



## Relation between morphology and native climate in the resistance of different *Pinus pinaster* populations to pitch canker disease caused by *Fusarium circinatum*

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### ABSTRACT

Maritime pine (*Pinus pinaster*) is a forest species of great ecological and economic interest in the European Union, and especially in the Iberian Peninsula. Currently, one of the main threats this species has to face is the spread of the quarantine pathogenic fungus (A2 list) *Fusarium circinatum*, causal agent of pine pitch canker disease. Since one of the main strategies for the control of the disease is the use of resistant clones, it is necessary to carry out extensive work on the susceptibility analysis of several *P. pinaster* populations against *F. circinatum*. To this end, we have studied 35 *P. pinaster* populations located throughout the natural distribution range. After inoculation with *F. circinatum* spores, the susceptibility of all populations was studied, correlating it with their morphological traits (height and stem diameter) and relating it with the climate of origin. The results obtained reported significant differences among populations in both the morphological traits and susceptibility to the disease, with a negative correlation between height and susceptibility. With respect to the climate of origin, a negative correlation between continental climate and resistance was established. Therefore, populations with the indicated characteristics could be selected for the establishment of programs of breeding for resistance to control the spread of *F. circinatum* in *P. pinaster* forests.

### 1. Introduction

Maritime pine (*Pinus pinaster* Aiton) is a conifer of great economic and ecological interest in its area of distribution. Forests of *P. pinaster* can mainly be found in its native areas of Southwest Atlantic and Mediterranean regions, in France, Italy, Spain, Morocco, and Portugal (Fonseca et al., 2022). In these areas, *P. pinaster* forests cover more than 3 million hectares, mainly in the Iberian Peninsula, where it represents the most abundant conifer and an important source of income for the local economy (Ribeiro et al., 2022). However, at present these forests can also be found in a testimonial form in other Eurasian countries (United Kingdom, Belgium, the Balkans or Turkey), African (South Africa), Oceania (Australia or New Zealand) or South American countries (Chile, Argentina or Uruguay) (Fonseca et al., 2022). This wide distribution is due to its capacity to develop under average annual temperatures of 13–15 °C, average rainfall of 800 mm/year and altitudes of up to

800 m above sea level (Fonseca et al., 2022).

*P. pinaster* forests are habitats of great interest for the European Union (EU) and, especially, for the Iberian Peninsula, due to their great ecological importance in the maintenance and conservation of biodiversity. For this reason, the EU classifies these forests as "Mediterranean pine forests with endemic Mesogean pines" (EU Council Directive, 1992). With respect to its industrial applications, *P. pinaster* forests are used in construction, furniture manufacturing and the paper industry. In addition, the bark is used as functional food and to obtain absorbents, resins and natural foams, due to the high presence of phenolic compounds and terpenes (Alonso-Esteban et al., 2022). The collection of the resin is an activity of great socio-economic importance in certain rural areas of the Iberian Peninsula, due to its subsequent use for the industrial production of varnishes, inks, paints and glues. Other less important uses of *P. pinaster* are the manufacture of charcoal and particleboard with needles and cones (Alonso-Esteban et al., 2022).

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Like other forest tree species, *P. pinaster* is facing the decline of Mediterranean pine forests, a problem that causes the disappearance of large forest stands, whose cause is not associated to a single origin, but to the combination of biotic factors (different pathogens and pests) and abiotic factors (increase in temperatures, drought, lack of fire, intensive grazing, etc.) (Morales-Rodríguez et al., 2024). One of the main threats faced by *P. pinaster* is pine pitch canker (PPC) disease, whose causal agent is the fungus *Fusarium circinatum* (Nirenberg & O'Donnell) (Drenkhan et al., 2020).

PPC mainly affects pines (*Pinus* spp.) and Douglas-fir (*Pseudotsuga menziesii*) and causes the mortality or decay in seedlings (damping-off), and the dieback of branches and stems in mature trees, in addition to the appearance of the most characteristic symptom known as "pitch" or production of copious resin from cankers (Drenkhan et al., 2020). *F. circinatum* is native to Mexico, although it is now widely distributed in 41 countries on all continents (Zamora-Ballesteros et al., 2019; Drenkhan et al., 2020). Currently, it is considered a quarantine pathogen by the European and Mediterranean Plant Protection Organization (EPPO), included in the A2 list, whose control and eradication is a primary focus of the plant health policy of the European Union (Vettraino et al., 2018). Current strategies to control the pathogen include the use of resistant genotypes in replanting, the use of biological control agents, the management of insect vectors, or the application of fungicides in nurseries, among others (Zamora-Ballesteros et al., 2019). With respect to the use of resistant genotypes, a thorough population study of susceptibility to

the pathogen is necessary. In this sense, several population susceptibility studies have been carried out against *F. circinatum* in a wide variety of coniferous species, such as *Pinus sylvestris* (Iturrutxa et al., 2013; Martín-García et al., 2017, 2018; Davydenko et al., 2018; Woodward et al., 2022), *P. nigra*, *P. pinaster*, *P. radiata*, *P. halepensis*, *P. pinea* (Iturrutxa et al., 2013), *P. mugo* (Martín-García et al., 2017), *P. muricata* (Schmale & Gordon, 2003), *P. densiflora*, *P. thunbergii*, *P. x rigitaeda*, *P. echinata*, *P. virginiana* (Kim et al., 2008), *Larix decidua* (Martín-García et al., 2018), *Picea abies* (Martín-García et al., 2017, 2018), *Widdingtonia schwartzii*, *W. cederbergensis*, *W. nodiflora* (Porter et al., 2009), *Podocarpus latifolia*, *P. elongatus*, *P. henkelii* (Porter et al., 2009) or *Pseudotsuga menziesii* (Iturrutxa et al., 2013). Specifically, in the case of *P. pinaster*, few works of population susceptibility against *F. circinatum* have been developed so far. These works have allowed to verify that there is a significant genetic variability at the three hierarchical levels: population, family and clone with respect to PPC disease (Vivas et al., 2012; Elvira-Recuenco et al., 2014). In addition, they highlighted the possible successful evolutionary adaptation of maritime pine to PPC disease (Elvira-Recuenco et al., 2014) and the use of less susceptible genotypes as a management strategy against the disease (Vivas et al., 2012).

