

BACTERIAL LEAF SCORCH CAUSED BY XYLELLA FASTIDIOSA: A KENTUCKY SURVEY; A UNIQUE PATHOGEN; AND BUR OAK, A NEW HOST

by J. R. Hartman, B. C. Eshenaur and U. E. Jarlfors

Abstract. Bacterial leaf scorch caused by *Xylella fastidiosa* was detected using an ELISA test from 1989 to 1993 in symptomatic pin, red, shingle, and white oak; sycamore; and red maple in 16 Kentucky cities and towns distributed statewide. The disease was also detected in southern Indiana and in Tennessee. Leaf petiole xylem elements of affected trees were partially occluded with bacteria. Examination of the causal agent by electron microscopy revealed bacteria with unusual scalloped or rippled cell walls, typical of *X. fastidiosa*. Bacterial leaf scorch was identified for the first time in bur oak (*Quercus macrocarpa*) in Lexington, Kentucky. Symptoms included marginal leaf necrosis, premature leaf browning, and defoliation. The disease was confirmed by detecting the pathogen with an ELISA test and by electron microscopic observation of the causal agent in leaf petiole tissues. Detection of bacterial leaf scorch has now progressed from coastal states to the U.S. interior.

Landscape trees have long been afflicted with leaf scorch symptoms caused by environmental factors such as root damage, road salt, and drought, and by wilt diseases caused by fungi (2,12). The association of xylem-limited bacteria with shade tree leaf scorch symptoms was first reported in 1980 (5). In 1987, the bacterium associated with leaf scorch was described as a new species, *Xylella fastidiosa* (13). Bacterial leaf scorch has been reported in coastal U.S. states from New York to Texas, and recently in Kentucky in pin, red, and shingle oak, and sycamore (1,3,4,7,10).

Bacterial leaf scorch symptoms in oak are characterized by dead margins with green tissues near the main veins and leaf petiole. Symptoms first appear in late summer in individual branches. The following spring, affected branches re-foliate and leaves remain green until scorch symptoms reappear in late summer. Many affected leaves drop prematurely. In succeeding years, as the

disease spreads, leaf scorch symptoms appear in late summer throughout the tree. Gradually, infected trees suffer a chronic decline with branch dieback affecting more of the tree each year. Figures 1 and 2 show the progress of decline of an infected pin oak over a five-year period. Secondary factors such as twig and branch cankers may contribute to the branch dieback, and eventually, the nearly dead tree needs to be removed. Tree decline, from first discovery of the disease to removal may take place over a period of five to ten or more years. It is not known how *X. fastidiosa* causes leaf scorch and defoliation of landscape trees, but water stress due to xylem occlusion seems to be a likely cause (6).

Although symptoms are often distinctive, additional evidence is usually needed to confirm diagnosis of this disease. Plant disease diagnostic specialists use an enzyme linked immunosorbent assay (ELISA) developed for *X. fastidiosa* ("Pathoscreen-XP", Agdia, Inc., Elkhart, IN) to detect the bacterium for accurate diagnosis (Figure 3) (11). Our plant disease diagnostic laboratory, in the University of Kentucky College of Agriculture, Plant Pathology Department, used ELISA for a survey of landscape trees with leaf scorch (4). Bacterial leaf scorch in Tennessee was also diagnosed with this ELISA test at their plant disease clinic (8). Used correctly, this assay is an excellent tool for diagnosing large numbers of samples.

The objective of this research was to determine where bacterial leaf scorch disease occurs in Kentucky urban forests. In addition, we wanted to observe *X. fastidiosa* in infected trees to reveal some unique characteristics of this new tree pathogen. Finally, this research reports the dis-



Fig. 1. Pin oak with bacterial leaf scorch in 1990.

cover of bacterial leaf scorch in bur oak.

