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Diseases Caused by Fastidious Xylem-Limite

The term "xylem-limited bacteria" (XLB) is now being used to describe prokaryotic plant pathogens difficult to isolate by standard bacteriologic procedures. These fastidious organisms require complex media for growth, occur in the xylem of infected plants, are transmitted by xylem-feeding leafhoppers, and cause difficult-to-control diseases of economically important crops. When first associated with plant diseases (5,10,14), these agents were referred to as "rickettsialike bacteria" because of ultrastructural similarities to animal rickettsiae. Recently, many XLB have been cultured in vitro, and pathogenicity has been demonstrated (3,19,22). Pure cultures of the bacteria have been used to study the guanine + cytosine (G + C) ratios and serologic relationships with other plant-pathogenic bacteria and rickettsiae, and XLB have been found to be unrelated to ricksettsiae and other plant pathogens.

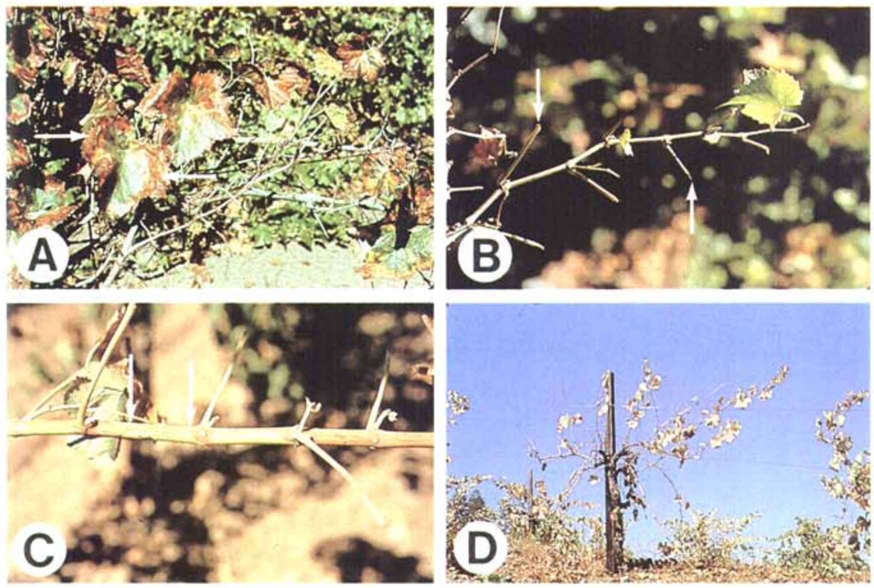

Fig. 1. Symptoms of Pierce's disease of grapevines: (A) Leaf scorching and bronzing, with chlorotic areas next to necrotic areas (arrows); (B) loss of leaf blades, with only petioles remaining (arrows); (C) irregular maturation of diseased stem, with patches of brown (arrows) and green tissues; (D) decline and death of the plant.

Diseases Caused by XLB

Pierce's disease of grapevines (*Vitis vinifera* L.), reported in California in 1892, brought the professional plant pathologist N. B. Pierce to the state. The disease was originally observed near Anaheim and was called California vine disease. Pierce studied the symptomatology and distribution of the disease extensively, but because bacteria isolation techniques were limited, he could only hypothesize that the disease was caused by a "minute microorganism."

When Pierce's disease was found to be graft-transmissible, the cause was attributed to a virus (7). This conclusion was strengthened by detection of insect vectors (8). While studying insect transmission, Hewitt et al (8) observed a high incidence of Pierce's disease in vineyards near alfalfa (*Medicago sativa* L.) fields with a high incidence of alfalfa dwarf disease. Later, Pierce's disease and alfalfa dwarf disease were shown to be caused by a similar pathogen (5).

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Even though no virus or viruslike agents were observed in diseased grape and alfalfa tissues and Koch's postulates were not fulfilled, the two diseases were considered to be caused by a virus until 1973, when pathologists in California and Florida independently found "rickettsialike" bacteria in the xylem of affected plants but not in that of healthy plants (5,10). Heat therapy studies supported the association of bacteria with Pierce's disease (5). After Davis et al (3) succeeded in formulating bacteriologic culture media and in vitro isolation techniques, the bacterium could be cultured, Koch's postulates were fulfilled, and new avenues were opened for the study of XLB biology. The Pierce's disease bacterium was also shown to cause almond leaf scorch disease in almond (*Prunus amygdalus* Batsch), responsible for severe losses in some almond cultivars in California, and the same insects were found to be the vectors of both diseases (13).

