IDENTIFICATION OF ALTERNATIVE HOSTS OF *XYLELLA FASTIDIOSA* IN THE WASHINGTON, D.C., AREA USING NESTED POLYMERASE CHAIN REACTION (PCR)

by Andrew J. McElrone[1], James L. Sherald[2], and Margaret R. Pooler[1]

Abstract. A survey of 27 herbaceous and woody plant taxa was conducted to identify possible alternative hosts of the xylem-limited bacterium, *Xylella fastidiosa*. Samples were collected from wild and cultivated plants inhabiting and surrounding the National Park Service Daingerfield Island Nursery in Alexandria, Virginia, and from national parks in Washington, D.C. These samples were analyzed using up to 3 different bacterial extraction methods and a 2-step, nested polymerase chain reaction (PCR) amplification using oligonucleotide primers specific to *X. fastidiosa*. Six out of the 27 sampled plant taxa tested positive. *Acer negundo*, *Aesculus* × *hybrid*, *Celastrus orbiculata*, *Cornus florida*, and *Hedera helix* have not been previously confirmed as hosts, while *Vitis* species have been described as wild and cultivated hosts in numerous studies. Eleven of the 27 taxa could not be tested due to failure to amplify *X. fastidiosa* DNA in the positive controls, regardless of the extraction method.

Key Words. *Xylella fastidiosa*; immunomagnetic separation; nested PCR; alternative hosts; xylem-limited bacteria.

Xylella fastidiosa (Wells et al. 1987) is a fastidious gram-negative, xylem-limited bacterium that has a very wide host range encompassing at least 30 families of monocotyledonous and dicotyledonous plants (Sherald and Kostka 1992). Many of the host plants are symptomless, but other hosts can be severely affected, exhibiting scorched leaves and stunted growth. Economically important plant diseases caused by *X. fastidiosa* include Pierce's disease of grapevine (Davis et al. 1978), alfalfa dwarf (Goheen et al. 1973), phony peach disease (Wells et al. 1983), periwinkle wilt (McCoy et al. 1978), leaf scorch of plum (Raju et al. 1982), pear (Leu and Su 1993), almond (Mircetich et al. 1976), mulberry (Kostka et al. 1986), elm, sycamore, oak (Hearon et al. 1980), maple (Sherald et al. 1987), and coffee (deLima et al., 1998), and citrus variegated chlorosis (Chang et al. 1993; Hartung et al. 1994).

Xylem-feeding insects—including leafhoppers, sharpshooters (family Cicadellidae), and spittlebugs (family Cercopidae)—transmit *X. fastidiosa* (Purcell 1995). Vector transmission of the bacteria from asymptomatic or symptomatic host plants to other plants is considered a primary means of pathogen spread (Freitag 1951; Hill and Purcell 1995). There is no adequate control of *X. fastidiosa* once it has infected a host; thus, the identification and subsequent removal of alternative hosts in the vicinity of susceptible hosts has been suggested as a possible management technique (Sherald 1995).

X. fastidiosa is of particular interest in the Washington, D.C., area due to the incidence of leaf scorch disease of elm (*Ulmus americana*) along the National Mall. Sherald et al. (1994) surveyed approximately 600 elms in this area over a 6-year period and found that 30% of these trees had leaf scorch symptoms. No study has been conducted to identify alternative hosts of *X. fastidiosa* in the Washington, D.C., area. It is unknown whether alternative hosts may be reservoirs of bacteria that infect the elms. However, polyphagous leafhopper insects in this region have tested positive for *X. fastidiosa* and are suspected of transmitting the bacterium between hosts (Pooler et al. 1997). Identification of alternative hosts could provide information needed to implement disease management techniques similar to those suggested for grape vineyard ecosystems (Raju et al. 1983; Hopkins and Adlerz 1988).

Here, we report additional natural hosts of *X. fastidiosa* surrounding the National Park Service Daingerfield Island Nursery in Alexandria, Virginia, near Washington, D.C., and in national parks in Washington, D.C. Detection assay methods included up to 3 different bacterial extraction and purification protocols, followed by a 2-step, nested polymerase chain reaction (PCR) amplification using previously

developed primers specific to *X. fastidiosa* (Pooler and Hartung, 1995).

