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Field performance of *Populus* expressing somaclonal variation in resistance to *Septoria musiva*

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Abstract

Over 1500 trees from two hybrid poplar clones regenerated from tissue culture and expressing somatic variation in leaf disease resistance in a laboratory leaf disk bioassay were field-tested for 5–11 years to examine their resistance to *Septoria* leaf spot and canker and to assess their growth characteristics compared with the source clones. Somaclones expressed a wide range of disease reactions and growth rates. Generally, the leaf disk bioassay did not predict the resistance of the trees to stem cankers in the field; however, several selected somaclones outperformed the source clones. Results from this study indicate that somaclonal selection for desirable tree traits, such as disease resistance in *Populus*, may be feasible if a highly effective selective bioassay can be employed and if the trait is stable in the field. Cell and tissue culture conditions themselves apparently increased the growth rate of some of the regenerated trees compared with the source clones. At this time the cause of the random variation in disease resistance and tree growth is unknown and obtaining disease resistance in *Populus* using somaclonal selection is difficult and unpredictable.
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Keywords: Tissue culture; Intraclonal variation; Disease resistance; Tree improvement

1. Introduction

Species and hybrids of *Populus* are of worldwide importance for the production of fiber and energy. Hybrid poplars are increasingly being grown as a row crop in several regions of the United States as a source of biomass for energy; pulp for paper production; and for the manufacture of various solid wood products. Unfortunately, the foliage and stem diseases caused by *Septoria musiva* (teleomorph: *Mycosphaerella populorum*) reduce biomass yields [1] and can kill highly susceptible poplar clones in the eastern United States [2].

Members of the genus *Populus* are amenable to all systems of in vitro regeneration and they have been widely utilized in model systems with various cell and tissue culture-based biotechnologies for tree improvement [3]. Manipulating plant cells in a culture cycle often results in spontaneous phenotypic and genetic variation in regenerated plants, referred to as somaclonal variation [4].

Although somaclonal variation for increased disease resistance has been recovered in many agronomic plants, only a few examples exist for woody plants. Lester and Berbee [5] observed changes in height, leaf morphology, and branching habit among hybrid poplars derived from callus cultures. Since then, there have been an increasing number of reports of somaclonal variation in numerous traits among poplars regenerated from callus and protoplast cultures [6]. Explant source and culture method can influence how frequently somaclonal plants are recovered [7,8].

In *Populus*, somaclonal variation in disease resistance among regenerated poplar plants has been reported for *Melampsora* leaf rust [9,10], *Hypoxylon mammatum* toxins [10] and *Septoria* leaf spot [11]. Other reported examples of somaclonal variation in various traits in *Populus* have been reviewed [12].

Evidence for the genetic basis of observed somaclonal variation in *Populus* is lacking and definitive tests to prove that changes are heritable in poplars are difficult owing to their long generation time. Also lacking is the

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evaluation of the stability of the reported changes. There have been no long-term field trials of poplar somaclones established to examine not only the stability of the trait of interest, but to determine if other cryptic changes may have taken place that could affect clonal performance. To be useful for tree improvement, somaclonal variants expressing valuable traits must be field-tested under local conditions to monitor their growth and resistance to disease and insect pests.

The objective of this study was to determine if the somaclonal variation in disease resistance expressed in the laboratory in trees regenerated from two hybrid poplar clones was also expressed in the trees planted in four field trials. The hypothesis tested was that somaclones exhibiting increased resistance to *Septoria* leaf disease in the laboratory bioassay would also be more resistant, relative to the source clones, to stem cankers caused by *S. musiva*.

2. Materials and methods

2.1. Source clones

Populus nigra var *betulifolia* × *P. trichocarpa* (NE 299) and *P. charkowiensis* × *P. incrassata* (NE 308) were propagated from hardwood cuttings obtained from a single stool in a nursery bed and grown in the greenhouse. Clone NE 299 has superior growth characteristics and clone NE 308 has good early growth and is tolerant to drought, but both are susceptible to *Septoria* leaf spot and canker [2], often with stem breakage resulting from stem infections within 4 years after planting. The stock plant of each clone was hedged in the greenhouse every 4–6 weeks to stimulate lateral branching and served as the source of explants for callus cultures and cuttings for propagation of the control plants.

