Screening European Elms for Resistance to *Ophiostoma novo-ulmi*

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Abstract: Resistance breeding of the native elms against Dutch elm disease, caused by the fungus *Ophiostoma novo-ulmi* Brasier, is a major objective in Europe for the conservation of this tree species. More than 2,500 cuttings of 324 elm clones (*Ulmus minor* Miller, *U. glabra* Huds., *U. laevis* Pall., *U. pumila* L., *U. minor* x *U. glabra*, and *U. minor* x *U. pumila*) from eight European countries, planted in several randomized two-block designed plots were inoculated with various *O. novo-ulmi* strains. Crown wilting and dieback were recorded during the first year after inoculation. The wilting of the control trees varied among the plots, making the results difficult to compare, but analysis of variance (ANOVA) within each plot showed significant differences in disease severity among the clones tested, allowing study of the variation of the response among elm taxa. Results showed that 19 European inoculated clones recovered from *O. novo-ulmi* attack, giving hope for the reestablishment of native elms in countryside hedges and forests. FOR. SCI. 51(2):134–141.

Key Words: *Ophiostoma ulmi*, *Ulmus*, plant breeding.

ELMS occur, both naturally and cultivated, throughout much of the temperate world. In Europe, the field elm (*Ulmus minor* Miller), wych elm (*U. glabra* Huds.), and European white elm (*U. laevis* Pall.) are considered the native species (Collin et al. 2000). As a result of hybridization between *U. minor* and *U. glabra*, some hybrid trees have been selected and extensively planted (i.e., *U.* x *hollandica* Mill. in Holland and Belgium). A fourth species, the Siberian elm (*U. pumila* L.), was probably introduced in Spain during the 16th century as an ornamental tree (Gil et al. 2003) as well as in Italy during the 1930s. Natural hybridization with *U. minor* trees in these countries has produced new elm individuals, leading to a complex taxonomy (Cogolludo-Agustín et al. 2000).

The spread of the vascular wilt disease of elms, Dutch elm disease (DED), has resulted in two massive, destructive pandemics in which most mature European elms have died (Brasier 2000). The first pandemic, caused by *Ophiostoma ulmi* (Buisman) Nannf., occurred as this pathogen spread during the 1920s through the 1940s from northwest Europe into eastern Europe, and westward to the United Kingdom and Portugal. The current pandemic is caused by *O. novo-ulmi* Brasier, and has been spreading into the regions previously affected by *O. ulmi*. Owing to the enormous number of elms lost to DED, conservation of the native elm genetic resources is a major concern in Europe and considerable effort is being put into breeding disease-resistant elms (Heybroek 1993, Mittempergher and La Porta 1993, Solla et al. 2000).

In 1997, a 5-year European Union (EU) project started to coordinate the conservation of the elm genetic resources among European countries (Collin et al. 2000). One objective of the project was to screen native elms for low susceptibility to *O. novo-ulmi*. The present investigation reports an extensive field experiment, in which the differential response of elms from eight EU countries to *O. novo-ulmi* was measured. It also examines the diversity within European elm species in their recovery after inoculation and searches for potential clones to be used in landscape gardening projects, restoration programs for field hedges, and forest stands and as parents in breeding programs.

Materials and Methods

Plant Material

From 1996 to 1999, 324 elms belonging to *Ulmus minor*, *U. glabra*, *U. laevis*, *U. pumila*, *U. minor* x *U. glabra*, and *U. minor* x *U. pumila* were selected and propagated (Table

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1, Figure 1). The selection has been made from natural forests, rural areas, roadsides, parks, and gardens. The selection criteria were good sanitary status (i.e., putative DED tolerance for those trees that survived within an affected elm stand), geographic and ecologic diversity, and ornamental characteristics. To include a high degree of genetic variability, elms were selected from detached places scattered throughout the whole national territories. When available, isozyme analyses (Cogolludo-Agustín et al. 2000) and molecular markers applied to nuclear and chloroplast DNA (Cogolludo-Agustín et al. 2003) excluded elms with similar genetic patterns. Ten institutions participated in plant propagation by means of root cuttings (Tchernoff 1963) or soft and hardwood cuttings (Mittempergher et al. 1991) (Table 1). At least eight ramets were obtained per clone.

Seven inoculation plots were installed at different locations (Table 2). Plots were designed in two-blocks, with random experimental units of three to four ramets per block. Spacing was 0.5 m between plants and 1.5 m between rows, and an elm line border was included to avoid side effects. To assure plant growth, the soil was fertilized (Osmocote Plus, Scotts; once a year) and the plants were watered, if necessary. Saplings were fastened to supports to avoid being shaken by wind.

