ORIGINAL ARTICLE

Hiroyuki Muraoka · Yanhong Tang · Hiroshi Koizumi
Izumi Washitani

Effects of light and soil water availability on leaf photosynthesis and growth of *Arisaema heterophyllum,* a riparian forest understorey plant

Abstract The effects of soil-water availability on leaf light acclimation and whole-plant carbon gain were examined in *Arisaema heterophyllum* Blume, a riparian deciduous forest understorey plant. Photosynthesis, above-ground morphology and ramet biomass accumulation (relative growth rate: RGR of a corm for a full leaf life-span) were measured on plants raised under three light treatments combined with two soil water conditions. The two higher light treatments during growth (high: max. 550 µmol photons $m^{-2} s^{-1}$; medium: 150 µmol photons $m^{-2} s^{-1}$) resulted in a twofold increase in RGRs, 30% higher photosynthetic capacities and 20% less photosynthetic low-light use efficiency than those under a low light condition (50 µmol photons $m^{-2} s^{-1}$). Leaf area was the smallest and leaf mass area ratio was the largest under the high light treatment. Water stress decreased both photosynthetic rate and leaf area and, hence, RGR in all the light regimes. However, water stress did not alter the general patterns of physiological and morphological responses to different light regimes. We estimated that higher photosynthetic low-light use efficiency and larger leaf area in the low light leaf would lead to a threefold carbon gain as compared with the high light leaf under simulated low light conditions. Both experimental and simulation results suggest that the physiological and morphological acclimations tend to be beneficial to carbon gain when light availability is low, whereas they favor increased water use efficiency when light availability is sufficiently high.

H. Muraoka (✉) · H. Koizumi
Institute for Basin Ecosystem Studies, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
Fax +81-58-2932062
e-mail: muraoka@green.gifu-u.ac.jp

Y. Tang
Division of Environmental Biology, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan

I. Washitani
Institute of Agriculture and Life-Science, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Introduction

Morphological and physiological plasticity to the light environment have been suggested to be beneficial for plant growth (Björkman 1981; Givnish 1988; Pearcy and Sims 1994). Light acclimation responses of plants can be roughly divided into two categories: (1) changes in biomass allocation patterns, and (2) change in carbon economy through physiological and/or morphological adjustments (Hunt and Lloyd 1987; Givnish 1988; Osunkoya and Ash 1991; Pearcy and Sims 1994). These responses can be found in shoot/root ratio and shoot architecture (King 1991; Lambers and Poorter 1992; Walters et al. 1993a; Sims and Pearcy 1994; Valladares and Pearcy 1998; Pearcy and Valladares 1999), and leaf anatomy and physiology (Nobel 1976; Björkman 1981; Sims and Pearcy 1989; Chazdon and Kaufmann 1993; Terashima and Hikosaka 1995). Many previous studies have revealed that these acclimation responses contribute to acquisition and utilization of light. However, adaptive significance of such responses have to be evaluated quantitatively in terms of fitness components such as biomass accumulation through measurement of actual growth (Jurik and Chabot 1986; Popma and Bongers 1988; Rice and Bazzaz 1989; Thompson et al. 1992a, b; Walters et al. 1993b; Kitajima 1994; Laing et al. 1995; Huante and Rincon 1998), or estimation of carbon gain (Rice and Bazzaz 1989; Sims et al. 1994).

Our previous study with *Arisaema heterophyllum* Blume (Araceae), a threatened perennial herbaceous species grown in riparian forest understorey (diffuse transmittance of <30%) and in neighboring deforested open sites (>80%), showed that leaf morphological and photosynthetic characteristics acclimate to the given light environment (Muraoka et al. 1997). Moreover, some plants in the understorey could reach a similar biomass accumulation as those in the

open site despite the large difference in light availability (Muraoka et al. 1997). This suggests leaf acclimation responses compensate for the shortage of light in the understorey environment.

In natural habitats, however, concurrent change of water availability with light environment (Osmond 1983; Valladares and Pearcy 1997) and among-year variation in meteorological conditions (Abrams et al. 1994) affect leaf morphological and physiological properties. Our previous study with *A. heterophyllum* showed that responses of leaf morphological and physiological characteristics and whole plant biomass accumulation to light environments were affected highly by the amount of precipitation (Muraoka et al. 1997). In the deforested open site, light-saturated photosynthetic rate (A_{max}), leaf mass per area (LMA), and individual biomass accumulation [relative growth rate (RGR) of corm] were reduced in a dry year by 33%, 30%, and 62%, respectively. In the forest understorey, A_{max} and RGR were reduced by 27% and 65%, respectively (no significant change in LMA). Because there were no significant changes in leaf longevity between the wet and dry years in both sites, the reduction in RGR was expected to be due to reduced photosynthetic capacity. Under drought conditions, carbon gain during the life of a leaf can be reduced mainly by low photosynthetic capacity because of inhibited leaf development and low stomatal conductance (e.g., Hsiao 1973; Davies and Gowing 1999), and by reduced leaf area.

The objective of this research was to evaluate the effects of water availability on light environment, leaf morphological and physiological characteristics, and whole plant carbon gain in *A. heterophyllum*. Water stress could (1) alter the leaf acclimation responses to light, and/or (2) reduce whole-plant carbon gain by physiological and morphological changes. Evaluating these possibilities could help us to understand the relationship between the ecophysiological properties and a suitable environment for growth for this threatened species. We raised *A. heterophyllum* plants under controlled light and water conditions using a growth cabinet, and measured leaf photosynthetic characteristics, leaf and shoot morphology, and individual biomass accumulation. Model estimation was also used to examine the effects of leaf morphological and physiological responses to light and water availability to the potential carbon gain of the whole plant.

Materials and methods

Plant materials and growth conditions

Arisaema heterophyllum is a threatened perennial herbaceous species that grows in humid floodplains throughout eastern Asia, including Honshu and Kyushu Island in Japan (Environmental Agency 1997). The species is found mainly in riparian deciduous broadleaf forest understorey, but there are some plants in neighboring deforested open sites (Oshima et al. 1997). A plant (ramet) of *A. heterophyllum*

has a single palmately compound leaf on a monopodial pseudostem, which emerges from a corm below ground in mid-spring (late April) and defoliates by mid-summer (mid-August). *A. heterophyllum* plants were collected from the forest understorey and deforested open sites of the Kokai River in Mitsukaido city (36°0′N, 140°1′E; altitude 10.5–12.5 m), Ibaraki Prefecture, Japan. About 40 corms were extracted in late August or early September in 1996, and were transferred to the laboratory and kept under moist conditions at 4°C until use. On 13 January 1997, the corms were potted into clay pots (15 cm diameter × 15 cm height, one corm per pot) with a mixture of field soil and vermiculite (2 : 1 vol/vol) and were transferred to a growth cabinet in the National Institute for Environmental Studies (about 10 km east of the natural habitat).

