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pH and osmotic potential of pine ash as post-fire germination inhibitors

Nava Henig-Sever, Amram Eshel and Gidi Ne'eman

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The dominant plant species in native pine forests in Israel *(Pinus halepensis, Cistus salviifolius* and *C. creticus)* regenerate from seeds after wildfires. The future structure of the regenerating forest is determined largely by the spatial distribution of the seedlings, which depends on the response of the seeds to conditions in the upper layer of the soil. The pH and osmotic potential (π) of the soil water in this layer is strongly affected by the ash which covers the burned forest floor. The effects of pH and π on the germination of the wild species noted above and the effect of pH on the germination of two crops, radish *(Raphanus sativus radicula)* and oat *(Avena sativa)* were studied in a growth chamber. Bis-Tris propane and CAPS [3-(cyclohexylamino)-1-propanesulfonic acid] buffer solutions (pH 6–11), as well as mannitol solutions (down to –1.5 MPa) were used. The upper soil layer from a recently burned forest had pH 9 and π –0.08 MPa. Under this pH the germination of *P. halepensis* and *C. creticus* was reduced by ca half, and of *C. salviifolius* by 40%. Germination of radish and oats was reduced by ca 80%. Osmotic potential of –0.1 MPa did not have a significant effect on the germination of any of the species studied.We conclude that the high pH of the soil, caused by ash, is an important environmental factor that controls the regeneration of the forest plant community. Seed adaptation to the high pH may be decisive in determinig the plants' fate in fire-prone ecosystems.

Key words – Ash, *Avena sativa*, *Cistus*, fire, germination, oat, osmotic potential, pH, pine, *Pinus*, radish, *Raphanus sativus radicula*.

N. Henig-Sever and A. Eshel, Dept of Botany, George S. Wise Faculty of Life Sciences, Tel Aviv Univ., Ramat Aviv 69978, Israel; G. Ne'eman (corresponding author, e-mail zeac619@uvm.haifa.ac.il), Dept of Biology, Univ. of Haifa at Oranim, Tivon 36006, Israel.

Introduction

Mediterranean ecosystems exhibit a high degree of resilience to frequent wildfires (Naveh 1994, Trabaud 1994). The dominant plants in these ecosystems have been selected for their capability to regenerate after a fire. These species have been classified according to their post-fire regeneration behavior as either seeders or resprouters (Trabaud 1987, Keeley 1991, 1994). Most perennial species of the sclerophyllous North and East Mediterranean vegetation are resprouters that regenerate vegetatively. Nevertheless, the dominant species of native and planted pine forests, *Pinus halepensis* and *Cistus* spp. are obligate seeders. The mature plants of these species cannot survive fire, and spontaneous regeneration occurs only by post-fire germination from seed stocks in the soil or in serotinous cones (Naveh 1975, Trabaud and Oustric 1989, Thanos et al. 1992).

Every forest is a mosaic of trees and bushes of various sizes interspersed with smaller plants and rocks. The uneven distribution of live and dead organic matter causes fire intensity and the amount of residual ash to vary among locations in the forest. Big trees supply most of the fuel for the fire, and in their vicinity high temperatures occur during the fire. A thick cover of ash is found under the burned canopy of each big pine tree but it diminishes with distance from the stump. We have previously observed that seedling of annuals were rare and that seedling density of *Pinus* and *Cistus* was much lower under the burned canopies than elswhere (Izhaki et al. 1992, Ne'eman et al. 1992).

Seeds of *Pinus halepensis* are mostly stored in seroti-

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nous cones and released by the hot and dry air during the fire, germinating readily in the following rainy season (Thanos and Skordilis 1987, Keeley 1994). Because most of the viable seeds are dispersed after the fire when air currents are weak, a decreasing gradient in seed density with distance from the burned stumps is expected. However, density of pine seedlings under burned pine trees is low. Direct effects of ash on seed germination have been suggested to be responsible for this seedling distribution pattern in the regenerating forest (Thomas and Wein 1990, 1994, Ne'eman et al. 1993).

