IMPACT OF MULTIPLE-STRAIN INFECTIONS OF *XYLELLA FASTIDIOSA* ON ACQUISITION AND TRANSMISSION BY THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
In this project, studies were conducted to 1) develop a method to detect and differentiate multiple strains of *Xylella fastidiosa* in individual glassy-winged sharpshooters (GWSS,) and 2) determine the relative ability of an individual GWSS to simultaneously retain or transmit two strains of *X. fastidiosa*. Strain-specific primers were developed that can detect and differentiate the Pierce's disease strain (PD) and the oleander strain (OLS) present in extracts from individual insects fed on *X. fastidiosa*-infected grape and oleander plants. After feeding on infected grape and oleander source plants for one day each, 76% of surviving adults tested positive for one or both strains of *X. fastidiosa.* The majority of individuals tested positive for only one strain of the pathogen (29% PD, 41% OLS), and only 7% tested positive for both strains; 24 % tested negative. Overall, individual insects transmitted the pathogen 39% of the time (13% PD, 26%OLS). Thus, only about half the insects that tested positive for *X. fastidiosa* actually transmitted the pathogen to a susceptible host. Although each individual used in transmission studies was exposed to both strains of the pathogen and both types of test plants, in all cases an individual insect transmitted only one strain of the pathogen, never both.

OBJECTIVES
Assess the ability of glassy-winged sharpshooter exposed to two strains of *X. fastidiosa* to transmit either strain of the pathogen.

INTRODUCTION
Xylella fastidiosa is a bacterial plant pathogen that causes a variety of diseases in a broad range of plant hosts including Pierce's disease of grapevines, almond leaf scorch, alfalfa dwarf, citrus variegated chlorosis, leaf scorch of live oak, pear leaf scorch, and oleander leaf scorch (Brlansky et al., 1982; Hopkins, 1989; Hartung et al., 1994; Purcell and Hopkins, 1996; Purcell et al., 1999). The genetic diversity of additional strains has been examined (Pooler and Hartung, 1995; Albibi et al., 1998; Chen et al., 1992; da Costa et al. 2000; Hendson et al. 2001).

Two strains of this pathogen that are presently causing severe economic losses in California are the Pierce's disease (PD) strain that infects grape and other hosts, and the oleander leaf scorch (OLS) strain that infects oleander (Blua et al., 1999; Purcell and Saunders, 1999, Purcell et al., 1999). The PD strain does not infect oleander and the OLS strain does not infect grape. The grape strain appears to have a broader host range than the oleander strain, however the complete host range of each strain is not really known.

The pathogen is spread from plant to plant by leafhoppers. Several leafhopper vectors transmit this pathogen, but the dominant vector in Southern California is the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Blua et al., 1999; Sorensen and Gill, 1996, Purcell and Saunders 1999). This insect feeds on a very broad range of plant hosts and is capable of transmitting both the grape and oleander strain of the pathogen (Purcell, 1990; Purcell et al., 1999; Costa et al. 2000). The high mobility of this insect, and its utilization of large number of host plants provide this vector with a great opportunity to be exposed to more than one strain of the *Xylella* pathogen in its lifetime. In this project, studies were conducted to determine the relative ability of an individual glassy-winged sharpshooter to simultaneously retain or transmit two different strains of *X. fastidiosa*.

RESULTS
DNA extraction and amplification
Both the commercial DNA extraction kit and immunocapture techniques we used effectively extracted enough bacterial DNA from plant and insect tissue to allow detection by PCR, however, the commercial kit extraction method was preferred because it was faster and less complicated than the immunocapture techniques. The mixing of strain specific primers in a single reaction minimized the number of samples needed and amount of handling required to get a strain specific identification. The use of the mesh-lined sample homogenization bags for extraction also greatly increased the speed of processing samples compared to using liquid nitrogen or grinding in microcentrifuge tubes. In addition, the use of the Ready-To-Go® beads

simplified and standardized the reagents required to perform PCR. Thus the processing of samples could be performed quickly, with relatively little specialized training if personnel had basic laboratory skills and the necessary equipment was available.

Use of strain specific primers on insects

Strain-specific primer sets were developed to detect and differentiate the PD and OLS strains in individual insects. Extractions of individual insects fed on *X. fastidiosa*-infected oleander that were amplified with a mixture of the PD and OLS specific primer pairs produced only a 638 bp band, which is the size of the oleander-specific product. When extracts from individual insects that fed on infected grapevine were amplified with the same mixture of primers, only a 412 bp band was produced, which is the size of the PD specific product. In some cases, when individual insects were allowed to sequentially feed on both infected grapevine and infected oleander, the products of both primers pairs were produced.

