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EFFECTS OF MICROHABITAT ON LEAF TRAITS IN *DIGITALIS GRANDIFLORA* L. (VERONICACEAE) GROWING AT FOREST EDGE AND INTERIOR

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Abstract – The morphological, anatomical and biochemical traits of the leaves of yellow foxglove (*Digitalis grandiflora* Mill.) from two microhabitats, forest interior (full shade under oak canopy) and forest edge (half shade near shrubs), were studied. The microhabitats differed in the mean levels of available light, but did not differ in soil moisture. The mean level of light in the forest edge microhabitat was significantly higher than in the forest interior. Multivariate ANOVA was used to test the effects of microhabitat. Comparison of the available light with soil moisture revealed that both factors significantly influenced the morphological and anatomical variables of *D. grandiflora*. Leaf area, mass, leaf mass per area (LMA), surface area per unit dry mass (SLA), density and thickness varied greatly between leaves exposed to different light regimes. Leaves that developed in the shade were larger and thinner and had a greater SLA than those that developed in the half shade. In contrast, at higher light irradiances, at the forest edge, leaves tended to be thicker, with higher LMA and density. Stomatal density was higher in the half-shade leaves than in the full-shade ones. LMA was correlated with leaf area and mass and to a lesser extent with thickness and density in the forest edge microsite. The considerable variations in leaf density and thickness recorded here confirm the very high variation in cell size and amounts of structural tissue within species. The leaf plasticity index (PI) was the highest for the morphological leaf traits as compared to the anatomical and biochemical ones. The nitrogen content was higher in the "half-shade leaves" than in the "shade leaves". Denser leaves corresponded to lower nitrogen (N) contents. The leaves of plants from the forest edge had more potassium (K) than leaves of plants from the forest interior on an area basis but not on a dry mass basis; the reverse was true for phosphorus.

Key words: Light reaching, microhabitat, morpho-anatomical leaf traits, plasticity index; soil moisture

INTRODUCTION

Plants exhibit a capacity to modify their phenotypes, so-called phenotypic plasticity, depending on the environment in which they grow (Weiner, 2004). The phenotypic plasticity of plant structure is an important aspect of adaptation, enabling adaptation to heterogeneous environments (Crick and Grime, 1987; Via et al., 1995; Guo et al., 2007). Certain theories have predicted that greater levels of environmental heterogeneity should select for higher magnitudes of phenotypic plasticity (Bradshaw and Hardwick, 1989; Alpert and Simms, 2002). The morphological traits of plants are affected by both abiotic (e.g., light and shade, temperature, pH, water and nutrients availability) and biotic factors (e.g., pathogens, parasites, *predators,* pollinators, *competitors)* (Chen et al., 1992; Schlichting, 2002; Schlichting and Smith, 2002).

Morphological plasticity plays an important role in resource acquisition by plants. The knowledge of

plasticity could provide useful biological information for the conservation of rare and very rare native species, endangered or protected species (Noel et al., 2007; Kostrakiewicz, 2009; Stachurska-Swakoń and Kuż, 2011). Many studies have examined phenotypic plasticity as an individual model of plant adaptation to the environment (Platenkamp, 1990; Sultan and Bazzaz, 1993; Oyama, 1994; Via et al., 1995; Galloway, 1995; Ackerly et al., 2000; Donohue et al., 2001).

The forest edge and forest interior are commonly considered as typically contrastive habitats. The magnitude of microhabitat factors generally decreases from forest boundaries into the interior. Due to increased solar radiation and wind, forest edges often have higher and more variable air and soil temperatures, higher evapotranspiration rates and higher vapor pressure deficits compared to forest interiors (Brothers and Spingarn, 1992; Matlack, 1993; Young and Mitchell, 1994; Murcia, 1995; Chen et al., 1995; Didham and Lawton, 1999; Gehlhausen et al., 2000; Herbst et al., 2007). Because of the higher evapotranspiration rates, soil and litter moisture content is lower at the forest edge than in the interior (Didham and Lawton, 1999; but cf. Riutta et al., 2012). Relative to the interior, the forest edge tends to show a high density of saplings, greater shrub cover, the production of adventitious limbs by canopy trees and invasion by species more typical of open habitats (see Matlack, 1994). The forest edge is a zone of transition between the wide climatic fluctuation of a canopy opening, and the relatively stable environment of the undisturbed forest (Collins and Pickett, 1987). Changes in microclimate at the forest edges, for example, may favor a plant community that is different from the one found in the forest interior (Farris, 1984; Noss and Cooperrider, 1994; Gehlhausen et al., 2000).

