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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.
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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 phonological stage satage) were steam distilled

for 3 h using a Clevenger-type system. The oil volume was measured directly in the extraction burette. Samples were dried with anhydrous sodium sulfate and kept in amber vials at 4 °C until chromatographic analysis. Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter.

2.4. Gas chromatography

Samples of 0.1 μL were subjected in triplicate to analysis by capillary gas chromatography. A Hewlett-Packard 5890 gas chromatograph (GC) (Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm HP-5 (cross-linked Phenyl-Methyl Siloxane) column with 0.25 μm film thickness (Hewlett-Packard, Palo Alto, CA, USA), was used for this study. The FID and the injector were maintained at 280 and 250 °C, respectively. Helium was used as carrier gas, the flow through the column was 1 mL/min, and the split ratio was set to 100:1. The column was maintained at 60 °C for 4 min, increased to 64 °C at a rate of 1 °C/min, then increased to 155 °C at a rate of 2.5 °C/min and finally raised from 155 to 250 °C at a rate of 5 °C/min.

For the identification of the compounds, retention times and retention index were confirmed with commercially available standard compounds (Acros Organics BVBA/SPRL, Fisher Scientific S.A. and Sigma Aldrich Química S.A.).

2.5. Mass spectrometry analysis

Gas chromatography–mass spectrometry (GC–MS) was used for the identification of volatile components in thyme essential oil. For this portion of the work, a Hewlett-Packard 5890 Series II Plus gas chromatograph (GC), equipped with a 30 m × 0.25 mm HP-5 column with 0.25 μm film thickness, was used. The GC was linked to a Hewlett-Packard Model 5972 mass spectrometry detector. Mass spectrometer was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m/z* 50 to 500 at 3.21 scan/s. The ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. The electron multiplier voltage was maintained at 1300 V. The chromatographic conditions were identical to those used for gas chromatography analysis.

2.6. Qualitative and quantitative analysis

The individual peaks were identified by retention times and retention index (relative to C_6–C_{17} *n*-alkanes), compared with those of known compounds, and by comparison of mass spectra of their corresponding commercially available standard components, except for tricyclene, α-thujene, verbenene, γ-cadinene, 3-methyl-2-buten-1-ol, (*Z*)-sabinene hydrate, (*E*)-verbenol, 3-heptanone, and pinocarvone, which were tentatively identified considering the NBS75K library (United States, National Bureau of Standards, 1986) spectra and their corresponding retention index. Percentage compositions of samples were calculated according to the area of the chromatographic peaks.

2.7. Statistical analysis

For comparison of the plant dry matter production, essential oil productions and mean values of each component in the essential oils, Duncan's test was used.

3. Results and discussion

It is known that genetic constitution and environmental conditions influence the yield and composition of volatile oil produced by thyme plants. Correlations between chemotype polymorphism, sexual polymorphism and the environment have been detected (Gouyon et al., 1986). These are some of the reasons why it is very difficult to determine the best harvesting period of thyme plants in order to obtain an essential oil with better quality and quantity from plants without any kind of pre-selection. In the present work clones of *T. hyemalis* and *T. vulgaris* plants were used to monitor real changes happened during vegetative cycle.

3.1. T. hyemalis essential oil

Results concerning seasonal variations of *T. hyemalis* essential oil yield and composition are shown in Tables 1–6. Due to the complexity of the results, a total of 99 components have been identified in the volatile profile of this thyme species, analytes have been grouped on the basis of their functional group. In Table 2, 24 compounds represent the terpenic hydrocarbons profile being *p*-cymene, γ-terpinene, α-pinene, α-terpinene, α-thujene and camphene the components quantified at highest relative concentrations. Regarding the evolution of these compounds through the vegetative cycle, and taking into account only the components that have shown statistical significant differences among relative concentrations, it can be stated that the most volatile components (from tricyclene to myrcene) along with limonene have shown their maximum concentrations at the beginning of the vegetative cycle. Other components including, α-

Table 1
Evolution of *T. hyemalis* and *T. vulgaris* essential oil yield during the whole vegetative cycle

	Phenological stages, yield (%w/v)				
	VEG.	FB	FB–FR	FR	A. FR
T. hyemalis	3.13 ± 0.03	3.39 ± 0.05	3.65 ± 0.02	3.13 ± 0.03	2.14 ± 0.01
T. vulgaris	4.73 ± 0.02	3.94 ± 0.01	4.05 ± 0.03	2.5 ± 0.02	2.17 ± 0.01

VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation.

Table 2
Thymus hyemalis essential oil composition (evolution of terpenic hydrocarbons profile during the whole vegetative cycle)

