Pathogenicity of *Xylella fastidiosa* in American Elm and Failure of Reciprocal Transmission Between Strains from Elm and Sycamore

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ABSTRACT

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Pathogenicity of *Xylella fastidiosa* was demonstrated in American elm seedlings. Ten 4-mo-old seedlings were stem-inoculated with a pure culture of *X. fastidiosa* obtained from a naturally infected elm. One year after inoculation, all inoculated seedlings developed leaf scorch symptoms characteristic of naturally infected trees. *X. fastidiosa* was isolated from six inoculated seedlings but not from 10 symptomless control seedlings. Isolates of *X. fastidiosa* obtained from a naturally infected elm and a previously inoculated sycamore were pathogenic in seedlings of elm and sycamore, respectively, but not in reciprocal hosts.

The fastidious, xylem-inhabiting bacterium *Xylella fastidiosa* Wells et al (20) has a wide host range that includes both monocotyledonous and dicotyledonous plants (4,9). Many species are asymptomatic, while others such as grape (Pierce's disease of grape), peach (phony disease of peach), and almond (almond leaf scorch) are severely affected. Although *X. fastidiosa* is classified as a single species (20), there is a complex and barely defined relationship between hosts and strains of this unique pathogen (8).

Chronic leaf scorch symptoms in American elm (*Ulmus americana* L.) (6), red oak (*Quercus* spp.) (1,5,6,11), red maple (*Acer rubrum* L.) (19), red mulberry (*Morus rubra* L.) (13), and American sycamore (*Platanus occidentalis* L.) (15) have been associated with *X. fastidiosa*. Bacterial leaf scorch is widespread in the mid-Atlantic and southeastern states, but its distribution and significance in forest and amenity trees is not well understood (16).

Elms, red oaks, sycamores, and mulberries infected with *X. fastidiosa* are commonly found growing close to each other. Therefore, it may be that these species are affected by the same strain or strains of *X. fastidiosa*. Pathogenicity of *X. fastidiosa* isolated from oak, mulberry, and sycamore has been demonstrated in the respective hosts by mechanical inoculation (1,13,15,17). Although graft transmission of *X. fastidiosa* has been demonstrated in elm, attempts at mechanical inoculation have not been successful (6,10,21). This study was undertaken to demonstrate the pathogenicity of *X. fastidiosa* in elm by mechanical inoculation and to determine if strains of *X. fastidiosa* isolated from elm and sycamore are pathogenic in the reciprocal host. A preliminary report has been published (14).

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MATERIALS AND METHODS

Pathogenicity in elm. A strain of *X. fastidiosa* was obtained from a naturally infected nursery elm that showed leaf scorch symptoms the previous year. Stem sections (1–1.5 × 15–20 cm) were collected on 18 July 1988 from branches with leaf scorch symptoms. Three or four wood chips (0.5 × 1.5 cm) were aseptically removed and incubated at 28 C in test tubes with 25 ml of modified periwinkle broth medium (PWM) (2,15). Broth cultures were examined after 10 days by phase contrast microscopy (1,000×) for bacteria characteristic of *X. fastidiosa*. Bacteria were 0.3–0.5 × 1–3 μm and often had dark regions at one or both ends of the organism. Positive cultures were transferred to semisolid PWM by flooding the center of two or three petri plates with 0.5 ml of broth from one positive culture. After incubation for 2 wk at 28 C, bacteria were rinsed from plates with a phosphate-buffered citrate magnesium solution (PBCM) (3). Inoculum was standardized in PBCM at an OD of 0.07–0.10 at 560 nm (10^7–10^8 cells per milliliter) with a Bausch & Lomb Spectronic 710 spectrophotometer.

Ten American elm seedlings (4 mo old, 20 cm tall) were inoculated with the elm strain on 12 August 1988. Ten control seedlings were treated similarly with PBCM. A drop of inoculum (approximately 0.025 ml) was placed with a syringe at three points on the stem. A scalpel incision was made into the stem through the drop in a spiral direction along the stem axis. The inoculum was usually absorbed immediately by the stem.

Seedlings were grown in a potting mixture of ProMix BX, perlite, and potting soil (1:1:1, v/v) in 10-cm-diameter plastic pots. Plants were held in the greenhouse (7–35 C) from September 1988 to May 1989, when they were transferred to 5.6-L plastic containers and placed outside under trickle irrigation. Leaf scorch–affected leaves and unaffected leaves were counted on each seedling biweekly from 2 June to 31 August 1989 and again on 4 October 1989. Seedling height was measured in June, July, and October. Stem caliper was measured in October 1989 with a micrometer at 7.5 cm above the soil line.