All plants were raised in one growth cabinet (ca. 2.5 × 5 × 2.5 m) in which light, air temperature, humidity, and CO_2 concentration could be controlled. Six corms with similar biomass (11.0 ± 3.3 g fresh weight; mean ± SD) were placed in each of the six growth regimes prepared by combining three light and two soil-water conditions. The light regimes were high light with a maximum photosynthetically active photon flux density (PPFD) of 550 μmol m^{-2} s^{-1} and a daily PPFD of 26.8 mol m^{-2} day^{-1} (approximately 77% of daily PPFD at the deforested site), medium light with 150 μmol m^{-2} s^{-1} and 8.0 mol m^{-2} day^{-1}, and low light with 50 μmol m^{-2} s^{-1} and 2.7 mol m^{-2} day^{-1}. The daily PPFD of the medium light condition corresponded to the light environments at the forest edge and the well-illuminated forest understorey, and that of the low light condition corresponds to deeply shaded forest understorey in the natural habitat. Light was supplied by halogen lamps (400 W Metal halide lamps; Toshiba Corporation, Tokyo, Japan) for 16 h per day, in which light incidence was increased or decreased in a stepwise manner (four steps) in the "morning" or "evening", respectively. About 30 min was provided for each light level in the "morning" and "evening" in the growth cabinet. A neutral density filter (shade cloth) was used to produce the medium and low light treatments. Soil water regimes were "wet" with daily irrigation and "dry" with intermittent irrigation per 7–10 days. Soil water content (vol/vol) was 26.3 ± 3.4% in the wet regime and 6.4 ± 3.6% in the dry regime at the evening of the day before irrigation. The dry regime was kept "wet" for 2 days after irrigation. Hereafter, the plants grown in wet regimes are referred to as high light, wet (HW), medium light, wet (MW), and low light, wet (LW) plants, and those grown in the dry regimes are as high light, dry (HD), medium light, dry (MD), and low light, dry (LD) plants. Nutrients were supplied in about 1-week interval for all plants at 1/1,000 strength solution of a commercial fertilizer, HYPONeX (Murakami Bussan CO., Tokyo, Japan). CO_2 concentration, temperature, and relative humidity of the air inside the growth cabinet were kept at 350 μmol mol^{-1}, 25/20 °C (day/night), and 70%, respectively. To avoid the influences of heterogeneity of environmental conditions in the growth cabinet to plant growth (Potvin and Tardif 1988; Potvin et al. 1990), the position of individual plants was rotated in the cabinet once a week.

Plant growth and morphology

Fresh weight of the corms was measured immediately before they were potted (fw_1) and after leaf senescence (fw_2). The fw_2 thus included the fresh weight of both the maternal corm and its daughter corms. Relative growth rate (RGR) of the corm was calculated as an index of individual biomass accumulation for one growth period. Dry weight (dw) of the corms was estimated from fw as $dw = 0.243 \times fw$ (Muraoka et al. 1997), and RGR (g g^{-1} one growth period^{-1}) was calculated as,

$$RGR = \ln dw_2 - \ln dw_1 \qquad (1)$$

Although the difference in moisture content could affect the ratio of dw to fw of the corms after leaf senescence and such a difference could affect the values of RGR, dw of the corms after leaf senescence was not measured by drying because we did not want to reduce the population size of this threatened species; the corms were reburied into the surface soil in the natural habitat after all the experiments were finished.

The processes of leaf bud break (started on 1 February 1997), leaf emergence, and senescence (yellowing) were monitored for the individual plants at intervals of 2 to 3 days. Leaf longevity was determined as the number of days between the date of leaf emergence and the date when more than 90% of whole leaf had senesced.

Leaf area (LA), basal diameter (BD), and length of pseudostem (L) were measured for the fully matured plants. Because we planned to measure the whole plant biomass accumulation for a full leaf life-span, we did not measure plant biomass directly during the life of a leaf. Leaf area was measured by tracing the leaf shape onto a transparent plastic sheet, which was then photocopied to a sheet of paper. The paper was cut along the photocopied leaf edge and then the area of the pieces were measured by a leaf area meter (AAM-7, Hayashi Denko, Japan). A single leaflet was harvested from each plant before the leaf yellowed, to minimize the effect of decreasing leaf area on whole plant carbon gain. After measuring the area of the leaflet with the leaf area meter, it was dried at 70 °C for more than 24 h and weighed to obtain LMA. LMA was used to estimate the gas-exchange parameters on a leaf dry mass basis and total leaf biomass {$M_{leaf} = LMA \times LA$}. The basal diameter and length of the pseudostem were used to estimate its biomass (M_{stem}), by an empirical equation {$M_{stem} = 0.034 \times [3.14 \times (BD/2)^2 \times L]$; $r^2 = 0.972$}. Root biomass was not measured to avoid destructive damage to plants.

