INFLUENCE OF HIGH TEMPERATURES ON SEED GERMINATION OF A SPECIAL *Pinus pinaster* STAND ADAPTED TO FREQUENT FIRES.

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Abstract

This study examines the effect of fire on the germination of *Pinus pinaster* seeds from a population with special adaptations to this type of disturbance, due to the high frequency of fires to which they have been subjected. The action of fire was simulated in the laboratory using thermal shocks. Temperatures of 60, 90, 120, 200 and 300 ºC were used for exposure times of 1 and 5 min. The viability of seeds of this species from the soil seed bank of a population subjected to a natural fire was also evaluated. The results show that germination is not increase by a thermal treatment. The effect is negative at temperatures above 90ºC and exposure times of 1 min. with a significant decrease in, or even no, germination. Therefore the seeds cannot stand high temperatures and the reproductive effort is placed on serotinous cone development and the production of high numbers of seeds. The high viability of the seeds from the area burned by natural fires, immediately after the fire and one year later, show the soil seed bank high potentiality to participate in the regeneration of the burned area.

Introduction

Recurring fires are the most significant disturbances which the vegetation in the Mediterranean region is subjected to (Tárrega & Luis 1992). Over the last few decades more than 200000 ha., 41.2% of which were woods, have been burned annually by fires in Spain (Pérez & Moreno 1998). Two factors characterize the strength of a fire: the duration of the burn and the temperature reached (Reyes & Casal 1995).

Pinus pinaster stands are amongst the communities, which frequently suffer the passage of fire, are *Pinus pinaster* stands. According to Trabaud & Campant (1991) pine communities are more inflammable than isolated specimens of this species. The litter of these communities is usually inflammable during dry periods, producing flames, which can reach the upper vegetation strata. The effects of the fires on coniferous forest ecosystems have been widely studied by various authors (Naveh 1974; Castro *et al.* 1990; Archibold 1989, Trabaud & Campant 1991; Thanos & Marcou 1991; Martínez Sánchez 1994; Pérez & Moreno 1998).

The recovery rate and the post-fire composition of the burned communities are determined by the nature of the original cover and by the type and intensity of the fire, because these factors determine the size and vigour of the residual flora (Archibold 1979). According to Fenner (1995), in order to carry out an appropriate administration of the resources, it has become more and more necessary to model and predict the regeneration processes (Red *et al.* 1998)

In the case of *Pinus pinaster,* as in many other pine species, the only possibility of regeneration is from seed (Martínez Sánchez 1994). The advantage of germination is that it increases the genetic variability and stability of the populations (Baskin & Baskin 1998). However, obligate seeders are rare and at a disadvantage in comparison with species that can resprout vegetatively and so occupy the area more quickly (Naveh 1975; Trabaud 1987, Valbuena 1995). In the obligate seeders the seedlings reappearing after a fire come from the soil seed bank, by dispersion from neighbouring areas or from seeds held within cones in the canopy (serotiny) (Lamont *et al.* 1991). Many studies verify the influence of fire on seed germination in *Pinus* species (Castro *et al*. 1990; Martínez-Sánchez *et al*. 1995; Reyes & Casal 1995; Thanos & Marcou 1991).

The population studied is in an area which suffered from frequent fires. Tapias *et al*. (1998) indicate that in this mountain region, 30 fires were caused by lightning alone between 1978 and 1998. The magnitude of the problem, the historical importance and frequency of fires is evident from a summary of the most significant fires, which occurred in 1922 (8000 hectares were burnt), 1949 (400 hectares), 1979 (2000 hectares), 1991 (2000 hectares), 1993 (400 hectares), 1994 (700 hectares), 1997 (400 hectares) y 1998 (3000 hectares). The latter was classed as a large fire because more than 3000 ha. burned. It is estimated that two fires per year are started by lightning in this area. Because of these frequent fires, the mass of *Pinus pinaster* in this area has acquired a series of morphological and physiological characteristics that are absent from the nearest populations (150 km away). Amongst the characteristics which distinguish this group from other *P. pinaster* populations in the Iberian Peninsula, the population studied was classified as very serotinous (more than 80% of cones are serotinous). These serotinous cones accumulate in the crown of the trees and only open as a result of thermal increases (temperatures above 45ºC) (Tapias *et al*. 2001). Another important and distinctive characteristic of this population is precocious flower production, which can begin in 4 year old trees, although the production of fertile seeds starts between 10 and 15 years (Sanchez 1999). In addition, *Pinus pinaster* has a characteristic aerial seed bank, and is capable of storing fertile seeds in closed cones for up to 50 years (Tapias *et al*. 1998).

