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# DEVELOPMENT OF NONLIGNIFIED FIBERS IN LEAVES OF *GNETUM GNEMON* (GNETALES)[1]

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Leaves of *Gnetum gnemon* have an extensive anastomosing network of thick-walled cellulosic fibers that permeate mesophyll tissues. Brochidodromus venation is precise with major veins originating by uniseriate plate meristems. In mesophyll differentiation, laticifers appear before fibers and more or less parallel to major veins. Fiber initials appear later, mostly within the subhypodermal mesophyll cell layers, but otherwise adjacent to the leaf margin or the major veins. Fibers are early binucleate and sometimes become four-nucleate. Fiber initials extend by symplastic but mainly intrusive apical growth, become irregular, little branched and interpenetrate other mesophyll layers. They make frequent contact with other fibers forming the anatomosing system, but remain thin-walled until leaf expansion is complete. Sclereids are little developed, thus fibers become the main mechanical system of the mature leaf. Once expansion is complete, maturation of fibers involves rapid formation of a cellulosic but unlignified secondary wall that is non-lamellate and almost occludes the cell lumen. These fibers are contrasted with the gelatinous (tension) fibers developed eccentrically in stems of *Gnetum*. Apart from their mechanical function, fibers may also have a hydraulic function in maintaining a highly hydrated internal leaf atmosphere.

**Key words:** brochidodromous venation; fibers; Gnetales; *Gnetum*; intrusive growth; leaf development; mesophyll differentiation.

*Gnetum gnemon* L. is an understory tree species of southeast Asian rain forests that is contrasted in its habit with the usual lianescent form of most *Gnetum* species. Architecturally it provides a precise example of Roux's model in the Hallé-Oldeman system of tree architecture (Hallé et al., 1978). Phyllotaxis is decussate throughout. There is pronounced axis dimorphism, with an erect orthotropic trunk (Fig. 1) with radial symmetry producing a continuous series of plagiotropic axes with dorsiventral symmetry that branch infrequently to produce a flattened complex (Fig. 2). It has been shown that displaced trunk axes can be re-erected by eccentric development of gelatinous fibers in the primary cortex and secondary phloem, the fibers ("tension fibers") having the same secondary wall features (a thick cellulosic, lamellate, and loosely attached innermost layer of secondary wall or Sg layer) as is found in the tension wood of dicotyledonous stems and roots (Tomlinson, 2001, 2003). Similar fiber development occurs in the plagiotropic axes and maintains the quite precise horizontal position of these axes.

An extensive system of nonlignified fibers has been repeatedly mentioned for this species in the lamina mesophyll of *Gnetum gnemon*, e.g., George (1930), Rodin (1967), Martens (1971), so that a relevant question is to ask whether these mesophyll fibers also function as tension fibers. We show here that they are distinctive in their development, with neither the shape nor wall characteristics of tension fibers. They still have an important mechanical function but more significantly may show unusual water-conducting properties that facilitate the maintenance of a highly hydrated internal air-space system that characterizes the mesophyll of these leaves (N. M. Holbrook and M. A. Zwieniecki of Harvard University, personal communication).

## MATERIALS AND METHODS

Four adult specimens, the largest ~10 m high, were available at the Kampong Garden of the National Tropical Botanical Garden, Coconut Grove, Miami, Florida, USA, together with saplings grown from seed in pots for experimental purposes (e.g., Tomlinson, 2001). Most leaves were taken from plagiotropic shoots, but leaves from orthotropic shoots were shown not to differ in any structural ways. Material was examined either fresh or after fixation in F.A.A. (5 parts 40% formalin-10 parts glacial acetic acid-85 parts 50% ethanol), followed by sectioning, maceration, or clearing.

***Sectioning***—Sections of unembedded material were cut either free-hand with a single-edge razor blade or on a sliding microtome at a thickness of 20–30 μm. Appropriate stains were used to show the distribution of starch (iodine-potassium iodide), lignin (concentrated HCl and phloroglucinol), lipids (saturated alcoholic Sudan IV) and 0.1% aqueous toluidine blue as a general metachromatic stain. Sections were examined in the hydrated state in glycerine : water (1 : 1). Distribution of crystals and birefringent cell walls was observed using polarizing optics.