I examined leaf structure variation in *D. grandiflora* L. plants that grew in two microhabitats with contrasting light regimes: in the forest interior (full shade under oak canopy), and at the edge of an oak forest (half shade). I hypothesized that i) microclimatic differences at the forest edge and interior will cause differences in the leaf structure and biochemistry; and that ii) differences between leaf structure and biochemistry at the forest edge and interior are mainly driven by light limitation and soil moisture in the forest interior. The primary aim was to quantify the microclimate of forest edges and forest interior, and to assess the effects of the available light reaching and soil moisture on the morphological, anatomical and biochemical parameters of leaves of *D. grandiflora*.

MATERIALS AND METHODS

Study species

Digitalis grandiflora, commonly known as the "yellow foxglove", is a member of the Veronicaceae (Olmstead et al., 2001). It is an herbaceous biennial or perennial plant with glossy green, veined leaves, whose flowering stem can reach a heights ranging from 30-100 cm. Its leaves (70-250×20-60 mm) are ovate-lanceolate, finely serrated, usually glabrous and shining green above and sparsely pubescent underneath (Heywood 1972a,b). Flowers are zygomorphic and arranged in one-sided racemes. The mean number of flowers per inflorescence in the Polish material is 35 (Kołodziejek, unpublished data). The calyx is five-lobed and shorter than the corolla. The corolla (40-50 mm) is ochre-yellow, dark veined underneath, with a cylindrical-tubular-to-globose tube; it is often constricted at the base and the limb is more or less two-lipped. The upper lip is usually shorter than the lower, which is spotted or veined on the inside (Bräuchler et al., 2004). This species occurs from central to eastern Europe and southwards to northern Greece and northwards to Estonia (Heywood 1972a, b). It grows in deciduous woods and glades and on mountain meadows. In Poland, flowering starts in mid June, and the average flowering period is between 3 and 4 weeks (Kucowa, 1963). *Digitalis grandiflora* is a protected plant in some countries (e.g., Germany, Poland).

Study area

The study was carried out in a 100-150 year-old oak stand in the Niewiesz Forest, about 50 km E of Lodz,

central Poland (51°91'72"N, 18°89'42"E). The average annual temperature is 8.8°C (July 18.7°C, January -1.8°C); the average annual precipitation is 570.1 mm, with a maximum in June-July; the average length of the growing season is 210-220 days. The forest stand is primarily (84%) *Quercus robur* L. (Fagaceae), some of which are 150-200 years old, with 12% *Pinus sylvestris* L. (Pinaceae) and 4% *Carpinus betulus* L. (Betulaceae). Common species of the herb layer include *C. betulus*, *Frangula alnus* Mill. (Rhamnaceae), *Corylus avellana* L. (Betulaceae) and *Q. robur* seedlings. *Betonica officinalis* L. (Lamiaceae), *Veronica officinalis* L. (Plantaginaceae), *Carex montana* L. (Cyperaceae), *Fragaria vesca* L. (Rosaceae), *Poa nemoralis* L. (Poaceae), *Campanula persicifolia* L. (Campanulaceae), *Luzula pilosa* (L.) Willd. (Juncaceae) and *Melica nutans* L. (Poaceae) were the most common understory woody plants. The oak stand grows on a luvisol, developed on a deep loam.

Two plots ~0.1 ha (200×50 m) were chosen for the study. One was along the forest edge, within 5 m of the southern boundary, and the other was within the forest interior at least 200 m from the forest edge. The southern boundary of Niewiesz Forest was bordered by a crop (*Zea mays*) field at the time of the study. The length of the crop-forest edge was estimated to be 400 m. The forest edge plot had a greater understory vegetation cover than the forest interior plot, ranging from 40% at the beginning of the growing season to 100% in early summer, while the understory cover for the forest interior plot ranged from 20% to 90%. The interior microhabitat had significantly larger trees (stem diameter at breast higher DBH, X±SD *Q. robur* 69.4±5.8 cm) than the forest-edge microhabitat (DBH: *Q. robur* 24.9±3.8 cm; $P < 0.001$ for all two comparison, *t*-tests on long-transformed data). Dominant trees are typically 20-25 m tall.