The following clones were used as controls: Sapporo [supplied by CEM, France, with high resistance to *O. novo-ulmi* (Smalley and Lester 1973)]; Lobel [supplied by IBW, Belgium, intermediate resistance (Heybroek 1976)]; Commelin [supplied by IBW, low resistance (Heybroek 1961)]; CEM077, CEM085, and CEM262 (French clones supplied by CEM, low resistance); and CNR118 (Italian clone supplied by CNR, Italy, low resistance). If available, eight ramets per control clone were planted in each plot.

Inoculations

Plants less than 1.8 m tall or affected by natural infections were discarded from inoculation. Plants were inoculated after 3 years of being propagated, and at least 1 year of growth elapsed after transplantation. Inoculations were carried out during the end of April or May for plots located in the Mediterranean countries, and during the end of May or June for plots located at higher latitudes (Table 2). Due to differences in propagation success and growth of the plantlets in Nogent-sur-Vernisson, Hann. Münden, and Madrid, inoculations were undertaken in 2 years. Plants were inoculated about 15–30 days after full leaf development, but under different meteorological conditions (i.e., in Madrid, spring occurred relatively earlier in 2001, and maximum temperatures were about 32°C 3 days before inoculation and during the following 2 weeks; spring in 2002, however, was characterized by intense rain and maximum temperatures were about 22°C before inoculation and during the incubation period).

Local strains of *Ophiostoma novo-ulmi* were used for inoculations. For example, in Firenze and Madrid, well characterized isolates obtained from a freeze-preserved material (at −20°C) were used for the preparation of inoculum (Table 2). The inoculum consisted of a bud-cell suspension of the fungi grown in Tchernoff's medium (Tchernoff 1965). Conidia were centrifuged to eliminate the medium, and suspended in sterile distilled water (10^6 spores/mL). Two droplets of inoculum were introduced into the xylem by drawing them into the lower part of the upper third of the main stem from the tip of a syringe while cutting transversally through the bark into the wood.

Data Collection, Criteria for Selection, and Statistics

Plant height was measured on dormant trees before inoculation. The percentage of the crown showing wilting or death of the foliage was visually estimated 4, 10, and 16 weeks after inoculation, using a 5% interval. A final assessment of the percentage of the crown showing dieback was carried out 1 year after inoculation.

Sapporo and CEM085 control clones were inoculated in most plots, and due to the consistency of symptoms among trials, they were used to compare the severity of inoculations. The degree of severity was assigned for each trial:

Table 1. Plant material specifications

Origin	Propagation		Clones per species*						Total
	Type†	Yr	M	G	L	P	MG	MP	
Belgium (IBW††)	S, H	1999	13	1	2	—	5	—	21
France (CEM)	S	1997/1998	6/17	—/6	12/18	—	7/13	—	79
Germany (FVA)	S	1998	—	2	7	—	1	—	10
Germany (HLF)	S	1998	1	25	2	—	—	—	28
Germany (NFV)	S	1998/1999	1	3	—	—	1	—	5
Greece (FRI)	R	1998	8	—	—	—	—	—	8
Italy (CNR)	H	1996	64	4		2	3	11	84
Portugal (EFN)	R	1997	9	—	—	—	—	—	9
Spain (UPM)	R, H	1997/1998	33/32	—	—/1	—	—	1/4	71
Sweden (SKS)	S	1998	2	5	2	—	—	—	9

* *Ulmus minor* (M), *U. glabra* (G), *U. laevis* (L), *U. pumila* (P), *U. minor* x *U. glabra* (MG), *U. minor* x *U. pumila* (MP).

† Softwood cuttings (S), hardwood cuttings (H), root cuttings (R).

†† Institutes participating in propagation: Inst. voor Bosbouw en Wildbeheer (IBW), Cemagref (CEM), Forstliche Versuchs- und Forschungsanstalt (FVA), Hessen-Forst FIV (HLF), Niedersächsische Forstliche Versuchsanstalt (NFV), Forest Research Institute (FRI), Consiglio Nazionale delle Ricerche (CNR), Estaçao Florestal Nacional (EFN), Universidad Politécnica de Madrid (UPM), The National Board of Forestry (SKS).

