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1 **Multi-environment evaluation of *Pinus pinaster* provenances: evidence of genetic**
2 **trade-offs between adaptation to optimal conditions and resistance to the maritime**
3 **pine bast scale (*Matsucoccus feytaudi*)**

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24 Short title: *Pinus pinaster* resistance to *Matsucoccus feytaudi*

27 **Abstract**

28 Maritime pine (*Pinus pinaster* Ait.) is currently threatened by the scale insect *Matsucoccus*
29 *feytaudi* Duc., which feeds exclusively on this species. Some *P. pinaster* provenances from the
30 western Mediterranean are known to be resistant to *M. feytaudi*. The primary resistance
31 mechanism is the existence of anatomical defenses (i.e., resin ducts), but the production of these
32 defenses may come at the cost of resource allocation trade-offs with other functions, including
33 growth. We analyzed a multi-environment trial in central Italy including eight representative
34 provenances of *P. pinaster*. One trial was severely attacked by *M. feytaudi*, whereas three trials
35 remained insect-free at tree age 20. Genotype by environment interactions for growth and
36 mortality were analyzed using the Additive Main effects and Multiplicative Interaction (AMMI)
37 model. Strong correlations were found between AMMI genotypic scores for tree height and
38 diameter and *M. feytaudi* nymph density at the infested trial. Particularly, provenances
39 exhibiting specific adaptation to near-optimal conditions showed high susceptibility to the
40 insect; conversely, those origins better adapted to poorer conditions were much less affected by
41 the outbreak. This study demonstrates that a potentially adaptive divergence in above-ground
42 growth among *P. pinaster* provenances is related to resistance to the insect *M. feytaudi*.

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51 **Keywords:** maritime pine, growth-defense trade-offs, G×E interaction, genetic constraints,

52 AMMI model

53 **Introduction**

54 Maritime pine (*Pinus pinaster* Ait.) is a species of high economic interest that has long been
55 grown for its copious production of resin, which is tapped after the fashion of rubber trees in the
56 western Mediterranean region (Devesa 1997, Blondel and Aronson 1999). It is also used for a
57 variety of forestry-related purposes: landscape enhancement, erosion control of torrents and
58 slopes, soil protection, atmospheric CO₂ storage and ecosystem and social services (Ruiz-
59 Peinado et al. 2013). This species ranks among the most frequently used in large-scale
60 reforestation plans across the Mediterranean basin (Valbuena-Carabaña et al. 2010). However,
61 maritime pine is currently threatened by the scale insect *Matsucoccus feytaudi* Duc. (the
62 maritime pine bast scale), which feeds exclusively on this species. *M. feytaudi* is considered as a
63 primary pest because it causes the early weakening of colonized trees by subtraction of phloem
64 sap from trunk and posterior release of toxic substances causing tissue necrosis (Roversi et al.
65 2013). It predisposes *P. pinaster* to attack by secondary xylophagous insects such as pine
66 weevils and bark beetles.

67 Originally distributed through the western Mediterranean basin, *M. feytaudi* is currently found
68 across most natural range of *P. pinaster*, including its eastern edges (e.g. central Italy).
69 Significant damages by *M. feytaudi* have neither been observed in the Atlantic forests of the
70 French Landes and Portugal nor in most native or artificial pinewoods of Morocco and Spain,
71 where the insect is considered to be endemic (Bariteau 2005, Rodrigo et al. 2013). Conversely,
72 *M. feytaudi* represents the primary pest in the eastern range of the species distribution (south-
73 eastern France and central Italy), probably due to the absence of natural enemies in these areas,
74 with the exception of the anthocorid *Elatophilus nigricornis* Zetterstedt (Jactel et al. 2006). The
75 earliest severe outbreaks following accidental introduction of the insect occurred in Provence
76 pinewoods (Carle and Pontivy 1968, Schwester 1971), where about 120,000 ha of forest were
77 devastated around the late 1960s. Afterwards it spread over the natural and artificial pinewoods
78 of the Maritime Alps (northern Tyrrhenian region of Italy), which are characterized by fragile
79 and simplified ecosystems (Binazzi 2005). Finally the attacks reached Liguria (Masutti 1973,

80 Fabre 1980, Binazzi and Covassi 1989), Tuscany (Binazzi 2005, Roversi et al. 2013) and
81 Corsica (Jactel et al. 1998). These areas of the eastern range of *P. pinaster* are characterized by
82 favorable conditions for the epidemiological development of the invasive herbivore, i.e. micro-
83 stational characteristics and local winds that support the scattering of the first instar nymphs
84 (Roversi et al. 2013).

85 Previous studies performed in French common garden trials have shown that some *P. pinaster*
86 provenances from Morocco (e.g. Tamjoute) and eastern Spain (e.g. Cuenca) are resistant to *M.*
87 *feytaudi* (Schvester and Ughetto 1986). Schvester (1988) pointed out that the low susceptibility
88 of the Tamjoute provenance was related to the impossibility for *M. feytaudi* to reproduce up to
89 injurious levels. In particular, different resistance mechanisms involved in the interactions
90 between the invasive insect and *P. pinaster* have been proposed, thus resulting in different
91 damage levels for similar pest densities. For instance, the existence of mechanical factors
92 related to stembark structure (e.g. bark thickness) constitutes a first obstacle for the crawlers'
93 settlement that presents both a genetic basis and can be influenced by site conditions (Yanchuk
94 et al. 2008). Also, physiological variations in the biochemical content of plant tissues, which is
95 known to be genetically determined (Gerber et al. 1995), may govern tree
96 resistance/susceptibility to the insect (Riom 1979, Schvester and Ughetto 1986, Schvester
97 1988). But the primary resistance mechanism in conifers, and especially so in the case of *P.*
98 *pinaster*, is the existence of a system of canals exuding resin, which acts as both a mechanical
99 and a chemical defense against natural enemies (Franceschi et al. 2005). Resin ducts are
100 constitutive (i.e., produced and present before natural enemies attack) and induced (formed after
101 damage) anatomical defenses that supposedly increase in number wherever a high risk of
102 herbivory exists (Sampedro 2015). However, although investment in defenses increases plant
103 fitness, the production of these defenses usually comes at the cost of resource allocation trade-
104 offs with other important functions (Stamp 2003, Ferrenberg et al. 2015). In this regard, recent
105 observations of genetic trade-offs between growth and resin canal formation in *P. pinaster*
106 (Moreira et al. 2015) are symptomatic of the vegetative costs associated with defense. This

107 could imply that resistant populations such as Tamjoute exhibit limited growth performance
108 under near-optimal conditions (i.e., adaptive syndrome). On the other hand, this provenance has
109 been identified as the most tolerant to water and saline stresses across the distribution range of
110 maritime pine (Saur et al. 1993, Guehl et al. 1995).

