MONITORING THE SEASONAL INCIDENCE OF *XYLELLA FASTIDIOSA* IN GLASSY-WINGED SHARPSHOOTER POPULATIONS

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ABSTRACT
The incidence of *Xylella fastidiosa (Xf)* in GWSS populations was monitored between August 2004 and February 2005 using various analytical techniques as indirect measures and transmission to grapevines as a direct measure of GWSS inoculativity. Field collections of GWSS made between August 2004 and February 2005 showed an increasing proportion of the population positive for *Xf*. The mean titer of *Xf* in the field samples also increased through the fall months, but then diminished from peak levels during 3 collections made in the winter months of December and February. Differences among collection locations were observed in the proportion of the respective populations positive for *Xf*. Results from a transmission experiment conducted 6 February 2005 demonstrated that field-collected adults not only test positive for *Xf* by ELISA, but also transmit *Xf* to grapevine test plants (var. Chardonnay). An initial evaluation of xylem fluid collected from multiple branches per test plant revealed 11 plants out of 15 exposed to individual field-collected STSS adults and 5 out of 8 plants exposed to GWSS adults became infected with *Xf*. Analysis of the STSS and GWSS adults used in the 6 February 2005 transmission tests yielded absorbance readings in the lower positive range similar to levels observed in the *Xf* monitoring of the general population study. Further tests of the titers of *Xf* in these test insects and future test insects will be conducted once a real-time PCR test has been successfully developed.

INTRODUCTION
Information on the prevalence of GWSS adults positive for *Xf* and the rate they transmit to grapevines is among the most fundamental pieces of knowledge required to improve understanding of *Xf* epidemiology. The paucity of information regarding the degree of *Xf* incidence in GWSS populations is partly responsible for rampant speculation about the future of the GWSS/PD crisis in California. Certain fears have been expressed that even low densities of GWSS in a region could have a significant impact on the incidence of PD or other diseases caused by *Xf*. While adoption of worst case scenarios may be considered prudent and defensive, it can also lead to important policy decisions that, in the absence of accurate information, result in unnecessary and expensive actions. A compilation of data from many sources has contributed to a decent understanding of the distribution of GWSS populations within California and the relative intensities of regional infestations. Additional information on the proportions of individuals within these populations that are inoculative with *Xf* will help to complete a more realistic picture of the risks of *Xf* spread associated with various levels of GWSS infestation.

Although GWSS nymphs are capable of transmitting *Xf* (Almeida and Purcell, 2003), it is the transmission of the bacterium by adults that is of greatest concern in the epidemiology of *Xf*. GWSS adults are flight mobile and capable of moving long distances across the landscape, and therefore represent a potential threat of primary spread of *Xf* from an external host plant into an uninfected vineyard. To estimate the rate that such events may be occurring requires large numbers of GWSS adults to be collected in the field and tested to determine the proportion that transmit *Xf*. Estimates of the rate that field-collected GWSS adults transmit *Xf* can be made by both direct and indirect methods. Direct methods involve the classical approach of confining one or more live insects onto an uninfected test plant, holding them for a period of time on the plant before removing, then retaining the plant for a sufficient period of time to allow disease development. When carried out well, this approach provides the most accurate determination of the natural rate that GWSS adults transmit *Xf* to uninfected host plants. In contrast, indirect methods are capable of detecting the presence of *Xf* in a vector, but do not necessarily represent a measure of the rate of transmission to a plant. Analytical tests such as ELISA and PCR are being used to detect and quantify the titer of *Xf* in GWSS adults. In addition, *Xf* culturing media is being used to assess whether the bacterium was present in a test insect.

