Screening Hybrid Poplars In Vitro for Resistance to Leaf Spot Caused by *Septoria musiva*

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ABSTRACT

Ostry, M. E., McRoberts, R. E., Ward, K. T., and Resendez, R. 1988. Screening hybrid poplars in vitro for resistance to leaf spot caused by *Septoria musiva*. Plant Disease 72:497-499.

An in vitro bioassay was developed to screen hybrid poplars (*Populus* spp.) for resistance to leaf spot caused by *Septoria musiva*. Leaf disks of clones inoculated with conidia of *S. musiva* displayed disease resistance similar to that found in field trials. This method shortens the time needed for evaluating the relative disease resistance of poplar clones.

Septoria musiva Peck, which causes a leaf spot and stem canker disease of many *Populus* spp. and hybrids, is one of the most damaging pathogens of poplars in plantations (2). Chemical and cultural control of Septoria leaf spot and canker is difficult and only partially effective (1).

Populus clones differ in their susceptibility to *Septoria*. Some clones are so susceptible that they cannot be grown successfully in the north central United States (3). The best way to prevent this disease is to plant highly resistant clones. Planting clones with high resistance to Septoria leaf spot also can reduce the incidence of Septoria canker.

Field testing poplars for resistance to *Septoria* is time-consuming and expensive. In addition, results may vary because of inconsistent inoculum concentrations and widely fluctuating weather conditions critical for infection and disease development. Other biotic and abiotic diseases also may complicate field evaluations.

This study was undertaken to develop an in vitro bioassay using *Populus* leaf disks inoculated with conidia of *S. musiva* to compare clones with respect to differences in resistance to Septoria leaf spot. A set of *Populus* clones with known field reactions to *Septoria* was tested, and

Accepted for publication 21 January 1988 (submitted for electronic processing).

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results of the in vitro bioassay were compared with results obtained from field trials. This technique is similar to the one described by Spiers (4) to assess resistance of poplars to leaf spot caused by *Marssonina brunnea* (Ell. & Ev.) Magnus.

MATERIALS AND METHODS

Isolates of *S. musiva* were obtained from hybrid poplar leaves collected from a plantation near Rosemount, MN. Diseased leaf pieces approximately 5 mm^2 in size were surface-sterilized, placed onto a potato-dextrose agar medium, and incubated at 20 C under continuous light (3,000 lx). To induce sporulation, cultures were transferred to an agar medium containing 180 ml/L of V-8 juice, 2 g/L of $CaCO_3$, and 20 g/L of agar. Sporulating cultures were maintained at 20 C under continuous light (3,000 lx) and subcultured weekly onto the V-8 juice medium.

Stock plants of 10 *Populus* clones were grown in the greenhouse (18–30 C, 18-hr photoperiod). The relative resistance of these clones to *Septoria* in field trials in Minnesota, Wisconsin, and Iowa is presented in Table 1. Young, recently fully expanded leaves (third or fourth from apex) were collected from each clone for use in the bioassay. Leaves were rinsed thoroughly with distilled water and 18-mm-diameter disks were removed with a cork borer. Holes of the same diameter were made in 2% water agar in petri plates and the leaf disks were placed into them with the abaxial surface up.

Conidia were removed from 7- to 10-day-old *S. musiva* cultures of a single isolate by flooding the plates with distilled deionized water and gently agitating the plates. The conidial suspension was adjusted to concentrations ranging from 1×10^2 to 1×10^6 conidia/ml by serial dilutions and use of a hemacytometer. Each leaf disk was inoculated with 0.1 ml of the conidial suspension. Distilled deionized water was applied to leaf disks that served as controls. In each trial, from six to 12 leaf disks of each clone were inoculated, and two leaf disks served as uninoculated controls. Leaf disks were incubated in a continuously lighted growth room (20–25 C, 2,000 lx). Spore germination percentages were determined for each trial by inoculating two petri plates of 2% water agar with 0.1 ml of the conidial suspensions and incubating them for 24 hr at 20–25 C under continuous light (2,000 lx). Germinated spores were counted in a random sample of 100 conidia on each plate.

The area of necrosis on leaf disks was measured beginning the fourth day after inoculation using a dot grid (25 dots/1.8 cm^2). Measurements were made every 2 days and continued for 32 days or until the control leaf disks started to become necrotic. The number of bioassays run on each clone ranged from six to 94. Measurements from each trial were pooled for analysis.

