
JOURNAL

OF THE

ARNOLD ARBORETUM

VOL. XXXVI

JANUARY 1955

NUMBER 1

A FOURTH TYPE OF NODAL ANATOMY IN DICOTYLEDONS, ILLUSTRATED BY CLERODENDRON TRICHOTOMUM THUNB.

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With one plate and seventeen figures

INTRODUCTION

IN 1914, Sinnott demonstrated by an analysis of 34 orders and 164 families that there are three significant forms of foliar nodal anatomy in dicotyledons, viz. (1) the *unilacunar* form in which the vascular supply of the leaf is related to a single gap in the stele, (2) the *trilacunar* form in which the vascular strands are related to three distinct and more or less widely separated gaps, and (3) the *multilacunar* form in which five or more vascular strands are related to as many independent gaps. Although various dicotyledonous families are prevailingly unilacunar, trilacunar or multilacunar — thus providing significant evidence in the identification and classification of plants — there is a considerable number of families and many orders in which transitions between the different forms of nodal anatomy occur. Sinnott concluded that the trilacunar condition is primitive in angiosperms, the multilacunar form (much modified in monocotyledons) having arisen by amplification of the number of independently attached lateral strands, and the unilacunar form (a) in certain families by reduction and elimination of the two lateral strands and (b) in other families by the approximation of the median and lateral strands forming an aggregation of three strands that is related to a single gap in the stele.

Using this conclusion as a basis, Sinnott and Bailey (1914, 1915) attempted to demonstrate by statistical correlations that the primitive angiospermic leaf was simple and palmately veined. Subsequently, Eames (1931) adopted the concept of a primitive trilacunar condition as a working hypothesis in the study of floral morphology. Thus, nodal anatomy became part of the foundation for considerable phylogenetic investigation.

Recently, studies of the Austrobaileyaceae (Bailey and Swamy, 1949),

* This investigation was supported in part by a fellowship from the English-Speaking Union, and by the Fanny Bullock Workman Fellowship from Radcliffe College. Part of this paper represents a portion of a thesis submitted for the Ph.D. degree in Radcliffe College to the Department of Biology, Harvard University.

Monimiaceae (Money, Bailey and Swamy, 1950) and Chloranthaceae (Swamy and Bailey, 1950; Swamy, 1953a) have revealed patterns of foliar vasculature which necessitate a comprehensive reassessment of the significance of nodal anatomy in the phylogeny of the angiosperms.

The leaves of the majority of dicotyledons, as indicated by Sinnott, are vascularized by what appears at the nodal level to be an odd number (1, 3, 5, 7, etc.) of discrete vascular strands. On the contrary, the leaves of *Austrobaileya*, *Trimenia*, and *Ascarina* are vascularized by two independent strands that are related to a single gap at the nodal level. Since similar structures occur in various gymnosperms and in the cotyledonary node of many dicotyledons, it is essential to determine whether the foliar unilacunar nodes of dicotyledons have been derived in all cases from a trilacunar one.

Most of the work in the past has dealt with comparative investigations of fully matured structures at nodal levels. Such comparative studies of end-products without comprehensive developmental investigations at successive levels of the shoot and leaf, may be misleading. What is needed in the study of the evolution of various plant organs, as well as of the plants as a whole, is "a phylogeny of successively modified ontogenies." Therefore it is essential to obtain an accurate developmental picture of plants which have a double leaf trace at their unilacunar nodes. Being unable to obtain viable seeds of such ranalian genera as *Austrobaileya*, *Trimenia* and *Ascarina*, it seemed desirable to make a comprehensive study of the vascularization of *Clerodendron trichotomum* Thunb. of the Verbenaceae, which has at maturity a similar type of nodal anatomy.

MATERIAL AND METHODS

Adult plant: Abundant material was obtained from shrubs of *Clerodendron trichotomum* Thunb. in the Rare Plants section of the Arnold Arboretum. Weekly collections of buds, nodal material, and young and mature leaves were made from March 20 to about September 15, 1952, and after this, collections of buds were made irregularly until March, 1953.

The morphology of *C. trichotomum* has been described by Lam (1919). The material used for our studies, however, does not completely agree with his description, since the three-lobed leaves which he records have never been observed in adult specimens, but large-lobed juvenile leaves have been observed on two-year-old plants grown from seed. Specimens of the plants used in these investigations have been placed in the herbarium of the Arnold Arboretum.

The plants of *C. trichotomum* are shrubs 8 to 10 ft. in height, which branch profusely, particularly from the base of the main stem. The leaves are arranged in an opposite and decussate manner; they are simple, lanceolate to ovate, slightly serrate and have reticulate venation. The lamina of adult leaves is approximately 8 cm. long, while that of the juvenile ones is approximately 12 cm. long. The petioles are frequently as long as the leaves.

In the axil of each leaf there is a lateral bud complex. This consists of one main bud with one pair of subsidiary buds, lateral to it, and usually one additional sub-lateral bud. From these aggregations of buds lateral branching often occurs near the base of the shoot, in one- and two-year-old plants.

The inflorescence is a many-flowered cymous panicle, and is trichotomous. Each of the lateral cymes arises in the axil of a foliage leaf. Reduced scale-like bracts subtend the higher divisions of the inflorescence; these are caducous.

Seedling: Originally, supposedly fresh seed of *C. trichotomum* was obtained through a commercial seed house. Some of this seed was planted in seed pans in the greenhouses of the Biological Laboratories, Harvard University. Other samples of the same seed were presoaked, scarified, or given cold treatment at 5 degrees C. in moist peat for 6–8 weeks to allow after ripening. In all cases germination occurred in less than 3% of the seeds planted.

The next fall ripe fruits were collected directly from the shrubs in the Arnold Arboretum. The seeds were removed, immediately planted on the surface of soil, and covered by damp sphagnum moss. They were planted in mid-October and were kept in the greenhouses of the Arboretum. By mid-December approximately 10% of the seeds planted had germinated. Germination continued until by mid-January approximately 70% of the seeds had germinated.

There appears to be no description of the fruit, seed, or seedling of *C. trichotomum* in the literature, although the seed and seedling of *C. Kaempferi*, Fisch. has been described by Lubbock (1892). This description differs so markedly from *C. trichotomum* (probably because Lubbock failed to recognize that *C. Kaempferi* is hypogeal in germination) that the following description of *C. trichotomum* is given here.

The fruit is formed from a gynoeceium which consists typically of four carpels. There is a single loculus and each carpel usually bears two ovules; however, in the fruit there are never more than four seeds and frequently only two or three. Each seed has an extremely hard and sclerotic testa which is sculptured on one side, and smooth with a deep longitudinal groove on the other side. Through this groove the fleshy funicle attaches the seed to the placenta. On germination the radicle emerges through this longitudinal groove, which also marks the line of weakness along which the testa eventually splits. The seed is non-endospermic and the cotyledons are large and fleshy.

Germination is hypogeal (see PLATE I:3). Shortly after the radicle emerges through the longitudinal slit, the shoot appears above the surface of the ground with its tip bent over (PLATE I:3c, d). The epicotyl straightens, elongates and carries the first pair of leaves upwards (PLATE I:e, f). The fleshy cotyledons remain underground enclosed in the testa; after about three months they shrivel and become detached.

The hypocotyl is minute, 2–3 mm. long, 2 mm. thick, and glabrous. The epicotyl (i.e., between the point of attachment of the cotyledons and the

first pair of leaves) is 4.5–6 cm. long, pale green and pubescent. The cotyledons are stalked (petiole 3–5 mm. long, lamina 8–10 mm. long), white, fleshy, ovate, entire, without obvious venation; they are enclosed in the testa. The root is a tap root bearing many lateral branches. The first internode of the stem is 4.25–6 cm. long, the second 2–3 cm. long and subsequent internodes are 1 cm. long or less. The stem is cylindrical, bright green, pubescent. The leaves are simple, opposite and decussate, exstipulate and petiolate. The petiole is very long; the lamina and petiole are pubescent on both surfaces. Leaves at the first node are suborbicular, pale green, larger than the cotyledons with margins irregularly toothed towards the apex. At the second node leaves are rhomboidal to ovate and the margins are irregularly toothed. The third pair of leaves resembles the adult leaves which are ovate and irregularly toothed.

Histological techniques: Seedling, foliar and nodal material was fixed in formal acetic alcohol, medium chromo-acetic and strong chromo-acetic alcohol (Johansen, 1940). A modified Zirkle's n-butyl alcohol method for dehydrating refractory plant material was employed and subsequently the paraffin-tissuemat method described by Pratt and Wetmore (1951) was used for embedding the material. Hard tissues were presoaked in water before sectioning. Embedded material was sectioned on a Spencer rotary microtome at 8 to 10 micra. Egg albumen fixative was used for mounting the serial sections, and preparatory to staining the slides were coated with a 1/2% solution of parlodion in ether.

Staining combinations used for serial sections were as follows: — Heidenhain's iron-alum haematoxylin with safranin as a counterstain (Jeffrey, 1917); 1% safranin counterstained with picro-aniline blue (1 gm. water soluble aniline blue to 100 cc. of 80% ethyl alcohol with 1 cc. of saturated aqueous picric acid); 1% safranin and a saturated solution of cotton blue in lactophenol. In very young material where it was desired to emphasize meristematic tissues, as for example in seed and seedling sections, Foster's (1934) tannic acid and ferric chloride technique was employed.

Foliar material was cleared by use of a 5–8% NaOH solution (Bailey and Nast, 1943). In the case of seedling material, clearing methods involving the use of lactic acid (Debenham, 1939) have been found to be more satisfactory than the NaOH method. A basic fuchsin stain was used in some cases to accentuate the vascular pattern (De Tomasi, 1936; Kumar et al., 1942).

VASCULARIZATION OF SEED AND SEEDLING

Seed: Seed obtained directly from shrubs in October was examined by means of serial sections since the fleshy cotyledons are difficult to clear without damage to the delicate epicotyl and radicle. At the cotyledonary node each cotyledon possesses two distinct procambial¹ strands (FIG. 1:A). Four vascular strands differentiate behind the shoot apical meristem as it

¹The criteria used by Esau (1942, 1943a, and 1943b) for the identification of the procambium, first-formed xylem, and first-formed phloem have been followed in this investigation.

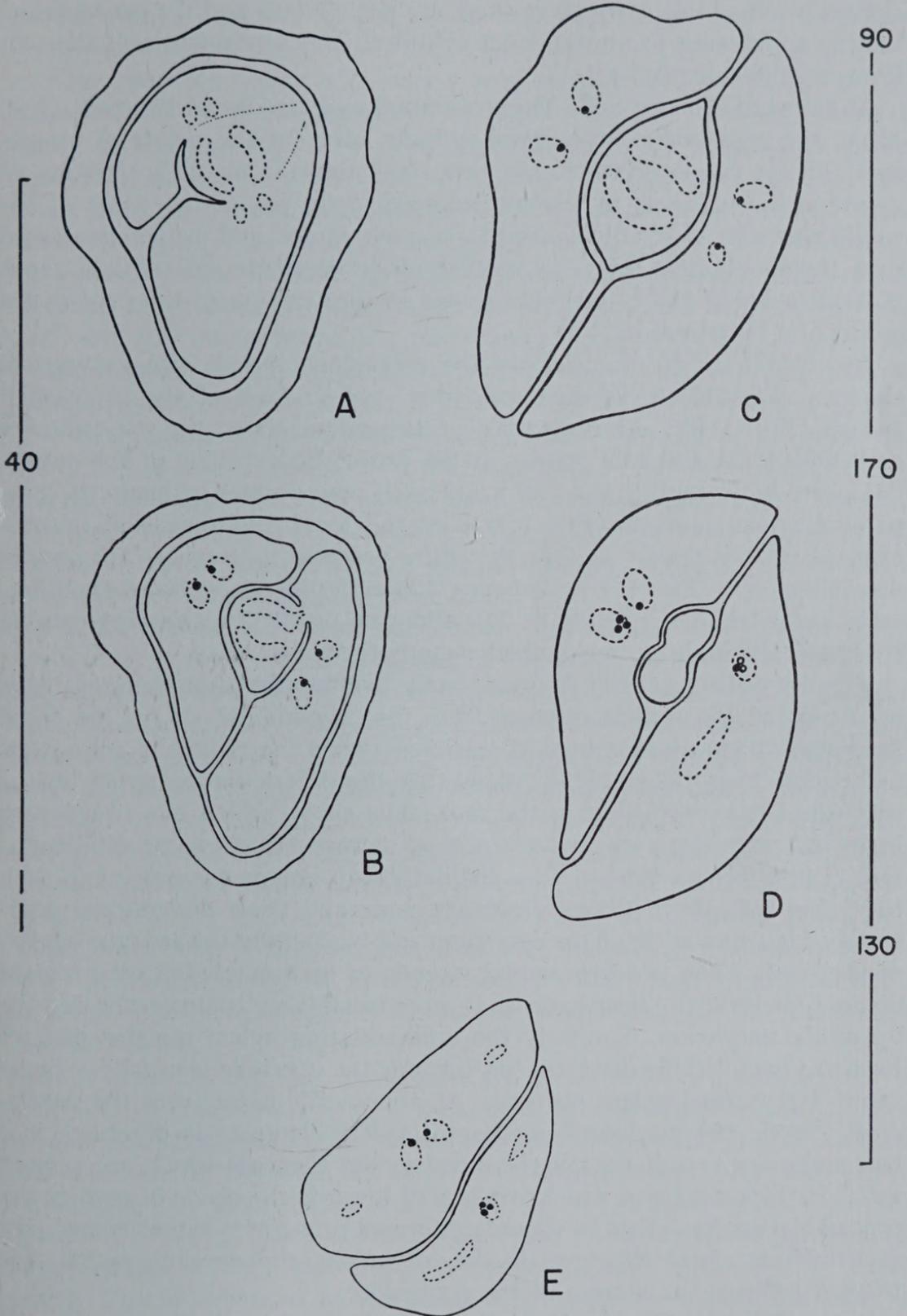


FIGURE 1

SERIES OF SECTIONS OF THE SEED OF *Clerodendron trichotomum*. Procambium is represented within the dotted lines, and first xylem elements as black dots. The distance in micra between sections is shown on the left and right.

elongates but at this early stage these are not obvious and the procambium has the appearance of an indistinct cylinder. The procambium of the root forms a solid central core.

At the cotyledonary node the procambial cylinder forms two arcs (FIG. 1:A); the gaps in the procambial cylinder occur at the points of attachment of the two cotyledons. At this stage there is no differentiation of xylem in the epicotyl, in the cotyledonary node nor in the bases of the cotyledons. In the radicle, which is much larger and better developed than the shoot, four spiral or annular xylem elements differentiate about 800 micra below the cotyledonary node in the position of the protoxylem points of a tetrach root.

Approximately 50 micra above the cotyledonary node two protoxylem elements differentiate in each cotyledon, one in each of the procambial groups (FIG. 1:B). At a distance of 100 micra above the cotyledonary node additional elements appear in the procambial strands of the cotyledons, and between 100 and 250 micra each may possess as many as three parallel xylem elements (FIG. 1:D). At this level, and above, lateral procambial strands depart so that the entire vascular pattern of each cotyledon is laid out (FIG. 1:E). Between 250 and 300 micra, however, differentiation is less, until finally at 350 micra no protoxylem elements can be observed, although the procambial strands remain distinct.

First seedling stage: Approximately two months after the seeds have been planted the radicle emerges from the longitudinal slit in the testa. Seedlings which are examined at this stage when the radicle is only a few millimeters long (PLATE I:3b) show that the differentiating xylem in the cotyledons links with that in the root. The shoot, which has grown very little, still possesses a distinct procambial pattern but shows no differentiation of first-formed xylem. Two distinct xylem groups are present in each cotyledon well above the cotyledonary node and these differentiate acropetally towards the tip of the cotyledon and basipetally towards the cotyledonary node. The two procambial strands in each cotyledon may remain distinct for its entire length, or, as is more usual, they coalesce towards the tip of the cotyledon. Similarly the differentiating xylem remains distinct for some considerable distance, but towards the tip there is usually a single spiral first-formed xylem element. At about 400 micra from the cotyledonary node the maximum number of xylem elements is observed, and here there are two distinct first-formed xylem elements which are usually spiral in thickening and which are located towards the inside of each of the procambial groups. Two or three late-formed primary xylem elements may also differentiate at this level in the cotyledons and development is centrifugal towards the margin of the cotyledon.

Later differentiation between the region of initial maturation in the cotyledons and the cotyledonary node is basipetal. Frequently the newly formed xylem nearer the cotyledonary node is scalariform and therefore probably "late formed primary xylem."² Spiral and annular xylem ele-

² It is well known that the term protoxylem is used to denote the first-formed xylem in plants, and that although this frequently exhibits spiral and annular thicken-

ments always drop out of the cotyledonary traces as they are followed downwards through the node.

The *transition region* of a very young seedling before epicotyl elongation exhibits much the same form as that in an older seedling where the shoot becomes vascularized. The procambium in the epicotyl is divided into four distinct strands (FIG. 2:1). At the cotyledonary node each trace from the cotyledons passes in towards a separate procambial strand from the epicotyl (FIG. 2:2) and joins with it (FIG. 2:2). Below the cotyledonary node the four partially differentiated vascular bundles remain distinct; if any protoxylem is present the arrangement is endarch. Between 300 and 700 micra below the cotyledonary node any spiral or annular xylem related to the cotyledonary traces drops out, and four distinct annular xylem elements appear in the position of the protoxylem poles of the root, i.e., in the gaps between the vascular bundles of the hypocotyl (FIG. 2:4, 5, 6). These protoxylem traces are in no way connected with the cotyledonary traces but extend upwards from the protoxylem points of the root protostele. They usually extend farther up in the gaps at right angles to the median axis of the cotyledons than in the gaps between the two traces of each cotyledon (FIG. 2:5).

At the cotyledonary node the phloem lies on the same radius as the meta- and protoxylem and remains in the same position throughout the hypocotyl and into the root. In the hypocotyl the internal annular elements along this radius drop out, leaving collateral bundles of phloem and scalariform xylem (FIG. 2:4-7). Passing downwards these bundles approach each other about the center of the axis until a solid xylem core is formed surrounded by the same four strands of phloem (FIG. 2:7, 8). Meanwhile new protoxylem strands have appeared along radii alternate with the collateral bundles (FIG. 3); these become the protoxylem poles of the tetrach root (FIG. 2:8). Hence the first-formed xylem of the shoot is not continuous with that of the root, but joins with metaxylem from the root at a level in the hypocotyl at which protoxylem strands from the root protoxylem poles have already been differentiated. This fact is illustrated in figures 2 and 3.

