Transmission of *Xylella fastidiosa* to almonds by the glassy-winged sharpshooter

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Almond leaf scorch (ALS) is caused by the xylem-limited bacterium *Xylella fastidiosa*. This pathogen also causes Pierce's disease of grapevines (PD). ALS has generally had a low incidence and economic importance in California, with the exception of an outbreak in the late 1970s and a few orchards with persistent problems. The invasion by the glassy-winged sharpshooter (GWSS, *Homalodisca coagulata*) into California during the 1990s increased interest in ALS because this insect is a vector of *X. fastidiosa* to almonds and has been associated with epidemics of PD in Orange and Kern Co. A fundamental difference between previous PD epidemics and epidemics associated with GWSS is that disease spread appears to be very rapid (exponential). Furthermore, the spatial patterns of PD spread by GWSS appear to be less influenced by the proximity of vector source areas such as citrus groves. Both of these observations suggest that the vine to vine spread of *Xylella fastidiosa* by vectors is important with GWSS. This has not been the case for traditional PD vectors in California. The feeding ability of GWSS on woody hosts such as trees are two major differences from traditional vectors in California. The first is the tendency of GWSS to feed on woody branches and canes. Nymphs and young adult GWSS prefer young green shoots, but many adult GWSS have been observed feeding on woody tissues. The second different feeding trait is that GWSS will feed on a variety of dormant vines and trees during winter months. This requires feeding on woody tissues, as these are the only tissues available on dormant trees. We studied various aspects of ALS ecology, especially evaluating prospects for GWSS as a future major vector for ALS.

TRANSMISSION OF *X. fastidiosa* TO ALMONDS BY GWSS

Experiments to determine the efficiency GWSS with which acquired *X. fastidiosa* from infected almonds were done by confining non-infected individuals on almonds for 1, 2 and 4 days of acquisition access period (AAP). After this period we transferred the insects to healthy almond seedlings for 4 days of inoculation access period (IAP). Three, 2 and 1 out of 30 plants per period became infected for 1, 2 and 4 days AAP respectively. These results show that acquisition of *X. fastidiosa* by GWSS was much efficient from almond compared to grapevines, with rates below 10%.

To study GWSS inoculation efficiency to almonds, we confined insects for 4 d on infected plants, and transferred groups of 4 individuals to healthy seedlings for 1, 2 and 4 d. Figure 1 shows our results. Although transmission rates per group increased over time (solid line), the estimated transmission rate (Purcell 1981) per individual per day (dashed line) remained constant, at about 4%. These results are consistent with the low acquisition rates and suggest that GWSS transmission of *X. fastidiosa* from almonds to almonds has low efficiency.

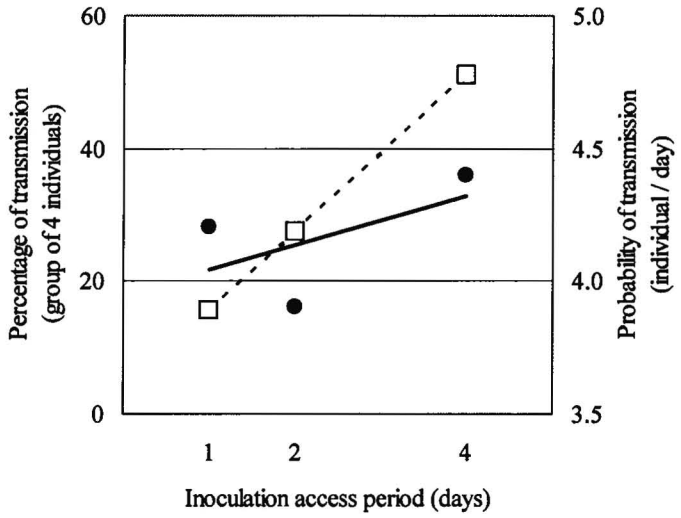

Figure 1. GWSS transmission of *X. fastidiosa* to almonds after 4 d of acquisition access period on source plant, followed by various periods of inoculation access period on healthy almonds. Open squares represent inoculation rates by groups of 4 insects caged per plant; dashed line is the regression for those data points (transmission probability y = 3.922 + 11.765 access time x, $r^2 = 1$, $P < 0.001$). Solid circles represent the same data converted to estimates of transmission rates per individual per day (Purcell 1981); solid line is the regression ($P > 0.05$) for those points.

Because GWSS is often found feeding on woody tissue, we tested the possibility of this vector transmitting *X. fastidiosa* to 1-year old almond wood. All groups that transmitted the bacterium to wood also transmitted to young shoots; 5 groups inoculated only shoots. Sixteen of 30 groups transmitted to shoots, not significantly different from the 11 groups that also transmitted to 1-year old wood (Chi-square test with Yates correction factor for continuity, $P > 0.05$, 1 df).There were no differences in survival rates, an average of 3.87 of 4 insects survived the 4 d IAP on both tissues.

