

Effects of altitude and season on glandular hairs and leaf structural traits of *Nepeta nuda* L.

George KOFIDIS and Artemios M. BOSABALIDIS*

Department of Botany, School of Biology, Aristotle University, Thessaloniki 54124, Greece

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ABSTRACT. The effects of altitude (950, 1,480 and 1,760 m) and season (April to October) on some morphological (including glandular hairs), anatomical, and ultrastructural leaf traits of *Nepeta nuda* were studied. During the whole vegetative period, plants at 1,760 m were significantly shorter than plants at the lower elevations. At all altitudes, the leaves obtained their maximal surface in July. Leaves emerging in the autumn were smaller than summer leaves. Remarkable fluctuations were noticed in leaf thickness at the three altitudes during the growing period. Stomata occurred in higher density on the abaxial leaf surface than on the adaxial one. As related to altitude, leaves of 1,480 m plants possessed more stomata than leaves of plants at the two altitudinal extremes. Non-glandular hairs were denser on the adaxial leaf surface (no significant differences were noticed between plants of the three populations). However, leaves at 950 m were the less pubescent on the abaxial leaf surface during the whole sampling period. Glandular hairs of *N. nuda* were of two morphologically distinct types, i.e. capitate hairs (composed of a basal cell, a stalk cell and a head cell) and peltate hairs (composed of a basal cell, a stalk cell and a voluminous head of 4 cells). The density of capitate hairs tended to increase from spring to autumn on both leaf surfaces, in all populations examined. Peltate hairs were numerous on the abaxial leaf surface, but they could hardly be observed on the adaxial one. Leaves of 950 m had significantly higher peltate hair density on early summer compared to leaves of the higher altitudes (1,480 m and 1,760 m), but later on, in late summer and early autumn, just the opposite held true. At all three altitudes, summer leaves contained phenolics in their epidermal cells while autumn leaves seemed devoid of such substances. Differences also existed in mesophyll chloroplasts of plants at the three populations. In all altitudinal populations, the relative volume of starch grains became decreased from summer to autumn, when starch grains occupied only a small portion of the chloroplast stroma. On the other hand, during the same period, the relative volume percentage of grana per chloroplast increased.

Keywords: Altitude; Chloroplasts; Glandular hairs; Leaf structure; *Nepeta nuda*; Season.

INTRODUCTION

Aromatic plants of Labiatae are economically important due to the essential oils they produce. The exclusive sites of essential oil production are the glandular hairs (Mc Caskill and Croteau, 1995), which are epidermal structures covering the aerial parts of the plants. Two distinct types of glandular hairs are distinguished, the peltate hairs and the capitate hairs, which mainly differ in the volume of their secretory head. The density of these epidermal structures on the leaves has been found to be positively correlated with the essential oil content of the plants (Bosabalidis, 2002). Efforts have been made to determine the factors affecting the essential oil production (Chalchat et al., 1997; Hudaib et al., 2002), as well as the effects of various abiotic stresses on it (Yamaura et

al., 1989; Karousou et al., 1998; Panou-Filothou et al., 2001). However, little information is available about the morphological, cytological, and physiological features of aromatic plants associated with their adaptation to their natural environment (Kofidis et al., 2003, 2007).

The genus *Nepeta* (Labiatae) includes approximately 250 species, many of which are used for pharmaceutical purposes. Terpenoids, mainly of the nepetalactone type, are usually the principal constituents of *Nepeta* species, commonly known as catmints. Nepetalactones have been found to possess antimicrobial and insect repellent activities, but they are also strong feline attractants (De Pooter et al., 1987; Handjieva et al., 1996). These substances are observed to accumulate in the subcuticular chamber of the peltate glandular hairs (Clark et al., 1997).

Nepeta nuda is the most widespread species of the genus *Nepeta* in Greece. It is divided into two morphologically and geographically distinct subspecies, i.e. subsp. *nuda* and subsp. *albiflora*, mainly differing in their corolla colour and their geographic distribution

*Corresponding author: E-mail: artbos@bio.auth.gr; Tel: +30-2310-998365; Fax: +30-2310-998389.

(Kokkini and Babalonas, 1982). *Nepeta nuda* plants mostly occur in forest clearings and meadows, at montane and subalpine altitudes up to 2,100 m (Baden, 1991). Essential oils of *N. nuda* have been found to exhibit significant variations in their composition in relation to the plants' origin (De Pooter et al., 1987; Handjieva et al., 1996; Kökdil et al., 1996; Kökdil et al., 1998).

The present work deals with the seasonal variability (April to October) of some morphological (including glandular hairs), anatomical, and ultrastructural leaf traits of *Nepeta nuda* in three populations along an altitudinal gradient (950 m, 1,480 m and 1,760 m). The observed fluctuations are discussed in relation to growth and adaptation of *N. nuda* to its native habitat.