111 Following the widespread outbreaks of *M. feytaudi* in northern Italy, a multi-environment trial
112 (MET) involving different provenances of *P. pinaster* was established countrywide in the mid
113 1980's, which essentially cover the area ecologically favorable for the cultivation of maritime
114 pine and predicted to be affected by the insect (i.e., Liguria, Latium and Sardinia) (Figure 1).
115 Here we analyze four testing sites located in central Italy (two located in Sardinia, one in
116 Latium and one in Liguria) where trees have reached an age of 20 years. A first phytosanitary
117 monitoring revealed heavy infestations by *M. feytaudi* at one site located in the Liguria region
118 (Fusaro 1997). This study takes advantage of this severe outbreak affecting one of the four sites
119 including in the MET in order to: (i) determine the extent of genetic variation in resistance to
120 the scale insect *M. feytaudi* among provenances of contrasting geographic origin in the infested
121 site, (ii) characterize genotype \times environment interactions and adaptation patterns for tree
122 growth (total height, diameter at breast height) and mortality across the three healthy sites, and
123 (iii) examine and interpret potential genetic trade-offs across provenances between adaptability
124 to the abiotic environment (i.e., growth potential and survival under insect-free conditions) and
125 resistance to *M. feytaudi*. Currently there is an urgent need for a better understanding of the
126 genetic and environmental factors underlying the resistance mechanisms to the pest and also of
127 the management strategies for the protection of these forest ecosystems. Our general aim is to
128 provide information on suitable genetic resources of *P. pinaster* for use in afforestation and for
129 restoring pinewood stands affected by *M. feytaudi*. This study is part of a long-term research
130 activity on maritime pine launched across Italian pinewoods where *M. feytaudi* infestations have
131 occurred or are expected to occur in the near future.

132

133 **Materials and Methods**

134 *Plant material, site conditions and experimental layout*

135 Field testing was carried out in four *P. pinaster* common garden provenance trials. Three trials
136 were located in central Italy: Rome (Latium region) and Vallermosa and Domusnovas (island of
137 Sardinia). The fourth trial was established in northern Italy (Bordighera, Liguria region) (Figure
138 1, Table 1). At each trial, eight *P. pinaster* provenances originating from Spain (five
139 provenances), Morocco, Italy and France (island of Corsica) were tested. The exception was
140 Coca, a Spanish provenance that was not included in the Domusnovas trial. Estimates of mean
141 annual temperature and precipitation at origin were obtained from the FORADAPT database
142 (<http://w3.avignon.inra.fr/ForSilvaMed/>) and the high-resolution CRU TS3.22 dataset (Harris et
143 al. 2014), respectively, for the period of 1985–2006. The geographical and climatic
144 characteristics of each provenance are reported in Table 2. Provenances can be grouped into two
145 distinct classes according to mean annual precipitation (MAP): low-precipitation provenances
146 (MAP < 700 mm; most Spanish populations plus the Morocco origin) and high-precipitation
147 provenances (MAP ≥ 1300 mm; from Italy and Corsica plus the Spanish “Arenas de San Pedro”
148 population).

149 Seeds were provided by FAO, INRA (France) and local forestry services for the establishment
150 of a provenance trial network on Mediterranean conifers promoted by FAO/Silva Mediterranea.
151 Open-pollinated seeds from at least 20 trees, spaced at least 100 m apart, were obtained within
152 each population and subsequently bulked into population seedlots. Seedlings were grown in
153 nursery for one year and then planted in the field during 1985 at a spacing of 3 x 3 m and a
154 density of 1,111 trees ha⁻¹. Site preparation included removal of competing vegetation and
155 tilling with a disc ripper before planting. Neither irrigation nor fertilization treatments were
156 applied during the establishment. The experimental layout at each trial was a completely
157 randomized block design with four (Rome), five (Vallermosa and Domusnovas) and ten
158 (Bordighera) replicated plots. Each plot or experimental unit consisted of 25 (Rome, Bordighera
159 and Vallermosa) and 36 trees (Domusnovas). Trial surfaces were 0.70, 1.0, 1.5 and 3.0 ha at
160 Rome, Vallermosa, Domusnovas and Bordighera, respectively. Mean annual precipitation

161 ranged from 689 mm (Vallermosa) to 820 mm (Rome), with differences in summer drought
162 period (i.e., 2-3 months at Rome and Bordighera and 3-4 months at Vallermosa and
163 Domusnovas) (Table 1). Vallermosa was the driest trial (aridity index, or ratio of precipitation
164 to potential evapotranspiration, of 0.76) and Rome had the highest mean annual temperature
165 (16.0 °C). Climate records correspond to the period of 1985 (year of plantation) to 2006 (last
166 year of field monitoring) and were obtained from nearby meteorological stations located within
167 a radius of 1 km of each experimental site. Soil characteristics are outlined in Table 1.

168

169 *Field data*

170 In this study, we analyzed field measurements taken once trees reached the age of 20 years.
171 Particularly, results on tree mortality, tree height and diameter at breast height (dbh) are
172 reported. Total height was measured using a PM-5 clinometer (Suunto, Sylvan Lake, MI, USA)
173 and diameter at breast height was determined using a logger's tape. Trees showing bifurcated
174 and/or heavily bent trunks were not included in the analysis. Tree mortality was expressed as
175 percentage of dead trees at the plot level. The Bordighera trial was not included in the analysis
176 of growth and mortality because it resulted heavily affected by *M. feytaudi*. This trial, however,
177 became an ideal setting for the phytosanitary monitoring of the pest because it was located in an
178 area hosting the first severe outbreak of *M. feytaudi* in Italy (Fusaro et al. 2005). On the other
179 hand, the three remaining trials remained insect-free during the evaluation period.

180

181 *Phytosanitary monitoring*

182 Visual phytosanitary observations and entomological samplings were conducted at the
183 Bordighera trial at age 10 (i.e., once the outbreak occurred) and included monitoring tree
184 phytosanitary status and estimating larval population density. When an outbreak of *M. feytaudi*
185 occurs, sap sucking by larvae induces resin exudation and tree weakening, which often translate
186 into growth decrease and dieback (Jactel et al. 1996, Jactel et al. 1998). The first instar nymphs

187 develop actively since the early spring season, sucking tree sap by inserting their stylets into the
188 phloem. They usually remain at the settling site for the rest of its nymphal development. The
189 mortality rate of the first instar nymphs is critical for the future development of the population,
190 as 90-98% of crawlers usually die due to adverse climatic conditions (i.e., rain episodes) or
191 intense resin production (Riom 1979). Nymph population density was estimated by taking
192 small-sized bark samples (approximately 7×7 cm) of trees at around half their height during
193 the spring season and counting the number of nymphs with a binocular microscope.
194 Additionally, visual observations were also conducted to assign a score to each tree based on
195 infestation symptoms and associated phytosanitary status due to the outbreak, i.e. abundant resin
196 flow from trunk, yellowing and reddening of the foliage, and, ultimately, needle loss. The
197 following evaluation grid was used: (A) no visible symptoms observed, individual with good
198 vegetative condition, (B) some visible symptoms already observed, but individual keeping an
199 adequate condition, (C) abundant visible symptoms observed, tree heavily damaged,
200 functionality compromised and/or in biological decay and/or died. The phytosanitary evaluation
201 was conducted in at least 1-2 trees per provenance and experimental unit. All trees included in
202 the trial, except those of Tamjoute, died during the subsequent years following the phytosanitary
203 monitoring.

