DOCUMENTATION AND CHARACTERIZATION OF *XYLELLA FASTIDIOSA* STRAINS IN LANDSCAPE HOSTS

Project Leaders:

Frank Wong
Dept. of Plant Pathology
University of California
Riverside, CA 92521
frank.wong@ucr.edu

Donald A. Cooksey
Dept. of Plant Pathology
University of California
Riverside, CA 92521
donald.cooksey@ucr.edu

Heather S. Costa
Dept. of Entomology
University of California
Riverside, CA 92521
heather.costa@ucr.edu

Cooperators:

James Downer, UCCE, Ventura County
Mike Henry, UCCE, Riverside County
John Kabashima, UCCE, Orange County
John Karlik, UCCE, Kern County
Michelle LeStrange, UCCE, Tulare County
David Shaw, UCCE , San Diego County

Reporting Period: The results reported here are from work conducted from October 15, 2003 to October 12, 2004.

ABSTRACT

To better understand the impact of *Xylella fastidiosa* on the urban environment and the potential for ornamental hosts to serve as reservoirs for agronomically important diseases caused by the bacteria, a survey project was initiated to document and characterize strains of the bacteria harbored in landscape plants. Targeted sampling of 122 landscape species either symptomatic for bacterial scorch or testing positive for *X. fastidiosa* by ELISA in 2003 was performed. Of the 830 samples, 321 tested positive by ELISA (representing 77 of the 122 species tested). *X. fastidiosa* was also detected in 23 species by PCR-amplification using *X. fastidiosa* specific primers. Twenty-seven isolates from 13 host species were obtained from samples testing positive by ELISA. Isolates from plants not previously reported as hosts in southern California urban environments included mulberry, heavenly bamboo, magnolia, day lily, western redbud, jacaranda and peach. Genetic characterization of these isolates by 16S-23S rDNA sequencing distributed these isolates amongst previously characterized strain groups: almond leaf scorch (crape myrtle, ornamental plum, liquidambar, gingko, olive), Pierce's disease (magnolia, peach, western redbud), mulberry leaf scorch (mulberry, heavenly bamboo), and oleander leaf scorch (magnolia, jacaranda, day lily). The role of some *X. fastidiosa* strains in their ability to cause disease is presently being tested by fulfilling Koch's postulates in glasshouse experiments. The data collected from this study strongly suggest that *X. fastidiosa* is causing a number of scorch diseases in the urban landscape, and that strains of agronomic importance may be harbored in this environment.

INTRODUCTION

Xylella fastidiosa (*Xf*) is a xylem-limited, insect-vectored, plant pathogen that can cause severe damage to a wide range of host plants. Diseases caused by this pathogen include Pierce's disease of grapevine (PD), oleander leaf scorch (OLS) and almond leaf scorch (ALS). In 2003, a survey of landscape plants in five urban locations in southern California was initiated to document the incidence of the *Xf* infection in landscape ornamental hosts and to characterize strains existing in these hosts that may prove a threat to landscape ornamentals or crops of agronomic importance. Two hundred twenty one samples (29%) representing 48 species tested positive by ELISA. Ten isolates of *Xf* were obtained from eight plant species (*Fatsia japonica, Ginkgo biloba*, *Lagerstroemia indica*, *Liquidambar styraciflua*, *Morus alba*, *Nandina domestica*, *Olea europea*, and *Prunus cerasfiera*) not previously described as hosts of *X. fastidiosa* in southern California.

Based upon the results of the first year, targeted sampling of host species testing positive by ELISA was performed primarily in the Riverside and Redlands areas in order to obtain additional isolates for characterization. To prove the role of *Xf* in causing disease in previously identified hosts, test plants were inoculated in glasshouse experiments to fulfill Koch's postulates for these isolates, and to determine if they were able to cause disease in grapevine and oleander.

OBJECTIVES

1. Use laboratory methods to identify landscape host species that are infected with *X. fastidiosa.*
2. Secure isolates from these hosts to document infection and provide material for genetic characterization of the *X. fastidiosa* strain(s) involved.
3. Genetically characterize the strains of pathogen in landscape plant species.
4. Confirm pathogenic infection through inoculation studies with specific isolates.
5. Test ability of new strains to infect agricultural crops including grape, olive, and almond.

RESULTS

Objective 1

In 2004, 830 samples from 122 landscape plant species were collected. Sampling focused on plant species that were symptomatic or had tested positive by ELISA in 2003 surveys. Three hundred twenty one samples (39%), tested positive by ELISA. At least one sample from 77 of the 122 species tested was positive by ELISA (63%). Attempts to isolate the

pathogen from these positive samples yielded only a small number of isolates (see next section). PCR testing (Minsavage 1994) was performed on a subset of the samples collected using a modification of the published methodology. Briefly, petioles and midveins from leaves were chopped in sterile water, tissues were allowed to sit in the water for several minutes to allow for the release of *Xf* from the tissues and then DNA extracted from the water. Results were greatly improved using this method, and *Xf* was detected in 23 species tested (Table 1). PCR testing of additional species testing positive by ELISA is continuing on species from which isolates could not be obtained.

