

Document downloaded from:

<http://hdl.handle.net/10251/64620>

This paper must be cited as:

Taïbi, K.; Campo García, ADD.; Mulet Salort, JM.; Flors, J.; Aguado, A. (2014). Testing Aleppo pine seed sources response to climate change by using trial sites reflecting future conditions. *New Forests*. 45(5):603-624. doi:10.1007/s11056-014-9423-y.



The final publication is available at

<http://dx.doi.org/10.1007/s11056-014-9423-y>

Copyright Springer Verlag (Germany)

Additional Information

1 **Testing Aleppo pine seed sources response to climate change by using trial sites reflecting**  
2 **future conditions**

3 Taïbi, K<sup>1,2,3</sup>, del Campo, A D\*<sup>1</sup>, Mulet, J M<sup>2</sup>, Flors, J<sup>4</sup>, Aguado, A<sup>4</sup>

4

5 <sup>1</sup>Research Group in Forest Science and Technology (Re-ForeST), Department of Hydraulic  
6 Engineering and Environment, Universitat Politècnica de València. Cami de Vera, S/N 46022  
7 Valencia, Spain.

8 <sup>2</sup>Instituto de Biología Molecular y Celular de Plantas (IBMCP), Universitat Politecnica de  
9 Valencia-Consejo Superior de Investigaciones Cientificas, Avd. de los Naranjos s/n, Valencia,  
10 46022, Spain.

11 <sup>3</sup>Faculty of Life and Natural Sciences, Ibn Khaldoun University, BP 78, Campus Karman, Tiaret,  
12 14000, Algeria.

13 <sup>4</sup>Centro Nacional de Recursos Genéticos Forestales de Alaquàs. Ministerio de Agricultura,  
14 Alimentación y Medio Ambiente, Valencia, Spain.

15 \*corresponding author: [ancamga@upv.es](mailto:ancamga@upv.es)

16

17 **Abstract**

18 Large-scale biogeographical shifts in forest tree distributions are predicted in response to the  
19 altered precipitation and temperature regimes associated with climate change. Adaptive forest  
20 management to climate change experienced in either stable or rapidly changing environments  
21 must consider this fact when carrying out reforestation programs or specifically assisted  
22 population migration for conservation purposes. The aim of this study was to compare field  
23 performance of eleven seed sources of Aleppo pine outplanted in core and marginal habitats and  
24 to assess their phenotypic plasticity for further screening under specific conditions in particular  
25 reforestation areas. We hypothesize that current marginal habitat due to low temperature is  
26 shifting toward conditions found on the core habitat and that current core habitat will shift  
27 toward warmer and drier marginal habitat. Our study reproduced real conditions of reforestation

28 in potential future climatic conditions. Results suggest that it is difficult to predict Aleppo pine  
29 provenances' performance in different natural sites from their performance at a single location,  
30 even though 'Levante interior' and 'La Mancha' seed sources showed the best overall response  
31 among sites. On a site basis, provenances were matched in groups according to their survival and  
32 growth responses. Seedlings grown from local seed sources or seed orchards performed better on  
33 the core habitat. However, as conditions shifted to marginal habitats, seedlings from climatically  
34 similar regions performed better than local sources at least in the short term; in this sense, current  
35 plantations in areas already affected by global change could have better establishment.

36

37 **Keywords**

38 *Pinus halepensis*, plantation performance, core and marginal habitats, growth, survival, assisted  
39 population migration.

40

## 41 **1. Introduction**

42 Mediterranean regions, transitional climatic zones between arid and humid regions of the world,  
43 are areas where global climate change is expected to have the greatest impacts. These areas are  
44 particularly prone to degradation because of the combination of climate change and human  
45 activities leading to increased habitat fragmentation, deforestation and land abandonment  
46 (Cortina et al. 2013). Climate change is triggering vegetation range-shifts in Europe where these  
47 changes are expected to result in the northward shift of Mediterranean ecosystems (IPCC 2001).  
48 Quézel and Médail (2003) pointed that global change should induce an altitudinal shift from one  
49 vegetation stage to another over the Mediterranean basin and thus, the theoretical limit between  
50 species should quickly shift in elevation and in latitude with climate changes (Vennetier et al.  
51 2007). Moreover, Metzger et al. (2008) reported that Mediterranean North and Mediterranean  
52 Mountain environments are predicted to shift, respectively, to South and North Mediterranean,  
53 indicating a potential expansion of deserts habitats, and an increased risk of forest fires and  
54 drought.

55 Individual species responses to such changes will depend on ecological and evolutionary factors  
56 such as niche breadth, competitive ability, phenotypic plasticity and adaptability (Soto et al.  
57 2010). The responses of long-lived plant species to changes in environmental conditions are  
58 determined by the capacity of individuals to alter their structure and function to novel  
59 environments (i.e. phenotypic plasticity), adapt through natural selection or migrate (Matesanz  
60 and Valladares 2013). In this sense, mitigation and adaptation to climate change is of paramount  
61 importance in current and future restoration plans (Atzmon et al. 2004; Vallejo et al. 2012).

62 Climate change adaptation strategies such as assisted migration, defined as the movement of  
63 species and populations to facilitate natural range expansion in direct management response to  
64 climate change, have gained more interest during last decade (Williams and Dumroese 2013).  
65 Effective implementation of assisted migration can only occur if target transfer guidelines are  
66 developed because current seed transfer guidelines, established to guide the movement of plant

67 materials, lack inherent spatial and temporal dynamics associated with climate change (Potter  
68 and Hargrove 2012).

69 Restoration of the Mediterranean regions is currently carried out using many native tree species  
70 taking into account other objectives than productivity, i.e. plant biodiversity and functionality,  
71 soil conservation, climate change mitigation, among others (Hernández et al. 2010; Vallejo et al.  
72 2012). Aleppo pine (*Pinus halepensis* Mill.) is preferentially chosen for reforestation because of  
73 low-technical requirements for nursery production and high-resistance to drought and degraded  
74 environments (del Campo et al. 2007a). Although it is considered a pioneer species and favours  
75 establishment of late successional, Aleppo pine is also considered part of the climax community  
76 (Quézel and Médail 2003). However, forest restoration with Aleppo pine in the Mediterranean  
77 presents high variability in the establishment success, mainly due to variation in site quality and  
78 stock quality (del Campo et al. 2007b). In addition, the use of unsuitable seed sources for  
79 afforestation dramatically decreases the chances of success (Zobel and Talbert 1984), especially  
80 on cold and drought-prone sites (Oliet et al. 2013).

81 In Spain, *P. halepensis* has a total of twenty regions of provenance (Alía et al. 2009), defined as  
82 territories subjected to almost uniform ecological conditions and consisting of populations which  
83 present analogous phenotypic or genotypic features (Esteban et al. 2010). Gandullo and Sánchez-  
84 Palomares (1994) reported climatic, physiographic and edaphic variables throughout the  
85 geographic area occupied by the species in 131 experimental sites. They defined marginal  
86 interval as that one encompassing the lowest or highest 10% of the values in a variable, whereas  
87 the core interval encompasses the 10-90% range. On a site, the higher the number of key  
88 ecological variables falling out of the central interval the higher the marginality of that habitat  
89 for the species.

90 The existence of genetic differentiation between populations is a key aspect of adaptation to  
91 different environments in forest trees (Schiller and Atzmon 2009). Marked differences among  
92 Aleppo pine populations in terms of growth and survival have been reported (Bariteau 1992;

93 Atzmon et al. 2004). Common garden studies assessing Aleppo pine intraspecific variability  
94 revealed adaptive variation to climate (Voltas et al. 2008; Atzmon et al. 2004), which  
95 demonstrated the selective role of climate variables in determining populations fitness in this  
96 species (Climent et al. 2008). Agúndez et al. (1997) reported that Aleppo pine selection for  
97 drought and frost tolerance has led to adaptation of provenances. The contribution of local  
98 adaptation and plasticity across populations to the persistence of species under present and future  
99 climate change could be decisive (Benito-Garzón et al. 2011). High adaptive phenotypic  
100 plasticity would decrease the risk of species loss due to climate change by improving phenotypes  
101 suited to the new environments (Matesanz and Valladares 2013). Identifying how populations'  
102 growth and survival respond to climate constitutes a key element for a deeper understanding of  
103 ecosystems dynamics in a changing environment and should be considered in reforestation  
104 activities (Oliver et al. 2012; Potter and Hargrove 2012).

105 Reforestation guidelines that focus on local seed sources may become irrelevant, as these stocks  
106 will not be adapted to the new environmental conditions (Williams and Dumroese 2013). The  
107 need to analyse the plant material responses to environmental conditions in order to develop  
108 effective reforestation policies and to cope with the new environmental cues from climate change  
109 has long been put into writing and is currently among the research priorities for most  
110 international organizations (EFI 2009; United Nations et al. 2011; FAO 2013). In this sense,  
111 investigation of how different seed sources (i.e. plant material from which forest reproductive  
112 material is derived) of Aleppo pine respond to extreme environmental conditions in strongly  
113 contrasting environments renders valuable information for the current afforestation programmes  
114 of degraded regions and to manage the prospect assisted migration of this species.

115 We address the question of whether current core habitats for Aleppo pine can shift towards more  
116 marginal habitats where drier provenances could perform better than local provenances in such  
117 situations. At the same time, current marginal habitats for the species (high altitude belts) could  
118 be suitable in the near future, as they become core habitats (and thus, marginal for other species).

119 The aim of this study is to build and expand the knowledge of several Aleppo pine seed sources  
120 by assessing their performance (survival, growth, and the ecophysiological behaviour) and their  
121 phenotypic plasticity under three different and contrasting climatic conditions within the natural  
122 distribution of the species, and to provide knowledge on seedling adaptive transfer in ecological  
123 restoration, tree breeding and conservation of genetic resources. Three sites, representing the dry  
124 situation (shift from core to drier marginal habitat), the cold situation (shift from cold marginal  
125 to core habitat) and the current core habitat for the species, are compared. The predicted response  
126 of growth and survival of the different seed sources under the expected climate change in the  
127 core and marginal habitats was also addressed throughout this study. In this context, our results  
128 allow for further screening for specific conditions, such as drought and low temperature events,  
129 to help reforestations programs and provide knowledge to manage assisted migration efforts.

