DISCUSSION

4 DISCUSSION

Leaves of most angiosperms are determinate in their growth and have limited life span. There is a basic pattern of development which is common to leaves in general and the difference in the final form of a mature leaf can be explained as variations of one morphogenetic theme (Wetmore and Steeves, 1971). Upon and within the apex of the stem a leaf primordium is distinguishable in a definite relationship to the other primordia which is expressed eventually as the phyllotaxy of leaves on a stem. This arrangement may be established as alternate, opposite or whorled. Both Gmelina arborea and Tabebuia rosea show opposite and decussate phyllotaxy. Gmelina has a simple leaf type and Tabebuia leaf is pentafoliate type.

4.1 Elongation of the petiole

The leaf primordium undergoes a period of growth establishing a foliar axis. In the course of development, a leaf enlarges and attains its ultimate form. The petiole is interpolated in the development of many leaves. Masuda (1933) studied the elongation of the petiole in woody and herbaceous plants and revealed three patterns. Type 'a' had each zone elongate almost equally which was regarded the most common type. In 'b', the upper zone showed a greater amount of elongation and in type 'c', the lower zone showed a greater amount of elongation. Growth was completed in a short span of 6 days or over a long period of 51 days. However, he has not

included the leaf base or pulvinal areas in the observations.

Howard (1974) revealed that almost all the leaves completed the elongation of the petiole in a period of 12 to 15 days, and that the petioles appeared to increase in length in such a way that the middle of the total length of the petiole increased fastest in length and stopped its growth earliest, i.e., expansion and cessation proceeded in a wave from the middle of petiole towards each end. He considered the pulvinus base separately and showed that it has the least elongation.

I have made a similar study of petiole in Gmelina and Tabebuia. In both the plants pulvinus base has been included with the petiole base for measurements. Gmelina belongs to 'a' type of pattern wherein the three regions of the petiole elongate simultaneously and the elongation is completed in a month. Tabebuia belongs to 'b' type pattern wherein the distal portion of the petiole elongates more and the petiole ceases elongation in 15 days.

4.2 Evergreen versus deciduous habit

One of the most universal characteristics of trees and shrubs is more or less recurrent shedding of both vegetative and reproductive portions of their plant body (Kozlowski, 1973). Shedding of leaves in the life of an individual tree represents a reduction in its photosynthetic and transpiring tissues, except when leaves are produced and

lost steadily throughout the year (Perry, 1971), i.e., throughout the growth period of the plant old leaves abscise and new ones develop; in this way the plant maintains a relatively constant leaf area which is photosynthetically active.

How to distinguish an evergreen from a deciduous tree has been the subject of much argument and confusion (Longmann and Jenik, 1974; Tomlinson and Zimmerman, 1978). Trees of some species achieve maximum leaf area early in the season and do not produce any leaves during the rest of the year, whereas others add new leaves, either by continuous production and expansion of new leaf primordia, or by several intermittent 'flushes' of growth involving recurrent formation and opening of buds during the growing season followed by expansion of their contents (Kramer and Kozlowski, 1979).

Deciduous trees and shrubs in temperate regions shed the bulk of their leaves nearly simultaneously during the autumn. Deciduous tropical trees and lianas lose leaves both in season and throughout the year. So called evergreen species in both temperate and tropical trees shed leaves after one or more years of growth and development (Ewers and Schmid, 1981), but all the leaves on an individual plant do not abscise at the same time, therefore the tree always bears foliage.

Thus basically it is a matter of the relative timing of bud break and abscission that distinguishes an evergreen tree from a deciduous tree. But again, the shoot growth of tropical trees is not always synchronized, so that some branches may be in full flush, others may be leafless, while some again may be in flushing phase. Taking all these into consideration Longmann and Jenik (1974) recognized four patterns of leaf retention and shedding in tropical forest trees and have pointed out that it was difficult to draw sharp lines among these classes.

(i) Periodic growth, Deciduous type: Leaf shedding occurs well before bud opening. The life span of leaves is about 4 to 11 months. The entire tree or branch is leafless for several weeks to months. Leaf shedding and bud opening do not appear to be related.

(ii) Periodic growth, Leaf exchanging type: Leaf shedding is related to bud opening. The life span of leaves often is about 6 to 12 months. The new leaves emerge approximately when the old ones are shed.

(iii) Periodic growth, Evergreen type: Leaf shedding occurs long after bud opening. The life span of leaves is 7 to 14 months. The branch or tree is definitely evergreen.

(iv) Continuous growth, Evergreen type: Leaf initiation and loss occur continuously. No dormant buds are formed. The life span of leaves is variable but may be upto 14 months.

Leaf production and shedding are irregular and vary with environmental factors.

Gmelina is of periodic growth, deciduous type. Leaf fall begins in the month of December/January and in the month of February the tree is entirely leafless. Young shoots come up only in May/June. Tabebuia is grouped as the periodic growth, evergreen type wherein leaf shedding occurs long after bud opening and there is no simultaneous leaf fall.

4.3 Nodal anatomy

The node is commonly defined as the position on the stem at which the leaf occurs. Variations within this definition allow for the node to have one leaf or two leaves as opposite condition or several in a whorled condition associated with a single node. Many have defined the node in broad terms (Mitra and Majumdar, 1952; Howard, 1970, 1974; Dickison, 1975), yet the actual boundaries of the node are ill defined. Nodal region and leaf base are used here to the approximate lower and upper bounds respectively of the commonly accepted interpretation of the node.

The three widely accepted nodal types, on the basis of which species are often classified are unilacunar, trilacunar or multilacunar (Sinnott, 1914). The three are categorized on the basis of the number of lacunae (leaf gap) that occur at each node when the leaf traces depart the stem axis. The leaf gaps are formed by acropetal closing of the procambial

strands above a departing leaf trace (Majumdar, 1942; Larson and Pizzolato, 1977). Esau (1977) defined 'leaf gap' as a parenchymatous region in the vascular cylinder of the stem occurring where a leaf trace deviates from the vascular cylinder of the stem to the leaf.

