Recessive Resistance to Septoria Stem Canker of Hybrid Poplar

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ABSTRACT

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Stem canker, caused by *Septoria musiva*, is the most serious disease limiting intensive hybrid poplar culture in eastern North America. *Populus deltoides* (D) is itself resistant, but the susceptibility of western black cottonwood, *P. trichocarpa* (T) is apparently dominant in the F₁ generation. To test a hypothesis of recessive inheritance of canker resistance, a three-generation T × D pedigree was deployed in the field in Iowa and Minnesota. In both sites and in keeping with expectations, *P. trichocarpa* and its F₁ hybrids were susceptible to canker, whereas the *P. deltoides* parent was resistant. In Iowa, 10 of 70 F₂ (TD × TD)

individuals were free of canker, suggesting that a single recessive gene might control resistance. In the third year in Minnesota, more resistant individuals than expected were seen in the F₂ generation and in TD × D and T × TD backcross progenies due to disease escape. By the fifth year, this was no longer an issue, but winter injury may have eliminated many clones. Qualitatively, however, evidence for recessive inheritance of resistance was still obtained. The only canker-free clones were in the TD × D backcross and the F₂ generation, the two progenies in which they were expected. However, conclusive evidence that recessive canker resistance is conferred by a single gene was not obtained in this field study.

Additional keywords: cottonwood, *Mycosphaerella populorum*.

Stem canker of poplar in North America is caused by *Septoria musiva* Peck. It is a serious disease that prevents the cultivation of susceptible hybrid poplar in much of the Mississippi and St. Lawrence River drainages (7,9). Stem breakage can result from cankers, and short-rotation plantations of cankered hybrid poplar have proven to be commercial failures (6,7).

Populus deltoides Marshall, the Eastern cottonwood, is known to be resistant to stem canker (7). *S. musiva* does cause leaf spots on *P. deltoides*, but this disease is seldom associated with serious damage. However, hybrids of *P. deltoides* with species in *Populus* section *Tacamahaca* are typically susceptible to stem canker. In particular, *P. trichocarpa* Torr. & Gray × *P. deltoides* F₁ hybrids have proven susceptible in many locations in eastern North America (7). The susceptibility of *P. trichocarpa* itself has also been demonstrated many times in various trials where stem canker occurs.

In regions such as the Pacific Northwest, stem canker of genetically susceptible hybrid poplar and *P. trichocarpa* does not occur, for reasons that are still not understood (5). Although the absence of *S. musiva* in the Pacific Northwest is suggestive, *S. populicola* Peck does occur in the region (5) and it can cause cankers in the field (11) and in greenhouse assays (M. Ostry, unpublished data).

P. trichocarpa × *P. deltoides* (T × D) F₁ hybrids are desirable commercial clones in the Pacific Northwest. Rooting ability and resistance to leaf rust caused by *Melampsora medusae* are contributed by *P. trichocarpa* to its interspecific hybrid offspring. With adequate resistance to stem canker, T × D hybrids would be an important additional resource for intensive forestry in eastern North America.

T × D F₁ hybrids are uniformly susceptible to stem canker; therefore, we hypothesized that the resistance of *P. deltoides* is recessive. According to this hypothesis, *P. trichocarpa* is homozygous dominant for susceptibility, and thus the interspecific F₁ progeny are always susceptible. To test the hypothesis, a three-

generation T × D pedigree was deployed in the field in Minnesota and Iowa. We expected that up to one-quarter of the F₂ progeny of susceptible F₁ parents would be resistant. In Minnesota, the reciprocal backcross progenies of the three-generation pedigree were added to the trial. Up to one-half of a TD × D backcross progeny was expected to be resistant, whereas a T × TD backcross progeny was expected to be entirely susceptible like the F₁ generation.

MATERIALS AND METHODS

Field trials of a three-generation hybrid poplar pedigree.

The pedigree plantings near Ames, IA, and Rosemount, MN, were established in 1995 and 1996, respectively. They were designed for the study of growth and development of *P. trichocarpa* and T × D hybrid poplars in environments where stem canker is known to occur. Trees were planted at a spacing of 0.3 m within rows and 2.8 m between rows in a modified randomized complete block design. The Ames planting contained three replicates, each of which consisted of two-tree plots of 70 genotypes or clones of F₂ family 331, its F₁ parents, and its *P. trichocarpa* and *P. deltoides* grandparents, '93-968' and ILL-129, respectively. In Rosemount, the reciprocal backcross progenies, families 342 (TD × D) and 354 (T × TD), were included. Pedigree structure is shown in Figure 1. Previous articles have dealt with the importance of this pedigree as a focus for coordinated research efforts in the genus *Populus* (1,8,10).

