PHOTOSYNTHETICA 44 (1): 11-16, 2006

Morpho-anatomical and physiological leaf traits of two alpine herbs, *Podophyllum hexandrum* and *Rheum emodi* in the Western Himalaya under different irradiances

S. PANDEY*, N. KUMAR, and R. KUSHWAHA

Department of Biotechnology, Institute of Himalayan Bioresource Technology, Palampur-176 061 (H.P.), India

Abstract

Morpho-anatomical leaf traits and photosynthetic activity of two alpine herbs, *Podophyllum hexandrum* (shade-tolerant) and *Rheum emodi* (light-requiring), were studied under field (PAR>2 000 μmol m^{-2} s^{-1}) and greenhouse (PAR 500 μmol m^{-2} s^{-1}) conditions. Mesophyll thickness, surface area of mesophyll cells facing intercellular spaces (S_{mes}), surface area of chloroplasts facing intercellular spaces (S_c), intercellular spaces of mesophyll cells (porosity), photon-saturated rate of photosynthesis per unit leaf area (P_{Nmax}), and ribulose-1,5-bisphosphate carboxylase/oxygenase activity decreased in the greenhouse with respect to the field and the decreases were significantly higher in *R. emodi* than in *P. hexandrum*. *P. hexandrum* had lower intercellular CO_2 concentration than *R. emodi* under both irradiances. The differences in acclimation of the two alpine herbs to low irradiance were due to their highly unlikely changes in leaf morphology, anatomy, and P_{Nmax} which indicated that the difference in radiant energy requirement related to leaf acclimation had greater impact under low than high irradiance.

Additional key words: alpine plants; leaf anatomy; leaf mass per unit leaf area; leaf physiology; mesophyll; net photosynthetic rate; ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

There has been a long-standing interest in the effects of altitude on photosynthesis, because of the possibility of acclimation and adaptation to particular conditions (Körner 1999). With increasing altitude, atmospheric pressure and air temperature decrease, and they are associated with high irradiance, wind velocity, and great diurnal fluctuation of these environmental factors (Larcher 1980, Friend and Woodward 1990). Low temperature at high elevations is responsible for small organ size (small leaves and shoots), increased cell wall thickness, and leaf thickness. High irradiance results in altered stomatal density (Körner *et al.* 1989). Thus, low temperature and strong irradiance determine leaf structure at high altitude. The enhanced photosynthetic efficiency of high altitude plants is linked to the increase of nitrogen content per unit leaf area (Oleksyn *et al.* 1998) and the higher ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity per unit protein as well as per unit leaf area (Bunce 1986). The higher photosynthetic efficiency is maintained when plants from higher altitudes are grown at lower altitude (Körner and Diemer 1994).

Shade-tolerant deciduous species are expected to have higher photosynthetic rates under low irradiance than the light-demanding species (Givnish 1988). However, leaf mass per unit leaf area (LMA) in shade-tolerant species is higher as compared to light demanding ones under low irradiance (Rijkers *et al.* 2000). In general, shade-tolerant species are more drought sensitive than light-demanding ones (Abrams 1994) and stomata impose a relatively larger constrain on their photosynthesis (Roden and Pearcy 1993). Thus, leaf morphology, anatomy, and photosynthetic biochemistry are fundamental aspects of plant

Received 25 January 2005, *accepted* 9 June 2005.
*Author for correspondence; present address: Department of Bioinformatics & Information Technology, Shoolini Institute of Life Sciences & Business Management, The Mall, Solan-173 212 (H.P.), India; fax: +91 1792 226364, e-mail:spandey66@yahoo.co.in
Abbreviations: C_i – intercellular CO_2 concentration; Chl – chlorophyll; F_v/F_m – photochemical capacity of photosystem 2 in the dark adapted state; g_s – stomatal conductance; LMA – leaf mass per unit leaf area; P_{Nmax} – net photosynthetic rate per unit leaf area at saturation irradiance; PAR – photosynthetically active radiation; PS – photosystem; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; S_c – surface area of chloroplasts facing intercellular spaces; S_{mes} – surface area of mesophyll cells facing intercellular spaces.
Acknowledgements: We thank Dr. P.S. Ahuja, Director, IHBT for providing necessary facilities. Financial assistance from CSIR, New Delhi to SP under pool scientist scheme is gratefully acknowledged.

acclimation to different irradiances.

