VASCULAR ANATOMY AS A SELECTION CRITERION FOR VERTICILLIUM WILT RESISTANCE IN NORWAY MAPLE (ACER PLANTANOIDES)1/

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ABSTRACT

Norway maples resistant to Verticillium wilt were found to have roots with significantly lower vessel and vessel group densities and to have vessels and vessel groups of larger diameter (all at the .001 level) When compared to susceptible trees. Stems of resistant trees were also significantly more on (a higher vessel area per square millimeter), again at .001 level. Of these variables, the vessel density and vessel group density of roots appear to be heritable. This study has also demonstrated that, by using multivariate techniques, over 99% of the variation in vascular streaking could be accounted for using parent branch and root xylem anatomical variables. These xylem variables have potential for indirect selection of Verticillium resistance in this species.

Keywords: Anatomy, resistance, Acer platanoides, Verticillium dahliae, selection, genetic improvement

INTRODUCTION

The localization process, which generally serves to protect the host from extensive fungal invasion, is a three—step resistance mechanism (Beckman,1955). Mobile cells or spores are first screened out of the transpiration stream and trapped on end walls, porous perforation plates, and pits within the vascular system. Temporary occlusion of infected vessels by Bells follows and finally overgrowth of vasicentric parenchyma cells occurs forming tyloses which permanently seal the infected region. Infections seldom extend far into the vascular system because parasites can usually be contained by spore trapping and gel formation long enough for the infected vessels to be sealed by tyloses. Progress of the infection is permanently stopped and since the plant has an excess of transport capacity, function is usually unimpaired.

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Determinants of successful localization are not well known. The focus in annuals has been on pathogen recognition (Browning, 1979) and tolerance to fungal metabolites (Beckman et al., 1972), processes that are generally controlled by one to few genes. Although these processes are also important in woody plants, it appears vascular anatomy may also play a critical role in localization. Variation in vessel size and distribution has been correlated with resistance to Dutch elm disease in Ulmus (Elgersma, 1957; Elgersna, 1970; McNabb et al., 1970; Sinclair et al. , 1972) , and with can par tmentalization in hybrid poplar (Populus del to ides Marsh. X P.trichocarpa Hook.) (Eckstein et al., 1979; Wittberg, 1981) and bigtooth aspen (P.grandidentata Michx.) (Wittberg and Eckert, 1982).

Because anatomical features may be valuable in selecting resistant individuals, root and stem anatomy of Norway maple was investigated to find correlations with resistance to Verticillium wilt.

MATERIALS AND METHODS

Forty 4-year-old maternal half-sib Norway maple families in two replications were used in this study. Each replication of this planting had originally consisted of three treatments (Valentine et al., 1981), two of which were inoculated with Verticillium propagules and a third that served as a control. There were approximately eight trees Der family per treatment per replication. In this study, each tree was root inoculated in June of 1982 by forcing a dandelion digger into the soil on two sides of each tree and discharging Verticillium dahliae inoculum into the root zone (Treatment 2 of Sinclair et al., 1981). In late August of 1982, two trees per family per replication in each original control treatment were selected randomly and lifted. Two 2.5cm long segments were cut from each tree, the first 10cm above the root collar and the second 5cm below. A 20 micrometer section was cut with a sliding microtome from each segment. Seven anatomical variables were measured three times in the outermost ring of each section and six other variables were calculated (Table 1).

Verticillium infection was judged two different ways. For the two trees per family per replication examined under the microscope, the classification of the presence or absence of infection was based on the stem symptoms of vascular occlusion, ring width, and barrier zones (or false rings). Investigation has demonstrated that these symptoms can be used with reliability in predicting Verticillium infection (Wittberg, unpublished). Vascular streaking, the presence of elongated, necrotic regions of stem xylem, was used to compare families for relative resistance. This has also been show to be an acceptable criterion for disease condition evaluation (Valentine et al., 1981). Each tree in every family was checked for the presence of streaking and an arc-sin transformation was performed on the proportion of the family streaked to develop a resistance rating for each family.

