Article

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How dehydration affects stem bending stiffness and leaf toughness after sampling of the liana *Amphilophium crucigerum* (L.) L.G.Lohmann (Bignoniaceae)

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ABSTRACT – (How dehydration affects stem bending stiffness and leaf toughness after sampling of the liana *Amphilophium crucigerum* (L.) L.G.Lohmann (Bignoniaceae)). Lianas are woody climbers and their stems and leaves deal with different environmental pressures such as resistance to mechanical damage and dehydration. The damage resistance of plants can be biomechanically evaluated by their stiffness, bending and toughness. Despite the well-known relationship between physical resistance and moisture of plant organs in woody plants, this relationship is uncertain and has not been previously evaluated in lianas. Thus, this study investigated experimentally the effect of stems and leaf dehydration on the structural Young's modulus in the stem and fracture toughness in leaves across time in the liana *Amphilophium crucigerum* (Bignoniaceae). Ten stem and leaf samples were collected and assigned to two distinct conditions: (i) samples kept moist and (ii) samples underwent gradual dehydration with natural moisture loss by air exposition. Successive measures of structural Young's modulus and fracture toughness were taken every 4 hours during a 48-hour period for both conditions. Stem and leaf samples which underwent gradual dehydration showed greater bending stiffness and fracture toughness, respectively, while the samples kept moist presented no changes in any studied biomechanical features during the entire experiment. We concluded that the moisture of both stem and leaf samples are critical factors to estimate the biomechanical properties of lianas stem and leaves.
Keywords: climbing plant, leaf fracture, plant biomechanics, stem flexibility, Young's modulus

RESUMO – (Como a desidratação afeta a flexibilidade do caule e a tenacidade das folhas pós-coleta na liana *Amphilophium crucigerum* (L.) L.G.Lohmann (Bignoniaceae)). Lianas **são** trepadeiras lenhosas e seus caules e folhas lidam com diferentes pressões ambientais, como a resistência aos danos mecânicos e de desidratação. A resistência ao dano das plantas pode ser biomecanicamente avaliada pelas propiedades de dureza, flexão e tenacidade. Apesar da conhecida relação entre resistência física e umidade dos órgãos das plantas em espécies lenhosas, essa relação não foi avaliada anteriormente e é incerta em lianas. Assim, este estudo investigou experimentalmente o efeito da desidratação de caules e folhas na estimativa do módulo estrutural de Young do caule e da tenacidade à fratura da folha ao longo do tempo, na liana *Amphilophium crucigerum* (Bignoniaceae). Dez amostras de caules e folhas foram coletadas e distribuídas em duas condições distintas: (i) amostras mantidas úmidas e (ii) amostras em processo de desidratação gradativa com perda natural de umidade quando expostas ao ar. Medidas sucessivas do módulo de Young e da resistência à fratura dos órgãos foram tomadas a cada 4 horas durante um período de 48 horas em ambas as condições. Amostras de caule e folhas que sofreram desidratação gradual apresentaram maior rigidez à flexão e tenacidade à fratura, respectivamente, enquanto as amostras mantidas úmidas não alteraram essas características durante o experimento. Concluímos que a umidade das amostras de caules e folhas em lianas também é um fator crítico para estimar as propriedades biomecânicas desses órgãos em seu ambiente natural.
Palavras-chave: trepadeira, fratura de folha, biomecânica vegetal, flexibilidade do caule, Módulo de Young

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Introduction

Plants deal with different environmental pressures, resisting damage, stress and maintaining structural support along their entire life span. The damage resistance and structural support of stems and leaves of woody plants can be biomechanically evaluated by their stiffness, bending and toughness (Niklas 1992, Speck 1994, Rowe & Speck 1996, Speck & Rowe 1999, Niklas et al. 2006, Rowe et al. 2005). Interestingly, biomechanical properties have also been focus of studies cross-evaluating possible associations between animal's feeding behavior and the biomechanical properties of the specific plant parts these animals consume. The physical properties of leaves and stems were shown to be related to the primate's masticatory structure (Strait 1997), and ultimately, suggested to be coevolving in animals and plants (Thiery et al. 2017). In those studies, the biomechanical properties of plant structures are measured by mechanical tests. For example, structural Young's modulus measures the relation of bending stiffness by the sample's geometrical property of area (Niklas 1992). On the other hand, toughness is the material's or plant tissue resistance to fracture, corresponding to the energy required to fracture an object. In this case, the area under a stress-strain diagram provides the measure of a material's toughness in terms of the work done per unit volume (Vincent 1992, Niklas 1992). These properties depend on the composition, shape, and hydric status of the material (Niklas 1992, Vincent 1990a). Although mechanical properties are well known for homogeneous materials, such as plastic or iron, the same is not true for the heterogeneous biological materials, such as plant structures.

