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Is hemiepiphytism an adaptation to high irradiance? Testing seedling responses to light levels and drought in hemiepiphytic and non-hemiepiphytic *Ficus*

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The epiphytic growth habit in many *Ficus* species during their juvenile stages has commonly been hypothesized to be an adaptation for avoiding deep shade in the forest understory, but this has never been tested experimentally. We examined growth and ecophysiology in seedlings of three hemiepiphytic (Hs) and three non-hemiepiphytic (NHs) *Ficus* species grown under different irradiance levels. Both Hs and NHs exhibited characteristics of high light requiring species, such as high plasticity to growth irradiance and relatively high maximum photosynthetic assimilation rates. Diurnal measurements of leaf gas exchange showed that Hs have much shorter active photosynthetic periods than NHs; moreover, leaves of Hs have lower xylem hydraulic conductivity but stronger drought tolerance as indicated by much lower rates of leaf diebacks during the drought treatment. Seedlings of NHs had 3.3- and 13.3-fold greater height and biomass than those of Hs species after growing in the nursery for 5 months, indicating a trade-off between growth and drought tolerance due to the conflicting requirements for xylem conductivity and cavitation resistance. This study does not support the shade-avoidance hypothesis; rather, it suggests that the canopy regeneration in Hs is an adaptation to avoid alternative terrestrial growth-related risks imposed to tiny *Ficus* seedlings. The NHs with terrestrial regeneration reduce these risks by having an initial burst of growth to rapidly gain relatively large seedling sizes, while in Hs seedlings more conservative water use and greater drought tolerance for surviving the canopy environment are intrinsically associated with slow growth.

Abbreviations – HMR, height to aboveground biomass ratio; Hs, hemiepiphytic *Ficus* species; LA, leaf area; LAR, leaf area ratio; LCP, light compensation point; LMA, leaf mass per area; LMF, leaf mass fraction; LSP, light saturation point; NHs, non-hemiepiphytic *Ficus* species; PNUE, photosynthetic nitrogen use efficiency; PPFD, photosynthetic photon flux density; RGR, relative growth rates; RMF, root mass fraction; SMF, stem mass fraction; WUE, water use efficiency; XTBG, Xishuangbanna Tropical Botanical Garden.

Introduction

Irradiance is a critical factor affecting growth and survival of plants beneath the canopy of tropical and subtropical forests (Denslow and Hartshorn 1994, Fetcher et al. 1994, Chazdon et al. 1996). Incoming solar radiation is absorbed progressively by successive leaf layers and thus irradiance decreases exponentially from the top of canopy to the forest understory (Larcher 2003). In dense tropical forest only about 1–3% of sunlight reaches the understory (Chazdon 1988, Clark et al. 1996). Many tropical forest species have evolved epiphytic growth habit to escape the very low irradiance in the forest understory. Similarly, some hemiepiphytic plants, such as strangler *Ficus* species, show epiphytic growth habit during their juvenile stage. Hemiepiphytism is commonly hypothesized as an adaptation to exploit the higher irradiance in the forest canopy (Dobzhansky and Murca-Pires 1954, Ramirez 1977, Putz and Holbrook 1986, Laman 1995, Williams-Linera and Lawton 1995), but this hypothesis has not been tested experimentally. With about half of 800 species having hemiepiphytic growth habit *Ficus* is the largest genus containing woody hemiepiphytic species (Berg and Corner 2005, Harrison 2005). The coexistence of species with and without hemiepiphytic growth within this single genus makes *Ficus* an ideal system for ecophysiological comparative studies and for interpreting the adaptive significance of plant hemiepiphytism in tropical rainforests.

Ecophysiological studies on hemiepiphytic *Ficus* species (Hs) have focused on water relations (Holbrook and Putz 1996a, 1996b, Hao et al. 2010), while the potential importance of light adaptation remains largely unknown especially for juvenile plants, although it is crucial for the regeneration of these plants (Grubb 1977). Water and light availability are thought to be inversely associated in natural environments for *Ficus* regeneration (Benzing 1990, Coxson and Nadkarni 1995, Laman 1995, Holbrook and Putz 1996a, 1996b, Swagel et al. 1997), and thus these two environmental factors may interact differently in affecting seedling adaptations in species of different growth forms. Growth of Hs seedlings shows a strong positive response to light level when water is abundant (Laman 1995), but it is unknown whether sympatric non-hemiepiphytic *Ficus* species (NHs) would respond in a similar manner.

In the previous studies conducted on adult trees grown in a common garden, we found substantial differentiation in functional traits related to both water and light responses between Hs and NHs (Hao et al. 2010, 2011a, 2011b). Even greater differences are likely to occur for juveniles between the two growth forms, which experience contrasting environmental conditions when establishing under natural conditions. Here, using a subset of Hs and NHs, we conducted detailed tests of Hs and NHs in seedling ecophysiology. Comparative study on seedlings of these two growth forms grown under a gradient of irradiance levels would allow us to test whether irradiance played an important role in niche differentiation (canopy vs ground) between Hs and NHs. Furthermore, the inclusion of plant hydraulics and drought tolerance in the study enables us to evaluate the potential interactions between water and light in determining environmental adaptations of *Ficus* seedlings, considering that the two factors are often antagonistically related in regenerating habitats of *Ficus*.

We hypothesize that Hs and NHs are different in response to different growth irradiance during juvenile stage, with Hs showing characteristics of higher irradiance requirement. We further hypothesize that in Hs more conservative water use and stronger drought tolerance that are crucial for canopy regeneration have restricted their photosynthetic assimilation and growth under favorable water conditions. Contrastingly, in NHs the more prodigal water use would be related to higher growth and competitiveness but lower drought tolerance. Adaptation to water and light environmental conditions in *Ficus* is strongly coordinated in determining the characteristics of plants during the regeneration stage.