Table 1. Norway maple xylem anatomical variables and descriptions[1]

Variable	Explanation
Average number of vessels per group	the number of vessels in 50 groups were counted and averaged. a[2], b, d, e, h
Vessel density	the number of vessels in a .5mm tangential ring segment were counted and divided by .5mm and ring width to get number of vessels/mm^2. d
Average number of rays per mm	the number of rays per mm were counted. i
Average distance between rays	the distance between 10 consecutive rays were measured and averaged. i
Average number of large rays per mm	the number of rays 3 cells wide were counted per mm. i
Average vessel diameter	the diameters of 25 vessels were measured and averaged. a, c, d, e, g, h
Average ring width	of outermost annual ring. i
Sample diameter	of root or stem segment. i
Average vessel group diameter	= average number of vessels per group X average vessel diameter. e
Vessel group density	= vessel density/average number of vessels per group (or number of vessel groups per mm^2. i
Average number of vessels per compartment	= vessel density X ring width X average distance between rays (a compartment is a term taken from Shigo's CODIT model and is defined by the area between two consecutive rays in one growth ring). f
Openness	= vessel density X (average vessel diameter)^2 X 0.7854 (or vessel area as a percent of the total area). h
Previous growth	= sample diameter - (2 X ring width). i

[1]All measures were taken on both stem and root segments.

[2]Variables are referenced by letters as follows: a-Eckstein et al., 1979; b-Elgersma, 1967; c-Elgersma, 1970; d-Kucera and Bosshard, 1973; e-McNabb et al., 1970; f-Shortle, personal communication; g-Sinclair et al., 1972; h-Wittberg and Eckert, 1982, i-unreferenced.

Twenty-two parent trees (all located in Sraouse, N.Y.) were also sampled and the same variables as described previously for the progeny were measured on two segments, one from a lower branch and one from an arbitrarily selected root.

Narrow sense single tree heritabilities were estimated two different ways for each variable. On the two trees per family per replication sampled, an analysis of variance method (Becker, 1957) was used:

heritability =4VF/(VP+VE)

where VF=variance attributed to female family group

VE=variance attributed to error

The other method is based upon the regression of standardized family variable means on standardized parent means (Becker, 1967). Variables were standardized by subtracting the mean and dividing by the standard deviation. The heritability estimate is equal to two times the regression coefficient.

Data analysis was performed using the analysis of variance, stepwise multi ple linear regression, simple linear regression, and stepwise discriminant analysis programs from the Statistical Package for the Social Sciences (SPSS by Nie etal, 1975) and Statistical Analysis System (SAS by Barr et al., 1975).

RESULTS

Nine variables were found to be significantly different between resistant and susceptible trees, five of which were significantly different at the 0.001 level. These results are presented in Table 2. Differences appeared to be more substantial in the root than in the stem. In the root, vessel density, average vessel diameter, average vessel group diameter, and vessel group density were all very highly significantly different between infected and noninfected trees whereas only the variable openness was significantly different at the 0.001 level in the stem.

The heritability estimates for each variable that has been associated with Verticillium resistance are presented in Table 3. On the basis of the two methods of estimating heritability, root and stem estimates of average number of vessels per group, vessel density, and vessel group density appear to be heritable.

Means and standard deviations for each variable are presented in Table 4.

Differences between study and control trees significant at the .05 level were found for only one xylem variable: stem vessel group density (mean=61.8 vessel groups per mm^2). No other variable was found to be significantly

Table 2. Comparison of means of anatomical variables associated with Verticillium resistance in Norway maple

Variable	Variable mean values in: Resistant trees	Susceptible trees
Vessel density (stem)	128 vessels/mm^2	115 **[1]
(root)	48.0	59.8 ***
Average vessel diameter (stem)	0.0277 mm	0.0257 *
(root)	0.0340	0.0297 ***
Average vessel group diameter (stem)	0.0494 mm	0.0440 **
(root)	0.0593	0.0503 ***
Vessel group density (root)	27.6 vessel groups/mm^2	35.5 ***
Openness (stem)	7.91 %	6.11 ***
Previous growth (stem)	4.52 mm	4.05 *

[1]*, **, *** - Significant at 0.05, 0.01, 0.001 levels respectively.