130

## 131 **2. Materials and methods**

### 132 *Plant material*

133 Eleven seed sources (basic materials) of *Pinus halepensis* were selected considering not only  
134 spatial distribution but also environmental heterogeneity, prioritizing those that represent  
135 contrasted environments (Table 1). This selection covers most of the climatic and ecologic  
136 regions of the natural range of this species in Spain with a wide spectre of molecular and  
137 phenotypic variation (Climent et al. 2008; Voltas et al. 2008; Grivet et al. 2009). These seed  
138 sources correspond to nine Spanish provenances defined for the species (Alía et al. 2009) and  
139 two seed orchards (Table 1).

140 Seed lots were collected as open-pollinated bulk populations either from seed sources or stands  
141 and grown as one-year-old container stock in a public nursery located in Centro Nacional de  
142 Mejora Genética Forestal de Alaquàs, Valencia (Spain), in growing years 2008 and 2009.

143 Seedling lots were grown as one-year-old container stock under the standard conditions for the  
144 species (shade-house, 33 weeks, 200 cm<sup>3</sup> container, sphagnum peat and a 15-4-7.5 slow release

145 controlled fertilizer added at  $7 \text{ g l}^{-1}$ ). During nursery culture, plant materials were arranged in a  
146 randomized block design with four replicates in order to avoid important differences in stock  
147 quality associated with microsite differences in the nursery benches. However, stock differed  
148 slightly among seed sources. Stock dimensions (average and standard deviation) in the 2008  
149 stock were height:  $35.4 \pm 8.0 \text{ cm}$ , stem diameter at root collar:  $4.4 \pm 0.59 \text{ mm}$ , total dry biomass:  
150  $8.38 \pm 2.51 \text{ g}$ , shoot/root:  $3.18 \pm 1.18 \text{ g g}^{-1}$ , N concentration (needles):  $2.21 \pm 0.13 \%$ , root growth  
151 potential:  $43 \pm 24 \text{ mg}$  of new roots in optimum conditions (del Campo et al. 2007a). The values  
152 for the 2009 stock were respectively:  $18.2 \pm 2.1 \text{ cm}$ ,  $2.97 \pm 0.32 \text{ mm}$ ,  $2.88 \pm 0.63 \text{ g}$ ,  $1.73 \pm 0.34 \text{ g g}^{-1}$ ,  
153  $1.91 \pm 0.08 \%$ ,  $8.3 \pm 4.1 \text{ mg}$ , much lower due to the cooler climate conditions during that year in  
154 the nursery.

155

#### 156 *Site selection and characterization*

157 The study was performed in three climate-contrasting sites in Eastern Spain (table 2),  
158 encompassing zones in both core and more marginal habitats of this species in Spain (as defined  
159 by Gandullo and Sánchez-Palomares 1994). The three sites have similar soil characteristics  
160 (especially depth) and naturally regenerated Aleppo pine trees (Table 2).

161 The Granja d'Escarp site is located in the province of Lleida, NE Spain, and represents the driest  
162 site. It was chosen to represent the shift from current core habitat to marginal dry habitat. This  
163 area has a dry continental Mediterranean climate with cold winters and hot dry summers. The  
164 site is located in the provenance region of Aleppo pine 'Cataluña Interior' and borders 'Ibérico  
165 Aragónés' and 'Monegros Depresión del Ebro' provenances.

166 The Tramacastiel site is located in Monte UP 283, south of Teruel province (central-eastern  
167 Spain), and represents a current marginal habitat for the species (cold) that could become core  
168 habitat with climate change, and thus marginal habitat for species, such as *Pinus nigra* or  
169 *Juniperus thurifera*. The site is characterized by a continental mountain climate where frosts are  
170 common from November to April. Summer drought is mitigated by altitude and storms. Before a



171 wildfire in 1994, this area was occupied by a natural stand of *Pinus nigra*, *P. pinaster* and  
172 *Juniperus thurifera*, whereas *P. halepensis* was minority. Since the fire, minimal natural  
173 regeneration of these species has occurred whereas Aleppo pine saplings are becoming present.  
174 This site is located in the Aleppo pine provenance region ‘Maestrazgo-Los Serranos’.  
175 The third site acts as a control site (La Hunde, Ayora, Valencia, SE Spain) and belongs to a core  
176 habitat of the species according to Gandullo and Sanchez Palomares (1994). Aleppo pine forest,  
177 either naturally regenerated or planted, is the most common vegetation type on the landscape.  
178 This site is located in the Aleppo pine provenance region ‘Levante Interior’. Climatic variables  
179 for the three sites were monitored from weather stations located in the vicinity of the  
180 experimental plots.

181

#### 182 *Site preparation, planting works and experimental layout*

183 The trials Granja d’Escarpi and Tramacastiel were established in February 2010 whereas the trial  
184 site La Hunde was established in February 2009. The sites selected for trials were all considered  
185 suitable for forest field experimentation. Seedlings were outplanted using standard reforestation  
186 techniques.

187 Before outplanting, sites were disked and each planting hole (0.5 x 0.5 x 0.4 meters) was opened  
188 with a backhoe excavator. Experimental layout was a randomized complete block design with  
189 three blocks. Each seed source was represented by thirty-three trees per block for a total of  
190 ninety-nine trees per experimental site (1089 seedlings per site). Seedlings were outplanted in  
191 rows and spaced 2.5 m apart.

192

#### 193 *Plantation monitoring and measurements*

194 We measured survival and growth expressed as the monthly relative growth rate in height (RGR-  
195 H) and stem diameter (RGR-D) three times during the first year: at outplanting (before spring  
196 growth), before summer (June-July, after shoot elongation and at the early summer drought

197 period), and after summer (September-October, once the late growth period concluded). Survival  
198 and growth were also measured in the spring (May) of the third year for all sites. By this way,  
199 the first three years of growth and the first two summer periods were recorded; sufficient data to  
200 evaluate early plantation performance (Landis et al. 2010). Appending 1, 2, 3, or T to the RGR  
201 variables indicate the measurement period: 1 corresponds to growth from first to second  
202 assessment, 2 from second to third assessments, 3 from third to fourth assessments and T (total)  
203 from first to fourth assessments. Seedling height was measured to the nearest 0.5 cm from soil to  
204 the terminal. Seedling stem diameter was measured at 1.0 cm above the soil surface. Survival  
205 was assessed by determining the status (alive or dead) of each individual.

206 Transpiration and photosynthesis were measured on May 2012 under similar atmospheric  
207 conditions on the same tissues of five plants per seed source per site (randomly selected in one  
208 block) using an LCpro+ Portable Photosynthesis System with leaf chamber (ADC Bioscientific  
209 Ltd. Hoddesdon, Hertz, EN11 0DB) with alight unit; ambient CO<sub>2</sub> was set at 360 ppm, air  
210 humidity at 60–70%, temperature at 25°C and photosynthetic photon flux density (PPFD) at 800  
211  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . These conditions were selected because they yielded maximal photosynthetic rates.  
212 *In vivo* measurements were performed on intact needles with no visible symptoms of damage.  
213 After each measurement, needles were cut and put into plastic bags on ice until analysis of leaf  
214 area, which was performed with a numeric scanner connected to the software WinRhizo (Regent  
215 instruments Inc, Quebec, Canada). Net photosynthetic (PN,  $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ) and transpiration  
216 (E,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) rates were expressed on a needle area basis. The efficiency of  
217 photosynthetically active radiation utilization (PAR) was measured by quantifying photon flux  
218 density incident on needles surface area ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The efficiency of photochemical  
219 reactions driving photosynthesis was assessed by chlorophyll fluorescence measurements.  
220 Minimal fluorescence (F<sub>0</sub>) yield was obtained upon excitation with a weak measuring beam  
221 from a pulse light-emitting diode, while maximal fluorescence yield (F<sub>m</sub>) was determined after  
222 exposure to a 0.8 s saturating pulse [ $>10.000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ ] of white light. The maximal

223 PSII photochemical efficiency ( $F_v/F_m$ ) was then estimated as the ratio of variable ( $F_v = F_m - F_0$ )  
224 to maximal fluorescence.

225

### 226 *Statistical analysis*

227 Geographic and climatic variables with recognized influence on ecotypic variation of  
228 Mediterranean pines (Climent et al. 2008; Voltas et al. 2008) were selected (Tables 1,2) to  
229 calculate the transfer distance from the trial site to the corresponding seed source native site  
230 (Aitken et al. 2008; Reich and Oleksyn 2008; Benito-Garzón et al. 2011). Correlations between  
231 measured (growth and survival) and seed sources (geographic coordinates, climatic variables and  
232 transfer distance) variables were determined based on the coefficient of *Pearson*. Seed sources  
233 from seed orchards (Alaquàs and El Serranillo) were excluded in these analyses ( $n=9$ ) because  
234 they are grown from various sources. The Kaplan-Meier method (Kaplan and Meier 1958) was  
235 used to compare the survival functions of the different seed sources on a site basis. The statistics  
236 of LogRank, Breslow and Tarone-Ware were used to detect significant differences either among  
237 overall seed sources or for pairwise comparisons when significant differences appeared. The  
238 initial seedling height was not homogenous among seed sources (in spite of the nursery  
239 experimental design), which can affect field performance (del Campo et al. 2011). To control for  
240 this factor, initial height was introduced as a covariate in a Cox prediction hazard model together  
241 with the categorical predictor (seed source), resulting in this variable having no significant  
242 influence on the survival function. Regarding growth, differences among seed sources were  
243 tested on the RGR-H and the RGR-D, determined every two consecutive measurements, as well  
244 as the overall RGR value (last and first measurements). For this purpose, a multivariate analysis  
245 of variance (MANOVA) using the Wilks' Lambda statistic was performed on all RGR variables  
246 (height and diameter) as dependent variables and block and seed sources as factors. Follow-up  
247 analyses (ANOVA) were performed to assess whether differences among groups on the  
248 population means for each dependent variable were significant. ANOVA was also used to test

249 for differences in physiological parameters. Post-hoc comparisons of means were made using  
250 Tukey's test. In all cases, data were examined for normality and homogeneity of variances; we  
251 identify any violations of assumptions. Variance components estimation was performed to  
252 partition the phenotypic variance into its seed source, site and seed source by site components  
253 using the restricted maximum likelihood procedure for all the measured traits and was expressed  
254 as a percentage of variation explained by each fact.

255 Multivariate screening of seed sources was carried out to make additional comprehensive  
256 assessments of the overall performance. Canonical discriminant analysis was used to summarize  
257 the intra-site among seed sources variability on survival (mean survival time, first post-winter,  
258 first post-summer and final survival), growth (final height, final diameter and RGR for the first  
259 spring (1), first summer (2) and total period (T)) and physiological traits showing statistically  
260 significant differences between seed sources at the considered site according to ANOVA  
261 analyses. The multivariate generalized distances of Mahalanobis were computed later on the  
262 same variables used for the multivariate analysis and were used to cluster seed sources into  
263 groups with similar performance in terms of growth and survival (physiological traits not  
264 included, see the results section). The agglomerative hierarchical clustering procedure based on  
265 Ward's method was used. Data were analysed with SPSS© 17.0 at a significance level of p-  
266 value<0.05 for all analyses.

