

ECOPHYSIOLOGY OF SEED GERMINATION IN *PINUS HALEPENSIS* AND *P. BRUTIA*

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Introduction

Aleppo and the east Mediterranean (or brutia) pines (*Pinus halepensis* and *P. brutia*, respectively) are obligate seeders; therefore, the regeneration of both these two species and of their pine forests is totally dependent upon seedling recruitment (particularly in post-fire conditions; Thanos 1999). Every year, mature pine trees produce prolific numbers of seeds, a large fraction of which remain enclosed within their cones (canopy seed bank) and maintain their viability for long periods (Cucui *et al.* 1996, Daskalaku and Thanos 1996).

In their discourse on serotiny (mostly for Southern Hemisphere plants), Lamont *et al.* (1991) put forth (and discussed) 9 adaptive postulates. Five of these adaptive characteristics may be applicable to the Mediterranean pines: 1. Protection of seeds (from both consumers and fire). 2. Maximisation of seed availability after a fire. 3. Efficient and homogeneous, post-fire, wind seed dispersal. 4. Minimisation of post-dispersal hazards (seed colour mimicry and elimination or reduction of predator populations). 5. Optimisation of seed germination and early seedling growth substrate (nutrients added by fire and considerably decreased competition for light and water). In regard to the latter, water and light seem to be most important, as pines are generally well adapted to nutrient poor soils (Mirov 1967). Hanley and Fenner (1997), working with seedlings of *P. brutia* over the first 12 weeks of growth, showed that deprivation of any single macronutrient had no effect on growth.

In addition to the above, the character of partial cone serotiny (or seed bradychory, Thanos 1999), shared by both *P. halepensis* and *P. brutia* (Daskalaku and Thanos 1996), confers a dual strategy of regeneration. Besides the *en masse* post-fire germination, their seeds can exploit and colonise open and disturbed areas virtually every year, also in fire-free conditions.

Seed Morphology

Despite their close phylogenetic relationship, seeds of *P. halepensis* and *P. brutia* show marked differences in both their structure and, as will be discussed in the next section, germination behaviour.

Firstly, the average seed weight is significantly higher in brutia than in Aleppo pine, e.g., Nahal (1983) refers to a gross average seed weight of 50 mg for *P. brutia* as opposed to 15-20 mg for *P. halepensis*. In an extensive survey of 50 Turkish provenances of *P. brutia* seeds, Sefik (1965) determined an average seed weight of 56 mg (range 38-68 mg); Isik (1986) found a higher average (63.8 mg) from 6 Turkish provenances whereas Panetsos (1981) reported a considerably lower value from various Greek provenances (40.5 mg). Similarly, Aleppo pine seed weight has been found to vary widely among collections (12-30 mg) according to several sources (e.g., Debazac and Tomassone 1965, Pelizzo and Tocci 1978, and Thanos *et al.* 1995). Figure 1 shows the linear regression curves between average seed weight and average number of cotyledons in numerous accessions of both species. The grand average values for seed weight are 20 mg for *P. halepensis* and 51 mg for *P. brutia* and there is minimal overlap between the ranges of values. The grand average number of cotyledons are 7.4 and 8.4, respectively; a considerably steeper slope is obtained in Aleppo pine than in brutia.

Seed weight has been shown to vary 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, on the one hand, and seed weight and germinability, on the other. Mean seed weight of *P. brutia* was considerably more variable among trees than within a tree, and it was almost constant within a single cone (Thanos and Daskalaku 1993). Finally, seed weight may correlate positively with tree and cone size; in a nursery study (Matziris 1998), the cones produced by 10-year-old Aleppo pine saplings showed particularly high values of cone and seed weights (28.7 mg), which were attributed to the favourable growing conditions.

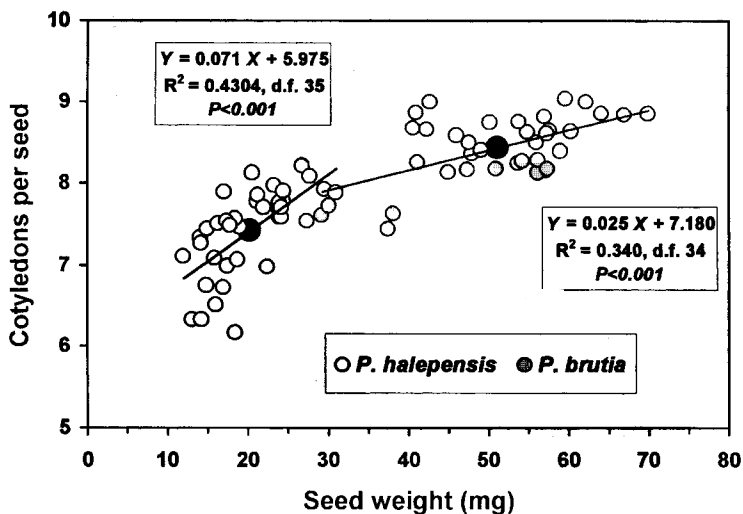


Fig. 1. The average cotyledon number as a function of the average seed weight in 37 and 36 provenances of *Pinus halepensis* and *P. brutia*, respectively. Straight lines are best fits of linear regression (equations are given in the insets) and the 2 black circles represent the gross mean value for each species (Based on compiled data from Debazac and Tomassone 1965, Pelizzo and Tocci 1978 and Thanos *et al.* 1995).