2.2. Production, recovery, and multiplication of somaclones

Plants of NE 299 for field planting were recovered using various regeneration methods and tissue sources [7]. Callus [11], protoplast [13], micro cross-section [14], and axillary bud cultures [7] were used to produce somaclones.

Callus cultures of NE 299 were initiated from in vitro cultured stem internode explants. Explants were surface-sterilized in 1.5% NaOCl and 0.33^{-3} μM Tween 20, grown at 25 °C in the dark on woody plant medium (WPM) [15] plus 2% (w/v) sucrose, 0.6% Difco Bacto agar and 2.3 μM 2,4-dichlorophenoxyacetic acid (2,4-D). Proliferating callus was cultured on WPM containing 0.45 μM 2,4-D and subcultured every 3–4 weeks onto fresh medium. Adventitious bud formation and shoot proliferation were induced on WPM with 1.1 μM 1-naphthalenacetic acid (NAA), and cultures were maintained at 25 °C with a 16 h photoperiod (57 μmol m^{-2} s^{-1}). Elongated shoots were excised, rooted in a growth chamber at 20–25 °C under constant light (37 μmol m^{-2} s^{-1}). Rooted plants were maintained in the greenhouse (18–30 °C, 18 h photoperiod).

Callus cultures of NE 308 were initiated from stem internode sections excised from the source clone. The sections were surface-sterilized in a beaker containing 1.5% NaOCl with 0.33^{-3} μM Tween 20. After 20–25 min, the beaker was decanted and the segments were rinsed three times with sterile water. The sections were cut into 1 cm segments and placed horizontally in shell vials containing 10 ml of WPM with 0.7% Difco Bacto agar and 0.45 μM 2,4-D. Cultures were incubated in the dark at 25 °C.

The callus was subcultured every 3–4 weeks onto fresh medium and returned to the incubator. After two subcultures, the callus was transferred to WPM with 0.7% agar, 0.89 μM 6-benzylaminopurine (BA) and 0.05 μM NAA to induce shoot production. Cultures were maintained at 25 °C and with a 16 h photoperiod (60 μmol m^{-2} s^{-1}). After 3–4 weeks, shoot elongation and proliferation were induced by transferring the cultures onto WPM with 0.7% agar and 0.44 μM BA; or Murashige and Skoog medium (MS) [16] with 0.6% agar and 0.44 μM BA. Some cultures were incubated for 7 days in the dark and then 14 days in the light to induce rooting.

Elongated shoots were excised and rooted in sand at 100% humidity in covered plastic trays under continuous light (15 μmol m^{-2} s^{-1}) at 20–25 °C. The plants were gradually acclimated to lower humidity and were transferred to pots containing 2:1:1 peat:perlite:sand on a greenhouse misting bench after 4 weeks. When sufficient growth was attained, the rooted shoots were transplanted to larger pots and placed on an automatic watering system. Plants were fertilized bi-weekly with a 20–20–20 fertilizer.

Micro cross-sections were prepared from midveins of fully expanded leaves and were cultured on 10 ml of WPM supplemented with 2% sucrose, 0.89 μM BA, 0.05 μM NAA and solidified with 0.7% agar. Cultures were maintained under 16 h of cool white fluorescent light (60 μmol m^{-2} s^{-1}) at 25 °C. Shoots were acclimated in a growth room and transferred to the greenhouse.

Leaf protoplasts were isolated from sterile shoot cultures and cultured using the techniques of Russell and McCown [13]. After adventitious shoot formation on solidified MS medium, shoots were excised and placed into Techniculture™ peat plugs enclosed in a plastic rooting chamber. Rooted shoots were acclimated in a growth room and transferred to the greenhouse.

To determine if the tissue culture cycle induced somaclonal variation in poplars, axillary buds were excised, surface-sterilized and placed onto 10 ml of

WPM with 0.89 μM BA and 0.05 μM NAA in shell vials. Shoots were induced and rooted under the same conditions as used for adventitious shoot proliferation from callus cultures.

2.3. Laboratory screening for somatic variation

A total of 3801 trees (3128 NE 299 and 673 NE 308) were screened for resistance to *Septoria* leaf spot using a laboratory leaf disk bioassay [17]. Excised leaf disks from plants propagated by softwood cuttings of the source clone and plants regenerated in vitro from various tissue sources were inoculated with 0.1 ml of a spore suspension (1×10^6 conidia per ml of water) of *S. musiva*. Disease progression was recorded by measuring the necrotic area on each disk every 2 days using a dot grid (25 dots per 1.8 cm^2). A regression analysis of the pooled leaf disk measurements for all regenerates from a single explant source was performed using percent green leaf area and elapsed time as the dependent and independent variables, respectively. Plants were rated as resistant if the bioassay indicated $\geq 50\%$ green leaf area at 32 days. The bioassay was generally repeated three times for each somaclone rated as resistant. Most somaclones rated as susceptible were discarded after one bioassay.

