American Journal of Botany
Celebrating 100 years 1914–2014

American Journal of Botany 101(6): 925–934. 2014.

DIFFERENT BIOMECHANICAL DESIGN AND ECOPHYSIOLOGICAL STRATEGIES IN JUVENILES OF TWO LIANA SPECIES WITH CONTRASTING GROWTH HABIT[1]

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- *Premise of the study:* Lianas constitute a major functional type in tropical zones. While some liana species start climbing immediately after germination (shade-avoidance), others have a long self-supporting phase (shade-tolerance). The morphophysiological characteristics of these two growth habits are unknown.
- *Methods:* We quantified growth traits, biomass allocation, mechanics, anatomy, and hydraulics for saplings of *Ventilago calyculata* (an immediate obligate climber) and *Ziziphus attopensis* (having a long self-supporting phase), both in the family Rhamnaceae. The mechanics, anatomy, and hydraulics for the mature individuals of the two species were also evaluated.
- *Key results:* In the juvenile stage, *V. calyculata* had a higher slenderness ratio, height growth rate, and photosynthetic rate but similar biomass growth rate compared with *Z. attopensis*. In contrast, *Z. attopensis* had a higher leaf area growth rate, specific leaf area, and leaf mass fraction. *Ziziphus attopensis* had stiffer, but less conductive stems than *V. calyculata.* Stem rigidity of saplings decreased from base to apex in *Z. attopensis*, but increased in *V. calyculata*. Both species had similar resistance to xylem embolism. However, the leaves of *V. calyculata* were able to resist greater water deficits. At the mature stage, wider and longer vessels emerged in the xylem, and both species increased stem specific conductivity and drought resistance in stems and leaves. *Ventilago calyculata* had significantly higher specific conductivity and was more drought tolerant than *Z. attopensis*.
- *Conclusions:* The two lianas differed significantly in growth, biomass allocation, anatomy, mechanics, ecophysiology, and hydraulic properties in line with their growth habits and shade adaptation strategies.

Key words: cavitation resistance; drought tolerance; hydraulic conductivity; liana; life history; Rhamnaceae; trade-off; *Ventilago calyculata*; water relations; *Ziziphus attopensis*.

Lianas (woody climbers) are an important component of tropical rainforests. Nearly 40% of the woody individuals and up to 25% of the woody species in some tropical forests are lianas (Gerwing and Farias, 2000; Chave et al., 2001; Schnitzer et al., 2012). In some mature tropical forests, liana seedlings may account for 25% of the seedling community (Putz, 1984; Caballé, 1998). Although lianas are often considered to be light-demanding because they tend to be relatively abundant in gaps and along the margins of forests, many lianas can establish and persist in shaded understories, exhibiting a shade-tolerance syndrome during their juvenile phase (Schnitzer and Bongers, 2002; Valladares et al., 2011). For example, some liana species initially grow like small self-supporting shrubs or saplings of trees in the forest understory and later rely on other plants for support to reach the upper canopy (Putz, 1984; Putz and Mooney, 1991; Rowe and Speck, 2005). It has been suggested that the self-supporting phase is a supporter-waiting period (Gianoli, 2002, 2003; Gianoli and González-Teuber, 2005), and it may last for many years in the absence of supporters (Caballé, 1998). During the transition from the self-supporting to the climbing stage, lianas undergo a transformation in morphology, anatomy, mechanical strength, and hydraulics (Gallenmüller et al., 2004; Isnard and Silk, 2009). Instead of increasing the mechanical demand with plant size, the stem diameter and stiffness decrease after the self-supporting phase (Isnard et al., 2003; Isnard and Silk, 2009). Additionally, climbing stems develop specialized, long, wide long vessels with high hydraulic efficiency (Ewers and Fisher, 1991; Gartner, 1991; Chiu and Ewers, 1992). These contrasts between self-supporting juvenile saplings and climbing mature individuals have been found in some liana species (Isnard and Silk, 2009; Angyalossy et al., 2012). However, it is unclear whether lianas that have a very short or no self-supporting phases show such morphological, anatomical, and mechanical contrasts between juvenile and mature stages that result in differential hydraulic performance.

Because light is one of the most limiting factors in many tropical rainforests, understory plants have to adjust their morphology and physiology to maximize light capture while minimizing construction and maintenance costs. The strategies used by lianas to survive under such understory conditions have been poorly investigated (Gilbert et al., 2006; Selaya et al., 2007; Valladares et al., 2011; Letcher and Chazdon, 2012). Lianas also make the transition from self-support to climbing, with

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[1]Manuscript received 2 December 2013; revision accepted 24 April 2014.

This research was supported by National Nature Science Foundation of China (grant 31100291) and the CAS 135 program (XTBG-T01, XTBG-F01). The authors thank Hong Ma for assistance with measurements. They also thank M. K. Bartlett for constructive comments and S. Isnard for sharing her equipment and technical knowledge.

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doi:10.3732/ajb.1300429

concomitant structural and physiological changes. Many lianas have extremely fast axial growth rates (Putz and Mooney, 1991) that allow them to escape the shaded understory by elongating their stems quickly toward more favorable positions. Other lianas can persist in the understory. Therefore, the self-supporting phase of lianas is related to a shade-tolerance strategy, whereas climbing growth is associated with a shade-avoidance strategy. A liana that does not have a self-supporting phase would therefore employ an avoidance strategy, whereas a liana with a self-supporting phase would switch from a tolerance to an avoidance strategy. Here, we compared the morphophysiological characteristics of two related liana species that differ in their life history strategies related to climbing and understory persistence. Most studies related to life history of woody plants focus on the differences between lianas and trees (Cai et al., 2007b, 2008; Selaya et al., 2007; Selaya and Anten, 2008). There have been few tests of the differences in strategies between lianas with different juvenile life history traits. We investigated the ecophysiological strategies displayed by a liana that tolerates the shaded forest understory and those of a liana that avoids the shaded forest understory during the juvenile stage.

In tropical seasonal rainforests, most plants experience severe water deficit in the dry season. Lianas typically have wide, long vessels and thus achieve high hydraulic conductivity (Gartner, 1991; Isnard and Silk, 2009; Zhu and Cao, 2009; Sande et al., 2013). Some studies have shown that lianas have lower cavitation resistance than trees in the juvenile (Sande et al., 2013) and the mature stages (Zhu and Cao, 2009; Johnson et al., 2013) and therefore have a higher risk of xylem dysfunction than trees under water deficit. We expect species lacking a self-supporting phase to be less constrained by mechanical demands than those that undergo a self-supporting phase. Instead, we expect that such lianas develop an efficient hydraulic system in the juvenile stage that ensures fast growth and allows them to quickly escape the shaded understory. However, this strategy may be a trade-off against the costs of resistance to cavitation because of the demand to have large vessels and thus softer wood (Preston et al., 2006; Poorter et al., 2010; Fan et al., 2012). Theoretically, species with a long self-supporting phase must, like trees and shrubs, increase stem stiffness to meet increasing mechanical demands (Isnard and Silk, 2009). They may develop dense wood with a high ratio of fibers and with narrow vessels, resulting in relatively low conductivity, but high resistance to cavitation caused by water deficit. Therefore, from the perspective of hydraulic safety, the obligate climber strategy is presumably more risky than the self-supporting strategy.

