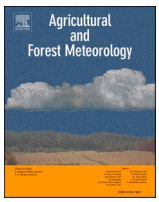
Agricultural and Forest Meteorology 359 (2024) 110294

Contents lists available at ScienceDirect

Agricultural and Forest Meteorology

journal homepage: www.elsevier.com/locate/agrformet



Genotype-by-environment interaction in Dutch elm disease resistance

Jorge Domínguez [a,c], David Macaya-Sanz [b], José Alberto Ramírez-Valiente [b], Juan A. Martín [c,*]

[a] Centro Nacional de Recursos Genéticos Forestales Puerta de Hierro. TRAGSA., Ctra. de La Coruña, Km 7.5, 28040, Madrid, Spain
[b] Department of Forest Ecology & Genetics, Institute of Forest Sciences (ICIFOR-INIA), CSIC, Ctra. de La Coruña, Km 7.5, 28040, Madrid, Spain
[c] ETSI Montes, Forestal y del Medio Natural. Universidad Politécnica de Madrid, c. José Antonio Novais 10, 28040, Madrid, Spain

ARTICLE INFO

Keywords:
Dutch elm disease
Breeding
Resistance
Ulmus
Ophiostoma novo-ulmi
Phenotypic plasticity

ABSTRACT

Dutch elm disease (DED) is a devastating forest disease. Recently, the deployment of native resistant cultivars has prompted initiatives of elm reintroduction in Europe and North America. It is known that DED resistance varies with the tree genotype and is influenced by climatic factors. However, genotype-by-environment interactions in DED resistance remain largely unexplored. In this work, we examined whether there is genetic variation in DED resistance plasticity and the roles of tree growth, phenology and anatomical traits in plastic responses. We established two experimental plots with 12 *Ulmus minor* genotypes in two environmentally contrasting locations in Spain: Madrid, under an inland continental climate, and Valencia, under a coastal Mediterranean climate. We monitored growth and phenology detecting high plasticity in both traits. In the inland plot, genotypes were taller and showed a more synchronized phenology than in the coast. A first DED-pathogen inoculation was carried out 45 days after the average flushing date in each location, after which trees exhibited more symptoms inland. A second inoculation was carried out by dividing the coastal plot trees into early and late flushing trees and inoculating each group at 45 days after its average flushing date. Therein, susceptibility rose to a level close to the inland plot. In both inoculations, we detected a significant genotype-by-location interaction in DED resistance. The xylem anatomy revealed high plasticity and a significant genotype-by-location interaction in most traits. In the coastal trial, trees formed narrower vessels and stored more starch before inoculation. The synchrony of leaf phenology, higher growth rate, lower starch reserves and higher structural vulnerability of earlywood to DED possibly favored susceptibility in the inland plot. The varying responses of genotypes in phenology, growth and anatomy at both locations were likely related to the differences in DED resistance, which can have important consequences for elm reintroduction.

1. Introduction

In Southern Europe, the most severely degraded elm species as a consequence of the Dutch elm disease (DED) pandemics is *Ulmus minor* Mill. (field elm), which before the pandemics was widely present in riparian formations and floodplains (Martín et al., 2019). It was more abundant in the Mediterranean region where it frequently played a role as a dominant riparian tree (Mackenthun 2000). In this region, characterized by summer drought, the microclimatic, geological, and orographic diversity of the Mediterranean areas determines the existence of a wide spectrum of riparian communities. The Iberian populations of *U. minor* constituted the least demanding riparian communities in soil moisture availability. For this reason, their position in the transverse zoning was the furthest from the riverbed, and can be considered a mesophyte in the proximity of the zonal vegetation.

The dramatic consequences of the DED pandemics on European and North American elm populations since the last 100 years have motivated profuse research on elm resistance mechanisms against the DED pathogens *Ophiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier (e.g. Peace 1960; Heybroek 1993; Collin et al. 2020; Mittempergher and Santini 2004; Martín et al. 2021). These vascular pathogens spread effectively within the xylem vessels causing massive vessel dysfunction and rapid foliage wilting (Ouellette et al. 1962), and are transmitted from infected to healthy trees by elm bark beetles in the genera *Scolytus* and *Hylurgopinus* (Collins et al. 1936). Tree resistance to DED is associated with constitutive factors such as the anatomy of xylem vessels (Elgersma 1970; McNabb et al. 1970; Moravčík et al. 2022) and with the ability of the plant to activate effective defense mechanisms after infection, an ability which is influenced by environmental factors. Phenotypic traits, such as tree growth, vessel diameter and length

* Corresponding author.
E-mail address: juan.martin.garcia@upm.es (J.A. Martín).

https://doi.org/10.1016/j.agrformet.2024.110294
Received 15 April 2024; Received in revised form 25 October 2024; Accepted 28 October 2024
Available online 2 November 2024
0168-1923/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(Elgersma 1970; McNabb et al. 1970; Sinclair 1975; Martín et al. 2013; Beier and Blanchette 2020), foliar phenology (Santini et al. 2005), plant chemistry, oxidative stress control and several other physiological traits (Büchell et al. 2016) have frequently been related to DED resistance. Other factors depending on both the plant genotype and the environment, such as the tree mycobiome composition, also influence the tree resistance level against biotic and abiotic stress (Martinez-Arias et al. 2021; Macaya-Sanz et al. 2023). In addition, environmental parameters, such as light intensity, temperature, soil moisture or nursery growing conditions (e.g. pots vs ground) have also been associated with DED resistance (Kais et al. 1962; Sutherland et al. 1997; Solla and Gil 2002).

In spite of the valuable advances in the characterization of DED resistance mechanisms, there is a lack of knowledge on the phenotypic plasticity of DED resistance, that is, the ability of genotypes to express contrasting DED resistance levels under different environmental conditions. Because the plasticity of a given trait is a property of the genotype, it might be heritable, exhibiting genetic variation. These genetic differences in phenotypic plasticity are also known as genotype-by-environment interactions. Thus, elm reintroduction actions using DED resistant genotypes should take into account possible genotype-by-environment interactions in resistance, which, to our knowledge, has never been studied. Each elm genotype should be only planted in areas where the timely activation of resistance mechanisms against DED pathogens is not compromised by environmental factors inducing enhanced susceptibility. Among the main factors affecting DED resistance is the timing of bud burst (Sinclair and Campana 1978; Ghelardini and Santini 2009). Therefore, different temperature regimes during spring affecting leaf emergence phenology, such as the divergent maritime and continental climates, could affect tree resistance.

In this work, our goal was to evaluate whether contrasting environmental conditions affect the DED resistance level (i.e. plasticity in DED resistance), whether there is genetic variation in such plasticity (i.e. the genotype-by-environment interaction), and to what extent plasticity in DED resistance is related to changes in tree growth, bud burst, and xylem anatomical traits. For this purpose, we evaluated these parameters in several *U. minor* genotypes under two common garden experiments under different environmental conditions, with an emphasis on two contrasting climatic regimes: i) an inland continental Mediterranean climate (hereafter inland environment), and ii) a coastal Mediterranean climate (hereafter coastal environment). The two experiments also varied in terms of soil properties (sandy vs clay soil) and other experimental conditions (see Materials and methods). However, how these specific climatic, edaphic, or experimental conditions individually affected tree phenotypes was beyond the scope of this work.

We hypothesize that environmental differences between the two trials have important consequences for DED resistance. We also hypothesize that there is genetic variation in the plasticity of the resistance (i.e. significant genotype-by-environment interaction) associated with the plasticity of tree growth, phenology, and xylem anatomical traits.

2. Materials and methods

2.1. Plant material and study sites

We selected eleven *U. minor* genotypes, seven of which originated from controlled crosses among four *U. minor* trees (M-DV4/4, J-CA2, GR-DF3 and AB-AL1). The susceptibility to DED of these four parent trees was determined through several inoculation trials conducted during the regular resistance screening tests carried out by the Spanish elm breeding program between 2009 and 2019. The results of these inoculation trials concluded that these four genotypes can be considered moderately susceptible to DED. Specifically, MDV4/5 showed foliage wilting ranging from 35.0 % to 62.1 %; J-CA2 ranged from 30.8 % to 57.1 %; GR-DF3 ranged from 42.2 % to 50.9 %; and AB-AL1 ranged from 35.5 % to 70.6 % (authors, unpublished results). The other four genotypes were straight clonal replicates from lingering trees collected from four locations across Spain. Both the parents and the ortets are kept in an *ex situ* conservation plot in Madrid, Spain (Table 1). These eleven genotypes were propagated through aerial winter stem cuttings in 2013 and remained in the nursery (in 3 l pots) until their plantation in the two experimental field plots. Generally, between ten and twelve clonal replicates per genet were planted in each plot (Table 1). Additionally, at least ten ramets of the resistant hybrid cultivar Sapporo Autumn Gold (*U. davidiana* var. *japonica* × *U. pumila* with high DED resistance) were planted in each plot as a negative control. The coastal plot was placed in November 2014 at the breeding center of Alacuás (39°27′03″ N; 0°28′43″ W; 40 m.a.s.l.; Valencia, Spain), whereas the inland plot was deployed in January 2015 at the center of Puerta de Hierro (40°27′24″ N; 3°45′0″ W; 700 m.a.s.l.; Madrid, Spain) (Fig. 1a). The coastal plot in Valencia is characterized by a coastal Mediterranean climate with lower thermal amplitude, higher mean temperature and slightly higher precipitation than the inland plot in Madrid, which has a continental Mediterranean climate (Fig. 1; Table S1). Soil in the coastal experimental plot has a silty clay loam texture and a higher active calcium carbonate and organic matter content than the soil in the inland plot (Table S1).

The two experimental plots were not significantly irrigated by any water course. Therefore, the plots received drop irrigation from April to July to avoid water stress (around 70–100 l per plant and year). The plants in both plots were distributed following a randomized complete block design with two blocks and a spacing between plants of 2.0 × 2.0 m in the coastal plot and 1.25 × 1.25 m in the continental plot. A border line of trees was used to avoid border effects in both plots.

