

GELATINOUS FIBERS AND VARIANT SECONDARY GROWTH RELATED TO STEM UNDULATION AND CONTRACTION IN A MONKEY LADDER VINE, *BAUHINIA GLABRA* (FABACEAE)¹

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- *Premise of the study:* Some of the most striking stem shapes occur in species of *Bauhinia* (Fabaceae) known as monkey ladder vines. Their mature stems are flattened and develop regular undulations. Although stems have variant (anomalous) secondary growth, the mechanism causing the undulations is unknown.
- *Methods:* We measured stem segments over time (20 mo), described stem development using light microscopy, and correlated the changes in stem shape with anatomy.
- Key results: Growing stems are initially straight and bear tendrils on short axillary branches. The inner secondary xylem has
 narrow vessels and lignified fibers. As stems age, they become flattened and increasingly undulated with the production of two
 lobes of outer secondary xylem (OX) with wide vessels and only gelatinous fibers (G-fibers). Similar G-fibers are present in the
 secondary phloem and the cortical sclerified layer. In transverse sections, the concave side of each undulation has a greater area
 and quantity of G-fibers than the opposite convex side. Some older stems are not undulated and have less lobing of OX. Undulation
 causes a shortening of the stem segments: up to 28% of the original length.
- Conclusions: Uneven distribution of G-fibers produces tensions that are involved in the protracted development of undulations. While young extending shoots attach by lateral branch tendrils, older stems may maintain their position in the canopy using undulations and persistent branch bases as gripping devices. Flattened and undulated stems with G-fibers produce flexible woody stems.

Key words: anomalous secondary growth; *Bauhinia*; G-fiber; gelatinous fiber; liana; *Phanera*; reaction wood; *Schnella*; stem contraction; tension wood; vine.

The stems of many lianas (woody climbers) have unusual shapes and variant (also called anomalous) secondary growth (Carlquist, 1988; Fisher and Ewers, 1992; Beck, 2010). Possibly the most striking liana stems are those of the monkey ladder vines of the genus *Bauhinia* (sensu lato, Fabaceae): notably B. guianensis Aubl., B. glabra Jacq., B. microstachya Macbr., B. scandens L., B. surinamensis Amshoff, and others that are closely related phylogenetically (although they do not appear to form a monophyletic group; see Hao et al., 2003; Sinou et al., 2009; Queiroz, 2006; Wunderlin, 2010a). Old stems of these plants are flattened (at least during an intermediate stage of their development) and show extreme undulations that develop independently of the presence or absence of supporting structures in their immediate surroundings. In fact, the young stems of these lianas have modified short lateral branches bearing tendrils by which they attach to supports in their early extension growth. The undulations are produced in a very regular pattern (Figs. 1-6; also see Schenck, 1893; Wagner, 1946; Rowe and Speck, 2005; fig. 9A in Isnard and Silk, 2009).

¹Manuscript received 5 November 2013; revision accepted 7 March 2014.

The authors thank Prof. S. Ellis and Prof. L. Samuels for the use of their laboratory equipment and supplies and K. Fisher for photographic help in the field. M.A.B. thanks the Organization for Tropical Studies for post-course and graduate research grants for conducting fieldwork in Palo Verde National Park in 1999–2001.

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doi:10.3732/ajb.1300407

A cross section of the stem reveals uneven secondary growth with two lobes of secondary xylem (Fig. 18; also see Schenck, 1893; Wagner, 1946; Basson and Bierhorst, 1967; Roth and Ascensio, 1977). However, it remains unclear how the undulations are formed and what is the nature of the relationship between undulations and the variant anatomy. We present information on the development of stems in one species, *Bauhinia glabra*, and correlate the changes in stem shape to anatomy.

MATERIALS AND METHODS

Wild plants of *Bauhinia glabra* Jacq. were observed at Palo Verde National Park, Guanacaste Province, Costa Rica (10°20'N, 85°20'W, Fig. 1; population vouchered by *Blanco 1866* in the CR, FLAS, and USJ herbaria). A detailed description of the site was provided by Hartshorn (1983). This species is one of several that are commonly called escalera de mono or monkey ladder in Costa Rica, and bejuco de cadena or chain liana in other parts of Latin America.

The nomenclature of *Bauhinia* is complex with much synonomy (Wunderlin, 1976). The Neotropical lianescent species have more recently been placed in either the genus *Phanera* (Lewis et al., 2005; Queiroz, 2006) or *Schnella* (Wunderlin, 2010a, b) as part of the division of the large and paraphyletic genus *Bauhinia*. As seemingly there is no consensus yet on the generic circumscription of the various segregates of *Bauhinia* sensu lato, we opt to treat the genus *Bauhinia* in the wide sense in the present article.

