

ANATOMY OF THE PALM RHAPIS EXCELSA, VIII.
VESSEL NETWORK AND VESSEL-LENGTH DISTRIBUTION
IN THE STEM

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THE CONCEPT of the vessel, a conducting unit consisting of a series of vessel elements lined up end to end, has been known for well over a century (e.g., Hartig, 1878). The fact that vessels are of limited length is of considerable functional importance. If a vessel is damaged (for example, by an insect), air is drawn into its lumen as water withdraws into neighboring tissue, because xylem water is normally under less than atmospheric pressure. The damaged vessel is thus permanently lost as a functioning unit. Numerous small vessels therefore represent a conservative and safe water-conducting system, while wide and long vessels are much more vulnerable, although a great deal more efficient (Zimmermann, 1978).

Vessels are far too long to be seen in their entirety in single microtome sections. Macerations may reveal individual elements that can be interpreted as vessel ends (see Bierhorst & Zamora, 1965; Handley, 1936), but terminal elements are thus seen only in isolation. Length and arrangement of vessels within the xylem is of interest to anyone studying the hydraulic construction of a plant. It was not really known how and where vessels end until the method of three-dimensional cinematographic analysis first described in the initial paper of this series (Zimmermann & Tomlinson, 1965) had been developed. Cinematographic analysis proved to be a powerful tool that enabled us to sort out the most complex vascular systems with relative ease. In previous papers of this series as well as in many other publications, we described the course of entire vascular bundles. Vascular bundles are, of course, the pathways of both xylem and phloem transport. The direction and pathway of phloem transport has been studied by following thousands of autoradiographs through the *Rhapis* stem (Zimmermann, 1973). In this paper, attention is focused on the xylem, with descriptions of the precise layout of vessels within vascular bundles and of the functional significance of their arrangement.

MATERIALS AND METHODS

Individual vessels were followed in some of the motion picture films that we had made earlier for the analysis of the vascular system of *Rhapis* (see

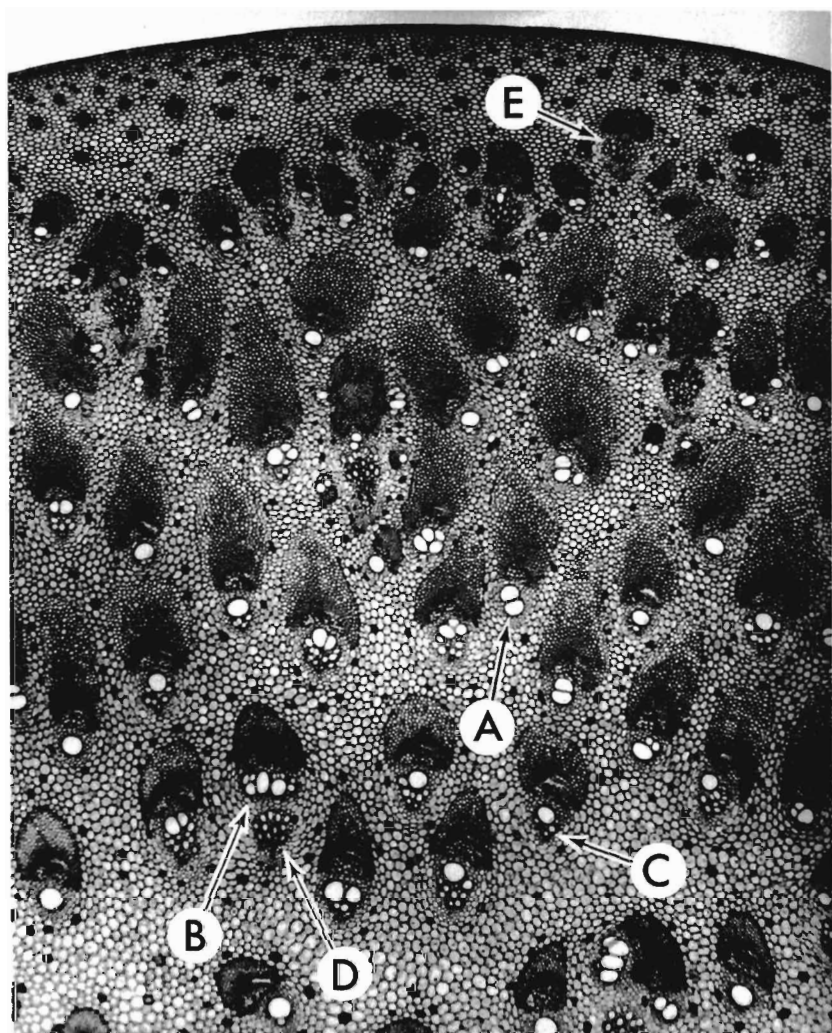


FIGURE 1. Transverse section of stem of *Rhaps excelsa*, $\times 35$. Axial bundles more than 10 cm below leaf contact have no protoxylem. Two metaxylem vessels overlapping (A). Leaf traces containing small amount of protoxylem (C), much protoxylem (D) and metaxylem (B), and only protoxylem (E).

