

Is the benefit of larger seed provisioning on seedling performance greater under abiotic stress?

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Abstract

Seed size is a relevant predictor of seed survival, seedling phenotype and species recruitment and it is generally accepted that larger seeds give rise to seedlings with improved performance. In pioneer tree species the success in seedling survival is strongly determined by resource availability, which largely varies at diverse spatial scales. Here, using a pioneer Mediterranean pine species as a model (Maritime pine, *Pinus pinaster* Ait.), we hypothesized that the positive effect of seed provisioning on seedling establishment and early performance may be stronger under environmental harassment, and that the progeny of lineages investing in bigger seeds would be favored in such stressful conditions. We explored the effect of seed mass on seedling traits related to germination, survival, growth and biomass partitioning by sowing pre-weighted seeds of known genetic background and culturing the seedlings under a range of abiotic stress conditions by experimentally modifying phosphorus (four levels, 5260 seeds) and water availability (three levels, 975 seeds) in two independent experiments. Phenotypic variation in seed mass was extremely large, with more than a tenfold change between the smallest and the largest seed. Seed mass variation showed a strong genetic component with large and significant variation both between and within populations. As expected, seed mass was a positive predictor of germination time and early seedling performance. However, contrary to our expectations, populations from more stressful sites produced smaller seeds, and the positive effect of seed mass on seedling performance was not magnified under resource limitation. Our results thus does not support that early seedling performance is favored by greater seed provisioning under resource limiting conditions.

Key Words: seed mass, seedling survival, intraespecific variation, maternal effects, drought stress, nutrient availability

Introduction

Seed mass is a relevant life history trait with well-documented effects on early seedling phenotype, affecting crucial processes of species recruitment such as dispersal (Debain et al., 2003), germination (Cendán et al., 2013; Mishra et al., 2014), seedling growth (Zas et al., 2013), seedling survival (Larson et al., 2015; Lebrija-Trejos et al., 2016; Moles and Westoby, 2004), seed predation (Fricke and Wright, 2016) and tolerance to different stressors, including shade (Paz and Martínez-Ramos, 2003; Quero et al., 2007), drought (Khurana and Singh, 2000; Metz et al., 2010), limited nutrient availability (Bergholz et al., 2015; Hanley et al., 2007), or fire (Calvo et al., 2016; Escudero et al., 2000). Because of these large effects on the transition across generations, seed mass is considered to be a crucial functional trait linked to plant life history (Leishman et al., 2000).

Although, comparatively much less studied than in annual plants, seed mass is also known to affect the offspring performance of long-lived plants, at least during the early establishment (Bladé and Vallejo, 2008; Castro, 1999; Cendán et al., 2013; Gómez, 2004; Milberg and Lamont, 1997; Parker et al., 2006; Ramirez-Valiente et al., 2009; Surles et al., 1993; Wennstrom et al., 2002; Zas et al., 2013). Because seedling establishment is the most critical life stage of trees, during which extremely large mortality rates can occur (Petit and Hampe, 2006), seed mass is also assumed to have high adaptive relevance in trees (Zas and Sampedro, 2015). Particularly, seed size effects are expected to be especially relevant in sun-loving pioneer tree species, in which regeneration usually occurs at high densities in large forest gaps over a short period of time, resulting in a high competitive environment. Any little

advantage at the establishment can, thus, have strong fitness consequences (Castro, 2006). Previous results on pine trees have confirmed the relevance of seed mass on recruitment processes (Castro, 1999; Cendán et al., 2013; Parker et al., 2006; Zas et al., 2013). However, empirical evidences are not completely conclusive, with a number of studies failing to detect significant effects of seed mass on germination rate, germination time or seedling performance (Bladé and Vallejo, 2008; Calvo et al., 2016; Escudero et al., 2000; Wahid and Bounoua, 2013). Differences in the species considered, the age at which seedling traits are assessed or the environmental conditions in which seeds were tested, all can contribute to this variability.

Of particular relevance is the potential effect of the abiotic environment as a potential modulator of the relationship between seed mass and seedling performance: seedlings

developing from larger seeds, containing more nutrients and carbon-based reserves, could take advantage of a better provisioning leading to an improved ability to uptake the scarce resources and support respiration longer under carbon starvation (Leishman et al., 2000; Westoby et al., 1992; Westoby, 2002). Consequently, the effect of seed mass on seedling survival and performance may be more evident under conditions of abiotic stress or limited resource availability. This prediction has been reported for tolerance to shade (Paz and Martínez-Ramos, 2003; Quero et al., 2007) and intraespecific competition (Lebrija-Trejos et al., 2016). However evidences in the case of nutrient limitation and drought are limited and almost restricted to annual grasses (Bergholz et al., 2015; Hanley et al., 2007).

A larger effect of seed mass on seedling performance under stressful conditions may impose a positive

selection pressure on seed mass, leading to heavier seeds when resources are scarce (Leishman et al., 2000; Westoby et al., 1992). This has been widely studied at the interspecific level, searching for abiotic gradients in seed mass, in which large-seeded species are commonly associated with unfavorable environmental conditions (Dainese and Sitzia, 2013; Metz et al., 2010; Moles et al., 2007; Qi et al., 2014). However, whether the intraespecific variation in seed mass across populations or lineages may influence the ability of the emerging seedlings to survive and grow under stressful conditions has received comparatively much less attention (Khurana and Singh, 2000; Quero et al., 2007; Völler et al., 2012). Populations from harsher environments have been reported to provide heavier seeds (Guo et al., 2010; Konarzewski et al., 2012; Völler et al., 2012) suggesting that this could be relevant also within species. However, opposite results, with seed

mass decreasing across populations as the environmental conditions become more stressful have also been reported (Lázaro and Traveset, 2009; Pluess et al., 2005). In the particular case of pine trees, seed mass is known to largely vary both within (Bilir et al., 2008; Zas and Sampedro, 2015) and across populations (Calvo et al., 2016; Correia et al., 2014; Wahid and Bounoua, 2013), with some authors relating the variation in seed mass across populations with the variation in their ability to cope with different stresses. For example, Wahid and Bounoua (2013) suggested that heavier *P. pinaster* seeds are better adapted to drought conditions, while Correia et al. (2014) found *P. pinaster* seeds from high-altitude origins to be larger and germinate earlier than seeds from lowland origins.

The aim of the present study was to test, using a pioneer pine species (*Pinus pinaster* Ait.) as a model, i)

whether the effect of seed mass on seedling performance is more relevant when seedlings experience reduced resource availability, and ii) whether populations from abiotic stressed environments bear larger seeds in order to help seedlings to cope with that stress. We explored the effect of seed mass on seedling traits related to germination, survival, growth and biomass partitioning by sowing pre-weighted seeds of known genetic background and culturing the seedlings under a range of abiotic stress conditions by manipulating nutrient (four levels of phosphorous availability, 5260 seeds) and water availability (three levels, 975 seeds) in two independent experiments. We hypothesize that i) seeds from origins experiencing more abiotic stress will be heavier than seeds from origins with more favorable environmental conditions. And that ii) the influence of seed mass will be more relevant under

conditions of limiting resource availability, or, in other words, that seedlings developing from large seeds will be less dependent on the availability of soil nutrients and water.

Materials and methods

This study includes two independent greenhouse experiments. In the first one, *P. pinaster* seedlings derived from pre-weighted seeds of 39 half-sib families of one population were submitted to four different levels of phosphorous availability. In the second, seeds from 18 half-sib families of 3 contrasting populations, which are known to strongly differ in their tolerance to drought (Corcuera et al., 2011; Corcuera et al., 2012), were grew under different levels of water availability, from full field capacity to severe water deficiency. After one growing period subjecting the seedlings to the stress treatments, we measured different seedling traits in order to

analyze how seed mass affected seedling performance under abiotic stress.

Maritime pine (*Pinus pinaster* Ait.) is a species with a high level of population differentiation (González-Martínez et al., 2002) and for which seed mass is known to be extremely variable (Wahid and Bounoua, 2013; Zas and Sampedro, 2015) and to have important impacts on seedling traits (Cendán et al., 2013; Escudero et al., 2000; Zas et al., 2013). Drought is a main limiting factor and a major driver of population differentiation for this Mediterranean pine species (Gaspar et al., 2013; Lamy et al., 2014). Phosphorus limitation is one of the most important factors limiting pine productivity in the Atlantic and humid area of Northwest Spain (Martíns et al., 2009).

Experiment 1: Nutrient stress experiment

Plant material. Open pollinated seeds from 39 mother trees selected within the Atlantic Coastal-Galicia (CGAL) population (Northwest Spain) were used in the nutrient stress experiment. The region has a temperate humid climate with mean annual precipitation around 1500 mm, low summer drought, and mean annual temperatures of about 11°, typically ranging between 4° and 25°C (min and max daily means). Soils in this area are thin, sandy and acidic, with high organic matter content, high total nitrogen and very low phosphorous concentration. Soil phosphorus availability has been identified as one of the most relevant drivers of forest productivity in the region (Martíns et al., 2009; Zas and Serrada, 2003).