We compared the morphophysiological characteristics of two Rhamnaceae species: *Ziziphus attopensis* Pirre (Cai et al., 2007a) and *Ventilago calyculata* Tul. (see Fig. 1). Both species are hook climbers and are large, evergreen, late-successional lianas. However, the two species differ in their growth habits during the juvenile stage. *Ziziphus attopensis* has a long self-supporting period, while *V. calyculata* starts climbing soon after seed germination. Mature individuals of both species can grow to 20–30 m in length and more than 10 cm in basal diameter. We evaluated the growth, biomass allocation, leaf gas exchange, wood anatomy, mechanical properties, and stem and leaf hydraulic properties for saplings of the two species. In addition, selected traits of mature individuals were examined to evaluate whether these characters remain constant over the life history of each liana. We addressed the following questions (1) do the two strategies differ in growth, biomass allocation, xylem anatomy, mechanical properties, or stem and leaf hydraulic properties? (2) How do these traits reflect two contrasting liana adaptive strategies in the understory? We hypothesized that *V. calyculata* would be more flexible in morphology and physiology and have a searcher (avoidance) strategy with long explorative runners. This strategy is risky in the dark understory, but rewarding when the plant rapidly reaches a better-lit position. Additionally we expected *V. calyculata* to have wider and longer vessels than *Z. attopensis* that result in higher vulnerability to cavitation than *Z. attopensis* because the maximization of elongation growth and conducting efficiency may compromise resistance to water deficit.

MATERIALS AND METHODS

Experimental design—This study was conducted at Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences, Yunnan, southwestern China (21°41′N, 101°25′E, altitude 570 m a.s.l.). This region is dominated by the southwest monsoon with three distinct seasons: a wet season from May to October, a cool season from November to January, and a dry season from February to April (Cao et al., 2006), with heavy fog during the dry and cool seasons (Liu et al., 2004). Mean annual temperature is 22.9°C, and mean precipitation is 1500 mm.

We compared plant functional and growth characteristics of *V. calyculata* and *Z. attopensis* in a sapling experiment under controlled greenhouse conditions. Additionally, we compared selected plant functional (mainly anatomical, mechanical, and hydraulics) characteristics of juvenile individuals with those of adults of the same species. For the juveniles, we used shadehouse plants. Mature individuals for the mechanical and hydraulic comparison were collected from a nearby seasonal rainforest at XTBG. We defined the mature individuals as those that reached the canopy and had typical climbing behavior with thick, flexible main stems. The anatomy, mechanics, and hydraulics of juvenile main stems were compared with those of the branches of mature individuals to ensure that "stems" of similar size were compared. To understand the general mechanical characteristics of the two growth habits under investigation, we collected saplings of six additional common species from the rainforest understory (4–6 individuals of each species; Appendices S1–S3, see Supplemental Data with the online version of this article). All of these species are large, evergreen, late-successional lianas. We assessed the variation in the mechanical strength along the main stem of each sapling. The development of two species [*Cudrania fruticosa* (Roxb.) Wight ex Kurz, and *Acacia pennata* (L.) Willd.] was characterized by a long self-supporting period in the juvenile stage, whereas the other four species [*Gnetum montanum* Markgr., *Bauhinia glauca* subsp. *tenuiflora* (Watt ex C. B. Clarke) K. Larsen & S. S. Larsen, *Bauhinia aurea* H. Lév., and *Hiptage benghalensis* (L.) Kurz] did not appear to have a self- supporting period.

For the sapling experiment, we collected seedlings (1–2 yr old) of the two study species from a nearby seasonal forest in July 2010 at the start of the rainy season and planted them in a nursery with nearly 10% full sunlight. After 1.5 mo of adaptation to the nursery in 10% light, 12 randomly selected individuals were moved to shadehouses. In the shadehouses, neutral-density screens that permitted nearly 4% of the sunlight to pass were used to simulate the light conditions in the forest understory. The mean height of seedlings (±1 SD, n = 10) of *Z. attopensis* was 26.4 cm ± 1.52 and 37.7 cm ± 0.98 for *V. calyculata*. Seedlings were grown in pots with substrates consisting of forest surface soil mixed with river sand 2:1 by volume (see Chen et al., 2008). All pots were rotated at a 20-d interval to avoid rooting into the ground and any effects of local variation in light availability. We fertilized all pots with 20 g NPK slow-release compound fertilizer (Osmocote, Scotts, Marysville, Ohio, USA) every 3 mo after transplanting and watered all pots on days without rain to maintain the soil near field capacity. Weeds were removed regularly, and insecticides were used when necessary.

Gas exchange—After 14 mo, a photosynthetic light-response curve (A-PPF curve) was determined for fully expanded healthy new leaves (6 leaves on 3 individuals) between 0900 and 1100 hours. Irradiance dosages were from 0 to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 10 steps created with an built-in-LED-B light source, using a portable Li-6400 photosynthesis system (Li-6400, LI-COR, Lincoln, Nebraska, USA) under constant CO_2 concentration (ca. 400 ppm). Photosynthetic variables (light-saturated photosynthetic rate [P_{max}], apparent