267

### 268 **3. Results**

#### 269 **3.1. Climate variation among sites**

270 Climatic variables in the three experimental sites during the first and second years after planting  
271 showed some deviation from their historical means (Table 2), although the underlying hypothesis  
272 for this work was maintained that temperatures and precipitation in La Hunde were more suitable  
273 for the species than Granja d'Escarp (dry) and Tramacastiel (cold) (Gandullo and Sanchez-  
274 Palomares 1994) (Table 2). For instance, seedlings grown in the dry site of Granja d'Escarp

275 received only 310 mm in 2011, which falls into the marginal value of precipitation for Aleppo  
276 pine (less than 10% of the Spanish populations of Aleppo pine grow in this threshold). Contrary,  
277 in the cold site of Tramacastiel, mean temperatures in the first year were considerably below the  
278 historical record, reaching a monthly average of lows of -3.2°C in January 2010 (0.16°C in  
279 March when plantation was finished), whereas summer drought was mitigated in this site.

280

### 281 **3.2. Plantation performance**

282 Decomposition of variance indicated that site was the most important factor affecting survival  
283 and growth traits (total variation attributed to site ranged from 84.6 to 99.8%). However, seed-  
284 source effects and the seed sources by site interaction explained an extremely low proportion of  
285 the total variance (Table 3).

#### 286 *Survival response*

287 Seedling survival varied considerably among sites (Fig. 1). La Hunde seedlings had the highest  
288 final survival among sites (93%), with a mean survival time of 26.4 months over 28 and no  
289 significant differences in the Kaplan Meyer analysis. In the pairwise comparisons, however, the  
290 statistics differed significantly between the survival of 'Litoral levantino' (lowest survival at the  
291 end of this study, 85%) and other seed sources with final survival more than 93% (Fig. 1).

292 Survival rate was maintained up to 94% throughout the first year (2010) at Granja d'Escarp site.  
293 However, survival decreased by the spring of 2012 (third year) to an average of 77%; the mean  
294 survival time in this site was 23.2 months out of 28 months. Survival among seed sources  
295 differed (p-value<0.01); 'Ibérico Aragonés' and 'H.S. El Serranillo' had lower survival (68%)  
296 than 'Litoral Levantino' and 'La Mancha' (84%) (Fig. 1). At Tramacastiel, survival decreased  
297 for all seed sources after the first winter of plantation (51%), and no additional mortality was  
298 observed during the first summer at this site. A further decrease till an average of 38% was  
299 recorded between October 2010 and May 2012. Differences among survival for seed sources  
300 were highly significant (p-value<0.001), with 'H.S. El Serranillo', 'Alcarria' and 'Levante

301 interior' showing the highest survival after the first winter (about 60%) and 'Ibérico Aragonés'  
302 and 'Bética Septentrional/Sur' showing the lowest survival (about 35%) (Fig. 1). The mean  
303 survival time was 13.8 months out of 28 months.

304 In general, 'Levante Interior', 'La Mancha' and 'H.S. El Serranillo', showed the best inter-site  
305 survival, whereas 'Ibérico Aragonés', 'Bética Septentrional/Sur' and 'Cataluña' showed the  
306 highest inter-site mortality. Significant correlations between climatic variables (Table 1) and  
307 survival were found in La Hunde and in Granja d'Escarp. In La Hunde, post-summer survival  
308 during the first year (2009) was positively correlated with altitude ( $p\text{-value}<0.01$ ), negatively  
309 correlated with annual mean temperature ( $p\text{-value}<0.01$ ) and negatively correlated with  
310 minimum daily temperature of the coolest month ( $p\text{-value}<0.01$ ). Moreover, survival in May  
311 2011 was negatively correlated with annual mean temperature ( $p\text{-value}<0.05$ ). In Granja  
312 d'Escarp, latitude and survival in May 2012 were negatively correlated ( $p\text{-value}<0.05$ ).

313

#### 314 *Growth response*

315 Relative growth rates differed according to site and time (Fig. 2). During the first summer, RGR-  
316 H was greater on the coldest site, Tramacastiel, than on La Hunde and Granja d'Escarp by at  
317 least  $0.080\text{ cm cm}^{-1}\text{ month}^{-1}$ ; for La Hunde and Granja d'Escarp, maximum RGR-H was reached  
318 during the first spring (RGR-H1:  $0.038$  and  $0.020\text{ cm cm}^{-1}\text{ month}^{-1}$  respectively), although, they  
319 were lower than those at Tramacastiel (RGR-H1:  $0.057\text{ cm cm}^{-1}\text{ month}^{-1}$ ). Consequently, RGR-  
320 HT at Tramacastiel was two to three times higher than La Hunde and Granja d'Escarp,  
321 respectively. RGR-D showed similar site and temporal patterns (Fig. 2). In the intra-site  
322 comparison of growth among seed sources (MANOVA), Wilks' Lambda statistic was significant  
323 ( $p\text{-value}<0.001$ ) for RGR-H and RGR-D in La Hunde and Granja d'Escarp, but not in  
324 Tramacastiel. ANOVA and Tukey tests showed that RGR-D differences were only due to the  
325 first spring rate (RGR-D1) at La Hunde and Granja d'Escarp and the RGR-DT (total) in Granja  
326 d'Escarp (Table 3, Fig. 2). Moreover, total growth rates (height and diameter) between

327 'Maestrazgo-Los Serranos' and 'Ibérico Aragonés' planted in Tramacastiel were significantly  
328 different. Thus, only height growth differed among seed sources. In La Hunde, the ranking was  
329 almost constant, with 'H.S. El Serranillo', 'H.S. Alaquàs' and 'Alcarria' showing higher  
330 performance (height and diameter growth) across time than 'Cataluña' and 'Ibérico Aragonés'.  
331 In Granja d'Escarp, temporal differences were less constant, although 'Bética septentrional/sur',  
332 'La Mancha' and 'Maestrazgo-Los Serranos' showed better growth performance than 'Los  
333 Monegros Depresión del Ebro', 'Litoral Levantino', 'Cataluña' and 'Ibérico Aragonés'.  
334 Significant correlations were found between climatic variables from table 1 and growth on the  
335 three sites. In the core habitat, La Hunde, height growth during the first summer (RGR-H2) was  
336 significantly correlated with mean maximum daily temperature of the warmest month (p-  
337 value<0.05\*) whereas RGR-D2 showed opposite correlations to those found for post-summer  
338 survival in this site: altitude (-), annual mean temperature (+) and mean maximum daily  
339 temperature of the warmest month (+). In the marginal dry habitat of Granja d'Escarp, there were  
340 negative correlations between height growth and latitude (RGR-H1, p-value<0.05; RGR-H3 and  
341 RGR-HT, p-value<0.01) and height growth and longitude (RGR-H2, RGR-H3 and RGR-HT, p-  
342 value<0.05). In addition, diameter growth (RGR-D1, RGR-D3 and RGR-DT, p-value<0.05) was  
343 positively correlated with latitude transfer distance and negatively correlated with altitude and  
344 longitude transfer distance. In the marginal cold habitat of Tramacastiel, there was a negative  
345 correlation between height growth and latitude (RGR-H3, p-value<0.05) and between diameter  
346 growth and altitude (RGR-D2 and RGR-D3, p-value<0.05).

347

#### 348 *Physiological response*

349 No significant differences were detected in the physiological variables among seed sources  
350 within each site (p-value>0.05). PSII maximal photochemical efficiency, estimated by the  
351 fluorescence parameter Fv/Fm, was unaffected by seed sources and ranged between 0.59 and  
352 0.82; this ratio was more stable in Tramacastiel (0.79 to 0.82) than in La Hunde (0.59 to 0.70)

353 and Granja d'Escarp (0.70 to 0.79). Regarding transpiration rates, water loss at date of  
354 measurement was higher in Tramacastiel ( $3.39 \pm 0.3 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) and La Hunde ( $3.2 \pm 0.5 \text{ mmol}$   
355  $\text{m}^{-2} \text{ s}^{-1}$ ), while it was lower under the driest conditions of Granja d'Escarp ( $1.09 \pm 0.2 \text{ mmol m}^{-2} \text{ s}^{-1}$ ).  
356 On average, PAR was similar between Tramacastiel and La Hunde and lower in Granja  
357 d'Escarp. The net photosynthesis rate PN was similar among the three sites and ranged between  
358 7.9 and  $11.9 \mu\text{mol m}^{-2} \text{ s}^{-1}$ .

359

### 360 **3.3. Multivariate screening of seed sources**

361 Canonical discriminant analysis (CDA) was performed for each site on survival and growth traits  
362 (Fig. 3). In the three cases, the first canonical axis, Can1, had higher discriminant power (more  
363 than 50%) than the second one, Can2.

364 Seed sources outplanted at La Hunde were separated by Can1, accounting for 81.5% of the  
365 variability. RGR-H accounted for 84% of the variability whereas RGR-HT was responsible for  
366 48% of the variability. A clear separation among seed sources can be observed according to this  
367 axis: 'Maestrazgo-Los Serranos', 'Cataluña', 'Bética septentrional/sur' and 'Ibérico Aragonés'  
368 are on the right side of the axis while 'H.S. El Serranillo' and 'H.S. Alaquàs', characterized by  
369 the highest growth rates, appeared on the opposite side. Separation on Can2 (10.3% of  
370 variability) was explained mostly by stem diameter variation (39.6%) from the whole variability  
371 ( $r^2=0.87^{**}$ ), while RGR-H explained about 20% ( $r^2=0.4^*$ ). 'Alcarria' and 'Maestrazgo-Los  
372 Serranos' manifested a high stem relative growth rates and appeared on the opposite side of  
373 'Cataluña', and 'Ibérico Aragonés' seedlings.

374 Can1 described around 61% of the whole variability of growth and survival observed in the dry  
375 and warm site of Granja d'Escarp. Around 46% of this variability was due to height growth  
376 (RGR-HT: 26% and RGR-H2: 19%), while 27% of the whole variability was due to survival  
377 variations among seed sources (survival time: 14.5% and rate: 13.5%). 'Maestrazgo los  
378 Serranos', 'Litoral levantino' and 'Bética septentrional/sur' manifested high relative growth rates



379 and appeared on the right of the axis, whereas ‘Cataluña’, ‘Los Monegros Depresión del Ebro’  
380 and ‘Levante interior’, with lower performance, are on the left side. Can2 (17.7% of total  
381 variability) was described by total height relative growth rate (30%) and did not show a clear  
382 seed source separation.

