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Complex dormancy in the seeds of *Hypericum philonotis*

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ABSTRACT

Species of the genus *Hypericum* have elicited great interest in pharmacological research, therefore, their propagation is necessary. However, only a low germination percentage has been achieved to date. A population of *Hypericum philonotis* (Cham. & Schlecht.) grows every 2 or 5 years in the Parque Ecológico de la Ciudad de México (PECM). To understand seed germination and the population dynamics of *H. philonotis* in the PECM, we studied their seed germination to identify the dormancy type. We assessed the effect on germination of light, constant temperatures (5–35 °C) and fluctuating (25/35 °C, 18/6 h, with the highest temperature at noon, photoperiod 12/12), cold stratification, scarification (with HCl); gibberellins and seed immersion in acetone or hexane. We also studied the morphology and structure of the seeds, and the allelopathic potential of the pigments present in the seed coat on its own seed germination and early seedling growth. Light, gibberellins, a combination of temperature with gibberellins, and immersion in acetone increased seed germination. Alternating temperatures replaced the effect of gibberellins. Immersion in acetone eliminated a pigment from the seed coat, enhancing full seed germination. The acetone extract from *H. philonotis* seeds partially inhibited seed germination and early growth. The endosperm was reduced, and the main reserve in cotyledons was proteins. The *H. philonotis* seeds exhibited physiological dormancy. Gibberellins increased the embryo growth potential, reducing the constraint of the thick seed cover and any inhibiting effect of the acetone extract. The requirement of light and temperature fluctuation acts as an environmental cue for the successful germination of these minuscule seeds (0.5–0.8 mm in length).

Introduction

The genus *Hypericum* (L.) (Hypericaceae) includes 484 herbaceous or shrubby species widely distributed in temperate and subtropical regions (Crockett and Robson, 2011). Some of the species, such as *H. perforatum* and *H. avicullariifolium*, have elicited great interest in pharmacological research (Çirak et al., 2007) due to the following reasons: (1) the use of hyperforin and adhyperforin to treat moderate depression (hyperforin also exhibits antibiotic and antitumoral properties) (Karppinen et al., 2007); (2) the antiretroviral activity of *H. triqetrifolium* hypericin and pseudohypericin (Meruelo et al., 1988); and (3) the use of *H. silenoides* and *H. philonotis* extracts to produce weight loss in obese rats (García-de la Cruz et al., 2013). Hyperforin, quercetin, rutin and chlorogenic acid have been identified in aqueous extracts of these two species (García-de la Cruz et al., 2013). Additionally, in this genus has been identified 14 essential oils, amongst them: alkanes, alkanols, monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene

Abbreviations: PECM, Parque Ecológico de la Ciudad de México.

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hydrocarbons, oxygenated sesquiterpenes (García-de la Cruz et al., 2015). To maintain the seed populations of *H. canadense* and *H. hirsutum*, their viability in gene banks (Walsh et al., 2003) and the germination of *H. gramineum* (Willis et al., 1997) and *H. erectum* (Tsuyuzaki, 2010) in the soil seed bank have been studied.

Some *Hypericum* species are potential invaders, such as *H. perforatum*, whose interpopulation germination behaviour has been studied (Pérez-García et al., 2006; Tisdale et al., 1959). By contrast, the herbaceous *H. philonotis* (Cham. and Schlecht.) (Synonym = *H. paniculatum* H.B.K., *H. submontanum* Rose) is a species that is occasionally found growing every two or five years. It forms a reddish stand in a forest gap of the Parque Ecológico de la Ciudad de México (A. Orozco-Segovia, personal observations). This behaviour has also been reported in *Ambrosia artemisiifolia* in other ecosystems (Willemsen and Rice, 1972). In general, the plant population dynamic of plant species is initially determined for the germination response and the seedling growth and recruitment (Zobel et al., 2000). The seeds of a total of 59 *Hypericum* species have been reported as orthodox. In gene banks, the seeds of *H. androsaeum* maintain its viability for at least 11 years (Royal Botanic Gardens, Kew, 2015) and *H. canadense* and *H. hirsutum* maintain it for at least 7 years (Walsh et al., 2003). In the soil bank, the seeds of *H. gramineum* and *H. erectum* maintain seed viability for 18 months and >5 years, respectively (Tsuyuzaki, 2010; Willis et al., 1997). In the laboratory, seeds of *H. philonotis* can remain dormant for more than five years (M.E. Sánchez-Coronado, personal observations). Therefore, the life cycle of this species in the field may be related with a deep seed dormancy.

