SEASONAL TRANSMISSION OF *XYLELLA FASTIDIOSA* BY THE GLASSY-WINGED SHARPSHOOTER FROM GRAPEVINES INFECTED FOR VARIOUS LENGTHS OF TIME

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ABSTRACT
This study is part of our larger project aimed at understanding the feeding biology of the glassy-winged sharpshooter (GWSS) as it relates to acquisition and transmission of *Xylella fastidiosa (Xf)*. GWSS feeding biology was studied in three seasons (summer, fall, winter) on mature Cabernet Sauvignon and Chardonnay grapevines using choice and no-choice studies. 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 *Xf* that has been associated with GWSS. In no-choice 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. Future work will evaluate GWSS feeding behavior when confronted with PD-infected grapevines.

INTRODUCTION
Pierce's disease (PD), a disease of grapes caused by the bacteria, *Xylella fastidiosa* (*Xf*) 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. 2005a, 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 *Xf* 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. 2005a, Hill 2006). Understanding details of primary and secondary spread of *Xf* 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. (2005b) demonstrated that GWSS could even transmit *Xf* to dormant vines in the field. 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, Feil et al. 2003, Hill 2006).

OBJECTIVES

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 *Xf* 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 *Xf* for 2, 3, or 4 years and determine the subsequent transmission from these acquisitions.
3. Determine the relationship between *Xf* inoculation by GWSS at different times of the year and the development of the vine as a source for further acquisition by GWSS.

In order to proceed with Objectives 2 and 3, we first must determine where GWSS feed on mature vines and this is the focus of the current report.

RESULTS

Choice Tests for Grapevine Tissue Selection

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. The grapevines were from a mixed field-grown vineyard at the University of California in Riverside (UCR) that was covered with 60% shade-cloth to protect them from PD. We made hourly observations during daylight hours over three consecutive days to determine the location of each GWSS. This experiment was executed twice in the fall of 2007, twice in the winter 2008 and once in summer 2008.

Results of the two fall trials were pooled, as were the results of the two winter trials. In the fall, GWSS were found on the cage in 14% and 16% of our observations on Cabernet Sauvignon and Chardonnay vines, respectively (**Figure 1**). We also found that a high proportion (35%) of GWSS, averaged across variety and gender, switched from one tissue to another each hour (data not shown). Clearly, GWSS moved frequently among the vegetation, important for the spread of bacteria within and among vines. When GWSS were present on the canes, they utilized all tissues with no consistent preference for any type. However, over the course of the trial and averaged across both varieties, GWSS were found more frequently on young tissue (18.2%) than on old tissue (10.7%). Looking further at the data, GWSS were found more frequently on young stems, petioles and leaves (28.5%, 6%, and 20%, respectively) than on old stems, petioles and leaves (7.5%, 7.5%, and 14.5%, respectively). Interestingly, the insects spent the least amount of time on petiole tissue of any age than on any other tissue type. There also were some interesting results with respect to variety. GWSS were found more frequently on leaves (old and young) of Cabernet Sauvignon compared to the leaves of Chardonnay while the reverse was seen for petioles and stems (old and young). These results suggest that the two grapevine varieties vary in the xylem components that are important for GWSS feeding, a result that could impact the location where *Xf* cells are introduced into healthy grapevines. To finish the discussion of this trial, there appeared to be little difference between sexes in their selection of feeding sites (**Figure 1**).

In the winter trial, GWSS were found on the cage walls in 49% of our observations. At this time of year, 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 fall 2007 trials. There was little difference in the tendency of GWSS to change positions among variety or sexes.

The summer trial again 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 fall and winter also occurred in the summer. 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.

