Regulation of the germination of Aleppo pine (*Pinus halepensis*) by nitrate, ammonium, and gibberellin, and its role in post-fire forest regeneration

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Aleppo pine (*Pinus halepensis* Mill.) regenerates after wildfires only by germination. This post-fire germination is controlled by temperature, light and the chemical characteristics of the ash-bed. The extremely high pH caused by the ash inhibits germination of Aleppo pine as well as of many other species. Here we report that ash extracts at neutral pH stimulate *P. halepensis* germination. The effects of ash extracts, nitrate, ammonium (in concentrations present in the ash) and GA₃ on the germination of *P. halepensis* seeds and on lipase activity at early germination were studied under controlled conditions. Sub-optimal concentrations of nitrate- and ammonium-stimulated germination and the activity of lipase in the seeds. It is proposed that nitrate increases seed sensitivity to GA₃ while ammonium directly affects lipase activity. The balance between the inhibition of germination by the high pH of ash and its stimulation by the nitrate and ammonium present in the ash determines the overall effect of the ash on post-fire germination of Aleppo pine. The pine seedlings appear in high density among the trees but because of the effects of the ash they appear in sparse stands under the projection of the burned pine canopies. These seedlings benefit from weak inter- and intra-specific competition as well as improved mineral nutrition and have good chances to grow into large trees that will form the regenerating forest; therefore, the enhancement of *P. halepensis* germination by nitrate and ammonium and its inhibition by high pH can be considered as ecophysiological adaptations to post-fire regeneration.

Introduction

Aleppo pine (*Pinus halepensis* Mill.) forests in the Mediterranean basin are subjected to periodical wildfires (Trabaud et al. 1985, Naveh 1990). Germination is the first stage in the regeneration process of pines as well as other post-fire obligate seeders and its control is a key factor in post-fire regeneration of the plant community. Optimal germination of *P. halepensis* occurs at 20°C in darkness (Calamassi et al. 1984, Thanos and Skordilis 1987). Heat shock is not needed for germination, moderate heat shock (up to 150°C for 1 min) does not inhibit germination, but severe heat shock (more than 150°C for 5 min) is lethal (Martínez-Sánchez et al. 1995, Trabaud 1995). Germination is promoted by daytime white light or continuous red light and inhibited by far-red light, by high osmotic stress and by stratification (Falusi et al. 1983, Thanos and Skordilis 1987, Skordilis and Thanos 1995, 1997). These results indicate that *P. halepensis* seeds are not dormant and they germinate optimally at the average temperatures of the rainy season in areas where the

species grows (Skordilis and Thanos 1997). Aleppo pine germination is not dependent on fire related cues (Trabaud 1995), but it is abundant in post-fire conditions (Arianoutsou and Ne'eman 2000).

The spatial distribution and the size of pine trees before the fire affect post-fire germination of *P. halepensis* as well as other perennial and annual species. Seedling density of most species under the burned pine canopies, where a thick ash layer usually accumulates, is lower than their density in the gaps (Ne'eman et al. 1992). The high pH resulting from dissolution of carbonates, oxides and hydroxides present in the ash inhibits germination (Henig-Sever et al. 1996) and is probably the cause of the low seedling density under the burned pine canopies.

Heat, charred wood and smoke are 3 fire-related factors known to stimulate germination (Keeley 1994). Stimulation of germination by charred wood was reported for several species from the chaparral in California (Wicklow 1977,

Keeley and Pizzorno 1986, Keeley 1991), from the fynbos in South Africa (Brown 1993a), from Australia (Bell et al. 1987, 1993) and for one east Mediterranean species (Keeley 1994). Organic thermodegradation products of cell wall hemicellulose, such as certain oligosaccharides, were proposed as the germination-stimulating factors in charred wood (Keeley and Pizzorno 1986). The germination of several chaparral, fynbos and Australian species was stimulated by smoke (Brown 1993a,b, Baldwin et al. 1994, Baxter and Van Staden 1994, Keeley 1994, Dixon et al. 1995). It was suggested that NH_3, nitrogen oxides (NO_x), acids or ethylene are the active compounds in smoke that are responsible for this stimulating effect (Van de Venter and Esterhuizen 1988, Brown 1993a, Keeley 1994, Sutcliffe and Whitehead 1995, Thanos and Rundel 1995, Keeley and Fotheringham 1997, 1998a,b).