Zimmermann & Tomlinson, 1965). Most of these films are made up of individual frames that show 50- μm -thick transverse sections spaced at 250- μm intervals. Magnification in these films is high enough that individual vessels are seen clearly. In some cases it is easy to distinguish vessels from vessel elements. In an axial bundle a metaxylem-vessel end may overlap the end

of the continuing vessel by 2 cm—the length of about 40 elements. Transverse sections show the vessel pair (e.g., at A in FIGURE 1). To be certain that one is dealing with vessels and not merely with element ends—especially in areas such as leaf-trace departures, where vessels are crowded (e.g., at B in FIGURE 1)—the film must be run back and forth in order to ascertain vessel continuity.

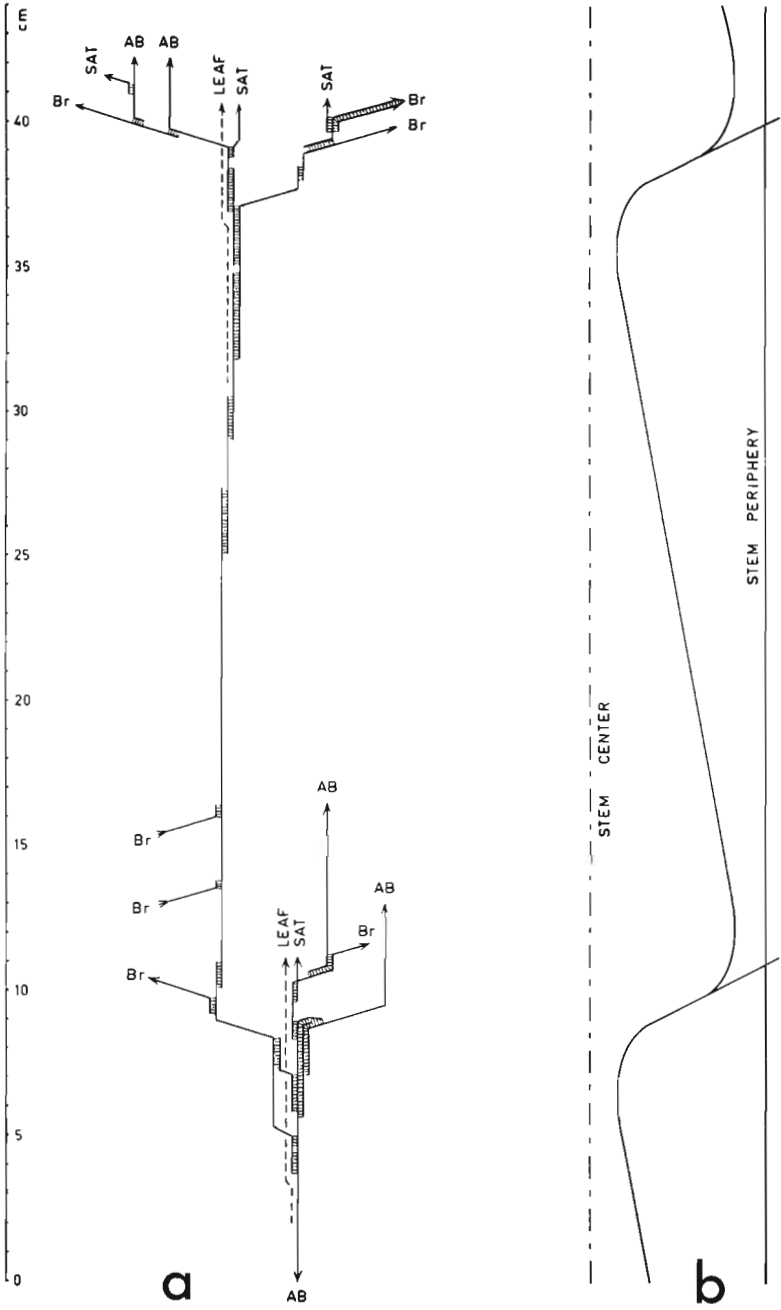
Vessel-length distribution measurements were made with the latex-paint infusion technique (Zimmermann & Jeje, 1981). Fresh stems of plants grown at the Harvard Forest greenhouse and outdoors at the Fairchild Tropical Garden were cut at the base (just above the root system), trimmed cleanly with a razor blade or microtome, and vacuum infiltrated with water to remove any air that might have been drawn into the vessels by cutting the stem. The plant was then allowed to transpire in a horizontal position, taking up a dilute suspension of latex paint particles, until wilted (1–2 weeks). This procedure fills all vessels that are cut open at the basal end with paint particles. When no more liquid was taken up by the plant, the stem was cut into 5-cm-long segments. The ends were trimmed with the microtome, the paint-containing vessels were counted, and the vessel-length distribution was calculated.