The stem of each plant was removed at 10 cm above the ground on 4 October 1989, and wood chips were removed for isolation of bacteria as described above. Strains were examined by phase contrast microscopy for bacteria characteristic of *X. fastidiosa*. Positive cultures were maintained on semisolid PWM and sent to Agdia, Inc., Elkhart, Indiana, to be tested with the ELISA kit Pathoscreen xf, developed for the detection of *X. fastidiosa* (18).

Reciprocal transmission. Inoculum was produced from strains obtained from a naturally infected elm that had shown severe leaf scorch for 4 yr and from a nursery-grown American sycamore that had been mechanically inoculated in 1981 with *X. fastidiosa*. The sycamore had shown leaf scorch symptoms annually since 1982.

On 10 August 1989, 4-mo-old elm (95 cm tall) and sycamore (1.10 m tall) seedlings were inoculated at five to seven points on the stem as described previously. Ten seedlings of each species were inoculated with strains from elm and sycamore. Control seedlings were inoculated with PBCM. Seedlings were overwintered in the greenhouse (4–37 C) from September 1989 to May 1990. Plants were then transferred to 11-L containers and placed outside under trickle irrigation until October 1991. Plants were evaluated for leaf scorch symptoms biweekly from May through August in 1990 and 1991. Stem sections were collected from all seedlings between August and October 1991 for bacterial isolation and testing as described above.

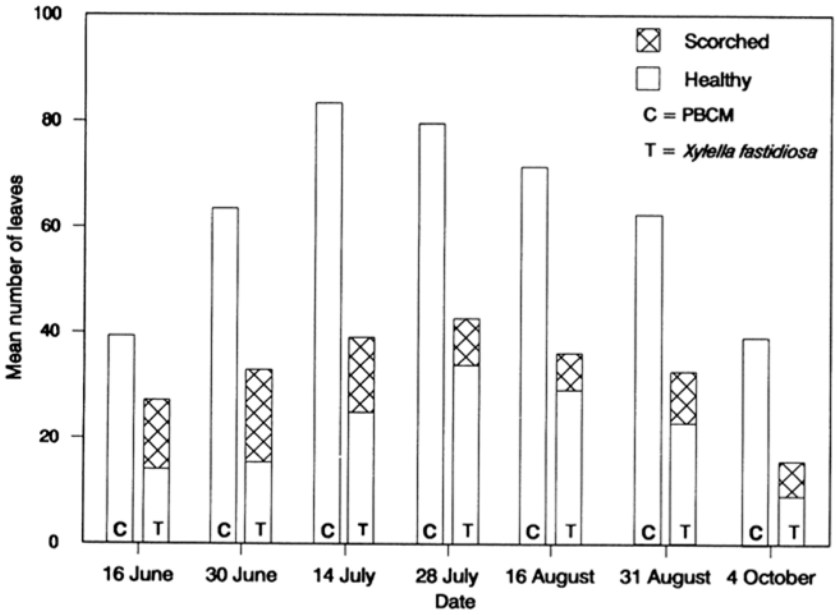

Fig. 1. Number of scorch-affected and unaffected leaves of 10 American elm seedlings inoculated with either *Xylella fastidiosa* or phosphate-buffered citrate magnesium (PBCM). Seedlings were treated in August 1988 and evaluated from June through October 1989.

Fig. 2. Elm seedling showing severe marginal necrosis, leaf curl, and abscission 1 yr after being inoculated with *Xylella fastidiosa*.

RESULTS

Pathogenicity in elm. Leaf scorch symptoms were first observed 2 June 1989 on seven of 10 elm seedlings inoculated in August 1988. All 10 seedlings developed symptoms by 16 June, with over 50% of their leaves affected (Fig. 1). The seedlings showed symptoms characteristic of naturally infected trees, first an olive drab discoloration and later an irregular marginal necrosis separated from green tissue by a narrow chlorotic halo. Symptoms were most severe on the older leaves toward the bottom of the stem and decreased in severity on more recently developed leaves at the growing tip (Fig. 2). Severely scorched leaves curled and abscised by late June. New growth, which began to form in July, developed symptoms by mid-August. All control seedlings remained symptomless. Leaf abscission beginning in July exceeded new leaf development and accounted for the decline in mean number of leaves examined in control and treated plants from July to October. By October, 14 mo after inoculation, terminal elongation of treated seedlings was reduced 32% compared to controls (Fig. 3). The caliper of treated seedlings was 0.48 cm, significantly less than the 0.70 cm of the controls ($P = 0.05$; PROC MEAN, t test; SAS System, SAS Institute, Cary, NC).

Bacteria characteristic of *X. fastidiosa* were isolated from six of the 10 inoculated seedlings. Strains isolated from five of the seedlings yielded positive ELISA reactions for *X. fastidiosa* (OD 4.000–0.834) and another strain gave a weakly positive reaction (OD 0.143). Bacteria could not be isolated from four of the inoculated seedlings or from any of the control seedlings.

