Effects of Light/Darkness and Inhibitory Components on the Germination of *Pinus pinaster*

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Abstract

As with many other species of pine, the only possibility of regeneration for *Pinus pinaster* is from seed. The advantage of germination is that it increases the genetic variability and stability of populations. This species has an important natural recovery rate in the field after a wildfire. However, no germination or survival of seedlings under adult trees with a capacity for producing viable seeds has been seen to exist in the field. The hypothesis of this paper is that germination under the cover of the adult trees could be controlled by the following ecological factors: availability of light, inhibition by substances produced by the aboveground mass of the tree or inhibition produced by substances emitted by decomposing fallen litter. The effect of nutrient availability on germination was analysed. The germination percentages were high in all of the treatments carried out, with a positive effect appearing in the germination of leaf exudates and a lower percentage in the seeds left in total darkness. However, no significant differences are observed among the total germination percentages. The main difference between the effects of the different treatments appears in the mean time required to germinate: it was shortest in the seeds treated with leaf exudates and longest in the seeds left in total darkness.

Keywords: germination, *Pinus pinaster*, light/darkness, inhibitory components, nutrient effects

Introduction

Maritime pine (*Pinus pinaster* Ait.) is an important species in the Mediterranean basin and is found throughout the western range, from France to Morocco and from Portugal to Tunisia. Also, *P. pinaster* diverges significantly from the common origin in different races in the Iberian Peninsula. The Iberian populations belong to 7 of these geographical races (Salvador et al. 2000). This indicates the extremely wide variety of ecological conditions under which they can develop - soils ranging from calcareous to sandy and climates from Mediterranean to Atlantic - resulting in specific adaptations of the species in terms of growth pattern and survival (Alía et al. 1995). For this reason it is difficult to extrapolate the results from research in different areas.

The studied population is in NW Spain and has especially adapted to frequent disturbances such as fire. Amongst these adaptations the capacity to bloom and bear fruit at an early stage in addition to possessing an aerial seed bank and being capable of storing fertile seeds in the closed cones for up to 50 years need to be emphasized (Tapias et al. 1998). *Pinus pinaster* is an obligate seeder (Martínez Sánchez et al. 1995). The advantage of germination is that it increases the genetic variability and stability of the populations (Baskin and Baskin, 1998). One of the observations made in the field is that no seedlings appear under adult populations of *Pinus pinaster* as a result of germination. This has had an influence on studying the possible hypotheses which identify the factors influencing the low rate of population recruitment in the field in a species like this with a high yield of seeds of great viability over long periods of time (Velez 2000).

As occurs in other Mediterranean species (Williamson 1990; Gallet 1994; Pellissier 1994) natural regeneration can be limited by the presence of determined chemical substances which act as germination inhibitors. In many other species the inhibitory effect of exudates produced by leaves on the germination of their own seeds has been demonstrated (Chaves et al. 1997; Li-Jun et al. 1997; Peñuelas and Llusiá 1998, Robles et al. 1999). López Mosquera and Guillen (1993) recorded the presence of allelopathic substances in *Pinus pinaster* both in the bark, where it acts as an inhibitor of various herbaceous species, and in the leaves. Similarly Fernández de Simón et al. (2001) have defined the variation in the composition of the terpenes and different acids of the *Pinus pinaster* acicules. These substances could be considered responsible for seed germination inhibition in mature forests. Gallet and Pellissier (1997) also showed that determined phenol compounds, which inhibit germination, are also persistent in the humus of forests limiting growth and germination at the fallen leaf and humus layer level. Pellissier (1994) documented how the germination and growth of seedlings of some conifers like *Picea abies* are inhibited by the phenol compounds of the humus. Thus, this could partially explain the natural regeneration difficulties of these species in the absence of fire, since fire would eliminate those substances and allow germination.