It is quite obvious that the transformation of vascular pattern from the true root to true stem condition described for *Clerodendron trichotomum* in no way corresponds to the "transition types" postulated by Van Tieghem (1891); nor has any twisting, rotation or inversion been observed such as is described by Eames and MacDaniels (1947), Lenoir (1920), or Van Tieghem (1891). These workers regard the vascular system of the seedling plant as a single unit which is morphologically equivalent in all its parts. The observations in this study seem rather to uphold the work of Dangeard (1889, 1913) in which he indicates that the seedling system is initially discontinuous. He considers that this system consists of a ra-

ings, this is not necessarily always the case. However, spiral and annular elements usually differentiate in tissues which are undergoing elongation, and scalariform and pitted elements in tissues which have ceased such growth (as recently indicated by the work of Smith and Kersten, 1942).

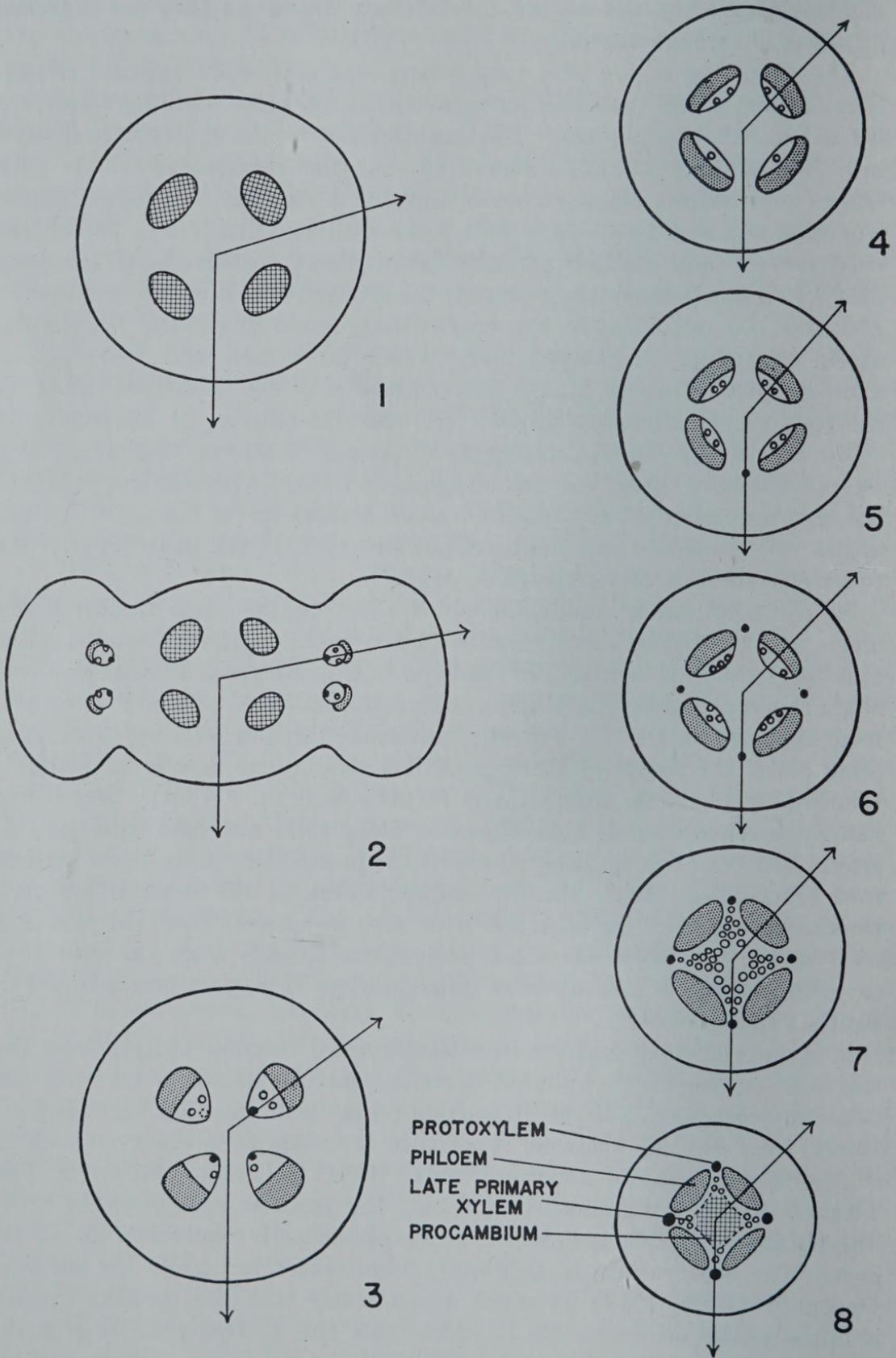


FIGURE 2

SERIES OF SECTIONS THROUGH THE TRANSITION REGION OF A SEEDLING OF *Clerodendron trichotomum*. The arrows indicate the direction of the diagram in figure 3.

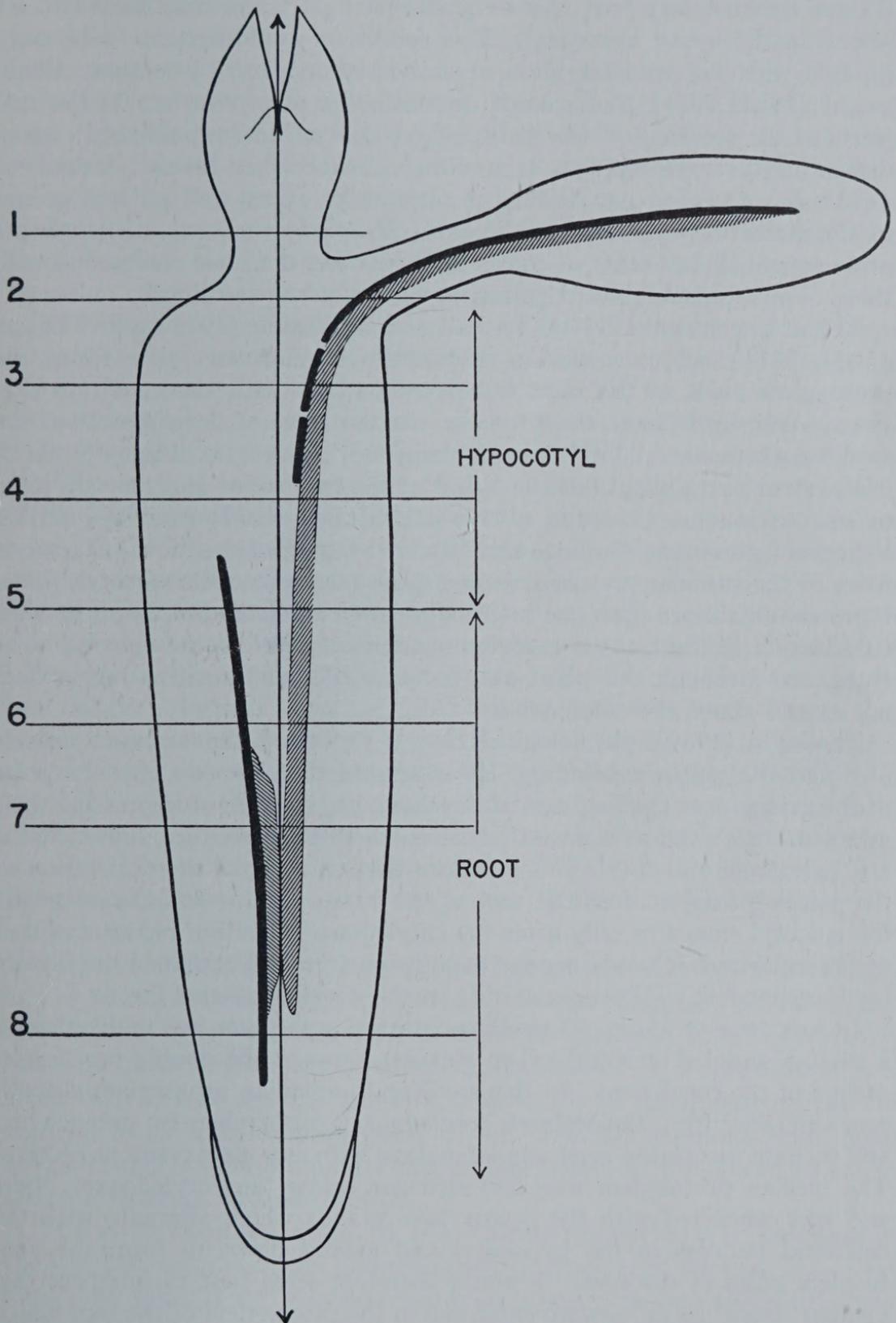


FIGURE 3

LONGITUDINAL SECTION OF THE SEEDLING OF *C. trichotomum* (DIAGRAMMATIC) SHOWING THE POSITION OF THE SECTIONS IN FIGURE 2. First xylem elements are shown in black, later formed xylem is striped. The direction of the diagram is shown on the transverse sections in figure 2.

dicular hypocotylary part and a cotyledonary part and that these two are joined in the upper hypocotyl. This condition would appear to be comparable with the vascular ontogeny observed in *C. trichotomum*. Chauveaud (1911, 1921) also records discontinuity of protoxylem in the majority of the seedlings which he investigated. Paxson (unpublished) found discontinuity of protoxylem in seedlings of *Cucurbita texana*. Lehmborg (1923-24) observed no twisting or rotation of xylem and phloem groups in the transition region of seedlings of *Helianthus annuus*, although the arrangement and number of traces in *Helianthus* does not correspond with those observed in this investigation on *Clerodendron* seedlings.

The arrangement observed by Hill and de Fraine (1913) and Thomas (1907, 1914), where a median protoxylem cotyledonary pole forms the protoxylem point in the root, is not comparable with the condition previously described for *C. trichotomum*. At the stage of development of the seedling where the epicotyl has not elongated, there is no differentiation of protoxylem in the shoot but the xylem of the cotyledons and root are more or less continuous. Compton (1912) and Muller (1937) examined certain hypogeal leguminous seedlings and observed that the transitional characteristics of the vascular system extended quite far up into the epicotyl, sometimes through more than one internode. In *C. trichotomum*, however, vascularization of the transition region is completed before the epicotyl elongates, and although this plant also is hypogeal, the transition region does not extend above the cotyledonary node.

Thoday's (1939) physiological theory explained the separate centers of differentiation in a seedling. He suggested that the two opposite poles of the axis, viz. of the root and of the shoot, are capable of impressing their own pattern on the meristematic tissues which they produce, and that the leaf primordia and cotyledons influence the structure of the upper part of the seedling axis, as does the root at the base. In *C. trichotomum* where the epicotyl elongates only after the cotyledons, transition region and root are vascularized, it would appear that the pattern is determined principally by (independent) influences arising in the cotyledons and the root.

In only one of about 30 seedlings studied was there any indication of a median annular or spiral xylem element between the double procambial groups of the cotyledons. In this seedling the median protoxylem element was separated from the endarch cotyledonary protoxylem by parenchyma and was not associated even at a later stage with any metaxylem or phloem. The median protoxylem was also stronger below the cotyledonary node, and was connected with the protoxylem strands which alternate with the collateral bundles in the hypocotyl and extend down to form the protoxylem poles of the root. It would therefore seem best to interpret this median "trace" as an upward extension of the protoxylem of the root which ordinarily terminates in the hypocotyl, and not as a true cotyledonary trace. It is possible that such a condition would arise when the root elongates slowly permitting the center of differentiation in the root to impress its pattern at a higher level in the hypocotyl than is ordinarily the case (see Thoday, 1939).

Vascularization of the seedling shoot: Serial sections and cleared specimens of seedlings which possess only one pair of leaf primordia exhibit two distinct procambial strands in each of the young leaves. In the epicotyl the procambium differentiates in four distinct bundles and shortly after these are formed the first phloem starts to differentiate. At the base of each of the leaf primordia a spiral or annular xylem element differentiates in each of the two procambial groups, although this may not occur simultaneously in the four procambial strands. Differentiation of these first-formed xylem elements then proceeds towards the leaf tip, and simultaneously, down the epicotyl towards the cotyledonary node.

By following serial transverse sections through the first pair of leaves and shoot apex, one observes that additional elements, spiral, annular and pitted, may extend approximately 600 micra above the level of the apical meristem (FIG. 4:A). At the base of each leaf primordium there are always two separate groups of elements (FIG. 4:C, D), even though the two may fuse and/or branch higher in the leaf. Above the point of departure of the leaf traces there are only two arcs of procambial tissue in the stem apex (FIG. 4:D). The gaps lie opposite the leaf primordia and each pair of leaf traces passes in through one gap in the stem procambium (FIG. 4:D). The two traces from each leaf join the procambial arcs of the stem, and then approach each other and become connected tangentially by procambium; so that a cylinder of procambium with four endarch strands of protoxylem is formed (FIG. 4:E). Below the node this opens to give four separate procambial strands (FIG. 4:F), down which the protoxylem is progressively differentiating towards the cotyledonary node. It should be pointed out here that the procambial pattern of the shoot appears to be laid down by continuous acropetal differentiation of epicotylary procambial bundles close behind the apical meristem. This is to be contrasted with the strongly discontinuous differentiation of the first-formed xylem described above.

Each procambial group in the epicotyl shows the differentiation of only one or two xylem elements. In cleared specimens it appears that the spiral and annular xylem in most cases is discontinuous, and in this very early stage the pitted xylem can also be discontinuous. As differentiation proceeds the vascular strands become continuous from the leaves through the epicotyl to the cotyledonary node. In all seedlings examined each foliar strand connects with a separate procambial strand of the epicotyl and becomes a separate vascular bundle.

The cotyledonary node in the young condition: In seedlings of the age just described, the differentiation of spiral and annular xylem between the epicotyl and cotyledonary node is not complete and continuous. It is not until the second pair of leaves become vascularized that the xylem of the epicotyl becomes connected with that in the cotyledonary node. No spiral or annular elements differentiate in this region probably since elongation of tissues in the cotyledonary near nodal region is complete, so that only xylem of the pitted type is formed. Moreover, the first scalariform

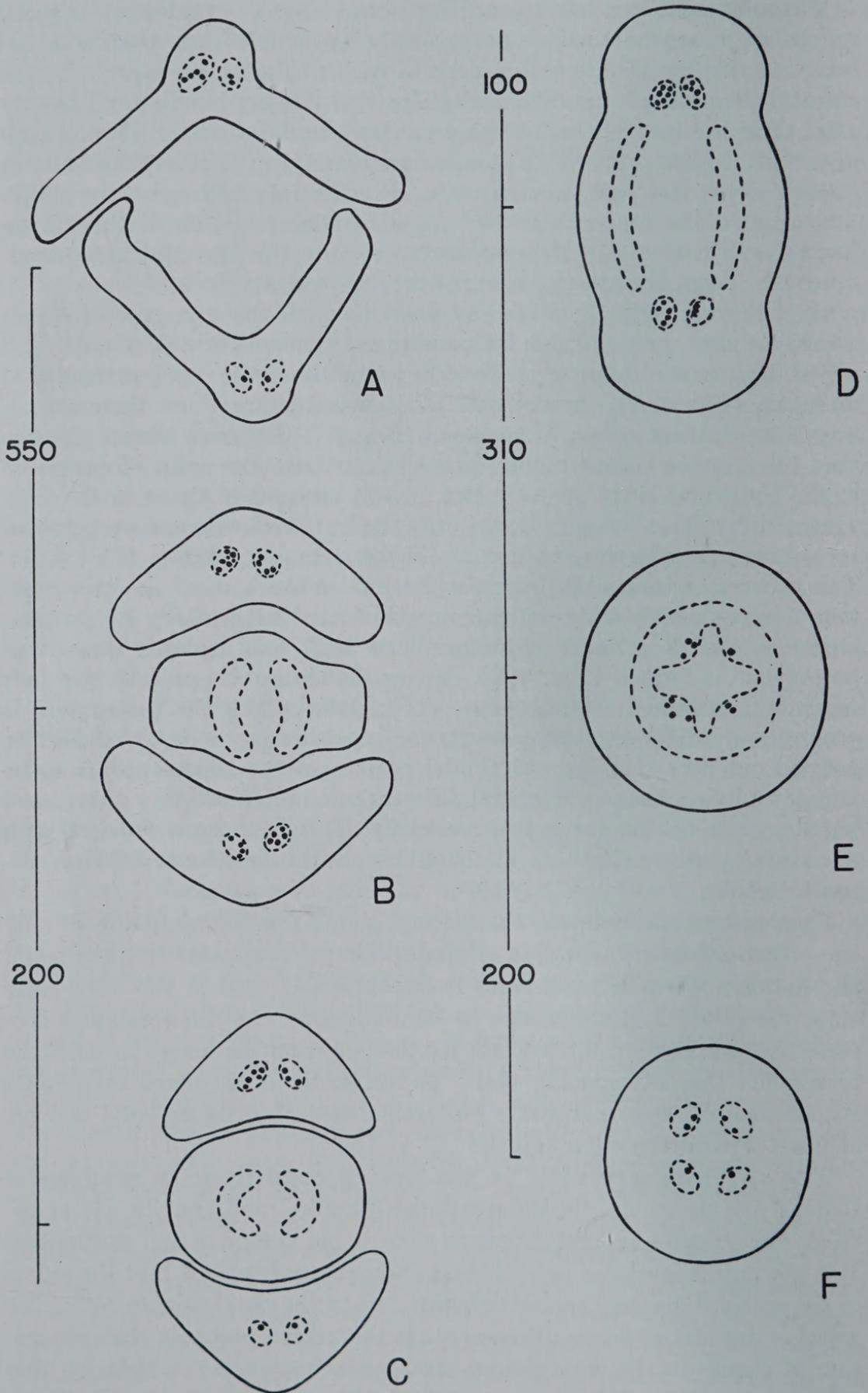


FIGURE 4

A SERIES OF SECTIONS THROUGH THE EPICOTYL OF A YOUNG SEEDLING OF *Clerodendron trichotomum*. Procambium is shown within the dotted lines. First formed xylem elements are represented as black dots. The distance in micra between sections is shown on the left.

xylem to differentiate at the base of the epicotyl becomes continuous with the late-formed primary xylem in the upper portion of the hypocotyl.