Terpenic hydrocarbons	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
Tricyclene	929	0.03 ± 0.00^{c}	0.02 ± 0.00$^{a.b}$	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{b}
α-Thujene	940	1.16 ± 0.10^{b}	0.95 ± 0.01^{a}	1.05 ± 0.01^{ab}	1.17 ± 0.02^{b}	1.04 ± 0.21^{ab}
α-Pinene	949	2.98 ± 0.36^{c}	1.14 ± 0.01^{b}	0.64 ± 0.09^{a}	0.70 ± 0.03^{a}	1.14 ± 0.50^{b}
Camphene	969	1.08 ± 0.11^{d}	0.66 ± 0.02^{b}	0.61 ± 0.01^{ab}	0.54 ± 0.01^{a}	0.75 ± 0.07^{c}
Verbenene	976	0.10 ± 0.01^{c}	0.07 ± 0.00^{a}	0.06 ± 0.01^{a}	0.08 ± 0.01^{ab}	0.10 ± 0.03^{bc}
Sabinene	1001	0.13 ± 0.01^{d}	0.09 ± 0.00^{c}	0.07 ± 0.01^{b}	0.05 ± 0.01^{a}	0.04 ± 0.02^{a}
β-Pinene	1007	0.20 ± 0.01^{c}	0.09 ± 0.01^{b}	0.08 ± 0.01^{ab}	0.09 ± 0.01^{ab}	0.07 ± 0.01^{a}
Myrcene	1026	0.99 ± 0.10^{b}	0.86 ± 0.01^{b}	0.53 ± 0.26^{a}	0.46 ± 0.03^{a}	0.51 ± 0.20^{a}
α-Phellandrene	1042	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}	0.18 ± 0.02^{a}	0.17 ± 0.02^{a}	0.15 ± 0.04^{a}
Δ3-Carene	1048	0.07 ± 0.00^{a}	0.06 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}
α-Terpinene	1058	1.27 ± 0.09^{a}	1.45 ± 0.02^{b}	1.37 ± 0.01^{ab}	1.29 ± 0.03^{a}	1.24 ± 0.23^{a}
p-Cymene	1068	33.52 ± 2.20^{b}	30.43 ± 0.03^{a}	32.41 ± 0.04^{b}	41.39 ± 0.27^{c}	40.28 ± 0.65^{c}
Limonene	1073	2.74 ± 0.11^{d}	1.52 ± 0.01^{c}	1.20 ± 0.02^{a}	1.32 ± 0.13^{ab}	1.43 ± 0.25^{bc}
(*Z*)-β-Ocimene	1086	0.08 ± 0.03^{b}	0.09 ± 0.01^{b}	0.06 ± 0.00^{ab}	0.04 ± 0.01^{a}	0.08 ± 0.03^{b}
(*E*)-β-Ocimene	1097	0.31 ± 0.09^{c}	0.77 ± 0.01^{d}	0.35 ± 0.02^{c}	0.16 ± 0.01^{a}	0.22 ± 0.03^{b}
γ-Terpinene	1109	12.64 ± 1.29^{bc}	14.31 ± 0.02^{c}	11.45 ± 0.05^{b}	7.46 ± 0.26^{a}	12.09 ± 2.63^{b}
Terpinolene	1141	0.17 ± 0.00^{b}	0.15 ± 0.01^{a}	0.14 ± 0.01^{a}	0.18 ± 0.02^{b}	0.20 ± 0.02^{c}
(*E*)-Caryophyllene	1419	0.34 ± 0.02^{a}	0.76 ± 0.03^{c}	0.95 ± 0.02^{e}	0.86 ± 0.05^{d}	0.49 ± 0.06^{b}
Aromadendrene	1440	0.14 ± 0.03^{c}	0.10 ± 0.01^{b}	0.19 ± 0.01^{d}	0.24 ± 0.03^{e}	0.02 ± 0.01^{a}
α-Humulene	1457	0.01 ± 0.01^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{c}	0.03 ± 0.00^{c}	0.02 ± 0.00^{ab}
Alloaromadendrene	1465	0.03 ± 0.01^{a}	0.05 ± 0.01^{b}	0.06 ± 0.01^{c}	0.06 ± 0.01^{bc}	0.05 ± 0.01^{b}
Valencene	1510	0.13 ± 0.01^{a}	0.18 ± 0.02^{c}	0.16 ± 0.01^{b}	0.12 ± 0.02^{a}	0.15 ± 0.02^{b}
γ-Cadinene	1537	0.03 ± 0.00^{b}	0.01 ± 0.01^{a}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}
δ-Cadinene	1553	0.04 ± 0.00^{a}	0.06 ± 0.01^{b}	0.08 ± 0.01^{c}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}

Values within rows with uncommon superscripts (a–e) were significantly different ($P < 0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

Table 3
Thymus hyemalis essential oil composition (evolution of phenolic profile during the whole vegetative cycle)

Phenols	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
Thymol methyl ether	1265	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Carvacrol methyl ether	1272	0.54 ± 0.06^{a}	1.07 ± 0.04^{b}	1.17 ± 0.01^{b}	1.31 ± 0.03^{c}	1.53 ± 0.16^{d}
Thymol	1308	16.09 ± 0.98^{a}	26.22 ± 0.19^{c}	29.27 ± 0.03^{d}	25.93 ± 0.26^{c}	20.74 ± 2.14^{b}
Carvacrol	1314	1.17 ± 0.08^{b}	1.05 ± 0.04^{a}	1.32 ± 0.01^{c}	1.31 ± 0.04^{c}	1.19 ± 0.06^{b}
Eugenol	1358	0.01 ± 0.01^{ab}	0.01 ± 0.00^{a}	0.03 ± 0.01^{c}	0.04 ± 0.01^{d}	0.02 ± 0.01^{bc}
Methyl eugenol	1408	0.01 ± 0.00^{c}	tr^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Isoeugenol	1451	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{b}	0.02 ± 0.01^{b}

Values within rows with uncommon superscripts (a–d) were significantly different ($P < 0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

Table 4
Thymus hyemalis essential oil composition (evolution of alcoholic profile during the whole vegetative cycle)