Materials and methods

Seedling growth

Seeds of three Hs (*Ficus concinna*, *Ficus tinctoria* and *Ficus virens*) and three NHs (*Ficus hispida*, *Ficus racemosa* and *Ficus semicordata*) were collected from four to six individuals in 2007 at the Xishuangbanna Tropical Botanical Garden (XTBG) of the Chinese Academy of Sciences (see Hao et al. 2010 for site description and species information). We excluded climbers and shrubs from our study and all the selected species have a tree or tree-like growth form at adult stage and are all commonly found in Xishuangbanna. In January 2008, seeds were placed in Petri dishes for germination in a growth chamber with fluorescent light and temperature controlled at 25°C. When seedlings had grown to approximately 1 cm height, they were transplanted to a nursery, which was partially shaded using neutral-density shade cloth transmitting 20% of daylight. Sufficient water and nutrients were given to seedlings during the nursery growth stage.

In June 2008 after a 5-month growth in the nursery, 10 seedlings of each species were randomly selected from the nursery for initial harvest, and seedling heights were measured, and soil was washed carefully from roots and plant dry mass was determined after oven drying for 48 h. Eighty seedlings of medium sizes for each species were transplanted to individual 7 l pots and randomly sorted into one of the four growth houses of 11 m length × 5.5 m width × 2.5 m height (20 seedlings per species per growth house). Different irradiance levels were obtained by covering the growth houses with neutral-density shade cloth of different densities (no cover for full daylight treatment). The diurnal changes of photosynthetic photon flux densities (PPFDs) in the growth houses were measured six times from the beginning through the end of the experiment using three LI-190SA quantum sensors (one 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). The irradiance treatments were 100%, 50% (measured range 47.3–51.3%), 25% (23.5–25.2%) and 5% (4.5–5.2%) sunlight. Light level of each growth house was measured once a month throughout the experimental period and no significant changes in shade level were found in all the three shaded growth houses.

All plants were watered to field capacity (i.e. until water dripped from the bottom of pots) every other day on days without rainfall. Seedlings were treated every 2 months with N–P–K slow-release fertilizer. Each growth house was divided into four areas and plants were relocated every month among areas and within each area to minimize the effect of irradiance variation within growth houses.

Leaf xylem anatomy

Leaves of six seedlings of each species under each light condition were collected during November and December of 2008 for anatomical analysis. All these leaves were newly formed and matured after seedlings transferred to different light conditions. Only one leaf was sampled from each seedling to minimize the influence of leaf loss on plant growth. After excision, leaves were sealed in plastic bags. In the laboratory, the area of individual leaves was measured using a leaf area (LA) meter (LI-3000; LI-COR) and cross-sections were taken from the petiole immediately below the lamina insertion and vessel lumen diameter and vessel numbers were determined under an optical microscope (YS100, Nikon Corp., Tokyo, Japan). LA normalized theoretical axial hydraulic conductivity of the petiole ($K_{t,}$ mmol m^{-1} s^{-1} MPa^{-1}) was calculated based on the anatomy of petiole xylem (following methods described by Hao et al. 2010).

Leaf gas exchange measurements

Light saturated net CO$_2$ assimilation rate per LA (A_n) and stomatal conductance (g_s) was measured for each irradiance treatment during midmorning on well-watered plants at the beginning of March 2009, using a portable infrared gas analyzer (LI-6400; LI-COR). Also, diurnal changes of gas exchange were measured on plants grown under full sunlight for each species during consecutive clear days. Instantaneous water use efficiency (WUE) was calculated as A_n/g_s. Measurements were made for three leaves for each of five individuals per species under each irradiance treatment. Further, light response curves of A_n were determined during the late dry season, March to April 2009 (measured on one leaf per individual, for three to five individuals per species under each irradiance treatment). All light response measurements were conducted between 08:30 and 11:30 h during clear days. Using the leaf chamber inbuilt light source, leaves were first illuminated at a PPFD of 1000 μmol m^{-2} s^{-1} until a steady state of net CO$_2$ fixation. The PPFD was then increased to 1800 μmol m^{-2} s^{-1} and light response was conducted with PPFD decreasing in a stepwise manner (1800, 1500, 1200, 1000, 800, 600, 400, 200, 100, 50, 10 and 0 μmol m^{-2} s^{-1}). The leaf cuvette temperature was maintained at 30°C and chamber CO$_2$ at ambient levels. At each irradiance level, measurement was taken after stabilization of photosynthetic rate, usually within 2–4 min.

Light response curves were fitted with the algorithm $Y = Y_{max} - ae^{-bx}$ (SIGMAPLOT 10.0; Systat Software Inc., San Jose, CA), 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 net assimilation rate (A_{max}). 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 PPFDs (0–100 μmol m^{-2} s^{-1}). The quantum yield for CO$_2$ assimilation (α_{JCO2}) was calculated as the slope of the linear part of light response curve and dark respiration rate ($R_{d\text{-}area}$) was calculated as the y-axis intercept.

Drought treatment

In March 2009, two chambers with transparent polyethylene film on top were built as a rain shelter in the open site, one of which was covered with neutral-density shade to obtain 5% daylight. The plastic shelters were built over 2 m above ground with the four radial walls open in order to maintain a similar inner temperature as the ambient air. Plants grown under full sunlight and 5% sunlight treatments were transferred to two rain shelters with corresponding irradiance levels (five randomly selected individuals per species per irradiance treatment). Under each shelter, plants were arranged into five blocks with each block containing one seedling per species. The location of the blocks and the position of the plants within each block were re-randomized each week. All pots were watered to field capacity in the evening on the day before the start of drought treatment (day zero) and weighed early in the early morning of the next day (day 1). Since then, all the pots were weighed every other day in the early morning to estimate the water loss by evapotranspiration during the 2 days. For each pot, half the amount of the lost water between the two weighing events was added and a similar rate of soil water content decrease was obtained among different plants. Plant mass change was neglected because it is much smaller compared with the soil mass. Watering was based on repeated weighing throughout the drought experiment to allow a relatively slow decrease in soil moisture over a period of 11 days, during which the average soil water content decreased from 0.55 to 0.18 g [H_2O] g^{-1} [soil]. Photosynthetic net assimilation rate was measured every 2–4 days during midmorning on each of the five plants of each species in the two irradiance treatments (using a LI-6400; CO_2, irradiance and temperature set at ambient levels). Under each irradiance treatment, drought treatment was continued until the average A_n dropped below 20% of the original maximum values.

