Adaptive Significance of Leaf Morphological and Physiological Acclimations in *Arisaema heterophyllum* to Contrasting Light Environments

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Contents

Abbreviations

A, A_{area}	photosynthetic rate on a leaf area basis
A/g_l	instrinsic water use efficiency
A_{mass}	photosynthetic rate on a leaf dry weight basis
A_{max}	light saturated rate of photosynthesis on a leaf area basis
A_{350}	photosynthetic rate at Ci of 350 μmol mol^{-1}
ANOVA	analysis of variance
α	inclination angle of a leaflet
β	angle of leaflet folding
Ca	CO_2 concentration of air entering the leaf chamber
Cc, $Ci*$	CO_2 mole fraction at the catalystic site of Rubisco
CE	carboxylation efficiency
Ci	intercellular CO_2 concentration
$Ci^{\#}$	'true' intercellular CO_2 concentration
D	direction of a light receiving surface
D_{Leaf}	mean direction of individual leaflets on a single plant
D_{max}	direction for maximizing diffuse light capture at a given microsite
DR	dark respiration rate
dw	dry weight
E	transpiration rate
F	steady-state chlorophyll fluorescence yield under actinic light
Fm	maximum fluorescence yield of a dark-adapted leaf
Fm'	maximum fluorescence yield under the actinic light
Fo	minimal fluorescence yield of a dark-adapted leaf
Fv	variable fluorescence yield of a dark-adapted leaf
Fv/Fm	maximum photochemical efficiency (quantum yield of PS II)
$\Delta F/Fm'$	effective quantum yield of PS II electron transport
fw	fresh weight
g_{cw}	cuticular conductance to water vapor
g_{ic}	leaf internal conductance to CO_2
g_{lw}, g_l	leaf conductance to water vapor
g_{sc}	stomatal conductance to CO_2
g_{sw}	stomatal conductance to water vapor
g_{tc}	conductance of CO_2 from boundary layer through stomata
$\Gamma*$	CO_2 compensation point in the absence of non-photorespiratory CO_2 evolution
ISL	index of stomatal limitation to assimilation rate
J_C	electron flow devoted to carboxylation of RuBP
J_O	electron flow devoted to oxygenation of RuBP

J_T	total linear electron flow
LCE	light capture efficiency
LCP	light compensation point
LMA	leaf mass (dry weight) to area ratio
O	O_2 mole fraction in air
PPFD	photosynthetically active photon flux density
PS II	photosystem II
QE	apparent quantum yield of photosynthetic rate
q_N	non-photochemical quenching coefficient
θ	azimuth
Rd	'day' respiration
RGR	relative growth rate
R-PPFD	relative PPFD under diffuse light conditions
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	ribulose-1,5-bisphosphate
S	specificity factor of Rubisco
T_L	leaf temperature
V_C	carboxylation rate of RuBP
V_O	oxygenation rate of RuBP
VPD	(leaf to air) vapor pressure deficit
WUE	water use efficiency calculated as A/E

Abstract

All the stages in plant life-cycle, i.e., seedling establishment, biomass accumulation, and both vegetative and sexual reproductions depend on photosynthetic matter production in leaves. Light, as energy resource for photosynthesis, thus has a great importance for any autotrophic plants. Since a plant is not able to relocate itself for favorable light condition, acclimation to the local light environments would have high adaptive significance. The objective of the present study is to clarify the adaptive significance of leaf-level acclimation to a given light environment through several types of ecophysiological approaches such as *in situ* measurements, laboratory experiments and computer simulations. I focused on the acclimation of *Arisaema heterophyllum* Blume (Araceae) to contrasting light environments of an understory of a riparian forest and an adjacent deforested open site. Investigations were made to understand how the acclimation occurs at several levels from biochemistry to geometry, and what the ecological significance of the acclimation is for the growth and survival for *A. heterophyllum.*

In chapter 1, photosynthetic characteristics, leaf longevity and biomass accumulation were studied for *A. heterophyllum* at the forest site and deforested site for three years to understand the combined influence of light and water availability. Precipitation varied considerably among the three years. Despite the difference in water availability among the years, dry years of 1994 and 1996, and a wet year of 1995, the species showed a strong acclimation to the contrasted light environments: a higher light saturated assimilation rate on the leaf area basis, and a larger leaf-mass to area ratio (LMA) and a higher relative growth rate (RGR) were recorded for the plants at the deforested site. While the positive correlation between individual RGR and microsite light availability was found in the wet year, no correlation was found in the dry years, and mean RGR was significantly lower in the dry year for both sites. Leaf longevity,

photosynthetic capacity on the leaf mass basis, dark respiration rate and leaf conductance to water vapor, especially in the plants at the deforested site, varied considerably from year to year, probably depending on the water availability of the year. The deforested site plants showed a lower photosynthetic rate and leaf conductance under the unwatered than under the watered conditions. These results suggest that the yearly water availability may strongly affect light-acclimation and annual RGR of the herbaceous species, even in natural habitats under a mesic climate condition.

In chapter 2, effects of flexible leaflet orientations on light capture and photosynthesis were investigated for *A. heterophyllum* grown in two contrasting light environments: the forest site and the deforested site. Leaf orientations are determined by inclination of leaflet midvein and folding of leaflet blade. Leaves were flatter with smaller angles of inclination at the forest site than at the deforested site. Directions (angular altitude and azimuth) of leaf surfaces of the forest site plants were close to those predicted to maximize diffuse light capture at each microsite, which were determined by the analysis of a hemispherical canopy photograph. Mean light capture efficiency (the ratio of actual diffuse light capture at a leaf to maximal receivable light) reached 98 %. In contrast, marked leaflet folding occurred at the deforested site. The degree of folding varied diurnally with the maximum around noon. Computer simulations showed that PPFDs (photosynthetically active photon flux density) over the photosynthetic saturation level of *A. heterophyllum* can be effectively reduced by increasing the slope of leaflet surfaces. The importance of decreasing excess irradiance to avoid photoinhibition and to maintain high rates of photosynthesis was confirmed by artificially constraining horizontal leaves at the deforested site.

In chapter 3, *A. heterophyllum* plants grown at the forest and deforested sites were transferred to an unshaded experimental garden in early summer and diurnal changes in photosynthetic gas exchange and chlorophyll fluorescence were measured to reveal stomatal and non-stomatal limitations to diurnal assimilation. Assimilation rate (A) and leaf conductance to water vapor reached their maximum in the morning and then decreased to about half the maximum values, but often showed a small recovery in the late afternoon. A at internal CO_2 concentration of 350 µmol mol^{-1} (A_{350}) was estimated from linear electron transport rate (J_T) estimated fluorometrically, and specificity factor of Rubisco (S), and an index ($1 - A / A_{350}$) was used to quantitatively evaluate the stomatal limitation to assimilation. In sun leaves under photosynthetically saturated light conditions, ($1 - A / A_{350}$) after the 'peak' of diurnal assimilation rate was 70 % higher than that before the 'peak', while in shade leaves the value after the 'peak' was about 20% higher than that before the 'peak'. Photochemical efficiency of photosystem II ($\Delta F/Fm'$) and J_T were considerably lower in shade leaves than in sun leaves, especially at and after the assimilatory 'peak'. In shade leaves, A positively depended on J_T at PPFD above 500 µmol m^{-2} s^{-1} throughout the day, but this was not the case in sun leaves. Partitioning of electron flows between the carboxylation (J_C) and oxygenation (J_O) of RuBP was estimated from A and J_T. In sun leaves, the J_O/J_T ratio was significantly higher after the assimilatory 'peak', while, in shade leaves, little difference was observed between before and after the 'peak'. Photorespiratory CO_2 efflux in the absence of air CO_2 was about three-times higher in sun leaves than that in shade leaves. The present data suggest that the midday depression of assimilation rate was mainly attributed to the increased rate of photorespiration brought about stomatal closure. The large capacity of photorespiration, which is accompanied with photosynthetic capacity, is necessary for leaves to avoid photoinhibitory damages and/or to tolerate high leaf temperature and water stress often occurring under excess light, and the light regime during leaf development strongly affects the avoidance response ability of the leaf.

From these investigations, I conclude that adaptive significance of leaf-level acclimation to the given light environment is the abilities to enable plants to minimize the habitat-specific stresses on photosynthetic productivity, i.e., shortage of light in the forest understory or stresses accompanying excess irradiance at the deforested site. The most important data obtained in the present study are those suggesting that the various aspects of acclimation to natural high light environment were significant to avoid photoinhibitory damages and severe water deficit and to maintain a high leaf photosynthetic activity.

General introduction

All the stages in plant life-cycle, i.e., seedling establishment, biomass accumulation, and both vegetative and sexual reproductions depend on photosynthetic matter production in leaves. Light, as energy resource for photosynthesis (photosynthetically active photon flux), thus has a great importance for any autotrophic plants. Since a plant is not able to relocate itself for acquiring light, the strategies for acquirement and utilization of light resource other than relocation become critical for the plant to photosynthesize to grow or to survive. Leaves exposed to sunlight at the canopy surface can receive sufficient or even excessive sunlight, while those in the forest understory, or deep within canopies often receive limited amount of light depending on their location since light decreases exponentially in its transmittance through canopy (Monsi and Saeki 1953; Anderson 1964). In some cases, light transmittance can be as low as less than 1 % in understories (Baldocchi and Collineau 1994; Tang 1997). Moreover, leaves often experience a large spatial and temporal change of light due to heterogeneity in canopy structure and foliage dynamics (Tang 1997). Light capture largely depends on the geometrical features of a plant body, while utilization depends on leaf anatomical, biochemical and physiological characteristics. Thus phenotypic responses to light environments of the plants inhabiting relatively wide range of light regime can be shown at various levels of plant organizations ranging from membrane structure to community, and at various time scales varying from less than a second to years in nature (Osmond and Chow 1988).

It has been long suggested that plants develop their morphological and physiological features in a way to maximize the photosynthetic productivity under a given light environment. This type of plasticity has been called 'light acclimation' (for reviews see Boardman 1977; Björkman 1981; Anderson and Osmond 1987; Givnish 1988; Pearcy and Sims 1994; Björkman and Demmig-Adams 1995; Terashima and

Hikosaka 1995). The studies on plant acclimation to light extend over wide ranges of plant traits and responses, including leaf anatomical (Nobel 1980; Sims and Pearcy 1992; Chazdon and Kaufmann 1993), biochemical and physiological responses (Bazzaz and Carlson 1982; Terashima and Inoue 1984, 1985; Koizumi 1985; Koizumi and Oshima 1985; Pearcy 1987; Field 1988; Harrington, Brown and Reich 1989a; Sims and Pearcy 1989, 1991; Chazdon 1992; Evans 1993; Tang *et al.* 1994; Hikosaka and Terashima 1995; Valladares and Pearcy 1997), their interactions (Björkman and Holmgren 1963; Nobel, Zaragoza and Smith 1975; Bauer and Thoni 1988; Sims and Pearcy 1989; Terashima 1989; Nishio, Sun and Vogelmann 1994), geometry and biomass allocation (King 1991; Takenaka 1994; Ackerky and Bazzaz 1995), and their consequences on leaf longevity and/or potential of whole plant carbon gain (McCree and Troughton 1966; Williams, Field and Mooney 1989; Sims, Gebauer and Pearcy 1994; Ackerky and Bazzaz 1995).

However, few studies have addressed how the light acclimation of leaves enables a plant to maximize the photosynthetic production and biomass accumulation under a given natural environmental condition (Chazdon 1986; Popma and Bongers 1988; Harrington *et al.* 1989a, b). In the present study I have attempted to clarify the ecological significance of leaf-level acclimation to a given natural light environment through several types of ecophysiological approaches such as *in situ* measurements, laboratory experiments and computer simulations.

Physiological and morphological acclimation to low light condition

For the leaves under low light availability such as those in a forest understory, which are often referred to as 'shade leaves', it would be important to maximize light capture and to utilize the light efficiently. To maximize light capture physiologically and/or biochemically, more nitrogen tends to be invested for light harvesting systems in shade leaves compared to those in sun leaves (Evans 1989; Hikosaka and Terashima 1995; Kimura *et al.* 1998).

Anatomically, shade leaves tend to be thinner with a few leaf mesophyll layers, which may contribute to the efficient transferring of light energy into the chloroplasts located in the lower side of the leaf (Nobel and Hartsock 1981; Sims and Pearcy 1992; Nishio *et al.* 1994). Morphological responses to increasing light capture have been also appreciated with respect to whole plant architecture. For example, plants under low light conditions tend to invest their biomass for enlarging leaf area i.e., light capturing surface area (Rice and Bazzaz 1989a; King 1991, 1994; Popma and Bongers 1991). Since light availability of the leaves in the forest understory microsites depends on over-story canopy architecture which is always spatially heterogeneous (Baldocchi and Collineau 1994; Bazzaz and Wayne 1994), light capture depends largely on the geometry of the plants regarding leaf position and inclination (Ehleringer and Werk 1986). Significance of plant geometry with respect to leaf and/or branch arrangements in effectual light utilization under a heterogeneous light environment has been recently emphasized through the investigations of relationships between plant architecture and directionality of light incidence (Chazdon, Williams and Field 1988; Takenaka 1994; Ackerly and Bazzaz 1995; Pearcy and Yang 1996; Takenaka, Inui and Osawa 1998).

In order to further deepen our understanding of the adaptive significance of the leaf morphological and/or physiological acclimation to the low light environment, it is necessary to evaluate the contribution of leaf responses to photosynthetic productivity and/or biomass accumulation *in situ* in the natural habitat for the plant where there are great spatial and temporal heterogeneities of light incidence.

Physiological and morphological acclimation to high light condition

Leaves exposed to a high light environment such as those in the uppermost layer of canopy or in an unshaded place, i.e., so called 'sun leaves', have been thought to tend to develop morphologically and physiologically in a way to enhance the photosynthetic capacity as to utilize the high light incidence sufficiently (Björkman 1981; Givnish 1988). Increasing leaf thickness may contribute to increasing photosynthetic apparatus per leaf area (Pearcy and Sims 1994). Leaf nitrogen tends to be invested to increase the capacities of electron transport and carbon fixation rather than that of the light harvesting system (Björkman 1981; Evans 1989, 1993; Hikosaka and Terashima 1995).

However, excess light energy that was not utilized for photosynthesis may cause physiological damages especially in photosystem (PS) II and thus may result in reduced photosynthetic carbon gain (Powles 1984; Anderson and Osmond 1987; Osmond 1994). Moreover, a high light incidence inevitably results in increase in both leaf temperature and surrounding air temperatures. Excess rising of leaf temperature further reduces efficiencies of PS II photochemistry and carbon fixation reaction (e.g., Weis and Berry 1987; Gamon and Pearcy 1990; Valladares and Pearcy 1997). High leaf and air temperatures also enhance transpirational water loss, which may result in larger water deficit in the plants (Hsiao 1973; Schulze and Hall 1982). Restricted stomatal aperture can prevent leaves from a high transpirational water loss under a low soil water availability or an excess rising of leaf temperature (Hsiao 1973; Chaves 1991). Stomatal closure, however, inevitably results in reduced CO_2 supply into the leaf and thus in a low photosynthetic activity (Farquhar and Sharkey 1982), leading to the excess light energy in leaf. Excessive light energy has been suggested to have deleterious influence on the photosynthetic productivity of forest understory plants that happen to be exposed to large canopy gaps (Mulkey and Pearcy 1992; for reviews see Powles 1984; Anderson and Osmond 1987; Osmond 1994). In order to further understand the effects and consequences of natural excessive light conditions on photosynthetic productivity, it is necessary to investigate the combined effects of light, water availability and temperatures on plant performances such as leaf phenology and biomass accumulation with leaf morphological and physiological responses.

In the recent two decades, biochemical effects of excess irradiance on photosynthetic apparatus and the biochemical/physiological mechanisms to avoid photoinhibitory damages have been investigated intensively (Powles 1984; Demmig-Adams and Adams 1992; Osmond 1994). Non radiative (thermal) dissipation of excess irradiance, which occurs as down regulation of PS II photochemistry, i.e., 'dynamic' photoinhibition, is now recognized to serve as a 'photon protective' system (Demmig-Adams and Adams 1992; Long, Humpreis and Falkowski 1994; Osmond 1994). In addition, consumption of excitation energy by photorespiration (RuBP oxygenation) and the Mehler-ascorbate peroxidase reaction have been suggested to play important roles in maintaining electron flow and thus avoiding damages of the two photosystems, i.e., 'chronic' photoinhibition (Osmond 1981; Osmond and Grace 1995; Asada 1996; Lovelock and Winter 1996; Logan *et al.* 1998). Photorespiration should be especially important in a situation where excitation energy is not fully used in photosynthesis due to low internal CO_2 induced by stomatal closure that frequently occurs in the natural high light environments (Valentini *et al.* 1995). Comparing photosynthetic responses between sun and shade leaves under natural light conditions should contribute to understanding the significance of suggested mechanisms of the leaf acclimation to high light.

Ecological roles of anatomical and morphological responses in avoiding high light stresses have been also well appreciated. Thicker sun leaf with thicker epidermis, palisade and spongy tissues contribute not only to increasing photosynthetic capacity per leaf area, but also to avoiding whole leaf chronic photoinhibition (Nishio *et al.* 1994). Since the incident photons are largely absorbed by the chloroplasts located in the upper layer inside the leaf, light reaching the chloroplasts in the lower layer is reduced in quantity and far from saturation (Terashima 1989). This light gradient inside the leaf may contribute to prevent the photosynthetic apparatus in the lower layers from photoinhibitory damages (Nishio *et al.* 1994). At the level of leaf organization, plasticity of leaf orientation enables plants to regulate light capture (Ehleringer and Forseth 1980). For instance, in beans (*Macroptilium atropurpureum* DC and *Glycine max* Merr.), kudzu (*Pueraria lobata* (Wild.)) and wild grape (*Vitis californica* Benth.), paraheliotropic orientation of leaves under sunny conditions have been reported to avoid excess irradiance, which further results in the decrease in leaf temperature, the increase in water use efficiency and the decrease in photoinhibition (Ludlow and Björkman 1984; Forseth and Teramura 1986; Gamon and Pearcy 1989; Kao and Forseth 1991, 1992; Saitou *et al.* 1994). These morphological mechanisms for avoidance of high light stresses would be more important in plants whose leaves have low or suppressed physiological tolerance to environmental stresses.

Scope of the present study

Importance of leaf- to whole plant-level ecophysiological studies in situ

As described above, adaptive features of leaf acclimation to the growth light regimes appear in various aspects ranging from leaf biochemistry to plant geometry. However, understanding of their ecophysiological functions is still insufficient, and the hypothesis that leaf acclimation contributes to maximizing photosynthetic production and plant growth under a given light environment has never been tested under natural conditions (but see Chazdon 1986; Popma and Bongers 1988; Harrington *et al.* 1989a, b; Epron, Dreyer and Breda 1992; Mulkey and Pearcy 1992; Ögren and Rosenqvist 1992; Valentini *et al.* 1995; Valladares and Pearcy 1997).

The necessity of the ecophysiological studies *in situ* also arises from the fact that environmental factors such as light, temperature and water conditions always interact each other in much complicated manners in natural habitats, where acclimation has been evaluated as an adaptation to environmental variability and heterogeneity.

The present study focuses on the acclimation of *Arisaema heterophyllum* Blume (Araceae) to contrasting light environments, i.e., a deciduous forest understory and an adjacent deforested open site. Investigations were made to understand how the acclimation occurs at several levels from biochemistry to geometry, and what the ecological significance of the acclimation is for the growth and survive of *A. heterophyllum* in the natural habitats.

Plant species and study site

A. heterophyllum is a perennial herbaceous species that tightly associated with humid floodplain throughout eastern Asia including Japanese mainland and Kyushu Island (Ohwi and Kitagawa 1983). A plant has one palmately compound leaf for photosynthetic production, one pseudostem to support the leaf, and one corm for storage. In late April to early May, *A. heterophyllum* emerges from the underground corm and starts to expand its palmately compound leaf. Each leaf is composed from five to 21 leaflets. The plant sheds its leaf in mid-summer and overwinters as a corm. Like other *Arisaema* species (Ewing and Klein 1982; Kinoshita 1986; Clay 1993), size-dependent sex expression is known for the species. The plant changes from asexual (no flower), through male to monoecious with increasing individual corm size. In addition to reproduction by seeds, the plant reproduces also vegetatively by forming cormlets around the maternal corm.

The investigations were mainly carried out in the floodplain of the Kokai River in Mitsukaido city (36°0'N, 140°1'E; altitude 10.5-12.5 m), Ibaraki prefecture, 45 km north-east of Tokyo. The plants growing in two contrasting habitats with respect to light availability, a riparian forest understory site and an adjacent deforested open site

(Oshima, Tang and Washitani 1997), were used for the measurements. The relatively wide range of growth light regimes and the simple architecture of *A. heterophyllum* provide me with a good chance to study the contribution of leaf-level light acclimation to whole plant growth.

The organization of this thesis

In Chapter 1, I reported field investigations and laboratory experiments to clarify the combined effects of light and water availability on morphological and physiological traits of leaves and biomass accumulation of individuals of *A. heterophyllum* grown at the forest and deforested sites. I found that leaf photosynthesis and plant growth depend on microsite light availability in the forest understory while at the deforested site they are limited by excess irradiance, drought and high leaf temperature. The study suggested that water availability largely affects the leaf acclimation to natural high light environments.

In Chapter 2, I showed the plasticity of leaf orientation in the two contrasting light environments and its contribution to reducing the habitat specific stresses, i.e., shortage in light in the understory and those accompanying high light at the deforested site, through experimental measurements of leaf orientation, photosynthesis and light environments, and computer simulations. The study demonstrated that leaves in the forest understory orient toward the direction in which leaf can maximize light capture, while those at the deforested site incline and tentatively fold so as to avoid excess irradiance and photoinhibition. In both habitats, the changes in leaf orientation contributed to higher photosynthetic productivity than fixed leaf orientation.

Chapter 3 describes the diurnal changes of photosynthesis under natural high light conditions using data from gas exchange and chlorophyll fluorescence measurements for the leaves of *A. heterophyllum* plants grown at the forest site and deforested site. Objectives of the study were to clarify the possible mechanisms involved in stomatal limitation and photoinhibitory limitation of photosynthesis in natural high light environments, and to understand the physiological details of leaf acclimation to natural 'stressful' high light environments. I paid a particular attention to the midday depression of photosynthetic responses and afternoon recovery from it because these are among the most important events to understand light acclimation of photosynthesis in the natural high light environments. The study suggested the ecological importance of photorespiratory reaction in dissipating excess energy in avoiding photoinhibition of photosynthesis, which was recently well discussed among the plant physiologists (Osmond *et al.* 1997).

Lastly, I discussed the ecological significance of leaf morphological and physiological acclimations to light environment based on my findings. In addition, suitable habitats for this threatened species (EAGC 1997) are discussed.

Chapter 1

Combined effects of light and water availability on photosynthesis and growth of *Arisaema heterophyllum* in the forest understory and an open site.

Introduction

Comparative studies on the photosynthesis and leaf characteristics of plants grown at high and low light levels can provide crucial insights into leaf level acclimation to sun and shade environments (Boardman 1977; Björkman 1981; Chow *et al.* 1988; Givnish 1988). A high-light or sun leaf usually has a higher photosynthetic capacity per unit leaf area and a higher stomatal conductance than a low-light leaf or shade leaf. Sun leaves also tend to be thicker and with a higher leaf-mass to area ratio (LMA) as compared to shade leaves. Other differences between sun and shade acclimated leaves can be found at biochemical, anatomical, and ultrastructural levels and also in the architecture of the plants at the canopy level (e.g., Fetcher *et al.* 1983; Givnish 1988). Many studies have tried to explain how and why plants change their leaf traits to acclimate or adapt to different light environments (see reviews by Horn 1979; Björkman 1981; Anderson and Osmond 1987; Pearcy and Sims 1994; Björkman and Demmig-Adams 1995). In most of these studies, the acclimation to photon flux density has been considered to 'aimed at' increased dry-matter production through the increase in utilization of light resources (Chapin *et al.* 1987; Pearcy and Sims 1994).

However, a plant in a high light regime tends also to be subjected to stress resulting from a high temperature and a low soil and/or air water contents. Any trait acclimating to a light environment would be modified or constrained by the other environmental factors (e.g., Osmond 1983; Mulkey and Pearcy 1992). Therefore, understanding the effects of other environmental factors on light acclimation is critical for further understanding the significance of a certain type of light acclimation of plants in natural environments. Moreover, whole-plant performances depend not only on the leaf-level acclimation, but also on the morphology, geometry and dynamics of the plant canopy. It is therefore necessary to scale up the consequences of leaf-level acclimation to whole-plant performance in order to fully comprehend adaptive significance of the light acclimation at the leaf-level. For these reasons, this study investigates light acclimation 1) under contrasting natural environments, 2) from leaf-level to whole-plant.

The simple architecture (i.e., one leaf, one pseudostem and one corm) and a brief growth season (spring to mid-summer) of *Arisaema heterophyllum* provide a good model for evaluations of light availability, leaf longevity, photosynthetic characteristics and annual biomass accumulation for individual plants.

In this chapter, I examined photosynthetic responses, leaf performance and biomass accumulation in *A. heterophyllum* for three meteorologically different years in two riparian habitats with contrasting light conditions, i.e., a forest understory and a deforested open site. The questions investigated were 1) how does *A. heterophyllum* respond physiologically and morphologically to the contrasting light environments? 2) how is light acclimation affected by soil water availability and temperature? 3) how does light acclimation at the leaf level contribute to whole-plant performance?

Materials and methods

Study site

The investigation took place in the floodplain of the Kokai River in Mitsukaido city (36°0' N, 140°1' E; altitude 10.5-12.5 m), Ibaraki prefecture, 45 km north-east of Tokyo.

Meteorological data were obtained at the Nagamine Meteorological Observatory (36°0' N, 140°1' E; altitude 25 m), which is about 10 km east-northeast of the study site. Plants growing in two habitats contrasting with respect to light availability, the forest site and the deforested site, were used for the measurements. The plants were considered to be genetically homogeneous since there is little variation within and between the two sites for a number of glycolysis enzymes (T. Yahara and T. Kawahara, personal communication).