Leaf vascularization in older seedlings: In seedlings possessing two pairs of leaves on an epicotyl from 4.5–6 cm. long, the complete vascular connection between the epicotyl and root has occurred. As in the first pair of seedling leaves, the leaves of the second pair each possess two procambial strands. Near the level of the apical mound differentiation of vascular tissue occurs in the second leaf pair in a manner identical with that in the first pair of leaves. A few hundred micra above their bases, these leaves possess several spiral, annular and scalariform elements which may or may not be arranged in two distinct groups. But approximately 200 micra above the leaf base and from here downwards, the differentiating xylem groups are quite distinct. About 200 micra below the apical meristem the leaves attach to the stem and the two strands of each leaf, with 1–4 differentiated xylem elements, join with shoot tip procambium through one gap opposite the leaf base. A small bud procambial trace appears in the axil of each leaf primordium (FIG. 5:D).

At about 120 to 150 micra below the origin of the youngest leaf primordia the leaf trace procambium merges with the epicotyl procambium (FIG. 5:E, F) to form (approximately 100 micra below) a complete cylinder (FIG. 5:G). The protoxylem traces of the youngest pair of leaves now form the protoxylem points of the tip of the epicotyl, and each trace from each bundle of the leaf again forms a separate conducting system which never fuses nor runs together with the other strands for their entire course through the shoot.

Approximately 600 micra below this, the procambial cylinder opens and two procambial bud traces pass out to minute buds in the axil of the first pair of seedling leaves (i.e. the oldest pair of leaves). The shoot at this level now possesses two procambial arcs, each with two xylem groups (consisting of 3 or 4 elements from the youngest pair of leaves) which are continuing to develop acropetally towards the region of maximal area of the second node. 150 to 200 micra below this, the region of maximal area (of the first-formed node) is encountered and at this level two xylem traces of 5–8 elements enter the stele from each leaf base through a unilacunar gap. Now eight distinct xylem groups appear in the procambial cylinder of the shoot and these remain very obvious for about 600 to 800 micra below the region of maximal area of the first node. In cleared specimens the four traces from the youngest pair (second pair) of leaves do not appear to extend much below this point.

Secondary condition superimposed: The picture becomes extremely difficult to interpret in subsequent stages since seedlings possessing more than two pairs of leaves show initiation of cambial activity. Seedlings with only two pairs of leaves have been examined in which cambial activity has already occurred.

The eight xylem groups appearing in the procambial cylinder as described above eventually link with each other at the base of the epicotyl to form four strands just above the cotyledonary node. They do, how-

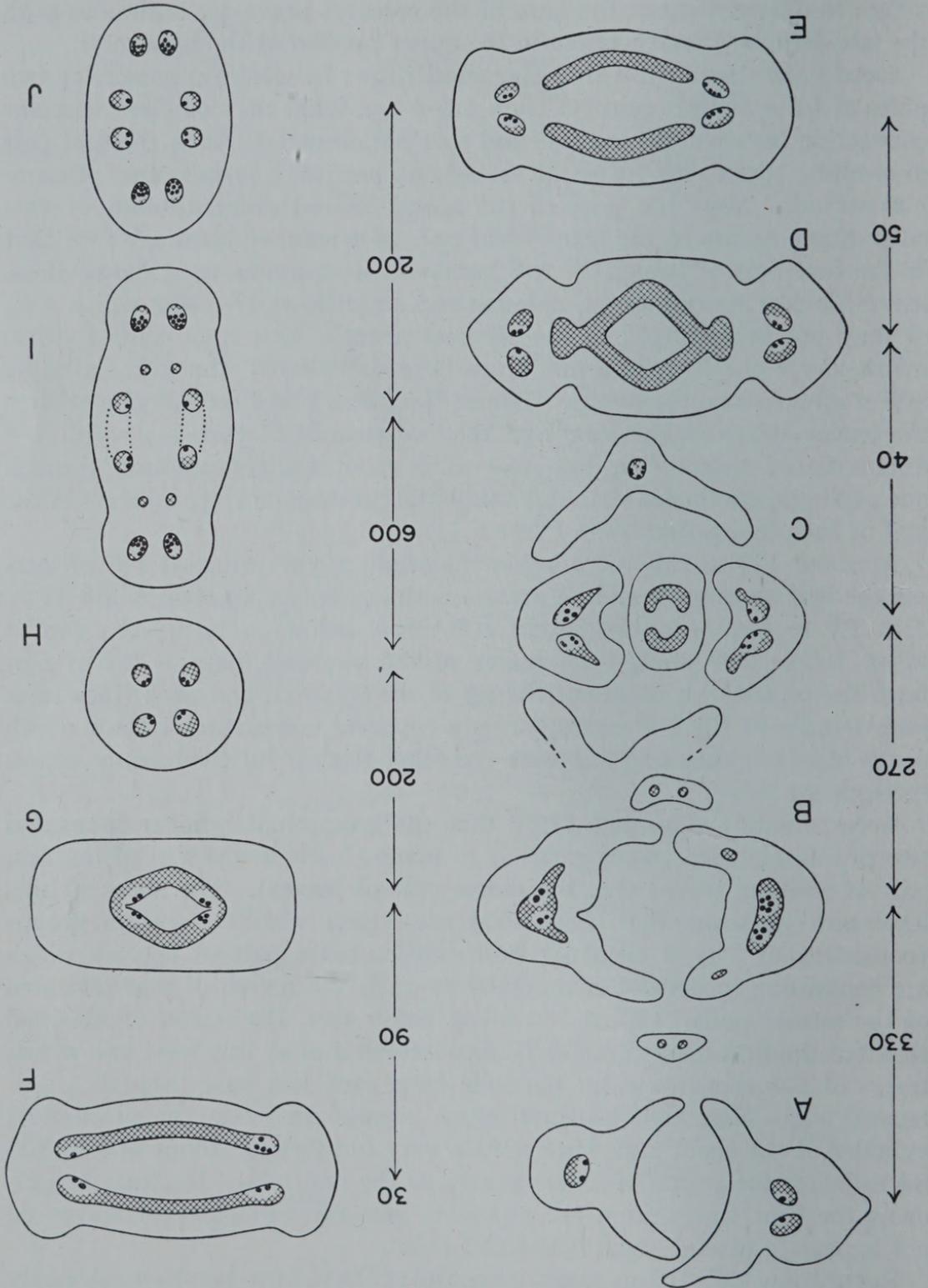


FIGURE 5

SERIES OF SECTIONS THROUGH THE SEEDLING SHOOT TIP WITH TWO PAIRS OF LEAF PRIMORDIA. (*C. trichotomum*). The procambium is shown cross-hatched. First xylem elements are represented in black. The distance in micra between sections is shown on the left.

ever, become connected as a result of secondary activity at a higher level in the epicotyl when a xylem cylinder is formed.

Vascular connection at the cotyledonary node: Careful study of cleared seedlings has shown that the acropetally differentiating pitted elements at the base of the epicotyl become continuous with the cotyledonary traces just below the point of attachment of the cotyledons. The differentiation of spiral and annular elements ceases about 1000 to 1500 micra above this region. However, the pitted elements continue in their acropetal differentiation and two or three parallel elements are apparent in each of the four stem strands. Towards the cotyledonary node the number of pitted elements is reduced so that only one or two elements of each strand become continuous with the pitted elements of the cotyledonary traces. The epicotylary traces join the cotyledonary traces on the inside. In many cases this junction with the cotyledonary traces may be coincident with the differentiation of further strands of pitted xylem centripetally in the lower hypocotyl to form the typical exarch root which possesses a solid core of pitted xylem.

Summary: The important points arising from this ontogenetic study of the seedlings of *C. trichotomum*, which should be emphasized at present, are as follows:

1. In the case of the cotyledons and the first and second pairs of seedling leaves there are two distinct procambial strands which differentiate two annular or spiral elements, and later, at least in the lower one third of these leaves, two xylem bundles.
2. In all these leaf types the two leaf strands are associated with a unilacunar gap in the stele in both the earlier and later stages of differentiation.
3. Each of the two traces from each of the pairs of leaves examined becomes associated with an entirely separate and independent portion of the procambium and later of the vascular eustele.
4. In all cases examined the first-formed xylem of the leaf primordia, the epicotyl, cotyledons and root are discontinuous and therefore neither the protoxylem differentiating from the cotyledons nor that differentiating from the shoot are connected to, or become, the four protoxylem points of the root.
5. The transition region observed in *C. trichotomum* does not correspond with any of the transition types recorded by Van Tieghem or Sargent (1900).
6. A median protoxylem strand does not usually occur in the cotyledons. When one is present it is (1) separate from the two cotyledonary traces and their endarch protoxylem, (2) not associated with any metaxylem or phloem, and (3) stronger below and connected with the protoxylem strands which alternate with collateral bundles in the hypocotyl and extend down to form the protoxylem poles of the root. Therefore it is best interpreted as an upward extension of the protoxylem of the root, which ordinarily terminates in the hypocotyl, and is not a true cotyledonary trace. The

latter is truly double in nature and imposes on the hypocotyl its characteristic fourfold eustelic symmetry.

Further consideration of *C. trichotomum* in comparison with other types of seedlings will be included in the discussion.

NODAL ANATOMY AND VASCULATURE OF LEAVES

Nature and arrangement of foliar buds: As in plants of *Syringa*, *Betula* and *Euptelea* (Garrison, 1949a, 1949b), the buds of *Clerodendron trichotomum* are of two generations; these have been designated by Garrison as "primary" and "secondary" buds for the plants mentioned above, and the same terminology has been adopted here. The term "primary" is applied to the mature buds which bear embryonic "secondary" buds in the axils of their leaf primordia.

An examination of the primary terminal and axillary buds of the young shoot of adult plants of *C. trichotomum*, shoots of two-year-old plants (with the juvenile habit), and primary buds of seedlings 4 to 6 months old, has been made. The general arrangement of buds in all these stages of development is essentially the same. In *Clerodendron trichotomum* both the terminal and axillary buds are arranged in complex groups (PLATE I:1, 2). In the axil of each leaf there is one main axillary bud, one pair of lateral accessory buds, and usually a single accessory bud below the main axillary bud (PLATE I:1, 2). Additional pairs of lateral accessory buds may be present.

Terminal and axillary buds were collected at various times during the year. In their winter condition, the terminal buds are small, dull red and extremely hairy. Between the middle of April and the beginning of May, the buds open and the new year's growth is formed. At this time many of the axillary buds grow into lateral branches also (PLATE I:1, 2). Winter buds usually consist of one or two pairs of cataphylls, and two or three pairs of leaf primordia. In the spring condition the number of leaf primordia increases giving between 6 and 12 pairs of leaf primordia.

In many cases a delay in the opening of the terminal buds has been observed and the axillary buds at the first node elongate rapidly at the beginning of the new season producing the much branched condition of the adult plants. Shortly after the new growth of terminal and axillary buds, the cataphylls (PLATE I:1) drop off (PLATE I:2); the first two foliage leaves (the transitional leaves), which are much reduced in size (PLATE I:2), are also shed after a time. The terminal and lateral branches elongate rapidly producing, in the mature plant, adult leaves (PLATE I:1, 2) and in the seedling plants (1 to 2 years old) the enlarged juvenile leaves.

Origin, development and vascularization of buds: During the elongation of the terminal buds, and of those axillary buds destined to become lateral branches, the leaf primordia mature into leaves. The apical meristem of such shoots retains its meristematic properties, producing the leaf primordia of a new primary bud in the late spring and summer. The

apical meristem and the production of leaf primordia and secondary buds is similar in terminal and axillary (primary) buds.

From the time of its initiation, the tissues of the first pair of minute leaf primordia and of the apical mound are all meristematic. As the second pair of leaf primordia are initiated, a small mound of meristematic tissue becomes obvious in the axil of the leaf primordia of the second node. This meristematic mound is delimited from the terminal meristematic tissues in the leaf of whose axil it occurs during the next plastochrone³ by a layer of cells which are cambial-like in appearance; these form what is known as the shell-zone (Schmidt, 1924). Hence the first secondary bud is detached in primary buds in the axil of the third leaf in *Clerodendron trichotomum*. As this secondary bud assumes a position at the fourth node from the apex, it becomes more obvious due to the extensive vacuolation of surrounding cells in the cortical and pith zones. Each bud primordium which is formed in this manner has been termed a "detached meristem" (Wardlaw, 1943).

In these detached meristems during the third to fifth plastochrones of the leaves in whose axils they occur obvious procambial tissue is differentiated continuously and acropetally in connection with that in the axis, before these secondary buds produce leaf primordia. Two strands of procambium differentiate on each side of the secondary bud primordium, giving four procambial strands in all. These bud traces continue to differentiate acropetally into the first pair of leaf primordia which are produced by the secondary bud later in the season; the bud traces thus become the strands of the first pair of cataphylls.

From the time that the apical mound of meristematic tissue of a secondary bud is delimited from the meristematic tissues of the primary bud, to the elongation of the mature bud (now primary) with all of its leaf primordia, 16 or more months elapse. Detached secondary bud meristems become obvious in September or October. Both primary and secondary buds remain dormant until the following February or March, and only then are the first leaf primordia produced by the secondary bud meristem. One or two pairs of cataphylls, one pair of transitional leaves, and one or two pairs of foliage leaves are produced between March and April of this year. In March, the production of these bud primordia is preceded by marked enlargement of the bud primordia due to rapid cell division. The first pair of leaf primordia of the secondary bud develop at right angles to the subtending leaf primordium of the primary bud. Subsequent pairs of leaf primordia are initiated in an opposite and decussate arrangement. The first two or three leaf primordia thus produced become cataphylls. By the time the primary buds open and the axis starts to elongate the cataphylls and the first pairs of leaf primordia of the secondary buds

³ Askenasy (1880) used this term to denote the interval of time between the appearance of two successive leaf primordia on the shoot apex. It has been used here also for successive intervals of time in the development of any one leaf primordium, viz. a leaf primordium may be 2, 3, 4, etc. plastochrones old. The interval of time for the formation of a leaf primordium is its first plastochrone.

have been produced. By September the remaining pairs of leaf primordia have been produced. These buds, now "primary," elongate the following spring.

The sequence of events in the development of primary and secondary buds of *Clerodendron trichotomum* described above is essentially the same as that observed by Garrison (1949a, 1949b) for *Syringa*, *Betula*, and *Euptelea*.

Vascularization of buds: Esau (1943b) has pointed out the lack of information on the development and vascularization of axillary buds, and the need for more complete investigations of this kind. She emphasizes (1953) the importance of considering vascular differentiation in both transverse and longitudinal directions, since these occur simultaneously. This investigation has been primarily concerned with the differentiation of xylem in connection with the foliar and nodal anatomy, but as far as it has been possible, procambial and phloic differentiation have also been considered. The following description of differentiation applies to both terminal and axillary buds unless otherwise stated.

Longitudinal course of differentiation: PROCAMBIUM. — Procambial tissue is not easily distinguished from the cells of the meristematic region and its course can only be followed with difficulty. Frequently procambium is not observed when sections are cut obliquely, or when the procambial strands themselves run obliquely at the nodes due to leaf gaps. In this study procambial tissue has been recognized by (1) its ability to stain more densely than the surrounding tissues, and (2) its narrower cells, which are elongated parallel to the long axis of the bud (cf. Esau, 1942, 1943b and 1953).

The differentiation of procambium during bud formation in *Clerodendron trichotomum* is continuous and acropetal in both primary and secondary buds. The procambium differentiates in connection with the previously existing procambium of the axis, acropetally into the leaf primordia and also into the secondary bud primordia (before they produce leaf primordia). The meristematic tissue of the apical mound and the first pair of leaf primordia is in continuity. When the production of leaf primordia is rapid, procambium does not differentiate into a pair of leaf primordia until the next plastochrone, when secondary buds are initiated in their axils. If, however, leaf primordia are initiated more slowly (at the beginning of leaf production in March and April), then procambium differentiates acropetally into the first leaf primordia during the second plastochrone. This acropetal differentiation of procambium is in accordance with procambial development in *Phlox* (Miller and Wetmore, 1946), *Linum* (Esau, 1942), *Syringa*, *Betula* and *Euptelea* (Garrison, 1949a and b).

In *Clerodendron trichotomum*, two distinct procambial strands (FIG. 6:B) differentiate into each leaf primordium during the third to fifth plastochrone after its formation. Four separate procambial strands differentiate into each secondary bud primordium (FIG. 6:B). Precocious procambial production such as described for *Linum perenne* by Esau (1942) has not been observed in *C. trichotomum*. No difference has been observed

in the differentiation of procambium to cataphylls, transitional leaves and foliage leaves.

FIRST-FORMED PHLOEM. — As in the seedling, the phloem is the first vascular tissue to differentiate in the vegetative buds of the mature plants of *Clerodendron trichotomum*. The first-formed phloem differentiates continuously and acropetally, following the pattern mapped out for it by the procambium. The first-formed phloem elements have been recognized in this study by their early vacuolation, loss of nuclei, thickening of the cell wall (nacré appearance) and the formation of sieve plates. Differentiation of the first-formed phloem may occur in the bases of the first leaf primordia, if production of leaf primordia is slow. During the third plastochrone of this leaf primordium, differentiation of phloem is completed (FIG. 7:B). If the production of leaf primordia is more rapid the first phloem elements can be recognized in the second pair of leaf primordia.

Two separate strands of first-formed phloem differentiate acropetally into the base of each leaf primordium, one on the abaxial surface of each procambial strand. Similarly, four strands differentiate into each secondary bud primordium, about the time that the first pair of leaf primordia are produced in that bud. The first-formed phloem elements differentiate to the base of each of the first pair of leaves, so providing the bud primordium with a vascular connection to the main axis.

FIRST-FORMED XYLEM. — The first-formed xylem elements differentiate considerably later than the first-formed phloem elements. The timing of their differentiation is extremely variable and is connected with the rate at which leaf primordia are produced. If production of leaf primordia is slow, some xylem may be produced in a leaf primordium during its third plastochrone (FIGS. 6:B, 7:B). However, during more rapid production of leaf primordia the first xylem elements may not be differentiated in a leaf primordium until its fourth or fifth plastochrone. In either case, differentiation of the first-formed xylem elements is by-directional and discontinuous. A strand of first-formed xylem appears on the adaxial side of each of the two procambial strands; these xylem elements were recognized by the use of the criteria Esau has cited (1942). They are initiated at the base or some distance above the base in the leaf primordium. In either case differentiation occurs in two directions simultaneously, viz. acropetally towards the tip of the leaf primordium, and basipetally towards the main axis of the bud. The basipetally differentiating first xylem elements meet and join with acropetally differentiating first-formed xylem in the main axis of the bud. The actual union of these traces does not occur until about the fifth plastochrone of the leaf primordium in whose axil the bud occurs (FIGS. 6:B, 7:B).

