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Vascular construction and development in the stems of certain Pandanaceae

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By means of serial sections and cinematographic methods the vascular system in the stems of certain Pandanaceae is analyzed, in both mature axes and developing crowns. This serves to establish the three-dimensional basis for the bi- or tri-polar vascular bundles which are apparent in single sections of stems of all species of Pandanaceae which so far have been examined. The basic "*Rhapis*-principle", which characterizes woody monocotyledons, is here expressed in a distinctive way which can be seen as an important developmental variation.

CONTENTS

INTRODUCTION

In recent analyses of the construction and development of the vascular system in the stems of larger monocotyledons, palms have been used as a model. The principles of vascular construction established for the palm family have also been shown to operate in the stems of other arborescent monocotyledons in other families (Zimmermann & Tomlinson, 1965, 1967,

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1968 1969). In this article we turn our attention to the Pandanaceae and account for the way certain distinctive diagnostic features of stem anatomy of this family, evident in transverse sections of its stems, have an explanation in the three-dimensional distribution and the developmental pattern of vascular strands.

It has long been recognized that, in the anatomy of their stems, Pandanaceae can be distinguished from those of other woody monocotyledons by the frequent presence of compound (bi- or tripolar) vascular bundles, i.e. two or three distinct conducting strands enclosed by a common bundle sheath. The three-dimensional distribution of these strands has been investigated with considerable insight by Carano (1906a) and Meyer (1933). Meyer emphasized strongly that there was no continuity of vascular tissue between different strands within the compound bundle, an important functional point. Our own contribution carries the story further by investigating the vascular system more thoroughly with the aid of newer techniques and by adding a developmental perspective. This shows that Meyer's view has to be modified.

THE FAMILY PANDANACEAE

Taxonomy

The Pandanaceae is one of the most natural families of monocotyledons, with a distinctive and very specialized arrangement of the reproductive parts, which do not resemble typical flowers and so remain largely uninterpreted by comparative morphologists. Information regarding the morphology of the "flowers" of Pandanaceae is given in Warburg (1900) and there is an interesting discussion of putative homologies by Stone (1968). The naturalness of the family is further emphasized by a distinctive complex of microscopic anatomical characters in the vegetative parts, of which the compound stem bundles mentioned above are but one (Sólereder & Meyer, 1933). Earlier, Carano (1906b) had emphasized that even a fragment of a *Pandanus* can be recognized by its microscopic anatomy.

The family is large (over 1000 species) and widely distributed in the tropics of the Old World. The three genera are distinct from each other and differ widely in the number of species they contain. *Pandanus* is the largest, with species variously estimated between 500 and 1000; *Freycinetia* has of the order of 200 species. *Sararanga* is a little-known genus of three species (Stone, 1961).

Habit

Growth habit in the family is diverse and bears some comparison with the diversity in the habit of palms. However, in contrast to most palms, pandans typically have branched aerial stems. Since we have tried to encompass this wide range of form in our comparative observations, habit is described in some detail. This variety of habit, furthermore, is accomplished without the development of any secondary vascular tissue. The earlier reports by Strasburger (1906) and Warburg (1900) of secondary thickening by means of a cambium in certain species of *Pandanus* is incorrect and appears to be based on the observations of radial series of dividing cells developed close to the root insertions, which superficially resemble cells derived from a cambium. Since they lack secondary tissues the pandans suffer from the basic restrictions which this deficiency imposes on branching and habit (Holttum, 1955), i.e. an inability to develop a mechanically and physiologically much-branched shoot system on a trunk of fixed diameter and an inability to augment the root system effectively by branching of the radicle alone. Nevertheless, the pandans are often much-branched, indicating that they have overcome these deficiencies in effective ways. Essentially branching is accomplished by precocious development of lateral meristems, usually in association with sympodial growth, while the root system is augmented by the ready development of abundant and often massive adventitious roots. The following paragraphs describe the major growth forms in each genus with the aid of the diagrammatic illustrations of Figs 1 and 2.

Sararanga (at least the most familiar species, *S. sinuosa* of New Guinea and the Solomon Islands) has a massive columnar stem up to 70 cm in diameter, with an overall height up to 30 m (Fig. 2F). It is sparingly branched and lacks aerial roots. The leaves are 4-ranked and not 3-ranked as in *Pandanus* and *Freycinetia* (Stone, 1961).

Freycinetia generally grows as a root-climber (Fig. 2H); the relatively slender stems are anchored to the supporting tree by clasping, adventitious roots and contact with the soil is made by thicker feeding roots which are most abundant on the basal stem parts. Stems are sparingly branched with the branches usually narrower than the parent axis (e.g. Fig. 2D). Casual field observations by one of us (P.B.T) suggests that in some species shoots are dimorphic with flowering restricted to plagiotropic branches which have leaves smaller than those on the orthotropic main axis. In a minority of species of *Freycinetia*, as observed in a specimen of *F. cumingiana* cultivated at Fairchild Tropical Garden, the plant is a low shrub with numerous vigorous erect, distally-branched shoots arising from a "rootstock", a habit which recalls that of the bamboos in some ways (Fig. 2A). It is probable that growth habits in *Freycinetia* are more diverse than is presently appreciated, since detailed descriptions of the aspect of the plant are neglected by collectors.

Pandanus species occupy a wide variety of ecological situations (van Steenis, 1954) and this is no doubt related to the diversity of growth form (e.g. Stone, 1970a). Typically the base of the stem is provided with, and often in part supported by, thick aerial roots ("stilt-roots") which relate to the manner of establishment growth described below. Some species do lack these aerial roots, however (Fig. 2G). The main stem is sparsely to frequently branched (Figs 1A- C and 2E,G). Where the stem is massive, columnar and with aerial roots in a dense cluster at the base together with regularly-produced narrow branches, probably of determinate growth, a characteristic "coniferoid" habit results (Fig. 2G), as in several Madagascan species described by Stone (1970b). With a similar but shorter main axis but frequent wide-spreading branches of two or more successive orders and more extended growth, a low, spreading tree with a well-developed and symmetrical crown is formed, as in the familiar cultivated *P. utilis* (Fig. 1B). In very many species the branches are low-spreading but then more obviously supported by aerial roots ("prop-roots") which arise from the lower surface of the branches (Fig. 1A). With age these branches may become disconnected at the base from the main axis and, since they have an