Experimental design. The greenhouse experiment followed a randomized split-plot design replicated in four blocks, with four levels of phosphorus availability (complete fertilization, and

three levels of phosphorus-limited fertilization) as the whole factor and 39 open-pollinated half-sib families as the split-plot factor. In total we sowed 5260 seeds, approximately 135 of each family, of which 3389 finally germinated. During this germination phase, we maintained common environmental conditions for all the seeds. Approximately one month after sowing we arranged the plants following the final experimental design and began the fertilization treatments. A total of four plants per family, randomly selected among the available germinated seedlings, were included in each whole plot (phosphorus treatment by block combination). The experiment included, thus, a total of 2496 seedlings (4 blocks × 4 phosphorus treatments × 39 families × 4 plants).

Greenhouse conditions and nutrient availability treatments. Individually weighed seeds (at 0.0001 g) were sown

in 2 L pots filled with sterilized perlite and covered with 1 cm layer of sand. In order to prevent pathogen infections, both seeds and seedlings were treated with a fungicide (Fernide, Syngenta Agro, Madrid, Spain) every two months. Nutrient availability treatments were applied by subirrigation every two days, starting one month after sowing. The complete fertilization treatment was a balanced solution of N: P: K: Ca: Mg in a concentration of 100:20:70:7:9 mg L⁻¹ (P20), and the necessary amounts of micronutrients and trace elements. This complete fertilizer solution is similar to that used in local nurseries for optimize pine growth. In the other 3 nutrient availability treatments we maintained the concentration of all macro and micronutrients except that of phosphorus, which was reduced to 10 (P10), 5 (P5), and 2 (P2) mg L⁻¹. A more detailed explanation of culture conditions can be consulted in Moreira et al. (2012) and Sampedro et al. (2011).

Previous results indicated that low P availability in treatment P2 promoted growth deficiencies similar to those observed at field in young Maritime pine stands (Martíns et al., 2009). Fertilizer solutions were prepared freshly every two-week and the pH adjusted to 6.5. The experiment was carried out in a greenhouse with controlled temperature (10°C minimum at night and 25°C maximum during day) and light (12 h of light per day) at the Lourizán Forest Research Centre (Xunta de Galicia, Pontevedra, Spain).

Sampling and measurements. The germination time (assessed as the time for seedling emergence in days since sowing) was determined by periodical inspections every 2-3 days. Plant height was measured every 15 days during 4 months, starting three months after sowing. Height and stem basal diameter was also measured before harvest at the end of the experiment, when plants

were approximately six months old. Plants were cleaned of foreign matter and substrate, separated into needles, shoots and roots fractions, oven-dried (72 h at 65°C) and weighed. Aerial dry weight (ADW), total dry weight (TDW) and Root to Shoot Ratio (RSR) were derived from those values.

Experiment 2: Drought stress experiment

Plant material. Seeds were collected from open pollinated trees in natural stands of three different provenances covering a wide range of environmental conditions, from low to high summer drought: San Ciprián in Northwest Spain (SCRI) with quite high annual precipitation (1330 mm) and mean temperature (12.5 °C) and very low summer drought; Coca (COCA) in the center of Spain, with 470 mm of annual precipitation, mean temperature of 12 °C, and large periods of high temperatures and low water availability

in summers; and Oria (ORIA) in the coastal Mediterranean region of Southeast Spain, with 350 mm of annual precipitation, mean temperature of 14.5 °C, and extremely severe summer drought (Corcuera et al., 2012).

Experimental design. The experiment followed a randomized split-plot design, replicated in four blocks, with three levels of water availability (control, moderate water stress, and severe water stress) as the whole factor, and 6 open-pollinated half-sib families from each population as the split-plot factor. In total we sowed 975 seeds, between 50 and 60 for each family, of which 625 finally germinated. Between 12 and 14 plants per family and treatment were finally included in the experimental setup (3 plants per plot), except for the families of the COCA population for which the low germination rates obligated to reduce these numbers. For this population only control and

moderate water stress treatments were applied. A total of 491 plants were included in the experiment, of which 352 survived and were sampled for biomass determinations.

Watering treatments and greenhouse conditions. Individually weighed seeds (0.0001 g) were sown in 2 L pots filled with a mixture 1:1 (v:v) of peat (Humin-substrate N3, Klasmann-Deilmann GmbH, 49744 Geeste, Germany) and sterilized river granitic sand (very fine grain), and covered with a 1 cm layer of sand on which seeds were buried. Seeds were treated with a fungicide (Fernide, Syngenta Agro, Madrid, Spain) before sowing in order to prevent pathogen infections. During the germination phase, a fungicide (Previcur, Bayer Chem., Madrid, Spain) was applied every two weeks and a Fe-fertilizer (Sequestrene, 138 Fe-G100, Novartis Agro, Madrid, Spain) once a month. During the germination phase

the seedlings were cultivated under common environmental conditions and when they were 8 months old, were arranged according the experimental design and subjected to the different watering treatments for 4 months. All pots were first watered to field capacity and the maximum water content of the substrate estimated. The drought stress treatments consisted on maintaining different amounts of water in the substrate: 80-95% of field capacity in the unstressed control treatment (corresponding to a water potential in the substrate of about $\Psi = -0.001$ MPa), 35-50% of field capacity in the moderate water stress treatment ($\Psi = -0.01$ MPa), and 25-35% in the severe water stress treatment ($\Psi = -0.1$ MPa) (Fig. S1). To estimate the need of watering and the amount of water to be added for each treatment a series of extra control pots (12 per treatment) were weighed every 2-3 days, before and after watering (Fig. S1). Watering

treatments were applied by drip irrigation. At each of these dates, all plants in each treatment were irrigated with the same amount of water, as estimated by the weights of the control pots (Fig. S1). The experiment was carried out in a greenhouse with controlled temperature (settled above 11/18 °C at night/day during the germination period, and above 11/20 °C night/day during the water stress treatments) and light (12 h of light per day) at the Misión Biológica de Galicia (CSIC, Pontevedra, Spain).

Sampling and measurements. Germination (seedling emergence) was evaluated every two days. Twelve months after sowing height and basal diameter were measured and plants were harvested, cleaned of foreign matter and substrate, and the fresh weight of needles shoots and roots determined. Aliquots of each sample were weighted separately and then

oven-dried 72 h at 70 °C in order to obtain the humidity percentage, which was then used to estimate the biomass of each fraction. Aerial dry weight, total dry weight and root to shoot ratio were derived from those data.

Statistical analyses

First, intraespecific genetic variation in seed mass was analyzed by means of a mixed model assuming population as a fixed factor and family within population as a random factor. Then, independent but equivalent analyses were carried for the two experiments. Germination time was analyzed with similar mixed models but including also seed mass as a covariate. In the case of the nutrient stress experiment, the interaction of seed mass with population was also included in the model to explore whether the covariation of germination with seed mass differed among the different pine origins. All these mixed models were fitted using

the Proc Mixed procedure of the SAS System (Littell et al., 2006). Similarly, the ability of each seed to germinate was analyzed fitting generalized mixed models using the Proc Glimmix procedure of the SAS System, including the same fixed and random factors as described before, and assuming a binomial probability distribution and a logit link function (Littell et al., 2006).

To test whether the effect of the seed mass depends on the abiotic stress we followed an ANCOVA strategy using the Proc Mixed procedure of SAS (Littell et al., 2006). For each trait and experiment, we first fitted a heterogeneous slope model in which we included the covariation with seed mass and its interaction with both the stress treatments and the pine populations (i.e. allowing for different slopes across treatments and populations). If these interactions terms were not significant we then fitted a common slope model just including the covariation with seed

mass but none of the previous interaction terms. All these models included the stress treatments, the pine population and their interaction as fixed factors, and block and family effects (the latter nested within populations) as random factors. The statistical significance of the variance components for each random factor was assessed using likelihood ratio tests. When it was needed, normality was achieved by log-transformations, and residual heterogeneity models were used when significant deviations were found. In all cases, the statistical significance of the fixed factors (Stress Treatment, Population and their interaction) was assessed by means of F-ratio tests whereas that of the random factors (Family within Population) was assessed using likelihood ratio tests (Littell et al., 2006).

Seed mass variation within and between populations

Phenotypic variation in seed mass in the two experiments was extremely large, with more than a tenfold change between the smallest and the largest seed. Statistical analyses indicated that this variation showed a strong genetic component with large and significant variation both between (Fig. 1B) and within populations (Fig. 1A). Variation in seed mass within populations was high, with around a two-fold change between family means (Fig. 1A). Across populations, seed mass variation followed an Atlantic-Mediterranean gradient, with seed mass tended to diminish from the humid and favorable conditions of the Coastal-Galicia population in Northwest Spain to the driest and drought-stressed environment of the Oria Population in Southeast Spain (Fig. 2).