Alcohols	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
Butanol	742	tr^{ab}	tr^{a}	0.01 ± 0.00^{c}	tr^{ab}	0.01 ± 0.00^{b}
3-Methyl-1-butanol	767	tr^{a}	tr^{a}	tr^{b}	0.01 ± 0.00^{b}	tr^{a}
3-Methyl-2-buten-1-ol	789	n.d.^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{b}	n.d.^{a}
(*Z*)-3-Hexen-1-ol	852	0.01 ± 0.01^{b}	n.d.^{a}	0.01 ± 0.00^{b}	0.01± 0.00^{c}	tr^{a}
Hexanol	865	n.d.^{a}	0.02 ± 0.00^{b}	0.02 ± 0.01^{b}	0.04 ± 0.00^{c}	0.02 ± 0.01^{bc}
1-Octen-3-ol	1009	0.11 ± 0.01^{c}	0.05 ± 0.01^{a}	0.08± 0.01^{b}	0.05 ± 0.01^{a}	0.07 ± 0.00^{b}
3-Octanol	1031	0.03 ± 0.01^{c}	0.02 ± 0.00^{b}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
2-Octanol	1037	0.01 ± 0.00^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
(*E*)-Sabinene hydrate	1151	0.63 ± 0.06^{c}	0.51 ± 0.02^{b}	0.64 ± 0.03^{c}	0.43 ± 0.03^{a}	0.48± 0.08^{ab}
Octanol	1123	n.d.	n.d.	0.01 ± 0.00	n.d.	n.d.
(*Z*)-Sabinene hydrate	1151	0.19 ± 0.01^{d}	0.14 ± 0.01^{b}	0.16 ± 0.01^{c}	0.12 ± 0.02^{a}	0.15 ± 0.02^{bc}
Linalool	1152	0.60 ± 0.02^{a}	0.86 ± 0.01^{b}	1.02 ± 0.02^{c}	1.20 ± 0.19^{d}	0.79 ± 0.15^{b}
Fenchol	1163	0.20 ± 0.01^{c}	tr^{a}	0.09 ± 0.01^{b}	0.10 ± 0.03^{b}	0.19 ± 0.03^{c}
(*E*)-Pinocarveol	1186	0.21 ± 0.01^{c}	0.13 ± 0.01^{ab}	0.12 ± 0.02^{a}	0.13 ± 0.01^{ab}	0.14 ± 0.01^{b}
(*Z*)-Verbenol	1188	0.59 ± 0.02^{d}	0.58 ± 0.02^{cd}	0.51± 0.02^{b}	0.45 ± 0.06^{a}	0.54 ± 0.04^{bc}
(*E*)-Verbenol	1193	0.06 ± 0.00^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.07 ± 0.01^{b}
Isoborneol	1203	0.01± 0.00^{b}	n.d.^{a}	n.d.^{a}	tr^{b}	n.d.^{a}
Borneol	1213	3.32 ± 0.37^{c}	2.26 ± 0.02^{a}	2.26 ± 0.04^{a}	2.09 ± 0.08^{a}	2.65 ± 0.10^{b}
Menthol	1217	0.03 ± 0.00^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Terpinen-4-ol	1220	0.50 ± 0.03^{ab}	0.47 ± 0.02^{a}	0.52 ± 0.02^{b}	0.74 ± 0.04^{d}	0.59 ± 0.04^{c}
p-Cymen-8-ol	1227	0.32 ± 0.03^{b}	0.27 ± 0.01^{ab}	0.26 ± 0.02^{a}	0.29 ± 0.04^{ab}	0.45 ± 0.08^{c}
α-Terpineol	1231	0.68 ± 0.03^{c}	0.39± 0.02^{a}	0.33 ± 0.02^{a}	0.36 ± 0.04^{a}	0.52 ± 0.15^{b}
Carveol	1252	0.03 ± 0.02^{b}	0.03 ± 0.00^{b}	n.d.^{a}	0.02 ± 0.01^{b}	n.d.^{a}
Nerol + Citronellol	1260	0.06 ± 0.00^{c}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{b}
Geraniol	1280	0.01± 0.00^{ab}	0.02 ± 0.00^{bc}	0.01± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.01^{c}
Spathulenol	1640	0.22 ± 0.01^{a}	0.25 ± 0.03^{a}	0.25 ± 0.01^{a}	0.22 ± 0.02^{a}	0.25 ± 0.08^{a}

Values within rows with uncommon superscripts (a–d) were significantly different ($P < 0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

Table 5
Thymus hyemalis essential oil composition (evolution of aldehydic profile during the whole vegetative cycle)

Aldehydes	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
(*E*)-2-Butenal	735	n.d.^{a}	tr^{ab}	tr^{bc}	tr^{c}	tr^{a}
2-Methyl-butanal	741	n.d.^{a}	0.01 ± 0.01^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	n.d.^{a}
Ventanal	754	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	tr^{a}
Hexanal	802	tr^{a}	tr^{b}	tr^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}
Furfural	832	0.01 ± 0.00^{d}	0.01 ± 0.00^{c}	tr^{a}	tr^{ab}	tr^{bc}
(*E*)-2-Hexenal	849	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	tr^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}
Heptanal	904	tr^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	tr^{a}
(*E,E*)-2,4-Hexadienal	921	n.d.^{a}	n.d.^{a}	0.01 ± 0.00^{b}	n.d.^{a}	n.d.^{a}
Nonanal	1157	0.14 ± 0.02^{d}	0.09± 0.01^{b}	0.06 ± 0.01^{a}	0.08 ± 0.01^{b}	0.12 ± 0.02^{c}
Citronellal	1201	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.01 ± 0.00^{b}
Decanal	1243	0.05 ± 0.00^{b}	0.21 ± 0.01^{e}	0.17 ± 0.01^{d}	0.14 ± 0.02^{c}	0.03 ± 0.01^{a}
Neral	1270	n.d.^{a}	n.d.^{a}	0.05 ± 0.01^{b}	0.06 ± 0.01^{bc}	0.07 ± 0.02^{c}
Geranial	1291	0.16 ± 0.04^{b}	0.06 ± 0.01^{a}	0.17 ± 0.01^{b}	0.29 ± 0.03^{c}	0.31 ± 0.02^{c}

