IMPORTANCE OF GROUND VEGETATION IN THE DISPERSAL AND OVERWINTERING OF *XYLELLA FASTIDIOSA*

Project Leaders:
Kent M. Daane
Division of Insect Biology, Dept. of Environmental Science, Policy, & Management
University of California
Berkeley, CA 94720

Alexander Purcell
Division of Insect Biology, Dept. of Environmental Science, Policy, & Management
University of California
Berkeley, CA 94720

Cooperators:
Glenn Yokota
Division of Insect Biology
University of California
Berkeley, CA 94720

Elaine Shapland
Division of Insect Biology
University of California
Berkeley, CA 94720

Christina Wistrom
Division of Insect Biology
University of California
Berkeley, CA 94720

Tarcisio Ruiz
Division of Insect Biology
University of California
Berkeley, CA 94720

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ABSTRACT
The purpose of this project is to determine the ability of alternate host plants, specifically "weeds," in almonds and vineyards to serve as reservoirs for *Xylella fastidiosa* (*Xf*) and for new inoculations by the glassy-winged sharpshooter (GWSS). We collected and analyzed weed and GWSS samples in and around commercial vineyard and almond fields for the presence of *Xf* on a monthly basis. *Xf* has been recovered from weeds collected during February and March, while no collected weeds tested positive for the presence of *Xf* between April and September. Monthly ground cover sampling will continue through the winter, as this time period may prove most important in the persistence of *Xf* over consecutive growing seasons. GWSS collected from alternate host plants have also been processed for *Xf* and have shown that adults collected on many species harbor *Xf* in their mouthparts. Results from these experiments will help to identify what time of year and what ground cover species are of most concern to growers wanting to control the spread of PD with minimal environmental impact.

INTRODUCTION
The economic viability of California's vineyards and almonds has received considerable attention of late because of the expanding range of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* , which can vector the xylem-limited bacterial pathogen, *Xylella fastidiosa* (*Xf*) (Goodwin & Purcell 1992, Redak et al. 2004). *Xf* is the causal agent of Pierce's disease (PD) and almond leaf scorch (ALS) as well as other plant diseases. The arrival of GWSS has dramatically changed the epidemiology of *Xf* and its associated diseases in California (Redak et al. 2004). GWSS may not be an "efficient" vector of PD (Almeida & Purcell 2003a,b; Purcell & Saunders 1999a,b), but it presents a more serious threat, in part, because of its wide host range (Redak et al. 2004) and dispersal abilities (Blua et al. 2003). Of importance here is that the wide host range of *Xf* commonly overlaps with plant species visited by GWSS. Our proposed research will focus on the common host range of both vectors and pathogen, with an emphasis on potential annual weeds that may provide an overwintering reservoir for *Xf* and a spring feeding site for vectors of PD and ALS.

How can this work impact control decisions? An excellent example of an overlooked insect-pathogen-host triangle is stinging nettle (*Urtica urens*), a common weed throughout the Central Valley. In our 2003 survey, we found that stinging nettle was a common host for GWSS in springtime, and recent DNA extraction showed the presence *Xf* in 60% of stinging nettle collected near a Kern County PD-infected vineyard. Whether or not *Xf* titer is high enough in these weeds for GWSS acquisition and transmission is not known, and is one aspect of the proposed study. Regardless, management of common hosts may be a critical component of epidemiology and area wide management of PD and ALS (Redak et al. 2004). With over 145 natural or experimental host plants for *Xf* that can cause PD, the insect/pathogen relationship is far too diverse a subject for one study. For this reason, we are studying the common landscape and ground vegetation found near vineyards and almonds in the San Joaquin Valley.

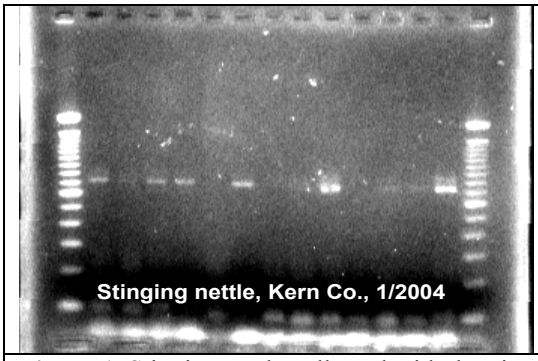

Figure 1. Stinging nettle collected with the vine rows of a PD-infected vineyard showed 9 of 12 samples positive for *Xf*.

OBJECTIVE

1. Determine the presence of *Xylella fastidiosa* in alternate host plants that are commonly visited by glassy-winged and native sharpshooters in selected ecosystems in the San Joaquin Valley; with samples representing different seasons and annual or perennial hosts.

RESULTS

Insect and Plant Samples

GWSS and native sharpshooter (Feil and Purcell 2001) visitation on common non-agricultural crops were monitored to determine the importance of the seasonal period as a component of PD epidemiology. Newly molted adult vectors need to acquire *Xf* from overwintering reservoirs in order to spread PD. GWSS displays seasonal preference for different plant hosts (Daane et al. 2003, 2004), which are often related to host plant phenology or condition (Anderson et al. 1992). We have observed that in winter and early spring, GWSS preferentially feed on perennial weeds such as stinging nettle, filaree (*Erodium* sp.) and common groundsel (*Senecio vulgaris*) in or near vineyards.

