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Effect of seed mass and number of cotyledons on seed germination after heat treatment in *Pinus sylvestris* L. var. *iberica* Svob.

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Abstract

Aim of study: We investigated the combined effect of seed mass and number of cotyledons on the seed germination of *Pinus sylvestris* var. *iberica* (Iberian Scots pine) in simulated fire conditions.

Material and methods: We used 3,600 fresh seeds extracted from 158 cones obtained from 10 pine trees located at the Biological Station of the Complutense University (Guadarrama mountains, Madrid region). All the seeds were individually weighed and assigned to one of the two following seed mass classes: class I (1.6-12.5 mg) and class II (12.6-145.0 mg). Germination capacity (GC) and mean germination time (MGT) were studied in combined experiments of four different temperatures (100°, 125°, 150° and 175°C) and two exposure times (1 and 5 minutes) together with a control (no treatment). Four replicates of 50 seeds each were used for each treatment and hydrated daily for 14 days to germinate under constant illumination. The number of cotyledons was counted in seedlings.

Main results: Germination was depressed at above 125°C for 5 min. GC and MGT were negatively related, and were influenced by temperature and exposure time. Seed mass was found to have a significant effect on GC at some moderate heat treatment but not on MGT. The number of cotyledons was positively correlated to seed mass but there was not found correlated with germination after seed heat treatments.

Research highlights: In the case of the Iberian Scots pine, higher seed mass mitigate the negative effects of temperature on seed germination after moderate heat treatment simulating fire.

Key words: fire disturbance; germination; Mediterranean mountains; *Pinus sylvestris* L. var. *iberica;* temperature.

Introduction

Pinus sylvestris L. (Scots pine) is the most representative pine tree in the mountain areas of entire Europe and one of the most widely distributed in the Iberian Peninsula, where it covers an area of 1,280,000 ha, of which 47% is natural forest and the rest is planted stands (Montero *et al.*, 2001a). It plays a key role in Spanish forestry due to its economic, ecological and social importance (Cañellas *et al.*, 2000; Palahí *et al.*, 2002). Scots pine forests reach their south-westernmost geographical limit in the high Mediterranean mountains of the Iberian Peninsula where *Pinus sylvestris* has diversified into several geographically-structured microtaxa, among which *P. sylvestris* var. *iberica* (Iberian Scots pine hereafter) occupies the high central mountains (Amaral Franco, 1986; Peinado and Rivas-Martínez, 1987).

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Spanish mountains, included Scots pine forests, are affected by repeated and frequently human-induced fires (Martínez *et al.*, 2009; Montero *et al.*, 2001b). Due to its sensitivity to high temperatures, it has been characterized as a rarely pyrophytic species (Reyes & Casal, 1995; Núñez & Calvo, 2000). Unlike certain Mediterranean pines such as *P. halepensis* Mill. and *P. pinaster* Aiton (Mirov, 1967), *P. sylvestris* does not have serotinous cones which open after a fire (Tapias *et al.*, 2004) therefore implying a lower resistance to the high temperatures reached in fires. However, fire prepares an appropriate seedbed by changing the soil characteristics, and eliminates herbaceous plant competitors (Hille & Den Ouden, 2004) which explain the increasing seedling density of Iberian Scots pine after fires (Castoldi *et al.*, 2013).

Seed mass is a maternal plant effect and —as with other seed characteristics— it influences seed dispersal and germination (Roach & Wulff, 1987; Castro, 1999). Specifically, seed mass has a positive influence on early seedling growth in Scots pine (Wennström *et al.*, 2002). The number of cotyledons is also related to a maternal effect due to their relation with seed mass (Squillace, 1964). In Scots pine, the higher the number of cotyledons, the greater the growth height (Reich *et al.*, 1994). The number of cotyledons has been positively related to seed size and hence to early plant survival in pine species (Buchholz, 1946; Isik, 1985) other than Scots pine, of which the same behaviour could be expected. Southern European populations of Scots pine have heavier seeds than central and northern populations (Reich *et al.*, 1994). It is therefore important to know whether these maternal factors (seed mass and number of cotyledons) are an advantage against fire in southern European populations where fire is one of the key stressors. The interaction between seed size-number of cotyledons and germination in a range of thermal-shock temperatures simulating fires in Scots pine is unknown.

