Dutch Elm Disease in Clones from White Elms Resistant and Susceptible to Ceratocystis ulmi

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This work was supported in part by a grant from the Allegheny Foundation via the Elm Research Institute. We thank Sharon Condon and John Wickham for technical help.

Accepted for publication 26 November 1973.

ABSTRACT

Ramets of Ulmus americana and U. laevis 4 mo to 2 yr old, from ortets resistant and susceptible to Ceratocystis ulmi, were inoculated with cell suspensions of C. ulmi in greenhouse tests. Ramets from resistant ortets showed less severe foliar symptoms and less extensive internal spread of C. ulmi than those from susceptible ortets when inoculated in mainstems after the annual period of maximum susceptibility or if inoculum was placed in xylem of current-season shoots. When mainstems of ramets were inoculated 4-5 wk after budbreak, no clones displayed resistance.

Additional key words: vascular wilt disease, disease resistance.

White elms, Ulmus americana L. and U. laevis Pall., are highly and quite uniformly susceptible to infection by Ceratocystis ulmi (Buis.) C. Moreau (1, 6, 15, 19, 20). Elm selection and breeding programs have generally avoided these species because of the scarcity of putatively resistant phenotypes and technical difficulties associated with the tetraploidy of U. americana (3, 4, 20, 23). A selection program first reported by Smucker (16) and later reviewed by Whitten and Swingle (22) and Clapper (2) yielded only two resistant trees from among ca. 35,000 that were artificially inoculated with C. ulmi. Moreover, these trees were later found to be susceptible to phloem necrosis (2).

Ouellet and Pomerleau (6) found no resistant individuals among artificially inoculated seedlings from open-pollinated U. americana in Quebec. The female parent trees were growing in areas where Dutch elm disease (DED) had killed most elms. Appreciable resistance was found, however, in a few seedlings among ca. 150,000 from seed subjected to X-radiation or thermal neutron bombardment (6). Lester and Smalley (4) showed low transmission of resistance in U. americana for both seed and clonally propagated materials from phenotypically resistant parents in Wisconsin.

Townsend (18) and Wester (21) reported the discovery of phenotypically resistant trees of U. laevis and U. americana, respectively. The susceptibility of these trees to other diseases, however, was not reported. Moreover, the inoculation test by which Wester detected resistant American elms was not rigorous.

Sinclair et al. (11) selected 17 white elms putatively resistant to DED from among ca. 21,000 seedlings representing several provenances. The selected trees withstood repeated systemic DED infections following inoculation of C. ulmi into the trunks. The resistance of some of the trees was associated with very slow rates of growth. However, Zahand and Sinclair (24) showed independent variation of apical growth rates and resistance levels within the selected group.

We sought to confirm the resistance of the selected trees by artificial inoculation of ramets (individual members of clones) from resistant and susceptible ortets (original trees from which clones were derived) (17). Here we report a series of greenhouse tests in which the resistance of clones from the selected trees was confirmed and shown to be strongly dependent upon test conditions.

MATERIALS AND METHODS.—Plant materials.—Thirty-five clones of U. americana and one of U. laevis were used. The latter and eight clones of the former were from putatively resistant ortets selected previously (11). The remaining 27 clones were from ortets that had survived as stump sprouts after being cut or killed to ground level as a result of DED infections in the same selection program. Ramets of 15 to 24 clones were inoculated in each of 10 experiments; nine clones were used in an eleventh test. Two to five ramets per clone were inoculated in each test.

The ramets were rooted from softwood cuttings. Rooted cuttings were transplanted into plastic pots containing ca. 2 kg of a mixture of peat moss, sand, and clay-loam soil (1:1:1, v/v/v). They were grown in a greenhouse under natural light supplemented with fluorescent lighting to provide 16-h daylength, and were fertilized at irregular intervals with a dilute solution of 20-20-20 fertilizer. They were stored for winter dormancy in a cold room at ca. 0°C or in a cold frame.

