

Seed quality characteristics of *Pinus halepensis* – seed germination strategy and early seedling growth

Thekla K. Tsitsoni

Tsitsoni, T. K. 2009. Seed quality characteristics of *Pinus halepensis* – seed germination strategy and early seedling growth. – Web Ecol. 9: 72–76.

Pinus halepensis is a Mediterranean tree species occupying areas of high tourist interest, where it forms aesthetic and recreational forests. However, intense human pressure, adverse climatic conditions and overgrazing degrade Aleppo pine forest ecosystems and render the natural regeneration of this species difficult. The ecological, landscape, recreational and soil conservation uses of *P. halepensis* along with its aesthetic value, make this species important for landscape planning and multi-purpose forestry. For these reasons, artificial regeneration may be required in order to render ecosystem restoration faster. Although *P. halepensis* is characterized by a high germination capacity and a constant temperature of 20°C is considered optimal for germination, no research has dealt with the germination behaviour and early growth of seedlings under alternative temperature conditions similar to those dominating outdoors. Moreover, little research was conducted on seed quality characteristics of this species. Thus, in this study seed quality of *P. halepensis* was estimated by measuring purity, number of seeds per kg, weight of 1000 seeds, average seed weight, seed moisture content and percentage of empty seeds. Also, seed germination capacity, germination rate, percentage of infected and not germinated viable seeds, abnormal seedlings as well as the total seedling length were studied under laboratory (alternative temperature) and chamber (constant temperature) conditions with the same photoperiod. Results showed that the percentage of empty seeds and abnormal seedlings was extremely low and the total germination percentage was very high (87–90%) in both environments. Germination capacity, germination rate and the total length of seedlings did not show any differences among the two growth environments.

T. K. Tsitsoni (tsitsoni@for.auth.gr), Dept of Silviculture, Faculty of Forestry and Natural Environment, Aristotle Univ. of Thessaloniki, PO Box 262, GR–54 124 Thessaloniki, Greece.

Aleppo pine (*Pinus halepensis*) is the most widely distributed pine species in the Mediterranean Basin, where it is subjected to recurrent wildfires (Trabaud et al. 1985, Navéh 1990, Tsitsoni 1997). It regenerates easily after fires, but also in lack of fires, under suitable stand conditions (Tsakaldimi et al. 2004). Germination is therefore a key factor in post-fire regeneration of the plant community.

Intense human pressure, adverse climatic conditions and overgrazing degrade Aleppo pine forests and render

the natural regeneration of this species difficult. In addition, the ecological role and landscape, recreational and aesthetic values of *P. halepensis* make this species important for landscape planning and forestry. Thus, in many cases, artificial regeneration is required in order to make ecosystem restoration faster.

Seed germination represents a risky transition from the most tolerant environmental condition stage (i.e. the seed) to the weakest and most vulnerable stage in plant develop-

ment, the seedling (Castro et al. 2005). *Pinus halepensis* is characterized by a high germination capacity. The optimal seed germination occurs at 20°C in darkness, though rather slowly (Thanos and Skordilis 1987). Germination is promoted by daytime light or by continuous red light and is inhibited by far-red light, by high osmotic stress and by stratification (Falusi et al. 1983, Thanos and Skordilis 1987, Skordilis and Thanos 1995). Aleppo pine seeds are generally non-dormant (Thanos 2000).

Germination rate may vary with geographical origin of seeds. *Pinus halepensis* seeds showed a great heterogeneity in their germination, ranging from 15 to 85% for Greek provenances, around 90% for Sierra de los Donceles (Spain) seeds, 50% for Mt Carmel (Israel), and 60% for Italian provenances (Petruzzelli 1984, Martinez-Sanchez et al. 1995, Henig-Sever et al. 2000, Thanos 2000).

However, because Aleppo pine germination in the field takes place 'en masse' almost exclusively after fire, most of the available information derives from studies of burned sites (Thanos 1999). No research has dealt with germination behaviour and early growth of seedlings under alternative temperature conditions that mimic environmental conditions. Moreover, little research has been conducted on seed quality characteristics (quality, weight, moisture, empty seeds etc.) of the species.

