

Seed mass variation, germination time and seedling performance in a population of *Pinus nigra* subsp. *salzmannii*

P. A. Tíscar Oliver^{1*} and M. E. Lucas Borja²

¹ Centro de Capacitación y Experimentación Forestal. C/ Vadillo-Castril, s/n. 23470 Cazorla (Jaén). Spain

² Instituto de Investigación en Energías Renovables. Grupo de Medio Ambiente y Recursos Forestales. Universidad de Castilla-La Mancha. 02071 Albacete. Spain

Abstract

Seed size and germination time are related to factors affecting natural regeneration of forest stands. Sources of seed mass variation and the effect of seed mass on seedling performance were investigated in a *Pinus nigra* subsp. *salzmannii* population. From eight maternal plants, seed mass variation was characterized for two crops at the within-individual level by mean, coefficient of variation, skewness and kurtosis values. Fifty seeds were planted in each of two substrate types, to monitor emergence, and survival and seedling performance during one growing season. Most variation in seed mass occurred within trees (c. 61%), rather than between them (c. 39%). Seed mass influenced emergence, depending on substrate type and mother plant. There were between-tree differences in the date of emergence, and a negative relationship was found between seed mass and date of emergence for the pine-seeds sown in peat substrate. The growth of seedlings was positively related to seed mass. It was argued that successful establishment of seedlings appears to be dependent on mother plant identity associated to seed traits, and that *Pinus nigra* stands could achieve regeneration by two different alternatives, depending on whether environmental variance overwhelms genetic variation.

Key words: maternal effects; seedling growth; kurtosis; skewness; forest regeneration.

Resumen

Variación del peso y del tiempo de emergencia de las semillas, y crecimiento de las plántulas en una población de *Pinus nigra* subsp. *salzmannii*

La variación del peso de las semillas y la relación entre el peso de las semillas y el crecimiento de las plántulas se investigaron en una población de *Pinus nigra* subsp. *salzmannii*. La variación del peso se analizó en dos cosechas recogidas en ocho árboles, utilizando los valores medios, y los coeficientes de variación, simetría y kurtosis. La variación dentro de los árboles fue del 61% y entre los árboles del 39%. Dos muestras de 50 piñones se sembraron en sendos tipos de sustrato para seguir la emergencia, la supervivencia y el crecimiento de las plántulas durante un período vegetativo. El peso de las semillas influyó en la emergencia, dependiendo del tipo de sustrato y de la planta madre. Hubo diferencias entre árboles en la fecha de emergencia, que se correlacionó negativamente con el peso de las semillas sembradas en turba. El crecimiento de las plántulas sí se correlacionó positivamente con el peso de los piñones. Se concluyó que el establecimiento de las plántulas depende de la identidad de la planta madre concomitantemente con el peso de las semillas, y que la regeneración natural de *Pinus nigra* podría conseguirse mediante dos estrategias dependiendo de si la variabilidad ambiental supera la genética o no.

Palabras clave: efectos maternos; crecimiento de plántulas; asimetría, curtosis; regeneración natural.

Introduction

Regeneration of Mediterranean pines is limited by factors acting at different stages of the regeneration process, *i.e.* seed, seedling and sapling. At the seed stage, dispersal capacity, environmental conditions du-

ring germination and predation affect the reproductive success of pines, whilst summer drought has been quoted as the main factor causing mortality at the seedling stage (Tíscar, 2007; Castro *et al.*, 2004). All the mentioned factors can be related to seed size.

Thus, seed mass is found to be negatively correlated to dispersal capacity in *Pinus sylvestris* (Debain *et al.*, 2003), and it is generally accepted that, relative to large ones, small seeds usually emerge less frequently and

* Corresponding author: pedroa.tiscar@juntadeandalucia.es
Received: 13-04-10; Accepted: 21-07-10.

more slowly (Tripathi and Kahn, 1990; Rice *et al.*, 1993). As far as we are aware, the relationship between seed mass and the probability of being predated has not been investigated for pine species. Gómez (2004) found that the predation rate is higher for large acorns of Mediterranean evergreen oaks, but a similar relationship does not apparently exist in the sympatric species *Pinus nigra* (Tíscar, unpublished data).

Different studies have shown seedlings from larger seeds to withstand stress or disturbance better (Westoby *et al.*, 1997; Leishman *et al.*, 2000). For instance, seedling survival has been correlated with seed mass in Mediterranean shrubland species, due to a higher root allocation in seedlings originating from bigger seeds (Lloret *et al.*, 1999).

As a result, seed mass and germination behaviour are found to be related to plant fitness and subject to selection in natural conditions (Simons and Johnston, 2000; Gómez, 2004; Halpern, 2005). This would be expected to affect the regeneration process of Mediterranean pine-forests in future climate scenarios (see IPCC 2007), with implications in the vulnerability and adaptive capacity of this type of forest. Therefore, a better understanding of the sources of seed mass variation, and its consequences in the natural regeneration process could help the improvement of silvicultural methods.

According to Reich *et al.* (1994), pine-seed mass has been shown to vary depending on collection year, age of the seed tree, cone size, location of the seed within the cone and the cone upon the tree, nutrient availability and stand density. This means that observed variation in the weight of pine-seeds would respond mainly to environmental variability, although genetic factors are also important (Castro, 1999). Three genetic components are thought to determine seed size: the pollen donors, the seed-bearing plant, and the genetic constitution of the embryo (Castro *et al.*, 2006).