Materials and Methods

To find locations where bacterial leaf scorch was active in Kentucky, leaves with scorch symptoms were collected from landscapes in 19 Kentucky cities and towns during September and October, 1989-1993. Samples for the survey also included specimens sent to the plant disease diagnostic laboratory by concerned tree owners. Samples normally consisted of one or two affected twigs per tree with 10 - 12 leaves attached and were refrigerated for up to two weeks, if necessary, for later analysis. The pathogen was detected with the "Pathoscreen-Xf" ELISA test kit specific for *X. fastidiosa*. Following a protocol provided with the test kit, excised leaf petioles immersed in an extraction buffer prescribed by the manufacturer



Fig. 2. Progress of the disease in 1994.

were crushed using a mortar and pestle and assayed for bacteria. Leaf petiole tissues of landscape trees showing symptoms were used for all assays and included negative and positive controls from trees near the laboratory. Data on the status of bacterial leaf scorch in nearby states were obtained from university plant disease diagnosticians.

Whenever bacterial leaf scorch was detected in a species representing a new Kentucky record, additional evidence was obtained to confirm the finding (3,4). Thus, first samples of pin oak, red oak, sycamore, red maple, and shingle oak collected in Kentucky found positive by ELISA were subsequently examined by electron microscopy (3,4). To prepare samples for microscopic viewing, pieces of leaf petiole no larger than 2 mm were fixed in 3% glutaraldehyde in 0.1 M cacodylate

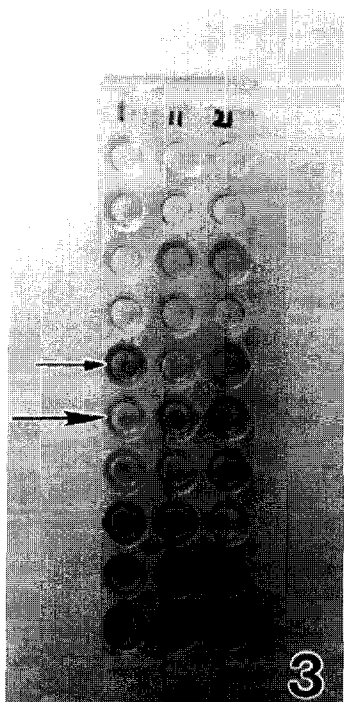


Fig. 3. *X. fastidiosa* detection using ELISA; small arrow indicates positive and large arrow indicates negative test result.

buffer for 2 hr, post fixed in 1% osmium tetroxide in the same buffer for 2 hr, dehydrated in ethanol and embedded in Spurr's medium. One micron sections (stained with toluidine blue) for light microscopy and 80 nm sections (stained with uranyl acetate and lead citrate) for electron microscopy were cut on an LKB Ultratome III and viewed in a Zeiss Photomicroscope II and a Philips 400 Transmission Electron Microscope, respectively.

In October, 1993, a 15 year old bur oak (*Quercus macrocarpa*) in Lexington, Kentucky with symptoms of bacterial leaf scorch was tested by using ELISA and examined microscopically for *Xylella*.

Results and Discussion

Kentucky survey. In symptom- and ELISA-based surveys of trees from 1989 to 1993, bacterial leaf scorch was found in 16 Kentucky cities and towns distributed statewide (Figure 4). Using the ELISA test, the pathogen was detected in symptomatic pin, red, shingle, and white oaks and in red maple and sycamore. We also detected the

pathogen outside Kentucky in symptomatic pin oaks in Knoxville, (Knox Co.) Tennessee and in Rockport (Spencer Co.) Indiana. Affected Kentucky counties include: Boyle, Caldwell, Campbell, Christian, Daviess, Fayette, Garrard, Hardin, Henderson, Hopkins, Jefferson, Jessamine, McCracken, Oldham, Pulaski, Union, Warren. In our survey, bacterial leaf scorch was detected only in trees growing in urban landscapes; a forest survey was not attempted.

Bacterial leaf scorch has been found widely distributed in Tennessee (19 counties) on 12 tree species based on symptoms and ELISA tests of specimens submitted to plant and pest diagnostic center (8). Although not part of the Kentucky survey, this distribution is also shown in Figure 4. Affected Tennessee counties include most of the major metropolitan areas. In addition to tree species previously reported, the Tennessee detections of bacterial leaf scorch include white, scarlet, post, water, swamp, chestnut, and willow oaks and sweetgum (8). These ELISA detections had not been corroborated with microscopy or bacterial cultures. Bacterial leaf scorch has not otherwise been confirmed by state university plant pathologists in Illinois, Indiana, and Missouri.

