

Early Screening of Elms for Resistance to *Ceratocystis ulmi*

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ABSTRACT

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The repeatability of an early screening technique to evaluate resistance to *Ceratocystis ulmi*, the cause of Dutch elm disease, was tested with selected elm clones in their first year of growth. In controlled-environment chambers, significant differences among elm clones, incubation temperatures, and fungus strains were detected. American and English elms and the cultivar Belgica showed low levels of resistance; the cultivars Groeneveld, Dodoens, Lobel, and Plantyn showed moderate resistance; and the cultivars Regal, Christine Buisman, and Sapporo Autumn Gold showed high resistance. Aggressive and nonaggressive strains of *C. ulmi* were clearly differentiated by the range of clones and incubation temperatures employed.

Development of elm cultivars resistant to *Ceratocystis ulmi* (Buism.) C. Moreau, the cause of Dutch elm disease, is a major goal of elm-breeding programs but is normally a long process requiring several evaluation periods to characterize relative resistance levels among candidate

trees (8,9,11,12,23). Attempts have been made to accelerate resistance screening, and screening of juvenile trees appears to be useful in the early evaluation of elm breeding stocks (1,21). Controlled-environment growth chambers can provide suitable conditions for repeatable trials while also providing an opportunity to determine optimum conditions for infection and the study of host-pathogen interactions (22). Use of controlled-environment facilities also permits testing of candidates or cultivars, including some not hardy in Wisconsin, to establish performance levels for use on a national

level. This study investigates the feasibility of using controlled-environment growth chambers for screening elm clones for resistance to *C. ulmi* and provides a resistance ranking of selected elm cultivars.

MATERIALS AND METHODS

Inoculum. Isolates of *C. ulmi* were drawn from a collection assembled by E. B. Smalley in 1970 and maintained on elm wood chips at -26 C. A typical aggressive form (strain 70-99, Brown County, MN) and a typical nonaggressive form (strain 70-116, Orono, ME) were used in this study at an inoculum concentration of 10^6 spores per milliliter of distilled water (5).

Plant materials. Various elm species and cultivars were used in two trials (Table 1). American elm and the cultivar Sapporo Autumn Gold were used in both 1982 and 1983. In addition, Regal and Christine Buisman were used in the 1982 trial; Belgica, Dodoens, Groeneveld, Lobel, and Plantyn and one clone of English elm were used in 1983. All named cultivars and English elm were clonally propagated (3,20) (Table 1); the American

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elms were grown from seed of two local trees.

Inoculation procedure. Trees were wounded by drilling a hole 3 mm deep with a hand drill using a 0.8-mm bit. Inoculum was injected into each wound until runoff with a hypodermic syringe fitted with a 27-gauge needle. Each wound was then sealed with masking tape to serve as a marker and to prevent desiccation and contamination (1,10). Trees used in 1982 were inoculated 1.5–2 cm above the soil line; those used in 1983 were inoculated 3 cm above the scar of the primary bud that produced the stem.

Experimental procedure. All plants were grown in a sterilized soil mixture of two parts muck to one part sand, pruned to a single vigorous bud to form a single-stem tree (14), and staked. The plants were raised in a growth chamber in which fluorescent and incandescent lights produced about $248 \mu\text{E m}^{-2} \text{s}^{-1}$ for 12 hr per day at a constant temperature of 24 C.

At the end of 60 days, the plants were randomized, divided into five groups (1982 trial), and transferred to growth chambers maintained at 16, 20, 24, 28, and 32 C with illumination as noted before. At this point, plant materials were very uniform in height, caliper, and apparent vigor (Fig. 1). In 1983, only four temperatures were used, 20, 24, 28, and 32 C. The growth chamber set at 16 C was omitted from the 1983 trial because a few clones showed rapid onset of leaf abscission and dormancy at this temperature. Trees were allowed to acclimate to these temperatures for 1 wk before inoculation. On the date of inoculation, 20 plants of each clone were again randomized and divided into two groups. Half were inoculated with *C. ulmi* strain 70-99 and half with strain 70-116.

