Research paper

Regeneration responses to water and temperature stress drive recruitment success in hemiepiphytic fig species

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Mechanisms for surviving water and temperature stress in epiphytes are essential adaptations for successful regeneration in forest canopies. Hemiepiphytes start their life cycle as epiphytes, eventually establishing aerial root connections to the ground. This strategy allows for greater light capture, while benefitting from minimized risk of fire, flooding and damage by terrestrial herbivores, but exposes the vulnerable seedling stage to heat and drought stress. However, the response to temperature and water stress during early regeneration in hemiepiphytes is not known. In this study, we tested the effect of temperature (15/5, 25/15 and 35/25 °C; day/night diurnal variation) and water availability, as substrate moisture (0.00, −0.20 and −0.35 MPa) and water vapor (18.5–99.5% relative humidity), on seed germination, seedling emergence and survival in six hemiepiphytic and nine non-hemiepiphytic *Ficus* species. Under high-temperature conditions (35/25 °C), hemiepiphytes had higher gemination and seedling survival, achieved peak germination slower and extended germination. Greater water stress (−0.35 MPa) in the growth substrate resulted in higher germination of non-hemiepiphytes; hemiepiphytes, in contrast, took a shorter time to complete germination, but had higher seedling emergence and survival. Hemiepiphytes germinated at 99.5% relative humidity more readily compared with non-hemiepiphytes. These findings provide the first comprehensive evidence that hemiepiphytic *Ficus* species are better adapted to drier and warmer conditions during the critical transition from seed to seedling. Through greater flexibility in achieving peak germination and duration of regeneration activity, hemiepiphytes modulate their recruitment process to be more resilient under abiotic stressors. This may allow them to be more successful in regenerating in forest canopies under ambient conditions that are transient. These results support previous work showing greater drought tolerance of hemiepiphytic *Ficus* species in larger size classes and extend this finding to show that physiological adaptations for drought and heat tolerance start from the early seedling emergence stage.

Keywords: aridity, drought resilience, growth habit, heat stress, recruitment limitation, water potential.

Introduction

A greater understanding of factors that influence recruitment can significantly enhance our comprehension of the ecological processes and physiological mechanisms driving plant species assembly and survival in contrasting environments. A series of interconnected and complex demographic processes determine recruitment success (Muller-Landau et al. 2002). The regeneration process begins with germination, the transition from a quiescent or dormant seed to a germinated seed. Seedling emergence, the transition from a germinated seed to an emerged seedling, is followed by establishment, the transition from a newly emerged seedling to an autotrophic seedling (Baskin and Baskin 2014). Barriers to germination and emergence are the reasons for the majority of mortality events impacting seedling survival, defined as the seedling's ability to persist through the initial growing conditions (Zeiter et al. 2006, Eriksson and Ehrlén 2008). However, we only have a fragmented understanding of these interconnected processes (James et al. 2011, Larson et al. 2015), limiting the critical first step toward isolating underlying physiological and morphological traits that influence recruitment outcomes.

The plant regeneration process shows strong adaptation to environmental conditions, particularly to temperature and water availability, determining the ability to perform and survive in contrasting environments. This can result in differentiation among even closely related species (for e.g., in figs: Hao, et al. 2011*a*, 2011*b*, 2013). The genus *Ficus* (commonly known as figs; family Moraceae), one of the largest woody plant genera with ~800 species widely distributed from tropical and subtropical regions, is a good example of this; it consists of various growth forms differentiated as trees, shrubs, climbers, epiphytes and hemiepiphytes (Berg 1989, Berg and Corner 2005, Harrison 2005). Figs that are distributed in high latitudes and at the northern range limits may be more adapted to regeneration under cooler temperatures, whereas the tropical and subtropical species in lower latitudes may be adapted to germinate and emerge under warmer conditions (Zhang et al. 2014). Although substrate moisture can determine successful recruitment (Zhao et al. 2015), moisture in the form of relative air humidity (RH) is also an important driver of drought-resilient traits such as sap flow and water balance in epiphytic species (Darby et al. 2016). In the genus *Ficus*, studies conducted on seedlings, saplings and adult terrestrial 'tree-like' growth forms, have shown that hemiepiphytic species display a more drought-resilient cluster of traits, such as: substantially lower xylem hydraulic conductivity and stomatal conductance, higher instantaneous photosynthetic water-use efficiency, significantly smaller leaves, higher leaf mass per area and smaller xylem vessel lumen diameters (Hao, et al. 2011*a*, 2011*b*, 2013). These traits are attributed to be adaptations to drought-prone canopy growth during the recruitment of hemiepiphytes. Yet, drought adaptation has not been investigated as a mechanism determining recruitment success

of hemiepiphytes in canopy branches, when transitioning from seed to seedling, in comparison with non-hemiepiphytic (NH) forms.

Figs are rare in species assessments, showing low population densities under natural conditions, as potential microsites for colonization are strongly undersaturated (Laman 1995*a*). Although rare species such as figs comprise the bulk of the diversity and endemism in tropical forests and face greater extinction risks, their ecophysiological mechanisms that determine recruitment success are not well understood (Leitão et al. 2016, Humphreys et al. 2019). The primary limitation that determines rarity in figs could be obstacles to recruitment (Galil and Meiri 1981, Laman 1996, Harrison 2005). Both hemiepiphytic and NH growth forms have small seeds and high fecundity that are essential, in particular for hemiepiphytes, to find recruitment success in suitable host canopy branches, which are infrequent and widely dispersed recruitment opportunities (Harrison 2001, 2005, 2006). In comparison with their terrestrial counterparts, the hemiepiphytes face drier and warmer conditions due to higher light and temperature, limited soil for water retention and windy conditions in the canopy recruitment sites that could quickly desiccate regenerating juveniles (Laman 1996, Putz and Holbrook 1986, Harrison 2006). In the canopy environment, ambient conditions such as water availability after a rainfall event may be transient, providing a limited time window to complete the regeneration process. Hence, for epiphytes, the speed with which they can achieve germination and the duration taken to complete a recruitment event may be an important ecophysiological mechanism that determines their success in the aerial growth phase.