383 At the cold Tramacastiel site, Can1 explained 52.3% of the total variability and was positively  
384 correlated to survival traits ( $r^2=0.26$ , 38% from 52.3% of variability) and negatively correlated to  
385 height ( $r^2=-0.5^*$ , 42% from 52.3% of variability) and stem relative growth rates ( $r^2=-0.4^*$ , 20%  
386 from 52.3% of variability). ‘Bética septentrional/sur’ seedlings had greater RGR-HT than ‘Los  
387 Monegros/Depression del Ebro’ and ‘Ibérico Aragonés’ seedlings. Can2 (24.8% of total  
388 variability) explained seedlings’ survival by 80% ( $r^2=0.69^{**}$ ). ‘H.S. El Serranillo’ (high time and  
389 rate of survival) appeared close on the axis to ‘Levante interior’ and ‘La Mancha’ while ‘Litoral  
390 levantino’, ‘Cataluña’ and ‘Ibérico Aragonés’ seed sources characterized by low survival were  
391 opposite.

392 Dendrograms constructed using the Mahalanobis distances between pairs of seed sources  
393 revealed many similar associations (Fig. 4). Similar distances demonstrated by seed sources  
394 indicated similar performance of their seedlings in the site when measured variables showed  
395 significant differences. In the core habitat of La Hunde, seed sources are divided into two main  
396 groups. The first group was characterized by seed sources with high height and moderate  
397 diameter growth (‘H.S El Serranillo’ and ‘H.S Alaquàs’) or by moderate height and high  
398 diameter growth (‘Levante interior’, ‘Litoral levantino’ and ‘La Mancha’ subset). The second  
399 group was characterized by low height growth and moderate diameter growth. ‘Alcarria’ and  
400 ‘Maestrazgo-Los Serranos’ emerged as single clusters in the second group, even though they had  
401 better survival and growth (high, moderate and low magnitudes are presented in Fig. 2).

402 In Granja d’Escar, ‘Maestrazgo-Los Serranos’ (good growth and survival scores) was separated  
403 from the other seed sources, leaving two groups. One group included seed sources with moderate  
404 to good performance (‘Bética septentrional/sur’, ‘La Mancha’ and ‘Litoral levantino’) and the

405 other group included seed sources with bad to moderate performance ('Ibérico Aragonés', 'Los  
406 Monegros Depresión del Ebro' and 'Cataluña'). 'Levante interior', which had good survival and  
407 growth, appeared connected to the latter group, probably due to its low summer height growth.  
408 In Tramacastiel four groups emerged. The first group comprised 'Ibérico Aragonés' and 'Los  
409 Monegros Depresión del Ebro' seed sources characterized by low performance. The second set  
410 included 'Alcarria', 'Levante interior', 'La Mancha' and 'H.S El Serranillo', which manifested  
411 higher survival rates with good growth. The third set including 'Litoral levantino', 'Cataluña'  
412 and 'H.S Alaquàs' showed worse performance than the previous group. The fourth cluster  
413 included the remaining seed source 'Bética septentrional/sur' which had the lowest survival rate  
414 in the site despite suitable growth.

415

#### 416 **4. Discussion**

417 We analyzed the variability in growth and survival among eleven Aleppo pine seed sources and  
418 their phenotypic plasticity across three contrasting environmental sites in eastern Spain for  
419 subsequent assisted migration to expected climate change. Site was the most important factor  
420 affecting growth and survival reflecting that high phenotypic plasticity is widespread in this  
421 species. However, seed-source effects and the seed sources by site interaction explained low  
422 proportion of the variance, indicating a similar plastic response among seed sources. Phenotypic  
423 plasticity allows species to live across a wider range of environments than those with stable  
424 phenotypes (van Kleunen and Fischer 2007; Aitken et al. 2008; Schiller and Atzmon 2009).  
425 Generally, tree species are considered to express moderate to high plasticity in their responses to  
426 environmental stress (Climent et al. 2008) and studies on some species suggest that plasticity can  
427 be highly trait dependent (Aitken et al. 2008; Benito-Garzón et al. 2011).  
428 As expected, seedlings planted in its core habitat at La Hude showed the highest final survival  
429 (93%) in contrast to seedlings grown at the two sites representing marginal habitats. Our results  
430 for Aleppo pine at La Hude were similar to that previously reported for this area (del Campo et

431 al. 2007a,b). Survival differences were minimal, although seed sources from colder and higher  
432 altitude provenances survived more (tables 1 and 2). Growth difference among seed sources was  
433 significant at La Hunde, with trees from warmer provenances having faster growth rates than  
434 those from cooler provenances. Similar finding was found by Climent et al (2008). This trade-off  
435 between cold tolerance and growth potential has also been observed in different populations of  
436 other conifer species, such as *Pinus taeda* (Schmidting 1994) and *Pinus contorta* (Rehfeldt et al.  
437 2002). *Pinus patula* populations differed significantly for seedling growth and exhibited a  
438 significant although weak altitudinal clinal pattern, with populations from lower altitudes having  
439 on average taller seedlings than populations from higher altitudes (Sáenz-Romero et al. 2011).  
440 Environmental stress response usually prompts plant cells to use energy that would otherwise be  
441 used for growth. So, surviving under stressful conditions usually results in a concomitant  
442 penalty, that is, reduced growth (Aitken et al. 2008). Several descriptions of molecular or  
443 physiological processes show that a response to stress hinders growth and vice versa, both in  
444 model organisms and in plants (Mulet et al. 2006). In our case, provenances moved from slightly  
445 different transfer distance metrics were the best performers. Longer growing seasons, as those  
446 observed in La Hunde in 2009 and 2011 (not shown), would have favoured seed sources from  
447 warmer provenances, although freezing events that likely occurred at this site could have  
448 decreased survival. In this sense, seed sources that were genetically improved (both seed  
449 orchards) or from this site ('Levante Interior') or from neighbouring regions with similar  
450 conditions ('La Mancha') could be the best choice for optimum reforestation results (early  
451 performance). Incidentally, these seed sources are grouped together according to their overall  
452 performance (Fig. 3,4). 'Litoral levantino' was also included in this group but survived poorly;  
453 thus, it is not recommended for this site. We cannot discard that epigenetic factors or maternal  
454 effects may have contributed to the high performance of both seed orchards at the first stages of  
455 development since they were improved from the local provenance of 'Levante Interior', 'Litoral  
456 levantino' and 'Maestrazgo los Serranos'.

457 The environmental conditions of the marginal dry site Granja d'Escarp led to a high  
458 differentiation among seed sources in survival and growth. In contrast to La Hunde, differences  
459 in survival among seed sources were related more closely to the ability of seedlings to cope with  
460 dry and warm conditions (Villar-Salvador et al. 2012). These responses could also be partially  
461 linked to latitude of seed sources (Table 1). Similarly, *Pinus sylvestris*, exhibits clinal trends  
462 associated with the latitude of seed origin (Persson 1994). Clinal variation in Aleppo pine in  
463 relation to latitude does occur (Agúndez et al. 1997; Climent et al. 2008). Growth rates were the  
464 lowest on this site. Our results show that growth performance of the seed sources were not  
465 opposite to that of survival (as occurred in La Hunde). In fact, correlations between performance  
466 and origin characteristics yielded the same result for either growth or survival: seed sources from  
467 lower latitudes performed best. This finding was expected as the distribution area of Aleppo pine  
468 in Spain presents a clinal increase of aridity towards the South, indicating that southern seed  
469 sources would perform better in drier and warmer sites (Ferrio and Voltas 2005; Aitken et al.  
470 2008; Reich and Oleksyn 2008). In our data set, latitude and summer precipitation were  
471 significantly correlated ( $p$ -value $<0.05$ , not shown); moreover, in the specific harsh conditions of  
472 Granja d'Escarp (not representative from the larger Cataluña's provenance region, see tables 1  
473 and 2) the home provenance performed worse. Growth needs to be coupled to the functional  
474 traits allowing a plant to survive in water-restricted environments. As consequences, seedlings  
475 may significantly reduce height and diameter growth as an adaptive strategy (Voltas et al. 2008).  
476 This is known to occur in other *Pinus* species, e.g. *P. sylvestris* and *P. taeda* (Cregg and Zhang  
477 2001). Previous experiments involving the exposure of Aleppo pine to water stress resulted in  
478 decreased stem volume growth and aboveground biomass in association with decreased carbon  
479 assimilation rates (Villar-Salvador et al. 2012). Tognetti et al. (1997) reported similar results in  
480 Aleppo pine, with drought resistance (higher leaf conductance and transpiration rate) increasing  
481 from mesic to xeric sites of origin in water-stressed seedlings, but without geographical  
482 discrimination in well-watered seedlings. Less favourable growing conditions could enhance

483 intraspecific differences between seed sources (Voltas et al. 2008). Populations are locally  
484 adapted when they have their highest relative fitness at their provenance sites and lower fitness  
485 in other parts of their range (Soto et al. 2010). Our results show that populations do not always  
486 present higher average fitness when grown under environmental conditions similar to those in  
487 their original provenance region. Multivariate grouping of seed sources at Granja d'Escarp is an  
488 example of how local provenances ('Cataluña', 'Ibérico Aragonés' and 'Monegros Depresión del  
489 Ebro') might be less suitable in environments expected to get drier and warmer due to climate  
490 change (Potter and Hargrove 2012; Williams and Dumroese 2013). Granja d'Escarp is  
491 characterized by lower precipitation (annual and summer) than the average of its provenance  
492 region, a fact that could be the general case with climate change. Precipitation is the main factor  
493 influencing growth of pines species planted in semiarid Mediterranean conditions (Atzmon et al.  
494 2004; Schiller and Atzmon 2009; Villar-Salvador et al. 2012).

