

Dear Author

Here are the proofs of your article.

- You can submit your corrections **online**, via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For **fax** submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
- Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL:

<http://dx.doi.org/10.1007/s13595-015-0527-0>

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information, go to:

<http://www.link.springer.com>.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us, if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

1	Article Title	Provenance and seed mass determine seed tolerance to high temperatures associated to forest fires in <i>Pinus pinaster</i>	
2	Article Sub- Title		
3	Article Copyright - Year	INRA and Springer-Verlag France 2015 (This will be the copyright line in the final PDF)	
4	Journal Name	Annals of Forest Science	
5	Corresponding Author	Family Name	Calvo
6		Particle	
7		Given Name	Leonor
8		Suffix	
9		Organization	University of León
10		Division	Area of Ecology, Facultad de Ciencias Biológicas y Ambientales
11		Address	León 24071
12		e-mail	leonor.calvo@unileon.es
13	Author	Family Name	Hernández
14		Particle	
15		Given Name	Verónica
16		Suffix	
17		Organization	University of León
18		Division	Area of Ecology, Facultad de Ciencias Biológicas y Ambientales
19		Address	León 24071
20		e-mail	
21	Author	Family Name	Valbuena
22		Particle	
23		Given Name	Luz
24		Suffix	
25		Organization	University of León
26		Division	Area of Ecology, Facultad de Ciencias Biológicas y Ambientales
27		Address	León 24071
28		e-mail	
29	Author	Family Name	Taboada

30		Particle	
31	Given Name	Angela	
32	Suffix		
33	Organization	University of León	
34	Division	Area of Ecology, Facultad de Ciencias Biológicas y Ambientales	
35	Address	León 24071	
36	Organization	University of León	
37	Division	Institute of Environmental Research (IMA)	
38	Address	León 24071	
39	e-mail		
<hr/>			
40	Received	17 June 2015	
41	Schedule Revised		
42	Accepted	27 October 2015	
<hr/>			
43	Abstract	<p>Key message: We show that <i>Pinus pinaster</i> provenance affects both seed germination and seedling recruitment after fire. This information is crucial for managers to select the best seed-provisioning populations when implementing reforestation programs to assist the natural post-fire regeneration of the species.</p> <p>Context: Reforestation programs to assist natural regeneration of maritime pine (<i>Pinus pinaster</i> Aiton) forests after wildfires very often neglect the fire-adaptive traits of the selected seed-source populations, threatening future stand resilience to fire.</p> <p>Aim: We investigate the influence of seed provenance and weight on the rate and timing of seed germination, and on early seedling growth of <i>P. pinaster</i> after fire.</p> <p>Methods: We simulated conditions during fire by experimentally submitting free seeds from three <i>P. pinaster</i> provenances with contrasting levels of serotiny (from <6 to >58 % of trees bearing serotinous cones within the population) to six combinations of temperature and exposure time. We fitted GLMs and LMs to test for differences among provenances in reproductive performance after the thermal shock treatments.</p> <p>Results: Heavy seeds derived from the very low serotinous population exhibited greater tolerance to heat (higher germination rate, lower germination time, and higher seedling length) than the lighter ones from the highly serotinous population.</p> <p>Conclusion: The seeds enclosed in serotinous cones are, however, the only ones protected from high fire-induced temperatures, delaying release until favourable post-burn conditions for germination and seedling establishment. Therefore, selecting the best fire-adapted populations with the optimal serotiny level as seed-provisioning sources should be encouraged to ensure forest regrowth in fire-prone areas.</p>	
44	Keywords separated by ' - '	Post-fire regeneration - Seed germination - Seedling growth - Seed weight - Maritime pine	
<hr/>			
45	Foot note information	<p>Handling Editor: Eric Rigolot</p> <p>L. Calvo: coordinating the research project, experimental design, supervising</p>	

AUTHOR'S PROOF

the lab work, and writing and editing the paper

V. Hernández: lab work and editing the paper

L. Valbuena: experimental design, help in lab work, and help in editing the paper

A. Taboada: running the data analysis, and writing and editing the paper

Q1 4 Provenance and seed mass determine seed tolerance
5 to high temperatures associated to forest fires in *Pinus pinaster*

Q2 6 Leonor Calvo¹ · Verónica Hernández¹ · Luz Valbuena¹ · Angela Taboada^{1,2}
7

8 Received: 17 June 2015 / Accepted: 27 October 2015
9 © INRA and Springer-Verlag France 2015

10 **Abstract**

11 • **Key message** We show that *Pinus pinaster* provenance
12 affects both seed germination and seedling recruitment
13 after fire. This information is crucial for managers to se-
14 lect the best seed-provisioning populations when
15 implementing reforestation programs to assist the natural
16 post-fire regeneration of the species.
17 • **Context** Reforestation programs to assist natural regenera-
18 tion of maritime pine (*Pinus pinaster* Aiton) forests after wild-
19 fires very often neglect the fire-adaptive traits of the selected
20 seed-source populations, threatening future stand resilience to
21 fire.
22 • **Aim** We investigate the influence of seed provenance and
23 weight on the rate and timing of seed germination, and on
24 early seedling growth of *P. pinaster* after fire.
25 • **Methods** We simulated conditions during fire by experimen-
26 tally submitting free seeds from three *P. pinaster* provenances
27 with contrasting levels of serotiny (from <6 to >58 % of trees
28 bearing serotinous cones within the population) to six

combinations of temperature and exposure time. We fitted 29
GLMs and LMs to test for differences among provenances 30
in reproductive performance after the thermal shock 31
treatments. 32
• **Results** Heavy seeds derived from the very low serotinous 33
population exhibited greater tolerance to heat (higher germi- 34
nation rate, lower germination time, and higher seedling 35
length) than the lighter ones from the highly serotinous 36
population. 37
• **Conclusion** The seeds enclosed in serotinous cones are, 38
however, the only ones protected from high fire-induced tem- 39
peratures, delaying release until favourable post-burn condi- 40
tions for germination and seedling establishment. Therefore, 41
selecting the best fire-adapted populations with the optimal 42
serotiny level as seed-provisioning sources should be encour- 43
aged to ensure forest regrowth in fire-prone areas. 44

Keywords Post-fire regeneration · Seed germination · 45
Seedling growth · Seed weight · Maritime pine 46

Handling Editor: Eric Rigolot

Contribution of the co-authors L. Calvo: coordinating the research
project, experimental design, supervising the lab work, and writing and
editing the paper
V. Hernández: lab work and editing the paper
L. Valbuena: experimental design, help in lab work, and help in editing
the paper
A. Taboada: running the data analysis, and writing and editing the paper

✉ Leonor Calvo
leonor.calvo@unileon.es

¹ Area of Ecology, Facultad de Ciencias Biológicas y Ambientales,
University of León, 24071 León, Spain

² Institute of Environmental Research (IMA), University of León,
24071 León, Spain

1 Introduction 47

The maritime pine (*Pinus pinaster* Aiton) is a very inflamma- 48
ble conifer species with high resin content that constitutes one 49
of the principal fire-prone forest ecosystems of the Iberian 50
Peninsula (Vasques et al. 2012). During 2012, 41 large wild- 51
fires (ranging from 511 to 28,879 ha) were recorded in Spain, 52
which burned 135,580 ha in total (representing 63 % of the 53
total forested area burned in that year, a surface 300 % higher 54
than the average of the last decade), primarily affecting many 55
large *P. pinaster* stands (Ministerio de Agricultura, 56
Alimentación y Medio Ambiente 2014). As other Mediterra- 57
nean pine species, such as *Pinus halepensis* Mill. and *Pinus* 58
brutia Ten. (De las Heras et al. 2012), *P. pinaster* is an obligate 59

60 seeder, i.e. non-resprouter species that relies only on regener- 113
61 ation from seeds for post-fire recovery (Pausas et al. 2004). 114
62 The natural post-fire regeneration of *P. pinaster* occurs con- 115
63 tinuously until 3 years after a fire (Fernandes and Rigolot 116
64 2007; Madrigal et al. 2005), thanks to the long-lasting storage 117
65 of seeds in serotinous cones (i.e. cones that release the seeds 118
66 only after exposure to high temperatures) within the canopy 119
67 level (Álvarez et al. 2005, 2007; Calvo et al. 2013; Fernandes 120
68 and Rigolot 2007; Tapias et al. 2001, 2004; Torres et al. 2006). 121
69 In the last 20 years, however, the post-fire recovery of 122
70 *P. pinaster* has been undermined by the increased severity, 123
71 extent, and recurrence of wildfires (with an interval between 124
72 fires of less than 10–15 years, the minimum required time for 125
73 adult pines to reach their reproductive maturity; Pausas et al. 126
74 2008; Tapias et al. 2001), together with extreme summer 127
75 droughts and intense competition with resprouting species 128
76 (Madrigal et al. 2011; Rodrigo et al. 2004). In this new situa- 129
77 tion, and given the commercial importance of the species, the 130
78 Spanish National and Regional Forest Administrations devel- 131
79 op seed collection programs, in which *P. pinaster* seeds are 132
80 harvested from pre-selected outstanding trees in specific pop- 133
81 ulations and preserved for silvicultural purposes. Seeds are 134
82 then used in reforestation programs (either by direct seeding 135
83 or by growing and planting new saplings), to enhance the 136
84 recovery and ensure the survival of the stands devastated by 137
85 high-intensity fires (see Gil et al. 2009). 138