Microclimate

During the 2012 growing season, midday microenvironmental measurements were made in each microhabitat at 7-14-day intervals ($n = 10$) between May 14 and August 11 on relatively cloud-free days. The environmental variables measured are in two categories: attributes of the soil environment (soil moisture), and an estimate of the amount of light hitting the forest floor (percent canopy openness). Soil moisture was measured gravimetrically two weeks after rainfall. The gravimetric soil water of the upper 15 cm of soil was measured on four replicate samples from each microsite on each date. To estimate the light reaching the ground layer, I recorded canopy openness (i.e., % of full sun) through hemispherical photograph analysis (Canham, 1988) taken in each microhabitat on overcast days between 11:00 and 13:00 h using a Nikon Coolpix 800 digital camera equipped with a Nikon UR-E6 180° fisheye lens. The camera was mounted approximately 1 m off the ground with a tripod and aligned to the magnetic north. The aperture was set at F/8 and shutter speed to 1/30 second in order to standardize the exposure. Each image was processed using Gap Light Analyzer (GLA) software (Frazer et al., 1999) to calculate percent canopy openness.

Leaf morphology

In the plots, 60 plants were tagged (30 in the forest interior and 30 at the forest edge) and measured as to vertical height and number of flowers. On 30 June 2011, one leaf (always the fifth from the bottom) per individual from each shoot × site combination was collected for morphological, structural and leaf nutrient analyses. Basal leaves wither rapidly, so during the flowering period they are already withered, and therefore their variability was not taken into account. Leaves were removed with scissors, immediately placed between wet sheets of paper, placed into sealed plastic bags and kept in a portable cooler to minimize water loss during transport to the laboratory. After being transported to the lab, leaf samples were placed in water in the dark at 5°C for 12 h (Garnier et al., 2001). This procedure ensured full leaf rehydration. Leaf outlines were digitized with a video camera and then the outlines were analyzed using image-analysis software, which converted the scanned drawing to a bitmap image and determined leaf surface area (LA, mm^2) (one side) and leaf perimeter (LP, mm) of the resulting image. After

determination of the area, leaves were dried for at least 48 h at 80°C and dry mass (LDMC, mg) was determined. From the primary data the following variables were derived: leaf shape (=[4π×area]/ [perimeter]^2 ratio), specific leaf area (SLA, mm^2 mg^{-1}; one-sided leaf area per leaf dry mass), and leaf mass per unit leaf area (LMA, g cm^{-2}; leaf dry mass per leaf area).

Leaf anatomy

Only fully expanded, undamaged fresh leaves of *D. grandiflora* without serious herbivore or pathogen damage, as recommended previously (Reich et al., 1992; Garnier et al., 2001), were chosen for sectioning. From these measurements, I estimated the proportion of each tissue in the transversal section area of the lamina. Care was taken to measure the leaf blade in between the leaf nerves. To examine stomatal density, leaf peels were taken using a razor to skim the surface of the upper and lower epidermis (SDADE and SDABE, stomata mm^{-2}). Each peel was placed on a slide, immersed in a drop of water underneath a coverslip and observed at × 200 magnification. Leaf thickness was measured for the same leaves used for leaf area determination. To gauge the leaf thickness, leaf cross-sections were prepared for microscopic examination. One 0.5 × 1.0-cm strip was taken from the middle portion of the lamina across the midrib, each from a different leaf. Preliminary work had shown that replicate measurements on the same leaf varied very little (see also Shipley, 1995). Strips were immediately fixed in a mixture of 40% formaldehyde:glacial acetic acid:50% ethanol (5:5:90 by volume) for several days. The material was then dehydrated in an ethanol series and embedded in wax. Semi-thin leaf sections (5 μm thick) were obtained with a rotation microtome and stained with toluidine blue O (Jensen, 1962) and observed under a light microscope. The main anatomical parameters were measured by Video Image Analysis using a digital camera mounted on light microscope. Leaf tissue density (LTD, μg mm^{-3}) was obtained according to Witkowski and Lamont (1991) as the ratio between LMA and total leaf thickness. The mean leaf thickness for each sample of ten leaves was calculated.

Leaf-nutrient analyses

Ten leaf samples from ten plants per microhabitat were harvested from each nutrient treatment. Plant nitrogen (N), phosphorus (P) and potassium (K) concentrations were determined at the Regional Agrochemical Station in Lodz, Poland. Total leaf N concentration was determined using a micro-Kjeldahl digest and an auto-analyzer. Phosphorus (P) and potassium (K) concentration were determined by emission spectrometry in an inductively coupled plasma. Nitrogen (N), phosphorus (P) and potassium (K) contents are expressed on the bases of both dry mass and leaf area.