Field observations of plant growth and development—Plants of Bauhinia glabra were observed in Palo Verde to gain an understanding of the development of its stems. Stems of this species go through a complex series of developmental changes (briefly described here, and in more detail in Results). Young stems are straight and almost circular to quadrangular in cross section (stage 1); later, they become flattened and undulated (stage 2); after this, the stem wings thicken while the original central portion remains thin (stage 3). In very old

American Journal of Botany 101(4): 608–616, 2014; http://www.amjbot.org/ © 2014 Botanical Society of America



Figs. 1–6. Stems of *Bauhinia glabra* and one related species, *B. guianensis*. **1.** Plants growing in the seasonally dry forest of Palo Verde National Park, Costa Rica, when canopy trees are deciduous. **2.** Live old stem of *B. guianensis* (a species that develops the same type of stem undulations as *B. glabra*) hanging over a tree branch in La Selva Biological Station, Costa Rica. Note that the *Bauhinia* stem has secured the tree branch in the concave side of one of its undulations, which reduces its risk of becoming dislodged from the support. **3.** Young shoots with youngest expanded leaf and expanding leaves subtending fully elongated lateral branches (arrows) with tendrils. Leaves removed from stem on right for clarity. These young stems are straight. **4.** Thicker stems showing start of undulations and older leaves. On left, lateral branch (arrow) lacks tendril and points toward base of stem. On right, an older undulated stem with basal remnants of axillary branches after these and their leaves and tendrils have abscised. **5.** Old stem, about 3 cm thick, viewed perpendicular to plane of undulation. **6.** Another old stem, about 2 cm thick, viewed in plane of undulation. In Figs. 3–6, the apical end of the main stem is always located on top. Scale bar = 50 mm (in 3, 4).

stems, the thickened wings (stem lobes) grow straighter, while the original central part remains thin and undulated (stage 4).

Measurements to calculate cross-sectional area (CSA), degree of undulation, and changes in length were performed in 49 stem segments, initially in September 1999. These segments were marked, and the same measurements were repeated on the surviving stems in May 2000 and in May 2001 (8 and 20 mo after the initial measurements, respectively). Each segment consisted of 10 nodes plus one quarter of the lower internode and three quarters of the upper internode (chosen this way because of the mode of development of the undulations; see Results). At least 10 segments in each developmental stage were selected (stages assigned subjectively, based on the external appearance of the stem). Segments with visible mechanical damage or developmental abnormalities were avoided.

An indirect method of measuring the CSA was required because the stems were not cut but left intact for continued growth. Each segment consisted of 10 undulations. The CSA of stems in stages 1 and 2 was calculated by multiplying the diameter measured in one direction by the diameter measured in a perpendicular direction (as if the section approached a rectangular shape). CSAs of stems in stage 3 were calculated by multiplying two perpendicular radiuses (diameters divided by half) by π for each of the two thickened lateral lobes (as if their section approached an ellipse), and adding to these the area for the thin central section (calculated as explained for stages 1 and 2). CSAs of stems in stage 4 were calculated by adding the area of the two thickened lateral lobes (calculated as explained for stage 3); the area of the central thin section was negligible in comparison and difficult to measure at this stage. For each stem segment, these measurements were made at three different points (proximal end, middle, and distal end of segment), and the three calculated CSAs were averaged to obtain a single CSA value representative of that particular segment. These CSA values are rough approximations of the real CSAs, but they provide a good indication of the stem size.

A contortion index (CI) was used to quantify the degree of curvature of the stem undulations. For each stem concavity, the length (L, defined as the distance between the crests of the two adjacent undulations) and the depth (D,defined as the distance between the bottom of the concavity and the L line, and perpendicular with the later) were measured. The contortion index was calculated by dividing the depth over the length (CI = D/L), so that more pronounced undulations have higher CIs. The CI values of the 10 undulations of each stem segment were averaged to obtain a mean contortion index (MCI) for that segment. Contortion indices were recorded as 0.00 for stems in stage 1 because they are virtually straight, and L and D are nearly impossible to measure without deforming the then-flexible stem in the process. To illustrate the variation in contortion index values, the MCI of the two young stems in Fig. 3 would be 0.00 (no measurable contortion); 0.05 for the stem to the left in Fig. 4; 0.28 for the thicker stem to the right in Fig. 4, and 0.55 for the mature stem in Fig. 5 (the most pronounced undulation near the bottom of that photograph has an individual CI of 0.82).

The length of each stem segment was measured to determine whether a contraction of the stem occurred as the undulations formed. The segment length was measured as the sum of the distances between the 11 points of inflection of the 10 undulations (the points where the curves change direction) or the corresponding points in stage 1 stems (see Results). The upper and lower limits of each segment were marked with red wax pencil to ensure that the measurements were repeated in the same portion of stem during each successive visit.

