Anatomical Marker for Resistance of Ulmus americana to Ceratocystis ulmi

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ABSTRACT

Vessels in the second xylem ring outward from the pith of small branches of American elms resistant to *Ceratocystis ulmi* were found to have significantly smaller mean radial diameters than comparable vessels in branches of susceptible trees. This relationship was exploited by development of a vessel diameter index (VDI) that can be determined with few measurements. VDI = mean percentage of vessels of diameter $\geqslant 50\ \mu m$ among total vessels intersected by arbitrary radii in the second and third xylem rings outward from the pith. American elms known to be resistant to *C. ulmi* and others found healthy among dense populations of elms killed by Dutch elm disease had lower VDI's than trees known to be susceptible. Severe Dutch elm disease symptoms were not observed in trees with VDI's of 12 or less. VDI may be useful for rapid assessment of possible resistance in American elms that survive Dutch elm disease epidemics.

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Among and within species of *Ulmus* that have been examined, the level of resistance to *Ceratocystis ulmi* (Buis.) C. Moreau, incitant of Dutch elm disease, is negatively correlated with dimensions of xylem vessels (4, 5, 9, 12). Treatments that prevent or retard normal development of vessels can enhance resistance (1, 2, 3, 19). Localization of infection, a mechanism of resistance in elms (9) is thought to be more rapid and effective in species such as *U. pumila* that have small and/or well-separated xylem vessels than in species like *U. americana,* characterized by large vessels that occur in groups. Even within *U. americana,* however resistant trees are known (16) and localization of infection is a general mechanism of resistance (18).

The work reported here was part of a study of characters associated with resistance in American elms. The objective was to identify a "marker" by means of which the possibility of resistance in nontested trees could be rapidly and conveniently assessed. A preliminary account has appeared (17).

MATERIALS AND METHODS.—Three or more branches were cut from diverse parts of the crown of each sampled tree. Lengths of internodes were measured so that rates of apical growth could be related to xylem characters. From each branch, a segment 1 to 5 cm long was cut from an internode having at least three annual layers of xylem. Cross-sections 10 to 30 μm thick were cut with a table microtome and hand-held knife from fresh or fixed [weak formalin - acetic acid - ethyl alcohol solution (13)] segments. For some parts of the work, fixed segments were dehydrated and embedded in celloidin or synthetic resin and sectioned with a sliding microtome.

Sections stained with safranin and fast green or nonstained were examined microscopically for determination of thickness of xylem rings, mean diameters of vessel elements, frequency distribution of vessels of various diameters, and mean numbers of contiguous vessels per group in the first, second, and third annual xylem increments outward from the pith, hereafter designated as rings 1, 2, and 3, respectively.

Preliminary sampling showed that reliable data could be obtained by examining xylem elements intersected by three arbitrary radii per segment. This was done by sampling along one arbitrary radius in each of three randomly oriented sections per segment. Radii for sampling were established by the position of the scale axis of a filar micrometer, with which all microscopic measurements were made. If an arbitrarily chosen radius happened to overlie a medullary ray, a slight adjustment to one side was made so that vertical xylem elements would be intersected. Vessels per group were counted whenever one or more vessels in a group of contiguous vessels were intersected by a sampling radius.

Vessel diameter index.—After an inverse relationship between resistance and vessel diameter was detected, a vessel diameter index (VDI) was developed to facilitate measurements. VDI = mean percentage of vessels of diameter $\geqslant 50$ μm among vessels intersected by arbitrary radii through rings 2 and 3 in elm branches. Determinations were based upon one radius in each of three randomly oriented sections from each of three branches per tree. Several groups of American elms, including some of known resistance or susceptibility to *C. ulmi,* were compared with respect to mean VDI and proportion of trees having low VDI's.

