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Original Research

Ecophysiological and phytochemical characterization of wild populations of *Inula montana* L. (Asteraceae) in Southeastern France

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ABSTRACT

Inula montana is a member of the family Asteraceae and is present in substantial numbers in Garrigue country (calcareous Mediterranean ecoregion). This species has traditionally been used for its anti-inflammatory properties as well as *Arnica montana*. In this study, three habitats within Luberon Park (southern France) were compared regarding their pedoclimatic parameters and the resulting morpho-physiological response of the plants. The data showed that *I. montana* grows in south-facing poor soils and tolerates large altitudinal and temperature gradients. The habitat conditions at high elevation appear to affect mostly the morphology of the plant (organ shortening). Although the leaf contents of total polyphenols and flavonoids subclass essentially followed a seasonal pattern, many sesquiterpene lactones were shown to accumulate first at the low-elevation growing sites that suffered drought stress (draining topsoil, higher temperatures and presence of a drought period during the summer). This work highlights the biological variability of *I. montana* related to the variation of its natural habitats which is promising for the future domestication of this plant. The manipulation of environmental factors during cultivation is of great interest due to its innovative perspective for modulating and exploiting the phytochemical production of *I. montana*.

1. Introduction

The sessile living strategy of terrestrial plants, anchored to the ground, forces them to face environmental variations. Plants have developed complex responses to modify their morpho-physiological characteristics to counteract both biotic and abiotic factors (Suzuki et al., 2014; Rouached et al., 2015). Altitude is described as an integrative environmental parameter that influences phytocoenoses in terms of species distribution, morphology and physiology (Liu et al., 2016). It reflects, at minimum, a mixed combination of temperature, humidity, solar radiation and soil type (Körner, 1999). In addition, the plant age, season, microorganism attacks, competition, soil texture and nutrient availability have been proven to strongly influence the morphology and the secondary metabolite profile of plants (Seigler, 1998). Altitudinal gradients are attractive for eco-physiological studies to decipher the mechanisms by which abiotic factors affect plant biological characteristics and how those factors influence species distribution (Graves and Taylor, 1988). For instance, a summer increase of nearly 10% in solar irradiance per 1000 m in elevation has been demonstrated in the European Alps. This increase was also characterized by an 18% increase in UV radiation (Blumthaler et al., 1997). Considering the reliefs of the Mediterranean basin, plants must confront both altitude and specific climate, namely high summer temperatures, infrequent but abundant precipitation, and wind (Bolle, 2012). Moreover, plants that live at higher elevation must also survive winter conditions characterized by low temperatures and high irradiance. All together, these factors force the plants to develop dedicated short- and long-term phenological, morphological and physiological adaptations (Kofidis et al., 2007). Many of these adjustments are protective mechanisms against photoinhibition of photosynthesis (Guidi and Calatayud, 2014; Sperlich et al., 2015), and most of them involve the synthesis of secondary metabolites (Ramakrishna and Ravishankar, 2011; Bartwal et al., 2012).

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The genus *Inula* (Asteraceae) includes more than 100 species that are widely distributed in Africa and Asia and throughout the Mediterranean region. These plants have long been collected or cultivated around the world for their ethnomedicinal uses. They synthesize and accumulate significant amounts of specific terpenoids and flavonoids. Secondary metabolites (including sesquiterpene lactones) from *Inula* spp. have shown interesting biological activities such as antitumor, anti-inflammatory, antidiabetic, bactericidal, antimicrobial and antifungal activities, and these plants have also been used for tonics or diuretics (Reynaud and Lussignol, 1999; Seca et al., 2014).

The species *Inula montana* is a hairy rhizomatous perennial (hemicryptophyte) herb with a 10–40 cm circumference and solitary capitulum (5–8 cm diameter) of yellow florets (long ligules) positioned at the top of a ≈20 cm floral stem. It grows at altitudes of 50–1300 m from eastern Italy to southern Portugal and is frequent in Southeast France. This calcicolous and xerophilous plant can be locally abundant, particularly in the Garrigue-type lands (Gonzalez Romero et al., 2001; Girerd and Roux, 2011; Tela Botanica, 2016). In the south of France, *I. montana* was incorrectly called “Arnica” because it was used in old traditional medicine as an alternative drug to the well-known *Arnica montana* (Reynaud and Lussignol, 1999). Due to herbivory pressure, loss of habitat and the fact that it is mainly harvested from the wild, *A. montana* is cited in the Red List of Threatened Species (IUCN). In Europe, more than 50 t of dried flowers are traded each year (Sugier et al., 2013). Although many efforts are currently underway to domesticate *A. montana* and to correctly manage its habitats, the opportunity to find an alternative plant would therefore be of considerable interest.

