

Effects of Water Stress on Colonization of Poplar Stems and Excised Leaf Disks by *Septoria musiva*

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ABSTRACT

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Septoria musiva causes leaf spot and canker diseases of trees in the genus *Populus*, and is one of the most damaging fungal pathogens of hybrid poplar in eastern North America. The effect of host water stress on *Septoria* canker development was studied in two separate greenhouse experiments. Hybrid poplar clones NM6, NC11396, and NE308 were stressed by withholding water until predawn water potential fell below -1.0 MPa. Stems were treated by removing a leaf and applying agar plugs that were either colonized by *S. musiva* (inoculated) or sterile

(control) to the wound. Cankers on inoculated water-stressed trees were significantly larger than those on nonstressed trees. A leaf disk assay also was conducted three times with the NM6 and NE308 trees. We cut two disks from each of 120 stressed and 120 well-watered trees, placing them on water agar in 24-well tissue culture plates. A conidial suspension was applied to one disk in each pair and sterile water to the other. Inoculated disks from water-stressed trees developed less necrosis than those from well-watered trees. These results demonstrate that environmental influences on host condition must be considered in evaluating resistance of clones proposed for widespread culture of hybrid poplar.

Additional keywords: drought, *Mycosphaerella populorum*.

The anticipation of shrinking petroleum and forest product supplies is stimulating the development of short-rotation intensive culture of woody plants as an alternative fuel and fiber resource (26). In the United States, research has focused on the use of *Populus* species for this purpose, because they are among the fastest growing trees in the temperate zone. Many agents, however, limit the ability of poplars to achieve their potential. Among these is the fungus *Septoria musiva* Peck (teleomorph = *Mycosphaerella populorum* G.E. Thompson).

S. musiva is found in eastern and central North America, (3) as well as in the Crimea and the Caucasus region of Asia (31). The fungus overwinters in leaf debris, producing ostiolate perithecia in the spring that give rise to airborne ascospores at budbreak (21). Ascospores can penetrate leaves through stomata (20), but little information is available on their role in stem infection. The fungus also produces pycnidia that exude cirrhi containing conidia. Conidia are rain splashed (21) and infect leaves and stems (10).

S. musiva causes leaf spot and canker diseases and is one of the most serious agents of damage to hybrid poplars in the eastern United States and Canada (3,16,21,32,33). Leaf spotting can reduce the photosynthetic area of the tree and can cause premature defoliation (32). Cankers can predispose the tree to wind damage (16) and can girdle the tree (33), killing the portion of the tree above the infection site. McNabb et al. (15) reported biomass losses as high as 63% in a plantation.

Little information is available about the potential effects of host condition on diseases caused by *S. musiva*. Field trials have been conducted to screen poplar clones for resistance to the fungus, but disease incidence and severity have been observed to vary from site to site (22,24). In a field study, *Septoria* canker incidence was more severe on a site in which the water table was low (1). How-

ever, other factors including the concentrations of various nutrients and the presence of poplar-and-willow borer (*Cryptorhynchus lapthi*) also differed, making it difficult to draw definite conclusions. In laboratory inoculations of dormant *Populus tremuloides* Michx. and *P. trichocarpa* Torr. & A. Gray stem cuttings, lower bark moisture was associated with greater colonization of stems by *S. musiva* (4). However, direct experimental information is lacking on the effects of specific environmental variables, including water potential, on canker development by intact, growing plants under controlled conditions.

Our objective was to determine the effect of moisture stress on the colonization of *Populus* hybrid stems by *S. musiva*. We also evaluated the influence of host moisture stress on the response of inoculated leaf disks in a bioassay that has been used to estimate relative resistance of clones to *S. musiva*. The responses evaluated included canker development in wounded, inoculated shoots and necrosis of excised leaf disks. Three hybrid poplar clones and two isolates of *S. musiva* were used in greenhouse trials.

MATERIALS AND METHODS

Experiment 1, stem inoculation. We conducted a greenhouse trial using rooted greenwood cuttings of two hybrid poplar clones with differing levels of resistance to *S. musiva*. Single cuttings of NM6 (*P. nigra* L. \times *P. maximowiczii* Henry), a moderately resistant clone (30), and NC11396 (*P. nigra* \times *P. beroliniensis* Dippel), a more susceptible clone, were planted in Fafard mix #2 (Fafard Inc., Agawam, MA) in paper cartons measuring 7 \times 7 \times 18 cm. Plants were placed in a greenhouse with ambient light supplemented with 16 h of light from 400-W sodium vapor lamps.

Two different watering regimens were used to create differences in host moisture status. We watered the trees to field capacity either three times per week (nonstressed), or after the soil water potential fell below -1.0 MPa (stressed). Moisture status was monitored by measuring predawn leaf water potential (Ψ_{PD}) for one tree per treatment three times per week using a pressure chamber

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(PMS Instruments, Corvallis, OR). If the ψ_{PD} of any leaf sampled was below -1.0 MPa, we immediately measured leaves from three trees in each treatment. We intensively sampled to allow statistical comparisons of treatment means using the general linear model (proc GLM) in SAS (SAS Institute, Cary, NC). The design was a two-way factorial using Fisher's least significant difference to test for significant differences among treatment means at $\alpha = 0.05$.