The aims of the present study are to examine: a) the seed quality characteristics from seed lots of *P. halepensis*, b) the effect of alternative temperature conditions similar to environmental conditions (in laboratory environment) on germination behaviour and early growth of seedlings; and c) to compare these results with those obtained from the constant temperature (20°C) conditions (in a growth chamber environment).

Material and methods

Seeds of *Pinus halepensis* were obtained from the Forestry Division, Ministry of Agriculture, Greece. Throughout the experimentation period, seeds were hermetically stored in a refrigerator (3–5°C) in darkness (Skordilis and Thanos 1995).

Before the germination tests, seeds were examined for quality according to the ISTA rules (ISTA 1996). Tests included the precision of authenticity and purity analyses, the weight of 1000 seeds, their number per kg and their moisture content. The essence of good seed testing is the application of reliable standard methods of examination to

ensure that uniform and reproducible results are obtained (Turnbull 1975).

In order to study seed germination behaviour two experiments were conducted: the first experiment took place in a growth chamber, under constant temperature 20°C with a photoperiod of 12 h light/ 12 h dark. The second experiment took place in a laboratory environment, with approximately the same photoperiod conditions (during March) and alternative temperature (room temperature).

In both experiments, germination tests were performed using eight replications, each containing 100 seeds, per petri dish (diameter 9 cm) lined with two sheets of filter paper, moistened with deionised water (Skordilis and Thanos 1995, Raccuia et al. 2004) and covered to prevent evaporation. Water was added daily. In order to avoid fungal infection and to keep petri dishes clear, filter papers were replaced every third day. Seeds were separated from each other in order to reduce the chance of cross-contamination by micro-flora (Ganatsas and Tsakalidimi 2007). Germination was evaluated every seven days. The seeds were considered germinated when their radicle showed positive geotropism and their shoot exhibited normal growth and morphology (Bonner and Vozzo 1987, Fahrettin and Alptekin 2006). The following were recorded: weekly germination percentage, accumulative germination percentage, germination rate and total length of seedlings. The germination rate (T_{50}) was estimated as the time needed for the manifestation of half of the final cumulative germination (Skordilis and Thanos 1995). Abnormal seedlings were not included in the germination experiment because they rarely survived to produce plants (FAO 1987), but were retained to the end of the experiments and recorded. At the end of the experimental period, I cut all remaining ungerminated seeds and the number of fresh, firm, possibly viable seeds and infected seeds were recorded (Bonner 1974).

Statistical differences between treatments were analysed by t-test at $p < 0.05$ level of significance with SPSS software.

Results and discussion

The purity percentage of *P. halepensis* seeds was high (98.8%), the weight of 1000 seeds was 18.94 g, the number of pure seeds per kg was 52.798, the mean weight of seeds was 0.019 g, the moisture content was 7.9% and the percentage of empty seeds was very low (0.9%) (Table 1). The

Table 1. Seed quality characteristics. Values given in the table represent means, $n = 2500$ seed units.

Purity (%)	Weight of 1000 seeds (g)	Number of pure seeds per kg	Moisture content (%)	Mean fresh weight of seed (g)	Empty seeds (%)
98.83	18.94	52.798	7.9	$0.019 \pm 3 \times 10^{-4}$	0.9

Table 2. Percentage of abnormal seedlings, viable seeds that did not germinate and infected seeds. Values represent an average of 8 replicates from 100 seeds in each experiment and their standard error in parenthesis. * = significant differences ($p < 0.05$, t-test).

Treatment	Abnormal seedlings (%)	Viable seeds that did not germinate (%)	Infected seeds (%)
Chamber conditions	0.25 (0.16)	4.75 (0.75)*	4.25 (0.90)*
Laboratory conditions	0.00 (0.00)	8.88 (1.29)*	2.25 (0.48)*

number of seeds per kg and the mean seed weight found in this study agree with the results of previous studies. Piotto and Di Noi (2001) reported 50 000–100 000 pure seeds of *P. halepensis* per kg, while Thanos and Skordilis (1987) reported an average seed weight of 0.022 g. However, *P. halepensis* seed weight has been found to vary widely among collections (12–30 mg; Pelizzo and Tocci 1978, Thanos et al. 1995) and among locations and altitudes. Comparing various Italian provenances of *P. halepensis*, Cuccui et al. (1996) found positive and statistically significant correlation coefficients between site altitude, seed weight and germinability, respectively.