MATERIALS AND METHODS

Plant material and sampling

Native populations of *Nepeta nuda* L. subsp. *nuda* (Labiatae) were studied at three sites along the altitudinal gradient of Mt. Pangeon, N. Greece. The two sites at lower elevations (40°55' N / 24°11' E, 950 m above sea level and 40°54' N / 24°07' E, 1,480 m a. s. l.) occur within the beech forest where soil is poor in inorganic elements (Ca, K, Mg, Fe, Mn). The upland site (40°55' N / 24°06' E, 1,760 m a. s. l.) is located within the alpine meadow vegetation, where soil is rich in Mg and Mn. Average temperatures for July are 19, 16 and 14°C, and for October 10, 7 and 5°C, respectively, from the lowland to the upland site. The mean annual rainfall on the mountain varies between 600-800 mm, with the maximum values being observed at the highest elevation. Light intensities also increase with altitude, and other environmental factors like wind exposure, ozone concentration, and partial CO₂ pressure may differ between altitudes. Collections and measurements were performed repeatedly in the years 1998-1999 during the growing period (April to October). Weather conditions were more or less the same over the two years. 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 top) of annual stems were used. Leaves sampled were from different plants, had the same age and a south side exposure.

Leaf blade area

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 *in situ* (between 9.00 and 9.30 a.m.) for 3 h with 5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2. After being washed in buffer, the specimens were postfixed for 2 h with 2% osmium tetroxide, same 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), the tissue was afterwards embedded in Spurr's (1969) resin. Semithin sections for LM were obtained with a Reichert Om U₂ ultramicrotome, stained with toluidine blue O and photographed in a Zeiss III photomicroscope. Histochemical identification of the phenolic compounds was conducted according to Reeve (1951). Free-hand sections were treated with equal volumes of reagents in the following succession: 10% sodium nitrate, 20% urea, 10% acetic acid and after 3-4 minutes with two volumes of 2N NaOH. Positive reaction produces a range of colours from red to yellow.

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 assessment of the relative volume of the leaf phenolic compounds, a transparent sheet bearing a square lattice of point arrays, 10 mm apart, was laid over light micrographs of leaf cross-sections ($\times 800$). The point-counting analysis technique was then applied (Steer, 1981). Similar 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.

Statistical analysis

The data were subjected to analysis of variance (ANOVA). For comparisons of the means, the Duncan's multiple range test was employed.

RESULTS

Populations of *Nepeta nuda* subsp. *nuda* at three successive altitudinal levels (950 m, 1,480 m, 1,760 m) were studied. In the population at 950 m, the vegetative period starts in April, and those at 1,480 m and 1,760 m, two months later, in June. Growth for all populations ends in October. At the beginning of their growing cycle, plants of all three populations are short, and they progressively become taller, reaching a maximum height from mid-

Table 1. Effects of altitude and season on plant height, leaf area, leaf width / length ratio, and leaf thickness.

	Altitude (m)	April	May	June	July	August	September	October
Plant height (cm)	950	14.5	33.0 a	40.1 a	66.8 a	64.1 a	64.0 a	58.0 a
	1480	*	*	33.3 b	58.9 a	67.3 a	71.3 a	64.2 a
	1760	*	*	24.7 c	45.7 c	61.7 a	50.7 c	46.0 c
Leaf area (mm ²)	950	149	801 a	949 a	1010 a	909 a	848 a	804 a
	1480	*	*	906 a	1122 a	845 a	807 ab	735 a
	1760	*	*	999 a	1022 a	832 a	700 b	709 a
Leaf width/length ratio	950	0.39	NM	0.40 a	NM	0.36 a	NM	0.44 a
	1480	*	*	0.40 a	NM	0.41 ab	NM	0.43 a
	1760	*	*	0.40 a	NM	0.47 b	NM	0.43 a
Leaf thickness (µm)	950	202	NM	276 a	NM	277 a	NM	163 a
	1480	*	*	208 b	NM	200 b	NM	238 b
	1760	*	*	299 a	NM	197 b	NM	228 b

n=50 (for leaf thickness n=10). Means of the same column 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.

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

summer until early-autumn (Table 1). Later on, a slight reduction in height is observed, mainly due to grazing. During the whole vegetative period, plants at 1,760 m are significantly shorter than those at 950 m and 1,480 m.

The leaves of *N. nuda* at 950 m are small in the early spring (April), but they grow fast (with a 4-fold increase in leaf area from April to June), obtaining their maximal surface in July (Table 1). At all altitudes, the leaves emerging in the autumn are smaller than the summer leaves. The shape of leaves differs in the three populations only in August, when the higher altitude leaves are more rounded (higher width/length ratios) than those at the 950 m altitude. The thickness of the leaves at the three altitudes exhibits remarkable fluctuations during the vegetative period (Table 1).