Results

Effect of seed mass on germination and germination time

Seed mass was a positive and significant predictor of germination success in the two experiments (Table 1) with bigger seeds germinating more than smaller ones ($F_{1,954} = 93.52$; $p < 0.001$) (see also Fig. 2). This effect differed among the three pine populations in the water-stress experiment as indicated by the significant interaction between seed mass and population (Table 1; Fig. 2). Germination ability also significantly varied between families within populations for both experiments (results not shown).

Germination time was also significantly affected by seed mass in the two experiments (Table 1), with heavier seeds tending to germinate earlier than lighter ones. Germination time was also variable between populations in the water-stress experiment (Table 1), with seeds from

the larger seeder populations from Coastal-Galicia and San Ciprián germinating earlier than those from Oriá and Coca (Fig. S2). Seeds from the Coca population showed a lower germination success and required a longer time to emerge.

Effect of seed mass on seedling performance under abiotic stress

Both the nutrient and the water stress treatments significantly affected the phenotype and growth rate of seedlings ($P < 0.001$; Tables S1 and S2, respectively), except in the case of Root: Shoot Ratio in the water-stress experiment. In the two experiments, seedlings from the control (unstressed) treatments were taller, thicker and accumulated more aerial, root and total biomass than seedlings from the stressed treatments (Fig. S3).

The effect was dose-dependent in the two experiments although whereas in the nutrient stress experiment only the

highly P-deprived treatment (P2) generated large differences in seedling performance regarding the control treatment, in the water-stress treatments, seedling performance was similarly affected by the two levels of stress (Fig. S3). In the nutrient stress experiment, almost all seedlings survived in all the treatments. Contrastingly, mortality in the severe and moderate drought treatments was 80% and 25%, respectively.

In the nutrient-stress experiment, the covariation with seed mass across treatments (common-slope ANCOVA) was significant for all seedling traits except for the Root: Shoot Ratio (Table S1; Fig. 3). In the water-stress experiment, however, the covariable was only significant for seedling height, although it was marginally significant ($P<0.10$) in the case of the aerial and total biomass (Table S2). In both cases, the correlations between seed mass and seedling traits were positive, with

heavier seeds leading to bigger and heavier seedlings (Fig. 3). The relationship between seed mass and seedling traits was especially evident at the family level (Fig. 3b, d), and relatively weaker at the individual phenotypic level (Fig. 3a, c).

The heterogeneous slope ANCOVA models revealed that the covariation between seed mass and seedling traits did not differ across the different nutrient and water treatments (Table 2; Fig. 3). In both cases the interaction between seed mass and the stress treatments was no significant for all traits (Table 2), indicating parallel relationships between seed mass and seedling traits across treatments (Fig. 3). Similarly, the covariation between seed mass and seedling traits did not vary across pine populations, as revealed by the lack of a significant population \times seed mass interaction in the water-stress experiment (Table 2). Populations did differ, however, for

several seedling traits (height, aerial dry weight and Root: Shoot ratio) and even in their plasticity to the water-stress treatments (significant water-treatment \times population interaction for height, diameter and Root: Shoot ratio) (Table S2, Fig. S3).

Discussion

Three main findings can be derived from this study. First, seed mass largely varied across and within populations. The pattern of variation, however, does not support our hypotheses, as seeds from favorable environments were heavier than those from sites with environmental harassment. Second, seed mass significantly affected germination and early seedling performance, although these positive effects tended to diminish along time. And third, not supporting our initial hypothesis, the positive effect of seed mass on seedling performance was not significantly greater when seedlings

experienced reduced nutrient or water availability. That is, we found no evidences of advantages for larger seeders when seedlings grew under resource scarcity in this pine species.

Intraespecific variation in seed mass

In agree with previous findings (Calvo et al., 2016; Correia et al., 2014; Wahid and Bounoua, 2013), our results indicated that seed mass largely vary among Maritime pine populations. However the pattern of variation was opposite to our initial expectations. Based on the supposedly stronger benefits of larger seeds when resources are scarce (Leishman et al., 2000), we hypothesized that seed mass should be greater in populations living on stressful conditions as an adaptation to cope with that stress. However, the results presented here indicated that seed mass decreased as the environmental conditions become more stressful, following an Atlantic-Mediterranean

gradient. Several non-exclusive explanations can help to understand this unexpected pattern.

On the one hand, increasing seed mass under unfavorable conditions can be constrained by the lack of enough resources for seed provisioning that must be shared with other life functions (Lázaro and Traveset, 2009; Pluess et al., 2005). Genetic and plastic forces governing seed size variation among species or among populations within species across different environments are acting, thus, in opposite directions (Qi et al., 2014). By sampling seeds at origin, most studies analyzing how environmental conditions may determine seed mass variation failed to differentiated whether the differences in seed mass among species or populations are of genetic or plastic nature (Lázaro and Traveset, 2009; Pluess et al., 2005; Qi et al., 2014; Ramirez-Valiente et al., 2009; Völler et al., 2012). Common garden

tests would be needed for this (Konarzewski et al., 2012). Some studies reporting bigger seeds under limited resource conditions suggest, however, that genetic factors are prevailing, as environmental factors should go in the opposite direction (Lázaro and Traveset, 2009; Ramirez-Valiente et al., 2009). Meanwhile, Soper G. et al (2016) have reported that intraespecific patterns for seed traits were remarkably inconsistent and that the scale of climate variation in seed traits is highly species-specific. In other studies, however, genetic and plastic forces balance out one another resulting in no significant differences in seed mass among species or populations (Qi et al., 2014). Our results are consistent with the idea that constraints imposed by limited resource availability predominated and determined the observed variation across populations, in which seed mass showed a trend to

diminish from favorable to unfavorable growing conditions.

Indirect effects of the considered environmental factors on regeneration may also contribute to explain population differentiation in seed mass across environmental gradients. In instance, high resource availability may indirectly led to stronger competition, whose negative effects for seedling establishment could compensate the direct benefits of the abundance of resources (Bergholz et al., 2015). Selection pressures on seed mass may thus depend on the relative predominance of direct or indirect effects of greater resource availability on regeneration processes (Bergholz et al., 2015). On the other hand, differential selection processes during recruitment depending on the relative importance of seed mass on seed predation, dissemination, germination, and seedling development can also contribute to modulate differentiation

patterns across environmental gradients (Lázaro and Traveset, 2009).

Finally, common evolutionary histories shared by species or populations within species may be also confounded with environmental gradients in seed mass (Dainese and Sitzia, 2013; Guo et al., 2010; Pluess et al., 2005). Many phylogenetic-controlled studies comparing species seed mass across environmental gradients, suggest, however, an adaptive role of seed mass. For example, after controlling phylogenetic autocorrelation among species, soil fertility remained the most important predictor for explaining seed mass variation in mountain grasslands (Dainese and Sitzia, 2013).

Conflicting pressures on seed mass at a given environment (in instance seed predation and early seedling performance) may explain the maintenance of large within population variation in seed mass in this pine

species. The large variation among families within populations observed here agrees with previous findings (Cendán et al., 2013; Zas and Sampedro, 2015) and could be interpreted as a lack of evidence of an adaptive role of seed mass in this species. In the present study, we cannot distinguish, however, whether the observed variation in seed mass among families is due to true genetic differences or to environmental effects associated to the mother plants. Evolutionary forces such as gene flow, environmental heterogeneity or trade-offs with other life history traits may be also contributing to maintain large within population variation in seed mass.

Taking all together, although our results suggest that the observed population differentiation do not follow any clear adaptive pattern and seems more related to direct phenotypic responses to environmental variation,

further studies in common garden tests are needed to determine whether the observed variation in seed mass across populations (and families) has a genetic basis and can be related to local adaptation processes.

Seed mass effects on germination and seedling traits

The positive effect of seed mass on germination rate and germination time, with heavier seeds germinating easier and earlier, was consistent across the two experiments. Previous studies suggest, however, that the relation between seed mass and germination is highly context specific (Moles and Westoby, 2004), depending on both the environment and the species considered (Bergholz et al., 2015). Particularly on pines, some studies have reported that seed mass is a relevant driver of germination rate (e.g. Calvo et al., 2016; Escudero et al., 2000) and germination time (e.g. Cendán et al., 2013; Parker et

al., 2006; Tiscar Oliver and Lucas Borja, 2010) but others have not (e.g. Bladé and Vallejo, 2008; Calvo et al., 2016; Wahid and Bounoua, 2013). Understanding how seed mass influences germination is extremely relevant, as most of the variation in seed to seedling survival is commonly explained by differences in germination and emergence (Larson et al., 2015). However, it seems that, at least in pine species, no general pattern can be depicted for this relationship. Besides the possible influences of the species and the environmental conditions in which seeds are germinated (Bergholz et al., 2015), the effect of seed mass on seedling establishment can also differ among populations of the focal species as occurred in the present study (Lázaro and Traveset, 2009; Wennstrom et al., 2002).

