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Water Relations and Carbon Economy of Hemiepiphytic and Non-hemiepiphytic Ficus Tree Species in Southwest China

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UNIVERSITY OF MIAMI

WATER RELATIONS AND CARBON ECONOMY OF HEMIEPIPHYTIC AND NON-HEMIEPIPHYTIC *FICUS* TREE SPECIES IN SOUTHWEST CHINA

By

Guangyou Hao

A DISSERTATION

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of the University of Miami
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WATER RELATIONS AND CARBON ECONOMY OF HEMIEPIPHYTIC AND NON-HEMIEPIPHYTIC *FICUS* TREE SPECIES IN SOUTHWEST CHINA

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Water Relations and Carbon Economy of Hemiepiphytic and Non-hemiepiphytic *Ficus* Tree Species in Southwest China (May 2010)

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Hemiepiphytes are important components of tropical forests and are attractive to scientists due to their unique epiphytic growth habit during some period of their life cycle. Unique characteristics in plant water relations and carbon economy have been found in hemiepiphytic plants; however, to further understand this group of species on an evolutionary basis it is necessary to carry out comparative studies between hemiepiphytes and their close relatives. In this dissertation I conduced a comparative study in a suite of functional traits related to plant water relations and photosynthesis between hemiepiphytic and non-hemiepiphytic tree species from a single genus—*Ficus*. Great differentiation in functional traits has been found between species of the two growth forms both during juvenile and adult stages. Seedlings of hemiepiphytic *Ficus* species (H) had significantly lower xylem hydraulic conductivity, stomatal conductance, net light saturated CO_2 assimilation, and higher water use efficiency than congeneric non-hemiepiphytic species (NH), which are adaptive to a drought-prone epiphytic growth conditions under natural conditions. The conservative water use adaptation in H species is likely crucial to the drought tolerance and survival in the forest canopy but is related to much lower growth rates than NH species. Species of the two growth forms both showed relatively large plasticity in responding to variation in light level as in typical light-

demanding species. Surprisingly, the NH species showed characteristics related to higher light demand than H species, which is opposite from the prediction that H species are more light-demanding than NH species. Thus, although commonly accepted, it is likely that light was not the selective pressure for the evolution of hemiepiphytism in *Ficus*. Using adult trees grown in a common garden, I found that H species showed characteristics of more conservative water use even after they established connections to the soil. Moreover, H species showed significantly different traits in photochemistry compared to NH species due to hydraulic-photosynthetic coordination. The evolution of an epiphytic growth habit during the juvenile stage of a life cycle in the hemiepiphytic *Ficus* species thus involved changes in a suite of functional traits that persist during their terrestrial growth stages.

TO THE MEMORY OF MY GRANDPARENTS

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Finally, I would like to acknowledge my family for their invaluable support during this whole process.

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CHAPTER 1

INTRODUCTION

Background knowledge of hemiepiphytes

Hemiepiphytic plants are a unique group of species that spend one part of their life cycle as epiphytes on their hosts and the other part of life cycle as plants with connections to the ground (Williams-Linera and Lawton 1995). Some species begin their life as epiphytes and eventually establish connection with the ground by sending down aerial roots (primary hemiepiphytes), while others start as terrestrial plants and then become epiphytic by severing the connections with the ground (secondary hemiepiphytes) (Kress 1986). There are 25 families and approximately 59 genera containing hemiepiphytes, with at least 823 species of primary hemiepiphytes and 649 species of secondary hemiepiphytes (Williams-Linera and Lawton 1995). *Ficus* (Moraceae) is the largest group of woody primary hemiepiphytes and within this genus the hemiepiphytic habit has evolved independently a number of times (Putz and Holbrook 1986). There are about 800 *Ficus* species, of which about 500 species are hemiepiphytic (H) and 300 species are non-hemiepiphytic species (NH), including shrubs, small to large trees, and climbers (Berg and Corner 2005). All NHs start their life-cycles as terrestrially rooted seedlings. *Ficus* are one of the most important components of tropical lowland rainforests throughout the world (Harrison 2005) and are ecologically important due to their interactions with many frugivorous animals and other plant species in ecosystems (Shanahan et al. 2001).

It is commonly considered that there are several potential advantages to start the life cycle as an epiphyte. The most important advantage is that the forest canopy is better lit than the forest understory (Williams-Linera and Lawton 1995). It has been suggested that hemiepiphytes evolved from plants that colonized rocky areas as an adaptation to access high light environments in the canopy (Dobzhansky and Murea-Pires 1954; Ramirez 1977; Putz and Holbrook 1986; Todzia 1986; Laman 1995; Williams-Linera and Lawton 1995). Also they may benefit from minimizing the risk of fire, flooding, damage by terrestrial herbivores (Holbrook and Putz 1996b) and coverage by falling debris. The advantages of spending the initial part of the life cycle as an epiphyte can be offset by the potential limitation of water and nutrient availability (Benzing 1990; Coxson and Nadkarni 1995; Holbrook and Putz 1996a,b; Swagel et al. 1997). From a physiological point of view hemiepiphyties are a fascinating group of plants (Putz and Holbrook 1986) and a topic ripe for further investigation (Harrison et al. 2003).

Because of the radical changes in rooting environment between the two growing phases, developmental and physiological plasticity is important for these species. The change from epiphyte to tree is accompanied by a dramatic shift in rooting volume and characteristics of the rooting zone (Holbrook and Putz 1996b). Epiphytes are likely to be under severe water deficit even in areas with very humid climate (Benzing 1990). In seasonally dry climate, hemiepiphytes at the epiphytic stage experience even more severe drought during the dry season, when frequent and severe periods of low water availability can occur (Benzing 1984; Sinclair 1984). Besides drought, epiphytic phase plants are also likely exposed to other environmental stresses such as over-excitation to PSII by strong irradiance, overheating of leaves by sunlight and shortage of nutrients. How can these plants cope with the multiple environmental stresses related to the specialized habitat is an important and interesting issue in plant eco-physiology. In hemiepiphytic species, the naturally existing two different growth phases with substantially different environmental conditions make it interesting and convenient for comparative study in plant ecophysiology, particularly in plant water relations.

***Ficus* as the model plants for studying plant water relations**

Within the genus *Ficus*, the hemi-epiphytic habit has most likely evolved four times in sections Urostigma, Sycidium and Pharmacosycea and in a closely related group comprised of sections Conosycea, Galoglychia, Americana and Malvanthera (Harrison 2005). From the point of view of evolutionary biology it is important to know the main environmental factors that resulted in this specialized growing habit. Comparative study in ecophysiology between hemi-epiphytic and non-hemiepiphytic *Ficus* species will provide valuable information about the main differences between these two groups in seedling/sapling and adult trees in adaptation to the environments, which will allow us to infer the major selective forces for the evolution of the hemiepiphytic habit.

Previous studies have found that nutrient availability does not exert a major limitation to the epiphytic phase of hemiepiphytic *Ficus* (Putz and Holbrook 1989). Measurement of stomatal conductance and leaf phenological studies on the other hand indicated that water availability is frequently a major constraint as compared to terrestrially rooted trees of the same species (Holbrook and Putz 1996a,b). Some hemiepiphytes rely on CAM metabolism (such as species in the genus *Clusia*). But all the species of *Ficus* studied so far showed only C_3 photosynthesis (Ting et al. 1987). Epiphytic strangler *Ficus* species were found to avoid water deficits by a combination of strong stomatal control of transpirational water losses and maintenance of relatively high leaf water potentials (Holbrook and Putz 1996a). During both the rainy season and the dry season, stomatal conductance of epiphytic strangler *Ficus* species are lower than conspecifics, and the stomata of epiphytic *Ficus* species are only open during the early morning throughout the dry season (Holbrook and Putz 1996a). Furthermore, epiphytic phase *Ficus* plants can better control water loss from leaf surfaces than tree-phase figs after stomata are closed (Holbrook and Putz 1996b). It is considered that the water loss after the stomatal closure occurs mainly from surfaces of guard cells around the stomata that are more conductive to water (Muchow and Sinclair1989). The smaller guard cell surface area due to lower stomata density enables the epiphytic *Ficus* to lose water relatively slowly compared with tree phase plants of the same species. Due to these water conservative traits, leaf water potentials of epiphytic stage *Ficus* plants were found to be similar or even less negative than conspecific tree-phase individuals (Holbrook and Putz 1996a).

In *Ficus*, intrinsic developmental changes are important for the shift from epiphytic to tree phase (Holbrook and Putz 1996b). When both epiphytic phase and tree phase *Ficus* were well supplied with water, the epiphytic leaves exhibited significantly lower stomatal conductance and much lower water loss rate from leaf surfaces (Holbrook and Putz 1996 a,b). Hemiepiphytic *Ficus* have several-fold lower leaf mass per area (LMA) and 2-to 4-fold lower stomatal densities than conspecific trees.

Ficus species have evolved to encompass a variety of species with different life histories including hemiepiphytes and non-hemiepiphytic tree species, but *Ficus* species, as a whole, have many traits that are typical of pioneer species such as small seeds, high net CO_2 assimilation and growth rates (Harrison 2005). Neotropical *Ficus* (including species of both functional groups) exhibit relatively high capacity to conduct water per unit cross section of wood compared to other tropical trees (Patiño et al. 1995) and the photosynthetic rates of the leaves of a freestanding species *Ficus insipida* was found to be among the highest of any C_3 tree measured under natural conditions (Zotz et al. 1995). Generally speaking, *Ficus* species as a group appear to be light demanding species and have traits characteristic of pioneer species (Harrison 2005). The hemiepiphytic *Ficus* species germinate on microhabitats on host trees that are better lit than the forest understory and thus can establish even in a climax forest community with very shaded understory. But the intrinsic differences between hemiepiphytic and non-hemiepiphytic *Ficus* growth forms in terms of regenerating light requirements are still not clear and are awaiting for further investigation.

Many of the studies on the ecophysiology of hemiepiphytic *Ficus* species have focused on the comparison in water relations between plants of different growth phases (i.e. epiphytic and terrestrially established tree stage) within the same species, but very few studies have been conducted with the objective to compare hemiepiphytic and non-hemiepiphytic *Ficus* species. One single study carried out in Neotropical forests on established adult individuals showed that freestanding *Ficus* species have more cross-sectional wood per unit leaf area, slightly more conductive wood and consequently greater long distance water transport capacity per leaf area than hemiepiphytic stranglers (Patiño et al. 1995). Also, no study has been done on the photosynthetic characteristics of *Ficus* species of these two growth forms. To better understand their intrinsic differences, it is necessary to do more detailed studies in plant water relations and photosynthesis.

Also the investigation should include not only adult plants but also individuals at the early growing stages when the differences are probably most distinct between the two functional groups.

The coordination between plant water relations and photosynthesis

Results from the literature showed that across a variety of plant species xylem hydraulic conductivity is positively coordinated with plant photosynthetic capacity represented either by maximum apparent electron transport rates of photosystem II or maximum net CO_2 assimilation rates (Brodribb and Field 2000; Brodribb et al. 2002; Campanello et al. 2008; Santiago et al. 2004a,b; Zhang and Cao 2009). For a given soil water potential, higher xylem hydraulic conductivity allows plants to transport water more efficiently during transpiration and thus maintain higher stomatal conductance (Meinzer et al. 1995; Sperry 2000; Meinzer 2003; Ackerly 2004; Santiago et al. 2004a; Zhang et al. 2008). If resistance to water transport in the xylem is high plants need to have lower stomatal conductance and transpiration rates to prevent leaf water potential dropping to critical values, which may cause cavitation in vascular conduits (Brodribb and Holbrook 2004). However, lower stomatal conductance increases the resistance of atmospheric CO_2 diffusion into leaves through stomata and causes lower intercellular CO_2 concentration, which can limit photosynthetic CO_2 assimilation (Katul et al. 2003).

The coordination between plant hydraulics and photosynthesis can also be studied by the comparison in hydraulic conductivity between species with different photosynthetic pathways. In plants with C_4 photosynthetic pathway, water use efficiency

(WUE) is generally two to three times higher than that of C_3 plants (Larcher 2003). Consistent with their differences in WUE, significant differences in stem xylem structure and stem hydraulic conductivity were found between C_3 and C_4 species. In woody plants, C_4 species on average have higher wood density, shorter and narrower vessels, lower sapwood specific hydraulic conductivity (K_s) and leaf area specific hydraulic conductivity (K_l) (Kocacinar and Sage 2004; Kocacinar et al. 2008). The C_4 species were also found to have greater leaf area per unit xylem area and thus even in few cases K_s of some C_4 species was similar to that of C_3 species, their K_l was still significantly lower than C_3 species (Kocacinar and Sage 2004). In herbaceous plants, Kocacinar and Sage (2003) found similar patterns in stem xylem structure and hydraulic conductivity when comparing C_3 and C_4 species in general. In addition, hydraulic traits of C_4 species that grow in different environments can be greatly different in hydraulic traits. The C_4 species from arid environments have narrower and shorter vessels, lower K_s, and higher resistance to drought induced cavitation in the xylem tissue; while C_4 species from resource-rich regions have xylem hydraulic characteristics similar to that of C_3 species but showed significantly higher leaf area per xylem area (Kocacinar and Sage 2003; Sage 2004).

In plants with CAM metabolism, stomata are only open during nighttime to uptake CO_2 and can thus avoid higher potential transpiration rate during the daytime when VPD, temperature and irradiance are high (Larcher 2003). Some hemiepiphytic species, such as species in the genus *Clusia* (Clusiaceae), can switch their photosynthetic pathways between CAM and C_3 with changes in environmental condtions (Ting et al. 1987; Winter et al. 1992; Zotz et al. 1994). During the epiphytic growth stage this species can have CAM metabolism, but at the terrestrial growth stage with reliable water sources it can switch to C_3 metabolism (Ting et al. 1987). Having different photosynthetic pathways at different growth phases helps this hemiepiphytic plant to maintain water balance at different phases with contrasting water availabilities. However, *Ficus* species have C_3 photosynthetic pathway during both epiphytic and terrestrial growth stages although the epiphytic growth phase has much less water availability (Ting et al. 1987). To maintain an adequate water balance, epiphytic phase *Ficus* have to restrict transpiration by having much lower stomatal conductance than conspecific terrestrial individuals (Holbrook and Putz 1996a). Also, some studies showed that hemiepiphytic species tend to have lower stem hydraulic conductivity even after they are terrestrially rooted (Patiño et al. 1995; Zotz et al. 1997). If this is true, tree phase hemiepiphytic *Ficus* species, may have significant different characteristics in photosynthetic traits compared to their congeneric non-hemiepiphytic species.

Introduction to the study site

Xishuangbanna is a Dai Autonomous Prefecture of Yunnan Province, China (21º 09' to 22° 36' N and 99° 58' to 101° 50' E) with a total area of 19,125 km^2 (Fig. 1.1). It is bordered by Myanmar to the southwest and Laos to the south and southeast. It is at the margin of tropics and has slightly lower annual mean temperature and lower precipitation than typical tropical rainforests.

Basically, the area has a mountain-valley topography with altitudes ranging from 550 m at the bottom of Lancang River Valley to 2429.5 m at the top of the highest mountain. The regional climate is controlled by a typical tropical monsoon climate system with a distinct alternation of wet and dry seasons. In those areas of lower hills and valleys (< 900 m) covered by tropical rain forest, the annual mean temperature is 21°C to 22.8 °C. The annual precipitation varies from 1200mm to 1800 mm, of which more than 80% falls during the rainy season from May until the end of October. The tropical rainforest accounts for about 18% of the total area and forms a mosaic with Montane evergreen forests and semi-evergreen forests. This region is well-known for its high biodiversity and is the only place in China that still maintains large tracts of tropical rain forests. However, in the last few decades this area was undergoing severe deforestation and the remnant natural forests are fragmented to a great extent. These changes also caused significant changes in the microclimate of local habitats (Zhu et al. 2004).

Xishuangbanna is one of the regions in the world with high *Ficus* species diversity (Harrison 2005). There are about 67 *Ficus* species and about half of them have hemiepiphytic growth habit. *Ficus* trees are considered to be holy trees by the Dai people living in this region and are well protected. Most of the study will be conducted in and around the Xishuangbanna Tropical Botanical Garden (XTBG; http://english.xtbg.cas.cn/) (21° 56' N, 101° 15' E, 600m elevation; Fig. 1.1) of the Chinese Academy of Sciences (CAS). It has a total area of 900 ha and a large part of it is protected and kept in its natural conditions. The mean annual temperature is 21.7^{o}C, mean temperature of the hottest moth (July) is 25.3 ^{o}C and the mean temperature of the coolest month (January) is 15.6^{o}C (Liu and Li 1996). Mean annual precipitation is 1557 mm with about 83% of the rain falling from May to October (Fig. 1.2).

Ficus species in Xishuangbanna and their importance to the local ecosystem

In Xishuangbanna region, there are 46 *Ficus* species that naturally occur in the forests as well as two subspecies and 19 varieties (Zhu et al. 1996). Within the 67 *Ficus* taxa (including subspecies and varieties), 48 taxa are trees, 15 taxa are shrubs, and 4 taxa are lianas (Table 1.1). Among the 67 *Ficus* taxa, the 48 tree species, including both hemiepiphytic and non-hemiepiphytic tree species (thereafter they are named as H and NH species; 23 and 25 species, respectively), are most important to the local ecosystem in terms of total biomass. The H species are commonly known as stranglers or banyan trees (Fig. 1.3). Both of the H and NH species are very important to the local ecosystem not only in species numbers and biomass but also because of their interactions with many animals (e.g. fig wasps and fig fruit-eating animals) and other plant species. For example, many epiphytic species grow on organic materials trapped by hemiepiphytic *Ficus* trees. Thus ecophysiological study of *Ficus* species is also relevant to conservation biology.

Table 1.1 *Ficus* species that naturally occur in Xishuangbanna and their bio-ecological characteristics. Adapted from Zhu et al. (1996).

Species name	*Epiphytic phase*	*Adult growth form*	*Aerial roots*	*Leaf texture*
Ficus abelii		shrub		papery
F. altissima	+	large tree	+	leathery
F. annulata	+	large tree	+	thinly leathery
F. asperiuscula		shrub		thinly leathery
F. auriculata		small tree	+	thickly papery
F. benjamina	+	tree	+	thinly leathery
F. benjamina var. *nuda*	+	tree	+	thinly leathery
F. callosa	+	large tree		thickly leathery
F. caulocarpa		tree		thinly leathery
F. chapaensis		small tree		papery
F. chrysocarpa		small tree		papery
F. concinna	+	large tree	+	leathery
F. concinna var. *subsessilis*	+	large tree	+	leathery
F. curtipes	+	tree	+	thickly leathery
F. cyrtophylla		small tree		papery
F. drupacea		small tree	+	leathery
F. esquiroliana		tree		papery
F. fistulosa		small tree	+	papery
F. gasparriniana var. *lacerate-folia*		shrub		thinly leathery
F. gasparrinana var. *viridescens*		shrub		thinly leathery
F. glaberrima		tree	+	thinly leathery
F. glaberrima var. *pubescens*		tree	+	thinly leathery
F. hederacea	+	liana	+	thickly leathery
F. heterophylla		shrub		papery
F. hirta	+	shrub		papery
F. hirta var. *brevipila*		shrub		papery
F. hirta var. *imberbis*		shrub		papery
F. hirta var. *roxburghii*		shrub		papery
F. hispida		tree		papery
F. hookeri		large tree		leathery
F. ischnopoda		shrub		papery
F. kurzii		large tree		thinly leathery
F. laevis	+	liana		papery
F. langkokensis	+	small tree		papery
F. maclellandii	+	tree		leathery
F. macleliandi var. *rhododendrifolia*	+	tree		leathery
F. microcarpa	+	tree	+	thinly leathery
F. neriifolia		small tree		papery
F. nervosa		tree		leathery
F. oligodon		tree		papery
F. orthoneura		tree		leathery
F. pisocarpa	+	tree		leathery
F. pubigera		liana		leathery
F. pubigera var. *anserina*		small tree		leathery
F. pubigera var. *maliformis*		small tree		leathery

Table 1.1 Continued

F. pubigera var. reticulata		*small tree*		*leathery*
F. pyrformis		shrub		leathery
F. racemosa		large tree	+	thinly leathery
F. racemosa var. *miquelli*		large tree	+	thinly leathery
F. religiosa	+	large tree	+	leathery
F. sagittata	+	tree	+	leathery
F. sarmentosa var. *lacrymens*		liana	+	leathery
F. semicordata		small tree		papery
F. squamosa		shrub		papery
F. stenophylla		shrub		papery
F. stricta	+	tree	+	thinly leathery
F. subincisa		shrub		papery
F.subinicisa var. *paucidentata*		shrub		papery
F. subulata	+	small tree	+	papery
F. superba var. *japonica*		small tree	+	papery
F. tinctoria ssp. *gibbosa*	+	tree	+	leathery
F. tinctoria ssp. *parasatica*	+	tree	+	thinly leathery
F. variegata var. *chlorocarpa*		tree		papery
F. variolosa		small tree		leathery
F. vasculosa		small tree	+	leathery
F. virens	+	large tree	+	leathery
F. virens var. *sublanceolata*	+	large tree	+	leathery

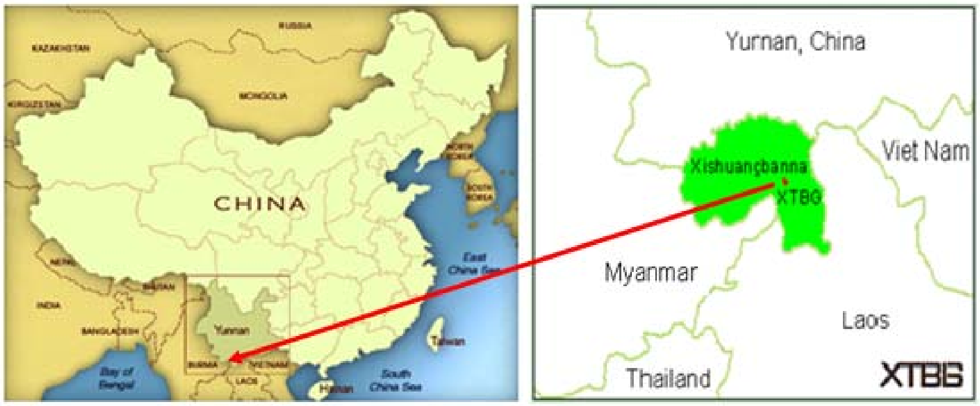

Figure 1.1 The location of Xishuangbanna region, Yunnan Province, China

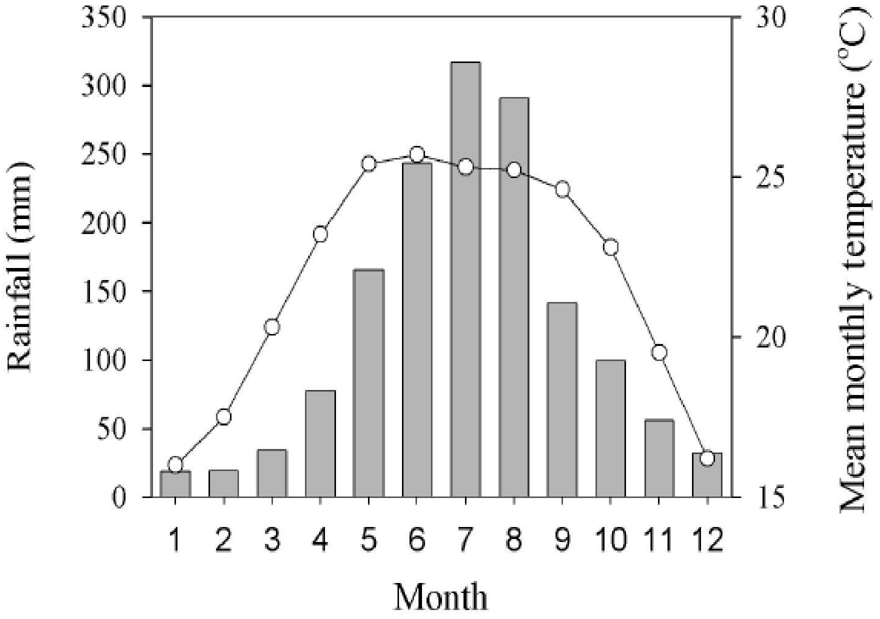

Figure 1.2 Monthly mean rainfall (bars) and temperature (open circles) of Xishuangbanna. Meteorological data are from Xishuangbanna Rainforest Ecological Station located in the XTBG.

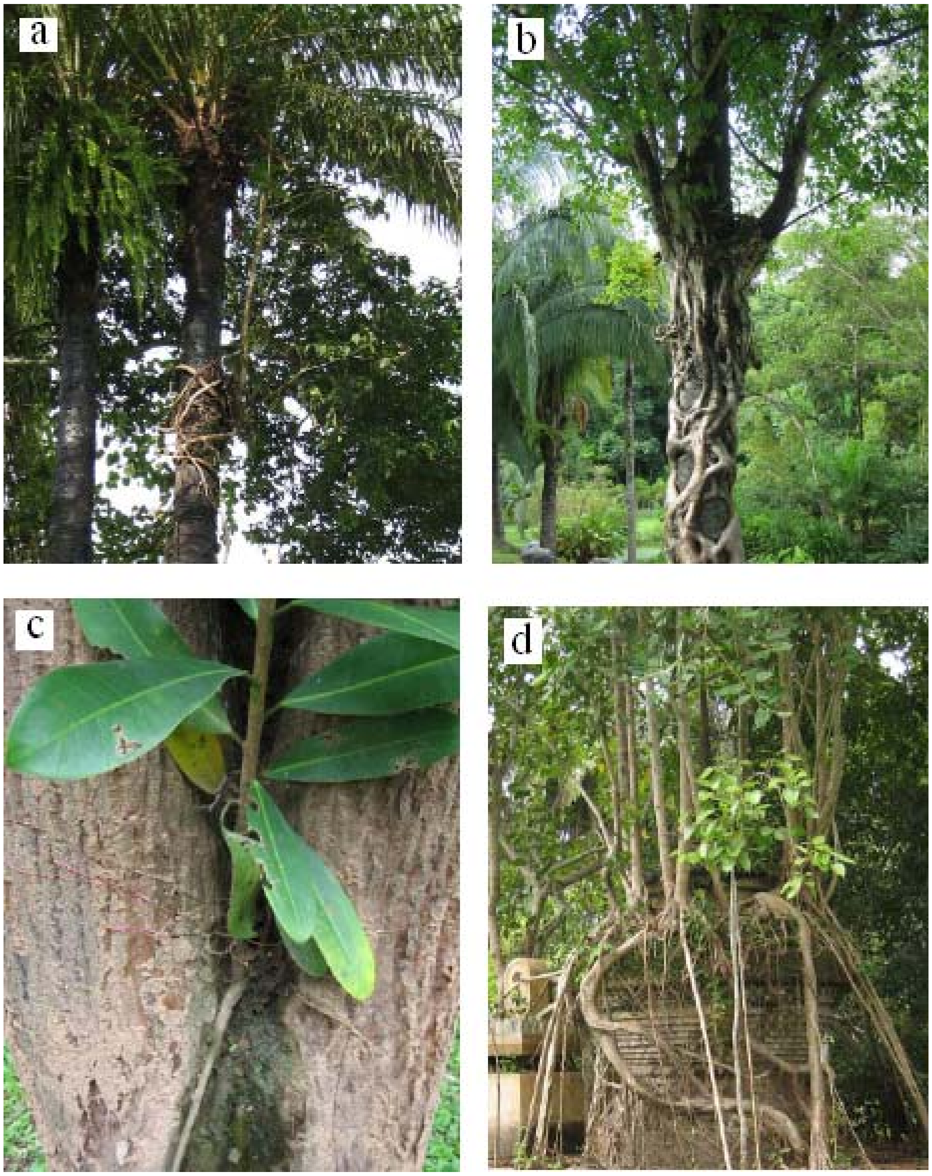

Figure 1.3 Photos of two hemi-epiphytic *Ficus* species grown in XTBG showing different growth stages. (a) epiphytic-phase *Ficus concinna* (Miquel) Miquel growing on a palm tree; (b) terrestrial-phase *F. concinna* strangling a palm tree; (c) epiphytic-phase *F. curtipes* Corner grown on a host tree; (d) terrestrial-phase *F. curtipes* wrapping a tower.

CHAPTER 2

DIFFERENTIATION OF LEAF WATER FLUX AND DROUGHT TOLERANCE TRAITS

Summary

Leaf structural and physiological traits are associated with growth form and habitat, but little is known of the specific traits associated with hemiepiphytes, important components of tropical rainforests. The hemiepiphytic life history includes a drought-prone epiphytic phase and a terrestrial phase that may benefit from different leaf function relative to terrestrial species. Traits related to the flux of water through the leaf and to drought adaptations were studied in five hemiephiphytic (H) and five non-hemiepiphytic (NH) *Ficus* tree species grown in a common garden to determine genetically based differences. Leaves of H and NH species differed substantially in structure and physiology; on average, H species had smaller leaves with higher LMA, thicker epidermis, smaller vessel lumen diameters in petioles, and lower petiole hydraulic conductivity. Leaf traits also indicated stronger drought tolerance in H species, including lower epidermal conductance and turgor loss point, and earlier stomatal closure with desiccation than NH species. Across H and NH species, water flux traits were negatively correlated with traits related to drought tolerance. The divergences in hydraulics and water relations between growth forms for these closely related species reflected specialization according to contrasting habitat and life form. Conservative water use and increased ability of leaves to persist under severe drought would provide an advantage for H species, especially

during the epiphytic phase, while the higher water use of NH species would be associated with higher assimilation rates and potential competitiveness under high water supply.

Introductory remarks

Much classical and recent work has focused on the importance of leaf traits in plant life history, and several studies have shown leaf traits to be linked with plant growth form (e.g., Givnish 1987; Reich et al. 2004; Waite and Sack 2010). However, relatively few such works have been done on hemiepiphytes that have unique life form different from both terrestrial and epiphytic plants. Hemiepiphytes are very abundant in lowland tropical rainforests, and in lower montane and midmontane cloud forests (Putz and Holbrook 1986; Williams-Linera and Lawton 1995). They may play a central role in the development of tropical forest canopy structure both by competing with trees and by stabilizing mats of epiphytic organic soil and thus affecting rainfall interception and ecosystem hydrological properties (Veneklaas et al. 1990; Williams-Linera and Lawton 1995).

This study focuses on the leaf traits linked with hemi-epiphytism in the genus *Ficus* (Moraceae), one of the most conspicuous hemiepiphytic groups in terms of habitat breath, number of species, and biomasss (Dobzhansky and Murca-Pires 1954; Putz and Holbrook 1986; Holbrook and Putz 1996a). *Ficus* consists of about 500 hemiepiphytic and 300 non-hemiepiphytic species and it has been shown that hemiepiphytic growth habit evolved at least four times in this genus (Harrison 2005).

Hemiepiphytic *Ficus* species are adapted to an epiphytic phase during which water availability is very limited. For five New Word *Ficus* species, the epiphytic growth phase exhibited more conservative water use and enhanced drought tolerance than the terrestrial phase, including stronger stomatal control and slower epidermal water loss (Holbrook and Putz 1996a,b). Studies of adult trees of hemiepiphytic (H) and non-hemiepiphytic *Ficus* species (NH) indicated significantly lower stem hydraulic conductivity for the hemiepiphytic species (Patiño, Tyree and Herre 1995). Differences in stem hydraulic conductivity are likely paralleled by differences in leaf hydraulic traits between H and NH species. We aimed to determine the differences in leaf traits associated with water relations for *Ficus* species of the two growth forms.

The leaf is a major bottleneck in the whole-plant water flow pathway, with a hydraulic resistance that accounts on average for 30% (and up to >90%) of the whole-plant resistance (Nardini and Salleo 2000; Brodribb, Holbrook, and Gutierrez 2002; Sack et al. 2003). Recent work has identified the leaf hydraulic system as a key determinant of whole plant hydraulic responses and water relations (Sack and Holbrook 2006; Brodribb and Cochard 2009). The leaf hydraulic conductance varies over an order of magnitude across species and was found to correlate with "flux" traits (Sack et al. 2003), including stomatal pore area, maximum stomatal conductance and photosynthetic rate per leaf area (Aasamaa, Sober and Rahi 2001; Sack et al. 2003; Brodribb and Holbrook 2004; Brodribb et al. 2005). Leaves are also more vulnerable than stems to drought-induced cavitation and thus species growing in environments with distinctly different water availability may differ more strongly in leaf hydraulic traits than in that of stems (Hao et al. 2008). We hypothesized that the differences in life form groups (i.e. H and NH) would be associated with strong differences in leaf water flux traits.

