Septoria musiva on Hybrid Poplar in Southern Ontario

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ABSTRACT

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Septoria canker, caused by *Septoria musiva* (teleomorph: *Mycosphaerella populorum*), was found in plantations of hybrid poplar at three locations in southern Ontario in 1983 and 1984. This disease had not been found in surveys between 1978 and 1982. Isolates from Ontario and the United States were similar in cultural morphology, temperature requirements, and virulence in artificial inoculation tests.

Septoria canker, incited by *Septoria musiva* Pk. (teleomorph: *Mycosphaerella populorum* G. E. Thompson) has decimated certain hybrid poplar plantations in the United States (3,5,6). The fungus is common in Ontario as a leaf-spotting organism on native poplars, and early reports also indicated the presence of Septoria canker in the province (1). Exploratory programs in poplar management were initiated by the Ontario Ministry of Natural Resources in the 1960s, and hybrid poplar is expected to play an important role in future forest industries (12). If these hopes are to be realized, the impact of Septoria canker must be determined and control methods developed. We report the results of disease surveys of hybrid poplar plantations in southern Ontario and characterization of morphology, temperature requirements, and pathogenicity of *S. musiva* isolates from southern Ontario.

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MATERIALS AND METHODS

Disease surveys were done twice a year, in June and August, from 1978 to 1982 in plantations in southern Ontario (Table 1). Levels of disease for each poplar clone at each locality were expressed as disease rating, the summation of (disease severity × proportion in severity class) over the range of severity classes. Disease severity is the amount of leaf or stem surface affected by disease, expressed on a scale of 1–5, where 1 = 0–20%, 2 = 20–40%, 3 = 40–60%, 4 = 60–80%, and 5 = 80–100%. The higher of the two yearly assessments was assumed to be the better representation of disease level, because premature shedding of infected leaves or severe attack by another pathogen would have caused erroneously low disease ratings for lesions caused by *S. musiva*. After 1982, the annual surveys were discontinued.

During 1983 and 1984, visits were made in late summer to nurseries at Maple, Kemptville, and Brockville,

Ontario, as well as to plantations at locations 1, 2, 4, 5, 7, 8, and 12 (Table 2) and younger plantations (planted in 1979 or later) near Brockville. The presence of leaf spot was noted, and intensive searches for Septoria cankers were carried out. Overwintered leaves were collected in hybrid poplar plantations and natural stands of *P. balsamifera* L. and *P.* × *jackii* Sarg. in early spring of 1983 and 1984 and examined for the presence of the teleomorph, *M. populorum*.

Single-spore or multispore isolates were obtained from ascospore casts or conidial suspensions on cornmeal agar containing 300 mg of chloramphenicol and 50 mg of streptomycin per liter. Germinating spores were transferred to poplar leaf agar (PLA), prepared by a modification of the method of Luley (4) as follows: 25 g of fresh poplar leaves were boiled in 500 ml of distilled water for 25 min and the leaf debris removed by filtration. Fifteen grams of agar, 300 mg of chloramphenicol, and enough distilled water to make 1 L were added, and the medium was sterilized at 121 C and 6.8 kg of pressure for 15 min. Fifty milligrams of streptomycin per liter was added after autoclaving. This medium enhanced growth of *S. musiva* and inhibited growth of aerial contaminants. Isolations from cankers were done following the method of Long (3), except PLA was used instead of malt agar. Wood chips were surface-disinfested in 10% sodium hypochlorite for 30 sec. Spore measurements were done in 1% cotton blue in lactophenol. For each collection or isolate, 25–50 spores were measured.

Growth studies were done using potato-dextrose agar (PDA), malt extract agar (MEA), Leonian's agar (2), PLA amended with 5 g of malt extract per liter (PLAam), or a medium containing (per liter) 10 g of glucose, 2 g of yeast extract, 1 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.2 mg of Fe^{++}, 0.2 mg of Zn^{++}, and 0.1 mg of Mn^{++}, with pH adjusted to 4.8 before autoclaving (GYM). Cultures were incubated in the dark at 10, 17, 21, 25, or 33 C, and colony growth along two perpendicular diameters was measured.