86 *P. pinaster* forests exhibit a high geographic structuration 139
87 of genetic variability at the intraspecific level in their whole 140
88 distribution area (Bucci et al. 2007). Such variability is possi- 141
89 bly related to the great variety of ecological conditions under 142
90 which these forests develop, resulting in different life history 143
91 strategies and peculiar population adaptations in terms of re- 144
92 production (Escudero et al. 2002; Gil et al. 2009; see also 145
93 Bramlett et al. 1983), growth and survival (Alía et al. 1995), 146
94 and response to fire (Ghildiyal et al. 2009). Variability in the 147
95 reproductive traits (e.g. number of reproductive trees, cone 148
96 production, and sapling and seedling density) of *P. pinaster* 149
97 from different provenances also determines disparities in fire 150
98 resilience among populations (Gil et al. 2009). Of the three 151
99 genetic groups identified for *P. pinaster* (i.e. Atlantic, Medi- 152
100 terranean-European, and Maghrebian; Tapias et al. 2004), the 153
101 Atlantic one shows the highest intra- and inter-population vari- 154
102 ability in fire adaptive traits, especially in the serotiny level 155
103 (i.e. the percentage of serotinous cones varies among individ- 156
104 uals of the same population and among different populations) 157
105 (Tapias et al. 2001, 2004), which is a heritable character 158
106 (Hernández-Serrano et al. 2014) closely related to the type 159
107 of fire regime affecting each population (Hernández-Serrano 160
108 et al. 2013; Tapias et al. 2004). Serotiny acts as an advanta- 161
109 geous reproductive adaptation of *P. pinaster* populations in 162
110 fire-prone environments, because (1) the canopy seed bank 163
111 of the species is relatively small in non-serotinous populations 164
112 (Tapias et al. 2001), and (2) the soil seed bank is insufficient 165

and temporary and ends up irreversibly damaged either by 113
fire-induced temperatures or by post-dispersal predation (De 114
las Heras et al. 2012; Ruano et al. 2015; Torres et al. 2006). 115
Populations with high levels of serotiny can retain closed 116
cones up to 40 years and preserve seed viability for 30 years 117
(Tapias et al. 2004) and are, therefore, more likely to survive 118
frequent crown fires and achieve successful post-fire recruit- 119
ment (i.e. high rates of seed germination and seedling emer- 120
gence) (Fernandes and Rigolot 2007; Gil et al. 2009; Tapias 121
et al. 2004). In contrast, non-serotinous or weakly serotinous 122
populations display alternative strategies of fire adaptation 123
(e.g. earlier and more abundant flowering, and the formation 124
of a thick bark to protect the tree against lethal temperatures) 125
that enable them to withstand low intensity fires (Fernandes 126
and Rigolot 2007; Gil et al. 2009; Tapias et al. 2001, 2004). 127
Such striking divergences in fire resilience and post-fire seed- 128
ling recruitment among populations evidence the necessity of 129
selecting the best fire-adapted *P. pinaster* provenances as seed 130
sources in post-fire reforestation programs (Gil et al. 2009; 131
Pausas 2015). Particularly in *P. pinaster* forests threatened 132
by recurrent fires, the introduction of genetic material from 133
populations with advantageous fire adaptive strategies (e.g. 134
high levels of serotiny) is expected to improve natural regen- 135
eration in fire-devastated stands (Gil et al. 2009). However, 136
there is a general lack of information on the species linking the 137
serotiny level of the maternal population from which seeds are 138
harvested and the resultant post-fire tree recruitment (Vega 139
et al. 2008). 140

Moreover, extremely high variability in seed mass exists 141
within and among *P. pinaster* provenances (Escudero et al. 142
2000; Zas and Sampedro 2015; Zas et al. 2013), similarly to 143
other pine species (e.g. Court-Picon et al. 2004; Ganatsas et al. 144
2008; Liu et al. 2013; Parker et al. 2006). Seed mass is often 145
positively associated with post-fire seedling establishment, 146
growth, and survival (Álvarez et al. 2007; Bladé and Vallejo 147
2008; Escudero et al. 2000), but no consensus exists on the 148
relationship between seed mass and germination rates follow- 149
ing fire (Escudero et al. 2002; Hanley et al. 2003; Reyes and 150
Casal 1995, 2004; Vasques et al. 2012). In the case of 151
P. pinaster, heavier and larger seeds are less sensitive to high 152
temperatures and more resistant to fire (Escudero et al. 1999, 153
2000; Reyes and Casal 1995, 2001) and are, therefore, thought 154
to experience lower mortality during severe fires. Indeed, 155
heavy seeds insulate the embryo better and preserve seed vi- 156
ability and survival, also favouring seedling performance, 157
when submitted to diverse thermal shock treatments reproduc- 158
ing the effects of fire (Álvarez et al. 2007; Escudero et al. 159
2000; Reyes and Casal 2001). However, the effects of seed 160
mass on post-fire recruitment may be more complex and 161
very likely mediated by seed provenance, i.e. by the mater- 162
nal environment and genotype (Cendán et al. 2013; Zas 163
et al. 2013), from which seeds are harvested in reforestation 164
programs. 165

166 In this study, we aim to analyse the importance of the geo-
 167 graphic origin and the variation in weight of the seed material
 168 used in reforestation programs for the post-fire regeneration of
 169 *P. pinaster* stands devastated by recurrent fires. In particular,
 170 we intend to evaluate the influence of (1) seed provenance and
 171 (2) seed weight on the reproductive performance (i.e. rate and
 172 time of seed germination, and early seedling growth and sur-
 173 vival) of *P. pinaster* after fire. To achieve this aim, we simulate
 174 temperature conditions experienced during a wildfire by ex-
 175 perimentally exposing the seeds from *P. pinaster* populations
 176 with contrasting serotiny levels to a number of heat shocks for
 177 different exposure times (Álvarez et al. 2005; Escudero et al.
 178 1999, 2000; Martínez-Sánchez et al. 1995; Reyes and Casal
 179 2001; Reyes et al. 2015). We expect the seeds obtained from
 180 highly serotinous populations to exhibit enhanced rate of seed
 181 germination at higher thermal shock temperatures (Fernandes
 182 and Rigolot 2007; Goubitz et al. 2003; Tapias et al. 2004). We
 183 also expect heavy seeds to germinate better (Reich et al. 1994;
 184 Reyes and Casal 2001) and produce larger seedlings (Castro
 185 1999; Escudero et al. 2000; Reyes and Casal 2004) than light
 186 ones after exposure to the thermal treatments.

187 2 Materials and methods

188 2.1 Seed material

189 The biological material used in this study consisted of
 190 *P. pinaster* seeds collected from three different provenances
 191 belonging to the Atlantic genetic group (Tapias et al. 2004):
 192 (1) Tabuyo del Monte, henceforth 'Tabuyo', (León, Spain; 42°
 193 16' 35" N/06°11' 52" W, altitude 900 m a.s.l., mean annual
 194 temperature 9.7 °C, annual rainfall 752 mm); (2) Sierra de
 195 Oña, henceforth 'Oña', (Burgos, Spain; 42° 44' 33"
 196 N/03°22' 10" W, 700 m, 10.8 °C, 685 mm); and (3) Meseta
 197 Castellana–Coca, henceforth 'Coca', (Segovia, Spain; 41° 14'
 198 31" N/04° 29' 60" W, 810 m, 11.4 °C, 474 mm). The three
 199 provenances differ in their life histories related to fire adapta-
 200 tion, particularly in the serotiny level (i.e. the percentage of
 201 trees with serotinous cones within the population) (Tapias
 202 et al. 2004): Tabuyo is classified as a highly serotinous pro-
 203 venance (more than 58 % of serotinous trees), Oña as a mod-
 204 erately serotinous provenance (12–20 % of serotinous trees),
 205 and Coca as a very low serotinous provenance (less than 6 %
 206 of serotinous trees).