Microscopic characteristics of the causal agent. For any new species suspected of being infected with *X. fastidiosa*, the ELISA assay in its present form could yield a "false positive" (or even a "false negative") reaction because a positive control is not yet known. Thus, when a new host is detected, we use an additional test to corroborate ELISA results. Bacterial leaf scorch can be further verified by culturing the causal bacterium from infected tissues (7) or by examining infected tissues microscopically for evidence of the pathogen. We observed *Xylella* under the electron microscope in leaf petioles of pin oak, red oak, sycamore, red maple, and shingle oak following their respective first positive ELISA reactions (3,4).

Bacteria morphologically indistinguishable from *X. fastidiosa* were consistently observed using microscopy. Groups of bacteria were detected in leaf petiole xylem vessels (Figure 5). Electron microscopic views of the bacteria associated with xylem vessels are shown in Figure 6. Bacteria appear to invade, but in this case, not cross the

BACTERIAL LEAF SCORCH IN THE MIDWEST

(1988 -1993)

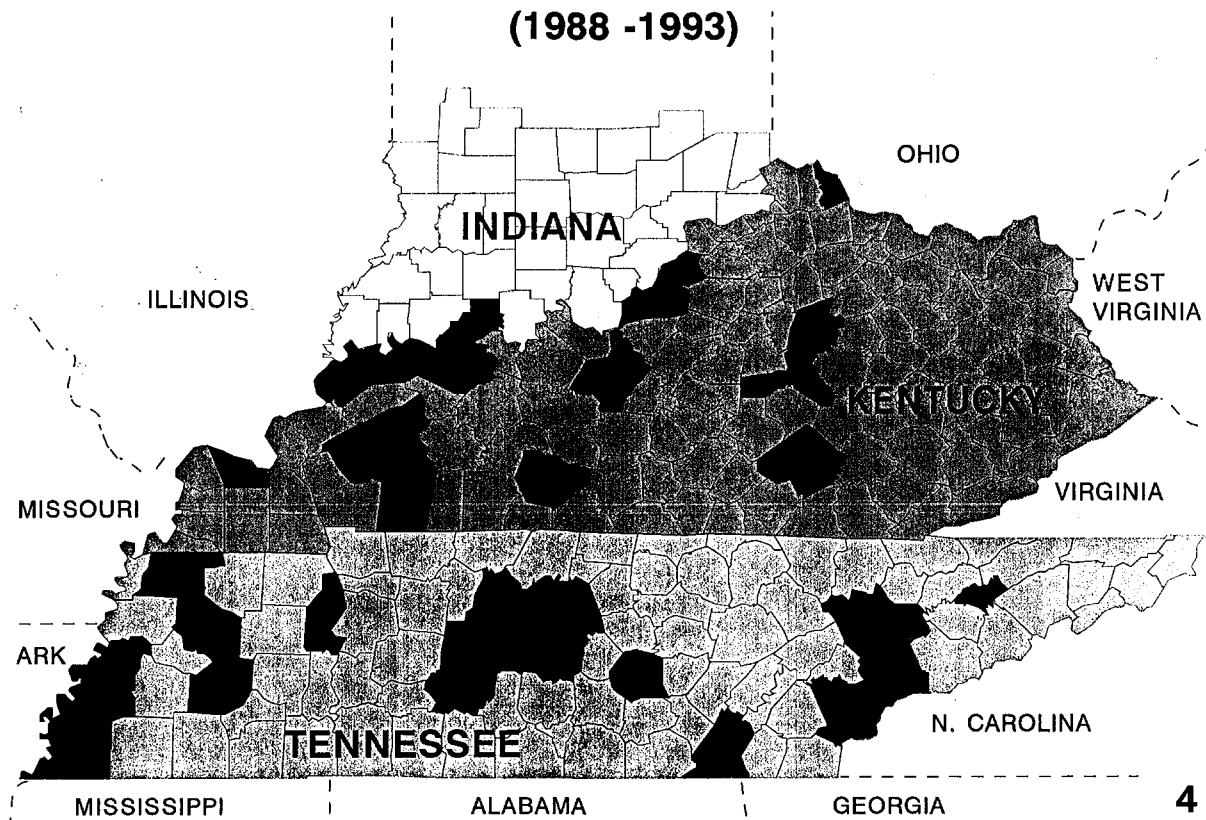


Fig. 4. Distribution of bacterial leaf scorch in Kentucky, Tennessee, and Indiana (counties with positive trees in black).

