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Germination response to fire-related factors of seeds from non-serotinous and serotinous cones

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Abstract

Pinus halepensis, a Mediterranean pine tree, is a partially serotinous species: individual trees of this species carry both non-serotinous and serotinous cones. Serotinous cones open mainly after fire, whereas non-serotinous cones open in absence of fire. In this study we addressed the question, whether or not this cone response is linked with the germination response of seeds to fires. Two main factors associated with fire are heating of seeds and soil pH. A combination of high heat and high pH simulates a scenario with fire, whereas low heat and low pH simulates a scenario without fire. We assessed the separate and combined effects of heat and pH on the germination rate and the percentage of germination of seeds from non-serotinous cones and two age classes of serotinous cones of *P. halepensis*. Heat had no effect on the percentage of germination of seeds from any of the cone types, but did positively affect the germination rates of seeds from both age-classes of serotinous cones. High pH negatively affected the germination rate of seeds from all cone types as well as the percentage of germination of seeds from non-serotinous cones. The combinations of heat and pH had different effects on the three cone types: percentage of germination and rate of germination of seeds from non-serotinous cones was higher in the combination high heat-high pH than in the combination low heat-low pH. In the combination high heat-high pH, seeds from serotinous cones germinated better than seeds from non-serotinous cones. The different germination responses of seeds from non-serotinous and serotinous cones could not be attributed to differences in cone age. Our results indicate that the cone response is linked to the germination response of the seeds in *P. halepensis*, with seeds from serotinous cones being more tolerant to fire related factors.

Introduction

Many species in Mediterranean-type ecosystems are resilient to frequent wildfires (Keeley 1994; Trabaud 1987). Some species survive by post-fire resprouting, but do not have post-fire seedling recruitment. Other species have adults that do not survive fires, and post-fire germination and seedling establishment is required for post-fire regeneration (Whelan 1995; Bond and van Wilgen 1996). Seed germination can be affected by several fire-related factors such as heating temperature (Herranz et al. 1998; Nunez and Calvo 2000), ash (Henig-Sever et al. 1996; Reyes and Casal 1998), nitrogen supply (Broncano et al. 1998), litter cover (Lamont et al. 1993; Trabaud and Renard 1999), smoke (Keeley and Fotheringham 1998), ethylene (Ne'eman et al. 1999) and soil water potential (Thanos and Skordilis 1987). Seeds can germinate after fire from soil seed banks, like members of the *Cistaceae* and many legumes (Thanos 2000; Hanley and Fenner 1998) or from canopy seed banks, like some Conifers (Lamont 1991; Keeley and Zedler 1998). Canopy seed storage, or serotiny, is a common phenomenon in species growing in fire-prone areas (Cowling et al. 1987; Lamont 1991; Whelan 1995; Enright et al. 1998). Seeds stored in serotinous cones or fruits are released after fire and may profit from improved post-fire conditions for seed germination and seedling establishment (Ne'eman et al. 1993; Henig-Sever et al. 2000).

The degree of serotiny varies among species and populations (Cowling and Lamont 1985; Tinker et al. 1994; Whelan et al. 1998; Midgley 2000). This variation is often attributed to variation in fire frequency (Gauthier et al. 1996; Bradstock et al. 1994; Enright et al. 1998). The degree of serotiny can also vary among individuals of the same species (Lamont et al. 1991; Gauthier et al. 1993). Some individuals are partially serotinous to allow regeneration also in absence of fire. A good example is *Pinus halepensis* Mill., a lowland Mediterranean pine, with individual trees carrying both non-serotinous and serotinous cones (Panetsos 1981). The partial serotiny of *P. halepensis* reflects its dual life strategy with two different seedling recruitment scenarios (Nathan and Ne'eman 2000). The first deals with recruitment of seedlings from seeds of non-serotinous cones, which are released annually under warm and dry 'Sharav' weather conditions (Nathan et al. 1999). These seeds germinate in open or disturbed habitats, like the forest edge (Richardson 2000). In the second scenario, seeds from serotinous cones, which are released after fire, germinate in the post-fire environment. However, part of the serotinous cones open also in the absence of fire, under warm and dry weather conditions (Nathan et al. 1999). Therefore, Nathan et al. (1999) hypothesised that serotiny in *P. halepensis* might have evolved in synchrony with dry weather conditions rather than being associated with fire. This seems supported by several studies on *P. halepensis*, which demonstrated a negative correlation between germination and fire related factors, such as seed heating temperature and exposure time (Martínez-Sánchez et al. 1995; Hanley and Fenner 1998; Escudero et al. 1999; Nunez and Calvo 2000) and the high pH of the soil (Henig-Sever et al. 1996). A negative influence of fire related factors on germination contradicts the idea that serotiny is an adaptation to fire.