Inbreeding depression, caused by a reduction in the number of pollen donors (Bañuelos and Obeso, 2003) or by the occurrence of self-pollination (Alonso, 2005), usually lowers seed weight. Nevertheless, pollen quality, along with the genetic constitution of the embryo, appears to have a weak influence in determining seed size, the effect exerted by the seed-bearing plant being much more important (Castro *et al.*, 2006). This means that genetic differences among maternal plants could explain most of the inter-individual seed mass variation observed in pines growing in the same stand (Castro, 1999).

Environmental factors include biotic and abiotic conditions during the time of seed maturing. For ins-

tance, Hódar *et al.* (2003) found that *Pinus sylvestris* produces smaller and fewer seeds per cone when the tree has been defoliated by processionary caterpillar (*Thaumetopoea pityocampa*).

Nutrient fertilization is known to increase seed mass in cultivated plants, but nutrient availability does not change much in forests from one year to another. As a consequence, soil fertility should have a minor influence in inter-annual variability of seed mass, although it can be a source of variation between forest stands.

Drought is known to be a major factor in reducing the size of pine-seeds. For example, *Pinus sylvestris* from Sierra Nevada (Southeast Spain) consistently produces heavier seeds when cones ripen in rainy years (Castro, 1999). Additionally, the interaction of water availability with nutrient uptake cannot be disregarded.

Along with water stress, temperatures above or below the optimum at flowering or during seed maturing may also be detrimental to seed size (Prasad *et al.*, 2002). This temperature effect could explain seed mass variation across populations of the same tree species growing at different altitudes or latitudes (Aizen and Woodcock, 1992; Oleksyn *et al.*, 1998). For instance, Tíscar (2002) quoted a smaller mean seed mass for trees growing at higher altitude in a study of the reproductive capacity of *Pinus nigra* subsp. *salzmannii* in relation to mother plant age.

Tree age can be an important factor in determining seed size, because the ability to gather nutrients and water might be dismissed in ageing individuals. In this respect, it is worth remembering that new silvicultural systems recommend management strategies which favour currently under-represented age classes and old trees (Tíscar, 2002). Thus, it seems necessary to investigate the contribution of old trees in forest stand regeneration.

Mean values are normally used when describing seed mass and germination behaviour, although variation around the mean might have an adaptive value of equal importance (Herrera, 2009). In the case of wind-dispersed pines, where seed mass is associated with dispersal distance (Debain *et al.*, 2003), variability of seed mass leads to variable seed shadows, which may constitute a form of bet-hedging strategy to withstand unpredictability and heterogeneity in Mediterranean environments (Crean and Marshall, 2009).

Apart from variance, skewness and kurtosis could be important descriptors of plant phenotype, particularly in species producing large numbers of seeds (Herrera, 2009). For example, Castro (2006) related

early emergence to post-summer seedling survival in a Mediterranean population of *Pinus sylvestris*. Therefore, it is possible to argue that a pine tree showing a negative skewed distribution of germination times would have greater fitness, because a fraction of its seedlings would be more likely to survive drought. Similarly, a negative kurtosis would be expected in a well-adjusted bet-hedging strategy.

Considering all these implications, the aims of this study were to analyze (i) within- and among-tree variability in seed mass and germination time, and (ii) the effect of seed mass on seedling emergence and performance in a population of *Pinus nigra* subsp. *salzmannii*.

Materials and methods

Species and study area

Seeds were collected in a natural stand of Spanish black pine (*Pinus nigra* Arn. subsp. *Salzmannii* (Dunal) Franco) located at an altitude of 1,800 m above sea level in Sierra del Pozo, a calcareous mountain range in Southeast Spain. At the study site, *Pinus nigra* forms an open forest with an understorey of *Juniperus communis*, *J. sabina*, and *Erinacea anthyllis*. The climate is oromediterranean and humid (Rivas-Martínez, 1987). Mean annual precipitation is 1,400 mm, although summer drought is pronounced, and mean annual temperature 7°C.

Pollination of black pine occurs during the month of May in the study area, and seeds are mature in December of the second year. We analysed seed mass variation in seeds collected in January 2000 and 2003 (see later). Total precipitation from May 1998 to December 1999, and from May 2001 to December 2002 was computed to analyse the possible influence of rainfall in seed mass. Values were 1,272 mm and 1,414 mm for the first and second period respectively, with no statistical difference between them (Wilcoxon test of matched samples; $Z = 0.765$; $n = 21$; $P = 0.445$).

Experimental design

Eight mature trees with a similar competitive status were arbitrarily chosen, covering the range of diameters found in the studied population of black pine (ca. 4 ha). At this stage, diameter was used as a surrogate of age. A core was extracted from each tree to count

the number of annual growth rings. This allowed us to order trees from youngest to oldest (trees no. 1 to 8 from here onwards). We counted: 55, 64, 119, 204, 398 and 743 rings at a height of 1,30 m. The ages of the other two trees were already known, and they were approximately 900 and 1,100 years old (see Creus, 1998).

