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Daniel Moya, Jorge Heras, Rossella Salvatore, Edelmira Valero, Vittorio Leone. Fire intensity and serotiny: response of germination and enzymatic activity in seeds of *Pinus halepensis* Mill. from southern Italy. *Annals of Forest Science*, 2012, 70 (1), pp.49-59. 10.1007/s13595-012-0236-x . hal-01201457

HAL Id: hal-01201457

<https://hal.science/hal-01201457>

Submitted on 17 Sep 2015

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Fire intensity and serotiny: response of germination and enzymatic activity in seeds of *Pinus halepensis* Mill. from southern Italy

Daniel Moya · Jorge De las Heras · Rossella Salvatore · Edelmira Valero · Vittorio Leone

Received: 13 March 2012 / Accepted: 9 August 2012 / Published online: 11 September 2012
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Abstract

- **Context** The Mediterranean Basin is a fire-prone area. *Pinus halepensis* Mill. is a naturally growing conifer which is frequently used for reforestation and restoration as it displays some degree of adaption to fire. One of the traits conferring tolerance to fire is the frequent presence of serotinous cones that are thought to protect seeds from fire damage.
- **Aim** We checked for the physiological responses of seeds to different intensities of fire and related them to the degree of serotiny of the cones.
- **Methods** Germination percentage, mean germination rate and enzymatic activity (α -amylase and protease) were recorded

for seeds burned either free or enclosed in cones. We included as experimental factors the following: fire intensity, serotiny and time during which seeds were stored in cones after harvesting and germination stage.

- **Results** Burned seeds (released and enclosed) developed in serotinous cones exhibited higher heat insulation. In released seeds, germination was decreasing according to increasing fire intensity, although we found differences depending on site, serotiny and time stored after harvesting. The enzymatic activity was also found to be related to serotiny and fire intensity.

- **Conclusion** Serotiny is an adaptive trait increasing the tolerance to fire which should be promoted in natural and restored populations.

Handling Editor: Eric Rigolot

Contribution of the co-authors All co-authors wrote, revised and contributed significantly to this manuscript. Jorge De las Heras and Vittorio Leone devised and designed the experiment and supervised and coordinated the work. Edelmira Valero implemented the enzymatic experiment and developed and improved the chemical analysis. Daniel Moya and Rossella Salvatore sampled in the field, carried out the tests described in methods and analysed the results. Daniel Moya wrote the manuscript, ran statistical analyses, built tables and implemented contributions from all co-authors.

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Keywords Aleppo pine · Serotinous cones · Resilience · Heat insulation · Germination · Enzymatic activity

Abbreviations

G	germination
MGT	mean germination time
SEROT	serotiny
MAR	mean annual rainfall
MAT	mean annual temperature
S	serotinous
NS	non-serotinous

1 Introduction

In fire-prone areas, plants have developed adaptive and exaptive traits, with two main strategies: resprouting and fire-cued seed recruitment (Keeley et al. 2011). Soil or serotinous fruits protect seeds against the high temperatures of wildfires (Ne'eman et al. 2009; Moya et al. 2008b), but even released seeds were found to survive after such heat (Martinez-Sanchez

et al. 1995; Nuñez et al. 2003; Reyes and Casal 2002). The term serotiny has been used to describe reproductive structures that retain seeds or delay their release from canopy banks (Lamont et al. 1991). However, opening fruits and seed dispersal are independent processes despite the ecological conditions for their occurrence being similar, and the term bradychory has been used to name the process which delays seed release (Thanos 2004). In most species, heat influences fruit/cone opening and has positive effects on seed release, but both effects do not necessarily relate directly (Clarke et al. 2010). However, life history has a major effect on dehiscence and subsequent variation in seed release by avoiding hasty dispersal of seed in hostile environments (heat, ash, ph, etc.) and improving recruitment expectations (Clarke et al. 2010).

Pinus halepensis Mill. is the main coniferous tree species covering drier, lower-altitude areas in western parts of the Mediterranean Basin (Quezel 2000). It has developed adaptive traits to drought and fire and exhibits early cone production (Tapias et al. 2001) and a dual life strategy to bear both serotinous and non-serotinous cones (Ne'eman et al. 2009). In *P. halepensis*, seeds in serotinous cones show better biological quality, mimicry (lower predation risk) and opportunistic seed release delay (poor competition) (Saracino et al. 1997). In addition, Aleppo pine cones offer seeds fire insulation and protection (Habrouk et al. 1999), similarly to other Mediterranean pines (Reyes and Casal 2002). Anatomical and morphological characters (thick woody scales) provide heat insulation, in addition to special characteristics found in seeds enclosed in serotinous cones (thick coats covered with wax; insulation properties conferred by Si and Ca) (Moya et al. 2008b; Salvatore et al. 2010). The germination ecology of the Aleppo pine seeds enclosed in cones has been related to fire severity (temperature and heating time) (Martinez-Sanchez et al. 1995) and cone type (Salvatore et al. 2010), and displays less reduced germination in the seeds enclosed in serotinous cones. Moreover, the germination of the seeds enclosed in serotinous cones is less affected by fire (heating and pH increase) than those stored in non-serotinous cones (Goubitz et al. 2003). This scenario has been related to internal seed processes, such as proteolytic enzymes (Salmia 1981a). In *Pinus* species, the onset of seed germination has been related to enzyme activity, particularly α -amylase and protease (Wang and Cao 2010), and has been found to be dependent on environmental factors, mainly temperature (Acevedo and Cardemil 1997). Hence, closed cones confer protection against increased temperature and light access by maintaining low moisture values to diminish enzymatic activity (Bewley and Black 1994). This enzymatic activation partially explains delayed seed germination, which is not fully understood (Thanos 2000).

This study mainly aims to highlight the relationship between serotiny of cones and resilience in fire-prone areas. During a fire event, natural regeneration is promoted by the heat insulation provided by cones. In addition, the ecological

and physiological characters that enhance fire resistance develop in the seeds enclosed in serotinous cones. So, we examined the response of Aleppo pine seeds (enclosed and released) to heating (germination percentage and rate, and α -amylase and protease activity) by including the seeds from serotinous and non-serotinous cones from S Italy. We tested the effects of heat on seed germination and whether exposure to extreme heat leads to population variation since more extreme fire weather is predicted; hence, fire intensity is likely to increase. We first tested whether there were temperature and heating time thresholds for reduced germination with different fire intensities. These characteristics could differ depending on the cone type in which they developed and were enclosed. Then we tested whether the percentage and rate of germination related to enzymatic activity by selecting α -amylase and protease as the enzymes involved in germination processes.

