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Seasonal variations in leaf structure, morphometry and essential oils of two *Mentha spicata* populations grown at altitudinal extremes

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The structure (LM, SEM, TEM), morphometry and essential oils of *Mentha spicata* leaves were studied in two altitudinally extreme populations (200 m and 950 m) of Mt Pangeon, a NATURA 2000 site of NE Greece (GR 1150005), along the seasonal gradient. Plants at 200 m of altitude (LA, low altitude) are taller and have smaller leaves compared to those at 950 m (HA, high altitude) throughout the vegetative period. Leaf thickness does not significantly differ. Glandular hairs are denser in the leaves of the LA plants, while non-glandular hairs are denser in the leaves of the HA plants. Glandular and non-glandular hairs are always more numerous on the abaxial leaf surface and increase in number from spring to summer. The density of stomata is generally higher in the HA plants and increases from spring to summer. Stomatal population is significantly greater on the lower leaf surface. Phenolic compounds occur more often at LA and their amounts peak in August. Mesophyll chloroplasts ordinarily have larger starch grains at HA than at LA, but in both cases starch volume decreases from spring to autumn. Season and altitude seem furthermore to play an important role in leaf essential oil content. Summer leaves have a higher yield in essential oil than autumn leaves for both altitudes. Leaves of LA plants are richer in essential oil (major components linalool and piperitenone), while leaves of HA plants are poorer (major components *cis*-piperitone oxide and piperitenone oxide).

Key words: *Mentha spicata*, NATURA 2000, leaves, anatomy, ultrastructure, morphometry, essential oils.

INTRODUCTION

Mountains of the Mediterranean region are of particular interest because of the climatic variations they exhibit along their altitudinal gradient. Plants growing at different altitudes undergo different abiotic pressures to which they respond by alterations in the morphological and anatomical patterns of their organs. Low-altitude (LA) plants have to withstand summers with high temperatures and minimal precipitation, whereas high-altitude (HA) plants mainly face low temperatures, strong winds and high UV-irradiance.

Mentha spicata is an aromatic herb belonging to the Labiatae family. It is a polymorphic species, both in morphology and essential oils content. In Greece, it is the most widespread native *Mentha* species. Different vernacular names are used for the plants of the species, reflecting the chemical variation, i.e. the distinctive smell of the four chemotypes that are found in Greece. These chemotypes are characterized by the presence of i) linalool, ii) carvone or dihydrocarvone, iii) piperitone oxide and/or piperitenone oxide and iv) menthone, isomenthone and pulegone, respectively (Kokkini & Vokou, 1989). Carvone-scented plants, commonly known as spearmint, are widely cultivated in many places around the world, but also linalool and menthone-scented plants are of high economic significance.

The aim of the present work was the assessment of the alterations in structure (morphology, anatomy, cytology), morphometry, chlorophyll fluorescence and essential oils of *M. spicata* leaves due to the combined

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effects of altitude and season. Statistical analysis was carried out in order to find out whether the leaf characteristics measured could distinguish the populations examined and if so, which characteristics greatly contribute to this distinction.

MATERIALS AND METHODS

Plant material and sampling

Mt Pangeon (40˚55′ N, 24˚14′ E) is a dominating mountain of NE Greece and is a NATURA 2000 site (GR 1150005). Native populations of *Mentha spicata* L. (Labiatae) plants were studied at two extreme altitudes (200 and 950 m) of Mt Pangeon. At 200 m of altitude (LA), the machie vegetation dominates, while at 950 m (HA) the beech forest. As concerns the soil, this is thick at smooth slopes (~ 1.20 m in depth) and has a sandy-clay constitution. At steep slopes, the soil layer is thin and often the mother rock (limestone) emerges. Collections and measurements were performed during the growing period (April to October). Sampling and biometrics were conducted on the same sites of the populations, so that results are comparable. Fully-expanded leaves from the fifth node (from the tip) of annual stems were used.

Leaf blade area measurement

Leaf blade area was measured with an MK2 area meter (Delta-T Devices Ltd, Cambridge, UK) connected to a TC7000 Series Camera (Burle Industries Inc., Lancaster, PA, USA).

Microscopy (LM, TEM, SEM)

Small pieces of leaves were initially fixed for 3 hrs with 5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2. After washing in buffer, the specimens were postfixed for 2 hrs with 2% osmium tetroxide, similarly buffered. The temperature in all solutions was kept at 0˚C to avoid leaching of phenols during fixation. Samples were then dehydrated in an alcohol series followed by propylene oxide.

For light microscopy (LM) and transmission electron microscopy (TEM), leaf segments were afterwards embedded in Spurr's epoxy resin (Spurr, 1969). Semithin sections for LM were obtained in a Reichert Om U_2 microtome, stained with toluidine blue O and photographed in a Zeiss III photomicroscope. Ultrathin sections for TEM were cut using a Reichert-Jung Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate and examined in a JEM 2000 FXII transmission electron microscope.

For scanning electron microscopy (SEM), the specimens, after fixation and dehydration, were critical point dried in a Balzers CPD 030 device and then coated with carbon in a JEE-4X vacuum evaporator. Observations were made with a JSM 840-A scanning electron microscope.