The most characteristic symptom of Pierce's disease of grapevines is leaf scalding or scorching. Another early sign is sudden drying of part of a green leaf. Affected areas of a leaf usually turn brown, and adjacent tissue turns yellow or red. Usually, scorching begins at the leaf margin and progresses inward (Fig. 1A). Sometimes only the tip of a leaf appears scorched; other times an entire leaf is affected and the blade drops off, leaving the petiole (Fig. 1B). Diseased stems often mature irregularly, with patches of brown and green tissues (Fig. 1C). During the second year, growth is delayed, dwarfing occurs, fruit yields are reduced, the root system declines, and the plant dies (Fig. 1D). Hopkins (9) suggested that scorching results from restricted flow of water and nutrients owing to partial or complete plugging of xylem vessels by bacteria. We have suggested that bacterial toxins induce the symptom.

Symptoms of almond leaf scorch are

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similar to those of Pierce's disease (Fig. 2). Alfalfa under field conditions, however, does not show scorching; instead, affected plants show decline and stunting (Fig. 3).

Phony peach disease was known to pathologists even before Pierce's disease was observed in California, having caused serious losses in peach (*P. persica* (L.) Batsch) orchards in the southeastern United States as early as 1890 (11). Because of graft- and insect-transmissibility, the disease was attributed to a virus, until 1973, when "rickettsialike" bacteria were detected by electron microscopy in the xylem tissues of affected peach (14). Wild *Prunus* spp. were found to be hosts. In 1977, a leaf scald disease of Japanese plum (*P. saliciana* Lindl.) was reported from Georgia and Alabama. Plum leaf scald also occurs in Argentina, Brazil, and Paraguay. Recently, bacteria morphologically and ultrastructurally similar to the Pierce's disease bacterium were isolated in pure culture (2,23) from plant material affected with phony peach disease and plum leaf scald, and Koch's postulates were fulfilled (19,22). Pathogenicity tests showed the diseases to be caused by the same pathogen.

Peach trees with phony peach disease do not show scorching or scalding but are severely stunted and have a compact growth habit (Fig. 4). The trees do not die but produce small, distorted fruit with no market value. Diseased plum trees, however, show leaf scorching or scalding similar to that of grapevines with Pierce's disease.

In recent years, XLB have been shown to be the causal agents of, or associated with, several plant diseases for which no etiologic agents or abiotic causes were previously known (6,20). These include ragweed (*Ambrosia artemisiifolia* L.) stunt, periwinkle (*Vinca minor* L.) wilt, and leaf scorch of elm (*Ulmus americana* L.) (Fig. 5A), oak (*Quercus rubra* L.) (Fig. 5B), mulberry (*Morus rubra* L.), and sycamore (*Platanus occidentalis* L.). Diseased ragweed has been reported only in Florida; no symptoms were apparent in nature, but reduced growth and stunting were observed under greenhouse conditions. Similarly, periwinkle wilt has been found in a greenhouse in Florida but not observed in nature; symptoms included chlorosis and yellowing of foliage, stunting, and wilt. Leaf scorch symptoms on elm, oak, mulberry, and sycamore have been observed in the northeastern and southern United States and are similar to those of Pierce's disease of grapevines.

Insect Vectors

XLB are transmitted by leafhoppers known as sharpshooters and spittlebugs, including *Draeculacephala minerva* Ball, *Carneocephala fulgida* Nottingham, *Helochara communis* Fitch, *Homalodisca coagulata* (Say), and *Oncometopia nigricans* (Walker) (15). The observation that Pierce's disease of grapevines was severe near alfalfa fields and decreased with distance led to the discovery of insect vectors (8). Some sharpshooters, e.g., *H. coagulata* and *O. nigricans*, transmit not only the Pierce's disease bacterium but also phony peach disease, plum leaf scald, and periwinkle wilt bacteria.

In insect transmission tests on 100 plant species, Freitag (4) identified 75 symptomless hosts of the Pierce's disease bacterium. The leafhoppers usually overwinter and breed on the symptomless wild hosts, then spread the pathogen to and within cultivated crops. A recent study (18) showed the importance of wild plants as sources of the Pierce's disease

Fig. 2. Almond leaves with scorching symptom typical of almond leaf scorch disease.

Fig. 3. (Left) Stunting and compact growth of alfalfa plant with alfalfa dwarf disease; (right) healthy plant. (Courtesy D. Hall)

Fig. 4. (Left and center) Stunting, short internodes, and compact growth of peach trees with phony peach disease; (right) healthy tree.

