Determining the Role of Bacterial Leaf Scorch, Canker Stain, and Botryosphaeria Canker in the Dieback of Plantation Sycamores in the Southeastern United States

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In recent years, there has been renewed interest in growing American sycamore (*Platanus occident&)* in plantations throughout the southeastern United States. Sycamore has good commercial value because of its rapid growth and excellent pulping qualities for paper product production. Sycamore typically grows in bottomlands where wintertime access for harvesting is limited by wet soils and the accompanying environmental concerns. Occasionally, sycamore is a pioneer species on upland old-field sites, but it usually does not grow well on these sites (24). Forest industries are beginning to grow sycamore in plantations on upland sites that can be accessed at any time of the year. The advent of fiber-farming technology is allowing industry growers to take advantage of the sycamore's fast initial growth on even relatively infertile soils or other upland sites and to foster continued rapid growth by using irrigation and liquid fertilization (i.e., fertigation). However, several diseases that can occur in the first 3 to 5 years often hamper successful plantation establishment of this bottomland species on upland sites.

Surveys conducted in the early and mid-1970s to determine the severity of sycamore dieback in the Southeast focused on leaf scorch, dieback, and cankers and found a complex of pathogens including *Ceratocystis fimbriata* f. sp. *platani* (Ellis and Halst.) J.M. Walter, *Botryosphaeria rhodinu* (Cooke) Arx, and two conidial stages of *Apiognomoniu veneta* (Sacc. and Speg.) Hõhn, which causes sycamore anthracnose (7,11,15). These reports generally agreed that branch dieback and mortality were caused by *C. fimbriata* f. sp. *platuni* and *B. rhodinu* and that leaf scorching occurred in all locations surveyed. Leaves were described as scorched, eventually turning completely brown, but not shedding prematurely. These symptoms describe bacterial leaf scorch (1,12,20) but, at the time, were attributed to late-summer expressions of anthracnose, with a species of *Colletotrichum* as the suspected pathogen (15). Sherald and

Kostka (20) stated that tree diseases caused by *Xylella fastidiosa* Wells et al. were hard to diagnose because (i) diagnosticians were unfamiliar with the pathogen; (ii) symptoms were easily confused with those caused by other biotic and abiotic factors such as moisture stress; and (iii) due to its fastidious nature, the presence of the bacterium was difficult to confirm using routine laboratory techniques. The advent of enzyme-linked immunosorbent assay (ELISA) testing has made it fairly easy to test for X. *fastidiosa* in plants and facilitated the discovery of bacterial leaf scorch in sycamore throughout the Southeast (2,4,10,19).

Bacterial leaf scorch of sycamore, caused by X. *fustidiosu,* was widespread in plantations (Plate 19) surveyed during 1996, 1997, and 1998 in Alabama, Georgia, Florida, Illinois, Kentucky, North and South Carolina, and Virginia (4). X. *fastidiosa* infections cause leaf scorching (Plate 18), branch dieback, and can eventually kill trees (12). Botryosphaeria cankers, caused by *B. rhodina*, were recorded in these same plantations and states during the same years. Canker stain disease, caused by C. *fimbriata* f. sp. *platani*, was found in Illinois, Kentucky, and North and South Carolina. One of these three pathogens may predominate in a plantation, or they may occur in some combination, sometimes with other leaf or canker diseases of lesser importance, as a complex of pathogens causing dieback and decline throughout the plantation. Bacterial leaf scorch also was documented in sycamore shade trees in Alabama, Georgia, and Mississippi during 1996 to 1998 (*unpublished data*).

The name X. *fustidiosu* was proposed to describe all known strains of fastidious, Gram-negative, xylem-limited bacteria (23). The bacterium is known to cause disease in numerous hardwoods including American sycamore, London and oriental plane (20), red maple (*Acer rubrum*), American elm (*Ulmus americana*), several species in the red oak group (*Quercus* spp.), and red mulberry *(Moms rubru)* (1,22). Diseases caused by X. *fastidiosa* in important agronomic crops include Pierce's disease of grape, peach phony disease, plum leaf scald, citrus blight, almond leaf scorch, alfalfa dwarf (22), citrus variegated chlorosis, and coffee leaf scorch (6). X. *fustidiosu* was associated recently with pecan fungal leaf scorch (17). The natural host range of X. *fastidiosa* also includes a number of woody shrubs and vines and many annual herbaceous species (8,20). Xylem-feeding spittlebugs (family Cercopidae) and sharpshooter leafhoppers (family Cicadellinae) are known vectors of X. *fustidiosu* to grapes and fruit trees (16). Although not yet proven, it is assumed that similar insect vectors exist for sycamore.

