HOST PLANT PREFERENCE AND NATURAL INFECTIVITY OF INSECT VECTORS ON COMMON WEEDS KNOWN TO HOST *XYLELLA FASTIDIOSA*

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ABSTRACT
Common weed species can harbor *Xylella fastidiosa* (*Xf*) and its insect vectors. Should weed control be part of a Pierce's disease control program? To address this question, we will survey weed species in agricultural areas known to host *Xf,* and determine the level of insect vector activity and the proportion of potential vectors that carry *Xf*. In the laboratory, we will compare three techniques to detect *Xf* in insects. Current methods produce mixed results; assessment of each method's accuracy will improve comparison of research projects and field survey results. This project will provide information for control decisions by investigating the importance of vegetation management in reduction of insect populations and inoculum potential.

INTRODUCTION
The emergence of Pierce's disease (PD) of grape in the General Beale Road area in Bakersfield in 2001 and 2002 exemplifies the threat posed by the glassy-winged sharpshooter (GWSS). While GWSS populations and PD are currently managed by an area-wide insecticide spray program and diseased vine removal, endemic GWSS populations are still present. This may be especially true in weedy fields, abandoned vineyards, and along roadsides and windbreaks where PD and GWSS are not managed (J. Hashim - personal communication). Numerous common weeds and windbreak species are hosts of *Xylella fastidiosa* (*Xf*) in greenhouse studies (Purcell and Saunders 1999, Costa et al. 2004, Wistrom and Purcell 2005). More importantly, nine weed species common in the Central Valley were found to be naturally infected with *Xf* (Shapland et al. 2006). Since so many different plants can harbor *Xf* to some extent, more information on sharpshooter host plant use in the field is required before those studies can be translated into concrete recommendations to growers. While GWSS have been observed feeding on a wide range of ornamental and weedy species (CDFA host list at www.cdfa.ca.gov/phpps/pdcp/index), the quantitative data on the numbers and host plant preference of GWSS in agricultural settings focused mainly on presence on citrus, grapes and urban areas, with insect choice determined on plants provided in pots (Naranjo and Toscano 2003; Perring and Gispert 2004; Daane and Johnson 2004; Phillips et al. 2004).

The first objective of this study is the identification of preferred feeding and oviposition hosts by GWSS, among plants already identified as hosts of *Xf* in agricultural areas. Year-round information on sharpshooter presence on host plants would provide information about the need for vegetation removal or modification in and around vineyards. For example, the identification of major breeding hosts of blue-green sharpshooters (BGSS) in northern California enabled the development of a riparian management plan. When the major breeding and feeding hosts of BGSS were removed and replanted with other plants less attractive to BGSS, large reductions in sharpshooter populations and Pierce's disease in adjacent vineyards resulted (Purcell et al. 1999). Similarly, in Central Valley almond orchards affected by almond leaf scorch, the identification of common sharpshooter, treehopper, and spittlebug insect vectors has just been completed (Daane et al. – unpublished data), as well as concurrent assessment of *Xf* presence in sampled weeds and sharpshooters (Shapland et al. 2006).

Field-based data is critical for practical application of treatment thresholds in development (Perring 2004), for GWSS control in areas with endemic sharpshooter populations. One important variable in the infectivity model is the proportion of vectors carrying the pathogen. This second objective of this study will determine the proportion of field-collected sharpshooters, in the San Joaquin Valley, that carry *Xf* in agricultural areas. With a functional treatment threshold, growers can predict the relationship between GWSS population and Pierce's disease potential, and better plan insecticide applications for GWSS control. The natural infectivity of BGSS captured in riparian area was highly variable, ranging from 5 to >40% (A. Purcell -

unpublished data), while in greenhouse studies, between 10 and 20 % of glassy-winged sharpshooters transmitted *Xf* (Almeida and Purcell 2003).

The third objective of this study is to compare the sensitivity and convenience of four techniques to detect *Xf* in insects in a side-by-side comparison. *Xf* transmission to grapes has only been correlated reliably with and bacterial presence in the precibarial region of sharpshooter mouthparts (Almeida and Purcell 2006). PCR-based vacuum-extraction (Bextine 2004a,b) of *Xf* in sharpshooter heads enabled more rapid, efficient, and convenient bacterial detection, in comparison to transmission tests with live plants and vectors (Purcell and Finlay 1980), or insect head culture (Newman et al. 2004). However, lyophilization and maceration, followed by chloroform/phenol extraction, also sensitively detects *Xf* in sharpshooter heads (R. Groves.- personal communication). *Xf* transmission to grapevines is highly sensitive, so sharpshooter infectivity can be assessed when endogenous bacterial populations are below the detection thresholds of culture or PCR (Hill and Purcell 1995). Culture determines the population of living bacteria, whereas PCR is a rapid technique that allows detection of bacterial DNA after the insect has died. Objective three will compare accuracy of detection, cost, turnaround time, and ease of processing between live insect transmission to plants, sharpshooter head culture, vacuum-extraction PCR, and lyophilization-maceration PCR.