Differentiation of the first xylem elements in the secondary bud primordia does not occur until the fifth plasochrone of the leaf in whose axil it occurs although mature phloem may be present much earlier. A secondary bud at this time has two or three pairs of leaf primordia, into which the procambium and phloem have already developed acropetally, as described above. The first xylem elements differentiate acropetally into the base of

the secondary bud in continuity with the xylem trace to the subtending leaf primordium of the primary bud. Subsequently, in the secondary bud itself, the first xylem elements to differentiate are observed, as in the primary buds, at or near the base of the third or fourth pair of leaf primordia. Further differentiation is as described for the primary buds.

Transverse course of differentiation: PROCAMBIUM. — The first procam-

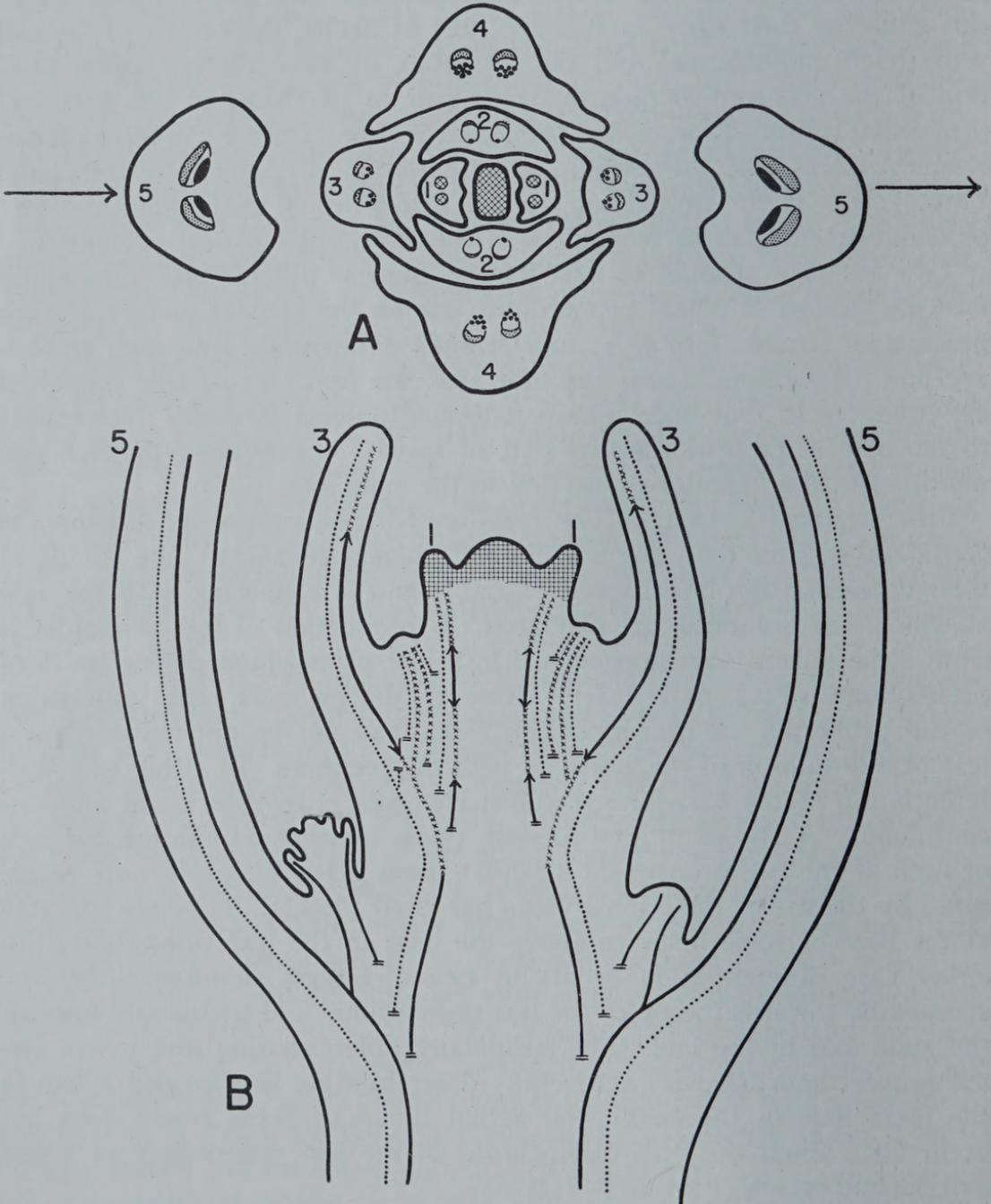


FIGURE 6

THE COURSE OF VASCULAR DIFFERENTIATION IN THE FIRST, THIRD AND FIFTH LEAF PRIMORDIA OF A BUD OF *Clerodendron trichotomum*. Crosses indicate the procambial strands. Phloem is shown as a dotted line. Xylem is represented as a solid black line. The arrows indicate the direction of differentiation. Traces terminating in a pair of transverse lines pass out of the plane of section.

bium differentiates in the main axis below the youngest pair of leaf primordia. In transverse sections it appears as four small groups of densely staining cells, these represent the four procambial traces to the youngest pair of leaves (two strands to each leaf). At this level they appear to be linked laterally by cells with stain-ability intermediate between procambium and ground tissue. These cells constitute what has been termed "less determined meristematic tissue," and will become parenchyma of the interfasc-

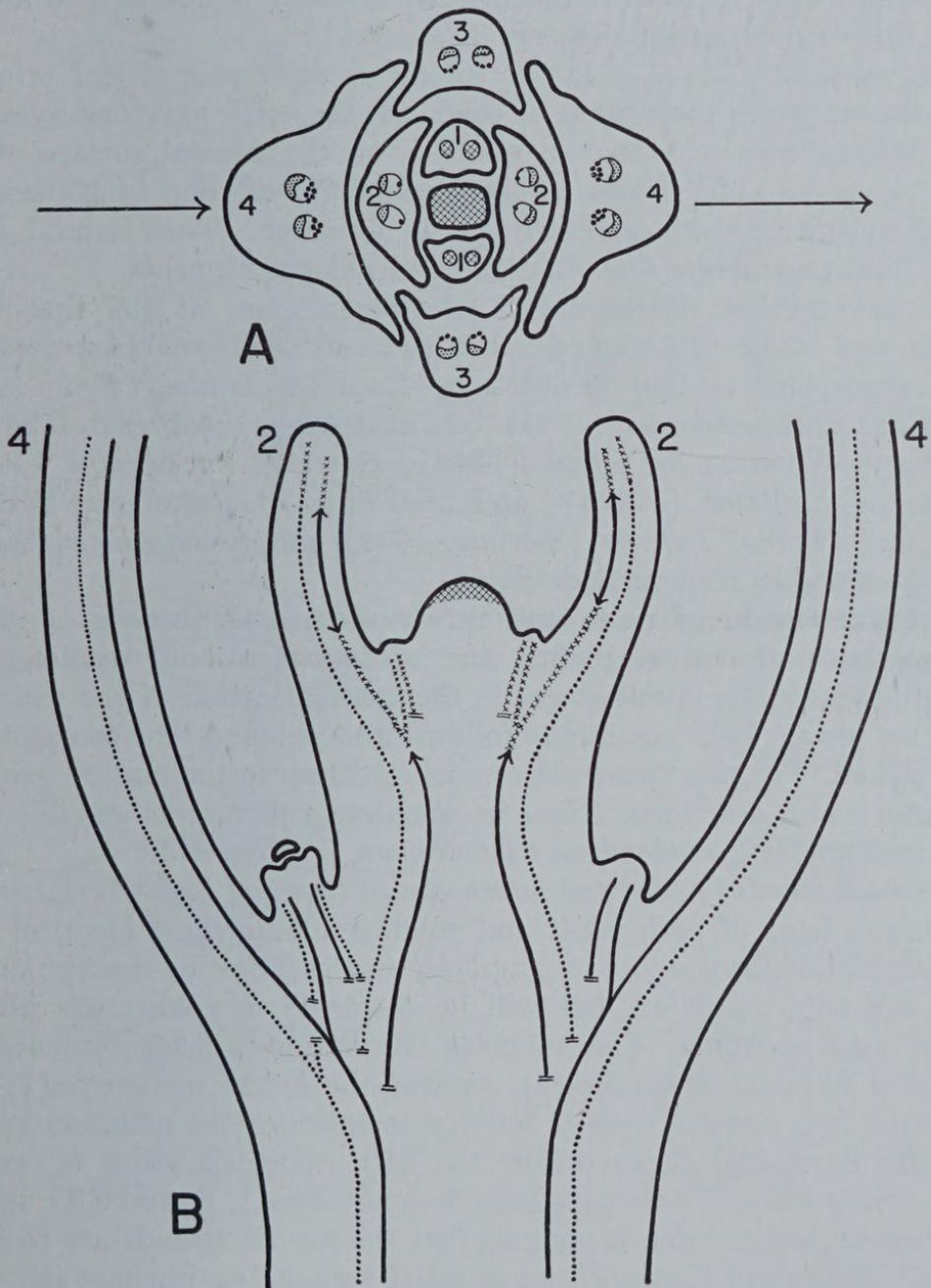


FIGURE 7

THE COURSE OF VASCULAR DIFFERENTIATION IN THE SECOND AND FOURTH LEAF PRIMORDIA OF A BUD OF *Clerodendron trichotomum*. Symbols are the same as in figure 6.

circular regions and leaf gaps (cf. Esau, 1943b and 1953). The nests of procambium in the main axis divide both anticlinally and periclinally, so building up the four distinct groups in the main axis.

FIRST-FORMED PHLOEM. — The acropetally differentiating first-formed phloem appears in the main axis below the youngest node, and in the second pair of leaf primordia. Its differentiation is initiated on the outside of the axis procambial trace and on the abaxial surface of each of the procambial strands to the leaf primordia (FIG. 6:A, 6:B). Subsequent development of the first-formed phloem is centripetal; laterally it develops in advance of the first-formed xylem elements (FIG. 6:A).

FIRST-FORMED XYLEM. — In the second or third pair of leaf primordia some distance from their point of origin on the main axis, one xylem element differentiates in a median position, on the adaxial surface of each of the two procambial strands. Subsequent differentiation from these first-formed xylem elements is centrifugal in direction. Their lateral spread lags behind that of the first differentiated phloem elements.

The direction of differentiation of procambium, of the first-formed phloem, and of the first-formed xylem elements in *Clerodendron trichotomum* corresponds to that recorded by Esau for *Linum* (1942, 1943a), *Helianthus* and *Sambucus* (1945) (cf. also Esau 1943b and 1954); by Miller and Wetmore for *Phlox* (1946); Garrison for *Syringa* (1949a), *Betula* and *Euptelea* (1949b); and in *Ginkgo* (Gunckel and Wetmore, 1946a and 1946b), *Sequoia* (Sterling, 1945) and *Pseudotsuga* (Sterling, 1947) at least in a transverse direction.

Vascularization of node and internode: Serial sections of primary terminal buds of mature plants, and of four-month-old seedlings have been studied for the initial stages in the vascularization of the main axis. It has not always been possible to follow, in these stages, the course of each foliar strand. For this reason, the nodal structure in the mature two-year-old plant is also described. Thus, as complete a picture as possible of the nodal anatomy of *Clerodendron trichotomum* is given.

Vascularization of nodes and internodes in terminal buds: Serial sections of terminal buds of both adult and seedling plants show identical nodal conditions. For the purpose of simplicity in the following description, bud traces are not considered, but will be discussed in connection with the mature nodal anatomy. The following description is made from the fifth node of a terminal bud upwards towards the apical meristem (FIG. 8). Below the fifth node secondary activity complicates the nodal anatomy.

In the internodal region below the fifth node, six pairs of vascular strands are present. These have been designated as A, B, and C (FIG. 8:1). At a slightly higher level, it appears that the four B strands are double in nature. The A and C strands move apart forming an obvious gap in the eustele as the fifth node is approached, and at this node, the two pairs of A traces pass out to the fifth pair of leaves (from the apical meristem) (FIG. 8:2). At this level also the lens-shaped axillary bud primordia constrict the stele pushing the B and C pairs to the opposite side of the stele, and now four new small strands, D, separate from the two pairs of B

strands. The node, then, at this level, still contains six pairs of vascular strands (FIG. 8:2, 3).

In the internode above the fifth node, the eustele closes and the six

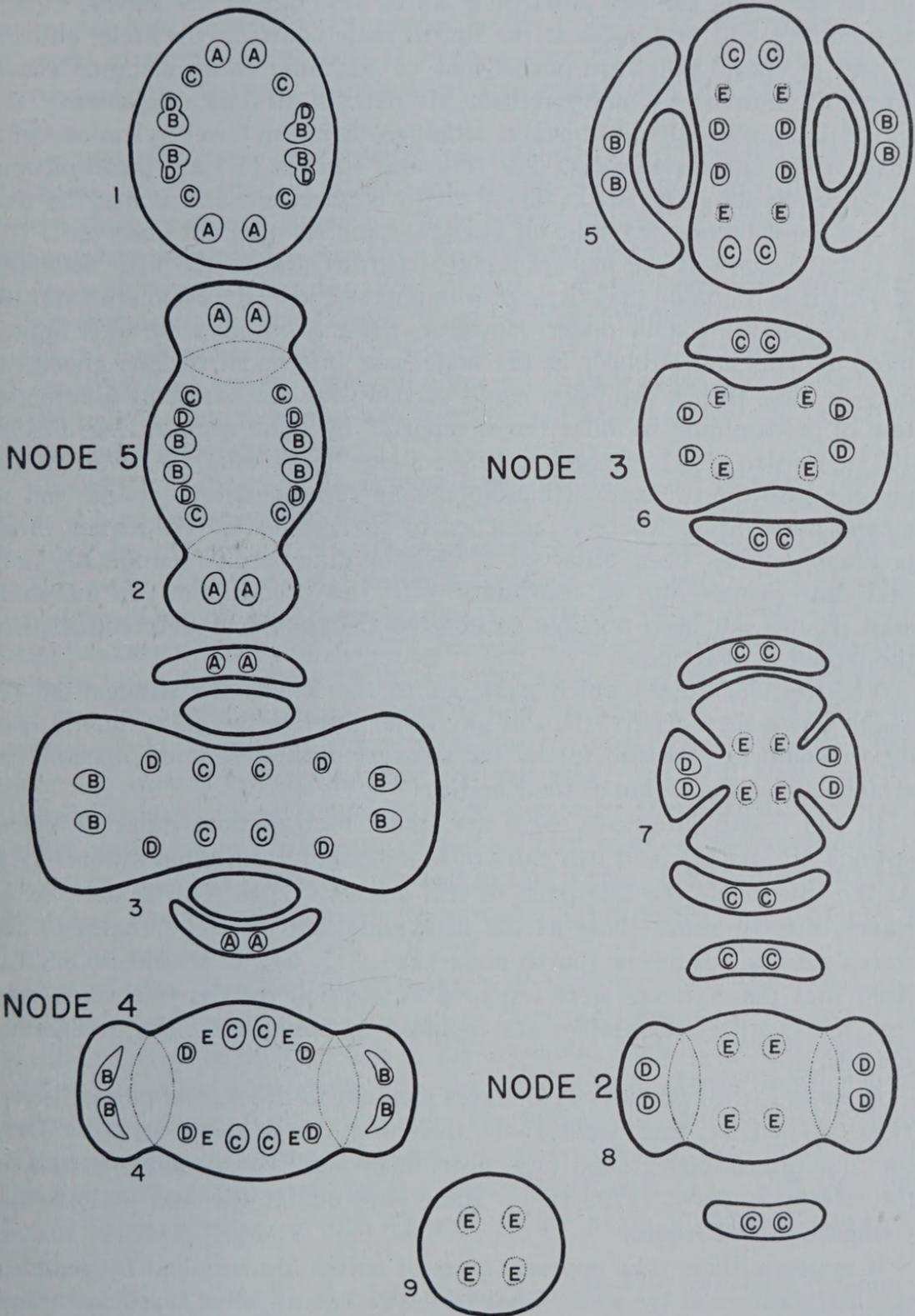


FIGURE 8

A SERIES OF SECTIONS THROUGH THE PRIMARY TERMINAL BUD OF *Clerodendron trichotomum*. Explanation in text.

pairs of strands resume a symmetrical arrangement. The fifth pair of leaves has detached from the axis and each contains two A strands. As the fourth node is approached the B and D pairs separate at either end of the stele, and the two pairs of B traces pass out to the leaves. Again, the axillary bud primordia at the fourth node constrict the stele, and the C and D strand pairs are pushed out of position. Some distance above, where the normal symmetry returns, six pairs of strands are present. The C and D bundle pairs are obvious although there are fewer xylem elements than in the internode below. The four new strands (E) are inconspicuous and procambial (FIG. 8:4). Their origin is uncertain and it may be that at the fourth node they split off obliquely and so were not observed.

From a study of the mature nodal structure, and of the fifth node (cf. above) it is probable that these procambial strands (E) originate from the C traces at the fourth node. However, these procambial strands appear more obvious and stronger in the nodal and internodal regions above. It may be that such a condition could account for the basipetal differentiation of procambium in foliar traces reported by some workers, e.g. Foster, 1935; Kaplan, 1937; Grégoire, 1938; Boke, 1940, etc. However, in the present case the procambial bundle E may run an oblique course and so escape observation where it attaches to the strand C. At higher levels procambium has been observed always to differentiate acropetally into each leaf primordium in continuity with the procambium in the main axis. It has not been possible to observe the course of differentiation of the strand E at its base.

The vascular traces which pass out to the leaves are stronger at the nodes where they depart, than at any other level in the axis. Such a condition would be provided for by the stronger development of procambium strands above the region of their origin.

In the fourth internode there are, then, four distinct pairs of xylem strands (C and D), and two pairs of less distinct procambial strands (E). At the third node the two pairs of leaf traces, C, pass out to the pair of leaves directly above those at the fifth node, and at right angles to the traces passing out at the fourth node (FIG. 8:5, 6). It should be emphasized that the A traces were replaced in position by the C strands, and that the C traces themselves are replaced in position by the procambial strands, E.