We argue that fire related selection for serotinous cones should be accompanied by a selection for seeds that tolerate high temperatures and high pH, caused by fire. We hypothesize that the cone response is linked to the seed germination response to fire. If this is the case, experiments on germination responses to fire related factors should make a clear separation between seeds from serotinous and non-serotinous cones. We use *P. halepensis* as a model to examine if fire affects germination success of seeds from serotinous and non-serotinous differently. Germination success of *P. halepensis* seeds depends not only on the percentage of germination, but also on the germination rate. The rate is important because competition for water with other (fast) germinating species is crucial to their survival in the post-fire environment (Ne'eman 1997). We use seed heating temperature and pH as fire related test factors. Heat and pH occur in specific combinations: low heat and low pH co-occur in natural conditions without fire, whereas high heat and high pH co-occur in case of a fire. We use these specific combinations to simulate two scenarios: with and without fire. To be able to assess the separate and combined effects of heat and pH on germination rate and the percentage of germination we also use crossed combinations of heating and pH. We expect the combination high heat-high pH to have no negative effect, or at least a smaller negative effect, on the germination rate and the percentage of germination of seeds from serotinous cones in comparison to seeds from non-serotinous cones. To check for cone age related differences we use young serotinous cones, of similar age as non-serotinous cones, and older serotinous cones.

Methods

This study was carried out in a natural population of *P. halepensis* in Israel, located in the Carmel National Park on Mount Carmel. This area has a typical Mediterranean climate with moderate wet winters and hot dry summers. Cones were collected from mature trees at the end of October 2000, when the season of seed dispersal had ended. Mature cones of *P. halepensis* can be classified as three groups.

1. Mature cones of the current crop that open readily in the same season (non-serotinous cones).
2. Mature cones of the current crop, which remain closed for at least one more growth season (new serotinous cones).
3. Old mature closed cones, which have already been stored in the canopy for more than one year (old serotinous cones).

The young cones could easily be distinguished from older cones by their brown color, as older cones were gray. Young mature cones were defined as non-serotinous when scales were partially opened or when they could be opened under light hand-pressure. Cones, of which more than 25% of the scales were open, were excluded from cone collection. Young mature brown cones that had no opened scales and could not be opened by hand pressure were defined as new serotinous cones. Gray closed cones of more than 4 years old were defined as old serotinous cones. Cones always grow out on new shoots, therefore cone age was determined by counting the wood-rings of the attached twig.

We collected at random 100 cones of each type from 25 trees at several locations. Cones were placed in an oven at 80 °C for 10 minutes, enough to break the scale connections, but too short to allow bending of the scales. Thus, heat-shock to the seeds was avoided. Subsequently all cones were placed in an oven, in individual paper bags, at 40 °C for 2 days to allow full cone opening and seed retrieval. Per cone type, we divided the cones in 14 groups of 4 cones and selected from each group 80 seeds, which were divided over the four different treatments (n = 20 seeds per treatment). Each group of 4 cones represented one replicate in each treatment.

The treatments consisted of different combinations of seed-heating temperature and pH values. Heating values were set at 40 °C, a temperature similar to the normal outside temperature in the research area in summer, and 100 °C, a temperature assumed to be present within cones in a fire (Fraver 1992). These temperatures are within the range of many other studies on effects of seed heating in pines (Martínez-Sánchez et al. 1995; Reyes and Casal 1995; Izhaki et al. 2000). The pH values were chosen based on field data from the research area; pH 7 as the value for unburned soil and pH 10 as the value of the ash layer after a forest fire (Henig-Sever et al. 1996). Heat treatment included heating the seeds (after being retrieved from the cones) at 40 °C or 100 °C for 5 minutes. The pH treatment included a 5 ml solution of pH 7 (made from 0.05 *M* Bis-Tris propane buffer) or a 5 ml solution of pH 10 (made from 0.05 *M* CAPS buffer). Henig-Sever et al. (1996) showed that these are the pH values of the soil in the field and that there is no difference between germination in distilled water and the solution with pH 7 (made from the Bis-Tris propane buffer at 0.05 *M*). Subsequently, 14 petri dishes per treatment with 20 seeds each were placed in a growth chamber (Biotronette model 845–2, Lab-Line, IL). The growth chamber was set at optimum germination conditions of 20 °C and 12 hours of light (a mixture of 20 W cool white fluorescent light and 40 W incandescent lamps) (Henig-Sever et al. 1996). Two of the treatments represent the two main germination scenarios: heat 40 °C combined with pH 7, representing a scenario without fire, and heat 100 °C combined with pH 10, representing the scenario with fire. The crossed treatments allow us to separate the effects of heat and pH and examine their interactions.

The number of germinated seeds per petri dish was counted weekly up to 8 weeks, when no more seeds germinated. Seeds were selected visually on being ‘filled’ before being submitted to the treatments. Seeds that had not germinated after 8 weeks were dissected to check for contents. All seeds were found to contain regular seed contents and were assumed to be viable. The accumulated percentage of germinated seeds of the total number of seeds per dish per week was calculated. The final number of germinated seeds as a percentage of the of the total number of seeds per dish after 8 weeks represents the total percentage of germination. The slope of the relation between the weekly number of germinated seeds and time represents the germination rate. Thus the more seeds germinated each week, the higher the germination rate and the faster the final germination percentage was reached.