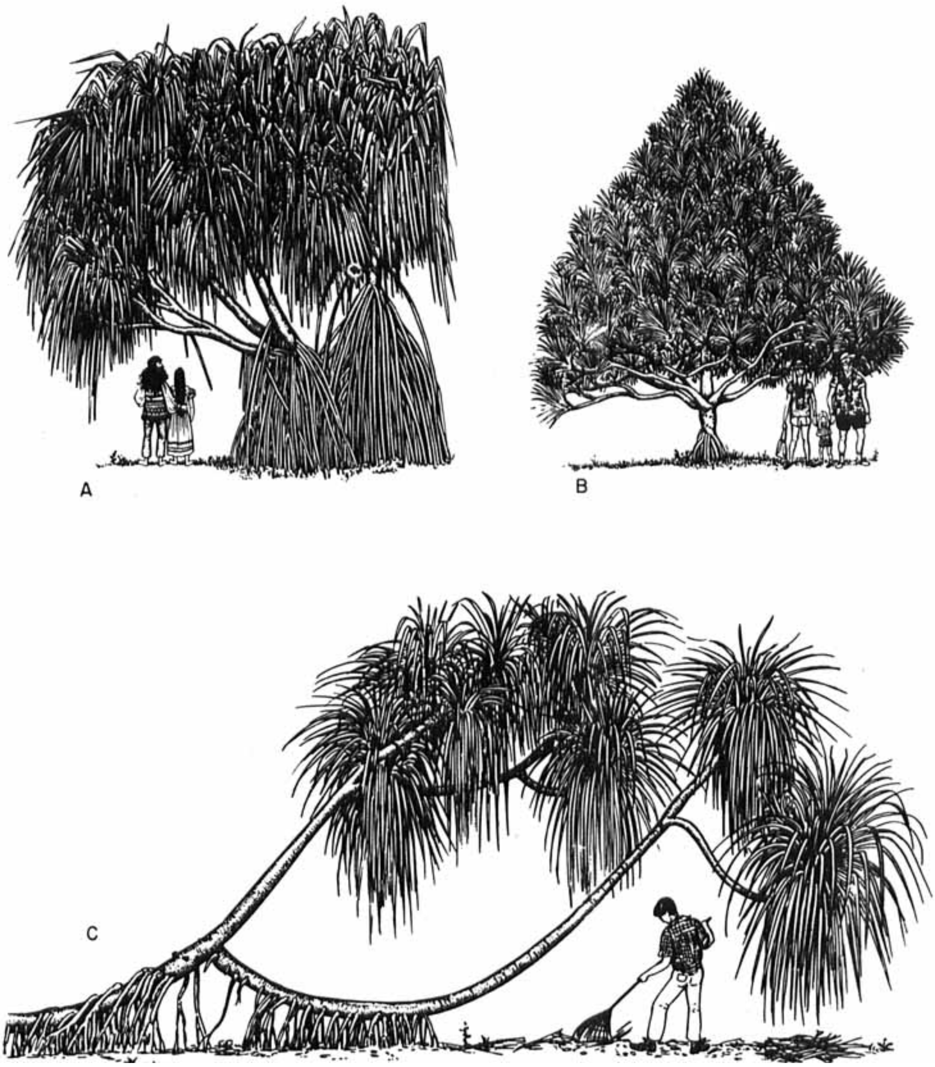

Figure 1. Habit variation in *Pandanus*. A, *Pandanus* sp. (cf. *P. baptisii*) specimen cultivated at Fairchild Tropical Garden, Miami, Florida to show erect tree with well-developed aerial roots; B, *Pandanus utilis*, specimen cultivated at Fairchild Tropical Garden, showing regular form and aerial roots restricted to base of trunk; C, *Pandanus* sp. (cf. *P. tectorius*) specimen cultivated at U.S.D.A. Experiment Station, Old Cutler Road, Miami, Florida, showing sprawling habit and supporting aerial roots from lower branches.

Figure 2. Habit and morphology in Pandanaceae. A-D, *Freycinetia cumingiana:* A, habit of part of a clump, developing shoots to the right, branched, developed shoot to the left (x 1/30); B, dormant lateral bud (x $1\frac{3}{8}$); C, developing lateral bud, piercing the base of the subtending leaf (x 4/10); D, base of a lateral branch showing transition from scale to foliage leaves (x4/15). E-H. Habit in various genera of Pandanaceae: E, *Pandanus* sp. (cf. *P. julianettii*), drawn from photographs—young unbranched individual to right, older branched individual to the left; F, *Sararanga sinuosa*, drawn from photographs—young unbranched individual to the right, older branched individual to the left; G, *Pandanus* sp. with "coniferoid" habit, after Stone (1970b); H, *Freycinetia* sp. (cf. *F. banksii*) root-climber, drawn from a photograph.

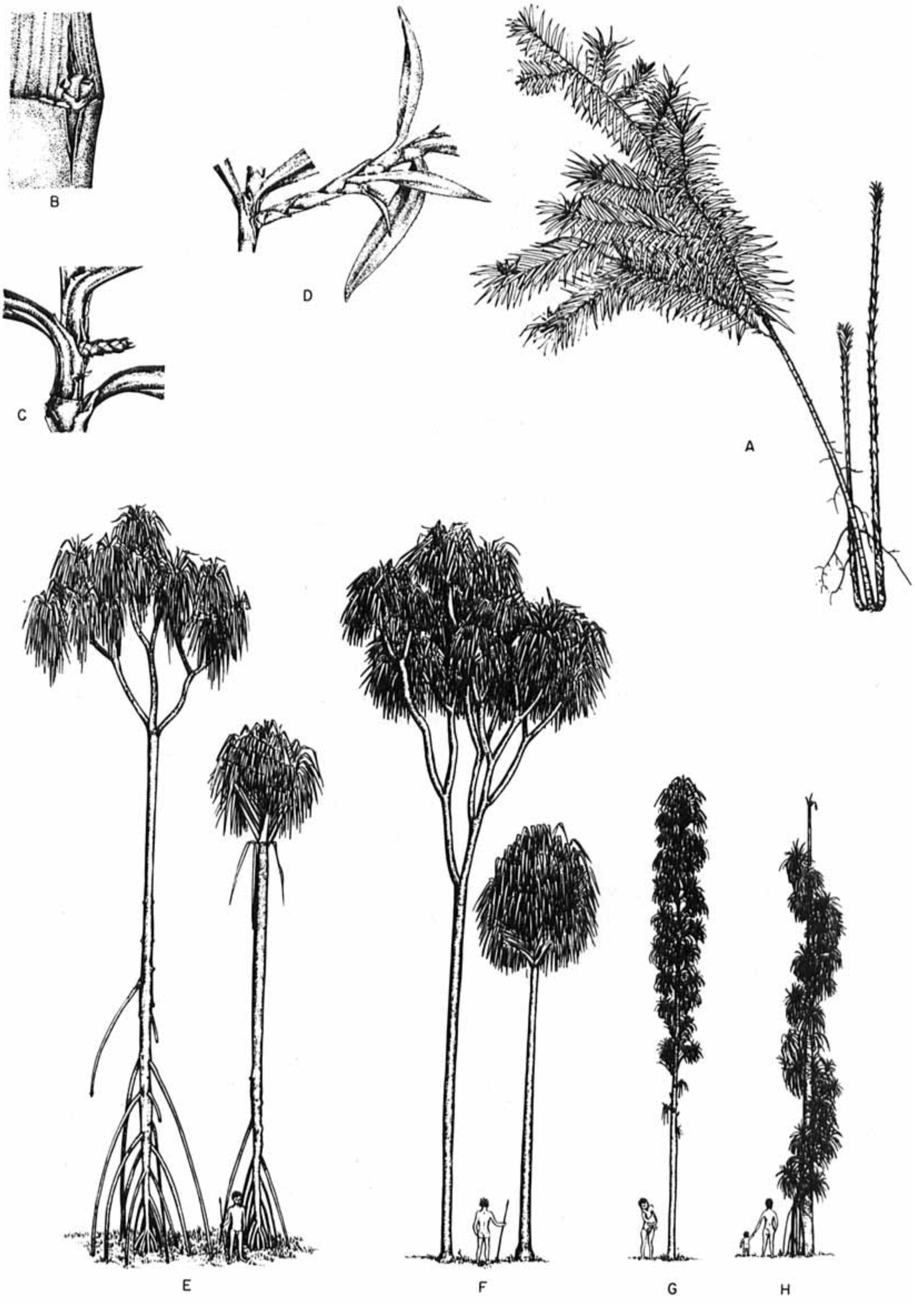

Figure 2.

independent root system, they can persist independently of the parent axis (Fig. 1C). By this means a certain amount of vegetative spread is achieved, as in the West African *P. candelabrum* (Hallé & Oldeman, 1970: 76). This sprawling habit is also characteristic of the widely distributed species of coastal sea-shores in the Pacific which is referred to *P. tectorius.* Intermediates between these erect and more decumbent growth forms occur. No information is available about the habit of the few epiphytic *Pandanus* species.

Schoute (1906) showed that in *Pandanus* branching is usually sympodial below a terminal inflorescence. Where the sympodium remains simple, the original axis is replaced by a single lateral branch; but should two or more laterals replace the original inflorescence the sympodium multiplies and the axis is visibly branched or bifurcated. Branching in these woody axes without secondary thickening growth is possible because of the precocious development of the lateral branch in such a way that vascular continuity between parent and daughter axis is maintained entirely via primary vascular tissues (cf. Tomlinson, 1973). Morphological details of this process are well described by Schoute (1906).

Lateral buds in *Pandanus* and *Freycinetia* (probably also in *Sararanga*) arise singly at each node and normally in the axil of each foliage leaf (Fig. 2B,C). This is in contrast to palms, which usually lack vegetative buds on their aerial parts. In *Pandanus* the lateral buds are strongly inhibited, except during the normal process of sympodial growth when one (sometimes more) immediately beneath an inflorescence is released from inhibition. In the exceptional *P. gemmiferus* described by St. John (1962), where numerous laterals develop, these are short-lived and insignificant. Branching in *Freycinetia* is more frequent and the branches may grow out quite belatedly (Fig. 2C), a phenomenon which requires further investigation.

Flowering in *Pandanus* is always such that shoots are determinate with terminal inflorescences on all branches (but in the apparently monopodial types, probably not of the main axis itself). Exceptional species may have inflorescences which could be described as "lateral", i.e. as short specialized flowering lateral shoots produced on a monopodial main axis. This habit has been seen by one of us (P.B.T.) in an un-named species of *Pandanus* in Fiji. Quite obviously, however, the difference between *Pandanus* with terminal and those with "lateral" inflorescences is one of degree only

The habit of many of the larger Pandanaceae, as illustrated in Figs 1B and 2E,F, gives the appearance of a tree with secondary thickening growth because there is a wide trunk and a crown made up of progressively narrower branches or, in the "coniferoid" habit, of short narrow branches. This appearance, however, is the result of decreased primary thickening growth in lateral branches, producing narrower shoots. By this mechanism a mechanically and physiologically efficient tree is produced, even without a vascular cambium. The potentially dimorphic shoot system of *Freycinetia* species is therefore not very dissimilar from the "coniferoid" pandans when regarded in a general comparative way (cf. Fig. 2G,H).

