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Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. essential oils composition

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Abstract

Thymus hyemalis L. and Spanish *Thymus vulgaris* L. shrubs are characterized by a large chemical intraspecific variability among the plants. This fact makes it difficult to detect real changes occurring in their essential oil composition during the vegetative cycle. Based on this, the clones of *T. hyemalis* and Spanish *T. vulgaris* were used in this present work to monitor seasonal variations in the composition of the essential oil. Shrubs were harvested at five different phenological stages during the vegetative cycle. The volatile profile of the essential oil samples was determined by capillary GC/MS analyses. This technique identified 99 and 98 components in *T. hyemalis* and *T. vulgaris* essential oils, respectively.

For the Spanish *T. vulgaris* essential oil, the major components quantified were 1,8-cineole, followed by terpenyl acetate, borneol, linalool, β-pinene, α-terpineol, and camphor. With respect to the concentrations of some of the most abundant components, the mid-vegetative stage seems to be the most appropriate harvesting time for this species. Cineol, borneol, monoterpenic hydrocarbons, and camphor exhibited their maximum relative concentrations at this phenological stage. In contrast, terpenyl acetate, α-terpineol, and linalool, probably components that are associated with the fresh aroma in the oil, were mostly concentrated from full bloom to advanced fruit formation. Correlations were detected among the concentrations of the most abundant components in this essential oil. Thus, terpenyl acetate and cineol concentrations varied during the entire vegetative cycle. The same behaviour was observed between sabinene and linalool.

For the *T. hyemalis*, the thymol, which defines the chemical type and the essential oil quality, and its precursors γ-terpinene and *p*-cymene, showed synchronized patterns of variation during the entire vegetative cycle. In this way, the maximum relative concentration of γ-terpinene, a precursor of *p*-cymene, was achieved at the full bloom (FB) phenological stage that coincided with the minimum concentration detected for *p*-cymene, a precursor of thymol. However, the maximum relative concentration of thymol was detected at full bloom/at the beginning of fruit maturation (FB–FR). From these observations, we can conclude that between FB and FB–FR stages could be the period beyond which the sequence γ-terpinene → *p*-cymene → thymol begins. On the other hand, the essential oil exhibited the highest amount in alcohols, ketones, and esters at the vegetative stage.

Keywords: *Thymus hyemalis*; *Thymus vulgaris*; Thyme; Essential oil; Phenological stages

1. Introduction

The genus *Thymus*, extended in the Iberian Peninsula, is mostly composed by typical Mediterranean plants, which are adapted to alternative dry and rainfall periods. The progressive forest degradation has influenced the continuous expanding of these plants. It is known that thyme shrubs have a great incidence in the colonization of eroded lands (Sotomayor, 1998).

In the present work, two *Thymus* species, *Thymus hyemalis* L. and *Thymus vulgaris* L. (ssp. *vulgaris*),

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(endemic over the Southeastern Iberian Peninsula) were considered for the analysis of their volatile profile.

Thymus hyemalis Lange, winter thyme, can be found mainly in Alicante, Murcia and Almeria. This plant is normally present in siliceous and calcareous extensions, from sea level to 400–700 m above sea level. It is able to resist long dry periods, but its winter flowering condition (November through May) does not allow it to grow in areas having very cold weather.

The chemical variability of essential oil from *T. hyemalis* of the South-eastern Iberian Peninsula has been reported (Adzet et al., 1976; Elena-Rosello, 1984; Cabo et al., 1986, 1987; Jiménez et al., 1989; Blanca et al., 1993; Sáez, 1995; Sotomayor, 1998; Jordán et al., 2003). These researchers stated that thymol, carvacrol, borneol and linalool were the chemotypes most abundant in this area.

T. vulgaris, also known as Spanish common thyme, has its flowering period between March and July. It can be normally located from sea level to 2000 m above sea level (Morales, 1986). Studies related to the chemical composition of this essential oil species have stated a 1,8-cineol chemotype (Adzet et al., 1977; Sotomayor, 1998).

Some benefits in human and animal welfare have been related to the use of thyme essential oil by industry (Youdim et al., 2002). At this point, this product can be considered as a potential impulse of the new trends in food, pharmaceutical and cosmetic industries (Echeverrigaray et al., 2001). High demands of natural products by society justify the increments observed in oil production for the last 10 years (ESTACOM, 2000).

The antifungal, pesticide and antibacterial activities of *Thymus* essential oil have been demonstrated by several researchers (Higes and Llorente, 1996; Daferera et al., 2000; Kalemba and Kunicka, 2003; Bagamboula et al., 2004). Studies related to the antioxidant properties of thyme oil have shown the potential uses of this product by the food industry and its effectiveness as a dietetic supplement (Youdim and Deans, 1999).

The species of this genus are characterized by having an excellent richness in essential oil. However, the easy hybridisation exhibited among thyme species having geographical proximity and coincident full bloom period leads to a great intraspecific variability, which affects the homogeneity of oil yield and its chemical composition. Natural populations are usually heterogeneous, composed of plants of different chemotypes (Echeverrigaray et al., 2001).

Due to the high demand for thyme essential oil and the great variability that it exhibits (Jordán et al., 2003), the next step in our investigation was to cultivate clones of *T. hyemalis* and *T. vulgaris* in order to monitor real seasonal variations in the composition of the essential oil. This will provide information on optimum harvesting period to commercial growers, based on industrial requirements.

2. Material and methods

2.1. Crop experimental design

This study was performed in an experimental area of the IMIDA (Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario) at Torreblanca (37°47′N–0°54′W and 30 m above sea level) in the region of Murcia (Spain). Soil texture in the first 30 cm of the cultivation area can be defined as clayey, with a composition of sand (14.41%), silt (33.98%), and clay (51.61%). This soil shows a field capacity of 39% (by volume) and a wilting point of 21%. Semi-arid climatic conditions are characterized by an annual average temperature of 18.2 °C and average rainfall of 308.3 mm/year. A total of 25 cloned plants for species studied were cultivated in this area with a planting distance of 0.3 m × 0.4 m. A watering level equivalent to the 60% of the corresponding evapotranspiration (Eto) for cultivation of Graminaceous species (660 mm/year) was applied using a drip irrigation system.

2.2. Plant material

Thyme species were propagated by top cuttings of plants already cultivated in this experimental area. For that, stems were planted in alveolar trays and they were kept in greenhouse conditions until the total taking roots was achieved. All the plants were transplanted in the experimental area in autumn 2001. Harvesting of five shrubs per species was made in each of the five phenological stages during the entire vegetative cycle: the middle of the vegetative phase, full bloom, full bloom/at the beginning of fruit maturation, fruit maturation, and advanced fruit maturation stages.

2.3. Essential oil extraction

Five plants from each thyme species were harvested in every phenological stage, making a total of 25 plants per species. Plant material was dried in a forced-air drier at 35 °C for 48 h, until it reached a constant weight.

Aerial parts of dry individual plants (90 ± 10) g, including leaves, stems, flowers, and fruits (depending on the phenological stage satage) were steam distilled