The aim of this study is to analyse the effect of the high temperatures produced during a fire on the germination of *Pinus pinaster* seeds belonging to a population with special adaptations to the recurring fires. This study will be complete with the analysis of the viability of the seeds present in the soil seed bank after a wildfire.

Materials and methods

The biological materials used in this study were seeds of *Pinus pinaster* Aiton. The seeds were collected in a natural *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 precipitations of 650 to 900 mm. (Ministerio de Agricultura, 1980). An interesting feature of the local climate is the frequency of dry storms and very low precipitation in spring (mean annual spring precipitation is 180mm) and summer, (mean annual summer precipitation is 14.65 mm) which often produces crow fires (Sánchez 1999).

Seeds were collected in July 2000, coinciding with the natural dispersal period of this species. Fruits were collected from different specimens throughout the stand of *Pinus pinaster*. The seeds were stored in open paper bags, which permitted ventilation, and at laboratory temperature in a dry place until they were used.

In order to determine the effect of fire on germination, a method widely used by various authors (Tárrega *et al.* 1992; Trabaud & Casal 1989) was employed. This method consists of exposing non-selected seeds to high temperatures for short periods of time in order to simulate the action of fire under conditions as natural as possible. According to Trabaud (1979), the heat in a fire operates on a concrete point for only a short period of time (between 5 and 15 min.) and the temperatures reached at 2.5 cm below the soil surface vary between 44º and 150º.

Based on these facts, we selected these combinations of temperature and exposure times in order to simulate fire action on the seeds: 60, 90, 120, 200 and 300 ºC for 1 min. and 5 min.; the seeds were heated in a hot air chamber.

Immediately after treatment the seeds were sown in 8.5 cm diameter Petri dishes on four layers of filter paper saturated with demineralized water. There were 6 replicates of 15 seeds for each treatment. These treatments were compared with another group of 6 replicates, which was not subjected to thermal shock. 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, following other germination studies where the temperature between 20ºC and 23ºC (Trabaud & Oustric 1989). Fungus attacks were avoided by using a fungicide (Benlate) at a very low concentration of 0.5g/l, which has been proven to have no effect on seed germination.

The seeds were examined every week. 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 17 weeks.

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).

In order to identify the viability of the *Pinus pinaster* seeds present in the soil seed bank, which have suffered a natural fire, and maintenance of this viability over time, three plots (B1, B2 and B3) of 30 x 10 metres established in the area affected by the 1998 fire. A control plot established in an unburned area. Ten 20x25 cm soil samples were randomly collected to a depth of 5 cm in each plot (B1, B2, B3 and control). Sampling was carried out immediately after the fire (1 month), before the first rains and one year later in order to examine the maintenance of viability over time. The direct method was used to obtain the seeds from the soil seed bank (Roberst 1981). The Tetrazolium test (Besnier Romero 1989) was used to assess the viability of the seeds collected from the plots.

Data analysis

The results of germination were assessed using an analysis of variance comparing the percentage of germination to the different treatments. Scheffe's test (1959) was used to detect any significant differences (α=0.05) in the comparison between the pairs of treatments. Percentage germination in each treatment in time was compared by a two-way (treatment and time, repeated measure) ANOVA. The significance of the results was assessed using Scheffe's test (1959). Prior to this, we checked sampling normality following David *et al*. (1954) and the homogeneity of the variances following Cochran (1941).

