Leaf structural features of Mediterranean perennial species: plasticity and life form specificity

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We studied leaf structural traits of eight perennial Mediterranean species (Arbutus unedo, Quercus coccifera, Pistacia lentiscus, Myrtus communis, Lavandula stoechas, Cistus incanus, Calamintha nepeta, and Melissa officinalis) co-occurring in the same area and representing different lifeform groups. In summer leaves, we measured the thickness of leaf lamina, cuticles and epidermises of adaxial and abaxial leaf surfaces, as well as of the palisade and spongy parenchymas. We also estimated the specific leaf mass (SLM), the density of stomata, the density of glandular and non-glandular hairs and the relative volumes of the leaf tissues and of the intercellular spaces of the mesophyll. While the evergreen-sclerophyllous species are characterized by thicker leaves, thicker cuticles, and more compact mesophyll, the seasonally dimorphic and the non-woody perennials bear hairs. All species had a high stomatal density on the abaxial leaf surface. Phenolic compounds were only observed in A. unedo, Q. coccifera and P. lentiscus. Well known features of xeromorphic leaves, like the development of the palisade tissue at the expense of the spongy one, were not found in the species examined; in the evergreen-sclerophyllous M. communis, in particular, there was an exceptional development of the spongy parenchyma over the palisade one. SLM, leaf thickness and relative contribution of the two mesophyll components show considerable plasticity enabling species to cope with different environmental regimes. Traits of presumably high discriminant value, like the sclerophylly index (SLM), cannot clearly separate the representatives of the different life-form groups.

Key words: Leaf anatomy, xerophytes, sclerophylly, trichomes, stomata, phenolics.

INTRODUCTION

The mediterranean-type climate is characterized by a marked seasonality, typified by the alternation of a cold and relatively wet period with a hot and dry one (Aschmann, 1973; Nahal, 1981). This seasonality, associated with low temperatures when water is available and high temperatures when water is scarce, is considered to limit plant growth and concomitantly productivity of mediterranean-type ecosystems (Mitrakos, 1980; Specht, 1987). The distribution of the latter is largely determined by the severity of the summer drought: maquis, dominated by evergreensclerophyllous species, occur at the wet end of the precipitation gradient, whereas phrygana (syn. garigue), dominated by seasonally dimorphic (semideciduous) species, occur at the dry end (Margaris, 1981). High temperatures, vapor pressure deficit of the air and high irradiance during summer are additional stressful factors acting in parallel to water stress (Mendes *et al.*, 2001).

The structure of leaves has important implications for the performance of plants in different habitats (Garnier *et al.*, 1999). A number of leaf features exhibit high plasticity (Gutschick, 1999). Taxonomically different species co-occurring in the same habitat often share common morphological and physiological traits. This similarity reflects a convergent evolution in response to common environmental factors affecting them. Many leaf features have been recognized as protective mechanisms that enable

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plants to tolerate different levels of stressful factors, such as drought, high air temperature, UV-B radiation, etc. (Christodoulakis & Bazos, 1990; Karabourniotis *et al.*, 1992; Stephanou & Manetas, 1997).

Sclerophylly is a recurrent plant trait in the mediterranean-climate region; it serves protective functions useful under conditions of drought stress and results from selection for leaf longevity under resource paucity (Turner, 1994; Salleo & Nardini, 2000). Sclerophylly increases in conditions of stress, due to intense radiation, drought or soil infertility (Gutschick, 1999). High leaf mass per unit area, high leaf tissue density or small leaf area are traits of evergreen-sclerophyllous species that improve their resistance to drought (Abril & Hanano, 1998; Castro-DTMez *et al.*, 1998; Gratani & Bombelli, 1999; Werner *et al.*, 1999).

Seasonal dimorphism, on the other hand, is the adaptive strategy of many woody species of phrygana, in response to climatic seasonality (Orshan, 1964; Aronne & De Micco, 2001; Margaris, 1981; Margaris & Vokou, 1982). These plants are characterized by seasonal reduction of their transpiring surface, which they achieve by shedding the larger winter leaves and developing small summer leaves. Transition between the two leaf forms has been found to be induced by photoperiod, although leaf water relations seem to play a major role (Kyparissis & Manetas, 1993; Kyparissis et al., 1997). Other noticeable features of seasonally dimorphic species include greater leaf folding along the midrib (Gratani & Bombelli, 2000), dense leaf pubescence, deeply developed stomata and accumulation of mucilage and other secondary metabolites in the mesophyll (Ludlow, 1989; Save et al., 2000).