Table 1. ELISA, isolation and PCR results for 23 of 122 species tested for *Xf*.

Plant Name	Common Name	#Tested	#ELISA(+)[a]	Culture(+)[b]	PCR(+)[c]
Albizia julibrissin	Silk Tree	6	5		yes
Cercis occidentalis	Western Redbud	4	3	yes	yes
Ginkgo biloba	Maidenhair Tree	15	6	yes	yes
Hemerocallis	Day Lily	9	5	yes	yes
Jacaranda mimosifolia	Jacaranda	49	24	yes	yes
Juglans	Walnut	2	2	no	yes
Lagerstroemia indica	Crape Myrtle	17	5	yes	yes
Lavandula dentata	Lavender	4	4	no	yes
Ligustrum lucidum	Glossy Privet	7	5	no	yes
Liquidambar styraciflua	Liquidambar	19	7	yes	yes
Magnolia grandiflora	Southern Magnolia	31	18	yes	yes
Morus alba	White Mulberry	3	2	yes	yes
Nandina domestica	Heavenly Bamboo	20	3	yes	yes
Nerium oleander	Oleander	3	3	yes	yes
Olea europaea	Olive	6	5	yes	yes
Phoenix reclinata	Senegal Date Palm	2	2	no	yes
Prunus cerasifera	Ornamental Plum	12	7	yes	yes
Prunus dulcis	Almond	3	3	yes	yes
Prunus persica	Peach	5	2	yes	yes
Rosmarinus officinalis	Rosemary	13	8	no	yes
Vitis labrusca 'Concord'	Concord Grape	2	2	yes	yes
Vitis vinifera 'Red Flame'	Red Flame Grape	2	2	yes	yes
Vitis vinifera 'Thompson Seedless'	Thompson Seedless Grape	5	5	yes	yes

[a] denotes number of samples testing positive using a commercial *Xf*-specific ELISA kit
[b] denotes if an *Xf* isolate was successfully obtained from at least one sample
[c] denotes if PCR-amplification using RST31/33 primers from plant tissue was successful for at least one sample

Objective 2

Twenty-seven isolates (from 13 host species) were obtained from samples testing positive by ELISA (Table 2). Isolation of the pathogen from samples, even those testing strongly positive from ELISA, was not always possible. Briefly, samples were washed in soapy water, soaked for 1 min in 70% ethanol, 1 min in 20% bleach, then triple rinsed in sdH_2O. Samples were then sliced into 1-2 mm pieces and soaked in PBS. Fifty microliters of the PBS buffer was then plated onto PW media with or without the addition of 25 ppm of cycloheximide. The failure to obtain isolates from all samples testing positive by ELISA suggests that specific methodologies need to be determined for specific tissue types from different hosts as a general isolation protocol may be inadvertently killing the pathogen, the pathogen may be highly irregularly distributed in host tissues, or the commercially available ELISA kit may be generating a high number of false positives due to non-specific interactions with host tissue.

Objective 3

Collected isolates were confirmed as being *Xf* by extraction of the DNA from the cultures using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA) and subsequent PCR amplification with the RST31/33 primer pair. Isolates were further characterized by amplification and sequencing of the 16S-23S ribosomal DNA intergenic spacer region as described by Hendson et al. 2001. All the 16S-23S rDNA sequences were aligned using the clustalX program (Thompson *et al*., 1994) and their relationship was analyzed with the PHYLIP program (Felsenstein, 1995) with the sequence of the *Xanthomonas vesicatoria* (AY288080) as an outlying group (Figure 1).

Two strains isolated from mulberry (Morus024 and Morus012) showed 99.41% identity with the previously reported mulberry-VA strain from the eastern U.S. (Huang and Sherald, 2004), while Nandina065, Morus059 and Morus063 showed a 100% of identity with the same strain. For the two peach isolates, Peach018 showed 100% identity with previously reported Pierce's disease strains (AO5) while Peach018 showed a little less identity (99.41%), but both grouped with PD strains. The Cercis050 strain also grouped with PD strains (99.61% identity). Strains isolated from Magnolia showed just 98.44 % identity between them. Since Magnolia038 was more closely related to Oleander leaf scorch (OLS) (99.02% identity) while

Magnolia002 showed more identity (99.41%) to PD strains. For isolates from Hemerocallis and Jacaranda, they showed 100% identity between them and showed to be more closely related to oleander strains (99.22%)
Gingko, olive, liquidambar and some ornamental plum strains showed to be closely related to the Dixon almond leaf scorch strain (100% identity). Some ornamental plum strains showed divergence amongst them (97.86% identity) and from ginkgo, olive and liquidambar, but all of them grouped together with the Dixon strain. Lastly, the strain isolated from a yet to be identified host (nicknamed "*negrito"*) showed slight differences from the ornamental plum, liquidambar and olive isolates. None of the isolates grouped with plum leaf scald, phony peach, oak leaf scorch group or with citrus variegated chlorosis and coffee leaf scorch strains.