2.2. Tree height, bud burst phenology, and O. novo-ulmi inoculation

In both experimental plots, plant stem height was measured in December 2016 (4-year-old trees) using a measuring pole with millimeter precision. The DED pathogen *O. novo-ulmi* was inoculated in two consecutive years in each plot. Since *U. minor* symptomatology after *O. novo-ulmi* inoculation increases until the age of 4, and then stabilizes (Solla et al. 2005a), the first inoculation year was 2017 in Valencia, the coastal plot, (4-year-old trees) and 2018 in Madrid, the inland plot (5-year-old trees). The bud burst phenology was characterized in both locations before inoculation, in the second week of April. The leaf development was classified into three stages: 1) dormancy (dormant or swelling buds but with closed flakes); 2) bud break (flakes open and the leaf ends visible in the apex of buds); and 3) leaf expansion (two or more

Table 1
Ulmus minor material used in the experiment. Genotypes used in the anatomical study are marked in bold.

Plant genotype	Parental tree (♀ × ♂)	Origin in Spain	Number of replicates in experimental plots	
			Inland	Coastland
AB-AM2.4	–	Almansa (Albacete)	12	12
L-TE3.3	–	Termens (Lérida)	12	12
MA-PD2	–	Pedrizas (Málaga)	10	12
M-DV1	–	Madrid (Madrid)	12	9
b9	M-DV4/5 × J-CA2	Madrid (Madrid) x Cazorla (Jaén)	12	12
46	J-CA2 × M-DV4/5	Cazorla (Jaén) x Madrid (Madrid)	10	12
5	GR-DF3 × AB-AL1	Deifontes (Granada) x Alatoz (Albacete)	12	11
117	M-DV4/5 × J-CA2	Madrid (Madrid) x Cazorla (Jaén)	12	12
n2	J-CA2 × M-DV4/5	Cazorla (Jaén) x Madrid (Madrid)	10	12
n4	J-CA2 × M-DV4/5	Cazorla (Jaén) x Madrid (Madrid)	10	12
97	M-DV4/5 × GR-DF3	Madrid (Madrid) x Deifontes (Granada)	10	12
Sapporo	–	–	11	10

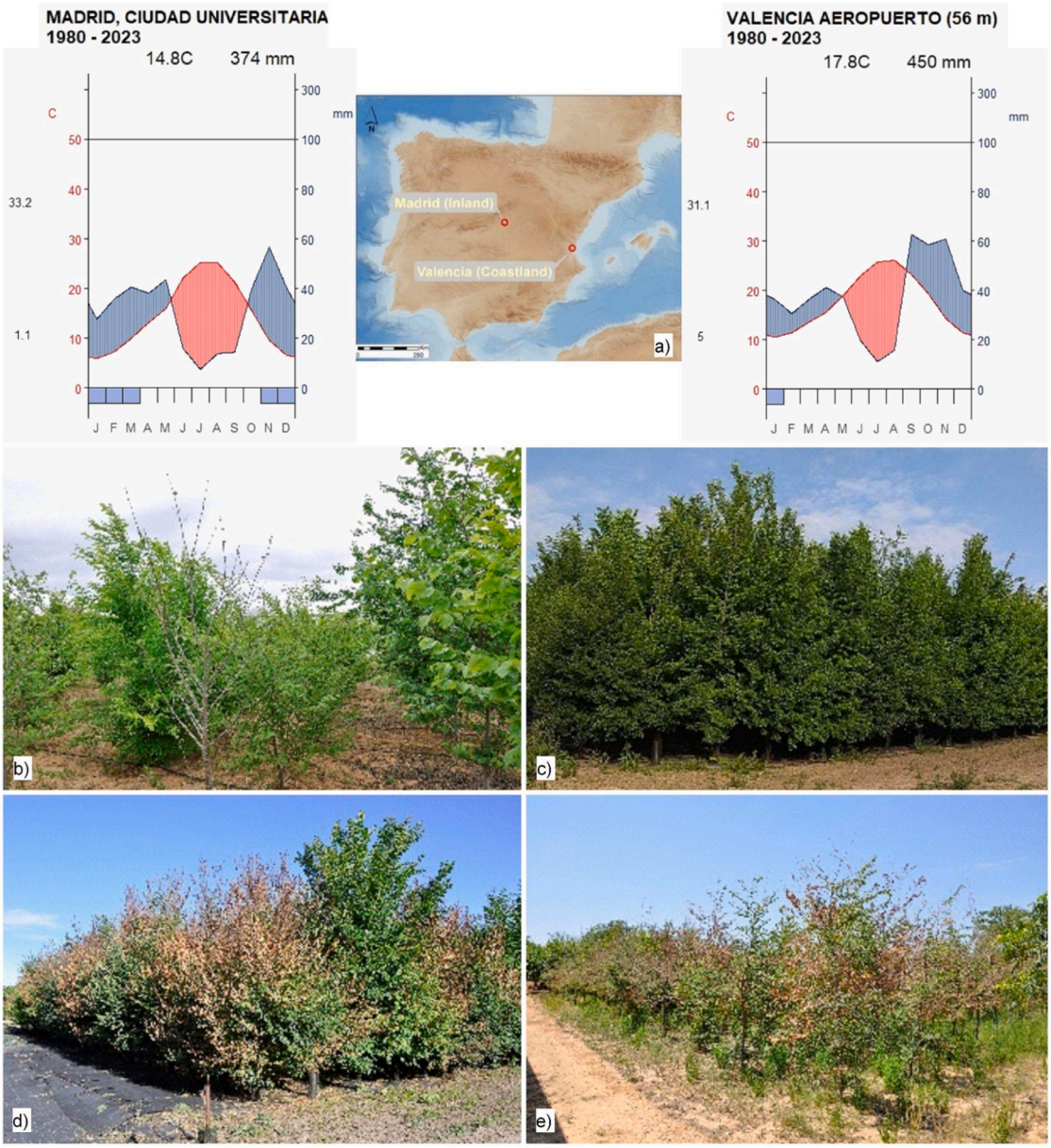


Fig. 1. (a) Location of the two study plots in Spain and climographs for the nearest weather stations to the experimental plots. Values above the graph represent long-term annual mean temperature and accumulated annual mean rainfall. Top left situated temperature value is the mean of the average daily maximum temperature of the hottest month and bottom left value is the mean of the average daily minimum of the coldest month. Months marked in blue in the bottom are those with average minimum temperatures above 0 °C, but with absolute minimum temperatures below 0 °C (probable frost). The area shaded in red represents the drought period. (b) Marked differences in leaf phenology among genotypes in Valencia. (c) Synchronized leaf phenology in Madrid. Foliage wilting symptoms after *O. novo-ulmi* inoculation in Madrid (d) and Valencia (e). Climographs were prepared using the R package "Climaemet" developed by Pizarro et al. (2021).

leaves fully expanded). We observed that the bud break phenology differed significantly between the two plots, with pronounced bud break asynchrony among genotypes on the coast but not inland (Fig. 1b, c). In the coastal plot, five genotypes were in bud break phase at the assessment week and were classified as early flushing genotypes, whereas the remaining seven genotypes were in leaf expansion phase so they were classified as late flushing genotypes. In contrast, in the inland plot, the 12 examined genotypes were in the bud break phase at the assessment week. In the first inoculation year, inoculation was carried out in early spring, 45 days after the average date in which trees reached the bud break phase in each location (Table 2), the date at which susceptibility is usually at its maximum (Tchernoff 1965; Solla and Gil 2003). In order to study the effect of leaf phenology asynchrony on DED susceptibility, in the second inoculation year each phenology group in the coastal plot

Table 2
Experimental design: date of inoculation with *O. novo-ulmi*, number of genotypes (N), and total number of trees (in brackets) inoculated each year in both experimental locations (Madrid and Valencia). EF = early flushing genotypes; LF = late flushing genotypes.

Location	First inoculation		Second inoculation	
	Date	N	Date	N
Inland (Madrid)	May 9, 2018	12 (132)	May 10, 2019	12 (132)
Coastal (Valencia)	May 10, 2017	12 (137)	April 24, 2018 (EF)	7 (78)
			May 11, 2018, (LF)	5 (59)

was inoculated 45 days after the average bud break date within each group, which resulted in an offset of 15 days between inoculations of each group. In the inland plot, the second inoculation (2019) was performed similarly to the first inoculation; i.e. 45 days after the average bud break date of all genotypes (Table 2).

For the inoculations, we used two *O. novo-ulmi* Spanish isolates obtained from diseased mature elm trees: M-RT16 (from Madrid) and Z-BU1 (from Zaragoza). Both isolates showed a similar high virulence (> 70 % of foliage wilting on susceptible control trees) in previous screening tests of the Spanish elm breeding program (Martín et al. 2015; Li et al. 2016; Dominguez et al. 2022). In 2017, we inoculated the first isolate and in 2018–2019 we inoculated the second. Two months before inoculations, isolates were cultured on 2 % malt extract agar medium (MEA) in Petri dishes at 22 °C in the dark and sub-cultured every week. One week before inoculations, mycelial plugs were immersed in Tchernoff liquid medium (Tchernoff 1965) to induce sporulation under constant orbital shaking during four days. The inoculum consisted of a suspension of *O. novo-ulmi* blastospores in distilled sterilized water, adjusted to 10^6 spores ml^{-1} using a Thoma hemocytometer. Inoculations were performed following a modified Dutch inoculation method (Heybroek 1993; Buiteveld et al. 2015). Two drops of the blastospore suspension were delivered into the vascular xylem at the base of the trunk using a sharp blade. Disease symptoms were evaluated by three independent observers who visually estimated the percentage of foliage wilting at 60 days post inoculation (dpi).