Four stem treatments were applied on November 1, 1993 (day of year 305): nonwounded control, wounded control, and wounding followed by inoculation with one of two *S. musiva* isolates. We marked the fourth fully expanded leaf on the nonwounded controls. For the other three stem treatments, this leaf was removed to create a fresh leaf scar. To this wound we applied either a sterile 5-mm plug of *S. musiva* on poplar leaf decoction agar (PLDA) (13), or one cut from the margin of a 10-day-old culture on PLDA. The plug was held in a depression in a piece of plastic foam and bound to the stem with Parafilm; foam and Parafilm were removed 7 days later. The cultures were obtained from single-conidium isolates from the same leaf spot, and designated 92-49A and 92-49C. Wounded control and inoculated treatments were randomly assigned to eight or seven trees per combination of watering regimen (stress), and stem treatment (inoculation) for NC11396 and NM6, respectively. The remaining seven NC11396 and six NM6 trees were used as nonwounded controls.

Response of trees was evaluated after 80 days. Canker length was measured, to the nearest millimeter, as the length of either the wound or of the associated discoloration, whichever was larger. Percentage of the stem girdled was visually estimated to the nearest percent. In trees treated with sterile agar plugs, subsequently produced callus tissue was the response evaluated. Because nonwounded controls showed no response, they were omitted from statistical analysis. We used the GLM procedure in SAS to perform a three-way factorial analysis of the data. The full analysis of variance (ANOVA) models for canker length and estimated girdling percentage showed large differences between wounded control and *S. musiva* inoculated treatments. However, because the wounded control treatment means had much smaller standard deviations, we performed separate analyses to avoid violating the assumption of equal variances.

Experiment 2, stem inoculation. In this experiment, resistant clone NM6 was used again, but clone NE308 (*P. nigra* var. *charkowiensis* \times *P. berolinensis*), more susceptible to *S. musiva*, replaced NC11396. In a greenhouse, six rooted cuttings of each

clone were planted together in each of 30 plastic boxes measuring $56 \times 46 \times 28$ cm. Trees were planted in an alternating grid in boxes instead of individual pots so that the two clones could be exposed to the same soil environment. The soil mix was 1:1 Fafard mix #2 and sandy loam field soil plus 2 kg m^{-3} Osmocote 14-14-14 (Sierra Chemical Co., Milpitas, CA). We applied Liquid Iron (containing 0.05% Cu, 3.25% Fe, 0.15% Mn, and 0.15% Zn; Fertilome, Bonham, TX) as a foliar spray at planting, and 0.03 kg m^{-3} Peter's 20-20-20 (Scott Sierra, Marysville, OH) as a soil application both at planting and subsequently once per month. Lighting was the same as in experiment 1.

The watering regimens were the same as in experiment 1, except that water was applied to boxes instead of to individual containers, and the monitoring scheme for soil moisture was modified. We monitored ψ_{PD} at low, moderate, and high intensities. Low intensity sampling involved measuring ψ_{PD} on one leaf of each clone in one well-watered and one less frequently watered box (four leaves). When low intensity sampling revealed a ψ_{PD} below -0.5 MPa for any of the leaves, we sampled from three boxes (moderate intensity, 12 leaves). When moderate intensity sampling revealed that the ψ_{PD} had fallen below -1.0 MPa, we sampled two trees of each clone for every box (high intensity, 120 leaves). All boxes were then watered to field capacity.

Three trees of each clone in every box were inoculated with *S. musiva* isolate 92-49A; the other three of each clone were used as wounded controls (nonwounded controls, which produced no response in experiment 1, were omitted). The inoculation procedure was the same as described above except that we used the second fully expanded leaf from the shoot apex. Inoculum plugs were removed from plates containing PLDA that had been inoculated 7 days earlier by spreading 10^6 conidia of *S. musiva* suspended in 1 ml of sterile water on the agar surface. Response of trees to the fungus was evaluated and statistically analyzed as described for experiment 1.

Stem segments were harvested after 75 days, and kept at 4°C until we could examine them for signs of the fungus and reisolate from them. We examined each stem segment, including the site of inoculation and resulting canker, under a dissecting microscope for the presence of pycnidia with visible cirrhi. Cirrhi were placed on microscope slides and examined under a compound microscope to confirm the presence of *S. musiva* conidia. A modified procedure of Long (12) was used to reisolate the fungus. We dipped stem segments into 95% ethanol, flamed them for 5 s, and cut

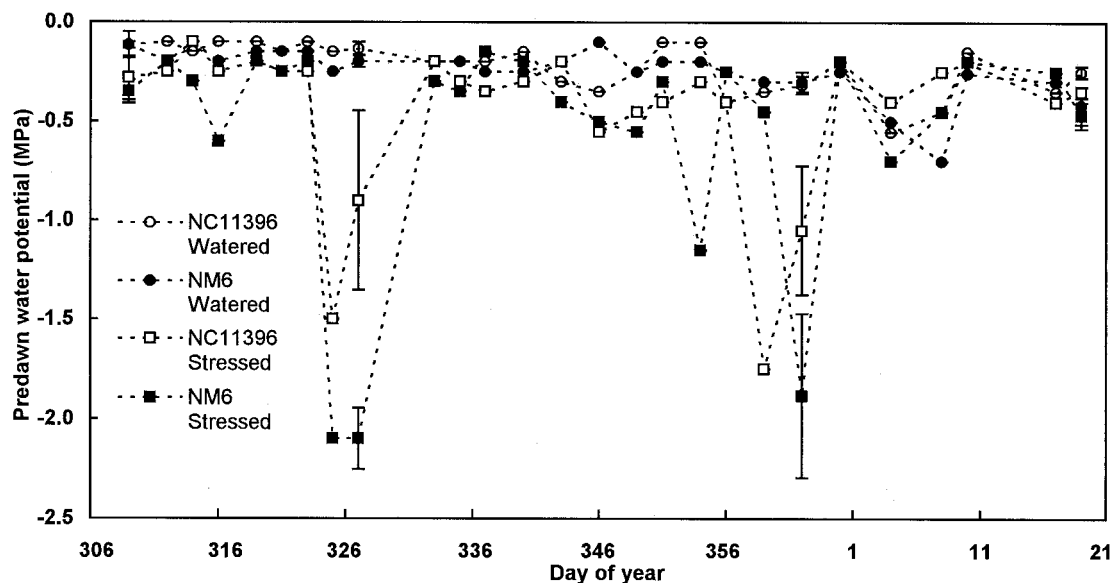


Fig. 1. Predawn leaf water potentials of hybrid poplar as affected by water stress and clone over time. Watered trees were watered three times per week. Stressed trees were watered only after mean ψ_{PD} fell below -1.0 MPa. Error bars represent one standard error above and below the mean for three observations per treatment combination.