Fixed developing and mature leaves were embedded in paraffin in the usual way, and serial sections were cut 8–15 μm thick in both transverse and paradermal planes. Sections were stained either in safranin and fast green or in safranin : alcian green (4 : 1, aqueous solution). The last two stains produced the best, most consistent contrast.

***Maceration***—Material of young leaf primordia (up to ~10 mm long) was macerated overnight in dilute HCl (1 part : 3 parts $H_2O$ in a 55°C oven). Older material was cut into thin slivers, boiled for 2–3 min in 10% aqueous KOH, rinsed well in tap water, followed by 20% chromic acid for 15–25 min. Macerated material was again rinsed well in water, teased apart in dilute glycerin, and examined either unstained or stained in 0.1% aqueous toluidine blue. The epidermis in these preparations usually separated as a continuous sheet, and cytological details (e.g., presence of nuclei) were preserved as in Fig. 17.

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Figs. 1–2. Habit of *Gnetum gnemon*. **1.** Young tree (Roux's model), orthotropic trunk with four orthostichies of plagiotropic axes. **2.** Plagiotropic branch complex with diffuse branching and dorsiventral symmetry. Scale bars = 10 cm.

***Clearing***—Cleared preparations were best made from fixed material of either whole young leaf primordia or portions of mature ones after washing well in water. Initially, they were soaked for 1 or 2 d in alcoholic sodium hydroxide (equal volumes of 95% ethyl alcohol and 10% aqueous NaOH), the reaction speeded overnight in a 55°C oven, for older leaves. The alkaline solution was removed by repeated washing in tap water, followed by 50% ethyl alcohol and then 85% lactic acid. Specimens were either examined unstained in lactic acid, or stained in various ways, of which Bismarck brown (1% in 70% ethyl alcohol) was the most successful. Some stained specimens were dehydrated and mounted permanently in Permount (Fisher Scientific, Fair Lawn, New Jersey). Distribution of fibers in cleared preparations was revealed best by viewing the specimen with polarizing optics.

***Photography***—Illustrations from these various preparations were made using a Nikon (Nikon Corp., Tokyo, Japan) Coolpix 4500 digital camera with an attached Leitz Periplan lens. Images were processed via Adobe Photoshop 9 (Adobe Systems Inc., San Jose, California), with the original color form translated into black-and-white.

***Scanning electron microscopy (SEM)***—The SEM images (Figs. 19, 20) were produced from transverse and paradermal sections of leaves fixed in 70% ethanol, critical point dried, and coated with platinum. These were photographed at 5 kV on an FEI Quanta 200 Environmental Science and Engineering microscope (FEI Company, Hillsboro, Oregon) at the Center for Mesoscale Structure in the Harvard University Herbarium.

## RESULTS

Results are presented in a development sequence represented in transverse sections in the series in Figs. 6–8 and Figs. 14–16, with the mature leaf in Figs. 18–19. Important details in paradermal view are shown in Figs. 10–13 and Fig. 20.

***Lamina development***—Each apical bud is enclosed within the pouched bases of the terminal pair of leaves, with additional protection from epidermal hairs at the mouth of the pouch. The enclosed bud includes only two leaf pairs below the shoot apex. Extension growth of the shoot normally involves elongation, by means of a rib meristem, of the single internode below the second enclosed leaf pair (Johnson, 1950). At the same time, this leaf pair expands, initially protruding through the mouth of the pouch. During this extension, a new leaf pair is initiated by the apical meristem to maintain a constant leaf number in the bud. Any shoot may make several of these extensions, with a resting period between, during the more active growing season in South Florida, but there is little synchronous development throughout the crown. On vigorous orthotropic shoots, there may be continuous extension of two or three internodes at one time.

Leaf development begins with a somewhat cylindrical leaf axis that develops a marginal meristem as a precursor to the blade, with the retention of the thickened midrib. Succeeding growth of the lamina establishes a number of cell layers, varying between 10 and 13 (including the epidermis), with fewest at the leaf margin and apex. These cell layers remain constant throughout blade expansion because each layer functions as a plate meristem, with cell division in several anticlinal directions occurring longest in the hypodermal layer. In this way, a single compact adaxial hypodermal palisade is formed, together with a somewhat less well-defined abaxial hypodermal layer.