We examined hydraulics and water relations traits in five H and five NH species grown in a common garden to determine genetically based differences between growth forms and whether these were aligned with the substantial differences in stem hydraulic conductivity between the two growth forms (Patiño, Tyree and Herre 1995). Specifically, we hypothesized that compared to NH species (1) H species would have leaves with lower values for flux related traits, such as xylem hydraulic capacity and maximum gas exchange rates; (2) H species would have tighter stomatal control of water loss; (3) leaves of H species would have stronger ability to persist under unfavorable water conditions. Such patterns would affect the physiological and ecological performance of species of the two growth forms.

Materials and methods

Study species

The study was conducted in Xishuangbanna Tropical Botanical Garden (see Chapter 1 for more detailed description). All the measurements were conducted at the beginning of the wet season of 2008 (May and June). Five H and five NH species that naturally occur in the region were used. The five H species are from three sections and the five NH are from four sections (Table 2.1). *Ficus tinctoria* is a hemiepiphyte that usually does not form a self-supporting trunk and falls after its host tree rots, *F. benjamina*, *F. concinna*, and *F. curtipes* are stranglers that can eventually form free-standing trunks, and *F. religosa* can form a massive trunk and canopy up to 30 m. The five NH species usually regenerate in forest margins and gaps and are more commonly found in secondary forests: *F. racemosa* can grow to 30 m, and the other four species typically grow to 10 m.

Samples were taken from adult trees ranging 15-60 cm in diameter at breast height, with hemiepiphytic species in their free-standing terrestrial growth stage. Six trees were sampled for each of 10 species. For the five dioecious species (i.e., *F. auriculata, F. esquiroliana, F. hispida, F. semocordata,* and *F. tinctoria*), samples were taken from three male and three female individuals; no significant differences were found for any trait between individuals of different sexes (data not shown) and data were pooled for further analyses. The use of common garden plants minimized plastic adjustments to local site conditions, allowing the detection of genetically based species differences. All leaves were sampled from sun-exposed branches.

Leaf morphological and anatomical measurements

Leaf area (*A*) was measured with a leaf area meter (LI-3000, LI-COR Inc., Lincoln, Neb., USA) and leaf dry mass (*M*) was determined after oven-drying for 48 hours at 70ºC. Leaf mass per unit area (LMA) was calculated as *M*/*A*. Leaf saturated water content (SWC) and leaf lamina density (ρ_{leaf}) were measured on leaf disks rehydrated overnight as, respectively, (rehydrated mass – dry mass) / dry mass, and dry mass/ rehydrated volume, with volume determined by water-displacement using a balance.

To determine lamina and petiole xylem traits we made microscope observations of cross-sections from the middle of the leaf lamina avoiding major veins and from the petiole immediately below the lamina insertion point, respectively. Measurements were made of the thickness of the lamina, adaxial epidermis, palisade mesophyll, spongy mesophyll, abaxial epidermis (three leaves from each of six sampled trees) and petiole vessel lumen diameters (one leaf per tree) under an optical microscope (YS100, Nikon Corp., Tokyo, Japan).

We determined theoretical petiole xylem hydraulic conductivity as a proxy of leaf hydraulic capacity. Direct measurements of leaf hydraulic conductance (K_{leaf}) using typical techniques (e.g. Sack et al. 2002) were not possible due to the copious latex in these species. Leaf area-normalized petiole hydraulic conductivity was expected to be a reasonable proxy of K_{leaf} across species (Sack et al. 2003; Sack and Frole 2006). For petiole anatomical traits we measured the number of vessels contained in each sectioned petiole, and for 50 randomly chosen vessels, determined theoretical axial hydraulic conductivity treating the vessels as ellipses:

$$K_i = \frac{\pi a^3 b^3}{64\eta(a^2 + b^2)} \qquad (2.1)$$

where K_{i} is the theoretical axial hydraulic conductivity of an individual vessel, a and b are the long and short axes of the vessel lumen, and η the viscosity of water at 25°C (Lewis and Boose 1995). The leaf area normalized theoretical axial hydraulic conductivity of the petiole ($K_{\text{t},}$ mmol m^{-1} s^{-1} MPa^{-1}) was then calculated as (Cochard, Nardini and Coll 2004; Sack and Frole 2006):

$$K_t = \frac{n}{50A}\sum_{1}^{50} K_i \qquad (2.2)$$

where n is the total number of vessels in a petiole, and A is the lamina area.

Leaf water loss after excision

Measurements were made of water loss from detached leaves to determine the minimum rate of water loss through epidermis after stomatal closure (Holbrook and Putz 1996b). Terminal branches were collected in the evening, immediately re-cut under water and rehydrated overnight wrapped in plastic bags to determine leaf saturated mass (SM). For each species, six leaves were excised and placed on the lab bench under dim light (ca. 3 μmol m^{-2} s^{-1} PPFD; LI-1400 data logger and quantum sensor, LI-COR Inc., Lincoln, Neb., USA), with temperature approximately 20ºC and average vapor pressure deficit 2.3 kPa (range 1.9-2.6 kPa), and leaf fresh mass (FM) for each leaf was measured periodically. One H and one NH species were randomly paired and measured simultaneous. At the end of the experiment leaves were oven-dried at 70ºC for 48 hours before determining dry mass (DM). Leaf relative water content (RWC = (FM-DM) / (SM-DM) × 100%) was calculated following the dehydration process and plotted against the time interval (ΔT) from leaf excision to each FM measurement. The water loss rates between RWC of 90% to RWC of 60% for all the studied species were most stable during the dehydration process and were used to calculate mean epidermal transpiration rates (E_{min}; Muchow and Sinclair 1989; Holbrook and Putz 1996b). The time required for a saturated leaf to drop to RWC of 70% (T_{70}), a mean threshold for physiological damage (Lawlor and Cornic 2002) was determined from RWC versus ΔT regressions. Epidermal conductance (g_{min}; mmol m^{-2} s^{-1}) was calculated by dividing E_{min} by the daily average value for mole fraction VPD (VPD / atmospheric pressure).

Pressure-volume relationships

Leaf pressure-volume curve parameters were quantified using the bench drying method (Tyree and Hammel 1972). For each species a branch was collected from each of six individuals at predawn and allowed to rehydrate for 2-3 hours. Leaf mass and water potential (Ψ_l, using a pressure chamber; PMS1000; Corvallis, Oregon, USA) were measured periodically during desiccation. At higher water potentials, latex made precise determination of Ψ_l difficult, and the first measurement (> -0.3 MPa) for each leaf was not used. Leaf absolute capacitance (C_{leaf}) per leaf area (mmol m^{-2} MPa^{-1}) was determined by fitting the linear relations of $1/\Psi_l$ versus RWC before turgor loss and was normalized by leaf area (Koide et al. 1991; Brodribb and Holbrook 2003):

$$C_{leaf} = \Delta RWC/\Delta\Psi_l \times (DM/LA) \times (WM/DM)/M \qquad (2.3)$$

where LA is leaf area (m^2), WM the mass of leaf water at 100% RWC (WM = FM - DM, in g), and M the molar mass of water (g mol^{-1}).

Stomatal response to leaf water potential

A portable LI-6400 photosynthetic system (LI-COR Inc., Lincoln, Neb., USA) was used to measure stomatal conductance (g_s) in response to Ψ_l, *in situ* from 900 to 1600 hours. Measurements were made at ambient temperature, VPD, PPFD and CO_2 concentration (ranging 26-38^oC, 0.6-4.5 kPa, 100-1600 µmol m^{-2} s^{-1} and 370-430 ppm, respectively). After g_s measurement, leaves were removed, numbered and sealed immediately in plastic bags with wet tissue papers, and kept in a cooler before Ψ_l measurement in the laboratory within one hour (previously found not to affect the Ψ_l; data not shown). To determine g_s

under extreme drought, measurements were also conducted on excised branches (Brodribb and Holbrook 2003). The relation between g_s and Ψ_l was fitted using a sigmoid function.

Statistics

One-way ANOVA was used to test differences between N and NH species means (df = 1 and 8 for growth form and error term, respectively). Correlations among species means for all traits were analyzed with Pearson correlation using SPSS version 15.0 (SPSS, Inc., Chicago IL). We tested correlations that were hypothesized *a priori* (see ''Introductory remarks''), and additionally we present a correlation matrix to reveal the intercorrelative structure for all tested variables, but not to reach any conclusions about non-hypothesized relationships (Givnish, Montgomery and Goldstein 2004; Edwards 2006; Dunbar-Co, Sporck and Sack 2009). As a general test of the degree of inter-relationship of the measured traits, and whether this differed from what might occur due to chance, we tested whether correlations were significant in more than 5% of cases (using a proportion test; Minitab Release 15, College Park, Pennsylvania, USA; Waite and Sack 2010). Linear and nonlinear regression analyses between traits were performed using Sigmaplot 10.0 (Systat Software, Inc., San Jose, CA, USA).

Results

Leaf characteristics differed significantly between *Ficus* species of the two growth forms. All the H species had leathery leaves while four out of five of the NH species had papery leaves (Table 2.1). H species had on average 71% thicker leaves, with 120% thicker spongy mesophyll, 85% thicker upper epidermis, 159% thicker lower epidermis, and 53% lower palisade/spongy ratio (Table 2.2). Given their greater thickness, and similar leaf densities on average, H species had 50% higher LMA than NH species (Table 2.3).

The NH species had anatomical traits indicating capacity for higher leaf water flux rates. NH species had 30% larger vessel lumen diameters (D_v) than H species ($P < 0.01$; Table 2.3), while the number of vessels per petiole, normalized by leaf area, did not differ significantly between NH and H species (1.69 and 2.65 cm^{-2}, respectively; $P = 0.12$). NH species had a K_t on average 104% higher than H species ($P < 0.05$, one-way ANOVA; Table 2.3).

The growth forms also differed strongly in maximum stomatal conductance (g_{max}) and in the response of g_s to water potential change. NH species had significantly higher g_{max} and higher turgor loss point (π^0) than H species (Table 2.3). The leaf water potential at which g_s fell to 50% and 20% of maximum values ($\Psi_{gs50\%}$ and $\Psi_{gs20\%}$) did not differ significantly between H and NH species (Table 2.4). However, at turgor loss point, H species had a smaller degree of stomatal openness on average compared to NH species. In H species g_s dropped to values close to their minima at turgor loss point (Fig. 2.1a-e), whereas in NH species g_s remained around 50% of g_{max} even after turgor loss (Fig. 2.1f-j). Thus, in H species $\Psi_{gs50\%}$ were 0.18 to 0.37 MPa higher than π^0 while in NH species $\Psi_{gs50\%}$ were lower than π^0 in three out of five species and in the other two species, *F. esquiroliana* and *F. racemosa*, $\Psi_{gs50\%}$ was higher than π^0 by only 0.11 and 0.04 MPa respectively.

Leaves of H species were much more resistant than NH species to water loss after excision. NH species had on average four times higher g_{min} than that of H species (10.7 and 2.1 mmol m^{-2} s^{-1}, respectively; Table 2.3). The T_{70} in H species (17.2 hours) was on average ten times that of NH species (1.7 hours; Table 2.3). For two H species (i.e. *F. benjamina* and *F. curtipes*), T_{70} was 32.7 and 36.1 hours respectively, several times longer than the other species (Table 2.3). Several processes can affect the water loss rate of excised leaves and thus influence the T_{70}, which depends on the period in which the stomata remain initially open, their rate of closure, and the rate of water loss after stomata are closed. Thus, all else being equal (1) a higher g_{max} should reduce T_{70}; (2) faster stomatal closure with leaf desiccation should increase T_{70}; and (3) a lower g_{min} should increase T_{70}. Analyses of correlations between T_{70} and these possible underlying traits showed that T_{70} was uncorrelated with g_{max} (Fig. 2.2a), but was negatively correlated with $g_{\mathrm{s\text{-}TLP}}/g_{\mathrm{max}}$ (where low values represent effective stomatal closure during desiccation) and negatively correlated with g_{min} (Fig. 2.2b,c).

Coordination among traits related to xylem water supply and transpiratory demand was observed (Table 2.5). Among all 19 tested leaf traits, there were 46 significant correlations of 171 tested (27%), greater than the 5% to be expected due to chance ($P <$ 0.001, proportion test), indicating a significant tendency for coordination among these functional traits. Thus, K_{t} was positively correlated with D_{v}, g_{max} (with the exception of outlier *F. hispida*) and $g_{\mathrm{s\text{-}TLP}}/g_{\mathrm{max}}$ (Fig. 2.3a-c). Negative correlations of leaf water flux traits and drought tolerance traits were also found across species of the two life forms. Across species, K_{t} and D_{v} were negatively correlated with T_{70} and was negatively correlated with the differences between $\Psi_{\mathrm{gs50\%}}$ and π^0 ($\Psi_{\mathrm{gs50\%}}$- π^0; Fig. 2.4a,b,c). These correlations indicated that leaves of species with lower hydraulic conductivity tended to desiccate more slowly after excision, due to lower cuticle and stomatal leakiness and wider safety margins, and closing stomata earlier during desiccation, even before losing turgor. The $g_{\text{s-TLP}}/g_{\text{max}}$ and π^0 were positively correlated across species with H species and NH species segregated along the regression line (Fig. 2.4d).

Discussion

Traits linked with maximum flux through the leaf

We found strong differences between H and NH species, such that H species had lower values for leaf water flux-related traits. These findings parallel earlier findings that hemiepiphytes tended to have lower hydraulic conductivity in stems (Patiño, Tyree and Herre 1995; Zotz, Patiño and Tyree 1997). The significantly higher D_{v}, K_{t}, g_{max}, C_{leaf} and $g_{\text{s-TLP}}/g_{\text{max}}$ all support NH having higher leaf water flux rates, which would support higher gas exchange and, for a given level of leaf allocation, higher growth rates (Sack and Holbrook 2006). Our greenhouse growth experiment showed seedlings of NH species to have significantly higher growth rates than H species (see Chapter 4).

The lower values for flux-related traits in H species are consistent with a lower requirement for water per leaf area. Indeed just as H species had significantly lower g_{max} compared with NH species (Table 2.4), midmorning measurements of gas exchange showed that H species had significantly lower g_{s} and higher intrinsic water use efficiency (net CO_2 assimilation / g_{s}) than NH species (see Chapter 3). Higher C_{leaf} in NH species may be important for buffering higher rates of water flux to minimize transient fluctuations in mesophyll water potential as found in temperate woody species (Sack et al. 2003), paralleling the function of capacitance in wood (Meinzer et al. 2009). Also, the correlation between C_{leaf} and K_{t} (Table 2.5) may be related to the finding that excised leaves of higher leaf hydraulic conductance close their stomata relatively slowly (Aasamaa and Sober 2001; Sack et al. 2003), further confirmed in our study with NH species having a weaker stomatal control of water loss than H species during leaf desiccation.

These differences between H and NH species extend the finding that species adapted to different environments tend to differ in a cluster of numerous flux-related traits (Sack et al. 2003; Sack, Tyree and Holbrook 2005; Dunbar-Co, Sporck and Sack 2009). These traits would contribute to NH species having greater water and nutrient transport efficiencies and a higher potential growth capacity per investment in leaf mass (Sack and Holbrook 2006; Brodribb, Field and Jordan 2007). Thus, the differences in leaf flux-related traits between H and NH species may underlie the NH species' having higher photosynthetic capacity per investment in leaf dry mass (see Chapter 3).

Traits conferring drought tolerance

We found H species to possess traits linked with stronger drought tolerance than NH species. H species had significantly higher LMA compared to NH species (Table 2.3), typical in many cases of species adapted to drier environments (Hoffmann et al. 2005; Hao et al. 2008; Poorter et al. 2009). Leaves of H species had significantly thicker epidermis for both upper and lower sides compared to NH species (Table 2.2). The densely arranged multi-layered epidermal cells in H species, while not contributing to a higher C_{leaf}, may play important localized roles in water storage, or slowing down the rate of water loss from inner mesophyll cells.

The H species reduced their g_s to minimum values at π^0 (Fig. 2.1a-e), while all the NH species kept g_s at relatively high values even when Ψ_l dropped bellow π^0 (Fig. 2.1f-j). It has been suggested that such a narrow "safety margin" between stomatal closure and turgor loss point, or the depression of K_{leaf}, can benefit plants by allowing the maintenance of gas exchange and thus optimizing return on xylem investment (Brodribb and Holbrook 2004). By contrast, the effective stomatal closure in H species is consistent with a conservative water use that would reduce the risk of catastrophic hydraulic failure under drought stress (Brodribb and Holbrook 2004).

The H species were conservative not only in their stomatal response to leaf desiccation but also in their water loss rate after the stomata had closed, which may indicate a greater ability to persist during severe drought. The strong correlations of T_{70} with both $g_{\text{s-TLP}}/g_{\text{s-Max}}$ and g_{min} across species (Fig. 2.2b,c) indicated that leaf desiccation avoidance was determined by both the stomatal response to water deficits and the water retention of leaves after stomata closure, which relates to the resistance to water loss of cuticle and closed stomata (Muchow and Sinclair 1989; Holbrook and Putz 1996b). Notably, the H species had more negative π^0, consistent with greater drought tolerance, but their C_{leaf} was also lower (Table 2.4). Consequently, the product of the two parameters ($\pi^0 \times C_{\text{leaf}}$), which represents the amount of water released per unit area between saturation and turgor loss point, was not significantly different between species of the two groups ($P = 0.81$, one-way ANOVA; mean values are -735 and -703 mmol m^{-2}, respectively). Thus, the absolute amount of stored water per leaf area does not contribute to the greater drought tolerance of H species. However, due to their significantly tighter stomatal control and lower cuticle conductance, H species would better preserve turgor, and hydrated cells, during natural drought, consistent with field observation that leaf curvature and diebacks are commonly found in NH but not H species during the peak of dry seasons.

Importance of trait differences between H and NH species

The H and NH growth forms possess leaf traits contributing to contrasting adaptation in water transport and use. The NH species had higher xylem water transport capacity, associated with their larger vessels, and these species also showed weaker stomatal control of water loss. By contrast, H species had lower xylem water transport capacity, but showed tighter stomatal control and greater ability of leaves to persist under drought. Thus, across species traits related to leaf water flux were negatively correlated with those of drought tolerance. Our finding of a negative relationship of maximum flux-related traits and drought tolerance traits for H and NH species seems analogous to the trade-off between hydraulic conductivity and xylem cavitation resistance in stems (Martinez-Vilalta et al. 2002). Such a trade-off may affect the distribution of species: species distributed in relatively wet environments are usually more vulnerable to xylem embolism than species adapted to dry environments (Maherali, Pockman and Jackson 2004). The existence of a compromise between the ability to cope with water stress and the ability to grow at high rates under more favorable water conditions can partially explain why drought-tolerant plant species are displaced from mesic and humid habitats (Orians and Solbrig 1977). The higher growth rates of plants dominating more humid environments compared to species subject to frequent drought may be partially explained by their higher hydraulic conductivity that affects the uptake of water from the soil, higher stomatal conductance, and higher CO_2 assimilation rates (Salleo et al. 2000; Santiago et al. 2004). We note that the negative correlation across species between hydraulic conductivity and xylem cavitation resistance is not always found (Bhaskar, Valiente-Banuet and Ackerly 2007; Chen et al. 2009), and thus is not necessarily intrinsic or necessary, but may typically arise due to contrasting selection scenarios—i.e., there may be selection of high water flux and low drought tolerance in moist environments, where high assimilation rates would repay the investment in constructing xylem of high conductivity. On the other hand, there may be selection of low water flux and high drought tolerance in dry environments, where investment in high conductivity would not be repaid. Such co-selection of traits may also explain the clustering of flux-related traits, and drought-tolerance traits in H and NH species, and their negative correlation with each other. A negative correlation of flux traits and drought tolerance traits was not found across phylogenetically diverse species (Sack et al. 2003), and thus may not necessarily arise from any intrinsic genetic or structural trade-off. The negative correlation of these traits would arise within a single genus due to contrasting adaptation, given that high flux and low drought tolerance is advantageous for NH species, and low flux and high drought tolerance for H species.

The existence of the epiphytic growth stage, more frequently subjected to water deficits, in H species apparently led to a greater benefit for xylem composed of smaller vessels, with lower hydraulic transport efficiency, which persists even in the terrestrial growth stage studied here. The divergences in hydraulics between H and NH species are analogous to the differences between xeric and humid-grown species (Bhaskar, Valiente-Banuet and Ackerly 2007), indicating that species within a single genus, that co-exist in the same community can diverge significantly in numerous aspects of leaf structure and function, according to their micro-habitat in the regeneration phase. The evolution of an epiphytic growth habit at the beginning of their life history enabled H species to successfully regenerate in the canopy of dense forests with extremely shaded understory (Harrison et al. 2003). By contrast, the regeneration of NH species in dense rainforests depends on the formation of high irradiance forest gaps; in these species a higher xylem water transport efficiency would enable higher rates of carbon assimilation and growth rates and thus greater competitiveness in resource acquisition, given a reliable water supply and high irradiance (Brodribb, Holbrook and Gutierrez 2002; Santiago et al. 2004; Brodribb et al. 2005; Zhang and Cao 2009).

Conclusions

The two growth forms of *Ficus* are consistent with the two generalized contrasting types of water use for plants according to Passioura (1982). The H species have leaf traits conferring conservative water use, of particular advantage to plants growing under harsh environmental conditions, whereas the NH species show leaf traits conferring prodigal water use that would be advantageous under competitive situations (Heilmeier et al. 2002). The patterns identified in this study indicated that the existence of an epiphytic habit during the juvenile stage in H species involved a suite of leaf water flux and drought tolerance traits of clear functional importance, contrasting with those of congeneric NH species, and that persist to a large degree in the terrestrially rooted adults.

Table 2.1 Five hemiepiphytic (H) and five non-hemiepiphytic (NH) *Ficus* tree species investigated in this study. Nomenclature follows Wu and Raven (2003).

Species name	Code	Growth form	Section	Leaf size (cm^2)	Leaf texture
F. benjamina Linnaeus	BE	H	Conosyce	24.2 ± 2.0	Leathery
F. concinna (Miquel) Miquel	CO	H	Urostigma	25.7 ± 1.6	Leathery
F. curtipes Corner	CU	H	Conosyce	71.3 ± 12.4	Leathery
F. religosa Linnaeus	RE	H	Urostigma	189.6 ± 24.6	Leathery
F. tinctoria Frost. f. subsp. gibbosa (Bl.) Corner	TI	H	Syzidium	48.3 ± 5.9	Leathery
F. auriculata Loureiro	AU	NH	Sycomorus	624.4 ± 75.8	Papery
F. esquiroliana H. Léveillé	ES	NH	Eriosycea	690.2 ± 131.1	Papery
F. hispida Linnaeus	HI	NH	Sycocarpus	136.4 ± 15.1	Papery
F. racemosa Linnaeus	RA	NH	Sycomorus	44.5 ± 4.1	Leathery
F. semicordata Buchanan-Hamilton ex Smith	SE	NH	Hemicardia	180.8 ± 21.1	Papery

Table 2.2 Thickness of leaf lamina tissues in transverse sections. Epidermis includes hypodermis layers. Data are means ± SE (n = 6). "*", "^{ms}" and "^{ns}" following a parameter indicate statistical difference between hemiepiphytic (H) and non-hemiepiphytic (NH) growth forms at $P < 0.05$, $0.05 \leq P \leq 0.1$ and $P > 0.1$ level, respectively (one-way ANOVA). Species code are given in Table 2.1.

Species	Upper* epidermis (UE)	Palisade ^{ns} mesophyll (PA)	Spongy* mesophyll (SP)	Lower* epidermis (LE)	Palisade/spongy^{ms} ratio (P/S)
BE	18.6 ± 0.5	24.4 ± 2.2	31.3 ± 1.6	7.3 ± 0.2	0.78 ± 0.05
CO	11.9 ± 0.3	27.3 ± 1.4	22.3 ± 2.8	6.0 ± 0.3	1.27 ± 0.09
CU	29.4 ± 1.5	37.9 ± 2.5	75.8 ± 3.7	17.1 ± 0.3	0.50 ± 0.02
RE	18.7 ± 0.5	31.7 ± 1.8	29.6 ± 1.0	8.2 ± 0.2	1.07 ± 0.04
TI	10.5 ± 0.3	22.3 ± 1.8	49.5 ± 2.8	5.5 ± 0.2	0.45 ± 0.03
H mean	**17.8 ± 3.7**	**28.7 ± 3.1**	**41.7 ± 10.8**	**8.8 ± 2.4**	**0.81 ± 0.18**
AU	11.1 ± 0.4	29.7 ± 0.8	33.6 ± 1.9	3.3 ± 0.1	0.89 ± 0.03
ES	6.9 ± 0.3	19.8 ± 2.2	5.8 ± 0.3	2.4 ± 0.1	3.42 ± 0.31
HI	11.1 ± 0.2	21.7 ± 1.2	16.5 ± 0.7	3.4 ± 0.1	1.32 ± 0.05
RA	10.0 ± 0.3	27.6 ± 1.4	22.1 ± 0.9	4.6 ± 0.1	1.25 ± 0.03
SE	8.8 ± 0.3	29.3 ± 2.0	17.0 ± 1.2	3.4 ± 0.1	1.73 ± 0.03
NH mean	**9.6 ± 0.9**	**25.6 ± 2.3**	**19.0 ± 5.0**	**3.4 ± 0.4**	**1.72 ± 0.50**

Table 2.3 Leaf structural and physiological traits of five hemiepiphytic (H) and five non-hemiepiphytic (NH) *Ficus* species. LMA, leaf dry mass per area; D_v, average vessel lumen diameter in leaf petioles; K_t, theoretical hydraulic conductivity of petioles; SWC, leaf saturated water content; ρ_{leaf}, density of leaf lamina; T_{70}, time required for bench drying of fully saturated leaves to 70% relative water content; g_{min}, leaf epidermal conductance. Data are mean ± SE (n = 6). "**", "*" and "^{ns}" following a parameter indicate statistical difference between H and NH growth forms at $P < 0.01$, $0.01 \leq P < 0.05$, and $P \geq 0.05$ level, respectively (one-way ANOVA). Species code are given in Table 2.1.

Species	LMA* (g cm^{-2})	D_v** (μm)	K_t** (mmol m^{-1} s^{-1} MPa^{-1})	SWC^{ns} (g g^{-1})	ρ_{leaf}^{ns} (g cm^{-3})	T_{70}* (hour)	g_{min}** (mmol m^{-2} s^{-1})
BE	99.2 ± 7.0	16.6 ± 0.4	0.61 ± 0.09	1.71 ± 0.09	0.35 ± 0.01	32.7 ± 11.1	0.60 ± 0.17
CO	87.3 ± 5.3	18.5 ± 0.8	0.68 ± 0.12	1.59 ± 0.04	0.37 ± 0.01	5.1 ± 0.5	3.22 ± 0.36
CU	154.7 ± 4.7	20.0 ± 1.0	0.62 ± 0.11	1.60 ± 0.07	0.34 ± 0.01	36.1 ± 3.7	0.71 ± 0.08
RE	85.2 ± 6.1	23.9 ± 2.2	0.83 ± 0.14	2.21 ± 0.24	0.45 ± 0.02	3.2 ±0.7	4.30 ± 0.31
TI	83.1 ± 4.0	18.3 ± 0.5	0.52 ± 0.05	2.49 ± 0.11	0.27 ± 0.01	8.9 ± 2.4	1.83 ± 0.26
H mean	**101.9 ± 15.1**	**19.5 ± 1.4**	**0.65 ± 0.06**	**1.92 ± 0.20**	**1.06 ± 0.08**	**17.2 ± 7.9**	**2.13 ± 0.06**
AU	67.4 ± 6.6	26.9 ± 1.6	1.02 ± 0.21	1.83 ± 0.15	0.25 ± 0.01	1.9 ± 0.1	9.14 ± 0.30
ES	48.7 ± 6.7	23.1 ± 1.3	0.79 ± 0.07	2.46 ± 0.10	0.15 ± 0.01	2.6 ± 0.1	7.65 ± 0.47
HI	93.6 ± 5.3	26.5 ± 2.2	1.96 ± 0.25	2.07 ± 0.14	0.34 ± 0.01	1.6 ± 0.1	16.76 ± 1.00
RA	50.2 ± 7.1	24.8 ± 1.1	1.24 ± 0.15	2.81 ± 0.20	0.34 ± 0.01	1.3 ± 0.4	7.26 ± 1.42
SE	80.6 ± 5.3	25.0 ± 1.9	1.64 ± 0.38	1.87 ± 0.05	0.34 ± 0.01	1.0 ± 0.1	12.55 ± 0.61
NH mean	**68.1 ± 9.7**	**25.3 ± 0.8**	**1.33 ± 0.24**	**2.21± 0.21**	**1.24 ± 0.21**	**1.7 ± 0.3**	**10.67 ± 0.22**

Table 2.4 Comparison of leaf functional traits of plant water relations. π^0, osmotic potential at turgor loss point; C_{leaf}, leaf absolute capacitance; g_{max}, maximum stomatal conductance; $\Psi_{\mathrm{gs50\%}}$, leaf water potential at 50% g_{max}; $\Psi_{\mathrm{gs20\%}}$, leaf water potential at 20% g_{max}; $\Psi_{\mathrm{gs50\%}} - \pi^0$, difference between $\Psi_{\mathrm{gs50\%}}$ and π^0; $g_{\mathrm{s\text{-}TLP}}/g_{\mathrm{s\text{-}Max}}$, the ratio of stomatal conductance at turgor loss point to g_{max}. Error bars are not provided as species' values were derived from pressure volume curves with points pooled for all individuals, or from fitted regressions. "**", "*" and "$^{\mathrm{ns}}$" following a parameter indicate statistical difference between H and NH growth forms at $P < 0.01$, $0.01 \leq P < 0.05$, and $P \geq 0.05$ level, respectively (one-way ANOVA). Species code are given in Table 2.1.

Species	π^0* (MPa)	C_{leaf}* (mmol m^{-2} MPa^{-1})	g_{max}** (mol m^{-2} s^{-1})	$\Psi_{\mathrm{gs50\%}}{}^{\mathrm{ns}}$ (MPa)	$\Psi_{\mathrm{gs20\%}}{}^{\mathrm{ns}}$ (MPa)	$\Psi_{\mathrm{gs50\%}} - \pi^0$** (MPa)	$g_{\mathrm{s\text{-}TLP}}/g_{\mathrm{max}}$**
BE	-1.65	416.1	0.287	-1.32	-1.79	0.33	0.267
CO	-2.32	332.8	0.15	-1.97	-2.14	0.35	0.149
CU	-1.47	290.3	0.257	-1.1	-1.25	0.37	0.116
RE	-1.69	515.5	0.26	-1.51	-1.65	0.18	0.191
TI	-1.82	505.3	0.256	-1.6	-1.71	0.22	0.156
H mean	**-1.79**	**412.0**	**0.242**	**-1.50**	**-1.71**	**0.29**	**0.176**
AU	-0.86	532.9	0.326	-0.94	--	-0.08	0.583
ES	-1.15	517.5	0.402	-1.04	-1.42	0.11	0.378
HI	-1.23	531.5	0.281	-1.39	-1.51	-0.16	0.541
RA	-1.44	550.9	0.412	-1.48	-2.17	-0.04	0.529
SE	-1.52	666.2	0.437	-1.48	-2.1	0.04	0.462
NH mean	**-1.24**	**559.8**	**0.372**	**-1.27**	**--**	**-0.03**	**0.499**

Table 2.5 Pearson correlation coefficient (r) for leaf trait relationships for 10 *Ficus* species. Values in bold are statistically significant ($P < 0.05$). * $P < 0.05$; ** P < 0.01. Abbreviations for plant traits are as defined in Table 2.2, 2.3 and 2.4.