Changes in temperature and water affect the recruitment process of a species through altering seed persistence, and preventing, delaying or enhancing the processes and mechanisms of seed germination, seedling development and seedling establishment (Walck et al. 2011). Comparative empirical studies on terrestrial and epiphytic orchid species' seed traits have shown that seeds of the terrestrial species had lower water loss rates, smaller activation energies for water loss and absorbed water from lower relative humidity conditions (Yoder et al. 2000). Further, epiphytes lack the enhanced water retention capacity associated with the terrestrials (Yoder et al. 2000) and their seeds are smaller, lighter, and are more porous (Yoder et al. 2010), implying that epiphytic orchids are capable of germinating quickly under adequate substrate moisture. However, there are no empirical, comparative studies that demonstrate higher resilience to temperature and water stress in epiphytic species in the probability and the speed or timing of regeneration outcomes, especially under simulated aerial growth conditions relevant for epiphytic regeneration.

Here, we studied the effects of temperature and water availability from substrate and air humidity, two key factors that affect

regeneration (Walck et al. 2011), on the critical demographic processes driving the recruitment probability and response times in two divergent growth forms—the hemiepiphytic and NH species—in the genus *Ficus*. Focusing on congeneric species for a robust experimental design, we tested the following hypotheses. (i) The hemiepiphytes will have greater resilience, with greater probability of successful recruitment under higher temperature conditions and lower water availability compared with non-hemiepiphytes. (ii) Under greater water and temperature stress, hemiepiphytes will achieve peak germination times faster compared with non-hemiepiphytes. (iii) However, hemiepiphytes will stay in active germination longer compared with non-hemiepiphytes.

Methods

Study species and seed collection

Mature drupelets of 15 *Ficus* species (six hemiepiphytic species and nine NH species), distributed along a tropical to subtropical belt, were collected from south China from Yunnan and Guangxi provinces during 2016–19 (Table 1). The genus *Ficus* has >380 species of hemiepiphytes (Berg 1989, Berg and Corner 2005, Harrison 2005) that are located in two subgenera, *Urostigma* and *Sycidium* (Cruaud et al. 2012). Five of our six hemiepiphytic species belong to the subgenus *Urostigma*. The sixth hemiepiphyte, *Ficus tinctoria*, belongs to the subgenus *Sycidium*. Thus, in our species selection we have representative species for both subgenera containing hemiepiphytic species. The NH *Ficus* species mainly belong to five subgenera of three system branches, and our materials contain representative groups of two main branches of two subgenera (Cruaud et al. 2012).

To obtain seeds, drupelets were collected from a minimum of five trees for each species and from randomly chosen mature syconia (i.e., fruits). All species were identified to the species level and seeds were extracted by hand and cleaned with water by removing pulp and any empty seeds that floated during washing. Seeds for each species were mixed together and were air dried for a day at 25 °C. Then they were transported to Plant Ecophysiology and Evolution Laboratory of Guangxi University, where they were stored at 10 °C in paper bags until further experimentation.

To account for differences in seed lot viability, prior to each experiment, seeds were removed from storage and empty seeds and any debris were further separated from filled seeds by fanning. To test for viability after storage, a subset of seeds was germinated in agar plates made using18 g of 1300 g cm^{-2} agar dissolved in 1 l of water (Coolaber, Beijing Cool Technology Co., Beijing, China) placed in germination chambers (HTR-3X100 germination chambers, He Tian Equipment Co. Ltd, Shanghai, China; 12/12 h light/ dark conditions with photosynthetic photon flux density 400 µmol m^{-2} s^{-1} emitting visible light, i.e.,

400–700 nm; ~ 60% RH; 25 °C constant temperature) after surface sterilization using 1% sodium hypochlorite (NaClO) for 3 min (Method S1 and Figure S1 available as Supplementary data at *Tree Physiology* Online). Seed lots with >98% viability were used for the following three experiments.

The effect of temperature on regeneration responses

In June 2019, we examined the effect of fluctuating day and night temperatures on recruitment responses and response times using 15 *Ficus* species (Table 1). A total of 14,400 seeds were separated into 20 seeds per Petri dish, and under sterile conditions, each was placed on a Petri dish containing agar medium, which was prepared by using18 g of 1300 g cm^{-2} agar dissolved in 1 l of water (Coolaber, Beijing Cool Technology Co., Beijing, China). To avoid pseudo replication, each temperature condition was replicated in four independent chambers, with each chamber containing four Petri dishes for each species (Sileshi 2012). We simulated a 10 °C variation in temperature between day (12 h) and night (12 h) under three temperature treatments; day and night temperature variation of 15/5, 25/15 and 35/25 °C. These three treatments represent temperature variation observed across south China from low elevations to mountain tops.

Effect of water availability on regeneration responses

We examined the influence of water availability in the growing media on regeneration responses in February 2019 using the same 15 *Ficus* species. The successful germination under 25/15 °C 12/12 h cycle in the temperature simulation experiment was chosen as the chamber temperature condition for this experiment conducted under the same light and humidity conditions as above. To simulate differences in water availability, we used a polyethylene glycol (PEG; PEG 8000, Bio Base Laboratory Products, Jinan, shandong, China) infused plate system, which was modified from Van Der Weele et al. (2000) after preliminary testing (Method S1 available as Supplementary data at *Tree Physiology* Online). The first water availability treatment contained media with water potential (ψ_w) of 0.00 MPa, which was prepared by dissolving18 g of 1300 g cm^2 agar in 1 l of water (R Life Sciences). The second treatment was set as $\psi_w = -0.20$ MPa and the ψ_w of the third treatment was set at −0.35 MPa, which was prepared using sterilized and solidified agar solution. The first, second and third water availability treatments were overlaid with 0 g, 250 g and 350 g of dissolved PEG in 1 l of water (3:2, v/v), respectively to obtain the relevant ψ_w. The pH of the agar solution was stabilized using 2-(*N*-morpholino) ethanesulfonic acid (MES; 0.48 g in 1 l of water) and was adjusted to 5.7 pH with KOH. Once the plates were equilibrated overnight, the liquid was carefully poured out from each Petri dish allowing the solidified agar to remain. The ψ_w of the agar solutions were verified using a dewpoint potentiometer (WP4C01256, Decagon, USA) by

Table 1. Characteristics of six hemiepiphytic (H) and nine NH *Ficus* species investigated in this study[1].

