A Test of the Validity of Screening Poplar Clones for Long-Term Canker Disease Damage by Responses to Inoculation with *Septoria Musiva*

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Summary

Septoria musiva (*S. musiva*) causes a stem canker disease that severely damages susceptible hybrid poplars in Eastern North America. An earlier field trial demonstrated the potential for short-term responses of poplar stems to inoculation with *S. musiva* to be predictive of long-term canker disease damage. In the summer of 2000, additional poplar clones primarily selected by a forest industry cooperator on the basis of growth potential (plus the resistant and susceptible standard clones used in the similar field trial in 1998) were inoculated in a test of the validity of the screening procedures. Trees were inoculated during their first season of growth by removing the fourth or fifth fully expanded leaf and placing an agar plug colonized by an aggressive isolate of *S. musiva* over the resulting wound. Four months after inoculation, incidence of cankers, canker length, and percentage of stem circumference affected (girdle) were recorded. Although incidence of cankers was slightly lower on the two standard clones than was observed in the earlier field trial, analyses of canker length and girdle data from both years showed that these clones responded similarly in the two trials. In the current trial, the 15 clones varied greatly in canker incidence (17-96 percent), mean canker length (6-55 mm, 0.24-2.17 in), and mean girdle (10-91 percent). Logistic regression analysis was used to compare these inoculation responses with canker disease damage categories assigned on the basis of subsequently obtained information from longer-term field studies. Incidence, canker length, and girdle data were all informative, allowing correct prediction of assigned canker disease damage categories, respectively, for 11, 13, or 12 of the 15 clones. In addition, when using these inoculation response data the probabilities of placement of clones that had been assigned to the high canker disease damage category (based on longer-term field studies) into the low category

were extremely low. Thus, it appears unlikely that clones that would be severely damaged by canker disease in a commercial rotation of 8-10 years or longer would not be detected in screening using these procedures. In addition to providing information about the likelihood of canker disease damage to clones of commercial interest in the Northcentral United States, these results validate previous work indicating the potential benefits of screening juvenile poplar clones for responses to inoculation with *S. musiva* before extensive field trials and release to growers.

Introduction

The fungal pathogen *Mycosphaerella populorum* (anamorph = *Septoria musiva*) causes leafspot and canker diseases that affect poplar species and hybrids in the eastern United States and Canada (Bier 1939; Lo and others 1995; Ostry and McNabb 1985; Ostry and others 1989; Strobl and Fraser 1989; Waterman 1954). Significant damage can result from cankers on the branches and main stems of susceptible poplar clones. Cankers can cause stem defects that reduce economic value, may kill portions distal to girdling cankers, lead to stem breakage, and result in death of highly susceptible trees. In this region, Septoria canker has been a major barrier to the success of intensively managed poplar plantations as sources of fuel, fiber, and lumber.

Poplar clones are reported to differ greatly in responses to inoculation with *S. musiva* or in the amount of damage resulting from cankers that sometimes have been attributed to *S. musiva* (Bier 1939; Farmer and others 1991; Filer and others 1991; Hansen and others 1994, Lo and others 1995; Maxwell and others 1997; Mottet and others 1991; Netzer and others 2002; Newcombe and Ostry 2001; Ostry and McNabb 1985; Strobl and Fraser 1989; Waterman 1954; Zalasky 1978). Observations of Bier

(1939) and Waterman (1954) indicate an apparent relationship between responses of some clones to inoculation and disease in the field. Weiland and others (2003) compared the incidence and severity of cankers 4 months after inoculation of young poplar trees in the field. Clones had been selected to represent a range in canker disease damage already observed in longer-term field studies. Incidence of cankers resulting from inoculation of these 27 clones varied from 28-98 percent, mean canker length 10-53 mm (0.39-2.09 in), and mean percentage of stem circumference affected (girdle) from 14-94 percent. Results of logistic regression analyses indicated that these responses to inoculation with *S. musiva* were predictive of canker disease damage that had been observed in the longer-term studies. Thus, the potential benefit of screening poplar clones was demonstrated.