Data analysis and leaf plasticity index

Analysis of variance (ANOVA) was used in the field study to test for differences between the two microsites. A multivariate ANOVA, main factors: light reaching [L], moisture [SM], and microhabitat [M], was initially used to test the hypothesis, i.e. whether there were significant L×M or SM×M interactions. Data for all statistical tests were $\log_{10}$ transformed as necessary to meet the assumptions of normality and homogeneity of variances implicit in parametric statistical procedures. Pearson's correlation coefficients were calculated between leaf specific mass (LMA) and various leaf traits (LA, LDMC, LTH, LTD) in the whole set of individuals. The normality of the data distribution was tested with the Chi-squared test.

The index of leaf plasticity (Valladares et al., 2000) was calculated as the difference between the maximum and the minimum mean value divided by the maximum mean value (per trait). The leaf plasticity index ranged from 0 to 1. The median for the plasticity index was determined by the Mann-Whitney U rank sum test.

For flowering individuals, Spearman's rank correlation tests were used to investigate the relationship between plant height and the number of flowers per individual. The software package STATISTICA

Table 1. Multivariate ANOVA of the *Digitalis grandiflora*. Main factors: light reaching, moisture, and microhabitat (forest edge and interior) (df = 7.56). The anatomical traits are the five traits presented in Table 2. Main factors: light reaching, soil moisture, and microhabitat (forest edge and Interior) (df = 4.21). Numbers in the table are F ratios. Significance levels: *, $P < 0.05$*; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

	Wilks' λ	
	Morphological traits	Anatomical traits
Microhabitat	0.146***	0.273**
Light	0.563**	0.646*
Soil moisture	0.762^{NS}	0.356^{NS}
Microhabitat × light	0.965^{NS}	0.154^{NS}
Microhabitat × soil moisture	0.642^{NS}	0.265^{NS}
Light × soil moisture	0.674^{NS}	0.489^{NS}
Microhabitat × light × soil moisture	0.897^{NS}	0.123^{NS}

PL. ver. 10 was used for all mentioned numerical analyses.

RESULTS

Microclimate

There were clear differences among the forest edge and interior microsites throughout the growing season. The soil moisture level in the forest interior was elevated, but forest edge and interior levels did not differ significantly. In parallel, percent light reaching (as estimated from forest canopy openness) differed significantly between sites ($P < 0.001$) being higher at the forest edge (61.84±5.27) and lower in the shaded forest interior site (11.33±1.16) (Fig. 1). Both the forest edge and interior microhabitats were temporally homogenous in terms of light reaching ($F = 2.934$, $P > 0.37$; repeated-measures ANOVA) and for soil moisture ($F = 0.932$, $P > 0.54$; repeated-measures ANOVA).

The multivariate ANOVA to test the effects of microhabitat, light reaching and soil moisture showed that microhabitat ($P < 0.001$ or $P < 0.01$), light reaching ($P < 0.01$ or $P < 0.05$) and soil moisture ($P < 0.01$ or $P < 0.01$) significantly influenced the *D. grandiflora* morphological and anatomical variables measured. There were no significant interaction terms (microhabitat × light, $P = 0.872$ or $P = 0.329$; microhabitat × soil moisture, $P = 0.184$ or $P = 0.126$; light × soil moisture, $P = 0.846$ or $P = 0.729$; microhabitat × light × soil moisture, $P = 0.821$ or $P = 0.221$) (Table 1).

Leaf morphology

Full-shade and half-shade leaves differed significantly in their morphological and anatomical traits. Analysis of variance indicated that LA, leaf shape, DM, LMA, SLA, LTD, SDADA and SDABE were significantly influenced by the habitat. Mature *D. grandiflora* plants growing in the forest interior plot had greater LA ($F = 123.42$ $P < 0.05$), LDMC ($F = 176.65$, $P < 0.001$) and higher values of SLA ($F = 2.09$, $P < 0.01$) than plants in the forest edge plot. In contrast, plants from the forest edge had denser leaves, with higher values of both LSI ($F = 0.93$, $P < 0.01$) and LMA ($F = 126.64$, $P < 0.001$) than the leaves of plants from the interior (Table 2).