We statistically tested the effects of cone type, heat, and pH on the germination rate patterns over the 8-week period by submitting the data to a Cox-regression in a survival analysis of a standard SPSS package. This test is designed specifically for comparing differences in rates, taking into account the repeated-measure structure of the experiment, the not normal distribution of data and censored data (Fox 1993). The output of this test is comparable to that of regular regression tests (Underwood 1997). To test for the effects of cone type, heat and pH on the percentage of germination we used a 3-way ANOVA. Finally, we assessed the differences in the final percentage of germination and in germination rates between the combinations of high heat-high pH and low heat-low pH for each cone type and among cone types for each combination by one-way ANOVAs (Underwood 1997).

Results

Germination rate

The multiple Cox regression showed that germination rate was affected by cone type, heating temperature and pH separately as well as by their interaction (Table 1). Analyses of the effects of heating temperature and pH per cone type, showed that heating tempera-

Table 1. The results of a multiple Cox-regression in a survival analyses on the separate and interactive effects of cone type, heating and pH on germination rate. The score is similar to an F-value.

Variable	Test statistic	df	p
Cone type	9.4732	1	0.009*
Heat	7.6515	1	0.006*
pH	55.0265	1	< 0.001*
Cone × heat	2.9264	2	0.232
Cone × pH	18.7731	2	0.001*
Heat × pH	2.7798	1	0.096
Cone × heat × pH	6.5251	2	0.038*

* Significant with $p < 0.05$

ture had no effect on the germination rate of non-serotinous cones, but higher temperature increased the germination rate for both new and old serotinous cones at pH 10 (Table 2A). In general, pH 10 slowed down the germination rate for seeds from all cone types in comparison to pH 7 (Table 2A).

Subsequent analyses showed a significantly higher germination rate in the combination low heat-low pH (heating 40 °C and pH 7) than in the combination high heat-high pH (heating 100 °C and pH 10) for non-serotinous cones (Figure 1A, Table 2B). Both serotinous cone types showed no significant difference in germination rates between the two combinations (Figs. 1B and 1C, Table 2B).

Differences in germination rates of seeds of the three different cone types were not significant in the combination low heat-low pH (Figure 2A, Table 2C), whereas in the combination high heat-high pH, seeds from both serotinous cone types showed higher germination rates than seeds from the non-serotinous cones (Figure 2B, Table 2C).

Percentage of germination

The final percentage of germination after 8 weeks was not affected by cone type, heating temperature or pH separately, but only by the interaction of cone type and pH (Table 3). Heating temperature had no effect in any interaction and thus data were pooled over heating conditions for subsequent analyses. In that analysis, the effect of pH was checked per cone type. The results showed a significant inhibiting effect of the higher pH on the final percentage of germination of seeds from non-serotinous cones, whereas pH did not affect the final percentage of germination of seeds from serotinous cones (Table 4A).

Final percentage of germination of seeds from non-serotinous cones was higher in the combination low heat-low pH that in the combination high heat-high pH (Figure 1A, Table 4B). Seeds from both serotinous cone types germinated equally well in both combinations (Figures 1B and 1C, Table 4B).

Final percentage of germination did not differ significantly among seeds from the three different cone types in the combination low heat-low pH (Figure 2A, Table 4C), whereas in the combination high heat-high pH, seeds from both serotinous cone types showed a higher percentage of germination than seeds from the non-serotinous cones (Figure 2B, Table 4C).

Discussion

Our results showed that seeds from non-serotinous cones and serotinous cones reacted differently in terms of germination rate and percentage of germination to combinations of low heat-low pH and high heat-high pH. While differences were small they were significant throughout. Although several studies on partially serotinous conifers have focussed on effects of heat and pH on seed germination, possible differences between seeds of serotinous and non-serotinous cones have been largely ignored (Martínez-Sánchez et al. 1995; Reyes and Casal 1995; Escudero et al. 1999). When comparing our results with other studies, one should keep in mind that in other studies mixtures of serotinous and non-serotinous cones may have been used. Even when it is stated that only new cones were used (Hellum and Pelchat 1978), this crop may still consist of a mixture of non-serotinous and new serotinous cones, as defined in our methods.

Heat stimulates germination of Mediterranean plants with dormant seeds, such as certain species of the *Rhamnaceae*, *Cistaceae* and *Fabaceae* (Keeley 1994). Seed dormancy can be broken by heat shock (Keeley 1994), chemicals leached from charred wood (Keeley 1987; Keeley and Bond 1997) and smoke (Keeley and Fotheringham 1998). Seeds of the genera *Cupressus* and *Pinus* are not dormant but seeds are retained in their cones, which remain closed after seed maturation. Heat shock is often essential for opening serotinous cones (Johnson and Gutsell 1993) and thus for seed dispersal. Seeds stored in serotinous cones are protected by the cone from overheating (Beaufait 1960; Fraver 1992; Despain et al. 1996; Habrouk et al. 1999). However, several studies have found a strong decrease in percentage of germination