Following our previous study (Weiland and others 2003), we were asked to characterize responses to inoculation of an additional group of poplar clones of commercial interest in the Northcentral United States, primarily selected by a forest industry cooperator on the basis of high potential productivity. At that time, little or no long-term performance data was available for the majority of these clones. Since then, however, field observations have allowed us to categorize these clones according to longer-term canker disease damage. Therefore, we were interested in whether analyses of these data would validate our methods (i.e., whether the range of responses of juvenile poplar clones to inoculation with *S. musiva* in this second, independent test could be indicative of canker disease damage in the longer-term field trials). Resistant and susceptible standard clones used in our previous study and 13 additional clones were included. The responses of the standard clones were compared to those obtained previously, and the predictive potentials of response data from all 15 clones were compared.

Materials and Methods

Clonal selection, propagation, and establishment. Clones DN34 and NC11505 were considered resistant and susceptible standards, respectively, based on responses to inoculation in a previous study (table 1) (Weiland and others 2003) and ratings of canker disease damage reported in the literature (table 2). Clone MWH13 was selected as

Table 1. *Resistant (DN34) and susceptible (NC11505) poplar clone standards and responses to inoculation[a] with* Septoria musiva *in 1998 and 2000 field experiments.*

Clone	Year	Incidence[b]		Canker length[c]							Girdle (%)[c]			
					mm				in					
		%	(n)	(n) mean	range	SE	mean	range	SE	(n)	mean	range	SE
DN34	2000	17	(42)	(7) 6	4-9	0.69	0.24	0.16-0.35	0.03	(7)	10	10-10	0.00
	1998	35	(40)	(14) 11	5-17	0.99	0.43	0.20-0.67	0.04	(14)	17	10-20	1.25
NC11505	2000	80	(44)	(35) 55	34-74	1.59	2.17	1.34-2.91	0.06	(35)	91	70-100	1.70
	1998	98	(40)	(26)[d] 53	43-68	1.01	2.09	1.69-2.68	0.04	(39)	94	70-100	1.49

[a] Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium on the resulting wound. Responses were evaluated approximately 4 months later.

[b] Chi-squared tests (for each clone separately) supported the conclusion that canker incidence was not independent of the year (for DN34, p=0.06, for NC11505, p=0.01).

[c] Analyses of variance indicated effects of clone on canker length and on girdle (values of p≤0.01), but not effects of the year or clone by the year interactions on canker length or on girdle (values of p≥0.10). Analyses for percentage data were performed after applying the arcsine of the square root transformation to the proportions.

[d] Due to an error, data for calculation of mean length were obtained for only 26 individuals of this clone in 1998.

Table 2. *Poplar clones, parentage, assigned canker disease damage categories, references, and responses to inoculation[a] with* Septporia musiva *in a 2000 field experiment (in order of increasing severity of girdle).*

Clone (synonyms)	Parentage[b]	Assigned[c] damage category	References[d]	Incidence % (n)	(n)[e]	Canker length mm			Canker length in			Girdle (%)		
						mean	range	SE	mean	range	SE	mean	range	SE
DN34 (NC5326, Eugenei)	dxn	Low	2,3,4,6,7,8	17(42)	(7)	6	4-9	0.69	0.24	0.16-0.35	0.03	10	10-10	0.00
I-476	dxn	Low	2,3,6,7	23(13)	(3)	10	7-14	2.11	0.39	0.28-0.55	0.08	10	10-10	0.00
NE264	dxn	Low	3,6,8,9	24(34)	(8)	7	4-12	1.04	0.28	0.16-0.47	0.04	11	10-20	1.25
I45/51	dxn	Low	2,3,6,7,8	54(41)	(10)	12	4-31	1.54	0.47	0.16-1.22	0.06	16	10-40	2.04
DN2	dxn	Intermediate	1,3,6	58(40)	(23)	16	5-68	2.79	0.63	0.20-2.68	0.11	17	10-50	2.01
DN170	dxn	Low	3,6	70(10)	(7)	19	9-31	3.16	0.75	0.35-1.22	0.12	19	10-30	2.61
NC14105	dxm	Intermediate	OA	67(42)	(28)	13	4-20	0.90	0.51	0.16-0.79	0.04	21	10-70	2.67
NC14106	dxm	Low	OA	67(36)	(24)	15	7-38	1.77	0.59	0.28-1.50	0.07	29	10-70	3.61
113.64	dxm	High	OA	74(31)	(23)	19	8-42	1.59	0.75	0.31-1.65	0.06	30	10-60	3.02
NC14103	dxm	High	OA	95(38)	(36)	20	10-35	0.93	0.79	0.39-1.38	0.04	45	10-90	2.99
MWH14	dxm	High	OA	68(40)	(27)	26	4-48	2.37	1.02	0.16-1.89	0.09	50	10-80	3.94
MWH13	dxm	High	OA	90(31)	(28)	25	12-55	1.70	0.98	0.47-2.17	0.07	53	20-80	2.85
313.55	dxm	High	OA	87(39)	(34)	30	10-48	1.37	1.18	0.39-1.89	0.05	56	30-50	2.49
25	dxm	High	OA	96(28)	(27)	43	32-63	1.50	1.69	1.26-2.48	0.06	67	40-90	2.20
NC11505 (Kingston, NE388)	mxt	High	4,5,8	80(44)	(35)	55	34-74	1.59	2.17	1.34-2.91	0.06	91	70-100	1.70

