# BACTERIAL LEAF SCORCH OF LANDSCAPE TREES CAUSED BY XYLELLA FASTIDIOSA<sup>1</sup>

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The bacterium *Xylella fastidiosa*, first described in 1987 (44), has been associated with leaf scorch and decline of American elm (*Ulmus americana*) (13,26,37), American sycamore (*Platanus occidentalis*) (13,38,39), red mulberry (*Morus rubra*) (31), red maple (*Acer rubrum*) (41), and several species of red oak (*Quercus*) (2,11,13,18,28). This article reviews what is known and unknown about this unique pathogen and its effect on landscape trees.

X. fastidiosa has a diverse and extensive host range encompassing over 30 families of monocotyledonous and dicotyledonous plants (Table 1). While most hosts are asymptomatic, there are a number of species in which symptoms occur and some that are severely affected (Table 2). Pierce's disease of grape and phony disease of peach are the two most thoroughly studied diseases caused by X. fastidiosa. Epidemics of Pierce's disease were first observed in California in the 1880's. The disease is now known to be endemic in the southeastern United States where it is the major factor limiting grape culture (15). Pierce's disease causes leaf necrosis, decline, and eventually death of the vine. Phony disease of peach was first observed in Georgia during the same period and is found predominantly in the southeastern United States. The characteristic symptoms of peach phony are dwarfing accompanied by profuse lateral branching and flattened dark green foliage. Trees live for many years, but fruit size, number, and quality are reduced (3).

Early efforts to isolate the pathogens from grape or peach were unsuccessful. However, the causal agents of both Pierce's disease and peach phony were transmitted by xylem feeding leaf-hoppers (14,22,42,43) or by grafting using tissues that included xylem and not bark alone (3,7,23).

Such observations supported the hypothesis that both Pierce's disease and peach phony were caused by xylem inhabiting viruses, an unusual occurrence since viruses were known to occur only in the phloem and parenchyma tissue and not in the xylem.

The first evidence that a bacterium rather than a virus was involved in these diseases occurred in 1971 when symptoms of Pierce's disease were suppressed by treating plants with the antibiotic tetracycline (21). Electron microscopy later con-

Table 1. Selected list of natural hosts of J	Kylella fastidiosa.
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American elderSambucus canadensis L.18Blue elderS. caerulea Raf.8Boston ivyParthenocissus tricuspidata PlancVirginia creeperP. quinquefolia (L.) Planch.18	_
Boston ivy Parthenocissus tricuspidata Planc	_
	_
	_
	3
Peppervine Ampelopsis arborea (L.) Koehne <sup>11</sup>	-
Porcelain berry A. brevipedunculata (Maxim.)Trau	tv a
American beautyberry Callicarpa americana L.18	
Eastern baccharis Baccharis halimifolia L. <sup>18</sup>	
Coyote brush B. pilularis DC. <sup>8</sup>	
Sumac Rhus sp. <sup>18</sup>	
Goldenrod Solidago fistulosa Mill. <sup>18</sup>	
Blackberry Rubus sp. <sup>8,18</sup>	
California mugwort Artemisia vulgaris L. var. heteroph Jepson <sup>8</sup>	ylla
Ladino clover <i>Trifolium repens</i> L. var. <i>latum</i> McCarthy <sup>8</sup>	
Bermuda grass Cynodon dactylon (L.) Pers. <sup>8</sup>	
Hairy crabgrass Digitaria sanguinalis (L.) Scop. <sup>8</sup>	
Dallis grass Paspalum dilatatum Poir.8	

<sup>a</sup> Sherald, J. L., unpublished.

#### Table 2. Diseases associated with Xylella fastidiosa.

Pierce's Disease of Grape (9,19) Citrus Blight (16)		
Almond Leaf Scorch (33)	Elm Leaf Scorch (13)	
Alfalfa Dwarf (9)	Sycamore Leaf Scorch (38)	
Peach Phony Disease (20)	Maple Leaf Scorch (41)	
Plum Leaf Scald (24)	Mulberry Leaf Scorch (31)	
Periwinkle Wilt (32)	Oak Leaf Scorch (2,13)	

<sup>1</sup> Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Interior and does not imply approval to the exclusion of other products or vendors that also may be suitable.

firmed the presence of small, rippled walled bacteria that resembled rickettsia in the xylem of plants affected with Pierce's disease and peach phony (9,19,20,34). In 1978 a medium was developed which facilitated the isolation of the pathogen (6). Wells et al. (44) later examined 25 strains isolated from 10 species and determined that they were phenotypically and genotypically similar, and that they formed a distinct bacterial species unrelated to rickettsia. The name *X. fastidiosa* was chosen to reflect the xylem habitat and fastidious growth requirements.