Establishment growth

This term was coined as a modification of the German term "Erstarkungswachstum" to describe the way in which the originally narrow seedling axis of arborescent monocotyledons increases its primary diameter with age by means of a progressively wider crown (Tomlinson & Zimmermann, 1966). Corresponding to this overall increase in stem diameter, the total number of vascular bundles progressively increases at successively higher levels. The method of establishment growth in Pandanaceae varies in different genera so that, although all have an obconical axis, the overall shape is very different, depending largely on the amount of internodal elongation.

In the scandent species of *Freycinetia* the transition from the narrow seedling axis takes place very gradually, often over a distance of several metres. This is possible mechanically because the axis is not self-supporting. Representative measurements for the axis of a specimen of *F. banksii* in New Zealand are given in Table 1 (see also Tomlinson & Esler, 1973). In many species of *Pandanus*, e.g. *P. utilis*, the juvenile phase of growth produces a short obconical and subterranean axis, as in most palms. However, unlike most palms, the base of the columnar trunk produces massive aerial roots which help to support the plant (Fig. 1B). In other species of *Pandanus* successive internodes are elongated as well as widened, but the obconical trunk so produced is stabilized by aerial "prop-roots" (Fig. 2E). There is no detailed information for *Sararanga*, but the few seedlings supplied to us (see Appendix) suggest that there is no morphological specialization of the juvenile axis; it thus resembles that of a palm.

Table 1. *Freycinetia banksii* seedling dimensions (Material in Waitakere Hills, nr. Auckland, New Zealand)

	Height (cm)	Diameter of internode (cm)	No. of nodes below	Average length of internode (cm)
Apex				
	160	2.6	57	1.31
	85	2.0	49	1.0
	35	1.4	31	0.8
	10	0.8	22	0.45
Base	0	0.3		

It is probable that other types of establishment growth will be recognized in the Pandanaceae; these brief notes are intended to draw attention to the subject.

In considering the anatomy of axes in the Pandanaceae in subsequent sections, the wide variety of growth forms, which has been briefly outlined, must be considered. Apart from the variation likely to be found along a single axis, which is most obvious in the transition from seedling to adult, there is considerable variation in the size and type of adult or distal branches. This is reflected in considerable quantitative variation, but we do believe this does not affect the principles of construction described below.

MATERIAL

Sources of material are listed in the Appendix. *Sararanga sinuosa* was provided in quantity for us by G. F. C. Dennis as formalin-preserved specimens of seedling and adult main stem. *Freycinetia banksii* has been studied in some detail in New Zealand by one of us (P.B.T.), supplemented by casual observation of unnamed species in New Guinea. A specimen of *F. cumingiana* at Fairchild Tropical Garden supplied additional material. In addition to *Pandanus utilis,* commonly cultivated in the Miami area, we had access to several unnamed species of *Pandanus* cultivated at the U.S.D.A. Plant Introduction Station (Chapman Field), Old Cutler Road, Miami, Florida. Further specimens from Malaysia, West Africa and Australia respectively have been supplied by Dr B. C. Stone, Mr J. B. Hall and Dr V. I. Cheadle.

METHODS

Sectioning

For comparative studies sections of unembedded material or of material embedded in celloidin were cut on a Reichert "OME" sliding microtome and stained and mounted by standard methods. The longest specimen that can be held in the standard clamp of this microtome is of the order of 8 cm. Since it is usually necessary to analyse much longer stems, we had two special clamps designed and constructed (Brookfield Machine Co., West Brookfield, Mass.) to accommodate pieces of stem of indefinite length and maximum diameters of 2.5 and 5 cm respectively. In the preparation of sequential series of sections for cinematographic analysis the methods described by Zimmermann & Tomlinson (1965, 1966) have been followed. Serial sections of developing crowns of *Pandanus utilis* and *Freycinetia banksii* were cut from specimens embedded in Paraplast after dehydration via the usual tertiary-butyl alcohol series. Prolonged periods of treatment were necessary for the infiltration of the larger buds of *Pandanus.* In this material the resulting sections were so large that only two could be mounted on a single 5 x 7.5 cm slide. Such series of sections were used for vascular analysis with the optical shuttle.

Cinematographic analysis

As is now routine in our procedure, analysis and three-dimensional interpretation of the adult and developing stems was carried out using cinematographic techniques, as described in detail in a number of articles (Tomlinson, 1970; Zimmermann & Tomlinson, 1965, 1966, 1967, 1969). Whenever possible we used the Wild M20 shuttle microscope. The lowest magnification of this covers a rectangular area measuring 3.5 x 5 mm. A specially constructed shuttle allowed us to use a yet lower magnification, including an area of the section up to 22 mm wide. (This instrument is illustrated on p. 12 of the Annual Report of the Harvard Forest, 1970-71.)

For direct photography of planed surfaces the methods described in detail in Tomlinson (1970) were used. A variation of the technique applied to certain very hard *Pandanus* stems was to play a jet of steam on to the surface of the specimen as it was cut. The camera had to be protected by asbestos boards from excess heat and the microtome operator had to be protected by asbestos gloves.

Certain restrictions on this method of direct photography are imposed by the texture of *Pandanus* stems. The centre is often too spongy to be planed smooth and only the more woody peripheral basal parts could be filmed.

DIAGNOSTIC FEATURES OF STEM ANATOMY IN PANDANACEAE

The following section deals with the general stem anatomy of the three genera, as visible in single sections, and is supplemented by the photomicrographs on Plates 1 and 2.

The following account emphasizes only features diagnostic for the family:

Epidermis sometimes becoming sclerotic, as in *Freycinetia* (Plate 2A). Periderm (Plate 2A,B) typically developed in *Pandanus* and often in *Freycinetia*, the phellogen appearing earliest at the node in association with leaf abscission. *Cortex* (Plate 1A) narrow, largely without an independent vascular system except for transitory leaf traces but including short, rarely anastomosing fibrous strands (Plate 2B). *Endodermis* sometimes present at inner limit of cortex, but usually absent. *Central cylinder* otherwise abruptly delimited from cortex by a peripheral zone of congested vascular bundles (Plate 2A,B), central vascular bundles often much more diffuse. Vascular bundles either (a) *simple* each with (1-)2-3 wide metaxylem vessels or tracheids and varying amounts of protoxylem, the narrow phloem strand sheathed by thick- or thin-walled fibres; or (b) *compound*, bi-(tri-) polar with 2(-3) separate conducting strands with the separate phloem strands more or less facing each other and enclosed by a common fibrous sheath, the phloem not usually contiguous in mature bundles (but observed e.g. in seedlings *Sararanga* (Plate 1C)). Compound bundles most commonly more or less restricted to a region just within the congested peripheral zone. *Ground parenchyma* sometimes becoming sclerotic, especially between the congested peripheral vascular bundles and throughout the older, basal parts of larger stems; otherwise developing an extensive series of air-lacunae in the centre of the central cylinder so that the ground tissue becomes very spongy (Plates 1B and 2A). Lacunae originating by early, localized enlargement of ground parenchyma cells, itself causing a type of diffuse secondary growth of the stem. Subsequent collapse of these enlarged cells produces lysigenous cavities. *Calcium oxalate* abundant in either raphide-sacs or isodiametric crystal cells. Raphide-sacs abundant throughout ground tissues, often the site of initiation of a lacuna (R in Plate 3B); crystal cells in longitudinal files next to sheathing fibres of vascular bundles. Vessels present in metaxylem of stem of *Pandanus* and *Freycinetia*, the elements often very long and with oblique scalariform perforation plates. Vessels not observed in adult stem of *Sararanga*, the metaxylem tracheids then very long, tapering and without definite end walls. Sieve tubes of metaphloem with long oblique compound sieve plates.

The possession of primary wood with frequent compound vascular bundles, crystal cells associated with vascular bundles, each crystal cell with a single cubic calcium oxalate crystal and lysigenous air-lacunae provides a combination of diagnostic features whereby stem tissues of Pandanaceae can be distinguished from the tissues of other woody monocotyledons.

The variation in stem structure between the three genera of Pandanaceae has not been investigated in sufficient detail to indicate whether there are diagnostic features of stem anatomy at the generic level. *Sararanga* in our experience does seem distinct in lacking vessels from the metaxylem of the stem. Quantitative differences between different species are large; for example there seems to be a positive correlation between stem diameter and diameter of individual vascular bundles. This variation can be seen in a single species; for example in *Froraranga sinuosa* the average diameter of vascular bundles in the adult stem (Plate 1B) is several times larger than those of the vascular bundles in the seedling (Plate 1C).