5-mm square chips of bark from the inoculation point and from the basal and apical margins of the canker. We also collected three bark chips from our controls: one chip from the site of wounding and two chips from 1 cm on either side of the wounding site. Each bark chip was placed in a sieve-bottomed crucible and dipped for 2 s in 95% ethanol, for 30 s in a 0.5% NaOCl solution, and then for 2 s in sterile distilled water. We placed the crucibles on a blotting surface to remove excess moisture, and placed the bark chips in slants of 2% water agar (WA). We subsequently examined these slants for the presence of *S. musiva* spores and *S. musiva*-like mycelium. To confirm that the mycelium was accurately identified, we randomly chose 10% of the cultures that had *S. musiva*-like mycelia to further attempt identification of the pathogen. This was done by subculturing until conidia were produced or the cultures became overgrown with other organisms

and no *S. musiva*-like mycelium was visible. Frequencies of identification among the different treatments were compared by means of χ^2 analysis in the FREQ procedure of SAS.

Experiment 2, leaf-disk bioassay. A leaf-disk bioassay was performed three times during the course of experiment 2. Before dawn on the last morning of each drying cycle (before watering), we removed the second fully expanded leaf from the shoot apex. Before measuring the ψ_{PD} as described above, we used a cork borer to cut two 15-mm-diameter disks from each leaf for use in a bioassay. Disks were kept in moist chambers on ice until they could be transported to the lab and kept at 4°C until they were used the same day.

The leaf-disk bioassay was modified from those of Spiers (29) and Ostry et al. (25). Leaf disks were randomly placed, abaxial surface up, on top of 1 ml of 2% WA in wells of 24-well tissue

TABLE 1. Analysis of variance for the effects of clone^a and water stress on canker length and estimated percentage of the stem girdled for control, and *S. musiva*-inoculated^b trees after 80 days

Source	df	Control			df	<i>S. musiva</i> -inoculated		
		Type III MS	F	P		Type III MS	F	P
Canker length								
Total	29				58			
Clone	1	43.07	10.72	0.0030	1	3.11	0.05	0.8241
Stress	1	0.21	0.05	0.8189	1	636.89	10.24	0.0024
C × S	1	2.21	0.55	0.4644	1	0.36	0.01	0.9398
Inoc					1	1205.16	19.37	0.0001
C × I					1	228.25	3.67	0.0610
S × I					1	312.38	5.02	0.0294
C × S × I					1	74.07	1.19	0.2803
Error	26	4.02			51	62.21		
Stem girdling (%)								
Total	29				58			
Clone	1	318.94	8.65	0.0068	1	50.17	0.10	0.7562
Stress	1	5.95	0.16	0.6911	1	1597.45	3.10	0.0842
C × S	1	50.75	1.38	0.2513	1	43.17	0.08	0.7733
Inoc					1	11231.16	21.81	0.0001
C × I					1	1363.28	2.65	0.1098
S × I					1	1166.28	2.27	0.1385
C × S × I					1	447.22	0.87	0.3557
Error	26	36.87			51	514.84		

^a For each stress and isolate treatment combination, seven NM6 (*P. nigra* × *P. maximowiczii*) and eight NC11396 (*P. nigra* × *P. berolinensis*) trees were used. Water stress was induced by withholding water from pots until the mean ψ_{PD} fell below -1.0 MPa. Well-watered trees were watered to field capacity three times per week.

^b After removing the fourth fully expanded leaf, we placed a poplar leaf decoction agar plug that was either sterile, or colonized by *S. musiva* isolate 92-49A or 92-49C, over the wound. We performed separate analyses on data from control and inoculated trees, because the variance was much larger for inoculated trees. Nonwounded controls were not included in this analysis because of the extremely low variability, and because any measurable response was due to normal leaf abscission processes and not any experimental treatment.

TABLE 2. Analysis of variance for the effects of clone^a and water stress on canker length and estimated percentage of the stem girdled for control, and *S. musiva*-inoculated^b trees after 75 days

Source	df	Control			df	<i>S. musiva</i> -inoculated		
		Type III MS	F	P		Type III MS	F	P
Canker length								
Total	178				177			
Stress ^c	1	115.96	0.67	0.4207	1	4532.04	15.33	0.0005
Box err.	28	4861.22			28	295.63		
Clone	1	269.42	8.31	0.0045	1	13172.44	63.49	0.0001
C × S	1	0.93	0.03	0.8660	1	479.11	2.31	0.1308
Error	147	32.44			146	207.47		
Stem girdling (%)								
Total	178				177			
Stress	1	466.60	14.39	0.0007	1	6267.48	20.58	0.0001
Box err.	28	32.42			28	304.50		
Clone	1	548.30	22.16	0.0001	1	70798.02	139.80	0.0001
C × S	1	365.09	14.75	0.0002	1	6509.07	12.85	0.0005
Error	147	24.75			146	73938.63		

^a We watered 180 greenwood cuttings each of clones NM6 (*P. nigra* × *P. maximowiczii*) and NE308 (*P. nigra* var. *charkowiensis* × *P. berolinensis*) either three times per week or after the mean ψ_{PD} fell below -1.0 MPa.

