Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*

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Abstract

The ecophysiology of germination in *Pinus brutia* and *P. halepensis* was studied in seeds collected from different areas of Greece. In regard to the temperature range of germination, both *P. halepensis* and the southern provenance (Lasithi, Crete) of the East Mediterranean pine, *P. brutia*, follow a typical Mediterranean pattern. In the latter species, dramatic differences in the degree of dormancy were noted among the three provenances investigated; in all seed lots however, 20°C was clearly the optimal temperature for germination. Stratification resulted in a considerable promotion of *P. brutia* seed germination. Nevertheless, the inductive effect of stratification was shown to differ among the three provenances used, escalating from a simple increase of germination rate (in the southern seed lot from Lasithi, Crete) through a broadening of the temperature range of germination (in the intermediate lot from Thasos Island) to, finally, a dramatic release from a particularly deep dormancy (in the northern lot from Soufli). These deeply dormant seeds of the latter provenance displayed an absolute stratification requirement; prolonged illumination or seed coat scarification could not substitute for the promotive effect of prechilling. A considerable interaction between far-red light and stratification was revealed in the dormant seeds of *P. brutia* (Soufli provenance); far-red pulses during stratification could either cancel or diminish the germination promotion induced by low temperatures. The differences observed in the germination behaviour among the various *P. brutia* provenances 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).

Keywords: dormancy, seed germination, *Pinus brutia*, *Pinus halepensis*, seed provenance, stratification, temperature range of germination.

Introduction

Pinus brutia Ten. (East Mediterranean pine) and *P. halepensis* Mill. (Aleppo pine) are the commonest pine species around the Mediterranean region. Both occur abundantly in Greece, *P. halepensis* in most of the mainland and the neighbouring islands and *P. brutia* in the north-eastern mainland and the larger islands of the Aegean Sea, Crete included (Fig. 1). There is a well defined spatial separation in the natural distributions of the two species, the shortest distance (about 50 km) being in northern Greece (Panetsos, 1975). Both species are a national resource of great economic and environmental importance.

Gradually increasing human impact, expressed either directly (e.g. land use change) or indirectly (e.g. fires, overgrazing), is leading to a loss of vast forest areas in the Mediterranean Rim. Current efforts for reforestation have not been particularly successful, which may be attributed partly to the use of inappropriate seed sources for the specific reforestation areas.

Relatively little research has been carried out on the seed germination of *P. brutia* and *P. halepensis* (e.g. Magini, 1955; Shafiq, 1978, 1979; Calamassi *et al.*, 1980; Calamassi, 1982; Thanos and Skordilis, 1987). Seeds of both species germinate optimally at 20°C in darkness, though rather slowly. Continuous red light or diurnal white light always promote germination rate while far-red light always inhibits germination (Thanos and

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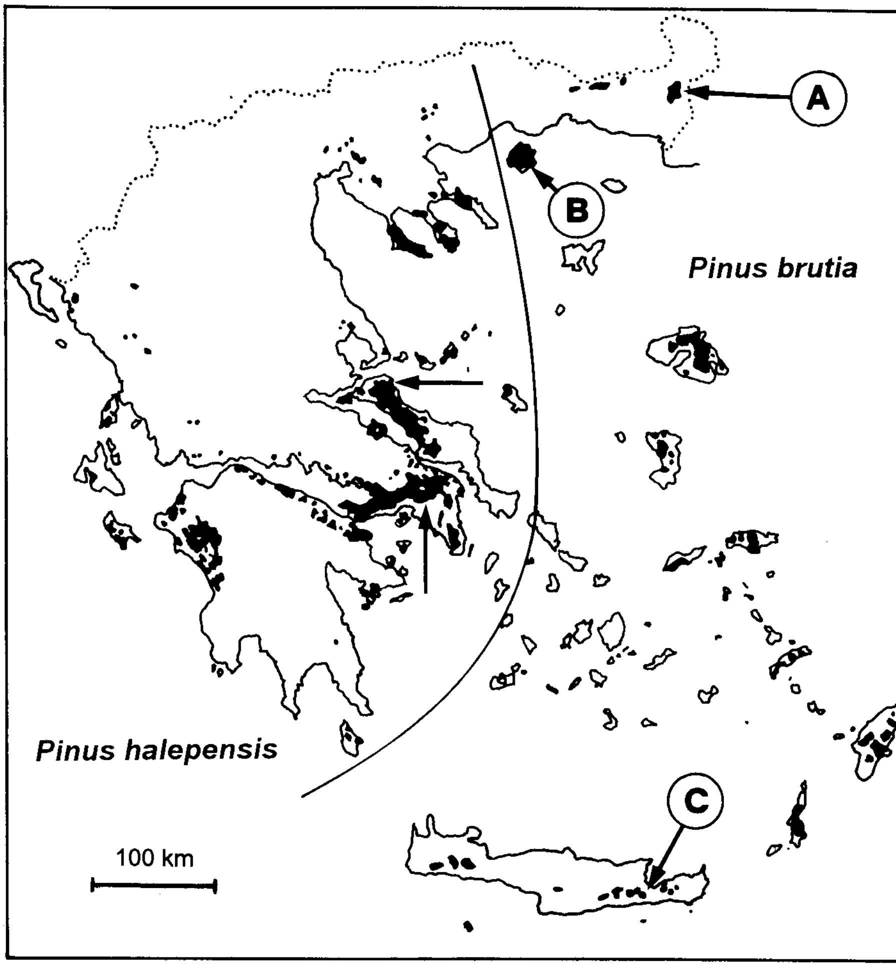