2.3. Anatomical traits

Four replicates of three elm genotypes – (30,1)n2; (32,1)n4; (36,02) 97 – were selected for anatomical characterization (24 trees in total). For genotype selection, we excluded four genotypes showing severe wilting symptoms (M-DV1, n2, 97 and b9), as the observation of anatomical traits in transverse sections from heavily diseased trees is hampered by the tissue damage caused by the wilt process. Then, three genotypes were randomly selected among the remaining seven *U. minor* genotypes. Within each tree, three growth rings were measured: the ring formed the year before inoculation, the ring formed during the first-inoculation year, and the ring formed during the second-inoculation year (72 growth rings measured in total).

For these anatomical analyses, a 3-cm long segment of the main trunk was sawn off 10 cm above the inoculation wound in 2020. Wood samples were conserved in 70 % ethanol and once in the lab they were cut in transverse sections (30 μm thick) using a sledge microtome. Sections were treated with the phloroglucinol-HCl method (Martín et al. 2005) and observed under a microscope Olympus BX51 with a 4x/0.13 objective using tungsten light to observe lignified tissues, and under UV light to quench the autofluorescence of lignin, allowing the visualization of suberin-like compounds. Other sections were stained with potassium iodide to observe the content of starch in the xylem (Begum et al. 2007). Sections were photographed with a camera attached to the microscope and the anatomical traits were measured using the ImageJ software (Schneider et al. 2012). The following anatomical traits were measured: mean vessel area (μm^2) in earlywood (EW) and latewood (LW), density of vessels (N/mm^2) in EW and LW, radial ring width (μm) in EW, LW and the total ring, percentage of occluded vessels in EW, percentage of the xylem area with starch reactions, and percentage of the xylem area with suberin-like reactions. In addition, the following parameters were calculated: i) theoretical hydraulic conductance (THC, μm^2) in EW and LW (EW_THC, and LW_THC, respectively), predicted by the Hagen-Poiseuille equation, as the sum of the fourth power of internal vessel radii (Σrn^4) divided by the sector area; ii) vessel transectional area (VTA, %) in EW and LW (EW_VTA and LW_VTA, respectively), as the area occupied by vessels in a sector (wall excluded) divided by the total area of the sector, then multiplying by 100; and iii) the ratio between LW and EW radial width (LW/EW). In total, 15 anatomical traits were obtained per growth ring.

2.4. Statistical analyses

First, a linear mixed model was developed to test the effects on height of genotype, location (enclosing the contrasting environments) and their interaction. Second, to evaluate which factors were affecting DED susceptibility, several Generalized Linear Mixed Models were built. Using Akaike Information Criterion, we found that the model that best fitted the data was the one using a Beta distribution to model errors, with a logit link function, over a probit link function or a plain Gaussian distribution (equivalent to a Linear Mixed Model). These models were run considering the wilting percentage at 60 dpi each year as the response variable. Since that variable is a ratio ranging from 0 to 100 %, and given that Beta distribution range excludes 0 and 1, the following linear transformation of the variable was done: $y_2 = (y \bullet (N - 1) + 0.5) / N$, being y and y_2 the observed and transformed phenotypic values, and N the number of values (Smithson and Verkuilen 2006). The first of such models was run with tree height, genotype, location and the interaction between the two latter as fixed-effect factors, to evaluate the effect genotype-by-environment effects. A second set of models tested the effect of tree height on foliage wilting at 60 dpi separately for both inoculation years and locations. Finally, the effect of bud burst phenology on DED susceptibility was tested in a model where the phenology group (early and late) and height were included as fixed-effect factors. Even though block as a factor was not significant in either trials, it was included as a random effect in all the above-mentioned models to absorb spatial effect and prevent statistical bias.

Phenotypic variance of height and DED susceptibility was partitioned between genetic and environmental components to assess whether location and inoculation year affected the proportion of genetic variance (i.e. heritability) and to compare with previous studies. Genetic and environmental variance components of height and foliage wilting after first and second inoculations were therefore calculated for the whole experiment but also independently for each trial, by means of one-way analyses of variance, with genotype as the only factor. Although, as mentioned before, a Beta distribution modeled more adequately foliage wilting than a linear model, we opted to estimate heritability assuming normality, because the phenotype distributed rather normally, but also for comparative purposes, since it has been traditionally estimated assuming normality, and, more importantly, because heritabilities estimated from non-linear models are difficult to interpret and apply, especially in breeding. Broad-sense heritability was then estimated as: $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$ and $\sigma_g^2 = [MS(G) - MS(E)]/r$, where r is the mean number of replicates per genotype, and $MS(G)$ and $MS(E)$ are the mean sums of squares for genotype and residual error obtained from the analyses of variance.

Anatomical traits of the three selected genotypes were analyzed separately for the three examined growth rings (pre-inoculation, 1st inoculation and 2nd inoculation) in each trial. To analyze these traits ($N = 15$), we first analyzed each trait separately through a generalized linear model (GLM) with a logit link function and gamma distribution for the error, where the genotype, location, and their interaction were included as fixed-effect factors. Second, we conducted principal component analyses (PCAs) on the 15 anatomical traits for the 24 sampled trees. We performed one PCA per growth ring (pre-inoculation, first inoculation and second inoculation). Third, to test the significance of the grouping found between locations in the first two principal component (PC) score plots, we analyzed the scores of the first two PCs as dependent variables, while location, genotype and their interaction were included as fixed-effect factors. To simplify, the first two principal components (PCs) were analyzed together using a multivariate analysis of variance (MANOVA). The MANOVA results were further corroborated using univariate ANOVAs for each PC axis separately. Statistical analyses were conducted using Statistica 10.0 and R version 4.1.0 (R Core Team, 2022) in RStudio version 2022.02.1 + 461 (RStudio Team, 2022) and EnvStats (Millard 2013), lme4 (Bates et al. 2015), glmmMTB

(Brooks et al. 2017) and Heritability (Kruijer et al. 2023) packages.

3. Results

3.1. Tree height and DED susceptibility

The results showed significant effects of the genotype, location and genotype-by-location interaction on tree height. On average, all genotypes exhibited a greater height in the inland location but the rank of genotypes changed between locations (Table 3; Fig 2).

The Beta regression model showed significant effects of the genotype and the genotype-by-location interaction on DED resistance in both the first- and the second-inoculation years, while the effect of the location was significant in the first-inoculation year (Table 4). Genotypes responded differently in both environments, with significantly higher overall foliage wilting values in the inland plot in the first inoculation year (Fig. 3; Table 4). In the second inoculation year, foliage wilting values in the coast, though still being lower than inland on average, rose to a similar level (Fig. 3; Table 4).

Beta regressions also showed that, in the first inoculation year, foliage wilting significantly increased with tree height in the inland ($\chi^2_1 = 14.88$; $P < 0.001$) but not in the coastland ($\chi^2_1 = 0.01$; $P = 0.930$) trial (Fig. S1). In the second inoculation year, foliage wilting significantly increased with tree height in both locations ($\chi^2_1 = 5.74$; $P = 0.016$; and $\chi^2_1 = 7.24$; $P = 0.007$, in the inland and coastland trial, respectively) (Fig. S1).

3.2. Bud burst phenology

Two phenology groups of genotypes were established based on the marked asynchrony in bud burst observed in the coastland plot. Such groups were used to partly stratify the second inoculation. In the inland trial, where bud burst phenology was rather synchronized and all trees were inoculated on the same date, Beta regressions showed that no differences between groups were observed in the first inoculation ($\chi^2_1 = 1.18$, $P = 0.277$; Fig. 4a), and in the second inoculation ($\chi^2_1 = 1.81$, $P = 0.178$; Fig. 4b). In the coast, the two groups also showed no significant differences in the first inoculation ($\chi^2_1 = 1.16$, $P = 0.282$; Fig. 4c), but in the second inoculation, which was done in two distinct dates, one per phenology group and set apart two weeks, the early flushing genotypes showed clearly higher DED symptoms than the late flushing genotypes ($\chi^2_1 = 18.74$, $P < 0.001$; Fig. 4d). It is worth highlighting the marked increase in symptomatology observed in both locations from the first to the second inoculation, particularly in the early flushing group (40.0 % increase in the coastland and 32 % in the inland plot).

3.3. Heritability estimates

Broad-sense heritability values, estimated as clonal repeatability, of tree height and foliage wilting at 60 days after first inoculation ranged from $H^2 = 0.51$ to 0.59 (Table 5) both inland and in the coast, and were higher than the overall estimations ($H^2 = 0.20$ and 0.32, respectively). In the second-inoculation year, foliage wilting broad-sense heritability increased to $H^2 = 0.75$ in the coastland trial but remained in the same range in the inland trial ($H^2 = 0.55$).

Table 3
Results of Wald chi-square test on the fixed factors affecting tree height before inoculation. The linear mixed model had location (L) (inland and coastland), genotype (G) ($N = 12$), and their interaction ($G \times L$) as fixed categorical factors; and block as random factor.

Factor	df	Chi-square	P-value
G	11	233.60	< 0.0001
L	1	548.88	< 0.0001
$G \times L$	11	110.61	< 0.0001

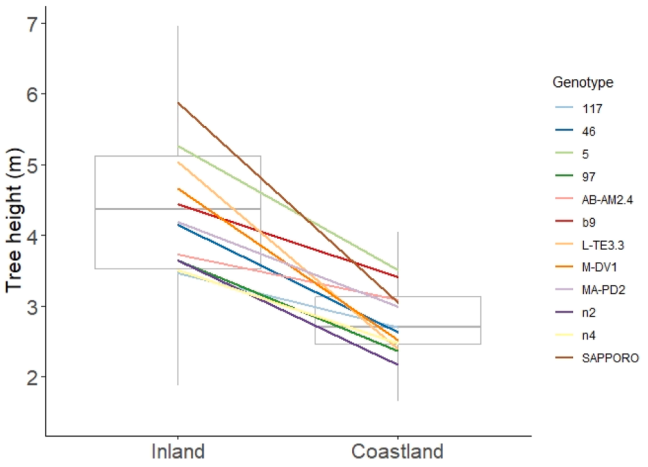


Fig. 2. Tree height of 12 elm genotypes grown in the inland (Madrid) and the coastland (Valencia) common gardens. Height was measured when trees were 4 years old. In box-plots, the black horizontal lines represent median values, while the lower and upper hinges correspond to the first and third quartiles. Vertical bars represent minimum and maximum values not considering outliers. Colored lines between locations represent average reaction norms for each genotype.