There are several studies dealing with leaf morphological traits of Mediterranean species. However, attempts to interpret each feature in a functional way, in terms of response to specific environmental factors, should be carefully assessed (Reich, 1993; Smith *et al.*, 1998).

This study focuses on Mediterranean perennial plant species that grow wild in the same area and are, therefore, subject to common climatic and other pressures. Our aim is (i) to examine whether there are structural leaf traits that are life-form specific or whether species exhibit common traits, irrespective of their life forms, and (ii) given the information available on some woody species from other Mediterranean countries, to examine the level of leaf similarity of geographically distinct populations of the same species.

MATERIALS AND METHODS

Plant material

Leaf samples were collected from wild growing plants in Sithonia peninsula, Halkidiki (northern Greece). The species examined co-occur in the same area and are major components of the vegetation. They are: Arbutus unedo L, Quercus coccifera L, Pistacia lentiscus L., Myrtus communis L., Lavandula stoechas L., Cistus incanus L., Calamintha nepeta (L.) Savi and Melissa officinalis L. The first four are evergreen-sclerophyllous species, commonly found in the Greek maquis. L. stoechas and C. incanus are seasonally dimorphic shrubs; the latter species is commonly found in phrygana but also in maquis, under the canopy of taller shrubs. C. nepeta and M. officinalis are non-woody perennial species; in the studied area, the first occur in open spaces, whereas the latter exclusively along a seasonal stream. M. communis, L. stoechas, C. nepeta and M. officinalis belong also to the group of aromatic plants. Information on the species life forms and habitats is given in Table 1.

In August 2001, mature leaves of these species were sampled from randomly chosen plants (three individuals per species) representing the population; five samples were taken from each individual. Thus, there were fifteen samples per species.

Meteorological data

The meteorological station in the vicinity of our study site operated only during the years 1968-1975. To have an estimate of the climatic variability during the study period, we used data from the meteorological station of Thessaloniki. Exploratory analysis for this eight-year period proved that data from the Thessaloniki station reflect adequately the climatic situation in the study area. Comparison of temperature data from the two stations over the period 1968-1975 showed a very high degree of correlation (R = 0.99, p < 0.001), while rainfall data were also considerably correlated (R = 0.54, p < 0.001). On average, the two stations did not differ in temperature, but the Thessaloniki station was slightly wetter.

Light microscopy and morphometry

Immediately after collection, leaves were cut into small pieces and subsequently fixed with 5% glu-

Species	Family	Life form ¹	Habitat ²
Arbutus unedo	Ericaceae	Phanerophyte	Wood margin, scrub, rocky slopes
Quercus coccifera	Fagaceae	Phanerophyte caespitose	Dry places, on limestone and siliceous soils
Pistacia lentiscus	Anacardiaceae	Phanerophyte scapose	Dry open woods and scrub on dry hills
Myrtus communis	Myrtaceae	Phanerophyte caespitose	Scrub, usually calcifuge
Lavandula stoechas	Lamiaceae	Nanophanerophyte	Dry, usually sunny places
Cistus incanus	Cistaceae	Nanophanerophyte	Dry scrub or open woodland
Calamintha nepeta	Lamiaceae	Hemicryptophyte scapose	Dry river bed bank, phrygana, grassland, scree, open areas
Melissa officinalis	Lamiaceae	Hemicryptophyte scapose	Forest clearings, scrub, macchie, rocky slopes and crevices, streamsides

 TABLE 1. Plant species, life forms and habitats

¹ Pignatti (1982).

² Tutin et al. (1964), Tutin et al. (1968, 1972); Polunin & Huxley (1981); Davis (1982).

taraldehyde in 0.05 M phosphate buffer (pH 7.2) for 3 h. After rinsing in buffer, the specimens were postfixed for 4 h with 1% OsO_4 (similarly buffered). The temperature in all solutions was kept at 0 °C to avoid leaching of phenolics during fixation. Specimens were dehydrated in graded ethanol series (50-100%), then treated with propylene oxide, and finally infiltrated and embedded in Spurr's resin (Spurr, 1969). Semi-thin sections (1 µm thick) were obtained with a Reichert OM U₂ ultramicrotome; they were stained with 1% toluidine blue O in 1% borax and photographed using a Zeiss III photomicroscope.

For each species, the total leaf thickness and the thicknesses of the leaf histological components, viz. of the adaxial and abaxial cuticles, the adaxial and abaxial epidermises, and the palisade and spongy parenchymas were measured from 15 light micrographs of leaf cross-sections (\times 800). The relative volumes of the leaf tissues and of the leaf intercellular spaces were further estimated by overlaying the micrographs with a transparent sheet bearing a square lattice of point arrays, 10 mm apart, and applying the point-counting analysis technique (Steer, 1981). The density of stomata was assessed using micrographs of paradermal leaf sections.

