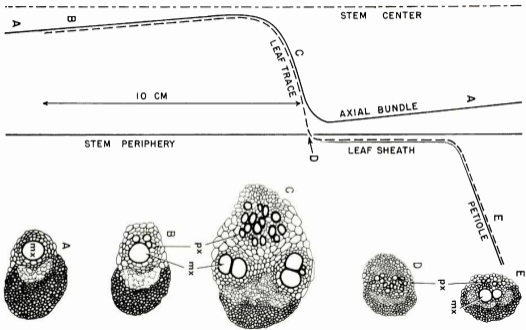ANATOMY OF THE PALM RHAPIS EXCELSA. IX.
XYLEM STRUCTURE OF THE LEAF INSERTION

Martin H. Zimmermann and John S. Sperry

Stems of perennial plants, particularly trees, represent a considerable investment in biomass. Trees can survive even under the most adverse conditions, but only if the hydraulic integrity of the stem is preserved. A very important and vulnerable part of the stem is the xylem. As water is pulled into the top of the tree, a period of excessive drought can drop xylem pressures to such low negative values that water columns break (cavitation). The tracheary elements in which this happens are permanently lost as functional parts unless positive pressures refill them within a very short time (perhaps hours). We have recently found that the xylem of trees is constructed in such a way that under conditions of severe drought the hydraulic integrity of the stem is favored at the expense of leaves or even branches (Zimmermann, 1978). The principle of this construction works as follows: the resistance to flow in the tracheary elements is much lower in the stem than in the peripheral parts (leaves, twigs, branches). Under transpirational conditions pressures therefore drop to much lower levels in the peripheral organs than in the stem. If pressures drop to disastrously low levels, water columns break first in leaves, later in lateral axes, and last in the stem. This quantitatively adjusted xylem construction is referred to as the hydraulic architecture of the plant (Zimmermann, 1978).

Although hydraulic architecture appears to be important as a mechanism for controlling cavitation in dicotyledonous and coniferous trees, such trees at least have the advantage of being able to renew lost xylem by cambial activity. In contrast, palms and other arborescent monocotyledons that have no secondary growth are entirely dependent on primary vascular stem tissue throughout their entire life. Prevention of permanent embolism in the stem is therefore of paramount importance for the survival of palms. From our earlier investigations of palm-stem anatomy (Zimmermann & Tomlinson, 1965), we know that the xylem that connects stem and leaves consists of very narrow tracheary elements. This indicates, at least qualitatively, that the leaf insertion represents a hydraulic bottleneck. In this paper qualitative and quantitative anatomical analysis of the stem-to-leaf vascular connection will provide the basis for discussing the hydraulic architecture of *Rhapis* and how it may work to preserve the functionality of the stem. According to our hypothesis, the hydraulic bottleneck in the leaf insertion is absolutely essential for the survival of palms. In this series of papers we have used *Rhapis* as a model for all palms—a reasonable assumption supported by substantial comparative evidence (Zimmermann & Tomlinson, 1974).

MATERIALS AND METHODS

Greenhouse-grown *Rhapis excelsa* (Thunb.) Henry plants from the Harvard Forest in Petersham, Massachusetts, and field-grown plants from the Fairchild Tropical Garden in Miami were used in this study.

The anatomy of tracheary elements in the stem-to-leaf vascular connection was studied in macerated material. Macerations were made from mature tissue of the peripheral stem area and the base of the leaf sheath and were examined for those elements of narrow diameter that connect stem and leaf sheath (see FIGURE 1, at D). The stem macerations necessarily contained both stem-to-leaf xylem and xylem from narrow peripheral axial bundles. The macerations from the leaf sheath, however, contained only xylem from the stem-to-leaf connection. From the macerated material, elements could be classified as vessel members or tracheids. Secondary-wall structure, length, and outside diameter of the elements were recorded, and many elements were photographed. Although inside diameter is relevant for hydraulic analysis, it is difficult to use in macerated material.

