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The effects of light, temperature and osmotic stress on the germination of *Pinus halepensis* and *P. brutia* seeds

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Summary

Seeds of the mediterranean pines, *Pinus halepensis* and *P. brutia*, germinate optimally at 20 °C in darkness. Their temperature range of germination is rather narrow and their rate very slow. When seeds are osmotically stressed the rate becomes even slower, while final germination percentage is inhibited only at very low osmotic potentials. Continuous red light or diurnal white light always promote germination rate and sometimes maximum percentage, as well. Intermittent far-red light not only inhibits germination in both species, but also induces a secondary dormancy, shown to be under phytochrome control. Experiments under daily alternating conditions of light and temperature resembling those which are naturally met, lead 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.

Résumé

Effets de la lumière, de la température et de la pression osmotique sur la germination des semences de Pinus halepensis et P. brutia.

Des semences de pins méditerranéens, *Pinus halepensis* et *P. brutia*, ont une germination optimum à 20 °C à l'obscurité. Leur gamme de températures de germination est assez étroite et leur vitesse de germination très lente. Lorsque les semences sont soumises à une pression osmotique, cette vitesse devient même encore plus faible, tandis que le pourcentage final de germination n'est affecté qu'à potentiels osmotiques très faibles. Une lumière rouge continue ou une lumière blanche diurne favorisent toujours la vitesse de germination et parfois également le pourcentage maximum. Une lumière intermittente rouge-lointain, non seulement inhibe la germination des deux espèces, mais elle induit également une dormance secondaire, démontrée être sous contrôle du phytochrome. Des expérimentations sous des conditions quotidiennes de lumière et de température alternées, comparables à celles que l'on rencontre naturellement, conduisent à la conclusion que la germination en champ est possible tout au long de la saison pluvieuse dans les climats de type méditerranéen et qu'elle est fortement favorisée dans les sites ouverts et ensoleillés.

Zusammenfassung

Der Einfluß von Licht, Temperatur und osmotischem Streß auf die Keimung der Samen von Pinus halepensis *und* P. brutia.

Samen der mediterranen Kiefern *Pinus halepensis* und *P. brutia* keimen optimal bei 20 °C im Dunkeln. Ihr Keimtemperaturbereich ist ziemlich eng und ihre Keimgeschwindigkeit sehr langsam. Wenn Samen osmotisch gestreßt werden, wird die Geschwindigkeit noch langsamer, wobei der erreichbare Prozentsatz der Keimfähigkeit schon bei geringen osmotischen Potentialen vermindert wird. Dauerrotlicht oder täg-

liches Weißlicht fördert die Keimgeschwindigkeit stets und manchmal gleicherweise auch die maximale Keimfähigkeit. Intermittierendes Dunkelrotlicht hemmt nicht nur bei beiden Arten die Keimung, sondern induziert auch eine sekundäre Dormanz, die nachweislich unter Phytochromkontrolle steht. Untersuchungen mit täglich wechselnden Licht- und Temperaturbedingungen, die den natürlich gegebenen entsprechen, lassen den Schluß zu, daß ein Feldaufgang während der gesamten Regenzeit des mediterranen Klimatyps möglich und in offenen, sonnigen Lagen stark begünstigt ist.

Introduction

Pinus halepensis P. Miller (Aleppo pine) and *Pinus brutia* Tenore (Calabrian pine) are by far the commonest pine species around the Mediterranean basin. Although it is evident that both species are well adapted to the mediterranean-type climate there is a clear cut separation in their natural distributions. *P. brutia* is found in the relatively smaller NE corner while *P. halepensis* grows all over the rest of the mediterranean region as well as on the Moroccan Atlantic coast. The natural distribution of *P. halepensis* in Greece is restricted to the large part of the mainland and the neighbouring islands. On the other hand, *P. brutia* grows in the north-eastern part of the mainland, the islands of the east Aegean sea and Crete (Panetsos, 1981).

The climatic pressure exerted on the plants growing in regions with a mediterranean-type climate (especially the drought stress) is accompanied by a gradually increasing human impact expressed either directly (e.g. land management) or indirectly (e.g. fires, overgrazing). Therefore, the investigation of the adaptive mechanisms concerning seed germination and seedling establishment and survival are of great importance to both conservation and regeneration, natural or artificial, of the mediterranean pine ecosystems.

Experimental data about *P. halepensis* and *P. brutia* seed germination are scarce. Nevertheless the effect of light operating via the phytochrome system on the promotion of seed germination, especially after prechilling, in some American pine species as well as in *P. sylvestris* is well documented (Toole, 1973).

