Neth. J. Pl. Path. 76 (1970) 196–204

Anatomical factors in resistance to Dutch elm disease[1]

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Accepted 11 November 1969

Abstract

The localizing of pathogen (*Ceratocystis ulmi*) establishment in Dutch elm disease-resistant hosts is hypothesized as the major mechanism of resistance to this disease. Four factors are proposed that, singly or in all possible combinations, could regulate this general mechanism. We tested one of these factors, vessel size and number of contiguous vessels in the functional xylem at the point of natural inoculation. Using 23 selections from the Ulmaceae, we examined a constant area of second-year xylem of 2-year-old twigs. These anatomical data indicate that a correlation exists between increasing disease susceptibility and increasing vessel group size (the product of average vessel diameter and average number of contiguous vessels). Other evidence suggests that lateral pathogen movement is at first confined to the vessel group or groups of initial inoculation. Therefore, in a given limited time period, tylosis production could more effectively block vertical pathogen movement in vessel groups of small size. Relationships between vessel group size and the extent of sapwood discoloration are discussed. Vessel size and arrangement also is discussed in relationship to the resistance reported for young, greenhouse-grown trees. The use of vessel group size is suggested in screening programs for disease resistance. Recent research on oak suggests chemical modification of xylem tissue to lessen elm susceptibility.

Introduction

The family Ulmaceae contains species that vary in reaction to *Ceratocystis ulmi* (Buism.) C. Moreau from immunity in *Celtis occidentalis* L. to extreme susceptibility in *Ulmus americana* L. Research in The Netherlands during the last four decades has produced a number of F_1 and F_2 *Ulmus* interspecific hybrids. These hybrids and their parents exhibit a wide range of reactions to *C. ulmi*. Therefore, the plant material available in this family from natural or breeding material is ideal for basic studies on the mechanisms of resistance in woody-plant wilt diseases.

We hypothesize two general mechanisms of resistance in Dutch elm disease. First, resistant hosts limit establishment of the pathogen, thereby preventing the systemic development of the disease. For example, *U. pumila* L. may become diseased, but both the extent of host colonization by the pathogen and the development of external symptoms, if any, usually are localized. Second, the external symptoms of disease are limited, but systemic establishment of the pathogen parallels that found in susceptible

[1] Journal Paper No. J-6392 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1706. Appears also as Mededeling 106 of the Forest Research Station "De Dorschkamp".
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hosts. For example, discoloration and pathogen presence in the current year's xylem of diseased *Hemiptelea davidii* (Hance) Planch. may become systemic, but few external symptoms are produced (Holmes and Demaradzki, 1960). Thus, the host–parasite relationship may become more neutral than pathogenic.

Of these two proposed general mechanisms of resistance, the localizing of pathogen establishment appears to fit the majority of cases. Therefore, we shall consider this hypothesis further and propose four host factors that, singly or in all possible combinations, could regulate this general mechanism. These four factors are: (1) Vessel size and grouping in the functional xylem, a relationship suggesting that a group of contiguous vessels constitutes the initial establishment site for the pathogen; (2) Relative time required for plugging of the vessel group or individual vessel, through tyloses, gums or increased viscosity of the xylary fluid; (3) Relative rate of fungous growth, reproduction, and/or "toxin" production as affected by gross nutrient level of the xylary fluid, pre- and postinfection; and (4) Inhibition or promotion of fungous growth, reproduction, and/or "toxin" production by hormone-like host metabolites, pre- and postinfection.

The relationship among the factors may be expressed by a mathematical model, where these four factors are additive and where a, b, c, and d are coefficients that relate the relative importance of the factors to each other for each situation:

$$R = aV + bT + cN + dH$$

Our study is an attempt to test the hypothesis that vessel size and grouping are correlated with resistance to Dutch elm disease.

