Genetic Variation in Resistance to Canker Disease of Young American Sycamore

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ABSTRACT

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Nine percent of trees in a sycamore progeny test on a Mississippi River delta site exhibited leaf scorch, dieback, and lethal cankers by age 4. This represented an increase from 5% affected trees in May to 9% affected in September of the fourth growing season. Southern seed sources were least susceptible. Disease symptoms were negligible in similar tests at three locations outside the delta. Six-month-old seedlings representing some progeny families in the field tests were inoculated with *Botryodiplodia theobromae* in a greenhouse. Significant genetic variation among families within sources but not among sources was obtained for disease development. Correlations between greenhouse and field results were positive but low.

Canker diseases are killing American sycamore trees (*Platanus occidentalis* L.) in commercial plantations and natural stands. Symptoms include leaf scorch, twig dieback, and lethal trunk cankers (5). Although several fungi have been implicated, losses have most frequently been associated with *Ceratocystis fimbriata* (Ell. and Halst.) Davids. f. *platani* Walter in natural stands and *Botryodiplodia theobromae* Pat. in plantations (9). Sycamore decline, as the disease is sometimes called, has been observed in Illinois (10) and throughout the South (5), but mortality appears greatest in the lower Mississippi River valley. Up to 20% of the trees in several plantations in that area were killed between 1971 and 1973.

The use of disease-resistant planting stock will be the most cost-effective measure for preventing canker disease losses in plantations established in high risk areas. Cooper et al (3) found southern seed sources to be more resistant than northern sources and some progenies within sources to be more resistant than the source mean. Artificial inoculation of seedlings might provide rapid screening for resistance and hasten development of resistant stock.

This article reports disease incidence in 3- and 4-yr-old sycamore progeny tests at four locations in Mississippi. Seed source and family differences are evaluated. Greenhouse inoculation results are compared with natural disease incidence on the same progeny families in the field.

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MATERIALS AND METHODS

Field studies. Open-pollinated progenies from 200 sycamore trees representing 16 midsouth seed sources in a Mississippi State University tree improvement program and five sources across the South were planted in both 1975 and 1976 at three Gulf Coastal Plain sites and one Mississippi River delta site in Mississippi (Fig. 1). The 16 midsouth sources were each represented by 10 mother-tree families (five from each of two stands), and the other sources contained 4–10 families per source.

Percent mortality from trunk cankers and incidence of trees with leaf scorch or top dieback were scored during May and September 1978 in the 4-yr-old progeny tests and during September 1978 in the 3-yr-old tests. Significance of differences among seed sources was tested by analyses of variance of percent infected trees; differences among families within sources were tested by the Friedman nonparametric test (2) using the individual-tree disease scores. Fungi were identified from diseased branch tissues that were collected at the delta site in November 1978. These infected tissues were placed

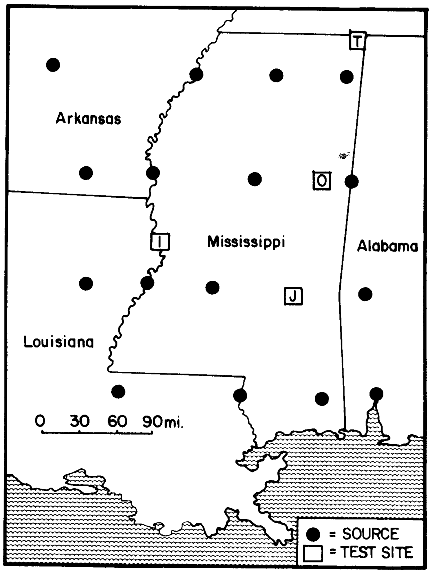

Fig. 1. Midsouth seed sources and planting sites used to study genetic variation in sycamore disease resistance. Planting sites: I = Issaquena County, J = Jasper County, O = Oktibbeha County, and T = Tishomingo County, Mississippi.

on potato-dextrose agar and incubated at 25 C.