The distinction between metaxylem and protoxylem in the leaf insertion was made from both structural and developmental information. We define protoxylem as the primary xylem that has reached maturity before the surrounding tissue has ceased elongation, and metaxylem as that maturing after elongation is complete, even though it may begin to differentiate much earlier. Secondary-wall structure of tracheary elements provides only circumstantial evidence for distinguishing between protoxylem and metaxylem as thus defined. In the macerations tracheary elements with annular or helical secondary thickenings were regarded as protoxylem, and elements with pitted walls were assumed to be metaxylem.

To demonstrate the distribution of metaxylem and protoxylem in the leaf insertion more conclusively, we studied the relationship of xylem maturation to leaf elongation. Rate of elongation for the sheath, petiole, and subtending stem combined was determined from repeated measurements of the distance between the insertion of the lamina on the petiole and a reference point on the mature stem. These measurements were made on four uppermost leaves with expanded laminae. Elongation of the petiole was similarly determined from reference points marked on it in waterproof ink. Extension growth of the sheath or stem alone was impossible to measure; one cannot dissociate the extension of the leaf sheath from the extension of its associated internode because of the overlapping leaf bases. Leaf axes (sheath and petiole) or petioles that had recently ceased elongation were sectioned by hand, and the sections were stained in phloroglucino¹-concentrated HCl. In a given section of the

FIGURE 1. Diagrammatic representation of departure of leaf-trace bundle in *Rhapis excelsa*. Solid lines in curves A-B-C-A and D-E indicate presence of metaxylem vessels; dashed lines, narrow protoxylem tracheids. Transverse sections of vascular bundles (A-E, right) correspond to positions A-E in curves on left: mx = metaxylem, px = protoxylem. Structure shown in diagram repeats itself axially, as indicated by 2 positions marked A.

nonelongating tissue, metaxylem was indicated by immature tracheary elements (those with thin and nonstaining walls). Any fully mature elements (those with thick, stained walls) in the same section must represent protoxylem. Partially mature elements (those with intermediate wall characteristics) in the section were assumed to be transitional between protoxylem and metaxylem. These were always located between the mature and immature elements.

Vessel- and tracheid-length distributions were measured with the latex paint–infusion technique of Zimmermann & Jeje (1981). Dilute latex paint was injected into a cut stem for several days; paint-filled vessels were then counted with the aid of a stereo microscope on transversely cut surfaces at various distances from the point of injection. Vessel-length distribution in the vessel population of the stem was calculated from these counts. In our experimental material protoxylem elements were so narrow that they could not be resolved by the stereo microscope in transverse view. We therefore prepared relatively thick transverse sections so that paint-filled elements could be counted with the compound microscope. Each transverse section was cut into ca. 2-mm-wide strips; by counting piece after piece, it was possible to keep track of which parts of the section had already been counted.

Relative conductance per unit length of the xylem was determined at points along the vascular path from stem to lamina base in the following manner (see FIGURE 10, below). Petioles of leaves that had completed their extension were sectioned transversely from the base of the sheath to the lamina at 2-cm intervals. From these sections, inside diameters of all tracheary elements wider than ca. 12 μm were measured (smaller-diameter elements are hydraulically insignificant). The sums of all fourth powers of inside tracheary diameters were then calculated for each transverse section to yield a figure proportional to the conductance per unit length, according to the Hagen-Poiseuille equation of laminary flow. This assumes that all conduits are ideal capillaries, which is not strictly true (Zimmermann, 1983). Relative conductances along the vascular path were then plotted, based on the conductance arbitrarily taken as equal to one at the point of leaf attachment. Because the xylem connection between stem and leaf is extremely complex (Zimmermann et al., 1982), the relative conductance value of the stem is based on the comparison of a single large metaxylem vessel of an axial bundle with a single leaf trace at the leaf insertion. This comparison is possible because we know that each leaf trace is ultimately continuous with a single axial bundle (see FIGURE 1). We did not determine the relative conductance of the leaf traces between the base of the sheath (D in FIGURE 1) and their junction with the metaxylem of axial bundles (approximately C to B in FIGURE 1) because of the difficulty in distinguishing the total complement of these traces from other bundles in the stem.