Cistus spp. regenerate after fire by germination from the soil's seed bank (Naveh 1975, Thanos and Georghiou 1988). Like many post-fire obligate seeders, *Cistus* spp. have small, refractory, hard seeds (Keeley 1991). This is typical for all members of the Cistaceae (Thanos et al. 1992). Most of the seeds of *C. salviifolius* and *C. creticus* have a water-impermeable, hard seed coats and require intense heat or another treatment that will crack the coat and induce germination (Thanos and Georghiou 1988, Aronne and Mazzoleni 1989). Temperatures in the range of 100–200°C, which occur during fire in the upper soil layers (Raison et al. 1986), may be responsible for such a heat shock and inducing germination. The role of ash and seed bank mortality in the seedling establishment of these species under the large burned pine trees has yet to be studied.

Inhibitory effects of wood ash on seed germination have been demonstrated for *Pinus banksiana* (Thomas and Wein 1990, 1994), *P. halepensis*, *Cistus* spp. and several annual species (Ne'eman et al. 1993). It was assumed that either high pH (Thomas and Wein 1990), suppression of water uptake by the embryo resulting from low osmotic potential, or the effect of toxic ions was responsible for such inhibition (Ne'eman et al. 1993). High pH can affect germination by inhibiting proteolytic enzymes involved in the metabolism of seed storage compounds (Mayer and Poljakoff-Mayber 1989). The hydroxyl ion may also interfere with uptake of essential anions (Fitter and Hay 1987) and so affect membrane potential. Inhibition of enzyme activity and changes in membrane potential may in turn inhibit seed germination. High pH is also known to inhibit root cell elongation (Tang et al. 1993). Acidification of cell walls may be required for their loosening and extension (Taiz 1984). Therefore high pH may inhibit radical elongation of germinating embryos and thereby reduce germination.

Here we report the values of pH and osmotic potential of ash and soil samples collected after fire in a burned pine forest. Our objective was to assess the effect of these conditions on post-fire seed germination of the dominant species of the East Mediterranean pine forest, *Pinus halepensis, Cistus salviifolius* and *C. creticus*. The effect of pH on the germination of two annual crops, radish (*Raphanus sativus radicula*) and oat (*Avena sativa*) was also studied.

Materials and methods

pH and osmotic potential of ash and soil

Samples of ash and upper soil layer (0–5 cm) were collected one week after a wildfire at a pine forest near Amirim, Upper Galilee, Israel, in June 1993. Both ash and soil were collected at the same site again in January 1994. In June 1994 only soil was sampled because all the ash was eroded. The samples were collected from high intensity fire sites where the tree trunks were burned down to their roots, from medium intensity fire sites where stems remained unburned after the fire, and from an adjacent unburned site. Ten samples of ash and of soil were collected at each site. The samples of each group were then mixed, sieved (2 mm), and one subsample out of each group was taken for analysis. Ash collected immediately after the fire on June 1993 from the high intensity fire site, was divided and 4 subsamples were heated at 250, 350, 550 and 1 000°C for 24 h in an electrical furnace.

The samples, of soil and ash, were mixed with distilled water (1:2, w:v, solid to water) for pH and osmotic potential (π) measurements. The pH was measured with a pH meter (model PBS 730, El-Hama Instruments, Mevo-Hama, Israel). Osmotic potential (π) was measured with a vapor pressure osmometer (model 5100C, Wescor Inc., Logan, UT, USA).

Effect of pH and osmotic potential on germination

Pinus halepensis Mill., *Cistus salviifolius* L. and *Cistus creticus* L. are dominant components of East Mediterranean pine forest vegetation. Seeds of *Cistus* spp. and *P.halepensis* cones were collected (September 1993) at natural stands on Mt. Carmel, Israel. Seeds of *P. halepensis* were removed from the cones by drying them at 40°C for 48 h. Seeds of *Cistus* spp. were preheated at 100°C for 15 min. Preliminary experiments have shown that this was required in order to achieve maximal germination in the controlled conditions. Seeds of radish (*Raphanus sativus radicula* Pers.) and of oats (*Avena sativa* L.) were obtained commercially. These were chosen as models for annuals representing dicotyledonous and monocotyledonous species. All seeds were surface-sterilized in dilute sodium hypochlorite solution for 15 min, and rinsed thoroughly in distilled water before incubation for germination.