Pathogenicity tests were done using hybrid poplar clones DN 55 (*P.* × *euramericana* (Dode) Guinier), NE 216 (*P. deltoides* Marsh. × *P. trichocarpa* Torr. & Gray), and NE 252 (*P. deltoides* var. *angulata* (Michx.) Sarg. × *P. trichocarpa*). Clone DN 55 was rated resistant to Septoria canker, and NE 216 and NE 252 were rated susceptible in previous studies (6,7,9). Plants were grown from 15-cm stem cuttings in a 3:1:1 mixture of peat moss/perlite/vermiculite in a greenhouse. After roots were well formed, the plants were fertilized at weekly intervals with a soluble fertilizer (16:8:12:5, NPKMg). The plants were transferred to a growth chamber maintained at 24 C and 75% relative humidity for inoculation. Inoculum consisted of plugs cut with a 5-mm cork borer from 21-day-old colonies on PLA; controls were plugs of PLA. After surface disinfestation of the stem with 80% ethanol, a plug was placed under a bark flap made with a sterilized scalpel, and the wound was wrapped with paraffin film. Each stem had two inoculations and one control, separated by at least 10 cm and arranged in a helix around the stem. The isolate-clone combinations were replicated five times in a randomized block design. After 30 days, the length of discolored epidermis was measured and reisolations carried

Table 1. Poplar plantations in Ontario surveyed for *Septoria* diseases between 1978 and 1982

Location (region, township)	Date planted	No. of replicates	No. of clones
Algonquin, Monck	1973	5	20
Central, Flesherton	1970	2	35
Central, Flos[a]	1969	4	25
Central, Innisfil	1971	2	9
Central, Innisfil[b]	1972	3	20
Central, Vespra[a]	1970	4	7
Eastern, Augusta	1975	2	20
Eastern, Augusta[a,b]	1976	4	30
Eastern, Elizabethtown[a]	1976	4	30
Eastern, Gloucester[a]	1975	4	27
Eastern, Haldimand[a,b]	1977	4	30
Southwestern, Malahide	1973	4	20

[a] Not surveyed in 1982.
[b] Not surveyed in 1978.

Table 2. Septoria leaf spot disease ratings for susceptible clones in southern Ontario poplar plantations from 1978 to 1982[a]

Poplar clone	Sites surveyed[b]	Year				
		1978	1979	1980	1981	1982
AK 41	1,12	0.1	0.0	0.0	0.0	0.0
CAG 23	1, 5, 12	1.0 (2)[c]	0.0	0.0	0.0	0.0
D 37	1, 5, 12	0.1 (2)	0.0	0.0	0.0	0.0
D 38	1, 5, 7, 11, 12	0.0 (3)	0.0	0.0	0.3	0.0 (4)
DJac 4	8, 9	0.0 (1)	0.9	0.3	1.5	NS[d]
DJac 6	8, 9	0.0 (1)	0.3	1.1	1.3	NS
DJac 10	10	0.0	0.5	0.0	0.0	NS
DJac 12	10	0.0	0.4	0.9	0.0	NS
DJac 14	11	NS	0.0	1.7	0.0	NS
DJac 26	8	NS	2.0	2.4	3.3	NS
DJac 27	8, 9	0.0 (1)	0.5	1.3	0.8	NS
DN 60	9	0.0	0.3	0.0	0.0	NS
DN 103	7	0.0	0.0	1.5	0.0	0.0
DN 108	7	0.0	0.0	1.5	0.0	0.0
DN 109	7	0.0	0.0	1.5	0.0	0.0
DN 141	7	0.0	0.0	1.6	0.0	0.0
DN 144	8, 9	0.0 (1)	0.0	0.6	0.0	NS
DN 159	7	0.0	0.0	1.5	0.0	0.0
DTac 2	10	0.4	0.8	0.2	0.0	NS
DTac 4	10, 11	0.6 (1)	0.9 (1)	1.5	2.5	NS
Jac 4	1, 5, 10, 11, 12	1.6 (3)	1.6	1.5	1.0	1.1 (3)
Jac 7	10	0.5	0.8	0.0	2.0	NS
Jac 16	8	NS	1.2	1.7	1.0	NS
Jac 22	8	NS	0.0	3.7	3.0	NS
Jac 27	8	NS	0.7	3.3	0.0	NS
Jac 28	8	NS	0.0	5.0	4.0	NS
Jac 30	10	0.0	0.0	0.2	0.0	NS
JacN 5	8, 11	NS	0.4	1.2	0.0	NS
JacN 7	10	0.0	2.2	0.0	0.0	NS
JacN 11	8	NS	0.0	3.0	0.0	NS
JacN 12	8, 10	0.0 (1)	1.8	0.6	0.0	NS
JacN 13	8	NS	1.0	0.3	0.0	NS
JacN 14	8	NS	3.0	2.0	0.0	NS
JacN 15	8	NS	0.0	1.8	0.0	NS
JacN 16	10	0.0	1.0	0.7	0.0	NS
JacN 18	8	0.0	1.8	1.4	0.0	NS
JacN 22	8	NS	1.5	2.0	0.0	NS
JacN 24	8	NS	2.0	3.5	2.5	NS
JacN 31	10	0.0	0.0	0.9	4.0	NS
OP 45	11	NS	1.0	0.0	2.0	NS

