The influence of seed age on germinative response to the effects of fire in *Pinus pinaster*, *Pinus radiata* and *Eucalyptus globulus*

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Abstract – The aim of this study was to understand the germinative response to fire of three species of seeds of different ages. An experiment was designed in which mature seeds of *Pinus pinaster*, *P. radiata* and *Eucalyptus globulus* were matured and stored in four consecutive years (1990, 1991, 1992 and 1993) and then subjected to high temperatures, the addition of ash, and both factors together. A control treatment for the seeds of each age and species was also performed. Significant differences were observed between the species. The germinative behaviour of seeds subjected to different factors involved in forest fires varied according to the age or the year of collection. This variation was more pronounced in some species than in others. For *E. globulus* the germination rate followed biannual cycles in which a year with low values followed a year with high values. In nearly all the species, sensitivity to factors related to forest fires increased with age.

**P. pinaster** / **P. radiata** / **E. globulus** / germination / fire / seed age

1. INTRODUCTION

Forest fires are one of the most serious environmental problems in Galicia. Of the total surface area (2,926,23 ha), two thirds are dedicated to shrublands and woodlands. In this region, between 1970 and 1999, 71% of these areas were burned. Within the wooded areas, the problem of fires particularly affects populations of the three species studied, as they are both the most abundant and the most combustible species in the region.

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In both pines and eucalyptus, the seeds are found within hard structures (cones in the case of pines and capsules in eucalyptus) which protect them from predators, fungi and pests, as well as from fire. The period of time in which the mature seeds remain enclosed in these cones or capsules depends on the species, the environmental conditions in which they grow and their degree of serotinility. Many studies have described several species of Pinus and Eucalyptus as serotinous [1, 2, 3, 4, 9, 10, 12, 14, 19, 28, 30, 33, 42, 46], although the degree of serotinility is not a constant characteristic. It can vary from one population to another and between individuals in the same population, even to the point where a non-serotinous tree may become serotinous as it gets older [30].

Once they have been liberated, the viability of the seeds depends to an important degree on the conditions where they are found [5, 29]. On the ground, both pine and eucalyptus seeds are quickly detected by seed gatherers or attacked by ground-dwelling fungi or microorganisms. In good storage conditions, P. pinaster seeds remain viable for 11 years, P. radiata for 21 years [7] and E. globulus for 10 years [39].

It is recognised that the germinative capacity of Pinus and Eucalyptus seeds varies over a period of time depending, above all, on the age of the seed and environmental conditions. The effect of fire on the germinative behaviour of stored seeds, whether in a canopy seed bank or in laboratory conditions, has not been studied a great deal in the past. This study was designed to define the germinative behaviour of the seeds of P. pinaster, P. radiata and E. globulus after storage for different periods of time, when subjected to some of the most important effects of forest fires. The degree that their response to the effects of fire may be influenced by the age of the seed was also considered to be of interest.

2. MATERIALS AND METHODS

For this experiment, seeds of P. pinaster, P. radiata and E. globulus were obtained from plantations in Galicia in the years 1990, 1991, 1992 and 1993. The fruit was collected and seeds obtained between the end of summer and the beginning of autumn. The seeds were stored until 1994, at laboratory room temperature in well-ventilated conditions. Therefore, when the germination test was carried out the seeds had been in storage for between one and four years. As the method of collection and selection of the seeds used the same criteria, it is assumed that the seeds of each species were of the same age when collected and stored.

The fire factors tested in this experiment were those of thermal shock and ash. Four treatments were chosen: a control treatment, a thermal treatment at 90 °C for 5 minutes, a treatment with an ash concentration of 1 g L⁻¹ in sprinklers, and a combined treatment in which the seeds were subjected to thermal shock and watering with ash solution. These four treatments were applied to all of the groups of seeds collected in the 4 years mentioned. All of the treatments consisted of six replicates of 30 seeds each, using the methodology of previous studies [36, 37, 38].

Placing the seeds in a hot air oven for 5 minutes performed the thermal shock treatment. The temperature was previously selected and checked for constancy. Only one replicate of each species was introduced at a time. In this way, a breakdown in the apparatus or its handling would not cause repercussions in all of the replicates, and lead to erroneous results.