In plants, samples of stems and leaves are made up of complex tissues linked with different functions (Niklas et al. 2006, Vincent 1992, Wegst & Ashby 2004). For example, fibers and sclerenchyma in different tissues are related to mechanical support, while parenchyma functions primarily in storage, but also directly influences the flexibility of the woody stems, increasing it (Niklas 1992, Speck & Rowe 1999, Ménard et al. 2009, Gerolamo et al. 2020). Different tissue and cell types combinations and arrangements are present in stems, roots, and leaves, generating a diversity of mechanical properties directly related to the types of stresses that occur on each plant organ (Niklas 1992). The amount of water available in complex tissues is also relevant in determining its mechanical properties (Niklas 1992, Vincent 1990a). In general, dehydration of biological materials causes structural changes in tissues and organs, modifying their mechanical properties (Caliaro et al. 2013, Niklas 1992, Niklas et al. 2006, Vincent 1990b). For example, grass leaves had a gradual increase in stiffness and toughness due to loss of water content (Vincent 1983). Similarly, in the stem of self-supporting species, the bending stiffness increases with the loss of wood tissue moisture content, and dry branches became more brittle and can fracture more easily when compared to their moistened counterparts (Niklas 1992). More recently, experimental evidence showed that petioles of *Caladium bicolor* lost their bending stiffness with the increase of water deficit, causing wilting (Caliaro et al. 2013).

It is well known that the effect of dehydration of stem and leaves increases the stiffness and toughness of these organs in self-supporting species (Boldrin et al. 2018, Caliaro et al. 2013, Niklas 1992). In addition, to avoid dehydration in plant structures of fresh samples, such as stems, roots and leaves, they are generally taken to the laboratory for biomechanical tests right after collection (Gallenmüller et al. 2004). Experimental evidence showing the size of the effect of dehydration on biomechanical properties is rare, and in lianas it has never been done. Lianas (wood climbers) are important and intriguing forest components in the tropics worldwide, accounting for about 25% of woody stem species diversity (Gentry 1991, Schnitzer & Bongers 2011), playing an essential role in forest dynamics (Schnitzer & Bongers 2002), and as food and canopy pathways for animals (Putz & Mooney 1991). In disturbed areas and forest edges, liana stems are abundant, and their stems are usually thin and flexible, forming dense liana tangles (Schnitzer & Bongers 2011). Lianas have unique stem anatomies, related to highly flexible stems, often with abundant parenchymatous tissue that helps torsion and resist damage after injuries (Rowe et al. 2004, Read & Stokes 2006, Rocha et al. 2020). We hypothesized that fresh liana stem and leaf samples kept moist will retain its original bending stiffness and fracture toughness of the stems and leaves, respectively, while gradual dehydration of samples exposed to air will increase stiffness and toughness a few hours. To explore this knowledge gap, we chose one liana species as a model to test whether: (i) stem structural Young's modulus and leaf fracture toughness increase during gradual dehydration of the samples over time, and (ii) how long stems and leaves maintain constant biomechanical properties after field sampling.

Materials and Methods

Plant species and study site – The stems and leaves samples used in this study were collected from the neotropical widespread liana species *Amphilophium curcigerum* (L.) L.G.Lohmann (Bignoniaceae; common name: Monkey's comb) in the Forest Reserve of the Institute of Biosciences of the University of São Paulo (23° 33'S, 46° 43'W), São Paulo, Brazil. We have immediately taken the samples to the Plant Anatomy Laboratory of the Institute of Biosciences with a controlled environments (average temperature: 25.35 ±SD 0.03 °C; relative humidity: 55.23 ± 0.53%).

Experimental design – Ten straight stem segment, approximately 20 cm long and 5-10 mm in diameter, were selected for biomechanical tests. Stem samples with a tapering percentage greater than 10 % were not considered

(Rowe et al. 2005). Also, ten mature sun leaves without herbivory marks or damage were sampled in the same individual. All of them, stem, and leaf samples, were initially weighed with a precision weighing balance and tested as described below to obtain the structural Young's modulus and fracture toughness in time 0 (natural condition), respectively.

To investigate the role of the dehydration process on the biomechanical properties of stems and leaves, we carried out a 48hours experiment manipulating the hydric status of the samples. The stem and leaf samples were randomly and evenly assigned to two distinct hydric conditions: (i) samples kept moist in a tray covered with wet tissue paper and (ii) samples undergoing dehydration gradually with natural moisture loss to the air. During the experiment, the samples were weighed every four hours and subjected to biomechanical tests.