Podophyllum hexandrum Royle is a hypostomatous perennial succulent herb growing under sun and shade conditions in the inner ranges of the Himalayas at altitudes of 3 000–4 200 m. *Rheum emodi* Wall is an amphistomatous perennial herb growing in sun conditions in the Himalayan range at 2 000–4 500 m.

Numerous studies consider relationships between leaf photosynthesis and shade tolerance, but little attention has been given to the relationship between leaf anatomy and shade tolerance in alpine species. The objective of present study was to compare morpho-anatomical and physiological leaf traits of two alpine species, *P. hexandrum* (shade-tolerant) and *R. emodi* (light-requiring) under low irradiance.

Materials and methods

Field site: The study was carried out in the upper part of the Chandra river valley of the Western Himalayan region (Northern India) in the alpine zone above the timberline. Individuals of *Podophyllum hexandrum* were collected at Koksar (32°22'21"N; 77°14'05"E; 3 350 m above sea level) and individuals of *Rheum emodi* at lower part of Rohtong pass (32°22'19"N; 77°14'46"E; 3 500 m a.s.l.).

Meteorological data were collected at fortnightly interval at Koksar and Rohtong pass during the growth period (April–September). At Koksar and Rohtong, mean soil temperature was 12.2 and 11.4 °C, mean air temperature 9.2 and 7.7 °C, relative humidity (RH) 61 and 67 %, atmospheric pressure 70 and 64 kPa, and photosynthetically active radiation (PAR; measured during clear sky between 09.00 and 11.00) >2 000 μmol m^{-2} s^{-1}, respectively. The vegetation cover was 50–60 % in both the sites.

Measurements were carried out at 09.00–11.00 h, under clear sky during July 2001 (in the field) and September 2002 (in greenhouse).

Plants of *P. hexandrum* Royle and *R. emodi* Wall were collected during July 2001 and transferred to the Palampur campus (1 300 m a.s.l., 32ºN, 76º33'E). Plants were grown in pots filled with the native soil under controlled greenhouse conditions (photoperiod: 14 h; mean air temperature: 24 ºC, RH 65 %, PAR: 500 μmol m^{-2} s^{-1}, and atmospheric pressure 87.6 kPa).

Gas exchange was measured using an open gas system IRGA (*Li-6400*, *LiCor*, Lincoln, NE, USA) equipped with an automated barometer. P_{Nmax}, VPDL, g_s, and C_i were measured on mature leaves (the second leaf from the top) at a PAR of 1 500 μmol m^{-2} s^{-1} (obtained from PAR response curve at irradiance saturated photosynthesis), ambient CO_2 concentration (355 mol mol^{-1}), and 25 ºC. Mature leaves (5 per species) were selected from five different plants. PAR response curve of photosynthesis per unit leaf area was determined at PAR ranging from 0 to 2 500 μmol m^{-2} s^{-1} at constant CO_2 (355 μmol mol^{-1}) by a "cool light" source (*6400–02* LED). Carboxylation efficiency was determined as the ratio of P_{Nmax} to C_i under saturating irradiance (1 500 μmol m^{-2} s^{-1}) using a *6400-01* CO_2 injector.

Chlorophyll (Chl) fluorescence of photosystem 2 (PS2) was measured with a portable pulse-modulated fluorescence monitoring system (model *FMS2*, *Hansatech Instruments*, UK) on mature leaves. After 30 min of dark adaptation the ratios between variable and maximum fluorescence (F_v/F_m) were determined with 100 % of the available "actinic light".

Leaf morphology and anatomy: LMA was calculated from leaf dry mass and one sided leaf area. Dry mass was determined by drying 30 leaves at 70 ºC to constant mass whereas leaf area was measured using leaf area meter *CI-203* (*QC CID, USA*). Stomatal density [number mm^{-2}] was measured from surface impressions by coating the both upper/lower surface of the leaves with clear enamel nail polish, avoiding veins (Meidner and Mansfield 1968). Stomata were counted with a haemacytometer (1×1 mm) under microscope at 40× magnification in six fields of view from each peel taken from four leaves of four different plants.

Leaf segments (3×7 mm) from the middle of the lamina were fixed in FAA (formaldehyde : acetic acid : 50 % ethanol, 5 : 5 : 90) and dehydrated in a *t*-butyl alcohol series. Leaf sections were stained with safranine-fast green, and the slides were mounted in *DPX* [80–10 g *Distrene* (British resin product), 5 cm^3 dibutylpthalate, and 35 cm^3 xylene]. Micrographs were made using a *Nikon* (*Biophot*) No. *78508*, Japan at 100× magnification and a digital camera (*Nikon DXM 1200*). Epidermis thickness, palisade parenchyma thickness, mesophyll cells, and porosity were measured with the software NIH image (National Institute of Health).