Table 4
Results of Wald chi-square test on the fixed factors affecting foliage wilting (%) shown by 12 *U. minor* genotypes after two inoculations (60 days post each inoculation) with *O. novo-ulmi* in inland and coastland experimental plots, considering total height before inoculation (H) as covariate. The Beta regression had as fixed categorical factors the location (L) (inland and coastland), the genotype (G) ($N = 12$), and their interaction ($G \times L$); and block as a random factor.

Year of inoculation	Factor	df	Chi-square	P-value
1st	H	1	23.623	< 0.0001
	G	11	269.163	< 0.0001
	L	1	23.945	< 0.0001
	$G \times L$	11	66.445	< 0.0001
2nd	H	1	20.227	< 0.0001
	G	11	281.209	< 0.0001
	L	1	2.801	0.09421
	$G \times L$	11	55.128	< 0.0001

3.3. Xylem anatomy

Concerning foliage wilting, the three genotypes selected for the wood anatomical analysis showed higher percentage of foliage wilting in the inland plot compared to the coastal plot (25.4 ± 4.1 % vs 12.6 ± 6.6 % after the first inoculation, and 42.6 ± 6.4 % vs 28.8 ± 3.1 % after the second inoculation; mean ± SE).

The microscopic examination of wood sections evidenced significant plasticity in several anatomical traits. We first analyzed the effect of the location and the genotype on each anatomical trait separately through a GLM (Table S2; Fig. 5). Concerning the location effect, this analysis revealed that in the pre-inoculation growth ring, anatomical traits related to vessel size in earlywood (EW vessel area, EW_VTA, EW_THC), and the ratio LW/EW width were higher inland than in the coast (Table S2). In the same ring, trees in the coast grew more radially (EW width, total ring width) and stored more starch than inland (Table S2; Fig. 5). In the first-inoculation growth ring, trees in the coastal plot also grew more radially (EW, LW and total ring), while inland trees showed higher vessel density in both EW and LW (Table S2). The second-inoculation growth ring was characterized by higher vessel density (in EW and LW) and LW_VTA in the inland plot, and higher LW/EW width in the coastal trial (Table S2; Fig. 5).

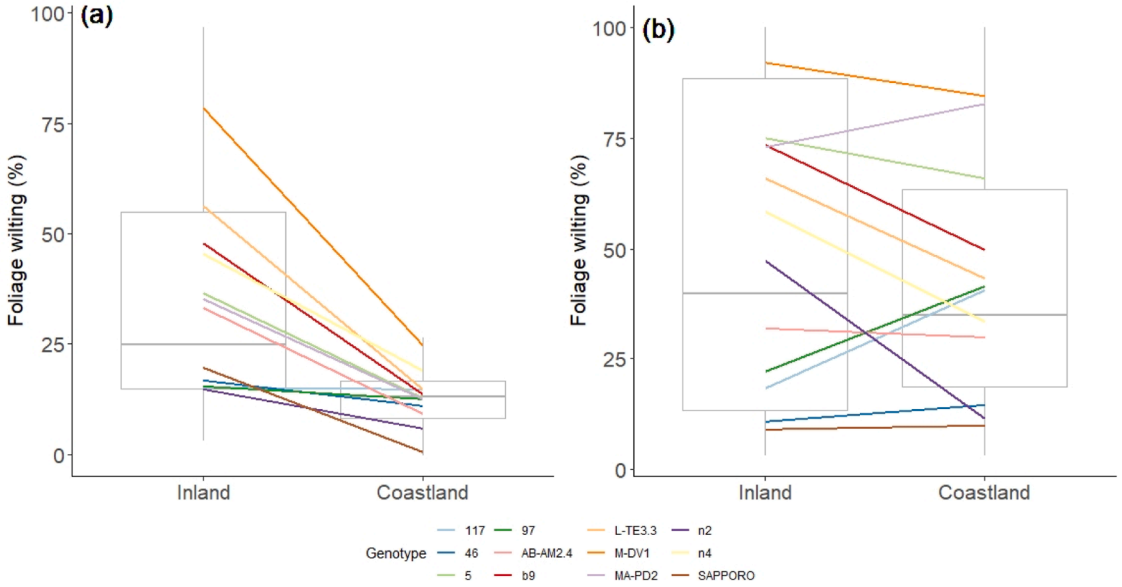


Fig. 3. Foliage wilting of 12 elm genotypes 60 days after inoculation with *O. novo-ulmi* in inland (Madrid) and coastal (Valencia) common gardens in the first-inoculation year (a) and second- inoculation year (b). In box-plots, black horizontal lines represent the median value, while the lower and upper hinges correspond to the first and third quartiles. Vertical bars represent minimum and maximum values not considering outliers. Colored lines between locations represent average reaction norms for each genotype.

Concerning the genotype effect on these anatomical traits, it was, in general, low (Wald 2; $P > 0.05$), with some exceptions (e.g., EW width in the pre-inoculation year, or EW_THC in the first-inoculation year) (Table S2). Significant genotype-by-location interactions were observed for several traits. For example, in the pre-inoculation ring, the variables related to vessel area, EW_THC and EW_VTA showed significant genotype-by-location interaction (Table S2, Fig. 5). However, the most significant interactions were observed after inoculation, e.g. in LW/EW width in the first-inoculation ring, or EW vessel density in the second-inoculation ring (Table S2). Some of the mentioned anatomical differences can be visually observed in Fig. S2 and associated supplementary text.

Second, to analyze the xylem anatomy we also conducted PCAs on the 15 anatomical traits for the 24 sampled trees, performing one PCA per growth ring. The spatial distribution of the scores of the first two PCs showed a clear distinction of the two locations for the three measured growth rings (pre-inoculation, first inoculation and second inoculation), with the highest differentiation between locations in the pre-inoculation growth ring (Fig. 6). Distinction among locations was mainly due to PC1 (Fig. 6). The MANOVA performed to analyze the effect of the location, the genotype and their interaction on the two first PCs confirmed that the highest differentiation between locations was found in the pre-inoculation growth ring (Table 6). Furthermore, the MANOVA revealed that genotype-by-location interaction in xylem anatomy was significant for the rings formed in both inoculation years, but not in the pre-inoculation year (Table 6).

4. Discussion

4.1. DED resistance variation between inoculation sites

Our results support previous research which already evidenced that DED resistance is a plastic trait with moderate to high levels of heritability. Previous studies have shown that several climatic and edaphic factors influence DED resistance (Kais et al. 1962; Sutherland et al. 1997; Solla and Gil 2002). The plasticity in DED resistance of English elm (*U. minor* var. *vulgaris*) was observed in a series of inoculation trials established at the same location in England, in which three different pathogen isolates were inoculated during 14 years with variable climatic conditions (Sutherland et al. 1997). Although the virulence ranking of the three isolates was consistent across the years, the level of the symptoms varied with the inoculation year, most likely as a consequence of the climatic influence on tree resistance. In our work, the average susceptibility of the 12 evaluated genotypes in the coastal environment was significantly lower than in the inland environment after the first inoculation, indicating that DED resistance can vary with environmental conditions (i.e. it is a plastic trait).

Although the plasticity of DED resistance has been documented previously (Kais et al. 1962; Sutherland et al. 1997; Solla and Gil 2002), to our knowledge, the existence of genetic differences among elm genotypes in plasticity in DED resistance (i.e. genotype-by-environment interaction) has not been formally investigated so far. Previous studies have provided evidence of potential genotype-by-environment interaction in DED resistance. In particular, Solla et al. (2005b) evaluated the performance of seven elm genotypes in controlled experimental plots in six European countries. The disease ratings of the seven cultivars were different depending on the location and the inoculation year, suggesting that DED resistance was plastic. In addition, although the significance of the genotype-by-environment interaction on DED resistance was not specifically estimated, their results showed that two genotypes exhibited a contrasting susceptibility ranking in Italy and Germany, suggesting that genotype-by-environment interactions in DED resistance can occur. In our work we observed a genotype-by-location interaction in DED resistance, which means that the tree response to DED under contrasting environments is under genetic control. The lower values of overall heritabilities for wilting compared to the within-trial heritability estimates also suggest the involvement of a significant phenotypic plasticity (Table 5). In addition, our heritability estimates of the independent plots (Table 5) are in line with previously reported values, with moderately high broad-sense estimates ($H^2 = 0.71 \pm 0.22$; Solla et al. 2014) and narrow-sense estimates ranging from $h^2 = 0.45$ - 0.63 (Dominguez et al. 2022).

In the following sections, by analyzing the plasticity of phenotypic traits that have been frequently associated with DED resistance (tree growth, leaf phenology or xylem anatomical traits) we aim at disentangling which of these traits are more prone to phenotypic variation and could be associated with plasticity in resistance.