The forest site is dominated by *Quercus acutissima* Caruth. Shrub layers are absent due to human interventions. The understory herbaceous layer is dominated by *Amsonia elliptica* Roem. et Schult, and contains many other herbaceous species such as *Euphorbia adenochlora* Morr. et Decne., *Viola raddeana* Regel and *Thalictrum simplas* L. var. *affine* Regel. Evergreen perennial bamboo grass *Pleiobrastus chino* Makino has recently invaded and now dominates the northern part of the forest. At this site, there are more than 500 individuals of *A. heterophyllum* in an area of approximately 1300 m^2. The deforested site is about 150 m downstream of the forest site. Several herbaceous species such as *Humulus scandens* Sieb. et Zucc., *Solidago altissima* L. and *P. chino* dominated during the summer in 1994. In February of 1995, *P. chino* was selectively mowed in order to make a sunny condition for *A. heterophyllum*. At this site, about 70 individuals inhabit in a small plot of about 80 m^2.

The meteorological conditions during the growing seasons of *A. heterophyllum* for the study years 1994, 1995 and 1996 are summarized in Fig. 1-1. In all three years, mean monthly air temperature increased from 13 °C in April to 27 °C in August. Precipitation patterns differed greatly among the years, and monthly precipitations in 1995 was 50-60 % higher than in 1994 or in 1996. Correspondingly, mean duration of sunshine in 1995 was much shorter than in 1994 or 1996.

Measurements

Microsite light availability and soil surface temperature

To characterize the microsite light availability, photosynthetically active photon flux density (PPFD) was measured under diffuse light conditions on densely overcast days three to four times during the growing season. The measurements were made using a hand-held measuring bar with four quantum sensors (IKS-25, Koito Industry, Japan), at the height of the leaves of *A. heterophyllum*. PPFD in the open was measured simultaneously at the height of 3 m near the forest. Before the measurement, these sensors were calibrated against a LI-190SB (Li-Cor Inc., Lincoln, Neb., USA) quantum sensor under a variety of natural light conditions from dark to full sunlight. All the sensors were connected to a data logger (Thermodac-E, Eto-Denki Inc., Japan). Relative PPFD (R-PPFD), which is the ratio of PPFD at the leaf height of individual plants to the open sky PPFD, was calculated and used for an index for microsite light availability (Tang *et al.* 1989; Washitani and Tang 1991). Soil surface temperatures were measured using max-min thermometers. The readings of each thermometer were recorded every 10-14 days during the period from late April to mid August.

Plant growth

The fresh weight of the corm of each plant was measured just before leaf emergence (fw_1) in March and after leaf senescence (fw_2) in July to August. The corms were excavated, and quickly weighed with a portable scale and reburied at the original depth. Thus fw_2 included the fresh weight of both the maternal corm and its daughter corms. To estimate the dry weight, I obtained both dry and fresh weight from 9 corms ($r^2 = 0.987$) after leaf senescence in August, and a linear regression equation was determined as follows,

$$dw = 0.243 fw \qquad (1)$$

where dw and fw are the dry and fresh weight of the same corm, respectively.

Relative growth rate (RGR) for a growing season was calculated as,

$$\mathrm{RGR} = \ln dw_2 - \ln dw_1 \qquad (2)$$

where dw_2 and dw_1 are the dry weights for fw_2 and fw_1, respectively.

The processes of leaf emergence and senescence (yellowing) were monitored for the individual plants at intervals of 2 or 3 days. Aboveground growing period (i.e., leaf longevity) of *A. heterophyllum* was determined as the number of days between the time of budbreak and the time when more than 90 % of whole leaf area had senescenced. A single leaflet was harvested from the individual plants just before the leaf senescence in 1995 and in 1996. After weighing fresh weight and measuring the area of the leaflet, leaves were dried at 70 °C for 24 h and weighed to obtain leaf-mass to area ratio (LMA). Leaf area was measured with a leaf area meter (AAM-7, Hayasi Denko, Japan).

Gas exchange measurements

In early April, three to five plants were dug up carefully from each of the two sites and transferred into clay pots (15 cm diameter × 15 cm depth) filled with field soil. A 3 cm diameter hole in the bottom of each pot permitted a similar water status within and outside of the pot. The potted plants were replaced at the ground at the microsite from which they came. At 7- to 10-day intervals during the growing period, leaf gas exchanges were measured in the laboratory. The potted plants were transferred to the laboratory in the evening, well watered and then allowed to stand overnight in the dark before measurement. For the plants from the deforested site in 1995, photosynthesis was measured before and after watering in order to study the effects of *in situ* soil water availability on the photosynthetic parameters. After each measurement, the water content of the pot soil was measured by weighing a small part of soil sample (50 cm^3) before and immediately after drying at 70 °C for 24 h.

Leaf age was defined as the days from the time when the leaf emerged (about 3 - 7 days after budbreak). Gas exchange parameters expressed on the basis of leaf dry weight were based on LMA of the plants for gas exchange measurements. Leaflets for calculating LMA were collected just before leaf senescence, since preliminary measurements showed that the leaf area of a fully-expanded leaf was relatively constant during the season. I assumed that there was little variation of LMA during the measurement.

Leaf photosynthesis was measured with a single attached leaflet using an open gas exchange system with CO_2/H_2O analyzer (LI-6262, Li-Cor Inc., Lincoln, Neb., USA). PPFD was adjusted by inserting or removing a piece of white cheesecloth between the leaf chamber and the light source, a 1000-W Xenon arc lamp (MLBO250C-U, Mitsubishi, Japan). Dark respiration rates were measured after the measurements of photosynthesis in 1994 and 1995. Since dark respiration rate can be stimulated by the amount of photosynthates in the leaf (Azcon-Bieto and Osmond 1983), leaves were kept in darkness until the respiration rate became constant. In 1996, dark respiration rate was measured before the measurements of photosynthesis. A quantum sensor (LI-190SB, Li-Cor Inc., Lincoln, Neb., USA) was used to measure PPFD at the chamber height. The air containing 350 μmol mol^{-1} of CO_2 was supplied by an air-balanced gas cylinder (Takachiho Industry, Japan). Air flow entering and leaving the chamber was measured using mass flow meters (Kofloc-1600R, Kojima, Japan). Humidity was controlled by bubbling the air through a water bath with a temperature-controlled condenser (Coolnit C80, Taiten, Japan). Relative humidity and temperature of the air entering the chamber were measured with a humidity-temperature probe (HMP 35A, Vaisala KK., Japan). During the measurements of light-photosynthetic responses, air temperature within the chamber was kept within the range from 24 - 27 °C by a 2-cm-depth water bath placed between the light source and the chamber. A thermo-control system (PWM and BIPOLAR Control System, Thermocontroller Type MT 702-1206, Netsu-Denshi, Japan) attached to the underside of the chamber was also used for rapid adjustment of air temperature in the chamber. Leaf and air temperatures within the chamber were measured with 0.1 mm diameter copper-constantan thermocouples. Leaf to air water vapor pressure deficit (VPD) during the light-response measurement varied between 1.0 and 1.5 kPa. Temperature-dependency of photosynthesis was examined under various temperatures between 17 and 45 °C and a constant PPFD of 600 μmol m^{-2} s^{-1}. Prior to the measurement of the temperature dependency, the plants were watered. VPD was allowed to increase with the increase in leaf temperature during the measurements since more natural responses of the leaves can be expected with VPD varying spontaneously with leaf temperature.

Gas exchange parameters were calculated according to von Caemmerer and Farquhar (1981). The initial slope (apparent quantum efficiency) of the PPFD-dependency of assimilation (*QE*) was obtained by fitting a linear regression to the data at low light from 0 to 60 μmol photons m^{-2} s^{-1}. Intrinsic water use efficiency was calculated from the ratio of photosynthetic rate (A) to leaf conductance (g_l) (Osmond *et al.* 1980). Since the difference in evaporative demand between the inside of the leaf chamber and that which prevailed before sealing the leaf inside will immediately be reflected in transpiration rate (E), but not in leaf conductance, A/g_l should provide more consistent estimates of water use efficiency than A/E (Meinzer *et al.* 1990).

Statistical differences in mean values for the parameters for plant growth, leaf longevity and gas exchange between the sites on a same year were tested by Student's *t*-test, and those among the years in a same site were tested by Scheffe's a posteriori test using Statview J-4.5 software (Abacus Concepts Inc. 1995).

Results

Microsite light availability

Light availability for individual plants averaged within the site was 2- to 4-fold greater at the deforested site than at the forest site throughout the growing season (Fig. 1-1). Microsite light availability gradually decreased with the closure of the forest canopy and the growth of understory plants. At the forest site, microsite light availability declined from 30 % at the time of leaf expansion of *A. heterophyllum* in late April, to 15 % at the time of leaf senescence from late July to mid August.

At the deforested site, microsite light availability declined from 42 % in late April to 35 % in mid June in 1994. In 1995 and 1996, it declined from 90 % in late April to 53 % in mid July. I observed the maximum irradiance of approximately 2000 μmol photons m^{-2} s^{-1} at sunny midday. The low microsite light availability in 1994 was due to the relatively dense coverage of *P. chino*, which was mown in February 1995.

On the forest floor, weekly maximum of soil surface temperature was constantly below 30 °C even during summer, while it exceeded 35 °C in the deforested site throughout the growing season except for rainy periods (Fig. 1-1). The temperature fluctuation was considerably greater in the deforested site than in the forest site.

Plant phenology and biomass accumulation

Leaves of *A. heterophyllum* started to emerge in late April to early May and fully expanded within 7 - 15 days (Table 1-1). Leaf emergence was 3 - 6 days earlier at the forest than at the deforested site. The time of full expansion of leaves was similar at the two sites in 1995, but significantly earlier at the forest site in 1994 and 1996. The time of leaf emergence and full expansion also varied among the three years. Leaf senescence occurred considerably earlier at the deforested site than at the forest site in 1994, while in 1995 with abundant precipitation during the season, senescence occurred 10 days earlier in the forest site than in the deforested site. In spite of significantly less precipitation in 1996 than in 1995, leaf senescence occurred almost the same time at both sites.

Leaf longevity was therefore fluctuated between the sites and among the three years (Table 1-1). At the forest site, the yearly fluctuation was small, but there was a significant difference between 1995 and 1996. At the deforested site, leaf longevity was significantly shorter ($p < 0.0001$, Scheffe's a posteriori test) in 1994 than in 1995 or in 1996.

RGR of individual plants (corms) considerably varied between and within the habitats, and between the years (Table 1-2, Fig. 1-2). In the drier year 1994, mean RGRs were -0.22 and 0.10 g g^{-1} one growing $season^{-1}$, while they were 0.12 and 0.22 g g^{-1} one growing $season^{-1}$ in 1996 for the forest and the deforested site plants, respectively. In 1995, with abundant precipitation during the growing season, plants achieved much greater RGR and the mean RGRs were 0.34 and 0.58 g g^{-1} one growing $season^{-1}$ for the forest and deforested sites, respectively. At the forest site, RGR of the plants whose initial weights were ≤ 6 g tended to increase with the increase of microsite light availability in 1995 and in 1996. A similar trend was also observed in 1995 when all the individuals were concerned (Table 1-2). No clear correlation between RGR and microsite light availability was observed in 1996.

Mean LMA was significantly higher at the deforested than at the forest site either in 1995 or in 1996. At the deforested site, significant difference was also observed between the years (44.19 ± 6.00 g m^{-2} in 1995 and 30.67 ± 5.19 g m^{-2} in 1996; mean ± SD, $p < 0.0001$) (Table 1-3).

Photosynthetic characteristics

Leaf age dependency

The relationships between leaf age and photosynthetic capacity were markedly different between the two sites in the drier year 1994 but rather similar in the other two years (Fig. 1-3). In 1994, photosynthetic capacities (A_{max}) of the forest site plants increased gradually to the maximum rate at a leaf age of around 30 days, and then declined gradually until the leaves senescenced at the leaf age of about 75 days. At the deforested site, photosynthetic capacity increased rapidly to the maximum rate at the age around 20 days, and then decreased rapidly until the leaves etiolated at around 40 days (Fig. 1-3a). In this year, photosynthetically active period for the deforested site plants was about half that of the forest site plants. Photosynthetic rates of the deforested site plants after watering was almost twice that of the unwatered plants (Fig. 1-3c), except for the measurements during the rainy season which starts approximately at a leaf age of 40 days in 1995.

In general, dark respiration rates gradually declined during the first one-third of the photosynthetically active period and then remained constant throughout the remaining period (Fig. 1-3). In 1994, dark respiration rate for the deforested site plants was consistently higher than that of the forest site plants. However in 1995 and in 1996, there was no significant difference between the sites.

Leaf conductance indicated similar changes with photosynthetic capacity (Fig. 1-3). As a whole, leaf conductance of the deforested site plants was slightly higher than that of the forest site plants in both years. In the drier year 1996, leaf conductance at both sites were slightly lower than those in 1995.

Light-responses of photosynthetic parameters

Light-saturated photosynthetic rate per unit leaf area, at the leaf age when the photosynthetic capacity was reached maximum (maximum A_{area}), for the forest site plants was significantly lower than that for the deforested site plants in 1995 and in 1996 (Table 1-3).

However, mean light-saturated photosynthetic rates per unit leaf dry weight (maximum A_{mass}) was not necessarily higher in the deforested site plants. A_{mass} was significantly higher in the forest site in 1995 (0.536 and 0.425 μmol CO_2 g^{-1} s^{-1} for the forest site and the deforested site plants, respectively, $p < 0.01$), but A_{mass} was similar in the two sites in 1996. Dark respiration depended on years as well (Table 1-3). There was no difference for apparent quantum yield (*QE*) and light compensation point (LCP) between the sites (Table 1-3).

Leaf conductances of the forest site plants at the maximum A_{area} were consistently lower than those of the deforested site plants (Table 1-3). Although not statistically significant, intrinsic water use efficiencies tended to be slightly higher in the forest site plants than in the deforested site plants (Table 1-3).

In addition to the differences between the sites, significant differences in gas exchange parameters were also observed between the wet year 1995 and the dry year 1996. As also shown in the leaf age dependency (Fig. 1-3), photosynthetic capacity and leaf conductance in 1996 were 10 - 20 % lower than in 1995 (Table 1-3).

Effects of soil water status or temperature on photosynthetic characteristics

At the deforested site plants, watering (i.e., increase in soil water contents from 25 to 40 %) caused a 2-fold increase in the light-saturated photosynthetic rates from 6.57 ± 2.26 to 12.30 ± 1.82 μmol CO_2 m^{-2} s^{-1} ($p < 0.01$, Student's *t*-test) (Fig. 1-4a) and in g_l from 0.090 ± 0.054 to 0.247 ± 0.057 mol H_2O m^{-2} s^{-1} ($p < 0.05$) (Fig. 1-4b). *QE* was also enhanced from 0.032 ± 0.005 to 0.041 ± 0.006 μmol CO_2 μmol^{-1} photons ($p < 0.05$).

Photosynthetic rates were almost constant at the leaf temperatures below 30 °C, but decreased with increasing temperature above 30 °C in both sites (Fig. 1-5a). Between 18 °C and 35 °C, photosynthetic rates were slightly higher in the deforested site plants, but the slope of drop in the photosynthetic rates at higher temperatures above 35 °C was greater in the deforested site plants. Leaf conductance reached the maximum at 22 °C irrespective of the growing environments, and then declined with increasing temperature (Fig. 1-5b).

Discussion

Effects of water availability and high temperature on photosynthetic acclimation

It has been well documented that photosynthetic rates are higher in sun than in shade leaves on the leaf area basis (Boardman 1977; Björkman 1981; Givnish 1988; Pearcy and Sims 1994). Under sufficiently watered conditions, photosynthetic rates on the leaf area basis of *A. heterophyllum* tended to be slightly higher under high- than under low-light conditions. However, decreasing water availability in natural environments causes stomatal closure, which may also raise leaf temperatures, decreasing water use efficiency (Gauhl 1979; Osmond 1983; Björkman and Powles 1984; Gildner and Larson 1992). In addition to the short-term variation in soil water availability, yearly difference in rainfall may also significantly affect the leaf phenology and photosynthetic capacity. Influences of water availability on photosynthetic capacity and leaf conductance are likely to be stronger in high-light than in low-light acclimated leaves, and this trend may reflect higher evaporative demands in the former.

In response to drought, plants typically reduce transpiration rates by partial stomatal closure; this reduces water loss more than photosynthetic carbon gain, increasing water use efficiency (Chapin *et al.* 1987; for reviews see Hsiao 1973; Chaves 1991). The high intrinsic water use efficiency under drought condition and rapid recovery of photosynthetic rate after watering in *A. heterophyllum* would be effective responses to the large variation of water availability in the sunny habitat. Such physiological traits may be among the important components of drought tolerance of the species.

In addition to water stress, temperature variation may also be important in the photosynthetic acclimation of *A. heterophyllum*. Temperature fluctuation is inevitable under variable light conditions, which should affect the photosynthetic capacity considerably (Berry and Björkman 1980; Ludlow and Björkman 1984; Gamon and Pearcy 1990). The rather flat response of photosynthetic rates to temperature observed in the range from 18 °C to 30 °C may help *A. heterophyllum* to maintain efficient carbon gain in fluctuating temperatures. However, unlike the forest site plants, the plants at the deforested site showed a greater drop in photosynthetic rate and leaf conductance at the temperatures above 35 °C. Higher sensitivities to higher temperatures of the leaves of *A. heterophyllum* from the deforested site may contribute to reduce damage by water loss in stressful high light conditions.

Stressful conditions are known to have different effects on apparent quantum yields under different light regimes, while sun and shade leaves do not differ in their quantum yields under a favorable environment (see Pearcy and Sims 1994). In *A. heterophyllum*, although quantum yield was not different between the leaves under well-watered conditions, there was a significantly lower quantum yield at the deforested site plants when water availability was low, due to the drought stress and high temperature. The result suggests that light acclimation of quantum yield may be considerably affected by water availability and high temperature stress. Photosynthetic responses of *A. heterophyllum* leaves from the natural habitats revealed that although light is the major determinant of the 'potential' photosynthetic capacity, soil moisture as well as ambient temperature considerably limit the achievement of maximum photosynthesis under high light.

Photosynthetic capacity of sun and shade leaves

Although photosynthetic rates of *A. heterophyllum* tended to be higher in sun than in shade leaves on the leaf area basis under sufficiently watered conditions, photosynthetic performance of sun leaves may perform similar or even worse than shade leaves on a leaf mass basis. This is mainly due to the higher LMA in sun than in shade leaves (Table 1-3). Some recent studies also reported such a 'disadvantage' of matter economy in sun leaves (Rice and Bazzaz 1989b; Sims and Pearcy 1991, 1994; Sims *et al.* 1994; Pearcy and Sims 1994). However, the high investment of dry-matter in sun leaves has been considered to contribute to increased photosynthetic capacity through increment in mesophyll thickness and contents of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) per unit area (e.g., Björkman 1981; McMillen and McClendon 1983; Sims and Pearcy 1991, 1992; for reviews see Givnish 1988; Pearcy and Sims 1994). Further studies on leaf anatomy and biochemical component variation under different light regimes in *A. heterophyllum* are needed in the future. Although it has been also suggested that thicker or heavier leaves may be better to tolerate stress conditions such as soil water deficits, high temperature or high light stresses than thinner leaves (Rice and Bazzaz 1989; Taylor 1989; Raven 1989; Chazdon and Kaufmann 1993; Sims *et al.* 1994), these problems have not been addressed experimentally (but see Nishio *et al.* 1994).

Phenological response to different light and water availability

In addition to various physiological responses, *A. heterophyllum* showed contrasting leaf longevity in response to different light and water availabilities. As has been well documented, light availability is the major environmental factor affecting leaf longevity (Chabot and Hicks 1982; Williams *et al.* 1989; Sims and Pearcy 1991). Leaf longevity of *A. heterophyllum* was found to be shorter at the deforested site in the dry year 1994, while no between-site difference was observed in 1996. It is also known that leaf longevity decreases with water deficit due to reduced photosynthetic capacity (e.g., Wolfe *et al.* 1988). For perennial herbs, biomass accumulation in the current year may considerably affect the construction of aboveground parts in the next year. Therefore, longer leaf longevity of *A. heterophyllum* at the forest site may contribute to increasing carbon gain under light limitation. A marked difference in leaf longevity observed at the deforested site among years with a great difference in precipitation indicates strongly that water availability is also an important determinant of the leaf longevity. In spite of the low precipitation in 1996, leaf longevity was not significantly different between the sites. The slightly longer leaf longevity in 1996 may be partly related to the improved soil water availability, which was caused by the decrease in *P. chino* foliage after the selective mowing.

Environmental constraints on RGR and their ecological implication

At the whole-plant level, a positive dependence of RGR on microsite light availability at both sites in 1995 and at the forest site in 1996 indicate that light is an important environmental factor determining the growth of *A. heterophyllum* in the natural environments (Fig. 1-2). Biomass accumulation (i.e., RGR of corm) in *A. heterophyllum* and its dependence on microsite light availability tended to be higher in smaller individuals (Table 1-2). Size-dependent variation in biomass accumulation of *Helianthus tuberosus* has been reported by Kondo and Oshima (1981) and of three Betulaceae species has been reported by Walters *et al.* (1993). A high allocation to leaves and rapid translocation may result in a high RGR in small plants (Kondo and Oshima 1981). A maximum corm size may be another reason for the lower dependence in larger individuals. Measurements of photosynthetic capacity and plant growth in the natural habitats in the years contrasting in rainfall, provided me with insights for the possible effects of water availability. Among the factors other than light, water availability is likely to be the most important for light acclimation, and also annual RGR in natural habitats even under a mesic climate since the correlation coefficient between RGR and microsite light availability tended to be higher in the wet year (1995) than in the drier years (1994 and 1996).

Table 1-1. Leaf phenology of *Arisaema heterophyllum* grown at a forest and a deforested site during three years from 1994 to 1996. Leaf longevity is defined as the period (days) from bud break to leaf senescence. Bud break, full expansion and leaf senescence were measured by days from 1st April in each year. Mean ± SD of measurements were shown. Sample numbers are indicated in parenthesis. Different capitals and small letters on the right-top of each mean indicate a significant difference between the two sites in the same year ($p < 0.05$, Student's *t*-test) and among years in the same site ($p < 0.05$, Scheffe's a posteriori test), respectively.

	Forest site		
	1994 (n=54)	1995 (136)	1996 (139)
Leaf longevity (days)	$86.7^{Aab} \pm 20.0$	$84.8^{Aa} \pm 20.0$	$91.3^{Ab} \pm 19.9$
Bud break	$23.6^{Aa} \pm 5.4$	$20.8^{Ab} \pm 3.9$	$27.2^{Ac} \pm 5.1$
Full expansion	$35.5^{Aa} \pm 4.8$	$31.9^{Ab} \pm 3.3$	$43.0^{Ac} \pm 6.9$
Leaf senescence	$108.8^{Aa} \pm 19.6$	$104.7^{Aa} \pm 19.6$	$118.5^{Ab} \pm 19.8$

	Deforested site		
	1994 (22)	1995 (59)	1996 (51)
Leaf longevity (days)	$69.1^{Ba} \pm 10.7$	$94.6^{Bb} \pm 9.5$	$89.8^{Ab} \pm 18.4$
Bud break	$29.4^{Ba} \pm 4.0$	$22.3^{Bb} \pm 2.5$	$30.3^{Ba} \pm 5.2$
Full expansion	$43.1^{Ba} \pm 2.9$	$32.8^{Ab} \pm 1.8$	$48.6^{Bc} \pm 3.6$
Leaf senescence	$98.3^{Ba} \pm 10.9$	$115.9^{Bb} \pm 9.4$	$120.2^{Ab} \pm 16.6$

Table 1-2. Relative growth rate (RGR, mean ± SD), and the coefficients of correlation between microsite light availability and RGR in *A. heterophyllum* in three years from 1994 to 1996. Microsite light availability is the transmittance of photosynthetic photon flux density at the height of *A. heterophyllum* plants under diffuse light conditions. Sample numbers are indicated in parenthesis. Different capitals and small letters on the right-top of each mean indicate a significant difference between the two sites in the same year ($p < 0.05$, Student's *t*-test) and among years in the same site ($p < 0.05$, Scheffe's a posteriori test), respectively.

	Forest site		
	1994	1995	1996
RGR (g g^{-1} one growing season^{-1})**	$-0.22^{Aa} \pm 0.43$	$0.34^{Ab} \pm 0.36$	$0.12^{Ac} \pm 0.40$
Correlations coefficients for			
plant corms initially larger than 6.0 g	0.379 (22)	0.297 (31)	0.081 (47)
plant corms initially less than or equall 6.0 g	0.247 (29)	0.264* (74)	0.672* (44)
all plants	0.249 (51)	0.354* (105)	0.171 (91)

	Deforested site		
	1994	1995	1996
RGR (g g^{-1} one growing season^{-1})**	$0.10^{Ba} \pm 0.48$	$0.58^{Bb} \pm 0.30$	$0.22^{Aa} \pm 0.36$
Correlations coefficients for			
plant corms initially larger than 6.0 g	0.820 (4)	0.683* (20)	0.244 (30)
plant corms initially less than or equall 6.0 g	0.100 (15)	0.366 (23)	0.625 (9)
all plants	0.367 (19)	0.526* (43)	0.311 (39)

*: The coefficient of correlation between microsite light availability and RGR which is significant at $p < 0.05$.

**: One Growing season : from April to August.

Table 1-3. Mean ± SD of light-saturated assimilation rate (A_{max}), dark respiration, apparent quantum yield (*QE*), light compensation point of assimilation rate (LCP), leaf conductance (g_l), intrinsic water use efficiency (A/g_l), and leaf-mass to area ratio (LMA) in *A. heterophyllum* grown at a forest and a deforested site in three years from 1994 to 1996. Plants of 3-5 individuals were used for gas exchange measurements. Sample numbers for LMA are indicated in parenthesis. Different capitals and small letters on the right-top of each mean indicate a significant difference between the two sites in the same year ($p < 0.05$, Student's t-test) and among years in the same site ($p < 0.05$, Scheffe's a posteriori test), respectively. Dark respiration in 1996 were not compared with those in other years since the procedure of the measurement was different.