It should be noted that, *D. grandiflora* plants from the forest interior were taller (height: 42.1±6.4 cm) and had greater flower production per plant (38±2.63) than plants in the forest interior (27±2.46), with the result that the number of flowers increased with the increasing height of these individuals (Spearman's test, $r_s = 0.657$, $P < 0.0001$, $n = 60$, pooled across forest edge and interior). Flowering individuals were greater than those of non-flowering plants were. Leaf area generally was significantly greater for

Table 2. A multivariate ANOVA of morphological, anatomical and biochemical traits (total $n = 720$) of *Digitalis grandiflora*. Numbers in the table are F ratios. The last column presents the difference between the highest and lowest microhabitat means. N_{mass}, P_{mass}, K_{mass} were arcsine transformed, the other leaf traits were log_{10}-transformed prior to analysis. *, $P < 0.05$*; **, $P < 0.01$; ***, $P < 0.001$. Interaction effects between microhabitat and light level, microhabitat and soil moisture could not be evaluated for N_{area}, N_{mass}, P_{area}, P_{mass}, K_{area} and K_{mass}. †LA, leaf surface area; LSI, leaf shape index; LDMC, leaf dry matter content; LMA, leaf mass per unit leaf area; SLA, specific leaf area; LTD, leaf tissue density; LTH, leaf thickness; MTH, mesophyll thickness; SDADE, stomatal density adaxial epidermis; SDABE, stomatal density abaxial epidermis. N_{area}, nitrogen concentration per unit leaf area; N_{mass}, nitrogen content per unit leaf mass; P_{area}, phosphorus concentration per unit leaf area; P_{mass}, phosphorus content per unit leaf mass; K_{area}, potassium concentration per unit leaf area; K_{mass}, potassium content per unit leaf mass.

Parameters†	Microhabitat (M)	Light (L)	Soil moisture (SM)	M×L	M×SM	L×SM	M ×L×SM	Plasticity index
LA (mm^2)	123.42*	45.84***	11.23	5.85	0.24	3.61	0.87	0.27
LSI	0.93**	0.54*	0.18	1.68	0.35	1.23	0.52	0.71
LDMC (mg)	176.65***	67.65*	12.16	1.87	3.46	0.96	0.36	0.51
LMA (μg mm^{-2})	126.64***	26,64*	10.03	0.81	0.76	2.37	0.49	0.46
SLA ($mm^2 mg^{-1}$)	2.09**	0.89**	0.42	0.57	0.51	0.65	0.25	0.84
LTD (μg mm^{-3})	337.65***	92.34*	27.90	1.89	3.21	1.72	0.36	0.45
LTH (μm)	118.71*	16.43*	11.15	0.63	2.40	0.50	0.64	0.38
MTH (μm)	8.63*	0.31*	2.34	2.56	1.54	0.41	0.72	0.14
SDADE (stomata mm^{-2})	9.21**	0.49*	0.78	1.68	5.34	3.74	0.96	0.26
SDABE (stomata mm^{-2})	5.28***	0.36*	0.15	0.35	4.83	0.79	0.51	0.27
N_{area} (mg g^{-1})	6.35*	0.32	0.47					0.14
N_{mass} (g cm^{-2})	5.87*	0.15	0.21					0.22
P_{area} (mg g^{-1})	16.43*	2.75	0.54					0.24
P_{mass} (g cm^{-2})	12.38*	1.89	0.32					0.30
K_{area} (mg g^{-1})	4.65*	0.62	0.48					0.11
K_{mass} (g cm^{-2})	3.98*	0.36	0.36					0.27

Table 3. Pearson correlation coefficients between leaf specific mass (LMA) and various leaf traits of *Digitalis grandiflora* (abbreviations as in Table 2). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

Parameters	Microhabitat	
	Forest edge	Interior forest
LA	–0.89**	–0.38*
LDMC	-0.92***	-0.44*
LTH	0.32*	0.36^{ns}
LTD	0.15**	0.61^{ns}

the taller plants that grew at the forest edge than for plants from the forest interior (P <0.05, ANOVAs on leaf area).

Leaf anatomy

Microhabitat significantly affected all the examined anatomical traits. The leaves of plants from the forest edge were thicker (LTH, F = 118.71, P< 0.05), and had higher stomatal density in both adaxial (SDADA, F = 9.21, P <0.01) and abaxial epidermis (SDABE, F = 5.26, P <0.001) than those from the interior forest.