2.4. Field trials

2.4.1. Rhinelander 1986 NE 299 planting

The original rooted somaclones from NE 299 expressing increased resistance to *Septoria* leaf spot in the laboratory bioassay and clonally multiplied plants of the source clone using hardwood cuttings were established in single-tree plots in a field near Rhinelander, WI in 1986. The planting included 243 NE 299 somaclones and 70 NE 299 source plants. Tree spacing was 1.2×1.2 m, weeds were controlled chemically and water was applied as necessary during the first 2 years.

2.4.2. Rosemount 1991 NE 299 planting

To determine stability of the resistance trait after clonal multiplication, selected NE 299 somaclones and source plants were clonally propagated using softwood cuttings and established in a replicated planting at the University of Minnesota's Agricultural Experiment Station near Rosemount, MN in 1991. Included in the planting were 20 newly generated somaclones from callus (10), protoplast (3), and micro cross-section (7); plants from axillary bud cultures and rooted hardwood cuttings of the source clone; and 19 somaclones selected for resistance or tolerance to *Septoria* canker from the Rhinelander planting. In most cases, 12 trees of each somaclone and the source clone were planted in four-tree plots in a randomized complete block design. Trees were planted at 3.6×3.6 m spacing, weeds were controlled mechanically, and water was applied as necessary during the first 2 years.

2.4.3. Rosemount 1996 NE 299 planting

A second replicated NE 299 planting of unrooted hardwood cuttings of the source plant and 23 somaclones that had expressed resistance or tolerance to *Septoria* canker in the Rhinelander planting was established at the Rosemount Experiment Station in 1996. The objective was to test the plants for their performance at close spacing in a nursery stool bed. Ten of the somaclones were propagated using cuttings collected from trees that had been established in the 1991 Rosemount planting and 13 additional somaclones based on their ability to callus-over cankers were propagated from the Rhinelander planting. Thirty trees of each somaclone, trees from axillary bud culture and the source clone were planted in ten-tree plots in a randomized complete block design. Trees were planted 0.3 m apart within rows with 3.6 m spacing between rows. Weeds were controlled mechanically and water was applied as necessary during the first 2 years.

2.4.4. Rosemount 1995 NE 308 planting

Thirty somaclones of NE 308 expressing more resistance to *Septoria* leaf spot than the source clone in the laboratory bioassay were clonally multiplied using softwood cuttings and established in a replicated planting at the Rosemount Experiment Station in 1995. Also included were plants from axillary bud cultures and plants clonally propagated from the source clone using hardwood cuttings. Twelve trees of each line were planted in four-tree plots in a randomized complete block design. Trees were planted at 3.6×3.6 m spacing, weeds were controlled mechanically and water was applied as necessary during the first 2 years.

2.5. Tree growth and disease assessments

Plants were assessed annually for survival, growth, tree form, incidence and severity of foliar and stem damage from insect pests and disease, and production of callus at canker margins that stopped canker expansion. Tree heights were measured using a height pole and diameter was measured at breast height (DBH). Foliar disease was rated as 0, none; 1, few infected leaves; 2, infection throughout crown; 3, premature defoliation in lower crown; and 4, defoliation throughout crown. Stem disease was rated as 0, none; 1, branch canker(s) only; 2, stem canker(s); and 3, stem dieback and breakage associated with cankers.

2.5.1. Data analyses

Growth and disease data were analyzed with ANOVA and Fisher's Least Significant Difference test using SYSTAT (SPSS Science). Survival data were analyzed

with the Likelihood-Ratio Test using STATXACT-3 (Cytel Software Corp.). NE 299 somaclone lines and the source plant common to two or three plantings were ranked by the formula:

$$R = \frac{\sum(c \times d)}{n}$$

where R = mean rank, c = rank in stem canker number from lesser to greater, and d = rank in diameter from large to small, and n = number of plantings.