Seed mass also affected seedling growth rate and biomass accumulation, in agreement with

previous findings in this and other pine species (Bladé and Vallejo, 2008; Castro, 1999; Escudero et al., 2000; Parker et al., 2006; Surles et al., 1993; Tiscar Oliver and Lucas Borja, 2010; Wennstrom et al., 2002; Zas et al., 2013). We found that this effect was significant for almost all the studied traits in the nutrient stress experiment but only for height growth (and marginally for aerial and total biomass) in the water stress experiment. Differences between the two experiments may arise, among other reasons, because of different populations and different seedling ages at assessment (6 months since sowing in the nutrient stress experiment versus 12 months in the water stress experiment). Access to seed reserves has been reported to be ephemeral and lasting only for a short period of time since germination in tanoak and maize (Kenedy et al., 2004; White and Veneklaas, 2012). Accordingly, the

effect of seed mass on early seedling performance typically diminishes as seedling ages (Castro, 1999; Debain et al., 2003; Wennstrom et al., 2002). Indeed, this seems to be the case in our study. Judging from the F ratios of the covariable (Table S1), the relation of seed mass with seedling height in the nutrient stress experiment tended to diminish from the first (3 month-old) to the final assessment (6 month-old). However, in pine trees the effect of seed mass on seedling traits commonly remains significant for a much longer period than the explored here, and it has been reported to last up to 2 years in *P. halepensis* (Bladé and Vallejo, 2008), 5 years in *P. sylvestris* (Wennstrom et al., 2002), and up to 15 years in *P. elliottii* (Surles et al., 1993). This discrepancy may be explained by the way the seed-seedling relationship is analyzed. Most of the cited studies explored the relation between seed mass and seedling traits on a family or seed lot basis, analyzing

the correlations between family or lot means. Instead, we analyzed the relationship at the individual phenotypic level, which tend to be weaker than family correlations (see Fig. 3).

Seed-seedling relationships were not magnified under limited resource conditions

The results of the two experiments consistently indicated that the effect of seed mass on seedling performance was not altered by the abiotic stress and resource availability in which the seedlings grew, with parallel seed mass to seedling performance relationships across environments. These results refute our hypothesis and suggest that the benefit of larger seeds is not magnified under stressful situations, at least regarding the two stresses considered, nutrient deprivation and drought, and the intensity of the stress imposed by our experimental treatments.

Previous results exploring how the environment modulates seed-seedling relationships are not conclusive, especially regarding these two environmental factors (Bergholz et al., 2015; Hanley et al., 2007; Khurana and Singh, 2000). Some studies did found that the seed-seedling relationships were stronger under impoverished resource conditions. For example, Khurana and Singh (2000) found that seedlings from smaller seeds of a tropical tree were much more susceptible to severe water stress. Contrary to our results they found a significant interaction between seed mass and water stress for several seedling traits including diameter, leaf area and root: shoot ratio. Accordingly, Caddick and Linder (2002) found that larger seedlings of African Restionaceae, produced by larger diaspores, are less likely to succumb to drought-induced mortality than the smaller ones. In other cases, however,

the effect of the offspring environment on the seed-seedling relationships was not evident or was context-dependent. For example, Hanley et al. (2007) found a positive effect of seed mass on seedling growth for Australian Fabaceae species grown under nutrient limitation but not for British species, whereas Bergholz et al. (2015) found nutrient environment to modulate the effect of seed mass on survival of grass land species, but not on growth. Metz et al. (2010) found that seed-seedling relationships were not altered across a rainfall gradient, and suggested that the benefits of heavier seeds under limited resources conditions could be counterbalanced by a minimization of competition in these sites in comparison with more favorable environments. However, this indirect effect of the environmental stress must be discarded in our study as seedlings were cultivated in individual pots.

Few studies have explored this question in pine trees, and the scarce available results are again variable, with environmental conditions modulating the seed-seedling relationship in some cases (Surles et al., 1993; Wennstrom et al., 2002) but not in others (Parker et al., 2004; Reich et al., 1994). Analyzing open pollinated families of *Pinus elliottii* Engelm, Surles et al. (1993) found stronger correlations between seed and seedling traits under low N conditions than under high N availability. These authors analyzed the relationships between family means and explored the differences across N treatments by comparing the r coefficients but they did not analyze the differences in the slope of the seed-seedling relationships. Higher r coefficients at low nutrient availability may be just an indirect consequence of lower variability in the seedling traits because of reduced seedling size when resources are limiting. Wennstrom et al. (2002) also

found higher correlation coefficients between *Pinus sylvestris* L. seed mass and seedling growth under harsh field conditions than under a more favorable nursery environment. However in this case, they also found that the relative benefit of larger seeds was greater under harsh conditions. Opposite results were found by Parker et al. (2006) in *Pinus strobus* L, for which seed mass effects on seedling growth was unaffected by light conditions.

In summary, although it seems difficult to generalize across the *Pinus* genus, based on our results, we must refute the idea that the benefits of larger pine seeds are magnified under limited resource conditions. In other words, we found no evidences that seedling from larger seeders take advantage from improved seed provisioning for a better seedling performance under abiotic harassment in this pioneer pine species. Taking all together, although bigger seeds produce better seedling

performance in Maritime pine, other forces acting on seed mass such as dispersal, pre- and post- dispersal seed predation, and even biotic resistance should be considered for understanding the adaptive value of seed mass in this species.

Acknowledgments

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References

- Bergholz, K., Jeltsch, F., Weiss, L., Pottek, J., Geißler, K., Ristow, M., 2015. Fertilization affects the establishment ability of species differing in seed mass via direct nutrient addition and indirect competition effects. *Oikos* 124, 1547-1554.
- Bilir, N., Prescher, F., Lindgren, D., Kroon, J., 2008. Variation in cone and seed characters in clonal seed orchards of *Pinus sylvestris*. *New Forest* 36, 187-199.
- Bladé, C., Vallejo, V.R., 2008. Seed mass effects on performance of *Pinus halepensis* Mill. seedlings sown after fire. *Forest Ecology and Management* 255, 2362-2372.
- Caddick, L.R.L., P. L. , 2002. Evolutionary strategies for reproduction and dispersal in African Restionaceae. *Australian Journal of botany* 50, 339-355.
- Calvo, L., Hernández, V., Valbuena, L., Taboada, A., 2016. Provenance and seed mass determine seed tolerance to high temperatures associated to forest fires in *Pinus pinaster*. *Annals of Forest Science* 73, 381-391.
- Castro, J., 1999. Seed mass versus seedling performance in Scots pine: a maternally dependent trait. *New Phytologist* 144, 153-161.
- Castro, J., 2006. Short delay in timing of emergence determines establishment success in *Pinus sylvestris* across microhabitats. *Annals of Botany* 98, 1233-1240.
- Cendán, C., Sampedro, L., Zas, R., 2013. The maternal environment determines the timing of germination in *Pinus pinaster*. *Environmental and Experimental Botany* 94, 66-72.
- Corcuera, L., Cochard, H., Gil-Pelegrín, E., Notivol, E., 2011. Phenotypic plasticity in mesic populations of *Pinus pinaster* improves resistance to xylem embolism (P50) under severe drought. *Trees* 25, 1033-1042.
- Corcuera, L., Gil-Pelegrín, E., Notivol, E., 2012. Differences in hydraulic architecture between mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiology* 32, 1442-1457.
- Correia, I., Santos, L., Faria, C., Nóbrega, C., Almeida, H., David, T., 2014. Cone to seedling variation between *Pinus pinaster* provenances from contrasting altitudes. *Forest Science* 60, 724-732.
- Dainese, M., Sitzia, T., 2013. Assessing the influence of environmental gradients on seed mass variation in mountain grasslands using a spatial phylogenetic filtering approach. *Perspectives in Plant Ecology, Evolution and Systematics* 15, 12-19.