Buffer solutions with adjusted pH values in the range 6–11, were used to examine the effect of pH on germination. For pH 6, 7, 8 and 9 we used 0.05 *M* Bis-Tris propane buffer solutions, and for pH 10 and 11 we used 0.05 *M* CAPS [3-(cyclohexylamino)-1-propanesulfonic acid]. Distilled water served as a control.

Mannitol solutions were used to examine the effect of osmotic potentials (π) of –0.1, –0.2, –0.3, –0.4, –0.5, –0.7, –1.0, –1.2 and –1.5 MPa on germination. Distilled water served as a control. Mannitol was used because in preliminary experiments even low concentrations of polyethylene glycol (PEG 6000) had a negative effect on germination of these seeds (see also Leshem 1966).

Germination experiments of all species were replicated 10 times: 20 seeds per Petri dish (diameter 9 cm) lined with one Whatman no. 1 filter paper, moistened with 5 ml of either distilled water or one of the pH or π treatment solutions. The seeds were incubated in a temperature-controlled plant growth chamber (Biotronette model 845-2, Lab-Line, IL, USA) at 20 ± 1°C, and exposed to 11 h white light daily (a mixture of 20 W cool white fluorescent lamps, Sylvania, Danvers, MA, USA, and 40 W incandescent lamps with a total energy flux of 30.3 μmol $m^{-2} s^{-1}$). Radical emergence from the seed coat was used as the criterion for germination. Germinated seeds were counted and removed once a week, until no more germination was observed. Germination of the annual species was completed within 2 weeks and that of perennials after 6 weeks.

The pH and osmotic potential experiments were performed sequentially and not in parallel, therefore each set of experiments has its own distilled water control.

Data analysis

The effect of treatments, species and the interaction between them, on seed germination was analyzed by a two-way analysis of variance (ANOVA). For the comparison of germination among species, the total germination was expressed as the percentage of the respective control. ANOVA was done on the transformed (arcsin square root) final fraction of germinated seeds. The effect of treatments, within each species, was further analyzed by one-way-ANOVA. The results of significant effects were followed by a Duncan's multiple range test (P<0.05) to compare individual treatments. All analyses were performed using the GLM procedure of SAS (SAS Institute 1990).

Results

pH and osmotic potential of ash and soil

Up to the first 6 months after fire, ash samples had pH values of about 10. The π value of these samples increased from –0.26 to –0.11 MPa. During the first post-fire year, soil samples varied from pH 8.3 to pH 9, increasing with time, and their π varied from –0.01 to –0.04 MPa, reaching the lowest values during winter (Tab. 1).

The pH values and osmotic potential of heated pine ash are presented in Tab. 2. The pH values increased, and π decreased progressively until 550°C, but at 1 000°C there was a great change in pH and π, but in opposite directions.

Effect of pH and osmotic potential on germination

A decrease in total percentage of germination with increasing pH was observed for all species tested (Fig. 1). The total germination at pH 6–7 was not significantly different from that in water (Fig. 1), indicating that the buffer solution itself had no effect on germination. At pH 10, the pH level of unheated ash (Tab. 2), germination of *P. halepensis* was reduced by 88% (Fig. 1A). At the same pH, germination of *C. salviifolius* was reduced by 73% and that of *C. creticus* by 77% (Fig. 1B,C) but the germination of radish was reduced by 96%, and oat did not germinate at all (Fig. 1D,E). The decline in germination percentage of *P. halepensis* and *Cistus* spp. was noted at pH values above 8, whereas the decline in germination of oat and radish seeds started at pH 7.