Another possible effect on germination may be determined by nutrient availability, which has been shown to have a positive effect on the germination and growth of different conifers (Ferm et al. 1992). This could explain the higher germination in the field in burned areas, since the ashes would act as a source of nutrients in a readily assimilated form, whilst the unburned areas would have lower nutrient contributions.

Our intention was to contrast the hypothesis that natural regeneration, under the canopy of mature *Pinus pinaster*, is controlled either by abiotic factors, like availability of light or by the presence of inhibitory substances from the exudates of leaves or the remains of decomposition. We also tried to determine the effect of nutrient availability on germination.

Materials and methods

The biological materials used in this study were seeds of *Pinus pinaster* Aiton. The seeds were collected in a *Pinus pinaster* stand situated in the Sierra del Teleno, SW León province (M.T.U.29TQG2984), at an approximate altitude of 1100 m. The climate is Mediterranean with 2-3 months' summer dryness and annual precipitation of 650 to 900 mm. (Ministerio de Agricultura 1980).

The study area is a natural *Pinus pinaster* stand with the total size of the original wood being 11,500 ha and its anatomical and physiological characteristics distinguish it from any other natural population of the species in the Iberian Peninsula.

Seeds were collected in July 2000, coinciding with the dispersion period of these species. The seeds were stored in open paper bags, which permitted ventilation, and at laboratory temperature in a dry place until they were used. *Pinus pinaster* leaves and litter used in the treatments of autotoxicity were collected from the *Pinus pinaster* population, located in the Sierra de Teleno.

In order to discover the effect of light/dark, inhibitory components and nutrient availability on germination, a method widely used by various authors (Trabaud and Casal 1989; Tárrega et al. 1992) was employed. A total of 10 seeds per treatment were placed on Petri dishes, thus producing 5 replicates / treatment. The treatments were:

Complete darkness, the Petri dishes were completely covered so that no light could enter and were watered with demineralized water;

Effect of leaf exudates: the seeds were watered with an aqueous extract obtained by macerating *Pinus pinaster* leaves for 48 hours.

Effect of humus exudates: the seeds were watered with an aqueous extract of humus obtained by macerating humus for 48 hours.

Effect of nutrients on germination: the seeds were watered with a solution of ashes, which represents the nutrient contribution to the seeds. Ash was obtained by burning leaves and thin branches that were colleted from the same population. The quantities of ash were (0.5 g/l; 1 g/l; 1.5 g/l) and were based on the amounts of ash/m2 collected by Soto (1993) after controlled fires.

Control: the seeds were watered with demineralized water.

The dishes were placed in a controlled environment cabinet at a temperature of 20ºC ± 1ºC with photoperiods of 15 hours' light/9 hours' dark. A temperature of 20ºC was used, as in other germination studies varying between 20ºC and 23ºC (Trabaud and Oustric 1989). The seeds were examined every day. A seed was considered to have germinated when the radicle could be seen with the naked eye (Côme 1970). The experiment was continued in this way for two months.

The average time for germination was also estimated using the expression:

$$t_m = \frac{N_1 T_1 + N_2 \cdots + N_n T_n}{N_1 + N_2 \cdots + N_n}$$

Where N_1 is the number of seeds which have germinated between time T_1 and T_2, and so on (Côme, 1970).

Before carrying out the previously mentioned treatments a viability test was also carried out on a sample of 100 seeds from the same population. The Tetrazolium test (Besnier Romero 1989) was used for the viability analysis.

Data analysis

The results of germination were statistically analysed by analysis of variance to determine the significance of the differences found between the treatments applied to the seeds. The Scheffe test (1959) was used to detect any significant differences (α=0.05) in the comparison between the pairs of treatments. Prior to this the sampling normality was checked by the David test (David et al. 1954) and the homogeneity of the variances by the Cochran test (Cochran 1941).

Results

The viability percentages of the seeds of this *Pinus pinaster* were 100%, which means that all the seeds collected in the field are able to germinate and that the possible delays in germination may be due to the existence of factors extrinsic to the seed itself.