- Debain, S., Curt, T., Lepart, J., 2003. Seed mass, seed dispersal capacity, and seedling performance in a *Pinus sylvestris* population. *Ecoscience* 10, 168-175.
- Escudero, A., Nunez, Y., Perez-Garcia, F., 2000. Is fire a selective force of seed size in pine species? *Acta Oecologica* 21, 245-256.
- Fricke, E.C., Wright, S.J., 2016. The mechanical defence advantage of small seeds. *Ecology Letters* 19, 987-991.
- Gaspar, M.J., Velasco, T., Feito, I., Alía, R., Majada, J., 2013. Genetic variation of drought tolerance in *Pinus pinaster* at three hierarchical levels: a comparison of induced osmotic stress and field testing. *Plos One* 8(11), e79094.
- Gómez, J.M., 2004. Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* 58, 71-80.
- González-Martínez, S.C., Alía, R., Gil, L., 2002. Population genetic structure in a Mediterranean pine (*Pinus pinaster* Ait.): a comparison of allozyme markers and quantitative traits. *Heredity* 89, 199-206.
- Guo, H., Mazer, S.J., Du, G., 2010. Geographic variation in seed mass within and among nine species of Pedicularis (Orobanchaceae): effects of elevation, plant size and seed number per fruit. *Journal of Ecology* 98, 1232-1242.
- Hanley, M.E., Cordier, P.K., May, O., Kelly, C.K., 2007. Seed size and seedling growth: differential response of Australian and British Fabaceae to nutrient limitation. *New Phytologist* 174, 381-388.
- Kenedy, P.G., Hausmann, N.J., Wenk, E.H., Dawson, T.E., 2004. The importance of seed reserves for seedling performance: an integrated approach using morphological, physiological and stable isotope techniques. *Oecologia* 141, 547-554.
- Khurana, E., Singh, J.S., 2000. Influence of seed size on seedling growth of *Albizia procera* under different soil water levels. *Annals of Botany* 86, 1185-1192.
- Konarzewski, T.K., Murray, B.R., Godfree, R.C., 2012. Rapid Development of Adaptive, Climate-Driven Clinal Variation in Seed Mass in the Invasive Annual Forb *Echium plantagineum* L. *PLoS One* 7(12), e49000.
- Lamy, J.B., Delzon, S., Bouche, P., Alía, R., Vendramin, G.G., Cochard, H., Plomion, C., 2014. Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. *New Phytologist* 201, 874-886.

- Larson, J.E., Sheley, R.L., Hardegree, S.P., Doescher, P.S., James, J.J., 2015. Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. *Journal of Applied Ecology* 52, 199-209.
- Lázaro, A., Traveset, A., 2009. Does the spatial variation in selective pressures explain among-site differences in seed mass? A test with *Buxus balearica*. *Evolutionary Ecology* 23.
- Lebrija-Trejos, E., Reich, P.B., Hernandez, A., Wright, S.J., 2016. Species with greater seed mass are more tolerant of conspecific neighbours: a key driver of early survival and future abundances in a tropical forest. *Ecology Letters* 19, 1071-1080.
- Leishman, M.R., Wright, I.J., Moles, A.T., Westoby, M., 2000. The evolutionary ecology of seed size. In seeds-the ecology of regeneration in plant communities., 2nd ed. CAB International, pp 31-57.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS System for mixed models, second edition. SAS Institute, Cary, NC.
- Martíns, P., Sampedro, L., Moreira, X., Zas, R., 2009. Nutritional status and genetic variation in the response to nutrient availability in *Pinus pinaster*. A multisite field study in Northwest Spain. *Forest Ecology and Management* 258, 1429-1436.
- 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. *Journal of Ecology* 98, 697-704.
- Milberg, P., Lamont, B.B., 1997. Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. *New Phytologist* 137, 665-672.
- Mishra, Y., Rawat, R., Rana, P.K., Sonkar, M.K., Mohammad, N., 2014. Effect of seed mass on emergence and seedling development in *Pterocarpus marsupium* Roxb. *Journal of Forestry Research* 25, 415-418.
- Moles, A.T., Ackerly, D.D., Tweddle, J.C., Dickie, J.B., Smith, R., Leishman, M.R., Mayfield, M.M., Pitman, A., Wood, J.T., Westoby, M., 2007. Global patterns in seed size. *Global Ecology and Biogeography* 16, 109-116.
- Moles, A.T., Westoby, M., 2004. Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology* 92, 372–383.

- Moreira, X., Zas, R., Sampedro, L., 2012. Genetic variation and phenotypic plasticity of nutrient re-allocation and increased fine root production as putative tolerance mechanisms inducible by methyl jasmonate in pine trees. *Journal of Ecology* 100, 810-820.
- Parker, W.C., Noland, T.L., Morneau, A.E., 2004. Effect of seed mass on early seedling growth of five eastern white pine (*Pinus strobus* L.) families under contrasting light environments. *Canadian Journal of Botany* 82, 1645-1655.
- Parker, W.C., Noland, T.L., Morneau, A.E., 2006. The effects of seed mass on germination, seedling emergence, and early seedling growth of eastern white pine (*Pinus strobus* L.). *New Forests* 32, 33-49.
- Paz, H., Martínez-Ramos, M., 2003. Seed mass and seedling performance within eight species of *Psychotria* (Rubiaceae). *Ecology* 84, 439-450.
- Petit, R., Hampe, A., 2006. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution and Systematics* 37, 187-214.
- Pluess, A.R., Schütz, W., Stöcklin, J., 2005. Seed weight increases with altitude in the Swiss Alps between related species but not among populations of individual species. *Oecologia* 144, 55-61.
- Qi, W., Guo, S., Chen, X., Cornelissen, J.H.C., Bu, H., Du, G., Cui, X., Li, W., Liu, K., 2014. Disentangling ecological, allometric and evolutionary determinants of the relationship between seed mass and elevation: insights from multiple analyses of 1355 angiosperm species on the eastern Tibetan Plateau. *Oikos* 123, 23-32.
- Quero, J.L., Villar, R., Marañón, T., Zamora, R., Poorter, L., 2007. Seed mass effects in four Mediterranean *Quercus* species (Fagaceae) growing in contrasting light environments. *American Journal of Botany* 94, 1795–1803.
- Ramirez-Valiente, J.A., Valladeres, F., Gil, L., Aranda, I., 2009. Population differences in juvenile survival under increasing drought are mediated by seed size in cork oak (*Quercus suber* L.). *Forest Ecology and Management* 257, 1676-1683.
- Reich, P.B., Oleksyn, J., Tjoelker, M.G., 1994. Seed mass effects on germination and growth of diverse European Scots pine populations. *Canadian Journal of Forest Research* 24, 306-320.
- Sampedro, L., Moreira, X., Zas, R., 2011. Costs of constitutive and jasmonate-induced pine tree chemical defenses emerge only under low nutrient availability. *Journal of Ecology* 99, 818-827.

- Soper Gorden, N.L., Winkler, K.J., Jahnke M.R., Marshall, E., Horky, J., Huddelson, C., Etterson, J.R. 2016. Geographic patterns of seed mass are associated with climate factors, but relationships vary between species. Americal Journal of Botany 103 (1), 60-72.
- Surles, S.E., White, T.L., Hodge, G.R., Duryea, M.L., 1993. Relationships among Seed Weight Components, Seedling Growth Traits, and Predicted Field Breeding Values in Slash Pine. Canadian Journal of Forest Research 23, 1550-1556.
- Tiscar Oliver, P.A., Lucas Borja, M.E., 2010. Seed mass variation, germination time and seedling performance in a population of *Pinus nigra* subsp *salzmannii*. Forest Systems 19, 344-353.
- Völler, E., Auge, H., Prati, D., Fischer, M., Hemp, A., Bossdorf, O., 2012. Geographical and land-use effects on seed-mass variation in common grassland plants. Basic and Applied Ecology 13, 395-404.
- Wahid, N., Bounoua, L., 2013. The relationship between seed weight, germination and biochemical reserves of maritime pine (*Pinus pinaster* Ait.) in Morroco. New Forest 44, 385-397.
- Wennstrom, U., Bergsten, U., Nilsson, J.E., 2002. Effects of seed weight and seed type on early seedling growth of *Pinus sylvestris* under harsh and optimal conditions. Scandinavian Journal of Forest Research 17, 118-130.
- Westoby, M., Jurado, E., Leishman, M., 1992. Comparative evolutionary ecology of seed size. Trends in Ecology and Evolution 7, 368-372.
- Westoby, M.F., D. S.; Moles, A. T.; Vesk, P. A.; Wright, I. J., 2002. Plant ecological strategies: some leading dimensions of variation between species. Annual Review Ecological Systems 33, 125-159.
- White, P.J., Veneklaas, E.J., 2012. Nature and nurture: the importance of seed phosphorous content. Plant and Soil 357, 1-8.
- Zas, R., Cendán, C., Sampedro, L., 2013. Mediation of seed provisioning in the transmission of environmental maternal effects in *Pinus pinaster*. Heredity 111, 248-255.
- 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.
- Zas, R., Serrada, R., 2003. Foliar nutrient status and nutritional relationships of young *Pinus radiata* D. Don plantations in north-west Spain. Forest Ecology and Management 174, 167-176.