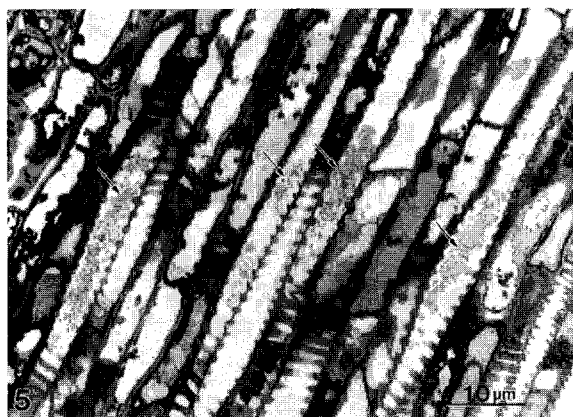


Fig. 5. Clumps of *X. fastidiosa* (arrows) in xylem vessels of pin oak leaf petiole seen through the light microscope.

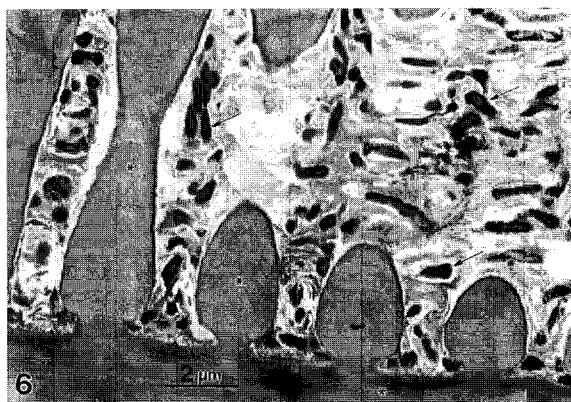


Fig. 6. An electron microscope view of the same tissues as Fig. 5. Asterisks denote xylem wall structural elements.

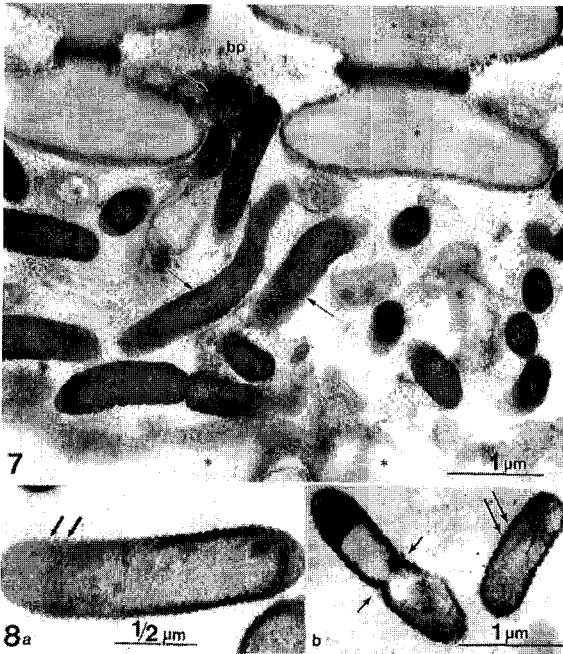


Fig. 7. A bordered pit (bp) region of pin oak xylem vessel showing bacteria (arrows) as seen with the electron microscope. Asterisks indicate vessel walls.