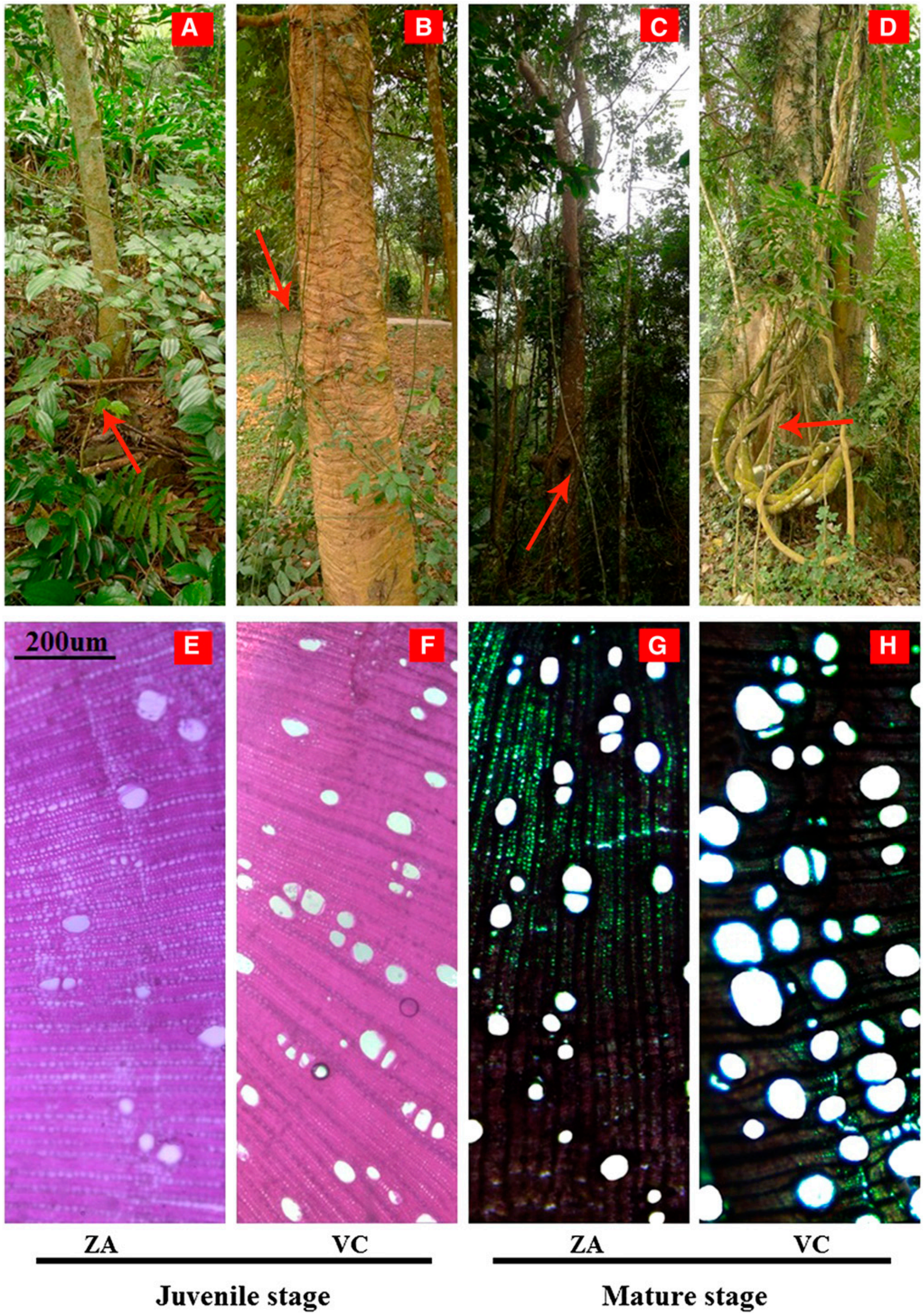

Fig. 1. Morphology and xylem anatomy for (A, C, E, and G) *Ziziphus attopensis* (ZA) and (B, D, F, and H) *Ventilago calyculata* (VC) juvenile (left four) and mature (right four) individuals.

quantum efficiency [AQE], dark respiration rate (R_d), and light compensation point (LCP), light saturation point (LSP)) were calculated from the A-PPF curves.

Biomass allocation and growth traits—At the beginning of the experiment (July 2010), 10 plants per species were harvested and separated into roots, stems, and leaves. Samples were oven dried at 80°C for 48 h to determine dry mass.

After 14 mo, all saplings were harvested and separated into roots, stems, and leaves. Roots were washed in tap water. Height and diameter (10 cm from the base) of fresh stems were measured. Leaf areas were measured using a leaf area meter (LI-3100A, LI-COR). All tissues were dried at 80°C for 48 h. Slenderness ratio (stem height/diameter, $cm \cdot cm^{-1}$), specific leaf area (SLA, $cm^2 \cdot g^{-1}$), leaf area ratio (LAR = leaf area/plant mass, $cm^2 \cdot g^{-1}$), leaf mass fraction (LMF = leaf mass/plant mass, $g \cdot g^{-1}$), stem mass fraction (SMF = stem mass/plant mass, $g \cdot g^{-1}$), root mass fraction (RMF = root mass/plant mass, $g \cdot g^{-1}$) and R/S ratio (belowground mass/aboveground mass, $g \cdot g^{-1}$) were calculated.

We calculated the following growth variables for each sample: relative growth rate in dry biomass ($RGR_B = (\ln W2 - \ln W1)/(T2 - T1)$, where W2 and W1 are the final and the initial total dry mass per plant, respectively, and T2 − T1 is the time interval, in months), relative growth rate in height (RGR_H), and relative growth rate in leaf area (RGR_{LA}) (formulas as for RGR_B with mass replaced by height and leaf area, respectively).

Biomechanical traits—The stem stiffness (Young's modulus, MPa) was determined by the three-point bending method using the protocol of Isnard et al. (2005). For the bending protocol and determination of span length (the distance between two supports during bending to avoid shear stress during measurements), we followed the methods of Isnard and Rowe (2008). We sampled all saplings from the base to the apex of the main stem to represent the axial variation of mechanical strength along the main stem. The stem stiffness of saplings and long branches of mature individuals for *Z. attopensis* and *V. calyculata* were also compared.

Stem hydraulic properties—Maximal vessel length was measured by injecting a 0.1 MPa air into the cut end and then cutting the distal end at 1 cm intervals until air bubbles were found (Brodribb and Feild, 2000). The maximal vessel length for saplings of *Z. attopensis* ranged from 0.21 to 0.34 m and from 0.37 to 0.62 m for *V. calyculata*. However, the maximal vessel length of mature individuals ranged from 0.48 to 1.1 m for *Z. attopensis* and from 0.64 to 1.73 m for *V. calyculata.*

Segments used for hydraulic measurement were longer than the maximum vessel length to avoid the presence of open vessels. We measured stem hydraulic traits of 4–6 segments from four individuals from each species. Sapwood specific conductivity (K_S, $kg \cdot m^{-1} s^{-1} \cdot MPa^{-1}$) was determined using the steady-state flowmeter method (Sperry et al., 1988). Stem segments were cut under water with a fresh razor blade and then flushed for 1 h using degassed ultra-filtered KCl solution (10 $mmol \cdot L^{-1}$). Vulnerability curves were established using an air-injection method (Cochard et al., 1992). Maximal conductivity (K_{max}, $kg \cdot m^{-1} \cdot s^{-1} \cdot MPa^{-1}$) was measured for each segment. Air seeding was then induced by compressed air at different pressures. Conductivity (K, $kg \cdot m \cdot s^{-1} \cdot MPa^{-1}$) was measured after each pressurization. K was calculated as: $K = J_v/(dP/dx)$. Sapwood specific conductivity (K_S, $kg \cdot m^{-1} \cdot s^{-1} \cdot MPa^{-1}$) was determined by dividing K by the cross-section area of the sapwood. Percentage loss of conductivity (PLC) was calculated as: $PLC = 100 \cdot (K_{max} - K)/K_{max}$. The vulnerability curve was obtained as the function of PLC vs. air seeding pressure and fitted by a 3-parameter sigmoidal equation.