Stimulation of germination by nitrate and ammonium has been documented for several species (Mayer and Poljakoff-Mayber 1982, Cohn 1989, Adkins and Adkins 1994). It was suggested that induction of germination by nitrate is mediated by gibberellic acid (Saini et al. 1985, Hilhorst 1995, Bianco et al. 1996). Gibberellic acid induces degradation of storage products in the seeds via specific metabolic pathways at early germination stages (Mayer and Poljakoff-Mayber 1982, Brits et al. 1995, Karssen 1995, Ranjan and Lewak 1995). This is true not only for starch-storing seeds but also for lipid-storing ones, such as pine (Hammer and Murphy 1993). GA_3 was shown to induce and stimulate lipase activity at early germination stage in lipid-storing seeds of several species (Gonzalez 1978, Gonzalez and Delsol 1981, Fincher 1989, Ranjan and Lewak 1995). However, as far as we are aware, no information exists about lipase activity and control in germinating seeds of *P. halepensis*.

Ammonium and nitrate were previously proposed to regulate post-fire germination of some Californian species (Thanos and Rundel 1995, but see Keeley and Fotheringham 1998a). The germination of *Helichrisum stoechas*, which inhabits fire-prone areas in Greece, was also promoted by nitrate (Doussi and Thanos 1997). Ammonium and nitrate are released by wet pine ash to the topsoil (N. Henig-Sever 1997. Thesis, Tel Aviv University, Israel); therefore, these ions may take part in regulation of germination during post-fire forest regeneration. The aim of the present study was to examine the effects of ammonium and nitrate on germination of *P. halepensis*, and to determine the physiological mechanism of their action in relation to the activity of gibberellic acid and lipase in the seeds.

Materials and methods

Plant material

Pinus halepensis Mill. cones were collected annually in late summer at natural stands on Mt. Carmel, Israel. Seeds were released from the cones by drying them at 45°C for 48 h.

Ash extracts

Ash was collected around burned pine trees 1 week after a wildfire at a pine forest near Amirim, Upper Galilee, Israel in June 1993. The ash was sieved (2 mm) and in order to simulate various fire intensities, 1 kg sub-samples were heated at 250°C, 350°C, 550°C and 1000°C for 24 h, in an electric furnace. Aqueous extracts were prepared from unheated and heated ash by rinsing the ash packed in a 2.34 cm diameter column (100 g ash) in 300 ml of distilled water. This ratio was estimated to resemble an average annual precipitation of 700 mm. Nutrient and organic matter contents were determined by standard methods by the Extension Service laboratory at Newe Yaar.

Germination experiments

Prior to germination, the seeds were surface sterilized in 3% Na hypochlorite solution for 15 min followed by a thorough rinse in distilled water.

The effects of ash extracts, NO_3^- and NH_4^+ solutions, and GA solutions on seed germination were tested in Petri dishes (9 cm) lined with one layer of Whatman no. 1 filter paper, moistened with either 5 ml of distilled water (control) or one of the treatment solutions. Each Petri dish contained 20 seeds, and each treatment was replicated 10 times. The seeds were incubated in a temperature-controlled growth chamber (Biotronette model 845-2, Lab-Line, IL, USA) at 20 ± 1°C. The seeds were exposed daily to 11 h of white light (a mixture of 20 W cool white fluorescent lamps, Sylvania, Danvers, MA, USA and 40 W incandescent lamps) at a total photon flux density 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 additional germination was observed for 4 weeks. The pH of the germination media was measured at the beginning and at the end of the experiments with ash extracts.

The effect of the extract of heated ash (550°C) on germination was tested in the presence and in the absence of CO_2. Half of the extract was kept in a sealed bottle in order to maintain its basic pH, and the other half was bubbled with air until its pH decreased to pH 8. Half of the seed plates treated with the basic pH extract were incubated within transparent gas-proof plastic boxes that were supplied constantly with CO_2-free air. All other plates were incubated in identical boxes supplied with normal air.

The effects of NO_3^-, NH_4^+ singly and in combination were tested using solutions of KNO_3, $Ca(NO_3)_2$, NH_4HCO_3 and NH_4OH. The solutions contained NO_3^- and NH_4^+ in

Table 1. Organic matter, nitrate (NO_3^-) and ammonium (NH_4^+) content in extracts of unheated and heated ash.