	LMA	D_v	K_t	SWC	ρ_{leaf}	T_{70}	g_{min}	TLP	C_{leaf}	g_{max}	$\Psi_{gs50\%}$	$\Psi_{gs20\%}$	$\Psi_{gs50\%} - \pi^0$	$g_{s\text{-TLP}}/g_{max}$	UE	PA	SP	LE	P/S
LMA				*		**			**						**		**	**	
D_v	-0.42		*			*	**	*	*				**	**					
K_t	-0.21	**0.76**					**		*				**	*					
SWC	**-0.67**	0.30	0.12																
ρ_{leaf}	0.40	-0.08	0.13	-0.28															
T_{70}	**0.78**	**-0.68**	-0.52	-0.50	0.13		*		*				*		**		*	**	
g_{min}	-0.38	**0.84**	**0.94**	0.17	-0.12	**-0.66**			*				**	**					
TLP	-0.23	**0.70**	0.40	0.20	-0.50	-0.16	0.52				**		*	*					
C_{leaf}	**-0.66**	**0.67**	**0.64**	0.52	-0.14	**-0.70**	**0.69**	0.43		*			*	*	*			*	
g_{max}	-0.52	0.55	0.45	0.49	-0.40	-0.31	0.48	0.62	**0.75**					*					
$\Psi_{gs50\%}$	0.04	0.36	0.02	-0.01	-0.54	0.23	0.14	**0.89**	0.10	0.47		*							
$\Psi_{gs20\%}$	0.48	-0.02	-0.18	-0.10	-0.30	0.42	-0.09	0.51	-0.33	-0.14	**0.73**								
$\Psi_{gs50\%} - \pi^0$	0.56	**-0.90**	**-0.81**	-0.44	0.20	**0.70**	**-0.88**	**-0.69**	**-0.76**	-0.57	-0.28	0.12		**				*	
$g_{s\text{-TLP}}/g_{max}$	-0.57	**0.81**	**0.76**	0.29	-0.28	-0.55	**0.82**	**0.73**	**0.70**	**0.67**	0.39	-0.26	**-0.92**					*	
UE	**0.88**	-0.39	-0.40	-0.50	0.46	**0.83**	-0.57	-0.15	**-0.69**	-0.44	0.17	0.48	0.60	-0.58		*	**	**	
PA	0.60	0.04	-0.15	-0.45	0.50	0.38	-0.30	-0.05	-0.34	-0.15	0.11	0.07	0.30	-0.27	**0.73**		*	*	
SP	**0.79**	-0.42	-0.49	-0.32	0.17	**0.71**	-0.61	-0.12	-0.59	-0.41	0.16	0.42	0.52	-0.56	**0.80**	**0.64**		**	*
LE	**0.88**	-0.45	-0.46	-0.43	0.38	**0.80**	-0.63	-0.25	**-0.73**	-0.43	0.09	0.46	**0.66**	**-0.67**	**0.96**	**0.74**	**0.86**		
P/S	-0.58	0.31	0.21	0.31	-0.55	-0.45	0.40	0.28	0.35	0.52	0.21	0.06	-0.26	0.32	-0.56	-0.48	**-0.73**	-0.53	

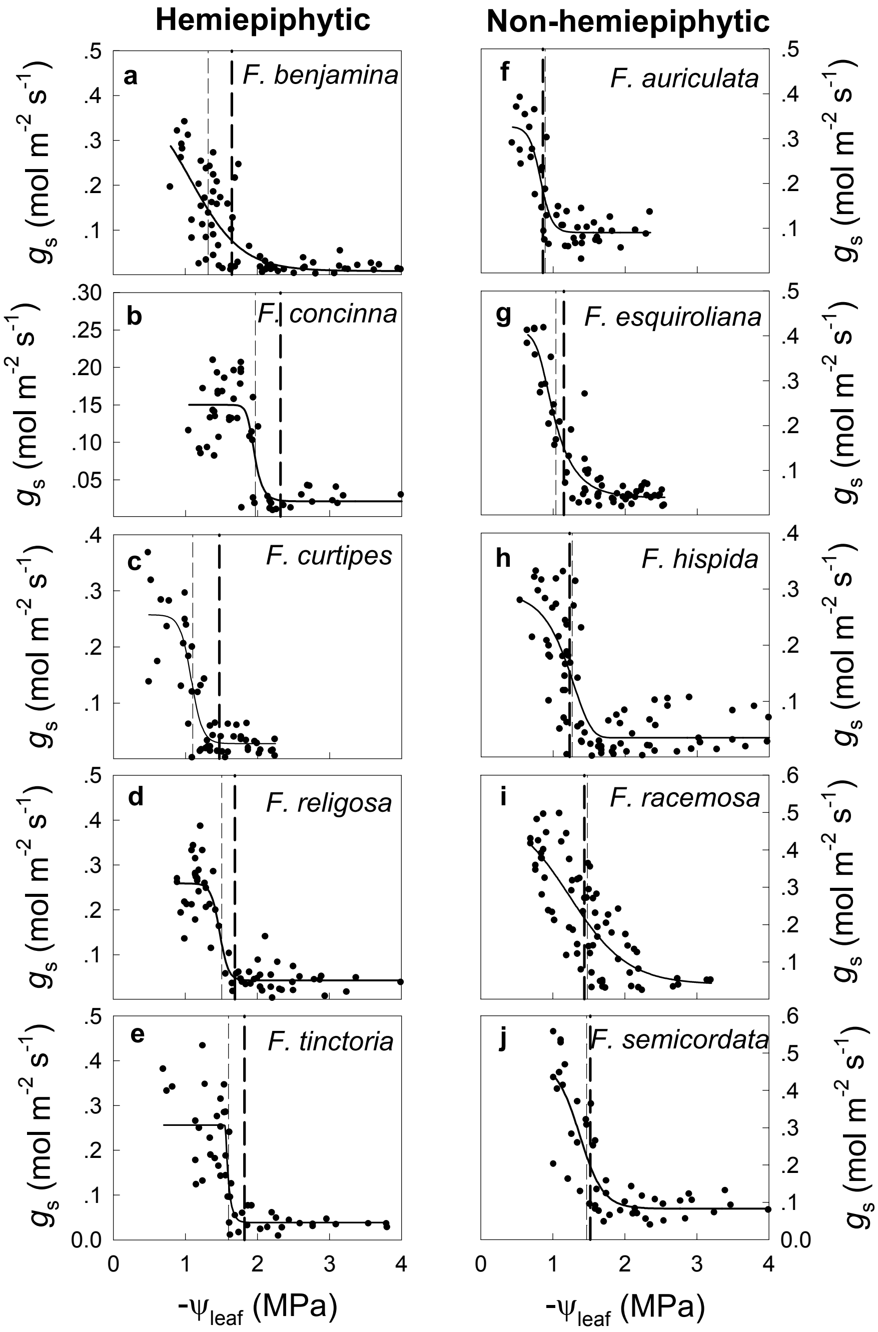

Figure 2.1 Stomatal conductance (g_s) in response to change of leaf water potential (Ψ_l) in five hemiepiphytic (a to e) and five non-hemiepiphytic (f to j) *Ficus* species. A sigmoid function was fitted to the data ($y = a/(1+\exp(-(x-x_0)/b))$). Vertical dashed lines show Ψ_l at 50% of maximum g_s and the heavy dashed lines show Ψ_l at turgor loss.

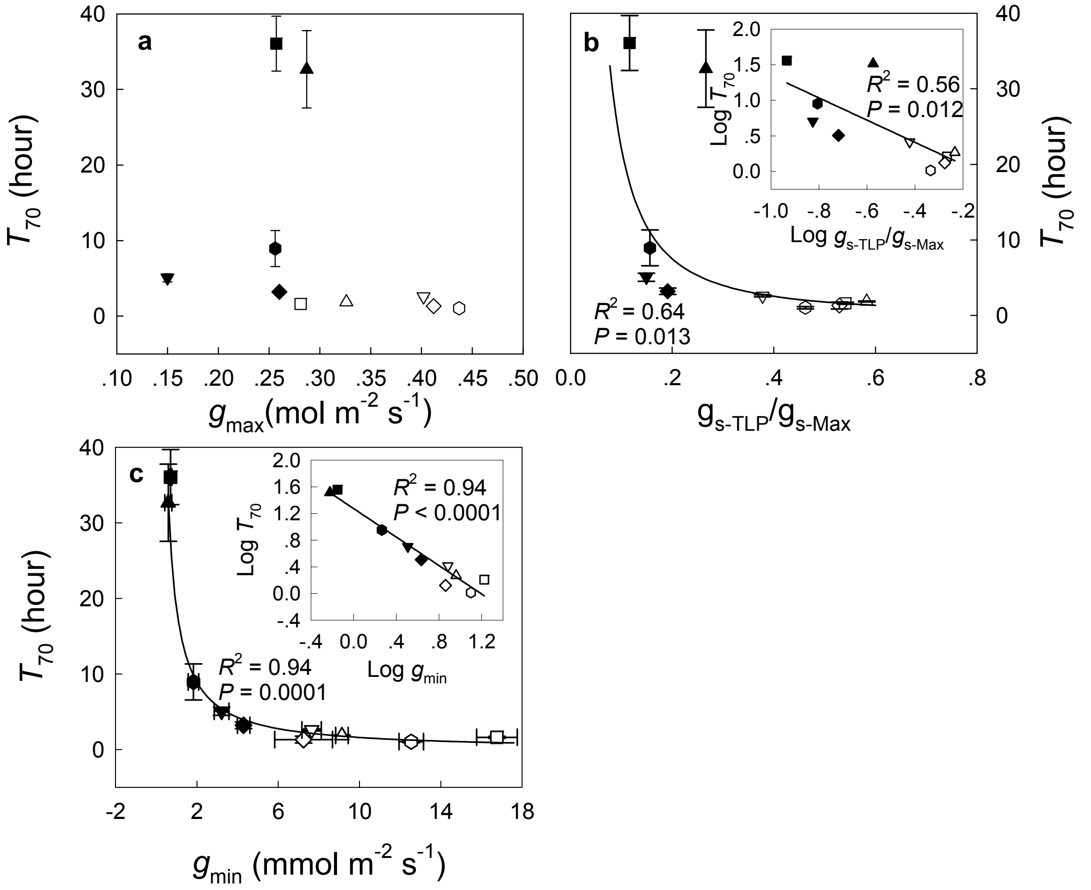

Figure 2.2 Correlations between time required for saturated leaf to drop to a relative water content of 70% (T_{70}) and (a) maximum stomatal conductance (g_{max}); (b) ratio of stomatal conductance at turgor loss point to g_{max} ($g_{s\text{-}TLP}/g_{max}$); (c) leaf epidermal conductance (g_{min}) across five hemiepiphytic (H; filled symbols) and five non-hemiepiphytic (NH; open symbols) *Ficus* species. Data in plot b and c were fitted with power functions ($y = ax^b$) and the insets show linear regressions fitted to $\log_{10}$-transformed data. *Ficus benjamina* (▲), *F. concinna* (▼), *F. curtipes* (■), *F. religosa* (◆), *F. tinctoria* (⬢), *F. auriculata* (△), *F. esquiroliana* (▽), *F. hispida* (□), *F. racemosa* (◇), *F. semicordata* (⬡).

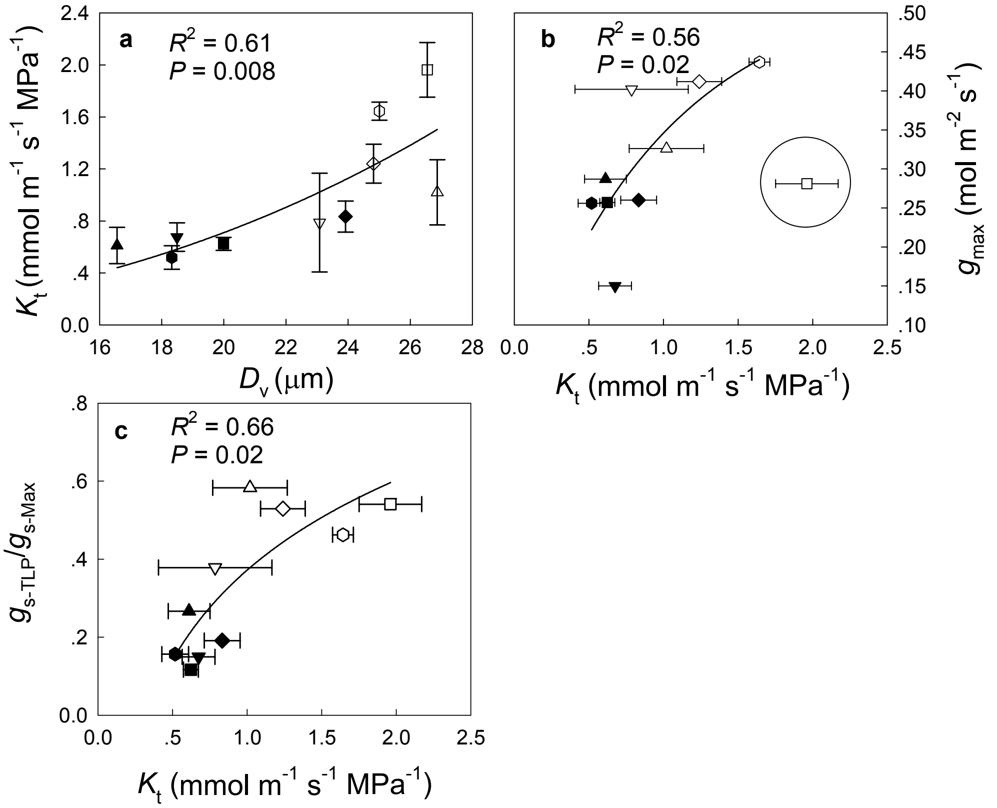

Figure 2.3 (a) Relationship between average vessel lumen diameter in leaf petiole (D_v) and petiole theoretical hydraulic conductivity (K_t); (b) relationship between K_t and maximum stomatal conductance (g_{max}); (c) relationship between K_t and ratio of stomatal conductance at turgor loss point to g_{max} ($g_{s\text{-TLP}}/g_{max}$) across five hemiepiphytic (H; filled symbols) and five non-hemiepiphytic (NH; open symbols) *Ficus* species. Data are fitted using power functions ($y = ax^b$ for a and b; $y = y_0 + ax^b$ for c). In plot b, outlier *F. hispida* was excluded from the regression (the circled symbol). Symbols are as defined in Figure 2.2.

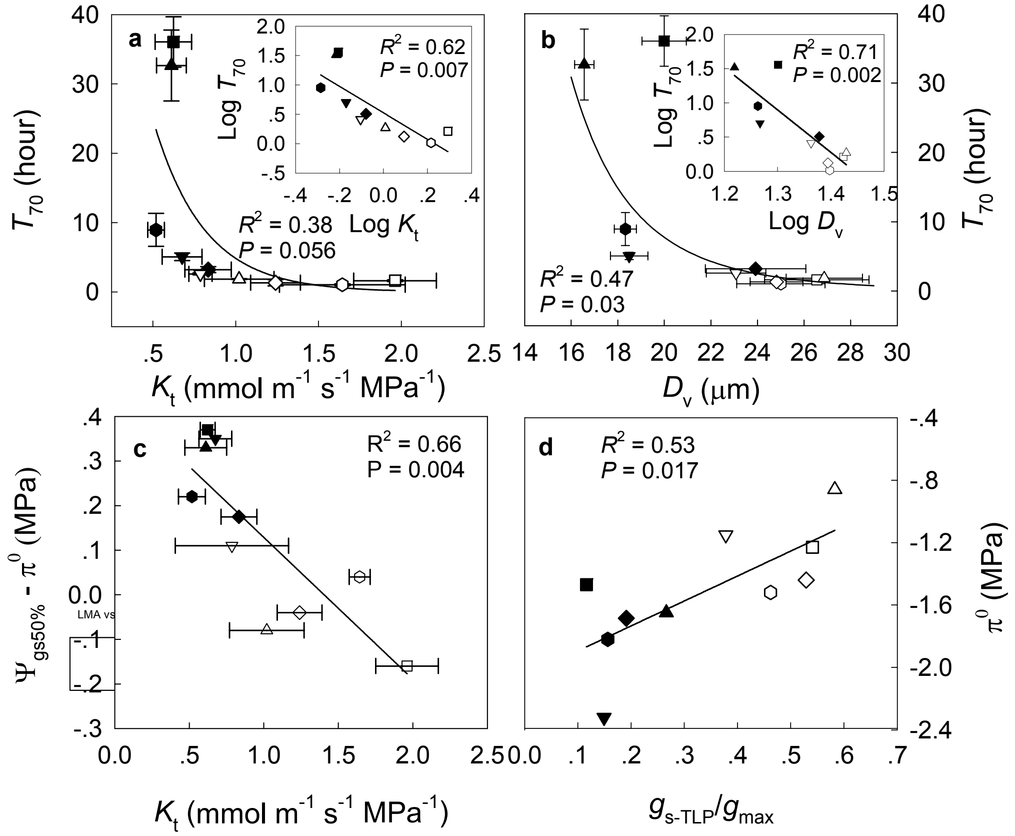

Figure 2.4 (a) Correlations between time required for a saturated leaf to drop to a relative water content of 70% (T_{70}) and theoretical petiole hydraulic conductivity (K_t); (b) between T_{70} and average vessel lumen diameter (D_v) in petiole xylem; (c) between the difference in leaf water potential from 50% of maximum stomatal conductance to leaf osmotic potential at turgor loss ($\Psi_{gs50\%} - \pi^0$) and K_t; (d) between the ratio of stomatal conductance at turgor loss to maximum stomatal conductance ($g_{s\text{-}TLP}/g_{max}$) and leaf osmotic potential at turgor loss point (π^0) across five hemiepiphytic (H; filled symbols) and five non-hemiepiphytic (NH; open symbols) *Ficus* species. Data in plot a and b were fitted with power functions ($y = ax^b$) and the insets show linear regressions fitted to $\log_{10}$-transformed data. Data in plot c and d were fitted with a linear regression. Symbols are as defined in Figure 2.2.

CHAPTER 3

DIFFERENTIATION OF PHOTOCHEMISTRY, PHOTORESPIRATION AND CO_2 ASSIMILATION

Summary

Hemiepiphytic *Ficus* species (H) possess traits of more conservative water use compared to non-hemiepiphytic *Ficus* species (NH) even during their terrestrial growth phase, which may cause significant differences in photosynthetic light use between these two growth forms. Stem hydraulic conductivity, leaf gas exchange, and leaf fluorescence was studied in adult trees of five Hs and five NHs grown in a common garden. Hemiepiphytic *Ficus* species showed significantly lower xylem water transport efficiency, stomatal conductance, photosynthetic N and P use efficiency but higher intrinsic water use efficiency compared to NHs. To avoid photoinhibition at high irradiance, Hs tend to dissipate a higher proportion of excessive light energy through thermal processes compared to NHs, while NHs have relatively higher photorespiration rates to partition excessive electron flow. Stem xylem hydraulic conductivity is positively correlated with maximum electron transport rate but is negatively correlated with quantum yield of non-photochemical quenching across the 10 studied *Ficus* species. These findings indicate that a canopy growth habit during early life stage in Hs resulted in substantial adaptive differences from common congeneric terrestrial species not only in water relations but also in photosynthetic light use and carbon economy. The development of new growth habits, even for part of their life history, involves profound evolutionary changes in a

suite of inter-correlated ecophysiological traits of the *Ficus* trees that persist for the whole life of the species, which contribute to species diversification.

Introductory remarks

It is commonly considered that there are several potential advantages to start the life cycle as an epiphyte. The most important one is that the forest canopy is better lit than the forest understory (Williams-Linera and Lawton 1995). It has been suggested that hemiepiphytic habit evolved in response to the scarcity of light on the forest understory (Dobzhansky and Murea-Pires 1954; Ramirez 1977; Putz and Holbrook 1986; Todzia 1986; Laman 1995; Williams-Linera and Lawton 1995). Also by growing in the canopy young individuals of hemiepiphytes may benefit from minimizing the risk of fire, flooding, damage by terrestrial herbivores (Holbrook and Putz 1996b) and coverage by falling debris. The advantages of spending the initial part of the life cycle as an epiphyte, on the other hand, can be offset by the potential limitations of water and nutrient availability (Coxson and Nadkarni 1995; Holbrook and Putz 1996a,b; Swagel et al. 1997).

When both epiphytic phase and tree phase *Ficus* were well supplied with water, the epiphytic leaves exhibited significantly lower stomatal conductance and much lower water loss rate from leaf surfaces, indicating the existence of an intrinsic water-use-related developmental change from epiphytic to tree phase (Holbrook and Putz 1996a,b). Despite the existence of a phase transition in Hs related to water availability from canopy to terrestrial growth (Holbrook and Putz 1996a,b), even after being terrestrially established, Hs still retain traits that confer relatively low stem xylem water transport efficiency (Patiño et al. 1995; see Chapter 5). Due to the potential coordination between stem xylem water transport and leaf gas exchange (Brodribb and Field 2000; Melcher et al. 2001; Brodribb et al. 2002; Santiago et al. 2004; Brodribb et al. 2005; Franks 2006; Zhang and Cao 2009), Hs in the terrestrial stage are expected to have lower stomatal conductance and higher water use efficiency than NHs.

The low stomatal conductance related to conservative water use on the other hand can limit the influx of CO_2 and thus photosynthetic carbon assimilation, resulting in an increased risk of overexcitation of photosystem II (PSII) reaction centers and thus photoinhibition when plants are exposed to excessive irradiance (Osmond 1994). This is especially the case as most *Ficus* species are light demanding species. Therefore, mechanisms balancing the use of absorbed light for photosynthesis and the safe dissipation of potentially harmful excess light energy can be of critical importance in Hs to overcome the potential photoinhibitory damage under conditions of high irradiance. Furthermore, in C_3 plants, such as *Ficus* species, photorespiration at high irradiance levels is considered to be an important process that recycles CO_2 in the Calvin-Benson cycle and consumes a considerable portion of electron flow when CO_2 influx is restricted (Valentini et al. 1995; Kozaki and Takeba 1996; Muraoka et al. 2000; Franco and Lüttge 2002; Zhang et al. 2008).

In the present study, we examined leaf gas exchange and chlorophyll fluorescence in response to different irradiance levels in five hemiepiphytic and five non-hemiepiphytic *Ficus* species with all the hemiepiphytic plants being adult trees with roots well connected to the ground. Two questions were addressed: (1) do Hs and NHs differ significantly in photosynthetic traits, such as net CO_2 assimilation rate, efficiencies of non-photochemical energy dissipation of PSII (Φ_{NPQ}), and photorespiration rates (R_l) under conditions of excessively high irradiance? (2) Is xylem hydraulic conductivity an important factor underlying photosynthetic light use and carbon assimilation by affecting stomatal openness? The use of closely related species growing under similar environmental conditions guaranteed that the interspecific variations in plant hydraulics, photochemistry, and carbon assimilation are attributable to functional type (NH versus H) rather than phylogeny or growth environment.

Materials and methods

Study species

Measurements were carried out during clear days in April and May 2008, during which intermittent rainfall ensured that the plants were not under drought conditions. *Ficus tinctoria* Frost. f. subsp. gibbosa (Bl.) Corner is a hemiepiphyte that usually does not form a firm self-supporting trunk and falls down soon after the host tree rots. *Ficus benjamina* L., *F. concinna* (Miquel) Miquel, and *F. curtipes* Corner are stranglers that can form free-standing trunks. *F. religosa* L. is a species that starts its growth on a host and later on forms a massive trunk and canopy up to 30 meters tall. Among the five non-hemiepiphytic *Ficus* species (i.e. *F. auriculata* Loureiro, *F. esquiroliana* H. Léveillé, *F. hispida* L., *F. racemosa* L., and *F. semicordata* Buchanan-Hamilton ex Smith), *F. racemosa* can growth up to 30m tall and the other four species are trees of medium size usually no more than 10m tall. All plants were growing under similar environmental conditions at XTBG and had branches reachable from the ground or by using a ladder.

The DBH of the studied trees ranged from 15 to 60cm with individuals of the hemiepiphytic species all at the terrestrial growth stage.

Gas exchange and fluorescence analyses

A portable LI-6400 photosynthetic system with a 6400-40 Fluorescence Chamber (Licor, Inc., Lincoln, Neb., USA) was used to measure leaf net CO_2 assimilation and fluorescence simultaneously in response to different irradiance levels in intact leaves. The night before carrying out the measurements, nighttime respiration rate (R_n), nighttime leaf temperature (T_n) were measured in three leaves of each of five to six different trees per species. These were followed by measurements of chlorophyll fluorescence yield (F_m) after illuminating the same leaves with a pulse of saturation irradiance provided by the fiber optics of the fluorescence chamber. Photosynthetic light response curves were obtained the following day in one of the marked leaves of each tree used for nighttime measurements. All light response measurements were made between 8:00 AM and 11:30 AM (solar time) when photosynthesis was most active. Leaves were illuminated at a PPFD of 1000 μmol m^{-2} s^{-1} until a steady state of net CO_2 fixation and a stable fluorescence yield were reached. The PPFD was then increased to 2000 μmol m^{-2} s^{-1} and then decreased step-wise from 2000 to 0 μmol m^{-2} s^{-1}. The leaf cuvette temperature was controlled at 30 ºC. At each irradiance level, net CO_2 assimilation rate, chlorophyll fluorescence yield under illumination (F_s), and maximum fluorescence yield during illumination (F_m') were measured after a five-minute equilibration period.

The allocation of photons absorbed by photosystem II (PS II) antennae to photosynthetic electron transport and thermal dissipation were assessed from the light response measurements. The total absorbed light energy was considered to be partitioned to four different quenching pathways, i.e., to photochemistry ($\Phi_{PS\ II}$), regulated (Φ_{NPQ}) and constitutive (Φ_D) non-photochemical energy dissipation and fluorescence (Φ_f), respectively. These parameters summed up to unity when expressed in fluorescence terms (Hendrickson et al. 2004):

$$\Phi_{PSII} = 1 - \frac{F_s}{F_m'} \qquad (3.1)$$

$$\Phi_{f,D} = \Phi_f + \Phi_D = \frac{F_s}{F_m} \qquad (3.2)$$

$$\Phi_{NPQ} = \frac{F_s}{F_m'} - \frac{F_s}{F_m} \qquad (3.3)$$

We also estimated the rate of photorespiration (R_l) following the approach of Valentini et al. (1995):

$$R_l = \left[\alpha_L (ETR) - 4(A_n + R_d)\right]/12 \qquad (3.4)$$

where α_L is the average absorptance (species means ranging from 0.909 to 0.926) of photosynthetic active irradiance of the leaves measured for each species using a spectrometer (USB 4000, Ocean Optics Inc., Florida, USA), ETR is the apparent electron transport rate, A_n is net photosynthetic CO_2 assimilation rate, and R_d is dark respiration rate during the day, which was estimated from nighttime measurements of dark respiration rate and leaf temperature using the following equation (Valentini et al. 1995; Larcher 2003):

$$R_d = R_n Q_{10}^{(T_d - T_n)/10} \qquad (3.5)$$

where Q_{10} is the temperature coefficient that is expected to be around 2.3 for tropical trees (Cavaleri et al. 2008), T_d is leaf temperature during the day when A_n were measured, R_n and T_n are night time respiration rate and leaf temperature, respectively.

Light response curves were fitted by the algorithm $Y = Y_{max} - ae^{-bx}$, where Y is ETR or A_n, x is PPFD and a×b is the initial slope of the light response curve (Iqbal et al. 1996). Dark respiration (R_d) and light compensation point (LCP) were calculated by fitting a linear regression to the relationship between A_n and PPFD at lower levels of PPFD (from 200 and 0 μmol m^{-2} s^{-1}).

Besides light response measurements, A_n and stomatal conductance (g_s) were also measured using the LI-6400 between 9:00AM and 11:00AM under a controlled PPFD of 1000 μmol m^{-2} s^{-1} to examine the performance of gas exchange under optimal conditions. Leaf intrinsic water use efficiency (WUE) was calculated as the ratio of A_n to g_s.

Stem hydraulic conductivity and leaf chemical analysis

Stem hydraulic conductivity was measured on three branches per tree of six to eight individuals per species. Branches were collected in the morning, re-cut immediately under water to avoid embolisms and transported to the laboratory with the cut end immersed in water and the free end tightly covered with opaque plastic bags. Distilled and degassed water was used as the perfusion fluid and a hydrostatic pressure generated by a constant and small hydraulic head of 50 cm was applied to avoid refilling of embolized vessels (Bucci et al. 2004). Because *Ficus* species produce latex which can cause clogging of vessels, both ends of a segment were shaved with a sharp razor blade immediately before each measurement of flow rate.

Total leaf N concentration was determined with an auto Kjeldahl unit (K370, BÜCHI Labortechnik AG, Flawil, Switzerland) after samples were digested with concentrated H_2SO_4. Total leaf N concentration was analyzed using an inductively coupled plasma atomic-emission spectrometer (IRIS Advantage-ER, Thermo Jarrell Ash Corporation, Massachusetts, USA) after samples were digested with concentrated HNO_3-$HClO_4$. Leaf N and P content were also expressed on leaf area basis (N_a and P_a; mol m^{-2} and mmol m^{-2}) based on leaf mass per area (LMA) measurements. The photosynthetic nitrogen use efficiency (PNUE) and photosynthetic phosphorous use efficiency (PPUE) were calculated by dividing A_n with N_a and P_a, respectively.

Results

Under optimal field conditions (mid-morning; PPFD range from 900 to 1300 μmol m^{-2} s^{-1}), Hs had significantly lower g_s than NHs but A_n was not significantly different between *Ficus* specie of the two growth forms (Table 3.1). Hs showed significantly higher WUE and dark respiration rates compared to NHs (Table 3.1 and 3.2). Consistent with the midmorning measurement of A_n, maximum A_n calculated from light response curves were not significantly different between species of the two growth forms; however, maximum apparent ETR were significantly higher for NHs (Table 3.2). Leaf mass per area of Hs was significantly higher than that of the NHs and thus leaf dry mass based maximum net CO_2 assimilation rate (A_m) was on average significantly higher in NHs than in Hs (Table 3.1).

The partitioning of light energy among the three dissipation components ($\Phi_{f,d}$, Φ_{NPQ}, and Φ_{PSII}) showed substantial differences between the two growth forms (Fig. 3.1). At higher PPFD levels, Hs as a group tended to dissipate a larger proportion of absorbed light energy through non-photochemical quenching (Φ_{NPQ}) compared to NHs (wider shaded area in upper panels than in lower panels in Fig. 3.1, except for *F. auriculata*; on average 10% higher shaded area in upper panels). In all the species, $\Phi_{f,d}$ was maintained at around 0.2 irrespective of the changing irradiance levels; while Φ_{NPQ} increased with a compensating decrease in Φ_{PSII} from low to high PPFD levels.

The two growth forms exhibited, at 30 ^{o}C leaf temperature and controlled irradiances, similar rates of maximum A_n, which were attained at similar PPFD´s (Fig. 3.2a; Table 3.2). On the other hand, ETR and photorespiration were significantly higher in NHs than those of Hs at saturating PPFD levels (Fig. 3.2b,c). When PPFD ranged from 800 to 2000 μmol m^{-2} s^{-1}, R_l in NHs accounted for 60% to 62% of the total linear electron flow and the R_l/A_n ratio in NHs ranged from 0.57 to 0.61 (Fig. 3.3a,b). Under the same PPFD levels, estimated electron flow to photorespiration ranged from 48% to 59% and the ratio of R_l/A_n ranged from 0.35 to 0.55 (Fig. 3.3a,b) in Hs, which were both significantly lower than in NHs.

Under saturating irradiance (800, 1000, 1500 and 2000 μmol m^{-2} s^{-1}) R_l was negatively related to Φ_{NPQ} (Fig. 3.4a), while R_l was positively correlated to ETR, across all the species (Fig. 3.4b). Significant correlations between xylem hydraulic conductive capacity and photosynthetic traits were also observed. For example, leaf area specific hydraulic conductivity (K_l) was negatively correlated to Φ_{NPQ} but was positively correlated to ETR (Fig. 3.5a,b). In addition, K_l was also negatively related to LMA and positively related to g_s across the 10 studied species (Fig. 3.5c,d).

Hs had significantly higher concentration of leaf N but similar leaf P compared to NHs (Table 3.3). Both PNUE and PPUE were significantly lower in Hs than in NHs (Table 3.3) and negative relationships between photosynthetic intrinsic water use efficiency (A/g_s) and both PNUE and PPUE were found across the 10 studied species (Fig. 3.6a,b).

Discussion

In the present study, significant differences in a suite of photosynthetic traits between terrestrially rooted hemiepiphytic *Ficus* species and non-hemiepiphytic species of the same genus were found. These differences include quenching of exited light energy through photochemical and non-photochemical pathways (i.e. among $\Phi_{f,d}$, Φ_{NPQ} and Φ_{PSII}), partitioning of electron flow through assimilative and non-assimilative pathways, photosynthetic N and P use efficiencies, g_s and WUE. Photosynthetic traits of *Ficus* species were strongly influenced by xylem hydraulic conductive capacity and leaf water use, which differed significantly between Hs and NHs. The hydraulic-photosynthetic coordination found in the present study provides the basis for expanding the understanding of the influence of xylem hydraulic conductivity on photosynthetic capacity (Brodribb and Field 2000; Melcher et al. 2001; Brodribb et al. 2002; Santiago et al. 2004; Brodribb et al. 2005; Franks 2006; Campanello et al. 2008; Zhang and Cao 2009) and gives mechanical explanations for the linkage between hydraulics and photosynthesis, as it will be explained below.