Specific leaf mass (SLM) was estimated according to Reich *et al.* (1992) as the ratio of leaf dry mass to leaf area. Dry leaf mass was determined by oven drying the leaves at 70°C for 72 h (Bussotti *et al.*, 2002), and leaf area by using a leaf area meter (Eijkelkamp, Agrisearch equipment, Netherlands).

Measurement of glandular and non-glandular hairs

To determine the density of glandular and non-glan-

dular hairs, stereoscopic photographs of the adaxial and abaxial leaf surfaces were used. The number of hairs was determined over a defined area on the photograph. The final density was computed according to the magnification of the photograph and was expressed as number of hairs per square millimeter. In the case of the very pubescent *L. stoechas*, the density of non-glandular hairs was estimated by counting them over a fixed area under the stereoscope.

Statistical analyses

All statistical tests were performed using the statistical software package SPSS for Windows (11.5.1, SPSS Inc., USA). Differences in means of anatomical and morphological leaf variables were assessed with analysis of variance (ANOVA) and Tukey's B test for multiple comparisons. Test assumptions were checked and data were transformed, when necessary. Correlation analysis was used to examine relationships among leaf variables. Principal component analysis (PCA) was further applied. For both correlation and principal component analyses, the variable values for each species were the averages per individual. Thus, for every variable, there were three data points (each being the average of five) per species. Since all species did not bear hairs on their leaves, in PCA, the value 1 was assigned to species having no hairs and the value 2 to those bearing them.

RESULTS

The climatic variability, during the period 2000-2001, is shown in Fig. 1. The climate is typical mediterranean. The spring 2001, before our sampling (Au-



FIG. 1. Mean monthly air temperature (dotted line) and total monthly rainfall (solid line) during the period 2000-2001; data from the meteorological station of Thessaloniki.

gust), was quite wet. Rainfall during March, April, and May 2001 not only exceeded the respective value in 2000, but also the respective average for the eight-year period (1968-1975), during which both the Thessaloniki and Halkidiki meteorological stations were in operation.

All leaf variables examined differ significantly among species (Table 2, Figs 2-9). *Q. coccifera* has the thickest leaves (310.4 μ m), whereas *M. officinalis* the thinnest ones (99.7 μ m). The average leaf thickness of the four evergreen-sclerophyllous species ranges between 229 μ m in *M. communis* and 310.4 μ m in *Q. coccifera*; it never attains such high values in the other species. The cuticle and the epidermis thicknesses differ significantly (p < 0.05) between the adaxial and abaxial leaf surfaces, in all species; both cuticle and epidermis are thicker in the adaxial leaf surface.

Among the evergreen-sclerophyllous species, cuticle thickness of either the adaxial or the abaxial leaf surface is highest in *A. unedo* and lowest in *P. lentis*cus and *M. communis* (Table 2). Both adaxial and abaxial epidermal layers are also thickest in *A. unedo* and thinnest in *M. communis*. Between the two seasonally dimorphic species, thicker are the leaves of *L. stoechas*; this holds true for all leaf tissues exam-

TABLE 2. Average thickness (μ m) of leaf tissues in cross-sections. AdC (adaxial cuticle), AdE (adaxial epidermis), PP (palisade parenchyma), SP (spongy parenchyma), AbE (abaxial epidermis), AbC (abaxial cuticle), LT (total leaf thickness). Different letters in the same column indicate significant differences among species at p < 0.05 (Tukey's B test of homogeneity, $n = 15, \pm SE$)