S_{mes} was calculated from photographs. To convert length in cross section to surface area, a curvature factor was determined assuming that the palisade cells are cylinders with flat ends and the spongy cells are spheroid (Thain 1983). S_c was determined as: $S_c = (L_c/L_{mes})\ S_{mes}$, where L_c and L_{mes} were the total lengths of chloroplasts and mesophyll cells facing the intercellular space in the section.

Chl content was determined using 10 leaf discs (0.79 cm^2 each) from the second leaf (from 3 plants per species) of plants in the field and in the greenhouse. Chl *a* and *b* contents were determined from absorbances at 642.5 and 660.0 nm.

Measurement of RuBPCO activity: Leaves of *P. hexandrum* and *R. emodi*, collected either in the field or in the greenhouse between 09:00–11:00 were frozen in liquid nitrogen and stored at –70 ºC. Extractions were made in chilled 50 mM Tris-Cl buffer (pH 7.5) containing $MgCl_2$ (1 mM), DTT (5.0 mM), PMSF (1 mM), insoluble PVP (2 %, m/v), glycerol (10 %, v/v), and *Triton X-100* (0.1 %, v/v) using pre-chilled mortar and pestle. The extract was centrifuged at 12 000×*g* for 10 min at 4 ºC, and enzyme activity was determined in the supernatant. Enzyme was activated according to Kumar and Kumar (2000). Samples were counted in a scintillation cocktail (*Sisco Research Laboratories*, India) using a *Beckman* Scintillation Counter *LS 6000TA* (USA).

Statistical analysis: Differences between the species and among leaf traits were analyzed using two-way analysis of variance (ANOVA) and differences among means were tested using Duncan's multiple range test (p<0.01).

Results

Anatomical and morphological leaf traits: *R. emodi* and *P. hexandrum* had a greater mesophyll and palisade parenchyma thickness and a larger S_{mes} and S_c in the field than in the greenhouse; nevertheless, the differences were significant only in *R. emodi* (Table 1). *R. emodi* had significantly thicker epidermis than *P. hexandrum* in the field. The mesophyll porosity was significantly larger in *R. emodi* than in *P. hexandrum* in the greenhouse. A higher ratio of S_c/S_{mes} was measured in the field for the two considered species; nevertheless, it was significantly different only for *R. emodi*.

LMA was significantly higher in *R. emodi* in the field and in the greenhouse than in *P. hexandrum;* differences were not significant in *P. hexandrum* comparing field and greenhouse measurements. Stomatal density was greater in both the considered species in the field than in the greenhouse (Table 1).

Table 1. Morphological, anatomical, and physiological characteristics of leaves of *P. hexandrum* and *R. emodi* developed in greenhouse and field conditions. *Different letters in superscript* following the values in rows showed significant differences at p<0.01.

Leaf characteristic	*P. hexandrum*		*R. emodi*	
	Field site	Greenhouse	Field site	Greenhouse
LMA [g m^{-2}]	48.5^{b}	43.4^{b}	54.3^{a}	44.6^{b}
Stomatal density adaxial	absent	absent	46.0^{a}	35.0^{b}
abaxial [number mm^{-2}]	44.6^{c}	39.5^{c}	198.8^{a}	110.4^{b}
Leaf thickness [mm]	0.27^{b}	0.25^{b}	0.34^{a}	0.26^{b}
Upper epidermis thickness [µm]	40.4^{b}	38.2^{b}	44.2^{a}	38.5^{b}
Mesophyll thickness [mm]	0.20^{b}	0.18^{b}	0.26^{a}	0.19^{b}
Palisade thickness [mm]	0.08^{b}	0.07^{b}	0.10^{a}	0.07^{b}
S_{mes} [m^3 m^{-2}]	20.4^{b}	18.2^{b}	27.8^{a}	19.0^{b}
S_c [m^3 m^{-2}]	16.5^{b}	14.5^{b}	23.0^{a}	15.0^{b}
Porosity [m^3 m^{-3}]	0.25^{b}	0.35^{b}	0.26^{b}	0.46^{a}
Chl [g m^{-2}]	0.26^{b}	0.27^{a}	0.25^{c}	0.27^{a}
Chl *a/b*	2.5^{a}	1.6^{b}	2.5^{a}	1.4^{c}
F_v/F_m	0.84^{a}	0.84^{a}	0.86^{a}	0.85^{a}
Carboxylation efficiency [µmol(CO_2) m^{-2} s^{-1} kPa^{-1}]	0.09^{b}	0.08^{bc}	0.12^{a}	0.07^{c}
C_i [µmol mol^{-1}]	199^{d}	255^{b}	230^{c}	271^{a}
S_c/S_{mes}	0.81^{b}	0.80^{bc}	0.83^{a}	0.79^{c}