Current understanding of the mechanisms of acquisition and inoculation of *Xf* by GWSS adults, either in the controlled conditions of the laboratory and greenhouse, or in the more challenging setting of their natural habitat, are in reality quite limited While the laboratory approach can provide essential answers to questions regarding the rate of acquisition and efficiency of transmission, it ultimately reflects the conditions imposed by the researcher. For example, the type and age of the acquisition source plant, the isolate of *Xf* used and period of time that the acquisition source plant has been infected, as well as the source of the experimental GWSS individuals and the conditions under which they are provided access to the *Xf* source plant are all variables controlled by the researcher. A dual approach that balances the findings from the laboratory with monitoring information from the field will improve our understanding of how epidemics of *Xf* occur in vineyards and elsewhere. A compilation of data from many sources has contributed to a good understanding of the distribution of GWSS populations within California and the relative intensities of regional infestations. By evaluating the proportion of individuals within these populations infected with *Xf*, a critical deficiency in our understanding of *Xf* epidemiology will be addressed.

OBJECTIVES

1) Monitor GWSS adults from citrus and other sources year-round to determine the proportion positive for *Xf* using ELISA, PCR, and media culturing techniques.
2) Perform transmission experiments on a portion of the field-collected adults using grapevine seedlings to determine the seasonal transmission rate.
3) Quantify the titer of *Xf* in GWSS adults that transmitted *Xf* to grape seedlings using quantitative ELISA and RT-PCR, and determine the relationship between transmission rate and titer in the vector.

RESULTS

Collections of live GWSS adults began in August 2004 and were made in Riverside and Redlands at bimonthly or monthly intervals until densities dropped in February to levels too low to sample. Numbers of GWSS adults were particularly low through the late winter and early spring period of 2005 and collection attempts were hampered by wet weather. Sampling resumed in July 2005 as the spring generation of adults emerged to repopulate citrus and the surrounding landscape, although not nearly at levels seen in previous years. The discrete nature of GWSS generations, i.e. the nearly synchronous emergence of adults beginning in mid-June and continuing through mid-July, results in what is effectively a single generation per year that emerges and then ages through time until the following spring when a relatively few remaining adults from the previous summer give rise to the next generation. The second, or summer generation of GWSS in essence fails to materialize due to heavy parasitism and other mortality factors. The contribution of the second generation to the total population appears to be rather small based on data collected in field 5 of Ag Ops in Riverside during 2001-02. Hence, the present evaluations of the incidence of *Xf* in GWSS adults have assumed that systematic samplings of GWSS adults from the time of their beginning emergence in mid-June represents an aging population of adults with only limited perturbation of the age structure due to a subsequent emergence of the summer generation of adults.

Results from the 2004-05 (Figure 1) season support previous data from 2002-03 (Naranjo et al., 2003) concerning the incidence of *Xf* in populations of GWSS adults. While both data sets indicated that the proportion of the adult population positive for *Xf* increased through time, a clear trend of increasing mean titers of *Xf* in GWSS adults was apparent during the 2002-03 season only. In 2004, the mean titers of *Xf* also increased from the time of the first collections in late summer through early November. Subsequent collections in December and February 2005, however, yielded lower mean titers compared to the fall 2004 samples (Figure 1b). While a decline in mean titers may represent no more than a sampling phenomenon, it could also represent an environmental interaction whereby growth of *Xf* within foreguts of GWSS adults is reduced relative to other times of the year. Hence, acquisition of *Xf* by individual GWSS adults may not necessarily result in colonization of the foregut and progressive growth thereafter, but instead may produce both increases and decreases in *Xf*

colony growth depending on nutritional or perhaps temperature conditions. Colder temperatures may affect *Xf* growth within foreguts of GWSS adults through reduced feeding by the insects or by altering the nutritional quality of xylem fluid that

