Variability in *Septoria musiva* in Aggressiveness

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ABSTRACT

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In growth studies on five different culture media, spore production of *Septoria musiva* was best on V-8 juice with phytone peptone agar and yeast malt extract agar. A spore concentration of 1×10^6 spores/ml and an incubation time of 48 hr were found to promote good symptom development in glasshouse inoculations. Different levels of aggressiveness were detected for *S. musiva* in glasshouse inoculations of five *Populus* clones with local leaf isolates, local canker isolates, and regional leaf isolates from North Dakota, South Dakota, and Minnesota. Isolates with high and low levels of aggressiveness were selected from the local leaf isolates, the local canker isolates, and the regional leaf isolates and compared. High and low levels of aggressiveness were confirmed for the three types of isolates. Canker isolates were found to be as aggressive as leaf isolates. The local leaf and local canker isolates were found to be as aggressive as isolates that were obtained from a more widespread geographic area.

Populus species are used for windbreak plantings in the Great Plains because of their rapid growth rate. Trees of this genera and hybrids were planted extensively throughout the northern and central Great Plains during the early years of settlement (1,5,6).

Septoria musiva Peck (teleomorph, *Mycosphaerella populorum* Thompson) can cause serious disease problems on *Populus* in the United States and Canada (1,3,8,12,13,15,17,19). Early work by Bier demonstrated that *S. musiva* caused serious foliar and canker diseases in windbreak plantings as well as in plantation plantings (1). Biomass losses as high as 63% have been reported for a highly susceptible hybrid clone (11). Considering that *S. musiva* is widely distributed (1,19), has the ability to infect a wide range of germ plasm (12,13), and has the potential to cause disease losses (11), selecting for resistance to *S. musiva* should be part of a tree improvement program for *Populus* species. Planting disease-resistant clones could be an important factor in limiting disease problems in long-term plantings such as windbreaks or plantations (12,13).

The objectives of this study were to determine if isolates have different levels of aggressiveness and if regional isolates are as aggressive as local isolates. Inoculation techniques for *S. musiva* also were investigated. The development of inoculation techniques required studies to identify a substrate that would produce a large number of spores for inoculations and to determine the conidia concentration and the incubation time needed for good symptom development.

MATERIALS AND METHODS

Isolates of *S. musiva* were obtained from lesions on leaves collected locally from nursery propagation beds and experimental plantations in central North Dakota and from windbreaks and native stands in Minnesota, North Dakota, and South Dakota (8). Leaf sections were cut from leaves, dipped in 70% ethanol, placed for 3 min in a 1% sodium hypochlorite solution containing a surfactant, rinsed in sterile distilled water, and placed on water agar. The leaf sections were incubated at room temperature (24 ± 3 C) 33 cm below fluorescent light (F40 CW). After 7 to 9 days, cirrhi were transferred to 18% V-8 juice agar (V-8A) containing 10 ppm of gentamicin sulfate to reduce bacterial contamination. Canker isolates were obtained by surface sterilizing cankers with 70% ethanol, cutting the bark off, cutting wood chips from canker margins, and placing the wood chips on water agar. Plates were incubated and isolations were made as above. Cultures were maintained on V-8A in a controlled-temperature room (21 ± 1.5 C) 30 cm below continuous fluorescent light (F40 CW). For long-term storage, spore suspensions were frozen and maintained at −90 C.

Medium effects on growth and sporulation. Four isolates of *S. musiva* were plated onto five selected media that promoted good spore production in two initial studies in which 19 cultural media were compared. The five media used and their composition per liter were as follows: 1) Czapek-Dox with V-8 juice (CDV-8A): 35 g of Difco Bacto Czapek-Dox broth (Difco Laboratories, Detroit, MI), 20% V-8 juice, and 3 g of calcium carbonate (2); 2) oatmeal agar (OMA): 72.5 g of Difco Bacto oatmeal agar; 3) V-8 juice agar (V-8A): 18% V-8 juice and 2 g of calcium carbonate; 4) V-8 juice agar and phytone peptone (V-8PPA): 18% V-8 juice, 5 g of BBL phytone peptone (Becton-Dickinson & Co., Cockeysville, MD), and 2 g of calcium carbonate; and 5) yeast malt extract agar (YMA): 4 g of Sigma yeast extract (Sigma Chemical Co., St. Louis, MO), 4 g of Sigma malt extract, and 4 g of sucrose. Agar concentration of all media was 2%, and the final volume of all media was obtained by adding distilled water.