## **Xylella fastidiosa and Landscape Trees**

In 1959 Wester and Jylkka described the similarity between a leaf necrosis of elm and Pierce's disease of grape (45). On the basis of successful graft transmission studies using scion wood containing xylem tissue as a source of inoculum, they proposed that the Pierce's disease virus may be the causal agent of elm scorch. Twenty years later, Wester and Jylkka's hypothesis was supported when electron microscopic examinations found bacteria, morphologically similar to the Pierce's disease bacterium, in the tracheary elements of scorched elm leaves (13). Similar organisms were subsequently observed in leaves of oak, maple, sycamore, and mulberry exhibiting chronic, late summer leaf scorch (13.31.41). Strains of X. fastidiosa have now been cultured from all five tree species and strains isolated from sycamore, mulberry, oak, and elm have been found to be pathogenic in their respective hosts (2,31,37,38,39).

Diagnosis of tree diseases caused by X. fastidiosa has been difficult for several reasons:

- 1. Only recently has *X. fastidiosa* been recognized as a pathogen of landscape trees. Consequently, many tree care professionals are not familiar with the disease.
- Symptoms may be easily confused with other disorders, particularly moisture stress.
- 3. Because of the fastidious nature of *X. fastidiosa*, it has been difficult to confirm presence of the bacterium via routine laboratory culture.

Over the last decade considerable progress has been made in describing the symptoms associated with *X. fastidiosa* infection of landscape trees and in developing improved diagnostic tools and methods (10,36,40).

**EIm.** Symptoms of elm bacterial leaf scorch first become apparent in mid-summer and progress in severity throughout late summer and fall. Leaves develop an undulating necrotic region along the leaf margin which spreads toward the midvein. Necrosis is preceded by a chlorotic band of tissue of varying width. Symptoms appear first on the lower, older leaves on a branch and develop on the newer leaves later in the season. Terminal leaves sometimes remain symptomless. Some severely affected leaves may curl upward, while others remain expanded or occasionally curl downward. Premature abscission is common.

Bacterial leaf scorch can affect elms of any age. Symptoms progress slowly throughout the canopy over many years, with affected trees exhibiting branch dieback and reduced twig elongation. Fewer flower buds may develop on affected branches and those buds that are present may fail to open. Leaf buds of affected branches are slower to break and expand than those of unaffected branches (25,30).

Although a cursory observation of bacterial leaf scorch symptoms may suggest a Dutch elm disease infection, the two diseases are readily distinguishable. Dutch elm disease causes a true wilt where leaves become flaccid before necrosis begins, whereas leaves with bacterial scorch develop marginal necrosis but do not wilt. In contrast to Dutch elm disease, no vascular streaking is associated with bacterial leaf scorch. Elms with bacterial leaf scorch exhibit chronic symptoms over many years, while Dutch elm disease kills trees in one to two years. Elms weakened by X. fastidiosa are more likely to be attractive to the elm bark beetle which transmits Dutch elm disease. Wester and Jylkka reported that elms with leaf scorch symptoms, characteristic of those now associated with X. fastidiosa, were more likely to contract Dutch elm disease than unaffected elms (46).

Leaf scorch affected elms were found to be widespread in the southeastern United States as early as 1957-58 (45). The disease has been observed in Baltimore, MD and as far south as New Orleans, LA. In addition to wild type American elms, *X. fastidiosa* has been isolated from leaf scorched 'Augustine Ascending' American elms as well as from symptomatic Wych (*U. glabra*), and Siberian elms (*U. pumila*) (25,27).