RESULTS

The structure of the leaf-trace complex in the stem has been studied previously (Zimmermann & Tomlinson, 1965; Zimmermann et al., 1982) and is summarized for a single leaf-trace bundle in FIGURE 1. A vascular bundle at A contains only metaxylem vessels (mx) and is called an axial bundle. These
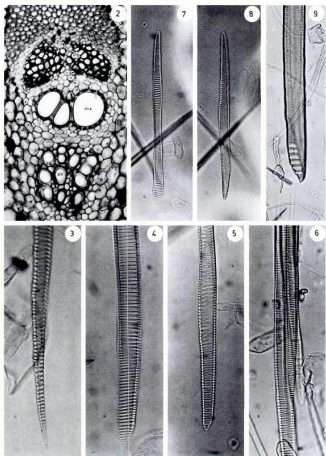
FIGURES 2–9.   2, transverse section of leaf trace in stem. Large metaxylem vessels (mx) continuous within stem; smaller tracheids (px) connect stem with petiole (F = fibers, Ph = phloem), × 260. 3, 4, tips of protoxylem tracheids with helical secondary-wall thickening, × 130. 5, tracheid with helical secondary wall at tip and reticulate secondary wall along main part of body, × 130. 6, cluster of tracheids (note matching position of their secondary walls), × 130. 7, 8, vessel element in macerate of leaf base shown from 2 different angles; scalariform perforation plate at lower end at very tip of element, upper one about ⅓ down from tip, × 230. 9, relatively wide vessel element of leaf sheath near insertion, × 230.

normally contain a single vessel, except where vessel ends overlap. If one follows such an axial bundle acropetally, one eventually depicts a few protoxylem elements (px). This point is located at least 10 cm below the departure of the leaf trace. At B a few narrow px elements are present. Some of these px elements are in lateral contact with the mx vessel.

As one moves acropetally along the bundle from B to C, the number of px elements seen in transverse section increases. This is true to a lesser extent of the mx vessels as well. Lateral contact between px and mx is present to a point somewhere between B and C. At C the px is completely isolated laterally from the mx, even though both systems are within the same vascular bundle.

The isolation of px below C marks the beginning of the leaf trace proper. FIGURE 2 shows a leaf trace in a stem transverse section corresponding to level C in FIGURE 1. If one follows such a leaf trace acropetally, one can see that the large metaxylem vessels (mx) continue up the stem within an axial bundle branch (A in FIGURE 1) and bridges (not shown in FIGURE 1, but see Zimmermann & Tomlinson, 1965), and that the narrow protoxylem (px) tracheary elements continue as a leaf-trace bundle that enters the leaf base (at D in FIGURE 1) (Zimmermann *et al.*, 1982). Thus, the leaf base is supplied by protoxylem only.

This paper primarily concerns the xylem of the leaf trace in the leaf-insertion region. This is the area of the leaf trace between its beginning as isolated px in the vascular bundles of the stem (C in FIGURE 1; FIGURE 2) and its course through the basal part of the leaf sheath. The term "leaf insertion" refers to the organographic junction of leaf and stem (D in the vicinity of the vascular junction. The vascular junction of leaf and stem is the region of contact between px and mx in the vascular bundles of the stem (between B and C in FIGURE 1). The xylem of the leaf trace in the leaf-insertion region contains narrow tracheary elements that suggest the presence of a hydraulic constriction for xylem sap entering the leaf.