The venation corresponds to that termed brochidodromous by Hickey (1973) or perhaps more precisely "festooned brochidodromous" in the terminology of the Leaf Architecture Working Group (Wing, 1999). This involves early establishment of about seven major second-order veins on each side of the mid-rib, the veins joined together by submarginal loops (Fig. 3). Lamina expansion then involves increase in the area between the major veins and development of third- and fourth-order veins of progressively narrower diameter (Fig. 4). These delimit progressively smaller areoles that enclose the ultimate veins, some of which are blind-ending. Although fibers are initiated early, they only become obvious in later stages and are best revealed by polarizing optics (Fig. 5). Cell divisions within each layer progressively expand the mesophyll, which is segmented into discrete areoles delimited by the procambial strands of the ultimate veins (Fig. 6; Fig. 11, PC). The autonomy of each cell layer of the lamina is indicated in paradermal sections that show the nuclei in a single plane (Fig. 11). Procambium is continuous as the lamina expands and is followed by protophloem and protoxylem, the last often at first discontinuous in the ultimate vein order.

In early stages, there is little mechanical tissue, other than that of the tracheary elements (Fig. 4); most support comes from the compact epidermis whose anticlinal walls ultimately interlock in the manner of jig-saw puzzle pieces. The final stages of epidermal maturation involve the development of a thick and uniformly cutinized outer wall (Fig. 16). Stomatal differentiation is exclusive to the abaxial epidermis and begins early in leaf differentiation, with new initials continually added as the leaf enlarges; these new initials account for their random orientation (Fig. 4).

Late expansion of the leaf, once cell division has ceased, involves cell separation and enlargement in the middle 6–8 layers so as to produce the spongy mesophyll. In paradermal view, this is seen as a reticulum of elongated and lobed cells enclosing circular cavities (Figs. 13, 20). These cavities are continuous with narrow intercellular spaces of the abaxial hypodermal one or two layers. The adaxial palisade layer remains uniformly compact; its subhypodermal layer is initially somewhat compact but is eventually disrupted by the development of fibers (Fig. 12).

Vein size is determined by order and time of maturation. The second-order veins have an indistinct compact sheath of elongated parenchyma cells within which some fibers are concentrated (Figs. 7, 15). In progressively smaller veins of higher order, the sheath is narrower; the ultimate veins show limited sheath development.

***Histological development***—Earliest cell differentiation in the lamina produces laticifers within the still compact mesophyll. They remain thin-walled and unbranched and extend apparently by symplastic rather than intrusive growth because their ends are always blunt. They can be recognized by their dense granular or amorphous cytoplasm that stains readily with Sudan IV indicating lipid or resinous contents (Figs. 7, 8). Laticifers are nonarticulate although the regular contraction of the dense cytoplasm may give a superficial impression of a multicellular condition. This may account for the description by Behnke and Herman (1978) of articulate laticifers in the stem of *Gnetum*, said to be formed by breakdown of transverse walls in early stages of laticifer formation in the lamina. Laticifers reach lengths of several millimeters to the extent that they are never retained in their entirety in macerations and cannot be measured precisely. Their overall course tends to be parallel to each other and to the larger veins, but they rarely anastomose. Because of their persistent thin walls and loss of cell contents, they are ultimately difficult to identify in thin sections (cf. Figs. 7, 8).