The seeds of *H. philonotis* are between 0.5 and 0.8 mm in length (M.E. Sánchez-Coronado, personal observation). Seeds of several *Hypericum* species require light and fluctuating temperatures for germination, which is a common requirement of small-sized seeds (Thompson et al., 2001). The pharmacological utility of *Hypericum* species has generated the necessity of propagating or cultivating them. However, although the germination of several *Hypericum* species has been studied, only low germination percentages have been achieved and its dormancy type has not been characterised. The seed germination of *H. philonotis* has not been studied. Therefore, the aim of the present work was (1) to understand the sporadic presence of *H. philonotis* in the field and (2) to identify their seed dormancy type and germination requirements. With this purpose, we characterised the morphology and structure of the seeds and determined their light and temperature germination requirements, which might be based on seed morphology and the possible chemical composition of the pigments in the seeds. We applied acetone and hexane treatments to the seeds, and we eliminated the most external layers of the seed coat with HCl to induce seed germination. Finally, we tested the allelopathic potential of the reddish pigments present in the seed coat.

Materials and methods

Seed collection area

The Parque Ecológico de la Ciudad de México is located in southern México City at 2400–2800 m a.s.l., in the Ajusco volcano. Mean annual precipitation is 717–918 mm (~80% falls between June and October). Mean annual temperature is 15.6 °C (with extreme values from –3.3 to 0.5 °C in winter and from 27.9 to 30.2 °C in the summer, Ecoguardas Meteorological Station). The area is a lava field that is 1650–2000 years old (Siebe, 2000). Vegetation includes a xerophylous shrubland with shallow and undeveloped soils. The *Quercus* forests grow in the deeper soils (González-Hidalgo et al., 2001). *H. phillonotis* grows in a large forest gap with deep soil (terrace). This area was cultivated until the 80 years and its origin is probably prehispanic (Soberón et al., 1991). The area is covered by grasses and isolated small *Q. rugosa* individuals. Grass density is reduced when a quasi mono-specific reddish stand of *H. philonotis* is established every 2, 3 or 5 years. The seeds were collected during two shedding seasons, in June 2003 and in June 2005. In the laboratory, seeds were manually removed from the floral remnants and kept in glass jars at 23–25 °C and 20–50% of relative humidity.

General procedures

Seeds were sown in Petri dishes on 1% agar plates (Bioxon, México) and incubated in growth chambers (Lab-Line Instruments, Inc., 844, IL, USA) (five replicates of 50 seeds per Petri dish, per treatment). Germination was determined by the observation of the protruded radicles under a stereoscopic microscope (Stereo Star, American Optical Corporation, Phoenix, Arizona, USA). To test seed imbibition, the longitude and width of 30 seeds immersed in water (during 24 h) and 30 dry seeds were individually measured using an optical microscope (Olympus BX 41, Japan).

Effect of light on germination

For white light treatments, growth chambers were provided with cool white fluorescent lights (F20T12/CW, Sylvania, 20 W). The photoperiod was 12 h day^{-1} and photosynthetic photon flux density (PPFD, 400–700 nm) was 33.21 $\mu mol\ m^{-2}\ s^{-1}$ (measured using a LI 185A quantometer LI-COR, Inc., 18 NE, USA; provided with quantum sensor LI-190SA; 400–700 nm). For the dark treatments, Petri dishes were wrapped with two layers of aluminium foil. In the white light treatments, germination was recorded every day for one month. Germination in darkness was recorded after one month.

Seeds collected in 2003

Effect of temperature, scarification and gibberellins

Immediately after collection, the seeds were scarified with hydrochloric acid (HCl 37.2%, J.T. Baker, México) for 0–5 min. After that seeds were washed and the germination response was tested at six constant temperatures (5, 15, 20, 25, 30 or 35 °C), and at a fluctuating temperature (25/35 °C, 18/6 h, with the highest temperature at noon, 12/12 h photoperiod). These treatments were combined with the addition of 0 or 2000 mg L^{-1} of gibberellins (GA_3 G-7645 Sigma Chemical Co., USA). Control seeds were not scarified. Seeds were germinated at 18 h light at 25 °C/6 h darkness at 35 °C.

Seeds collected in 2005

Effect of gibberellins, solvents and light on germination

Immediately after collection, the seeds were pre-treated by immersion in: (a) acetone (J.T. Baker, México) (3 min), (b) concentrated HCL (3 min), (c) hexane (J.T. Baker, México) (1 min), (d) hexane (3 min) or (e) seeds were placed in a mesh bag and washed with running tap water (10 min). Control seeds were not treated. Gibberellins (0, 1000 or 2000 mg L^{-1}) were added separately to each treatment and Petri dishes were placed in growth chambers at 25 °C in the light (12/12 h photoperiod) or dark.

Effect of cold stratification on germination

Another set of seeds was treated as above and exposed to 0–4 weeks of cold stratification at 4 °C and 12 h of fluorescent light in a refrigerator (American, México, D.F.). After that, seeds were incubated at 25 °C in growth chambers with light.