Species	Adult growth form[2] (max height in m)	Distribution elevation (m a.s.l.)	Growth area	Distribution	Habit[3]	Subgenus[4]	Section[4]
Ficus auriculata Lour.	Trees (4–10)	100–2100	Forests in moist valleys	China (S Guangdong, Guangxi, SW Sichuan, SW Guizhou, Hainan, Yunnan), Bhutan, India, Myanmar, Nepal, Pakistan, Sikkim, Thailand, Vietnam	NH	Sycomorus	Neomorphe
F. beipeiensis S.S.Chang	Trees (15)	300–500	Limestone areas	China (Chongqing)	NH	Sycomorus	Neomorphe
F. hispida L.f.	Shrubs or small trees	700–1500	Along streams, plains	China (Guangdong, Guangxi, Guizhou, Hainan, Yunnan), Bhutan, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, New Guinea, Sikkim, Sri Lanka, Thailand, Vietnam, Australia	NH	Sycomorus	Sycocarpus
F. oligodon Miq.	Trees (5–10)	200–2100	Valleys, along streams, moist soil areas	China (Guangxi, Guizhou, Hainan, SE Xizang, Yunnan), Bhutan, India, Malaysia, Myanmar, Nepal, Sikkim, Thailand, Vietnam	NH	Sycomorus	Neomorphe
F. prostrata Wall.ex Miq.	Small trees	1200–1500	Dense forests	China (Yunnan), Bangladesh, NE India, Sikkim, Vietnam	NH	Sycomorus	Hemicardia
F. racemosa L.	Trees (25–30)	100–1700	Moist areas, beside rivers and streams, occasionally in streams	China (Guangxi, Guizhou, Yunnan), India, Indonesia, Myanmar, Nepal, New Guinea, Pakistan, Sri Lanka, Thailand, Vietnam, Australia	NH	Sycomorus	Sycomorus
F. semicordata Buch.-Ham. ex Sm.	Trees (3–10)	600–2800	Forest margins, valleys, along trails	China (Guangxi, Guizhou, SE Xizang, Yunnan), Bhutan, C India, Malaysia, Myanmar, Nepal, Sikkim, Thailand, Vietnam	NH	Sycomorus	Hemicardia
F. subulata Blume	Shrubs	Below 800–1600	Sparse forests	China (Guangdong, Guangxi, Guizhou, Hainan, Xizang, Yunnan), Bhutan, Indonesia, Malaysia, Myanmar, Nepal, New Guinea, Sikkim, Thailand	NH	Sycidium	Palaeomorphe
F. variegata Bl. var. chlorocarpa (Benth.) King	Trees (7–15)	Low to medium elevations	Valleys	China (Fujian, Guangdong, Guangxi, Hainan, Taiwan, S Yunnan), India, Indonesia, Japan, Malaysia, Myanmar, Philippines, Thailand, Vietnam, Australia, Pacific Islands	NH	Sycomorus	Neomorphe
F. benjamina L.	Trees (20)	500–800	Moist mixed forests	China (SW Guangdong, Guangxi, Guizhou, Hainan, S Taiwan, Yunnan), Nepal, Sikkim, Bhutan, India, Myanmar, Thailand, Vietnam, Malaysia, Philippines, Solomon Islands, N Australia	H	Urostigma	Conosycea
F. concinna (Miq.) Miq.	Trees (15–20)	900–2400	Dense forests, near villages	China (Fujian, Guangdong, Guangxi, Guizhou, S Jiangxi, SE Xizang, Yunnan, S Zhejiang), Bhutan, India, Laos, Malaysia, Myanmar, Philippines, Sikkim, Thailand, Vietnam	H	Urostigma	Urostigma
F. glaberrima Bl.	Trees (20)	500–800	Moist mixed forests	China (SW Guangdong, Guangxi, Guizhou, Hainan, S Taiwan, Yunnan), Bhutan, India, Indonesia, Myanmar, Nepal, Sikkim, Thailand, Vietnam	H	Urostigma	Conosycea
F. maclellandii var. rhododendrifolia Corner	Trees (15–20)	400–1200	Along streams, plains	China (Yunnan), Bangladesh, Bhutan, NE India, Malaysia, Myanmar, Sikkim, Thailand, Vietnam	H	Urostigma	Conosycea

(Continued)

Table 1. Continued.

Species	Adult growth form[2] (max height in m)	Distribution elevation (m a.s.l.)	Growth area	Distribution	Habit[3]	Subgenus[4]	Section[4]
F. religiosa L.	Trees (15–25)	Low to high elevations (mostly planted)	Cultivated	China (Guangdong, Guangxi, S Yunnan), native to N India, Nepal, Pakistan	H	Urostigma	Urostigma
F. tinctoria Forst. f. subsp. gibbosa (Bl.) Corner	Trees or shrubs	700–1900	Moist valleys, on rocks	China (Fujian, Guangxi, Guizhou, Hainan, Taiwan, SE Xizang, Yunnan), Bhutan, India, Indonesia, Malaysia, Myanmar, Nepal, New Guinea, Philippines, Sri Lanka, Thailand, Vietnam, Australia	H	Sycidium	Palaeomorphe

[1] Information obtained from Flora of China (Chang et al. 1998).
[2] Adult growth form was determined based on species' habit of reproduction or maturity level.
[3] Habit was determined based on regeneration strategy. All species that begin seed germination and seedling emergence in the forest canopy and later establish root connections to the ground forming strangling root trunks and take the tree or shrub like adult growth form were considered as H species. All species that begin their regeneration on the forest floor and had an adult growth form of tree or shrub were considered as NH species. Note that F. subulata adult growth form and habit are complex. The male plant is a free-standing shrub or small tree. The female plants regenerate as epiphytes closer to the forest floor and are stoloniferous climbers in their early regeneration phase that develop into shrubby epiphytic thickets. However, they do not develop root trunks as in hemiepiphytic stranglers. Hence, we have classified this species as a NH species (Corner 1976).
[4] Subgenus and section information was obtained using the phylogeny published by Cruaud et al. (2012).

using solidified agar discs (four replicates of 10 mm diameter per treatment) that were placed at 25 °C until the temperature was equilibrated. The measured variation in selected random samples of the second and third treatments ranged from −0.19 to −0.20 and −0.34 to −0.35 MPa, respectively and there was no variation observed of the $\psi_w = 0.00$ MPa treatment. Then, each agar sample was immediately transferred to the sample chamber of the potentiometer and allowed to equilibrate until a stable instrument reading was obtained. We placed a total of 14,400 seeds with 20 seeds per Petri dish, and four Petri dishes for each species and each water treatment, in four replicate germination chambers.