2 Methodology

2.1 Study site and sampling

Sampling focused on two mature (ca. 70 years old) natural coastal Aleppo pine stands located in S Italy with similar fire regimes and serotiny percentages above 53 % (cones closed after maturation divided by the total number of cones produced):

- Taranto (40°27'43" N, 16°56'09"E; 5 m amsl) 253 ha on sand dunes, close to the Ionian Sea. The Emberger Index value was 60.13 (535 mm MAR; 15.8 °C MAT (averaging 1987–2007)) corresponding to a Mediterranean semiarid and temperate climate (Emberger 1930). Even-aged stand (251 tree ha⁻¹; mean trunk diameter 30.65 cm and mean height 9 m) with typical shrub species *macchia Mediterranea* (*Phillyrea angustifolia* L., *Pistacia lentiscus* L., *Rosmarinus officinalis* L. and *Cistus salvifolius* L.).
- Salerno (40°00' N, 15°22' E; 250 m amsl) 350 ha on dolomitic calcareous soils, close to the Tyrrhenian Sea. The Emberger Index value was 150.86 (792 mm MAR; 16.7 °C MAT (averaging 1987–2007)), a Mediterranean humid climate (Emberger 1930). Even-aged stand (694 tree ha⁻¹; mean trunk diameter 24.75 cm and mean height 16 m) whose understory was composed of mainly evergreen sclerophyllous shrub species (*Juniperus phoenicea* L., *Pistacia lentiscus* L., *Rosmarinus officinalis* L., *Vitex agnus-castus* L. and *Rhamnus alaternus* L.).

In July 2007, 20 mature average-sized trees (sensu Ayari et al. 2011) were chosen and cut down at each site. All the cones in the crop were counted, and those of a similar shape were sampled to avoid any cone size influence (10 cm long, 4 cm wide). The selected cones were taken to a laboratory. Those which ripened in 2007 (brownish) were selected to

avoid any cone age influence (Goubitz et al. 2003). In the laboratory, cones were opened, and their seeds were manually released to consider their serotiny. The seeds enclosed in the (closed) cones and released seeds were heated at different temperatures and for varying exposure times. Then, germination tests were done to check the percentage and rate of germination. We repeated some heating exposures with released seeds to check any enzymatic activity variations. Three different germination tests were done: control (with no heat), heated seeds enclosed in cones and heated released seeds.

2.2 Heating enclosed and released seeds

The experimental burning in two fire scenarios was replicated to simulate the seed's condition. Crown fires affecting closed cones (*enclosed seed scenario*) and surface or crown fires affecting seeds directly (opened cones or seeds on soil) (*released seed scenario*) were simulated (Habrouk et al. 1999; Martinez-Sanchez et al. 1995; Moya et al. 2008b). The influential factors studied were as follows:

- sampling site (STAND), referred to as ombroclimate (HUMID or SEMID);
- fire intensity (HEAT), represented as the heat index (temperature and exposure time);
- cone type in which seeds were stored according to *serotiny* (SEROT); and
- storage period (STORE) of seeds inside the cones after harvesting, applicable to released seeds.

Serotiny was characterised according to cones opening at the 45 °C turning temperature (Goubitz et al. 2003; Tapias et al. 2001). Cones were put in an oven (31 °C and <20 % relative humidity), and the temperature was increased 2 °C per day to reach 45 °C in a week, which did not affect seed germination (Moya et al. 2008b). Cones were checked daily; those with a slight opening (scale separation) were removed and classified as non-serotinous (NS); those which remaining closed were serotinous (S). In the released seed scenario (see below), *time of seed storage* in the cone from harvest to release was included, and there were four levels: no storage (<10 days), short period (<1 month after harvest), medium period (6 months) and long period (24 months).

The *heat index* was calculated by multiplying temperature (in degrees Celsius) and the natural logarithm of the exposure time plus one (in minutes) (Paula and Pausas 2008). Following DeBano (1982), moderate and mild burn severity above the surface were simulated, and the following temperature (50 °C increases) and exposure time thresholds were selected:

- enclosed seed scenario 70–270 °C; 1, 2 and 5 min; and
- released seed scenario 70–220 °C; 1, 5, 10 and 20 min.

In the enclosed seed scenario, S and NS cones were placed into a muffle furnace (12PR/300, $T_{\max}$ 1,200 °C)

while controlling temperature and exposure time. After heating, all the cones were opened, and seeds were manually extracted. In the released seed scenario, S and NS cones were opened by sun exposure (30 days) (Moya et al. 2008b). Then, seeds were extracted manually, counted and placed into a laboratory muffle furnace (12PR/300, $T_{\max}$ 1,200 °C) to accomplish the target temperature and exposure time. From each set, four replicates (100 seeds each) were randomly sampled to do the germination tests. In all, 25,600 seeds from the enclosed seed scenario (16 sets replicated per site and cone type) and 83,200 seeds from the released seed scenario (1,600 seeds as a control set (not heated and stored for less than 10 days), and the 17 sets (replicated per site and cone type) (Table 1) were tested according to the heat indices.

When seeds were available, they were placed on paper discs in petri dishes and placed onto a Jacobsen table to maintain optimal irradiance, temperature and humidity values (20.0±0.5 °C, 8-h photoperiod (white light, 1,250 lx)). Replicates were monitored each 2–3 days (for 28 days), and germinated seeds (geotropic radicle >1 mm) were recorded and removed. We calculated the germination percentage (G) as the ratio between germinated seeds and the total number of seeds (multiplied by 100). If G was higher than 5 %, we calculated the mean germination time (MGT) following Bewley and Black (1994):

$$\text{MGT} = \sum_i \left(\frac{n_i * d_i}{N} \right)$$

where n_i is number of seeds germinated; d_i , time after sowing began (in days); and N , total number of seeds tested.