	Forest site		
	1994	1995	1996
Amax			
($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	12.94^{Aa} ±1.25	11.75^{Aa} ±0.67	8.55^{Ab} ±0.42
($\mu mol\ CO_2\ g^{-1}DW\ s^{-1}$)		0.536^{Aa} ±0.057	0.304^{Ab} ±0.024
Dark respiration			
($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	0.36^{Aa} ±0.14	0.55^{Aa} ±0.14	0.32^{A} ±0.05
($\mu mol\ CO_2\ g^{-1}DW\ s^{-1}$)		0.025^{A} ±0.006	0.011^{A} ±0.001
QE ($\mu mol\ CO_2\ \mu mol^{-1} photons$)	0.054^{Aa} ±0.008	0.045^{Aa} ±0.002	0.045^{Ba} ±0.001
LCP ($\mu mol\ photons\ m^{-2}\ s^{-1}$)	6.45^{Aa} ±2.75	12.21^{Ab} ±3.08	5.19^{Aa} ±1.06
g_l ($mol\ H_2O\ m^{-2}\ s^{-1}$)		0.183^{Aa} ±0.019	0.100^{Ab} ±0.027
A/g_l ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)		64.51^{Aa} ±6.02	91.05^{Aa} ±33.13
LMA ($g\ m^{-2}$)		22.66^{Aa} ±7.79 (n=74)	23.52^{Aa} ±5.51 (92)

	Deforested site		
	1994	1995	1996
Amax			
($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	13.68^{Aab} ±3.31	15.69^{Ba} ±1.97	10.50^{Bb} ±0.66
($\mu mol\ CO_2\ g^{-1}DW\ s^{-1}$)		0.425^{Ba} ±0.022	0.320^{Ab} ±0.044
Dark respiration			
($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	0.94^{Ba} ±0.15	0.69^{Aa} ±0.12	0.31^{A} ±0.08
($\mu mol\ CO_2\ g^{-1}DW\ s^{-1}$)		0.019^{B} ±0.002	0.009^{A} ±0.003
QE ($\mu mol\ CO_2\ \mu mol^{-1} photons$)	0.065^{Aa} ±0.020	0.044^{Aa} ±0.005	0.043^{Ba} ±0.005
LCP ($\mu mol\ photons\ m^{-2}\ s^{-1}$)	13.24^{Aa} ±1.98	15.62^{Aa} ±1.86	7.07^{Ab} ±1.76
g_l ($mol\ H_2O\ m^{-2}\ s^{-1}$)		0.284^{Ba} ±0.067	0.138^{Ab} ±0.042
A/g_l ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)		57.55^{Aa} ±13.17	79.21^{Aa} ±17.26
LMA ($g\ m^{-2}$)		44.19^{Ba} ±6.00 (47)	30.67^{Bb} ±5.19 (40)

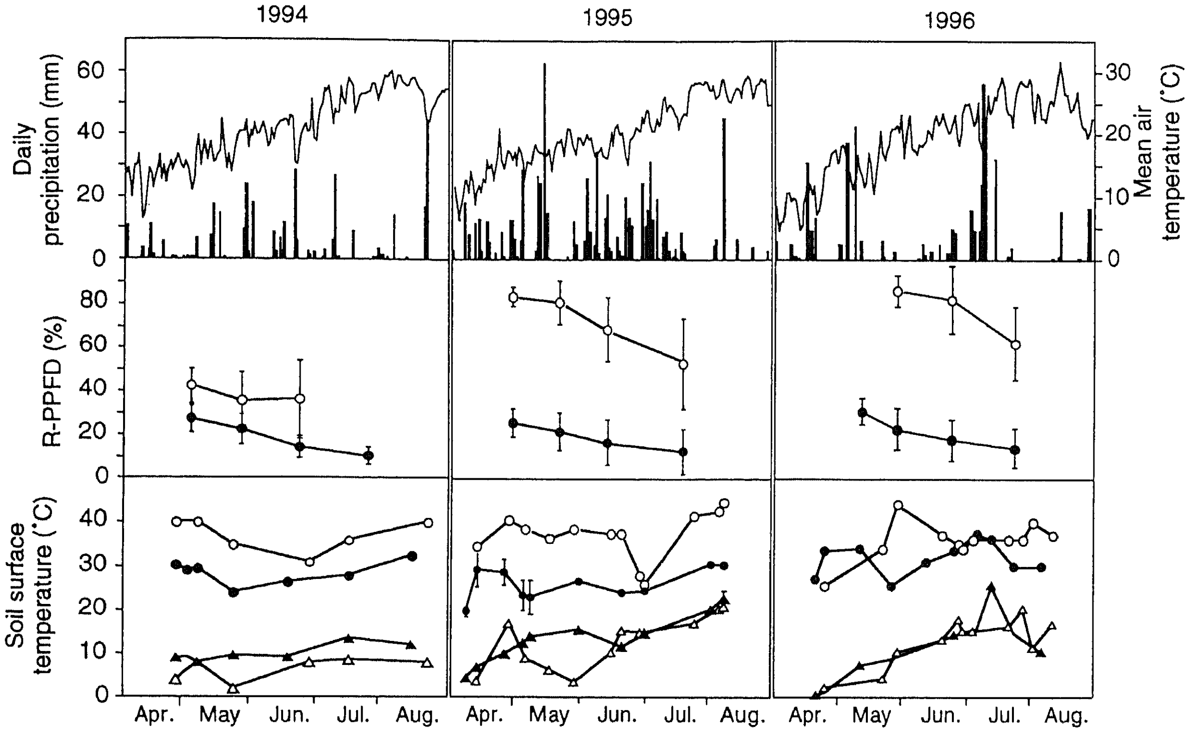

Figure 1-1. Seasonal changes in daily precipitation (solid bars) and daily mean air temperature during the growing season of *A. heterophyllum* (top), microsite light availability (middle) and maximum and minimum soil surface temperatures (bottom) at the forest site (● and ▲) and deforested site (○ and △) in the years 1994, 1995 and 1996. Symbols with error bars represent mean ± SD. Data on daily precipitation and mean air temperature were obtained from Nagamine Meteorological Observatory.

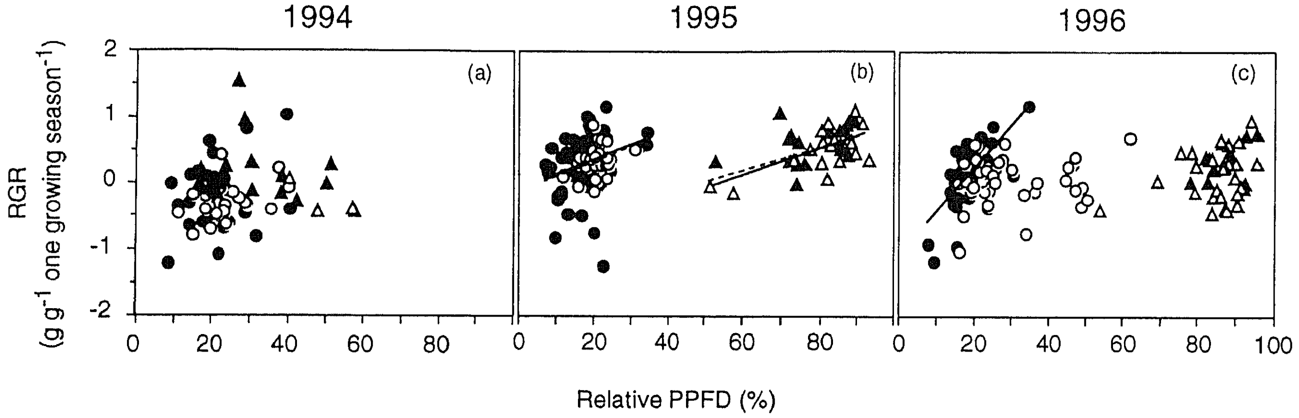

Figure 1-2. Relationship between microsite light availability in late May and annual relative growth rate of corm at the forest (circles) and at the deforested site (triangles) in 1994 (a), 1995 (b) and 1996 (c). Filled symbols indicate the individuals whose initial fresh weight of corm was less than or equal to 6g, and open symbols indicate the individuals weighed more than 6g initially. Solid lines represent the significant relationships for the corms smaller or equal to 6g at the forest site and for the corms larger than 6g at the deforested site. Dashed lines represent the significant relationships for the all plants. The details of correlation coefficients between microsite light availability and RGR are shown in Table 1-2.

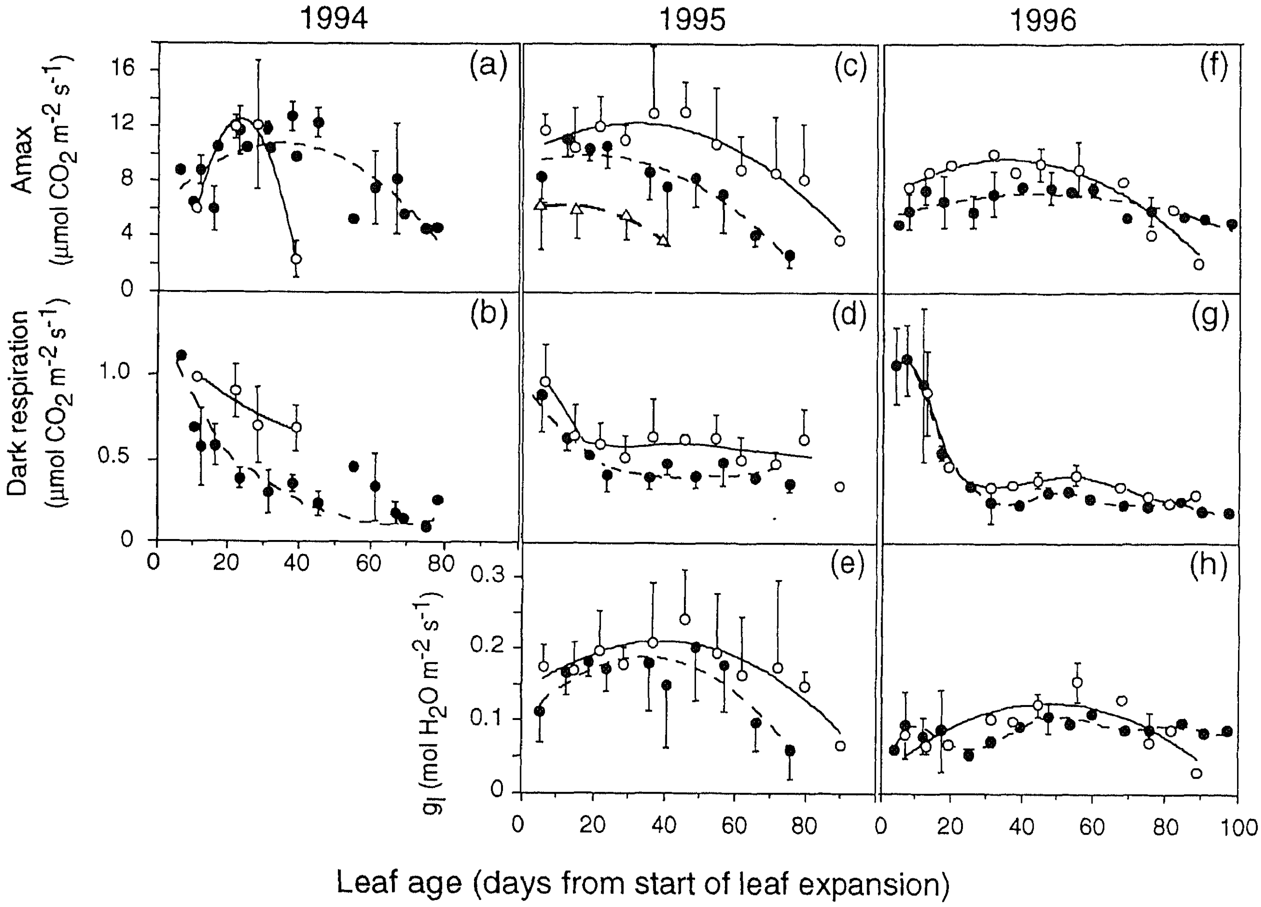

Figure 1-3. Leaf-age dependency of light saturated photosynthetic rates (A_{max}) in 1994 (a), in 1995 (c) and in 1996 (f), dark respiration rates in 1994 (b), in 1995 (d) and in 1996 (g) and leaf conductance in 1995 (e) and in 1996 (h) for the leaves of *A. heterophyllum* grown at the forest site (●) and the deforested site (○: watered, △: unwatered). Symbols with error bars (± SD) represent means of 3 to 5 replicate plants. A_{max} was measured at PPFD of over 1000 μmol m^{-2} s^{-1}, leaf temperature of 25 - 27 °C, relative humidity of 70 % and ambient CO_2 concentration of 350 μmol mol^{-1}. Dark respiration was measured at leaf temperature of 25 °C, relative humidity of 70 % and ambient CO_2 concentration of 350 μmol mol^{-1}.

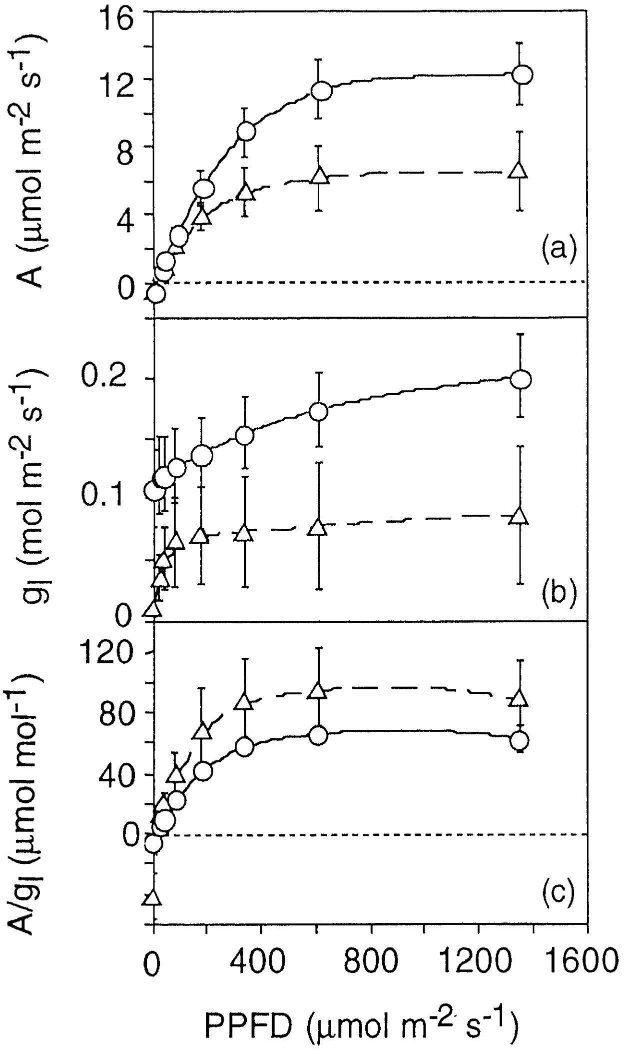

Figure 1-4. Effects of soil water contents on light-photosynthetic rates (a), leaf conductance (b) and intrinsic water use efficiency (c) of *A. heterophyllum* grown at the deforested site in 1995. The data are plotted for watered (○:40 % for soil water content) and unwatered (△:25 % for soil water content) regimes. Symbols with error bars (± SD) represent means of four replicate plants. The measurements were made at the leaf age of 15 days.

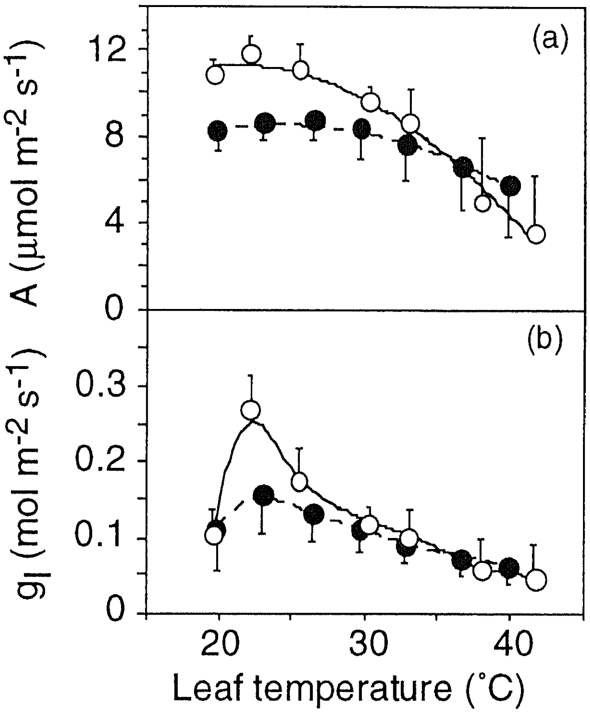

Figure 1-5. Temperature dependence of photosynthetic rates (a) and leaf conductance (b) at 600 μmol photons m^{-2} s^{-1} of *A. heterophyllum* grown at the forest (●) and deforested site (○) in 1995. Symbols with error bars (± SD) represent means of triplicate plants. The measurements were made at the leaf age of 26 days for the forest site plants and 37 days for the deforested site plants, respectively.

Chapter 2

Flexible leaf orientations of *Arisaema heterophyllum* maximize light capture in a forest understory and avoid excess irradiance at a deforested site.

Introduction

Leaf physiological and morphological characteristics of *Arisaema heterophyllum* were shown to acclimate to the contrasting light environment: photosynthetic capacity and LMA (leaf-mass to area ratio) were approximately 30 % higher at the deforested open site than in the forest understory (Chapter 1). Moreover, leaf geometries differed markedly between the two habitats: leaflets are held almost flat in the forest understory, while they are more inclined and folded at the deforested site. Leaflet folding shows diurnal changes.

Photosynthetic productivities of forest understory plants are often limited by insufficient light resources (e.g., Popma and Bongers 1988; Pfitsch and Pearcy 1992; Sims and Pearcy 1993). In a forest understory, microsite light availability of leaves depends largely on forest size, canopy density and the distribution of canopy openings (Baldocchi and Collineau 1994; Bazzaz and Wayne 1994). For leaves experiencing limited light availabilities, the increment of light capture by orientating the leaf surface to the canopy openings contributes greatly to increasing photosynthetic productivity (Chazdon 1986; Ackerly and Bazzaz 1995). In a previous study, photosynthetic productivity and individual biomass accumulation of *A. heterophyllum* plants were found to depend significantly on microsite light availability (Chapter 1). The almost flat leaflet orientation of *A. heterophyllum* in the forest understory should play an important role in increasing light capture under conditions of low light availability.

In an open habitat, excess PPFDs and co-occurring stresses, such as drought and high leaf temperature, inhibit photosynthetic activity, especially around midday on a sunny day (e.g., Berry and Björkman 1980; Powles 1984; Lange 1988). Paraheliotropic leaf orientation under sunny conditions has been reported to decrease incident radiation which further results in a decrease in leaf temperature, an increase in water use efficiency and a decrease in photoinhibition (Ludlow and Björkman 1984; Forseth and Teramura 1986; Gamon and Pearcy 1989; Jurik, Zhang and Pleasants 1990; Kao and Forseth 1991, 1992). I have shown in Chapter 1 that drought and high leaf temperature caused by high light incidence at the deforested site considerably limit leaf photosynthesis *in situ* in *A. heterophyllum*. In this situation, inclination and folding of leaflets should minimize the amount of excess PPFDs received and hence maintain higher photosynthetic activity. Moreover, changing leaf orientation in response to varying water or nutrient availability has been reported to contribute to the maintenance of an efficient photosynthetic rate by regulating light capture (Shackel and Hall 1979; Kao and Forseth 1991, 1992). Diurnal changes in leaflet folding in *A. heterophyllum* may contribute to the balance between the capture of light resource and photosynthetic activity, which tends to be influenced by leaf temperature and soil water availability.

Aims of this chapter are: 1) to make a quantitative evaluation of the leaf geometry and its diurnal variation at the forest and deforested open sites; 2) to investigate whether *A. heterophyllum* adjusts the spatial orientation of its leaf in a way that maximizes light capture in the forest understory, while avoiding excess irradiance at the deforested site; and 3) to confirm the effects of leaf orientation on photosynthesis by artificially constraining leaves in a horizontal position at the deforested site.

Materials and Methods

Study site

Investigations were carried out in the floodplain of the Kokai River in Mitsukaido city (36°0'N, 140°1'E; altitude 10.5 - 12.5 m), Ibaraki prefecture, 45 km north-east of Tokyo. The plants growing in two habitats of contrasting light availability, a forest site and a deforested site, were used for the measurements (see Fig. 1-1 for micrometeorological data).

The forest site is dominated by *Quercus acutissima* Caruth. Shrub layers are absent due to human interventions. The understory herbaceous layer is dominated by *Amsonia elliptica* Roem. et Schult. Evergreen perennial bamboo grass *Pleiobrastus chino* Makino dominates the northern part of the forest. The deforested site is about 150 m downstream of the forest site and *A. heterophyllum* plants coexist with several herbaceous species such as *Humulus scandens* Sieb. et Zucc., *Solidago altissima* L. and *P. chino*. *A. heterophyllum* plants are not shaded by these herbs except when the sun is close to the horizon.

Measurements of leaf morphology

Inclination angle (α) and azimuth (θ) of the leaflet midvein were measured for individual plants using equipment described by Norman and Campbell (1989). Leaflet folding (β) was calculated from the widths of the leaflets in folded and unfolded states (Fig. 2-1). Since a twist angle around the midvein of a leaflet was almost negligible, maximal slope of leaflet surface (ϕ) can be calculated as

$$\cos\phi = \cos\alpha \cdot \cos\beta.$$

Plants with 10 to 17 leaflets and 30 to 60 cm tall were selected for the measurements; this size range includes most of the plants in the habitat. At the deforested site, leaflet inclination and folding for a total of 16 plants were measured around noon on sunny days (24, 27 June and 4 July) in 1996. Repeated measurements on 10 of 16 plants were made of the three experimental days. In addition, diurnal changes in β were measured for five leaflets from five plants on 12 June (after a few rainless days) and for four plants on 16 June 1996 (next day of rain). On each measuring day except 12 June, β was measured for three leaflets for each sample plant. At the forest site, α and θ of 35 plants were measured in mid-May 1997 when the leaves had just expanded fully. In order to assess the ability of the leaflets to re-orientate with seasonal changing in microsite light availability, the 32 surviving plants of the original 35 plus an additional three plants were measured again in late June when the forest canopy was fully closed.

Estimation of light capture

Directionality of light at the forest site

Immediately after leaf orientation was measured, light environments were assessed with 180° hemispherical photographs taken with 8 mm fish-eye lens (Nikon, Tokyo) just above the individual plants at the forest site. Measurements were performed on 34 plants in mid-May and on 35 plants in late June 1997. Photographs were taken under diffuse light conditions. The image of the hemisphere was divided into 200 cells of identical solid angles using a computer (Fig. 2-2). Canopy openness was determined for each cell at 1 % precision as the proportion of 'open' pixels included in the cell.

Light capture of hypothetical light receiving surfaces at various directions in the forest understory was estimated basically following the approach of Chazdon and Field (1987). SOC (Standard Overcast Sky; Moon and Spencer 1942) was assumed for diffuse light distribution over the sky. By multiplying the sky brightness distribution and the light transmittance through the canopy openness distribution, and by assuming diffuse PPFD of 1000 μmol photons m^{-2} s^{-1} above the canopy, directional distribution of diffuse radiation coming through the canopy was estimated. After the cosine-correction for light incident angle from each cell to a light receiving surface, PPFD on the surface was calculated as the sum of PPFD values from the 200 cells on the sky hemisphere. Light incident angle was determined assuming that the light comes from the center of each cell. Orientation of the light receiving surface was varied over 201 directions corresponding to the centers of 200 cells and the zenith. In addition, the direction of the surface maximizing the light capture was determined: the direction is referred to as $D_{\max}$ hereafter.

Leaf geometry and light capture at the deforested site

The system developed by Takenaka, Inui and Osawa (1998) to estimate light capture of a model plant was used to assess light capture of *A. heterophyllum.* The basic idea of the model is essentially the same as that of the YPLANT model of Pearcy and Yang (1996). The three-dimensional structure of a plant is reconstructed in a computer from geometric information of the elements of a plant canopy. The light capture of the model plant is estimated incorporating directionality of the light source, mutual shading among plant parts, and incident angles of light to the leaves. Somewhat different algolism was adopted to assess the obstruction by other plant parts in the two systems; looking down on the model plant from the sky in YPLANT, but looking up to the sky from plant parts in the system of Takenaka *et al.* (1998). Further discussion on the calculation procedure is in Takenaka *et al.* (1998).

Model plants of *A. heterophyllum* have standard-shaped with 15 leaflets. Each leaflet is composed of two halves divided by the midvein. Each half leaflet is assumed to be a flat surface. Various geometries of leaves were reconstructed by computer by varying the inclination (α) and folding (β) of leaflets (Fig. 2-3). Seasonal daily frequency distributions of incident PPFDs were estimated for a total of 90 observation points located over the leaf (three points for each half leaflet, cf. Fig. 2-1) which was assumed to be under completely open sky.

In order to estimate the absolute light incidence for the above calculation, a model for the light source is needed. I used a model from Takenaka (1985), which was based on continuous measurements of irradiance near the present study site. In the model, sky conditions were classified either clear, well-illuminated cloudy (daily maximum light incidence is 50 % of clear day) or heavily cloudy (10 % of clear day). Frequency of each weather type and the maximum light incidence within a day were determined for each month from the observed data. SOC was assumed for the distribution of diffuse light on cloudy days. Light distribution on a clear day is the summation of diffuse light following SOC and a direct incidence from the sun which contributes 80 % of total light incidence. Diurnal variation in the sky brightness was calculated assuming the maximum at the solar noon and zero at sunrise and sunset, and was proportional to the cosine of the difference of the time angle from the solar noon.

Model light capture was calculated for the period from May to July, the main growing season of *A. heterophyllum*. Hemisphere as the light source was also divided into 200 cells of identical solid angles and brightness of each cell was determined using the model mentioned above. After the cosine-correction for the light incident angle from each cell to the leaf surface, PPFD on a leaf was calculated as the summation of the PPFD values from the 200 cells. Direct light incidence on the clear day was calculated by including the brightness of the sun in the brightness of a cell just at the direction of the sun. Mutual shading among leaflets was also considered, assuming leaf light transmittance of 10 %. Details of the calculation of mutual shading within a plant are described in Takenaka *et al.* (1998). Various azimuth angles were included for the model plants (cf. Fig. 2-3) but no significant differences were observed among the directions when the light capture was evaluated at a whole leaf level. Thus results were shown for the plants facing south (Fig. 2-3).

Gas exchange measurements

In order to clarify the effects of continuous exposure to high light on photosynthetic activity at the deforested site, leaves of seven plants with 15 to 17 leaflets and about 50 cm in height were unfolded and constrained horizontally using thin wire in early June 1996. Diurnal patterns of leaf gas exchange of these plants were measured on 12 (three plants) and 16 (four plants) June 1996 using portable photosynthesis measuring system (Model LI-6400, Li-Cor Inc., Lincoln, Neb., USA). A single attached leaflet was used for each sample plant. On each day of the measurements, three control plants (α: 20 - 30°, maximum β: 35 - 70°) were also measured , and these leaves are termed 'naturally oriented leaves' hereafter.