At the end of the germination process a negligible percentage of abnormal seedlings was observed both in the chamber and the laboratory environment (Table 2). The percentage of viable seeds that did not germinate and the percentage of infected seeds were also low. However, non-germinated viable seeds were significantly less in the chamber environment (4.75%) than in the laboratory (8.88%), which means that constant temperature enhanced the earlier germination of viable seeds. Also, Skordilis and Thanos (1995) reported that the mean percentage of unsound *P. halepensis* seeds was around 10% for all studied seed lots.

The cumulative seed germination percentage was high enough (87–90%) and did not show significant differences among the two experiments (chamber vs laboratory environment, Fig. 1). This shows that the seeds of *P. halepensis* do not present any dormancy nor difficulty

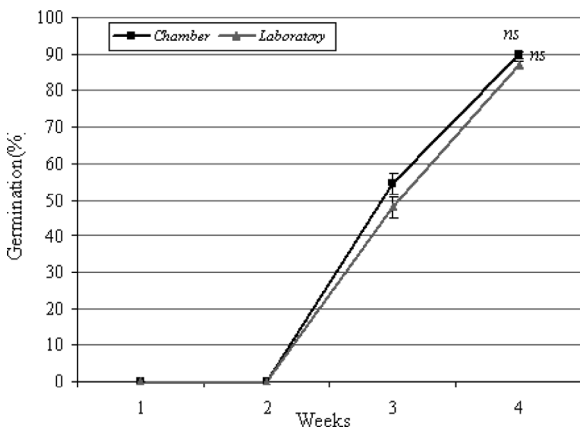


Figure 1. The germination percentage (weekly and total) in the two experiments.

to germinate, even in natural spring conditions. These results agree with those obtained from Skordilis and Thanos (1997), reporting that *P. halepensis* seeds are not dormant and germinate optimally at average temperatures of the rainy season in areas where the species grows. Similarly, Martinez-Sanchez et al. (1995) found high total germination percentage (90%) for *P. halepensis* seeds of Spanish origin. Loisel (1966) studied Aleppo pine seed germination in the field using a phytosociological approach. He sowed pine seeds in 9 different vegetation associations and monitored seedling emergence and survival. Germination was satisfactory in 7 of 9 vegetation types.

The germination rate (T_{50}), however, was relatively slow (15.6–16 d) in both treatments, (Fig. 2). Skordilis and Thanos (1995) found that the germination rate of *P. halepensis* seeds from Greek origin was 10.3 d at 20°C in dark. Calamassi et al. (1984), working on 9 circum-Mediterranean Provenances of Aleppo pine, found a slow pace of dark germination; overall average T_{50} values were 12, 9.5 and 10 days at the optimal temperatures of 15, 18 and 21°C, respectively. This 'delay' mechanism of seed germination in *P. halepensis* and *Pinus brutia* (and in numerous other Mediterranean plants) is still unknown. However, it should not be attributed to a seed-coat-imposed, restraining effect because de-coating in both Aleppo and brutia pine seeds resulted in only a slight enhancement of germination rate (Skordilis 1992, Thanos et al. 1995).

No significant differences were observed between chamber and laboratory environment for total length of the new seedlings (4 weeks old), which fluctuated between 67.7 and 68.8 mm (Fig. 3).

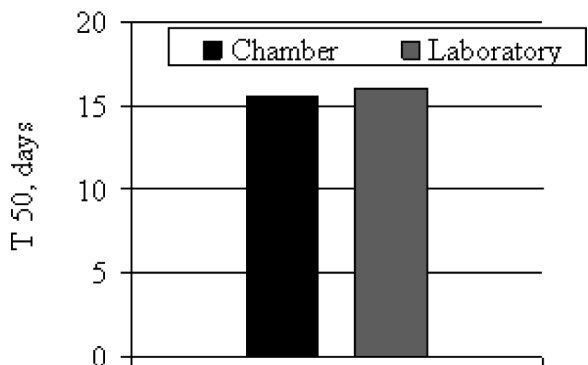


Figure 2. The germination rate (T_{50} , days) in the two experiments.