The relative pathogenicities of X. *fastidiosu, C. fimbriata* f. sp. *platani*, and *B. rhodina* were studied using sycamore seedlings in greenhouses. Our objective was to begin to understand how these three common pathogens of American sycamore interact with each other within their host. This is a first step toward developing management guidelines for sycamore dieback and decline given the seemingly infinite number of combinations of pathogens, geographic locations, soil types, host genotypes, and cultural practices to which sycamore is exposed across the region.

MATERIALS AND METHODS

One-year-old sycamore seedlings were planted in April 1998 prior to bud break in 16-liter plastic pots using a local sandy clay loam soil. The study was replicated in greenhouses in Stoneville, MS and Athens, GA using soil native to each location. Seedlings were grown from open-pollinated seed from a seed orchard parent that was known to be sensitive to sycamore dieback. Pathogens were inoculated singly, or in various combinations, into 15 seedlings for each of 16 treatment levels, a total of 240 seedlings. Initial inoculations with *C. fimbriata* f. sp. *plantani*, *B. rhodina*, or agar were made on some seedlings in July 1998 and second inoculations of these same treatments occurred in August 1998 on the same seedlings according to the sequences given in Table 21 .1. Second or third inoculations with fungi or agar, following September 1998 bacterial inoculations were delayed until July 1999 to allow the bacteria to become established in the host.

Preceding fungal inoculations a cork borer was used to remove a 6- or 8-mm-diameter bark disk, depending on the seedling stem diameter, from a seedling at a point *30* cm above the soil. *C. fimbriata* f. sp. *platani* and *B. rhodina*, grown on acidified potato dextrose agar (APDA), were inoculated into seedlings using agar disks 6- or 8-mm in diameter. Agar disks were placed on the xylem in the hole made with the cork borer and covered with paraffin film. Controls were established in the same manner except that sterile APDA disks were used. One of three isolates of X. *fastidiosa*, collected from sycamore in Mississippi and Alabama, was introduced into plants in

TABLE 21. 1. Inoculation sequences for three pathogens[a] on sycamore

First inoculation		Second inoculation		Third inoculation	
Inoculant	Date	Inoculant	Date	Inoculant,	Date
Cerarocysfis	7/98	Control	8/98		
Ceratocystis	7/98	*Botryosphaeria*	8/98		
Ceratocysfis	7/98	***Xylella***	9/98		
Ceratocystis	7/98	*Xylella*	9/98	***Botryosphaeria***	7/99
Xylella	9/98	Control	7/99		
Xylella	9/98	*Botryosphaeria*	7/99		
Xylella	9/98	***Ceratocystis***	7/99		
Xylella	9/98	***Ceratocystis* and *Botryosphaeria***	7/99		
Botryosphaeria	7/98	**Control**	8/98		
Botryosphaeria	7/98	***Ceratocystis***	8/98		
Botryosphaeria	7/98	***Xylella***	9/98		
Botryosphaeria	7/98	*Xylella*	9/98	*Ceratocystis*	7/99
Control	7/98	Control	8/98		
Control	7/98	***Botryosphaeria***	8/98		
Control	7/98	*Xylella*	9/98		
Control	7/98	***Cerafocystis***	8/98		

[a] ***Ceratocystis*** *fimbriata* **f.** sp. *platani*; ***Botryosphaeria*** *rhodina*; and ***Xylella*** *fastidiosa*

periwinkle wilt (PW) broth medium (5) using a l-ml syringe with a 21-gauge needle. A droplet of PW broth, containing approximately 1 x 10^7 cfu per ml of bacteria, was placed on the bark of a green shoot that was then pricked with a syringe needle until the droplet was sucked into a xylem element via the transpiration stream. A single drop was inoculated into each of three shoots per seedling. Control seedlings-were similarly treated with PW broth only.