Leaf gas exchange

Photosynthetic light-response curves and dark respiration (R) were measured for leaflets with a portable gas exchange measuring system (LI-6400; Li-Cor Inc., USA). Measurements were conducted periodically (7–10-day intervals) to find the maximum capacity of photosynthesis during the life of a leaf. For the plants in the dry regimes, measurements were taken both before and 1 day after the irrigation. Here-after, the measurements before the irrigation are referred to as HDd, MDd and LDd, whereas those after the irrigation are referred to as HDw, MDw, and LDw. Net CO_2 assimilation rate (A) and leaf conductance for water vapor (g_{lw}) were calculated according to Field et al. (1991). The clamp-on leaf chamber, which has a transparent window, allowed us to measure photosynthesis using the light (PPFD < 600 µmol m^{-2} s^{-1}) provided in the growth cabinet. Such measurements enabled us to measure photosynthesis and leaf conductance under the growth light qualities quickly for many sample plants (20–30 plants on a day). Incident PPFD beside the leaf chamber was measured with a photon sensor (LI-190SA). Incident PPFD to the leaf chamber was changed by combining the stepwise decrease of incident light in the growth cabinet and the shade cloth. Because high light incidence over 600 µmol m^{-2} s^{-1} in the growth cabinet increases the leaf temperature, we used the red-blue LED light source of the LI-6400 to measure the photosynthetic rate at high light intensities. Preliminary measurements showed there was no difference in photosynthetic rate between the two light sources in high light, if the leaf temperatures were similar. Leaves were illuminated at each level of PPFD at least 15 min before gas exchange measurements were made. CO_2 concentration, air temperature, and relative humidity of the air entering the leaf chamber were adjusted to those in the growth cabinet. Apparent quantum yield (α) was calculated by linear fitting of the photosynthetic light response curves at low PPFDs of 0–60 µmol m^{-2} s^{-1}. The light compensation point (LCP) was calculated as the x-intercept of this linear-regression. R was measured during the "night" (20 °C) in the growth cabinet.

Chlorophyll content

Measurements of chlorophyll content and chlorophyll a/b ratio were taken for the fully matured leaves. Leaf discs (0.785 cm^2) were collected and then transferred to a deep freezer (–80 °C) until use. In order to extract chlorophylls, the leaf discs were transferred to 4.9 ml of N,N-dimethylformamide, and held in darkness at 4 °C for 3 days. Chlorophyll concentration in the extract was measured with a spectrophotometer (UV-200S, Shimadzu, Japan), using the method of Porra et al. (1989).

Estimation of photosynthetic carbon gain

To evaluate the effect of physiological plasticity on carbon gain, the daily net carbon gain was estimated from the empirical data of leaf photosynthesis, dark respiration, and the leaf area of this study. The light-photosynthesis curves were fitted by the equation of Thornley (1976), as shown below:

$$A = \frac{\alpha I + GA_{max} - \sqrt{(\alpha I + GA_{max})^2 - 4\theta\alpha I GA_{max}}}{2\theta} - R \qquad (2)$$

where A is the net CO_2 assimilation rate on a leaf area basis at a given PPFD (I) in the growth cabinet, R is the dark

respiration rate, α is the apparent quantum yield of the light–photosynthesis curve, GA_{max} is the light-saturated rate of gross photosynthesis (i.e., $A_{max} + R$) and θ is the convexity factor. We assumed that A responded quickly to the changes of incident light in the growth cabinet. Moreover, based on a preliminary measurement, we also assumed that A did not decrease significantly at midday, as was found in natural high light (Muraoka et al. 2000). Night time (8 h) respiration was calculated by assuming that R was constant for 24 h. We did not take into account the influence of leaf orientation on light interception of the plants kept at high light intensities (Muraoka et al. 1998) because these plants did not incline their leaflets remarkably.

The effect of physiological light acclimation on the potential daily carbon gain on a leaf area basis (A_{day}/area) in the growth cabinet was evaluated by simulating the daily net photosynthesis of each leaf type under every light condition provided in the growth cabinet. In addition, the combined effects of photosynthetic and morphological light acclimations on the daily whole leaf carbon gain (A_{day}/plant) was examined using the data of gas exchange parameters averaged for three to five sample leaves with the leaf area of six plants from each of the growth regimes. These estimations were conducted for the wet treatment.

Influence of water stress on carbon gain through physiological changes were evaluated by calculating the daily carbon gain (A_{day}/area) of the HW, MW, LW, and watered or unwatered HD, MD and LD leaves under their original light conditions. The combined influence of physiological and morphological changes because of water stress on whole leaf carbon gain was evaluated by estimating A_{day}/plant from the gas exchange parameters averaged for three to five sample leaves and the leaf areas of six individual plants for each growth regime.

Results

Leaf longevity and biomass accumulation

Leaf longevity was shorter under higher light conditions and ranged from 105 (low-light plants) to 85 days (high-light plants), but a statistical significance was observed only between low-light and high-light plants ($P < 0.001$, Table 1). There was no significant difference in leaf longevity between wet and dry regimes.

RGR of both water treatments responded similarly to light availability (Table 1). RGR was the lowest in low-light plants, either in wet or dry regimes. RGR was slightly higher in MW plants than in HW plants, though the difference was not statistically significant. Drought treatment resulted in 30–85% reduction of RGR in all light regimes ($P < 0.0001$).

Table 1. Summary of plant growth characteristics grown under three light (low, medium, and high) and two soil water (wet and dry) conditions. Mean ± SD for six plants are represented for each growth regime (the data do not have SD values when they are for only one sample plant). *Bold values* indicate significant difference between the soil water regimes for each light regime (Student's t-test, $P < 0.05$). The lower case letters represent a significant difference among the light regimes for each soil water regime (Scheffe's a posteriori test, $P < 0.05$)