Anatomical observations-Seeds from the Palo Verde site were germinated in 2001 and grown both in the ground and in pots for more than 5 yr at Fairchild Tropical Botanical Garden (Coral Gables, Florida, USA) and at The Marie Selby Botanical Gardens (Sarasota, Florida, USA). Stem segments from these plants were fixed in 70% ethanol or FAA (formalin-alcohol-acetic acid) (Ruzin, 1999). Transverse sections were made using a sliding microtome or by hand using a razor blade. Sections were stained for the presence of lignin with phloroglucinol-HCl (= Wiesner reagent) that stains red for cinnamaldehyde units (Clifford, 1974). To check for lignin with syringyl units, sections were treated with Mäule reagent (Meshitsuka and Nakano, 1979). Sections were treated with 1% (w/v) aqueous KMnO₄ for 5 min, washed thoroughly with water and then treated with 3% HCl until the color changes to beige. Finally, concentrated NH₄OH was added to develop purple-red to brownish coloration for presence of syringyl lignin. For general histology, sections were stained with aqueous toluidine blue O (Ruzin, 1999). Areas of tissues in transverse sections were measured from captured digital images using ImageJ software (National Institute of Mental Health, Bethesda, Maryland, USA; http://rsb.info.nih.gov/ij/).

RESULTS

Field observations of plant growth and development—In Bauhinia glabra, a new vertical (orthotropic) stem of a sapling or established plant is self-supporting at first, and later forms a searcher shoot with delayed leaf expansion (Fig. 3). The leaves are distichous, with short axillary branches (short shoots) that bear a single tendril each; these short shoots are sylleptic (they grow out and mature before their subtending leaves fully expand). The short shoots are borne at every node of the long shoots; each short shoot typically produces 2 to 5 visible internodes, of which the basal most (hypopodium) is the longest one (1.5–3.0 cm). The tendril is in turn a modified branch subtended by one of the prophylls (both prophylls are reduced to minute scales; some other lianescent species of Bauhinia have two tendrils per short shoot (see fig. 1J of Isnard and Silk, 2009). The stem axis of the long shoot is straight, and the tendrils attach upon contact with supports. The apical 4-5 cm segment of the main (long) shoot is always pendulous, irrespective of the orientation of the shoot. These straight, almost cylindrical, juvenile stems are here assigned to stage 1 of development.

Farther back from the shoot apex (at least 0.5 m, but more typically several meters, in stem portions that are at least 1 yr old), the main stem becomes flattened throughout its length (due to the appearance of "wings" perpendicular to the plane of phyllotaxy) and

develops undulations in the plane of phyllotaxy (stage 2 of development). Each undulation encompasses one node, three quarters of the upper internode, and one quarter of the lower internode; the next undulation reverses the direction of curvature (Fig. 4). The undulations increase their curvature as the stem ages and thickens (Figs. 4, 5). Most short shoots abort and abscise, but their basal portion (roughly corresponding to the hypopodium) persist as thickened, reflexed stumps that resemble thorns. The crest of each undulation usually occurs a few centimeters above the node; thus, each stump appears as a hook located on the upper part of each concavity (Figs. 4, 5). The tendrils are usually shed with their short shoot, unless they have secured a support (in which case they become thickened and lignified, and can persist for a few years). The intensity of the undulations is variable, with slow-growing shoots tending to be less undulated than vigorous shoots.

As the stems become older, the undulations become more pronounced, and the outer edges of the wings become visibly thicker while the central part of the stem (adjacent to the pith) stays thin (stage 3 of development). These newly formed lateral lobes of the stem grow more toward the concave side of each undulation, so that the stem loses its flattened appearance, and becomes approximately U- or V-shaped in cross section in the peak of each undulation. This leaves the central part of the stem inside a narrow slit (produced by the lateral lobes) in the concave side of each undulation, but visible as a subtle ridge on the opposite, convex side (Fig. 6). In one case, an old stem showed physical injury that either removed or aborted one lobe along the length of the stem. The remaining stem continued to grow, but the surviving lobe became contorted with the remaining cylinder of inner xylem running along its flank.

In very old stem portions, the lateral lobes become almost straight (due to compensating cambial activity on alternating undulations), while the central part of the stem becomes hidden (stage 4 of development, not shown in figures). In this advanced stage, the inactive central part eventually dies out and disintegrates, and the two lobes produced by a single stem can become detached from each other and hang separately for a variable length up to 3 m (see a photo of the same phenomenon in a different species of *Bauhinia* in Fig. 1A of Isnard and Silk, 2009). Speck and Rowe (1999) reported the same phenomenon happening in old stems of *B. guianensis*.