At node 2, the two pairs of D traces pass out to the second pair of leaves (FIG. 8:7, 8), i.e. four nodes above their origin from the B strands. Only the four procambial groups (E), now much more conspicuous, remain in the internode above (FIG. 8:9). These pass out at the first node to the youngest pair of leaves.

It appears, then, that opposite pairs of leaves are supplied by separate vascular systems of the stele. That is, the vascular system supplying traces to the pairs of leaves at the fifth, third and first nodes (A, C, and E, respectively) are quite separate from the system supplying leaves at the fourth and second nodes (B and D, respectively). In fact, each leaf in

a separate phyllotactic position is supplied by two quite separate strands in the eustele.

In each case it appears that the traces for any one pair of leaves originate as vascular strands of the eustele at one node, pass upwards through two nodes, and depart to the pair of leaves at the next node above. For example, at the fifth node the D strands arise from the B traces; at the fourth node the B traces pass out; at the second node the D traces pass out.

Vascular structure at the node in adult plants: The nodal and internodal conditions in the adult plant are comparable with those described above, except that secondary tissue has been added. In this condition the vascular strands described above have been identified by their primary xylem groups which project into the pith. At each internode twelve such primary xylem masses can be recognized. Eight of these are always more obvious; these are the strands which will become foliar traces at the next two nodes above. The four smaller primary xylem groups are those which separated, at the node below, from the two pairs of leaf traces which will depart at the node immediately above.

The series of diagrams in figures 9 and 10 illustrates this condition at three successive nodes, A, B and C. Only the primary xylem groups are shown here. At node A, the traces marked 1 depart to the pair of leaves at this node. In near nodal sections the stele separates to form a single gap through which the traces gradually move outwards towards the leaf base; successive pairs of strands become detached from this, and pass into the base of the petiole. Three or four pairs of strands pass out to the leaf from these two separate groups (1) in the axis (FIG. 9). Finally the two traces (1) completely detach themselves from the stele and remain separated from it by parenchyma. At this stage, two bud traces are detached at each side of the axis, from the same traces that supply the leaves, and pass out obliquely to the axillary buds. The vasculature of accessory buds has not been followed in this study. Finally the remainder of strand 1 passes out laterally to vascularize the wings of the petiole as shown in figure 14.

At the same time that the bud traces become detached, two small pairs of primary xylem groups (4) separate from the sides of the larger xylem groups (2) (i.e. in the direction of the departing strands, see FIG. 9) which will depart at the node above (B). In the internode above, the stele again has twelve groups of primary xylem. The four smaller groups (3) (FIG. 9), of the internode below node A, now enlarge considerably and move to close the gap made in the stele by the departing strands (1). Now the eight larger primary xylem groups (3 and 2) are again in opposite positions (FIG. 9).

Similarly, at node B, the four strands, numbered 2, depart through a unilacunar gap in the stele in a similar manner. The number of primary xylem groups is again renewed by the larger traces (3) detaching four small primary groups (5) (FIG. 9). The smaller groups, numbered 4, in-

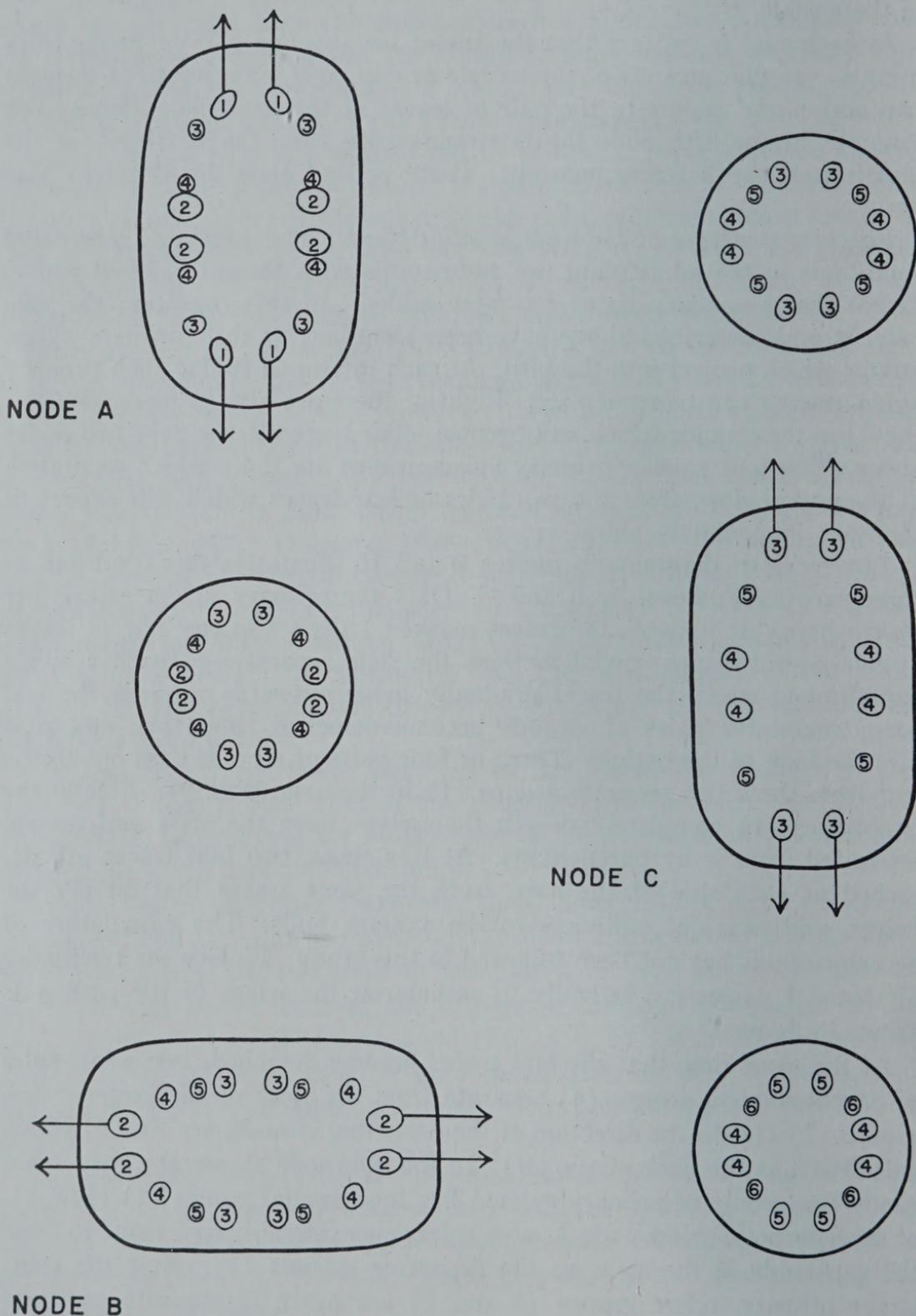


FIGURE 9

DIAGRAMMATIC SERIES THROUGH THE ADULT NODE OF *C. trichotomum*. Explanation in text.

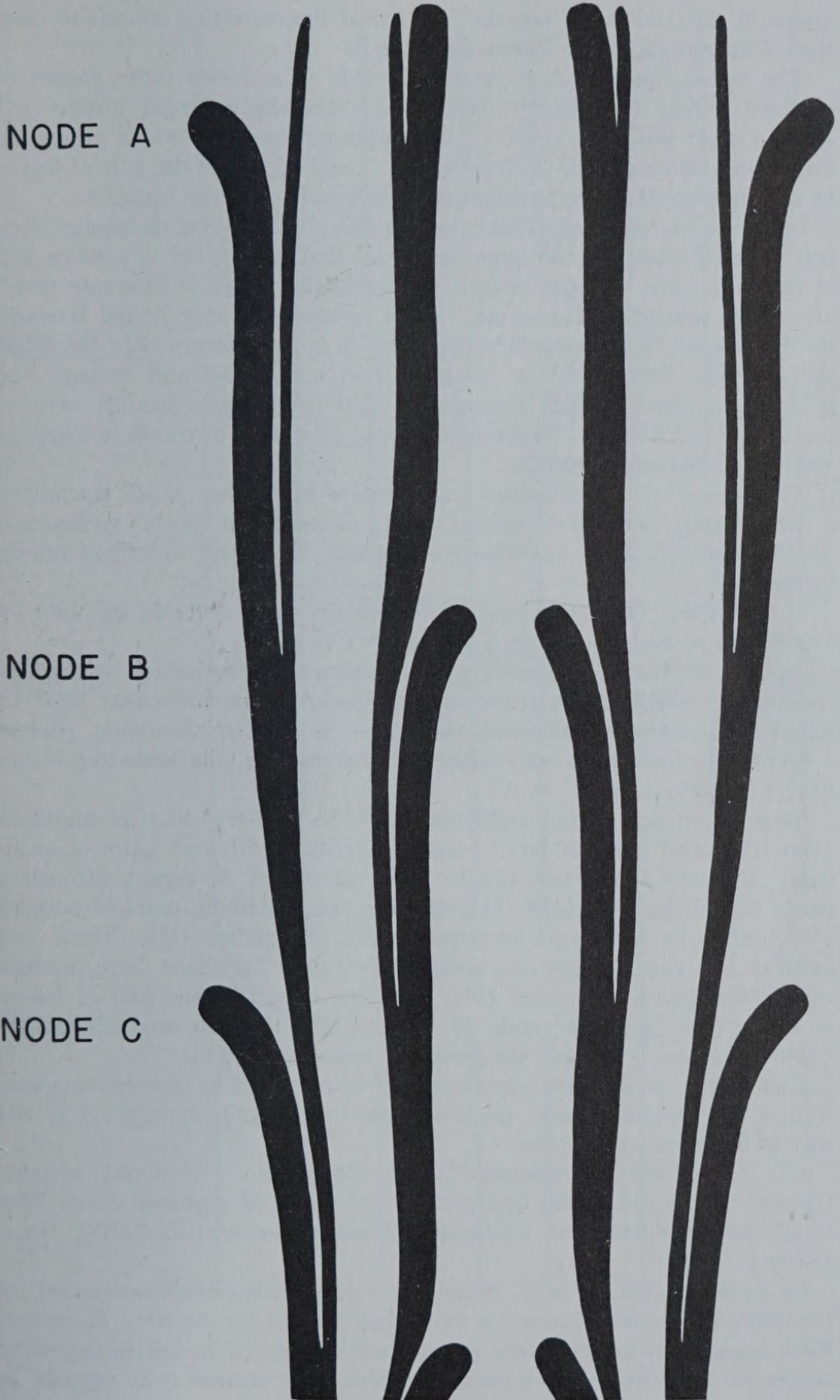


FIGURE 10

THE LONGITUDINAL COURSE OF THE PRIMARY VASCULAR STRANDS IN THE STEM OF *Clerodendron trichotomum*. Only the vascular strands on the near side of the stem are shown.

crease in size and move into the position of the departing strands to close the unilacunar gap in the internode above B.

The traces, numbered 3, depart at node C and two more groups of primary xylem (6) detach themselves from the enlarged groups (4) (FIG. 9). At the node above C, the traces numbered 4, which originated four nodes below at node A, from traces 2, will depart to the pair of leaves in the same phyllotactic position as those supplied by the traces 2.

In *Clerodendron trichotomum*, where the phyllotaxy is decussate, each leaf is vascularized by two discrete strands that are related to a single gap in the stele. The vascular strands supplying the leaves at alternate nodes arise from independent systems. These systems are only linked laterally by the production of secondary tissue which is not concerned in the foliar supply. The leaves are vascularized from an eight-strand system: the primary vascularization is a system of eight independent strands, consisting of four pairs which independently supply the pairs of traces to leaves in the four phyllotactic positions.

Comparison of Clerodendron trichotomum with other plants possessing a similar nodal anatomy: Several plants possessing a similar unilacunar, double trace condition have been examined. These are described briefly below.

(1) *Ephedra* (By examination of stem tip serial sections supplied by Dr. Taylor A. Steeves, Harvard University) (FIG. 11).

As in *C. trichotomum*, the leaves of *Ephedra* are vascularized by two discrete traces which depart from the eustele through a unilacunar gap. In the slides of *Ephedra* examined, the leaves, as in *C. trichotomum*, possess a decussate phyllotaxy, but, unlike *C. trichotomum*, the scale-like leaves have a sheathing base.

Each internode contains eight bundles in its primary vascular condition (FIG. 11); two pairs of large bundles alternate with two pairs of small ones. At node 1, the two smaller pairs of traces, A, depart through a single gap in the stele (FIG. 11), and the two remaining pairs of bundles (B) branch to form two additional pairs of bundles (C). These two bundles (C) run through one node above (node 2) where these produce two further pairs of bundles (D), and then depart to the pair of leaves at the next node above (node 3). Meanwhile, the two original bundles (now leaf traces B) supply the leaves at node 2 (FIG. 11).

The leaves of *Ephedra* are therefore vascularized at successive nodes from a four-strand system. Each of these four strands supply one of the pair of leaves at every node.

(2) *Austrobaileya scandens* (From examination of serial sections through the terminal bud, first and second nodes of a young shoot. Material from the Harvard University Wood Collection; H-27800, Brass 18160.)

In *Austrobaileya*, as in *C. trichotomum*, the leaves are vascularized by two pairs of strands, through a unilacunar gap in the eustele. Moreover, these arise from two separate procambial strands, although during later stages in differentiation of vascular tissues the strands may appear to

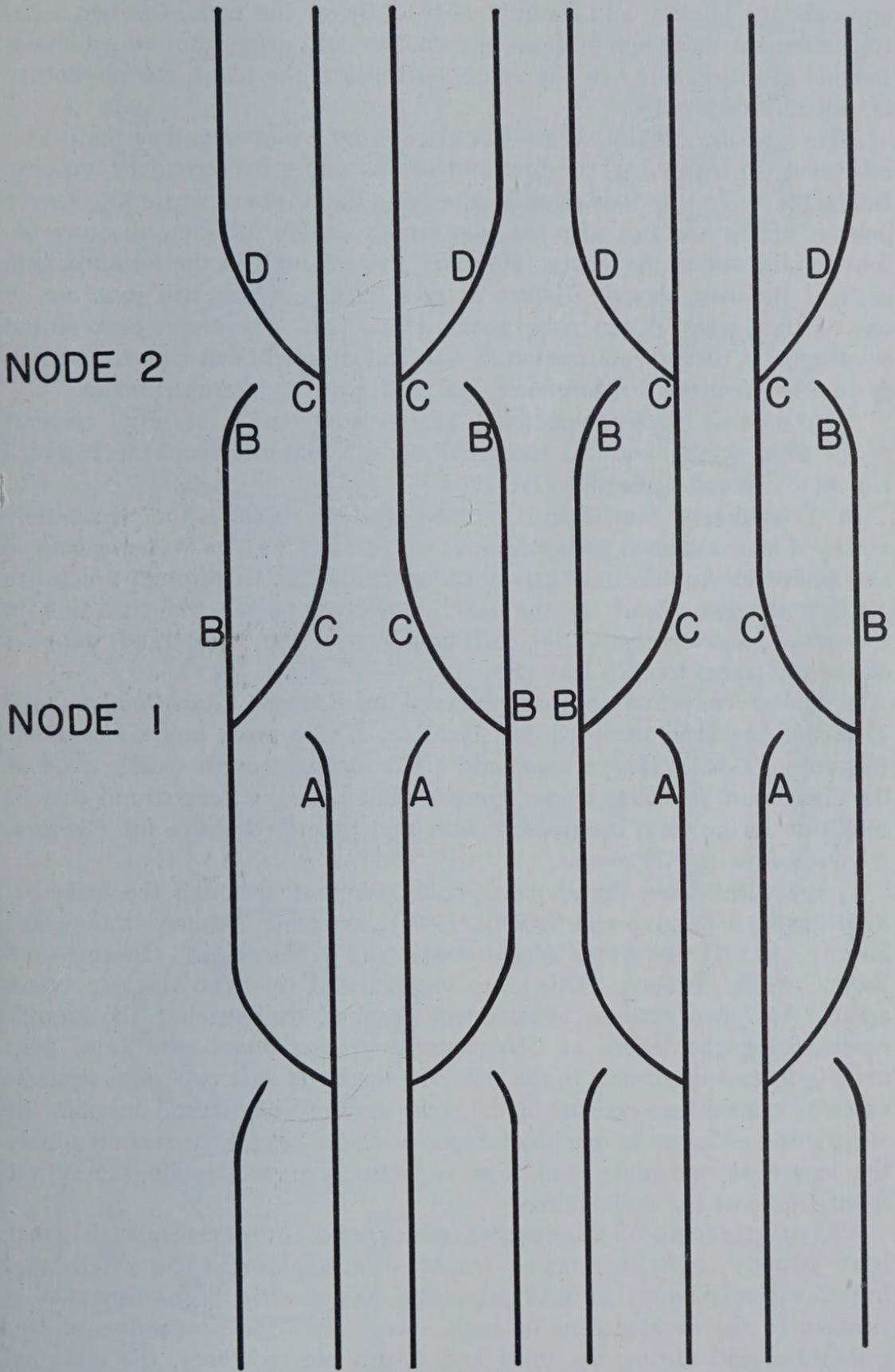


FIGURE 11

THE LONGITUDINAL COURSE OF THE PRIMARY VASCULAR STRANDS IN THE STEM OF *Ephedra*, AS IF SPREAD OUT IN ONE PLANE.

approximate (Bailey and Swamy, 1949). Below the node, the two foliar traces remain independent from one another and arise from two different strands of the eustele. In the young portions of the plant, the phyllotaxy is typically decussate.

As in *Ephedra*, the leaves are vascularized by a four-strand system. This condition is obscured at the first and second nodes by secondary activity. Below the nodes, the four strands supplying the leaves separate into twelve pairs of traces and run with the four-strand system for some distance before passing out to the leaves. However, at each node in the terminal bud, each of the four strands divides to give a trace which will pass out to one of the leaves at the node above (FIG. 12). Therefore, each strand supplies half the vascularization of one leaf at successive nodes, and not, as in *Clerodendron trichotomum*, one leaf only at alternate nodes.

(3) *Trimenia weinmanniaefolia* (From examination of serial sections of the stem through several successive nodes. Material from the Harvard University Wood Collection, H-27965.)