[a] Disease rating is the summation of disease severity (amount of leaf surface covered by lesions, expressed on a scale from 1 [least disease] to 5 [most disease]) and disease occurrence (percentage of each clone showing a given degree of disease severity). The values for disease ratings represent either the first or second yearly observation, whichever was higher, averaged over all sites evaluated.
[b] Site locations: 1 = Algonquin: Monck. Central: 2 = Flesherton, 3 = Flos, 4 = Innisfil (planted 1971), 5 = Innisfil (planted 1972), and 6 = Vespra. Eastern: 7 = Augusta (planted 1975), 8 = Augusta (planted 1976), 9 = Elizabethtown, 10 = Gloucester, and 11 = Haldimand. Southwestern: Malahide.
[c] Numbers in parentheses indicate how many sites contributed to the average disease rating if not all sites containing the clone were evaluated.
[d] Not surveyed.

out by the method described for canker isolations. Significance was measured by the Ryan-Einot-Gabriel-Welsch multiple F test (8).

RESULTS

Septoria leaf spot was common in poplar plantations between 1978 and 1982 (Table 2) and was observed primarily on poplar clones derived from *P. balsamifera*. Septoria leaf spot was not detected on 122 other clones, and Septoria canker was not found in any of the 1978–1982 surveys. Previously surveyed plantations visited in 1983 and 1984 were still free of Septoria canker, but cankers matching descriptions of Septoria cankers (3,11) were observed in nurseries and younger plantations. Fourteen to 29 cankers were collected from each of six sites (a stool bed at Maple, a 5-yr-old planting at Kemptville, and four 3- to 5-yr-old plantations at Brockville), and isolation yielded typical *S. musiva* cultures from 8 to 75% of cankers from a single site. Teleomorphic fruiting structures were abundant on overwintered leaves in natural stands of *P. balsamifera* and *P.* × *jackii* and also in hybrid poplar plantations.

Isolates from cankers, conidia, and ascospores were similar and produced typical *S. musiva* conidia that averaged 40 × 2.5 μm (range: 26.8–54.7 × 1.7–3.5 μm) with one to four septa. Colonies on PDA and MEA were dense, raised, and feltlike with white, gray, or olive-green centers and white, even margins. Colonies on PLA and PLAam were less dense, had little aerial mycelium, and were uniformly white to grayish. Pycnidia formed most frequently on Leonian's agar or GYM and produced conidia in pink or yellow droplets. Ascospores from natural ascocarps on overwintered leaves averaged 17.2 × 4.4 μm (range: 11.6–26.8 × 2.3–5.8 μm). Conidia produced in pycnidia on leaf spots averaged 43.2 × 2.6 μm (range: 27.9–62.9 × 1.7–3.5 μm) and had two to four septa. In culture, neither single-spore nor multispore isolates produced teleomorphic structures. Cultures from Ontario and the United States were morphologically indistinguishable.