In January 2000, a minimum of 30 pinecones were collected from each tree, using a 7 m pole. The pinecones were completely opened in an oven set at 40°C, then a sample of 100 sound seeds were randomly chosen from each tree and individually weighed in a 2 mgr precision scale. These seeds were used in the sowing experiment that year (see later). In 2003, a variable number of pinecones were again collected from the same trees (0, 1, 4, 6, 7, 8, 10 and 12 cones depending on the tree), in order to determine patterns of seed-mass variation across years. Studied pine trees did not produce cones in 2001, and only trees no. 1, 7 and 8 produced pinecones in 2002 (data not included in the analysis).

Sowing experiments began on March 21st 2000. Two types of substrate were used: natural soil collected in the study site, and a commercial peat («Vapo-Gro Substrato superior»). From each mother-tree, 50 seeds were distributed at random within each substrate type and sown at a depth of 1 cm in 54-cell seedling trays of 400 cm³ (18 cm in height). Trays were placed in a forest gap next to Centro de Capacitación y Experimentación Forestal, and regularly watered until the end of emergence on May 13th. From this date onwards, only the peat trays were watered until substrate saturation every other day. Emergence was monitored in both types of substrate, whilst survival of seedlings was monitored in natural soil only. Performance (belowground and aboveground dry biomass, height and leaf area) was measured in seedlings from the peat trays. A belowground to aboveground biomass ratio (B/A) was calculated too.

The percentage of empty seeds was measured, and the viability of filled seeds checked in a growth chamber before starting the sowing experiments. There was no difference in the percentage of germination between the growth chamber and outdoors (Wilcoxon test of matched samples; $Z = 1.40$; $n = 8$; $P = 0.161$).

Seedling emergence was monitored daily. In this respect, every seedling was marked as emerged when it had straightened up. The height of each seedling was measured in mid-June (89 d after sowing), when the dry season begins in an average year, and once again

in mid-October at the end of the growing season (216 d after sowing). Seedlings were then uprooted, leaf area was measured in a Li-Cor 3000 portable area meter (Li-Cor Inc., Lincoln, NE, USA), and belowground dry biomass (root + hypocotyl, due to difficulties in distinguishing root collar) and aboveground dry biomass (epicotyl) was weighed after placing plants in an oven (80°C, 48 h).

Data analysis

Analysis of seed mass and germination time variation within and among trees were performed with ANOVAs. The effect of seed size on emergence and survival was analyzed for each tree in the two types of substrate by using a logistic regression. As seed mass was weighed in 2 mg intervals, logistic regressions were carried out with a Generalized Linear Model, using a binomial distribution for the error term, logit as the link function and the deviance as the overdispersion parameter. Mother plants were pooled to carry out additional logistic regressions with the residuals from an ANOVA of maternal plant and seed mass as independent variable. This procedure was followed to ensure the independence of seeds with a common origin, performing the logistic regression with the part of variability explained by the seed mass. The relation between seed mass and date of emergence was similarly analyzed for all maternal plants pooled by a regression in which the independent variable was the residuals from a previous analysis of variance between the maternal plant and seed mass, for each type of substrate. The log likelihood ratio was used as an indicator of model fit in the analysis of logistic regression (Menard, 2000).

Seedling performance variables were analyzed with analysis of covariance (ANCOVAs), with seed mass as a covariate. When the categorical and continuous predictor variables interacted, influencing responses on the outcome, a separate slope design was used. The correlations between mean size of seeds produced by each maternal plant, the mean values of growth parameters and mother plant age were analyzed by using the Spearman rank correlation coefficient.

In parametric analyses, the maternal plant was considered to be a random factor, and data were transformed to reach normality and homocedasticity when necessary (Zar, 1996). Type III sum of squares were used owing to the unbalanced nature of the data. All the

analysis were performed with the computer software Statistica 5.5 (StatSoft, 1999). Throughout the paper, values are means \pm SE.

Results

Sources of seed mass variation

Mean seed mass varied amongst trees, amongst cones within the same tree and between fruiting years (Table 1). Differences amongst trees were found in both 2000 ($F_{7,786} = 73.07$; $P = 0.000$) and 2003 ($F_{6,1105} = 46.99$; $P = 0.000$). No relationship was observed between number and mean mass of seeds borne in the same pinecone ($F_{1,46} = 0.87$; $P > 0.05$).

Mean seed mass was higher for 5 of the 7 trees producing pinecones in 2003, but the rank of means among maternal plants remained fairly constant between 2000 and 2003 (Fig. 1). On the other hand, coefficients of variation, skewness and kurtosis varied with a less recognizable pattern. Extreme values of seed mass remained fairly constant between years, ranging from 8 to 32 mg in 2000 and from 8 to 36 mg in 2003, although the mode values rose in 2003 with respect to 2000, causing the noted increase in the values of means (recognized by the more negative values of skewness, Table 2).

$14.2 \pm 2\%$ of the seeds sampled in 2000 and $20.6 \pm 2.7\%$ of the overall seeds collected in 2003 were empty, the difference being significant (Wilcoxon test of matched samples; $Z = 2.19$; $n = 7$; $P = 0.027$). The proportion of empty seeds was not related to mother plant age (Spearman correlation; $P > 0.10$ for both years), but a negative

Table 1. Results of ANOVA testing for seed mass differences among trees, crop years and cones within a tree

Source	df	Type III SS	F	P
<i>Years 2000 and 2003</i>				
Tree	7	6,789.7	80.39	0.000
Year	1	2,705.7	224.24	0.000
Error	1,889	22,793.2		
<i>Year 2003</i>				
Tree*	5	3,370.9	4.02	0.005
Cone(Tree)	40	8,153.4	40.43	0.005
Error	1,053	5,253.6		

* Data from tree no. 1 were not included in this analysis, because they came from one single cone.