The clamp-on leaf chamber allowed for natural illumination of the upper leaf surface during the measurements. Incident PPFD beside the leaf chamber was measured with a photon sensor (LI-190SA; Li-Cor Inc., Lincoln, Neb., USA). CO_2 concentration of the air entering the leaf chamber was controlled at 360 μmol mol^{-1}. Air temperature in the chamber was adjusted manually to match that outside of the system, referring to the readings of copper-constantan thermocouples mounted at a height of 1 m above ground. Relative humidity in the chamber was similar to that outside. PPFD at a height of 3 m above the ground was measured with a photon sensor (IKS-25, Koito Industry, Japan) mounted horizontally. Environmental data were stored in a data logger (Thermodac-E, Eto-Denki Inc., Japan) at 1-min interval.

Results

Leaf morphology in two contrasted habitats

Mean inclination angles of leaflets of plants at the forest understory site in both May and June were considerably smaller than these of the plants at the deforested site (ANOVA, $p < 0.0001$, Table 2-1). No leaflet folding was observed ($\beta = 0$) at the forest site. The maximum slope of the leaflet surfaces of the deforested site plants was significantly greater than that of the forest site plants (ANOVA, $p < 0.0001$, Table 2-1). At the deforested site, β changed diurnally with the maximum around noon (Fig. 2-4, Table 2-1).

For each plant, leaf surface direction (D_{Leaf}) was calculated as the mean direction (angular altitude and azimuth), D, of all halves of leaflets. The half leaflet divided by its midvein was used as a unit because it can be assumed to be a flat surface. Variations in D of each half leaflet around D_{Leaf} represent the 'departure from flatness' of the whole leaf; smaller the variation, the flatter the whole leaf. Mean differences between D of each half of leaflets and D_{Leaf} of the deforested site plants were significantly larger than those of the forest site plants (ANOVA, $p < 0.0001$, Table 2-1).

Leaf orientation and light capture

Forest understory

Relationships between the D_{Leaf} (mean direction of individual leaflets) and light incidence were analyzed with the hemispherical canopy photographs taken just above each of the *A. heterophyllum* plants. During the period from May to June, maximal diffuse light incidence on the leaves relative to that above the forest canopy changed from 24.8 ± 6.7 % in May (mean ± SD for 34 plants) to 17.2 ± 5.2 % in June (35 plants). Because of the heterogeneous distribution of canopy openings over the hemisphere, light capture of a leaf surface depends on its orientation. Fig. 2-5 shows the distributions of D_{Leaf} and $D_{\max}$ (direction maximizing diffuse light capture at given microsite) in May and June 1997. Most D_{Leaf} values are distributed close to $D_{\max}$ with the angular deviations of 24.9 ± 10.4 and 25.6 ± 15.2 degree in May and June, respectively. Light capture efficiency (LCE), the ratio of total diffuse irradiance incident actually on a leaf to that facing $D_{\max}$, was shown to be as high as 98 % (Table 2-1). Directional dependency of LCE and spatial distributions of each half of leaflet for the plants in a microsite with $D_{\max}$ biased from the zenith, and for those in a microsite with $D_{\max}$ most close to the zenith (indicated with arrows in Fig. 2-5b) are shown in Fig. 2-6. Directional dependency of LCE represents the relative amount of photons received at a light receiving surface facing various directions over the hemisphere. Fig. 2-6 shows that most leaflets are orientated to achieve LCEs of more than 90 %.

Leaf geometry and estimated light capture at open site

Daily light capture estimated for a model leaf under clear open sky was largest for a leaf with horizontal leaflets, and an increase in the slope of leaf surface (ϕ) resulted in decreasing light capture (Fig. 2-7a). A leaf with 47 degree slope, which is the mean slope of the leaflets at the deforested site, received only 75 % of the light captured by a horizontal leaf (Table 2-1).

Photosynthetic rate of *A. heterophyllum* saturates at about 600 μmol photons m^{-2} s^{-1} (Chapter 1). Increase in the slope of leaflet surface increased the duration with PPFDs below this light level, while the duration with a higher PPFD decreased as the slope increased (Fig. 2-7b). Effects of leaf angle on duration with light above a threshold PPFD became larger for a higher PPFD. For example, the duration of a leaf with a slope of 47 degree decreased by 20 % for PPFDs above 600 μmol photons m^{-2} s^{-1}, but it decreased to zero for PPFDs above 1600 μmol photons m^{-2} s^{-1}. β increases up to 80 degree around noon, while α took a constant value in individual plants ranging from 10 - 40 degree.

Gas exchange at the deforested site

In both naturally oriented leaves and horizontally constrained leaves, net photosynthetic rates (*A*) and stomatal conductance to water vapor (g_{sw}) reached the maximum in the morning with a midday depression on both experimental days (Fig. 2-8). *A* recovered from this midday depression in both treatment leaves in the late afternoon of 12 June, but only in the naturally oriented leaves on 16 June.

It was occasionally cloudy on 12 June, but soil water availability was low. On this day, leaves of the two treatments showed a similar diurnal pattern in gas exchange, but the horizontally constrained leaves showed a slightly larger midday depression of *A* (Fig. 2-8b). On 16 June it was sunny all day, but soil water condition was considerably better due to previous day's rain. On this day, *A* and g_{sw} of the naturally oriented leaves were slightly higher than those on 12 June, while the horizontally constrained leaves showed a lower maximum *A* and g_{sw}, and a severer midday depression than on 12 June (Fig. 2-8g, h). Moreover, *A* and g_{sw} of the horizontally constrained leaves were considerably smaller than those of the naturally oriented leaves.

Water use efficiency (the ratio of *A* to transpiration rate) tended to be higher at midday, but no significant difference was found between the treatments (Fig. 2-8d, i). Calculated intercellular CO_2 concentrations (*Ci*) were similar in the two treatments (Fig. 2-8e, j).

Discussion

Calculations on model leaves demonstrated that actual leaf orientations of *A. heterophyllum* plants in the forest understory were such that the leaves receive approximately 96 to 98 % of maximal diffuse light receivable at their microsites. If the leaves were held horizontally, light capture would decrease by 2.8 ± 3.6 % as compared to actual leaves. Especially for 22 plants with $D_{\max}$ biased more than 10 degrees from the zenith, light capture would decrease by 3.8 ± 4.1 % if assumed to face the zenith. Therefore, adjusting leaf orientations to maximize light capture is a significant morphological response in *A. heterophyllum* inhabiting forest understory with low light availability. Small departure from flatness of a whole leaf of the forest site plants can be interpreted as the result of adjusting the orientation of each leaflet to the same direction, $D_{\max}$. The high LCEs of *A. heterophyllum* plants are realized by regulating the angular geometry of leaflets in response to the directionality of canopy openings.

As shown by model simulations (Fig. 2-7), leaflet inclination and folding at the deforested site effectively decreased the duration of excess irradiance with PPFDs above photosynthetic saturating level, and should contribute to maintaining efficient photosynthesis under a high, stressful irradiance.

In the model estimation of daily light capture, I assumed that there are no diurnal changes in leaflet inclination angle nor folding. However, leaves of *A. heterophyllum* change their geometry to enhance light capture under conditions of relatively low light incident light and moderate air temperature in morning and late afternoon, and to suppress light capture under high incident light with highly rising air temperature at midday by varying the degree of leaflet folding. Diurnal regulation of leaf light capture by the flexible leaf surface orientation appears to maximize carbon gain over the life of the leaf. This flexible and rapid regulation of orientation of the leaf surface through leaflet folding may be caused by turgor pressure changes responding to the leaf water status (e.g., Koller 1990; Saitou, Inamura and Ishihara 1994).

The artificial horizontal constraining of *A. heterophyllum* leaves at the deforested site showed striking effects of leaf orientation on photosynthetic performances, i.e., substantial decrease of A and g_{sw} in the horizontally constrained leaves on the sunny day of 16 June. There are two possible mechanisms for the reduced daily maximum A and severe midday depression; stomatal closure and photoinhibitory damages. It is well known that stomata close with increasing leaf to air vapor pressure deficit and transpirational water loss (Tenhunen, Lange and Braun 1981; Farquhar and Sharkey 1982; Maier-Maercker 1983; Mott and Parkhurst 1991). It is also well documented that excess irradiance and high leaf temperature cause photoinhibitory damages, i.e., inactivation of photosynthetic reactions associated with photosystem II, and/or reduced carboxylation efficiency (Powles 1984; Tenhunen *et al.* 1984; Demmig-Adams *et al.* 1989; Demmig-Adams and Adams 1992; Cheeseman and Lexa 1996; Valladares and Pearcy 1997). If the former is the case, Ci should be decreased by photosynthetic consumption of CO_2. In the latter case, A at a given Ci should decrease while Ci may not decrease. Since values of Ci in the horizontally constrained leaves of *A. heterophyllum* did not differ from those in the naturally oriented leaves which showed high A all the day (Fig. 2-8), photoinhibitory damage is mainly responsible for the depression of A in the horizontally constrained leaves.

Midday depression in the horizontally constrained leaves was less on 12 than on 16 June. The lower irradiance with intermittent low light period on 12 June caused less photoinhibitory damages in the horizontally constrained leaves. This result indicates that the photoinhibitory reduction of A in the horizontally constrained leaves depends on the daily light condition, rather than the accumulation of damage due to continuous exposure to high light incidence for about 10 days. The similar values of early morning

A of the two treatment leaves on both experimental days suggest that the horizontally constrained leaves can recover from the photoinhibitory damage over night. This result is consistent with the recent reports that photoinhibitory effects on photosynthesis are generally short-lived (e.g., Gamon and Pearcy 1990; Krause, Virgo and Winter 1995; Krause and Winter 1996).

Increased leaf angles in response to a drier conditions are reported in soybean (Meyner and Walker 1981; Kao and Forseth 1991; Saitou *et al.* 1994) and other plants (e.g., Forseth and Ehleringer 1980; Comstock and Mahall 1985). As transpirational leaf cooling depends on water availability (Schulze 1982), decreasing light capture by means of flexible leaf orientation helps maintain moderate leaf temperatures, with less transpirational water loss under drought conditions (Cowan 1982). Decreasing light capture of *A. heterophyllum* by the flexible paraheliotropic leaflet movements should be advantageous at the deforested site, where leaf photosynthesis is highly limited by shortage of soil water (Chapter 1).

Table 2-1. Summary of leaf morphology and light capture for *Arisaema heterophyllum* plants at the forest understory and deforested open site. Leaflet folding was measured around noon. 'Departure from flatness of a whole leaf' was calculated as the variations in the direction of each half of leaflet around mean direction of whole leaf (D_{Leaf}). This was calculated assuming β=40 in the deforested site plants. Relative light capture at the forest understory indicates the ratio of whole light capture at actual orientation (D_{Leaf}) to maximal potential light capture facing $D_{\max}$ at each microsite, i.e., light capture efficiency. At the deforested site, it indicates the ratio of estimated daily light capture for the measured leaflet orientations (ϕ) to that of horizontal leaflet (cf. Fig. 2-7a). Values are mean ± SD except leaflet folding of the forest site plants. Sample plant numbers of the departure from flatness and relative light capture of the forest site plants, and sample leaflet numbers of α, β, ϕ, and of relative light capture of the deforested site plants are indicated in the parenthesis, respectively.

	Forest understory		**Deforested site**
	May 1997	June 1997	June 1996
Inclination of leaflet midvein (α)	17.3±10.5	19.5±10.8	24.1±10.9
	(407)	(414)	(143)
Leaflet folding (β)	0	0	40.7±22.4
	(407)	(414)	(65)
Max. slope of leaflet surface (ϕ)	17.3±10.5	19.5±10.8	47.2±18.2
	(407)	(414)	(65)
Departure from flatness	13.6±3.2	14.0±3.1	41.1±5.0
of a whole leaf	(35)	(35)	(10)
Relative light capture (%)	95.6±2.2	97.7±2.5	75.1±15.8
	(34)	(35)	(65)

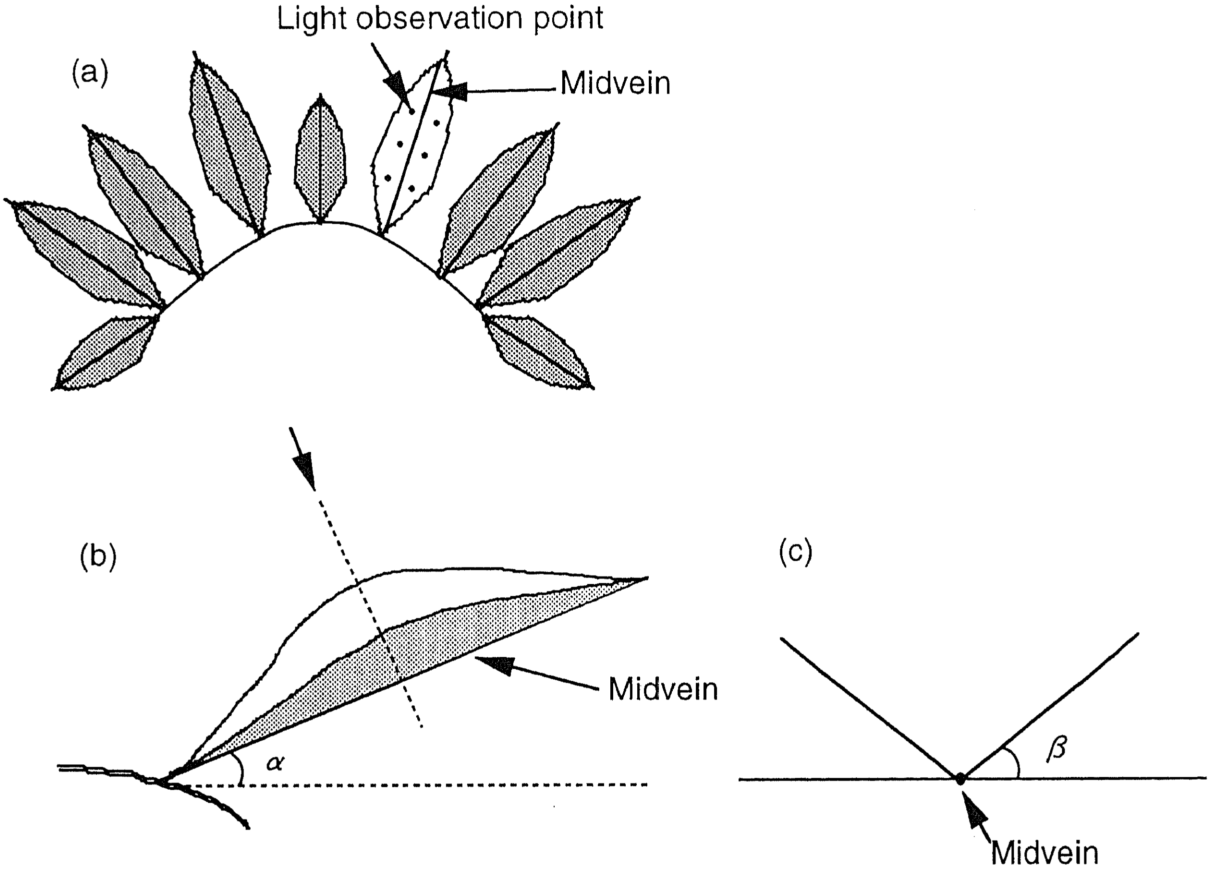

Figure 2-1. Vertical view of a leaf of *A. heterophyllum* (a) and parameters of leaflet geometry measured in the present study. Inclination angle (a) of midvein (b) and folding (b) of leaflet blade (c). (c) represents the cross section of the leaflet indicated with the dotted line in (b).

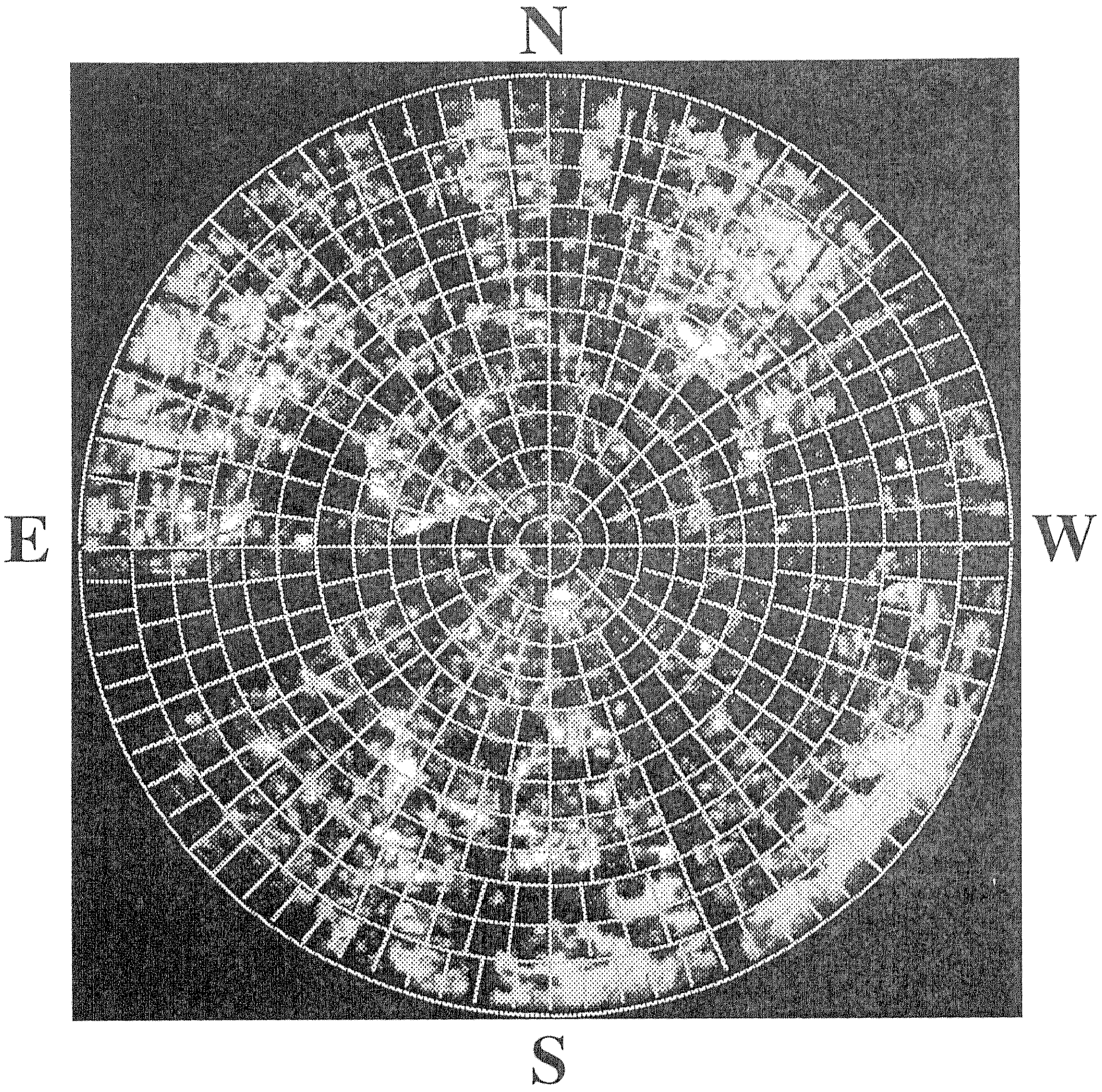

Figure 2-2. A grid to divide the sky hemisphere into 200 cells of identical solid angles. This virtual grid is superimposed in a computer on the images of hemispherical photographs of forest canopy to assess spatial distribution of canopy openness over the hemisphere.

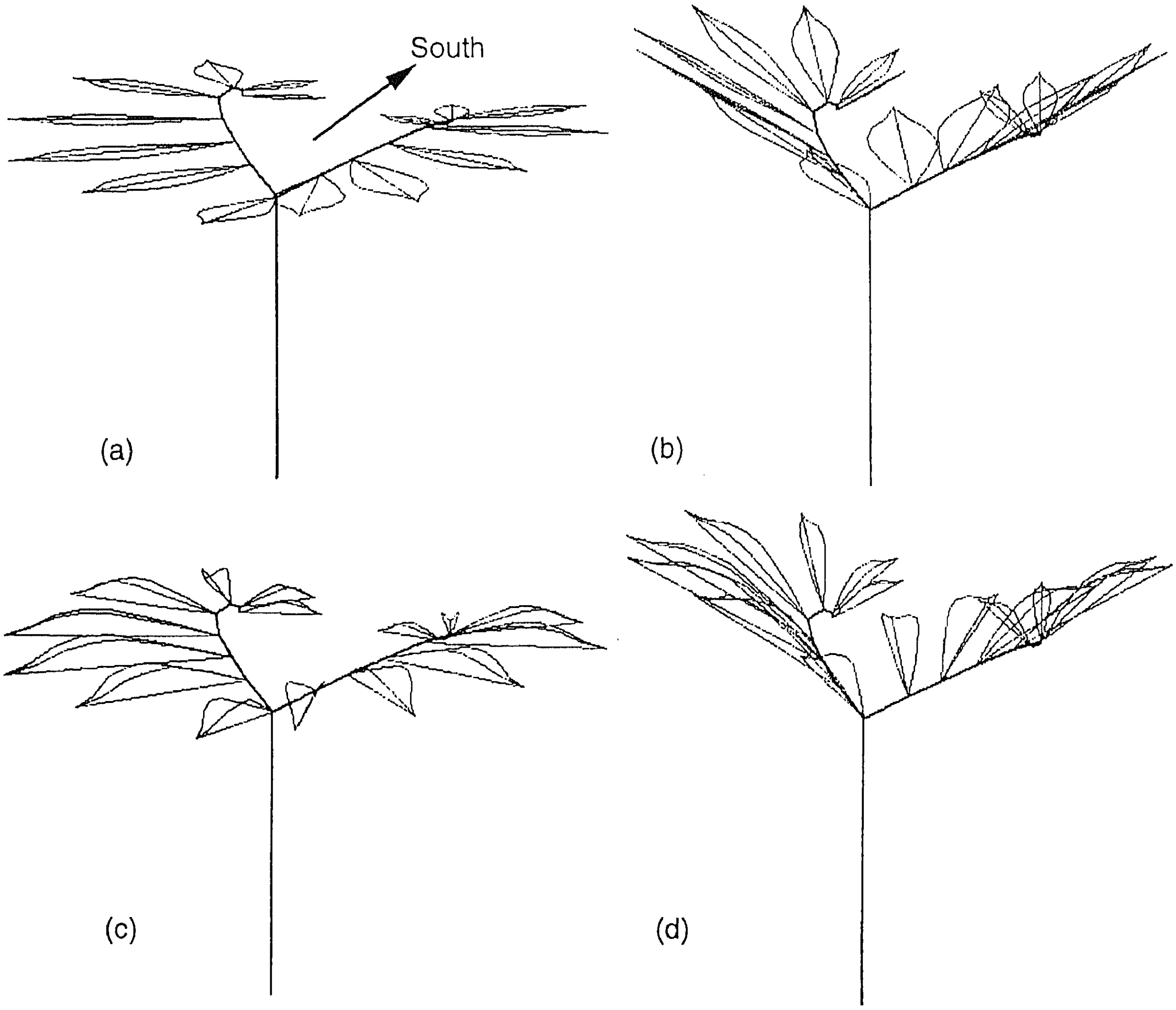

Figure 2-3. Images of *A. heterophyllum* plants reconstructed by computer. Plants with horizontal (a), inclined (b), folded (c), and inclined and folded (d) leaflets. Arrow in (a) indicates the azimuth of a whole plant (see text).

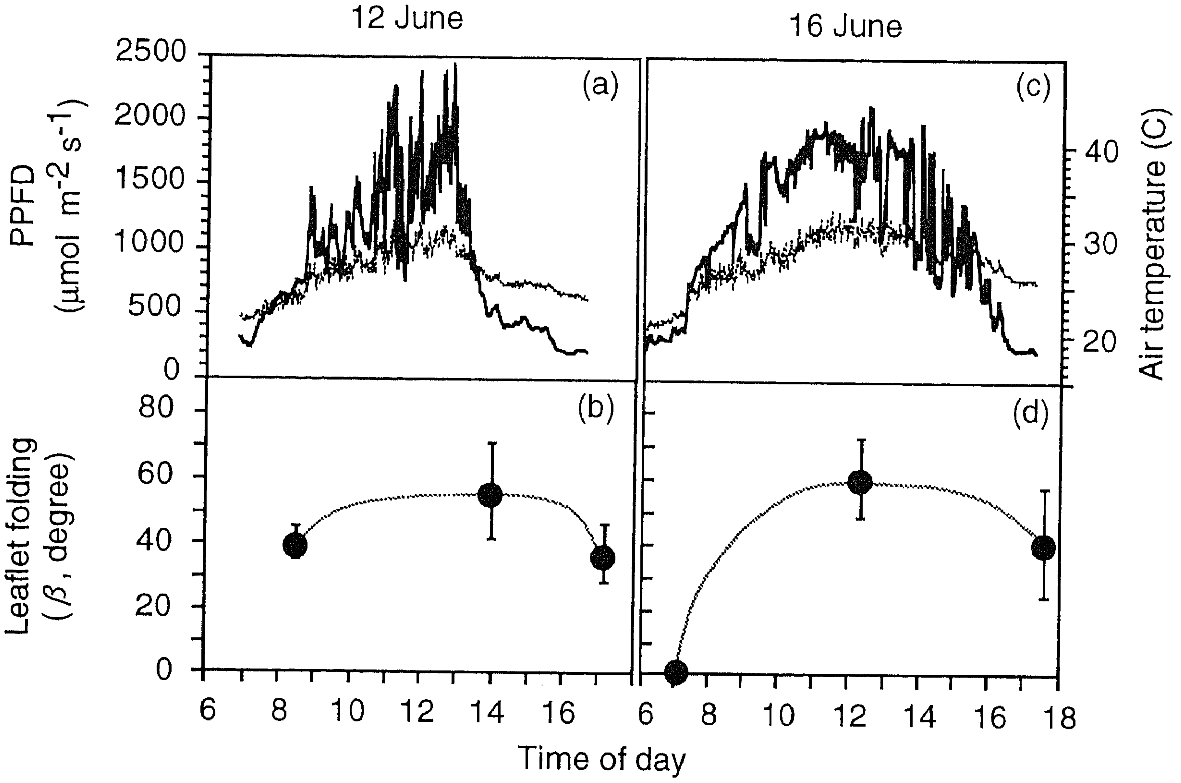

Figure 2-4. Diurnal changes of leaflet folding at the deforested site on 12 (b) and 16 (d) June 1996. Mean ± SD of five (on 12 June) and 12 (on 16 June) leaflets are plotted. Lines in (b, d) were fitted by eye. PPFD at 3 m height horizontal plane (thick line) and air temperature at 1 m height (thin line) were also measured (a, c).