Xylem anatomical analyses—The same segments were used for measurement of hydraulic and anatomical traits. Transverse sections that were 20 μm thick were cut using a sliding microtome (AO860, Lambert Tech., Buffalo, New York, USA). The sections were stained with safranin after bleaching and rinsing with water. Sections were observed with a Leica DM2500 microscope (Leica Microsystems, CMS GmbH, Wetzlar, Germany), and six images of each segment were captured using a Leica DFC295 digital camera. Mean and maximum vessel diameters, vessel density, and the fraction of lumen area were determined using image analysis software Image J 1.47 (National Institutes of Health, Bethesda, Maryland, USA).

Leaf pressure–volume curve—Pressure–volume (P-V) curves were produced using the bench dry method (Lenz et al., 2006). Eight small terminal branches from at least four individuals per species were sampled from both seedlings and mature individuals (leaves were from the same individuals as used for the stem hydraulics measurement). The branches were recut underwater and hydrated until leaf water potential recovered to greater than −0.05 MPa. Samples were left to dry on the bench, and fresh leaf mass and water potential were measured periodically during slow desiccation. Leaf desiccation continued until leaf water potential began to rise due to cell damage. Then the parameters for the P-V curves were estimated, including osmotic potential at full turgor (Π_o, MPa), the turgor loss point (Ψ_{TLP}, MPa), and the bulk modulus of elasticity (ε, MPa).

RESULTS

Allometry, biomass allocation, and gas exchange variables—After 14 mo of growth, *Z. attopensis* was 80.3 ± 7.3 cm long (mean ±1 SE), with a mean biomass of 58.9 ± 9 g and mean leaf area of 3829.3 ± 786.8 cm^2. *Ventilago calyculata* was 259.7 ± 42.1 cm long, with a mean biomass of 119.9 ± 8.8 g and mean leaf area of 2947.5 ± 436.7 cm^2.

The two species had comparable RGR_B (RGR_B: 7.5 ± 0.4 $mg \cdot g^{-1} \cdot d^{-1}$ for *Z. attopensis* and 7.7 ± 0.2 $mg \cdot g^{-1} \cdot d^{-1}$ for *V. calyculata*; $t = 1.24$, df = 8, $P = 0.25$). However, the two species differed in RGR_H and RGR_{LA} (Fig. 2). Compared with *Z. attopensis*, *V. calyculata* exhibited significantly higher RGR_H (RGR_H: 4.5 ± 0.4 $mm \cdot cm^{-1} \cdot d^{-1}$ for *V. calyculata* and 2.6 ± 0.2 $mm \cdot cm^{-1} \cdot d^{-1}$ for *Z. attopensis*; $t = 6.37$, df = 8, $P < 0.001$) but lower RGR_{LA} (RGR_{LA}: 6.5 ± 0.4 $mm^2 \cdot cm^{-2} \cdot d^{-1}$ for *V. calyculata* and 7.5 ± 0.5 $mm^2 \cdot cm^{-2} \cdot d^{-1}$ for *Z. attopensis*; $t = -2.65$, df = 8, $P = 0.029$).

Significant differences in morphology and biomass allocation were found between the two species (Fig. 3). *Ventilago calyculata* had a slenderness ratio that was 6.6 times that of *Z. attopensis.* However, *Z. attopensis* had a significantly higher

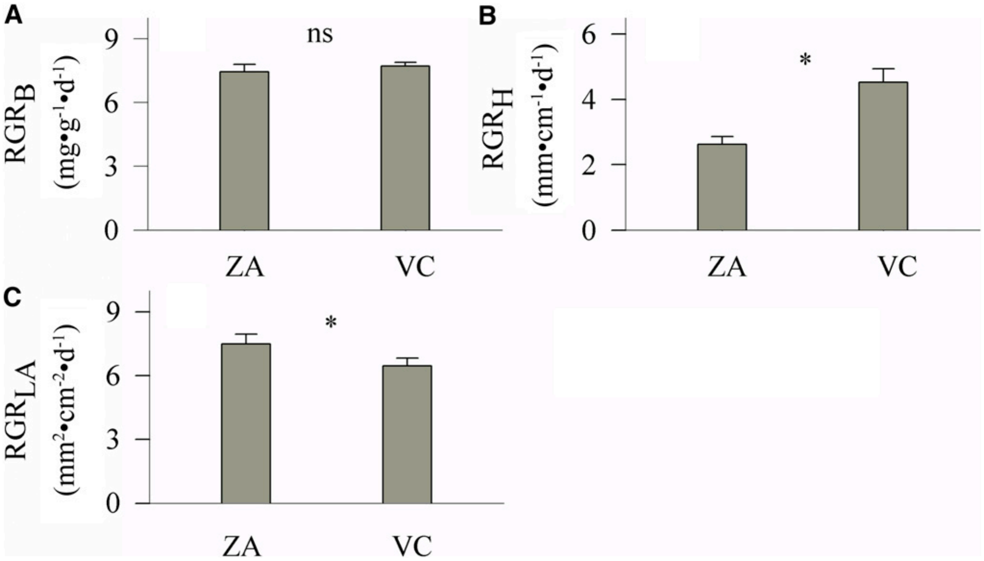

Fig. 2. Relative growth rate in (A) dry biomass (RGR_B), (B) height (RGR_H), and (C) leaf area (RGR_{LA}) of *Ziziphus attopensis* (ZA) and *Ventilago calyculata* (VC) saplings. Values are means ±SE; the asterisk (*) represents significant difference at $P < 0.05$ and "ns" indicates no significant difference.

