Final Research Report, May 2002

Project # 01-AP-00

Project Title: Transmission of Xylella fastidiosa to almonds by the glassy-winged sharpshooter

Project Leader: Alexander H. Purcell

## **Objectives:**

- 1. Determine the efficiencies of acquisition and inoculation of *X. fastidiosa* by the GWSS to almonds.
- 2. Quantify populations of X. fastidiosa in infected almonds in the field throughout a season.
- 3. Determine the ability of the GWSS to inoculate *X. fastidiosa* to mature (> 1 year) and dormant woody tissues of almond.

The glassy-winged sharpshooter (GWSS) is a newly introduced vector of *Xylella fastidiosa*, the causal agent of almond leaf scorch (ALS) and Pierce's disease (PD) of grapes. GWSS poses a threat to the almond and grape industries, due to its large populations in citrus groves and irrigated ornamental landscapes, its rapid dissemination and wide host range. GWSS has already been found in Southern San Joaquin Valley, and will probably continue to move north in the coming years. ALS was also detected south of Bakersfield during the last season; an area without previous problems with ALS, and its spread was probably due to the presence of GWSS. This proposal intends to determine the efficiency of transmission of *X. fastidiosa* to almond by GWSS. We will also measure natural populations of the bacterium within almonds, since this is probably an important factor in transmission. The results of this research will have direct applicability in the future management of ALS in the Central Valley given the presence of GWSS.

We studied the effect of time on *X. fastidiosa* inoculation by infective GWSS, by caging insects on source (infected) almond plants for an 'acquisition access period' (AAP) of 4 days, then transferring groups of 4 insects to healthy almonds (Peerless) for 1, 2 and 4 days of 'inoculation access period' (IAP). The infected status of the almond sources was evaluated by culturing (dilution plating) on PWG medium. Insects survived poorly on source plants, but well on healthy ones. We tested 51 groups of 4 GWSS per IAP, the transmission results are shown in Figure 1.

GWSS groups transmitted X. *fastidiosa* with 1 day of IAP, and transmission rates increased up to 50% after 4 days. If the transmission rates are calculated for 1 individual for 1 day of plant access time, the efficiency is approximately 4.5%.

When we studied the effect of time on *X. fastidiosa* acquisition efficiency by GWSS, individuals were used instead of groups. A total of 31 insects were tested per acquisition period (1, 2 and 4 days). After AAP, adults were transferred to healthy seedlings for 4 days. Three, 2 and 1 plants became infected for 1, 2 and 4 days of AAP respectively. These results suggest that acquisition efficiency from almonds is low, around 5% per individual per day or lower.



Figure 1. *Homalodisca coagulata* transmission of *Xylella fastidiosa* to almonds after 4 days of acquisition access period on source plant, followed by various periods of inoculation access period on healthy almonds. Open squares represent inoculation rates by groups of 4 insects caged per plant; dashed line is the regression for those data points (y = 3.922 + 11.765 x, r<sup>2</sup>=1, P<0.01). Solid circles represent the calculated transmission rates per individual per day (Purcell 1981); solid line is the regression for those points.

In our research with *X. fastidiosa* transmission with grapevines, we found that bacterial populations within plants are correlated with vector acquisition efficiency (Hill and Purcell 1995). Because GWSS acquisition rates on almonds are lower than those we observed on grapes, we tested the bacterial population hypotheses. We collected almond leaves from plants with almond leaf scorch symptoms in an orchard at Davis various times last year (2001). Bacterial populations were found to be between log 4 to log 7 colony forming units (CFU) / gram of tissue. These populations are 100-1000 fold lower than on grapes with Pierce's disease, suggesting that this may be the cause for low vector acquisition efficiency on almonds. To further test this hypotheses, this year we are collecting samples once again at the Davis orchard; but will also collected samples from various varieties from different locations in the Central Valley, to check if the results are similar for different varieties and environmental conditions.

GWSS has been found to feed on woody tissues of almonds in Kern County. To test if GWSS can infect plants after feeding on tissues >1-year old, we groups of insects on wood or young shoots of Peerless plants. The results are presented on the table below (Table 1). GWSS transmits *X. fastidiosa* to both shoots and 1-year old wood (no statistical difference). Insects also survived well on both tissues.

Table 1. Transmission of *Xylella fastidiosa* to 1-year-old wood and shoots of almonds. Groups of 4 *Homalodisca coagulata* adults were used per plant. Insects had 4 d of acquisition on grapevine source of *X. fastidiosa* and 4 d of inoculation access period on susceptible almonds ('Peerless').

	Tissue inoculated	
	Shoot	1-year old wood
# plants	30	30
# infected plants	16 <sup>a</sup>	11 <sup>a</sup>
Average H. coagulata / plant	3.87	3.87
Transmission rate/group (%)	53	37
Individual transmission rate per day (%) <sup>b</sup>	4.9	3.0

<sup>a</sup> Values did not differ by Chi-square test at 95% level of significance.
<sup>b</sup> Calculated according to Purcell (1981).

Because GWSS also feeds on dormant plants, we performed a preliminary test under laboratory conditions to see if dormant plants can be inoculated with *X. fastidiosa*. We know that the insects survived well on the dormant plants, but the results of these experiments are still pending the incubation period of the disease in our greenhouse.