A sterile transfer loop with a spore suspension was used to streak the medium in a line across the center of the petri dish. Twelve dishes of each media were plated (three replications × four isolates). All petri dishes were maintained in a controlled-temperature room (21 ± 1.5 C) 30 cm below continuous fluorescent light (F40 CW). Both mycelial growth and spore production were measured. Mycelial growth was determined on the 14th day by measuring the colony width at the widest point. Spore production was determined on the 15th day. The surfaces of the plates were flooded, rubbed with a rubber spatula, and rinsed with distilled water. The washings were poured into a blender, mixed at low speed for 30 sec, and poured through four layers of cheesecloth. A spore suspension was poured into a graduated cylinder, and the volume was adjusted to 100 ml. Six samples were counted on a hemacytometer to determine the number of spores per milliliter. Growth rate and spore production data were analyzed separately by standard analysis of variance procedures, and statistical comparisons were made with the Student-Newman-Keuls' test (16).

Inoculation techniques. Five *Populus* clones (*P.* × *euramericana*

'Carolina,' 'Norway,' or 'Eugenei'; *P. deltoides* 'Siouxland'; *P.* × *jackii* 'Northwest'; *P.* × *euramericana*. 'Robusta'; and *P.* × *deltoides* 'Walker' were used. Cuttings (15–20 cm long, 6–9 mm in diameter) of *Populus* clones were planted in a peat moss vermiculite mixture (1:1) in Styrofoam block containers (29.5 × 35 × 20 cm). Only 15 of the 30 cavities (5 × 5 × 18 cm) in the containers were used; empty cavities were left between cuttings. Three cuttings of each clone were planted in each container. For the first two studies (SM-1, SM-2), containers were placed in a mist bed for 2–3 wk to initiate growth. For the remaining studies (SM-3 through SM-10), containers were placed on nursery heating pads and watered with warm water to initiate growth. Plants were grown in a glasshouse and fertilized with a balanced, macro- and micro-nutrient fertilizer. Sodium vapor lamps (400 W) were used to supplement the natural light and provide a 16-hr light period. After 7 wk, the *Populus* cuttings were inoculated.

Pycnidiospores for inoculations were produced by flooding V-8A plates. A suspension of spores, obtained by flooding a sporulating culture with sterile distilled water and rubbing the surface with a sterile glass rod, was transferred to media plates with a sterile pipet. The plates were rotated to evenly distribute the suspension and were maintained in a controlled-temperature room (21 ± 1.5 C) 30 cm below continuous fluorescent light (F40 CW). After 14 days of growth, a spore suspension was obtained as described above. Inoculum concentrations which were standardized for each inoculation study ranged from $1–5 \times 10^6$ spores/ml. Two drops of Tween 20/100 ml was added to the suspensions.

All 15 plants in a container were atomized to runoff with 200 ml of inoculum (13.3 ml/plant average). Plants were maintained in a high-humidity chamber (9) for 48 hr and moved to a glasshouse bench (4,17,20). All inoculations were done in a fume hood which was rinsed and aired after each individual isolate was used. Leaves were visually assessed for overall severity of infection (percentage necrosis) 3 wk after inoculation. A disease rating system (1–16) which was based on visually comparing the leaves with herbarium pressed leaves also was used when comparing isolates. Herbarium leaves were visually selected to illustrate a range of symptoms, which was based on combinations of lesion number (very few, few, intermediate, and numerous) and lesion size (very small, small, medium, and large). Leaves with no symptoms were rated 1. Combinations of lesion number and lesion size provided ratings from 2 (very few, very small lesions) through 16 (numerous, large lesions). The combination of numerous, very small lesions was not used. Ratings of three leaves were averaged for each of three replications. These three leaves included the youngest leaf that was inoculated and the next two older leaves on the same stem. For each study an analysis of variance was conducted on the transformed percentage necrosis data and the disease rating data. Statistical comparisons were made with the Student-Newman-Keuls' test (16).