THE COURSE OF VASCULAR BUNDLES ON THE MATURE AERIAL STEM

Carano (1906a) had an understanding of the course of vascular bundles in the *Pandanus* stem which surprises us today, considering the relatively primitive methods he used. His study was based upon living material from 19 species which had been growing in the botanical gardens of Rome and Florence. He correctly described the path of vascular bundles. At the point where a leaf trace departs from the stem centre towards the periphery (the leaf insertion), it branches in an upward direction. The branch is the continuing axial bundle which gradually, on its distal path, "moves" first toward the stem periphery and then back towards the stem centre where it finally becomes a leaf trace again, repeating the cycle. The compound bundles are temporary unions of two or more axial bundles. In the mature stem there is no functional vascular connection between the two. Meyer (1933) reinvestigated the problem, observing further details, but added little to the information that had been gathered by Carano. In our own investigation, carried out during the course of several years, a number of surface-view sequences and microscopic analyses of serial sections of several species have been made. This paper is based upon information contained in about ten films of 4000 frames each. Since we photograph surface views with single frames and microscopic views with two frames each, the number of individual transverse views on record is of the order of 30,000.

The compound bundles are usually restricted to the congested subperipheral zone of the central cylinder. This region is widest and most pronounced in the trunk-like stems of larger species but less distinct in the distal shoots and in smaller species. As one follows a specific bundle up and traces its course on the moving transverse plane in the film, the overall path evidently resembles that of a bundle in a palm stem like *Rhapis,* though it is somewhat more complicated (cf. Fig. 3A,B). The leaf-trace departure is quite palm-like: at the level where it adopts the nearly radial outward course, it branches and produces the continuing axial bundles as well as several bridges (upwardly-pointing connections to neighbouring bundles which are not shown in Fig. 3A,B). Bridges are usually short, but in addition to these short ones, one occasionally finds longer ones which form an "anastomosing bridge system" of the kind described in the seedling axis of *Rhapis* (Tomlinson & Zimmermann, 1966).

As in some palms, axial bundles of some *Pandanus* species describe left- and right-turning helical paths. Cinematographic analysis makes this visible in the projected transverse sections as a rotating motion: bundles in the subperipheral zone of compound bundles move in a tangential direction one way, the bundles nearer the stem centre move in the opposite direction.

The bundle path in the Pandanaceae (Fig. 3B) differs from that of the palm *Rhapis* (Fig. 3A) in that it shows repeated temporary associations of axial bundles with each other in a more or less regular way. The overall path of an axial bundle begins at its point of origin where it branches off the leaf trace (at a in Fig. 3B). The axial bundle, immediately upon its release from the leaf

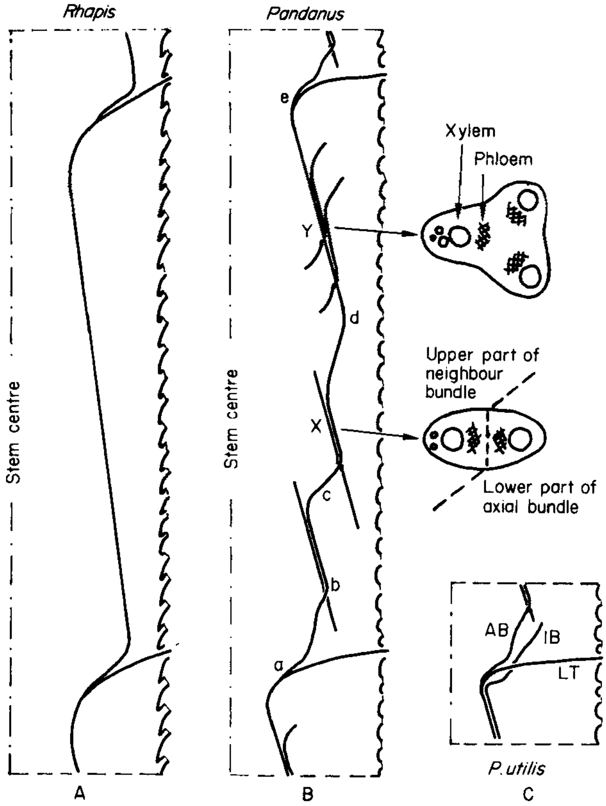

Figure 3. Diagrams of course of vascular bundles in *Rhapis* and *Pandanus* compared. A, Course of a vascular bundle in the palm *Rhapis* represented diagrammatically in one plane (after Zimmermann & Tomlinson, 1972); B, course of a vascular bundle in *Pandanus*, represented diagrammatically in one plane (letters a-e indicate levels referred to in the text, diagrams to the right represent a bipolar bundle in transverse section at level X and a tripolar bundle in transverse section at level Y); C, detail of axial bundle branch (AB) from leaf trace (LT) shortly above departure of last associated inverted bundle (IB) of bipolar bundle. To simplify B and C, bridges are not shown.

trace, follows a short path towards the stem periphery, where it makes a 180° turn around its own axis and associates (at b in Fig. 3B), in an inverted position (phloem side towards the stem centre), with a neighbouring axial bundle, thus forming a compound bundle. The fibrous sheaths of the two individual bundles are fused, but there is no continuity of vascular tissue. A little further up the stem, the axial bundle becomes independent again and "moves" further towards the stem periphery, where it joins another axial bundle in the same way (at c in Fig. 3B). It may do this once, twice or more times before reaching its outermost position, but not necessarily the very periphery of the central cylinder. From there it gradually regains the stem centre, temporarily again forming compound bundles with one or more other bundles, but this time it remains normally orientated, on the inside of the compound bundle, xylem towards the inside (above d in Fig. 3B). When the bundle has reached its innermost position it becomes a leaf trace and turns abruptly into a near-radial path, branching and "moving" out into a leaf (at e in Fig. 3B). The total distance from leaf-trace to leaf trace (a-e) has been called the "leaf-contact distance" by Zimmermann & Tomlinson (1965). The regularity of this arrangement lies in the fact that the inverted member of a compound bundle is always the lower part of the axial bundle, always on its "way" out (radially) towards the stem periphery, whereas the normally-orientated member of the compound bundle is always the upper part, on its "way" in (radially) towards the stem centre, nearer its level of becoming a leaf trace. These two contrasted regions are represented by a-d and d-e in Fig. 3B. In a single transverse section one can see that the normally-orientated part of a compound bundle has always more protoxylem than the inverted part, because, as in *Rhapis*, the number of protoxylem elements increases as one follows a bundle upwards. This is related to differences in time of development.

Compound bundles with three members are found in positions where a normally-orientated bundle acquires a second inverted bundle before it loses the first one. An example of this has been drawn into Fig. 3B at Y; an actual example is shown in Plate 1B.

As in *Rhapis*, we can distinguish between major, intermediate and minor bundles. The bundle path is either more centrally or more peripherally located, the former are comparable to the major, the latter to minor bundles in *Rhapis*. Unlike *Rhapis*, major bundles do not necessarily reach the periphery of the central cylinder, so that major bundles in *Pandanus* may not have the longest leaf-contact distance. In fact, this seems to be quite variable and reflects a developmentally less regular system than in *Rhapis*. Bundles outside the zone of compound bundles, at the very periphery of the central cylinder, run parallel to the axis for a long distance before penetrating towards the interior of the central cylinder. Developmentally this area is derived *Rhapis*-like from the outermost part of the meristematic cap, as we will show later.

In a monocotyledonous stem of the *Rhapis* type, all vascular bundles run more or less parallel to each other with the exception of departing leaf traces. This is relatively simple to draw on a two-dimensional diagram. However, in plants of the family Pandanaceae, there are, in addition to the more or less parallel distal-and-inward bundle paths (d-e in Fig. 3B) as in *Rhapis*, the distal-and-outward paths in the lower part of axial bundles (a-d in Fig. 3B). This makes two-dimensional representation difficult. With some imagination the reader may draw a more complete picture of the *Rhapis* stem simply by inserting a major and minor leaf trace continuous into every leaf. We have done this in an earlier paper (Zimmermann & Tomlinson, 1972; Fig. 1). This could, of course, also be done for *Pandanus*, but the result would be a very much more confusing picture. This is why, in Fig. 3B, only short lengths of the bundles associated with the axial strand are shown.

A further complication is the varying helical path of bundles along the stem. The bundles never lie neatly in a radial plane as shown in Fig. 3B, but describe different helical paths in the peripheral, subperipheral and central regions.

Our most detailed information was obtained from a series of 1400 sections, spaced at 80 μm, of a 4 cm thick piece of stem of *Pandanus utilis*. Leaf traces (LT) in this series depart invariably from a compound bundle, leaving the axial bundle branch (AB) and the associated inverted bundle (IB) behind (Fig. 3C). Also in *Pandanus utilis* we have found occasional splitting leaf traces. In one instance the leaf trace was seen to split and send two traces out to the leaf immediately above the axial bundle branch. In another instance the leaf trace part of a triple bundle split, each part retaining one of the inverted bundles. This type of behaviour further demonstrates that *Pandanus* is much more variable than *Rhapis*.