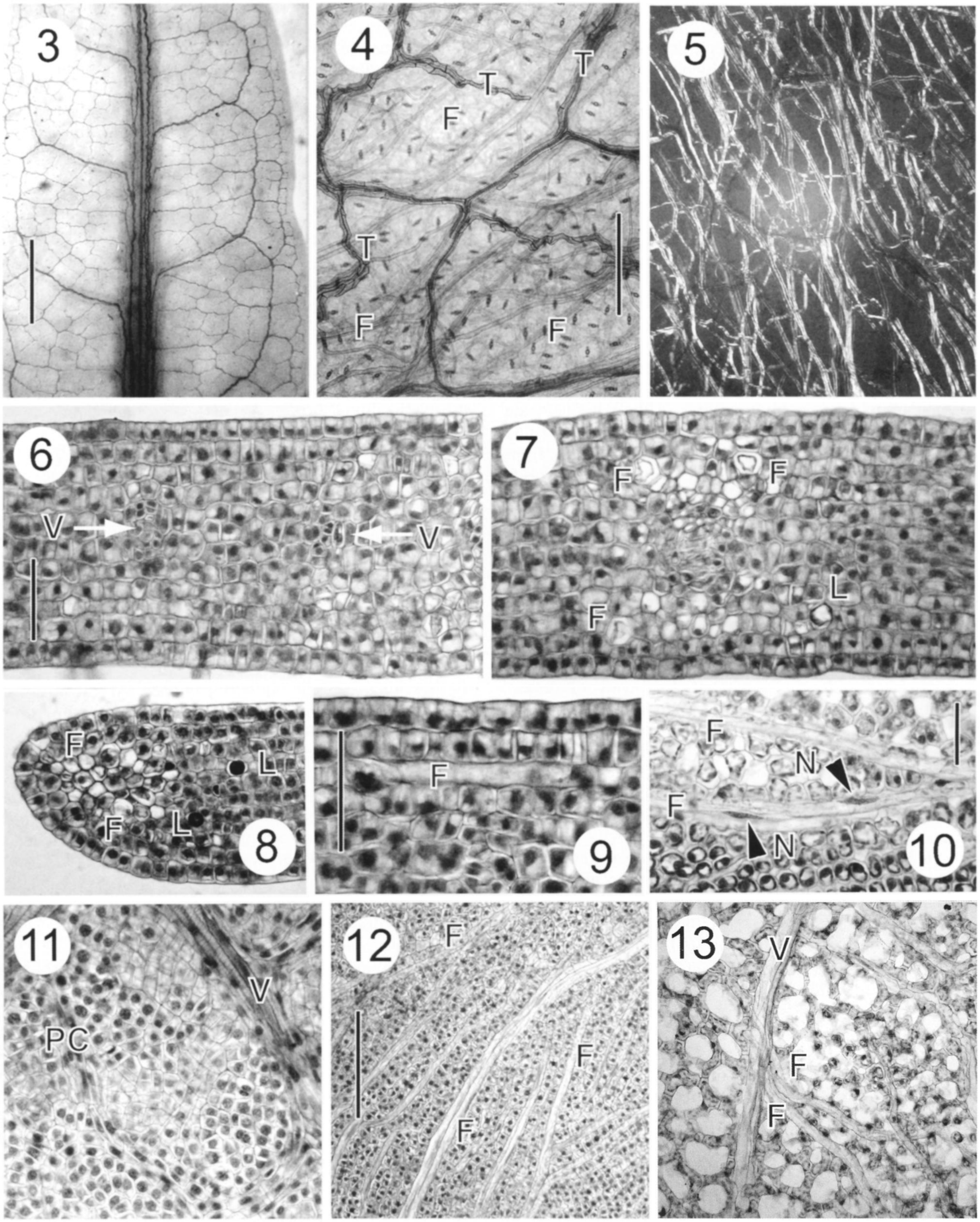
Astrosclereids, which are common in the stem, are little developed in the leaf except for the ground tissues of the petiole, midrib, and larger veins. This contrasts with the leaves of most of the scandent species of *Gnetum* in which the mesophyll has well-developed sclereids that are diagnostically useful (Rodin, 1966).

***Fiber development***—Fibers mostly originate from cells of the second hypodermal layer of each surface, and they are tentatively identified first as elongated subhypodermal initials, i.e., separated from the epidermis by a single layer of cells (Fig. 9). Otherwise, they originate from cells accompanying the larger veins (Fig. 7) and at the leaf margin (Fig. 8). They are seen first in the mesophyll in leaves about 8 mm long as cells larger than adjacent cells of the same layer (Fig. 9). Their presence soon becomes unequivocal as they extend by intrusive growth either within the same layer (Fig. 12) or often extending into the spongy mesophyll (Fig. 13). Less commonly they may reach the epidermis.

The most distinctive feature of the fibers is their binucleate condition, which is evident very early (Fig. 17). Occasional examples are four-nucleate, as is seen in macerated material. The cytoplasm is limited to a thin peripheral layer, normally collapsed in fixed material and surrounding the central vacuole. In early stages, nuclei are spherical, as in ordinary mesophyll cells, but they soon become elongated and finally spindle-shaped (Fig. 10) as they migrate along the extending fiber. Elongated nuclei are seen in early procambial cells (Fig. 11), but they are never spindle-shaped.