Effect of RH on regeneration responses

In a separate experiment, we evaluated how the water availability in the form of water vapor in air would affect the regeneration responses and response times of the same set of 15 *Ficus* species. We designed this experiment to mimic the canopy air humidity conditions from 18.5 to 99.5% RH and tested the effect of humidity on seed germination and seedling emergence inside sealable plastic containers (SPC: 15.5 cm in diameter and 8.5 cm in height; further details are in Method S2 and Figure S2-S3 available as Supplementary data at *Tree Physiology* Online). Seeds were placed in the SPC saturated with chemical and water solutions to adjust the relative humidity within each container. Nine relative humidity conditions were created using saturated solutions of a series of chemicals following Winston and Bates (1960). The chemicals and their respective RH were: NaOH 18.5%, LiCl 20.5%, $MgCl_2$ 34%, K_2CO_3 44.5%, $Mg(NO_3)_2$ 50.5%, NaCl 80.5%, KCl 90%, K_2SO_4 99% and H_2O 99.5%. The RH in still air inside the SPCs were measured using a hygrometer (Thermostat YHZ90268 Centaury Harvest, China), at saturation under room temperature (25 °C) before commencing experiments. Seeds were placed in folded tin foil boxes (1.5 cm × 1 cm), which were then placed inside a Petri dish at the top of a glass ashtray (10.3 cm in diameter and 3.5 cm in height) turned up-side-down, inside the SPC. This system allowed the seeds to be placed in tin boxes but suspended above the solutions experiencing the RH for each chemical at saturation. Each SPC for each RH level was replicated four times with each tin boat containing 50 seeds of each species totaling 27,000 seeds for this experiment. Seeds were not subjected to sterilization as in the previous experiments to avoid water absorption into the seed through an imbibition response. This procedure ensured that the only water available for seed germination during this experiment was through absorption from air humidity.

Assessment of regeneration responses

For all experiments, *Ficus* seeds that successfully reached or passed each life stage were scored as a successful event. Those individuals that failed to reach or pass the same event

were scored as a failure. Seeds were scored as germinated when the emerging radicle length was at least 1 mm long. Seedling emergence was determined when germinated seeds showed the hypocotyl growth followed by the expansion of two cotyledons. Emerged seedlings were considered as survived if cotyledons turned green and did not wither or die until the end of the experimental period of 90 days. Seedlings were confirmed as dead if observed as dried, decayed or turned gray or black in color and remained so for 20 days after completing the experiment. For all experiments, except the RH experiment, we assessed germination success, seedling emergence and seedling survival once in every 2 days in the first 60 days and every 4 days thereafter for the total experimental duration of 90 days. For the RH experiment, we assessed germination success and seedling emergence up to 60 days and scored the final germinated seeds and emerged seedlings, and also monitored fungal growth as it occurred in the 99.5% RH condition. Once no new germination events occurred, seeds were scored as viable if they germinated or if seeds were firm, with white embryos, and brown and shiny seed coat. Seeds were scored as unviable if seeds did not germinate and showed deterioration, or were dry with black or gray seed coat and inner material was found dissolved upon applying pressure. All calculations below were conducted on viable seeds following exact final counts after completing each experiment.

Calculation of regeneration responses

All regeneration response data were calculated at the growth chamber level.

Regeneration response probabilities Regeneration response probabilities were calculated for each regeneration stage by using binary response variables (1 = reached or passed the life stage, 0 = failed to reach or pass the life stage) for the specific treatment being assessed. The probability of seed germination (P_G) was calculated as the ratio between the number of seeds that successfully reached or passed seed germination and the total number of seeds sown. The probability of seedling emergence (P_E) was calculated as the ratio between the number of emerged seedlings and the total number of seeds sown. Likewise, the probability of cumulative seedling survival (P_{CSS}) was calculated as the ratio between the number of survived seedlings and total number of seeds sown. Probability data are presented as values between 0–1. We calculated the binary response for regeneration probability using the *cbind* function in R (version 3.6.1; R Development Core Team 2019). For the RH experiment we limited our calculations to P_G and P_E.

Regeneration response times Regeneration response times were calculated for germination and emergence as follows. The time (d) duration of germination activity (GT_A: the number

of days from the first successful germination event to the last successful germination event during the experiment), time (d) to reach peak germination (GT_P: the number of days to achieve the highest daily germination rate from the start of the experiment), and time (d) taken for 50% seedling emergence of total viable seeds sown (ET_{50}) were assessed using scored germination and seedling emergence time data. In instances where <50% seedling emergence was detected during the experimental period, ET_{50} values were replaced by the emergence percentages at 90 days, the longest duration of the experiment.

Statistical analysis

All statistical analyses were conducted in R (version 3.6.1; R Development Core Team 2019) using *glmmTMB* and *lme4* package. Figures were produced through the *ggplot2* package (Wickham 2009). To assess the relative importance of factors affecting P_G, P_E and P_{CSS}, we used generalized linear mixed models with temperature, water availability, growth form of the species as categorical fixed factors and species as a random factor assuming binomial distribution and a log-link function (Sileshi 2012, Hay et al. 2014). We used linear mixed-effect models to assess the effect of the same fixed factors on GT_P, ET_{50} (log$_2$-transformed for all three) and GT_A (sqrt-transformed), assuming normal distribution. Each main experiment was analyzed separately. The full model had the following form:

$$Y \sim \beta_0 + \beta_1 GC_i + \beta_2 GF + \beta_3 GC_i GF + \varepsilon_{sp} + \varepsilon_{residual},$$

where Y is the regeneration response (P_G, P_E, P_{CSS}, GT_P, GT_A or ET_{50}), GC_i is the regression parameter for the growth condition (temperature or water availability), GF is species' growth form (hemiepiphytic or NH), GC_iGF is the interaction of each growth condition with the species' growth form. The random effect of species is denoted by ε_{sp} and $\varepsilon_{residual}$ is the residual error.