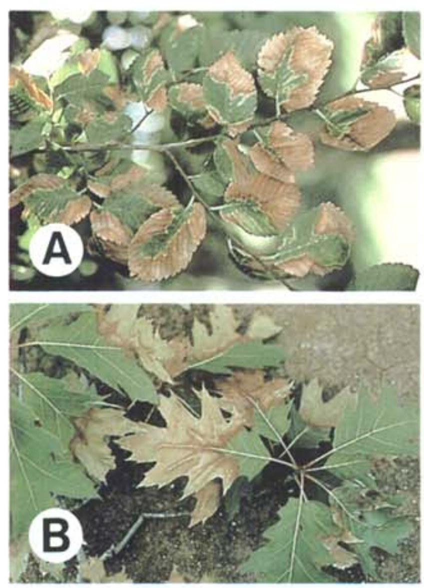

Fig. 5. Leaf burning and necrosis of (A) elm and (B) red oak with leaf scorch disease. (Courtesy S. Kostka)

bacterium for leafhoppers. Differences in vector efficiency may be related to the vectors' host preferences. For example, *D. minerva* feeds primarily on grass and only occasionally on grapevines and almond trees, whereas *Graphocephala atropunctata* (Signoret) is found primarily on woody perennials, including wild and cultivated grape.

Nymph and adult sharpshooters are equally efficient in transmitting XLB. The minimum latent period is 2 hours or less; in some instances, the vectors transmit the bacterium almost immediately after acquisition. The insect remains infective until molting. XLB do not circulate via the hemolymph to the salivary glands, as do mycoplasmalike organisms and spiroplasmas, but do propagate in the foregut, which is shed during molting.

On the basis of scanning electron microscopy studies, Purcell et al (16) proposed the following mechanism of transmission for the Pierce's disease bacterium: Bacterial cells taken up by xylem-feeding insects from diseased plants attach to the floor of the cibarium and the apodemal groove of the diaphragm and multiply, forming a bacterial plaque (Fig. 6); during subsequent feeding, the bacteria are flushed from the foregut by egestion of the sucking pump and enter the host's xylem tissues. Similar observations were made with other XLB and their insect vectors (1).

Unlike the Mollicute plant pathogens (i.e., those with no cell walls), XLB cannot be transmitted by leafhoppers through Parafilm, even though the bacteria can be readily injected mechanically into the plant. Fimbriaelike structures are easily observed by electron microscopy in XLB in plant and insect hosts but are rarely seen in XLB from pure cultures. Possibly, these structures are necessary for vector transmission.

The Bacteria

XLB are rod-shaped with distinctive rippled cell walls (Fig. 7). The bacteria are nonflagellate, do not form spores, measure 0.3–0.5 μm in diameter and 1–5 μm in length, and occur only in xylem tissues of affected plants. The fastidious bacteria cannot be cultured on conventional bacteriologic media. The recently developed BCZE medium (19), as well as some others, can be used to isolate and grow several XLB. The bacteria grow well at 20–25 C and pH 6.7–7.0. The tolerances for salt and carbon dioxide are 1 and 2.5%, respectively. Some type of hemin chloride seems to be essential for growth. Primary colonies are generally white or greenish white, are mostly circular with smooth or rough margins, and can be seen 1–2 weeks after the primary isolation.

Phony peach disease and plum leaf scald bacteria are gram-negative and are positive for catalase, gelatinase, and hippurate and negative for acid fastness,

Fig. 6. Scanning electron micrograph of inoculative leafhopper cibarium with dense growth of Pierce's disease bacterium. (Courtesy A. Purcell)

oxidase, coagulase, β-galactosidase, H_2S production, urease, phosphatase, indole production, and acid formation from glucose (21). Studies with various XLB show the G + C ratio to be about 50.5 mol%, with genome molecular weights of $1.4 \times 10^9 + 0.2 \times 10^9$ (12). No genetic relatedness has been shown between XLB and other plant-pathogenic bacteria.

Serologic studies have indicated, and pathogenicity tests have confirmed, two distinct XLB groups: the Pierce's disease group and the phony peach disease group. Morphological, ultrastructural, genetic, serologic, and pathological studies have identified XLB as a distinct group of plant pathogens not related to other organisms. Hence, a new name, *Xylemella fastidiosum*, has been proposed for the Pierce's disease bacterium (21), with this bacterium as the type strain for the genus *Xylemella*.