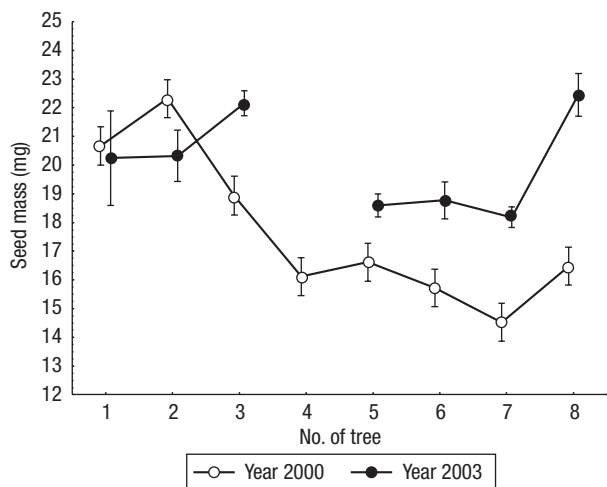


Figure 1. Masses (mean ± SE) of seeds collected from the eight trees used in the the study.

almost significant correlation existed between mean seed mass and mother plant age ($r_{\text{Spearman}} = -0.714$; $n = 8$; $P = 0.054$ for 2000; $r_{\text{Spearman}} = -0.071$; $n = 7$; $P = 0.861$ for 2003). No relationship was found between mother plant age and other moments of seed mass distribution (Spearman correlation; $P > 0.10$ for all the cases).

Seedling emergence and mortality

A greater proportion of seeds ($94.5 \pm 1.5\%$) emerged in the peat substrate than in the natural soil ($90 \pm 1.5\%$), but this difference was not significant (K-S test; $DN = 0.5$; $P = 0.271$). Mean emergence time varied from 24.3 ± 0.8 d to 31.5 ± 1.8 d after sowing in the natural soil, and from 23.7 ± 0.6 d to 31 ± 0.6 d in the peat substrate (Table 3). There were between-tree differences in the date of emergence in both the natural soil experiment ($F_{7,351} = 5.28$; $P = 0.000$) and the commercial peat one ($F_{7,369} = 18.41$; $P = 0.000$). A negative relationship was found between seed mass and date of emergence for the pine-seeds sown in peat substrate ($F_{1,375} = 4.98$; $P = 0.026$), although the proportion of variance explained by this independent factor was very low ($r^2 = 0.01$). No relationship was found for seeds sown in natural soil ($F_{1,357} = 2.66$; $P = 0.103$). However, a positive correlation existed between tree age and kurtosis of germination time ($r_{\text{Spearman}} = 0.714$; $n = 8$; $P = 0.059$).

Logistic regressions for each plant showed that seed mass was positively related to seedling emergence for three trees in the commercial peat sowing experiment,

Table 2. Descriptive statistics fo seed mass (mg) per maternal plant

Tree number	No. of seeds		Range		Mean weight		Coefficient of variation		Skewness		Kurtosis	
	2000	2003	2000	2003	2000	2003	2000	2003	2000	2003	2000	2003
1	100	16	10-28	14-22	20.67	20.25	15.23	11.36	-0.59	-1.48	1.71	2.32
2	100	55	8-32	8-36	22.32	20.33	13.26	47.91	-0.83	-0.10	5.04	-1.87
3	100	235	12-30	8-30	18.93	22.16	16.27	13.76	0.31	-0.76	0.80	2.31
4	100	—	8-26	—	16.12	—	26.36	—	0.54	—	-0.60	—
5	100	275	8-24	8-24	16.62	18.59	16.00	14.46	-0.14	-0.44	0.82	0.40
6	100	106	10-22	12-24	15.72	18.77	14.18	16.78	0.28	-0.40	0.28	-0.58
7	100	338	8-22	8-24	14.52	18.18	16.94	13.86	0.42	-0.65	0.49	0.77
8	100	79	8-26	8-28	16.48	22.46	23.24	15.36	0.13	-1.12	-0.54	2.78

Table 3. Descriptive statistics of time to emerge (days) per maternal plant

Tree	Natural soil substrate				Commercial peat substrate			
	Mean	C.V.	Skewness	Kurtosis	Mean	C.V.	Skewness	Kurtosis
1	24.30	21.24	0.245	-1.306	23.67	17.66	0.517	0.674
2	26.35	18.18	-0.517	-0.666	24.98	18.09	0.318	0.674
3	30.57	18.03	0.915	2.889	29.77	18.24	-0.219	-0.754
4	28.48	19.31	0.298	1.409	26.13	19.21	0.048	-1.003
5	27.38	21.21	0.308	0.611	24.00	18.46	0.452	-0.579
6	29.52	22.06	1.010	2.831	27.20	16.32	-0.195	-0.682
7	29.74	18.88	0.146	1.921	29.47	12.62	-0.416	-0.046
8	31.48	39.39	3.869	21.029	31.00	12.23	-0.961	0.681

Table 4. Results of the logistic regressions performed to analyse the relation between seed mass and emergence and survival in each type of substrate