204

205 *Statistical analyses*

206 As a starting point, separate analyses of variance (ANOVAs) were fitted at the trial level for
207 growth traits, and also for nymph population density at the Bordighera trial. We used an
208 individual-tree mixed model consisting of a block effect, a provenance effect, an intra-block
209 error term (or provenance \times block interaction), and a random term accounting for the between-
210 tree within-plot variation (or intra-plot error). Inspection of the range of plot error variances
211 indicated that population effects for nymph density had been estimated with unequal precision
212 across populations at the Bordighera trial. Therefore, a log transformation was selected as the

213 method to remedy error plot variation. Besides, mortality rates (mostly ranging between 0% and
 214 30% at the plot level) were also squared-root transformed to stabilize variances (Bowley 1999).
 215 Since forest genetic trials are commonly large and strongly heterogeneous, adjustments for
 216 micro-environmental heterogeneity often become compulsory (Zas 2006, Ye and Jayawickrama
 217 2008). When spatial heterogeneity is present, near neighbors are more similar than far
 218 neighbors, i.e. records are spatially autocorrelated, and the requirement of data independence in
 219 standard parametric statistics is violated. We explored the presence of spatial autocorrelation in
 220 the studied traits, with the exception of survival data since individual records of tree mortality
 221 were not available. Residuals of each variable after subtraction of provenance effects were used
 222 to investigate the spatial structure of the data at each trial, that is, height and *dbh* at Rome,
 223 Vallermosa and Domusnovas, and log (nymphs cm⁻²) at Bordighera. Previously, the *x-y*
 224 coordinates of each tree were obtained by dividing the plantation lines in *n-1* equal-length
 225 segments, where *n* is the number of trees in a particular line.

226 The spatial structure of the resulting residuals was modeled using a semivariogram, which plots
 227 the semi-variance among trees as a function of their distance. The semivariance $\lambda(h)$ was
 228 calculated as:

$$229 \quad \lambda(h) = \frac{1}{2n} \sum_{i=1}^n [z(s_i) - z(s_{i+h})]^2 \quad (1)$$

230 where *n* is the number of observation pairs separated by distance *h* (called the lag distance),
 231 $z(s_i)$ is the value for a tree located at s_i and $z(s_{i+h})$ is the value for a tree located at a distance
 232 *h* from s_i . For randomly distributed data, little change in the semivariance will be obtained
 233 when *h* increases, and the semivariogram will be essentially flat. If spatial dependence is
 234 present, the semivariance will be lower at short distances, it will increase for intermediate
 235 distances and it will typically reach an asymptote for long distances. The distance at which the
 236 asymptote begins, if present, indicates the range or patch size of heterogeneity below which
 237 data are stochastically dependent (Cressie 1993).

238 We fitted isotropic exponential, Gaussian and spherical models to the experimental
239 semivariograms (Littell et al. 2006). The best fitted model was then used to partition the
240 variation of residuals into spatially autocorrelated variation and random error by integrating the
241 spatial variability into mixed models. Starting values of spatial dependence for each mixed
242 model were suggested by the estimates from the corresponding best model fitted to the
243 experimental semivariogram. Further details on the procedure used can be found in Hong et al.
244 (2005) and Littell et al. (2006). Three types of models were compared: a) Randomized
245 Complete Block with independent errors (default model), b) Randomized Complete Block with
246 correlated errors, and 3) Correlated Errors models (i.e., dropping block effects from the model).
247 The Akaike Information Criterion was used to compare the relative goodness-of-fit among
248 candidate models. If relevant, the impact of spatial adjustment on the inference of provenance
249 effects was assessed by comparing *F*-ratios and standard errors of population means for
250 unadjusted (Randomized Complete Block with independent errors) and spatially adjusted
251 models. Finally, we computed Tukey's HSD post-hoc tests for means separation among
252 provenances for the studied traits. The experimental semivariograms were computed with the
253 VARIOGRAM procedure implemented in SAS version 9.4 (SAS Institute Inc. 2013). The
254 theoretical semivariograms, either spherical or exponential, were fitted to the experimental
255 semivariogram using the NLIN procedure in SAS. Variance components and best linear
256 unbiased estimators (BLUEs) of provenance effects were estimated using the restricted
257 maximum likelihood method of the MIXED procedure in SAS.

258 Best linear unbiased estimators (BLUEs) of genotypic (i.e., provenance) effects at the trial level
259 (i.e., genotype-environment means) were then carried forward to a second stage for the analysis
260 of the multi-environment trial. Mixed 2-way ANOVAs were fitted to growth and (squared-root
261 transformed) mortality data with the following treatment structure: genotype (G), environment
262 (E) and G×E interaction. The genetic effects were considered as a random factor and the relative
263 relevance of G×E interaction was estimated by the ratio σ_G^2/σ_{GE}^2 , where σ_G^2 and σ_{GE}^2 are the

264 provenance and provenance \times site interaction variance components estimated from the multi-
265 environment ANOVA.

266 A common extension of ANOVA for studying G \times E interaction is the additive main effects and
267 multiplicative interaction (AMMI) model (Gauch 1992). AMMI was fitted to the 2-way G \times E
268 table for tree growth (height, dbh) and mortality. AMMI is a special case of biadditive model,
269 that is, models that are characterized by terms that consist of products of row and column
270 parameters (Denis and Gower 1994, van Eeuwijk 1995). The general formulation for the AMMI
271 model for a trait Y of genotype i in environment j is:

$$272 \quad Y_{ij} = \mu + g_i + e_j + \sum_k \lambda_k c_{ik} d_{jk} + \rho_{ij} \quad (2)$$

273 where μ is the overall mean; g_i and e_j are genotypic and environmental main effects; λ_k is the k^{th}
274 singular value from the singular value decomposition (Gabriel 1978) of the matrix of interaction
275 residuals; c_{ik} and d_{jk} are the genotypic and environmental scores, respectively, for the k^{th}
276 multiplicative term; and ρ_{ij} is a residual term after the extraction of the multiplicative interaction
277 effects. AMMI generates a family of models based on the number of multiplicative terms, K ,
278 necessary for an adequate description of the interaction. The AMMI0 model indicates that no
279 multiplicative terms are included and the model is equivalent to the additive (main effects only)
280 ANOVA. AMMI1 includes the first multiplicative term, and so on, while AMMI F is the full
281 model that includes all axes and is equal to the raw data. The maximum number of
282 multiplicative terms, K , for a given dataset is one less the minimum number of genotypes or
283 environments ($K=2$ in our case) (note that the Spanish provenance Coca could not be included
284 in the AMMI analyses because it was not evaluated at Domusnovas). The best AMMI model
285 was assessed by first transforming the eigenvalues (=sum of squares) corresponding to the
286 individual terms to mean squares (Gollob 1968). The mean squares were obtained by dividing
287 the eigenvalues by degrees of freedom: $I+J-3$ for the first term, $I+J-5$ for the second, etc.
288 Subsequently, significance for these mean squares was assessed with an F -test. Typically, more
289 parsimonious models, containing a few multiplicative axes, are often preferred to summarize

290 G×E patterns. AMMI models are most helpful when the multiplicative terms have biological
291 interpretability. AMMI was fitted using SAS/Interactive Matrix Language (IML) software (SAS
292 Institute Inc. 2013).