Our working hypothesis is that seed size and number of cotyledons influence the germination of seeds after being subjected to temperatures simulating fire conditions. The specific objectives address the following questions: a) how does the combined effect of seed mass and heat influence germination? and b) how does the combined effect of number of cotyledons and heat influence germination?

Material and methods

Seed source

Scots pine cones were collected in the Biological Field Station of the Complutense University located in the foothills of Guadarrama Mountains (Madrid region, 1,200 m a.s.l., 40° 54' N, 3° 52' W). In November 2011 we selected at least 10 cones each —before opening— from 10 dominant, healthy wild trees of similar size, obtaining 158 cones as a result from which 3,600 seeds were selected.

The area is flat with a negligible difference in altitude. The plant landscape is mainly a semi-natural forest consisting of patches of young *Quercus pyrenaica* Willd. mixed with stands of Iberian Scots pine (Castoldi & Molina, 2012). *Pinus sylvestris* trees reach an average height of 22 m and an average diameter at breast height of 48 cm. Open woodlands of *Fraxinus angustifolia* Vahl and subhumid meadows of *Agrostis castellana* Boiss. & Reuter occur in wetter soils. The area has undergone numerous burns as the consequence of prescribed slash-pile fire management (1,708 slash-pile burns carried out in the last ten years, A. Canencia, pers. comm.). The climate in the study area is sub-Mediterranean, with 895 mm of mean annual precipitation and a mean annual temperature of 10.1°C (Elías Castillo & Ruiz Beltrán, 1977). Soils correspond to brown soils on silicate rocks (Guerra *et al.*, 1966). Soils are developed mainly on glandular gneisses and the average of pH soils in the area is 7.5 (Castoldi, unpublished data).

Experimental design

The seeds were previously removed from the cones by placing in a dry air oven at 45°C for 24 hours. We extracted the seeds from the cones manually and removed the seed wings. We used fresh seeds (taken directly from the cone and not from the ground) collected 23 days before the experiment without any prior selection in order to reproduce natural forest conditions. All the seeds were individually weighed with a precision balance and assigned to one of two seed mass classes (class I and class II, Table 1) defined for the bimodal distribution found in the data. Seed mass distribution was normal for each class (class I: 1.6-12.5 mg, mean and SD: 8.9 ± 0.1; class II: 12.6-145.0 mg, mean and SD: 15 ± 0.3). Since the correlation between seed weight and seed-coat weight in pines is highly significant (Yeatman, 1966) we considered only seed weight in our experiment. Combinations of four different temperatures (100°, 125°, 150° and 175°C) and two exposure times (1 and 5 minutes) were studied together with a control (no treatment). Treatments were the following: 1) 100°C, 1min, I class; 2) 100°C, 1 min, II class; 3) 100°C, 5 min, I class; 4) 100°C, 5 min, II class; 5) 125°C, 1 min, I class; 6) 125°C, 1 min, II

Table 1. Classes established to rank the fungal colonization in each Petri dish

Nb of infected seeds	Fungi cover (%)	Class
0	0	1
1	2	2
2-5	3-10	3
6-12	11-25	4
13-25	26-50	5
26-37	51-75	6
38-50	76-100	7

class; 7) 125°C, 5 min, I class; 8) 125°C, 5 min, II class; 9) 150°C, 1 min, I class; 10) 150°C, 1 min, II class; 11) 150°C, 5 min, I class; 12) 150°C, 5 min, II class; 13) 175°C, 1 min, I class; 14) 175°C, 1 min, II class; 15) 175°C, 5 min, I class; 16) 175°C, 5 min, II class; 17) control, I class; 18) control, II class. These temperatures were chosen because they are commonly used in Scots pine germination studies to reproduce natural fire conditions (Habrouk *et al.*, 1999; Escudero *et al.*, 1997; Núñez & Calvo, 2000). Four replicates of 50 seeds each were used for each treatment and placed on Petri dishes.