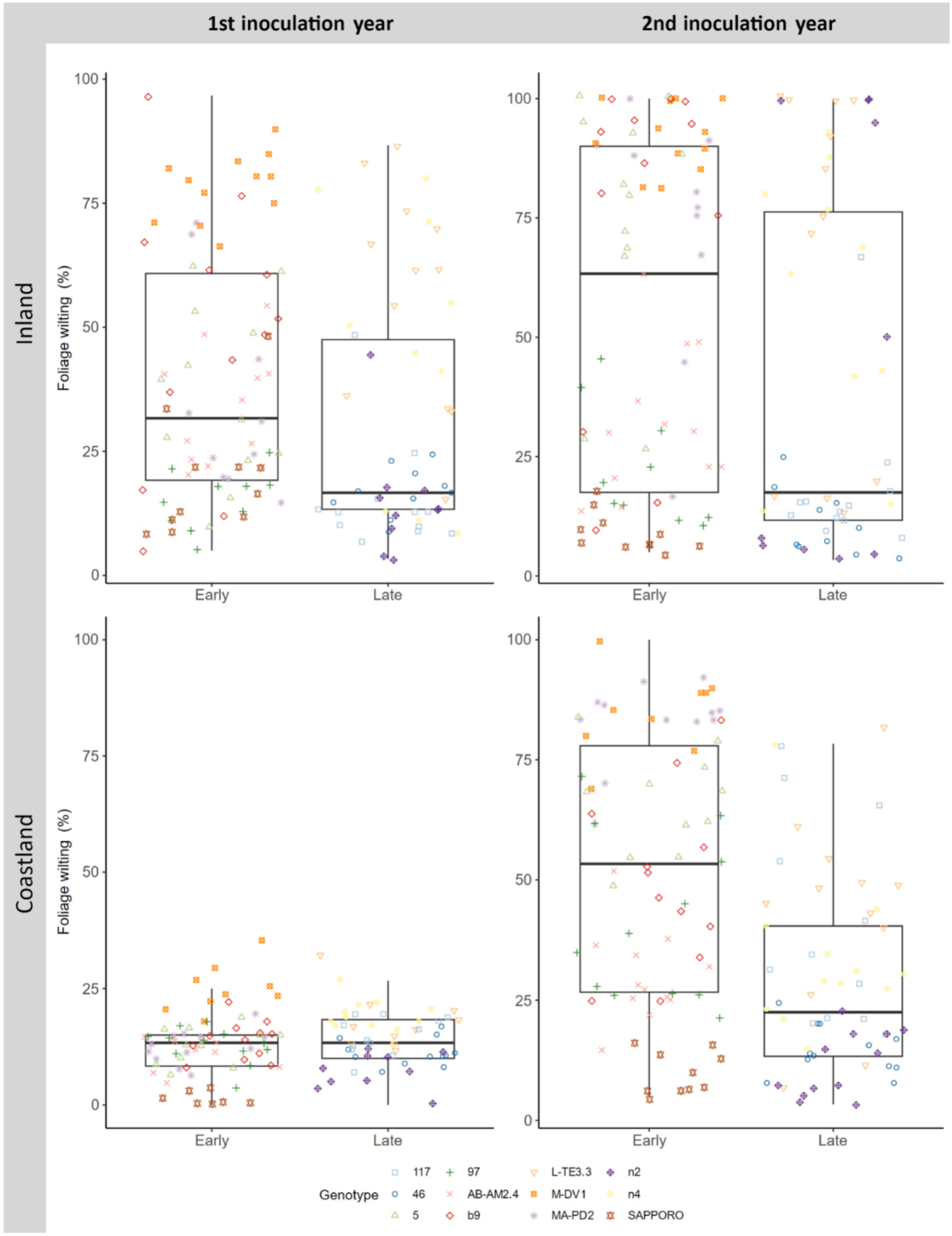


Fig. 4. Foliage wilting values observed each year in inland and coastland inoculation plots according to the two bud burst phenology groups of genotypes: early and late flushing genotypes. In box-plots, the black horizontal line represents the median value, while the lower and upper hinges correspond to the first and third quartiles. Vertical bars represent minimum and maximum values not considering outliers.

4.2. Tree height variation and relation with DED resistance

The highly significant effects of the location and the genotype-by-location interaction on tree height support the hypothesis of remarkable phenotypic plasticity of height growth and is consistent with a previous work which reported genotype-by-environment interaction in height growth in *Ulmus* spp. (Santini et al. 2010). Height growth and leaf phenology, two traits reported to be under strong genetic control (Bradshaw et al. 1995; Howe et al. 2000; Friedman et al. 2011), have been suggested to have a significant influence on elm resistance to *O. novo-ulmi* (Ghelardini L. 2007; Solla et al. 2014), and could be underlying the observed plasticity in DED resistance in our study. Although not always consistent, a direct association between height growth and DED susceptibility has been observed in several DED-inoculation trials (Heybroek 1957; Kais et al. 1962; Townsend et al. 2001; Santini et al. 2005; Martín et al. 2021; Domínguez et al. 2022). The causes behind this

Table 5
Variance partition estimates, including broad-sense heritability, of the foliage wilting at 60 dpi for the two inoculation years and tree height before the first inoculation. H^2: broad-sense heritability. *CI*: confidence interval. S_g^2: estimated genetic variance. S_e^2: estimated residual variance. CV_g: coefficient of genotypic variation. CV_e: coefficient of experimental variation.

Variance components and heritability estimates		Foliage wilting (1st inoc.)	Foliage wilting (2nd inoc.)	Tree height
Inland	H^2	0.58	0.55	0.51
	CI	0.37–0.81	0.35–0.79	0.31–0.76
	S_g^2	380.20	773.53	0.56
	S_e^2	279.42	623.93	0.55
	CV_g(%)	55.02	56.92	17.33
	CV_e(%)	47.17	51.12	17.13
Coastland	H^2	0.59	0.75	0.57
	CI	0.39–0.81	0.59–0.90	0.37–0.80
	S_g^2	30.11	583.80	0.17
	S_e^2	21.18	190.46	0.13
	CV_g(%)	43.27	57.97	15.06
	CV_e(%)	36.29	33.11	12.99
Overall	H^2	0.32	0.56	0.20
	CI	0.19–0.59	0.38–0.79	0.09–0.45
	S_g^2	149.70	612.60	0.26
	S_e^2	325.22	473.50	1.04
	CV_g(%)	51.30	54.75	14.49
	CV_e(%)	75.61	48.14	28.80

association are unclear but might be related to trade-offs between resources allocated to growth or to defense mechanisms under the influence of environmental conditions, a theory evaluated and discussed in different works (e.g. Herms and Matson 1992; Villari et al. 2014; Zust and Agrawal 2017). According to our results, it seems that elm trees under the environmental and experimental conditions prevailing in the coast allocated less resources to height growth and more to defense (as suggested by the lower symptoms induced by the pathogen) and to carbon storage (evidenced by the higher starch content in xylem tissues). A tree with enough carbon stored in the stems could invest these resources in chemical and physical defenses upon infection, such as barrier zone formation or vessel occlusion (Shigo 1981; Martín et al. 2005a), without severe impairment of other basic physiological functions. Although the occlusion and embolism of xylem vessels is one of the main causes of tree physiological failure due to DED infection, carbon starvation also contributes to tree death (Li et al. 2016). DED infection drastically reduces the starch reserves stored in xylem tissues (Shigo et al. 1986) most likely due to the activation of defense mechanisms and the restriction in photosynthesis caused by pathogen infection (Li et al. 2016). Consistent with this hypothesis, higher starch content in xylem tissues before infection has been associated with higher resistance to DED (Martín et al. 2005b; 2008) and with higher capacity of the tree to recover after DED infection (Shigo et al. 1986). In our work, the relationship between height growth and susceptibility was significant except for the first inoculation year in the coastal trial, possibly due to the low symptomatology shown in that year. However, trees in that trial tended to grow more radially than the inland ones (Fig. 5), revealing that the trade-off hypothesis between growth and defense is complex to assess. It is plausible that, according to the shade-avoidance response (Keuskamp et al. 2010), the lower tree density in the coast likely reduced light competition between plants, leading to greater radial growth and reduced height growth. Furthermore, the significant genotype-by-environment interaction in height growth found in our study indicates that the environmental effect on tree resource allocation can strongly vary according to the tree genotype.

4.3. Influence of leaf phenology on DED susceptibility

The phenology of leaf formation was markedly different in both locations. The continental temperature regime with colder winters and sharper temperature rises in early spring in the inland trial might have favored the sprouting synchrony among genotypes in comparison to the more gradual sprouting over a broader flushing period observed in the coastal plot, driven by a gentler temperature increase in early spring (Faticov et al. 2020). The effect of the continental climate was also noticeable in the transition between early and latewood, which was more abrupt than in the coast. There, trees formed a wider earlywood increment relative to the total growth ring, leading to a lower LW/EW ratio in the pre-inoculation ring (Fig. 5). These results indicate that bud burst phenology exhibits a strong plasticity despite being considered a canalized trait under strong genetic control (Ghelardini et al. 2006). The influence of leaf phenology on DED resistance relies on the association between leaf sprouting and the initiation of earlywood formation. In European elms, the period of maximum susceptibility to DED is around 40–50 days after bud break (Tchernoff 1965; Solla and Gil 2003). By this time, elms, with a marked ring-porous structure, have already formed the wide earlywood vessels suitable for a high-efficiency water conduction in spring, coinciding with high water availability in soil at this period. These large spring vessels favor pathogen spread, but, at this time, latewood has not been formed yet, or at least not a large proportion of it (Santini et al. 2005). Latewood is largely formed by small vessels, parenchyma cells and fibers with thick cell walls and small lumens, rendering this tissue unsuitable for pathogen spread (Shigo and Tippet 1981; Bonsen et al. 1985). Latewood also serves as a constitutive barrier against radial pathogen spread towards the cambial area (Shigo 1984; Martín et al. 2021), preserving the capacity of the cambium for producing new healthy wood. Therefore, if DED infection occurs either when earlywood vessels are still not totally functional, or when a large proportion of latewood has been formed, the spread of the pathogen would be restricted and the tree would have more chance to survive. In the first inoculation year, we applied the same criterion for choosing the inoculation date in both inland and coastland trials, i.e. 45 days after the average bud break in the plot. However, due to the marked phenological asynchrony in the coast, the genotypes were probably inoculated outside their peak susceptibility window, resulting in a low symptomatology (Fig. 4). In the second-inoculation year, the inoculation in the coastal plot was carried out in two dates, one for early and another for late flushing trees. Taking the varying phenology of the genotypes into account by adapting the inoculation timing rose the susceptibility in that plot to a level close to the inland one, particularly in early flushing genotypes, and probably was the main cause of the marked increase in the coastal plot's broad-sense heritability of the resistance after the second inoculation. In the late flushing genotypes, the increase in susceptibility in the coastland with the second inoculation was lower, possibly because either these genotypes are genetically more resistant or because the second inoculation was still slightly offset to the peak susceptibility of these genotypes.