Species	AdC^1	AdE ²	\mathbf{PP}^1	SP	AbE ²	AbC ²	LT
Arbutus unedo	8.9 ± 0.2 a	24.5 ± 1.2 b	74.6 ± 2.1 b	$131.2 \pm 3.4c$	17.5 ± 1.0 a	6.2 ± 0.1 a	263.1 ± 4.2 c
Quercus coccifera	6.4 ± 0.4 b	11.8 ± 0.2 e	104.6 ± 4.3 a	173.9 ± 5.4 a	9.4 ± 0.6 c	4.3 ± 0.1 b	310.4 ± 6.2 a
Pistacia lentiscus	4.2 ± 0.2 c	16.8 ± 0.3 cd	95.6 ± 4.1 a	147.3 ± 3.9 b	13.5 ± 0.6 b	$3.0 \pm 0.3c$	280.5 ± 3.9b
Myrtus communis	$4.5 \pm 0.3c$	$7.2 \pm 0.4 f$	41.9 ± 1.0 de	165.8 ± 1.6 a	6.1 ± 0.3 d	$3.5 \pm 0.3c$	229.0 ± 1.3d
Lavandula stoechas	$2.7 \pm 0.1 c$	33.8 ± 1.7 a	67.3 ± 3.3 bc	82.7 ± 4.5 d	17.6 ± 1.2 a	$1.3 \pm 0.1 e$	$205.6 \pm 6.4 e$
Cistus incanus	1.2 ± 0.1 d	20.9 ± 1.1 bc	45.3 ± 1.4 d	$45.1 \pm 2.3 f$	11.9 ± 0.3 b	0.5 ± 0.0 g	124.9 ± 2.1g
Calamintha nepeta	$4.4 \pm 0.1c$	16.9 ± 0.7 cd	58.7 ± 3.3 c	70.1 ± 2.8 e	12.5 ± 0.9 b	2.3 ± 0.3 d	$164.9 \pm 3.7 f$
Melissa officinalis	$1.8 \pm 0.1 \mathbf{d}$	16.9 ± 1.2 d	36.9 ± 1.2 e	33.7 ± 1.0 f	9.6 ± 0.5 c	$0.8 \pm 0.1 \text{ef}$	99.7 ± 1.8 h

¹Transformed in $\log_{10} (x + 1)$

² Transformed in $\log_{10}(x)$

ined (Figs 6, 9; Table 2). Between the two non-woody perennials, *C. nepeta* exceeds *M. officinalis* in the thickness of the leaf and in the thickness of all indi-

vidual tissues, except for that of the adaxial epidermis (16.9 μ m in both) (Figs 7-8).

The relative volumes (%) of the leaf tissues ex-



FIGS 2-9. Light microscope view of leaf cross-sections (× 170). 2 = A. unedo, 3 = Q. coccifera, 4 = P. lentiscus, 5 = M. communis, 6 = L. stoechas, 7 = C. nepeta, 8 = M. officinalis; 9 = C. incanus. Note the absence of hairs in the first four (2-5) species.

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Species	AdC	AdE	РР	SP	AbE	AbC
Arbutus unedo	4.8 ± 0.4	7.6 ± 0.4	31.0 ± 1.0	47.8 ± 1.4	5.9 ± 0.3	2.9 ± 0.3
Quercus coccifera	2.9 ± 0.1	4.1 ± 0.2	32.6 ± 0.7	54.8 ± 0.9	3.4 ± 0.3	2.2 ± 0.2
Pistacia lentiscus	2.4 ± 0.2	5.5 ± 0.3	31.9 ± 0.7	54.6 ± 0.9	3.9 ± 0.3	1.7 ± 0.2
Myrtus communis	2.1 ± 0.2	3.3 ± 0.3	18.6 ± 0.6	71.8 ± 0.6	2.7 ± 0.1	1.5 ± 0.3
Lavandula stoechas	2.9 ± 0.5	12.6 ± 0.4	36.4 ± 1.2	40.5 ± 1.1	6.1 ± 0.5	1.5 ± 0.3
Cistus incanus	3.3 ± 0.4	15.3 ± 0.8	28.1 ± 1.2	43.1 ± 1.1	8.1 ± 0.5	2.1 ± 0.3
Calamintha nepeta	3.9 ± 0.3	7.7 ± 0.3	36.7 ± 0.5	44.2 ± 0.6	4.8 ± 0.4	2.7 ± 0.1
Melissa officinalis	4.5 ± 0.7	14.1 ± 0.6	35.3 ± 0.6	34.4 ± 0.7	9.3 ± 0.7	2.4 ± 0.4

TABLE 3. Relative volume (%) of leaf adaxial cuticle (AdC), adaxial epidermis (AdE), palisade parenchyma (PP), spongy parenchyma (SP), abaxial epidermis (AbE), and abaxial cuticle (AbC) ($n = 15, \pm SE$)

amined do not show marked differences among species (Table 3). *M. communis* is the species with the lowest relative volume of the palisade parenchyma (18.6%) and the highest of the spongy parenchyma (71.8%). In *M. officinalis*, the palisade and spongy tissues have very similar relative volumes. The maximum values of cuticle relative volume are recorded in two unrelated species, *A. unedo* and *M. officinalis* (Table 3).