The ISTA Rules (ISTA, 1976) prescribe for the seed germination of *P. halepensis:* 20 °C and white fluorescent light for at least eight hours per day. Recent works on *P. brutia* by Shafiq (1979) and Calamassi (1982), have shown that there is also a significant promotive effect of light on seed germination. Thalouarn (1975, 1976) reported a dramatic increase in the germination rate of *P. halepensis* seeds by the successive administration of mercuric and chloric ions. This effect can be attributed to the physical and chemical properties of the seed coat. Thus, the seed coat could be considered responsible for the significant delay of germination. Falusi, Calamassi and Tocci (1983) studied the water potential effects on seed germination and root growth of *P. halepensis* from different districts and found that their behaviour under stress was very different.

In the present work the effect of various factors on seed germination of *P. halepensis* and *P. brutia* are examined. These factors comprise temperature (constant or alternating), light (white or chromatic) and water (osmotic) stress.

Materials and methods

Seeds of *P. halepensis* and *P. brutia* were obtained from the Forestry Division, Ministry of Agriculture, Greece. They had been collected from Istiaia and Thasos respectively (1983 harvest). Throughout the experimentation period, seeds were stored at room temperature (20 ± 5 °C) in darkness. At the beginning, seeds were dusted with fungicide (Thiram). Its use, though obviously not inhibitory to germination, was necessary for the prevention of fungal infection, especially at high temperatures. Before the germination tests, only damaged and insect-infested seeds were discarded but not the empty ones, since no floating method had been applied. The mean weights of *P. halepensis* and *P. brutia* seeds of the lots used in this work were 0.0220 ± 0.0004 g and 0.0601 ± 0.0010 g respectively (n = 200).

Germination tests were performed with 25 seeds per petri dish (diameter 9 cm), each lined with two layers of filter paper, moistened with 5 ml of either deionised water or osmotic solution. Osmotica were mannitol (Merck) solutions and their Ψ_s was estimated by the van 't Hoff equation. Experiments were carried out in temperature controlled chambers (Heraeus BK 5060 EL.W. Germany), where in all cases the temperature was kept constant within ± 1 °C. The chambers were equipped with white light and broad band red (R) and far-red (FR) light sources. The broad band ones have already been described elsewhere (Georghiou and Thanos, 1983). The white light source consisted of seven fluorescent (Philips TLD 18W/33) and four incandescent (Philips Philinea 6276 × 60W) tubes. The emission spectrum was quite similar to natural daylight and the total fluence rate was 15 $W.m^{-2}$.

The criterion of germination was visible radicle protrusion. Measurements were taken either once or twice a week as indicated and germinated seeds were removed. The measurements ended when no additional seeds germinated and the final experimental values shown in figures represent maximal germination percentages. Germination values are means of six replicates. Vertical lines and ± values represent standard error.

Results

The time course of dark germination of *P. halepensis* seeds as a function of temperature is shown in figure 1. It is clear that at 20 °C, germination is optimal with respect to both rate and final percentage of germination. At 15 °C there is a slight decrease in rate and final percentage compared with 20 °C, while at 25 °C a significant inhibition of germination is observed.

Corresponding results of *P. brutia* seed germination are presented in figure 2. Similarly to *P. halepensis*, germination is again optimal (though at lower rate) at 20 °C. At 15 °C or 25 °C though, *P. brutia* germination is dramatically decreased, and especially at 15 °C where it is almost fully inhibited.

Figure 3 shows the germinability of *P. halepensis* seeds (counted every week) as a function of an external osmotic stress, at 15 °C in darkness. As expected, the lower