Sycamore. Decline of sycamores caused by X. fastidiosa and characterized by leaf scorch and dieback has been reported in Washington, D.C., Louisiana, North Carolina, South Carolina, Texas, and Florida, and is most likely widespread throughout the mid-Atlantic and southeastern United States (12,18,38). In mid-summer, leaves of affected branches develop an interveinal olive discoloration which later turns tan. Necrotic areas are preceded by a zone of reddish tissue. Severely affected leaves curl upwards and generally remain attached to the tree. Symptoms initially develop in older leaves and then progress to newer ones, often leaving tufts of unaffected leaves at the branch tips. Leaf expansion is delayed, growth is reduced, and affected trees set less seed. Symptoms reoccur each year involving progressively more of the tree canopy. In advanced stages of the disease, dieback decreases a tree's aesthetic value necessitating early removal.

Strains of *X. fastidiosa* isolated from sycamore have been found to cause leaf scorch in London plane (*P. x acerifolia*) and oriental plane (*P. orientalis*) as well as American sycamore (*P. occidentalis*) (38,39, Sherald, unpublished).

Bacterial leaf scorch may be confused with the leaf blight phase of sycamore anthracnose caused by the fungus *Apiognomonia veneta*. However, anthracnose leaf blight occurs primarily in the first few weeks of growth, develops first along the veins, and then progresses into interveinal tissue. Also, since leaf scorch occurs in late summer, symptoms may be mistakenly attributed to early fall senescence.

**Oak.** Several species in the red and black oak group have been found to be affected by *X*. *fastidiosa. Quercus rubra, Q. coccinea, Q. falcata, Q. palustris, Q. laurifolia*, and *Q. nigra* have all been reported as hosts (2,13,18,28). Although the disease has been observed as far north as Long Island, it is most common in urban areas of the mid-Atlantic and southeastern United States. Cases have been reported in Washington, D.C., Pennsylvania, Delaware, Virginia, Georgia, and Florida (2,18,28). Widespread occurrence of oak bacterial leaf scorch has been recently reported in Kentucky and the disease has also been confirmed in Indiana and Tennessee (11, J. Hartman, personal communication).

Symptoms either appear throughout the crown or in distinct branches of old or newly planted trees. Necrosis progresses from the leaf tips and margins toward the midvein and petiole. Tissues turn dull green and later become necrotic with a narrow band of chlorotic or reddish brown tissue separating the necrotic and healthy tissues. Several concentric zones or waves of alternating light and reddish brown tissue may occur in severely scorched leaves. In oak, all leaves on a branch are affected simultaneously in contrast to the symptom progression from older to younger leaves observed in elm and sycamore. Although early leaf abscission occurs, many scorched leaves remain attached.

Symptoms recognized late in the summer or early fall can easily be dismissed as early senescence. We have noted that symptoms on pin oaks are particularly obscure and are often discounted simply as a consequence of a variety of stress factors.

Bacterial leaf scorch of oak may be also confused with oak wilt, but there are several distinguishing characteristics. Oak wilt usually kills trees in a single year, while oaks affected by bacterial leaf scorch decline over many years. Vascular discoloration occurs in oak wilt, but not in oaks infected with *X. fastidiosa*.

As in elm and sycamore, bacterial leaf scorch affected oaks progress through chronic decline with more of the crown affected each year and dieback occurring in trees with long-term infections. Since older trees may succumb only after a long period of infection, other stress factors, such as insects and pathogens, are likely to contribute to the eventual death of the tree. The obvious presence of some secondary factors may obscure the possible role of *X. fastidiosa* as the primary pathogen.

**Red mulberry and red maple.** Red mulberry, *M. rubra*, and red maple, *A. rubrum*, are also affected by *X. fastidiosa* (31,41). Infected mulberries may first show desiccation over a large portion of the leaf with only a slight change in color. Later, the tissue turns necrotic and leaf margins curl upward. In other cases leaves may first develop a diffuse marginal chlorosis which turns necrotic. Necrosis progresses toward the center and base of the leaf and is separated from green tissue by a narrow band of reddish brown tissue and a more diffuse chlorotic zone. As in elm and sycamore, symptoms develop from older to younger leaves resulting in branches with leaves in progressive stages of symptom severity. Severely affected leaves fall early, often resulting in otherwise bare branches with tufts of symptomless leaves at the tips. Although dieback occasionally occurs, infected trees do not appear to be severely debilitated by the disease. The disease is common in northern Virginia and has been found as far north as southern New York (31). Recently, the disease was confirmed in Nebraska and Missouri (S. Kostka, unpublished).