Biomechanical parameters – We calculated the stem bending stiffness using a universal testing machine (MBioI, Biopdi, São Paulo, Brazil) with a 5 N force transducer and a constant speed of 1 mm min^{-1}, applying a three-point bending test to each stem segment, following the protocols described by (Vincent 1990b) and Rowe & Speck (1996). Previous analyzes showed that the shear effect is minimized in the three-point bending test when the span-to-depth ratio values are greater than 20 in this wood species (Supplementary material Figure S1). Therefore, we standardized the span-to-depth ratio to 30 to avoid the shear effect on the measured bending, following Vincent (1990b) and Gerolamo et al. (2020). We evaluated the displacement-force curve of each measurement interval per sample, using software MBioI coupled to the universal testing machine. The stem samples were placed on the two vertical supports, with small displacement (2 mm), ensuring that the measurements would be entirely within the elastic range of the stem to minimize possible shear influence. The typical duration for bending tests here was approximately five minutes. The flexural stiffness (*EI*; in N mm^2) of each segment was calculated using the formula [$EI = l^3$ß / 48], where *l* (mm) is the distance between the two vertical supports and ß is the slope (N mm^{-1}) of the best-fitting regression of the displacement-force curve ($R^2 > 0.98$). We calculated the axial second moment of area (*I*; in mm^4) using the diameter of each segment in three positions, in the middle and approximately 15 mm from both ends. We approximate the cross-section shape of each segment as an ellipse using the formula [$I = \pi a^3 b / 4$], where *a* (mm) represents the vertical radial diameter and *b* (mm) the diameter perpendicular to the direction of the applied force, respectively. Lastly, structural Young's modulus (*E*; in M Nm^{-2}) was calculated as [EI / I] of each sample and at each sampling time (every 4 hours), totaling 13 measurements for each stem sample.

We calculated the fracture toughness of leaves lamina using the Lucas Scientific FLS-1 portable mechanical tester (FLS-1, São Paulo, Brazil), based on a standard scissors test in which the sample is put placed between the blades of a pair of scissors (Lucas & Pereira 1990, Lucas et al. 2001). Every 4 hours, a new flat portion of the center of the leaf lamina, parallel and between secondary veins, was excised with a razor blade. Leaf lamina strips of 2.5 mm long and 1.5 mm wide were mounted perpendicularly between the blades of a pair of scissors attached to the equipment for mechanical tests. The crosshead of the tester drives the blade handle down with the load monitored at the same point to cut, in the leaf section, over a given displacement (work is done – W_c). In sequence, the scissors are returned to their original position, and the leaf sample is removed. The scissors are driven down again over the same displacement, and we recorded the work done against friction between the blades themselves (frictional work – W_f), which is termed "empty pass." The work frictional in the empty pass is deduced from the work done with the leaf sample, which gives fracture work. In the tests reported here, the scissor blades are cleaned before the test to remove any trace of the previous test. Lastly, we measure the cut length (L; mm) and its thickness of the leaf lamina sample (t; mm) using a sensitive dial caliper, and the cleaved area was calculated as the product of the leaf thickness and the length of the slit. The fracture toughness (R; J m^{-2}) of each leaf lamina sample was calculated as the work of fracture divided by the area cleaved, using the formula [$R = (W_c - W_f) / Lt$].

To obtain the relative water content (RWC; %), we used five samples of stems and leaves with the same dimensions as those used in biomechanical tests. The relative water content (%) in the stem and leaf at each dehydration level i was calculated as: [$(w_i - w_d) / (w_t - w_d)$] x100, where w_i is the mass (in g) of the stem or leaf sample at dehydration level i (i.e., at each interval of time), w_t is the mass of the sample at full hydration, and w_d is the mass of the dry stem or leaf (Scoffoni et al. 2014). To obtain the full hydration and dry mass of the stems and leaves, samples were floated in distilled water in a tray with damp papers for 24 h and weighed at least three times until stabilizing the mass. At the end of the imbibition period, samples were placed in the oven-drying, at 60 ˚C for 24h, and weighed at least three times until mass stabilization to obtain the dry mass (Turner 1981).

Anatomical procedures and analyses – The stem and leaf sample were cut in transverse sections, using a sliding microtome. Before sectioning, the samples were gradually embedded in polyethylene glycol-PEG 1500 (Rupp 1964). To avoid tearing apart phloem and xylem during the sectioning, expanded polystyrene dissolved in butyl acetate was brushed on the stem samples, and an adhesive tape was attached before a section was cut (Barbosa et al. 2010). The stems and leaf sections were double stained in 1 % w/v Astra blue and 1% w/v safranin, helping sort cells with cellulose from those with cellulose and lignin (Bukatsch 1972, Kraus & Arduin 1997), and permanent slides were prepared with Canada balsam.

Statistical analysis – To test the effect of gradual dehydration overtime on structural Young's modulus, fracture toughness, and relative water content in stems and leaves, we constructed linear and nonlinear mixed model, estimating the parameters by restricted maximum likelihoods (REML). In these models, time was used as an independent variable separately, and samples were always included as a random term. The random term herein explicitly describes aspects of our sampling design, i.e., repeated measurements of each sample over time. Similarly, nonlinear mixed models were constructed to evaluate the direct effect of relative water content on structural Young's modulus of stems and fracture toughness of leaves undergoing gradual dehydration. Exponential nonlinear models were used to represent best-fitted curves, similar to those applied by Boldrin et al. (2018) and Scoffoni et al. (2014). The models were submitted to residual analysis, checking model assumptions graphically (Crawley 2007). Finally, the models were tested by analyses of variance (ANOVA), considering P-value ≤ 0.05 as the threshold for significance (Zar 1996). We performed all statistical analyses using R version 3.6.1 (R Core Team 2019) and the R packages lme4 (Bates 2010).