^b After removing the second fully expanded leaf, we placed a poplar leaf decoction agar plug that was either sterile, or colonized by *S. musiva*, over the wound. We performed separate analyses on data from control and inoculated trees, because the variance was much larger for inoculated trees.

^c Because watering treatment was applied at the level of box, we used box-to-box variability to test for the effect of stress.

culture plates. We applied 0.1 ml of sterile water to one randomly selected disk in each pair, and 0.1 ml of a suspension of 10^4 conidia/ml to the other. The tissue culture plates containing leaf disks were sealed with Parafilm and incubated at 21°C, 25 cm below continuous fluorescent lights for 9 weeks. We also inoculated 2%

WA plates to quantify spore germination. Incubation conditions for spore germination plates were the same as for the leaf disks, except that they were only incubated for 24 h. After 24 h, we determined the percentage of spore germination by examining 100 conidia. Spores were considered to be germinated if the germ tube was one-fourth as long as the conidium.

We measured relative disease severity for each leaf disk at various intervals. Images of each leaf disk were acquired using a Sony 3CCD color video camera (Sony Corp. of America, Montvale, NJ) and a Targa+ frame grabber board (Truevision, Inc., Indianapolis, IN) and saved on an optical disk as JPEG compressed files. Later, Optimas 4.1 (Bioscan, Inc., Redmond, WA) was used to assess the relative severity of disease symptoms for each disk. We wrote a macro for this program that semi-automated the process of distinguishing darkly colored necrotic tissue from healthy green tissue, which resulted in a rapid (3 min/sample), accurate, and nondestructive measure of symptom development. The GLM procedure in SAS was used to test the effects of watering regimen (stress), disk treatment (inoculation), and clone. The full ANOVA models for percentage of leaf disk necrosis showed a large difference between control and inoculated treatments. However, the variance for control disks was much smaller than for inoculated disks. Therefore, we performed separate analyses.

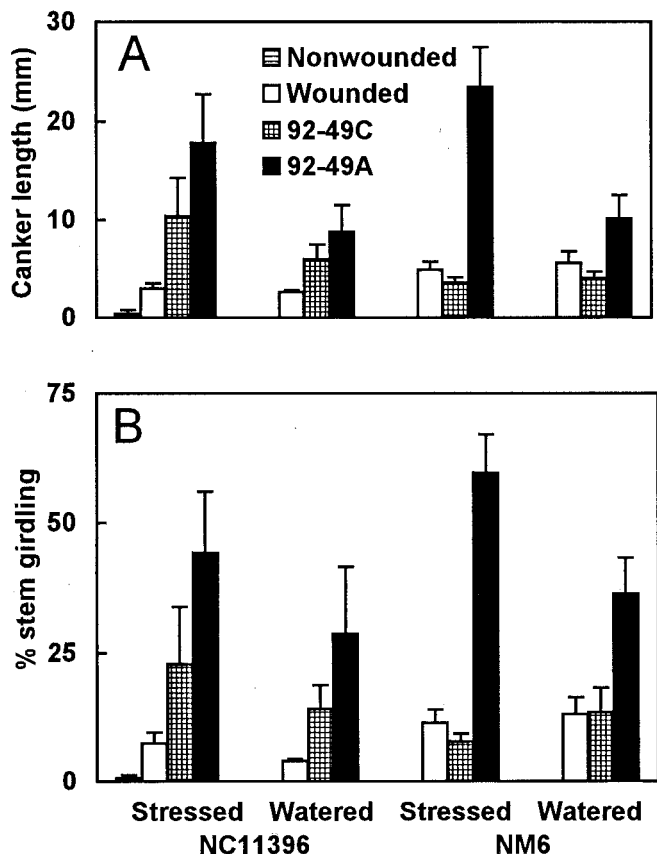


Fig. 2. A, canker length and B, percentage of stem girdling of rooted cuttings of hybrid poplar clones 80 days after inoculation with *Septoria musiva*. Trees were treated by removing the fourth fully expanded leaf and placing either a sterile poplar leaf decoction agar plug or one colonized by *S. musiva* 92-49A or 92-49C over the wound. For each stress and isolate treatment combination, seven NM6 (*Populus nigra* × *P. maximowiczii*) and eight NC11396 (*P. nigra* × *P. berolinensis*) trees were used. Error bars represent one standard error above the mean.

TABLE 3. Numbers of poplar trees inoculated^a with *Septoria musiva* showing the presence or absence of conidia 75 days after stem inoculation as affected by clone and water treatment^b

Water treatment ^c	Clone	Conidia	
		Absent	Present
Well watered	NE308	15	30
	NM6	41	4
Stressed	NE308	20	25
	NM6	33	12

^a After removing the second fully expanded leaf from 180 greenwood cuttings each of clones NM6 (*P. nigra* × *P. maximowiczii*) and NE308 (*P. nigra* var. *charkowiensis* × *P. berolinensis*), we placed a colonized poplar leaf decoction agar plug over the wound.

^b Trees were watered either three times per week or after mean ψ_{PD} fell below -1.0 MPa.

^c For each water treatment, χ^2 analysis indicates lack of independence of clone and presence or absence of spores. For the well-watered treatment $P = 0.005$; for stressed $P < 0.001$. χ^2 analysis also shows a significant three-way interaction between watering, clone, and presence or absence of spores ($P = 0.017$).