THE PATTERN OF DEVELOPMENT OF THE VASCULAR SYSTEM

The crown of two species has been investigated using serial paraffin sections. One series consisted of 700 sections. (10 μm thick) of the crown of *Pandanus utilis*, mounted on 5 x 7.5 cm slides, each slide containing two consecutive sections from the ribbon. The other included 330 sections (10 μm thick) through the meristematic crown of *Freycinetia banksii*. Since the vascular patterns in the two crowns are similar, this discussion will be restricted to *Freycinetia*, which was studied somewhat more thoroughly. Examples of the type of preparation used are shown in Plate 3, from sections at successively lower levels. In this type of analysis bundle continuity was sought, not individual sections of high quality.

The course of the strands in each of the two crowns was plotted in a way which is now standard for us. The procedure always begins with the measurement of the outline of the crown region in a radial plane, and the establishment of the scales of representation on graph paper (slide numbers as ordinate and ocular scale units as abscissa, both later to be converted to millimetres). In previous investigations major strands could always be plotted on a single sheet of graph paper because the *Rhapis* type of developmental pattern is relatively simple (Zimmermann & Tomlinson, 1967, 1968, 1969).

The zig-zag path of the lower parts of bundles in the Pandanaceae would make representation of too many strands confusing on a single sheet, the strands were therefore plotted on separate individual sheets for the sake of clarity. Some typical examples are shown in Fig. 4. As before, all positions within the stem are shown in a single radial plane.

The general topography of the *Freycinetia* crown is similar to those of previously investigated monocotyledons and is shown in Fig. 4 (cf. also Plates 2C,D and 3). Axial bundles, running more or less parallel to the stem axis in the mature region in the crown, turn about 90° towards the apical meristem and there assume a near-radial path. As one follows an axial bundle in a distal direction it is less and less differentiated until, as a procambial strand, it loses its identity and fuses into the meristematic cap (marked in Fig. 4 with an arrow and the letter C). Leaf traces, on the other hand, running more or less radially from the stem interior toward the stem periphery and so into leaves are orientated more or less axially in the crown region. This reflects the way in which the younger leaves are arranged as a concentric series on the crown (Plate 2D). Primary stem growth, when complete, therefore involves a 90° reorientation of all stem tissue.

The most interesting question to be answered is whether Pandanaceae have a mode of vascular development different from that of other monocotyledons, or if their developmental pattern is only a modification of the *Rhapis* principle. This is important since we regard the *Rhapis* principle as the fundamental pattern of monocotyledonous vascular development. In *Freycinetia* there is in fact uncommitted growth of axial bundles from a "meristematic cap", as in *Rhapis*. The bundle becomes committed as a leaf trace when it differentiates,

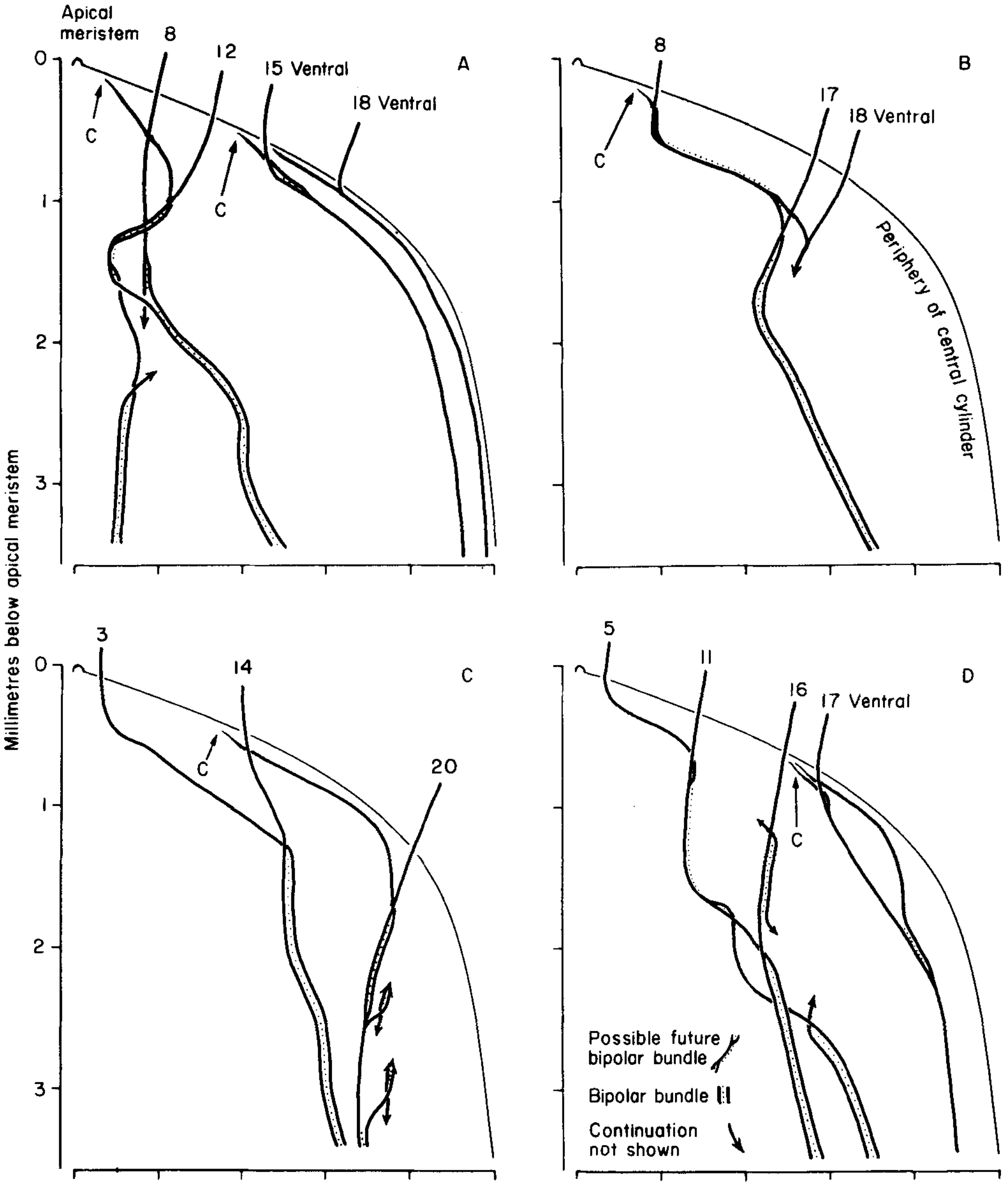

Figure 4. Plot of course of developing vascular bundles, represented in one plane, obtained from serial transverse sections by the methods described in the text. Because of the complexity of the pathway, four diagrams are used to illustrate bundles at different stages of development. Different bundles to the same leaf (e.g. P_8 in A and B) may be at different stages of development. Late-developing bundles, towards the ventral side of the leaf, are shown to P_{15}, P_{18} and P_{17} in A, B and D, labelled "ventral". The ends of procambial strands ending in the cap are marked with an arrow and the letter c.

or we might say is "captured" by newly arising leaf strands. This is not surprising because, as has been shown in the diagram of the mature stem (Fig. 3B), the course of the upper part of the axial bundle is like the course of bundles in *Rhapis*. Figure 4 makes it clear that this portion of the axial bundle in *Freycinetia* develops from a cap as in *Rhapis*. Several uncommitted axial bundles (arrows pointing from c in Fig. 4) are shown in the cap region.

The extent of the meristematic cap can be determined in two ways. As one follows, in a distal direction, a bundle at the very periphery of the central cylinder, a point is reached where the procambial strand loses its identity. This is the outer edge of the cap, in our example approximately below leaf primordium 16, as shown in Fig. 4D.

Another measure of the extent of the cap is to examine the position, during leaf development, when the full complement of vascular traces has been acquired by the leaf. In order to do this, we simply count the number of recognizable traces in all leaf primordia at the point of their insertion in the stem, and plot their numbers against leaf position. This is shown in Fig. 5. The number of leaf traces increases very rapidly from P1 to P4, which has about 20 (cf. Plate 2D); further traces are then initiated only gradually until P18, when the full complement of about 35 is present.

In many other monocotyledons we have found that the leaf base continues to initiate traces even outside the cap; these, unable to make vascular contact with the central cylinder, give rise to the outer cortical system, whose bundles are usually fibrous. We have found no outer system in the Pandanaceae, or at least only a very rudimentary one where there are very short bundles, discontinuous with each other, but usually connecting to older leaves.