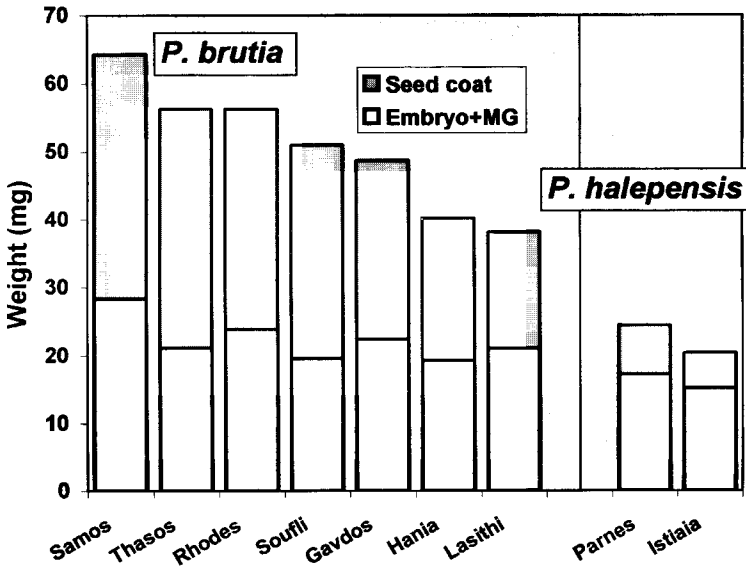


Fig. 2. Average seed weight (total length of bars) and the contribution of the two components (seed coat and embryo plus megagametophyte) in seeds of *Pinus brutia* and *P. halepensis* (7 and 2 Greek provenances, respectively; after Thanos *et al.* 1995).

A second notable difference between seeds of Aleppo and brutia pines is seed coat thickness. As illustrated by Figure 2, a substantial fraction of *P. brutia* seed weight is contributed by the seed coat (55.5% on average) whereas the corresponding contribution is only half this value (27.4%) in Aleppo pine. As a consequence, the difference in embryo (plus megagametophyte) weight is considerably less marked than between whole seeds. Expressed on an embryo weight basis, the seed coat is 5 times greater in *P. brutia* than in *P. halepensis*.

Finally, seed coloration is an interesting feature. Saracino *et al.* (1997) detected a fine dark dust inside closed cones that covers the seeds externally; the dust remains on the seeds when they are released after a fire. Therefore, this dark coloration is postulated to be an adaptation of mimicry that reduces the predation risk of Aleppo pine seeds (and presumably those of brutia as well) against granivorous birds on the ash-covered soil.

Physiology of Seed Germination

Apart from a certain number of 'early' works (e.g., Oppenheimer 1933 and Mouloupoulos 1939), Magini (1955) and Sefik (1965) should be praised as the pioneers of seed germination research in *P. halepensis* and *P. brutia*, respectively.

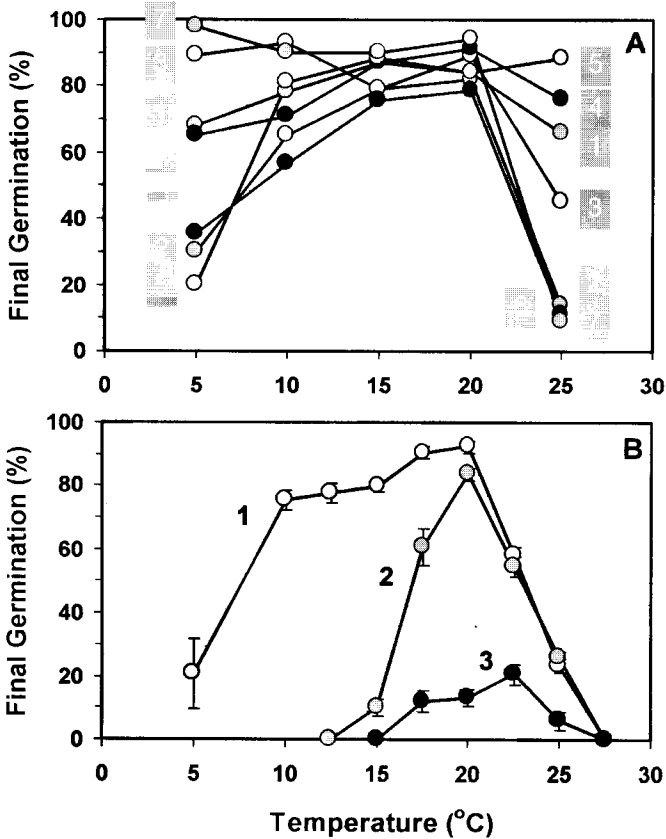


Fig. 3. Final dark germination as a function of temperature in *Pinus halepensis* (A) and *P. brutia* (B). Each curve corresponds to a different, Greek seed provenance: 7 for Aleppo pine and 3 for brutia; 1: Lasithi (eastern Crete), 2: Thasos Island (Aegean Sea), and 3: Soufli (northern Greece); Vertical lines represent S.E. (After Thanos *et al.* 1995 and Skordilis and Thanos 1995).

Temperature Range of Germination

Magini (1955) studied germination as a function of temperature and placed the optimum for *P. halepensis* seeds at 18.5°C; high and low temperatures (25–28°C and below 10°C) retarded or inhibited germination. In an extensive survey of 50 Turkish provenances of *P. brutia* seeds, Sefik (1965) determined an average germinability of 78% at 20°C.

Several studies with Greek provenances confirmed the optimum temperature range (15–20°C) for Aleppo pine seed germination in darkness (Thanos and Skordilis 1987, Skordilis 1992, Skordilis and Thanos 1995). Figure 3A, in addition to the optimal range (15–20°C), illustrates the suboptimal cool temperatures (around 10°C) and the 'ambiguous' extremes 5 and 25°C. Thus a particular provenance may germinate fully at only a single end of the temperature range (e.g., provenances 5 and 7) whereas other provenances follow a less clear pattern. Calamassi *et al.* (1984)

studied the effects of temperature on 9 circum-Mediterranean provenances and concluded that dark germination is optimal in the range 15-21°C (average means 82-83%); at 24°C germination is considerably decreased to a gross average of 58%.