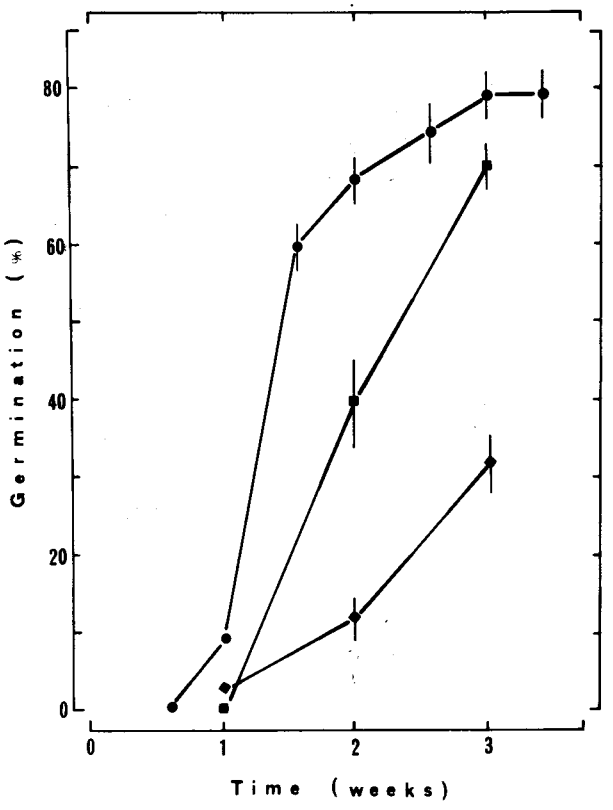


Figure 2. Time course of *P. brutia* seed germination in darkness, at 15°C (■), 20°C (●) and 25°C (◆).

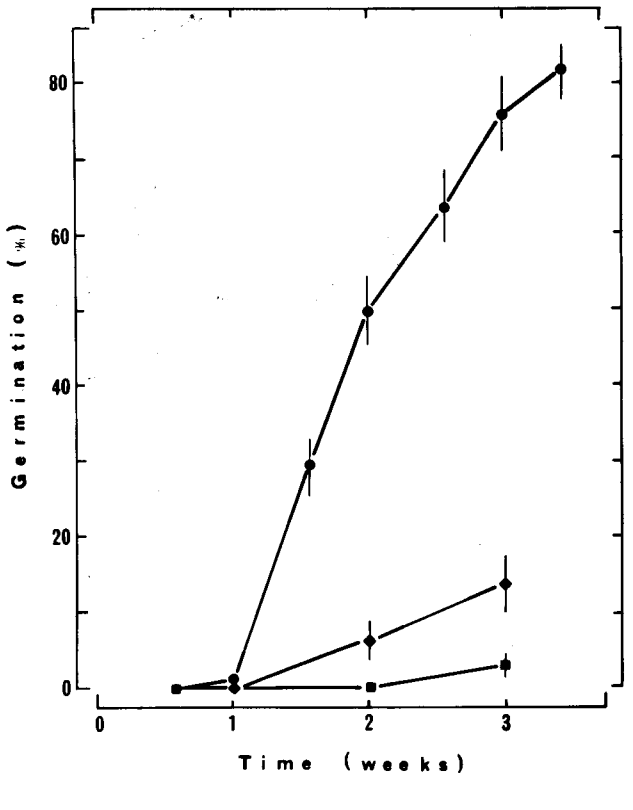


Figure 1. Time course of *P. halepensis* seed germination in darkness, at 15°C (■), 20°C (●) and 25°C (◆).