All our global models were simple models not constrained by the influence of multiple variables on the response variable (Grueber et al. 2011). Therefore, we retained the global model as our final model for all experiments and for all response variables. In all experiments for all response variables assessed, we assessed the best-fit model among the binomial model, observation-level random effects model and the beta-binomial model using Akaike's information criterion (ΔAIC) value. We found that the beta-binomial models, which quantifies and models the excess variation from overdispersion, were the best to explain the variation in our data (Harrison 2015). Models were evaluated using the variance inflation factor <2 for collinearity among model predictor variables using the *car* package (Fox and Weisberg 2019). We evaluated model fit to the full data sets using marginal and conditional R^2 values (R^2 marginal and R^2 conditional, respectively), developed for mixed effect models
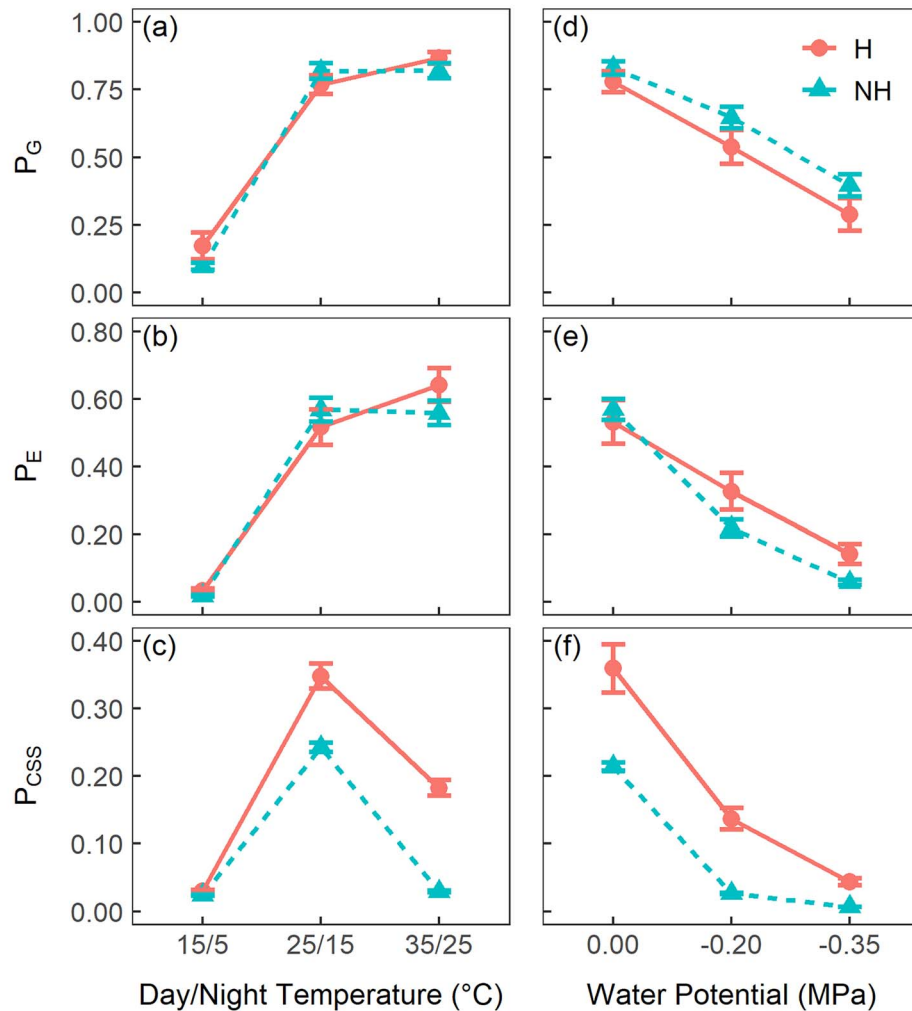
Figure 1. The effects of variation in simulated atmospheric temperature and water potential on regeneration responses of six hemiepiphytic (H: circles connected with solid line) and nine NH (NH: triangles connected with dashed lines) species of *Ficus* from Southwest China. The effect of fluctuating day and night temperature on (a) probability of seed germination—P_G, (b) probability of seedling emergence—P_E and (c) probability of cumulative seedling survival—P_{CSS} are shown. The response variable water potential (d–f) is identically shown in the left panels.

(Nakagawa and Schielzeth 2013). Within the context of each fitted full model, the influence of fixed factors was tested using the *car* package.

Results

Using a total of 55,800 seeds, we evaluated the influence of two major environmental parameters, i.e., temperature and water availability on seed germination, seedling emergence and subsequent survival of 15 *Ficus* species from south China. In all three experiments, we saw significant differences in regeneration responses and response times that were partitioned across each temperature and water availability condition, allowing for differentiation in timing and abundance of germinated seeds and emerged seedlings (Figures 1 and 2, and Figure S1 available as Supplementary data at *Tree Physiology* Online).

Effect of fluctuating day and night temperature on regeneration responses

As species progressed in the regeneration process, the probability of success at arriving or passing each life stage declined (germination, seedling emergence and seedling survival percentages in 35/25 °C = 85.24 ± 2.04, 59.07 ± 3.28 and 11.11 ± 2.45; 25/15 °C = 80.92 ± 2.45, 54.45 ± 3.57 and 27.15 ± 2.38; and 15/5 °C = 15.26 ± 3.14, 4.13 ± 1.34 and 4.13 ± 1.34, respectively). Generally, hemiepiphytic species, compared with NH species, were more resilient during the regeneration process when experiencing higher temperature conditions (Figure 1a–c). Temperature affected on P_G, P_E and P_{CSS} of all species (all $P < 0.0001$; Table 2) with these responses being fairly well explained by the variation in temperature and species growth form across all species ($R^2 = 0.69$, 0.64 and 0.35, respectively, at least $P < 0.05$ in all cases,
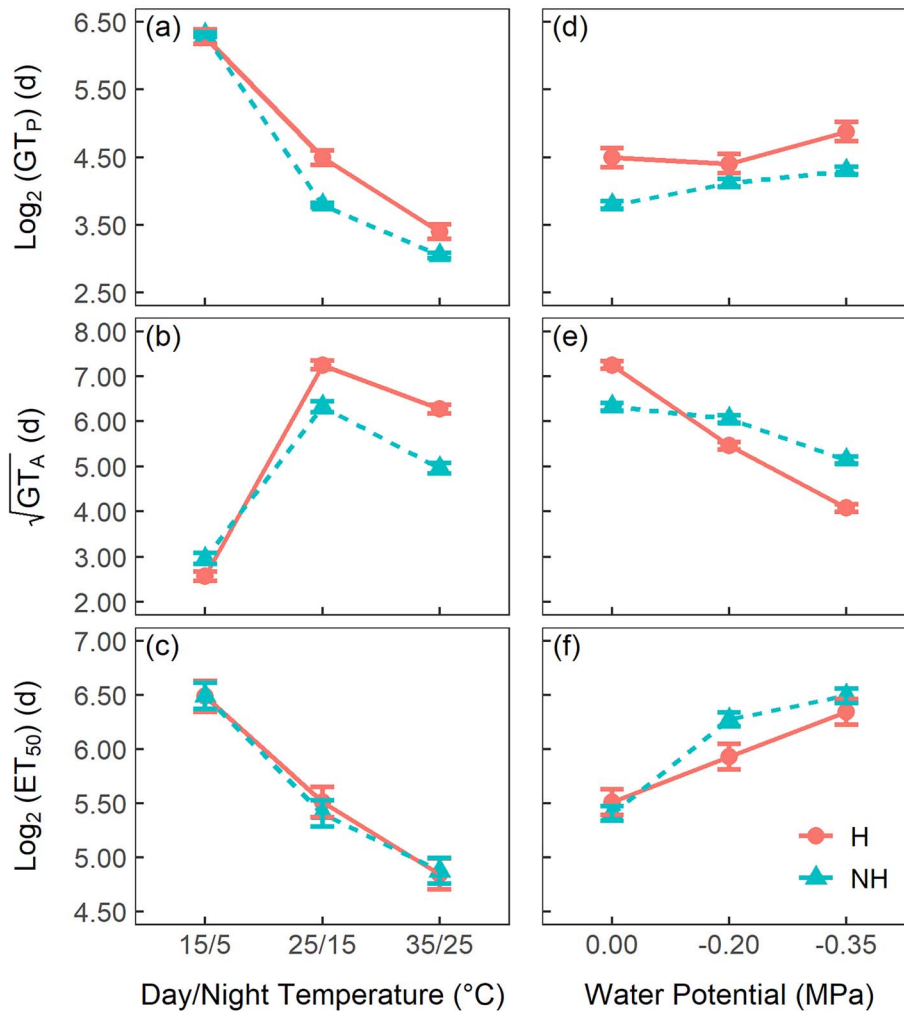
Figure 2. The effects of variation in simulated atmospheric temperature and water potential on regeneration responses of six hemiepiphytic (H: circles connected with solid line) and nine NH (NH: triangles connected with dashed lines) species of *Ficus* from Southwest China. The effect of fluctuating day and night temperature on (a) time of days taken to achieve seed germination peak—$Log_2 (GT_P)$, (b) duration of seed germination activity—$\sqrt{GT_A}$ and (c) time of days taken to achieve 50% seedling emergence—$Log_2 (ET_{50})$ are shown. The response variable water potential (d–f) is identically shown in the left panels.