Seeds were placed on filter papers moistened with purified water. Germination was run at a constant temperature of 20°C and 28% HR (air) and 22°C and 56% HR (germination tables) under constant illumination of 20 µE m-2s-1 (fluorescent lamp F 40 W/33 RS cool white light). Germination was checked daily at the same hour for 14 days. This period is considered sufficient since it has been reported that 98% of Scots pine seeds germinate in the first five days under constant light and temperature —20°C— (Nygren, 1987). Come's criterion (1970) was followed to determine the germinated seeds: we considered that a seed had germinated when its radicle could clearly be observed outside the tegument, and its size was equal to the size of the seed. Seeds that were considered germinated were removed and the number of cotyledons counted in order to study the relation between it and seed size (length and width). After the experiment we investigated the un-germinated seeds by the cutting test and divided them into filled or empty seeds in order to study seed viability. Secondary fungi contamination was che-cked by recording the percentage of fungi cover in the Petri dishes the 4th, 8th and the 14th day and allocated to seven classes (Table 1). Fungi were taxonomically determined up to the genus level (Seifert *et al.*, 2011). All germination tests and seed weightings were performed in the Seed Laboratory at the Swedish University of Agricultural Sciences, Department of Southern Swedish Forest Research Centre, Alnarp (Sweden).

Statistical analysis

Germination capacity (GC) and mean germination time (MGT) were calculated for each treatment as follows: GC (%) = (N° germinated seeds/total N° of seeds sown) * 100; MGT (days) = Σ ($n_i * i$)/N, where n_i is the number of seed germinated on day i and N is the total number of seeds germinated along the study period (Bewley and Black, 1994).

Data were checked for normality or transformed if necessary, and ANOVA was performed to test the effects of heat and seed mass on germination (both GC and MGT). A Student's t-test was applied to identify statistically significant differences in GC and MGT between the control and the treated seeds. One-way ANOVA was performed to determine significant differences between heated and control seeds, among heat treatments, between seed mass classes and between heated and control seeds in fungi second contamination. Three-way ANOVA was used to consider the temperatures (H), exposure times (T) and seed mass (S) together. Spearman's coefficient was used to relate the GC and MGT variables and number of cotyledons and seed size (length and width used as independently variables). A generalized regression model (GRM) with stepwise procedure was performed to investigate the interactions between seed mass and temperature and exposure time. Spearman's rank correlation coefficient and linear regression analysis were used to relate seed size to number of cotyledons. Student's t-test was used to compare the number of cotyledons in seedlings after heat treatment and in control. Statistical analyses were done using SPSS 13.0 and STATISTICA software.

Results

Over the total sowed seeds the 15% germinated. On cutting the un-germinated seeds, we found the 85.8 % were full. Thus the majority of the seed material was healthy and failed to germinate due to the treatments. Treated seeds showed the highest GC at 100°C for 1 min (53%), and decreased with higher temperatures and longer exposure times (H-100 T-5; H-125 T-1) (Fig. 1). GC was depressed at above 125°C for 5 min. There were significant differences in GC between treatments and controls and among the treatments (in both cases $p < 0.001$). GC was negatively influenced by temperature ($F_{2,23} = 12.61$, $p < 0.001$) and exposure time ($F_{2,23} = 26.91$, $p < 0.001$). The less aggressive heat treatment (100°C, 1 min) already shows significant differences in GC in comparison to the control ($F_{1,22} = 17.31$, $p < 0.001$). The seed mass had a significant effect on GC when considering heat treatment at 100°C for 5 minutes ($F_{1,6} = 9.375$, $p = 0.02$) and the con-

