ANATOMY OF THE PALM RHAPIS EXCELSA, X. DIFFERENTIATION OF STEM CONDUCTING TISSUE

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DESPITE THE APPARENT VASCULAR COMPLEXITY of the palm, its vascular development, structure, and function can be perceived quite readily. Compared with branched trees, it is architecturally simple (Hallé *et al.*, 1978). Metaxylem and protoxylem are structurally and topographically very distinct. Vascular tissues are radially as well as tangentially separated (unlike those of dicotyledons), and due to the relatively massive meristematic region and the long time course of differentiation, successive events are widely separated in time as well as in space. Finally, primary structures are not obscured by later development of secondary vascular tissues. In dicotyledons recent advances in our understanding of vascular developmental processes have required very precise analyses of serial thin sections, as in the work of Larson (1982; and earlier papers cited therein) on *Populus*.

In this paper we consider the sequence of initiation of xylem and phloem within the procambial template and the changes within these tissues as both radial and axial extension take place. Special attention is given to xylem differentiation in traces to a given leaf at various stages of its development, a topic being investigated physiologically by John Sperry at Harvard Forest.

In the first paper in this series (Zimmermann & Tomlinson, 1965), a cinematographic method of analysis was used to describe the course of vascular bundles in the mature aerial stem of the small palm *Rhapis excelsa* (Thunb.) Henry. In a topographic sense, traces serving a given leaf (leaf traces) usually branch to give an axial bundle that becomes a leaf trace at a higher level. Axial bundles in the region of leaf-trace departure are connected by short bridge bundles. In addition to establishing the principle of vascular continuity in the stem, that paper demonstrated the changes that take place in individual mature bundles throughout their length. It provided a basic, general framework (since termed the "*Rhapis*-principle"—see Zimmermann & Tomlinson, 1972; Tomlinson, 1983) for understanding the vasculature of monocotyledonous stems, which may be described as a regular pattern of outgoing leaf traces branching to generate topographically axial bundles and other derivatives. To add to the topographic analysis, in the fourth paper of the series (Zimmermann & Tomlinson, 1967), the sequence of initiation of strands within the developing crown was analyzed in terms of their inception as procambial strands. The principle of vascular development that has been shown to be generally applicable to monocotyledons was demonstrated. Procambial strands that connect to young leaf primordia are initiated within a cap of meristematic tissue. They are

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continuous with an existing leaf trace but are at first uncommitted in a distal direction to a specific future leaf primordium. These processes are difficult to visualize in static diagrams but are shown clearly in recently produced demonstration films (Zimmermann & Mattmüller, 1982).

We believe that the basic nature of the *Rhapis* type of construction has wide relevance to an understanding of monocotyledonous vascular development (Tomlinson, 1983). Our overall objective is to present an understanding of the palm crown in terms of anatomy, development, and physiology.

METHODS

The analysis involved two successive steps (see FIGURE 1) because the developing region of the shoot (the crown) is an extended structure. First, the sequence of development of vascular tissue within individual bundles was studied at early stages of development (see FIGURE 3). In this phase radial expansion predominates. Second, a less-detailed analysis was carried out of late stages of development; dye-infusion techniques were used and unembedded material of long apical portions was sectioned (see FIGURE 4). In this phase longitudinal expansion predominates. Although technically necessary, such separation into phases is artificial since growth is a continuous process.

The first method provides information about the development of the vascular connection, via protoxylem, between stems and young leaves until initiation of predominant axial extension of the internode below a leaf with an expanded blade. This initial analysis can be done with stem pieces (including the shoot apex) up to a length of about 1.5 cm (FIGURE 1, A). The second method gives information about the establishment of connection between the leaf and the metaxylem of the stem during the period when the leaf blade is fully expanded but the intercalary extension of the leaf sheath is still incomplete (FIGURE 1, B). Final maturation of metaxylem occurs as soon as internodal extension is complete. Again, separation into phases of development is artificial because growth is continuous, but it is important to appreciate that blade expansion precedes maturation of the leaf sheath and its associated internode. The "first fully mature leaf" is therefore the first leaf in which extension of all these regions is complete.

The stage at which a given developmental event occurs varies by as much as three plastochrones, depending upon the size of the crown. A leaf usually first becomes visible externally somewhere between plastochrones P8 and P11 (P1 is the first microscopically visible leaf primordium). Internodal extension is generally complete somewhere between P14 and P17. For purposes of describing the position of leaves that are only visible externally, the youngest visible leaf may be referred to as leaf 1, the next youngest as leaf 2, etc. Without dissection the numbering of such leaves by the plastochrone (P) system cannot be done. In the crown analyzed we recognized about seven leaf primordia enclosed within the apical bud and not visible externally (as in FIGURE 5, where the position of P8 corresponds to the "spear leaf"—i.e., the first externally visible leaf in the crown, its blade still unexpanded).

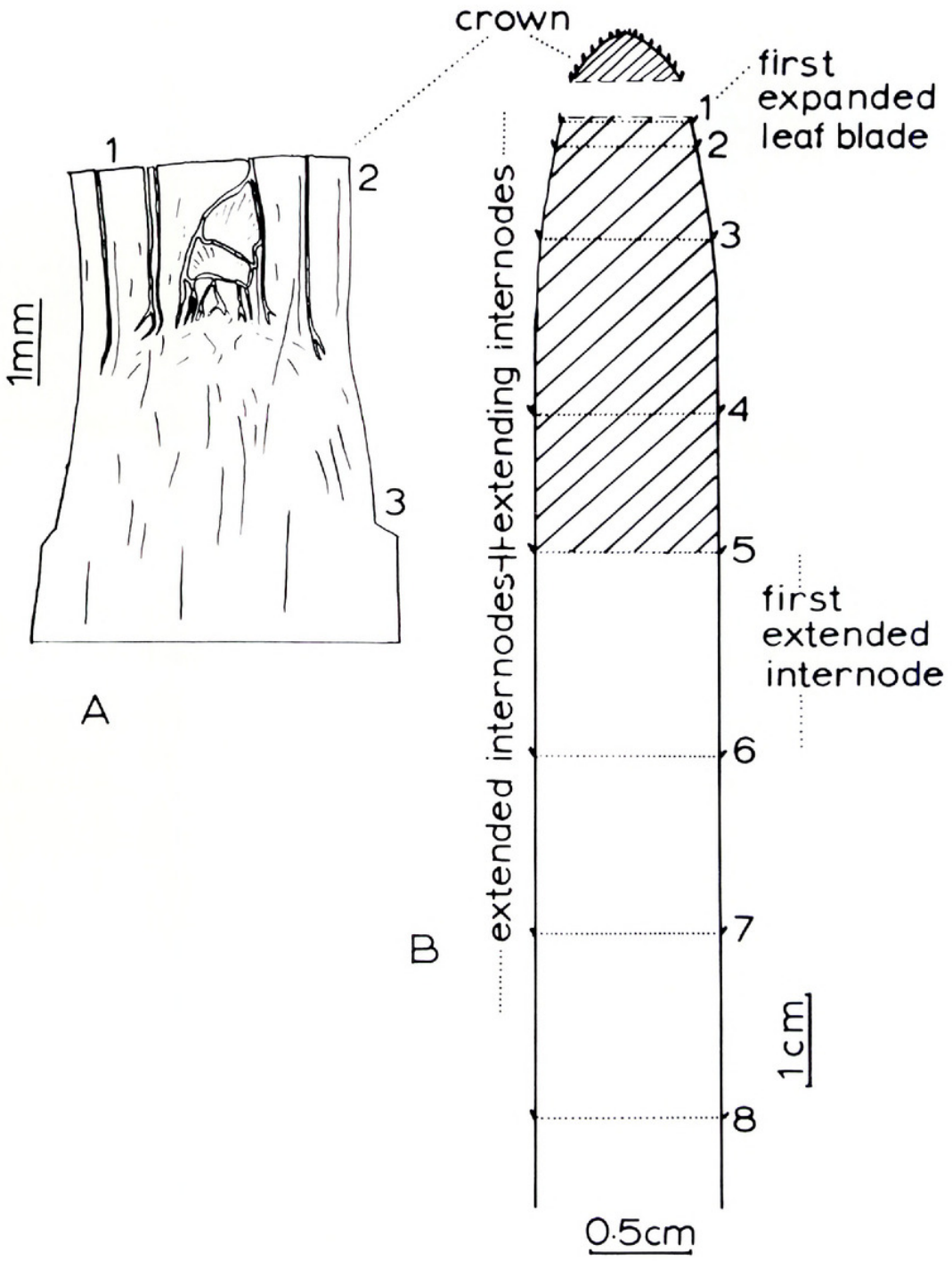

FIGURE 1. *Rhapis excelsa*, diagrams showing size of specimens used to analyze vascular development in shoot (2 methods): A, camera lucida drawing of median longitudinal section of apex about same size as that used in analytical plots (see FIGURE 5); B, diagram of axis used in experimental dye injection of single leaf, foreshortened 50%. Numbers refer approximately to corresponding expanded leaves in visible crown.