Red maples do not appear to be as commonly affected as the other tree species. Affected trees have only been reported in northern Virginia (41). Leaves develop normally in the spring but begin to die in mid- to late July. They develop irregular necrotic patterns of light brown and reddish brown tissue separated from green tissue by a narrow but distinct chlorotic border (41). This contrasts with the uniform marginal browning so commonly found on leaves of maples affected by drought. Dieback has not been observed in the few trees examined and leaf symptoms can be easily mistaken for early senescence.

### Diagnoses

Procedures are now available for isolating X. fastidiosa from affected trees as well as for detecting X. fastidiosa in tissue extracts. In some species such as grape, X. fastidiosa is isolated by simply expressing sap from petioles on to semisolid media developed for X. fastidiosa. Since it is difficult to express sap from leaf petioles of trees, we have developed a procedure for isolating X. fastidiosa from stems. Wood chips, 0.5 X 1-2 cm, are removed aseptically, as is done for isolation of other vascular pathogens, and two-three chips are incubated in tubes containing 20-25 ml of one of the media developed for X. fastidiosa. A medium that we have found consistently effective in isolating X. fastidiosa from trees is given in Table 3. After incubation for two-four weeks at 28 C, bacteria can be readily

seen under phase contrast microscopy at 1000 X. X. fastidiosa is a small (0.25-0.35 X 0.9-3.53  $\mu$ m), gram-negative rod which frequently occurs in clumps in wet mounts prepared from broth cultures.

An enzyme linked immunosorbent assay (ELISA) has been developed for X. fastidiosa ('PATHOSCREEN-Xf', Agdia Inc. Elkhart, IN). ELISA can be used to confirm the identity of isolated strains as well as to detect X. fastidiosa in extracts of buds, leaf veins, petioles, and other tissues (40). Kits can be purchased and used directly by diagnosticians and tree care experts, or samples can be submitted to the manufacturer for testing. In either case, control samples from symptomless trees should be tested simultaneously. If samples yield negative reactions, then trees should be sampled and tested again. Since the ELISA kit can only detect X. fastidiosa at levels greater than 10° cells/ ml, caution must be exercised in interpreting negative results. If the ELISA reaction is negative and there is still suspicion that the tree is infected, then an

Table 3. Modified formulation of the PERIWINKLE WILT MEDIUM (PW) (5) used in isolating *Xylella fastidiosa* from landscape trees.

Distilled Water	905 ml	
Soytone	4.00 g	
Tryptone	1.00 g	
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	0.85 g	
КН <sub>2</sub> РО <sub>4</sub>	1.20 g	
K2HPO4	1.00 g	
Hemin Chloride	15 ml	
MgSO₄	0.80 g	
Potato starch	2.00 g	
Histidine	1.00 g	
BSA fraction V	6.00 g	
L-2-Glutamine	4.00 g	
Agar <sup>a</sup>	12.00 g	
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All components except BSA and glutamine are mixed and autoclaved. BSA and glutamine are filter sterilized and added after the medium has cooled. BSA is solubilized in 30 ml of distilled water by stirring slowly for 2-3 h before passing through a filter series of 1.20  $\mu$ m, 0.80  $\mu$ m, 0.45  $\mu$ m, 0.20  $\mu$ m and a sterile 0.20  $\mu$ m disposable nitrocellulose filters. Glutamine is solubilized in 50 ml distilled water by heating to 50 C before passing through a 0.20  $\mu$ m sterile disposable nitrocellulose filter. BSA and glutamine are combined before adding to the medium. Hemin chloride stock solution is prepared by adding 0.10 g of hemin chloride to 100 ml of 0.05N NaOH.

a For semisolid medium.

attempt should be made to isolate the pathogen.

## Management

Shade and forest trees are probably not new hosts of *X. fastidiosa*. More likely, diseases caused by *X. fastidiosa* have been misdiagnosed or overlooked in the past. Tree care professionals should emphasize to clients that diseases caused by *X. fastidiosa* have only recently been recognized and that our knowledge is limited, particularly in the areas of managing spread and treating infected trees.