Therefore, it is necessary to characterize the population susceptibility of *P. pinaster* to *F. circinatum*, in order to develop breeding programs with resistant populations to the disease. Specifically, this work has studied the susceptibility of various populations of *P. pinaster* to the disease, relating them to their morphological characteristics (of the

**Table 1**

*Pinus pinaster* populations evaluated for resistance to *Fusarium circinatum*, along with associated geoclimatic data. Lat and Long: Latitude and longitude, in decimal degrees (DD, WGS 84); Alt: altitude, in m.a.s.l.; T: annual mean temperature, in °C; P: annual precipitation, in mm.

Population code	Provenance region / seed orchard	Provenance / seed orchard name	Geographic group*	Lat (DD)	Long (DD)	Alt (m)	T (°C)	P (mm)
HSQ26001	HSQ-26001	HSQ-26001	Atlantic coast of the Iberian Peninsula	43.3	-7.93	610	12.8	1350
HSFRVG006	HS-FR-VG-006	HS-FR-VG-006	France-Catalonia region	44.2	-1.19	52	13.2	1052
PTES01a	1a	Galician coast	Atlantic coast of the Iberian Peninsula	42.83	-8.54	289	13.6	1580
PTES01b	1b	Galician coastal interior	Atlantic coast of the Iberian Peninsula	42.63	-7.3	605	11	1445
PTES02	2	Telero	Central Iberian Peninsula	42.25	-6.08	947	11	793
PTES03	3	Oña	Central Iberian Peninsula	42.85	-3.47	782	11.2	836
PTES04	4	Gata-Hurdes	Central Iberian Peninsula	40.29	-6.54	764	13.1	867
PTES05	5	Tiétar	Central Iberian Peninsula	39.97	-6.03	375	15.3	566
PTES06	6	Gredos	Central Iberian Peninsula	40.27	-4.91	877	12.1	515
PTES07	7	Guadarrama	Central Iberian Peninsula	40.59	-4.35	1055	10.3	504
PTES08	8	Meseta Castellana	Central Iberian Peninsula	41.16	-4.68	868	11.7	388
PTES09	9	Soria-Burgos	Central Iberian Peninsula	41.87	-3.07	1156	9.3	505
PTES10	10	Sistema Ibérico Central	Central Iberian Peninsula	41.31	-1.43	954	11.7	428
PTES11	11	Rodenaes de Molina	Southeast Iberian Peninsula	40.96	-2.26	1179	10.8	542
PTES12	12	Serranía de Cuenca	Southeast Iberian Peninsula	39.94	-1.82	1009	11.9	465
PTES13	13	Albarraçin	Southeast Iberian Peninsula	40.31	-1.21	1251	11.1	428
PTES14	14	Maestrazgo	Southeast Iberian Peninsula	40.27	-0.73	1041	10.7	447
PTES15	15	Espadán	Southeast Iberian Peninsula	39.84	-0.27	653	15.6	431
PTES16	16	Levante	Southeast Iberian Peninsula	39.09	-0.85	766	13.1	397
PTES17	17	Segura-Alcaraz	Southeast Iberian Peninsula	38.25	-2.66	1111	13.5	443
PTES18	18	Moratalla	Southeast Iberian Peninsula	38.14	-2.01	1130	12.2	459
PTES19	19	Almijara-Nevada	Southeast Iberian Peninsula	36.93	-3.8	1245	13	548
PTES20	20	Bermeja	Southeast Iberian Peninsula	36.51	-4.99	572	15.8	680
PTESA	A	Benicasim	Southeast Iberian Peninsula	40.08	-0.01	339	15.7	448
PTESB	B	Pradell	Southeast Iberian Peninsula	40.99	0.86	535	15.5	596
PTESC	C	Litoral Catalán	France-Catalonia region	41.85	2.63	327	12.7	806
PTESD	D	La Safor	Southeast Iberian Peninsula	38.88	-0.24	422	15.8	362
PTESE	E	Fuencaliente	Southeast Iberian Peninsula	38.42	-4.28	1011	14.3	518
PTESF	F	Oria	Morocco-Oria region	37.52	-2.41	1331	12.2	466
PTESG	G	Serranía de Ronda	Southeast Iberian Peninsula	36.66	-5.97	576	17.7	617
PTFR800	PPA 800	Corsica	North - Italy	42.23	9.12	100	8.9	919
PTITRA	Arno River	Arno	North - Italy	43.93	10.27	100	14.3	817
PTMAATLA	High Atlas	High Atlas	Morocco-Oria region	32.34	-5.22	1000	9.7	319
PTMAATLM	Medium Atlas	Medium Atlas	Morocco-Oria region	33.96	-4.44	1000	15.6	411
PTPORPIV	PNB-RPIV	IV	Atlantic coast of the Iberian Peninsula	40.36	-8.74	55	15	884

\* Geographic groups based on Jaramillo-Correa et al. (2015). 1 1.5 generation seed orchard from Galician breeding program which comes from the PTES01a provenance. 2 Second-generation seed orchard from French breeding program in Landes.

inoculated seedlings) and climatological parameters of origin, being the first work that relates these aspects.