Inoculations.—In most experiments, the trees inoculated were whips 30-90 cm tall. Inocula consisted of suspensions containing approximately equal numbers of bud cells from one to six isolates of C. ulmi grown 5-7 days in malt extract shake culture. Usually a single drop of inoculum was administered to the xylem in the mainstem 2-6 cm above soil line via a gouge having a V-shaped blade 5 mm wide. The drop, carried at the tip of the gouge, was drawn into the stem as the blade was forced through the bark into the wood. Cell suspensions and drop volumes were calibrated so that each inoculum drop contained 10^5 to 10^6 germinable cells, depending upon the experiment. Cell numbers were determined by haemocytometer. Numbers of germinable propagules were determined by dilution plate technique.

Evaluation of symptoms and internal spread of the pathogen.—Weekly records of the development of external symptoms on each tree were kept. These
symptoms included epinasty, browning of tissues within veins (visible in transmitted light), temporary loss of turgor, desiccation, and browning of parts of leaf laminae or entire leaves, yellowing and premature abscission, and death of shoots or older parts of stems. The presence or absence of symptoms on each first-order lateral branch was noted and the proportion of branches free of symptoms on each tree was recorded. Data were later converted to (1- proportion healthy). Symptom data based upon first-order branches had been found in a preliminary trial to be strongly correlated with data based upon all leaves and shoots \( r = +.99 \). For record-keeping the terminal shoot was counted as a first-order lateral. If dieback and loss of branches occurred, the base for such proportional data was the number of branches initially present. A branch was judged symptomatic if any leaf on it displayed symptoms. In three tests involving 4-mo-old whips without lateral branches, the leaves were considered as first-order laterals.

The distribution of C. ulmi within inoculated trees was determined by the method of Pomerleau and Pelletier (9) using 1.5% water agar containing 200 mg of cycloheximide per liter.

Scoring of clones.—For evaluation of the relative susceptibility of a clone through the series of experiments, percentile scores were derived. The score for a given clone in an experiment was determined by: (i) ranking clones from best to worst according to each of several criteria such as proportion of branches symptomatic or time elapsed from inoculation to display of foliar symptoms; (ii) determining the number of clones that ranked lower than the given clone for each criterion and expressing this number as a percentage of the total number of clones in the experiment; (iii) averaging these percentile scores for several criteria to obtain a mean score for the clone for the experiment. The more resistant a clone, the higher its score.

Limitation of report.—All 36 clones in this study represented ortets that had survived DED infections following mainstem inoculations, although most of these trees were quite susceptible and persisted only as sprouts from stumps. As many as five of the nine clones from putatively resistant ortets (selected clones) were inoculated in a given experiment. For contrast with the selected clones in reporting results, we chose up to five clones from a group of seven representing ortets that were killed to ground level after two consecutive annual mainstem inoculations and were therefore thought to be of ordinary susceptibility. These are termed nonselected clones. Data from the remaining 20 clones, which were from ortets of intermediate susceptibility to DED, are not presented.

RESULTS.—Susceptibility related to stage of growth.—Symptoms developed relatively slowly and were less severe in selected than in nonselected clones if mainstem inoculations were made 8 wk or more after budbreak (Fig. 1). At that time apical growth had slowed or stopped and a sheath of new summerwood was forming in the stems. Mainstem inoculations during the period of maximum susceptibility (7, 13) resulted in rapid display of symptoms by both groups of clones (Fig. 1).

In one experiment, ramets of 21 clones including five each from the selected and nonselected groups were inoculated within the proximal 2 cm of a terminal shoot 5 wk after budbreak. The subsequent development of symptoms primarily reflected basipetal spread of the pathogen. Symptoms developed slowly during the 4-mo course of the experiment and were more extensive in the nonselected clones at all times (Table 1, Fig. 1-C).

Few symptoms developed as a result of inoculation of 4-mo-old whips during active growth, and the distinction between selected and nonselected clones in these tests was slight (Table 1).

The differences in average percentile scores between selected and nonselected groups of clones were greatest in experiments involving inoculation of mainstems at 8 to 11 wk, or terminal shoots 5 wk after budbreak (Table 1).

