Ann. For. Sci. 59 (2002) 327–334

DOI: 10.1051/forest:2002028

Original article

Effect of high temperatures on cone opening and on the release and viability of *Pinus pinaster* and *P. radiata* seeds in NW Spain

Otilia Reyes* and Mercedes Casal

Área de Ecología, Dpto de Biología Fundamental, Fac de Biología, Univ de Santiago de Compostela, 15782 Santiago de Compostela, Spain

(Received 5 February 2001; accepted 29 August 2001)

Abstract – *Pinus* genus is characterized by woody cones able to open even after a forest fire, which also protect seeds from damages during the fire. The aim of the present study is to analyze the effect of high temperatures on pine cones opening as well as the releasing and viability of the seeds of *P. pinaster* and *P. radiata*, throughout a selection of different combinations of temperatures and time exposures. During a forest fire, extremely high temperatures have a very low remanence. 26 different combinations were selected, beginning by 500 ºC/1 min and then gradually increasing time exposure whereas the temperature, on the opposite, was set lower and lower. This process was applied up to combinations of relatively low temperatures and long lapses of time such as 100 ºC/30 min. 5 cones from each species were tested with each combination, a total of 260 cones were finally set under study. *P. pinaster* species showed a scales' opening of 50% on average whereas *P. radiata* neared 90%. The rate for *P. radiata* seeds' releasing is also higher than *P. pinaster's*. Finally, the viability of the seeds remained unchanged under the influence of thermal shocks for both *Pinus* species.

fire / high temperatures / pine cone opening / *P. pinaster* / *P. radiata*

Résumé – Effet des hautes températures sur l'ouverture des cônes, la dissémination et la viabilité des semences de *P. pinaster* et *P. radiata* du NO d'Espagne. Le genre *Pinus* présente des cônes ligneux qui protègent les semences du feu et qui s'ouvrent même après le feu. Le but de cette étude est de connaître l'effet des hautes températures sur l'ouverture des cônes, ainsi que sur la dissémination et la viabilité des semences des espèces *P. pinaster* et de *P. radiata* au travers des différentes combinaisons de températures et temps d'exposition. Lors d'un feu de forêt les très hautes températures ont un temps de remanence très peu élevé ; on a fait une sélection de 26 différentes combinaisons de températures et temps d'exposition, à partir de 500 ºC/1 min et en augmentant progressivement le temps d'exposition. L'on a fait décroître la température, jusqu'à des combinaisons de températures relativement basses avec de longs laps de temps (100 ºC/30 min). Chacune de ces combinaisons de facteurs a été appliquée à 5 cônes de chaque espèce, un total de 260 cônes a été étudié. L'espèce *P. pinaster* a présenté un taux moyen d'ouverture d'environ 50 % de ses écailles, alors que le *P. radiata* s'approche de 90 %. Le taux de semences disséminées est aussi plus élevé pour *P. radiata* que pour *P. pinaster*. Finalement, la viabilité des graines n'a pas changé sous l'influence des chocs thermiques et ce, dans aucune des deux espèces de *Pinus*.

feu / hautes températures / ouverture des cônes / *P. pinaster* / *P. radiata*

* Correspondence and reprints
Tel. 34 981 563 100; Fax. 34 981 596 904; e-mail: bfreyes@usc.cs

1. INTRODUCTION

Some species in the genus *Pinus* are characterized by an aerial seed bank [1, 5, 11, 15, 16, 31]. That is, seeds remain inside the cones on the parent tree for a long time until conditions are suitable for dispersal and germination. In the event of fire, seeds already shed from the cones may be burned and prove useless for reproduction. Hence, the seeds most likely to survive are those that remain inside the cones and are dispersed after the fire, thus avoiding destruction, or those that are buried in the soil.

Pinus seeds last for a very short time on the soil surface as they are eaten or attacked by many different organisms [2, 12, 13, 18, 19, 22, 24, 26]. Therefore, soil surface seed banks are quite ephemeral.