207 Seeds were provided by the Forest Germplasm Bank of the
 208 National Centre of Forest Genetic Resources (Spanish Minis-
 209 try of Agriculture, Food and Environment). Mature (i.e.
 210 brown-coloured) cones were randomly and extensively har-
 211 vested in 2010 from pre-established stands within each popu-
 212 lation that are used as permanent seed-provisioning sources
 213 for research, conservation, and silvicultural management pur-
 214 poses by the Forest Germplasm Bank (total assigned

harvesting area in each population: Tabuyo, 1779 ha; Oña, 215
 820.7 ha; and Coca, 4187.4 ha; Ministerio de Agricultura, 216
 Alimentación y Medio Ambiente 2015). Since the stands have 217
 been selected by the experts of the National Centre of Forest 218
 Genetic Resources as representative of the genetic material of 219
 each population, we can, therefore, presume that they are as 220
 well equivalent to the studied populations in terms of the se- 221
 rotiny level (Tapias et al. 2004). All harvested cones were 222
 submitted to temperatures surpassing 45 °C by progressive 223
 drying by sun exposure, a procedure generally used to melt 224
 the resin seal that closes the scales and release the seeds with- 225
 out damage. Immediately after cone opening, the seeds were 226
 carefully extracted manually. Since both types of *P. pinaster* 227
 cones open below the attained temperatures (Tapias et al. 228
 2001), the seed material obtained from each population may 229
 thus consist of a mixture of seeds released from non- 230
 serotinous and serotinous cones. This procedure for opening 231
 the vast majority of harvested cones and removing the seeds is 232
 assumed not to interfere with the results of the subsequent 233
 thermal shock treatments (see e.g. Habrouk et al. 1999; Moya 234
 et al. 2013; Reyes and Casal 2002; Salvatore et al. 2010). The 235
 released seeds were stored until their acquisition, under con- 236
 trolled optimal conditions to impede germination and preserve 237
 viability by the Forest Germplasm Bank. After purchase, we 238
 kept the seeds in open paper bags, which permitted ventila- 239
 tion, at laboratory temperature in a dry place until they were 240
 used. 241

242 Prior to the heat treatments, we characterised seed mass by
 243 weighting individually each of the seeds that were used in the
 244 experiment (i.e. 700 seeds per provenance, 2100 seeds in to-
 245 tal). We then randomly divided the seeds in 105 groups of 20
 246 seeds (i.e. 35 groups per provenance) and calculated the aver-
 247 age seed weight value for each group or replicate for statistical
 248 analyses. The groups of seeds were randomly assigned to the
 249 thermal shock treatments.

250 Also, seed viability assays on 100 additional seeds per
 251 provenance (i.e. five replicates of 20 seeds) were performed
 252 using the tetrazolium test according to standard procedures
 253 (Besnier Romero 1989). The seeds were sectioned to expose
 254 the embryo, placed in five Petri dishes (i.e. 20 seeds per dish),
 255 and submerged in 1 % solution of tetrazolium. After 24 h,
 256 seeds whose embryos had stained red were classified as via-
 257 ble. The percentage of seed viability was determined for each
 258 of the five replicates of 20 seeds per provenance.

259 2.2 Thermal shock treatments

260 In 2012, a total of 600 seeds per *P. pinaster* provenance (i.e. 30
 261 groups of 20 seeds per provenance) were submitted to differ-
 262 ent thermal shocks and another 100 seeds (i.e. five groups of
 263 20 seeds per provenance) were used as a control. Seeds were
 264 subjected to six combinations of temperature (100, 150 and
 265 200 °C) and exposure time (1 and 5 min), using a dry air oven.

266 These combinations were selected after Trabaud (1979) and
 267 Moya et al. (2008), to simulate heat conditions acting in the
 268 course of a stand-replacement fire (that affects both the surface
 269 and the crown layer) on free seeds released from non-
 270 serotinous cones at the ground surface and on seeds enclosed
 271 in serotinous cones at the canopy level (see also Moya et al.
 272 2013; Reyes et al. 2015). According to Trabaud (1979), tem-
 273 peratures reached at the soil surface during a natural fire can
 274 vary between 60 and 150 °C for a short period of time (be-
 275 tween 1 and 15 min). According to Moya et al. (2008), tem-
 276 peratures reached at seed location inside serotinous cones of
 277 *P. halepensis* Mill within the canopy layer can raise up to
 278 70 °C during a few seconds after 3 min of fire exposition.
 279 The seeds from all provenances were submitted to the thermal
 280 treatments at a time, in pre-heated ceramic capsules containing
 281 20 seeds each (i.e. 5 ceramic capsules per provenance, 15
 282 ceramic capsules per treatment in total). There were, therefore,
 283 five replicates of 20 seeds per thermal shock and control treat-
 284 ment in each provenance (i.e. sample size=5 replicates×7
 285 treatments×3 provenances=105).

286 Immediately after the thermal shocks, each group of 20
 287 seeds per heat shock and provenance was sown in an 8.5-
 288 cm-diameter Petri dish on four sheets of filter paper satu-
 289 rated with demineralised water to avoid desiccation (105
 290 dishes in total). The dishes were placed in a controlled
 291 environment cabinet at a temperature of 20±1 °C with
 292 photoperiods of 15-h light/9-h dark, watered twice per
 293 week, and examined once per week to determine the rate
 294 and time of seed germination. The use of a temperature of
 295 20 °C is comparable to other germination studies in which
 296 temperatures varied between 20 and 23 °C (Trabaud and
 297 Oustric 1989). A seed was considered to have germinated
 298 when the radicle could be seen with the naked eye (Côme
 299 1970). Seeds were inspected for germination for 63 days in
 300 total, using the method proposed by Martínez-Sánchez
 301 et al. (1995), according to which the experiment ends after
 302 approximately 10 days without germination. We calculated
 303 the percentage of seed germination and the average seed
 304 germination time after the thermal treatments for each of
 305 the five replicates of 20 seeds per treatment in each prov-
 306 enance. The average germination time (t_m) was calculated
 307 using the following formula (Côme 1970):

$$t_m = \frac{N_1 T_1 + N_2 \dots + N_n T_n}{N_1 + N_2 \dots + N_n},$$

308 where N_1 is the number of seeds that have germinated
 309 during time T_1 , N_2 is the number of seeds that have germi-
 310 nated between time T_1 and time T_2 , and so on.

312 As soon as a seed had germinated, it was individually
 313 transplanted into a plastic pot (7.5×7.5×8 cm), placed in a
 314 controlled temperature area, and watered once per week
 315 with demineralised water. Due to mortality risks, and to

ensure that a minimum of five seedling replicates per treat- 316
 317 ment and provenance combination would survive until the
 318 end of the growing period, the first 12 germinated seeds
 319 from each thermal treatment (except 200 °C during 5 min,
 320 after which insufficient seed germination occurred) and
 321 provenance were transplanted (i.e. 216 seedlings in total).
 322 Five weeks after the germination of each individual seed,
 323 we measured the length of both the aerial part of the stem
 324 and the subterranean root of every seedling that had sur-
 325 vived using a digital calliper. We determined the total
 326 length of every seedling by summing up the aerial and
 327 subterranean length measurements, and we randomly se-
 328 lected five total seedling length values per thermal treat-
 329 ment in each provenance for statistical analyses.

2.3 Statistical analysis 330

331 We first analysed the differences in seed viability and
 332 weight among the three *P. pinaster* provenances (Coca,
 333 Oña, and Tabuyo) by performing a generalised linear mod-
 334 el (GLM) and a linear model (LM), respectively. We
 335 modelled seed viability data following a quasi-Poisson er-
 336 ror distribution, using the logit link function, and seed
 337 weight data following a Gaussian error distribution using
 338 the identity link function.

339 We also used GLMs and LMs to test for differences in seed
 340 germination rate, seed germination time and seedling length
 341 after the thermal shock treatments. We modelled seed germi-
 342 nation rate data following a quasi-Poisson error distribution to
 343 account for overdispersion, and seed germination time and
 344 seedling length data following a Gaussian error distribution.
 345 The predictor variables in the models were seed weight (i.e.
 346 the average seed weight value for each replicate), seed prov-
 347 enance, thermal shock treatment (i.e. control, 100 °C for
 348 1 min, 100 °C for 5 min, 150 °C for 1 min, 150 °C for
 349 5 min, 200 °C for 1 min, and 200 °C for 5 min), and the
 350 interaction between seed provenance and thermal shock treat-
 351 ment. The average seed weight value was included as the first
 352 predictor variable in the models to account for inter-
 353 provenance variability in seed weight (see below) and test
 354 whether seed provenance still affects seed tolerance to heat
 355 shocks after removing the main effect of seed weight. The
 356 predicted values of seed germination rate after the different
 357 thermal shock treatments were obtained from the GLMs for
 358 the observed range of seed weight values in each provenance.
 359 The predicted values of seed germination time and total seed-
 360 ling length after the different thermal shock treatments were
 361 obtained from the LMs for the mean seed weight of all three
 362 provenances.

363 All data analyses were carried out with R software, version
 364 3.1.1 (R Core Team 2014) using the ‘stats’ and ‘MASS’
 365 (Venables and Ripley 2002) packages.

366 **3 Results**

367 **3.1 Seed viability and weight**

368 We found no significant differences ($\chi^2=0.29, P=0.867$) in
 369 seed viability among the three *P. pinaster* provenances. Al-
 370 most all seed viability values were higher than 95 % indicating
 371 that nearly all the seeds subjected to the thermal shock treat-
 372 ments were capable of germinating. There were significant
 373 differences ($F=47.34, P<0.001$) in seed weight among prov-
 374 enances, with the highest values measured in Coca and the
 375 lowest in Tabuyo (Fig. 1).

