# SEPTORIA CANKER ON NURSERY STOCK OF POPULUS DELTOIDES

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# Abstract

Septoria musiva Peck is capable of establishing itself on unwounded first-year stems of eastern cottonwood. Natural infections have been observed since 1969 in three forest nurseries in Mississippi, and inoculations have confirmed that both conidia and ascospores are capable of causing stem infections.

This paper offers evidence that the fungus <u>Septoria</u> <u>musiva</u> <u>Peck</u> is capable of causing cankers on unwounded stems of eastern cottonwood, <u>Populus</u> <u>deltoides</u>.

P. deltoides is the most widely distributed Populus species in the Southern United States; it occurs in natural stands along major rivers and its use in plantations is increasing. Throughout the United States range it is attacked by a leaf spot disease caused by S. musiva (2), but it has been regarded as resistant to stem infection by this fungus. Bier (1) reported that the fungus caused cankers on P. balsamifera (= P. deltoides) when inoculations were made in stem wounds, but he found no infection when unwounded stems were inoculated.

The present authors observed naturally occurring stem cankers during 1969 in an experimental cottonwood nursery at Stoneville, Mississippi. Later in the year, cankers were found at Winona and Mount Olive, in State nurseries growing cottonwood cuttings for tree planters.

The information reported here includes observations on the severity of the disease at the Stoneville and Winona nurseries, and on the manner in which stem infection occurs in spring. In addition, inoculations were made to identify the fungus and to test its pathogenicity.

## MATERIALS AND METHODS

<u>Nursery Observations</u>: Cottonwood nurseries are usually started by planting cuttings in late winter or early spring. The cuttings take root rapidly and send up shoots that may reach heights of 10 to 14 feet by the end of the first nursery season. Cuttings for field planting are then made from the aboveground parts, and the rootstocks are left for another year's production.

Stoneville: The Stoneville nursery, which is maintained by the Southern Hardwoods Laboratory, had been in production for about a decade and contained rootstocks of various ages and provenances. During 1969, 1000 leaves were examined for the presence of pycnidia. The leaves were from the current season's shoots on rootstocks in the first, second, or third year of growth.

In addition, stem cankers were studied on 20 clones planted in 1968. These clones had been collected within a 50-mile radius of Stoneville and represented both a natural population and selections with above-average growth potential. All stems on these rootstocks were examined in July 1969 and June 1970 -- a total of 10,296 stems in 2 years. Pycnidia or pustules on the cankers were taken as evidence that the fungus was present. Cankers were randomly selected for microscopic examination and culture isolations.

Winona: Cuttings taken at random from the natural population had been planted at Winona in  $19\overline{63}$ , 1968, and 1969. All stems (12,232) and 750 randomly selected leaves were examined in July 1969 to determine the relation between age of rootstock and incidence of infection. Random samples of cankers were collected for isolations and spore measurements.

Inoculations: In May 1970, 190 leaves on 19 potted cuttings were inoculated with a single-spore Septoria isolate obtained from a canker. A spore-mycelium suspension was applied to leaves with an air brush. After 48 hours of incubation in a mist chamber at 90-98% relative humidity (RH), plants were transferred to a greenhouse maintained at 23-30°C, 65-85% RH.

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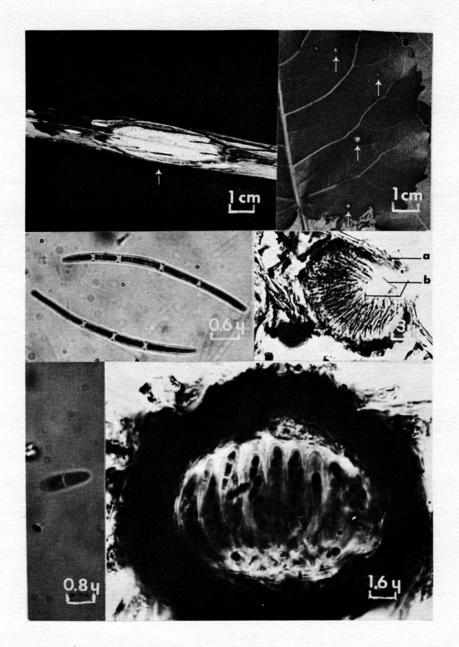


FIGURE 1. Symptoms and signs caused on <u>Populus</u> <u>deltoides</u> by <u>Septoria</u> <u>musiva</u> and its ascogenous form Mycosphaerella populorum.

Upper left: Septoria canker on 6-month-old cottonwood stem.

Upper right: Septoria spots on green cottonwood leaf.

Center left: Conidia isolated from canker of inoculated cottonwood stem.

Center right: Cross section of (a) pycnidium and (b) conidia in leaf tissue.

Lower left: Ascospore of M. populorum isolated May 1970 from cotton-

wood leaf collected in October 1969.

Lower right: Perithecium, asci, and ascospores of M. populorum produced in cottonwood leaf collected October 1969 and stored in a lath house until March 1970.

Additional leaf inoculations on 24 cuttings were made with 12 single-spore isolates obtained from cankers. Each isolate was tested on one dorsal and one ventral leaf surface of each cutting. The inoculum was in the form of 2-mm agar disks containing spores and mycelium and affixed with masking tape. Infection counts were made after 60 days in the greenhouse.

A pathogenicity test on stems was conducted by making 200 inoculations on 47 potted cuttings. One hundred inoculations were made by placing a 2-mm agar disc containing spores and mycelium beneath the bark in a stem wound  $0.25 \times 0.5$  inch in diameter, and 100 inoculations were made on bark surfaces of unwounded stems. All inoculation points were covered with masking tape. The inoculum was from single-spore isolates of cottonwood leaf spots in the Stoneville nursery. Percentage of infection was tabulated after 30 days.