2.3 Enzymatic activity

In addition to *serotiny* and the heat index, the *germination stage* was included as a main factor rather than rejected *site*

Table 1 Values obtained according to heat index (temperature (in degrees Celsius) multiplied by the natural logarithm of exposure time plus one (in minutes)) for both the simulated fire scenarios (seeds enclosed in cones and released) according to the selected temperatures and exposure times

Time (min)	70 °C	120 °C	170 °C	220 °C	270 °C
1	48.52	83.18	117.84	152.49	187.15
2	76.9	131.83	186.76	241.7	296.63
5	125.42 ^a	215.01 ^a	304.6 ^a	394.19 ^a	483.78 ^a
10	167.85	287.75	394.19	527.54	–
20	213.12	365.34	517.57	669.8	–

Italicised values indicate the heat indices used in the released seed scenario

^a Border heat indices used in the enclosed seed scenario

and storage *period*. Based on previous studies (Acevedo and Cardemil 1997; Thanos 2000), four germination stages (GERMSTAGE) were selected after counting the days after seeds had been sown in petri dishes: 0, 5, 10 and 15 days.

Cones' serotiny was characterised (as previously described), and released seeds were obtained (about 5,000 seeds from SEMID). Seeds were sampled randomly to conform sets by weighing 15 g (about 700 seeds), which were placed in the muffle furnace; heat at 70, 120 and 170 °C was applied for 1 min (Salvatore et al. 2010). Sets were obtained (replicated for both cone types) with four heat indices: 0.00, 48.52, 83.17 and 117.84. Some 2.5 g of non-germinated seeds from each germination stage were sampled, including 2.5 g of germinated seeds (geotropic radicle >1 mm) for day 15. α -amylase and protease activities were assessed in a BIO-TEK EL-340i spectrophotometer (540 and 280 nm absorbance), following Bernfeld (1951) and Firenzuoli et al. (1968):

- α -amylase activity: 48 subsamples were prepared (crushed, mixed with cold buffer and centrifuged). The lipid fraction covering the supernatant was removed and stored (2 °C), and a solution containing 1 % starch (1 g starch, and phosphate buffer 20 mM, pH6.9) was added. Activity was expressed as α -amylase units per seed gram (a unit of α -amylase releases 1 μ mol of soluble groups (maltose) from soluble starch per minute (at 25 °C, pH6.9)).
- protease activity: haemoglobin (5 g) was used in urea solution, and 50 ml phosphate buffer was added (250 mM, pH7.0). Activity was expressed as the number of protease units per gram of seeds (amount of enzyme required to increase a unit in absorbance (at 280 nm) under the assay conditions).

2.4 Statistical analysis

General linear models were used to assess an overall model including variables that contribute significantly to prediction. Relationships were checked to select independent variables and their interactions, but non-realistic effects were excluded. In the enclosed seed scenario, sampling site (STAND) and serotiny (SEROT) were considered categorical factors; heat index (HEAT) was included as a quantitative factor. In addition, storage time (STORE) was added as a quantitative factor in the enclosed seed scenario. For the enzymatic activity experiment, germination stage was added (GERMSTAGE) as a quantitative factor.

Kruskal–Wallis tests were used to check the mean significant differences among the independent variables. Significant differences between groups were tested using simple ANOVA using the Duncan's test method (showing a low rate for error type I, but a high error type II). Single regression models were

used with dummy variables to compare regressions (interception point and slope) and to obtain relationships between the recorded parameters (G, MGT and enzymatic activity) and the heat index variable, including the influential factors obtained. Values were transformed using logarithms, square roots and inverse functions to maximise Pearson's correlation coefficient (r) to achieve normality assumptions and homoscedasticity. However, values were shown as untransformed, along with a standard error ($\pm$ SE). Statistical analyses were performed by the Statgraphics CENTURION XV software, using a critical p value of <0.05.

3 Results

3.1 Enclosed seed scenario

The protective role of the cones simulating a crown fire was checked, and germination tests were done with the seeds heated inside the cones (Table 2). GLM showed the significant influential factors relating to G ($R^2=44.08\%$) and MGT ($R^2=81.51\%$), which were STAND, SEROT, and interactions STAND $\times$ SEROT, STAND $\times$ HEAT and STAND $\times$ SEROT $\times$ HEAT. According to the Wilks' lambda value, STAND, STAND $\times$ SEROT and the latter interaction explained the majority of variability.

An analysis of variance and multiple regressions were done to obtain the relationships of influential factors with germination. By considering STAND, SEROT, HEAT and the STAND $\times$ SEROT interaction, ANOVAs (Table 2) were done and showed positive relationships between the burning and germination responses at both sites. When heat was absent, higher G in S cone seeds sampled in SEMID than in HUMID were obtained, but no differences were noted in those from NS cones. Likewise, germination was delayed (2 days) in the seeds from the S cones sampled in HUMID, with no differences found relating to STAND from those seeds in NS cones. After heating, the G values were above 70 % (the lowest value was 68.71 ± 1.77), which is usual in this species. In general, heating enclosed seeds affected neither G nor MGT, and even G increased with a higher heat index. A delay in the germination rate was related to SEROT, and the differences were even higher after heating.

Multiple regression analyses were done for G and MGT by taking the STAND $\times$ SEROT interaction and combining HEAT to conform a linear regression as the dummy variables (Table 3). The analysis with triple interactive factors ($R^2=47.63\%$) showed no differences for G in S cone-enclosed seeds. However, G differed for the seeds enclosed in NS cones in accordance with STAND, showing the highest value for the interception point, but the lowest value for slope than the regression representing the NS cone seeds sampled in HUMID. The regression lines representing MGT showed no

Table 2 Germination percentage and mean germination time (days) obtained in *P. halepensis* seeds in S Italy after heating closed cones (enclosed seed scenario) from two sites (HUMID and SEMID) in differentiating serotinous and non-serotinous cones according to the potential opening when cones were exposed to 45 °C