ANATOMY OF THE TRACHEARY ELEMENTS

The macerated tissues from both the peripheral stem area and the lowermost part of the leaf sheath included parenchyma cells, fibers, and tracheary elements—the latter the objects of our attention. Even in stem macerations one can easily recognize protoxylem elements of outgoing leaf traces by their narrow diameter and annular or helical secondary wall structure, although there may be macerations from the basal part of the leaf sheath, we are dealing exclusively with xylem that is continuous from stem to leaf.

The macerated material from the stem periphery and the leaf sheath contained many tracheids about 1 mm long. The shortest one recorded was 425 μm; the longest (in the leaf base), 2.8 mm. Tracheids tended to be somewhat longer in the leaf sheath than in the stem, and their outside diameters ranged from ca. 23 to ca. 40 μm. Most tracheids had helical secondary walls throughout their length and were assumed to be protoxylem (FIGURES 3, 4, 6). There were others, however, with helical secondary walls at their tips and reticulate walls

in their middle sections (FIGURE 5). These tracheids are apparently transitional between metaxylem and protoxylem. No tracheids with entirely reticulate walls were found. We did find reticulate-walled vessel elements of narrow diameter, which we classified as metaxylem. Some were of narrow, long, tracheidlike shape with scalariform perforation plates (FIGURES 7, 8); others were wider, but still with scalariform perforation plates (FIGURE 9). In the stem preparations we could not be sure whether these apparent metaxylem vessels were from stem-to-leaf xylem or from narrow peripheral axial bundles. Their presence in the leaf base, however, suggested that both protoxylem and metaxylem contribute to the bottleneck of the leaf insertion.

Extension-growth studies provided more direct evidence for the presence of metaxylem in the leaf-insertion region. As expected, elongation of the petiole ceased basipetally, and this was assumed to be the case for the sheath as well. Total elongation of the petiole, the leaf sheath, and the subtending stem ceased in the second-youngest expanded leaf. Anatomical examination of the leaf insertion of the second-youngest expanded leaf revealed narrow metaxylem elements (as indicated by their immaturity) in the nonelongating tissue). Such elements are presumably destined to become those that we saw as narrow, reticulate-walled vessel members in the maceration of the mature leaf base. This metaxylem extends from the very base of the sheath into the petiole (solid line from D to E in FIGURE 1). Comparative structural examination indicated that metaxylem maturation is basipetal and is not complete until the leaf is the fourth expanded one in the crown. There was no indication of metaxylem in the area of the leaf trace between the axial bundle junction and the base of the sheath (ca. C to D in FIGURE 1).

Length distributions of the wide metaxylem vessels of the central cylinder of the stem have been reported before (Zimmermann et al., 1982). In another set of latex paint-infusion experiments, we measured the length distribution of the tracheids and vessels in the peripheral stem area. We counted a total of 1843 perfused narrow conducting units (tracheids and vessels) in the stem. Counts at 1-mm intervals yielded the following length distribution: 55 percent, lengths shorter than 1 mm; 35 percent, 1–2 mm; and 10 percent, longer than 2 mm and up to several cm. From our maceration results it was obvious that the two shortest length classes (0–1 and 1–2 mm) concerned tracheids and the longer length classes concerned primarily vessels, although there are a few tracheids exceeding 2 mm in length. Length distributions of the conducting units in the sheath and petiole concerned only metaxylem vessels and were similar in both regions. Roughly 85 percent of the vessels were shorter than 1 cm; the remaining were mostly shorter than 5 cm, with a few as long as 9 cm.