Figure 1. Geographical distribution of natural populations of *Pinus halepensis* and *P. brutia* in Greece. The two arrows in the distribution of the former species correspond to Istiaia (northern) and Attica (southern) provenances, In *Pinus brutia*, the three provenances used are indicated by arrows marked A, B and C (Soufli, Thasos and Lasithi, respectively).

Skordilis, 1987). Information on the germination behaviour of different provenances within the same species is particularly scarce (Calamassi *et al.*, 1980).

In some tree species both germination rate and final amount, as well as timing under field conditions, may vary among provenances (Fowler and Dwight, 1964; Bramlett *et al.*, 1983). The taxonomic records, indicate that *P. brutia* (as well as its variants *eldarica*, *pithyusa* and *stankewiczii*) is an extremely variable species (Mirov, 1967; Panetsos, 1981; Nahal, 1983). Different provenances of *P. halepensis* showed a considerably greater homogeneity in their germination response towards light and temperature than a number of provenances of *P. brutia* seeds (Calamassi, 1982).

Seed source influences the efficacy of chilling treatments in various conifers (Borghetti *et al.*, 1986, 1989; Leadem, 1986). Significant interactions were observed between altitude and stratification requirement in *P. brutia* seeds (Isik, 1986). However, latitudinal variation has not yet been investigated; this variation might prove of even greater interest in view of the prolonged isolation of numerous populations of *P. brutia*, including several island provenances.

The present study investigates the effects of stratification on germination in several *P. brutia* and *P. halepensis* provenances. Since the regeneration of both species depends exclusively upon seeds, their particular ecophysiological characteristics may prove of great importance to the conservation of Mediterranean pine ecosystems.

Materials and methods

Seeds of *Pinus brutia* and *P. halepensis* were obtained from the Forestry Division, Ministry of Agriculture, Greece. In the present study, seeds of *P. brutia* from three different regions of Greece were used: a northern continental provenance from Soufli, Thrace (latitude N 41° 12'), a North Aegean provenance from Thasos Island (N 40° 47') and a southern provenance from Lasithi, Crete (N 35° 12'). Damaged, insect-infected and undersized seeds were removed by hand from each seed lot. The mean seed weight (n = 200) for each of the lots used was 52.9 ± 0.9, 60.1 ± 1.0 and 38.2 ± 0.7 mg, respectively.

Seeds of *P. halepensis* originated from Istiaia, Euboea Island (latitude N 38° 58') and Attica (N 37° 54'). The mean seed weight (n = 200) of the above seed lots was 22.0 ± 0.4 and 20.3 ± 0.4 mg, respectively. All five seed provenances used were of low altitude (0–200 m a.s.l.) and are indicated on the map in Figure 1.

Prior to storage the seeds were dusted with fungicide (Thiram) in order to prevent fungal infection upon subsequent sowing, especially at high temperatures. The seeds were hermetically stored in a refrigerator (3–5°C) and no differences were observed in the germination characteristics of each particular lot, during the two-year long experimentation period.

Germination tests were performed with 6 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 ± 1°C. The chambers were equipped with white and broad-band far-red (FR) light sources, described elsewhere (Thanos *et al.*, 1991). Cold-moist stratification was accomplished by maintaining, for varying durations, imbibed seeds at a temperature of 2–4°C in the dark. Seed scarification was achieved by abrasion with sandpaper at the micropylar end.

Germination was recorded every two days and was considered complete when no additional seeds germinated. The criterion of germination was radicle protrusion; seeds exhibiting abnormal germination were excluded from germination counts. All germination percentages were based on the filled seed portion only, determined from cutting tests performed after completion of the experiment; the mean percentage of unsound seeds was around 10% for all the seed lots studied. T_{50} is the time needed for manifestation of half of the final germination level and it was calculated from the two median values.

Climatic data (used in the ombrothermic diagrams of Fig. 7) refer to the closest meteorological station (for each particular seed provenance) and are compiled from information for a period of at least 25 years (Andreakos, 1978).