Marsden and Bailey (1955) recognized a fourth type of nodal anatomy in which two traces with independent origin in the vascular cylinder are associated with a unilacunar node termed as the 'double trace' unilacunar node. Double trace strands are common in angiosperms particularly in the species exhibiting decussate phyllotaxy (Bailey, 1956). Howard (1970) recognized the 'split lateral' or 'common gap' condition in which, the outermost lateral traces of opposite leaves in a decussate phyllotaxy share a common gap.

Some members of the family Verbenaceae show varying stages of nodal condition. Unilacunar node with double trace condition has been reported in Clerodendron trichotomum (Marsden and Bailey, 1955). Shah et al. (1969) reported varying stages of nodal condition in three species of genus Clerodendron. In C. splendens the node is unilacunar with a compound single trace formed by the fusion of 2 independent strands and others medianly situated smaller strands. In C. inerme the compound single trace is formed by the fusion of two or three strands, but in C. phlomidis the two strands arise independently near the node as branches of a single axial strand.

In Gmelina, each leaf is served by a double trace formed by 5 composite strands, which merge to form a single trace before leaving a single gap. That is, the unilacunar leaf trace is a compound single trace which again trifurcates at the base of the petiole. Ramji and Parmeswaran (1961) have reported such a condition in the stem of Clerodendron aculeatum.

In Tabebuia the node is unilacunar with 5 traces departing from the internodal vascular system, i.e., it is a multitrace unilacunar nodal type. This condition has been reported earlier in cotyledonary nodes (Kato, 1966; Sugiayama, 1976) and in adult leaves with unilacunar node (Watari, 1934, 1936, 1939; Benzing 1967; Kato, 1967). This condition is most frequent in species with unilacunar nodes (Sinnott, 1914; Philipson and Philipson,1968, Dickison, 1975; Neubauer,1981), however, trilacunar nodes also show this condition (Howard, 1970; Sehgal and Paliwal, 1974; Neubaver,1979).

Added to the basic types of nodal structure, Metcalfe and Chalk (1950) listed 55 families of dicotyledons in which cortical and/or medullary bundles are known. Gmelina shows cortical strands extending the entire petiole but Tabebuia lacks them. They have varied role in their relationship to particular vascular system and in their contribution to the vascular supply of the leaf (Fahn and Bailey, 1957; Balfour and Philipson, 1962).

The general pattern of relationships of the cortical vascular system to the leaf supply includes (i) a cortical system may run all through the length of the stem without association with the main supply of the leaf, (ii) the cortical system may run the length of the stem giving rise to girdling branches at each node, while other branches enter the petiole, (iii) the cortical system may originate just above the node and enter the leaf at the next node (Howard, 1974). In Gmelina cortical strands are absent in the axial vasculature. The cortical vascular system in the petiole develops later than its principal vascular system. It develops as a divergence from the lateral strands at the base of the petiole and traverses independently or branches in the entire petiole in the cortical region on either side of the adaxial groove. At the distal end of the petiole the cortical strands extend along the margin of the lamina.

4.4 Petiole vasculature

A variation in the number, position and arrangement of vascular strands is noticed in sections from different regions of the same leaf/petiole. In general, leaf trace strands depart from the principal vascular system within the length of the internode that is represented superficially by the petiole base or leaf scar, i.e., the vascular strands make an abrupt angle and structure and organization of petiolar strands are indeed complex. In Gmelina the single leaf trace trifurcates giving rise to a median and two

laterals which subdivide and traverse the petiole with or without anastomosing and fusion. The median strand is the largest and it remains mostly undivided and discrete upto the distal end of the petiole. In Morus the node is reported trilacunar, three trace and the median trace directly enters the base of the petiole undivided (Govindaiah, et al., 1990). In Gmelina it is the laterals which divide and are mainly involved in the formation of the petiole vasculature. No definite pattern of anastomosing or fusion was noticed. Subdivision of traces and approximation occur randomly at any level in the petiole. A similar observation has been reported in the petiole of a deciduous tree, Crataeva (Murukesan,1993). At the laminar base the petiolar strands are redistributed and dispersed as the laminar veins. The median strand traverses to the apex of the lamina as the primary vein. No predictable pattern of bifurcation or fusion was observed. In Tabebuia the petiole and laminar base serve as the regions of extreme vascular diversity. The three leaf trace strands exit the node and enter the leaf base which serves as a major centre where the strands are subdivided equally and oriented in various ways. The strands in the petiole continue unaltered in the entire petiole upto the distal end. The laminar base serves as a centre for redistributing and dispersing the petiolar strands into the petiolule. The petiolar strands equally subdivide on their either side and are reorganized before being distributed as petiolule strands. A similar kind

of observation of the petiolar strands equally subdividing and traversing without much variation has been reported in the petiole of Salvadora, an evergreen tree (Murukesan, 1993). The median petiolules vascular system separates out the last in Tabebuia.

The leaves of Gmelina when compared to the evergreen leaves of Tabebuia have a short life span. Within the short life span of the leaves the assimilates are to be transported to the other parts of the plant before their senescence for which the petiole serves as an intermediate organ and hence the enhanced functional requirements are met by the numerous vascular strands. The increase in the number of vascular strands by subdivision of leaf traces results in an increase in the vascular area (Larson, 1984a). The more number of vascular strands indicates that there are many channels for translocation thereby increasing the translocating efficiency of the leaf.

Although anastomoses and approximation of strands are observed to a greater extent in Gmelina than in Tabebuia the identity of principal vascular strands is conspicuous even in a mature petiole. In a mature petiole of Gmelina it was possible to recognize the individuality of principal vascular strands because they were discrete even at maturity, though the interfascicular parenchyma is lignified. In Tabebuia though many trace strands depart the principal vascular cylinder through a single gap in the young petiole the median

strand maintains its identity. In a mature petiole because of the development of the interfascicular cambium and its derivatives to form a closed cylinder the identity of individual strands is later lost.

The arrangement of vascular strands in the petiole varies in different species. The crescent or arc pattern is one of the two main vascular pattern found in petioles (Howard, 1979b). In Gmelina the strands are separate and arranged in the form of an arc. A ring of bundles which is found in many other plants (Watari, 1934; Howard, 1979a) is the second pattern. The vasculature of Tabebuia consists of separate strands arranged in a ring.

