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Effect of light conditions and salinity on germination behaviour and early growth of umbrella pine (*Pinus pinea* L.) seed

By P. P. GANATSAS* and M. N. TSAKALDIMI
Aristotle University of Thessaloniki, School of Forestry and Natural Environment,
Institute of Silviculture, P.O. Box. 262, 54124 - Thessaloniki, Greece
(e-mail: pgana@for.auth.gr)
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SUMMARY

Pinus pinea L. (umbrella pine) is a tree species distributed around the Mediterranean basin. The ecological, landscape, recreational and soil conservation uses of *P. pinea*, and its aesthetic value, make this species important for landscape planning and multipurpose forestry. In this study, seeds of *P. pinea* were collected from the two principal forests where the species is found in Greece (the Strofylia Forest, southern Greece, and the Sithonia Peninsula, northern Greece), in order to examine the effects of light conditions and soil salinity, two important environmental factors, on seed germination behaviour and early growth. The effect of light alone on seed germination was ascertained under different light regimes; while, to investigate the effect of salinity, seeds maintained in continuous light were watered with varying concentrations of NaCl and, after germination, the early growth of seedlings was monitored. The findings showed that the percentage germination of *P. pinea* seed is high and does not depend on light or dark conditions, while salinity markedly inhibited seed germination at NaCl concentrations of 0.05 M and above. Seedlings raised under saline conditions (0.02 M NaCl) were significantly smaller than those of the controls, especially in their below-ground structures.

Pinus pinea L. (umbrella pine or Italian stone pine) is a tree species that is widespread around the Mediterranean basin (Barbero *et al*., 1998). It is a dramatic ornamental tree, widely planted in parks and gardens throughout the World (Fady *et al*., 2004). The ecological, landscape, recreational, and soil conservation uses of *P. pinea*, the high economic yield of its two principal products, wood and pinyon nuts, its ability to withstand low intensity fires (Tapias *et al*., 2004), and its high aesthetic value, make this species important for landscape planning and multipurpose forestry. As a consequence, the interests of landscape designers, forest managers, growers and researchers in the ecology of the species have increased (Calama *et al*., 2003).

P. pinea is propagated readily from seeds, which have been reported to be resilient and long-lived (Ranaldi *et al*., 2003). However, the species faces regeneration problems in many localities. While the reasons for this phenomenon have not yet been identified, several hypotheses have been proposed (Moussouris and Regato, 1999). Although the species is considered highly light-demanding throughout its life (Moulopoulos, 1962), the effects of light conditions on seedling emergence have not yet been studied. In addition, the geographical distribution of *P. pinea*, as well as its aesthetic use mainly along coasts, and thus in saline soil, could pose possible problems for species establishment. Along the coastline of Tuscany, low concentrations of Na^+ and Cl^- ions have been found in the needles and woody tissues of *P. pinea* trees, indicating absorption of salt (Teobaldelli *et al*., 2004). In general, salinity inhibits seed germination (Lombardi *et al*., 1998, Tobe *et al*., 1999; Koslowski, 2002), primarily by lowering the osmotic potential of the soil solution, thus inhibiting water absorption by seeds (Katembe *et al*., 1998), but also through salt toxicity to the embryo. High ion contents in plant cells induce changes in protein hydration (Zekri, 1993).

Little is known about the effects of environmental conditions on seed germination and early growth in *P. pinea* (Baskin and Baskin, 1998). Previous studies (Skordilis and Thanos, 1997; Escudero *et al*., 2002) reported a high variability in seed germination of *P. pinea* that was related to environmental conditions and variations in populations.

Thus, the aim of this study was to examine the effect of light conditions and salinity on seed germination and early growth in two populations of *P. pinea*, from northern and southern Greece. The hypothesis was that environmental factors would have a significant effect on seed germination, and thus an analysis of their influence could contribute to a better understanding of the ecology of the species.