Morphometry

For the morphometric assessments, a transparent sheet bearing a square lattice of point arrays, 10 mm apart, was laid over light micrographs of leaf cross-sections (×800). The point-counting analysis technique was then applied (Steer, 1981). Such sections were used to estimate leaf lamina thickness. The densities of stomata, glandular and non-glandular hairs on both leaf surfaces were determined using leaf paradermal sections and SEM micrographs. The technique of point-counting analysis was further applied to TEM micrographs to assess the volume fraction of chloroplasts per cell and the volume fractions of starch grains, plastoglobuli and grana per chloroplast.

Leaf chlorophyll assay

To determine the leaf chlorophyll α content, 1 cm^2 of fresh leaf material was homogenized with liquid N in 90% acetone, remained for 24 hrs at –10˚C and then centrifuged at 10000 g for 15 min. The absorbance of the supernatant was measured at 664 nm and 647 nm with an LKB Ultraspec II spectrometer. Chlorophyll α content was calculated using the coefficients given by Jeffrey & Humphrey (1975).

Chlorophyll a fluorescence

Chlorophyll α fluorescence was measured with a portable fluorometer model Plant Stress Meter (BioMonitor AB, Sweden). Leaves were dark-adapted for 30 min. For the excitation of fluorescence energy, actinic light of 400 μmol m^{-2} s^{-1} was used for 10 s. The fluorescence values F_o, F_v, F_m, F_v/F_m and $t_{1/2}$ of the fast induction kinetics were calculated. Each value represents the mean of eight measurements.

Essential oil isolation and analysis

Plant material was air-dried at room temperature for 10 days and then grossly pulverized. The essential oils were isolated by hydrodistillation for 2 hrs, using a Clevenger apparatus. The essential oil content was expressed in ml 100 g^{-1} d.w. GC and GC/MS analyses

were carried out according to the procedure described in Kofidis *et al.* (2004).

Statistical analysis

The data were subjected to analysis of variance (ANOVA). For comparisons of the means, the Duncan's multiple range test was employed. Principal component analysis (PCA) was additionally applied to examine the interrelationships between populations grown at different altitudes and season. The variables used are listed in Tables 1 to 5.

RESULTS

Two populations of *Mentha spicata* at the altitudinal extremes (200 m and 950 m) of Mt Pangeon (NE Greece, GR 1150005) were studied. In the population at 200 m (LA), the vegetative period starts in April, while in that at 950 m (HA), one month later, in May. Both populations terminate their growth in October. In spring (April-May), plants of both populations are short, while they progressively become taller reaching a maximum height in late summer-early autumn (Table 1). A slight reduction in height is then observed, mainly due to grazing. Plants at HA are steadily shorter than those at LA, during the summer months and also in September.

The leaves of *M. spicata* are small in early spring, but they quickly obtain their maximal surface (in May at 200 m and June at 950 m) (Table 1). The leaves emerging in autumn are much smaller than those emerging in spring and summer. The thickness of the leaves at 200 m does not exhibit any significant fluctuation during the vegetative period, whereas such a fluctuation is observed at 950 m (Table 1).

Leaves are covered on both sides with numerous non-glandular and glandular hairs. Non-glandular hairs are much denser on the abaxial leaf surface than on the adaxial one (Fig. 1) in such a degree that other epidermal structures (glandular hairs, stomata, etc.) are hardly discerned. Non-glandular hairs are ordinarily simple, uniseriate, but they may be also branched. Their density is greater in the summer leaves than in the spring and autumn leaves (Table 2). Among non-glandular hairs, glandular hairs also occur. They are the sites of essential oil biosynthesis. They are anatomically composed of a single large basal cell, a single flattened stalk cell and a voluminous head of 12 cells (4 central small cells and 8 peripheral larger cells) (Fig. 2). The essential oil accumulates in a space between the head cells and the detached cuticle (Fig. 2A). Glandular hairs are more numerous on the abaxial leaf surface (Table 2). In most months, HA leaves have significantly thinner glandular hair pubescence compared to LA leaves for both leaf surfaces. As concerns the seasonal distribution, the density of glandular hairs is low on the surface of the spring leaves, it greatly increases in the summer leaves, while later in the autumn leaves it becomes more or less stabilized (Table 2).

Leaves of *M. spicata* bear stomata on both surfaces. Stomata on the abaxial leaf surface are locally projecting (Fig. 2C). Generally, stomata occur in a higher density on the abaxial leaf surface than on the adaxial one (Table 2). Observations on stomatal density related to elevation, showed that, generally, LA

TABLE 1. Mean values of plant height, leaf blade area, leaf width/length ratio and leaf thickness of *Mentha spicata* at different altitudes during the sampling period. Means of the same raw marked with the same letter are not significantly different ($p < 0.05$). Bold letters indicate significantly different values ($p < 0.05$) compared to the previous measurement of the same raw. n = 50 (for leaf thickness n = 10)