Review of literature

The movement of *C. ulmi* spores within xylem vessels of *U. americana* has been amply demonstrated. Rapid distal movement of spores through large earlywood vessels was shown when trees were bole inoculated with a massive number of spores (Banfield, 1941). An opposite movement at a much slower rate has also been shown (May, 1935; Banfield, 1938; Hart, 1960; Brown et al., 1963; Campana, 1967; Neely, 1968). Other researchers have suggested that the rapid distal movement of spores demonstrated by Banfield (1941) represented a highly artificial condition. Under conditions of beetle infection, spore movement would occur at a much lesser rate. The beetle carrier, *Scolytus multistriatus* Marsh. while feeding in twig crotches, could only inoculate with a limited number of spores. Artificial twig-crotch inoculations documented these suggestions (Brown et al., 1963; Campana, 1967). Kerling's (1955) observations on European elm likewise indicated that distal spore movement occurred at a slower rate. P^{32}-labelled *C. ulmi* spores have been observed to move into petioles and leaves of *U. americana* (Pomerleau and Mehran, 1966). Undoubtedly, spore movements within xylem vessels have appeared erratic, because the transpiration stream is erratic (Buisman, 1936; Banfield, 1938; Hart, 1960).

Vascular invasion by *C. ulmi* is known to be influenced both by time and locus of infection. The dissolution of the end walls of earlywood vessels has been correlated closely with initiation of the period of susceptibility of *U. americana* (Banfield, 1948; Campana and Hyland, 1970). Observations relating early cambial activity and the susceptibility period of elms were made in Quebec, Canada (Pomerleau, 1965, 1966).

Similarly, the increase in resistance during the growing season appeared closely related to the onset of latewood production (Pope, 1943; Banfield, 1968).

The locus of infection was shown to be a factor in vascular distribution of *C. ulmi* spores (Banfield, 1947). Vascular invasion was limited when trees 1.5–4.0 inches in diameter at breast height were inoculated in crowns; however, extensive invasion occurred where inoculations were made in boles or roots. Histological studies revealed that the extent of vascular invasion was conditioned by vessel length at the point of inoculation (Banfield, 1941). Vessels in new shoots were only a few centimeters long in contrast to those in the bole, which reached lengths of several meters (Priestley et al., 1935; Banfield, 1941; Greenidge, 1952).

Lateral movement of *C. ulmi* also must be involved in diseased trees if complete colonization is to occur. Information on such lateral movement is limited. Lateral distribution seems to be mainly from one vessel element to another and is accomplished by direct fungous growth through bordered pit membranes and cell walls Wilson, 1965; (Pope, 1943; Ouellette, 1962; MacDonald and McNabb, 1970).

The preceding discussion suggests a relationship between vascular anatomy and resistance. Wood structure had no effect on the movement of spores through either resistant or susceptible European elm (Buisman, 1936). Subsequent investigators (Dimond et al., 1949) found no difference in vessel length or vessel diameter between *U. americana* and *U. pumila*, thus agreeing with Buisman that resistance cannot be accounted for on anatomical grounds. Comparisons between the vascular anatomy of *U. pumila* and *U. americana* by Pope (1943), however, suggested an anatomical relationship to resistance. He concluded that *U. pumila* had a short susceptibility period because of its early initiation and subsequent production of latewood. In *U. americana* latewood production was delayed. He further characterized latewood by the lack of interconnections between vessels and bundles of vessels. He concluded that the fungus was isolated in that portion of the wood where inoculation was made. Elgersma (1970a) found a slower movement of water through xylem of the resistant clone 390 as compared with a susceptible *U. carpinifolia*, and suggested that narrow and short vessels might be a factor in resistance.

Materials and methods

Observations associated with other studies of ours gave the initial hint of a possible relationship between elm anatomy and resistance to Dutch elm disease. We then made preliminary observations on collections of a wide variety of material from The Netherlands and the Ames, Iowa, area. These collections included samples of Dutch hybrids (taken in early April, late June, early August and late November), of field-grown material in Ames (mid-June and mid-August) and of greenhouse plants of rooted-cutting origin (early November). When these observations further indicated a correlation between elm anatomy and resistance, the study reported herein was initiated.

Trees from the Ulmaceae representing a wide range of disease reactions were selected for critical study (Table 1 and 2). Growing conditions in The Netherlands are very different from those found in the Ames area. Therefore, the selections were divided into two groups according to the location of the collection.

A resistance rating code was developed (Table 3) based on results from artificial inoculation trials on these selections in The Netherlands.

Table 1. Data on elm material from The Netherlands.

