Sycamore Leaf Scorch: Culture and Pathogenicity of Fastidious Xylem-Limited Bacteria from Scorch-Affected Trees

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ABSTRACT

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Morphologically identical gram-negative bacteria serologically related to the Pierce's disease (PD) and elm leaf scorch bacteria were cultured from 24 of 25 scorch-affected sycamores (*Platanus occidentalis*) in Washington, DC, Richardson, TX, and New Orleans, LA, by incubating wood chip samples in a liquid medium similar to that used for culture of the periwinkle wilt agent. Isolates then grew readily on media developed for the PD bacterium. Roots of 19 sycamore seedlings were inoculated with one isolate from scorched sycamore. Five months after inoculation, bacteria were reisolated from 11 of the seedlings, seven of which showed leaf scorch symptoms. No bacteria were isolated from 20 control trees.

Leaf scorch commonly affects sycamores in natural stands, plantations (2,7,16), and urban landscapes (9,16). Xylem-limited bacteria resembling the Pierce's disease (PD) pathogen have been associated with leaf scorch in elm (9,11,12,13), red oak, and sycamore in Washington, DC (9). Bacteria vacuum-extracted from American elms and red oaks affected with leaf scorch were serologically related to the PD bacterium in indirect immunofluorescent antibody serological tests (IFAS) (9).

In this paper, we report 1) the isolation and culture of bacteria from scorch-affected sycamores in Washington, DC; Richardson, TX, a suburb of Dallas, TX; and New Orleans, LA; 2) the serological relationship between sycamore leaf scorch bacteria and the PD and elm leaf scorch (ELS) bacteria; and 3) the pathogenicity of one isolate upon inoculation to sycamore seedlings. A preliminary report has been published (17).

MATERIALS AND METHODS

Isolation and culture of bacteria. Stem samples were collected from scorch-affected and symptomless sycamores (20–90 cm diam. at 1.4 m above the ground) in Washington, DC, and Richardson, TX, from March through September 1980–1981 and from scorch-affected trees in New Orleans, LA, in August and November 1981. Most of the trees examined were American sycamores, *Platanus occidentalis* L. Some, however, had a few paired fruits, a characteristic of the London plane tree, *P.* × *acerifolia* (Ait.) Willd., and their classification was uncertain. Bacteria were vacuum-extracted from stem sections (8) in phosphate-buffered saline (pH 7.0) and examined by phase-contrast microscopy.

Stem sections measuring 1–1.5 × 15–20 cm were rinsed with 70% ethanol and flamed. The outer bark was removed, then two or three wood chips measuring 0.5 × 1.5 cm were excised from each stem and incubated at 28 C in 25 ml of periwinkle wilt (PW) broth medium (5) supplemented with 0.85 g $(NH_4)_2HPO_4$, 2 g potato starch, 1 g histidine, and 25 mg cycloheximide per liter. Occasionally, wood chips were incubated in S-8 medium developed for the ratoon stunt bacterium (4). Isolates were subcultured onto semisolid PW medium supplemented in the same manner as the PW broth and then maintained through biweekly transfers on semisolid PD-2 or PD-4 media (3) at 28 C.

Serology. Indirect IFAS was performed as described previously (9). Smears of stem extracts and/or cultured isolates from each locality were reacted with antisera produced to the PD and ELS bacteria. Smears of cultured PD and ELS bacteria were treated in the same manner. Control slides were reacted with rabbit preimmune serum.

Pathogenicity tests. Sycamore seedlings about 60 cm tall were inoculated in June 1981 with a bacterial isolate obtained in January 1981 from a scorch-affected sycamore in Washington, DC. Bacteria were maintained on PD-4, rinsed from plates of this medium 14 days after transfer, and washed once in phosphate-buffered citrate-magnesium solution (PBCM) (5) by centrifugation at 7,500 *g* for 10 min in an SS 34 rotor. Concentration was adjusted to 1×10^8 cells per milliliter of PBCM based on cell counts made with a Petroff-Hausser Bacteria Counter.

