

Association of Fastidious, Xylem-Inhabiting Bacteria with Leaf Scorch in Red Maple

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ABSTRACT

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Trees of red maple (*Acer rubrum* L.) were examined that showed a late-summer, marginal leaf scorch characteristic of diseases caused by fastidious, xylem-inhabiting bacteria (FXIB). Bacteria morphologically identical to the Pierce's disease bacterium and to other FXIB were found in the tracheary elements of scorch-affected leaves but not in leaves of an adjacent symptomless tree. Isolations were made from scorch-affected trees by incubating wood chips in an amended formulation of the periwinkle wilt broth medium used previously for isolating the sycamore and mulberry leaf scorch FXIB. A strain from maple cultured for serological and biochemical tests reacted positively in indirect ELISA with monoclonal antibodies specific to the FXIB, and the cellular fatty acid composition was characteristic of that of the FXIB.

Leaf scorch is a common symptom of subacute moisture stress in shade trees. It is usually caused by abiotic factors such as drying winds, road salt, and confined, limited root systems (8). Recently, however, fastidious, xylem-inhabiting bacteria (FXIB), previously referred to as rickettsialike bacteria, have been associated with leaf scorch symptoms in elm, red oak, sycamore, and mulberry (8,9,19,26). FXIB are a recently recognized and taxonomically unclassified group of bacteria that are causal agents of a number of diseases including Pierce's disease of grape (PD) (4,7,11), almond leaf scorch (5,23), plum leaf scald (16), peach phony disease (12,24), alfalfa dwarf (7,28), periwinkle wilt (22), and ragweed stunt (29). The bacteria are small ($0.25-0.50 \times 1.0-4.0 \mu\text{m}$), gram-negative, and distinguished by their ultrastructural characters, including thick, rippled cell walls and their inability to grow on conventional bacteriological media (6,10).

Bacteria ultrastructurally similar to PD and other FXIB have been observed

in ultrathin sections of tracheary elements of scorch-affected leaves from elm, sycamore, red oak, and mulberry (9,19) and have been isolated consistently from scorch-affected trees in media developed for FXIB (1,17-19,26). Strains obtained from four shade tree species were serologically related to the PD bacterium (9,19,26). The pathogenicity of FXIB strains from sycamore and mulberry has been confirmed with their respective hosts (19,26,27).

Recently, a leaf scorch of red maple (*Acer rubrum* L.) was observed in northern Virginia. Leaf scorch developed in late summer as an irregular marginal necrosis and appeared similar to the leaf scorch associated with FXIB in other shade trees. The purpose of this study was to determine if FXIB were associated with leaf scorch in red maples.

MATERIALS AND METHODS

Isolation and culture of bacteria. Stem sections ($1-1.5 \times 15-20 \text{ cm}$) were collected from affected branches of three trees showing leaf scorch from August to October 1983. Stem sections were also collected from two symptomless trees growing adjacent to one affected landscape tree. Bacteria were isolated as reported previously (19,26). Wood chips ($0.5 \times 1.5 \text{ cm}$) were aseptically removed from each stem and incubated at 28 C in

25 ml of periwinkle wilt (PW) broth medium (3) supplemented with 0.85 g of $(\text{NH}_4)_2\text{HPO}_4$, 2 g of potato starch, 1 g of histidine, and 25 mg of cycloheximide per liter (26). Cultures were examined periodically by phase-contrast microscopy at $1,000 \times$ for cells characteristic of FXIB. Strains were subcultured on semisolid, supplemented PW medium. Subculturing also was attempted on PD4 (6) and nutrient agar. One strain, MPS-1, was cloned from a single colony on BCYE (buffered charcoal-yeast extract) agar (31) and maintained on BCYE for serological and fatty acid analyses.

Electron microscopy. Leaves were collected from one scorch-affected landscape tree and an adjacent symptomless tree in September 1983. Primary and secondary veins were excised from nonnecrotic tissue adjacent to necrotic tissue of scorch-affected leaves and from comparable tissue of symptomless leaves. Tissue was prepared for ultrastructural examination as described previously (9).