[a] Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium on the resulting wound. Responses were evaluated approximately 4 months later.

[b] Letters refer to *Populus* species as follows: d=*deltoides*, m=*maximowiczii*, n=*nigra*, t=*trichocarpa*.

[c] Canker disease damage categories were assigned based on information from past field trials, as referenced in the next column.

[d] Numbers refer to references except OA, which refers to observations by the authors of the present study of trees in clonal field trials located at Arlington, WI (unpublished): 1=Boysen and Strobl 1991; 2=Hansen and others 1983; 3=Hansen and others 1994; 4=Lo and others 1995; 5=Long and others 1986; 6=Netzer and others 2002; 7=Ostry and McNabb 1985; 8=Ostry and others 1989; 9=Schreiner 1972.

[e] Number of cankers, for both canker length and girdle.

a second likely susceptible clone, having been observed by the authors of the current study to exhibit high incidence and severity of naturally occurring cankers. Twelve test clones of commercial interest in the Northcentral United States were included at the request of a forest industry cooperator, based primarily on field observations suggesting high-potential productivity.

Dormant cuttings were planted in spring 2000 on a spacing of approximately 0.6 m x 2.4 m (2 ft x 8 ft) in a completely randomized design into each of two plots at the University of Wisconsin-Madison West Madison Agricultural Research Station. Soil at the site is well-drained to moderately well-drained Plano silt loam that previously had been planted in alfalfa, but was used to grow poplars during previous 2 years. The second plot was planted 1 week after the first plot. Trees were mulched with 0.91 m x 0.91 m (3 ft x 3 ft) squares of perforated black plastic (Vispore tree mats, Treessentials Co., Mendota Heights, MN) and each plot was surrounded by a 2-row border of clone DN34. Plots were sprayed immediately after planting with the preemergent herbicides imazaquin and pendimethalin. Subsequent maintenance included both mechanical and chemical (glyphosate) management of competing vegetation and pruning of the trees to maintain a single leader.

Inoculation. Inoculum was produced from a single conidial isolate of *S. musiva* (isolate 92-49A, DAOM 229444) obtained from a hybrid poplar leaf lesion. This isolate has induced cankers after inoculation of poplar stems in previous studies (Maxwell and others 1997; Stanosz and Stanosz 2002; Weiland and others 2003). Conidia stored frozen in sterile water were streaked in three equally spaced lines on the surface of malt extract agar (MEA) in Petri dishes and allowed to grow for approximately 2 weeks at 21ºC (70ºF) under continuous fluorescent light. Plugs of inoculum 5 mm (0.20 in) in diameter were then cut from colony margins.