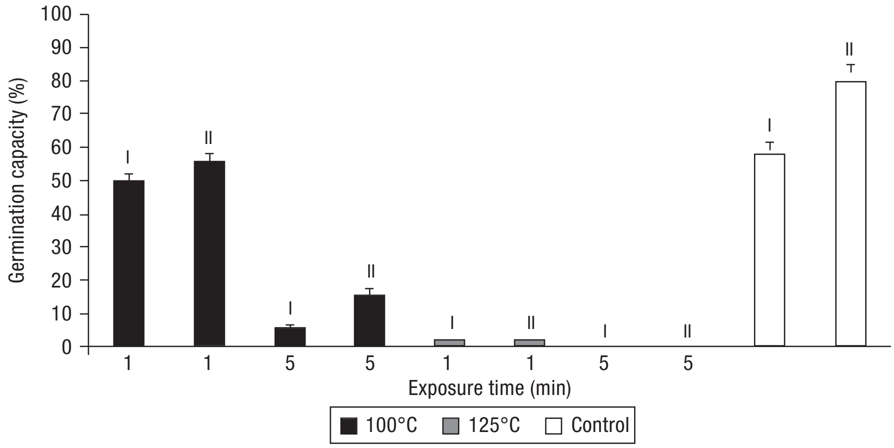

Figure 1. GC of Iberian Scots pine seeds in response to heat treatments and control. Labels correspond to seed mass classes (I and II class) and exposure minutes (1 or 5 minutes). Values are mean ± SD. Differences between treatments and control are significant for all treatments, except for the first one (100°C, 1 min, I seed mass class, first column; $p = 0.199$). Heat treatments that avoid germination capacity are not shown with the exception of the treatment 125°C, 5 min.

trol ($F_{1,6} = 23.45$, $p = 0.003$). In contrast, seed mass had no significant effect on GC when considering heat treatment at 100°C for 1 minute ($F_{1,6} = 1.652$, $p = 0.246$), or when all the treatments were considered together ($F_{1,24} = 1.14$, $p = 0.29$).

The first seedlings were observed in the control 4 days after sowing, with the highest seedling counts seen after 5 days. Table 2 shows that the lowest MGT was observed in the control, with no exposure to heat. MGT was influenced by temperature ($F_{2,23} = 27.59$, $p < 0.001$) and exposure time ($F_{2,23} = 54.79$, $p < 0.001$). MGT differences between all heat treatments and the control were not supported statistically ($F_{1,70} = 1.82$, $p = 0.181$) but they were when considering the less aggressive heat treatments of 100°C for 1 min ($F_{1,14}=74.12$, $p < 0.001$) and 100°C for 5 min ($F_{1,14} = 993.08$, $p < 0.001$). The difference in MGT between the heat treatment of 125°C for 1 min and the control was not significant ($F_{1,14} = 1.06$, $p = 0.321$). MGT varied among the different heat treatments ($p < 0.001$). The seed mass had no significant effect on MGT ($F_{1,24} = 0.008$, $p = 0.93$). Generalized Regression Model showed that there are not significant interactions between seed mass, temperature and exposure time in MGT (Table 3). The stepwise procedure kept the two first variables in the model (T and H). The germination capacity (GC) and mean germination time (MGT) were negatively correlated, meaning that lower MGT corresponds to a high GC ($r = –0.9143$, $p < 0.001$, Fig. 2).

Descriptive statistics of cotyledons number, seed width and length for treated and control seeds are shown in Table 4. The number of cotyledons in 14-day-old seedlings was positively correlated to seed size ($r = 0.358$, $p < 0.001$). The number of cotyledons was

Table 2. Mean germination time (days) of seeds of Iberian Scots pine in response to different exposure time to dry heat at different temperatures and times (Mean ± SD)

Time	Seed mass	100°C	125°C	150°C	75°C
1 min	I	8.84 ± 2.7	14 ± 0	0	0
	II	9.59 ± 2.1	14 ± 0	0	0
5 min	I	13.60 ± 0.9	0	0	0
	II	13.20 ± 1	0	0	0
		No treatment			
Control	I	5.83 ± 2.7			
	II	5.89 ± 4.6			

Table 3. Generalized Regression Model

	Steps	Degr. of freedom	F to remove	P to remove	F to enter	P to enter	Effect status
T	Step Number 3	2	60.25485	0.000000			In
H		2	35.07437	0.000000			In
S		1			0.17895	0.676795	Out
T * S		2			1.10146	0.352695	Out
H * S		2			0.08896	0.915258	Out

H: temperature. T: exposure times. S: seed mass.

positively correlated to seed length ($r = 0.1203$, $p = 0.005$) and to seed width ($r = 0.0949$, $p = 0.002$). This result was confirmed by a linear regression analysis between seed size and the number of cotyledons that was highly significant ($F = 13.927$, $\beta = 0.358$, $p < 0.001$). The number of cotyledons in seedlings after heat treatment did not differ significantly from the number of cotyledons in the control seeds ($p = 0.1034$). (t-test heat treatment mean = 6.7, control mean 7.1, $p = 0.1069$).