Rating canker incidence and severity due to *S. musiva*.

Canker categories were as follows: 0 = none, 1 = branch canker or cankers only, 2 = stem canker or cankers, 3 = stem dieback and breakage associated with cankers, and 4 = killed by canker.

Rating leaf spot severity due to *S. musiva*. Leaf spot categories were as follows: 0 = none; 1 = slight, with few infected leaves; 2 = moderate, with infection throughout crown; and 3 = moderate, with premature defoliation in the lower crown. Secondary, conidial inoculum of *S. musiva* is produced throughout the growing season in leaf spots (6). Cankers can result from secondary inoculum.

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Statistical analysis. Genetic hypothesis testing: χ^2 -squared tests were performed to test Mendelian ratios expected for a single recessive gene for resistance. Analysis of variance was used to test for family differences.

RESULTS

Canker incidence in Iowa after the third year of growth. We expected the *P. trichocarpa* clone, 93-968, and its F_1 hybrids, '53-242' and '53-246', to be cankered. Canker incidence for these three clones was 100% (a combined 26/26 cankered ramets). In contrast, and as expected, the *P. deltoides* clone, ILL-129, was not cankered (0/13 cankered ramets). The distribution of canker incidence in the F_2 generation is shown in Figure 1. Ten clones were free of canker (a combined 0/43 ramets), whereas 60 sibs were cankered. This segregation is compatible with the simple hypothesis of a single recessive gene for canker resistance (Table 1). The 60 susceptible clones varied in mean canker incidence among ramets, from 16 to 100%.

Canker incidence and severity in Minnesota after the third year of growth. Canker incidence and severity in the 24 F_1 clones in 1998 (third year of growth) were close to expected values. The median and mean canker severities were 2.0 (i.e., stem cankers were present) and 1.86, respectively. Under the hypothesis of a single recessive gene for resistance from *P. deltoides*, no canker-free clones were expected, but one was observed. It is an academic point given the other results, but 1/24 does not constitute a significant deviation from the expected 24/24 (Yates' corrected χ^2 -squared and Fisher exact tests; $P = 1.0$, Table 1).

A large deviation from expectation was observed in the F_2 progeny (TD $\times$ TD, $n = 49$ clones). Under the hypothesis of a

single recessive gene for resistance from *P. deltoides*, only one-quarter of the F_2 clones (i.e., 12 or 13) were expected to be canker-free, but 35 were observed, leading us to conclude that disease escape was a factor at this point in the experiment. The median and mean canker severities were zero and 0.61, respectively. The four severely cankered clones (i.e., ≈ 3.0) were outside values (≈ 2.7 standard deviations from the mean) in the F_2 distribution.

More resistant clones than expected were also observed in the progeny of the backcross to *P. deltoides* (TD $\times$ D, $n = 31$ clones). Under the hypothesis of a single recessive gene for resistance from *P. deltoides*, 15 or 16 canker-free clones were expected, but 25 were observed. The median and mean canker severities were zero and 0.36, respectively.

Similarly, canker incidence was less than expected in the progeny of a backcross to *P. trichocarpa* (T $\times$ TD, $n = 20$). Under the hypothesis of a single recessive gene for resistance from *P. deltoides*, no canker-free clones were expected, but five were observed. This deviation bordered on significance: insignificant in Yates' corrected χ^2 -squared test ($\chi^2 = 3.66$, $P = 0.056$), but significant according to the Fisher exact test ($P = 0.047$). The median and mean canker severities were 2.0 and 1.70, respectively.

Family was a significant factor in canker severity after 3 years of growth in Minnesota (F ratio = 18.29, $P < 0.001$) (Table 2). The hypothesis of recessive inheritance of canker resistance was also confirmed in that the F_2 generation was significantly more resistant than the F_1 (mean difference of 1.25; Bonferroni Adjusted $P < 0.001$).

