



Plant-derived smoke and temperature effects on seed germination of five *Helianthemum* (Cistaceae)[☆]



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ABSTRACT

Plant-derived smoke promotes germination in Mediterranean-like environments, but its effect is unclear in the Mediterranean Cistaceae. This article investigates the role of smoke in the comparative germination ecology of five *Helianthemum* taxa. Laboratory germination experiments were conducted using seeds collected in the field and stored in a seed bank. All seeds were mechanically scarified prior to testing. Various pre-treatments with smoke solutions and gibberellins were applied, and seeds were incubated in three germination temperature regimes (30/20 °C, 22/12 °C, 14/4 °C). In *H. cantabricum*, *H. nummularium*, *H. oelandicum* and *H. urriense* germination was very high at the three temperatures, and no effect of smoke was detected. In *H. tinetense* germination was generally poor and a significant effect of smoke was found, but the effect size was small. The thermal niche of *Helianthemum* appears to follow an opportunistic strategy, being limited only by physical dormancy at the time of dispersal. *H. tinetense* follows a more conservative strategy, with physiological dormancy retarding germination. In the Mediterranean basin and surrounding areas, smoke appears to be one of a multitude of environmental signals controlling germination. The smoke effect might be more relevant in certain species such as *H. tinetense*.

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1. Introduction

In ecosystems subjected to frequent fire disturbances, heat and smoke are major signals for seed germination (Cushwa et al., 1968; Jefferson et al., 2014). Heat may break the seed coat of physically dormant seeds (Baskin and Baskin, 2014), allowing imbibition during the next rainfall (Herranz et al., 1998). Smoke, produced by burning vegetation, has an important role by itself (van Staden et al., 2000). It carries chemicals, e.g. karrikins (Flematti et al., 2004; Guo et al., 2013), which can overcome physiological dormancy (Baskin and Baskin, 2014). Germination promotion by smoke is common in Mediterranean-like climates where fire plays a central role, like the South African fynbos (Brown, 1993), the Californian chaparral (Keeley and Fotheringham, 1998) and Western Australia (Dixon et al., 1995). Although earlier studies failed to detect a smoke

response in the Mediterranean basin (Keeley and Babr-Keeley, 1999; Reyes and Casal, 2006; Reyes and Trabaud, 2009; Rivas et al., 2006), recent work suggests that smoke also promotes germination in the flora of the Mediterranean and surrounding areas (Crosti et al., 2006; Keeley et al., 2011; Mojzes et al., 2015; Moreira et al., 2010; Paula et al., 2009; Pérez-Fernández and Rodríguez-Echeverría, 2003).

Cistaceae Juss. are largely linked to the Mediterranean environment (Watson and Dallwitz, 1992). Physical dormancy is a common feature of the family (Ferrandis et al., 1999; Thanos et al., 1992); and post-fire seedling emergence has often been reported (Baskin and Baskin, 2014). A response to smoke has only been found in some species of genera such as *Cistus* L. and *Fumana* (Dunal) Spach. (Chen, 2014; Jefferson et al., 2014). In the diverse *Helianthemum* Mill. genus, heat breaks physical dormancy (Pérez-García and González-Benito, 2006), but attempts to find a germination response to smoke have been unsuccessful so far (Moreira et al., 2010). Five *Helianthemum* taxa occur in the Cantabrian Mountains of northern Spain (Carlón, 1998; López González, 1993). Seeds of these Cantabrian *Helianthemum* germinate massively after coat scarification, with the notable exception of the endemic *H. tinetense*, whose germination percentages are generally low. The special ecology of *H.*

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tinetense, closely linked to frequently burnt slopes (Carlón, 1998), makes it a good candidate to find a response to smoke (Mojzes et al., 2015). If such response exists in *H. tinetense*, it could be interpreted as one of the traits that allowed it to colonize its highly disturbed niche.

Thus, characterizing the germination response to smoke in these closely related *Helianthemum* can help to clarify the role of this signal in the functional ecology of the Mediterranean Cistaceae. For this reason, we conducted a comparative seed germination study with five taxa of *Helianthemum*, focusing on *H. tinetense*. It was our goal to test the hypothesis that, in these taxa, and especially in *H. tinetense*, smoke promotes seed germination.

2. Materials and methods

2.1. Plant material

In this work we studied the germination of the five *Helianthemum* (Cistaceae) which occur in the Cantabrian Mountains: *Helianthemum apenninum* subsp. *cantabricum* (M. Laínz) G. López [= *Helianthemum croceum* subsp. *cantabricum* M. Laínz], henceforth *H. cantabricum*; *H. apenninum* subsp. *urriense* (M. Laínz) G. López [= *Helianthemum urriense* (M. Laínz) Nava & Fern. Casado], henceforth *H. urriense*; *Helianthemum nummularium* (L.) Mill.; *Helianthemum oelandicum* subsp. *incanum* (Willk.) G. López [= *Helianthemum canum* subsp. *cantabricum* (Font Quer & Rothm.) Rivas Mart.], henceforth *H. oelandicum*; and *Helianthemum tinetense* Mayor & Fern. Benito. All taxa occupy dry scrub and grasslands at different altitudes. *H. tinetense* is the only one exclusive of acidic poor soils, usually in fire-prone slopes and road edges (Carlón, 1998).