Tree	Emergence										Survival				
	Natural soil					Commercial peat					Natural soil				
	Estimate	SE	χ^2	P	R ²	Estimate	SE	χ^2	P	R ²	Estimate	SE	χ^2	P	R ²
1	0.045	0.118	0.14	0.705		0.888	0.118	0.14	0.705		-0.319	0.155	4.21	0.040	0.11
2	-0.322	0.135	5.66	0.017	0.12	0.412	0.101	16.70	0.000	0.36	-0.289	0.170	2.90	0.088	
3	0.064	0.122	0.28	0.595		-0.897	0.243	13.66	0.000	0.35	0.023	0.114	0.43	0.837	
4	-0.061	0.086	0.50	0.478		0.648	0.212	9.364	0.002	0.29	-0.128	0.091	2.00	0.157	
5	-0.029	0.157	0.03	0.854							-0.117	0.147	0.63	0.427	
6	0.353	0.212	2.77	0.096							0.246	0.190	1.67	0.196	
7	0.063	0.165	0.14	0.705		0.314	0.167	3.52	0.061		-0.025	0.149	0.28	0.866	
8	0.105	0.112	0.88	0.348		0.565	0.184	9.42	0.002	0.26	-0.189	0.095	0.65	0.420	
All	-0.006	0.054	0.013	0.909		0.311	0.081	14.70	0.000	0.11	-0.074	0.042	3.03	0.082	

but negatively related for one tree in the commercial peat and one in the natural soil. Interestingly, emergence of mother plant no. 2 was both positive and negatively affected by seed mass, depending on the type of substrate. An overall positive relationship persisted in the peat substrate when all maternal plants were pooled (Table 4). An additional logistic regression for the combined natural soil-commercial peat dataset showed that seedling emergence was related to the type of substrate ($\chi^2 = 14.25$, $P = 0.0001$) and seed mass ($\chi^2 = 20.71$, $P = 0.0000$). The interaction substrate \times seed mass was also significant ($\chi^2 = 20.70$, $P = 0.0000$)

Seedling survival in mid-July, after 9 weeks of dry weather, was only studied in the natural soil sowing experiment. Here, seedling survival ranged from 45% to 88%. Seed size was only found to be related to seedling survival for one tree, and no relationship appeared when all seeds were pooled (Table 4). Only two seedlings originating from the same maternal plant (tree no. 2) survived after the first growing season.

Seedling performance

ANCOVAs showed that early growth, namely seedling height in June, was affected by seed mass and maternal plant identity, and that their effect was still present by the end of the first growing season. Thus, mother plant and seed mass factors were still affecting seedling height, when it was measured in October, along with all the biomass, and leaf area measurements carried out shortly afterwards. Only the belowground/aboveground ratio of biomass was not affected by seed mass (Table 5).

On the other hand, mean values of seed mass per maternal plant were positively correlated with mean growth values for all the parameters measured, excepting leaf area and seedling height October. Belowground biomass, aboveground biomass and seedling height in October were negatively related to mother plant age ($r_{\text{Spearman}} < -0.710$; $n = 8$; $P < 0.05$ in all the cases).

Discussion

Seed mass varied up to 4-fold within individuals of the *Pinus nigra* study population. As a consequence, most variation in seed mass occurred within trees (c. 61%), rather than between them (c. 39%). Different studies have documented extensive levels of within-plant variability in seed size (Herrera, 2009). For example, Bladé and Vallejo (2008) also reported up to 4-fold seed mass variation in families of *Pinus halepensis*, but within individual variation of seed traits appears to be generally limited in pine species (García *et al.*, 2009).

Results showed that skewness and kurtosis of the within-plant distribution of seed mass were either positive or negative, depending on the mother plant. However, the sign seemed to change arbitrarily across the two crops considered (Table 2), suggesting a developmental instability or plastic response to environmental conditions as fruits develop. The temporal variation of mean seed mass across years would confirm this effect of resource availability in determining seed size. Although weather conditions were similar during the

Table 5. Summary of ANCOVAs on seedling performance estimates

	Source	df	Type III SS	F	P
Seedling height June ($R_{adj}^2 = 0.46$)	Tree	7	3,267	126.58	0.0000
	Mass	1	3,958		0.0000
	Error	367	11,474		
Belowground biomass ($R_{adj}^2 = 0.67$)	Tree	7	18,399	28.67	0.0000
	Mass	1	15,761	171.93	0.0000
	Error	342	31,352		
Aboveground biomass* ($R_{adj}^2 = 0.56$)	Tree	7	2,561	2.74	0.0088
	Tree × mass	8	31,132	29.16	0.0000
	Error	334	44,572		
Seedling biomass* ($R_{adj}^2 = 0.75$)	Tree	7	6,372	7	0.0002
	Tree × mass	8	92,038	8	0.0000
	Error	334	73,968		
B/A ratio of biomass ($R_{adj}^2 = 0.10$)	Tree	7	0.5977	5.507	0.0000
	Mass	1	0.0001	0.006	0.9361
	Error	341	5.287	14.92	
Seedling height October ($R_{adj}^2 = 0.38$)	Tree	7	3,235	8.78	0.0000
	Mass	1	3,974	75.47	0.0000
	Error	359	18,900		
Leaf area ($R_{adj}^2 = 0.52$)	Tree	7	4.835	8.742	0.0000
	Mass	1	7.383	93.435	0.0000
	Error	148	11.694		

* Separate-slopes model.

maturing time of both 2000 and 2003 crops, and nutrient availability was unlikely to change remarkably, crop sizes proved to be very different. According to criteria described by Tíscar (2009), the crop size from 2000 was «abundant» in comparison with the «scarce» crop size observed in 2003, *i.e.* the latter crop shared a similar quantity of resources (overlooking the possibility of resource accumulation) among a much smaller number of pine-seeds which, in turn, increased their size.