Thirty days after inoculation, individual elms were cut off below the inoculation point. The bark was peeled with a razor blade, working toward the apex until the length of discoloration was determined (13,14). Random isolations from non-discolored stem sections failed to recover *C. ulmi*, whereas such isolations from discolored stem sections routinely recovered the strain used for inoculation. A discoloration index (DI) was estimated for each stem as the ratio of discolored stem length to total stem length. In addition, the percentage of cross-sectional area of discoloration was estimated at the midpoint of the discolored portion of the stem. These data, though not included in this report, yielded results and cultivar rankings essentially the same as those from the DI data.

Statistical analyses. Conventional statistical procedures were used for all data analyses (18). Following an angular transformation ($\arcsin \sqrt{p}$) of the percentage data, analyses of variance were performed using the ANOVA package of the Statistical Analysis

System (6). Duncan's multiple range test was used to compare treatment means (19).

RESULTS

Vascular discoloration developed in most stems in response to inoculation by *C. ulmi*, with the presence and extent of discoloration being dependent on the clone, temperature, and strain of *C. ulmi* used. Discoloration varied from a small, brown, localized spot to brown streaks to total discoloration of the entire stem.

Analysis of variance indicated significant variation among elm clones and between the two strains of *C. ulmi*, but temperature of incubation was only

significant in the 1983 trial (Table 2).

Clones. In 1982, American elm was characterized by a high DI value (45.3%) after inoculation by either strain of *C. ulmi* and incubation at all temperatures. Under the same conditions, Sapporo Autumn Gold and Regal elms had DI values of 4.0 and 2.2%, respectively, and Christine Buisman had the smallest DI value (0.6%) (Fig. 2A, Table 3).

In 1983, the American and English elms yielded the highest DI values (92.1 and 87.2%), with Belgica only slightly lower (74.7%). The Dutch cultivars, Groeneveld (30.3%), Lobel (25.0%), Dodoens (22.5%), and Plantyn (22.0%), had only moderate DI values, whereas

Table 1. Elms inoculated in this study and their origin, including genetic background for hybrids

Clone	Trial	Origin	Propagation method ^a
American elm	1982, 1983	<i>Ulmus americana</i> L. University of Wisconsin campus	Seed
Sapporo Autumn Gold	1982, 1983	<i>U. pumila</i> × <i>U. japonica</i>	Leaf-bud
Regal	1982	(<i>U. hollandica</i> 'Vegeta' × <i>U. carpinifolia</i> No. 1) × (<i>U. pumila</i> × <i>U. carpinifolia</i> Hoersholmiensis)	Leaf-bud
Christine Buisman	1982	<i>U. carpinifolia</i> Gleditsch.	Leaf-bud
English elm	1983	<i>U. procera</i> Salisb. National Park Service, Washington, DC	Leaf-bud
Belgica	1983	<i>U. hollandica</i> Mill.	Graft
Dodoens	1983	(<i>U. glabra</i> 'Exoniensis' × <i>U. wallichiana</i> No. 39) × (open-pollinated)	Graft
Groeneveld	1983	<i>U. × hollandica</i> (<i>U. glabra</i> No. 49 × <i>U. carpinifolia</i> No. 1)	Graft
Lobel	1983	(<i>U. glabra</i> 'Exoniensis' × <i>U. wallichiana</i> No. 39) × (<i>U. hollandica</i> 'Bea Schwartz' × (selfed))	Graft
Plantyn	1983	(<i>U. glabra</i> 'Exoniensis' × <i>U. wallichiana</i> No. 39) × (<i>U. carpinifolia</i> No. 1 × <i>U. carpinifolia</i> No. 28)	Graft

^a Cultivars used as grafts were grafted onto rootstocks of *U. pumila*.



Fig. 1. View of elm plants in controlled-environment chamber just before inoculation; note uniformity of size.

Table 2. Analyses of variance (ANOVA) for the 1982 and 1983 trials

Source	1982				1983			
	df	ANOVA SS	F value	PR > F	df	ANOVA SS	F value	PR > F
Elm	3	90,382.2	88.3	0.0001	7	231,694.7	114.6	0.0001
Temperature	4	2,114.4	1.6	0.1900	3	10,857.0	12.5	0.0001
Elm × temperature	12	4,850.5	1.2	0.2900	21	11,744.2	1.9	0.0080
Strain	1	1,454.1	4.3	0.0400	1	3,906.5	13.5	0.0003
Elm × strain	3	847.6	0.8	0.4800	7	2,167.0	1.1	0.3800
Temperature × strain	4	5,810.5	4.3	0.0020	3	4,467.5	5.2	0.0020
Elm × temperature × strain	12	6,975.7	1.7	0.0600	21	7,918.0	1.3	0.2000