From 4 to 22 trees per clone in each plot (depending on availability of cuttings, survival, and size) were inoculated. Dates of inoculation were 24-25 July and 31 July - 1 August 2000, respectively, for plots 1 and 2. Each stem was inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of inoculum on the resulting wound with the mycelium side toward the stem. The inoculum plug was held in place by wrapping the plug and stem with Parafilm (American National Can, Menasha, WI), but the piece of plastic foam used to hold

the plug in place on the stem in previous studies (Maxwell and others 1997; Stanosz and Stanosz 2002; Weiland and others 2003) was omitted. Additionally, up to three trees of each clone per plot were used as controls by applying a sterile MEA plug onto the fresh wound. Parafilm was removed 2 weeks after inoculation and a small spot of latex paint was applied above each inoculated and control leaf scar to aid in finding the inoculation site during harvest. The appearance of cankers that developed in response to inoculation was noted at intervals during the growing season.

Canker evaluation. Responses of clones were evaluated following harvest of plots 1 and 2 during the week of 19 November and 26 November 2000, respectively. A segment of each stem 30 cm (12 in) long, centered on the inoculation point, was collected and stored for up to 4 weeks in a plastic bag at 5ºC (41ºF) until the presence or absence of a canker on each segment was recorded. When a canker was present, the outer bark was carefully peeled away, working from healthy bark toward the inoculation point to reveal the canker margin. The length of the canker, as indicated by darkly discolored and/or necrotic tissue, was measured to the nearest 1 mm (0.04 in) along the stem axis. The percentage of the stem circumference affected by the canker, as indicated by darkly discolored and/or necrotic tissue (hereafter referred to as girdle), was visually estimated to the nearest 10 percent.

Canker damage category assignment. Based on information about incidence and severity of cankers in longer-term studies described in the literature or from observations made in field trials located at Arlington, WI, by the authors of the current study, each clone was placed into one of three canker disease damage categories (low, intermediate, and high) (table 2). For example, the resistant standard clone DN34 (assigned to our low damage category) previously received canker disease ratings of 1.2 and 0.2 (for harsh and good sites, respectively, in the Northcentral United States) on a 0 (low) to 3 (high) scale (Hansen and others 1994). In addition, Lo and others (1995) assigned DN34 canker disease ratings of 0.1 and 0.4 (at ages 3 and 9 years, respectively, in plots in New York). In contrast, the susceptible standard clone NC11505 (assigned to our high damage category) received a canker disease rating of 2.2 on a scale of 0 to 3 at age 3 years in the same New York plots, and was no longer present at 9 years (Lo and others 1995). Field trials at Arlington, WI, were established in 1995 and 1997, and canker disease symptoms have been noted in these plots

starting the year after establishment through 2003 (unpublished data of second author).

Statistical analyses. *Comparison of DN34 and NC11505 responses in 1998 and 2000.* Responses of clones DN34 and NC11505 were compared with data obtained for these clones during a similar study in the same location in 1998 by Weiland and others (2003). For each of these two clones and years, incidence data were analyzed using the Chi-square test of independence (Sokal and Rohlf 1995) to test whether canker incidence (i.e., each stem cankered or not cankered) was independent of plot. For each of these two clones and years, severity data (canker length and girdle) also were analyzed to test for an effect of plot. Girdle data were converted into proportions (percentage of stem circumference girdled/100) and transformed by the arcsine of the square root before analyses. Because there was not strong evidence of effects (values of p>0.05 for all tests), data from the two plots in each year were pooled for further analyses and presentation. Pooled incidence data were analyzed using the Chi-square test to test whether canker incidence was independent of the year (1998 or 2000). Pooled severity data were also analyzed using analysis of variance to test for effects of clone, year, and their interaction. These analyses were performed using Minitab Statistical Software release 14 (Minitab Inc., State College, PA).

Comparison of responses of all clones in 2000. Data collected for all clones were analyzed to compare responses to inoculation in 2000. Incidence data, regardless of clone, were analyzed using the Chi-square test to test whether canker incidence was independent of plot. Because there was not strong evidence for effect of plot on canker incidence (p=0.158), data were pooled for presentation. Severity data (canker length and girdle) were analyzed using analysis of variance to test for effects of clone, plot, and their interaction. Girdle data were converted into proportions (percentage of stem circumference girdled/100) and transformed by the arcsine of the square root before analyses. There was not strong evidence for effect of plot or clone by plot interaction on canker length, or effect of clone by plot interaction on girdle. Although there was evidence for a plot effect on girdle (p=0.02), the difference in mean girdle was only slightly greater in plot 1 than in plot 2 (47 percent vs. 40 percent), and this difference might be explained by the later inoculation of trees in plot 2. Therefore, data from the two plots were pooled for further analysis and presentation. Pooled length data and pooled girdle data were analyzed using analysis of variance to test

for effect of clone. Girdle data were transformed as described above. In addition, the relationships among incidence, mean canker length, and mean girdle for each clone were examined by calculating Pearson correlation coefficients. These analyses also were performed using Minitab.