Values within rows with uncommon superscripts (a–e) were significantly different ($P < 0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

Table 6
Thymus hyemalis essential oil composition (evolution of ketonic, esters, epoxidic and ether profile during the whole vegetative cycle)

	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
Ketones						
2-Butanone	729	n.d.	n.d.	tr	tr	tr
3-Heptanone	888	tr	tr	n.d.	n.d.	n.d.
3-Octanone	1019	0.04 ± 0.00^{c}	0.03 ± 0.01^{a}	0.04 ± 0.01^{bc}	0.03 ± 0.01^{a}	0.03 ± 0.01^{ab}
β-Thujone	1158	0.06 ± 0.01^{c}	0.04 ± 0.01^{b}	0.04 ± 0.01^{b}	0.03 ± 0.01^{a}	0.05 ± 0.01^{bc}
Camphor	1192	1.67 ± 0.06^{d}	1.07 ± 0.01^{b}	0.86 ± 0.04^{a}	0.88 ± 0.08^{a}	1.21 ± 0.12^{c}
Menthone	1200	n.d.	tr	n.d.	n.d.	n.d.
Pinocarvone	1208	0.03 ± 0.01^{c}	0.02 ± 0.01^{b}	0.01 ± 0.00^{a}	0.02 ± 0.01^{b}	0.02 ± 0.00^{b}
Dihydrocarvone	1235	0.16 ± 0.01^{b}	0.12 ± 0.01^{a}	0.13 ± 0.02^{a}	0.16 ± 0.03^{b}	0.12 ± 0.01^{a}
Verbenone	1245	9.59 ± 0.36^{c}	5.76 ± 0.01^{b}	4.85 ± 0.04^{a}	4.35 ± 0.13^{a}	6.03 ± 1.14^{b}
Carvone	1271	0.07 ± 0.01^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Thymoquinone	1276	0.07 ± 0.01^{c}	0.02 ± 0.01^{a}	0.05 ± 0.01^{b}	0.05 ± 0.01^{b}	0.04 ± 0.02^{b}
β-Ionone	1497	n.d.^{a}	n.d.^{a}	0.03 ± 0.01^{b}	n.d.^{a}	n.d.^{a}
Esters						
Ethyl acetate	732	n.d.^{a}	n.d.^{a}	tr^{b}	tr^{c}	n.d.^{a}
Benzyl acetate	1214	0.07 ± 0.01^{d}	0.05 ± 0.01^{ab}	0.06 ± 0.01^{cd}	0.05 ± 0.01^{bc}	0.04 ± 0.01^{a}
Ethyl caprilate	1238	0.06 ± 0.00^{c}	0.05 ± 0.01^{ab}	0.05 ± 0.01^{ab}	0.04 ± 0.01^{a}	0.05 ± 0.01^{bc}
Octyl acetate	1248	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.09 ± 0.02^{b}
Fenchyl acetate	1253	0.05 ± 0.01^{d}	n.d.^{a}	0.02 ± 0.00^{b}	n.d.^{a}	0.03 ± 0.01^{c}
Linallyl acetate	1281	0.02 ± 0.01^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Bornyl acetate	1302	n.d.^{a}	0.12 ± 0.01^{b}	0.13 ± 0.02^{bc}	0.13 ± 0.01^{b}	0.14 ± 0.02^{c}
Isobornyl acetate	1304	0.16 ± 0.01^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Terpenyl acetate	1353	0.02 ± 0.01^{b}	n.d.^{a}	n.d.^{a}	0.01 ± 0.00^{a}	n.d.^{a}
Citronellyl acetate	1356	0.04 ± 0.01^{a}	0.07 ± 0.01^{c}	0.06 ± 0.01^{bc}	0.06 ± 0.01^{bc}	0.06 ± 0.03^{b}
Neryl acetate	1366	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.03 ± 0.01^{b}
Geranyl acetate	1386	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	tr^{b}
Epoxides						
(*Z*)-Linalool oxide	1123	n.d.^{a}	0.01 ± 0.00^{b}	n.d.^{a}	0.01 ± 0.00^{b}	0.01 ± 0.01^{b}
(*Z*)-Limonene oxide	1173	1.14 ± 0.09^{c}	0.04 ± 0.01^{a}	0.49 ± 0.02^{b}	0.03 ± 0.01^{a}	0.05 ± 0.02^{a}
Caryophyllene oxide	1650	0.20 ± 0.02^{a}	0.22 ± 0.02^{ab}	0.24 ± 0.01^{b}	0.25 ± 0.03^{b}	0.46 ± 0.03^{c}
Ether						
Cineol	1076	0.03 ± 0.0^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.05 ± 0.01^{b}	0.03 ± 0.01^{a}

Values within rows with uncommon superscripts (a–d) were significantly different ($P < 0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

terpinene, (*E*)-β-ocimene and valencene were detected at their greatest concentrations at the full bloom stage. Contrary, components with less volatility including from (*E*)-caryophyllene to δ-cadinene increased their presence between full bloom–beginning of fruit maturation and fructification.

It is known that γ-terpinene is the precursor of *p*-cymene and this at the same time of thymol (Piccaglia and Marotti, 1993). According to the results showed in Tables 2 and 3, the concentration of these components showed synchronized patterns of variation during the entire vegetative cycle. The phenological stages in which the conversion among components begins can be considered between FB and FB–FR, point beyond which γ-terpinene decreased its concentration and *p*-cymene increased its presence in the essential oil. Thymol, phenolic component which define the essential oil quality, showed its maximum concentration at the FB–FR phenological stage (Table 3), from this period to advance fructification stage it decreased its presence in the essential oil, being coincident with an increase in *p*-cymene relative concentration.