Table 2. The results of a Cox-regression test on differences in germination rate (the statistic test value and probability). **A)** The, per cone type, effects of heating temperature and pH on the germination rate, at given factors (df = 1). **B)** The, per cone type, effect of combination (low heat-low pH and high heat-high pH) on the germination rate (df = 1). **C)** Effects of cone type on the germination rate in a combination of low heat-low pH and high heat-high pH (df = 2)

Variable	**Factor**	**Statistics per cone type**		
		Non-serotinous	**New serotinous**	**Old serotinous**
A				
Effect of heating at:	pH=7	Stat. = 2.3972	Stat. = 0.0228	Stat. = 0.5336
		p = 0.121	p = 0.880	p = 0.465
	pH=10	Stat. = 2.5699	Stat. = 5.1003	Stat. = 10.4273
		p = 0.109	p = 0.024*	p = 0.001*
Effect of pH at:	Heat = 40 °C	Stat. = 13.7627	Stat. = 0.0683	Stat. = 7.2264
		p = 0.002*	p = 0.794	p = 0.007*
	Heat = 100 °C	Stat. value = 51.4091	Stat. = 10.4273	Stat. = 7.8318
		p < 0.001*	p = 0.001*	p = 0.005*
B				
Effect of Combination (pH × heat)		Stat. = 63.5350	Stat. = 2.3391	Stat. = 4.2023
		p < 0.001*	p = 0.206	p = 0.100
C				
Variable		**Statistics per combination**		
		Low heat-low pH	**High heat-high pH**	
Effect of cone type		Stat. = 4.2453	Stat. = 16.2095	
		p = 0.119	p = 0.003	

*Significant with $p < 0.05$

of *P. halepensis*, when these seeds were heated at temperatures expected to be found within the cones (ranging between 100–200 °C) (Nunez and Calvo 2000; Martínez-Sánchez et al. 1995; Escudero et al. 1999). They concluded that *P. halepensis* is not really adapted to fire regimes. We argued that the germination response of serotinous seeds might be linked with the cone response. Because serotinous cones open mainly after fire and post-fire recruitment depends entirely on the seeds from serotinous cones (Daskalakou and Thanos 1996), we expected seeds from serotinous cones to be tolerant to high temperatures. Our results showed that heating had no effect on the percentage of germination, but it had a positive effect on the germination rate of seeds from serotinous cones, whereas it did not affect seeds from non-serotinous cones. This corresponds to a study of Despain et al. (1996) on *P. contorta*, which indicated that seeds from serotinous cones reacted positively to heating (outside the cones), whereas seeds from non-serotinous cones were not or negatively affected by heating. Also Hanley and Lamont (2000) found serotinous seeds of some serotinous species to have a higher germination rate and higher percentage of germination after heating treatments (100–120 °C). An experiment in which seeds were heated within woody *Callistemon* fruits showed an increased percentage of germination after heating (Whelan and Brown 1998). The fact that heating stimulates seed germination rates of seeds from serotinous cones enhances the competitive power of *P. halepensis* seedlings in the post-fire environment, when competition for water and light among seedlings of several species is strong (Ne'eman 1997).

The ash layer, after fire, causes the high pH of the topsoil. A high pH can negatively affect germination by inhibiting proteolytic enzymes (Mayer and Poljakoff-Mayber 1982), affecting the membrane potential (Fitter and Hay 1987) or inhibiting root cell elongation (Tang et al. 1993). Our results only partly confirm this negative effect of high pH on germination. The germination rates of seeds from all cone types were negatively affected by high pH. Similarly, percentage of germination of seeds from non-serotinous