Non-photochemical quenching, carbon assimilation, and photorespiration

An important mechanism of photoprotection is non-photochemical quenching of excited energy that takes place in the light-harvesting complexes of PSII that reduces the supply of energy to photochemical processes by diverging part of the excitation energy to thermal dissipation. This is achieved by a high trans-thylakoidal pH gradient (ΔpH) and the reversible de-epoxidation of the components of the xanthophyll cycle (Osmond 1994; Gilmore 1997). In the studied *Ficus* species, energy that is dissipated via ΔpH and xanthophyll-mediated processes ranged from 20% to 60% of the total light energy absorbed by PSII antennae as PPFD experienced by leaves varied between 500 and 2000 μmol m^{-2} s^{-1}. The two growth forms of *Ficus* species differ significantly in non-photochemical quenching with Φ_{NPQ} on average being significantly higher in Hs than NHs (Fig. 3.1), which caused significantly lower maximum ETR (ETR$_{max}$) in Hs (Fig. 3.2b). However, maximum net CO$_2$ assimilation rate (A_{max}) was not significantly different between Hs and NHs. For a given Φ_{PSII} or ETR$_{max}$, leaves can have different maximum net CO$_2$ assimilation rate depending on the partitioning of photosynthetic electron flow between assimilative and non-assimilative pathways, such as photorespiration. For example, in the uppermost-canopy leaves of four dipterocarp species Zhang et al. (2008) found high level of ETR throughout the day but the net CO$_2$ assimilation rate exhibited sustained depression from midmorning onward.

Photorespiration as an important energy sink in C_3 plants (Heber et al. 1996) is another important mechanism involved in protecting PSII from potential damaging effects of photoinhibition when plants are exposed to excessive irradiance (Franco and Lüttge 2002; Zhang et al. 2008). At high irradiance levels, photorespiration in Hs was significantly lower than that of NHs, which can largely explain why A_n was similar between the two growth forms, although NHs had significantly higher ETR. The significantly higher photorespiration rates at saturating irradiance in NHs may serve as an adaptive mechanism for maintaining higher levels of electron flow. However, in Hs photorespiration showed a clear saturation or even decreased at PPFDs higher than 1000 μmol m^{-2} s^{-1}. Thus, in Hs non-photochemical quenching was probably a more important process compared to photorespiration in controlling the safe dissipation of excitation energy at high irradiance.

The negative correlation between R_l and Φ_{NPQ} under saturating irradiance (Fig. 3.4a) suggested that species that tended to dissipate more energy via thermal dissipation involved a lower degree of photorespiration, thus the two mechanisms (photorespiration and non-photochemical quenching) complemented each other in avoiding photoinhibition under potentially harmful high irradiance. The positive correlation observed between R_l and ETR suggested that under saturating PPFD the utilization of light energy to drive CO_2 assimilation was more strongly constrained in species with higher ETR. Higher ETRs due to higher photochemical energy efficiencies in NHs did not result in higher net carbon assimilation rates than in Hs, but rather a higher proportion of electron flow was diverged to non-assimilatory processes in NHs, such as photorespiration.

Links between photosynthesis and hydraulics and its mechanisms

Photosynthetic traits correlated with K_l in a consistent manner across the 10 *Ficus* species belonging to two distinct growth forms. ETR_{max} was positively correlated to K_l, which is consistent with the relationships found between xylem hydraulic conductivity and photosynthetic capacity measured as ETR_{max} or Φ_{PSII} in other species (e.g. Brodribb and Field 2000; Brodribb et al. 2002; Campanello et al. 2008). No such correlation was found across the studied *Ficus* species when photosynthetic capacity was expressed on an area-based maximum photosynthetic rate, although this correlation has been found across a wide variety of species (Santiago et al. 2004; Zhang and Cao 2009). Differences in partitioning electron flow between assimilative and non-assimilative pathways (mainly photorespiration) makes the correlation between xylem hydraulic conductivity and photosynthesis more complicated to interpret. The positive correlation between K_l and ETR_{max} across a certain group of species does not necessarily result in a positive correlation between K_l and maximum carbon assimilation rate.

The correlation between photosynthetic traits and hydraulic conductivity may not be direct but rather linked via a suite of other leaf traits, such as LMA and g_s, which are affected strongly by plant hydraulics and differed significantly between species of the two growth forms of *Ficus* species. Plants adapted to drought-prone environments tend to have leaves with higher LMA (Bucci et al. 2004; Hoffmann et al. 2005; Hao et al. 2008), which is the case in the present study with Hs having significantly higher LMA compared to NHs. Higher LMA involves longer distances and larger resistances for CO_2 diffusion from stomata to chloroplasts (Parkhurst 1994), which in turn limits photosynthetic carboxylation. Under adequate soil water supply, high stem hydraulic conductivity allows quick water transport to leaves to compensate for transpirational water loss and consequently maintain higher leaf water potential and higher maximum g_s (Meinzer et al. 1995; Sperry 2000; Meinzer 2003; Ackerly 2004; Santiago et al. 2004; Zhang et al. 2008). The extension of the relationship between xylem hydraulic conductivity and g_s to leaf CO_2 assimilation rates through the photosynthesis-g_s-intercellular CO_2 concentration equations of Farquhar et al. (1980) offered a theoretical basis for understanding the hydraulic-photosynthetic coordinations (Katul et al. 2003). However, in the present study despite a significant positive correlation between K_l and ETR_{max} across the *Ficus* species the coordination between K_l and A_{max} is not significant from a statistical point of view, which is caused by differences in photorespiration among species. Although species with higher K_l do have higher ETR_{max} (potentially higher photosynthetic rate), but higher ETR_{max} are not related to higher A_{max} in *Ficus* species because species with higher ETR_{max} also showed higher photorespiration rates.

Trade-offs between water use efficiency and photosynthetic nutrient use efficiency

To overcome the conflicts between water conservation and uptake of CO_2, some woody hemi-epiphytic plants, such as species in the genus *Clusia* (Clusiaceae), evolved facultative CAM metabolism to cope with the conflict between water conservation and efficient CO_2 uptake for photosynthesis (Ting et al. 1987; Borland et al. 1992). The epiphytic phase of these species has CAM metabolism opening stomata at night to fix exogenous CO_2 while reducing water loss by closing stomata during the daytime. The photosynthetic metabolism can switch to C_3 to maximize CO_2 assimilation when the plant is rooted and soil water availability is relatively high (Ting et al. 1987). While in hemiepiphytic *Ficus* species, which maintain C_3 metabolism during all life stages (Ting et al. 1987), the tight stomatal control over water loss protects plants from dropping their water potentials to critical values during drought periods and hence avoiding hydraulic failure (Brodribb and Holbrook 2004).

Tight stomatal control has a carbon cost because low g_s unavoidably limits the influx of CO_2 and thus photosynthetic carbon assimilation. Leaf N and P contents of the studied Hs were significantly higher than those of NHs, but investments to the carboxylation processes were apparently lower than in NHs as indicated by their significantly lower PNUE and PPUE values (Table3). Across all the studied *Ficus* species, a trade-off between photosynthetic WUE and nutrient use efficiency (PNUE and PPUE) seems to exist with Hs having on average significantly higher WUE but lower PNUE and PPUE compared to NHs (Fig. 3.6a,b). Such a trade-off between water use efficiency and leaf nutrient use efficiency has also been found in other groups of species (e.g. Santiago et al. 2004; Zhang et al. 2009) and may imply a commonly existing conflict between leaf water conservation and effective photosynthetic carbon assimilation. The trade-off is likely mediated by differences among species in stomatal openness and characteristics of photosynthetic systems, such as photorespiration and non-photochemical quenching. However, Hs tend to produce leaves with higher LMA, which are more persistent under severe drought (see Chapter 2), indicating higher investment in non-photosynthetic leaf structures and longer leaf life spans. Thus, by producing more persistent leaves under unfavorable environmental conditions with potentially longer leaf life spans Hs compensate their lower photosynthetic nutrient use efficiency with a longer period of carbon fixation which helps to maintain balances between water conservation and plant carbon economy.

Conclusions

Overall, the results of this study imply that *Ficus* species of the two growth forms had different mechanisms for avoiding risks from photoinhibition. Under high irradiance levels, Hs on average dissipated a larger proportion of absorbed energy through non-photochemical pathways compared to NHs while NHs showed a higher ability to partition electron flow through photorespiration. Non-photochemical energy quenching and photorespiration seemed to be two photoprotective mechanisms compensating for each other with a significant negative correlation between R_l and Φ_{NPQ} across the studied species. Differences in efficiencies for long distance water transport and stomatal control of water use are involved in the underlying mechanisms explaining these differences in photosynthetic traits across all the studied congeneric *Ficus* species. The relatively large variation in functional traits both within and between growth forms and trade-offs among functional traits explains, at least in part, life history trait differentiation among *Ficus* species and their co-existence in the Xishuangbanna region of SE Asia.

Table 3.1 Leaf photosynthetic traits of 5 hemiepiphytic (H) and 5 nonhemiepiphytic *Ficus* species (NH). Net CO_2 assimilation rate on area (A_n) and mass (A_m) basis, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and intrinsic leaf water use efficiency (WUE) measured at midmorning. Each is value is mean ± SE (n = 5–6). "**" and "*" imply significant differences between H and NH species at $P < 0.01$ and 0.05 levels (t-test), respectively and "^{ns}" implies non-significance.

Species (code)	LMA* (g m^{-2})	A_n^{ns} (μmol m^{-2} s^{-1})	A_m* (μmol g^{-1} s^{-1})	g_s** (mol m^{-2} s^{-1})	WUE** (μmol mol^{-1})
F. benjamina (BE)	99.2 ± 7.0	11.30 ± 1.48	0.114 ± 0.015	0.255 ± 0.031	44.7 ± 3.9
F. concinna (CO)	87.3 ± 5.3	11.75 ± 1.23	0.135 ± 0.014	0.263 ± 0.041	46.2 ± 3.4
F. curtipes (CU)	154.7 ± 4.7	15.35 ± 0.74	0.099 ± 0.005	0.332 ± 0.024	46.9 ± 4.0
F. religosa (RE)	85.2 ± 6.1	15.26 ± 0.68	0.179 ± 0.008	0.442 ± 0.028	34.7 ± 1.3
F. tinctoria (TI)	83.1 ± 4.0	14.48 ± 0.32	0.174 ± 0.004	0.281 ± 0.025	52.7 ± 4.2
H	**101.9 ± 15.1**	**13.63 ± 0.98**	**0.140 ± 0.018**	**0.315 ± 0.039**	**45.0 ± 3.3**
F. auriculata (AU)	67.4 ± 6.6	16.01 ± 0.93	0.238 ± 0.014	0.462 ± 0.037	34.8 ± 1.3
F. esquiroliana (ES)	48.7 ± 6.7	12.52 ± 0.61	0.257 ± 0.013	0.433 ± 0.063	30.0 ± 3.4
F. hispida (HI)	93.6 ± 5.3	13.09 ± 0.59	0.140 ± 0.006	0.366 ± 0.019	35.9 ± 1.4
F. racemosa (RA)	50.2 ± 7.1	16.57 ± 0.63	0.330 ± 0.013	0.477 ± 0.041	35.2 ± 1.8
F. semicordata (SE)	80.6 ± 5.3	12.64 ± 0.81	0.157 ± 0.010	0.368 ± 0.053	35.5 ± 4.1
NH	**68.1 ± 9.7**	**14.16 ± 0.98**	**0.224 ± 0.039**	**0.421 ± 0.026**	**34.3 ± 1.2**

Table 3.2 Dark respiration rate (R_d), photosynthetic light compensation point (LCP), photosynthetic photon flux density at 90% of maximum net assimilation rate and electron transport rate ($PPFD_{90\%A}$ and $PPFD_{90\%ETR}$), maximum net CO_2 assimilation rate (A_{max}) and maximum apparent electron transport rate (ETR_{max}) calculated from photosynthetic light response curves. Each datum is the mean value ± SE (n = 5–6). Abbreviations are as defined in Table 3.1.

	R_d* (μmol m^{-2} s^{-1})	LCP^{ns} (μmol m^{-2} s^{-1})	$PPFD_{90\%A}{}^{ns}$ (μmol m^{-2} s^{-1})	$PPFD_{90\%ETR}{}^{ns}$ (μmol m^{-2} s^{-1})	$A_{max}{}^{ns}$ (μmol m^{-2} s^{-1})	ETR_{max}* (μmol m^{-2} s^{-1})
BE	1.45 ± 0.21	26 ± 4	633 ± 49	635 ± 31	12.84 ± 1.08	136.9 ± 11.1
CO	2.04 ± 0.41	37 ± 7	984 ± 182	853 ± 68	15.36 ± 3.21	168.0 ± 19.2
CU	2.63 ± 0.84	55 ± 20	1230 ± 170	799 ± 47	16.56 ± 1.72	162.8 ± 11.6
RE	1.12 ± 0.04	21 ± 1	956 ± 156	695 ± 69	15.46 ± 1.32	130.9 ± 13.7
TI	1.42 ± 0.40	31 ± 10	823 ± 115	1069 ± 26	13.02 ± 2.89	135.3 ± 22.4
H	**1.73 ± 0.30**	**34 ± 7**	**925 ± 110**	**810 ± 84**	**14.65 ± 0.82**	**146.8 ± 8.6**
AU	1.23 ± 0.06	28 ± 4	627 ± 42	736 ± 71	12.87 ± 1.54	165.2 ± 17.8
ES	0.76 ± 0.26	18 ± 6	698 ± 71	844 ± 6	12.61 ± 1.06	152.3 ± 3.6
HI	0.85 ± 0.08	22 ± 3	803 ± 32	939 ± 88	13.05 ± 1.34	198.9 ± 20.3
RA	1.12 ± 0.12	24 ± 3	884 ± 71	954 ± 82	19.02 ± 2.12	193.1 ± 18.6
SE	1.40 ± 1.00	28 ± 1	760 ± 101	896 ± 57	15.65 ± 3.45	175.4 ± 21.5
NH	**1.06 ± 0.13**	**24 ± 2**	**754 ± 49**	**874 ± 44**	**14.64 ± 1.37**	**177.0 ± 9.6**

Table 3.3 Leaf N and P percentage contents (*N* and *P*), leaf N and P contents on area basis (N_a and P_a), and photosynthetic N and P use efficiencies (PNUE and PPUE) in the 10 studied *Ficus* species. Each datum is the mean value ± SE (n = 5). Abbreviations are as defined in Table 1.

	N* (%)	P^{ns} (%)	N_a** (mol m^{-2})	P_a* (mmol m^{-2})	PNUE* (μmol CO_2 s^{-1} mol^{-1} N)	PPUE* (mmol CO_2 s^{-1} mol^{-1} P)
BE	2.05 ± 0.10	0.111 ± 0.003	0.145 ± 0.007	3.56 ± 0.09	77.7 ± 10.2	3.17 ± 0.47
CO	2.53 ± 0.16	0.148 ± 0.008	0.158 ± 0.010	4.18 ± 0.21	74.4 ± 7.8	2.81 ± 0.29
CU	1.46 ± 0.08	0.083 ± 0.004	0.161 ± 0.009	4.16 ± 0.22	95.4 ± 4.6	3.70 ± 0.18
RE	2.44 ± 0.08	0.190 ± 0.015	0.148 ± 0.005	5.23 ± 0.42	102.8 ± 4.6	2.92 ± 0.13
TI	2.61 ± 0.13	0.235 ± 0.012	0.155 ± 0.008	6.30 ± 0.33	93.5 ± 2.0	2.30 ± 0.05
H	**2.22 ± 0.24**	**0.154 ± 0.030**	**0.153 ± 0.003**	**4.69 ± 0.54**	**88.7 ± 6.1**	**2.98 ± 0.25**
AU	1.86 ± 0.10	0.132 ± 0.012	0.089 ± 0.005	2.87 ± 0.26	179.1 ± 10.4	5.57 ± 0.32
ES	1.79 ± 0.14	0.200 ± 0.003	0.062 ± 0.005	3.14 ± 0.20	201.0 ± 9.8	3.99 ± 0.20
HI	2.11 ± 0.09	0.151 ± 0.013	0.141 ± 0.006	4.55 ± 0.38	92.9 ± 4.2	2.88 ± 0.13
RA	1.41 ± 0.06	0.144 ± 0.005	0.051 ± 0.002	2.33 ± 0.08	327.0 ± 12.5	7.12 ± 0.27
SE	1.78 ± 0.22	0.136 ± 0.007	0.102 ± 0.013	3.54 ± 0.19	123.6 ± 7.9	3.57 ± 0.23
NH	**1.79 ± 0.12**	**0.152 ± 0.013**	**0.089 ± 0.018**	**3.28 ± 0.42**	**184.7 ± 45.2**	**4.63 ± 0.86**

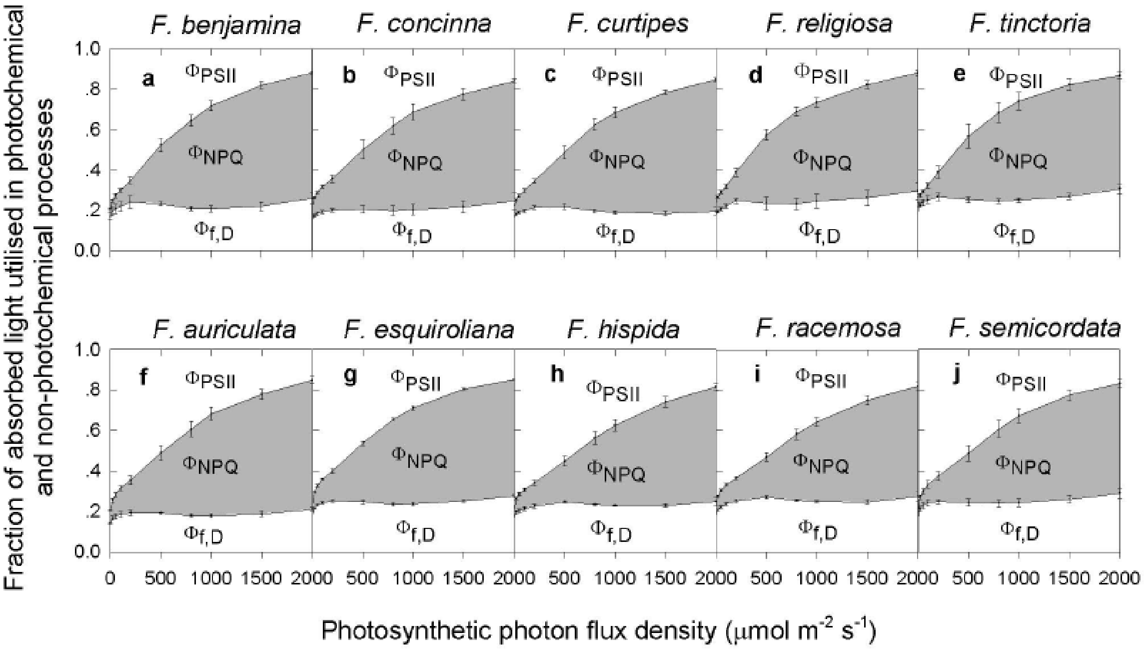

Figure 3.1 Estimated fraction of absorbed irradiance consumed via photochemistry (Φ_{PSII}), ΔpH- and xanthophyll-regulated thermal dissipation (Φ_{NPQ}), and the sum of fluorescence and constitutive thermal dissipation ($\Phi_{f,D}$) in five hemiepiphytic (a-e) and five non-hemiepiphytic (f-j) *Ficus* species. Each point is the mean ± SE of five to six leaves from different trees

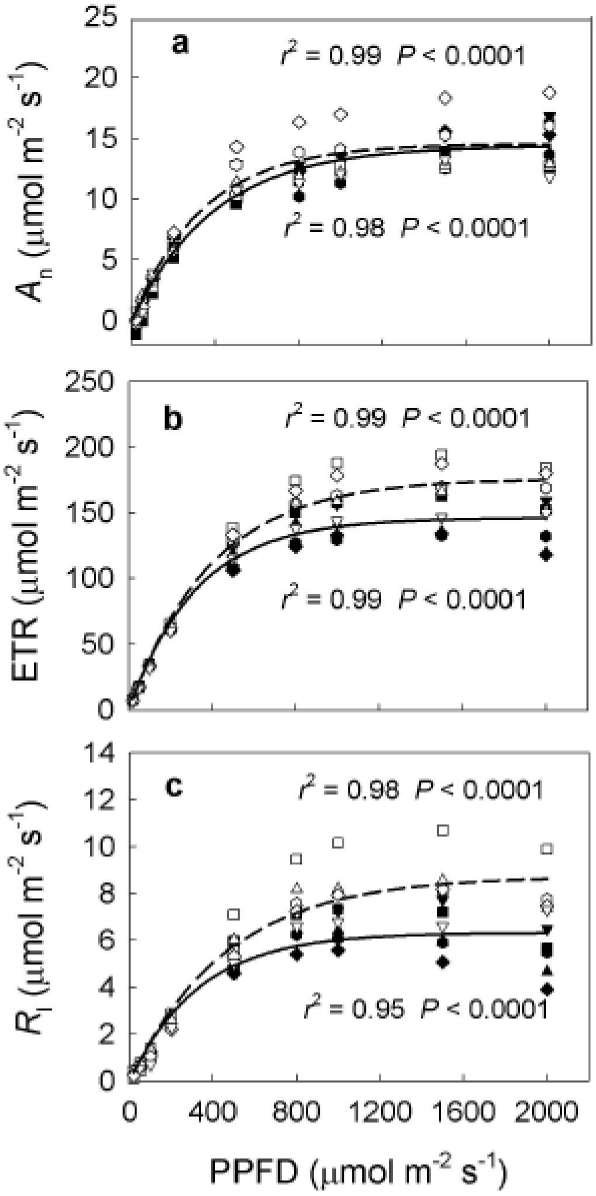

Figure 3.2 Response of (a) net CO_2 assimilation rate (A_n), (b) apparent electron transport rate (ETR), and (c) photorespiration rate (R_l) to photosynthetic photon flux density (PPFD) in five hemiepiphytic (filled symbols) and five non-hemiepiphytic (open symbols) *Ficus* species. Each point is the mean of five to six leaves from different trees of a species. Light response curves were fitted with the model $Y = Y_{max} - ae^{-bx}$ through mean values of five species within each growth form. *Ficus benjamina* (filled triangle up), *F. concinna* (filled triangle down), *F. curtipes* (filled square), *F. religosa* (filled diamond), *F. tinctoria* (filled hexagon), *F. auriculata* (open triangle up), *F. esquiroliana* (open triangle down), *F. hispida* (open square), *F. racemosa* (open diamond), *F. semicordata* (open hexagon)

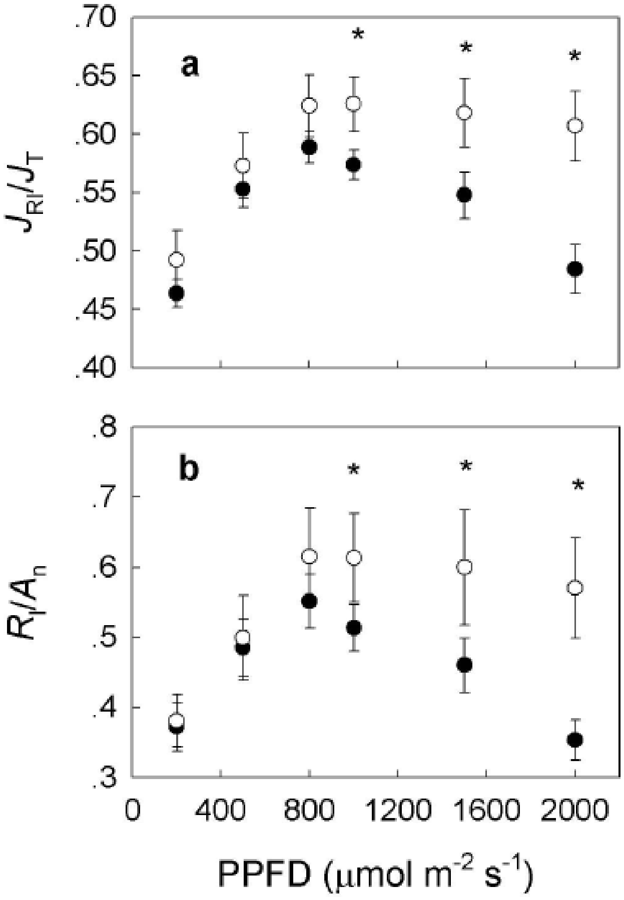

Figure 3.3 (a) Ratio of electron use attributable to photorespiration ($J_{Rl} = 12R_l$) to total electron flow ($J_T = 12R_l + 4A_n + 4R_d$) and (b) ratio of photorespiration to net CO_2 assimilation rate (R_l/A_n) at different levels of photosynthetic photon flux density (PPFD) for hemiepiphytic (filled circles) and non-hemiepiphytic (open circles) growth forms (mean ± SE; n = 5). R_d is dark respiration rate. "*" atop of data points indicate significant difference between the two growth forms of *Ficus* species ($P < 0.05$)

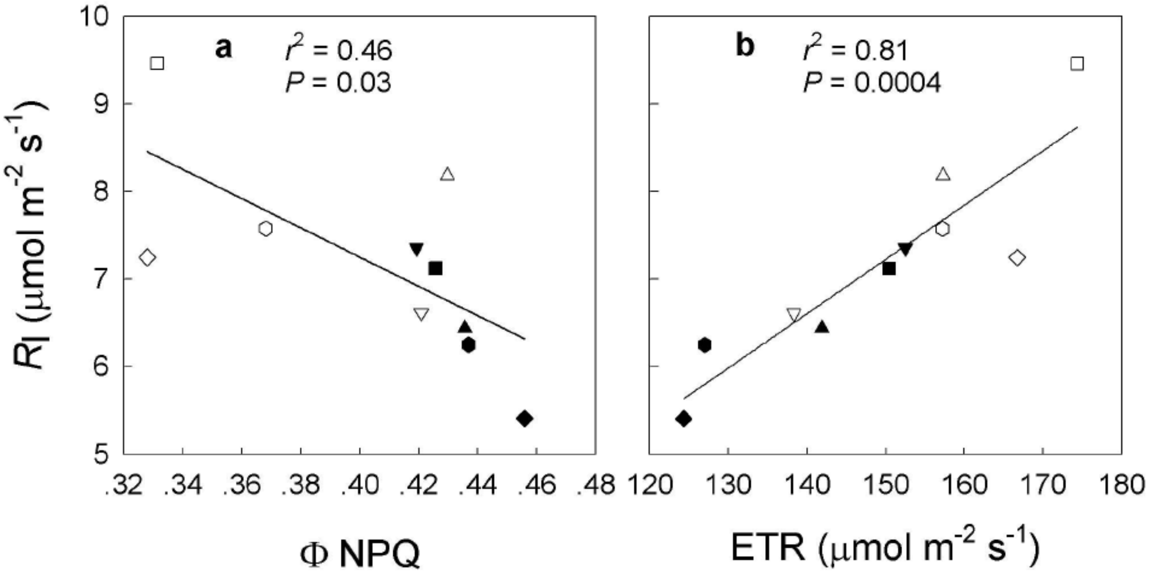

Figure 3.4 (a) Relationship between photorespiration rate (R_l) and efficiency of PSII thermal dissipation (Φ_{NPQ}) and (b) relationship between R_l and apparent electron transport rate (ETR) at PPFD of 800 μmol m^{-2} s^{-1} for 10 *Ficus* species. Symbols are as defined in Figure 3.2. Each point is the mean value of one species (n = 5–6)

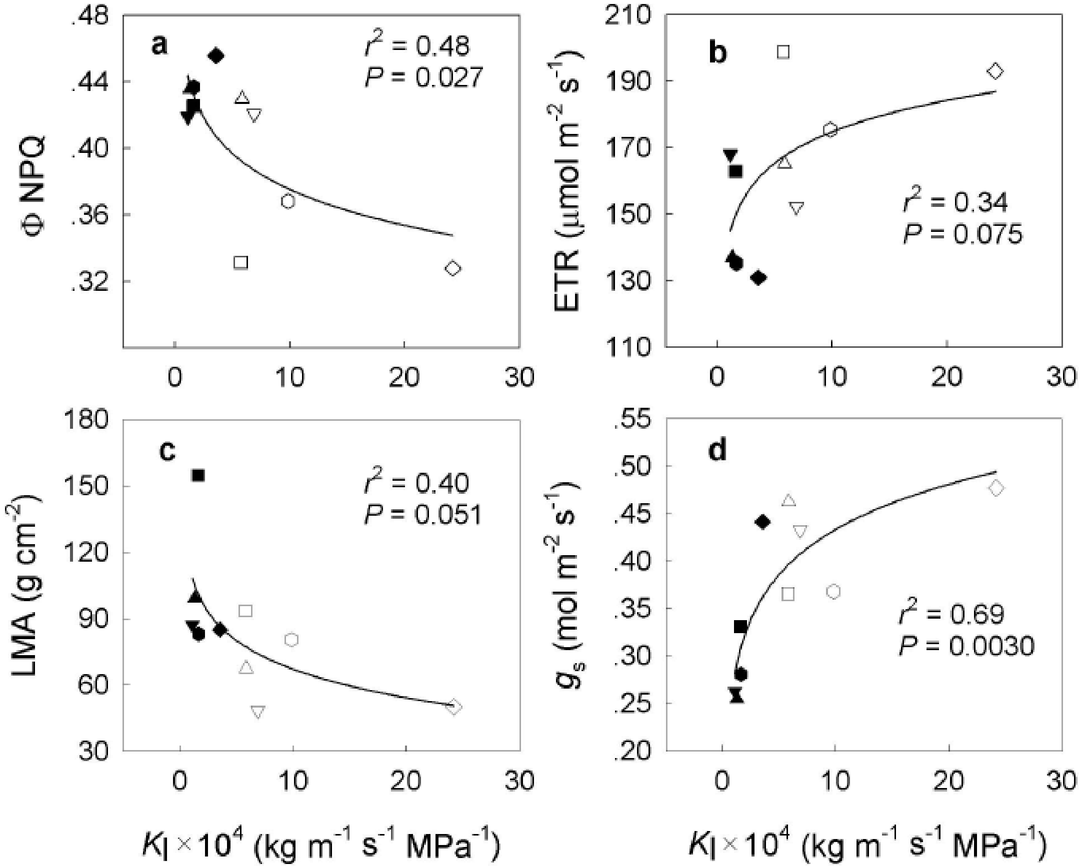

Figure 3.5 Relationship between leaf specific hydraulic conductivity (K_l) and (a) efficiency of PSII thermal dissipation (Φ_{NPQ}), (b) between apparent electron transport rate (ETR), (c) leaf dry mass per area (LMA), and (d) maximum stomatal conductance (g_s). ETR and Φ_{NPQ} were measured at a saturating PPFD of 800 µmol m^{-2} s^{-1}. Data were fitted with the logarithm functions in SigmaPlot 10.0. Symbols are as defined as in Figure 3.2.