293

294 **Results**

295 *Mortality*

296 Overall, mortality rates 20 years after planting were low (mean mortality across trials = 13%).
297 However, tree mortality was significantly ($p<0.05$) higher in Vallermosa (17.4%) than in Rome
298 and Domusnovas (9.6% and 8.1% respectively) (Table 3). We also found significant differences
299 among provenances ($p<0.05$) at the trial level, with Tamjoute, Val Freddana and Siles
300 consistently showing lower mortality (<10%) compared with the rest of provenances (Table 3).
301 In particular, the Coca provenance showed the highest mortality rates, with 45% and 26%
302 mortality at Vallermosa and Rome respectively. The ranking of provenances for mortality rate
303 was quite consistent across trials, as also indicated by the high ratio of G to G×E variance
304 components ($\sigma^2_G/\sigma^2_{GE}= 3.9$).

305

306 *Growth traits*

307 Modeling spatial heterogeneity at the trial level did not improve the estimation of genetic effects
308 for growth traits (height and *dbh* at age 20). The only exception was Vallermosa, which slightly
309 benefitted from spatial adjustment by using a Gaussian function fitted to the height data. In this
310 case, the range or patch size for total height was ~15 m (i.e., the distance below which height
311 values were spatially dependent). As a consequence of the spatial adjustment (using a
312 Randomized Complete Block Design with correlated errors) the F-ratio for provenance effects
313 increased from 8.1 to 9.9 and the standard error of differences between provenances decreased
314 from 0.33 m to 0.31 m.

315 We found significant genetic effects ($p<0.05$) for both height and diameter at the trial level. At
316 the Vallermosa trial, differences in mean *dbh* among provenances ranged from 19.1 cm

317 (Tamjoute) to 20.6 cm (Vivario), whereas mean total height varied between 8.93 m (Coca) and
318 10.57 m (Vivario) (Table 3). At the Rome trial, mean *dbh* ranged from 18.9 cm (Tamjoute) to
319 23.8 cm (Vivario) and mean total height from 9.73 m (Tamjoute) to 14.50 m (Vivario). Finally,
320 at Domusnovas mean *dbh* varied between 20.4 cm (Arenas de San Pedro) and 24.3 cm (Siles)
321 and mean total height between 10.46 m (Arenas de San Pedro) and 13.09 m (Vivario). Overall,
322 trees were significantly ($p < 0.05$) taller at Rome and Domusnovas as compared with Vallermosa.
323 However, they reached a higher *dbh* at Domunosvas than at Rome, followed by Vallermosa.
324 The ranking of provenances across trials for height and diameter was much less consistent than
325 for mortality as suggested by the much smaller ratios of G to G×E variance components
326 ($\sigma^2_G/\sigma^2_{GE} = 1.1$ and 0.38 for height and *dbh* respectively). This result pointed to sizeable G×E
327 effects affecting growth traits. At the trial level, the ranking of provenances for growth traits at
328 the juvenile (age 5) and adult stage (age 20) was quite inconsistent (e.g. Spearman's rank
329 correlation for height: $r = -0.09$, $p = 0.82$ at Rome; $r = 0.13$, $p = 0.78$ at Domusnovas; $r = 0.60$, $p = 0.11$
330 at Vallermosa).

331

332 *Phytosanitary status*

333 Unlike for growth traits, residuals after subtracting provenance effects clearly showed
334 nonrandom spatial structures for *M. feytaudi* nymph population density at Bordighera. In this
335 case, the spherical theoretical semivariogram fitted well to the observed semivariogram ($r^2 =$
336 0.66) (Figure 2), indicating that data from near neighbors were more similar than those from far
337 neighbors. Particularly, the range or patch size for $\log(\text{nymphs cm}^{-2})$ was ~ 16 m, clearly lower
338 than the block size (~ 0.25 ha), which indicates a spatial heterogeneity within blocks that violates
339 the block design assumptions. By using a Randomized Complete Block Design with correlated
340 errors the F-ratio for provenance effects hugely increased from 11.6 to 25.2 and the standard
341 error of differences (s.e.d.) between provenances decreased from 0.16 to 0.11. There were some
342 noticeable switches in provenance ranking after the spatial adjustment. For example, the
343 tolerance to *M. feytaudi* was overestimated for Graja de Campalbo (initially ranking 2th in

344 tolerance, shifting to 4th after spatial adjustment) and underestimated for Cuenca (initially
345 ranking 3rd in tolerance, shifting to 2nd after spatial adjustment).

346 Provenance means for log-transformed records of nymph population density are shown in Table
347 4. Tamjoute showed the lowest mean (0.60 nymphs cm⁻²), whereas the highest values were
348 observed for Vivario and Val Freddana, with 2.05 and 1.83 nymphs cm⁻² respectively. These
349 provenances were also severely damaged according to visual observations. The Spanish
350 provenances showed intermediate values, ranging from 1.14 to 1.59 nymphs cm⁻². The visual
351 assessment also indicated no evident vegetative damages in the Tamjoute provenance,
352 suggesting complete resistance to the bast scale *M. feytaudi* (Figure S1).

353

354 *AMMI analysis*

355 The AMMI1 model accounted for 63.2%, 78.7% and 70.8% of the G×E sum of squares for total
356 height, *dbh* and mortality, respectively, with only 7 (out of 18) G×E degrees of freedom. Thus,
357 an AMMI model with just one multiplicative term sufficed to capture most G×E sum of squares.
358 The outcome of the AMMI analyses was summarized with the aid of biplots, in which the
359 additive main effects (provenance and trial) were plotted on the *x*-axis in the corresponding
360 units, and the scores of the first multiplicative term were plotted on the *y*-axis, which have units
361 expressed as the square root of the original unit. In this way, the relationships between main
362 effects and interaction could be observed simultaneously.

363 The biplot of the AMMI1 model for total height is shown in Figure 3A. The vertical reference
364 line represents the grand mean height of the dataset and the horizontal line is placed at zero for
365 the scores of the first multiplicative axis, where points farther away from this line indicate
366 provenances or trial environments with larger interactions. Provenances and environments
367 having the same sign for their AMMI1 scores have positive interactions (i.e. specific adaptation
368 of a provenance to a particular environment), whereas opposite signs denote negative
369 interactions. In particular, most Spanish provenances plus the Moroccan origin showed specific
370 adaptation to the Sardinian trials (Vallermosa and Domusnovas) (i.e., better performance

371 relative to what it would be expected based on the ranking of genotypic means across trials),
372 whereas the Italian (Val Freddana) and Corsican (Vivario) provenances plus the Spanish origin
373 Arenas de San Pedro were better adapted to the Rome trial. This behavior seemed independent
374 of the average provenance performance in terms of total height. For instance, Vivario showed
375 the largest height across trials, followed by Graja de Campalbo and Cuenca (x-axis; Figure 3A).
376 Whereas the former showed specific adaptation to Rome, Graja de Campalbo and (especially)
377 Cuenca were better adapted to the Sardinian environments. The two most extreme provenances
378 in terms of interaction patterns were Vivario and Tamjoute, as they occupied perfectly opposite
379 positions in the biplot (Figure 3A).