The ash was obtained by complete combustion (for approximately 20 minutes) of dry material (mainly small branches and leaves) from each of the species studied. The seeds from each species were only treated with ash produced from biomass obtained from individuals of their own species. The ash treatment was obtained by diluting the corresponding amount of ash in distilled water.

The temperature and time periods selected, as well as the ash treatment used in this experiment are similar to those used in other studies that examine the effect of forest fires on the germination of different species [21, 22, 28, 31, 35, 37, 38, 41, 43, 44, 45].

The seeds were sown under laboratory conditions (20–22 °C) on the 4th of May 1994, in 9-cm diameter Petri dishes on a double layer of filter paper. A count was made every two days until the germination process was completed after a total of 46 days. It was considered that a seed had germinated when the radicle protruded from the teguments by one or more millimetres [11]. Each germinated seed was removed in order to avoid confusion in the count. The seeds were periodically watered, either with distilled water or with the ash solution according to the treatment, to keep them moist at all times.

The average germination time, as well as the percentage of germination, was also calculated (tₘ). This parameter was calculated using the equation:

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tₘ = \frac{N₁T₁ + N₂T₂ + \ldots + NₙTₙ}{N₁ + N₂ + \ldots + Nₙ}
\]

where \(N_i\) is the number of seeds germinated in the period of time \(T_i\), \(N_i\) is the number of seeds which have germinated between \(T_i\) and \(T₂\), etc. [11].
Statistical treatment

The data obtained was statistically treated using Multivariate Variance Analysis. In order to increase normality, the germination percentage data was transformed using an Arc-sine Transformation, and the average germination time data using the log (mean germination time). The Test of Tukey [40] was used to analyse the differences between pairs of treatments, with a 95% degree of confidence.

Because of the design complexity of the experiment and the different germinative behaviour of each species, the effect of age and treatment with fire on each one of them was analysed separately.

3. RESULTS

The germinative response of these three species to treatment with heat and ash was analysed by using the rate of germination, average germination time and the period of time over which germination was distributed.

3.1. Rate of germination

The rate of germination, expressed as a percentage, varied in each species according to the age of the seed, the harvest site, and the treatment used (figure 1). The average germination percentage of P. pinaster was 54.65%, of P. radiata 62.81% and of E. globulus 51.59%.

P. pinaster reaches its highest average rate of germination in the seeds from 1991 (57.50%), followed by the seeds from 1993 (56.80%), 1990 (54.30%) and 1992 (50.00%). However, the differences between them are not significant. When comparing the results by treatments, the highest percentages of germination were found in the treatments with Ash and Ash + Temperature. The average values for these two treatments in the four age groups, were 57.19% and 57.22% respectively. Control with 54.44% followed this and the lowest rate of 49.72% was obtained by treatment with Temperature alone. However, neither are the differences between treatments found to be significant.

In P. radiata significant differences were found between ages (p < 0.0001) but not between the treatments. According to the Test of Tukey, these differences are due to the seeds from 1990 (43.05%) and 1991 (59.16%). The seeds from 1992 (72.50%) and 1993 (76.52%) are homogenous and germination was considerably higher than for the other years. Within the age groups, the differences between treatments are of little relevance. The treatment that reaches the highest average rate is that of Ash + Temperature with 66.52%, followed by Temperature (65.55%), Control (64.99%) and finally Ash (62.91%).

The average germination percentage for E. globulus is 51.59%, with a variation in germination rates of between 10.55% and 86.66% at the upper limit of the values obtained. Highly significant differences were detected between ages (p < 0.0001) and treatments (p < 0.0001). The highest germination percentage corresponded to the seeds from 1993 (83.88%), and the lowest to those from 1990 (24.86%). However, the reduction in germinative capacity over a period of time is not linear, as the seeds from 1991 have a higher percentage (57.91%) than the seeds from 1992 (39.72%). Statistically, all ages are significantly different from each other. The treatment that has the highest average value is that of Ash (62.22%), although it is very close to the value of the Control (59.72%). The treatments with Temperature and Ash + Temperature gave considerably lower values than the others (43.19% and 41.25% respectively). The Test of Tukey found that the results of the Control and Ash treatments are the same as each other, and different from Temperature and Temperature + Ash. In all ages studied, treatments involving the application of thermal shock obtained lower rates of germination than those registered in the Control group or those treated with Ash.