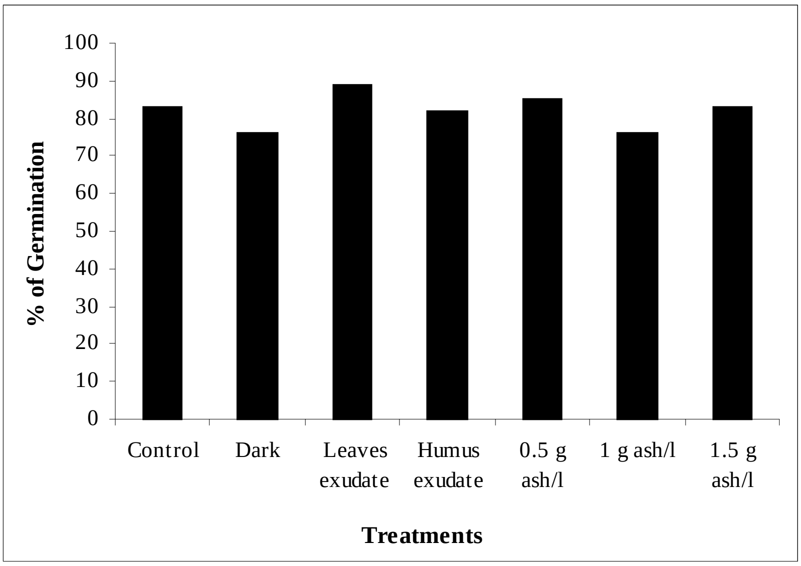

Figure 1. Germination percentages of *Pinus pinaster* seeds in the control situation and after different treatments: (Darkness, Inhibitory effects of leaf exudates; Inhibitory effects of humus exudates, and effects of three different amounts of ash (0.5 g/l; 1 g/l, 1.5 g/ l).

The total germinated *Pinus pinaster* seeds (Fig. 1) subjected to different treatments shows that there are high germination percentages in all the treatments: over 70% in all cases. The seeds watered with leaf exudate present the highest germination percentage and those watered with nutrient concentrations of 1 gram of ash per litre have the lowest germination percentages. However, no significant differences can be observed among the germination percentages in any of the treatments (Table I).

Table I. Results of analysis of variance to compare differences in percentage of germination of *Pinus pinaster* among different treatments.

Source	DF	Sum of Squares	F-test
Between groups	6	22.57	0.554
Within groups	28	190	P=0.762
Total	34	212.57	

Temporal distribution of germination (Fig. 2) shows that control, leaf exudates and humus exudates have very similar germination patterns. The first germination occurs 4 days after sowing in the leaf exudate treatment. In contrast the seeds kept in total darkness need at least 10 days to start to germinate. The control as well as those watered with humus exudates start after 7 days and those watered with different ash concentrations require 8 days. Another difference recorded among them is the maximum germination peak. This appears after 12 days in the control, those watered with leaf and humus exudates and at the opposite end are those kept in complete darkness. The seeds watered with different concentrations of ash are in an intermediate situation, as they require 15 days to reach the maximum germination peak. The most notable germination peak appears in the control situation. The germination of this species under the treatments to which they were subjected shows a certain irregularity, as different germination peaks appear in the different treatments throughout the study period (60 days). The treatments with the highest ash concentrations (1 g ash/l and 1.5 ash/l) showed the most marked peaks of germination in time.

