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Sycamore Canker Caused by Botryodiplodia theobromae

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ABSTRACT

In the Mississippi Delta, *Botryodiplodia theobromae* placed beneath the bark of sycamore trees incited cankers on 99% of trees inoculated in July, and on 66% of those inoculated in September. Infection occurred following inoculations made throughout the year, but was most common when the fungus was introduced during warm weather. The quality of the site for sycamore growth did not significantly influence disease development. Eighty-four per cent of trees inoculated on good sites, and 95% of those on poor sites, developed cankers.

The fungus caused no cankers unless it was placed beneath the bark. Ninety per cent of the inoculated wounds formed enlarged cankers. Insects probably spread the fungus to noninfected trees. Phytopathology 59:76-78.

A canker disease of American sycamore trees (*Plantanus occidentalis* L.) was first reported from near Greenville, Miss., by Toole in 1956 (6). A fungus isolated from naturally occurring cankers produced cankers on inoculated healthy sycamores. It was reisolated from the artificially produced cankers, but was not identified.

A dieback of sycamore (*Diplodia natalensis* Evans = *Botryodiplodia theobromae* Pat.) was described in Georgia by Thompson (5).

The present paper reports the results of inoculations beneath the bark with four fungi isolated from diseased sycamores in natural stands. *B. theobromae* is shown to cause the canker disease. A preliminary account has been published (2).

MATERIALS AND METHODS.—Fungi were isolated from diseased sycamore trees growing along the edge of the Mississippi River near Greenville, Vicksburg, and Natchez, Miss. Single hyphal-tip isolates were grown on various media to induce spore production for identification. Hagem's modified medium (3) was best for pycnidia and pycnidiospore production. The most common isolates were *B. theobromae, Cephalosporium* sp., *Phomopsis* sp., and *Fusarium solani* (Mart.) Appel.

Greenhouse tests.—Eighty 1-yr-old sycamore seedlings were transplanted into 12-inch pots and placed in a greenhouse on 19 December 1963. Six seedlings for each of the four fungi were inoculated on 10 March 1963. A 9-mm mycelium agar disc was placed behind a 1-inch bark flap made by cutting through the bark to the cambium. Six seedlings were wounded but not inoculated. To prevent drying, half of the wounds were covered with moist cotton and bound with masking tape, and half were covered with Vaseline. Ten additional seedlings for each fungus were inoculated in May 1964.

Field tests.—To test the four fungi on older trees, 20-year-old trees on the Delta Experimental Forest were inoculated by the method described above.

A more comprehensive test was established 8 miles west of Greenville, Miss., in a natural stand of several hundred 20-year-old sycamore trees. The site was rated poor for the growth of sycamore according to Broadfoot's criteria (1). Fifty trees were inoculated with *B. theobromae* as in the greenhouse tests, but all wounds were covered with moist cotton and tape, because the Vaseline appeared to be phytotoxic in the greenhouse study. Ten trees were wounded but not inoculated. Inoculations were made on the bole 1.4 m aboveground on the sides facing the four cardinal directions in September and November 1964. Results of the September inoculations were observed in November 1964. Final observations were made in July 1965.

In 1965, another 50 trees on the area near Greenville were inoculated in July, September, and November by the methods described. The boles of the trees were sprayed in November above and below the inoculation point with chlordane to reduce contamination of wounds by material from ants and other crawling insects.

A final field test was established to determine if *B. theobromae* could penetrate the bark, and to test the influence of site on infection and disease development.

At the Greenville site in June 1965, 20 additional trees were wounded and inoculated as already described. Without making wounds, inoculum was placed on the trunk surface of 20 other trees. At a site near Rosedale, Miss., rated excellent for sycamore (1), 40 wounded trees and 40 trees without wounds were inoculated. Twenty trees at each location were wounded but not inoculated. The boles of the trees at Greenville were sprayed with chlordane; those at Rosedale were not.

To determine whether the sexual stage of the fungus was produced, host material containing the fungus was collected monthly from September 1964 to September 1965, and weekly from October 1966 to 1 July 1967. Isolates were grown on artificial media.