PPFD (mol m^{-2} day^{-1})		2.7 (Low)	8.0 (Medium)	26.8 (High)
Leaf longevity (days)	Wet	104.0 ± 10.3a	96.8 ± 9.2a	81.2 ± 15.1b
	Dry	107.0 ± 7.0a	96.0 ± 8.6b	88.0 ± 5.1c
RGR (g g^{-1} one growth period^{-1})	Wet	**0.52 ± 0.23a**	**1.09 ± 0.25b**	0.87 ± 0.36a,b
	Dry	**0.08 ± 0.27a**	**0.33 ± 0.26a,b**	0.55 ± 0.28b
LMA (g m^{-2})	Wet	24.7 ± 0.7a	26.8 ± 5.7a	45.6 ± 6.1b
	Dry	24.7 ± 4.0a	28.7 ± 2.0a	39.0 ± 8.2b
LA (cm^2)	Wet	255.9 ± 28.5a	270.9 ± 64.9a	**190.4 ± 47.9b**
	Dry	227.4 ± 38.0a	194.8 ± 58.0a	**125.3 ± 25.3b**
LA/M_{corm} (cm^2 g^{-1})	Wet	**502.5 ± 85.5a**	**463.0 ± 69.3a**	242.7 ± 40.7b
	Dry	**371.0 ± 80.6a**	**317.5 ± 49.7a**	201.7 ± 60.6b
M_{leaf} (g)	Wet	0.63 ± 0.08a	0.78 ± 0.22a	**0.87 ± 0.25a**
	Dry	0.55 ± 0.09a	0.54 ± 0.24a	**0.48 ± 0.14a**
M_{leaf}/M_{corm} (g g^{-1})	Wet	**1.24 ± 0.19a**	1.28 ± 0.45a	**1.10 ± 0.35a**
	Dry	**0.91 ± 0.22a**	0.87 ± 0.12a	**0.75 ± 0.16a**
L (cm)	Wet	58.2 ± 8.1a	55.2 ± 7.8a	35.2 ± 3.1b
	Dry	60.6 ± 3.3a	50.4 ± 5.0b	30.3 ± 4.9c
M_{stem} (g)	Wet	0.96 ± 0.14a	1.03 ± 0.21a	0.89 ± 0.14a
	Dry	1.06 ± 0.14a	0.94 ± 0.15a	0.37
M_{stem}/M_{corm} (g g^{-1})	Wet	0.44 ± 0.03a	0.40 ± 0.05a	0.26 ± 0.03b
	Dry	0.41 ± 0.03a	0.36 ± 0.02a	0.15
L/BD (cm cm^{-1})	Wet	75.6 ± 8.7a	70.1 ± 8.2a	37.9 ± 5.3b
	Dry	76.5 ± 7.9a	62.6 ± 9.0b	29.5
LA/BA (cm^2 cm^{-2})	Wet	559.7 ± 109.6a	**559.4 ± 139.2a**	274.5 ± 74.7b
	Dry	459.8 ± 85.1a	**368.1 ± 45.3b**	181.4

Leaf gas exchange characteristics and chlorophyll content

The light-saturated rate of net CO_2 assimilation on a leaf area basis (A_{max}/area) showed its maximum at a leaf age of 40 days. Figure 1 shows the light-response curves of A/area and the leaf conductance for water vapor (g_{lw}) of 40-day-old leaves. A/area was saturated at PPFD of approximately 300, 400, and 800 μmol m^{-2} s^{-1} in LW, MW and HW leaves, respectively (estimated by eye). The light response of g_{lw} also differed among the leaves from the different light regimes. If PPFD was <300 μmol m^{-2} s^{-1}, then the g_{lw} of MW and LW leaves increased rapidly with increasing incident PPFD as compared with HW leaves. If PPFD was >600 μmol m^{-2} s^{-1}, then g_{lw} of MW and LW leaves tended to decrease. Water stress reduced both A/area and g_{lw} at PPFD > 100 μmol m^{-2} s^{-1} for plants in all the light regimes.

Table 2 shows the effects of light and water regimes on the leaf physiological characteristics. In the leaves from wet regimes, A_{max}/area ($P = 0.0001$), R/area ($P < 0.05$), and LCP (NS) were the lowest under low light conditions. Apparent quantum yield tended to be higher under low light conditions. These values did not differ between high light and medium light conditions. Across the light conditions, A_{max}, on a leaf mass basis (A_{max}/mass), g_{lw}, and intrinsic water-use efficiency (A_{max}/g_{lw}) at light saturation did not differ significantly. In HDw, MDw and LDw leaves, A_{max}/area, A_{max}/mass, and g_{lw} were 30% lower than in HW, MW, and LW leaves ($P < 0.01$). Under Dw conditions, A_{max}/area tended to be higher at the higher light regime ($P < 0.05$). A_{max}/area, A_{max}/mass, and g_{lw} in the Dd regimes were about 50% or less than those in the wet regimes ($P < 0.001$). Chlorophyll content was higher at lower light regimes ($P < 0.0001$), but there was no effect from soil water regimes. The chlorophyll a/b ratio did not show a significant relationship with light and water treatments.

Biomass allocation and allometry of the aboveground parts

LMA increased with an increase in light availability and there was a significant difference between high-light and low-light plants ($P < 0.0001$, Table 1). Water availability did not affect LMA. LA was the smallest under high light conditions in both wet and dry regimes ($P < 0.0001$). LA in HD and MD plants were smaller than those in HW and MW plants by about 30% ($P < 0.05$, 0.06, respectively). The ratio of LA to initial corm biomass (M_{corm} = dw$_1$), which represents the investment in leaf area of the whole plant (we used M_{corm} as an index of plant size because we did not measure the plant biomass during plant growth), was also the smallest under high light conditions ($P < 0.001$). Water stress reduced it by 24% in low-light ($P < 0.05$) and by 30% in medium-light ($P < 0.01$) plants, but this was not the case in
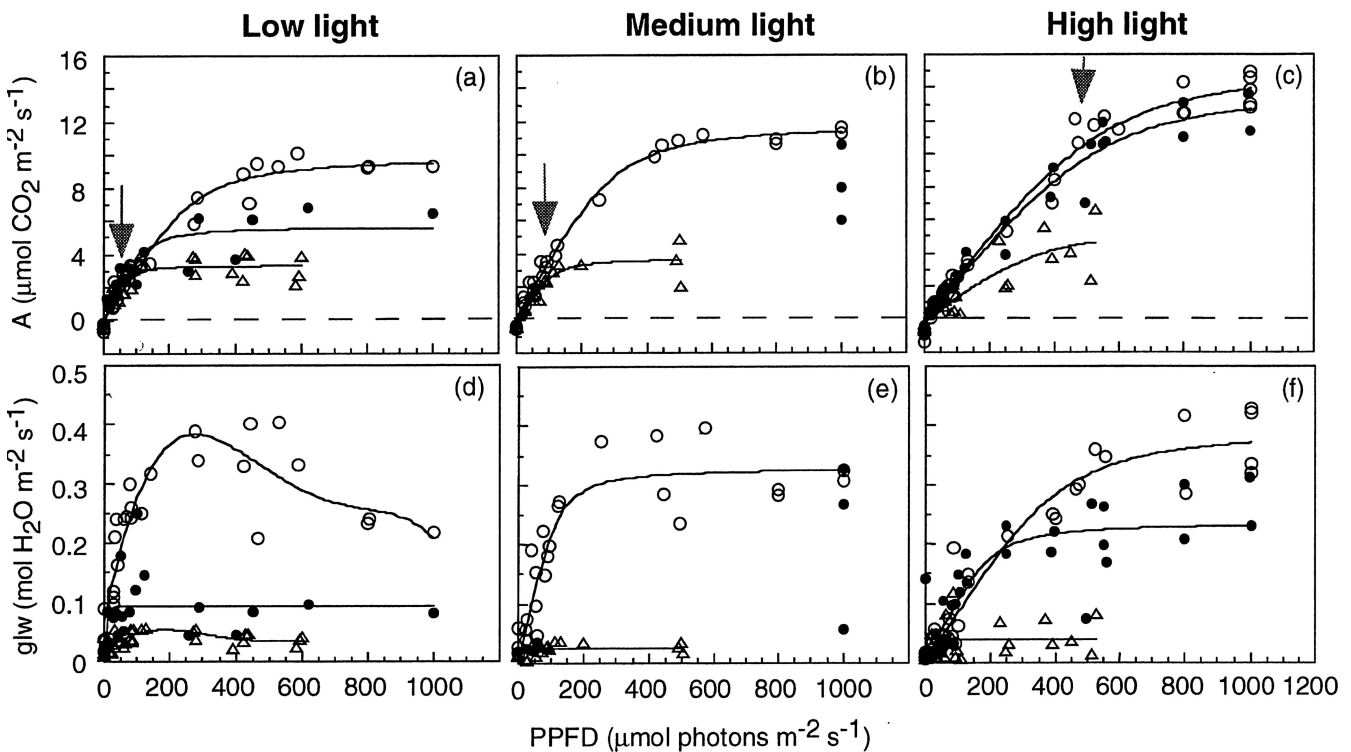