Results

According to the analyses and the different test, The germination results for the *Pinus pinaster* seeds allow three treatment groups to be differentiated (Fig. 1) with significant differences among them ($p<0.0001$). The first one consists of the control, with a percentage of 86.58%, 60ºC for 1 and 5 min. treatment and, lastly, the 90ºC-1 min. treatment with a recorded maximum of 88.82% germination. The percentages are very similar within this group and no significant differences are found among them.

A second group discriminate the 90ºC and 5 min treatment as an intermediate situation as regards percentage of germinated seeds (24.44%). There are significant differences between this group and the other two ($P>0.001$).

The third groups is formed by the highest temperature treatments: 120ºC, 200ºC and 300ºC and exposure times of 1 and 5 min. Independent of the exposure time, percentage germination in this group falls more markedly or is even zero. No significant differences are recorded among them.

In general, *Pinus pinaster* germination is not significantly affected by temperatures from 60ºC to 90ºC. However, when temperatures are higher than 90ºC and exposures times are elevated, it has a significant negative influence on germination

In relation to the speed of germination, we have evaluated the extent of variation in the percentage og germination over seventeen weeks (Fig. 2). Temporal patterns in germination after each thermal treatment show that the control, 60ºC-1 min., 60ºC- 5 min. and 90ºC-1 min. attain germination peak (approximately 40%) in the second week. From the second week, there is a there is a significant ($p < 0.0001$) decrease in germination. However, when either temperature or exposure time increase, maximum percentage of germination is lower than the first group (90ºC-5 min: 7.77 % in the sixth week) (Fig 2). It is obvious that treatments above 90ºC-5 min have a negative impact on germination, since there is either no germination, or only 1 seed germinates, as in the case of 120ºC-1min and 300ºC-1min.

Most treatments: control, 60ºC-1 min.-5 min.; 90ºC-1 min. and 120ºC-1 min., show an average germination time (Table I) of approximately three weeks. However, 90ºC-5 min, 120ºC-5min and 300ºC-1min attain the highest average germination times at more than 5 weeks.

The information provided by these results must be completed with that concerning what happens in the soil after a natural fire; this depends on the viability of the seeds existing in the soil. For this reason, we studied the viability of seeds collected from the natural soil seed bank after a wildfire. Immediately after a fire, the percentage viability of the seeds of this *Pinus pinaster* stand is very high, exceeding the 77% in the plots B1, B2 and B3 (Table II). As regards the control zone, the number of seeds found is very small (4 seeds), so the data obtained in the viability test are not representative of what happens in the soil seed bank and have not been considered in the results obtained as a whole.

These percentages decrease one year after the fire, showing important differences among areas. This reduction results from the germination of some seeds, which thus from the seed bank, or are eaten by herbivores. As a result, if there is no new addition to the seed bank, as occurred in zone B1, numbers decreased, as in B2 and B3. B1 maintains its viability at very high levels one year after the fire presumably because new seeds which were deposited by trees surviving a fire (personal observation) and which were viable. However the percentage viability in B2 and B3 one year after the fire, was significantly reduced. This way be because no new seeds were contributed by surviving trees where crow damage was high. In addition, it is possible that seeds present in the seed bank became less viable after one year in the soil.

Discussion

It is well established that the fire regime has important implications for plant community composition within ecosystems (Williams *et al*. 1994). As indicated by Hanley and Fenner (1998), the growth responses of seedling from seeds that have been subjected to thermal treatment may provide some clues as to how fire-adapted regeneration strategies influence the patterns of recruitment observed in post-fire plant communities. It this study area, no significant differences were observed between the germinations of seeds which were not subjected to heat treatment and those which received up to 90ºC-1 min., but higher temperatures damage the embryo and germination falls sharply. Therefore the temperature increase did not produce an increase in percentage germination. Similar results were obtained in other populations of the same species (Escudero *et al.* 1999; Martínez-Sanchez *et al.* 1995) and in other species of the genus *Pinus* (*P. halepensis* and *P. sylvestris*) from nearby areas (Nuñez & Calvo 2000). However, other authors, such as Reyes & Casal (1995), working with *P. pinaster* population in Galicia (NW Spain), did register differences between percentage germination of control (58%) and thermally treated seeds (approximately 35 %). However, the percentages reached in the Galicia study are very far from the 90 % attained in our study.