Clone number	Parents* or selection name and origin	Year of cross	Year first vegetatively propagated
28	*Ulmus carpinifolia* Gled. from France		
P 38	*Ulmus hollandica* Mill. 'Vegeta'		
P 39	*Ulmus wallichiana* Planch. from India		
49	*Ulmus glabra* Huds. from England		
62	*Ulmus hollandica* 'Bea Schwarz' from France		
202	P265 × P39	1938	1944
215	*U. pumila pinnato-ramosa* Henry × P268	1939	1944
248	P39 × 1	1939	1947
P265	*Ulmus glabra* 'Exoniensis'		
274	P38 × 1 ('Commelin' elm)	1940	1949
P275	*Ulmus hollandica* 'Belgica'		
283	62 × 1	1942	1949
296	49 × 1 ('Groeneveld' elm)	1941	1949
390	62 open pollinated	1948	1957
476	248 × P275	1955	1962
496	202 × 302	1954	1962
497	283 × P275	1954	1962
519	215 × 274	1956	1963
579	P275 × 248	1955	1966

* Data on parent clones not used in study:
1 = *Ulmus carpinifolia* from France
302 = 1 × 28
P268 = *Ulmus carpinifolia* 'Hoersholmiensis'

Tabel 1. Gegevens betreffende iepemateriaal uit Nederland.

All xylem material came from 2-year-old twigs growing on sapling-size or larger trees. The use of 2-year-old twigs duplicated the area of natural fungous inoculation by *Scolytus multistriatus*. The twigs were of comparable size. The material from The Netherlands was collected in early April and that from Ames, in mid-June. Lengths of twigs adjacent to twig crotches were killed and fixed in formalin-alcohol-acetic acid solution (FAA) (Sass, 1958). Isopropyl alcohol was used in The Netherlands and ethyl alcohol in Iowa. All twig material was stored in FAA for up to 2 years before cross-sections were made.

For sectioning, the fixed twigs were wrapped tightly in paper towelling and cut 15 to 20 μm thick with a sliding microtome. The cross-sections were stored in distilled water at 4 °C before anatomical observations were made. Three of the Ames selections were also embedded in paraffin, cut 10 μm thick on a rotary microtome and stained with saffranin-fast green (Sass, 1958). The latter was done to check the accuracy of vessel determination in the thicker, unstained sections.

Observations were made on images of twig cross-sections projected on white card-board using a projection mirror mounted on a compound microscope. With the microscope placed at stand-up bench height, the image was projected to sit-down table height. An enlargement of × 248 was obtained thereby.Determination of vessels was aided by the appearance of bordered pits common to these cells. Vessel lumen

Table 2. Data on Ulmaceae material from Ames, Iowa, U.S.A.

Species code	Species (and selection)	Location in Ames
C.o.	*Celtis occidentalis*	ISU Campus
U.a.	*Ulmus americana*	ISU Horticulture Farm
24	*Ulmus carpinifolia* 'Christine Buisman' from Spain	ISU Horticulture Farm
U.p.	*Ulmus pumila* 'Chinkota'	ISU Horticulture Farm

Tabel 2. Gegevens betreffende Ulmaceeën-materiaal uit Ames, Iowa, U.S.A

Table 3. Resistance rating code for the 23 selections or clones used for anatomical study.

Rating code	Degree of resistance
7	Immune
6	Highly resistant
5	Resistant
4	Fairly resistant
3	Slightly resistant (or slightly susceptible)
2	Fairly susceptible
1	Susceptible
0	Highly susceptible

Tabel 3. Schaal van de graden van resistentie van de 23 Ulmaceeën-vormen.

diameter in the projected image was measured in millimeters, giving a relative unit of measure for comparison among selections.

The data represent the vessel characteristics of a constant area of xylem grown the year prior to collection (second-year growth). All earlywood and most, if not all, latewood were included in this area. For each cross-section observed, data were recorded for four different areas. Two cross-sections were checked from each of two branches collected from each elm selection. Only one tree for each selection was used. The data from the sixteen areas for each elm selection were averaged. Although variability within a selection was present, it did not affect statistically the final comparisons among the different selections.

Results and discussion

The anatomical data suggest a trend of increasing disease susceptibility correlated with increasing average size of vessel groups (Table 4). The average size of a vessel group is defined as the product of the average vessel diameter and the average number of contiguous vessels. As evidence indicates that lateral distribution of the pathogen is from one vessel element to another, we think that the vessel group constitutes a more significant unit than the single vessel (Elgersma 1970b, in this issue). Lateral movement would at first be confined to the vessel group or groups initially inoculated.

Spread of the fungus from one vessel group to another may mainly happen where

Table 4. Correlation of resistance rating and anatomical data for 23 selections of Ulmaceae collected from The Netherlands or from Ames, Iowa, U.S.A.