A similar optimal temperature range (15-20°C) has been repeatedly observed for *P. brutia* seeds (Sefik 1965, Isik 1986, Thanos and Skordilis 1987, Skordilis 1992). Nevertheless, in several provenances of this species a more or less pronounced dormancy has been observed. Figure 3B illustrates germinability as a function of temperature in three representative lots of brutia pine seed.

Rate of Germination

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 (time needed for 50% of final germination) were 12, 9.5 and 10 days at the optimal temperatures of 15, 18 and 21°C, respectively. A similarly 'sluggish' germinability was also observed in several additional cases with both pine species (Thanos and Skordilis 1987, Skordilis 1992, Skordilis and Thanos 1995). This 'delay' mechanism of seed germination in these pines (and numerous other Mediterranean plants) is still unknown; however, it should not be attributed to a seed-coat-imposed, restraining effect because decoating in both Aleppo and brutia pine seeds resulted in only a slight enhancement of germination rate (Skordilis 1992, Thanos *et al.* 1995).

Successive applications of Hg^{2+} and Cl^- ions resulted in a dramatic promotion of the germination rate of Aleppo pine seeds; T_{50} was decreased to only 2 days from a value of 9 days for the untreated seeds (Thalouarn and Heller 1977). This stimulation has been attributed to modified water permeability because an early increase of embryo (plus megagametophyte) imbibition was observed in treated seeds followed by both reserve mobilisation and radicle growth (Pargney and Thalouarn

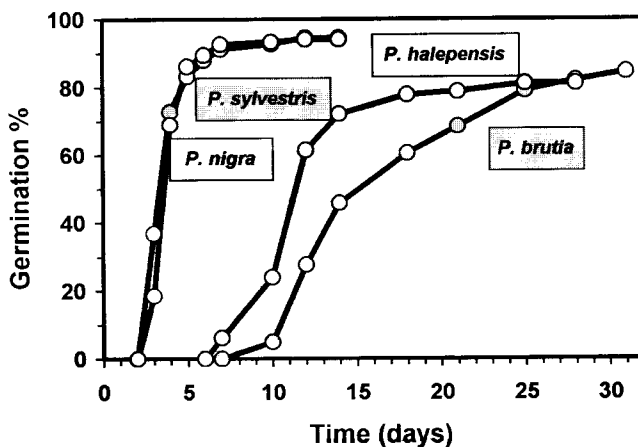


Fig. 4. Germination time courses for coastal (*Pinus halepensis*, *P. brutia*) and mountainous (*P. sylvestris*, *P. nigra*) pine species (all seed provenances from Greece) at optimal conditions (20°C, 12 h white light photoperiod) (After Skordilis and Thanos 1997).

1978). Although the mode of action of Hg^{2+} ions remains elusive, the presence of the seed coat is required for the manifestation of germination induction.

Among the 13 pines belonging to the assembly of the Mediterranean pines (according to Mirov 1967 and Klaus 1989), *P. halepensis* and *P. brutia* are placed (on an evolutionary basis of morphological characters, Klaus 1989) in the group of 'Mediterranean shore and island pines' (together with *P. canariensis*, *P. pinea* and *P. pinaster*). The remaining species are grouped in two distinct classes of mountainous pines (the first of which comprises the *Diploxylon* species). These three classes seem to conform well with seed germination behaviour (Skordilis and Thanos 1997); in particular, the maritime Mediterranean pines, *P. halepensis* and *P. brutia*, show a very slow germination rate whereas the mountainous species germinate considerably faster (Fig. 4).

The Effect of Light on Germination

The role of light and the mediation of the phytochrome photoreceptor system is generally well studied in pine seeds and has been known for some time (Toole, 1973). Nevertheless, and despite the well known photophilous nature of both species (Trabaud 1995), until recently only preliminary results by Shafiq (1979a) and Calamassi (1982) had revealed an overall inductive role of light on seed germination of *brutia* and Aleppo pine, respectively. In a number of detailed studies on the subject, the physiological and ecological importance of light was investigated (Thanos and Skordilis 1987, Skordilis 1992, Skordilis and Thanos 1995, Thanos *et al.* 1995). It was found in both species that continuous red (R) light or diurnal white light always promotes the germination rate and sometimes the maximum percentage as well. Continuous or intermittent far-red (FR) light not only inhibits germination in both species, but also induces a secondary dormancy; the relief of this photodormancy is under phytochrome control as shown by R-FR reversibility.

Dormancy and Stratification

Aleppo pine seeds are generally non-dormant; on the other hand, several provenances of *P. brutia* exhibit considerable and varying degrees of dormancy. Moreover, Isik (1986) was able to show that among six populations from different elevations (southern Turkey) the high elevation provenances germinated more slowly and had lower germination percentages. In another case with three Greek provenances from contrasting latitudes, dramatic differences in the degree of dormancy were noted (Fig. 3B). Stratification (1-3 months) resulted in a considerable promotion of *P. brutia* seed germination (Fig. 5A). Nevertheless, the inductive effect of stratification was shown to differ among the three provenances, escalating from a simple increase of germination rate (in the southern seed lot from Crete) through a broadening of the temperature range of germination (in the intermediate lot from Thasos Island) to a dramatic release from a particularly deep dormancy (in the northern lot from Thrace). These deeply dormant seeds of the latter provenance displayed an absolute stratification requirement; prolonged illumination or seed coat scarification could only slightly substitute for the promotive effect of pre-chilling. Moreover, a considerable interaction between far-red light and stratification was revealed in the dormant seeds of *P.*