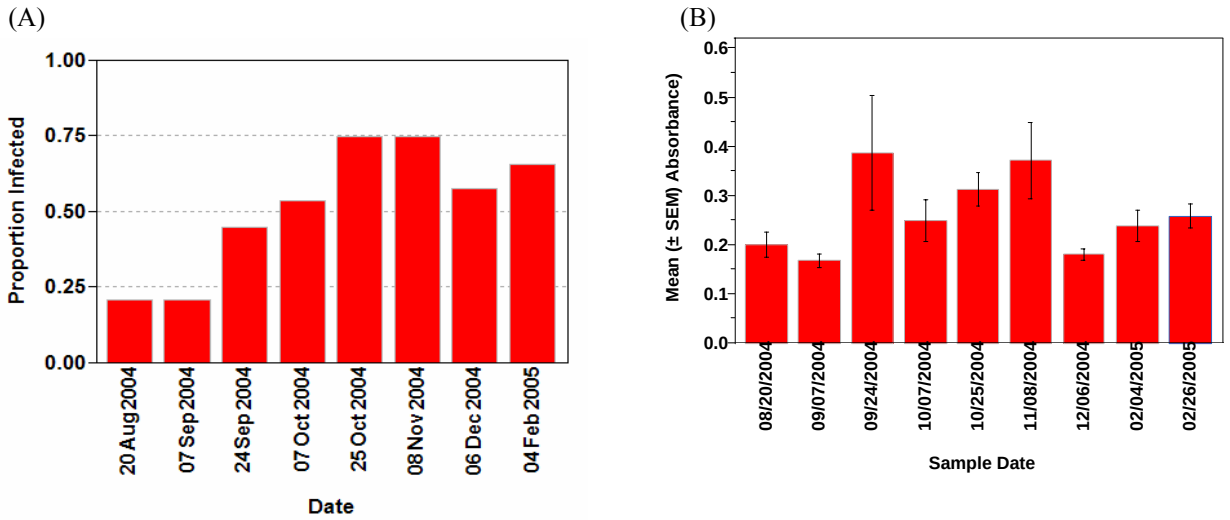

Figure 1. Incidence of *Xf* in GWSS adults collected from citrus orchards at UC Riverside's Ag Ops from August 2004 through February 2005. The proportion of GWSS positive for *Xf* (A) is based on ELISA absorbance values for field collected individuals in excess of the mean absorbance + 4 standard deviations of non-infected control GWSS adults. The mean titer of *Xf* (B) is based on the mean absorbance of individual GWSS adults collected each date using an *Xf*-specific ELISA test (n=18 for each date).

nourishes both GWSS and *Xf* colonies that may be present in their foreguts. Alternatively, because GWSS insects are ectodermic, colder ambient temperatures and colder xylem fluid ingested by the GWSS adult hosts of *Xf* could lead to

reduced colony growth and a temporal pattern as observed in Figure 1a. Closer attention will be paid to environmental conditions in 2005 as they may relate to changing titers of *Xf* within GWSS adults.

Figure 2. Vegetatively propagated grapevines (var. Chardonnay) grown in perlite within a misted propagation chamber.

Transmission tests are currently in progress using field-collected GWSS and STSS adults that are given 3 day inoculation access periods to grapevine test plants. A propagation chamber is being used to grow experimental grapevines to serve as test plants in the transmission studies. Lateral branch shoots consisting of 4-5 leaves are being cut from certified disease-free parental grapevines (var. Chardonnay) and placed in propagation media until roots are generated (Figure 2). These are transplanted to 8" pots (Figure 3) and allowed a minimum of 3-4 weeks to establish before being used in transmission experiments. Ventilated corsage cages are then used to enclose each grapevine plant and provide full access to the entire plants by GWSS adults (Figure 4). Following the 3 day IAP, tests insects are collected from each plant and frozen (-80°C) for subsequent PCR and ELISA analysis. Grapevine test plants are held for 4 months to allow disease development. Xylem fluid collected from each plant for PCR and ELISA analyses is then used as an independent and sensitive evaluation to compare with the visual assessments. An essential component of this approach is the availability of clean GWSS (in rearing) to serve as ELISA and PCR controls. Experimental and analytical results will be collated to determine which analytical procedure provides the closest agreement with transmission test results and help provide essential perspective.

Figure 3. Established grapevines vegetatively propagated from certified disease free Chardonnay grapevines that have been used in transmission experiments with field collected GWSS adults.

Figure 4. Ventilated corsage cages use to enclose field-collected sharpshooter adults on test grapevines.