Physiological leaf traits: P_{Nmax}, g_s, and RuBPCO activity were significantly different between the two species in the field. In the greenhouse only RuBPCO activity was significantly different between the two considered species (Fig. 1).

Chl content was significantly different in the field between the two species (Table 1) and Chl *a/b* ratio was significantly different in the greenhouse. F_v/F_m did not change significantly for both the species either in the greenhouse or in the field. The carboxylation efficiency of the two species decreased by 45 and 16 % in *R. emodi* and *P. hexandrum*, respectively, in the field with respect to the greenhouse (Table 1).

The relationship between P_{Nmax} and Chl was used to evaluate the acclimation responses of the two species (Fig. 2). P_{Nmax}/Chl decreased by ~50 % in the greenhouse compared to the field in *R. emodi;* the decline was lower (~12 %) in *P. hexandrum* and it was not significant.

Correlation analysis: In both the species, variations in leaf anatomy affected photosynthetic capacity (Fig. 3*A,B*) and RuBPCO activity (Fig. 4). The increase in mesophyll thickness showed a significant positive correlation with RuBPCO activity in *R. emodi* (r = 0.96; p<0.01) but in-

significant one in *P. hexandrum* ($r = 0.73$; $p<0.01$). Similarly, P_{Nmax} positively correlated with mesophyll surface area facing intercellular spaces (S_{mes}) and surface area of chloroplasts facing intercellular spaces (S_c). This positive correlation was highly significant in *R. emodi* but insignificant in *P. hexandrum* indicating that S_{mes} and S_c changed very little in the latter in the greenhouse (low irradiance) than in the former.

Discussion

The results show different morphological, anatomical, and physiological responses of the two considered alpine herbs to low (greenhouse) and high (field) irradiance. The higher P_{Nmax} in *R. emodi* in the field than in the greenhouse were associated with the increase of S_{mes} and S_c (Table 1). Higher S_{mes} and S_c values (larger surface area of chloroplasts) are advantageous for CO_2 flux from the mesophyll surface to the stroma of chloroplasts (Terashima *et al.* 2001). This is further supported by the larger decrease of mesophyll thickness, S_c, and S_{mes} in *R. emodi* under greenhouse conditions resulting in a sharp decline in P_{Nmax}. The highest mesophyll thickness and S_c value in the field enhances photon capture efficiency (Evans 1999, Nobel 1991).

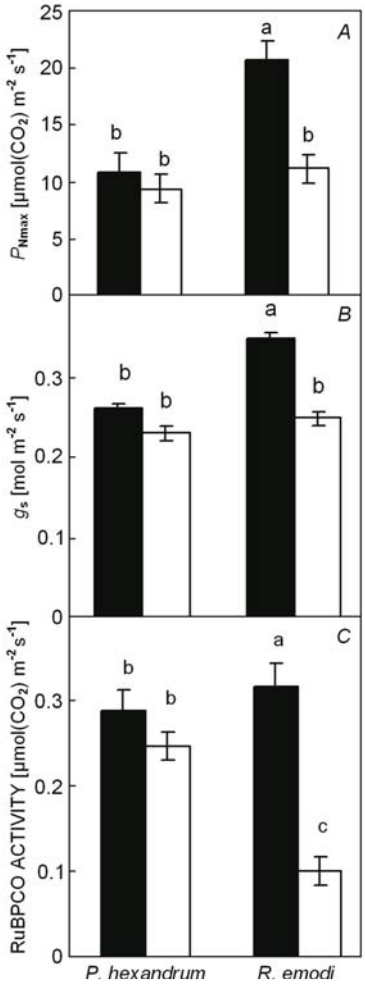

Fig. 1. (*A*) Irradiance saturated net photosynthetic rate, P_{Nmax}, (*B*) stomatal conductance, g_s, and (*C*) ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity of *P. hexandrum* and *R. emodi* grown under field and greenhouse conditions. *Different letters above the bars* show significant differences among means at $p<0.01$.