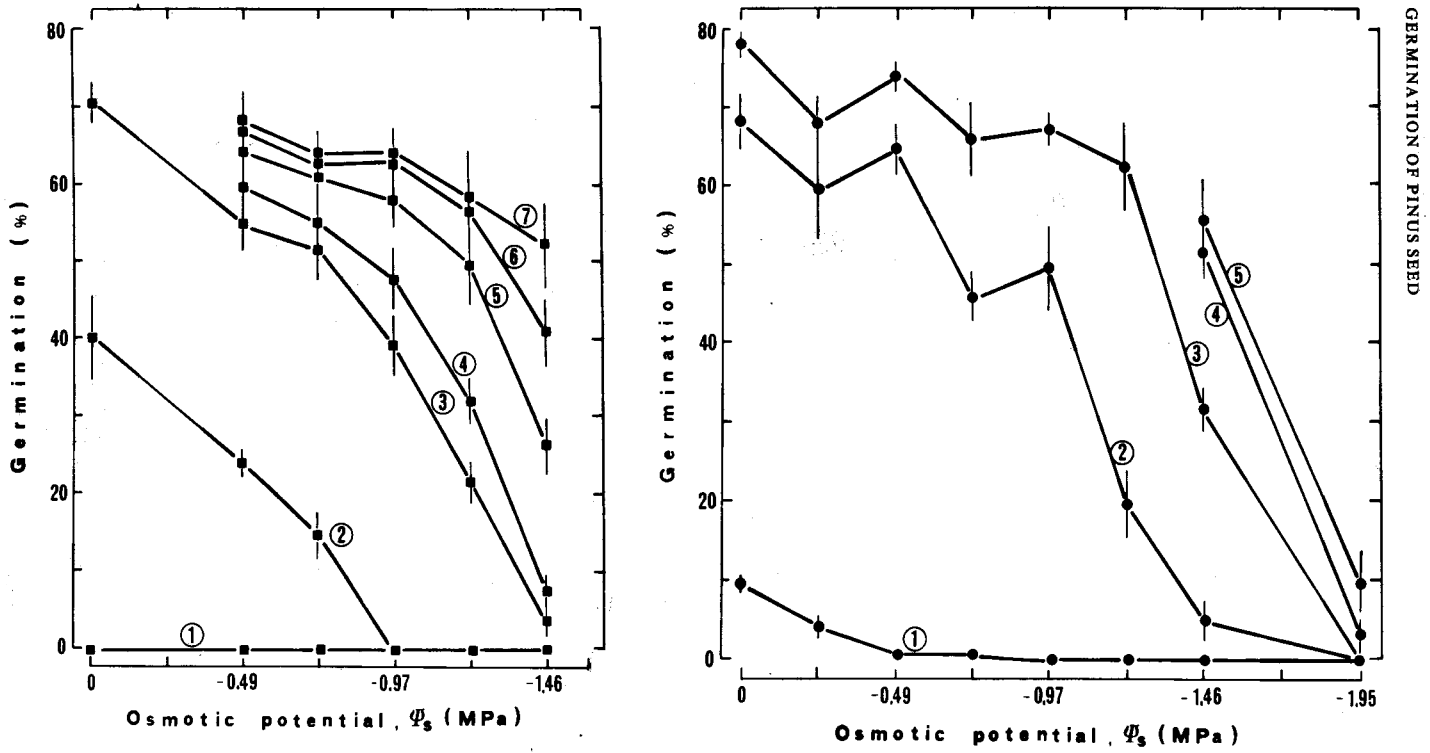
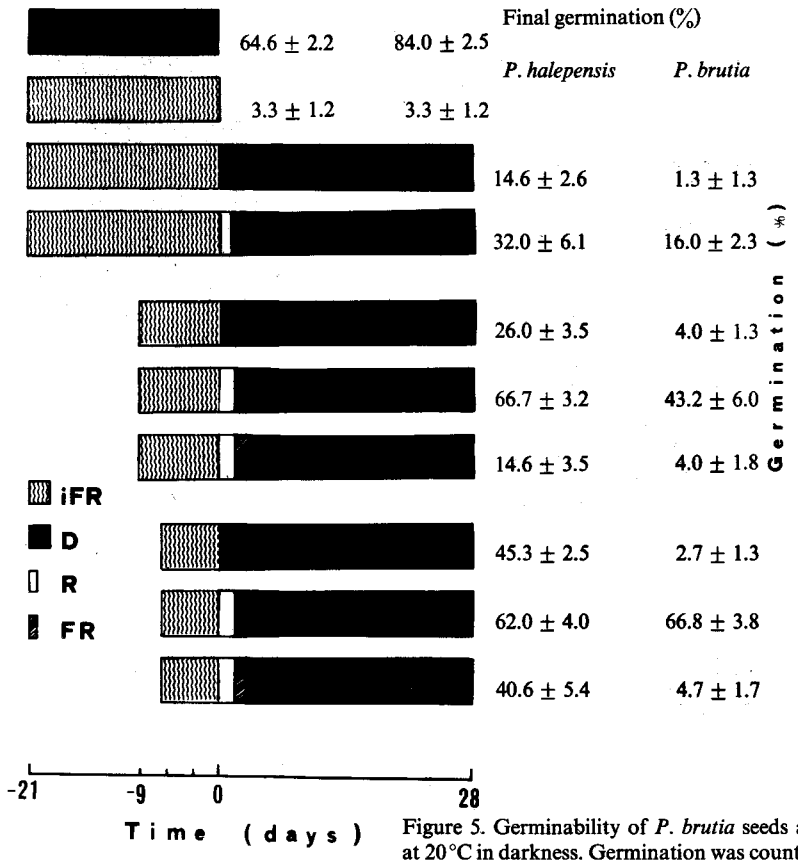


Figure 3. Germinability of *P. halepensis* seeds as a function of the osmotic potential of mannitol solutions, at 15 °C in darkness. Germination was counted 1–7 weeks after start of imbibition in the osmoticum (curves 1–7 respectively).

Figure 4. Germinability of *P. halepensis* seeds as a function of the osmotic potential of mannitol solutions, at 20 °C in darkness. Germination was counted 1–5 weeks (curves 1–5 respectively) after start of imbibition in the osmoticum.