Table 2. *Xf* isolates collected in 2004 surveys.

Host Scientific name	Common Name	Isolate designation
Cercis occidentalis	Western Redbud	Cercis050
Hemerocallis	Day Lily	Hemerocallis034
Jacaranda mimosifolia	Jacaranda	Jacaranda028
Liquidambar styraciflua	Liquidambar	Liquidambar020
Magnolia grandiflora	Magnolia	Magnolia038
Magnolia grandiflora	Magnolia	Magnolia 002
Morus alba	White Mulberry	Morus012
Morus alba	White Mulberry	Morus024
Nerium oleander	Oleander	Oleander031
Nerium oleander	Oleander	Oleander028
Prunus cerasifera	Ornamental Plum	Pcerasifera057
Prunus cerasifera	Ornamental Plum	Pcerasifera086
Prunus cerasifera	Ornamental Plum	Pcerasifera047
Prunus cerasifera	Ornamental Plum	Pcerasifera052
Prunus cerasifera	Ornamental Plum	Pcerasifera053
Prunus dulcis	Almond	Almond036
Prunus persica	Peach	Peach018
Prunus persica	Peach	Peach.019
Unknown species	'negrito'	Negrito005
Vitis labrusca 'Concord'	Grape	Grape153
Vitis labrusca 'Concord'	Grape	Grape154
Vitis vinifera 'Red Flame'	Grape	Grape155
Vitis vinifera 'Red Flame'	Grape	Grape156
Vitis vinifera 'Thompson Seedless'	Grape	Grape149
Vitis vinifera 'Thompson Seedless'	Grape	Grape150
Vitis vinifera 'Thompson Seedless'	Grape	Grape151
Vitis vinifera 'Thompson Seedless'	Grape	Grape152

Objectives 4 and 5

Eight characterized strains of *Xf* collected from the landscape in 2003, plus an oleander and a grape strain, were inoculated into their host plants of origin in glasshouse assays. Strains used were Almond276, Ginkgo, Lagestroemia02 (crape myrtle), LiquidambarUI12 (liquidambar), Morus069 (mulberry), Nandina065, Olive AC12 , Pcerasifera076 (ornamental plum), Riverside3 (oleander), GrapeA05. These same eight strains were also used to inoculate grapevine and oleander. Briefly, isolates were grown on PW media for two weeks from which a suspension of 1 x 10^9 CFU in sterile phosphate buffer was obtained. Plants were needle inoculated on three to four sites per plant using the needle-stab technique described by Hill and Purcell (1995). Approximately 25 plants were used for the inoculation studies. All plants were tested by ELISA prior to inoculation to ensure that they were *Xf* free. Starting approximately three months after inoculation, plants were ELISA tested and attempts were made to isolate the pathogen from positive plants. *Xf* cultures have been obtained from some hosts testing positive by ELISA and have been confirmed as *Xf* by PCR, namely those from mulberry inoculated with the Morus069 isolate. Isolation and characterization studies from these test inoculations are currently underway for the rest of the test plants and *Xf* isolates.

CONCLUSIONS

The results of the study do indicate that there are a number of landscape hosts that are harboring different strains of *Xf* in southern California. Of the new isolates characterized, it appears that new hosts have been identified for a number of strain groups: Pierce's disease (magnolia, peach, western redbud), oleander leaf scorch (magnolia, jacaranda, day lily), mulberry leaf scorch (heavenly bamboo), and almond leaf scorch (ornamental plum, crape myrtle, liquidambar, gingko, olive). Inoculation tests appear to have confirmed the role of *Xf* in causing mulberry leaf scorch in California, while other tests await completion. It does appear that new methodologies will have to be developed to successfully obtain or test for *Xf* in a number of ornamental plant species. The role of *Xf* infections in landscape hosts does appear to have a significant impact on

several species; however, additional studies must be completed to further elucidate the role of this pathogen in causing widespread disease in the urban setting as well on crops of agronomic importance in California.