Fiber initials are always fewer in the abaxial subhypodermal layer (Figs. 14, 16), possibly because this layer develops an intercellular space system earlier than the corresponding adaxial layer (Figs. 14, 16). The difference is obvious in the mature leaf (Fig. 19). Because of their intrusive growth, the path of the fibers is irregular, and they frequently double back on themselves. They remain thin-walled until after leaf expansion is complete but rarely branch, the branch always being short (Fig. 5). The tip of the main fiber and the branches taper to a rounded, irregular point as the fiber conforms to the outline of the intercellular space it penetrates. Fibers originating in vein tissue can remain straight and parallel to the vein, without evidence of intrusive growth, but their frequent extension into the mesophyll and anastomoses with mesophyll fibers (Fig. 13) shows that their tips extend in much the same way by intrusive growth and contributes to the overall interlinked fiber system. Fiber length, even in macerated material, cannot be measured accurately because of their irregular course, but clearly exceeds 10 mm in length.

Although conspicuous in paradermal view, fibers (Fig. 12)



Figs. 3–13. Leaf development of *Gnetum gnemon*. Figs. 3–5, 10–13, paradermal views; 6–9, transverse sections (TS). **3.** Cleared portion of a young leaf stained in fast green. **4.** Abaxial detail of cleared leaf stained in Bismarck brown, fibers (F) with thin primary walls; veins including protoxylem tracheids (T); stomata randomly arranged and out of focus. **5.** Cleared lamina in lactic acid under polarizing optics showing anastomosing fibers with birefringent primary walls. **6.** Lamina from leaf 8 mm long, cell layers (12) without obvious differentiation except for early procambium of two small veins (V). **7.** Lamina with larger vein and early fibers (F) with vacuolated contents; laticifers (L) with more densely stained cytoplasm. **8.** Lamina margin with numerous fiber initials (F); laticifers (L) with densely stained contents filling cell lumen. **9.** Early fiber initial (F) in adaxial subhypodermal layer. **10.** Later fiber (F) differentiation as

in both freehand and paraffin sections of young leaves can be difficult to recognize because of their thin walls and by loss of cell contents (e.g., Figs. 14–16). This condition persists until leaf expansion is complete, at which time the leaf is very flaccid. Support is then provided by cell turgor, the interlocked epidermal cells, and tracheary elements of the vein system. Protoxylem tracheids are at first the only lignified cells, subsequently augmented by the occasional astrosclereids associated with larger veins. Cleared preparations reveal the extensive juxtaposition of many fibers along their long axis as they permeate the mesophyll (Fig. 5). Fibers tend to run parallel to each other and parallel to the larger veins (Figs. 4, 12), suggesting that they follow a path of least resistance as the mesophyll expands.

Fibers only complete their development after the leaf becomes fully expanded and its subtending internode completes extension, i.e., before a succeeding leaf pair begins extension, a consequence of the articulate method of shoot growth. Final development involves the formation of a thick secondary cellulose wall that almost occludes the cell lumen (Figs. 18–20). This wall is not obviously stratified, unlike the tension fibers of the stem, but like them remains unlignified as shown in Fig. 18 in which the xylem of the midvein is the only dark-stained tissue in the lignin reaction.

At maturity the lamina becomes rigid and the mechanics of the leaf is completed, with supplementary rigidity provided by the final thickening and cutinization of the outer epidermal wall. Detached mature leaves do not wilt but retain their rigidity for several days or longer in detached shoots.

***Mature lamina***—The mesophyll of the fully expanded leaf includes a single compact adaxial hypodermal palisade-like layer (Fig. 16) and the less compact adaxial subhypodermal layer in which most fibers originate. The abaxial hypodermal layer is interrupted by the substomatal cavities that communicate with the air-space system of the central mesophyll so that a distinct dorsiventral symmetry exists (Fig. 19). The bulk of the mesophyll consists of horizontally extended curved or lobed cells that collectively enclose the characteristic circular lacunae and produce the areolate appearance seen in paradermal view (Figs. 13, 20). The whole mesophyll tissue is permeated by fibers, although these are concentrated in the surface layers, reflecting their place of origin (Fig. 19).

