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Effect of cold storage, heat, smoke and charcoal on breaking seed dormancy of *Arctostaphylos pungens* HBK (Ericaceae)

Efecto de almacenamiento en frío, calor, humo y carbón vegetal para romper la latencia de *Arctostaphylos pungens* HBK (Ericaceae)

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Abstract. We evaluated the effect of cold storage and fire-related cues on seed germination of *Arctostaphylos pungens* HBK (Mexican Manzanita), a common shrub in poorly managed pine-oak forests in Durango, Mexico. Because this shrub has a high density in previously burnt forests, we investigated the effect that high temperatures, smoke and charcoal might have on seed germination of this species. Seeds were collected fresh from the shrubs. The highest germination was 30% for seeds that had been cold stored, heat shocked, past through smoke and watered with diluted charcoal.

Keywords: Seed ecology; Germination.

Resumen. En este estudio se evaluó el efecto del almacenamiento en frío y factores relacionados con el fuego en la germinación de *Arctostaphylos pungens* HBK (Manzanita Mexicana), un arbusto común en bosques de pino-encino mal manejados en Durango, México. Debido a que este arbusto presenta una alta densidad en bosques que han sido incendiados, investigamos el efecto de alta temperaturas, humo y carbón sobre la germinación de las semillas. Se trabajó con semillas recién colectadas de los arbustos. El porcentaje de germinación más alto que se obtuvo fue de 30%, para semillas que se almacenaron en condiciones frías, con tratamiento de shock térmico, pasadas por humo y regadas con dilución de carbón vegetal.

Palabras clave: Ecología de semillas; Germinación.

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INTRODUCTION

In ecosystems adapted to fire, two main strategies can be found for plant reproduction. These strategies include heat resisting (often underground) structures, and heat resistant seeds (Keeley, 1991). *Arctostaphylos* is a genus with 150 species, many of which inhabit fire-prone ecosystems like chaparrals, and cold and temperate forests (Berg, 1974; Weise et al., 1991). Species in this genus can survive fire as adult plants or germinate after fire (Weise et al., 2005).

For seeds that germinate after fire, several factors can trigger germination or break dormancy: heat (Álvarez et al., 2007; Luna et al., 2007; Paula & Pausas, 2008); smoke (Brown, 1992; Keeley & Fotheringham, 1997; Flematti et al., 2004; Crosti et al., 2006; Tieu et al., 1999); charred wood residues (Brown, 1992; Pérez-Fernández & Rodríguez-Echeverría, 2003), or a combination of them (Thomas et al., 2003, 2007; Gilmour et al., 2000). Other factors such as late seed maturation (after-ripening), and over-wintering can influence germination of plants from temperate environments (Baskin and Baskin, 1998).

Mexican Manzanita (*Arctostaphylos pungens* HBK) is a widespread shrub common to pine and pine oak forests and chaparrals in Southern United States and Mexico (Márquez-Linares et al., 2005a). This species has some medicinal use in Mexico (González-Elizondo, 1984; Bye, 1986), and is used as ornamental in California (Márquez-Linares et al., 2005c). Its establishment occurs after fire under natural conditions, although natural fire regimes are poorly understood (Rzedowsky, 1978; Casas et al., 1995; Fulé et al., 2000; Márquez-Linares et al., 2005a,b).

Seeds of *Arctostaphylos* resemble a small nut from 1 to 3 mm length with 3 to 7 seeds and a hard endocarp. While the mesocarp is edible, the exocarp is a thin paper-like structure. Seeds are water permeable; however, seed germination of this genus is not clearly understood, since it does not germinate in contact with water (Bliss, 1958; Berg, 1974). Seeds from *Arctostaphylos pungens* have none or very low germination (Martínez-Pérez et al., 2006) even after applying conventional treatments such as mechanical scarification, cold storage and different light regimes, and using seeds either from different ages or collected from dung of dispersal agents (Márquez-Linares, 2004). Perhaps variables associated with fire (such as high temperature and smoke) break seed dormancy. However, no studies have investigated the effect of fire on the germination of Mexican Manzanita. We explored whether germination of *Arctostaphylos pungens* might be induced by cold storage, heat, smoke and charcoal. This would allow to better understand field conditions that might result on the encroachment of this shrub in Durango forests and chaparrals.