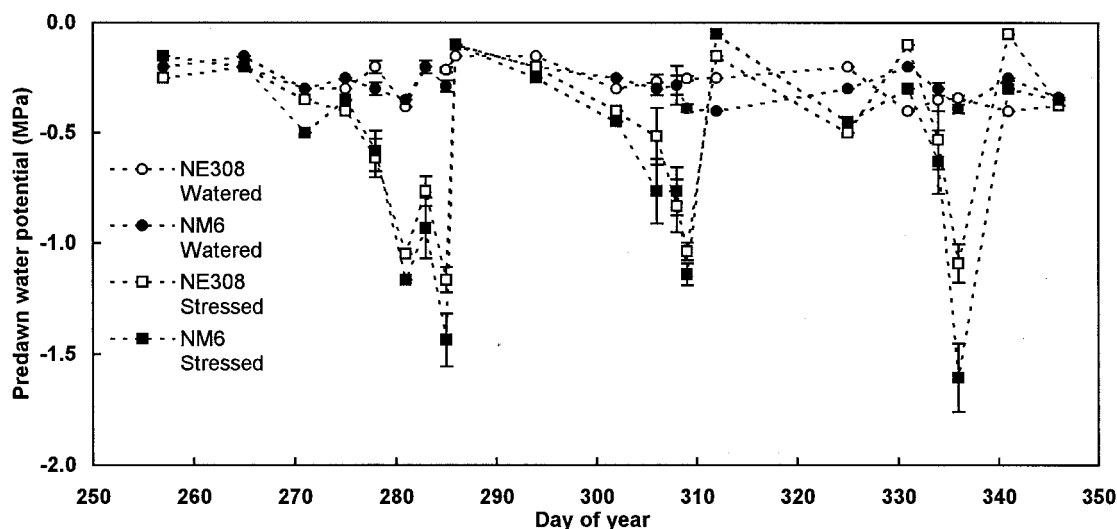


Fig. 3. Predawn leaf water potentials of hybrid poplar as affected by water stress and clone over time. Watered boxes were watered three times per week; stressed boxes were watered only after ψ_{PD} fell below -1.0 MPa. Error bars represent one standard error above and below the mean for three observations per treatment combination on days of the year 278, 283, 306, 308, and 334; and for 30 observations on days 285, 309, and 336.

RESULTS

Experiment 1, stem inoculation. Watering regimen influenced ψ_{PD} for both clones. Among the four dates when ψ_{PD} was most intensively measured, well-watered trees had a significantly higher ψ_{PD} than water-stressed trees on days of year 327 ($P = 0.0005$) and 362 ($P = 0.0024$). A clonal difference was also evident. Water-stressed NM6 trees showed significantly more negative mean ψ_{PD} than NC11396 trees (Fig. 1) on days of year 327 ($P = 0.0077$) and 362 ($P = 0.0567$). No significant differences in ψ_{PD} between watering regimens were detected on days of year 309 and 19 (Fig. 1).

Inoculation treatment (Table 1) affected both canker length (Fig. 2A; $P < 0.0001$) and estimated girdling percentage (Fig. 2B; $P < 0.0001$). Averaged across all treatments, inoculation with either isolate produced larger cankers than the responses of the wounded controls. Additionally, isolate 92-49A produced larger cankers than 92-49C.

Clones interacted differently with different inoculation treatments (Table 1) in terms of both canker length ($P = 0.0457$) and estimated girdling percentage ($P = 0.1356$). In response to isolate 92-49A, clone NM6 produced larger cankers than NC11396. By contrast, when 92-49C was the inoculum, NC11396 developed larger cankers than NM6. For NM6, wounded controls actually produced a slightly greater response than trees inoculated with isolate 92-49C.

When inoculated, water-stressed trees developed larger cankers than well-watered trees, which was indicated by a significant stress \times inoculation interaction for both canker length ($P = 0.0006$) and percent stem girdling ($P = 0.0870$). Mean canker length (Fig. 2A) and percentage of girdle (Fig. 2B) of stems on

water-stressed trees of either clone inoculated with isolate 92-49A were greater than those on well-watered trees inoculated with this isolate. The same response occurred for isolate 92-49C on NC11396 only.

Experiment, 2 stem inoculation. The two watering regimens again resulted in significantly different ψ_{PD} for watered and stressed trees. All moderate and high intensity sampling dates had more negative ψ_{PD} for nonwatered trees than for well-watered trees (Fig. 3; $P < 0.0001$). In addition, at the three high intensity sampling dates, we detected differences between clones when water-stressed: NM6 was more water-stressed, with P values of 0.0447, 0.1129, and 0.0045 for the first, second, and third high intensity sampling dates, respectively.

NE308 responded differently than NM6, both when wounded and when inoculated (Table 2). When inoculated, NE308 trees developed larger cankers than NM6 in terms of both canker length (Fig. 4A, $P < 0.0001$) and stem girdling percentage (Fig. 4B, $P < 0.0001$). For wounded controls, the wound response was larger on NM6 than on NE308 ($P = 0.0045$ for canker length, $P < 0.0001$ for girdling percentage).

When inoculated, water-stressed trees developed larger cankers (Table 2). This was true for both clones in terms of canker length (Fig. 4A, $P = 0.0005$). However, this was true only for NM6 in terms of girdling percentage (Fig. 4B). NE308 developed the same amount of girdling regardless of its moisture status (Table 2, $C \times S$ of ANOVA for stem girdling; $P = 0.0005$).