Leaves of *N. nuda* bear stomata on both surfaces (Figure 1). Stomata on the abaxial leaf surface are locally projecting. Generally, stomata occur in a higher number on the abaxial leaf surface (400-700 st. mm⁻²) than on the adaxial one (110-250 st. mm⁻²) (Table 2). Observations on stomatal density related to elevation showed that leaves at 1,480 m have more stomata on both of their surfaces compared to leaves at the two altitudinal extremes (950 m and 1,760 m). Seasonally, the density of stomata does not appear to significantly fluctuate on the abaxial leaf surface for any of the three populations (Table 2).

Apart from stomata, leaves also bear numerous epidermal non-glandular hairs (Figure 1). These hairs are multicellular, composed of 4-7 cells in line, with the apical cell acute. At their basis, they are surrounded by 4-6 radially arranged and locally projecting epidermal cells.

Table 2. Effects of altitude and season on stomatal density on the adaxial [$D_{st(ad)}$] and abaxial [$D_{st(ab)}$] leaf surfaces and on non-glandular hair density on the adaxial [$D_{h(ad)}$] and the abaxial [$D_{h(ab)}$] leaf surfaces.

	Altitude (m)	April	June	August	October
$D_{st(ad)}$ (No/mm ²)	950	120	170 a	153 a	113 a
	1480	*	235 b	250 b	192 b
	1760	*	178 a	151 a	188 b
$D_{st(ab)}$ (No/mm ²)	950	400	426 a	414 a	410 a
	1480	*	635 b	645 b	701 b
	1760	*	440 a	443 a	499 c
$D_{h(ad)}$ (No/mm ²)	950	8.4	10.2 a	11.0 a	10.6 a
	1480	*	11.0 a	11.4 a	11.4 a
	1760	*	10.2 a	10.8 a	11.2 a
$D_{h(ab)}$ (No/mm ²)	950	2.5	3.0 a	2.8 a	2.8 a
	1480	*	4.6 b	5.0 b	4.8 b
	1760	*	4.2 b	4.0 c	4.2 b

n=12. Means of the same column 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.

* Plants have not yet started growing.

Non-glandular hairs are much denser on the adaxial leaf surface than on the abaxial one. No significant differences were noticed in the hair density on the adaxial leaf surface between the three populations, but leaves at 950 m were the less pubescent on the abaxial leaf surface, during the

whole sampling period (Table 2).

Among non-glandular hairs, glandular hairs also occur (Figure 1). They are the sites of the essential oil production, and they are of two morphologically distinct types. The smaller ones, the capitate hairs, are composed