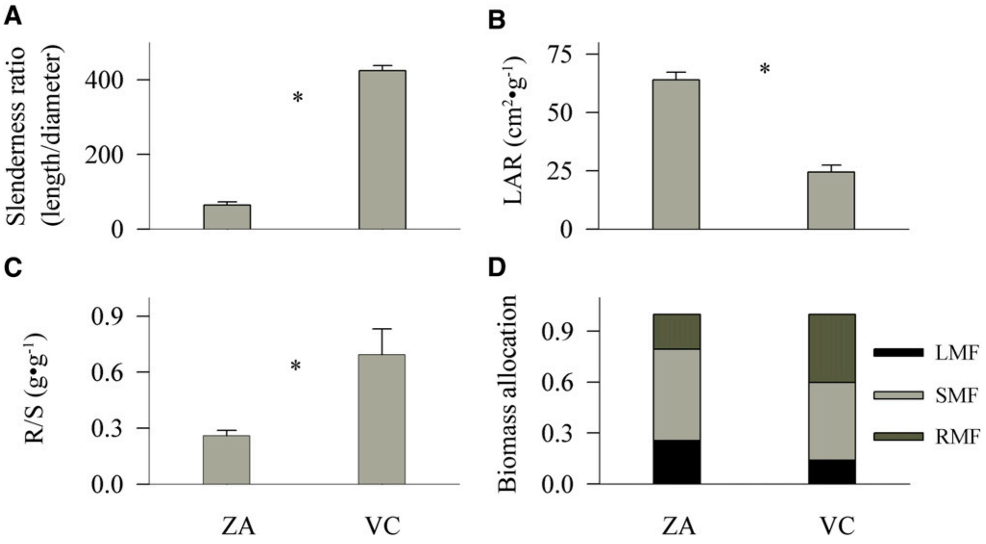

Fig. 3. (A) Slenderness ratio, (B) leaf area ratio (LAR), (C) belowground/aboveground mass ratio (R/S), and (D) biomass allocation of *Ziziphus attopensis* (ZA) and *Ventilago calyculata* (VC) saplings. LMF, leaf mass fraction; SMF, stem mass fraction; RMF, root mass fraction. The asterisk (*) represents a significant difference at $P < 0.05$.

LAR (*Z. attopensis*: 64 ± 3.3 $\text{cm}^2 \cdot \text{g}^{-1}$, *V. calyculata*: 24.5 ± 2.9 $\text{cm}^2 \cdot \text{g}^{-1}$; $t = -15.79$, df = 8, $P < 0.001$), leaf mass ratio, and stem mass ratio than did *V. calyculata*. This resulted in R/S in *V. calyculata* that was 2.7 times that in *Z. attopensis*. *Ziziphus attopensis* had significantly lower P_{max}, Rd, and LCP than did *V. calyculata* (Table 1).

Morphology, xylem anatomy, and biomechanical traits—In general, the two species showed distinctive patterns in morphology, xylem anatomy, and biomechanical traits. For example, the SLA of *Z. attopensis* was nearly 1.6 times that of *V. calyculata* (Table 1). The diameter tapered sharply from stem base to the apex in *Z. attopensis*, whereas tapering was not significant in *V. calyculata.*

Ventilago calyculata had a larger fraction of lumen area and vessels that were wider, longer, and more dense relative to *Z. attopensis*, particularly in the juvenile stage (two-way ANOVA, species: $F_{1,51} = 29.25$, $P < 0.001$ for the fraction of lumen area; $F_{1,51} = 7.0$, $P = 0.002$ for vessel diameter; $F_{1,41} = 11.84$, $P = 0.001$ for maximal vessel length; $F_{1,51} = 6.24$, $P = 0.016$ for vessel density; Fig. 4, Table 2). Moreover, saplings and mature individuals differed significantly in xylem anatomical properties (two-way ANOVA, species: $F_{1,51} = 11.32$, $P = 0.002$ for vessel diameter; $F_{1,41} = 59.24$, $P < 0.001$ for maximal vessel length; $F_{1,51} = 37.59$, $P < 0.001$ for vessel density; $F_{1,51} = 12.89$, $P = 0.001$ for the fraction of lumen area; Fig. 4, Table 2). Vessels were wider and longer in the mature individuals than in the juvenile individuals (Fig. 4C, D). The widest vessels in the xylem of mature individuals of *Z. attopensis* and *V. calyculata* were 92 µm and 115 µm in diameter, respectively. In saplings, the widest vessels were 83 µm and 101 µm in diameter, respectively. The longest vessels in mature *Z. attopensis* and *V. calyculata* were 1.1 m and 1.73 m, respectively. However, the longest vessels were only 0.34 m and 0.62 m long for saplings of *Z. attopensis* and *V. calyculata.*

Ziziphus attopensis had a stiffer stem than *V. calyculata* did (two-way ANOVA, species: $F_{1,125} = 29.13$, $P < 0\ 0.001$; Table 2). There was no significant difference in stiffness between juvenile stems and the mature-stage branches of *Z. attopensis* (6525 ± 259.9 MPa for saplings and 6559.8 ± 345.8 MPa for branches of mature individuals; two-way ANOVA, stage: $F_{1,125} = 1.27$, $P = 0.26$; Fig. 4, Table 2). In contrast, *V. calyculata* had lower stiffness in the mature-stage branches than in juvenile stems (4637 ± 137.7 MPa for mature branches and 5324.1 ± 196 MPa for saplings). In the juvenile stage, the stem stiffness from base to apex first increased slightly and then decreased in *Z. attopensis*, whereas in *V. calyculata* the pattern was reversed (Fig. 5). Stiffness increased with stem diameter in *Z. attopensis* ($r^2 = 0.53$, $P < 0.0001$) but decreased in *V. calyculata* ($r^2 = 0.56$, $P < 0.0001$; Fig. 5E, F). All of these patterns for the two contrasting growth strategies were consistent among five of six additional liana species that are common in the Xishuangbanna region (online Appendices S1–S3).

Leaf and stem hydraulic traits—We found that K_S was higher in mature plants than in juvenile plants (K_s increased from 0.2 ± 0.1 to 1.3 ± 0.2 $\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$ for *Z. attopensis* and from 3.9 ± 0.9 to 7.6 ± 1.2 $\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}$ for *V. calyculata*; two-way ANOVA, $F_{1,20} = 9.82$, $P = 0.005$; Table 2). K_s was higher in *V. calyculata* than in *Z. attopensis* both for juvenile and mature individuals (two-way ANOVA, $F_{1,20} = 44.21$, $P < 0.001$; Fig. 4A, Table 2).

TABLE 1. Photosynthetic variables and specific leaf area (SLA) in *Ziziphus attopensis* and *Ventilago calyculata* saplings.

Variable	*Z. attopensis*	*V. calyculata*	*t*	df	*P*
P_{max}	3.97 ± 0.29	4.88 ± 0.32	−2.57	10	0.028
AQE	0.04 ± 0.00	0.05 ± 0.01	−0.61	10	0.55
R_d	0.05 ± 0.02	0.45 ± 0.15	2.93	10	0.003
LCP	1.51 ± 0.54	8.53 ± 1.94	−4.27	10	0.002
LSP	416.65 ± 23.84	551.75 ± 126.52	−1.29	10	0.23
SLA	270.32 ± 18.32	170.51 ± 6.52	5.42	13	<0.001

Notes: P_{max}, light-saturated photosynthetic rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); AQE, apparent quantum efficiency; R_d, dark respiratory rate ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); LCP, light compensation point ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); LSP, light saturation point ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