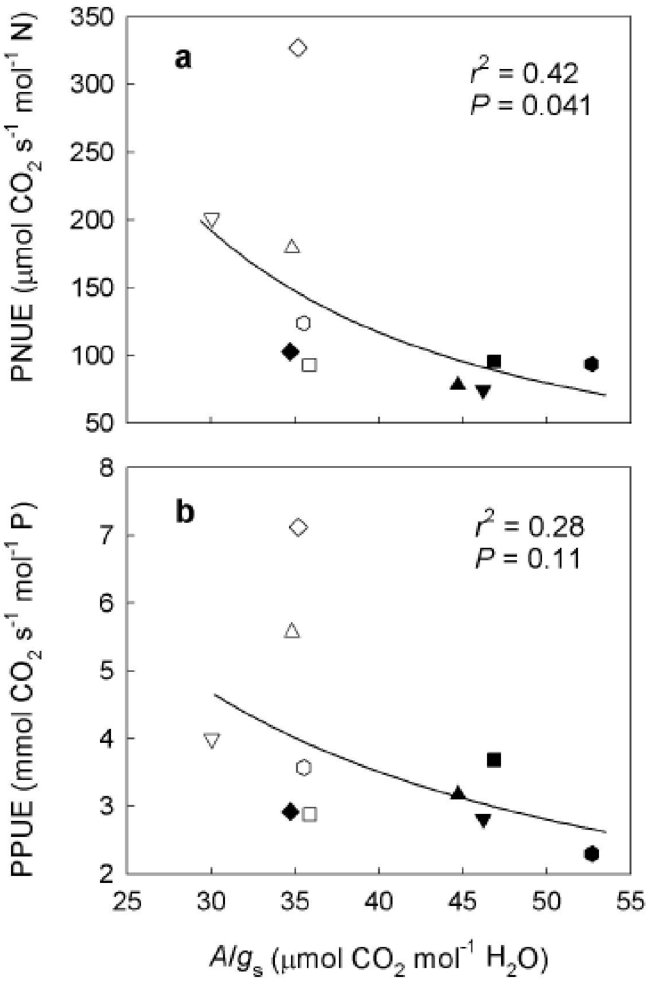

Figure 3.6 Relationship between photosynthetic (a) nitrogen, (b) phosphorous use efficiency (PNUE and PPUE) and intrinsic water use efficiency (A/g_s). Data were fitted with the model $Y = ax^{-b}$. Symbols are as defined in Figure 3.2

CHAPTER 4

COMPARISON BETWEEN GROWTH FORMS IN LIGHT ACCLIMATION AND DROUGHT TOLERANCE DURING THE JUVENILE STAGE

Summary

It has been suggested that the evolution of a hemiepiphytic growth habit in the early stage of their life cycle in some tropical plant species was an adaptation to avoid deep shade of the forest floor. Although commonly accepted, this hypothesis is not supported by data from ecophysiological investigations. Hemiepiphytic (H) and non-hemiepiphytic (NH) *Ficus* seedlings cultivated from seeds were grown under four different light levels in growth houses with irradiance ranging from full to 5% sunlight to study the light requirement for regeneration of the two growth forms. Results showed that seedlings of hemiepiphytic *Ficus* species had substantially lower growth rates than non-hemiepiphytic congeneric species under all light treatments. Despite the great differences in growth rate, both H and NH species exhibited considerably high plasticity in most functional traits in response to growth light level. In few other traits, both H and NH seedlings showed relatively low degree of plasticity, such as biomass allocation among leaf, stem and root tissues. NH species had significantly higher photosynthetic capacity than H species under all light treatments. Although not statistically significant, NH species tended to have higher light compensation points and light saturation points than H species, suggesting that they are slightly more light-demanding than H species. These results are contrary to

intuition considering the fact that H species regenerate in the canopy, which is commonly believed to be better lit than the forest floor. Although H species regenerate in the canopy, the micro-habitats at which they germinated may be shaded to a relatively large degree by host plants while NH species regenerate in forest gaps. Thus, it is possible that the two growth forms did not differ substantially in regenerating light levels or even the NH species experience higher light levels under natural conditions. The most distinct difference between both growth forms occurred in traits related to xylem water transport and leaf transpirational water use, which is consistent with findings in adult trees showing H species having smaller vessels, lower xylem hydraulic conductivity and more conservative water use. Our results suggest that in the genus *Ficus* the driving force for the evolution of epiphytism at the regeneration stage is likely to be factors other than light that affect the success of seedling establishment on the forest floor.

Introductory remarks

Solar radiation is a critical factor affecting reproduction, survival and growth of plant species in tropical and subtropical forests (Denslow and Hartshorn 1994; Fetcher et al. 1994; Chazdon et al. 1996). The incoming solar radiation is absorbed progressively in its passage through the different leaf layers of a forest. Thus light decreases exponentially from the top of the canopy to the forest understory (Larcher 2003) and even leaves relatively close to the upper canopy can receive substantially lower irradiance relative to full sunlight. In dense tropical forest the sunlight reaching the forest understory can be as low as 1% (Chazdon 1988; Clark et al. 1996). Light also varies considerably in forests horizontally with gaps receiving higher irradiance than the forest understory; tropical forests have canopy openings receiving 2–5% of full sun and few large gaps with more than 5% of total solar radiation (Nicotra et al. 1999). Seedlings of shade tolerant tropical tree species can grow under very low irradiance in the understory where pioneer species can not survive (Denslow 1980; Tuomela et al. 1996; Myers et al. 2000). In relatively large gaps, pioneer or light-demanding tree species with higher growth rates and ecophysiological plasticity have competitive advantages over shade tolerant tree species (Denslow 1980).

Most ecophysiological researches on hemiepiphytic plants focused on plant water relations (e.g. Holbrook and Putz 1996a,b) but the ecophysiology of hemiepiphytic plants in terms of light adaptation during the regenerating stage (or the first stage of the life cycle after germination) remain largely unknown. It has been suggested that the hemiepiphytic habit evolved as an adaptation to access high light environments in the forest canopy (Dobzhansky and Murea-Pires 1954; Ramirez 1977; Putz and Holbrook 1986; Todzia 1986; Laman 1995; Williams-Linera and Lawton 1995). However, by living in the canopy hemiepiphytes at the canopy growth stage are likely to experience severe water deficits especially during the dry season in areas with strong seasonality in rainfall (Benzing 1984; Sinclair 1984). Thus it has been suggested that the suitability of hemiepiphytic *Ficus* species establishment sites occurs along antagonistic gradients of water and light availability (Laman 1995; Benzing 1990; Coxson and Nadkarni 1995; Holbrook and Putz 1996a,b; Swagel et al. 1997). Strangler *Ficus* seedlings show a strong growth response to higher light levels when water and nutrients are abundant (Laman 1995), which should lead to a trade-off between access to light and consistent and adequate water supply. However, whether seedlings of closely related non-hemiepiphytic species also respond to light levels in a similar manner is not known. Whether in nature the light levels for hemiepiphytic and non-hemiepiphytic *Ficus* species regeneration sites are substantially different, and thus substantial differences in adaption to light levels at the juvenile stage between H and NH growth forms do exist are uncertain.

It is thus far from conclusive that light is the main driving force for the evolution of the commonly found hemiepiphytism in *Ficus*. Besides light, other factors may be responsible for the evolution of canopy growth at the beginning of their life cycle. For example, by regenerating in the canopy these plants may benefit from minimizing the risk of fire, flooding, damage by terrestrial herbivores and coverage by falling debris (Holbrook and Putz 1996a).

Using adult trees grown in a common garden, our previous studies found substantial differentiation in light and water related functional traits between H and NH species (Hao et al. 2010a,b). But knowledge in plant responses to environmental conditions at the seedling and small sapling stage is likely to be more important in interpreting the differences in regeneration requirements between the two growth forms, which will contribute to understand the evolution of hemiepiphytism. Also it is more likely that greater differences between the two growth forms exist at the juvenile stage, during which the largest differences in environmental factors are experienced by H and NH species under natural conditions. The first goal of the present study was to examine whether there are intrinsic differences between H and

NH species in acclimating to different growth light levels during seedling and small sapling stages. The second goal of the study was to investigate leaf functional traits related to xylem water transport and gas exchange in small saplings of H and NH species grown under similar conditions with sufficient soil water supply. We hypothesized that seedlings of H species will have traits of more conservative water use than NH species even under good soil water availability. Whether their regenerating light requirement is different from NH species, the initial epiphytic growth in H species involves more frequent and severe water deficits, which may have acted as a selective pressure for more conservative water use in H species during the juvenile stage.

Materials and Methods

Seedling growth

Seeds of six *Ficus* species used in the present study were collected from several individuals (n = 4-6) during the year of 2007 at the Xishuangbanna Tropical Botanical Garden (XTBG) of the Chinese Academy of Sciences. In January of 2008, seeds of the six *Ficus* species were placed in Petri dishes for germination in a growth chamber with a fluorescent light source. Temperature of the growth chamber was controlled at 25°C. After the seeds germinated and seedlings grew to about 1 cm, they were transplanted to 7L pots containing a mixture of 2/3 forest surface soil and 1/3 river sand. Seedlings of each species were placed in pots at the same density to avoid the effects of different densities on seedling growth. The nursery was partially shaded using neutral-density shade cloths with about 20% of the solar radiation.

On June of 2008, 10 seedlings of each species were randomly selected from the nursery and harvested to determine seedling height and total biomass. Then 80 seedlings with medium sizes for each species were transplanted to 7L individual pots and 20 pots of each species were randomly placed into one of the four growth houses (11m × 5.5m × 2.5m; length × width × height). Different degrees of shade in each growth house were obtained by coving the growth houses using neutral-density shade cloths with different densities and different layers and the full sunlight treatment growth house was not covered. There were no trees or buildings in close proximity to the growth houses and the four shadehouses were relatively far away apart to avoid shading to one another. The PPFDs of the fully exposed growth house and the three shaded growth houses were measured multiple times periodically during clear days from the beginning through the end of the experiment using three LI-190 SA quantum sensors (1 placed in the fully exposed site and two in different locations of a shaded growth house) connected to a LI-1400 data logger (Li-Cor, Lincoln, NE, USA). The average PPFD relative to the sunlight for each of the four growth houses were maintained relatively constant around 100%, 50%, 25% and 5% of full sunlight, respectively (total PPFD per day measured on consecutive clear days of March 2009 were 29.9, 13.8, 7.6, and 1.7 mol m^{-2} day^{-1}, respectively).

All plants were watered to full saturation until water started draining every other day on days without rainfall. Seedlings were treated every two months with N, P, K slow-release fertilizer. Plants within each growth house were relocated every month to minimize the effect of light level variation within the growth houses. Every three to four weeks, the heights of all the plants were measured.

Leaf gas exchange measurements

Light saturated net CO_2 assimilation rate (A_a) and stomatal conductance (g_s) were measured (in three leaves per individual and five individuals per species) under each light treatment during midmorning on four consecutive clear days at the beginning of March 2009, using a portable infrared gas analyzer (LI-6400, Li-Cor). Besides measurements of A_a and g_s, light response curves of CO_2 net assimilation rate (A_n) were conducted during the dry season of 2009 from March to April (three to five curves per species under all light treatments). All light response measurements were done between 0830 and 1130 hours during clear days. The PPFD varied from 1800 μmol m^{-2} s^{-1} to 0 μmol m^{-2} s^{-1} in a decreasing step-wise manner (1800, 1500, 1200, 1000, 800, 600, 400, 200, 100, 50, 10, 0). The leaf cuvette temperature was set at 30ºC. At each irradiance level, measurements were taken after CO_2 readings stabilized, which was usually achieved in about 2 to 4 minutes.

Light response curves were fitted with the algorithm $Y = Y_{\max} - ae^{-bx}$ using Sigmaplot 10.0 (Systat Software, Inc., San Jose, CA, USA), where Y is A_n, x is PPFD and a×b is the initial slope of the light response curve (Iqbal et al. 1996). Y^{max} is the maximum value of A_n. The PPFD level leading to 90% saturation of A_n was taken as light saturation point (LSP). Light compensation point (LCP) was calculated by fitting a linear regression to the relationship between A_n and PPFD at low levels of

PPFD between 50 and 0 μmol m^{-2} s^{-1}. The quantum yield for CO_2 assimilation (α_{JCO2}) was calculated as the initial slope of the light response curves.

Biomass allocation

At the end of April 2009, 10 saplings per species under each light treatment were randomly selected. Seedling height (H) was measured for each of the selected saplings. Soil of the rhizosphere was washed out carefully using tap water avoiding breakage of fine roots, then each plant was divided into leaves, stems, and roots. Total leaf area (LA) of each harvested plant was measured using a leaf area meter (LI-3000A, Li-Cor). Further, for each plant new leaves that were fully expanded were selected for calculating average leaf size (LS) and were bagged separately for later use in leaf chemistry analysis. All leaves, stems and roots of the saplings were oven-dried for 48 hours and then dry mass of each plant compartment was determined.

From the above measurements, we calculated leaf area ratio (LAR; total leaf area/total seedling biomass), leaf mass per area (LMA; total leaf dry mass avoiding major veins/total leaf area), leaf mass fraction (LMF; total leaf dry mass/total seedling biomass), stem mass fraction (SMF; stem dry mass/total seedling biomass), root mass fraction (RMF; root dry mass/total seedling biomass), root to shoot ratio (R/S; root dry mass/shoot dry mass). To determine the sapling height growth relative to aboveground biomass, we also calculated the height to aboveground biomass ratio (HMR).

Leaf petiole xylem anatomy

Leaves of each species were collected in the wet season of 2008, by sampling six seedlings per species grown under each light condition. Only one newly matured, healthy, and fully expanded leaf was selected per sampled seedlings to minimize the effect of sampling on plant growth. After being cut from the seedling, leaves were sealed in plastic bags and transported to the laboratory. Leaf area for each leaf was measured using a leaf area meter, cross sections were made from the petiole immediately below the lamina insertion point. Based on the measurement of vessel lumen diameter and vessel numbers, leaf area normalized theoretical axial hydraulic conductivity of the petiole ($K_{t,}$ mmol m^{-1} s^{-1} MPa^{-1}) was calculated. For details about the measurements and calculation please see Chapter 2 Material and Methods.

Leaf chemistry analysis

After the measurements of tissue dry mass, the newly matured leaves (separated before oven-drying) of each species for each of the four light treatments were ground and sent to the Biogeochemistry Laboratory of XTBG for chemistry analysis. Leaf C and N concentration was determined with an auto Kjeldahl unit (K370, BÜCHI Labortechnik AG, Flawil, Switzerland). Total P and K contents were analyzed using an inductively coupled plasma atomic-emission spectrometer (IRIS Advantage-ER, Thermo Jarrell Ash Corporation, Massachusetts, USA) after samples were digested with concentrated HNO_3-$HClO_4$. The mass-based net assimilation rate (A_m), photosynthetic nitrogen use efficiency (PNUE), and photosynthetic phosphorous use efficiency (PPUE) were calculated by dividing A_a with LMA, leaf N and P concentrations, respectively.

Statistics

For each trait measured (Table 4.1), an arithmetic average was calculated per species grown under each of the four light treatments. Data were log-transformed to improve normality and homoscedasticity prior to analysis (c.f. Markesteijn et al. 2007). To test the effect of growth form, species and light level on all the plant traits measured, data were analyzed using a three-way nested ANOVA with growth form and light level as fixed factors, and species as a random factor nested within the factor of growth form. A plasticity index of each trait in response to growth light level variation was calculated as the difference between the largest average value under one light treatment and the smallest average value under the other light treatment, divided by the largest average value, and multiplied by 100% (Valladares et al. 2000). All statistical analyses were performed with SPSS 15.0.1 (SPSS Inc. Chicago, USA).

Results

Light dependent trait variation and trait plasticity

Most of traits were strongly affected by growth light levels as indicated by relatively high plasticity indexes, and species of H and NH growth forms exhibited similar plasticity in all traits in responses to variation in growth light levels; traits that have higher plasticity in H species also have higher plasticity in NH species (Table 4.2). The three-way nested ANOVA results showed that growth light level had significant effects on most traits measured (Table 4.3). Under lower light levels, all species tended to have lower leaf xylem hydraulic conductivity, g_s, LSP, and A_{max}, resulting in a lower growth rates and lower water use efficiency. When growth irradiance was low, saplings of all species tended to have lower $R_{d\text{-area}}$, LCP, and higher PNUE, indicating physiological acclimation to shaded environments. Further, leaf morphology was strongly affected by light. Under lower light levels, all species tended to have larger leaf size, higher LAR, and lower LMA. One exception was that under the 5% light treatment H species on average had smaller leaves than under 50% and 25% light treatments, which were caused by the very slow growth rate and the overall small size of *F. virens* seedlings under the 5% light treatment (Fig. 4.1a).

The final sapling biomass was significantly affected by growth light level. Most species had the largest biomass under full sunlight expect *F. racemosa* and *F. semicordata* that had the largest biomass under the intermediate (50%) light treatment (Fig. 4.1 a). However, in all species final plant height was similar under all light levels except in one species, *F. virens*, whose height was much lower than for any other species under the three partially shaded growth houses. At lower light levels all species tended to reach a higher height per unit aboveground biomass (Fig. 4.1b).

Comparison in growth rates between growth forms

The initial growth rates of H and NH species in the nursery for the first four months showed significant differences (Fig. 4.3); NH seedlings had 3.3 and 13.3 times higher height and biomass, respectively, than seedlings of H species sowed on the same day. At the end of the experiment, the biomass of H species was also significantly lower than that of NH species under all of the four light treatments (Table 4.2). However, after the seedlings were transplanted to individual pots H species showed higher relative growth rates and height relative growth rates (Fig. 4.2a,b) and at the harvest time there was no statistical difference in seedling height between H and NH species, but H species still showed significantly lower plant biomass (Fig. 4.1a) due to their much smaller initial sizes than NH species. H species had an overall higher height to biomass ratio than NH species (Fig. 4.1b).

Comparison in morphology and biomass allocation between growth forms

Most traits related to morphology and biomass allocation were not significantly different between the two growth forms (three-way nested ANOVA; Table 4.3). Biomass allocation between photosynthetic tissues (leaves) and non-photosynthetic tissues (stem and roots) were not significantly different between growth forms, although H species tended to have higher LMF, RMF, R/S and lower SMF. However, H species did show significantly higher HMR than NH species under all light conditions showing that H species tended to reach higher height with the same amount of aboveground biomass (Table 4.2 and 4.3). Under 100%, 50% and 25% sunlight treatments, HMR did not show large differences, but at the 5% sunlight treatment, HMR dramatically increased to significantly higher values in all species

(Fig. 4.1b). At the end of the experiment, under the same growth light levels the average basal stem diameter was on average three times larger in NH species compared to H species.

Differences in gas exchange between growth forms

Results of gas exchange measurements during mid-morning indicated that H species had consistently lower A_m, g_s, and higher WUE but lower PNUE under all light conditions (Table 4.2), which were consistent to patterns found in adult trees grown in a common garden (Hao et al. 2010a). Area based net CO_2 assimilation rate was also found to be higher in NH species under all light conditions, contrary to observations in adult trees.

Photosynthetic light responses

A_{max} and LSP were significantly higher in NH species (three-way nested ANOVA; Table 4.3). LCP and α_{JCO2} were not statistically different between growth forms, but under the three partially shaded light treatments they tended to be higher in NH species, while under the full sunlight treatment both parameters were similar between growth forms (Table 4.2). Light had a larger effect on photosynthetic light responses than the other two factors (growth form and species) and consistent changes in leaf photosynthetic light curves were found in all the studied species along the irradiance gradient (Fig. 4.4a-d). Generally, under shaded growth conditions, light saturated photosynthetic CO_2 assimilation was lower and reached saturation at lower PPFDs (Table 4.2). Under full sun light, all NH species exhibited high photosynthetic capacity than H species, while under partially shaded conditions overlaps in photosynthetic capacity were always observed between H and NH growth forms.

Diurnal courses of gas exchange

The diurnal gas exchange showed that in H species A_n reached to a maximum around 11:00AM and declined gradually thereafter towards the end of the day (Fig. 4.5c). In NH species different patterns were found. A_n reached a maximum at approximately the same time in the morning, but unlike the H species there was a second peak of A_n in the afternoon after a relatively small midday depression (Fig. 4.5d). Although the daily maximum values of A_n were not significantly different between H and NH species, the cumulative CO_2 assimilation (area below the diurnal curve) during a day in NH species was 46.5% higher than that of H species.

Responses of CO_2 net assimilation to the water withholding treatment

Following water withholding of well irrigated plants, A_n of H and NH species declined in a similar fashion although one H species (*F. virens*) exhibited a smaller decrease in A_n than other species (Fig 4.6 and Fig. 4.7). However, H and NH species had different rates of recovery from the A_n depression caused by drought stress. In NH species, it took a longer time to recover to maximum values similar to those before the drought treatment. More importantly, drought treatment did affect more strongly the NH saplings under both high (100% sunlight) and low light (5% sunlight) treatments in terms of leaf loss (Fig. 4.8). Under high and low light conditions, NH species on average lost 30% and 70% of their leaves during the drought treatment, respectively. While in H species, no obvious leaf shedding happened during the drought treatment except for a 10% leaf loss in *F. tinctoria* grown under full sunlight treatment (Fig. 4.8).

Functional convergences

Important functional convergences found in adult trees were also found in seedlings across species despite plants being grown under different light levels. The positive correlations between K_t and leaf gas exchange traits (g_s and A_a) were also significant in juvenile plants (Fig. 4.9a,b). Although the negative correlation between K_t and WUE was not statistically different, the tendency that H had higher WUE than NH species was evident from the inspection of the scatter diagram (Fig. 4.9c). Also, K_t and final plant biomass at harvest showed a significant positive correlation (Fig. 4.9d). LMA was highly correlated to leaf gas exchange traits (Fig. 4.10a,b). Further, LMA was found to scale with LAR and growth (Fig. 4.10c,d). More interestingly, correlations between LMA and these traits (A_a, PPUE and BIOM) had similar slopes but different intercepts for the two growth forms ($P < 0.05$; ANCOVA); NH species had higher A_a, PNUE and BIOM than H species at a given LMA (Fig. 4.10a,b,d).

Contrasts between growth forms in juvenile and adult trees

Our previous investigation showed that in adult trees most leaf functional traits related to gas exchange and carbon economy were significantly different between H and NH species (Table 4.4). For the controlled experiments on juvenile plants the number of species included in this study was smaller compared to studies on adult trees (6 vs. 14 species), but still significant differences were found between H and NH species in traits related to leaf xylem hydraulic conductivity and leaf gas exchange. Under all light levels, H species had lower K_t, A_m, g_s, and higher WUE than NH species, which showed the same patterns in comparisons between the two growth forms using adult trees (Table 4.4). Also, A_a and A_{max} were higher under all light treatments in NH species, although in adult trees they were not significantly different between the two growth forms.

Discussion

In the six studied *Ficus* species, light had a large effect in shaping most plant functional traits, including physiological and morphological traits, growth, and biomass allocation. All these traits responded in a similar manner to growth light levels in H and NH species; traits that had higher plasticity in H species also had higher plasticity in NH species. It has been suggested that traits have higher plastic response as a function of growth irradiance are important for plant functioning in different light environments (Bongers and Popma 1988), while traits that showed a small degree of plasticity with respect to light may not have an important adaptive value for plants in tropical forests where light changes dramatically in both vertical and horizontal directions.

Morphology, xylem water transport and water use in response to light treatment

In shaded environments when light becomes a limiting resource plants enhance their light interception by allocating more biomass to leaves (higher LMR) and producing thinner leaves with lower LMA (Popma and Bongers 1988; Osunkoya et al. 1994; Poorter 1999). In both H and NH species, LMA decreased with the decrease in growth light level and both growth forms showed relatively high plasticity, indicating that LMA is important for plants in adjusting to different light levels.

At high light, plants in general reduce transpirational water loss by producing small leaves with thinner boundary layers, which can better avoid over heating through better convective cooling (Parkhurst and Loucks 1972; Givnish 1984). In all the studied *Ficus* species, leaf size significantly decreased from low to high growth light levels. Further, NH species tended to have larger leaves than H species under all light conditions. Under high light conditions, large leaves in NH species may require higher transpiration for leaf cooling, which was consistent with the finding that NH species had significantly higher g_s compared to H species. Under low light conditions, such as the 5% light treatment, leaf overheating by strong irradiance may not be relevant, but NH species also showed substantially higher g_s indicating that higher transpiration rates have other functions besides latent heat dissipation. One possibility is that higher g_s was related to higher net CO_2 assimilation rates, which might be crucial for the fast growth of NH seedlings in forest gaps as in other light-demanding species (Kubiske et al. 1996).

High light conditions are often accompanied by high leaf temperatures that result in high leaf-to-air vapor pressure deficits (VPD) and higher transpiration demand. Higher K_t was always found under higher light conditions in all the studied *Ficus* species indicating the acclimation of leaf vascular system to different irradiances. The higher WUE in higher light levels in both H and NH species may indicate that under high irradiance, increasing WUE is more important for avoiding leaf wilting and to increase net carbon gain rather than merely increasing net assimilation rates (Hanba et al. 2002). Furthermore, H species had significantly higher WUE than NH species under all light conditions even when all species were provided with sufficient soil water, which suggests genetically based differences in leaf water use as an adaptation to prevailing water conditions in natural regeneration habitats.

Light response curves and shade tolerance

Leaves expanded in the shade tend to have higher apparent quantum yield, lower respiration rates, light-saturated photosynthetic rates, light compensation and saturation points than leaves expanded in high light environments (Langenheim et al. 1984; Oberbauer and Strain 1985; Davies 1998). Although A_a and $R_{d\text{-area}}$ were significantly higher under high than under low light treatments, due to the high LMA plasticity, $R_{d\text{-mass}}$ was not found significantly different among light treatments. Even higher A_m were found under low light levels as found in other studies and was likely due to a lower ratio of structural to photosynthetic leaf tissue (Strauss-Debenedetti and Berlyn 1994) and a greater leaf surface area for CO_2 diffusion in plants grown under lower light levels (Field and Mooney 1986; Reich and Walters 1994).

The light-demanding and shade-tolerant species sometimes show contrasting photosynthetic response to light. When grow in high light, $A_{\max}$ of light-demanding species are generally higher than those of shade-tolerant species (Kozlowski and Pallardy 1997). Under the highest light treatment, NH species had higher $A_{\max}$ than H species but $A_{\max}$ of H species was also relatively high and comparable or even higher than seedlings of light-demanding pioneer species (e.g. Oberbauer and Strain 1984; Turnbull 1991; Davies 1998). It has also been found that early successional or pioneer species have high plasticity in ecophysiological and morphological traits in response to growth light level variation (Bazzaz and Pickett 1980). The high degree of plasticity in response to light levels, further suggests that both H and NH *Ficus* species have ecophysiological traits similar to pioneer or light-demanding species. There seems to be little difference in regeneration light requirements or acclimation to light levels between the two growth forms. The higher $A_{\max}$ in NH species than H species probably represents an adaptation to drought-prone canopy environment rather as an adaptation to differences in light levels.

Growth rate and biomass allocation

Under all light treatments NH species always grew faster than H species. This finding was consistent with results from other studies. For example, using 15 rainforest species Poorter (1999) found that fast growing species in high light treatments were also fast growing under low light treatments and that the light-demanding species always grow better than shade tolerant species. Fast-growing species have certain characteristics that enable them to outgrow other species, such as high LAR in low-light environments and high net assimilation rate in high-light environments (Poorter 1999). In the present study, on the other hand, it seems that the differences in growth rates between H and NH species depends more on traits related to leaf physiological traits (hydraulic conductivity and gas exchange) than on whole plant biomass allocation. Fast growth for NH seedlings regenerating in gaps might be crucial to compete for light resources with other species. It has been found that the larger the seedling is in a forest gap the more likely that it will survive and eventually reach the canopy (Brown and Whitmore 1992; Boot 1996; Zagt and Werger 1998). For H seedlings, survival through the harsh abiotic environment experienced at the epiphytic stage is probably more important than competition for limited resources between species. It has been found that seedlings of hemiepiphytes have low survival rates and very low densities in the forest canopy and only very few of the potential epiphytic habitats are occupied (Corner 1940; Laman 1995). Thus low competition among hemiepiphytic species is expected. More importantly, between *Ficus* tree species of the two growth forms, differences in light requirements may play a minor role in affecting their differences in growth rates as indicated by relatively small differences in light responses between H and NH species. Other environmental factors, such as water deficit during juvenile stage of H species, might exert a large selective pressure for conservative water use to increase the ability to survive under drought (Hao et al. 2010a), but at the cost of low growth rates.

Biomass allocation between aboveground and belowground plant tissues is important for determining the shade tolerance of plants. Species that are more shade-tolerant are expected to have higher biomass allocation to leaves and will gain maximum light and CO_2 capture by having a larger leaf area (Poorter 1999; Poorter 2001;Markesteijn 2007). Plants in the shade also tend to have a higher stem length per unit stem biomass thus increasing height growth to escape from low-light environment (Sasaki and Mori 1981). It has been suggested that such a plastic response is restricted to light demanding species regenerating in short-stature gap vegetation (Bazzaz 1979; Bazzaz and Pickett 1980; Bazzaz and Wayne 1994). All the studied *Ficus* specie showed a high degree of plasticity in stem height growth with HMR becoming substantially higher in low light levels (Fig. 4.1b), further indicating that all the *Ficus* species (both H and NH species) are relatively light demanding. H species showed a substantially higher HMR than NH species under all light treatments. However, this may not occur as an adaptation to light, but may be related to differences in the requirement of physical support for stems. The H species use host trees for support especially at the early stages of the life cycle and thus thick and strong stems or branches are selected against during evolution since they are costly. In NH species with gap regeneration, faster height growth and stronger stem physical strength have a positive adaptive value compared to H species.

Comparison in regenerating light requirements

Avoidance of shaded environments in the forest floor was hypothesized to be the main driving force for the evolution of epiphytic habit during the juvenile stage in hemiepiphytes (Dobzhansky and Murea-Pires 1954; Ramirez 1977; Putz and Holbrook 1986; Todzia 1986; Laman 1995; Williams-Linera and Lawton 1995). Following that logic one would expect seedlings of H species to be less shade-tolerant or more light-demanding than congeneric NH species. However, our results showed that both of the two growth forms grow better under high light levels. The NH species even showed characteristics of more typical light-demanding species, such as high photosynthetic capacity under high light treatments (Walter et al. 1993; Kozlowski and Pallardy 1997; Kital et al. 2000). H species possess some traits of pioneer species, but their unique life history with an epiphytic growth stage at the early stages of the life cycle selected for water conservative use and drought tolerance (Holbrook and Putz 1996a,b).

The small differentiation in growth and physiology in response to light levels between growth forms may be caused by phylogenetic inertia because we are comparing species of the same genus (*Ficus*), which in general possess typical traits of pioneer species—small seeds, high assimilation and growth rates, etc. (Harrison 2005). The NH seedlings are not found to grow in deep shade in the forest floor but rather are found in forest gaps with relatively high light level (personal observation); H seedlings and saplings grow on the host trees above the forest floor, and are usually partially shaded by the host canopy before they gain a substantially large size and extrude above the host tree canopy. In nature, the light conditions for *Ficus* species of the two growth forms may not be significantly different and thus substantial differences in response to light level were not evolved. Although not many differences in responses to light level were observed, differences in drought tolerance related to life history traits may still result in great divergences between H and NH species. H and NH species were well separated by traits related to xylem water transport and leaf water use (Fig. 4.9a-d). While lack of differences in traits and behavior may be caused by phylogenetic inertia as we are using closely related species within the same genus, differences in water transport efficiency between both growth forms strongly suggest that the differences are the results of distinct selective pressures imposed by the environment.

Leaf water use strategy and drought tolerance

The daily maximum A_a was higher in NH species than H species and an even larger difference was seen when the diurnal course of A_a was compared between the two growth forms. The differences in diurnal course of A_a between the two growth forms was probably a consequence of the tighter stomatal control of leaf water loss in H species. Epiphytic stage H species under natural conditions were found to only open their stomata during the early morning in the dry season (Holbrook and Putz 1996a). In the present study, even when they were well watered, still low stomatal conductances were observed and the active CO_2 assimilation periods were much shorter in H than in NH species. The stronger stomatal control in H species is likely related to their intrinsically low xylem hydraulic conductivity. By having a tighter stomatal control net assimilation rates were also lower, which is consistent with the hypothesized trade-off between hydraulic conductivity and resistance to hydraulic failure (Martinez-Vilalta et al. 2002). The commonly found leaf diebacks in NH species during drought treatment indicated that they are less drought tolerant than H species and their luxurious water use can only be beneficial when soil water is sufficient. Conservative water use of H species, although resulting in intrinsic low assimilation and growth rates, may enables them to survive in the drought-stressed environments typical of epiphytic microhabitats.

Comparison among ontogenetic stages

For the first few months after germination, seedlings of H species in the nursery had extremely low growth rates in both height and biomass. But after a few months, the H species showed a higher height growth than NH species and at the end of the experiment no significant differences were found in height between the two growth forms, although NH were still significantly larger in biomass than H species. It is possible that the largest difference between H and NH species exists at the very early stage of life cycle. Low growth rate of the seedlings after germination may be related to lower survival rates due to all kinds of biotic and abiotic constraints if grown on the forest floor (Holbrook and Putz 1996a). For example, a very small seedling with low growth rates may be easily covered by a single leaf and die. Avoiding these potential damages rather than shade-intolerance may be one of the selective pressures for the evolution of hemiepiphytism in *Ficus*.

When comparisons were made between small sapling and adult stages, most consistent patterns were found in traits related to water transport and use while other differences were not as significant as in adult trees (Table 4.4). Even with a much smaller sample size, H species still showed lower K_t, A_a, A_m, g_s, A_{max} and WUE, which were all consistent to findings in adult trees, indicating intrinsically more conservative water use and growth in hemiepiphytic species during the juvenile stage.