Fig. 8. *X. fastidiosa* as seen with the electron microscope. a) A grazing section reveals the wall ridges (arrows) extending across the bacterium. b) A dividing bacterium (opposing arrows); ridged wall (double arrows).

membrane of a bordered pit (Figure 7). Individual *Xylella* cells with characteristic rippled cell wall features were seen with the electron microscope (Figure 8a), as was a *Xylella* cell in the process of dividing (Figure 8b). Electron micrographs revealed similar features of bacteria associated with almond leaf scorch disease in California (9). Figure 9 shows fibrous strands associated with *Xylella* in the xylem vessels. The function of these fibrils is unknown (6). Unlike other species of plant pathogenic bacteria, *Xylella* has a rippled cell wall and lives and grows entirely within xylem tissues. These features make *Xylella* a unique pathogen.

Bur Oak, A New Host

The disease was identified in a 15 year old bur oak (*Quercus macrocarpa*), in Lexington in October, 1993 using the ELISA test. Symptoms included marginal leaf necrosis (Figure 10), pre-

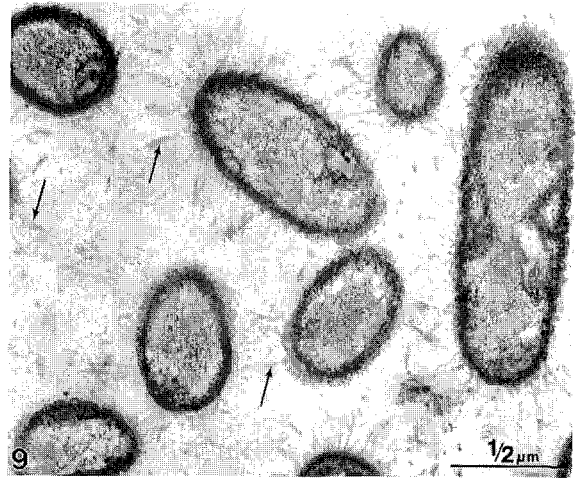


Fig. 9. Bacteria from pin oak xylem are bordered by filamentous structures (arrows).



Fig. 10. Bacterial leaf scorch of bur oak with green leaf tissues (arrowheads) and brown, scorched leaf tissues (arrows) shown.



Fig. 11. Diseased bur oak, (left), displaying thinning of foliage evident in early summer before scorch symptoms appear compared with adjacent healthy bur oak tree.

mature leaf browning, and defoliation. Microscopic examination of leaf petiole tissues revealed partially occluded xylem elements and xylem-limited bacteria with typical scalloped or rippled cell walls. Compared to its neighbors the affected tree was beginning to show early decline symptoms (Figure 11).

This is the first U.S. report of bacterial leaf scorch of bur oak. Detection of bacterial leaf scorch of landscape trees has now progressed from coastal states to the U.S. interior. In the absence of effective controls, this disease may represent a threat to bur oak and other widely planted susceptible trees of midwestern states.

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Résumé. La brûlure bactérienne des feuilles causée par *Xylella fastidiosa* a été identifiée sur le chêne à gros fruits (*Quercus macrocarpa*) à Lexington dans le Kentucky. Les symptômes présents incluaient une nécrose marginale des feuilles, un brunissement prématuré des feuilles et une défoliation. La présence de la maladie se confirme par la détection du pathogène avec un test spécifique d'ELISA pour *Xylella* et par l'observation, au microscope électronique, de l'agent causal dans les tissus du pétiole de la feuille. L'examen microscopique des tissus du pétiole de la feuille révèle des éléments fermés de xylème. Ceci constitue le premier rapport sur la brûlure bactérienne des feuilles du chêne à gros fruits.

Zusammenfassung. Bakterielle Blattdürre, verursacht durch *Xylella fastidiosa*, wurde in Moosbecher-Eichen (*Quercus macrocarpa*) in Lexington, Kentucky, identifiziert. Die Symptome beinhalteten marginale Blattnekrosen, verfrühte Blattverbräunung und Blattfall. Diese Krankheit wurde durch den Nachweis des Pathogens mit einem ELISA-Test, spezifisch für *Xylella*, und durch elektromikroskopische Untersuchung der verursachenden Träger im Blattstieltgewebe bestätigt. Die mikroskopische Untersuchung der Blattstieltgewebe enthüllte teilweise verstopfte Xylemantelle. Dies ist der erste US-Bericht über Bakterielle Blattdürre an Moosbecher-Eichen.