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RESEARCH ON FACTORS LEADING TO THE ABSENCE OF NATURAL REGENERATION OF *PINUS PINEA* L. IN THE STROFYLIA FOREST, WESTERN PELOPONISOS, GREECE

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Abstract

The Pinus pinea forest in Strofylia, western Peloponisos, belongs to a wider system of coastal dunes and wetlands that are included in the RAMSAR convention and the Natura 2000 European Network. Great regeneration problems of the species in the area, have been reported since 1954. Since then, several hypotheses have been made to explain the reasons for the species regeneration failure. However, the causes for this phenomenon have not been determined yet. This study is a part of the Life Project 2002/Nat/Gr/8491 and examines the hypothesis that species regeneration absence may be attributed to the one of the following three conditions: 1) insufficient seed supply, 2) insufficient seed germination under the mother stands and 3) seedling growth is prohibited or it is very low. The first condition was checked by measuring cone and seed production of the stands. The second condition was checked by: i) examining germination of the seed produced by mother stands in the laboratory, ii) seed germination testing under different light conditions similar to understory environment, iii) seed germination testing under high salt concentrations and iv) in situ seeding. The third condition was checked by i) in situ planting seedlings and monitoring their field performance ii) monitoring the germinated seeds and iii) examining grazing effect. Even though some of the experiments are still in progress, the findings showed that: Seed production of the mother stands is very low, seed quality is high, light conditions do not affect seed germination, high salt concentrations prohibit seed germination, seeding in situ was sufficiently successful, planting in situ was very successful and grazing effect is very important to seedling establishment and growth.

INTRODUCTION

Pinus pinea is a Mediterranean tree species whose natural range is 'impossible to be determined' due to early human use and expansion [1]. It is a species that has commonly been used from ancient times for wood and (mainly) for nuts production [2, 3]. It is geographically distributed along the Mediterranean basin while its habitat is included in the Annex I of the Directive 92/43 of European Union for nature conservation.

Pinus pinea is considered to be a species easily propagated by seeds. *P. pinea* seed is an extremely resistant one retaining remarkable possibilities for good germination for years [4], while the seedlings grow well and artificial tree establishment is generally easy [5]. However, absence of species regeneration has been recorded in some places such as in the Strofylia forest, Peloponisos [6] and in Shinias, Marathonas, Greece [7]. In those cases, even though several hypotheses have been made, the reasons for this phenomenon have not yet been identified. Additionally, species habitat restriction by invasion of the species *Pinus halepensis* is observed in many cases around the Mediterranean basin [8, 9]. Measures to solve the problems are needed in order to conserve the populations of the species for the future.

Pinus pinea forest of Strofylia is located within a wide system of dunes and wetlands that belongs to the RAMSAR convention, as well as to the NATURA 2000 European network and to the Special Protected Areas for birds. Also, the forest is almost the only pure *P. pinea* forest in Greece and it is considered as one of the possible natural habitats of the species worldwide. The problem of species regeneration in the area is considered very serious since: 1) absence of *P. pinea* regeneration was recorded for the first time many decades ago (1954); this absence, in combination with the high regeneration rate of *Pinus halepensis*, causes a restriction of *P. pinea* habitat and a forest succession by the gradual replacement of *P. pinea* by *P. halepensis,* 2) all the stands are heavily degraded due to several reasons such as: individual tree death by secondary attacks of *Marcallina helenica* and *Lithodromus pinastri,* tree uprooting due to the shallow root system that the species forms in the area, overgrazing and high human touristic pressures. This study is a part of a Life Project and examines the hypothesis that absence of species regeneration may be attributed to one of the following three conditions: i) insufficient seed supply, ii) insufficient seed germination under the mother stands and iii) seedling growth is prohibited or it is very low.

The first condition was checked by measuring cone and seed production of the stands.

The second condition was checked by i) examining germination of the seed produced by mother stands in the laboratory, ii) seed germination testing under different light conditions similar to understory environment, iii) seed germination testing under high salt concentrations (since soil salinity is a possible problem, [10]) and iv) in situ seeding

The third condition was checked by i) planting seedlings and monitoring their field performance ii) monitoring the germinated seeds and iii) examining grazing effect.