Tables and Figures

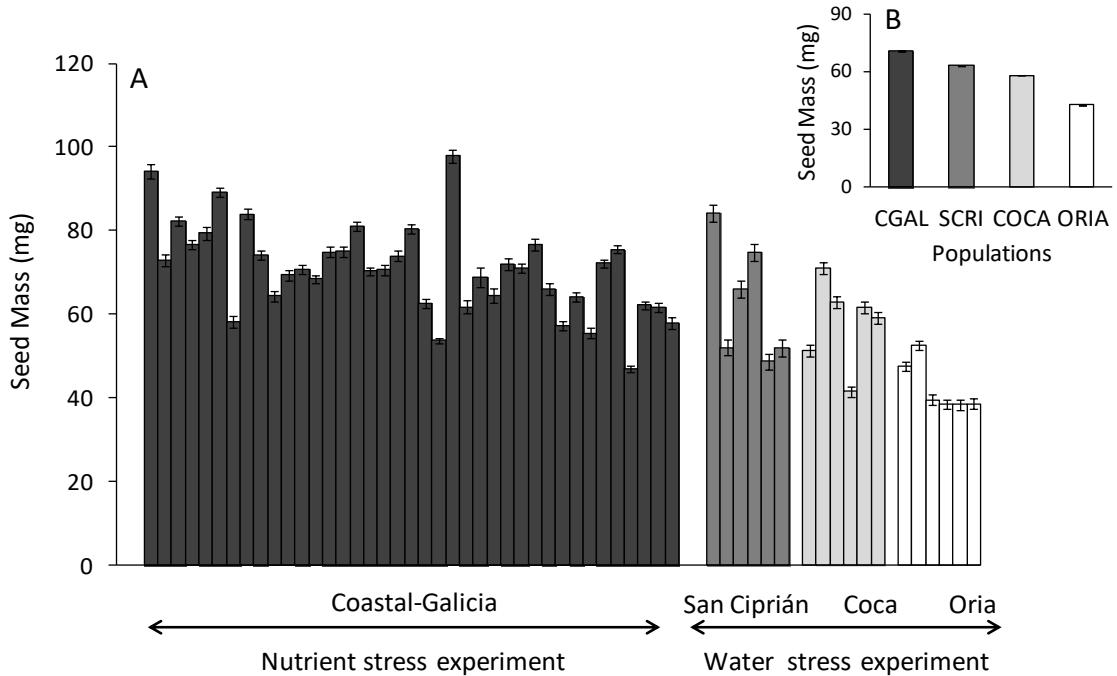


Fig. 1. Seed mass variation between the open-pollinated families (A) and between the four Maritime pine populations (B) included in the two independent experiments of this study. CGAL (Galicia-Coastal), SCRI (San Ciprián), Oria and Coca. Mean \pm standard error. In total, 6235 seeds were tested. Seed mass significantly varied both among populations ($F_{3, 53} = 13.0$, $p < 0.001$) and among families within populations ($\chi^2_1 = 2505$, $p < 0.001$).

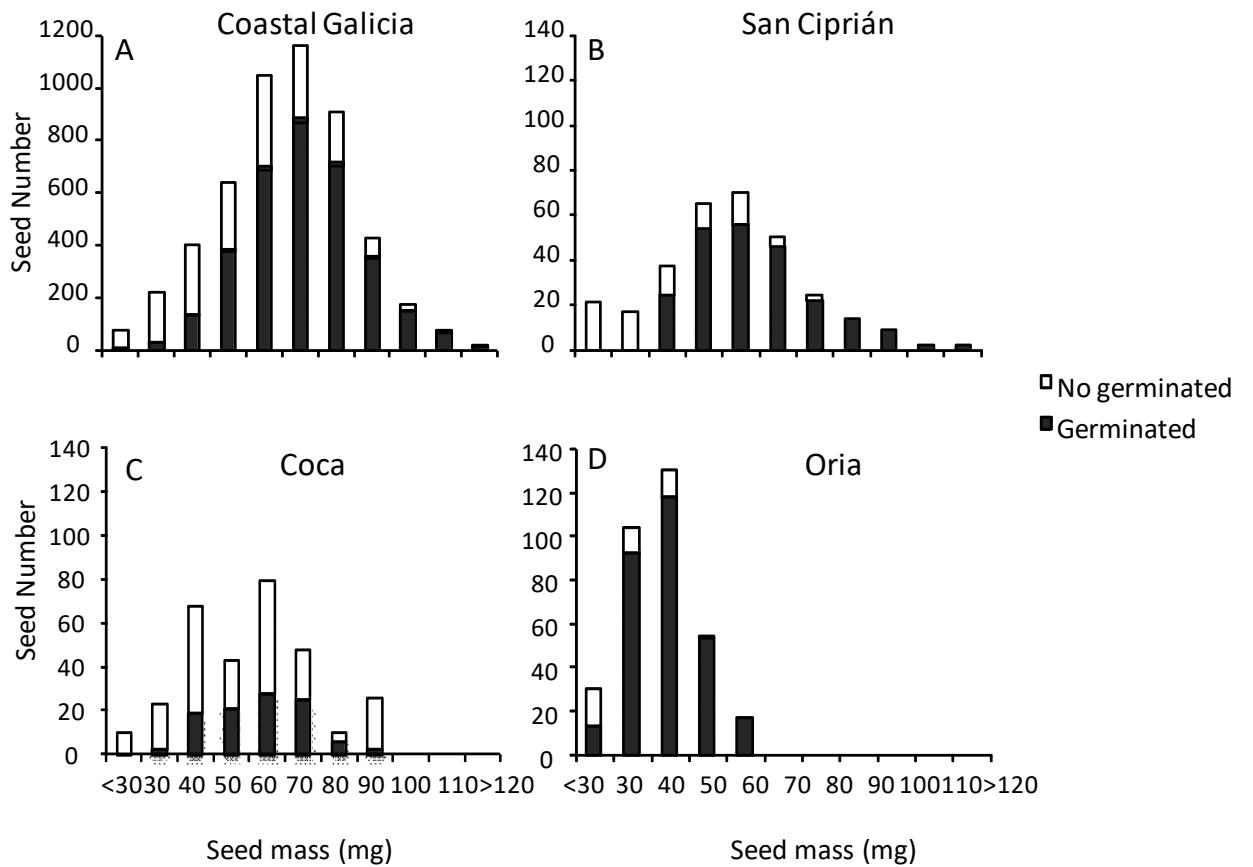


Fig. 2. Distribution of seed mass and influence of seed mass on germination success within four contrasted Maritime pine populations (Coastal Galicia, San Ciprián, Coca and Oria). Number of germinated and no germinated seeds for each seed mass class are showed.

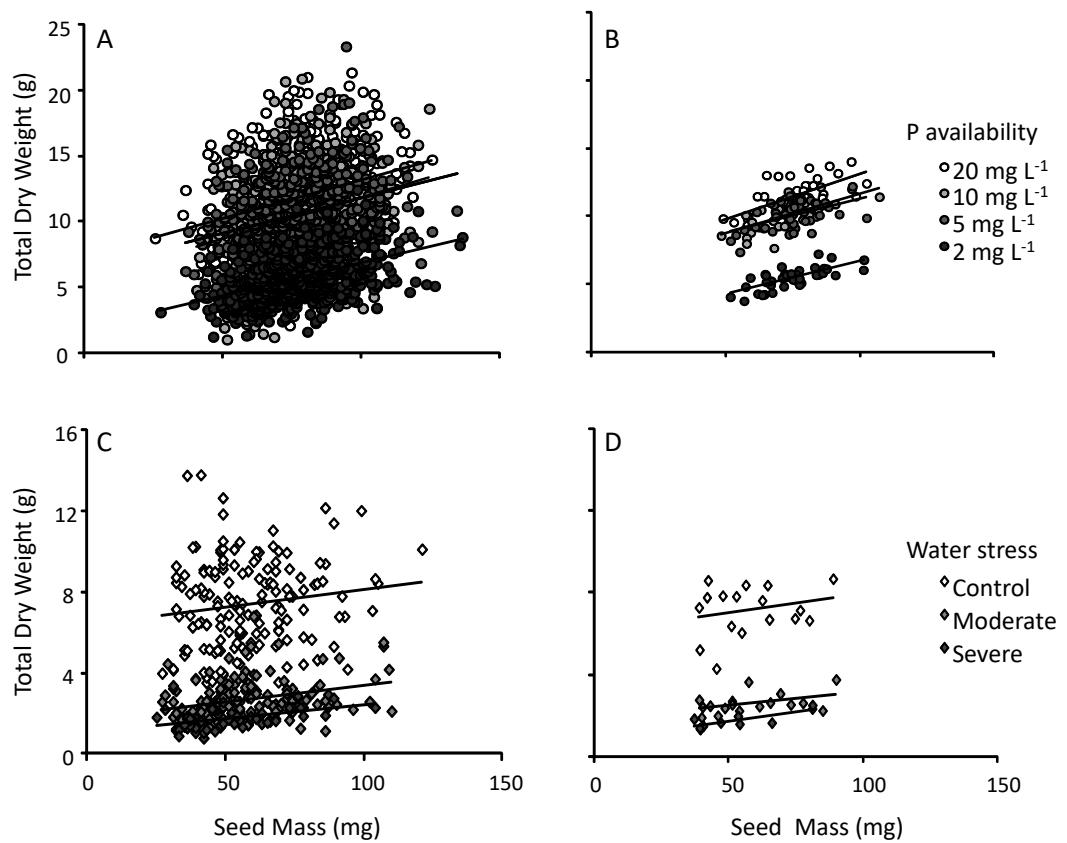


Fig. 3. Relationship between seed mass and seedling biomass in the nutrient stress experiment (panels A and B) and the water stress experiment (panels C and D). Left panels (A and C) show the relationships at the individual phenotypic level ($N = 479\text{-}600$ seedlings for the nutrient experiment and $N = 48\text{-}158$ for the water stress experiment). Right panels show the relationships at the family level for each treatment in the nutrient and water stress experiments ($N=39$ and $N=18$ families respectively). There were fewer individuals for the severe water stress treatment due to the high mortality. All relationships were statistically significant ($p<0.05$). Please note the parallel slopes across treatments in both experiments (ANCOVA $P > 0.05$).