376 **3.2 Seed germination rate**

377 The percentage of seed germination after the thermal shocks
 378 was significantly affected by seed weight, seed provenance,
 379 and the type of thermal shock treatment applied (Table 1). The
 380 significant effect of provenance after removal of the main
 381 effect of seed weight indicates that there may be relevant fea-
 382 tures related to the origin of seed populations other than dif-
 383 ferences in seed weight that are very much affecting seed
 384 germination success. The effect of the thermal shocks on the
 385 rate of seed germination differed among provenances (i.e. sig-
 386 nificant interaction term). In general, the rate of seed germi-
 387 nation increased with increasing seed weight in all three prov-
 388 enances (Fig. 2). Also, the provenance with the heaviest seeds
 389 (Coca) showed the highest seed germination rates after all the
 390 thermal treatments, while the provenance with the lightest
 391 seeds (Tabuyo) showed the lowest. The percentage of seed
 392 germination decreased consistently across all three prov-
 393 enances after subjecting the seeds to the heat treatments of
 394 100, 150 and 200 °C for 5 min. Moreover, no seed germina-
 395 tion was detected in Oña after the heat treatment of 200 °C for
 396 5 min. Compared to the control, the percentage of seed germi-
 397 nation increased only after the heat treatments of 100 and
 398 150 °C for 1 min in Coca and Tabuyo, and 200 °C for 1 min in
 399 Coca.

400 **3.3 Seed germination time**

401 The average seed germination time after the thermal shock
 402 treatments was significantly affected by seed provenance
 403 and the type of thermal shock treatment applied, but not by
 404 seed weight (Table 2). Other features related to seed prov-
 405 enance besides differences in seed weight are, therefore, very
 406 likely affecting the time at which the seeds germinate. We also
 407 found a significant interaction between seed provenance and
 408 thermal treatment. The lowest germination time was found in
 409 Coca (except after the heat treatment of 200 °C for 5 min),
 410 where the predictions of seed germination time ranged be-
 411 tween 14 and 20 days (Fig. 3). Compared to the control, the
 412 average germination time consistently increased after all

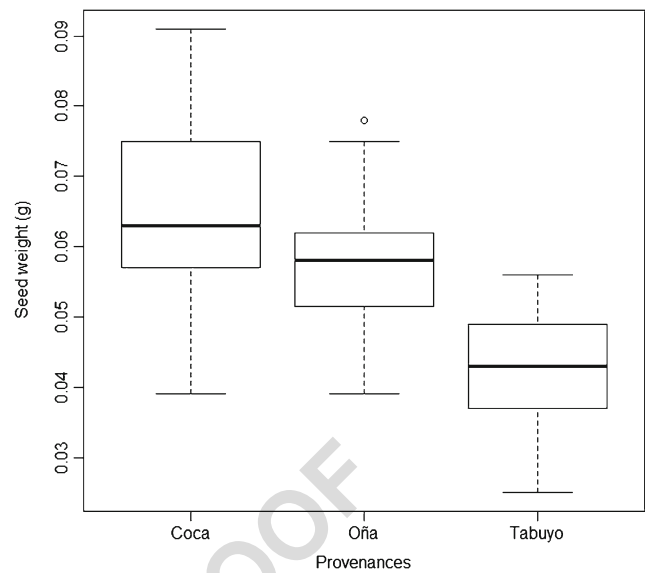


Fig. 1 Boxplot diagram of the seed weight values of *Pinus pinaster* for each provenance (Coca, Oña, and Tabuyo) ($N=35$ weight values \times 3 provenances = 105). Each seed weight value was calculated by averaging the individual weight values of a group of 20 seeds (see text for further details). The horizontal line shows the median seed weight, while the bottom and top of the box show the 25th and 75th percentiles, respectively (i.e. the location of the middle 50 % of the data, also called the first and third quartiles)

thermal treatments (except 200 °C for 5 min) in Oña, where
 the predicted seed germination time ranged between 19 and
 26 days. The greatest variability in seed germination time
 corresponded to Tabuyo (predicted values between 14 and
 32 days), where no clear pattern of response was found in
 relation to the thermal treatments. Under control conditions,
 we detected a negative relationship between germination time
 and seed weight.

3.4 Seedling length

Germinated seeds from the three provenances that had been
 exposed to the heat treatment of 200 °C for 5 min failed to
 grow and survive under laboratory conditions, and, therefore,
 not enough seedlings were obtained from this particular treat-
 ment. Seedling length was significantly affected by seed prov-
 enance, but not by seed weight or by the thermal shock treat-
 ments to which the seeds were subjected (Table 3). Therefore,
 excluding differences in seed weight, other relevant factors
 related to seed provenance are, in all probability, having an
 effect on seedling length. The response of seedling length to
 the different seed thermal treatments showed no general trend
 and varied among seed provenances (i.e. significant interac-
 tion term; Table 3 and Fig. 4). Compared to the control, seed-
 ling length decreased after the thermal treatments in the case
 of Coca, while it primarily increased in the case of Oña. The
 greatest reduction in seedling length was recorded in Tabuyo,
 when submitting the seeds to the heat treatments of 100 and

t1.1 **Table 1** Generalised linear model (GLM) results for the effects of seed weight, seed provenance (i.e. the geographic origin of the maternal seed population), seven different thermal shock treatments, each consisting in a combination of temperature and exposure time (i.e. control, 100 °C for 1 min, 100 °C for 5 min, 150 °C for 1 min, 150 °C for 5 min, 200 °C for 1 min, and 200 °C for 5 min), and the interaction between seed provenance and thermal shock treatment, on the rate of seed germination of *Pinus pinaster*

t1.2	Variable	Df	Deviance	Residual Df	Residual deviance	χ^2 value	P value
t1.3	Null			104	2194		
t1.4	Seed weight	1	23.8	103	2170	9.1	0.003
t1.5	Seed provenance	2	162.4	101	2008	62.4	<0.001
t1.6	Thermal shock treatment	6	1513	95	494.8	581.1	<0.001
t1.7	Seed provenance * thermal shock treatment	12	272.6	83	222.2	104.7	<0.001

Each seed weight value was calculated by averaging the individual weight values of a group of 20 seeds that make up each of the 5 replicates per heat treatment and provenance. $N=5$ replicates \times 7 treatments \times 3 provenances = 105. Df=degrees of freedom

439 150 °C for 5 min. Under control conditions, we detected a
 440 positive relationship between seedling length and seed weight.

441 **4 Discussion**

442 Our results evidenced a clear effect of seed provenance on the
 443 rate and timing of seed germination and on early seedling
 444 growth, thus confirming the relevance of the geographic ori-
 445 gin of the mother trees selected in reforestation programs for
 446 the regeneration of *P. pinaster* after fire. Prior research on the
 447 species showed strong effects of the maternal environment
 448 (i.e. the conditions where the mother tree grows) on seed ger-
 449 mination (Cendán et al. 2013) and seedling performance (Zas
 450 et al. 2013), partly mediated by differences in seed weight
 451 between mother trees, as well as on seedling resistance to
 452 biotic stress (Vivas et al. 2013). Research further indicates that
 453 the origin of seeds sown after a fire eventually determines the
 454 canopy structure and the amount of cone production of a re-
 455 generated stand, as a result of contrasting adaptive traits be-
 456 tween mother tree populations, particularly the serotiny level
 457 (Gil et al. 2009; Vega et al. 2008). In our study, the three
 458 populations exhibiting distinct serotiny levels significantly

differ in their average seed weight and responded dissimi- 459
 larly not just under control conditions but also after the 460
 thermal shocks simulating the effects of fire. The popula- 461
 tion with the highest percentage of trees bearing serotinous 462
 cones had significantly lighter seeds of possibly lower 463
 quality (Álvarez et al. 2007; Bladé and Vallejo 2008) and 464
 underwent poorer natural regeneration under control con- 465
 ditions (i.e. lower rate of seed germination, higher seed 466
 germination time, and lower seedling length). Correspond- 467
 ingly, the population with the lowest percentage of trees 468
 with serotinous cones had significantly heavier seeds that 469
 performed better under control conditions (i.e. higher rate 470
 of seed germination, lower seed germination time, and 471
 higher seedling length). Subjecting the seeds to the thermal 472
 shocks accentuated such differences to a certain degree, 473
 since heavier seeds displayed even greater germination rate 474
 and lower germination time than the lighter ones, but did 475
 not produce seedlings with markedly higher length. As a 476
 consequence, and contrary to our expectations, the popu- 477
 lation with the highest percentage of serotinous trees did 478
 not respond better to the different heat shocks under labo- 479
 ratory conditions than the other two populations with lower 480
 serotiny levels. 481