In contrast, seeds stored in cones form a seed bank that is protected against predators. Likewise, in the event of a forest fire, *Pinus pinaster* Aiton and *Pinus radiata* D. Don cones protect their seeds. A few days after a fire, cones slowly open their scales and release the seeds. Surface fires do not usually affect the opening of pinecones since the crowns are not sufficiently heated. However, in crown fires flames can sometimes reach temperatures close to 1000 ºC in a short space of time [9], which leads to cone combustion. The role of fire in the opening of cones and seed dispersal has been studied in some *Pinus* species [4, 9, 10, 16–18, 24, 27, 32]. It is within this context that we proposed to study the effect of a wide spectrum of temperature-heat residence time combinations on the opening of *P. pinaster* and *P. radiata* cone scales, on the release of seeds, and on their viability.

We chose *P. pinaster* and *P. radiata* from among all the species of the genus *Pinus* because both are widely used in reforestation, both frequently suffer crown fires and demonstrate different degrees of serotiny: low in *P. pinaster* and high in *P. radiata*.

2. MATERIALS AND METHODS

2.1. Experimental design

To carry out this experiment we selected mature and apparently intact *P. radiata* and *P. pinaster* cones from populations in Galicia (NW Spain). Ten cones were collected from 13 individuals of each species, their colour and position was not taken into account. A total of 260 pinecones were harvested and grouped into 26 lots of 5 cones from each species. Each treatment was applied to 5 replicates of one cone from each of the two species.

Given that the high temperatures produced during a forest fire last for a relatively short time [8], we found that when the closed cones were subjected to temperatures or exposure times of over 500 ºC/1 min ignition occurred. In order to cover the widest possible range, we selected 26 different temperature-time combinations. Starting at 500 ºC/1 min, we gradually increased exposure times and reduced temperatures until relatively low temperatures and long residence times were reached.

The following combinations of temperature-exposure were tested:

500 ºC/0 min, 500 ºC/1 min

400 ºC/0 min, 400 ºC/1 min

350 ºC/0 min, 350 ºC/1 min, 350 ºC/5 min

300 ºC/0 min, 300 ºC/1 min, 300 ºC/5 min, 300 ºC/10 min

250 ºC/0 min, 250 ºC/1 min, 250 ºC/5 min, 250 ºC/10 min, 250 ºC/15 min

200 ºC/0 min, 200 ºC/1 min, 200 ºC/5 min, 200 ºC/10 min, 200 ºC/15 min, 200 ºC/20 min

150 ºC/0 min, 150 ºC/5 min, 150 ºC/10 min, 150 ºC/15 min, 150 ºC/20 min, 150 ºC/25 min

100 ºC/0 min, 100 ºC/10 min, 100 ºC/15 min, 100 ºC/20 min, 100 ºC/25 min, 100 ºC/30 min

Once the selected oven temperature was stabilised, five pinecones of each species were introduced. These pinecones were removed after the specified exposure time and the process was repeated for each treatment.

The number of open scales, dispersed seeds, and their viability, was recorded for each cone subjected to thermal shock.

The percentage of open scales for *P. pinaster* and *P. radiata* cones caused by induced heat was obtained by counting all the open scales on each cone after the thermal treatment had been applied. The scales were counted manually and marked with a felt-tip pen to avoid confusion. The value obtained refers to the maximum number of scales capable of opening. To obtain this maximum figure, the same cones were subjected to another thermal shock, at 100 ºC for 2 hours, two days after the treatment and the open scales were counted on the following day. Prior to this, we tested different combinations of temperatures below 200 ºC and over prolonged periods and checked that the *P. pinaster* and *P. radiata* cones that had undergone 100 ºC for 2 hours had reached their maximum level of opening. This maximum level does not signify that all the scales open (the smallest and close to the base never open). The total numbers of open scales were counted after thermal shock and after subjection to 100 ºC over two hours. One value was expressed in relation to the other, thus obtaining a percentage of open scales. The test for viability followed a commonly used method, which consists of imbibing the seeds in 1% tetrazole in darkness for 24 hours [23]. Live seed embryos finally become reddish while those of dead seeds do not change colour. This test was only applied to full seeds. Empty seeds were counted and their percentage was calculated.