Table 3. Heritability estimates for anatomical variables associated with resistance to Verticillium wilt in Norway maple

Variable	ANOVA heritability estimate	Regression heritability estimate
Average number of vessels per group (stem)	0.29	0.56
(root)	0.29	0.48
Vessel density (stem)	0.19	0.47
(root)	0.39	0.17
Average vessel diameter (stem)	0.32	0.05
(root)	0.26	0.15
Average vessel group diameter (stem)	0.42	0.07
(root)	0.22	0.00
Vessel group density (stem)	0.19	0.35
(root)	0.56	0.12
Openness (stem)	0.37	0.06
(root)	0.00	0.00

Table 4. Means and standard deviations of Norway maple progeny xylem variables

Variable		Mean	Standard deviation
Average number of vessels per group	(stem)	1.77 vessels/group	0.202
	(root)	1.74	0.220
Vessel density	(stem)	125 vessels/mm^2	26.9
	(root)	50.8	13.5
Average number of rays per mm	(stem)	9.48 rays/mm	1.34
	(root)	8.72	1.48
Average distance between rays	(stem)	0.093 mm	0.016
	(root)	0.102	0.019
Number of large rays per mm	(stem)	0.161 rays/mm	0.418
	(root)	0.110	0.334
Average vessel diameter	(stem)	0.0272 mm	0.0044
	(root)	0.0329	0.0054
Average ring width	(stem)	1.38 mm	0.884
	(root)	0.798	0.415
Sample diameter	(stem)	7.10 mm	1.35
	(root)	6.83	1.64
Average vessel group diameter	(stem)	0.0481 mm	0.0091
	(root)	0.0517	0.0105
Vessel group density	(stem)	71.0 vessel groups/mm^2	14.0
	(root)	29.5	8.33
Average no. vessels per compartment	(stem)	15.9 vessels/compart.	10.6
	(root)	3.98	2.10
Openness	(stem)	0.0749 vessel area/mm^2	0.0288
	(root)	0.0427	0.0128
Previous growth	(stem)	4.35 mm	1.45
	(root)	5.23	1.70

different.

Discriminant analysis was used to check the ability of xylem anatomical variables to discriminate among families and was found to reclassify 30,3% of the cases correctly (Table 5). One significant (0.001 level) function was generated using seven variables in which average number of vessels per group in the root, root vessel density, and root vessel group density were the most important variables. All but two of the families had a significant multidimnsional distance from at least one other family and several distances were found to he very highly significant.

Multiple regression of progeny streaking on parent anatomy (Table 5) generated a 18 variable equation significant at 0.001 level with r=0.999 and the percent of the variation in progeny streaking explained by parent anatomy equal to ,.998. This equation was judged to be the best on the basis of a minimum standard error (0.75). The four most important variables in the regression were average vessel diameter of the root, root openness, average vessel group diameter in the root, and stem vessel group density.

Table 5. Standardized canonical discriminant function coefficients in order of inclusion on ability of xylem anatomical variables to predict family membership

Variable	Coefficient
Stem vessel density	0.501
Average distance between rays in stem	0.647
Average number of vessels per group in root	1.528
Root vessel density	-2.153
Average number of rays per mm in root	-0.054
Average vessel group diameter in stem	0.198
Root vessel group density	1.781

Canonical correlation = 0.671.
Percent of cases reclassified correctly = 30.3%.

Table 6. Standardized and unstandardized multiple regression coefficients of standardized[1] parent anatomical variables used in regression of progeny family streaking on parent anatomy and simple correlations (r) of streaking with these variables[2]

Variable (in order of inclusion)	Unstandardized regres. coef.	Standardized regres. coef.	r
Average vessel diameter (stem)	1838.9	0.798	0.317
Sample diameter (root)	0.517	0.200	-0.263
Sample diameter (stem)	4.962	1.302	0.256
Vessel group density (stem)	0.461	2.122	0.024
Average vessel diameter (root)	2353.7	3.296	-0.012
Average distance between rays (root)	444.9	1.359	0.291
Vessel density (root)	0.498	1.924	0.254
Average no. vessels per compart. (root)	1.834	0.838	0.096
Average distance between rays (stem)	-287.8	-0.871	0.142
Ring width (stem)	-15.78	-1.029	0.035
Openness (root)	-2.870	-2.337	0.111
Vessel density (stem)	-0.122	-1.007	0.027
Average vessel group diameter (root)	-876.2	-2.378	0.009
Average no. vessels per group (root)	33.81	1.070	0.063
Average no. large rays per mm (root)	2.272	0.227	-0.100
Average number of rays per mm (root)	1.124	0.234	-0.192
Average no. large rays per mm (stem)	-0.721	-0.107	-0.193
Average number of rays per mm (stem)	-0.378	-0.067	0.102
(Constant)	-217.0		

[1]Variables standardized by subtracting the mean and dividing by the standard deviation

[2]Multiple correlation coefficient = 0.999; r^2 = 0.998; significant at 0.001 level.

[3]No variable is significantly correlated with streaking in progeny at 0.05 level.