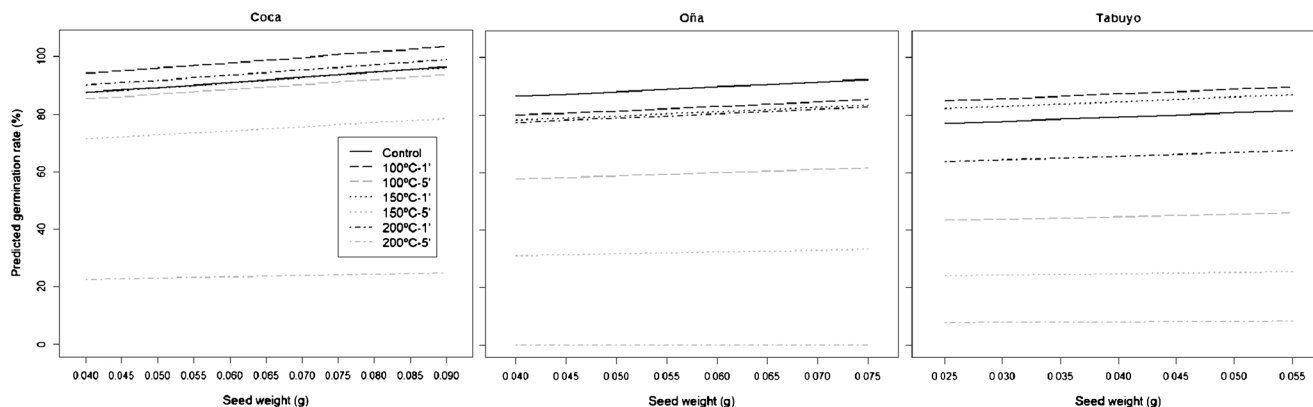


Fig. 2 Mean predicted values of the seed germination rate of *Pinus pinaster* after the different thermal shock treatments in each provenance (Coca, Oña, and Tabuyo). Generalised linear model (GLM) predictions were obtained for the observed range of seed weight values in each provenance

Pine seed provenance affects recruitment after fire

t2.1 **Table 2** Linear model (LM) results for the effects of seed weight, seed provenance (i.e. the geographic origin of the maternal seed population), seven different thermal shock treatments, each consisting in a combination of temperature and exposure time (i.e. control, 100 °C for 1 min, 100 °C for 5 min, 150 °C for 1 min, 150 °C for 5 min, 200 °C for 1 min and 200 °C for 5 min), and the interaction between seed provenance and thermal shock treatment, on the average time of seed germination of *Pinus pinaster*

t2.2	Variable	Df	Sum of squares	Mean squares	F value	P value
t2.3	Seed weight	1	43.7	43.7	2.6	0.110
t2.4	Seed provenance	2	176.3	88.2	5.3	0.007
t2.5	Thermal shock treatment	6	1526	254.4	15.2	<0.001
t2.6	Seed provenance * thermal shock treatment	12	1937	161.4	9.7	<0.001
t2.7	Residuals	83	1389	16.7		

Each seed weight value was calculated by averaging the individual weight values of a group of 20 seeds that make up each of the 5 replicates per heat treatment and provenance. $N=5$ replicates \times 7 treatments \times 3 provenances = 105. Df = degrees of freedom

482 Previous empirical observations have demonstrated the
 483 benefits of yielding bigger seeds under stressful environments
 484 (e.g. Metz et al. 2010; Moles and Westoby 2004; Stock et al.
 485 1990; Violle et al. 2009; Zas et al. 2013). Favouring seeds as
 486 heavy as possible to the detriment of a higher number of light
 487 seeds allows the plant to develop bigger embryos with greater
 488 vitality and amount of reserves (Delgado et al. 2001) and to
 489 give rise to more vigorous seedlings with lower mortality rate
 490 (Castro 1999; Escudero et al. 2000; Moles and Westoby 2004;
 491 Reyes and Casal 2001; but see Moles and Westoby 2006).
 492 Heavy seeds are also thought to exhibit higher resistance to
 493 heat (Álvarez et al. 2007; Delgado et al. 2001; Reyes and
 494 Casal 1995), providing the plant with an advantageous adap-
 495 tive trait against frequent fires (Escudero et al. 1999; Keeley
 496 1977; Reyes and Casal 2001). Yet, our findings do not support
 497 the role of the fire regime as a strong selective pressure for the
 498 evolution of great seed mass, since the bigger seeds derived
 499 from the moderately serotinous and very low serotinous pop-
 500 ulations evolved under less recurrent fires (Tapias et al. 2004),
 501 and the lighter ones from the highly serotinous population
 502 subjected to frequent crown fires (Tapias et al. 2004).

503 Our results are, instead, consistent with the differences in
 504 weight between seeds stored in serotinous and non-serotinous
 505 cones earlier described in other Mediterranean pines (De las
 506 Heras et al. 2012; Salvatore et al. 2010): The population with
 507 the highest number of trees bearing serotinous cones
 508 displayed the lowest average seed weight value, and vice
 509 versa. Even though the seed material in our study may consist
 510 of a mixture of seeds harvested from serotinous and non-
 511 serotinous cones, it seems reasonable to assume that the ma-
 512 jority of seeds harvested in one population derive from the
 513 prevailing type of cone in that population (e.g. seeds collected
 514 from the population with the highest percentage of serotinous
 515 trees would be mostly harvested from serotinous cones). It
 516 might therefore be the case that the poorer response of the
 517 lighter seeds harvested from the population with the highest
 518 serotiny level to the heat shocks under laboratory conditions
 519 was the result of them being protected from high fire-induced
 520 temperatures in the field by serotinous cones. Serotinous

cones favour seed physiological and anatomical resistance to
 fire (Moya et al. 2013; Salvatore et al. 2010), provide effective
 seed mechanical and thermal defence (Fernandes and Rigolot
 2007; Moya et al. 2008), guarantee seed viability in the course
 of a high-severity fire (Reyes and Casal 2002) and promote
 rapid post-fire establishment of seedlings, owing to a massive
 discharge of seeds triggered by heat (De las Heras et al. 2012).
 The seeds enclosed in serotinous cones are released several
 days after a fire, delaying germination until temperature at
 surface level is decreased, and thus benefiting from optimal
 post-burn conditions for germination and seedling establish-
 ment (i.e. high availability of space and nutrients, low compe-
 tition for water and light and low predation risk) (Hernández-
 Serrano et al. 2013; Saracino et al. 1997). Oppositely, the
 seeds stored in non-serotinous cones may be very adapted to
 germinate promptly and grow under less favourable

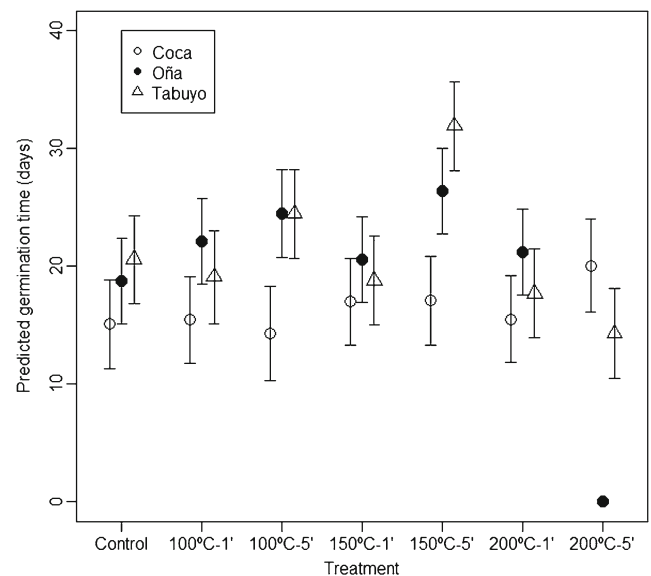


Fig. 3 Predicted values (mean \pm 95 % confidence intervals) of the seed germination time of *Pinus pinaster* after the different thermal shock treatments in each provenance (Coca, Oña, and Tabuyo). Linear model (LM) predictions were obtained for the mean seed weight of all provenances

t3.1 **Table 3** Linear model (LM) results for the effects of seed weight, seed provenance (i.e. the geographic origin of the maternal seed population), six different thermal shock treatments, each consisting in a combination of temperature and exposure time (i.e. control, 100 °C for 1 min, 100 °C

for 5 min, 150 °C for 1 min, 150 °C for 5 min and 200 °C for 1 min), and the interaction between seed provenance and thermal shock treatment, on the total seedling length of *Pinus pinaster*

t3.2	Variable	Df	Sum of squares	Mean squares	F value	P value
t3.3	Seed weight	1	1.6	1.6	0.3	0.578
t3.4	Seed provenance	2	36.9	18.4	3.5	0.035
t3.5	Thermal shock treatment	5	61.0	12.2	2.3	0.052
t3.6	Seed provenance * thermal shock treatment	10	174.0	17.4	3.3	0.001
t3.7	Residuals	71	373.0	5.3		