Table 1. Summary of the generalized and general mixed models for the analysis of the sources of variation for germination rate and germination time in two independent experiments. Degrees of freedom (DF), F-ratios and p-values of the effects of the pine population, the covariation with the individual seed mass and their interactions are shown. Statistically significant effects ($p<0.05$) are highlighted in bold font. Please note that these variables were taken before applying the stress treatments.

Germination	Nutrient stress treatment			Water stress treatment		
	DF	F	p>F	DF	F	p>F
Seed Mass	1,5216	544.8	<0.001	1,954	93.5	<0.001
Provenance				2,15	1.0	0.393
Seed Mass*Provenance				2,954	6.4	0.002
Germination Time						
Seed Mass	1,3389	14.5	<0.001	1,619	7.7	0.006
Provenance				2,15	4.7	0.026
Seed Mass*Provenance				2,619	0.7	0.497

Table. 2. Summary of the ANCOVA models for testing whether the effect of seed mass on different seedling traits differed across the stress treatments and the pine population in the two experiments. F ratios and associated probability levels are shown for the seed mass (SM) by nutrient treatment interaction in the nutrient stress experiment (left), and for the seed mass by water stress treatment and seed mass by population interactions in the water stress experiment.

	Nutrient stress experiment		Water stress experiment			
	SM × Nutrient treatment		SM × Water treatment		SM × Provenance	
	F	p > F	F	p > F	F	p > F
Height at age 1	1.3	0.262				
Height at age 2	1.5	0.216				
Height at age 3	1.1	0.330				
Height at age 4	1.5	0.204				
Height at age 5	1.6	0.190				
Height at age 6	2.0	0.116				
Final height	1.6	0.179	0.1	0.890	0.5	0.614
Diameter	2.1	0.105	2.2	0.116	1.3	0.268
Total Dry Weight	1.2	0.323	0.1	0.897	0.1	0.877
Aerial Dry Weight	1.8	0.138	0.7	0.484	0.0	0.981
Root Dry Weight	0.5	0.681	0.2	0.826	0.4	0.692
Root Shoot Ratio	1.0	0.396	1.0	0.380	0.5	0.593

Supplementary material

Is the benefit of larger seed provisioning on seedling performance greater under abiotic stress?

Estefanía Suárez-Vidal, Luis Sampedro, Rafael Zas*

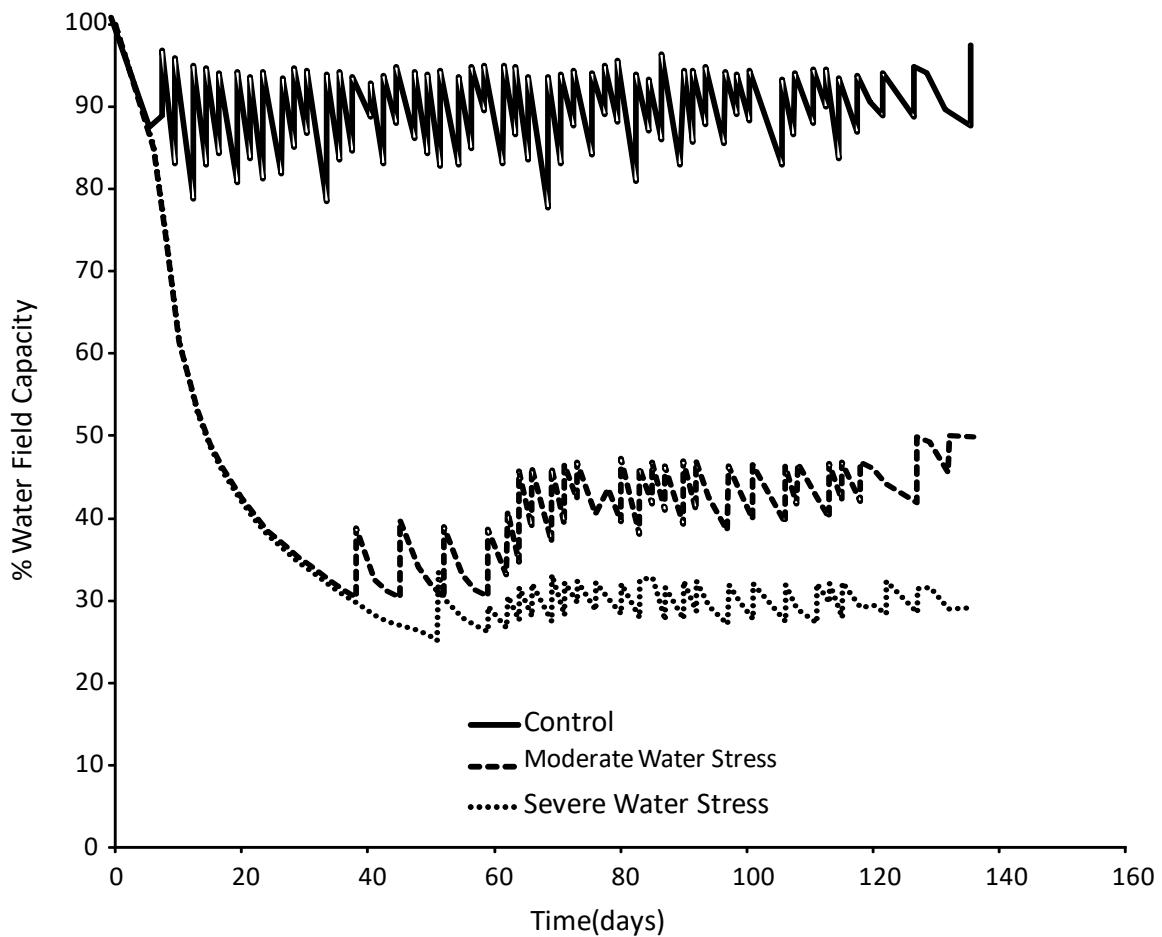


Fig. S1. Percentage of water field capacity in the different water stress treatments.

Water availability in each treatment (Control = 80-95% of field capacity; Moderate water stress = 35-50% of field capacity, Severe water stress=25-35%) was maintained by differentially watering the plants every 2-3 days. Mean values of N = 12 pots.

