Localization of Dutch Elm Disease in 10-yr-old White Elm Clones from Resistant Parents

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ABSTRACT

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The ability of ramets from some white elms (*Ulmus americana* and *U. laevis*) to resist *Ceratocystis ulmi* by localizing Dutch elm disease (DED) near sites of inoculation increases with age. A comparison of DED in artificially inoculated 10-yr-old ramets from localizing and normal parents showed that symptoms developed slower and were less extensive and recurrences were fewer in ramets from the localizing group. Such differences had not been demonstrable in 1- and 2-yr-old ramets of the same clones.

Some white elms (*Ulmus americana* L. and *U. laevis* Pall.) resist *Ceratocystis ulmi* (Buism.) C. Moreau, the incitant of Dutch elm disease (DED) (3,4,7). The pathogen moves less rapidly in such trees, whether naturally or artificially inoculated, than in elms of ordinary susceptibility (8), and infection becomes localized (2) near the site of inoculation.

This localizing response was questioned when small ramets from localizing and normal parent trees showed more similarity in DED than was expected on the basis of parental behavior (6,8). Young ramets from localizing parents were relatively resistant in greenhouse tests only if inoculated after the seasonal peak of susceptibility (6). In a nursery, 2- and 3-yr-old ramets from normal and localizing parents developed comparable DED symptoms but showed different degrees of entrapment of propagules of *C. ulmi* (7). We speculated, therefore, that small ramets do not provide an arena of sufficient size for localization of infection and that with increasing age and size of ramets, the distinction between localizing and normal clones would become greater.

MATERIALS AND METHODS

Ramets from normal and localizing trees of *U. americana* and from one localizing tree of *U. laevis* were grown from cuttings during 1967–1968, planted in a nursery during 1969–1970, and inoculated with *C. ulmi* in 1978. Localizing clones were defined as those that in field and greenhouse tests during 1967–1969 had been more resistant than the median of all clones tested. Each clone had a percentile rank based on many measurements. Of nine localizing clones available, eight were from trees previously selected as resistant to *C. ulmi* (7). The localizing clones, represented by 41 trees, had percentile ranks of 50–72 (av, 61). Seven normal (nonlocalizing) clones, including 42 trees, were available, and these had ranks of 19–35 (av, 29). In the trees selected for inoculation, localizing and normal groups were represented by equivalent size distributions: 2–8 m tall, 3–19 cm diameter 30 cm above ground.

An isolate of *C. ulmi* (T1037) obtained in 1977 from a wilting American elm was grown in 1% malt extract in shake culture for 3 days to a concentration of 9.35×10^7 cells per milliliter and used as inoculum. This was applied by syringe at the rate of 10 μl (about 935 cells) to each of two inoculation sites within 20 cm of one another on one twig per tree. The inoculation sites were prepared to simulate two types of injuries made by elm bark beetles (*Scolytus multistriatus* Marsh.). One type was a groove about 1.6 mm wide, made by pressing the fluted shaft of a battery-powered drill bit against a twig crotch and spinning the bit until it wounded the xylem. The other type was a 1.6-mm hole drilled through the twig about 5 mm proximal to another crotch so that xylem of the main axis of the twig was wounded. Trees were inoculated 6 June 1978 when, on the basis of new xylem development, they were at the annual peak of susceptibility. Whenever possible, the sites of inoculation were on dominant apical twigs. On the tallest trees, however, inoculum was applied to the leaders of major lateral branches about 5 m above ground.

Observations were then made of foliar symptoms, maximum diameters of twigs or branches killed (related to tangential progress of *C. ulmi*), recurrence of foliar symptoms in 1979, and (after dissection on 4 June 1979) linear extent of xylem discoloration caused by *C. ulmi* downward from the lowermost site of inoculation. A sample of discolored xylem was collected from each living tree, and 25 of these samples were drawn at random for attempted recovery of *C. ulmi* on malt agar. Data were subjected to chi-square analyses or, where appropriate, to analyses of variance for unequal numbers of observations; differences with probability less than 0.05 were considered significant.

RESULTS AND DISCUSSION

DED developed in all 83 inoculated branches. Symptoms developed slower and were less extensive in ramets from localizing parents than in those from normal parents (Fig. 1). On 1 August 1978, when the final observations of foliar symptoms were made for that year, DED "flags" (the portion of the tree with foliar symptoms) in the ramets from localizing parents averaged 1.84 m long and those in the normal group averaged 2.41 m long; the difference between groups was significant (Table 1). Nine elms in the localizing group and one in the

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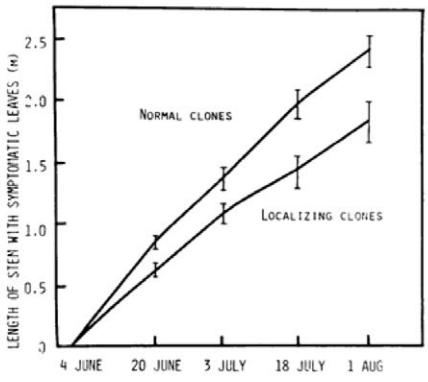

Fig. 1. Development of foliar symptoms of Dutch elm disease in ramets from localizing and normal white elms. Standard errors are indicated.