Results

Anatomical structure – The stems are slender (average = 8.4 mm ±SD1.35) and cylindrical (figure 1a) in *Amphilophium curcigerum* (L.) L.G.Lohmann (Bignoniaceae). These stems have a cambial variant known as xylem interrupted by phloem wedges (figure 1b). The secondary xylem has wide vessels and broad rays that can be easily noticed in macroscopic transverse section (figure 1b). The leaves are slightly asymmetrical, with cordiform shape, venation pinnately and leaf mesophyll is dorsiventral with two layers of palisade parenchyma and about three layers of lacunose parenchyma (figures 1c-d).

Biomechanical structure – On average, the structural Young's modulus in stems kept moist is similar over time ($F_{df(59)}$ = 0.001; P = 0.96; figure 2 a), with a value around 4360 ± SD 316 MNm^{-2} and the median close to 4331 MNm^{-2}, while stems undergoing gradual dehydration increased structural Young's modulus over time ($F_{df(59)}$ = 21.54; P < 0.001; figure 2 b; Supplementary material table S1). Indeed, relative water content decreased exponentially with time in stem undergoing gradual dehydration ($F_{df(59)}$ = 215.3; P < 0.001; figure 2 c; Supplementary material table S2) and structural Young's modulus showed a negative exponential relationship with stem relative water content ($F_{df(59)}$ = 11.46; P = 0.001; figure 3; Supplementary material table S3). For example, at 12 % moisture content, the structural Young's modulus of the stem in *A. crucigerum* is on average 4500 MNm^{-2}. With 24 % moisture content, the structural Young's modulus is 4400 MNm^{-2}, a reduction of 4 % in bending stiffness. Shortly after collection, the structural Young's modulus is about 4034 MNm^{-2} in stems under gradual dehydration condition, maintaining approximately this value during the first 12 hours (figure 2 b) and up to 80 % of moisture content (figure 3 a).

On average, the fracture toughness in leaves lamina kept moist is similar over time ($F_{df(59)}$ = 1.71; P = 0.19; figure 2 d), with a value around 206 ± 53 Jm^{-2} and the median close to 197 Jm^{-2}, while leaves undergoing gradual dehydration increased fracture toughness over time ($F_{df(59)}$ = 12.57; P < 0.001; figure 2 e; Supplementary material table S4). In addition, leaf relative water content decreased exponentially with time in the leaf samples undergoing gradual dehydration ($F_{df(59)}$ = 208; P < 0.001; figure 2 f; Supplementary material table S5) and leaf fracture toughness showed a negative exponential relationship with stem relative water content ($F_{df(59)}$ = 9.35; P = 0.003; figure 3 b; Supplementary material table S6). For example, at 12% moisture content, the fracture toughness of leaf lamina in *A. crucigerum* is on average 270 Jm^{-2}. In contrast, at 24 % moisture content, the fracture toughness is on average 230 Jm^{-2}, a reduction of 15 % in leaf fracture toughness.

Discussion

Although not previously described for liana species, our results highlight that dehydration significantly affects biomechanical properties of stems and leaves of the widespread liana *Amphilophium curcigerum* (L.) L.G.Lohmann (Bignoniaceae), increasing the bending stiffness and fracture toughness, respectively. The increase of bending stiffness makes the stems of lianas more prone to rupture, limiting flexion and torsion capacity. Moreover, keeping samples moist after fieldwork allows a proper estimation of biomechanical properties, as discussed below.

It is well known that the temperature and humidity of the environment directly affect the hydric conditions of plant structures and that both anatomical composition and hydric condition affect biomechanical properties of each plant structure (Niklas 1992, Vincent 1992, Rowe et al. 2005). For this reason, most studies with biomechanics on plants keep the samples moist right after collection to obtain the closest results from those of living plants in the natural environment. However, until the present study, it was not known the magnitude of the dehydration effect on the values of bending stiffness and fracture toughness of lianas stems and leaves, respectively.

The dehydration effect in plant organs formed predominantly by parenchymal tissues has been recently described (Caliaro et al. 2013, Kampowski et al. 2018). These authors found that the loss of water content of petioles and hypocotyls causes a decrease in bending stiffness. On the other hand, for stems and woody roots in self-supporting plants predominantly formed by fibers and conducting cells (vessel and/or tracheid), the dehydration increases the bending stiffness of the material, making branches more