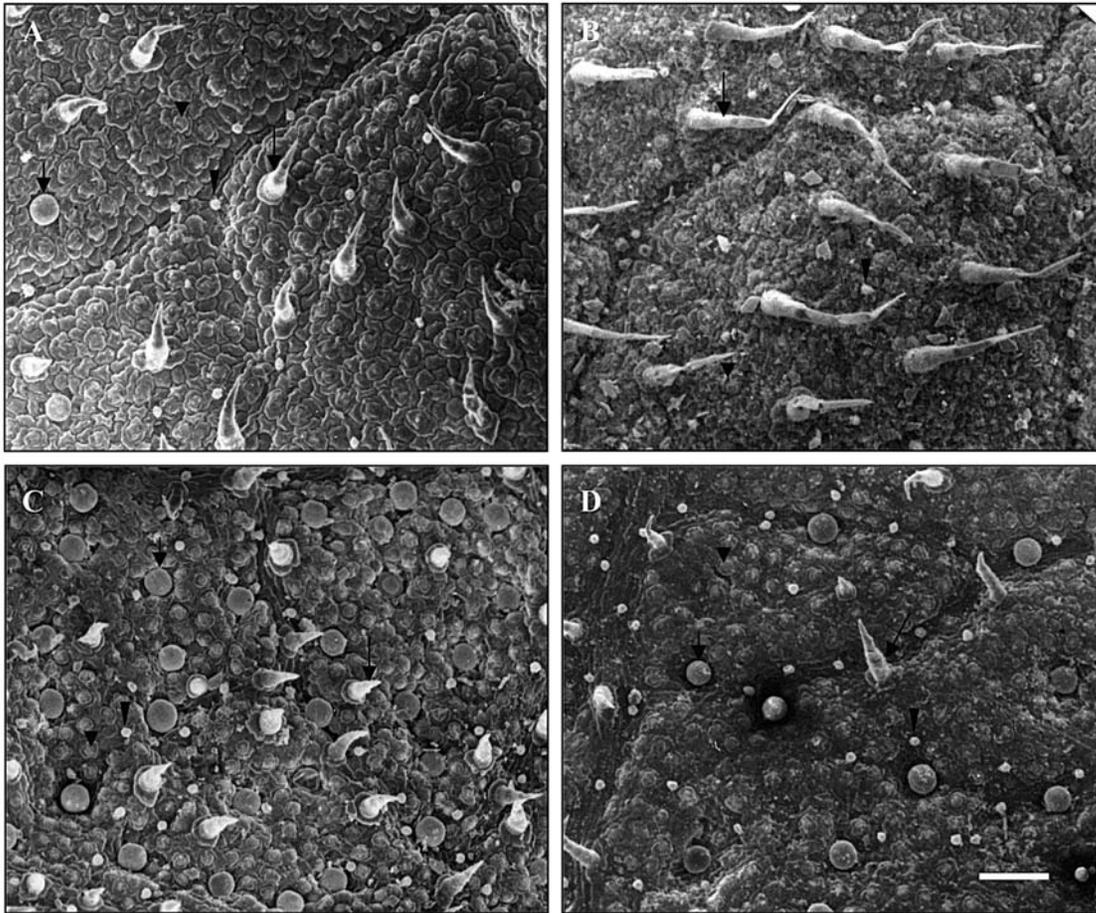


Figure 1. SEM micrographs of leaf surfaces of June plants at the two altitudinal extremes showing stomata (short arrowheads), capitate hairs (long arrowheads), peltate hairs (short arrows) and non-glandular hairs (long arrows). A, 950 m, adaxial surface; B, 950 m, abaxial surface; C, 1,760 m, adaxial surface; D, 1,760 m, abaxial surface. (Bar=100 μ m).

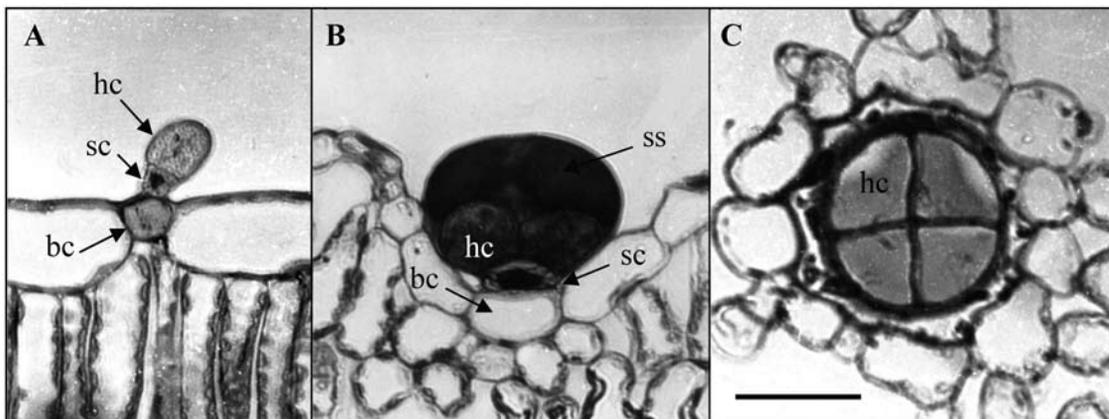


Figure 2. LM micrographs of glandular hairs. A, A capitate hair (leaf cross section) consisting of a basal cell (bc), a stalk cell (sc) and a head cell (hc); B, A peltate hair (leaf cross section) consisting of a basal cell (bc), a stalk cell (sc) and head cells (hc). A subcuticular space (ss) filled with essential oil is over the head; C, A peltate hair (leaf paradermal section) at the level of the head consisting of four cells (hc). (Bar=30 μ m).

of a single basal cell, a stalk cell, and a head cell (Figure 2A) and are present in high densities on both leaf surfaces. There is a trend toward a seasonal increase in their density from spring to autumn on both leaf surfaces, in all populations examined (Table 3). The other type of glandular hairs, the peltate hairs, are anatomically composed of a single large basal cell, a single flattened stalk cell, and a voluminous head of four cells (Figures 2B and C). The essential oil accumulates in a space between the secretory head cells and the detached cuticle (Figure 2B). Peltate hairs are numerous on the abaxial leaf surface, but they can hardly be observed on the adaxial leaf surface (Figure 1; Table 3). Leaves of 950 m have significantly higher peltate hair density in early summer compared to leaves of the higher altitudes (1,480 m and 1,760 m), but later on, in late summer and early autumn, just the opposite holds true (Table 3).