Prediction of assigned categories using responses to inoculation. Using procedures employed in our similar, previous study (Weiland and others 2003), collected data (canker incidence, length, and girdle) also were analyzed as potential explanatory variables to determine whether they were predictive of the assigned long-term canker disease damage categories (low, intermediate, high). These categories are viewed as ordered categorical data requiring specific methods of analysis. Thus, methods for multinomial models with ordinal responses were used (Agresti 1996). Such models are an extension of standard (binary) logistic regression to the case with three or more ordered categories. A key underlying assumption of these models is that of proportional odds; this assumption effectively means that there is a common slope (in the logistic regression model) separating each pair of categories. A p-value of less than 0.05 would indicate a failure of the proportional odds assumption (i.e., the collected data do not support the ordinal logistic model). To assess the performance of the multinomial models (given that the proportional odds assumption was met), we calculated "prediction accuracy" as the proportion of the cases (poplar clones) that were successfully predicted by the particular model. A proportion close to 1 (e.g., 12/15) suggests high model prediction accuracy. The effects of the predictor variables (incidence, length, girdle) are quantified by a p-value (calculated by maximum likelihood) where each p-value indicates the impact of a given variable after all other terms have been accounted for. A p-value less than 0.05 indicates statistical significance.

In the implementation of model fitting to our data, means of incidence, length, and girdle for each clone were each used as predictors in logistic regression where the "outcome" variable was the disease damage category. All three predictors, each possible pair of predictors, and each predictor separately were tested for each of the two experiments. Logistic regression analyses were performed using SAS version 8 (SAS Institute, Cary, NC).

Results

Canker characteristics. Cankers that developed on inoculated trees closely resembled cankers produced in

response to inoculation in our previous study (Weiland and others 2003), and those attributed to natural infection of poplars by *S. musiva* in the field. No control trees developed cankers.

Comparison of DN34 and NC11505 responses in 1998 and 2000. Chi-squared tests (for each clone separately) supported the conclusion that canker incidence was not independent of the year (for DN34, p=0.06; for NC11505, p=0.01) (table 1). For NC11505, 98 and 80 percent of the inoculated trees developed cankers in 1998 and 2000, respectively. For DN34, 35 and 17 percent of the inoculated trees developed cankers in 1998 and 2000, respectively. Analysis of variance indicated effects of clone (values of p<0.001) on both canker length and girdle, but not effects of the year or interaction (values of p>0.05) on either. Mean canker lengths and girdle in each of these 2 years were approximately 5 to 10 times greater for the susceptible standard NC11505 than for the resistant standard DN34.

Comparison of responses of all clones in 2000.
Canker incidence. Incidence of cankers varied greatly among clones (table 2). Clones with relatively low incidence (<25 percent) were DN34, I-476, and NE264. Incidence was less than or equal to 90 percent for clones NC14103, MWH13, and 25, and was 80 percent for clone NC11505. Incidence by clone was positively correlated to mean canker length (r=0.69, p=0.005) and mean girdle (r=0.74, p=0.002).

Canker length. Analysis of variance indicated an effect of clone on mean canker length (p<0.001) (table 2). Mean canker length was relatively short (<10 mm, 0.39 in) for clones DN34, I-476, and NE264. Mean canker length was greatest (55 mm, 2.17 in) for clone NC11505.   Mean canker length by clone was positively correlated to mean girdle (r=0.96, p<0.001).

Canker girdle. Analysis of variance indicated an effect of clone on mean canker girdle (p<0.001) (table 2). Mean canker girdle was relatively small (10 percent) for clones DN34 and I-476. Mean canker girdle was greater than or equal to 50 percent for clones MWH14, MWH13, and 25, and was 91 percent for clone NC11505.