Growth and biomass allocation

At the end of April 2009, 7–10 saplings per species under each irradiance treatment were harvested for growth analysis. Final height of plant at harvest (H_{final}) was first measured and the soil of the rhizosphere was washed out carefully, avoiding breakage of fine roots, and each plant was divided into leaves, stems and roots. Total LA of each plant was measured using a LA meter (LI-3000A; LI-COR). For each plant, newly matured leaves were selected for determination of average leaf size and bagged separately for leaf chemistry analysis. All leaves, stems and roots were oven-dried to constant weight at 70°C for at least 48 h and then the dry masses were determined.

From the above measurements, we calculated LA ratio (LAR; total LA/seedling biomass), leaf mass per area (LMA; leaf dry mass/area), leaf mass fraction (LMF; total leaf/seedling dry mass), stem mass fraction (SMF; stem/seedling dry mass), root mass fraction (RMF; root/seedling dry mass) and the height to aboveground biomass ratio (HMR; height/stem and leaf dry mass). Mean relative growth rates between initial and final harvest were calculated on both mass and height basis (RGR_{mass} and RGR_{height}) as

$$\mathrm{RGR} = \frac{\ln(X_{final}) - \ln(X_{initial})}{\text{growth time}}$$

with the plant dry mass or height.

Leaf chemistry analysis

After the measurements of dry mass, three to six newly matured leaves from each individual of each species from each irradiance treatment were ground and sent to the Biogeochemistry Laboratory of XTBG for chemical analysis. Leaf C and N concentrations were determined with an auto Kjeldahl unit (K370, BÜCHI Labortechnik AG, Flawil, Switzerland), and P and K concentrations were determined using an inductively coupled plasma atomic-emission spectrometer (IRIS Advantage-ER; Thermo Jarrell Ash Corporation, Franklin, MA) 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 phosphorus use efficiency were calculated by dividing A_n by LMA, leaf N and P concentrations, respectively.

Statistical analysis

To test the effect of growth form, species and irradiance on all the plant traits measured (Table 1), data were analyzed using a three-way nested ANOVA with growth form and irradiance level as fixed factors, and species as a random factor nested within the factor of growth form (Table 2). Data were log-transformed before analyses to improve normality and homoscedasticity (Sokal and Rohlf 1995, Markesteijn et al. 2007). A plasticity index of each trait in response to growth irradiance was calculated as the difference between the largest and smallest average values for given irradiance treatments, divided by the largest average value, and multiplied by 100% (Valladares et al. 2000). All statistical analyses were performed using SPSS 15.0.1 (SPSS Inc., Chicago, IL).

Results

Growth rates and biomass allocation

The initial growth in the nursery for the first five months resulted in significantly different seedling sizes between *Ficus* species of the two growth forms; NHs seedlings had 3.3- and 13.3-folds greater height and biomass, respectively, than Hs seedlings (Fig. 1A, B). However, over the 11-month growth period after transferred to individual pots, Hs showed higher RGR than NHs on

Table 1. Plasticity of functional traits in response to growth irradiance levels in hemiepiohytic (Hs) and non-hemieiphytic (NHs) *Ficus* seedlings after growing in different light treatments for 9–11 months. Plasticity values higher than 30% are shown in bold face.

Traits	Abbreviations	Plasticity (%)	
		Hs	NHs
Theoretical axial hydraulic conductivity of petioles	K_t (mmol m^{-1} s^{-1} MPa^{-1})	**58.9**	**56.9**
Net CO_2 assimilation rate on area basis	A_n (μmol m^{-2} s^{-1})	**37.3**	**30.7**
Net CO_2 assimilation rate on mass basis	A_m (μmol g^{-1} s^{-1})	**36.8**	**46.3**
Dark respiration rate on area basis	$R_{d\text{-}area}$ (μmol m^{-2} s^{-1})	**63.0**	**57.8**
Dark respiration rate on mass basis	$R_{d\text{-}mass}$ (μmol g^{-1} s^{-1})	**38.1**	**43.8**
Stomatal conductance	g_s (mol m^{-2} s^{-1})	25.4	25.3
Instantaneous water use efficiency (A_n/g_s)	WUE (μmol CO_2 mol^{-1} H_2O)	**43.1**	**30.0**
Maximum net assimilation rate on area basis	A_{max} (μmol m^{-2} s^{-1})	**50.0**	**51.6**
Quantum yield for CO_2 assimilation	α_{JCO2} (mol mol^{-1})	13.4	16.7
Light compensation point	LCP (μmol quanta m^{-2} s^{-1})	**67.4**	**73.0**
Light saturation point	LSP (μmol quanta m^{-2} s^{-1})	**59.3**	**56.6**
Leaf carbon percentage concentration	C (%)	5.0	6.3
Leaf nitrogen percentage concentration	N (%)	25.3	8.7
Leaf phosphorus percentage concentration	P (%)	**38.7**	**33.1**
Leaf potassium percentage concentration	K (%)	**60.3**	**54.6**
Leaf nitrogen content per unit area	N_{area} (mol m^{-2})	23.9	10.9
Leaf phosphorus content per unit area	P_{area} (μmol m^{-2})	**60.8**	**60.4**
Leaf carbon to nitrogen ratio	C/N (g g^{-1})	**46.4**	**59.7**
Leaf nitrogen to phosphorus ratio	N/P (g g^{-1})	**36.3**	**34.0**
Photosynthetic nitrogen use efficiency	PNUE (μmol CO^2 s^{-1} mol^{-1} N)	**40.5**	**43.7**
Photosynthetic phosphorus use efficiency	PPUE (mmol CO_2 s^{-1} mol^{-1} P)	**32.1**	**48.8**
Leaf size	LS (cm^2)	**55.6**	**54.9**
Leaf mass per area	LMA (g g^{-1})	**62.9**	**55.9**
Leaf area ratio	LAR (cm^2 g^{-1})	**60.3**	**62.7**
Leaf mass fraction	LMF (g g^{-1})	25.5	**33.0**
Stem mass fraction	SMF (g g^{-1})	23.3	18.3
Root mass fraction	RMF (g g^{-1})	13.5	18.1
Relative growth rate	RGR_{mass} ($\times 10^{-3}$ g g^{-1} day^{-1})	32.8	29.3
Height relative growth rate	RGR_{height} ($\times 10^{-3}$ m m^{-1} day^{-1})	19.3	24.0
Total biomass at harvest	$BIOM_{final}$ (g)	**82.1**	**68.1**
Height at harvest	H_{final} (cm)	**38.2**	28.3
Height aboveground biomass ratio	HMR (cm g^{-1})	**61.3**	**65.7**