## 2. Materials and methods

### 2.1. Biological material

As plant material, we used 6-month-old seedlings from 35 *P. pinaster* populations. Populations originated from stands belonging to 33 provenance regions and from two seed orchards with two breeding populations (HSQ26001 from the Galician breeding program and HSFRVG006 from the French breeding program) (Table 1, Fig. 1). The 33 stands were located all around the *P. pinaster* distribution area, Spain (28), Portugal (1), France (1), Italy (1) and Morocco (2). We clustered the 35 populations into six geographic groups based on the study of molecular markers related to climate adaptation done by Jaramillo-Correa et al. (2015) (Table 1). The seeds used to obtain the seedlings were collected in each population from 30 trees per population (origin). Seedlings were grown in the nursery of the Centro de Investigación Forestal de Lourizán (Pontevedra, Spain), using trays of 60 cells of 200 cc capacity, with a mixture of peat moss and vermiculite 9:1 (v/v) as substrate.

As pathogen, strain FcCa6 of the fungus *F. circinatium* (belongs to mating type 2), isolated from a *P. radiata* specimen with symptomatology of PPC disease, located in Comillas (Cantabria, Northern Spain; GPS: 4\_17017.706" W; 43\_2005.033" N; 265 m above sea level) was used (Martín-García et al., 2017). The fungus was routinely grown on PDA (Potato Dextrose Agar) medium at 25°C in the dark.

### 2.2. Experimental design and pathogenicity tests

The experiment was carried out under controlled conditions in the biological containment level greenhouse of the Instituto de Investigación en Agrobiotecnología (CIALE, Salamanca, 41° 59' 18.6" N, 4° 30' 51.3" W, Spain). *P. pinaster* seedlings were grown in 200 cc plastic pots with a mixture of peat moss and vermiculite 9:1 (v/v) as substrate. The seedlings had a mean height of 12.2 cm at the moment of inoculation. The temperature during the whole experiment and seedling growth was between 15 and 33°C, with a mean temperature of 18.2°C. Watering by drip irrigation occurred on a weekly basis during the assay.

Inoculation with the pathogen was carried out on 6-month-old plants. The experiment followed a randomized complete block design with 35 *P. pinaster* populations, 4 blocks and one experimental unit consisting of 4 *F. circinatium*-inoculated seedlings and 4 water-inoculated seedlings (controls). Therefore, we used 32 plants per population, being 1120 plants for the whole experiment. The duration of the experiment was up to 95 days post-inoculation.

A total of 560 seedlings were inoculated with the strain FcCa6 of the fungus *F. circinatium*. First, a small wound was made in the bark of each plant with a scalpel (lifting only the lignified part, without reaching the vessels), at a height of about 5–7 cm above the soil surface. Subsequently, each wound was inoculated with 20 µL with 10<sup>6</sup> spores/mL of the pathogen, sealing the inoculated wound with Parafilm. Control seedlings were inoculated with 20 µL of sterilized distilled water using the same method.

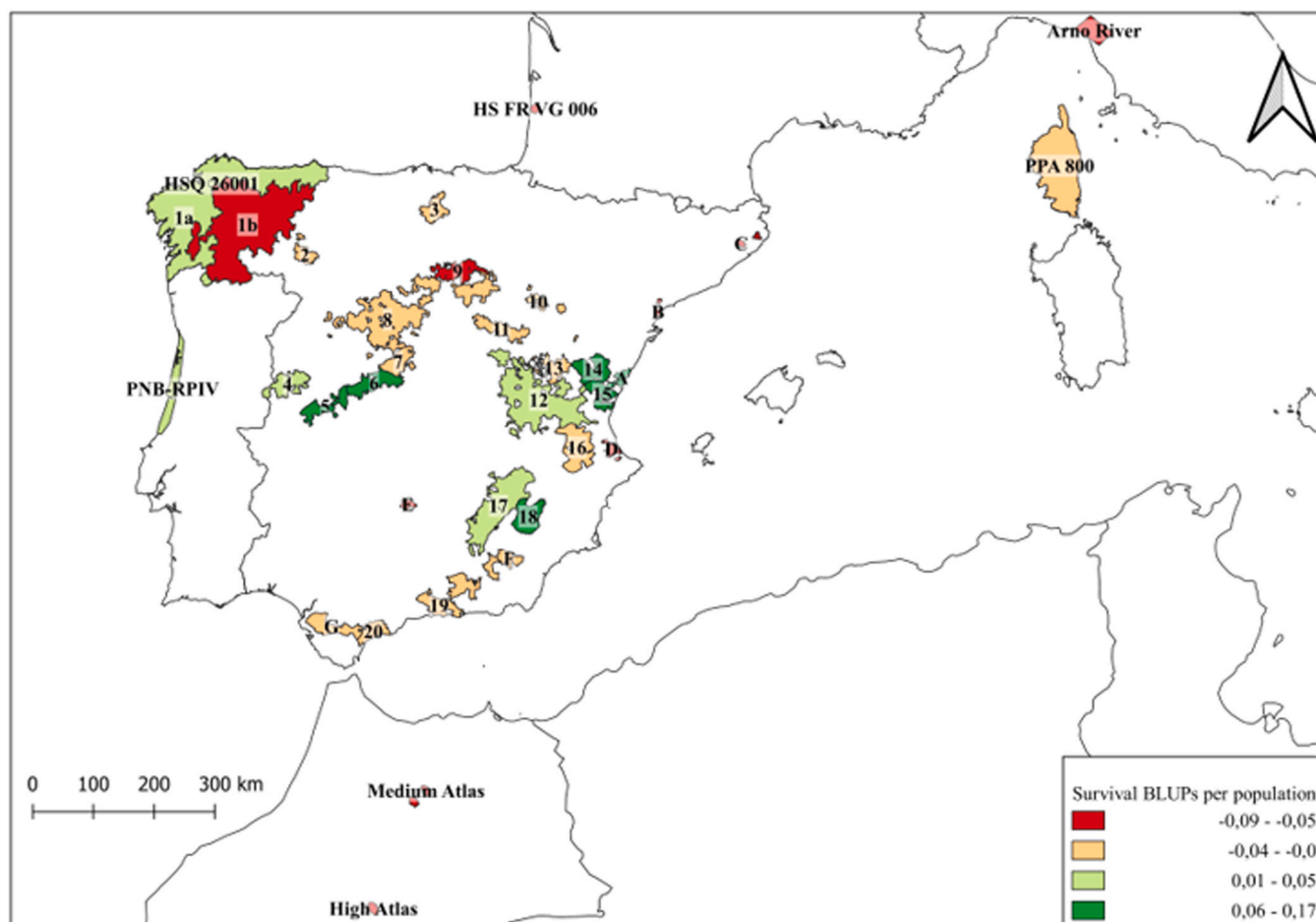


Fig. 1. *Pinus pinaster* populations evaluated in the experiment. The color gradient represents the different intervals of the survival BLUPs.