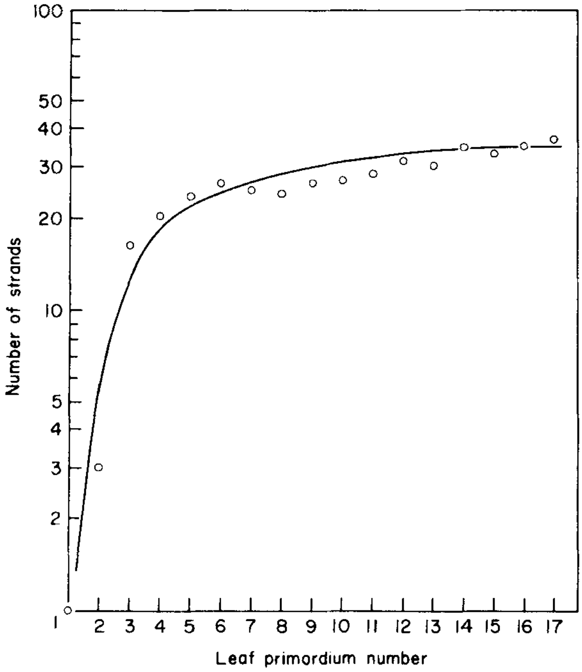

Figure 5. *Freycinetia banksii*. Plot of total number of vascular bundles at each leaf insertion (on a logarithmic scale) against leaf number, in the developing crown.

With the developmental pattern of the upper part of each vascular strand being comparable to that in *Rhapis, Prionium* and *Dracaena* described in published accounts as well as in other monocotyledons (unpublished), the really interesting question in *Pandanus* is then how and where the axial bundle branches off the leaf trace and how the bi- and multipolar bundles arise. One point is clear from the investigation of the course of bundles in mature stems: an axial bundle branching off a minor leaf trace near the periphery of the central cylinder never assumes an inverted position; it follows a path and orientation exactly as in *Rhapis.* However, if the axial bundle branches off a major leaf trace nearer the stem centre, its lower part assumes one or more times an inverted position, forming a bipolar bundle in association with an older bundle. Major (central) bundles do not necessarily show the most numerous inversions, because they often do not reach the periphery of the central cylinder (level a-d in Fig. 3B) before assuming the "*Rhapis*-type" path (level d-e in Fig. 3B).

It is evident that only the upper, normally-orientated bundle part, represented by level d-e in Fig. 3B, develops from the cap. The lower, inverted part (represented by level a-d in Fig. 3B), which forms in association with the normally-orientated bundles, develops much later, below the cap region. As progressively younger bipolar bundles were investigated it became clear that their middle part develops last. The sequence of events seems to be as follows. A branch from a leaf trace may form a procambial bridge to a neighbouring bundle, which itself may or may not already be continuous into a leaf as a leaf trace further up. This upper leaf-trace part may in turn itself form such a bridge, or send a strand into the cap. When this happens the two new branches seek to join each other, the upper one in the basipetal, the lower one in the acropetal direction. This late connection takes place along the phloem side, opposite the xylem, and gives rise to the inverted part of a bipolar bundle. Three such future connections are shown in Fig. 4, below leaf trace 8 in Fig. 4B, leaf trace 11 in Fig. 4D and leaf trace 12 in Fig. 4A. The connection to leaf trace 12 is almost complete and had nearly differentiated throughout as a bipolar bundle. In an early stage of development, where the central part of the inverted bundle is not yet continuous (e.g. between leaf trace 8 and 17 in Fig. 4B), it is difficult to recognize whether the branch from the leaf trace will become a bridge, or an inverted bundle. We believe that an inverted bundle develops only when an upper and a lower branch appear simultaneously in a position favourable for connection. We consider axial bundle branches, bridges and diverging inverted bundles to be homologous, because in an early stage of development they are indistinguishable. A procambial strand branching from a leaf trace initially fuses with a neighbouring axial bundle; along this strand a separate strand then becomes gradually differentiated until, in the mature stem, the two form a bipolar bundle, the two units fusing back-to-back within their common fibrous sheath. Stages in the development of the bipolar bundle, as seen in transverse section are shown diagrammatically in Fig. 6. In early stages of vascular development there is phloem continuity; subsequently, by obliteration of protophloem and its replacement by fibres together with the differentiation of metaphloem, the separation of bundles takes place.

From these findings we can make a few generalized statements. The normally-orientated part of a bipolar bundle is always developmentally earlier than the inverted part: it always develops from the cap. The inverted part of a bipolar bundle is developmentally later than the normally-orientated part of a bipolar bundle: it always develops below the cap along the already existing normally-orientated strand. This is quite evident from the plots in Fig. 4.

In summary then, the pattern of vascular differentiation is one step more complicated than that of *Rhapis*. Axial strands originating as branches from leaf traces *may* differentiate entirely from the cap *Rhapis*-like if they arise from minor bundles at the cap periphery. In *Rhapis*, the zone where axial bundles, bridges and satellites arise from leaf traces is quite sharply confined to the narrow area along the cap periphery, which, in effect, regenerates the cap and produces the *Rhapis*-type vascular pattern. The same situation exists in the Pandanaceae, but in addition bridges can arise at lower points along leaf traces as far as 1.5 mm below the cap in the case of *Freycinetia* cf. Fig. 4. Such late-developing bridges may connect with bridges or axial-bundle branches higher up as an inverted bundle along the existing normally-orientated one. Compared with *Rhapis* the vascular system of Pandanaceae therefore takes place in a less restricted volume and consequently produces a more complex three-dimensional vascular pattern.

DISCUSSION

Systematic conclusions

The structural analyses reported in this article establish two points about the systematic position of the Pandanaceae. First, the presence of the same essential features of construction as are found in other groups of arborescent monocotyledons (e.g. Palmae, certain Agavaceae) suggests that the Pandanaceae are closer to these groups than is traditionally accorded in systematic arrangements of monocotyledons. Second, the unusual "*Pandanus*-principle" confirms the relatively isolated position of the family in the monocotyledons. This theme of interrelationships has to be developed further by study of other woody monocotyledons, and provides a strong incentive for this study.

Developmental conclusions

Analysis of the pattern of vascular development has shown that the vascular system of the Pandanaceae is similar to that of *Rhapis*, but one step more complicated. The upper part of the vascular bundles is orientated normally in the stem, with the xylem on the inside and phloem on the outside. This part develops as in *Rhapis* from a meristematic cap. The inverted bundles which form bi- and multipolar bundles with normally-orientated ones develop later, below the cap. This is possible because in the Pandanaceae bridge-formation is not restricted to a narrow zone peripheral to the cap. When a bridge forms somewhere below the cap from, say, a leaf trace of P_{18} to a trace to P_8 (as shown in Fig. 4B), and at the same time the trace 8 itself produces an axial bundle branch or a bridge higher up, the two may connect in the form of an inverted bundle along the normally-orientated bundle which is, in its upper part, a leaf trace to P_8. We have therefore here a situation where a single procambial strand matures into a double vascular bundle. Functionally this means that there is an initial phloem connection between leaf trace 18 and bundle 8 (a simple bridge), but during differentiation into mature tissue this initial vascular connection is severed and the two strands become independent, as shown in Fig. 6. This interpretation is supported by the observation of protophloem remnants in the fibrous sheaths between the two units of a bipolar bundle. In *Sararanga*, phloem continuity may still be visible in bipolar bundles of the mature seedling axis (Plate 1C).

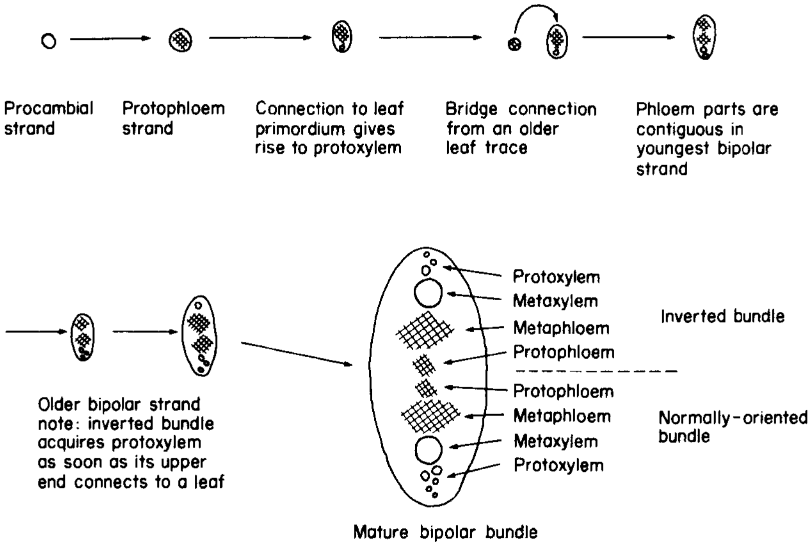

Figure 6. Diagrammatic representation of the development of a bipolar bundle.