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In the evergreen-sclerophyllous species, the palisade parenchyma is composed of cell layers varying in number: one in *M. communis*, two in *A. unedo* and *P. lentiscus* and two to three in *Q. coccifera* (Figs 2-5). The relative proportion (%) of intercellular spaces in the palisade parenchyma is lowest in *M. communis* (9.2%) and highest in *P. lentiscus* (12.4%). Regarding the spongy parenchyma, it is most compact in *Q. coccifera* (Table 4, Fig. 3). Specific leaf mass (SLM) varies significantly among species (p < 0.001); it is highest in *Q. coccifera* (15.57 mg cm⁻²) and lowest in *M.* communis $(7.67 \text{ mg cm}^{-2})$ (Table 4).

Regarding the seasonally dimorphic species, *C. incanus* has one cell layer of palisade parenchyma and *L. stoechas* has two. In *C. incanus*, the relative volume of intercellular spaces in the palisade parenchyma is higher than that in *L. stoechas*, while the opposite holds true for the spongy parenchyma. *C. incanus* has also higher specific leaf mass (SLM) (Table 4).

The two non-woody perennial species, *C. nepeta* and *M. officinalis*, have only one cell layer of palisade parenchyma. They are very similar in compactness of both the palisade and the spongy parenchyma, but they differ in the specific leaf mass, which is higher in *C. nepeta* (Table 4).

A. unedo (Figs 2, 10,18) and *Q. coccifera* (Figs 11, 19) contain phenolic substances, seen as dark spots, in both the adaxial and abaxial epidermal cells. In *P. lentiscus*, these compounds are present only in the mesophyll cells of palisade and spongy parenchymas

TABLE 4. Relative volume (%) of intercellular spaces (palisade and spongy parenchymas) and specific leaf mass (\pm SE, n = 15)

Species	Intercell	SLM	
	Palisade parenchyma	Spongy parenchyma	
Arbutus unedo	11.1 ± 1.0	37.5 ± 0.7	10.32 ± 0.02
Quercus coccifera	10.9 ± 0.8	26.9 ± 1.9	15.57 ± 0.08
Pistacia lentiscus	12.4 ± 0.8	32.5 ± 0.9	13.63 ± 0.03
Myrtus communis	9.2 ± 1.4	36.0 ± 0.6	7.67 ± 0.01
Lavandula stoechas	17.5 ± 1.1	37.6 ± 1.8	11.53 ± 0.00
Cistus incanus	26.0 ± 2.1	33.5 ± 2.0	13.32 ± 0.15
Calamintha nepeta	14.8 ± 1.1	27.4 ± 1.6	9.21 ± 0.00
Melissa officinalis	15.6 ± 2.1	28.8 ± 2.1	6.11 ± 0.05

¹ Volume of intercellular spaces in palisade and spongy parenchyma tissues were estimated relative to their respective tissues.

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FIGS 10-17. Light microscope view of leaf paradermal sections (adaxial leaf surface) (× 170). 10 = A. unedo, 11 = Q. coccifera, 12 = P. lentiscus, 13 = M. communis, 14 = L. stoechas, 15 = C. nepeta, 16 = M. officinalis; 17 = C. incanus. Stomata are not detectable on the adaxial leaf surface of any of the species.



FIGS 18-25. Light microscope view of leaf paradermal sections (abaxial leaf surface) (× 170). 18 = A. unedo, 19 = Q. coccifera, 20 = P. lentiscus, 21 = M. communis, 22 = L. stoechas, 23 = C. nepeta, 24 = M. officinalis; 25 = C. incanus. Stomata are clearly visible.

(Fig. 4). Phenolic compounds were not detected in any of the other species.

In all species studied, stomata are present only on the abaxial leaf surface (Figs 10-25). Some stomata are present on the adaxial surface of *L. stoechas, C. incanus, C. nepeta,* and *M. officinalis,* but their extremely low number makes density calculation impractical. The highest stomatal density over all species is recorded in *L. stoechas* (533 per mm²) (Table 5).

The evergreen-sclerophyllous species that we examined seem to be devoid of hairs (Table 5, Figs 2-5), whereas the phryganic and non-woody species bear both non-glandular and glandular hairs (in *C. incanus*, we were unable to discern glandular hairs) (Figs 6-9). Over all hair-bearing species and for either leaf surface, the density of both types of hairs, glandular or not, is highest in *L. stoechas*. The density of both glandular and non-glandular hairs is higher on the abaxial leaf surface of all species, except for *M. officinalis*, where the density of non-glandular hairs does not differ between the two surfaces (Table 5).