PLOTTING OF EARLY STAGES

To plot the early stages of vascular bundles, we examined three section series, including one described in a previous paper (Zimmermann & Tomlinson, 1967). These series were derived from apices from which all but the youngest enclosed leaves were trimmed. They were fixed, embedded, sectioned at 8–15 μm, stained in safranin and Delafield's haematoxylin, and mounted in the usual way, but with only a single section on each slide. Plotting was carried out by the drawing and shuttle methods of Zimmermann and Tomlinson (1966)—i.e., either with a drawing attachment to a Wild M-20 microscope or with two separate microscopes connected by an optical bridge. These methods provided precise alignment for successive sections. Single major leaf traces belonging to successively older leaves were followed, starting with P1 (the youngest leaf primordium), in a basipetal direction from the level of insertion of the leaf. In *Rhapis* the crown is a shallow cone (FIGURE 1, A; see also Zimmermann & Tomlinson, 1967, *fig. 4*), so it is possible to follow traces continuously in one direction. Positions of each trace measured at regular intervals were plotted on graph paper, with only the radial coordinate considered, the helical or tangential displacement being ignored for diagrammatic purposes (see Zimmermann & Tomlinson, 1967, *fig. 2*).

At each plotted level the number of differentiated xylem elements was counted. To facilitate recognition of differentiated protoxylem elements, transverse sections were viewed through partly crossed polarizing filters; the birefringence of cell walls of fully mature elements rendered them conspicuous. It was considerably more difficult to recognize protophloem elements at very early stages of vascular differentiation in bundles that were cut somewhat obliquely. The criteria that we used were presence of cell walls densely stained with haematoxylin, and cell contents relatively unstained or even clear. Sometimes simple sieve plates could be seen in face view, making the identification of sieve elements unequivocal. The principal objective was to establish whether or not phloem was continuously acropetal in its differentiation.

GENERAL ANALYSIS OF LATE STAGES

To show late stages of xylem differentiation and particularly to identify the vascular contribution of a given leaf in its final stages of development, dye ascent and descent experiments were conducted using Schiff's reagent (reduced-acid fuchsin) after a preliminary perfusion with 0.5 percent periodic acid as an aldehydic mordant. This was similar to the method used by Priestley and colleagues (1935) to trace bundles from specific leaves in *Alstroemeria* with Magdala red as a marker stain but without any mordant. In the ascent experiment the stem was cut about 20 cm below the shoot apex. The cut end was immersed in dye, and suction was applied to a cut leaf. Distally, dye moved preferentially into the traces of the leaf to which suction was applied. In the more-informative descent experiments, a flask containing the dye was attached via an air-tight seal to the cut petiolar stump of a given expanded leaf of a detached shoot. Dye moved only into the traces of the injected leaf. On different shoots successive leaves from the youngest to the fifth-youngest expanded

leaves were infiltrated. The dye penetrated the shoot over a period of about 24 hours; it could then be recognized in its colored, oxidized form in the cell walls of the leaf-trace xylem continuous from the cut leaf, as seen in sections cut at successively lower levels (e.g., FIGURE 4). In this way the trace system from a given leaf could be recognized in freehand sections of the shoot at successively lower levels. Sections were dehydrated and mounted in Permount without further staining. Traces were followed to a maximum distance of 36 cm—i.e., up to about 15 internodes below the morphological level of insertion of the injected leaf. Additional sections cut at intervals from other shoots, stained in phloroglucinol and concentrated HCl, showed the general progression of late stages of maturation of lignified tracheary elements, but without reference to a particular leaf. These sections were useful for recognizing the highest level of maturation of metaxylem.

RESULTS

GENERAL FEATURES OF VASCULAR DIFFERENTIATION

EARLY STAGES (RADIAL EXPANSION PREDOMINANT). Observation of single sections gives general information about the progress of vascular differentiation at a single level. FIGURE 2 represents the appearance of a vascular bundle at an approximately comparable level in successive plastochrones, so that ontogenetic events in a single bundle are simulated. The illustrations were produced by selecting an arbitrary standard level (a major leaf trace at its most central location) and, with the aid of the drawing apparatus, making a drawing for a comparable bundle serving a leaf at successive plastochrones. ("Standard level" refers to an equivalent position below successive older leaves.) The drawings are thus illustrative of the general process of vascular differentiation at that particular level. Other sequences at other levels are shown photographically in FIGURE 3. Precise information about developmental events in different parts of representative individual bundles is included in the plots (FIGURES 5, 6).

The procambial strand is visible in transverse section as a group of narrow elements that retain a meristematic appearance in contrast to the surrounding vacuolated cells (FIGURES 2, A; 3, A). We have not been concerned in this study with the details of the appearance of procambial cells or with the method by which the diameter of the vascular strand is increased, although this is an important early process in the development of the vascular bundle (e.g., the changes within FIGURES 2, A–E, and 3, A, B). Cell divisions in later stages of procambial development are mainly longitudinal. In longitudinal section the strand comes to have a characteristic "tiger-tail" appearance because nuclei of adjacent, recently divided procambial cells lie at the same level. Cells in the center of the bundle vacuolate early, in contrast to those in the inner (xylic) and outer (phloic) regions, but cell division still continues in them and produces a temporary "cambiumlike" condition seen in transverse section (FIGURE 2, D).

At the standard level illustrated (FIGURE 2), the first appearance of vascular

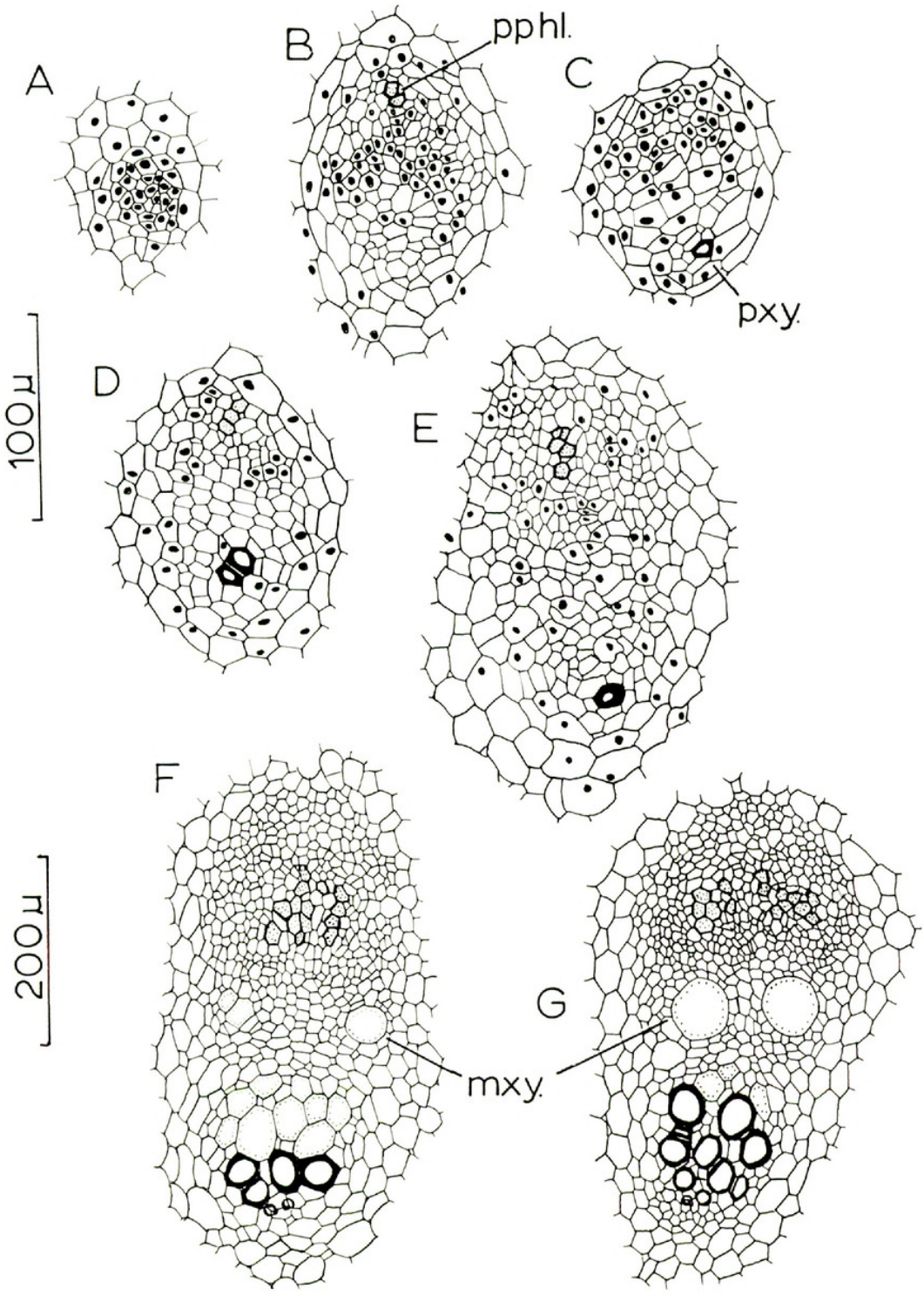

FIGURE 2. *Rhapis excelsa*, transverse sections of developing leaf traces to progressively older leaves (drawn from series used in preparing FIGURE 5). Bundles represented at different axial distances below shoot apex and estimated to serve leaf primordia (P) indicated: A, 0.45 mm (P3), no differentiated vascular tissue; B, 1 mm (P5), protophloem (pphl.) only; C, 1 mm (P5), protoxylem (pxy.) only; D, 1 mm (P6), protoxylem and protophloem; E, 1.8 mm (P7), protoxylem and protophloem; F, 2.8 mm (P9), first differentiation of metaxylem (mxy.); G, 12.8 mm (P11), differentiating metaxylem vessels