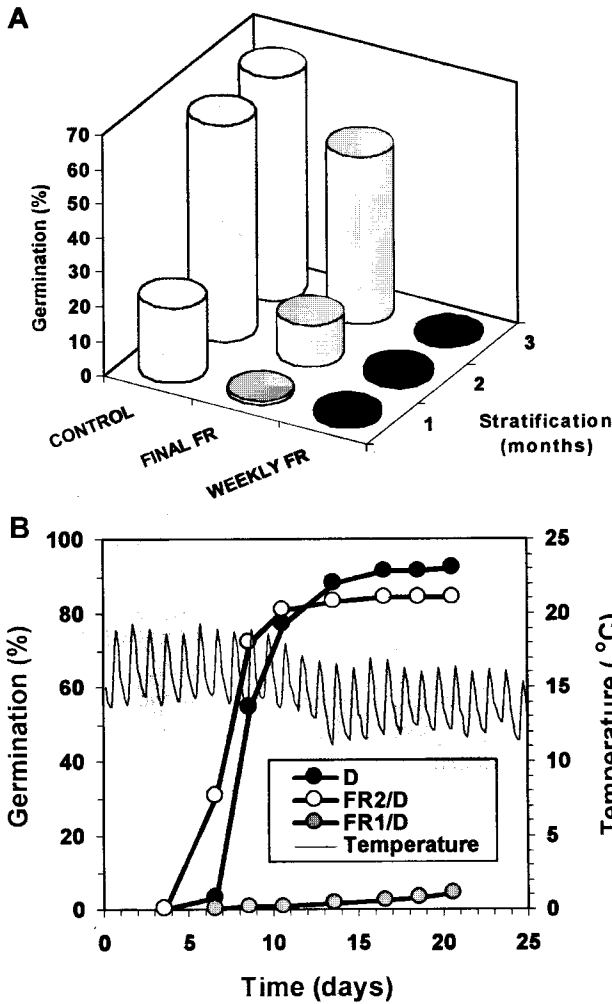


Fig. 5. *A*. Final germination of *Pinus brutia* seeds (Soufli provenance) at 15°C and darkness, after various durations of a stratification pre-treatment. Control: seeds kept in darkness throughout. Final FR: seeds given a single 30-min long far-red pulse at the end of the chilling pre-treatment. Weekly FR: seeds given weekly, 30-min long, far-red irradiations throughout pre-chilling. (After Skordilis and Thanos 1995). *B*. Time course of *Pinus halepensis* seed germination in simulated autumn conditions under 3 different light regimes: diurnally alternating far-red (FR) – darkness (“intense”, FR1; “mild”, FR2) and continuous darkness (D). The fluctuating lines represent the average daily temperature at a representative, semi-arid Mediterranean environment (Athens Airport); time 0 corresponds to November 8. The seeds were extracted from freshly-matured cones (0 years old) collected from the forest of Villia, Attica (After Daskalaku and Thanos 1996).

brutia (Fig. 5A); far-red pulses during stratification could either cancel or diminish the germination promotion induced by low temperatures (Skordilis and Thanos 1995).

In the survey of 50 Turkish provenances of *P. brutia* (Sefik 1965), stratification was found to decrease germination rate and increase germinability (above the gross

average 78%; this indicates that a few provenances may have been dormant). In addition, Shafiq and Omer (1969) found that stratification in the sand for 45 days at 4°C increased the germinability of *P. brutia* seeds considerably. Similarly, Falusi (1982) found a markedly higher rate of germination in stratified seeds of *P. brutia*. Although the dormancy observed in brutia pine seeds evidently belongs to the embryonic type, there may be some interaction with the seed coat, as implied in the previous paragraph. Shafiq (1970/71) treated *P. brutia* seeds with dense sulphuric acid and observed a decrease of germinability (obviously as a result of embryo damage) while the mechanical scarification of the seed coat led to a significantly improved germination at 25°C (Shafiq 1979b).

Calamassi *et al.* (1984) studied the effects of stratification on 9 circum-Mediterranean provenances of Aleppo pine and an overall suppression of germinability was obtained (from a grand mean of 83% for untreated seeds to 77% and 69% after 1 and 2 months respectively). Although not all provenances respond similarly (some are unaffected by stratification), the rate of germination is enhanced (overall means of T_{50} decreased by 5 and 6 days, respectively). Similarly, Skordilis (1992) and Skordilis and Thanos (1995) found that a stratification treatment for longer than 1 month resulted in a considerable decrease in germinability of Aleppo pine; this decline was attributed to a gradual necrosis of embryos in cold temperatures.

Moisture Stress, pH and Inhibitors

Germination in both species is gradually suppressed with increasing concentrations of osmotic solutions (moisture or hydric stress). Nevertheless, the data obtained were greatly variable not only among provenances but also among researchers (Calamassi *et al.* 1980, Falusi and Calamassi 1982, Falusi *et al.* 1983, Thanos and Skordilis 1987, Skordilis 1992, Henig-Sever *et al.* 1996). According to Thanos and Skordilis (1987), when seeds are osmotically stressed the slow rate of germination becomes even slower, although the final germination percentage is inhibited only at a range of very low osmotic potentials. Ne'eman *et al.* (1993) and Henig-Sever *et al.* (1996) studied the role of ash and pH on seed germination of Aleppo pine and found a considerable suppression at values 9-10, similar to those encountered under post-fire conditions in the alkaline ashes. Recently, Henig-Sever *et al.* (2000) found that this inhibition may be counteracted by an enhancement of seed germination brought forth by nitrate and ammonium ions (also known to occur in the soil of a recently burned forest). ABA was found to inhibit seed germination in *P. brutia* and its action was reversed by a subsequent application of GA_3 , whereas kinetin and benzyladenine were ineffective (Kabar 1998). The application of three systemic insecticides was toxic to both seeds and seedlings of Aleppo pine (Olofinboba and Kozlowski 1982).