Transmission experiments

Mortality rates of insects feeding on infected plants were higher than expected (57%). Few insects managed to survive exposure to both infected source plants and subsequent exposure to test plants, likely because of repeated handling during the course of the experiments. Only insects that were alive at the end of the experiment were analyzed. After feeding on infected grape and oleander source plants for one day each, 76% of surviving adults tested positive for one or both strains of *X. fastidiosa.* The majority of individuals tested positive for only one strain of the pathogen (29% with PD, 41% OLS), 7% tested positive for both strains, and 24 % tested negative. Overall, surviving insects transmitted one the pathogen 39% of the time (PD 13%, OLS 26%). Thus, only about half the insects that tested positive for *X fastidiosa* actually transmitted the pathogen to a susceptible host. No individual insect transmitted both strains of the pathogen.

CONCLUSIONS

The strain-specific detection method we have described is a practical tool that can be used to differentiate strains of *X. fastidiosa* when multiple strains are being used in controlled experiments. In addition, it will also be useful to screen field-collected insects to determine which strains of the pathogen local populations are carrying. Although false negatives may occur, this method can still provide an indication that infected insects are present in an area, and provide an estimate of the relative numbers that are infected with each strain. Additional strain-specific primer pairs could be designed to detect additional strains of this pathogen that may be present in different geographic areas.

In our studies, the detection of the pathogen in an individual insect using PCR did not always indicate the propensity to transmit the pathogen to a test plant; many insects that tested positive did not transmit the pathogen. The reasons for this are not known. Other studies using media culture to isolate bacteria from insects also found that neither the detection of bacteria in insect heads, nor the estimated numbers of bacteria present, predicted the propensity of the insect to transmit *X. fastidiosa* (Hill and Purcell 1995, Almeida and Purcell 2003). One of the more interesting results observed was that although all surviving individuals were exposed to both strains of the pathogen, and both types of susceptible test plants, in no case did a single individual transmit both strains of the pathogen. Thus, there must be other factors that are contributing to an individual's ability to successfully retain and transmit the pathogen after exposure.

REFERENCES:

Albibi, R., J. Chen, O. Lamikanra, D. Banks, R.L. Jarret, and B.J. Smith. 1998. RAPD fingerprinting *Xylella fastidiosa* Pierce's disease strains isolated from a vineyard in north Florida. FEMS Microbiol. Letters 165: 347-352.

Almeida, R.P.P., and A.H. Purcell. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). J. Econ. Entomol. 96: 264-271.

Blua, M.J., P.A. Philips, R.A. Redak. 1999. A new sharpshooter threatens both crops and ornamentals. Cal. Ag. 53: 22-25.

Brlansky, R.H., L.W. Timmer, and R.F. Lee. 1982. Detection and transmission of a gram-negative, xylem-limited bacterium in sharpshooters from a citrus grove in Florida. Plant Dis. 66, 590-592.

Chen, J., C.J. Chang, R.L. Jarret, and N. Gawel. 1992. Genetic variation among *Xylella fastidiosa* strains. Phytopathology 82: 973-977.

Costa, H.S., M.S. Blua, J.A. Bethke, and R.A. Redak. 2000. Transmission of *Xylella fastidiosa* to oleander by the glassywinged sharpshooter, *Homalodisca coagulata*. HortScience 35: 1265-1267.

da Costa, P.I., C.F. Franco, S. Vicente, S. Miranda, D.C Teixeira, and J.S. Hartung, 2000. Strains of *Xylella fastidiosa* rapidly distinguished by arbitrarily Primed-PCR. Curr. Microbiol. 40: 279-282.

Hartung, J.S., M.J.G. Beretta, R.H. Brlansky, J. Spisso, and R.F. Lee. 1994. Citrus variegated chlorosis bacterium: axenic culture, pathogenicity, and serological relationship with other strains of *Xylella fastidiosa*. Phytopathology 84: 591-597.

Hendson, M., A.H. Purcell, D. Chen, C. Smart, M. Guilhabert, and B. Kirkpatrick. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. Applied and Environ. Microbiol. 67: 895-903.

Hill, B.L., and A.H. Purcell. 1995. Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. Phytopathology. 85: 209-212.

Hopkins, D.L. 1989, *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Ann. Rev. Phytopathology 27: 271-290.

Pooler, M.R., and J.S. Hartung. 1995. Specific PCR detection and identification of *Xylella fastidiosa* strains causing citrus variegated chlorosis. Curr. Microbiol. 31: 377-381.

Purcell, A.H., 1990. Homopteran transmission of xylem-inhabiting bacteria. pp. 243-266 in: Advances in disease vector research, Vol. 6. Springer-Verlag, New York.

Purcell, A.H., and D.L. Hopkins. 1996. Fastidious xylem-limited bacterial plant pathogens. Annu. Rev. Phytopathol. 34: 131-151.
Purcell, A.H., and S.R. Saunders. 1999. Glassy-winged sharpshooters expected to increase plant disease. Cal. Ag. 53: 26-27.
Purcell, A.H., S.R. Saunders, M. Hendson, M.E. Grebus, and M.J. Henry. 1999. Causal role of *Xylella fastidiosa* in oleander leaf scorch disease. Phytopathology. 89: 53-58.
Sorensen, J.T., and R.J. Gill. 1996. A range expansion of *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) to southern California. Pan-Pacific Entomol. 72: 160-161.

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