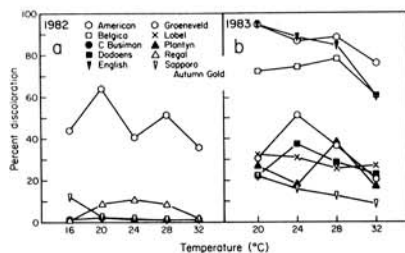


Fig. 2. Stem discoloration of selected elm clones incubated at several temperatures after inoculation with *Ceratocystis ulmi*: (A) 1982 trial and (B) 1983 trial.

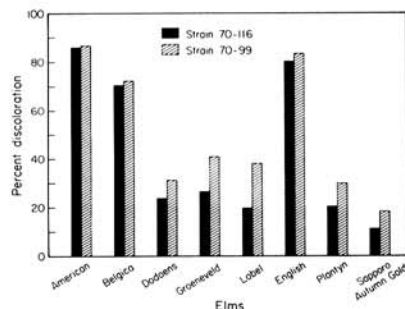


Fig. 3. Stem discoloration of selected elm clones after inoculation with two strains of *Ceratocystis ulmi* during the 1983 trial.

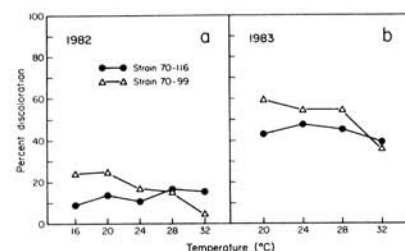


Fig. 4. Stem discoloration produced after elm inoculation with two strains of *Ceratocystis ulmi*: (A) 1982 trial and (B) 1983 trial.

Sapporo Autumn Gold had the smallest DI value (11.2%) (Fig. 2B, Table 3). No statistical comparisons are possible between years, but we attribute the increases in DI values for American elm and Sapporo Autumn Gold in 1983 to improvements in experimental procedures.

Temperatures. The DI estimate for each clone after inoculation with either strain of *C. ulmi* varied in response to temperature in the 1983 trial. The highly susceptible elms (American, English, and Belgica) yielded higher DI values at the

Table 3. Duncan's multiple range test applied to clonal means for percentage of stem length showing vascular discoloration

Clone	Means ² (%)
1982	
American elm	45.3 a
Regal	4.0 b
Sapporo Autumn Gold	2.2 bc
Christine Buisman	0.6 c
1983	
American elm	92.1 a
English elm	87.2 a
Belgica	74.7 b
Groeneveld	30.3 c
Lobel	25.0 c
Dodoens	22.5 c
Plantyn	22.0 c
Sapporo Autumn Gold	11.2 d

²Means followed by the same letter are not significantly different ($P < 0.05$).

lower temperatures after inoculation with aggressive strain 70-99. Their response to strain 70-116 was the opposite, with DI values increasing at the higher temperatures. The moderately resistant elms (Groeneveld, Lobel, Dodoens, and Plantyn) produced this same pattern of response but with lower levels of discoloration. However, the elms with the highest resistance (Christine Buisman, Sapporo Autumn Gold, and Regal) showed little differential response to either strain at any temperature; their DI values were uniformly low at all temperatures.

Strains of *C. ulmi*. In both years, strain 70-99 produced higher DI values among the elms studied, except for the highly susceptible American, Belgica, and English elms; this group showed a uniformly high response to both strains (Fig. 3).

Interactions. A temperature × strain interaction existed, with strain 70-116 producing a fairly constant level of discoloration at the lower temperatures, then increasing slightly above 28 C. Strain 70-99 caused most discoloration at 20–28 C; this decreased at 32 C (Fig. 4).

The susceptible elms (American, English, and Belgica) had slightly lower discoloration at the higher temperatures. This was also true for all the other elms, except Dodoens, which had higher discoloration at 32 than at 20 C, and Lobel, which showed no differential response to temperature.

DISCUSSION

Elms can be tested for resistance to Dutch elm disease at a very early age through use of controlled-environment facilities. This testing permits preliminary screening for disease resistance and in this study provided an opportunity to examine the effects of incubation temperatures and fungus strains and their interactions with various elm clones.