	Altitude (m)	April	May	June	July	August	September	October
Plant height (cm)	200	13.4	18.4 ^{a}	37.7 $^{\mathbf{b}}$	43.2 ^{b}	44.5 ^{b}	45.1 ^{b}	40.0 ^{a}
	950	*	18.2 ^{a}	21.1 ^{a}	31.4 $^{\mathbf{a}}$	37.8 ^{a}	36.2 ^{a}	35.0 ^{a}
Leaf blade area (mm^2)	200	250	343 $^{\mathbf{a}}$	335 ^{a}	297 ^{a}	273 ^{a}	226 $^{\mathbf{a}}$	172 ^{a}
	950	*	270 ^{a}	533 $^{\mathbf{b}}$	340 $^{\mathbf{a}}$	314 ^{a}	204 $^{\mathbf{a}}$	188 ^{a}
Leaf width/length ratio	200	0.47	NM	0.40 ^{a}	NM	0.58 $^{\mathbf{b}}$	NM	0.42 $^{\mathbf{a}}$
	950	*	NM	0.38 ^{a}	NM	0.45 $^{\mathbf{a}}$	NM	0.43 ^{a}
Leaf thickness (μm)	200	161	NM	162 ^{a}	NM	168 ^{a}	NM	161 ^{a}
	950	*	NM	190 ^{b}	NM	153 $^{\mathbf{a}}$	NM	161 ^{a}

* Plants have not yet started growing, NM = not measured

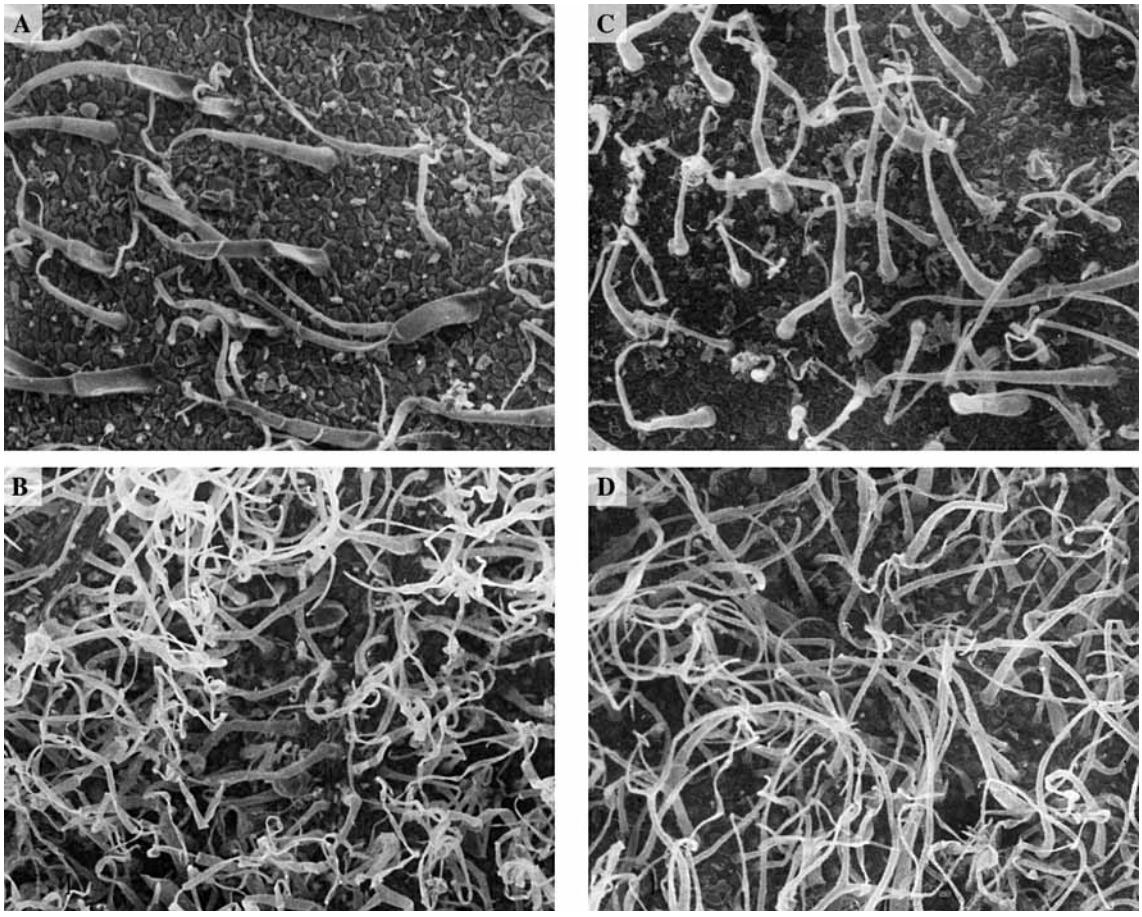

FIG. 1. SEM micrographs of non-glandular hairs on both leaf surfaces of *Mentha spicata* plants grown at altitudinal extremes (200 m and 950 m) (×125). A. 200 m, adaxial leaf surface. B. 200 m, abaxial leaf surface. C. 950 m, adaxial leaf surface. D. 950 m, abaxial leaf surface.