The internode - node - leaf continuum (Howard, 1974) is exemplified in Gmelina and Tabebuia by the continuity of the vascular tissues in the internode, node, petiole and lamina. The vascularization of leaves in both these plants conforms to a general pattern. Leaf trace strands exit the node and enter the petiole base where they are subdivided and mixed in various ways as in Gmelina or without much anastomosing as in Tabebuia. The reoriented strands traverse the main part of the petiole with (as in Gmelina) or without (Tabebuia) branching and at the base of the lamina the petiolar strands are distributed as veins.

4.5 Functional significance of vascular strands in the petiole

The vascular strands of the petiole and midrib are extensions of the original trace strands from the internode (leaf trace strands) and hence together provide vascular continuity between stem and the leaf. As each strand in the petiole is continuous with a specific portion of the lamina they may provide the independent channel for rapid translocation as suggested by Bailey (1956).

4.6. Primary vascular differentiation

The primary vascular system is initiated in the form of its meristem, the procambium (or provascular tissue). Vascularization proceeds developmentally from a primary to a secondary vascular system with the primary serving as both a structural and an organizational template for the secondary (Larson, 1980a). Primary vascular organization within the shoot can be most conveniently analyzed in terms of the leaf trace concept (Esau, 1965a). According to this concept, vascularization of the stem and leaf is developmentally inseparable. The primary vascular system of the stem consists of an aggregation of leaf traces which do not terminate at the leaf base but they develop through the petiole and finally into the lamina to form a ramifying vein system. The leaf trace system is therefore, a functional as well as a developmental continuum between the stem and the leaf.

The procambium is the logical starting point for discussing the vascularization because it serves not only as a precursor of the cambium but also a template for all subsequent vascular tissues. Anatomists generally agree that the procambial system develops continuously and acropetally in continuity with the older procambial strands in the stem (Esau, 1943b, 1954, 1965a; Sterling, 1945; Philipson and Balfour, 1963; Cutter, 1971; Devadas and Beck 1971; Wetmore and Steaves, 1971; Shininger, 1979; Larson, 1982). The structure of procambial strands in Gmelina and Tabebuia verifies their acropetal and continuous development from older procambium below. New procambial strands that diverge from the parent trace acropetally in the main axis and develop into the petiole are both larger and more differentiated at their points of divergence than those at their acropetal fronts.

The acropetally developing procambial strand that first establishes contact with a new primordium to become a leaf trace continues its acropetal development as the primordium develops in both Gmelina and Tabebuia. Invariably the median trace develops first and its main derivative develops as a midvein to the tip of the mature lamina in Gmelina and the median petiolule in Tabebuia. This observation confirms with those of earlier workers (Lersten, 1962; Ramji, 1975; Larson, 1984a).

In Populus deltoides other than the acropetally developing strands, there are strands which originate at the node as subdivisions of the leaf trace and develop either basipetally in the stem, acropetally in the leaf or both, and have been designated as the 'subsidiary strands' (Larson, 1975). Acropetally they contribute to the petiole vasculature and lamina venation. Basipetally they contribute to the integration of the stem vasculature. In Gmelina in the basal region of young elongating petiole a few procambial strands develop independently from the interfascicular parenchyma by its dedifferentiation and redifferentiation as procambial cells and then develop both acropetally and basipetally. These strands have been termed as subsidiary strands which are comparable to those of Populus except that they differ in their origin. The subsidiary strands observed in the young elongating petiole of Gmelina may supplement the additional vascular requirements in the developing petiole.

4.6.1 Vascular meristem

The procambium in the stem differentiates from derivatives of the apical meristem which is defined as that part of the shoot apex distal to the youngest leaf primordia or the youngest node (Esau, 1965a). The subapical region in which the procambial strands are first evident has been interpreted in many different ways. In most apices there is a ring of meristematic tissue in which procambial strands can be first detected when serial sections are followed downward

from the apex. This meristematic region has been variously referred to as prodesmogen, meristem ring, procambial ring, provascular tissue or residual meristem (Esau, 1943b) and its histogenic significance varied accordingly. The residual meristem is considered a derivative of the apical meristem (Esau, 1954) or a continuation of the eumeristematic part of the apical meristem (Esau, 1965b).

The petiole develops away from the shoot apex and its procambium develops from older procambial strands present below in the axis. It is in no way related to the apical meristem and hence none of these terminologies and concepts can be generally applicable in the petiole. The development of procambium and establishment of the primary vascular system in the petiole are independent of stem-leaf relationship. In Gmelina the meristematic arc in which acropetally developing procambial strands are not yet discernible has been termed as the 'vascular meristem'. The vascular meristem assumes histological significance when the acropetal procambial strands developed within it. It may be considered as the procambial front or the region of the 'procambial strand fade out' as proposed by Larson (1975).

In the subjacent region procambial strands can be first distinguished from the adjacent uncommitted cells of the vascular meristem. Cells of the vascular meristem not committed to the strand development become the interfascicular vascular meristem (Esau, 1965a;

Devadas and Beck, 1971). One effect of the acropetally developing procambial strands is to block out pattern in the vascular meristem by separating the fundamental tissues of the pith and cortex (Wetmore and Steeves, 1971), i.e., the acropetal procambial strands block out the prospective vasculature in the vascular meristem and the organized procambial system thus formed serves as a template for further vascularization.

4.6.2 Procambium

The anatomical approach to vascular development in shoot apical meristem has generated some controversy concerning what should properly be called procambium. By definition procambium shows the first signs of differentiation towards vascular tissue by the derivatives of the apical meristem (Esau, 1965a). Difficulty in identifying the first procambial cells has been expressed (McGahan, 1955; Larson, 1982). Shininger (1979) also emphasized the absence of good cytological and biochemical evidence for identifying procambium. At the microscopic level it is seldom possible to recognize anatomically single procambial cell in either transverse or longitudinal plane. Groups or islands of procambial cells can be recognized by their dense staining and small size relative to the adjacent cells in transverse plane and by their elongated appearance in the longitudinal plane. Few details are known of procambial ultrastructure (Catesson, 1974) and no distinguishing characters have been

observed during morphological differentiation even in culture systems (Phillips, 1976). Attempts have been made to biochemically characterize procambium particularly in shoot apex (Van den Born, 1963; McLean and Gahan, 1970). Mueller (1991) identified procambium in the primary root of Trifolium pratense by using esterase activity as a histochemical marker in the early stage of vascular differentiation. Hernandez and Driss Ecole (1990) identified the cells at the origin of the procambium in Lycopersicon esculentum during leaf initiation after 3H thymidine incorporation and high resolution autoradiography. They were identified by their position, which consists in a selective area found at the edge of the already differentiated procambium and by their ability to synthesize DNA.