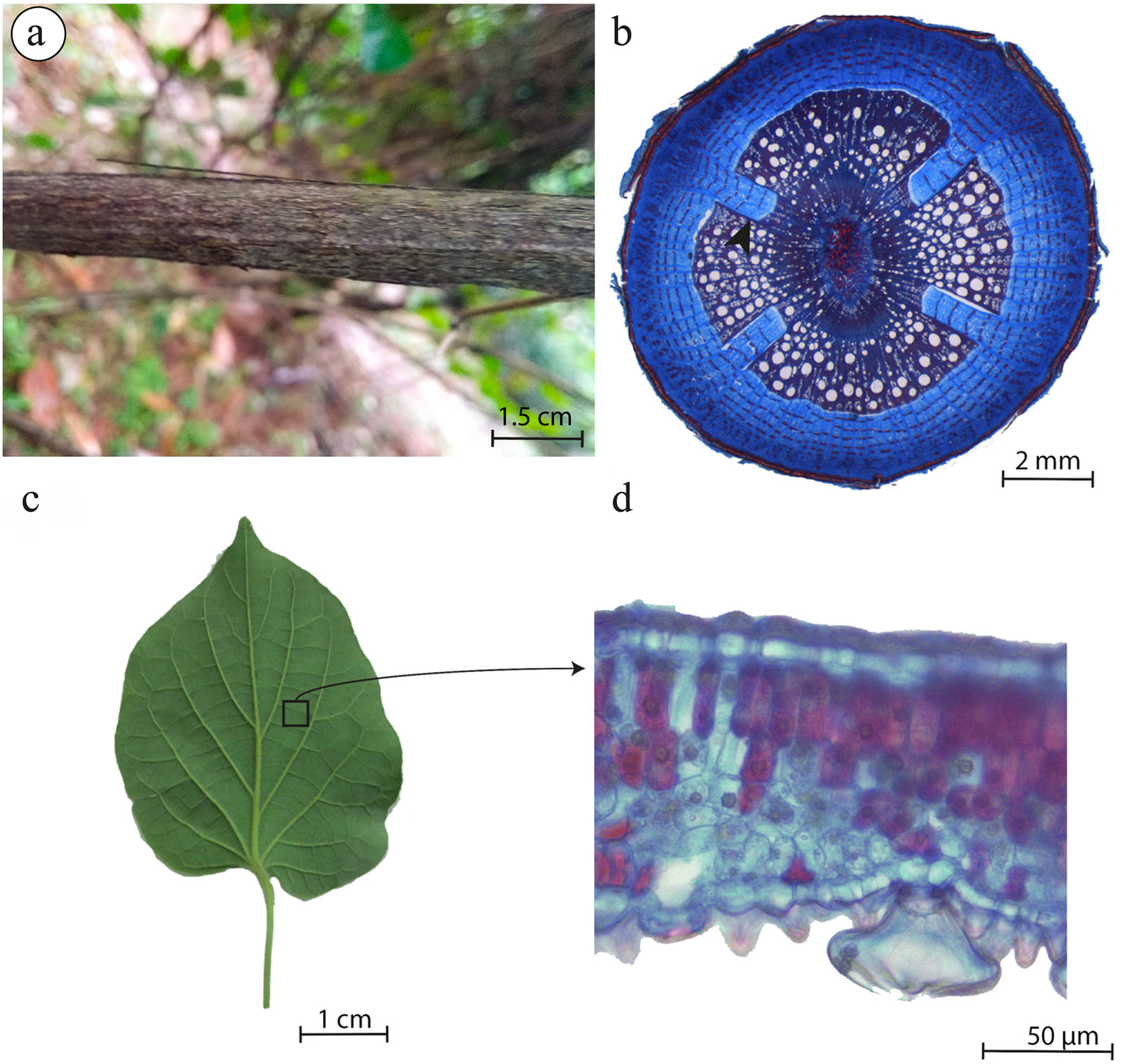

Figure 1. Morphology and anatomy of stems and leaves of *Amphilophium curcigerum* (L.) L.G.Lohmann (Bignoniaceae). a. slender, straight, and woody stem. b. anatomy of the stem transverse section, showing the four wedges of the phloem (arrowhead) and lianescent wood with wide vessels and broad rays. c. leaflets are ovate with cordate base and venation reticulate. d. anatomy of the leaf transverse section, showing the thin mesophyll.

brittle (Niklas 1992, Wang & Wang 1999, Niklas & Spatz 2010, Boldrin et al. 2018). For example, in *Picea sitchensis,* there is a 23% reduction in the value of bending stiffness when the moisture content rises from 12 to 24% in woody stems (Niklas 1992). In general, our results concur with previous studies with trees and expand this approach for liana stems. Also, the effect size of *A. crucigerum* stem dehydration on bending stiffness is six times smaller than that found for stems of *Picea sitchensis*. These smaller changes in bending stiffness with the stem dehydration on the liana may occur due to the lianescent anatomical structure. Lianas generally have wide vessels, few fibers, and a greater percentage of parenchyma cells and soft tissue, increasing the capacity to store water (Tyree & Yang 1990, Campanello et al. 2016) and forming compliant liana stems (Rowe 2018, Gerolamo et al. 2020). Indeed, the analyzed species *A. crucigerum* have phloem wedges, large vessels, and wide rays, as can be seen in figure 1, increasing the stem flexibility and reducing the effect of dehydration.