The differentiation of two distinct vascular bundles out of a single procambial strand does not seem to be unusual. It has been found in the leaves of *Cladium* (Cyperaceae; Fisher, 1971) and leaves of *Gynerium* (Gramineae; Tomlinson, unpublished). We strongly suspect that a similar "basipetal extension of the *Rhapis* system" takes place in other monocotyledonous stems, although the end product is not multiple bundles, but groups. Distinct bundle groups, associated with leaf traces (but not to be confused with satellite bundles) have been observed in certain large species of palms and in members of the Strelitziaceae and Araceae. The developmental patterns of these plants are under investigation and we shall report on them in due time. The scope and nature of this work is such that we are approaching a systematic appreciation of major groups of monocotyledons.

SUMMARY

Growth habit and shoot construction is outlined in the three genera of Pandanaceae, *Freycinetia*, *Pandanus* and *Sararanga*. Stem anatomy is essentially the same in all three and reflects the naturalness of the family. The most distinctive diagnostic feature is the presence of compound (bi- and tripolar) bundles, i.e. vascular bundles with two or three discrete vascular strands restricted to certain regions of the stem. The phloem parts of the separate

strands of a compound bundle are near each other so that only one strand has a normal orientation. This feature distinguishes Pandanaceae from most other monocotyledons.

Analysis of stem vasculature by cinematographic methods showed that the principle of construction conforms to the one first described accurately for the palm *Rhapis* and later for other arborescent monocotyledons. For each strand there is a continuing axial bundle which splits from a diverging leaf trace, together with longer or shorter bridges which connect leaf traces upwardly with adjacent axial bundles. Traced in an upward (acropetal) direction the axial bundle repeatedly joins, in an inverted position, other strands to form bi- or multipolar bundles, until it becomes normally orientated, regains the stem centre, and becomes a leaf trace. The normally-orientated part of a compound bundle has more abundant protoxylem than the inverted part, it is developmentally older, and is nearer the level of its divergence into a leaf.

Normally-orientated bundles differentiate *Rhapis*-like from a cap. Inverted bundles differentiate later, below the cap, as an upper and a lower bridge connect along an existing strand. The phloem of the two parts of a bipolar bundle is originally contiguous but becomes separated during later growth by obliteration of protophloem. Sometimes phloem continuity is maintained even in mature bundles, as in seedlings of *Sararanga.*

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APPENDIX

Stem material of Pandanaceae examined during the course of this investigation

(Only material with voucher specimens or for which a reasonably reliable name is available has been cited. This list therefore excludes several collections of *Freycinetia* and a larger number of *Pandanus* species of which stem material has been examined but for which no specific identification has been possible).

Freycinetia.

F. arborea Gaud. Poamoho Trail, Oahu, Hawaii. P. B. Tomlinson. 27.VIII.61

F. banksii A. Cunn. Waitakere Hills, Auckland, New Zealand. P. B. Tomlinson. 26.II.69.

F. excelsa F. Muell. Cairns, Queensland, Australia. V. I. Cheadle. CA 216 & 230.

F. mariannensis Merrill Guam. B. C. Stone. 4217.

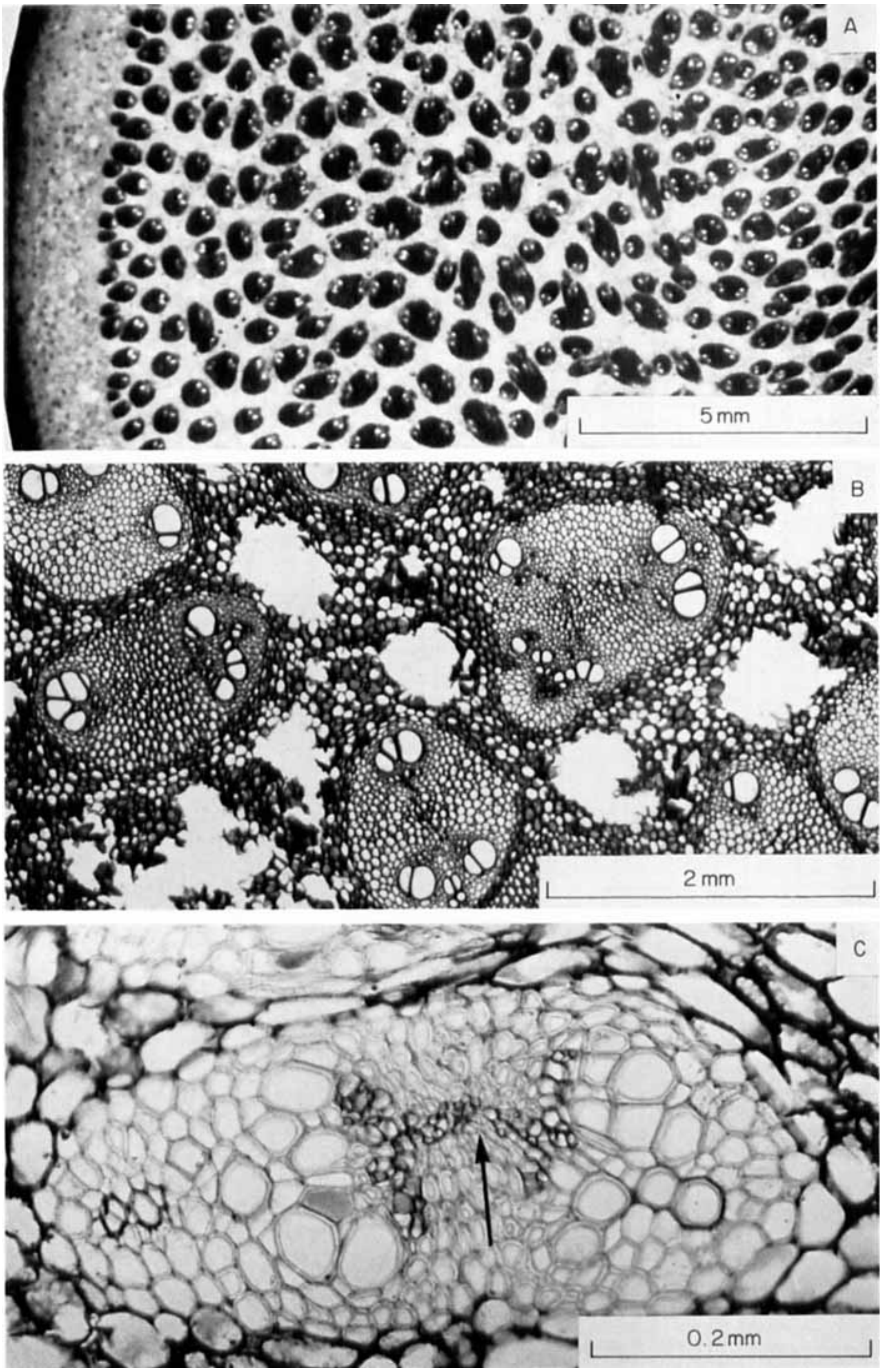
F. cumingiana Gaud. Cultivated Fairchild Tropical Garden.