These statements agree with those published by Hudaib et al. (2002). For these authors generally thyme essential oil from *T. vulgaris* was found to be rich in the active monoterpene phenols (thymol and carvacrol) and their corresponding terpenic hydrocarbons precursors (*p*-cymene and γ-terpinene), which collectively showed synchronized patterns of variation during the different collection periods and in different season.

A total of 27 alcohols constitute the alcoholic aromatic profile (Table 4) of this thyme species. It is interesting to remark that almost all of them have shown statistical significant differences among concentrations during the whole vegetative cycle (except for spathulenol). The presence of alcohols in the essential oil profile is favoured in the vegetation phenological stage, since 15 of them reached their maximum relative concentration in this phenological stage. Indeed, some of them including isoborneol and menthol were only detected in the oil extracted during this period. Other components that are also related to the quality of the oil, reached their maximum concentration in the phenological stage of fructification as linalool and terpinen-4-ol.

Related to aldehydes (Table 5), 13 components were identified. Most of the compounds were present at trace level, with nonanal, decanal and geranial detected at the highest relative concentrations. Pentanal and citronellal were only present in samples harvested at the phenological stage of advance fructification, coinciding with hexanal, neral and geranial, components that showed their greatest relative concentration in this stage.

Evolution of ketones, esters, expoxides and the ether concentrations are shown in Table 6. These components have shown behaviour similar to alcohols, since most of them were present at their greatest concentrations at the beginning of the vegetative cycle.

Studies carried out by Hudaib et al. (2002) about evaluation of thyme (*T. vulgaris*) oil composition and variations during the vegetative cycle showed that the oil collected in May/June (phenological stage of FB–FR) was characterized by significantly lower levels of monoterpene hydrocarbons mainly γ-terpinene and the

Table 7
Spanish *Thymus vulgaris* essential oil composition (evolution of terpenic hydrocabons and ether profile during the whole vegetative cycle)

	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
Terpenic hydrocarbons						
Tricyclene	929	0.12 ± 0.00^{c}	0.12 ± 0.02^{bc}	0.12 ± 0.02^{c}	0.09 ± 0.02^{a}	0.10 ± 0.02^{ab}
α-Thujene	940	0.25 ± 0.01^{b}	0.15 ± 0.03^{a}	0.16 ± 0.02^{a}	0.13 ± 0.03^{a}	0.16 ± 0.06^{a}
α-Pinene	949	2.99 ± 0.07^{c}	2.33 ± 0.23^{b}	2.45 ± 0.11^{b}	1.92 ± 0.39^{a}	2.09 ± 0.45^{ab}
Camphene	969	3.20 ± 0.02^{b}	2.85 ± 0.22^{ab}	2.87 ± 0.24^{ab}	2.55 ± 0.34^{a}	2.65 ± 0.36^{a}
Verbenene	976	0.03 ± 0.01^{a}	0.03 ± 0.02^{a}	0.03 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}
Sabinene	1001	3.28 ± 0.16^{b}	2.18 ± 0.21^{a}	2.25 ± 0.14^{a}	2.11 ± 0.27^{a}	2.31 ± 0.44^{a}
β-Pinene	1007	3.67 ± 0.04^{c}	2.53 ± 0.32^{ab}	2.71 ± 0.20^{b}	2.32 ± 0.30^{a}	2.56 ± 0.45^{ab}
Myrcene	1026	2.63 ± 0.06^{a}	3.16 ± 0.20^{b}	3.03 ± 0.01^{b}	2.71 ± 0.41^{a}	2.95 ± 0.29^{ab}
α-Phellandrene	1042	0.06 ± 0.02^{b}	0.06 ± 0.02^{b}	0.05 ± 0.01^{ab}	0.04 ± 0.00^{a}	0.06 ± 0.01^{b}
Δ_3-Carene	1048	0.01 ± 0.01^{b}	n.d.[a]	n.d.[a]	n.d.[a]	0.01 ± 0.00^{b}
α-Terpinene	1058	0.19 ± 0.08^{a}	0.21 ± 0.06^{a}	0.20 ± 0.04^{a}	0.10 ± 0.08^{a}	0.18 ± 0.07^{a}
p-Cymene	1068	1.00 ± 0.50^{a}	0.57 ± 0.08^{a}	0.58 ± 0.03^{a}	0.77 ± 0.32^{a}	0.60 ± 0.25^{a}
(*Z*)-β-Ocimene	1086	0.15 ± 0.02^{a}	0.37 ± 0.08^{c}	0.28 ± 0.03^{bc}	0.27 ± 0.12^{b}	0.26 ± 0.02^{b}
(*E*)-β-Ocimene	1097	1.11 ± 0.25^{a}	4.10 ± 0.31^{c}	3.82 ± 0.23^{c}	2.44 ± 1.58^{b}	2.72 ± 0.26^{b}
γ-Terpinene	1109	1.05 ± 0.38^{c}	0.61 ± 0.08^{ab}	0.64 ± 0.04^{ab}	0.38 ± 0.31^{a}	0.72 ± 0.31^{bc}
Terpinolene	1141	0.21 ± 0.06^{a}	0.36 ± 0.11^{b}	0.34 ± 0.11^{b}	0.16 ± 0.09^{a}	0.26 ± 0.06^{ab}
α-Copaene	1378	0.47 ± 0.04^{a}	0.39 ± 0.07^{a}	0.39 ± 0.03^{a}	0.43 ± 0.05^{a}	0.40 ± 0.04^{a}
(*E*)-Caryophyllene	1419	1.20 ± 0.06^{a}	2.11 ± 0.22^{c}	2.00 ± 0.19^{c}	2.13 ± 0.33^{c}	1.59 ± 0.29^{b}
Calerene	1432	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	n.d.[a]	0.03 ± 0.02^{b}	0.01 ± 0.01^{ab}
Aromadendrene	1440	0.02 ± 0.01^{a}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{ab}
α-Humulene	1457	0.07 ± 0.01^{a}	0.10 ± 0.03^{a}	0.08 ± 0.01^{a}	0.11 ± 0.02^{a}	0.33 ± 0.54^{a}
Alloaromadendrene	1465	0.08 ± 0.01^{a}	0.15 ± 0.03^{bc}	0.13 ± 0.04^{bc}	0.17 ± 0.03^{c}	0.12 ± 0.04^{ab}
Elemene	1481	0.29 ± 0.03^{b}	0.16 ± 0.04^{a}	0.18 ± 0.03^{a}	0.32 ± 0.09^{b}	0.42 ± 0.03^{c}
β-Selinene	1485	n.d.[a]	0.40 ± 0.04^{b}	n.d.[a]	n.d.[a]	n.d.[a]
Valencene	1510	1.98 ± 0.30^{a}	2.89 ± 0.21^{a}	2.76 ± 0.27^{a}	2.20 ± 1.07^{a}	2.13 ± 0.11^{a}
γ-Cadinene	1537	0.14 ± 0.02^{a}	0.10 ± 0.03^{a}	0.11 ± 0.03^{a}	0.14 ± 0.03^{a}	0.13 ± 0.02^{a}
δ-Cadinene	1553	0.05 ± 0.00^{a}	0.13 ± 0.02^{c}	0.12 ± 0.02^{c}	0.12 ± 0.02^{c}	0.09 ± 0.02^{b}
(*Z*)-α-Bisabolene	1572	n.d.[a]	0.01 ± 0.00^{bc}	0.01 ± 0.01^{b}	0.02 ± 0.01^{c}	n.d.[a]
Ether						
Cineol	1076	36.42 ± 1.00^{c}	31.13 ± 0.85^{b}	30.64 ± 0.21^{ab}	29.17 ± 1.91^{a}	30.94 ± 2.21^{ab}