### 2.3. Morphological traits

Prior to inoculation, the following morphological traits were measured: initial height (HI, in cm), and initial root-collar diameter (DI, in mm).

### 2.4. Disease development and survival

Wilting symptoms (D95) were assessed once a week until the end of the assay. For disease level assignment, the scale described by Menéndez-Gutiérrez et al. (2018) was followed: a four-level scale corresponding to the percentage of affected needles from 1 (no external symptoms) to 4 (all needles brown and wilted). The determination of disease survival and development in plants was based on this wilting symptoms scale.

In the case of survival at the end of the trial (S95), seedlings with wilting symptoms between 1 and 3 were considered alive (assigned a binary value of 1), while those with a value of 4 were considered dead (assigned a binary value of 0). On the other hand, three different variables were considered to analyze the development of the disease. First, the start of wilting symptoms (SW) was analyzed, noting the day after inoculation (DAI) when the wilting symptoms reached level 2. Subsequently, when the wilting symptoms reached level 4 was considered the end of wilting symptoms (EW). Finally, the difference in days between SW and EW was noted as the duration of wilting symptoms (DW).

In addition, it was tested whether the dead plants were a consequence of the presence of *F. circinatum* in their tissues. Following Koch's postulates, at the end of the trial, the pathogen was reisolated in 6 plants from each of the different populations of *P. pinaster*, confirming the presence of *F. circinatum* in all the plants analyzed.

### 2.5. Statistical analysis

#### 2.5.1. Statistical summary

The average and standard deviation of each variable were calculated using the experimental units.

#### 2.5.2. Mixed models

Morphological variables (i.e., HI and DI) were analyzed by a mixed model where the population was considered as random effect. On the other hand, symptomatic variables (i.e., SW, DW, EW, D95 and S95) were analyzed by a mixed model with population and block considered random effects. A logarithmic transformation was applied to SW, EW and DW variables in order to meet the assumptions of normality and homoscedasticity of the residuals.

Then, populations were grouped into 6 geographical groups (Table 1) based on the gene pool reported by Jaramillo-Correa et al., (2015). Subsequently, S95 was analyzed again considering the geographic group as a fixed effect and the populations, nested within each geographic group, as well as the block as random effects. Finally, to know if there are differences among populations within each geographic group, S95 was reanalyzed for each geographic group considering the populations of the corresponding geographic group and the block as random effects. The likelihood ratio test (LRT) was used to determine the significance of the random effects in each model and Tukey's test was used to look for differences between geographic groups.

#### 2.5.3. Correlations between variables

Using the best linear unbiased predictors (BLUPs) of each mixed model for each variable, Pearson correlations between BLUPs were calculated. Then, type A genetic correlations ( $rAa$ ) between all the variables were estimated using the variance and covariance from a multivariate mixed model with population and block as random effects:

$$rAa = \frac{S_{xy}}{\sqrt{S_x^2 \cdot S_y^2}}$$

Where  $S_{xy}$  is the estimated family covariance between trait "x" and trait "y",  $S_x^2$  and  $S_y^2$  are the estimated family variances of traits "x" and "y", respectively.

#### 2.5.4. Climate analysis

Using the central coordinate of each population, 19 bioclimatic variables between the period 1970 and 2000 were downloaded from the WorldClim climate database (<https://www.worldclim.org/data/worldclim21.html>; Supplementary Table 1). The variables measured in the trial were classified into two groups: morphological variables (HI and DI) and symptomatic variables (SW, DW, EW, D95 and S95). Similarly, a first group of bioclimatic variables that correlated with any of the morphological variables (bio 2, bio 4, bio 6, bio 7, bio 12, bio 13, bio 16, bio 17 and bio 19) and a second group of bioclimatic variables that correlated with any of the symptomatic variables (bio 1, bio 8, bio 9, bio 10, bio 11, bio 14, bio 15, bio 17 and bio 18) were made. Description of each bioclimatic variable is available in Supplementary Table 1. Both groups of bioclimatic variables were analyzed with principal component analysis (PCA; Supplementary Table 1).

A first PCA was performed for the bioclimatic variables related to the morphological variables (PCA-morf). Then a linear regression was performed for each morphological variable (HI and DI; dependent variables) with the principal components (PC) obtained from PCA-morf (independent variables).

A second PCA was performed for the bioclimatic variables related to the symptomatic variables (PCA-sint). A linear regression was then performed for each symptomatic variable (SW, DW, EW, D95 and S95; dependent variables) with the principal components (PC) obtained from PCA-sint (independent variables).

#### 2.5.5. Software used

R software (R Core Team, 2020) was used to perform the statistical analyses. Mixed models were estimated using the "lme4" package (Bates et al., 2015). LRT test was applied through the "lmerTest" package (Kuznetsova et al., 2017). Multivariate mixed models, as well as genetic correlations, were calculated through the "BreedR" package (Muñoz & Sanchez, 2023).