both mass and height basis under all irradiances (Fig. S1). At final harvest, Hs had achieved heights similar to NHs but still had lower plant biomass than NHs under all four irradiances (Fig. 2A, B). The higher RGR in Hs was largely attributable to their smaller initial sizes than NHs and does not point to an advantage of Hs in realized growth given that NHs remained larger even at final harvest. Significant ontogenetic fluctuations in RGR may have occurred along the relatively long time between the two harvests; therefore, RGR may not be very meaningful for this study (Poorter 1989). We thus used the final plant biomass to infer the differences in realized growth that would affect seedling performance in the field (Sack 2004). After 11-month growth under the four irradiance treatments, biomass allocation as estimated by LMF, SMF and RMF, did not differ significantly between growth forms (Tables 2, Table S1). The Hs achieved the same height with lower aboveground biomass (i.e. higher HMR) than NHs under all irradiances (Fig. 2C). For all species, the HMR was similar among the 100, 50 and 25% daylight treatments but significantly greater under 5% daylight than under other three irradiances.

Trait variation and plasticity in response to irradiance

Most traits were strongly affected by growth irradiance (three-way nested ANOVAS; Table 2). The two growth forms exhibited similar degrees of plasticity in most traits and consistent patterns were found between the two growth forms; traits with high plasticity in Hs also had high plasticity in NHs (Table 1). Under lower growth irradiance, all species tended to have lower leaf area, normalized petiole hydraulic conductivity, net assimilation rates, respiration rates, LSP and LCP and WUE, indicating physiological acclimation to shade (Table S1). Furthermore, leaf morphology was strongly affected

Table 2. Three-way nested ANOVA with the factor of growth form (n = 2), species (nested within growth form; n = 6) and light level (n = 4). Plant functional traits *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 1.

	Growth form		Species		Light	
	F	*P*	F	*P*	F	*P*
K_t	5.79	0.074*	11.5	0.000***	13.0	0.000***
A_n	20.1	0.011**	0.951	0.462 ns	31.0	0.000***
A_m	4.77	0.094*	4.47	0.014**	40.4	0.000***
$R_{d\text{-area}}$	0.086	0.784 ns	2.71	0.070*	42.2	0.000***
$R_{d\text{-mass}}$	0.006	0.941 ns	4.27	0.017**	2.98	0.065*
g_s	6.79	0.060*	15.7	0.000***	2.65	0.087*
WUE	4.99	0.089*	7.33	0.002***	5.28	0.011**
A_{max}	6.47	0.064*	1.56	0.235 ns	9.60	0.001***
α_{JCO2}	2.08	0.223 ns	0.890	0.494 ns	0.422	0.740 ns
LCP	2.59	0.183 ns	3.60	0.030**	20.4	0.000***
LSP	6.89	0.058*	0.527	0.718 ns	12.6	0.000***
C	0.258	0.638 ns	21.3	0.000***	29.6	0.000***
N	0.065	0.811 ns	3.87	0.024**	0.861	0.483 ns
P	16.0	0.016**	1.56	0.236 ns	14.0	0.000***
K	2.06	0.225 ns	2.95	0.055*	31.3	0.000***
N_{area}	0.003	0.962 ns	4.29	0.016**	53.8	0.000 ***
P_{area}	5.14	0.086*	1.93	0.157 ns	16.9	0.000***
C/N	0.169	0.702 ns	6.55	0.003***	2.04	0.152 ns
N/P	11.8	0.026**	1.35	0.298 ns	8.95	0.001***
PNUE	1.56	0.280 ns	6.38	0.003***	24.0	0.000***
PPUE	0.496	0.520 ns	2.94	0.056*	8.39	0.002***
LS	1.67	0.266 ns	4.83	0.011**	0.590	0.631 ns
LAR	0.729	0.441 ns	6.10	0.004***	20.4	0.000***
LMA	0.029	0.874 ns	4.21	0.018**	112	0.000***
LMF	2.03	0.228 ns	3.58	0.031**	1.26	0.323 ns
SMF	2.91	0.163 ns	15.8	0.000***	1.50	0.255 ns
RMF	1.97	0.233 ns	2.53	0.084*	1.21	0.341 ns
RGR_{mass}	10.8	0.030**	12.5	0.000***	28.8	0.000***
RGR_{height}	11.3	0.028**	15.7	0.000***	0.174	0.912
$BIOM_{final}$	6.73	0.060*	7.45	0.002***	22.8	0.000***
H_{final}	0.322	0.601 ns	14.2	0.000***	0.339	0.797 ns
HMR	15.1	0.018**	6.95	0.002***	19.0	0.000***

by shade; all species tended to have larger leaves, higher LAR and lower LMA under the lower irradiance treatments. The only exception was that under 5% daylight the Hs had smaller leaves on average than under 50 and 25% daylight, which was because of the overall small seedling sizes of *F. virens* under the 5% daylight (Fig. 2A). The plant biomass at harvest was significantly affected by growth irradiance (Table 2). All species had the largest biomass under higher irradiance levels, i.e. full daylight (*F. concinna*, *F. tinctoria*, *F. virens* and *F. hispida*) or 50% daylight (*F. racemosa* and *F. semicordata*) (Fig. 2A). Final plant height, however, was not significantly affected by irradiance (Table 2).