Protocols of Germination

Seed germination rules by the International Seed Testing Association, ISTA (1993) include instructions for Aleppo pine, only. They recommend germination at a constant temperature of 20°C, first count at 7 – last count at 28 days, with no further comments. Similarly, Young and Young (1992) suggest 20°C for 28 days but they add a note that seeds are sensitive to warm temperatures. Light is not mentioned and

no suggestions are furnished at all for *P. brutia*. On the basis of the experimental evidence described previously, it is suggested that germination protocols be finally formulated as follows:

- *Pinus halepensis* TP, 20°C, 7-28 days (first-last count), white light.
- *Pinus brutia* TP, 20°C, 7-28 days (first-last count), white light; if final germination is below 70-80% repeat the test after a 2-month-long pre-chilling treatment. [TP: on top of paper]

Ecology of Seed Germination

In a speculative but far-reaching discussion, Magini (1955) emphasised the difference in the optimum temperature for seed germination between Aleppo and umbrella pines (on the one hand) and a number of mountainous pines. This difference was attributed to diverging life history adaptations, namely seedling emergence and survival in relation to their corresponding, critical (restrictive) seasons: dry and hot summer for the former and frozen winter for the latter. Thus germination is assumed to take place during fall and winter in the former and spring in the latter and Magini should be acknowledged as the pioneer of pine seed germination ecology.

As stressed in the introduction, post-fire regeneration of both species depends exclusively upon the canopy seed bank, due to the short life span of their soil seed banks and the consumption by fire of all the seeds that happen to be found in the soil (Daskalakou and Thanos 1996). Therefore, considerably long seed longevity is of paramount importance, and indeed it seems that this is the case for both species. Viability of Aleppo pine seeds within serotinous cones was found to decrease at a slow pace with cone age (Cuccui *et al.* 1996, Daskalakou and Thanos 1996). In *P. brutia*, germination tests showed that seeds remaining in closed cones had high germination ability (Eler 1990); moreover, Selik (1958) reported that even nine-year-old cones contain germinable seeds.

Cones confer a noteworthy protection to seeds against the lethal temperatures of fire, as shown by several field and laboratory studies. For instance, Trabaud and Oustric (1989) found that high temperatures in a simulated fire in the lab killed all Aleppo pine seeds. Martinez-Sanchez *et al.* (1995) applied short heat treatments to *P. halepensis* seeds and concluded that germination is suppressed by the temperature increase that can be reached in soils during a fire. Similarly in *P. brutia*, the germination percentage of the seeds decreased significantly when 'bare' seeds were exposed to high temperatures from 70-90°C (Neyisci 1988). Seedling survivorship and growth in *P. brutia* was reduced after the seeds had been exposed to temperatures exceeding 90°C (Hanley and Fenner 1998); with a thermal pre-treatment above 110°C, a rapid decline in germination was also observed. On the other hand, when seeds were left inside the cones that were heated to 125°C, the seeds remained viable (Cengiz 1993).

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. In 7 out of 9 vegetation types germination was satisfactory whereas significantly lower germination levels were observed in 2 of them that were dominated by the grass *Brachypodium ramosum*.

Because pine seed germination in the field takes place *en masse* almost exclusively in post-fire conditions, most of the available information derives from studies of burned sites (Thanos 1999). Pine seed germination and seedling emergence take place almost exclusively during the first post-fire wet season; more specifically, their occurrence usually takes the form of a massive wave early in the period (October-January), closely following the start of the wet season, as shown by both field (Daskalakou 1996) and laboratory studies (Thanos and Skordilis 1987; Skordilis and Thanos 1995). During the second post-fire rainy period no additional seedlings are usually observed. In *P. brutia*, it was postulated that this pattern may be modified in certain regions with more severe winters; the seedling recruitment profile is shifted to the late winter or early spring months, possibly as a frost-avoiding mechanism of the vulnerable seedlings (Skordilis and Thanos 1995). Thus the differences observed in the germination behaviour among the various *P. brutia* provenances (Fig. 3B) may be attributed to a variable ecophysiological strategy in regard to the temporal pattern of seedling emergence and establishment. According to the variants of this strategy, seed germination is timed to occur during either spring (in regions with relatively cold and moist climates) or autumn and early winter (in southern, mild and dry areas) or both (in intermediate conditions).

The above postulated strategy is based on a particular kind of seed dormancy that can be manipulated by stratification. Falusi (1982), based on a study of the stratification effect on seeds of *P. brutia* from different provenances, was the first to suggest differential timing of autumn *versus* spring germination in relation to seedling survival during the critical winter season. Although much remains to be done to validate this hypothesis, certain field data and observations do support it. Eron (1987) reported that germination of *P. brutia* generally starts in November at lower elevations (250-300 m), in February at middle elevations (350-650 m) and in April at higher ones (700-800 m). Ozdemir (1977) found that germination of *P. brutia* in the Antalya region starts in mid-January and mid-March at the lower and higher elevations, respectively. In two other cases, an initial small part of the population germinated during the winter months, November to February, and a 'burst' of germination occurred in March and April (Thanos *et al.* 1989, Eler and Senergin 1990).

Because pine seed germination takes place only during the first post-fire wet season, it is of paramount importance for the survival of the ensuing population that erratic germination be reduced or eliminated. Therefore, the slow rate of germination is considered an adaptation to delay germination until well into the wet season and in this way reliable water availability is ensured.