Nevertheless, the range of individual seed masses was stable across crop years (Fig. 1), the increase of mean values being rather due to a rise of mode values, and coefficients of variation remained quite similar across crop years (see later). This constancy of seed mass suggests that maternal effects (see Roach and Wulff, 1987) are more important than embryo or parental genotype in determining seed mass of pines (Castro, 1999; Castro *et al.*, 2008).

The high coefficient of variation estimated for tree no. 2 in year 2003 (Table 2) was attributable to the abnormal morphology of the few cones (6) collected from that tree; space constraints due to cone morphology are known to affect seed mass (McGinley *et al.*, 1990; García *et al.*, 2009). Similarly, 2003 data from

tree no. 1 came from a single pinecone, but more than five cones were collected from the other trees, which might be enough to obtain reliable results (see García *et al.*, 2009). In any case, it is clear that the occurrence of mast- and non-mast years in *Pinus nigra* constitutes an additional source of seed mass variation, including the production of empty seeds as denoted by the higher percentage of empty seeds collected in the «scarce» crop from 2003; the amount of empty seeds is mediated by the frequency of self-pollination in pines (Kärkkäinen *et al.*, 1999).

As skewness and kurtosis values of seed mass distributions changed from one crop to the other, it is unlikely that this parameter holds any potential adaptive value for the studied species, but different findings could result when comparing «abundant» crops to each other. In this respect, it is necessary to take into account that «abundant» crops or mast-years probably contribute most to the natural regeneration of *Pinus nigra* (Tíscar, 2007, 2009).

Results on skewness and kurtosis of seed mass distributions would still conform to a bet-hedging strategy (Crean and Marshall, 2009), for example through a variation in dispersal capacity (Debain *et al.*, 2003).

This way, seeds would be able to reach the whole variety of microhabitats present in a stand, finding the more suitable ones for establishment (Tíscar, 2003). Similarly, germination time variability and higher moments of this trait could be adaptive for *Pinus nigra*, if they translate into higher regeneration success during the pine's lifetime. In fact, these parameters varied among trees (Table 3), so there would be an opportunity for natural selection. For instance, seedlings of *Pinus sylvestris* emerging earlier are more likely to survive summer drought in the mountains of Southeast Spain (Castro, 2006); from that point of view, the right-skewed distribution of germination times found for most of the studied individuals of *Pinus nigra* was unexpected. The present study found germination time to be largely determined by the mother plant, since the germination time differed between the type of substrate, and among trees, but there was no relationship with seed mass. A variety of studies have also found a significant maternal effect on the time to seedling emergence (Castellanos *et al.*, 2008, and references therein).

Seedling emergence has been either unrelated or positively related to seed mass in Mediterranean populations of pines. Thus, final emergence of *Pinus halepensis* has proved to be unaffected by seed mass (Bladé and Vallejo, 2008), but a positive relationship was found for *Pinus sylvestris* subsp. *nevadensis* (Castro, 1999). Here, results reflected both (i) seedling emergence being unaffected by seed mass, when seeds had been sown in natural soil, and (ii) seed mass exerting a positive effect upon emergence of seeds sown in commercial peat substrate (Table 4). Certainly, analyses showed a negative effect in two cases, being interpreted that heaviest seeds had failed to emerge because they possessed excessively thick coats.

Two reasons, both considering maternal effects, would explain the positive effect found in the commercial peat sowing experiment. First, peat substrate could have been a more stressful environment for emerging seedlings, compared to natural soil. In fact, larger seed size might favour germination under high environmental stress, even when this relationship does not appear under less stressful conditions (Castro, 1999, and references therein). This finding could be interesting from the point of view of *Pinus nigra* seedlings emerging naturally on pine-litter.

Second, and on the contrary, it has been argued that maternal effects are easier to detect under very homogeneous environmental conditions (Nuismer and Gandon,

2008). As commercial peat apparently constitutes a more homogeneous substrate than natural soil, the results would be reflecting that probability of emergence is related to mother plant (some plants being affected and others not), although driven by differences in seed mass. However, both explanations are mutually exclusive, and rather speculative. Therefore, more research should be carried out in this direction.

This study found no relationship between seed mass and survival of *Pinus nigra* seedlings 115 d after sowing, with the exception of tree no. 1, suggesting a possible maternal effect (Table 4). This impression is reinforced by the fact that the only two seedlings still alive after the first growing season came from the same pine tree, and originated from medium size seeds (20 and 22 mg).

Seedling performance of *Pinus nigra* was largely determined by seed mass (Table 5), this effect being present at the moment of harvest, 7 months after sowing. Debain *et al.* (2003) reported similar results from their experiment on *Pinus sylvestris*, but, working with the same species, Castro (1999) found that influence of seed mass on seedling performance had ceased after the first growing season. Again, these contradictory results obtained from different populations suggest that maternal origin must be an important determinant in the early seedling growth of pine species. In fact, tree identity influenced *Pinus nigra* seedling performance irrespective of seed mass, and the factors «Tree» and «Mass» interacted for several seedling traits (Table 5), indicating that seed mass acts concomitantly with mother identity in determining first-year growth.