Cross-sections of *N. nuda* leaves showed the typical anatomy of the dicot leaf (Figure 3). At all three altitudes, summer leaves appear to contain phenolics (up to $10.0 \pm 4.4\%$) in their epidermal cells (Figure 3A) while autumn leaves seem devoid of such substances (Figure 3B).

Observations on the chloroplasts of the leaf mesophyll cells disclosed some differences between the three populations (Table 4). The highest relative volume of chloroplasts per cell was noticed in the June leaves of 1,760 m. The most remarkable differences on chloroplasts were found in the relative volume of starch grains within the chloroplast stroma. In all altitudinal populations, the relative volume of starch grains decreases from summer to autumn, when they occupy only a very small portion of the chloroplast stroma (Figure 4; Table 4). On the other hand, values of the relative volume percentages of grana per chloroplast increase from summer to autumn. As concerns the chloroplast plastoglobuli, these were more numerous in the August leaves of 950 m. In all populations, plastoglobuli appear more developed in

August and October. In addition, they seem to negatively correlate with chloroplast grana (Table 4).

DISCUSSION

Plants growing along an altitudinal gradient exhibit differences in their height which generally become

Table 3. Effects of altitude and season on peltate hair density on the adaxial [$D_{ph(ad)}$] and abaxial [$D_{ph(ab)}$] leaf surfaces and on capitate hair density on the adaxial [$D_{ch(ad)}$] and the abaxial [$D_{ch(ab)}$] leaf surfaces.

	Altitude (m)	April	June	August	October
$D_{ph(ad)}$ (No/mm ²)	950	0.4	0.7 a	0.5 a	0.4 a
	1480	*	0.5 ab	0.8 b	0.6 b
	1760	*	0.4 b	0.5 a	0.5 ab
$D_{ph(ab)}$ (No/mm ²)	950	14.0	23.5 a	20.5 a	18.4 a
	1480	*	11.2 b	23.1 ab	25.2 b
	1760	*	10.6 b	27.3 b	22.8 b
$D_{ch(ad)}$ (No/mm ²)	950	22.4	28.6 a	29.6 a	30.5 a
	1480	*	25.0 a	32.5 a	34.2 a
	1760	*	17.0 b	30.5 a	32.4 a
$D_{ch(ab)}$ (No/mm ²)	950	30.0	40.0 a	45.2 a	45.6 a
	1480	*	39.0 a	46.8 a	51.5 ab
	1760	*	36.7 a	50.2 a	54.2 b

n=12. Means of the same column 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.

* Plants have not yet started growing.

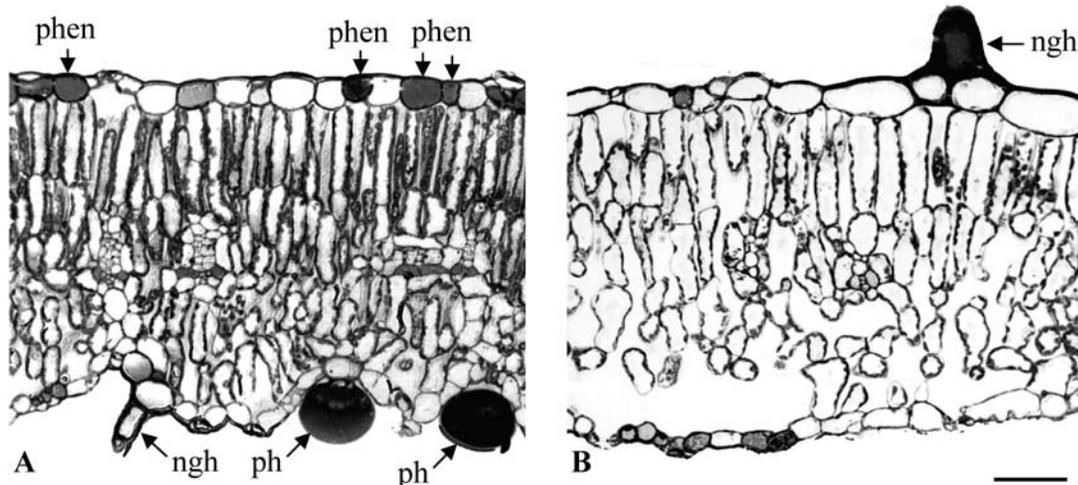


Figure 3. LM micrographs of leaf cross sections showing epidermal cells filled with phenolics (phen). ph= peltate hair, ngh= non-glandular hair. A, 1,760 m, June; B, 1,760 m, October. (Bar=50 μ m).

Table 4. Effects of altitude and season on 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}).