Major veins of the lamina have an indistinct sheath of elongated parenchyma cells, which is progressively reduced in higher-order veins so that there is a continuum of vein types, beginning with the distal part of the midrib (Fig. 18). Limited development of xylem and phloem occurs; the xylem of the larger veins includes narrow vessels, mostly with the foraminate perforation plates that are characteristic of the Gnetales. Laticifers, a conspicuous cell type in early leaf development, are inconspicuous in mature leaves except in the larger veins.

***Leaf senescence***—Leaves on older shoots show changes that may reflect a senescent condition. This includes accumulation of starch and fine crystals, together with the deposition of large oil-bodies in all cell layers. Senescent leaves may have secondary expansion of mesophyll cells that become spherical and occlude the air-space system. However, there is no increased lignification, and tannin is not developed.

## DISCUSSION

Rodin (1967) makes generalized statements about leaf development in *Gnetum* based on four species, one of which is *G. gnemon*. He used only cleared specimens and so emphasizes only the origin of the venation, which he mistakenly presents as a dichotomizing system. However, the arrangement better conforms to the type referred to as brochidodromous by Hickey (1973), with the further development of the ultimate vein system, which includes some free vein endings, corresponding to that described by Pray (1963) for angiosperm leaves. Thus, the oft-repeated comment (e.g., Martens, 1971) about the dicotyledonous appearance of the leaves of *Gnetum* (Fig. 2) is confirmed by detailed developmental study. A comparison of *Gnetum gnemon* with at least the American species of the genus (Rodin, 1966) shows that its leaves largely lack lignified elements, other than the tracheary elements, whereas in the scandent species of *Gnetum*, Rodin suggests that lignified sclereids, which are often abundant, are diagnostic at the specific level.

In the present account, we emphasize the mesophyll fibers of *G. gnemon* because of their unusual method of development and cell wall structure. Fiber initials appear very early in leaf development and usually in the subhypodermal layer of both surfaces, but more numerous toward the adaxial surface. They initiate intrusive apical growth immediately, at a time when the mesophyll layers are still compact. As the intercellular space system appears, fiber initials increasingly penetrate other mesophyll layers, sometimes producing short branches. This extension growth is carried out while the primary wall remains thin, the cell highly vacuolated, but also with two, sometimes more, nuclei produced by cell division without wall formation. The fibers are produced in such abundance and grow with such extensive elongation that the whole mesophyll becomes permeated by an extensively anastomosing system, with frequent contact made to similar cells that run adjacent to and parallel to the main veins.

Because developing fibers remain thin-walled, the leaf in its early development is very flaccid. Only when the leaf has reached full size does secondary wall formation begin in fibers. Previous lamina support is provided simply by the interlocking cells of each epidermis together with the vein system. Once fibers produce their thick non-lamellate walls, the stiffness of the lamina is much increased, but it remains flexible because of the absence of lignin. However, N. M. Holbrook and M. A. Zwieniecki of Harvard University (personal communication) have shown an additional function in that fibers conduct water readily, as seen in the movement of fluorescent dyes, as will be reported in a separate publication. This apo-

