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**1 Among-population variation and plasticity to drought of Atlantic,
2 Mediterranean and interprovenance hybrid populations of Maritime pine**

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4 Short title: Variation and plasticity to drought in maritime pine

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15 **Autos contribution:** RM, EM and RZ conceived the paper; EM performed and
16 coordinated hybridizations; RM and EM coordinated vegetative propagation,
17 plant development and treatment application; RM coordinated assessments,
18 processed the data and performed the statistical analysis; EM and RZ achieved the
19 funds; RM coordinated the writing of the manuscript; EM and RZ helped in
20 writing by subsequent revisions.

21

22 **3 Tables + 4 Figures + 3 Electronic Supplementary Materials**

23

24 **N° characters:** 53,740

25 Abstract

26 Maritime pine grows naturally under a wide range of climatic conditions, from
27 strongly Atlantic to strongly Mediterranean. Aiming to improve our understanding
28 of the genetic structure and inheritance of drought resistance strategies in the
29 species, we conducted an environmentally controlled experiment to assess the
30 genetic variation and plasticity to drought of Atlantic and Mediterranean
31 populations, and the interprovenance hybrids between them. Hybridization could
32 also help to provide new genetic material for use in transitional areas between the
33 two regions, for which reproductive materials of good quality are generally
34 lacking. Plastic responses to water stress appeared to be highly conserved among
35 populations, with a common conservative isohydric strategy based on promoting
36 growth when water was abundant, and stopping it when water became limiting.
37 We found, however, a strong intraspecific variation in biomass allocation patterns.
38 The Atlantic populations showed a risky growth-based strategy with a larger
39 amount of juvenile needles, whereas Mediterranean populations showed a more
40 conservative strategy, minimizing aerial growth and increasing the proportion of
41 adult needles that are more resistant to water loss. Hybrid populations performed
42 more similarly to the Mediterranean parent, suggesting a dominance of the
43 Mediterranean-like characteristics. Some of the tested hybrid populations,
44 however, combined high growth with traits of drought adaptation, and thus
45 represent potentially interesting materials for use in transitional regions between
46 the two climate zones.

47

48 **Keywords:** Phenotypic plasticity; drought stress; *Pinus pinaster*; interprovenance
49 hybrids; biomass allocation; optimal partitioning theory

501. Introduction

51 Water availability is one of the most important limiting resources for recruitment
52 in Mediterranean forest tree species (Pigott and Pigott 1993), especially at early
53 growth stages when the small root system of juvenile plants makes them highly
54 vulnerable to water deficit. The seedling stage is, in fact, the most critical step of
55 the tree's life cycle and when most relevant selection pressures occur (Leck et al.
56 2008). Consequently, adaptive mechanisms for drought resistance in juvenile trees
57 are expected to underlie the geographic distribution of Mediterranean populations
58 along natural gradients of water availability (Florian et al. 2013). The
59 physiological mechanisms that enable plants to face water stress are assembled
60 around three main strategies: drought evasion (shifts in phenology so that major
61 periods of physiological activity do not coincide with periods of water-deficit),
62 desiccation tolerance (mechanisms that minimize or remove the damage caused
63 by water stress, such as osmotic adjustment and antioxidant or repairing systems)
64 and desiccation avoidance (mechanisms to prevent or minimize water stress in the
65 tissues, either by maximizing water capture or minimizing water loss) (Larcher
66 1995). Biomass allocation patterns are key to this last strategy and can be
67 especially relevant in juvenile trees (Leck et al. 2008). Allocation patterns largely
68 determine the ability of plants to capture primary resources and tolerate abiotic
69 stress, but also to compete with neighbours and, ultimately, to survive and
70 reproduce (Poorter et al. 2012). Specifically, high investment in roots and low
71 investment in leaves is expected to confer drought resistance by maximizing water
72 capture and minimizing water losses through transpiration (Ludlow 1989).

73Accordingly, several studies on pine trees have found higher root investment (e.g.
74Nguyen and Lamant 1989) and lower specific leaf area (e.g. Fernández et al.
751999) in populations from the driest sites.

76 Maritime pine (*Pinus pinaster* Ait.) is an important forest tree species of
77the Western Mediterranean basin, with many small isolated populations highly
78differentiated in various phenotypic traits with a likely adaptive value (González-
79Martínez et al. 2004). Maritime pine is considered a drought-avoiding species
80with a high stomatal sensitivity to dry conditions (Fernández et al. 2000), but it
81also shows drought tolerance mechanisms such as osmotic adjustment (Nguyen-
82Queyrens et al. 2002) and changes in biomass partitioning (Aranda et al. 2010;
83Sánchez-Gómez et al. 2010). Naturally occurring in a wide range of
84environmental conditions, inter-population variation has been observed for several
85traits related to drought resistance, such as biomass allocation (Aranda et al. 2010;
86Chambel et al. 2007), vulnerability to cavitation (Corcuera et al. 2011), hydraulic
87architecture (Corcuera et al. 2012), water use efficiency (Corcuera et al. 2010),
88and osmotic adjustment (Nguyen and Lamant 1989; Nguyen-Queyrens et al.
892002). As well as the strong differentiation between populations, large individual
90variation within populations in terms of resistance to water stress has also been
91observed (Aranda et al. 2010; Correia et al. 2008; Gaspar et al. 2013; but see
92Lamy et al. 2014), suggesting that the costs associated with these resistance
93mechanisms, interactions with other environmental factors and/or high levels of
94gene flow prevents their fixation (White et al. 2007). On the other hand, this intra-
95population variability allows populations to evolutionarily respond to

96environmental pressures such as extreme drought events (Florian et al. 2013). A
97high phenotypic plasticity to drought also characterizes this species, with
98significant changes in stomatal transpiration, osmotic adjustment and even
99biomass reallocation in response to water deficit (Chambel et al. 2007; Fernández
100et al. 1999, 2000; Nguyen-Queyrens et al. 2002).

101 Along its natural distribution range, Maritime pine grows under a wide
102range of climatic conditions, from genuinely Mediterranean areas (Central and
103South Spain, South France and North Africa) with severe and prolonged summer
104drought stress, to Atlantic humid climates (coastal areas of Portugal, Spain and
105France) with no or little water deficit over the year. Although several breeding
106programs have been launched in both areas, they have only successfully
107progressed in the Atlantic areas. In many Mediterranean areas, and especially in
108transitional regions, Maritime pine could potentially attain high productivity rates
109but good quality local reproductive material is lacking (de la Mata and Zas 2010a,
1102010b). Looking for alternative materials that perform well in these areas has
111become a primary objective. On the other hand, local climate models predict an
112increase in mean temperature coupled with important changes in rain distribution
113over the course of the year with a strong intensification of water stress (IPCC
1142007). Improving our understanding of how *P. pinaster* populations face water
115stress would help to predict the adaptation and resilience of the Atlantic stands to
116this climate change.