## 3. Results

### 3.1. Morphological variables

In both morphological variables (HI and DI) significant differences were reported among the *P. pinaster* populations (Table 2). Population means for HI ranged between 9.00 cm (provenance PTMAATLM) and 13.44 cm (provenance PTPORPIV). Regarding the DI, population means ranged between 1.82 mm (population PTES02) and 2.31 mm (population PTMAATLA). Regarding the controls, the mean HI of the seedlings was  $12.17 \pm 2.93$  cm (while the mean HI of the inoculated seedlings was  $11.36 \pm 1.36$ ) and the DI was  $1.94 \pm 0.32$  mm (while the mean DI of the inoculated seedlings was  $2.06 \pm 0.20$ ).

### 3.2. Symptomatic variables

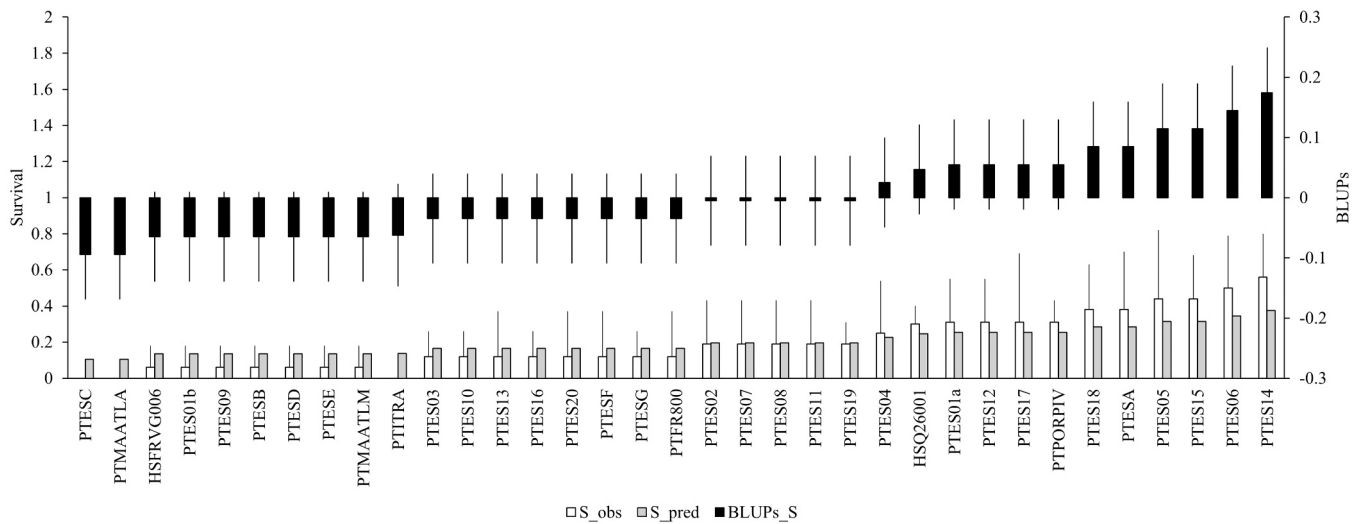
Significant differences between populations were reported both for S95 ( $\chi^2_{LRT} = 5.86$ ;  $p$ -value = 0.016) and D95 ( $\chi^2_{LRT} = 4.64$ ;  $p$ -value = 0.031) (Table 2; Fig. 2). At the end of the experiment, the mean survival was 20 %, ranging between 56 % (provenance PTES14) and 0 % (provenances PTESC, PTITRA and PTMAATLA), while the mean symptom's level was 3.48, ranging between 2.44 (PTES14) and 4 (provenances PTESC, PTITRA and PTMAATLA).

When grouping populations into geographic groups, there were no

**Table 2**

Mean values  $\pm$  standard deviations (SD), variance components ( $\sigma^2$ ), standard error (se), likelihood ratio significance test ( $\chi^2$ LRT). HI: initial height (cm), DI: initial diameter (mm), SW and EW: start and end of damage symptoms (days after inoculation, DAI), DW: duration of damage symptoms (days), D95: damage symptoms at final date (scale 1–4), S95: survival at final date (scale 0–1).

Variable	Mean $\pm$ SD	Random effect					
		Populations			Blocks		
		$\chi^2$ LRT	$\sigma^2$ (se)	$p > \chi^2$	$\chi^2$ LRT	$\sigma^2$ (se)	$p > \chi^2$
HI	11.36 $\pm$ 1.36	17.72	0.62 (0.23)	<0.001	–	–	–
DI	2.06 $\pm$ 0.20	5.54	0.007 (0.004)	0.018	–	–	–
SW	17.70 $\pm$ 5.43	14.84	0.02 (0.008)	<0.001	0	<0.001 (<0.001)	1
EW	45.92 $\pm$ 10.35	1.70	0.005 (0.004)	0.190	0	<0.001 (<0.001)	1
DW	28.22 $\pm$ 10.81	3.87	0.02 (0.01)	0.049	0	<0.001 (<0.001)	1
D95	3.48 $\pm$ 0.64	4.64	0.07 (0.04)	0.031	0	<0.001 (<0.001)	1
S95	0.20 $\pm$ 0.24	5.86	0.01 (0.06)	0.016	0	<0.001 (<0.001)	1

