observations

from 8am to 5pm over three consecutive days to document the cane (infected or non-infected) on which the GWSS fed.

Figure 2. Acetate cage uses to evaluate GWSS feeding preference for infected (see yellow marker on lower cane) and non-infected grapevine tissue. Notice GWSS feeding in center of infected cane.

At the conclusion of each trial, we conducted a variety of procedures to verify the infection status of the cane tissue to which the GWSS were exposed. All living GWSS were collected and surface sterilized, after which their heads were macerated and plated to determine if they were carrying *X. fastidiosa*. Each section of cane that was inside the acetate cages was removed from the vine, marked and planted into pots. Following growth of these cuttings, we will conduct ELISA and culturing to determine the infection status of the section of cane to which GWSS was exposed. In addition, a small section of each of these canes (0.5 in) was macerated and subjected to ELISA immediately after the trial was concluded.

Results

While the results of these experiments are preliminary at this time (due to verification of *X. fastidiosa* infection) they are quite interesting. In both experiments, GWSS showed a strong preference for infected grapevine tissue (Figure 3). Over three days of feeding, in which we monitored the location of insects every hour, 50% (trial 1) and 54% (trial 2) of the time, GWSS were found on the infected tissue. This compared to 17% and 14% on the non-infected canes in trials 1 and 2, respectively. Females and males had nearly identical preference for infected tissue in trial 1 (49% and 52%), but in trial 2 a substantially higher proportion of females (66%) than males (41%) were found feeding on infected tissue. In both trials, a higher proportion of females than males were able to feed on grapevine tissue, regardless of the infection status. In trial 1, 74% of females fed on grapevine tissue compared to just 60% for males and in trial 2, 78% of the females compared to 57% of males fed on grapevine tissue. This may reflect the larger size of females and the need they have for feeding to produce eggs. 15 and 6% of the females died in trials 1 and 2, respectively, compared to 23 and 19% of the males in the two trials (Figure 3).