TABLE 2. Mean values of glandular hair density (D_g), non-glandular hair density (D_{ng}) and stomatal density (D_{st}) at the adaxial (ad) and abaxial (ab) leaf surfaces of *Mentha spicata* at different altitudes during the sampling period. Means of the same raw marked with the same letter are not significantly different ($p < 0.05$). Bold letters indicate significantly different values ($p < 0.05$) compared to the previous measurement of the same line. n = 12

	Altitude (m)	April	June	August	October
$D_{g(ad)}$	200	1.6	2.6 **b**	4.1 **a**	4.5 b
(No mm^{-2})	950	*	1.9 a	3.0 **a**	2.7 a
$D_{g(ab)}$	200	3.5	7.1 **a**	8.4 b	8.7 b
(No mm^{-2})	950	*	6.0 a	6.4 a	6.0 a
$D_{ng(ad)}$	200	15	19 a	22 a	10 **a**
(No mm^{-2})	950	*	21 a	25 a	13 **a**
$D_{ng(ab)}$	200	50	200 **a**	200 a	20 **a**
(No mm^{-2})	950	*	180 a	200 a	50 **b**
$D_{st(ad)}$	200	29	35 a	44 a	42 a
(No mm^{-2})	950	*	104 b	100 b	109 b
$D_{st(ab)}$	200	478	412 a	559 **a**	601 a
(No mm^{-2})	950	*	638 b	720 b	659 a

* Plants have not yet started growing

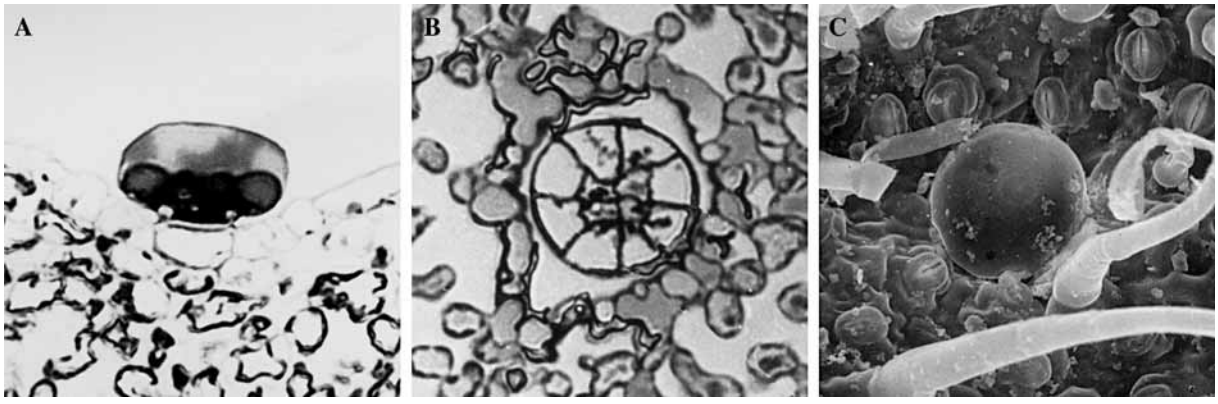

FIG. 2. *Mentha spicata*. A. A glandular hair (peltate) in leaf cross section (×200). B. The head of the glandular hair in leaf paradermal section. It is composed of 12 cells (×200). C. SEM micrograph of a glandular hair (×200).

TABLE 3. Mean values of the relative volume percentages of chloroplasts per cell (RV_{chl}), grana per chloroplast (RV_{gr}), starch grains per chloroplast (RV_{sg}) and plastoglobuli per chloroplast (RV_{pg}) of *Mentha spicata* at different altitudes during the sampling period. Means of the same raw marked with the same letter are not significantly different ($p < 0.05$). Bold letters indicate significantly different values ($p < 0.05$) compared to the previous measurement of the same line. n = 10

	Altitude (m)	April	June	August	October
RV_{chl}	200	42.0	52.0 [a]	20.9 **[a]**	30.5 **[b]**
	950	*	42.0 [a]	29.0 **[a]**	18.4 **[a]**
RV_{gr}	200	17.0	19.6 [a]	22.8 [a]	26.8 [a]
	950		20.6 [a]	16.0 [a]	38.4 **[b]**
RV_{sg}	200	50.5	53.8 [a]	29.0 **[a]**	34.6 [b]
	950	*	50.5 [a]	56.0 [b]	16.5 **[a]**
RV_{pg}	200	0.3	2.6 **[b]**	3.9 **[b]**	3.7 [b]
	950	*	1.3 [a]	1.8 [a]	1.0 **[a]**

* Plants have not yet started growing

TABLE 4. Mean values of leaf chlorophyll *α* content (chl*α*), initial fluorescence (F_o), variable to maximal fluorescence (F_v/F_m), and half-rise time ($t_{1/2}$) from initial to maximal fluorescence of *Mentha spicata* at different altitudes during the sampling period. Means of the same raw marked with the same letter are not significantly different ($p < 0.05$). Bold letters indicate significantly different values ($p < 0.05$) compared to the previous measurement of the same line. n = 8

	Altitude (m)	April	June	August	October
chl*α* ($\mu g\ cm^{-2}$ f.w.)	200	27.8	23.4 **[a]**	22.5 [a]	25.8 **[b]**
	950	*	22.0 [a]	23.9 [a]	21.4 [a]
F_o	200	0.20	0.20 [a]	0.19 [a]	0.21 [b]
	950	*	0.19 [a]	0.18 [a]	0.18 [a]
F_v/F_m	200	0.735	0.673 **[a]**	0.704 **[a]**	0.790 **[a]**
	950	*	0.765 [b]	0.764 [b]	0.778 [a]
$t_{1/2}$ (msec)	200	167	159 [a]	132 [a]	123 [a]
	950	*	170 [a]	171 [b]	149 [b]