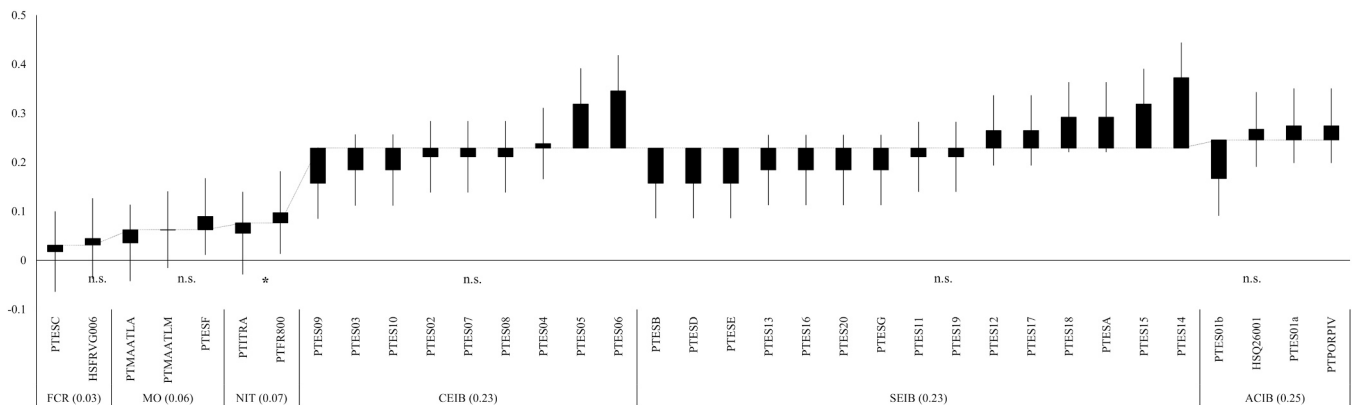


**Fig. 2.** Black columns represent BLUPs (right axis), white columns show observed survival (scale 0–1, left axis) and grey columns indicate predicted survival (scale 0–1, left axis) for each population. Values were estimated from a mixed model for survival at 95 DAI with population and block as random effects. Vertical lines are standard errors of each variable.

differences among geographic groups for S95 ( $F$ -value = 1.68;  $p$ -value = 0.17; data not shown), although there were significant differences among populations within geographic groups ( $\chi^2$ LRT = 3.95;  $p$ -value = 0.047; Fig. 3; data not shown). On the other hand, when S95 analysis was performed for each geographic group, it was found that there were

differences only within the Italian geographic group ( $\chi^2$ LRT = 3.99;  $p$ -value = 0.049; Fig. 3; data not shown). The rest of the geographic groups showed no differences among their populations (Fig. 3).

Regarding the development of symptoms, significant differences were obtained among populations for SW and DW ( $p$ -value < 0.001 and



**Fig. 3.** Survival ranking of populations by geographic group at 95 DAI. Values were calculated from a mixed model with geographic group as a fixed effect and populations within geographic groups and blocks as random effects. The predicted survival for each geographic group is shown in brackets. Solid horizontal line represents best linear unbiased estimates (BLUES) of each geographic group on the logit scale. Black bars represent best linear unbiased predictors (BLUPs) of each population on the logit scale. Vertical lines are standard errors of BLUPs. ACIB, Atlantic coast of the Iberian Peninsula; CEIB, Central Iberian Peninsula; FCR, France-Catalonia region; MO, Morocco-Oria region; Cor, Corsica Island; SEIB, southeast the Iberian Peninsula.



p-value = 0.049, respectively). However, there were no significant differences with respect to EW (p-value = 0.19) (Table 2).

### 3.3. Correlations

S95 correlated strongly with D95, DW and EW. In addition, S95 also correlated with HI at genetic level, while BLUPs correlations showed a marginal positive correlation between both of them (p-value = 0.08). Accordingly, D95 correlated negatively with HI and DI, although not significantly. BLUP correlations showed that D95 correlated negatively with DW and EW. In addition, DW correlated negatively with SW and positively with EW. Finally, HI also correlated strongly with DW and EW, both at BLUP and genetic levels. Non-convergence problems did not allow to calculate the genetic correlation between D95 with DW and EW and did not allow to calculate the standard error of HI with DW (Table 3).

### 3.4. Climatic pattern

With respect to climate, for the bioclimatic variables related to the morphological variables, PC1 is positively influenced by thermal oscillations (diurnal, seasonal and annual thermal oscillations; bio 2, 4 and 7; Table 4, Supplementary Table 1) and negatively by the average minimum temperature of the coldest month (bio 6), and the annual, winter and summer precipitations (bio 12, 13, 16, 17, and 19). Therefore, positive values of PC1 indicated greater thermal oscillation and low summer precipitation, which is related to greater continentality, and vice versa.

Only the HI showed a significant and negative relationship with PC1 (Table 4; Fig. 4). For this trait, populations from MO geographic group had the lowest BLUPs and the highest PC1 values, whereas populations from ACIB geographic group showed high BLUPs and the lowest PC1 values.

Regarding the bioclimatic variables related to symptomatologic variables, PC1 was positively influenced by the average annual temperature and the average temperature of the winter and summer months; and negatively influenced by the precipitation of the summer months. Therefore, positive values of PC1 indicate greater summer aridity and mild winters. By contrast, PC2 was positively influenced by almost all variables. High PC2 values implied less continentality, because it implied both high temperatures and high summer precipitation.

Linear regression showed that D95 and S95 were not related to any of these PCs (p-value > 0.05). However, SW was significantly and positively related to PC1, whereas DW was significantly and negatively related to PC1 and positively related to PC2. Thus, populations whose climates of origin present greater summer aridity and milder winters tend to survive fewer days, once they have shown symptoms, than populations whose climates of origin are more humid. Conversely, populations from less continental climates tend to survive more days. EW was significantly and positively related to PC2 (Table 4; Fig. 4),

**Table 3**

Type A genetic correlations (above the diagonal) and Pearson's correlation among BLUPs (below the diagonal) between pairs of traits. Standard errors of genetic correlations are given in parenthesis; + non-convergence problem; ns: no significant. (X): unable to estimate standard error.