Values within rows with uncommon superscripts (a–c) were significantly different ($P < 0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

Table 8
Spanish *Thymus vulgaris* essential oil composition (evolution of alcoholic profile during the whole vegetative cycle)

Alcohols	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
3-Methyl-3-buten-2-ol	737	tr^{a}	n.d.^{a}	n.d.^{a}	tr^{a}	n.d.^{a}
Butanol	742	n.d.^{a}	tr^{a}	tr^{a}	tr^{a}	tr^{a}
1-Penten-3-ol	747	n.d.^{a}	tr^{a}	n.d.^{a}	tr^{a}	n.d.^{a}
3-Methyl-1-butanol	767	n.d.^{a}	n.d.^{a}	tr^{a}	0.01 ± 0.00^{a}	tr^{a}
3-Penten-2-ol	784	tr^{a}	tr^{a}	tr^{a}	n.d.^{a}	tr^{a}
3-Methyl-2-buten-1-ol	789	tr^{b}	tr^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}
(*Z*)-3-Hexen-1-ol	852	0.03 ± 0.02^{b}	0.02 ± 0.01^{ab}	0.04 ± 0.01^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{ab}
(*E*)-2-Hexen-1-ol	863	n.d.^{a}	0.04 ± 0.01^{c}	0.04 ± 0.00^{b}	n.d.^{a}	n.d.^{a}
Hexanol	865	0.02 ± 0.00^{b}	n.d.^{a}	n.d.^{a}	0.03 ± 0.01^{c}	0.02 ± 0.01^{bc}
1-Octen-3-ol	1009	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.07 ± 0.02^{b}	0.06 ± 0.01^{a}	0.05 ± 0.01^{a}
3-Octanol	1031	n.d.^{a}	tr^{bc}	tr^{c}	0.01 ± 0.00^{ab}	n.d.^{a}
(*E*)-Sabinene hydrate	1151	0.72 ± 0.22^{b}	0.35 ± 0.05^{a}	0.38 ± 0.03^{a}	0.65 ± 0.13^{b}	0.62 ± 0.08^{b}
(*Z*)-Sabinene hydrate	1151	0.27 ± 0.03^{c}	0.18 ± 0.08^{ab}	0.14 ± 0.04^{a}	0.22 ± 0.03^{bc}	0.19 ± 0.01^{ab}
Linalool	1152	1.57 ± 0.05^{a}	3.70 ± 0.20^{c}	3.38 ± 0.34^{bc}	3.58 ± 0.3^{c}	3.23 ± 0.26^{b}
Fenchol	1163	0.02 ± 0.00^{b}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}	n.d.^{a}	n.d.^{a}
(*E*)-Pinocarveol	1186	0.13 ± 0.01^{b}	0.09 ± 0.04^{a}	0.08 ± 0.02^{a}	0.10 ± 0.02^{a}	0.08 ± 0.03^{a}
(*Z*)-Verbenol	1188	0.27 ± 0.02^{c}	0.17 ± 0.03^{b}	0.11 ± 0.02^{a}	0.13 ± 0.02^{a}	0.14 ± 0.04^{ab}
(*E*)-Verbenol	1193	0.01 ± 0.01^{a}	0.02 ± 0.01^{bc}	0.03 ± 0.01^{c}	0.01 ± 0.01^{a}	0.01 ± 0.01^{ab}
Isoborneol	1203	0.06 ± 0.02^{bc}	0.07 ± 0.01^{c}	0.04 ± 0.04^{b}	n.d.^{a}	n.d.^{a}
Borneol	1213	4.77 ± 0.24^{a}	4.38 ± 0.40^{a}	4.63 ± 0.26^{a}	4.57 ± 0.35^{a}	4.34 ± 0.09^{a}
Terpinen-4-ol	1220	0.91 ± 0.20^{b}	0.69 ± 0.13^{a}	0.68 ± 0.13^{a}	0.60 ± 0.14^{a}	0.70 ± 0.08^{a}
p-Cymen-8-ol	1227	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.05 ± 0.04^{a}
α-Terpineol	1231	2.47 ± 0.12^{a}	2.83 ± 0.27^{ab}	2.77 ± 0.18^{ab}	2.83 ± 0.47^{b}	3.07 ± 0.11^{b}
γ-Terpineol	1237	0.02 ± 0.00^{a}	0.03 ± 0.01^{bc}	0.02 ± 0.01^{abc}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{c}
Carveol	1252	0.03 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.02^{a}
Nerol + Citronellol	1260	0.06 ± 0.01^{b}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}
Geraniol	1280	0.83 ± 0.08^{b}	0.51 ± 0.07^{a}	0.54 ± 0.05^{a}	0.47 ± 0.05^{a}	0.46 ± 0.10^{a}
Spathulenol	1640	1.01 ± 0.23^{bc}	0.60 ± 0.07^{ab}	0.53 ± 0.05^{a}	1.41 ± 0.62^{c}	1.00 ± 0.33^{bc}