Results

Final dark germination of *P. brutia* seeds as a function of temperature (Fig. 2A) was found to vary considerably according to seed origin. Seeds of the southern provenance (Lasithi) showed a temperature range of germination significantly wider than the other lots; germination was almost maximal at 10–20°C. Based on the rate of germination (T_{50}: 15.7 d at 20°C compared with 21.1 and 39.6 d at 15 and 10°C, respectively) 20°C could be considered the optimal temperature of seed germination for the Lasithi seeds. At the marginal temperature of 5°C, germination was very slow (T_{50}: 70 d) and the final value of germination was obtained 3 months after sowing. On the other hand, at the supraoptimal temperature range (22.5–27.5°C), final germination values declined steeply. Tem-

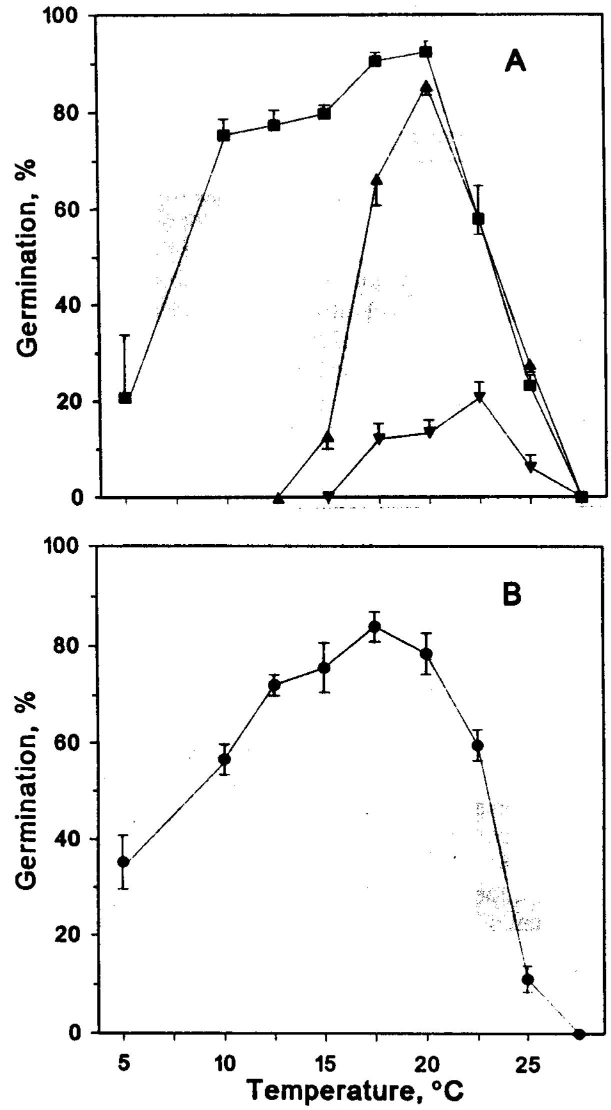

Figure 2. Final dark germination as a function of temperature in *Pinus brutia* (**A**) and *P. halepensis* (**B**). The three curves in (**A**) correspond to the seed provenances of *P. brutia*: Soufli (▼), Thasos (▲) and Lasithi (■). Vertical lines represent either 1 or 2 S.E. (**A** and **B**, respectively).

peratures around 20°C were also found to be optimal for the other two provenances of *P. brutia*; T_{50} was 12.3 and 19.5 d for Thasos and Soufli lots, respectively. In the case of Thasos provenance, germination was restricted to a very narrow range of temperatures; below 20°C, the final germination percentage was dramatically decreased compared with the Lasithi provenance, while at higher temperatures (22.5–27.5°C) germinability was not affected. In the third provenance (Soufli), dark germinability was only 20% for a rather warm range of temperatures while it was completely inhibited at 15°C and below.

Corresponding results for *P. halepensis* seeds are presented in Figure 2B. The two seed lots used showed, throughout the experiments, identical germination characteristics and therefore means of the two lots are displayed. The temperature range of *P. halepensis* final dark germination percentage was very similar to that of *P. brutia* from Lasithi (southern provenance). Nevertheless, germination was comparatively faster in the former case; T_{50} was 10.3, 14.7 and 32.5 d at 20, 15 and 10°C, respectively.

Stratification of *P. brutia* seeds for 1, 2 or 3 months resulted in a considerable, gradual improvement of both final germinability (where not optimal) and rate of germination (as illustrated by the T_{50} graphs on the right column of Fig. 3). The enhancing effect of stratification on subsequent seed germination was dramatic in the dormant seeds of Soufli provenance; a very long period of chilling was required for full promotion of germination, at both temperatures tested, while shorter treatments resulted in intermediate results. In the Thasos provenance, stratification was particularly effective in broadening the temperature range; germination at the suboptimal temperature of 15°C was increased from 10% in untreated seeds to 73, 84 and 98% in seeds stratified for 1, 2 and 3 months, respectively. In the third provenance of Lasithi, where final germination percentages were maximal at both 15 and 20°C, only a significant increase in germination rate was obtained by stratification. On the other hand, final germination percentages of *P. halepensis* seeds showed a slight but steady decline with increased prechilling periods. Based on morphological evidence, this decrease could be attributed to loss of viability in a fraction of the seed population, brought about by the low temperatures during stratification. However, much greater germination rates, at both 15 and 20°C, were obtained with longer stratification periods.