Correlation analysis revealed significant relationships between the leaf traits examined. Specific leaf mass (SLM) is positively related to thickness of the palisade parenchyma, and total leaf thickness. Adaxial cuticle, abaxial cuticle, palisade parenchyma, spongy parenchyma and total leaf thickness are all positively correlated. Adaxial and abaxial cuticle thicknesses are positively correlated with total leaf thickness. Adaxial and abaxial epidermis thicknesses are also positively correlated. No significant relationship was found between the epidermal and cuticle layers (Table 6).

Results of the principle component analysis of

Species	Stomata		Hairs				
			Non-g	landular	Glandular		
	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface	
Arbutus unedo	0	459.2 ± 17.3 bc	0	0	0	0	
Quercus coccifera	0	409.3 ± 22.8 bcd	0	0	0	0	
Pistacia lentiscus	0	244.9 ± 6.2 f	0	0	0	0	
Myrtus communis	0	394.7 ± 15.2 cd	0	0	0	0	
Lavandula stoechas	nd	532.8 ± 21.9 a	150.2 ± 4.3	$247.6 \pm 6.6^{***}$	14.3 ± 0.5	$17.9 \pm 1.0^{**}$	
Cistus incanus	nd	317.4 ± 12.9 e	7.2 ± 0.3	$8.7 \pm 0.3^{**}$	nd	nd	
Calamintha nepeta	nd	465.8 ± 12.7 b	6.49 ± 0.3	$21.0 \pm 0.8^{***}$	4.2 ± 0.3	$12.9 \pm 1.2^{***}$	
Melissa officinalis	nd	369.6 ± 21.1 de	2.5 ± 0.2	2.3 ± 0.2	nd	4.1 ± 0.2	

TABLE 5. Density (No. mm⁻² \pm SE) of stomata and hairs on the adaxial and abaxial leaf surfaces (n = 15)

Different letters in the same column show significant differences among species at p < 0.05. Asterisks (associated with abaxial surface) indicate significant differences between adaxial and abaxial leaf surfaces at p < 0.01 (**) and p < 0.001 (***); nd = non-detectable.

TABLE 6. Pearson correlation matrix of leaf traits (thickness of different leaf tissues and SLM). Asterisks indicate significance at p < 0.05 (*), p < 0.01 (**) and p < 0.001 (***)

	Adaxial cuticle	Adaxial epidermis	Palisade parenchyma	Spongy parenchyma	Abaxial epidermis	Abaxial cuticle	Leaf
Adaxial epidermis	0.09						
Palisade parenchyma	0.50*	0.03					
Spongy parenchyma	0.59**	-0.43*	0.63**				
Abaxial epidermis	0.38	0.86***	0.28	-0.19			
Abaxial cuticle	0.95***	-0.14	0.54**	0.71***	0.17		
Leaf	0.69***	-0.15	0.85***	0.93***	0.12	0.76***	
Specific leaf mass	0.16	0.12	0.77***	0.38	0.24	0.19	0.57**



FIG. 26. Component loadings of the first two principal components of PCA of leaf anatomical and morphological traits. The first axis accounts for 49.6% of the total variance and the second for 24.0%. Variable abbreviations are in Table 2. SLM = specific leaf mass, ST = stomatal density, GH = density of glandular hairs, NGH = density of non-glandular hairs.

the leaf traits examined are shown in Figures 26 and 27 (for traits and species, respectively); the first two components explain 73.7% of the total variance. Leaves with low leaf specific mass tend to have thin laminas and cuticles, thin palisade and spongy parenchymas but higher densities of glandular and non-glandular hairs (Fig. 26). Species are clearly separated along the first axis, which explains 50% of the total variance (Fig. 27). All evergreen-sclerophyllous species (*A. unedo, Q. coccifera, P. lentiscus, M. communis*), located on the right side of the graph, are clearly separated from the phryganic and non-woody species (*L. stoechas, C. nepeta, M. officinalis, C. incanus*), found on the left. The traits responsible for



FIG. 27. Projection of the eight Mediterranean species on the first two axes of PCA of leaf anatomical and morphological traits. The first and second axes account for 49.6 and 24.0% of the total variance, respectively.

this separation are the absence of hairs and the distinctly thicker lamina, cuticles, palisade and spongy parenchymas. Along the second axis, which accounts for 24% of the total variance, *A. unedo* is separated from the other three evergreen-sclerophyllous species. Separation of the species of the other mixed group is not consistent with their habit: each of the two subgroups that are formed contains one representative of the seasonally dimorphic and one of the non-woody species. The traits responsible for the separation along the second axis are the thickness of the adaxial and abaxial epidermises, and the density of stomata.