495 In Tramacastiel, the high seedling mortality during the first winter, regardless of seed source,  
496 was important. This mortality was attributed to the minimal temperatures recorded immediately  
497 after outplanting in February (average: -3.2°C; range -7.8 to 4.8°C) and March 2010 (average:  
498 0.16°C; range -7.2 to 4.1°C); such temperatures are known to affect establishment of Aleppo pine  
499 (Puértolas et al. 2005). Extreme winter temperatures cause frequent freeze-thaw events, which  
500 induce xylem embolism and frost drought (Mayr 2007). Our results indicate that a good choice  
501 of seed source can increase survival by 20%. Although no correlations were found between the  
502 environmental conditions of the seed source origin and survival on this site, 'H.S. El Serranillo',  
503 'Alcarria' and 'La Mancha' yielded good survival and they are originally from continental areas  
504 with the lowest minimum temperatures during the coldest months. In fact, the ecological  
505 constraints of Tramacastiel (altitude, low winter temperature, continentality, etc) closely  
506 resemble those found for 'H.S. El Serranillo', 'Alcarria' and 'La Mancha' than those of the  
507 corresponding Aleppo pine region 'Maestrazgo-Los Serranos'. In turn, growth differences  
508 among seed sources were minimal and correlations indicated reduced height growth (RGR-H3)

509 with increasing origin latitude. A remarkable finding was the low mortality in the first summer  
510 coupled with very high RGR (H2 and D2), usually, mortality peaks and growth decreases for  
511 outplanted seedlings during the summer (del Campo et al. 2007a,b). Also, the sustained (long  
512 term) RGR-HT doubled and tripled that of La Hude and Granja d'Escarp, respectively. This is  
513 mainly attributed to the lack of summer drought and the subsequent favourable growth  
514 conditions on this site, pointing out its marked influence in the growth of the species (Matesanz  
515 and Valladares 2013). Ontogenetic variation might also contribute to the phenotypic variation of  
516 growth (Climent et al. 2008).

517 The previous knowledge (Agúndez et al. 1997; Atzmon et al. 2004; Climent et al. 2008; Voltas  
518 et al. 2008; Schiller and Atzmon 2009) on the geographic variation of *P. halepensis* has led to  
519 expect an ecotypic differentiation for survival and growth performance. Our results have shown  
520 an intraspecific variability in the expression of the measured traits: low survival differences  
521 along with high growth variation among seed sources were found in the core habitat against an  
522 opposite pattern in the marginal cold habitat. However, the harsh environmental conditions of the  
523 marginal dry habitat led to high differentiation for both survival and growth among seed sources.  
524 This is implying clearly the need to assist the migration of the species under the predictable  
525 climate change conditions (Aitken et al. 2008; Williams and Dumroese 2013). Some traits might  
526 vary in their sensitivity to the environment being more plastic than others (Matesanz and  
527 Valladares 2013) as was the case in our experiment where not only phenotypic plasticity but also  
528 variation among seed sources was higher for the considered traits at each situation of core and  
529 marginal habitats. Ecological marginality can reduce ecosystem resilience under adverse climate  
530 conditions (Rehfeldt et al. 2002), although high phenotypic plasticity could be expected for  
531 species subjected to greater variation in precipitation and extreme temperatures (Sultan and  
532 Spencer 2002; van Kleunen and Fischer 2007).

533 We measured different physiological parameters in the experimental process, but we were unable  
534 to observe significant differences among seed sources on outplanting sites. This is probably due

535 to the fact that these measurements were performed in late spring, when environmental  
536 conditions were favourable across all sites. The maximal photochemical efficiency of PSII  
537 ( $F_v/F_m$ ), which can be used to detect the occurrence of photo-inhibitory damage in response to  
538 environmental stresses (Maxwell and Johnson 2000), ranged among trial sites from 0.70 to 0.82,  
539 slightly lower than the values obtained by Demming and Björkman (1987) for optimum growth  
540 conditions (from 0.78 to 0.85). Maki and Colombo (2001) and Percival (2004) noted that  
541 photosynthesis and, as a result, growth were not negatively affected when  $F_v/F_m$  values were  
542 more than 0.6, which suggest that our seedlings were not adversely affected at time of  
543 measuring.

544 Lack of physiological differences among seed sources has led us to use survival and growth as  
545 the main variables for multivariate analysis and screening. Survival is regarded as one of the key  
546 variables when analysing tree provenance trials because it indicates the adaptability of the  
547 provenance to the environment at the trial site. It should be noted that survival reflects only the  
548 conditions experienced during the first years of the trial and not necessarily the climatic extremes  
549 and conditions that may be experienced during the life-span of a tree (Ræbild et al. 2003).

550 Regarding growth, tree height is considered an important variable in the evaluation of species  
551 and provenances (Bariteau 1992); height may be seen as a measure of the adaptability of trees to  
552 the environment, such that tall trees are better adapted to the site than short provenances/trees  
553 (Ræbild et al. 2003). This interpretation need not always be true, however, as examples exist  
554 where the tallest provenances are suddenly affected by stress with a subsequent die-off of the  
555 trees (McDowell et al. 2008). Stem diameter is often used as a measure of the productivity of  
556 stands because it is correlated to radial growth and wood production (Ræbild et al. 2003). By  
557 using these variables we were able to determine which seed sources, from different provenances,  
558 will have the highest chances to succeed in a reforestation programme under the selection  
559 pressure of future climate change throughout the assisted migration. Population structure is  
560 expected to determine species behaviour (Benito-Garzón et al. 2011), in this case, the use of

561 plasticity and genetic differentiation among populations for adaptive transfers and assisted  
562 migration would be critical to improve efficiency in reforestation programs under conditions of  
563 global warming.

564

## 565 **5. Conclusions**

566 Our results indicate that ecological constraints present on our trial sites drove the overall  
567 response of seed sources, with those belonging to regions of similar ecological (climate)  
568 constraints performing better, independently of the proximity or vicinity between trial site and  
569 provenance regions. The use of local seed sources or qualified seeds seems more reliable on sites  
570 where conditions of core habitat remain and are present (i.e. local seed sources are best under  
571 local climate conditions). As climatic conditions shift, however, to more marginal habitats  
572 (temperatures and precipitation) seed sources from climatically similar regions could perform  
573 better than local sources, at least in the short term. In this sense, current plantations in areas  
574 already affected by global change (high and consistent deviation from historical climate records)  
575 could have better establishment.

576 The differences in survival and growth found in our study among some seed sources at each site  
577 during the experiment indicate a high phenotypic plasticity within this species and that these  
578 parameters were partly conditioned by the specific genetic status of each seed source. At the  
579 same time, some variables did not differ significantly among other seeds sources, indicating that  
580 the differences in genetic constitution were masked by the effect of site conditions. However,  
581 seed source and seed source by site interaction indicated a similar plastic response among seed  
582 sources.

583 The results suggest that it is difficult to predict the performance of Aleppo pine provenances  
584 under different natural sites from their known performance at a single location. However, this  
585 study only encompassed a very limited and specific set of likely future scenarios for reforestation  
586 with Aleppo pine, that coupled with the variation within this species and local adaptation



587 understanding, provide evidence to screen a broader spectrum of conditions in order to provide  
588 specific recommendations and to significantly improve the ability of the selected trees to survive  
589 under unfavourable conditions. Even though the use of seedlings from groups of performing seed  
590 sources, we can conclude that ‘Levante interior’ and ‘La Mancha’ seedlings showed the best  
591 overall response and may be used for reforestation in contrasting environments similar to those  
592 tested here. Seed sources from southern provenances are more specifically suitable for sites  
593 adversely affected by climate change. Also, the present study clearly emphasises the need for  
594 broad selection programs for Aleppo pine on the molecular level. The genomic approaches are  
595 becoming necessary to confirm our findings and to aid in selecting climatically appropriate  
596 genotypes.

597

## 598 **Acknowledgments**

599 This study is a part of two research projects: “Application of molecular biology techniques in  
600 forest restoration in Mediterranean environments, PAID-05-11” funded by the Universitat  
601 Politècnica de València (UPV), program for supporting R&D of new multidisciplinary research  
602 lines; and the contract subscribed between the UPV and the Ministry of Environment, Rural and  
603 Marine affairs (Centro Nacional de Recursos Genéticos Forestales de Alaquàs) through its public  
604 partnership TRAGSA titled: “Study of seedling quality and field performance of 12 seed sources  
605 of *Pinus halepensis* Mill.” The authors are grateful to Amparo Pedros-Marí for field work in La  
606 Hunde, to the Valencia Regional Government (CMAAUV, Generalitat Valenciana) and  
607 VAERSA staff for their support in allowing the use of the experimental forest of La Hunde. We  
608 thank Dr. Kasten Dumroese from USDA Forest Service, Rocky Mountain Research Station for  
609 his critical and valuable comments on the draft manuscript. Also, we thank the anonymous  
610 referees for their comments, which significantly improved the final manuscript.

611

612

613 **References**

- 614 Agúndez ID, Degen B, von Wuehlisch G, Alia R (1997) Genetic variation of Aleppo pine (*Pinus*  
615 *halepensis* mill.) in Spain. For Gen 4(4):201-209.
- 616 Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or  
617 extirpation: climate change outcomes for tree populations. Evol App 1:95-111.  
618 doi:10.1111/j.1752-4571.2007.00013.x
- 619 Alía R, Garcia del Barrio JM, Iglesias S, Mancha JA, de Miguel J, Nicolas JL, Perez F, Sanchez  
620 de Ron D (2009) Regiones de procedencia de especies forestales en España. Organismo  
621 Autonomo Parques Nacionales, Madrid, 363 pp
- 622 Atzmon N, Moshe Y, Schiller G (2004) Ecophysiological response to severe drought in *Pinus*  
623 *halepensis* Mill. trees of two provenances. Plant Ecol 171:15–22.
- 624 Bariteau M (1992) Variabilité géographique et adaptation aux contraintes du milieu  
625 méditerranéen des pins de la section halepensis : résultats (provisoires) d'un essai en  
626 plantations comparatives en France. Ann Sci For 49:261-276
- 627 Benito-Garzón M, Alía R, Robson TM, Zavala MA (2011) Intra-specific variability and  
628 plasticity influence potential tree species distributions under climate change. Global Ecol  
629 Biogeogr 20:766–778.
- 630 Climent J, Prada MA, Calama R, Chambel MR, Sánchez de Ron D, Alía R (2008) To grow or to  
631 seed: ecotypic variation in reproductive allocation and cone production by young female  
632 Aleppo pine (*Pinus halepensis*, Pinaceae). Americ J Bot 95(7): 833–842.
- 633 Cortina J, Vilagrosa A, Trubat R (2013) The role of nutrients for improving seedling quality in  
634 drylands. New For DOI 10.1007/s11056-013-9379-3.
- 635 Cregg BM, Zhang JW (2001) Physiology and morphology of *Pinus sylvestris* seedlings from  
636 diverse sources under cyclic drought stress. For Ecol Manage 154:131-139.

637 del Campo AD, Hermoso J, Flors J, Lidón A, Navarro-Cerrillo RM (2011) Nursery location and  
638 potassium enrichment in Aleppo pine stock 2. Performance under real and hydrogel-mediated  
639 drought conditions. *Forestry* 84(3):235-245.