4.4. Variation of anatomical traits between inoculation sites

Differences in anatomical traits between locations were largest in the year before the first inoculation and lowest in the second inoculation year. This pattern was probably related to a reduction in tree growth after the inoculations and an investment of resources in defense responses, such as vessel occlusion or deposition of opaque matter in parenchyma cells, possibly phenolic compounds (Ouellette and Rioux 1992; Martín et al. 2008; Büchel et al. 2016). These processes strongly constrained the formation of a regular xylem, leading to a lower influence of environmental factors on xylem structure. Interestingly, the genotype-by-location interaction in anatomical traits (estimated through the MANOVA), which was non-significant in the pre-inoculation growth ring, became significant during the inoculation years, evidencing a different anatomical response to DED of the genotypes in both locations. In the pre-inoculation ring, inland trees showed higher EW vessel area, EW_VTA and EW_THC. All these parameters have been associated with enhanced susceptibility to DED (Elgersma 1970;

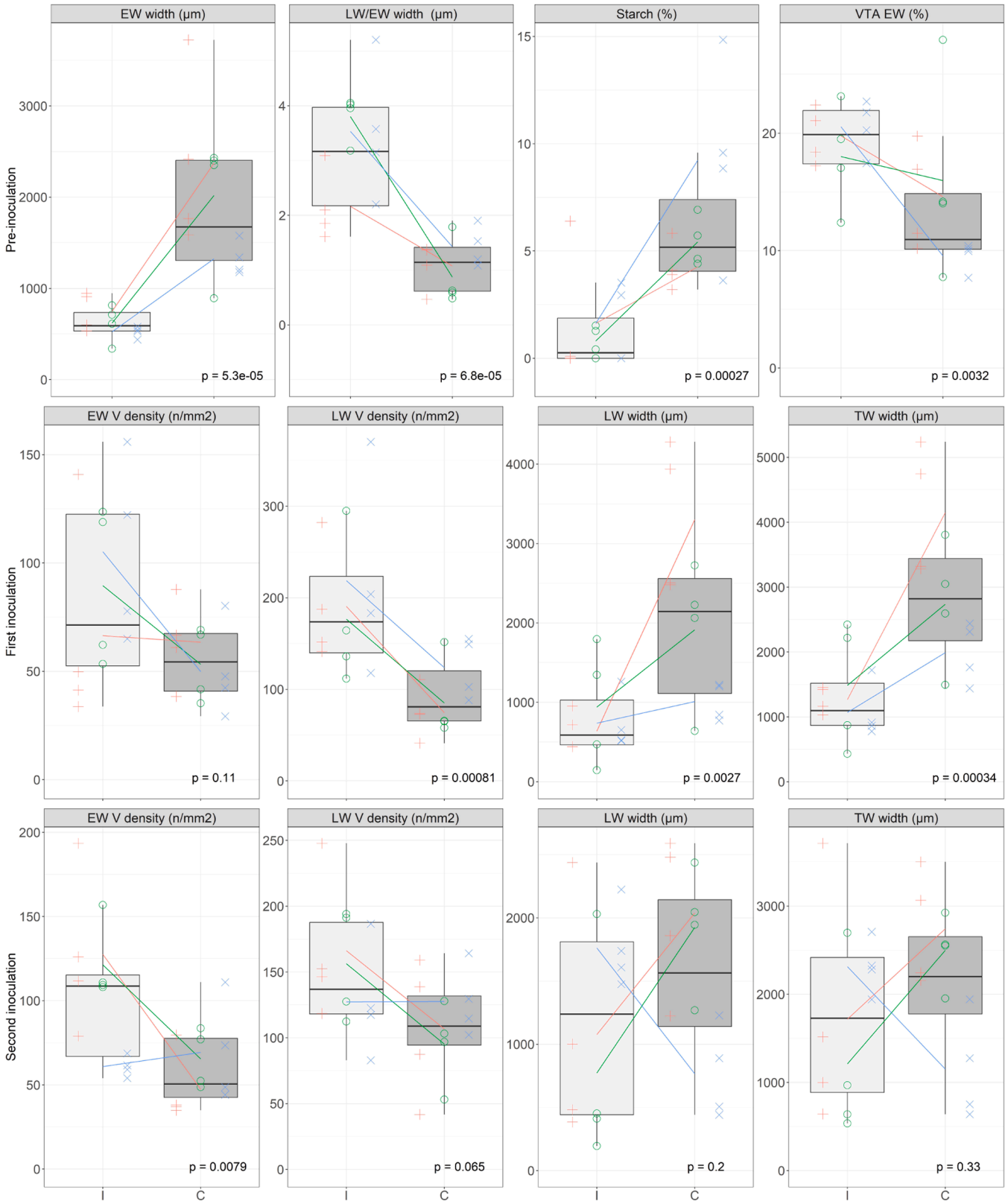


Fig. 5. Box plots of the anatomical traits of n2 (green), n4 (blue) and 97 (red) genotypes showing the biggest differences among inland (I) and coastland (C) locations for each measured growth ring: pre-inoculation year (upper panels), first-inoculation year (middle panel) and second-inoculation year (lower panel). For each growth ring, the four traits providing the most significant distinction between locations (see Table S2 for further details) are displayed. In box-plots, black horizontal lines represent median values, while lower and upper hinges correspond to the first and third quartiles. Vertical bars represent minimum and maximum values not considering outliers. Colored lines between locations represent average reaction norms for each genotype. EW: early wood. LW: late wood. V: vessels. VTA: vessel transectional area.

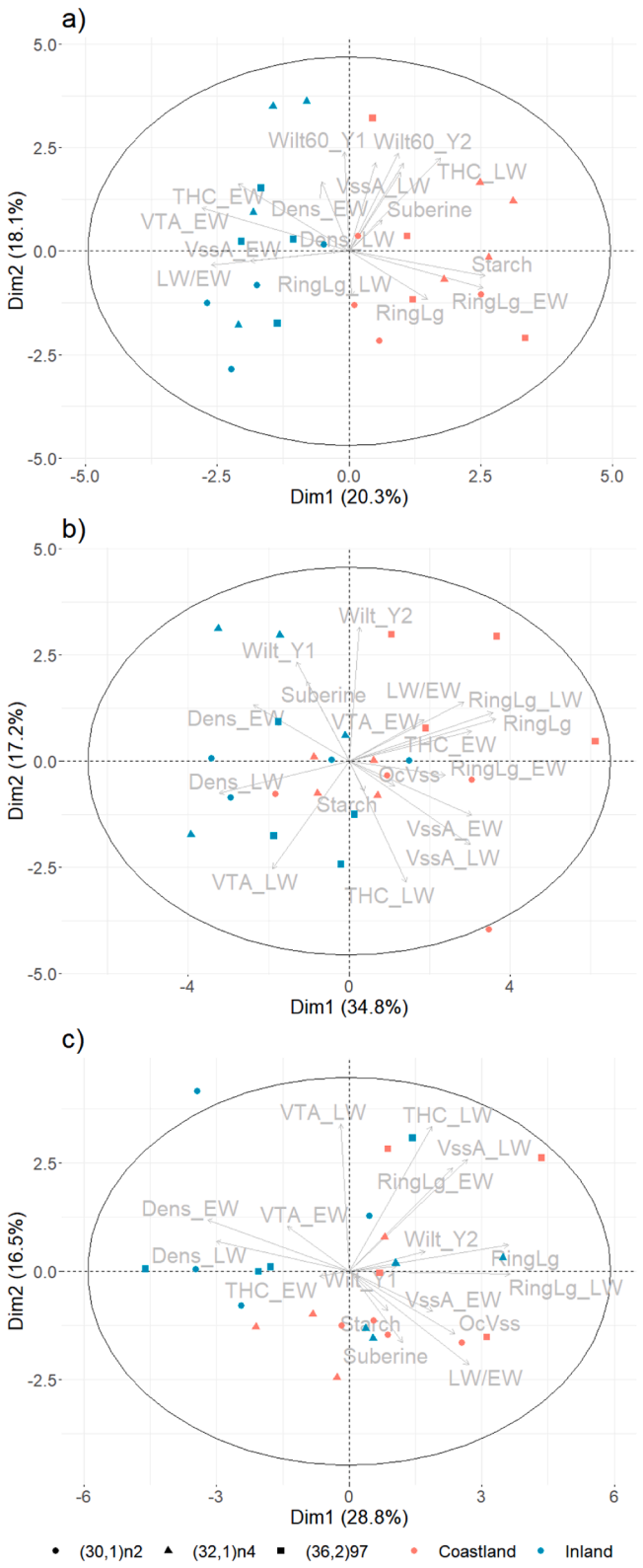


Fig. 6. Score plots and variable projection of the two first principal components (PCs) obtained from the principal component analysis of the anatomical traits measured in *U. minor* xylem tissues. (a) Growth ring formed the year before the first inoculation with *O. novo-ulmi* (i.e. wood not affected by inoculation). (b) Growth ring formed during the first-inoculation year. (c) Growth ring formed during the second-inoculation year. Red dots depict trees growing in the coastal trial and blue dots trees growing in the inland trial. The different shapes of the dots correspond to different genotypes according to the legend.

Table 6
Results of the MANOVA of the first two principal components resulting from the PCA of the anatomical traits ($N = 15$) measured in *U. minor* xylem. Three growth rings were measured: the year before the first inoculation with *O. novo-ulmi* (pre-inoculation), and the two inoculation years (first and second inoculation, respectively). The categorical factors in the model were location (L) (inland and coastland, $df = 1$), genotype (G) (three genotypes, $df = 2$), and their interaction ($L \times G$, $df = 2$).