RESULTS AND DISCUSSION

THE VESSEL NETWORK

Representation of three-dimensional vascular systems in two-dimensional illustrations is always difficult. Some simplification is usually necessary in order to bring out a specific feature. In the past we have shown the path of vascular bundles in monocotyledonous stems projected onto a radial plane (radial coordinate projection), as in FIGURE 2, b. These plots were usually foreshortened five to ten times to enhance radial displacement. In order to show vessel contacts, we have projected the vascular system here radially onto a tangential plane (FIGURE 2, a). In this case radial displacement in the stem is ignored. The diagrams show the axial extent of vessels and vessel overlaps—the feature of specific interest here. Horizontal distances in the drawing are shown greatly expanded and without scale. In visualizing dimensions, one has to keep in mind that the width of the entire leaf-trace complex shown in FIGURE 2, a, is, at the most, 1 mm. In other words, if the horizontal scale were the same as the vertical one, the whole leaf-trace complex would not occupy much more space than the thickness of the vertical line illustrating a vessel!

FIGURE 2, a, shows the vessels in a major vascular bundle over an entire leaf-contact distance. Solid lines indicate metaxylem (mx) vessels. Hatching between two parallel-running vessels indicates that the two are connected by intervessel pitting. Protoxylem (px) vessels and tracheids are shown as dashed lines. A single dashed line indicates presence of px, regardless of whether it is a small group of px elements at the lower end of a leaf trace (such as in FIGURE 1 at C) or a larger group near the leaf-trace departure



(such as in FIGURE 1 at D). All wide mx vessels remain within the central cylinder of the stem; it is the narrow px only that connects the vascular system of the stem with the leaf base (FIGURE 1 at E) (Zimmermann & Tomlinson, 1965).

The situation shown in FIGURE 2, a is summarized in another way in FIGURE 3. As we follow an axial bundle up the stem, we make the following observations (FIGURE 2, a). In its lowest portions, near the stem periphery, the bundle has a single mx vessel. The bundle gradually approaches the stem center. At some point (at 2 cm on the scale, FIGURE 2, a), we observe the appearance of px (position 3 in FIGURE 3). We are now about 10 cm below the leaf contact. Additional mx vessels appear, and the bundle becomes wider (position 4 in FIGURE 3). Usually, but not always, new mx vessels appear next to an existing mx vessel. However, a px vessel may "grow" to become an mx vessel as we follow the vascular bundle upward.

At ca. 9 cm on the scale (FIGURE 2, a), the leaf trace begins to break up, as shown in position 5 of FIGURE 3. The branches are bridges, connecting to neighboring axial bundles (outward-pointing arrows in FIGURE 2, a), axial bundles continuing their way up and repeating the cycle, or satellites connecting to the inflorescence. The leaf trace proper, containing px only, enters the leaf base (as also shown in FIGURE 1, at E).

The leaf trace shown in FIGURE 2, a, at 10 cm has three axial bundles, two bridges, and one satellite bundle. The film follows the axial bundle on the left on the diagram. The vessel of the axial bundle ends, and a new one begins with an overlap of only about 1 cm. Two bridges are then "received" from neighboring leaf traces (position 1 in FIGURE 3). Before the bundle increases in size to become a leaf trace again, the vessel ends twice more and is replaced by a new one. The longest vessel in the diagram extends from levels 10 to 27 cm, a length of 17 cm. The leaf-contact distance of this bundle is about 30 cm. The upper leaf-trace complex breaks up into three bridges, three satellites, and two axial bundles.

Extended continuity along individual bundles, like that shown in FIGURE 2, a, is difficult to obtain on film since it requires a continuous series of about 2000 high-quality transverse sections. We have only one such 40-cm-long section series. In order to look at vessels of some other stems, we plotted the vessel network in a number of shorter series, some of which are given in FIGURE 4. FIGURE 4, a-d, shows the lower parts of four axial bundles, each of them "receiving" bridges from departing leaf traces.