Fig. 1. Light-response curves of net CO_2 assimilation rate (A) and leaf conductance for water vapor (g_{lw}) for the leaves ($n = 3$–4) of *Arisaema heterophyllum* plants grown under three light and two soil water conditions. Data obtained at the leaf age of maximum photosynthetic capacity are plotted. *W* Wet, *empty circle*; *Dw* watered dry, *filled circle*; *Dd* dry, *triangle*. Lines for the net CO_2 assimilation rate were fitted by Eq. 3 of Thornley (1976) and those for leaf conductance were fitted by eye. *Arrows* indicate the maximum PPFD in each growth light regime

Table 2. Summary of leaf characteristics related to photosynthetic gas exchange for plants grown under three light and two soil water regimes (wet and dry). Leaf gas exchange measurements for the plants in dry regime were made before (*Dd*) and after (*Dw*) hydration. Mean ± SD for three to four leaves are represented for each light and soil water condition. The different lower case letters indicate the significant difference among the growth light regime under each soil water condition. Data that represent only a value have less than three sample leaves

PPFD (mol m^{-2} day^{-1})		2.7 (Low)	8.0 (Medium)	26.8 (High)
A_{max}/area (µmol m^{-2} s^{-1})	Wet	9.61 ± 0.34a	12.03 ± 0.64b	13.30 ± 1.05b
	Dw	6.74 ± 0.93a	8.20 ± 2.32a	11.20 ± 1.49b
	Dd	3.71 ± 0.56a	3.75 ± 1.16a	5.23 ± 1.33a
A_{max}/mass (µmol g^{-1} s^{-1})	Wet	0.40 ± 0.02a	0.52 ± 0.14b	0.31 ± 0.03a
	Dw	0.26 ± 0.05a	0.29 ± 0.08a	0.32 ± 0.12a
	Dd	0.14 ± 0.02a	0.13 ± 0.04a	0.115 ± 0.03a
R/area (µmol m^{-2} s^{-1})	Wet	0.47 ± 0.19a	0.54 ± 0.34a	0.85 ± 0.15b
	Dw	0.25	0.34	0.63 ± 0.24
	Dd	0.25 ± 0.07a	0.36 ± 0.14a	0.36 ± 0.20a
α (µmol CO$_2$ µmol^{-1} photons)	Wet	0.061 ± 0.019a	0.048 ± 0.001a	0.042 ± 0.010a
	Dw	0.067	0.039	0.034 ± 0.008
	Dd	0.060 ± 0.020a	0.029 ± 0.003a	0.018 ± 0.006a
LCP (µmol m^{-2} s^{-1})	Wet	9.1 ± 2.7a	10.4 ± 3.7a	17.1 ± 5.0a
	Dw	3.7	8.7	15.7 ± 7.3
	Dd	4.1 ± 2.7a	9.7 ± 4.7a	14.5 ± 6.6a
g_{lw} (mol m^{-2} s^{-1})	Wet	0.249 ± 0.052a	0.284 ± 0.036a	0.234 ± 0.059a
	Dw	0.107 ± 0.042a	0.172 ± 0.105a	0.223 ± 0.063a
	Dd	0.041 ± 0.014a	0.041 ± 0.021a	0.055 ± 0.020a
A_{max}/g_{lw} (µmol CO$_2$ mol^{-1} H$_2$O)	Wet	38.6 ± 6.5a	42.4 ± 17.8a	56.8 ± 17.8a
	Dw	63.0 ± 22.1a	47.8 ± 22.1a	50.2 ± 23.7a
	Dd	90.5 ± 40.0a	91.5 ± 55.2a	95.1 ± 66.5a
Chl $a+b$ (mmol m^{-2})	Wet	0.645 ± 0.149a	0.693 ± 0.123a	0.460 ± 0.070b
	Dry	0.711 ± 0.121a	0.672 ± 0.08a	0.470 ± 0.073b
Chl a/b	Wet	3.14 ± 0.26a	3.77 ± 1.13a	3.62 ± 0.56a
	Dry	3.48 ± 0.54a	2.95 ± 0.23a	3.33 ± 0.14a

high-light plants. Leaf biomass (M_{leaf}) did not differ among the light regimes, though it was decreased by 13–44% because of water stress at all light regimes (Table 1). The index of biomass investment to leaf, expressed as the ratio of M_{leaf} to M_{corm}, did not differ among the light regimes. However, M_{leaf}/M_{corm} in dry plants were 30% lower than in the wet treatment at all light regimes ($P < 0.05$).

Stem biomass (M_{stem}) did not differ among the light and water regimes (Table 1), but M_{stem}/M_{corm} was the lowest in the high light regime. Stem length (L) and the ratio of L to BD, which represents stem morphology, were smaller under higher light conditions ($P < 0.0001$), though they were not affected significantly by water stress. The ratio of LA to stem basal area (BA), which represents the load for water transport from the roots through the stem to the leaf (i.e., the inverse of the "Huber value"; Tyree and Ewers 1991), was lower under higher light ($P < 0.001$) and in dry regimes ($P < 0.01$).