As mentioned before, most axillary branches develop as short shoots that abort, and their thickened bases persist as reflexed stumps. However, some branches located at seemingly random positions continue their apical growth and become long shoots; they follow the same developmental sequence outlined above and produce tendrilled short shoots themselves. The first 30–70 cm of the main trunk on each plant (i.e., the most basal stem portion of the plant) grows more or less cylindrical, and never becomes undulated; after this point, there is a short transitional segment where the undulations become progressively more developed.

Undulations became more pronounced as the stems grew older. The MCI ranged from 0.00 (in all stage 1 stems) to a maximum of 0.68 (in a stage 3 stem); they increase rapidly with increase in cross sectional area, but their final value is highly variable (Fig. 7A). All stems with a cross-sectional area >1.00 cm² had CIs > 0.10 (and in most cases, <0.50). Calculated cross-sectional areas ranged from 0.02–0.14 cm² for stage 1 stems, 0.12–0.30 cm² for stage 2 stems, 0.38–2.44 cm² for stage 3 stems, and 1.91–12.02 cm² for stage 4 stems.

On average, MCI values increased by 0.024 after 8 mo (n = 39) and 0.051 after 20 mo (n = 28), but this increment varied with the developmental stage of the stem (Fig. 7B). Smaller

0.80

0.70

0.60

0.50 0.40

0.30

0.20

0.10

0.00

100

90

70

60

50

40

30

20

10

0

0

2

4

6

Stem cross-sectional area (cm²)

8

10

12

(cm) 80

Stem segment length

Α

Contortion index

С



Decrease 2

1

0

10 3

Stage 1

Stage 2

Stem developmental stage

Fig. 7. Morphometric changes of Bauhinia glabra stem segments, each one consisting of 10 undulations (each undulation includes one node, and ca. three quarters of the upper internode and one quarter of the lower internode). (A) Relationship between cross-sectional area and contortion index; only data from 1999 (time = 0) are shown. (B) Average increment in mean contortion index after 8 and 20 mo, according to the developmental stage of the stems at time = 0. Stage 1 stems showed no measurable undulation after 8 mo. (C) Relationship between cross-sectional area and stem segment length; only data from 1999 (time = 0) are shown. (D) Average decrease in segment length after 8 and 20 mo, according to the developmental stage of the stems at time = 0. Vertical lines in (B) and (D) represent standard deviations. Numbers in bars of (B) and (D) denote sample sizes.

14

MCI increments occurred during the last developmental stage (as the undulations approach their final curvatures). The maximum MCI increments were registered in one stem of stage 2 (0.16 after 8 mo, and 0.24 after 20 mo, for a final recorded MCI of 0.33).

As the stem segments became more undulated, they also became shorter. The average length of the segments decreased with increasing cross-sectional area (Fig. 7C). Furthermore, individual stem segments became increasingly shorter with time (Fig. 7D); the largest decrease in length occurred in stems of stages 2 and 3, which also showed the largest increase in MCI (Fig. 7B). One segment actually lost 20.5 cm over the 20-mo period (went from 72.5 to 52 cm, a decrease of 27.8% of its original length). An unexpected result is the lack of change in length of stem segments of stage 1 after 20 mo (Fig. 7D); this is probably an artifact of the low sample size (only three of 16 stems of stage 1 survived this period).

Anatomical observations-At the level of the first fully expanded leaf (Fig. 8), the internode has a cross-shaped pith and a narrow layer of vascular tissue composed of a few tracheary elements of primary xylem that merge into a narrow band of secondary xylem (Fig. 9). This early-formed secondary xylem is called inner xylem (IX) and has narrow vessels and normal lignified fibers (Fig. 10). There is a band of early secondary phloem and a cortex with a middle layer of lignified fibers and sclereids (Fig. 11).

At the level of an older, thicker, undulated internode (stages 2 and 3, Fig. 12), there are two lobes of outer secondary xylem (OX) and a thicker ring of IX between the pith and OX (Fig. 13). The change in secondary xylem begins at about the level of the first fully expanded leaf, the vascular cambium begins to produce wide vessels and G-fibers of the OX, but only in arcs at right angles to the plane of phyllotaxy. These arcs of active vascular cambium vary slightly in peripheral position according to their longitudinal position within the internode. The vascular cambium between these arcs of cambial activity becomes dormant and stops producing secondary xylem and phloem. Thus, the stem becomes either flattened (Fig. 18) with cambial arcs 180° apart or lobed (Fig. 19) with cambial arcs <180° apart, depending upon the level of section within the internode (i.e., flattened in the straight segment adjacent to a node vs. lobed in the curved part of each undulation near the center of the internode). The arrangement of the OX varies within one internode from symmetrical in the upper region (Fig. 18 to asymmetrical in the lower region (Fig. 19) and at the node itself (Fig. 20). In older internodes (stages 2 and 3), the lobes continue to enlarge, but OX is not produced between the lobes, i.e., in the plane of phyllotaxy (Fig. 12). The OX has many wide vessels and mainly gelatinous fibers (G-fibers) mixed with xylem parenchyma (Figs. 14, 15). The inner walls of these fibers are unlignified while the thin outer secondary walls color red to pink with both phloroglucinol and Mäule reagent. Darker staining is seen