Natural spore discharge and infection was used in May 1970 to test the pathogenicity of the ascogenous stage of <u>S. musiva</u> (<u>Mycosphaerella populorum</u> G. E. Thompson) (2). Leaves containing Septoria infections were collected in October 1969 at the Stoneville nursery and stored overwinter in a lath house. In March 1970 they were placed to completely cover the soil below cuttings growing in a flat measuring 24 x 32 inches. The flat was kept in the mist chamber at 90-98% RH for 7 days, then transferred to a greenhouse maintained at 23-27°C, 65-85% RH, and 50% shade. Infection was computed from the number of exposed stems that had cankers after 90 days. A total of 25 stems on 18 rootstocks were exposed.

#### RESULTS

The fungus isolated from cankered stems at the Stoneville and Winona nurseries is similar to S. musiva Peck as described by Waterman (3) and Bier (1) on other Populus species (Fig. 1). The spores averaged 34.2 x 2.2 $\mu$  (range 20-52 x 2-3.2 $\mu$ ). They were mostly 3 septate, but ranged from 1-4 septate. Pycnidia averaged  $107\mu$  (range 47-143 $\mu$ ). The average spore size is smaller than that reported by Thompson (2) but is within limits mentioned by Bier (1) and Waterman (3). When single-spore isolates from the cankers were transferred to an artificial medium they produced spores  $3.0\mu$  wide.

The disease symptoms on cottonwood are similar to those on other poplars (1,3). The cankers on 3-month-old stems averaged 1/2 inch wide and 1 1/2 inches long and had black pycnidia protruding through the epidermis. Most infections were less than 3 feet above ground.

In the Stoneville nursery natural infection of the 20 clones (current year's stems on 2-year-old roots) in 1969 varied from 5 to 53% and averaged 26%. In 1970, when rootstocks were 3 years old, the infection varied from 10 to 79% and averaged 48%. Two clones showed apparent resistance to the fungus; they averaged 16 and 14% infection in 1969, and 10 and 19% in 1970.

Natural infection of leaves on 1-, 2- and 3-year-old rootstocks averaged 15, 29, and 24%, respectively (Table 1).

At Winona the number of cankers on 1-year stems in 1969 varied with age of rootstock (Table 1). Of 5,555 stems on 7-year-old stumps, 22% had cankers. Cankering averaged 0.5% on 2-year-old rootstock and less than 0.1% on 1-year rootstock.

Leaf infection at Winona averaged 16% in the 1- to 2-year-old rootstock and 40% on leaves growing on 7-year-old rootstock. Thus both stem cankers and leaf infections were most numerous on trees from the old rootstock.

<u>Inoculations</u>: Sixty-three percent of the leaves became infected when sprayed with the suspension of conidia and 100% infection occurred when single-spore cultures were placed on the dorsal or ventral surfaces of leaves. No artificial wounds were needed to initiate infection on leaves. Minute necrotic spots appeared 7 days after inoculation. These spots enlarged until they coalesced with adjacent infections; on some leaves 50% of the tissue was infected.

Table 1. Canker infection of stems and leaves of Populus deltoides in 1969.

Age of	:		:		:		:		:	
rootstock (years)	:	Nursery location	:	No. Leaves	:	% Infected	:	No. Stems	:	% Infected
		Stoneville		250		15				
2		Winona		250		16		4,710		. 5
		Stoneville		500		29		4,630		26.0
3		Stoneville		250		24				
7		Winona		250		40		5,555		22.0

Date			Total no.	%
inoculated	Source of inoculum	Inoculation location	inoculated	Infected
May 14	Canker conidia	Sprayed on leaves	190	63
May 27	Canker single-spore isolates	Taped on leaves	24	100
July 8	Greenhouse leaf spot isolates	Unwounded stems	100	20
July 8	Greenhouse leaf spot isolates	Wounded stems	100	73
May 20	Asci on old leaves	Below plants on soil surface	25	60

Table 2. Septoria inoculations of Populus deltoides in greenhouse, August 8, 1970.

Pycnidia were evident after 21 days. Reisolations from leaves contained conidia that were smaller than those described for <u>S. musiva</u>. When conidia from leaves were cultured on artificial media, however, the spores fitted Bier's description.

Conidia can cause infection on young cottonwood stems in 31 days (Table 2). Some cankers were formed 14 days after inoculation. When an artificial mode of entry was made, 73% infection occurred. Without a wound as an entry point, infection from the conidia averaged 20%.

The technique used to determine infection from natural ascospore discharge demonstrated that the fungus overwinters on old leaves and can infect new growth. Sixty percent of the stems were infected. At least half of the infected stems had multiple cankers. Pycnidia and spore tendrils were evident, and <u>S</u>. <u>musiva</u> conidia were isolated from the new cankers 45 days after inoculations.

#### DISCUSSION

For rootstocks of equal age, the percent of infected stems was lower (0.5 vs. 26) at Winona than at Stoneville (Table 1). Nursery practices employed at Winona may account for the difference. At Winona furrows are plowed between rows in winter and spring, the purpose being to control weeds until the shoots are approximately 3 feet high. Plowing covers a large amount of inoculum that overwinters on the leaves. No winter or spring cultivation is attempted at Stoneville, and the amount of infection on stems after the first year is probably related to amount of overwintering inoculum.

Although the development of cankers on older stems has not been studied, diseased stock seems obviously undesirable for establishing either clonal nurseries or commercial plantations. The Winona nursery culled 200,000 cuttings for cankers in 1969 alone. In addition, many of the cuttings that were classed as merchantable had small, callused-over cankers that may still have been a source of inoculum. On smooth-barked clones, the roughness caused by the callus may serve as spots for oviposition by boring insects (Paranthrene spp.).

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