	HUMID						SEMID						
	C=73.21±1.76	70 °C	120 °C	170 °C	220 °C	270 °C	C=89.55±2.24	70 °C	120 °C	170 °C	220 °C	270 °C	
G													
S	1 min	71.50±2.15	81.88±4.20	68.71±1.77	82.00±3.47	82.13±1.84	1 min	94.38±1.68	91.50±3.03	91.45±3.28	92.08±1.40	92.67±2.24	
	2 min	79.50±2.04	78.00±2.38	83.25±3.48	75.88±3.29	82.13±2.07	2 min	92.75±2.30	90.58±4.07	94.25±1.26	96.00±1.20	93.08±1.40	
	5 min	74.20±3.15	86.88±2.39	87.75±1.92	81.75±3.51	79.25±1.01	5 min	96.00±1.15	96.33±1.14	90.91±2.90	92.50±2.05	84.25±4.31	
NS	C=81.91±0.98	70 °C	120 °C	170 °C	220 °C	270 °C	C=81.00±0.80	70 °C	120 °C	170 °C	220 °C	270 °C	
	1 min	77.75±2.26	79.92±2.53	76.75±2.10	82.42±1.36	85.00±1.58	1 min	67.40±2.16	74.08±1.70	64.50±1.55	77.42±2.67	83.42±2.18	
	2 min	82.00±1.43	79.58±1.18	81.58±2.32	82.92±1.98	80.83±1.88	2 min	71.00±3.11	70.33±1.33	75.33±2.33	71.09±2.11	86.08±2.13	
	5 min	78.92±2.12	83.00±2.40	79.92±1.74	81.33±1.18	87.42±1.46	5 min	74.67±1.51	71.33±1.96	81.55±1.56	74.25±1.75	84.42±2.32	
MGT													
	C=15.97±0.72	70 °C	120 °C	170 °C	220 °C	270 °C	C=13.94±0.38	70 °C	120 °C	170 °C	220 °C	270 °C	
S	1 min	12.63±0.35	14.07±0.38	13.56±0.28	13.46±0.24	13.76±0.12	1 min	16.43±0.12	14.25±0.13	15.21±0.13	14.89±0.17	14.15±0.26	
	2 min	12.93±0.31	13.31±0.39	12.84±0.18	14.13±0.48	13.44±0.12	2 min	15.69±0.21	14.94±0.10	15.88±0.17	14.01±0.28	14.09±0.54	
	5 min	13.26±0.86	13.14±0.21	13.62±0.21	13.20±0.26	13.54±0.36	5 min	15.53±0.07	15.32±0.05	16.37±0.29	13.20±0.32	13.51±0.41	
NS	C=11.06±0.20	70 °C	120 °C	170 °C	220 °C	270 °C	C=10.65±0.10	70 °C	120 °C	170 °C	220 °C	270 °C	
	1 min	10.56±0.24	9.88±0.13	10.36±0.16	11.25±0.27	10.50±0.24	1 min	8.92±0.32	9.07±0.14	9.35±0.31	9.92±0.22	9.61±0.12	
	2 min	10.21±0.20	10.49±0.17	10.39±0.21	11.46±0.25	10.37±0.22	2 min	9.11±0.10	8.97±0.14	9.22±0.19	9.33±0.23	9.92±0.27	
	5 min	10.02±0.22	10.22±0.14	9.66±0.12	10.88±0.28	10.82±0.21	5 min	9.23±0.10	9.34±0.22	9.21±0.25	9.59±0.21	10.24±0.30	

Averaged values (mean±standard error) without transformation are shown depending on the temperature reached and exposure time. The control values are included in the top left cell of each set (C).

G germination, *MGT* mean germination time, *HUMID* humid ombroclimate, *SEMID* semiarid ombroclimate, *S* serotinous, *NS* non-serotinous

Table 3 Multiple regressions relating the heat index and significant factors SEROT and STAND (as dummy variables) to obtain the germination percentage (%) and the mean germination time (days) of *P. halepensis* seeds for the enclosed and released seed scenarios

Enclosed seed scenario										
Factor	α_g	SE	β_g	SE	$G = \alpha_g + \beta_g \text{ HI}$	α_{mgt}	SE	β_{mgt}	SE	$MGT = \alpha_{mgt} + \beta_{mgt} \text{ HI}$
S*HUM	80.29	0.55	0.011	0.002	$p \text{ value} < 0.01$	13.25	0.08	-0.003	0.000	$p \text{ value} < 0.01$
S*SEM						10.51	0.11			
NS*HUM	94.34	0.99	0.001	0.000	$F\text{-ratio} = 146.19$	15.46	0.16			$F\text{-ratio} = 696.95$
NS*SEM	77.64	0.63	0.011	0.002	$R^2 = 47.63 \%$	9.58	0.12			$R^2 = 81.26 \%$
Released seed scenario										
Factor	α'_g	SE	β'_g	SE	$G' = \alpha'_g + \beta'_g \text{ HI}$	α'_{mgt}	SE	β'_{mgt}	SE	$MGT' = \alpha'_{mgt} + \beta'_{mgt} \text{ HI}$
S*HUM	61.05	1.76	-0.139	0.005	$p \text{ value} < 0.01$	18.41	0.87	-0.034	0.003	$p \text{ value} < 0.01$
S*SEM	70.90	2.11				11.93	1.49	0.027	0.006	
NS*HUM	80.06	2.11			$F\text{-ratio} = 238.01$	14.20	1.23	-0.022	0.003	$F\text{-ratio} = 73.44$
NS*SEM			-0.1116157	0.002	$R^2 = 54.00 \%$	18.38	1.23	-0.017	0.004	$R^2 = 41.56 \%$
1*HUM	72.05	1.64	-0.132	0.005	$p \text{ value} < 0.01$	19.92	0.65	-0.022	0.001	$p \text{ value} < 0.01$
1*SEM						14.46	0.83			
6*HUM	61.05	2.19			$F\text{-ratio} = 293.47$	19.92	0.65	-0.036	0.002	$F\text{-ratio} = 59.92$
6*SEM	72.05	1.64				13.26	0.83	-0.022	0.001	
24*HUM	60.33	2.19			$R^2 = 52.02 \%$	14.39	0.88			$R^2 = 33.18 \%$
24*SEM	72.05	1.64				13.40	0.83			
1*S	67.37	1.62	-0.132	0.005	$p \text{ value} < 0.01$	16.73	0.56	-0.026	0.002	$p \text{ value} < 0.01$
1*NS	72.73	2.19						-0.017	0.003	
6*S	67.37	1.62			$F\text{-ratio} = 402.10$			-0.304	0.002	$F\text{-ratio} = 140.04$
6*NS								-0.022	0.001	
24*S					$R^2 = 49.73 \%$	14.88	0.74	-0.026	0.002	$R^2 = 27.78 \%$
24*NS						12.64	1.38	0.036	0.007	

G germination percentage for enclosed seed scenario; α_g interception point ; β_g slope of linear regression; MGT mean germination time for enclosed seed scenario; G' germination percentage for released seed scenario; α'_g : interception point ; β'_g : slope of linear regression; MGT' : mean germination time for released seed scenario; SE standard error; HI heat index; S serotinous cones; NS non-serotinous cones; HUM humid ombroclimate; $SEMID$ semiarid ombroclimate; $1ST$ time of storage lower than one month; $6ST$ seeds enclosed in cones for 6 months; $24ST$ seeds enclosed in cones for 2 years.

differences for the slope value, but the interception point was dependent on both STAND and SEROT with high values for those seeds from S cones at each site.