Fungal colonies of cosmopolitan and ubiquitous fungi taxa such as *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., and *Mucor* sp. were found infecting the Petri dishes. The highest percentages of fungi cover (25% or more seeds infected) were recorded in seeds submitted to temperatures above 125°C. There was no significant difference in fungi cover between heated and control seeds for each measuring day (day 4th: $F_{1,70} = 0.679$, $p = 0.413$; day 8th: $F_{1,70} = 1.22$, $p = 0.272$; day 14th: $F_{1,70} = 2.13$, $p = 0.149$).

Discussion

As expected from the background literature our results showed that germination is depressed by temperature and long exposure. A temperature range between 120°C and 150°C has been reported in failed GC of Scots pine seeds from Spain (Habrouk *et al.*, 1999;

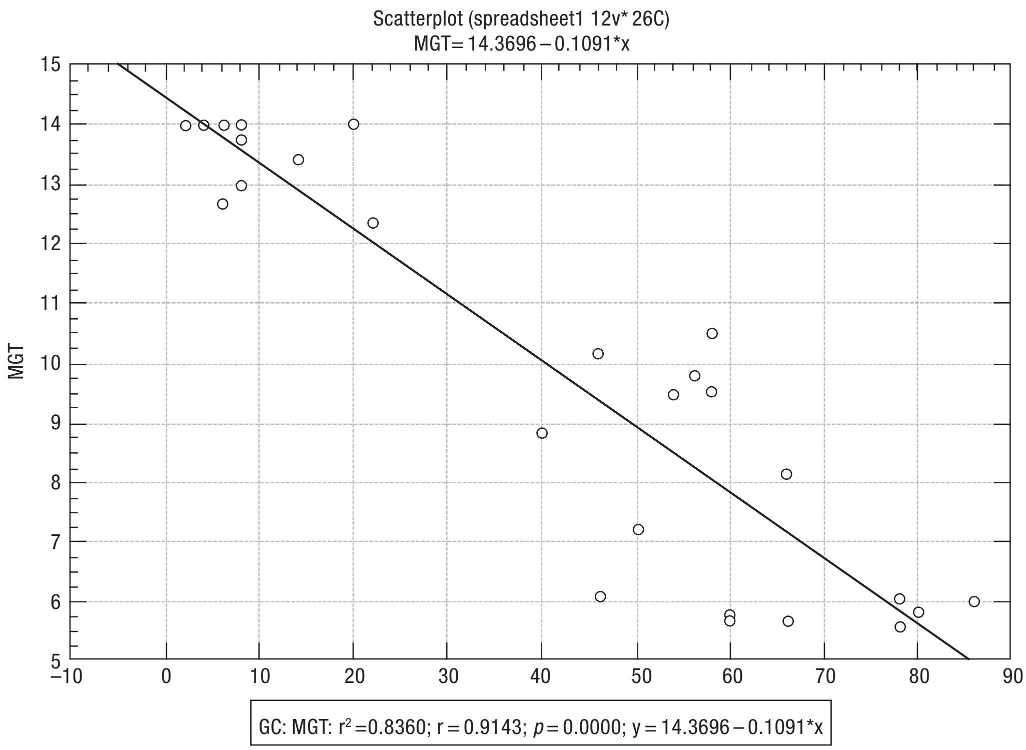

Figure 2. Spearman's correlation between the dependent variables Germination Capacity (GC) and Mean Germination Time (MGT).

Table 4. Descriptive statistics of seed traits (seed mass class, number of cotyledons, width and length of seed) for heated (all treatments) and control seeds

		Mean	Min	Max	SD
Heated seeds					
Seed mass class I (N =40)	N° cotyledons	6.675	5	9	0.79703
	Width (mm)	53.15	44	61	3.886532
	Length (mm)	27.775	23	35	3.166228
Seed mass class II (N = 40)	N° cotyledons	6.8	5	8	0.790975
	Width (mm)	56.175	50	64	3.685644
	Length (mm)	29.05	22	33	2.406721
Control seeds					
Seed mass class I (N = 10)	N° cotyledons	7.3	6	8	0.674949
	Width (mm)	55.1	48	62	4.629615
	Length (mm)	55.1	25	30	1.523884
Seed mass class II (N = 10)	N° cotyledons	7	6	8	0.666667
	Width (mm)	58.1	53	62	3.142893
	Length (mm)	28.4	25	31	1.712698

Escudero *et al.*, 1997; Núñez & Calvo, 2000). Our results showed that heat treatments at 125°C for 5 min on healthy Iberian Scots pine seeds resulted in null germination. Moreover, long exposure (5 min) notably decreases GC even at the lowest temperature studied in this work (100°C). The slight differences seen by authors in the temperature threshold for germination can be explained by experimental conditions such as light time exposure or the seed provenance. Significant delays have been reported in germination time after heat treatments (Escudero *et al.*, 1997) increasing the MGT both temperature and exposure time (Habrouk *et al.*, 1999) Our results support this pattern and show that temperature and exposure time affect MGT even at the lowest temperature shock of 100°C in our experiment.