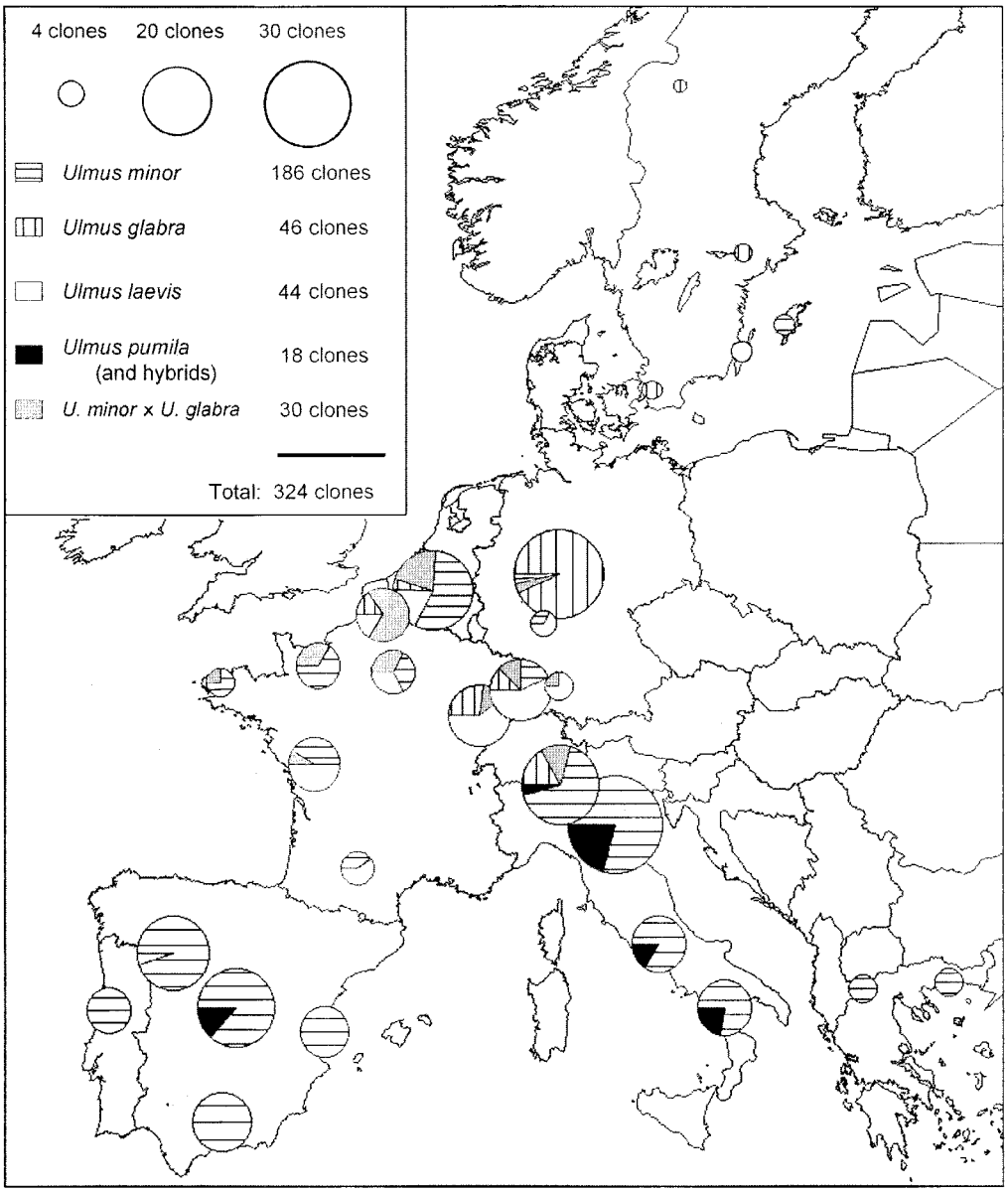

Figure 1. Origin of the elm clones tested for resistance to *Ophiostoma novo-ulmi*.