Values within rows with uncommon superscripts (a–c) were significantly different ($P<0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

highest levels of oxygenated monoterpenes linalool and borneol, monoterpene phenols (mainly thymol) and their derivates (mainly, carvacrol methyl ether), sesquiterpenes (mainly β-caryophyllene) and their oxygenated derivates (caryophyllene oxide) in comparison with all other samples. These results agree in part with those found at the present work. Although, in general the most volatile monoterpenes decreased in relative concentra-

Table 9
Spanish *Thymus vulgaris* essential oil composition (evolution of aldehidic profile during the whole vegetative cycle)

Aldehydes	Phenological Stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
2-Methyl-butanal	741	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.01 ± 0.00^{a}	tr^{a}
Ventanal	754	n.d.^{a}	n.d.^{a}	tr^{b}	n.d.^{a}	n.d.^{a}
(*E*)-2-Hexenal	849	tr^{a}	n.d.^{a}	n.d.^{a}	0.01 ± 0.00^{a}	n.d.^{a}
Heptanal	904	n.d.^{a}	tr^{b}	tr^{ab}	n.d.^{a}	n.d.^{a}
Nonanal	1157	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}
Citronellal	1201	0.01 ± 0.00^{a}	0.01 ± 0.01^{a}	n.d.^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Decanal	1243	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}
Neral	1270	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.01 ± 0.01^{a}
Geranial	1291	0.04 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}

Values within rows with uncommon superscripts (a and b) were significantly different ($P<0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

tion from vegetation to FB–FR, the oxygenated terpenes did not increase their presence in the oil in FB–FR, even component as isoborneol was only quantified in samples at the beginning of the vegetative cycle and advance fructification was the stage in which caryophyllene oxide was detected at its greatest concentration.

3.2. Spanish *T. vulgaris* essential oil

This thyme species is characterized by having a 1,8-cineol chemotype, being endemic in the Iberian Peninsula. The chromatographic analysis yield a total of 97 compounds identified in the volatile profile of this thyme essential oil (Tables 7–10). Major components identified were 1,8-cineol and terpenyl acetate which represent 36.42%, and 25% of the essential oil composition at the vegetative and fructification stages, respectively (Table 5). Studies carried out by Granger and Passet (1973) showed the wide range in which concentrations of components including linalool (3–67%), terpenyl acetate (2–62%) and the phenols thymol and carvacrol (0.2–72%) can be detected in French *T. vulgaris*. The component terpenyl acetate has never been described before as a volatile component in Spanish *T. vulgaris* essential oil. Taking into account that the seeds were obtained from a commercial greenhouse, is it easy

Table 10
Spanish *Thymus vulgaris* essential oil composition (evolution of ketonic, esters, phenolic and epoxidic profile during the whole vegetative cycle)