Inoculation studies. Studies were conducted in the glasshouse to determine the spore concentration of the inoculum and incubation time required to obtain disease symptoms on the foliage. Because there were no statistical differences among spore concentrations ranging from $1–20 \times 10^6$ spores/ml in preliminary tests, five spore concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, and 5×10^6 spores/ml) were compared in one inoculation study. In a study to determine the incubation time required for disease development, a spore concentration of 2×10^6 spores/ml was used, and the plants were incubated for 24, 48, 72, 96, and 120 hr. Four *Populus* clones (Carolina, Siouxland, Northwest, and Robusta) were used. The inoculum was a composite of five isolates.

Comparison of isolates. The relative aggressiveness of isolates of *S. musiva* was studied in 10 inoculation studies (SM-1 through SM-10). In 1985, 12 regional isolates were compared in two studies (SM-1, SM-2). Five studies were conducted in 1986: 16 regional isolates were compared in two studies (SM-3, SM-4); eight local isolates from different clones in a plantation were compared in study SM-5; eight local isolates from different clones in a propagation bed were compared in study SM-6; and six local isolates obtained from cankers in a local preparation bed were compared in study SM-7 with two aggressive leaf isolates, selected from SM-1 and SM-2.

Three studies were conducted in 1987 to confirm the results of the earlier studies, SM-1 through SM-7. Eight isolates were selected from the 28 regional isolates that were compared in studies SM-1 through SM-4. Two isolates, one with a low level and one with a high level of aggressiveness, were selected from each of the first four studies and compared in study SM-8. Eight isolates were selected from the 22 local isolates that were compared in studies SM-5 through SM-7. Four isolates from leaves, two with a low level and two with a high level of aggressiveness, were selected from studies SM-5 and SM-6. Four isolates obtained from cankers, two with a low level and two with a high level of aggressiveness, were selected from study SM-7. In the final study (SM-10), three aggressive regional isolates were compared with three aggressive local isolates to determine if the local isolates are as aggressive as those selected from a more diverse area. One isolate (5841) with a low level of aggressiveness was included.

Four *Populus* clones (Northwest, Carolina, Robusta, and Siouxland) were used in all inoculation studies in which isolates were compared. A fifth clone, Walker, was included in studies SM-3 through SM-10. Leaf sections from inoculations with 12 isolates (two isolates from each of six studies) were plated on water agar to reisolate the fungus. A preliminary report was made (7).

RESULTS AND DISCUSSION

Medium effects on growth and sporulation. The best medium for spore production was V-8PPA (1.19×10^6 spores/ml). YMA (1.10×10^6) was statistically similar to V-8PPA and V-8A. V-8A (0.95×10^6) and CDV-8A (0.91×10^6) were statistically similar to each other and ranked intermediate in spore production. OMA (0.6×10^6) gave the least spore production. Mean spore production of the four isolates over all media was statistically similar (0.9 to 1.1×10^6).

After 14 days, the best growth rate was on CDV-8A (1.86 mm/day). V-8PPA (1.64 mm/day) and V-8A (1.57 mm/day) were statistically similar to each other and rated second for growth rate. OMA (1.35 mm/day) and YMA (1.21 mm/day) were statistically separated and ranked third and fourth. The growth rate of the four isolates varied from 1.36 mm/day to 1.71 mm/day in rate of growth. *S. musiva* had faster growth rates on the above media at 20 C than Spielman et al reported on Leonian's agar (0.56 mm/day) and amended poplar leaf agar (0.65 mm/day) which were maintained at 25 C for 43 days (15). Because of the slow growth rate of *S. musiva* in culture with standard transfers, pycnidiospores for inoculations were produced more efficiently by flooding media plates as described above.

