69. Comparative Ecophysiology of Seed Germination Strategies in the Seven Pine Species Naturally Growing in Greece

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Abstract

Seven out of the eleven European pine species grow naturally in Greece. *Pinus halepensis, P. brutia* and *P. pinea* are low-altitude, typical Mediterranean species. The Greek populations of *P. nigra, P. heldreichii, P. sylvestris* and *P. peuce* grow in high altitudes and are located at the southern limits of their natural world distributions.

Seed germination data concerning temperature dependence as well as light and/or stratification requirements are presented and discussed in relation to the individual species characteristics. Fire resilient *P. halepensis* and *P. brutia* are characterized by quite low germination in the dark, throughout their optimal temperature range (10–20°C); in addition, their germination is photosensitive (white-light promoted and far-red inhibited). Prolonged stratification is always beneficial to *P. brutia* but detrimental to *P. halepensis* seeds. Germination of *P. pinea* seeds is also slow but indifferent to light conditions and restricted to a very narrow temperature range, around 20°C. *P. nigra* seeds are fast germinating over a wide temperature range and rather indifferent to light. Germination of the deeply dormant *P. heldreichii* seeds as well as of the less dormant ones of *P. sylvestris* requires light and/or stratification.

By combining germination data for each species with the particular seasons of seed dispersal and the climatic conditions of the respective habitats, timing schedules of seed germination and subsequent seedling emergence in nature are proposed.

Introduction

Despite the relatively small area of Greece, the majority of European pine species grow naturally in the country, in the form of mixed or unmixed forests and/or isolated stands. *Pinus halepensis* Miller and *P. brutia* Ten. are common, low-altitude pine species, covering extended areas around the Mediterranean basin. *P. brutia* largely replaces *P. halepensis* in the north-eastern part and there is a well defined spatial isolation in their natural distributions; the shortest distance (in northern Greece) is about 50 km (Panetsos, 1975). *P. pinea* L. also a typical Mediterranean pine, is found in lowland areas often on sandy ground near the sea. Its natural range is currently uncertain, since this species has been widely planted for centuries due to its edible seeds. *P. nigra* Arnold, is a sub-Mediterranean pine, which ranges widely through southern Europe. Its natural

variability is reflected in its great taxonomic complexity at the subspecies and variety levels. *P. heldreichii* Christ *(P. leucodermis* Antoine) is a montane and subalpine pine confined to high elevations in the Balkan peninsula and in southern Italy, often replacing *P. nigra* at high altitudes. *P. sylvestris* L. is a widespread European Siberian pine; throughout its extensive distribution, this variable montane species has been divided into several subspecies and varieties. *P. peuce* Griseb. is a montane Balkan species surviving today only in a few humid mountain systems. The latter four species hardly reach the Mediterranean coast and their populations in Greece are located more or less at the southern limits of their natural world distributions.

Figure 1 shows the natural distribution of all pine species in Greece while a list of their main characteristics is presented in Table 1 (data from Pozzera, 1959; Little and Critchfield, 1966; Critchfield and Little, 1969; Krugman and Jenkinson, 1974; Panetsos, 1981; Greuter *et al.*, 1984; Strid, 1989; Ministry of Agriculture, Greece, 1992; Gaussen *et al.*, 1993).

Despite their economic and ecological significance, the ecophysiological diversity of pines is often not fully appreciated. Yet the variation among species
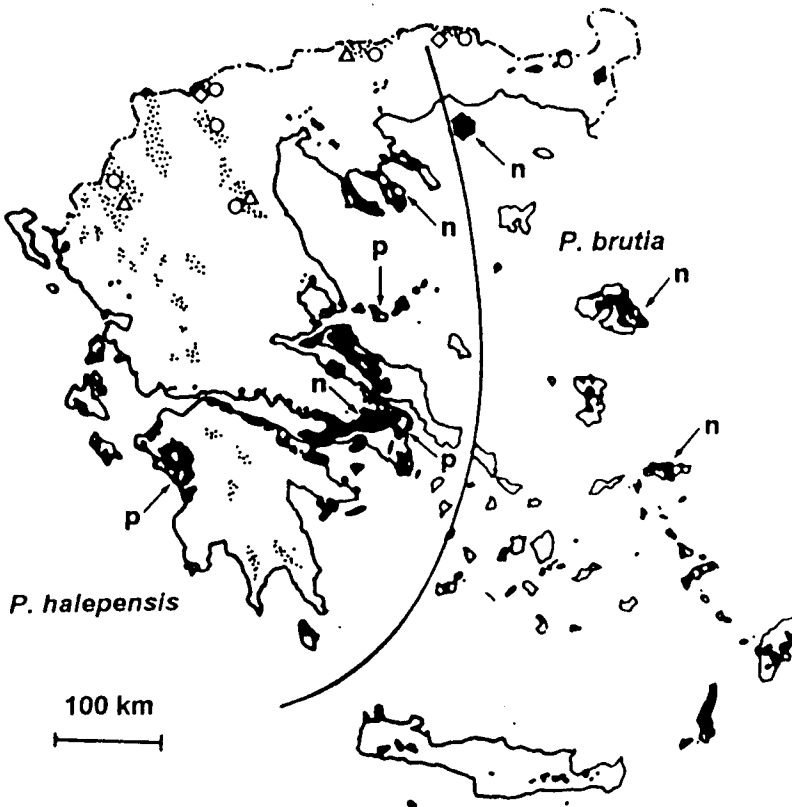