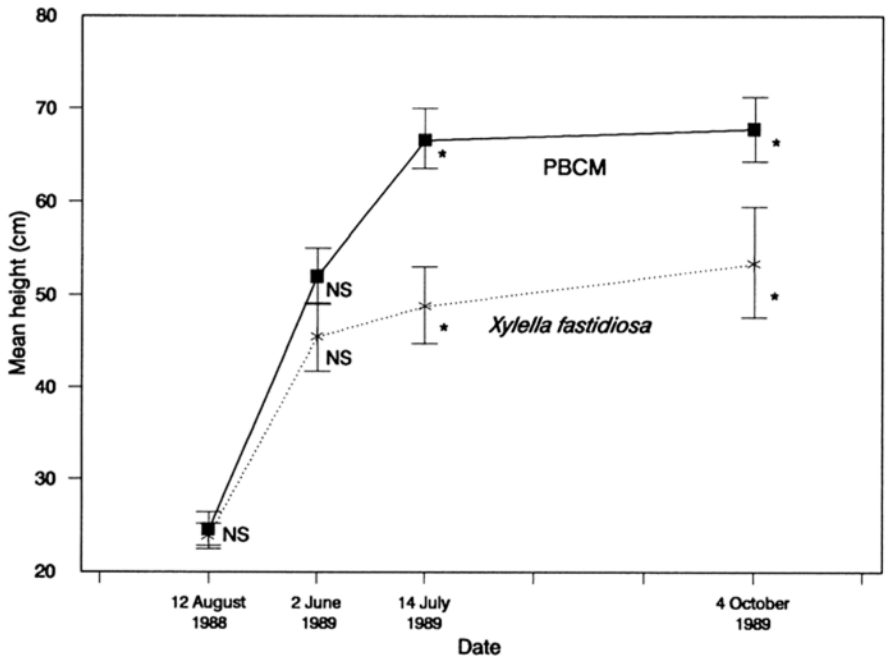

Fig. 3. Mean height of 10 American elm seedlings inoculated with either *Xylella fastidiosa* or phosphate-buffered citrate magnesium (PBCM). Vertical bars represent standard errors. NS = no significant difference; * = significant difference ($P \leq 0.05$) between treatments on that date according to analysis of variance.

Reciprocal transmission. All elm seedlings inoculated in 1989 with *X. fastidiosa* isolated from a naturally infected elm developed leaf scorch symptoms for 2 yr after inoculation (Table 1). Leaf scorch first appeared in June 1990 and progressed in severity throughout the summer. Leaves developed an irregular marginal necrosis with a chlorotic border typically found on naturally affected elms. Symptoms developed more rapidly in the second year after inoculation. All seedlings showed symptoms by 12 July. Affected leaves of two seedlings abscised and new growth remained symptomless on 29 August 1990 (Table 1). *X. fastidiosa* was isolated from seven of the 10 seedlings. Six of the seven strains were tested with the ELISA and gave positive reactions (OD 0.353–0.261). None of the elm seedlings inoculated with PBCM or with the sycamore strain developed leaf scorch symptoms in 1990. In 1991, however, two PBCM-treated controls developed symptoms in 20 and 30% of their crowns, and

X. fastidiosa was isolated from one of these trees (Table 1). One elm seedling inoculated with *X. fastidiosa* from sycamore developed leaf scorch symptoms in 5% of the crown in 1991, but *X. fastidiosa* could not be isolated from this tree (Table 1). All other elm seedlings treated with PBCM or the sycamore strain remained symptomless in 1991 (Table 1), and *X. fastidiosa* could not be isolated from them.

Eight of 10 sycamore seedlings inoculated with *X. fastidiosa* isolated from sycamore developed leaf scorch symptoms in 1990 and 1991. Two other inoculated seedlings remained symptomless in both years. Leaves developed typical marginal and interveinal necrosis bordered by a reddish band of tissue. Symptom severity progressed from older to younger leaves, with most of the affected leaves remaining attached. In contrast to elms, symptoms developed faster on sycamore the first year after inoculation than the second. In 1990, symptoms were first apparent on 15 June, and all affected trees showed symptoms by 9 July. In 1991, symptoms were not seen until 12 July, and the eight affected seedlings were not all symptomatic until 9 August (Table 1). *X. fastidiosa* was isolated from seven of the eight symptomatic trees but not from the two symptomless trees. The remaining symptomatic tree died and could not be sampled. All seven isolates responded with a positive ELISA reaction for *X. fastidiosa* (OD 1.534–0.266). None of the sycamores inoculated with buffer or with the elm strain developed leaf scorch symptoms, and *X. fastidiosa* could not be isolated from any of these seedlings 2 yr after inoculation.

