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Short Communication

An efficient method for evaluating black walnut for resistance to walnut anthracnose in field plots and the identification of resistant genotypes

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With 2 tables

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Abstract

Black walnut is native to the eastern USA and prized for its high-quality timber. Walnut anthracnose, the most important foliar disease of black walnut, is caused by *Gnomonia leptostyla*. There is no germplasm available that is resistant to the disease. Ramets of 42 black walnut clones, comprising about one-third of the Midwestern USA black walnut timber breeding stock, were rated for susceptibility to walnut anthracnose from 1974 to 1982. Significant differences in susceptibility were identified among clones. A method of truncation selection was able to identify the most resistant clones in less time and with a smaller commitment of resources than methods based on means. Truncation selection permitted the identification of the most susceptible clones even in years when the severity of anthracnose was low.

Key words: *Juglans nigra* — anthracnose — truncation selection — disease resistance

The most widespread and serious foliar disease of black walnut is walnut anthracnose, caused by *Gnomonia leptostyla* (Fr.) Ces. & de Not. The fungus has a world-wide distribution (Berry 1981) and is known to infect other members of the genus *Juglans* (Black and Neely 1978a). Symptoms develop on the current year's leaves, nuts and stems as irregular necrotic areas, usually less than 5 mm in diameter, that are often surrounded by small chlorotic halos. In severe cases, these lesions may coalesce and cause premature defoliation, fruit drop, or poorly filled nuts (Black and Neely 1978b). Repeated defoliations caused by walnut anthracnose may have a deleterious effect on plant growth (Funk et al. 1981, Todhunter and Beineke 1984), but no controlled experiments have been performed.

There is significant variation in susceptibility to the disease (Black and Neely 1978a, Funk et al. 1981) but no specific genotype has ever been reported to be highly resistant or immune. Identifying walnut anthracnose-resistant black walnuts in field plantings is costly and complicated because of the large size and long juvenility of the trees, and the lack of a uniform method of rating trees for resistance (Funk et al. 1981, Neely 1981, Cline and Neely 1984, Todhunter and Beineke 1984). In this paper, results from 9 years of rating 42 black walnut clones in the field for susceptibility to walnut anthracnose, and a simple, efficient method for identifying the most walnut anthracnose-resistant clones in field trials are presented.

The 42 black walnut, *Juglans nigra* L., clones (unique genotypes) in this study were selected on the basis of timber form from wild populations in the Midwestern USA (Table 1). Two clones (138 and 139) were open-pollinated progeny of clones already under evaluation. The clones were grafted onto seedling rootstock and planted in the Martell Forest, a research facility in Tippecanoe County, 12.87 km west of West Lafayette, IN, USA. At least three and as many as eight ramets of all 42 clones were planted arbitrarily within a clone bank. The ramets were all propagated from mature trees and had mature foliage, but not all of the ramets were the same age or size. The ramets were rated for symptoms of walnut anthracnose from 1972 to 1982. Disease severity ratings were assigned on a scale from 1 = no or a few lesions, to 5 = leaves completely senescent or abscised, based on the appearance of the entire tree (Todhunter and Beineke 1984). A balanced subset of the data — ratings of three random ramets of 42 clones from 1974 to 1982 — was used for more detailed analysis (Table 1). Ranks for disease resistance were assigned for each clone in each year, based on the mean disease ratings of the three ramets per clone. The ranks for each clone in 1974–82 were analysed using ANOVA to determine if rank differences among clones were significant. The yearly ranks from 1974 to 1982 were rescaled from 1 to 42 to correct for the manner in which ties were resolved.

Three methods were compared for their efficiency in identifying the most anthracnose-resistant clones: 'mean disease rating', 'cull on three', a type of truncation selection, and 'fewest threes'. The balanced data subset was evaluated using all three methods until the most resistant 10% of the clones were identified. For the 'mean disease rating' method, the most resistant clones were identified based on the lowest overall mean disease rating from 1974 to 1982. For the method of 'cull on three', a clone was evaluated until any ramet of that clone was rated ≥ 3 for disease severity, at which point the clone was considered unacceptably susceptible. The method was evaluated using one, two or three ramets per clone per year. There were three replications of the 'cull on three' method using one ramet per clone per year. The method of 'fewest threes' identified the most resistant clones by counting the number of times ramets of a clone were rated ≥ 3 for the duration of the evaluation.

Analyses were performed using SAS software (SAS Institute, Cary, NC, USA) vs. 8.0. Differences in rates of culling of clones were analysed using PROC LIFETEST, with the number of observations as an independent variable and the number of clones removed as the dependent variable.