In *Trimenia*, a four-strand vascular system supplies the decussately arranged leaves at each node with four traces (FIG. 13), as in *Austrobaileya* and *Ephedra*. A unilacunar gap is formed in the eustele through which two distinct strands depart to the leaf. However, unlike the condition in *Clerodendron trichotomum*, at each node one of the four strands supplies one of the traces to each leaf (FIG. 13).

A similar condition to that observed in *Ephedra*, *Austrobaileya* and *Trimenia* has been recorded for *Ascarina*, *Hedyosmum* and *Chloranthus* (Swamy, 1953a). *Hedyosmum* and *Chloranthus* show a modification in the division of the foliar traces, however, but possess a four-strand eustelic condition in the stem comparable with that described above for *Ephedra*, *Austrobaileya* and *Trimenia*.

It is evident from the above comparisons that although the leaves of *Austrobaileya* (Bailey and Swamy, 1949), *Trimenia* (Money, Bailey and Swamy, 1950), *Ascarina*, *Hedyosmum* and *Chloranthus* (Swamy and Bailey, 1950; Swamy, 1953a) are vascularized by "two discrete traces arising from two entirely independent parts of the eustele," the strands vascularizing the leaves of *Clerodendron trichotomum* arise from even more independent strands in the stele. In the latter case two quite separate vascular systems are present in the stele, and any one strand supplies the vasculature of leaves in one phyllotactic position only. Four strands supply the leaves at one node, and four entirely separate strands supply the foliar traces at the node above.

Vascularization of vegetative leaves, and comparison with other leaf types: Adult vegetative leaves—development: The origin and initial vascularization of leaf primordia has already been discussed in relation to the development of buds. Soon after the production of leaf primordia, and during the third and fourth plastochrones, the marginal and submarginal initials are formed. The subsequent development of these embryonic layers is similar to that described in detail by Esau (1953, pp. 442–447) for ordinary dicotyledonous leaves having an expanded blade,

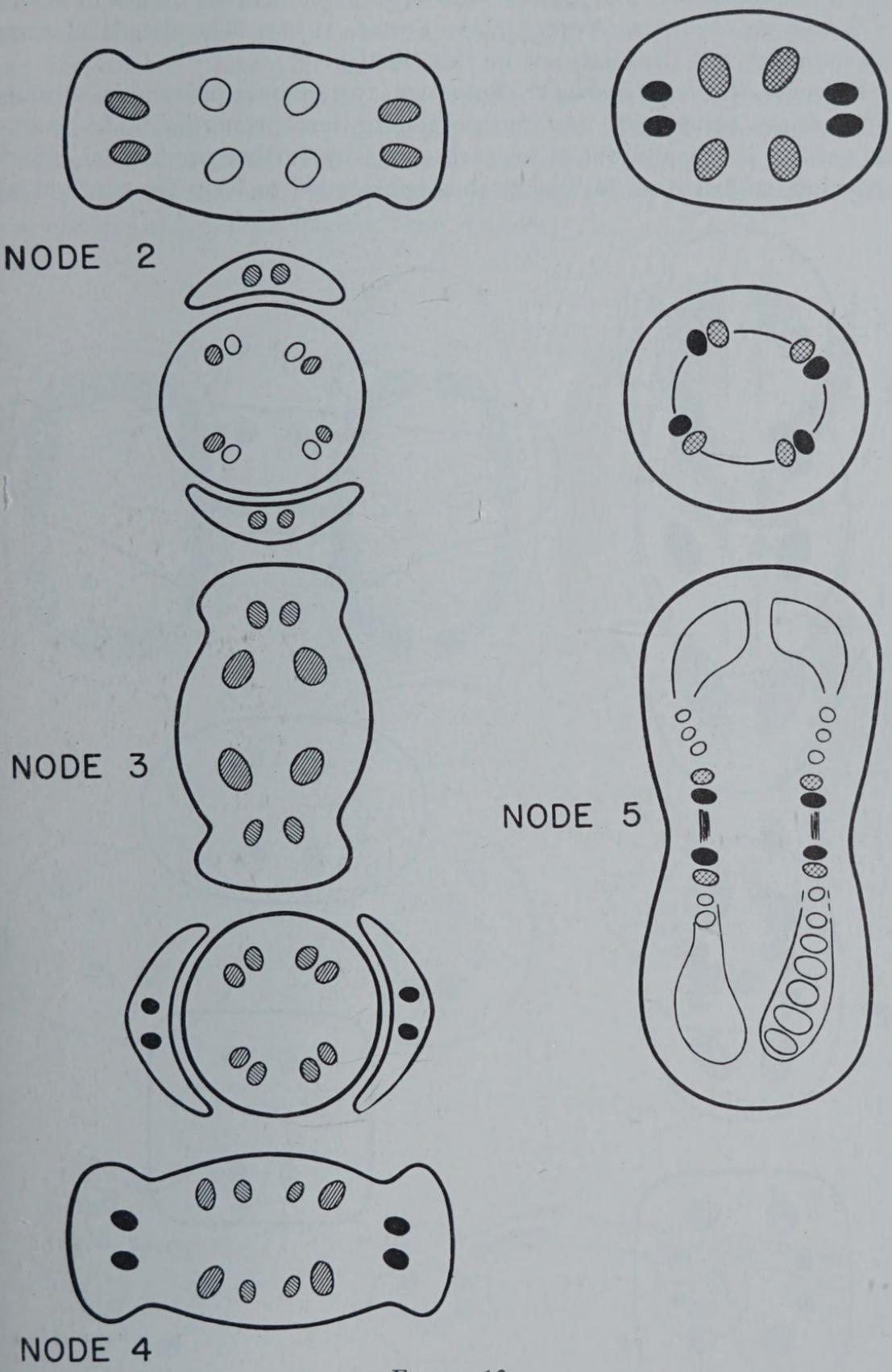


FIGURE 12

THE NODAL ANATOMY OF *Austrobaileya*.

and a petiole, and is comparable with the development of tissues in leaves of *Nicotiana tabacum* (Avery, 1933; Foster, 1936). The details of their development will therefore not be described here.

However, in *Clerodendron trichotomum*, two distinct procambial strands differentiate acropetally into the developing leaf primordia. The vascular pattern is mapped out at an early stage by further procambial differentiation, and each of the two strands is extended to form the procambial

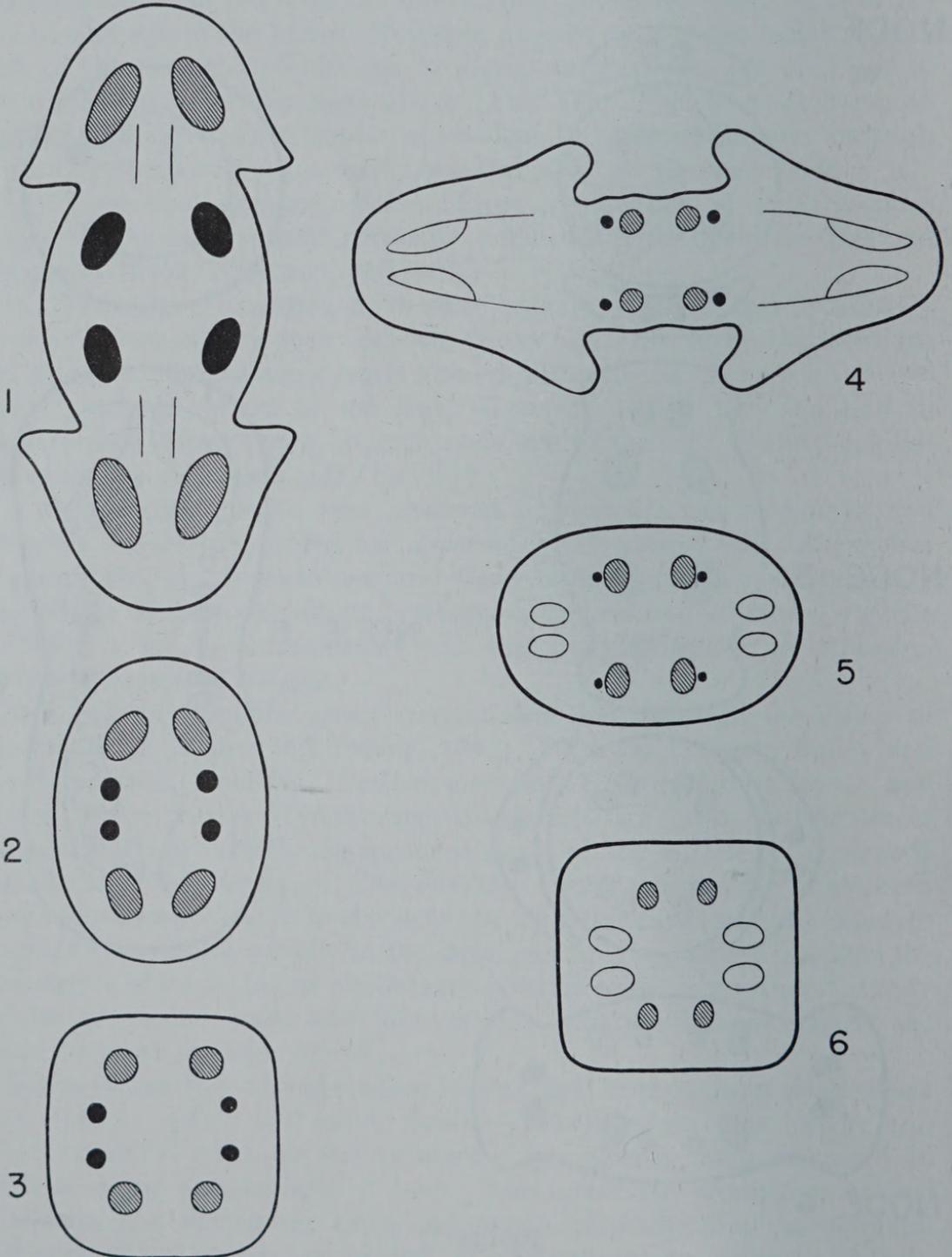


FIGURE 13
THE NODAL ANATOMY OF *Trimenia*.

network of half the leaf. The first vascular tissue to differentiate in the leaves is phloem, and this develops acropetally in continuity with the existing first-formed phloem in the axis. The first phloem is formed in the leaf during its first or second plastochrone. The first xylem elements usually differentiate in a leaf during its fourth or fifth plastochrone. The differentiation of xylem first occurs at the base of the leaf primordium, and is bidirectional and discontinuous. Differentiation of late-formed primary xylem and phloem has not been studied.

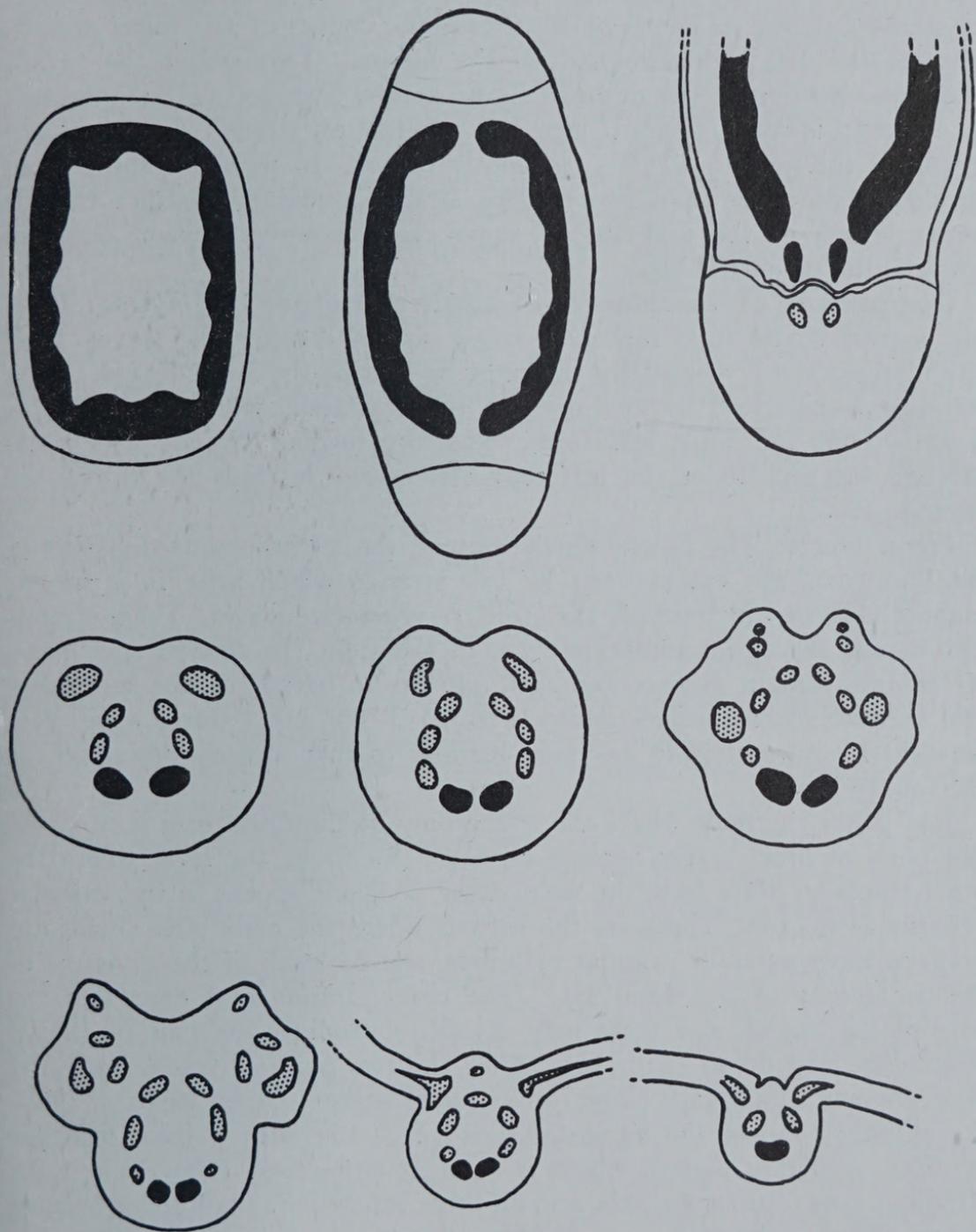


FIGURE 14

A SERIES OF SECTIONS THROUGH THE NODE AND LEAF OF *Clerodendron trichotomum*.

Mature adult leaf — vasculature: In the mature leaf the two leaf traces divide before departing through a unilacunar gap in the stele. The main traces divide to form three or four pairs of subsidiary strands and a pair of bud traces, and finally depart to the base of the petiole. The first two traces to depart to the leaf base form the main strands (shown in black in FIG. 14) of the mid-rib, and these remain distinct throughout the petiole and mid-rib of the lamina, until finally they approximate towards the leaf apex (FIG. 14). The last two strands departing through the single gap in the stele, vascularize the wings of the petiole (FIG. 14). The series of sections shown in figure 14 illustrates the course of the foliar strands through the petiole and mid-rib of the lamina. Throughout the petiole and lower portion of the mid-rib the bilaterally symmetrical arrangement is evident; towards the leaf tip, the number of strands decreases and fusion of the median strands frequently occurs. In many of the cleared specimens examined, the two median strands remained distinct for the entire length of the mid-rib; in some cases, however, fusion occurred towards the tip of the leaf.

Comparison of vasculature of adult and other leaf types: *Juvenile leaves:* Apart from the much larger size of the juvenile leaves, their vascular pattern is essentially the same as that of the adult leaves. They are also vascularized by two discrete foliar strands, which subdivide as described for the adult leaf form. The two median strands which run through the mid-rib of the leaf may also merge towards the tip of the juvenile leaves.

Floral bracts: The bracts which subtend the trichotomous divisions of the floral axis are vascularized by two strands which arise in a similar manner to those which supply the ordinary vegetative leaves. These strands also depart through a unilacunar gap in the stele. In cleared specimens, the strands remain distinct and each appears to consist of one spiral and two or more scalariform elements (FIG. 15). The bract traces arise from an eight-strand system in a similar manner to that already described for the foliage leaves.

Just below the node where the trichotomy of the floral axis occurs, the two pairs of bract traces become obvious. At about the level where the bract traces separate from the stele, other divisions appear in the vascular cylinder (FIG. 16). These are the regions where the main stele divides to produce three separate vascular cylinders, one for each of the divisions of the trichotomy of the floral axis. The bract strands now depart to the base of the bracts, and these may divide to produce one pair of lateral strands as they do so (FIG. 16). After the two pairs of traces leave the stele, the unilacunar gaps close, forming a horseshoe-shaped vascular cylinder at either end of the elongated axis (FIG. 16) with a third vascular cylinder, medianly placed, which is open at either end (FIG. 16). Constrictions now form in the axis, and all three future floral axis steles become closed. Finally, the stem itself divides into three floral branches (FIG. 16).

In all the cleared specimens of bracts which have been examined, the two median strands remain distinct throughout their length. They do not

appear to merge towards the tip of the bracts, as is sometimes the case in the foliage leaves. The bracts differ from the foliage leaves in their reduced size and narrowly ovate shape. Their vascularization, however, is similar in that they are supplied by two discrete traces through a unilacunar gap in the stele.

Cataphylls: The vascularization of the bud scales has already been discussed earlier in this chapter in connection with leaf primordia. As in the vegetative leaves, and bract scales, the cataphylls are supplied by two separate procambial traces, which arise from the eight-strand system in the same way as the ordinary foliar traces. The subsequent development of the cataphylls is similar to that described by Esau (1953). The vasculature of the cataphylls of *Clerodendron trichotomum* appears, as in the floral bracts, to be limited to the two median strands with occasionally one pair of smaller lateral strands. The cataphylls and floral bracts drop off shortly after the primary vegetative and floral buds elongate.

Transitional leaves: In *C. trichotomum* each bud usually possesses one pair of transitional leaves, just above the cataphylls, which have a more expanded lamina, are green, and lack the great abundance of hairs and cells containing phenolic compounds found in the bud scales. They are, however, reduced in size and transitional in shape between the bud scales and adult foliage leaves. Like the cataphylls and floral bracts, they are shed shortly after the primary buds open. The transitional leaves are supplied with two discrete traces through a single gap in the stele. These traces arise from the eight-strand stelar system in a similar manner to those supplying the foliage leaves and bud scales. However, in the transitional leaves two pairs of laterals are usually produced by the main strands; these latter strands may approximate towards the transitional leaf apex, as in the case of the regular foliage leaves. Hence all these leaf types, the adult and the juvenile foliage leaves, the floral bracts, cataphylls and transitional leaves are similar in their vascularization.