MATERIALS AND METHODS

Pinus pinea seeds were collected by hand from the Strofylia Forest, a coastal forest located in the western Peleponisos, in southern Greece, and from the Sithonia Peninsula, Chalkidiki, in northern Greece. At each site, cones were collected from five different locations, and placed in the sun to open. The seeds were removed and stored at 4ºC until germination tests. Prior to germination, seeds were immersed in water and any that floated were removed. Seeds were then dusted with fungicide (Captan, Mercap 83 WP; Papaeconomou Agrochemica, Thessaloniki, Greece).

*Author for correspondence.

In order to examine the influence of light conditions on seed germination, four treatments were applied: continuous light as a control, light for 10 min d^{-1}; light for 30 min d^{-1}; and complete darkness.

The effect of salinity on seed germination was estimated using four salt treatments. Seeds were first watered with 0.02 M, 0.05 M, 0.2 M, or 0.5 M NaCl solution. The control seeds were watered with deionised water only.

For all treatments, germination tests were performed using ten replicates, each containing ten seeds, per 90 mm-diameter glass Petri dish, lined with two sheets of Whatman No 1 filter paper (Skordilis and Thanos, 1997; Raccuia *et al*., 2004). Seeds from each site were kept separate from each other. Approx. 5 ml deionised water or salt solution, depending on the treatment, were added at the beginning of the test to moisten the two sheets of filter paper in each Petri dish. Then, as much deionised water as was needed to replace the losses from evaporation was added daily. However, in order to avoid any fungal infection, and to keep the Petri dishes clear we replaced the filter papers, and the salt solutions, with fresh filter papers and fresh salt solutions at the original concentration every third day.

The seeds were separated from each other in the Petri dishes in order to reduce the chance of cross-contamination by micro-flora. Germination tests were carried out in a plant growth chamber under continuous light with a photon flux density of 55 – 65 µmol m^{-2} s^{-1}, where the temperature was kept constant at 20º ± 1ºC. This temperature was recommended for laboratory germination trials of *P. pinea* and seedling production in nurseries by International Seed Testing Association (1993) guidelines. Germination was recorded every 2 d and was considered complete when no additional seeds germinated. The criterion for germination was protrusion of the radicle through the seed coat (Skordilis and Thanos, 1995; Ranaldi *et al*., 2003; Raccuia *et al*., 2004). The measurements made were: germination energy (the first count that can be used as an indicator of vigour (Bonner *et al*., 1994), mean time to complete germination (MGT), percentage germination (after every 2 d of germination), and total germination. Germination percentage was calculated as the ratio between the number of germinated seeds at a given time and the total number of seeds sown (ISTA, 1993). MGT was calculated as follows (Hartmann *et al*., 1997; Raccuia *et al*., 2004):

$$MGT = (N_1T_1 + N_2T_2 + ... + N_xT_x) \ / \text{ Total number of seeds germinating}$$

where N_i = number of seeds germinating within consecutive intervals of time, and T_i = the time between the beginning of the test and the end of the particular interval of measurement.

At the end of the salinity experiment, the pre-germinated seeds from all treatments were sown in 350 ml black plastic containers (Rootrainers; Ronaash Ltd., Kelso, UK) containing sand, initially moistened with the respective concentration of NaCl used for the salt treatment, or with deionised water for the control. Thereafter, we added deionised water every 2 d. The containers were placed in a plant growth chamber with day/night temperatures of 20º/15ºC, and daylight supplemented by high-energy sodium lights to obtain a 16 h photoperiod (Croser *et al*., 2001). Forty-eight pots (16 pots × 3 replications) were sown for each treatment. After 4 weeks, the seedlings were harvested and washed in deionised water. Seedling shoot and central root lengths, the number of laterals > 10 mm, and shoot and root dry weights (DW) were recorded. For DW measurements, shoots and roots were oven-dried at 70ºC for 48 h, then weighed.

All results were analysed by ANOVA (SPSS software; SPSS Inc., Chicago, IL, USA), and the Waller-Duncan criterion was used to compare means between the different treatments. Distributions were tested for normality by the Kolmogorov-Smirnov criterion, and the homogeneity of variances by Levene's test. Percentages were transformed to arsine square root values, before analysis (Snedecor and Cochran, 1988; Norusis, 1994).