The results of two-way ANOVA show that total percentage of germination was significantly affected by pH. The differences among species and the interaction between species and pH were also significant (Tab. 3). The effect of pH on the species tested was found to be significant in one-way ANOVA (Tab. 4).

Total germination percentages of *P. halepensis*, *C. salviifolius* and *C. creticus* at osmotic potentials ranging from 0 to –1.5 MPa are presented in Fig. 2. Gradual, but significant (Tab. 4) decreases in germination with decreasing π were found for all species. Although seeds of *P. halepensis* germinated (40%) even at a π value of –1.5 MPa, germination of seeds of *C. salviifolius* was completely inhibited at –1 MPa, and that of *C. creticus* was completely inhibited at –0.7 MPa. At –0.3 MPa, which is near the π value of unheated ash (Tab. 2), the reduction in germination of *P. halepensis*, relative to control, was not significant (Fig. 2A). The reduction, relative to control, in germination of seeds of *C. salviifolius* (by 17%) and of *C. creticus* (by 64%) was statistically significant (Fig. 2B,C). The results of two-way ANOVA indicate that germination was significantly affected by π,

Tab. 1. Changes in pH and osmotic potential (π, MPa) of *P. halepensis* ash and soil at the Amirim wildfire site (June 1993) 1 week, 7 months and 1 year after the fire. Soil was collected from an unburned site (1), a medium-intensity fire site (2) and a high-intensity fire site (3). – , No ash left.

Sample	1 week		7 months		1 year	
	pH	π	pH	π	pH	π
Ash	10.0	–0.26	9.8	–0.11	–	–
Soil 1	7.0	–0.04	7.3	–0.05	7.4	–0.02
Soil 2	8.3	–0.01	8.8	–0.07	8.8	–0.03
Soil 3	8.5	–0.02	9.1	–0.08	9.0	–0.04

Tab. 2. pH and osmotic potential (π) of *P. halepensis* ash collected from the Amirim wild-fire site (July 1993) and heated at various temperatures for 24 h.

Treatment	pH	π (MPa)
Unheated	10.0	–0.26
250°C	10.4	–0.21
350°C	10.7	–0.18
550°C	11.7	–0.16
1000°C	13.0	–0.45