DISCUSSION

This study has clearly shown that anatomy of Norway maples resistant and susceptible to Verticillium wilt is different (Table 2). Several significant differences were found between stems of resistant ant susceptible trees, but these differences were not as dramatic as those found in roots. Resistant individuals generally had fewer vessels and vessel groups in a given area of root tissue than did susceptible trees and these vessels and vessel groups were usually of a larger diameter. It is thus possible that anatomy may make an important contribution to resistance because of a localization advantage it imparts to the resistant individual. For example, the more vessels in a given area, the more, on the average, that will become infected when a root is cut. If every infe.tted vessel has a certain probability of becoming systemic, the plant that has the most infected root vessels will be the most susceptible. Thus, Norway maples with a low vessel density will be naturally more resistant to Verticillium wilt.

These results are in line with previous findings in cotton (Garber and Houston, 1957) and olive (Wilhelm, 1975) that indicated the importance of the root in resistance to Verticillium wilt. Besides the four important root variables mentioned above, three of the four most important variables in the multiple regression equation were root variables, two of which (vessel diameter and vessel group diameter in the root) are among the group with differences between resistant and susceptible Norway maples significant at the 0.001 level. Finally, the three most important variables in the discriminant analysis were root vessel density, root vessel group density, and average number of vessels per group in the root. The first two are also among the important root variables in Verticillium resistance. In sum, anatomical differences among Norway maple families reside largely in the root, and these root differences can be used to explain variation in Verticillium wilt resistance.

In the heritability calculations, the stem and root measures of average number of vessels per group, vessel density, and vessel group density all appear to be heritable. Of these, the average number of vessels per group has been shown previously to be the most heritable xylem characteristic in hybrid poplar stems (Wittberg, 1931). In Norway maple, however, it does not appear to be an important factor in resistance to Verticillium wilt. Of the other variables, root vessel density and root vessel group density were both found to be very important variables with differences between resistant and susceptible trees significant at the 0.001 level. Stem vessel density was also found to have significant differences (0.01 level) between these two groups.

The accuracy of these heritability estimates is undoubtedly questionable. Because open-pollinated seed was used in this study, progeny are a mixture of full- and half-sibs. This would tend to inflate the heritability estimate. Furthermore, the accurate estimation of heritability requires a large number of progeny per family (Wright, 1976) whereas only four individuals per family were sampled in this study. This shortcoming further decreases the accuracy of the estimate by increasing the confidence limits. Nevertheless, these

figures can serve as a rough estimate of the actual value.

Multivariate techniques, however, may be a more accurate method of examining xylem anatomy and links with resistance than simple techniques. One reason for this can be seen in the success of the multiple regression Of progeny family streaking on parent anatomy. This regression (Table 5) demonstrated that, taken together, parent anatomical variables were able to explain 99.8% of the variation in progeny streaking (significant at 0.001 level). Yet, not one variable by itself was significantly correlated (0.05 level) with progeny family streaking. Thus, multiple regression techniques clearly indicate that a relationship exists between parent anatomy and progeny resistance, but this relationship would have been ignored if simple techniques alone had been employed. Further evidence of the superiority of multivariate over simple techniques in examining anatomical relationships can be seen in the discriminant analysis results (Table 5). The discriminant analysis was able to reclassify 30.3% of the individuals in the proper family on the basis of xylem anatomy (also significant at 0.001 level). This is not very high, but it is 27.8% better than would be expected assigning the individuals to families at random. The discriminant analysis success indicates that xylem anatomy is significantly different among the families, yet simple techniques indicate that only one variable, root vessel group density, is significantly different (0.05 level) among the families (this is reflected by the high heritability estimate for this variable through the analysis of variance method in Table 3). In other words, multivariate methods were also better than simple techniques in detecting family differences.

Verticillium wilt is becoming an increasingly serious problem in urban environments. According to Pirone (1973), it has resulted in the death of more maples in the past 35 years than any other disease. But with the loss of American elm from Eastern city streets over the past 20 years, Norway maple has risen to a position of prominence. it is often the most common old street tree and is a widely planted replacement tree (Valentine et al., 1978 and Santamour and McArdle, 1982). Breeding resistant Norway maples is one possible way to attack the Verticillium problem (Valentine et al., 1981). This study has indicated that vessel system variation may play a very important role in Verticillium resistance in this species and that xylem differences can be used with reasonable accuracy to select Norway maples that will produce resistant offspring.

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