Each seed weight value was calculated by averaging the individual weight values of a group of 20 seeds that make up each of the 5 replicates per heat treatment and provenance. Germinated seeds that had been exposed to the heat treatment of 200 °C for 5 min failed to grow and survive under laboratory conditions, and, therefore, no seedlings were obtained from this particular treatment to be included in the analyses. $N=5$ replicates \times 6 treatments \times 3 provenances = 90. Df = degrees of freedom

537 conditions (Hernández-Serrano et al. 2013), since these cones
 538 open at relatively low temperatures, easily reached through
 539 summertime in the Mediterranean basin (Tapias et al. 2001).
 540 Where subjected to a crown fire, non-serotinous cones very
 541 likely release the seeds before the arrival of the flame front,
 542 when air temperature rises from ambient to hundreds of de-
 543 grees in a few seconds (Odion and Davis 2000), melting the
 544 resin seal that closes the scales (see Alexander and Cruz
 545 2012). The seeds at the soil surface might then be exposed
 546 to extremely high flame temperatures (Certini 2005), which
 547 could have acted as a major selective force favouring specific
 548 seed resistance mechanisms to heat damage. These seeds
 549 might, too, have compensated the lack of protection in non-
 550 serotinous cones by obtaining greater embryo insulation via
 551 increased seed mass and a thicker seed coat (Escudero et al.
 552 1999; Salvatore et al. 2010). Such adaptations of seeds
 553 enclosed in non-serotinous cones are perhaps responsible for
 554 the better response to the heat shocks of the seeds harvested
 555 from the population with the lowest number of trees bearing
 556 serotinous cones.

557 Under laboratory conditions, seed viability after the thermal
 558 shocks was preserved in all three populations even when tem-
 559 peratures reached 200 °C, except when seeds were submitted
 560 to prolonged times of temperature exposure (i.e. 5 min) that
 561 also generally caused a sharp decrease in germination. These
 562 findings agree with previous results by Álvarez et al. (2005)
 563 and Torres et al. (2006), but somewhat contradicted those by
 564 Escudero et al. (1999) and Martínez-Sánchez et al. (1995).
 565 When translating these results to real wildfire scenarios, how-
 566 ever, the viability of seeds may not be preserved at the surface
 567 level, where both fire intensity and duration very often exceed
 568 lethal values and destroy the majority of free seeds that had
 569 earlier been released from non-serotinous cones (Habrouk
 570 et al. 1999). Seed viability appears, hence, to be safeguarded
 571 solely inside those serotinous cones that remain closed within
 572 the canopy level and are not ignited or scorched by direct flame
 573 contact (Alexander and Cruz 2012; Fernandes and Rigolot

2007; Reyes and Casal 2002). Yet, variation in cone serotiny
 among individual trees of the same population provides
P. pinaster and other Mediterranean pines with a dual strategy
 for ensuring (1) tree recruitment in fire-free periods, and (2)
 fire-heat resistance and post-fire successful regeneration (De
 las Heras et al. 2012; Gil et al. 2009; Goubitz et al. 2004; Moya
 et al. 2008, 2013). Changing the percentage of trees bearing
 serotinous cones in the population could, therefore, result in
 the failure of tree regeneration and the loss of fire resilience,
 leading to important difficulties in forest regrowth in the frame-
 work of future, more severe and frequent fires and more ex-
 treme weather conditions (Espelta et al. 2011; Moriondo et al.
 2006; Pausas 2004). For this reason, the first priority of post-
 fire restoration programs designed to assist natural

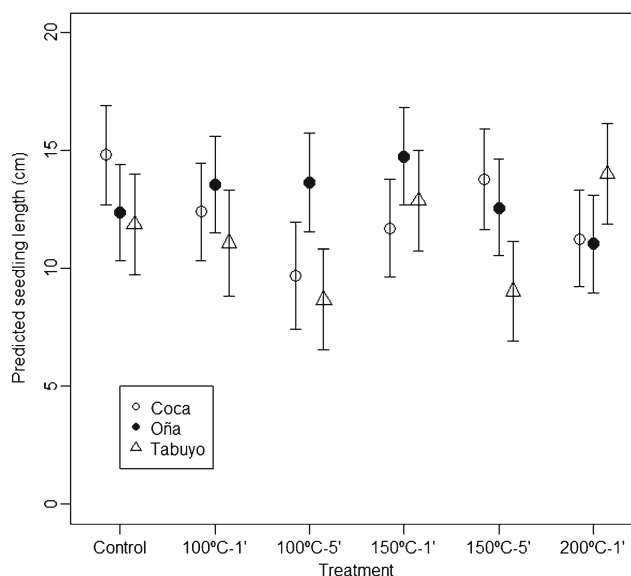


Fig. 4 Predicted values (mean \pm 95 % confidence intervals) of total seedling length of *Pinus pinaster* after the different thermal shock treatments in each provenance (Coca, Oña, and Tabuyo). Linear model (LM) predictions were obtained for the mean seed weight of all provenances

588 regeneration in fire-prone areas should be to preserve pine
 589 stand resistance and resilience to new fires, by selecting the
 590 best fire-adapted populations with the optimal serotiny level as
 591 seed-provisioning sources (De las Heras et al. 2012; Gil et al.
 592 2009; Moya et al. 2013; Pausas 2015). Restoration programs
 593 should further try to maximise natural pine recruitment, by
 594 preferentially harvesting the seeds from mother trees growing
 595 in non-stressful environments within the selected populations
 596 (i.e. mother trees producing heavy seeds with proven superior
 597 germination and seedling growth) (see Espelta et al. 2011; Zas
 598 et al. 2013). To what extent these practices aimed at selecting
 599 the best seed sources in post-fire restoration programs may
 600 allow fire-prone pine ecosystems to cope with new fire regime
 601 scenarios in the Mediterranean basin is still unknown and certainly
 602 deserves future attention (Pausas 2015).

603 **5 Conclusions**

604 Free seeds harvested from *P. pinaster* populations with con-
 605 trasting levels of serotiny responded differently to fire: heavy
 606 seeds from the very low serotinous population had much
 607 greater resistance to the thermal shocks than the lighter ones
 608 from the highly serotinous population. These unexpected find-
 609 ings may be, above all, explained by the distinct reproductive
 610 and fire-adaptive strategies of trees predominantly bearing
 611 non-serotinous and serotinous cones in these populations
 612 (Goubitz et al. 2003). Seeds enclosed in non-serotinous cones
 613 are released annually under warm and dry weather conditions
 614 and eventually display prompt germination to withstand com-
 615 petition. In the event of a fire, the discharge of seeds from non-
 616 serotinous cones is triggered by heat, causing most of these
 617 seeds at the ground surface to end up being killed by the fire
 618 (Habrouk et al. 1999). The seeds retained inside serotinous
 619 cones are otherwise protected from high fire-induced temper-
 620 atures and encounter optimal germination conditions when
 621 massively released several days after fire.

622 Where post-fire reforestation programs are developed to
 623 assist natural regeneration, selecting the best fire-adapted pop-
 624 ulations with the optimal serotiny level as seed-provisioning
 625 sources may be, therefore, crucial for these fire-prone pine
 626 ecosystems to cope with the predicted future, more catastroph-
 627 ic, fire scenarios (Pausas 2015; Pausas et al. 2008).

628 **Acknowledgments** Two anonymous reviewers provided very valuable
 629 comments on the manuscript. The study was financially supported by the
 630 Spanish Ministry of Economy and Competitiveness, and the European
 631 Regional Development Fund (ERDF), in the frame of the GESFIRE
 632 project (AGL2013-48189-C2-1-R); and by the Regional Government of
 633 Castilla and León in the frame of the FIRECYL project (LE033U14).

634 **Compliance with ethical standards**

636 **Funding** The study was financially supported by the Spanish Ministry
 637 of Economy and Competitiveness, and the European Regional

Development Fund (ERDF), in the frame of the GESFIRE project 638
 (AGL2013-48189-C2-1-R); and by the Regional Government of Castilla 639
 and León in the frame of the FIRECYL project (LE033U14). 640

641 **References**

642 Alexander ME, Cruz MG (2012) Modelling the effects of surface and 644
 crown fire behaviour on serotinous cone opening in jack pine and 645
 lodgepole pine forests. *Int J Wildland Fire* 21:709–721. doi:10. 646
 1071/WF11153 647

648 Alía R, Gil L, Pardos JA (1995) Performance of 43 *Pinus pinaster* prov- 648
 enances on 5 locations in Central Spain. *Silvae Genet* 44:75–81 649

650 Álvarez R, Valbuena L, Calvo L (2005) Influence of tree age on seed 650
 germination response to environmental factors and inhibitory sub- 651
 stances in *Pinus pinaster*. *Int J Wildland Fire* 14:277–284. doi:10. 652
 1071/WF04066 653

654 Álvarez R, Valbuena L, Calvo L (2007) Effect of high temperatures on 654
 seed germination and seedling survival in three pine species (*Pinus* 655
pinaster, *P. sylvestris* and *P. nigra*). *Int J Wildland Fire* 16:63–70. 656
 doi:10.1071/WF06001 657