640 del Campo AD, Navarro-Cerrillo RM, Hermoso J, Ibáñez AJ (2007a) Relationships between root  
641 growth potential and field performance in Aleppo pine. *Ann For Sci* 64:541-548.

642 del Campo AD, Navarro-Cerrillo RM, Hermoso J, Ibáñez AJ (2007b) Relationships between site  
643 and stock quality in *Pinus halepensis* Mill. reforestation on semiarid landscapes in eastern  
644 Spain. *Ann For Sci* 64:719-731

645 Demming B, Bjorkman O (1987) Comparison of the effect of excessive light on chlorophyll  
646 fluorescence (77K) and photon yield of O<sub>2</sub> evolution in leaves of higher plants. *Planta*  
647 171:171-184.

648 Esteban GL, Martín JA, de Palacios P, Fernández FG, López R (2010) Adaptive anatomy of  
649 *Pinus halepensis* trees from different Mediterranean environments in Spain. *Trees* (2010)  
650 24:19–30. DOI 10.1007/s00468-009-0375-3.

651 European Forest Institute (2009) A Mediterranean Forest Research Agenda-MFRA. 2010-2020.  
652 Mediterranean Regional Office–EFIMED.  
653 [www.efimed.efi.int/files/attachments/efimed/mediterranean\\_forest\\_research\\_agenda\\_2010-](http://www.efimed.efi.int/files/attachments/efimed/mediterranean_forest_research_agenda_2010-2020.pdf)  
654 [2020.pdf](http://www.efimed.efi.int/files/attachments/efimed/mediterranean_forest_research_agenda_2010-2020.pdf)

655 FAO (2013) State of Mediterranean Forests 2013, [www.fao.org/docrep/017/i3226e/i3226e.pdf](http://www.fao.org/docrep/017/i3226e/i3226e.pdf)

656 Gandullo J, Sánchez Palomares O (1994) Estaciones ecológicas de los pinares españoles,  
657 ICONA, Ministerio de Agricultura, Pesca y Alimentación, Madrid.

658 Grivet D, Sebastiani F, González-Martínez SC, Vendramin GG (2009) Patterns of polymorphism  
659 resulting from long-range colonization in the Mediterranean conifer Aleppo pine. *New Phytol*  
660 184:1016–1028.

661 Hernandez EI, Vilagrosa A, Pausas JG, Bellot J (2010) Morphological traits and water use  
662 strategies in seedlings of Mediterranean coexisting species. *Plant Ecol* 207:233-244.

663 IPCC (2001) The Scientific Basis. In: Climate Change 2001 (eds Houghton JT, Ding Y, Griggs  
664 DJ, Noguer M, Van Der Linden PJ, Dai X, Maskell K, Johnsson CA), pp. 881. Cambridge  
665 University Press, Cambridge.

666 Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. J Am Stat  
667 Assoc 53:457-481.

668 Landis TD, Dumroese RK, Haase DL (2010) The container tree nursery manual: Seedling  
669 processing, storage, and outplanting. vol.7. USDA For. Serv., Agr. Handbook. 674,  
670 Washington, DC. 192 p.

671 Maki DS, Colombo SJ (2001) Early detection of the effects of warm storage on conifer seedlings  
672 using physiological tests. For Ecol Manage 154:237-249.

673 Matesanz S, Valladares F (2013) Ecological and evolutionary responses of Mediterranean plants  
674 to global change. <http://dx.doi.org/10.1016/j.envexpbot.2013.09.004>.

675 Maxwell K, Johnson GN (2000) Chlorophyll fluorescence-a practical guide. J Exp Bot 51:659-  
676 668.

677 Mayr S (2007) Limits in water relations. Trees at their upper limit. Tree life limitation at the  
678 Alpine timberline (ed. by G. Wieser and M. Tausz), pp. 145–162. Springer, Berlin.

679 McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West  
680 A, Williams DG, Yezzer EA (2008) Mechanisms of plant survival and mortality during  
681 drought: why do some plants survive while others succumb to drought? New Phytol 178:719-  
682 739.

683 Metzger MJ, Bunce RGH, Leemans R, Viner D (2008) Projected environmental shifts under  
684 climate change: European trends and regional impacts. Environ Conserv 35(1):64-75.

685 Mulet JM, Martin DE, Loewith R, Hall MN (2006) Mutual antagonism of target of rapamycin  
686 and calcineurin signaling. J Biol Chem 281(44):33000-33007.

687 Oliet JA, Puértolas J, Planelles R, Jacobs DF (2013) Nutrient loading of forest tree seedlings to  
688 promote stress resistance and field performance: a Mediterranean perspective. *New For*  
689 44:649–669. DOI 10.1007/s11056-013-9382-8.

690 Oliver J, Bogino S, Spiecker H, Bravo F (2012) Climate impact on growth dynamic and intra-  
691 annual density fluctuations in Aleppo pine (*Pinus halepensis*) trees of different crown classes.  
692 *Dendro* 30:35-47.

693 Percival GC (2004) Evaluation of physiological tests as predictors of young tree establishment  
694 and growth. *J Arboric* 30(2):80-92.

695 Persson B (1994) Effect of provenance transfer on survival in nine experimental series with  
696 *Pinus sylvestris* (L.) in northern Sweden. *Scand J Forest Res* 9:275-287.

697 Potter KM, Hargrove WW (2012) Determining suitable locations for seed transfer under climate  
698 change: a global quantitative method. *New For* DOI 10.1007/s11056-012-9322-z

699 Puértolas J, Gil L, Pardos JA (2005) Effects of nitrogen fertilization and temperature on frost  
700 hardiness of Aleppo pine (*Pinus halepensis* Mill.) seedlings assessed by chlorophyll  
701 fluorescence. *Forestry* 78(5) doi:10.1093/forestry/cpi055.

702 Quézel P, Médail F (2003) *Ecologie et biogéographie des forêts du bassin méditerranéen*. Elsevier  
703 (Collection Environnement), Paris, 573 p.

704 Ræbild A, Graudal L, Khan SR (2003) Evaluation of a *Prosopis cineraria* provenance trial at  
705 Dagar Kotli, Pakistan. Trial no. 23 in the arid zone series Results and Documentation No. 28.  
706 Danida Forest Seed Centre, Humlebaek, Denmark.

707 Rehfeldt GE, Tchebakova NM, Parfenova YI, Wykoff WR, Kuzmina NA, Milyutin II (2002)  
708 Intraspecific responses to climate change in *Pinus sylvestris*. *Glob Change Biol* 8:912-929.

709 Reich PB, Oleksyn J (2008) Climate warming will reduce growth and survival of Scots pine  
710 except in the far north. *Ecol Lett* 11:588–597. doi: 10.1111/j.1461-0248.2008.01172.x.

711 Sáenz-Romero C, Beaulieu J, Rehfeldt GE (2011) Altitudinal genetic variation among *P. patula*  
712 in growth chambers simulating global warming temperatures. *Agrocien* 45:399-411.

713 Schiller G, Atzmon N (2009) Performance of Aleppo pine (*Pinus halepensis*) provenances  
714 grown at the edge of the Negev desert: A review. *J Arid Env* 73:1051-1057.

715 Schmidting RC (1994) Use of provenance tests to predict response to climatic change: loblolly  
716 pine and Norway spruce. *Tree Physiol* 14:805-817.

717 Soto A, Robledo-Arnuncio JJ, González-Martínez J, Smouse BE, Alía (2010) Climatic niche  
718 and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective  
719 View. *Mol Ecol* (2010) 19, 1396–1409 doi: 10.1111/j.1365-294X.2010.04571.x

720 Sultan SE, Spencer HG (2002) Metapopulation structure favors plasticity over local adaptation.  
721 *Americ Naturalist* 160:271-283.

722 Tognetti R, Michelozzi M, Giovannelli A (1997) Geographical variation in water relations,  
723 hydraulic architecture and terpene composition of Aleppo pine seedlings from Italian  
724 provenances. *Tree Physiol* 17: 241-250.

725 United Nations, United Nations Economic Commission for Europe & Food and Agriculture  
726 Organization of the United Nations (2011) The European Forest Sector Outlook Study II  
727 2010-2030. 107 pp. <http://www.fao.org/docrep/016/ap406e/ap406e00.pdf>

728 Vallejo et al. 2012 Vallejo VR, Smanis A, Chirino E, Fuentes D, Valdecantos A, Vilagrosa A  
729 (2012) Perspectives in dryland restoration: approaches for climate change adaptation. *New*  
730 *For* 43:561–579. DOI 10.1007/s11056-012-9325-9

731 van Kleunen M, Fischer M (2007) Progress in the detection of costs of phenotypic plasticity in  
732 plants. *New Phytol* 176:727–730.

733 Vennetier M, Vilà B, Liang E, Guibal F, Taahbet A, Gadbin-Henry C (2007) Impact of climate  
734 change on pines forest productivity and on the shift of a bioclimatic limit in Mediterranean  
735 area. In: Leon e V. (ed.), Lovreglio R. (ed.). Proceedings of the international workshop  
736 MEDPINE 3: conservation, regeneration and restoration of Mediterranean pines and their  
737 ecosystems. Bari : CIHEAM, 2007. p.189-197 (Options Méditerranéennes : Série A.  
738 Séminaires Méditerranéen s; n. 75)

739 Villar Salvador P, Puértolas J, Cuesta B, Peñuelas JL, Uscola M, Heredia-Guerrero N, Rey  
740 Benayas JM (2012) Increase in size and nitrogen concentration enhances seedling survival in  
741 Mediterranean plantations. Insights from an ecophysiological conceptual model of plant  
742 survival. *New For* DOI 10.1007/s11056-012-9328-6.

743 Voltas J, Chambel MR, Prada MA, Ferrio JP (2008) Climate-related variability in carbon and  
744 oxygen stable isotopes among populations of Aleppo pine grown in common-garden tests.  
745 *Trees* 22:759-769.

746 Williams MI, Dumroese RK (2013) Preparing for climate change: forestry and assisted  
747 migration. *J For* 111(4):287-297

748 Zobel B, Talbert JJ (1984) *Applied forest tree improvement*. John Wiley and Sons, New York,  
749 pp 75-116.

750

751 **Table 1.** Site descriptions for the eleven seed sources of *Pinus halepensis* Mill. (from Alía et al. 2009). T<sub>M</sub>: annual mean temperature; T<sub>MC</sub>: mean  
752 minimum daily temperature of the coolest month; T<sub>MW</sub>: mean maximum daily temperature of the warmest month; DM: drought months; SP: summer  
753 precipitation; AP: annual precipitation.