Figure 1. The geographical distribution of *Pinus brutia* (■), *P. halepensis* (■), *P. nigra* (▦ and n: isolated occurrence), *P. pinea* (p), *P. heldreichii* (Δ), *P. sylvestris* (○) and *P. peuce* (◇)

Table 1. A list of characteristics for the seven pine species naturally growing in Greece

	P. brutia	*P. halepensis*	*P. heldreichii*	*P. nigra*	*P. peuce*	*P. pinea*	*P. sylvestris*
Common name	East Mediterranean pine	Aleppo pine	Heldreich pine	Austrian pine	Balcan pine	Umbrella pine	Scotch pine
Natural distribution	East Mediterranean, Black Sea, Syria, Iran	Central and Western Mediterranean	Southern Balkans and Central Italy	Southern Europe and Western Asia	Southern Balkans (limited distribution)	Around Mediterranean basin and Black Sea	Europe and North Asia
Altitude (m)	0–850 (1200)	0–600	1300–2300	600–2150	~1700	0–150	1100–1800
Total cover in Greece (ha)	196 000	372 000	8300	282 000	10	200	21 000
Mature tree height (m)	15–20 (30)	10–20 (30)	20–30	40–50	20–30	15–25	20–40
Seed bearing age (y)	7–10	7–10	?	15–40	12–30	?	5–15
Seed bearing intervals (y)	1	1	?	2–5	3–4	3–6	4–6
Flowering dates	March–April	March–April	May–July	May–June	May–June	April–May	April–May
Cone ripening dates	April–May	April–May	Aug.–Sept.	Sept.–October	Sept.–October	Nov.–Dec.	Sept.–October
Seed production (years after flowering)	3	3	2	2	2	2.5	2
Seed dispersal dates	May–June (some remain closed)	May–July (many remain closed)	Sept.–October	March–April	Sept.–October	May–June	Dec.–March
Mean seed weight (mg)	35–90	~20	~30	~50	~30	~850	~10

in morphology and life history is quite large. Concerning seed germination, relatively little research has been carried out in the Mediterranean species *P. brutia, P. halepensis* and *P. pinea* (e.g. Magini, 1955; Calamassi *et al.*, 1980; 1984; Thanos and Skordilis, 1987; Skordilis and Thanos, 1995). On the other hand, seed germination data available for *P. sylvestris, P. nigra, P. heldreichii* and *P. peuce* refer to northern provenances. The regeneration of all these pines depends exclusively upon their seeds; therefore a study on their particular ecophysiological adaptations may contribute considerably to the conservation of these species as well as of their habitats in Greece.

Materials and Methods

All seeds were either hand collected by the authors or offered by the Forestry Division, Ministry of Agriculture, Greece. Seed provenances used in the present study were, respectively: *P. sylvestris* from Kozani, *P. heldreichii* from Konitsa, *P. nigra* from Kastoria, *P. pinea* from Attica, *P. peuce* from Mt Voras, *P. brutia* from Thasos, Samos, Rodos and Lasithi and *P. halepensis* from Halkidiki, K. Vourla, Attica, Istiaia and Steni.

Germination tests were performed with 5 replicates of 25 seeds per Petri dish (diameter 9 cm, lined with two discs of filter paper and moistened with 5 ml of distilled water). Experiments were carried out in plant growth chambers (W.C. Heraeus GmbH BK Model 5060 EL, Germany) where the temperature was kept constant within $\pm 0.5°C$. The germination experiments presented in Figure 3 were carried out on a temperature- and light-programmable growth cabinet, model GB48 (Conviron, Canada) equipped with a lamp canopy of 48 incandescent bulbs (Sylvania 50A19, 50 W, 227 V) and 10 fluorescent tubes (Sylvania Cool White FR96T12/CW/VHO-235/1). Temperature was kept constant while a photoperiod of 12 h was applied. The broad-band far-red (FR) light source, was obtained through a FR filter (one blue and 2 red plexiglas layers, Rohm: No. 627 and No. 501, respectively). Cold moist stratification was accomplished by maintaining imbibed seeds in the dark, at a temperature of $3 \pm 1°C$.