Figure 1 represents the evolution of the germination rate in the different treatments applied to P. pinaster, P. radiata and E. globulus according to the age of the seeds. Although the trajectory of P. pinaster is not totally linear, there are no important variations in the germination rate over a period of time, or within the different treatments applied.

The evolution of the germination rate of P. radiata at different ages increases continuously and significantly from 1990 until 1993. However, the differences between treatments at a given age are minimal.

In E. globulus the percentages obtained in the Temperature and Temperature + Ash treatments gradually decreased as storage time or seed age increased, although not always gradually, as the seeds from 1991 had a higher value than seeds from 1992. In the Control and Ash treatments, the seeds from 1993 and 1991 offered very high values with little difference between them, whereas the seeds from 1990 and 1992 had very low germination values for the same treatments. For the Temperature and Temperature + Ash treatments, germination rates nearly always increased as seed age decreased.
Figure 1. Percentage of germination obtained in each of the treatments applied to the seeds collected and stored from 1990, 1991, 1992 and 1993.

Figure 2. Average germination time in days reached by different aged seeds of *P. pinaster*, *P. radiata* and *E. globulus* subjected to different treatments.
3.2. Average germination time

Average germination times vary according to the species, treatment and age studied (figure 2).

*P. pinaster* has an average germination time which varies greatly according to the age of the seeds or the treatments applied. The differences found between different ages has a very high significance value \(p < 0.0001\) and these differences are due to the average germination time of the seeds from 1993, which are different from all the others. These seeds have the shortest average germination time, with an average value of 6.36 days. A second, slower, group of seeds from 1991 has an average of 11.19 days. Seeds from 1990 have a rate of 12.27 days, and seeds from 1992 have a rate of 13.77 days. The differences that exist between the seeds from 1991 and 1992 are also significant. Significant differences were also found between the different treatments \(p < 0.05\). The Control and Ash + Temperature treatments being responsible for this.

In *P. radiata* there were no significant differences between seeds of different ages, although it did offer quite important differences between treatments \(p < 0.001\). In agreement with the Test of Tukey, the Temperature treatment is significantly different from Ash and Ash + Temperature. The Temperature treatment presents the shortest average germination time in seeds of all ages (15.01 days on average), followed by Control (17.03 days), Ash + Temperature (17.24 days), and finally Ash (18.71 days).

For *E. globulus* there are no significant differences, either between the treatments or between ages. The average germination time is approximately 15 days. The seeds from 1993 had the shortest average germination time, with the longest corresponding to those from 1990 (figure 2), although between them there is only a difference of two days. In none of the treatments are differences in average germination time greater than two days.

3.3. Temporal distribution of germination

The temporal distribution of germination is fairly concentrated for the three species, although particularly so in *E. globulus*. This species also has the most important and most clearly defined germination peaks.

In both *P. pinaster* and *P. radiata* most of the treatments place the peaks of maximum germination between days 7 and 25. These peaks are sharper in *P. radiata* than in *P. pinaster* (figure 3).

Neither the treatments applied to *P. pinaster* nor the fact that the seeds came from different harvests, caused important variations in the time-period distribution of germination (figure 3).

In turn, *P. radiata*, has much sharper germination peaks in the Control treatments than in the others (figure 3). Furthermore, these peaks increase in importance, as the age of the seed decreases from 1990 to 1993.

The distribution of germination in *E. globulus* is conditioned by the year in which seeds were collected. The seeds collected in 1990 and 1992 were characterised by a more continuous rate of germination spread over the time-period and less important peaks. Whereas the seeds from 1991 and 1993 had a germination period concentrated in a few days, with very sharp peaks (figure 3).

The seeds from most of the treatments began to germinate on Day 5, although the seeds from 1993 began on Day 3. The last seeds germinated on Day 41, although the majority germinated in the first few days. In the seeds from 1990 and 1991, the most important germination peaks appeared between days 5 and 15. A moderately important level of germination continued until Day 23 in the seeds from 1992 and germination occurred slightly earlier, concentrated between days 3 and 9 in the seeds from 1993. It is important to note that, regardless of the age of the seeds, the Temperature and Ash + Temperature treatments lower the intensity of the germination peaks.