In both Gmelina and Tabebuia the procambial strands were identified in accordance with the structural definition given by Esau (1965a), i.e., by their dense staining and greater cell activity which distinguish them clearly from the ground meristem cells which show an early and increased vacuolation. The procambial cells undergo repeated longitudinal divisions expanding transversely to a limited degree and hence in a longitudinal view appear as dense and narrow cells, elongated parallel with the longitudinal axis of the cell. The procambial strand enlarge by cell divisions within the strand and by acquisition of cells from the vascular meristem. The procambial cells elongate by passive accommodative growth during the early elongation

of the petiole.

4.6.3 Metacambium

Many authors have attempted to distinguish the vascular cambium from procambium. The commonly accepted concept is that procambium and cambium represent sequential developmental stages of the same vascular meristem (Esau, 1943b, 1965b; Cutter, 1971; Steeves and Sussex, 1972; Fahn, 1982; Iqbal, 1990). The two terms procambium and cambium refer to those parts of the continuum associated with primary and secondary growth of the plant body respectively and the primary growth of the vascular system merges gradually into the secondary growth (Sterling, 1946; Eames and McDaniels, 1947; Philipson and Ward, 1965). Most of the works on the ontogeny of the vascular system, however,have concentrated on either the procambium or the cambium in the shoot and do not include a precise description of transition between them (Esau, 1942; Gunckel and Wetmore, 1946; McGahan, 1955). Cells from widely separated parts of the meristem can be readily identified but the characteristics of the procambial cells during their transition into the cambium are not easily distinguishable. Sterling (1946) found no sharp transition between procambium and cambium in Sequoia. Catesson (1964) claimed that the formation of the cambium can be defined clearly, especially by the appearance of vascular rays in tangential sections. Anatomists have recognized and acknowledged changes in the procambial derivatives by

distinguishing metaxylem and metaphloem from protoxylem and protophloem, although not necessarily by universally accepted definitions (Esau, 1965a,b; Fahn, 1982). However, similar stages have neither been recognized nor proposed for the lateral meristem especially in the petiole.

Larson (1976) tried to resolve this dilemma by identifying an intermediate stage between the procambium and the cambium in the seedlings of Populus deltoides. The recognition of a metacambium stage does not contravene the original concept of procambium-cambium continuum. Metacambium is distinguished from procambium both by cell division planes and by products of these divisions.

An intermediate stage between procambium and cambium was traceable only in Tabebuia. In the petiole of Gmelina the secondary vascular meristem cells do not resemble the cambial cells, and are termed as the 'transit cells'. A true cambium is absent. Even based on the characteristics of the derivatives an intermediate stage could not be identified because the events rapidly overlap in the successive stages in the petiole of Gmelina.

In Gmelina the procambium is homogeneous in its early stage of development. Radial seriation of procambial cells resulting from early periclinal divisions occurs when a petiole is very young. In the late procambial stage the procambium micromorphologically consists of two types of

cells, the rectangular and polygonal ones.

In Tabebuia the procambium is homogeneous and observed in discrete strands. The metacambium is fully established when the petiole is 2 cm long. The metacambium develops within the vascular strands replacing the irregularly distributed procambial cells with a tangentially oriented meristem. This meristem is preceded by the periclinal divisions in the isolated cells of the procambium. As the periclinal divisions increase in frequency a continuous band of metacambium eventually appears across the strand first and when the division process spreads laterally into the interfascicular region between the strands, a complete metacambial cylinder is formed.

In the stem the linking of the fascicular vascular meristem in each strand usually occurs following the onset of secondary growth by the development of sectors of interfascicular cambium from the parenchyma cells between each primary vascular strand (Butterfield, 1976). In the petiole of Tabebuia when metacambium is fully established within the vascular strands periclinal divisions are noticed in the interfascicular parenchyma. The formation of this interfascicular metacambium is discrete when a petiole is 5cm long. Initially primary xylem (metaxylem) develops in the interfascicular region until a complete cylinder of xylem is formed later.

Cumbie (1967), Soh (1974a,b) and Butterfield (1976) report the procambium in shoots of some species forming an almost complete cylinder from its early or late appearance. They do not, however, indicate the presence of the intermediate metacambial stage. In Tabebuia during the procambial development the vascular strands are discrete, until the metacambium is fully established within the vascular strands. With the development of the interfascicular metacambium and its derivatives it forms a complete cylinder.

Metacambium is also identified on the basis of its derivatives. Although protoxylem elements differentiate from irregularly oriented procambial cells, they occasionally may occur in short disjunctive radial files. Metaxylem elements are formed from derivatives of radially arranged metacambium cells, and therefore occur in consecutive radial files. Some metaxylem elements do differentiate from procambium cells before the radially seriated meristem is evident in the strands. In Tabebuia the xylem elements arising from the interfascicular metacambial derivatives have been designated as metaxylem, because they appear simultaneously with the metaxylem elements formed from the metacambium in the vascular strand. These elements can also be termed as late metaxylem elements because they appear after the differentiation of protoxylem and early metaxylem of the vascular strand. So the differentiation of primary xylem occurs in two stages. Initially, a number of protoxylem and

metaxylem elements develop within the strand. These are gradually linked into a complete cylinder of primary xylem by the differentiation of the interfascicular metacambial derivatives.

The micromorphology of the metacambium in Tabebuia later becomes organized into two cell systems, one of elongated cells and other of short cells in the strands, when viewed in tangential sections. Differentiation of two cell systems has been reported in the early development of procambium in the stem of Canavalia (Cumbie, 1967) and in the late development of procambium in the shoots of Ginkgo, Acer, Aucuba and Weigela (Catesson, 1964; Soh, 1972, 1974a,b). Though they have reported the formation of two cell systems during the early or late development of procambium, from the study of photographs and diagrams of transverse and longitudinal sections of these species I feel that procambium stage precedes the differentiation of the two cell systems and that the stage at which differentiation of two cell system occurs resembles the metacambial stage,described in the present work. The differentiation of two cell systems has also been reported in cotyledonary node of seedlings in Pinus koraiensis (Hong and Soh, 1993).