Table S1a available as Supplementary data at *Tree Physiology* Online). Greater probability of seedling survival of hemiepiphytic species compared with NH species was observed in 35/25 and 15/5 °C conditions ($P = 0.0070$ and 0.0018, respectively, Figure 1a–c, Table 2 and Table S1 available as Supplementary data at *Tree Physiology* Online). Irrespective of the species growth form, the best condition for fast achieving ET_{50} was 35/25 °C condition ($P < 0.0001$; Figure 2c and Table S1a available as Supplementary data at *Tree Physiology* Online). There were also interaction effects between species growth form and temperature condition in GT_P and GT_A (Figure 2a and b); the NH species had greater decrease in GT_P from 15/5 to 25/15 °C condition but hemiepiphytic species had greater decrease in GT_P when temperature further increased in the 35/25 °C condition ($P = 0.0028$; Figure 2a and Table S1a

available as Supplementary data at *Tree Physiology* Online). The increase in GT_A for hemiepiphytic species was greater compared with NH species when temperature increased from 15/5 to 35/25 °C condition ($P = 0.0287$; Figure 2b and Table S1a available as Supplementary data at *Tree Physiology* Online).

Only the P_G model indicated that species, specified as a random factor, had significant explanatory power for the variation in our data in this experiment (P_G, P_E to P_{CSS} R^2 conditional—R^2 marginal = 0.21, 0.13 and 0.04, respectively; Table S1a available as Supplementary data at *Tree Physiology* Online). At 35/25 °C all species showed >50% germination and cumulative survival percentage was >10 for six species: *Ficus maclellandii*, *Ficus glaberrima*, *Ficus benjamina*, *Ficus concinna*, *F. tinctoria* and *Ficus semicordata*. With >10% cumulative seedling survival, the resilient species at 15/5 °C were, *F. concinna*,

Table 2. Chi-square and P-values for the effects of temperature, water availability, species' habit and their interaction on the regeneration response probabilities (P_G = seed germination, P_E = seedling emergence, P_{CSS} = cumulative seedling survival) and regeneration response time (GT_P = time taken to achieve the highest daily germination count, GT_A = duration of germination, ET_{50} = time for 50% seedling emergence, all data transformed). Responses depicted in bold are significant at $P < 0.05$.

Experiment	Factor	χ^2	P	χ^2	P	χ^2	P
		P_G		P_E		P_{CSS}	
Fluctuating day and night temperature							
	Temperature	613.85	**<0.0001**	299.94	**<0.0001**	96.92	**<0.0001**
	Habit	0.07	0.7811	0.03	0.8699	7.12	**0.0076**
	Temperature*Habit	7.22	**0.0270**	7.45	**0.0241**	11.47	**0.0032**
Water availability							
	Water	405.39	**<0.0001**	283.11	**<0.0001**	162.65	**<0.0001**
	Habit	0.34	0.5628	0.02	0.8892	4.86	**0.0275**
	Water*Habit	5.75	0.0564	4.43	0.1090	7.70	**0.0212**
	Factor	$\mathrm{Log}_2\,(GT_P)$		$\sqrt{GT_A}$		$\mathrm{Log}_2\,(ET_{50})$	
Fluctuating day and night temperature							
	Temperature	3219.29	**<0.0001**	110.49	**<0.0001**	158.64	**<0.0001**
	Habit	2.43	0.1191	1.30	0.2540	0.00	0.9551
	Temperature*Habit	40.46	**<0.0001**	5.36	**0.0685**	0.30	0.8617
Water availability							
	Water	51.37	**<0.0001**	53.61	**<0.0001**	101.28	**<0.0001**
	Habit	3.21	0.0734	0.41	0.5245	0.20	0.6582
	Water*Habit	10.37	**0.0056**	14.22	**0.0008**	4.81	0.0905

F. racemosa and *F. benjamina* (Figures S2–S4 available as Supplementary data at *Tree Physiology* Online).

Effect of water availability on regeneration responses

Overall, as species progressed in the regeneration process, the probability of P_G, P_E and P_{CSS} declined with greater water stress with 0.00 MPa being the best moisture condition for successful recruitment (germination, seedling emergence and seedling survival percentages were 81.25 ± 2.41, 54.87 ± 3.57 and 27.45 ± 2.43, respectively; Figure 1d–f). Generally, hemiepiphytic species, compared with NH species, were more resilient during the regeneration process when experiencing greater water stress resulting in higher P_{CSS} ($P = 0.0275$; Figure 1d–f and Table 2).