RESULTS.—We noted that the typical ring-porous wood of American elm is not formed in a given internode until the second or sometimes third growing season. Secondary xylem in new shoots is typically diffuse-porous and vessels are of much smaller diameter than

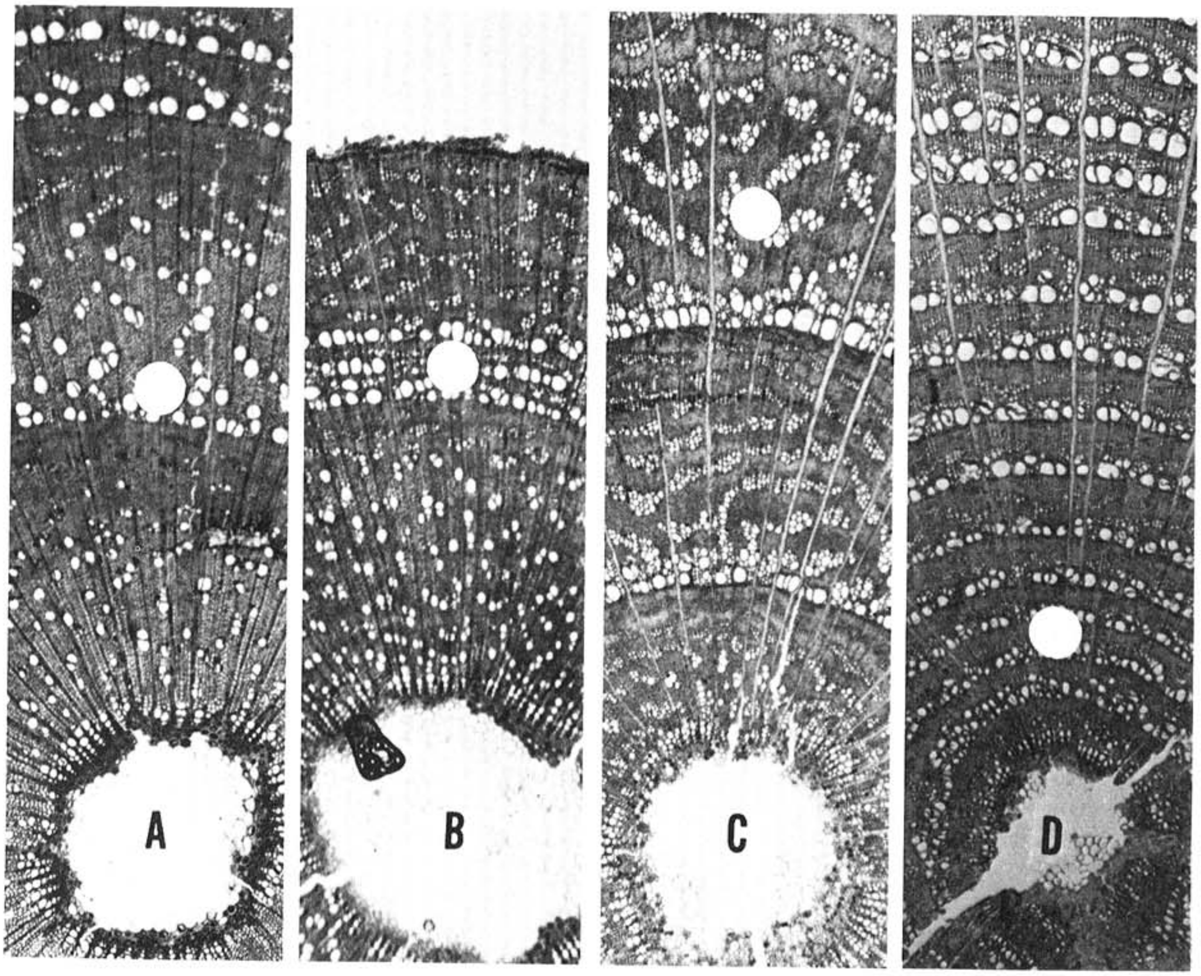

Fig. 1-(A to D). Transverse sections of branches of *Ulmus americana* showing variation in radial growth rates and xylem vessel diameters. White markers identifying ring 2 are scaled at 235 μm in diameter. Vessel diameter indices (VDI's) of the source trees were: A - 41, B - 35, C - 6, D - 5. VDI's determined from four radii immediately adjacent to size standards in these sections are: A - 35, B - 45 (marker overlies rings 2 and 3), C - 9, D - 0.

those formed in succeeding years (Fig. 1). The tendency toward diffuse-porous wood sometimes persists through the second growing season (Fig. 1-A), and springwood vessels of increasingly larger diameter may be formed for several years (Fig. 1-D).