3.2 Released seed scenario

GLM showed significant factors relating to G ($R^2 = 58.19 \%$) and MGT ($R^2 = 65.84 \%$). The significant influential factors found were STAND and HEAT, in addition to interactions STAND $\times$ SEROT, STAND $\times$ HEAT, SEROT $\times$ HEAT, SEROT $\times$ STORE and STAND $\times$ SEROT $\times$ HEAT. STORE also related significantly to MGT . ANOVAs were done using G and MGT as variables, and with STAND, SEROT, HEAT and interaction STAND $\times$ SEROT as factors (Table 4). By performing germination tests with non-heated seeds, it was confirmed that the highest G was found in the seeds from S cones sampled in SEMID than in HUMID, but no differences were obtained in the seeds from NS cones. Delayed germination was encountered in the seeds sampled in SEMID and in those enclosed in S cones. When considering STORE, the highest G value was found for short-term storage ($35.34 \pm 2.06 \%$), which lowered the longer storage

time became (30.88 ± 1.97 and $29.47 \pm 1.91 \%$ for 6 and 24 months, respectively). After heating, and without including STORE, G decreased the higher the heat index became. In HUMID, G dropped below 30 % when the heat index exceeded 215 (120 °C, 5 mins), meanwhile G in SEMID was above 50 % with heat index values of 365 (120 °C, 20 mins). MGT was higher in seeds from HUMID, irrespectively of SEROT. For each site, in general, the seeds enclosed in S cones had a higher MGT than those in NS.

The multiple regression analysis for G and MGT included interactions STAND $\times$ SEROT $\times$ HEAT and STAND $\times$ STORE $\times$ HEAT as the dummy variables (Table 3). The analysis with triple interactive factors ($R^2 = 47.63 \%$) showed no differences for G in the seeds enclosed in S cones, irrespectively of STAND. However, the G of those seeds enclosed in NS cones differed according to STAND, showing the highest interception point value, but the lowest value for slope, for the seeds from NS cones sampled in HUMID. The regression lines representing MGT showed no differences according to the slope value, but interception was dependent on both, with higher values for those seeds from S cones at each site.

Table 4 Germination percentage and the mean germination time (days) obtained in *P. halepensis* seeds in S Italy after heating seeds directly (released seed scenario) from two sites (HUMID and SEMID) in differentiating serotinous and non-serotinous cones according to the potential opening when cones were exposed to 45 °C

	HUMID					SEMID				
		70 °C	120 °C	170 °C	220 °C		70 °C	120 °C	170 °C	220 °C
G	C=70.18±1.91					C=83.31±1.61				
	2 min	38.25±6.21	52.67±3.32	0.00±0.00	0.00±0.00	2 min	70.33±2.17	72.92±2.65	57.00±2.32	0.00±0.00
	5 min	48.92±6.23	32±2.57	0.00±0.00	0.08±0.08	5 min	67.25±2.45	71.25±2.21	0.33±0.26	0.00±0.00
	10 min	46.83±4.12	6.33±3.04	0.00±0.00	0.00±0.00	10 min	72.33±2.38	68.25±2.05	0.17±0.17	0.00±0.00
	20 min	53.42±6.50	1.42±0.83	0.00±0.00	0.00±0.00	20 min	79.50±2.25	41.75±2.36	0.17±0.17	0.00±0.00
NS	C=87.40±1.55					C=81.00±0.65				
	2 min	79.50±2.66	62.67±5.97	5.08±1.39	10.00±4.37	2 min	61.17±1.84	49.33±3.33	0.08±0.08	0.00±0.00
	5 min	66.92±5.76	55.25±5.66	0.08±0.08	1.67±0.74	5 min	56.08±2.34	44.75±2.27	0.00±0.00	0.00±0.00
	10 min	69.08±5.86	14.00±3.67	0.00±0.00	0.00±0.00	10 min	55.33±3.09	40.00±4.17	0.00±0.00	0.00±0.00
	20 min	69.50±6.93	1.75±0.63	0.00±0.00	0.00±0.00	20 min	60.17±2.83	51.17±2.85	0.00±0.00	0.00±0.00
MGT	C=15.38±0.45					C=12.17±0.35				
	2 min	18.81±1.17	18.37±0.78	170 °C	220 °C	2 min	11.31±0.22	10.52±0.20	170 °C	220 °C
	5 min	16.41±1.14	21.47±0.54	–	–	5 min	11.58±0.19	11.48±0.07	14.46±0.17	–
	10 min	16.61±0.77	22.20±1.05	–	–	10 min	11.84±0.60	17.28±0.10	21.00±0.00	–
	20 min	18.42±1.14	28.00±0.00	–	–	20 min	12.12±0.63	20.21±0.35	22.00±0.00	–
S	C=13.39±0.40					C=10.65±0.08				
	2 min	14.86±0.62	16.97±0.77	170 °C	220 °C	2 min	70 °C	120 °C	170 °C	220 °C
	5 min	13.62±0.94	16.59±0.91	24.92±1.17	21.20±0.53	5 min	12.04±0.38	10.39±0.32	25.00±0.00	–
	10 min	15.01±0.71	23.64±0.88	28.00±0.00	25.95±1.07	10 min	10.64±0.30	11.15±0.57	–	–
	20 min	14.70±0.83	24.70±0.77	–	–	20 min	9.45±0.23	17.18±0.39	–	–

Averaged values (mean±standard error) without transformation are shown depending on the temperature reached and exposure time. The control values are included in the top left cell of each set (C)
G germination, *MGT* mean germination time, *HUMID* humid ombroclimate, *SEMID* semiarid ombroclimate, *S* serotinous, *NS* non-serotinous

The analysis relating STAND and SEROT to G ($R^2=54.00\%$) found differences for interception point and slope (Table 3). The seeds enclosed in NS cones had a higher interception point, while those sampled in SEMID had a higher slope, thus confirming the results obtained in the previous paragraph. When relating STORE and STAND to G ($R^2=52.02\%$), the same slope was found, but the highest interception point was for the seeds sampled from SEMID, irrespectively of STORE. According to interaction STORE $\times$ SEROT ($R^2=49.73\%$), differences were found only in the seeds from NS cones with short-term storage, showing a higher interception point. The relationships with MGT had low variation percentages since the sets with non-germinated seeds presented no MGT values. The results differed for almost all sets, although relevant differences were observed. Only a positive slope, when relating heat index and MGT, was found in the seeds from S cones in SEMID. According to STORE and STAND, the seeds from HUMID had higher MGT values than those from SEMID during all the storage periods. In addition, the interception point decreased in long-term storage periods.