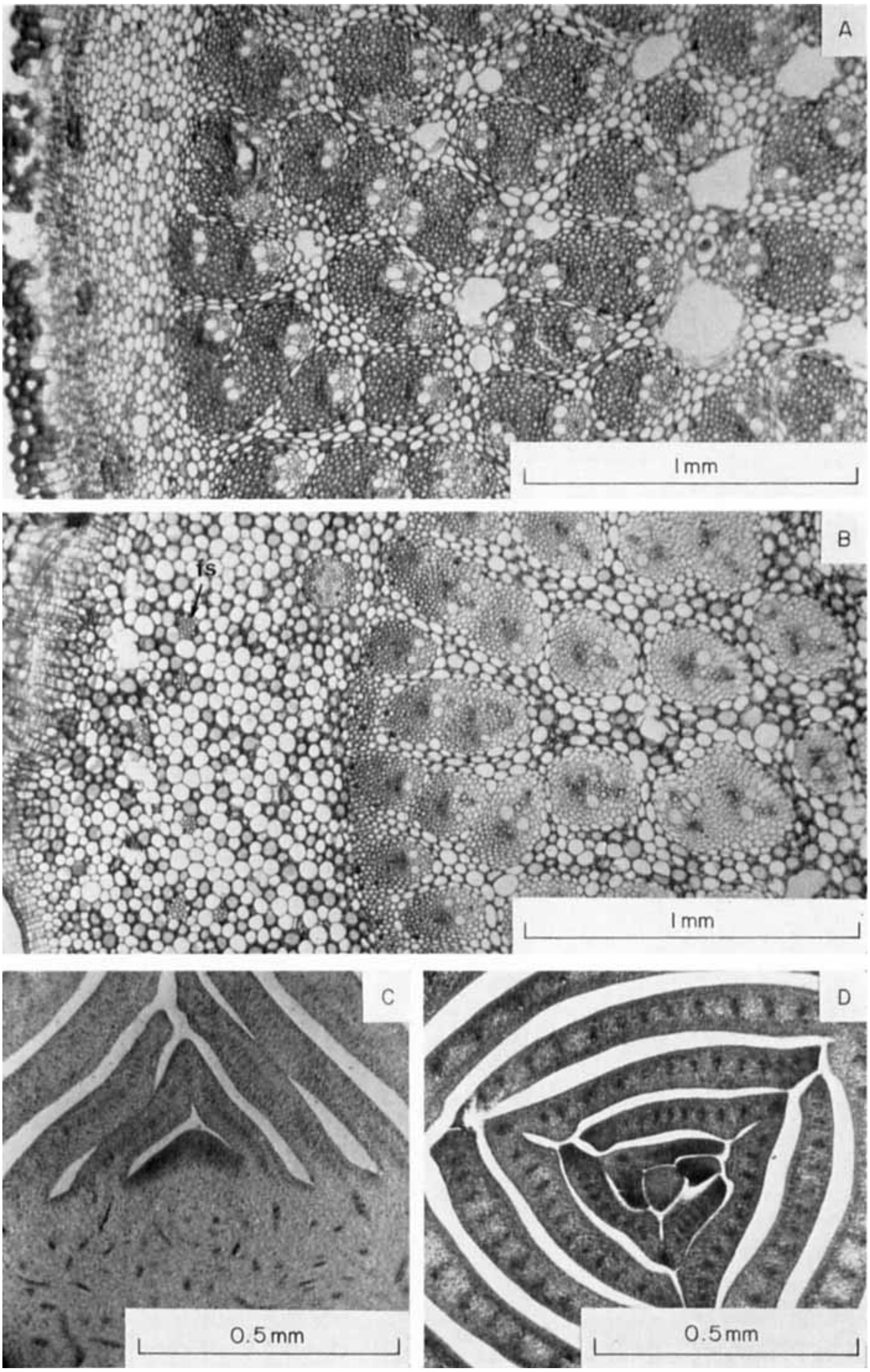
Pandanus.

P. attenuatus St. John B. C. Stone. 6426.

P. aurantiacus Ridl. B. C. Stone. 6130.

Plate 1

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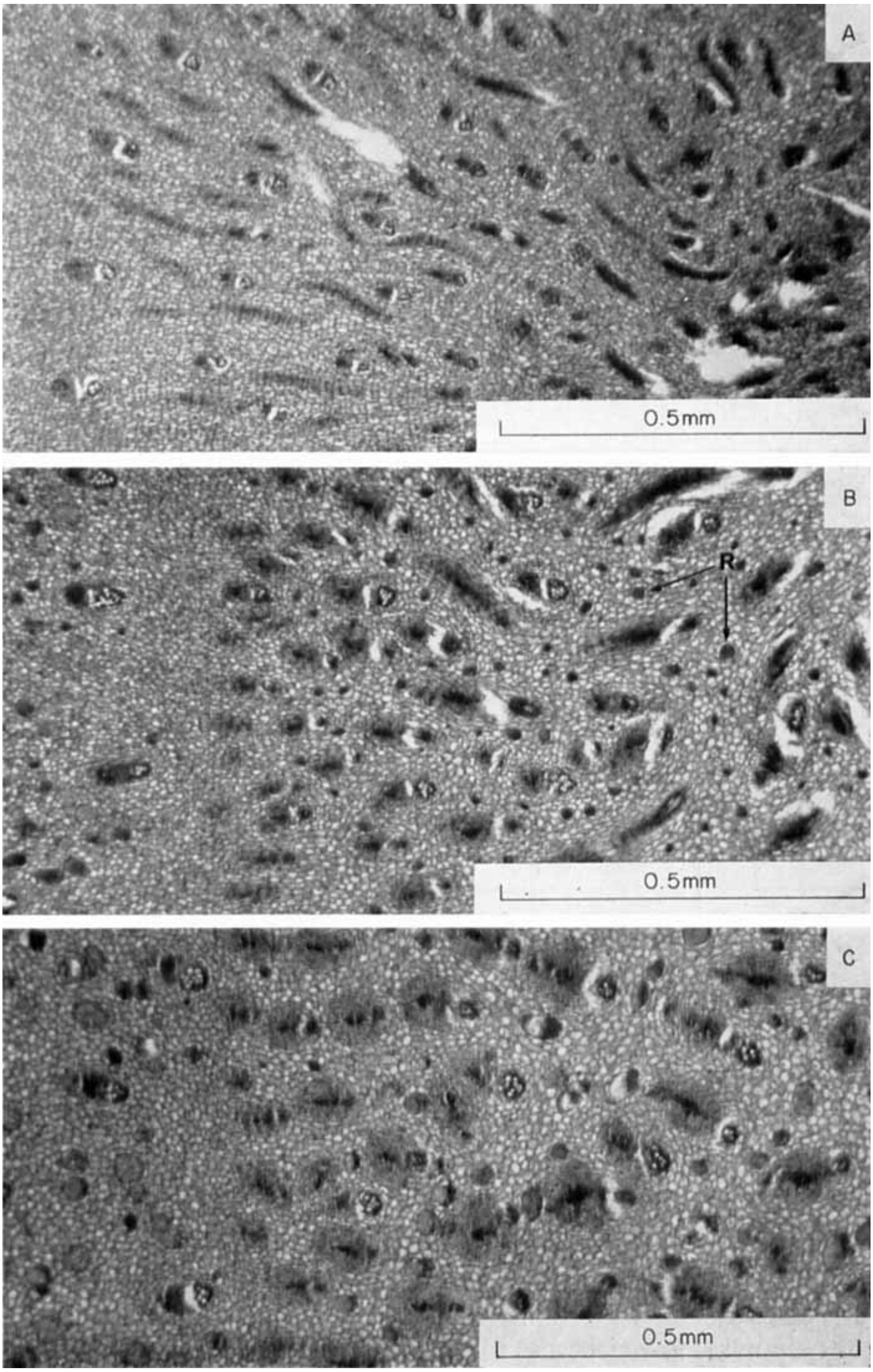

M. H. ZIMMERMANN

P. candelabrum Beauv. Ako Fokyir, Cape Coast. Ghana. J. B. Hall. s.n.
P. utilis L. Cultivated Fairchild Tropical Garden
P. yvanii Solms-Laub. Kassim s.n. (Supplied by Dr B. C. Stone.)

Sararanga.

S. sinuosa Hemsl. Tulagi Island. British Solomon Islands. G. F. C. Dennis 340.
S. sinuosa Tenaru, Guadalcanal, British Solomon Islands. G. F. C. Dennis s.n.

EXPLANATION OF PLATES

PLATE 1

A. ***Pandanus utilis.*** Peripheral sector from transverse section of a distal branch. This represents the type of preparation used in the analysis of the vascular system of the mature parts of this species, using the low-magnification shuttle.

B. ***Sararanga sinuosa.*** Transverse section of part of mature stem of adult plant, including one tripolar bundle and some bipolar bundles. Some indication of the obliterated protophloem can be seen in the centre of the compound bundles. Air-lacunae in the ground parenchyma.

C. ***Sararanga sinuosa.*** Transverse section of a single bipolar vascular bundle from a seedling, with protophloem of the individual vascular bundles evidently contiguous (arrow). Normally-orientated vascular bundle with more protoxylem on the left, inverted vascular bundle on the right.

PLATE 2

A. ***Pandanus attenuatus.*** Peripheral sector from transverse section of a lateral branch 6 mm in diameter.

B. ***Freycinetia banksii.*** Peripheral section from transverse section of a narrow distal branch 9 mm in diameter. Cortical fibrous strands (fs) are common.

C. ***Freycinetia banksii.*** Approximately median longitudinal section of shoot apex at centre of broadly conical crown.

D. ***Freycinetia banksii.*** Transverse section of crown at level of shoot apex.

PLATE 3

Freycinetia banksii. Peripheral sector from transverse sections of crown at the following levels below the shoot apex: A, 0.5 mm; B, 1 mm; C, 2 mm. Level A is just below the meristematic cap. The cortex is to the left in each photograph. Raphide-sacs are numerous in B and C (e.g. at R).

The tearing within vascular bundles is an artefact which is almost universal in this type of preparation, involving a large mass of meristematic tissue.