Germination was recorded every 1 or 2 days (d) and was considered complete when no additional seeds germinated. The criterion of germination was visible radicle protrusion; seeds exhibiting abnormal germination were excluded from germination counts. All germination percentages were based on the filled seed portion only; empty seeds determined by dissection tests, performed after completion of each experiment, were not counted. The mean percentage of unsound seeds was less than 5% for *P. heldreichii, P. peuce, P. pinea* and *P. sylvestris* seedlots, around 10% for all *P. halepensis* and *P. brutia* seedlots and around 25% for *P. nigra*. Data concerning *P. brutia* and *P. halepensis* seed germination, shown in Figures 2 and 3, represent mean germination values for all the seedlots used.

Results

Final dark germination, in the range of 5 to 25°C, was found to vary significantly between the different pine species (Fig. 2). These differences can be grouped and summarized as follows: *P. brutia* and *P. halepensis* germinated promptly in a rather wide temperature range, 10–20°C. On the basis of germination rates, 20°C could be considered as the optimal temperature. Germination of both these species could also take place at the marginal temperatures, 5 and 25°C, but to a significantly lower level; in addition, the germination rate at 5°C was extremely low, while at 25°C it was relatively fast. Similar results were obtained for *P. nigra,* although the maximum germination percentages were scored in the narrower range of 15–20°C. Once more, 20°C was the optimal germination temperature. In comparison with the two previous species, the temperature-dependence germination curve of *P. nigra* seems to have shifted towards higher temperatures.

Dark germination of *P. pinea* seeds was feasible only at 20°C; its inability to germinate at a wider range seems to be a characteristic of the species.

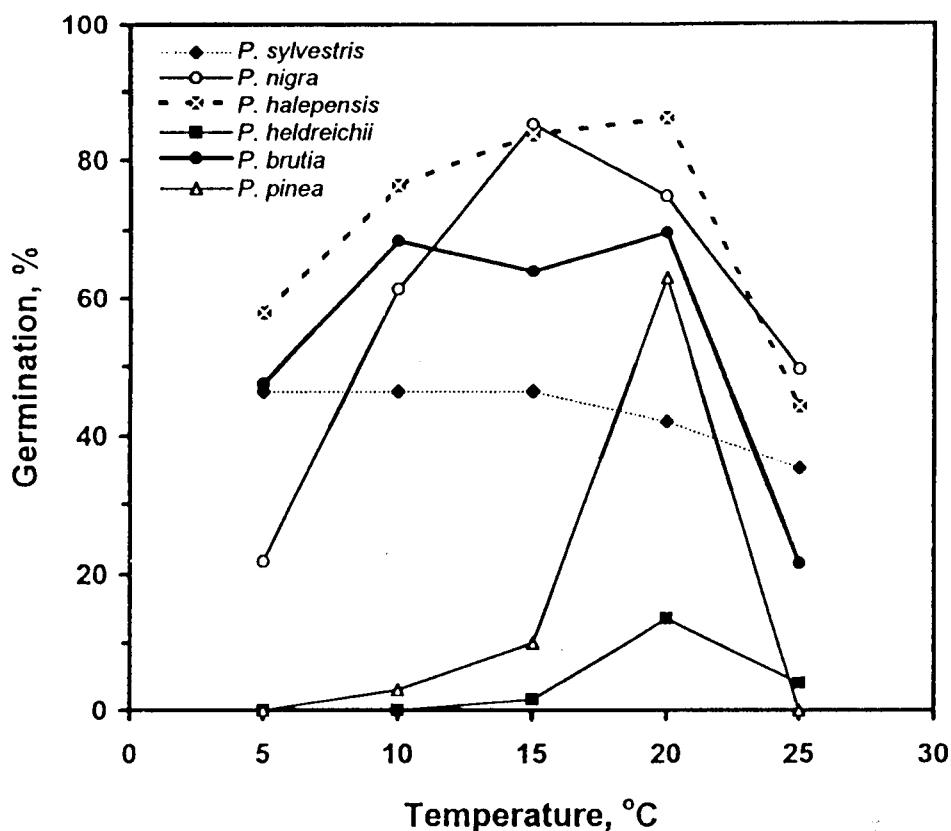*P. sylvestris* and *P. heldreichii* exhibited a primary seed dormancy expressed