	Phenological stages					
	R.I.	VEG.	FB	FB–FR	FR	A. FR
Ketones						
2-Butanone	729	n.d.^{a}	0.07 ± 0.01^{b}	tr^{a}	0.02 ± 0.01^{a}	n.d.^{a}
3-Heptanone	888	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.01 ± 0.00^{b}	n.d.^{a}
3-Octanone	1019	0.01 ± 0.00^{ab}	0.01 ± 0.00^{d}	0.01 ± 0.00^{cd}	0.01 ± 0.00^{bc}	n.d.^{a}
β-Thujone	1158	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.02 ± 0.01^{a}
Camphor	1192	2.17 ± 0.05^{b}	1.88 ± 0.29^{a}	1.81 ± 0.19^{a}	1.71 ± 0.18^{a}	1.81 ± 0.17^{a}
Pinocarvone	1208	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.04 ± 0.01^{a}	0.02 ± 0.03^{a}
Dihydrocarvone	1235	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.05 ± 0.03^{a}	0.06 ± 0.01^{a}
Verbenone	1245	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.07 ± 0.03^{a}	0.05 ± 0.01^{a}
Carvone	1271	n.d.^{a}	0.01 ± 0.00^{ab}	tr^{ab}	0.01 ± 0.00^{b}	0.01 ± 0.01^{b}
Thymoquinone	1276	n.d.^{a}	0.01 ± 0.00^{bc}	0.01 ± 0.00^{ab}	0.01 ± 0.01^{b}	0.02 ± 0.01^{c}
(*Z*)-Jasmone	1404	0.02 ± 0.01^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
α-Ionone	1426	n.d.^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{ab}	0.02 ± 0.01^{c}	0.01 ± 0.01^{b}
β-Ionone	1497	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	tr^{a}	0.01 ± 0.00^{a}	n.d.^{a}
Esters						
Ethyl acetate	732	tr^{a}	tr^{a}	tr^{a}	0.01 ± 0.00^{b}	tr^{a}
Ethyl butyrate	804	n.d.^{a}	tr^{cd}	tr^{bc}	tr^{b}	tr^{d}
Ethyl caprilate	1238	0.01 ± 0.00^{ab}	tr^{bc}	tr^{c}	n.d.^{a}	0.01 ± 0.00^{ab}
Bornyl acetate	1302	1.10 ± 0.19^{b}	0.67 ± 0.11^{a}	0.58 ± 0.13^{a}	1.17 ± 0.36^{b}	1.50 ± 0.15^{c}
Terpenyl acetate	1353	18.17 ± 0.81^{a}	23.20 ± 0.74^{b}	23.52 ± 0.38^{b}	25.04 ± 2.26^{b}	23.41 ± 3.05^{b}
Neryl acetate	1366	0.02 ± 0.01^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.01^{ab}	0.01 ± 0.01^{ab}
(*E*)-Mirtanyl acetate	1381	tr^{b}	n.d.^{a}	tr^{a}	n.d.^{a}	n.d.^{a}
Geranyl acetate	1386	0.17 ± 0.05^{b}	0.08 ± 0.02^{a}	0.12 ± 0.02^{a}	0.19 ± 0.06^{b}	0.20 ± 0.03^{b}
Butyl caprilate	1387	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}	n.d.^{a}
Phenols						
(*E*)-Anethole	1303	n.d.^{a}	n.d.^{a}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	n.d.^{a}
Thymol	1308	0.10 ± 0.02^{a}	0.07 ± 0.02^{a}	0.05 ± 0.01^{a}	0.04 ± 0.04^{a}	0.09 ± 0.08^{a}
Carvacrol	1314	0.02 ± 0.01^{a}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
Eugenol	1358	0.04 ± 0.02^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.01 ± 0.01^{a}
Isoeugenol	1451	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	n.d.^{a}	0.02 ± 0.02^{a}
Epoxides						
(*Z*)-Linalool oxide	1123	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}
(*Z*)-Limonene oxide	1173	n.d.^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}
(*E*)-Limonene oxide	1188	0.02 ± 0.02^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}
Caryophyllene oxide	1650	0.49 ± 0.07^{a}	0.48 ± 0.07^{a}	0.42 ± 0.05^{a}	0.87 ± 0.36^{b}	0.70 ± 0.27^{ab}

Values within rows with uncommon superscripts (a–d) were significantly different ($P < 0.05$). ±, Standard deviation; VEG., vegetation; FB, full bloom; FB–FR, full bloom–beginning of fruit maturation; A. FR, advance fruit maturation. R.I., Kovat's Index (HP-5).

to think that we are working with hybrids between Spanish and French *T. vulgaris* species.

Among the 28 terpenic hydrocarbons identified, α-pinene, camphene, sabinene, β-pinene, γ-terpinene showed their greatest amount at the beginning of the vegetative cycle. Other major terpenic hydrocarbons identified including myrcene, (*E*)-β-ocimene, and (*E*)-caryophyllene reached their maximum concentrations at the FB and FB–FR phenological stages. Regarding the evolution of the alcohols (Table 8), the terpenic alcohols fraction seems to represent the most abundant section among these components. Borneol, linalool and α-terpineol were the alcohols quantified at the greatest relative concentrations. Borneol, the most abundant alcohol identified, did not change in concentration throughout the vegetative cycle. However, linalool and α-terpineol increased their presence in the oil from the FB to FR phenological stages.

Aldehydes were not abundant in the volatile profile of this thyme species (Table 9). Among 13 ketones identified (Table 10), camphor was the most abundant ketone found, being present at its maximum concentration at the beginning of the vegetative cycle. After this stage its concentration did not change throughout the cycle. Regarding the rest of the components analyzed, phenolic components (Table 10) were quantified at low concentrations and they did not modify their presence in the whole vegetative cycle. On the other hand, caryophyllene oxide was the epoxide with greatest relative concentration quantified.

Correlations among relative concentrations of related components were studied. In this way, it is interesting to notify the negative correlation detected between terpenyl acetate and cineol ($P = 0.00$) and sabinene-linalool ($P = 0.00$). In both cases their concentrations varied in accordance during the whole vegetative cycle.

4. Conclusions

Regarding the essential oil yield and the components which define the quality of the oil, in *Thymus hyemalis*, full bloom to the beginning of fruit maturation stage is the most adequate period for its harvesting, since yield and phenolic components reached their highest concentrations at this phenological stage. At the same time, between full bloom and full bloom/beginning of fruit maturation could be the period beyond which the sequence of precursors and their final component γ-terpinene → *p*-cymene → thymol begins.

For the Spanish *T. vulgaris* essential oil yield and composition, at the phenological stage of vegetation the oil exhibited its greatest yield and relative concentration of its major component 1,8-cineol along with low molecular weight components. However, other components also associated to the fresh aroma of this essential oil, terpinyl acetate and linalool, as the most abundant, increased their concentration from full bloom to advance fruit maturation. For this species and attending to the aromatic profile, the harvesting period can only be defined by the necessities of the industry according to its application.

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