We used seeds from two sources. First, to compare the germination of the five taxa, we used seeds that had been banked in the Seed Bank of the Jardín Botánico Atlántico (Xixón, Spain). All seed accessions had been collected in natural populations of the Cantabrian Mountains (Supplementary material 1). They had been preserved by drying to 15% RH and freezing at -13°C (ENSCONET, 2009) and then stored for different time periods (10–1 years depending on the taxon, Supplementary material 1). Twenty-four h before the experiments began, we removed seeds from the storage freezers and allowed them to equilibrate to ambient temperature and humidity.

Second, to analyse germination of fresh seeds of *H. tinetense*, during August 2014 we collected ripe capsules from three natural populations: (1) Tinéu ($43^{\circ}17'12''\text{N}$, $06^{\circ}31'22''\text{W}$; 500 m above sea level); (2) Ayande ($43^{\circ}16'11''\text{N}$, $06^{\circ}35'30''\text{W}$; 650 m); and (3) Ibiás ($43^{\circ}02'25''\text{N}$, $06^{\circ}51'59''\text{W}$; 300 m). In each site, we sampled all individuals bearing mature capsules. Capsules stayed in our laboratory (c. 21°C , 50% RH) for two weeks before the start of germination experiments.

To produce a plant-derived smoke solution, we also collected plant remains from the Tinéu population, belonging to the dominant shrubs at the site: *Calluna vulgaris* (L.) Hull, *Cytisus striatus* (Hill) Rothm. and *Erica cinerea* L. We burnt these remains in controlled conditions, and during 5 min we impregnated the smoke produced by the combustion into (1) filter paper held 1 m above the fire (henceforth paper-smoke) and (2) an aerosol of distilled water passed through the smoke column (henceforth water-smoke).

2.2. Germination experiments

2.2.1. General conditions

To break the seed coat and remove the well-known physical dormancy of *Helianthemum* (Pérez-García and González-Benito, 2006; Baskin and Baskin, 2014; Royal Botanic Gardens Kew, 2016), we scarified all seeds with sandpaper before any further manipula-

tion. Afterwards, we exposed seeds to one of four pre-treatments: (1) paper-smoke, 24 h immersion in 40 mL of distilled water with four 6 cm discs of paper-smoke, prepared as described above; (2) water-smoke, 24 h immersion in 30 mL of distilled water mixed with 10 mL of water-smoke, prepared as described above; (3) GA₃, 24 h immersion in 40 mL of distilled water with gibberellic acid GA₃ (0.5 g/L); and (4) control, 24 h immersion in 40 mL of pure distilled water. The purpose of the GA₃ application was to confirm whether the low germination in *H. tinetense* was due to physiological dormancy. Then we sowed seeds in 6 cm Petri dishes holding 1% agar, and sealed the dishes with Parafilm to prevent agar from drying. We sowed 4 dishes with 25 seeds each per experimental treatment.

We incubated these dishes at one of three alternating temperature regimes programmed inside growth chambers (GROW-360, Ing Climas, Barcelona): (a) $30/20^{\circ}\text{C}$, a situation of soil heating; (b) $22/12^{\circ}\text{C}$, typical summer temperatures; and (c) $14/4^{\circ}\text{C}$, typical autumn or spring temperatures. Temperature regimes were coupled to a photoperiod of 12 h of light (c. $20\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ produced by six fluorescent tubes) followed by 12 h of darkness. Incubations lasted 28 days, during which we scored germination daily, as radicle emergence. After the incubations, we opened non-germinated seeds with a scalpel and classified them as apparently normal, non-scarified (i.e., hard and not imbibed), empty and contaminated by fungi. We removed non-scarified, empty and infected seeds from further calculations. Thus, we considered that the number of potentially germinable seeds in each dish was the sum of germinated and non-germinated but apparently normal seeds. The cumulative number of germinated seeds in each scoring day, and the potentially germinable seeds in each dish, are provided in Supplementary material 2.

2.2.2. Comparative effect of temperature and smoke in *Helianthemum*

To compare the effect of temperature in the five taxa, and to test if the low germination of *H. tinetense* was due to a non-optimal germination temperature being used, we incubated non-treated (control) seeds at $30/20^{\circ}\text{C}$, $22/12^{\circ}\text{C}$ and $14/4^{\circ}\text{C}$. We sowed both non-stratified (sown directly) and cold-stratified seeds (previous 12 weeks at 3°C in darkness and 1% agar), in order to assess the potential break of physiological dormancy by overwintering. However, in the end the effect of cold stratification could not be considered, because all taxa germinated during stratification (i.e. at 3°C in darkness), with similar percentages to those of non-stratified seeds. In addition, to compare the germination response to smoke in the five taxa, we sowed seeds pre-treated with water-smoke at $22/12^{\circ}\text{C}$.

2.2.3. Effect of temperature and smoke in *H. tinetense*

For *H. tinetense*, we performed a factorial experiment to analyse the effect of smoke and temperature on the germination of fresh seeds from the three natural populations. In this experiment we incubated control, paper-smoke, water-smoke and GA₃ treated seeds at $30/20^{\circ}\text{C}$, $22/12^{\circ}\text{C}$ and $14/4^{\circ}\text{C}$.

2.3. Statistical analyses

We calculated statistics using R (R Development Core Team, 2008). We analysed the effect of the different experimental treatments on the final germination percentage (germinated/potentially germinable) after 28 days of incubation. For this we used Generalised Linear Models (GLM) with binomial error distribution and logit link function. In some treatments that had zero variance because all seeds germinated during the experiment, we subtracted one germinated seed from the fourth Petri dish. We began by fitting three fully factorial models to analyse (a) the effect of taxon and temperature in the genus; (b) the effect of taxon and smoke in

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