Greenhouse study. Six-month-old seedlings from 48 mother trees representing 12 of the midsouth seed sources were inoculated with a virulent strain of *B. theobromae* isolated from a dying sycamore tree at College Station, TX (6). Each source was represented by two mother-tree families from each of two stands. Thirty-five of these families were represented in the field studies.

Cultures of the fungus were grown on Czapek's media at 25 C for 3 days and stored for 11 days at 10 C. Seedlings were inoculated with 3-mm diameter agar hyphal plugs in stem flap wounds that were 5–9 mm long, and the inoculated wounds were sealed with petroleum jelly. Four of the five seedlings in a family plot were inoculated. The fifth was wounded and covered with jelly to serve as a control.

Seedling height and ground-line stem diameter were recorded at the time of inoculation. Canker lengths were measured at 3-day intervals for 60 days, after which survival and canker length (expressed as a fraction of seedling height) were recorded.

Analyses of variance of a randomized complete block design were used to test significance of genetic variation among seed sources, stands within sources, and families within stands for rate of canker elongation during the 12 days following inoculation, maximum canker length as a fraction of seedling height, and arcsin of the square root of percent survival. The effect of seedling size on damage sustained by seedlings was examined by regression analysis. Field and greenhouse results were compared using Spearman's coefficient of rank correlation (11) for ranked family and source means.

RESULTS

Field studies. Incidence of diseased trees at the delta site nearly doubled from 5 to 9% during the fourth growing season (Table 1). The adjacent 3-yr-old test, containing the same families, had only one-third the disease incidence of the 4-yr-old test. Less than 0.25% of the 4-yr-old trees in three similar progeny tests on Gulf Coastal Plain sites showed symptoms.

Fungi isolated from diseased trees at the delta site were *B. theobromae*, *Phomopsis scabra* (Sacc.) Trav., *Fusarium rigidiusculum* (Berk. and Brme.) Sacc., *Alternaria* sp., and *Nigrospora* sp. Trees with extensive leaf scorch had orange discoloration of inner bark.

Percentages of diseased trees (leaf scorch, top dieback, or mortality) differed significantly among sources at the delta site (Table 2). Most diseased trees were derived from seed from the more northern sources. Variation among families within sources was not significant according to Friedman's test.

Greenhouse study. Cankers developed within 3 days on seedlings inoculated with *B. theobromae*. Canker length increased linearly with time (r = 0.95), averaging 12.9 mm/day during the first 12 days after inoculation. The first seedlings died between day 12 and day 15. Control seedlings remained symptomless.

Canker development and mortality varied with seedling size. Seedling size was measured by ground-line stem diameter and seedling height at the time of inoculation. Larger seedlings had slower canker elongation during the first 12 days (r = −0.69 with stem diameter and −0.56 with seedling height), smaller maximum canker lengths (expressed as a fraction of total seedling height) (r = −0.73 with diameter and −0.65 with height), and greater survival percentages (r = 0.76 and 0.63 respectively). Equations and details are presented by Coggeshall (1).

Differences among half-sib families within stands were significant and much greater than differences among stands within sources or among sources for seedling height, rate of canker elongation, maximum canker length, and percent survival (Table 3). Tests of significance for the three disease measures were changed little by covariance adjustment for seedling size effects. Genetic correlations among the measures were high for families within stands, being 0.84 for rate of elongation vs. maximum canker length, 0.80 for rate of elongation vs. percent survival, and 0.91 for maximum canker length vs. percent survival.

Although the greenhouse and field results agreed poorly for importance of seed source variation, much better agreement occurred when only the 35 families common to both studies were considered. A Spearman correlation coefficient of 0.54 was obtained between ranked source means for field disease score and maximum canker length expressed as a fraction of seedling height.

Table 1. Incidence of sycamore trees with "sycamore decline" at ages 3 and 4 on a delta site in Issaquena County, Mississippi

Age of trees and year planted	Total number of trees observed	Percent of trees: Dead	Percent of trees: Showing signs of decline[a]
End of third growing season (1976 planting)	3,533	0.4	3.3
Start of fourth growing season (1975 planting)	3,429	0.5	5.4
End of fourth growing season (1975 planting)	3,429	1.9	9.3

[a]Includes both dead trees and trees with dieback or severe leaf scorch.