FIGURE 2. a, vessels of vascular bundle (plotted from film) over complete leaf-contact distance of 40 cm, projected radially onto tangential plane. Metaxylem vessels, solid lines; parallel-running vessels with vessel-to-vessel pitting between them, connected by hatching; dashed line indicates that bundle contains protoxylem (regardless of amount). AB = axial bundle, Br = bridge, SAT = satellite bundle (to inflorescence). Arrows indicating bridges always shown pointing away from leaf trace. Diagram greatly expanded laterally (leaf-trace complex ca. 1 mm wide). b, same bundle, radial coordinate projection.

FIGURE 4, e, shows a leaf trace complex that branches into three continuing axial bundles and no bridges. The axial bundle branch shown on the right "receives" three bridges from neighboring departing leaf traces. FIGURE 4, f, shows another leaf-trace complex, which breaks up into three bridges and one axial bundle. Two bridges are "received" by that axial bundle. In this

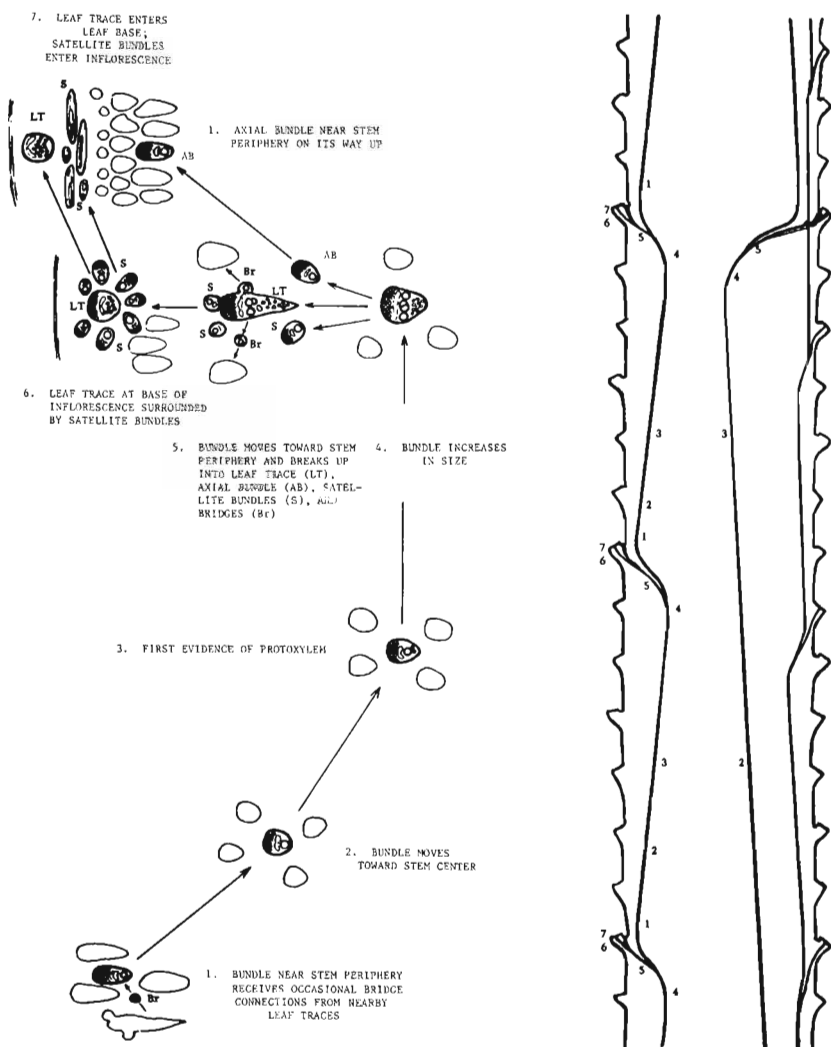


FIGURE 3. Course of vascular bundles in stem of *Rhaps*. In diagram on right, stem axis foreshortened four times in relation to stem diameter. (Slightly modified from Zimmermann & Tomlinson, 1965.)

particular series (FIGURE 4, e, f) it was possible to determine the position of the nodes; they are given as horizontal lines next to the scale. The axial bundles shown in FIGURE 4, a-d, could easily be the continuation of the axial bundles at the upper ends of FIGURE 4, e, f. In summary, the results represented in FIGURE 2 and 4 are very similar.

FIGURE 5 is a three-dimensional representation of a leaf-trace departure.