117 Interprovenance hybridization has been used in several forest tree species
118as a tool for improving traits of productive interest by taking advantage of hybrid

119vigor or by combining specific desirable characteristics (Bolstad et al. 1990) .
120While the gains obtained by heterosis have rarely been promising, most studies
121with conifer hybrids have shown an intermediate performance between their
122parents (Eriksson and Ilstedt 1986; Kaya and Lindgren 1992). Accordingly,
123interprovenance hybridization has successfully combined the high growth rates of
124the Atlantic *P. pinaster* Landes population with the good stem forms and the high
125resistance to the insect *Matsucoccus feytaudi* Duc. typical of the Corsican origin
126(Harfouche et al. 1995; Harfouche and Kremer 2000). Exploring water stress
127resistance patterns in hybrid populations between high growth rate Atlantic
128populations and drought-tolerant Mediterranean populations will not only help to
129find new genetic material sources that combine desired properties, but also to
130improve our understanding of the genetic structure and inheritance of drought
131resistance strategies.

132 In this study we assessed the genetic variation and the plasticity to drought
133of Atlantic and Mediterranean *P. pinaster* populations and the interprovenance
134hybrids between them. We conducted a greenhouse controlled experiment with
135two experimental treatments of water availability and we assessed survival,
136growth and biomass allocation among different plant functional tissues as
137indicators of drought resistance. Specifically, we aimed i) to quantify the
138intraspecific variation in functional traits related to water stress resistance, ii) to
139determine the extent to which plasticity patterns to water stress varied among
140populations, iii) to explore whether interprovenance hybrids between Atlantic and
141Mediterranean origins exhibited an intermediate performance between that of

142their parents and iv) to select hybrid materials that combine the high growth
143potential of Atlantic origins with the drought tolerance of Mediterranean
144populations, which may represent a suitable material for planting in transitional
145regions between both climates.

146

1472. **Materials and methods**

1482.1 **Genetic material**

149The genetic material studied consisted of 12 *Pinus pinaster* populations, five of
150which were pure provenances and seven of which were interprovenance hybrids
151between them (see Online Resource 1). The five pure populations included one of
152pure Atlantic origin (from the coastal area of Galicia, NW Spain), one from the
153Mediterranean region (from Montaña de Soria de Burgos, Central Spain), and
154three populations selected from an Atlantic area with a slight Mediterranean
155influence (from an inland area of Galicia). The NW Atlantic population was a
156representation of the Coastal-Galicia breeding population (CG), and was collected
157from a first generation clonal seed orchard located in Sergude (A Coruña, Spain).
158Similarly, the Montaña de Soria Burgos population (SB) was collected from a first
159generation clonal seed orchard of this provenance located in Valsaín (Segovia,
160Spain). The three inland Galicia populations correspond to three selected stands of
161the Northwest Interior provenance region, located in Carballiño (IN1; Ourense,
162Spain), Guitiriz (IN2; Lugo, Spain) and Sober (IN3; Lugo, Spain). From each of
163these populations, three genotypes were selected based on their genotypic and/or

164phenotypic superiority (growth, straightness and branch habit), and open-
165pollinated seeds and pollen were collected.

166 In addition to the five open pollinated populations, we obtained seven
167interprovenance hybrid populations by means of controlled pollinations carried
168out in 2003 within the two aforementioned seed orchards (Table 1). In every case,
169pollinations were performed on the same individuals from which open-pollinated
170seeds had also been collected. In the Valsaín seed orchard (SB), the three
171genotypes were pollinated with pollen mixtures from the selected genotypes of the
172four Atlantic populations (CG, IN1, IN2 and IN3). In the Sergude seed orchard,
173the three selected genotypes were pollinated with pollen mixtures from the three
174inland Galicia populations (but only pollinations with IN1 were successful) and
175also from two extra French provenances: Landes (L), an Atlantic origin with good
176resistance to drought (Corcuera et al. 2010) and Corsica, a genuine Mediterranean
177provenance (see Online Resource 1). The pollen from the Landes population was
178collected from a random selection of five trees within a plantation of this origin
179located in La Garganta (Ciudad Real, Spain; 38° 29' N 4° 27' W). For the Corsica
180population, pollen was collected from a selection of five trees in a *P. pinaster*
181provenance trial planted in 1967 and located in Espinoso del Rey (Toledo, Spain;
18239° 36' N 4° 49' W).

183 The genetic material used in this study was part of a larger long-term
184experiment involving other trials under controlled and field conditions. Because
185available seeds were insufficient to meet all our needs for plant material, we

186clonally replicated each individual by vegetative propagation using mini-cuttings
187(see Online Resource 2 for details).

188

189**2.2. Experimental design, treatments and assessed variables**

190The study was conducted in a greenhouse of the Centro de Investigación Forestal
191de Lourizán (Xunta de Galicia, Pontevedra, Spain; 42° 24' N 8° 40' W; 50 m
192a.s.l.) under natural light and temperatures ranging from 12 to 30 °C. The final
193containers were individual 650 cm³ pots situated on trays with 6 × 3 pots per tray.
194Each pot was filled with 840 g of washed and sieved river granitic sand, mixed
195with 1 g of gradual release fertilizer (Multicote 8, NPK 7:15:15 + 2MgO +
196micronutrients, Haifa®).

197 Plants were arranged following a split-plot design replicated in seven
198blocks, with two water availability treatments acting as the main factor (whole
199plots) and the 12 populations as the split factor (split-plots). In four of the blocks,
200each population was represented in each whole-plot by one individual of each of
201the three (open or control pollinated) families. In the remaining three blocks, and
202due to lack of material, each population was represented by two families. Thus,
203the trial included 36 plants per population (2 treatments × (3×4 + 2×3) = 36) and a
204total of 432 plants.

205 After cuttings were planted out into the final pots, they were watered every
2062-3 days to full capacity. Five months after transplanting (August 10th, 2007),
207when the plants had a mean height of 15.5 ± 3.9 cm and a diameter at the root
208collar of 3.7 ± 0.6 mm (mean ± standard deviation), the application of both water

209availability treatments was started over a period of six months (180 days). Half of
210the plants were subjected to a stress-free water regime, while the other half
211suffered a water deficit intensified over time to allow plants to develop adaptive
212responses gradually. The two watering regimes were established based on the
213saturation level of the substrate. In the well-watered treatment the substrate was
214kept at a mean saturation level of 80-90% throughout the whole experimental
215time, while in the drought-stressed treatment the substrate was kept at 30-40% of
216the mean saturation level for the first two months and then reduced to 20-30% for
217the last four months. The amount of water to be supplied -the same for all plants
218of the same treatment- was determined daily by weighing a tray of each block ×
219treatment combination. When the water content dropped below the previously
220calculated threshold, manual watering was individually performed with a test-tube
221(accuracy 1 ml) to achieve the corresponding level for each treatment. The
222reference weight corresponding to the saturation level was adjusted for the
223increase in plant size over the experiment. To this end, fresh plant total mass was
224modelled based on stem length and diameter at the root collar using an extra sub-
225sample of plants from all sizes and populations. Stem length and diameter were
226measured in all the experimental plants every 15 days, and plant fresh mass
227estimated based on this equation.