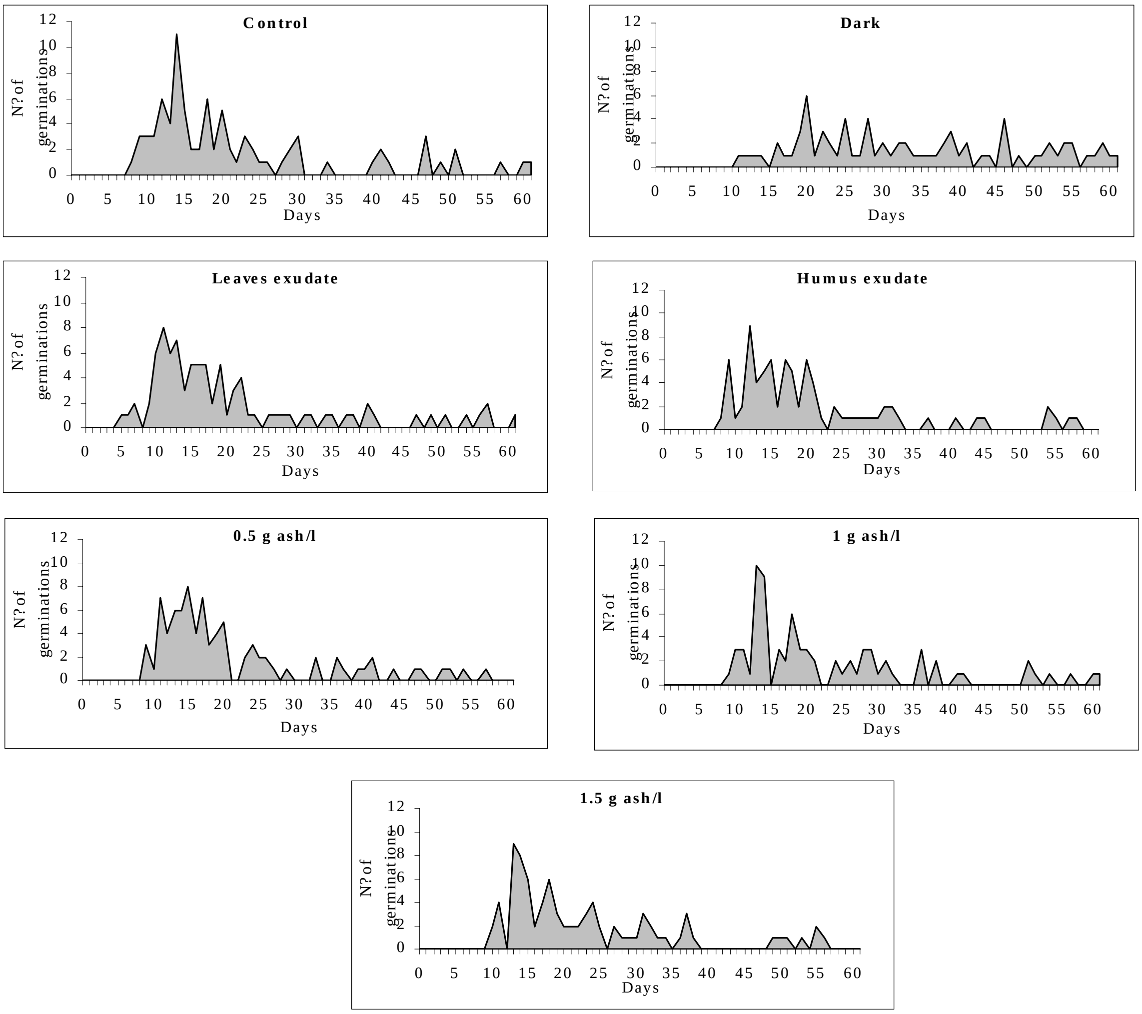

Figure 2. Distribution of germination times of *Pinus pinaster* in each treatment (Darkness, Inhibitory effects of leaf exudates; Inhibitory effects of humus exudates, and effects of three different amounts of ash (0.5 g/l; 1 g/l, 1.5 g/ l).

These results are complemented by the analysis of the mean germination times (Table II). The lowest mean germination time corresponds to the lowest concentration of ash treatment (0.5 g ash/l) (20.17 ± 4.43 days) followed by leaf exudate (20.96 ± 1.85 days). The treatment, which delayed germination most, was darkness (33.95 ± 6.02). Significant statistical differences were found among the mean germination times in the different treatments (Table III). The Scheffe test showed that the treatments responsible for these differences were darkness, with the longest germination time, and the rest of the treatments. No significant statistical differences were found among the rest of treatments.