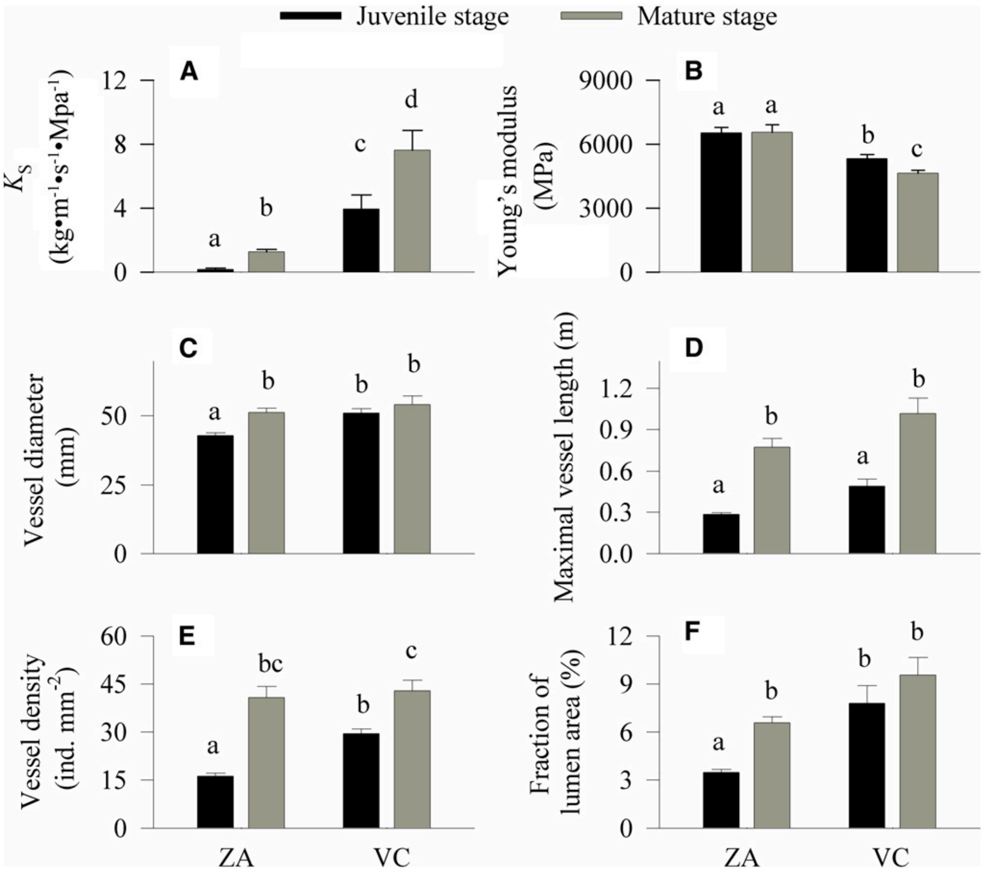

Fig. 4. Comparison of (A) specific stem hydraulic conductivity (K_s), (B) Young's modulus, and (C–F) xylem anatomical properties in the juvenile and mature stages for *Ziziphus attopensis* (ZA) and *Ventilago calyculata* (VC). Values are means ±SE. Different letters represent a significant difference at $P < 0.05$.

In the juvenile stage, the two species had similar resistance to embolism (P_{50} was −0.87 MPa and −0.83 MPa for *Z. attopensis* and *V. calyculata*, respectively; Fig. 6), but in the mature stage, *V. calyculata* was more resistant to embolism than *Z. attopensis* (P_{50} was −0.91 MPa and −1.27 MPa for *Z. attopensis* and *V. calyculata*, respectively).

In the mature stage, the two species had more negative Π_o and Ψ_{TLP} (Π_o: from −0.68 ± 0.08 MPa to −1.04 ± 0.06 MPa and from −1.1 ± 0.08 MPa to −1.71 ± 0.14 MPa for *Z. attopensis* and *V. calyculata*, respectively; Ψ_{TLP}: from −0.78 ± 0.08 MPa to −1.34 ± 0.06 MPa and from −1.28 ± 0.08 MPa to 1.94 ± 0.13 MPa for *Z. attopensis* and *V. calyculata*, respectively; two-way

TABLE 2. Two-way ANOVA comparing stem stiffness (Young's modulus), specific conductivity (K_s), variables derived from P-V curves and xylem properties between *Ziziphus attopensis* and *Ventilago calyculata*, stage, and their interaction.

	Stem stiffness			K_s			Π_0			Ψ_{TLP}			ε		
Factor	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Species	29.13	1	**0.000**	44.21	1	**0.000**	29.35	1	**0.000**	30.46	1	**0.000**	6.96	1	**0.014**
Stage	1.27	1	0.26	9.82	1	**0.005**	25.14	1	**0.000**	38.31	1	**0.000**	0.01	1	0.905
Species × Stage	1.55	1	0.22	2.89	1	0.105	0.01	1	0.917	1.03	1	0.318	3.7	1	0.065

	Vessel diameter			Maximal vessel length			Vessel density			Fraction of vessel area		
Factor	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Species	6.99	1	**0.002**	11.84	1	**0.001**	6.24	1	**0.016**	29.25	1	**0.000**
Stage	11.32	1	**0.002**	59.24	1	**0.000**	37.59	1	**0.000**	12.9	1	**0.001**
Species × Stage	2.05	1	0.159	0.09	1	0.762	3.29	1	0.076	0.99	1	0.326

Notes: Boldface *P* values indicate significant effects. Π_o, osmotic potential at full turgor; Ψ_{TLP}, the turgor loss point; ε, bulk modulus of elasticity.