228 Plant height was measured before starting the treatment applications and
229six months after (February 5th, 2008), computing thereafter height growth
230increment (ΔH , cm) for both stressed and non-stressed plants. Survival was also
231recorded by means of a binary variable (SUR) at the end of the experiment

232(February 5th 2008). The 324 surviving plants were then harvested and roots were
233carefully washed to separate them from the substrate. The harvested plants were
234then partitioned into five fractions based on the different functional properties of
235tissues, and then oven-dried (80° C to constant biomass) for biomass
236determination and weighed (± 0.001 g) to obtain the dry weights of stems (SDW),
237coarse roots (≥ 2 mm diameter, CRDW), fine roots (< 2 mm diameter, FRDW),
238adult needles (ANDW) and juvenile needles (JNDW). The following variables
239were derived: total dry weight (TDW), adult-juvenile needles dry weight ratio
240(AJR= ANDW/JNDW, g g^{-1}) and the fractions of total biomass represented by
241each of the different plant functional tissues [stems (SMF= SDW/TDW), coarse
242roots (CRMF= CRDW/TDW), fine roots (FRMF= FRDW/TDW), adult needles
243(ANMF= ANDW/TDW) and juvenile needles (JNMF= JNDW/TDW)]. Using
244biomass fractions instead of dry biomass or other ratios between parts (e.g. root-
245shoot ratio) has the advantage of greater independence from the overall size of the
246plant (see next section), and this approach forms an integral part of the concept of
247growth analyses and carbon economy (Poorter et al. 2012 and references therein).

248

2492.3. Statistical analyses

250For characterizing adaptive plastic responses we selected two growth-related
251variables (ΔH and TDW), five variables related to biomass partitioning (SMF,
252CRMF, FRMF, ANMF, JNMF) and one related to ontogenetic development
253(AJR). For all these variables an analysis of variance (ANOVA) was carried out
254based on a linear mixed model that took into account the experimental split-plot

255 layout. The main factors (watering treatment (T), population (P) and their
256 interaction (T×P)) were considered fixed factors, whereas the blocks (B) and the
257 T×B and T×B×P interactions (representing the variation among whole and split
258 plots, respectively) were considered random factors in order to consider the
259 appropriate error terms for each fixed factor (Littell et al. 2006). The mixed model
260 was fitted with the MIXED procedure of SAS (Littell et al. 2006). A significant
261 effect of the watering treatment would indicate significant phenotypic plasticity to
262 water stress for a particular variable, whereas a significant treatment × population
263 interaction would indicate significant differences among populations in the
264 phenotypic plasticity for that variable.

265 Dependent variables were $\log_{10}(x)$ or \sqrt{x} transformed to meet the model
266 assumptions when necessary. To analyze the survival binary variable (SUR) a
267 generalized linear mixed model with the same terms as before was fitted using the
268 GLIMMIX procedure of SAS, assuming a binomial probability distribution and a
269 logit link function (Littell et al. 2006). Adjusted means for treatments, populations
270 and treatment × population combinations were estimated using the LSMEANS
271 statement of the MIXED or GLIMMIX procedures. Pearson correlations were
272 estimated between population means using the CORR procedure in SAS. Specific
273 contrasts between groups of populations (Atlantic vs Mediterranean) were carried
274 out with the CONTRAST statement of the MIXED procedure.

275 Resource allocation patterns are known to change with plant size as it
276 grows, and it has been argued that some observed changes in allocation after the
277 application of a specific treatment are in fact just the consequence of changes in

278plant size rather than a direct response to that treatment (Coleman et al. 1994). For
279example, biomass investment in mechanical support tissues increases more with
280size than does the investment in photosynthetic tissues (Niklas 1994), especially
281in woody species (Poorter et al. 2012). Because water availability is expected to
282substantially affect plant growth, it is convenient to apply an allometric analysis to
283account for possible size differences between plants of different treatments
284(Coleman et al. 1994; Poorter and Nagel 2000). To analyze the size effect on
285allocation variables, linear regression models relating the different biomass
286fractions and the decimal logarithm of total dry biomass were fitted for both
287treatments jointly (Poorter and Nagel 2000). In addition, to compare allometric
288trajectories of the two treatments, linear regression models of the decimal
289logarithm of the dry weight of each fraction on the decimal logarithm of the dry
290weight of the rest of the plant were fitted (Müller et al. 2000). Differences
291between the slopes and intercepts of these regressions were tested by means of an
292analysis of covariance (ANCOVA, with total plant biomass (\log_{10} units) as the
293covariate), using the MIXED procedure in SAS (Littell et al. 2006). In this
294allometric analysis, if both linear relationships are significant and overlap, slopes
295and/or intercepts will only differ if the water stress treatment was responsible for
296the changes in biomass allocation.

297 To study the plastic responses of populations to water availability we also
298constructed environment by environment biplots in which we graphically
299represented the position of each population in the space defined by its mean
300phenotype under the water stress treatment (x -axis) and under the non-stress

301treatment (y -axis) (Pigliucci and Schlichting 1996). When the two axes are
302presented at the same scale, this biplot can illustrate interesting biological
303properties. The main diagonal represents the line of null phenotypic change, and
304the magnitude of the plasticity is defined by the tangent (slope) of the angle (α)
305formed between the diagonal and the line connecting each population to the
306origin, referred as angular phenotypic change index (APCI). The advantage of this
307index, when compared to most common methods that are based on the differences
308between mean phenotypic values in each environment (represented in this
309environment \times environment biplot by the orthogonal distance to the main
310diagonal) is that the slope is reflecting the phenotypic change in relative terms,
311and also reflects the direction of the response, providing greater biological
312significance.

313 In order to integrate the information from the different assessed variables,
314we also implemented a principal component analysis (PCA) using the main
315variables selected for the univariate analyses. This PCA analysis allowed us to
316identify potentially homogeneous population groups in terms of biomass
317allocation, growth and ontogenetic performance. To perform this analysis we used
318the PRINCOMP procedure in SAS.

319

3203. Results

321*Plastic responses to water stress*

322The generalized mixed model for survival did not converge when including the
323treatment \times population interaction term. When this term was removed from the

324model, the treatment effect was highly significant, as expected, while no
325significant differences were detected between populations (Table 2). The survival
326rates varied between 96.5% in the non-stressed treatment and 52.5% in the water
327stressed treatment (Table 3). Mortality in the stressed treatment occurred mainly
328between the 20th and 60th day after starting treatment applications, thereafter the
329mortality rate decreased and remained almost constant from 100th day until
330harvesting. Survival rates for each population under water stress conditions varied
331widely, from 76.5% for SB×IN3 to 27.8% for SB, but the differences were not
332statistically significant (results not shown).

333 The watering treatment also strongly affected plant growth (Table 2), with
334well watered plants gaining twice the height and accumulating 40% more dry
335mass than water stressed plants (Table 3). Significant differences among
336provenances were also detected for both variables (Table 2). Regarding biomass
337allocation, significant differences between treatments were detected for the stems,
338coarse roots and young needles fractions, whereas the population effect was
339highly significant for all variables (Table 2). The coarse roots fraction was nearly
340twice greater in non-stressed than in stressed plants, and the stem fraction was
341also slightly higher. In contrast, the juvenile needles fraction was greater in
342stressed plants (Table 3). The population × treatment interaction effect was only
343significant for height growth (Table 2). Plants from pure Atlantic populations
344showed significant differences from those with at least one Mediterranean parent
345for all traits except for survival, and coarse and fine roots fractions (Table 2).