The fracture toughness of mature leaves of *A. crucigerum* (average 206 ±SD 53 Jm^{-2}) was at least three times lower than the values found for mature leaves of other dicotyledonous

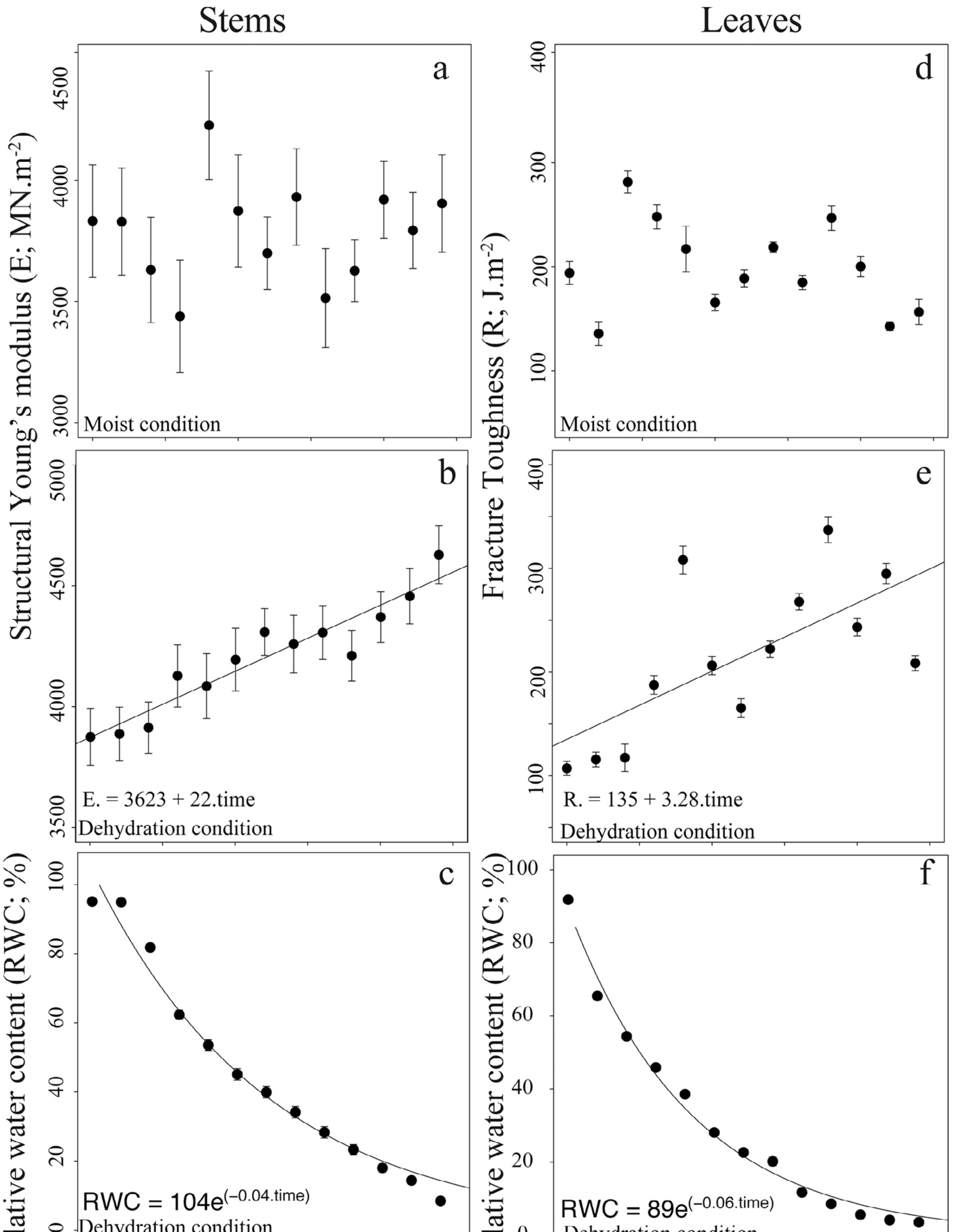

Figure 2. Biomechanical descriptors of stems and leaves of *Amphilophium curcigerum* (L.) L.G.Lohmann (Bignoniaceae) over time. a. relationship between the structural Young's modulus and time (hours) for stems kept moist. b. for stems undergoing gradual dehydration. c. relationship between the relative water content (RWC) and time for stems undergoing gradual dehydration. d. relationship between fracture toughness and time for leaves lamina kept moist, and e. for leaves lamina undergoing gradual dehydration. f. relationship between the relative water content (RWC) and time for leaves undergoing gradual dehydration. Dots and bars represent average ± standard error of five replicates per sampling time. Lines represent best-fitted curves, and best-fit equations are included in the plots and detailed in Supplementary material Tables S1-S6.

species (Lucas & Pereira 1990) but similar to that found for *Piper kadzura*, *Morus australis* and *Trema orientalis* (Hill & Lucas 1996). The lower toughness can occur due to the difference in leaf anatomy and thickness. The fracture toughness increases with the leaf thickness and percentage of fibers, but decreases with the greatest contribution of