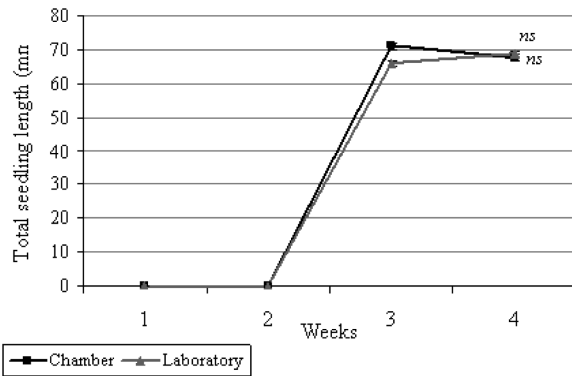


Figure 3. The growth and total length (mm) of new seedlings in the two experiments.

Previous experiments under daily alternating conditions of light and temperature resembling natural conditions led to the conclusion that field germination is feasible throughout the rainy season of the Mediterranean-type climate and is strongly favored by open, sunny sites (Daskalakou and Thanos 1996). In addition, the results of the current study fit well with field observations of natural seed germination where a 'burst' of germination occurred in March and April (Thanos and Skordilis 1987).

Thus, based on this study, it is concluded that in case anyone wants to examine *P. halepensis* germination capacity from various seed lots or from various provenances, there is no need to test the seeds in a controlled environment such as a growth chamber, because laboratory conditions simulate natural environmental spring conditions, and provide similar results. From a practical point of view, the results of the current study are useful and convenient for nursery managers and scientists, as they ensure lower laboratory costs and save time.