In an attempt to investigate further the type of primary dormancy observed in the *P. brutia* seeds from Soufli, two of the commonest methods for breaking dormancy in conifer seeds (besides stratification) were applied: prolonged white-light illumination and seedcoat scarification (Fig. 4). Germination tests were at the optimum temperature of 20°C and both treatments resulted in only a meagre promotion of germination, about 20% above the control levels. The levels attained by both treatments were comparable to the promotion of germination obtained by only one month of stratification (Fig. 3).

The prolonged exposure of the non-dormant *P. brutia* seeds (Lasithi provenance) to intermittent broadband far-red irradiation (iFR) resulted in an induction of secondary dormancy; the preilluminated seeds were virtually unable to germinate upon a subsequent transfer to darkness (Fig. 5). However, an intervening period of stratification could partly relieve this secondary dormancy and the longer the duration of chilling the higher the final germination percentage (Fig. 5).

An additional aspect of the interaction between FR light and stratification was investigated in the dormant seeds of *P. brutia* (Soufli provenance). At 15°C in darkness, untreated seeds could not germinate at all while various prechilling durations resulted in gradually increased germination values (Fig. 6). When a single, final FR pulse was given following the stratification treatment, and upon transfer of seeds to 15°C and darkness, full inhibition of germination was obtained after 1- or 2-month long stratification treatments. However, the inductive effect of a 3-month long prechilling could be only slightly reversed by a final FR treatment. On the other hand, a FR pulse given once a week, throughout the duration of the stratification treatment, cancelled completely any promotion of germination caused by chilling.

Discussion

Seeds of Aleppo pine, *P. halepensis*, are well known to germinate without particular difficulties while different provenances show relatively homogeneous germination patterns (e.g. Magini, 1955; Calamassi *et al.*, 1984; Thanos and Skordilis, 1987; Daskalakou and Thanos, 1994; A. Skordilis and C.A. Thanos, unpublished). In regard to the temperature range of germination, *P. halepensis* seeds (as well as the southern provenance of *P. brutia* used in this work) follow a pattern very common among species growing in regions with a Mediterranean climate (Thompson, 1973; Thanos *et al.*, 1991). In agreement with this pattern, Aleppo pine seed germination takes place at a relatively cool temperature range, between 5 and 25°C; a final value *ca.* 80–100% is attained between 10 and 20°C in darkness, the optimum being around 15–20°C. It is assumed that these particular temperature requirements have evolved as an adaptation to the autumn temperatures of the Mediterranean climate: seed germination is accomplished early enough during the rainy season so that the developing seedlings exploit most of the mild winter and following spring prior to the harsh, water-stressed conditions of summer (Thanos *et al.*, 1991).