Table 2. Incidence of diseased 4-yr-old sycamore trees grown from 21 geographic seed sources in an Issaquena County, Mississippi, plantation

Geographic seed source: Location	Latitude	Means (%) by[a] Seed source: Dead	Seed source: Decline[b]	Latitude: Dead	Latitude: Decline[b]
Baldwin County, Alabama	30°45′	0.6 AB	6.9 abc		
George County, Mississippi	30°45′	0.6 AB	4.6 abc	0.4 A	4.5 a
Pearl River County, Mississippi	30°45′	0.0 A	2.9 a		
Pointe Coupee Par., Louisiana	30°45′	0.6 AB	3.5 a		
Choctaw County, Alabama	32°00′	0.0 A	5.3 abc		
Copiah County, Mississippi	32°00′	0.0 A	4.5 abc	1.0 A	4.5 a
Jefferson County, Mississippi	32°00′	3.0 AB	4.2 abc		
Catahoula Parish, Louisiana	32°00′	1.2 AB	3.9 ab		
Pickens County, Alabama	33°15′	0.0 A	9.0 abc		
Attala County, Mississippi	33°15′	1.7 AB	9.9 abc	1.3 A	13.1 b
Bolivar County, Mississippi	33°15′	0.6 AB	10.6 abc		
Drew County, Arkansas	33°15′	2.9 AB	23.1 d		
Itawamba County, Mississippi	34°30′	4.5 B	16.0 bcd		
Marshall County, Mississippi	34°30′	2.8 AB	12.8 abcd	3.1 B	13.6 b
Phillips County, Arkansas	34°30′	1.8 AB	12.7 abcd		
Saline County, Arkansas	34°30′	3.4 AB	13.0 abcd		
I. P. Company, Mississippi	31°50′–32°50′	0.6 AB	4.6 abc		
Seminole County, Georgia	30°54′	1.8 AB	8.9 abc		
Eastern Texas	30°15′–33°30′	1.0 AB	13.5 abcd		
Catawba Company, South Carolina	35°00′–35°55′	4.9 B	10.3 abc		
Payne County, Oklahoma	36°00′	8.8 C	16.3 cd		

[a]Averages followed by the same letter are not significantly different at the $P = 0.05$ level.
[b]Includes both dead trees and trees with dieback or severe leaf scorch.

Table 3. Seedling size, rate of canker elongation, maximum canker length, and percent survival of 6-mo-old sycamore seedlings artificially inoculated with *Botryodiplodia theobromae*

Geographic seed source	Seedling height (cm) at inoculation		Canker elongation (mm/day) for 12 days after inoculation		Maximum canker length as fraction of seedling height		Percent survival	
	Source mean	Family range	Source mean	Family range	Source mean	Family range	Source mean	Family range
Baldwin County, Alabama	76.2	71.3–83.0	11.8	6.9–15.5	0.32	0.30–0.37	76.6	68.8–81.3
George County, Mississippi	67.7	65.4–70.3	11.2	2.2–23.2	0.32	0.17–0.54	76.6	50.0–93.8
Pointe Coupee Parish, Louisiana	71.7	67.7–73.9	13.9	10.7–20.2	0.45	0.38–0.61	68.8	43.8–81.3
Choctaw County, Alabama	76.2	65.4–84.1	14.3	9.9–21.4	0.46	0.32–0.55	59.4	50.0–81.3
Copiah County, Mississippi	68.2	62.1–75.4	15.7	9.6–23.4	0.43	0.31–0.59	64.1	37.5–81.3
Jefferson County, Mississippi	68.8	59.2–85.5	15.6	4.8–21.8	0.47	0.23–0.59	59.4	43.8–81.3
Pickens County, Alabama	75.7	61.2–85.6	9.5	6.2–19.7	0.33	0.30–0.41	78.1	68.8–87.5
Attala County, Mississippi	69.8	59.5–78.6	14.4	8.2–24.2	0.44	0.27–0.68	68.8	37.5–87.5
Bolivar County, Mississippi	79.5	73.4–94.7	12.5	6.3–15.4	0.39	0.12–0.52	73.4	56.3–93.8
Itawamba County, Mississippi	69.5	60.7–86.0	8.8	5.8–15.3	0.26	0.17–0.42	81.3	62.5–93.8
Marshall County, Mississippi	75.5	71.0–78.9	14.4	10.0–22.6	0.39	0.35–0.48	73.4	62.5–87.5
Phillips County, Arkansas	70.1	61.9–80.1	12.4	6.8–16.4	0.44	0.25–0.63	67.2	50.0–81.3
Overall average	72.4	59.2–94.8	12.9	2.2–24.2	0.39	0.12–0.68	70.6	37.5–93.8