The positive relationship between seed mass and seedling performance conforms to the idea that seedlings from larger seeds withstand stress better (Westoby *et al.*, 1997; Leishman *et al.*, 2000). In this respect, the relationship between drought resistance —drought is the main stress factor causing mortality in *Pinus nigra* seedlings (Tíscar, 2007)— and seed mass was indirectly evaluated through B/A. The hypothesis was that bigger B/A values would mean higher allocation to roots, which in turn can be correlated to seedling survival in water-stressed environments (Lloret *et al.*, 1999). Results showed a B/A variation among trees with no effect of seed mass, suggesting that some mother plants allocated more to roots, irrespective of seed size. Nevertheless, seed size effects should not be disregarded, because seedlings were watered during the experiment, and major root allocation is unlikely to occur under wet conditions (Lloret *et al.*, 1999). Additionally, Tíscar (2002) found between-population differences in root

allocation, because seed size and root length of *Pinus nigra* covariate with altitude.

This study did not evaluate seedling survival and seed size correlates under field conditions, although there is wide evidence in literature that seedling performance is positively related to seedling survival (Bladé and Vallejo, 2008; Westoby *et al.*, 1997; Leishman *et al.*, 2000, and references therein). Thus, it can be summarized that successful establishment of *Pinus nigra* seedlings appears to be dependent on mother plant identity associated to seed traits.

It is difficult to state the implications of maternal effects in the silviculture of *Pinus nigra*, apart from the fact that they highlight the importance of the natural regeneration method in a sustainable forestry context. The main reason for that difficulty is water deficit during summer, which is expected to increase due to climate change. Drought had caused a 99.44% seedling mortality by the end of the experiment, matching figures of natural mortality frequently found under field conditions (Tíscar, 2007).

Depending on whether environmental variance overwhelms genetic variation or not, *Pinus nigra* could achieve successful regeneration under hardened summer conditions in two different ways: (a) restricting regeneration to years with optimal conditions for seedling establishment, (b) or using a bet-hedging strategy to track favorable conditions across microhabitats. The first case would select for mother plants with high mean and low variance of seed size. In the second case, higher moments of seed mass and germination time distributions would be of importance. For these reasons, that are extensive to other Mediterranean plant species, researchers are now being encouraged to examine shifts in variance, skewness and kurtosis (Herrera, 2009; Castellanos *et al.*, 2008; Crean and Marshall, 2009), as done in the current study. Results from this paper do not provide support for either alternative hypothesis: (a) or (b), but show a way for future research. Whatever the alternative, it would provide additional information when selecting silvicultural treatments.

Finally, results confirmed that reproductive function works throughout the lifecycle of *Pinus nigra* (see Tíscar, 2002), and veteran to old-senescent individuals (extramatured) still contribute to natural regeneration of forest stands. Although no correlation was found in this study between the frequency of empty seeds and mother plant age, Tíscar (2002) found a negative relationship between these two variables in a previous study that included 34 trees. Therefore, the non-signifi-

cant correlation reported here for some Spearman correlation analyses could be a consequence of using a limited number of maternal plants. Interestingly, seed mass was correlated to mother plant age, suggesting there are changing reproductive alternatives during the tree's lifetime.

Acknowledgements

Consejería de Medio Ambiente facilitated the means to carry out the research. We are also grateful to Luis García del Moral (University of Granada) who provided facilities for using the planimeter.

References

- AIZEN M.A., WOODCOCK H., 1992. Latitudinal trends in acorn size in eastern North American species of *Quercus*. *Canadian Journal of Botany* 70, 1218-1222.
- ALONSO C., 2005. Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*. *American Journal of Botany* 92, 1264-1269.
- BAÑUELOS M.J., OBESO J.R., 2003. Maternal provisioning, sibling rivalry and seed mass variability in the dioecious shrub *Rhamnus alpinus*. *Evolutionary Ecology* 17, 19-31.
- BLADÉ C., VALLEJO R., 2008. Seed mass effects on performance of *Pinus halepensis* Mill. seedlings sown after fire. *Forest Ecology and Management* 255, 2362-2372.
- CASTELLANOS M.C., MEDRANO M., HERRERA C.M., 2008. Subindividual variation and genetic *versus* environmental effects on seed traits in a European *Aquilegia*. *Botany* 86, 1125-1132.
- 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.
- CASTRO J., ZAMORA R., HÓCAR J.A., GÓMEZ J.M., 2004. Seedling establishment of a boreal tree species (*Pinus sylvestris*) at its southernmost distribution limit: consequences of being in a marginal Mediterranean habitat. *Journal of Ecology* 92, 266-277.
- CASTRO J., HÓDAR J.A., GÓMEZ J.M., 2006. Seed size. In: *Handbook of seed science and technology*. Ed A Basra, Haworth's Food Products Press, New York. pp. 397-427.
- CASTRO J., REICH P.B., SÁNCHEZ-MIRANDA A., GUERRERO J.D., 2008. Evidence that the negative relationship between seed mass and relative growth rate is not physiological but linked to species identity: a within-family analysis of Scots pine. *Tree physiology* 28, 1077-1082.