tissue is the development of protophloem toward the outside (peripherally oriented) part of the procambial strand (FIGURE 2, B). In the permanent preparations used in this analysis, differentiated protophloem sieve elements have somewhat thickened angular walls. These walls stain densely with haematoxylin, and either the protoplast stains lightly or the cell lumen appears completely empty. Simple transverse sieve plates are often evident. Protophloem elements of this kind have a diameter of about 8–10 μm. At the given level sieve-element (phloem) differentiation scarcely precedes protoxylem differentiation and in some strands even follows it (FIGURE 2, C). We found no precise evidence for discontinuous sieve-element differentiation, although in a few bundles uncertainty in recognizing phloem elements sometimes gave this impression within a few adjacent sections.

Later differentiation of sieve elements at any one level was always in a centripetal direction and involved elements somewhat wider (10–15 μm) than those first detected (FIGURE 3, B). In association with these changes, there was evidence of disruption and collapse of the youngest protophloem elements. In transverse appearance the lumen of the first-formed sieve elements becomes irregular and is finally occluded by expansion of surrounding cells. In still later stages the position of the protophloem is marked by a densely stained region of cell-wall material. In the axial portion of a mature bundle, the protophloem region is obscure and included within the innermost bundle-sheath fibers. It proved difficult in the kind of sections used to quantify (in terms of numbers of functional sieve tubes) the sequence of events involved in early phloem differentiation because of the problems of recognizing both early stages of sieve-element differentiation and their later collapse.

In transverse sections of young leaf traces, protoxylem can first be recognized unequivocally as enlarged cells (ca. 10–15 μm diameter) with thick, lignified walls that stain with safranin (FIGURE 2, C, D). Polarizing optics confirmed the existence of a thickened birefringent cell wall. In addition the cell lumen always appears empty. The first protoxylem element occurs on the inner side of the procambial strand, remote from the protophloem, and is followed by a succession of elements that appear in centrifugal order. Later-formed elements are somewhat wider and more conspicuous; they form a circular or wedge-shaped group of cells in transverse section (FIGURE 2, G). The intervening area between protoxylem and protophloem is occupied by procambial cells, and differentiation within this region varies depending on the level at which one examines a given strand, as is indicated later (FIGURE 3, C, D). The distance between first-formed xylem and phloem elements actually increases as the bundle matures, due to the increase in number of procambial cells by the continued longitudinal divisions already mentioned.

We have not followed developmental changes in detail in longitudinal sec-

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near maximum diameter. (Black circles = nuclei, stippled areas = sieve tubes of protophloem, solid black walls = protoxylem, lumen outline dotted = differentiating protoxylem and metaxylem elements.)

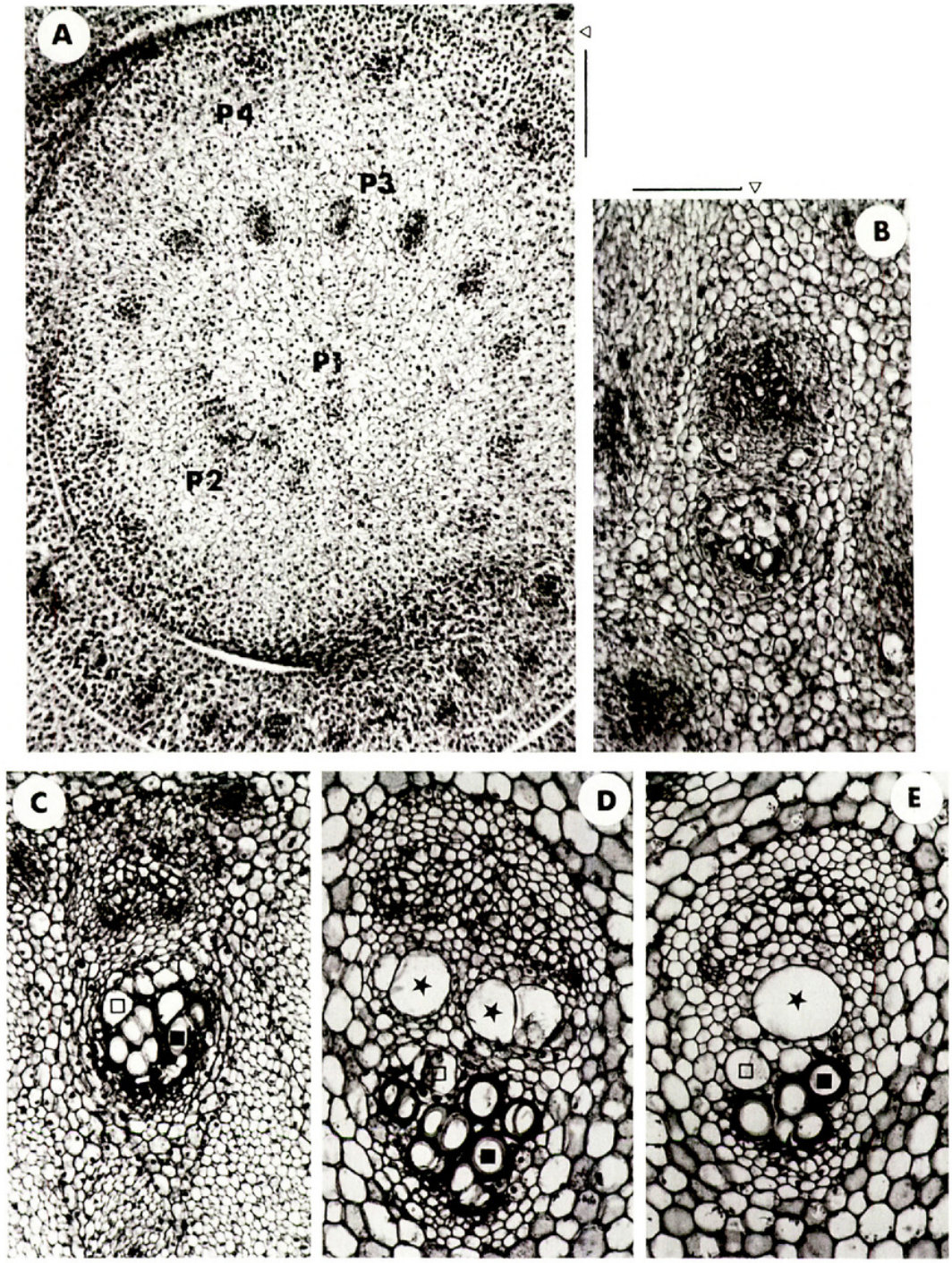

FIGURE 3. *Rhapis excelsa*, photomicrographs of 15-μm-thick transverse sections from paraffin-embedded material. A, 0.3 mm below shoot apex: leaf-trace system to P1, P2, and P3 still discretely recognizable within stem, trace system to P4 within leaf base. B, 1.8 mm below shoot apex: major leaf trace to P8; protoxylem and protophloem well developed, early metaxylem differentiation evident. C, 5.7 mm below shoot apex: major leaf trace to P10 close to periphery of central cylinder; protoxylem and protophloem still incompletely differentiated (no metaxylem in this part of leaf trace). D, 15 mm below shoot apex: major leaf trace; protoxylem almost completely differentiated, metaxylem elements not in contact with protoxylem almost completely expanded but still thin walled. E, 13.8 mm below shoot apex: axial bundle; single incompletely differentiated metaxylem

tions. Serial sections show that the protoxylem elements are short, but with annular or helical wall thickenings, and are thus presumably extensible. Protophloem elements are also short.

At the standard level illustrated in FIGURE 2, metaxylem differentiation begins at a very early stage when there is little mature protoxylem (see FIGURE 3, B). In transverse view the first evidence for metaxylem differentiation is the enlargement and vacuolation of a pair of cells toward the middle of the bundle but laterally widely separated from each other (FIGURE 2, F). These initials subsequently widen considerably and block out the characteristic metaxylem pattern. The walls of the enlarging vacuolating cells remain unthickened and contrast with the lignified, thick, birefringent walls of the protoxylem (FIGURE 2, G). Maturation of metaxylem occurs very late, well below the crown, and only in bundles that are directly in association with older expanded leaves and that run through fully extended internodes (i.e., in the unshaded part of FIGURE 1, B), as is described later.