Serology. Serological tests were by indirect enzyme-linked immunosorbent assay (ELISA), using biotinylated antimouse immunoglobulin (IgG), an avidin-biotin-peroxidase complex (Vector Lab, Burlingame, CA) (13), and an EIA Gilford PR-50 automatic analyzer system (Gilford Instrument Labs, Oberlin, OH). Antigen was strain MPS-1, harvested at log phase by centrifugation at 13,000 g for 5 min, washed twice in carbonate buffer, pH 6.9, and sonicated for 2 min with a Fisher Sonic Dismembrator (Fisher Scientific, Springfield, NJ). Fourteen other strains of FXIB, including strains of the PD bacterium, PCE-RR and PCE-GG, were used as reference antigens (15). Strain accession numbers at the American Type Culture Collection (ATCC), Rockville, MD, or at the Plant Disease Division, Department of Scientific and Industrial Research (PDDCC), Auckland, NZ, are: PCE-RR (ATCC 35879), PCE-GG

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(ATCC 35877), ALS (PDDCC 8739), PLM-G83 (ATCC 35871), PLM-84 (PDDCC 8743), PCH-83 (PDDCC 8744), PWT-22 (ATCC 35878), PWT-100 (ATCC 35880), RGW-R (ATCC 35876), ELM-ES6 (ATCC 35873), ELM-2 (ATCC 35872), OAK (ATCC 35874), SYC (PDDCC 8740), and MUL-1 (ATCC 35868). Antigens were standardized at 10–20 μg of protein per well (0.2–0.4 mg/ml) by Bio-Rad protein assay. Antibodies were secreted from two monoclonal hybridomas produced from fusion of NS-1 myeloma cells and spleen cells of BALB/C mouse immunized with a strain of the PD bacterium by procedures described by Lin and Chen (20). Protein concentration of antibody was standardized at 1–5 μg of protein per well (20–100 μg /ml). Coating, blocking, and incubation procedures with serum, antigen, IgG, reagents, and substrate were as described by Lin and Chen (20). Results of ELISA were expressed as average optical density readings at 640 nm from duplicate runs. Values greater than 0.1 were considered positive reactions.

Fatty acid analysis. Total cellular fatty acid composition of isolate MPS-1 was compared by gas-liquid chromatography (GLC) with those of a collection of reference strains of the FXIB, including the 14 strains described earlier. Cells were grown on BCYE agar for 30 days at 25 C, saponified, and methylated by a modification of the method of Maas et al (21). The hexene-diethyl ether extract was concentrated under a stream of prepurified N_2 gas and 1 μl of concentrate injected into the GLC. A Varian Model 3700 Gas Chromatograph (Varian Associates, Sunnydale, CA) was used with a flame-ionization detector and a fused silica

capillary column (15 M \times 0.25 μm). Chromatography conditions were: helium carrier gas flowing at 30 ml/min, air at 300 ml/min, hydrogen at 30 ml/min, and temperature programmed at 100–225 C at 8 C/min. Data were processed with a Model 4270 Integrator (Varian) and expressed as percentage of total peak area. Individual fatty acids were identified and chemically confirmed by methods previously described (30).

RESULTS

Symptomatology. Three red maple trees were found showing leaf scorch in 1982 and 1983 in Alexandria, VA. Two trees were landscape specimens (20 and 40 cm in diameter at 1.4 m above the ground), and the third tree was a wild sapling.

Leaf scorch localized in individual branches had been observed in one landscape tree since 1982. Each year, additional branches were affected. In the other landscape tree, a large portion of the canopy showed leaf scorch when first observed in 1983. Only a few small branches were affected by leaf scorch in the wild sapling.

In the spring, leaves developed normally on previously affected branches; however, symptoms began appearing in mid to late July and intensified as the summer progressed. Leaf discoloration occurred initially at the leaf margins and migrated with an undulating front toward the midrib and base of the leaf. Necrotic leaf tissue was composed of light brown areas frequently bordered by narrow to wide zones of dark reddish brown tissue. Necrotic tissue was separated from green tissue by a narrow but distinct chlorotic border (Fig. 1).