Cotyledons: The vascularization of the cotyledons was discussed earlier. These also are supplied with two discrete strands, although the condition at the cotyledonary node differs from the nodes of the epicotyl. However, the two cotyledonary traces do arise from two separate procambial strands, as is the case in the foliage leaves, cataphylls, transitional leaves and floral bracts.

Considering all the leaf types mentioned above, it is apparent that the vascular supply in all these cases is comparable. Each leaf, regardless of size and form, is vascularized by two discrete strands. In adult and juvenile leaves, in floral bracts, cataphylls and transitional leaves, these two traces arise from the eight-strand stelar system in the same manner, and depart to the leaves, in each case, through a unilacunar gap in the stele. In all six types of foliar appendages the two vascular strands differentiate from two discrete procambial strands in a comparable manner. Hence these leaf types are basically the same and have been modified according to their position and function in relation to the other parts of the plant.

The initiation of an eight-strand vascular system in the seedling: In the

seedling of *Clerodendron trichotomum* there is a four-strand system at the cotyledonary node, which is comparable to the situation observed in adult plants of *Austrobaileya*, *Trimenia* and *Ephedra*. However, in *C. trichotomum*, the cotyledonary traces pass out to the cotyledons directly at their node of origin, and not one node above as in the species mentioned above.

Examination of the epicotyl in seedlings of *Clerodendron* with one pair of leaves only, also demonstrates a four-strand condition. But, between

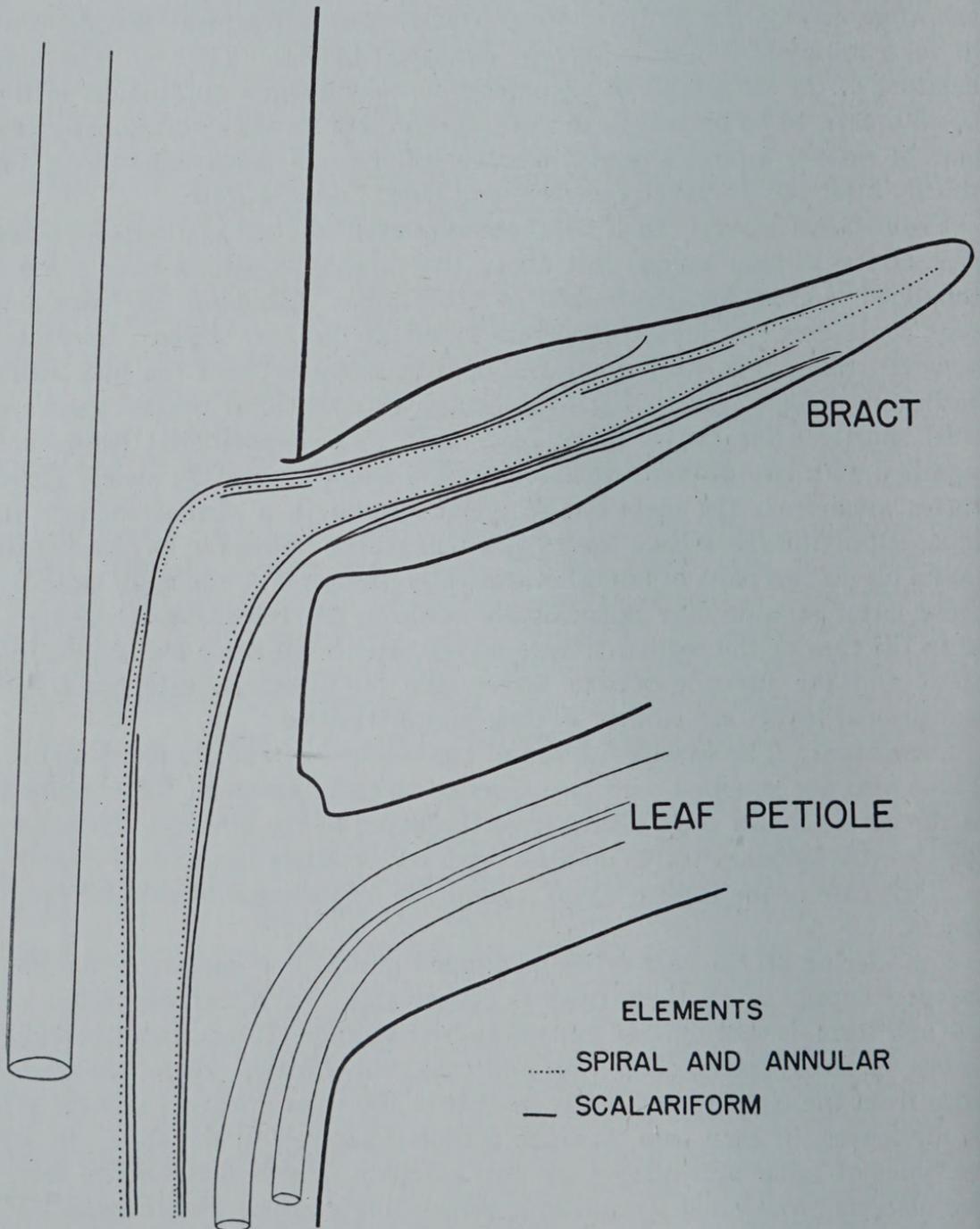


FIGURE 15

DIAGRAM OF A CLEARED FLORAL BRACT SHOWING THE XYLEM ELEMENTS.
(*Clerodendron trichotomum*)

the four first-formed xylem groups of the first pair of leaves, four additional xylem groups differentiate from the next pair of leaf primordia which are produced, so forming the eight-strand system apparent in the adult plant. The xylem of the second group of foliar traces differentiates basipetally down the entire length of the epicotyl to join the late-formed xylem just above the cotyledonary node. There is some variation in the position of attachment of the second group of foliar traces, but in all cases they attach to the late-formed primary xylem between the cotyledonary node, and approximately 1000 micra above. Seedlings with more than two pairs of leaf primordia were not examined and it is therefore not possible to say how the traces to the third pair of leaf primordia arose without further investigation. Foliar traces to subsequent leaf primordia have not been observed in older seedlings in the epicotyl below the first pair of leaf

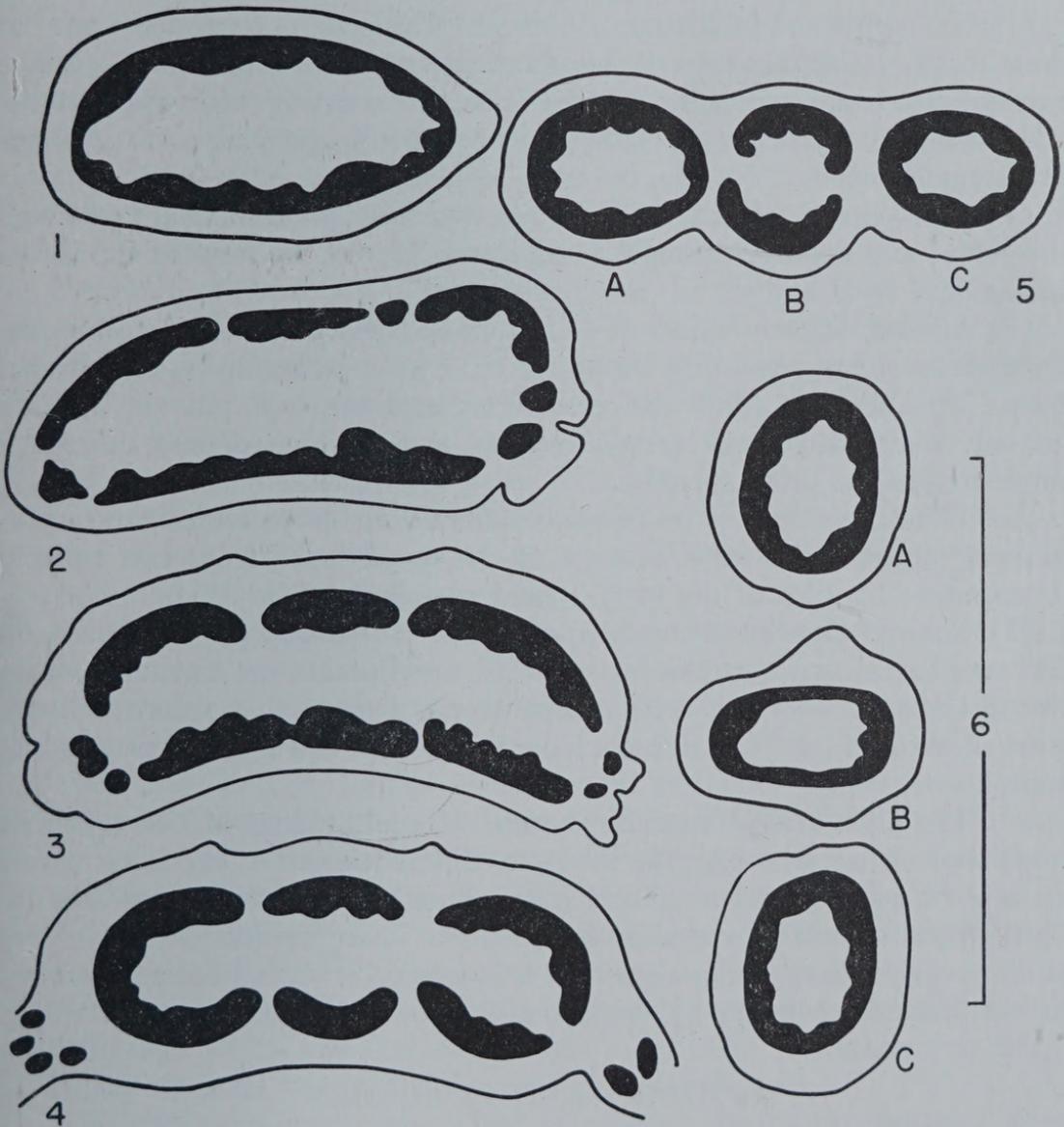


FIGURE 16

A SERIES OF SECTIONS THROUGH THE FLORAL TRICHOTOMY OF *C. trichotomum*, SHOWING THE DEPARTURE OF THE BRACT TRACES.

primordia, and traces to the third pair of leaves may have arisen at the first-formed node from the foliar traces to this pair of leaves.

Summary: (1) In the primary buds: (a) two separate procambial strands differentiate continuously and acropetally into each leaf primordium during the first and second plastochrones. (b) First-formed phloem similarly differentiates acropetally during the second plastochrone. The phloem differentiates first on the abaxial surface of each procambial strand and spreads centripetally. (c) The first-formed xylem differentiates discontinuously (both acropetally and basipetally, simultaneously) during the third and fourth plastochrones, on the adaxial surface of each procambial strand and spreads in a centrifugal direction.

(2) The secondary buds are formed from "detached meristems" during the third plastochrone; differentiation of vascular tissues occurs in a similar manner to that in the primary buds.

(3) An eight-bundle eustelic condition develops in the node; four of these supply foliar traces to the leaves at one node, and four quite separate and independent bundles supply the leaves alternating with these at the node above, i.e. there are two independent systems supplying pairs of leaves at alternate nodes.

(4) Each pair of foliar traces arises at one node, passes upwards through two nodes and departs through a unilacunar gap in the stele to the leaves at the next node above.

(5) All the different types of foliar appendages examined in *Clerodendron trichotomum*, viz. adult leaves, juvenile leaves, cataphylls, transitional leaves, floral bracts (and also cotyledons and the first pair of seedling leaves), are vascularized by two discrete traces which depart through a single gap in the primary stele.

(6) These foliar traces in all cases differentiate from two separate procambial strands, and arise (except to the cotyledons) from two entirely independent bundles of the eight-strand eustele.

(7) The examination of nodal material of *Austrobaileya*, *Trimenia* and *Ephedra* has shown that the leaves in all these plants are vascularized by two discrete traces which arise independently from a four-strand primary stele. The foliar traces in all cases have arisen from two separate procambial strands.

(8) The eight-strand vascular system in adult plants of *Clerodendron trichotomum* has arisen by the basipetal differentiation of the foliar traces of the first two pairs of seedling leaves. Thus, a four-strand, and then an eight-strand system is formed in the epicotyl. There is a four-strand system at the cotyledonary node, since the epicotylary strands become attached to late-formed secondary xylem just above the cotyledonary node.

SUMMARY AND DISCUSSION

Since his survey of the foliar nodal anatomy of dicotyledons (Sinnott, 1914), a fourth type of nodal arrangement, previously known in cotyledonary nodes (Thomas, 1907) but not considered by Sinnott, has recently

been demonstrated to occur at the foliar nodes of a number of dicotyledons (Bailey and Swamy, 1950; Money, Bailey and Swamy, 1949; Swamy, 1953).

The three types of foliar nodal anatomy recognized by Sinnott are as follows:

(a) The *trilacunar* condition, in which three bundles supply the leaf, each causing a gap of its own in the primary vascular cylinder.

(b) The *unilacunar* condition, where one or three leaf traces depart through a single gap in the primary stele.

(c) The *multilacunar* condition where more than three bundles are related to a corresponding number of independent gaps in the primary stele.

Sinnott regarded the trilacunar nodal condition as a very ancient type in the angiosperms, since it occurs in a great majority of the Archichlamydeae. He thought that the unilacunar form was derived from this in some families (1) by the reduction and elimination of the two lateral strands, and in other families (2) by the approximation of the median and lateral strands forming an aggregation of three strands related to a single gap in the stele. The multilacunar form he thought to have arisen by amplification of the number of independently attached lateral strands.

Further, in their series of investigations of the phylogeny of angiosperms, Sinnott and Bailey (1914) regarded the primitive angiosperm leaf as a simple one with palmate venation, possibly stipulate, and provided with three main bundles arising separately at the node. Leaves with parallel venation and those with sheathing leaf bases were observed to be associated with the multilacunar type of node, whereas exstipulate leaves usually exhibited a unilacunar nodal condition. Both of these observations tended to substantiate Sinnott's theory of the derivation of other nodal types by amplification and reduction.

Many dicotyledonous families are predominantly tri-, uni-, or multilacunar but in some families much variation occurs and transitions have been recorded among these three types. The fact that a fourth type of foliar nodal anatomy is now known to occur in the Austrobaileyaceae (Bailey and Swamy, 1949), Monimiaceae (Money, Bailey and Swamy, 1950), and Chloranthaceae (Swamy and Bailey, 1950; Swamy, 1953 a and b), coupled with the common occurrence of such a type of structure in cotyledonary nodes makes it essential to determine whether all types of unilacunar nodes are derived from trilacunar ones since the possibility exists that the fourth type of nodal anatomy may actually be a primitive rather than a reduced one. This fourth type of structure exhibits a single gap through which two discrete strands supply the leaf base, a condition that characterizes many of the gymnosperms and ferns.

The trilacunar arrangement was considered the more primitive condition in the angiosperms since it occurred most frequently in the Ranales and Rosales, which have been regarded as similar to the ancestral angiosperms in other anatomical features. Since the greater number of angio-

sperm families are trilacunar, and since the other nodal forms were thought to be modifications of this by reduction and amplification, its primitiveness has not been questioned until the unilacunar double-trace condition was demonstrated in several ranalian families.

There appears to be insufficient evidence to support the theory that the unilacunar condition is derived in all cases by reduction, especially when nodal arrangements in the vascular plants as a whole are considered. Examination of the transitional forms indicates that the double-trace unilacunar form may be the most primitive arrangement in the angiosperms.

If the unilacunar nodal condition was thought to be derived in some cases by the fusion, or by the loss, of one pair of lateral traces and gaps, could not the single trace of the unilacunar form also have arisen from the fusion of two (unilacunar) traces or by the loss of one trace? In this case, could the primitive number of foliar strands be even rather than odd?

It has been well established that within the woody Ranales primitive morphology of a given plant part is usually accompanied by specialization of other structures, so that no one taxonomic group is conspicuously the most primitive. The primitive characters seem to be divided among a diverse group of families. Therefore, it is not possible to establish which type of nodal anatomy is primitive by correlation with other primitive features; moreover, all four types of nodal anatomy occur in this group. This situation renders dubious Sinnott's correlative argument for the primitiveness of the trilacunar node, and suggests that valid comparative evidence for the primitive nodal condition in angiosperms must consider the Pteropsida as a whole, living and fossil, in an attempt to distinguish the basic nodal pattern in the complex out of which the angiosperms seem to have arisen.

It appears that among the ferns, Cycadofilices, Cordaitales, Bennettitales, Coniferales, Ginkgoales and *Ephedra*, the foliar strand, although often much divided in the petiole and lamina, departs from the primary body of the stele through a single gap. In these groups, the foliar bundles seem often to be *double* in nature.

In most of the Osmundaceae (Tansley, 1907; Scott, 1923; Sinnott, 1911) there is a single foliar strand departing from a single gap in the primary stele. In *Osmunda javanica* Blume (Posthumus, 1924), there is a double leaf trace, each strand with a distinct protoxylem group. The Gleicheniaceae and Ophioglossaceae possess a single gap with one trace, although young stages frequently exhibit a double trace in their first leaves (Bower, 1899, 1908). In all the members of the Marattiaceae, even in young plants of *Angiopteris* though not in the older plants, the condition at the base of the petiole is a leaf trace consisting of *two bundles*, each with a distinct protoxylem group, which depart through a single gap in the stele (Sinnott, 1911). In the fossil ferns of the Carboniferous, *Psaronius* and members of the Botryoptideae, Scott (1900) reports two distinct foliar traces.

Potonié (1896) and Scott (1900) have recorded that most of the Cycadofilices have two distinct foliar traces although fusion of these frequently occurs in the petiole. Sections of the fossil stems of *Lyginodendron* Old-

hamium, *Calamopitys Saturni* and *Medullosa anglica* show two distinct traces passing through the cortex to the base of each petiole, with fusion of these two strands occurring at various levels in the petiole.

The adult plants of the living Cycads exhibit an extremely complex vasculature of the petiole and in some cases the nodal structure is confused by numerous pairs of lateral traces, each of which causes a gap of its own in the stele.

In the Cordaitales, *Mesoxylon* (Traverse, 1950), *Poroxyton* and *Cordaites* (Williamson, 1898) all possessed a double leaf trace departing through a single gap in the stele, which seems to have been typical of the group as a whole. In the Bennettiales, although the vascular supply to the adult leaves is much more simple than in the living and fossil Cycads, the beautifully preserved embryos exhibit the double nature of the vascular strand, and at the cotyledonary node two distinct vascular strands supply each cotyledon, dividing in the tissues of the leaf to form as many as six traces to each embryo leaf (Wieland, 1916). In *Williamsoniella*, however, there is a double trace in adult leaves also (Thomas, 1915).