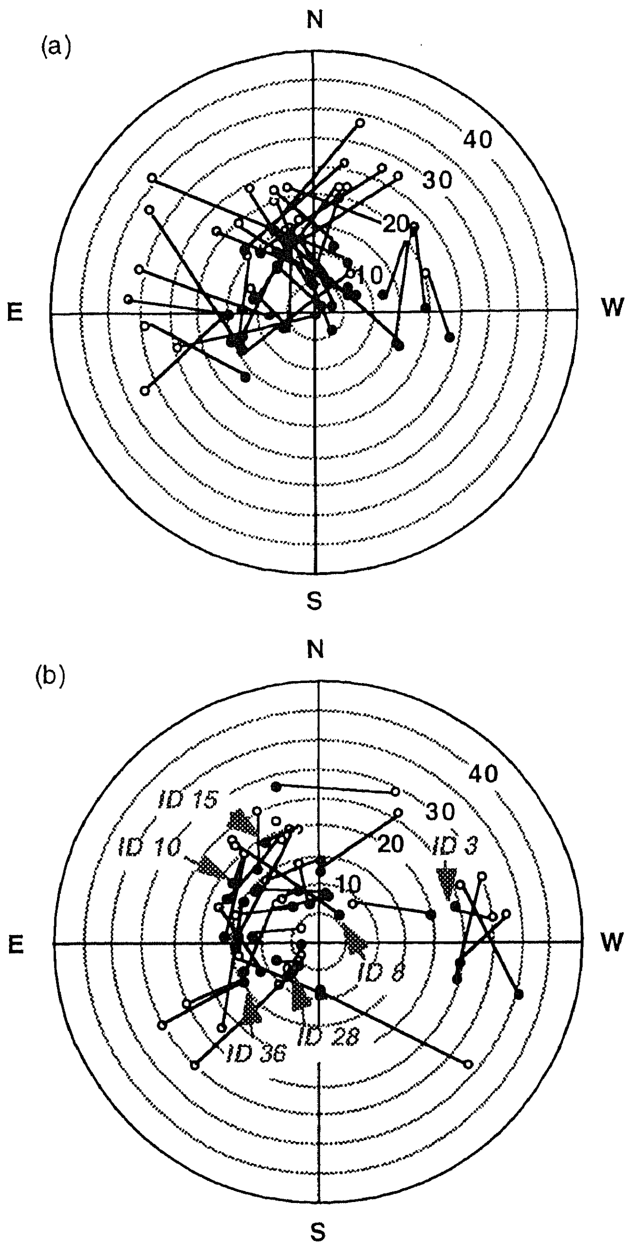

Figure 2-5. Spatial distributions of D_{max} (direction of the leaf surface for maximizing diffuse light capture; ○) and D_{Leaf} (direction of whole leaf; ●) for *A. heterophyllum* plants at the forest understory in May (a) and June (b) 1997. Lines connect the two vectors for each plant. Numbers on the circles represent zenith angle. Distributions of D_{leaflet} (actual directions of leaflets) and directional dependence of leaf light capture are shown in figure 2-6 for the plants with arrows in (b). Note that the east and west are reversed as in hemispherical canopy photographs.

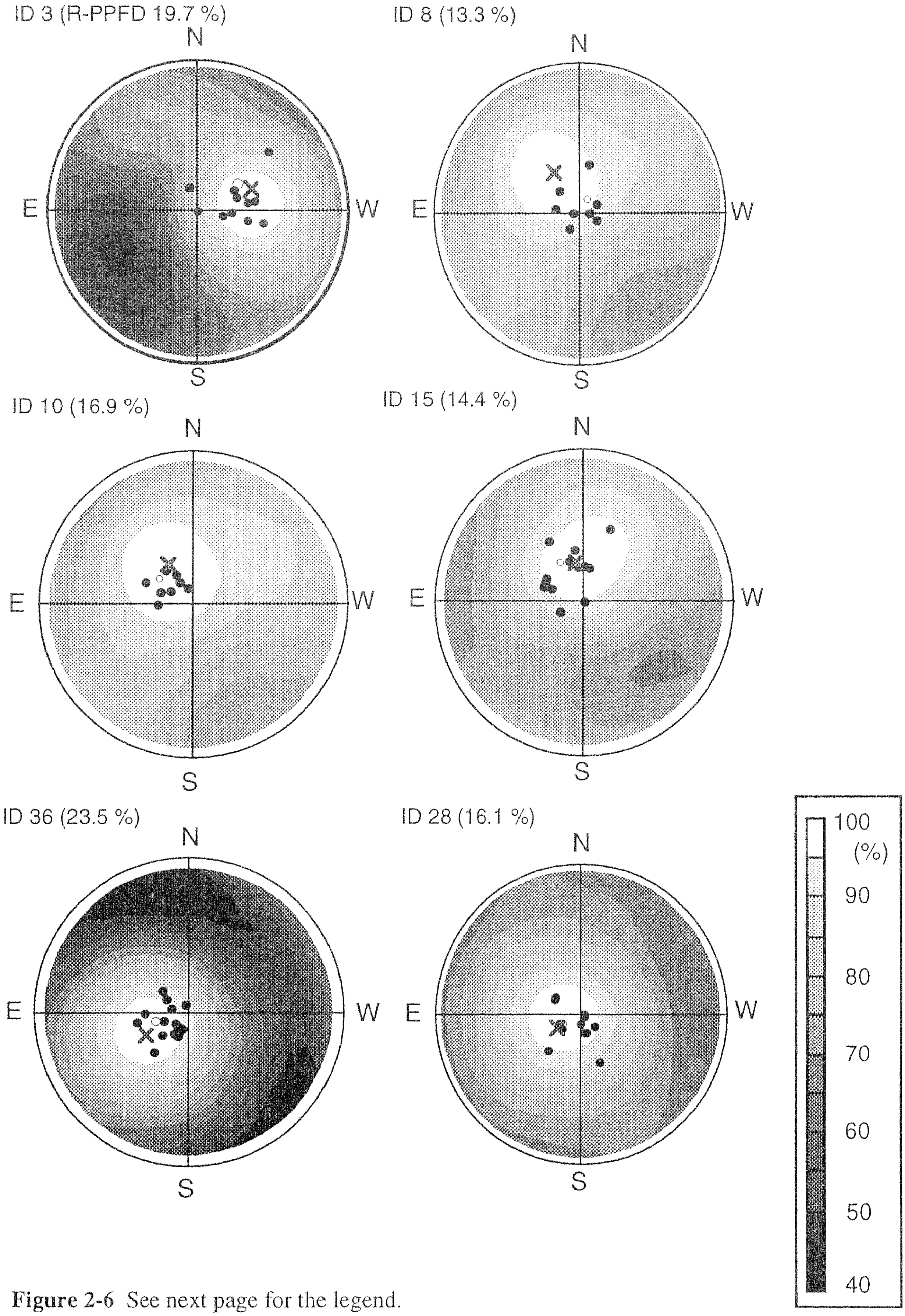

Figure 2-6 See next page for the legend.

Figure 2-6. Counter map of light capture efficiency (LCE) on a light receiving surface facing various directions over the hemisphere for the *A. heterophyllum* plants at the forest site measured in June 1997. LCEs are plotted as percentages (5 % intervals) to the predicted maximum diffuse light capture when the whole leaf surface faced to $D_{\max}$ (×). D_{Leaf} (○) and D_{leaflet} (●) are also plotted. Relative PPFD (the ratio of leaf light capture at the actual orientation to PPFD above the forest canopy) are indicated in the parenthesis by the ID numbers. Zenith angle of $D_{\max}$ and D_{Leaf} were; 23.8 and 30 degree for ID 3, 5.7 and 23 degree for ID 8, 17.8 and 22 degree for ID 10, 19.8 and 20 degree for ID 15, 13.6 and 25 degree for ID 36, and 8.2 and 7 degree for ID 28, respectively. Zenith angles are proportional to the distance of a point between the zenith and the outer circle, which represents a zenith angle of 90.

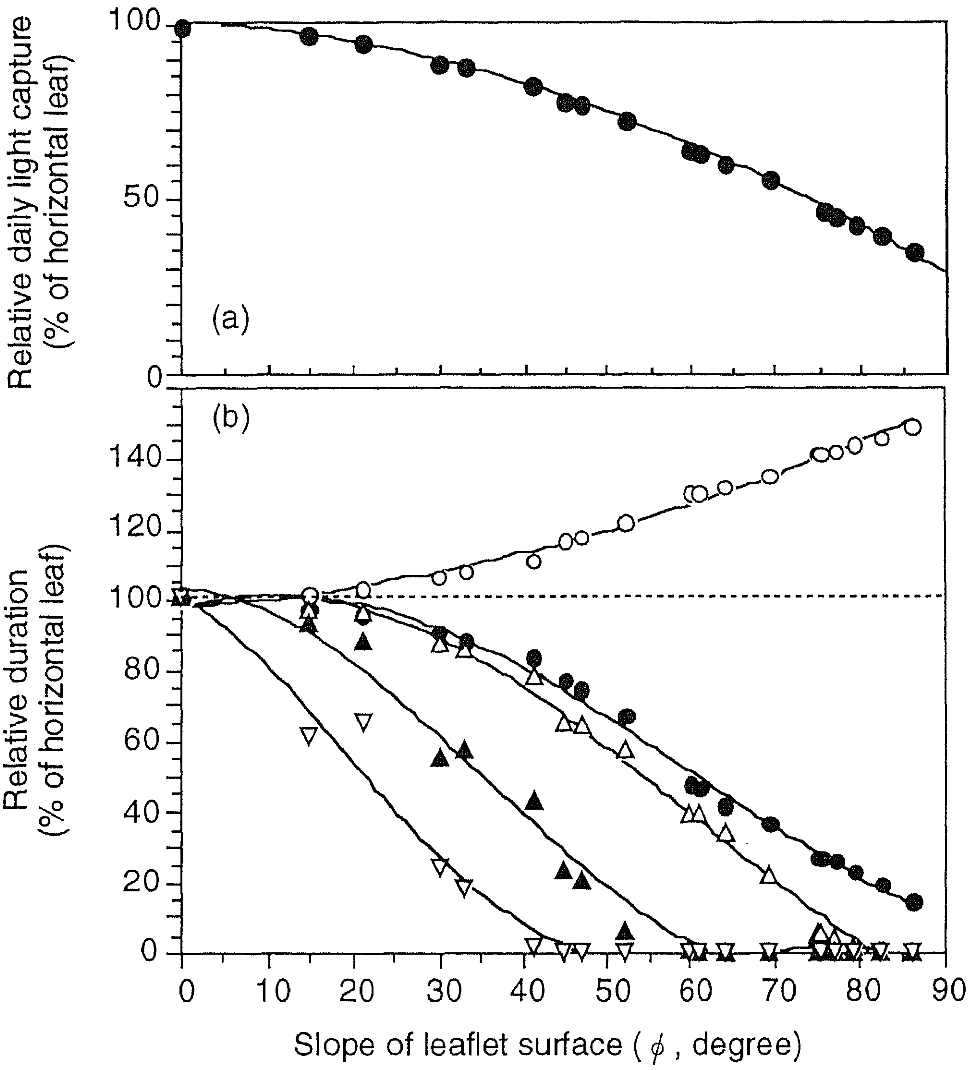

Figure 2-7. Effects of the slope of leaflet surface (ϕ, calculated from α and β) on (a) daily light capture and (b) relative duration of light capture (accumulated time frequency in a day) for PPFDs below photosynthetic saturating light level (600 μmol photons m^{-2} s^{-1}) and PPFDs above 600 μmol photons m^{-2} s^{-1}, i.e., 'excess' light incidence. Symbols in (b) are; ○ for PPFD < 600 μmol photons m^{-2} s^{-1}, ● for PPFD > 600 μmol photons m^{-2} s^{-1}, △ for PPFD > 1000 μmol photons m^{-2} s^{-1}, ▲ for PPFD > 1400 μmol photons m^{-2} s^{-1} and ▽ for PPFD > 1600 μmol photons m^{-2} s^{-1}.

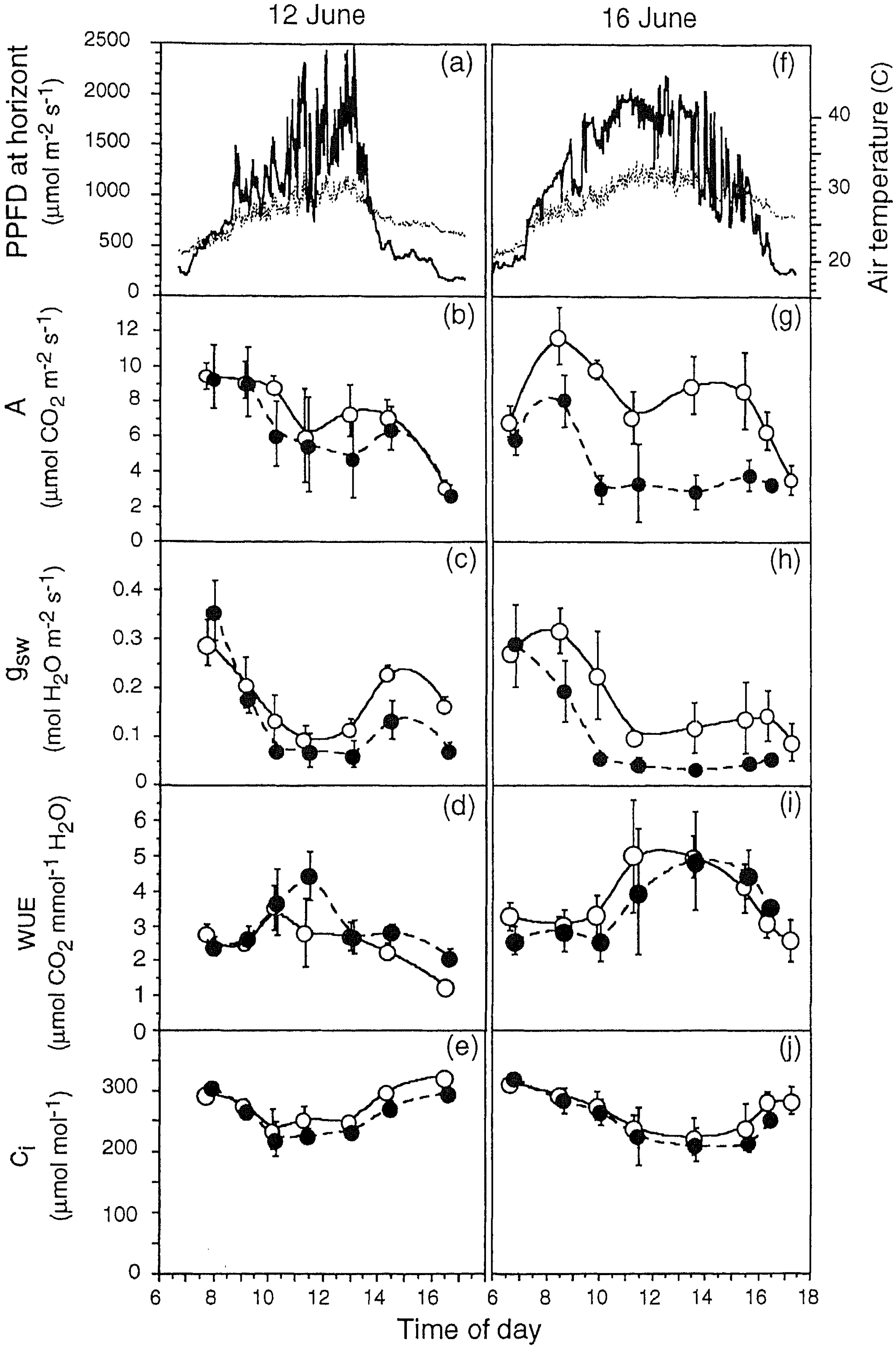

Figure 2-8. Diurnal changes of photosynthetic rates (b, g), stomatal conductance to water vapor (c, h), water use efficiency (d, i) and calculated intercellular CO_2 concentration (e, j) for the naturally orientated (○) and horizontally constrained (●) leaves at the deforested site on 12 and 16 June 1996. Mean ± SD of three or four leaflets are plotted. Reference PPFD at 3 m height horizontal plane (thick line) and air temperature at 1 m height (thin line) were also measured (a, f).

Chapter 3

Stomatal and non-stomatal limitations to diurnal photosynthesis under natural high light conditions in *Arisaema heterophyllum* plants acclimated to sun and shade environments.

Introduction

In the previous chapters, physiological and morphological evidence showed that *Arisaema heterophyllum* is highly plastic to light environments (Chapters 1 and 2). Leaves developing at the deforested site exhibited a significantly higher light saturated assimilation rate and a higher leaf-mass to area ratio (LMA) as compared with the leaves from the forest site (Chapter 1). *A. heterophyllum* can also change its leaf orientation to increase light availability in light-limited microsites as well as to avoid excess incident light at the deforested site (Chapter 2). These studies indicate that leaves of *A. heterophyllum* are able to both "tolerate" the high light and "avoid" excess light in a sunny environment to some extent. In the present chapter, I further explore physiological mechanisms involved in light acclimation of the leaves in the species.

Plant ecophysiologists have made intensive studies on light acclimation of photosynthesis (for reviews see Björkman 1981; Givnish 1988; Pearcy and Sims 1994; Terashima and Hikosaka 1995). Some of the studies suggest that high-light leaves have physiological features favorable to utilize high light or to tolerate excess PPFD (photosynthetically active photon flux density) as compared to low-light leaves (Ferrer and Osmond 1986; Adams *et al.* 1988; Correia, Chaves and Pereira 1990; Mulkey and Pearcy 1992; Ögren and Rosenqvist 1992). However, few studies have evaluated high light acclimation of leaf physiological characteristics in detail under natural light regimes (but see Epron, Dreyer and Breda 1992; Mulkey and Pearcy 1992; Valentini *et al.* 1995;

Valladares and Pearcy 1997). Further studies on photosynthetic characteristics in response to high light stress and other stressful environmental factors co-occurring in the natural high light habitats would provide an insight into deeper understanding of acclimation of plants to high light environments.

Excess light, often co-occuring with high leaf temperature and/or water deficit, is demonstrated to be the major environmental stress causing the midday depression of photosynthesis (Schulze *et al.* 1980; Tenhunen, Lange and Braun 1981; Tenhunen, Lange and Jahner 1982; Tenhunen *et al.* 1984; Chaves *et al.* 1987; Downton, Grant and Loveys 1987; Demmig-Adams *et al.* 1989b; Correira *et al.* 1990; Epron *et al.* 1992; Valladares and Pearcy 1997). Stomatal closure and photoinhibitory damages could be among the major physiological factors responsible for the midday depression. Stomatal closure, which can be induced by a large VPD (leaf to air water vapor pressure deficit) or transpirational demand (Maier-Maercker 1983; Mott and Parkhurst 1991), would lead to a decrease in intercellular concentration of CO_2 (*Ci*) (Tenhunen *et al.* 1981; Lange, Tenhunen and Beyschlag 1985; Roessler and Monson 1985; Raschke and Resemann 1986; Wise *et al.* 1990). In *A. heterophyllum*, stomatal closure may be one of the most important factors limiting CO_2 assimilation in high light environment, since I found that photosynthesis of the plants under drought was largely limited by stomatal closure (Chapter 1).

Photoinhibitory damages include the inactivation of photosynthetic reactions associated with photosystem (PS) II (Powles 1984; Adams *et al.* 1988; Ögren 1988; Demmig-Adams *et al.* 1989b; Demmig-Adams and Adams 1992; Osmond 1994), and/or reduced carboxylation efficiency (Tenhunen *et al.* 1984; Quick *et al.* 1992; Cheeseman and Lexa 1996). In addition to excess light, high temperature and water deficit may also enhance further inactivation of photosynthetic reactions (Ludlow and Björkman 1984; Raschke and Resemann 1986; Gamon and Pearcy 1990; Valladares and Pearcy

1997). The inactivation of PS II includes two means of photoinhibition, i.e., 'dynamic' and 'chronic' photoinhibition (Osmond 1994). The former is rapidly reversible and dominated by a down regulation due to ΔpH-dependent, zeaxanthin associated processes in PS II antenna that promote thermal dissipation of excitation (Demmig-Adams and Adams 1992). The latter is slowly reversible and dominated by PS II reaction center core processes involving disequilibrium of the D1 protein cycle and generation of non-functional centers that promote thermal dissipation (Osmond 1994). I have already found that leaf photosynthesis of *A. heterophyllum* decreased considerably at high leaf temperatures (Chapter 1) and continuous exposure of leaves to excess irradiance resulted in the reduction of assimilation rate at given *Ci* (Chapter 2). Photoinhibitory effects therefore may be also involved in the midday depression of the species.

In natural high light environments, limitations occurring at each process of photosynthetic carbon fixation should affect each other (Foyer *et al.* 1990; Cheeseman and Lexa 1996). For instance, a CO_2 deprivation at the chloroplast level, induced by stomatal closure under elevated temperature and high irradiance, could enhance the sensitivity of photosynthetic apparatus to high light stresses and promote damage to PS II photochemistry (Powles 1984; Peterson, Sivak and Walker 1988; Cornic and Massacci 1996). In sun leaves of *A. heterophyllum* that may frequently experience stomatal closure under excessive PPFDs, physiological capacities such as thermal dissipation and photorespiration can be well increased to avoid chronic photoinhibition by excess excitation light energy, as compared to shade leaves.

In order to clarify the possible mechanisms involved in stresses-induced reduction of photosynthesis under natural high or excess light conditions, and also in avoidance of photoinhibitory damages, I made quantitative evaluations of stomatal and non-stomatal limitations to photosynthesis and of their interactions by measuring diurnal changes of photosynthetic responses using gas exchange and chlorophyll fluorescence techniques in the leaves of *A. heterophyllum* plants grown in two contrasting light environments, the forest site and the deforested site. I paid a particular attention to the midday depression of photosynthetic responses because it is likely to be one of the most important keys to understand light acclimation of photosynthesis in the natural environments.

Materials and methods

Measurements of leaf gas exchange and chlorophyll a fluorescence

All measurements were carried out using two to four plants of *A. heterophyllum* grown at the forest site and the deforested site. In mid-May 1997, sample plants with 10 to 15 fully matured leaflets were dug up carefully not to damage their roots and transplanted into clay pots (15 cm diameter × 15 cm depth) filled with field soil. The potted plants were transferred to an experimental garden of the Agricultural and Forestry Research Center, in the University of Tsukuba, about 15 km north-east of the natural habitat of *A. heterophyllum* during the evening, watered well and allowed to stand overnight. The potted deforested site plants were kept under a light condition of approximately 90 % of full sunlight in the experimental garden during the experiments to avoid destructive damage due to flooding in the natural habitat, while I used newly collected forest site plants for each measurement day. The potted forest site plants could be kept at their growing habitat since there was less influence of flooding because of its slightly higher altitude (12 m) than that of the deforested site (10 - 11 m).

Leaf gas exchange and chlorophyll *a* fluorescence were measured at the experimental garden, on clear days of 28 May, 7 and 24 June 1997. For each sample plant, three to four single attached leaflets having various inclination angles (0 - 30 degree) were chosen and used for the measurements.

Diurnal patterns of leaf gas exchange were continuously measured using a portable photosynthesis measuring system (LI-6400; Li-Cor Inc., Lincoln, Neb., USA). Gas exchange parameters such as *A* (net CO_2 assimilation rate), g_{lw} (leaf conductance) and *Ci* were calculated according to Field, Ball and Berry (1991). The clamp-on leaf chamber allowed natural illumination of the upper leaf surface during the measurements. During the periods when leaflets were inserted to the chamber, they were oriented to be fully exposed to the direct irradiance. Incident PPFD beside the leaf chamber was measured with a photon sensor (LI-190SA; Li-Cor Inc., Lincoln, Neb., USA). CO_2 concentration of the air entering the leaf chamber (*Ca*) was controlled at 350 μmol mol^{-1}. Air temperature in the chamber was adjusted manually to the air temperature outside the system, which was measured by copper-constantan thermocouples placed at 1 m above ground. Relative humidity in the chamber was adjusted to be similar to that in the outside air. PPFD at a height of 3 m above ground was measured with a photon sensor (IKS-25; Koito Industry, Japan) mounted horizontally. Data of reference PPFD and air temperature were recorded on a data logger (Thermodac-E; Eto-Denki Inc., Japan) at 1-min interval.

Concurrent measurements of chlorophyll fluorescence yield (Genty, Briantais and Baker 1989; Bilger, Schreiber and Bock 1995) were performed on the same leaves monitored for gas exchange using a portable pulse-amplitude modulated fluorometer (PAM-2000; Heinz Walz, Effeltrich, Germany). The end of the optical fibre was inserted into the upper cover of the leaf chamber (LI-6400-06) of LI-6400 through a hole. Care was taken to avoid shading of the leaf surface. Sealing of the chamber was tested by exposing the chamber to higher CO_2 gas than that inside the chamber. The central unit of the fluorometer was shaded against direct sunlight to avoid overheating of the equipment. The following parameters were obtained through the measurement; basic (*Fo*) and maximum (*Fm*) fluorescence before dawn to obtain photochemical efficiency of open reaction centers as [$Fv/Fm = (Fm - Fo) / Fm$], and steady-state (F) and maximum (Fm') diurnal values of fluorescence under natural illumination to obtain photochemical efficiency of PS II as [$\Delta F/Fm' = (Fm'-F) / Fm'$], according to Genty *et al.* (1989). Fm and Fm' were measured by illuminating a strong flash of about 6000 µmol photons m^{-2} s^{-1} for 800 ms. Pre-dawn Fv/Fm was measured on 7 and 24 June. On 7 June, Fv/Fm was also measured around 1700 h after 15 - 20 min dark-adaptation.