DIMENSIONS OF THE STEM-PETIOLE CONNECTION

Cursory observation revealed that there are more narrow tracheary elements at the leaf insertion than in the petiole. In a typical case the total number of tracheary elements (per transverse section) at the leaf insertion was a little over 200, but in the first 2 cm of the leaf sheath above the insertion it decreased to about half that number and then remained roughly constant along the length
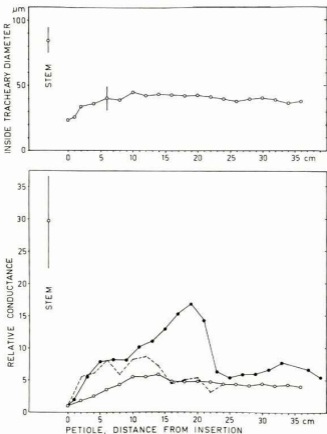
Figure 10. Above: average inside diameters of all tracheary elements wider than 12 μm, from insertion at stem (0 cm) along petiole (narrower ones hydraulically insignificant). Average diameter of large vessels in stem given at left for comparison. Vertical bars = standard deviations. Below: calculated relative conductances (per unit length) of xylem at points along vascular path from stem to lamina base, with conductance = 1 at leaf insertion (0 cm). Values for petioles (including leaf sheath) from 3 different plants. Average value for stem shown on left.

of the petiole. The diameters of the tracheary elements in each bundle increased from an average of 24 μm at the insertion to ca. 35 μm within the petiole (FIGURE 10). Absolute values of these dimensions varied from leaf to leaf, and among different shoots, but relationships within a plant were quite constant.

FIGURE 10 also shows the relative conductance along a typical mature stem-to-leaf connection for three leaves. Plainly, the observed bottleneck at the insertion is quantitatively significant. Conductance is highest in the stem and lowest at the insertion; it increases distally in the petiole to a value somewhat below that of the stem. The magnitude of the constriction would probably be even greater if the area of the leaf trace that is solely protoxylem (between C and D in FIGURE 1) could have been measured. The bottleneck is consistently present in all leaves examined, although its magnitude varied somewhat from leaf to leaf. The meaning of this variation is unknown, but it may be correlated with leaf position. Leaves low on the stem seem to have less of a bottleneck (Sperry, unpubl.).

Although based on precise measurements, FIGURE 10 shows a somewhat theoretical result. We know that the hydraulic constriction at the leaf insertion must be greater than FIGURE 10 indicates. There are four reasons for this. 1) The area of the leaf trace (roughly 4 cm) between C and D in FIGURE 1 could not be included in the calculations for technical reasons mentioned above. Because this stretch is constituted wholly of protoxylem, it probably has a conductance as low as or lower than that determined for the base of the leaf sheath. 2) The region between B and C in FIGURE 1, where water must pass from the large metaxylem vessels to the narrow contiguous protoxylem tracheids, offers additional resistance to flow. The amount of resistance afforded by this crossover is unknown, but it may be considerable. 3) The xylem of the insertion consists primarily of tracheids, while in the stem and petiole most of the water moves through vessels. Tracheids are less efficient conductors than vessels, regardless of diameter. 4) Some of the protoxylem tracheids are certainly nonfunctional because they have been stretched and superseded by later-formed tracheids and vessels. These should not have been counted, but it was not possible to recognize nonfunctional tracheids in transverse sections. We must therefore assume that the actual hydraulic constriction at the leaf insertion is greater than is indicated by FIGURE 10.

DISCUSSION

Our observations provide evidence for a considerable hydraulic constriction in the xylem path between stem and petiole. It is caused in part by the absence of metaxylem from the area of the leaf trace between the junction of the axial bundle and the base of the sheath (the dashed line between C and D in FIGURE 1). Small metaxylem vessels, however, continue the constriction at the base of the leaf sheath (the solid line immediately above D in FIGURE 1). From the base of the leaf sheath to the petiole, conductance increases due to the presence of ever-wider metaxylem vessels (FIGURE 10). The fact that metaxylem contributes to the structure of the constriction suggests that this constriction is not

merely a consequence of a developmental constraint (the inability of the plant to make wide protoxylem), but that it is an integral part of the vascular design and has adaptive value. We suggest that this hydraulic constriction at the leaf insertion plays a critical role in confining cavitation and subsequent conduction failure to areas beyond the stem, thereby insuring the functional integrity of the stem xylem.