* Plants have not yet started growing

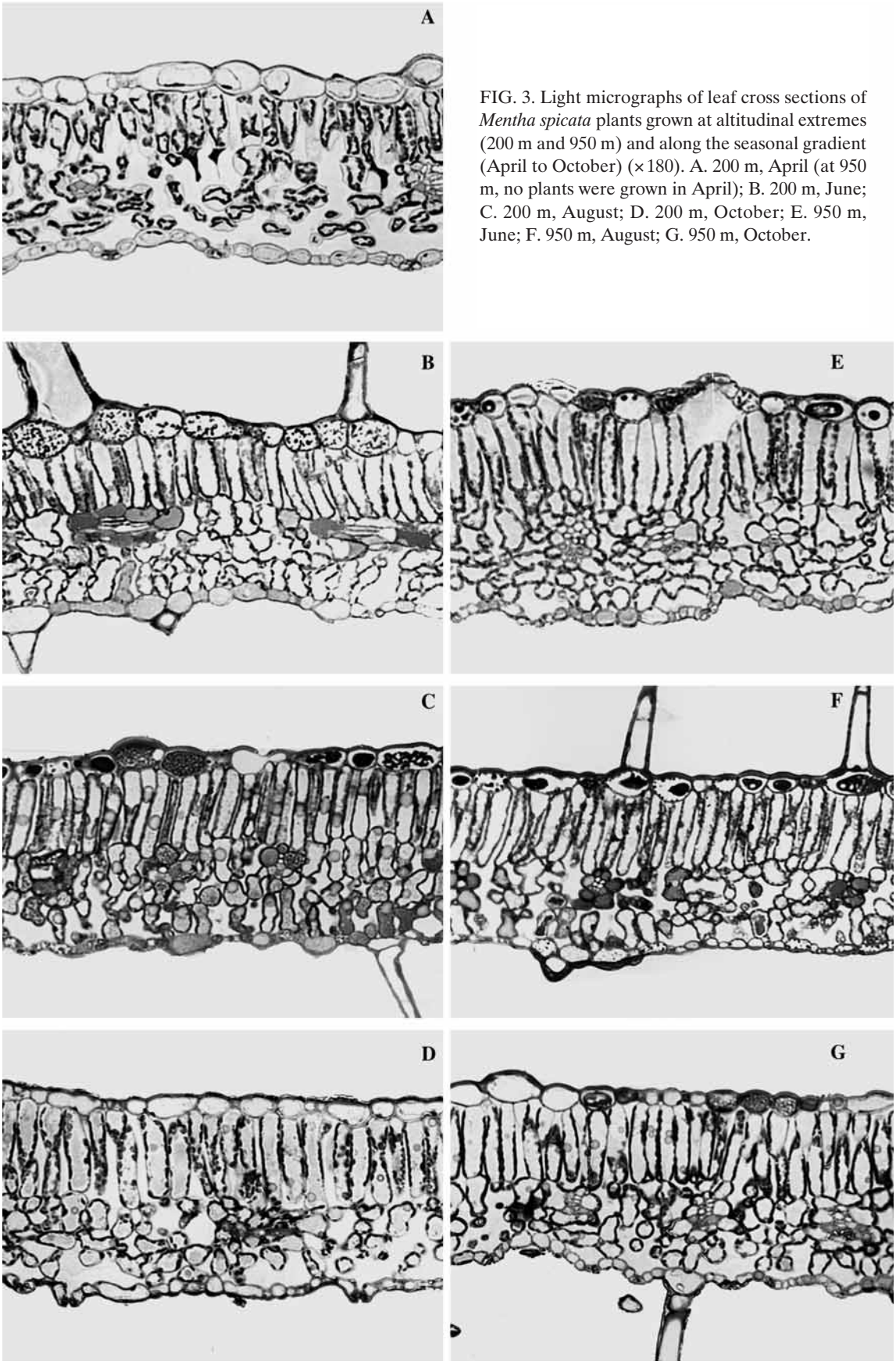

FIG. 3. Light micrographs of leaf cross sections of *Mentha spicata* plants grown at altitudinal extremes (200 m and 950 m) and along the seasonal gradient (April to October) (×180). A. 200 m, April (at 950 m, no plants were grown in April); B. 200 m, June; C. 200 m, August; D. 200 m, October; E. 950 m, June; F. 950 m, August; G. 950 m, October.

leaves have fewer stomata on both leaf surfaces compared to HA leaves (Table 2). Seasonally, the density of stomata does not appear to significantly fluctuate, particularly on the adaxial leaf surface.

Cross-sections of *M. spicata* leaves showed the typical anatomy of the dicot leaf (Fig. 3). In LA plants, April leaves do not appear to contain any phenolics, whereas August leaves are filled with phenolics in their epidermal and mesophyll cells (Fig. 3A, C). By the end of October, phenolics occupy only a small portion of the epidermal tissue or they are entirely lacking (Fig. 3D). In HA plants, phenolics exist in small quantities in the epidermal cells of the June leaves, whereas in August they fill the epidermal cells and occasionally the mesophyll cells (Fig. 3E, F). Leaves of October bear phenolics only in their epidermis (Fig. 3G).

Observations on the chloroplasts of the mesophyll cells showed that these organelles are generally different in the leaves of the two altitudinal populations (Fig. 4). At LA, the starch grains in chloroplast stroma initially occupy about the half of the chloroplast volume (April, June) (Fig. 4A), while later (August, October) about one third of it (Fig. 4C, Table 3). At HA, starch grains occupy more than the half of the chloroplast volume during the summer (Fig. 4D), while later in the autumn, their relative volume becomes highly decreased (Fig. 4F, Table 3). As concerns the chloroplast plastoglobuli, these were more numerous in the LA leaves than in the HA leaves (Fig. 4, Table 3). In both populations, plastoglobuli appear more developed in August.