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 favoured in open, sunny sites (Daskalakou and Thanos 1996). Figure 5B shows that white light irradiation during the warm part of simulated autumnal day results in a marked promotion of germination, whereas intense far-red light (simulating a dense canopy cover) causes a significant inhibition, in agreement with previous similar results (Thanos and Skordilis 1987).

Henig-Sever *et al.* (1996) confirmed the relatively high resistance of pine seed germination towards osmotic stress (previously reported by Thanos and Skordilis 1987) and suggested that this may constitute an adaptation to the post-fire, ash-

enriched (i.e., low osmotic potential) substrates. According to Saracino *et al.* (1998), ashes and particularly lipids produced during fire by combustion of various plant materials may affect Aleppo pine seed germination. This postulated effect is attributed to the modified hydrological properties of the burned soil and eventually suppresses seedling emergence and affects spatial patterns. Henig-Sever *et al.* (1996, 2000) concluded that increased pH and substantial concentrations of nitrate and ammonium, in the burned soil, are important factors for seed germination and forest regeneration.

Conclusions and Suggestions for Future Research

Based on several vegetative and taxonomic traits, Mirov (1967) and, later, Panetsos (1981) have granted to *P. brutia* the attribute of 'variable' whereas *P. halepensis*, despite its postulated close evolutionary relationship to the former and inversely to its large geographical distribution, is considered 'stable'. Depending upon the altitude and latitude of the provenance, this variability of *P. brutia* is also displayed by seed morphological characters (seed size and seed coat contribution) as well as by the 'plasticity' of seed germination (degree of dormancy, stratification requirements and, eventually, timing of seedling emergence).

Small seed size in *Pinus* has been correlated with-both invasibility (Richardson *et al.* 1990) and fire-resilience (McCune 1988). Considering the factors which contribute to the invasive potential of exotic pines in South African mountain fynbos, Richardson *et al.* (1990) concluded that the most successful species (*P. halepensis*, *P. pinaster* and *P. radiata*) are fire-resilient, have small seeds, low seed-wing loading, short juvenile periods, moderate to high degrees of serotiny and are relatively poor fire-tolerant as adults. By examining 33 spp. (34 taxa) of North American pines, McCune (1988) has described 5 adaptive modes; his group 4 comprises 11 fire-resilient spp. (12 taxa) that are precocious reproducers and produce small (and light) seeds (9.7 mg, the smallest value among the 5 groups), often in serotinous cones (by far the highest value among the 5 groups). Although both Aleppo and brutia pine species might be placed in the fire-resilient group of McCune (1988) and the relevant one of Richardson *et al.* (1990), it could be argued that the significantly higher (and extremely plastic among provenances) seed weight of *P. brutia* and its lower (though not well documented) degree of serotiny might be an indication for a rather marginal participation of the latter species in the above-mentioned groups. Moreover, the 'adoption' of an embryonic dormancy relieved by chilling and its postulated association with a 'radiating' seedling recruitment strategy might be considered as evidence of an evolutionary divergence from dry and fire-prone habitats (typical of Aleppo pine) towards colder and wetter ones.

In conclusion, long-lasting canopy seed banks are formed in both species whereas their soil seed banks are transient and rather unimportant. Seed longevity within cones has been found satisfactory, and cone scales protect seeds from the lethal heat of fire. Seeds are dispersed in summer or after a fire and germination takes place at a relatively slow pace and preferably at open, sunlit sites under the cool temperatures of autumn that coincide with the onset of the wet season. Exceptions to this rule are several dormant provenances of *P. brutia* that may show either an extensive recruitment pattern covering most of the wet season or a spring-peaked pattern in

extremely cold habitats (northernmost latitudes or highest altitudes). Stratification may prove harmful for *P. halepensis* seeds but is almost always beneficial for the seeds of the latter species.

Some Targets of Future Research

- The quantification of the protection against the heat of fire for the enclosed seeds by surrounding cone tissues.
- Storability and longevity of seeds – both under controlled environments (in the lab or gene banks) and natural conditions (within the cones and in the soil).
- Germination ecotypes of *P. halepensis* in regard to the temperature range of seed germination.
- Germination ecotypes of *P. brutia* in regard to stratification requirements, timing of germination and seed coat properties.
- The possible role of various chemicals on germination at fire-free (e.g. litter) and post-fire conditions (e.g. nitrates, charate, smoke, etc).
- The detrimental role of stratification in Aleppo pine seeds; underlying mechanisms and ecological implications.