Table 2. Location of plots and inoculation dates

	Coordinates	Altitude (m)	Plant origin	Installation (yr)	Inoculation date (strain)
Geraardsbergen, Belgium (IBW*)	50°47′N 03°56′E	30	Belgium	1999	05/31/01† (local)
Nogent-sur-Vernisson, France (INRA)	47°51′N 02°45′E	148	France	1999	06/06/01 (local) and 05/22/02 (local)
Freiburg, Germany (FVA)	47°59′N 07°51′E	278	Germany	2000	05/31/01 (local)
Hann. Münden, Germany (HLF)	51°26′N 09°38′E	132	Germany, Sweden	2000	06/05/02 (local) and 06/11/03 (local)
Thessaloniki, Greece (FRI)	40°30′N 23°05′E	90	Greece	1999	05/13/02 (ASP1)
Firenze, Italy (CNR)	43°47′N 11°15′E	50	Italy	1998	05/17/01 (H328)
Madrid, Spain (UPM)	40°27′N 03°46′W	660	Spain, Portugal	1998–99	05/21/01 (V-NG) and 04/29/02 (OR-VR)

* Institutes in charge of inoculation: Inst. voor Bosbouw en Wildbeheer (IBW), Institute National de la Recherche Agronomique (INRA), Forstliche Versuchs- und Forschungsanstalt (FVA), Hessen-Forst FIV (HLF), Forest Research Institute (FRI), Consiglio Nazionale delle Ricerche (CNR), Universidad Politécnica de Madrid (UPM).

† Month/day/yr.

low, medium, or high, if average wilting of both clones (10 weeks after inoculation) was 0–20%, 21–40%, or 41–100%, respectively. If one of these clones was not present within a trial, comparisons of wilting among other control clones were used. Clones were selected as resistant if their maximum wilting (10 weeks after inoculation) and dieback were less than 15%, 35%, and 45% for trials assigned with a low, medium, and high degree of severity. These thresholds were established to yield a desired number of resistant clones.

An angular transformation of the wilting and dieback percentages (x) was performed to normalize data before statistical analysis [$y = (x/100)^{1/2}$] (Sokal and Rohlf 1995). For each trial, plant height, wilting, and dieback were analyzed using a multifactor analysis of variance (ANOVA), considering the block and the species as factors. Tukey tests were applied to compare averages ($P \leq 0.05$ and $P \leq 0.01$). Regression analyses were made and correlation coefficients (r) calculated among values of height, wilting, and dieback for each trial and species. Statistics were carried out using Statgraphics Plus version 5.1.

Results

Considering all trees in a trial, moderate to high correlation coefficients were found between symptom expression variables ($r > 0.60$; $P \leq 0.01$). Considering species, wilting values occurring in elms 4 and 10 weeks after inoculation were strongly correlated ($r > 0.70$; $P \leq 0.0001$). In some trials, wilting values 10 weeks after inoculation were significantly correlated with preinoculation height values (Table 3). Regression analyses between wilting values 10 weeks after inoculation and dieback values 1 year after inoculation provided coefficients ranging from −0.30 to 0.80, some of them nonsignificant (Table 3). For most of the trials, maximum wilting percentages were observed 10 weeks after inoculation (not for *U. laevis* clones in Nogent-sur-Vernisson, showing maximum wilting 16 weeks after inoculation). In general, wilting 4 weeks after inoculation underestimated disease response, whereas assessment at 16 week was somewhat confounded by re-sprouting or leaf damage during summer (i.e., *Xanthogaleruca luteola* Müller in Thessaloniki). Since wilting 10 weeks after inoculation provided the highest F-ratio values in the ANOVAs (results not shown), this measurement was used for further statistical analyses.

The height and susceptibility of the control clones varied within the trials (Figure 2), making the results difficult to compare. Significant interactions location x clone were found because of inconsistent wilting of CEM262 and Commelin clones in Hann. Münden and in Firenze, respectively. However, the wilting ranking of the other control clones remained consistent regardless of the location and the year. Low wilting values were observed not only for Sapporo but also for CEM085 in Madrid$_{2001}$ (Figure 2B), this trial being unique as lowest in severity. Inoculations in Nogent-sur-Vernisson, Freiburg, Thessaloniki, and Firenze resulted in symptoms of medium severity, and inoculations in Geraardsbergen, Hann. Münden, and Madrid$_{2002}$ produced very severe symptoms.

Average wilting values for *U. glabra* clones were higher than for *U. minor* and *U. laevis* clones ($P \leq 0.05$) (Figure 3), except in the Geraardsbergen trial, in which only one *U. glabra* clone was inoculated. In all trials, average wilting values for *U. minor* clones were similar or lower than for *U. laevis*. For the hybrids, average wilting values for *U. minor* x *U. glabra* were similar or intermediate to those for *U. minor* and *U. glabra*. However, average wilting values for *U. minor* x *U. pumila* were lower than the average wilting for *U. minor*, the two *U. pumila* clones included in Italy (Figure 3; Firenze$_{2001}$), and similar to Sapporo in Spain (Madrid$_{2001}$).