Gas exchange under sufficient water supply

The Hs seedlings had consistently lower A_m, g_s and higher WUE but lower PNUE than those of NHs under all irradiances (Table S1), consistent with comparisons made in adult trees grown in a common garden (Hao et al. 2011b). Area-based net CO_2 assimilation rate was also lower in Hs seedlings than in NHs seedlings under all irradiance treatments.

Irradiance had a larger effect on photosynthetic light responses than the other two factors, namely growth form and species. Consistent changes in A_{max}, LCP and LSP were found in species of both growth forms across the irradiance treatments (Table S1); light saturated photosynthetic CO_2 assimilation was lower and reached saturation at lower PPFDs under shade. The Hs had marginally lower A_{max} and LSP than NHs while LCP and α_{JCO2} did not differ significantly between growth forms (three-way nested ANOVAs; Table 2).

Diurnal gas exchange measurements showed that A_n reached a maximum around 11:00 h in both growth forms. In Hs, the A_n declined gradually thereafter until the end of the day (Fig. 3B), whereas in NHs, A_n kept at high levels until around 14:00 h or even displayed

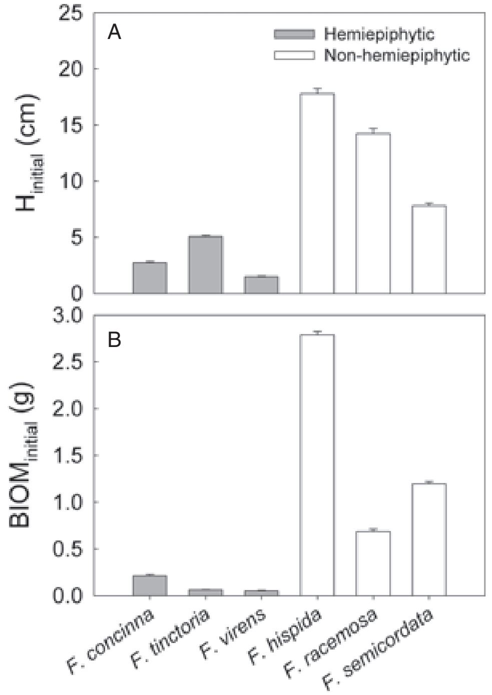

Fig. 1. (A) Initial seedling height ($H_{initial}$) and (B) biomass ($BIOM_{initial}$) of hemiepiphytic and non-hemiepiphytic species after growing in the nursery for 5 months. Error bars show +1 SE (n = 7−10).

a second afternoon peak after the midday CO_2 uptake depression (Fig. 3C). Although the daily maximum values of A_n did not differ between Hs and NHs, the cumulative CO_2 assimilation (area below the diurnal curve) during a day in NHs was 46.5% higher than for Hs, a substantial difference in total carbon assimilation, on a daily basis (Fig. 3B, C).

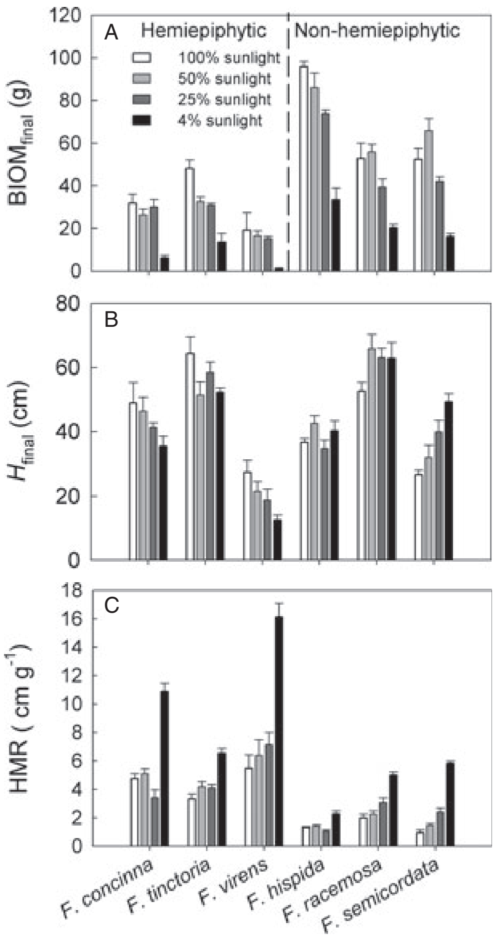

Fig. 2. (A) Plant biomass ($BIOM_{final}$), (B) height (H_{final}) and (C) height to aboveground biomass ratio (HMR) at final harvest for three hemiepiphytic (left) and non-hemiepiphytic (right) *Ficus* species grown under four irradiance levels. Error bars show +1 SE (n = 7−10).

Responses to drought

Following the drought treatment, A_n and g_s decreased gradually in both Hs and NHs. When A_n was plotted against g_s, contrastingly different patterns were seen between Hs and NHs under both irradiance treatments (Fig. 4A, B). Under a given g_s value, Hs had substantially higher A_n indicating a higher intrinsic WUE. Notably, under both high (100%) and low irradiances (5%), with the decrease of g_s following the drought treatment, the slope of the fitted curves increased in Hs indicating increases in photosynthetic WUE, whereas the correlations between A_n and g_s were nearly linear in NHs (Fig. 4A, B). Gas exchange measurements on well-watered (control) plants showed that A_n did not change significantly with time (data not shown). Drought had much stronger effects on NHs than on Hs under both high and low irradiance as indicated by the leaf loss (Fig. 5A, B). The NHs on average lost 30 and 70% of their leaves during the drought treatment under high and low irradiance, respectively, whereas Hs did not shed leaves except for a 10% leaf loss in *F. tinctoria* under full daylight (Fig. 5A, B).

Correlations between xylem water transport and plant functioning

We found a positive correlation between K_t and leaf gas exchange (g_s and A_n) in seedlings grown under different irradiance treatments (Fig. 6A, B). The negative correlation of K_t and WUE was not statistically significant, although a higher WUE in Hs than NHs was evident (Fig. 6C). Also, K_t was positively correlated with plant biomass at final harvest (Fig. 6D) indicating the coordination between water transport and CO_2 assimilation in determining seedling growth.

