TESTING VERTICILLIUM WILT RESISTANCE IN URBAN NORWAY MAPLES[1,2]

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Abstract. Wilt symptoms in Norway maple half-sib families from open-pollinated seed from a random of street trees in Rochester and Syracuse, N.Y. inoculated with *Verticillium dahliae* were analyzed for the reliability in predicting disease, variability in the parent population, and heritability. Inoculum was prepared from 11 isolates of diverse origin from maples and reisolates from Norway maple previously inoculated with these stocks. Two replications were inoculated with two inoculum concentratons (2.5×10^6 and 0.5×10^6 propagules/ml) and a distilled water control by dunking wounded roots of 2-0 nursery stock. Disease was verified by isolating the pathogen from stems. Thirteen families averaging 3.5 trees/family/treatment/rep (274 total) were tested in 1979, and 40 families averaging 7.8 trees/family/treatment/rep (1875 total) in 1980. Vascular streaking (dark greenish lesions) and two measures of foliage necrosis (% Crown Necrotic and % Necrosis, Worst Leaf) proved most reliable for predicting infection. These traits are recommended for screening for resistence. Three height growth measures did not prove reliable. Considerable variability is present in the parent populations, but estimates of heritabilities for each trait are quite variable, requiring further study. Increasing the incidence of infection is also important.

Healthy, vigorously growing trees play a major role in providing an aesthetically pleasing and physically comfortable environment for urban dwellers. A tree that is diseased and in declining health, however, is an "eyesore" and an economic burden to the community. Urban Norway maple (*Acer platanoides*) exhibit health and disease problems of serious magnitude in cities (Manion 1981a, Pirone 1978, Valentine et al. 1978, and Wilson 1977). Verticillium wilt, caused by the cosmopolitan soil-borne fungus *Verticillium dahliae*, has resulted in the death of more maples in the past 35 years than any other disease according to Pirone (1978). In a New Jersey survey he found Norway maple the second most susceptible maple species to this disease with silver maple (*A. saccharinum*) having the dubious distinction of ranking first. This is a serious disease in the urban environment because of two important attributes of this ubiquitous pathogen. First is its wide host range among hundreds of herbaceous and woody plant species including most shade and ornamental trees (Engelhard 1957, Himelick 1969, McCain et al. 1970, and Smith and Neely 1979), and the second, its ability to persist as a saprophyte on dead host roots or as microsclerotia for several years in soil once occupied by diseased plants. Sinclair et al. (1981) attributed the increase in occurrence of Verticillium infection in nurseries and landscape plantings in the last 20 years to the establishment of many shade tree nurseries on land where infected herbaceous crops once grew. Zeller (after Martin 1929) and Bedwell and Childs (1938) reported 50% to 85% mortality, respectively, in Norway maple nursery stock planted on land formerly in potatoes. A more insidious effect is the spread of the pathogen from infected planting stock that exhibits no disease symptoms in the nurseries to new planting sites. The widespread occurrence of the disease among dead or dying Norway maple in Metropolitan New York City and adjoining New Jersey reported by Pirone (1959) could be due to either the persistence of the pathogen in urban soils or the planting of infected nursery stock.

At present no reliable treatment of the disease has been developed, according to Pirone (1978), so that the only sound management practice is to cut trees showing a general and severe infection, completely destroy it and as much of the root as possible, and replace with a species not known to be susceptible to the fungus. An attractive alternative is replacement with Verticillium-resistant planting stock or cultivars grafted on resistant rootstock. This holds promise as a biological control system that will remain effective over time

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since new virulent forms of the pathogen that could infect resistant maples are not likely to persist in the populations of *V. dahliae.* This is due to the fact that the extensive range of hosts in the urban environment and the effect of stabilizing selection in the saprophytic and pathogenic phases of the pathogen's life cycle will favor genotypes of "average" virulence, ones already comprising the major part of the indigenous population of the pathogen according to Day (1974) and Manion (1981b).