4.7 Vascular cambium

Radial seriation of cells and their derivatives is the characteristic most commonly associated with the vascular cambium. The occurrence of radial seriation of cells during

the procambial development is common among dicots and has been documented in many species (Esau, 1943b; Fahn et al., 1972; Soh, 1990). In the petiole of Gmelina and Tabebuia the procambium as well as the cambium cells show radial seriation. Despite its rather common occurrence, radial seriation of protoxylem and protophloem cannot be considered either as a criterion of or even as an indicator of impending cambial development. Not only do these events occur during early petiole elongation but they originate from cells that can in no way be considered as belonging to a tangentially oriented meristem.

One of the most characteristic features of the vascular cambium is the presence of two types of meristematic cells, elongated fusiform initials being responsible for the production of the axial elements of secondary xylem and phloem and the more or less procumbent ray initials being responsible for the production of the horizontal ray system (Philipson and Ward, 1965). In the petiole of Gmelina though all the characteristics attributed to secondary growth are present the secondary vascular meristem cells do not resemble the typical cambial cells. A comparative study on vascular cambium in the stem of 12 species of Verbenaceae has revealed that the cambium is composed of both fusiform and ray cells (Ghouse et al., 1980; Rao and Dave, 1981). Even in the stem of Gmelina the cambium showed two types of cells (Dave and Rao, 1982). Hence in Gmelina petiole I have termed

the secondary vascular meristem cells as the 'transit cells', i.e., an intermediate stage between the procambium and the cambium.

The two types of meristematic cells were noticed in the vascular cambium of the petiole in Tabebuia. During the later stages of petiole elongation the procambium in Gmelina and metacambium in Tabebuia assume characteristics resembling those of procambium-cambium transition. The vascular strands at this stage possess both the general appearance and many of the structural characteristics attributed to cambium when viewed in transverse plane. The metacambium in Tabebuia appears as a heterogeneous tissue in tangential view. Both intrusive growth of the elongated metacambial cells (the incipient fusiform cells) and blocking out of the prospective ray tissue occur apparently during the late phase of petiole elongation. None the less neither the meristematic tissue nor its derivatives assume the true characteristics attributed to cambium until the petiole elongation has ceased. The two distinct system of cells in the petiole during the metacambial stage are therefore still a part of the primary body and at the most be considered as transitional or incipient stages of cambium.

Events leading to the identification of cambium occur gradually. Several investigators described events like the intrusive growth of the cells, blocking out of prospective ray tissue etc., that occurred during the final stages of

primary growth in the internode and attempted to define cambium in terms of these events (Catesson, 1964; Enright and Cumbie, 1973; Butterfield, 1976). Fahn et al. (1972) also described these events in the stem of Ricinus but they did not relate them to primary growth.

None of the attempts to distinguish cambium from procambium were successful when based exclusively on either the appearance or structure of the meristematic cells. It is a sum of events together which signal the differentiation of cambium.

The most definitive criterion of distinguishing the vascular cambium is the nature and structure of its derivatives. Bailey (1944) observed an abrupt decrease in the length of tracheary elements during the transition from primary to secondary xylem and suggested this to be a criterion to distinguish the two growth regions. Bailey also observed that the metaxylem vessels elongate considerably during differentiation, but the secondary xylem vessels do not; they remain essentially of the same length as that of their cambial precursors. In analysing the procambium-cambium transformation in the petiole of Gmelina and Tabebuia, these observations have been taken into consideration.

At the termination of petiole elongation the secondary growth complements the primary growth. During the transition considerable overlap in the events occur. None the less tissues of the secondary body maintain both developmental and

functional continuity with those of the primary body. Larson (1980b) in his investigation in the seedlings of Populus deltoides concluded that metaxylem is not transformed in to secondary xylem. Rather, metaxylem vessels anastomose with secondary xylem vessels during the transition and functional continuity is maintained. In Gmelina and Tabebuia while metaxylem vessels are maturing secondary xylem vessels are observed differentiating.

Although secondary elements are observed during the final stages of internodal elongation in Populus, they do not mature until the internodal elongation has ceased. At this time the elements attain their final lengths and their walls begin to lignify. Lignified elements can be readily detected by their birefringent walls in polarized light. Because fibers are associated with secondary vessels but not with metaxylem vessels, the primary-secondary transition was judged to occur when fibers with birefringent walls were first detected both within and between adjacent traces forming the vascular cylinder. Such an observation was very well confirmed in Tabebuia. In Gmelina xylem fibers are absent, but lignification of protophloem fibers, xylem parenchyma and decrease in the length of vessel elements were noticed and were found to be a useful critera to judge the transition between procambium and the secondary vascular meristem.

That the primary (procambium) and secondary (cambium/transit cells) vascular meristem are sequential stages of the same meristem is well illustrated in my studies in petiole of Gmelina and Tabebuia. Beginning with the earliest detectable procambium in the vascular meristem, the procambium passes imperceptibly through a series of stages. Although differences were noted when the two discrete stages of the vascular meristem are compared, none the less the transitional stage between them presents a continuum.

4.8 Interfascicular cambium

When procambial strands are first discernible in the vascular meristem, they appear as discrete groups or islands of cells in transverse sections. The cells immediately surrounding and interjacent to the procambial cells have been referred to as interfascicular residual meristem (Larson, 1975). I have referred to this region as the interfascicular vascular meristem in the petioles of Gmelina and Tabebuia.

In the petioles of Gmelina and Tabebuia when the primary vascular system is well established, the vascular strands are discrete with interfascicular parenchyma between them. Many cells of the interfascicular vascular meristem are incorporated in the developing and enlarging procambial strands. The cells which do not contribute to the development of fascicular tissue become highly vacuolated and differentiate as interfascicular parenchyma. In Gmelina these

cells remain parenchymatous throughout the developmental stages of the petiole. On maturity of the petiole these cells assume additional characteristics of undergoing lignification. In Tabebuia interfascicular cambium develops between the vascular strands.