Analyses of variance were performed on symptom data from five experiments in which all clones were represented by the same number of ramets. In general, variation among ramets within clones was so great that only a few of the least and most severely affected clones differed significantly from one another at the 5% probability level. The selected and nonselected groups of clones, however, showed little overlap in severity of symptoms if inoculated after the period of maximum susceptibility or if current-season shoots were inoculated (Table 2). In the experiment involving inoculation of shoots, for example, only one of the five selected clones showed more extensive symptoms than the least affected of the five nonselected clones (Table 2).
TABLE 1. Influence of time and locus of inoculation with *Ceratocystis ulmi* on display of symptoms, and on percentile scores for symptoms shown by selected and nonselected clones of *Ulmus americana* 6 wk after inoculation

<table>
<thead>
<tr>
<th>Locus and time of inoculation</th>
<th>Branches symptomatic (%)</th>
<th>Average percentile scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mainstems 8 to 11 weeks after budbreak; 5 tests</td>
<td>Selected 24</td>
<td>Nonselected 59</td>
</tr>
<tr>
<td>Mainstems 4 to 5 weeks after budbreak; 2 tests</td>
<td>Selected 28</td>
<td>Nonselected 45</td>
</tr>
<tr>
<td>Proximal ends of current shoots 5 weeks after budbreak; 1 test</td>
<td>Selected 6</td>
<td>Nonselected 26</td>
</tr>
<tr>
<td>Mainstems of growing, 4-month-old cuttings; 3 tests</td>
<td>Selected 18</td>
<td>Nonselected 28</td>
</tr>
</tbody>
</table>

*a*Selected clones were from ootets that withstood systemic infections induced by inoculation of *C. ulmi* into mainstems in several different years. Percentile scores indicate the proportion of clones in one or more experiments that ranked lower (less resistant) than a given clone on the basis of symptom expression.

TABLE 2. Final proportion of branches symptomatic and percentile scores for symptom expression for selected and nonselected clones of *Ulmus americana* inoculated with *Ceratocystis ulmi* in mainstems after, or in current-season shoots during, the period of maximum susceptibility

<table>
<thead>
<tr>
<th>Group and clone</th>
<th>Experiments (no.)</th>
<th>Branches symptomatic at end of experiment (%)</th>
<th>Avg percentile score</th>
<th>Branches symptomatic 26 days after inoculation (%)</th>
<th>Percentile score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R9-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14 a</td>
<td>95</td>
</tr>
<tr>
<td>R23-35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32 ab</td>
<td>81</td>
</tr>
<tr>
<td>R18-2</td>
<td>5</td>
<td>24</td>
<td>81</td>
<td>55 b-c</td>
<td>57</td>
</tr>
<tr>
<td>R28-32</td>
<td>1</td>
<td>28</td>
<td>67</td>
<td>58 b-e</td>
<td>47</td>
</tr>
<tr>
<td>P26-12</td>
<td>5</td>
<td>45</td>
<td>62</td>
<td>71 c-f</td>
<td>42</td>
</tr>
<tr>
<td>R30-1</td>
<td>2</td>
<td>34</td>
<td>49</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R29-6</td>
<td>2</td>
<td>13</td>
<td>46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R21-38</td>
<td>1</td>
<td>70</td>
<td>42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R9-2</td>
<td>1</td>
<td>61</td>
<td>37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean</td>
<td>59</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonselected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R23-1</td>
<td>5</td>
<td>61</td>
<td>38</td>
<td>57 b-c</td>
<td>52</td>
</tr>
<tr>
<td>R30-25</td>
<td>3</td>
<td>58</td>
<td>46</td>
<td>93 f</td>
<td>5</td>
</tr>
<tr>
<td>R23-17</td>
<td>2</td>
<td>86</td>
<td>5</td>
<td>73 c-f</td>
<td>33</td>
</tr>
<tr>
<td>R24-84</td>
<td>2</td>
<td>62</td>
<td>4</td>
<td>80 d-f</td>
<td>29</td>
</tr>
<tr>
<td>R20-8</td>
<td>3</td>
<td>55</td>
<td>23</td>
<td>82 d-f</td>
<td>24</td>
</tr>
<tr>
<td>P9-5</td>
<td>3</td>
<td>61</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R22-19</td>
<td>4</td>
<td></td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean</td>
<td>63</td>
<td>21</td>
<td>77</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

*a*Selected clones were from ootets that withstood systemic infections induced by inoculation of *C. ulmi* into mainstems in several different years. Percentile scores indicate the proportion of clones in one or more experiments that ranked lower (less resistant) than a given clone on the basis of symptom expression.