Functional convergences

Despite seedlings being grown under large differences of irradiance, significant functional convergences were still found using pooled data. Stomatal conductance, net CO_2 assimilation rate, and final plant biomass were all positively correlated with xylem hydraulic conductivity, indicating that xylem hydraulic conductivity capacity has an overall controlling effect on plant's performance. The scaling between LMA and other functional traits may due to the high plasticity of the LMA in response to growth light conditions, while change of LMA strongly affects leaf gas exchange, carbon economy and plant growth.

Conclusions

Different from predictions based on commonly accepted hypotheses, seedlings of hemiepiphytic and non-hemiepiphytic *Ficus* species did not exhibit much difference in adaptations to different light levels suggesting that factors, other than light, may be the driving force for the evolution of hemiepiphytism in *Ficus*. On the other hand, seedlings of the two growth forms did differ in water transport and leaf water use in a similar manner as in adult trees. No matter whether there are differences in light levels between natural habitats of H and NH species, water deficit is certainly more frequent and severe to H seedlings than NH seedlings. Seedlings of H species showed consistently lower xylem hydraulic conductivity and more conservative water use, which is an adaptation to survive the drought prone canopy growth environment.

Table 4.1 Plant traits included in the study and the abbreviations used.

Traits	Abbreviations	Units
Theoretical axial hydraulic conductivity of petioles	K_t	mmol m^{-1} s^{-1} MPa^{-1}
Net CO_2 assimilation rate on area basis	A_a	μmol m^{-2} s^{-1}
Net CO_2 assimilation rate on mass basis	A_m	μmol g^{-1} s^{-1}
Dark respiration rate on area basis	$R_{d\text{-}area}$	μmol m^{-2} s^{-1}
Dark respiration rate on mass basis	$R_{d\text{-}mass}$	nmol g^{-1} s^{-1}
Stomatal conductance	g_s	mol m^{-2} s^{-1}
Instantaneous water use efficiency (A_a/g_s)	WUE	μmol CO_2 mol^{-1} H_2O
Maximum net CO_2 assimilation rate on area basis	A_{max}	μmol m^{-2} s^{-1}
Quantum yield for CO_2 assimilation	α_{JCO2}	mol mol^{-1}
Light compensation point	LCP	μmol quanta m^{-2} s^{-1}
Light saturation point	LSP	μmol quanta m^{-2} s^{-1}
Leaf carbon content	C	%
Leaf nitrogen content	N	%
Leaf phosphorous content	P	%
Leaf potassium content	K	%
Leaf carbon to nitrogen ratio	C/N	g g^{-1}
Photosynthetic nitrogen use efficiency	PNUE	μmol CO^2 s^{-1} mol^{-1} N
Photosynthetic phosphorous use efficiency	PPUE	mmol CO_2 s^{-1} mol^{-1} P
Leaf size	LS	cm^2
Leaf area ratio	LAR	cm^2 g^{-1}
Leaf mass per area	LMA	g g^{-1}
Leaf mass fraction	LMF	g g^{-1}
Stem mass fraction	SMF	g g^{-1}
Root mass fraction	RMF	g g^{-1}
Root to shoot ratio	R/S	g g^{-1}
Total biomass	BIOM	g
Height	H	cm
Height aboveground biomass ratio	HMR	cm g^{-1}

Table 4.2 Mean values of functional traits for hemiepiphytic (H) and non-hemiepiphytic *Ficus* (NH) species grown under four different light conditions and the plasticity of functional traits in response to changes in irradiance.

Traits	100% sunlight		50% sunlight		25% sunlight		5% sunlight		Platicity	
	H	NH	H	NH	H	NH	H	NH	H	NH
K_t	1.68	3.02	1.48	3.20	1.00	2.92	0.77	1.54	58.9	56.9
A_a	14.34	16.14	13.96	15.58	11.73	14.25	9.00	11.00	37.3	30.7
A_m	0.160	0.171	0.193	0.239	0.205	0.242	0.254	0.320	36.8	46.3
$R_{d\text{-area}}$	1.27	0.95	1.04	1.09	0.58	0.69	0.47	0.46	63.0	57.8
$R_{d\text{-mass}}$	14.42	10.49	14.46	17.14	10.14	11.69	13.37	13.46	38.1	43.8
g_s	0.25	0.48	0.31	0.51	0.24	0.49	0.26	0.40	25.4	25.3
WUE	60.69	38.10	50.41	33.86	55.42	31.36	36.43	31.64	43.1	30.0
A_{max}	15.1	19.4	11.4	16.2	11.0	14.9	7.9	9.6	50.0	51.6
α_{JCO2}	0.059	0.060	0.054	0.060	0.058	0.061	0.055	0.061	13.4	16.7
LCP	24.0	23.8	37.7	41.1	16.5	27.6	9.0	11.8	67.4	73.0
LSP	807.0	985.9	797.6	851.4	617.1	813.3	366.2	433.2	59.3	56.6
C	44.25	43.71	44.46	44.42	43.87	43.45	42.24	41.63	5.0	6.3
N	2.79	2.55	2.41	2.58	2.41	2.60	2.65	2.70	25.3	8.7
P	0.148	0.204	0.129	0.163	0.179	0.234	0.200	0.242	38.7	33.1
K	1.61	1.28	0.82	0.74	1.43	1.29	2.10	1.64	60.3	54.6
C/N	16.54	17.31	18.92	17.52	18.49	16.99	16.43	15.85	23.9	10.9
PNUE	83.57	93.65	113.73	130.76	120.57	131.75	135.16	167.05	40.5	43.7
PPUE	3.39	2.72	4.69	4.65	3.58	3.26	3.97	4.09	32.1	48.8
LS	40.61	70.83	55.10	88.78	57.85	90.84	43.05	107.36	55.6	54.9
LAR	33.95	27.69	40.79	31.46	39.63	43.28	92.51	59.30	62.9	55.9
LMA	89.99	97.16	72.45	65.49	57.94	58.83	35.65	34.40	60.3	62.7
LMF	0.253	0.193	0.254	0.204	0.259	0.252	0.292	0.217	25.5	33.0
SMF	0.246	0.359	0.244	0.321	0.274	0.353	0.241	0.335	23.3	18.3
RMF	0.501	0.448	0.502	0.475	0.467	0.411	0.467	0.448	13.5	18.1
R/S	1.08	0.89	1.05	0.96	0.91	0.74	1.00	0.92	32.1	32.6
BIOM	33.07	67.11	25.18	69.21	25.39	51.76	6.97	23.30	82.1	68.1
H	46.82	38.53	39.67	46.71	39.46	45.88	32.24	50.73	38.2	28.3
HMR	4.53	1.42	5.22	1.72	4.89	2.18	11.19	4.37	61.3	65.7

Table 4.3 Three-way nested ANOVA with the factor of growth form ($n = 2$), species (nested within growth form; $n = 6$) and light level ($n = 4$). F-values, P-values, and the level of significance are given ("ns": $P \geq 0.1$; "*": $0.05 \leq P < 0.1$; "**": $0.01 \leq P < 0.05$; "***": $P < 0.01$). Abbreviations are as defined in Table 4.1.

	Growth form			Species			Light		
	df	F	p	df	F	p	df	F	p
K_t	1	5.785	0.074 *	4	11.490	0.000 ***	3	13.006	0.000 ***
A_a	1	20.068	0.011 **	4	0.951	0.462 ns	3	30.972	0.000 ***
A_m	1	4.772	0.094 *	4	4.468	0.014 **	3	40.381	0.000 ***
$R_{d\text{-area}}$	1	0.086	0.784 ns	4	2.706	0.070 *	3	42.240	0.000 ***
$R_{d\text{-mass}}$	1	0.006	0.941 ns	4	4.269	0.017 **	3	2.980	0.065 *
g_s	1	6.785	0.060 *	4	15.672	0.000 ***	3	2.646	0.087 *
WUE	1	4.992	0.089 *	4	7.331	0.002 ***	3	5.275	0.011 **
A_{max}	1	6.473	0.064 *	4	1.563	0.235 ns	3	9.598	0.001 ***
α_{JCO2}	1	2.077	0.223 ns	4	0.890	0.494 ns	3	0.422	0.740 ns
LCP	1	2.590	0.183 ns	4	3.597	0.030 **	3	20.353	0.000 ***
LSP	1	6.895	0.058 *	4	0.527	0.718 ns	3	12.621	0.000 ***
C	1	0.258	0.638 ns	4	21.319	0.000 ***	3	29.615	0.000 ***
N	1	0.065	0.811 ns	4	3.874	0.024 **	3	0.861	0.483 ns
P	1	16.034	0.016 **	4	1.559	0.236 ns	3	14.006	0.000 ***
K	1	2.060	0.225 ns	4	2.949	0.055 *	3	31.296	0.000 ***
C/N	1	0.169	0.702 ns	4	6.547	0.003 ***	3	2.039	0.152 ns
PNUE	1	1.558	0.280 ns	4	6.376	0.003 ***	3	24.025	0.000 ***
PPUE	1	0.496	0.520 ns	4	2.940	0.056 *	3	8.390	0.002 ***
LS	1	1.670	0.266 ns	4	4.827	0.011 **	3	0.590	0.631 ns
LAR	1	0.729	0.441 ns	4	6.097	0.004 ***	3	20.383	0.000 ***
LMA	1	0.029	0.874 ns	4	4.208	0.018 **	3	111.991	0.000 ***
LMF	1	2.027	0.228 ns	4	3.576	0.031 **	3	1.261	0.323 ns
SMF	1	2.913	0.163 ns	4	15.802	0.000 ***	3	1.501	0.255 ns
RMF	1	1.967	0.233 ns	4	2.528	0.084 *	3	1.209	0.341 ns
R/S	1	2.358	0.199 ns	4	1.399	0.282 ns	3	0.931	0.450 ns
BIOM	1	6.734	0.060 *	4	7.445	0.002 ***	3	22.828	0.000 ***
H	1	0.322	0.601 ns	4	14.160	0.000 ***	3	0.339	0.797 ns
HMR	1	15.119	0.018 **	4	6.950	0.002 ***	3	19.045	0.000 ***

Table 4.4 Contrasts in leaf functional traits between hemiepiphytic and non-hemiepiphytic *Ficus* species at juvenile and adult stages.

Traits	Saplings				Adult trees			
	H		NH	*p*	H		NH	*p*
K_t		<		*		<		*
A_a		<		**				ns
A_m		<		*		<		**
$R_{d\text{-area}}$				ns		>		*
$R_{d\text{-mass}}$				ns				ns
g_s		<		*		<		**
WUE		>		*		>		**
A_{max}		<		*				ns
α_{JCO2}				ns		>		*
LCP				ns				ns
LSP		<		*				ns
C				ns		>		*
N				ns		>		*
P		<		*				ns
K				ns		>		*
C/N				ns				ns
PNUE				ns		<		**
PPUE				ns		<		**
LS				ns		<		*

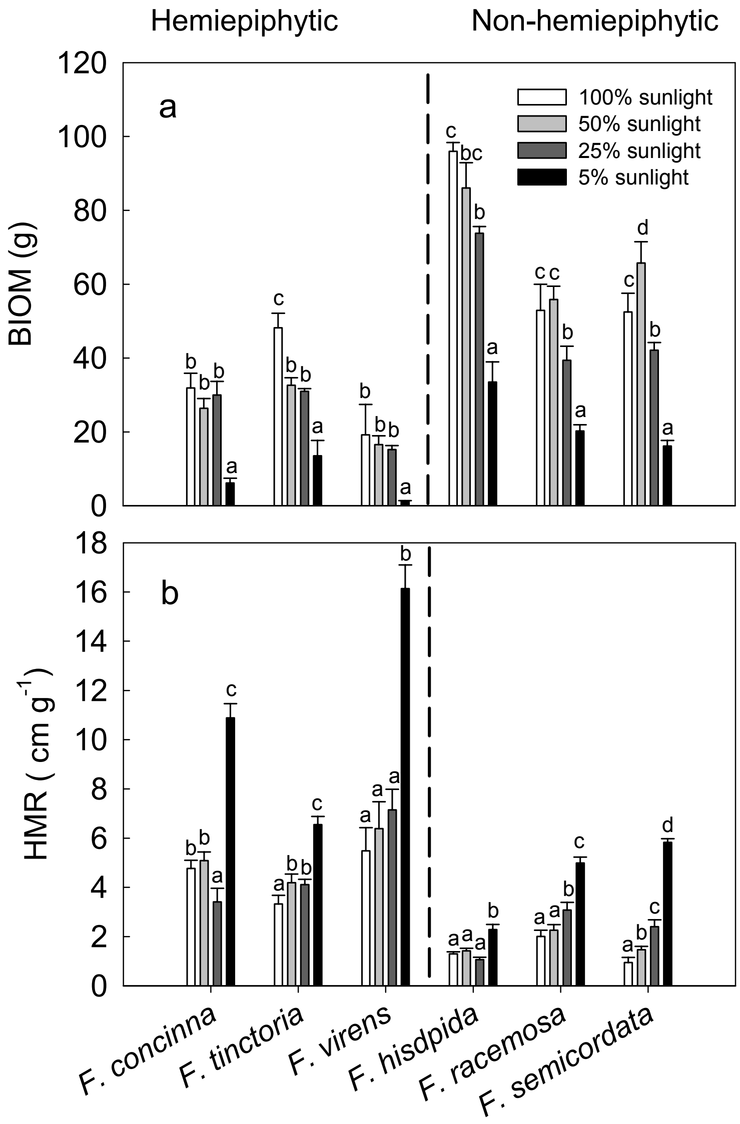

Figure 4.1 (a) Total seedling biomass (BIOM) of hemiepiphytic and non-hemiepiphytic *Ficus* species at the end of the experiment; (b) seedling height aboveground biomass ratio (HMR). Vertical dashed lines separated hemiepiphytic species from non-hemiepiphytic species.

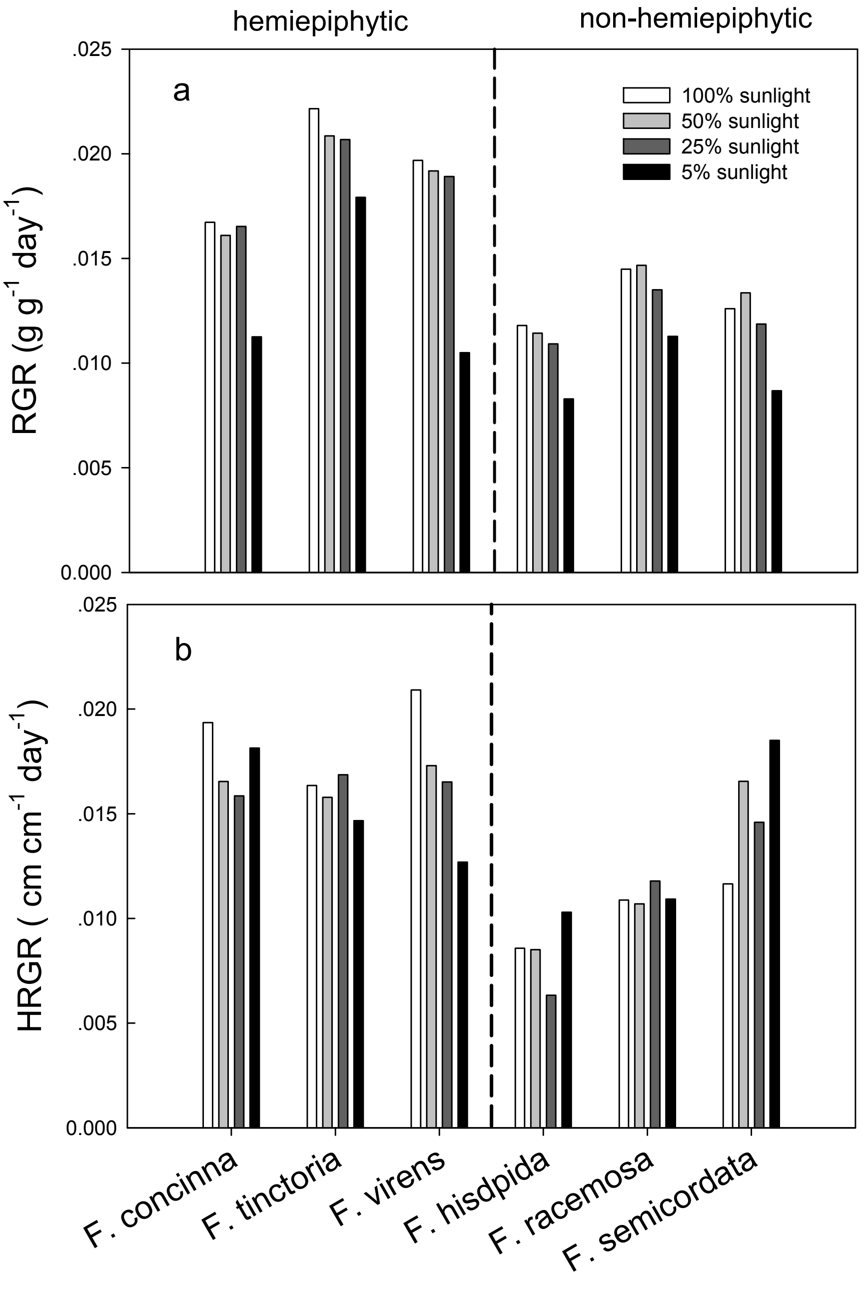

Figure 4.2 (a) Relative growth rates (RGR) and (b) height relative growth rates (HRGR) of the six *Ficus* species after transplanted to four light growth conditions. Vertical dashed lines separated hemiepiphytic species from non-hemiepiphytic species.

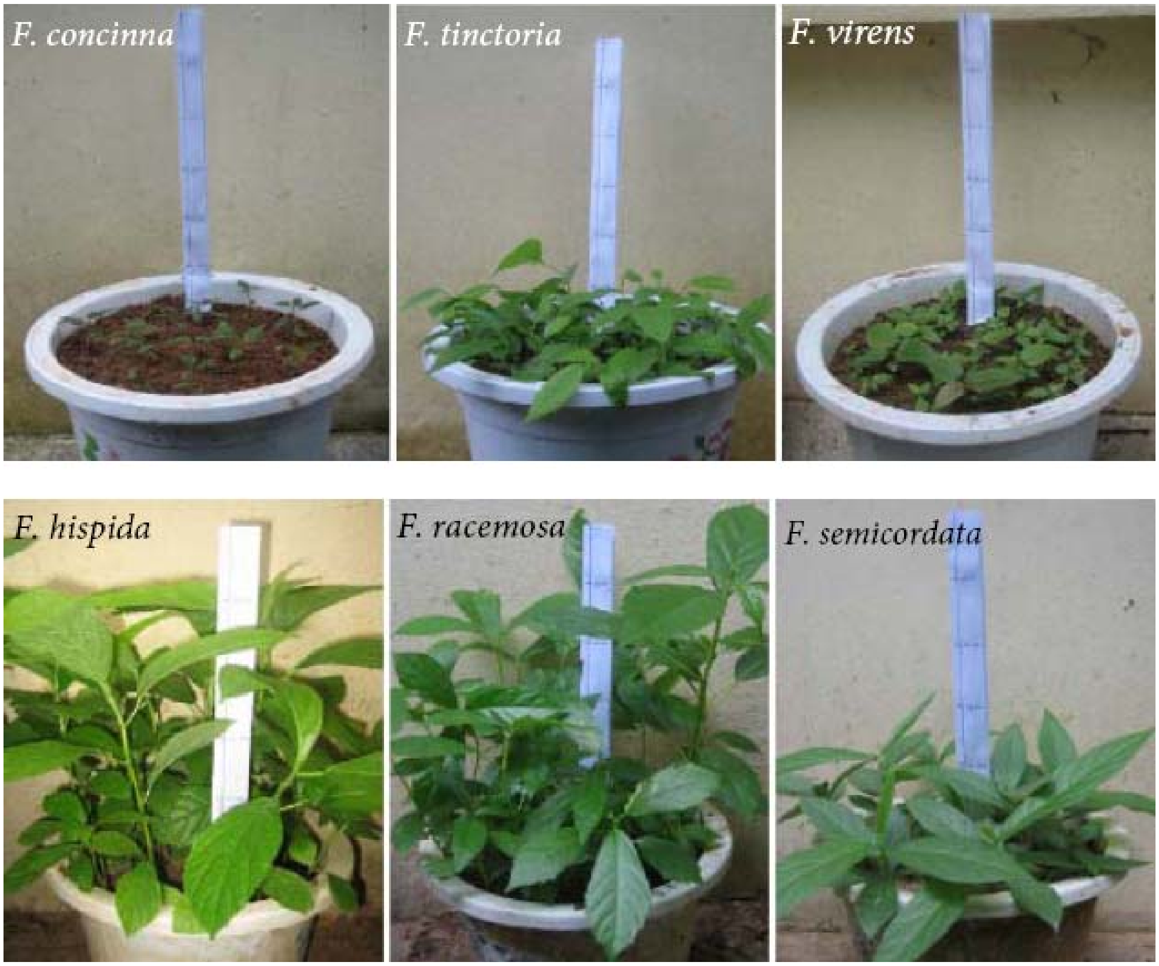

Figure 4.3 Photos showing the seedling sizes of hemiepiphytic (upper panels) and non-hemiepiphytic (lower panels) *Ficus* species in the nursery before being transplanted to individual pots.

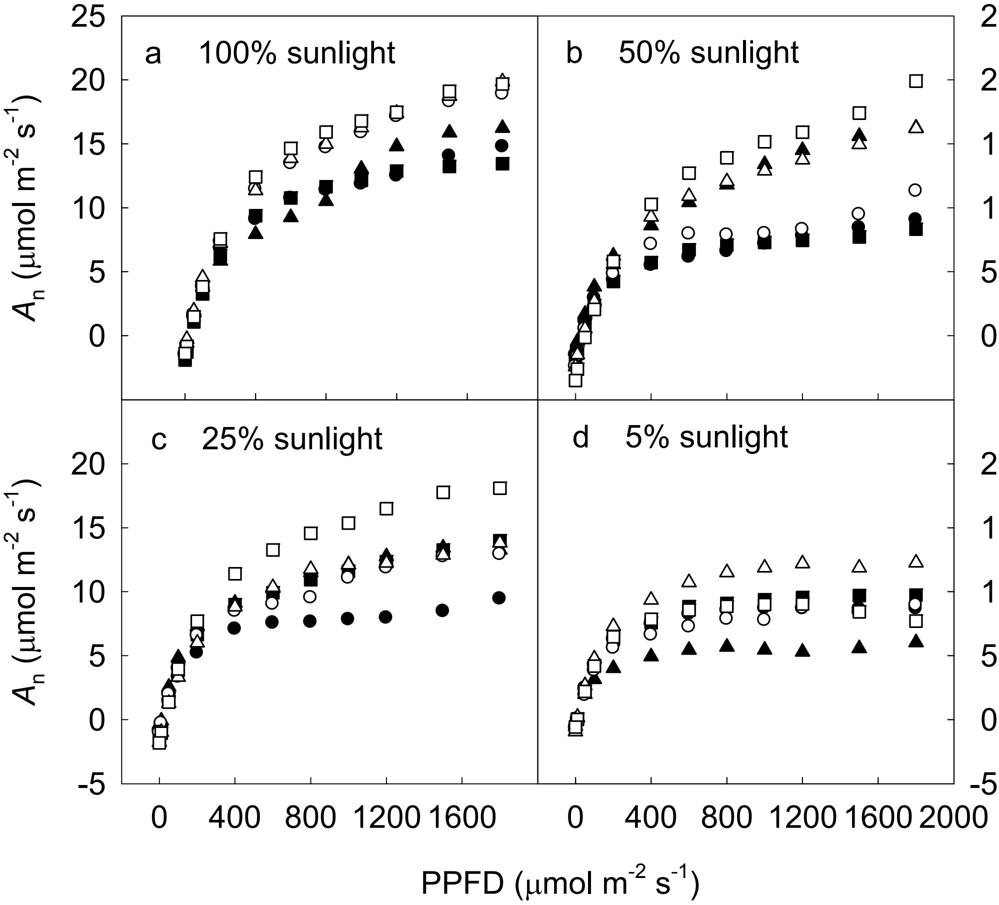

Figure 4.4 Leaf photosynthetic light response curves of three hemiepiphytic (filled symbols) and three non-hemiepiphytic *Ficus* tree seedlings (open symbols) grown under four different light levels (a-d). *Ficus concinna* (●), *F. tinctoria* (▲), *F. virens* (■), *F. hispida* (○), *F. racemosa* (△), *F. semicordata* (□).

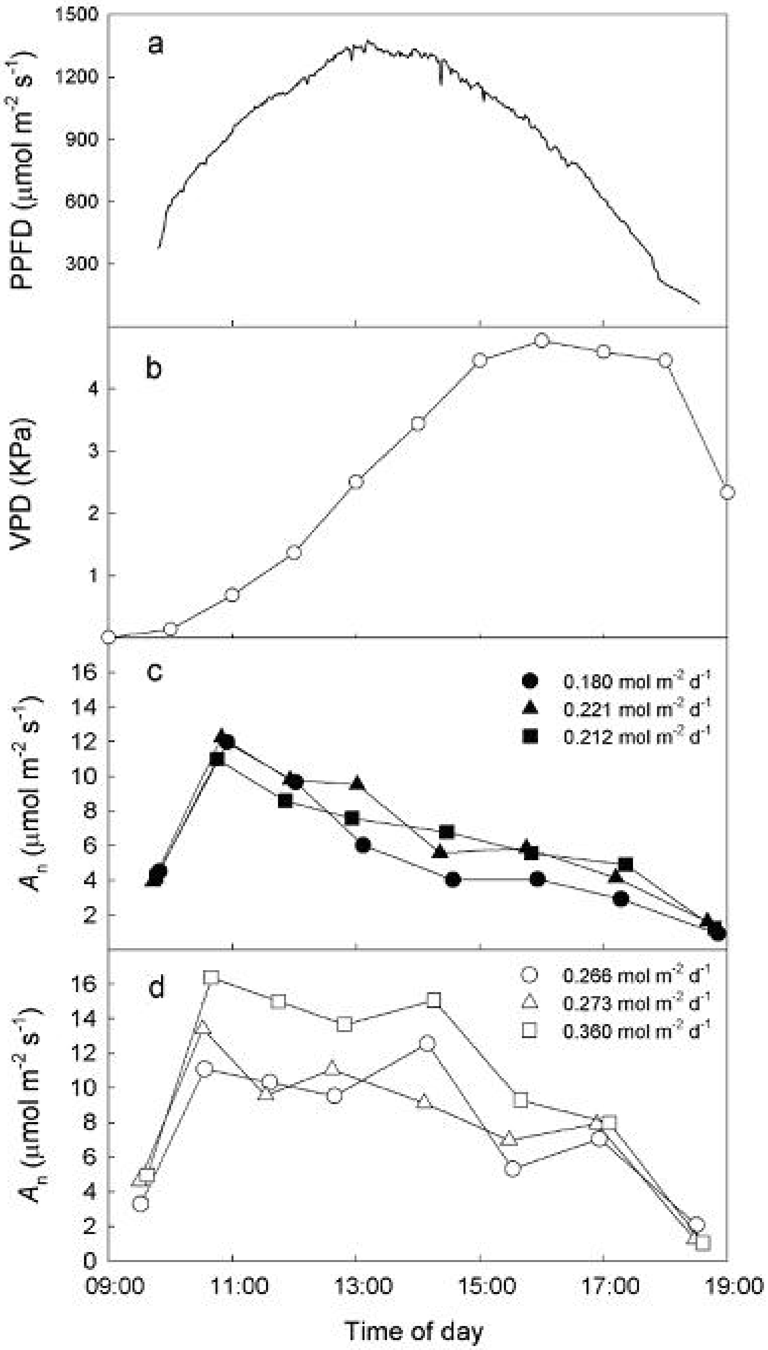

Figure 4.5 Diurnal courses of (a) environmental photosynthetic photon flux density (PPFD); (b) air saturation vapor pressure deficit; and leaf net CO_2 assimilation rate (A_n) of (c) hemiepiphytic and (d) non-hemiepiphytic *Ficus* seedlings grown under full sunlight. Symbols are as defined in Fig. 4.4.

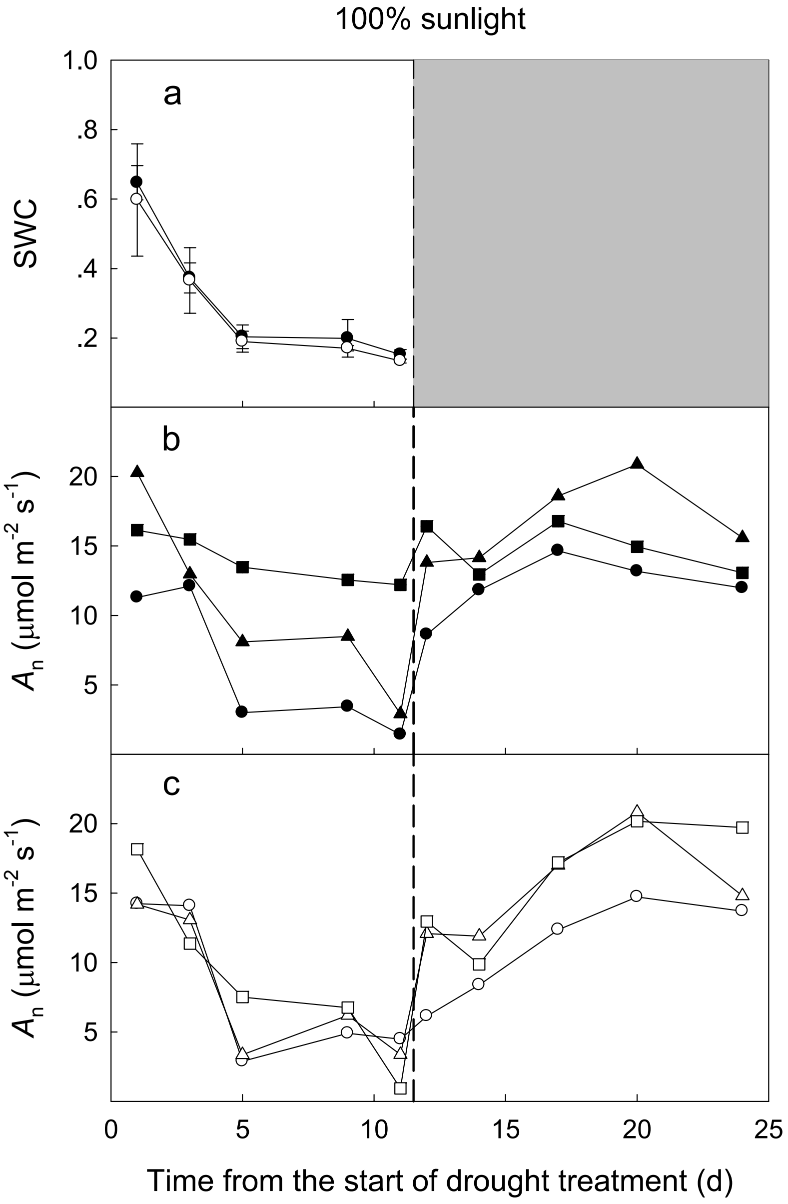

Figure 4.6 (a) Soil water content (SWC) of the potted seedlings following a water withholding treatment (shaded area indicates re-watering of plants to field capacity everyday); changes of mid-morning leaf net CO_2 assimilation rate (A_n) following the water withholding-re-watering treatments in hemiepiphytic (b) and non-hemiepiphytic (c) *Ficus* seedlings grown under the full sunlight treatment. Vertical dashed lines indicate the start of re-watering treatment. Symbols are as defined in Fig. 4.4.