Depending on the level examined, protoxylem may or may not be in direct lateral contact with the metaxylem (cf. FIGURE 3, D and E); in the distal part of any axial bundle, at the level of its divergence toward a leaf, protoxylem is separated from metaxylem by a broad region of procambial cells that mostly mature as parenchyma cells. In late stages of differentiation, the cells of this conjunctive parenchyma become somewhat radially seriated (FIGURE 2, D), indicative of further late, regular tangential division, which is completed before metaxylem differentiation begins. Evidence for the time and regularity of division is provided by the identical length and the coincident end walls, at any one level, of the wide vessel elements and the metaphloem sieve tubes (Parthasarathy & Tomlinson, 1967). However, the situation is complicated by the late differentiation of tracheary elements on the outer face of the protoxylem, as is described later.

Fiber differentiation outside the protophloem is initiated early but is long continued. Maturation of fibers begins in the region of the protophloem, but the pattern within the future fibrous sheath of the vascular bundle becomes complex. The extent of apical intrusive growth of differentiating fibers is not known. However, in larger palms there are regular trends of change of fiber length throughout single stems, indicating a high degree of endogenous control of fiber length but some correlation with internode length (Tomlinson & Zimmermann, 1967).

LATE CHANGES (INTERNODAL EXTENSION PREDOMINANT). In any one leaf trace followed basipetally, the gap between protoxylem and metaxylem decreases, and at some level incompletely differentiated protoxylem elements are seen to be contiguous with incompletely differentiated metaxylem elements (FIGURE 3, E). Fully differentiated contiguous protoxylem and metaxylem elements

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vessel contiguous with incompletely differentiated (presumed) protoxylem elements. (Empty squares = differentiating protoxylem, solid squares = mature protoxylem, solid stars = differentiating metaxylem. Scale for A = 50 μm, for B–E = 100 μm.)

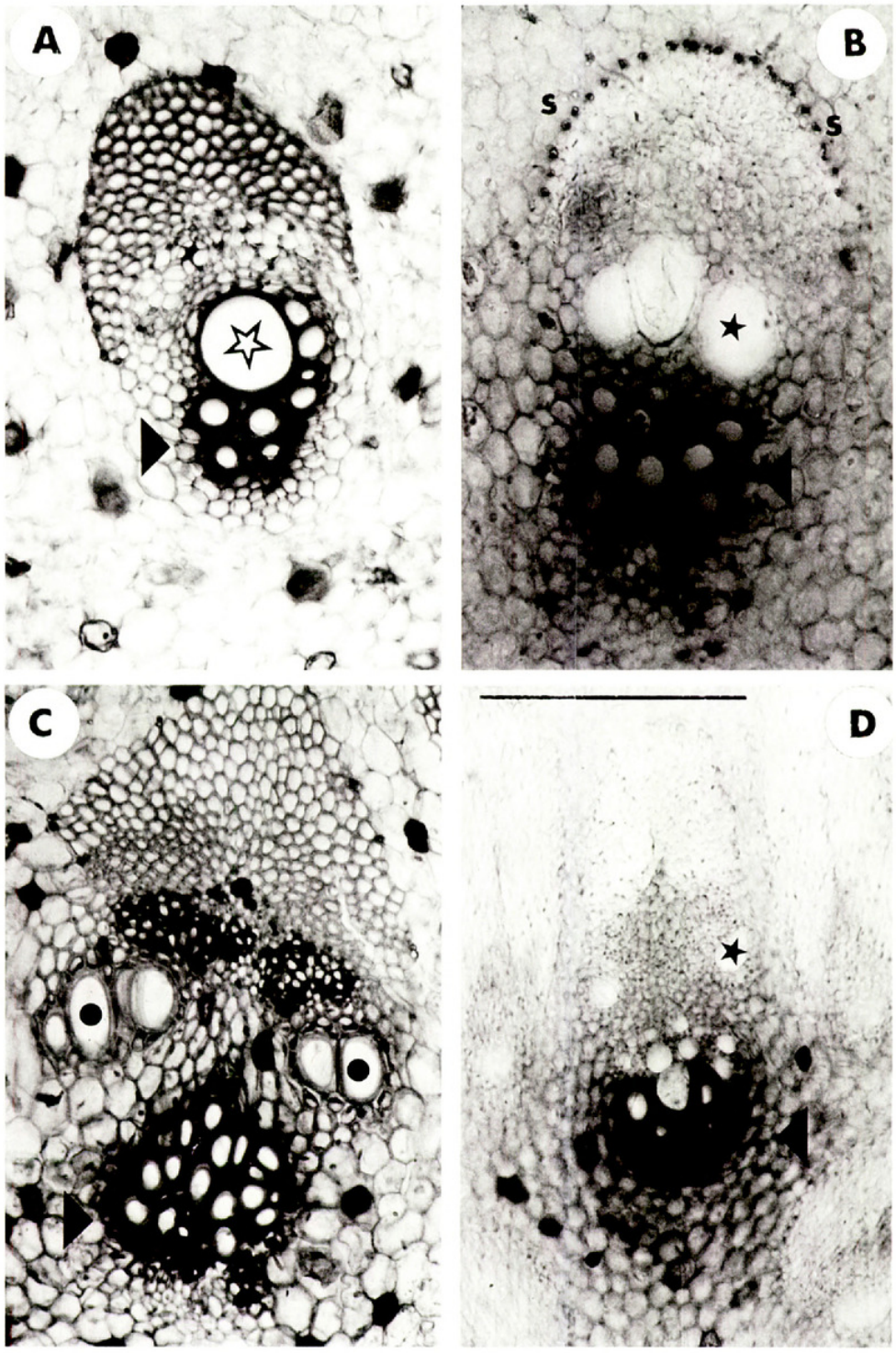

FIGURE 4. *Rhapis excelsa,* transverse sections about 50 μm thick from fresh, unembedded material after dye-descent injection experiments. Dark staining indicates presence of Schiff's reagent in xylem. A, major leaf trace to first fully expanded leaf 3.9 cm below its morphological insertion: protoxylem and metaxylem fully differentiated and closely contiguous so dye moves from former to latter (downward in this experiment). B, major leaf trace to second fully expanded leaf 3 cm below its morphological insertion: protoxylem fully differentiated and conducts dye readily downward; metaxylem incompletely differentiated and not contiguous with protoxylem so dye not conducted. C, major

occur only in internodes in which extension is complete (FIGURE 4, A). Transport of water is then possible between the two types of element, as the dye descent and ascent experiments show. In axial bundles a single metaxylem element is differentiated in the absence of any protoxylem. This construction also applies to bridge bundles (and presumably to branch traces, although we have not examined their development in detail).

The peripheral part of each bundle differentiates as the fibrous cap, the extent of which is directly related to the proximity of the bundle to the stem center: peripheral bundles develop a wide fibrous sheath, central bundles a narrow one. Stegmata (silica cells) are conspicuous in early stages of maturation of the fibers (e.g., s in FIGURE 4, B).

Although we have not followed individual bundles directly over long distances, the overall maturation of conducting elements can be seen in sections at progressively lower levels. In developmental terms there is thus an advancing "front" of maturation so that overall vascular differentiation is at about the same level of advancement in all bundles.

VASCULAR DIFFERENTIATION IN THREE DIMENSIONS

Changes in vascular pattern with bundle position and age may first be examined from plots of major bundles to leaves at successive plastochrone intervals. These positions are plotted collectively in FIGURE 5, with details of the apical region enlarged in FIGURE 6. We should emphasize that quantitative information relates to the one crown from which the plot was made. Different crowns give different absolute values, but the relative values are much the same. The differences (largely quantitative) between intermediate and minor bundles are explained later.

This reductionist approach demonstrates the basic pattern for all bundles, but is free of the topographic complexity occurring in the crown due to the large number of bundles involved. It should be reemphasized that, according to the earlier study of Zimmermann and Tomlinson (1967), differentiation of the axial bundle is continuously acropetal in relation to the meristematic cap just below the shoot apex. Evidence that some vascular tissue develops in a continuous acropetal direction comes from the observation that undifferentiated vascular tissue is continuous below with differentiating and ultimately

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leaf trace to fifth fully expanded leaf 2 cm below its morphological insertion: protoxylem and metaxylem both fully mature but only protoxylem conducts dye downward; metaxylem not contiguous with protoxylem at this level and remains unstained. D, major leaf trace to first fully expanded leaf 0.8 cm below its morphological insertion: only differentiated protoxylem (dark stained) conducts dye downward; undifferentiated protoxylem and metaxylem remain unstained (compare A, showing comparable bundle at lower level; hydraulic constriction apparent). (Open star = mature metaxylem vessel contiguous with protoxylem; solid triangles = protoxylem with densely stained walls; solid circles = metaxylem not in contact with protoxylem, unstained in this experiment; solid stars = differentiating and nonconducting metaxylem; S = stegmata. Scale bar = 200 μm.)