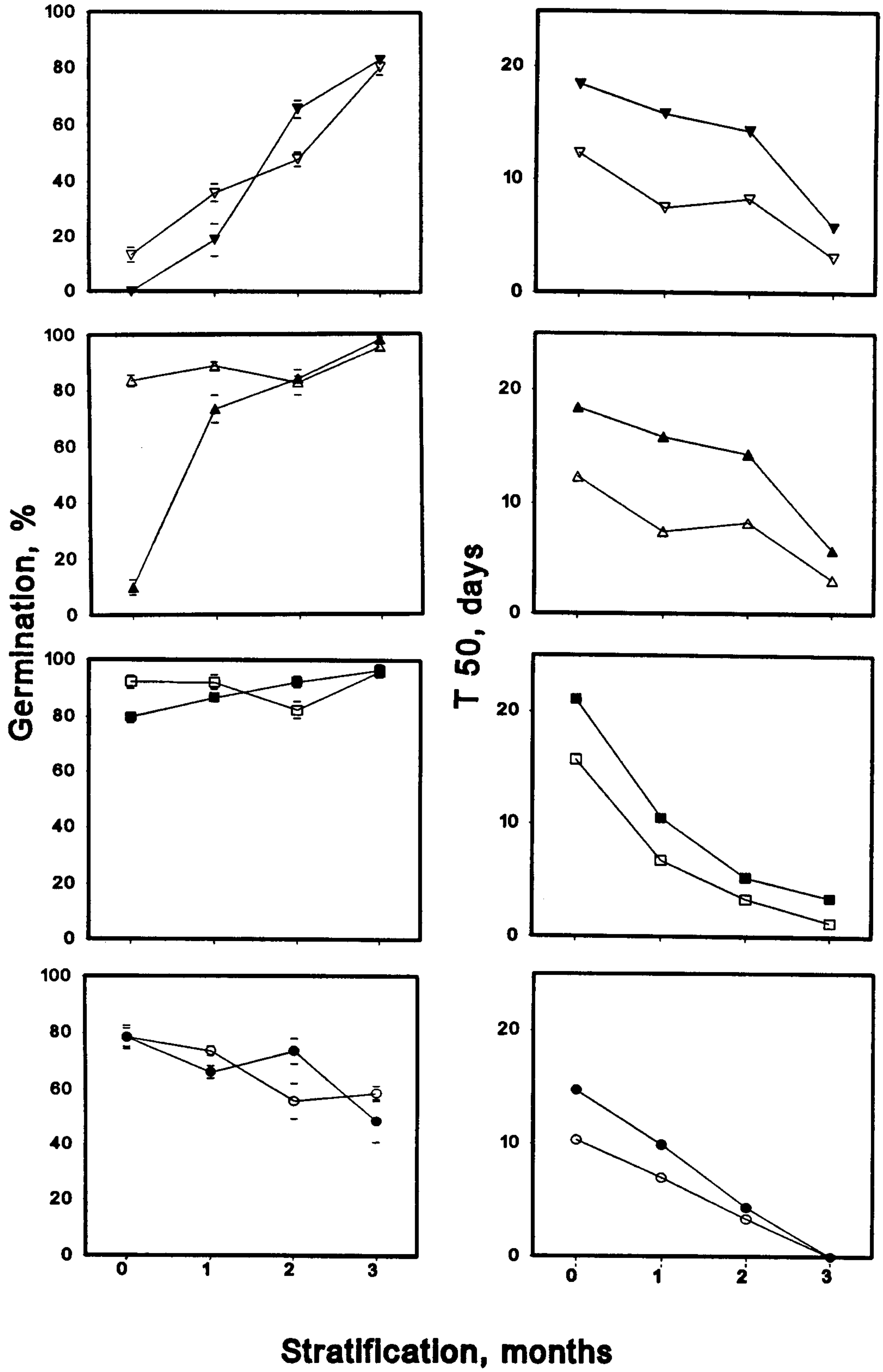

Figure 3. The effect of seed stratification on subsequent final dark germination and T_{50} values of *Pinus brutia* (Soufli: ▼, Thasos, ▲, Lasithi: ■) and *Pinus halepensis* (●) at 15 and 20°C (closed and open symbols, respectively).

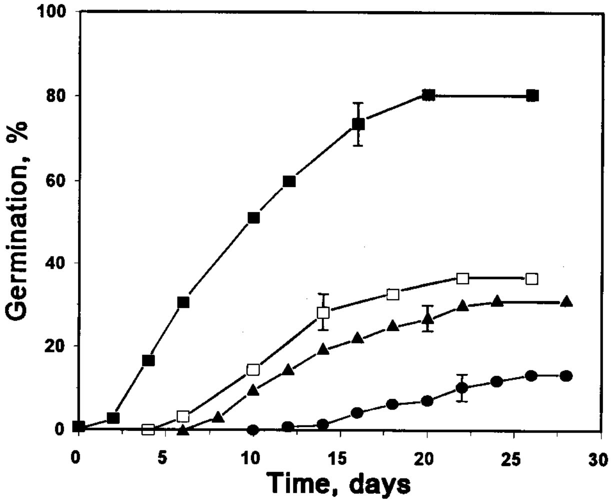

Figure 4. Time course of seed germination in *Pinus brutia* (Soufli provenance) at 20°C in the dark. Seeds were untreated (●), scarified (▲) or stratified for 3 months (■). An additional sample (□) imbibed under diurnally alternating temperatures (14 h 20°C/10 h 15°C, white light during the warm period). Vertical lines represent the maximum value of S.E. within each time course.

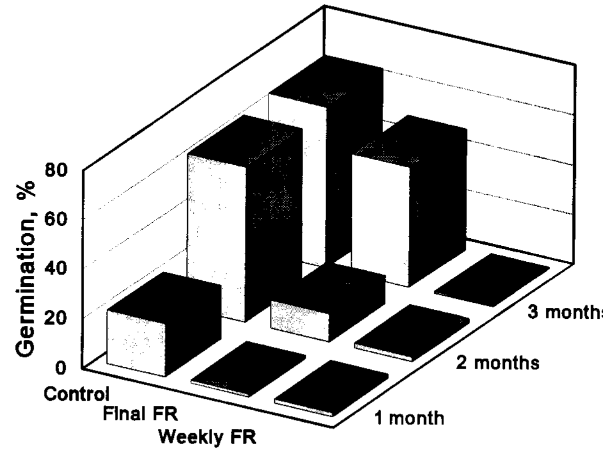

Figure 6. Final germination of *Pinus brutia* seeds (Soufli provenance) at 15°C and darkness, after various durations of a stratification pretreatment. Control: seeds kept in darkness throughout. Final FR: seeds given a single 30-min far-red pulse at the end of the chilling pretreatment. Weekly FR: seeds given weekly, 30-min far-red irradiations throughout prechilling.