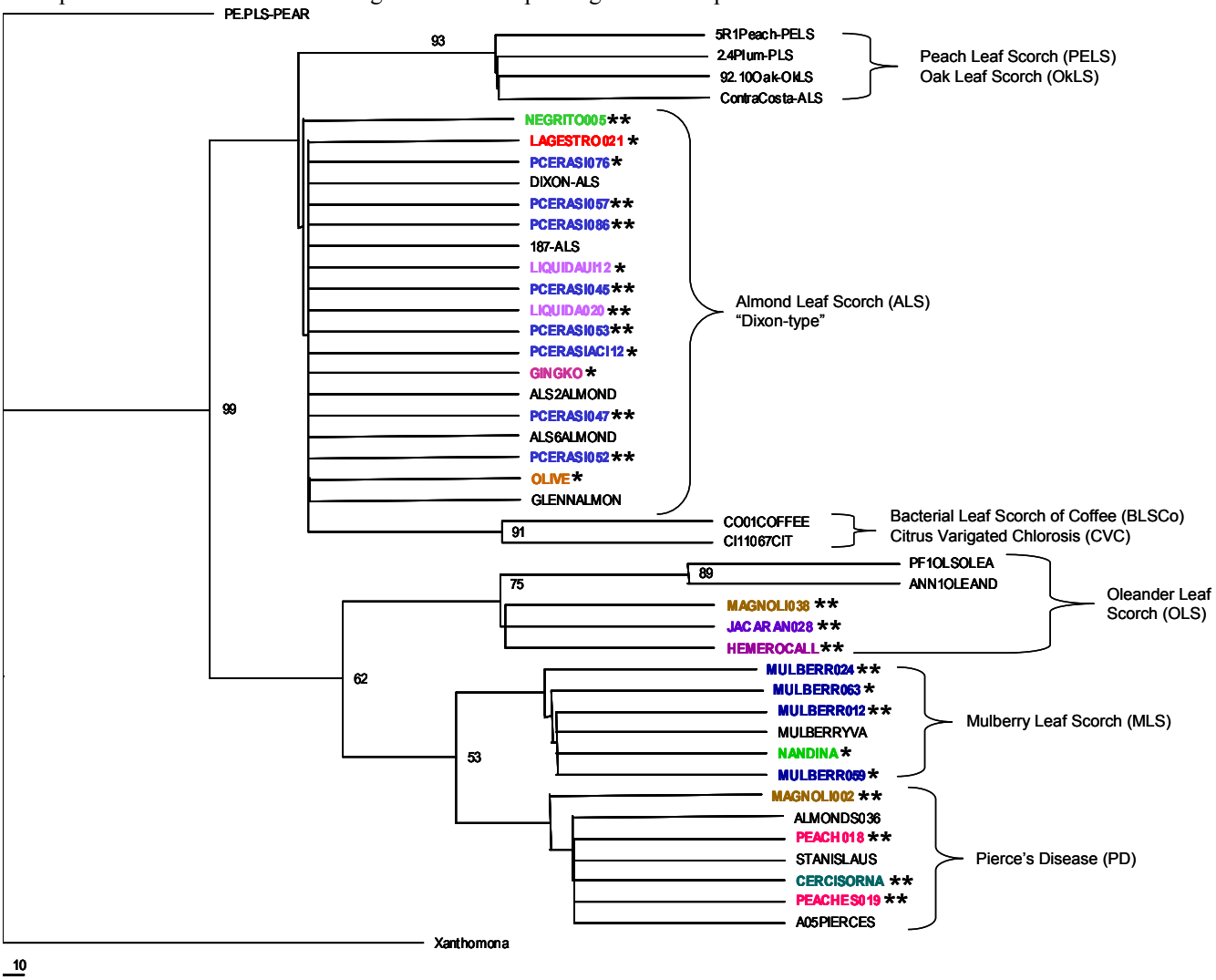

Figure 1. Preliminary phylogenetic tree constructed using the neighbor joining method, based on 16S rDNA sequence data for *Xylella fastidiosa* with the sequence of *Xanthomonas vesicatoria* (AF203392) as the outgroup. The numbers above the branches represent bootstrap values obtained for 100 replications. * Indicates isolates collected in 2003, ** indicates isolates collected in 2004.

REFERENCES

Felsenstein, J. (1995). PHYLIP version 3.57 manual. U.Washington, Seattle.

Hendson, M., Purcell, A. H., Chen, D.,Smart, C., Guilhabert, M., and Kirkpatrick, B. (2001) App. and Env. Microbio. 67:895-903.

Hill, B. L. and Purcell, A. H. (1995) Phytopath. 85:1368-1372.

Huang,Q. and Sherald,J.L. (2004) Curr. Microbiol. 48 (1), 73-76.

Minsavage, G. V., Thompson, C. M., Hopkins, D. L., Leite, R.M.M.V.B.C., and Stall, R. E. (1994) Phytopath. 84: 456-461.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) Nucleic Acids Research, 22:4673-4680

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, with additional contributions made by the California Association of Nurseries and Garden Centers' CANERS Foundation.