DISCUSSION

The pathogenicity of *X. fastidiosa* has been demonstrated previously in American sycamore, red oak, and red mulberry (1,13,15,17). Seedlings of each species were successfully inoculated with strains of *X. fastidiosa* isolated from naturally infected hosts; several mechanical inoculation techniques were used. Sycamore seedlings were successfully inoculated either by infusion of inoculum through a severed root or by injection of the stem with a syringe (15,17). Seedling oaks have been successfully inoculated by the same root infusion technique used with sycamore (1). A combination of root and stem infusion and stem injection was used to demonstrate pathogenicity of *X. fastidiosa* in red mulberry (13). Pathogenicity has not been demonstrated in red maple. In elm, *X. fastidiosa* has been transmitted only by grafting (6,21). Previous attempts to inoculate elm with *X. fastidiosa* by root infusions, stem infusions, and stem injections have been unsuccessful (10). Failure to demonstrate pathogenicity in elm was attributed to several possible factors, including juvenile resistance of elm seedlings, loss of virulence of the strains used, inappropriate inoculation route, and/or a lack of critical edaphic or environmental factors (10).

These studies demonstrated that American elm seedlings are susceptible to *X. fastidiosa* and that stem inoculation is an effective and reliable inoculation procedure. In the two experiments performed in 1988 and 1989, all 20 elm seedlings inoculated with strains of *X. fastidiosa* developed leaf scorch symptoms typical of naturally infected trees. Terminal elongation of inoculated seedlings was significantly reduced, similar to that observed in naturally infected trees (12). Bacteria morphologically identical to the inoculum were isolated from 13 of the 20 seedlings and confirmed as *X. fastidiosa* by ELISA.

Successful demonstration of pathogenicity may be attributed to the short period (24 days) that bacteria were maintained in culture before inoculation. Some strains of the Pierce's disease pathogen have been shown to lose pathogenicity in culture (7). Although *X. fastidiosa* has been described as a single species, there are at least two host-specific pathovar groups. Pierce's disease of grape, almond leaf scorch, and alfalfa dwarf are all caused by the same strain or strains. Similarly, there is cross pathogenicity between strains causing plum leaf scald and peach phony disease. There is, however, no evidence of cross pathogenicity between these two groups. The relationship of other strains to these and possibly other pathovar groups is unclear.

Because leaf scorch-affected elms, oaks, sycamores, and mulberries commonly occur close to each other in landscape settings and on the edge of natural areas, it was suspected that these tree species were affected by the same or closely related strains. This study, however, showed that strains from elm and sycamore were only pathogenic in their respective hosts and not in the reciprocal species. There was also no evidence that bacteria multiplied in the reciprocal species 2 yr after inoculation. It is likely that the two symptomatic control elms and the one symptomatic elm inoculated with the sycamore strain became naturally infected with a strain pathogenic to elm. All seedlings were held in the same bed, and other elm nursery stock maintained near this bed also became infected.

This study further illustrates the host/pathovar complexity of *X. fastidiosa*. Clearly, more work is needed to unravel the host range and relationship of strains affecting amenity trees. Likewise, the relationship between strains from trees and those of other known hosts should be examined. Recognition of other species, herbaceous or woody, that harbor strains pathogenic to amenity trees may be useful in developing disease management strategies. For example, in the Napa Valley of California, Pierce's disease of grape is controlled by keeping grapes 100 m from permanent bodies of water where weeds are known to harbor the pathogen and leafhopper vectors (8). Also, strain-specific diagnostic procedures based on monoclonal antibodies and nucleic acid probes would be useful in unraveling the complex host/pathovar relationships associated with this unique species of bacterium.

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Table 1. Number of elm and sycamore seedlings showing leaf scorch symptoms 1 and 2 yr after inoculation with strains of *Xylella fastidiosa* isolated from elm and sycamore or with phosphate-buffered citrate magnesium (PBCM)[a]

	Symptomatic elm seedlings			Symptomatic sycamore seedlings		
Date	PBCM	Elm strain	Sycamore strain	PBCM	Elm strain	Sycamore strain
1990						
15 May	0	0	0	0	0	0
15 June	0	1	0	0	0	2
29 June	0	3	0	0	0	7
9 July	0	7	0	0	0	8
19 July	0	9	0	0	0	8
1 Aug.	0	10	0	0	0	8
29 Aug.	0	8	0	0	0	8
1991						
21 May	0	0	0	0	0	0
14 June	0	5	0	0	0	0
28 June	0	9	0	0	0	0
12 July	1	10	1	0	0	1
26 July	1	10	1	0	0	4
9 Aug.	1	10	1	0	0	8
22 Aug.	2	10	1	0	0	8

[a] In August 1989, suspensions of *X. fastidiosa* were injected into stems of 10 4-mo-old elm seedlings and 10 4-mo-old sycamore seedlings; PBCM was injected into 10 control seedlings.

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