RESULTS

Influence of light conditions on seed germination behaviour

The final percentage germination of *P. pinea* seeds was high under the different light conditions, and did not differ significantly ($P > 0.05$) between treatments (Figure 1). Also, only small differences were recorded between seeds originating from southern and northern Greece (the differences in percentage germination were less than 1% for all treatments). Seeds exposed to continuous light exhibited the lowest percentage germination (87.2%), while seeds exposed to light for 30 min d^{-1} gave 100% germination. Germination was complete within 30 d for all light treatments, after which no late seed emergence was observed in any case. The mean time to complete germination did not differ statistically among the light treatments, and ranged from 13.4 – 15.6 d. Darkness delayed germination the most (15.6 d). The temporal distribution of germination (Figure 2) showed that seeds from the different light treatments did not give similar patterns of germination. First seed germination occurred 8 d after sowing in treatments with light, while seeds in the dark took 10 d after sowing to start germination. Another difference recorded was the time to reach the peak of maximum germination. This occurred after 10 d in seeds exposed to continuous light,

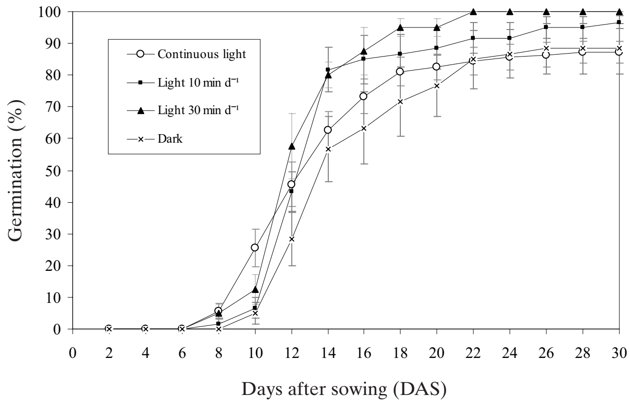

FIG. 1

Percentage germination of *P. pinea* seeds as a function of time in continuous light (○), light for 10 min d^{-1} (■), light for 30 min d^{-1} (▲), or in darkness (×). Vertical bars represent the standard error of the mean.

