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Pathogenicity of Cytospora, Phomopsis, and Hypomyces on Populus deltoides

T. H. Filer, Jr.

Plant Pathologist, Southern Forest Experiment Station, Forest Service, USDA. Assigned to the Southern Hardwoods Laboratory, which is maintained at Stoneville, Mississippi, in cooperation with the Mississippi Agricultural Experiment Station and the Southern Hardwood Forest Research Group.

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ABSTRACT

Cytospora chrysosperma, Phomopsis macrospora, and *Hypomyces solani* are pathogenic on cottonwood (*Populus deltoides*). These canker-causing fungi were most virulent in November, when rains were frequent and temperatures were between 20 and 30 C. Trees growing on an unfavorable site were more susceptible to *C. chrysosperma* than those on a favorable site.

In 1963, thousands of trees in 2- to 3-year-old cottonwood plantations near Fitler, Miss., were killed by cankers (3). Fungi consistently isolated from the edge of diseased bark tissue were subsequently identified as *Cytospora chrysosperma* Fr., *Phomopsis macrospora* Kobayashi & Chiba, and *Hypomyces solani* (Mart.) Snyd. & Hans. Past studies indicated that any of the three might have been the causal organism (2, 4, 6, 7, 9). The present study was initiated in 1963 to determine which of the fungi are pathogenic on cottonwood and what influence weather and seasons have on disease development. Potted cuttings and trees in plantations were inoculated.

MATERIALS AND METHODS.—*Pot inoculations.*—Fifty-two recently rooted cottonwood cuttings were planted in individual clay pots in June 1963. The cuttings averaged 23 cm in length and 1.5 cm in diam. After 3 months, a 13-mm-long wound was made in the stem of each cutting, and a 9-mm agar disc was placed beneath the bark. In this manner, 13 of the cuttings were inoculated with isolates of *C. chrysosperma,* 13 with *P. macrospora,* and 13 with *H. solani.* Isolates had been grown on potato-dextrose agar plates for 3 weeks. Sterile agar discs were inserted under the bark of the remaining 13 cuttings, which served as checks. The wounds were covered with moist cotton held in place with masking tape.

Four replications (16 trees) were placed in a greenhouse where temperature varied from 10 to 38 C, and relative humidity from 40 to 100%. Screens provided 50% shade. Soil moisture was maintained at near field capacity.

Four replications were put in a lathhouse where temperature varied with outdoor conditions. The remaining five replications were placed in a growth chamber where temperature was 26 C, relative humidity 50%, light intensity 18,000 lumens/m^2, and day length 12 hr. Plants in the lathhouse and growth chamber were watered daily.

After 30 days, tape was removed from all wounds, and any cankers that had formed were measured. The cankers were reexamined after 12 months, tree mortality was noted, and fungi were reisolated from the cankers.

Field inoculations.—Three 20-tree plots were in 7- to 10-year-old cottonwood plantings near Stoneville, Miss., and three in 2- to 3-year-old plantings near Fitler, Miss. On each plot, five trees were inoculated with *H. solani,* five with *P. macrospora,* and five with *C. chrysosperma;* the remaining five were untreated controls. Trees were inoculated in November 1963 and reinoculated at different places on the stem in January, March, May, July, and September 1964, in the same manner as the potted cuttings. Isolates were prepared in the same way as for the inoculations of cuttings.

The relative turgidity of two bark samples from five inoculated trees on each plot was determined every 2 months according to Bier's method (1). Bier reported a correlation between low bark moisture and a high percentage of infection for several canker diseases.

In November 1964, 100 2-year-old trees at Fitler were inoculated with *C. chrysosperma.* Half were on a good cottonwood site; they averaged 6.1 m in height and 11.4 cm in diam. Half were on a poor site and averaged 3.05 m in height and 5.1 cm in diam. On each site, 50 additional trees were selected as checks.

Weather.—Temperature and precipitation records were obtained from the U.S. Weather Bureau at Onward, Miss., which is approximately 5 miles east of Fitler, and from the U.S. Weather Bureau at Stoneville. Data from adjacent weather stations elsewhere in the Mississippi Delta indicate that rainfall and mean temperature between Onward and Fitler probably differed less than 2.5 cm and 1° for the year of the study.