9

Stage 3

Stage 4



Figs. 8-21. Stem transverse sections of Bauhinia glabra from plants under cultivation. 8-11. Sections of young stem at level of first fully expanded leaves and beginning of secondary growth (ix). 8. Internode with cruciform pith (p) and young inner xylem (ix), phloroglucinol stain. 9. Edge of internode; pith (p) at top, epidermis (e) at bottom, toluidine blue stain. 10. Inner secondary xylem with thick-walled lignified fibers and pith (p) at top, phloroglucinol stain. 11. Cortical ring of sclerenchyma, epidermis (e) at bottom, phloroglucinol stain. 12–17. Older undulated internodes in region of matured and dehiscing leaves, phloroglucinol stain. 12. Internode of thick undulated stem. Cruciform pith (p) surrounded by ring of inner xylem (ix) and two lobes of outer xylem (ox), phloroglucinol stain. 13. Inner secondary xylem with lignified fibers and small vessels (ix), pith (p) at top, outer secondary xylem with wide vessels (ox) at bottom, phloroglucinol stain. 14. Outer secondary xylem vessel (ox) with G-fibers and xylem parenchyma in bottom region, phloroglucinol stain. 15. Outer secondary xylem with greater lignification of the thin outer cell walls of G-fibers (in three clusters) and walls of xylem parenchyma near vessel, phloroglucinol stain. 16. Secondary phloem with scattered G-fibers with bright unstained walls, phloroglucinol stain. 17. Cortical sclerenchyma layer (red) with cluster of unlignified fibers in focus. The periderm (per) on bottom and phloem with G-fibers [like those seen in Fig. 16] on top are not in focus in this image. 18-21. Sections for the same undulated 55-mm-long internode used for measurements in Table 1. Sections are oriented with bottom side to the right, toluidine blue stain. 18. Top of internode (Stem 1 in Table 1) 10 mm below the upper branch on right side. 19. Near base of internode (Stem 1 in Table 1) 10 mm above the lower branch on left side of section. 20. Base of internode (Stem 1 in Table 1) at the insertion of the branch on left side. 21. Upper part of 75-mm-long, straight internode (Stem 3 in Table 1) with wide ring of inner secondary xylem, cut 5 mm below upper branch. Abbreviations: e, epidermis; io, inner xylem; ox, outer xylem; p, pith; per, periderm; Scale bar = 1.0 mm (in 8, 12, 18-21); 0.1 mm (in 9, 13); 0.05 mm (in 10, 11, 14-17).

at the cell corners in the middle lamella region near cell contact points. The walls of vessels and their adjacent parenchyma cells (Figs. 14, 15) have rose to cherry red color, typical of lignin staining in angiosperms. Deeper red-violet to brown color with Mäule reagent was never observed. Within the OX, lignified cell walls were found in vessels and adjacent parenchyma and in occasional fibers. Ray parenchyma and arcs of xylem parenchyma are unlignified (Figs. 14, 15).

The secondary phloem also has scattered clusters of G-fibers having wall structure similar to xylem G-fibers but frequently lack clear lignin staining of outer secondary walls (Fig. 16). Although a periderm develops (Fig. 12 and at bottom of Fig. 17), the cortex remains intact with groups of G-fibers dispersed as clusters within the sclerified cortical ring (Fig. 17).

Although all young stems (stage 1) have straight internodes (Fig. 3), some older stems are not undulated and have a series of straight or only slightly curved internodes. These internodes have a thick ring of IX and less developed symmetrical lobes of OX containing the typical G-fibers for this tissue (Fig. 21).

