Pecan Bacterial Leaf Scorch: Cultivar Susceptibility and Pathogen Transmission

R. S. Sanderlin and Rebecca A. Melanson

Louisiana State University Agricultural Center Pecan Research-Extension Station, P. O. Box 5519, Shreveport, LA 71135

Subject Category: Plant Pathology

Pecan Bacterial Leaf Scorch: Cultivar Susceptibility and Pathogen Transmission

Additional index words. *Carya illinoinensis*, *Xylella fastidiosa*, graft-transmission, insect vectors

Abstract. Pecan bacterial leaf scorch (PBLS) and the pathogen *Xylella fastidiosa* were identified in 20 pecan cultivars observed in Louisiana commercial orchards over a two-year period. The disease occurred in the majority of orchards observed and was distributed throughout the pecan growing areas of the state. Leaf scorch was most prevalent in the Cape Fear cultivar. A few other cultivars sometimes exhibited disease severity similar to that typical of Cape Fear. The disease was also identified in a few ungrafted trees in some orchards. An experimental plot was established to test the possibility of *X. fastidiosa* transmission through scion wood. At the end of two growing seasons following grafting, over 16% of the seedlings grafted with scions from infected trees had developed leaf scorch. The bacterium was also transmitted through 66% of infected seedlings used as rootstock into newly developing trees that were grafted with scion wood from healthy trees. The pecan spittlebug, *Clastoptera achatina*, was tested as a possible vector of *X. fastidiosa* to pecan. About 3000 spittlebug nymphs and 150 adults were collected from infected trees and placed onto seedlings of Cape Fear. Through the first growing season none of the 100 plus seedlings exposed to the spittlebugs have developed PBLS.

Pecan bacterial leaf scorch is caused by the xylem-limited bacterium *Xylella fastidiosa*, the pathogen that causes Pierce's disease of grapes and scorch-type diseases in numerous other hosts including many hardwood tree species. Affected leaflets of trees with PBLS begin to turn tan to brown at the tips and/or edges, and the necrosis progresses toward the midrib of leaflets followed by abscission of affected leaflets. The symptoms usually begin to appear in mid-summer following leaf maturity. Disease symptoms may occur throughout a tree or be confined to individual limbs. The disease is chronic and symptoms tend to occur annually with variation in the degree of severity. The Cape Fear cultivar is known to have a high incidence and severity of PBLS but nothing is known of the susceptibility of other cultivars to the disease or how the pathogen is transmitted to trees. Information is reported here on the occurrence of the disease in other cultivars and on attempts to transmit the pathogen through grafting and insect vectors.

Materials and Methods

Cultivar survey for PBLS. To determine if PBLS occurs in cultivars other than Cape Fear, a survey was conducted over a two-year period for the disease in cultivars found in commercial pecan orchards in Louisiana. Symptomatic leaves were collected for serological assay from trees that appeared to have the disease. Likewise, some trees without symptoms were assayed for the bacterium. A commercial ELISA kit (Agdia, Inc.) was used for all tests to detect the pathogen throughout this work.

Graft-transmission of X. fastidiosa in pecan. In 2003, an experimental plot of 200 seedlings was planted at the Pecan Research-Extension Station at a 3 m X 3 m spacing.

One-half of the seedlings in the plot were grafted to scion wood collected from cv. Cape Fear trees infected with the bacterium, fifty seedlings were grafted to scion wood collected from trees believed to not be infected, and fifty seedlings were left ungrafted. The plot was arranged in a complete-randomized block design with ten blocks. Scion wood for the plot was collected in January 2003. Stem sections from one or two season's growth were cut into pieces 15 to 20 cm in length for individual scions and then sealed with a melted mixture of resin and beeswax. The scion wood was wrapped in moist paper towel and stored in plastic bags at 4 to 7^0C until it was used for grafting in May. All grafts were made using the four-flap technique and were wrapped with plastic grafting tape. The plot was monitored for scorch symptoms throughout the summer and all trees that survived were assayed for infection by ELISA in September 2003.

In addition to transmission through scions, the possibility of transmission through infected rootstock was evaluated. Twenty-five potted seedlings of different cultivar types that had previously been infected by needle puncture inoculation and twelve seedlings that were uninfected were grafted (four-flap) to scions collected from healthy trees. The scion wood was collected in the manner described previously and the seedlings were grafted in May 2004. Disease development was monitored through the summer and the trees were assayed for *X. fastidiosa* by ELISA in the fall.