MATERIALS AND METHODS

The stands conditions of *Pinus pinea* forest in the area were evaluated by taking a sample of 20 plots of 0.6 ha along the area that the species occupies. In each plot the following data were collected:

Tree dimensions (DBH and height) of all the trees with DBH above of 4 cm were measured.

Tree age was measured in 3 trees by taking tree cores and measuring tree annual ring in the laboratory.

Based on the stand characteristics and in combination with the stand discrimination carried out by Papamichos [6] three main stand categories were distinguished in the area, mature closed stands, mature open stands and mature high degraded stands.

Cone and seed production measurements

In order to estimate the cone production, thirty (30) dominant and semi-dominant trees were selected from each stand category; the trees were randomly selected at a distance of at least 50 m apart [11] and the number of cones per tree was recorded.

From the above trees, ten (10) trees per stand category were selected which bore at least 10 mature closed cones and finally five (5) cones per tree were collected for the estimation of seed production. However, in the case of high degraded stands, we collected cones from five (5) trees because it was impossible to find more trees with cones. Afterwards, all the cones (125 cones totally) were put under the sun to open and then they were completely dissected to determine seed yields per cone [12]. The seeds were stored at 4 °C until the germination test.

Laboratory tests

Seed germination research included: i) performance of germination test in order to determine the germination capacity of the seeds produced in the area, ii) investigation on the effect of light conditions on seed germination behaviour and iii) research of any possible effect of high salt concentrations on seed germination. Thus, three experiments were conducted at the laboratory:

Prior(1st) experiment

Seed germination was tested taking a random sample of 100 seeds from the mixture of all collected fully developed seeds. Standard conditions were used [13], those suggested by ISTA [14] for *P. pinea* laboratory germination trials and seedling production in the nurseries [15].

2nd experiment

In order to discover the effect of light and darkness on seed germination, seeds were exposed to: i) continuous light (control), ii) light for 10 min. daily, iii) light for 30 min. daily and iv) continuous darkness.

The treatments ii) and iii) were tested because we supposed that these conditions may be similar to those under the forest canopy in the stand floor due to the contribution of sunflecks to total illumination [16].

3rd experiment

In order to discover the effect of salinity, seeds were watered with 0.02M, 0.05M, 0.2M and 0.5M NaCl solution daily. The control was the same as in the 2nd experiment (seeds were watered only with deionized water).

Prior to germination, seeds from all three experiments were immersed in water and any floating seeds were removed. Then, the seeds were dusted with fungicide (Captan) in order to prevent fungal infection. In all three experiments, germination tests were performed with 10 replicates of 10 seeds per glass petri dish (diameter 9 cm, lined with two sheets of filter paper and moistened with 5 ml of distilled water) per treatment, [17, 18]. In the petri dishes the seeds were separated from each other in order to reduce the chance of cross contamination by micro-flora [19]. Germination tests were carried out in a plant growth chamber under continuous light with a photon flux density of 55-65 $\mu E.m^{-2}.s^{-1}$, where the temperature was kept constant at 20 1 ^{o}C; this temperature value is suggested from ISTA [14] for *P. pinea* laboratory germination trials and seedling production in the nurseries. Germination was recorded every two days and was considered complete when no additional seeds germinated. The criterion of germination was radicle protrusion through the seed coat [20, 4, 18].

Field research

Field research included the examination of the in situ seeding, the estimation of the plantation results, the seedlings' growth in the field and the grazing effect on species regeneration.

In situ seeding

An amount of collected seeds was sown in the field in patches (40cmX40 cm) under the *P. pinea* stands; there were three seeds per patch. Totally 1620 seeded patches (90 patches x 18 different locations) were introduced across the forest. The distance between the patches was 4 m. The number of germinated seeds as well as the germinants development were recorded in May after seeding, in August and in November of the same year.

Plantation results

Plantations were applied in 6 different plots across the forest. Two thousand five hundred (2500) two-year old container seedlings of *P. pinea* were manually planted in November. Planting spacing was 4 m. Seedling survival was estimated before summer (May) and after the summer drought (October) of the next year. Also, seedling annual height growth was monitored from 30 randomly selected seedlings per plot. Additionally, the seedling performance of five-year old plantations (planting space 2m X 2m and average initial seedlings' height 50 cm) was estimated. These had been planted in the area by the Forest Service. In three randomly selected plots of 100 m^2 in three 3 different locations, seedling survival was estimated by measuring the number of seedlings. Seedling growth was also estimated by measuring their total height.