3. Results

3.1. Rhinelander 1986 NE 299 Planting

The single-tree plots of somaclones were treated in the analysis as a group and compared with the source group. *Septoria* leaf spot and canker were first detected in 1987 and the mean foliage and stem disease ratings of somaclone and source clone groups were similar throughout the study period. There were no significant differences in tree diameter or tree survival between the source clone group and the somaclone group.

Severity of leaf disease in the leaf disk bioassay was not correlated to either tree growth or canker severity. By 1996, 23 somaclones and seven source clones were free of stem cankers; however, 76% of the infected somaclones with stem cankers had completely closed or walled-off the cankers with callus compared with only 38% of the infected source trees. Mean number of cankers per tree was similar for somaclones (2.9) and the source clone (3.1) and mortality caused by cankers was similar; however, canker incidence was more variable among somaclones than among source plants (Table 1a).

3.2. Rosemount 1991 NE 299 planting

The incidence of winter dieback caused by extreme cold weather in 1993 was high throughout the planting. In addition, tree mortality caused by mice girdling stems was periodically high and by 1998 overall tree survival was less than 10% precluding statistical testing. Although some somaclones had from 2 to 4 times greater mean growth than the source clone, growth of the somaclones varied much more than the source clone (Table 1b). The somaclones had fewer stem cankers than the source (Table 1b) and the majority of cankers on somaclones were walled-off with callus whereas few source trees produced callus around cankers. Mortality resulting from stem breakage at *Septoria* cankers was 21% for somaclones and 27% for the source clones.

Prior to the weather- and rodent-related tree mortality, six of the 19 selected somaclones propagated from the 1986 Rhinelander planting outgrew the source clone and had fewer *Septoria* cankers. Two somaclones regenerated from callus culture consistently out-performed other somaclones and the source clone in growth. By 1996, they had twice the growth of the source clone and had no cankers, whereas the source clone had stem cankers and canker-related dieback. Five other somaclones also had greater mean growth compared with the source clone and they had no cankers in 1996. Somaclones with the greatest survival, growth and field disease resistance compared with the source clone were produced from callus (40% greater growth) and micro cross-section culture (10% greater growth).

3.3. Rosemount 1996 NE 299 planting

The high incidence of weather-related mortality of the source clone prevented valid statistical comparisons of continuous variables between source and somaclones at the end of the experiment (Table 1c). Fourteen of the somaclone lines had greater survival ($P \leq 0.05$) and nine had similar survival to the source clone. It is not known why the weather-related mortality was so much greater in the source clone than in the somaclone lines.

The tallest eight somaclones were similar in mean height as were the shortest five clones. Somaclone 4–19 had significantly greater mean height ($P \leq 0.05$) than 12 of the somaclones, and somaclones 3–4 and 1–56 were significantly taller than seven somaclones (Table 2).

There was less separation among the somaclones in diameter growth than height growth with somaclone 1–43 being significantly larger than five somaclones (Table 3). Canker incidence among the somaclones was similar except for somaclone 85–507 that had significantly ($P \leq 0.01$) more cankers and also exhibited a mutant peeling bark morphology. Based on a combination of the best survival, disease resistance, and height and diameter growth, somaclones 4–19, 3–4, 1–56, 1–43, and 85–79 warrant further testing.

3.4. Rosemount 1995 NE 308 planting

Somaclone lines with fewer than four surviving trees were included in the analysis of somaclones as a group, but not in the analysis of individual somaclone lines. Survival of source and axillary plants was similar to each other and greater than the somaclone lines (Table 1d). There were no significant ($P = 0.10$) differences in the number of stem cankers between somaclone lines, source plants or plants regenerated from axillary shoot cultures. Results from the leaf disk bioassay did not correlate well with canker incidence, and fewer somaclones exhibited greater disease resistance than the source clone compared with somaclones of NE 299.