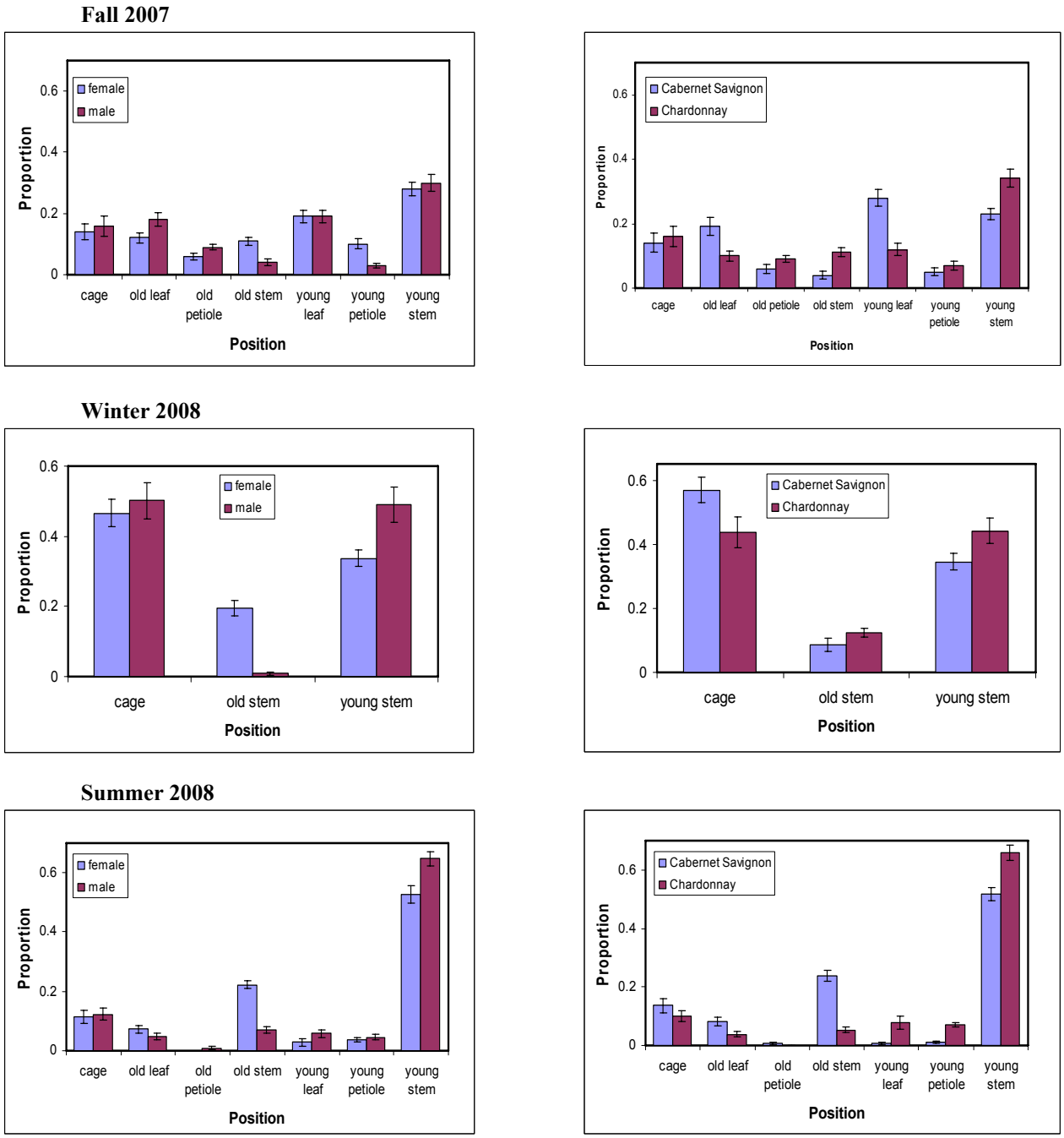


Figure 1. GWSS preference on field-grown Cabernet Sauvignon and Chardonnay grapevines in choice experiments initiated on 29 August and 11 September 2007 (Fall 2007), 16 January and 6 February 2008 (Winter 2008) and 1 July 2008 (Summer 2008). Bars represent average proportions of GWSS (±SE) observed on various tissue types for the two varieties and for the two GWSS genders.

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.