380 Very similar interaction patterns were found for *dbh* (Figure S2), with the Spanish provenance
381 Arenas de San Pedro showing the largest affinity for the conditions encountered in the Rome
382 trial, followed by Val Freddana. Tamjoute, on the other hand, showed the best relative
383 performance in Domusnovas (i.e., the most negative AMMI1 genotypic score). This similarity
384 in G×E trends is reflected in the significant correlation between AMMI1 genotypic scores for
385 height and *dbh* ($r= 0.86$, $p<0.01$; $n=7$). On the other hand, G×E interaction patterns for tree
386 mortality were essentially independent of those found for growth traits. For instance, Arenas de
387 San Pedro and Vivario showed relatively low mortality rates in the Sardinian trials, whereas
388 Cuenca and Graja de Campalbo performed better at Rome (Figure S3). The independence of
389 adaptation patterns for growth traits and tree mortality is also demonstrated by the lack of
390 significant relationships between AMMI1 genotypic scores involving height and mortality ($r= -$
391 0.36 n.s.) or *dbh* and mortality ($r= -0.40$ n.s.).

392 Finally, we attempted to relate the abovementioned genotypic mean performances and
393 interaction patterns for growth traits and tree mortality with the reaction of the provenances to
394 the outbreak of *M. feytaudi* at the Bordighera trial. To this end, provenance means across trials
395 and AMMI1 genotypic scores were correlated with the nymph population density at the
396 genotype level following the insect attack. First, the associations between genotype means of
397 total height, *dbh* or tree mortality and nymph abundance were non-significant (Figure 3B,

398 Figure S2B, Figure S3B). However, differences in adaptive patterns for growth traits, as
399 explained by the AMMI1 scores, were significantly correlated to nymph density across
400 provenances. This was especially evident for total height (Figure 1C), but also for *dbh* (Figure
401 S2C), suggesting that growth adaptation to particular conditions underlay the relative genotypic
402 tolerance to *M. feytaudi*. In particular, provenances exhibiting specific growth adaptation to
403 Rome (e.g. Vivario) showed high susceptibility to the insect; conversely, those origins better
404 adapted to the Sardinian environments (e.g. Tamjoute) were less affected by the outbreak. On
405 the other hand, adaptation patterns for tree mortality were found to be unrelated to genotypic
406 differences in tolerance to *M. feytaudi* (Figure S3C).

407

408 **Discussion and Conclusions**

409 Maritime pine shows a very wide array of adaptation patterns to contrasting edaphic and
410 climatic conditions along its distribution range (González-Martínez et al. 2002). Among
411 Mediterranean pines, *P. pinaster* also displays high levels of phenotypic plasticity, modulating
412 its performance according to the environment where it grows (Chambel et al. 2007). In the
413 present study we have characterized G×E interactions for growth and survival using a
414 representative set of maritime pine provenances, demonstrating that a potentially adaptive
415 divergence in above-ground growth among genetic origins is related to resistance to the scale
416 insect *M. feytaudi*.

417

418 *Adaptive divergence in growth performance*

419 The maritime pine provenances studied here originate from different bioclimatic areas. Arenas
420 de San Pedro, Val Freddana and Vivario thrive in Mediterranean humid and sub-humid zones
421 characterized by mild to cool winters, annual precipitation exceeding 1,000 mm and moderate
422 summer drought (Corcuera et al. 2011, Desprez-Loustau and Dupuis 1994). Tamjoute, Coca and
423 Siles grow in semi-arid zones at relatively high elevations, moderate annual precipitation (<700
424 mm) and a more pronounced summer drought period. Graja de Campalbo and Cuenca are found

425 under intermediate conditions, that is, relatively cold temperatures and moderate annual
426 precipitation (Nicolas and Gandullo 1967). The sizeable G×E interactions for growth traits
427 indicated differential genotypic reactions to site conditions. This outcome has been extensively
428 documented for *P. pinaster* populations in Italy (Tognetti et al. 2000) and Spain (Alía et al.
429 1997) and also among families of a breeding population of the species (de la Mata et al. 2012).
430 According to Shelbourne (1972), G×E interaction may be defined of “practical relevance” in
431 tree genetics if the variance component of G×E effects reaches 50% or more of the genotypic
432 variance component (that is, $\sigma^2_G/\sigma^2_{GE} < 2$). In this study, G to G×E variance component ratios
433 for growth traits clearly exceeded this threshold. This result anticipated important changes in
434 provenance ranks depending on the testing site (*cf.* Table 3). However, the importance of G×E
435 effects for tree mortality was less relevant, as opposed to previous results on a large MET
436 analysis including over 50 provenances of maritime pine evaluated in Spain (Alía et al. 1997).
437 In this study all provenances showed the lowest tree size and highest mortality at the driest site
438 (Vallermosa). However, the relative ranking of provenances was not kept across sites.
439 According to previous studies (Corcuera et al. 2012, Gaspar et al. 2013, de la Mata et al. 2014),
440 populations from mesic sites show higher plasticity and grow better under optimal water
441 availability, whereas populations originating from harsher climates show less responsiveness to
442 water availability in terms of growth. This observation is fully confirmed by the AMMI
443 analysis: Arenas de San Pedro, Vivario and Val Freddana, originating from humid and low-
444 altitude sites, performed better at the lowest and, in principle, most productive trial (i.e., Rome);
445 however, they suffered under relatively arid (i.e., Vallermosa) or cold (i.e., Domusnovas)
446 conditions. On the other hand, high-altitude, drier-than-average provenances such as Tamjoute,
447 Graja de Campalbo and Siles grew better, in relative terms, in dry or cold sites. Other works
448 have reported that differences among populations in root morphology, whole-plant conductance,
449 carbon allocation, plant growth and water-use efficiency are significant and consistent with
450 dryness at origin, with xeric populations exhibiting lower growth and a conservative water-use
451 efficiency (Tognetti et al. 2000, Corcuera et al. 2012). In any case, Vivario consistently

452 outperformed most provenances across trials, exhibiting exceptional growth potential and high
453 plasticity.

454

455 *Insect resistance trades-off with growth adaptation to optimal conditions in P. pinaster*

456 The spatial analysis allowed detecting the patchy distribution of the insect attack, with a range
457 value of spatial dependence of about 15 m. Forest genetic trials can greatly benefit from spatial
458 adjustment (Zas 2006, Ye and Jayawickrama 2008), especially in the case of screening resistant
459 material to pests and diseases, where the evaluation of genetic entries can be seriously
460 compromised by the patchy distribution of the infection or outbreak (e.g. Zas et al. 2007). In our
461 case, comparisons among provenances for resistance to *M. feytaudi* greatly improved due to
462 higher accuracy of the provenance effect estimations (i.e., reduction of s.e.d. of ca. 30%).

463 The growth conditions encountered at the Rome trial can be regarded as optimal for maritime
464 pine. Rome is located at about sea level and has the highest precipitation and the highest mean
465 annual temperature of all sites, which suggests a very long growing season and a favorable tree
466 water status across the year (Alía et al. 1997). However, this theoretical climate optimum did
467 not always translate into a clear growth advantage as compared with one Sardinian trial
468 (Domusnovas). Anyhow, Domunosvas and Vallermosa (the second Sardinian trial) elicited
469 similar adaptive growth responses, probably owing to their higher altitude and lower mean
470 annual temperature and precipitation. Notably, the divergent adaptive responses to site condition
471 were highly related to the importance of the scale bast attack at the provenance level as shown
472 in an independent trial (Bordighera) heavily affected by *M. feytaudi*.