Statistical analyses of data revealed significant negative correlations between LMA and LA at the forest edge (r = –0.89, P <0.01) or forest interior (r=

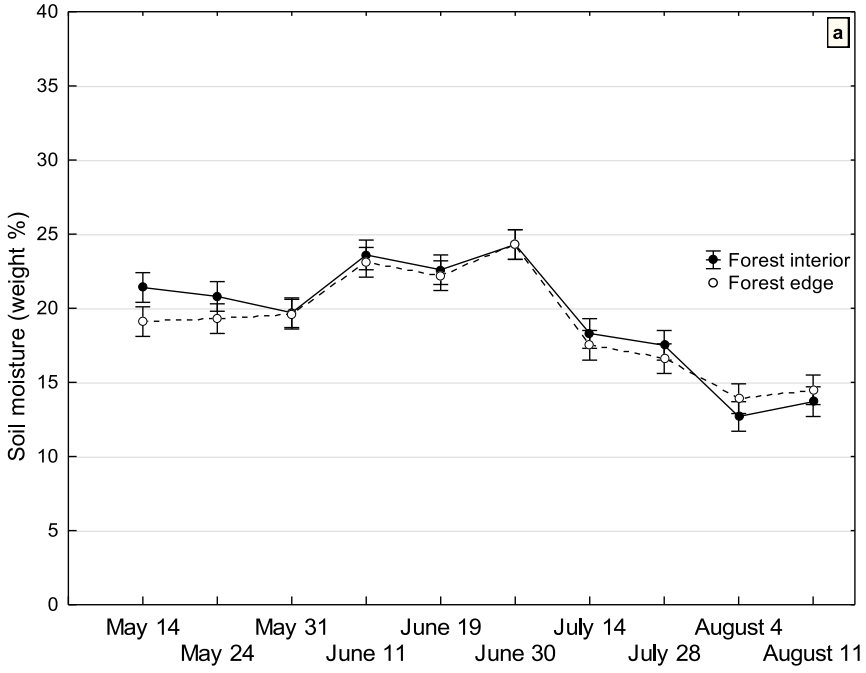

Fig. 1. Percentage of soil moisture and light reaching measured in the course of the growth season from May to August at the forest edge and interior. Each point (mean ± SE) is the average of five recordings; a – soil moisture (weight %) at 15 cm depth; b – light reaching (percent canopy openness 1 m above the ground level).

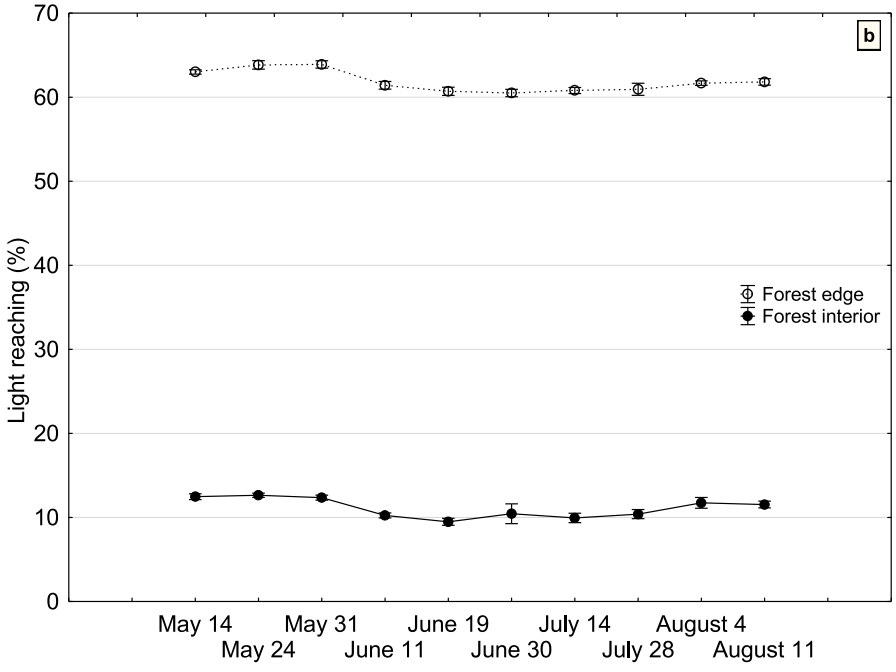

Fig. 2.

–0.38, P <0.05); another significant negative relationship was between LMA and DM (r = –0.92, P <0.001 or r = –0.44, P <0.05). In contrast, no significant interaction was found between LMA and LTH or LMA and LTD in the forest interior (r = 0.36 or r = 0.61, P >0.05 in both cases) (Table 3).