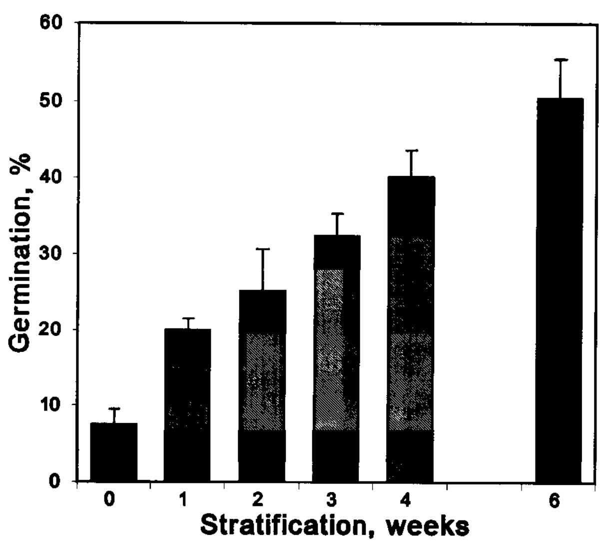

Figure 5. The effect of stratification on the release from far-red (FR) dormancy in *Pinus brutia* seeds (Lasithi provenance). Seeds were initially made dormant by a 7-day illumination treatment (1 min FR every 15 min). Afterwards, seeds were stratified for various periods in the dark and were eventually transferred for germination at 20°C and darkness. Vertical lines represent S.E.

East Mediterranean pine, *P. brutia*, is a significantly more variable species than *P. halepensis*; this variability is attributed to a geologically and climatically caused, long-term fragmentation and isolation of many populations within the original extensive range of the species (Panetsos, 1981). Such a noteworthy variability is also demonstrated in the different germination requirements displayed by the different seed provenances used in the present study.

In all three lots of *P. brutia* seeds investigated here, 20°C was clearly the optimal temperature, in agreement with the Mediterranean character of the species. At the relatively high temperature of 25°C, a low germinability was obtained both in the present and an earlier study (Thanos and Skordilis, 1987), in contrast to considerably higher values (70–85%) reported previously (Sefik, 1965; Shafiq and Omer, 1969; Shafiq, 1978, 1979). This discrepancy and its possible causes have to be resolved by future investigation.

Stratification has been repeatedly found to have a beneficial effect on subsequent germination performance in *P. brutia* seeds (Sefik, 1965; Shafiq and Omer, 1969; Isik, 1986). However, the promotion reported concerned mainly germination rate rather than final germination percentage. In seeds collected at high elevations both germination rates and final values were inferior to those from lower elevations and this partial dormancy could be eliminated by stratification (Isik, 1986). Nevertheless, no seed lot exhibited such a marked effect of stratification on final germination percentage, or in other words showed such a deep dormancy, as the population of *P. brutia* from Soufli (present study).

In the present work, the inductive effect of stratification was shown to differ among the three provenances used, from a simple increase of germination rate (in the southern seed lot) through a broadening of the temperature range of germination (in the intermediate lot) to, finally, a dramatic release from a particularly deep dormancy (in the northern lot). Light, which usually enhances pine seed germination in forest openings (Washitani and Saeki, 1986; Thanos and Skordilis, 1987; Thanos *et al.*, 1989), cannot substitute for the inductive effect of stratification on this deeply dormant northern latitude provenance. Furthermore, seed dormancy and germination in *P. brutia* have previously been found to remain unaffected by either chemical (Shafiq, 1970/71) or thermal scarification (Thanos *et al.*, 1989). On the other hand, despite the slight promoting action of either prolonged illumination (present study) or mechanical scarification (Shafiq, 1979; present study) it is obvious that these methods cannot substitute for the promotive effect of prechilling treatment in the release from primary dormancy of the Soufli provenance. We therefore conclude that the deep dormant seeds of the Soufli provenance display an absolute stratification requirement. Nevertheless, a considerable interaction between far-red light and stratification was revealed in these seeds. During the relatively shorter stratification durations, application of far-red pulses virtually cancelled the germination promotion induced by chilling, while for longer chilling treatments this effect was considerably weaker.

The broadening of the germination temperature range in *P. brutia* seeds from Thasos brought about by stratification (present study) or light (Thanos and Skordilis, 1987) is consistent with numerous similar results obtained with various temperate conifers, e.g. *Abies fraseri* (Adkins *et al.*, 1984) and *A. amabilis* (Leadem, 1986).