MATERIALS AND METHODS

Samples from 27 plant taxa (Table 1) were collected on June 9 and July 31, 1998 from natural and cultivated plants inhabiting and surrounding the National Park Service Daingerfield Island Nursery in Alexandria, VA, where bacterial leaf scorch commonly affects elm. Additional samples were collected from national parks in Washington, D.C. where diseased elm, oak, and sycamore occur. Initially, plants were sampled early in the season, while leaf scorching symptoms generally appear later in the summer. Therefore, we did not follow symptom progression in the sampled hosts, nor, with the exception of the *C. florida*, did we select plants for analysis based on symptom expression. We chose relatively abundant plant species in each community, assuming that more abundant plant species would have a higher frequency of insect vector feeding and a higher probability of harboring the bacterium.

Samples were collected using hand pruners, and pruner blades were surface disinfested with 70% ethanol between samples. Plant locations were recorded to prevent re-sampling of individuals later in the season. Samples were sealed in individual plastic sandwich bags and stored at 4°C (39°F) for up to 1 month until analysis. Each plant sample was analyzed individually

Table 1. Scientific and common names, sampling date, method used for extraction, and number of samples testing positive for *X. fastidiosa* per total number tested for 27 plant taxa sampled.

Scientific name	Common name	Sampling date(s)	Extraction method[z]	No. positive/ no. tested[y]	Host[x]
Acer negundo	box elder	6/9, 7/31	1	3/24	***
Aesculus × hybrid	buckeye	7/31	2	1/11	***
Ampelopsis brevipedunculata	porcelainberry	6/9, 7/31	1, 2, 3	NA	
Artemisia spp.	mugwort	6/9, 7/31	1, 2, 3	NA	
Campsis radicans	trumpet creeper	6/9, 7/31	2	0/16	
Celastrus orbiculata	bittersweet	6/9, 7/31	2, 3	5/19	***
Chionanthus virginicus	fringetree	7/31	1	0/8	
Cotoneaster spp.	cotoneaster	7/31	1	0/5	
Cornus florida	flowering dogwood	7/31	2	1/1	***
Cornus kousa	Chinese dogwood	7/31	1	0/5	
Euonymous alatus	winged burning bush	7/31	1	0/4	
Fragaria spp.	strawberry	6/9, 7/31	3	NA	
Hedera helix	English Ivy	6/9, 7/31	2	2/19	***
Ipomoea spp.	morning glory	6/9, 7/31	3	0/20	
Lonicera japonica	Japanese honeysuckle	6/9, 7/31	1, 2, 3	NA	
Parthenocissus quinquefolia	Virginia creeper	6/9, 7/31	2	0/14	
Phytolacca americana	pokeweed	6/9, 7/31	2	0/14	
Plantago spp. # 1	plantain	6/9	3	NA	
Plantago spp. # 2	plantain	6/9	3	NA	
Malus spp.	crabapple	7/31	2	0/11	
Rhododendron spp.	azalea	7/31	1, 2, 3	NA	
Ribes spp.	currant or gooseberry	6/9	1, 2, 3	NA	
Rosa multiflora	rose (multiflora)	6/9, 7/31	1, 2, 3	NA	
Rumex spp.	dock	6/9	1, 2, 3	NA	
Trifolium pratense	red clover	6/9	3	0/10	
Trifolium repens	white clover	6/9	3	NA	
Vitis spp.	wild grape	6/9, 7/31	2	2/18	

[z]See Materials and Methods for details. For all samples, we used the simplest method that amplified DNA from the positive control. The simplest method is method #1, followed by method #2, then method #3. Samples for which the positive control reaction failed (NA) were tested using all 3 methods.

[y]NA: Testing of these samples was not possible due to unsuccessful PCR amplification.

[x]Host: *previously reported host; ***new host (uncited).

in groups separated by taxa. Each group contained a positive and negative control. Positive controls consisted of approximately 10,000 *X. fastidiosa* (isolated from citrus by John Hartung, USDA/ARS/Fruit Lab, Beltsville, MD) added to the plant material or to xylem fluid extract at the beginning of the extraction procedure. Negative controls to test for contamination of reagents or labware did not contain plant tissue or xylem fluid. Detection of *X. fastidiosa* used 1 of the following 3 methods depending on success with the particular plant taxa and the relative woodiness of the plant tissue (Table 1).