The yearly means for walnut anthracnose disease ratings ranged from 1.8 to 2.8, in 1974 and 1978, respectively. The overall mean disease rating was 2.4 ± 1.0 (Table 1). Thus, a rating of 3 was used as a threshold level for culling (see

Table 1: Walnut anthracnose disease severity ratings for 42 black walnut clones and their ramets

Clone	Source[1]	Disease rating[2] Mean	SD
1	Tippecanoe	2.2	0.7
17	Montgomery	2.1	0.6
31	Cass Co., MI	3.4	0.9
34	Tippecanoe	2.4	0.6
36	Dubois	2.3	0.6
41	Brown	2.2	0.9
44	Lawrence	1.6	0.5
48	Knox	1.7	0.5
49	Lawrence	1.6	0.6
55	Montgomery	2.6	0.9
63	Wabash	3.5	0.2
68	Carroll	2.0	0.4
72	Carroll	3.7	1.2
82	Tippecanoe	2.9	0.6
86	Grant	2.4	0.6
92	Randolph	1.5	0.4
95	Crawford	1.0	0.0
98	Carroll	2.4	0.5
102	Tippecanoe	2.2	1.1
109	Fayette	1.4	0.4
113	Vermillion	2.4	0.6
115	St Joseph	2.7	0.6
116	St Joseph	2.0	0.7
117	St Joseph	4.1	0.7
118	Tippecanoe	3.0	0.9
119	Sullivan	2.0	0.4
123	Jackson	2.0	0.6
124	Jackson	1.5	0.6
126	Wabash	3.7	1.3
129	Montgomery	2.1	0.5
130	Tippecanoe	2.4	0.7
132	Morgan	1.7	0.5
133	Gibson	2.0	1.0
134	Hancock	2.4	0.5
135	Henry	1.2	0.4
136	Wayne	2.1	0.7
138	Progeny of 106	3.0	0.9
139	Progeny of 65	2.8	0.6
143	LaGrange	2.9	0.9
145	Whitley	1.8	0.5
147	Leavenworth, KS	1.7	0.6
153	Tippecanoe	1.9	0.6
Totals	131 Clones	2.4	1.0

[1] Sources are counties in Indiana unless noted otherwise.
[2] Mean of three ramets per clone rated annually for 9 years on a 1–5 disease severity scale, where 5 = most severe symptoms.

Materials and Methods), because ramets rated ≥ 3 for disease severity in any year had a greater than average susceptibility to walnut anthracnose. The data set contained 1134 observations (42 clones, three ramets per clone observed in 1974–82, or 27 observations per clone), of which 450 were ≥ 3 (39.7%).

Both extremes in susceptibility were found in the study population based on mean disease rating (Table 1). The clones with the lowest and highest mean disease rating were 95 (mean = 1.0; SD = 0.0) and 117 (mean = 4.1; SD = 0.70). An ANOVA on ranks for disease severity rating revealed significant differences among the clones ($F = 10.4$, d.f. = 41 336; $P < 0.0001$).

The clones were ranked for their disease severity rating in each year, and correlations among the yearly ranks were examined for trends. The rank correlations among years were generally moderate and highly significant, ranging from $r = 0.40$ to $r = 0.75$, $P < 0.01$. The amount of walnut anthracnose in any year was apparently not strongly influenced by disease levels the previous year, because the rank of clones for disease severity in any year was not more highly correlated with the rank in the immediately previous or subsequent year than with other years in the data set (not shown).

The data were evaluated using the cull on three and fewest threes methods of mean disease rating until four clones (best 10%) were identified. Each method yielded a similar, but not identical, list of clones, but the methods had important differences with respect to the number of observations, number of ramets, and the number of years required to determine the most resistant clones (Table 2).

The mean disease rating method required 1134 observations and identified clones 95 (mean = 1.0), 135 (mean = 1.2), 109 (mean = 1.4), 124, and 92 (mean = 1.5 for both) as most resistant (Table 1).

When one ramet per clone per year was rated, the cull on three method required field spaces for 42 trees (ramets) and either 7 years (replications 1 and 2) or 8 years (replication 3) of disease rating, for a total of about 160 observations. Compared with mean disease rating, the cull on three method using one ramet per clone per year required 1 or 2 years' less evaluation time, one third of the space, and only 14% of the observations to identify the most walnut anthracnose-resistant clones. The clones identified as most walnut anthracnose-resistant when one ramet per clone per year was rated were very similar to the clones identified using mean disease rating, with the addition of clones 48, 129 and 132. These clones had mean disease ratings of 1.7, 2.1 and 1.7, respectively.

Compared with using only one ramet per clone per year, rating two or three ramets per clone per year increased the number of observations and the amount of field space required, but decreased the number of years for the evaluation. Rating two or three ramets per year using the cull on three method identified the same clones as the most walnut anthracnose-resistant as the methods of mean disease rating and fewest threes (Table 2, and see below). Even when multiple ramets per clone per year were evaluated, the cull on three method required many fewer observations over fewer years than mean disease rating. The results of a Wilcoxon test indicated that rating three ramets per clone per year, compared with using one or two ramets, significantly increased the number of observations required to eliminate all but the best 10% of the clones ($\chi^2 = 18.3$, $P < 0.0011$).