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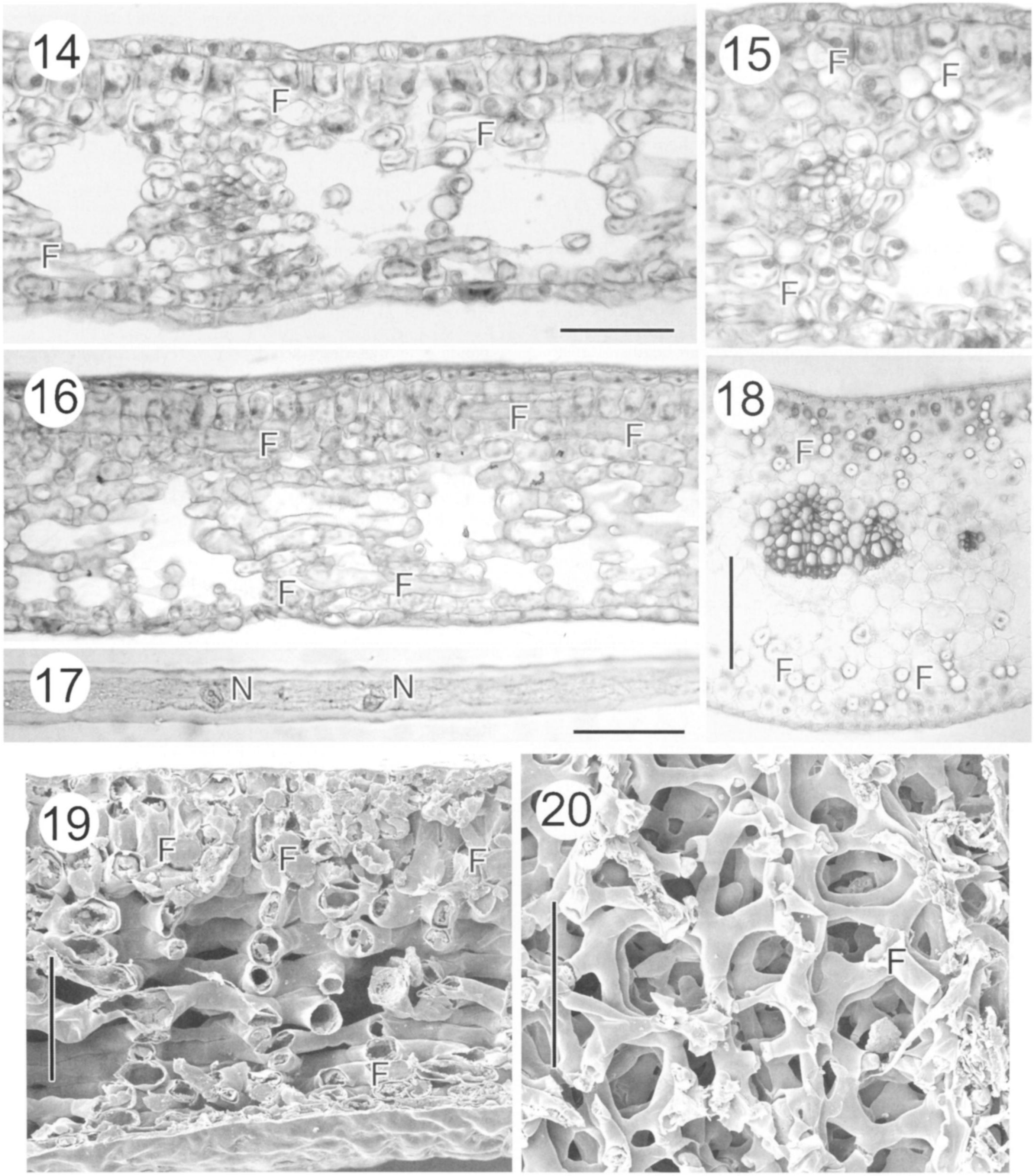
intercellular space system of mesophyll begins to enlarge; the two contiguous lower fibers each have a characteristic spindle-shaped nucleus (N) (cf. Fig. 17). **11.** Early development of mesophyll, with procambium (PC) of minor vein to left and larger vein (V) to right, with some early protoxylem, intervein mesophyll layer uniformly undifferentiated. **12.** Adaxial subhypodermal layer with numerous anastomosing fiber initials (F). **13.** Late stage of mesophyll differentiation with characteristic areolate mesophyll to left, abaxial more compact subhypodermal layer to right, fibers (F) diverging from vein (V). Fig. 3, scale bar = 1 mm; Figs. 4–5, scale bar = 400 μm; Figs. 6–9, scale bar = 50 μm; Figs. 10–11, scale bar = 100 μm; Figs. 12–13, scale bar = 200 μm.



Figs. 14–20. Maturing and mature fully expanded lamina of *Gnetum gnemon*. All TS except 17 and 20. **14.** Lamina immature but intercellular space system of mesophyll fully established, fibers (F) thin-walled and highly vacuolated and therefore obscure. **15.** Larger vein with numerous accompanying fibers (F) recognized by highly vacuolated condition (cf. Fig. 7). **16.** Lamina with cutinized and thicker-walled epidermis but fibers (F) still thin-walled and obscure. **17.** Portion of young fiber from maceration to show two nuclei (N); the collapsed cytoplasm is an artifact. **18.** Distal midrib from free-hand section of a mature leaf, stained in phloroglucinol and concentrated HCL; xylem is lignified, mature fibers with thick secondary walls are nonlignified. **19.** An SEM transverse view of leaf tissue with mature fibers (F). **20.** SEM, paradermal view showing areolate mesophyll with only one fiber (F) visible. Figs. 14–16, scale bar = 100 μm; Fig. 17, scale bar = 40 μm; Fig. 18, scale bar = 160 μm; Figs. 19–20, scale bar = 100 μm.

plastic transport system is responsible for maintaining a high internal humidity; consequently stomata tend to remain open, promoting more extensive gas exchange. This property may accord well with the status of *G. gnemon* as a forest understory species.