2.2. Statistical processing

Data on the percentage of open scales and percentage of dispersed seeds for both of the species was analysed using two-way ANOVAs, to determine whether there were any significant differences between the species and the applied treatments. The Arcsin(Sqrt(x)) transformation was performed on the open scale and liberated seed data. It was proved that significant interaction existed between the species and treatment factors. For this one-way ANOVAs were performed, analysing the data of each species separately. In those cases in which significant differences were detected, a Tukey test was performed to determine between which treatments these significant differences existed.

3. RESULTS

Figure 1 shows the percentage of scales that opened in *P. pinaster*, the seeds released, and their viability percentage. *Figure 2* shows the values of the same three variables for *P. radiata*. Given that the percentages of viability obtained in the treatments applied to both species were nearly 100%, in *figures 1* and *2* we assumed that the viability percentage of the seeds enclosed in the cones before opening was 100%. Similarly, the percentage of open scales and seeds dispersed in 0 time was 0.

3.1. Scale opening

The percentage of scales that opened as a result of each of the thermal shocks tested is considerably different when comparing *P. pinaster* and *P. radiata*. The former reveals a mean opening rate for scales of approximately 52%, while almost 90% of *P. radiata* scales opened. If the 200 ºC/1 min and 100 ºC/10 min treatments for both species and the 150 ºC-5 min treatment for *P. pinaster* are excluded, since they had no effect on the state of the scales, most of the opening rates for *P. pinaster* were below 60% while the lowest value obtained for *P. radiata* was 87.45 ± 4.87%.

Statistical analyses show large differences between *P. pinaster* and *P. radiata* and in the interaction between species and treatments this was highly significant (*table I*). For these two reasons we opted for the study of

Table I. Results obtained by applying two-way ANOVA to the values of scale opening data.

Source	Sum of Squares	*df*	Mean square	*F*-Ratio	*P*-Value
MAIN EFFECTS					
A : species	69189.2	1	69189.2	91.13	0.0001
B : treatments	63505.5	25	2540.22	3.35	0.0001
INTERACTIONS					
AB	424544.8	25	1698.19	2.24	0.0011
RESIDUAL	157918.0	208	759.219		
TOTAL (CORRECTED)	333067.0	259			