Seedling stress

In order to examine the causes for seedling mortality in the field, the following measurements were made; i) seedling water stress (predawn and midday water potential) was estimated by measuring a number of 30 seedlings four times during the year (February, May, August and October) and ii) soil water status in the surface soil (0-20cm) horizons was measured in three points in each plot with TDR methodology four times during the year.

Grazing

Grazing effect was examined by fencing the plots and examining the difference between the fenced areas and unfenced areas. However, due to the fact that this effect is not short-term, the effect of grazing on the old plantations was examined by taking three plots of 100 m2 in each case. In each selected plot, the seedling density was measured as well as the seedling total height.

Statistical analysis

All the results were analyzed by using ANOVA model (SPSS program) and the criterion Waller-Duncan was used for the comparison of the means. Distribution was tested for normality by Kolmogorov-Smirnov criterion and the homogeneity of variances was tested by Levene's test. The percentages were transformed to arsine square root values, before analysis [21, 22].

RESULTS

Stand conditions

The stands of *P. pinea* are even-aged and approximately 130 years old (Table 1). The stands density is very low, ranging from 40 to 140 trees per hectare. The average tree diameter ranged from 48.6 cm (in high degraded stands) to 57.8 cm (in mature open stands). Tree height also was found to be constant in the mature stands categories, reflecting the similar site conditions existing in the area occupied by *P. pinea* [6], while it was found lower in the high degraded stands.

Table 1. Stand characteristics. The values are means of the twenty plots.

	Mean	Min	Max
Tree diameter (cm)	52.6	30	110
Tree height (m)	15.8	14.5	24.5
Stand density (n/ha)	88.2	40	140
Tree age (years)	128.3	124	137

Cone and seed production

Cone production of *Pinus pinea* trees is very low, especially, in the high degraded stands and the closed mature stands (Table 2) which in combination with the very low tree density (Table 1) leads to an estimation of extremely low cone production of the stands. Number of filled seeds per cone is high, except for the case of high degraded stands where the number was 34.8%-44.0% lower. However, comparatively the seed production of *Pinus pinea* trees is greatly lower than that of *Pinus halepensis* trees which produce over 1000 cones per tree in the area.

Table 2. Cone and seed production of *P. pinea* stands in the Strofylia forest.

Stand type	Average cone production per tree	Number of filled seeds per cone
Closed mature	9.8	75.2
Open mature	62.7	87.5
High degraded	almost zero	49.0
Pinus halepensis	>1000	-

Seed quality

Seed germination behaviour

Pinus pinea seeds exhibited a high average germination rate 87.2% (Figures 1). Seed germination starts on the eighth day and is completed in four weeks while the greater percentage of seeds germinated in the period between 8th and 18th day (Figure 2).

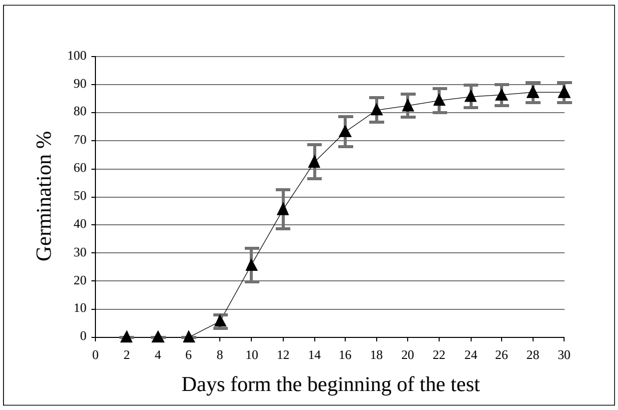

Fig. 1. Germination curve of *Pinus pinea* seeds of Strofylia forest in the laboratory conditions.