Inoculation studies. Inoculum with spore concentrations of 1×10^6 and 5×10^6 caused significantly more leaf necrosis (35 and 36%, respectively) than spore concentrations of 1×10^3 and 1×10^4 (15 and 20%, respectively). The spore concentration of 1×10^5 caused leaf necrosis (23%) statistically similar to the high and low spore concentrations. To insure good symptom development, inoculum containing 1×10^6 spores/ml was the minimum concentration used when isolates were compared.

Statistically there was more leaf necrosis associated with incubation times of 48–120 hr (46–62%) than with the 24-hr incubation time (18%). Because the incubation time of 48 hr was statistically similar to the longer periods of incubation, it was considered the minimum incubation time under our conditions to promote good symptom development and it was used when isolates were compared.

New succulent growth appeared to be the most susceptible (10). Even though the plants were not wounded, tip dieback and small stem cankers were found on some plants, particularly Northwest poplar, a highly susceptible clone. This indicates that *S. musiva* is capable of causing cankers on unwounded stems (4,20). This contrasts with one study in which no cankers developed when trees were atomized with a conidial suspension (10). A range of lesion types (1,14), some with pycnidia, was observed on the foliage of inoculated plants.

Comparison of isolates. Isolates were compared within each

individual study. Because of differences in the ability of isolates to cause disease, isolates were considered to differ in aggressiveness. There were significant differences among isolates in all 10 studies (SM-1 through SM-10).

In studies SM-1 through SM-4, symptoms were observed with all 28 regional isolates of *S. musiva* obtained from 14 counties in three states (Table 1). In each of these four studies, isolates that could be distinguished from one another were considered to differ in aggressiveness. When isolates with different levels of aggressiveness in studies SM-1 through SM-4 were compared in study SM-8, they again could be distinguished from one another (Table 1). Thus, differences in aggressiveness among isolates from diverse locations were confirmed. These differences among isolates should be considered when screening for resistance to *S. musiva*. The most aggressive isolates need to be used to prevent the future breakdown of selected germ plasm.

Different levels of aggressiveness also were detected among local isolates in studies SM-5 and SM-6 (Table 2). Differences among the canker isolates were evident in study SM-7 (Table 2). The more aggressive canker isolates were similar to the selected aggressive leaf isolates included in SM-7. Differences in aggressiveness in studies SM-5 through SM-7 were confirmed when local leaf and canker isolates were compared in study SM-9 (Table 2). Thus, differences among isolates also should be considered when screening for resistance with local isolates of *S. musiva*.

In study SM-10, the more aggressive local isolates (SM-5 through SM-7) were similar to the aggressive regional isolates (SM-1 through SM-4) (Table 3). Thus if one did not want to introduce regional isolates into local plantings, the most aggressive local isolates could be used for local field testing. However, these studies also indicate that, because the full range of levels of aggressiveness occur within one locality, the use of regional isolates would not introduce greater aggressiveness.

The *Populus* clones reacted differently from one another in 19 of 20 analyses of variance (10 analyses of percentage necrosis data and 10 analyses of disease rating data) when isolates were compared. Northwest was considered the most susceptible clone because it statistically had more symptoms than all other clones in 16 of the 20 analyses. Isolates of *S. musiva* were reisolated from leaf

TABLE 1. Differences among regional isolates of *Septoria musiva* in glasshouse inoculations of *Populus* clones[w]