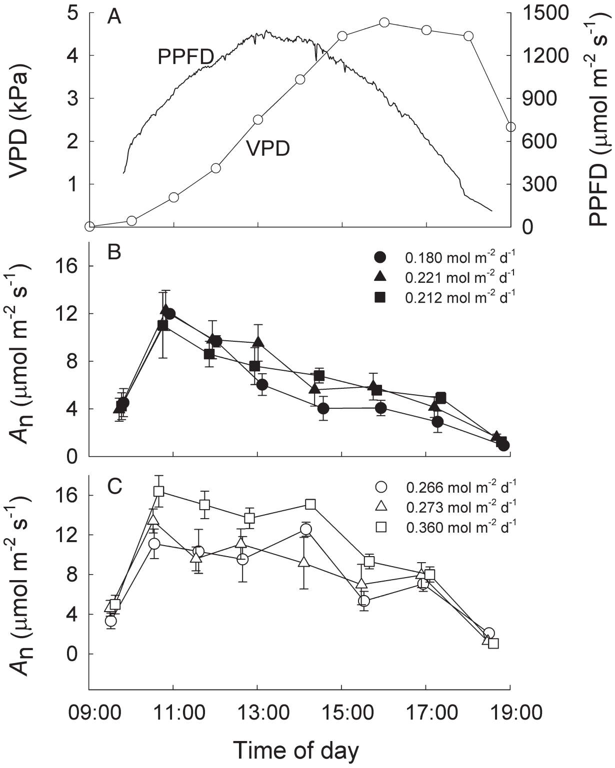

Fig. 3. (A) Diurnal courses of environmental photosynthetic photon flux density (PPFD) and air saturation vapor pressure deficit (VPD); leaf net CO_2 assimilation rate (A_n) of (B) hemiepiphytic and (C) non-hemiepiphytic *Ficus* saplings grown under full sunlight. Numbers in panel B and C indicate daily cumulative net assimilation. *Ficus concinna* (●), *Ficus tinctoria* (▲), *Ficus virens* (■), *Ficus hispida* (○), *Ficus racemosa* (▯), *Ficus semicordata* (□). Filled and open symbols indicate hemiepiphytic and non-hemiepiphytic growth form, respectively. Error bars show ±1 SE (n = 5).

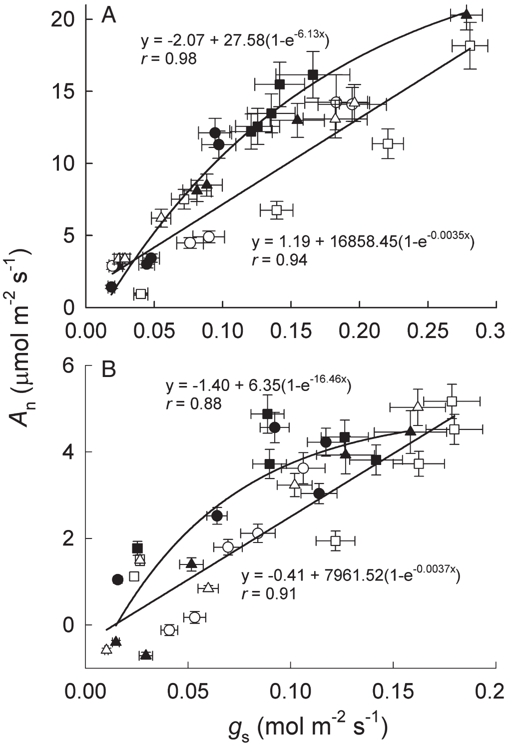

Fig. 4. Leaf net assimilation rate (A_n) vs stomatal conductance (g_s) following the drought treatment in plants grown under full sunlight (A) and 5% sunlight (B). Symbols for each species are as defined in Fig. 3. Filled and open symbols indicate hemiepiphytic and non-hemiepiphytic growth form, respectively. Error bars show ±1 SE (n = 5).

Discussion

Our study is not consistent with the conventional 'light' hypothesis for hemiepiphytic life form adaptation; seedlings of both Hs and NHs show characteristics of high light requirement. However, Hs and NHs seedlings show contrasting characteristics in growth, leaf water use and resistance to drought. Two contrasting 'strategies' are identified in *Ficus* seedlings of the two growth forms, which are likely to avoid tiny seedling size related risks: (1) slow growing epiphytic form that escapes the terrestrial-related risks at the cost of low water availability and (2) fast growing terrestrial form that gains a relatively large size in a relatively short period and becomes risk tolerant. Correspondingly, conservative and prodigal water uses have been selected respectively in these two forms of *Ficus* species, which are natively associated with contrasting photosynthetic characteristics and responses to drought.

Contrasts in seedling initial growth

A rapid initial growth would be crucial to NHs with terrestrial regeneration, which is especially true considering their tiny initial seedling sizes that are highly susceptible to physical damages from the forest ground. Larger seedlings in forest gaps are more likely to survive and eventually reach the canopy (Brown and Whitmore 1992, Boot 1996, Zagt and Werger 1998). By growing in the canopy, Hs seedlings can largely avoid these terrestrial originated damages and thus surviving the physiological stresses (i.e. low water and nutrients availability) experienced at the epiphytic stage is likely more important than fast growth and greater relative competitiveness. Seedlings of hemiepiphytes typically have low survival rates under natural conditions and only a few portions of the potential epiphytic microsites in the forest canopy are occupied (Corner 1940, Laman 1995). The struggle to succeed under strong interspecific competition in NHs seedlings may have shifted to a struggle to survive the harsh abiotic stresses in Hs, in which fast growth associated with prodigal resources use has been selected against.