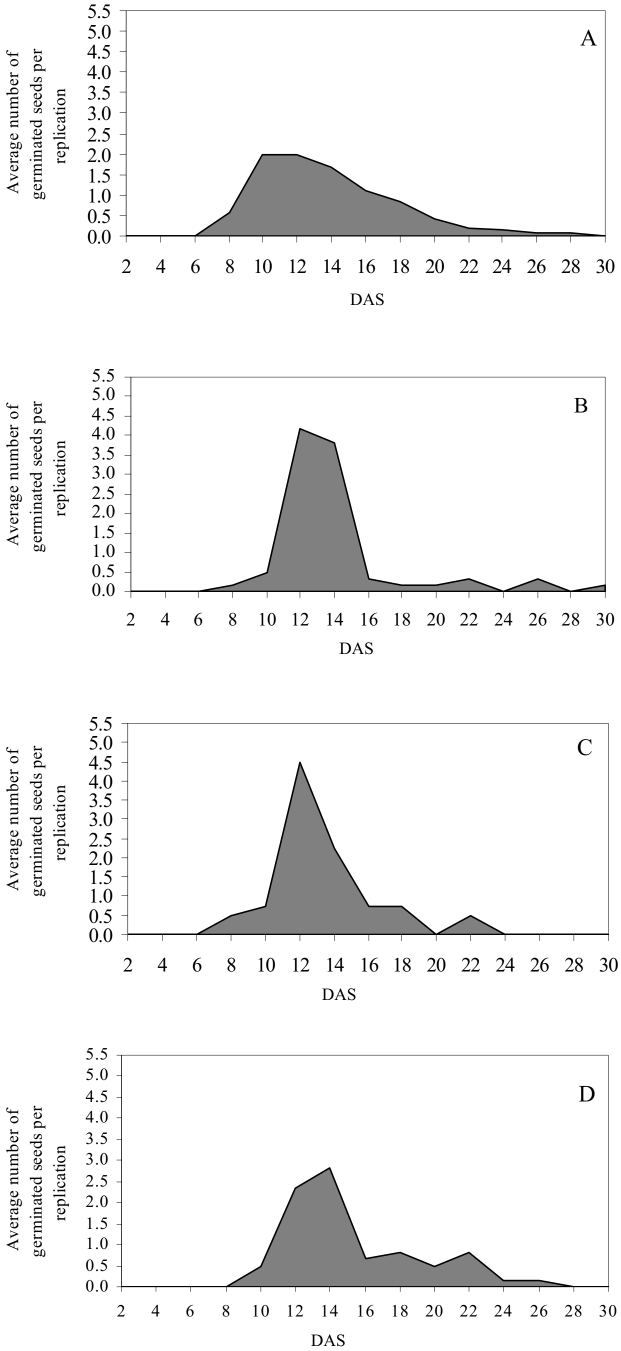

FIG. 2
Temporal distribution of germination of *P. pinea* seeds over time (days after sowing, DAS), in continuous light (control; Panel A), light for 10 min d^{-1} (Panel B), light for 30 min d^{-1} (Panel C), and in the dark (Panel D).

after 12 d in seeds exposed to light for 10 or 30 min d^{-1}, and after 14 d in seeds kept in the dark.

Influence of salinity on seed germination behaviour

Salinity represented a strong negative factor for germination of *P. pinea* seeds. Germination was completely inhibited in treatments with 0.05 M, 0.2 M or 0.5 M NaCl. Only at the lowest concentration of NaCl (0.02 M) did *P. pinea* seed exhibit a final germination percentage of 70%, while seeds treated with deionised water showed a significantly ($P < 0.01$) higher percentage germination (87.2%; Figure 3). Germination

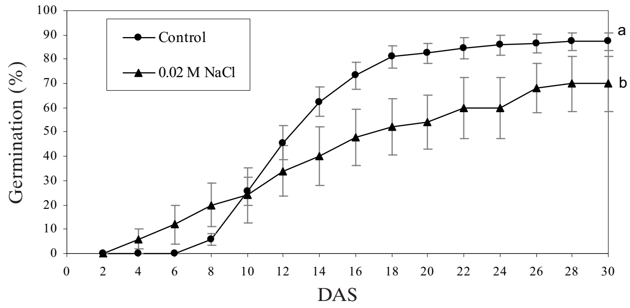

FIG. 3
Percentage germination of *P. pinea* seeds as a function of time in deionised water (control; closed circles) or in 0.02 M NaCl solution (closed triangles) under conditions of continuous light. Germination was zero in all other salt treatments. Vertical bars represent the standard error. Different lower-case letters (a,b) at the final percentage germination (30 DAS) indicate statistically significant differences ($P < 0.01$).

was complete in 30 d and, after this time, no seedling emergence was observed in any treatment. The mean time to complete germination did not differ statistically, and ranged from 13.6 d (in deionised water) to 14.8 d (in 0.02 M NaCl). The temporal distribution of germination showed that seeds in deionised water, or in 0.02 M NaCl, did not have similar patterns of germination (Figure 4). The first germination was recorded 4 d after sowing (DAS) in the 0.02 M NaCl treatment and 8 DAS in deionised water, while the peak of germination appeared earlier in the deionised water treatment (10 DAS), than in the NaCl treatment (12 DAS).

Early growth of seedlings

As no germinated seedlings existed in the 0.05 M, 0.2 M or 0.5 M NaCl treatments, the early growth of *P. pinea*