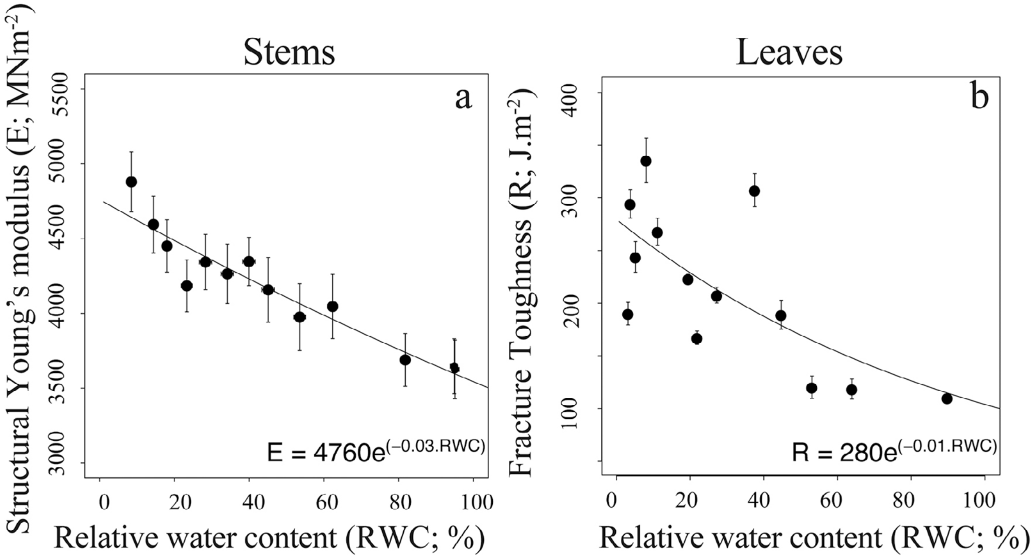

Figure 3. Biomechanical descriptors versus Relative water content for stems and leaves of *Amphilophium curcigerum* (L.) L.G.Lohmann (Bignoniaceae) undergoing gradual dehydration. a. show the relationship of structural Young's modulus for stems undergoing gradual dehydration versus relative water content. b. show the relationship of fracture toughness for leaves lamina undergoing gradual dehydration versus relative water content. Dots and bars represent average ± standard error of five replicates. Lines represent best-fitted curves, and best-fit equations are included in the plots and detailed in Supplementary material Tables S1-S6.

parenchyma cells in the mesophyll (Vincent 1990a, Lucas et al. 1995, Darvell et al. 1996). In our case, the leaf lamina of *A crucigerum* is thin and predominantly composed of parenchyma cells, as can be seen in figure 1, which probably contributed to the lowest toughness in this liana.

In addition, our results with lianas show that the fracture toughness of leaf laminas increases with drying, similar to that found in grass leaves in previous studies (Niklas 1992, Vincent 1990b, 1983). Indeed, as the leaves dry out, they become more brittle, i.e., more notch sensitive. For example, at 10% moisture content, the leaf fracture toughness of *A. crucigerum* is on average 280 Jm^{-2}. In contrast, at 50% moisture content, the fracture toughness is on average 200 Jm^{-2}, reducing 30% in fracture toughness. Detailed analysis of the effect of leaf dehydration on fracture toughness has been carried out for relatively few species (grasses: Vincent 1983, 1990b), and, therefore, we have expanded this approach to liana leaves.

The results shown here can have an important impact on evolution and diet studies of non-human primates. Plants' physical properties that are used or eaten by non-human primates can be a selective pressure behind morphology and behavior (Lucas et al. 2012, Youlatos 1999). Lianas are important structural elements of the forests, with resistant and flexible stems that stay hang connecting the tops of the trees and providing an access route to the locomotion of many animals (Rowe 2018, Lambert & Halsey 2014, Yanoviak 2015, Arroyo-Rodríguez et al. 2015). They can impact non-human primates' life and evolution that Youlatos (1999) suggested the locomotor anatomy of pygmy marmoset may be the results of feeding and traveling in liana forest. In addition, a lot of primates use many species of lianas as important food resources and can represent a large proportion in their diet, especially during periods of low availability of other foods (Marshall & Wrangham 2007). Therefore, it is reasonable to think that the low toughness of liana leaves is an important factor for the consumption of non-human primates. Accordingly, knowing the physical properties of lianas structures can be of important contribution to understanding the evolutionary impact in feeding and locomotor morphology of non-human primates (Youlatos 1999, Lucas *et al*. 2012).

In conclusion the dehydration stems and leaves of liana *A. crucigerum*, after 48 h field sampling, showed a significantly stiffer response compared to hydrated samples. In addition, the size of the dehydration effect is smaller on lianas compared to other habits, due to the unique anatomical structure of the lianas. Therefore, we recommend that to assess the biomechanical properties of plant organs in their natural condition, researchers should carry out their experiments with samples kept consistently moist or immediately after collection in the field. Our results improve our understanding about the biomechanical properties in the stems and leaves of lianas and provide support for current protocols used in biomechanics of plants.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author contributions

Caian S. Gerolamo: Conceived the ideas and experimental design; Performed the fieldwork, lab tests and data collection; Led the analyzed the data and writing of the manuscript; Reviewed and gave the final approval for publication.

Mariana D. Fogaça: Conceived the ideas and experimental design; Performed the fieldwork, lab tests and data collection; Reviewed and gave the final approval for publication.

Carolina L. Bastos: Conceived the ideas and experimental design; Performed the fieldwork, lab tests and data collection; Reviewed and gave the final approval for publication.