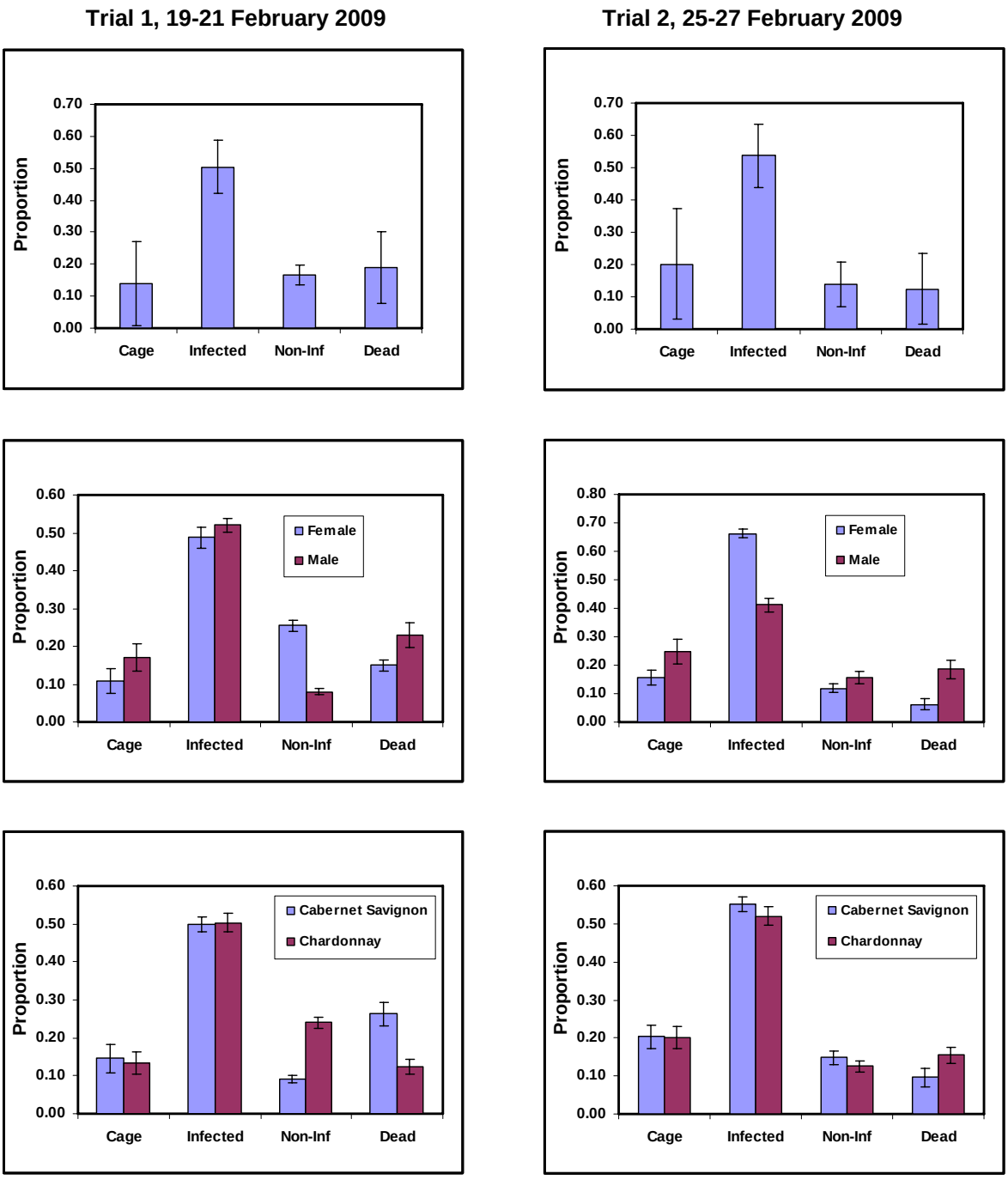

Figure 3. GWSS preference on field-grown Cabernet Sauvignon and Chardonnay grapevines in choice experiments initiated on 19 February and 25 February, 2009. Bars represent average proportions of GWSS (± SE) observed on the cage, feeding on infected or non-infected canes, or dead.

Looking at the data by grapevine variety, while a similar proportion of GWSS preferred infected tissue of both varieties, more GWSS died on the Cabernet Sauvignon then Chardonnay in the first trial. This did not appear to be the case in the second trial (Figure 3).

Following the confirmation of *X. fastidiosa* infection, we will conduct further analyses. Our plan is to repeat these experiments during the summer and fall 2009.

E. Intellectual Property Issues:

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

F. Appropriate References

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.

G. 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.

Perring, T.M., C.A. Farrar, and M.J. Blua. 2008. Seasonal transmission of *Xylella fastidiosa* by the glassy-winged sharpshooter from grapevines infected for various lengths of time. Pp. 43-48 in Esser, T. (ed.) Proceedings, 2008 Pierce's disease research symposium. California Department of Food and Agriculture, Sacramento, CA.

H. Research Relevance Statement

The detailed experiments that have been conducted in this project have tremendous implication in the movement of *X. fastidiosa* by GWSS. We have learned that both male and female sharpshooters prefer young tissue of Cabernet Sauvignon and Chardonnay grapevines, but they will feed on old stem tissue as well. While the percentage of time spent on these tissues is relatively small (7.5%, 11%, 15% in fall, winter, and spring trials, respectively), the older tissue is where *X. fastidiosa* has a higher probability of acquiring bacteria and it is also the tissue into which inoculation leads to chronic infection. We have seen that GWSS move frequently throughout the day, changing to different tissues in 35%, 14%, and 21% of the observations in the fall, winter and spring, respectively. This has serious consequence for moving *X. fastidiosa* around the vineyard at various times of the year. We also have learned that GWSS individuals were not able to feed on cordon tissue at any time of the year. No sharpshooters that were confined to this tissue survived. Aside from cordons, GWSS were able to feed on old and young stems, petioles, and leaves. However, the amount of feeding varied with the season. In the winter and summer, GWSS utilized old stems and young stems, while during the fall they were not able to feed on old stems. At this time of the year, the younger stems became hardened and woody, and survival and feeding on these stems was reduced.

In our most recent studies, we found that GWSS showed a strong preference for grapevine canes from putative infected vines. We are in the process of verifying infection in the cane sections to which they were confined. Should this result prove to be true, it reveals yet another feature of GWSS biology that contributes to its status as a vector of *X. fastidiosa* in grapevines. In addition, understanding the cause of this attraction may enable us to design methods to reduce it.