346

347 *Allometric trajectories*

348 Although statistically significant, the linear regression coefficients between
349 biomass fractions and total dry biomass were generally very low, except in the
350 case of juvenile needles which tended to show a moderate decrease as overall
351 biomass increased (Fig. 1, left figures). Allometric trajectories between the
352 biomass fraction of each plant functional tissue and the biomass of the rest of the
353 plant were always highly significant and had a high degree of overlapping
354 between treatments (Fig. 1, right figures), indicating their suitability for treatment
355 comparisons. Covariance analyses detected no significant differences between
356 treatments in the slopes of the allometric trajectories for any of the different plant
357 tissues, whereas significant differences among the intercepts were detected for
358 stems and coarse roots (Fig. 1, tables), suggesting direct plastic responses to water
359 stress in resource allocation for these two functional tissues.

360

361 *Variation at the population level*

362 Environment \times environment plots highlighted one group, formed by populations
363 CG, CG \times IN1, IN1, IN2 and SB \times IN1, which was characterized by greater height
364 increase, larger fractions of stems and juvenile needles (Fig. 2) and lower adult
365 needles when compared to the remaining populations. This group of populations
366 also showed a particularly high angular phenotypic change index (APCI) for ΔH
367 and JNMF. The common factor of these populations is their Atlantic origin,
368 except for SB \times IN1 which has a parent with moderate Mediterranean character.
369 Those other populations with at least one parent of Mediterranean origin (CG \times C,

370SB, SB×CG, SB×IN2 and SB×IN3) showed a contrasting performance, with a
371larger adult needles fraction and lower fraction of young needles and stems (Fig.
3722). These populations of Mediterranean character showed the highest levels of
373phenotypic plasticity for CRMF (Online Resource 3). Finally, the performances of
374the IN3 and CG×L populations were somewhat anomalous considering their
375Atlantic origin: the IN3 population behaved similarly to the group of populations
376with at least one Mediterranean parent, while the CG×L population was at an
377intermediate position between the two main groups, despite both parents being of
378Atlantic origin (Fig. 2 and Online Resource 3).

379 Hybrid populations performed mostly similarly to one of the parents,
380rather than being truly intermediate (Fig. 3). Hybrid populations with at least one
381parent from Mediterranean origin performed in a typically Mediterranean way. On
382the contrary, those hybrids that showed a typically Atlantic performance always
383derived from crosses where both parents were of Atlantic origin (with the
384exception of SB×IN1).

385 The principal component analysis effectively synthesized the information
386of the studied variables, with the two first principal components accounting for
38764% of the total variance (Fig. 4). The first principal component (PC1, 44% of
388total variance) was positively correlated with the adult needles mass fraction and
389the adult/juvenile needles ratio, and negatively correlated with the juvenile
390needles and stem mass fractions. The second component (PC2, 20% of total
391variance) was positively correlated with plant growth (ΔH and TDW). PC1 and
392PC2 clustered the populations in three main groups (Fig. 4). Again, one group was

393formed by the Atlantic populations CG, CG×IN1, IN1, IN2 and IN1×SB and was
394characterized by greater height growth and a larger fraction of stems and young
395needles. Another group, formed by SB, SB×IN3, SB×CG, CG×C and the Atlantic
396IN3, was defined by the opposite pattern, with higher fraction of adult needles, a
397higher adult-juvenile needles ratio and lower height growth. SB×IN2 and CG×L
398formed a third group with an intermediate performance between the first two. The
39912 populations are clearly aligned along a virtual axis defined by the stems and
400adult needles mass fractions, which can be interpreted as an Atlantic to
401Mediterranean gradient .

402

4034. Discussion

404Our analysis of the responses to water stress for growth and biomass allocation
405allowed us to identify two kind of adaptive strategies for coping with water deficit
406in juvenile plants of Maritime pine. On the one hand, seedlings showed common
407plastic responses to water stress, suggesting a conserved plastic strategy at the
408species level. However, divergent patterns at the population level were identified
409beyond the former plastic responses. These patterns of variation at the population
410level were related to environmental conditions in the origin and therefore can be
411considered as adaptive.

412

413*Plastic responses to water stress*

414The common strategy at the species level in the plastic responses to water stress is
415derived from the lack of population × treatment interaction for biomass allocation

416 variables (Table 2). This result indicates that there were no genetic differences
417 between populations in their phenotypic plasticity to water stress, which is in
418 accordance with previous water stress trials under controlled conditions for this
419 species (Aranda et al. 2010; Sánchez-Gómez et al. 2010; Lamy et al. 2014). The
420 interaction was significant, however, for height growth, and was originated by the
421 heterogeneity of variances between treatments as a consequence of a higher
422 responsiveness to drought in populations from wetter sites (Fig. 2). In agreement
423 with previous results for the species (Corcuera et al. 2010; Corcuera et al. 2011;
424 Gaspar et al. 2013), populations from more mesic origins displayed higher
425 plasticity for growth and increased height under water availability, whereas
426 populations originating from harsher environments showed less responsiveness to
427 water availability in terms of growth.

428 The results also indicate that all populations increased the leaf mass
429 fraction and decreased the root mass fraction when subjected to water stress. This
430 common strategy at the species level disagrees with previous studies in *P. pinaster*
431 populations in which no shifts in carbon allocation were detected (Aranda et al.
432 2010; Corcuera et al. 2012), probably because they used a shorter period or a
433 milder intensity of water stress. But the most intriguing result is that this
434 conserved strategy opposes the *functional equilibrium theory* (also known as
435 *balanced growth hypothesis* or *optimal partitioning theory*), which states that
436 plants allocate more resources to those organs involved in the acquisition of the
437 most limiting resource (Bloom et al. 1985). When water is the limiting resource,

438one could therefore expect an increased investment in root biomass and a reduced
439allocation to photosynthetic tissues (Ludlow 1989).

440 These unexpecting findings could be thought to be a consequence of using
441cuttings instead of plants from seeds. However, the long period for root
442development before treatment application lead us to think that this was not the
443case. For an alternative reliable explanation of these results we took advantage of
444a more detailed partition of biomass allocation between different plant fractions
445(not only considering roots, stems and leaves), which to our knowledge has not
446been performed before for the species. Our results show that the fine root fraction,
447which is responsible for water and nutrient absorption (Cody 1986), and the adult
448needle fraction, which includes the definitive photosynthetic tissue, did not differ
449significantly between treatments after the six-month stress period (Table 2).
450Therefore, it can be concluded that there was no phenotypic plasticity for these
451two fractions. This result combined with the high among population variation for
452these characters (Table 2), suggests that the two tissues responsible for immediate
453resource uptake were optimized for each population. However, the remaining
454biomass fractions showed plasticity to water stress, with stressed plants having
455reduced stem and coarse root fractions but a greater juvenile needle fraction
456(Table 3). While the stem and coarse root fractions had no relation with total dry
457weight and showed different allometric trajectories between treatments, the
458juvenile needle fraction tended to be lower in larger plants and a single allometric
459trajectory was detected for this variable regardless of the watering treatment (Fig.
4601). These results suggest that the increased investment in the stem and coarse