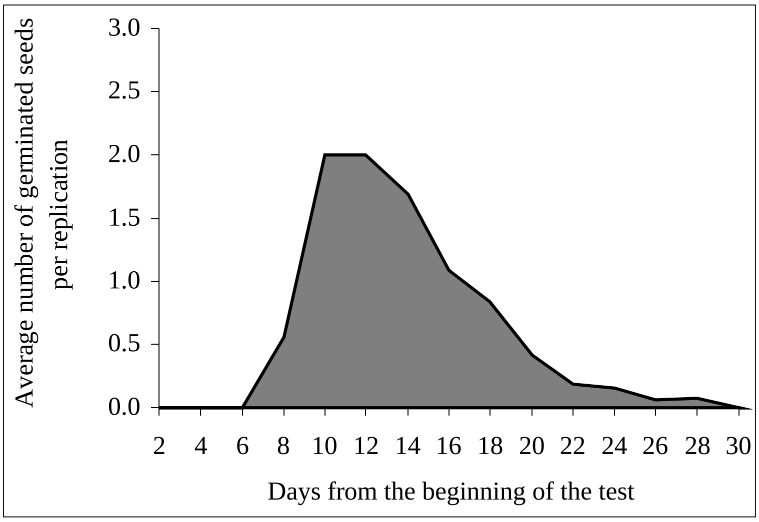

Fig. 2. Germination pattern of *Pinus pinea* seeds.

Light influence on seed germination behavior

No differences on seed germination of *P. pinea* were detected between the different light conditions (Figure 3). Final seed germination was high in all light treatments and varied from 87.2% to 100%. Seeds exposed to light for 30 min. daily exhibited the highest final germination percentage (100%) while seeds exposed to continuous light the lowest one (87.2%). The germination was completed within 30 days, for all light treatments; after this period no seed emergence was observed in any case. The first seed germination occurred 8 days after sowing in the treatments with light, while the seeds in darkness needed 10 days after sowing to start germination.

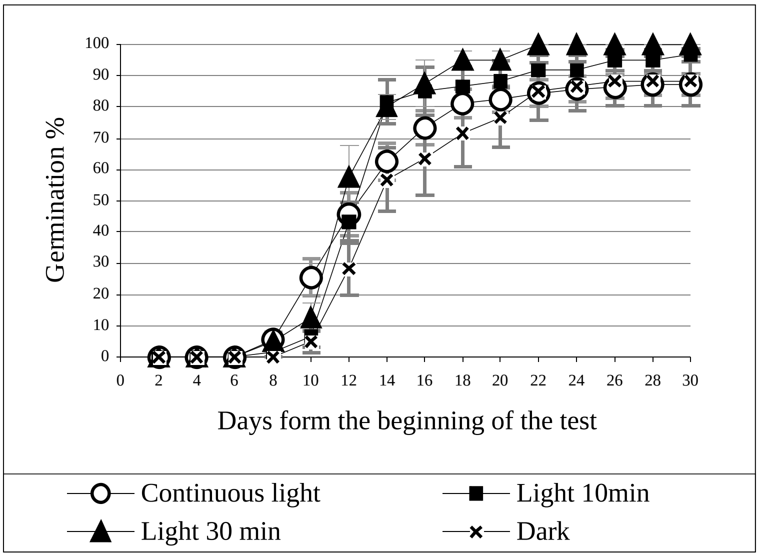

Fig. 3. Germination curves of *Pinus pinea* seeds under different light conditions.

Salinity effect on seed germination

High salt concentrations i.e. 0.05 M, 0.2M and 0.5M concentrations of NaCl, completely inhibited the seed germination. Only in very low NaCl concentration (0.02M) did *P. pinea* seeds exhibit 70% final germination percentage while the seeds treated with deionized water presented a significantly (P<0.01) higher percentage (87.2%) (Figure 4). The germination was completed in 30 days and after this period no seedling emergence was observed in any case. The first germination occurred 4 days after sowing in the NaCl (0.02M) treatment and 8 days in the deionized water.