Three regression analyses were performed with percent of green leaf area and elapsed time as dependent and independent variables, respectively. The first regression was linear with the intercept fixed at 100%; the second regression was linear with an estimated intercept; and the third regression was nonlinear using the logistic model $E(Y) = [1 + \exp(b_1 + b_2X)]^{-1}$ where Y = green leaf area and X = elapsed time.

Table 1. Resistance of *Populus* clones to *Septoria* in field trials and parameter estimates from bioassay analyses

Clone	Parentage	Disease resistance[a]	T.50[b]	R.50[c]
DN 34	*P.* × *euramericana* 'Eugenei'	H	133.4	.0036
NE 314	*P. nigra* var. *charkowiensis* × *P. nigra* var. *caudina*	H	33.0	.0180
NE 293	*P. nigra* var. *betulifolia* × *P. nigra* 'Volga'	H	21.7	.0230
NE 325	*P. balsamifera* var. *candicans* × (*P.* × *berolinensis*)	H	37.4	.0156
NE 41	*P. maximowiczii* × *P. trichocarpa* 'Androscoggin'	M	25.2	.0251
NE 252	*P. deltoides* var. *angulata* × *P. trichocarpa*	M	28.6	.0198
NE 386	*P. candicans* × (*P.* × *berolinensis*)	M	29.7	.0203
NE 319	*P. nigra* var. *charkowiensis* × *P. trichocarpa*	M	22.6	.0270
NE 1	*P. nigra* × *P. laurifolia* 'Strathglass'	L	19.2	.0260
NE 299	*P. nigra* var. *betulifolia* × *P. trichocarpa*	L	15.4	.0325

[a] H = Slight leaf spot, no defoliation; M = moderate leaf spot, premature defoliation in lower and midcrown; L = severe leaf spot, premature defoliation throughout crown.
[b] T.50 = Estimated no. of days to 50% necrosis.
[c] R.50 = Estimated rate of necrosis at T.50.

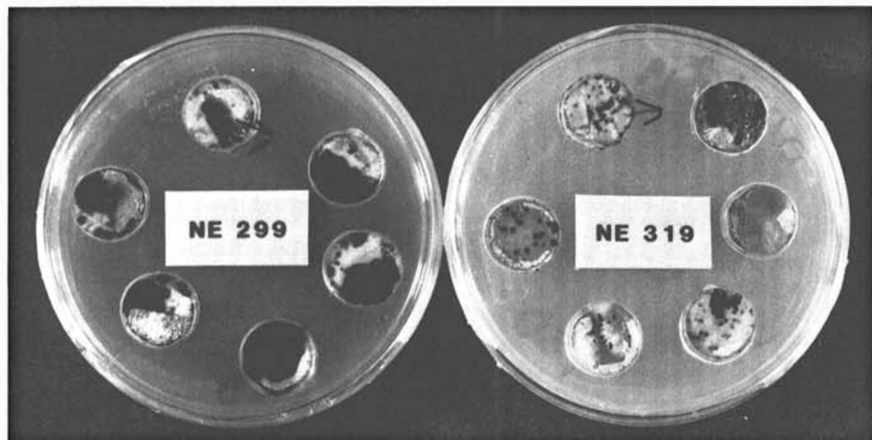

Fig. 1. Necrosis of leaf disks from moderately resistant (NE 319) and highly susceptible (NE 299) clones inoculated with conidia of *Septoria musiva*.

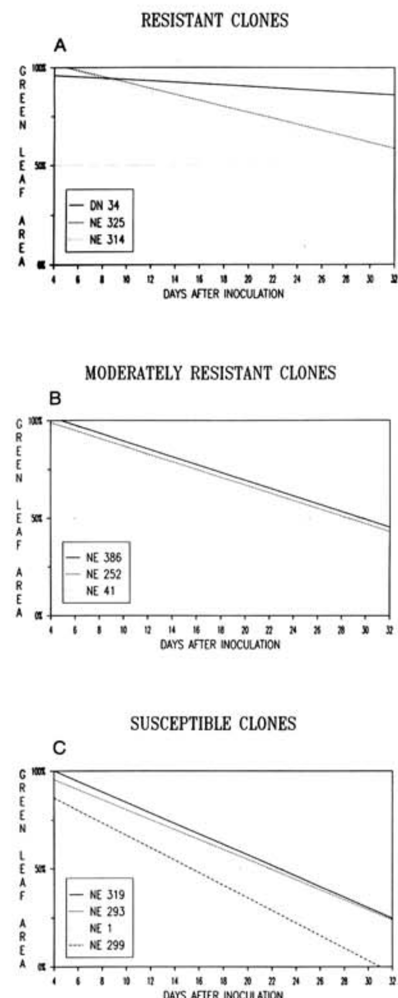

Fig. 2. (A–C) Estimated green leaf area-time curves from leaf disk bioassay of *Populus* clones ranging from highly resistant (DN 34) to highly susceptible (NE 299).

