The effects of fire-related cues on seed germination and viability of *Helenium aromaticum* (Hook.) H.L. Bailey (Asteraceae)

Los efectos de las señales del fuego sobre la germinación y la viabilidad de las semillas de *Helenium aromaticum* (Hook.) H.L. Bailey (Asteraceae)

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RESUMEN

Se evaluó el efecto de la temperatura y el humo sobre la germinación de *Helenium aromaticum*; una planta anual abundante en matorrales incendiados. Encontramos que las semillas son resistentes a la alta temperatura (100ºC) y al humo, pero su germinación no es estimulada por estos factores. Otras señales del fuego deben ser evaluadas.

Lightning-ignited burns are extremely rare in the matorral of Central Chile (25-37 ºS). This has led to the notion that natural fires have not played an important role in plant species evolution in the matorral (Armesto & Gutiérrez 1978, Muñoz & Fuentes 1989, Montenegro *et al.* 2004). This particular feature distinguishes the matorral from other Mediterranean-type ecosystems in which fire has shaped a wide variety of plant functional traits, such as germination stimulated by heat-shock or by the chemicals produced by smoke, ash or charred-wood (Keeley & Fotheringham 2000).

Anthropogenic fires affect thousands of hectares each year in Central Chile, and the matorral is the most damaged ecosystem (CONAF 2010). Severe fires strongly reduce the soil seedbank because native species seeds generally can not tolerate extreme temperatures (≥ 100ºC; Muñoz & Fuentes 1989, Gómez-González & Cavieres 2009). However, low-severity fires increase seed survival and seedling establishment in some matorral species (Segura *et al.* 1998). These superficial fires occur in open grasslands with low fuel load, where soil reaches non-lethal temperatures for seeds (< 40ºC) (Gómez-González & Cavieres 2009). In such conditions, chemicals in smoke or ash could be more relevant than heat in affecting seed germination. Gómez-González *et al.* (2008) reported smoke-stimulated germination in some pioneer woody species that are common in open matorral grasslands. Although smoke-stimulated germination in species from naturally fire-free habitats has been interpreted as the result of smoke chemicals acting on general germination regulators (see Pierce *et al.*1995, Van Staden *et al.* 2000), a selective role of anthropogenic fires in the evolution of this trait is also plausible.

Helenium aromaticum (Hook.) L.H. Bailey (Asteraceae) is an annual (or biannual) native herb that is widely distributed along the Mediterranean region of Chile (Bierner 1978). *Helenium aromaticum* is a pioneer plant that colonize disturbed and nitrified matorral sites (Gutiérrez 1993), where coexists with several other annual species (mainly aliens) forming seasonal grasslands that surround patches of woody vegetation. Gómez-González & Cavieres (2009) reported differential effects of fire severity on the emergence of *H. aromaticum* seedlings from the soil seed bank. They found no emergence of seedlings after applying intense fires on soil samples (around 100ºC at 2.5 cm soil depth) but significantly higher seedling emergence when fire was of low-intensity (reaching 35ºC at 2.5 cm soil depth). Field observations support these results, since we found monospecific stands (90% cover) of this species in lightly burned patches but low colonization on severely burned patches (Gómez-González, unpublished data). This suggests that *H. aromaticum* seeds are vulnerable to the high temperatures reached during severe fires, but when fire is superficial germination could be stimulated, presumably by smoke.

To assess this hypothesis we subjected *H. aromaticum* seeds to four treatments: i) heat-shock (using an oven at 100ºC during 5 min), ii) plant-derived smoke (cold smoke applied during10 min; see methodological details in Gómez-González *et al.* 2008), iii) combination of heat-shock and smoke, and iv) control. Each treatment was applied to 180 *H. aromaticum* seeds, which were distributed in 6 Petri-dishes (30 seeds in each one). After applying the treatments, Petri-dishes were placed in a growth chamber under favorable conditions (12 h light / 20ºC; 12 h dark / 10ºC), watered and checked for seed germination on a daily basis during 36 days. Afterwards, we performed a viability test using tetrazolium on non-germinated seeds (TTC 1% in phosphate buffer, pH 6.5-7.5, 24 h in darkness). We evaluated the effect of treatments (fixed factor) on the final germination percentage (number of germinated seeds after 36 days / 30 seeds) using a generalized linear mixed model (binomial GLMM), where Petri-dishes were considered as a random factor (R 2.8.0 software, R Development Core Team, 2008).