Heating temperature (°C)	Organic matter (%)	NO_3^- (µM)	NH_4^+ (µM)
Unheated	10.4	200	1784
250	8.3	192	578
350	4.3	135	1934
550	2.5	1.6	511
1000	0.0	0.0	0.0

concentrations identical to those found in the aqueous ash extracts (Table 1). The pH of all the solutions was not modified in any way before the experiment. NO_3^- (1.6–1600 μM) as KNO_3 and NH_4^+ (5.6–5556 μM) as NH_4HCO_3 solutions were used in order to find the optimal concentrations for pine seed germination. Solutions of gibberellic acid (GA_3 and GA_4) in concentrations of 0.01–1000 μM were used to test their effect on pine germination. The combined effects of NO_3^-, NH_4^+ and GA_3 on pine germination were tested using sub-optimal concentrations of NO_3^- (1.6 μM) and NH_4^+ (150 μM) in combination with GA_3 solutions (1, 10, 100, 1000 μM).

Lipase activity in pine seeds

The effects of NO_3^-, NH_4^+ and GA_3 on lipase activity during seed germination were assayed by germinating pine seeds in Petri dishes in the presence of distilled water (control) or 1.6 μM KNO_3, 150 μM NH_4HCO_3 or 100 μM GA_3. Lipase activity was assayed daily for 9 successive days (beginning at day zero – dry seeds, up to 1 day after radical emergence) in seeds germinated in water. Lipase activity was assayed only at the third day in the seeds germinated in the other solutions. Each treatment was replicated 5 times. Lipase was isolated according to Hammer and Murphy (1993). A homogenate was prepared from 500 mg pine megagametophyte tissue. The homogenate was centrifuged (1300 g, 10 min, 5°C) and separated into three fractions: the upper lipid layer containing the lipid bodies, the supernatant containing soluble cytoplasmatic proteins, and the pellet containing membrane fractions and other intracellular organelles. Lipase activity was assayed colorimetrically in each fraction by determining the amount of free fatty acids [according to Nixon and Chan (1979), with modifications by Huang (1985)].

Data analysis

The results of the germination experiments were analyzed by two- and one-way ANOVA on transformed (arcsin square root) data of final percentage of germination. The results of lipase activity experiments were analyzed by one-way-ANOVA on transformed (log) data of lipase activity in the seeds. The results of significant effects were followed by a Tukey multiple range test ($P < 0.05$) to compare individual treatments. The analyses were performed using SYSTAT 5 for windows (Wilkinson et al. 1992).

Results

In order to simulate various degrees of fire intensities, ash was heated to various temperatures up to 1000°C. The heating affected ash composition. The percentage of organic matter and the concentration of NO_3^- decreased gradually with the increase in heating temperature of the ash, whereas the decrease in the concentrations of NH_4^+ was less consistent (Table 1).

The initial pH of ash extracts was extremely high and correlated with heating temperature (Pearson r = 0.99, $P =$

Table 2. pH of germination media at the beginning and at the end of the experiments.

Germination medium	pH	
	Beginning	End
Distilled water	6.0	8.1
Extract of unheated ash	9.7	8.1
Extract of ash heated to 250°C	10.1	8.1
Extract of ash heated to 350°C	10.3	8.1
Extract of ash heated to 550°C	11.5	8.1
80 μM $Ca(NO_3)_2$	6.2	7.7
80 μM KNO_3	6.0	7.6
80 μM $NaNO_3$	5.3	7.8
556 μM NH_4HCO_3	7.2	7.0
556 μM NH_4Cl	5.9	7.4
556 μM NH_4OH	9.4	7.9

0.001), but it decreased almost to pH 8.0 in the course of the germination experiment (Table 2). Under vigorous air bubbling, dissolution of atmospheric CO_2 neutralized the basic pH of the extract within 24 h. In order to distinguish between effect of the ionic components of the ash and the negative effect of its high pH, we germinated seeds in water, and in basic and neutralized ash extracts. Germination in neutralized ash extracts (pH 7.2) reached 94.5% $\pm$ 6.9, while germination in distilled water control was only 50.5% $\pm$ 10.1. When the germination took place in a carbon dioxide-free atmosphere, the pH was 9.9 by the end of the experiment, and germination was drastically inhibited (9.0% $\pm$ 3.0).