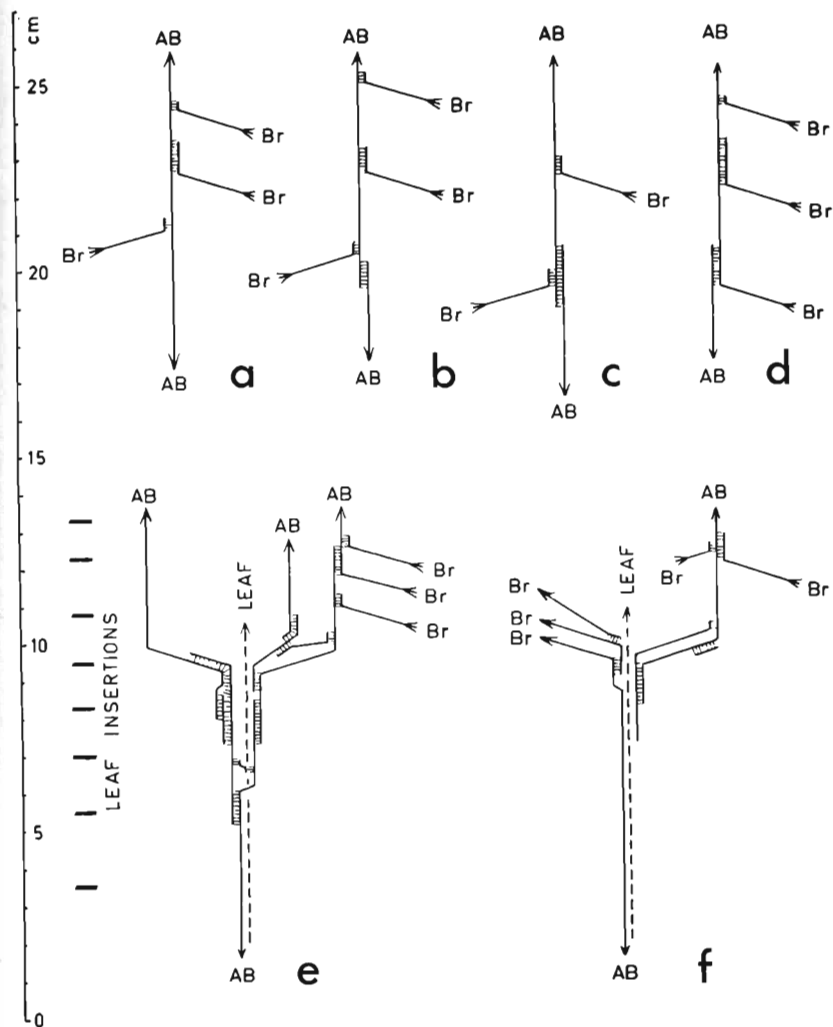


FIGURE 4. Plots of vessel distributions in shorter portions of vascular bundles: a-d, lower portions of axial bundles; e, f, leaf-trace complexes. Description as in Figure 2.

The axial scale is five times foreshortened; the actual axial extent of the section is 2.5 cm. Protoxylem is again shown as a single dashed line. At the lower end the leaf trace has three mx vessels. Where vessels run in close contact, we can assume vessel-to-vessel pit areas between them. The leaf trace gives off three bridges on the right to the neighboring axial bundles marked AB 1–3. The lower part of AB 3 was outside the field of view. An axial bundle branches off on the left (AB 4), and a small bridge connects to it (higher up). The leaf trace, containing px only, is seen leaving the central cylinder, accompanied by two satellites (S), at the upper surface of the block.

VESSEL-LENGTH DISTRIBUTION

FIGURE 2, a, shows eight vessels throughout their entire length; seven more are shown in FIGURE 3, f. If we classify these vessels according to their length, we find that nine (60%) are 0–5 cm long, five (33%) are 5–10 cm long, and one (7%) is 15–20 cm long. These percentages are vessel lengths within a given stem volume—namely, the volume covered by the plots. It must be emphasized that this is a very small statistical sample.

There are methods by which one can calculate the distribution of vessel lengths in a stem by recording distances to which paint particles are perfused. Paint particles, when sufficiently small, can pass scalariform perforation plates, but they cannot cross vessel-to-vessel pit membranes. The method, first described by Skene and Balodis (1968) and later modified by Zimmermann and Jeje (1981), assumes random distribution of vessels within the stem and yields percentages of vessels (per transverse-sectional area) of different length classes. Readers interested in the methods are referred to the above papers.

The method of Skene and Balodis works only if vessels are randomly distributed within the stem. Upon casual consideration, this does not seem to be the case: there may be more short bridge vessels in the nodal than in the internodal area. However, careful examination of FIGURE 4, e, f, reveals that each leaf-trace complex is spread out over a considerable axial distance; thus, the internal anatomy of the stem is not sharply segregated into nodal and internodal regions.