Twenty seedlings were unpotted and one large root of each was severed. The severed root was connected immediately by a short piece of Tygon tubing to a 10-ml pipette containing 10 ml of the bacterial suspension. Seedlings were repotted with the pipette reservoirs connected to the root. Roots of 20 control seedlings were similarly infused with PBCM. Most of the volume was absorbed in 3 days. The amount absorbed by each seedling varied, however, and was not measured. Plants were maintained in a greenhouse and watered sparingly through November 1981. In November, the upper half of each seedling was removed and segments (about 8 cm) were vacuum-extracted with 2 ml phosphate-buffered saline, pH 7.0. The extracts were centrifuged at 7,500 *g* for 10 min in an SS 34 rotor. Pellets were resuspended in 0.5 ml of 0.01 M phosphate buffer and examined under phase-contrast light microscopy or in sodium or potassium phosphotungstate negative stains and examined by electron microscopy. Wood chips from six control seedlings and the stem segments of seedlings in which bacteria were detected by examination of vacuum extracts were incubated in supplemented PW broth.

Electron microscopy. Leaf samples were collected in July 1980 in Richardson, TX, from four scorch-affected and four symptomless sycamores, about 30 cm diameter at 1.4 m above the ground, and

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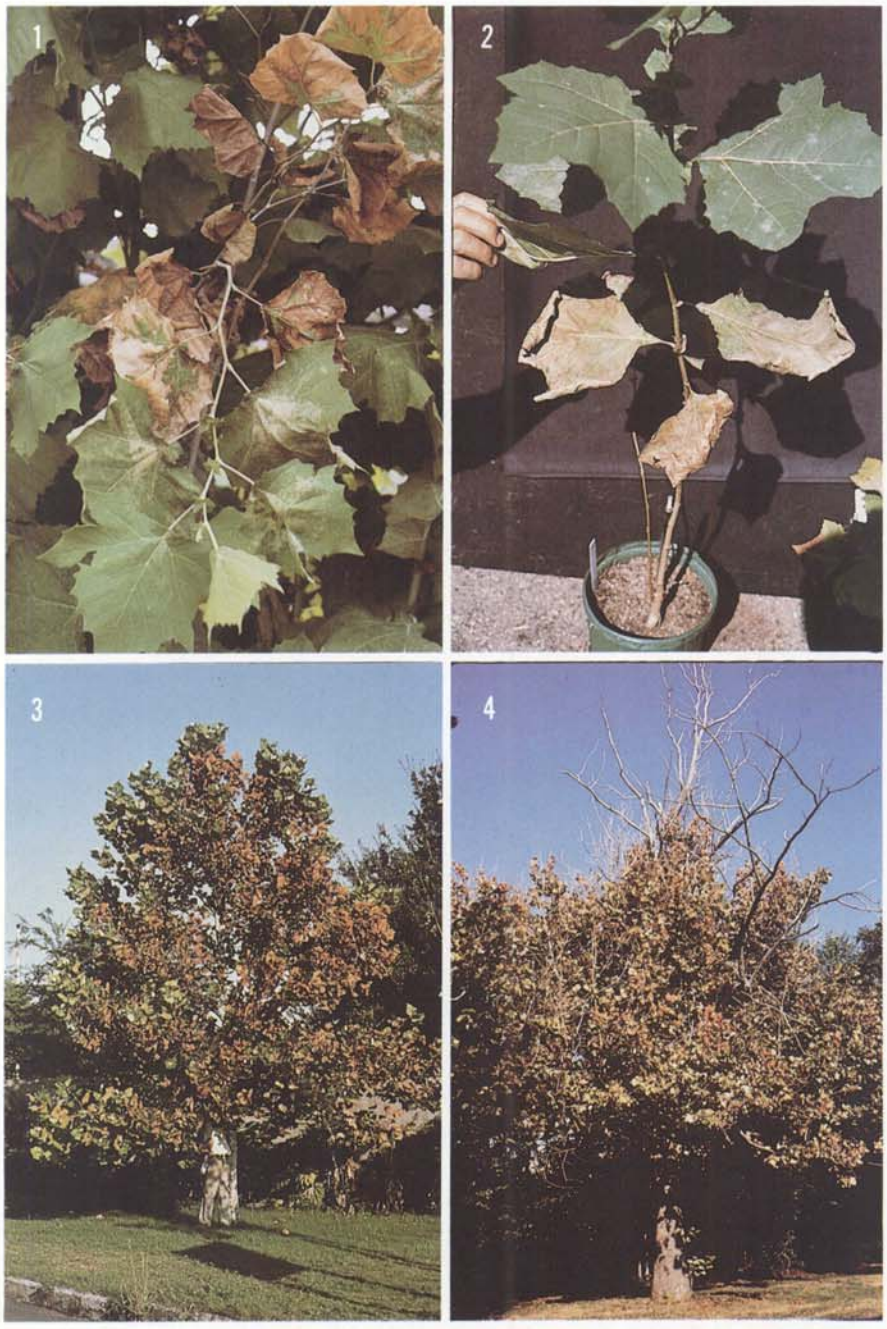
shipped to Beltsville, MD, for electron microscopy. Primary and secondary veins were excised from nonnecrotic tissue adjacent to scorched tissue and from comparable areas in symptomless leaves and prepared for ultrastructural examination (9). Ultrathin sections of the vascular tissue of one inoculated symptomatic sycamore seedling were similarly prepared and examined.