With increasing water stress NH species showed slightly higher P_G but this was reversed for P_E, with hemiepiphytic species showing greater success regeneration progressed to emergence and survival of seedlings (Figure 1d and e). Finally, when seedling survival was assessed hemiepiphytic species were clearly more likely to complete the regeneration process compared with the NH species under all water availability conditions ($P = 0.0275$; Figure 1d–f, Table 2 and Table S1b available as Supplementary data at *Tree Physiology* Online). The NH species achieved GT_P faster than hemiepiphytic species, but GT_A was longer among the NH species under water stress condition at −0.35 MPa ($P = 0.0056$ and $P = 0.0008$ respectively; Figure 2d and e and Table 2). The opposite trend was observed under no water stress. i.e., at 0.00 MPa (all $P < 0.0056$).

All our models indicated that species, specified as a random factor, provided lower explanatory power for the variation in P_G, P_E, P_{CSS} and P_{CSS} in the water availability experiment compared with the temperature experiment (R^2conditional—R^2marginal = 0.32, 0.25, 0.08; respectively; Table S1b available as Supplementary data at *Tree Physiology* Online). Based on species' regeneration responses and their final seedling survival, the hemiepiphytic species *F. concinna* could be considered as the most resilient to increased water stress with >10% of germinated seeds resulting in successful seedling survival at the strongest water stress, −0.35 MPa. At marginal water stress (−0.20 MPa), three other hemiepiphytic species, *F. benjamina*, *F. concinna* and *F. maclellandii*, were resilient to water stress with > 10% of germinated seeds and followed by successful seedling survival (Figures S5–S7 available as Supplementary data at *Tree Physiology* Online).

In the RH experiment assessing how water availability in the form of air humidity would affect P_G, P_E of hemiepiphytic and NH *Ficus* seeds, only the seeds placed at the 100% RH germinated, which began 10 days after the experiment was set up. Data pooled for all samples confirmed that at 100% RH, hemiepiphytic species had greater P_G and P_E compared with NH species (all $P < 0.0470$; Figure S1a and b, and Tables S1c and S2 available as Supplementary data at *Tree Physiology* Online). Because of fungal growth that occurred during this experiment, we analyzed the regeneration response of the seeds with and without fungi growth, which showed that regardless of the presence of fungi, the hemiepiphytic species' probability of seed germination was significantly higher

than NH species (P = 0.0063; Figure S1c–d and Tables S1d and S2 available as Supplementary data at *Tree Physiology* Online). However, seedling emergence was only observed in hemiepiphytes but was not facilitated by the presence of fungal mycelia (P = 0.0263; Figure S1b, and Table S1c and S2 available as Supplementary data at *Tree Physiology* Online).

Discussion

Epiphytic growth in forest canopies requires adaptations for survival under water and temperature stress, especially for the successful completion of the regeneration process. The genus *Ficus*, which has approximate 800 species, contains close to 380 species that are primary hemiepiphytes (Putz and Holbrook 1986, Berg and Corner 2005, Harrison 2005). They start their life cycle as epiphytes, eventually establishing aerial root connections to the ground (Kress 1986). This strategy benefits the seedlings allowing them greater light capture, minimizing risk of fire, flooding and damage by terrestrial herbivores (Holbrook and Putz 1996a). However, the canopy conditions render them more vulnerable to greater heat and drought stress.

Our study of the *Ficus* recruitment process assessing seed germination, seedling emergence and survival responses as well as response times, showed that hemiepiphytic species have greater probability of successful recruitment when faced with water and temperature stress compared with their NH counterparts. These results support our first hypothesis and complement the findings from later life history stages, i.e., seedlings, saplings and their terrestrial 'tree like' growth forms in *Ficus* species; pot grown 5-month-old seedlings of both life forms have high drought tolerance with more conservative use of water, but the hemiepiphytic figs were more tolerant to drought as indicated by their leaf traits even after transitioning from hemiepiphytic to terrestrial form (Hao et al. 2010). Our study adds evidence to hemiepiphytic species' adaptation to drought as shaping the habitat divergences between these two groups (Hao et al. 2013). Our study takes a step further, examining the drought tolerance hypothesis at the very beginning of regeneration process in these two groups, and we clearly demonstrate that hemiepiphytic figs have greater probability of successful recruitment under water and temperature stress during germination and seedling emergence, resulting in greater survival. Greater resilience to drought and temperature stress in hemiepiphytes, especially during the crucial regeneration phases (Eriksson and Ehrlén 2008), can provide greater success for recruitment in the canopy (Whigham et al. 2008).

Support for our second and third hypotheses was complex and depended on the abiotic stress, whether it was water or temperature. In our study, the proportion of emerged seedlings were the highest at the highest temperature condition. Although high temperature can promote germination, the high rate of water loss from the substrate at the high-temperature conditions

could be more limiting for seedling growth (Laman 1995b). Under temperature stress, hemiepiphytic species achieved peak germination slower and completed the active germination period in a longer time, whereas their peak germination remained the same under water stress but they took shorter time to complete the regeneration activity. In comparison with other tropical plants, *Ficus* species in general occupy typical traits of pioneer species with very small seeds and high growth rates (Harrison 2005). The ability to quickly secure growing space can be advantages for plant growth when competing for scares resources or when under biotic and abiotic stressors (Goodale et al. 2012, 2014). However, a quick response to current conditions can be detrimental if growing conditions quickly become adverse. Our results complements the findings from Hao et al. (2013), where 5-month-old seedlings of hemiepiphytic *Ficus* species were more conservative and slower growing compared with the non-hemiepiphytes they evaluated. They postulated that the ability to quickly achieve greater height and biomass was adaptive for the non-hemiepiphytes to avoid challenges in the understory.

Our results provide a more detailed understanding of the regeneration process. The data obtained from the very beginning of the regeneration process confirm the epiphytic and terrestrial germination contrast observed in dust-like seeds of orchids, where epiphytic orchids were capable of germinating quickly if there was adequate moisture in the substrate (Yoder et al. 2000). The ability to quickly germinate and become a seedling and secure the growing space on the canopy of a suitable microsite is essential for the successful recruitment of hemiepiphytes, as they are largely dependent on animal dispersers to find suitable microsites, which are probabilistically limited when combined with suitability of temperature and water availability for germination and emergence (Holbrook and Putz 1996b, Laman 1995b). However, under temperature stress, the hemiepiphytes seem to be able to reverse this strategy and extend the germination activity period, potentially allowing greater number of seeds to succeed under changing conditions. For the hemiepiphytic members of this genus, adaptations to regeneration in the epiphytic stage are likely more important than faster growth and greater relative competitiveness in the forest understory.