Several important aspects of the use of controlled-environment growth chambers for rapid screening are noteworthy. First, they allow for use of young plant material at a uniform stage of development. Tchernoff (21) noted that 1-yr-old elms showed internal discoloration when inoculated, and Birkholz-Lambrecht et al (1) noted the same for 8-mo-old seedlings. This study verifies the potential for using elm material less than 1 yr old for rapid screening. Early testing may overlook disease recovery mechanisms, but DI trends noted in this study correspond closely to the susceptibility differences found by Smalley and Lester (16,17) in field trials. Because controlled-environment and field results appear well correlated, we feel that true readings of resistance are being recorded even though trees are harvested before a ranking based on mortality can be constructed.

Second, environmental fluctuations that can produce variable (and inconclusive) readings of resistance are minimized. Kais et al (10) noted that environmental fluctuations affected the susceptibility of American elms to *C. ulmi*. Third, the controlled-environment growth chamber provides a relatively problem-free environment, allowing for production of vigorous trees that are at maximum susceptibility. Uniform age (within trials) of plant material and a controlled environment provide standard conditions for inoculation and disease development.

These test results demonstrate that the two strains of *C. ulmi* (70-99 and 70-116) produce consistent disease reactions at various temperatures. The aggressive strain (70-99) caused greater discoloration at lower temperatures, whereas the nonaggressive strain (70-116) produced greater discoloration at higher temperatures. When cultivar and species responses over the range of temperatures were averaged, the aggressive strain always caused more severe symptoms. In

the 1983 trial, strain 70-99 caused 52.3% discoloration, whereas strain 70-116 caused 42.9% (difference significant at $P < 0.05$). This confirmed the aggressive and nonaggressive classification (4). The critical temperatures for differentiating the responses of the clones to the two fungus strains appear to be 20 or 24 C for the aggressive strain (70-99) and 32 C for the nonaggressive strain (70-116). These results correspond closely to those of Brazier (2), who noted that the optimum temperature for growth was 30 C for his nonaggressive strain and 20–22 C for his aggressive strain.

European field tests showed the cultivar Belgica as highly susceptible (7), Groeneveld as moderately susceptible, Lobel, Dodoens, and Plantyn as having moderately high resistance, and Christine Buisman as highly resistant (8). American field tests show that Regal and Sapporo Autumn Gold also are highly resistant (15–17) but that American elm is almost always highly susceptible. Although earlier ratings were based on field tests made at various times by various people, the same relative rankings were established using controlled-environment growth chambers.

Comparisons can be made between certain species and the Dutch and American cultivars on the basis of our 1983 trial (Table 3, Fig. 3). American and English elms appeared to have the same approximate level of susceptibility, although we actually tested only a single clone of English elm, and Belgica had a slightly lower level of susceptibility. Groeneveld, Lobel, Dodoens, and Plantyn all had the same approximate level of resistance, but Lobel, Dodoens, and Plantyn were clustered (22–25%) at a level of susceptibility below that of Groeneveld. This lends support to the Dutch claim that the three former releases have greater resistance (8). Sapporo Autumn Gold had the highest level of resistance when compared with the Dutch cultivars. Regal is slightly more susceptible than Sapporo Autumn Gold to *C. ulmi* (controlled-environment growth chamber results; 16,17), and both

are slightly less resistant than Christine Buisman. Field testing of many of the Dutch clones is impossible in Wisconsin because they are not sufficiently hardy. Christine Buisman is only marginally hardy in much of the north central United States, but Regal and Sapporo Autumn Gold have shown excellent hardiness in south central Wisconsin.

Vascular discoloration can provide an accurate measure of differences in disease development (13,14). For young trees, it was a better measure than external symptoms (eg, wilting), which were often just beginning to develop 30 days after inoculation. Tchernoff (21) noted that lack of external symptoms was not sufficient proof of resistance. Indeed, in our controlled-environment screening, if visible symptoms alone had been used, a high level of resistance would have been suggested for most clones. In addition, use of vascular discoloration for ranking cultivars in terms of disease resistance correlates well with rankings obtained from earlier field trials. However, rankings obtained from controlled-environment trials are relative; therefore, standards of known response must be included in each evaluation for comparison.

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