Colonies of one Texas isolate growing on semisolid PD-2 medium were overlaid with a thin layer of 0.8% agarose in distilled water. Small blocks containing colonies sandwiched in medium and agarose were then fixed in 0.05 M phosphate-buffered glutaraldehyde-acrolein, postfixed in osmium tetroxide, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate or lead citrate only and examined by transmission electron microscopy.

In conjunction with phase-contrast studies, negative stain preparations were made of cultured bacteria and bacteria in vacuum extracts of naturally infected trees and inoculated seedlings. Bacterial cells were concentrated and washed by low-speed centrifugation and resuspension of pellets in 0.01 M phosphate buffer, pH 7. A drop of suspension was left on Formvar-coated grids for 1–2 min, mixed with an equal volume of negative stain, pH 7, and excess liquid was removed with filter paper after 15–30 sec. The grid was rinsed immediately with one or two drops of double-distilled water and allowed to dry.



Figs. 1–4. Sycamore leaf scorch: **(1)** Branch of scorch-affected tree. Basal leaves show marginal and interveinal necrosis, whereas terminal leaves are unaffected. Stippling on terminal leaves was caused by insects. **(2)** Sycamore seedling inoculated with a bacterial isolate from a scorch-affected sycamore. Lower leaves show scorch. **(3)** Severely scorched sycamore in New Orleans. Many terminal leaves are symptomless. **(4)** Crown dieback and severe leaf scorch in a tree in Washington, DC.

RESULTS

Symptomatology. Leaf scorch begins to develop in late June or July as an olive-green discoloration of marginal and interveinal tissues. Severely scorched leaves turn brown, curl, and remain attached to the branch (Figs. 1 and 2). Terminal leaves on the current year's growth are usually the last to scorch or may not scorch at all (Figs. 1 and 2). Leaf scorch may appear in individual branches or throughout the crown (Fig. 3). Sycamores with a history of leaf scorch are slow to foliate and produce fewer and occasionally smaller leaves than unaffected trees. Limb dieback (Fig. 4) and tree mortality occur in trees showing severe and prolonged leaf scorch. Symptoms appeared to be more prevalent and severe in Texas and Louisiana than in Washington, DC.

Isolation and culture of bacteria. Bacteria with identical morphological (Figs. 5 and 6) and cultural characteristics occurred in trees in Washington, Richardson, and New Orleans (Table 1). Bacteria were detected in vacuum extracts of 17 of the 18 scorched-affected and two of the 17 symptomless trees examined (Table 1). Bacteria were cultured from 24 of the 25 scorch-affected trees but from only one symptomless Texas tree, the same tree from which bacteria were vacuum-extracted (Table 1). Presence of bacteria in symptomless trees may indicate presymptomatic infection.

Bacterial growth was evident after wood chips were incubated for 2–4 wk in supplemented PW or S-8 broth. Growth in PD media was most frequent when new isolates were transferred to semisolid modified PW medium before being transferred to PD media. White, convex, entire colonies measuring 0.1–0.6 mm diameter developed on PD media after incubation for 2–4 wk. No differences were observed in colony development or morphology among isolates from the same or different locations. Growth was not observed on potato-dextrose or King's medium B agars. Isolates were gram-negative and occurred singly and in chains of two or three cells. Dark areas were observed at one or both ends of the organisms under phase contrast and in negative stain preparations and ultrathin sections (Figs. 5 and 6) observed by electron microscopy.

Serology. Extracted and cultured isolates from each locality fluoresced strongly when reacted with PD or ELS

antisera in indirect IFAS tests. PD and ELS bacteria also fluoresced strongly with either antisera. Isolates reacted with rabbit preimmune serum did not fluoresce.