If we assume that the likelihood of cavitation in a tracheary element increases with increasing sap tension and increasing tracheary diameter (given that the pore sizes in the tracheary walls are all the same (Zimmermann, 1983)), the quantitative anatomy presented in this paper indicates that vulnerability to cavitation is highest in the petiole, or possibly in the leaf blade. The reduced conductance at the insertion indicates a sharp pressure drop from stem to petiole when sap is flowing. Consequently, under transpirational conditions, xylem sap in the petiole will always be at considerably lower pressure than in the stem. Even in the absence of a constriction, xylem pressure in the petiole will be slightly lower than that in the stem, but the constriction introduces a sharp drop and thus greatly increases the pressure difference under conditions of flow. In addition to being at relatively low (negative) pressures, the sap in the petiole must flow in wide metaxylem vessels. Thus, relatively high tension is combined with wide vessels in the middle of the petiole. For this reason, we believe that in time of water stress, cavitations may be largely confined to the wide vessels of the petiole. Conduction in the xylem of the expendable leaf will be sacrificed to preserve the function of the xylem of the stem.

The leaf-trace structure illustrated for *Rhapis* in Figure 31 is similar to that in other palms (Zimmermann & Tomlinson, 1974). If the hydraulic architecture of other palms acts as we believe it does in *Rhapis*, it confines cavitations to the leaves. If no dependable mechanism is available to palms for the reversal of cavitation, the hydraulic constriction at the leaf base may be a necessary condition for the survival of palms. Less dramatically, it may also be responsible in part for observed patterns of leaf longevity in palms. Although some palms (e.g., arecoid palms with a crownshaft like *Roystonea*) abscise their leaves while they appear to be still healthy, when *Rhapis* and many other palms are grown under ideal (humid) conditions, the older leaves merely dry out. In these latter species, hydraulic architecture may be the mechanism responsible for the sequence of events. As a seedling palm grows taller, its stem must share the water supply with more and more leaves. Xylem pressures drop. Furthermore, if progressively more vessels in the petiole cavitate as the leaf ages, older leaves will have more vapor-blocked vessels than younger ones. This will increase the resistance to flow in old leaves, which may in turn increase the likelihood of cavitation. Hydraulic constriction may therefore favor young—and discriminate against old—leaves. Xylem failure in older leaves would happen more rapidly under unfavorable (dry) conditions. This would explain the fact that while *Rhapis* plants grown outdoors in the sun carry only a tuft of leaves at the top of the stem, those grown in a greenhouse and watered regularly retain leaves along a much greater length of the stem.

The hypothesis put forth in this paper is consistent with the available anatomical data and certain commonplace observations of palm growth. We hope

that it is a promising step toward a more complete analysis of how palm xylem functions in sap conduction and palm growth.

ACKNOWLEDGMENTS

LITERATURE CITED

ZIMMERMANN, M. H. 1978. Hydraulic architecture of some diffuse-porous trees. Canad. J. Bot. 56: 2286–2295.

———. 1983. Xylem structure and the ascent of sap. Springer series in wood science. Vol. I, Springer-Verlag, Berlin, Heidelberg, New York.

——— & A. A. JEJE. 1981. Vessel-length distribution in stems of some American woody plants. Canad. J. Bot. 59: 1882–1892.

———, K. F. McCUE, & J. S. SPERRY. 1982. Anatomy of the palm *Rhapis excelsa*. VIII. Vessel network and vessel-length distribution in the stem. J. Arnold Arbor. 63: 83–95.

——— & P. B. TOMLINSON. 1965. Anatomy of the palm *Rhapis excelsa*, I. Mature vegetative axis. J. Arnold Arbor. 46: 160–180.

——— & ———. 1974. Vascular patterns in palm stems: variations of the *Rhapis* principle. *Ibid.* 55: 402–424.

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