	Altitude (m)	April	June	August	October
RV_{chl}	950	15.0	18.0 a	26.0 a	30.0 a
	1480	*	24.0 a	25.5 a	27.2 a
	1760	*	40.2 b	20.0 a	28.4 a
RV_{gr}	950	22.6	20.0 a	9.0 a	26.2 a
	1480	*	16.6 a	26.7 b	31.2 ab
	1760	*	14.4 a	30.2 b	36.0 b
RV_{sg}	950	17.8	20.3 a	27.5 a	13.0 a
	1480	*	33.7 b	14.4 b	7.2 b
	1760	*	36.5 b	9.5 b	6.5 b
RV_{pg}	950	4.0	4.6 a	18.5 a	5.6 a
	1480	*	2.0 b	5.8 b	6.2 a
	1760	*	2.5 b	6.5 b	8.1 a

n=10. Means of the same column 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.

*Plants have not yet started growing.

expressed by a shortening of their stems at high elevations. Stem shortening allows plants to avoid the damaging effect of the strong winds blowing at high altitudes and to improve photosynthetic conditions by keeping the leaves closer to the warmer soil surface (Körner and Chochrane, 1983). In *Nepeta nuda*, a significant decrease in stem length of the upland plants was observed in all months during the growing period, except for the August stems. A similar reduction in plant height from the lowland to the upland habitats has been also observed in other Labiatae species grown wild on the same mountain (reduction by 45% and 37%, respectively) (Kofidis et al., 2003, 2007). Stem shortening may be due to the fact that high-altitude plants develop more slowly than low-altitude plants (Atkin and Day, 1990), a fact that is more obvious in the temperate and subarctic locations (Körner, 1989).

Because plants have to rapidly complete their growing cycle when their growth period is short, it is expected that leaf phenology may differ between the lowland and upland plants (Kudo, 1995). Indeed, many plants developing along altitudinal gradients have been found to have smaller leaves in their upland habitats (Morecroft and Woodward, 1996; Cordell et al., 1999; Kao and Chang, 2001; Kofidis et al., 2003). In *N. nuda*, no significant differences in leaf size were found as concerns altitude. However, the leaves of the plant were observed to undergo major alterations in relation to season. In all three populations, the leaves had the largest size by the middle of summer (in July). Along the growing season, leaves generally undergo a progressive decrease of their total surface from summer to autumn although the xerothermic conditions of summer are often associated with smaller leaves (Sutcliffe, 1979; Ristic and Cass, 1991).

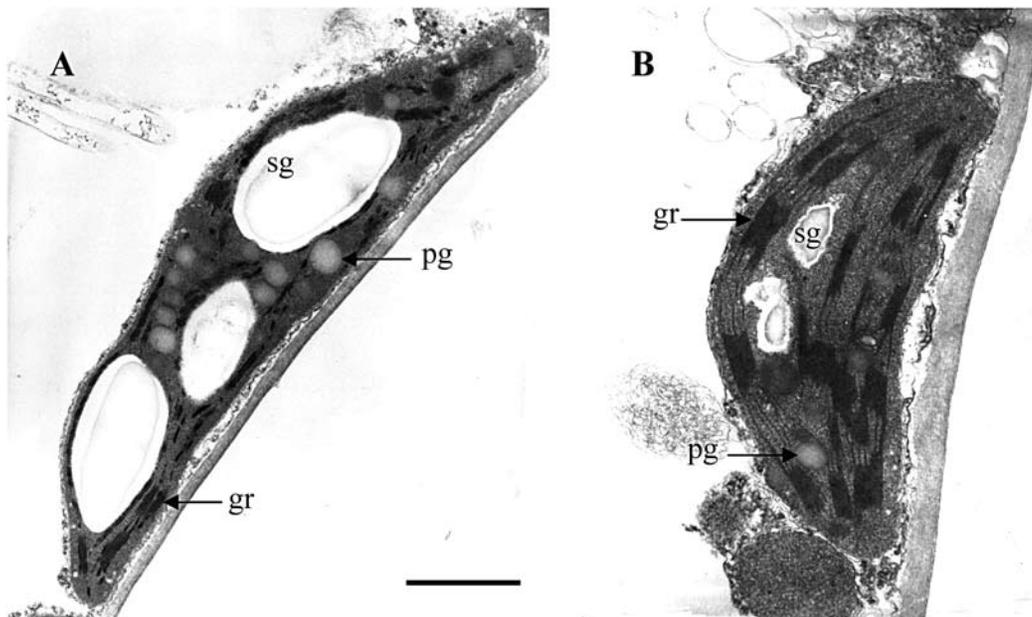


Figure 4. TEM micrographs of chloroplasts of mesophyll cells. A, A large portion of the chloroplast stroma is occupied by starch grains (sg) and plastoglobuli (pg), while grana (gr) possess only a small volume (950 m, August); B, Grana (gr) occupy most of the chloroplast stroma, while starch grains (sg) and plastoglobuli (pg) possess only a small volume (1,760 m, October). (Bar=1 μ m).