To find out whether nitrate and ammonium present in ash affect the germination of *P. halepensis*, we examined the effect of various concentrations of KNO_3 and NH_4HCO_3, in the range of those typical of unheated and heated ash extracts (Table 1). Both KNO_3 and NH_4HCO_3 significantly increased germination ($P = 0.001$) (Fig. 1). There were no significant ($P = 0.715$) differences among the effects of NO_3^- solutions with different complementary cations (Na^+, K^+ and Ca^{2+}) at an initial pH of 5.3–6.2 (Fig. 2, Table 2). There were also no significant ($P = 0.667$) differences among the effects of NH_4^+ solutions with various complementary anions (Cl^-, HCO_3^- and OH^-) at an initial pH of 5.9–9.4 (Fig. 2, Table 2).

In order to compare the separate and combined effects of ammonium and nitrate relatively to that of heated ash extracts, we germinated seeds in ash extracts and in nitrate and ammonium solutions of concentrations equal to those found in ash extracts, separately (Fig. 3A) and in combination (Fig. 3B). Aqueous extracts of pre-heated ash increased germination of *P. halepensis* significantly ($P = 0.0001$), with that of ash heated at 550°C having the maximal effect (Fig. 3B). Pure and mixed solutions of NO_3^- and NH_4^+, in concentrations equal to those in unheated and heated ash (250°C, 350°C and 550°C), enhanced pine germination significantly (Fig. 3, $P = 0.0001$). In most cases, single salt solutions of NO_3^- and NH_4^+ did not affect germination as much as the corresponding ash extracts (Fig. 3A). However, the effect of mixed $NO_3^- + NH_4^+$ solutions on germination was similar to that of the corresponding ash extracts (Fig. 3B). A comparison of the increase in germination under single (NO_3^- or NH_4^+) and mixed ($NO_3^- + NH_4^+$) solutions

at concentrations equal to ash heated to 350°C, where NO_3^- was at low concentration and NH_4^+ at its highest concentration, demonstrated that high NH_4^+ concentrations reduced the effect of NO_3^-. On the other hand, at sub-optimal concentrations (1.6 μM NO_3^- and 511 μM NH_4^+ equal to the concentrations in ash heated to 550°C) the effect of $NO_3^- + NH_4^+$ was additive (Fig. 3).

In preliminary experiments, we found that the effect of GA_3 and GA_4 on pine germination was the same with maximal enhancement at 100 μM (N. Henig-Sever 1997. Thesis, Tel Aviv University, Israel); therefore, the following experiments were performed with GA_3 only. In order to test the combined effects of NO_3^-, NH_4^+ and GA_3, we added 0–1000 μM of the hormone to the following germination media: water, sub-optimal concentrations of 1.6 μM NO_3^- and 150 μM NH_4^+ separately and in combined solutions. Addition of GA_3 to water gradually increased the germination up to 100 μM, while 1000 μM was already supra-optimal (Fig. 4). Two-way ANOVA indicated a significant effect of NO_3^-, NH_4^+ and $NO_3^- + NH_4^+$ ($P = 0.0001$), of GA_3 ($P = 0.0001$), and a significant interaction between them ($P = 0.0001$). Addition of NH_4^+ at sub-optimal concentration to GA_3 further increased germination rate, except in the supra-optimal level of 1000 μM GA_3 (Fig. 4, $P = 0.0001$). Germination enhancement by sub-optimal concentrations of
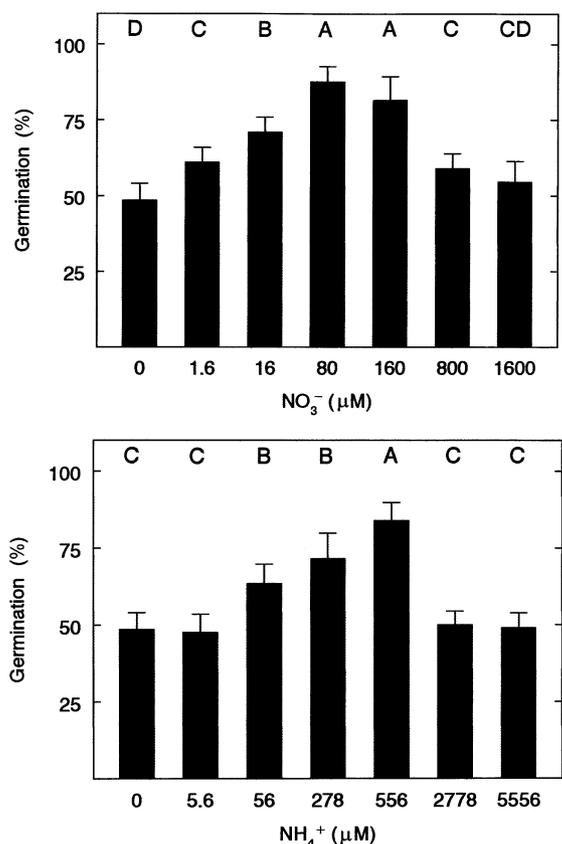