Ulmaceae selection (Table 1 and 2)	Resistance rating (Table 3)	Vessel diameter* (relative units)	Number of contiguous vessels*	Size of vessel group* (relative units)
		The Netherlands		
496	6	2.9	2.5	7.2
296	6	4.1	2.3	9.6
519	5	3.8	2.0	7.6
390	5	3.3	2.3	7.7
283	5	3.5	2.9	10.1
62	5	4.3	2.7	11.6
497	4	2.8	2.6	7.2
274	4	5.2	1.9	9.8
202	3	4.7	2.1	10.3
215	3	3.9	2.7	10.5
579	3	4.4	2.4	10.9
248	3	3.6	3.2	11.6
476	3	4.3	2.9	12.4
P 39	2	4.1	3.4	13.9
28	2	4.9	2.9	14.2
P 38	2	4.6	3.2	14.7
P265	2	4.3	3.4	14.7
49	2	4.9	3.3	16.0
P275	1	5.8	4.1	24.6
		Ames, Iowa, U.S.A.		
C.o.	7	4.7	1.4	6.5
U.p.	5	5.2	2.7	14.0
24	4	2.6	9.2	23.9
U.a.	0	8.0	9.1	72.8

* Average data represent 4 areas from each of 2 cross-sections from each of 2 branches.

Tabel 4. Correlatie tussen resistentiegraad en anatomische gegevens van 23 selecties van Ulmaceeën, verzameld in Nederland en in Ames, Iowa, U.S.A.

vessels or vessel groups have a non-parellel course, so that vessels that are separated by other tissue at one cross-section may be continuous higher up, and vice versa. Possible differences between elm selections for this important anatomical trait were not explored in this study.

In some selections average vessel diameter had the greater influence in reducing the size of the vessel grouping (Table 4). In others, the number of adjacent vessels was the greater contributor. For example, number of average contiguous vessels was high for *U. carpinifolia* 'Christine Buisman' (clone 24), but the average diameter of vessels was small. Many of the vessels in a group were not adjacent to parenchyma cells. Therefore we suggest that these vessels do not become plugged at this site with tyloses, thereby allowing for continued translocation of material. This could explain the rare breakdown of resistance in this selection. A visual examination and comparison of the sapwood browning symptoms between 24 and *U. americana* indicated isolated and narrow brown streaks in 24 and large, sometimes coalescing brown streaks in *U. americana.* Tchernoff (1965) used the terms "spreading" and "spotted" to describe the discolorations produced in diseased sapwood of susceptible and resistant Dutch selections, respectively.

The plugging of vessels by growth of tyloses (Beckman, 1966; Banfield, 1968), accumulation of gums (Elgersma, 1967) and a higher viscosity of xylary fluid (Dimond et al., 1949) were suggested as mechanisms of localizing or slowing the vertical distribution of the fungus. In a given period of time, the smaller the size of initial colonization (a small vessel group size), the more effective vessel plugging would be. In the comparison of the size of vessel groups between *U. americana* and *U. pumila* 'Chinkota', a five-fold difference is indicated (Table 4). Therefore, it is reasonable to conclude that, during a given time lapse after inoculation, the smaller size of vessel groups of the 'Chinkota' elm would be more effectively plugged. This would result in a localized disease reaction, the resistance exhibited by this elm selection.

In earlier preliminary observations, young greenhouse material with secondary xylem had much reduced size of vessel groups in comparison with that found in 2-year-old twigs from older trees of the same selection. This anatomical difference may contribute to the resistance (Caroselli and Feldman, 1951; Went, 1954; Heybroek, 1957) found in young trees. Sufficient young material was not available for further study.

Conclusions

Data obtained from xylem tissue representing a wide range of Ulmaceae selections clearly indicated a relationship between anatomy and susceptibility to Dutch elm disease. Failure of earlier observations (Buisman, 1936; Dimond et al., 1949) to recognize this relationship can be explained by the limited range of elm material examined. Present availability of hybrids and selections from the Dutch breeding program gave the needed range of resistant to susceptible hosts.

We would be amiss if we did not again credit Pope (1943) with suggesting a possible relationship between lateral vessel connections and latewood resistance. Although our study did not consider this phase of the problem, our thoughts relating lateral pathogen movement to lateral vessel connections led us to the concept of vessel group size.