DISCUSSION

Results regarding the leaf traits of the species that we studied are not always similar to those reported for the same species but from other countries. Gratani & Ghia (2002a) have reported a value of 395 um for the leaf thickness of A. unedo from Italy, which is 1.5 times higher than our observation. Despite this, the relative thicknesses of the leaf tissues examined are remarkably similar, except for those of the palisade and spongy parenchymas; these authors have found the palisade parenchyma to have a higher relative thickness than the spongy parenchyma, whereas the opposite holds true in our study. This explains why SLM values (Gratani & Ghia, 2002b) also differ; the lower value that we found is primarily due to the fact that the palisade parenchyma, the tissue that contributes most to leaf compactness, accounts for only 31% of the total leaf volume.

Q. coccifera leaf thickness is strikingly similar to

that of Q. ilex, from Italy (Gratani & Bombelli, 2000; 2001). The relative thicknesses of the leaf tissues are also similar, except for those of the two components of the mesophyll. As is the case of A. unedo, the palisade parenchyma of Q. coccifera has a lower relative thickness than the spongy parenchyma, whereas the opposite was found in Q. ilex. SLM also differs; it is higher in Q. ilex. Rather than be species-specific, these differences seem to reflect responses to different environmental factors, such as light intensity (Dengler, 1980) or water availability. It was found that Q. ilex plants from a xeric site differ from those from a mesic site in having significantly thicker epidermis, thicker palisade parenchyma, and concomitantly thicker leaf lamina, but thinner abaxial epidermis (Bussotti et al., 2002).

Differences in leaf traits were also observed between *P. lentiscus* plants from Greece and from Italy: leaf thickness and SLM values were lower than those reported for the species from Italy by Gratani & Bombelli (2001). These authors do not provide further information for the species as to the relative contribution of each leaf tissue. Nevertheless, these differences are probably due to a thicker and more compact palisade parenchyma, as is the case for the other species (*A. unedo* and *Q. ilex*) that the authors have studied and for which they give detailed information.

With respect to leaf morphological and anatomical features, M. communis diverges considerably from the other three evergreen-sclerophyllous species. In particular, the relative volume of its palisade parenchyma is very low (18.6%, vs 31.0-32.6% for the other three species), whereas that of the spongy parenchyma is very high (71.8% vs 47.8-55.8% for the other three species), and concomitantly SLM is low. In a study, in Portugal, in which M. communis plants growing under different light regimes were compared (Mendes et al., 2001), it was found that the palisade parenchyma in shade plants accounted for 18.6% and the spongy parenchyma for 72.4% of the leaf thickness. Both these values are strikingly similar to ours. Plants growing under the sun developed thicker leaves with comparatively thicker both the palisade and the spongy parenchymas. Nevertheless, the contribution of the palisade parenchyma was consistently smaller than that of the spongy parenchyma, as was the case for the shade plants.

Among all species, *L. stoechas* has the largest adaxial epidermal cells. Like *L. stoechas*, *C. incanus* has large adaxial epidermal cells, but its palisade parenchyma is less compact and its SLM is higher. In fact, SLM values of C. incanus are comparable to those of the evergreen-sclerophyllous species, A. unedo, Q. coccifera and P. lentiscus. Comparison of our results to those from studies of the same species in Italy show that the SLM values are quite similar $(13.3 \text{ mg cm}^{-2}, \text{ in our study}, 14.3 \text{ mg cm}^{-2}, \text{ in that by})$ Gratani & Bombelli, 2001), despite the fact that the contribution of the palisade and spongy parenchymas differ considerably; we found higher relative contribution of the spongy over the palisade parenchyma, whereas the opposite holds true for the plants from Italy (Gratani & Bombelli 2001). Regarding stomatal distribution and hairiness, our results agree with those by Aronne & De Micco (2001) for the same species in Italy; stomata are only found on the abaxial leaf surface, which in addition is more densely covered by hairs.

The two hemicryptophytes, *C. nepeta* and *M. officinalis*, both representatives of Lamiaceae, usually occur in shady places. *M. officinalis*, which is found only along a stream, has the thinnest leaves among all species examined. Low SLM values and large intercellular spaces in their palisade parenchyma characterize these two species, suggesting that they do not experience water stress.

Stomata regulate the gaseous exchanges between plants and their environment. All species examined have high stomatal density on the abaxial leaf surface and very low density or absence of stomata on the adaxial one. This stomatal distribution and density, also reported for other Mediterranean species like *Olea europaea*, leads to reduced evapotranspiration (Bosabalidis & Kofidis, 2002). The highest density of stomata recorded in *L. stoechas* is accompanied by a well-developed pubescence. These two features combined, ensuring enhanced CO_2 uptake (Mendes *et al.*, 2001) and drought tolerance (Save *et al.*, 2000), allow the species to prosper in open, sunny places.