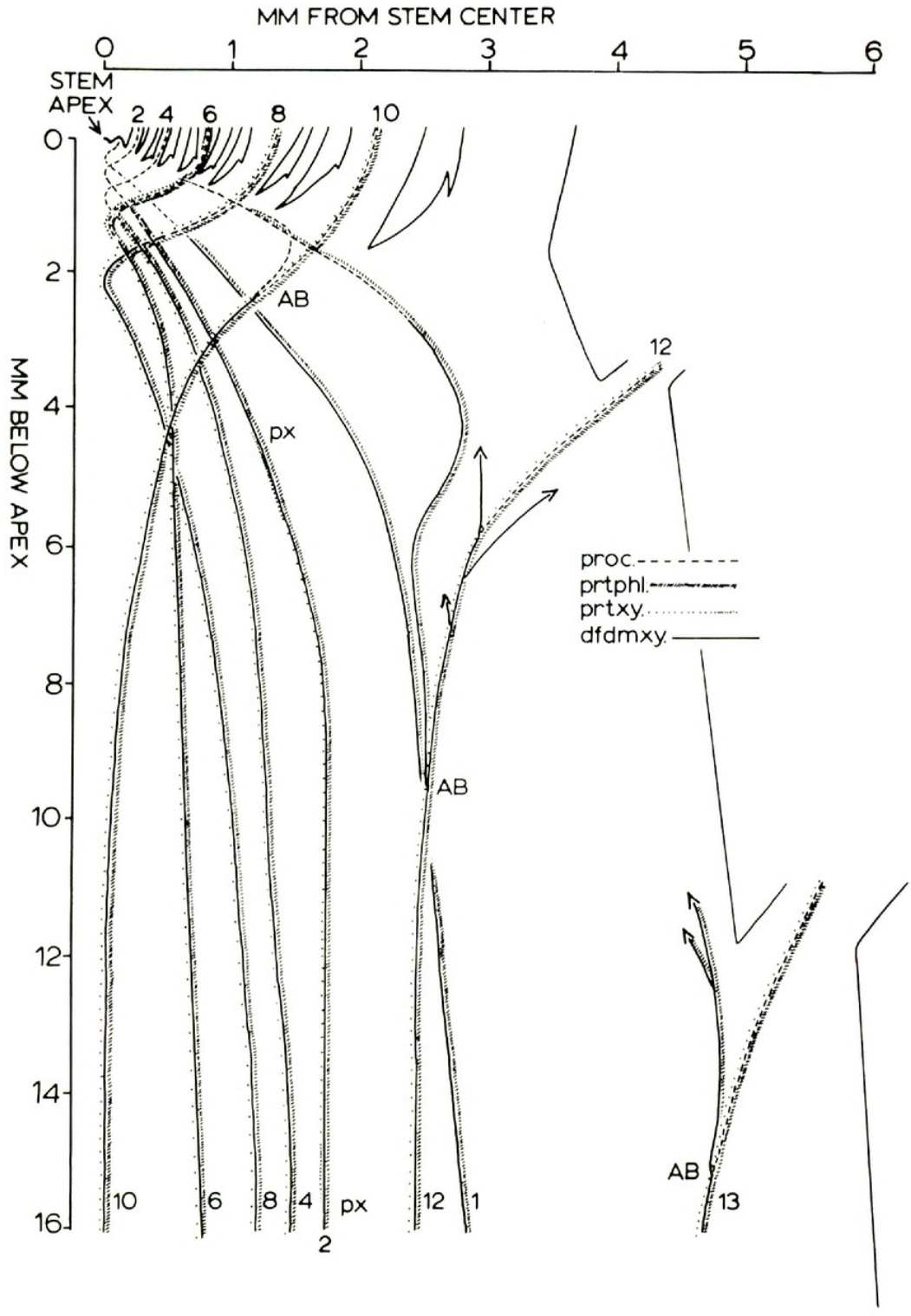

FIGURE 5. *Rhapis excelsa:* schematic longitudinal view of distribution of differentiating vascular tissues within major bundles to several successive leaves in developing crown, from same series used to produce FIGURE 2 (see FIGURE 6 for apical details). Axial dimension foreshortened about 50%. All leaves shown as if occupying single orthostichy instead of actual 2/5 spiral phyllotaxy; internal helical course of vascular bundles also ignored. Irregular width of leaf bases an artifact of plotting and display.

mature vascular tissue. These statements apply to protophloem and metaxylem, which are continuous acropetally. However, protoxylem is discontinuous and differentiates bidirectionally.

PROTOPHLOEM DIFFERENTIATION. Within the median leaf trace to P1, protophloem is not recognizable until nearly 1 mm below the shoot apex has been traversed (FIGURE 6). This is true of median major traces to the next four older leaf primordia (P2–P5). The precise level is somewhat uncertain because it is not easy to detect the first-formed protophloem element. Once located, however, protophloem can be traced continuously in a basipetal direction in the axial bundle. From sections of older vascular bundles, it seems that the differentiation of phloem, once initiated, is continuous at any one level, with new elements added in a centripetal direction (FIGURE 2, D–G). The distinction between elements differentiated during (protophloem) and after (metaphloem) elongation of the vascular procambium is somewhat arbitrary, but can be inferred indirectly by considering the level at which metaxylem first matures (which indicates cessation of organ extension). We have not investigated structural differences between protophloem and metaphloem established by this indirect method.

Protophloem continuity into a leaf is first evident in a trace to P6 and is then found in major traces to all older leaves (FIGURE 5).

PROTOXYLEM DIFFERENTIATION. Protoxylem was not detected at any level in a major trace to the youngest leaf primordium (P1) within the sectioned series. It can be seen in a trace to P2, but only at a considerable distance below the shoot apex—about 4 mm in an axial direction (FIGURE 5, upper px). Traced further in a basipetal direction, this protoxylem disappears at a level about 16 mm below the shoot apex (FIGURE 5, lower px). This is the developmental origin for the basipetal discontinuity of the protoxylem, evident in the mature stem. Since within a single plastochrone (P1 to P2) protoxylem appears and becomes elaborated over a distance of 12 mm, its differentiation is obviously very rapid. Further extension of this distance until protoxylem is continuous into the leaf base does not occur until the leaf is in position P6. In this leaf there is initially a short discontinuity within the leaf base (between px and px in FIGURE 6) representing the level of the intercalary leaf meristem; protoxylem can be seen in the leaf base above this level of discontinuity in the bundle investigated. Protoxylem continuity between leaf and stem is established in P7

Single major trace plotted to P1, 2, 4, 6, 8, 10, 12, and 13. In this crown P8 is youngest externally visible leaf ("spear leaf"); P9, first fully expanded leaf; P14 or P15 (outside this view), youngest leaf inserted above fully extended (mature) internode (see FIGURE 1). (Dashed lines = procambium (proc.), with or without differentiated vascular tissues; hatched lines = mature protophloem (prtphl.); dotted lines = mature protoxylem (prtxy.); solid lines = vascular bundle with differentiated (but still immature) metaxylem (dfdmxy.); AB = level of departure of axial bundle from leaf trace; px (above and below) = upper and lower limits of differentiated protoxylem in axial portion of major leaf trace to P2; arrows = bundles diverging from outgoing leaf trace with further extremity not plotted.)