Effects of photosynthetic light acclimation and soil water on carbon gain

Effects of light acclimation were examined for plants in the wet regime (Fig. 2a, b). Under simulated low light conditions, both A_{day}/area and A_{day}/plant were the largest for plants grown in the low light treatment compared with the other treatments. Under simulated medium and high light conditions, A_{day}/area did not differ among plants grown in any treatment, but A_{day}/plant was the smallest for plants grown in the high light treatment.

Soil water deficit resulted in significant decreases of 60% in A_{day}/area in HDd and MDd leaves compared with those in HW and MW leaves ($P < 0.0001$), but this was not the case for LDd leaves (Fig. 2c). The difference in estimated carbon gain among the three light conditions was significantly larger under wet or Dw conditions than under Dd conditions. The differences of A_{day}/plant between the water regimes tended to be larger at greater lighter intensities, and A_{day}/plant in HD and MD plants was lower than HW and MW plants by 70–80% ($P < 0.0001$, Fig. 2d). Under wet or Dw conditions, A_{day}/plant was larger in plants grown in higher light environments ($P < 0.0001$). However, under Dd conditions, A_{day}/plant was almost similar across the light conditions.

Discussion

Contribution of light acclimations to biomass accumulation

RGR and estimated carbon gain reached their maximum under medium light conditions and they were close to those

Fig. 2a–d. Effects of light acclimation responses (**a**, **b**) and soil water conditions (**c**, **d**) on potential carbon gain of *Arisaema heterophyllum* plants. Carbon gain was estimated on a leaf area basis (A_{day}/area, mean ± SD for three to five leaves) and in whole leaf (A_{day}/plant, mean ± SD for six plants). For panels **a** and **b**, the different *capital letters* indicate the significant difference among the different simulated daily PPFD for each leaf type (Scheffe's a posteriori test, $P < 0.05$) and the different *lower case letters* indicate significant difference among different leaf types under the same simulated daily PPFD (Scheffe's a posteriori test, $P < 0.05$). For panels **c** and **d**, the *different capitals* indicate significant difference among the growth light conditions for each soil water condition (Scheffe's a posteriori test, $P < 0.05$) and the different *lower case letters* indicate the significant difference among the soil water conditions for each growth light condition (Scheffe's a posteriori test, $P < 0.05$)
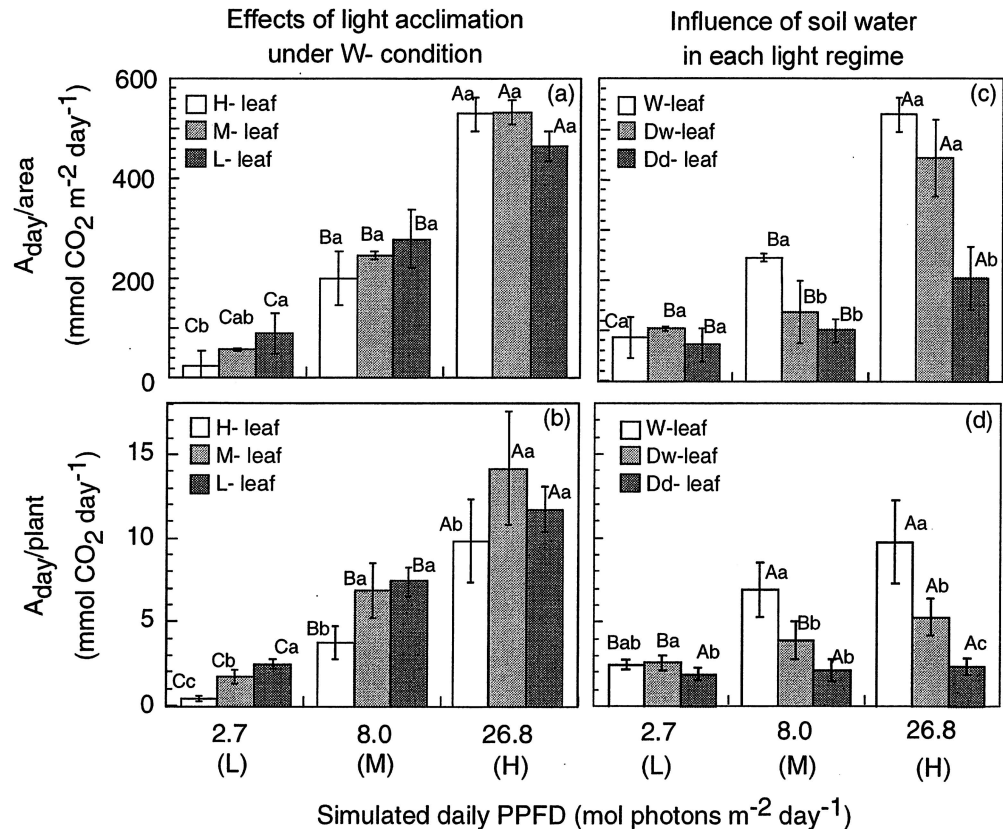
under high light conditions (Table 1, and Fig. 2). This result is consistent with our previous study in natural habitats, which showed that RGR reached its maximum level at 20–30% of diffuse transmittance (Muraoka et al. 1997). Similar results of whole plant biomass accumulation reaching its maximum at intermediate light environments have been reported for *Alocasia macrorrhiza* (L.) G. Don (Sims et al. 1994), for West African pioneer and non-pioneer shade tolerant tree species (Veenendaal et al. 1996), and for rain forest tree species (Poorter 1999; Valladares et al. 2000). The carbon gains under medium light and low light conditions were supported by the leaf photosynthetic and morphological adjustment to the light environment (Table 2, Fig. 2). The higher A/area at low PPFD and the lower R/area resulted in larger A_{day}/area of LW and MW leaves than that of HW leaves under simulated low light (by +200%) and medium light (by +40%) conditions (Fig. 2). These consequences suggest that a physiological acclimation to low light contributes to the whole-plant carbon gain. The high g_{lw} at low PPFD in low-light leaves is also important as an acclimation to low light environments because it increases light utilization efficiency.