Figure 1

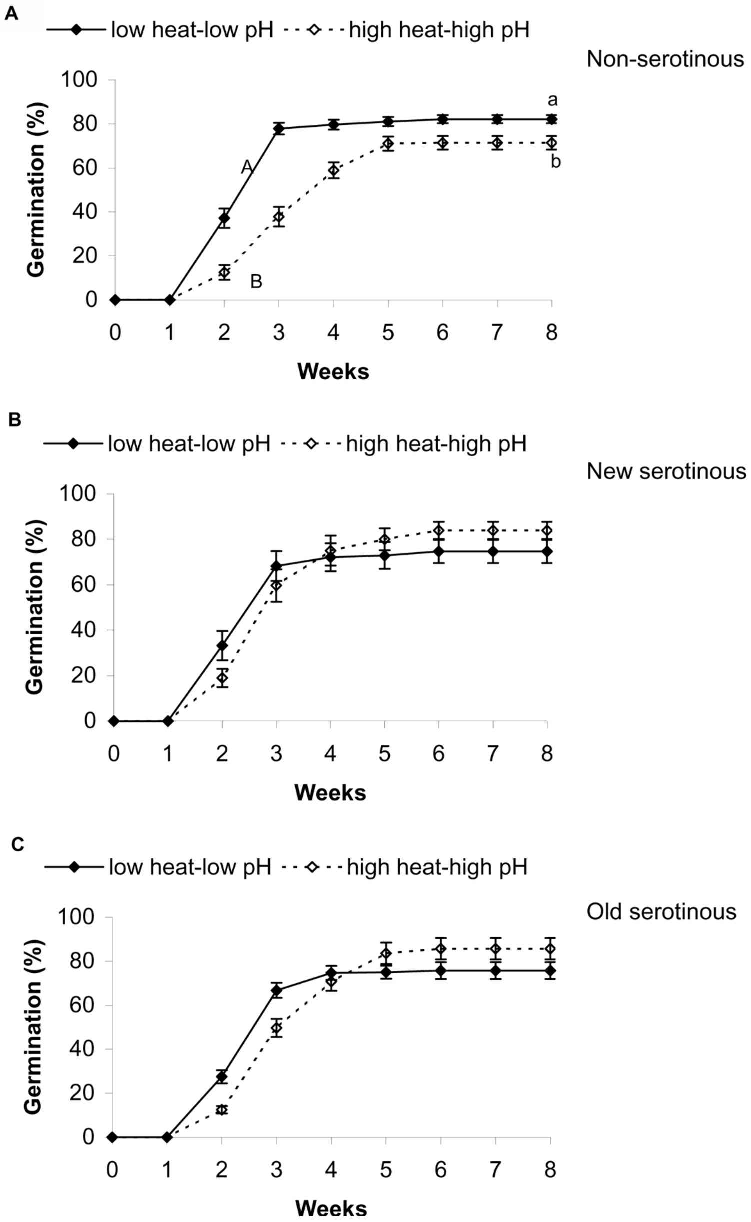

Figure 1. The germination percentage and rate of seeds from **1A)** non-serotinous cones, **1B)** new serotinous cones and **1C)** old serotinous cones over a 8 week period. Different letters indicate significant differences according to post-hoc tests in germination rate (A,B) and germination percentage (a,b) between the combinations low heat-low pH (40 °C and pH 7) and high heat-high pH (100 °C and pH 10). Error bars indicate standard errors.

cones decreased under higher pH. This corresponds to the results of Henig-Sever et al. (1996), who showed that pH 9 and 10 had a negative effect and pH 11 had a lethal effect on the percentage of germination of *P. halepensis* seeds from the same research area. However, our results also showed that the germination percentage of seeds from serotinous cones was not affected by high pH. This an advantage in the post-fire environment.

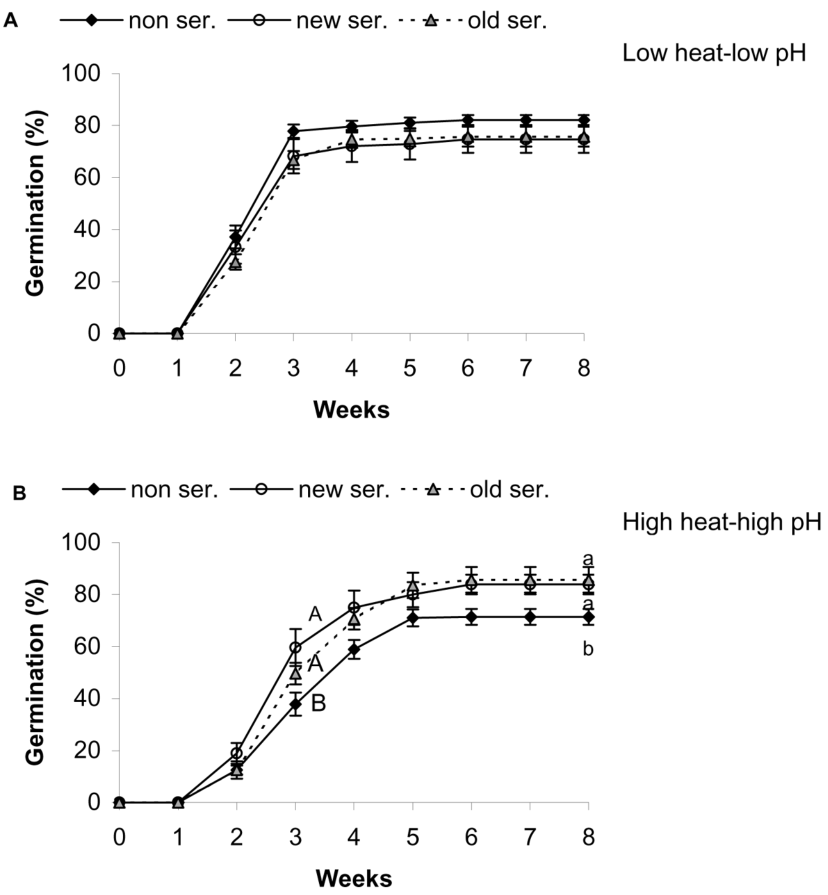

Figure 2. The germination percentage and rate in the combination **2A)** low heat-low pH (40 °C and pH 7) and **2B)** high heat-high pH (100 °C and pH 10) of seeds from non-serotinous, new serotinous and old serotinous cones over a 8 week period. Different letters indicate significant differences according to post-hoc tests in germination rate (A,B) and germination percentage (a,b) between different cone types (non-serotinous, new serotinous and old serotinous). Error bars indicate standard errors.