Martínez-Sanchez *et al.* (1995) state that the *Pinus pinaster* seeds from populations situated in the south of Spain are unlikely to be significantly affected at temperatures of up to 150ºC-1 min, and even register germinations at 200ºC-1 min. Escudero *et al* (1999) obtain identical results with seeds from the south of Spain. Reyes & Casal (1995) use seeds collected in the north of the peninsula and obtain germinations at temperatures of up to 150ºC. In contrast, in this *Pinus pinaster* stand studied here the temperature limit for germination was reduced to 90ºC-1 min; that is, these seeds had greater sensitivity to higher temperatures than any other population of the same species, despite having been subjected to frequent fires. Perhaps this population has adopted another strategy to adapt to the fires which consists of producing an abundant quantity of serotinous cones that remain closed in the tree, thus maintaining viability and ensuring survival of the species. As indicated by Hanley & Fenner (1998), a serotinous habit allows *Pinus* seeds to withstand high temperature or avoid its effects completely. Consequently, the seeds, as our results show, cannot stand high temperatures, and the reproductive effort goes into either cone serotiny or a high seed yield.

As regards the time required for germination to start, germinations was observed in this population from the first week after the thermal shock in those treatments which have no harmful effect on the seeds. At temperatures above 90ºC the time taken to start germination to start was longer. Similar results were observed by other authors (Escudero *et al.* 1999; Reyes & Casal 1995), but with the difference that all the seeds began to germinate at the same time independent of the thermal ranges. This supports the idea that this population is more sensitive to the thermal impacts.

The mean germination time was three weeks and was considered intermediate in comparison with those obtained by other authors: less than three weeks (Escudero *et al.* 1999; Reyes & Casal 1995) and longer periods in the studies carried out by Martínez Sanchez, *et al.*

(1995).

The heat to which seeds, either present in the soil or aerial bank, were subjected in the natural fire did not exceed 90ºC. This conclusion was arrived at by comparing the results of the viability of seeds from the area burned by natural fires and the results of experimental thermal shock.

If the seeds were in the soil, this is possible, as, as bibliographical data given by Trabaud (1979) and Marcos (1997) show that the soil temperature does not exceed 70ºC during a fire. If the seeds were in the aerial bank, the serotinous cones protected the pine seeds from high temperatures. The protective value of coniferous cones was demonstrated by Fraver (1994). A temperature increase is necessary to make the cones open (Francelet 1970). This increase can be attained during maximum summer and sprig temperatures in these latitudes; when natural dissemination occurs (Abbas *et al.* 1984; Catalán 1985; Francelet 1970; Loisel 1966; Vega 1977). *Pinus pinaster* does not need excessively high temperatures to make the cones open their bracts and disseminate the seeds from inside: an estimated at about 45ºCmay be sufficient (Tapia *et al* 1998).

The population belonging to the Sierra del Teleno presents some morphological and physiological adaptations that could have arisen from the particular environmental conditions which occur in the area, namely the frequent recurrence of fires. Among these adaptations, the most remarkable may be the existence of serotinous cones, which protect the seeds from the devastating effects of fire. This, together with the fact that that a high percentage of germination occurs very early in the life of the trees, allows us to predict that natural regeneration in this area will not suffer the problems found by Castro *et al.* (1990) in Portugal or Escudero *et al.* (1999) in south of Spain. As a result of these special adaptations, the thermal resistance of this population to the high temperatures is lower than that which other populations in the Iberian peninsula can withstand. Another special adaptation presented in this population is the appearance of fertile cones 8 years after the fire (unpublished data). This is not unique, since other researchers have recorded flowering in trees less than 6 years, but in conditions of reduced intraspecies or interspecies competition shrubs (Tapias *et al*. 2001).