In previous work, we observed that GWSS transmitted *X. fastidiosa* to dormant grapevines in the field and laboratory, and decided to test if the same can occur with almonds under controlled greenhouse conditions. We defined dormant almonds as plants that were taken outside the greenhouse late in the fall, lost leaves naturally, and finally moved to a 4°C refrigerated chamber. One day before GWSS had access to these plants, we brought them to the greenhouse. We found that after 1 day in the greenhouse GWSS survived well on our plants, but observed no positive root pressure (bleeding after puncture) on plants. Groups of 4 GWSS were caged per plant for 4 days, this IAP occurred within a 17°C growth chamber. Table 1 summarizes our results. GWSS survived well on these plants, and 30% of plants inoculated by infective groups (pre-tested on green vines) became infected with *X. fastidiosa*. We found that buds started to break about 5 days after moving plants from the cold box to the greenhouse

Table 1. Summary of GWSS transmission experiments to dormant almonds

No. experiment repetitions	3
No. grape pre-test plants	15
No. infected grape pre-test plants	9
No. dormant plants inoculated by infective groups	27
No. dormant plants inoculated by infective groups infected	9
Total no. *H. coagulata* starting experiment	60
H. coagulata survival rate on pre-test plants (%)	93.33
H. coagulata survival on dormant plants (%)	99.40

Finally, because of the concerns regarding movement of *X. fastidiosa* from vineyards to almond orchards by GWSS, we did an experiment to determine if one *X. fastidiosa* grape isolate from Napa Valley and another from Bakersfield can be acquired from grapes and transmitted to almonds. We found that GWSS acquired both isolates from grapes, and successfully transmitted them to grapes and almonds in successive IAPs.

X. fastidiosa POPULATIONS WITHIN ALMONDS

Populations of *X. fastidiosa* within grapevines and other plants have been correlated with vector transmission efficiency (Hill & Purcell 1995). Higher cell counts resulted in higher transmission efficiency. Because we found low transmission rates with GWSS and ALS, we hypothesized that bacterial populations in almond might be low, and this could be a reason for the low transmission rates observed. We quantified *X. fastidiosa* populations by a method described by Hill and Purcell (1995). Basically, a leaf petiole is surface sterilized, ground, and dilutions of the suspension plated on solid medium to estimate the number of *X. fastidiosa* colony forming units (CFU) per gram of tissue. We sampled petioles from symptomatic leaves from naturally infected areas in Stanislaus and Kern Co. Table 2 shows our results. These populations are approximately 100-fold lower than those found in grapevines with PD. All leaves with symptoms were positive for *X. fastidiosa*.

TABLE 2. Populations of *Xylella fastidiosa* within petioles of plants with almond leaf scorch symptomatic leaves from various locations in California.

Location	Collection date	n[a]	Mode[b]	Range
Modesto (Stanislaus Co.)	July 2001	28	$1.2x10^6$	$8.3x10^3$–$6.2x10^7$
Modesto (Stanislaus Co.)	August 2002	24	$1.3x10^7$	$5.0x10^5$–$4.0x10^7$
Ceres (Stanislaus Co.)	August 2002	24	$2.0x10^7$	$3.2x10^6$–$5.1x10^7$
Denair (Stanislaus Co.)	August 2002	24	$3.5x10^6$	$1.3x10^5$–$1.3x10^7$
Waterford (Stanislaus Co.)	August 2002	24	$2.7x10^7$	$5.9x10^6$–$9.5x10^7$
Bakersfield (Kern Co.)	September 2002	10	$2.6x10^7$	$1.2x10^7$–$6.3x10^7$

[a] Number of samples tested.
[b] CFU/g of tissue.

We did a similar experiment in an orchard at UC Davis (Armstrong orchard). In that area samples were collected during three months in 2001 and 2002, so that bacterial populations

could be surveyed during complete seasons (Table 3). Symptoms at Davis appeared in mid-June, as previously described (Moller et al. 1974).

TABLE 3. Populations of *Xylella fastidiosa* within petioles of plants with almond leaf scorch symptomatic leaves from Davis.

	April		June		September	
	2001	2002	2001	2002	2001	2002
No. samples tested	18	25	21	25	24	17
No. positive samples	2	3	4	18	4	17
Mode[a]	$3.5x10^5$	$2.1x10^4$	$2.5x10^5$	$1.5x10^6$	$1.2x10^6$	$1.5x10^7$

[a] CFU/g of tissue.

DISCUSSION

Populations of viable (cultivable) *X. fastidiosa* in almond were consistently lower than from comparable tissues in grape with PD. This might explain in part the lower acquisition rates of *X. fastidiosa* from almond by GWSS compared to grape (unpublished data). Our experiments demonstrated that GWSS can transmit both almond and grape strains of the bacterium. The lower or less efficient transmission rates not mean that GWSS will not be an important vector for ALS in California. Its flight ability and tendency to move often among plants and to switch host plants (sometimes several times per day), and its ability to survive on dormant trees during winter and feed on woody tissues are features we have not seen previously in vectors of *X. fastidiosa* in California. These could greatly expand the time during the year during which vectors can infect almonds. Infection of trees in larger branches may increase the over winter survival of *X. fastidiosa* in almond and increase the difficulty of eliminating new infections by pruning.

Further work is needed to field test the acquisition of *X. fastidiosa* by GWSS from dormant almonds and the ability of GWSS to inoculate *X. fastidiosa* into almond during winter and early spring (February), when GWSS have been most frequently observed in Central Valley almond orchards. Such tests in grape in 2002 showed that GWSS transmitted *X. fastidiosa* to grape during February in Kern County.

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