461 roots under high water availability was due to an active response to the treatment.
462 On the other hand, the relative reduction in juvenile needle growth was merely an
463 indirect consequence of increased size and the subsequent advance in
464 developmental state caused by the improved growing conditions. Therefore,
465 stressed plants do not redirect their photosynthetic resources towards appropriate
466 structures to deal with drought, but instead appear to simply adopt a more
467 conservative strategy in which investment in growth is reduced and ontogenetic
468 development is delayed, resulting in a greater fraction of juvenile needles. Well-
469 watered plants, on the other hand, increase their investment in structural tissues.
470 Increased stem and branch growth improves above-ground competition for light,
471 essential in sun-loving woody species (Shukla and Ramakrishnan 1986), while
472 increased coarse root growth enhances the prospecting for water and nutrients
473 needed to support future growth (Cody 1986). In summary, in our trial, *P.*
474 *pinaster* showed a development pattern that was common across all tested
475 populations. This pattern was based on investment in structural tissues when water
476 was available, and a cessation of that investment when water resources became
477 scarce, reducing carbon uptake and decreasing the ontogenetic development.
478 Inconsistent results with the *optimal partitioning theory* were also found in other
479 works with other drought adapted forest tree species (Osório et al. 1998) and also
480 with maritime pine (Danjon et al. 2009; Sánchez-Gómez et al. 2010). These
481 observed pattern essentially represents a drought avoidance strategy that is
482 characterized by stomatal closure before a significant decrease of leaf water

483potential (Martínez-Ferri et al. 2000), slowing down growth and adjusting plant
484size to the reduced amounts of assimilated carbon (Osório et al. 1998).

485

486*Variation at the population level*

487Despite the common pattern described above, the population effect was highly
488significant for height growth and biomass allocation to all plant fractions (Table
4893), indicating a high among population variation and adaptive differentiation
490processes to environmental conditions, as has been observed in other similar trials
491with contrasting populations of the species (Aranda et al. 2010; Corcuera et al.
4922012; Gaspar et al. 2013; Sánchez-Gómez et al. 2010; but see Lamy et al. 2014).
493Although the small number of families used to account for within population
494variation could be masking important information, the strong consistence and the
495clear patterns that were observed let us to think that the results were not
496excessively biased.

497 Two main groups of populations were clustered based on the mean
498phenotypic performance in each treatment (Figs. 2 and 4, and Online Resource 3).
499One group showed greater height growth and greater allocation of biomass to
500stems in both treatments, which is consistent with the general strategy followed by
501the species when water became available. This pattern of biomass partitioning
502corresponds to those populations with both parents from Atlantic origin, which are
503expected to be adapted to climates with higher water availability and only short
504periods of water deficit. This result is in agreement with previous results for mesic
505populations of the species (Corcuera et al. 2012; Tognetti et al. 2000). The other

506group consisted of populations with at least one Mediterranean parent, and
507showed lower height growth and a lower stem mass fraction. In contrast with
508previous results (Corcuera et al. 2012) these populations did not show a greater
509investment in tissues responsible for the absorption of water resources, although
510they did show the greatest plasticity to water stress in terms of their root system
511development (Table 2 and Online Resource 3). Coming from more water-
512restricted environments than those of Atlantic origin, the Mediterranean
513populations seem to adopt a more conservative resource allocation strategy,
514showing reduced growth and associated structural tissues when soil water reserves
515are limited (Corcuera et al. 2012; Nguyen and Lamant 1989; O'Brien et al. 2007).

516 Another interesting result was the strong variation observed between
517populations in the juvenile to adult needles ratio (Table 2). Under both treatments
518the Atlantic populations had a greater mass proportion of juvenile to adult needles
519than those of Mediterranean populations, thus showing a higher juvenility index
520(see Online Resource 3). Adult and juvenile needle fractions were inversely
521correlated across populations ($r = -0.88$, $p < 0.01$, $N = 12$), and those populations
522with a greater juvenile fraction also showed greater aerial growth in both
523treatments (greater shoot fraction ($r = 0.78$, $p < 0.01$, $N = 12$) and height growth (r
524= 0.86 , $p < 0.01$, $N = 12$)). Several studies have shown that patterns of ontogenetic
525development in juvenile pines, characterized either by an early heteroblastic
526change or by a prolonged juvenile phase, may be related to different adaptive
527strategies to face abiotic stress (Grotkopp et al. 2002; Strauss and Ledig 1985).
528Primary and secondary needles of Mediterranean pines (including *P. pinaster*)

529 differ in cuticular transpiration and frost tolerance (Climent et al. 2009; Pardos et
530 al. 2009). Compared with adult needles, juvenile needles use more water (higher
531 cuticular transpiration; Pardos et al. 2009) and are more sensitive to frost damage
532 (Climent et al. 2009), but are less expensive to construct and have a higher
533 photosynthetic efficiency due to their higher proportion of photosynthetic tissues
534 (Peters et al. 2003). Prolonging the juvenile stage and increasing the allocation to
535 juvenile needles would therefore be a cheaper but more risky strategy (Darrow et
536 al. 2002). This pattern of ontogenetic delay matches the one observed in the
537 present study for the *P. pinaster* populations from more mesic environments. The
538 lower cost of production of juvenile needles would allow plants to allocate more
539 resources to aerial growth, vital for competing with neighbours in favourable
540 environments such as those of the Atlantic area (Shukla and Ramakrishnan 1986).
541 However, the lower tolerance of juvenile needles to drought and frost damage
542 would make Atlantic populations more vulnerable to abiotic stress.

543 Two of the studied populations departed from the general phenotypic
544 segregation between Atlantic and Mediterranean origins. Contrary to what might
545 be expected, the CG×L and IN3 populations, both with a pronounced Atlantic
546 origin, showed intermediate and clearly Mediterranean performances,
547 respectively. The disparity of genetic origins, often unknown, that were used in
548 massive reforestations made in the Northwest Interior provenance region during
549 the mid 20th century (de la Mata and Zas 2010a), could explain the unexpected
550 performance of the IN3 population. In the case of the GC×L population, it seems
551 that trees from the French origin of Landes, at the northern limit of the species

552distribution range, on hybridising with the Coastal-Galicia origin population
553shifted the latter towards a more Mediterranean performance. This result is
554consistent with the high drought resistance shown by the Landes provenance in
555field trials, which was explained in terms of adaptation to sandy soils with low
556water retention capacity (Corcuera et al. 2010).

557

558*Performance of hybrid populations*

559Generally, tested hybrid populations appeared closer to the Mediterranean group
560when at least one parent was from this origin, while hybrids appeared within the
561Atlantic group only when both parents came from this climatic region (Figs. 3 and
5624). This result suggests dominance of the Mediterranean characteristics among the
563tested hybrids except for SB×IN1 population, which despite having a
564Mediterranean parent always appeared clustered with populations from Atlantic
565origin. It is also noteworthy that the performance of CG×C hybrid was the most
566Mediterranean in character, showing the strong Mediterranean influence of the
567Corsica provenance. These results indicate that the eventual mediating effect of
568the maternal environment was minimal, since there was no evidence of hybrids
569preferentially acquiring properties from the maternal environment where the seed
570was developed, as observed in other studies (e.g. Cendán et al. 2013). In fact,
571hybrids with either parent from Mediterranean origin showed this to be the more
572important factor, regardless of the maternal environment where the hybrid was
573collected.