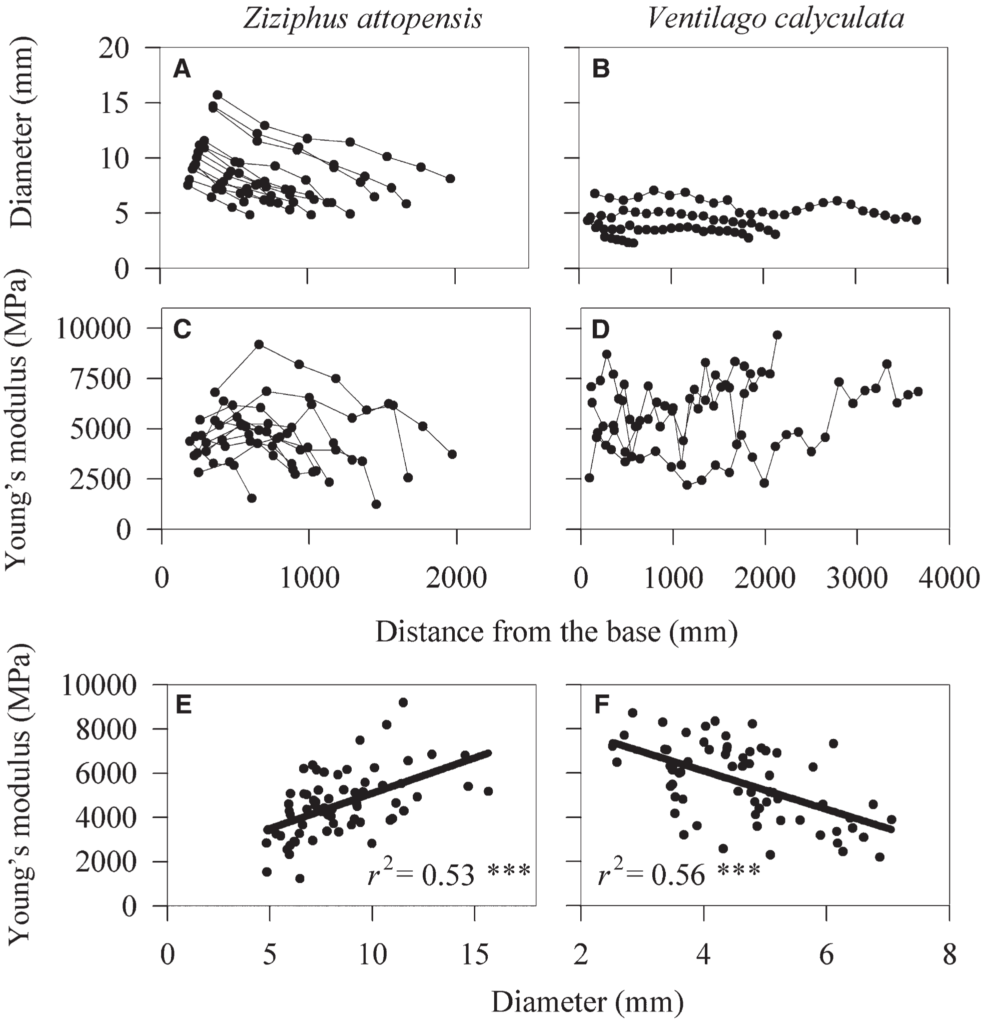

Fig. 5. Variation in the (A, B) diameter and (C, D) Young's modulus of the stems as a function of distance from the base and (E, F) the relationship between Young's modulus and stem diameter along the stems for saplings of *Ziziphus attopensis* (ZA) and *Ventilago calyculata* (VC).

ANOVA, stage: $F_{1,27} = 25.14$, $P < 0.001$ for Π_o; and $F_{1,27} = 38.31$, $P < 0.001$ for Ψ_{TLP}; Fig. 7, Table 2) as well as higher elastic modulus (*Z. attopensis*: 11.3 MPa and 8.7 MPa for saplings and mature individuals; *V. calyculata*: 13.1 MPa and 19 MPa for saplings and mature individuals; two-way ANOVA, stage: $F_{1,27} = 0.014$, $P = 0.91$ for ε; Table 2) than in the juvenile stage. Compared to *Z. attopensis, V. calyculata* had significantly lower Π_o, Ψ_{TLP} (two-way ANOVA, species: $F_{1,27} = 29.35$, $P < 0.001$ for Π_o; and $F_{1,27} = 30.46$, $P < 0.001$ for Ψ_{TLP}; Table 2) and a significantly higher ε, especially in mature stage (Fig. 7).

DISCUSSION

Growth habit influences allometry—The two species exhibited fundamental differences with regard to biomass allocation and growth traits. The two species showed similar RGR_B but differed significantly in RGR_H and RGR_{LA}, which indicates that different allocation strategies lead to their distinctive appearance and crown architecture. The different strategies employed by these two species are related to the trade-off between enhancing light interception in the understory and escaping the understory. Some liana species have poor leaf arrangement and display at the juvenile stage and therefore have a lower light-capturing ability on the basis of leaf area (Valladares et al., 2002). Another study showed that lianas with self-supporting saplings had light interception efficiency similar to the saplings of trees (Selaya et al., 2007). *Ventilago calyculata* enhanced the axial elongation rate to access a higher position and search for light at the cost of a broad crown to intercept more light. *Ziziphus attopensis*, in contrast, seemed to improve its ability to persist in shade, with much higher SLA, LAR, LMR, and a large crown area (data not shown), which is typical for lianas that have self-supporting saplings (Gilbert et al., 2006; Cai et al., 2007a; Selaya et al., 2007; Selaya and Anten, 2008; Valladares et al., 2011). *Ventilago calyculata* had greater root allocation (higher R/S and RMR) than *Z. attopensis* did, which might enhance access to relatively deep soil water, thereby decreasing the risk of water deficit in the dry season.

Mechanical stability and anatomical properties—The two juvenile liana species showed a distinctive pattern in their mechanical strategies that reflects a trade-off between stem elongation

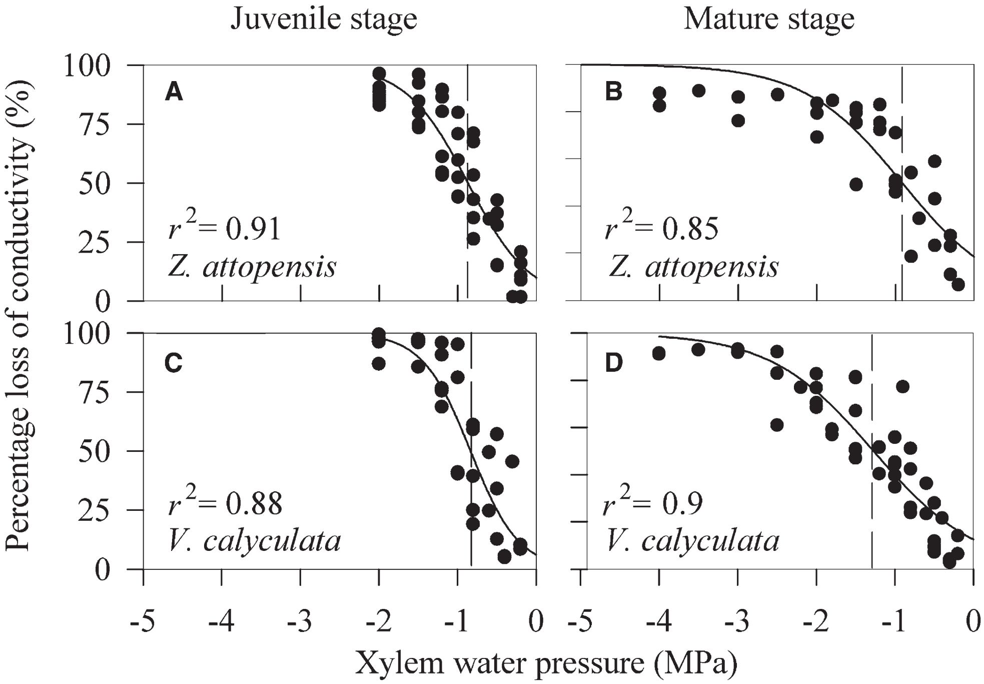

Fig. 6. Stem vulnerability curves for (A, B) *Ziziphus attopensis* and (C, D) *Ventilago calyculata* in the juvenile and mature stages. For all the regression lines, $P < 0.0001$. The vertical dashed lines indicate the xylem water potential at 50% loss of stem hydraulic conductivity (P_{50}).