Pathogenicity tests. One of 20 inoculated seedlings died shortly after inoculation. Seven of 19 seedlings developed leaf scorch 5 mo after inoculation (Fig. 2). Bacteria serologically related to the PD and ELS bacteria as demonstrated by indirect IFAS were vacuum extracted (Fig. 5B) and cultured from all seven scorch-affected seedlings. Bacteria were also detected in vacuum extracts from four of the symptomless bacteria-inoculated seedlings, but bacteria were cultured from only two of these four seedlings. All control seedlings remained symptomless and attempts to vacuum extract and culture bacteria from controls were negative.

Electron microscopy. Bacteria were abundant in the tracheary elements of leaf tissue collected from four scorch-affected Texas trees. No bacteria were observed in leaves from four symptomless trees. The distribution and morphology of bacteria in sycamore leaves from Texas were identical to those previously reported for bacteria in scorch-affected sycamore leaves from Washington, DC (9). Bacteria were rod-shaped (0.3–0.4 × 1–1.8 μm) and many were slightly tapered at one end. The cell wall was distinctly rippled. Peritrichous fimbriaelike projections were prevalent, particularly on the blunt end of the organism. Bacteria occasionally occurred in a lightly to moderately stained matrix. Interbacterial spaces in some elements contained strands with segments constricted into nodules of varying size. Smaller densely staining bodies, perhaps degenerate bacterial cells (9), were also observed. Bacteria identical in morphology and xylem orientation to those observed in scorch-affected trees were also observed in ultrathin sections of vascular tissue of an inoculated symptomatic seedling.

Bacteria morphologically similar to those observed in leaf tissue and in vacuum extracts of stems (Fig. 5) were observed in ultrathin sections of bacterial colonies in culture (Fig. 6). Bacteria in culture, however, commonly reached lengths of 4 μm, whereas bacteria in extracts and tissue generally measured 1–2 μm. Nodulated strands identical to those observed in the interbacterial space in tracheary elements were abundant between the organisms in the colony (Fig. 6), indicating bacterial rather than host origin.

DISCUSSION

Fastidious xylem-limited bacteria (FXLB), previously referred to as rickettsialike bacteria, have been associated with leaf scorch, decline, and/or wilt in many woody and herbaceous hosts (1,6,13). This study associates FXLB with sycamore leaf scorch in three locations and demonstrates the pathogenicity of one isolate. Leaf scorch disorders of shade trees, particularly in urban areas, are usually associated with such abiotic factors as excessive soil salinity or confined growing sites. The presence of bacteria serologically related to the PD bacterium in scorch-affected leaves in elm, oak (9), and sycamore, however, indicates that FXLB may be a significant factor causing or contributing to leaf scorch in shade trees.

Pathogenicity of the sycamore scorch bacterium was confirmed by isolating bacteria morphologically identical to the

Table 1. Vacuum extraction and culture of Pierce's disease-like bacteria from symptomless and scorch-affected sycamores in Washington, DC, Richardson, TX, and New Orleans, LA[a]

	Vacuum extraction		Culture	
Location	Symptomless	Scorch-affected	Symptomless	Scorch-affected
Washington, DC	1/13	12/13	0/13	13/13
Richardson, TX	1/4	4/4	1/4	7/8
New Orleans, LA	...[b]	1/1	...[b]	4/4
Total	2/17	17/18	1/17	24/25

[a] Number of trees positive/number of trees examined. Examinations were made by phase-contrast microscopy.
[b] Not examined.