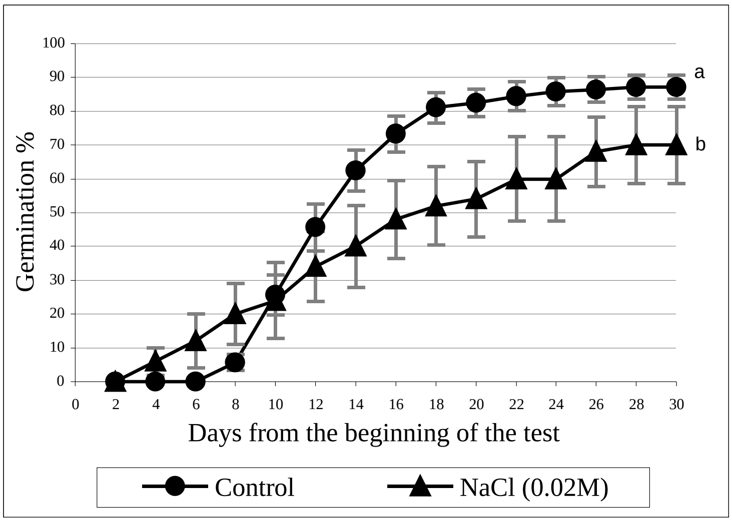

Fig. 4. Germination percentage as a function of time of *P. pinea* seeds in deionized water (control) and in 0.02M NaCl solution. Values followed by different letters are significantly different ($P<0.05$).

In situ seeding results

An average 0.7 seeds per patch germinated in spring that corresponds to 25% real germination (at the seedling stage). After germination, a satisfactory number of germinants (48%) managed to survive during the summer drought. Thus, from a practical point of view, seed germination capacity (seed quality) is not the factor to which the absence of species regeneration could be attributed, since the seeds germinate in the laboratory (and under different light conditions) as well as in the field under the ambient conditions.

Plantation results

Survival of the planted seedlings is extremely high (in most cases 100%) and seedling growth is satisfactory (Table 3). Concerning the seedling stress that cause the seedling mortality, based on the available data the mortality of both planted and naturally regenerated seedlings was observed only during the summer and mainly during the first summer of seedlings' life. Seedling death can be attributed to drought because soil water was only 2% (v/v) in many cases during the summer (Figure 5), and the predawn water potential of the seedlings was high, ranging from 1.2-1.8 MPa, during the summer.

Table 3. Field performance of the planted seedlings of *P. pinea* in the Strofylia forest.

Year of planting	Mean survival rate %	Mean annual height growth, in cm
2001	98.4	15.8
2004	94.8	7.6

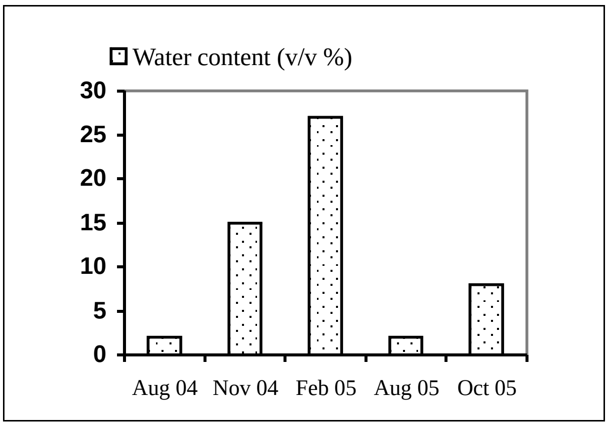

Fig. 5. Course of water content during the year in the upper 20 cm of the soil (TDR Methodology).

Grazing effect

Grazing greatly damages the existing seedlings, either directly by eating them or by injuring them during the livestock's passage through the forest (there are many field observations but no available quantitative data). It also negatively affects natural regeneration of the species, indirectly by increasing soil compaction and destroying soil porosity and in the long term view by changing the floristic composition of the stands. However, quantitative data for the above effect is not yet available.

However, based on the only field quantitative measurements on planted seedlings, it was found that the survival of *P. pinea* seedlings was much higher in the fenced areas (98.4%) while the average survival in the unfenced plantations ranged from between 32% to 50.5% depending on the grazing intensity.

DISCUSSION

Cone and seed production

The data analysis shows that seed production by the stands of *P. pinea* in the Strofylia forest is very low and in some cases extremely low; this causes a great problem for the species conservation on a long term scale. The available amount of species seeds is very low and it seems unable to secure species regeneration. In addition, comparatively the seed production of the species is greatly lower than that of *Pinus halepensis* trees, a competitive species for *P. pinea*, which produce over 1000 cones per tree. This comparison, in combination with the existence of a long and functional wing in *P. halepensis* seeds, suggests that this species will invade the biotope of *P. Pinea* and restrict the species habitat. A phenomenon that it is already taking place in the area, as well as in Shinias Marathonas [7] and elsewhere [9].

