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

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ABSTRACT

The xylem-limited bacterial pathogen *Xylella fastidiosa* causes a number of diseases in a wide range of hosts including Pierce's disease of grapevine and leaf scorch of oleander. Recently, the presence of the bacteria has been documented in a number of landscape ornamentals in southern California have showing symptoms typical of those caused by the pathogen. Plants such as sweet gum (liquidambar), olive and ornamental plum have been identified as susceptible hosts of the pathogen. During surveys conducted in the summer of 2003, over 500 samples from plants showing symptoms typical of *X. fastidiosa* infection from five cities in southern California were tested for the presence of the pathogen. Seventy-eight host species were represented in the samplings. Plants from 26 of the species represented tested positive for the presence of the pathogen by ELISA. Current work is focusing on the confirmation of the presence of the pathogen by PCR amplification and the collection of bacterial isolates from these suspected hosts for both genetic and host range characterization.

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 (Hopkins 1989; Hartung *et al*. 1994; Purcell and Hopkins, 1996; Purcell *et al*., 1999). Multiple strains of *X. fastidiosa* with different host ranges have been identified (Chen et al 1992, da Costa *et al*. 2000, Henderson *et al*. 2001), but little is known about the diversity of these populations in the urban landscape and their ability to cause loss in both plants of horticultural and agronomic importance.

Until recently, losses of ornamental plants resulting from *X. fastidiosa*-induced diseases were primarily limited to oleander. Since the symptoms of *X. fastidiosa* often mimic those caused by other pathogens or abiotic stresses, problems caused by this bacterium may have been frequently misdiagnosed in the urban environment. However, during recent surveys of potential alternate host plants of PD in southern California; liquidambar, olive, and ornamental plum were found to have symptoms of leaf scorch and tested positive for *X. fastidiosa* using ELISA and PCR analysis of plant tissue. None of these species have been reported as hosts of this pathogen before. In some areas, scorch symptoms were very common on these plants, i.e. in olive plantings in Riverside County, up to 33% of the trees exhibited symptoms. *X. fastidiosa* was isolated from each of these species on specialized media for genetic characterization. Preliminary studies of *X. fastidiosa* isolates from these hosts indicate that the strain(s) present differ from the PD and OLS strains found in grape and oleander respectively (D. Cooksey, *unpublished data*), however, their exact relationship to other previously identified strains, and the host range of these isolates, remains unknown. In order to develop strategies to manage this pathogen, it is essential to determine the potential of these strains to infect other host plants, particularly agricultural crops such as grape, almond, olive and citrus.

The broad host range of this pathogen, and its potential threat to California landscapes, agricultural and forestry crops makes it critical to document and characterize the strains of the pathogen that are present throughout California, and determine the plant host range of each of these strains. The result of this project will provide knowledge base of crop hosts that may be at risk of infection with new strains and will provide a database of ornamental species that can serve as inoculum sources of *X. fastidiosa.* This information is necessary to develop management practices that target the removal of infected plants and sources of inoculum near susceptible crops.

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 AND CONCLUSIONS

Objective 1

Over 500 samples from landscape plants showing symptoms of wilt, dieback or scorch were taken from five cities in distinct locations in southern California. Sampling locations consisted of areas several city blocks in size, from which both suspected host species and plants symptomatic of disease were taken (Table 1).

Originally, only 24 species of possible host plants were to be examined, but it was decided after an initial survey of plants showing symptoms typical of *X. fastidiosa* infection that all plants showing typical symptoms would be sampled. Thus, over 78 distinct landscape ornamental species were sampled in the survey.

A large number of plant samples indicated the presence of *X. fastidiosa* based upon ELISA results only (Table 2). Plants from 26 of the 78 species sampled tested positive. Within a tested species, the number of individual samples testing positive was variable, which was not unexpected. For example, 16 of 23 samples from *Agapanthus africanus* tested positive, while only 3 of 10 samples of *Jacaranda mimosifolia* tested positive. At this time, PCR confirmation using the RST31/RST33 primer pair (Minsavage *et al*. 1994, Pooler *et al*. 1997) for general amplification of *X. fastidiosa* has only been successfully completed for a small subset of these samples. Likewise, in only a few cases has the bacteria been successfully cultured on PD3 or PW agar.

Table 1. ***Origin of samples collected as of October 15, 2003.***

City	County	Samples	Species Represented[a]
Filmore	Ventura	105	45
San Diego	San Diego	102	32
Redlands	San Bernardino	117	37
Riverside	Riverside	97	40
Tustin	Orange	118	29
Total		*539*	*78*

[a] Actual number of plant species sampled per location. Some species sampled in multiple locations, depending of the presence of symptomatic specimens in the sampling area.

Table 2. Partial listing of plant species confirmed or likely to be hosts of *X. fastidiosa* in southern California urban landscapes.

Species	**Common Name**	**ELISA[a]**	**PCR[b]**	**Culture[c]**
Agapanthus africanus	Lily of the Nile	+	---	---
Alibiza julibrissin	Silk Tree	+	---	---
Cinnamonum camphora	Camphor Tree	+	---	---
Gingko biloba	Gingko	+	---	+
Impatiens spp.	Impatiens	+	---	---
Jacaranda mimosifolia	Jacaranda	+	---	---
Justica spicigera	Mexican Honeysuckle	+	---	---
Liquidambar styraciflua	Sweet Gum	+	+	---
Morus spp.	Mulberry	+	+	---
Nerium oleander	Oleander	+	+	+
Olea europa	Olive	+	+	---
Photina fraseri	Frasier Photina	+	+	---
Prunus dulcis	Almond	+	+	+

(+) indicates a positive test result, while (---) indicates a negative or inconclusive result.

[a] Positive reactions using a commercially available ELISA kit (Agdia)

[b] Amplification of product using RST31/RST33 primer set

[c] Isolation of *X. fastidiosa* from plant extracts on PD3 or PW media

Objective 2

So far, isolates available for characterization include those from Gingko, Oleander, and Sweet Plum. No additional work will be performed until a larger number of isolates becomes available.

The current focus of the work is to continue to collect and identify hosts of *X. fastidiosa* in landscape hosts, confirm the presence of the pathogen using the RTS31/RST33 PCR primers and obtain isolates of the bacterium from these hosts.

Objectives 3, 4 and 5
To be completed upon the successful collection of additional *X. fastidiosa* isolates.

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Section 4A: Bacteria-Insect and Bacteria-Plant Interactions