Symptom expression from tree to tree within each clone varied considerably, and so did the wilting and dieback values from clone to clone within each taxon (Table 4). Wider ranges of symptom expression among different clones were observed for *U. minor*, especially in plant material from France, Italy, and Spain. The lowest variation was observed for *U. glabra*, with wilting values ranging from 85 to 100% in the trial containing more *U. glabra* clones (Hann. Münden$_{2002}$, $N = 25$). In this trial, mortality

Table 3. Correlation significances between wilting values of elm trees (10 weeks after inoculation with *Ophiostoma novo-ulmi*) and other variables

Wilting, 10 weeks after	Plant origin	Pre-inoculation height						Wilting, 16 weeks after						Dieback, 1 yr after					
		M*	G	L	P	MG	MP	M	G	L	P	MG	MP	M	G	L	P	MG	MP
Geraardsbergen$_{2001}$	Belgium	ns†		ns		ns		+3		+2		+3		+3		+1		+2	
Nogent-sur-V.$_{2001}$	France	ns		ns		−2				+3				+3		−1		ns	
Nogent-sur-V.$_{2002}$	France	ns	ns	ns		ns		+3	+3	+3		+3		+3	+3	+3		+3	
Freiburg$_{2002}$	Germany								ns	+3					ns	+1			
Hann. Münden$_{2002}$	Germany	ns	+3	ns		ns		+3	+3	+3		+3		ns	+3	+3		ns	
	Sweden	ns	+2	+1				+2	+3	ns				ns	+3	ns			
Hann. Münden$_{2003}$	Germany	ns	+2					+3	+3										
Thessaloniki$_{2002}$	Greece	ns												+3					
Firenze$_{2001}$	Italy	+1	ns		−2	+3	−3	+3	+3		+3	+3	+3	+3	+3				
Madrid$_{2001}$	Spain	−3					+1	+3					ns	+3			+3	+3	+3
Madrid$_{2002}$	Spain	+3					+3	+3					+3	+3					+3
	Portugal	ns						+3						+3					

* *Ulmus minor* (M), *U. glabra* (G), *U. laevis* (L), *U. pumila* (P), *U. minor* x *U. glabra* (MG), *U. minor* x *U. pumila* (MP).
† Nonsignificant (ns); numbers indicate positive and negative correlations at $P \leq 0.10$ (1), $P \leq 0.05$ (2), and $P \leq 0.01$ (3).