Leaf spot incidence and severity in Minnesota after the third year of growth. Leaf spot caused by *S. musiva* was common after 3 years in the field in Minnesota. All clones in the F_1 , TD $\times$ D, and T $\times$ TD progenies were affected by leaf spot to varying degrees. In the F_2 generation, there were only five leaf spot-free clones. Leaf spot was not only common but generally moderate in severity (Fig. 2); relatively few clones were in the first category of having only slight leaf spot.

Fig. 1. Clone means (ramets per clone ≥ 4) for stem canker incidence in the family 331 (the F_2 progeny) in Ames, IA, in its third year of growth. Note 10 clones that were free of cankers (i.e., the "0" class).

TABLE 2. Matrix of pairwise mean differences (above diagonal) among pedigree progenies for resistance to *Septoria* canker in Minnesota after 3 years of growth, with Bonferroni Adjustment probabilities below the diagonal^a

Family	Family			
	F_1	F_2	TD $\times$ D	T $\times$ TD
F_1	...	<u>1.25</u>	<u>1.50</u>	0.16
F_2	<u>0.00</u>	...	0.26	<u>1.09</u>
TD $\times$ D	<u>0.00</u>	1.00	...	<u>1.35</u>
T $\times$ TD	1.00	<u>0.00</u>	<u>0.00</u>	...

^a *Populus trichocarpa* $\times$ *P. deltoides* = T $\times$ D. Underlined differences and probabilities are significant.

TABLE 1. Mendelian analyses of a putative, recessive resistance gene (rr) to poplar stem canker caused by *Septoria musiva*, based on third-year results from the field in Iowa (IA) and Minnesota (MN) with a three-generation *Populus trichocarpa* $\times$ *P. deltoides* (T $\times$ D) interspecific hybrid pedigree^a

Family identifier	Origin	Phenotype, or ratio of resistant:susceptible (R:S)		χ^2 probability ^b
		Expected	Observed	
ILL-129 (59-129-17)	<i>P. deltoides</i> , Illinois	R (IA), R (MN)	R (IA), R (MN)	...
93-968	<i>P. trichocarpa</i> , Washington	S (IA), S (MN)	S (IA), S (MN)	...
Fam. 53 ^c	F_1 (93-968 $\times$ ILL-129)	0:2 (IA), 0:24 (MN)	0:2 (IA), 1:23 (MN)	1.0 (IA), 1.0 (MN)
Fam. 331 ^d	F_2 (53-246 $\times$ 53-242)	17.5:52.5 (IA), 12.5:36.5 (MN)	10:60 (IA), <u>35:14 (MN)</u>	0.184 (IA), <u>0.00 (MN)</u>
Fam. 342 ^e	Backcross (53-246 $\times$ ILL-129)	15.5:15.5 (MN)	<u>25:6 (MN)</u>	<u>0.025 (MN)</u>
Fam. 354 ^f	Backcross (93-968 $\times$ 53-242)	0:20 (MN)	5:15 (MN)	0.056 (MN)

^a Significant deviations from expected ratios are underlined.

^b With Yates correction.

^c Resistant recessive homozygotes that are canker-free (R phenotype) like *P. deltoides* are not expected in the heterozygous F_1 generation.

^d A 1:3 R:S ratio was expected for the F_2 .

^e A 1:1 R:S ratio was expected for this TD $\times$ D backcross family. This family was not deployed in Iowa.

^f Resistant individuals were not expected for this T $\times$ TD backcross family that was also deployed only in Minnesota.

Relationship between leaf spot and canker severity in Minnesota after the third year of growth. Given the above-mentioned results of less-than-expected canker incidence and severity after 3 years, it is perhaps not surprising that leaf spot and canker severity were poorly correlated (Fig. 2; Spearman $r = 0.22$). In any case, abundant inoculum from leaf spots explains the absence of any further disease escape (i.e., by year 5).