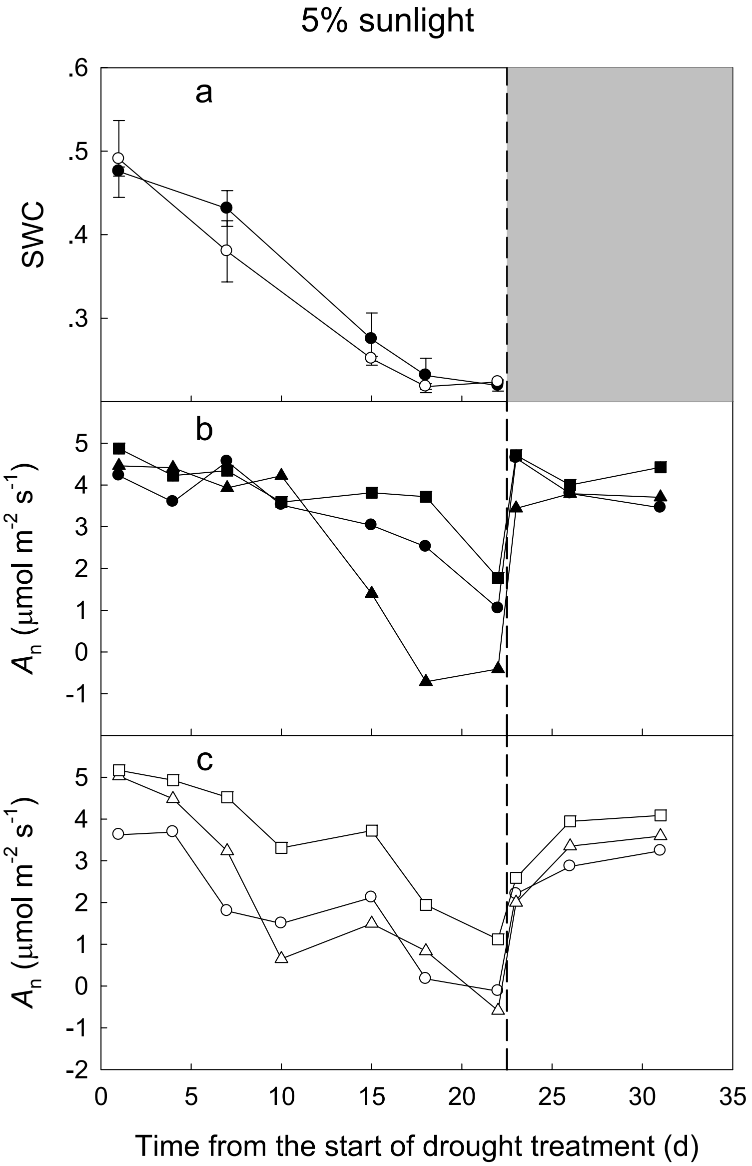

Figure 4.7 (a) Soil water content (SWC) of the potted seedlings following a water withholding treatment (shaded area indicates re-watering of plants to field capacity everyday); changes of mid-morning leaf net CO_2 assimilation rate (A_n) following the water withholding-re-watering treatments in hemiepiphytic (b) and non-hemiepiphytic (c) *Ficus* seedlings grown under 5% sunlight treatment. Vertical dashed lines indicate the start of re-watering treatment. Symbols are as defined in Fig. 4.4.

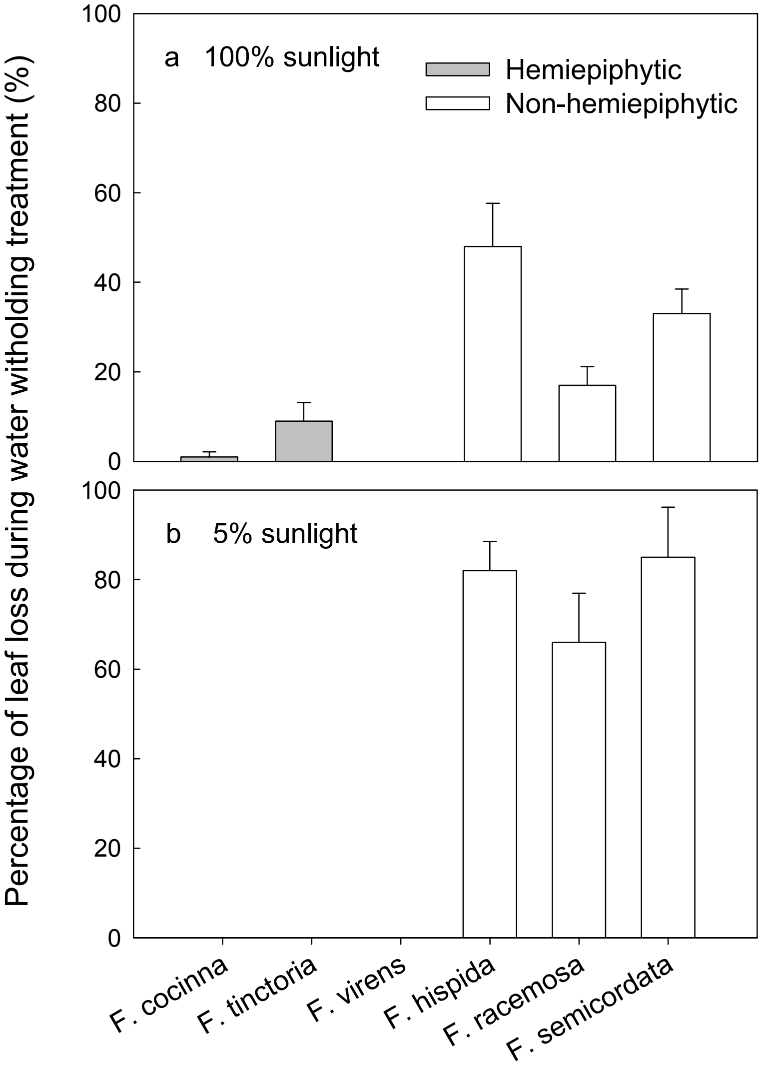

Figure 4.8 Percentage of leaf loss at the end of the water withholding-re-watering cycle for seedlings of hemiepiphytic and non-hemiepiphytic *Ficus* species grown under (a) full sunlight and (b) 5% sunlight treatment.

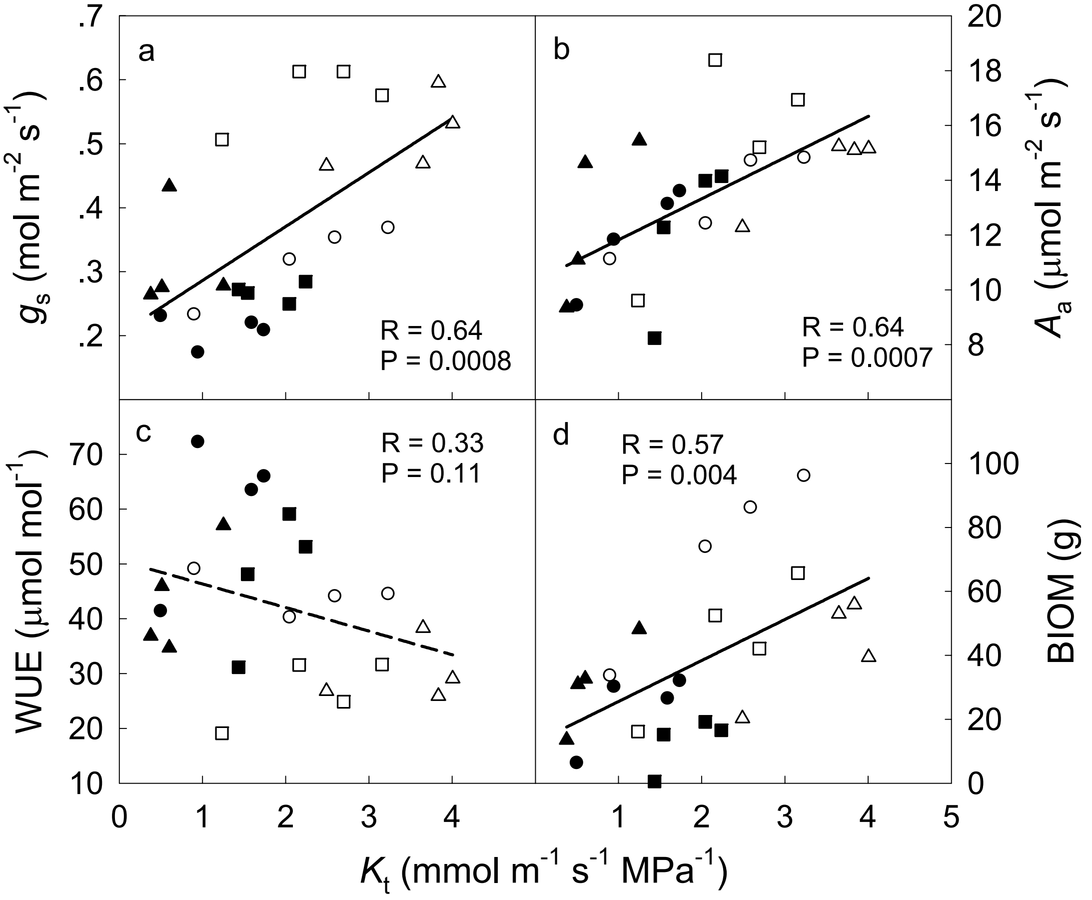

Figure 4.9 Correlations between theoretical axial hydraulic conductivity of leaf petioles (K_t) and leaf functional traits related to gas exchange and growth: (a) maximum stomatal conductance (g_s); (b) maximum net CO_2 assimilation rate (A_n); (c) leaf water-use efficiency (WUE); and (d) final plant dry mass (DM) in seedlings of hemiepiphytic (filled symbols) and non-hemiepiphytic *Ficus* species (open symbols). Measurements on seedlings grown under four different light conditions were used. Symbols are as defined in Figure. 4.4.

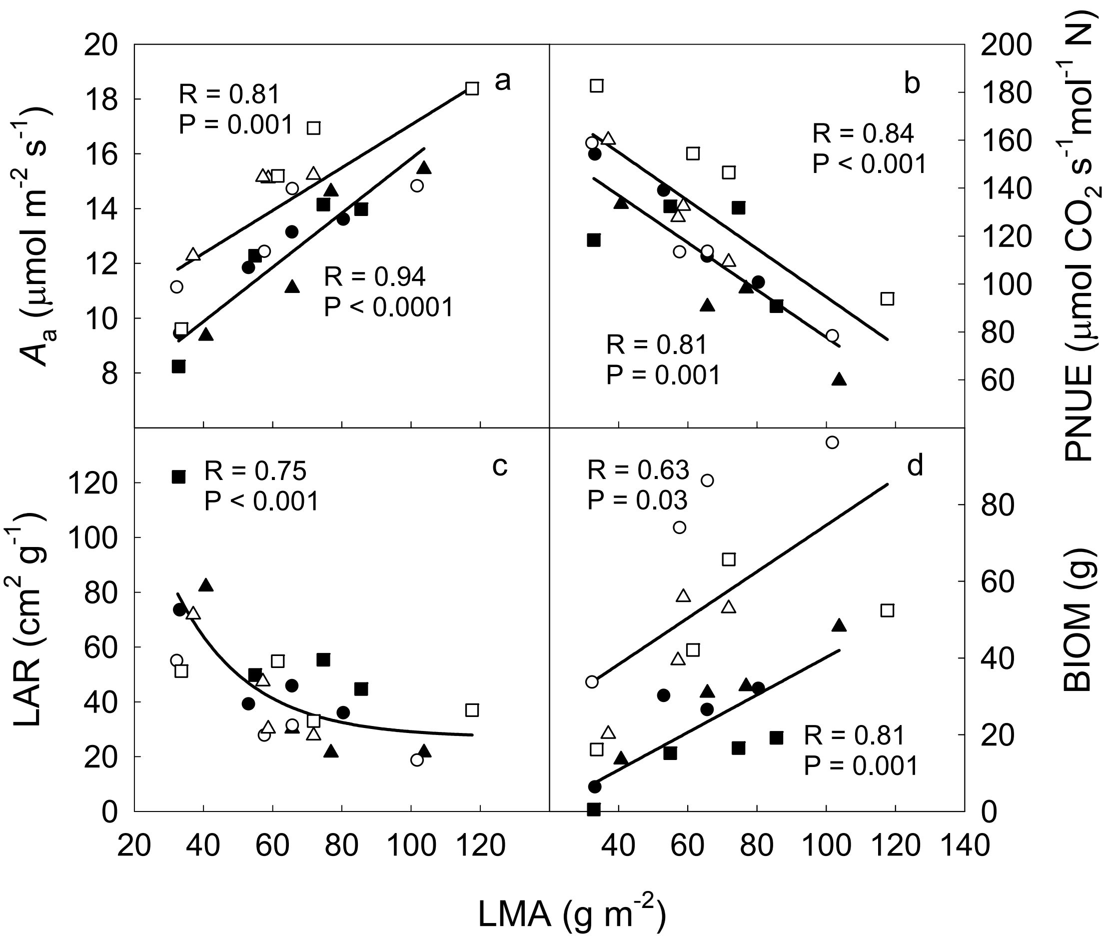

Figure 4.10 Correlations between leaf mass per area (LMA) and leaf functional traits related to gas exchange and growth: (a) maximum net CO_2 assimilation rate (A_n); (b) photosynthetic nitrogen use efficiency (PNUE); (c) leaf area ratio (LAR); and (d) final plant dry mass (DM) in seedlings of hemiepiphytic (filled symbols) and non-hemiepiphytic *Ficus* species (open symbols). Measurements on seedlings grown under four different light conditions were used. Regressions were fitted separately for hemiepiphytic and non-hemiepiphytic species in panels a, b and d. Symbols are as defined in Figure. 4.4.

CHAPTER 5

EVOLUTIONARY DIVERGENCE OF HYDRAULICS AND CARBON-ECONOMY

Summary

Hemiepiphytic *Ficus* species (H) differ from non-hemiepiphytic congeneric species (NH) in important life-history traits. However, there have been few studies comparing ecophysiological traits between species of the two growth forms, especially no such studies have been done within an explicit evolutionary framework. Using common garden plants, functional traits related to plant hydraulics and carbon economy of seven Hs and seven NHs were compared, and evolutionary correlations among these traits were examined by applying phylogenetic independent contrasts (PIC). *Ficus* species of the two growth forms differed mainly in their xylem hydraulic conductivity, leaf gas exchange, and carbon economy with Hs having significantly lower xylem water transport capacity and more conservative leaf water use than NHs. Across all the studied species, a suite of traits were correlated with stem xylem hydraulic conductivity and leaf mass per area when analyzed using both ahistorical and PIC methods, suggesting that these two traits are important in mediating a series of trade-offs in plant functioning. This study indicated that changes in plant-water relations accompanying the evolutionary transition from terrestrial to hemiepiphytic growth habit are responsible for the great divergences in ecophysiology between Hs and NHs.

Introductory remarks

Many studies have been conducted on the symbiosis between *Ficus* species and their pollinating wasps (e.g. Cook and Rasplus 2003; Herre et al. 2008), but ecophysiological studies on *Ficus* species are scarce (but see Patiño et al. 1995; Holbrook and Putz 1996a,b; Zotz et al. 1997). Hemiepiphytic *Ficus* species experience large structural and functional changes during the transition from the epiphytic to the terrestrial phase, in response to the change of environmental conditions (Holbrook and Putz 1996a; Holbrook and Putz 1996b). As a result of the limited rooting volume and characteristics of the rooting zone, epiphytic-phase *Ficus* frequently experience water limitation and thus are more conservative in terms of water use compared to terrestrially rooted individuals of the same species (Holbrook and Putz 1996a). Under seasonal climatic conditions, stomatal conductance of epiphytic-phase plants was significantly lower than conspecific terrestrially rooted individuals during both the wet and the dry seasons (Holbrook and Putz 1996a). Epiphytic-phase plants have better control of epidermal water loss from leaf surfaces than the tree-phase individuals of the same species (Holbrook and Putz 1996b). Due to the existence of an epiphytic phase that is commonly subjected to water deficit, it is likely that Hs have traits related to water-use that are substantially different from NHs, even after the plants have established contact with the ground. Therefore, we compared ecophysiological traits related to water-use efficiency and carbon economy among H and NH *Ficus* species. To rule out the effects of proximal environmental factors and compare intrinsic differences between species of the two growth forms, we only included terrestrial-phase individuals of Hs that were well rooted in the ground. For the same reasons, we only included trees in our NH sample, as shrubs and climbers may also experience very different proximal environments as adults.

Very few studies have been conducted to address the ecophysiological differences between Hs and NHs. One study comparing the hydraulic architecture of tropical woody plants in Neotropical forests did show that tree species of terrestrially rooted Hs tended to have less cross-sectional sapwood per unit leaf area than NHs (Patiño et al. 1995). However, most ecophysiological traits of Hs remain unknown. Moreover, ecophysiological studies of *Ficus* have yet to be conducted within an explicit evolutionary framework as phylogeny may explain a large proportion of variation among species in functional traits (Hao et al. 2008). Functional convergences among plant functional traits do not necessarily imply co-evolution of these traits, as they may be artifacts due to common ancestry (Felsenstein 1985; Ackerly and Reich 1999). To overcome the problem of non-independence of species in comparative studies, Felsenstein (1985) proposed the use of phylogenetic independent contrasts (PIC). Using PIC analysis on this group of closely related species enabled us to examine the correlated evolution of plant hydraulics and carbon economy related functional traits. Our results, therefore, have relevance beyond an understanding of the ecophysiological differences between growth-forms.

In the present study, we examined 18 functional traits related to plant hydraulics and carbon economy in 14 *Ficus* species native to Southeast Asia (seven Hs and seven NHs). The following questions were addressed: (1) Do tree-phase Hs differ significantly from NHs trees in xylem water transport, leaf water-use and leaf carbon economy? (2) If differences between the two growth forms do exist, what are the major functional traits that distinguish them from each other? (3) Are there evolutionary correlations among hydraulic and carbon economy related traits in the *Ficus* lineage?

Materials and Methods

Study site and plant material

The 14 study species are all from the genus *Ficus* (Moraceae) and included seven H and seven NH species that are commonly found in Xishuangbanna (Table 5.1). All of the plants used were adults growing under similar conditions at the XTBG. Individuals of the hemiepiphytic species studied had all reached their terrestrial stage with roots well established in the soil. It is ideal that all species used in the analysis of evolutionary differences are raised under similar environments (Garland and Adolph 1991), conditions that were met in this study. *Ficus tinctoria* is a hemi-epiphyte that usually does not form a self-supporting trunk and falls down if the host tree dies. The other six hemiepiphytic species can form firm trunks and become large independent trees. By growing supporting aerial roots from its branches, *F. altissima* can extend outwards and form extremely large canopies. *Ficus auriculata, F. esquiroliana, F. fistulosa, F. hispida,* and *F. semicordata* are dioecious species and thus traits were measured on trees of both sexes. No significant differences were found between female and male trees and data were pooled for further analyses.

Measurements of hydraulic conductivity

Stem hydraulic conductivity (K_h) was measured on three branches per tree of six to eight individuals per species. Early in the morning, terminal branches *ca.* 1 m in length were excised. The branches were re-cut immediately under water to avoid embolism and were transported to the laboratory shortly with the cut end immersed in water and the whole branches tightly wrapped in opaque plastic bags. In the laboratory, a 40- to 55-cm-long section of each branch was cut under water and both ends were smoothened with sharp razor blades. The stem segments were then connected to an apparatus with degassed and filtered 0.5 mmol l^{-1} KCl solution for determining K_h. A hydrostatic pressure for generating water flow through the stem segments was generated by a constant hydraulic head of 50 cm. The downstream end of the segment was connected to a graduated pipette and the time required for the meniscus in the pipette to cross a certain number of consecutive graduation marks was recorded. Hydraulic conductivity (kg m s^{-1} MPa^{-1}) was calculated as:

$$K_h = J_v / (\Delta P / \Delta L) \qquad (5.1)$$

where J_v is flow rate through the segment (kg s^{-1}) and $\Delta P/\Delta L$ is the pressure gradient across the segment (MPa m^{-1}). Sapwood area (A_{sw}) was determined at 1 cm from both ends of the segment using the dye staining method. The two values were averaged to represent the sapwood area of the segment. Total area of leaves distal to the stem segment (A_l) was measured using a LI-3000 leaf area meter (Li-Cor, Lincoln, NE, USA). Leaf area to sapwood area ratio (LA/SA) was calculated as the ratio of A_l to A_{sw}. Sap wood area specific hydraulic conductivity (K_s; kg m^{-1} s^{-1} MPa^{-1}) was calculated as the ratio of K_h to A_{sw} and leaf-specific hydraulic conductivity (K_l, kg m^{-1} s^{-1} MPa^{-1}) was calculated as the ratio of K_h to A_l.

Measurements of hydraulic related functional traits

For determining average leaf size (LS), leaf mass per area (LMA), leaf saturated water content (SWC), and leaf density (ρ_{leaf}), 10 to 20 sun-exposed mature leaves from each of six individuals of each species were collected. Total leaf area sampled from each individual was measured using a Li-Cor 3000 area meter and divided by leaf number to calculate LS. These leaves were oven dried at 70 °C for 48 hours to determine dry weight (DW) and then powdered using a coffee mill and sealed in plastic bags for later use in chemical analysis. Leaf disks sampled from another set of similar leaves were used to determine SWC and ρ_{leaf}. Leaf disks were fully saturated overnight in distilled water to determine leaf saturated weight (SW) on about 10 leaf disks and SWC was calculated as (SW-DW)/DW. The volume of leaf disks (LV) was measured using the water displacement method with a balance and ρ_{leaf} was calculated as DW/LV.

Measurements of leaf gas exchange

A portable photosynthetic system LI-6400 (Li-Cor Inc., Lincoln, NE, USA) was used to measure leaf gas exchange. All measurements were done between 8:30 AM and 11:30 AM during clear days in the wet season 2007. We used sun-exposed leaves that could be reached from the ground or with the help of a ladder. The photosynthetic irradiance supplied by red-blue LED lamps was maintained at 1200 μmol m^{-2} s^{-1} at the leaf surface and ambient CO_2 concentration was used. After gas exchange parameters stabilized, area-based net assimilation rate (A_a), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were obtained under ambient CO_2 concentrations. The intrinsic water-use efficiency (WUE) was calculated as the ratio of A_a to g_s. Three leaves from each of four to six individuals of each of the 14 studied species were measured.

Total leaf N concentration was determined with an auto Kjeldahl unit (K370, BÜCHI Labortechnik AG, Flawil, Switzerland) after samples were digested with concentrated H_2SO_4. Total P and K contents were analyzed using an inductively coupled plasma atomic-emission spectrometer (IRIS Advantage-ER, Thermo Jarrell Ash Corporation, Massachusetts, USA) after samples were digested with concentrated HNO_3-$HClO_4$. The mass-based net assimilation rate (A_m), photosynthetic nitrogen use efficiency (PNUE), and photosynthetic phosphorous use efficiency (PPUE) were calculated by dividing A_a with LMA, percentage of foliar contents of N and P, respectively.

Phylogenetic analysis of Ficus species

The phylogenetic relationships of the 14 *Ficus* species are inferred from the nuclear ITS sequences. The extraction of total DNA, PCR amplification and sequencing of ITS region were carried out according to Azuma et al. (2009). Alignments of the ITS sequences were performed using the multiple-alignment program MAFFT (Katoh et al. 2002). The sites including gaps were excluded from the aligned sequence data set; therefore, the final length of the sequence data set was 656 nt. The phylogenetic analyses based on the neighbor-joining (NJ) method and the maximum likelihood (ML) method was performed with PAUP 4.0b (Swofford 2001). Kimura's two-parameter (Kimura 1980) distance was used for NJ analysis. For ML analysis, heuristic searches were carried out using the HKY85 model of the nucleotide substitutions with tree-bisection-reconnection (TBR) branch-swapping under the default settings. The starting tree was obtained via stepwise addition, and the starting branch lengths were obtained using the Rogers-Swofford approximation method. Trees with approximate likelihoods 5% or further from the target score were rejected without additional iteration. The trees were evaluated using the bootstrap test based on 1000 replicates.

Data analysis

Principal component analysis (PCA) based on all species and all variables measured were performed using SPSS version 16.0 (SPSS, Inc., Chicago IL, USA). ANCOVA was used to examine the differences in ρ_{wood}-K_{h} (K_{s} and K_{l}) correlations between H and NH growth forms.

Based on the phylogeny tree of the 14 *Ficus* species, we tested the phylogenetic signal using the *K*-statistic and a randomization test implemented via phylogenetically independent contrasts (Blomberg et al. 2003) using R v2.8.1 (R Development Core Team 2008; package ‘picante’). As a high proportion of the traits exhibited a significant phylogenetic signal, we further used phylogenetically independent contrasts to examine relationships between ecophysiological traits (package ‘ape’). All data were $\log_{10}$-transformed before analyses.

Results

From the phylogeny tree of the 14 *Ficus* species, it is evident that species within each of the two growth forms (i.e. H and NH) are generally more closely related in phylogeny, which necessitates the use of PIC in analyzing correlations between functional traits to rule out the artifacts due to common ancestry. Despite this, at least three independent transitions from terrestrial to hemiepiphytic growth habit occurred in this subset of *Ficus* taxa (Fig. 5.1).

Compared to their congeneric tree species, Hs as a group had significantly lower K_s, lower K_l, and lower saturated leaf water content. Also, Hs showed significantly lower A_m, lower g_s, and higher intrinsic WUE than NH species (Table 5.2). Leaves of Hs had significantly higher N ($P < 0.05$; 2.11% and 1.69%, respectively) and K ($P < 0.05$; 1.70% and 1.14%, respectively) contents than those of NHs, while P concentration was not significantly different between the two groups (0.15% for both groups). Hemiepiphytic species also had significantly lower PNUE and PPUE than non-hemiepiphytic species ($P < 0.01$). Despite significant differences were found between H and NH growth forms for most functional traits measured, variation within each growth form and overlaps between the two groups forms are also relatively large for most functional traits (Fig. 5.3, 5.4, and 5.5).

In the PCA based on the 18 functional traits, the first three principal components explained 46.2%, 16.7% and 14.2% of the total variation, respectively. The distribution of the 18 functional traits along axis 1 and axis 2 is shown in Figure 1a and the correlation coefficient (*r*) of each functional trait with the first three PCA axes is given in

Table 5.4. Hs and NHs took different positions along the first PCA axis but species of the two growth forms did not separate along PCA axis 2 (Fig. 5.2b). Plant hydraulic traits (i.e. K_s and K_l) and leaf gas exchange related traits (i.e. g_s, C_i, A_a, A_m, PNUE and PPUE) clustered together (Fig. 5.2a) and were positively correlated with PCA axis 1 (Table 5.4). LMA, ρ_{wood} and WUE, on the other hand, were negatively correlated with PCA axis 1. Hs differed significantly from congeneric NHs in most of the ecophysiological traits related to plant hydraulics and leaf gas exchange (Table 5.4). The leaf N, P, and K contents were positively correlated to PCA axis 2 (Fig. 5.1a) and were the main contributors to this component (Table 5.4). PCA axis 3 was positively correlated with ρ_{leaf} and K content; negatively correlated with LA/SA, ρ_{wood} and leaf size (Table 5.4).

Wood density was not significantly different between Hs and NHs, although K_s and K_l were significantly higher in NHs (Table 5.2). Results of ANCOVA showed that the slopes of linear regressions of ρ_{wood} with both K_s and K_l were not significantly different between Hs and NHs but the intercepts of the regressions were ($P < 0.01$) (Fig 3a,b). A suite of traits related to gas exchange and carbon economy (e.g. g_s, WUE, PNUE) were found to be highly correlated with K_l (Fig.4) and LMA (Fig.5) across all the studied species. The same set of "rules" can be used to describe relationships between functional traits of across *Ficus* species belonging to two distinct growth forms (Fig. 5.5a,b), which may indicate significant functional convergence (Meinzer 2003; Bucci et al. 2004).

For 38% (seven out of 18) of the measured traits, we found strong phylogenetic signals using Blomberg et al. (2003)'s *K*-statistic ($K > 1$) or the randomization test ($P < 0.05$) (Table 5.4). Strong phylogenetic signals were found for the two traits of stem hydraulic conductivity (i.e. K_s and K_l) and for traits related to leaf carbon economy (i.e.

LMA, A_m, WUE, PNUE, and PPUE). When analyzed using PIC, more than half of the correlations between functional traits persisted (Fig. 5.4 and 5.5 right panels), but levels of significance declined substantially compared to correlations using the original data (Fig. 5.4and 5.5 left panels).

Discussion

Within the genus *Ficus*, the divergence of several major lineages is associated with changes in growth habit (Harrison 2005). Hemiepiphytic growth habit has probably evolved four times in *Ficus* with the three sections *Sycidium*, *Conosycea*, and *Urostigma* containing most species of hemiepiphytic figs distributed in the Indo-Pacific region (Harrison 2005). Consistent with the overall conclusion of multi-time evolution of hemiepiphytic growth form in the genus, phylogeny of this subset of *Ficus* species used in the present study also indicated three independent evolutionary transitions from terrestrial to hemiepiphytic growth habit (Fig. 5.1).

The hemiepiphytic growth habit is believed to have evolved by plants exploiting higher light in the canopy than the forest understory (Putz and Holbrook 1986; Laman 1995). This view was supported by the facts that hemiepiphytic *Ficus* seedlings were unable to survive in the forest understory and their growth was positively correlated with light intensity (Laman 1994; Laman 1995). Although the evolution of a canopy growth habit during youth stage enabled hemiepiphytic species to reach high light environment, new problems such as frequent and severe drought related to canopy growth at the epiphytic stage occurred for these species. Among the 14 *Ficus* species studied, a clear evolutionary shift in the transition from non-hemiepiphytic to hemiepiphytic life-form was evident in traits relating to xylem hydraulic capacity. The significantly lower xylem hydraulic conductivity and its related conservative water-use in Hs were likely evolved in response to their drought-prone environment experienced during the epiphytic phase. Although terrestrially rooted Hs may have as reliable soil water sources as NHs, and Hs are known to undergo dramatic ontogenic changes in both structure and physiology during the transition from epiphyte to rooted phase (Holbrook and Putz 1996a; Holbrook and Putz 1996b), some genetically based characteristics related to adaptation to the drought-prone epiphytic habitat clearly persist into the terrestrial phase and affect many aspects of plant functioning. The significant differences between H and NH species in leaf traits related to gas exchange and carbon economy may have resulted from the existence of a hydraulic-photosynthetic coordination commonly found in plants (Brodribb and Field 2000; Melcher et al. 2001; Brodribb et al. 2002; Santiago et al. 2004a,b; Franks 2006; Zhang and Cao 2009).

PCA axis 1, along which species of the two growth forms were well separated (Fig. 5.2b), revealed adaptive differences between Hs and NHs in xylem water transport, leaf water-use and photosynthetic traits. The positive values on the PCA axis 1 were associated with NH species, which had higher values of xylem water transport efficiency, photosynthetic capacity and leaf nutrient (N and P) use efficiency. Large negative values on PCA axis 1 reflected the higher ρ_{wood}, LMA and WUE of H species. PCA axis 1 thus reflected a trade-off between rapid carbon gain and high nutrient use efficiency against reduced water-use efficiency. PCA axis 2 did not separate the two growth forms, indicating that the major divergence between Hs and NHs was in plant hydraulics and correlated traits of leaf gas exchange.

The significantly lower hydraulic conductivity in Hs compared to NHs may reflect a trade-off between hydraulic conductivity and safety in the xylem against cavitation (Martinez-Vilalta et al. 2002). Species growing in relatively wet environments are usually more vulnerable to drought-induced cavitation. However, under favorable water conditions these species usually have high growth rates that are at least partially explained by their high water transport efficiency (Salleo et al. 2000; Santiago et al. 2004b). It has been argued that the existence of a compromise between the ability of plants to cope with drought stress and the ability to grow at high rates under more favorable water conditions explains why drought-tolerance species are displaced from mesic and humid habitats (Orians and Solbrig 1977). However, Hs are one of most successful groups of species inhabiting tropical lowland rainforests throughout the world (Harrison 2005). Their ability to colonize favorable light environments in the canopy as juvenile plants may compensate for lower rates of carbon gain and nutrient use efficiency, enabling them to successfully inhabit mature lowland rainforests (Harrison et al. 2003). Many NHs, including several species we studied, are confined to pioneer forest communities and do not attain a large size.

Xylem hydraulic conductivity is influenced by the xylem properties such as the density and diameter of the xylem conduits (Tyree et al. 1994), and thus closely related to ρ_{wood}. Wood density is negatively correlated with sapwood hydraulic conductivity, sap velocity and stomatal conductance (Roderick and Berry 2001; Margaret and Whitehead

2003; Bucci et al. 2004). However, a recent study indicated that ρ_{wood} and vessel traits are associated with different functions and that variation in vessel traits accounts for a large part of variation in wood density but not all (Preston et al. 2006). The differences in the ρ_{wood} and hydraulic conductivity correlations between the two growth forms of *Ficus* may also be attributable to differences in the properties of tissues surrounding vessels. Tension wood, with fibers of thickened and crystallized walls found in wood of hemiepiphytic figs (Fisher 1982), may explain the higher wood densities in Hs at a given level of hydraulic conductivity (Fig 2a,b).

