EPIDEMIOLOGY OF PIERCE'S DISEASE IN SOUTHERN CALIFORNIA: IDENTIFYING INOCULUM SOURCES AND TRANSMISSION PATHWAYS

Project Leader:
Donald A. Cooksey
Department of Plant Pathology
University of California,
Riverside 92521

Heather S. Costa
Department of Entomology
University of California,
Riverside 92521

Reporting Period: The results reported here are for the completed project from May 1, 2000 to July .31, 2003.

ABSTRACT
Xylella fastidiosa can cause a number of plant diseases in a variety of plant hosts including Pierce's disease of grapevines, almond leaf scorch disease, alfalfa dwarf, citrus variegated chlorosis, leaf scorch of live oak, pear leaf scorch, and oleander leaf scorch. In Southern California, the primary insect vector of concern is the glassy-winged sharpshooter, *Homalodisca coagulata* (Say). Previous studies of *Xylella fastidiosa*-induced diseases have described systems dealing with different primary vector species and different alternate host plants than those that are found in the Southern California systems. In this project, a variety of plant species found near a severe outbreak of Pierce's disease in vineyards in the Temecula valley of California were tested to identify potential sources of inoculum in the area. Plants were tested though three summer seasons using ELISA, culture on specialized media, and PCR methods to monitor for the presence of the pathogen. Plant species from the field that consistently tested positive for a grape strain of *X. fastidiosa* were the previously known hosts grape and almond, and two new hosts, Spanish broom, *Spartium junceum* and wild mustard, *Brassica spp.* Samples of oleander, *Nerium oleander*, also tested positive, however the strain of *X. fastidiosa* that infects oleander differs from the grape strain and does not appear to infect grape plants. Greenhouse transmission studies indicate that the glassy-winged sharpshooter was able to transmit a grape strain of the pathogen to *Spartium junceum*, *Brassica nigra* and other hosts.

INTRODUCTION
Diseases caused by *Xylella fastidiosa* threaten some of California's largest agricultural commodities, including the grape and wine industries, nursery, almond, and alfalfa production (Hopkins and Purcell 2002). Thus far, strains of *X. fastidiosa* that cause Pierce's disease of grapevines , almond leaf scorch, alfalfa dwarf, and oleander leaf scorch have been identified in California. The Pierce's disease (PD) strain and oleander leaf scorch (OLS) strain have caused devastating losses of grapevines and oleander plants respectively in California (Blua et al. 1999, Perring et al. 2001, Feil and Purcell, 2001). These two strains are genetically distinct (Hendson et al. 2001), and the strain of the pathogen that infects oleander does not infect grape, and vice versa (Purcell et al. 1999). The grape strain appears to have a broader host range than the oleander strain. However, the complete host range of each strain is not completely known (Hopkins and Purcell 2002).

In Southern California, the primary insect vector is the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), a recent introduction from the southeastern U.S. (Sorensen and Gill, 1996; Blua et al., 1999). This insect feeds on xylem tissue, and is reported to feed on over 75 species of plants in 35 families (Turner and Pollard, 1959). The feeding habits and host range of GWSS differ from other vector species in California that have previously been associated with this pathogen. Studies in Northern California suggest that the PD pathogen is primarily spread by leafhopper vectors that move into vineyards from outside habitats (Purcell, 1981; Purcell and Saunders, 1999). Thus, studies of alternative plant hosts and inoculum sources of *X. fastidiosa* conducted in Northern California concentrated on plants in riparian habitats surrounding vineyards (Purcell and Saunders, 1999). However, in grape- growing regions of Southern California, the habitats and plant hosts that surround the vineyards are much different than those found in Northern California. In addition to naturally occurring vegetation, vineyards in Southern California are frequently adjacent to citrus groves and suburban landscapes. Because *X. fastidiosa* has a broad host range and in some cases can be present in plant tissue without causing noticeable symptoms (Purcell and Saunders 1999), it is not always easy to identify plants that serve as alternate hosts and potential sources of inoculum.

Knowledge of the source of disease inoculum is essential to the development of effective disease management strategies. The objective of this study was to determine which plant species in Southern California are hosts of *Xylella fastidiosa* and serve as potential sources of inoculum for PD infection of grapevine. In addition, because more than one strain of *X. fastidiosa* is could be present in our sampling area, it was necessary to identify the strain of the pathogen that was present in positive samples to determine if they were PD strains that would be considered a threat to grapevines in the area. For example, a plant that tested positive for the oleander strain of *X. fastidiosa* would not be a threat to vineyards, since it does not infect grape.

OBJECTIVES
1. Determine which plant species near vineyards harbor *Xylella fastidiosa* and serve as potential reservoirs of inoculum for the spread of Pierce's disease to grapes.
2. Measure the ability of the glassy-winged sharpshooter to acquire and transmit *X. fastidiosa* to and from grape, citrus, almond, and other plant species identified as potential hosts and sources of inoculum for the spread of Pierce's disease.

3. Comparison of the sensitivity and specificity of various methods to screen large numbers of plant and insect samples for the presence of Pierce's disease.