Occasionally, isolated zones of discoloration also occurred in interveinal tissues. In late August, severely affected leaves began to abscise prematurely. Dieback was not observed in the three affected trees.

Isolation and culture of bacteria. Bacteria characteristic of the FXIB were isolated from all three scorch-affected trees but not from the two unaffected control trees. Bacteria were first observed in wood chip cultures after 2–11 wk of incubation in supplemented PW broth medium. Single cells and chains of two or three cells with dark areas in one or both ends of the organism were observed under phase-contrast microscopy. The bacterium was gram-negative. Subcultures grew on semisolid supplemented PW medium but not on PD4 or nutrient agars.

Electron microscopy. Bacteria resembling FXIB were readily observed in ultrathin sections of tracheary elements of leaves collected from the one scorch-affected tree examined but not in leaves collected from the adjacent symptomless tree. Tracheary elements were filled with bacteria surrounded by a lightly staining matrix (Fig. 2). Bacteria were rod-shaped and typically 0.4–0.5 \times 1–2 μm (Fig. 3); however, large polymorphic cells also were present. Numerous microsomes and DNA strands were clearly visible in most organisms. Cell walls were prominently rippled and consisted of five layers (Fig. 3). Walls were about 30 nm wide. Fimbriaelike projections and strands consisting of nodulated subunits projected from the cell walls and were also seen distributed throughout the matrix (Fig. 3).

Serology. Positive antigenic reactions



Fig. 1. Scorch-affected red maple leaf showing undulating marginal necrotic areas of light and reddish brown tissue separated from green tissue by a chlorotic border.

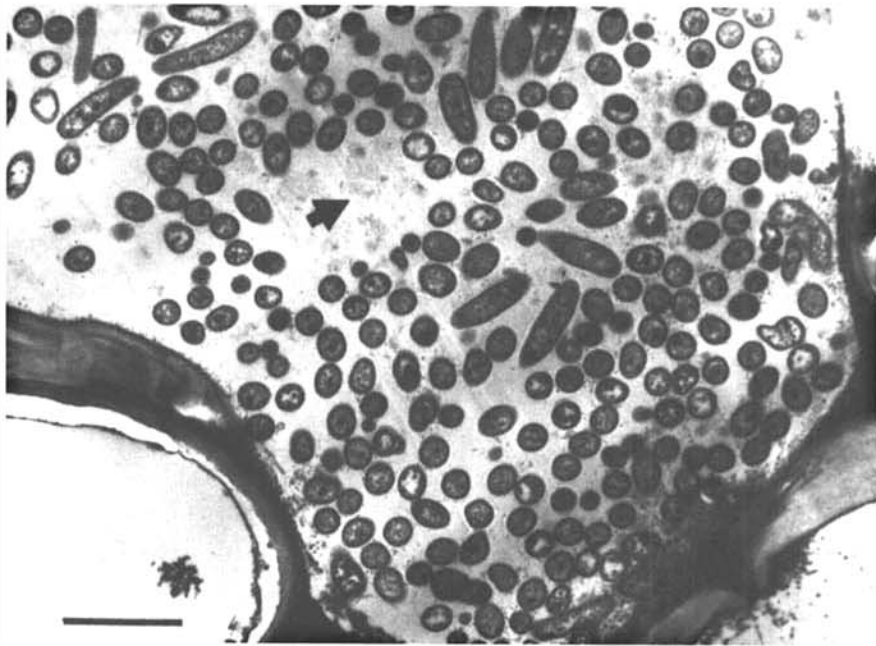


Fig. 2. Electron micrograph of bacteria in a cross section of the xylem of a scorched maple leaf. Bacteria occur in a lightly staining matrix (arrow). Scale bar = 2 μm .