Table 1. Size and disease characteristics of 10-yr-old white elm clones inoculated with *Ceratocystis ulmi* in June 1978

Characteristics	Ramets from: Elms that localized DED[a]	Ramets from: Elms of ordinary susceptibility[a]	Significance of difference[b]
Height (m)	5.3	5.5	F = NS
Stem diameter 0.3 m above ground (cm)	9.2	7.3	F = NS
Length of wilting or yellowing part of crown 1 August 1978 (m)	1.84	2.41	F = 8.01
Trees with "flags" <1 m long 1 August 1978	9	1	$\chi^2 = 6.25$
Trees wilting systemically 1 August 1978	7	17	$\chi^2 = 5.88$
Diameter of largest dead limb 25 May 1979 (mm)	12	19	F = 6.97
Trees that wilted in 1979	3	11	$\chi^2 = 5.11$
Extent of discolored xylem from point of inoculation 4 June 1979 (m)[c]	2.57	3.43	F = 15.94

[a] Judged on the basis of responses of parent trees to inoculations during 1967-1969.
[b] All differences significant at $P = 0.05$ except for height and stem diameter. NS = nonsignificant.
[c] Discoloration not measured below ground level.

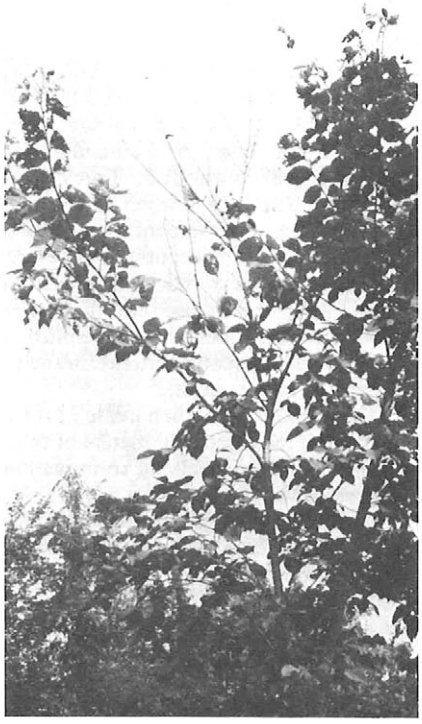

Fig. 2. Dutch elm disease in clone P26-12 of *Ulmus laevis* is restricted to small twigs of inoculated ramets.

normal group had flags less than 1 m long.

On 25 May 1979, the diameter of the stem at the proximal end of the part killed by *C. ulmi* was estimated to the nearest 2 mm. For localizing and normal groups, the mean diameters were 12 and 19 mm, respectively; this difference was also significant.

DED recurrence was recorded on 4 June 1979, before onset of symptoms from natural inoculations of that year. Wilt recurred in three trees (8%), all of one clone, of the localizing group and in 11 trees (26%), representing six clones, of the normal group. With 11 as the expected number of recurrent infections, chi-square was significant.

Discolored xylem extended 2.57 and 3.43 m in the localizing and normal groups, respectively. This difference, also significant, was conservatively estimated because measurements were made only to the ground line. Discoloration extended below ground level in 12 and 20 trees of the localizing and normal groups, respectively.

For each measurement, differences among clones within groups were insignificant owing to the amount of intraclonal variation. *C. ulmi* was recovered from 21 of the 25 samples of discolored xylem.

The significant differences in DED between the two groups of trees corroborate previous reports of resistance by localization of infection in white elms. *C. ulmi* traveled more than 2.5 m, on average, from points of inoculation in ramets from localizing parents, indicating that an arena provided by 1- or 2-yr-old ramets (6,8) may be too small for full expression of this form of resistance. Insufficiently large trees probably explains why 1- or 2-yr-old ramets from resistant parents did not localize DED (4,6).

If some American elms can localize DED, why do so few large trees persist where the disease has occurred for more than two decades? Intraclonal variation in this and previous experiments (8) suggests an explanation. In all white elms we have tested, by inoculating either multiple branches per tree or single branches of several trees per clone, some DED lesions became widespread. For example, the six ramets of one localizing clone in the present experiment had lesions averaging 2.5 m long. The extremes, however, were 0.6 m (infection confined to inoculated twig) and 5 m (infection extending into roots). Large DED lesions, especially those extending into roots, are more likely to coalesce and to lead to recurrent infection than small ones (1). The frequency with which infections are localized in small branches thus may determine longevity of trees that receive multiple natural inoculations annually. We envision the American elm that persists many years in the presence of DED as one in which most lesions are localized but which eventually succumbs because multiple lesions coalesce or an unusual single infection becomes large and recurrent.

One of the localizing clones, of *U. laevis* and designated P26-12, is of interest not only because it resists *C. ulmi* but also because it apparently resists the agent of elm yellows (phloem necrosis). This clone remained healthy after receiving bark patches from sources that caused yellows in *U. americana, U. carpinifolia,* and another clone of *U. laevis*. The parent tree had received multiple stem inoculations with *C. ulmi,* and foliar symptoms of DED never developed. In twig-inoculated ramets, damage was restricted to small twigs (Fig. 2). Such a clone could be a useful addition to disease-resistant *Ulmus* materials already developed in North America (5,9).

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