This correlation tested between the $P = 0.1$ and 0.05 significance levels as different from zero. Comparison of field disease score vs. maximum canker length for ranked family means gave a Spearman correlation of 0.37, which was significantly different from zero at the $P = 0.05$ level.

DISCUSSION

The twofold increase in sycamore decline during the fourth growing season at the delta site is sufficient to concern commercial sycamore planters in the lower Mississippi River valley. If the 3-yr results from the adjacent younger test are included, disease incidence may as much as triple in 12 mo. Leaf scorch and top dieback are usually indicative of future tree mortality. Therefore, 10% of the trees in the plantations at the delta site may be lost by age 5 or 6.

The reasons for sudden disease increase in the fourth year, and only at the delta site, are unknown. Cooper et al (3) observed similar disease development. Off-site planting and stress do not appear responsible, since the best sites for growth are in the delta. As an example, mean third-year height for the delta planting was 70% greater than the average for the three Coastal Plain plantings. Perhaps the differences among plantings are related to the time of crown closure, which occurred earliest at the delta site, or to differences in inoculum density and genotype.

Two of the fungi isolated (*B. theobromae* and *P. scabra*) were also found by Cooper et al (3) in two Mississippi plantations. They concluded that *B. theobromae* and *C. fimbriata* caused lethal bole cankers. *C. fimbriata* was not isolated in this study but may have been present, since tree trunks where the fungus is found were not sampled. *P. scabra* can cause severe bole cankers (8). Our observation of *F. rigidiusculum* is the first reported on sycamore, but *F. solani* (Mart.) Appel. has been found on diseased sycamore trees in natural stands near the delta site (4). The *Alternaria* sp. and *Nigrospora* sp. observed are common saprophytes. The orange discoloration that we observed in the inner bark of some dying trees has been found associated with *Hypoxylon tinctor* (Berk.) Cke. at other locations (3,7,10,12). However, either that orange discoloration can occur with other pathogens also, or *H. tinctor* was missed in the sampling procedure we used.

The north-south trend in disease resistance at the delta site confirms the earlier similar result for seed sources from the Mississippi and Chattahoochee rivers (3). The apparent lack of variation among families within sources in the field plantings is contrary to the reported variation found by Cooper et al (3). However, significant family variation may develop with increasing age.

The correlation between field symptoms and seedling response to *B. theobromae* inoculation was too low to justify use of the present procedure for rapid screening. Low correlations may have been due to age difference of hosts, circumvention of mechanical resistance at the plant surface by the inoculation method, differences among *B. theobromae* strains, differences in the composition and numbers of competitive microorganisms on the host plants, or other pathogens in the field.

Our study and that of Cooper et al (3) provide strong evidence for genetic variation in resistance to "sycamore decline," particularly among geographic seed sources. For high-risk areas in the lower Mississippi River valley, planting stock from the southern end of the valley should be used. The large number of different fungi isolated from diseased trees and the poor correlation of family susceptibilities in the field with results from *B. theobromae* inoculation in the greenhouse indicate that much work remains to be done to identify the specific mechanisms of genetic control of resistance.

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