Mean vessel diameters in susceptible and resistant trees.—In a preliminary study, we found no clear relationship between resistance and measured xylem characters in ring 1 of two resistant and two susceptible trees. Mean vessel diameters were 17.2 and 19.0 in the resistant trees, 19.7 and 20.5 μm in the susceptible trees. Long and short shoots on the same trees, moreover, showed no significant differences in vessel diameter. Further studies were concerned only with rings 2 and 3.

Mean diameters of vessels in ring 2 in three branches from each of eight elms previously selected as resistant to *C. ulmi* (16) and 10 susceptible elms were determined. For this purpose only vessels ⩾20 μm were counted and measured. The resistant and susceptible groups had significantly different ($P = 0.01$) mean vessel diameters of 39.9 and 49.6 μm, respectively. The ranges among individual trees were 35.3 to 42.8 μm in resistant trees, 41.1 to 58.9 μm in susceptible trees. Since the resistant trees were, as a group, less vigorous than the susceptible ones, a significant correlation ($r = .66$, $P = 0.01$) between mean vessel diameter and internode length was found when all 18 trees were considered. Within the resistant and susceptible tree groups separately, however, mean vessel diameter was not correlated with xylem ring width or with length of the internode produced in the same year as the xylem ring. Thus, although resistant trees had slower growth rates and smaller mean vessel diameters than susceptible trees, growth rate was not an important determinant of vessel diameter. Moreover, the number of vessels intersected per ring was not related to resistance or to vessel diameter.

In another test of possible relationship between growth rate and vessel diameter, the proportion of vessels with diameter ⩾60 μm in rings 2 and 3 was determined in branches having long (40-53 cm) and short (8-18 cm) internodes of the same age as the rings. Two branches from each of two resistant and two susceptible trees were studied. Branches from the resistant and susceptible trees had 6 to 12% and 26 to 38% of vessels ⩾60 μm in diameter, respectively. There was no relation between vessel diameter and internode length or ring width.

Vessel group size.—Vessel group size, the product of mean vessel diameter and mean number of contiguous vessels per group (9) was determined for ring 2 in eight resistant and 10 susceptible trees. There was no relation between number of contiguous vessels and resistance or susceptibility. Thus vessel group size was less clearly related to resistance than was mean vessel diameter alone.

Frequency distribution of vessel diameters; development of VDI.—Using data from ring 2 in eight resistant and 10 susceptible trees, we determined the percentage of intersected vessels with diameters exceeding 30, 40, 50, 60, and 80 μm. Resistant and susceptible groups were most easily separated on the basis of percentages of vessels in the large diameter classes (Table 1).

We wished to test the applicability of mean vessel diameter as a marker for resistance to *C. ulmi* in

TABLE 1. Size distribution of xylem vessel diameters in the second xylem ring outward from the pith of branches of American elms resistant and susceptible to *Ceratocystis ulmi*[a]

	Percent of vessels with diameters exceeding				
Tree group	30 μm	40 μm	50 μm	60 μm	80 μm
Resistant	68	38	21	9	1
Susceptible	77	59	42	28	8

[a]Means of vessels intersected by one radius in three sections from each of three branches from eight resistant and 10 susceptible trees. Vessels with diameters < 20 μm were not counted.

TABLE 2. Vessel diameter index (VDI)[a] of American elm trees as related to resistance and susceptibility to *Ceratocystis ulmi*

Tree group[b]	No. of trees	VDI Mean ± SD	Trees with VDI < 12 (%)
"Wilted"	38	25.7 ± 8.1	2.6
"Nonselected"	41	24.9 ± 7.5	2.4
"Specimen"	38	19.0 ± 6.8	13
"Resistant"	44	16.5 ± 10.3	41
"Naturally selected"	18	14.2 ± 5.8	39

[a]VDI = mean % of vessels of diam ⩾ 50 μm among vessels in the second and third xylem rings outward from the pith along an arbitrary radius in each of three cross-sections from each of three branches per tree.

[b]Trees in "wilted", "nonselected", and "specimen" groups are known or presumed to be susceptible to *C. ulmi.* Direct or circumstantial evidence exists for resistance of trees designated "resistant" and "naturally selected".