RESULTS AND CONCLUSIONS

Results

Field Samples

Over 60 species of plants, and more than 5000 samples were processed. Of the species tested in the Temecula valley, only grape, (*Vitis vinifera*), almond (*Prunus* spp.), Spanish broom (*Spartium junceum*), wild mustard (*Brassica spp*.) and oleander (*Nerium oleander*) consistently tested positive by two or more methods. Of these, wild mustard and Spanish broom were not previously reported as hosts for *Xylella fastidiosa*. *Brassica* plants tested positive by both ELISA and PCR, but we were unable to culture *X. fastidiosa* isolates from field samples for unknown reasons. We were, however, able to culture *X. fastidiosa* from *B. nigra* plants used in greenhouse transmission studies. Coyote brush, elderberry, citrus, and a few other samples occasionally tested positive with ELISA, however we were not able confirm those results with PCR or culture. Coyote brush and elderberry have previous been reported as hosts of *X. fastidiosa* when inoculated by insects under greenhouse conditions but tested negatively in field experiments (Purcell and Saunders 1999).

We were unable to detect the pathogen in grape and almond using ELISA in early spring. However, some samples of Spanish broom and oleander tested positive all year. Wild mustard plants tested positive in mid summer, and were generally not present in the field during the winter months.

Comparison of ELISA and IC/PCR for early season detection

n the first collection of grape samples in Spring (May), 15/25 samples from symptomatic field grapes, and 0/10 of the non-symptomatic plants tested positive by ELISA. Immunocapture PCR (IC/PCR) of the same plants did not detect any infected grape at that time. Both methods did detect infected oleander plants used as positive controls. In samples collected five weeks later (June), 5/21 symptomatic grape plants positive with ELISA, and these same plants also tested positive with IC-PCR. In this case, analysis of samples with IC/PCR early in the season did not help to detect any additional infected plants. Because ELISA was easier, less expensive and less time consuming, it was the best method we had for screening for the presence of *Xylella fastidiosa* in numerous plant samples. Samples testing positive could subsequently be confirmed with PCR.

Transmission Studies

Studies testing the ability of the GWSS to transmit *X. fastidiosa* from grape to 30 species of host plants found transmission of a PD strain to grape, black mustard, Spanish broom, almond, black sage, and Mexican elderberry. Previous studies have demonstrated that transmission of the grape strain to oleander did not occur (Purcell and Saunders 1999; Purcell et al. 1999). Similarly, in our studies no transmission from infected grape to oleander was observed.

Conclusions

Overall, few plant species could be documented as alternate host plants for the PD strain of *X. fastidiosa* in the Temecula valley. In the Temecula valley, it appears that infected grapevines, almond trees, Spanish broom, and wild mustard likely serve as major sources of PD inoculum. Thus, in addition to removing infected grapevines and almond trees, growers were advised to remove Spanish broom and wild mustard from areas surrounding the vineyards.

Citrus is a favored host of the GWSS, and is the most important year-long reproductive host in the Temecula valley (Perring et al. 2001, Hix et al. 2002). Although citrus plants were repeatedly sampled in the field, and exposed to infected insects in experimental transmission tests, we could never document infection in citrus in the field or in transmission test plants. Although citrus samples collected from the field occasionally tested weakly positive by ELISA, they could never be confirmed with PCR or culture. It is not clear if this is the result of occasional low levels of infection in citrus that are difficult to detect, or falsely positive ELISA results.

In our studies, we found that ELISA testing of plant samples appeared to be just as effective in detecting the pathogen in plant tissue as other method we used (PCR, media culture, IC/PCR). Thus, this method of analysis will continue to be extremely important when screening large numbers of plant samples. However, additional analysis of positive plants samples with other methods was necessary to eliminate the possibility of false positives with ELISA, and to identify the strain of the pathogen that was present. The strain-specific primers used were effective in giving a preliminary identification of *X. fastidiosa* strains. Comparative analysis using the 16-23s spacer region sequence was consistent with PCR results using the strain-specific primers. Additional strain-specific primer pairs could be designed to differentiate other strains of this pathogen that may be present in different geographic areas.

Some species of plants that tested positive for *X. fastidiosa* in field surveys and greenhouse transmission experiments in northern California (Raju et al 1983, Purcell and Saunders 1999) were never confirmed positive in samples collected in the Temecula valley even though they were present near infected grape plants. This could be a reflection of differences in the species of insect vectors present, the types of plant materials that dominate, or differences in host range of the *X. fastidiosa* isolates present in each location. Previous examination of strains of *X. fastidiosa* strains revealed genetic difference between northern and southern California strains of *X. fastidiosa* from grape (Hendson et al. 2001). More detailed testing is

being done to better characterize the PD strains of *X. fastidiosa* present in the host plants identified in the Temecula valley to determine if they differ from those found in other geographic areas.

REFERENCES

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

Feil, H., and A.H. Purcell. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. Plant Disease 85:1230-1234.

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

Hix, R., N. Toscano, R. Redak, and M. Blua. 2002. Area-wide management of the glassy-winged sharpshooter in the Temecula valley. Proceedings of the 2002 Pierce's Disease Research Symposium, San Diego, CA. pp. 157-158.

Hopkins, D.L., and A.H. Purcell. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Disease 86: 1056-1066.

Perring, T.M., C.A. Farrar, and M.J. Blua. 2001. Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. Calif. Agric. 55: 13-18.

Purcell, A.H., 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. Phytopathology 71: 429-435.

Purcell, A.H., and S.R. Saunders. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. Plant Dis. 83:825-830.

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.

Raju, B.C., A.C. Goheen, and N.W. Frazier. 1983. Occurrence of Pierce's disease bacteria in plants and vectors in California. Phytopathology 73: 1309-1313.

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.

Turner, W.F., and H.N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. U.S. Dept. Agr. Tech. Bul. 1188, 57pp.

FUNDING AGENCIES

Funding for this project was provided by the American Vineyard Foundation, the California Department of Food and Agriculture, and the University of California Agricultural Experiment Station.