Breeding for resistance to *Verticillium* spp. has been successfully used in large numbers of economically important crops including a Verticillium-resistant rootstock for olives (Wilhelm 1975). It appears feasible in Norway maple since variability in tolerance to the disease has been reported in red maple (Townsend and Hock 1973) and elm (Rauscher et al. 1974 and Lester 1975). Considerable phenotypic variability exists in urban Norway maple street tree populations that could serve as a base for selection (Valentine et al. 1978 and Smith et al. 1978), but more information is needed. In this report initial results in our program to develop resistant planting stock for future urban Norway maple populations will be presented. This includes (1) procedures for inoculating large numbers of maple seedlings, (2) the evaluation of disease symptoms and their reliability in predicting infection, (3) information on the genetic control of disease symptoms that is required in designing a breeding scheme, and (4) an assessment of the variability in susceptibility to this pathogen in urban street tree populations to determine whether sufficient variability is present to serve as the base for breeding Verticillium-resistant Norway maple.

Materials and Methods

Fifty-three half-sib families of Norway maple, derived from open-pollinated seed from street trees in Rochester and Syracuse, N.Y. were used in this study. Seed collected in 1976 was air-dried, stored at 4°C until stratified according to the procedures of Webb *et al.* (1973) and Pinfield *et al.* (1974), and planted in the nursery in late May 1977. Because of small family sizes due to poor germination, seed was again collected in 1977, air-dried and planted in late November 1977. Much larger families resulted the second year.

In April 1979 the first group of 2-0 stock was lifted, tops and roots pruned, bundled and stored at 4°C until field planted in mid-May. Similar procedures were followed in 1980 for the second group except root pruning was delayed until just prior to inoculation and planting in the field, according to the recommendation of Born (1974).

The Verticillium inoculum was derived from 12 cultures of diverse origin isolated from maples (5 from Illinois, 1 from Indiana, 4 from New York and 2 from Ohio) and reisolates of the fungus from Norway maple seedlings inoculated with the original isolates. In 1979, 11 of the original isolates and 11 reisolates were used, and in 1980, 6 of the original isolates and 11 reisolates from the 1979 test. These were cultured in Petri plates on PDA (potato dextrose agar) for approximately one month without light at 28°C. The mycelial mat was then scraped from the agar surface into distilled water, macerated in a blender, and diluted to concentrations of 2.5×10^6 propagules/ml for Treatment 1 and 0.25×10^6 propagules/ml for Treatment 2. Propagules include conidia and microsclerotia. A third treatment using distilled water instead of a fungal suspension was prepared for a control. Seedlings in a treatment were handled together to insure uniformity of treatment in the field. The roots were washed in tap water one minute, immersed in inoculum for one minute, packed in wet peat in buckets while carried to the field for planting at 0.6m × 2.4m spacing. Procedures in 1980 were similar except for root pruning in the field just prior to planting.

The field plots were planted according to a Randomized Complete Block design with three treatments, two replications and an unequal number of trees per family for both the 1979 and 1980 tests. The 1979 Test included 13 maternal half-sib families averaging 3.5 trees per family per treatment per rep, and the 1980 Test 40 half-sib families, 7.8 trees per family per treatment per rep. Seedlings were pruned to a single leader, and the initial height measured.

During the summer following planting, seedlings were checked at regular intervals for the develop-

ment of disease symptoms. Foliage traits were assessed twice each summer according to the numerical scales shown in Table 1. The traits "Best Leaf" and "Leaf Chlorosis" were omitted in 1980 since they were shown not to be reliable indexes of disease (Carlson 1980) in 1979.

Three measures of height growth have been analyzed since Townsend and Hock (1973), Rauscher *et al.* (1974) and Lester (1975) found height increment the best measure of the effect of Verticillium infection on seedlings. These are 3-yr-old total height, height increment 3rd year, and "relative growth" which is the height increment divided by the total height.

The presence of elongated, necrotic lesions in vascular tissue of the stem, hereinafter referred to as vascular streaking, was checked in late fall each year. A small cut was made using a sterilized knife near the base of the stem in the first year's growth. If the dark blue-green streaking, characteristic of Verticillium infection in maples was observed, a cut was made in the second year's height increment. If it also was found there, this was repeated in the next year's increment, etc. A seedling was scored 0, 1, 2, etc. according to the extent of the spread of the vascular discoloration in the stem.