574 None of the hybrids showed greater height growth than the Coastal-Galicia
575breeding population from Atlantic origin (Fig. 2), which agrees with the results
576found for hybrids of the species between the improved Landes Atlantic
577provenance and others sources of Mediterranean character (Corsica and Morocco)
578(Harfouche and Kremer 2000). These authors also found that certain hybrid
579crosses combined desirable traits from parental provenances, so that Landes
580provenance, which had the greatest vigor, benefited from a significant
581improvement in stem form and resistance to pests when hybridised with
582Mediterranean provenances. In our study, only the SB×IN1 population showed
583significant height growth while maintaining a resource allocation pattern best
584suited to drought conditions, which may show the suitability of this hybrid for
585planting in transition regions between Atlantic and Mediterranean climates. The
586CG×IN1 population showed a very similar vigor to the Coastal-Galicia breeding
587population but with a slightly more Mediterranean character, suggesting that this
588hybrid could be also considered for use in these transition zones (Fig. 2).

589

5905. Conclusions

591Three main findings can be derived from the results of this study. Firstly, plastic
592responses to water stress appeared to be highly conserved among the studied
593populations. Rather than reallocate resources to maximize water uptake and
594minimize water loss, as theoretically predicted, all populations showed a common
595conservative isohydric strategy based on promoting aerial growth when water is
596abundant, and stopping growth and delaying ontogenetic development when water

597 becomes limiting. Secondly, our results indicated a strong intraspecific variation
598 in biomass allocation patterns, with a clear differentiation between trees of
599 Atlantic and Mediterranean origins. The Atlantic populations showed a growth-
600 based strategy with greater investment in juvenile needles that are more
601 photosynthetically active but less resistant to water loss, which may make juvenile
602 plants more vulnerable to water stress. Mediterranean populations showed a more
603 conservative strategy, avoiding water stress by limiting water loss through
604 minimizing aerial growth and increasing the proportion of water-loss resistant
605 adult needles. Finally, hybrid populations, rather than show an intermediate
606 performance, generally performed similarly to the Mediterranean parent,
607 suggesting a dominance of the Mediterranean-like characters. Some of the tested
608 hybrid populations combined, however, the high growth of the Atlantic
609 populations with traits of drought adaptation, becoming, thus, interesting materials
610 for use in transitional regions between both climates.

611

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625

626**Data Archiving**

627Data has been submitted to the Spanish repository for forest resources (Red
628Nacional de Ensayos Genéticos GENFORED, www.genfored.es). Accession
629number will be supplied once available.

630

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775

776**Table 1** Code, name, climatic origin and pollination type of pure and hybrid
777tested populations

CODE	POPULATION	ORIGIN	POLLINATION
CG	Coastal-Galicia	Atlantic	Open
CG×C	Coastal-Galicia × Corsica	Atlantic × Mediterranean	Cross
CG×L	Coastal-Galicia × Landes	Atlantic × Atlantic	Cross
CG×IN1	Coastal-Galicia × Carballiño	Atlantic × Atlantic with Med influence	Cross
SB	Montaña de Soria-Burgos	Mediterranean	Open
SB×CG	Soria-Burgos × Coastal-Galicia	Mediterranean × Atlantic	Cross
SB×IN1	Soria-Burgos × Carballiño	Mediterranean × Atlantic with Med influence	Cross
SB×IN2	Soria-Burgos × Guitiriz	Mediterranean × Atlantic with Med influence	Cross
SB×IN3	Soria-Burgos × Sober	Mediterranean × Atlantic with Med influence	Cross
IN1	Galicia-Interior Carballiño	Atlantic with Med influence	Open
IN2	Galicia-Interior Guitiriz	Atlantic with Med influence	Open
IN3	Galicia-Interior Sober	Atlantic with Med influence	Open

778

779**Table 2** Results of the mixed model and specific contrasts between two groups of
 780populations of different origin (Atlantic (both Atlantic parents) versus
 781Mediterranean (at least one Mediterranean parent)) for the studied variables. F-
 782ratios and associate significance levels¹ are shown

Variable	Treatment (F _{1,6})	Population (F _{11,113})	Treat. × Pop. (F _{11,113})	Atlant. vs Medit. (F _{1,113})
Survival				
Alive/Died	51.85 ***	1.31 ^{ns}	-. ²	0.33 ^{ns}
Growth				
Height growth (cm)	108.79 ***	5.66 ***	1.98 *	18.57 ***
Total dry biomass (g)	54.68 ***	2.09 *	0.78 ^{ns}	5.10 *
Biomass allocation				
Stems mass fraction	13.58 *	6.14 ***	1.01 ^{ns}	19.41 ***
Coarse roots mass fraction	42.98 ***	2.62 **	1.30 ^{ns}	2.37 ^{ns}
Fine roots mass fraction	0.32 ^{ns}	2.91 **	1.67 ^{ns}	1.11 ^{ns}
Adult needles mass fraction	3.05 ^{ns}	7.23 ***	0.37 ^{ns}	21.69 ***
Juvenile needles mass fraction	25.64 **	5.63 ***	0.57 ^{ns}	16.69 ***
Adult/juvenile needles ratio	2.57 ^{ns}	6.37 ***	0.43 ^{ns}	24.45 ***

783

784¹ Significance levels: ***= P<0.001, **= P<0.01, *= P<0.05, ns= not significant

785² Because of convergence problems, the model implemented with the GLIMMIX

786procedure in SAS did not include the *Treatment* × *Population* interaction effect

787**Table 3** Least square means \pm standard errors in the two watering treatments for
788survival, growth and biomass allocation variables

789

Variable	No-stress	Stress
Survival		
Died/Alive (0/1)	0.96 \pm 0.02	0.52 \pm 0.10
Growth		
Height growth (cm)	12.40 \pm 0.80	6.20 \pm 0.80
Total dry biomass (g)	6.80 \pm 0.20	4.90 \pm 0.20
Biomass allocation		
Stems mass fraction	0.16 \pm 0.01	0.15 \pm 0.01
Coarse roots mass fraction	0.11 \pm 0.01	0.06 \pm 0.01
Fine roots mass fraction	0.26 \pm 0.01	0.25 \pm 0.01
Adult needles mass fraction	0.24 \pm 0.01	0.26 \pm 0.01
Juvenile needles mass fraction	0.19 \pm 0.01	0.24 \pm 0.01
Adult/juvenile needles ratio	1.30 \pm 0.10	1.10 \pm 0.10

790

791

Figure Captions

792

793**Fig. 1** Allometric regressions (adjusted- R^2 and significance level) between each
794biomass fraction versus total dry biomass in logarithmic scale for all treatments
795(left figures) [n= 324], and allometric trajectories for each watering treatment (no-
796stress: ● and continuous line [n= 206]; stressed: ○ and dotted line [n= 118])
797between the dry biomass of each fraction versus the remaining dry biomass of the
798plant, both on a logarithmic scale (right figures). Tables on the right show
799adjusted- R^2 and associated significance levels for the relationship of each
800allometric trajectory, and the results of the analysis of covariance for testing for
801differences between treatments in the slopes and intercepts of these trajectories
802(Porter and Nagel, 2000). Significance levels: *** = $p < 0.001$; ** = $p < 0.01$; * =
803 $p < 0.05$; ns = $p > 0.05$