The origin of the interfascicular cambium is still a matter of controversy. According to some authors the interfascicular meristem is a secondary meristem, i.e., it is a derivative of differentiated interfascicular parenchyma through the resumption of meristematic activity (Esau, 1977; Swamy and Krishnamurthy, 1980; Fahn, 1982). Siebers (1971) suggested that a direct ontogenetic continuity exists between procambium and interfascicular cambium. Some investigators have also suggested that the interfascicular cambium originates from the dedifferentiation of the interfascicular parenchyma (Catesson, 1964; Devadas and Beck, 1971; Phillips, 1976). Some have also argued that formation of cambial strips takes place in the interfascicular areas only in line with those strips of the fascicular sectors and that too under the influence of the fascicular cambium which originates earlier (Eames and MacDaniels, 1947; Catesson, 1964). According to Soh et al. (1989), in the hypocotyl of Ricinus, the tissue from which interfascicular cambium originates is structurally different from the parenchyma. The ontogenetic pattern of the interfascicular cambium is nearly the same as that of fascicular cambium.

Differentiation of cambium from the interfascicular parenchyma has been studied by many authors. It is concluded that by dedifferentiation and redifferentiation of interfascicular parenchyma vascular cambium is formed and the xylem and phloem elements derived are all considered secondary in nature. In the petiole of Tabebuia, I have interpolated a stage of metacambium which precedes the development of vascular cambium from the interfascicular parenchyma. I have justified elsewhere the reasons for such interpretation. And that is why xylem elements differentiated from this interfascicular metacambium have been considered as late metaxylem elements and not secondary elements.

In Tabebuia during the early metacambial development the vascular strands are discrete with interfascicular parenchyma. At a later stage the vascular strands are bridged by periclinal divisions in the interfascicular parenchyma that advance acropetally and laterally in line with the fascicular metacambial cells. The resultant derivatives have been termed as the 'interfascicular metacambial cells'.

Larson (1975) has reported periclinally dividing parenchyma cells bridging the primary vascular strands in the seedlings of Populus but he has not designated them as interfascicular metacambium. It is for the first time that interfascicular metacambial stage has been recognized during the development of interfascicular cambium in the petiole of Tabebuia. The interfascicular parenchyma by dedifferentiation

and redifferentiation give rise to the interfascicular metacambium. In tangential view these interfascicular metacambial cells are composed of two types like those of the fascicular metacambium, which later differentiate as the fusiform and ray cell system.

Whether the fascicular cambium originates before or after the interfascicular cambium is yet another matter of controversy. Fahn et al. (1972) presume that the interfascicular cambium is first to arise although belated in commencing activity thereby influencing the formation of fascicular cambium. The commonly held view is that the fascicular cambium precedes the interfascicular cambium. The lateral spread or advance of periclinal divisions from fascicular to interfascicular regions during primary growth is apparently common in many species (Esau, 1943b). This is true in the case of Tabebuia also. In the 2 cm long petiole metacambium is well established within the vascular strands and it is at this stage of the petiole that the periclinal cell divisions advance laterally in the interfascicular region. Consequently the interfascicular cambium is delayed in development when compared with the fascicular cambium.

4.9 Origin of rays

Based on the place of origin, the vascular rays could be categorized into two types in Tabebuia. Certain cells of the

primary body (i.e., the short cells in the metacambium) the primordial ray cells, give rise to the ray type cells. Secondary rays, on the other hand, originate during the development of the secondary body. Ray cells may arise from the fusiform cells. Philipson et al. (1971) have described the formation of ray initials from the fusiform cells in four ways, division off the side, division off the end, decline or segmentation. In Tabebuia fusiform cells with pseudotransverse divisions were noticed. Daughter cells formed in this way elongate to become fusiform cells or be transformed into ray cells. Segmentation of a part or of complete fusiform cell is also observed to give rise to ray cells.

The vascular cambium in the petiole of Crataeva and Salvadora, shows the presence of only the axial system, i.e., the fusiform cells (Murukesan, 1993). Ray cells are absent. As seen earlier, in the study of the transit cells/vascular cambium in the petiole of Gmelina and Tabebuia, only Tabebuia shows the presence of ray cells. They are absent in Gmelina.

Though rays are absent in the normal petiole of Crataeva, on wounding ray cells were observed within the wound cambium in the petiole. This has been explained due to the action of ethylene production on wounding (Murukesan, 1993). Formation of new rays followed by wounding in stems is reported earlier (Tippet and Shigo, 1981; Lowerts, et al., 1986).

Ray cells in the stem are known to serve in the radial transport of assimilates. The girth of the petiole when compared to the stem is very less and hence the demands of the radial transport are not very heavy. Moreover, the petiole has determinate growth. The absence of ray system in the petiole of Gmelina and its presence in the petiole of Tabebuia cannot, however, be explained simply on the necessity of the radial transport. The only explanation applicable is that development of the fusiform and ray cells in the normal development merely follows a blue print laid down in the procambium-cambium continuum which can be recognizable but not understandable.

4.10 Secondary growth in the petioles

Information on the secondary growth of leaves is meagre. Elliot (1937) found that in most evergreen and deciduous dicotyledons the leaves completed their development during the first year of growth. Shtromberg (1959) also compared deciduous and evergreen dicotyledons with regard to secondary tissues in leaves and used counts of procambial cells and of the resulting xylem cells to determine whether or not cambial activity had occurred. She observed more pronounced activity of cambium in the petiole, less in the mid vein, but was still noticeable in the lateral veins of first and second order.

In both Gmelina and Tabebuia the secondary vascular meristem (transit cells/cambial cells) produces considerable amount of secondary tissues during the post elongation period. In contrast to the middle region, distal and proximal regions of the petiole in both the species have very little secondary tissues. This suggests that the secondary growth is not uniform.

In Pinus longaeva Ewers (1982) found that uniseriate cambium is unidirectional in function. That is, only secondary phloem was noticed throughout the life span of the needles. In both presently studied taxa i.e., Gmelina and Tabebuia secondary xylem and secondary phloem are noticed.

4.11 Function of the leaf vascular cambium

The vascular cambium provides both secondary phloem and secondary xylem required for the maintenance of the leaf. It is likely that older leaves contribute significant amounts of photosynthates to the plant. Assuming that the sieve elements have a finite life span, new sieve elements must be produced to replace the old senescent sieve elements for the transport of photosynthates and other substances. The tracheary elements in the petiole are non-living at functional maturity and apparently remain functional in water transport throughout the life of the leaf, except those which are physically stretched during elongation of the petiole.