3.3 Enzymatic activity

α -Amylase and protease activities were recorded in non-heated seeds. GLMs analysed them to find that SEROT, GERMSTAGE and interaction SEROT $\times$ GERMSTAGE were significantly related to protease ($R^2=64.93\%$). Only interaction SEROT $\times$ GERMSTAGE influenced α -amylase ($R^2=72.38\%$). When the heating effect was included, GLM showed that all the factors influenced both activities, protease ($R^2=47.83\%$) and α -amylase ($R^2=55.44\%$), except GERMSTAGE $\times$ HEAT for the latter. ANOVAs were run, which significantly related both enzymes' activity to interaction SEROT $\times$ GERMSTAGE according to HEAT (Table 5). The non-heated seeds from NS cones displayed diminishing activity according to the post-sowing time. However, the α -amylase activity in the seeds obtained from S cones increased, although the effect was not significant for protease. Significantly greater α -amylase activity was found in the control than in the burned sets, while GERMSTAGE positively related to both enzyme activities in the seeds sampled from S cones but related negatively for those from NS. Generally after burning, α -amylase activity lowered if seeds were exposed to a higher heat index, but the opposite can be said for protease activity. In the seeds from both cone types, the general protease activity pattern changed when exposed to heat index 117.84 and increased according to the post-sowing time.

When comparing the regression lines for interaction SEROT $\times$ GERMSTAGE and HEAT ($R^2=65.88\%$ and $R^2=52.67\%$, respectively, for α -amylase and protease activity) (Table 6), HEAT positively related to α -amylase activity but negatively to protease activity. For α -amylase activity, higher slope values were found in the seeds from S cones sampled

Table 5 Averaged values for α -amylase (α) and protease (β) activities (units per gram) obtained in the *P. halepensis* seeds in S Italy in four different germination stages after heating seeds directly

SEROT	GERMSTAGE	HI=0		HI=48.52		HI=83.18		HI=117.84	
		α_0	β_0	$\alpha_{48.52}$	$\beta_{48.52}$	$\alpha_{83.18}$	$\beta_{83.18}$	$\alpha_{117.84}$	$\beta_{117.84}$
S	0	0.405 $\pm$ 0.011	0.101 $\pm$ 0.070	0.070 $\pm$ 0.001	0.035 $\pm$ 0.035	0.268 $\pm$ 0.017	0.035 $\pm$ 0.035	0.263 $\pm$ 0.003	0.082 $\pm$ 0.082
	1	0.193 $\pm$ 0.022	0.023 $\pm$ 0.022	0.013 $\pm$ 0.002	0 $\pm$ 0	0.087 $\pm$ 0.033	0 $\pm$ 0	0.047 $\pm$ 0.001	0.035 $\pm$ 0.022
	10	0.483 $\pm$ 0.107	0.043 $\pm$ 0.018	0.173 $\pm$ 0.040	0.035 $\pm$ 0.035	0.130 $\pm$ 0.038	0.078 $\pm$ 0.009	0.140 $\pm$ 0.002	0.106 $\pm$ 0.074
	15	0.668 $\pm$ 0.021	0.082 $\pm$ 0.014	0.097 $\pm$ 0.003	0.057 $\pm$ 0.043	0.086 $\pm$ 0.001	0.001 $\pm$ 0.001	0.028 $\pm$ 0.022	0.185 $\pm$ 0.071
NS	0	0.620 $\pm$ 0.087	0.488 $\pm$ 0.129	0.113 $\pm$ 0.016	0 $\pm$ 0	0.171 $\pm$ 0.039	0 $\pm$ 0	0.247 $\pm$ 0.039	0 $\pm$ 0
	1	0.233 $\pm$ 0.031	0.065 $\pm$ 0.028	0.016 $\pm$ 0.002	0 $\pm$ 0	0.105 $\pm$ 0.030	0 $\pm$ 0	0.049 $\pm$ 0.002	0.171 $\pm$ 0.082
	10	0.058 $\pm$ 0.000	0.132 $\pm$ 0.049	0.020 $\pm$ 0.001	0.029 $\pm$ 0.029	0.107 $\pm$ 0.011	0.240 $\pm$ 0.125	0.029 $\pm$ 0.000	0.417 $\pm$ 0.043
	15	0.049 $\pm$ 0.004	0.024 $\pm$ 0.007	0.014 $\pm$ 0.000	0.002 $\pm$ 0.002	0.088 $\pm$ 0.005	0.170 $\pm$ 0.018	0.0756 $\pm$ 0.029	0.248 $\pm$ 0.124

Averaged values (mean $\pm$ standard error) without transformation are shown

HI/heat index dependent on the temperature reached and exposure time, SEROT type of cones according to the potential opening when cones were exposed to 45 °C, S serotinous cones, NS non-serotinous cones, GERMSTAGE germination stage, days after seeds were sown in petri dishes (0, 1, 10 and 15 days)

Table 6 Multiple regressions relating the heat index and significant factors SEROT and STORE (as dummy variables) to obtain the enzyme activity for α -amylase (Amylase, units g^{-1}) and protease (Protease, units per gram) of the *P. halepensis* seeds sampled in S Italy

Factor	A_a	SE	B_a	SE	Amylase= A_a+B_a HI	A_p	SE	B_p	SE	Protease= A_p+B_p HI
S*0GS	0.252	0.038	0.003	0.001	p value<0.01	0.052	0.014	-0.004	0.001	p value<0.01
S*5GS	0.085	0.054								
S*10GS	0.413	0.077								
S*15GS	0.546	0.077	0.008	0.001	F -ratio=10.23					
NS*0GS	0.476	0.077	0.006	0.001		0.372	0.058			F -ratio=16.42
NS*5GS	0.101	0.054	0.003	0.001	$R^2=65.88\%$	0.052	0.014			$R^2=52.67\%$
NS*10GS	0.054	0.054						-0.002	0.001	
NS*15GS	0.057	0.054								

A interception point, B slope of linear regression, SE standard error, HI heat index, S serotinous cones, NS non-serotinous cones, $0GS$ beginning of germination stage, $5GS$ 5 days after seed sown, $10GS$ 10 days after seed sown, $15GS$ 15 days after seed sown

from the last germination stage and in the seeds from NS cones sampled immediately after sowing. The interception point value increased in the seeds from S cones but decreased in the NS cone seed in accordance to time that had passed since germination began. For protease activity, the same values in the S cones seeds were obtained, whereas for the NS cone seed, the highest interception point was noted in the first germination stage, with higher slopes in the last two stages.