Statistical tests were performed to determine the most appropriate model. From the parameter estimates of the most appropriate model, we estimated T.50, the elapsed time to 50% necrosis and R.50, the rate of necrosis at the time 50% necrosis was reached. Based on these two estimates, clones were classified as having high, moderate, or low resistance to *S. musiva*.

RESULTS

Germination of *S. musiva* spores averaged 98% in each trial. Spore concentration had a negligible effect on the classification of clones, so we used 1×10^5 condidia/.1 ml in all tests. Clone specificity was not detected among the *S. musiva* isolates, so all bioassays were done using a single isolate.

Disease symptoms on leaf disks from susceptible clones usually became visible within 6 days. Sporulation of *S. musiva* was common on infected leaf disks. Contamination by saprophytic fungi was not a problem, because uninoculated controls and leaf disks from resistant clones remained green throughout the trials. None of the clones tested using the leaf disk bioassay were immune to infection by *S. musiva*. Disease severity ranged from low to high (Fig. 1). Between the most resistant and the most susceptible clones, there was a difference of 22 days from the time of inoculation to the time when leaf disk necrosis reached 50%. Necrosis often failed to reach 50% on the highly resistant clone DN 34. The rate of necrosis of the most susceptible clone at the time 50% necrosis was reached was nine times greater than the rate of the most resistant clone.

Estimated green leaf area-time curves for the 10 clones are in Figure 2 and the parameter estimates using the leaf disk bioassay are in Table 1. A regression analysis with an estimated intercept was used. The classification of clones is similar for field trials and bioassays (Table 1). Exceptions are clones NE 293 and NE 319, classified low in resistance in the bioassay, but highly and moderately resistant, respectively, in the field.

DISCUSSION

The leaf disk bioassay effectively evaluated the relative resistance of poplar clones to infection by *S. musiva*. The test is easily repeatable and provides a rapid method to screen a large number of clones for disease resistance.

The leaf disk bioassay was sufficiently sensitive to distinguish among clones with high, moderate, or low resistance. Clones highly resistant or highly susceptible were most easily identified. There is a continuous range in the degree of resistance to *S. musiva* among hybrid poplars (3) and this leaf disk bioassay can rapidly determine approximately where a clone ranks. Disease severity on leaf disks of a clone varied somewhat in bioassay trials, but the variation did not change clonal classifications. The occasional variation among leaf disks within a petri plate may have been caused by an uneven dispersal of inoculum.

The leaf disk bioassay provides optimum conditions for infection by *S. musiva*. The possibility exists that some clones that are moderately or highly resistant in the field may be classified as being more susceptible in the bioassay. However, no clones that were susceptible in the field were classified as resistant by this method. This bioassay should, however, only serve as a preliminary screening technique before field tests.

Clones in the same field resistance category tend to group together in plots of R.50 vs. T.50 (Fig. 3). This suggests the possibility of developing a quantitative, multivariate criterion for distinguishing among clones on the basis of the parameters of the percent of necrosis and elapsed time models of this bioassay.

The generally high correlation of the bioassay results with those from the field means that this technique can be used to rapidly identify poplar clones highly resistant to *S. musiva*. These clones can then be used in further breeding and selection. Furthermore, as proposed by Spiers (4), this type of standardized testing procedure can be adopted internationally to more accurately assess disease resistance of poplar clones.

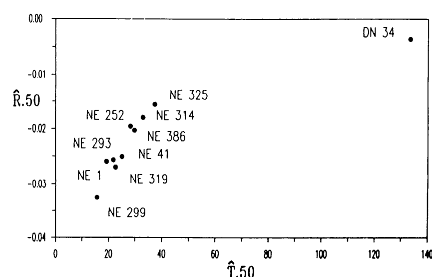

Fig. 3. Plot of clones based on estimates of T.50 (elapsed time to 50% necrosis) and R.50 (rate of necrosis at T.50) in leaf disk bioassays. In field tests, clones DN34, NE314, NE293, and NE325 were highly resistant to *Septoria*; clones NE41, NE252, NE386, and NE319 were moderately resistant; and clones NE1 and 299 were highly susceptible.

ACKNOWLEDGMENTS

This work was supported in part by USDA Competitive Research Grant No. 85-FSTY-9-0145.

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