Larger LA (and LA/M_{corm}) under low light and medium light conditions, a morphological response to low light, also contributed to whole-plant carbon gain. It has been frequently suggested that a high ratio of leaf area to plant biomass (leaf-area ratio) plays an important role in carbon gain, especially in a light-limited environment (Popma and Bongers 1988; Lambers and Poorter 1992; Walters et al.

1993b; Kitajima 1994; Sims and Pearcy 1994; Poorter 1999). Leaf size can give the plant a dilemma, i.e., increasing carbon gain, but also increasing water loss. With the same M_{leaf} per plant biomass, or R/mass, an increase in leaf area would increase the amount of light capture, which will lead to an increase of whole plant carbon gain. On the other hand, a larger LA inevitably increases transpirational water loss, especially in high light, if the leaf orientations are the same (Parkhurst and Louks 1972; Givnish 1979). *Arisaema heterophyllum* exhibited no significant change in leaf biomass allocation under different light regimes. In addition, A_{max}/mass did not differ. This species increased potential carbon gain through increasing LMA and A_{max}/area in response to high light. Our estimation showed that physiological and morphological acclimation to high light may result in an equivalent increase in A_{day}/area by 10% as compared with that in LW leaves when simulated under high light conditions (Fig. 2). Thus, the reduced leaf area in *A. heterophyllum* in high light seems to be beneficial to reduce transpirational water loss.

Leaf life-span also is one of the most important factors that determines the carbon gain of a leaf and a whole plant, especially in a light-limited environment (Chabot and Hicks 1982; Jurik and Chabot 1986; Williams et al. 1989; Kikuzawa 1991; Sims et al. 1994). In this study, leaf longevity responded to the given light environment, but there was no significant effect of water availability on leaf longevity (Table 1). This result is consistent with that observed in natural populations (Muraoka et al. 1997).

Influences of water availability on light acclimation and biomass accumulation

The drought treatment showed striking influences on photosynthetic capacity, morphology, and biomass accumulation of *A. heterophyllum*, but acclimation traits and the degree of influence differed among the light conditions. The causes of the reduction of RGR by water stress are different among the light regimes (Fig. 2). In the low light regime, LA was the factor that reduced RGR, whereas both photosynthetic rate and LA reductions affected RGR in medium and high light regimes in response to lower water availability. The reduction in LA as well as M_{leaf} is a general response of plants subjected to drought (Schulze 1986). In our experiment, reduction of RGR through water stress was the largest in the medium light regime and somewhat less in the low light regime. It is possible that, in the low and medium light regimes, the larger L/BD (Table 1) and/or larger LA/BA, which are the adaptive responses of shoot architecture to low light environment in an understorey vegetation, could have increased hydraulic resistance and reduced leaf water potential (Tyree and Ewers 1991) and thereby inhibited leaf growth.

The large decrease of A_{day}/plant in HD and MD leaves was caused by the low A_{max}/area due to small g_{lw} and LMA; g_{lw} affects both the instantaneous photosynthetic activity and the capacity, and LMA affects the capacity. The smaller LMA in HD plants should be due to the inhibited expansion of leaf cells during leaf development (Bradford and Hsiao 1982; Ellsworth and Reich 1992; Abrams et al. 1994). In contrast, as can be seen by the small effect of soil water on α in low-light leaves (Table 2), the A_{day}/area of LD plants was not decreased remarkably by drought.

Conclusion

In *Arisaema heterophyllum*, water stress reduced the morphological and photosynthetic capacity for carbon gain, but the plants did not alter their general response to light. The morphological and physiological plasticity of *A. heterophyllum* leaves increases carbon gain under low light conditions, while they are likely to save excess transpiration in high light or in drought. This plasticity to light and water availability is beneficial to survive in natural habitats where environmental conditions vary spatially and temporally.

Our results also suggest that a "suitable" environment for plant growth depends on the carbon-based cost-benefit relationship and water use in a plant. The low light environment that occurs below dense stands of *Pleiobrastus chino* Makino and *Solidago altissima L.* would lead to an extremely small or negative RGR in *A. heterophyllum*. In contrast, high light conditions, such as those in the open site, would strengthen the effect of water availability. Moreover, *A. heterophyllum* can not acclimate its leaf water relation characteristics to the contrasting light environments (H. Muraoka, personal observation). Thus, in *A. heterophyllum*, medium light and wet conditions are the most favorable for biomass accumulation, which can be found in the forest edge or relatively well illuminated forest understorey of the natural habitat (Muraoka et al. 1997).

References

Abrams MD, Kubiske ME, Mostoller SA (1994) Relating wet and dry year ecophysiology to leaf structure in contrasting temperate tree species. Ecology 75:123–133

Björkman O (1981) Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology I. (Encyclopedia of plant physiology, NS, vol 12A). Springer, Berlin Heidelberg New York, pp 57–107

Bradford KJ, Hsiao TC (1982) Physiological responses to moderate water stress. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology II (Encyclopedia of plant physiology, NS, vol 12B). Springer, Berlin Heidelberg New York, pp 263–324

Chabot BF, Hicks DJ (1982) The ecology of leaf life spans. Annu Rev Ecol Syst 13:229–259

Chazdon RL, Kaufmann S (1993) Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. Funct Ecol 7:385–394

Davies WJ, Gowing DJG (1999) Plant responses to small perturbations in soil water status. In: Press MC, Scholes JD, Barker MG (eds) Physiological plant ecology. Blackwell, Oxford, pp 67–89

Ellsworth DS, Reich PB (1992) Water relations and gas exchange of *Acer saccharinum* seedlings in contrasting natural light and water regimes. Tree Physiol 10:1–20

Environmental Agency, Government of Japan (1997) The Japanese plant red list. Tokyo

Field CB, Ball JT, Berry JA (1991) Photosynthesis: principles and field techniques. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW (eds) Plant physiological ecology. Chapman and Hall, London

Givnish TJ (1979) On the adaptive significance of leaf form. In: Solbrig OT, Jain S, Johnson GB, Raven PH (eds) Topics in plant population biology. Columbia University Press, New York

Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. Aust J Plant Physiol 15:63–92

Hsiao TC (1973) Plant responses to water stress. Annu Rev Plant Physiol 24:519–570

Huante P, Rincon E (1998) Response to light changes in tropical deciduous woody seedlings with contrasting growth rates. Oecologia 113:53–66