RESULTS AND DISCUSSION.—*Pot inoculations.*—All three fungi caused high mortality of cuttings in the greenhouse and lathhouse, but none caused mortality in the growth chamber (Table 1). All of the cuttings inoculated with *C. chrysosperma* developed cankers in the greenhouse and lathhouse and were girdled after 1 year. *P. macrospora* girdled 75% of the cottonwoods in the greenhouse and all those in the lathhouse. *H. solani* killed all the cuttings grown in the greenhouse and half of those in the lathhouse.

The lack of mortality in the growth chamber suggests that light, temperature, and humidity, singly or in combination, strongly influence the rate and spread of necrosis. Long (6) and Schreiner (7) hypothesized that *C. chrysosperma* is not a serious pathogen unless trees are under stress. The same may be true for *P. macrospora* and *H. solani.*

Field inoculations.—All fungi produced the greatest amount of infection on trees inoculated in November (Fig. 1). At both Fitler and Stoneville, differences in infection by date of inoculation were significant at the 1% level for *C. chrysosperma* and *P. macrospora,* but

TABLE 1. Rooted cottonwood cuttings grown in pots and inoculated in the fall with *Cytospora chrysosperma*, *Phomopsis macrospora*, and *Hypomyces solani*

Location	Inoculum	Trees	Trees with cankers after 1 month	Trees with cankers after 12 months	Trees girdled after 12 months
		no.	%	%	%
Greenhouse	*C. chrysosperma*	4	100	100	100
	P. macrospora	4	50	100	75
	H. solani	4	100	100	100
	Check	4	0	0	0
Lathhouse	*C. chrysosperma*	4	75	100	100
	P. macrospora	4	100	100	100
	H. solani	4	100	75	50
	Check	4	0	0	0
Growth chamber	*C. chrysosperma*	5	60	0	0
	P. macrospora	5	0	0	0
	H. solani	5	20	0	0
	Check	5	0	0	0

not for *H. solani*. Natural infection by the first two fungi was also high in the fall, as indicated by signs and symptoms on check trees and isolations of the fungi in the laboratory. A higher percentage of trees were killed by inoculation with *C. chrysosperma* (Fig. 1) than by inoculations with the other fungi, but more cankers were produced by *P. macrospora* in most months.

Trees on the poor site were significantly more susceptible to *C. chrysosperma* than trees on the good site (Table 2). Since no trees on either site were growing rapidly when they were inoculated in November, physiological factors other than the rate of growth at the time of infection must account for disease resistance.

Imperfect stages of the three fungi were reisolated from cankers throughout the year. The sexual stage of *H. solani* was found in the fall, and spore tendrils con-