Chlorophyll α measurements showed that *M. spicata* LA leaves contain more chlorophyll in April (Table 4). During the summer months, the amount of chlorophyll becomes decreased and then it increases again in October. No significant seasonal fluctuation in the amount of chlorophyll α of the HA leaves was observed (Table 4). In general, no significant differences in chlorophyll α were noticed between the two extreme altitudes in the spring and summer months, with the exception of the month of October.

The initial leaf chlorophyll fluorescence (F_o) exhibited a relative stability during the entire vegetative period when comparing the LA and HA populations. Differences in F_o existed only between the October leaves (Table 4). As concerns the variable to maximal chlorophyll fluorescence (F_v/F_m), this was found to be highly stable in the HA leaves along the seasonal gradient (Table 4). In the LA leaves, F_v/F_m values

TABLE 5. Seasonal variation of essential oil content (%) and composition of *Mentha spicata* leaves collected at 200 m and 950 m of Mt Pangeon (Pangeon, GR 1150005)

Component	200 m			950 m		
	June	August	October	June	August	October*
α-pinene	0.3	0.6	0.2	2.1	0.2	
β-pinene	0.7	1.3	0.4			
sabinene	0.3	0.7	0.3	2.1	0.5	
myrcene	1.0	1.3	1.1	2.1	1.6	
limonene	0.9	1.4	0.6			
1,8-cineole	3.9	5.4	2.3		2.1	
linalool	**28.5**	**36.0**	0.1			
β-elemene				7.4	2.8	
β-caryophyllene				15.3	4.9	
***cis*-piperitone oxide**	3.2	1.7	7.3	**33.3**	**9.6**	
trans-piperitone oxide	13.9	8.3	6.9		0.5	
piperitone	1.1		0.4		1.3	
isopiperitone	0.2	0.6	0.3		0.1	
piperitenone	**31.0**	**25.4**	**36.2**		1.2	
piperitenone oxide		< 0.1	0.2	**37.3**	**57.9**	
caryophyllene oxide					0.5	
Total essential oil content (ml 100 g^{-1} dry weight)	1.2	**1.8**	0.5	0.4	**1.0**	< 0.1