References

- Calamassi, R. 1982. Effetti della luce e della temperatura sulla germinazione dei semi in provenienze di *Pinus halepensis* Mill. e *Pinus brutia* Ten. L' Italia Forestale e Montana 37: 174-187.
- Calamassi, R., Falusi, M. and Tocci, A. 1980. Variazione geografica e resistenza a stress idrici in semi di *Pinus halepensis* Mill., *Pinus brutia* Ten. e *Pinus eldarica* Medw. Annali dell' Istituto Sperimentale per la Selvicoltura (Arezzo) 11: 193-230.
- Calamassi, R., Falusi, M. and Tocci, A. 1984. Effets de la température de germination et de la stratification sur la germination des semences de *Pinus halepensis* Mill. Silvae Genetica 33: 133-139.
- Cengiz, Y. 1993. Heat and ash effects on germination ability and subsequent seedling growth of *Pinus brutia* Ten. seeds (in Turkish). In: Papers of International Symposium on *Pinus brutia* Ten., Ministry of Forestry (Turkey), Marmaris, 18-23 October, 1993, pp. 90-98.
- Cuccui, I., Maltoni, A. and Tani, A. 1996. Indagini su semi e semenzali di provenienze di *Pinus halepensis* Mill. Monti e Boschi 47: 56-62.
- Daskalidou, E.N. 1996. Ecophysiology of the postfire regeneration of Aleppo pine (*Pinus halepensis*) (in Greek). Ph.D. Thesis, University of Athens, Greece, 181 pp.
- Daskalidou, E.N. and Thanos, C.A. 1996. Aleppo pine (*Pinus halepensis*) postfire regeneration: the role of canopy and soil seed banks. International Journal of Wildland Fire 6: 59-66.
- Debazac, E.-F. and Tomassone, R. 1965. Contribution à une étude comparée des pins Méditerranéens de la section Halepensis. Annales des Sciences Forestières 22: 213-256.
- Eler, U. 1990. The conditions of the seeds unshed but remained in mature cones during the following years in Calabrian pine (*Pinus brutia* Ten.) (in Turkish). Turkish Forest Research Institute, Technical Report 55: 147-168.
- Eler, U. and Senergin, S. 1990. Possibilities of making use of unripe *Pinus brutia* cones (in Turkish). Turkish Forest Research Institute, Technical Bulletin 223: 9-28.
- Eron, Z. 1987. Ecological factors restricting the regeneration of *Pinus brutia* in Turkey. Ecologia Mediterranea, 13: 57-67.
- Falusi, M. 1982. Variazione geografica e germinazione in semi di *Pinus brutia* Ten. Annali dell' Accademia Italiana di Scienze Forestali 31: 157-178.
- Falusi, M. and Calamassi, R. 1982. Effetti degli stress idrici su germinazione e crescita della radice in provenienze di *Pinus brutia* Ten. Annali dell' Accademia Italiana di Scienze Forestali 31: 99-118.
- Falusi, M., Calamassi, R. and Tocci, A. 1983. Sensitivity of seed germination and seedling growth to moisture stress in four provenances of *Pinus halepensis* Mill. Silvae Genetica 32: 4-9.

- Hanley, M.E. and Fenner, M. 1997. Seedling growth of four fire-following Mediterranean plant species deprived of single mineral nutrients. *Functional Ecology* 11: 398-405.
- Hanley, M.E. and Fenner, M. 1998. Pre-germination temperature and the survivorship and onward growth of Mediterranean fire-following plant species. *Acta Oecologica* 19: 181-187.
- Henig-Sever, N., Eshel, A. and Ne'eman, G. 1996. pH and osmotic potential of pine ash as post-fire germination inhibitors. *Physiologia Plantarum* 96: 71-76.
- Henig-Sever, N., Eshel, A. and Ne'eman, G. 2000. Regulation of germination of Aleppo pine (*Pinus halepensis* Mill.) by nitrate, ammonium, and gibberellin, and its role in post-fire forest regeneration. *Physiologia Plantarum* (in press).
- International Seed Testing Association (ISTA) 1993. International rules for seed testing. Annexes 1993. *Seed Science and Technology* 21 supplement: 83-288.
- Isik, K. 1986. Altitudinal variation in *Pinus brutia* Ten.: seed and seedlings characteristics. *Silvae Genetica* 35: 58-67.
- Kabar, K. 1998. Comparative effects of kinetin, benzyladenine, and gibberellic acid on abscisic acid inhibited seed germination and seedling growth of red pine and arbor vitae. *Turkish Journal of Botany* 22: 1-6.
- Klaus, W. 1989. Mediterranean pines and their history. *Plant Systematics and Evolution* 162: 133-163.
- Lamont, B.B., Le Maitre, D.C., Cowling, R.M. and Enright, N.J. 1991. Canopy seed storage in woody plants. *The Botanical Review* 57: 277-317.
- Loisel, R. 1966. Germination du Pin d'Alep au niveau de certaines associations végétales de Basse-Provence. *Bulletin de la Société Botanique Française* 113: 324-330.
- Magini, E. 1955. Sulle condizioni di germinazione del pino d'Aleppo e del pino domestico. *L'Italia Forestale e Montana* 40: 106-124.
- Martinez-Sanchez, J.J., Marin, A., Herranz, J.M., Ferrandis, P. and De las Heras, J. 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.
- Matziris, D. 1998. Genetic variation in cone and seed characteristics in a clonal seed orchard of Aleppo pine grown in Greece. *Silvae Genetica* 47: 37-41.
- McCune, B. 1988. Ecological diversity in North American pines. *American Journal of Botany* 75: 353-368.
- Mirov, N.T. 1967. The genus *Pinus*. Ronald Press, New York.
- Mouloupoulos, C. 1939. Studies on cover layer thickness and seed germination of Aleppo, Brutia and Corsican pine, Cypress and hybridogenous silver fir (in Greek). *Laboratory of Silviculture, University of Thessaloniki, Thessaloniki*, pp. 60-69.
- Nahal, I. 1983. Le pin brutia (*Pinus brutia* Ten. subsp. *brutia*). Première partie. *Forêt Méditerranéenne* 5: 165-171.
- Ne'eman, G., Meir, I. and Ne'eman, R. 1993. The effect of ash on the germination and early growth of shoots and roots of *Pinus*, *Cistus* and annuals. *Seed Science and Technology* 21: 339-349.
- Neyisci, T. 1988. A study on the germination ecology of the seeds of *Pinus brutia* Ten. (in Turkish). *Ormançilik Arastirma Enstitusu Yayinlari* 34: 81-89.
- Olofinboba, M.O. and Kozlowski, T.T. 1982. Effects of three systemic insecticides on seed germination and growth of *Pinus halepensis* seedlings. *Plant and Soil* 64: 255-258.
- Oppenheimer, H.R. 1933. Studies on germination and seedling development in Aleppo pine and *Quercus coccifera* (in German). *Gartenbauwissenschaft* 7: 308-364.
- Ozdemir, T. 1977. Studies on the possibilities of natural regeneration of *Pinus brutia* Ten. in Antalya region (in Turkish). *I. U. Orman Fakultesi Dergisi*, A/2: 239-290.
- Panetsos, C. 1981. Monograph of *Pinus halepensis* Mill. and *P. brutia* Ten. *Annales Forestales (Zagreb)* 9: 39-77.
- Pargney, J.C. and Thalouarn, P. 1978. Etude cytologique de l'embryon du Pin d'Alep lors de la germination: influence d'un traitement stimulant par administration successive d'ions mercure et chlore. *Canadian Journal of Botany* 56: 2931-2936.
- 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.
- Richardson, D.M., Cowling, R.M. and Le Maitre, D.C. 1990. Assessing the risk of invasive success in *Pinus* and *Banksia* in South African mountain fynbos. *Journal of Vegetation Science* 1: 629-642.
- Saracino, A., Pacella, R., Leone, V. and Borghetti, M. 1997. Seed dispersal and changing seed characteristics in a *Pinus halepensis* Mill. forest after fire. *Plant Ecology* 130: 13-19.