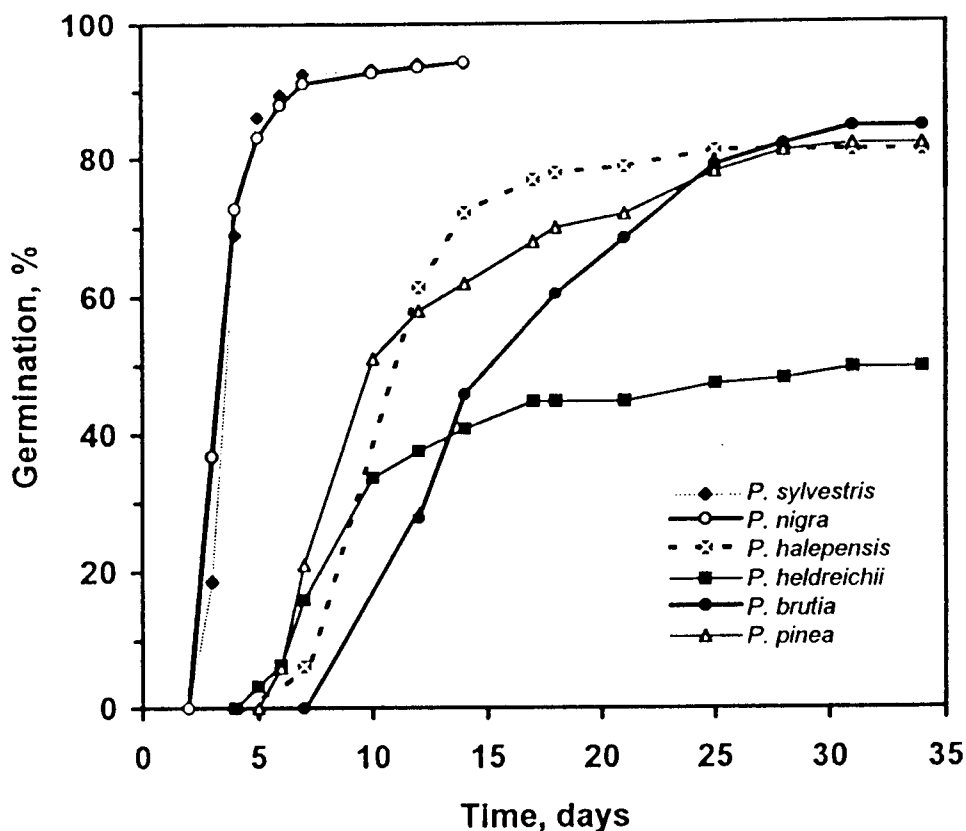*Figure 2.* Final dark germination as a function of temperature

Figure 3. Time course of seed germination at 20°C in a 12 h white light photoperiod

by a restricted germinability throughout the temperature range. However, the degree of dormancy differed significantly among the two species. *P. sylvestris* final dark germination values were quite constant, around 40%, for all temperatures tested and germination rate was optimal at 20°C. *P. heldreichii* seeds, on the other hand, were deeply dormant since the highest germination percentage (obtained at 20°C) was less than 15%.

Germination time courses of all pine species are shown in Figure 3. Germination took place at the optimal temperature of 20°C and, in addition, a 12 h white light photoperiod was applied. Diurnal light resulted in a general increase of germination rate; in each species tested, germination curves were advanced (compared to dark controls, data not shown in the figure) by 1 to 4 d, depending on the species. Moreover, a significant promotion of final germination was observed in *P. sylvestris* and *P. heldreichii*. Primary dormancy was fully released by white light in *P. sylvestris* seeds but only partially in the more dormant ones of *P. heldreichii* (cf. Fig. 2).

Concerning germination rate, *P. nigra* and *P. sylvestris* are relatively fast germinators since their almost identical time course curves were completed

Table 2. The effects of light (WL: white light; FR: far-red) and prechilling (one month at 3°C on the final level (G) and rate (R) of germination at the optimum temperature (20°C), as compared to dark controls

	WL promotion		FR	Chilling promotion	
	G	R	inhibition	G	R
P. brutia	(+)	+	+	(+)	+
P. halepensis	–	+	+	(–)	+
P. heldreichii	+	+	–	+	+
P. nigra	–	+	–		+
P. peuce	–	–		+	+
P. pinea	–	+	–		
P. sylvestris	+	+	–	+	+

(+), In dormant seedlots of *P. brutia*, the final germination percentage is improved significantly by WL and/or chilling

(–), Prolonged prechilling treatments (exceeding 2 months) are detrimental to *P. halepensis* seeds

within a week. On the other hand, the germination of the four other species, even at these optimal conditions, was considerably slower.