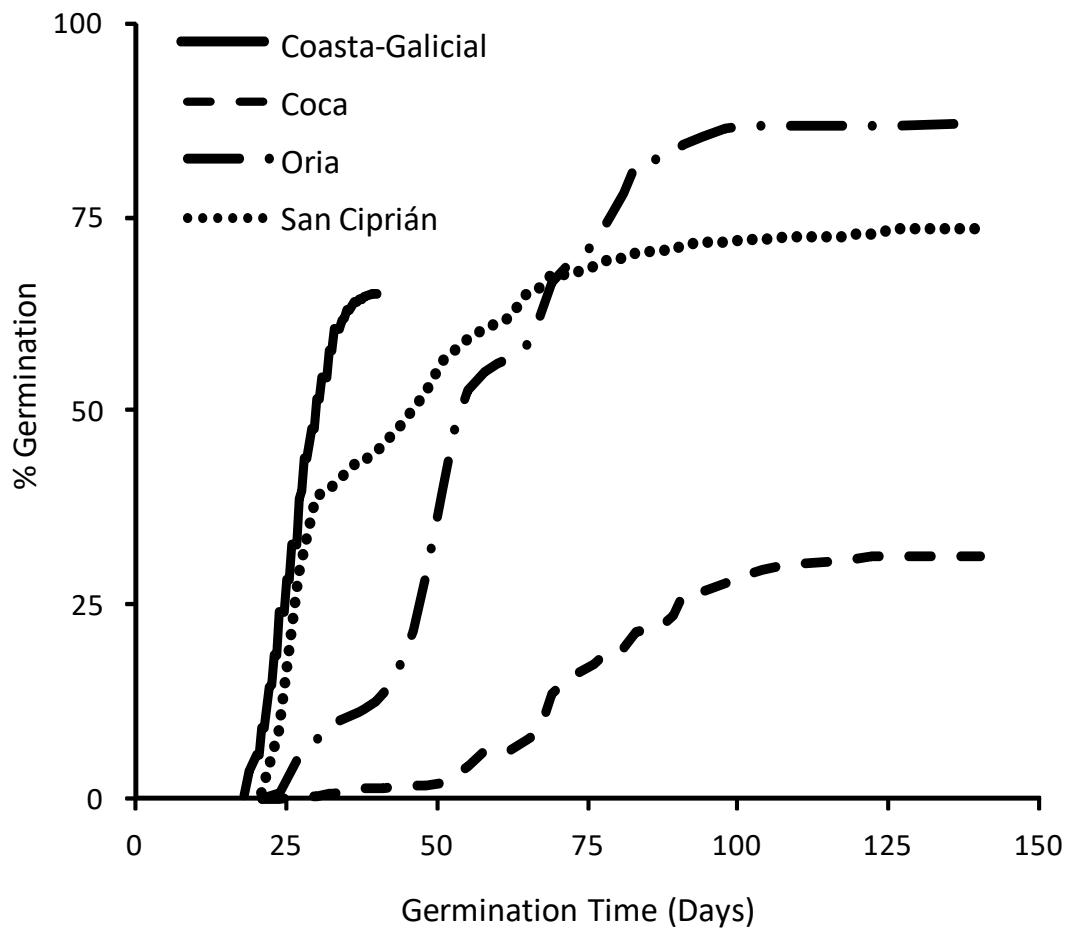


Fig. S2. Variation in germination time (days since sowing) across *Pinus pinaster* populations. In the case of the Coastal Galicia population, assessment of seed germination was done in a different independent experiment and stopped in day 35.

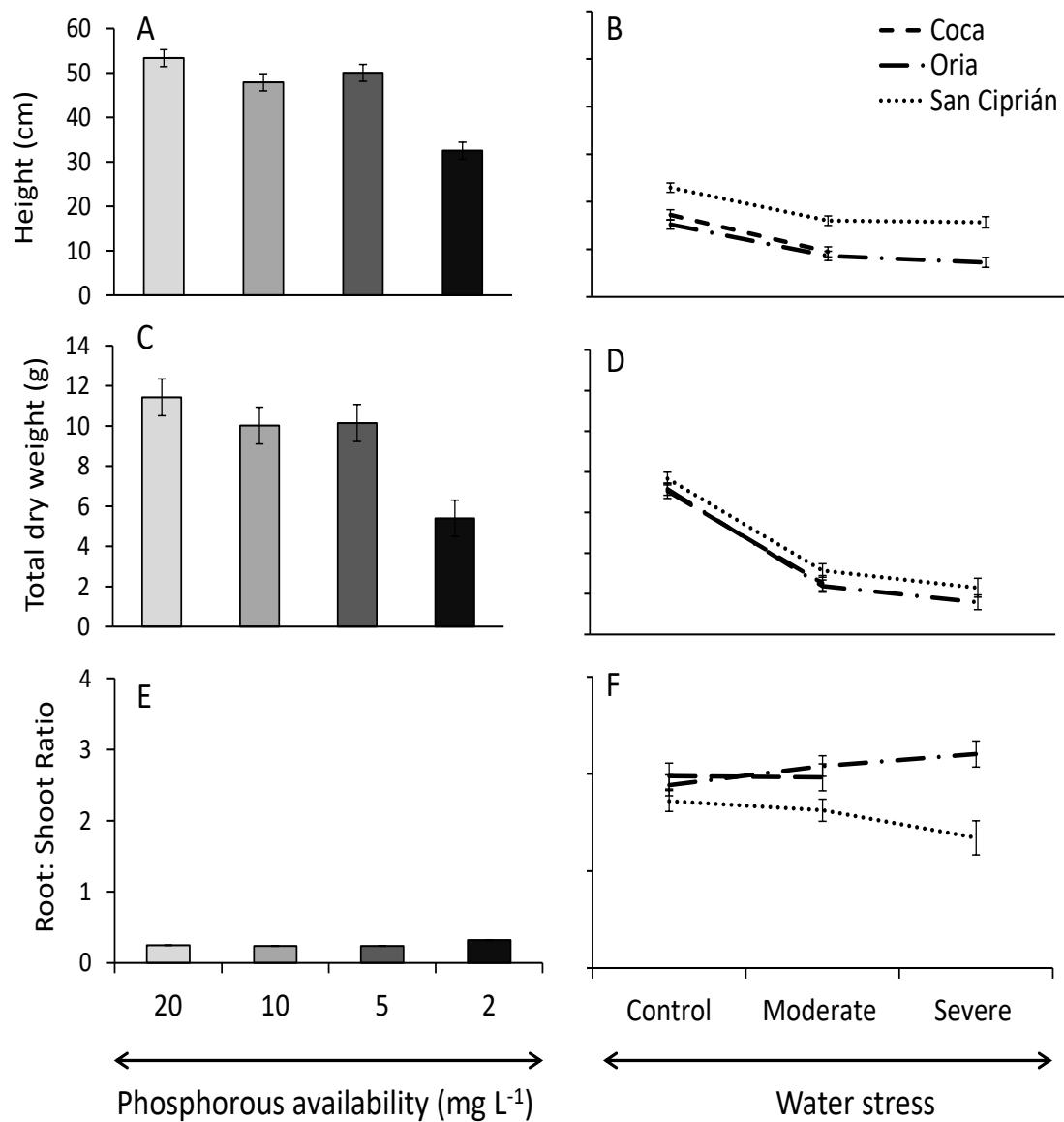


Fig. S3. Effect of the nutrient and water stress treatments on growth, total biomass and allocation priorities. Height (A, B), total dry weight (C, D) and root: shoot ratio (E, F) for the different nutrient stress levels (Phosphorus availability = 20 (control), 10, 5, and 2 mg L⁻¹) on the left, and for the different water stress levels (control, moderate and severe) and provenances (San Ciprián, Oria and Coca) on the right.

Table S1. Summary of the common-slope ANCOVA model for the analyses of different seedlings traits in the nutrient stress experiment. Degrees of freedom (DF), F ratios (F) and associated probability levels ($p>F$) are shown for the fixed effect of the nutrient treatment and the covariation with the seed mass, assuming a common covariation across the different treatments. Significant effects ($p<0.05$) are highlighted in bold font.

Seedling trait	Nutrient treatment			Seed Mass		
	DF	F	$p>F$	DF	F	$p>F$
Height 1	3, 9	29.3	<0.001	1, 2305	344.1	<0.001
Height 2	3, 9	38.9	<0.001	1, 2305	332.6	<0.001
Height 3	3, 9	41.0	<0.001	1, 2305	253.8	<0.001
Height 4	3, 9	46.4	<0.001	1, 2304	164.9	<0.001
Height 5	3, 9	59.4	<0.001	1, 2302	99.9	<0.001
Height 6	3, 9	70.1	<0.001	1, 2214	48.9	<0.001
Final height	3, 9	64.5	<0.001	1, 1980	26.0	<0.001
Diameter	3, 9	78.1	<0.001	1, 1980	108.9	<0.001
Total Dry Weight	3, 9	56.9	<0.001	1, 1979	220.0	<0.001
Aerial Dry Weight	3, 9	60.4	<0.001	1, 1979	199.6	<0.001
Root Dry Weight	3, 9	39.4	<0.001	1, 1981	211.1	<0.001
Root Shoot Ratio	3, 9	108.1	<0.001	1, 1979	0.1	0.783

Table S2. Summary of the common-slope ANCOVA model for the analysis of different seedlings traits in the water stress experiment. The effects of the water treatment, the provenance, the interaction between both and the covariation with seed mass. Significant effects ($p < 0.05$) are highlighted in bold font.

	Water treatment			Provenance			Twater*PROC			Seed Mass		
	DF	F	p>F	DF	F	p>F	DF	F	p>F	DF	F	p>F
Height	2, 322	281.1	<0.001	2, 14	51.4	<0.001	3, 322	10.7	<0.001	1, 322	4.8	0.029
Diameter	2, 326	187.3	<0.001	2, 14	1.4	0.278	3, 326	4.7	0.003	1, 326	0.0	0.908
Total Dry Weight	2, 326	535.5	<0.001	2, 14	1.7	0.212	3, 326	1.7	0.169	1, 326	3.3	0.068
Aerial Dry Weight	2, 326	417.6	<0.001	2, 14	3.9	0.045	3, 326	2.2	0.092	1, 326	3.5	0.063
Root Dry Weight	2, 326	406.1	<0.001	2, 14	0.0	0.994	3, 326	0.5	0.721	1, 326	2.1	0.145
Root Shoot Ratio	2, 326	0.5	0.621	2, 14	12.3	0.001	3, 326	3.5	0.016	1, 326	0.4	0.534