Several months after the initial inoculations, seedlings treated with C. *fimbriata* f. sp. *platani* that exhibited stem dieback in the Athens greenhouse were mistakenly pruned to the root collar. Several seedlings died as a result and the damage was felt to have compromised the Athens study. Stem dieback and canker data recorded up to that time are reported here. Stem heights in the Stoneville study were measured in August, 1 month after the first inoculations. No dieback had occurred at that point, so these data represent initial heights. Stem heights were measured again in September, 1 month after the second inoculations occurred. Periodically, lengths and widths of cankers were measured, and estimates were made of percent crown dieback and percent defoliation. In May and June 1999, seedlings inoculated with X.

and the controls were assayed for the bacterium using ELISA and were visually evaluated for bacterial leaf scorch symptoms.

RESULTS

In the Stoneville study, the average heights of seedlings inoculated with C. *fimbriata* f. sp. *platani* in July as initial inoculations or in August as second inoculations and measured in September were significantly ($P < 0.05$) less than those of seedlings not inoculated with C. *fimbriata* f. sp. *platani* (Fig. 21.1). Seedlings inoculated in July with C. *fimbriata* f. sp. ***plutuni*** had not yet experienced dieback in August, so there were no treatment differences in August stem heights. C. *fimbriata* f. sp. *platani* was lethal to the main stems between 30 and 60 days after the July inoculations and within 30 days after the August inoculations. The resulting stem dieback produced average stem heights, as measured on the tallest lateral branch, that were about 30% of those of seedlings not inoculated with C. *fimbriata* f. sp. *platani* (Fig. 21.1). Seedlings inoculated with C. *fimbriata* f. sp. *platani* had the greatest amounts of dieback and defoliation (data not shown) compared with other treatments. The average length of stem dieback (49.2 cm) caused by C. *fimbriata* f. sp. ***plutuni*** in the Athens study was greater ($P < 0.05$) than the dieback recorded for *B. rhodina*-treated (4.8 cm) or control (0.1 cm) seedlings. Seven of eleven dead seedlings in the Stoneville study were inoculated with ***C. fimbriata*** f. sp. *platani*. ***B. rhodinu*** infections in the Stoneville study caused some branch dieback, and 2- to 3-cm-long cankers that were sunken and partially callused over. Botryosphaeria cankers in the Athens study were sunken and water-soaked, but no callus developed.

X. fustidiosa was introduced into seedlings in September 1998 in Stoneville, MS; symptom expression occurred in many seedlings the following May. In June 1999, 71 of 120 seedlings inoculated with X. *fas-* but none of the 15 PW-control seedlings, showed symptoms of

bacterial leaf scorch. ELISA testing in late May and mid-June 1999 indicated the presence of X. *fastidiosa* in 29 of the 120 seedlings (24%) inoculated with ***X. fastidiosa*** and in none of the seedlings inoculated with periwinkle broth. Twenty-three of the twenty-nine ELISA-positive seedlings had bacterial leaf scorch symptoms, whereas the other six seedlings lacked those symptoms. One of the three *X. fastidiosa* isolates did not produce positive ELISA tests, but produced bacterial leaf scorch symptoms in more seedlings than either of the other two isolates.

DISCUSSION

The three pathogens tested in gteenhouse-grown sycamore seedlings produced host responses and symptoms similar to those seen in older trees in the field. As of early August 1999, X. *fastidiosa* produced fairly typical leaf scorch symptoms in 59% of the seedlings inoculated with the bacterium. However, it may be too early to determine how successful ***X. fastidiosa*** inoculations were overall, since some seedlings that tested positive for the bacterium did not express symptoms and vice versa. Sherald et al. (21) reported leaf scorching on only 15 of 23 sycamore seedlings 17 months after inoculation with ***X. fastidiosa***, but on 22 of 23 seedlings 3.5 years after inoculation. In addition, stem heights and diameters of *X. fastidiosa*-infected seedlings were less than those of controls. ***B. rhodina*** caused small stem