- CASTRO J., ZAMORA R., HODAR J.A., 2002a. Mechanisms blocking *Pinus sylvestris* colonization of Mediterranean mountain meadows. *Journal of Vegetation Science* 13, 725-731.
- CREAN A.J., MARSHALL D.J., 2009. Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. *Phil Trans R Soc B* 364, 1087-1096.
- CREUS J., 1998. A propósito de los árboles más viejos de la España peninsular: los *Pinus nigra* Arn. ssp. *salzmannii* (Dunal) Franco de Puertollano-Cabañas sierra de Cazorla, Jaén. *Montes* 54, 68-76.
- DEBAIN S., CURT T., LEPART J., 2003. Seed mass, seed dispersal capacity, and seedling performance in a *Pinus sylvestris* population. *Ecoscience* 10, 168-175.
- GARCÍA R., SIEPIELSKI A.M., BENKMAN C.W., 2009. Cone and seed trait variation in whitebark pine (*Pinus albicaulis*; Pinaceae) and the potential for phenotypic selection. *American Journal of Botany* 96, 1050-1054.
- GÓMEZ J.M., 2004. Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* 58, 71-80.
- HALPERN S.L., 2005. Sources and consequences of seed size variation in *Lupinus perennis* (Fabaceae): adaptive and non-adaptive hypotheses. *American Journal of Botany* 92, 205-213.
- HERRERA C.M., 2009. Multiplicity in unity. Plant subindividual variation and interactions with animals. The University of Chicago Press.
- HÓDAR J.A., CASTRO J., ZAMORA R., 2003. Pine processionary caterpillar *Thaumetopoea pityocampa* as a new threat for relict Mediterranean Scots pine forests under climatic warming. *Biological Conservation* 110, 123-129.
- IPCC, 2007. Climate change, fourth assessment report. Cambridge University Press, London, UK.
- KARKKAINEN K., SAVOLAINEN K., KOSKI V., 1999. Why do plants abort so many developing seeds. Bad offspring or bad maternal genotypes. *Evol Ecol* 13, 305-317.
- 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* (Fenner M., ed). CABI Publishing, Wallingford. pp. 31-57.
- LLORET F., CASANOVAS C., PEÑUELAS J., 1999. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. *Functional Ecology* 13, 210-216.
- MCGINLEY M.A., SMITH C.C., ELLIOTT P.F., HIGGINS J.J., 1990. Morphological constraints on seed mass in lodgepole pine. *Functional Ecology* 4, 183-192.
- MENARD S., 2000. Coefficients of determination for multiple logistic regression analysis. *The American Statistician* 54, 17-24.
- NUISMER S.L., GANDON S., 2008. Moving beyond common-garden and transplant designs: insight into the causes of local adaptation in species interactions. *The American Naturalist* 171, 658-668.
- OLEKSYN J., MODRZYNSKI J., TJOELKER M.G., ZYTKOWIAK R., REICH P.B., KAROLEWSKI P., 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology* 12, 573-590.
- PRASAD P.V.V., BOOTE K.J., ALLEN L.H., THOMAS J.M.G., 2002. Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (*Phaseolus vulgaris* L.). *Global Change Biology* 8, 710-721.
- 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.
- RICE K.J., GORDON D.R., HARDISON J.L., WELKER J.M., 1993. Phenotypic variation in seedlings of a keystone tree species (*Quercus douglassii*): the interactive effects of acorn source and competitive environment. *Oecologia* 96, 537-547.
- RIVAS-MARTÍNEZ S., 1987. Mapa de las Series de Vegetación de España. ICONA, Madrid.
- ROACH P.A., WULFF R.D., 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18, 209-235.
- SIMONS A.M., JOHNSTON M., 2000. Variation in seed traits of *Lobelia inflata* (Campanulaceae): sources and fitness consequences. *American Journal of Botany* 87, 124-132.
- STATSOFT, INC, 1999. STATISTICA for Windows (Computer program manual). Tulsa, OK: StatSoft, Inc, 2300 East 14th Street, Tulsa, OK 74104
- TÍSCAR P.A., 2002. Capacidad reproductiva de *Pinus nigra* subsp. *salzmannii* en relación con la edad de la planta madre. *Invest Agrar: Sist Recur For* 11, 357-371.
- TÍSCAR P.A., 2003. Condicionantes y limitaciones de la regeneración natural en un pinar oromediterráneo de *Pinus nigra* subsp. *salzmannii*. *Invest Agrar: Sist Recur For* 12, 55-64.
- TÍSCAR P.A., 2007. Dinámica de regeneración de *Pinus nigra* subsp. *salzmannii* al sur de su área de distribución: etapas, procesos y factores implicados. *Invest Agrar: Sist Recur For* 16: 124-135.
- TÍSCAR P.A., 2009. La vecería del pino salgareño (*Pinus nigra* subsp. *salzmannii*): un análisis a partir de datos recogidos por Enrique Mackay Monteverde. *Revista Montes* 96, 39-45.
- TRIPATHI R.S., KHAN M.L., 1990. Effects of seed weight and microsite characteristics on germination and seedling fitness in two species of *Quercus* in a subtropical wet hill forest. *Oikos* 57, 289-296.
- WESTOBY M., LEISHMAN M., LORD J., 1997. Comparative ecology of seed size and dispersal. *Plant life histories, ecology, phylogeny and evolution* (Silvertown J., Franco M., Harper J.L., eds). Cambridge University Press, Cambridge, UK. pp. 143-162.
- ZAR J.H., 1984. *Biostatistical analysis*. Prentice-Hall, Inc, New Jersey. 718 pp.