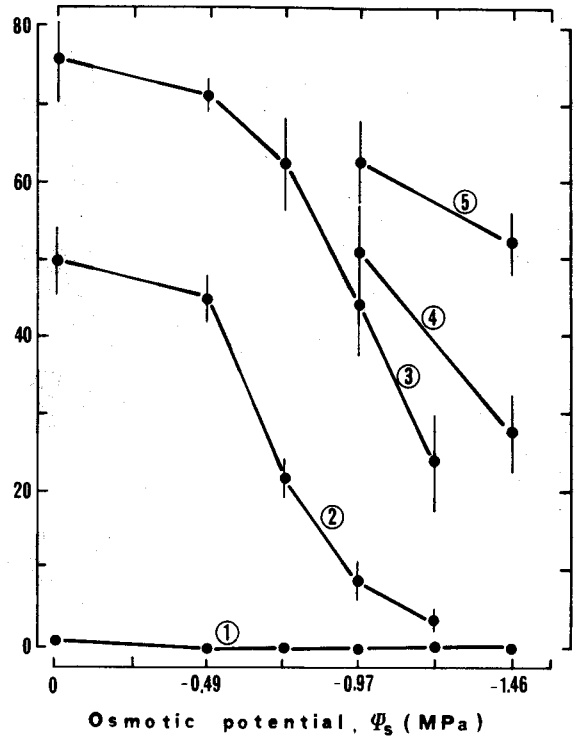
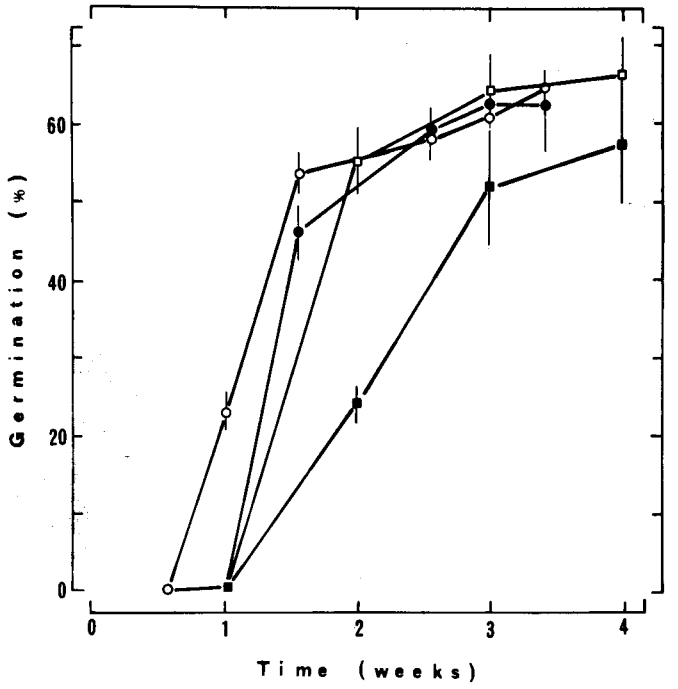


Figure 5. Germinability of *P. brutia* seeds as a function of the osmotic potential of mannitol solutions, at 20 °C in darkness. Germination was counted 1–5 weeks (curves 1–5 respectively) after start of imbibition in the osmoticum.

▲ Figure 6. Secondary dormancy of *P. halepensis* and *P. brutia* seeds induced by iFR (one minute FR/30 minutes) and regulated by subsequent brief (30 minutes) R and FR irradiations (t = 20 °C).