The temperature curves of seed germination in Aleppo pine and the southern *P. brutia* provenance may look almost identical but a major difference in germination behaviour was obtained in regard to the effect of chilling temperatures. Although short periods of stratification may improve germination rates, long term chilling of Aleppo pine seeds eventually led to gradual loss of viability. Similar results were previously reported by Calamassi *et al.* (1984) for several provenances of *P. halepensis* seeds. On the other hand, for seeds of *P. brutia* from Crete, stratification was shown to be beneficial to both germination percentage and T_{50} values and the longer the treatment the better the enhancement. In addition, in Lasithi seeds, the previously reported (Thanos and Skordilis, 1987) induction of secondary dormancy by far-red irradiation (a situation that in the ecological context might arise under a dense pine canopy) could be relieved, at least partly, by a subsequent stratification treatment.

The differences observed in the germination characteristics and the stratification requirements among the three *P. brutia* seed provenances used in this work are dramatic. A discussion about germination ecotypes seems rather preliminary but we can explore the consequences of these different germination responses for germination timing at different latitudes.

The left column of Figure 7 shows the ombrothermic diagrams for the particular seed provenances; along with the emphasis placed on the xerothermic period, data on frost occurrence are also presented. It is obvious that freezing temperatures, are, on average, encountered day after day during the three winter months in Soufli; the situation is milder in Thasos during the same period (frost scored once every four days). On the other hand, in Crete and Attica the climate is virtually frost-free. The distribution patterns, illustrated on the right column of Figure 7, represent postulated timing schedules of seedling emergence for the respective pine seed populations. These patterns are based on both laboratory germination behaviour and climatic data, particularly water availability and prevailing temperatures. Thus, in *P. brutia* from Soufli, the inherent (primary) seed dormancy completely prohibits autumn germination; the seeds are expected to germinate in spring after having experienced the inductive, low temperatures of winter. This delay of field germination may be viewed as an evolutionary result of selection by a rather long and severe winter, and may prevent young seedlings from being damaged by freezing temperatures. A similar mechanism has been reported for seeds of *Fraxinus excelsior*; seeds collected from latitudes with longer and colder winters require longer stratification periods than do seeds collected in regions characterized by less severe winters (Bewley and Black, 1982). A similar (but only moderate) negative correlation between germination capacity and altitude has already been reported for *P. leucodermis* (Borghetti *et al.*, 1986, 1989).

On the basis of the laboratory data, field germination in Thasos Island may exhibit a bimodal pattern; a fraction of the seed population may start germinating soon after the onset of the rainy season (mid September till the end of October), when temperatures are warm enough. Afterwards, germination may be suppressed by cool temperatures and a second burst in germination is expected in early spring when temperatures warm up again and after the ungerminated seeds have, additionally, experienced the promotive winter temperatures. The advantage of autumn- over spring-germinating seedlings in regard to the development of an efficient root system, may be counterbalanced by the much lower T_{50} values of the stratified seeds, in agreement with the observations of a similar study (Falusi, 1982) which indicate a considerable capacity for rapid seedling growth. The ratio of autumn- to spring-germinating seeds will vary according to the meteorological conditions of each particular year.

In the extremely mild climatic regime of Lasithi area, it is evident that the non-dormant *P. brutia* seed population of Crete will encounter field temperatures