In a considerable number of species, upland plants have been found to have smaller leaves, a fact principally attributed to the low air temperature (Woodward, 1983; Cordell et al., 1998; Kao and Chang, 2001). The photosynthetic process itself does not appear to be associated with the reduction of leaf size in the high-altitude plants since these plants have been measured to have photosynthetic rates close to those of the low-altitude plants (Körner and Diemer, 1987).

Leaf thickness in *Nepeta nuda*, does not seem to follow a common pattern in reference to altitude. In general, altitude and leaf thickness show no clear correlation. Thus, in some cases leaves of high-altitude plants were found to be thicker than those of low-altitude plants (Kofidis et al., 2003; Cordell et al., 1998; Codignola et al., 1987) while in some others just the opposite held true (Suzuki, 1998; Morecroft and Woodward, 1996). This fact is undoubtedly associated with genetics and environmental pressure.

Accumulation of phenolics in the summer leaves is a common characteristic of Mediterranean plants (Christodoulakis and Bazos, 1990; Kofidis et al., 2003). At low elevations, where the drought conditions during summer are more stressful, this phenomenon is more pronounced than at high elevations. In *N. nuda* (the plant of our study, occurring at mid and high elevations), phenolic compounds are present only in the epidermal cells of the June and the August leaves. Their presence, except for a defensive role against pests, may also contribute to the protection of the mesophyll chloroplasts and nuclei from the excessive UV-B radiation (Karabourniotis et al., 1998).

Anatomical (LM) and morphological (SEM) studies disclosed that *N. nuda* leaves bear stomata on both of their surfaces. Stomata, which have their guard cells raised in relation to the epidermal level, are more numerous on the abaxial leaf surface. Leaves of 1,480 m exhibited higher stomatal densities for both surfaces than leaves of 950 and 1,760 m. Generally, altitude has been found to be positively correlated to stomatal density, and this beneficial effect of altitude on stomatal density has been also reported for other plants like *Clinopodium vulgare* (Kofidis et al., 2007) *Origanum vulgare* (Kofidis et al., 2003), *Sedum atratum* (Codignola et al., 1987), *Miscanthus* ssp. (Kao and Chang, 2001), *Picea crassifolia* (Qiang et al., 2003) and some C₃ plants of New Zealand (Körner et al., 1986). The probable cause for stomatal increase at high altitudes is the higher solar intensity and not CO₂ concentration, which might significantly fluctuate (Furukawa, 1997; Apel, 1989). On the other hand, the low stomatal density at low altitudes presumably reflects the arid conditions (higher temperature, lower humidity) dominating at the foot of the mountains (Kofidis et al., 2003). In the case of *N. nuda* leaves, it would be expected for plants at 1,760 m to have a higher stomatal density than those at 1,480 m. However, this is not true, probably due to site and microclimate attributes of this elevation (an open site and thus more xeromorphic than the 1,480 m elevation).

Leaves of *N. nuda* were found to possess more numerous non-glandular hairs on their adaxial leaf surfaces regardless of altitude and season. No significant differences in non-glandular hair density were observed between the leaves of the three populations, except for the case of the 950 m population, in which leaves bore fewer non-glandular hairs on their abaxial surface compared to the leaves of the 1,480 and 1,760 m populations. Leaves at higher altitudes have to be more protected from excessive UV-B irradiance, and the flavonoid-containing non-glandular hairs aid this (Karabourniotis et al., 1998). Apart from UV-B protection, the dense leaf cover with non-glandular hairs at higher altitudes may further contribute to protection from low temperatures. Seasonally, the number of non-glandular hairs does not fluctuate during the growing period from spring to autumn. Since non-glandular hair density on leaves is generally considered to be positively correlated with xerothermic conditions, it would be expected that their number would be higher during summer. However, even in summer, climatic conditions at 950 m and along this elevation are not so stressful as those dominating at the foot of the mountain.