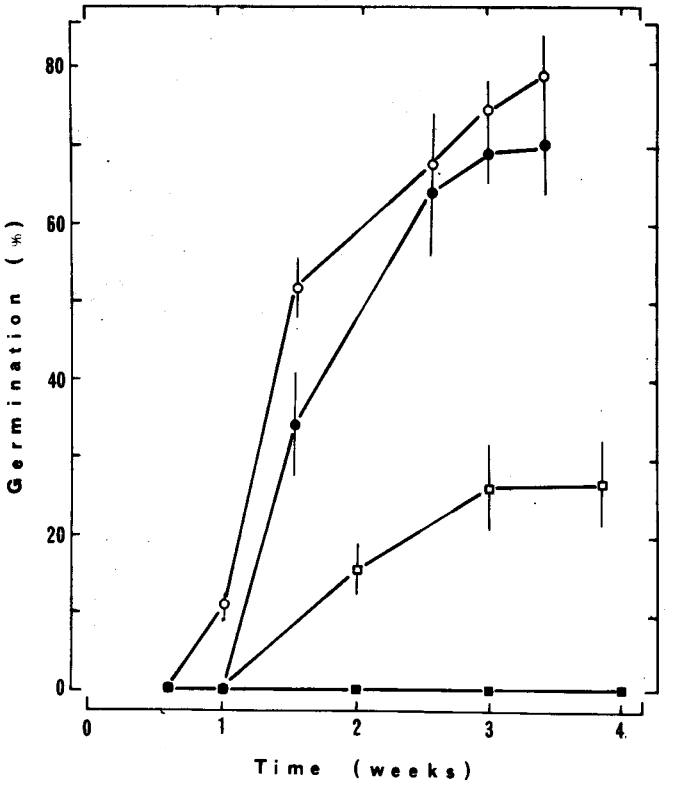


Figure 7. Time course of *P. halepensis* seed germination in darkness (closed symbols) and under cR (open symbols) at 15°C (■, □) and 20°C (●, ○).

Figure 8. Time course of *P. brutia* seed germination in darkness (closed symbols) and under cR (open symbols) at 15°C (■, □) and 20°C (●, ○).

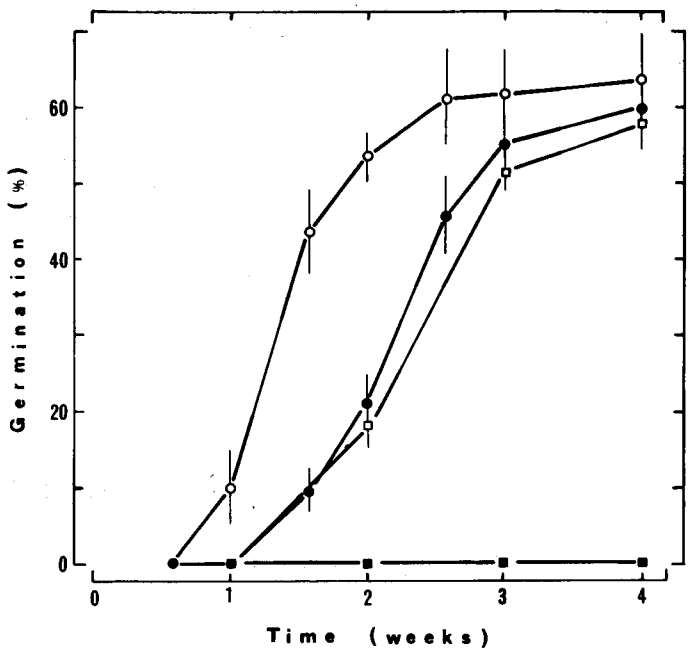


Figure 9. Time course of germination in *P. halepensis* seeds, imbibed at daily alternating temperatures: 14 hours 20 °C/10 hours 15 °C (○, ●); 10 hours 15 °C/14 hours 10 °C (□, ■). Open symbols represent samples illuminated with continuous white light throughout the 'warm' period; closed symbols are dark controls.

the Ψs, the slower the manifestation of germination. It is interesting to note that there is a significant increase until the third week for osmotica higher than −0.97 MPa, while for lower ones a remarkable rise in germination is observed later on. Thus, for a mannitol solution of 0.6 M (−1.46 MPa), final germination is about 50% (more than 70% of the water control). Similar, though faster, germination is observed at 20 °C (figure 4). The 2nd-week-curve closely resembles the 3rd-week one at 15 °C. The remarkably delayed rise in germination can be noticed again at low osmotic potentials, though at −1.95 MPa, germination after five weeks is restricted to only about 10%. From the data of figures 3 and 4 it is evident that the germination potential (i.e. the absolute value of the osmotic potential required for inhibition of germination in 50% of seed population) in *P. halepensis* seeds has a value between 1.46 and 1.95 MPa, at both 15 °C and 20 °C.

Figure 5 contains corresponding data for *P. brutia* at 20 °C. The curves presented are quite similar (though delayed by about one week) compared with those of *P. halepensis* (figure 4). In this case too the germination potential is higher than 1.46 MPa.