All seedlings, including the controls, were checked for vascular streaking. One or more stem segments 2 to 3 cm in length were taken from each tree with vascular streaking for laboratory verification of Verticillium infection. In addition, a number of seedlings not showing streaking in Treatments 1 and 2 and in the control (Treatment 3) were sampled and checked in the laboratory. The stem segments were surface sterilized by immersing in 2% sodium hypochlorite for one minute, cut into small disks which were transferred to PDA petri plates. The cultures were incubated at 28°C and checked periodically for *V. dahliae.* In 1980 seedlings lacking streaking in 1979 and seedlings with streaking in 1979 but not confirmed as having Verticillium infection were checked. All seedlings in the 1980 Test were checked.

SAS (Statistical Analysis System) computer programs were used in the analyses of the data (Helwig and Councils 1979). These include an analysis of variance (ANOVA) for an unbalanced designed with the sums of squares computed by the type IV method, Duncan's Multiple Range Test, simple correlation analyses, and heritability estimates based upon variance components calculated from type I sums of squares.

Table 1. Verticillium disease symptoms

Trait	*Scale*
Leaf chlorosis	1 = None or little to 5 = Wholly chlorotic.
Foliage necrosis	
Crown—per cent necrotic	0 = No leaves necrotic to 9 = 90 per cent or more
Best leaf—per cent necrotic	0 = None to 9 = 90 per cent or more.
Worst leaf—per cent necrotic	Ibid
Vascular streaking—extent	0 = None, 1 = 1st year's growth only, 2 = 1st and 2nd year, etc.
Stem height growth	
Total height	cm.
Annual increment	cm.
Relative growth	Annual incr./total height.

Results

The analysis of the assessment of vascular streaking in 1979 showed highly significant differences between treatments ($F = 13.52$, d.f. = 2 and 145, $P < .001$, between replications ($F = 11.88$, d.f. = 1 and 145, $P < .001$) and between REP X TREATMENT interaction ($F = 8.03$, d.f. = 2 and 145, P .001) (Carlson 1980). The difference in seedling response to Verticillium inoculation and to control treatment is expected, but not that between replications and the REP X TREATMENT interaction. The incidence of vascular streaking in this test is presented in Table 2. The between reps and the REP X TREATMENT interaction differences are likely due to the widely disparate incidences in streaking in the two reps, i.e. 39% and 7%, respectively, in Treatment 1, and 4% and 0 in Treatment 2. Since the exact reason for this difference is not known, the Rep 2 data will be omitted in further analyses of the results in the 1979 Test. The Treatment 2 data were also omitted in comparisons of inoculated to non-inoculated seedlings in the 1979 and 1980 tests because of the low frequency of infection.

Table 2. Incidence of vascular streaking in the 1979 Norway maple Verticillium test.

Inoculation treatment	Inoculum Concentration x[6] propagules /ml	Rep	Total no. trees	Trees with vasc streaking No.	Trees with vasc streaking Incidence
1	2.5	1	46	18	39%
		2	45	3	7%
		Total	91	21	23%
2	0.25	1	46	2	4%
		2	44	—	—
		Total	90	2	2%
3	0	1	46	0	—
		2	48	0	—
		Total	94	0	—

Table 3. Correlation of foliage necrosis and height growth with vascular streaking in verticilium inoculated seedlings.

Trait	1979 Test N	1979 Test R	1980 Test N	1980 Test R
Leaf Necrosis				
% Crown: 3-yr-old trees	46	.16	622	.38***
4-yr-old trees	37	.38*	—	—
% Worst leaf: 3-yr-old trees	46	.34*	622	.47***
4-yr-old trees	38	.30 (P=.06)	—	—
Height Growth: 3-yr-old trees				
Total height	46	.01	623	.09*
Annual increment	46	.04	623	.05
Relative growth (ann incr/ht)	46	.08	623	.02

*Statistically significant at the .05 level of probability.
***Statistically significant at the .001 level of probability.