Table 3. The results of a 3-way ANOVA test on the separate and interactive effects of cone type, heating and pH on final germination percentage.

Variable	F-value	df	p
Cone	0.804	2	0.450
Heat	2.247	1	0.136
pH	0.652	1	0.421
Cone × heat	1.337	2	0.266
Cone × pH	4.588	2	0.012*
Heat × pH	1.200	1	0.275
Cone × heat × pH	1.272	2	0.283

*Significant with $p < 0.05$

When seeds from serotinous cones disperse into neighboring unburned stands, they experience a combination of high heat and low soil pH. Conversely, when seeds from unburned stands disperse into a burned stand they experience a combination of low heat and high pH. However, most post-fire recruitment depends on seeds from serotinous cones from the burned stand. In a fire scenario high heating temperatures are combined with high pH. Germination rate and percentage of germination of seeds from non-serotinous cones were lower in the combination high heat-high pH than in the combination low heat-low pH, due to the negative effect of high pH. In the case of seeds from serotinous cones, the combination type did not affect the germination rate and the percentage of germination. In case of the percentage of germination this is due to the fact that there was simply no effect of either heat nor pH. In the case of the germination rate this is due to the opposing stimulating effect of high heat and inhibiting effect of high pH, which ‘neutralized’ each other. An opposing effect of heat and pH was found also in a study on *Rhus coriaria* (Ne’eman et al. 1999). The combined effect of

Table 4. The results of one-way ANOVAs on differences in germination percentage (the F-value and probability). **A)** Effects of pH on the germination percentage per cone type (df = 1). **B)** Effects of the combination low heat-low pH and high heat-high pH on the germination percentage per cone type (df = 1). **C)** Effects of cone type on the germination percentage per combination (df = 2).

Variable	**Statistics per cone type**		
	Non-serotinous	**New serotinous**	**Old serotinous**
A			
Effect of pH	F-value = 13.524 p = 0.001*	F-value = 1.032 p = 0.314	F-value = 0.111 p = 0.740
B			
Effect of combination (pH × heat)	F-value = 6.390 p = 0.001*	F-value = 0.555 p = 0.647	F-value = 1.604 p = 0.200
C			
Variable	**Statistics per combination**		
	Low heat-low pH	**High heat-high pH**	
Effect of cone type	F-value = 1.509 p = 0.227	F-value = 4.345 p = 0.016*	

*Significant with $p < 0.05$

heat and pH reduced the percentage of germination of all taxa in the soil seed bank of Mediterranean pine forests (Izhaki et al. 2000). Our results showed that the combined effect of high heat and high pH does not negatively affect the germination rate nor the percentage of germination of seeds from serotinous cones. Thus seeds from serotinous cones are more tolerant to fire than seeds from non-serotinous cones.

Conclusions

In conclusion, our results indicate that there is a difference in the germination response to fire between seeds from non-serotinous and serotinous cones. The difference could not be attributed to a difference in cone age, as no differences were found between new and old serotinous cones. Thus age, up to 8 years, did not affect the germination rate and the percentage of germination in our study. This suggests that seeds from non-serotinous cones are physiologically different from seeds of serotinous cones. Differences have been found between serotinous and non-serotinous cones (Leone et al. 1999), but no differences have been reported between seeds from these two cone types. Thus, individual *P. halepensis* trees can carry two cone types, which contain seeds that differ in their physiological control of germination. Such differences are also known from desert annuals (Gutterman 2001). The different germination responses to fire of seeds from non-serotinous and serotinous cones are linked with the different cone responses. Seeds from non-serotinous cones, which open in warm dry weather, germinate better in a combination of low heat and low pH, than in a combination of high heat and high pH. Seeds from serotinous cones, which open mainly after fire, germinate equally well in both combinations, while they germinate better than seeds from non-serotinous cones in a combination of high heat and high pH. This fits the dual life strategy of *P. halepensis* by facilitating the regeneration both in burned and unburned environments. The fact that seeds from serotinous cones germinate equally well without or with fire is consistent with the fact that some serotinous cones can open during warm and dry weather, in the absence of fire (Nathan et al. 1999). They thus have a chance to be exposed to a germination environment unaffected by fire. The results also show that although germination rate and percentage of germination of non-serotinous seeds is lower after fire, it is still high enough for recruitment in a post-fire environment from unburned trees neighboring a burned site.

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References

Beaufait W.R. 1960. Some effects of high temperatures on the cones and seeds of jack pine. Forest Science 6: 194–199.

Bond W.J. and van Wilgen B.W. 1996. Fire and plants. In: Usher M.B., DeAngelis D.L. and Manly B.F.J. (eds). Chapman and Hall, London.

Bradstock R.A., Gill A.M., Hastings S.M. and Moore P.H.R. 1994. Survival of serotinous seedbanks during bushfires: comparative studies of Hakea species from southeastern Australia. Australian Journal of Ecology 19: 276–282.

Broncano M.J., Riba M. and Retana J. 1998. Seed germination and seedling performance of two Mediterranean tree species, Holm oak (*Quercus ilex* L.) and Aleppo pine (*P. halepensis* Mill.): a multifactor experimental approach. Plant Ecology 138: 17–26.