Hunt R, Lloyd PS (1987) Growth and partitioning. New Phytol 106:235–249

Jurik TW, Chabot BF (1986) Leaf dynamics and profitability in wild strawberries. Oecologia 69:296–304

Kikuzawa K (1991) A cost–benefit analysis of leaf habit and leaf longevity of trees and their geographical pattern. Am Nat 138:1250–1263

King DA (1991) Correlations between biomass allocation, relative growth rate and light environment in tropical forest saplings. Funct Ecol 5:485–492

Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. Oecologia 98:419–428

Laing WA, Greere DH, Schnell TA (1995) Photoinhibition of photosynthesis causes a reduction in vegetative growth rates of dwarf bean (*Phaseolus vulgaris*) plants. Aust J Plant Physiol 22:511–520

Lambers H, Poorter H (1992) Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. In: Begon M, Fitter AH (eds) Advances in ecological research. Academic Press, London

Muraoka H, Tang Y, Koizumi H, Washitani I (1997) Combined effects of light and water availability on photosynthesis and growth of

Arisaema heterophyllum in the forest understorey and an open site. Oecologia 112:26–34

Muraoka H, Takenaka A, Tang Y, Koizumi H, Washitani I (1998) Flexible leaf orientations of *Arisaema heterophyllum* maximize light capture in a forest understorey and avoid excess irradiance at a deforested site. Ann Bot 82:297–307

Muraoka H, Tang Y, Terashima I, Koizumi H, Washitani I (2000) Contributions of diffusional limitation, photoinhibition and photorespiration to midday depression of photosynthesis of *Arisaema heterophyllum* in the natural high light. Plant Cell Environ 23:235–250

Nobel PS (1976) Photosynthetic rates of sun vs. shade leaves of *Hyptis emoryi* Torr. Plant Physiol 58:218–223

Oshima K, Tang Y, Washitani I (1997) Spatial and seasonal patterns of microsite light availability in a remnant fragment of deciduous riparian forest and their implication in the conservation of *Arisaema heterophyllum*, a threatened plant species. J Plant Res 110:321–327

Osmond CB (1983) Interactions between irradiance, nitrogen nutrition, and water stress in the sun-shade responses of *Solanum dulcamara*. Oecologia 57:316–321

Osunkoya OO, Ash JE (1991) Acclimation to a change in light regime in seedlings of six Australian rainforest tree species. Aust J Bot 39:591–605

Parkhurst DF, Louks OL (1972) Optimal leaf size in relation to environment. J Ecol 60:505–537

Pearcy RW, Sims DA (1994) Photosynthetic acclimation to changing light environments: scaling from the leaf to the whole plant. In: Caldwell MM, Pearcy RW (eds) Exploitation of environmental heterogeneity by plants. Academic Press, San Diego, pp 145–174

Pearcy RW, Valladares F (1999) Resource acquisition by plants: the role of crown architecture. In: Press MC, Scholes JD, Barker MG (eds) Physiological plant ecology. Blackwell, Oxford, pp 45–66

Poorter L (1999) Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. Funct Ecol 13:396–410

Popma J, Bongers F (1988) The effect of canopy gaps on growth and morphology of seedlings of rain forest species. Oecologia 75:625–632

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochem Biophys Acta 975:384394

Potvin C, Tardif S (1988) Sources of variability and experimental designs in growth chambers. Funct Ecol 2:123–130

Potvin C, Lechowicz MJ, Bell G, Schoen D (1990) Spatial, temporal, and species-specific patterns of heterogeneity in growth chamber experiments. Can J Bot 68:499–504

Rice AS, Bazzaz FA (1989) Growth consequences of plasticity of plant traits in response to light conditions. Oecologia 78:508–512

Schulze ED (1986) Whole-plant responses to drought. Aust J Plant Physiol 13:127–141

Sims DA, Peracy RW (1989) Photosynthetic characteristics of a tropical forest understorey herb, *Alocasia macrorrhiza*, and a related crop species, *Colocasia esculenta* grown in contrasting light environment. Oecologia 79:53–59

Sims DA, Pearcy RW (1994) Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole-plant performance – 1. Carbon balance and allocation at different daily photon flux densities. Plant Cell Environ 17:881–887

Sims DA, Gebauer RLE, Pearcy RW (1994) Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole-plant performance. 2. Simulation of carbon balance and growth at different photon flux densities. Plant Cell Environ 17:889–900

Terashima I, Hikosaka K (1995) Comparative ecophysiology of leaf and canopy photosynthesis. Plant Cell Environ 18:1111–1128

Thompson WA, Kridemann PE, Craig IE (1992a) Photosynthetic response to light and nutrients in sun-tolerant and shade tolerant rainforest trees. I. Growth, leaf anatomy and nutrient content. Aust J Plant Physiol 19:1–18

Thompson WA, Kridemann PE, Craig IE (1992b) Photosynthetic response to light and nutrients in sun-tolerant and shade tolerant rainforest trees. II. Leaf gas exchange and component processes of photosynthesis. Aust J Plant Physiol 19:19–42

Thornley JHM (1976) Mathematical models in plant physiology. Academic Press, London

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol 119:345–360

Valladares F, Pearcy RW (1997) Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. Plant Cell Environ 20:25–36

Valladares F, Pearcy RW (1998) The functional ecology of shoot architecture in sun and shade plants of *Heteromeles arbutifolia* M. Roem, a Californian chaparral shrub. Oecologia 114:1–10

Valladares F, Wright SJ, Lasso E, Kitajima K, Pearcy RW (2000) Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. Ecology 81:1925–1936

Veenendaal EM, Swaine MD, Lecha RT, Walsh MF, Abebrese IK, Owusu-Afriyie K (1996) Responses of West African forest tree seedlings to irradiance and soil fertility. Funct Ecol 18:501–511

Walters MB, Kruger EL, Reich PB (1993a) Growth, biomass distribution and CO_2 exchange of northern hardwood seedlings in high and low light: relationships with successional status and shade tolerance. Oecologia 94:7–16

Walters MB, Kruger EL, Reich PB (1993b) Relative growth rate in relation to physiological and morphological traits for northern hardwood tree seedlings: species, light environment and ontogenic considerations. Oecologia 96:219–231

Williams K, Field CB, Mooney HA (1989) Relationships among leaf construction cost, leaf longevity, and light environment in rain-forest plants of the genus Piper. Am Nat 133:198–211