Leaf-nutrient concentration

Leaves of plants from the forest edge had a higher N content on a dry mass basis than leaves from plants in the forest interior (F = 6.35, P <0.05). Leaf N concentration with respect to leaf area was less in plants from the interior than in those from the forest edge (F = 5.87, P <0.05). Plants originating from the forest interior contained more P per unit plant dry mass than those from the forest edge (F = 16.43, P <0.05), whereas the reverse was true (higher content in the leaves of plant from forest interior, and on a dry mass basis but not on an area basis) for P (F = 12.38, P <0.05). Leaf K concentrations, expressed either with respect to surface area or dry weight, were greater in plants from the forest edge than in those from the interior (F > 3.98, P <0.05) (Table 2).

Plasticity index

The median of morphological, anatomical and biochemical traits was the highest for the morphological trait (0.50, P <0.05) and lowest for anatomical and biochemical ones (0.30 and 0.21, respectively; P <0.05 in both cases), and among them, for SLA and LSI (0.84 and 0.71, respectively; P <0.05 in both cases) (Table 2).

DISCUSSION

Previous investigations of microhabitat factors at forest edges and interiors examined the influence of environmental factors such as photosynthetically active radiation (PAR), humidity, vapor pressure deficit (VPD), soil moisture, soil and air temperature (Brothers and Spingarn, 1992; Matlack, 1993; Young and Mitchell, 1994; Chen et al., 1995; Honu and Gibson, 2006). In this study, the direct microhabitat factor light availability had significant effects on morpho-anatomical leaf traits, while soil moisture did not affect leaf traits. Herein I showed that the oak forest edge is characterized by higher light transmittance than the forest interior, which is similar to patterns found in many forest edge studies (Matlack, 1993; Young and Mitchell, 1994; Chen et al., 1995; Jose et al., 1996). Previous studies have shown that the effects of intermediate light levels (25-50% of full sunlight) affective plant morphogenesis without dramatically changing plant biomass (Loach, 1970; Poorter, 1999). In contrast, a reduction of biomass production has been observed at irradiance levels lower than 10% of full sunlight (Poorter, 1999; Navas and Garnier, 2002).

Light availability has been suggested as the driving force behind the responses of other microclimate variables, namely air temperature, relative humidity and soil moisture (Matlack, 1993). Here, the soil moisture level was lower at forest edge than forest interior, but forest edge and interior levels did not differ significantly. This phenomenon might be explained by the complex interaction among microclimatic variables and forest structure (Chen et al., 1993). For instance, this may have been caused by increased evaporation and transpiration in the forest edge area under high levels of direct radiation.

Light generated (1) smaller leaves, (2) higher LMA, (3), lower SLA, and (4) higher stomatal density. It is well known that LMA varies considerably between species, individuals and within plant canopies and may change in response to variations in nutrient and/or moisture availability, light reaching, temperature, altitude leaf gas exchange, with leaf pubescence, season and with leaf age (reviewed in: Witkowski and Lamony, 1991). Similar to *D. grandiflora* in this study, many other comparisons of a single species have revealed that half-shade leaves have higher LMA than shade leaves (e.g., Abrams and Mostoller, 1995; Castro-Diez, 2000; Mojzes et al., 2005), and that the degree of variation can be different. The different kinds of relationship among LMA, LTD and LTH reported by different authors (Witkowski and Lamont, 1991; Choong et al., 1992; Castro-Diez, 2000) suggest that all the above alternatives are possible (Castro-Diez

2000). In some cases, variations in LMA were due to changes in leaf density in particular or thickness, or both, while in others, density and thickness varied without a net effect on LMA (Witkowski and Lamont, 1991). At the forest edge LMA exhibited a highly significant and negative correlation with both LA and LDMC, but showed little correlation with both LTH and LTD. Similar results were found in wild herbaceous species by Thompson et al. (1997). The considerable variations in leaf density and thickness recorded here confirm the very high variation in cell size and amounts of structural tissue in species. Previous studies have suggested that more light could be captured by spreading a given amount of pigment-protein complexes over a greater area than by concentrating it in a given area (Evans and Poorter, 2001). For example, leaves with low LMA that have about half the Rubisco content per unit leaf area can presumably absorb twice the number of photons per unit leaf mass in comparison with the high-LMA leaves (Evans and Poorter, 2001).