473 Provenances showing specific adaptation to near-optimal growing conditions (e.g. Vivario and
474 Arenas de San Pedro in Rome) were severely attacked by the insect at the Bordighera trial.
475 Particularly, Arenas de San Pedro has been earmarked as seedstock due to its combination of
476 traits conferring increased growth and drought resistance (Corcuera et al. 2012). This
477 provenance, however, was not particularly fast-growing and showed high susceptibility to *M.*
478 *feytaudi*. On the other hand, provenances showing adaptation to less favorable conditions were

479 comparatively much less affected and one (Tamjoute) showed no visual symptoms of damage
480 (cf. Table 4, Fig. S3). In fact, Tamjoute was the only surviving provenance during the years that
481 followed the phytosanitary assessment. These results reinforce those of previous studies
482 showing that the Moroccan provenance exhibits a large inversion of root biomass (Corcuera et
483 al. 2012) and high osmotic adjustment associated to drought tolerance (Loustau et al. 1995), but
484 they also support the theoretical background predicting that optimal relative investment in
485 defenses should be greater in slow-growing species or lineages adapted to stressful, growth-
486 limiting environments due to greater construction costs (Sampedro 2015). In the absence of
487 infestation, Bordighera (a site of poor soil fertility) could be expected to elicit genotypic
488 differences in terms of growth similar to those of the Sardinian sites and, particularly, to
489 Vallermosa (the lowest growth site in the MET). In fact, the correlation between genotype
490 means of Vallermosa and Bordighera at age 8 (before the heavy infestation occurred) was 0.90
491 ($p < 0.05$) (results not shown), which suggests that provenances specifically adapted to poor
492 conditions are, in turn, those showing higher resistance to *M. feytaudi*. In this regard, the
493 examination of genotypic patterns of resistance to the scale insect should also be examined in
494 near-optimal sites as to confirm the prevalence of the trade-off across environmental conditions.
495 Our results add to a growing, albeit scarce, body of literature reporting on the existence of
496 genetic constraints between plant growth and resistance against pest and pathogens (e.g. Rosner
497 and Hannrup 2004, Moreira et al. 2014, Moreira et al. 2015). In a recent study on 18 *Pinaceae*,
498 Moreira et al. (2015) observed that constitutive chemical defenses traded-off with growth
499 potential such that slow-growing species invested more in constitutive defense, whereas fast-
500 growing species invested more in inducible defense. A similar pattern was found at the intra-
501 specific level for *P. pinaster* families growing under limited phosphorus availability (Moreira et
502 al. 2014). Although we did not directly measure defensive allocation (i.e., density of constitutive
503 resin canals, resin production or flow), the novelty of our study lies on the independent
504 assessment of adaptive patterns of *P. pinaster* populations (in terms of growth and survival) and
505 resistance to a severe insect outbreak under true field conditions. Indeed, we found evidence for

506 genetic trade-offs at the intra-specific level between growth adaptation to optimal conditions
507 and effective resistance to *M. feytaudi* by characterizing field performance of different genetic
508 origins in the adult stage. To the best of our knowledge, this is the first field study reporting on
509 adaptive divergence for growth traits in adult forest trees linked to the potential cost of resource
510 allocation to defense as a way to optimize plant fitness. Additionally, a greater effective
511 resistance could also be linked to other factors deserving further consideration and not
512 necessarily related to resource allocation, such as pest recognition and ability for signaling the
513 damage perceived (Sampedro 2014). Anyhow, this study highlights the importance of an
514 optimal use of intra-specific variability in deployment of genetic material to particular
515 production environments in a widely-used conifer such as *P. pinaster*, especially for those areas
516 potentially affected by the maritime pine bast scale.

517

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750 SUPPLEMENTARY MATERIALS

751

752 Supplement 1. Detail of the Bordighera trial showing the resistance of the Tamjoute provenance

753 to *Matsucoccus feytaudi*.

754 Supplement 2. Biplot of the AMMI1 model for diameter at breast height.

755 Supplement 3. Biplot of the AMMI1 model for tree mortality rate.

756 FIGURE CAPTIONS

757

758 Figure 1. Distribution map of the natural range of *Pinus pinaster* L. (dark shaded areas) and
759 geographical location of the eight studied provenances and the four Italian trials. The numbers
760 refer to provenances as reported in Table 2 while the stars identify the different field trials.

761

762 Figure 2. Semivariogram for nymph density of *M. feytaudi* in maritime pine adjusted for
763 provenance effects (Bordighera trial), i.e. provenance mean subtracted to each individual
764 observation.

765

766 Figure 3. A) Biplot of the AMMI1 model for total height; B) Relationship between provenance
767 means across trials for total height and provenance nymph density of *M. feytaudi* at the
768 Bordighera trial; C) Relationship between genotypic scores of the AMMI1 model for total
769 height and provenance nymph density of *M. feytaudi* at the Bordighera trial. The black squares
770 indicate the position of the three field trials and the white circles denote the position of the eight
771 provenances in the biplot. The numbers in panels B and C refer to the provenance codes as
772 reported in panel A and in Table 2

773

1 TABLES

2

3 Table 1. Main geographic and climatic features of the four Italian field trials of *P. pinaster*.

Trial	Municipality	Latitude	Longitude	Altitude (m a.s.l.)	Mean Annual Precipitation (MAP) (mm)	PET (mm)	Aridity index (MAP/PET)	Mean Annual Temperature (°C)	Soil
Bordighera (Liguria)	Imperia	43°48'N	07°40'W	500	741	~ 670	1.10	14.0	Sandy
Domusnovas (Sardinia)	Cagliari	39°24'N	08°35'W	600	745	~ 850	0.87	12.3	Alluvial
Vallermosa (Sardinia)	Cagliari	39°23'N	08°47'W	400	689	~ 900	0.76	15.3	Sandy-clay
Rome (Latium)	Rome	41°54'N	12°22'W	50	820	~ 1000	0.82	16.0	Sandy-loam

4 PET, potential evapotranspiration.

5 Table 2. Main geographic and climatic features of *Pinus pinaster* provenances.

Provenance (ID)	Country	Municipality	Latitude	Longitude	Altitude (m a.s.l.)	Mean Annual Precipitation (MAP) (mm)	PET (mm)	Aridity index (MAP/PET)	Mean Annual Temperature (°C)
Arenas de San Pedro (1)	Spain	Avila	40°15'N	05°15'W	650	1362	1163	1.17	12.2
Coca (2)	Spain	Segovia	40°13'N	04°31'W	775	462	1068	0.43	11.3
Cuenca (3)	Spain	Cuenca	40°24'N	02°13'W	1061	687	1136	0.60	11.3
Graja de Campalbo (4)	Spain	Cuenca	39°54'N	01°16'W	1097	590	1002	0.59	11.2
Siles (5)	Spain	Jaen	38°25'N	02°30'W	1250	684	1195	0.57	13.4
Tamjoute (6)	Morocco	Tahala	32°53'N	04°10'W	1400	634	1391	0.46	14.5
Vivario (7)	Corsica	Bastia	42°10'N	09°10'W	600	1420	734	1.93	12.3
Val Freddana (8)	Italy	Lucca	43°55'N	10°17'W	200	1300	1000	1.30	13.6

6 PET, potential evapotranspiration.

7 Table 3. Provenance means for growth traits (total height, dbh) and tree mortality at three *Pinus pinaster* trials located in Italy.