A total of six vessel-length distribution measurements were made with the paint-infusion technique, some with plants grown at the Harvard Forest greenhouse and some with taller plants grown outdoors at the Fairchild Tropical Garden. FIGURE 6 (left) shows the results calculated from the pooled counts of all six specimens. This includes 4264 metaxylem vessels to which paint was applied. We also calculated each of the experiments separately. FIGURE 6 (right) shows an example of a small specimen with a stem diameter of 8 mm. It is interesting to note that the percentage of the shortest vessels (75%) is about the same in this small specimen as it is in the larger, more vigorous ones (stem diameter 1.5–2 cm), but the distribution of the longer vessels is different: only the longer vessels are longer in the larger specimens.

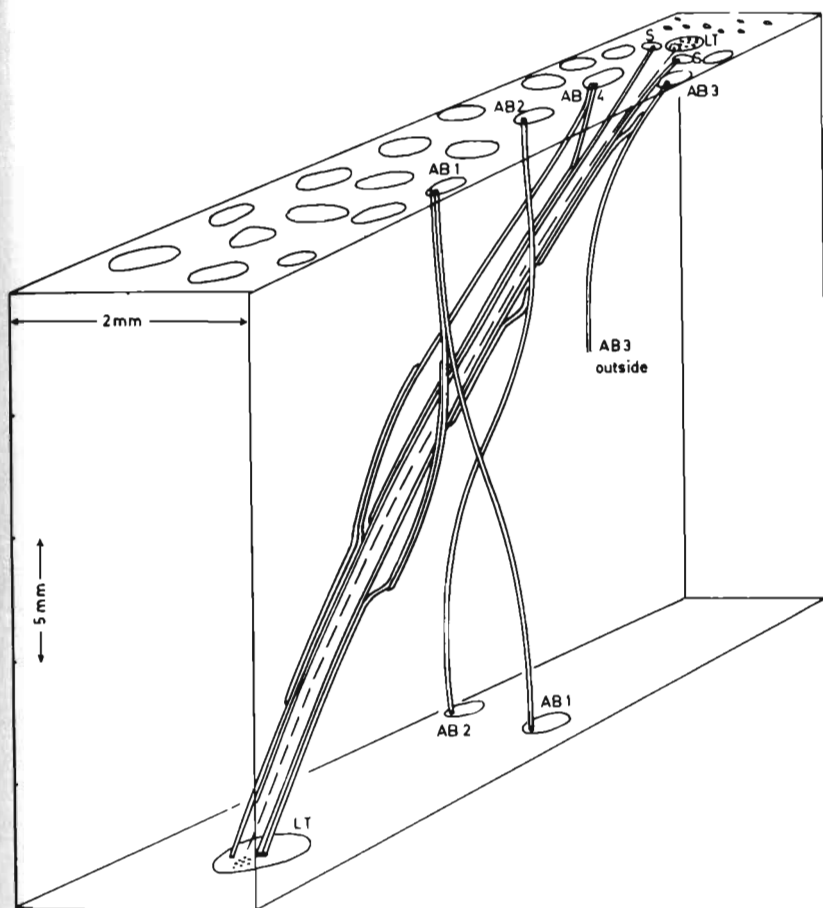


FIGURE 5. Reconstruction of three-dimensional arrangement of vessels associated with leaf-trace complex. Note that axial scale is five times foreshortened in comparison with horizontal scales.

This probably means that leaf-contact distances of major bundles are longer.

An interesting phenomenon that is not shown in FIGURE 6 is the fact that some vessels of the shortest length class are extremely short. This became evident when we recut the paint application surface with the microtome. The paint-containing vessels decreased very quickly to half or less when a few microtome sections were removed from the transverse surface of the stem. From this it is quite obvious that the shortest length-class contains the bridge vessels, and the longer length-classes contain axial-bundle vessels that vary in length depending upon leaf-contact distance.