The decrease of mesophyll thickness in *R. emodi* under low irradiance in respect to high irradiance is mainly due to the palisade parenchyma thickness decrease (Hanba *et al.* 2002, Pandey and Nagar 2002, Pandey *et al.* 2003). The increase in mesophyll, leaf, and epidermis thicknesses may explain the higher LMA in *R. emodi* in the field than in the greenhouse (cf. Kogami *et al.* 2001 for *Polygonum cuspidatum*; on the contrary, *P. hexandrum* maintains similar LMA values under both irradiances).

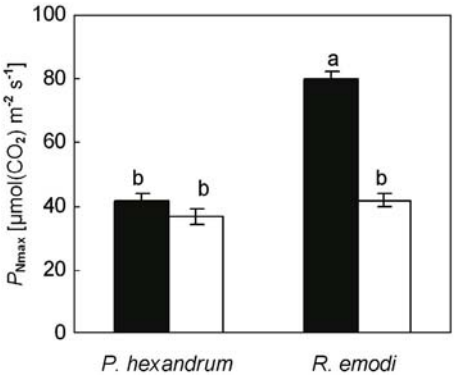

Fig. 2. Trend of ratios of net photosynthetic rate, P_{Nmax} to chlorophyll content in field and greenhouse for *P. hexandrum* and *R. emodi*. *Different letters above the bars* show significant differences among means at $p<0.01$.

Anatomical and physiological traits of *P. hexandrum* in the field and greenhouse conditions were unchanged. The positive correlation between mesophyll thickness and RuBPCO activity in *R. emodi* but not in *P. hexandrum* (Fig. 4) confirmed the results. Emphasizing the importance of leaf anatomical plasticity in determining photosynthetic acclimation to different irradiances, Niinemets *et al.* (1998) suggests that shade-intolerant temperate deciduous woody species have greater nitrogen investments in RuBPCO than shade-tolerant species. The greater RuBPCO activity in *R. emodi* than in *P. hexandrum* in the field suggested its higher photosynthetic potential. In addition, the correlation between P_{Nmax} and S_{mes}, and between P_{Nmax} and S_c in *R. emodi* (Fig. 3*A*,*B*) justified its highest carbon gain in the field (cf. Syvertsen *et al.* 1995, Evans 1998, 1999). The P_{Nmax} decrease in *R. emodi* in the

greenhouse was related to the significant decrease in g_s, RuBPCO activity, and stomatal density, supported by the significant correlation between g_s and stomatal density (Fig. 5), in accordance with the results of Woodward (1987) and Beerling *et al.* (1998). This could also be one of the reasons of unchanged g_s and P_{Nmax} values in the field and in the greenhouse for *P. hexandrum.* Nevertheless, altitude related variation of stomatal density is not yet clear (Hovenden and Brodribb 2000).

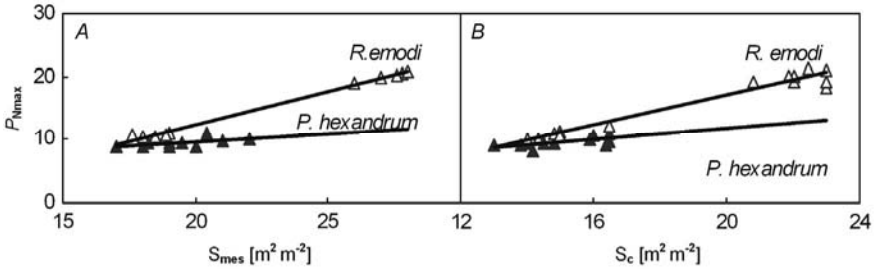

Fig. 3. Correlation between P_{Nmax} and anatomical characteristics (*A*) S_{mes} in *P. hexandrum* (r = 0.63; insignificant p<0.01) and *R. emodi* (r = 0.99; significant p<0.01) or (*B*) S_c for *P. hexandrum* (r = 0.65; insignificant p<0.01) and *R. emodi* (r = 0.97; significant p<0.01).