Prediction of assigned canker categories using data for responses to inoculation. Results of logistic regression analyses indicated how well canker incidence, length, or girdle data (or their combinations) from the field experi-

ment predicted the assigned long-term canker disease damage categories (low, intermediate, or high). For example, for the model using the mean canker girdle data as the sole predictor, clone DN34 had a much greater probability of placement in the low damage category (probability = 0.96) than in either the intermediate (probability = 0.04) or high (probability = 0.01) damage categories (table 3). In contrast, for the same model clone NC11505 had a probability of 1.00 of placement in the high damage category. For this model, the damage category with the highest probability matched the assigned damage category for 12 of 15 clones (tables 3 and 4). Further, for four of the six clones that had been assigned to the low damage category (i.e., literature reports or our observations indicated low canker disease damage in the field), the probability of placement in the low damage category based on the field experiment girdle data alone was greater than or equal to 0.79 (table 3). All seven of the clones that had been assigned the high damage category (i.e., literature reports or our observations indicated high canker disease damage in the field), the probability of placement in the low damage category based on the canker girdle data alone was less than or equal to 0.11 (table 3). For this model, the corresponding proportional odds assumption p-value was sufficiently high (p=0.1313) to satisfy the assumptions for this model and the maximum likelihood estimate p-value was low (p=0.0326), indicating the significant contribution of canker girdle to the model after all other terms were accounted for (table 4). Other models (using other variables singly or in combination) performed similarly to the model using girdle alone, satisfying model assumptions and accurately predicting the longer-term field performance of most clones. The two to four clones that were incorrectly predicted using the various models were limited to DN2, DN170, NC14105, and NC14106, each of which had moderate responses to inoculation (table 2). All other clones, which had either lesser or greater responses to inoculation, were never incorrectly predicted. Finally, when the model using all predictors (incidence, length, and girdle) was used, errors in prediction were never by more than one category. That is, clones assigned to the high canker disease damage category based on observations from longer-term field trials were never predicted to be in the low category based on inoculation responses, and vice versa.

Discussion

In addition to providing information indicating a wide range in the responses of clones of commercial interest in

Table 3. *Poplar clones, assigned canker disease damage categories, and probabilities of placement of respective clones in those categories as indicated by logistic regression analysis using percentage of stem circumference girdled by cankers resulting from inoculation[a] with* Septoria musiva *in a 2000 field experiment (in order of their appearance in table 2).*

Clone	Assigned damage category[b]	Probability of placement in category		
		Low	Intermediate	High
DN34	Low	0.96	0.04	0.01[c]
I-476	Low	0.96	0.04	0.01
NE264	Low	0.86	0.11	0.02
I45/51	Low	0.79	0.17	0.04
DN2	Intermediate	0.71	0.23	0.06
DN170	Low	0.65	0.28	0.07
NC14105	Intermediate	0.52	0.36	0.12
NC14106	Low	0.13	0.37	0.49
113.64	High	0.11	0.35	0.54
NC14103	High	0.01	0.03	0.96
MWH14	High	0.00	0.01	0.99
MWH13	High	0.00	0.01	0.99
313.55	High	0.00	0.00	1.00
25	High	0.00	0.00	1.0
NC11505	High	0.00	0.00	1.00

[a] Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium over the resulting wound. Responses were evaluated approximately 4 months later.

[b] Canker disease damage categories were assigned based on information from longer-term field trials (see table 2).

[c] Probabilities for a clone may total more or less than 1 due to rounding.

Table 4. *Results of logistic regression analyses of data from responses of 15 poplar clones of 3 canker disease damage categories to inoculation[a] with* Septoria musiva *in a 2000 field experiment.*

Predictor (s)	Proportion matched[b]	Proportional odds assumption p-value[c]	Maximum likelihood estimate p-value[d]		
			Incidence	Length	Girdle
Incidence, length, girdle	12/15	0.3095	0.6975	0.5768	0.4458
Incidence, length	12/15	0.1227	0.3530	0.2328	
Incidence, girdle	12/15	0.1659	0.5501		0.2163
Length, girdle	11/15	0.2814		0.4373	0.2245
Incidence	11/15	0.1320	0.0559		
Length	13/15	0.1388		0.0348	
Girdle	12/15	0.1313			0.0326

[a] Trees were inoculated by removing the fourth or fifth fully expanded leaf and placing a plug of medium bearing mycelium over the resulting wound. Responses were evaluated approximately 4 months later.