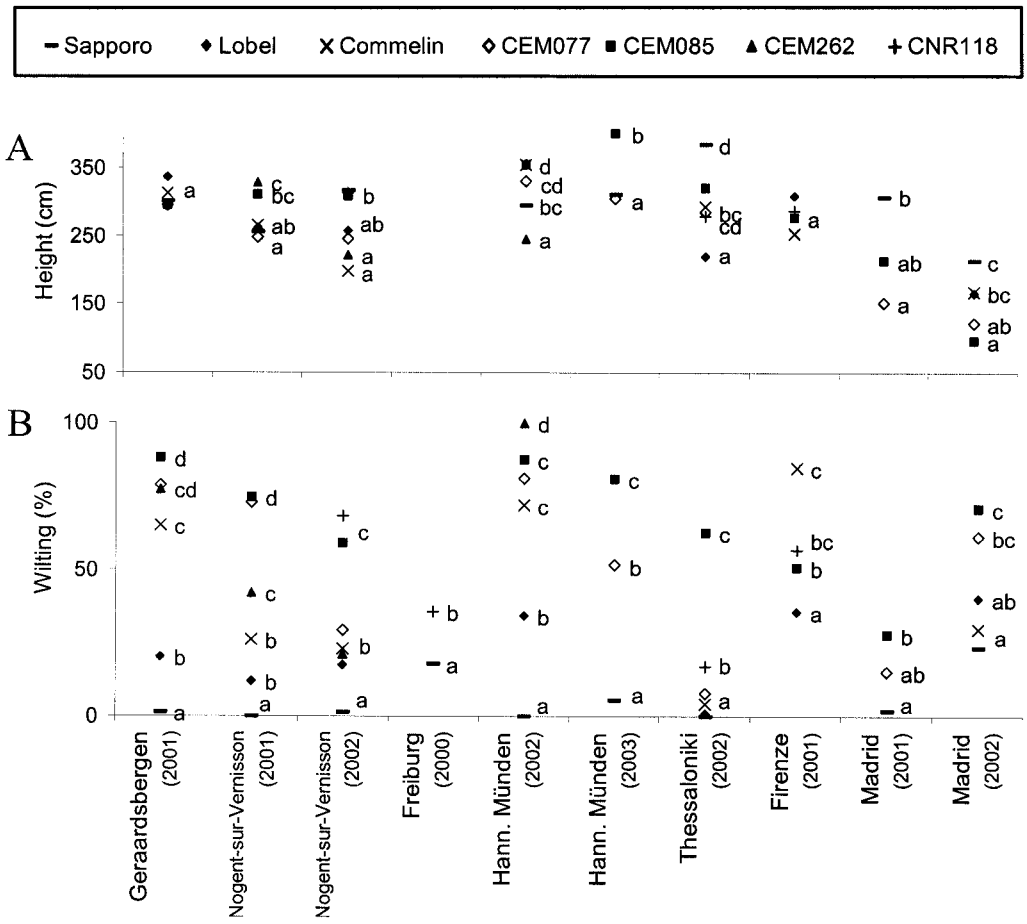

Figure 2. Average height of control clones before inoculation (A), and average wilting 10 weeks after inoculation (B), at different locations and years ($N > 6$). Different letters show differences between clones within the same trial ($P \leq 0.05$).

of individual *U. glabra* trees 1 year after inoculation was 68%. Several clones of *U. minor* x *U. glabra* (Nogent-sur-Vernisson$_{2001}$) and *U. minor* x *U. pumila* (Firenze) showed as much as 45% wilting 10 weeks after inoculation, but had fully recovered the following year (Table 4). According to the degrees of severity in each trial, 19 clones were selected because of their resistance to *O. novo-ulmi* (Table 5).

Discussion

The objective of the present investigation was to select from the native European elm gene pool a number of cultivars with superior resistance against DED. Several elm cultivars—the result of natural selection and conventional breeding—have been released in Europe (Heybroek 1993, Santini et al. 2002) or have been reported to be candidates for release due to their rooting ability, good growth and shape, and resistance to DED (Pinon et al. 1999, Solla et al. 2000). In this investigation, differential responses of elm clones to *O. novo-ulmi* allowed a first selection of putative resistant native elms.

Differences observed in the amount of defoliation among control clones could respond both to differences in the environment conditions and in the virulence of the fungal strains used. Time of inoculation can also affect symptom expression, as reported earlier (Lester and Smalley 1972b, Townsend et al. 1995, Townsend and Douglass 2001). According to the results, inoculation in Madrid$_{2001}$ (30 days after full leaf development) seems to have been performed late. The extent to which environmental conditions influence the variation of wilting (Smalley 1963, Sutherland et al. 1997, Solla and Gil 2002) highlights the importance of including into the trials well-defined control trees. The resistance of the cultivar Sapporo over Lobel supports previous results reported by Green and Guries (1985), and Pinon et al. (1999). The consistency in symptom expression of these clones, together with CEM085, makes them very good candidates as control standards for future screening work.

Differences in disease resistance among European elm species have already been reported (Townsend 1971, Brasier 1977), *U. glabra* being the most susceptible followed by *U. minor*, *U.* x *hollandica*, and *U. laevis*. In other studies, seedlings of *U. laevis* have also been reported to be more susceptible than seedlings of *U. glabra* and *U.* x *hollandica* (Went 1938, Smucker 1941). *Ulmus pumila* is well-known to have a high level of resistance (Lester and Smalley 1972c, Santamour 1973), and is currently used in Italy and Spain for breeding purposes. Results presented