RESULTS.—In the greenhouse all four fungi caused some cankers on sycamore seedlings. *Phomopsis* sp. and *F. solani* caused cankers on all wounds, but the symptoms were different from those found on naturally diseased trees. *Cephalosporium* sp. caused only an occasional canker.

One hundred per cent of the seedlings inoculated in the greenhouse in May with *B. theobromae* had symptoms similar to those observed in natural stands (Fig. 1). The cankers were about four times as long as

they were wide. The cambium and the outer xylem were discolored. Small cankers often appeared above or below points of initial infection. Although the bark between the old and new cankers appeared normal, the sapwood connecting the two cankers was discolored. The fungus was reisolated from this brownish sapwood.

Some cankers were produced by all four fungi in inoculations in the initial field test. *B. theobromae* caused cankers of the expected kind on 55% of the inoculated wounds (Table 1). *Phomopsis* sp. caused as many cankers, but they were not of the size and shape found naturally. It was therefore concluded that *B. theobromae* is the primary causal organism. Table 1 shows that infection can occur throughout the year.

In the more comprehensive tests in July, September, and November of 1964 and 1965, the results (Table 2) support the previous tests. Inoculations in November produced the least cankers, and those in the summer the most. The side of tree wounded did not significantly (0.05 level) influence the likelihood of infection (Table 2).

The width, length, and depth of cankers appeared to be the same in trees on good and poor sites, but the discolored sapwood adjacent to the wounds extended a

Fig. 1. Left, natural canker on sycamore tree. Right, canker on sycamore tree that was inoculated 10 months before with *Botryodiplodia theobromae*.

TABLE 1. Proportion of wounds that became infected on control trees and on those inoculated with *Botryodiplodia theobromae* from January through November in 1964

Month of inoculation	Inoculated	Control
	%	%
Jan.[a]	55	18
March[a]	18	8
May[a]	73	8
July[a]	64	18
Sept.[b]	66	20
Nov.[b]	20	0

[a] Twelve inoculated and 12 control trees.
[b] Fifty trees inoculated with the fungus at four points and 10 trees used as controls.

greater distance vertically from the point of infection on trees growing on the favorable sites. Ninety-five per cent of trees inoculated on poor sites and 84% of those inoculated on good sites developed cankers.

The fungus cannot penetrate the bark and needs some mode of entry before it can infect. No cankers were produced on 40 trees when the fungus was placed on the surface of the bark without wounds. Ninety per cent of the inoculated wounds were infected, and cankers developed.

At Rosedale, where no insecticide was used, 13% of the trees with noninoculated agar placed under the bark became infected. Inoculum was probably spread to these trees by crawling insects, primarily ants. Only 1% of the wounded but noninoculated trees sprayed with insecticide were infected at the Greenville site. Large numbers of ants were observed on untreated trees in both locations.

TABLE 2. Per cent of trees inoculated with *Botryodiplodia theobromae* that became infected, by date of inoculation and cardinal direction of wound, Greenville site[a]

Year	Month	Trees	North	South	East	West
		no.	%	%	%	%
1964	Sept.	50	60	66	78	60
1964	Nov.	50	14	26	20	20
1965	July	50	98	100	100	98

[a] There were 10 wounded check trees for each date. Check trees were infected only in September, when 20% of the wounds developed cankers.

Only the pycnidial stage was observed, and from this stage the fungus was identified as *B. theobromae*. Pycnidiospores were 25-29 μ × 12-15 μ. The asexual stage was isolated from diseased trees monthly in 1964-1965, and weekly from October 1966 to July 1967.

It is not possible to give a complete synonymy of the pycnidial stage at this time, but the name used here includes *D. paradisiaca* (Mont.) Wr. (pycnidiospores 20-50 μ × 10-22 μ) and agrees reasonably well with *D. natalensis* Evans (24 μ × 15 μ) (7). Stevens (4) describes the pycnidiospores of *Physalospora rhodina* (Berk. & Curt.) Cooke as 20-33 μ × 10-18 μ, with more than half 24-28 μ × 12-15 μ. This description fits *B. theobromae* very closely.

Since *B. theobromae* on sycamore could not be definitely linked with *P. rhodina*, the name for the pycnidial stage is used.

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