The transverse areas of various tissues were determined for several internodes (stages 2 and 3). Table 1 presents eight representatives of 16 internode sections that were measured. Symmetry was calculated by the ratios between top and bottom (T/B) and between left and right sides (L/R). Leaves were inserted on the top and bottom sides. Except for the sections made from Stem 1 (which were maintained in their original orientation), all other transverse sections were arbitrarily positioned so that OX lobes were on the bottom side. The results showed that the IX tissue was roughly symmetrical with L/R and $T/B \approx 1.0$ (actual range = 0.85-1.18) in all undulated and straight stems except for one internode with T/B = 0.45 (Fig. 20) which was cut at the node. Generally, the L/R symmetry in both OX and secondary phloem (Ph) was close to 1.0 (range 1.63–0.59). The T/B symmetry of OX and Ph was usually <0.5 or >2.0 within one internode, depending upon the convexity or concavity at the internode level being sectioned. When the two tissues containing G-fibers (OX + Ph) are summed, the T/B ratios ranged from 2.04 to 9.37 in the lobed regions of undulated internodes (but not including the flat regions as in Fig. 18). Within one internode (Stem 1 in Table 1), the lobes were on the inside of the undulation curve near the upper node, then the lobes were in one plane (i.e., flattened stem region) about one-fifth the distance below the node (Fig. 18), then the lobes were on the other side of the stem or the inside of the next undulation curve (Fig. 19 and at the lower node (Fig. 20). The sum of tissues containing G-fibers (OX + Ph) showed similar T/B ratios (Table 1). In the straight stem, the *T/B* ratio was near 1.0 (Table 1, Fig. 21).

DISCUSSION

Development of undulations—The formation of stem undulations in *Bauhinia glabra* is a product of a complex developmental process, as explained above. Growing shoots attach to nearby vegetation by tendrils on short shoots. Undulations develop along the entire length of the stem (except at the base of the plant), and their formation is not related to shoot twining, touching supports, stem orientation, or tendrils securing supports. Undulations develop routinely as the stem ages and thickens.

Young stems, including elongated searcher shoots, have an IX vascular cylinder composed of narrow vessels and lignified fibers, a feature found in the IX of other climbing species of

TABLE 1. Tissue cross-sectional areas and ratios in transverse sections of stems of *Bauhinia glabra*. Abbreviations: IX = inner secondary xylem; OX = outer secondary xylem; Ph = all tissues outside cambium; L/R = left to right ratio; T/B = top to bottom ratio. Internode lengths: Stem 1 = 55 mm; Stem 2 = 45 mm; Stem 3 = 70 mm.

Stem	Description	Tissue	Area (mm ²)	Ratio <i>L/R</i>	Ratio <i>T/B</i>	OX+Ph ratio <i>T/B</i>
1	Moderate undulation	Total stem	6.71			
	Cut 10 mm above	Pith	0.67			
	distal node					
	Distal bud on top	IX	1.05	1.09	1.15	
		OX	3.02	1.05	0.37	
		Ph	1.97	1.50	0.75	0.50
1	Cut at distal node	Total stem	8.02			
	Distal bud on top	Pith	0.65	1.02	1.10	
			1.27	1.03	1.19	
		OX Dh	3.33	0.84	0.32	0.27
	Cost 10 mm halans	Pn Tetel stere	2.56	0.70	0.45	0.37
1	distal node	Iotal stem	/.41			
	Distal bud on top	Pith	0.59			
	Fig. 18	IX	1.15	1.04	1.05	
		OX	3.01	0.89	0.89	0.00
		Ph	2.66	1.06	1.10	0.98
	Mid-internode	Total stem	7.25			
	distal node	Pith	0.56			
	Distal node on top	IX	1.04	1.04	1.01	
		OX	3.05	1.13	3.55	
		Ph	2.61	1.11	1.90	2.48
	Cut 10 mm above proximal node	Total stem	6.98			
	Proximal bud on bottom	Pith	0.59			
	Fig. 19	IX	1.02	1.02	1.04	
		OX	2.99	1.13	3.12	
		Ph	2.38	0.99	2.00	2.54
1	Cut at proximal node	Total stem	8.58			
	Proximal bud on bottom	Pith	0.69			
	Fig. 20	IX	1.71	1.18	0.45	
		OX	3.43	1.20	2.78	
		Ph	2.75	1.25	1.49	2.04
2	Large undulated stem	Total stem	28.30			
	Cut at mid-internode	Pith	0.60			
		IX	1.77	1.05	0.84	
		OX	15.34	1.22	11.24	
		Ph	10.59	1.12	7.49	9.37
3	Straight internode, distal end	Total stem	13.65			
	Cut 5 mm below distal node	Pith	1.56			
	Fig. 21	IX	5.16	1.00	1.01	
	-	OX	3.39	1.63	0.82	
		Ph	3.44	1.05	1.00	0.91

Bauhinia (Schenck, 1893; Wagner, 1946; Basson and Bierhorst, 1967; Roth and Ascensio, 1977; Ewers and Fisher, 1991; Fisher and Ewers, 1992). The changing placement of the OX is not related to the orientation of the internode. Therefore, the position of the two active arcs of the vascular cambium that produce the lobes of OX is under internal control, possibly related to the primary organization of the shoot (primary vascular pattern in nodes and internodes), as was shown in the different variant vascular pattern of *Macfadyena* (synonym: *Doxantha*; Dobbins, 1971). The beginning of undulation only occurs after the asymmetrical OX is present. Those thick stems that remain straight

614

or are only slightly undulated have a greater IX area and a relatively smaller OX area than in undulated stems.