4.12 Differentiation of phloem in the petiole

The vascular system delimited in the petiole of Gmelina and Tabebuia, in its meristematic state, differentiates in stages, first as procambial system and later into mature primary vascular elements (i.e., phloem and xylem). The relatively early delimitation of the procambial system is characteristic of the primary vascular differentiation in the shoot of many vascular plants (Esau, 1943b, 1965a, b; Wetmore, 1943; Steeves and Sussex, 1972).

A procambial strand in the petiole of Gmelina and Tabebuia is homogeneous in its cellular composition and does not give any evidence of its potentiality to differentiate into a sieve tube element, companion cell, parenchyma cells, tracheary or fiber cells. The present findings for the petiole of Gmelina and Tabebuia support the view expressed by Esau (1943a) in the shoot of Linum, that the differentiation of the first phloem element occurs before a procambial strand attains its final size and form.

4.12.1 Primary phloem

Primary phloem includes both protophloem and metaphloem. In the present investigation protophloem and metaphloem are distinguished mainly on the basis of two criteria (i) stage of elongation of the petiole (ii) micromorphological changes. In Gmelina there is no distinction of metacambium in the developmental stage of the procambium and hence the last

formed protophloem and first formed metaphloem elements have not been distinctly demarcated. Hence the stage of elongation of the petiole is the main criterion used to distinguish protophloem and metaphloem. Protophloem development occurs during the rapid elongation of the petiole while the metaphloem originates when there is a decrease in the rate of elongation. Another additional criterion to judge the development of metaphloem was the distinct radial seriation of cells in procambium and the development of metaxylem elements. In Gmelina protophloem development gradually merges with the metaphloem development. The phloem elements which were formed during the late phase of elongation were distinguished as metaphloem. In Tabebuia in addition to the rate of elongation of the petiole, presence of metacambium with micromorphologically two types of cells made it easy to categorize protophloem and metaphloem.

4.12.1.1 Protophloem

Both the first protoxylem and protophloem elements in the petiole of Gmelina and Tabebuia occur in the radial peripheral position in the procambial strand and towards the pith and cortex respectively. Subsequent vascular elements differentiate internal and lateral to them.

According to Esau(1943a) and McGahan(1955), in the stem commonly the first sieve elements differentiate in the second layer of procambial cells, that is they are separated from the cortex by one layer of procambial cells. My observations

in the petiole of Gmelina and Tabebuia support their findings. The first locus of differentiation of sieve tube element lies internal to the procambial strand, towards its radial periphery and separated from the cortex by a few procambial cells. In a procambial strand in the petiole of Gmelina and Tabebuia recognition of the sieve tube elements was facilitated by their thicker and more deeply staining walls than those of the associated procambial cells and by the lack of stainable contents in their lumen which makes it also conspicuous among the adjacent densely stained protophloem parenchyma cells, when viewed radially. Tangentially these sieve tube elements were more distinct because of the presence of the sieve plate and lateral sieve areas.

The primary phloem in Gmelina and Tabebuia consists of three types of cells, viz., sieve elements, companion cells and phloem parenchyma. In Gmelina they organize in the form of complexes separated by large parenchyma cells. Raju (1968) has reported arrangement of procambium and phloem derivatives in the form of complexes in the petiole of Mimusops elengi. In Tabebuia such complexes are not noted. The three types of cells are more or less uniformly distributed in the phloem region.

The first formed sieve tube elements in Gmelina and Tabebuia may or may not be associated with companion cell, but the later formed protophloem sieve tube elements are

associated with one to four companion cells in Gmelina and a single one in Tabebuia, aligned on any one of their lateral walls. It is also reported earlier that companion cells are absent in the protophloem in some shoots and roots, a fact which may be associated with the short life span of many sieve tube members in the earliest part of the primary phloem (Esau, 1969; Eleftheriou and Tsekos, 1982). Such a condition has been recently reported in the petiole of Crateava and Salvadora (Murukesan, 1993).

4.12.1.2 Metaphloem

The metaphloem which appears inward from the protophloem consists of groups of sieve tube elements, companion cells and phloem parenchyma cells. The distribution of various cells in the metaphloem shows no distinctive pattern. At the time of metaphloem differentiation the procambium shows a radial seriation of cells in the petiole of Tabebuia.

The metaphloem sieve tube elements in Tabebuia are associated with a single companion cell whereas in Gmelina one to eight companion cells are found associated with a single sieve tube element. A single companion cell or a longitudinal series of companion cells are aligned on any one or both the lateral walls of the sieve tube element. Companion cells aligned in a longitudinal series were designated as 'companion cell strands' by Chang (1954) and Huber and Graf (1955). According to Resch (1958) in

Vicia faba a single companion cell is restricted to protophloem whereas companion cells strands are characteristics of metaphloem and secondary phloem. In Gmelina both protophloem and metaphloem sieve elements show a single companion cell or a companion cell strand.

Esau (1969) suggested that when sieve tube elements are associated with companion cells on its either lateral walls, they sometimes differ in density of stainability of cytoplasm and nucleus, which Resch (1954) interpreted as a variability in expression of companion cell characteristics. No such differences were noticed between the companion cells aligned on either side of lateral walls of the metaphloem sieve tube elements in Gmelina.

The sieve elements are associated with parenchymatic elements in all vascular plants an association interpreted as a reflection of the close functional interdependence between these two cell types, and probably a result of the protoplasmic specialization of the sieve element, the highest level of specialization being between sieve tube member and companion cell (Esau, 1969; Evert, 1977, 1990; Cronshaw, 1981). Because of their numerous plasmodesmatal connections with the sieve tube members, it is believed that companion cells play a role in the delivery of assimilates to the sieve tube (Esau, 1969). In addition it has been suggested that companion cells maintain the enucleate sieve tube members through the transfer of informational molecules

(Gunning, 1976) or other substances such as ATP (Lehmann, 1979) from themselves in the sieve tube members via many connections in their common wall. Therefore, presumably, the increase in the frequency of the number of companion cells and/or its presence on either lateral wall in the metaphloem sieve tube elements of Gmelina indicates that these sieve tube elements are actively functional in the transport of assimilates.