Canker incidence in Minnesota after the fifth year of growth. These data are complicated by relatively poor tree growth and survival from the third to the fifth year. Of 24, 49, 20, and 31 clones in the F_1 , F_2 , $T \times TD$ backcross, and $TD \times D$ backcross generations, respectively, 23, 24, 14, and 25 were still alive at the end of the fifth year. Family differences in canker severity are shown in Table 3. Family was still a significant factor in canker severity after 5 years of growth in Minnesota (F ratio = 3.60, $P = 0.01$), as it was after 3 years, and in spite of complicating factors (e.g., winter injury) affecting survival. Although the F_2 generation was still more resistant than the F_1 (mean canker severities of 2.18 and 2.69, respectively), this difference was no longer significant (mean difference of 0.51; Bonferroni Adjusted $P = 0.135$). Still, resistant F_2 clones were evident; of 24 surviving F_2 clones, two clones (i.e., clones 331-1060 and 331-1127) were still canker-free (scoring '0'). Another nine F_2 clones had branch cankers only (scoring '1'), with no stem cankers, dieback, or breakage. In contrast, none of the 23 F_1 clones were canker-free, and only 1 F_1 clone (i.e., clone 53-747), represented by only one surviving ramet, showed branch cankers only. Other than the two F_2 clones, the only other canker-free clones were two $TD \times D$ individuals (342-855 and 342-904).

DISCUSSION

P. trichocarpa and its $T \times D$ F_1 progeny have invariably been susceptible to stem canker caused by *S. musiva* when planted in eastern North America. In contrast, *P. deltoides* is invariably resistant. These results were confirmed in this study. New to this study was the test and confirmation of the hypothesis that the resistance of *P. deltoides* is inherited recessively in its hybrids with *P. trichocarpa*. We discovered that canker-susceptible F_1 parents do produce some resistant F_2 progeny. After 3 years of growth in Iowa, 10 of 70 F_2 progeny were resistant, suggesting that a single recessive gene might be responsible for resistance. This simple hypothesis was not validated in Minnesota, where winter injury

and other factors may have complicated this experiment with an inbred pedigree. Nevertheless, the fact remains that there were only four canker-free clones in Minnesota after 5 years of growth, and these were in the two progenies (i.e., the F_2 and the $TD \times D$ backcross) predicted to segregate for resistance. Of the F_2 clones that were resistant in Iowa, only one (331-1127) survived into the fifth year in Minnesota, but it is noteworthy that it remained canker-free there also. Consistent with the hypothesis of recessive inheritance of canker resistance was the complete susceptibility of the F_1 generation and the $T \times TD$ backcross after 5 years in Minnesota.

There are two meaningful time frames in which to test hypotheses on the inheritance of resistance to canker caused by *S. musiva*. In the first time frame, one should observe cankers on all clones that are expected to be susceptible. In Iowa, 3 years was sufficient time for this first time frame. There, not only were *P. trichocarpa* and the F_1 hybrids cankered after 3 years, but the F_2 generation expectation of a 3:1 (susceptible:resistant) segregation was also confirmed. In Minnesota, 3 years was inadequate.

The second time frame might be considered to be from midrotation (3 to 5 years) to the end of the rotation (8 to 10 years in the Midwest). The resistance phenotype of *P. deltoides* is typically maintained in this second time frame to the end of the rotation. This phenotype is characterized by the complete absence of cankers. A second category of resistance phenotype is known, however. Among clones recommended for the Midwest are some that become cankered, but the cankers typically heal. This study did not clarify whether the resistance phenotype of the $TD \times TD$ or the $TD \times D$ clones is equivalent to *P. deltoides* in this second time frame. It is possible that additional genes in these hybrid classes modify the putative recessive gene for resistance, and that their resistance phenotype may involve development of branch cankers only or stem cankers that callus-over.

Although resistance from *P. deltoides* appears to be recessive, we have not yet concluded that it is conferred by a single gene. Evidence in support of the single-gene hypothesis was obtained in Iowa, but not in Minnesota owing to disease escape (after 3 years) and complicating factors affecting survival (after 5 years). Given the possibilities of disease escape and pathogenic variation (3) in the field, further testing of the hypothesis of a single recessive gene in this $T \times D$ pedigree should be conducted in the greenhouse with single-spore isolates.

Winter hardiness and slow growth rate may have been factors contributing to mortality of the field-deployed pedigree in Minnesota. The F_2 generation of this pedigree is inbred, and many genotypes grow slowly (1,10). The winter hardiness of coastal *P. trichocarpa* is marginal in Minnesota. Thus, we have initiated new field studies of the inheritance of canker resistance in hybrid poplar involving outbred progeny in which winter hardiness is expected to be adequate.