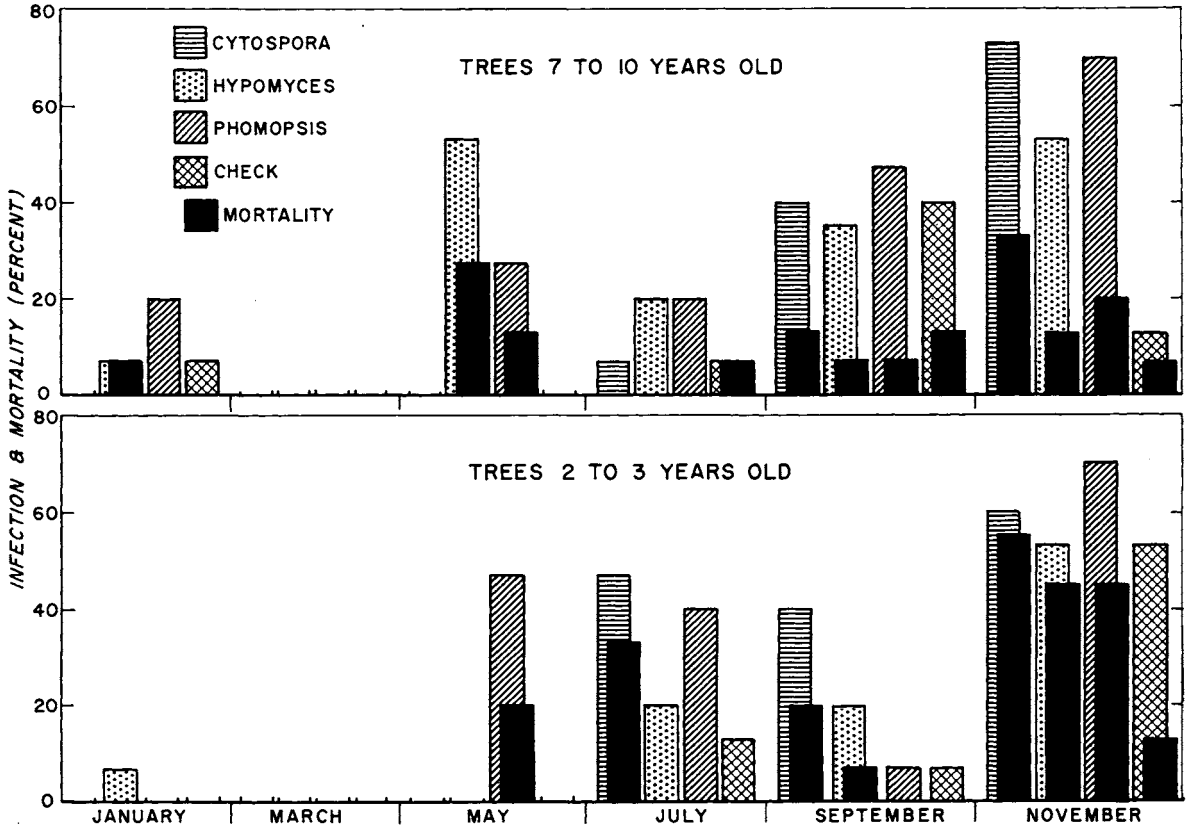

Fig. 1. Infection and mortality resulting from field inoculations in 1963-64.

TABLE 2. Effect of site quality on incidence of cankers caused by *Cytospora chrysosperma* at Fitler, Miss.

Treatment and result	Canker incidence[a]	
	Poor site	Good site
	%	%
Inoculation		
Infection	90	16
Girdling	66	2
Check		
Infection	32	8
Girdling	32	8

[a] Each value is the average for 50 trees.

taining viable conidia of *C. chrysosperma* and *P. macrospora* were observed in all months except January and February. The spore tendrils oozed from the pycnidia after 2-3 hr of relative humidity over 80%. All three fungi overwintered in the asexual and mycelial stages in diseased tissue.

Weather.—The optimum temperature for growth is 25 C for *Cytospora* (7) and *Phomopsis* (5), and 30 C for *Fusarium* (9). Sussman and Halvorson (8) state that most fungi require at least 60% relative humidity for germination. Temperatures of 20-30 C and high relative humidity from frequent rainfall were common in the fall prior to the epidemic years of 1963 (3) and 1965. In the fall prior to 1964, the temperature was near optimum, but precipitation was less than 2.5 cm in September and most of November. The precipitation in November fell in the last week, after the temperature had dropped below the minimum required for fungal growth. Consequently, there was no epidemic of cottonwood canker in 1964. Weather for the fall of 1965 was similar to that of the fall of 1963, and disease intensity for 1966 was similar to that of 1964.

Bark moisture.—There appears to be no simple, direct relationship between relative bark turgidity and the high rate of infection during November. Turgidity was lower in November than in some other months, but it was equally low, or lower, in other months when little or no infection occurred (Table 3).

CONCLUSIONS.—Inoculations, causing cankers that killed trees, and reisolations show that all three fungi are pathogenic on cottonwood. Even at Fitler, where a large amount of natural infection occurred, significantly more inoculated trees than check trees developed cankers. The fungi in question were isolated from natural infections as well as reisolated from trees that were inoculated.

TABLE 3. Average relative bark turgidity[a] at the time of inoculation

Stoneville		Fitler	
month	%	*month*	%
	[b]		
Jan.	80.3	May	88.3
May	79.7	Sept.	88.0
Nov.	71.7	Jan.	82.0
Mar.	71.3	Mar.	81.0
July	69.7	Nov.	77.0
Sept.	64.3	July	68.3

[a] Each value is the average of 30 samples from 15 trees.
[b] Figures connected by vertical lines are not significantly different at the 5% level according to Duncan's multiple range test.

Because it caused the most mortality, *C. chrysosperma* must be considered the most important of the three. *P. macrospora* caused more cankers but less mortality, and *H. hypomyces* caused little or no mortality except to trees growing on poor sites.

Any of the three fungi can infect cottonwood trees, but the size of canker and the possibility of mortality depend upon the quality of the site on which the tree is growing and upon environmental conditions during the attack. Frequent rains during the fall and temperatures above 20 C resulted in a disease epidemic on trees that were growing on poor sites.

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