Table II. Mean germination time (days) of the *Pinus pinaster* seeds for each of the treatments.

Treatments	**Mean**	**Standard deviation**
Control	21.8	2.25
Darkness	33.95	6.02
Leaf exudates	20.96	1.85
Humus exudates	21.17	3.56
0.5 g ash/l	20.17	4.43
1 g ash/l	22.76	3.78
1.5 g ash/l	22.20	3.38

Table III. Results of analysis of variance to compare differences in the mean germination time in the treatments.

Source	DF	Sum of Squares	F-test
Between groups	6	684.7	7.79
Within groups	28	411.3	P=0.0001
Total	34	1095.9	

Discussion

The germination percentage (83% in the control situation) of the *Pinus pinaster* population in León province is higher than the percentages given by Reyes and Casal (1995), who used *Pinus pinaster* seeds from the north of the Iberian peninsula, and recorded control situation percentages of 58%. However, in spite of the high germination percentage obtained in the laboratory and observed in the field after a fire (Luis et al. 2001), it is difficult to find pine seedlings in the undergrowth of a mature population. One possible explanation for this is that the amount of light reaching the soil surface, where the seeds are, is small due to the forest canopy effect. It has, nevertheless, been demonstrated that the seeds in this *Pinus pinaster* population are capable of germinating in the laboratory in conditions of complete darkness. Therefore, in this case light does not seem to be the abiotic factor controlling germination, in contrast to what occurs in other species (González 1993).

Various species present in the Mediterranean basin produce chemical substances which inhibit the germination of seeds of other species or their own (Reigosa et al. 1999). Many shrub species such as *Cistus albidus* (Robles et al. 1999), *Cistus ladanifer* (Chaves et al. 1997), *Thymus vulgaris* (Tarayre et al. 1995), *Lavandula stoechas* (Vokou 1992) show this effect. The inhibitory effect of arboreal species like *Quercus pubescens* (Hubert and Boglio 1989) and *Quercus ilex* (Li-Jun et al. 1997) has also been documented.

Other coniferous species have also been found capable of producing allelopathic substances, as occurs in the case of *Pinus halepensis*, which emits a considerable amount of terpenes (Peñuelas and Llusiá 1998), *Picea abies*, which emits phenol compounds from the green acicules to the soil solutions (Gallet and Pellissier 1997) and *Pinus pinaster*, which presents different amounts of terpenes and resinous acids in its acicules and bark (Fernández de Simón et al., 2001). These compounds act as powerful agents, which inhibit germination and the growth of some herbaceous species when found in the substratum (López-Mosquera 1993). Although this species is known to produce inhibitory substances in the field, it was observed that germination was not significantly inhibited in the laboratory when watered with the aqueous extract of acicules and humus. Therefore in principle it seems that, if these chemical compounds exist in the extract, they do not inhibit germination of seeds of their own species.

Likewise, the opposite effect, that is germination stimulation, could be conditioned by a high nutrient content such as that supplied by ashes. In this case of *Pinus pinaster*, germination does not seem to be affected by the different ash treatments carried out and only reaches a percentage slightly above that of the control in the case of the 0.5 g l^{-1} treatment, although the differences are not significant. Reyes and Casal (1998) recorded something similar in studies carried out on *Pinus pinaster*. Rather than a beneficial effect with high concentrations of ash, although this means a nutrient contribution, environment pH is increased considerably and this affects germination negatively.

It can be deduced from the results found that germination of the *Pinus pinaster* seeds in this population is not inhibited by factors such as light or substances emitted by their leaves or present in the humus. This induces us to think that the actions of herbivores consuming seeds of pines could be one of the causes of the reduction in the number of seeds and therefore of the non-presence of seedlings in the field. We also need to determine whether the seeds produced by adult trees penetrate the litter layer of the undergrowth and this makes epigeal growth of seedlings difficult in the field.

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