Upon examining the trees for the presence of *S. musiva* pycnidia and conidia immediately after harvesting, we found significant differences between control and inoculated trees and for the effects of clone and water treatment on inoculated trees. We observed evidence of sporulation on 40% of the trees that had

TABLE 4. Analysis of variance for the effects of water stress^a and clone on percent necrosis of leaf disks treated with either *S. musiva* or sterile water^b

Source	df	Control			df	<i>S. musiva</i> -inoculated		
		Type III MS	<i>F</i>	<i>P</i>		Type III MS	<i>F</i>	<i>P</i>
16 days after inoculation ^c								
Total	119				119			
Stress	1	12.22	1.77	0.1936	1	4835.40	4.99	0.0337
Box err.	28	6.89			28	969.68		
Clone	1	2.44	0.26	0.6095	1	6785.15	12.02	0.0008
$C \times S$	1	1.80	0.19	0.6605	1	40.11	0.07	0.7905
Error	88	9.27			88	564.67		
21 days after inoculation								
Total	119				119			
Stress	1	1.80	0.02	0.8895	1	2086.17	2.77	0.1074
Box err.	28	91.53			28	754.17		
Clone	1	339.70	3.62	0.0603	1	7115.42	12.05	0.0008
$C \times S$	1	26.71	0.03	0.5944	1	130.42	0.22	0.6396
Error	88	93.81			88	590.55		
44 days after inoculation								
Total	119				119			
Stress	1	477.60	6.37	0.0175	1	10502.86	9.18	0.0052
Box err.	28	74.93			28	1143.79		
Clone	1	65.42	0.67	0.4162	1	4342.95	3.00	0.0866
$C \times S$	1	48.64	0.50	0.4830	1	457.43	0.32	0.5752
Error	88	98.03			88	1445.67		
63 days after inoculation								
Total	119				119			
Stress	1	406.49	1.41	0.2456	1	4255.68	10.37	0.0032
Box err.	28	288.98			28	410.22		
Clone	1	2421.55	7.83	0.0063	1	7100.95	18.53	0.0001
$C \times S$	1	355.90	1.15	0.2862	1	251.14	0.66	0.4203
Error	88	309.10			88	383.15		

^a We used 180 greenwood cuttings each of clones NM6 (*P. nigra* \times *P. maximowiczii*) and NE308 (*P. nigra* var. *charkowiensis* \times *P. berolinensis*) that were watered either three times per week or after the mean ψ_{PD} fell below -1.0 MPa. Because watering treatment was applied at the level of box, we used box-to-box variability to test for the effect of stress.

^b We cut two 15-mm disks from the second fully expanded leaf and inoculated each with 0.1 ml of either sterile water or a suspension of 104 conidia/ml. Because the variance was much larger for inoculated disks, separate analyses were performed on data from control and inoculated disks.

^c Images acquired 16, 21, 44, and 63 days after inoculation were from disks excised on days of the year 336, 285, 306, and 336, respectively.

been inoculated, but never on the controls. *S. musiva* was observed to sporulate on NE308-inoculated stems significantly more frequently than on NM6 trees (Table 3). We observed evidence of sporulation on NM6 three times more frequently when the trees were water-stressed than when they were well-watered. Sporulation was evident on NE308 slightly less often in the stressed condition (25 times) than in the nonstressed condition (30 times).

After incubating surface-sterilized bark chips, we observed significant differences in frequency of recovery of *S. musiva* on control and inoculated trees, but no significant effects of water stress or clone (data not shown). We never observed *S. musiva* mycelia or spores on chips from control trees. On bark chips from inoculated trees, we observed *S. musiva* mycelia approximately 29% of the time. When we observed *S. musiva* mycelia, we also observed conidia approximately 2% of the time. In 14 randomly chosen tubes containing *S. musiva* mycelia, we were able to induce production of *S. musiva* conidia nine times (64% of the time).

Experiment 2, leaf disk bioassay. Responses of inoculated disks of the two clones were significantly different. Inoculated

leaf disks from NM6 developed more necrosis than those from NE308 (Fig. 5). This was true at all four times of measurement, with *P* values ranging from 0.0866 to 0.0001 (Table 4).

The response of leaf disks was also sensitive to host condition. Disks from well-watered trees developed more necrosis than those from water-stressed trees (Fig. 5). The *P* values ranged from 0.0052 to 0.0001 for the inoculated disks (Table 4).