American elms, and also to avoid laborious measurements and multicategorical classifications of xylem vessels. Accordingly, we counted the total number of vessels intersected by sampled radii and the number of such vessels with diameters equal to or greater than an arbitrary value, 50 μm. Measurements were required only for vessels having diameters so close to 50 μm that they could not be judged larger or smaller. The percentage of total vessels that exceeded 50 μm in diameter furnished a convenient index that was related to the mean diameter and could be used for anatomically diverse branches (Fig. 1).

Reliability of estimates.—Three branches per tree was assumed to be a practical minimum for reliable estimates of VDI. This assumption was not tested. However, sampling intensity and reliability of estimates from microscopic observations were tested. Estimates based on three radii per branch were not significantly different from those based on nine radii. Variation between two observers was tested. Their VDI estimates were significantly correlated ($r = 0.87$, $P = 0.01$). The mean difference between their estimates for 34 trees was 4.1%. For 25 of the sampled trees the estimates differed by 6% or less.

Correlation between VDI's of ortets and ramets.—VDI's were determined for ortets (original trees from which clones were made) and 4-year-old ramets (individual plants of clone) (20) of seven clones. The mean VDI's of ortets and ramets were 20 and 14, respectively, and were significantly correlated ($r = .81$, $P = 0.05$).

Application of VDI.—VDI's were estimated for 179 American elms in five groups: (i) "wilted" trees, dying of Dutch elm disease; (ii) "nonselected" trees – healthy roadside and campus trees presumed to be susceptible to *C. ulmi;* (iii) "specimen" trees brought to our attention by owners and others hopeful that they were resistant to *C. ulmi;* (iv) "resistant" trees, for which there is direct evidence of resistance to *C. ulmi,* including trees selected by Sinclair et al. (16), Ouellet and Pomerleau (10), F. W. Holmes *(unpublished),* and C. May (21); (v) "naturally selected" healthy trees, found among dense populations of elms killed by Dutch elm disease in upstate New York. The five groups had mean VDI's of 25.7, 24.9, 19.0, 16.5 and 14.2, respectively (Table 2). We found no trees with VDI less than 12 that wilted severely or were killed by Dutch elm disease. Thus, VDI of less than 12 may constitute an anatomical marker for resistance to *C. ulmi.*

Among the "naturally selected" trees, which were discovered in 1969-1971, seven developed systemic Dutch elm disease infections. These seven had a mean VDI of 18.7; the remaining 11 trees had a mean VDI of 11.4, and six had VDI's of 12 or less.

DISCUSSION.—Low VDI's seem dependably associated with resistance. Ramets of the clone L'Assomption (10), for example, had a VDI of 3. The converse relationship is less clear. Holmes' uniformly susceptible clone of *U. americana* (6) had a VDI of 47, but some trees for which there is direct evidence of resistance (16, 18) have VDI's of 20 to 32. Thus, if low VDI is used as a marker for screening potentially resistant elms, some resistant trees will be rejected. However, since small vessel diameter can theoretically contribute additively to resistance, the selection of trees having resistance *and* low VDI could be beneficial.

Why were rings 2 and 3 in small branches selected for intensive study of anatomical factors associated with resistance? First, young twigs and current-season shoots are important sites of pathogenesis in Dutch elm disease (8, 11), and small branches are also sites of localization of infection (18). Second, the anatomy of branch wood of American elm changes as succeeding annual sheaths of xylem are produced (Fig. 1-A, D). We reasoned that xylem characters associated with resistance should be sought in tissue where natural inoculations and localization of infection occur. Ring 3 was included only to increase sample size since xylem vessel diameters in rings 2 and 3 were significantly correlated ($r = .76$, $P = 0.01$), and ring 3 could be examined at the expense of relatively little time in addition to that required for examination of ring 2.

Determination of VDI may be useful for rapid assessment of possible resistance in American elms that survive Dutch elm disease epidemics. But, since such resistance is transmitted to seedlings and young clonal progenies poorly (7, 14), and since American elms resistant to *C. ulmi* may be subject to lethal attacks of phloem necrosis, such resistant trees seem to have little immediate usefulness. They have potential value if it is shown that clonal progenies develop resistance with increasing age and size, and in areas where phloem necrosis is thought to be excluded by climatic conditions (15).

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