4 Discussion

Special anatomical and morphological characteristics confer serotinous cones heat insulation (Moya et al. 2008b). The seeds enclosed in them also develop greater fire resistance, which is linked to thicker coats containing Si and Ca (Salvatore et al. 2010). Released and enclosed seed germination related to cone type and the time seeds in the cone were stored, which implies a staggered colonisation of obligate seeders adapted to xeric and fire-prone habitats (Herranz et al. 1999). Both cone types confer heat protection to the seeds enclosed inside them when simulating light and moderate fire severities, but effects vary depending on provenance (Goubitz et al. 2003; Habrouk et al. 1999; Moya et al. 2008b). In addition, the fact that seeds are enclosed in cones might improve the percentage and speed of germination in moderate fire severity conditions, similarly to obligate seeder species whose germination is stimulated by fire, which reveals that seedlings develop faster and healthier under post-fire conditions (Enright and Lamont 1989; Moreira et al. 2010).

As with other studies, increasing fire intensity reduces the percentage of germination and increases the germination rate of those seeds not enclosed in cones (Habrouk et al. 1999; Martinez-Sanchez et al. 1995; Nuñez et al. 2003). Released seeds are negatively affected by heat, and a lower germination rate is presented in seeds stored for a 2-year period. A heat index value of about 200 is the initial point for reduced germination, but non-living seeds can be found with a value

as of 550. The general pattern is similar to other serotinous species occurring in fire-prone areas (Ne'eman et al. 2009).

Heating also influences physiological variables and relates positively to α -amylase activity, but negatively to protease activity, mainly in the seeds obtained from serotinous cones. Enzymatic activities are also related to the post-sowing time of seeds, similarly to embryo development in other conifers (Misra 1994), showing greater α -amylase activity, but lower protease activity, in those seeds released from serotinous cones. In *Pinus sylvestris*, protease mobilises the protein reserve of germinating seeds (Salmia 1981a), while amylase breaks down starch reserves into sugars to supply energy to the embryo in early germination phases (Salmia 1981b). Therefore, protease and α -amylase activities in *P. halepensis* prevents early emergence of those seeds enclosed in serotinous cones, thus avoiding germination immediately after fire to promote seed survival. Both enzyme activities are negatively affected by heating, although the effect is noted more in those seeds enclosed in non-serotinous cones, mainly when the germination period begins, which is expected since the onset of germination launches sugar demands due to respiratory activities (Salmia 1981b). Protease activity has higher values in the two last germination stages because of seedling development (Acevedo and Cardemil 1997). Given the influence on enzyme activities, heating delays the germination of those seeds enclosed in serotinous cones and improves seedling survival since the environmental conditions immediately after fire may be unfavourable. It allows the number of safe post-fire sites to increase and promote Aleppo pine recruitment by improving the recolonisation ability as in *Banksia* species (Enright and Lamont 1989), which also exhibit gradual seed release by increasing the survival recruitment opportunities (Clarke et al. 2010).

In conclusion, we confirm the hypothesis that the dual life strategy in *P. halepensis* (Goubitz et al. 2003) is not based merely on serotinous and non-serotinous cones in the canopy; rather, it is an adaption to cope during short and long periods

between fire events. We also confirm that serotiny does not just play protective and insulating roles but also influences the ecological and physiological characteristics of seeds towards fire resistance. Our results reinforce the hypothesis that widespread post-fire Aleppo pine recruitment (Nathan and Ne'eman 2004) is an adaptive trait to fire (Keeley et al. 2011) since ecophysiological responses to heat depend on the cone type in which seeds develop and are enclosed. In addition, heating affects the germination percentage and rate of seeds enclosed in non-serotinous cones more negatively than that explained by differences in enzymatic activity. We propose to consider this in post-fire restoration; i.e., salvaged logging should be delayed to promote the release of those seeds encased in serotinous cones to improve natural regeneration (Leone et al. 2000). In order to also assist natural post-fire regeneration, for instance, early tree density reduction should be carried out to accelerate stand maturity and to promote a canopy seed bank composed mainly of serotinous cones (Verkaik and Espelta 2006; Moya et al. 2007, 2008a). In addition, the morphologic differences in seedlings related to the seeds enclosed in serotinous cones should be studied to improve the scientific basis employed in restoring arid and semiarid areas in order to cope with changing fire regimes in the Mediterranean Basin.

Acknowledgments We thank the Codra Mediterranea SRL personnel who helped in this research. We would also like to thank Helen Warburton for the language review.

Funds Thanks are given to the Spanish R&D+I National Programme CICYT-AGL 2008-03602/FOR and CONSOLIDER-INGENIO 2010: MONTES (CSD 2008-00040) for the support and funds provided.