658 Besnier Romero F (1989) Semillas. Biología y tecnología, Mundiprensa, 658
 Madrid 659

660 Bladé C, Vallejo VR (2008) Seed mass effects on performance of *Pinus* 660
halepensis Mill. seedlings sown after fire. *For Ecol Manag* 255: 661
 2362–2372. doi:10.1016/j.foreco.2007.12.039 662

663 Bramlett DL, Dell TR, Pepper WD (1983) Genetic and maternal influ- 663
 ences on Virginia pine seed germination. *Silvae Genet* 32:1–4 664

665 Buccì G, González-Martínez SC, Le Provost G, Plomion C, Ribeiro MM, 665
 Sebastiani F, Alía R, Vendramin GG (2007) Range-wide 666
 phylogeography and gene zones in *Pinus pinaster* Ait. revealed by 667
 chloroplast microsatellite markers. *Mol Ecol* 16:2137–2153. doi:10. 668
 1111/j.1365-294X.2007.03275.x 669

670 Calvo L, Torres O, Valbuena L, Luis E (2013) Recruitment and early 670
 growth of *Pinus pinaster* seedlings over five years after a wildfire 671
 in NW Spain. *Forest Syst* 22:582–586. doi:10.5424/fs/2013223- 672
 04623 673

674 Castro J (1999) Seed mass versus seedling performance in Scots pine: a 674
 maternally dependent trait. *New Phytol* 144:153–161. doi:10.1046/ 675
 j.1469-8137.1999.00495.x 676

677 Cendán C, Sampedro L, Zas R (2013) The maternal environment deter- 677
 mines the timing of germination in *Pinus pinaster*. *Environ Exp Bot* 678
 94:66–72. doi:10.1016/j.envexpbot.2011.11.022 679

680 Certini G (2005) Effects of fire on properties of forest soils: a review. 680
Oecologia 143:1–10. doi:10.1007/s00442-004-1788-8 681

682 Côme D (1970) Les obstacles à la germination. Mason, Paris 682

683 Court-Picon M, Galbin-Henry C, Guibal F, Roux M (2004) Dendrometry 683
 and morphometry of *Pinus pinea* L. in Lower Provenance (France): 684
 adaptability and variability of provenances. *For Ecol Manag* 194: 685
 319–333. doi:10.1016/j.foreco.2004.02.024 686

687 Ministerio de Agricultura, Alimentación y Medio Ambiente (2014) 687
 Incendios forestales en España año 2012. [Retrieved on: 28/09/ 688
 2014, available at: [http://www.magrama.gob.es/es/biodiversidad/ 689
 estadisticas/Incendios_default.aspx](http://www.magrama.gob.es/es/biodiversidad/estadisticas/Incendios_default.aspx)] 690

691 Ministerio de Agricultura, Alimentación y Medio Ambiente (2015). 691
 Catálogo Nacional de Materiales de Base para la producción de 692
 Material Forestal de Reproducción Identificado: *Pinus pinaster* 693
 Ait. [Retrieved on: 13/09/2015, available at: [http://www.magrama. 694
 gob.es/es/desarrollo-rural/temas/politica-forestal/recursos- 695
 geneticos-forestales/identificado_pinus_pinaster.aspx#](http://www.magrama.gob.es/es/desarrollo-rural/temas/politica-forestal/recursos-geneticos-forestales/identificado_pinus_pinaster.aspx#)] 696

697 De las Heras J, Moya D, Vega JA, Daskalidou E, Vallejo R, Grigoriadis 697
 N, Tsitsoni T, Baeza J, Valdecantos A, Fernández C, Espelta J, 698

699 Fernandes P (2012) Post-fire management of serotinous pine forests. In: Moreira F, Arianoutsou M, Corona P, De las Heras J (eds) Post-fire management and restoration of Southern European forests, managing forest ecosystems 24. Springer, Dordrecht, pp 121–150

700 Delgado J, Serrano J, López F, Acosta F (2001) Heat shock, mass dependent germination, and seed yield as related components of fitness of *Cistus ladanifer*. *Environ Exp Bot* 46:11–20. doi:10.1016/S0098-8472(01)00076-4

701 Escudero A, Sanz MV, Pita JM, Pérez-García F (1999) Probability of germination after heat treatment of native Spanish pines. *Ann For Sci* 56:511–520. doi:10.1051/forest:2001137

702 Escudero A, Núñez Y, Pérez-García F (2000) Is fire a selective force of seed size in pine species? *Acta Oecol* 21:245–256. doi:10.1016/S1146-609X(00)01083-3

703 Escudero A, Pérez-García F, Luzuriaga AL (2002) Effects of light, temperature and population variability on the germination of seven Spanish pines. *Seed Sci Res* 12:261–271. doi:10.1079/SSR2002116

704 Espelta JM, Aman X, Rodrigo A (2011) Non-fire induced seed release in a weakly serotinous pine: climatic factors, maintenance costs or both? *Oikos* 120:1752–1760. doi:10.1111/j.1600-0706.2011.19570.x

705 Fernandes PM, Rigolot E (2007) The fire ecology and management of maritime pine (*Pinus pinaster* Ait.). *For Ecol Manag* 241:1–13. doi:10.1016/j.foreco.2007.01.010

706 Ganatsas P, Tsakaldimo M, Thanos C (2008) Seed and cone diversity and seed germination of *Pinus pinea* in Strofilya Site of the Natura 2000 Network. *Biodivers Conserv* 17:2427–2439. doi:10.1016/j.jnc.2009.04.006

707 Ghildiyal SK, Sharma CM, Gairola S (2009) Environmental variation in seed and seedling characteristics of *Pinus roxburghii* Sarg. from Uttarakhand, India. *Appl Ecol Env Res* 7:121–129

708 Gil L, López R, García-Mateos A, González-Doncel I (2009) Seed provenance and fire-related reproductive traits of *Pinus pinaster* in central Spain. *Int J Wildland Fire* 18:1003–1009. doi:10.1071/WF08101

709 Goubitz S, Werger MJA, Ne’eman G (2003) Germination response to fire-related factors of seeds from non-serotinous and serotinous cones. *Plant Ecol* 169:195–204. doi:10.1023/A:1026036332277

710 Goubitz S, Nathan R, Roitemberg R, Shmida A, Ne’eman G (2004) Canopy seed bank structure in relation to: fire, tree size and density. *Plant Ecol* 173:191–201. doi:10.1023/B:VEGE.0000029324.40801.74

711 Habrouk A, Retana J, Espelta JM (1999) Role of heat tolerance and cone protection of seeds in the response of three pine species to wildfires. *Plant Ecol* 145:91–99. doi:10.1023/A:1009851614885

712 Hanley M, Unna J, Darvill B (2003) Seed size and germination response: a relationship for fire-following plant species exposed to thermal shock. *Oecologia* 134:18–22. doi:10.1007/s00442-002-1094-2

713 Hernández-Serrano A, Verdú M, González-Martínez SC, Pausas JG (2013) Fire structures pine serotiny at different scales. *Am J Bot* 100:2349–2356. doi:10.3732/ajb.1300182

714 Hernández-Serrano A, Verdú M, Santos-del-Blanco L, Climent J, González-Martínez SC, Pausas JG (2014) Heritability and quantitative genetic divergence of serotiny, a fire persistence plant trait. *Ann Bot* 114:571–577. doi:10.1093/aob/mcu142

715 Keeley J (1977) Seed production, seed population in soil, and seedling production after fire for two congeneric pairs of sprouting and non-sprouting chaparral shrubs. *Ecology* 58:820–829. doi:10.2307/1936217

716 Liu J, Bai Y, Lamb EG, Simpson D, Liu G, Wei Y, Wang D, McKenney DW, Papadopol P (2013) Patterns of cross-continental variation in tree seed mass in the Canadian boreal forest. *PLoS ONE* 8, e61060. doi:10.1371/journal.pone.0061060

717 Madrigal J, Hernando C, Martínez E, Guijarro M, Díez C (2005) Regeneración post-incendio de *Pinus pinaster* Ait. en la Sierra de Guadarrama (Sistema Central, España): modelos descriptivos de los factores influyentes en la densidad inicial y la supervivencia. *Invest Agrar: Sist Recur For* 14:36–51

718 Madrigal J, Hernando C, Guijarro M (2011) El papel de la regeneración natural en la restauración tras grandes incendios forestales: el caso del pino negral. *Boletín del CIDEU* 10:5–22. ISSN 1885–5237

719 Martínez-Sánchez JJ, Marín A, Herranz JM, Ferrandis P, Heras J (1995) Effects of high temperatures on germination of *Pinus halepensis* Mill. and *Pinus pinaster* Aiton subsp. *pinaster* seeds in southeast Spain. *Vegetatio* 116:69–72. doi:10.1007/BF00045279