Seed source <sup>(class)</sup>	Abbreviation	Coordinates	Altitude (m)	T <sub>M</sub> (°C)	DM	T <sub>MC</sub> (°C)	T <sub>MW</sub> (°C)	SP (mm)	AP (mm)
Alcarria <sup>1</sup>	Alc	40°24'N-2°24'W	869	12.4	2.8	-1.1	32.2	82	591
Bética septentrional/sur <sup>1</sup>	Bet	37°55'N-2°55'W	789	14.5	4.0	1.9	31.9	55	410
Cataluña <sup>2*</sup>	Cat	41°46'N-2°46'E	333	13.9	1.1	1.3	29.3	128	714
H.S. El Serranillo <sup>3</sup>	HSS	40°40'N-3°10'W	635	13.3	3.0	-0.4	33.0	46	380
H.S. Alaquàs <sup>3</sup>	HSA	39°27'N-0°28'W	43	17.2	4.0	5	31.1	58	444
Ibérico Aragonés <sup>1</sup>	Arg	40°47'N-0°04'W	555	14.0	2.7	1.5	30.2	91	497
La Mancha <sup>1</sup>	Man	39°55'N-2°14'W	856	13.1	3.2	-0.1	32.0	68	496
Levante Interior <sup>1</sup>	Lev	39°05'N-1°12'W	673	14.4	3.1	2.6	30.5	67	524
Litoral levantino <sup>1</sup>	Lit	39°45'N-0°18'W	200	16.5	3.2	4.8	30.3	64	599
Los Monegros-Depresión Ebro <sup>1</sup>	Mgr	41°53'N-0°52'W	401	14.2	3.7	0.9	32.3	79	399
Maestrazgo-Los Serranos <sup>1</sup>	Mst	39°47'N-1°11'W	763	13.6	2.5	1.4	30.0	93	546

754 **Note:** Class of seed source: 1, seed source (identified); 2, stand (selected); 3, seed orchard (qualified). \*This is a composite seed source from Cataluña  
755 interior and Alta Cataluña seed sources.



756 **Table 2.** Characteristics of three sites in Spain used to test survival, growth and ecophysiological conditions of Aleppo pine seed sources. Climatic  
757 variables are measured or historical value followed by first and second year weather station estimates in parentheses.  
758 (AP: annual precipitation, SP: summer precipitation, T<sub>M</sub>: annual mean temperature, T<sub>MW</sub>: mean maximum daily temperature of the warmest month,  
759 T<sub>MC</sub>: mean minimum daily temperature of the coolest month, PET: potential evapotranspiration).

Site, coordinates	Altitude (m)	AP (mm)	SP (mm)	T <sub>M</sub> (°C)	T <sub>MW</sub> (°C)	T <sub>MC</sub> (°C)	PET (mm)	Sum of water deficits (mm)	Texture (USDA)	Slope (%)	Aspect	S (°)
Lower marginal/core/higher marginal <sup>1</sup>	70- 350//970- 1375	243- 325//705- 901	9-23//133- 233	10.6- 12//16.2- 19.3	Not def.	Not def.	641- 692//846- 996	119- 261//551- 734	Not def.	0- 8//60 -75	Not def.	
La Hude <sup>2</sup> , Ayora- Valencia, 1°12'w; 39°05'N	800	477 (648;736)	80 (27;145)	13.5 (14;12.3)	31 (35.3;35.3)	0.9 (-2.7;-1.7)	765 (793;718)	-301 (-410;-256)	Silty loam	5	NE-SW	
Granja d'Escarp <sup>3</sup> , Lleida, 0°20'E; 41°23'N	310	353 (392;310)	47.7 (97;24)	14.2 (13.3;14.7)	31.3 (32.7;31.9)	1.0 (0;0.1)	779 (755;794)	-426 (-289;-484)	Silty loam	17- 44	S-NW	

Tramacastiel <sup>4</sup> , Teruel, 0°40'w; 39°12'N	1150	531 (586;445)	119 (162;94.1)	11 (10.3;11.9)	28.5 (30;29.5)	-1.6 (-3.2;-1.8)	659 (639;686)	-177 (-133;-241)	Loam	9	SW
---	------	------------------	-------------------	-------------------	-------------------	---------------------	------------------	---------------------	------	---	----

760 **Note:** 1, digits separated by “-” are the marginal ranges (low and high) and digits separated by “//” represent the core range. Type of habitat: 2, core

761 habitat; 3, marginal dry habitat; 4, marginal cold habitat.

762 **Table 3.** Sources of variation and percentage of explained variation of the survival and the relative growth rate (RGR) (height (H) and stem diameter  
763 (D)) of eleven Aleppo pine seed sources planted in three sites using the restricted maximum likelihood method (REML). Survival was measured during  
764 the first spring, during and after the first summer, and during spring of the 3<sup>rd</sup> year. RGR-H and RGR-D were measured during first spring (1), first  
765 summer (2), second/third year (3), and overall (T, Total growth).

<b>Traits</b>	<b>Components</b>	<b>1<sup>st</sup> survival/ 1<sup>st</sup> spring growth</b>	<b>2<sup>nd</sup> survival/ 1<sup>st</sup> summer growth</b>	<b>3<sup>rd</sup> survival/ 2<sup>nd</sup>/3<sup>rd</sup> year growth</b>	<b>4<sup>th</sup> survival/Total growth</b>
<b>Survival</b>	Site (S)	97.7	98.1	98.4	99.1
	Seed source (S.S)	0.0	0.3	0.4	0.1
	S x S.S	2.3	1.7	1.3	0.8
<b>RGR-D</b>	Site (S)	94.4	99.8	91.9	92.1
	Seed source (S.S)	0.0	0.0	5.1	1.0
	S x S.S	5.6	0.2	3.0	6.9
<b>RGR-H</b>	Site (S)	99.8	96.9	84.6	92.4
	Seed source (S.S)	0.0	0.2	3.8	1.7
	S x S.S	0.2	2.9	11.6	5.9

766

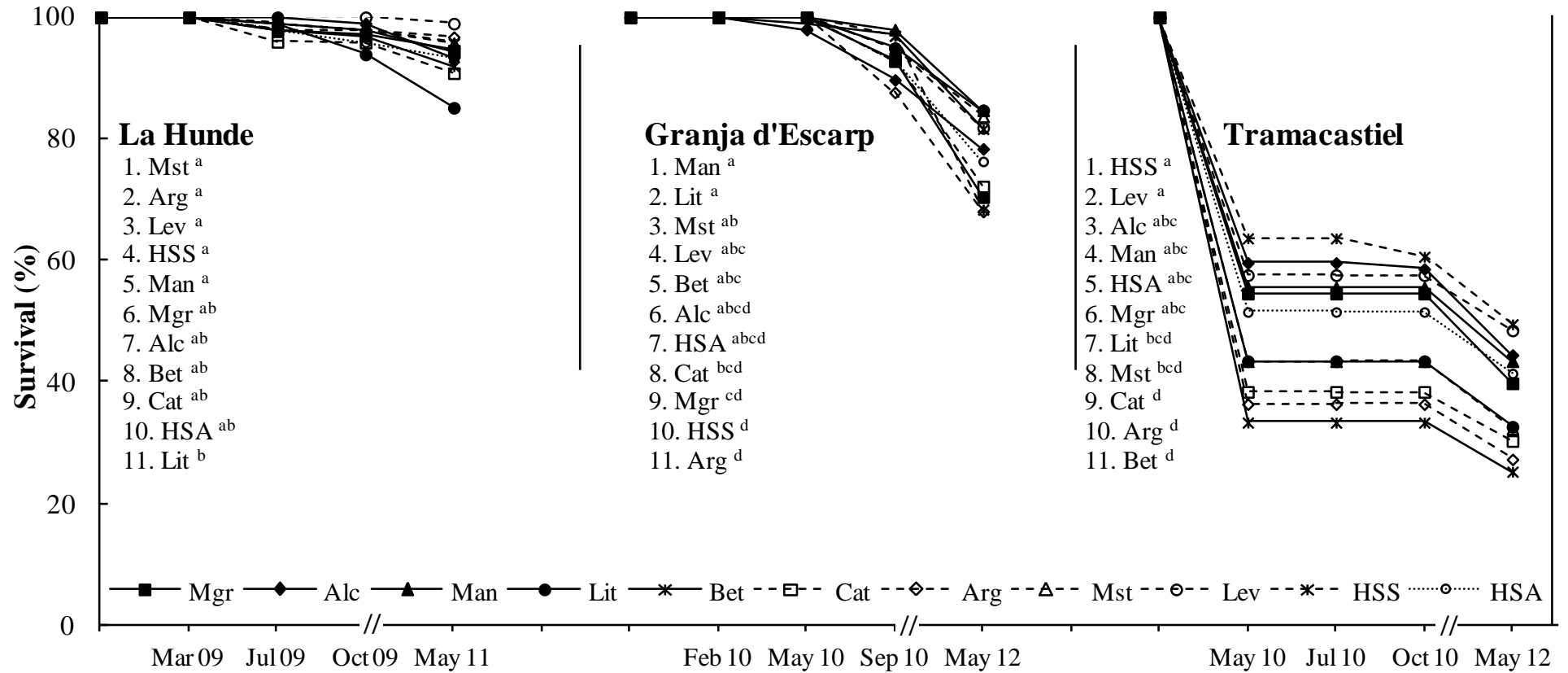
767

768 **Table 4.** ANOVA summary (F-value, Mean Square Error (MSE), degrees of freedom of error (df) and significance: \*p-value<0.05, \*\* p-value<0.01,  
769 \*\*\* p-value<0.001) of the relative growth rate (RGR) for height (H) or diameter (D) of eleven Aleppo pine seed sources planted in three sites. RGR-H  
770 and RGR-D were measured during first spring (1), first summer (2), second/third year (3), and overall (T, Total growth). †Homoscedasticity not  
771 achieved.