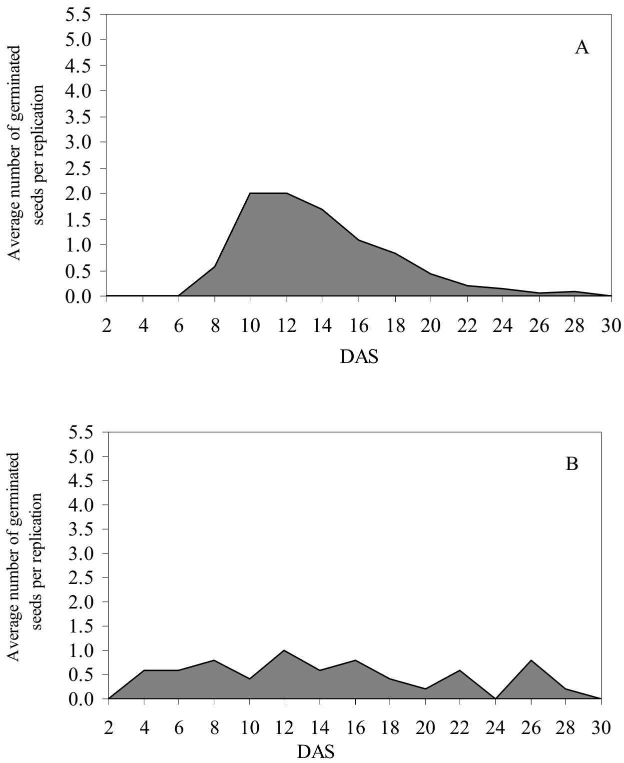

FIG. 4
Temporal distribution of germination of *P. pinea* seeds over time (DAS), under conditions of continuous light, in deionised water (Panel A) or in 0.02 M NaCl (Panel B).

seedlings could only be estimated from the control and 0.02 M NaCl treatments.

After 1 month in the growth chamber, an analysis of seedling morphology showed that salinity affected early seedling growth (Figure 5 A–C). The shoot and central root lengths, and shoot and root biomass of the seedlings, were found to be significantly lower in 0.02 M NaCl than in the controls. However, the greatest difference was observed in the number of lateral roots, with over three-times fewer in seedlings produced from NaCl-treated seeds.

DISCUSSION

Our findings show that seed germination in *P. pinea* is high (87.2 – 100%), regardless of the time for which seeds are exposed to light. The total percentage germination, as well as the mean time to complete germination, did not show any significant differences among seeds exposed to different light duration treatments or kept in the dark. The high percentage germination of seeds kept in the dark (88.3%) indicates that *P. pinea* seed can germinate below the soil surface, without light induction, even though the species is light-demanding throughout its life cycle after the germination stage (Fady *et al*., 2004). These results agree with Kozlowski (2002), who noted that seeds of most temperate-zone woody plants do not have a strict requirement for high intensity light for germination (e.g., seeds of *Pinus* require low illumination for germination). Skordilis and Thanos (1997) found that the percentage germination of *P. pinea* seeds from Attica (central Greece) in the dark at 20ºC was 63%; while with a 12 h photoperiod of white-light at 20ºC it was much greater (82%). It is possible that the time of collection and/or the seed storage conditions may explain these differences. However, in our study, the time required for seeds to reach maximum germination decreased, but not significantly, as the time of light exposure increased. Maintenance in the dark also decreased the "germination energy" of the seeds compared to the light treatments.

Concerning the origin of the seeds tested, there were negligible differences between the two sites (Strofylia Forest, southern Greece; Sithonia Peninsula, northern Greece), which can be attributed to the low genetic diversity of this species throughout the World (Fady *et al*., 2004).

Salt treatment had a strong negative influence on *P. pinea* seed germination. NaCl concentrations of 0.05 M and above completely inhibited seed germination, while 70% germination occurred in 0.02 M NaCl solution. This percentage was significantly lower than that of the control (87.2%). The peak of seed germination appeared earlier in the control (10 d) than in the 0.02 M NaCl treatment (12 d), while germination started earlier in the latter treatment (4 d *vs.* 8 d). The reduction in *P. pinea* seedling emergence may be attributed to the inability of less vigorous seed to overcome the external osmotic potential and take up water for embryo expansion because the hard seed coat of this species becomes impermeable in the presence of salt (Al-Niemi *et al*., 1992; Kozlowski, 1997; Song *et al*., 2005). An effect of osmotic constraints on germination has been reported in