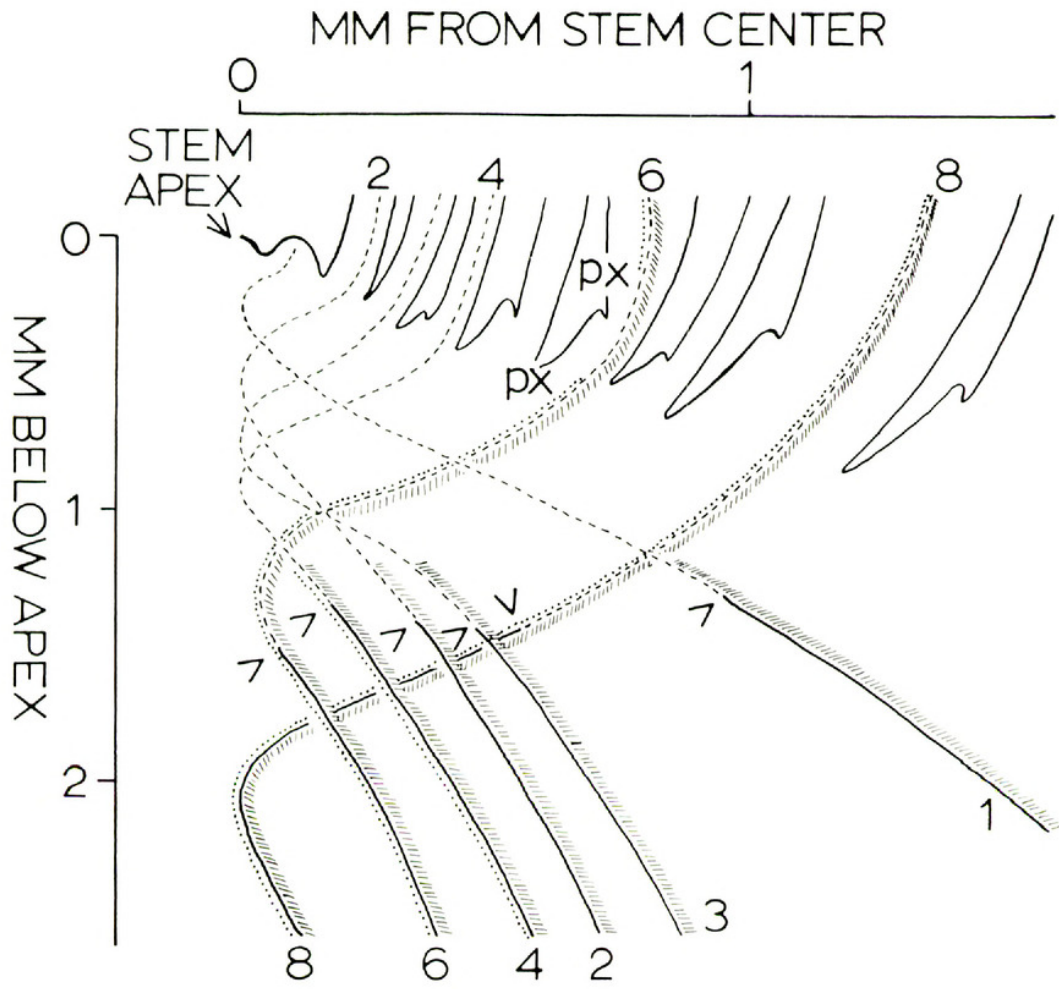

FIGURE 6. *Rhapis excelsa*, enlargement of crown region of FIGURE 5 to show early stages of vascular differentiation in major leaf trace to P1, 2, 4, 6, and 8. (Arrowheads = most distal level at which metaxylem differentiation first detected; discontinuity px to px in trace to P6 = discontinuity of mature protoxylem in leaf base. Shading conventions as for FIGURE 5.)

(not plotted in FIGURES 5 and 6) but is represented within the vascular bundle only by a single file of protoxylem elements at the leaf base. All older leaves (e.g., P8 in FIGURE 6) include protoxylem continuous from stem to leaf and in several overlapping files. This information demonstrates the intrinsic discontinuity of protoxylem. From the change in topography of a given bundle as a result of combined radial and axial displacement, it is reasonable to describe the further overall course of protoxylem differentiation as "bidirectional."

At any one level the amount of protoxylem progressively increases because differentiation of new elements proceeds faster than old elements are obliterated (cf. FIGURES 2, A and B; 3, B and C). Because of the protoxylem discontinuity, there is a basal decline in number of protoxylem elements that can be interpreted as evidence of the basipetal component of bidirectional differentiation. FIGURE 7, a plot of the total number of elements in all vascular bundles in the

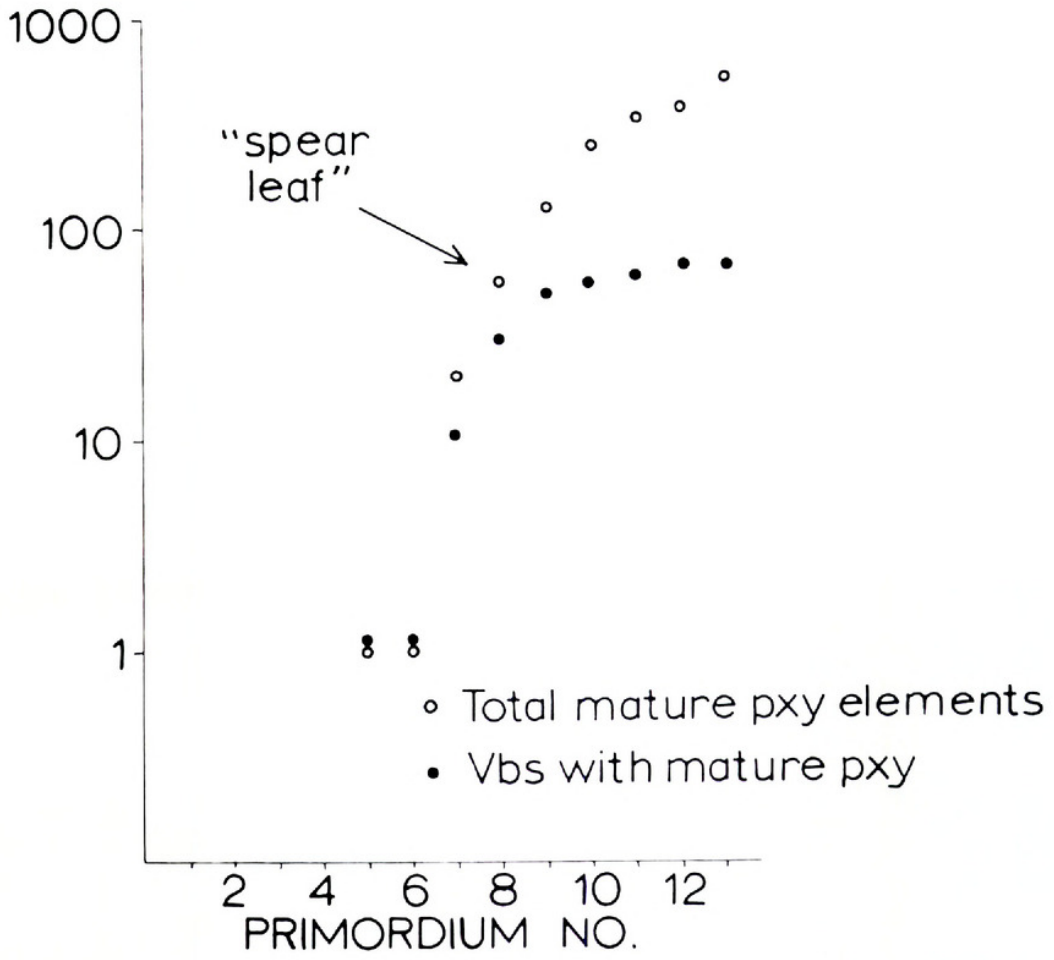

FIGURE 7. *Rhapis excelsa,* protoxylem development at leaf insertion of successively older leaves. (Closed circles = number of vascular bundles in leaf bases of successive primordia (P1 is youngest microscopically visible primordium); open circles = total mature protoxylem elements at same level.) P9 is first leaf with fully expanded blade; protoxylem maturation continues in leaf base for at least 4 plastochrones after leaf blade has expanded.

base of each leaf at successive plastochrones, indicates the extent of protoxylem connection of successive leaves but underestimates (because of protoxylem obliteration) total protoxylem produced. The information is sufficient to show that P8, the "spear leaf," is the first leaf with appreciable xylem contact with the stem, but that later leaves add considerably to their xylem transport capacity without adding new vascular bundles. This diagram can be compared with *fig. 4* in Zimmermann and Tomlinson (1967), which shows the total of all vascular bundles in successively older leaves.

METAXYLEM DIFFERENTIATION. Two stages of metaxylem differentiation can be recognized: early, when the conspicuous metaxylem vessels are first initiated (e.g., FIGURES 2, 3, 6); and late, when the cell contents are lost and wall thickening is completed (unshaded portion of FIGURE 1). In the mature vascular bundle at distal levels close to the level of branching as a leaf trace (e.g., lower

AB in FIGURE 5), the conspicuous metaxylem is represented by either a single vessel or a widely separated pair of vessels. Overlapping ends of vessels are commonly seen, especially in the region of attachment of bridges and branch traces (e.g., left-hand group of vessels in FIGURE 2, F). At any level where metaxylem is present, the elements are first visible as cells that vacuolate and enlarge conspicuously, in contrast to adjacent cells, most of which mature as narrow conjunctive parenchyma cells. This contrast occurs, for example, in a major trace to P1 at an axial distance of about 1.5 mm below the shoot apex (FIGURE 5). Significantly, this is well before protoxylem is differentiated in the same strand at this level. Protoxylem in the leaf trace does not occur much in advance of the differentiating metaxylem until about the time when the associated leaf is in position P5. After this, protoxylem is always continuous into the leaf base, as we have established. Metaxylem does not differentiate into the outgoing leaf trace but is continuous axially via bridges to adjacent axial bundles. These axial bundles themselves become recognizable as procambial strands in the crown just below the meristematic cap (Zimmermann & Tomlinson, 1966). However, bridges with early stages of metaxylem differentiation do not become recognizable until about the level of insertion of P12—i.e., almost 5 mm below the shoot apex proper in an axial direction. Such a leaf is the fourth-youngest fully expanded leaf in the crown illustrated in FIGURE 5. The relatively rapid advance of metaxylem differentiation within an axial bundle can be seen by comparing the procambial strand diverging from the leaf trace to P10 at the upper AB with that diverging to P12 at the middle AB in FIGURE 5.

Late stages of metaxylem maturation involve development of secondary wall layers and their lignification, together with loss of cell contents. Examination of free-hand sections stained with phloroglucinol and concentrated HCl shows lignified walls in wide metaxylem vessels at linear distances of about 3–4 cm below the shoot apex. This is about the level of insertion of the fifth leaf below the first fully expanded leaf and is always at the level where internode elongation has just ceased (FIGURE 1, B, unhatched portion). Maturation of metaxylem appears to be continuously acropetal.