Detection Methods

A method was devised as early as 1920 for detecting the phony peach disease agent. When stem sections were treated with acidified methanol, diseased xylem stained red (Fig. 8). This test worked well only with mature wood, however. Other XLB-detection methods that have been used, some with only limited success, are vacuum extraction of bacteria with potassium hydroxide, phase-contrast microscopy, direct and indirect immunofluorescence, and in situ immunofluorescence. More recently, enzyme-linked immunosorbent assay (ELISA) has been used extensively to detect XLB in diseased plants, symptomless hosts, and insects. With the help of in vitro isolation techniques and ELISA, several laboratories now routinely detect XLB in plants.

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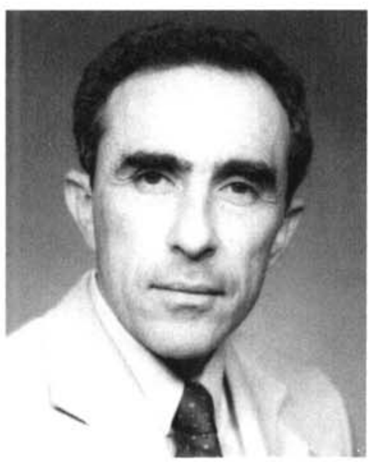

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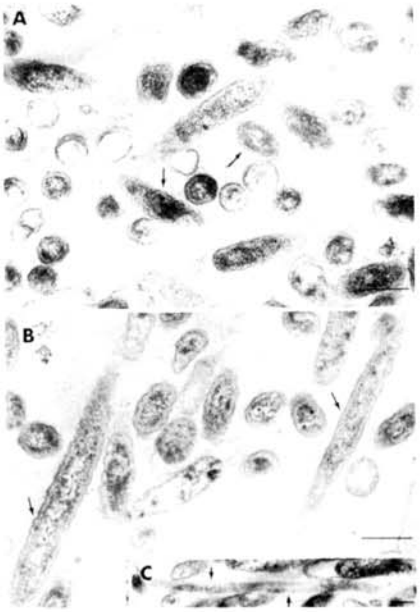

Fig. 7. Transmission electron micrographs of ultrathin sections of (A) phony peach and (B) plum leaf scald bacteria from culture; arrows indicate rippled cell walls. (C) Unusually long bacterial cell. Scale bar = 0.5 μm. (Courtesy S. Lowe)

Epidemiology

Pierce's disease of grapevines is endemic in symptomless wild plants throughout the southern United States and is the major limiting factor there in production of European-type and bunch grapes. In California, the disease occurs in "hot spots" adjacent to permanent water sources and several weed hosts. A recent study showed the importance of symptomless wild plants in the epidemiology of Pierce's disease in California (18). These plants not only support vast vector populations but also are excellent sources of the bacterium, which explains why removing affected grapevines has been of limited value in preventing disease spread in California vineyards. Similar observations have been made in Florida. Pierce's disease of grapevines has been reported from Costa Rica and Mexico but not from Europe.

Alfalfa dwarf disease has been observed only in the southern United States and in California, even though the insect vectors have also been found in midwestern and northern states. Almond leaf scorch has been reported only in California. Under greenhouse conditions, the same bacterium causes Pierce's disease of grapevines, alfalfa dwarf disease, and almond leaf scorch. When two different crop hosts are located near each other, however, only one is affected. For example, almond trees growing next to heavily diseased grapevines in California show no symptoms of almond leaf scorch, and vice versa. When mechanically inoculated with the bacterium in the field, however, almond trees show typical almond leaf scorch symptoms. Vector preference may be an important factor in this phenomenon.

Phony peach disease, plum leaf scald, ragweed stunt, and periwinkle wilt occur in the southern United States but not in California. Plum leaf scald, but not phony peach disease, has been reported from South America. In Argentina, peach trees adajacent to areas affected with plum leaf scald showed no symptoms of phony peach disease, even though the same bacterium causes both diseases under greenhouse conditions (19). In peach trees, populations of the phony peach disease bacterium are high in root tissues but low or absent in leaf and stem tissues (22), whereas in plum trees, populations are high in all three tissues. Peach, therefore, may be a dead-end host for the bacterium, since insect transmission from peach to peach is poor. Because plum leaves support good growth of the bacterium and stem tissue is excellent for graft and in vitro isolation studies, native and wild plums are considered important hosts for spread of phony peach disease.

Management Strategies

Because XLB infections are usually lethal and kill cultivated host plants within 2–3 years, the diseases are often self-eliminating. Some diseased plants serve as sources of inoculum for further spread, however. Diseased plants take up valuable space in an orchard but do not produce marketable fruit, even during the early stages of infection. Roguing infected plants and replanting with disease-tolerant cultivars is therefore important.