In addition to the above measurements of gas exchange and chlorophyll fluorescence yield in 1997, diurnal changes (0700 - 0900 h, 1000 - 1200 h and 1300 - 1500 h) in the A-Ci relation were measured on 28 May 1998 for the potted plants collected from the deforested and forest sites. Ca was changed in stepwise manner (0, 100, 200, 350, 600 and 800 µmol mol^{-1}). Chlorophyll fluorescence yield was also measured simultaneously. Measurements were carried out at the PPFD of 1300 - 1700 µmol m^{-2} s^{-1} and at natural air temperatures (20 - 27 °C). Leaf temperatures were in the range of 23 - 25 °C during the first measurement (0700 - 0900 h), but increased to 27 - 30 °C in the second (1000 - 1200 h) and to 28 - 31 °C in the third (1300 - 1500 h) measurements.

In order to clarify the 'potential' response of photochemistry, chlorophyll fluorescence yield was also measured under controlled light and temperature conditions in the laboratory with the plants grown at the deforested and forest sites in 1997. Fv/Fm was first measured for the attached leaflets of the sample plants which have been watered well and kept in the dark over night, and then $\Delta F/Fm'$ and non-photochemical quenching coefficient (q_N) were measured under several incident PPFDs. The q_N was calculated as [$(Fm - Fm') / (Fm - Fo)$], in which Fm and Fo, or Fm' were those obtained for the above measurements of Fv/Fm or $\Delta F/Fm'$, respectively. Leaves were illuminated at least for five minutes before fluorescence measurements at any given level of PPFD. This length of illumination was always found to be sufficient to reach a steady-state fluorescence yield. The light responses of chlorophyll fluorescence of the leaves from the deforested and forest sites were measured under controlled conditions with *Ca* of 350 µmol mol^{-1}, leaf temperature of 25 - 27 °C and relative humidity of 70 %.

Data analysis

Estimation of stomatal limitation to assimilation rate

In order to estimate the stomatal limitation to CO_2 assimilation rate under outdoor conditions, photosynthetic rate at *Cc* (CO_2 mole fraction at the catalystic site of Rubisco; ribulose-1,5-bisphosphate carboxylase/oxygenase) of 350 µmol mol^{-1} (A_{350}) was estimated by combining the following equations of Genty *et al.* (1989) and Farquhar, von Caemmerer and Berry (1980);

$$J_T = \Delta F/Fm' \times \mathrm{PPFD} \times 0.84 \times 0.5 \qquad (1)$$

$$J_T = 4V_C + 4V_O \qquad (2)$$

$$A = V_C - 0.5V_O - Rd \qquad (3)$$

where J_T is the total electron transport rate, V_C and V_O are carboxylation and oxygenation rate of RuBP, respectively, and A is the net CO_2 assimilation rate. Eqn (1) assumes an equal distribution of photons between the two photosystems and a leaf absorptance of 0.84. In the calculation, dark respiration rate was taken as the rate of 'day' respiration (*Rd*; µmol CO_2 m^{-2} s^{-1}), and I used the empirical equation, $Rd = 0.44 - 0.01\ T_L + 0.0006\ T_L^2$, to approximate *Rd* for both sun and shade leaves (not shown), where T_L is leaf temperature in °C. I found in a previous study that there is no significant difference in dark respiration rate between sun and shade leaves (Chapter 1).

The ratio of carboxylation versus oxygenation rate (V_C/V_O) is the product of the specificity factor of Rubisco (*S*; mol mol^{-1} in gas phase) and the ratio of CO_2 mole fraction (*C*) and O_2 mole fractions (*O*) at the carboxylation/oxygenation site (Laing, Ögren and Hageman 1974); here *O* is assumed to be equal to that in the air.

$$V_C / V_O = S\,(C / O) \qquad (4)$$

I estimated the leaf temperature dependency of S using the data of A-Ci relationship for *A. heterophyllum* (Fig. 3-8) following the method of Brooks and Farquhar (1985). CO_2 compensation point in the absence of non-photorespiratory CO_2 evolution (Γ^*) can be expressed from Eqn (4) when $V_C = 0.5 V_O$ as,

$$\Gamma^* = 0.5\, O / S \qquad (5)$$

Γ^* was estimated as an x-intercept of linear regression at low Ci of 15 - 140 μmol mol^{-1} of the relationship between $(A+Rd)$ and Ci (not shown). Thereby S was calculated using Eqn (5) substituting O of 210 mmol mol^{-1}. Measurements of the A-Ci relation with different leaf temperatures allowed me to obtain temperature dependency of S as,

$$S = 5570.2 - 139.8\, \mathrm{T_L} + 0.8\, \mathrm{T_L}^2 \qquad (6)$$

On the assumptions that J_T can be estimated from measured $\Delta F/Fm'$ and Eqn (1), and that electron flow to the Mehler-ascorbate peroxidase reaction is negligible, A_{350} can be estimated as follows. By substituting the value of S obtained from Eqn (6), 350 μmol mol^{-1} for CO_2 and 210 mmol mol^{-1} for O_2 into Eqn (4), V_C can be determined as

$$V_C = V_O / \phi \qquad (7)$$

where ϕ is the product of [(210000 / 350) / S].

Then substitution of Eqn (7) into Eqn (3) appears as

$$A_{350} = (1 / \phi - 0.5)\, V_O - Rd \qquad (8)$$

While, total electron transport rate (J_T) can be determined from Eqns (2) and (7) as

$$J_T = 4\,(1 / \phi + 1)\, V_O \qquad (9)$$

Since J_T is the product of measured $\Delta F/Fm'$ and Eqn (1), V_O can be obtained.

By substituting the determined value of V_O into Eqn (7) and the value of ϕ into Eqn (8), A_{350} can be calculated. Then I calculated the index of stomatal limitation (ISL; %) as;

$$\mathrm{ISL} = (1 - A / A_{350}) \times 100 \qquad (10)$$

where A is the measured rate of net CO_2 assimilation.

Estimation of the partitioning of photosynthetic electron flow between carboxylase and oxygenase reactions

Total electron flow (J_T) can be divided into two components J_C and J_O which are the electron flows devoted to carboxylation and oxygenation of RuBP, respectively. J_C and J_O were calculated from J_T, A and Rd with an assumption that electron flow to the Mehler-ascorbate peroxidase reaction is negligible, as follows according to Epron *et al.* (1995),

$$J_T = J_C + J_O \qquad (11)$$

$$J_C = 4A + 0.5J_O + 4Rd \qquad (12)$$

Eqn (11) represents that four electrons are required for one carboxylation and oxygenation cycle and one CO_2 is released every two oxygenation cycle in the glycine decarboxylation in the photorespiratory pathway (Laing *et al.* 1974).

From Eqns (11) and (12), J_C and J_O are calculated respectively as,

$$J_C = 1/3\ \{J_T + 8\ (A + Rd)\} \qquad (13)$$

$$J_O = 2/3\ \{J_T - 4\ (A + Rd)\} \qquad (14)$$

Curve fittings

Relationships between incident PPFD and A were line-fitted using an equation of rectangular hyperbora (Thornley 1976). For A-Ci curves measured on 28 May 1998, data were analyzed using the regression equation of $A = \mathrm{a} - \mathrm{b} \times exp\ (-\mathrm{c} \times Ci)$, and carboxylation efficiency (CE) was obtained as ($\mathrm{b} \times \mathrm{c}$).

Results

Diurnal patterns of leaf gas exchange rates and chlorophyll fluorescence yields

Daily assimilation rate (A) of both sun and shade leaves reached their maximum in the morning and then decreased during the day (Fig. 3-1). A tended to start to decrease at an air temperature around 25 °C. The maximum A was highly constant among the three different measurement days from 28 May to 24 June and it was around 15 and 10 μmol CO_2 m^{-2} s^{-1} for sun and shade leaves, respectively. Leaf conductance to water vapor (g_{lw}) exhibited a diurnal variation pattern more or less similar to A, but achieved its maximum earlier than A did depending on the measurement days, and it was around 0.25 and 0.15 mol H_2O m^{-2} s^{-1} for sun and shade leaves, respectively.

Pre-dawn *Fv/Fm* of *A. heterophyllum* was as high as 0.8 for the leaves from both sites. After a rapid decrease in the morning, *ΔF/Fm'* became relatively constant but increased again in the late afternoon. *ΔF/Fm'* around the midday was higher in sun leaves (0.3 - 0.4) than that in shade leaves (0.1 - 0.2). Total electron transport rate (J_T) showed a diurnal pattern contrasted to that of A and reached its maximum almost at the time when midday depression of A occurred. In sun leaves, J_T remained high during the midday depression of A.

Effects of PPFD, leaf temperature and VPD on diurnal assimilation rate

I examined the effects of environmental factors on photosynthetic performance before and after the 'peak' of A in the morning. Data obtained on 7 June 1997 were analyzed to evaluate the limiting factors of A *in situ*. The dependency of A on PPFD was markedly different before and after the 'peak' of the day (Fig. 3-2a, b). Light saturated A was significantly higher before than that after the 'peak' (ANOVA, $p < 0.0001$).

After the 'peak', light saturated A decreased approximately by 30 % in sun leaves and by 50 % in shade leaves, as compared to those before the 'peak'. The light saturated A, either before or after the 'peak', was higher in sun leaves than those in shade leaves.

In addition to the light incidence, ambient temperature varied greatly during the day. Assimilation rate reached its maximum at leaf temperature of about 25 °C and then decreased as the increase in leaf and air temperatures (Fig. 3-2c, d). The degree of reduction of A with rising leaf temperature was greater in shade leaves than that in sun leaves. Moreover, A decreased considerably with the increase of VPD (data not shown). The decrease in A with increasing VPD was more prominent at VPD > 1.5 kPa, especially in shade leaves in which VPD achieved higher than that in sun leaves.

Chlorophyll fluorescence

Light responses of chlorophyll fluorescence yield under optimal leaf temperature for photosynthesis (25 - 27 °C) (cf. Chapter 1) showed that $\Delta F/Fm'$ was slightly higher in shade leaves (Fig. 3-3a) while q_N was higher in sun leaves (Fig. 3-3b). Also in the plants under natural high light regime, $\Delta F/Fm'$ decreased almost linearly with the increase of PPFD, but was rather constant above PPFD of 700 µmol m^{-2} s^{-1} (Fig. 3-3c, d). Rising leaf temperatures up to 34 °C under the natural high light condition resulted in lower $\Delta F/Fm'$ than those at optimal leaf temperatures. Sun leaves showed by about two-times larger $\Delta F/Fm'$ than that in shade leaves at saturating PPFD and high leaf temperature. In sun leaves, $\Delta F/Fm'$ did not differ much between before and after the assimilation 'peak', while it decreased during the period of midday depression in shade leaves (ANOVA, $p < 0.001$).

In order to evaluate the effect of diurnal variation of PS II photochemical efficiency on A, I examined the relationship between A and J_T with the data at incident PPFD above 500 µmol m^{-2} s^{-1} where photosynthesis may not be limited by light availability

(Fig. 3-4). If the photoinhibitory limitation was responsible for the midday depression of A, a positive dependence of A on J_T would be expected. There was no positive relationship between A and J_T in sun leaves while an almost linearly positive dependence was observed in shade leaves.

Dissipation of excitation energy in photorepiration

Photorespiration was examined by the index J_O/J_T, where J_T is the total electron flow calculated from $\Delta F/Fm'$ and J_O is the fraction of electron flow needed for the photorespiratory path, obtained from Eqn (14). J_O/J_T increased linearly with increasing PPFD and reached a steady-state at PPFD above 500 μmol m^{-2} s^{-1}. In sun leaves, the J_O/J_T after the 'peak' of A was significantly higher than that before (ANOVA, $p < 0.0001$), but little difference was observed in shade leaves (Fig. 3-5a, b).

Photorespiration examined as J_O/J_T highly depended on leaf temperature in both sun and shade leaves (Fig. 3-5c, d). The slope of J_O/J_T, on leaf temperature was almost the same for the leaves of both types, or before and after the 'peak' of diurnal A.

When the J_O value obtained from total electron transport rate was converted into the percentage of photon flux density to the total absorbed incident light, it appeared that sun leaves dissipated about 10 - 15 % of photons absorbed while shade leaves did only 5 - 7 % through photorespiration (Fig. 3-5e, f). After the assimilation 'peak', the ratio of J_O to absorbed photons was significantly higher than that before the 'peak' in sun leaves (ANOVA, $p < 0.0001$), but less varied in shade leaves.

Stomatal limitation on assimilation

At PPFD above 500 μmol m^{-2} s^{-1}, which was the saturating light incidence, A depended linearly on g_{lw} in both sun and shade leaves as long as they were below g_{lw} of 0.2 mol H_2O m^{-2} s^{-1} (Fig. 3-6a, b). For sun leaves, however, A was relatively constant at g_{lw}

above 0.2 mol H_2O m^{-2} s^{-1}, indicating that stomatal aperture was not a major factor limiting A.

Variation in g_{tw} under 0.2 mol H_2O m^{-2} s^{-1} caused the change in the Ci that further resulted in changes in assimilation rate (Fig. 3-6c, d). Under saturated light and constant ambient CO_2 concentration of 350 μmol mol^{-1}, however, A inversely related to Ci. These relationships are also indicating that A at a given Ci of 200 μmol mol^{-1} decreased by 20 % in sun leaves and by 50 % in shade leaves, suggesting that carboxylation efficiencies (*CE*) are reduced.

An index of stomatal limitation was then used to further evaluate the stomatal limitation on A *in situ* quantitatively. I first estimated the specificity factor of Rubisco (S) (Fig. 3-7). Both the absolute value of S and its leaf temperature dependency thus obtained were close to those of purified spinach Rubisco measured *in vitro* by Jordan and Ogren (1984), and it allowed us to estimate the net CO_2 assimilation rate at Cc of 350 μmol mol^{-1} (A_{350}) from J_T. Thus I calculated the index of stomatal limitation ISL (Eqn 10). When the data on 7 June 1997 were closely examined, ISL increased with increasing PPFD in a similar magnitude in both leaves (Fig. 3-6e, f). However, at a given PPFD, ISL after the 'peak' of A was 70 % higher than that before in sun leaves (ANOVA, $p < 0.0001$), while the difference between before and after 'peak' was not significant and at best 20 % of before 'peak' in shade leaves.

Diurnal photosynthetic responses to variable CO_2 *concentrations*

Fig. 3-8 shows the diurnal changes in dependencies of A, $\Delta F/Fm'$, J_T and J_C/J_T on Ci measured under variable CO_2 concentrations on 28 May 1998.

In sun leaves, *CE* was higher in the morning (0700 - 0900 h, 0.10 ± 0.01; mean ± SD) and significantly decreased in the afternoon (1300 - 1500 h, 0.08 ± 0.01) by 20 % (ANOVA, $p = 0.0019$). While in shade leaves, *CE* decreased by 50 % before noon (from 0.04 ± 0.01 in 0700 - 0900 h to 0.02 ± 0.003 in 1000 - 1200 h; ANOVA, $p < 0.05$) and remained low during afternoon hours. CO_2 efflux from the leaves at *Ca* of 0 μmol mol^{-1} is the summation of photorespiration rate and dark respiration rate. I evaluated the photorespiration by subtracting the 'day' respiration rate (*Rd*) from the above rate of CO_2 efflux, taking the temperature dependency of *Rd* into the account. In sun leaves, this 'photorespiration' changed from 1.28 ± 0.11 μmol CO_2 m^{-2} s^{-1} in the early morning through 2.01 ± 0.20 in the late morning (ANOVA, $p < 0.001$) or 1.71 ± 0.09 in the afternoon (ANOVA, $p < 0.01$). While in shade leaves, they were 0.34 ± 0.16, 0.63 ± 0.03 and 0.62 ± 0.07 μmol CO_2 m^{-2} s^{-1} for the three periods, respectively, and the increase from early morning to other periods was significant (ANOVA, $p < 0.05$). 'Photorespiration' was significantly higher in sun leaves than in shade leaves throughout the day (ANOVA, $p = 0.0002$). With the decrease of *CE* and the increase of photorespiration rate, CO_2 compensation point increased from 30 to 60 μmol mol^{-1} in sun leaves, or to 80 μmol mol^{-1} in shade leaves.

In sun leaves, $\Delta F/Fm'$ showed a slight decrease under *Ci* below 200 μmol mol^{-1}, while it was relatively constant for *Ci* from 0 to 700 μmol mol^{-1} in shade leaves (Fig. 3-8c, d). High leaf temperatures decreased $\Delta F/Fm'$ and J_T by 40 % and 70 % in sun and shade leaves, respectively (Fig. 3-8c, d, e, f). J_O/J_T increased with decreasing *Ci* in both sun and shade leaves (Fig. 3-8g, h).

Discussion

Stomatal limitation of CO_2 assimilation rate

Several reports have emphasized the importance of stomatal closure in the mechanisms of photosynthesis suppression under natural high light environments (Tenhunen *et al*

1981, 1982, 1984; Roessler and Monson 1985; Raschke and Resemann 1986; Cheeseman *et al.* 1991). However, quantitative evaluation of stomatal limitation on photosynthesis has been scarcely studied *in situ*. In the present study, I attempted to estimate the stomatal limitation by combining the data of leaf gas exchange and chlorophyll fluorescence yield.

Stomatal and/or non-stomatal limitation of CO_2 assimilation has been evaluated mainly based on the *A*-*Ci* relation analysis (Farquhar and Sharkey 1982). However, overestimation of *Ci* due to patchy stomatal closure (Terashima 1992) or high cuticular transpiration (Kirschbaum and Pearcy 1988; Boyer, Wong and Farquhar 1997) was suggested to be apt to lead misunderstanding of the photosynthetic responses. For example in the present study, the method using Γ^* allowed me to obtain good estimation of *S* (Fig. 3-7) but another method using the ratio of J_C/J_O to *Ci/O* (cf Epron *et al.* 1995) led to an underestimation of *S* (Fig. 3-9). Since the apparent CO_2 flux around CO_2 compensation point is negligible and stomata should tend to open, careful calibration of the gas exchange system enabled me to obtain reliable *Ci* and to estimate Γ^* with little error from *A*-*Ci* relation measurements under low *Ca* conditions of 0 - 200 μmol mol^{-1}, and thus to obtain *S* as in the former case. The latter case is due to a large overestimation of *Ci* at relatively high *Ca* (ex. 350 μmol mol^{-1}).

I attempted to estimate the intercellular CO_2 concentration from *S* and linear electron transport rate (Eqn 10 - 13) as $[Ci = (1 / S) \cdot (J_C / J_O) \cdot O]$ (cf Eqn 4), and found that thus obtained value was half the value of *Ci* conventionally calculated based on the rate of transpiration at *Ca* above 200 μmol mol^{-1}. Hereafter I will refer to the intercellular CO_2 concentration calculated by the conventional method as *Ci*, and that obtained by my method as *Ci** to avoid confusion. Homobaric leaves of *A. heterophyllum* would not cause patchy stomatal closure. My further detailed analysis on the possible processes included in this large difference between *Ci* and *Ci**, i.e., large overestimation of *Ci*, suggested that cuticular transpiration is largely responsible even in early morning with mild transpirational demand, and its effect enhances with increasing transpirational demand and/or *Ca*, which induce stomatal closure (see "Appendix"). Thus data analysis using *Ci* calculated in the conventional methods often leads to a misunderstanding of the limiting processes on CO_2 uptake, especially under field conditions and/or water deficit. On the other hand, my method to evaluate Ci^* based on a leaf temperature dependency of S calculated from Γ^* and on the combination of electron flow theory as $[J_T = J_C + J_O]$ with gas exchange measurements would be useful in further data analysis.

Although my method estimates diffusional limitation rather than stomatal limitation itself, this method could be used widely in the evaluation of the relative limitation of assimilation rate throughout the day. The present study demonstrated that stomatal closure was an important factor responsible for the midday depression of photosynthesis. The stomatal closure caused 50 % decrease in assimilation rate in sun leaves of *A. heterophyllum*.

What is the major factor determining the stomatal limitation? Cowan (1982) indicated that rising transpirational demand results in deeper midday depression of stomatal conductance and hence, CO_2 uptake. Stomatal aperture has been demonstrated to respond to the transpirational demand that varies with leaf temperature and VPD (Maier-Maercker 1983; Hirasawa, Iida and Ishihara 1988; Mott and Parkhurst 1991). Since the potted plants in the present study were well watered before the measurements, the midday stomatal closure might be primarily the result of that in response to transpiration rate *per se* (Maier-Maercker 1983; Mott and Parkhurst 1991), but the stomatal closure induced by decreased leaf water potential (Hirasawa *et al.* 1988) due to rising transpirational demand would not be ruled out. In fact, the depression of A tended to begin at an air temperature of about 25 °C, when leaf temperature was about 25 - 27 °C (Figs. 3-1 and 3-2) with VPD of about 1.0 - 1.5 kPa. In the present study, the strength of midday depression was correlated with daily air temperature that is strongly related to transpirational demand (Fig. 3-1). Similar results were reported for some desert species (Schulze *et al.* 1980) and *Quercus suber* (Lange 1988). They additionally demonstrated the effects of soil water availability on the strength of midday depression of photosynthesis. But Raschke and Resemann (1986) could not find a clear relationship between the amount of transpirational water loss and reduced rates of net CO_2 assimilation during the midday depression.

In addition, drought highly limits leaf conductance and photosynthesis of *A. heterophyllum* leaves even under the constant leaf temperature of 25 °C and relative humidity of 70 % (Chapter 1). Low soil water availability associated with high light conditions in natural habitats should, therefore, also reduce stomatal aperture and then limit photosynthesis in the leaves of *A. heterophyllum* plants in those habitats like the deforested site.

Photoinhibitory effects on CO_2 assimilation rate

Continuous exposure of leaves to high light causing high leaf temperature of up to 34 °C decreased the photosynthetic rate at a given *Ci*, i.e., carboxylation efficiency (*CE*), by approximately 20 % in sun leaves and 50 % in shade leaves (Figs. 3-6 and 3-8), when the data were analyzed using *Ci* calculated in the conventional manner (when *Ci** was used, the decreases in *CE* were reduced about half in both sun and shade leaves). Two possible mechanisms should be responsible for the reduction of *CE* under the conditions of excess light and high leaf temperature (Tenhunen *et al.* 1984; Raschke and Resemann 1986; Chaves *et al.* 1987; Correira *et al.* 1990); reduced efficiency of PS II photochemistry and suppression of carboxylation activity of Rubisco (Powles 1984). A low *Fv/Fm* measured in the dark-adapted leaves in the late afternoon (the measurements conducted only on 7 June 1997) and reduced *ΔF/Fm'* in midday suggested the occurrence of photoinhibition in both sun and shade leaves, possibly due to high light incidence and rising leaf temperature. Simultaneous depression of photosynthetic CO_2 uptake and PS II photochemical efficiency under natural high light regimes have been also reported for several species (Demmig-Adams *et al.* 1989b; Epron *et al.* 1992; Epron and Dreyer 1993; Valladares and Pearcy 1997). However, in sun leaves of *A. heterophyllum*, *ΔF/Fm'* was relatively constant during the midday depression of *CE* (Fig. 3-3c, 3-8c) and *A* depended less on the variation of J_T (Fig. 3-4) after the assimilation 'peak'. On the other hand, in shade leaves, *ΔF/Fm'* decreased significantly after the 'peak' and *A* depended positively on J_T. These results indicate that the midday depression of *ΔF/Fm'* may not be responsible for the reduced carboxylation efficiency in sun leaves, and other changes in metabolism, i.e., the low activation state of Rubisco, is more likely to be the reason (Weis 1981; Sharkey 1985; Cheeseman and Lexa 1996).

Another factor responsible for the low *ΔF/Fm'* in sun leaves may be the high efficiency of non-photochemical quenching of excitation light energy (Fig. 3-3b). Non-photochemical quenching results in a decrease of PS II efficiency as a down-regulation to maintain a balance between electron flow and requirements of reducing power for both carboxylation and oxygenation (Demmig-Adams *et al.* 1989a; Krause and Weis 1991; Demmig-Adams and Adams 1992). However, it might be possible that the reduced PS II photochemical efficiency further increases excess energy in leaf and hence raises leaf temperature, which further decreases in Rubisco activity. This consequence might be more relevant in shade leaves that exhibited higher leaf temperature and severe photoinhibition.

Ecological importance of photorespiration and other processes

In sun leaves of *A. heterophyllum*, *A* after the diurnal 'peak' decreased and exhibited little variation with J_T. This suggests that the excitation energy is dissipated by some means other than CO_2 assimilation, and contributes to avoiding chronic photoinhibition. My analysis showed that photorespiration was significantly higher during the midday depression with high leaf temperatures, especially in sun leaves. A larger amount of excitation light energy was dissipated through the process of photorespiration during the midday depression (also see Valentini *et al.* 1995). Thus photorepiration possibly contributes to protecting the photosynthetic system against the deleterious effects of high light under low *Ci* condition (Osmond and Björkman 1972; Osmond 1981).

In addition, the Mehler-ascorbate peroxidase reaction (Asada pathway) has been recently recognized as the photon protective reaction (Osmond and Grace 1995; Asada 1996; Lovelock and Winter 1996). Although the calculated electron flow in the present study did not include the contribution of this reaction, recent reports are suggesting the possible quantitative importance in dissipating excess energy (Biehler and Fock 1996; Fryer *et al.* 1998). Thus the calculated ratio of J_O/J_T in the present study might overestimate the electron flow to photorespiration, and J_O should include 2/3 of the amount of electron flow to the Mehler-ascorbate peroxidase reaction (J_W) if J_T was expressed as $[J_C + J_O + J_W]$. Further detailed studies on the quantitative contributions of photorespiration, the Mehler-ascorbate peroxidase reaction and xanthophyll-cycle to dissipating excess light energy are needed (Wu *et al.* 1991; Osmond *et al.* 1997).

In contrast to the above characteristics of sun leaves, higher *ΔF/Fm'* at optimal leaf temperature in shade leaves (Fig. 3-3a) suggests their higher susceptibility to photoinhibition (cf Anderson and Osmond 1987). Larger chlorophyll content and lower chlorophyll *a/b* ratio of shade leaves than those of sun leaves (3.1 in shade leaves and 3.5 in sun leaves; H. Muraoka unpublished data) would be responsible for this consequence. Other factors related to the avoidance of severe photoinhibition in sun leaves are their higher stomatal conductance (Björkman 1981; Pearcy 1987) which contributes to efficient regulation of leaf temperatures (Schulze and Hall 1982), higher efficiency of recovery from photoinactivation (Mulkey and Pearcy 1992; Ögren and Rosenqvist 1992; Öquist *et al.* 1992), tolerance of photochemistry to heat (Valladares and Pearcy 1997) and increasing leaf thickness (Nishio, Sun and Vogelmann 1994). Physiological traits of the sun leaf as well as its morphology indicate that adaptive significance of acclimation to natural high light environments largely exists in their contributions to avoiding severe photoinhibitory damages and maintaining photosynthetic carbon gain.