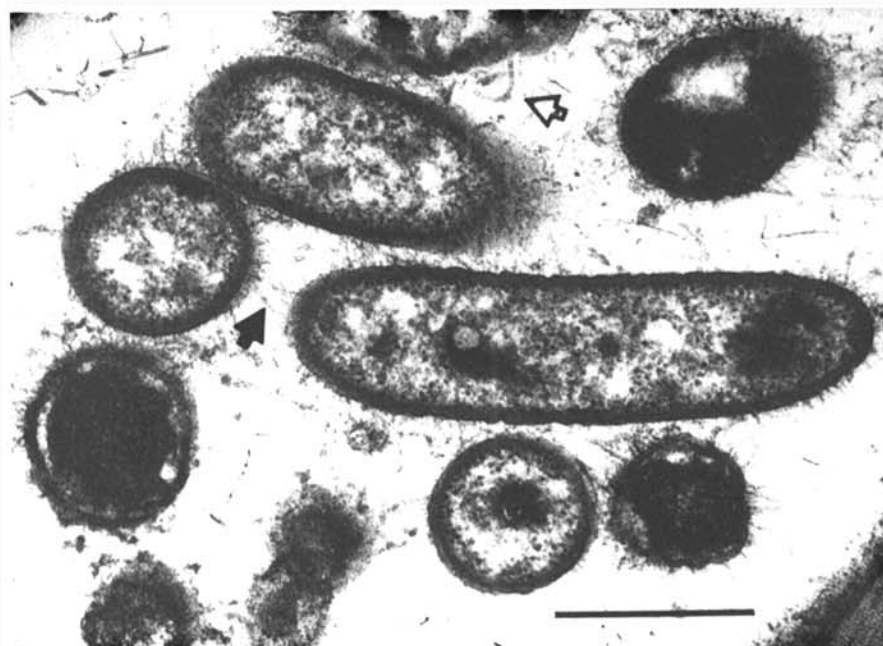


Fig. 3. Electron micrograph of cross and longitudinal views of bacteria in a scorched maple leaf. Fimbriae-like projections (solid arrow) and strands of nodulated subunits (open arrow) are associated with the bacteria. Scale bar = 0.5 μ m.

Table 1. Serological reactions by indirect ELISA of monoclonal antibodies specific to the fastidious, xylem-inhabiting bacteria (FXIB) with strain MPS-1 isolated from red maple and with bacteria representative of other bacterial genera

Antigen ^a	Monoclonal antibodies ^b	
	EF4-BE6	EF4-AC11
FXIB, reference strains ^c		
Range	0.197-0.571	0.180-0.403
Mean	0.310	0.308
MPS-1	0.327	0.292
<i>Erwinia amylovora</i>	0.017	0.010
<i>E. carotovora</i>	0.042	0.006
<i>Xanthomonas campestris</i>	0.005	0.001
<i>Pseudomonas syringae</i>	0.040	0.040
<i>Spiroplasma kunkelii</i>	0.011	0.005
<i>Rickettsia rickettsii</i>	0.030	0.038
<i>Legionella pneumophila</i>	0.002	0.010
Buffer check	0.030	0.028

^a Protein content standardized at 0.2-0.4 mg/ml.

^b Secreted from hybridomas produced from fusion of NS-1 myeloma cells and splenic cells of BALB/C mouse immunized against the Pierce's disease bacterium (20). Protein content standardized at 20-100 mg/ml.

^c Range and mean of optical density values from reactions with 14 FXIB strains isolated from 10 plant host species (including the Pierce's disease bacteria).

Table 2. Class analysis of cellular fatty acid composition of the maple leaf scorch isolate MPS-1 with that of known fastidious, xylem-inhabiting bacteria (FXIB)

Fatty acid class	MPS-1	Total percent	
		FXIB ^a	
		Mean	Range
Saturated, even-carbon straight chains	26.14	33.35	20.90-47.51
Saturated, odd-carbon straight chains	18.11	17.88	6.42-29.18
Unsaturated acids	40.38	34.60	22.03-50.03
Hydroxy-substituted acids	1.64	3.42	0.51-6.05
Branched-chain acids	12.59	8.32	1.65-18.07
Cyclic acids	0.00	0.02	0.00-0.33
Unidentified components	0.77	2.16	0.00-7.21
Total	99.63	99.75	
Ratio even/odd saturated straight chains	1.44	2.38	0.70-7.05
Ratio saturated/unsaturated acids	1.10	1.56	0.82-2.66

^a Mean and range of cellular fatty acid profile of 26 FXIB isolates from 10 plant hosts including grape, almond, plum, peach, oak, sycamore, elm, periwinkle, mulberry, and ragweed.