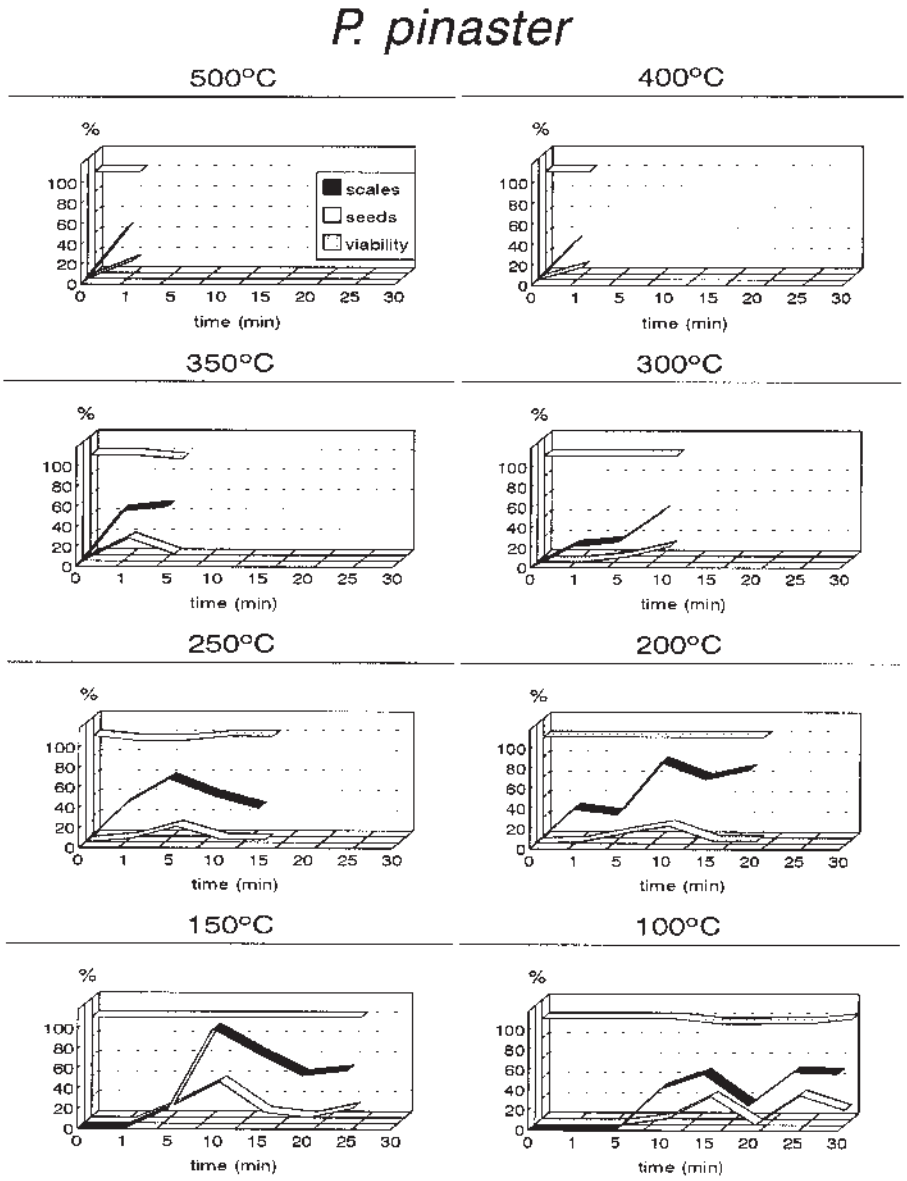

Figure 1. Percentage of open scales, released seeds and seed viability for *P. pinaster*. The variation of each percentage is shown in relation to exposure time for each of the selected temperatures.

each species separately. No marked differences were found between treatments in *P. pinaster*, but this was not so in the case of *P. radiata* (F = 60.68, df = 25, p = 0.0001). Differences in the latter were due to the 200 ºC/1 min and 100 ºC/10 min treatments in which the percentage of open scales was 0.0%.

On analysing the results of the thermal treatments for each species individually, we found that the increase in exposure time at a given temperature had no cumulative effect on the percentage of open scales. In *P. pinaster* (*figure 1*), the variations in the percentage of scales that opened at a given temperature, with increasing exposure times, were erratic. In contrast, in *P. radiata* with a relatively short exposure time, a threshold percentage (87.45%) of scale openings is obtained and remains more or less constant, even when exposure time is increased (*figure 2*).