- Saracino, A., Dinel, H., Fidanza F., Ruggiero, G. and Dumonter, S. 1998. Factors affecting post-fire regeneration under large *Pinus halepensis* trees. II. Influence of ashes and lipids on seed germination patterns. In: Trabaud, L. (ed), Fire Management and Landscape Ecology, International Association of Wildland Fire, Fairfield, WA, USA, pp. 213-222.
- Sefik, Y. 1965. Studies on the cone and seed of *Pinus brutia* (*Pinus brutia* Ten.) (in Turkish). Orman Genel Mudurlugu Yayinlaridan 420: 1-93.
- Selik, M. 1958. Botanical investigations on *Pinus brutia* Ten., especially in comparison with *Pinus halepensis* Mill. (in Turkish). Review of the Faculty of Forestry, University of Istanbul 8(2) A: 161-198.
- Shafiq, Y. 1970/71. The effect of sulphuric acid 98% on seed coat thickness and on germination of seeds of *Pinus brutia* Ten. Mesopotamia Journal of Agriculture 5-6: 41-46.
- Shafiq, Y. 1979a. Some effects of light and temperature on the germination of *Pinus brutia*, *Nothofagus obliqua* and *Nothofagus procera* seeds. Seed Science and Technology 7: 189-193.
- Shafiq, Y. 1979b. Effect of sulphuric acid, gibberellic acid, and scarification on the germination of *Pinus brutia* Ten. seeds. Beitrage zur tropischen Landwirtschaft und Veterinaermedizin 17: 207-210.
- Shafiq, Y. and Omer, M. 1969. The effects of stratification on germination of *Pinus brutia* seed. Mesopotamia Journal of Agriculture 4: 96-99.
- Skordilis, A. 1992. Seed germination and seedling development in *Pinus halepensis* and *P. brutia*. Physiological and ecological approach (in Greek). Ph.D. Thesis, Univ. Athens, Athens, 199 pp.
- Skordilis, A. and Thanos, C.A. 1995. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. Seed Science Research 5: 151-160.
- Skordilis, A. and Thanos, C.A. 1997. Comparative ecophysiology of seed germination strategies in the seven pine species naturally growing in Greece. In: Ellis, R.H., Black, M., Murdoch, A.J. and Hong, T.D. (eds), Basic and Applied Aspects of Seed Biology. Proceedings of the Fifth International Workshop on Seeds, Reading, UK, 1995, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 623-632.
- Thalouarn, P. and Heller, R. 1977. Action du chlorure mercurique sur l'imbibition des graines de *Pinus halepensis* Mill. Physiologie Végétale 15: 1-14.
- Thanos, C.A. 1999. Fire effects on forest vegetation, the case of Mediterranean pine forests in Greece. In: Eftichidis, G., Balabanis, P. and Ghazi, A. (eds), Wildfire Management. Proceedings of the Advanced Study Course on Wildfire Management, Athens, October 1997, Algosystems and European Commission DGXII, Athens, pp. 323-336.
- Thanos, C.A. and Daskalakou, E.N. 1993. Seed characteristics of *Pinus brutia* from various locations of Samos island. In: Papers of International Symposium on *Pinus brutia* Ten., Ministry of Forestry (Turkey), Marmaris, 18-23 October, 1993, pp. 295-302.
- Thanos, C.A., Marcou, S., Christodoulakis, D. and Yannitsaros, A. 1989. Early post-fire regeneration in *Pinus brutia* forest ecosystems of Samos island (Greece). Acta Oecologica / Oecologia Plantarum 10: 79-94.
- Thanos, C.A. and Skordilis, A. 1987. The effects of light, temperature and osmotic stress on the germination of *Pinus halepensis* and *P. brutia* seeds. Seed Science and Technology 15: 163-174.
- Thanos, C.A., Skordilis, A. and Daskalakou, E.N. 1995. Comparative ecophysiological study of the postfire regeneration of Mediterranean pines (in Greek). Technical Report of Research Project PENED 91/824, Athens.
- Toole, V.K. 1973. Effects of light, temperature and their interactions on the germination of seeds. Seed Science and Technology 1: 339-396.
- Trabaud, L. 1995. Modalités de germination des cistes et des pins Méditerranéens et colonisation des sites perturbés. Revue d'Ecologie (la Terre et la Vie) 50: 3-14.
- Trabaud, L. and Oustric, J. 1989. Influence du feu sur la germination des semences de quatre espèces ligneuses méditerranéennes à reproduction sexuée obligatoire. Seed Science and Technology 17: 589-599.
- Young, J.A. and Young, C.G. 1992. Seeds of Woody Plants in North America. Dioscorides Press, Portland, Oregon.