A similar IX and OX structure was noted by Speck and Rowe (2003: their fig. 1) in the stem of another monkey ladder vine, Bauhinia guianensis, which diverges from that of B. glabra in that young stems of the former are more ribbon-like during the early undulation phase (J. B. Fisher and M. A. Blanco, unpublished observations). The young, roughly cylindrical internode of B. guianensis is initially straight, then becomes flatter (much more so than *B. glabra*) rather than lobed as it develops undulations. After this flatter stem becomes undulated, pronounced cambial activity, and additional accessory and lobed vascular cambia develop on the two lateral edges of the stem. These two regions become thicker than the undulated part (= steps or rungs of the ladder) and eventually develop into the two straight longitudinal edges (= styles of the ladder) in the old large stems that truly look like ladders or stairs. Schenck (1893) provided a detailed description of the stem development of several lianescent species of Bauhinia, including B. "blumenaviana" (probably *B. guianensis*), but not *B. glabra*.

Speck and Rowe (2003) diagramed three developmental stages of the stem of *Bauhinia guianensis* in transverse section. Their young rigid searcher axis had a cylinder of dense IX (or more exactly with a cruciform pith similar to our Fig. 12). Their medium-sized flattened axis was more flexible and had OX in two wings. Their larger old basal stem was further flattened with additional vascular cambia in the two lateral wing regions that were now thicker in the center than in the earlier flattened stem. More detailed images of transverse sections of the IX and OX were presented by Rowe and Speck (2005: their fig. 1), which they referred to respectively as "dense, stiff wood" and "highly compliant wood" (p. 63), but they did not compare the fiber anatomy in these two wood types.

We have observed in *Bauhinia guianensis* that the IX had narrow vessels and thick-walled lignified fibers. The OX had wide vessels and mainly G-fibers, each with a thin lignified outer secondary wall (colored red with both phloroglucinol and Mäule reagent) and an inner nonlignified wall that filled the cell lumen. A few normal lignified fibers and lignified parenchyma were also scattered among the G-fibers, although large pockets of xylem parenchyma remained unlignified (J. B. Fisher and M. A. Blanco, unpublished observations).

Hoffmann et al. (2003) further described mechanical and chemical properties of the wood of *Bauhinia guianensis* but did not study its wood histology. They analyzed the IX (described as "wood type I formed during self-supporting growth phase", p. 33) and OX (described as "wood type II formed during nonself-supporting phase", p. 33). Because they did not indicate the size or shape of the older axis, we cannot be certain if their "type II" axis was only the xylem of the central undulating part of the stem (equivalent to undulating B. glabra stems, e.g., our Fig. 5) or if their stem was older and included the two straight longitudinal masses of xylem that develop laterally on either side of the undulating part. Hoffmann et al. (2003) found both the moduli of elasticity and torsion were greater in IX than in OX. Thus, IX is more rigid and less flexible than OX. Lignin content (syringyl and guaiacyl units in the cell wall residue) was lower in OX than IX. There was a strong indication that microfibril angles of OX were smaller (i.e., more parallel to the longitudinal axis) than those of IX, although with some uncertainty. They also concluded that moisture sensitivity (i.e., effect of rehydration) in their testing was greater in OX than IX. Therefore, the mechanical and chemical findings of Hoffmann

et al. (2003) were consistent with our findings that IX has only normal lignified fibers and OX has mostly G-fibers in *B. glabra*.

Role of G-fibers—G-fibers with their characteristic inner unlignified layers are found in OX, secondary phloem and cortical fiber layer. The thin outer secondary wall layer has staining properties of typical angiosperm fibers: strongly stained for cinnamaldehyde units with Wiesner reaction (Clifford, 1974) and no unambiguous staining for lignin with syringyl units with Mäule reaction (Meshitsuka and Nakano, 1979). All G-fibers appear to have a similar thick, inner G-layer and a thin, outer, lignified layer (we could not distinguish presumed $S_1 + S_2$ layers at the magnification used). Thus, both xylem and phloem have G-fibers (= tension fibers) that are like the reaction xylem and phloem fibers reported for some dicotyledons by Nakagawa et al. (2012).