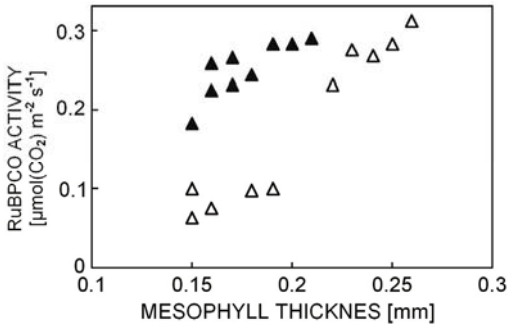

Fig. 4. Relationship between mesophyll thickness and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity per unit area in *P. hexandrum* (*solid triangles*; r = 0.73; significant p<0.01) and *R. emodi* (*open triangles*; r = 0.96; significant p<0.01) under greenhouse and field condition.

The higher g_s and P_{Nmax} in *R. emodi* in the field than in the greenhouse indicates that *R. emodi* grows better at high irradiance, similar to *Nothofagus cunninghamii* (Hovenden and Brodribb 2000). Furthermore, the highest P_{Nmax}/Chl in *R. emodi* in the field than in the greenhouse (Fig. 2) confirmed the better ability of *R. emodi* to utilize Chl for carbon gain under high irradiance and F_v/F_m values >0.8 indicated the species efficiency (Table 1). Some light-demanding temperate deciduous species have higher P_{Nmax} and g_s under high irradiance than shade-tolerant species (Kubiske *et al.* 1996, Niinemets *et al.* 1998, Kitao *et al.* 2000).

The highest C_i in *R. emodi* (amphistomatous) than *P. hexandrum* (hypostomatous) under both the environmental conditions is in accordance with the findings of Terashima and Evans (1988). The similar CO_2 assimilation rates and S_c/S_{mes} of *P. hexandrum* under both the considered irradiances indicate the low plasticity of this species underlining that a higher S_c value is advantageous because chloroplasts of such leaves are small and the amounts of RuBPCO per unit area of chloroplasts can be low (Terashima *et al.* 2001).

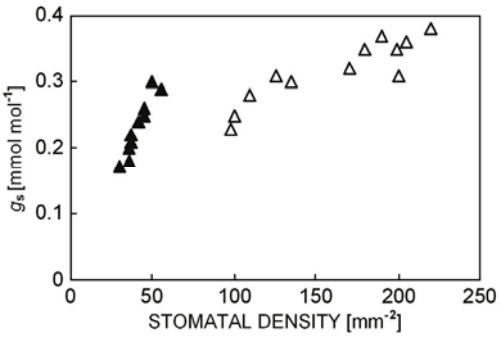

Fig. 5. Correlation between stomatal conductance (g_s) and stomatal density in *P. hexandrum* (*solid triangles*; r = 0.95; significant p<0.01) and *R. emodi* (*open triangles*; r = 0.90; significant p<0.01). Data obtained from the same leaves of both the species grown under field site and greenhouse conditions.

Thus the experiments suggest that the differences in radiation energy demand related to leaf acclimation have major impact on leaf anatomical and photosynthetic characteristics under low rather than high irradiance in light-requiring alpine herbs of Western Himalaya.

References

Abrams, M.D.: Genotypic and phenotypic variation as stress adaptation in temperate tree species. A review of several case studies. – Tree Physiol. **14**: 833-842, 1994.

Beerling, D.J., McElwain, J.C., Osborne, C.P.: Stomatal responses of the "living fossil" *Ginkgo biloba* L. to changes in atmospheric CO_2 concentrations. – J. exp. Bot. **49**: 1603-1607, 1998.