Presence of phenolics has been reported in the summer leaves of *Phlomis, Thymus, Ballota, Anthyllis, Sarcopoterium* and *Origanum*, all seasonally dimorphic species (Christodoulakis, 1989; Christodoulakis & Bazos, 1990; Christodoulakis *et al.*, 1990; Kofidis, *et al.*, 2003). We did not observe phenolic compounds in the tissues of the summer leaves of the two seasonally dimorphic species, *C. incanus* and *L. stoechas*. Instead, we found them in the epidermal and mesophyll tissues of *A. unedo, Q. coccifera* and *P. lentiscus* leaves, in fair amounts.

Principal component analysis of the morphologi-

cal and anatomical leaf traits clearly separated the group of evergreen-sclerophyll from the other species. While the evergreen-sclerophylls have, in general, thicker leaves with thicker cuticles and mesophyll, and in particular, more compact palisade parenchyma, as well as higher SLM, the seasonallydimorphic and non-woody perennials are densely covered by hairs, either both glandular and non-glandular or only non-glandular.

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Cowling & Campbell (1983) have proposed to consider the specific leaf mass (SLM) value of 7.0 mg cm⁻² as the borderline between malacophylls and sclerophylls. On the basis of this value, all species that we examined, despite their different life form, can be considered sclerophyllous, except for M. offic*inalis. M. communis*, with a value of 7.7 mg cm^{-2} is very close to the borderline. A. unedo, which has been reported as being at the borderline between semi-deciduous to drought and sclerophyllous species (Gratani & Ghia, 2002a, b), with an SLM value of 10.3 mg cm⁻², is clearly a sclerophyllous. We must also note that while PCA clearly separated A. unedo from the other evergreen-sclerophyllous species, it did not place it closer to the semi-deciduous ones. We can argue, therefore, that leaf traits of presumably high discriminant value, like the sclerophylly index (SLM), cannot clearly separate the representatives of the different life-form groups.

Various leaf traits have been associated with specific roles in response to environmental pressures. Leaf pubescence has been found to reduce transpiration, maintain favorable leaf temperature, and protect against photosynthetic inhibition by UV-B radiation (Save et al., 2000). High SLM is associated with reduced water loss (Specht, 1988) and thick cuticles with reduced cuticular transpiration (Bussotti et al., 2002). Phenolic compounds are suggested to protect cells from structural damages (Tevini, 1994) by absorbing UV-B radiation (Karabourniotis et al., 1992), and high contribution of the palisade parenchyma to be favoured under high irradiance (Dengler, 1980). Also, trait assemblages have been associated with specific plant groups. Thick walls and cuticles, high density of stomata, development of the palisade tissue at the expense of the spongy tissue, and hairs (Fahn, 1982; Bolhàr-Nordenkampf & Draxler, 1993) have been associated with xeromorphic leaves. But neither all these traits appeared en bloc in the species studied nor were they confined, as individual traits, in the woody species. As Fahn (1982) stated, xeromorphism is not confined to xerophytes, while not all xerophytes exhibit xeromorphic characters. Regarding the latter, the exceptionally high development of spongy over the palisade parenchyma in *M. communis*, is a notable example.

Almost all woody species that we studied seem not to posses one of the prominent xeromorphic features, the preponderance of the palisade over the spongy parenchyma. This is most accentuated in M. communis. In other studies of similar-to-ours species, this preponderance was detected. But in some cases, it disappeared when plants from less xeric sites were examined (Bussotti et al., 2002). We could, therefore, argue that the representatives of A. unedo, M. communis and C. incanus that we studied experience less arid conditions than their counterparts in Italy or Portugal (Gratani & Ghia, 2002a; Mendes et al., 2001; Gratani & Bombelli, 2001). However, this is not clearly substantiated by the climatic character of our study area. Comparison of the climatic data from Greece with those from the other two Mediterranean countries failed to prove that the Halkidiki site is considerably less arid than the respective study sites in the other countries. If we accept that the climatic data provided reflect the actual climatic conditions in the study sites, we have to admit that the reasons responsible for such leaf structural differences are more complicated than it is believed. Nevertheless, the high plasticity in the structure of the mesophyll in concert with other traits seems to enable these species to cope with different environmental regimes and therefore attain a wider habitat range.

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