Some traits related to plant water relations are found to be more evolutionarily conserved than others (Cavender-Bares et al. 2004; Preston et al. 2006; Hao et al. 2008). Few studies addressing the evolutionary lability of ecophysiological traits have been carried out using phylogenetically independent methods, which is important for ecological interpretations (Felsenstein 1985). Among the 14 *Ficus* species we studied, both K_s and K_l showed strong phylogenetic signals ($P < 0.05$), which is consistent with the finding that traits related to hydraulic architecture in the genus *Juniperus* were strongly influenced by the phylogenetic history of this group of species (Willson et al. 2008). Some previous studies showed that LMA was labile to evolutionary change (Chapin 1993; Hao et al. 2008), while the present study and the recent study on *Juniperus* (Willson et al. 2008) showed that LMA was highly evolutionarily conserved. Also, in contrast to the study of Preston et al. (2006), ρ_{wood} did not show strong phylogenetic signal in the present study. These discrepancies between studies indicate that the findings from one lineage do not necessarily apply to another, and great care should be taken in making generalizations about evolutionary patterns and ecological interpretations.

A large divergence among lineages, coupled with reduced divergence within lineages can explain correlations among traits using ahistorical analyses that diminish substantially with PIC correlations (Willson et al. 2008). On the other hand, traits with a higher degree of homoplasy usually evidence less discrepancy between ahistorical and PIC correlations. In the present study, species within each growth form (H and NH) tend to be more closely related to each other. For most of the measured traits, differences between H and NH species were large while differences within groups were relatively small, which is the main reason why all the correlations became weaker after using PIC analyses (Fig. 5.4, and 5.5). Nevertheless, the correlations among many of the functional traits remain significant when analyzed using PIC, indicating trait co-evolution or trade-offs between these functional traits.

The phylogenetically controlled correlations between K_l and other functional traits (Fig. 5.4g-j) indicated that many ecophysiological traits are evolutionarily related to plant hydraulics. Thus, hydraulics may be the main determinant of the ecophysiological differences among species, as well as between Hs and NHs. The positive correlations between K_l and leaf gas exchange parameters (i.e. A_m and g_s; Fig. 5.4g,h) indicated the importance of stem xylem water transport efficiency on leaf gas exchange. The negative correlation between K_l and WUE (Fig. 5.4i) suggests that species with high water transport efficiencies tended to use water more wastefully. However, significant negative correlations between WUE and nutrient (N and P) use efficiency across the studied species suggests that the costs of producing xylem of high water transport efficiency and a less conservative water-use is at least partially paid off by a higher efficiency of photosynthetic nutrient use.

LMA reflected the pattern of carbon allocation in leaves and is another variable often found to be correlated with many other functional traits, such as wood density and stem hydraulic conductivity (Bucci et al. 2004). Higher LMA was found to be adaptive to drier environments and was suggested to be an important predictor of growth habitats (Hoffmann et al. 2005; Wright et al. 2004). Negative correlations between LMA and A_m have been widely reported (e.g. Reich et al. 1997; Davies 1998), but this likely reflects correlations with other traits such as hydraulic architecture, stomatal behavior, leaf N and P content, rather than a causal relationship. The significant correlations found between LMA and photosynthetic traits using PIC (Fig. 5.5f,g,j) indicated that these correlations have an evolutionary basis.

Conclusions

This is the first ecophysiological study that compares congeneric species belonging to two distinct growth forms within an explicitly evolutionary framework. The patterns identified in this study indicate that the epiphytic growth habit during the juvenile stage in some *Ficus* species involved a suite of differences in hydraulic architecture, photosynthetic water-use, plant carbon and nutrient economies from non-hemiepiphytic species and these traits persist into the terrestrially rooted adults to a certain degree. Phylogenetic analyses revealed that many functional characteristics of H and NH *Ficus* species were phylogenetically conserved. Nevertheless, using phylogenetically independent contrasts this study showed that many plant functional traits are evolutionarily correlated. In particular, many functional traits are evolutionarily correlated with leaf specific hydraulic conductivity and leaf mass per area suggesting that they play a central role in plant functional convergence, and are prominent in affecting the ecological divergences among hemiepiphytic and non-hemiepiphytic *Ficus* species.

Table 5.1 List of species names, codes of species used in the present study, the *Ficus* section (subgeneric division), growth forms, and reproductive traits (monoecious versus dioecious) of the 14 *Ficus* species studied (Berg and Corner 2005). H stands for hemiepiphytic species and NH stands for non-hemiepiphytic species.

Species name	Code	Section	Growth form	Reproductive trait
F. altissima Blume	AL	Conosycea	H	Monoecious
F. benjamina Linnaeus	BE	Conosycea	H	Monoecious
F. concinna (Miquel) Miquel	CO	Urostigma	H	Monoecious
F. curtipes Corner	CU	Conosycea	H	Monoecious
F. religosa Linnaeus	RE	Urostigma	H	Monoecious
F. tinctoria Frost. f. subsp. gibbosa (Bl.) Corner	TI	Syzidium	H	Dioecious
F. virens Aiton	VI	Urostigma	H	Monoecious
F. auriculata Loureiro	AU	Sycomorus	NH	Dioecious
F. callosa Willdenow	CA	Oreosycea	NH	Monoecious
F. esquiroliana H. Léveillé	ES	Eriosycea	NH	Dioecious
F. fistulosa Reinwardt ex Blume	FI	Sycocarpus	NH	Dioecious
F. hispida Linnaeus	HI	Sycocarpus	NH	Dioecious
F. racemosa Linnaeus	RA	Sycomorus	NH	Monoecious
F. semicordata Buchanan-Hamilton ex Smith	SE	Hemicardia	NH	Dioecious

Table 5.2 Comparison of ecophysiological traits between hemiepiphytic (H) and non-hemiepiphytic (NH) *Ficus* species (values are means ± 1 SE; n = 7) and results of a two-way nested ANOVA with the factor of growth form and species nested within growth form. LA/SA, leaf area to sapwood area ratio; LS, leaf size; LMA, leaf dry mass per area; ρ_{leaf}, density of leaf lamina; SWC, leaf saturated water content; K_s, specific hydraulic conductivity; K_l, leaf specific hydraulic conductivity; A_a, light saturated net CO_2 assimilation rate on leaf area basis; ρ_{wood}, sapwood density; A_m, light saturated net CO_2 assimilation rate on dry mass basis; g_s, maximum stomatal conductance measured at midmorning; C_i, intercellular CO_2 concentration; WUE, intrinsic water-use efficiency; N (P, K) leaf N (P, K) content; PNUE, photosynthetic N use efficiency; PPUE, photosynthetic P use efficiency. "**", "*", and "ns" indicate statistical significance between the two growth forms at $P < 0.01$, $0.01 \leq P < 0.05$, and $P \geq 0.05$ levels, respectively.

Traits	H mean	NH mean	*F*	Sig.
LA/SA (cm^2 mm^{-2})	128.7 ± 18.0	115.2 ± 18.5	0.319	ns
ρ_{wood} (g cm^{-3})	0.50 ± 0.03	0.45 ± 0.03	1.850	ns
LS (cm^2)	84.8 ± 26.2	296.7 ± 106.6	4.394	*
LMA (g m^{-2})	107.0 ± 12.5	69.1 ± 6.5	8.407	**
ρ_{leaf} (g cm^{-3})	0.34 ± 0.02	0.29 ± 0.04	2.205	ns
SWC (g g^{-1})	1.88 ± 0.14	2.30 ± 0.15	4.763	*
K_s (kg m^{-1} s^{-1} MPa^{-1})	2.00 ± 0.22	7.06 ± 1.28	17.84	**
K_l (kg m^{-1} s^{-1} MPa^{-1})	2.28 ± 0.45	9.55 ± 2.71	8.184	**
A_a (μmol m^{-2} s^{-1})	13.0 ± 0.8	13.7 ± 0.8	0.383	ns
A_m (μmol g^{-1} s^{-1})	0.126 ± 0.016	0.210 ± 0.028	8.111	**
g_s (mol m^{-2} s^{-1})	0.301 ± 0.028	0.408 ± 0.021	11.016	**
C_i (μmol mol^{-1})	290.1 ± 3.5	305.2 ± 2.2	15.959	**
WUE (μmol mol^{-1})	44.6 ± 2.2	34.3 ± 1.2	19.334	**
N (%)	2.11 ± 0.18	1.69 ± 0.12	4.123	*
P (%)	0.15 ± 0.021	0.15 ± 0.009	0.011	ns
K (%)	1.70 ± 0.23	1.14 ± 0.13	5.375	*
PNUE (μmol CO_2 s^{-1} mol^{-1} N)	83.0 ± 5.9	181.6 ± 31.1	11.286	**
PPUE (μmol CO_2 s^{-1} mol^{-1} P)	2.71 ± 0.26	4.35 ± 0.60	7.285	*

Table 5.3 Results of the phylogenetic signal tests for 18 ecophysiological traits among 14 *Ficus* species and results of the principal components analysis (PCA) showing the correlation coefficients between traits and the first three PCA axes. Blomberg et al. (2003)'s *K*-statistic and *P*-value of observed vs. random variance of Phylogenetic Independent Contrasts (PICs) are calculated on $\log_{10}$-transformed data. $K > 1$, $P < 0.05$ and correlation coefficients greater than ± 0.5 are highlighted in bold face. Abbreviations are as defined in Table 5.2.

Traits	*K*-statistic	*P*-value	Correlations with PCA components		
			PCA 1	PCA 2	PCA 3
LA/SA	0.38	0.689	-0.281	0.386	**-0.732**
ρ_{wood}	0.44	0.401	**-0.583**	-0.328	**-0.551**
LS	0.66	0.175	0.440	0.128	**-0.719**
LMA	**0.81**	**0.032**	**-0.732**	-0.456	0.180
ρ_{leaf}	0.48	0.590	-0.250	-0.181	**0.727**
SWC	0.676	0.086	**0.757**	0.404	0.113
K_s	**1.18**	**0.008**	**0.845**	-0.254	0.126
K_l	**1.39**	**0.001**	**0.850**	-0.208	0.236
A_a	0.46	0.358	**0.536**	-0.089	0.403
A_m	**0.87**	**0.040**	**0.927**	0.192	-0.028
g_s	0.68	0.115	**0.896**	-0.043	0.028
C_i	0.78	0.063	**0.830**	0.302	-0.146
WUE	**1.05**	**0.011**	**-0.743**	0.064	0.235
N	0.55	0.338	-0.447	**0.714**	0.189
P	0.37	0.707	0.204	**0.935**	-0.003
K	0.71	0.056	-0.271	**0.751**	**0.523**
PNUE	**1.43**	**0.005**	**0.938**	-0.124	-0.014
PPUE	**0.95**	**0.021**	**0.840**	-0.333	0.036

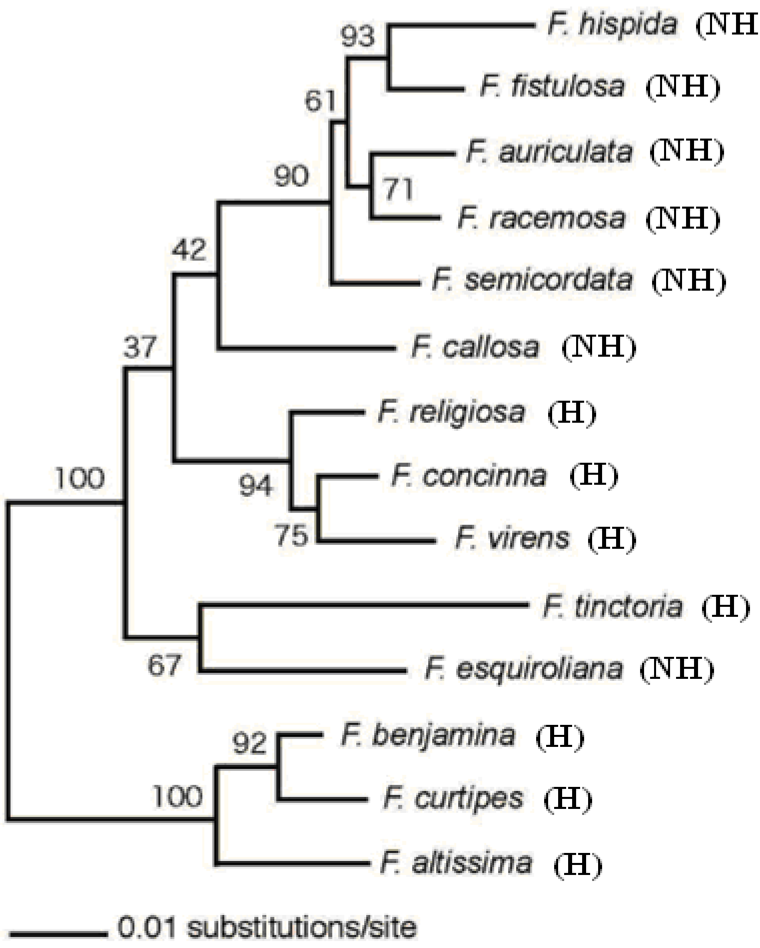

Figure 5.1 Bootstrap 50% majority-rule consensus tree constructed by ML method using the nuclear ITS sequences of 14 *Ficus* species. The bootstrap values were shown at branching points. For details, see Materials and Methods. “H” and “NH” following each species name represent for hemiepiphytic and non-hemiepiphytic growth forms.

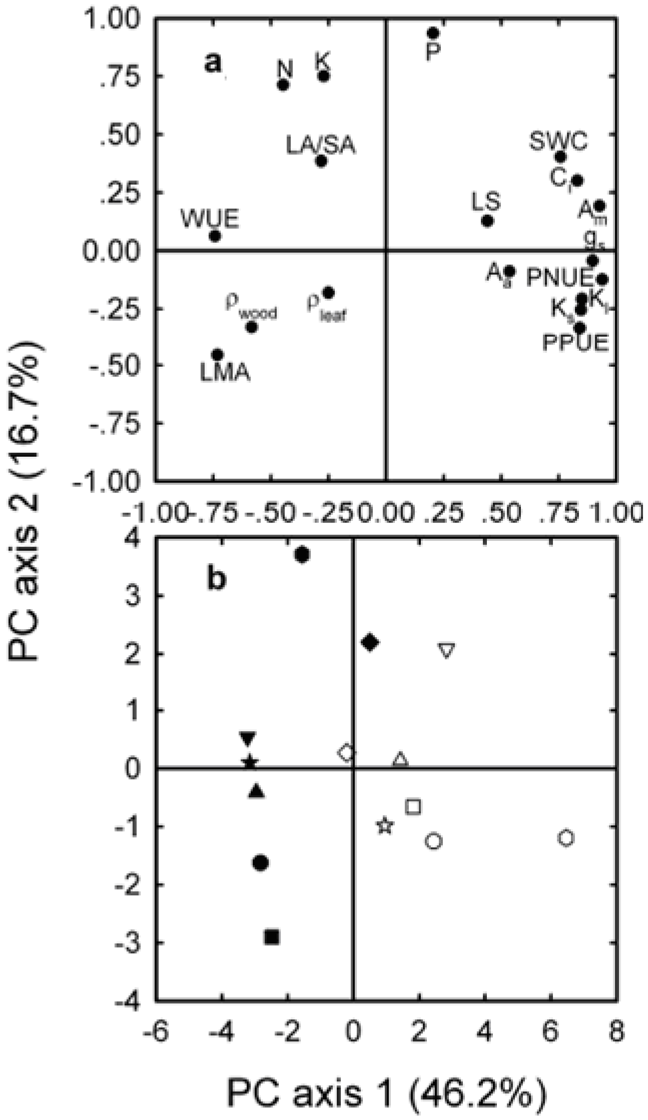

Figure 5.2 Principal component analysis (PCA) for 18 traits among 14 *Ficus* species. Loadings of plant traits and species along PCA axis 1 and 2 are shown in panel (a) and (b), respectively. Abbreviations for plants traits are the same as in Table 4. In panel b), filled and open symbols represent hemiepiphytic and non-hemiepiphytic species, respectively. *Ficus altissima* (●), *F. benjamina* (▲), *F. concinna* (▼), *F. curtipes* (■), *F. religosa* (◆), *F. tinctoria* (⬢), *F. virens* (★), *F. auriculata* (○), *F. callosa* (△), *F. esquiroliana* (▽), *F. fistulosa* (□), *F. hispida* (◇), *F. racemosa* (⬡), *F. semicordata* (☆).

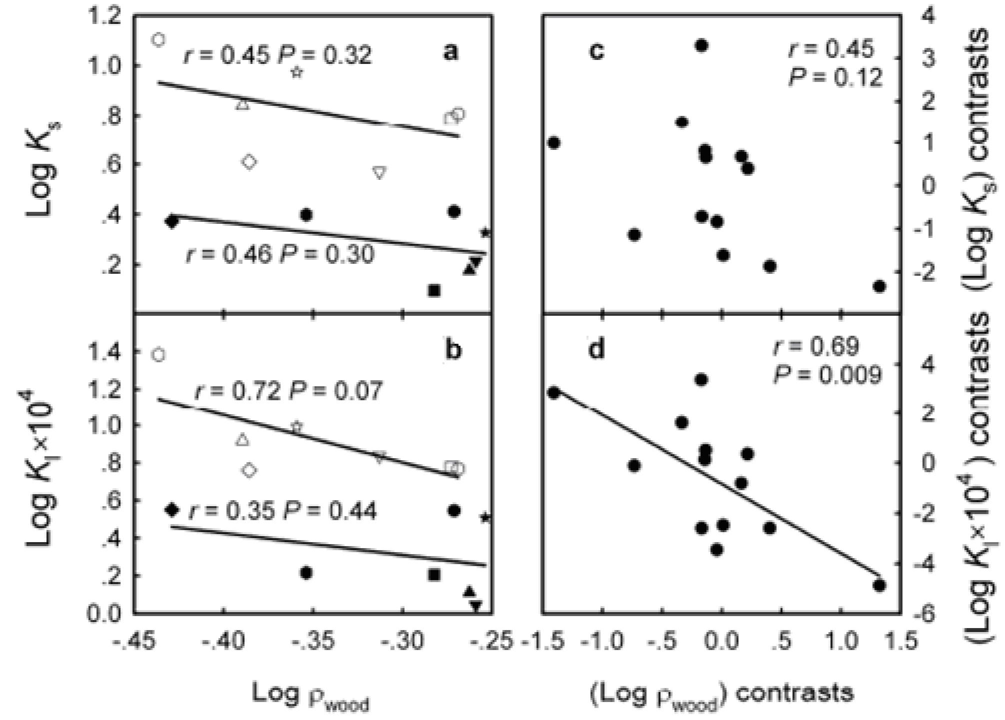

Figure 5.3 The correlations between wood density (ρ_{wood}) and (a) sapwood specific hydraulic conductivity (K_s); (b) leaf area specific hydraulic conductivity (K_l) in seven hemiepiphytic (filled symbols) and seven non-hemiepiphytic *Ficus* species (open symbols). Data are fitted separately for hemiepiphytic and non-hemiepiphytic species. Panels (c) and (d) are correlations between standardized independent contrasts of ρ_{wood} and standardized independent contrasts of K_s and K_l, respectively. Data are $\log_{10}$-transformed. Symbols are as indicated in Figure 5.1.

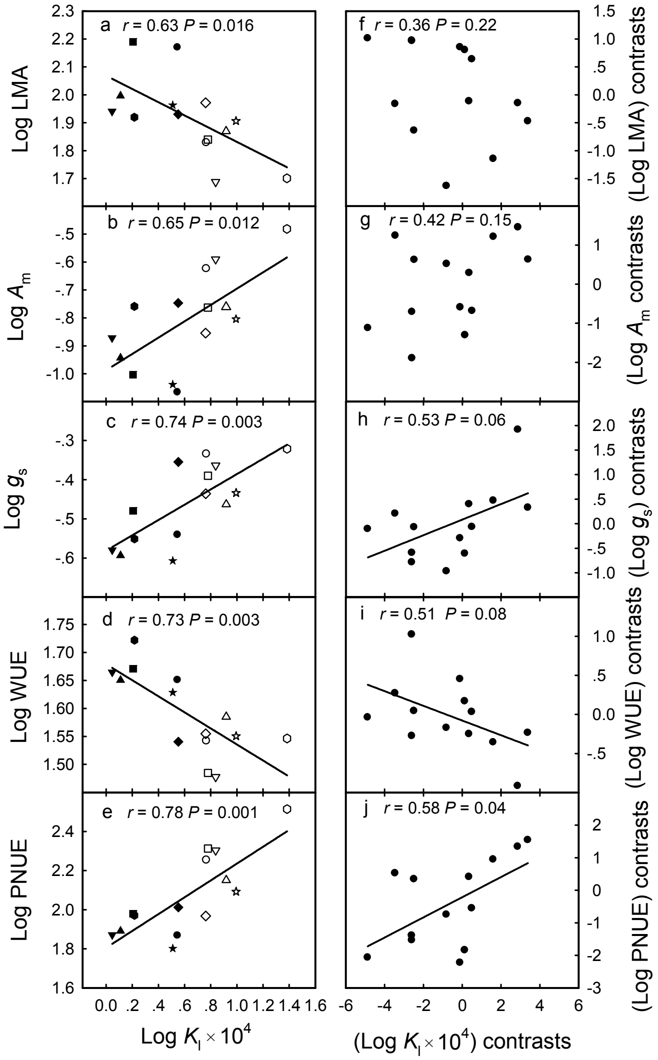

Figure 5.4 Correlations between leaf area specific hydraulic conductivity (K_l) and leaf functional traits related to hydraulics and carbon economy (i.e. leaf mass per area, LMA; maximum net CO_2 assimilation rate on mass basis, A_m; stomatal conductance, g_s; leaf water-use efficiency, WUE; and photosynthetic nitrogen use efficiency, PNUE) in seven hemiepiphytic (filled symbols) and seven non-hemiepiphytic *Ficus* species (open symbols). The left panels (a-e) show correlations of traits without considering phylogeny. Data are $\log_{10}$-transformed. Symbols are as defined in Figure 5.1. The right panels (f-j) show correlations of phylogenetic independent contrasts calculated using $\log_{-10}$ transformed data.

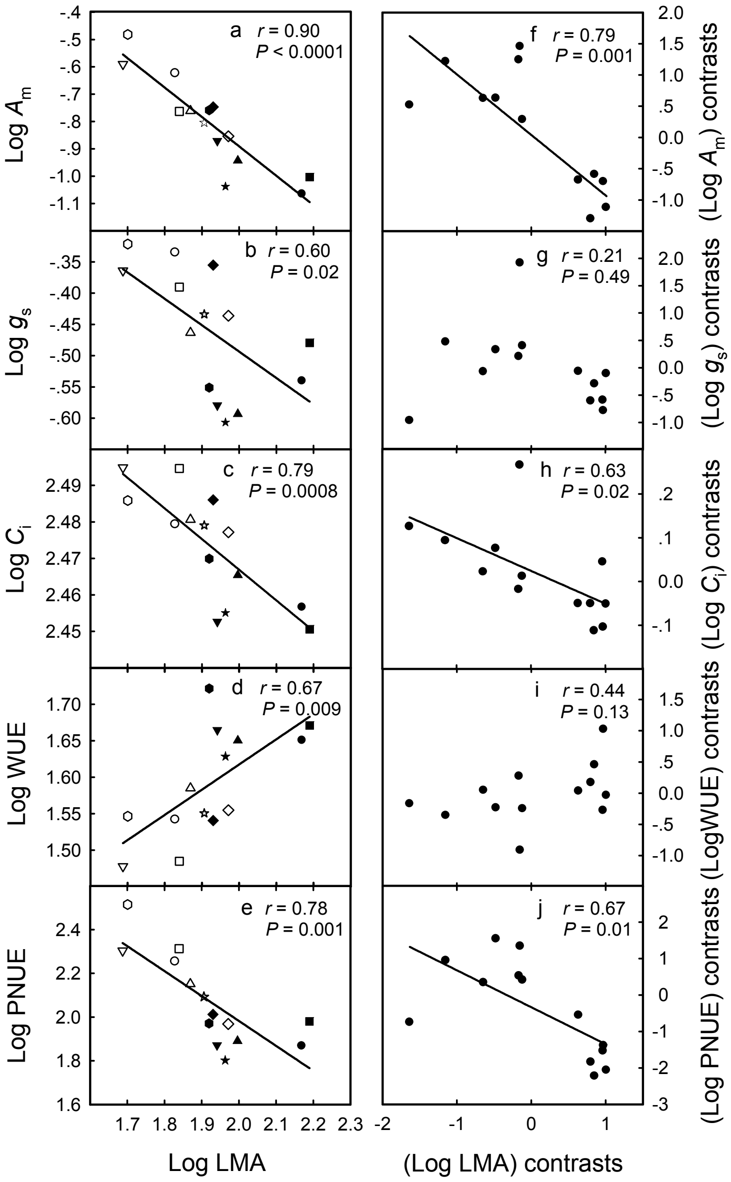

Figure 5.5 Correlations between leaf mass per area (LMA) and leaf functional traits related to hydraulics and carbon economy (i.e. leaf saturated water content, SWC; maximum net CO$_2$ assimilation rate on mass basis, A_m; stomatal conductance, g$_s$; leaf water-use efficiency, WUE; and photosynthetic nitrogen use efficiency, PNUE) in seven hemiepiphytic (filled symbols) and seven non-hemiepiphytic *Ficus* species (open symbols). The left panels (a-e) show correlations of traits without considering phylogeny. Data are log$_{10}$-transformed. Symbols are as defined in Figure 5.1. The right panels (f-j) show correlations of phylogenetic independent contrasts calculated using log$_{-10}$ transformed data.

CHAPTER 6

OVERALL CONCLUSIONS

H and NH species are different in water relations at the adult stage

Adult trees of hemiepiphytic *Ficus* species showed significantly lower stem hydraulic conductivity compared to congeneric non-hemiepiphytic species grown under similar conditions. Consistent with the difference in stem hydraulic conductivity between the two growth forms, they showed great differentiation in leaf morphological, anatomical, and physiological characteristics related to leaf water flux and drought tolerance. Compared to NH species, H species had leaves with higher LMA, thicker epidermis, tighter stomatal control over water loss, lower cuticular conductance, and more negative turgor loss point, which contribute to higher drought tolerance. On the other hand, NH species had vessels with higher lumen diameters resulting in higher xylem hydraulic conductivity, which enabled a higher stomatal conductance and more “wasteful” leaf water usage. It seems that *Ficus* species of the two growth forms had contrasting water use “strategies” with H species having more conservative water use and increased drought tolerance and NH species having increased leaf water flux but reduced drought tolerance.

H and NH species are different in photosynthesis at the adult stage

In adaptation to a drought-prone environment during the epiphytic stage, H species evolved traits for more conservative water use, and those traits still persist to a considerably large degree even during their terrestrially rooted stage. Although many traits in H species evolved mainly as an adaptation to lower water availability or lower hydraulic conductivity, such adaptive changes in leaf traits (e.g. higher LMA in H species compared to NH species) did affect leaf photochemistry and carbon economy due to hydraulic-photosynthetic coordination. Under high irradiance loads, H species tended to dissipate a higher proportion of excited light energy through non-photochemical quenching, which resulted in significantly higher light-saturated electron transport rates in NH species. Although significantly higher electron transport rates existed in NH species, their maximum net assimilation rates on area basis were not higher than H species due to the significantly higher photorespiration rates in NH species. But when net assimilation rates were calculated on leaf dry mass basis or on leaf nitrogen basis, NH species had significantly higher values than H species indicating higher efficiencies for carbon income relative to leaf construction costs.

Traits of stem xylem hydraulic conductivity and leaf water flux were highly coordinated with photosynthetic traits across the studied *Ficus* species, with H and NH species relatively well separated along these regression lines. The correlation between photosynthetic traits and hydraulic conductivity may not be direct but rather linked via a suite of other leaf traits, such as LMA and g_s, which are affected strongly by plant hydraulics and differed significantly between species of the two growth forms of *Ficus* species. It appears that the conservative water use in H species strongly influenced their carbon economy as shown by significantly lower photosynthetic N and P use efficiencies compared to NH species.

Plant functional traits are evolutionary coordinated

Phylogenetic analyses revealed that many functional characteristics of *Ficus* species were phylogenetically conserved. Nevertheless, using phylogenetically independent contrasts this study showed that many plant functional traits are evolutionarily correlated. In particular, across 14 studied species (7 H and 7 NH), a suite of traits were correlated with stem xylem hydraulic conductivity and leaf mass per area when analyzed using both ahistorical and phylogenetic independent contrasts, suggesting that these two traits are important in mediating a series of trade-offs in plant functioning. This study indicates that changes in plant water relations accompanying the evolutionary transition from terrestrial to hemiepiphytic growth habit are responsible for the great divergences in ecophysiology between Hs and NHs.

H and NH species are different in leaf hydraulic conductivity and water use at the juvenile stage

Consistent with our prediction, small saplings of H species had vessels with smaller lumen diameter, lower g_s, and higher WUE than those of NH species. Consequently, saplings of H species showed significantly lower photosynthetic capacity than those of NH species. Despite growth light levels differing substantially from full sunlight to 5% sunlight, the pattern of H species showing more conservative water use did not change. Diurnal measurements of leaf gas exchange showed that during a clear day, g_s in H species was kept at a maximum value only for a very short period during mid-morning and then decreased toward the end of the day, and diurnal courses of A_n showed a single peek. In NH species g_s was kept at a maximum value for a longer time period, from mid-morning to mid-afternoon, with an very small depression during the mid-day, and thus the diurnal courses either had a “plateau” or showed two peaks. Thus, the cumulative net CO_2 assimilation during a day was much higher in NH species compared to H species.

H species have a lower growth rate but are more drought tolerant than NH species at juvenile stage

During the juvenile stage, H species showed much lower growth rates than NH species under all growth light treatments. Especially during the first four months after germination, both height and biomass were several times higher in NH species than in H species. During a water-withholding-re-watering treatment, although maximum diurnal A_n dropped to minimum values at a similar rate in the two growth forms, A_n recovered faster in H species after re-watering. More importantly, during the water-withholding treatment NH species dropped a large proportion of their leaves but in H species almost all the leaves remained partially active through the drought period and resumed full photosynthetic activity soon after re-watering. From the point of view of carbon economy, drought can cause much greater harm to NH species than to H species. The advantage of fast growth in NH species can thus only occur under conditions of sufficient soil water supply.

H species are not more light-demanding than NH species at the juvenile stage

It is commonly believed that by growing in the forest, canopy juvenile stage hemiepiphytic *Ficus* species can benefit from higher irradiance than on the shaded forest floor, but results of the present study indicated that saplings of H species were not more light demanding than their congeneric NH species. Both H and NH species showed relatively large plasticity in functional traits in response to growth light levels with highest photosynthetic capacity occurring at high (100% or 50% sunlight) light levels, which are the same as in typical pioneer or light-demanding species. Thus both H and NH species were relatively light-demanding species, but NH species showed traits suggesting slightly higher degree of light demand than H species as indicated by their marginally higher light compensation and saturation points as well as significantly higher $A_{\max}$ than H species when grown under full sunlight.

Adaptation of hemiepiphytism in tropical forests

Our results showed that NH species are not more light-demanding than H species during the juvenile stage. All the *Ficus* species, including H and NH, have a suite of traits belonging to typical pioneer species. One of the most important traits that may strongly affect the regeneration of *Ficus* species is their tiny seed size. Seedlings at the very beginning after seed germination are very small in all species of this genus. These seedlings strongly rely on photosynthesis to survive and grow due to their very limited amount of nutrient and carbon storage in seeds. Thus it might be important for them to regenerate in places with relatively high irradiance, such as forest gaps or microhabitats in the canopy with relatively high irradiance. Seeds of H and NH species may have no differences in germination requirements, as our germination experiments showed that they can both germinate and grow in either petri dishes or directly in the soil of nursery pots, although H species had much lower growth rates during the first few months. The reason why H species usually regenerate on their host plants or rocks is likely because they died before reaching a relatively large size due to their intrinsically very low seedling growth rates. By regenerating in the forest canopy, many risks that can occur in the forest floor can be avoided. For example, damages from herbivores, flooding, covering by litter, and inter-specific competition for resources. Tolerating and surviving through harsh environmental stresses rather than fast growth and competition are more import traits and selected for in H species. While the seedlings of NH species are not able survive the drought stressed environment, such as the micro-habitats in forest canopy, even they may be dispersed to and germinate in the canopy habitats. However, their fast growth property under relatively high irradiance and more reliable soil water conditions allows NH species successfully regenerate in forest gaps. The divergence in hemiepiphytic and non-hemiepiphytic *Ficus growth* forms may have greatly contributed to the great success and high diversity of this genus in tropical forests.

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