Evaluation of pecan spittlebug as a vector of X. fastidiosa. The pecan spittlebug, *Clastoptera achatina*, was evaluated as a possible vector of the bacterium to pecan trees. Approximately 3000 spittlebug nymphs were collected from symptomatic terminals of

infected Cape Fear trees in a commercial pecan orchard. Terminals with spittlebug masses were cut and transported to the Pecan Research-Extension Station where the nymphs were removed with artist brushes and placed onto two-to-three-month old seedlings of Cape Fear. Generally, 10 to 30 nymphs were placed on a seedling. Similarly, adult spittlebugs were collected from symptomatic trees with an aspirator and transferred onto uninfected seedlings the same day. Over 200 adults were placed onto seedlings with 10 to 20 adults per seedling. An additional 2600 nymphs and 150 adults were tested serologically for the bacterium after collection from infected trees.

Results

Cultivar survey for PBLS. Pecan bacterial leaf scorch was present in the majority of orchards that were examined. Twenty susceptible cultivars were identified in the survey through symptom recognition and a positive assay for infection with the bacterium (Table 1) (Sanderlin, 2005). The disease was also identified in a few ungrafted trees present in some of the orchards surveyed. The Cape Fear cultivar had the highest incidence of disease of any of the cultivars observed. A few cultivars had some trees with disease severity similar to cv. Cape Fear. These included Barton, Cheyenne, and Pawnee. Trees without symptoms assayed negatively for *X. fastidiosa* in 70 of 75 trees across 17 cultivars. Four of the five trees without symptoms that assayed positive for infection were in cultivars that had other trees with symptoms of scorch that were infected. It is not unusual for asymptomatic trees that test positive for the bacterium to later develop visual symptoms. In the large majority of assays, trees with PBLS symptoms were verified as infected and trees without symptoms tested negative for infection.

Graft-transmission of X. fastidiosa in pecan. The pathogen survived in scion wood and was able to infect tissue that grew following grafting. After two growing seasons, 16.5% of the 85 surviving grafts with scion wood obtained from infected Cape Fear trees were positive for infection and developed symptoms of PBLS (Table 2) (Sanderlin, 2005). One of the 39 trees that developed following grafting with scions from uninfected trees developed scorch (Table 2). It is probable that infection was the result of scion wood that was collected from a tree that later was found to have one infected limb on it. However, other possible origins of the pathogen can not be ruled out including transmission through an infected rootstock or insect transmission. None of the other 38 trees that have grown from the uninfected source grafts developed infection during the two years following grafting. Likewise, none of the 48 surviving ungrafted seedlings have tested positive for the bacterium or developed disease over the two year period of the test.

Correspondingly, when infected seedlings were used as rootstock, the pathogen was able to pass into tissue developing from uninfected scions and establish disease in the new trees. By the end of the growing season, 66% of the trees that developed following grafting onto infected rootstock were infected (Table 3). None of the seven trees that survived following grafting with uninfected rootstocks and uninfected scions were infected.

Evaluation of pecan spittlebug as a vector of X. fastidiosa. None of the 103 seedlings exposed to pecan spittlebug nymphs or adults developed disease symptoms during the

growing season in which they were exposed. These plants are being maintained in a greenhouse for another year because it may take longer than one growth period for disease expression to occur. ELISA tests of the nymphs for the bacterium were negative. Most of the serological reactions with the adults were negative; however, three to five of the reactions were close to the criteria for a positive reaction and may represent a low concentration of the bacterium in individual adults.

Discussion

It was not possible to categorize cultivars into different levels of susceptibility in this study. There was a lot of variation within cultivars in the intensity of disease from orchard to orchard. For example, trees of the Pawnee cultivar were observed without symptoms in several orchards; however, in one orchard every one of 30 to 40 trees of Pawnee expressed a severe level of scorch symptoms, and the trees that were tested for infection by ELISA were positive. Orchard observations of a cultivar even when made at different locations over a period of more than a year are not adequate to accurately gauge the susceptibility of the cultivar. It is likely that many other cultivars, not commonly observed in this study, are susceptible to infection by the pathogen. Nothing is known of how environmental conditions or a pecan tree's physiological condition affect infection and disease development.

The results of the field plot to test for transmission of *X. fastidiosa* to scion wood indicate that the pathogen can survive a typical processing and storage technique for pecan scion wood. A graft-transmission rate of about 16% has been observed after two years of

observation; however, because the scion wood was collected from known infected trees but not from known infected limbs, it is possible that a higher rate of transmission would occur if all of the scion wood used in grafting was from infected limbs. Because the disease may move slowly from tree to tree (no clear spread to ungrafted trees or trees grafted to uninfected scions after two years in a 3 m X 3 m planting), the use of uninfected scion wood may provide a useful level of reducing introduction and spread of the disease into pecan orchards. To accomplish this, it would be necessary to identify trees without symptoms during late summer each year that scion wood would be collected from the trees. Not surprisingly, the pathogen was also readily transmitted to newly developing trees following grafting to infected rootstock. Thus, to avoid infection through grafting, it would be necessary to use only uninfected rootstock. The rootstock used in this study was infected through artificial inoculation; the frequency of natural rootstock infection is not known.