Factor	Pre-inoculation		First inoculation		Second inoculation	
	F	*P*	*F*	*P*	*F*	*P*
L	27.29	< 0.001	7.24	0.005	5.23	0.016
G	1.47	0.23	1.46	0.23	1.38	0.26
$L \times G$	0.87	0.48	2.99	0.03	3.95	0.009

Solla et al. 2005a; Martín et al. 2013). High values of vessel lumen, THC and VTA provide better conditions for *O. novo-ulmi* spread within vessels and from vessel to vessel (Solla et al. 2005b) and favor the process of embolism and occlusion of vessels, quickly limiting water transport capacity (McNabb et al. 1970; Venturas et al. 2013). In the coast, where the climate seemed to facilitate a more gradual transition between winter, spring and summer temperatures, the trees formed a thicker earlywood frequently including a transition zone with medium-size earlywood vessels (Fig. S2), which possibly made this xylem less prone to a rapid dysfunction in water transport due to DED. In the first-inoculation year, trees in the coastland plot showed higher radial growth, reflecting the lower impact of the inoculation at this site, while trees inland showed higher vessel density, a trait that similarly to VTA, possibly facilitates pathogen spread from vessel to vessel (Solla et al. 2005b), and contributed to the higher DED susceptibility at this site. In the second-inoculation year, trees in the continental plot still showed higher vessel density than in the coast and higher susceptibility. In sum, the effect of the environment on xylem anatomical traits, in parallel with the effect on tree phenology, seemed to reduce the susceptibility under the coastal conditions in Valencia where the trees formed more earlywood but with vessels of lower size and density, and accumulated higher levels of starch during the year before inoculation. The significant genotype-by-location interaction found for several anatomical traits indicates that we should not oversimplify the environmental effects on these traits. This interaction is consistent with the interaction found in DED resistance, meaning that the general trends observed are, however, genotype dependent.

4.5. Implications for elm reintroduction

Genotype-by-environment interactions in DED resistance can result in unintended consequences in forest restoration and urban forestry. Our results revealed that a genotype showing resistance under a certain environment could show susceptibility under different conditions, while other genotypes may show the opposite trend. In contrast, there are genotypes that seem to exhibit a canalized phenotype with either high or low DED susceptibility across environments (at least in the two studied conditions). Therefore, it is important to define as much as possible the conditions under which a resistant genotype performs well by means of inoculation trials under contrasting environments. Caution should be taken with the selection of the inoculation date when testing for DED resistance, because changes in tree phenology due to climatic conditions can profoundly affect the result of the inoculation trial. In those conditions, maximizing differences among genotypes in flushing dates (i.e. coastal conditions) the grouping of trees according to its foliar phenology is strongly advisable. The phenological monitoring of genotypes would also be advisable for its use as a covariate in the evaluation of resistance. In this regard, our results support those by Ghelardini & Santini (2009) who evidenced that flushing phenology can have a strong influence on DED symptomatology. In terms of reintroduction plantings, genotypes with low plasticity in resistance could be widely used in

different territories, while the ones with high plasticity should preferably be planted only in those conditions favoring resistance. For instance, the genotypes Sapporo and 46 performed well in both trials, showing low plasticity in DED resistance, while the genotypes 97 and 117 performed well under the continental conditions in Madrid, but not so well under the coastal conditions in Valencia.

In forest restoration and urban forestry, other pests, diseases and abiotic factors can compromise the performance of the selected DED resistant varieties. For example, the first resistant selection released by the Dutch elm breeding program in the first half of the 20th century, the clone Buisman from Spain, was not well adapted to the conditions of Central Europe due its susceptibility to cortical pathogens (Heybroek 1957). Therefore, although our work was focused on DED resistance, future studies should also pay attention to other factors, both biotic and abiotic, compromising the performance of the DED-resistant genotypes in different territories.

Funding

This work was supported by Dirección General de Biodiversidad, Bosques y Desertificación, Ministerio para la Transición Ecológica y el Reto Demográfico (MITECO, Spain) and the project PID2021–127347OA-I00 (Agencia Estatal de Investigación, Ministerio de Ciencia e Innovación, Spain). The position of Dr. Macaya-Sanz is mostly funded by the program "Atracción de Talento Investigador" of the Community of Madrid (ref. 2019-T2/BIO-12780).

CRediT authorship contribution statement

Jorge Domínguez: Writing – original draft, Methodology, Formal analysis, Conceptualization. **David Macaya-Sanz:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **José Alberto Ramírez-Valiente:** Writing – review & editing, Conceptualization. **Juan A. Martín:** Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank all who worked hard in the Spanish elm breeding program.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2024.110294.

Data availability

Data will be made available on request.

References

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J Stat Softw 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Begum, S., Nakab, S., Oribe, Y., Kubo, T., Funada, R., 2007. Induction of Cambial Reactivation by Localized Heating in a Deciduous Hardwood Hybrid Poplar (*Populus Sieboldii* × *P. grandidentata*) *Annals of Botany*, 100, pp. 439–447. https://doi.org/10.1093/aob/mcm130.

Beier, G.L., Blanchette, R.A., 2020. Xylem characteristics in *Ulmus americana* cultivars and their potential use as a preliminary screening method for Dutch elm disease resistance. For. Pathol. 50 (6), e12638. https://doi.org/10.1111/efp.12638.

Bonsen, K.J.M., Scheffer, R.J., Elgersma, D.M., 1985. Barrier zone formation as a resistance mechanism of elms to Dutch elm disease. IAWa J. 6 (1), 71–77. https://doi.org/10.1163/22941932-90000916.

Bradshaw, H.D., Stettler, R.F., 1995. Molecular genetics of growth and development in populus. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics 139 (2), 963–973. https://doi.org/10.1093/genetics/139.2.963.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9 (2), 378–400. https://doi.org/10.32614/RJ-2017-066.

Büchel, K., Fenning, T., Gershenzon, J., et al., 2016. Elm defence against herbivores and pathogens: morphological, chemical and molecular regulation aspects. Phytochem. Rev. 15, 961–983. https://doi.org/10.1007/s11101-015-9442-0.

Buiteveld, J., Van Der Werf, B., Hiemstra, J., 2015. Comparison of commercial elm cultivars and promising unreleased Dutch clones for resistance to *Ophiostoma novo-ulmi*. IForest. 8, 158–164. https://doi.org/10.3832/ifor1209-008.

Collin, E., Rondouin, M., Joyeau, C., Matz, S., Raimbault, P., Harvengt, L., Bilger, I., Guibert, M, 2020. Conservation and use of elm genetic resources in France: results and perspectives. IForest. 13, 41–47. https://doi.org/10.3832/ifor3065-013.

Collins, C.W., Buchanan, W.D., Whitten, R.R., Hoffmann, C.H., 1936. Bark beetles and other possible insect vectors of the Dutch elm disease *Ceratostomella Ulmi* (Schwarz) Buisman. J. Econ. Entomol. 29 (1), 169–176. https://doi.org/10.1093/jee/29.1.169, 1 February 1936.

Domínguez, J., Macaya-Sanz, D., Gil, L., Martín, J.A., 2022. Excelling the progenitors: breeding for resistance to Dutch elm disease from moderately resistant and susceptible native stock. For. Ecol. Manage. 511, 120113. https://doi.org/10.1016/j.foreco.2022.120113. ISSN 0378-1127.

Elgersma, D.M., 1970. Length and diameter of xylem vessels as factors in resistance of elms to *Ceratocystis ulmi*. Neth. J. Plant Pathol. 76, 179–182. https://doi.org/10.1007/BF01974328.

Faticov, M., Ekholm, A., Roslin, T., Tack, A.J.M., 2020. Climate and host genotype jointly shape tree phenology, disease levels and insect attacks. Oikos. 129, 391–401. https://doi.org/10.1111/oik.06707.

Friedman, J.M., Roelle, J.E., Cade, B.S., 2011. Genetic and environmental influences on leaf phenology and cold hardiness of native and introduced riparian trees. Int. J. Biometeorol. 55, 775–787. https://doi.org/10.1007/s00484-011-0494-6.

Ghelardini, L., 2007. Bud Burst phenology, Dormancy Release and Susceptibility to Dutch elm Disease in Elms (*Ulmus* spp.). Ph.D. Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Ghelardini, L., Falusi, M., Santini, A., 2006. Variation in timing of bud-burst of *Ulmus minor* clones from different geographical origins. Canadian J. Forest Res. 36 (8), 1982–1991. https://doi.org/10.1139/x06-092.

Ghelardini, L., Santini, A., 2009. Avoidance by early flushing: a new perspective on Dutch elm disease research. IForest. 2, 143–153. https://doi.org/10.3832/ifor0508-002.

Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol. 67, 283–335. https://doi.org/10.1086/417659.

Heybroek, H.M., 1957. Elm-breeding in the Netherlands. Sylvae Genet 6, 112–117.

Heybroek, H.M., 1993. The Dutch elm breeding program. Dutch Elm Disease Research. Springer, New York, NY, pp. 16–25.

Howe, G., Saruul, P., Davis, J., et al., 2000. Quantitative genetics of bud phenology, frost damage, and winter survival in an F2 family of hybrid poplars. Theor. Appl. Genet. 101, 632–642. https://doi.org/10.1007/s001220051525.

Kais, A., Smalley, E., Riker, A., 1962. Environment and development of Dutch elm disease. Phytopathology. 52, 1191–1196.

Keuskamp, D.H., Sasidharan, R., Pierik, R., 2010. Physiological regulation and functional significance of shade avoidance responses to neighbors. Plant Signal Behav. 5 (6), 655–662. https://doi.org/10.4161/psb.5.6.11401.

Kruijer, W., 2023. Contains Data Collected by Padraic Flood and Rik Kooke. Heritability: Marker-Based Estimation of Heritability Using Individual Plant or Plot Data. R package Version 1.4. https://CRAN.R-project.org/package=heritability</bib.