OBJECTIVES

1. Determine preference of insect vectors for common weeds known to host of *Xf* in the southern San Joaquin Valley.
2. Determine the proportion of collected insect vectors that carry *Xf*.
3. Compare the efficacy of *Xf* detection methods in insect vectors.

RESULTS

Funding for this project began last month, September 206. Here, we present our planned procedures for comments and discussion to improve future work.

Agricultural sites (e.g., citrus, olives) in the southern San Joaquin Valley (Tulare to Bakersfield, California) will be selected based on the presence of high populations of weeds known to be hosts of *Xf* and moderate populations of GWSS. Each site will be divided into 4 sections or grids, which will be sampled monthly for 1 year.

The percent cover for weeds that are major host species for *Xf* will be determined throughout the year, to account for seasonal changes in vegetation. The weed species sampled will be based on previously reported records of *X. fastidisoa* host status in weeds (Costa et al. 2004; Wistrom and Purcell 2005; Shapland et al. 2006). Sharpshooter abundance will be measured with sweep netting , sticky traps, and visual counts of insects and egg masses. Although we expect sharpshooter collections to be greatest with sweep netting, sticky traps will help assess sharpshooter movement within and adjacent to the site, and visual counts of egg masses and insects, on individually sampled weed species, will determine if hosts are preferred for feeding or breeding. After timed visual inspection of each site for GWSS presence, sharpshooter frequency will be measured with GWSS collections (sweep net samples) on common weed species, similar to surveys of BGSS in riparian habitats.

Sharpshooters captured by sweep netting will be assessed for *Xf*. Live sharpshooters will be placed individually on seedling grapevines for a 4-day inoculation access period (Purcell and Finaly 1980). After removal from test grapes, the sharpshooter cohort will be divided in two. Heads of one third of the insects will be analyzed for *Xf* by culture on PWG media (Davis et al. 1983), which detects multiple strains of *Xf* (Newman et al. 2004; Hill and Purcell 1995). One-third of heads will be analyzed for *Xf* by vacuum-extraction. *Xf* DNA is purified from the extraction buffer using the DNeasy kit (Qiagen Inc.- Valenica, CA.; Bextine et al. 2004b). The remaining one-third of heads will be lyophilized and macerated in liquid nitrogen with a small hand-held pellet pestle. *Xf* DNA will be purified with a modified phenol/chloroform/alcohol extraction (R. Groves 2006 – unpublished data). After vacuum or chemical extraction, the extracted DNA will be multiplied and detected using polymerase chain reaction (PCR), allowing selective detection of *Xf* (Minsavage et al. 1994). Multiple strains of *Xf* (including grape, almond and oleander) can be detected and identified with mulitplex primers (Minsavage et al. 1994). Sharpshooter-inoculated grapevines will be assessed for *Xf* presence by culture and symptoms after approximately 12 weeks.

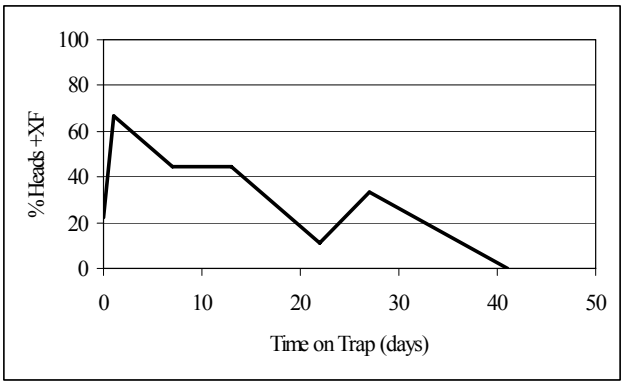

Figure 1: Recovery of *Xf* from blue-green sharpshooters with vacuum-extraction PCR, from sticky traps aged in the greenhouse (Daane and Shapland- unpublished).

Glassy-winged sharpshooters captured in sticky traps will also be analyzed for *Xf* presence using vacuum-extraction and PCR. Brief aging on sticky traps did not appear to affect the recovery of *Xf* from GWSS, (Figure 1; also Bextine et al. 2004a). If

infective sharpshooters are recovered, the weed hosts will be sampled for *Xf* presence with immunocapture PCR, which allows sensitive detection of *Xf* from plants without interference from plant-based PCR inhibitors PCR or contamination from other bacteria.