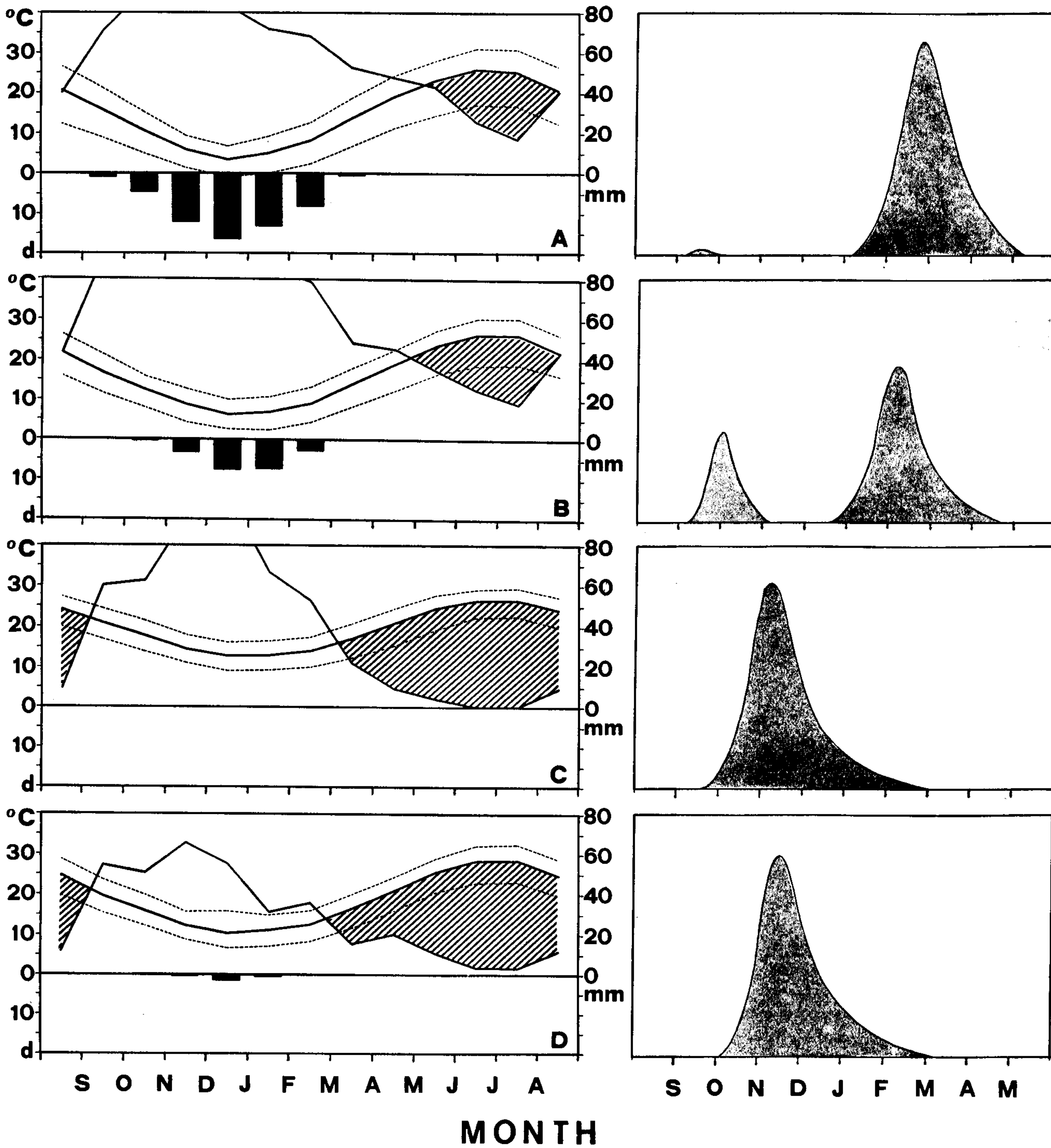

Figure 7. The ombrothermic diagrams of the four seed provenances; *Pinus brutia*: Soufli (A), Thasos, (B), Sitia (C) and *P. halepensis*: Ellinikon (D). The three concurrent lines represent (on the primary y-axis, in °C) the mean monthly temperature (solid line) and the monthly means of daily minimum and maximum temperatures (stippled lines). The secondary y-axis refers to mean monthly precipitation, in mm. Hatched areas and vertical bars represent xerothermic period and frost occurrence (days per month), respectively. Shaded areas on the right-hand graphs illustrate hypothetical distribution patterns of seed germination and seedling emergence in the field, for the respective populations.

suitable for germination as early as October, provided of course that water is available. By the end of December, it is almost certain that even the slowest germinators will already have entered the seedling stage. In this way, between seedling emergence and the onset of the long summer, at least four months would be available for the development of an efficient root system. Although no freezing temperatures occur in the Cretan lowland the possibility of responding to chilling is a relict trait of the species that still remains imprinted on the particular seed lot, in contrast to its virtual absence in Aleppo pine seeds.

The ombrothermic graphs of Attica and Lasithi are almost identical. Therefore, similarly to the southern *P. brutia* population, emergence of Aleppo pine seedlings is expected as soon as water is available. In this case there is an abundance of recent field measurements which confirm entirely the postulated pattern. According to these observations (Daskalakou and Thanos, in press; E.N. Daskalakou and C.A. Thanos, unpublished) germination of *P. halepensis* seeds in burned forests, during the first postfire year, starts soon after the onset of the rainy season (i.e. mid October–mid December, depending on the hydrological conditions) and is almost completed within two months. In the various populations of *P. brutia* there exists only circumstantial or preliminary evidence in favour of an early-peaked pattern in Chania, Crete (C.A. Thanos and C. Pendarakis, unpublished) as well as of either a two-peaked or a delayed-peaked pattern in Samos (Thanos *et al.*, 1989; C.A. Thanos and S. Marcou, unpublished) and at the high altitudes of Antalya region, Turkey (Ozdemir, 1977). It is therefore imperative that field data are collected in regard to the timing of seed germination and seedling establishment under natural conditions, at several locations. It is also important that germination characteristics are screened in the laboratory for a large and representative specimen of East Mediterranean pine populations. We may conclude that the observed *P. brutia* seed germination responses to stratification and temperature, reflect a climate-adapted strategy on the timing of seedling emergence and establishment, well known as the most vulnerable life phase (e.g. Thanos and Marcou, 1991; Daskalakou and Thanos, in press).

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