Previous studies on the influence of seed mass on germination in different pine species show contradictory results. Some report a positive correlation (Simak & Gustafsson, 1954; Debain *et al.*, 2003; Tíscas & Lucas, 2010), whereas other studies show no effect (Mikola, 1985; Zaborovskii, 1966; Parker *et al.*, 2006; Bladé & Vallejo, 2008). In Spanish Scots pines, seed mass has been positively correlated with germination (Castro, 1999). This agrees with our results on Iberian Scots pine showing that seed mass is positively related to germination when seeds are unheated. Furthermore, seed mass still positively influences GC in moderately heated seeds, which still retain the ability to germinate. Seed mass is also positively related to the number of cotyledons (Reich *et al.*, 1994) as our results also support. Thus it could be inferred that seeds with a higher number of cotyledons should have a better performance when germinating under moderate heat. However, due to the nature of the data this cannot be probed.

Southern European populations of Scots pine are known to have heavier seed mass (Reich *et al.*, 1994) —as our results confirm— and a higher number of cotyledons in relation to central and northern Scots pines. Since fire is one of the most important perturbations under the Mediterranean climate, these correlated characters could confer a maternal advantage against this stressor in low-intensity fires.

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References

Amaral Franco J, 1986. *Pinus* L. In: Flora Ibérica (Castroviejo S *et al.*, eds). CSIC, Madrid, Spain. Vol. 1:168-174.

Bewley JD, Black M, 1994. Seeds: physiology of development and germination. Plenum press, New York, USA.

Bladé C, Vallejo VR, 2008. Seed mass effects on performance of *Pinus halepensis* Mill. seedlings sown after fire. For Ecol Manage 255: 2362-2371.

Buchholz JT, 1946. Volumetric studies of seeds, endosperms and embryos in *Pinus ponderosa* during embryonic differentiation. Bot Gaz 108: 232-244.

Cañellas I, Martinez-Garcia F, Montero G, 2000. Silviculture and dynamics of *Pinus sylvestris* L. stands in Spain. Invest Agr Sist Recur For 1: 1-21.

Castoldi E, Molina JA, 2012. Vegetation composition of the UCM Biological Field Station "Finca de Ontalba". Obs Medioamb 15: 115-123.

Castoldi E, Quintana JR, García MR, Molina JA, 2013. Early post-fire plant succession in slash-pile prescribed burns of a sub-Mediterranean managed forest. Plant Ecol Evol 146: 272-278.

Castro J, 1999. Seed mass versus seedling performance in Scots pine: a maternally dependent trait. New Phytol 144: 153-161.

Come D, 1970. Les obstacles à la germination. Masson et Cie, Paris, France.

Debain S, Curt T, Lepart J, 2003. Seed mass, seed dispersal capacity and seedling performance en a *Pinus sylvestris* population. Ecoscience 10: 168-175.

Elías Castillo F, Ruiz Beltrán L, 1977. Agroclimatología de España. INIA. Cuaderno n° 7, Ministerio de Agricultura, Spain.

Escudero A, Barrero S, Pita JM, 1997. Effects of high temperatures and ash on seed germination of two Iberian pines (*Pinus nigra* ssp *salzmannii*, *P. sylvestris* var *iberica*). Ann For Sci 54: 553-562.

Guerra A, Guitián F, Paneque G, García A, Sánchez JA, Monturiol F, Mudarra JL, 1966. Mapa de suelos de España, escala 1: 1.000.000. CISC, Madrid, Spain.

Habrouk A, Retana J, Espelta JM, 1999. Role of heat tolerance and cone protection of seeds in the response of three pine species to wildfires. Plant Ecol 145: 91-99.