In addition to the wide metaxylem, there are late-differentiated tracheary elements on the outer surface of the protoxylem strand that may not be totally mature until the internode (and therefore the vascular bundle) is completely extended (FIGURE 3, D, E, open squares). This becomes obvious when late stages of vascular differentiation are examined, but it was not considered when the mature structure was described. Consequently, it was assumed that all narrow elements on the inner face of the leaf trace were protoxylem tracheids (Zimmermann & Tomlinson, 1965). The late-maturing elements in this position, however, must be metaxylem, and they possibly correspond to some of the narrow vessel elements observed by Zimmermann and Sperry (1983) in macerated material from stems. They occupy only a limited part of the leaf trace since they occur just below the level where the protoxylem first diverges from the metaxylem within a given bundle. However, they are functionally important because this is the region in which interchange of water between metaxylem and protoxylem occurs (FIGURE 4, A).

Establishment of the Vascular Connection

The level at which the "descending" protoxylem of the leaf trace becomes contiguous with the "ascending" mature metaxylem can be determined from dye descent experiments, since the downward-moving dye initially descends in the protoxylem and can only enter the metaxylem of the axial bundle when a) this is mature, and b) the two tissues are in direct contact (Figure 4). Because the dye is initially restricted to the traces connecting the injected leaf to the stem, the vascular supply to that leaf can be identified in the stem. The juxtaposition of the two types of xylem can also be readily recognized in sections.

Movement of dye from protoxylem to axial metaxylem occurs (see Figure 4, A) in the trace system of leaf P9 and can be observed in the internode below P13 or P14—i.e., the internode in which extension has just been completed. (This association is, of course, rational and a simple consequence of the definition of metaxylem, which cannot mature completely within an extending organ.) The area of overlap (vascular insertion) is also mature at this level. At a higher level in the same bundle, only protoxylem is fully mature and conducts the dye; metaxylem is still undifferentiated (see Figure 4, B) and cannot transmit the dye even if it is contiguous with the mature protoxylem. The same considerations apply when protoxylem and metaxylem are not contiguous, regardless of whether metaxylem is mature (see Figure 4, C) or not (see Figure 4, D).

Maturation of Intermediate and Minor Bundles

Each leaf is supplied with about 100 vascular leaf traces that connect with the central cylinder of the stem. These have been termed major, intermediate, and minor bundles according to their topographic arrangement and time of appearance in the leaf base (Zimmermann & Tomlinson, 1965). Major bundles appear earliest and diverge from the stem center; intermediate ones appear later and diverge from a position nearer the stem periphery; and minor ones appear last and are restricted entirely to the periphery of the central cylinder. These designations are arbitrary since there is a developmental continuum (Zimmermann & Tomlinson, 1967). There are corresponding differences in the leaf-contact distance, with major bundles many internodes long, and minor bundles only a few. In addition to the 100 vascular leaf traces, there are about 1000 cortical bundles. These appear last and, by definition, diverge only from the stem cortex. They are discontinuous basipetally or anastomose among themselves.

The "rules" that govern vascular development in major bundles also apply to later-formed bundles, except that maturation of cell types occurs later. Differentiation of vascular tissues in progressively more peripheral (minor) bundles takes place during shorter periods of extension; consequently, minor leaf traces have very little protoxylem. Metaxylem matures somewhat later than in major bundles but according to the same principles. Although it would be possible to quantify these statements with the techniques available, no fundamentally novel information would be obtained.

DISCUSSION

The information presented above, when considered together with our previous studies on the course and structure of vascular bundles in mature stems and the process of initiation of vascular bundles themselves, allows us to present a highly integrated view of vascular development in the palm crown. Vascular bundles establish continuity between stem and leaf as procambial strands and increase in number at the level of leaf insertion. When a bundle makes contact with a leaf in this way, protophloem and metaxylem elements are already differentiated in the lower part of its course; the differentiation and maturation of these tissues is continuous acropetally, but metaxylem matures in such a way that it diverges into bridges or branch traces and does not continue along the leaf trace proper into the leaf base.

Protoxylem first appears in the distal part of the trace when the associated leaf is in position P2. It is discontinuous both distally into the leaf and proximally into the axial portion of the leaf trace. Protoxylem continuous from stem to leaf first occurs when the leaf is at about position P7.

Metaxylem does not begin to mature until the subtending internode of a given leaf has ceased to elongate. However, younger leaves have an indirect vascular continuity with the axial metaxylem because mature protoxylem and metaxylem are contiguous in completely mature internodes, with the mature protoxylem distally continuous through actively extending tissues and into the leaf itself (FIGURE 8). Xylem contact of about four or five leaves with expanded blades is made through tissue extension in this way. This means that the xylem-to-xylem contact between stem and leaf is still developing after the leaf blade has expanded. Sperry (pers. comm.) has preliminary information showing that this change affects the transpirational capacity of the leaf in its early life as an expanded organ.

HYDRAULIC CONSIDERATIONS

Transport capacity in the xylem to the developing leaf is indicated approximately by FIGURE 7 as the total number of protoxylem elements in the leaf base at successive plastochrones, which is at first dependent on—and then subsequently independent of—the total number of vascular bundles that differentiate in the leaf base. The measure is incomplete and would be better indicated by the sum of the fourth power of the diameter of all elements (Zimmermann & Sperry, 1983). The basipetal connection of this protoxylem is dependent on the overlap between protoxylem and metaxylem at lower levels.

The vertical distance over which metaxylem and protoxylem are directly contiguous within any one vascular bundle is limited. It may be termed the *vascular insertion* of the leaf trace onto the axial system (Sperry, pers. comm.), in contrast to the *morphological insertion* of the leaf base at the node itself. Only in the region of overlap can water move directly from metaxylem into the protoxylem of leaf traces; this, therefore, is the significant level of leaf insertion as far as water transport is concerned. However, vascular continuity between axial and leaf-trace xylem is established very late in crown development, in internodes in which extension has just been completed. It is dependent

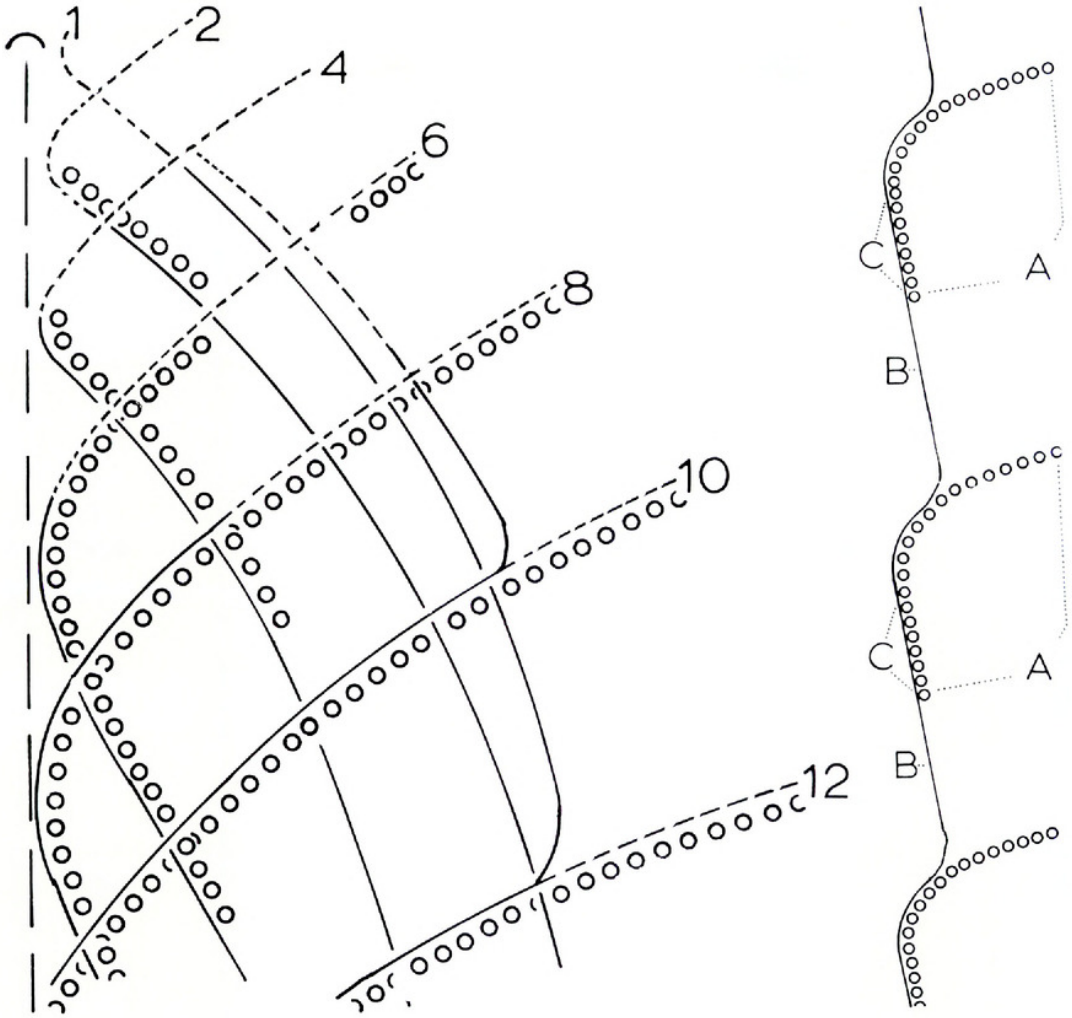

FIGURE 8. *Rhapis excelsa*, fundamental aspects of xylem development and topography. Left: developmental relationships between procambium (dotted lines), protoxylem (hollow circles), and differentiating metaxylem (solid line) in crown. (Numbers = leaf positions, topographic relationships schematic—see FIGURES 5, 6.) Right: relationship between protoxylem (hollow circles) and metaxylem (solid line) in mature stem; 2 complete leaf contacts of major stem vascular bundle. (A = "foliar" component of xylem system—protoxylem continuous into leaf base but discontinuous below in stem; B = "cauline" component of xylem system, permitting axial continuity in stem but not directly continuous into leaf; C = region of overlap between A and B where mature protoxylem and metaxylem in direct contact (region of "vascular" insertion within stem, in contrast to "morphological" insertion of leaf at node).)

on elements differentiating late as protoxylem, next to metaxylem elements in which maturation has just been completed. Distally, water moves into leaves only through protoxylem, as in the traces to P8, 10, 12, and 13 in FIGURE 5.