and mechanical stability. Stem elongation and mechanical stability were closely linked with xylem development. In the current study, the juvenile-phase mechanical properties of *Z. attopensis* and two other self-supporting saplings were consistent with those of self-supporting shrubs and trees for which mechanical demand commonly continues to increase during the self-supporting period due to increasing crown mass and size. For juvenile-phase *Z. attopensis*, the dense wood that was associated with relatively small vessels and a relatively low fraction of vessel area might be better optimized for a mechanical function than for hydraulic demand (Isnard and Silk, 2009; Angyalossy et al., 2012). In contrast, the juveniles of the climbing *V. calyculata*, free from mechanical demand, have soft, conductive stems as a result of their relatively wide, dense vessels and a large fraction of vessel area. These traits may impart high hydraulic conductivity to the thin stem. Additionally, the slim, flexible stem of *V. calyculata* reduces the risk of mechanical damage when climbing. Interestingly, the thin apex of *V. calyculata* juveniles had the highest rigidity. This high rigidity might be an important adaptation to search for potential supporters and

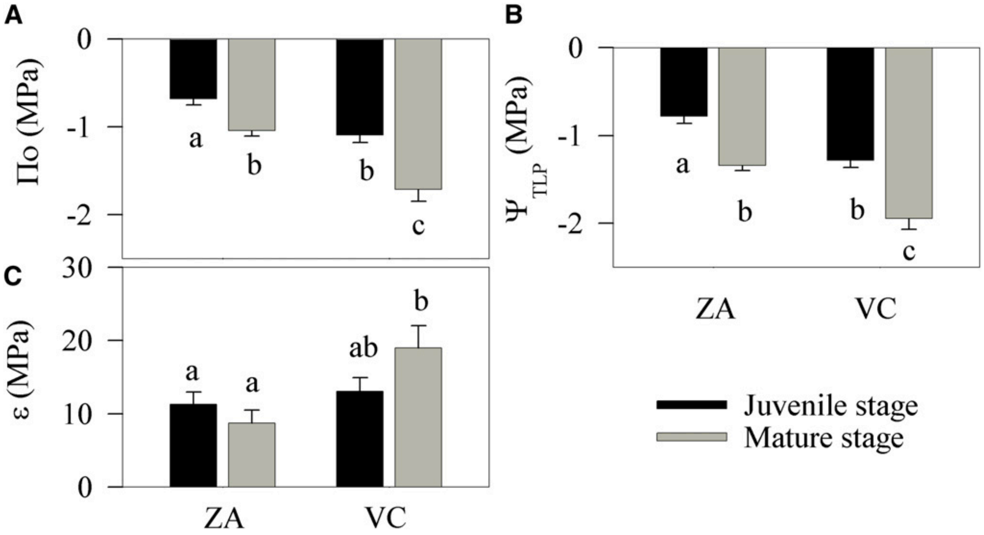

Fig. 7. Comparison of (A) osmotic potential at full turgor (Π_o), (B) turgor loss point (Ψ_{TLP}), and (C) bulk modulus of elasticity (ε) in the juvenile (full bars) and mature stages (open bars) of *Ziziphus attopensis* (ZA) and *Ventilago calyculata* (VC). Values are means ±SE. Different letters represent significant differences at $P < 0.05$.

may explain why some lianas can extend upward as far as 2 m above their last supporter (Putz, 1984).

Stem and leaf hydraulic traits—The wide, long vessels of lianas confer a very efficient transporting system compared with self-supporting woody species (Carlquist, 1985; Gartner, 1991; Chiu and Ewers, 1992; Feild and Balun, 2008; Isnard and Silk, 2009; Zhu and Cao, 2009; Jacobsen et al., 2012). However, our results indicated that self-supporting liana saplings have to cope with the paradoxical requirements for mechanical stability and hydraulic efficiency (Isnard and Silk, 2009). As expected, the lianoid *V. calyculata* exhibited a substantially higher K_s (Fig. 4A), wider and longer vessels, and a larger conducting area than in the self-supporting saplings of *Z. attopensis* (Fig. 4C–F). *Ziziphus attopensis*, constrained by the smaller and sparse vessels, even had a lower K_s than trees in the same region (Zhu and Cao, 2009; Fu et al., 2012). Similarly, a comparative study in Panama among eight liana species showed that the K_s of self-supporting saplings (ranging from 75.9 to 758.7 $\text{mol}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$) tended to be lower than that of climbing saplings (range from 179.2 to 3326.3 $\text{mol}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$) (Sande et al., 2013). The relatively low specific conductivity of *Z. attopensis* may represent a compromise between a high mechanical demand and stem conductivity (Isnard and Silk, 2009; Angyalossy et al., 2012).

The P_{50} values obtained in the current study were similar to those of other recent studies that showed that both juvenile and mature lianas were more vulnerable to embolism than were tree species (Zhu and Cao, 2009; Johnson et al., 2013; Sande et al., 2013). Our findings did not support the prediction that lianas without a juvenile self-supporting phase were more vulnerable to cavitation than lianas having self-supporting saplings due to their differences in xylem anatomy. Instead, our results indicated that the two species had similar resistance to xylem cavitation in the juvenile stage. The difference in P_{50} between liana saplings having the two growth habits was also not significant in a semideciduous, seasonally moist forest in Panama (Sande et al., 2013). The relatively low tolerance to leaf dehydration of *Z. attopensis* is balanced by its relatively high SLA. To persist in the understory, the self-supporting *Z. attopensis* must enhance light interception through the display of a high leaf mass ratio and thin leaves with high SLA. Both the stems and leaves of saplings of the two liana species studied are less resistant to dehydration-induced cavitation than those of mature individuals. Lianas are thought to have a dry season growth advantage and may benefit from seasonal drought in tropical forests (Schnitzer, 2005; Schnitzer and Bongers, 2011). However, it is not yet clear if seasonal drought favors juvenile lianas. The current study revealed that juvenile and mature individuals of the same species may react differently to water stress during drought incidents. The dry season growth advantage hypothesis (Schnitzer, 2005) may underestimate differences between growth stages and life history strategies. Additionally, liana species with a relatively long self-supporting phase during the juvenile stage may be more vulnerable to water stress in their leaves. Predicting the long-term effects of climate change on lianas may require accounting for hydraulic changes with their ontogeny.

Conclusion—The two liana species studied represent different life history strategies, with *Z. attopensis* employing a shade-tolerance strategy and *V. calyculata* representing a shade-avoidance strategy. As we predicted based on the growth habits of these species, they exhibited significant differences in their growth, biomass allocation, anatomy, mechanical properties, and leaf physiology, but not in hydraulics. However, to determine whether these are general characteristics related to growth habits, we need further study of more species.

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