Removal of alternate hosts has been recommended for Pierce's disease of grapevines and phony peach disease. The practice of removing diseased wild plums in Georgia dates back to 1930, and our studies in California indicate selective removal of symptomless hosts of Pierce's disease reduces availability of both bacterium and vector. Whether maintaining orchards free from weeds and wild plants helps control XLB-caused diseases is not known, but weeds and wild plants do support XLB vectors, so clean ground maintenance practices should be considered.

Practical control of all XLB vectors is difficult. Several studies have shown that insecticide sprays are not an economical means of controlling vectors of Pierce's disease of grapevines in California. During certain times of the year, however, insect populations increase drastically, and application of effective insecticides may be necessary.

Injections of antibiotics, particularly tetracycline hydrochloride, into diseased tree trunks adequately control some XLB-caused diseases. Plants with advanced disease have severely plugged xylem, however, and are difficult to treat in this manner. We tested the efficacy of several chemicals and antibiotics other

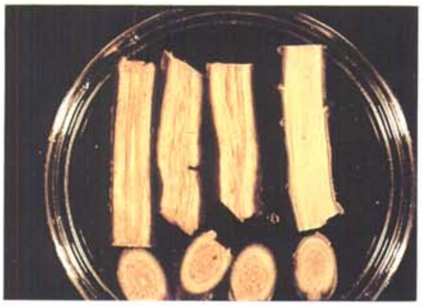

Fig. 8. When treated with acidified methanol, tissue of almond stems infected with Pierce's disease bacterium stains red, whereas healthy tissue (right) remains clear. (Courtesy S. Mircetich)

than tetracycline against some XLB and found that most are either phytotoxic to the host or cost-prohibitive. We also found that symptoms remitted only after injections, and not after foliar applications, and that treatment had to be given at least annually to maintain the remission. Therefore, unless highly effective new ones are developed, chemicals seem to offer little hope for controlling XLB-caused diseases.

Planting tolerant cultivars is one of the best strategies for long-range management of XLB-caused diseases, especially in areas where diseases occur in "hot spots." Several popular wine grape cultivars in California have been screened against the Pierce's disease bacterium under greenhouse and field conditions, and some tolerant ones have been identified (17). Grape cultivars tolerant to Pierce's disease have also been identified in Florida. Our recent studies indicate that almond is immune to phony peach disease and that peach is immune to Pierce's disease. Because survival of bacteria in roots is an important factor in chronic infection, these two hosts could be used as rootstocks. Almond rootstocks may be especially useful in controlling phony peach disease, since that bacterium survives mainly in the roots.

Future Directions

Innovative research in breeding and genetics is needed to achieve long-range control of diseases caused by XLB. A tissue culture system offers the greatest potential for developing XLB-tolerant plant material. For example, clones of grape resistant to Pierce's disease may be generated by using protoplast or callus cell cultures employing the bacterium or its products produced in culture. This approach may permit development of a "resistant clone" of a popular cultivar that is susceptible in the field.

Most of the XLB that cause plant diseases in the United States have been isolated and maintained in pure culture, yet their taxonomic status has not been defined. Taxonomic studies of XLB would improve communication among researchers.

The mechanism of pathogenesis is not fully understood. Research in this area would aid disease control and possibly open avenues for the use of nonharmful XLB or other agents in biocontrol programs.

For a better understanding of vector-pathogen-host relationships, we need to determine why vectors can easily transmit XLB from infected plants and mechanically injected culture-grown bacteria can cause disease in healthy plants but vectors feeding on cultures cannot transmit the bacteria. We also need to determine why plum leaf scald, but not phony peach disease, is a serious problem in several South American countries, and why phony peach disease and plum leaf scald are not seen in California despite the presence of both host and vectors. Why has Pierce's disease of grapevines been restricted to North and Central America, while other grape pathogens are distributed worldwide? The role of environmental factors in these phenomena and in the epidemiology of diseases caused by XLB needs to be elucidated.

Such serologic tests as ELISA with polyclonal antibodies have been of great help in detecting, characterizing, and identifying symptomless host plants and vectors of XLB. Development of monoclonal antibodies may enable researchers to define the exact serologic relationships of XLB, and use of DNA probes and dot-blot hybridization tests may further enhance the sensitivity of XLB detection.

In recent years, great progress has been made in developing media and techniques for isolating XLB from plants and insects and for demonstrating pathogenicity. Currently, the nutritional requirements of XLB need to be understood. Studies in this area may aid the development of media for other bacteria residing in the phloem and causing such diseases as citrus greening and clover club leaf.

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