The effect of intermittent broad band far-red irradiation (iFR) on *P. halepensis* and *P. brutia* seed germination is presented in figure 6. It is clear, that a long (3-week) iFR illumination applied immediately after onset of sowing, not only fully inhibits germination, but also induces a secondary dormancy in both species. This dormancy

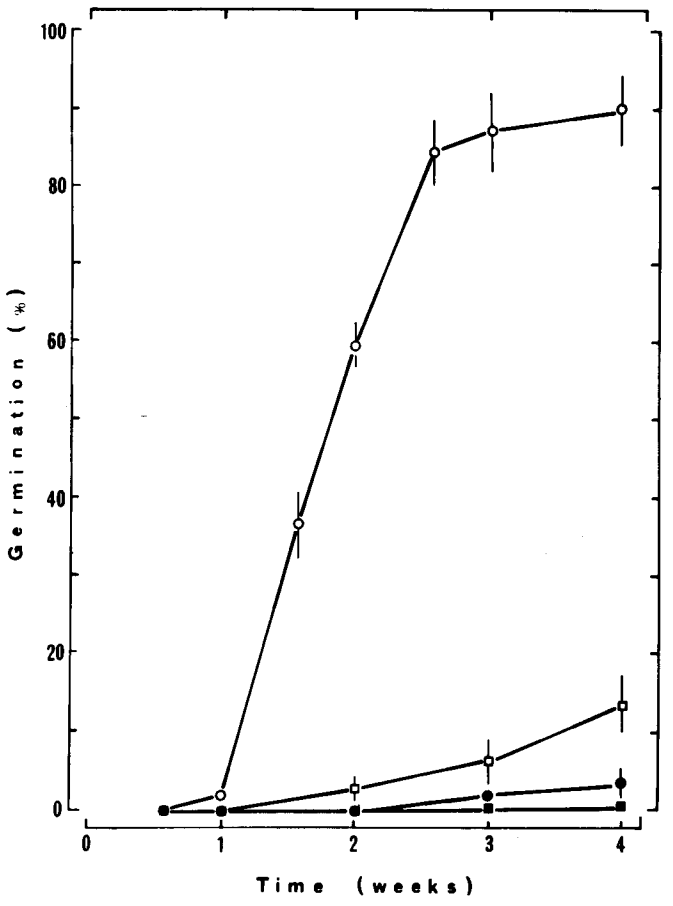


Figure 10. Time course of germination in *P. brutia* seeds imbibed at daily alternating temperatures: 14 hours 20°C/10 hours 15°C (○, ●), 10 hours 15°C/14 hours 10°C (□, ■). Open symbols represent samples illuminated with continuous white light throughout the 'warm' period; closed symbols are dark controls.

is manifested by the inability of the seeds to germinate subsequently in darkness. This deep secondary dormancy is only partly released by an intervening brief red (R) irradiation. In *P. halepensis* seeds, shorter exposures (nine or six days) to iFR result in a lesser degree of secondary dormancy which can be fully reversed by R irradiation. On the other hand, the exposure of *P. brutia* seeds to iFR for only six days results in a secondary dormancy which, though less deep than that imposed by 3-week iFR, nevertheless cannot be totally reversed by subsequent R irradiation. Brief FR irradiation given immediately after the brief R one, results in a complete reversion of the R promoting effect. This is a clear proof of phytochrome mediation in the secondary iFR-dormancy release.

The illumination of *P. halepensis* seeds with continuous red (cR) light results in a promotion of germination rate (figure 7). This promotion is rather slight at 20 °C while it is considerable (about one week) at 15 °C. The final percentages of germination are optimal in all cases presented in figure 7, though it must be noted that these experiments took place four months later than those of figure 1. This decrease (about 15%), observed in the final germination level, might thus be attributed to a loss of viability during storage (though no test of viability had been performed).

The same remark holds for *P. brutia* seeds too, as shown in figure 8. There too, there is a slight increase in germination rate by cR at 20 °C, while at 15 °C there is a more marked induction of germination by cR.

Figure 9 presents germination time courses of *P. halepensis* seeds imbibed at alternating temperatures. These two temperature regimes simulate those prevailing in natural conditions during early winter (mid-November) and late spring (end of April), which roughly coincide with the beginning and the end respectively of the rainy season in the mediterranean-type climate of Greece. It is evident from figure 9 that *P. halepensis* seed germination at both 20 °/15 °C and 15 °/10 °C is considerably promoted by the white light illumination provided during the warmer period. It is worthwhile noting that dark germination at 20 °/15 °C is quite similar to that at 20 °C in darkness (figure 7). Moreover, germination courses under alternating light and darkness at 20 °/15 °C and 15 °/10 °C are nearly identical to those at 20 °C and 15 °C in darkness respectively (figure 7).

The corresponding results of *P. brutia* seeds are shown in figure 10. Similar differences are observed in this case as well, while the inductive effect of light at both temperature regimes appears considerably increased compared with that observed in *P. halepensis* seeds.