Vascular streaking proved to be a reliable predictor of Verticillium infection. *Verticillium dahliae* was isolated from 12 of the 18 seedlings with streaking identified in 1979 and from five additional seedlings with streaking in the 1979 Test the following year. In 1980 two of the six trees which were not identified as Verticillium-infected in 1979 were found to be diseased the next year, leaving only four not verified as infected. Therefore, 83% of the trees with streaking have been verified as having *V. dahliae*. This contrasts with three successful isolations in 28 attempts (11%) from seedlings lacking vascular necrosis from all treatments. None of the three was among the control group. In the 1980 Test, the pathogen was isolated from 48 of the 62 seedlings with vascular streaking, or 77%. Clearly, the disease symptom vascular streaking is a good predictor of Verticillium wilt.

Two measures of foliage necrosis, the % Necrosis Worst Leaf and % Crown Necrotic, appear to have value in predicting disease incidence or resistance. The results of the correlation analyses of these traits with vascular streaking presented in Table 3 support this conclusion. The correlations given here are for the second of two foliage assessments made each year (July 24, 1979 and September 25, 1980). Those for the earlier assessments (July 11, 1979 and late August, 1980) are either not significant or are smaller than the ones shown in this table. The third foliage symptom, "Best Leaf" was dropped as it did not prove to be a good indicator of disease.

The three measures of height growth in this study exhibit a small, positive correlation with vascular streaking (see Table 3), but only that for Total Height of 3-yr-old seedlings in the 1980 Test is significant. None, therefore, appears to be a good predictor of disease. These traits are included in the remaining analyses, however, since growth was found to be a good indicator of tolerance to Verticillium in red maple (Townsend and Hock 1973) and in elm clones (Rauscher *et al.* 1974, and Lester 1975).

The results of the analysis of the variation in these six disease symptoms exhibited by trees in Rep 1 of Treatments 1 (inoculated) and 3 (Control) in the 1979 Test are presented in Table 4. The only significant difference between treatments is for vascular streaking in 1979 and the total streaking for both 1979 and 1980. The difference in the average spread of the streaking up the stem, given at the bottom of the Table, is obvious since none occurred in the Control seedlings. Results for the foliage necrosis traits for the two years are not significant in the analysis of variance, although % Necrosis Worst Leaf in 1979 approaches significance. The average is less for the inoculated seedlings than controls as would be expected, except for % Crown Necrotic in 1980. The results of the Duncan Multiple Range Test show significant differences for the 1979 % Necrosis Worst Leaf as well as for

vascular streaking. The differences among families is only significant for 3-yr-old height increment.

The results of the analysis of these same traits in the 1980 Test are presented in Table 5. Treatment mean squares and Treatment Means in Duncan's Multiple Range Test are statistically significant for all traits except 3-yr-old growth, supporting the conclusion that seedling response to the treatments are different. Differences between families are also either significant or approach significance (% Necrosis Worst Leaf, $P < .08$) except for vascular streaking. This indicates considerable variation exists among families, a characteristic of vital importance for family selection in a breeding program. The lack of difference between families for vascular streaking is likely due to the low incidence in the 1980 Test, which is about one-fourth that obtained in the Rep 1 Treatment of the 1979 Test. The mean squares for Reps are significant for most traits, but the lack of significance for vascular streaking is more noteworthy since it supports the conclusion that the average extent of the spread of streaking is the same in both reps. This similarity in response to inoculation is also supported by comparable incidences of streaking in the two reps (11% and 9%). It appears that whatever was responsible for differences in vascular streaking between the two replications in Treatment 1 in the 1979 Test did not occur in the 1980 Test. The interaction mean squares are significantly different for most traits except for Treatment X Rep. This lack of difference indicates that the expression of a trait in response to a treatment is the same in both reps. Finally, it should be noted that the mean values of the inoculated seedlings for foliage traits and vascular streaking are larger than for the controls, and for the three height growth traits, control means are larger than those for the inoculated seedlings. These are in accord with the expected responses of the seedlings to these treatments if these are reliable indexes of disease.