4. DISCUSSION

In this study it has been proven that the germinative behaviour of seeds of different ages, when related to the factor of forest fires, is different for each of the three species which were studied.

Germination rates vary according to the species and age of the seeds. In the cases where sensitivity to the treatments was detected, the same criteria were maintained regardless of the age of the seed. Germination was not significantly stimulated in any of the three species, either by the high temperature or by the ashes generated by a forest fire. Their effect on germination was either null or inhibitory. Similar results were obtained by [8, 15, 16, 26, 28, 31, 32, 35, 36, 37, 44], in different species of pines and eucalyptus.

*P. pinaster* is not sensitive to either high temperatures, ash or storage time. *P. radiata* is not sensitive to the effects of fire, although its germination rate decreases noticeably as storage time increases. This decrease in the
Figure 3. Germination time-period distribution of the seeds from 1990, 1991, 1992 and 1993 subjected to fire treatments (Control, Temperature, Ash, and Ash + Temperature) in each of the three species studied.
The germinative capacity of \textit{E. globulus} varied from year to year. The germination percentage did not diminish progressively over a period of time during the 4 years studied, but instead seems to follow biannual cycles in which a year of high fertility is followed by one of low fertility. Perhaps this conclusion is premature, after only 4 years of tests, but it could be a good starting point for the design of a new experiment capable of dealing with this question. Dale and Hawkins [12] confirmed that a good year of seed production in \textit{E. maculata} was preceded by at least two years of low production. These variations may be both due to the amount of photosynthate available for initiation and development of the floral buds and to successful pollination, damage caused by birds or insects, and/or the environmental conditions which were prevalent during development of the fruit and at maturity [5]. Consequently, the germinative characteristics of each species may also be subject to time-period variations.

Each species has an interval of time in which the seed remains viable and this capacity depends on storage conditions. In most forest species, the seed has to be conserved at low temperatures in a dry environment. In the case of \textit{Pinus} and \textit{Eucalyptus}, the best storage conditions are achieved at a temperature of between 2 and 4°C and humidity content of between 5 and 8%. As the storage temperature increases, the humidity content must be lowered [7]. Seeds of \textit{P. pinaster} which have been kept in cold storage may remain viable for 11 years, \textit{P. radiata} seeds for 21 years, and most species of \textit{Eucalyptus} for 10 years or more [39]. According to Catalán [7], if storage is at room temperature, viability decreases after the third or fourth year. In this study, it was proven that the seeds remain viable for at least 4 years. Houle and Filion [25] also verified that viability and germination rates vary from year to year, and that meteorological conditions are 74% responsible for the interannual variability of the production of viable seeds of \textit{P. banksiana}.

Seed size is different for each of these species, and probably represents a compromise between the energy required to disperse seeds and that used to establish the seedlings [18]. Small seed sizes facilitate long-distance dispersal, whereas storage of significant reserves in large seeds favours the later establishment of seedlings [47]. The average weight of the seeds (with coat) varies from 0.050 g in \textit{P. pinaster} to 0.030 g in \textit{P. radiata} and 0.002 g in \textit{E. globulus}. The differences in seed weight are notable, and furthermore the thickness of the seed coat is clearly differentiated, with \textit{E. globulus} seeds having the finest, followed by those of \textit{P. pinaster} and \textit{P. radiata}. Houle and Filion [25] found that in \textit{P. banksiana} the seed mass is positively correlated with the germination rate. The differences in seed weight and covering thickness between the three species may explain their different behaviour during the process of germination, and their differing sensibility to high temperatures, ash, and the effect of both of these together. \textit{E. globulus} is the species with the smallest seeds, and is the most sensitive to seed age and the effects of fire. Of the two species of pine studied, \textit{P. pinaster} is the least sensitive to seed age and the effects of fire, and also has a larger mass.

It is expected that the larger seeds, as well as being more resistant to fire [27], give rise to more vigorous seedlings and have a lower mortality rate than seeds of smaller size [17, 20, 23, 24, 34].

It is necessary to verify whether or not these responses occur with the same intensity, when the seeds are still stored in fruits on the mother plant.
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REFERENCES