Five somaclone lines, the source clone and trees regenerated from axillary shoot cultures had similar

Table 1
Summary of mean growth and canker incidence of 5- to 11-year-old NE 299 and NE 308 somaclones, and axillary and source plants

	Survival		Stem cankers/tree				DBH (cm)			
	n	(%)	Mean	Median	Variance	Range	Mean	Median	Variance	Range
(a) NE 299 somaclones and source plants in the 1986 Rhinelander, WI planting after 11 years growth										
Somaclones	243	42	2.9	2.0	8.03	0–11	8.4	8.7	5.84	2.5–14.2
Source plants	70	49	3.1	3.5	5.10	0–6	8.2	7.4	8.49	3.9–14.5
	Survival		Stem cankers/tree				DBH^2HT (1000 cm^3)			
	N	(%)	Mean	Median	Variance	Range	Mean	Median	Variance	Range
(b) NE 299 somaclones and source plants in the 1991 Rosemount, MN planting after 6 years growth										
Somaclones	388	8	0.7	0.0	1.72	0–5	9.5	7.7	4713.0	0.9–32.6
Source plants	12	17	2.5	2.5	12.50	0–5	5.6	5.6	5906.7	3.9–7.4
	Survival		Stem cankers/tree				DBH^2HT (1000 cm^3)			
	n	(%)	Mean	Median	Variance	Range	Mean	Median	Variance	Range
(c) NE 299 somaclones and source plants in the 1996 Rosemount, MN planting after 5 years growth										
Somaclones	675	35	0.5	0.0	1.74	0–13	10.9	10.6	34090.9	0.1–27.7
Source plants	30	7	2.5	2.5	0.50	1–5	5.0	5.0	1957.7	4.0–6.0
	Survival		Stem cankers/tree				DBH (cm)			
	n	(%)	Mean	Median	Variance	Range	Mean	Median	Variance	Range
(d) NE 308 somaclones and axillary and source plants in the 1995, Rosemount, MN planting after 6 years growth										
Somaclones	224	83	5.9	5.5	11.83	0–21	9.3	9.5	15.91	1.9–19.7
Axillary plants	12	92	6.5	7.0	14.07	2–13	12.4	12.7	12.05	4.4–17.8
Source plants	12	100	6.2	5.5	9.06	2–13	11.4	11.8	4.40	8.3–14.6

Table 2
Mean heights of somaclone lines with $n > 5$ surviving trees in the NE 299 1996 planting

Clone	Height (m)						
4–19	5.8	a[1]					
3–4	5.5	a	b				
1–56	5.4	a	b				
3–32	5.3	a	b	c			
1–43	5.2	a	b	c			
3–25	5.2	a	b	c	d		
85–79	5.2	a	b	c	d		
3–53	5.0	a	b	c	d	e	
1–48	5.2		b	c	d	e	
1–33	5.1		b	c	d	e	
85–65	5.1		b	c	d	e	
2–71	5.0		b	c	d	e	
1–15	5.0		b	c	d	e	
85–63	4.8			c	d	e	
85–573	4.8			c	d	e	
1–4	4.6			c	d	e	f
85–497	4.5				d	e	f
86–24	4.4				d	e	f
85–507	4.2					e	f
1–0	4.0						f

[1], Values followed by the same letter in a column are not significantly different at $P \leq 0.05$.

Table 3
Mean diameters of somaclone lines with $n > 5$ surviving trees in the NE 299 1996 planting

Clone	DBH (cm)			
1–43	5.0	a[1]		
1–56	4.8	a	b	
3–53	5.1	a	b	c
85–65	4.8	a	b	c
3–4	4.6	a	b	c
3–25	4.6	a	b	c
1–15	4.6	a	b	c
85–497	4.6	a	b	c
85–79	4.6	a	b	c
1–33	4.6	a	b	c
4–19	4.5	a	b	c
1–4	4.4	a	b	c
85–573	4.1	a	b	c
2–71	4.1	a	b	c
86–24	4.1	a	b	c
3–32	4.1		b	c
1–48	4.1		b	c
85–63	4.1		b	c
85–507	3.9			c
1–0	3.4			c

[1], Values followed by the same letter in a column are not significantly different at $P \leq 0.05$.

Table 4
Mean diameters of somaclone lines and axillary and source plants with $n > 3$ surviving trees in the NE 308 1995 planting

Clone	DBH (cm)									
92–48	13.6	a								
92–115	13.6	a								
92–310	13.3	a	b							
92–22	13.1	a	b	c						
Axillary	12.3	a	b	c						
92–45	11.8	a	b	c	d					
Source	11.4	a	b	c	d					
92–183	10.7		b	c	d	e				
92–253	10.3			c	d	e				
92–191	10.0			c	d	e	f			
92–261	10.0			c	d	e	f			
92–420	8.9				d	e	f	g		
92–421	8.8				d	e	f	g		
92–194	8.5					e	f	g		
92–30	7.7						f	g	h	
92–337	7.3						f	g	h	
92–443	7.2						f	g	h	i
92–358	7.0						f	g	h	i
92–439	7.0							g	h	i
92–410	6.8							g	h	i
92–274	6.7							g	h	i
92–84	4.6								h	i
92–282	3.9									i

[1], Values followed by the same letter in a column are not significantly different at $P \leq 0.05$.

mean stem diameters that were significantly ($P \leq 0.05$) greater than 16 of the other somaclone lines (Table 4). Trees of two somaclone lines developed a mutant, curled branching habit in the field that was not evident on the plants when they were propagated in the greenhouse.