We found that water was a significant factor that affected the regeneration responses of *Ficus* species. These findings are in parallel to the previous results that water was the most important limiting factor in the life cycle of *Ficus* (Holbrook and Putz 1996b, Laman 1995b). Both hemiepiphytic and NH species showed low regeneration probabilities under greater water stress (−0.35 MPa). Although hemiepiphytic species had lower germination probability compared with NH species, they did better at seedling emergence and survival compared with the NH species. These observations of 15 species significantly expand the seed germination study in *Ficus virens* that reduce

seed germination at low water potential (−1.2 MPa, Ji et al. 2018). Hao et al. (2010) indicated that hemiepiphytic species possess traits linked with greater drought tolerance compared with NH species, which is consistent with a conservative water use that would reduce the risk of catastrophic hydraulic failure under drought stress by effective stomatal closure (Brodribb and Holbrook 2004). Furthermore, forest canopies lose water easily due to strong winds on treetops. Thus, hemiepiphytic species are probably better able to preserve cell water during drought, which is on a par with our observation that hemiepiphytic species took a longer time to achieve peak germination, but spent a shorter time duration to complete active germination in contrast to NH counterparts.

Recent climate change models suggest that ambient temperature in humid tropics as well as in the summers in subtropical climates may increase, with the intensity of winter temperature also further increasing, especially at high elevations, associated with less predictable changes in rainfall and other climatic parameters (Deutsch et al. 2008, Walck et al. 2011, Feng et al. 2013, IPCC 2013). Our results suggest that under lower temperature conditions, the probabilities of germination and emergence were reduced for all *Ficus* species, resulting in very low probability of survival, indicating that seed germination and seedling emergence are primarily limited by cool temperatures. Therefore, even the *Ficus* species that have a year-round seed production may not germinate during cool temperatures, limiting their recruitment to warmer tropical conditions, which are under greater threat from habitat loss (ter Steege et al. 2015). In contrast, at high-temperature conditions, even though most of the seeds germinated and emerged as seedlings, only the hemiepiphytic seedlings showed high survival. These results indicate that hemiepiphytic species may have a greater survival advantage in warmer tropical regions in contrast to higher latitudes, but also provide an explanation as to why *Ficus* species, hemiepiphytic species in particular, are less common in higher latitudes. With the advancement of climate change, hemiepiphytic species may be favored in warmer climates owing to their successful seed germination and survival over NH counterparts. It is worth testing whether other taxa with these aerial and terrestrial divergent life forms may also show differences in resilience during recruitment in response to temperature and water stress, especially under expected climate change scenarios.

In this study, we simulated water stress also in the form of RH and found that there was a significant difference between germination probability of hemiepiphytic species and NH species. In particular, hemiepiphytic species' seed germination was higher than NH species at 99.5% RH condition. Outside of the experiment conducted by Yoder et al. (2000) on epiphytic and terrestrial orchids, this is the only experiment that we know of to test recruitment simulating aerial water availability. Germination took place only at 99.5% RH in the seeds floated above 100% water. During germination and seedling emergence, we observed clouds of fungal strains that covered germinating seeds and emerging seedling but allowed the cotyledons to reach above the mycelial layer. Our results showed that hemiepiphytes germinated at 99.5% RH more readily than non-hemiepiphytes but their seedling emergence was abetted by fungi. We attribute the fungal growth to not treating seeds with NaClO prior to experimentation, which we avoided to make sure that the seeds were not pre-imbibed and that the only water available for them to grow was through air humidity. It is important to also note that there was no fungal mycelial growth in any of the other relative humidity treatments (18.5–99.0% RH). Further experimental studies are required to assess whether there is true facilitation for germination from the fungal interaction.

As with any experimental study, our results need to be interpreted with several caveats in mind. Although the experiment focused on the regeneration phase, patterns of growth and survival can change in larger size classes. However, the regeneration phase is often a critical demographic stage significantly affecting future growth and survival (Grubb 1977), conferring importance to our findings relating to the recruitment process. Second, we observed high within-group variability for both growth forms, suggesting caution for generalizations at the group level. Third, our results could be confounded by phylogenetic relationships. Five of the six hemiepiphytic species studied by us belong to the subgenus *Urostigma*, and *F. tinctoria* belongs to the subgenus *Sycidium*, which represent both major branches in which hemiepiphytic growth form has been evolved. Eight of the nine NH species belong to the subgenus *Sycomorus*, and *F. subulate* belongs to the subgenus *Sycidium* (Cruaud et al. 2012). Here too, our sampling effort represent two main branches of two subgenera out of the five subgenera of three system branches in which NH species are located. We did not include phylogenetic considerations in our analysis, also because we did not find it to be meaningful given that the family is 850 species strong and we considered only 15 species for our experimental study. Even though our species selection is representative of the main branches in which each growth form has evolved, we recognize that our results could be also explained by phylogeny as well as growth form. Finally, other biotic factors such as seed predation and herbivory that operate under natural growing conditions could further modulate the observed regeneration responses. Nonetheless, the results presented here are the first that report regeneration response and response times under temperature and water stress for the two divergent growth forms. Studies monitoring how the impact of temperature and water stress during regeneration affects further growth and survival in larger size classes, combining the influence of biotic pressures, would provide more comprehensive information on the regeneration process of hemiepiphytic and NH growth forms.

Conclusions

Our study provides the first comprehensive evidence that hemiepiphytic *Ficus* species are better adapted to the drier and warmer conditions during the critical transition from seed germination, to seedling emergence and to seedling survival. It is the first study to demonstrate that the hemiepiphytes are more successful in seed germination and seedling emergence using only air humidity. Through greater flexibility in achieving peak germination and duration of regeneration activity, the hemiepiphytes can modulate their recruitment process to be more resilient under water and temperature stress, and regenerate under ambient conditions that may be more transient in forest canopies. This suite of high temperature and drought tolerance regeneration traits in hemiepiphytes are of functional importance in the forest canopy, contrasting with those of congenic NH species, which complete their recruitment in the forest understory.

Data availability statement

Data used in this study are available from Figshare Digital Repository: https://doi.org/10.6084/m9.figshare.13204343.v2.

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Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Author's contributions

H.C. was responsible for conceptualization, methodology, investigation, formal analysis and writing the original draft. N.G. undertook formal analysis, and review and editing. B.W. was responsible for methodology, and for review and editing. K.-f. participated in the methodology, and review and editing. U.M.G. undertook conceptualization, methodology, formal analysis, supervision, funding acquisition, and review and editing.

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Conflict of interest

The authors declare no conflicts of interest.

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