X. fastidiosa is transmitted in grape and other more widely recognized hosts by xylem-feeding spittle bugs, subfamily Cercopidae, and sharpshooter leafhoppers, subfamily Cicadillinae (17). The vectors responsible for transmitting X. fastidiosa in trees have not been determined. However, if leafhoppers are involved, they will be difficult if not impossible to control because they feed throughout the growing season.

Pruning has been a successful therapeutic technique for Dutch elm disease when symptoms are detected early and the infected branch is removed well below obvious symptoms (1). The same approach should be considered for trees where scorch symptoms are localized in a single limb and the pathogen has not entered the main trunk. Such a strategy would require careful scouting when symptoms become most apparent and prompt removal of affected branches well below symptomatic leaves. The chronic nature of scorch diseases suggests slow systemic spread of the pathogen and may allow sufficient opportunity for pruning therapy to be effective. To date, therapeutic pruning has not been tested in any of the tree species affected.

Therapeutic injections of oxytetracycline have been evaluated in elm and oak (2,29). Low volume injections have caused a remission of symptoms, but no cure. Further study of chemotherapy with larger volumes and multiple year treatments should be explored.

Improving tree vigor alone may prolong the life of trees infected with *X. fastidiosa*. Fertilization and irrigation, particularly when moisture is limiting, may extend the life and aesthetic quality of affected trees. Early removal of severely affected

trees should be considered since they may pose a threat to adjacent trees as a source of inoculum.

## **Questions Remain**

Although much has been learned about *X*. *fastidiosa* and its effect on landscape trees, many questions remain:

Host Range. What other species are affected by X. fastidiosa? X. fastidiosa is a versatile pathogen known to infect many species of monocotyledonous and dicotyledonous plants (8,18). It is likely that other hosts, including species of landscape trees and ornamental shrubs, will be found.

Geographic Distibution. What is the geographic distribution of X. fastidiosa in landscape trees? Without a systematic survey, it is not possible to accurately define disease distribution. It is likely that the diseases are most common and severe in warmer regions of the country, particularly the Southeast, where Pierce's disease and phony disease of peach are common. This is possibly a consequence of the longer growing season and greater opportunity for systemic spread and symptom expression (17). Affected trees are common in the mid-Atlantic and southeastern United States and oak leaf scorch has been found as far north as New York (28). To the west, sycamore leaf scorch is severe in the Dallas area of Texas and mulberry leaf scorch has been identified in Lincoln, Nebraska. Interestingly, X. fastidiosa has not been reported causing leaf scorch in landscape trees in California. Symptom awareness and the availability of diagnostic techniques will help define the geographic range of these diseases. To date X. fastidiosa has not been reported outside North, Central, and South America (17). This raises concern for the export of X. fastidiosa through international movement of infected nursery stock.

Transmission. How is X. fastidiosa transmitted in landscape trees? The xylem feeding sharpshooter leafhoppers are the principal vectors of X. fastidiosa. It is likely that they are the vectors of X. fastidiosa between trees and between trees and possible herbaceous hosts. Currently, vectors involved in the transmission of the pathogen in landscape trees are not known. Understanding the vectors and their host relationships may be important in disease management. Root graft transmission is a distinct possibility, particularly in elm and oak where graft transmission of fungal wilt pathogens is known to occur.

Pathogenesis. What role does X. fastidiosa play in the disease syndrome? Moisture stress caused by physical blockage of the xylem is generally believed to be the primary mechanism of action (30); however, growth regulator imbalance and toxins have also been proposed (17). Is X. fastidiosa a primary pathogen that induces a chronic decline promoted at various stages by other biotic and abiotic factors, or is X. fastidiosa an opportunist that only affects weakened or senescing trees? Some evidence points to a primary role for X. fastidiosa. Scorch affected elms are attacked by elm bark beetles transmitting Dutch elm disease, peaches affected by peach phony are less cold hardy, and some fungal cankers associated with tree declines are promoted by moisture stress (4,35,46). The specific role that X. fastidiosa plays in the decline of each tree species must be examined.

Although these and many other questions remain, we are at least now able to recognize *X*. *fastidiosa* as a pathogen of landscape trees. Undoubtedly future research will further our understanding of this unique pathogen and our ability to diagnose and manage the diseases it causes.

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