Beside non-glandular hairs, leaves of *N. nuda* also bear glandular hairs, which produce the essential oil. Glandular hairs are of two morphologically distinct types, the capitate hairs (smaller) and the peltate hairs (larger). The capitate hairs, are composed of a basal cell, a stalk cell, and a head cell. The peltate hairs, are composed of a single basal cell, a single stalk cell, and a large secretory head of four cells. These peltate hairs are also found in other members of Labiatae, like *Salvia blepharophylla*, *Ocimum basilicum*, *Teucrium chamaedris*, *Teucrium siculum*, *Sideritis syriaca*, and *Pogostemon cablin* (Henderson et al., 1970; Bini-Maleci and Servettaz, 1991; Karousou et al., 1992; Werker et al., 1993; Servetazz et al., 1994; Bisio et al., 1999). Capitate and peltate hairs have also been observed in *Nepeta racemosa*, where they are primarily located on the abaxial leaf surface (Bourett et al., 1994). The same holds true for *N. nuda*, with the capitate and peltate hairs being more numerous on the abaxial leaf surface. However, peltate hairs, which are believed to be responsible for the bulk of the essential oil secreted (Fahn, 1988), can hardly be observed on the adaxial leaf surface. Seasonally, leaves of 950 m have significantly higher peltate hair density in early summer compared to leaves of the higher altitudes (1,480 m and 1,760 m), but later on in summer and early autumn, just the opposite is true. As the vegetation period at 950 m starts early in spring, essential oil secretion, maturation and disintegration of the peltate hairs at this altitude occurs earlier than in the peltate hairs of 1,480 and 1,760 m plants. This is why the essential oil content of plants at 950 m peaks in June and why the content of plants at 1,480 and 1,760 m peaks at the end of summer (data not shown).

The submicroscopic examination of the leaf mesophyll chloroplasts of *N. nuda* disclosed some remarkable results. The chloroplast starch grains decrease in volume from summer to autumn, when they occupy only a small

portion of the chloroplast stroma. The diminishing of the chloroplast starch grains at high altitudes from August, and their disappearance in October, is in accordance with the results of other plant species growing along an altitudinal gradient (Zellnig and Gailhofer, 1989; Miroslavov and Kravkina, 1991). Starch degradation at high elevations reflects an adaptation to the cold conditions associated with increased respiration rates. Upland plants turn their growing cycle within a short period of time, and they thus develop higher respiration rates and possess more mitochondria than their lowland counterparts (Handley and Bliss, 1964). A negative correlation also seems to exist between the relative volumes of grana and plastoglobuli. This is in accordance with the fact that plastoglobuli represent a reservoir for excess amounts of plant lipids that cannot be stored in the thylakoids. The number and size of plastoglobuli increases, particularly in sun-exposed leaves and leaves that regularly receive enough light to perform photosynthesis at good rates (Lichtenthaler, 2007). On the other hand, the end of summer (August) and onset of autumn (September, October) are characterized by a decrease in the chlorophyll (grana):carotenoids (plastoglobuli) ratio.

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海拔高度及氣候因子對直齒荊芥的腺毛及葉部構造特徵之影響

George KOFIDIS and Artemios M. BOSABALIDIS

Department of Botany, School of Biology
Aristotle University, Thessaloniki 54124, Greece

本文研究海拔高度（950 公尺，1,480 公尺及 1,760 公尺）與季節（四月到十月）對直齒荊芥的葉片形態（含腺毛）、解剖及超微結構特徵的影響。在直齒荊芥植物的營養生長期，生長於 1,760 公尺處的植株明顯小於位於較低海拔生長者。以上三個不同海拔，葉的最大面積都在七月，秋季產生的葉子小於夏季者。三個不同海拔的直齒荊芥之葉片厚度在生長季裡有明顯的變動。葉下表面的氣孔密度高於上表面者；就海拔而言，長在 1,480 公尺處的植物葉片的氣孔比長在高於此處及低於此處海拔植物的氣孔數目為多。葉上表面非腺毛的密度較下表面為高（三處不同海拔的族群植物樣本並無顯著差異），然而在整個取樣期間，生長在海拔 950 公尺的植物葉片下表皮被毛少於其他海拔者。直齒荊芥的腺毛有兩種類型：頭狀毛（由一個基部細胞、一個柄細胞及一個頭細胞組成）及盾狀毛（由一個基部細胞、一個柄細胞及四個細胞形成的大頭組成）。由春天到秋天，所有測試族群植物葉部兩面的頭狀毛之密度都有增加的傾向；盾狀毛在葉下表面非常多，但在上表面則不容易見到；位於海拔 950 公尺的植物在初夏時葉面盾狀毛的密度遠高於較高海拔者（1,480 公尺與 1,760 公尺），但時至晚夏初秋則反是。分布在三個不同海拔的植物之夏季葉其表皮細胞中均含有酚類化合物，然而入秋後似乎就都檢測不到這種物質。似此差異亦見於三個不同海拔族群植物葉肉組織的葉綠體：自夏天至秋天，這三個不同海拔的族群植物澱粉粒在葉綠體基質含量少時，澱粉粒的相對容積也隨之下降；但在此時期，每個葉綠體之基粒的相對容積比例則上升。

關鍵詞：海拔高度；葉綠體；腺毛；葉片結構；直齒荊芥；季節。