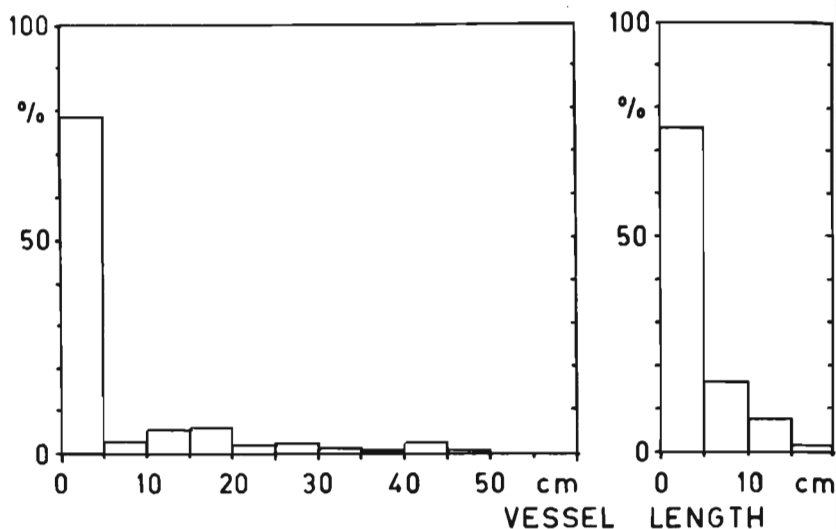
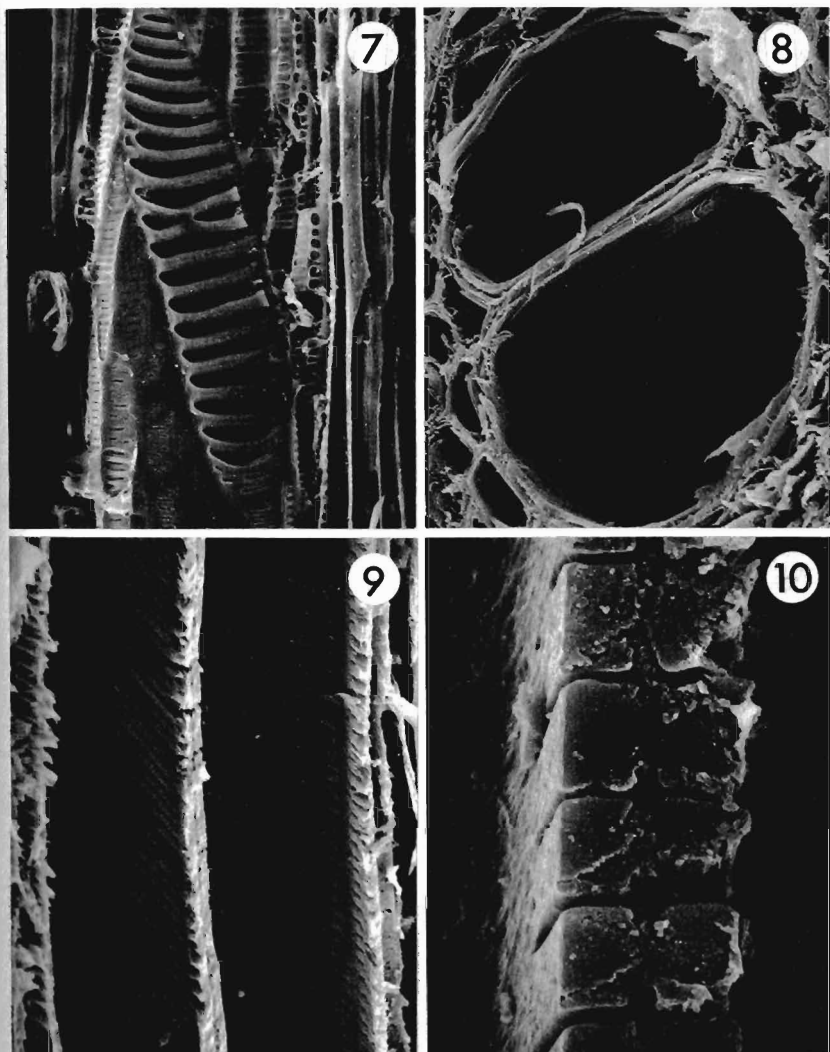


FIGURE 6. Metaxylem-vessel lengths in stem of *Rhaps*. Left diagram calculated from pooled results of six stems (total of 4264 metaxylem vessels). Right diagram figured from small greenhouse specimen with a stem diameter of 8 mm, which showed 323 metaxylem vessels on single transverse section.

DIMENSIONS IN RELATION TO WATER MOVEMENT

We looked at vessel-to-vessel contact areas with the scanning electron microscope in order to make certain measurements. An understanding of such areas is a prerequisite for the understanding of water movement in the palm stem.