- Extraction method 1: Xylem fluid samples were extracted from 3- to 4-cm (1.2- to 1.6- in.) plant stem segments by microcentrifuging in a Marathon MicroA microcentrifuge (Fisher Scientific, Pittsburgh, PA) in a 1.5-mL tube at maximum speed for 5 minutes; bark was removed for woody taxa, and the outer surface was shaved for herbaceous taxa prior to centrifugation. Centrifuge extraction was performed only on plants with stems sufficiently rigid to withstand high forces. This extraction method resulted in a ~100 µl drop of xylem fluid that was diluted with 800 µl of 50 mM Tris-Cl, pH 7.5 buffer and vortexed for 10 seconds. A 5 µl subsample of the buffered extract was analyzed directly in a 2-step nested PCR procedure using oligonucleotide primers specific to *X. fastidiosa*. Briefly, a first round of PCR was carried out in 10 µl volume with external primers (60°C [140°F] annealing temperature). This first-round PCR reaction was then used as the template for a second round of PCR in 85 µl volume with internal primers (Pooler et al.1997).
- Extraction method 2: Xylem fluid extraction by centrifugation as described above was also used in this method. However, DNA amplification was inhibited for some plant taxa when the buffered extract was used directly in PCR. For these plant taxa, we performed immunomagnetic capture using small, uniform, paramagnetic beads coated with antibodies (Dynal Corp., Lake Success, NY) to purify and concentrate the bacteria (Pooler et al. 1997). The bacteria/bead complex was then subjected to the 2-step nested PCR procedure described in method 1.
- Extraction method 3: Small 3- to 4-cm (1.2- to 1.6-in.) plant stem or runner segments were cut into 3- to 4- mm (0.12- to 0.16-in.) pieces with a #10 surgical scalpel; bark was removed for woody taxa, and the outer surface was shaved for herbaceous taxa prior to cutting. A new scalpel blade was used for each sample to prevent cross contamination. Plant pieces were placed in a 1.5-mL microfuge tube with 800 µl of 50 mM Tris-Cl, pH 7.5 buffer. Tubes were vortexed for 10 seconds then shaken vigorously on an orbital shaker for 15 minutes at 300 rpm. This vortex/shake sequence was repeated a second time. Samples were then vortexed a third time for 5 seconds immediately prior to transferring the buffered extract to a clean 1.5-mL microfuge tube, while the plant debris was discarded. This final vortexing step ensured that the bacteria were suspended in the buffer. The buffered extract was washed 3 times by microcentrifuging for 2 minutes at maximum speed, discarding the supernatant, and resuspending the pellet in 800 µl 50 mM Tris-Cl, pH 7.5 buffer. These washes were intended to remove or dilute any possible PCR-inhibiting plant compounds present in the suspension. From this point on, we followed the immunomagnetic capture and nested PCR protocol described in method 2 above.

RESULTS AND DISCUSSION

Xylella fastidiosa bacteria were detected in 6 of the 27 taxa sampled (Table 1). *Acer negundo*, *Aesculus x hybrid*, *Celastrus orbiculata*, *Cornus florida*, and *Hedera helix* have not been previously described as hosts, while *Vitis* species have been described as both wild and cultivated hosts in numerous studies (Davis et al. 1978; Hopkins 1995).

Celastrus orbiculata (oriental bittersweet), *Hedera helix* (English ivy), and *Vitis* species (wild grape), which all tested positive for the presence of *X. fastidiosa*, were very abundant throughout the disturbed forest habitat surrounding the nursery and may play an important role in pathogen spread due to their abundance. *Celastrus orbiculata* and *Hedera helix* are both invasive exotic species that were originally introduced as ornamentals from Asia and Europe, respectively. Both species have escaped cultivation and have become

established in open woods and thickets throughout northeastern deciduous forests (Gleason and Cronquist 1991). Three of 10 *Celastrus* plant samples collected on June 9 tested positive for *X. fastidiosa* (using method 3), while 2 of 9 samples collected on July 31 tested positive (using method 2). One of 10 *Hedera* samples collected on June 9, and 1 of 9 samples collected on July 31 tested positive. One of 9 *Vitis* plants sampled on both June 9 and July 31 tested positive for *X. fastidiosa*.