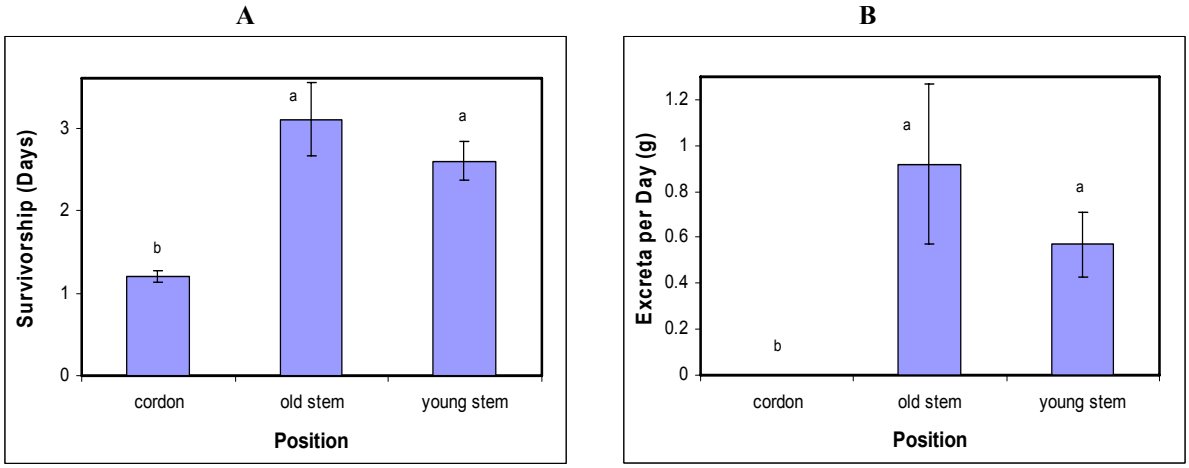
The day before the start of each test, GWSS adults were collected from citrus at Agricultural Operations, UCR, and placed in a cage with a potted rough lemon plant. The following morning, adults were isolated and sexed and then placed individually into the tube cages. Cages were inspected daily and the presence of excreta noted. Cages with dead GWSS were removed, and the amount of excreta was weighed. 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 or among the sexes. Survivorship in the winter trials averaged 2.04 days, and there were no significant differences in survivorship among varietals or 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.

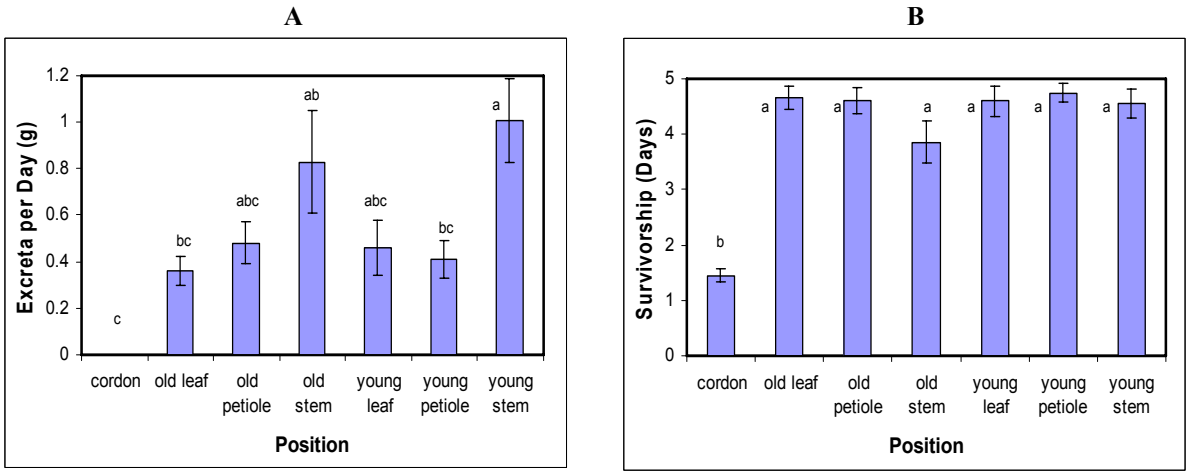
Sharpshooters fed on all tissues except cordons in the summer trial (**Figure 2**), averaging 0.51 g of excreta per day. Feeding on non-cordon tissues was highly variable, but there were some significant differences. Sharpshooters produced significantly more excreta on young stems than on young petioles, old leaves, and cordons. There were no significant differences among varietals, sexes, or tissue age (i.e. old leaves, petioles, and stems vs. 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 the fall trial, GWSS again fed on all tissues except cordons (**Figure 2**), averaging 0.229g of excreta per day (range 0-1.18g). This was less excreta than that produced by sharpshooters feeding in the winter (0.37g) and summer (0.51g) trials. While we are not sure why this reduction in feeding might occur, it may signal a natural decline in feeding as the sharpshooters enter the winter months. There was substantial variation among GWSS feeding in this trial (**Figure 2**). While it appears that GWSS feeding on old stems and young stems were nearly the same as the other non-cordon tissue, the means in this case are misleading. For the old stem, there were only 2 GWSS that survived longer than 1 day and of these 2 only 1 produced any measurable excreta (0.168g). On the young stems, only 5/20 GWSS survived longer than 1 day, and these insects produced an average of 0.24g of excreta per day (range 0.014-0.779g). This is a contrast to the summer trials, during which the insects survived well on the young stems. We noticed that in the fall trial, the young stem tissue had become hardened and woody, and while GWSS were able to feed on this tissue in the summer, they were not able to do so in the fall. It also is interesting that survival on old stem tissue seemed much better in the winter than in the fall. This may be due to the adaptability of GWSS that were field collected for our trials. In the winter months, GWSS may be better adapted for feeding on woody tissue than populations in the fall. Survival was consistently high on the leaves and petioles and production of excreta was consistent with this survival. The tissue yielding the most excreta was the young petiole (0.311g/day), followed by young leaves (0.233g/day), and old leaves (0.208g/day).