A scalariform perforation plate is shown in FIGURE 7. The perforations between the bars are approximately $8 \times 40 \mu\text{m}$. Such openings permit relatively unimpeded flow of water and, also important, permit the passage of an air-water interface. If air is admitted to the xylem via an injury, water recedes to the ends of the vessel, whereby the air-water interface passes through all perforation plates. An air-water interface passes through a wet pore if the pore diameter and the pressure gradient across the pore reach a certain magnitude. This is governed by the capillarity equation (Zimmermann, 1978). Individual vessels end by tapering out gradually, overlapping with one or more other vessels. The common walls of parallel-running vessels of the overlap region consist of bordered pit-pairs, which expose a large pit membrane area (the primary wall pair). The secondary wall arches over the pit cavities (FIGURES 8-10), thus reinforcing the pit membrane area against stress. The micropores in the pit membranes of vessels have never been measured, but we know that pores in other primary walls are ca. 25 nm in diameter (see Strugger & Peveling, 1961)—far too small to permit



FIGURES 7-10. Scanning electron micrographs of xylem as seen on cut stem surfaces: 7, scalariform perforation plate between vessel elements, $\times 240$; 8, transversely cut stem surface, $\times 700$, showing vessel pair and vessel-to-vessel pit area; 9, longitudinal section through vessel pair separated by scalariform vessel-to-vessel pits, $\times 540$; 10, vessel-to-vessel pits, longitudinal section (cutting across wall), $\times 3380$.

passage of an air-water interface. In other words, an air embolus remains confined to an individual vessel. Water, on the other hand, can readily pass through the bordered pits from vessel to vessel because the exposed pit membrane area is very large.

Let us look at some dimensions. If we take the vessel in FIGURE 8 to be circular with a diameter of 60 μm , the transverse-sectional area of the vessel lumen is $2.8 \times 10^{-3} \text{ mm}^2$. The width of the scalariform pit area is ca. 35 μm , as seen in FIGURES 8 and 9. The length of the vessel overlap may be 2 cm (FIGURES 2, 4) of which ca. 40 percent is membrane area and 60 percent is secondary-wall contact (FIGURE 10). From this we can calculate the total area of pit membrane through which water moves from one vessel to the next. It is ca. 0.28 mm^2 —about 100 times larger than the transverse-sectional area of the vessel. Considering that water has to move through the very small micropores of the pit membranes and that vessel-overlap distances are often much shorter, the resistance to flow from vessel to vessel must still be appreciable.

By comparing experimental flow rate through a piece of xylem with the calculated flow rate through ideal capillaries of the same diameters as the vessels, one can get an estimate of the resistance to flow across perforation plates and through vessel-to-vessel pits. Such measurements have been made with both coniferous and dicotyledonous wood. For dicotyledons the resistance to flow from one vessel to the next is about equal to the resistance to flow along the vessel (see the citations in Zimmermann & Brown, 1971). Such calculations are not very accurate and must be considered as estimates only. A small error in the measurement of the vessel diameter gives a large error in the flow-rate calculation because of the fourth-power relationship of the flow equation (Zimmermann, 1978). For example, a 10 percent overestimate of vessel diameter causes a flow-rate overestimate of 50 percent because $1.1^4 \cong 1.5$. For the same reason we can say that vessels that are only 50 percent efficient when compared with ideal capillaries are equal in performance to capillaries with 85 percent of the vessel diameter (i.e., $0.85^4 \cong 0.5$).

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LITERATURE CITED

- BIERHORST, D. W., & P. M. ZAMORA. 1965. Primary xylem elements and element associations of angiosperms. *Amer. J. Bot.* **52**: 657–710.
- HANDLEY, W. R. C. 1936. Some observations on the problem of vessel length determination in woody dicotyledons. *New Phytol.* **35**: 456–471.

- HARTIG, T. 1878. Anatomie und Physiologie der Holzpflanzen. 412 pp. Julius Springer, Berlin.
- SKENE, D. S., & V. BALODIS. 1968. A study of vessel length in *Eucalyptus obliqua* L'Hérit. J. Exp. Bot. **19**: 825-830.
- STRUGGER, S., & E. PEVELING. 1961. Die elektronenmikroskopische Analyse der extrafaszikulären Komponente des Transpirationsstromes mit Hilfe von Edelmetallsuspensionen adäquater Dispersität. Ber. Deutsch. Bot. Ges. **74**: 300-304.
- ZIMMERMANN, M. H. 1973. The monocotyledons: their evolution and comparative biology. IV. Transport problems in arborescent monocotyledons. Quart. Rev. Biol. **48**: 314-321.
- . 1978. Structural requirements for optimal water conduction in tree stems. Pp. 517-532 in P. B. TOMLINSON & M. H. ZIMMERMANN, eds., Tropical trees as living systems. Cambridge University Press, Cambridge, England.
- & C. L. BROWN. 1971. Trees: structure and function. Springer-Verlag, New York-Berlin-Heidelberg.
- & A. JEJE. 1981. Vessel-length distribution in stems of some American woody plants. Canad. J. Bot. **59**: 1882-1892.
- & P. B. TOMLINSON. 1965. Anatomy of the palm *Rhapis excelsa*. I. Mature vegetative axis. J. Arnold Arbor. **46**: 160-180.

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