The heritability estimates for the six symptoms of Verticillium wilt are presented in Table 6 for 3-yr-old seedlings in the 1979 and 1980 Tests and 4-yr-old seedlings in the 1979 Test (1980 data). Estimates for 3-yr-old trees in the 1979 Test based upon the data from both Reps 1 and 2 are given in parentheses following the estimate based on the Rep 1 data alone. Reasonably large heritability estimates are obtained for five of the six traits as one of the estimates for each trait. Four of these are based upon results in the 1979 Test (% Crown Necrotic in 4-yr-old trees, Vascular Streaking, Height Increment in 3-yr-old trees, and Growth in 3-yr-old trees), with the fifth, Total Height in 3-yr-old trees, in the 1980 Test. If the different heritability estimates for each trait are compared, it is obvious they vary considerably, except for the three estimates of % Crown Necrosis in 3-yr-old trees. This indicates that additional estimates are needed to obtain a reasonably reliable estimate of the true heritabilities for these traits in this population. Increasing the incidence of infection should improve these estimates and also minimize the chance that susceptible trees escape infection. The results of these tests, however, do suggest that several of these traits may have value in selecting for resistance to Verticillium wilt. Additional testing has been initiated with considerably more concentrated inocula that should provide more reliable results.

The results of the laboratory verifications of Verticillium infection by reisolation of the pathogen from inoculated seedlings exhibiting vascular streaking are presented in Table 7. Though the incidence of diseased trees is relatively low among all trees, i.e. 30% and 8% in the 1979 and 1980 Tests, respectively, the incidence among only those seedlings with vascular streaking is high, 78% and 77%, respectively. Information on the number of families with one or more diseased trees is given at the bottom of the Table. It should be noted 62% of the families in the 1979 Test and 75% in the 1980 have diseased members. Another family with a diseased tree in the 1979 Test was not included as it lacked vascular streaking. If it is included, the incidence in the 1979 Test would be close to 75% of the families, and the family incidence based on the pooled data from these two tests is 73.6%. If progeny performance in test populations, such as in this study, is to be used in identifying resistant parent trees, a higher incidence of families with susceptible progeny would be desirable. Increasing the incidence of disease using more concentrated inoculum might accomplish this.

Table 4. ANOVA of 1979 Verticillium test results: inoculated (N = 46) vs control (N = 46).

Source of Variation	DF	Mean Squares: % Crown necrotic 1979	% Crown necrotic 1980	% Necrosis worst leaf 1979	% Necrosis worst leaf 1980	Vascular streaking 1979	Vascular streaking Total	3-yr-old Ht (cm)	3-yr-old Incr (cm)	3-yr-old Growth Incr/ht
Betweeen families	12	—	—	—	—	—	—	—	108.2**	—
Between treatments	1	—	—	9.6	—	10.14**	13.25**	—	—	—
Fam x treat	12	—	—	—	—	—	—	—	—	—
Within plots										
(1979)	66	—	—	2.8	—	1.35	1.39	—	43.2	—
(1980)	57									
Duncan's test										
$\bar{x}$ Inoc.		1.6	2.5	1.5	2.6	.96	1.06	39.5	13.7	0.40
$\bar{x}$ Control		1.1	2.9	0.8	2.3	0	0	38.1	11.2	0.35
Difference	66	NS	NS	*	NS	*	*	NS	NS	NS

— or NS Not significant
* Significant at the .05 level of probability.
** Significant at the .01 level or probability.

Table 5. ANOVA of 1980 Verticillium test results: inoculated (N = 624) vs control (N = 626).