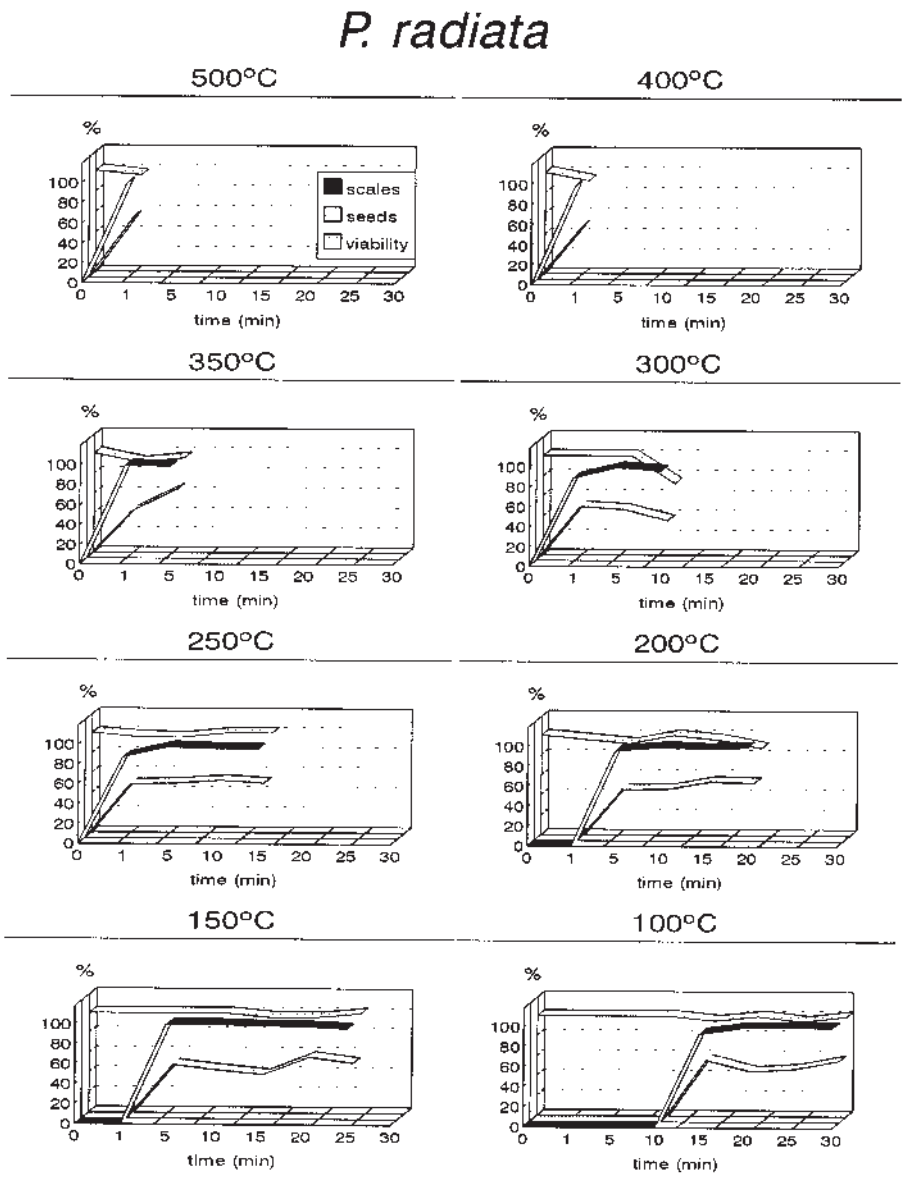

Figure 2. Percentage of open scales, released seeds and seed viability for *P. radiata*. The variation of each percentage is shown in relation to exposure time for each of the selected temperatures.

3.2. Seed release

Following the above pattern, the percentage of seed release is also greater in *P. radiata* than in *P. pinaster*. The latter released 11.91% of the seeds that could potentially have been released in view of the number of open scales. Two seeds could be released per scale. The mean dispersal rate for *P. radiata* was 50.41 ± 1.78% and reaches 54.61 ± 1.34% if the two cases in which no cones opened and hence no seeds were released (200 ºC/1 min and 100 ºC/10 min) are excluded.

In each of the tested temperatures, variation in exposure time is not linked to a gradual increase in the rate of seed release (*figures 1* and *2*), or in the rate of scale opening. In both *P. pinaster* (*figure 1*) and *P. radiata* (*figure 2*), the rates of seed release are invariably lower than the rate of scale opening, but follow the same pattern.