Hille M, Den Ouden J, 2004. Improved recruitment and early growth of Scots pine (*Pinus sylvestris* L.) seedlings after fire and soil scarification. Eur J For Res 123: 213-218.

Isik K, 1985. Altitudinal variation in *Pinus brutia* Ten.: seed and seedling characteristics. Silvae Genet 35: 58-67.

Martínez J, Vega-García C, Chuvieco E, 2009. Human-caused wildfire risk rating for prevention planning en Spain. J Environ Manage 90: 1241-1252.

Mikola J, 1985. Relationship between height growth differences of Scots pine full-sib families and variation in seed size, annual growth rhythm, and some foliage characteristics (Tiegerstedt PMA, Puttonen P, Koski V, eds). Helsinki University Press, Helsinki, Finland. pp: 233-243.

Mirov NT, 1967. The Genus *Pinus*. Ronald Press, New York, USA. 602 pp.

Montero G, Rojo A, Álvarez MF, Del Río M, 2001a. Aspectos selvícolas y económicos de los pinares de *Pinus sylvestris* L. en el Sistema Central. Estudios Agrosociales y Pesqueros 193: 27-56.

Montero G, Cañellas I, Ortega C, Del Río M, 2001b. Results from a thinning experiment in a Scots pine (*Pinus sylvestris* L.) natural regeneration stand in the Sistema Ibérico Mountain Range (Spain). For Ecol Manage 145: 151-161.

Nygren M, 1987. Germination characteristics of autumn collected *Pinus sylvestris* seeds. Acta forestalia fennica 201: Helsinki, Finland.

Núñez MR, Calvo L, 2000. Effect of high temperatures on seed germination of *Pinus sylvestris* and *Pinus halepensis*. For Ecol Manage 13: 183-190.

Palahí M, Miina J, Tomé M, Montero G, 2002. Stand-level yield model for Scots pine (*Pinus sylvestris* L.) in northeast Spain. Invest Agr Sist Recur For 11: 409-424.

Parker WC, Noland TL, Morneault AE, 2006. The effects of seed mass on germination, seedling emergence, and early seedling growth of eastern white pine (*Pinus strobus* L.) New Forest 32: 33-49.

Peinado M, Rivas-Martínez S, 1987. La vegetación de España. Serv Publ Univ Alcalá, Madrid, Spain. 544 pp.

Reich PB, Oleksyn J, Tjoelker MG, 1994. Seed mass effects on germination and growth of diverse European Scots pine populations. Can J For Res 24: 306-320.

Reyes O, Casal M, 1995. Germination behaviour of 3 species of the genus *Pinus* in relation to high temperatures suffered during forest fires. Ann For Sci 52: 385-392.

Roach DA, Wulff RD, 1987. Maternal effects in plants. Ann Rev Ecol Systematics 18: 209-235.

Seifert K, Morgan-Jones G, Gams W, Kendrick B, 2011. The Genera of Hyphomycetes. CBS Biodiversity Series 9. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Holand.

Simak M, Gustafsson Å, 1954. Seed properties in mother trees and grafts of Scots pine. Statens Skogsfor 44: 1-80.

Squillace AE, 1964. Geographic variation in Slash pine (*Pinus elliottii* Engelm.). PhD Dissertation. University of Florida, USA.

Tapias R, Climent J, Pardos JA, Gil L, 2004. Life histories of Mediterranean pines. Plant Ecol 171: 53-68.

Tíscas OP, Lucas BM, 2010. Seed mass variation, germination time and seedling performance in a population of *Pinus nigra* subsp. *salzamannii*. For Syst 3: 344-353.

Wennström U, Bergsten U, Nilsson JE, 2002. Effects of seed weight and seed type on early seedling growth of *Pinus sylvestris* under harsh and optimal conditions. Scand J For Res 17: 118-130.

Yeatman CW, 1966. Germinant size of jack pine in relation to seed size and geographic origin. Joint proceeding of the second genetics workshop of the Society of American Foresters and the seventh lake states forest tree improvement conference; Res Pap NC-6. St Paul, MN: US Department of Agriculture, Forest Service, North Central Forest Experiment Station. 28-36 pp.

Zaborovskii EP, 1996. Analysis of cones and seeds of a single Scots pine tree. Lesn. Zh. (Archangel, USSR) 9: 162-163.