GWSS were collected in May, June, July and August from urban ornamental plants that may serve as a host for transferring *Xf* from cities to agricultural land. Insects analyzed for the presence of *Xf* in their mouthparts with the DNeasy Tissue Kit from Qiagen (Bextine 2004). Adult GWSS collected from oleander, xylosma, Chinese elm and riparian zone plants tested positive for *Xf*, while insects collected from crape myrtle tested negative for *Xf*. Nymphal GWSS testing positive for *Xf* were found only on oleander during the month of June. Nymhal GWSS testing positive for *Xf* indicate from which plant the insects are acquiring the bacteria, but will not pose a threat for long since with each successive molt, the insects loose their ability to transmit *Xf*. Adult GWSS testing positive are more of a concern, as an adult GWSS can move between many plants during its lifetime, feeding and spreading *Xf*.

Presence of Pathogen

Non-agricultural plants commonly visited by sharpshooters were screened for the presence of *Xf*. While lists of *Xf* and sharpshooter host plants are available, there are some basic questions that have not been addressed for the San Joaquin Valley: How common is *Xf* in non-agricultural plants? How often do GWSS feed on *Xf* hosts?
Vineyards with heavy infestations of PD were sampled for ground vegetation weeds in and around the crops once a month from January through September. Collections focused on the most abundant variety of weeds, and three samples were taken from each weed species on each date. Samples were processed with either the selective media scheme of PWG and PD3, or with immunocapture DNA extraction and subjected to PCR with universal primers RST-31 and RST-33 (Minsavage 1994). Some weeds collected in January and February were found to contain *Xf*, but after early March, *Xf* was not detected in any weeds collected (Table 1).

Pathogen Population Levels

For GWSS to acquire and transmit *Xf*, the titer of *Xf* within plants typically should be equal to or greater that $\log 10^{4}$ (CFU per g), the threshold population required for acquisition for most sharpshooters (Almeida & Purcell 2003a,b). For chronic PD and ALS to develop, *Xf* infections must survive the winter, which can vary depending on temperature and the degree of plant dormancy (Almeida & Purcell 2003c, Feil & Purcell 2001) and the plant species.

Table 1. Winter/spring weed samples tested for the presence of *Xylella fastidiosa*.

Date	Abundant Weeds	*Xf*
4 February 2004	stinging nettle	+
11 February 2004	stinging nettle	+
3 March 2004	chickweed	+
	bluegrass	+
	shepherd's purse	+
	filaree	-
	alfalfa	-
10 March 2004	tall grass	-
	bluegrass	-

Preliminary analysis of ground cover weeds was conducted using selective media PWG and PD3. However, due to the large amounts of naturally occurring bacteria in wild weeds, all samples were contaminated beyond our ability to count *Xf* colony growth. The same samples were then processed using immunocapture DNA extraction and PCR, which did detect *Xf* in some weeds. When we no longer detected *Xf* in weeds after mid-March, we then tested the sensitivity our extraction methods and PCR. We found that using the immunocapture DNA extraction protocol for plants, we are able to detect at least 1.43×10^{-6} CFU/g of *Xf* DNA, which was satisfactory in ruling out faulty DNA extraction methods. The sensitivity of PCR to detect *Xf*

with RST-31 and RST-33 was also examined, and found to detect 6.5×10^{-5} µg/mL of DNA. In addition, an internal set of primers was developed so that nested PCR is now possible for samples appearing negative with traditional methods.

Pathogen Strain

A simple assay was conducted to categorize *Xf* by its common strains. Recent genetic and cross-inoculation studies showed that *Xf* had genetically distinct strains in different host plants (e.g., oak, oleander, grapes) (Almeida & Purcell 2003c, Chen et al. 1995, Henderson et al. 2001). Typically, *Xf* isolates from one plant species are genetically similar, despite different geographical origins. However, *Xf* isolated from almonds can be genetically separated into three distinct strains – with one ALS strain recovered in orchards in the northern San Joaquin Valley (ALS-*Xf*SJV) that is genetically more similar to grape strains than the two other ALS strains (ALS-*Xf*1, ALS-*Xf*2).

The few weeds samples that returned positive results in the winter and spring were analyzed using restriction enzyme digestion, and have so far been found to be all of the northern San Joaquin Valley (ALS-*Xf*SJV). The lack of positive results for *Xf* in vineyard weeds after mid-March prevented us from analyzing any changes (new inoculations) of *Xf* strains. However, we were able to analyze the strain of *Xf* in the mouthparts of the GWSS tested, and found that these insects were also found to be carrying *Xf* of the PD type. These results are consistent with previous findings that strains of *Xf* tend to be host-specific (Almeida and Purcell 2003c).

CONCLUSIONS

The results of this study indicate that the winter and spring weeds may be the most important reservoirs for *Xf* in vineyards infected with Pierce's Disease. We recovered *Xf* from four species of weeds that have either not been studied in depth (*Stellaria sp*. and *Capsella sp*.) or would benefit from further investigation (*Erodium sp*. and *Poa annua*). We seem to have caught the tail end of the season where *Xf* is abundant in weeds, so the next season's sampling scheme will focus more heavily on vineyard groundcover during the winter months of December, January and February. Future research along these lines could illuminate the importance of previously overlooked alternate host plant species.

One hypothesis for the importance of winter weeds for the persistence of *Xf* is that when symptomatic leaves senesce in late fall, they land directly on the groundcover, thus greatly enhancing the likelihood that any insect feeding there will transmit the bacteria to the weeds. Conclusive evidence of this hypothesis could provide a simple and low cost method for controlling the spread of PD.

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