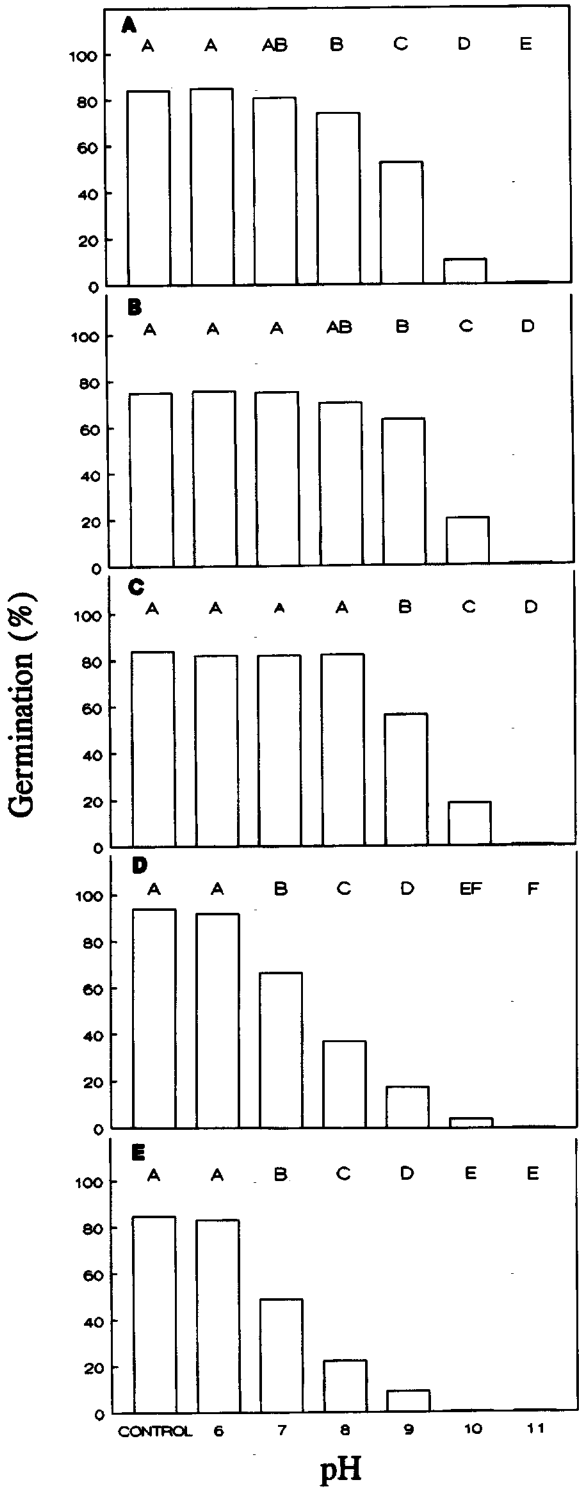

Fig. 1. The effect of pH on total germination percentages of seeds of *Pinus halepensis* (A), *Cistus salviifolius* (B), *C. creticus* (C), radish (D) and oats (E). Columns with a common letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

Tab. 3. Results of two-way ANOVA comparing (arcsin square root transformed) relative (control of each species set to 100%) germination of seeds, over different pH values and the different species, and over different osmotic potentials (π) and the different species.

Source of variation	R^2	df	*F*	*P*
pH				
Model	0.569	3	232.74	0.0001
pH		1	661.85	0.0001
Species		1	30.72	0.0001
pH × species		1	5.64	0.0179
Osmotic potential				
Model	0.585	3	110.05	0.0001
π		1	234.51	0.0001
Species		1	58.26	0.0001
π × species		1	28.37	0.0001

by differences among species, and by the interaction between species and treatments (Tab. 3).

Discussion

pH of wet ash was very high and reached extreme values after heating (Tab. 2), indicating a possible increase in ash pH with fire intensity. The high pH values of the ash also affected the pH of the underlying soil in which high values were reached and were increased with heating (Tab. 1); see also Kutiel and Shaviv (1989) and Giovannini et al. (1990). The pH of the soil solution increased during winter (Tab. 1), the time of massive post-fire germination. In the boreal forest, high pH values in soil may last for 2 years after fire (Thomas and Wein 1994). Since samples combined soil from 0–5 cm, it can be assumed that the actual pH at the uppermost soil horizon was even higher. Most post-fire germination occurs at the beginning of the rainy winter season, exposing seeds to elevated pH, according to the amount of ash at each site. pH values, equivalent to post-fire pH of ash and soil, almost completely inhibited seed germination in oats and radish and drastically reduced that of *P. halepensis* and of *Cistus* spp. (Fig. 1). Thus, high pH, caused by ash cover, can reduce all post-fire germination in the uppermost soil layer, affecting the tested species

Tab. 4. Results of one-way ANOVA for each of the species, comparing the percentage of germinated seeds (10 Petri dishes each with 20 seeds) over different values of pH (6–9=0.05 *M* Bis-Tris propane buffer, 10–11=0.05 *M* CAPS buffer), and osmotic potential (π, 0 to −1.5 MPa mannitol).

Species	R^2	df	*F*	*P*
pH				
Pinus halepensis	0.92	6	127.00	0.0001
Cistus creticus	0.95	6	182.96	0.0001
Cistus salviifolius	0.94	6	150.89	0.0001
Radish	0.97	6	287.52	0.0001
Oats	0.96	6	257.59	0.0001
π				
Pinus halepensis	0.55	9	12.18	0.0001
Cistus creticus	0.92	9	111.76	0.0001
Cistus salviifolius	0.93	9	126.51	0.0001