Winter 2008



Summer 2008



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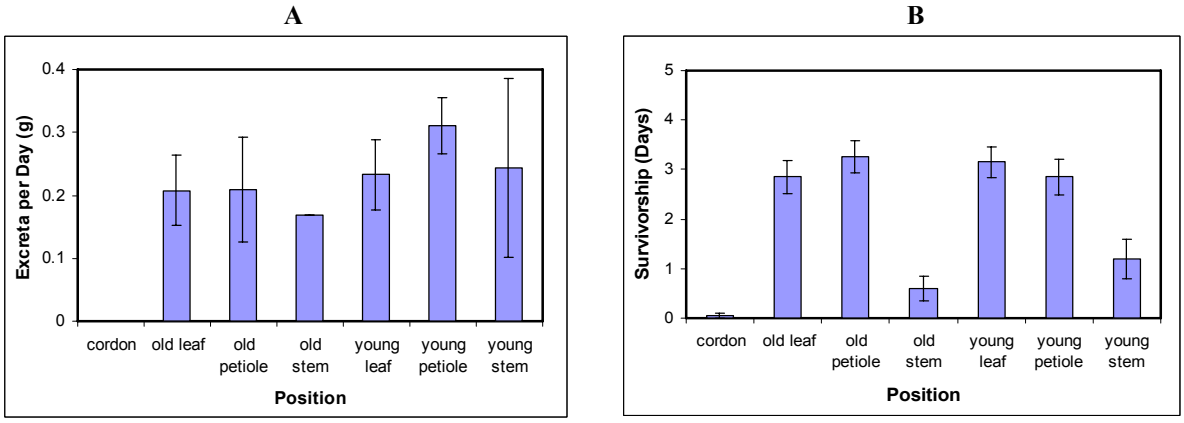

Figure 2. GWSS feeding on field-grown Cabernet Sauvignon and Chardonnay grapevines in no-choice experiments conducted in Winter 2008 (26 February, 4 March), Summer 2008 (15 July), and Fall 2008 (19 September). Bars represent A) average amount (g) of excreta per day (±SE) measured from various tissue 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). At the writing of this report, statistical analyses were not complete on the Fall trial, therefore only means (± SE) are presented.

CONCLUSIONS

Vine to vine spread of *Xf* by glassy-winged sharpshooter (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. GWSS landing and feeding behavior and tissue feeding capacity combine with grapevine phenology, and within-vine *Xf* distribution and phenology to make vine to vine spread possible. Our overall goal is to provide information on these various components to

enhance our understanding of vine to vine spread so that strategies can be defined to reduce widespread epidemics in other regions. We have conducted experiments in the fall, winter and summer in which we made hourly observations on the location of individual GWSS adults given access to mature tissue and young tissue on the same cane. Both males and females preferred young tissues (particularly the stems) to mature tissues on Cabernet Sauvignon and Chardonnay grapevines throughout the year. However, GWSS spent a substantial amount of time feeding on old stem tissue (7.5%, 11%, 15% in fall, winter, and spring trials, respectively), where *Xf* could potentially be transmitted leading to chronic infection. A significant finding is that GWSS moved frequently throughout the days of our studies, changing position in 35%, 14%, and 21% of the observations in the fall, winter and spring, respectively. This has serious consequence for moving *Xf* around the vineyard at various times of the year. Further characterization of GWSS feeding behavior was conducted in no-choice studies. We learned that at no time of the year, were individuals able to feed on the cordon tissue. While others have reported observing GWSS feed in this tissue, we were not able to demonstrate it in our trials on mature vines. 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. In addition, the young stems became hardened and woody, and survival and feeding on the young stems at this time of the year were reduced. Our goal is to integrate the information from the work reported here with planned studies on infected grapevines at different times of the year. Through this work, we will understand the interaction between feeding behavior on specific grapevine tissues that contribute to the spread of *Xf* from infected to healthy vines. With this knowledge, we can direct management strategies to mitigate vine to vine spread.

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