*only traces of essential oil were obtained

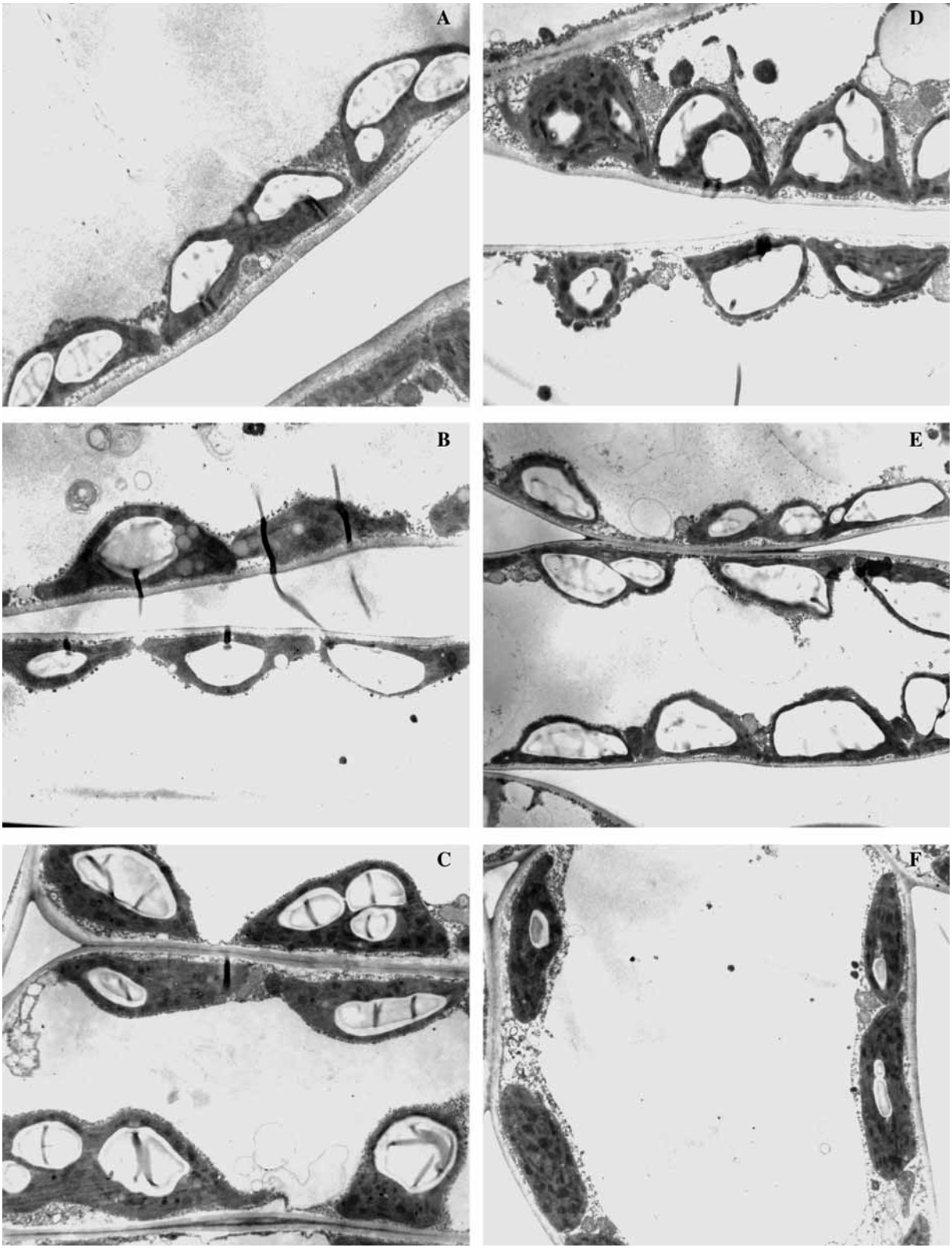

FIG. 4. TEM micrographs of mesophyll chloroplasts of *Mentha spicata* plants grown at altitudinal extremes (200 m and 950 m) and along the seasonal gradient (June to October) (×4500). A. 200 m, June; B. 200 m, August; C. 200 m, October; D. 950 m, June; E. 950 m, August; F. 950 m, October.

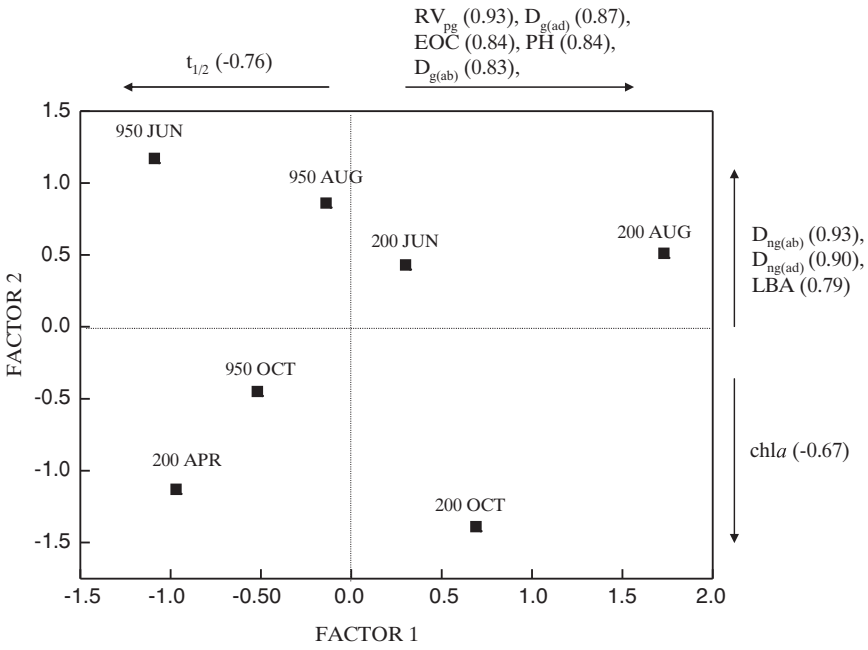

FIG. 5. Principal Component Analysis. Scatter diagram of *Mentha spicata* populations in the two main components. Arrows indicate the most important characters contributing to the discrimination. EOC = Essential oil content; PH = Plant height; LBA = Leaf blade area. Variables abbreviations are given in Tables 1-5.

showed an increasing tendency from summer to autumn. During the summer months, HA leaves exhibited the higher F_v/F_m values. Moreover, the half-rise time ($t_{1/2}$) from initial to maximal fluorescence appeared higher in the HA leaves compared to the LA leaves, by the end of summer and afterwards (Table 4).

Leaves are richest in essential oil in August, when plants are at full bloom (Table 5). At the beginning of autumn, there is a considerable reduction of the oil content. Altitude seems to play an important role, since HA leaves contain a much lower amount of essential oil compared to LA leaves. The qualitative and quantitative compositions of the oils obtained from *M. spicata* leaves collected in seasonal progress and at both altitudes are presented in Table 5. The main components of the essential oil of the LA leaves are piperitenone and linalool, with the latter being only at small percentage in the October leaves. The essential oil of the HA leaves was found rich in piperitenone oxide and *cis*-piperitone oxide. These two components present a negative correlation, with the former to increase from June to August and the latter to decrease during the same period.

The PCA, based on the characteristics (variables) presented in Tables 1 to 5, was then applied. Figure 5 shows the scatter plot of the *M. spicata* populations studied seasonally at the two altitudinal extremes. The total variance explained by the two factors was 34.3% and 25.1%, respectively. The variables that contributed to the distinction of the populations by factor 1 (on the x axis) were the relative volume of plastoglobuli, the glandular hair density on the adaxial and abaxial leaf surfaces, the essential oil content, the plant height and the $t_{1/2}$. The variables that contributed to the distinction of the populations by factor 2 (on the y axis) were the non-glandular hair density on the adaxial and abaxial leaf surfaces, the leaf blade area and the chlorophyll α content.