[b] Proportion of the 15 clones tested for which the canker disease damage category predicted by responses to inoculation matched the damage category assigned based on information from longer term field trials (see table 2).

[c] A value of less than 0.05 indicates a failure of the proportional odds assumption (i.e., collected data do not support the ordinal logistic model).

[d] A value of less than 0.05 indicates statistical significance (impact of a given variable after all other terms have been accounted for).

the Northcentral United States, our results support the validity of screening poplars for long-term canker disease damage by inoculation with *S. musiva*. Although the inoculation method used is artificial and could allow *S. musiva* to bypass mechanisms of resistance that might operate in naturally infected stems, processes that either facilitate or limit canker initiation and expansion appear to operate under the conditions of these tests. The wide range in responses among the tested clones to inoculation with *S. musiva* is consistent with results reported in our previous research (Weiland and others 2003). In both 1998 and 2000, clones exhibited a continuum of responses from very few and small cankers to very many and large cankers. Thus, rather than being qualitative (i.e., clones are either damaged severely or not damaged at all), responses to inoculation with *S. musiva* using these methods appear to be quantitative.

In spite of trees of each clone being genetically identical, there also was considerable variation in responses of different trees of the same clone. This tree-to-tree variation has important implications for application of these screening procedures to test new plant material. Instead of relatively few trees, many trees per clone needed to be inoculated to produce incidence data and reliable means for canker length and girdle. This variation also indicates that pure chance and environmental factors also may have great influence on development of individual cankers on individual trees.

The similarity of responses of DN34 and NC11505 to inoculation in two different years, however, supports their inclusion as standards. In each year these clones exhibited relatively low and high frequencies of cankers, respectively, although canker incidence for each was somewhat lower in 2000 than in 1998. Omission of plastic foam that had been

used to hold the plug in place on the stem in previous studies (Maxwell and others 1997; Stanosz and Stanosz 2002; Weiland and others 2003) may have resulted in reduced infection efficiency in 2000. Variation in other conditions, such as weather, also might have affected canker incidence. But for cankers that did develop on these two clones in either year, however, mean canker length and mean canker girdle were very similar. Thus, regardless of the year, these two clones represented the approximate extremes in the ranges of response to inoculation.

As seen in our earlier test, overall predictability of long-term canker disease damage from responses to inoculation with *S. musiva* was high. All responses (incidence, canker length, and girdle) can contribute to accuracy of prediction, and allow detection of clones most likely and least likely to be severely damaged. Clones that are intermediate in response might be continued in a breeding or selection program based on desirability of other characteristics of the clone and the degree of defect that can be tolerated (e.g., when used for pulpwood as opposed to lumber). Incorporation of screening for long-term canker disease damage from responses to inoculation with *S. musiva* will help to ensure that limited resources for further field testing in poplar clone development programs are used efficiently.

Conclusions

Stems of poplar clones of commercial interest in the Northcentral United States that were inoculated with the canker pathogen *S. musiva* during their first season of growth varied greatly in resulting canker incidence, canker length, and percentage of stem circumference affected. The responses of standard resistant and susceptible clones were consistent with previous results. Logistic regression analyses indicated that responses of the 15 clones generally were predictive of long-term canker disease damage categories assigned from information in the literature or observations of the authors of trees in longer-term trials.

Screening poplar clones for responses to inoculation with *S. musiva* allows detection of clones most likely and least likely to be severely damaged by canker disease in commercial rotations.

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Zalasky, H. 1978. Stem and leaf spot infections caused by Septoria musiva and S. populicola on poplar seedlings. Phytoprotection. 59: 43-50.Table 1. Resistant (DN34) and susceptible (NC11505) poplar clone standards and responses to inoculationa with Septoria musiva in 1998 and 2000 field experiments.