References

- Bonner, F. T. 1974. Seed testing. – In: Seeds of woody plants in the United States. Agriculture Handbook no. 450. For. Service, USDA, Washington DC, pp. 136–152.
- Bonner, F. T. and Vozzo, J. A. 1987. Seed biology and technology of *Quercus*. – USDA For. Service, New Orleans, LA GTR-SO-66.
- Calamassi, R. et al. 1984. Effets de la temperature de germination et de la stratification sur la germination des semences de *Pinus halepensis* Mill. – *Silvae Genet.* 33: 133–139.
- Castro, J. et al. 2005. Ecology of seed germination of *Pinus sylvestris* L. at its southern, Mediterranean distribution range. – *Invest. Agrar.-Sist. Recur. For.* 14: 143–152.
- Cuccui, I. et al. 1996. Indagini su semi e semenzali di provenienze di *Pinus halepensis* Mill. – *Monti Boschi* 47: 56–62.
- Daskalakou, E. N. and Thanos, C. A. 1996. Aleppo pine (*Pinus halepensis*) postfire regeneration: the role of canopy and soil seed banks. – *Int. J. Wildland Fire* 6: 59–66.
- Fahrettin, T. and Alptekin, U. C. 2006. Germination and seedling growth of *Quercus vulcanica*: effects of stratification, desiccation, radicle pruning and season of sowing. – *New For.* 32: 243–251.
- Falusi, M. et al. 1983. Sensitivity of seed germination and seedling growth to moisture stress in four provenances of *Pinus halepensis* Mill. – *Silvae Genet.* 32: 4–9.
- FAO forestry paper 20/2. (compiled by Willan, R. L.) 1987. A guide to forest seed handling. – FAO, Rome.
- Ganatsas, P. P. and Tsakalidimi, M. N. 2007. Effect of light conditions and salinity on germination behaviour and early growth of umbrella pine (*P. pinea* L.) seed. – *J. Hortic. Sci. Biotechnol.* 82: 605–610.
- Henig-Sever, N. et al. 2000. Regulation of the germination of Aleppo pine (*Pinus halepensis*) by nitrate, ammonium and gibberellin, and its role in post-fire forest regeneration. – *Physiol. Plant.* 108: 390–397.
- ISTA 1996. International rules for seed testing. Seed Science and Technology. Vol 24, Suppl. rules 1996. – International Seed testing Association.
- Loisel, R. 1966. Germination du Pin d'Alep au niveau de certaines associations vegetales de Basse-Provence. – *Bull. Soc. Bot. Francaise* 113: 324–330.
- Martinez-Sanchez, J. J. et al. 1995. Effects of high temperatures on germination of *Pinus halepensis* Mill. and *P. pinaster* Aiton subsp. *pinaster* seeds in southeast Spain. – *Vegetatio* 116: 69–72.
- Naveh, Z. 1990. Fire in the Mediterranean – a landscape ecological perspective. – In: Goldammer, J. G. and Jenkins, M. J. (eds), *Fire in ecosystem dynamics*. SPB Academic Publishing, pp. 1–20.
- Pelizzo, A. and Tocci, A. 1978. Indagini preliminari sui semi e semenzali di *Pinus halepensis* e *Pinus brutia-eldarica*. – *Annali dell' Istituto Sperimentale per la Selvicoltura (Arezzo)* 9: 111–130.
- Petruzzelli, L. 1984. The effect of ethanol on germination of Aleppo pine seeds (*Pinus halepensis* Mill.). – *Biol. Plant.* 26: 235–238.
- Piotto, B. and Di Noi, A. 2001. Seed propagation of Mediterranean trees and shrubs. APAT handbook. – APAT, Rome.
- Raccuia, S. A. et al. 2004. Intraspecific variability in *Cynara cardunculus* L. var. *syvestris* Lam. Sicilian populations: seed germination under salt and moisture stresses. – *J. Arid Environ.* 56:107–116.
- Skordilis, A. 1992. Seed germination and seedling development of *Pinus halepensis* and *P. brutia*. Physiological and ecological approach. PhD thesis. – Univ. of Athens, in Greek.
- Skordilis, A. and Thanos, C. A. 1995. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. – *Seed Sci. Res.* 5: 151–160.
- Skordilis, A. and Thanos, C. A. 1997. Comparative physiology of seed germination strategies in the seven pine species naturally growing in Greece. – In: Ellis, R. H. et al. (eds), *Basic and applied aspects of seed biology*. Kluwer, pp. 623–632.
- Thanos, C. A. 1999. Fire effects on forest vegetation, the case of Mediterranean pine forests in Greece. – In: Efthichidis G. et al. (eds), *Wildfire management. Proc. Adv. Study Course on Wildfire Management*, Athens, Oct 1997, Algosystems and European Commission DGXII, Athens, pp. 323–336.
- Thanos, C. A. 2000. Ecophysiology of seed germination in *Pinus halepensis* and *P. brutia*. – In: Ne'eman, G. and Traubad, L.

- (eds), Ecology, biogeography and management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean Basin. Backhuys Publishers, pp. 37–50.
- Thanos, C. A. and Skordilis, A. 1987. The effect of light, temperature and osmotic stress on the germination of *Pinus halepensis* and *P. brutia* seeds. – *Seed Sci. Technol.* 15: 163–174.
- Thanos, C. A. et al. 1995. Comparative ecophysiological study of the postfire regeneration of Mediterranean pines (in Greek). – Tech. Rep. Res. Project PENED 91/824, Athens.
- Trabaud, L. et al. 1985. Recovery of burned *Pinus halepensis* Mill. forests. II. Pine reconstruction after wild fire. – *For. Ecol. Manage.* 13: 167–179.
- Tsakalimi, M. et al. 2004. Aleppo pine (*Pinus halepensis*) natural regeneration, without fire, in the Kassandra Peninsula, northern Greece. – In: Arianoutsou, M. and Papanastasis, V. (eds), Proc. 10th MEDECOS Conference, Apr 25–May 1, 2004, Rhodes, Greece, Millpress, Rotterdam, pp. 1–8.
- Tsitsoni, T. 1997. Conditions determining natural regeneration after wildfires in the *Pinus halepensis* (Miller 1768) forests of Kassandra Peninsula (north Greece). – *For. Ecol. Manage.* 92: 199–208.
- Turnbull, J. W. 1975. Forest tree seed testing. – In: Report on FAO/DANIDA training course on forest seed collection and handling. Vol. 2, FAO, Rome.