DISCUSSION

Mountain Pangeon is a dominating mountain of NE Greece having a maximal altitude of 1956 m. *Mentha spicata* extends up to an altitude of 950 m (beech forest) and is also present at low altitudes of the mountain (200 m) in a macchie vegetation. Altitude exerts a significant influence on plant height, so that high elevation plants are generally shorter than low elevation plants. The low height of *M. spicata* at 950 m is principally associated with the shorter vegetative period, the lower temperatures, the limited disposal of soil water and nutrients, the higher UVB irradiation and the stronger winds (Woodward, 1979; Graves & Taylor, 1986; Cordell *et al.,* 1998; Kao *et al.,* 1998). Leaves of *M. spicata* are severely affected by season and altitude. Leaves emerging in spring have a larger size than those emerging in summer and autumn. The latter have the smallest size. Furthermore, LA leaves are smaller than the HA leaves. However, reports exist in which plants of low altitudes have larger leaves

than those of high altitudes (Morecroft & Woodward, 1996; Venema *et al.*, 2000). This reveals that particular plant species have specific adaptation abilities to various altitudinal environments.

The size of leaves is well correlated with the number of stomata on both leaf sides. In *M. spicata*, stomata are always more numerous in the HA leaves compared to the LA leaves. A similar increase of stomatal density parallel to increase of altitude was also observed in *Sedum atratum* (Codignola *et al.*, 1987), *Miscanthus* sp. (Kao & Chang, 2001) and *Picea crassifolia* (Qiang *et al.*, 2003). The reason for the decrease of stomatal density at 200 m might be associated with the xerothermic conditions prevailing at the foot of the mountain. Stomatal density, on the other hand, is associated with the rate of photosynthesis, and specifically the photochemical efficiency of PSII, which was found to be higher in the leaves of the 950 m population. The F_v/F_m values in this population are high during the whole developmental course of the plants.

Apart from stomata, other typical epidermal structures that occur on the leaf of *M. spicata*, are the non-glandular and the glandular hairs. Non-glandular hairs are denser on the abaxial leaf surface (where most stomata exist) and their number is higher during the summer period and at low altitude (200 m). These facts are in agreement with the views of many authors that non-glandular hairs constitute an adaptation to xerothermic conditions (blocking of water loss through stomata) and protection against insect attacks that are most often at low altitudes and during the summer (Johnson, 1975; Woodman & Fernandez, 1991; Fahn & Cutler, 1992). Glandular hairs on leaves of *M. spicata* are voluminous (their head is composed of 12 cells) and are the exclusive sites of essential oil biosynthesis (Bosabalidis, 2002). They are more numerous in the summer and autumn leaves than in the spring leaves. Although their density in the summer and autumn leaves is quite stable, summer leaves are found (by distillation) to have a much higher essential oil content than autumn leaves. This is due to the essential oil loss from summer to autumn, probably caused by the high summer temperatures and the heavy autumn rainfalls. As concerns altitude, LA leaves possess more glandular hairs than HA leaves. In accordance with that, LA leaves contain a much higher amount of essential oil than HA leaves.

Anatomical observations on *M. spicata* leaves showed that August leaves contain in the vacuoles of the epidermal and mesophyll cells the highest amounts of phenolic compounds. This event reflects a defensive mechanism of the plant by which the strong solar irradiation during August becomes absorbed by the phenolics (Christodoulakis, 1989), protecting thus the leaf cells from structural and functional damages (Tevini, 1994). Anatomical observations further showed that at high elevation (950 m), chloroplasts are shorter and contain fewer and smaller starch grains. An analogous effect of high elevation on chloroplast size and starch grain content has been also reported by Miroslavov & Kravkina (1991) in *Poa* sp. and *Oxytropis* sp. and by Zellnig & Gailhofer (1989) in *Picea* sp. The low starch content was attributed to the high rates of respiration of these plants confirmed by the existence of a great number of mitochondria within the mesophyll cells. Thus, HA plants because of the short vegetative period and the low temperatures are forced to develop higher respiratory rates (by consuming nutrients, particularly hydrocarbons) in order to complete their growth cycle.

The GC/MS analyses of the leaf essential oils obtained by the LA population revealed the occurrence of two distinct chemotypes in this population (the piperitone oxide and/or piperitenone oxide chemotype and the linalool chemotype). The existence of different chemotypes in the same *Mentha* population has been also mentioned by Kokkini (1991). A pure chemotype with a very high linalool content has been observed in the same mountain (Kofidis *et al.*, 2004). On the other hand, the HA population was found to belong to the piperitone oxide and/or piperitenone oxide chemotype which is frequently found in the members of the *Spicatae* group of the section *Mentha* (Kokkini, 1992). The major components of the leaf essential oils of this population were piperitenone oxide and *cis*-piperitone oxide. These two components presented a negative correlation, with the former to increase from June to August and the latter to decrease during the same period.

Based on Principal Component Analysis (PCA), the summer and autumn LA populations exhibited positive values on the x axis, whereas the summer and autumn HA populations (together with the spring LA population) exhibited negative values on the x axis. The characteristics which contributed more to this distinction were the relative volume of plastoglobuli, the glandular hair density on the adaxial and abaxial leaf surfaces, the essential oil content, the plant height and the $t_{1/2}$. On the y axis, summer plants of both altitudinal populations exhibited positive values, whereas spring and autumn plants of both altitudinal populations negative values. The characteristics which con-

tributed more to this distinction were the non-glandular hair density on the adaxial and abaxial leaf surfaces, the leaf blade area and the chlorophyll α content.

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