Site	Source	RGR-H				RGR-D			
		1 <sup>st</sup> spring	1 <sup>st</sup> summer	2 <sup>nd</sup> / 3 <sup>rd</sup> year	Total growth	1 <sup>st</sup> spring	1 <sup>st</sup> summer	2 <sup>nd</sup> / 3 <sup>rd</sup> year	Total growth
La Hunde	F-value	†6.91***	†5.59***	†20.77***	†23.78***	5.34***	0.78	1.27	1.45
	MSE(df)	6.2E-04(902)	5.2E-05(902)	5.1E-05(902)	4.0E-05(902)	1.2E-03(969)	3.1E-04(969)	4.4E-05(969)	3.7E-05(969)
Granja d'Escarp	F-value	†4.83***	†4.59***	5.23***	†6.60***	2.22*	0.71	1.64	2.79**
	MSE(df)	2.9E-04(793)	9.8E-05(793)	3.3E-05(793)	2.6E-05(793)	8.6E-04(819)	6.3E-04(819)	1.0E-04(819)	7.0E-05(819)
Tramacastiel	F-value	0.48	0.95	2.08*	1.31	†0.81	0.93	1.29	1.88
	MSE(df)	2.0E-03(180)	4.0E-03(180)	1.5E-04(180)	1.5E-04(180)	2.9E-03(181)	5.4E-03(181)	1.0E-04(181)	9.5E-05(181)

772

773



775

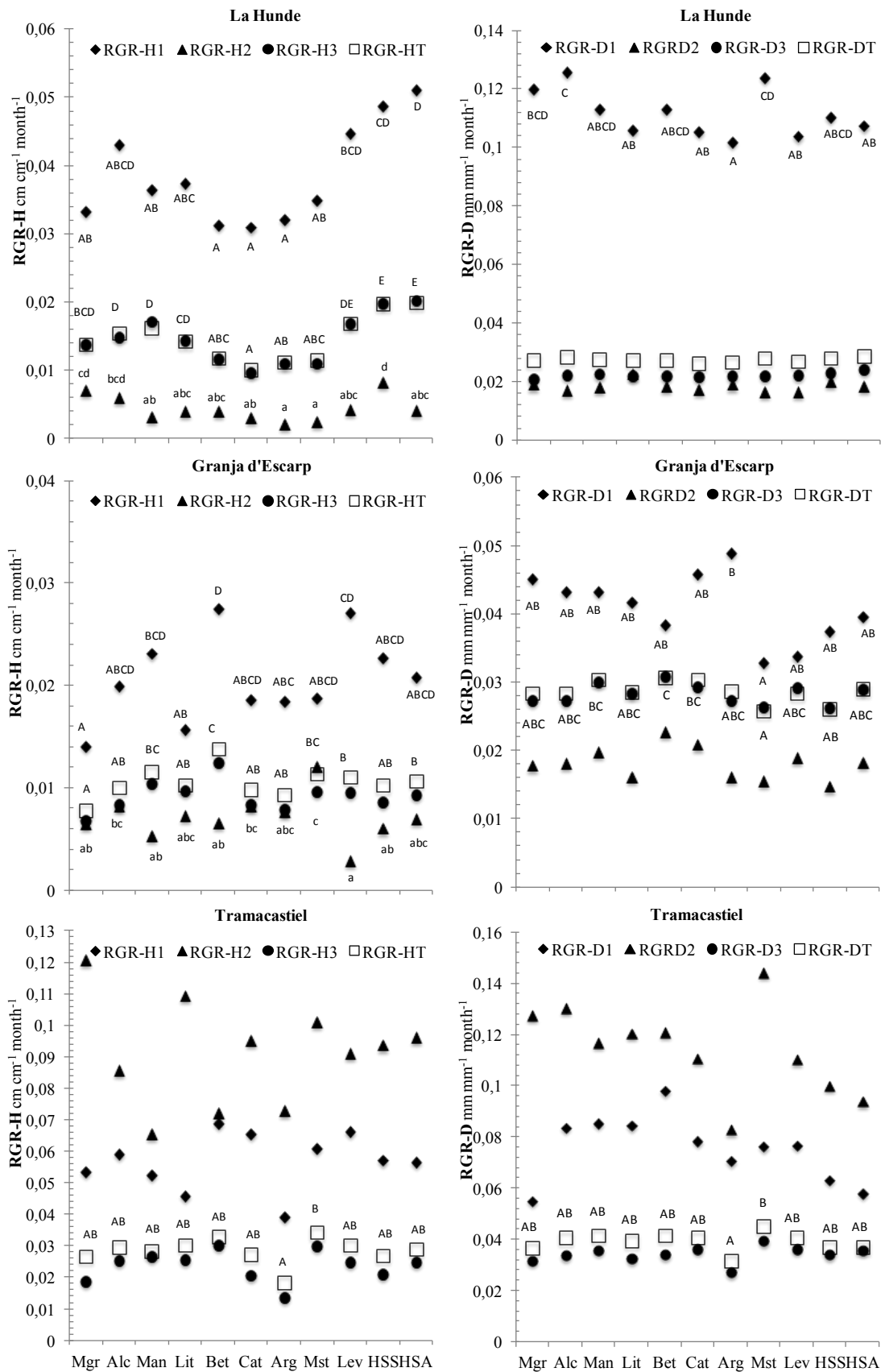
776

**Fig. 1.** Temporal survival of the eleven seed sources of Aleppo pine out-planted on the three trial sites. For each site, seed source abbreviations (see

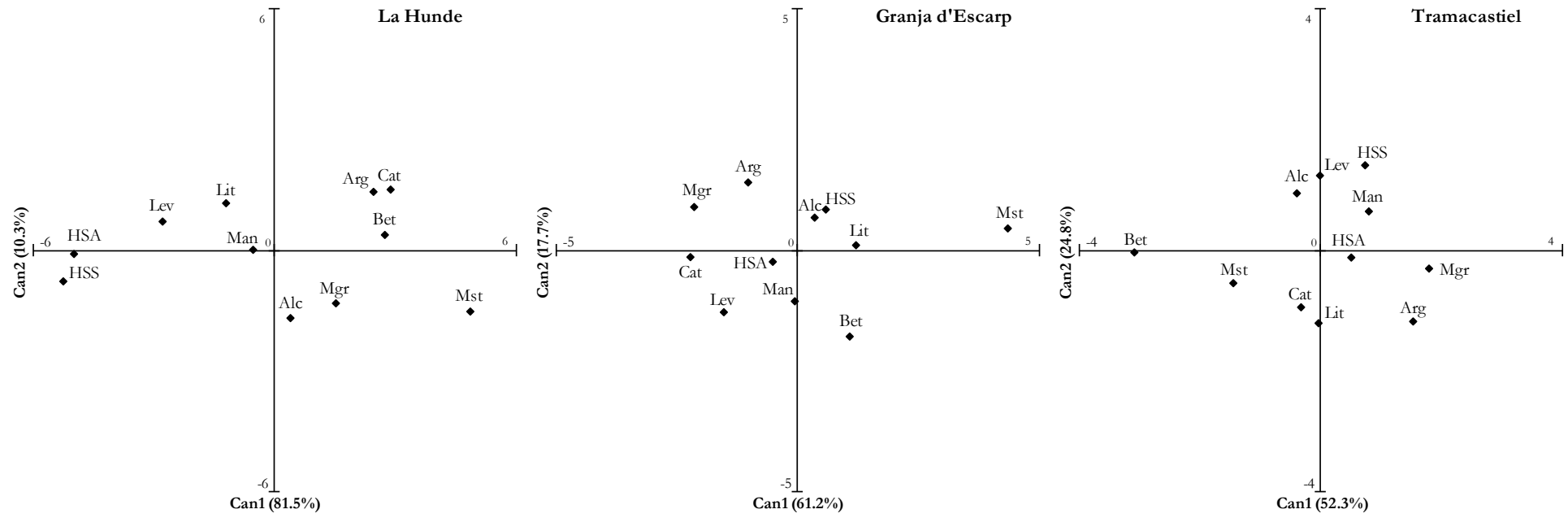
777

Table 1) followed by different letters indicate significant differences (p-value<0.05) in survival.

778

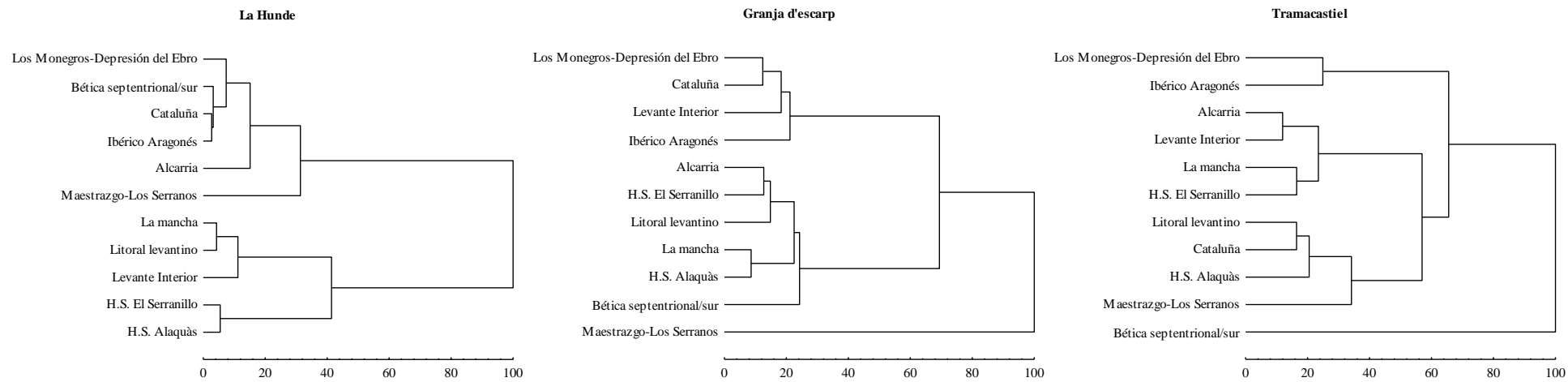


779  
 780 **Fig. 2.** Relative growth rates in height (RGR-H;  $\text{cm cm}^{-1} \text{ week}^{-1}$ ) and diameter (RGR-D;  $\text{mm mm}^{-1} \text{ week}^{-1}$ ) for the first spring (1), first summer (2), from first summer to 3<sup>rd</sup> May (3) and for  
 781 the total study period (T) of eleven Aleppo pine seed sources out-planted on three contrasting  
 782 sites. Within the same variable and site, different letters indicate significant differences at  $p$ -  
 783 value  $< 0.05$  (letters for RGR-H/D3 are not shown). Seed source abbreviations shown in Table 1.  
 784



786

787 **Fig. 3.** The scatter-plot of the first and the second Canonical axes obtained by Canonical Discriminant Analysis on eleven Aleppo pine seed sources at  
 788 the three trial sites in eastern Spain.



789

790 **Fig. 4.** Dendrograms based on Mahalanobis distances using clustering Ward's method among eleven Aleppo pine seed sources at the three trial sites in  
 791 eastern Spain.