Appendix

Estimation of the possible processes causing overestimation of Ci

Conventionally, *Ci* has been calculated from measured rate of transpiration using the equation as,

$$Ci = \{(g_{tc} - E / 2)\, \underline{C} - A\} \,/\, (g_{tc} + E / 2) \qquad (15)$$

in which g_{tc} is conductance for CO_2 diffusion from boundary layer through stomata to the stomatal cavity, *E* is transpiration rate and $\underline{C}$ is the mole fraction of CO_2 in the leaf chamber (I used this method in the data represented in "Results") or,

$$Ci = Ca - \quad A \,/\, (0.63\, g_{tw}) \qquad (16)$$

On the other hand, I attempted to estimate the intercellular CO_2 concentration from the specificity factor of Rubisco (*S*), which was calculated from Γ^* and $A + Rd$ (Eqn 1 - 5), linear electron transport (Eqn 9, 11 - 14) and Eqn (4) as

$$Ci = (1 / S)\, (J_C / J_O)\, O \qquad (17)$$

The value thus obtained was about half the value of *Ci* conventionally calculated as in Eqn (15) or (16) under a condition of high *Ca* over 200 μmol mol^{-1}. Hereafter I will refer to the intercellular CO_2 concentration calculated by the conventional methods as *Ci*, and that obtained by Eqn (17) as *Ci** to avoid confusion. Because of small CO_2 flux around Γ*, I could estimate the intercellular CO_2 concentration similar to that at carboxylation site, even if the conductance of CO_2 from intercellular airspace to carboxylation site is low, and hence estimate the *S* correctly. Thus I can approximate *Ci** to *Cc*.

Since in the homobaric leaves of *A. heterophyllum*, patchy stomatal closure might not occur, the large difference between *Ci* and *Ci** may be ascribed to an overestimation of *Ci* induced by cuticular transpiration and/or the possibility that conductance of CO_2 from intercellular airspace to carboxylation site (i.e., leaf internal conductance) is not infinitely high. It has been recently reported that the leaf internal conductance is not extraordinarily high (≈ 0.4 mol CO_2 m^{-2} s^{-1}: Epron *et al.* 1995), therefore, the latter could be responsible. I attempted to discriminate the contributions of these two possible effects using data of *A*-*Ci* relation of sun leaves of *A. heterophyllum* measured in early morning (Fig. 3-8) as follows.

Leaf internal conductance to CO_2 (g_{ic}) works as

$$A + Rd = g_{ic} (Ci - Cc) \qquad (18)$$

If *Ci* is calculated correctly or there is no cuticular transpiration, I can estimate g_{ic} from the measured values of *A*, *Rd*, and conventionally estimated *Ci*, and *Ci**. Here, I assume *Ci** = *Cc*. I obtained a value of 0.165 mol CO_2 m^{-2} s^{-1} for g_{ic} in a range of *Ca* from 0 to 100 μmol mol^{-1}. However, this value tended to decrease with increasing *Ca*.

The 'true' Ci (define as $Ci^{\#}$ hereafter) should be calculated as,

$$Ci^{\#} = Ca - A / g_{sc} \qquad (19)$$

where g_{sc} is stomatal conductance to CO_2. When cuticular conductance for water vapor (g_{cw}) is not negligible,

$$g_{sc} = 0.63 (g_{lw} - g_{cw}) \qquad (20)$$

Thus, if g_{cw} contributes largely to g_{lw}, which might occur under large transpirational demand and restricted stomatal aperture (Kirschbaum and Pearcy 1988; Boyer *et al.*

1997), Ci tends to be overestimated and g_{ic}, to be underestimated.

From Eqns (16), (19) and (20), an equation

$$Ci^{\#} = Ci - 1.6A\ \{1 / (g_{lw} - g_{cw}) - (1 / g_{lw})\} \qquad (21)$$

is obtained. The true g_{ic} is expressed as

$$g_{ic} = (A + Rd) / (Ci^{\#} - Cc) \qquad (22)$$

Substituting the Eqn (21) into Eqn (22) appears as

$$A + Rd = g_{ic}\ \{Ci - Cc - 1.6A\ g_{cw} / (g_{lw}^2 - g_{lw} g_{cw})\} \qquad (23)$$

Assuming both g_{ic} and g_{cw} are constant, as in the most previous studies, I tried to obtain these values from A-Ci relationships, but unsuccessfully. For instance, when I assume constant g_{ic} of 0.20 mol CO_2 m^{-2} s^{-1} in Eqn (23), g_{cw} increased from 0.01 to 0.14 mol H_2O m^{-2} s^{-1} with increasing Ca from 0 to 800 μmol mol^{-1}, which means the increase in g_{cw}/g_{lw} from 7 to 67 %. $Ci^{\#}$ thus calculated by Eqn (21) was similar to Ci at low Ca of 0 - 100 μmol mol^{-1}, but was lower than Ci by 25 - 60 % at high Ca of 200 - 800 μmol mol^{-1}. This should be due to that as g_{lw} tends to decrease or to be constant with increasing Ca, and since stomata would close with increasing Ca (Wong *et al.* 1978; Mansfield, Hetherington and Atkinon 1990), hence the contribution of g_{cw} to g_{lw} would increase with the increase in Ca, and thus overestimation of Ci increases.

Inversely, when I assume a constant g_{cw} of 0.02 mol H_2O m^{-2} s^{-1}, g_{ic} decreased from 0.23 to 0.04 mol CO_2 m^{-2} s^{-1} with increasing Ca from 0 to 800 μmol mol^{-1}. $Ci^{\#}$ appeared to be lower by 5 % than Ci. However, g_{ic} is not likely to change by such a large magnitude.

One possibility causing this discrepancy is that underestimation of Ci^* used as Cc. The Ci^* was estimated using J_C/J_O, which was obtained based on the assumption that total electron flow is only devoted to RuBP carboxylation and oxygenation. However, if the Mehler-ascorbate peroxidase reaction consumes a significant fraction of electron flow (J_W) and could be expressed as [$J_T = J_C + J_O + J_W$], J_C and J_O obtained in the present study would be overestimated (see "Discussion") especially under low Ca where photosynthetic energy consumption is restricted. This overestimation of J_C would lead to an underestimation of Ci^*, and hence to an overestimation of g_{ic} or an underestimation of g_{cw} at low Ca. However, even if 10% of J_T is assumed for J_W, Cc at low Ca did not change much, indicating that my methods to estimate S and Cc (i.e., Ci^*) and the relationship between g_{ic} and g_{cw} that I expected are robust. These are also suggesting that g_{cw} would change in response to Ca via the changes of stomatal aperture, and that g_{ic} would change in response to Ci.

Although both g_{ic} and g_{cw} have been considered to be almost constant for a given leaf, some changes in g_{ic} in response to Ci have been reported recently (Harley *et al.* 1992). Considering the above estimations and facts, the large difference between Ci and Ci^* would result from both small change of g_{ic} and large increase in g_{cw}/g_{lw} in response to increasing Ca. Moreover, these consequences are also responsible in the overestimation of Ci in the data on 7 June 1997, in which g_{cw}/g_{lw} would reach to as high as 50 % in midday, which led to a large misleading of S (Fig. 3-9). For further understanding of these consequences, responses of g_{ic} and g_{cw} to different environmental conditions are needed to be investigated.

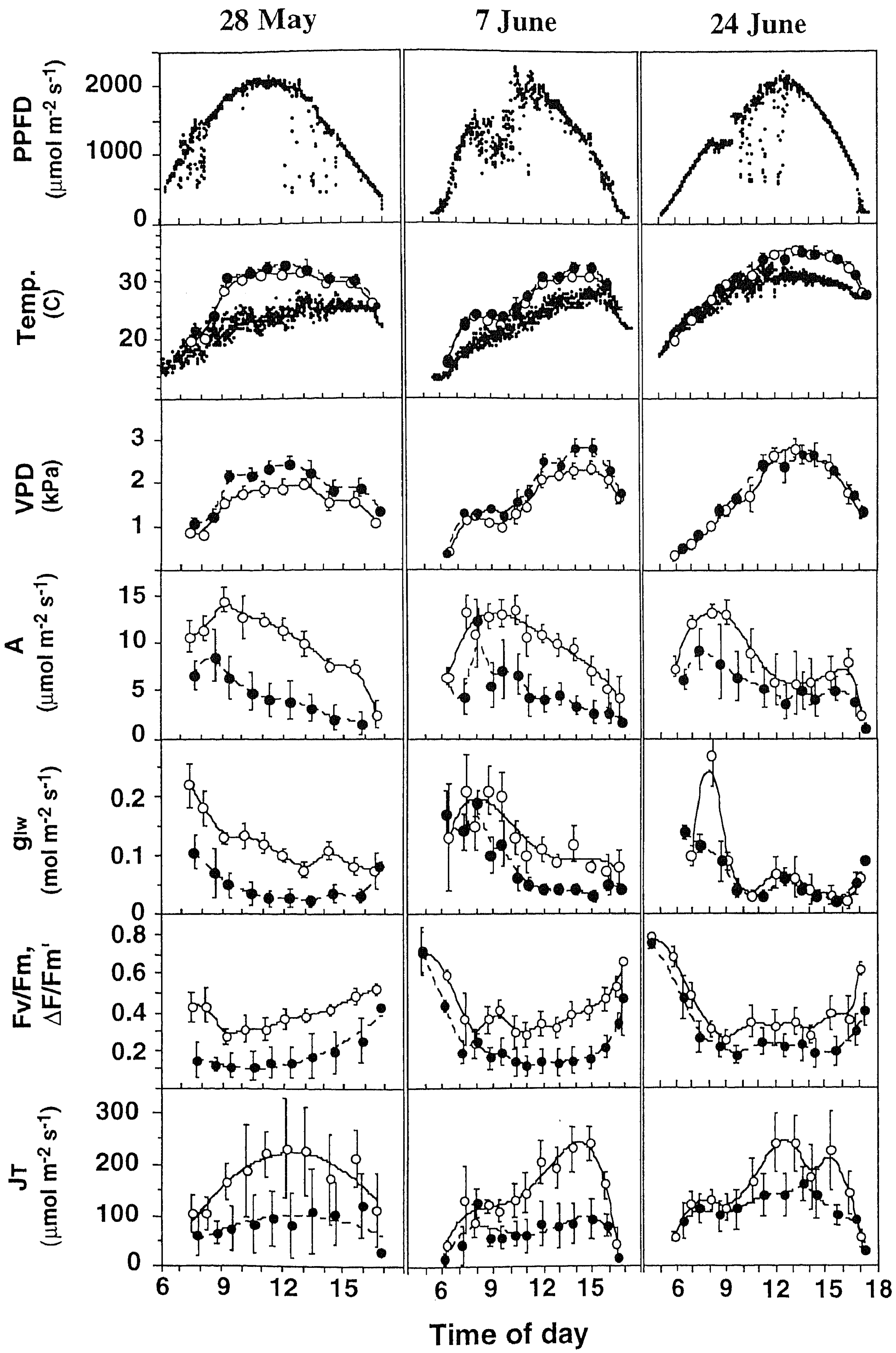

Figure 3-1 See next page for the legend.

Figure 3-1. Diurnal changes of gas exchange rates and photochemical efficiencies for the leaves of *Arisaema heterophyllum* grown at the deforested (○) and forest (●) sites. Measurements were carried out under sunny conditions at the open field on 28 May, 7 and 24 June 1997 (from left to right). PPFD at the horizontal plane, air and leaf temperature, leaf to air vapor pressure deficit, net CO_2 assimilation rate (A), leaf conductance to water vapor (g_{lw}), PS II photochemical efficiency (Fv/Fm, $\Delta F/Fm'$) and total electron transport rate (J_T) are plotted (from top to bottom). The first plots of PS II photochemical efficiency on 28 May, 7 and 24 June, and the last plot on 7 June indicate *Fv/Fm*. . Gas exchange and chlorophyll fluorescence measurements were carried out for two to four plants from the two sites and three to four attached leaflets were selected for each sample plant (data are represented as mean ± SD).

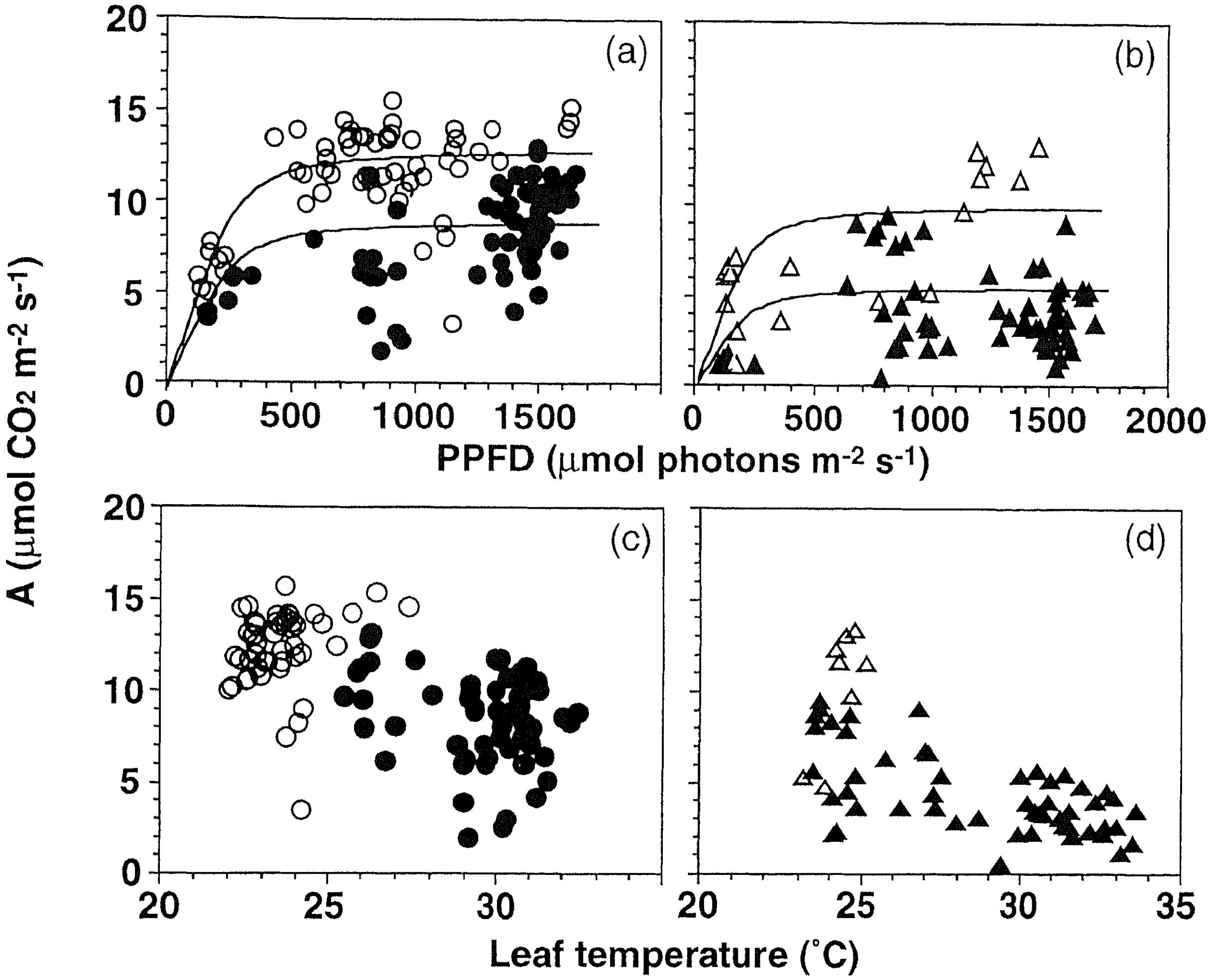

Figure 3-2. Dependences of net CO_2 assimilation rates (*A*) on incident PPFD (a, b) and leaf temperature (b, c) for the leaves of *A. heterophyllum* grown at the deforested (left column) and forest (right column) sites. Measurements were carried out under sunny conditions on 7 June 1997 (redrawn from Fig. 1). Open and solid symbols represent data obtained before and after the 'peak' of diurnal assimilation rates, respectively. In the dependence of *A* on leaf temperature, data above PPFD of 500 μmol photons m^{-2} s^{-1} are shown. Lines in (a) and (b) are fitted by rectangular hyperbora.

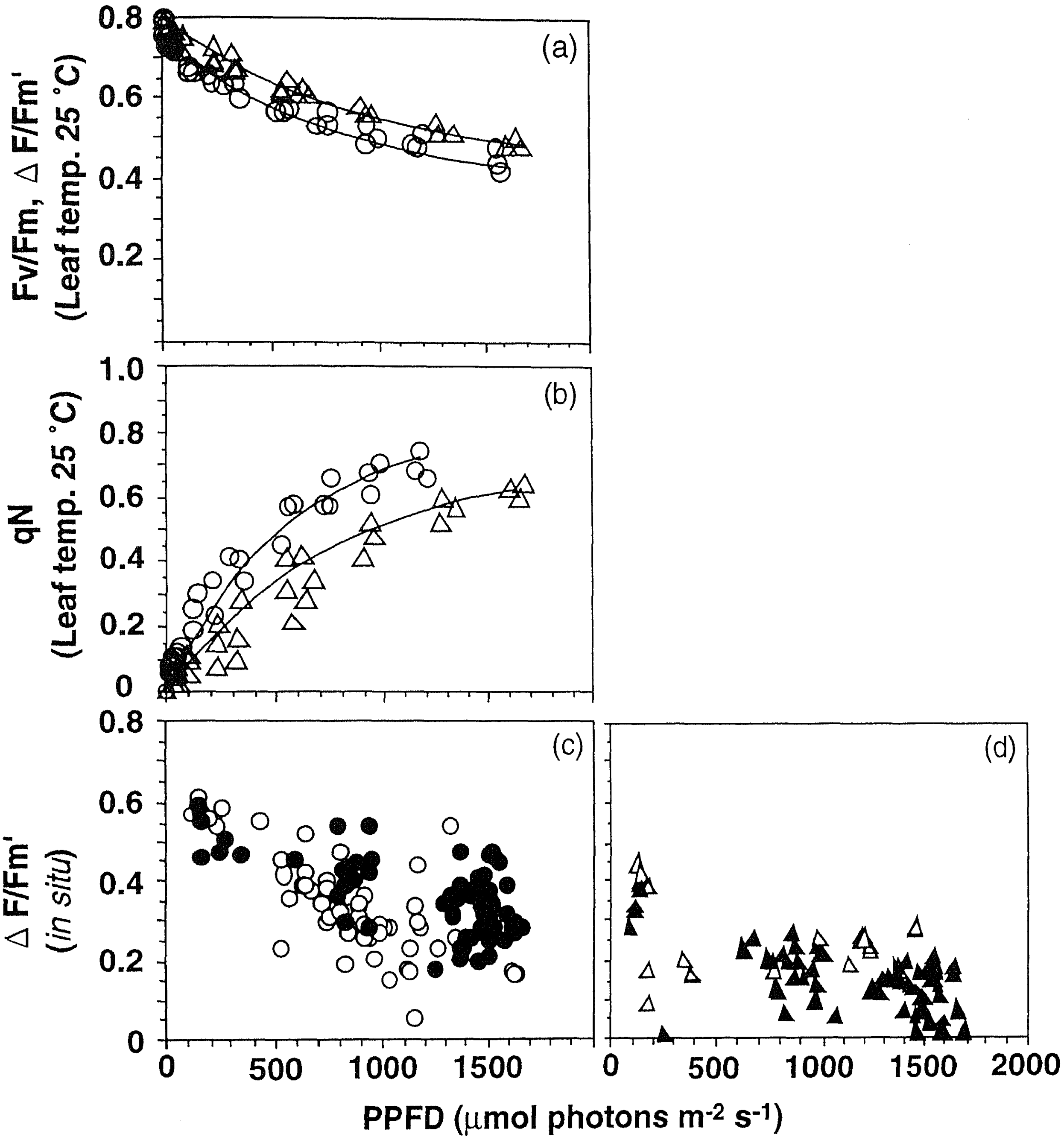

Figure 3-3. Light responses of effective quantum yields of PS II (*ΔF/Fm'*) under constant leaf temperature of 25 °C (a) and *in situ* (c, d), and q_N under leaf temperature of 25 °C (b) for the leaves of *A. heterophyllum* grown at the deforested (circles) and forest (triangles) sites. *In situ* measurements were carried out under sunny conditions on 7 June 1997 (redrawn from Fig.1). Open and solid symbols in plate c and d represent the data obtained before and after the 'peak' of diurnal photosynthetic rates, respectively.

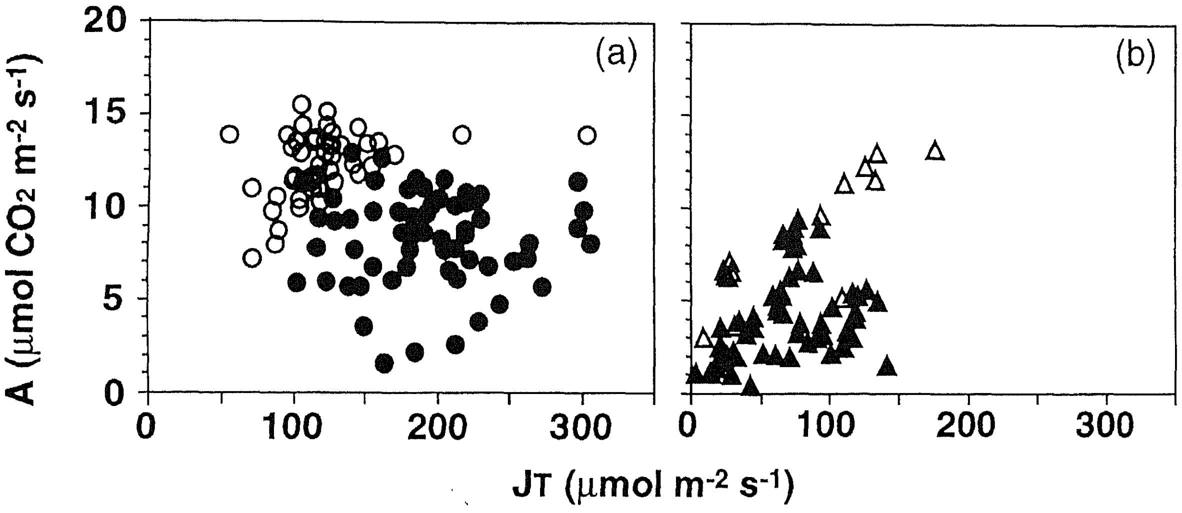

Figure 3-4. Relationships between net CO_2 assimilation rate (*A*) and total electron flow (J_T) calculated from *ΔF/Fm'* for the leaves of *A. heterophyllum* grown at the deforested (a) and forest (b) sites with incident PPFD above 500 μmol m^{-2} s^{-1}. Measurements were carried out at open field on 7 June 1997. Open and solid symbols represent data obtained before and after the 'peak' of diurnal photosynthetic rates, respectively.

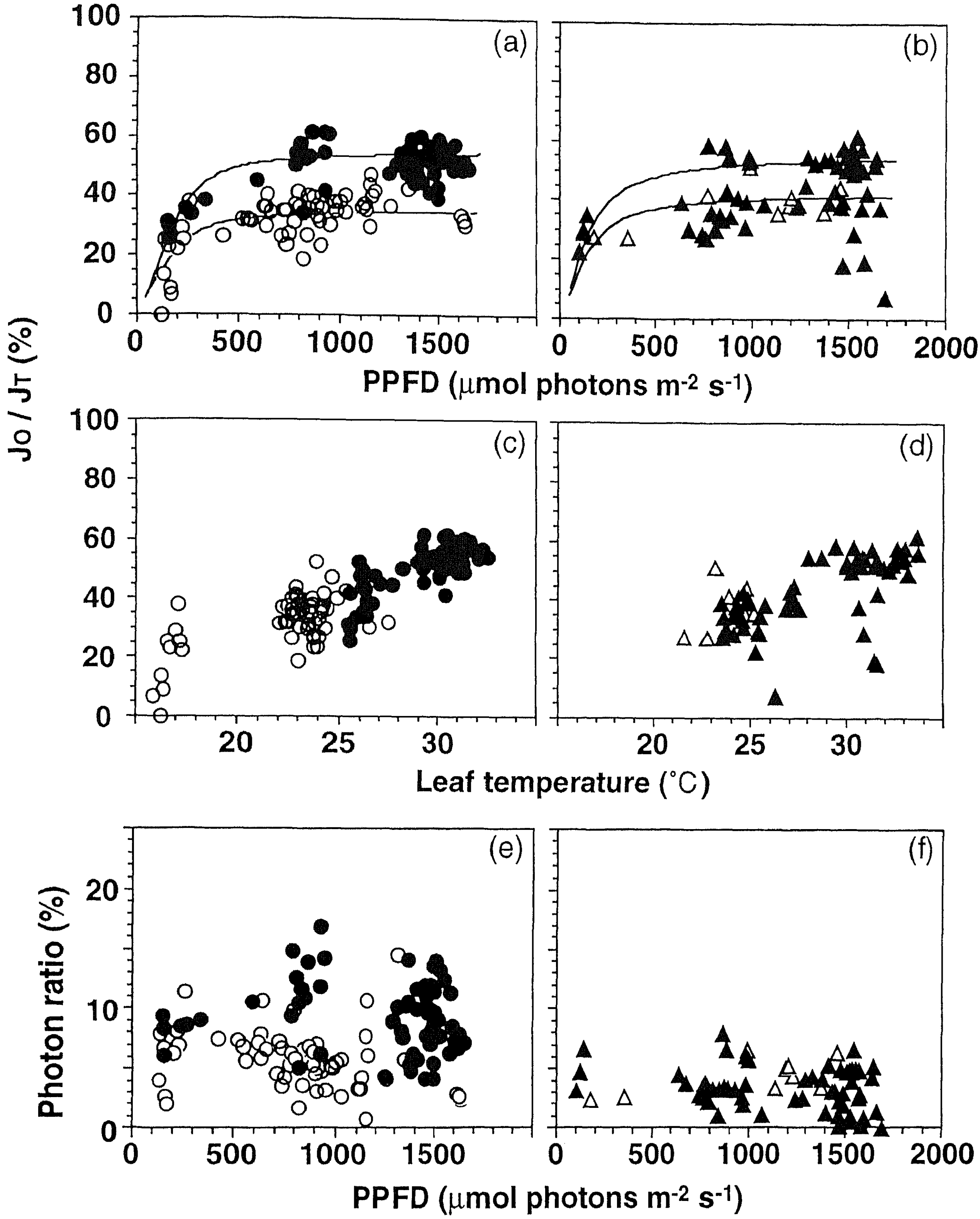

Figure 3-5. Dependences of relative light-driven electron flow to oxygenation (J_O) to total electron flow (J_T) on light (a, b) and leaf temperature (c, d) for the leaves of *A. heterophyllum* grown at the deforested (left column) and forest (right column) sites. Ratio of photons dissipated in oxygenation to total photons absorbed by the leaves was also represented (e, f). Measurements were carried out under sunny conditions on 7 June 1997. Open and solid symbols represent data obtained before and after the 'peak' of diurnal photosynthesis, respectively.