Fig. 1. The effect of various concentrations of KNO_3 (top panel) and NH_4HCO_3 (lower panel), including concentrations in the range found in ash, on germination of *P. halepensis* seeds (average of 10 replicates ± SD). Bars labeled with the same letter are not significantly different according to Tukey's multiple range test ($P < 0.05$).

Fig. 2. The effect of 80 μM NO_3^- (top panel) and 556 μM NH_4^+ (lower panel) accompanied by various ions on germination of *P. halepensis* seeds (average of 10 replicates ± SD). Bars labeled with the same letter are not significantly different according to Tukey's multiple range test ($P < 0.05$).
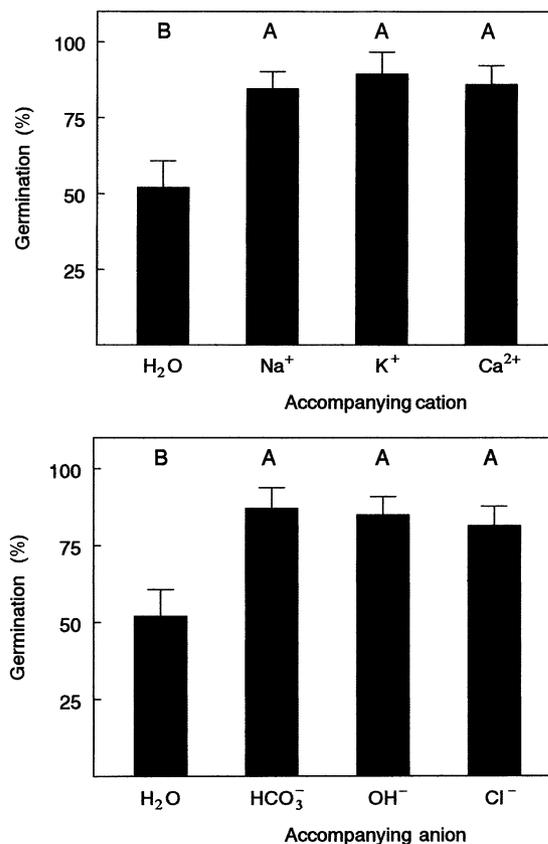
NO_3^- was evident only in the presence of sub-optimal concentrations of GA_3 (Fig. 4, $P = 0.0001$). However, combination of $NO_3^- + NH_4^+$ caused maximal germination even in the absence of GA_3, and the effect of GA_3 was completely masked (Fig. 4). These results indicate a synergistic effect of NO_3^- and NH_4^+ on pine germination at these concentrations that can be observed either in the presence or in the absence of GA_3.

In order to study the physiological mechanism of nitrate and ammonium influence on pine germination, we examined their effect on lipase activity in the seeds. Lipase activity in the lipid bodies of germinating *P. halepensis* seeds increased linearly during the first 9 days of germination. In contrast, a constant low lipase activity was found in the supernatant and in the pellet of the homogenized seeds (Fig. 5). The presence of NO_3^-, NH_4^+ and GA_3 significantly enhanced ($P = 0.0001$) lipase activity in the seeds on the third day of germination (Fig. 6). The effect of NO_3^-, NH_4^+ and GA_3 on lipase activity was similar to their effect on germination of these seeds (Fig. 6). Single ion solutions at sub-optimal concentrations significantly increased enzyme activity. However, GA_3 increased it to a higher level, as did the mixed solution of $NO_3^- + NH_4^+$ (Fig. 6). This indicates possible synergistic effects of NO_3^- and NH_4^+ on lipase activity in the germinating pine seeds.

Discussion

The inhibition of germination by ash (Ne'eman et al. 1992, 1993) and its relation to the extremely high pH of the ash have been described before (Henig-Sever et al. 1996), when it was found that the overall effect on germination is species specific. For example, in contrast with *P. halepensis*, *Rhus coriaria* only germinated after fire under large burned pine trees where ash covered the soil (Izhaki et al. 1992). Moreover, 2 cm of ash cover inhibited the germination of *P. halepensis*, *Cistus* and other species (Ne'eman et al. 1993), while it increased, by the release of ethylene, the germination of *R. coriaria*, in spite of the inhibition caused by the high pH of ash (Ne'eman et al. 1999).