MATERIALS AND METHODS

Freshly matured seeds of *Arctostaphylos pungens* were collected from randomly selected plants (n=20) on June 2003 at 2440 m above sea level. Seeds were cleaned in the lab from fruit remains. Seeds that (1) were malformed, (2) had insect perforations, and (3) were not easy to separate were discarded. Clean selected seeds were placed in water for one minute, and those that floated were considered unviable and discarded. A set of seeds was tested for viability using the tetrazolium test (Sutherland, 1998). Extra seeds were added so the number used for germination included at least 50 viable seeds per replicate.

Seeds were placed inside Petri dishes with filter paper. Cotton wool was placed under the filter paper to keep moisture and prevent excess available water. Seeds were positioned with the hypocotyl facing downward, in contact with the moist filter paper. Petri dishes were plastic sealed to keep moisture, and incubated at 20 °C. An emerging radicle was the indicator of a germinated seed. Germination was registered every three days for eight weeks, removing germinated seeds.

We tested the effect of (a) Heat at 100 °C and 120 °C: seeds were placed during five minutes inside a muffle furnace with an electronic temperature control. Temperature was set one degree higher than that reported for each trial to allow for cooling while opening the door. Temperature was independently registered (± 1 °C) using a thermometer with the bulb on the top of the seed tray; (b) Cold storage, by placing seeds 40 days at 4 °C; (c) Smoke: leaves and twigs of Mexican Manzanita were charred on a hot metal plate until they ignited without flame. Smoke was captured using an extractor, and directed toward seeds placed on a wire mesh for 5 minutes. Smoke temperature was < 38 °C when reaching the seeds. In this way, smoke effect was not confounded with heat effect, and (d) Smoke residues, where seeds were watered with burnt Mexican Manzanita residues following Keeley and Fotheringham (1998). These residues were obtained after twigs and leaves of Mexican Manzanita were charred on a hot metal plate. Residues were placed in a beaker with distilled water and stirred. After 12 h, this material was filtered using a 2 mm mesh and diluted to 5%. This dilution was used to water seeds inside the Petri dishes. A control group (e) of seeds kept at room temperature was also tested for germination. Four Petri dishes with 50 viable seeds each were used as replicates for each treatment.

Statistical analysis. Data were analyzed using a four-way ANOVA. Adjusting for viability, germination results were converted first to percentage, and then arc-sine transformed before ANOVA (GLM, SAS, 1989).

RESULTS

Seed exposure to 100 °C and 120 °C did not influence germination ($F = 2.95$; $d.f. = 2$; $P = 0.0608$). However, more seeds germinated after cold storage ($F = 10.33$; $d.f. = 1$; $P = 0.0022$), contact with smoke ($F = 9.81$; $d.f. = 1$; $P = 0.0028$) and extract of charcoal ($F = 10.73$; $d.f. = 1$; $P = 0.0018$) (Table 1). The interactions of charcoal extract and smoke, smoke and temperature, and those between smoke, temperature and charcoal extract were significant (Table 2). The highest germination occurred for cold-stored seeds that were exposed to smoke and watered with extract of charcoal (Table 1).

Table 1. Seed germination of *Arctostaphylos pungens* HBK (Mexican Manzanita) from a combination of treatments. Germination percentages are the mean from 4 Petri dishes with 50 viable seeds each. Equal lower case letters represent similar means ($p > 0.05$).

Tabla 1. Germinación de semillas de *Arctostaphylos pungens* HBK (Manzanita Mexicana) bajo una combinación de tratamientos. Los porcentajes de germinación son la media de 4 cajas de Petri con 50 semillas viables cada una. Letras iguales representan medias similares ($p > 0.05$).

Treatments				Germination
Smoke	Charcoal extract	Storage temperature	Heat exposure	Mean \pm sd (%)
no smoke	no	ambient	none	0.0 \pm 0.0a
no smoke	no	ambient	100 °C	1.1 \pm 1.1a
no smoke	no	ambient	120 °C	0.0 \pm 0.0a
no smoke	no	4 °C	100 °C	5.7 \pm 4.3b
no smoke	no	4 °C	120 °C	5.7 \pm 4.3b
no smoke	yes	ambient	100 °C	11.4 \pm 6.9b
no smoke	yes	ambient	120 °C	3.4 \pm 2.2ab
no smoke	yes	4 °C	100 °C	8.0 \pm 4.7b
no smoke	yes	4 °C	120 °C	3.4 \pm 2.2ab
Smoke	no	ambient	none	2.3 \pm 1.3a
Smoke	no	ambient	100 °C	1.1 \pm 1.1a
Smoke	no	ambient	120 °C	8.0 \pm 5.1b
Smoke	no	4 °C	100 °C	8.0 \pm 3.9b
Smoke	no	4 °C	120 °C	8.0 \pm 2.2b
Smoke	yes	ambient	100 °C	4.6 \pm 1.9ab
Smoke	yes	ambient	120 °C	12.6 \pm 7.1b
Smoke	yes	4 °C	100 °C	21.7 \pm 3.9c
Smoke	yes	4 °C	120 °C	29.7 \pm 8.4c