Bunce, J.A.: Measurements and modeling of photosynthesis in

field crops. – CRC crit. Rev. Plant Sci. **4**: 47-77, 1986.
Evans, J.R.: Photosynthetic characteristics of fast and slow-growing species. – In: Lambers, H., Poorter, H., Van Vuuren, M.M.I. (ed.): Inherent Variation in Plant Growth. Physiological Mechanism and Ecological Consequences. Pp. 101-119. Backhuys Publ., Leiden 1998.
Evans, J.R.: Leaf anatomy enables more equal access to light and CO_2 between chloroplasts. – New Phytol. **143**: 93-104, 1999.
Friend, A.D., Woodward, F.I.: Evolutionary and ecophysiological responses of mountain plants to the growing season environment. – Adv. ecol. Res. **20**: 59-124, 1990.
Givnish, T.J.: Adaptation to sun and shade: a whole-plant perspective. – Aust. J. Plant Physiol. **15**: 63-92, 1988.
Hanba, Y.T., Kogami, H., Terashima, I.: The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. – Plant Cell Environ. **25**: 1021-1030, 2002.
Hovenden, M.J., Brodribb, T.: Altitude of origin influences stomatal conductance and therefore maximum assimilation rate in Southern beech, *Nothofagus cunninghamii*. –Aust. J. Plant Physiol. **27**: 451-456, 2000.
Kitao, M., Lei, T.T., Koike, T., Tobita, H., Maruyama, Y.: Susceptibility to photoinhibition of three deciduous broadleaf tree species with differential successional traits raised under various light regimes. – Plant Cell Environ. **23**: 81-89, 2000.
Kogami, H., Hanba, Y.T., Kibe, T., Terashima, I., Masuzawa, T.: CO_2 transfer conductance, leaf structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and high altitudes. – Plant Cell Environ. **24**: 529-538, 2001.
Körner, C.: The nutritional status of plants from high altitudes. A worldwide comparison. – Oecologia **81**: 379-391, 1989.
Körner, C.: Alpine Plants Life. – Springer-Verlag, Berlin 1999.
Körner, C., Diemer, M.: Evidence that plants from high altitudes retain their greater photosynthetic efficiency under elevated CO_2. – Funct. Ecol. **8**: 58-68, 1994.
Körner, C., Neumayer, M., Menendez-Riedl, S.P., Smeets-Scheel, A.: Functional morphology of mountain plants. – Flora **182**: 353-383, 1989.
Kubiske, M.E., Abrams, M.D., Mostoller, S.A.: Stomatal and nonstomatal limitations of photosynthesis in relation to the drought and shade tolerance of tree species in open and understory environments. – Trees **11**: 76-82, 1996.
Kumar, N., Kumar, S.: Differential activation of ribulose-1,5-bisphosphate carboxylase/oxygenase in non-radiolabelled versus radiolabelled sodium carbonate. – Curr. Sci. **80**: 333-334, 2000.
Larcher, W.: Physiological Plant Ecology. 2nd Ed. – Springer-Verlag, Berlin – Heidelberg – New York 1980.
Meidner, H., Mansfield, T.A.: Physiology of Stomata. – McGraw-Hill Press, London 1968.
Niinemets, Ü., Kull, O., Tenhunen, J.D.:. An analysis of light effects on foliar morphology, physiology and light interception in temperate deciduous woody species of contrasting shade tolerance. – Tree Physiol. **18**: 681-696, 1998.
Nobel, P.S.: Physicochemical and Environmental Plant Physiology. – Academic Press, San Diego 1991.
Oleksyn, J., Modrzynski, J., Tjoelker, M.G., Zytkowiak, R., Reich, P.B., Karolewski, P.: Growth and physiology of *Picea abies* population from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. – Funct. Ecol. **12**: 573-590, 1998.
Pandey, S., Kumar, S., Nagar, P.K.: Photosynthetic performance of *Ginkgo biloba* L. grown under high and low irradiance. – Photosynthetica **41**: 505-511, 2003.
Pandey, S., Nagar, P.K.: Leaf surface wetness and morphological characteristics of *Valeriana jatamansi* grown under open and shade habitats. – Biol. Plant. **45**: 291-294, 2002.
Rijkers, T., Pons, T.L., Bongers, F.: The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. – Funct. Ecol. **14**: 77-86, 2000.
Roden, J.S., Pearcy, R.W.: Photosynthetic gas exchange response of poplars to steady-state and dynamic light environments. – Oecologia **93**: 208-214, 1993.
Syvertsen, J.P., Lloyd, J., McConchie, C., Kriedemann, P.E., Farquhar, G.D.: On the relationship between leaf anatomy and CO_2 diffusion through the mesophyll of hypostomatous leaves. – Plant Cell Environ. **18**: 149-157, 1995.
Terashima, I., Evans, J.R.: Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. – Plant Cell Physiol. **29**: 143-155, 1988.
Terashima, I., Miyazawa, S.I., Hanba, Y.T.: Why are sun leaves thicker than shade leaves? Consideration based on analyses of CO_2 diffusion in the leaf. – J. Plant Res. **114**: 93-105, 2001.
Thain, J.F.: Curvature correction factors in the measurement of cell surface areas in plant tissues. – J. exp. Bot. **34**: 87-94, 1983.
Woodward, F.I.: Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. – Nature **327**: 617-618, 1987.