Source of Variation	DF	Mean Squares: % Crown necrosis	% Necrosis worst leaf	Vascular streaking	Ht (cm)	3 yr old Incr (cm)	Growth incr/ht
Between families	39	10.9***	5.1*	—	494.3***	113.8***	.068***
Between treatments	1	23.3*	115.1***	21.31***	560.5**	121.8*	—
Replications	1	21.3*	19.9*	—	15,865.0***	552.2***	—
Fam x treat	39	9.3**	5.4*	—	127.1***	43.4**	.056**
Fam x rep	38	11.6***	—	—	218.7***	62.9***	.066***
Treat x rep	1	—	—	—	—	—	—
Fam x treat x rep	38	13.7***	5.0*	—	114.4**	63.5***	.069***
Within plots	1090	5.6	3.5	0.37	63.7	24.6	.030
Duncan's test:							
$\bar{x}$ Inoc.		2.14	1.60	0.27	23.3	5.5	0.21
$\bar{x}$ Control		1.86	1.00	0.01	24.7	6.2	0.22
Difference	1090	*	*	*	*	*	NS

— or NS Not significant
* Significant at the .05 level of probability.
** Significant at the .01 level of probability.
*** Significant at the .001 level of probability.

Discussion

Three symptoms of Verticillium infection in Norway maple nursery stock, vascular streaking and two measures of foliage necrosis (% Crown Necrotic and % Necrosis Worst Leaf), have been shown to be reliable measures of disease. Vascular streaking is the best, with 77.5% of the trees exhibiting this symptom, verified as diseased by isolation of the pathogen from stem segments exhibiting necrotic lesions. This most likely is an underestimate of the frequency since two of six trees with streaking, identified as non-diseased in 1979, were verified as diseased when retested in 1980. Sinclair *et al.* (1981) and Smith and Neely (1979) reported necrotic xylem extending beyond the spread of the fungus in the stem, which could in part explain the susceptible trees that escaped identification the first year. A portion of the 14 trees with streaking in the 1980 Test that are classified as non-diseased, no

Table 6. Variance components and heritability estimates of foliage traits, vascular streaking and height growth in Verticillium inoculated trees.

Trait and test	No. trees N	Variance components: Families V_F	Fam x rep V_{F*R}	Error V_E	Heritability: $\frac{4 \times V_F}{V_F+V_{F*R}+V_E}$
Crown Necrotic					
3-year-old trees					
1979 test	46	.007	—	2.24	.01 (.05)
1980 test	624	.07	.29	6.57	.04
% Crown necrotic					
4-yr-old trees					
1979 test	37	.68	—	3.67	.62
% Necrotic worst leaf					
3-yr-old trees					
1979 trees	46	−.41	—	5.31	— (.34)
1980 trees	622	.11	.14	5.10	.08
% Necrotic worst leaf					
4-yr-old trees					
1979 test	38	−.86	—	5.98	—
Vascular streaking:					
3-yr-old trees					
1979 test	46	.16	—	1.67	.35 (.20)
1980 test	624	.003	−.009	.72	.02
Total height					
3-yr-old trees					
1979 test	46	−.14	—	222.7	— (—)
1980 test	623	4.3	14.3	62.0	.21
Annual ht increment					
3-y-old trees					
1979 test	46	10.4	—	64.2	.56 (.98)
1980 tet	623	−.7	4.2	21.3	—
Rel. growth (ann. incr/ht)					
3-yr-old trees					
1979 test	46	.007	—	.095	.28 (.66)
1980 test	623	.003	−.009	.716	.02

doubt, will be verified as diseased upon rechecking this year. Therefore, the true incidence of disease among trees with vascular streaking should be somewhat higher than reported here. Some of these are likely infected with another fungus that causes stem lesions. Pirone (1978) reported isolating at least six other kinds of fungi from Norway, red and silver maples showing greenish discoloration and then reproducing the symptoms in healthy trees by inoculation with the fungi.

A low incidence of disease (11%) was found to occur among a sample of seedlings lacking vascular streaking from both inoculated and control groups. Therefore, other symptoms of disease should be used in conjunction with vascular streaking in screening for Verticillium-susceptible Norway maple seedlings. The two

traits measuring foliage necrosis could be used in this way.