	D95	S95	SW	DW	EW	DI	HI
D95		-0.99 (0.00)	-0.21 (0.17)	+	+	-20 (0.60)	-0.50 (0.54)
S95	-0.98***		0.10 (0.17)	0.90 (0.03)	0.99 (0.00)	0.24 (0.17)	0.53 (0.13)
SW	0.01 ns	-0.09 ns		-0.56 (0.12)	0.25 (0.75)	0.52 (0.66)	-0.06 (0.37)
DW	-0.34*	0.34*	-0.43*		0.65 (0.38)	0.11 (0.17)	0.89 (X)
EW	-0.45**	0.41*	0.19 ns	0.79***		0.62 (0.40)	0.99 (0.01)
DI	-0.13 ns	0.15 ns	0.17 ns	-0.02 ns	0.15 ns		-0.22 (0.42)
HI	-0.26 ns	0.30 ns	-0.16 ns	0.41*	0.39*	0.00 ns	

\* p-value < 0.001

\*\* p-value < 0.05

\*\*\* p-value < 0.01

**Table 4**

Summary of statistics from lineal regression used to describe the relationship between variables measured and climate. HI: initial height; SW: start of symptoms; DW: duration of symptoms; EW: end of symptoms; PC1: principal component 1; PC2: principal component 2; X: no significant relationship found.

Variable	Intercept	Estimate coefficient PC 1	Estimate coefficient PC 2	R2 adjusted	P-value
HI*	0.00	-0.10	-	0.14	0.015
SW**	0.00	0.03	-	0.23	0.002
DW**	0.00	-0.02	0.02	0.21	0.008
EW**	0.00	-	0.01	0.12	0.026

\* PC1-morf formed by bio 2, bio 4, bio 6, bio 7, bio 12, bio 13, bio 16, bio 17 and bio 19

\*\* PC1-sympt and PC2-sympt formed by bio 1, bio 8, bio 9, bio 10, bio 11, bio 14, bio 15, bio 17, bio 18

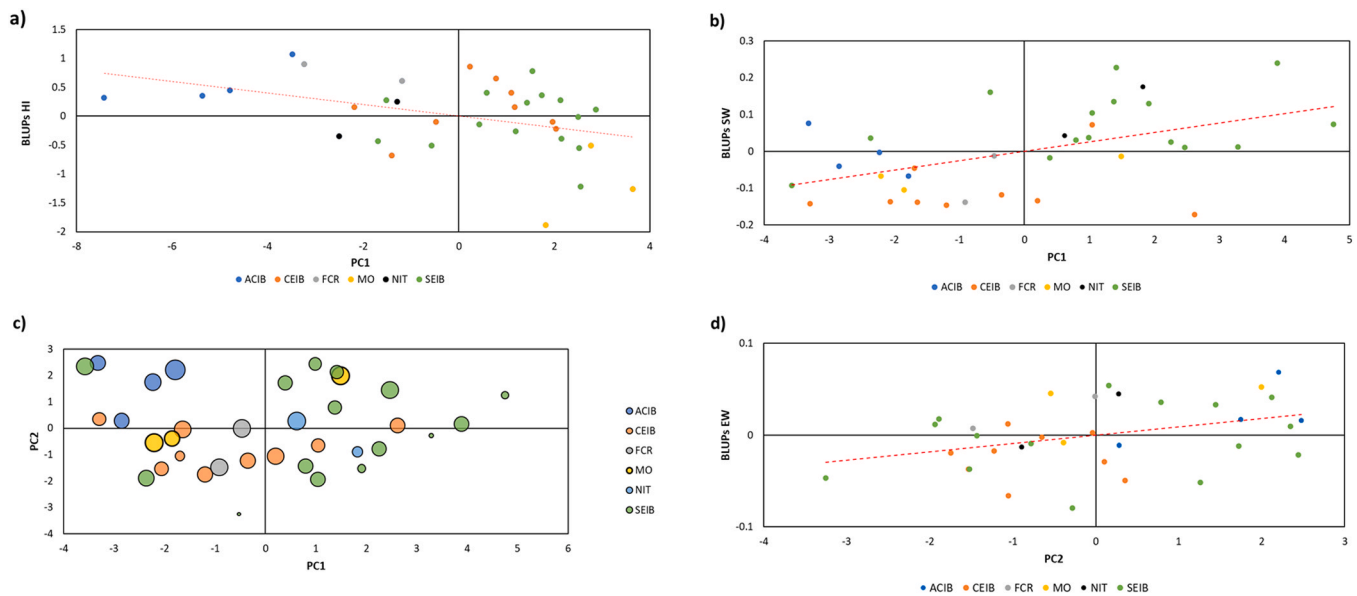
which means that populations coming from less continental climates tended to die later.

## 4. Discussion

The present study confirms the genetic variability of resistance of *P. pinaster* against *F. circinatum* at the population level. However, when the populations are grouped into genetic groups, the differences disappear, although there seems to be a trend of greater resistance of the Iberian groups (CEIB, SEIB and ACIB) compared to the non-Iberian groups (FCR, MO and NIT). The slight relationship found between resistance to *F. circinatum* and the rate of growth in height does not agree with other authors, since in the present study the most resistant population is the one with the highest growth in height. Finally, a clinal climatic pattern was not found for resistance to *F. circinatum*, but for some symptomatic variables, all of them related to aridity and/or continentality.

It has been known for decades that different geographic locations in forest species can give rise to patterns of genetic variation, being a consequence of natural selection and other genetic processes (Morgens-tern, 1996). In this sense, our study has confirmed the significant variability in susceptibility to *F. circinatum* in different *P. pinaster* populations. Our results are in accordance with other authors, who have already reported significant differences at population level in resistance to several diseases for different pine species, such as *P. pinaster* against *F. circinatum* (Elvira-Recuenco et al., 2014) and *B. xylophilus* (Menéndez-Gutiérrez et al., 2017a; Torres-Sánchez et al., 2023), *P. halepensis* against *Gremmeniella abietina* (Romeralo et al., 2016), or *P. sylvestris* against *Dothistroma septosporum* (Perry et al., 2016).