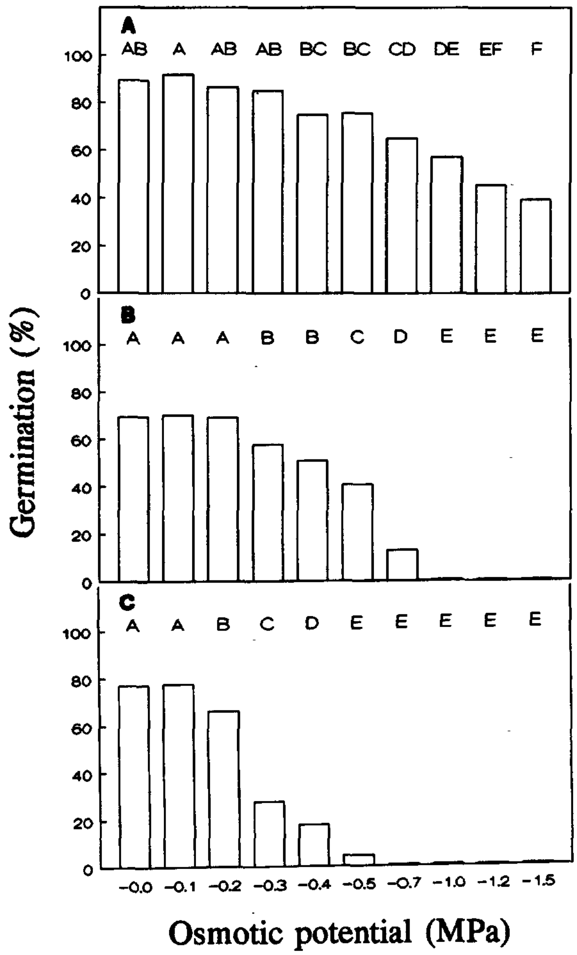

Fig. 2. The effect of osmotic potential (mannitol) on total germination percentages of seeds of *Pinus halepensis* (A), *Cistus salviifolius* (B) and *C. creticus* (C). Columns with a common letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

differently. Since *C. salviifolius* was found to be the most resistant species to ash (Fig. 1, Tab. 3), our results may offer an explanation to the puzzling fact that the density of seedlings of this species under the burned pine trees was higher than that of *P. halepensis*. They may also explain the almost complete absence of annual plant species at the same sites (Ne'eman et al. 1992). However, it should be kept in mind that, in nature, the effect of ash is combined with the effect of the high temperature at the soil surface during the fire, a combination which may reduce seed-bank viability.

We take the osmotic potential of wet pine ash to represent the osmotic potential under ash cover in the field after the first rainfall, when most seed germination takes place. From our results, it is evident that elevation in osmotic potential caused a differential reduction in seed germination of the tested species (Fig. 2, Tab. 3). However, the effects of these osmotic potential values were much smaller than those of pH levels of the ash and the underlying soil.

Our results agree with those of Thanos and Georghiou (1988) and Thanos and Skordilis (1987), who found higher seed germination at low osmotic potentials in *P. halepensis* than in *Cistus* spp. The ability of *P. halepensis* to germinate under low osmotic potentials was demonstrated also by Schiller and Waisel (1989) who proposed this trait as an adaptation to germination in arid areas.

We conclude that extreme pH values are the main factor in inhibiting seed germination by ash, while osmotic potential is of secondary importance. Thus, uneven distribution of ash on the forest floor might determine the distribution of seedlings in the regenerating plant community through these ecophysiological mechanisms. The resulting sparse density of *Pinus halepensis*, and other seedlings, at the sites of the big, burned pine trees, allows competition-free development of seedlings in a nutrient-rich environment. The dominant individual seedlings, which at these sites grow into young trees, are the most likely to succeed. In this way the spatial distribution of pine trees, which regenerate by seed germination, is maintained after a wildfire in this plant community.

The *Pinus* and *Cistus* spp. tested exhibit different degrees of adaptation to the conditions of high pH and osmotic stress. We intend to study other species, belonging to this community and to compare them on the basis of their degree of adaptation to germination under post-fire conditions. The physiological characteristics that are responsible for these adaptations may give new insight into germination control mechanisms in general.

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