Vallermosa¹			Rome¹			Domusnovas¹		
Provenance	height (m)		Provenance	height (m)		Provenance	height (m)	
Vivario	10.57	A	Vivario	14.50	A	Vivario	13.1	A
Cuenca	10.35	A B	Graja de C.	12.25	B	Graja de C.	12.7	B
A. de S. Pedro	9.72	B C	A. de S. Pedro	11.99	B C	Siles	12.2	B C
Graja de C.	9.68	B C	Coca	11.87	B C	Cuenca	11.7	B C
Siles	9.06	C	Siles	11.36	B C	Tamjoute	11.3	B C
Val Freddana	9.01	C	Cuenca	11.01	C	Val Freddana	10.5	C
Tamjoute	8.95	C	Val Freddana	10.99	C	A. de S. Pedro	10.5	C
Coca	8.93	C	Tamjoute	9.73	D	Coca	<i>n.a.</i>	

dbh (cm)			dbh (cm)			dbh (cm)		
Vivario	20.6	A	Vivario	23.8	A	Siles	24.3	A
Siles	20.2	A B	A. de S. Pedro	21.9	B	Graja de C.	24.0	A
A. de S. Pedro	19.9	A B	Coca	21.7	B C	Vivario	23.8	A B
Cuenca	19.8	A B	Siles	21.4	B C	Tamjoute	23.0	A B
Graja de C.	19.6	A B	Graja de C.	20.8	B C D	Cuenca	22.4	B C
Val Freddana	19.3	A B	Cuenca	19.8	C D E	Val Freddana	21.1	C D
Coca	19.2	A B	Val Freddana	19.4	D E	A. de S. Pedro	20.4	D
Tamjoute	19.1	B	Tamjoute	18.9	E	Coca	<i>n.a.</i>	

mortality (%)			mortality (%)			mortality (%)		
Tamjoute	5.6	A	Siles	4.0	A	Graja de C.	3.3	A
Siles	7.2	A	Val Freddana	5.0	A B	Tamjoute	3.3	A
Val Freddana	7.2	A	Tamjoute	6.0	A B	Val Freddana	3.9	B
Vivario	12.8	B	Graja de C.	6.0	A B	Siles	7.8	B
A. de S. Pedro	15.2	B	Cuenca	7.0	B C	Cuenca	10	C
Graja de C.	17.6	B	Vivario	9.0	C D	Vivario	11.7	C D
Cuenca	28.8	C	A. de S. Pedro	11.0	D	A. de S. Pedro	16.7	D
Coca	44.8	D	Coca	26.0	E	Coca	<i>n.a.</i>	

8 ¹Mean values with the same letter are not significantly different ($p > 0.05$) according to Tukey's HSD test. *n.a.*, not available.

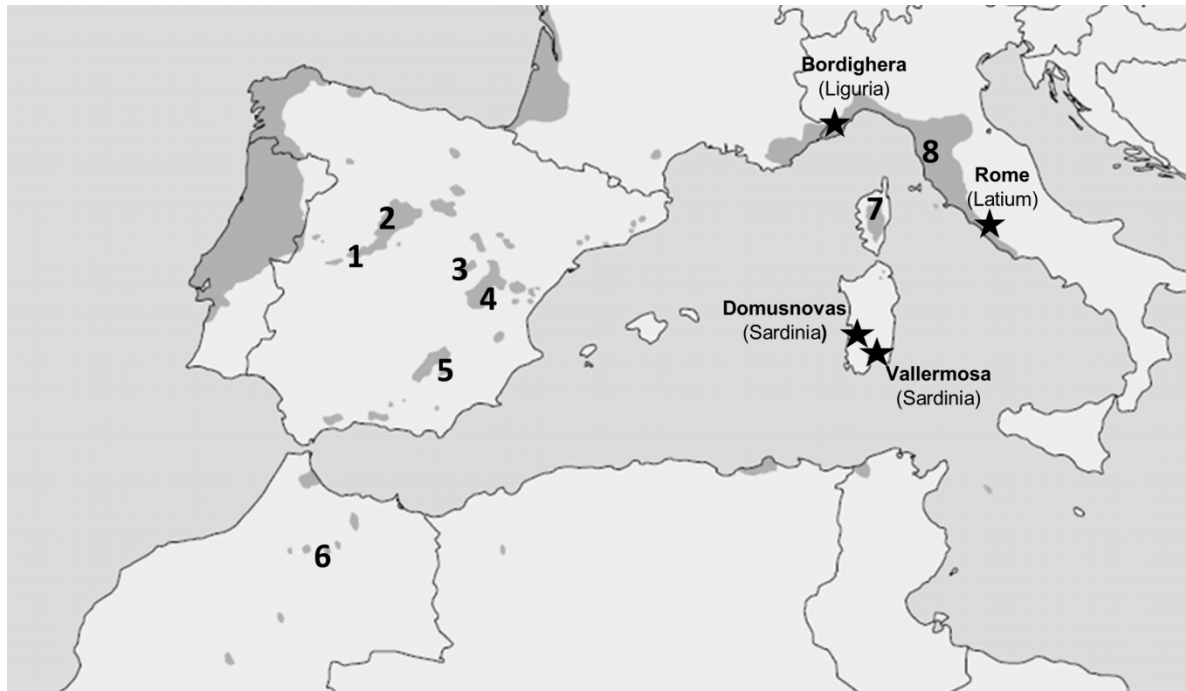
9 Table 4. Mean number of nymphs of *Matsucoccus feytaudi* per stembark surface unit and visual phytosanitary characterization at the Bordighera trial
 10 (for the visual assessment, the category having the greatest frequency of occurrence is indicated in the table).

Provenance	¹ Nymphs cm⁻²		Visual assessment (category)
Vivarior	2.05	A	(C) Foliar reddening, abundant trunk resin flow, loss of needles, trees strongly damaged or died
Val Freddana	1.83	A B	(C) Foliar reddening, abundant trunk resin flow, loss of needles, trees damaged or died
Arenas de San Pedro	1.59	A B C	(B) Foliar reddening, adequate vegetative conditions
Coca	1.46	B C D	(C) Foliar reddening, abundant trunk resin flow, loss of needles, trees damaged or died
Graja de Campalbo	1.26	C D	(B) Foliar reddening, adequate vegetative conditions
Siles	1.23	C D	(B) Foliar reddening, adequate vegetative conditions
Cuenca	1.14	D	(B) Foliar reddening, adequate vegetative conditions
Tamjoute	0.60	E	(A) No damages, good vegetative conditions

11 ¹Mean values with the same letter are not significantly different ($p > 0.05$) according to Tukey's HSD test.