Our results suggested that the variability in susceptibility is likely due to differences between a few populations from different geographic groups and between Northern Italian populations. The differences in susceptibility among populations reported in this work may be due to the fact that each population may develop specific adaptations to cope



**Fig. 4.** Regression of principal components for height (a), start of symptoms (b), duration of symptoms (c; DW: bubble size) and end of symptoms (d). ACIB, Atlantic coast of the Iberian Peninsula; CEIB, Central Iberian Peninsula; FCR, France-Catalonia region; MO, Morocco-Oria region; Cor, Corsica Island; SEIB, southeast the Iberian Peninsula; dashed red line represents the main trend.

with biotic and abiotic stresses (Ramírez-Valiente et al., 2022), even if all populations within the same geographical group share the same evolutionary history, as it is the case of Italian populations (Jaramillo-Correa et al. 2015). For example, Torres-Sánchez et al. (2023) suggested that the physical barrier of the Pyrenees mountains could lead to different adaptations, leading to different levels of susceptibility to *B. xylophilus* between the two populations of the FCR geographic group.

As in this work, grouping the populations based on the gene pools reported by Jaramillo-Correa et al. (2015) has been used previously by other authors when studying the resistance to other pathogens, such as *B. xylophilus* (Menéndez-Gutiérrez et al., 2017a; Torres-Sánchez et al., 2023), *Diplodia sapinea* and *Armillaria ostoyae* (Hurel et al., 2021; De Miguel et al., 2022). Unlike Torres-Sánchez, et al. (2023) adding the geographic group into the analysis did not help to explain part of the susceptibility variation. However, different sample sizes of each geographic group may have led to large variations in the errors of the applied models. Although not significant, it can be actually observed clear differences in susceptibility between the less represented geographical groups (FCR, MO and NIT) and those that are better represented (CEIB, SEIB, and ACIB). Therefore, we cannot discard the possibility of significant differences among geographic groups. Likewise, great genetic variability has already been described both at the population and family level for the susceptibility of *P. pinaster* against *F. circinatum* (Elvira-Recuenco et al., 2014; Vivas et al., 2012). Planning future trials with large, balanced sample sizes by geographic group would help us determine whether these differences are significant or not.

Regarding disease development (SW and DW), significant differences between populations were reported in this work, as other authors found differences between populations of *P. pinaster* infected by *F. circinatum* in the DW (Vivas et al. 2012). However, these differences among *P. pinaster* populations are not found in their infection by all types of pathogens, for example, in the case of the nematode *B. xylophilus* (Torres-Sánchez et al., 2023).

Regarding the susceptibility of *P. pinaster* populations to *F. circinatum*, the absence of significant differences in EW and the differences found in SW and DW indicate that all populations die in the same date range, but begin to show symptoms on different dates. In addition, our results suggested that symptom onset does not determine the susceptibility; whether one population starts showing symptoms

earlier than another does not determine whether it is more or less susceptible.

A positive correlation was obtained between HI and S95, showing that populations with more height growth show greater resistance to the disease. These results are contrary to those reported by other authors with other pathogens, where survival of *P. pinaster* populations against the nematode *B. xylophilus* was negatively related to growth rate (Menéndez-Gutiérrez et al., 2017, 2018; Torres-Sánchez et al. 2023). However, several works have demonstrated the direct relationship between the lower growth rate of *P. pinaster* and its higher phytochemical defensive capacity (Ferrenberg et al., 2015; Di Matteo & Voltas, 2016; Lombardero et al., 2016). As far as the populations-origin climate factor is concerned, a correlation was found with the height of *P. pinaster*, being taller the plants whose climate of origin had lower thermal oscillation and higher summer precipitation, therefore, less continental. It has been reported that greater growth rate is associated to habitats with stable temperatures, as in the coastal areas (Barrio-Anta et al., 2020). On the other hand, our work finds a correlation between the climate of origin of the populations and their behavior against *F. circinatum*. Populations with more arid summer climates and milder winters tend to develop symptoms later and die earlier than populations with more humid climates. Accordingly, populations from less continental climates tend to survive more days. These differences of how each population reacts to *F. circinatum* could be due to the fact that different temperatures and precipitation existing in each specific location of each *P. pinaster* population can generate an important adaptive differentiation (Correia et al. 2014). On the other hand, we have found a lack of correlation between survival and any PC. However, other works have found a clear relationship between the climate of origin of the pine population and its susceptibility to different diseases. Specifically, clinal patterns related to latitude, as in *P. halepensis* against the fungus *Gremmeniella abietina* (Romeralo et al., 2016), *P. sylvestris* against the fungus *Dothistroma septosporium* (Perry et al., 2016), or *P. pinaster* against the nematode *B. xylophilus* (Torres-Sánchez et al., 2023).

Finally, it should be noted that the models proposed in this work do not manage to explain all the population variation, so we should speak of a trend or a minor effect of the climate of origin. There may be other parameters that affect, such as pH, N, C, N/C ratio, as well as other nutrients.

As conclusions, there are significant differences in morphological

parameters and susceptibility to pine pitch canker disease among the different populations of *P. pinaster* analyzed, establishing a negative correlation height-susceptibility. In addition, the climate of origin also shows a clear negative correlation between continental climate and resistance to *F. circinatum*.

### Author contributions

R.D. and J.J.D. conceived, designed and supervised the experiments. T.S.G. performed the experiments. R.D., E.T.S. and J.M.G. analyzed the data. J.P. wrote the manuscript. R.D. and E.T.S. contributed to correcting and critically reading the manuscript. All authors read and approved the published version of the manuscript.

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### CRedit authorship contribution statement

**Raquel Díaz:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Esteban Torres-Sánchez:** Writing – review & editing, Formal analysis. **Jorge Poveda:** Writing – review & editing, Writing – original draft. **Jorge Martín-García:** Formal analysis. **Tamara Sanchez-Gomez:** Methodology, Investigation. **Julio J. Diez:** Writing – review & editing, Supervision, Funding acquisition.

### 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.

### Data Availability

Data will be made available on request.

### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.foreco.2024.121909](https://doi.org/10.1016/j.foreco.2024.121909).

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