References

- Acevedo E, Cardemil L (1997) Regulation of α -amylase isoenzymes expression in *Araucaria araucona* by gibberellic and abscisic acids. *Phytochem* 44:1401–1405
- Ayari A, Moya D, Rejeb MN, Ben Mansoura A, Garchi S, de las Heras J, Hanchi B (2011) Alternative sampling methods to estimate structure and reproductive characteristics of Aleppo pine forest in Tunisia. *For syst* 3:348–360
- Bernfeld P (1951) Enzymes of starch degradation and synthesis. In: Nord FF (ed) *Advances in enzymology*. Interscience Publications Inc, New York, pp 379–424
- Bewley JD, Black M (1994) *Seeds: physiology of development and germination*. Plenum, New York
- Clarke PJ, Knox KJE, Butler D (2010) Fire intensity, serotiny and seed release in 19 woody species: evidence for risk spreading among wind-dispersed and resprouting syndromes. *Aust J Bot* 58:629–636
- DeBano L (1982) Assessing the effects of management actions on soils and mineral cycling in mediterranean ecosystems. In: Conrad CE, Oechel WC (eds) *Proceedings of the symposium on dynamics and management of Mediterranean type ecosystems*. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, GTR PSW-58, pp 345–350
- Enright NJ, Lamont BB (1989) Seed banks, fire season, safe sites and seedling recruitment in five co-occurring *Banksia* species. *J Ecol* 4:1111–1122
- Firenzuoli AM, Vanni P, Mastronuzzi E, Zanobini A, Baccari V (1968) Enzymes of glyoxylate in conifers. *Plant Physiol* 43:1125–1128
- Goubitz S, Werger MJA, Ne'eman G (2003) Germination response to fire-related factors of seeds from non-serotinous and serotinous cones. *Plant Ecol* 169:195–204
- Habrouk A, Retana J, Espelta JM (1999) Role of heat tolerance and cone protection of seeds in the response of three pine species to wildfires. *Plant Ecol* 145:91–99
- Herranz JM, Ferrandis P, Martínez-Sánchez JJ (1999) Influence of heat on seed germination of nine woody Cistaceae species. *Int J Wildland Fire* 9:173–182
- Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA (2011) Fire as an evolutionary pressure shaping plant traits. *Trends Plant Sci* 16:406–411
- Lamont B, Lemaitre D, Cowling R, Enright N (1991) Canopy seed storage in woody-plants. *Bot Rev* 57:277–317
- Leone V, Saracino A, Trabaud L, Vélez R (2000) Fire prevention and management policies in West Mediterranean pine forests. In: Ne'eman G, Trabaud L (eds) *Ecology, biogeography and management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean Basin*. Backhuys, Leiden, pp 335–353
- Martínez-Sánchez JJ, Marin A, Herranz JM, Ferrandis P, De las Heras J (1995) Effects of high temperatures on germination of *Pinus halepensis* Mill. and *P. pinaster* Aiton subsp. *pinaster* seeds in southeast Spain. *Vegetatio* 116:69–72
- Misra S (1994) Conifer zygotic embryogenesis, somatic embryogenesis and seed germination: Biochemical and molecular advances. *Seed Sci Res* 4:357–384
- Moreira B, Tormo J, Estrelles E, Pausas JG (2010) Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Ann Bot* 105:627–635
- Moya D, Espelta JM, Verkaik I, López-Serrano FR, de las Heras J (2007) Tree density and site quality influence on *Pinus halepensis* Mill. reproductive characteristics after large fires. *Ann For Sci* 64:649–656
- Moya D, De las Hkeras J, López-Serrano FR, Leone V (2008a) Optimal intensity and age management in young Aleppo pine stands for post-fire resilience. *For Ecol Manag* 255:3270–3280
- Moya D, Saracino A, Salvatore R, Lovreglio R, De las Heras J, Leone V (2008b) Anatomic basis and insulation of serotinous cones in *Pinus halepensis* Mill. *Trees: Struct Funct* 22:511–519
- Nathan R, Ne'eman G (2004) Spatiotemporal dynamics of recruitment in Aleppo pine (*Pinus halepensis* Miller). *Plant Ecol* 171:123–137
- Ne'eman G, Ne'eman R, Keith DA, Whelan RJ (2009) Does post-fire plant regeneration mode affect the germination response to fire-related cues? *Oecologia* 159:483–492
- Núñez MR, Bravo F, Calvo L (2003) Predicting the probability of seed germination in *Pinus sylvestris* L. and four competitor shrub species after fire. *Ann For Sci* 60:75–81
- Paula S, Pausas JG (2008) Burning seeds: germinative response to heat treatments in relation to resprouting ability. *J Ecol* 96:543–552
- Quezel P (2000) Taxonomy and biogeography of Mediterranean pines (*Pinus halepensis* and *P. brutia*). In: Ne'eman G, Trabaud L (eds) *Ecology, biogeography and management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean Basin*. Backhuys, Leiden, pp 1–12
- Reyes O, Casal M (2002) Effect of high temperatures on cone opening and on the release and viability of *Pinus pinaster* and *P. radiata* seeds in NW Spain. *Ann For Sci* 59:327–334
- Salmia A (1981a) Proteinase activities in resting and germinating seeds of scots pine, *Pinus sylvestris*. *Physiol Plantarum* 53:39–47

- Salmia A (1981b) Peptidases, proteinases, and proteinase inhibitors in resting and germinating seeds of Scots pine, *Pinus sylvestris*. Department of Botany, University of Helsinki 7:1–33
- Salvatore R, Moya D, Pulido L, Lovreglio R, López-Serrano FR, De las Heras J, Leone V (2010) Advances on anatomy and morphology of Aleppo pine serotinous seeds. *New For* 39:329–341
- Saracino A, Pacella R, Leone V, Borghetti M (1997) Seed dispersal and changing seed characteristics in a *Pinus halepensis* Mill. forest after fire. *Plant Ecol* 130:13–19
- Tapias R, Gil L, Fuentes-Utrilla P, Pardos JA (2001) Canopy seed banks in Mediterranean pines of south-eastern Spain: a comparison between *Pinus halepensis* Mill., *P. pinaster* Ait., *P. nigra* Arn. and *P. pinea* L. *J Ecol* 89:629–638
- Thanos CA (2000) Ecophysiology of seed germination in *Pinus halepensis* and *P. brutia*. In: Ne'eman G, Trabaud L (eds) Ecology, biogeography and management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean Basin. Backhuys, Leiden, pp 37–50
- Thanos CA (2004) Bradychory—the coining of a new term. In: Ariannoutsou M, Papanastasis V (eds) Ecology, conservation and management of Mediterranean climate ecosystems. Proceedings 10th MEDECOS Conference. Millipress, Rotterdam, pp 1–6
- Verkaik I, Espelta JM (2006) Post-fire regeneration thinning, cone production, serotiny and regeneration age in *Pinus halepensis*. *For Ecol Manag* 231:155–163
- Wang X, Cao Z (2010) Water extract of *Alnus nepalensis* D. Don. to the seeds germination of *Pinus yunnanensis* Faranch. Effects of physiological and biochemical mechanism. In: Hu Z, Ma P (eds) 2nd International Workshop on Database Technology and Applications. Wuhan university, wuhan, pp 1–4. doi:[10.1109/DBTA.2010.5659070](https://doi.org/10.1109/DBTA.2010.5659070)