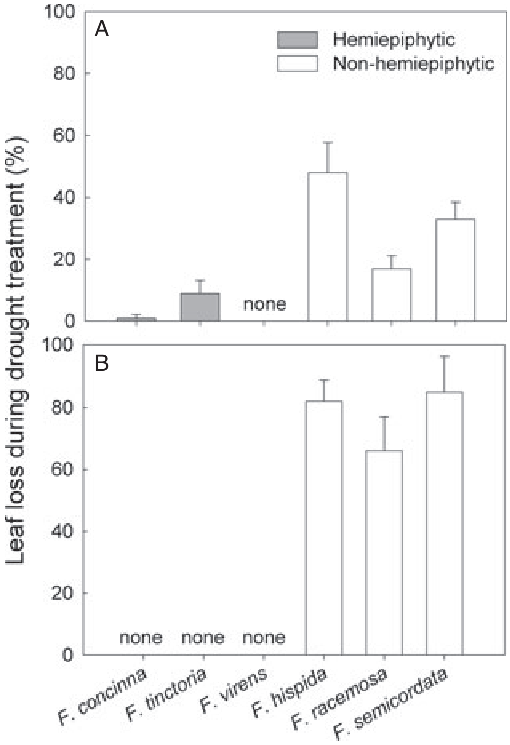

Fig. 5. Percentage of leaf loss at the end of the drought treatment for seedlings of hemiepiphytic and non-hemiepiphytic *Ficus* species grown under (A) full sunlight and (B) 5% sunlight treatment. Error bars show +1 SE (n = 5).

The substantially higher growth rates and larger seedling sizes in NHs during the first few months (nursery growth period) may be a critical factor for success, considering that small seedlings at initial stages of the life cycle are expected to be most vulnerable to risks related to the terrestrial growth. Contrasts in seedling biomass between the two growth forms, however, decreased with time. This is likely related to longer leaf lifespans in Hs seedlings (Hao personal observation), which allowed them to gain an increasingly higher LAR and LMF over time relative to NHs. Despite of this, Hs still have smaller plant biomass than NHs at the end of the experimental period.

Similarities in plastic responses to light

Light-demanding and shade-tolerant seedlings often show contrasting photosynthetic responses to irradiance but these contrasts were not found between Hs and NHs. When grown under high irradiance, A_{max} of light-demanding species are generally higher than those of shade-tolerant species (Kozlowski and Pallardy 1997, Valladares and Niinemets 2008). Under full daylight, both Hs and NHs had A_{max} values comparable with seedlings of typical light-demanding pioneer species (Oberbauer and Strain 1984, Turnbull 1991, Davies 1998). The Hs species even showed lower average A_{max} than NHs, which is opposite from what is expected if Hs were better adapted to higher irradiance. It has also been shown that early-successional or pioneer species that are light demanding for regeneration have high plasticity in physiological traits in response to growth irradiance (Bazzaz and Pickett 1980). The generally high plasticity in Hs and NHs traits may thus indicate that the two groups of *Ficus* species are both adapted to relatively high irradiance for regeneration. Similar patterns in response to irradiance have been found in Hs and NHs; physiological traits that had higher plasticity in Hs also had higher plasticity in NHs. These results further indicate that Hs and NHs are similar with respect to light adaptations.

More importantly, differences in light requirements or shade tolerance may play only a minor role in driving differences in growth rates between Hs and NHs seedlings. Instead, water deficit might have exerted the strongest selective pressure on Hs seedlings for conservative water use at a cost of low CO_2 assimilation rates as found in adult trees (Hao et al. 2010). Species that are more shade-tolerant are expected to allocate more biomass to leaves to gain maximum light and CO_2 capture with a larger LA (Poorter 1999, 2001, Markesteijn et al. 2007). However, the similarity in biomass allocation (i.e. LMF, SMF and RMF) suggested a lack of differences in light acclimation between Hs and NHs. Moreover, plants in the shade tend to have taller stem per unit stem biomass to escape from low irradiance in the understory (Sasaki and Mori 1981). Such a plastic response is especially strong in light-demanding species regenerating in gaps (Bazzaz 1979, Bazzaz and Pickett 1980, Bazzaz and Wayne 1994). All the studied *Ficus* species showed a high degree of plasticity in stem growth with HMR becoming substantially higher under low irradiance indicating acclimation for enhancing light capture. Although Hs showed a substantially higher HMR than NHs under all light treatments, this difference may not suggest adaptation to higher irradiance in Hs, but rather a lower requirement for physical support of stems compared with NHs. The Hs use host trees for support especially at the early stages of the life cycle and thus thick and strong stems or branches are not required from a biomechanical point of view.

Contrasting water use strategy and drought tolerance

Despite seedlings being grown under a wide range of irradiances, significant correlations were found