To date, a single transmission experiment using field-collected GWSS adults has been performed and analyzed for the presence of *Xf* in the test grapevines as well as the test insects. A major impediment to performing more transmission tests was the absence of GWSS adults in the field since early February 2005. In the one transmission experiment that has been completed, only 9 GWSS adults were collected, with a balance of 15 smoke-tree sharpshooters being used to complete the test. These insects were collected in the field and placed on the test grapevines on 6 February 2005. Additional attempts to collect GWSS adults through late winter and spring were defeated by the absence of GWSS adults. The test grapevines exposed to STSS and GWSS adults on 6 February were held in an insect free greenhouse for 3 months before taking samples to test for the presence of *Xf*. Up to 5 branches from each plant were sampled for xylem fluid using a pressure bomb. ELISA results for these samples indicate variability in absorbance readings among different branches even within a single plant, not to mention differences among plants in terms of being positive or negative for *Xf*. A higher number of positive readings occurred from branches collected from grapevines exposed to STSS adults than to GWSS adults (Figure 5).

Analysis of the STSS and GWSS adults used in the transmission test conducted 6 February 2005 revealed that more than 50% of both STSS and GWSS adults represented a statistical positive based on ELISA absorbance readings using clean GWSS insects as controls (Figure 6). Absorbance readings for each insect were not as high in the positive range as for some

insects collected in the field during fall 2004, but this is in accord with the earlier observation of reduced titers of *Xf* in adults collected during the winter compared to fall collected insects. Samples from these insects have been preserved for subsequent analysis by real-time PCR.

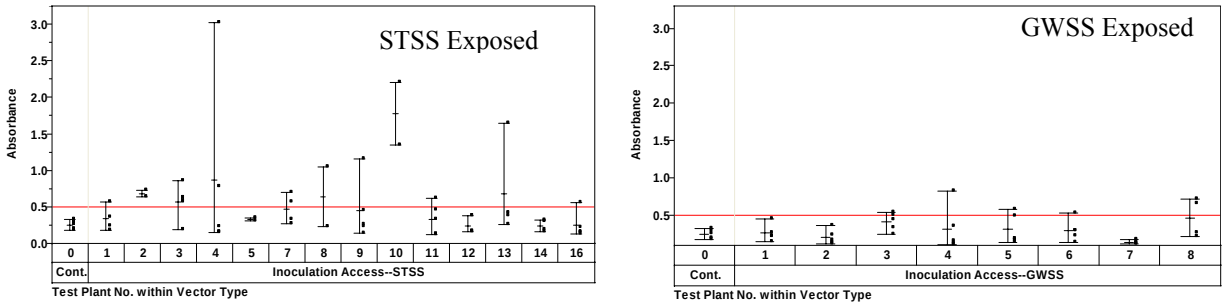

Figure 5. ELISA test results for the presence of *Xf* in xylem fluid collected from individual branches on grapevines exposed to either a single field-collected STSS adult or GWSS adult. Each point along the vertical range lines represent the absorbance$_{490}$ reading for a single branch with the mean absorbance for each plant represent by the horizontal dash at or near the midpoint of each vertical range line. Up to 5 branches were sampled from each plant, but some plants had only 2 branches (e.g. plant no. 8 in the STSS-exposed chart). The horizontal red line represents a reference line at twice the absorbance of the uninfected control grapevine xylem samples; any points above this line constitute a positive result for *Xf*.

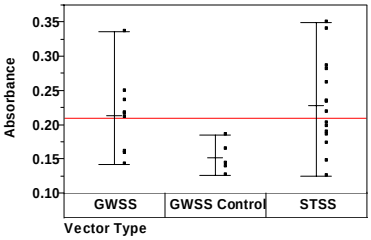

Figure 6. ELISA results for test insects used in the transmission test of 6 February 2005. Each point represents the absorbance for an individual insect with the horizontal dash representing the mean for each species. Points above the horizontal red line indicate statistical positives.

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