At the present time in North America, an intensive search is being made for resistant individuals of *Ulmus americana* and of other indigenous species. We suggest that vessel size and arrangement be added to the criteria used in screening suspected resistant trees. Care must be taken, though, to make anatomical comparisons among trees grown under comparable environmental conditions. In breeding programs anatomical comparisons could be used on those individuals that survive the initial mass-inoculation screening.

Chemical modification of xylem formation in oak was reported recently (Venn et al., 1968). Application of trichlorophenylacetic acid (TCPA) resulted in xylem consisting mainly of "parenchyma, fibers, fiber tracheids with few, small, scattered vessels". The possibility of elm reacting in a similar manner to TCPA should be investigated. Possibly this chemical will be found to modify elm xylem tissue to reduce vessel group size. Such a reduction should increase the resistance of a TCPA-treated tree to Dutch elm disease. Therefore, we propose research on TCPA and methods of its application to determine if disease resistance can be induced chemically in existing susceptible elms by this or similar chemicals.

Acknowledgments

These studies were initiated during the period Ir Heybroek was on leave from Stichting Bosbouwproefstation "De Dorschkamp" to hold a Visiting Lecturer position at Iowa State University. Dr McNabb is Professor of Forest Pathology and Mr MacDonald, a doctoral candidate holding a Graduate Research Assistant appointment. The method of sectioning was developed and sectioning done by Mr Marion E. Jones Jr, Graduate Research Assistant. The assistance of Dr J. G. Isebrands, North Central Forest Experiment Station, Rhinelander, Wisconsin, and Dr David Jowett, Department of Statistics, Iowa State University in statistical matters is acknowledged. Mr A. F. Dodge, U.S.D.A. Regional Plant Introduction Station, Ames; Mrs Marcia V. Angell, Graduate Teaching Assistant, Iowa State University; Mr A. J. Heydenburg Jr, Instructor, Grand Rapids, Michigan Junior College; and Mr A. M. van den Maagdenberg, Stichting Bosbouwproefstation "De Dorschkamp", Wageningen assisted in the field collections.

This research was supported in part by an Emergency Grant from the Executive Council of Iowa and the Elm Research Institute. Collection of material in The Netherlands was made possible through contributions from the Davey Tree Expert Co., Kent, Ohio; Perin Tree Service, Des Moines, Iowa and the Tree Research Institute.

Samenvatting

Anatomische factoren in de resistentie tegen iepeziekte

Als hypothese wordt gesteld, dat resistentie tegen de iepeziekte in de eerste plaats berust op het vermogen van resistente gastheren om de uitbreiding van de ziekteverwekker (*Ceratocystis ulmi*) te beperken. Er worden vier factoren genoemd die (alleen of in combinatie) dit vermogen zouden bepalen. Een van de vier werd getoetst, en wel afmeting van vaten en aantal aaneengrenzende vaten in de buitenste jaarring op de plaats waar natuurlijke infecties plaatsvinden. Er werden 22 iepeselecties en één andere Ulmacee gebruikt, waarin de vaten gemeten en geteld werden in een constant oppervlak van de dwarsdoorsnee van het tweede-jaars-hout van twee jaar oude twijgen. Deze anatomische gegevens tonen een correlatie tussen toenemende vatbaarheid voor de ziekte en toenemende vaatgroepgrootte, die hier gedefinieerd is als het produkt van de gemiddelde vatdiameter en het gemiddelde aantal aaneengrenzende vaten (zie Tabel 4). Uitbreiding van de schimmel in dwarsrichting van de boom is om te beginnen beperkt tot die vaatgroepen die oorspronkelijk geïnfecteerd waren. Daarom zal de uitbreiding van de schimmel in de lengterichting met meer effekt door thyllen geblokkeerd kunnen worden in vaatgroepen van geringere grootte.

De verhouding tussen vaatgroepgrootte en mate van houtverkleuring na infectie wordt besproken. Grootte en rangschikking van vaten wordt ook genoemd in verband met een zekere mate van resistentie van jonge, in de kas opgekweekte bomen. Het gebruik van vaatgroepgrootte wordt voorgesteld als een maatstaf bij het selecteren van

resistente individuen uit grote populaties. Recent onderzoek over het effect van de stof TCPA op de anatomie van eiken suggereert dat het mogelijk is met chemicaliën de opbouw van het houtweefsel van iep te wijzigen en zodoende zijn vatbaarheid voor de iepeziekte te verkleinen.

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