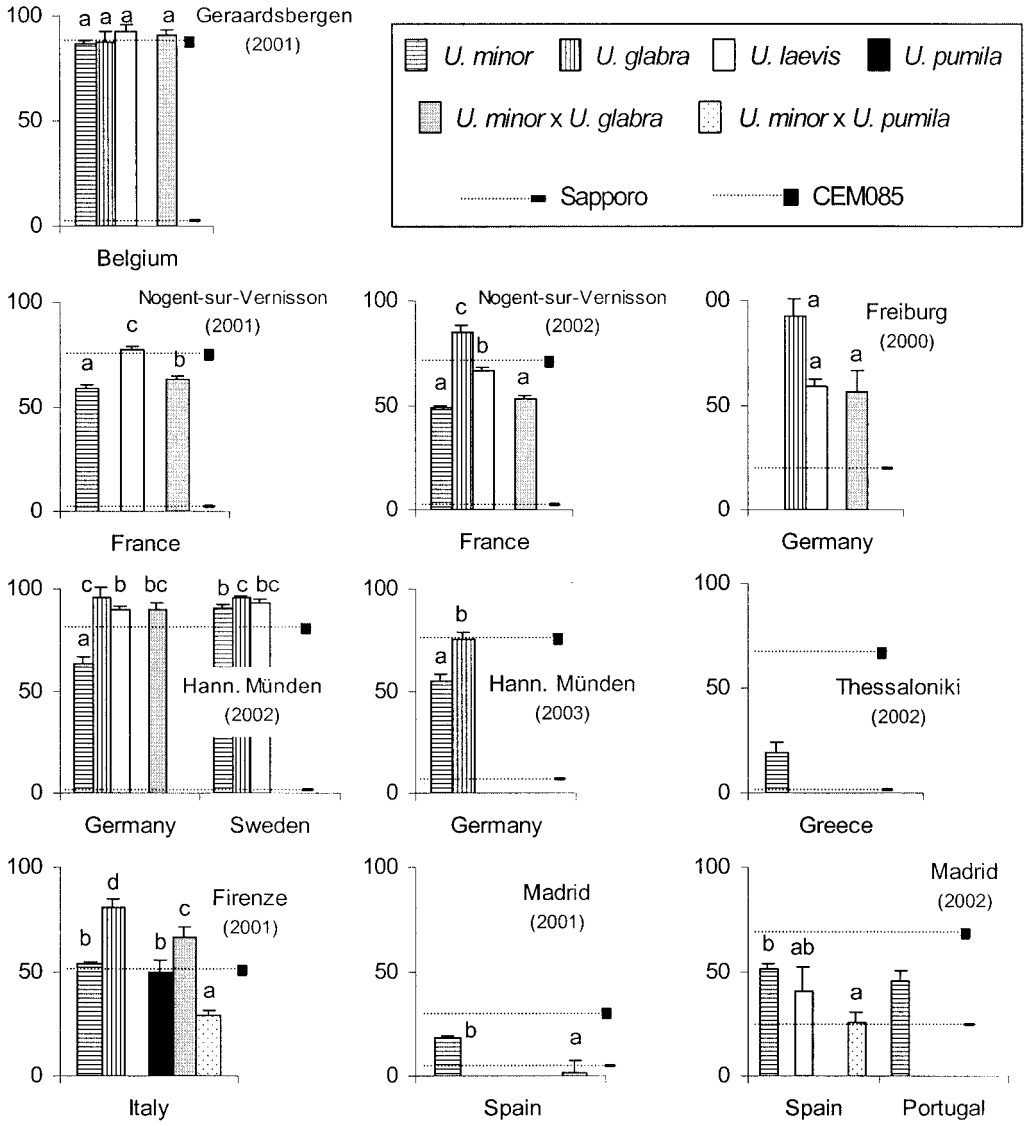

Figure 3. Average wilting 10 weeks after inoculation of elm species from different countries, being inoculated at different locations and years. Discontinuous lines in each trial indicate average wilting 10 weeks after inoculation of SAPPORO and CEM085 control clones. Vertical bars represent standard errors, and different letters show differences between taxa within the same trial ($P \leq 0.05$).

Table 4. Ranges of clone means for wilting and dieback percentages after inoculation with *Ophiostoma novo-ulmi*

Trial	Plant origin	Wilting, 10 weeks after (%)						Dieback, 1 yr after (%)					
		M*	G	L	P	MG	MP	M	G	L	P	MG	MP
Geraardsbergen$_{2001}$ (H†)	Belgium	60–95	85	90–95		80–100		20–100	100	85–90		50–100	
Nogent-sur-V.$_{2001}$ (M)	France	45–85		60–90		45–90		10–90		5–100		0–45	
Nogent-sur-V.$_{2002}$ (M)	France	10–80††	55–100	40–85		20–95††		5–45	40–80	25–65		10–70	
Freiburg$_{2002}$ (M)	Germany		100	85–100		85			100	55–100		85	
Hann. Münden$_{2002}$ (H)	Germany	40–55††	85–100	60–100††		90		35	65–100	30–100		90	
	Sweden	90	95–100	90–100				90–100	90–100	65–100			
Hann. Münden$_{2003}$ (H)	Germany	55	65–90										
Thessaloniki$_{2002}$ (M)	Greece	10–30††						35–90					
Firenze$_{2001}$ (M)	Italy	20–100††	70–100		30–70	60–80	5–75††	15–100	80–95		40–75	70–80	0–65
Madrid$_{2001}$ (L)	Spain	5–55††					0††	0–55					5
Madrid$_{2002}$ (H)	Spain	20–95††		40			5–60††	5–100		80			10–65
	Portugal	30–65						10–80					

* *Ulmus minor* (M), *U. glabra* (G), *U. laevis* (L), *U. pumila* (P), *U. minor* x *U. glabra* (MG), *U. minor* x *U. pumila* (MP).
† Degree of severity: high (H), medium (M), low (L).
†† Denote that clone selection was permitted (see Table 5).