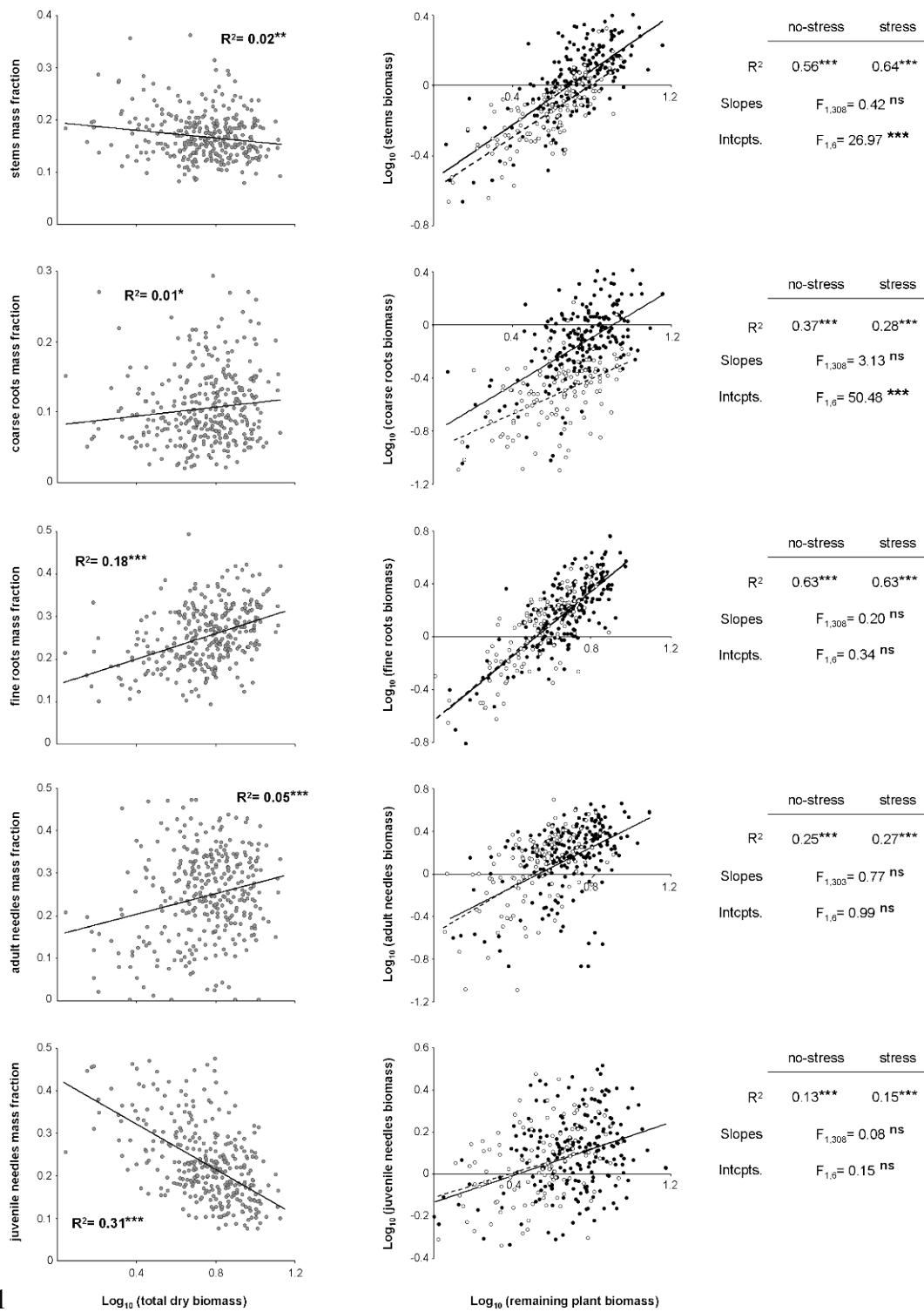
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805**Fig. 2** Environment \times Environment plots reflecting the position of each population
806in the space defined by their phenotypic means under stress and no-stress
807treatments. The diagonal line determines the stable performance or lack of
808phenotypic plasticity. The bars are standard errors. The legend shows the symbol
809used for each population according to the climatic origin (white for Atlantic, black
810for Mediterranean and gray for the hybrids) and the type of pollination (circles
811and triangles for open- and control-pollinated populations, respectively).
812Population codes are shown for several populations (see Table 1 for code
813definitions)

814**Fig. 3** Reaction norms under stress and no-stress water treatments for height
815growth and juvenile needle biomass fraction of different Atlantic (bold continuous
816lines) and Mediterranean (light continuous lines) *P. pinaster* populations and the
817hybrids between them (dashed lines). CG: Coastal-Galicia (Atlantic), SB: Soria-
818Burgos (Mediterranean), IN2: Galicia-interior (Atlantic with some Mediterranean
819influence).

820

821**Fig. 4** PCA results showing factor loadings of the variables included in the
822analysis (white squares) and populations on the space defined by the two principal
823components (PC1 and PC2, the proportion of variance explained by each
824component is also shown). The ellipses denote the three different groups that can
825be identified according to the relative position of populations on the plane. See
826Table 1 for explanations of codes and Fig. 2 for symbol legend of populations.
827**ΔH**: height growth (cm), **TDW**: total dry biomass (g), **CRMF**: coarse roots mass
828fraction, **FRMF**: fine roots mass fraction, **SMF**: stems mass fraction, **ANMF**:
829adult needles mass fraction, **JNMF**: juvenile needles mass fraction, **AJR**:
830adult/juvenile needles ratio.