Internode bending starts only after initiation of the arcs of OX, and these tissues are present as lobes in a flattened secondary stem. The degree of stem bending is related to the degree of asymmetry in the areas containing G-fibers, which we have quantified by the T/B ratio of tissue area. There is a wide recognition for the correlation between G-fibers and internal tensile forces that occur in tension wood (Yoshida et al., 2002; Clair et al., 2003; Fang et al., 2008), and this is supported by new molecular understanding of the mechanism for tension generation by G-fibers (Nishikubo et al., 2007; Goswami et al., 2008; Mellerowicz and Gorshkova, 2012). The consequence of the increased tensional stress in tissues is the movement of organs containing G-fibers including: branches and trunks (Fisher and Stevenson, 1981; although questioned by Tsai et al., 2012), some contractile hypocotyls and roots (Fisher, 2008; Fisher and Tomlinson, 2002; Zimmermann et al., 1968; Schreiber et al., 2010), and tendrils (Meloche et al., 2007; Bowling and Vaughn, 2009; Gerbode et al., 2012). Since we expect that tissues with G-fibers have the capacity to produce tension and contraction, we conclude that uneven distribution of G-fibers is involved in the progressive development of the undulations in Bauhinia glabra. The regularly changing position of the G-fibers along the length of the internode (much more abundant on the concave side of each undulation) results in the curvature observed on the stem side opposite the leaf insertion. The curvature continues to increase as the stem thickens and has more tissues (OX + Ph) containing G-fibers. Further support for our interpretation might come from future direct measurements of tissue tensile forces that correlate with the presence and quantity of G-fibers in Bauhinia stems, similar to what has been found in trees (Yoshida et al., 2002; Clair et al., 2003; Fang et al., 2008; Abasolo et al., 2009).

Function of undulations—There is a long history of correlating distinctive structural and physiological traits commonly associated with climbing or lianescent habit (most recently reviewed by Wyka et al., 2013). A reasonable question is: Do stem undulations have a direct adaption for the plant? Young elongating shoots attach to supports by tendrils on lateral branches long before the internodes begin to bend. Older stems may maintain their position by using the undulations that can lodge on other stems and branches. In addition, the persistent bases of the abortive short shoots (which are usually positioned just below the outer part or "crest" of each undulation) could act as hooks or thorns that aid in fastening to supports. This adaptive function for the undulated stems of some species of *Bauhinia*

was first suggested by Schenck (1893, p. 187). According to this interpretation, young stems of *Bauhinia glabra* behave as a tendril climbers, while mature stems behave as hook climbers (categories as per Putz, 1984); both climbing modes occur in different parts of adult plants. We further hypothesize that other monkey ladder species of *Bauhinia* s.l. use their stem undulations in the same manner.

An interesting observation is that the shoots of *Bauhinia* glabra experience a shortening with time (up to almost one third of the initial length in some segments). This contraction is a geometrical consequence of the undulation of the stem axis. This contraction must produce a pulling down of the stem, but the growing apices of the shoots presumably grow faster. In this case, axis contraction is indirectly related to G-fibers, rather than the more direct effect of G-fibers found in aerial root contraction of *Ficus* (Zimmermann et al., 1968). The biomechanical forces created by such stem contraction on distal points of attachment, and its consequences for the stability of the liana in the canopy, await further investigations.

We could also speculate that the undulations are adaptive by facilitating a flexible stem, which is less vulnerable to breakage and vascular disruption (Fisher and Ewers, 1989, 1991; Putz and Holbrook, 1991; Speck and Rowe, 1999; Rowe and Speck, 2005). The convoluted stem takes on a springlike shape that aids in the stems flexibility and ability to stretch in length when the supporting vegetation moves in the wind or breaks and falls. G-fibers occur widely in a number of life forms in the family Fabaceae (Höster and Liese, 1966; Fisher and Stevenson, 1981; Ranjani and Krishnamurthy, 1988; Yoshida et al., 2002; Fisher, 2008; Schreiber et al., 2010) and in other Bauhinia species (J. B. Fisher, unpublished observations) where they function in stem support or reorientation. These two putative adaptive functions of the stem undulation are not mutually exclusive. It is interesting (although not surprising) that this unusual and complex mode of stem development has evolved apparently only in the genus Bauhinia (although it is unclear whether it has evolved once or several times within the genus; several species of Bauhinia s.l. in both the New and Old World tropics have undulated stems, but many other lianescent species do not show this condition). In monkey ladder species of Bauhinia, the combination of variant secondary growth with G-fibers produces unique and remarkable stem shapes, but the undulations themselves should be considered as one part of an anatomical syndrome in lianas that results in great stem flexibility.

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