Inoculation test	Isolates	Source of isolate	State	Percentage necrosis[x]	Disease rating (1–16)[x,y]
SM-1	5926	Grand Forks Co.	ND	36 a	7.7 ab
	6415	Marshall Co.	SD	28 ab	7.7 ab
	5964	Pennington Co.	MN	25 b	8.3 a
	5947	Traill Co.	ND	23 b	5.9 bc
	5870	Ramsey Co.	ND	14 c	4.6 c
	6876	Morton Co.	ND	13 c	4.6 c
SM-2	6005	Cass Co.	ND	53 a	9.3 b
	5970	Pennington Co.	MN	51 a	10.6 a
	6004	Cass Co.	ND	40 b	8.7 bc
	5919	Nelson Co.	ND	30 c	6.7 d
	6877	Morton Co.	ND	28 c	7.6 cd
	5847	Foster Co.	ND	27 c	6.7 d
SM-3	5948	Traill Co.	ND	33 a	7.7 a
	5985	Norman Co.	MN	32 a	7.4 a
	5927	Grand Forks Co.	ND	29 ab	6.5 ab
	5991	Clay Co.	MN	28 ab	6.5 ab
	5872	Ramsey Co.	ND	27 ab	6.7 ab
	5848	Foster Co.	ND	24 ab	5.6 b
	5902	Towner Co.	ND	19 b	4.4 c
	5233	McHenry Co.	ND	6 c	2.0 d
SM-4	5921	Nelson Co.	ND	35 a	6.2 a
	5990	Norman Co.	MN	31 a	5.6 ab
	5946	Grand Forks Co.	ND	27 ab	5.5 ab
	5953	Traill Co.	ND	22 b	4.7 bc
	5913	Ramsey Co.	ND	14 c	3.8 cd
	5962	Pennington Co.	MN	14 c	3.1 d
	5955	Marshall Co.	MN	11 c	3.2 d
	5841	Morton Co.	ND	2 d	1.5 e
SM-8[z]	5921	SM-4, H	ND	27 a	8.4 a
	5926	SM-1, H	ND	25 a	7.5 a
	6005	SM-2, H	ND	21 ab	6.8 a
	5948	SM-3, H	ND	18 abc	7.0 a
	6876	SM-1, L	ND	17 abc	5.3 b
	5233	SM-3, L	ND	15 bcd	4.4 bc
	5847	SM-2, L	ND	9 cd	3.8 bc
	5841	SM-4, L	ND	6 d	3.0 c

[w] Four *Populus* clones used in all studies were Northwest, Robusta, Siouxland, and Carolina; Walker was included in studies SM-3 through SM-8. Analyses were done on transformed percentage necrosis data.

[x] Overall means were compared with the Student-Newman-Keuls' test; means followed by the same letter are not significantly different at $P = 0.05$.

[y] 1 = no symptoms; 2 = very few, very small lesions; 16 = numerous large lesions.

[z] Isolates selected for high and low aggressiveness from study SM-1 through SM-4. H = high level of aggressiveness in previous study; L = low level of aggressiveness.

TABLE 2. Differences among local isolates of *Septoria musiva* in glasshouse inoculations of *Populus* clones[t]

Inoculation test	Isolates	Source of isolate	Percentage necrosis[u]	Disease rating (1–16)[u,v]
SM-5[w]	6916	Leaf	22 a	5.4 a
	6909	Leaf	21 a	4.8 ab
	6907	Leaf	21 a	4.5 b
	6912	Leaf	17 ab	3.5 c
	6914	Leaf	16 ab	3.5 c
	6913	Leaf	14 b	3.7 c
	6915	Leaf	9 c	3.0 c
	6911	Leaf	6 d	2.3 d
SM-6[x]	6888	Leaf	38 a	6.8 a
	6023	Leaf	29 ab	5.2 bc
	6891	Leaf	28 ab	5.6 abc
	6887	Leaf	26 ab	6.4 ab
	6884	Leaf	26 bc	4.7 cd
	6024	Leaf	23 bc	5.2 bc
	6883	Leaf	17 bc	3.7 d
	6880	Leaf	15 c	3.6 d
SM-7[y]	5926	Leaf, SM-1	17 a	4.4 a
	6005	Leaf, SM-2	15 a	4.3 a
	7210	Canker	15 a	4.8 a
	7224	Canker	15 a	4.7 a
	7172	Canker	13 ab	4.3 a
	7190	Canker	9 b	3.2 b
	7192	Canker	6 c	3.0 b
	7218	Canker	5 c	2.6 b
SM-9[z]	7210	Canker, SM-7 H	32 a	7.8 a
	7224	Canker, SM-7 H	29 a	8.0 a
	6888	Leaf, SM-6 H	26 ab	8.0 a
	6916	Leaf, SM-5 H	23 ab	7.8 a
	7192	Canker, SM-7 L	18 bc	5.8 b
	7218	Canker, SM-7 L	16 bc	5.7 b
	6880	Leaf, SM-6 L	12 cd	4.1 c
	6911	Leaf, SM-5 L	8 d	3.4 c