Cowling R.M. and Lamont B.B. 1985. variation in serotiny of three *Banksia* species along a climatic gradient. Australian Journal of Ecology 10: 345–350.

Cowling R.M., Lamont B.B. and Pierce S.M. 1987. Seed bank dynamics of four co-occuring *Banksia* species. Journal of Ecology 75: 289–302.

Daskalakou E.N. and Thanos C.A. 1996. Aleppo pine (*Pinus halepensis*) postfire regeneration: the role of canopy and soil seed banks. International Journal of Wildland fire 6: 59–66.

Despain D.G., Clark D.L. and Reardon J.J. 1996. Simulation of crown fire effects on canopy seed bank in Lodgepole pine. International Journal of Wildland Fire 6: 45–49.

Enright N.J., Marsula R., Lamont B.B. and Wissel C. 1998. The ecological significance of canopy seed storage in fire-prone environments: a model for non-sprouting shrubs. Journal of Ecology 86: 946–959.

Escudero A., Sanz M.V., Pita J.M. and Perez-Garcia F. 1999. Probability of germination after heat treatment of native Spanish pines. Ann. For. Sci. 56: 511–520.

Fitter A.H. and Hay R.K.M. 1987. Environmental Physiology of Plants. Academic Press, London.

Fox G.A. 1993. Failure time analysis: emergence, flowering, survivorship, and other waiting times. In: Scheiner S.M. and Gurevitch J. (eds), Design and analysis of ecological experiments. Chapman & Hall, New York, pp. 253–289.

Fraver S. 1992. The insulating value of serotinous cones in protecting pitch pine (*Pinus rigida*) seeds from high temperatures. Journal of the Pennsylvania Academy of Science 65: 112–116.

Gauthier S., Bergeron Y. and Simon J.P. 1993. Cone serotiny in jack pine: ontogenetic, positional and environmental effects. Canadian Journal of Forestry Research 23: 394–401.

Gauthier S., Bergeron Y. and Simon J.P. 1996. Effects of fire regime on the serotiny level of jack pine. Journal of Ecology 84: 539–548.

Gutterman Y. 2001. Regeneration of plants in arid ecosystems resulting from patch disturbance. Geobotany 27, Kluwer Academic Publishers, The Hague, pp. 1–232.

Habrouk A., Retana J. and Espelta J.M. 1999. Role of heat tolerance and cone protection of seeds in the response of three pine species to wildfires. Plant Ecology 145: 91–99.

Hanley M.E. and Fenner M. 1998. Pre-germination temperature and the survivorship and onward growth of Mediterranean fire-following plant species. Acta Oecologica 19: 181–187.

Hanley M.E. and Lamont B.B. 2000. Heat pre-treatment and the germination of soil and canopy-stored seeds of south-western australian species. Acta Oecologica-International Journal of Ecology 21: 315–321.

Hellum A.K. and Pelchat M. 1978. Temperature and time affect the release and quality of seed from cones of lodgepole pine from Alberta. Canadian Journal of Forestry research 9: 154–159.

Henig-Sever N., Eshel A. and Ne'eman G. 1996. pH and osmotic potential of pine ash as post-fire germination inhibitors. Physiologia Plantarum 96: 71–76.

Henig-Sever N., Eshel A. and Ne'eman G. 2000. Regulation of the germination of Aleppo pine (*Pinus halepensis*) by nitrate, ammonium, and gibberellin, and its role in post-fire forest regeneration. Physiologia Plantarum 108: 390–397.

Herranz J.M., Ferrandis P. and Martinez-Sanches J.J. 1998. Influence of heat on seed germination of seven Mediterranean Luguminosae species. Plant Ecology 136: 95–103.

Izhaki I., Henig-Sever N. and Ne'eman G. 2000. Soil seed banks in Mediterranean Aleppo pine forests: the effect of heat, cover and ash on seedling emergence. Journal of Ecology 88: 667–675.

Johnson E.A. and Gutsell S.L. 1993. Heat budget and fire behaviour associated with the opening of serotinous cones in two *Pinus* species. Journal of Vegetation Science 4: 745–750.

Keeley J.E. 1987. Role of fire in seed germination of woody taxa in California chaparral. Ecology 68: 434–443.

Keeley J.E. 1994. Seed-germination patterns in fire-prone Mediterranean-climate regions. In: Arroyo M.T.K., Zedler P.H. and Fox M.D. (eds), Ecology and Biogeography of Mediterranean ecosystems in Chile, California and Australia. Springer-Verlag, New York, pp. 239–273.

Keeley J.E. and Bond W.J. 1997. Convergent seed germination in South African fynbos and Californian chaparral. Plant Ecology 133: 153–167.

Keeley J.E. and Fotheringham C.J. 1998. Smoke induced seed germination in California chaparral. Ecology 79: 2320–2336.

Keeley J.E. and Zedler P.H. 1998. Evolution of life histories in pines. In: Richardson D.M. (ed.), Ecology and biogeography of *Pinus*. Cambridge University Press, Cambridge, pp. 219–249.