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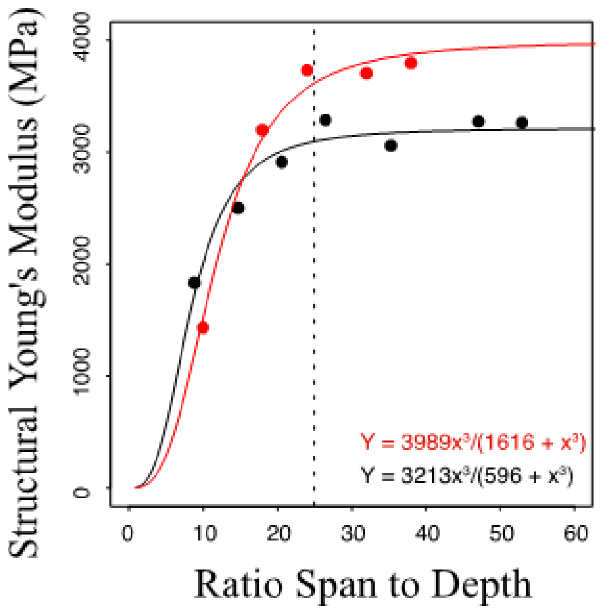
Supplementary material

Supplementary material figure S1. The nonlinear relationship between the structural Young's modulus and the span-to-depth ratio (that is, the ratios of length to diameter of tested samples) in *Amphilophium curcigerum* (L.) L.G.Lohmann (Bignoniaceae). In graph, colours of points and the predicted nonlinear model lines correspond to different samples of stems. The average size (± SD) of the stem diameter used was 5.0 ± 1.3 mm). In all cases, stiffness estimates above the 25 (dashed vertical line) of span-to-depth ratio had almost no shear effect.



Supplementary material table S1. Summary of linear mixed model of structural Young's modulus (response variable) measuring the effect of time on stem samples undergoing gradual dehydration.

Fixed effects	Coefficient	Std. Error	DF	t-value	P-value
Intercept	**3623**	680	59	5.32	< 0.001
Time	**22.75**	2.94	59	7.72	< 0.00
Random effects	Samples	Residual			
	1510	355.6			

Note: Significant coefficients ($p \leq 0.05$) are shown in bold.

Supplementary material table S2. Summary of nonlinear mixed model of relative content water (RWC: response variable), measuring the exponentially effect of time on stem samples undergoing gradual dehydration (RWC ~ a x $e^{(\text{time x b})}$).

Fixed effects	Coef.	Std. Error	DF	t-value	P-value
Intercept	**104.7**	1.77	59	58.87	0.0001
Time	**-0.04**	0.005	59	-7.94	0.0001
Random effects	Samples	Residual			
a	0.89	5.25			
b	0.01				

Note: Significant coefficients ($p \leq 0.05$) are shown in bold.

Supplementary material table S3. Summary of nonlinear mixed model of structural Young's modulus (E: response variable), measuring the exponentially effect of relative content water on stem samples undergoing gradual dehydration (E ~ a x $e^{(RWC \times b)}$).

Fixed effects	Coef.	Std. Error	DF	t-value	P-value
Intercept	**4578**	750	59	8.01	0.0001
Time	**-0.003**	0.0007	59	-3.38	0.0013
Random effects	Samples	Residual			
a	594	117.4			
b	0.002				

Note: Significant coefficients ($p \leq 0.05$) are shown in bold.

Supplementary material table S4. Summary of mixed linear model of fracture toughness (response variable), measuring the effect of time on leaf samples undergoing gradual dehydration.

Fixed effects	Coef.	Std. Error	DF	t-value	P-value
Intercept	**135.1**	24.28	59	5.56	0.0001
Time	**3.28**	0.85	59	3.82	0.0001
Random effects	Samples	Residual			
	0.005	103.6			

Note: Significant coefficients ($p \leq 0.05$) are shown in bold.

Supplementary material table S5. Summary of nonlinear mixed model of relative content water (RWC: response variable), measuring the exponentially effect of time on leaf samples undergoing gradual dehydration (RWC ~ a x $e^{(time \times b)}$).

Fixed effects	Coef.	Std. Error	DF	t-value	P-value
Intercept	**89.7**	2.12	59	40.54	0.0001
Time	**-0.06**	0.004	59	-14.42	0.0001
Random effects	Samples	Residual			
a	3.67	4.04			
b	0.008				

Note: Significant coefficients ($p \leq 0.05$) are shown in bold.

Supplementary material table S6. Summary of nonlinear mixed model of fracture toughness (R: response variable), measuring the exponentially effect of relative content water on leaf samples undergoing gradual dehydration (R ~ a x $e^{(RWC \times b)}$).

Fixed effects	Coef.	Std. Error	DF	t-value	P-value
Intercept	**276**	20.6	59	12.81	0.0001
Time	**-0.009**	0.002	59	-3.05	0.003
Random effects	Samples	Residual			
a	0.003	104.4			
b	0.0006				

Note: Significant coefficients ($p \leq 0.05$) are shown in bold.

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