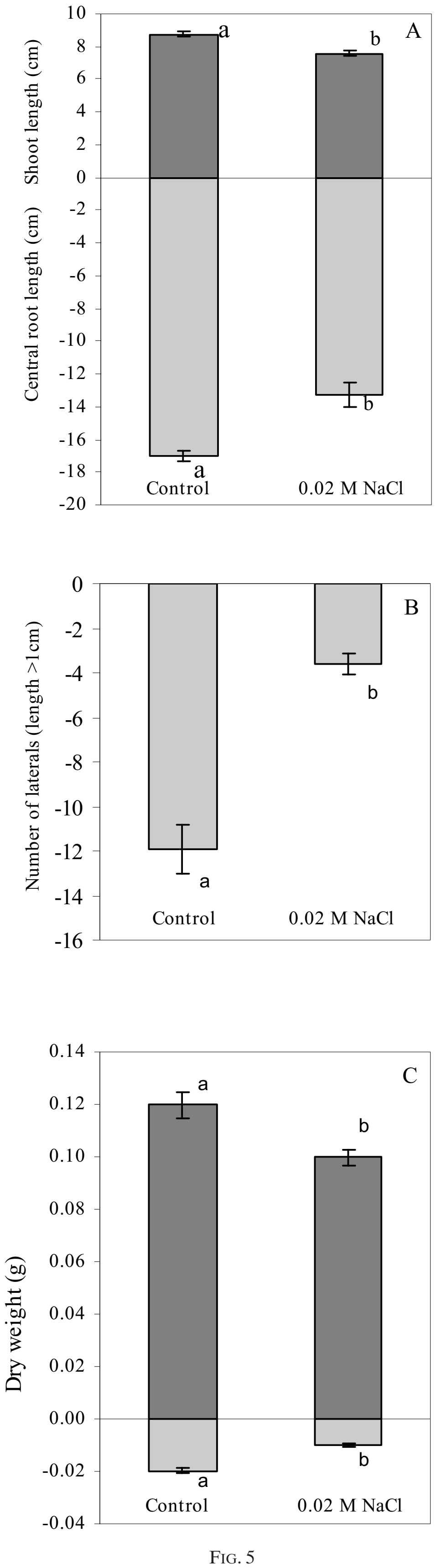

FIG. 5
Mean shoot (cm) and central root lengths (cm; Panel A), mean number of lateral roots with lengths >1 cm (Panel B), and mean shoot and root dry weights (DW in g; Panel C) of *P. pinea* seedlings in control (deionised water) or in 0.02 M NaCl, 1 month after sowing. Negative values are used to symbolise below-ground growth. Vertical bars represent the standard error of the mean and the lower-case letters represent significant differences between the treatments ($P < 0.05$).

many species, where salinity inhibited germination by limiting water uptake and hence postponed the initiation of germination (Dell'aquilla and Spada, 1993; Kozlowski, 1997; Croser *et al.*, 2001). In the present study, although germination initiated earlier under saline conditions, the total percentage of germination was significantly reduced.

Seedling growth was also affected by the presence of salt, even at 0.02 M NaCl. Seedlings raised under saline conditions were significantly smaller, especially in their below-ground structures. The number of lateral roots was over three-times lower in the salt-treated seedlings and the root DW was approx. 50% that of control seedlings. This shows that below-ground seedling growth depends strongly on the salinity of the medium, while above-ground growth is less influenced, probably because the aerial structures are not in direct contact with the medium.

Plant species differ in their sensitivity or tolerance to salinity (Huang *et al.*, 2003). Croser *et al.* (2001) found that when NaCl solutions of up to 0.05 M were used, seedling emergence in *P. banksiana* was not significantly different from that of the control. However, with a higher NaCl concentration (0.25 M), seedling emergence was only 13%, and no seedlings survived for longer than 1 week. In the same study, salt treatment had a more adverse effect on emergence in *Picea mariana* and *Picea glauca*, and no seed germination occurred in either species in 0.25 M NaCl. When NaCl solutions > 0.05 M were used, seedling survival decreased after 6 weeks in all species. Early seed tolerance to salt does not, therefore, preclude the possibility that, in later stages of development, seedlings may become more sensitive to salt (Kozlowski, 1997).

In conclusion, we report that light conditions are not a restricting factor for seedling emergence in *P. pinea*, while soil salinity is an important factor, which should be considered when using this species in areas with high salt concentrations. In such a case, species more tolerant to salt, such as *Tamarix* or *Eleagnus* spp. could be used.

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