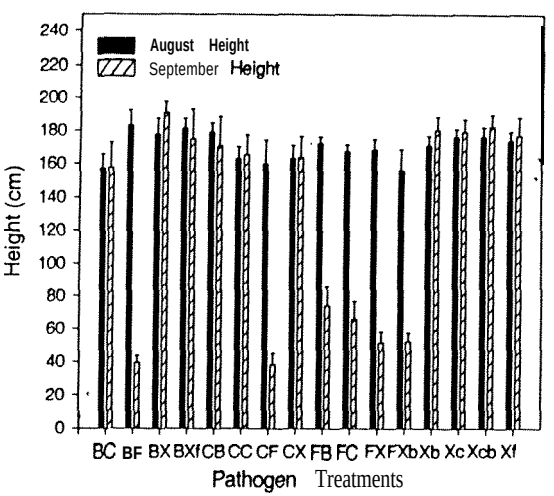

Fig. 21.1. Average (± standard error) stem heights of sycamore seedlings 1 (August) and 2 (September) months after inoculations with *Ceratocystis fimbriata* f. sp. *platani* (F), *Botryosphaeria rhodina* (B), *Xylella fastidiosa* (X), and agar (control [C]), separately or in combination. The first letters in each treatment indicate initial inoculations. The second, uppercase letters, indicate second inoculations, and lowercase letters indicate that 1999 growing season inoculations were applicable (cf. Table 21.1).

cankers and minor amounts of branch dieback, and callus formed over about a third of the canker area within a few months in the Stoneville study.

C. fimbriata f. sp. *platani* killed the main stem above the point of inoculation on all seedlings within 2 months. To date, 7 of the 11 dead seedlings were those inoculated with C. *fimbriata* f. sp. *platani*, another indication of the dramatic impact this fungus can have on young sycamore seedlings. In the midsouthern United States, canker stain has been reported to kill sycamores in I to 7 years depending on tree size and vigor (13), and l-year-old seedlings were killed in an average of 8 weeks after inoculation with *C. fimbriata* f. sp. *platani* (14). Except for those that died, the *C. fimbriata* f. sp. *platani*-inoculated seedlings in the Stoneville study produced a new leader from a shoot attached below the point of inoculation. However, infections have been observed moving down the stems, killing shoots below the inoculation point. Our study was designed to determine the relative aggressiveness of each pathogen in causing disease, and in contributing to the overall decline of sycamore seedlings, based on differences between treatments in data describing crown dieback over time, diameter and height growth, and biomass after 2 years. The importance of each pathogen to the dieback and decline syndrome in the field will depend on the prevalence of each organism in a given stand.

The pathogens *X. fastidiosa*, *C. fimbriata* f. sp. *platani*, and *B. rhodina* occur commonly throughout the southeastern United States. How do they interact with each other and various environmental factors to cause sycamore decline? McCracken and Burkhardt (14) reported that dieback in thin crowns caused by *B. rhodina* appeared more common on trees with canker stain disease. Britton et al. (3) reported that *B. rhodina* was commonly associated with *Xylella*-infected trees, but rarely found in plantations with *C. fimbriata* f. sp. *platani*. Mechanical wounding is an important factor in the transmission of canker stain (13) and is, therefore, important in disease reduction management programs in sycamore plantations (14). Growing sycamore on sites for which it is well suited and reducing wounding from equipment may ameliorate some problems caused by Botryosphaeria canker and canker stain. However, we lack information about the pathogenicity, singly and in combination with other pathogens, of *X. fastidiosa* in plantation sycamores. Limited evidence suggests that antibiotic injections can relieve temporarily (18) or delay the onset of (C. J. Chang, *unpublished data)* bacterial leaf scorch in sycamores, but not prevent or cure the disease. We need to do more studies and integrate the information into comprehensive management guidelines.

We also lack information about the insects that transmit *X. fastidiosa* to, and within, sycamore plantations. Direct control of insect vectors, such as leafhoppers and spittlebugs, is probably not practical, since these insects are common, abundant, and produce multiple generations throughout the growing season. Direct control of the alternate hosts of *X. fastidiosa* also is not feasible given the large number of common herbaceous plants in this category and the fact that the majority of these do not exhibit scorch symptoms (9). Probably the best management strategy will combine genetic resistance to the most pathogenic elements of the decline and include strategic and careful use of mechanical and chemical weed control and early chemical prophylaxis from diseases. A better understanding is required of the interactions between the major biotic and environmental factors involved-an understanding we have begun to develop through our current research and intend to continue developing in our future research.

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