4.12.2 Secondary phloem

The petioles of Gmelina and Tabebuia which have ceased to elongate show secondary phloem. The secondary phloem elements are derived from the secondary vascular meristem, the 'transit cells' in Gmelina and from the cambium cells in Tabebuia. Secondary phloem in Tabebuia reflects the structure of the vascular cambium and is ccmposed of two systems, the axial and ray, i.e., it consists of sieve tube elements, companion cells, axial and ray parenchyma. In Gmelina the ray parenchyma cells are absent.

The secondary phloem elements in both the plants agree in length with their precursors (transit cells/cambial cells). Each sieve tube element is associated with a companion cell. In Gmelina the sieve tube element with companion cells on both its lateral walls are present and their number varies from one to four on either wall. In Tabebuia the sieve tube element is generally associated with a single companion cell.

The differentiation of phloem from the secondary vascular meristem (transit/cambial) cells occurs by tangential divisions so that the derivatives occur in radial files. This is clearly evident in Tabebuia. In Gmelina only some strands show the phloem elements in the radial files and the primary phloem and secondary phloem complexes are demarcated by one or two layers of parenchyma cells. Schneider (1945, 1955) in the stem of Prunus and Esau (1965a) in Citrus observed a sharp delimitation between metaphloem and secondary phloem. After the formation of the metaphloem in these species, parenchyma cells differentiate from remaining procambial cells and separate the metaphloem from the secondary tissues.

Although there is a general resemblance in arrangement of cells of the cambium and the resulting secondary phloem, the differentiation of the cambial derivatives into phloem elements bring about changes that more or less further modify the phloem as compared with the meristem and each kind of element shows its specific course of ontogenetic development.

The derivatives of fusiform cambial initials produced by periclinal divisions towards the phloem usually do not directly differentiate into specific phloem cells but divide one or more times. It is appropriate, therefore, to speak of these derivatives as phloem mother cells or phloem initials (Cheadle and Esau, 1958). Evert(1963) reported that in Pyrus malus each derivative of the cambial initial divided at least

once. He based his interpretation on the occurrence of apparently recently formed tangential walls between partly differentiated phloem elements. In Gmelina and Tabebuia the radial files of cells in the secondary phloem have been analysed to determine the ontogenetic relationship of the secondary vascular meristem and its derivatives.

In Gmelina the precursor cell/mother cell first divides periclinally to give rise to two cells. The derivative cell may differentiate as a phloem parenchyma cell or may further divide once or twice (periclinally and/or anticlinally) to give rise to a sieve tube element and a companion cell or a sieve tube element, companion cell and a parenchyma cell. Zee (1968) has reported three patterns of division of cambial cell in the epicotyl of Pisum sativum. In Tabebuia, a division within the phloem mother cell gives rise to an assemblage (a group of cells derived from a cambial cell, or a phloem mother cell). An assemblage may consists of a pair of sieve tube elements and one or more companion cells or it may consist of a pair of sieve tube elements, companion cells and a parenchyma. Cheadle and Esau (1964) observed this pattern of divisions in the bark of Liriodendron tulipifera. Murukesan (1993) has reported that the mother cell undergoes periclinal and anticlinal divisions to give rise to a sieve tube element, companion cell and a phloem parenchyma, in the petiole of Crataeva nurvala.

4.12.3 Contents in the sieve elements

The question as to what constitutes the normal distribution of P-protein and other contents within the lumen of the mature sieve tube element has been the subject of numerous investigations. It has represented the most controversial aspect of sieve tube element structure (Evert, 1982). A parietal distribution of P-protein and other contents in mature sieve tube element has been reported for a number of species (Esau, 1969, 1978; Walsh, 1980; Evert, 1984; Behnke and Sjolund, 1990). Others describe the contents having more or less even distribution throughout the lumen of the mature sieve tube elements (Jarvis et al., 1973; De Maria and Thaine, 1974; Thaine et al., 1975).

The present study has convincingly shown that the contents of a mature sieve tube element at least during certain stage of development lie at the periphery with a distinct central cavity in Gmelina. Such sieve tube elements are presumed to be least affected by the surging artefact that occurs when sieve tube elements are severed, reflecting the normal distribution of the contents in the sieve elements.

In the petiole of Gmelina especially metaphloem and the secondary phloem sieve tube element contents show various morphological forms. The content of the sieve tube elements are lodged against one of the sieve plates or at times on

both to form the plugs. Plugs arise through the disruption of the normal parietally placed content, caused due to the severing and the subsequent accumulation of the content at the sieve plate. They are directly related to the peripheral content which undergo varying degrees of disturbances. A similar view has been expressed by Evert and Derr (1964). According to Cronshaw and Esau (1968) accumulation on the sieve plate resulted from a differential release of pressure in the severed sieve tube element, but the variations in the form of these accumulations are difficult to explain. Engleman (1965) suggested that the position of the plug may be taken as an indication of the location of the severing. A similar explanation can be given for the varying morphological appearances of the plugs in the present study. The varying nature of the plug reflects varying degrees of accumulation or condensation resulting from manipulation and fixation of tissues. The presence of central cavities is evident in plug formation. It may be due to the parietal position of the contents and the extensions of slime on both sides are the remnants of the parietal cytoplasm.

Plugs with more than one small cavities were also noted. The small cavities can be considered as portions of the central cavity either exposed due to plane of sectioning or the disturbance caused during the processing of the material. Some of the plugs show differential staining which indicates that there are varying degrees of condensation of the contents in the mature sieve tube elements during the plug

formation. Such a situation has also been noted by Raju (1968).

According to Cronshaw and Sabnis (1990) the relationship between morphologically distinct protein subunits fractionated by gel electrophoresis causes problem of terminology. All these proteins, however, are phloem-specific. Accordingly, they have proposed to use the term phloem-specific proteins for all the proteins that are characteristic of the phloem. For the group of these proteins that are morphologically distinct at the electron microscopic level and are observed in the cytosol are continued to be termed as P-proteins. In the present work the term slime is being used to mean the P-proteins and other contents collected at the sieve plate in the mature sieve elements.