Statistical analyses showed marked differences between the two species and also significant interactions (*table II*) between species and treatments. For this reason we analysed the effects of the treatments on seed dispersal for each species separately. The percentage of seeds released in *P. pinaster* is fairly homogenous for all treatments and the ANOVA did not detect any significant differences between these. In the case of *P. radiata* significant differences ($F = 12.00$, $df = 25$, $p = 0.0001$) were only found when comparing the 200 ºC/1 min and 100 ºC/10 min treatments (which showed no release) with the others.

3.3. Viability of seeds

The viability of the seeds released when the cones opened as a result of induced heat did not seem to be affected, as can be deduced by the data in *figures 1* and *2*. In most cases, viability is almost 100%. Apart from the analysis of the viability of full seeds, it was detected that the number of empty seeds represented 12.04 ± 2.07% of the total seeds released in *P. radiata*. The figure for *P. pinaster* was 15.05 ± 3.69%.

4. DISCUSSION

Species of the genus *Pinus* have, traditionally, been considered to be well adapted to fire. This, despite the fact that most of the species cannot resprout after fire [25, 29, 30]. This is the case with the species studied, *P. pinaster* and *P. radiata*, which only reproduce from ripe seeds. One of the main features of pines, as a species adapted to fire-prone ecosystems, is their capacity to produce a large number of seeds enclosed in cones [5, 7].

In this study, we found that high temperatures caused cones to open and the enclosed seeds to be released, scarcely affecting their viability. Pines have probably developed this adaptive feature and hence, their seeds can survive fires or long periods of drought [5,15].

The response to high temperatures is different in *P. pinaster* and *P. radiata*. Spontaneous dispersal of mature *P. pinaster* seeds in Galicia (NW Spain) coincides with the end of spring and lasts throughout the summer [32]. *P. pinaster* is a species which does not need excessively high temperatures for most of its cones to open their scales or bracts and disperse their seeds. The summer temperatures recorded in our latitudes are hot enough to allow for this process. According to Keeley and Zedler [16] *P. radiata*, in its zone of origin, can open its cones after fire or in response to normal temperature extremes. Long periods of hot and dry weather are not normal in Galicia and also fire frequency is very high. Both of these reasons could have caused the populations of *P. radiata* of this region to manifest themselves as pyriscent sensu Lamont et al. [18] and not as xeriscent sensu Nathan and Ne'eman [24]. As a result of these species differences in heat requirements, their responses to thermal shocks are also different.

Seed availability for germination is neither temporarily nor spatially the same for all the species. *P. radiata* can keep the seeds in its serotinous cones for a number of seasons [16, 32], as can *P. halepensis* [3, 19, 24], *P. banksiana* [4–6], *P. brutia* [28], *P. contorta* [17, 21], *P. mariana*, *P. resinosa* [6], *P. attenuata* and *P. muricata* [16, 20]. In these species the cones only open after fire thus ensuring regeneration of their populations.

Between the two species studied, *P. radiata* best favours high temperatures, since 90% of its scales opened. Only 50% of the *P. pinaster* scales opened in the same treatments. The former also requires exposure times of above 10 minutes at 100 ºC and above 1 minute at

Table II. Results obtained by applying two-way ANOVA to the values of seed release data.

Source	Sum of Squares	*df*	Mean square	*F*-Ratio	*P*-Value
MAIN EFFECTS					
A : species	64790.7	25	64790.7	306.77	0.0001
B : treatment	18152.2	1	726.089	3.44	0.0001
INTERACTIONS					
AB	13776.2	25	551.049	2.61	0.0001
RESIDUAL	43930.1	208	211.202		
TOTAL (CORRECTED)	140649.0	259			

200 ºC, while *P. pinaster* opens its cones at room temperature if humidity is low.