[t] Five *Populus* clones used in all studies were Northwest, Robusta, Siouxland, Carolina, and Walker. Analyses were done on transformed percentage necrosis data.

[u] Overall means were compared with the Student-Newman-Keuls' test; means followed by the same letter are not significantly different at $P = 0.05$.

[v] 1 = no symptoms; 2 = very few, very small lesions; 16 = numerous large lesions.

[w] Isolates from different clones at the same location—experimental plantation south of Northern Great Plains Research Laboratory, Mandan, ND.

[x] Isolates from different clones at the same location—nursery propagation beds located at Northern Great Plains Research Laboratory, Mandan, ND.

[y] Leaf isolates selected for high aggressiveness from study SM-1 and SM-2.

[z] Local isolates from leaves and cankers: SM# = previous study; H = high level of aggressiveness in previous study; and L = low level of aggressiveness.

TABLE 3. Comparison of regional and local isolates of *Septoria musiva* in study SM-10 in glasshouse inoculations of *Populus* clones[u]

Isolate	Source of isolate[v]	Previous study[w]	Aggressiveness level[x]	Percentage necrosis[y]	Disease rating (1–16)[y,z]
6415	Regional	SM-1	H	20 a	6.1 a
5926	Regional	SM-1, -7, -8	H	19 a	5.3 a
6909	Local	SM-5	H	18 a	5.6 a
5921	Regional	SM-4, -8	H	18 a	5.5 a
7210	Local	SM-7, -9	H	18 a	5.6 a
6916	Local	SM-5, -9	H	14 a	5.0 a
5841	Regional	SM-4, -8	L	5 b	2.9 b
Control				3 b	2.1 b

[u] Five *Populus* clones used were Northwest, Robusta, Siouxland, Carolina, and Walker. Analyses were done on transformed percentage necrosis data.
[v] Regional = isolates from diverse geographical locations; local = isolates from different clones near the Northern Great Plains Research Laboratory, Mandan, ND.
[w] Earlier tests in which isolates were used.
[x] Determined by previous tests: H = high level of aggressiveness; L = low level of aggressiveness.
[y] Overall means were compared with the Student-Newman-Keuls' test; means followed by the same letter are not significantly different at $P=0.05$.
[z] 1 = no symptoms; 2 = very few, very small lesions; 16 = numerous large lesions.

sections from the different studies. *S. musiva* was sporulating on 80% of the 96 leaf sections 8 days after they were plated.

The clone × isolate interaction was significant in 15 of the 20 analyses of variance. Statistically this would indicate the presence of specificity (18). But the relative magnitude of the mean square for the interaction was low compared to the mean square for the main effects of clones and isolates, and good evidence of clone × isolate interactions or specificity was not found.

Even though the value of comparing isolates among studies conducted over several years (1985–1987) in a glasshouse may be questioned, several comparisons were made with isolates that were used in more than one study. Disease severity values for a few combinations of isolates and clones stood out because they did not follow the overall pattern, but in most cases when these combinations were observed again in a later study with other isolates, they were not consistently different from the overall pattern. Even though inoculation conditions were standardized, it was difficult to demonstrate consistent specific clone and isolate interactions with most isolates under the present conditions. How the clones were ranked in each study for a particular isolate also was compared. A few isolates ranked clones similarly in all studies in which they were used, but this was not the case with all isolates. Thus strong support for specificity was not evident. The possibility of clone × isolate interactions deserves further study.

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