A further peculiarity of these leaves is that they lack tannin, but have a high content of fine crystals, which may serve as an alternate herbivore deterrent. However, leaves of *G. gnemon* are said to be eaten as a green vegetable in Malaysia (Burkill, 1966).

Our conclusion is that leaf fibers with dual functions of mechanics and hydraulics contrast with the gelatinous or reaction fibers of the cortex and secondary phloem of the stem, which have tensile properties (Tomlinson, 2001, 2003). Stem fiber maturation (cortical fibers) or initiation (phloem fibers) is apparently a gravimorphic response because they develop eccentrically in leaning or horizontal stems. The microfibrillar structure of the secondary wall of stem and leaf fibers also differs; leaf fibers do not have readily collapsed and lamellate walls, as do tension fibers (Tomlinson, 2003). Furthermore, leaf fibers are often shortly branched and have irregular shapes, neither of which is possible if they were to contract in the manner of tension fibers. *Gnetum* leaf fibers have uniform secondary walls that at maturity almost occlude the cell lumen. More detailed ultrastructural study of these contrasting fiber types in *G. gnemon* is desirable as is a comparative study of leaf development of scandent *Gnetum* species that develop abundant lignified sclereids (Rodin, 1966). A comparative biological approach should lead to a better understanding of fiber function in general. It should be emphasized that gymnosperms rarely develop nonlignified fibers (Napp-Zinn, 1966); their dual occurrence in *Gnetum* adds to the angiosperm-like features of this enigmatic genus.

## LITERATURE CITED

BEHNKE, H.-D., AND S. HERMANN. 1978. Fine structure and development of laticifers in *Gnetum gnemon* L. *Protoplasma* 95: 371–384.

BURKILL, I. H. 1966. A dictionary of the economic products of the Malay Peninsula, 2nd printing. Ministry of Agriculture and Cooperative, Kuala Lumpur, Malaysia.

HALLÉ, F., R. A. A. OLDEMAN, AND P. B. TOMLINSON. 1978. Tropical trees and forests: an architectural analysis. Springer Verlag, Berlin, Germany.

GEORGE, L. 1930. Contributions à l'étude des Gnétales. Thèse Faculté des Sciences de Paris, Nancy, France.

HICKEY, L. J. 1973. Classification of the architecture of dicotyledonous leaves. *American Journal of Botany* 60: 17–33.

JOHNSON, M. A. 1950. Growth and development of the shoot of *Gnetum gnemon* L. I. The shoot apex and pith. *Bulletin of the Torrey Botanical Club* 77: 354–367.

MARTENS, P. 1971. Les Gnétophytes. Handbuch der Pflanzenanatomie, Band 12, Teil 2. Gebrüder Borntraeger, Berlin, Germany.

NAPP-ZINN, E. 1966. Anatomie des Blattes. I. Blattanatomie der Gymnospermen. Handbuch der Pflanzenanatomie, Band VII, Teil I. Gebrüder Borntraeger, Berlin, Germany.

PRAY, T. S. 1963. Origin of vein endings in angiosperm leaves. *Phytomorphology* 13: 60–81.

RODIN, R. 1966. Leaf structure and evolution in American species of *Gnetum*. *Phytomorphology* 16: 56–68.

RODIN, R. 1967. Ontogeny of foliage leaves in *Gnetum*. *Phytomorphology* 17: 118–128.

TOMLINSON, P. B. 2001. Reaction tissues in *Gnetum gnemon*: a preliminary report. *International Association of Wood Anatomists Journal* 22: 401–413.

TOMLINSON, P. B. 2003. Development of gelatinous (reaction) fibers in stems of *Gnetum gnemon* (Gnetales). *American Journal of Botany* 90: 965–972.

WING, S. L. 1999. Manual of leaf architecture. Smithsonian Institution, Washington, D.C., USA. Available at website, http://www.peabody.yale.edu/collections/pb/MLA/.