720 Metz J, Liancourt P, Kigel J, Harel D, Sternberg M, Tielboerger K (2010) Plant survival in relation to seed size along environmental gradients: a long-term study from semi-arid and Mediterranean annual plant communities. *J Ecol* 98:697–704. doi:10.1111/j.1365-2745.2010.01652.x

721 Moles AT, Westoby M (2004) Seedling survival and seed size: a synthesis of the literature. *J Ecol* 92:372–383. doi:10.1111/j.0022-0477.2004.00884.x

722 Moles AT, Westoby M (2006) Seed size and plant strategy across the whole life cycle. *Oikos* 113:91–105. doi:10.1111/j.0030-1299.2006.14194.x

723 Moriondo M, Good P, Durao R, Bindi M, Giannakopoulos C, Corte-Real J (2006) Potential impact of climate change on fire risk in the Mediterranean area. *Clim Res* 31:85–95. doi:10.3354/cr031085

724 Moya D, Saracino A, Salvatore R, Lovreglio R, De las Heras J, Leone V (2008) Anatomic basis and insulation of serotinous cones in *Pinus halepensis* Mill. *Trees* 22:511–519. doi:10.1071/BT10193

725 Moya D, De las Heras J, Salvatore R, Valero E, Leone V (2013) Fire intensity and serotiny: response of germination and enzymatic activity in seeds of *Pinus halepensis* Mill. from southern Italy. *Ann For Sci* 70:49–59. doi:10.1007/s13595-012-0236-x

726 Odion DC, Davis FW (2000) Fire, soil heating, and the formation of vegetation patterns in chaparral. *Ecol Monogr* 70:149–169. doi:10.1890/0012-9615(2000)070[0149:FSHATF]2.0.CO;2

727 Parker WC, Noland TL, Morneau AE (2006) The effects of seed mass on germination, seedling emergence, and early seedling growth of eastern white pine (*Pinus strobus* L.). *New For* 32:33–49. doi:10.1007/s11056-005-3391-1

728 Pausas JG (2004) Changes in fire and climate in the eastern Iberian Peninsula (Mediterranean basin). *Clim Chang* 63:337–350. doi:10.1023/B:CLIM.0000018508.94901.9c

729 Pausas JG (2015) Evolutionary fire ecology: lessons learned from pines. *Trends Plant Sci*. doi:10.1016/j.tplants.2015.03.001

730 Pausas JG, Bradstock RA, Keith DA, Keeley JE, The GCTE (Global Change of Terrestrial Ecosystems) Fire Network (2004) Plant functional traits in relation to fire in crown-fire ecosystems. *Ecology* 85:1085–1100. doi:10.1890/02-4094

731 Pausas JG, Llovet J, Rodrigo A, Vallejo R (2008) Are wildfires a disaster in the Mediterranean basin? a review. *Int J Wildland Fire* 17:713–723. doi:10.1071/WF07151

732 R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, URL <http://www.R-project.org/>

733 Reich PB, Oleksyn J, Tjoelker MG (1994) Seed mass effects on germination and growth of diverse European Scots pine populations. *Can J For Res* 24:306–320. doi:10.1139/x94-044

734 Reyes O, Casal M (1995) Germination behaviour of three species of the genus *Pinus* in relation to high temperatures suffered during forest fires. *Ann For Sci* 52:385–392. doi:10.1051/forest:2001137

735 Reyes O, Casal M (2001) The influence of seed age on germinative response to the effects of fire in *Pinus pinaster*, *Pinus radiata* and *Eucalyptus globulus*. *Ann For Sci* 58:439–447. doi:10.1051/forest:19950408

736 Reyes O, Casal M (2002) Effect of high temperatures on cone opening and on the release and viability of *Pinus pinaster* and *P. radiata* seeds in NW Spain. *Ann For Sci* 59:327–334. doi:10.1051/forest:2001137

Pine seed provenance affects recruitment after fire

831 Reyes O, Casal M (2004) Effects of forest fire ash on germination and
 832 early growth of four *Pinus* species. *Plant Ecol* 175:81–89. doi:10.
 833 1023/B:VEGE.0000048089.25497.0c 864

834 Reyes O, García-Duro J, Salgado J (2015) Fire affects soil organic matter
 835 and the emergence of *Pinus radiata* seedlings. *Ann For Sci* 72:267–
 836 275. doi:10.1007/s13595-014-0427-8 865

837 Rodrigo A, Retana J, Picó FX (2004) Direct regeneration is not the only
 838 response of Mediterranean forests to large fires. *Ecology* 85:716–
 839 729. doi:10.1890/02-0492 866

840 Ruano I, Del Peso C, Bravo F (2015) Post-dispersal predation of *Pinus*
 841 *pinaster* Aiton seeds: key factors and effects on belowground seed
 842 bank. *Eur J For Res* 134:309–318. doi:10.1007/s10342-014-0853-z 867

843 Salvatore R, Moya D, Pulido L, Lovreglio R, López-Serrano FR, De las
 844 Heras J, Leone V (2010) Morphological and anatomical differences
 845 in Aleppo pine seeds from serotinous and non-serotinous cones.
 846 *New For* 39:329–341. doi:10.1007/s11056-009-9174-3 868

847 Saracino A, Pacella R, Leone V, Borghetti M (1997) Seed dispersal and
 848 changing seed characteristics in a *Pinus halepensis* Mill. forest after
 849 fire. *Plant Ecol* 130:13–19. doi:10.1023/A:1009765129920 869

850 Stock WD, Pate JS, Delfs J (1990) Influence of seed size and quality on
 851 seedling development under low nutrient conditions in five
 852 Australian and South African members of the Proteaceae. *J Ecol*
 853 78:1005–1020. doi:10.2307/2260949 870

854 Tapias R, Gil L, Fuentes-Utrilla P, Pardos JA (2001) Canopy seed banks
 855 in Mediterranean pines of southeastern Spain: a comparison between
 856 *Pinus halepensis* Mill., *Pinus pinaster* Ait., *Pinus nigra*
 857 *Am.* and *Pinus pinea* L. *J Ecol* 89:629–638. doi:10.1046/j.1365-
 858 2745.2001.00575.x 871

859 Tapias R, Climent J, Pardos JA, Gil L (2004) Life histories of
 860 Mediterranean pines. *Plant Ecol* 171:53–68. doi:10.1023/B:VEGE.
 861 0000029383.72609.f0 872

862 Torres O, Calvo L, Valbuena L (2006) Influence of high temperatures on
 863 seed germination of a special *Pinus pinaster* stand adapted to
 frequent fires. *Plant Ecol* 186:129–136. doi:10.1007/s11253-006-
 9117.4 873

Trabaud L (1979) Etude du comportement du feu dans la garrigue de
 chene kermes a partir des temperatures et des vitesses de propaga-
 tion. *Ann Sci For* 36:13–38. doi:10.1051/forest/19790102 874

Trabaud L, Oustric J (1989) Influence du feu sur la germination des
 semences de quatre espèces ligneuses méditerranéennes à reproduc-
 tion sexuée obligatoire. *Seed Sci Technol* 17:589–599 875

Vasques A, Maia P, Pedro M, Santos C, Vallejo VR, Keizer JJ (2012)
 Germination in five shrub species of Maritime Pine understory-does
 seed provenance matter? *Ann For Sci* 69:499–507. doi:10.1007/
 s13595-012-0206-3 876

Vega JA, Fernández C, Pérez-Gorostiaga P, Fonturbel T (2008) The
 influence of fire severity, serotiny, and post-fire management on
Pinus pinaster Ait. recruitment in three burnt areas in Galicia
 (NW Spain). *For Ecol Manag* 256:1596–1603. doi:10.1016/j.
 foreco.2008.07.005 877

Venables WN, Ripley BD (2002) Modern applied statistics with S, 4th
 edn. Springer, New York 878

Violle C, Castro H, Richarte J, Navas ML (2009) Intraspecific seed trait
 variations and competition: passive or adaptive response? *Funct*
Ecol 23:612–620. doi:10.1111/j.1365-2435.2009.01539.x 879

Vivas M, Zas R, Sampedro L, Solla A (2013) Environmental maternal
 effects mediate the resistance of Maritime pine to biotic stress. *PLoS*
ONE 8, e70148. doi:10.1371/journal.pone.0070148 880

Zas R, Sampedro L (2015) Heritability of seed weight in Maritime pine, a
 relevant trait in the transmission of environmental maternal effects.
Heredity 114:116–124. doi:10.1038/hdy.2014.76 881

Zas R, Cendán C, Sampedro L (2013) Mediation of seed provisioning in
 the transmission of environmental maternal effects in Maritime pine
 (*Pinus pinaster* Aiton). *Heredity* 111:248–255. doi:10.1038/hdy.
 2013.44 882

UNCORRECTED

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

- Q1. Springer offers the possibility to send copies of your article to colleagues. Please supply up to 10 e-mail addresses of colleagues, and we will send a PDF copy of your paper to each of **them.**
- Q2. Please check author name(s) and affiliation(s) if captured and presented **correctly.**

UNCORRECTED PROOF