The level of cone protection against heat varies from one species to another. Beaufait [4] found that *P. banksiana* protected its seeds until cone ignition and Despain et al. [9] found that in *P. contorta*, 88% of seeds remained viable after being exposed to 480 ºC for 30 seconds. Furthermore, Judd [14] suggests that insulation capacity depends on cone size and whether or not the fruit or cone contains internal divisions that increase its insulation capacity. None of the temperature-exposure time combinations tested, greatly affected the viability of the seeds enclosed in the cones in either of the species. Therefore, it seems certain that cones provide efficient insulation against the devastating effects of fire. Moreover, cone opening in both species did not occur immediately after the thermal shock, but rather the cones gradually opened 2 or 3 days after the induced heat treatment. Saracino et al. [27] observed this same behaviour in *P. halepensis*. This delay favours pines. Because when dispersal takes place, the fire is totally extinguished and the soil temperature has dropped to low levels. Hence seeds avoid burning or loss of viability after cones have opened. In this respect, forest fires could play a decisive role in the expansion and/or replacement of pine populations, the fire adaptive features of *P. radiata* being more successful against fire than those of *P. pinaster*.

Acknowledgements: We would like to thank Dr. E. de Luis Calabuig and the fire ecology team at the University of León for their help and for allowing us to carry out this experiment in their laboratory. This study was supported by a grant from the University of Santiago de Compostela to O. Reyes. We thank M.L. Picot, M. Dubois, V. Dubois and M. Fariña for their help.

REFERENCES

[1] Agee J.K., Fire and pine ecosystems, in: D.M. Richardson (Ed.), Ecology and Biogeography of *Pinus*, Cambridge University Press, 1998, pp. 193–218.

[2] Ahlgren C.E., Small mammals and reforestation following prescribed burning, J. Forest. 64 (1966) 614–618.

[3] Barbero M., Bonin G., Loisel R., Miglioretti F., Quezel P., Indice of exogenus factors on the regeneration of *Pinus halepensis* after fires, Ecol. Mediterranea Tome XIII (fascicule 4) (1987) 51–67.

[4] Beaufait W.R., Some effects of high temperatures on the cones and seeds of jack pine, For. Sci. 6 (1960) 194–199.

[5] Chandler C., Cheney P., Thomas P., Trabaud L., Willians D., Fire in Forestry, Vol. I. Wiley Interscience Public, New York, 1983.

[6] Chrosciewicz Z., Jack pine regeneration following postcut burning under seed trees in Central Saskatchewan, Forest. Chronicle (1988) 315–319.

[7] Clements F.E., The life history of lodgepole pine burn forests, Bulletin 79 Washington DC. United States Department of Agriculture, For. Serv. (1910) 56 p.

[8] Cowling R.M., Lamont B.B., Variation in serotiny of three *Banksia* species along a climatic gradient, Aust. J. Ecol. 10 (1985) 345–350.

[9] Despain D.G., Clark D.L., Reardon J.J., Simulation of crown fire effects on canopy seed bank in Lodgepole pine, Int. J. Wildland Fire 6(1) (1996) 45–49.

[10] Gauthier S., Bergeron Y., Simon J.P., Effets of fire regime on the serotiny level of jack pine, J. Ecol. 84 (1996) 539–548.

[11] Gill A.M., Adaptative responses of Australian vascular plant species to fires, in: Gill A.M., Groves R.H., Noble I.R. (Eds.), Fire and the Australian biota, Aust. Acad. Sci., Canberra, 1981.

[12] Hayashida M., Seed dispersal by red squirrels and subsequent establishment of Korean pine, For. Ecol. Manage. 28 (1989) 115–129.

[13] Janzen D.H., Seed predation by animals, Ecol. System 2 (1971) 465–493.

[14] Judd T.S., Seed survival in small myrtaceous capsules subjected to experimental heating, Oecol. 93 (1993) 576–581.

[15] Keeley J.E., Seed germination patterns in fire-prone Mediterranean-climate regions, in: Arroyo M.T.K., Zedler P.H., Fox M.D. (Eds.), Ecology and Biogeography of Mediterranean Ecosystems in Chile, California and Australia, 1994, pp. 239–273.