With greater light plants reduce transpiration losses and increase carbon gain by producing small-sized, thick leaves with a low SLA (Poorter, 1999). The larger and thinner leaves (higher SLA) are more advantageous for light capture under low light (Rychnowska, 1967; Björkman, 1981; Carpenter and Smith, 1981; De Lucia et al., 1996; Poorter 1999; Wilson et al., 1999). Small-sized sun leaves will provide less surface area for the loss of water through transpiration. In this way, less transpiration is needed for cooling down the leaf in a high-light environmental (Parkhurst and Loucks, 1972; Ashton and Berlyn, 1992; Gonçalves et al., 2008). This was observed, at least as a tendency, in the present study. Plants grown at the forest edge generally have thick leaves with a low SLA, due in part to an increase in number of cells and in part to longer palisade cells. The reduced leaf thickness in shady environments confirmed here was *similar to previous* research (e.g., Jackson, 1967; Fekete and Szujkó-Lacza, 1973; Nobel, 1977; Carpenter and Smith, 1981; Givnish, 1988; Abrams and Kubiske, 1990; Ashton and Berlyn, 1992; Rôcaas et al., 2001; Oguchi et al., 2003; Mojzes et al., 2005) and was probably due to shorter mesophyll cells and/or fewer mesophyll cell layers.

The mesophyll of the leaf of forest-edge *D. grandiflora* still shows no evidence of differentiation into palisade and spongy parenchyma. By contrast, individuals grown in mountains hayfields have heterogeneous mesophyll with assimilatory tissue differing in palisade and spongy parenchyma (György, 2009). Variation in the average number of mesophyll cell layers as well as in the mesophyll cell height indicated that leaves produced by *D. grandiflora* are sun-type in the exposed grassland and shade-type in the light-limited oak understory.

There is empirical evidence, consistent with my results, that the leaf nitrogen level is higher in sunny than in shady environments (Mooney and Gulmon, 1979; Reich and Walters, 1994; Reich et al., 1997; Ackerly et al., 2000; Gratani et al., 2006). Sun leaves allocated more nitrogen than shade leaves, reflecting an increase in carboxylating enzymes and proteins, responsible for photosynthetic electron transport in full sun (Reich and Walters, 1994; Walters and Reich, 1996). This might be explained by the fact that more than half of the total leaf nitrogen content was associated with the photosynthetic apparatus (Niinemets and Valladares, 2004; Takashima et al., 2004). Mooney and Gulmon (1979) concluded that optimal leaf nitrogen content should also be higher on moister or more fertile sites.

Shade tolerance is a term, most commonly used by foresters, to describe the relative ability of a species to grow and thrive under a forest canopy. A tolerant species can grow and thrive under tree canopy, whereas an intolerant species can_thrive only away from the main canopy or in the open (Muick, 1991). The shade tolerance of a species can vary over the lifetime of a plant and between individuals. Results presented here suggest that *D. grandiflora* is a shade-tolerant species. As pointed out by Rozendall et al. (2006) and Valladares et al. (2002), shade-tolerant species have higher plasticity in traits important for light harvesting (in this case reflected in high plasticity in SLA).

Although *D. grandiflora* populations can persist both along forest edges and under closed forest canopies, plants grown in higher light conditions have significantly greater biomass and reproductive output than plants grown in the low light conditions typical of those in a temperate forest interior (data not shown here). At the later stages, light is probably too limited to allow adequate accumulation of photosynthate for sexual reproduction. The positive relationship between light availability and the production of generative structures has been described in numerous studies (e.g., Meekins and McCarty, 2001; Gianoli, 2004; Stachurska-Swakoń and Kuź, 2011).

The great variations in morpho-anatomical leaf traits among individuals of *D. grandiflora* in different microhabitats may be response to the differences in light regimes in the microhabitats where they grow. The affect of light availability was more significant on leaf dimension than that of water availability in this study. Besides the effects of light, air temperature and humidity in the different forest habitats can also be expected to affect leaf morphological plasticity (Codarin et al., 2006; Koch et al. 2006; Xu et al., 2008; Stachurska-Swakoń and Kuź, 2011). Many previous studies have shown variations in leaf traits to be the result of adapting to growth habitats (Sisó et al., 2001; Pandey and Nagar, 2002; György, 2009).

Microclimate is made up of multiple variables, which can be studied either independently or in unison (Gehlhausen et al., 2000). In this study, I applied traditional microclimate variables, for example, soil moisture and light level. Collecting precise microclimatic data is difficult because of the high cost of instrumentation (see also Chen et al., 1993).

This study of leaf trait differences may contribute to our understanding of optimum habitat conditions and the ecophysiological adaptations of plants. *In conclusion*, the *results of the present study* may suggest that the resolution of taxonomy would require the consideration of heterogeneity within the same species based on leaf allometry and phenotypic plasticity (McLellan, 2000) and may provide evidence of adaptation and niche differentiation of coexisting species. However, I recognize that the limited geographical and phylogenetic scope in my research allows only a preliminary assessment of this expectation.

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