3.5. NE 299 somaclones and source plants common to two or three plantings

Plants from four NE 299 somaclone lines, and 38 source plants were common to the Rhinelander 1986, and Rosemount 1991 and 1996 plantings and plants from 22 somaclone lines were common to two of the three plantings (Table 5). The ranks in growth and *Septoria* canker resistance for several somaclones were consistently high across the plantings and 25 of 26 somaclone lines ranked higher than the source plant (Fig. 1). The somaclone lines that consistently ranked high in canker resistance and growth will be propagated and studied further in future field tests.

4. Discussion

The somaclone lines that were recovered from these two *Populus* clones expressed a wide range of reactions to *S. musiva* in the laboratory bioassay and to natural inoculum of *S. musiva* in the field. Several of the somaclones exhibited increased growth and disease resistance compared with the source clones (Table 5, Fig. 1) and warrant further testing. Increased growth rates, even in the absence of greater disease resistance, may shorten the time required for trees to reach merchantable size, reducing their risk of damage from stem breakage at cankers.

Somaclonal variation can be pre-existing in the tissues cultured or induced by the tissue culture regime, including potentially mutagenic formulations of the media used. Results of studies of the clones reported here suggest that genotype [11] and explant source and culture method [7] all may have influenced how frequently variant plants were recovered.

In this study, clone NE 308 was more recalcitrant in tissue culture, requiring modifications in growth media, but apparently disease resistance-susceptibility was more stable than NE 299 since fewer somaclones expressing variation in response to *S. musiva* were recovered. Except for the two NE 308 somaclones with mutant branching habit, no other mutant plants were recovered, unlike the many mutant phenotypes previously recovered with NE 299 [7]. However, with the exception of the above somaclones and the NE 299 somaclone with the peeling bark, other mutant plants of both clones died in the greenhouse.

Tolerance to stem infections expressed as callus production that walled-off cankers was evident among many of the somaclones of NE 299, but was less common in NE 308 somaclones and was absent in the source trees. Wood-rotting fungi often invade open *Septoria* cankers weakening the affected stems and predisposing them to breakage. Trees that are able to effectively wall-off cankers would be valuable in producing biomass for energy compared with trees that experience stem breakage at cankers before they reach harvest age.

Results from this work confirm that the increased disease resistance we detected among some of the regenerated trees of clone NE 299 in a laboratory bioassay was relatively stable through vegetative propagation, based on repeated bioassays, and was maintained in these trees for up to 11 years in field plots (Table 5, Fig. 1).

No significant differences in resistance or susceptibility to the various other leaf diseases and insect pests within the plantings were detected between somaclones and the source trees of either clone. The incidence of leaf disease caused by *Marssonina*, *Melampsora* and *Septoria* varied widely in different portions of the tree crowns, and severity varied by year and planting location. During the course of this study, trees were exposed to several years of severe winter weather with little snow cover and unusually cold temperatures that resulted in a high incidence of stem dieback and tree mortality that affected the test results.

Table 5
Mean diameter and canker incidence of 26 surviving 5- to 11-year-old NE 299 somaclone lines, and source plants common to two or three plantings in Minnesota and Wisconsin