The effects of light and prechilling on the final level and rate of germination at 20°C, as compared to dark controls are summarized in Table 2. Far-red light resulted in a total inhibition of *P. halepensis* and *P. brutia* seed germination, while no significant differences in the final germinability were observed in *P. nigra, P. pinea* and *P. sylvestris* seeds (detailed data not shown). The effect of prechilling prior to transfer to 20°C in the dark, was shown to deviate among the species tested (detailed data not shown), escalating from an increase of germination speed only (in *P. halepensis, P. brutia, P. nigra*) to a full release of dormancy in *P. sylvestris* and *P. heldreichii* seed germination.

P. peuce seeds were found to be extremely dormant. No germination was recorded at any temperature and light regime used. However, preliminary results indicate that a prolonged stratification (exceeding 4 months) is required for germination induction.

Discussion

A considerable array of differences was observed among the seven pine species concerning cone and seed characteristics, cone ripening and seed dispersal periods as well as germination behaviour (temperature dependence, germination speed, FR inhibition, chilling requirement). These differences may be attributed to varying ecophysiological strategies in regard to the temporal pattern of seedling emergence and establishment for each particular species.

According to the variants of this strategy, seed germination is timed either during autumn and early winter (in southern, mild and dry areas) or in spring (in regions with relatively cold and moist climatic conditions) or even throughout the wet season (in intermediate conditions), depending on the species.

The two typical, low-altitude Mediterranean, fire-resilient pines *P. halepensis* and *P. brutia* are distinguished by a high degree of cone serotiny (resulting in abundant canopy seed banks; Daskalakou and Thanos, 1994) as well as precocious reproduction. Laboratory germination is quite slow but feasible throughout the range of cool temperatures. Although short periods of stratification improve germination rates of both species, long term chilling of *P. halepensis* seeds was eventually proven detrimental (Skordilis and Thanos, 1995). The germination of the above species in the field is realized soon after the onset of the rainy season (mid October–November) and may continue to the end of the winter. The light sensitivity of both species (FR fully inhibits germination) indicates the 'invasive' potential of colonizing open habitats (pioneer species). It must be mentioned that populations of these species tend to survive as seeds through recurrent summer fires. Mature individuals have a low tolerance of fire but populations are fire-resilient through abundant seed reproduction and delayed seed release, similarly to the fire-resilient pines of North America (McCune, 1988). In addition, together with *P. pinea*, they are the only European pines where seeds are produced three years after cone initiation (two years for the other species, Table 1).

Our results concerning temperature dependence of *P. pinea* seed germination are in agreement with similar studies on other provenances (Magini, 1955). In contrast to the other pine species studied in the present work, the much heavier seeds of *P. pinea* are wingless; thus they are dispersed mainly under the canopy of the parent trees where seedling recruitment is eventually observed (Masetti and Mencuccini, 1991). Therefore, the indifference of *P. pinea* seed germination towards FR may be viewed as an adaptation to the below-canopy recruitment.

The deeply dormant seeds of *P. heldreichii* displayed an absolute stratification requirement, which could not be substituted by prolonged illumination. Analogous results were also shown by Borghetti *et al.* (1986, 1989) for Italian populations of this species. Under natural conditions, the presence of primary seed dormancy probably does not permit germination during autumn; thus germination takes place in spring, after seeds have experienced the low temperatures of winter, thus preventing young seedlings from being exposed to damaging freezing temperatures.

P. nigra seed germination was quite fast in a wide temperature range (also in agreement with populations of other origins; Paci, 1989), indicating that seed germination in nature will take place in spring, soon after dispersal. In contrast to the dry summer conditions prevailing in *P. halepensis* and *P. brutia* distribution areas, water availability in the mountainous habitats ensures the successful survival of the spring germinating *P. nigra* seedlings.

P. sylvestris seed dispersal, in nature, takes place at the end of winter. In this species, the promotive effect of stratification and light on the release from

primary dormancy, exhibited by a large proportion of the seed population, could be considered an adaptive strategy, which either delays germination if seeds are covered by snow or enhances germination at an open, snow-free position, suitable for seedling survival.

P. peuce, occurring as a Eurasiatic relic in some mountains of the Balkan peninsula (Wilhelm, 1987), is taxonomically very different from all other pine species studied in the present work (Prus-Glowaski *et al.*, 1985). The observed difficulties in germinating these extremely dormant seeds are in agreement with results from other provenances (Djordjeva, 1967). Due to its very extended prechilling requirement, germination in nature may take place the second or even third year following dispersal.

Acknowledgements

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