DISCUSSION

Seed germination of *A. pungens* was promoted by cold storage suggesting an after winter effect on seed germination. This is also the case in other species in temperate environments (Baskin & Baskin, 1998; Jurado & Flores, 2005). Seed germination was higher when using fire related stimuli: e.g., the combination of high temperature, presence of smoke and watering with charcoal. Post-fire germination is in agreement with other species in the genus for California. Keeley (1987) found that lixiviates of charred wood promoted seed germination of several California Chaparral species, including *A. glandulosa* and *A. patula*. He argued that germination is promoted by the presence of an oligosaccharide that results from heating hemicelluloses during mild fires; however; it has recently been found that germination is promoted by active butenolide present in plant-derived smoke (Flematti et al., 2004; Merritt et al., 2006; Daws et al., 2007; Stevens et al., 2007). Parker & Kelly (1989) found that seeds of *A. canescens* extracted from the soil germinated when watered with charred wood extract. The effect of smoke promoting germination has not been determined for *Arctostaphylos*, but it has been shown to promote seed permeability in several species of the Californian Chaparral (Keeley & Fotheringham, 1998). Heat did not stimulate germination by itself either for *A. viscida* and *A. mewukka* (Kauffman, 1986; Kauffman & Martin, 1991) or for *A. patula* and *A. glandulosa* (Keeley, 1987).

We found that several factors combined increased germination percentage of Mexican Manzanita seeds. This suggests a synergic effect in the combination of variables such as cold storage, smoke, and heat and charcoal. However, germination was relatively low (ca. 30%) even with a combination of cold storage and fire-simulating variables. It is possible that other factors such as fluctuating light regimes (Baskin & Baskin, 1998; Pons, 2000; Flores et al., 2006) might promote germination. However, it is also possible that only very few seeds of Mexican Manzanita germinate each year after winter, like other temperate climate species that form a seed bank (Jurado & Flores, 2005; Ibáñez et al., 2007). However, after a fire many seeds would germinate, like those from fire-adapted species (Álvarez et al., 2007; Luna et al., 2007; Paula & Pausas, 2008). The cold storage conditions, and the conditions made to simulate fire such as heat, smoke and charcoal used here, might have been only similar to natural winter temperature variations in the field, and the effects of a real fire. However, they were sufficient to trigger germination on a relatively small percentage of seeds. Further experiments manipulating the variables tested here, and others, might help to determine the natural conditions that trigger establishment of large numbers of Mexican Manzanita seedlings in Durango Forests and Chaparrals.

Table 2. Analysis of Variance on seed germination of *Arctostaphylos pungens* HBK (Mexican Manzanita) from a combination of treatments.
Tabla 2. Análisis de Varianza de la germinación de semillas de *Arctostaphylos pungens* HBK (Manzanita Mexicana) de una combinación de tratamientos.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Model	17	0.12762550	0.00750738	3.84	0.0001
Error	54	0.10551239	0.00195393		
Total	71	0.23313789			
Storage	1	0.02018976	0.02018976	10.33	0.0022
Heat	2	0.01152765	0.00576382	2.95	0.0608
Smoke	1	0.01915981	0.01915981	9.81	0.0028
Charcoal	1	0.02095787	0.02095787	10.73	0.0018
Storage*Heat	2	0.00132886	0.00066443	0.34	0.7133
Storage*Smoke	1	0.01145559	0.01145559	5.86	0.0189
Storage*Charcoal	1	0.00256729	0.00256729	1.31	0.2567
Heat*Smoke	1	0.01283446	0.01283446	6.57	0.0132
Heat*Charcoal	1	0.00004554	0.00004554	0.02	0.8792
Smoke*Charcoal	1	0.00483963	0.00483963	2.48	0.1214
Storage*Heat*Smoke	1	0.00188936	0.00188936	0.97	0.3298
Storage*Smoke*Charcoal	1	0.01583716	0.01583716	8.11	0.0062
Heat*Smoke*Charcoal	1	0.00482305	0.00482305	2.47	0.1220
Storage*Heat*Smoke*Charcoal	2	0.00016949	0.00008474	0.04	0.9576

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