Table 5. Clones selected after inoculation with *Ophiostoma novo-ulmi*

Trial	Plant origin	Clone	Species*	Pre-inoculation height (cm)	Wilting, 10 wks after (%)	Dieback, 1 yr after (%)
Nogent-sur-V.$_{2002}$ (M†)	France	CEM144	M	231	12	2
		CEM115	M	227	34	18
		CEM350	M	229	35	23
		CEM041	MG	297	35	29
Hann. Münden$_{2002}$ (H)	Germany	HLF374	M	292	42	29
Thessaloniki$_{2002}$ (M)	Greece	FRI902	M	224	9	10
		FRI910	M	212	12	13
		FRI905	M	288	13	10
Firenze$_{2001}$ (M)	Italy	CNR104	M	360	19	17
		CNR014	M	302	28	19
		CNR155	M	223	31	30
		CNR089	MP	417	7	1
		CNR196	MP	354	11	12
		CNR212	MP	351	15	14
		CNR029	MP	416	17	14
Madrid$_{2001}$ (L)	Spain	UPM093	M	241	4	0
		UPM130	M	227	5	2
Madrid$_{2002}$ (H)	Spain	UPM007	MP	281	21	25
		UPM026	M	183	27	36

* *Ulmus minor* (M), *U. minor* x *U. glabra* (MG), *U. minor* x *U. pumila* (MP).
† Degree of severity: high (H), medium (M), low (L).

here confirm the literature, but should be considered with caution because of the number of trees used. In some countries, the inoculated trees represent only a small sample of the diversity probably present. Since susceptibility to DED varies with plant age (Heybroek 1957, Smalley and Kais 1966) and this variation does not follow the same trend in all elm species (Townsend 1971), it is not easy to rank elm species according to their resistance.

The observed relation among symptom variables (Table 3) could suggest that disease development is fairly constant within a species and that the ranking of the trees remains consistent with time (Lester and Smalley 1972a). However, this did not happen in all cases. The wide degree of differences among elms in their ability to recover from inoculation as found in the present study demonstrates that it is important to assess the disease responses over a long period of time.

In previous studies, preinoculation height and symptom expression were reported to be positively related (Ouellet and Pomerleau 1965, Sinclair and Larsen 1980) or negatively related (Townsend and Douglass 2001). In the present work both situations occurred, i.e., in Madrid$_{2001}$ taller *U. minor* trees were associated with lower wilting values but the following year taller *U. minor* trees showed greater wilting. The biological basis for these differences warrants further investigation but the authors believe that climatic conditions may play an important role in this behavior.

European breeding programs have been successful in increasing DED resistance only through Asian hybridization. Up to now, no native clone has exhibited a sufficiently high level of resistance to *O. novo-ulmi*. The selection presented here has been identified on its short-term response to *Ophiostoma* infection, as most breeding programs do (Lester and Smalley 1972b, Ware 1992, Solla et al. 2000). The 19 selected elms should be propagated and tested again during two consecutive seasons in a common plot. It would be convenient to determine the long-term response of these elms to *O. novo-ulmi* through a study such as the one conducted by Townsend and Douglass (2001) for American elms. Their adaptability to different environments should be studied through trial plots in European countries (Heybroek 1983), and their vegetative and sexual propagation should be tested with the purpose of a definitive selection.

In summary, results presented here confirm on the one hand the high susceptibility of *U. glabra* and *U. laevis* to *O. novo-ulmi*, but on the other hand show promising levels of resistance of *U. minor* x *U. pumila* hybrids. Although most of the *U. minor* clones showed a high average wilting, variability within this species or within the hybrids with *U. glabra* and *U. pumila* appears sufficient to yield individuals with high levels of resistance. Thus, this level of resistance might be profitably used to select clones for the reestablishment of European elms, their release to the public, the restoration of landscapes, and for maintenance of the biodiversity of hedges and forests.

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