[16] Keeley J.E., Zedler P.H., Evolution of life histories in *Pinus*, in: Richardson D.M. (Ed.), Ecology and Biogeography of *Pinus*, Cambridge University Press, 1998, pp. 219–250.

[17] Knapp A.K., Anderson J.E., Effect of heat on germination of seeds from serotinous lodgepole pine cones, Amer. Mid. Natur. 104 (1980) 370–372.

[18] Lamont B.B., Le Maitre D.C., Cowling R.M., Enright N.J., Canopy seed storage in woody plants, The Botanical Review 57 (1991) 277–317.

[19] Leone V., Borghettii M., Saracino A. Ecology of postfire recovery in *Pinus halepensis* in southern Italy, in: Trabaud L. (Ed.), Life and Environment in the Mediterranean, WIT Press, Boston, 2000, pp.155–202.

[20] Linhart Y.B., Maintenance of variance cone morphology in California closed-cone pines: the roles of fire, squirrels and seed output, Southwestern Natur. 23(1) (1978) 29–40.

[21] Lotan J.E., The role of cone serotiny on lodgepole pine forests, Proceeding Symp Mgmt Lodgepole Pine Ecosystems, Washington State Univ. Coop. Exten. Serv. Publ. (1975) 471–495.

[22] Louda S.V., Effect of seed predation on plant regeneration: evidence from Pacific Basin Mediterranean communities, in: Arroyo T.M.K., Zeedler P.H., Fox M.D. (Eds.), Ecology and Biogeography of Mediterranean Ecosystems in Chile, California and Australia, Springer-Verlag, New York, 1995, pp. 311–344.

[23] Moore R.P., Manual de ensayos al tetrazolio. Ministerio de Agricultura, Pesca y Alimentación, 1985, Madrid.

[24] Nathan R., Ne'eman G., Serotiny, seed dispersal and predation in *Pinus halepensis*, in: Ne'eman G., Trabaud L. (Eds.), Ecology, Biogeography and Management of *Pinus halepensis* and *P. brutia* forest Ecosystems in the Mediterranean Basin, Backhuys Publishers, Leiden, The Netherlands, 2000, pp. 105–118.

[25] Naveh Z., Effects of fire in the Mediterranean Region, in: Kozlowski T.T., Algren C.E. (Eds.), Fire Ecosyst. Academic Press, New York, 1974, 401–434.

[26] Saracino A., Leone V., Ecological features and dynamics of seed dispersal after fire in a *Pinus halepensis* Miller forest, in: Trabaud L., Prodon R. (Eds.), Fire in Mediterranean Ecosystems, Commission of the European Communities, Banyuls-Sur-Mer, France, 1993, 151–160.

[27] Saracino A., Pacella R., Leone V., Borghetii M., Seed dispersal and changing seed characteristics in a *Pinus halepensis* Mill. forest after fire, Plant Ecology 130 (1997) 13–19.

[28] Thanos C.A., Christodoulakis D., Yannitsaros A., Early regeneration of *Pinus brutia* forest ecosystems of Samos island (Grece), Acta Ecologica, Oecologia Plantarum 10(1) (1989) 79–94.

[29] Trabaud L., Quelques valeurs et observations sur la phytodinamique des surfaces incendiées dans le Bas-Languedoc (I). (Premiers résultats), Naturalia Monspeliensis Ser. Bot. 21 (1970) 231–242.

[30] Trabaud L., Impact biologique et écologique des feux de végétation des zones de garrigues du Bas-Languedoc, Thèse de Doctorat, Université des Sciences et Technique du Languedoc, Montpellier, 1980.

[31] Trabaud L., Fire and survival traits of plants, in: Trabaud L. (Ed.), The Role of Fire in Ecological Systems, SPB Acad. Publi., The Hague, 1987, 65–89.

[32] Vega J.A., Influencias del fuego en los hábitos regenerativos del *P. pinaster* y *P. radiata* en Galicia (Noroeste de España), Monograph of ICONA 20 (1977) 47–49.