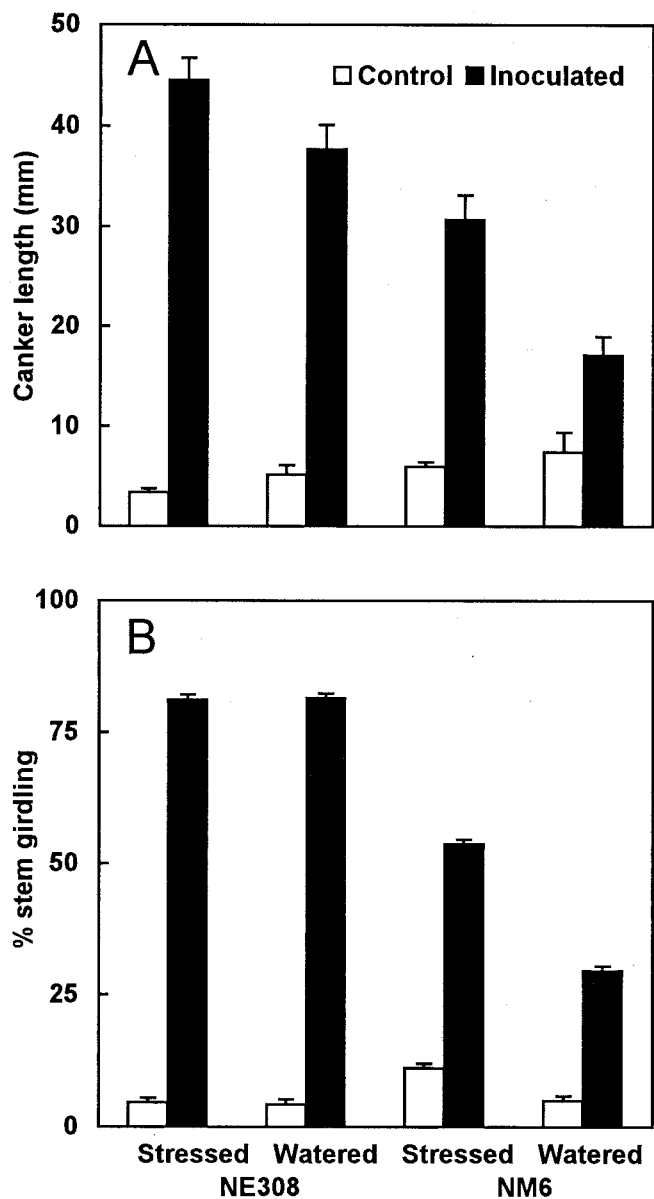


Fig. 4. A, canker length and B, percentage of stem girdling, of rooted cuttings of hybrid poplar clones 75 days after inoculating with *Septoria musiva*. We removed the second fully expanded leaf and applied either a sterile poplar leaf decoction agar plug or one colonized by *S. musiva* over the wound. Error bars represent one standard error above the mean for 45 observations per treatment combination.

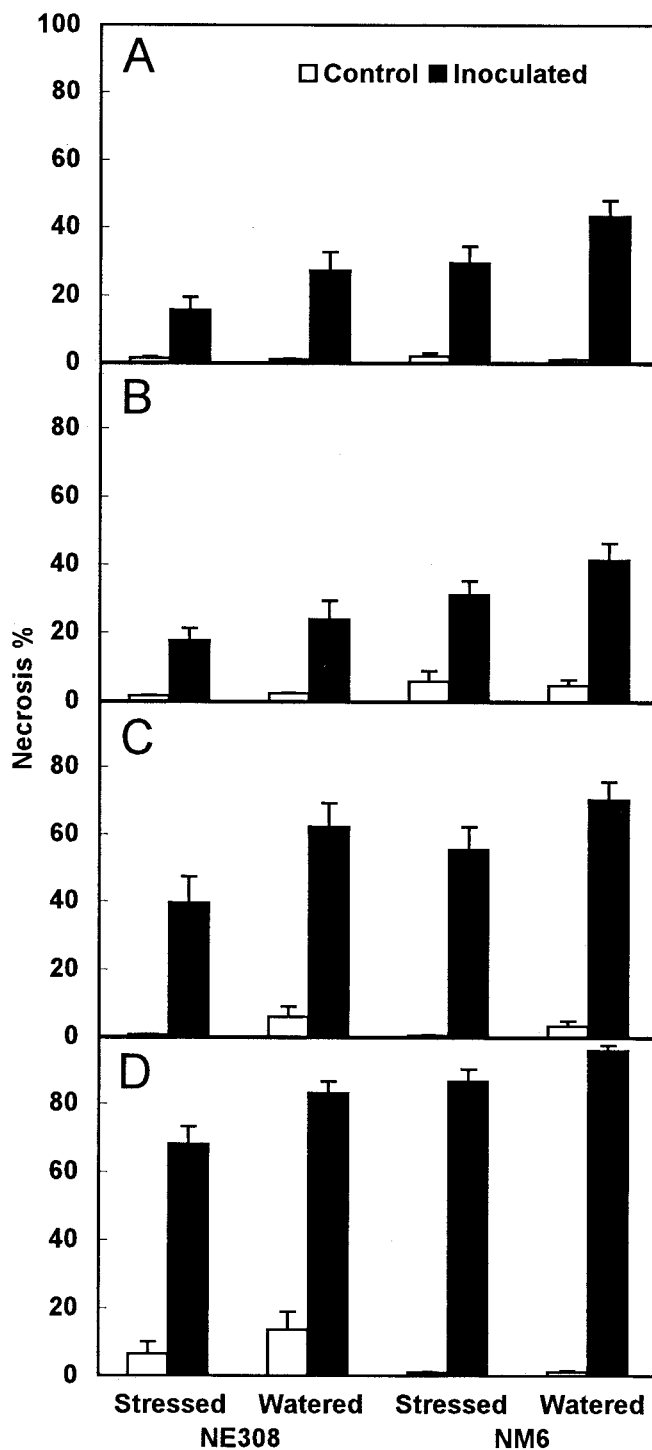


Fig. 5. Mean percentage of necrotic area on poplar leaf disks inoculated with *Septoria musiva* as affected by preinoculation water treatment and hybrid poplar clone. Leaf disks were excised from the second fully expanded leaf, placed abaxial surface up on 1 ml of 2% water agar in 24-well tissue culture plates, and treated with 0.1 ml of either sterile water or a suspension of 10^4 conidia/ml. Disks were excised on days of the year A and D, 336; B, 285; and C, 306. Images were captured after A, 16; B, 21; C, 44; and D, 63 days. Error bars represent one standard error above the mean for 30 observations per treatment combination.

DISCUSSION

This research establishes that host water stress enhances *Septoria* canker development in rooted cuttings of hybrid poplar. In previous studies of the interaction between hybrid *Populus* species and *S. musiva*, environmental factors have been ignored or not controlled. Environmental influences on host condition must be considered in screening for canker resistance and other future research.