In conclusion, the seeds of the *Pinus pinaster* stand studied show greater sensitivity to high temperatures than any other population of the same species. This population may have adopted an alternative strategy to adapt to fires, which consists of producing an abundant quantity of serotinous cones that remain closed in the tree, so maintaining viability and ensuring the survival of the species. As a result of this adaptation, post fire regeneration will be conditioned more by the presence of viable seeds in the soil seed bank that come from the aerial seed bank than by the high temperatures; that is to say, the cones which open as a result of temperatures reached during the fire. The density of seeds present in the soil is sufficient to ensure natural regeneration in this area during the first months after a fire. One year after the fire, the presence of seedlings is only possible if burnt trees in the area continue to contribute viable seeds to the ground.

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Figure legends

Fig. 1.- Percentage Germination of *Pinus pinaster* seeds in the original situation and after thermal shock treatments: 60, 90, 120, 200, 300ºC and exposure times of 1 and 5 min. different letters indicate significant differences between treatments.

Fig. 2.- Distribution of germination times of *Pinus pinaster* according temperatures (ºC) and times (minutes

Table 1.- Germination average time (weeks)

	Contro l	60º-1min	60º-5min	90º-1min	90º-5min	120º-1min	120º-5min	200º-1min	200º-5min	300º-1min	300º-5 min
Germination average time (weeks)	3.35	3.18	3.47	3.01	5.77	3.25	8	0	0	5.67	0

Table 2. Percentage of seed viability of seeds from the burnt plots (B1, B2 and B3) and the number of seeds found per square metre.

	B1	**B2**	**B3**
Time 0 After fire	85.39%	77.58%	84.78%
Seeds/m²	246 ± 31.13	202 ± 32.58	188 ± 30.32
Time 1 One year after fire	88.23%	27.77%	50%
Seeds/m²	432 ± 79.12	158 ± 24.49	136 ± 23.63

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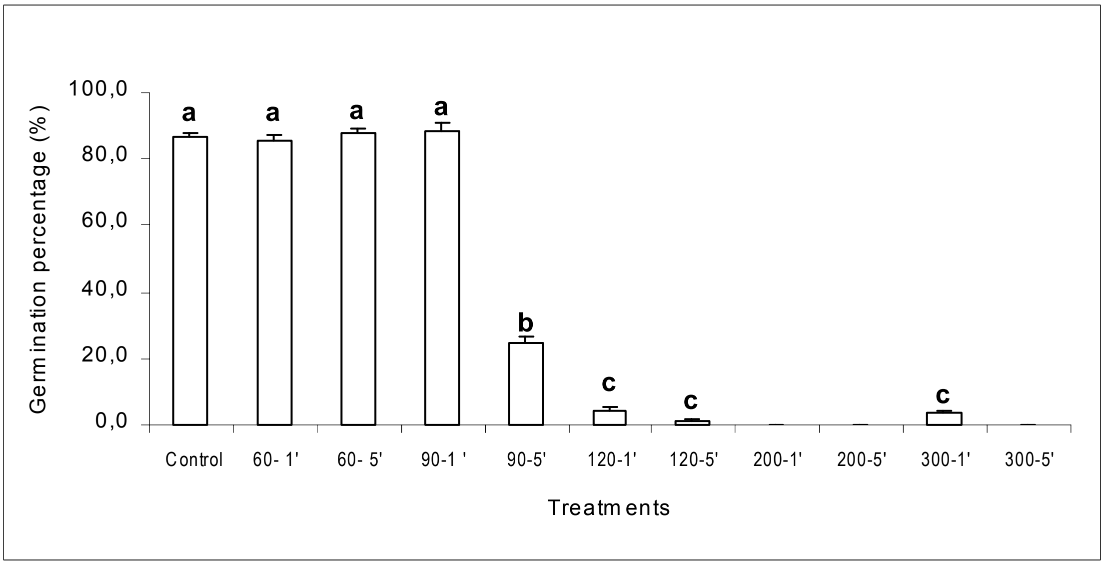