Acer negundo is a common lowland native species that occurs throughout most of the United States and was moderately abundant throughout the sampled area. Seven samples were collected on June 9, 3 of which tested positive for *X. fastidiosa*. Seventeen additional samples were collected on July 31, none of which tested positive.

The genus *Aesculus* includes several native species and cultivated hybrids throughout the United States The *Aesculus* hybrids were sampled on July 31 and 1 of 11 samples were found positive. This species could serve as an additional natural source of the bacterium for weedy plants such as the vines described above as well as for other tree species.

Cornus florida, the flowering dogwood, is a native species commonly used as an ornamental, and was the only species found positive in the national park areas. The dogwood, sampled on July 31, exhibited leaf scorch symptoms and was growing in close proximity to a red oak that previously had tested positive for *X. fastidiosa*. *X. fastidiosa* previously was isolated from a leaf scorch affected dogwood growing near other diseased trees including mulberry, oak, and sycamore (Sherald, unpublished). Leaf scorching of *C. florida* has generally been attributed to water stress during extended summer droughts (Sinclair et al. 1987). The association of *X. fastidiosa* with development of scorch symptoms in *C. florida*, and experimental demonstration of causality should be further investigated.

This study is by no means a comprehensive analysis of all plants of each species or all species found in the area surrounding the nursery. We intended this study to serve as a survey of a wide range of woody and herbaceous plants which can be used as a foundation for more detailed future studies.

Bacterial detection was not possible for 11 of the 27 plant taxa surveyed due to failure of the control PCR reaction (Table 1). We used all possible extraction methods for these 11 species, but we were unable to amplify DNA in the positive controls. We suspect inhibitory chemicals of plant origin were not completely removed during buffered extract cleaning steps, and subsequent amplification of DNA was blocked in the nested PCR. Pooler et al. (1997) overcame this problem by using immunomagnetic capture to concentrate and purify the bacteria from insect vectors, but that technique was not successful in this study. We did not attempt to analyze any of the samples using ELISA. Previous studies indicate that the nested PCR method used in this study has a detection limit of 5 bacteria per sample (Pooler et al. 1997), which represents at least a thousandfold increase in sensitivity compared to ELISA. It is possible that some of the samples in which the positive PCR control reactions failed could be adequately tested using ELISA.

Artemisia species (Freitag 1951, Hill and Purcell 1995) and *Fragaria* species (Raju et al. 1980) have been previously reported as hosts. However, we were unable to amplify bacterial DNA from these and other species. Our inability to detect *X. fastidiosa* in several plant species does not preclude their possible importance as reservoirs in this ecosystem. The wide geographic range of many of these "unanalyzable" species (e.g. *L. japonica*, *Artemisia* spp., *Ribes* spp., and *Rumex* spp.) suggests that, if susceptible to *X. fastidiosa* and attack by its insect vectors, they could be an important source of inocula in many habitats. Further work to perfect this detection technique to accommodate more plant species is necessary in order to gauge the potential role that these and other plants may play in the spread of *X. fastidiosa* to cultivated hosts. Despite its current limitations, this detection technique is very sensitive and, with modification, should prove useful in the future for detecting *X. fastidiosa* in a wide range of plant and insect species.

LITERATURE CITED

Chang, C. J., M. Garnier, L. Zreik, V. Rossetti, and J.M. Bove. 1993. Culture and serological detection of xylem-limited bacterium causing citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. Curr. Microbiol. 27:137–142.

Davis, M. J., A.H. Purcell, and S.V. Thomson. 1978. Pierce's disease of grapevines: Isolation of the causal bacteria. Science 199:75–77.

deLima, J.E.O., V.S. Miranda, J.S. Hartung, R.H. Brlansky, A. Coutinho, S.R. Roberto, and E.F. Carlos. 1998.

Coffee leaf scorch bacterium: Axenic culture, pathogenicity, and comparison with *Xylella fastidiosa* of Citrus. Plant Dis. 82: 94-97.

Freitag, J.H. 1951. Host range of the Pierce's disease virus of grapes as determined by insect transmission. Phytopathology 41:920–934.

Gleason, H.A., and A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. The New York Botanical Garden, Bronx, NY.