Although the inoculation method was artificial, it consistently produced cankers that resembled those attributed to *S. musiva* in the field. While this assay might not indicate how host factors influence the processes of penetration and establishment involved in infection through intact tissue, it did reflect differences in clonal susceptibility and environmental effects. Therefore, it is a tool that may be useful in examining environmental and clonal differences in the responses of hybrid poplar clones to *S. musiva*. The lack of consistency between the results of stem inoculation and of the leaf disk assay, with or without stress, indicates a potential limitation of the excised plant part assay or a danger in extrapolation from the assay to canker susceptibility. There may be very different mechanisms controlling responses of the host to the pathogen in different organs. However, responsiveness of the leaf disk assay to ψ_{PD} under which the host was grown prior to disk collection suggests that varying environmental conditions could change relative rankings of clones determined by this means. The fact that the response of control disks also was influenced by precollection ψ_{PD} indicates that the results of the leaf disk assay, to some degree, are influenced by the rate at which leaves become necrotic even in the absence of *S. musiva*.

Much of the focus of research on *S. musiva* has involved the search for clonal resistance (11,16,23,24). However, disease incidence and severity have varied from site to site (22,24), and it is not clear if this is because of environmental conditions or due to the distribution of the fungus. Ratings of canker incidence and severity for the same trees may also vary over time. Lo et al. (11) found that rankings of clones after 3 years of growth did not always predict rankings after 9 years. These inconsistencies could be due to age-related changes in host susceptibility, environmental factors, or both.

Our results are consistent with the general conclusion that environmental factors strongly influence canker development by fungal pathogens on woody plant hosts. In most cases, water stress leads to increased severity of cankering (27). *Lasioidiplodia theobromae* caused larger cankers on water-stressed dogwood (*Cornus florida*) (17). Similar responses have been observed on other angiosperms, including *Populus* species. Bagga and Smalley (2) demonstrated that *Hypoxyton pruinautum* induced larger cankers on water-stressed *Populus tremuloides*. Cuttings of *Populus* hybrid NE388 showed a similar response to *Cytospora chrysosperma* (6). Larger cankers may also develop on water-stressed gymnosperms. Examples include *Cytospora kunzei* on blue spruce (*Picea pungens*) (28) and *Sphaeropsis sapinea* on red pine (*Pinus resinosa*) (8). However, specific exceptions to this generalization must be noted. In field plots, water-stressed cypress (*Cupressus sempervirens*) trees inoculated with *Seridium cardinale* developed smaller cankers than well-watered trees (14). Likewise, Jacobi and Riffle (9) observed smaller cankers on water-stressed honey locust (*Gleditsia triacanthos*) inoculated with *Thyronectria austroamericana*. Thus, it is necessary to establish the effect of host water stress for each interaction.

Our results also are consistent with previously published work demonstrating enhanced canker development in trees exposed to ozone. Woodbury et al. (34) showed that chronic exposure to ozone significantly increased the incidence and severity of canker caused by *S. musiva* on *Populus* hybrid DN34. This suggests the potential for numerous environmental agents having many different mechanisms of effect to induce susceptibility. Alternatively,

more general processes that are influenced by a wide range of factors may regulate susceptibility. For example, phytoalexin production (35) and processes involved in the nonspecific wound-healing response (5) are inhibited in metabolically compromised hosts.

Previous studies in diverse plant-pathogen systems have indicated that more extreme water stress might be necessary to stimulate canker development. For instance, to study the effects of water stress on *Lasioidiplodia* canker of Dogwood, Mullen et al. (17) withheld water until 60% of the trees in their study showed incipient wilt; and Schoeneweiss (28) studied *Cytospora* canker of blue spruce in trees with ψ_{PD} between -2.0 and -3.0 MPa. Bier (4) suggested that there was a threshold level of bark moisture, 69 to 71%, below which *S. musiva* was active in unrooted cuttings of *Populus* species. In unrooted dormant cuttings of *Populus* hybrid NE388, Biggs et al. (6) measured ψ_{PD} to be -2.7 MPa when bark moisture was 68% and -1.9 MPa when bark moisture was 76%. Thus, Bier (4) was probably working with much more severely stressed plants (between -1.9 and -2.7 MPa) than those we used. Our results demonstrate that *S. musiva* is responsive to host ψ_{PD} even at more moderate stresses that routinely occur in the field.

The mechanism of resistance to development of cankers caused by *S. musiva* is unknown. However, the work of Woodbury et al. (34) demonstrated that susceptibility to *S. musiva* decreased rapidly with the passage of time. We might conclude from this that events in the bark, either soon after wounding or early in the process of pathogen invasion, might be critical to the resistance response. Factors that interfere with this process by either decreasing or delaying wound healing may increase susceptibility. One category of these responses that has been demonstrated to be inhibited by water stress is nonspecific wound healing (6), which involves the production of necrophylactic periderms and non-suberized impervious tissues (NIT). Necrophylactic periderms are probably initiated within hours after wounding (19) and are formed externally to NIT (7). The development of NIT may be involved in the host response to bark diseases (18). Biggs et al. (7) observed that, in the first week after inoculation, *Cytospora chrysosperma* easily penetrated lignified zones. Later, a lignified zone in advance of fungal ingress formed impervious qualities, a necrophylactic periderm formed around this zone, and fungal growth was restricted. Whether these processes are critical to the resistance response of poplars to *S. musiva*, and whether selecting for conditions that allow rapid phylloclerom responses will be useful in preventing this disease, might be the subject of future research.

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