1 FIGURES

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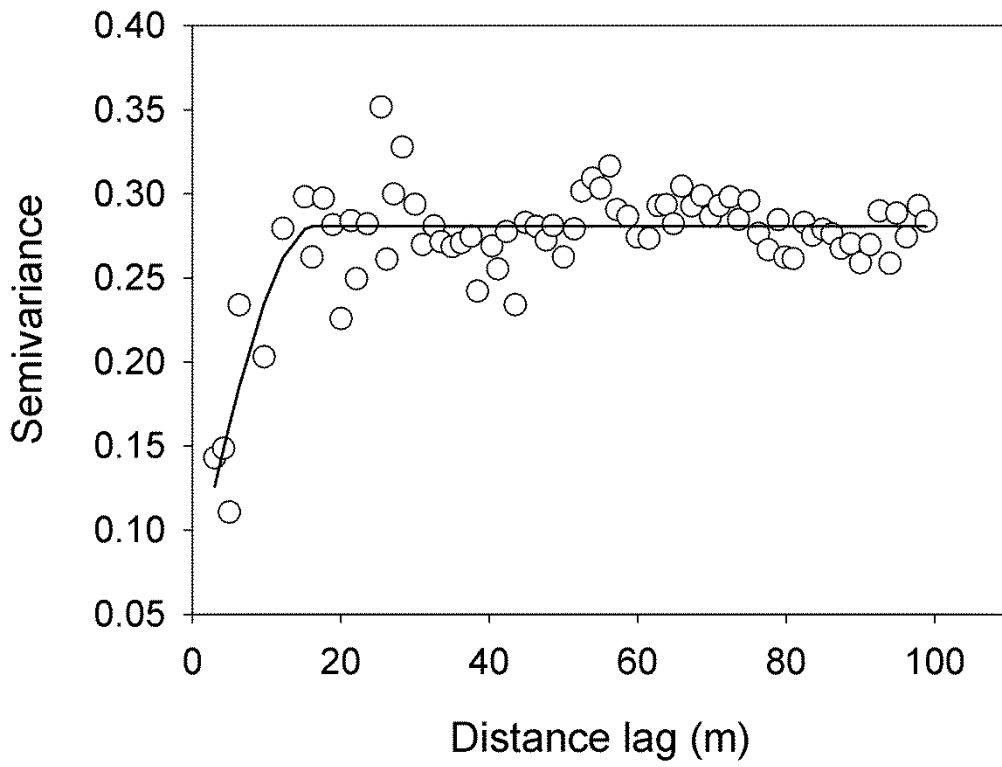
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5 Figure 1. Distribution map of the natural range of *Pinus pinaster* L. (dark shaded areas) and

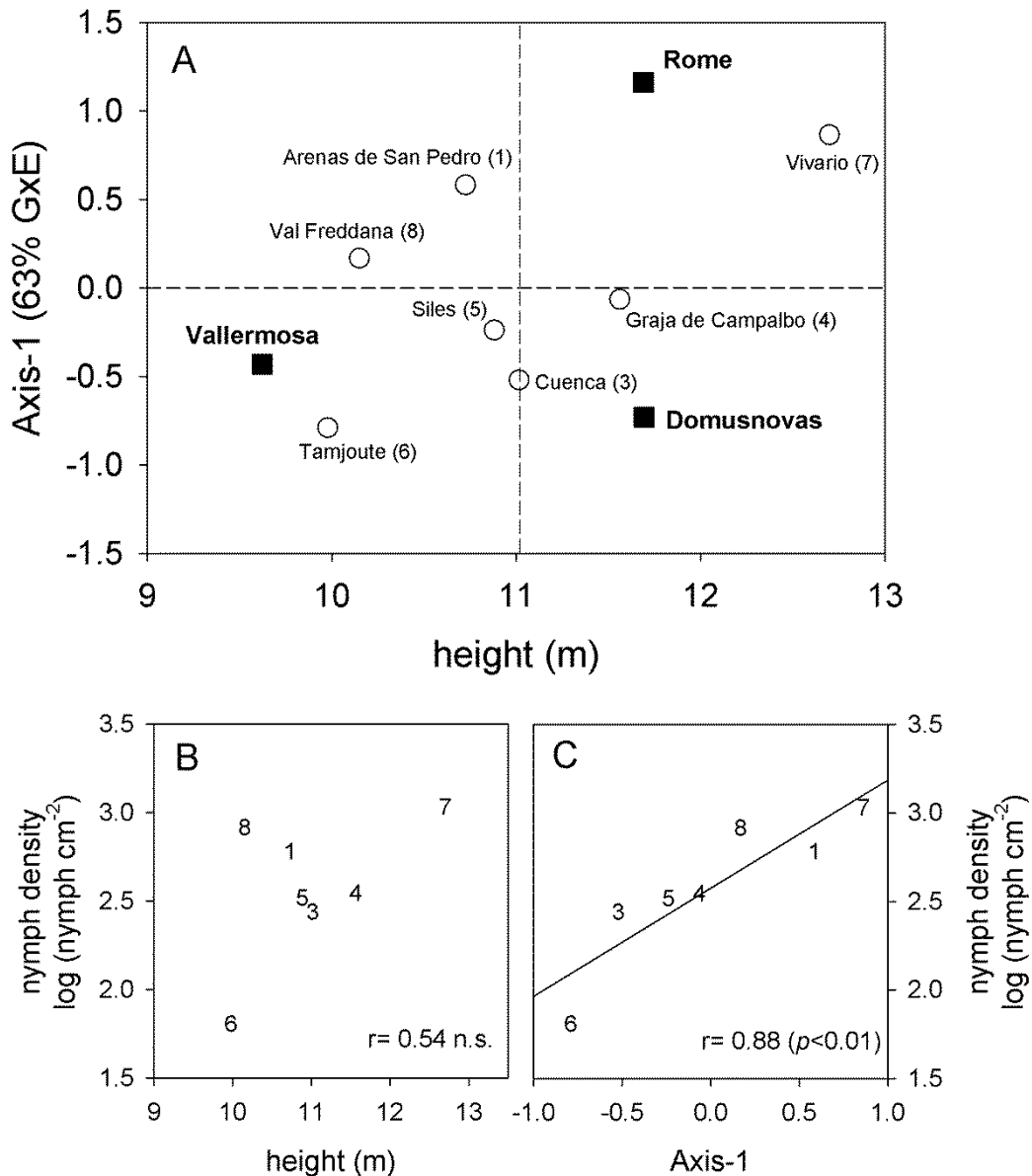
6 geographical location of the eight studied provenances and the four Italian trials. The numbers

7 refer to provenances as reported in Table 2 while the stars identify the different field trials.

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22 Figure 2. Semivariogram for nymph density of *M. feytaudi* in maritime pine adjusted for
23 provenance effects (Bordighera trial), i.e. provenance mean subtracted to each individual
24 observation.
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27 Figure 3. A) Biplot of the AMMI1 model for total height; B) Relationship between provenance
 28 means across trials for total height and provenance nymph density of *M. feytaudi* at the
 29 Bordighera trial; C) Relationship between genotypic scores of the AMMI1 model for total
 30 height and provenance nymph density of *M. feytaudi* at the Bordighera trial. The black squares
 31 indicate the position of the three field trials and the white circles denote the position of the eight
 32 provenances in the biplot. The numbers in panels B and C refer to the provenance codes as
 33 reported in panel A and in Table 2.