Goheen, A.C., G. Nyland, and S.K. Lowe. 1973. Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63:341–345.

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

Hearon, S.S., J.L. Sherald, and S.J. Kostka. 1980. Association of xylem-limited bacteria with elm, sycamore, and oak leaf scorch. Can. J. Bot. 58:1986–1993.

Hill, B.L., and A.H. Purcell. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology 85:1368–1372.

Hopkins, D.L. 1995. *Xylella fastidiosa*. In Singh, U.S., R.P. Singh, and K. Kohmoto (Eds.). Pathogenesis and Host Specificity in Plant Diseases: Histopathological, Biochemical, Genetic and Molecular Bases. Vol. 1: Prokaryotes. Elsevier Science, Inc. New York, NY.

Hopkins, D.L., and Adlerz, W.C. 1988. Natural hosts of *Xylella fastidiosa* in Florida. Plant Dis. 72:429–431.

Kostka, S.J., T.A. Tattar, and J.L. Sherald. 1986. Elm leaf scorch: Abnormal physiology in American elms infected with fastidious, xylem-inhabiting bacteria. Can. J. For. Res. 16:1088–1091.

Leu, L.S., and C.C. Su. 1993. Isolation, cultivation, and pathogenicity of *Xylella fastidiosa*, the causal bacterium of pear leaf scorch disease. Plant Dis.77:642 –646.

McCoy, R.E., D.L. Thomas, J.H. Tsai, and W.J. French. 1978. Periwinkle wilt, a new disease associated with xylem delimited rickettsialike bacteria transmitted by a sharpshooter. Plant Dis. Report. 63:1022–1026.

Mircetich, S. M., S.K. Lowe, W.J. Moller, and G. Nyland. 1976. Etiology of the almond leaf scorch disease and transmission of the causal agent. Phytopathology 66:17–24.

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.

Pooler, M.R., I.S. Myung, J. Bentz, J. Sherald, and J.S.Hartung. 1997. Detection of *Xylella fastidiosa* in potential insect vectors by immunomagnetic separation and nested Polymerase Chain Reaction. Lett. Appl. Microbiol. 25:123–126.

Purcell, A.H. 1995. Transmission and epidemiology. In Sherald, J.L., and A.B. Gould (Eds.). *Xylella fastidiosa* and associated diseases. Plant Diagn. Q. 16(3):111–115.

Raju, B.C., J.M. Wells, G. Nyland, R.H. Brylansky, and S.K Lowe. 1982. Plum leaf scald: isolation, culture, and pathogenicity of the causal agent. Phytopathology 72:1460–1466.

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.

Sherald, J.L. 1995. Leaf scorch of amenity trees. In Sherald, J.L. and A.B. Gould (Eds.). *Xylella fastidiosa* and associated diseases. Plant Diagn. Q. 16(3):119–123.

Sherald, J.L., J.M. Wells, S.S. Hurtt, and S.J. Kostka. 1987. Association of fastidious, xylem-inhabiting bacteria with leaf scorch in red maple. Plant Dis. 71:930–933.

Sherald, J.L., and S.J. Kostka. 1992. Bacterial leaf scorch of landscape trees caused by Xylella fastidiosa. J. Arboric. 18(2):57–63.

Sherald, J.L., E.N. Patton, T.M. Stidham, and C.L. Favre. 1994. Incidence and development of bacterial leaf scorch of elm on the National Mall. J. Arboric. 20(1):18–23.

Sinclair, W.A., H.H. Lyon, and W.T. Johnson. 1987. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, NY.

Wells, J.M., B.C Raju, and G. Nyland. 1983. Isolation, culture, and pathogenicity of the bacterium causing phony disease of peach. Phytopathology 73:859–862.

Wells, J.M., B.C. Raju, H.Y. Hung, W.G. Weisburg, L. Mandelco-Paul, and D.J. Brenner. 1987. Xylella fastidiosa: Gram-negative, xylem-limited, fastidious plant bacteria related to Xanthomonas. Int. J. Syst. Bacteriol. 37:136–143.

Acknowledgments. We are grateful for the financial support provided by the ISA Research Trust John Z. Duling Grant Program, which partially supported Andrew McElrone's internship at the U.S. National Arboretum. We also thank Erin Sweeny for technical assistance with sample preparation.