Clone	Minnesota				Wisconsin	
	5 years		6 years		11 years	
	Cankers	DBH (cm)	Cankers	DBH (cm)	Cankers	DBH (cm)
85–497	1	4.6	0	5.9	0	14.2
1–0	0	3.4	0	4.6	1	10.6
85–62			0	4.7	1	10.0
1–1			0	4.4	0	9.7
85–63	0	4.0	0	4.4	2	9.5
1–56	0	4.8	0	7.0	0	9.4
1–7			0	1.9	2	8.8
86–24	1	4.1			6	13.1
1–4	0	4.4			3	11.5
2–64	0	4.8			1	11.5
3–32	0	4.1			3	11.4
4–19	0	4.5			0	11.4
3–25	1	4.7			0	11.2
85–65	0	4.8			2	11.0
3–54	1	5.1			4	10.6
85–79	0	4.6			2	10.6
1–43	0	5.0			0	10.3
85–89	3	3.6			2	10.2
3–4	1	4.7			1	10.1
85–507	4	3.9			2	10.1
1–15	0	4.6			5	10.0
2–71	0	4.1			2	10.0
1–48	0	4.1			6	9.5
85–573	1	4.1			0	9.5
1–33	1	4.6			0	8.6
3–53	0	5.1			2	8.4
Source	3	3.5	3	3.8	3	8.2

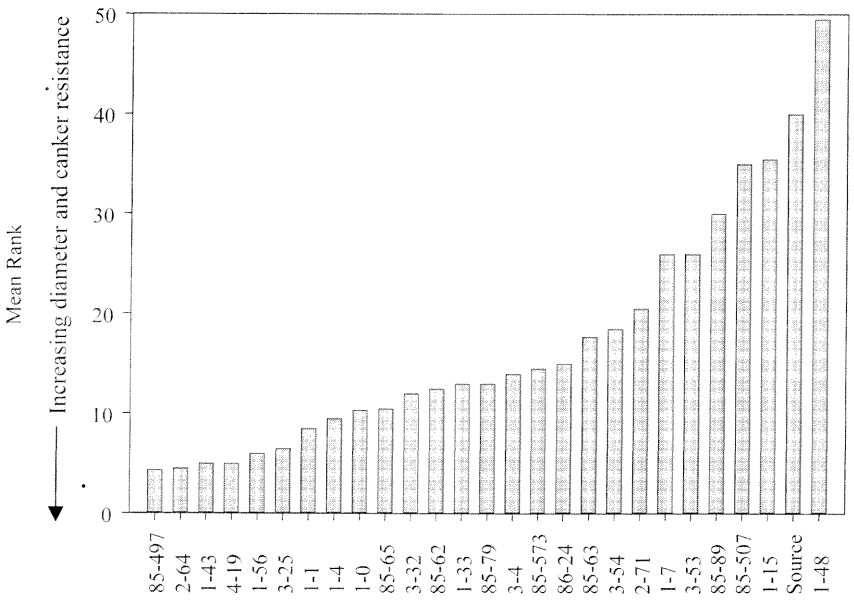

Fig. 1. Mean ranks of stem canker resistance and diameter growth of NE 299 somaclones and source plants common to two or three plantings in Minnesota and Wisconsin, $R = \Sigma(c \times d)/n$ where R = mean rank, c = rank in stem canker number from lesser to greater, d = rank in diameter from large to small, and n = number of plantings.

The leaf disk bioassay has been used successfully to screen poplars for their relative resistance to *Septoria* leaf spot [17]; however, it was not entirely predictive in regards to the level of field resistance to *Septoria* canker among individual somaclones. Some explanations can be offered for this. The leaf disk bioassay can effectively separate highly resistant from highly susceptible clones, but it does not clearly separate among moderately resistant clones. Somaclones from a single source clone are genetically similar to one another and perhaps the bioassay is not sufficiently sensitive. Preliminary Random Amplified Polymorphic DNA (RAPD) analyses using 20 primers (Operon Technologies) have revealed molecular genetic differences between the source clones, but not among the somaclones regenerated from individual source clones (Ostry and Ward unpublished data). Additionally, there is a poor correlation between susceptibility of individual clones to the two types of diseases [18]. Spores that develop on infected leaves are the inoculum source for stem cankers, and it may be that the leaf disease reduction on the somaclones based on the bioassay was not sufficient to reduce stem infections under the stressful weather conditions present during this field study. The incidence and severity of *Septoria* leaf disease is highly influenced by local weather conditions, and we have a poor understanding how various site conditions and stress factors affect the incidence of stem cankers.

We do not know the underlying cause of the somatic variation in disease resistance we recovered, or if this trait is heritable. However, a stable trait in poplars may have value even if it is not heritable since most poplars are easily propagated vegetatively. Our experience with these poplar clones underscores the difficulty of recovering a specific tree trait, such as disease resistance, using somaclonal selection. The somaclones expressing superior growth and resistance to canker in these field trials will undergo additional field tests to determine their suitability for larger-scale plantings.

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