Jeffrey (1905) indicated the prevalence of a double foliar trace in the Coniferales. In the Abietineae the central strand in the leaf is nearly always double in origin, and this is particularly marked in the early leaves. The two traces are clearly evident in *Araucaria*.

In many of these groups possessing a double foliar strand, for example *Mesoxylon*, *Poroxyton*, *Cordaites*, the Bennettiales and the Coniferales, it has been assumed that these leaf bundles arise from the same main vascular strand (Florin, 1931). These, then, exhibit the continual dichotomy of an initial main strand and uphold the "telome theory" (Zimmerman, 1930). However, in the Ginkgoales and Gnetales, and in several members of the Angiospermae which will be considered later, the two strands of the leaf depart through a single gap in the stele, and each of these is connected to a separate bundle of the eustele.

Ginkgo biloba is reported as having two distinct foliar traces in both long and short shoots (Gunckel and Wetmore, 1946a and b), and Thomas (1907) has observed a similar structure in the cotyledons. The base of the cotyledon is provided with a double bundle and the halves of this separate widely as they pass through the tissues of the embryonic leaves. Gunckel and Wetmore figure the vasculature of the stem and leaf systems emphasizing the connection of each strand of this double bundle with an entirely different and independent bundle of the eustele.

In the seedlings and young adult plants of *Ephedra andina* (Steeves, unpublished) two distinct foliar traces depart from the primary body of the stele through a unilacunar gap (as was also shown earlier in this thesis). These again arise from two entirely independent bundles of the eustele. Sinnott (1914) emphasized that in species of *Ephedra* two distinct gaps occur, but these are gaps in the secondary and not the primary body. This arrangement can therefore be discounted since it is of secondary modification. A similar condition occurs in *Trimenia* and occasionally in *Agathis*. The nodal condition in other members of the Gnetales is ex-

tremely complex in the adult forms, but in the seedlings of *Welwitschia mirabilis* (Rodin, 1953) the cotyledons show two longitudinal vascular bundles entering at the base in cleared seedlings, each dichotomises as it enters the tissue of the leaf and the two lateral bundles of these divide again.

In *Agathis* of the Araucariaceae, a condition similar to that observed in some *Ephedras* has been recorded by Thomson (1913) and the primary arrangement of two distinct vascular strands to the leaves is confused by the insertion of a small segment of secondary wood between the two bundles, causing what appear to be two separate strands in the stele.

This survey of Pteropsida exclusive of the angiosperms can be summarized as follows:

- (1) Almost all of these groups exhibit a unilacunar nodal condition.
- (2) Although frequently there is a single leaf trace, in the majority of the groups examined, two distinct foliar traces depart through the single gap in the primary stele.
- (3) In those cases where a complex condition has been observed in the older plant, the primary condition in the young plants or at the cotyledonary node in the seedling is almost always a double vascular supply to each young leaf or cotyledon from a single gap in the stele.
- (4) Occasionally two gaps occur in the secondary body, but the primary condition in these cases is a single gap with two foliar traces.

This prevalence of the unilacunar, double-trace condition in lower Pteropsida suggests that this nodal arrangement, rather than a trilacunar one, may have been the primitive nodal condition in ancestral angiosperms.

Since the nodal arrangements which occur more frequently in the angiosperms exhibit odd numbers of traces to the leaf base, the question arises as to how these one, three, five, etc. traces could have been derived from the two-trace condition. Since fusion occurs frequently between the two foliar traces of the angiosperms and lower Pteridophyta which possess the fourth type of nodal anatomy, it seems possible that in those cases where a single leaf trace supplies the petiole from a unilacunar gap, this trace may actually represent a fusion bundle.

In *Clerodendron*, *Austrobaileya*, *Trimenia* and *Ascarina* the two foliar traces may unite at varying levels in the petiole or lamina to form a single vein. In *Amborella trichopoda* (Bailey and Swamy, 1948) a single strand appears to depart from a unilacunar gap in the stele, but at sub-nodal levels this strand is seen to consist of two distinct parts which arise from independent portions of the stele (Bailey, unpublished). The fusion of foliar traces observed in the petiole or lamina of the plants cited above has been carried to sub-nodal levels in *Amborella* giving rise to a single trace by the fusion of two initial traces.

Ascarina and *Hedyosmum* of the Chloranthaceae (Bailey and Swamy, 1949; Swamy 1935a,b,c) possess two distinct strands related to a single gap in the stele. In *Hedyosmum* the two strands bifurcate and the two median strands of these then fuse to form a single median strand, while

the two lateral strands bifurcate again, producing a total of five strands. In *Chloranthus* and *Sarcandra*, two vascular strands depart from a unilacunar gap and these immediately bifurcate; the two median members fuse, forming three vascular strands, which become the mid-rib bundles of the leaf. Two smaller subsidiary veins vascularize the marginal portions of the leaf (cf. below).

In the Monimiaceae (Money, Bailey and Swamy, 1950), the family as a whole is uniformly unilacunar. The leaves of *Trimenia* are vascularized by two discrete strands which frequently remain separate throughout the entire petiole and costa of the lamina, each strand supplying by its subdivisions one half of the bilateral leaf, as in *Clerodendron trichotomum*. In some cases, in the leaves of *Trimenia* these two strands may merge to form a single midvein, particularly in the middle and upper portions of the leaf. In all cases the two independent strands arise from two distinctly separate portions of the eustele. In *Piptocalyx* there are two foliar strands which depart from the unilacunar gap, and which bifurcate to produce four strands. The two median strands of this group of four may fuse in the petiole to produce three strands, and in the lamina these coalesce, forming a single midvein. In other words, by a process of bifurcation and fusion, the number of strands has gone from an even number, 2 and 4, to an odd number, first 3 and then 1.

In *Hortonia angustifolia*, *Anthobembix Brassii* and *Mollinedia Rusbyana* (Money, Bailey and Swamy, 1950) (Monimiaceae) increasing odd numbers of foliar traces depart from the unilacunar gap at the node. The number of foliar traces is three in *Hortonia*, five in *Anthobembix* and seven in *Mollinedia*. In each case these arose initially from two distinct strands of the eustele, and this can be demonstrated by following the origin of the foliar traces through several nodes and internodes below.

From the examples cited above, it therefore appears certain that an odd number of foliar traces can arise by the division and fusion of an even number of strands. Particularly, it seems likely that the odd number (1 or 3) of traces related to the median gap of so many angiosperms could have been derived originally from the two-trace condition.

However, the cases of multiple-strand formation mentioned above are not examples of the formation of tri- or multilacunar from unilacunar nodes, because the odd number of strands departs from a single gap as did the primitive pair of traces. Can such multiple traces come to occupy separate gaps? Or is there evidence for the evolutionary addition of lateral traces with new gaps? Acceptance of the hypothesis that the unilacunar node is primitive requires that at least one of these possibilities be demonstrable.

It has been generally accepted since Sinnott's work that the multilacunar condition is specialized, and has been derived from the trilacunar by the addition of laterals with new gaps. It would seem possible for the unilacunar to give rise to the trilacunar in the same fashion. In other words, there is no particular reason to suppose that the kind of amplifica-

tion which resulted in the multilacunar node could not also produce the trilacunar node from the unilacunar one.

The addition of laterals in different gaps to a unilacunar system is adequately shown in the series of nodal forms of Chloranthaceae described by Bailey and Swamy. The primitive form here is undoubtedly the two-trace unilacunar type, as discussed above. However, in *Chloranthus* and *Sarcandra*, where the two traces branch and fuse to form three, as mentioned, two "subsidiary" traces which depart from gaps at right angles to the main gap also vascularize each leaf. The gaps in the eustele in which these traces arise are present but unoccupied in the less specialized Chloranthaceae.

It was mentioned above that in *Agathis* and some species of *Ephedra* a two-trace unilacunar condition becomes converted into an apparent "bilacunar" one by development of a wedge of secondary tissue in the gap parenchyma of the stele, between the two traces (Sinnott, 1914). It seems possible that multiple traces in one gap such as were described above for the Chloranthaceae and Monimiaceae could become separated in a similar way and, by extension of this phenomenon precociously to the primary body, come to occupy separate gaps. A tri- or multilacunar condition would thus have evolved.

It is of great interest that a double trace has been recorded in the cotyledons of so many angiosperms (Thomas, 1907) which have an odd number of traces in adult leaves. In the lower Pteropsida also, the double-trace condition is found in the cotyledons in many cases where a more complex and apparently specialized pattern appears at later nodes. On the other hand, among the plants whose mature nodes are unilacunar with two traces, no examples have been found in which the cotyledonary node does not exhibit similar structure (few, however, have been studied). It is tempting to consider that this cotyledonary condition represents the persistence of a primitive morphology. In cotyledons of *Clerodendron trichotomum*, which have been carefully studied with this question in mind, there occurs sporadically a median protoxylem strand which has arisen as an upward extension of a protoxylem pole of the young root and is apparently not a part of the intrinsic vascular pattern of the cotyledons. It seems likely that other cases where three cotyledonary strands have been observed may be instances of a similar complication.

However, seedlings of many dicotyledons (according to an unpublished survey by one of us) have an odd number of cotyledonary traces from a single gap, which are divided from two strands by branching and fusion in a manner similar to that described above for mature nodes of certain Monimiaceae. A common condition (in seedlings) is for the two original traces to branch once and the two median branches to fuse; giving a total of three traces in the petiole. This is found, for example, in Rosaceae, Leguminosae, *Hamamelis* and *Betula*. In others, e.g. *Impatiens* and *Acer*, a greater odd number of strands is produced by further branching. Laterals occupying separate gaps are present in Magnoliaceae and *Degeneria*, though the main gap shows a two-trace condition (Swamy, 1949). The tendency of the unilacunar pair of traces to fuse in the cotyledon, as ob-

served in *Amaranthus* and *Anchusa*, seems to be carried further to form a single unilacunar trace in the Cruciferae, Primulaceae, and *Celastrus scandens*. A pair of distinct parallel traces such as are found in *Clerodendron trichotomum* occurs commonly in Solanaceae and Labiatae.

The median bundle in the uni-, tri- and multilacunar types requires closer investigation. The whole question as to the exact nature of this bundle could be ontogenetic. Does the median bundle represent (a) two distinct procambial strands which have merged during differentiation; (b) two procambial strands, only one of which has differentiated, or (c) only a single procambial strand? And in those cases where there are two distinct foliar strands, are these developed from (a) two distinct procambial strands which have undergone normal differentiation, or (b) the lateral portions of one procambial strand, separated by a median layer which has failed to differentiate into vascular tissue?

In *Clerodendron trichotomum*, all the different leaf forms (excluding the floral appendages) are supplied in a similar manner, viz. by two discrete vascular strands from a single gap. In each case these two foliar traces arise separately from independent bundles of the eustele. In the foliage leaves, bud scales, floral bracts, and cotyledons these two traces originate from two distinct procambial strands. These represent, therefore, two separate procambial strands which have undergone normal differentiation. Similarly, in *Austrobaileya*, where each leaf is supplied by two discrete vascular traces arising from two independent portions of the eustele (Bailey and Swamy, 1949), these foliar traces have differentiated normally from two separate procambial strands. The two separate foliar traces of *Trimenia* (Money et al., 1950) also arise by the normal differentiation of two procambial strands. To the extent that this kind of evidence can indicate the nature of the foliar traces, the present observations suggest that the two leaf traces in the forms studied are distinct, and not the result of abnormal differentiation of a single strand.

This fourth type of nodal anatomy exhibited by *Clerodendron trichotomum*, which occurs in a great many dicotyledonous seedlings at the cotyledonary node, and in the adult plants of several ranalian genera, is a type of nodal structure which must be considered of phylogenetic importance. The double-trace, unilacunar condition does not conform with Sinnott's unilacunar form since it is not attained by modifications of a trilacunar node. The present survey of nodal anatomy, and the ontogenetic studies on *Clerodendron trichotomum* presented here, indicate that the fourth type of nodal structure may ultimately prove to be the nodal arrangement of ancestral angiosperms. In order that such a theory could become a working hypothesis upon which phylogenetic investigations could be based, further extensive ontogenetic studies are needed. It is tentatively suggested here that the unilacunar double-trace condition was the primitive structure of the node among the Tracheophyta, and that the three nodal forms described by Sinnott for the angiosperms are specializations of this. Accordingly, Sinnott's hypothesis of nodal evolution in dicotyledons can be modified as follows (FIG. 17): (a) The two-trace unilacunar condition

can give rise to a single trace by fusion; (b) the double-trace unilacunar condition can give rise to the trilacunar condition by fusion and the addition of one pair of lateral traces and gaps. The trilacunar form can give rise to a unilacunar condition, as it appears frequently to have done, by the loss of laterals, and to a multilacunar form by the addition of pairs of laterals as Sinnott has already postulated.

In conclusion, it should be emphasized that excessive attention has been focused in the past upon the addition or elimination of pairs of independently attached laterals, and to topographical features at the nodal level. Greater consideration should be given in the future to detailed investigations of vascular tissues related to the median gap, and to tracing these structures both downwards in the stem and outwards into the petiole and lamina of the leaf. Bifurcations and fusions of vascular strands, and transitions from an *even* to an *odd* number of such strands, occur at various levels

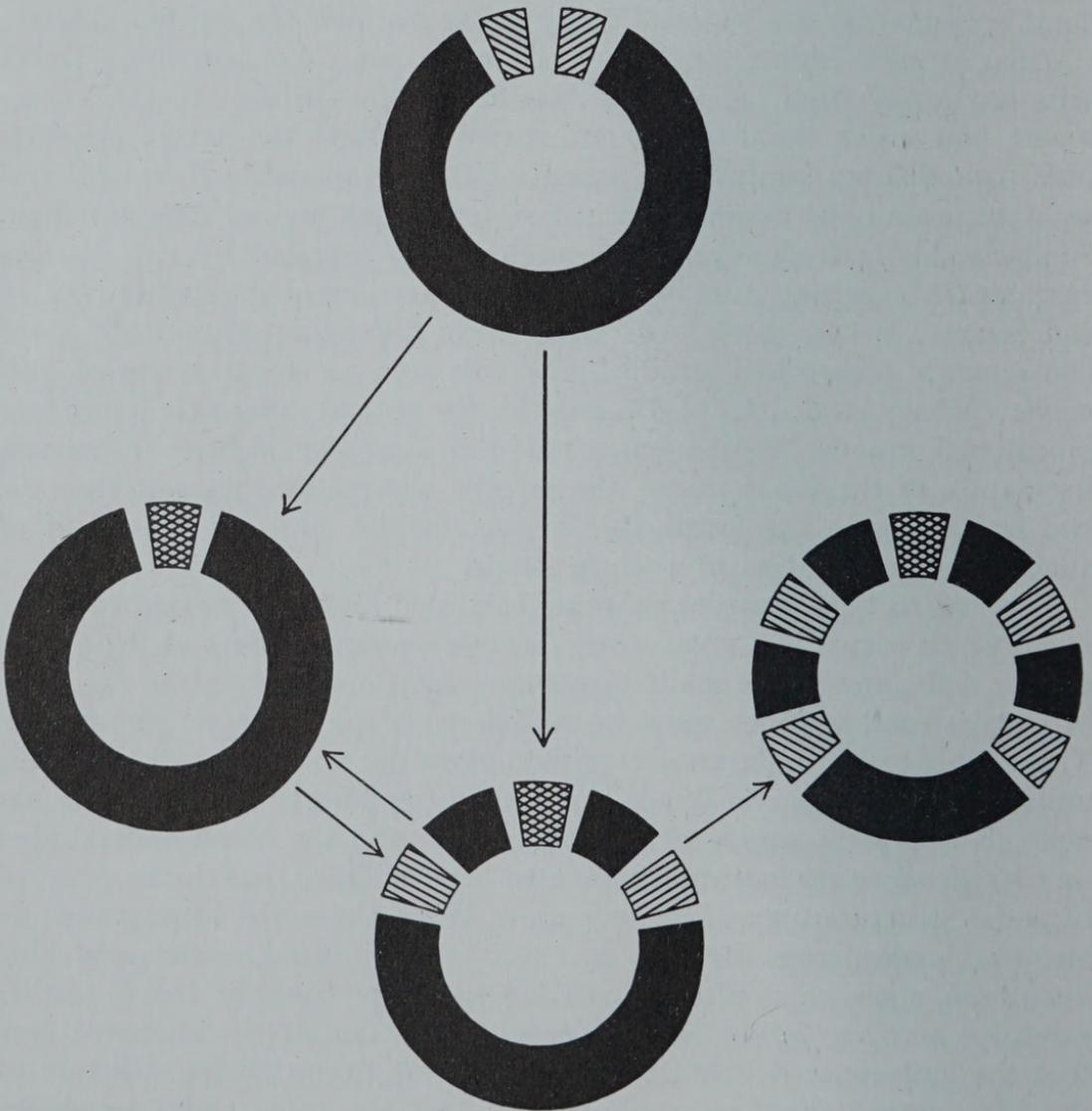


FIGURE 17

THE POSSIBLE DERIVATION OF THE DICOTYLEDONOUS NODAL FORMS FROM THE UNILACUNAR DOUBLE TRACE CONDITION.

of the stem and leaf. Structures which appear to be similar at the nodal level may not be truly homologous, and conversely differences which seem outstanding at the nodal level may acquire a different significance where comprehensive developmental studies at successive levels of the stem and leaf are made.

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PLATE I

1 and 2, expanding terminal and axillary buds of *Clerodendron trichotomum*, showing the cataphylls and transition leaves. Note the retarded growth of the terminal bud (2). $\times 2.5$. 3. Early stages in the germination of the seed of *C. trichotomum*, from left to right of the photograph: (a) Seed with testa removed. (b) Elongation of radicle. (c) Emergence of epicotyl. (d) Beginning of elongation of crook-shaped epicotyl. (e) and (f) Elongation and straightening of epicotyl. $\times 2$.



MARSDEN AND BAILEY, CLERODENDRON TRICHOTOMUM



Marsden, Margery P.F. and Bailey, Irving W. 1955. "A Fourth Type of Nodal Anatomy in Dicotyledons, Illustrated by *Clerodendron trichotomum* Thunb." *Journal of the Arnold Arboretum* 36(1), 1–51. <https://doi.org/10.5962/p.324644>.

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