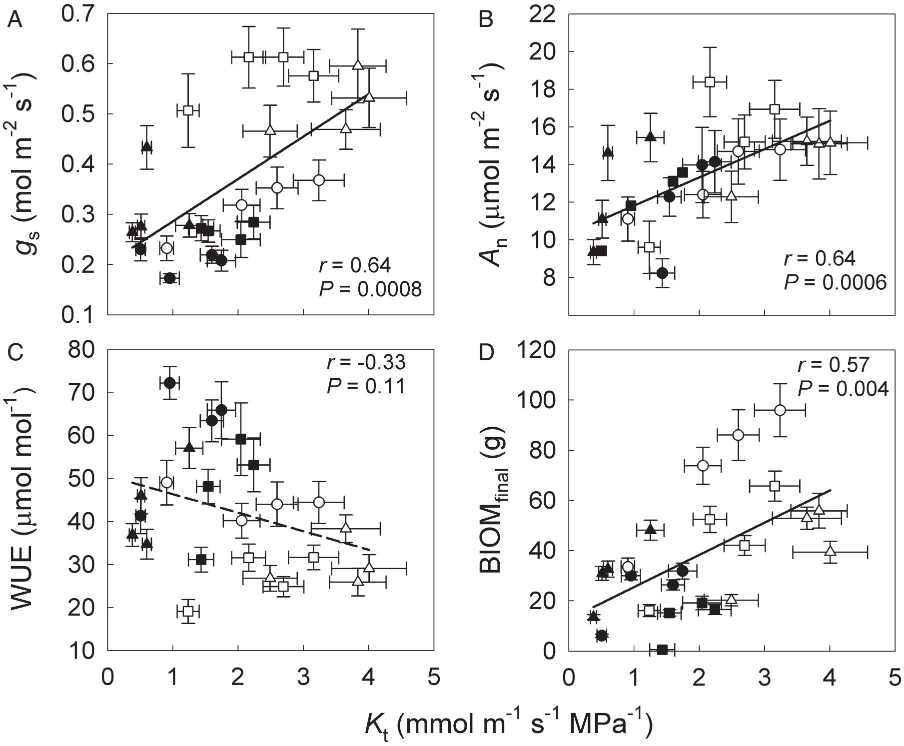

Fig. 6. Correlations between theoretical axial hydraulic conductivity of leaf petioles (K_t) and leaf functional traits related to gas exchange and plant growth. (A) maximum stomatal conductance (g_s); (B) maximum net CO_2 assimilation rate (A_n); (C) instantaneous leaf water use efficiency (WUE); and (D) final plant biomass (BIOM$_{final}$) across hemiepiphytic (filled symbols) and non-hemiepiphytic *Ficus* species (open symbols) grown under four different irradiance treatments. Symbols for each species are as defined in Fig. 3. Error bars show ±1 SE (n = 5).

between functional traits. Specifically, final plant biomass were positively correlated with xylem hydraulic conductance, indicating an intrinsic linkage between xylem water transport capacity and whole-plant performance, which is mediated by strong hydraulic-photosynthetic coordination as found in adult *Ficus* trees (Hao et al. 2011b).

The Hs and NHs were clearly distinct from each other when comparing traits related to xylem water transport and leaf water use. Higher leaf xylem hydraulic conductance in NHs indicates adaptations of the leaf vascular system to a higher transpiration demand (Sack et al. 2003), which is only advantageous under conditions of reliable water sources. The Hs had significantly higher WUE than NHs under all irradiances even when water was sufficient, suggesting constitutively conservative water use, consistent with adaptations to cope with environmental conditions in drier canopy habitats under natural regenerating conditions. The increase of WUE in Hs during the water withholding experiment is an evidence of physiological adjustments toward a more efficient water use under drought (Fig. 4A, B), whereas this adjustment is lacking in NHs. In Hs seedlings, more conservative water use and adjustments in WUE to drought have probably contributed to the protection of their leaf vascular systems from catastrophic xylem cavitation and hence avoided leaf diebacks because of hydraulic failure.

Difference in leaf gas exchanges between Hs and NHs was most significant when the diurnal courses were compared. Epiphytic stage Hs in their native habitats have been found to open their stomata only in the early morning during the dry season (Holbrook and Putz 1996b). In this study, even when plants were well watered, Hs had lower g_s and shorter active CO_2 assimilation periods than NHs, which is likely related to an intrinsically low xylem hydraulic conductivity in Hs (Hao et al. 2010). On the other hand, greater resistance to leaf wilting and loss under drought stress in Hs suggested a higher drought tolerance than NHs. The comparison between Hs and NHs is thus consistent with the hypothesized trade-off between hydraulic conductivity and resistance to hydraulic failure (Martinez-Vilalta et al. 2002, Wheeler et al. 2005). Conservative water use of Hs, although resulted in less efficient carbon assimilation, probably has contributed to their ability to survive the drought-stressed environments that are typical of epiphytic habitats.

Hemiepiphytism and *Ficus* regeneration in rainforests

The similarities in responses to growth irradiance between Hs and NHs seedlings found in this study do not support the conventional hypothesis that Hs habit is an adaptation to exploit higher light environment of the canopy. *Ficus* species in general possess typical traits of pioneer species – very small seeds, high assimilation and growth rates, high fecundity and flexible rooting habits (Harrison 2005). Besides the vertical change in the irradiance level in tropical forests, light intensity also varies considerably horizontally with much higher irradiance in gaps compared with forest understory (Nicotra et al. 1999). In relatively large gaps, pioneer or light-demanding tree species with higher RGRs and ecophysiological plasticity have competitive advantages over shade-tolerant tree species (Denslow 1980). The NHs seedlings of most species are not found in deep shade in the understory but rather in forest gaps with relatively high irradiance; Hs seedlings and saplings grow on the host trees are usually partially shaded by the host canopy before extending to high irradiance. Thus, in their natural habitats, the light conditions for seedlings of *Ficus* species of the two growth forms may not be very different, consistent with their lack of contrasting adaptations in physiology and morphology with respect to light requirements.

Epiphytic growth habit of Hs during early life stages may be an adaptation to avoid alternative risks related to terrestrial growth, such as high competition, flooding, terrestrial herbivores and coverage by falling debris (Holbrook and Putz 1996a), which are common in tropical rainforests. These risks may be especially high to *Ficus* with tiny seeds and small initial seedling sizes. To minimize these risks, two contrasting types of life history traits have been selected in this genus – terrestrial regeneration in gaps with an initial burst of growth to rapidly gain relatively large seedling sizes (NHs) and epiphytic regeneration with slow growth but enhanced ability for tolerating drought-prone canopy environment (Hs). By having epiphytic growth during their early life stage, Hs seedlings can establish in the canopy of tropical rainforests and their regeneration does not heavily depend on the formation of forest gaps as most late-successional species do. The high diversity and abundance of Hs *Ficus* species may thus be attributable, at least in part, to their canopy regeneration habit, which permits these species to inhabit forests of late-successional status despite the many traits of typical pioneer species (e.g. small seed sizes) they persist.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean values of functional traits measured under four irradiance levels.

Figure S1. Relative growth rates measured under four irradiance levels. (A) Relative mass growth rate ($\mathrm{RGR}_{\mathrm{mass}}$) and (B) relative height growth rate ($\mathrm{RGR}_{\mathrm{height}}$) of three hemiepiphytic (left) and three non-hemiepiphytic (right) *Ficus* species grown under four irradiance conditions. Error bars show +1 SE ($n = 5$).

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