In this context, we have developed a scientific program that aims to rehabilitate, domesticate and test *I. montana* as an efficient pharmaceutical substitute to *A. montana.* We have recently published a phytochemical investigation of the contents of leaves and flowers of *I. montana* (Garayev et al., 2017). Those data showed new compounds with associated anti-inflammatory activity. Here, we present the results of an ecophysiological study of *I. montana* that aimed to analyze the putative correlations between its morphology, its phytochemical production (with a focus on sesquiterpene lactones) and the characteristics (edaphic and climatic) of its natural habitats. It was expected that *I. montana* would face various abiotic stresses according to the large altitude gradient of its habitats. Assessing the response of the plant to its natural growing conditions will be helpful for its future domestication. In addition, a successful identification of environmental levers that could modulate the phytochemical production of this medicinal plant would be of great interest.

2. Material and methods

2.1. Luberon park

The present study was focused on *I. montana* populations growing in the French “Parc Naturel Régional du Luberon” (Luberon Park) that is located in southeastern France. The park (185,000 ha) is characterized by medium-sized mountains (from 110 to 1125 m high; mean altitude ≈680 m) that stretch from west to east over the “Vaucluse” and the “Alpes-de-Haute-Provence” regions (Supplemental file). Although the overall plant coverage of Luberon Park belongs to the land type “Garrigue” (calcareous low scrubland ecoregion), there are two significant climatic influences: first, the north-facing shady side is characterized by a cold and humid climate that supports the development of deciduous species such as the dominant white oak (*Quercus pubescens*). Second, the sunny south-facing side receives eight to ten times more solar radiation. On this side, the vegetation is typically Mediterranean with a majority of green oak (*Quercus ilex*), Aleppo pine (*Pinus halepensis*), kermes oak (*Quercus coccifera*) and rosemary (*Rosmarinus officinalis*). The ridges of the Luberon Park suffer from extreme climatic variations: windy during all seasons, intense summer sun, cold during the winter, dry atmosphere and spontaneous and intense rains. These conditions limit the spectrum of plant species to those most resistant to these conditions, such as the common juniper (*Juniperus communis*) and boxwood (*Buxus* sp.) (Gressot, 2010).

2.2. Sites of interest and sampling

Inula montana is present in highly variable amounts over Luberon Park. By exploring the south-facing sides we selected three sites of interest: Murs, Bonnieux and Apt (Supplemental file). At these locations, *I. montana* forms several small, sparse and heterogeneous groups of tens of plants per hectare. These sites were also selected for their similar presentation as grassy clearings (area from 4 to 9 ha) and for their uniform flatness and slight inclination (≈7%). The linear distance between the 3 sites is 21.4 ± 2 km. The Apt site is 500–600 m higher than both other sites. A preliminary phenological survey showed that the vegetative growth of *I. montana* extended from early April to late October, consistent with the hemicryptophytic strategy of the plant. Mid-June corresponded to the flowering period, which lasted ≈10 days. Accordingly, samples were synchronously collected from the three habitats at four consecutive periods during 2014: early April (early spring), mid-May (late spring), mid-June (summer) and late October (autumn).

2.3. Climatic and edaphic data

The measurements of climate characteristics (standard weather stations, 1.5 m height above soil surface) were accessed from the French weather data provider (meteofrance.fr, 2014, France) and supplemented with agronomic weather station data near each site (climate-data.org, 2014, Germany). The satellite-based solar radiation measurements (Copernicus Atmosphere Monitoring Service (CAMS)) were obtained from the solar radiation data service (soda-pro.com, 2014, MINES ParisTech, France). The measurements of the physical properties of the soils and of the chemical content of the aqueous extracts (cf. Table 1) were subcontracted to an ISO certified laboratory (Teyssier, Bordeaux, France) according to standards. Briefly, 10 g of raw soil were milled, dried (12 h at 45 °C, except for nitrogen determination) and sifted (2 mm grid). Samples were then stirred into 50 ml of demineralized water for 30 min at 20 °C and filtered. Organic matter was measured after oxidation in potassium dichromate and sulfuric acid. NH_4 and NO_3 were extracted with 1 M KCl. Organic matter, NH_4, NO_3 and water-extractable PO_4 were then determined by colorimetric methods. K, Mg, Ca, Fe, Cu, Mn, Zn and Bo were determined by atomic absorption spectroscopy.

2.4. Determination of growth parameters

Plant growth for each period evaluated was determined by using several parameters: fresh and dry weight, water content, leaf area, and height of floral stem at the flowering stage. For each period, ten plants were collected randomly from each of the three sites (Luberon Park). The fresh weight was measured immediately after harvest, and the leaves were scanned to measure their area with the ImageJ software (National Institutes of Health, USA). The collected plants were subsequently dried (80 °C, 24 h) to calculate the water content. Glandular trichome density was assessed on 10 leaves randomly collected at the flowering period from 10 different plants per site. This assessment was performed using a stereomicroscope (Nikon ZX 100, Canagawa, Japan) equipped with fluorescence (excitation 382 nm, emission 536 nm) and digital camera (Leica DFC 300 FX, Wetzlar, Germany). The captured images allowed the quantification of glandular trichomes using ImageJ.

2.5. Chlorophyll-a fluorescence measurements

Chlorophyll-a fluorescence was measured *in vivo* using a portable