Lamont B.B. 1991. Canopy seed storage and release- what's in a name? Oikos 60: 266–268.

Lamont B.B., Le Maitre D.C., Cowling R.M. and Enright N.J. 1991. Canopy seed storage in woody plants. Botanical Review 57: 277–317.

Lamont B.B., Witkowski E.T.F. and Enright N.J. 1993. Postfire litter microsites- safe for seeds, unsafe for seedlings. Ecology 74: 501–512.

Leone V., Logiurato A. and Saracino A. 1999. Serotiny in *Pinus halepensis* Mill: recent issues. Abstract in MEDPINE, International workshop on Mediterranean pines, February 1999, Beit Oren, Israel.

Martínez-Sánchez J.J., Maran A., Herranz J.M., Ferrandis P. and 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.

Mayer A.M. and Poljakoff-Mayber A. 1982. The Germination of Seeds. Pergamon Press, Oxford.

Midgley J. 2000. What are the relative costs, limits and correlates of increased degree of serotiny? Austral Ecology 25: 65–68.

Nathan R., Safriel U.N., Noy-Meir I. and Schiller G. 1999. Seed release without fire in *Pinus halepensis*, a Mediterranean serotinous wind-dispersed tree. Journal of Ecology 87: 659–669.

Nathan R. and Ne'eman G. 2000. Serotiny, seed dispersal and seed predation in *Pinus halepensis*. In: Ne'eman G. and Trabaud L. (eds), Ecology, Biogeography and Management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean basin. Backhuys publishers, Leiden, pp. 105–118.

Ne'eman G. 1997. Regeneration of natural pine forest-review of work done after the 1989 fire in Mount Carmel, Israel. International Journal of Wildland Fire 7: 295–306.

Ne'eman G., Meir I. and Ne'eman R. 1993. The effect of ash on the germination and early growth of shoots and roots of *Pinus, Cistus* and annuals. Seed Science and Technology 21: 339–349.

Ne'eman G., Henig-Sever N. and Eshel A. 1999. Regulation of the germination of *Rhus coriaria*, a post-fire pioneer, by heat, ask, pH and ethylene. Physiologia Plantarum 106: 47–52.

Nunez M.R. and Calvo L. 2000. Effect of high temperatures on seed germination of *Pinus sylvestris* and *Pinus halepensis*. Forest Ecology and Management 131: 183–190.

Panetsos K.P. 1981. Monograph of *Pinus halepensis* and *Pinus brutia*. Annales Forestales 9: 39–77.

Reyes O. and Casal M. 1995. Germination behaviour of 3 species of the genus Pinus in relation to high temperatures suffered during forest fires. Annales des Sciences Forestieres 52: 385–392.

Reyes O. and Casal M. 1998. Germination of *Pinus pinaster*, P-radiata, and *Ecalyptus globules* in relation to the amount of ash produced in forest fires. Annales des Sciences Forestieres 55: 837–845.

Richardson D.M. 2000. Mediterranean pines as invaders in the southern hemisphere. In: Ne'eman G. and Trabaud L. (eds), Ecology, Biogeography and Management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean basin. Backhuys Publishers, Leiden, pp. 131–142.

Tang C., Robson A.D., Longnecker N.E. and Greenway H. 1993. Physiological responses of lupin root to high pH. Plant Soil 155: 509–512.

Thanos C.A. 2000. Ecophysiology of seed germination of *Pinus halepensis* and *P. brutia*. In: Ne'eman G. and Trabaud L. (eds), Ecology, Biogeography and management of *P. halepensis* and *P. brutia* forest systems in the Mediterranean Basin. Backhuys Publishers, Leiden, pp. 37–50.

Thanos C.A. and Skordilis A. 1987. The effects of light, temperature and osmotic stress on the germination of *Pinus halepensis* and *P. brutia*. Seed Science and Technology 15: 163–174.

Tinker D.B., Romme W.H., Hargrove W.W., Gardner R.H. and Turner M.G. 1994. Landscape-scale heterogeneity in lodgepole pine serotiny. Canadian Journal of Forest Research.

Trabaud L. 1987. Fire and survival traits of plants. In: Trabaud L. (ed.), The role of fire in ecological systems. SPB Academic Publishing, The Hague, pp. 65–91.

Trabaud L. and Renard P. 1999. Do light and litter influence the recruitment of *Cistus* spp. stands? Israel Journal of Plant Sciences 47: 1–9.

Underwood A.J. 1997. Experiments in Ecology. Their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.

Whelan R.J. 1995. The Ecology of Fire. Cambridge University Press, Cambridge.

Whelan R. and Brown C.L. 1998. The role of Callistemon fruits and infructescences in protecting seeds from heat in fires. Australian Journal of Botany 46: 235–239.

Whelan R.J., de Jong N.H. and Von Der Burg S. 1998. Variation in bradspory and seedling recruitment without fire among populations of *Banksia serrata* (Proteaceae). Australian Journal of Ecology 23: 121–128.