Fig. 1.-

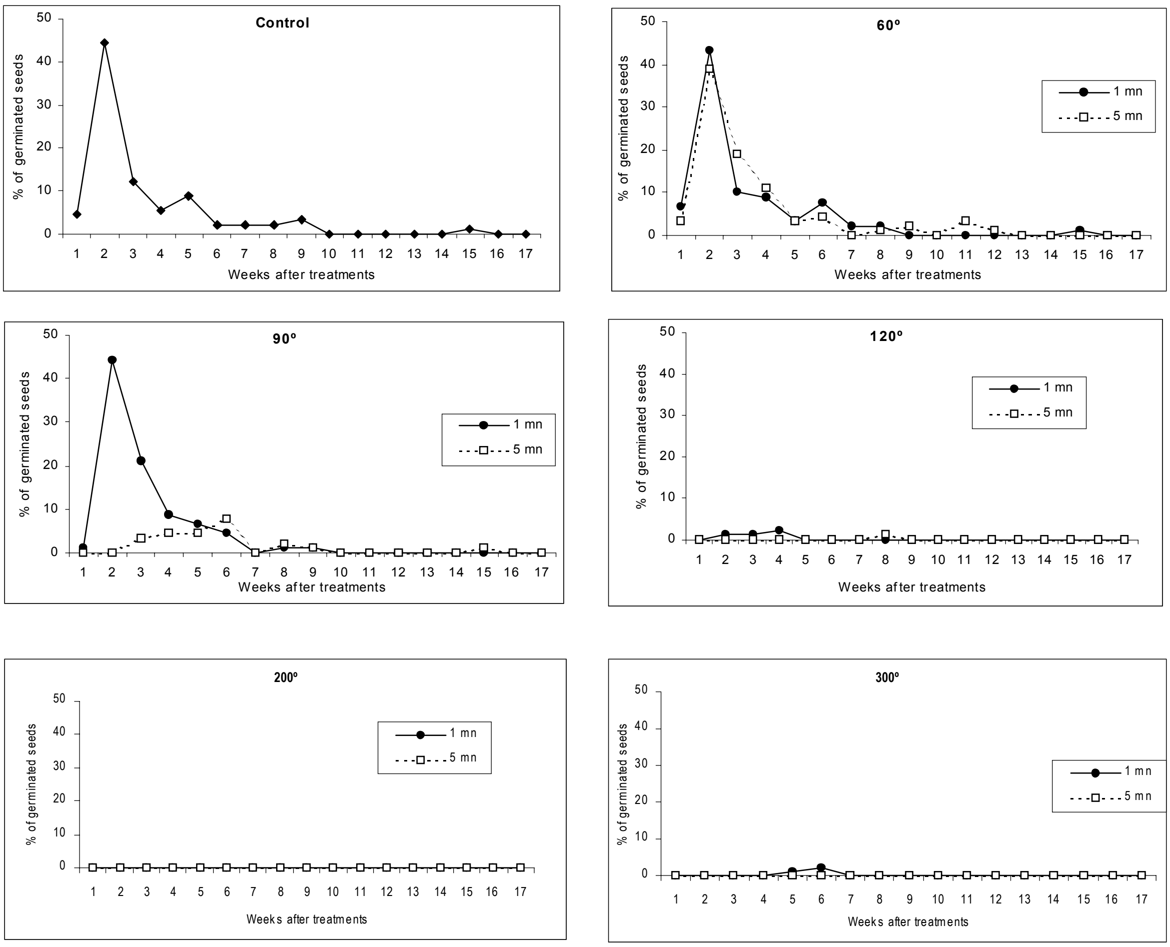

Fig. 2.-