Heat-shock decreased the final germination percentage of *H. aromaticum* from 80% to 67.7% ($z = -2.1$; $P = 0.039$; GLMM), while smoke or the combination of heat and smoke produced no effects (smoke: $z = -1.3$, $P = 0.176$; heat + smoke: $z = -1.4$, $P = 0.153$; GLMM) (Fig. 1). The tetrazolium test revealed that 100% of non-germinated seeds were viable after the germination essay ended (all treatments), and thus they were dormant seeds. Therefore, heat-shock treatment reduced germination but did not cause seed mortality. Furthermore, heat and smoke together did not affect seed germination, indicating that *H. aromaticum* seeds are resistant to high temperatures. These results support recent evidences demonstrating that native annual species have certain resistance to fire (see Figueroa *et al.* 2009, Gómez-González *et al.* 2011). Gómez-González *et al.* (2011) found increased richness of native annual species in burned matorral areas and suggested that the strong pressure of anthropogenic fires during the last centuries (Aravena *et al.* 2003) could have selected favorable traits on native species seeds such as harder seed coats. The evolutionary ecology of seed traits in relation to fire needs to be explored in native matorral species to test this hypothesis.

Our results contrast in some way to the pattern of seedling emergence after low- and high-intensity fires reported by Gómez-González & Cavieres (2009) for this species. Although high temperature did decrease *H. aromaticum* germination, the viability of *H. aromaticum* seeds was not affected. Furthermore, and contrary to expected, smoke did not stimulate seed germination. This could be due to other fire cues not evaluated in this study. The fire experiment performed by Gómez-González & Cavieres (2009) consisted in burning litter on soil seedbank samples, and thus, other factors such as ash and charred-wood were also involved. It has been shown that ash alone stimulates seed germination of many mediterranean species (Keeley & Fotheringham 2000) but its effects can be inhibitory when combined with high temperatures (González-Rabanal & Casal 1995). Therefore, the effect of these factors on seed germination of *H. aromaticum* should be addressed to reveal the mechanisms by which this native species increases its emergence and establishment only in burned grasslands of the Chilean matorral.

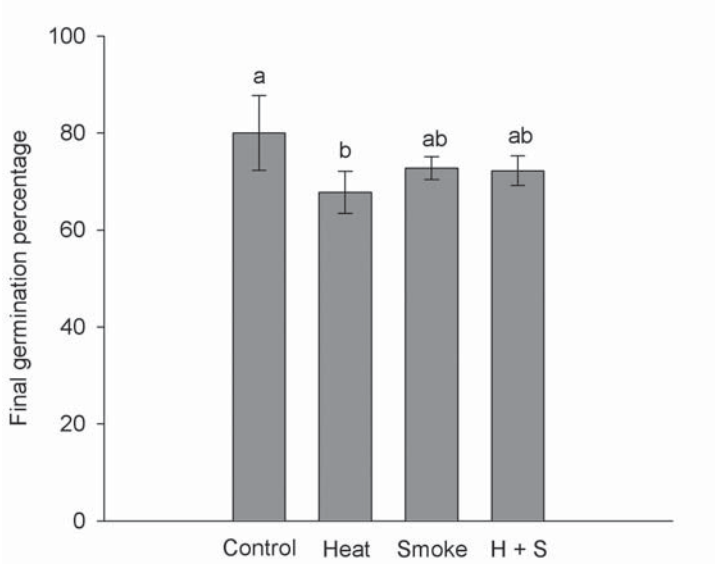

Figure 1: Effect of heat, smoke and the combination of both factors (H + S) on the final germination percentage of *H. aromaticum*. Mean values ± 2SE are shown, n = 6. Different letters denote significant differences among treatments ($P < 0.05$; GLMM).

Figura 1: Efecto del calor, el humo y la combinación de ambos factores (H + S) sobre el porcentaje de germinación final de *H. aromaticum*. Se muestran los valores medios ± 2EE, n = 6. Las letras diferentes denotan diferencias significativas entre tratamientos ($P < 0,05$; GLMM).

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