Stimulation of germination by aqueous ash extracts is reported here for the first time. The inverse relationship between organic matter content of the ash and its effect on germination (Fig. 3, Table 1) suggests that the stimulatory
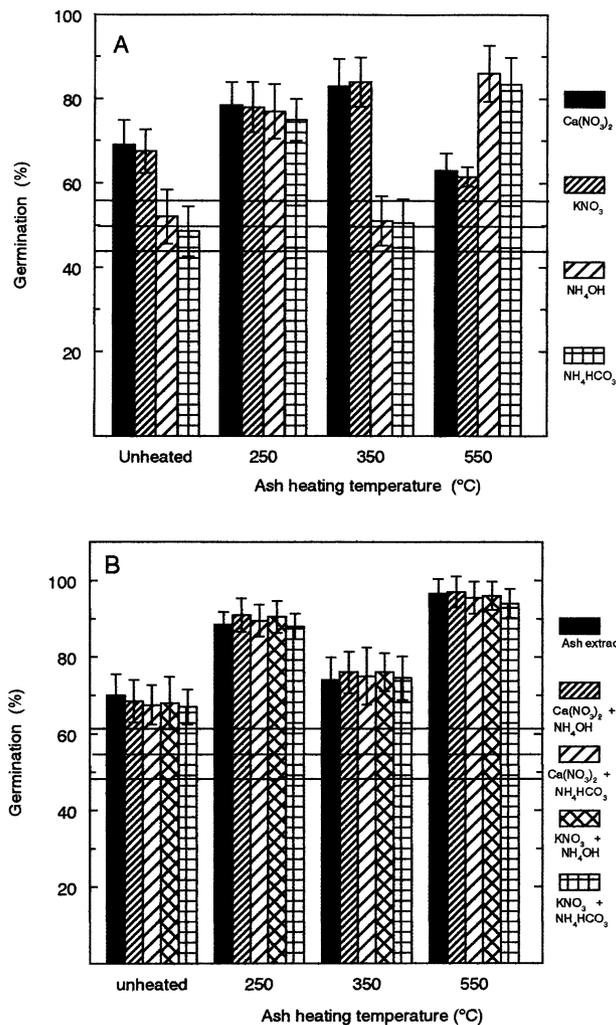
Fig. 4. Germination of *P. halepensis* seeds in distilled water and in solutions with sub-optimal concentrations of NO_3^-, NH_4^+, containing various concentrations of gibberellin (average of 10 replicates ± SD).

factor in ash extracts is inorganic. The high pH of ash extract had to be neutralized by atmospheric carbon dioxide in order to permit that stimulation to take place. If the high pH of the ash extract was retained by isolation from atmospheric CO_2, germination inhibition exceeded stimulation.

The results presented in Figs. 2 and 3 and Table 1 show that nitrate and ammonium ions are germination-stimulatory factors in ash extracts regardless of their complementary ions. Stimulation of germination by nitrate and ammonium has been documented for several other species and in some cases the enhancement of germination by a combined treatment of NO_3^- and NH_4^+ was higher than that of the separate ions (Cohn 1989, Karssen and Hilhorst 1992, Thanos and Rundel 1995, Doussi and Thanos 1997). Sub-optimal concentrations of $NO_3^- + NH_4^+$, similar to those in 550°C pre-heated ash extract, had an additive effect