Recessive resistance to canker caused by *S. musiva* can now be contrasted to the genetic control of resistance to leaf spots caused by *S. populicola*. In a study conducted in the field in western Washington with this same $T \times D$ pedigree, the 3 quantitative trait

Fig. 2. Poor correlation between stem canker and leaf spot caused by *Septoria musiva* at Rosemount, MN, in the third year of growth. Note confidence ellipse ($P = 0.68$), corresponding to Spearman $r = 0.22$, for 124 clones in the field-deployed, hybrid poplar pedigree. The distribution of each trait (i.e., canker and leaf spot) is also shown. For leaf spot from left to right: bar 1 = slight, with few infected leaves; 2 = moderate, with infection throughout crown; and 3 = moderate, with premature defoliation in the lower crown. For canker from left to right: 1 = branch canker or cankers only; 2 = stem canker or cankers; and 3 = stem dieback and breakage associated with cankers.

TABLE 3. Matrix of pairwise mean differences (above diagonal) among pedigree progenies for resistance to *Septoria* canker in Minnesota after 5 years of growth, with Bonferroni Adjustment probabilities below the diagonal^a

Family	Family			
	F_1	F_2	$TD \times D$	$T \times TD$
F_1	...	0.50	0.28	0.36
F_2	0.14	...	0.22	<u>0.86</u>
$TD \times D$	1.00	1.000	...	0.64
$T \times TD$	1.00	<u>0.02</u>	0.17	...

^a *Populus trichocarpa* $\times$ *P. deltoides* = $T \times D$. Underlined differences and probabilities are significant.

loci for resistance to *S. populicola* were all dominant and inherited from *P. deltoides* (4). Resistance to canker (*S. musiva*) in Iowa was not significantly correlated ($r = 0.02$) with resistance to leaf spot (*S. populicola*) in Washington. Thus, there are evidently distinct genes for resistance to these *Septoria* congeners (teleomorph *Mycosphaerella*) within the same host lineage.

Studies of natural hybrid zones have indicated that recessive resistance may be more common in undomesticated host-parasite systems than would be anticipated from the study of agricultural pathosystems (2). This study represents a confirmation of that expectation.

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LITERATURE CITED

1. Bradshaw, H. D., Jr. 1998. Case history in genetics of long-lived plants: Molecular approaches to domestication of a fast-growing forest tree: *Populus*. Pages 219-228 in: Molecular Dissection of Complex Traits.

- Chapter 16. A. H. Paterson, ed. CRC Press, Boca Raton, FL.
2. Fritz, R. S., Moulia, C., and Newcombe, G. 1999. Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. *Annu. Rev. Ecol. System.* 30:565-591.
3. Krupinsky, J. M. 1989. Variability in *Septoria musiva* in aggressiveness. *Phytopathology* 79:413-416.
4. Newcombe, G., and Bradshaw, H. D., Jr. 1996. Quantitative trait loci conferring resistance in hybrid poplar to *Septoria populicola*, the cause of leaf spot. *Can. J. For. Res.* 26:1943-1950.
5. Newcombe, G., Ostry, M., Hubbes, M., Mottet, M.-J., and Perinet, P. 2001. Poplar Diseases. Pages 249-276 in: *Poplar Culture in North America*. D. Dickmann and J. Isebrands, eds. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.
6. Ostry, M. E. 1987. Biology of *Septoria musiva* and *Marssonina brunnea* in hybrid *Populus* plantations and control of *Septoria* canker in nurseries. *Eur. J. For. Pathol.* 17:158-165.
7. Ostry, M. E., and McNabb, H. S., Jr. 1985. Susceptibility of *Populus* species and hybrids to disease in the north central United States. *Plant Dis.* 69:755-757.
8. Stettler, R. F., and Bradshaw, H. D., Jr. 1996. Pages 1-6 in: *Evolution, Genetics, and Genetic Manipulation. Part I, Overview*. R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley, eds. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.
9. Strobl, S., and Fraser, K. 1989. Incidence of *Septoria* canker of hybrid poplars in eastern Ontario. *Can. Plant Dis. Surv.* 69:109-112.
10. Wu, R., and Stettler, R. F. 1997. Quantitative genetics of growth and development in *Populus*. II. The partitioning of genotype $\times$ environment interaction in stem growth. *Heredity* 78:124-134.
11. Zalasky, H. 1978. Stem and leaf spot infections caused by *Septoria musiva* and *S. populicola* on poplar seedlings. *Phytoprotection* 59:43-50.