Discussion

The seeds of *P. halepensis* and *P. brutia* from the two regions used in this work, germinate in darkness without any prechilling treatment. Optimal percentage (ca. 80%) and germination rate (T_{50} about two weeks) is observed at 20 ° for both species. Seeds of *P. halepensis* show a wider range of temperature requirements than those of *P. brutia,* since the former germinate almost optimally at 15 °C as well. Nevertheless, it must be noted that germination in both species is restricted to a rather narrow range of temperatures, around 20 °C. These results are in accordance with both the prescription of ISTA Rules for *P. halepensis* (ISTA, 1976) and the experimental data reported previously for *P. halepensis* (Calamassi, Falusi and Tocci, 1984) and for *P. brutia* (Shafiq, 1979). The germination rate in both cases studied is noticeably low, a fact already observed in most *Pinus* species (ISTA, 1976). This delay might be attributed to the seed coat acting as a barrier to water penetration to embryo (Thalouarn, 1975; 1976).

The estimated values of the germination potential in osmotic stress, for both species, are extremely high (> 1.46 MPa). The manifestation of this property takes a considerably long period of time (e.g. seven weeks at 15 °C for *P. halepensis* seeds). It must

be noted that especially at the lower values of osmotic potential, germination rate is significantly decreased. For instance, at −1.46 MPa, germination of *P. halepensis* begins after two or four weeks depending on the temperature (20° or 15°C respectively). These results do not agree with recent data concerning the effect of water potential on the germination of *P. halepensis* seeds collected from four different provenances (Falusi *et al.*, 1983). Even their strongest seed lot (probably from the same region as ours) presented an osmotic inhibition of germination at values lower than −4 bar (−0.4 MPa) while the corresponding value in our data was lower than −10 bar. This disagreement may be due to a much higher resistance to water stress of the two seed lots used in the present work. The difference in water stress resistance might be attributed to the effect of either an inherent factor or seed age and storage conditions on seed vigour. It seems more probable though that the experimental time (30 days) was insufficient for full manifestation of germination under water stress. This is supported by the high value of mean germination time to germination, about 21 days, at −6 bar and about 25 days at −8 bar.

Light is generally considered as a promotor of *Pinus* spp. seed germination. In many cases there exists a primary dormancy which can be released by chilling and/or light (Toole 1973; ISTA, 1976). It has been proved that light control is exerted through the phytochrome system although its spectral characteristics in gymnosperms are a matter of dispute (Grill and Spruit, 1972). The promotive effect of white light in *P. brutia* seed germination has already been shown at constant temperature (20° or 21°C with light for eight or nine hours respectively; Shafiq, 1979; Calamassi, 1982). As far as *P. halepensis,* is concerned, data reported by Calamassi (1982) are contradictory to the ISTA Rules (1976) prescription, since nine hours of illumination at 21°C inhibit germination. The results presented in this work clearly show a marked promotion of germination in both seed lots caused by either continuous R light at constant temperatures (15° and 20°C) or diurnal white light at alternating temperatures (10 hours at 15°C and 14 hours at 20°C).

The germination of *P. halepensis* and *P. brutia* seeds, intermittently illuminated by FR is fully inhibited. When seeds are subsequently transferred to darkness, the duration of iFR pretreatment determines the extent of germinability, or in other words the degree of the secondary (FR-) dormancy induced. This secondary dormancy is under phytochrome control as shown by R/FR reversibility and is similar to that induced in many light inhibited seeds (e.g. *Amaranthus caudatus;* Kendrick and Frankland, 1969). It must also be noted that the germination of the *P. brutia* seed lot used seems to be more photosensitive in comparison with *P. halepensis.*

Experimental results of *P. halepensis* and *P. brutia* seed germination at alternating temperatures and light regimes have already been reported (Calamassi, 1982; Shafiq, 1979). However, the alternations chosen are quite unnatural, e.g. eight hours 30°C/16 hours 20°C or nine hours 21°C/15 hours 15°C with light during the warm period. On the other hand, the aim of the present experimental design was the simulation of the environmental conditions prevailing at the extremes of the rainy season. The germination of both species can be manifested in all cases studied, though with a lower

rate and level at cooler temperatures. These data fit well with field observations of natural seed germination (an initial small part of the population germinating during the winter months, November to February, and a 'burst' of germination occurring in March and April). Moreover, the promotive effect of light during the 'day' is clearly shown in the present work. This fact verifies the photophilous nature of these two species, a nature which allows their germination and establishment mainly in open, well-illuminated places.

Acknowledgement

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