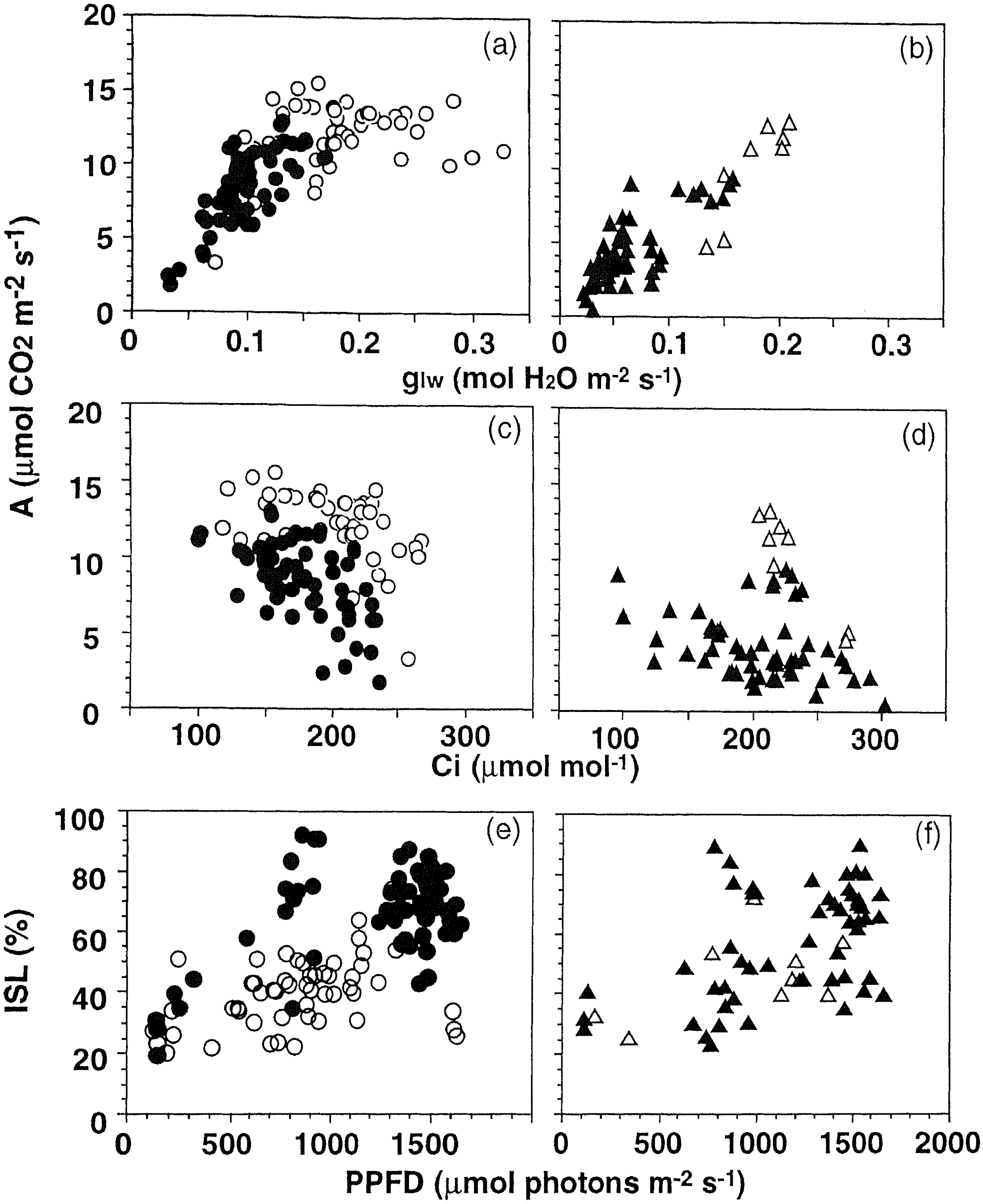

Figure 3-6. Dependences of net CO_2 assimilation rates (*A*) on leaf conductance to water vapor pressure (g_{lw}; a, b), intercellular CO_2 concentration (*Ci*) under the constant ambient CO_2 concentration of 350 μmol mol^{-1} (c, d) and light responses of stomatal limitation on *A* (ISL; e, f) for the leaves of *A. heterophyllum* grown at the deforested (left column) and forest (right column) sites. Measurements were carried out under sunny conditions on 7 June 1997 (redrawn from Fig. 1). Open and solid symbols represent data obtained before and after the 'peak' of diurnal assimilation rates, respectively. In the dependence of *A* on g_{lw} and *Ci*, data above saturated PPFD of 500 μmol photons m^{-2} s^{-1} are shown. A value of zero for ISL means that there is no stomatal limitation on assimilation rate.

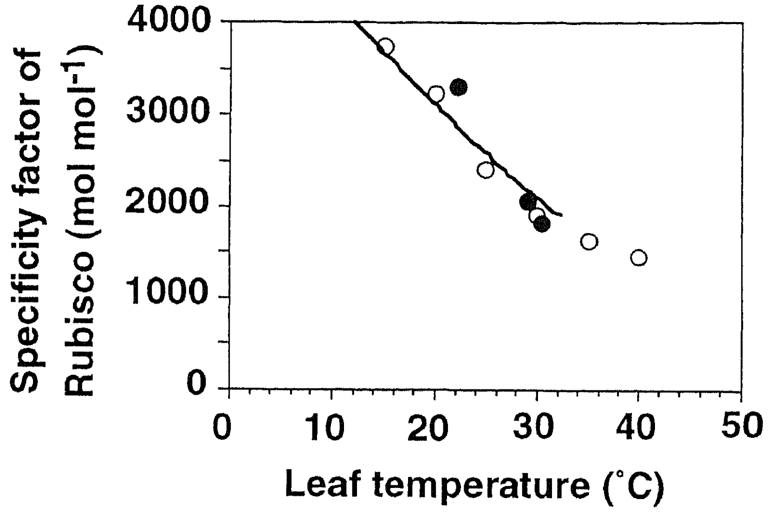

Figure 3-7. Dependence of the specificity factor of Rubisco (*S*) on leaf temperature (T_L). Closed circles represent *S* calculated from CO_2 compensation point in the absence of non-photorespiratory CO_2 evolution (Γ*) from the data of *A-Ci* relation measured at different leaf temperatures in sun leaves of *A. heterophyllum* (Fig. 8). Regression was supplied for these data and leaf temperature dependency of *S* was obtained as [$S = 5570.2 - 139.8\ T_L + 0.8\ T_L^2$]. Open circles represent the *S* of purified spinach Rubisco measured *in vitro* by Jordan and Ogren (1984).

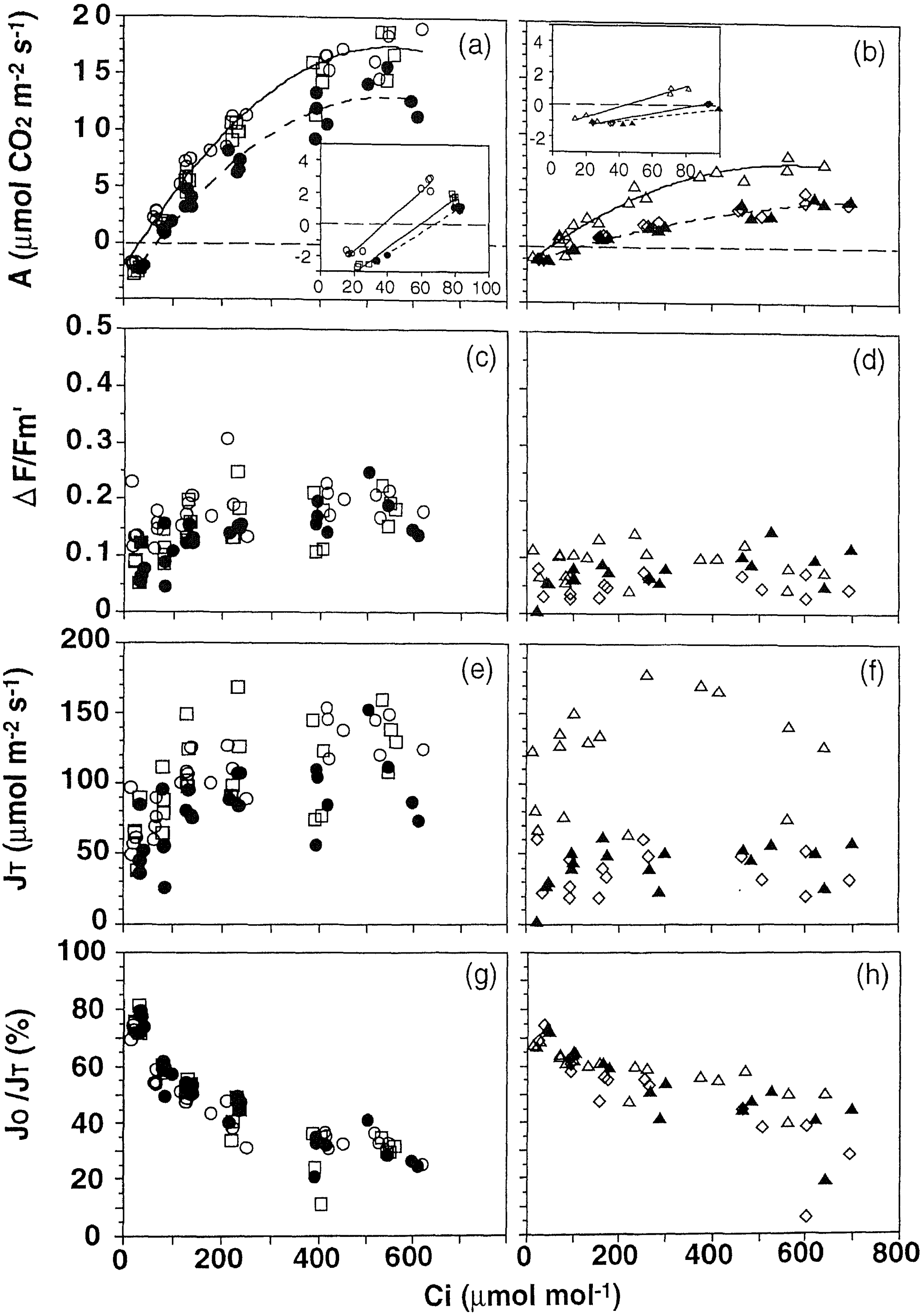

Figure 3-8 See next page for the legend.

Figure 3-8. Diurnal variations of relationships between *Ci* and *A* (a, b), *ΔF/Fm'* (c, d), J_T (e, f) and J_O/J_T (g, h) for the leaves of *A. heterophyllum* grown at the deforested site (left column) and forest site (right column). The insets in (a) and (b) represent *A* at low *Ci*. Measurements were carried out under sunny conditions (PPFD of 1300 - 1700 μmol photons m^{-2} s^{-1}) at the open field on 28 May 1998. Measurement periods are represented by different symbols as; 0700 - 0900 h (○, △), 1000 - 1200 h (□, ◇) and 1300 - 1500 h (●, ▲), respectively.

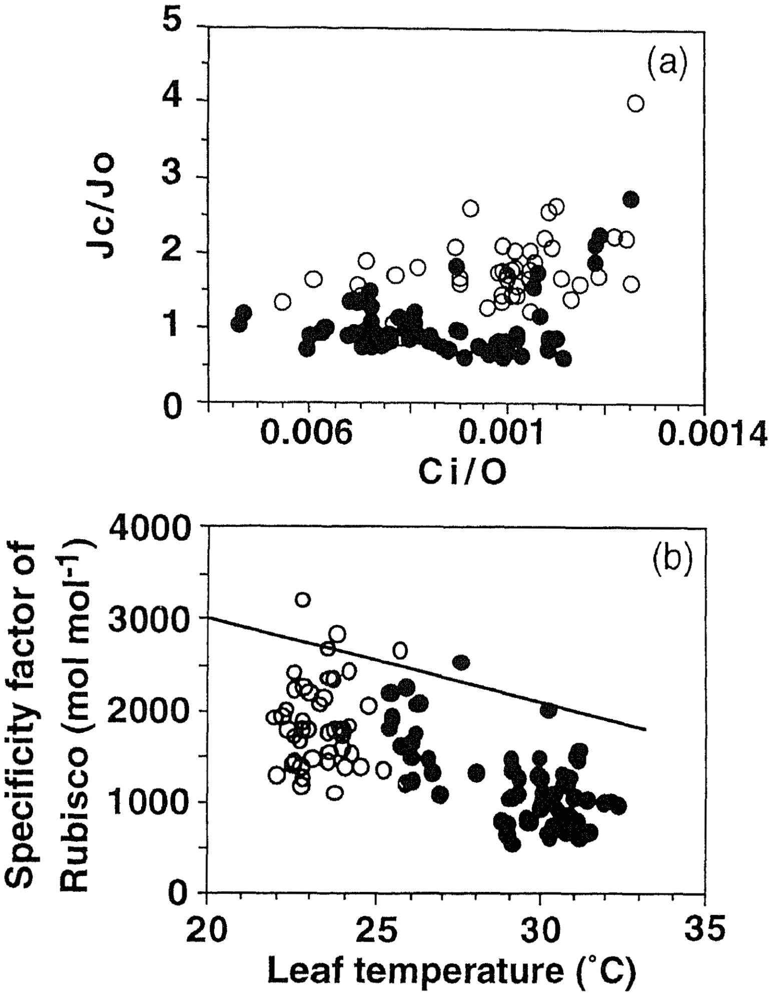

Figure 3-9. Relationships between the ratio J_C and J_O (electron transport rates devoted to the carboxylation and oxygenation of RuBP, respectively) and the ratio of intercellular CO_2 to O_2 mole fractions (*Ci/O*) (a), and between (J_C/J_O) / (*Ci/O*) and leaf temperature (b) for sun leaves. These data are obtained on 7 June 1997. Data above saturated PPFD of 500 μmol m^{-2} s^{-1} are plotted. Open and closed circles represent data for before and after 'peak', respectively. Epron *et al.* (1995) estimated *S* as the slope between J_C/J_O and *Ci/O*. The slope in (b) represents the leaf temperature dependency of *S* as in Fig. 7; $S = 5570.2 - 139.8\ T_L + 0.8\ T_L^2$.

General discussion

Light acclimation in leaf characteristics has long been one of the important topics in plant ecophysiology. However, it is not clear enough 'how' the light acclimation from biochemistry to geometry enables plants to maximize their photosynthetic productions in a given natural habitat where light, temperature and soil water conditions are always complicatedly interacted. Investigation on the light acclimation in natural environments in which adaptive evolution of light acclimation has occurred would provide us with further understanding on the ecological significance of leaf acclimation to various light regimes.

In Chapter 1, I demonstrated that individual biomass accumulation of *A. heterophyllum* depends on the microsite light availability in the forest understory where light limits photosynthetic rate, while at the deforested open site it may be highly limited by leaf temperature and soil water availability accompanying over-irradiance. In addition, morphological and physiological developments of leaves under a high light regime were shown to be significantly influenced by soil water availability.

In Chapter 2, I experimentally confirmed that the flexible leaf orientation can maximize light capture in the forest understory while it serves to avoid excess irradiance at the deforested site. The resulting leaf orientation at both sites contributes to minimizing the habitat-specific stresses for photosynthetic productivity.

In addition to the morphological avoidance of high light stresses, capacities of the thermal dissipation and photorespiratory consumption of excess excitation energy have been suggested to be important for the leaf acclimation to natural high light environment in which photoinhibitory damages reduce photosynthetic productivity (Chapter 3).

The most important data obtained in the present study are those suggesting that the various aspects of acclimation to natural high light environment was significant to avoid photoinhibitory damages and severe water deficit as to maintain a high leaf photosynthetic activity.

Interaction between water and light managements in leaf in high light environment

Acclimation to high light was largely influenced by soil water availability (Chapter 1). Water is also an important resource for plants and plays a critical role in photosynthesis, structure supporting and transpirational leaf cooling (Chaves 1991). Since plants under sunny condition as those of *A. heterophyllum* at the deforested site frequently suffer from water deficit due to large evapotranspirational water loss and largely fluctuated soil water availability, their water economies have considerable effects on photosynthetic production and growth in the natural high light environment. There are three ways for plants to regulate the water status under high transpirational demands due to high light incidence, rising leaf temperature and drought: 1) closing stomata to decrease transpirational water loss (Cowan 1982; Schulze and Hall 1982), 2) pulling water from the soil via rapid transpiration (Kramer and Boyer 1995) and 3) keeping water absorption by reducing the water potential via osmoregulation (Morgan 1984).

However, the most appropriate way to regulate water status may largely depend on the water relation traits of the species. In *A. heterophyllum*, reducing water potential due to high transpirational water loss and/or low soil water availability would result in severe water deficit because the species has a low capacity of osmoregulation (H. Muraoka, unpublished observation). Therefore, reducing water loss as efficient as possible through regulation of stomatal aperture would be one of the most effective strategies. In fact, stomata of *A. heterophyllum* leaves tend to be closed under low soil water availability (Chapter 1) and under a high transpirational demand due to rising leaf temperature (Chapter 3). But rehydration (Chapter 1) and the low transpirational demand in early morning and late afternoon (Chapter 3) could increase stomatal aperture and thus CO_2 uptake. The rapid recovery of stomatal aperture and photosynthetic CO_2 uptake should be brought about by the efficient avoidance of severe water deficit since over-hydration would cause large photoinhibitory damage and/or over-wilting of plant body. Thus the response of stomatal aperture in the sun leaves of *A. heterophyllum* to fluctuating water availability plays important roles in avoiding water deficit and maximizing daily photosynthetic carbon gain per transpirational water loss (cf Cowan 1982).

On the other hand, although stomatal closure prevents severe water loss from the plants, concomitant decrease of photosynthetic energy consumption would increase the amount of excess excitation energy, which might cause photoinhibitory damages (Powles 1984; Foyer *et al.* 1990; Cornic and Massacci 1996). In the sun leaves of *A. heterophyllum*, thermal dissipation and photorespiratory consumption of excitation light energy were significantly higher and photoinhibitory damage was suppressed to a much smaller extent as compared to the shade leaves (Chapter 3). Recent studies have clarified that sun leaves have a larger xanthophyll cycle pool and hence a larger capacity of thermal dissipation than shade leaves (e.g., Demmig-Adams *et al.* 1995; Demmig-Adams 1998). In addition, the present study demonstrated a higher photorespiratory capacity in sun leaves than that in shade leaves. Capacity of the Mehler-ascorbate peroxidase reaction in the consumption of excess excitation energy and promoting electron flow might be also enhanced in sun leaves (cf Logan *et al.* 1996). The present study thus emphasized the importance of biochemical and physiological aspects in the acclimation to natural high light environment which may cause compounded stresses comprising by excess irradiance, high temperature and water deficit. Further detailed studies on the quantitative contribution of photosynthesis and the above reactions to dissipating energy and avoiding photoinhibition are needed.

Plant geometry and stress avoidance

Acclimation to contrasting light environments was also remarkable in the leaf orientation and holding of *A. heterophyllum* (Chapter 2). In the forest understory, individual leaflets oriented in a way to maximize light capture. This would serve to maximize net carbon gain at their microsites. Recent studies have shown that tree species also arrange their leaves and branches in a way to maximize whole plant light capture and carbon gain (Takenaka 1994; Ackerly and Bazzaz 1995; Inui 1996; Pearcy and Yang 1996).

At the deforested site, inclination and folding of the leaflets of *A. heterophyllum* were shown to avoid photoinhibition due to over-irradiance successfully. The decrease in light absorption also contributes to decreasing transpirational water loss (Stevenson and Shaw 1971; Begg 1980; Forseth and Teramura 1986).

Although there are several studies on the function of leaf orientation in light capture and photosynthetic activity (for reviews see Ehleringer and Forseth 1980; Nilsen 1992), there have been few studies in which both low and high light natural environments were investigated for the same species. The present study would provide useful information required for clarifying the ecological significance of the plasticity of whole plant geometry under a wide range of light availability.

Adaptive significance of leaf morphological and physiological acclimation in understory plants to high light condition induced by canopy gap formation

Leaf morphological and/or physiological acclimation to a given light condition has been mainly studied with the plants growing in rainforest understories. Leaves in those plants experience a wide range of light environments from those of deeply shaded sites to those of partly exposed sites with small-sized canopy gaps (for a review see Chazdon *et al.* 1996). These studies showed that leaves are able to acclimate to rapid change in photon flux density, which is considered to be important to make efficient utilization of light energy and would thus increase photosynthetic production (Pearcy 1987; Popma and Bongers 1988, 1991; Chazdon 1992; Ellsworth and Reich 1992; Pfitsch and Pearcy 1992; Newell *et al.* 1993; Kuppers *et al.* 1996). However, few studies have addressed the combined effects of rising leaf temperature (Mulkey and Pearcy 1992) and/or drought (Osmond 1983; Mulkey, Wright and Smith 1993) with increasing light incidence. In the understory plants of tropical forests, an increase in incident light would often result in an increase in photosynthetic carbon gain. This is partly due to the efficient utilization of sunflecks, the direct light incidence from the canopy gaps (Chazdon 1988; Pearcy 1988), and partly due to the fact that other stresses, i.e., rising leaf temperature and/or water deficit associated to high light regimes, are negligible or not accompanied. Moreover, since the light availability for the understory plants is often very limited (sometimes less than 1 % of light in the canopy top), even a slight increase in light incidence would result in a considerable improvement of photosynthetic carbon gain (e.g., Pearcy 1988; Chazdon *et al.* 1996). In addition, because of a great height in canopy layer (30 - 60 m) of tropical forest, few sunflecks last continuously longer than 1 - 2 hours, even around noon (Chazdon 1988). This fact means that photoinhibition could be effectively avoided by thermal energy dissipation (Demmig-Adams *et al.* 1998) or repaired in the other periods with low diffuse light incidence (Krause and Winter 1996; Thiele, Krause and Winter 1996). Leaves in understories would thus not receive a severe reduction of photosynthesis during the period of high light or sunflecks. Limited duration of direct sunlight incidence is not likely to cause severe drought. In tropical rainforest understories, therefore, acclimation to high light may be largely confined to the increase in photosynthetic capacity and production so far.

In contrast, at the deforested site used in the present study, plants are exposed to direct sunlight for longer hours and the effects of high temperature and drought tend to be prolonged. In such a stressful situation, as I have shown with *A. heterophyllum*, both morphological and physiological mechanisms to avoid environmental stresses are quite important for photosynthetic matter production through the leaf life-span, and individual biomass accumulation. Biomass accumulation of the deforested site plants might be not only the result of reduced photosynthesis by high light stresses, but also the consequences of the morphological and physiological mechanisms to minimize the influences of multiple high light stresses.

Safe site for the growth of A. heterophyllum

In a year with high precipitation, most individuals of *A. heterophyllum* with relatively high microsite light availability above R-PPFD of about 10 % showed positive RGR (Chapter 1). Although plants at the deforested site with much higher light availability tended to show positive RGR, variation of yearly precipitation would significantly affect plant growth as shown in the difference between years 1995 and 1996. At the deforested site, shortage of light and water (including nutrient) resources due to the coverage by the dominance of high competitive herbaceous species such as *P. chino* and *S. altissima* would be the reason for low or negative RGR of *A. heterophyllum* plants in 1994. Considering the above results, a suitable habitat for *A. heterophyllum* is thought to be a forest edge or relatively well illuminated forest understory, where there is an adequate light incidence but water stress does not matter.

Future prospects

1) Partitioning of biomass between leaf and stem with respect to microsite light availability

In order to increase light capture under low light availability, it is also effective to hold the leaves at higher position than adjacent individuals by increasing the amount of biomass to construct stem. However, since there should be a trade-off in biomass investment between the stem elongation and leaf area expansion in *A. heterophyllum*, the balance of biomass investment may largely affect the whole plant light capture and net carbon gain. To answer the question "what is the signal of the determination of the balance between stem and leaf area in *A. heterophyllum* ?", may help us to further clarify the range of natural light environment in which this species can grow successfully.

2) Dynamics of the leaf ecophysiological responses under a wide range of light environment, and their implication in plant growth and population dynamics

Range of successful light acclimation in leaves should be an important factor that determines the dynamics and distribution of populations of a given species. Investigations of the plant responses from leaf biochemistry to whole plant geometry and growth under a wide range of natural light environment would provide us with fundamental informations to understand the range of light environment the species can utilize or withstand. Moreover, investigation of their responses to sudden change of light regimes would deepen our understanding of the dynamics of light acclimation. Incorporating these leaf and/or whole-plant level responses to heterogeneous light environment into the plant growth models would contribute to constructing more mechanistic models of plant population dynamics.

3) Ecological significance of biochemical/physiological light energy regulation in high light environment with other environmental stresses

Efficient regulation of absorbed light energy, which has a great importance in the life of a plant, is largely influenced by temperature and water conditions. These effects are remarkable in open habitats with extremely hot or cold climate. As I did in Chapter 3, concurrent measurements of gas exchange and chlorophyll fluorescence and an application of photosynthetic models such as Rubisco kinetics and electron transport enable us to evaluate the interaction of stomatal aperture, photochemistry, carbon fixation and photon protective mechanisms such as thermal dissipation and photorespiration. Further *in situ* studies with several species including sun plants such as inhabiting natural exposed habitats like gravelly floodplain and shade plants associated with various natural shaded conditions would help to deepen our understanding of the ecological significance of biochemical and physiological responses of plants to natural fluctuation and heterogeneity of light availability.

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