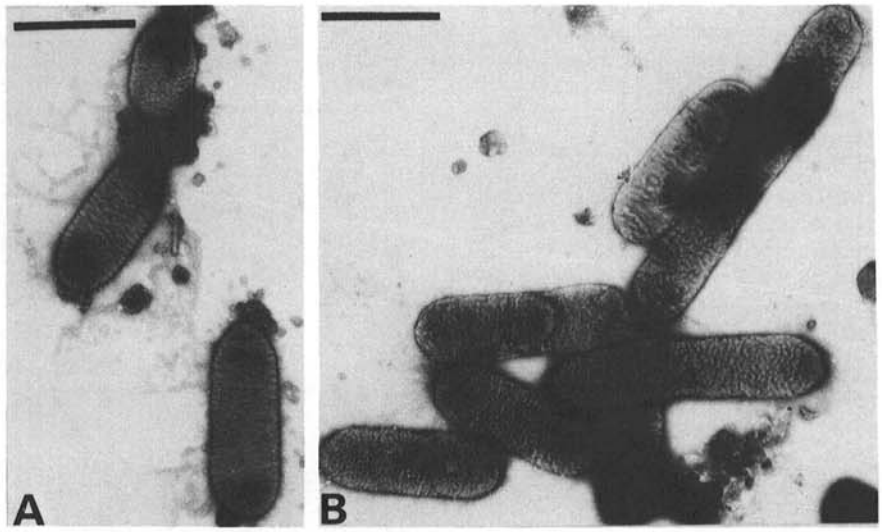


Fig. 5. Electron micrographs of negatively stained bacteria vacuum-extracted **(A)** from stem section of a leaf scorch-affected sycamore in Texas and **(B)** from a sycamore seedling inoculated with a cultured bacterial isolate from a naturally affected tree in Washington, DC. Note pointed end of cell, rugose cell wall, and densely staining polar regions. Scale bar = 1 μm.

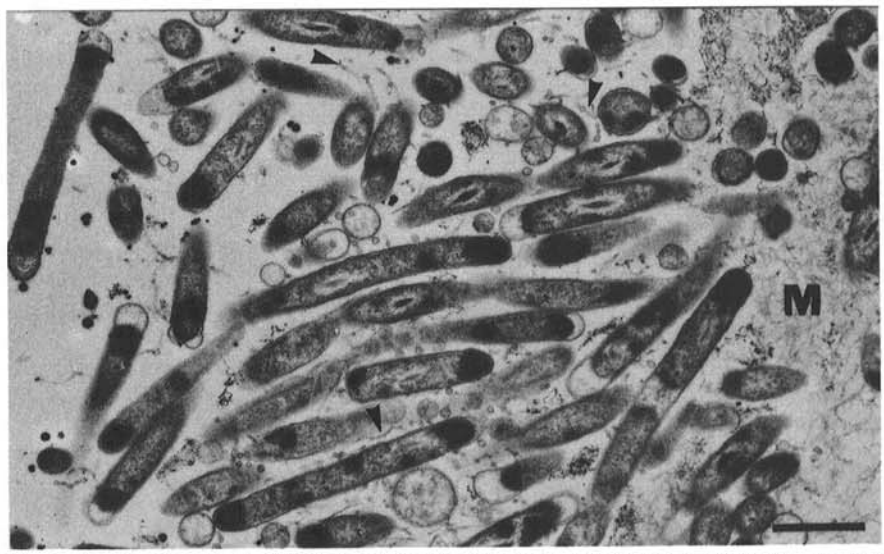


Fig. 6. Electron micrograph of bacteria cultured from a scorch-affected sycamore and growing in a colony on PD-2 medium. M = agar matrix. Arrows indicate nodulated strands. Note densely staining polar regions and DNA-like strands in centers of cells. Scale bar = 1 μm.

inoculum and serologically related to the PD bacterium from seven inoculated seedlings showing leaf scorch. The lack of symptom development in about two-thirds of the inoculated seedlings may relate to failure of the inoculation procedure or indicate that a longer incubation period and/or additional heat and drought stress are needed. Trees are being held a second year to test the first possibility. The role of heat and drought stress in symptom development has not been examined. The sycamore leaf scorch symptom may be caused by bacterial matrices restricting water movement in the tracheary elements (10) or possibly by toxins, which have been implicated in PD (14).

In addition to leaf scorch, bacterial impedence of water translocation in sycamore may be a predisposing or synergistic factor for other disease agents, particularly canker-causing fungi. Ricketts (16) described leaf scorch as the first symptom of sycamore decline. Several canker-causing fungi have been isolated from leaf scorch-affected trees (2,16) and some have been found particularly pathogenic on water-stressed plants (15,16).

Morphologically identical bacteria resembling the PD bacterium were consistently isolated from scorch-affected sycamores in Washington, Richardson, and New Orleans. Isolates from all three locations grew on media developed for the PD bacterium but not on conventional media. Isolates from each locality were serologically related to the PD and ELS bacteria; however, the degree of relatedness between isolates from different trees or different geographical locations or with other FXLB is not known.

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