The fewest threes method identified clones 95 (zero ramets rated ≥ 3), 109 (one ramet rated ≥ 3), 92, 124 and 135 (all with two ramets rated ≥ 3) as the most walnut anthracnose-resistant. This method was nearly identical to the mean disease rating method in terms of clones identified as most resistant, number of trees, years and observations required, but it has the advantage of simplicity because ramets could be rated as + or − (≥ 3 or not), and because it is non-parametric.

Clone 95 was clearly the most resistant to walnut anthracnose. The type of resistance observed in clone 95 might best be described as tolerance. Ramets of clone 95 had lesions caused by walnut anthracnose every year of the study, but no other obvious symptoms, such as senescence or defoliation, common in almost all other clones in most years.

Speed, simplicity, propagation costs and efficient land use are important considerations when evaluating a large number of clones for resistance in the field. The methods investigated in this paper for selecting more resistant genotypes were different in terms of the number of years, amount of space and number of

Table 2: Number of observations, ramets and years required to identify black walnut clones most resistant to walnut anthracnose using three methods

Method[1]	Number of observations	Number of ramets	Number of years	Best 10% identified
Mean disease rating	1134	126	9	95, 109, (92, 124)[2], 135
Cull on three[3]				
One ramet per clone				
Replication 1	142	42	7	95, 109, 132, 135
Replication 2	153	42	7	92, 95, 109, 129
Replication 3	171	42	8	48, 95, 109, 124
Two ramets per clone	175	84	5	95, 109, 124, 135
Three ramets per clone	292	126	4	95, 109, 124, 135
Fewest threes	1134	126	9	92, 95, 109, 124

[1] Three methods were used to evaluate the same data set (42 black walnut clones, three ramets per clone rated for walnut anthracnose from 1974 to 1982). For details of each method, see text.
[2] Clones 92, 124 had the same mean.
[3] The data were evaluated using the cull on three method with disease severity ratings from one, two or three ramets per clone per year. Three replications of the method using ratings from one ramet per clone per year were performed.

observations required, but they produced very similar lists of the most resistant clones. Cull on three was the most efficient method for identifying the most resistant clones in the shortest time, with the fewest observations and the least commitment of space. Rating one ramet per clone with the cull on three method required more years than rating two or three ramets per clone. This was because when multiple ramets per clone were rated in a year using the cull on three method, observations were made until any ramet rated ≥ 3, at which point disease rating for that clone ended. Thus, when two ramets per clone were rated in a year instead of one, the clone had twice as many opportunities in that year to be eliminated, and fewer years were needed to eliminate most of the clones. Rating two ramets per clone did not double the number of observations (relative to one ramet per clone), because sometimes the first of two ramets rated ≥ 3, and no additional observations were made for that clone. Truncation selection of this kind quickly eliminates the most susceptible clones so that time and space are not wasted making repeated observations on ramets of clones that have greater than average susceptibility. Truncation methods are easy to apply when many clones are undergoing initial screening because it is faster and easier to assign disease ratings that are + or − (above or below a threshold) than ratings based on a categorical scale. The cull on three method also increases the chances that every year can be used for evaluation, rather than waiting for years when disease pressure is very high. Even in the year with the lowest overall mean disease rating (1974, mean = 1.8), 17 of 42 clones (40%) had at least one ramet rated ≥ 3.

Every year, black walnut trees putatively resistant to walnut anthracnose are identified from wild populations and proposed for inclusion in the USDA Forest Service/Purdue University black walnut breeding programme. Prior to this analysis, there was no good estimate of how many clones to accept because it was not known how much time and space would be required to evaluate them. In this study, there was a 95% certainty that rating one ramet per clone per year for 7 years would be sufficient to observe a rating ≥ 3. In theory, only 5 years would be required for such an outcome (given the frequency of scores ≥ 3), and 8 years of evaluation would provide 99% certainty. The discrepancy between the theoretical value and the empirical data was probably due to variability in disease severity from year to year and because the distribution of the ratings was non-normal.

The importance of local environmental effects on these results was not estimated. There is no doubt that disease pressure was uneven within years, but the results of rank correlations from year to year indicated that the amount of inoculum in one year did not strongly influence disease ratings in the subsequent year, so the bias introduced by local variation in disease pressure may not have been large.

The study population described in this paper was mass selected for timber form, but it is likely that these clones closely approximated a random sample of the wild germplasm with respect to walnut anthracnose resistance. Linkage between genes related to resistance to walnut anthracnose and genes for traits such as straightness would limit the applicability of these results to a truly random sample.

Identifying the clones in our black walnut breeding programme that are the most and least susceptible to walnut anthracnose is a critical first step toward further genetic analysis of resistance to the disease.

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