Growth rates measured on two media were constant up to 43 days. At 25 C, average growth was 0.65 mm/day on PLAam and 0.56 mm/day on Leonian's agar. Optimal temperatures for growth ranged from 21 to 25 C for most isolates on GYM. One exceptional isolate (from Ontario) grew equally well at 17–25 C, whereas another (from Iowa) grew fastest at 17 C. Similar results were obtained for PLAam and Leonian's agar.

All isolates of *S. musiva* caused cankers, whereas none of the control inoculations did (Table 3). There were no significant differences in lesion length among isolates or clones, except for isolate 142 on clone NE 216. *S. musiva* was recovered from at least two lesions (average of four) in each treatment except the controls, from which the pathogen was never isolated.

DISCUSSION

The presence of Septoria leaf spot in hybrid poplar plantations during the entire period of this study is indicative of the endemic nature of the pathogen. Clones with balsam poplar parentage were the most susceptible, and leaf spot was most severe in 1979 and 1980. The abundance of teleomorphic fruiting structures in both natural and managed poplar stands demonstrates that the fungus is capable of going through its entire life cycle in Ontario. During 1978–1982, however, we did not find any Septoria canker, and observations in the following two years, albeit less systematic, revealed Septoria cankers at only three localities. This represents a far lower incidence of the canker phase of the disease than is found in other hybrid poplar-growing regions of North America. Among the possible explanations for this situation, differences in host resistance can be discounted because many of the same cultivars planted in the United States are present in Ontario plantations. Environmental differences giving rise to altered host-pathogen interactions could be responsible for lower disease levels in Ontario. This possibility is difficult to verify without introducing foreign pathogen strains into new regions, a tactic that would be unwise at our present state of knowledge.

Differences in the pathogen population could account for low disease severity, although our preliminary characterization provided no support for this hypothesis. Ascospore dimensions of Ontario isolates did not differ significantly from the pathogenic isolates studied by Thompson (16–28 × 4.5–6 μm) or Waterman (15–27 × 4–6 μm) (10,11). Neither do conidial dimensions differ from those reported previously (3,10,11). Ontario isolates were not distinguishable from U.S isolates on the basis of growth rate, cultural morphology, or temperature optima, and all isolates were equally capable of causing stem lesions. However, the inoculation procedure used in this study may have masked differences in isolate aggressiveness that would have been detected with gentler methods. The possibility of genetic differences among populations deserves further study.

Septoria canker of hybrid poplars may be on the increase in Ontario. The first detection of Septoria canker in 1983 and 1984 after several years of survey activity supports this hypothesis. Further evidence comes from B. A. Barkley of the Ontario Ministry of Natural Resources, Fast Growing Hardwoods Program, who provided data for plantations in eastern Ontario from 1984 showing an increase in Septoria-type cankers (*personal communication*). Although the causal agent was verified by isolation for only a few of these cankers, their morphology was very uniform and typical of Septoria cankers. Disease ratings for the 10 most susceptible clones ranged from 0.2 to 1.4 for Septoria-type cankers and from 0.1 to 0.7 for Septoria leaf spot, indicating an increase over the level of canker disease detected in our surveys. Such increases may be due to changing management practices, greater incidence of susceptible hosts, or changes in weather patterns. Hybrid poplar plantations in Ontario could be seriously damaged by Septoria canker, as has occurred in the United States. Further studies are needed on the distribution of Septoria canker in Ontario and the relationships among pathogen populations in Ontario and in other poplar growing regions.

Table 3. Mean lesion lengths for hybrid poplar clones inoculated with isolates of *Septoria musiva*

Isolate and source	Poplar clones		
	DN 55	NE 216	NE 252
126, Ames, IA (canker)	15.4[a]	17.6	15.4
127, Maple, Ont. (canker)	12.8	14.0	11.2
139, Stoneville, MS (leaf spot)	13.6	17.2	17.5
142, Brockville, Ont. (canker)	14.6	1.8[b]	11.4
145, Gutherie, Ont. (leaf spot)	9.4	20.9	18.0
151, Rhinelander, WI (leaf spot)	13.6	17.2	12.8
Control	0	0	0

[a] Length of discolored epidermis in millimeters (mean of five replicates). Control wounds were completely closed by healthy callus.

[b] Mean lesion length for isolate 142 on clone NE 216 was significantly smaller than all the others; no other differences are significant.

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