The mean percentage of necrotic leaf tissue for both % Crown Necrotic and % Necrosis Worst Leaf is significantly greater in diseased compared with non-diseased trees. Small differences between Treatment 1 (inoculated) and Treatment 3 (Control) means were found (see Tables 4 and 5), but when the same comparison was made between the diseased and non-diseased trees in families with one or more diseased tree, the differences are much larger. The difference between Treatment means for % Crown Necrotic, 0.28%, is about 1/13th that between diseased and non-diseased trees, 3.8%. And for % Necrosis, the differences are 0.6% and 3.8%, respectively, for the 1980 Test. The differences in the 1979 Test are not as marked. Clearly these traits will be of value in classifying trees for resistance to the disease. Results from the correlation of each of these traits with vascular streaking also support this conclusion as estimates of the true population values vary from r = .34 to r = .47.

The three measures of height growth appear to be of little value in assessing Verticillium infection in this study. Even though side branches and multiple leaders except one were pruned shortly after transplanting and bud break so that growth subsequent to inoculation would reflect any effect Verticillium infection might have, the results are disappointing. In the 1979 Test, the means of the inoculated treatment are larger than for the control treatment (see Table 4) which is unexpected even though the differences are not statistically significant. In the 1980 Test the control treatment means are larger than the inoculated group means and the differences are significant for Total Height and Height Increment, but the differences are small. Results from comparisons of the means of the diseased and non-diseased trees, as was done for foliage necrosis, however, are quite different. For all three traits, the means for the diseased are larger than the controls! The correlations of these traits with vascular streaking are also small (r = .09 or less), and only the correlation of Total Height and streaking is significant. If measurement of this trait were changed to include branch increments as well as leader elongation, perhaps it would prove to be a good index of disease, as reported by Townsend and Hock (1973) for red maple and by Rauscher *et al.* (1974) and Lester (1975) for elm clones.

Table 7. Vascular streaking and confirmed Verticillium disease among inoculated Norway maple trees.

	1979 Test	*1980 Test*
Number of trees:		
total	46	624
vasc streaking	18	62
incidence	39%	10%
Number of diseased trees		
incidence: among all trees	30%	8%
among trees with vascular streaking	78%	77%
Number of families:		
total	13	40
with diseased trees	8	30
incidence	62%	75%

Sufficient variation appears to be present in the urban Norway maple street tree populations used in this study to serve as a base for breeding Verticillium-resistant trees. This is indicated by the large variation among families in the 1980 Test (see Table 5) and by Coefficients of Variation greater than 78% for the foliage traits in the 1979 and 1980 Tests. Variability in the incidence of vascular streaking and disease must be expressed using parameters of a binomial distribution since these are "all or nothing" or qualitative differences. The variance is the appropriate statistic and is equal to: **pq/N**, where **p** is the frequency of diseased trees, for example, **q** the frequency of the non-diseased trees, and **N** the total number of trees. A maximum variance occurs when p = q = 0.5 for a given value of N. In this test, the population of interest is the female parent trees, so that N is 53 and the maximum variance is .0047. Variance estimates for the presence or absence of vascular streaking is .0035, or 75% as large as the maximum, and for diseased and non-diseased, .0037, or 79% of the maximum. It can be concluded that considerable variation exists in the street tree populations used in this study for these symptoms of Verticillium wilt and for resistance to the disease. This is in agreement with earlier estimates based upon other traits by Valentine *et al.* (1978) and Smith (1978). Comparable variability would be expected in other

cities, so that sampling urban populations for genetically superior trees appears feasible.

Two outcomes of this study are somewhat disappointing and require further testing. These are (1) the heritability estimates for the traits studied, and (2) the low incidence of disease. The one relatively large heritability estimate for vascular streaking (.35) and for each of the measures of foliage necrosis (.34 and .62) are encouraging, but the large differences among the two or three independent estimates for each trait are disturbing. Additional independent estimates must be obtained to resolve this dilemma. Perhaps increasing the incidence of disease would result in increases in the heritability estimates since increases in the variability among families should have this effect. An increase in disease incidence is also important to minimize susceptible genotypes that have escaped infection. It is critical in a breeding program to minimize "escapes" if maximum gains through selection are to be achieved. Modifications of both the inoculation procedure and age of stock are being tested in the hope of increasing the effectiveness in our screening for resistant trees. A program of re-inoculation of non-diseased trees in the inoculated treatments is also planned.

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