Fig. 3. Germination of *P. halepensis* seeds in solutions of NO_3^- and NH_4^+ (top panel), in ash extracts and in combined solutions of $NO_3^- + NH_4^+$ (lower panel) in concentrations equal to those of unheated and heated ash extracts (average of 10 replicates ± SD). The horizontal lines mark the average germination percentage in distilled water ± SD.
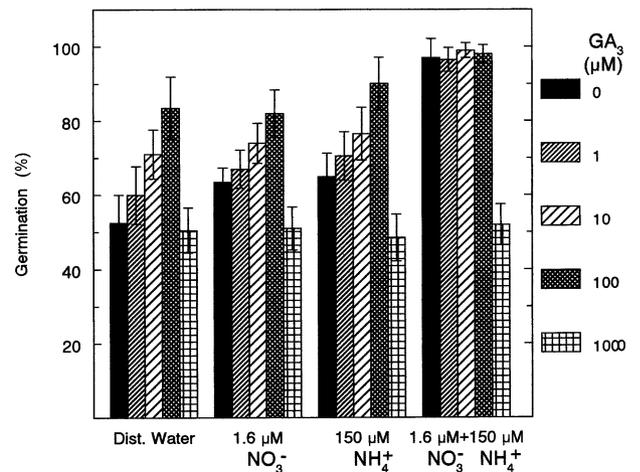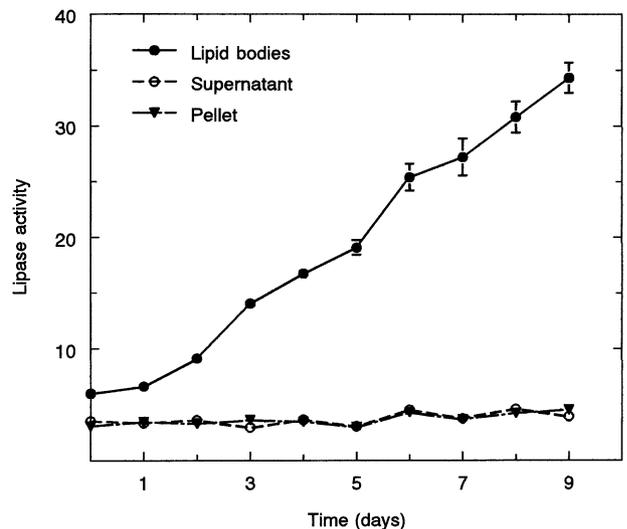
Fig. 5. Lipase activity (nmol fatty acid $seed^{-1}$ min^{-1}) in lipid bodies, supernatant and pellet of *P. halepensis* seeds at the first 9 days of germination (average of 5 replicates).
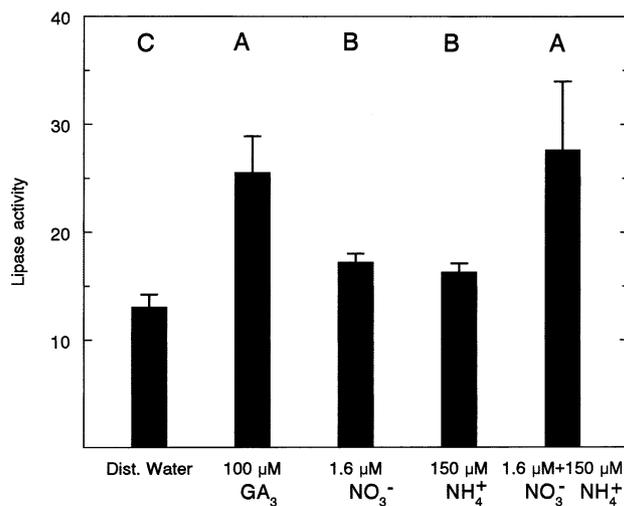
Fig. 6. Lipase activity (nmol fatty acid seed^{-1} min^{-1}) at the third day of germination in lipid bodies of *P. halepensis* seeds germinated in distilled water and solutions of GA$_3$ at optimal concentration and NO$_3^-$, NH$_4^+$ at sub-optimal concentrations (average of 5 replicates $\pm$ SD). Bars labeled with the same letter are not significantly different according to Tukey's multiple range test ($P < 0.05$).

on germination of pine seeds. On the other hand, higher concentrations of ammonium, similar to those found in other ash extracts, had an antagonistic effect to that of nitrate.

Germination of several species was induced or stimulated by nitrate in combination with other environmental factors, such as low temperature, temperature fluctuations or photoperiod (Hilhorst 1990a,b, Karssen and Hilhorst 1992, Carmona and Murdoch 1995, Egley 1995, Thanos and Rundel 1995). It was suggested that gibberellic acids play a role in this induction mechanism (Hilhorst and Karssen 1988, 1989, Whitehead and Sutcliffe 1995), by one of the following mechanisms: stimulation of gibberellin synthesis; transformation from an inactive into an active form; or increase in seed sensitivity to gibberellin (Mayer and Poljakoff-Mayber 1982, Hilhorst and Karssen 1988, 1989, Hilhorst 1995, Ranjan and Lewak 1995). From the results presented in Fig. 4 we also conclude that germination enhancement of Aleppo pine seeds by NO$_3^-$ is mediated by GA$_3$. In the presence of external GA$_3$, nitrate enhanced germination only at sub-optimal GA$_3$ concentration, while ammonium enhanced germination also at optimal GA$_3$ concentrations. These results are consistent with the assumption that NO$_3^-$ enhances the seed sensitivity to GA$_3$. However, because we measured the effect of external gibberellin on germination rather than its endogenous concentrations, we cannot exclude the possibility of induced GA$_3$ production or transformation.