were observed in indirect ELISA with isolate MPS-1 and monoclonal antibodies secreted by each of two hybridoma clones originally selected for FXIB specificity (15) (Table 1). Optical density values for the maple strain were similar to the mean optical density values of 14 known FXIB isolates reacting with the same antibodies at the same protein concentration. Negative reactions were observed with the buffer check and with bacteria of unrelated genera including *Erwinia amylovora*, *E. carotovora*, *Xanthomonas campestris*, *Pseudomonas syringae*, *Spiroplasma kunkelii*, *Rickettsia rickettsii*, and *Legionella pneumophila*.

Fatty acid analysis. The cellular fatty acid composition of the FXIB is distinct from that of other plant-pathogenic bacteria, particularly in the total absence of cyclic acids and in high percentages of saturated straight chains (30). The fatty acid composition of maple strain MPS-1 was similar to the mean fatty acid composition of the 26 FXIB isolates examined (Table 2) as well as to published values for FXIB (30). Saturated straight chains constituted 44% of the cellular fatty acids compared with a mean of 51% for the FXIB. The most abundant saturated straight-chain acids in MPS-1 (and other FXIB) were 15:0 (7%), 16:0 (28.7%), and 17:0 (10.1%). The proportion of even-carbon to odd-carbon straight-chain acids occurred at a ratio of 1.44 for MPS-1 and 2.38 (mean) for the FXIB as a group. Unsaturated fatty acids, predominantly 16:1 (27.2%) and 18:2 (13.2%), constituted 40% of the MPS-1 fatty acids compared with a mean of 34.6% for the FXIB. The ratios of saturated to unsaturated fatty acids were 1.10 and 1.56 for MPS-1 and FXIB, respectively. Branched-chain acids and hydroxy-substituted acids occurred in the same relative proportions in MPS-1 and the FXIB. Cyclic acids were not detected in MPS-1.

DISCUSSION

Although leaf scorch in red maple is commonly associated with moisture stress attributable to abiotic factors such as deicing salts or to anthracnose disease caused by *Discula* sp. (14), some leaf scorch disorders are associated with and may be caused by FXIB. The bacterium isolated from maple was morphologically and biochemically similar to that of FXIB isolated from other disease hosts, and it reacted strongly with monoclonal antiserum specific for the FXIB.

The general distribution and severity of FXIB-associated leaf scorch in red maple is not known. Several red maple trees with similar leaf scorch symptoms have been observed in the suburbs of Alexandria, VA, where the first affected trees were found. In no case did the disease appear to be particularly debilitating. Symptoms were usually sparse and occurred in late summer,

when they could be confused with early senescence. Dieback was not observed in the few trees examined.

The consequences of a chronic FXIB infection in red maple are not known. Although FXIB may not of themselves be lethal, the chronic nature of the infection may predispose maples to secondary stresses. For example, elms affected by elm leaf scorch are 12 times more susceptible to Dutch elm disease (32). Leaf scorch-affected elms are attractive brood trees for the European elm bark beetle (*Scolytus multistriatus*), a vector of *Ceratocystis ulmi*, the Dutch elm disease pathogen. Similarly, peaches affected by peach phony disease are more prone to cold injury (2). In urban landscapes, FXIB may enhance the vulnerability of infected trees to site-related stress factors such as confined growing space and exposure to deicing salts.

This report is the first of the association of the FXIB with red maple. The list of host plants for this new group of microorganisms has been expanding rapidly. Although FXIB may be only weakly pathogenic in maple, maple may be a reservoir for strains that are highly pathogenic in other hosts. There is experimental evidence for cross-pathogenicity of the PD bacterium between grape, almond, and alfalfa and for another FXIB between peach and plum (5,28,31). Leafhoppers, the primary vectors of FXIB (25), may transmit FXIB between maple and other host species. A complete understanding of the pathogenic relationships between strains of FXIB affecting different hosts and the vector relationships between host species awaits further study, however. Pathogenicity studies on the maple bacterium, particularly the fulfillment of Koch's postulates with maple, also need to be done.

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