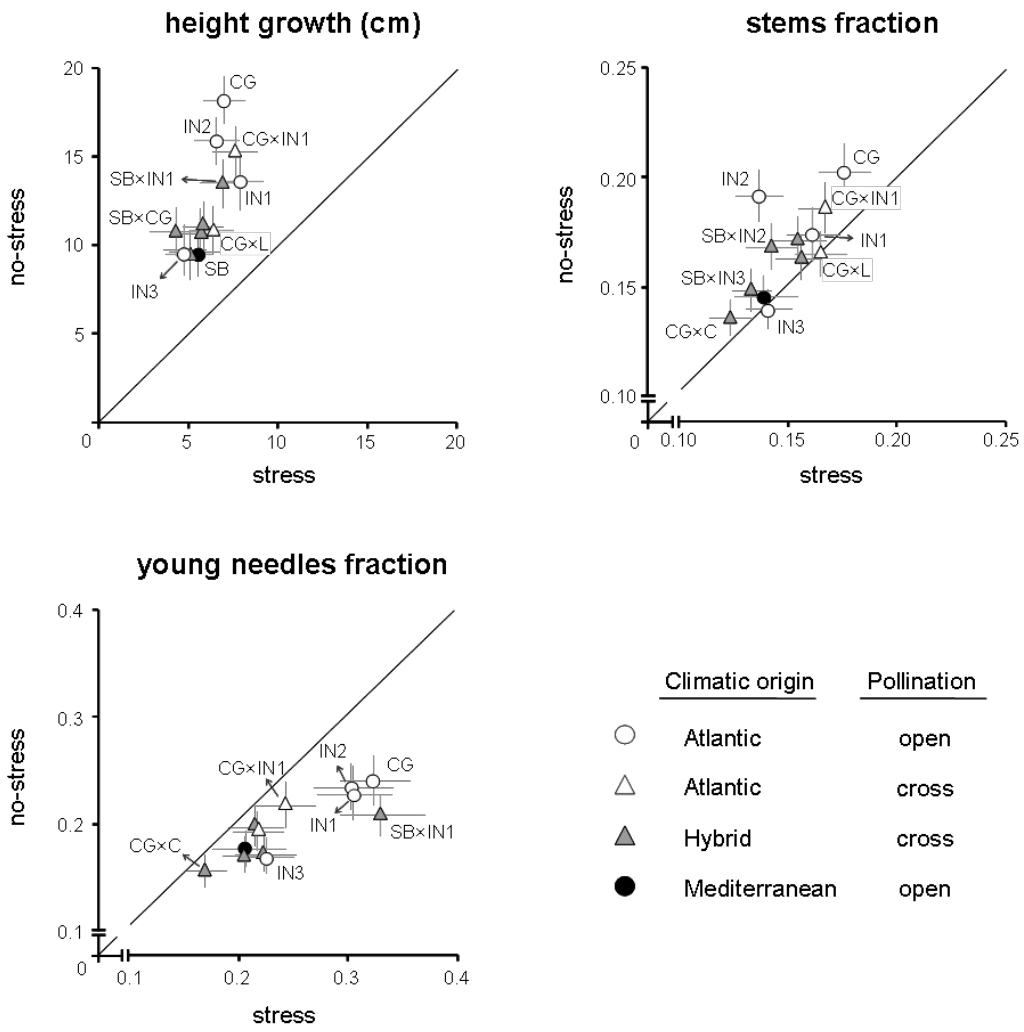


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833 Fig. 1

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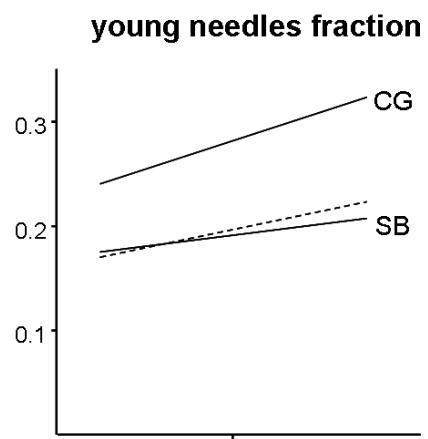
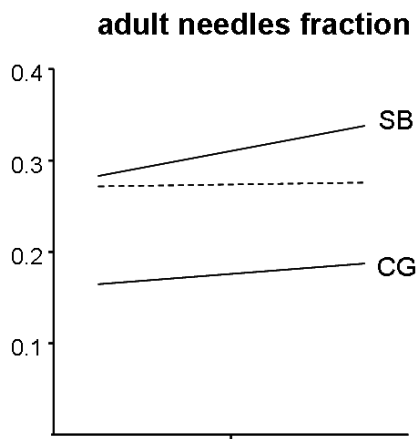
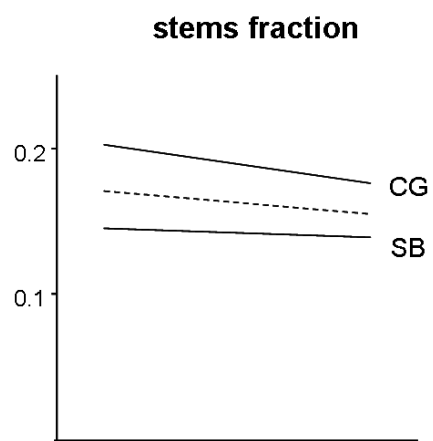
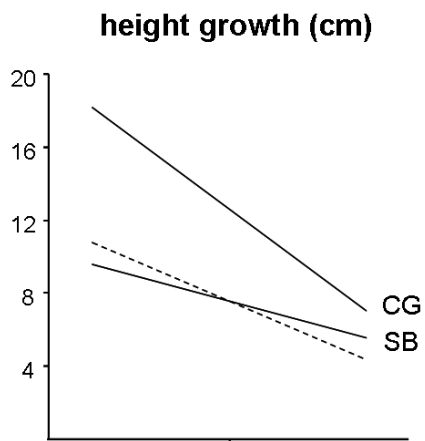
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841 Fig. 2

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844 No-stress Stress

No-stress Stress

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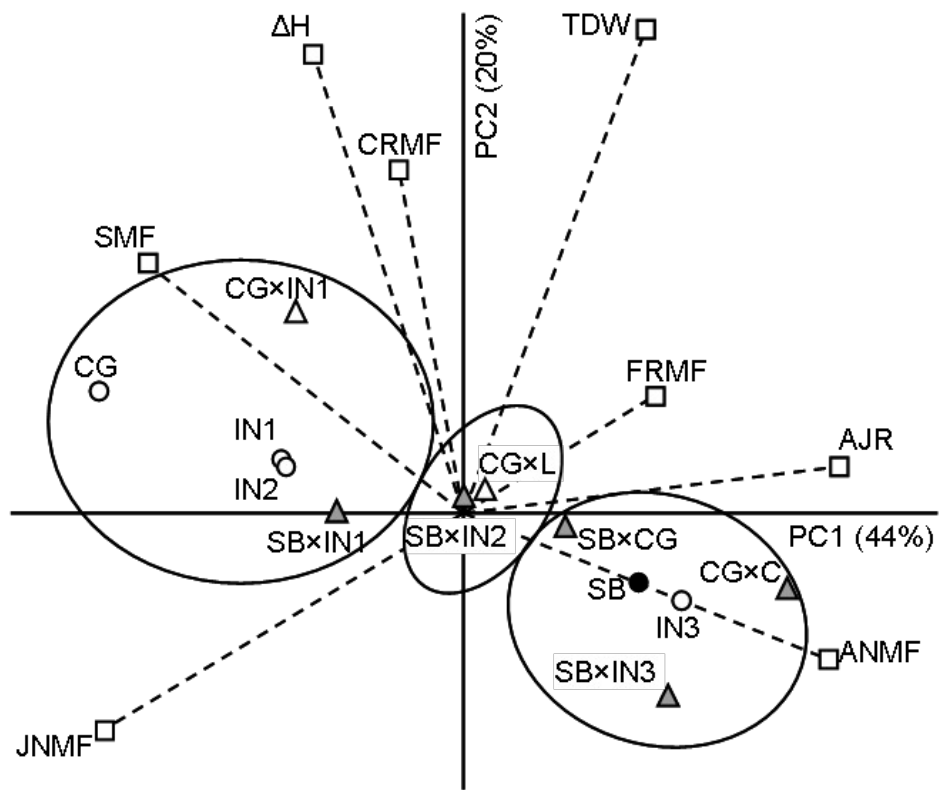
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851 **Fig. 3**

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865 Fig. 4