Seed quality

In contrast, the obtained laboratory results as well as the in situ seedling show that seed germination of *P. pinea* is high (87.2-100%) and does not depend on the time seeds are exposed to light. The high germination in the dark (88.3%) indicates that *P. pinea* seeds can germinate below the soil or without light induction. The results agree with the general aspect of Kozlowski [23] who notes that the seeds of most temperate-zone woody plants do not have a rigid light intensity requirement for germination, but a few do; for example, seeds of *Pinus* require very low illumination for germination. However, in earlier studies Skordilis and Thanos [17] in Attica, Greece, and Escudero et al. [24] in Spain, found that the germination of *P. pinea* seeds under dark conditions was lower. It is possible that the origin of the seeds or the time collection and the store conditions may explain these differences. The collected data confirm that light does not seem to be the abiotic factor controlling final seed germination percentage of *P. pinea*. However, the ability of seeds to germinate in the dark does not presuppose that the close canopy creates equally favorable conditions as the open canopy. From the findings, it can be suggested that *P. pinea* can more readily establish seedlings after canopy disturbance. Thus, the distribution of canopy trees after harvest should be a major consideration in harvest planning because of the strong negative influence of close canopy on the growth rate of seedlings [25].

Salinity negatively affected *P. pinea* seed germination. NaCl concentrations at 0.05M and above completely inhibited seed germination. Interestingly, in 0.02M NaCl concentration, *P. pinea* showed 70% seedling emergence but this was significantly lower than that of the control (87.2%). The reduction of *P. pinea* seedling emergence may be due to the inability of the seed to overcome the external osmotic potential and take up water for embryo expansion [26] because the hard seed coat becomes harder in the presence of salt. The effect of osmotic constraints on germination has been reported for many species, where the salinity inhibited germination by limiting water uptake and postponed initiation of germination processes [27, 28, 29]. However, plant species differ in their sensitivity or tolerance to salts; for example, the salt treatments affected the emergence of *Picea mariana* and *Picea glauca* more adversely and there was no seed germination in both species in 0.25M NaCl solution.

Regeneration in the field

The satisfactory seed germination in the field confirms the high physiological capacity of the seeds to germinate under the ambient conditions, regardless of the specific site conditions. Also, the young seedlings perform quite well and manage to survive in a satisfactory percentage (approximately 50%) during the summer drought. The good field performance of the planted seedlings also shows that seedling growth in the field is guaranteed if their growth is not prohibited by external reasons such as human actions or grazing. However, the problem of the regeneration absence can be mainly attributed to the low seed production of the stands.

On the contrary, grazing greatly damages the existed seedlings, either directly by eating them or by injuring them during the livestock' passage, and it contributes to regeneration absence. It also affects negatively natural regeneration of the species, indirectly by increasing soil compaction and destroying soil porosity and in the long term view by changing the floristic composition of the stands. The planted seedlings are also extremely vulnerable to the negative influence of grazing. The unfenced plantations exhibit very low results, while, in the case of the fenced plantations the seedling performance is extremely good and thus can contribute to habitat conservation of the species.

However, silvicultural measures are urgently needed in order to improve stand structure and consequently seed production which in turn will contribute to species regeneration [30]. This species regeneration can restrict the invasion of *Pinus halepensis* in the species habitat resulting in habitat conservation in the area.

CONCLUSIONS

Based on the results analysis the following conclusions can be extracted for *Pinus pinea* behaviour in the Strofylia forest, southern Greece:

Seed production of the stands is very low, especially in the case of high degraded and closed stands.

Seed quality is high and the seeds germinate under different light conditions as well as in the field while facing difficulties with high salt concentrations.

Seedlings growth is satisfactory in the field if the seedlings are protected from animal and human actions.

Artificial establishment of *Pinus pinea* is easy in the area.

Silvicultural measures are urgently needed to improve stand structure and seed production, to regenerate the stands and to stop the invasion of *Pinus halepensis.*

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