An increase in lipase activity was observed in the lipid bodies of *P. halepensis* seeds during early stages of germination. Lipase degrades lipids to fatty acids and is therefore a key factor in germination of fat-storing seeds. Controlling the activity of such a key enzyme may constitute an efficient regulation mechanism of seed germination. There are two mechanisms of lipase activation in seeds. In barley, the enzyme exists in an inactive form in the membrane of the protein bodies of the dry seeds. GA$_3$ activates the enzyme by induction of its transfer to the membrane of the lipid bodies at the onset of germination (Imeson et al. 1993, Ranjan and Lewak 1995). In such seeds, there is no increase in total lipase activity during the period of germination (Fernandez and Staehelin 1987). In other species, including some pines, an increase in lipase activity results from de novo synthesis of the enzyme at the early germination stage. In some of these species lipase does not exists at all in dry seeds (Wang and Huang 1987), while in others, such as *P. edulis*, the enzyme exists at a low level even in the lipid bodies of dry seeds (Hammer and Murphy 1993). The constant background degradation of lipids in the supernatant and the pellet fractions of the homogenate of germinating *P. halepensis* seeds were apparently non-enzymatic (Fig. 5). Moreover, the fact that the increase in lipase activity in the lipid bodies was not associated with a parallel decrease in the enzyme activity in the pellet, may indicate de novo synthesis of the enzyme.

GA$_3$, NO$_3^-$ and NH$_4^+$ enhanced lipase activity in the seeds at the early germination stage (Fig. 6). The similarity between the effects of GA$_3$, NO$_3^-$ and NH$_4^+$ on seed germination and on lipase activity may indicate that nitrate and ammonium enhance *P. halepensis* germination probably by affecting the lipase activity in germinating seeds. Optimal concentrations of nitrate and GA$_3$ enhanced both germination and lipase activity to a similar degree. In addition, sub-optimal concentration of nitrate in the presence of GA$_3$ enhanced germination only at sub-optimal concentrations of GA$_3$; therefore, we suggest that the enhancement of pine germination by NO$_3^-$ is a consequence of its effect on lipase activity, mediated by GA$_3$. In contrast, the enhancement of pine germination by NH$_4^+$ is a consequence of its direct effect on lipase activity.

The enhancement of *P. halepensis* germination by nitrate and ammonium was not related to acidic pH (Fig. 2, Table 2), as was found for some chaparral species (Keeley and Fotheringham 1997, 1998a,b) which have a semi-permeable sub-dermal cuticle that blocks the uptake of solutes. It was suggested that nitrogen oxides induce the germination of these species by oxidizing the sub-dermal cuticle and thus increasing its permeability (Keeley and Fotheringham 1998a,b).

The concentrations of NO$_3^-$ and NH$_4^+$ in the soil after a wildfire that occurred in the Judean Mountains in Israel in 1995 were within the range that enhanced germination as well as lipase activity of pine seeds in the laboratory experiments (N. Henig-Sever 1997. Thesis, Tel Aviv University, Israel). Induction of germination by similar NO$_3^-$ concentrations was found also for several Mediterranean, chaparral and fynbos species in Greece, California and South Africa (Baldwin and Morse 1994, Thanos and Rundel 1995, Doussi and Thanos 1997).

The balance between the inhibition of germination caused by the basic pH of ash cover and its enhancement by nitrate and ammonium present in the ash determines the final rate of post-fire *P. halepensis* germination. This mechanism explains the sparse appearance of pine seedlings under the projection of the burned pines and their higher density elsewhere. Pine seedlings that establish in low densities

under burned trees benefit from weak inter- and intra-specific competition as well as improved mineral nutrition enabling their rapid development. These seedlings have good chances to form the next generation of the forest trees. This ensures the occupation of good 'mother sites' by the generation of trees that emerge after the forest fire; therefore, it can be concluded that the enhancement of *P. halepensis* germination by nitrate and ammonium and its inhibition by high pH are ecophysiological adaptations to post-fire regeneration of the plant community.

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