*b*Means having any letter in common are not significantly different, *P* = 0.05.

*c*U. laevis.
Internal spread of the pathogen.—The extent of invasion of mainstems and laterals of inoculated ramets by *C. ulmi* was directly related to the proportion of branches symptomatic following inoculation of mainstems. The selected and nonselected groups averaged 54 and 80% of first-order laterals infected, respectively, in the three experiments that involved late mainstem inoculation and subsequent dissection and cultural detection of the pathogen within the tree. Immediately preceding dissection, 18 and 55% of first-order branches of the selected and nonselected groups, respectively, showed foliar symptoms. Mean percentile scores for resistance to invasion by *C. ulmi* in selected and nonselected groups of clones were 64 and 29, respectively. Corresponding scores based on symptom data were 65 and 29.

For the experiment in which terminal shoots were inoculated, the selected and nonselected groups had mean scores of 65 and 25, respectively, based on symptoms; 52 and 45, respectively, for data reflecting resistance to invasion. Thus, in this instance the two groups showed little difference in the extent of spread of *C. ulmi* into proximal parts, although they were clearly separated on the basis of display of foliar symptoms.

Another experiment confirmed that the pathogen might be present in nonsymptomatic foliage. All leaves distal to inoculation points on mainstems were stripped from 18 trees 14 days after inoculation and were judged symptomatic or nonsymptomatic. Then five leaves of each type from each tree were incubated to detect *C. ulmi*. The pathogen was found in an average of 40% of symptomatic leaves and 16% of nonsymptomatic leaves.

**DISCUSSION.**—The detection of *C. ulmi* in leaves free of external symptoms, and the absence of the pathogen in 60% of symptomatic leaves are in accord with the findings of Pomerleau (8) and MacHardy and Beckman (5), who noted that xylem dysfunction in shoots is a principal cause of foliar symptoms. Moreover, Pomerleau (8) showed that *C. ulmi* was commonly present in nonsymptomatic shoots of small, artificially inoculated trees. Thus the discrepancy between scores for symptoms and scores for resistance to invasion in one experiment of this study was not surprising.

The resistance of the selected clones relative to the nonselected group if inoculated 8 wk or more after budbreak, suggests that this resistance is due, in part, to a shorter-than-normal period of susceptibility. This could be determined by experiments of the sort reported by Smalley (13) and Smalley and Kais (14).

Less extensive spread of *C. ulmi* within mainstem-inoculated trees of the selected group than in the nonselected group was shown by the smaller proportion of sections cut from stems and branches in which the pathogen was detected. This smaller degree of invasiveness could be a reflection of the localization of infection shown by the orrets (12) but the difference between the two groups of clones is not great enough to justify much speculation.

The degree of intraclonal variation in display of symptoms in these tests may have been unavoidable. Schreiber and Roberts (10) showed that growth rates and some morphological characters of *U. americana* may vary as much within clones as between seedlings. So it is not surprising that the apparent susceptibility of ramets to DED, conditioned in part by growth rate and seasonal stage of growth (6, 13), showed variation.

The orrets selected by Sinclair et al. (11) had withstood systemic infections induced by introduction of suspensions containing ca. 10⁷ cells of *C. ulmi* into the xylem of mainstems during the period of peak susceptibility in several different years. It was surprising, therefore, that young clonal stock representing the selected orrets showed normal susceptibility when inoculated during the period of peak susceptibility in the greenhouse. In subsequent field trials, not reported here, young ramets from the selected orrets also displayed conventional susceptibility. Yet the selected and nonselected groups of clones in the greenhouse tests were differentiated on the bases of symptoms and internal distribution of the pathogen if inoculation was done after the period of maximum susceptibility. This provided confirmatory evidence for the resistance of the selected orrets.

Localization of infection occurs when branches of the resistant orrets are inoculated (12). However, we have observed symptoms leading to death of branches up to 2 cm in basal diam following natural inoculation in twig axes of these trees by *Scolythus multistriatus* Marsh. We therefore speculate that small ramets do not provide an arena of sufficient size for localization of infection; that with increasing age and size the ramets from resistant orrets will also display resistance to *C. ulmi.*

**LITERATURE CITED**

11. SINCLAIR, W. A., D. S. WELCH, K. G. PARKER, and L.