Li, M., López, R., Venturas, M., Martín, J.A., Domínguez, J., Gordaliza, G.G., Gil, L., Rodríguez-Calcerrada, J., 2016. Physiological and biochemical differences among *Ulmus minor* genotypes showing a gradient of resistance to Dutch elm disease. For. Path. 46, 215–228. https://doi.org/10.1111/efp.12247</bib.

Macaya-Sanz, D., Witzell, J., Collada, C., Gil, L., Martín, J.A., 2023. Core endophytic mycobiome in *Ulmus minor* and its relation to Dutch elm disease resistance. Front. Plant Sci. 14, 1125942. https://doi.org/10.3389/fpls.2023.1125942.

Mackenthun, G.L., 2000. Native Elms of Saxony, Germany. In: Dunn, CP (Ed.), The elms: breeding, conservation, and Disease Management. Kluwer Academic Publishers, Boston, pp. 305–314.

Martín, J.A., Solla, A., Woodward, S., Gil, L., 2005a. Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. Tree Physiol. 25 (10), 1331–1338. https://doi.org/10.1093/treephys/25.10.1331.

Martín, J.A., Solla, A., Coimbra, M.A., Gil, L., 2005b. Metabolic distinction of *Ulmus minor* xylem tissues after inoculation with *Ophiostoma novo-ulmi*. Phytochemistry 66, 2458–2467. https://doi.org/10.1111/j.1439-0329.2007.00542.x.

Martín, J.A., Solla, A., Domingues, M.R., Coimbra, M.A., Gil, L., 2008. Exogenous phenol increase resistance of *Ulmus minor* to Dutch elm disease through formation of suberin-like compounds on xylem tissues. Environ. Exp. Bot. 64, 97–104. https://doi.org/10.1016/j.envexpbot.2008.05.004, 2008.

Martín, J.A., Solla, A., Ruiz-Villar, M., Gil, L., 2013. Vessel length and conductivity of *Ulmus* branches: ontogenetic changes and relation to resistance to Dutch elm disease. Trees 27, 1239–1248. https://doi.org/10.1007/s00468-013-0872-2.

Martín, J.A., Solla, A., Venturas, M., et al., 2015. Seven *Ulmus minor* clones tolerant to *Ophiostoma novo-ulmi* registered as forest reproductive material in Spain. IForest. 8, 172–180. https://doi.org/10.3832/ifor1224-008.

Martín, J.A., Sobrino-Plata, J., Rodríguez-Calcerrada, J., et al., 2019. Breeding and scientific advances in the fight against Dutch elm disease: will they allow the use of elms in forest restoration? New. For. (Dordr) 50, 83–215. https://doi.org/10.1007/s11056-018-9640-x, 2019.

Martín, J.A., Domínguez, J., Solla, A., et al., 2021. Complexities underlying the breeding and deployment of Dutch elm disease resistant elms. New. For. (Dordr). https://doi.org/10.1007/s11056-021-09865-y.

Martínez-Arias, C., Sobrino-Plata, J., Ormeño-Moncalvillo, S., et al., 2021. Endophyte inoculation enhances *Ulmus minor* resistance to Dutch elm disease. Fungal. Ecol. 50, 101024. https://doi.org/10.1016/j.funeco.2020.101024.

McNabb, H.S., Heybroek, H.M., MacDonald, W.L., 1970. Anatomical factors in resistance to Dutch elm disease. Netherlands Jo. Plant Pathol. 76, 196–205.

Millard, S.P., 2013. EnvStats: An R Package for Environmental Statistics. Springer, New York. https://doi.org/10.1007/978-1-4614-8456-1. ISBN 978-1-4614-8455-4.

Mittempergher, L., Santini, A., 2004. The history of elm breeding. For. Syst. 13, 161–177. https://doi.org/10.5424/821.

Moravčík, M., Mamoňová, M., Račko, V., Kováč, J., Dvořák, M., Krajňáková, J., Ďurkovič, J., 2022. Different responses in vascular traits between Dutch elm hybrids with a contrasting tolerance to Dutch Elm Disease. J. Fungi 8 (3), 215. https://doi.org/10.3390/jof8030215.

Ouellette, G.B., 1962. Studies on the infection process of *Ceratocystis ulmi* (Buism.) C. Moreau in American elm trees. Canadian J. Bot. 40 (12), 1567–1575. https://doi.org/10.1139/b62-153.

Ouellette, G.B., Rioux, D., 1992. Anatomical and physiological aspects of resistance to Dutch elm disease. In: Blanchette, RA, Biggs, AR (Eds.), Defense Mechanisms of Woody Plants Against fungi. Springer series in Wood Science. Springer, Berlin, pp. 257–307. https://doi.org/10.1007/978-3-662-01642-8.

Peace, T., 1960. The status and development of elm disease in Britain. For Comm Bull 33, 44.

Pizarro, M., Hernangómez, D., Fernández-Avilés, G., 2021. climaemet: Climate AEMET Tools. https://doi.org/10.5281/zenodo.5205573. https://hdl.handle.net/10261/250390.

R Core Team, 2022. R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

RStudio Team, 2022. RStudio: Integrated Development for R. RStudio. PBC. http://www.rstudio.com/</bib.

Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L., Mittempergher, L., 2005. Variation among Italian and French elm clones in their response to *Ophiostoma novo-ulmi* inoculation. For. Pathol. 35, 183–193. https://doi.org/10.1111/j.1439-0329.2005.00401.x.

Santini, A., Pecori, F., Pepori, A.L., Ferrini, F., Ghelardini, L., 2010. Genotype × environment interaction and growth stability of several elm clones resistant to Dutch elm disease. Forest Ecol. Manag. Volume 260 (6), 1017–1025. https://doi.org/10.1016/j.foreco.2010.06.025.

Schneider, C., Rasband, W., Eliceiri, K., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089.

Shigo, A.L., 1984. Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. Annu Rev Phytopathol 22 (1), 189–214. https://doi.org/10.1146/annurev.py.22.090184.001201.

Shigo, A.L., Gregory, G.F., Campana, R.J., Dudzik, K.R., Zimel, D.M., 1986. Patterns of starch reserves in healthy and diseased American elms. Can. J. For. Res. 16, 204–210. https://doi.org/10.1139/x86-037.

Shigo, A.L., Tippett, J.T., 1981. Compartmentalization of American elm tissues infected by *Ceratocystis ulmi*. Plant Dis. 65, 715–718. https://doi.org/10.1094/PD-65-715.

Sinclair, W.A., 1975. Anatomical marker for resistance of *Ulmus americana* to *Ceratocystis ulmi*. Phytopathology. 65, 349. https://doi.org/10.1094/Phyto-65-349.

Sinclair, W.A., Campana, R.J., 1978. Dutch Elm Disease Perspectives After 60 years. (New York State Agric. Exp. Station, Cornell University.) Search Agr, 8, p. 52. Plant Pathology 1.

Smithson, M., Verkuilen, J., 2006. A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. Psychol. Methods 11 (1), 54–71. https://doi.org/10.1037/1082-989X.11.1.54.

Solla, A., Gil, L., 2002. Xylem vessel diameter as a factor in resistance of *Ulmus minor* to *Ophiostoma novo-ulmi*. For. Pathol. 32, 123–134. https://doi.org/10.1046/j.1439-0329.2002.00274.x.

Solla, A., Gil, L., 2003. Evaluating *Verticillium dahliae* for biological control of *Ophiostoma novo-ulmi* in *Ulmus minor*. Plant Pathol. 52 (5), 579–585. https://doi.org/10.1046/j.1365-3059.2003.00921.x.

Solla, A., López-Almansa, J., Martín, J.A., Gil, L., 2014. Genetic variation and heritability estimates of *Ulmus minor* and *Ulmus pumila* hybrids for budburst, growth and tolerance to *Ophiostoma novo-ulmi*. IForest. 8, 422–430. https://doi.org/10.3832/ifor1227-007.

Solla, A., Martín, J.A., Corral, P., Gil, L., 2005a. Seasonal changes in wood formation of *Ulmus pumila* and *U. minor* and its relation with Dutch elm disease. New Phytologist 166, 1025–1034. https://doi.org/10.1111/j.1469-8137.2005.01384.x.

Solla, A., Martín, J.A., Ouellette, G.B., Gil, L., 2005b. Influence of plant age on symptom development in *Ulmus minor* following inoculation by *Ophiostoma novo-ulmi*. Plant Dis. 89 (10), 1035–1040. https://doi.org/10.1094/PD-89-1035.

Sutherland, M.L., Pearson, S., Brasier, C.M., 1997. The influence of temperature and light on defoliation levels of elm by Dutch elm disease. Phytopathology. 87, 576–581. https://doi.org/10.1094/PHYTO.1997.87.6.576.

Tchernoff, V., 1965. Methods for screening and for the rapid selection of elms for resistance to Dutch Elm Disease 2. Acta Botanica Neerlandica 14, 409–452. https://doi.org/10.1111/j.1438-8677.1965.tb00204.x.

Townsend, A.M., Douglass, L.W., 2001. Variation among American elm clones in long-term dieback, growth, and survival following *Ophiostoma* inoculation. J. Environ. Horti.c 19 (2), 100–103. https://doi.org/10.24266/0738-2898-19.2.100.

Venturas, M., Lopez, R., Martín, J.A., Gascó, A., Gil, L., 2013. Heritability of *Ulmus minor* resistance to Dutch elm disease and its relationship to vessel size, but not to xylem vulnerability to drought. Plant Pathol. 63, 500–509. https://doi.org/10.1111/ppa.12115.

Villari, C., Faccoli, M., Battisti, A., Bonello, P., Marini, L., 2014. Testing phenotypic trade-offs in the chemical defence strategy of Scots pine under growth-limiting field conditions. Tree Physiol. 34 (9), 919–930. https://doi.org/10.1093/treephys/tpu063.

Züst, T., Agrawal, A.A., 2017. Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. Annu. Rev. Plant Biol. 68 (1), 513–534. https://doi.org/10.1146/annurev-arplant-042916-040856.