The lack of detection of transmission of *X. fastidiosa* to pecan by the pecan spittlebug thus far has been surprising. It was hypothesized that this insect would be a vector from pecan tree to pecan tree because spittlebugs are known to transmit the pathogen in other hosts (Frazier, 1965). It is believed that seedlings grown from cv. Cape Fear nuts would be susceptible to insect transmission of the bacterium because they have been infected in other tests by needle inoculation (Sanderlin and Heyderich-Alger, 2000). In addition to continuing to test the pecan spittlebug, other potential vectors such as leafhoppers will be tested when they can be captured.

An earlier study in which cv. Cape Fear seedlings were exposed for a period of a week from spring through summer in a commercial orchard with a high intensity of PBLS failed to result in any detectable infection of the seedlings over a three-year period (Sanderlin, unpublished). These results and observation of movement of the disease within orchards suggest that transmission from tree to tree is erratic and often slow. If this is an accurate view of the movement of the pathogen and disease within orchards, then the use of uninfected scions and rootstocks may provide a useful level of managing PBLS.

Some anomalies have been encountered in this work that warrant reporting but for which we do not currently have a verified explanation. Not everything that has scorch-like symptoms is caused by *X. fastidiosa*. Sometimes during late summer and fall, leaves develop symptoms but do not test positive for the bacterium. It is postulated that in some cases the symptoms are the result of leaf spot lesions such as zonate leaf spot coalescing to form large scorched areas on the leaflets. Such symptoms could also be the result of nutritional imbalances (potassium scorch) or drought (Gossard, 1961; Worley and Littrell, 1973). In one instance, a tree that had been uninfected (no symptoms and negative ELISA) for several years, developed scorch on one limb in 2003 and leaves from that limb tested positive for infection. However, in 2004, the same limb once again developed scorch but twice tested negative for infection by ELISA. Also in 2004, a few trees that had PBLS in previous years failed to produce symptoms and assayed negative for *X. fastidiosa*.

Literature Cited

Latham, A. J. 1969. Zonate leafspot of pecan caused by *Cristulariella pyramidalis*. Phytopathology 59:103-107.

Gossard, A. C. 1961. Some causes and effects of pecan leaf scorch. Proc. Southeast. Pecan Growers Assoc. 54:43-46.

Sanderlin, R. S. 2005. Cultivar and seedling susceptibility to pecan bacterial leaf scorch caused by *Xylella fastidiosa* and graft transmission of the pathogen. Plant Disease 89:446-449.

Worley, R. 1983. Effects of potassium and nitrogen on leaf scorch and yield of 'Desirable" pecans. Proc. Southeast. Pecan Growers Assoc.76:127-131.

Table 1. Pecan cultivars identified with pecan bacterial leaf scorch during a two-year survey of orchards in Louisiana.[z]

Barton	Melrose
Caddo	Moreland
Cape Fear	Navaho
Cherokee	Oconee
Cheyenne	Pawnee
Desirable	Schley
Farley	Schley-Barton
Forkert	Shoshoni
Jackson	Stuart
Kiowa	Sumner
Mahan	Ungrafted Trees

[z] Cultivars were identified as having pecan bacterial leaf scorch by symptoms and serological assay for infection with *Xylella fastidiosa*.

Table 2: Transmission of *Xylella fastidiosa* through scions.

Graft Combination	Trees Grafted	Tree Survival[z]	Trees Infected[y]	% Xylella Transmission
Healthy Scion and Rootstock	50	39	1	2.6
Infected Scion Source Healthy Rootstock	100	85	14	16.5
Healthy Seedlings	50	48	0	0

[z]Trees were grafted in May and the number of grafts and trees that survived were recorded in October.
[y]All infected trees developed leaf scorch.

Table 3: *Xylella fastidiosa* Rootstock Transmission in Pecan

	Effect on New Graft[z]	
Uninfected Rootstock[y]	PBLS Symptoms	Serology Test Seedlings
Barton	0/2	0/2
Cape Fear	0/1	0/1
Cheyenne	0/1	0/1
Kiowa	0/1	0/1
Schley	0/2	0/2
Infected Rootstock Seedlings		
Barton	0/2	1/2
Cape Fear	1/1	1/1
Cheyenne	1/2	2/2
Desirable	1/2	1/2
Kiowa	3/4	3/4
Schley	0/1	0/1

[z]The denominator is the number of trees in the test with successful grafts and the numerator is the number of trees that developed symptoms of PBLS and/or tested positive for infection with *X. fastidiosa* by serological assay.
[y]Grafts to uninfected rootstocks of Desirable seedlings were not successful.