The third objective will also require greenhouse-based controls, where field-collected GWSS and BGSS will be allowed to acquire *Xf* from Pierce's diseased plants, and then allowed a four-day inoculation access period to feed on a seedling test grapevine. After the inoculation period, heads of one-third of the cohort will be cultured, one-third will be analyzed with vacuum-extraction and PCR, and one-third will be analyzed with lyophilization and PCR. Because BGSS and GWSS differ in their transmission rates of *Xf*, it will be necessary to compare detection techniques with both sharpshooter species to fulfill this objective. In preliminary comparisons, *Xf* was recovered from 73% of heads with vacuum-extraction, while 43% of the same sharpshooters transmitted *Xf* to grape (K. Daane and E. Shapland – unpublished data). To detect a 40 % difference (at 95% confidence) with these 4 techniques (transmission to plants, culture from heads, head vacuum-extraction, and head lyophilization), we will need to sample 800 sharpshooters total.

CONCLUSIONS

As this is the first month of the study there are no conclusions to report at this time.

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EVALUATION OF SIGNAL SEQUENCES FOR THE DELIVERY OF TRANSGENE PRODUCTS INTO THE XYLEM

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ABSTRACT
Xylella fastidiosa (Xf), a gram-negative bacterium, is the causative agent of Pierce's disease (PD) in grapevines. Because *Xf* is xylem-limited, it will be essential that any anti-*Xylella* gene product be present in the xylem in an effective concentration. Work on understanding the mechanism of how proteins are targeted to this plant compartment will be relevant for the delivery of therapeutic proteins into the xylem. In addition, it will be a useful tool for *Xylella* and glassy-wing sharpshooter (GWSS) gene function studies.

We collected xylem exudate from grapevines and analyzed its protein composition by two-dimensional gel electrophoresis. Peptide spectrum and Blast analysis showed that the proteins found in the exudates are secreted proteins that share function similarities with proteins found in xylem exudates of other species. The corresponding cDNA sequences of 5 of them were found in the TIGR *Vitis vinifera* gene index. The signal sequences of xylem proteins Chi1b and similar to NtPRp27 were fused to the mature pear polygalacturonase inhibiting protein (pPGIP)-encoding gene. The expression of these chimeric genes will be evaluated in transient and permanent transformations in order to evaluate their ability to target pPGIP to the xylem. The results of this research will not only be applied in projects that test anti-*Xylella* gene products that should be delivered into the xylem but also in functional studies that are intended to target the products of *Xf* and GWSS genes to the xylem.

INTRODUCTION
Signal peptides control the entry of virtually all proteins to the secretory pathway, both in eukaryotes and prokaryotes. They comprise the N-terminal part of the amino acid chain and are cleaved off while the protein is translocated through the membrane of the endoplasmatic recticulum (Nielsen et al., 1997). Generally, signal peptides are interchangeable and secretion of non-secreted proteins becomes possible by the fusion of a signal peptide at the N-terminus of the mature protein; however, changing the signal sequence of recombinant proteins can affect the degree of protein production (Yoshida et al., 2004).

In previous research, we fused the sequence coding for the signal peptide of XSP30, a xylem-specific protein from cucumber (Masuda et al., 1999), to the green fluorescent protein (GFP) reporter gene. Contrary to what we expected, fluorescence was only detected inside the cells. Our results suggested that either the XSP30 signal peptide is not recognized by the grape secretory machinery or GFP is not secretion competent. If the first hypothesis is correct, signal sequences obtained from proteins present in grape xylem sap would constitute better candidates for delivery of transgene products to the xylem.

Interestingly, we have also found that the product of the pPGIP encoding gene from pear fruit, heterologously expressed in transgenic grapevines, is present in xylem exudates and moves through the graft union (Aguero et al., 2005). These results show that pPGIP is secretion competent in grapes and constitutes a good alternative to GFP. We intend to use the sequence encoding the mature pPGIP fused to the signal peptides for analysis.

We have collected xylem exudate from plants of *Vitis vinifera* 'Chardonnay' and analyzed its protein composition by two-dimensional gel electrophoresis. The purpose of this project is to fuse the signal sequences of these grape xylem sap proteins to the mature pPGIP-encoding gene in order to evaluate their ability to target pPGIP to the xylem.

OBJECTIVES
1. Obtain partial sequences of proteins found in grape xylem exudates and search cDNA databases for signal sequence identification and selection.
2. Design and construct chimeric genes by fusing the selected signal sequences to a sequence coding for a mature secreted protein (pPGIP).