[1*]*U.S. National Arboretum*
Floral and Nursery Plants Research Unit
United States Department of Agriculture
3501 New York Ave. NE
Washington, DC 20002

[2]*National Park Service*
National Capital Region
Center for Urban Ecology
4598 MacArthur Blvd. NW
Washington, DC 20007-4227

**Corresponding author: Margaret R. Pooler*

Résumé. Un inventaire sur 27 espèces de plantes herbacées et ligneuses a été mené pour identifier les hôtes alternatifs potentiels de la bactérie du xylème *Xylella fastidiosa.* Des échantillons ont été collectés sur des plantes sauvages et cultivées à l'intérieur et autour de la pépinière de Daingerfield Island du Service des parcs nationaux à Alexandria en Virginie ainsi que dans les parcs nationaux du district de Washington, et ce afin de les analyser en utilisant jusqu'à trois méthodes différentes d'extraction et une technique en deux étapes de réaction en chaîne de polymérase. La bactérie *X. fastidiosa* a été détectée sur six des 27 échantillons de plantes. *Acer negundo, Aesculus* × hybrid, *Celastrus orbiculata, Cornus florida* et *Hedera helix* n'avait pas encore été décrits comme hôtes, alors que les espèces de Vitis, cultivés ou sauvages, avaient déjà été décrites comme hôtes dans plusieurs études. Onze des 27 espèces n'ont pu être testées en raison de l'échec des tests de réaction en chaîne de polymérase. Les réactions positives de ces 11 espèces n'étaient pas valables peu importe la méthode d'extraction ; elles étaient probablement dues à l'inhibition de l'amplification d'ADN par des composés végétaux non identifiés.

Zusammenfassung. In einer Untersuchung von 27 krautigen Pflanzen und Gehölzen wurde versucht, mögliche, alternative Wirte für das xylem-limitierende Bakterium *Xylella fastidiosa* zu identifizieren. Die Proben wurden wilden und kultivierten Pflanzen in und um den Nationalparkdienst Daingersfield Island Nursery in Alexandria, VA und aus den Nationalparks in Washington DC. Entnommen und unter Anwendung von 3 verschiedenen Extraktionsmethoden und einer zweischrittigen Polymerase-Kettenreaktion-Technik (PCR) analysiert. *X. fastidiosa* wurde in 5 von 27 erprobten Pflanzenarten gefunden. *Acer negundo, Aesculus* × Hybriden, *Celastrus orbibulata, Cornus florida* und *Hedera helix* wurden bislang nicht als Wirtspflanzen beschrieben, während Vitis Arten in zahlreichen Studien sowohl als wilde, wie auch als kultivierte Pflanze als Wirt beschrieben wurde. Elf von den 27 Arten konnten wegen eines Fehlers bei der PCR-Technik nicht getestet werden. Die positiven Reaktionen dieser elf Pflanzen waren von der Extrakzionsmethode unbrauchbar und sie entstanden vermutlich hauptsächlich durch die Hemmung der DNA Amplifikation durch unbestimmte Pflanzenteile.

Resumen. Fue conducido un estudio de 27 especies de plantas herbáceas y leñosas para identificar los posibles hospederos alternativos de la bacteria del xilema, *Xylella fastidiosa.* Las muestras fueron colectadas del campo y plantas cultivadas que habitan y merodean el parque nacional Daingerfield Island en Alexandria, VA y de los parques nacionales en Washington, DC. Fueron analizadas usando tres diferentes métodos de extracción y una técnica de dos pasos de Reacción en Cadena de Polimerasa (PCR) anidada. Fueron detectadas bacterias de *X. fastidiosa* en 6 de las 27 plantas muestreadas. *Acer negundo, Aesculus* × hybrid, *Celastrus orbiculata, Cornus florida,* y *Hedera helix* no han sido descritas anteriormente como hospederos, mientras especies de Vitis han sido descritas como hospederos cultivados y silvestres en numerosos estudios. Once de las 27 especies no pudieron ser probadas debido a fallas de las reacciones de PCR. Las reacciones positivas de estas 11 especies fueron infructuosas, sin importar el método de extracción, y esto se debió probablemente a la inhibición del DNA por componentes de las plantas no identificados.