Major bundles that have extensive protoxylem connection with the leaf base continue to develop new tracheary elements between first-formed protoxylem and still-immature metaxylem within extending regions. Over the region of

vascular insertion (i.e., where protoxylem and metaxylem are laterally contiguous), differentiation of protoxylem continues from initials adjacent to immature metaxylem elements. The water-transport pathway is only completed when metaxylem finally matures.

Dissection of a number of crowns showed that there are four or five incompletely mature leaves (in which the leaf blade is expanded but the base is still immature) older than the spear leaf. The internodes below the node of insertion of each of these leaves is also still extending. Consequently, the first fully mature leaf associated with the first fully mature internode is about the fifth below the spear leaf and about P13 in this crown as a whole. All internodes below this level are completely extended and support fully mature leaves. The total number of leaves in the crown varies according to the position of the crown: fully exposed crowns include fewer visible and enclosed leaves (about nine of each), while those in the shade support more visible and enclosed leaves (about twelve of each). This range of quantitative variation has to be considered when absolute statements are made about the level of vascular differentiation below the shoot apex. Time of maturation of tissues may vary by as much as three plastochrones in different crowns. Nevertheless, similar conditions determine the development of the hydraulic connection established in each leaf as it develops.

Comparison with Other Monocotyledons

The most directly comparable study is that by Esau (1943) of the ontogeny of the vascular bundle in *Zea mays*. Although there are some common features, there are also appreciable divergences in the process of vascular bundle development between this grass and the palm studied here because of differences between them in size and general organization. A particular difference is in the axial meristem: it is interrupted in the stem of corn, and uninterrupted in the palm (see Fisher & French, 1976). The corn stem thus has a nodal plexus that is absent from the palm. Consequently, Esau was not concerned with the topographic differences between different bundles within the internode since these are largely controlled by the activity of the intercalary stem meristem, and she gave a detailed account of bundle development in leaves, which is not considered here. Despite this, similar features of development in the two plants include the method of early development of the procambium and the early appearance of protophloem and protoxylem at opposite poles of the strand (Figure 2). Increase in diameter of the bundle in both plants occurs by tangential longitudinal division that produces radial seriation of cells in transverse sections of the bundle (e.g., Figure 2, D). Esau considered it inappropriate to view this as a "cambium" (an opinion with which we concur) since this implies homologies with vascular organization that are not likely to exist in dicotyledons. That this is not a cambium is clear from the observation that division occurs during elongation of the vascular bundle. Figure 2, D, for example, in which radial seriation is particularly obvious, represents a level only 1 mm below the shoot apex, whereas extension occurs over an additional distance of several centimeters (cf. Figures 1 and 5).

Esau indicated that at any one level, protophloem always appears first, pro-

toxylem second. This sequence is common in stem bundles in *Rhapis,* but it is not always followed due to the greater topographic complexity. Protoxylem may precede protophloem (FIGURE 2, C) in the differentiation of leaf traces, and metaxylem initiation in axial bundles may even precede initiation of protophloem. Metaxylem initials, of course, are not totally mature until much later, after axial elongation is complete.

Despite these differences, some general features of vascular differentiation in *Rhapis*—including the continuous acropetal differentiation of phloem and metaxylem—correspond to those found in other angiosperms. In contrast, protoxylem is discontinuously differentiated within each bundle, originating in the distal part of each leaf trace and, in a relative sense, rapidly extending both acropetally and basipetally. The protoxylem of the proximal leaf trace must advance acropetally in the stem to make contact with the distal protoxylem of the bundle in the leaf base. Although it is reasonable to assume topographic advance of further protoxylem differentiation in a basipetal direction, we have not estimated this quantitatively against fixed reference points. There is never continuity between protoxylem of one trace and that of another. This discontinuity is a topographic consequence of the acropetal advance of the cessation of internodal elongation and a direct expression of stem growth via an uninterrupted meristem. Continuity of the xylem transport pathway is effected by the juxtaposition of metaxylem and the last-formed protoxylem. This is the basis for the "hydraulic constriction" at each leaf insertion, recognized by Zimmermann and Sperry (1983) and illustrated in Zimmermann *et al.* (1982), upon which the whole hydraulic architecture of the palm is based. This simple developmental arrangement is reinforced by the failure of metaxylem to differentiate in the outgoing leaf trace beyond the level of departure of the last bridge or axial bundle.

PHLOEM DIFFERENTIATION

Unlike Esau, in her study of *Zea* (1943), we have been unable to make a very clear distinction between protophloem and metaphloem. In *Zea* the latter is structurally distinct because only metaphloem includes companion cells and its elements are arranged in regular radial series. Such a distinction is not evident in *Rhapis.* However, if we accept that metaphloem, by definition, only matures after the bundle is completely elongated, it is clear from FIGURE 5 that all the phloem differentiated in the crown is protophloem. Obliteration of first-formed protophloem is extensive, but some passive extension can presumably be accommodated. Since metaphloem and protophloem are not topographically distinct (unlike protoxylem and metaxylem), continuity of development is evident.

LEAF TRACE AND AXIAL BUNDLE

We have used the terms "axial bundle" and "leaf trace" without precise definition in this series of papers. "Leaf trace" refers to the outwardly curved portion of a bundle shortly before its entry into the leaf base, and "axial bundle" to the portion of a bundle remote from its entry into a leaf. With progressive

increase in our understanding of the topography, initiation, and differentiation of vascular bundles, the terms can now be refined somewhat. In a topographic sense, the axial bundle can be said to become a leaf trace either at the point of its maximum level of penetration into the stem center or at the level of departure of the continuing axial bundle. The latter is unsatisfactory because this varies considerably in different monocotyledons: the leaf trace would be very short in some because the axial bundle departs close to the stem periphery, and in others because a distinct axial bundle is not always present (Zimmermann & Tomlinson, 1974, *fig. 9*).

In developmental terms the level at which the "basipetally" determined influence of the leaf conjoins the "acropetally" determined influence of the meristematic cap could represent the junction of the two portions of a subsequently continuous vascular bundle. This definition is difficult to apply, although it may have the most precise morphogenetic meaning. It corresponds closely to the first topographic definition.

In histological and functional terms, the "leaf trace" can be defined precisely as that portion of the stem bundle over which protoxylem differentiates (FIGURE 8). This definition is readily accommodated by structural analysis and has direct functional significance because it is the basis for the hydraulic constriction mentioned by Zimmermann and Sperry (1983). The definition differs from both preceding ones because the "axial bundle" (represented by the metaxylem) and the "leaf trace" (represented by the protoxylem) necessarily overlap considerably. The "axial bundle" is continuous along the stem and makes no direct contact with the leaf. We thus have a precise developmental and functional application of the abstract and much-debated notion of the "cauline bundle" (Esau, 1965). The "cauline" portion corresponds to the axial vasculature in which metaxylem is differentiated, since this is the pathway for axial movement of water up the stem (FIGURE 8, B). The "foliar" portion (FIGURE 8, A) is the leaf trace in the above protoxylic context and, at least in a palm, relates solely to the irrigation of the major appendages (the leaves) via the region of direct contact (FIGURE 8, C).

Protoxylem discontinuity appears to be a universal developmental feature of stem vascular tissues (Esau, 1965) but has not previously been explained in a functional sense. Hydraulic architecture gives a possible clue. At times of stress, xylem dysfunction is restricted to disposable plant parts while axial continuity is preserved (cf. Zimmermann, 1983).

From this analysis and its functional application, we can extract information that can be applied to vascular plants generally. For example, analyses of *Populus* show similarity in the time of appearance of vascular tissues and the development of hydraulic constrictions (Larson, 1976, 1982; Larson & Isebrands, 1978). However, these features are much condensed in the apical region: events that occur over distances of a few hundred microns in *Populus* (Larson, 1975) extend over centimeters and throughout many plastochrones in palms. This is the basis for our suggestion that palms are particularly suitable organisms for studying vascular development.

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