DISCUSSION

4 DISCUSSION

4.1 Deciduous vs. evergreen habit

One of the most universal characteristics of plants is the more or less recurrent natural shedding of both vegetative and reproductive portions of their plant body (Kozlowski, 1973). Deciduous tropical trees and lianas lose leaves both in season and throughout the year. In tropical evergreens growth of the shoot apex is more or less continuous, in which leaves are sequentially lost as new ones are produced. In the life of the individual tree the loss of leaves represents a reduction in photosynthetic and traspiring tissue, except when leaves are produced and lost steadily throughout the year (Perry, 1971).

How to distinguish an evergreen from a deciduous tree has been the subject of much argument and confusion (Longmann and Jenik, 1974; Tomlinson and Zimmermann 1978). Basically, of course, it is a matter of the relative timing of bud break and abscission, but there are two other points to be considered. In the first place, the average life span of tropical tree leaves varies about 4 to 14 months, so that 'evergreen' means something rather different from the condition, for

example, in north temperate evergreen plants, where leaves remain on coniferous trees for two years or more, sometimes even as long as 33 years (Ewers and Schmid, 1981). Secondly, the shoot growth of tropical trees is not always synchronized, so that some branches may be in full flush, other may be leafless, while some again may be in the flushing phase. Taking all these points into consideration, Longmann and Jenik (1974) have recognized 'four patterns of leafiness' in tropical trees, although no very sharp lines could be drawn between subclasses.

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 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 of a 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.

Accordingly, Crataeva nurvala is considered as a deciduous tree and Salvadora persica, an evergreen tree.

In tropical climates characterized by wet and dry seasons, flushing is seasonal (Kramer and Kozlowski, 1979). Experimental studies on the leaf-fall of tropical trees are very scarce, and it is therefore not possible to draw any general conclusions. The seasonal duration of leaf expansion varies greatly among species, the type of shoot, and environmental conditions, especially the temperature (Kozlowski,1971). The individual leaves of deciduous angiosperms as a group develop rather rapidly, usually requiring from a few days to few weeks. The petiole of Crataeva nurvala ceased to elongate within 10 to 15 days although lamina expansion continued for one or two days more.

Despite the rapid development of individual leaves of many deciduous angiosperms, the total amount of foliage per tree may nevertheless continue to increase over the growing season in certain species (Kozlowski, 1971). Whereas a number of species usually expand their total complement of foliage in one early season growth

flush, others such as Quercus, tend to produce an additional late seasonal growth flush to produce lammas shoots from opening of bud formed earlier in the current growing season (Kramer and Kozlowski, 1979). The Crataeva nurvala trees produce foliage twice in a year. The major flush occurs in the summer months i.e., in March/April and leaf fall occurs in August/September. Second flush of leaves immediately follows the first but complete defoliation takes place within 2 months and then tree enters a true dormancy period during the winter months.

Leaves of evergreen angiosperms expand much more slowly than those of deciduous angiosperms. For example, Citrus leaves grew in length and width for about 130 days (Scott et al.,1948). The rate of leaf expansion was uneven. Two periods of rapid expansion occurred and these coincided with the time of spring and fall growth flushes. Leaf growth of evergreen angiosperms usually is completed during the first growing season. Although no studies on petiole elongation is conducted in Salvadora it is observed that the leaf expansion is completed within a months time. Only one flushing is noticed and most of the new leaves are formed in the summer months i.e., March-May. Leaves remain on the trees for about one year.

4.2 Nodal anatomy

The actual boundaries of the node are ill-defined (Isebrands and Larson, 1977b) although the node has been defined in broad terms (Mitra and Majumdar, 1952; Howard, 1970, 1974; Dickison, 1975). Nodal region and leaf base are used here to the approximate lower and upper bounds, respectively, of the commonly accepted interpretation of the node. Leaf gaps are formed by acropetal closing of procambial strands above a departing leaf trace (Majumdar, 1942; Larson and Pizzolato, 1977). Species are often classified by the number of lacunae that occur at each node, the most common types being unilacunar, trilacunar and multilacunar (Sinnot, 1914). Many modifications of the basic nodal type have been recognized. One modification occurs when two traces with independent origin in the vascular cylinder are associated with a unilacunar node, the unilacunar, two-trace node (Marsden and Bailey, 1955), or the "split-lateral" or common gap condition (Howard, 1970). Such reports are most frequent in species with unilacunar nodes (Dickison, 1975).

As mentioned earlier, the node in Crataeva is unilacunar with many trace strands departing from the internodal vascular system. This condition has been reported earlier in cotyledonary nodes (Sugiayama, 1976) and in adult leaves with unilacunal node (Watari, 1934,

1936, 1939; Benzing, 1967 , Kato, 1967). Dickison (1975) emphasized that this condition is most frequent in species with unilacunar nodes, however, trilacunar nodes also showed this condition (Seghal and Paliwal, 1974).

Double leaf trace strands are common in angiosperms, particularly in species exhibiting decussate phyllotaxy (Bailey, 1956). In Salvadora persica each leaf is served by a double trace that enters the node via single gap. The two trace strands serving a leaf originate from and are in continuity with traces serving a leaf two nodes below. They develop acropetally and merge before leaving the single gap to serve a leaf. Thus, Salvadora essentially conforms to the same phyllotactic vascular pattern described for many other species with double leaf trace (Dormer, 1972).

Metcalfe and Chalk (1950) listed 55 families of dicotyledons in which cortical and/or medullary bundles are known. Medullary bundles have a varied role in their relationship to principal vascular system and in their contribution to the vascular supply of the leaf. Neither Crataeva nor Salvadora shows the presence of cortical or medullary hundles.

4.3 Petiole vasculature

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 the structure and organization of petiolar strands are indeed complex. Because of the developmental approach I was able to follow the ontogeny of each petiolar strand. Examination of mature petioles alone would not have yielded this information.

In the petiole the vascular strands from the node may assume a variety of configurations (Schofield, 1968). Open, closed and intermediate systems dominate the literature (Beck et al.,1982) as far as the arrangement of primary vascular system in the shoot is concerned. In open systems the sympodia are entirely discrete, i.e., with random interconnections consisting of only minor or accessory bundles. In closed systems anastomoses occur between leaf traces and axial bundles or between axial bundles so that a reticulate vascular pattern results. Intermediate systems are partly open and partly closed. This terminology is also applicable to the vasculature of leaves as proposed by Sporne (1958) and Schmid and Beck (1971).

Despite the various problems accompanying diagrammatic representation of steles or primary vascular systems, such diagrams present in readily accessible

form a large amount of information that is usually much more comprehensive, than it would be if present solely in written descriptions. It is with this idea that I tried to plot a two-dimensional diagram of the internode-node-petiole continuum.

The arrangement of vascular strands in the petiole varies in different species. The crescent, or arc pattern is one of two main vascular patterns found in petioles (Howard, 1979b). A ring of bundles, which is found in many other plants (Watari, 1934; Howard, 1979a) is the second pattern. The vascular system in the petiole of Crataeva is a closed one. The strands are arranged in the form of a ring. Clearly separated bundles with almost a closed cylinder are reported in the petiole of Steriphoma ellipticum (DC.) Spreng of the family Capparaceae, to which Crataeva also belongs (Metcalfe and Chalk,1950). In Crataeva religiosa these authors have reported solitary arc with the ends detached so as to appear as distinct strands. In contrast, the present work clearly shows the vasculature consisting of separate strands arranged a ring in Crataeva nurvala. Crataeva religiosa Forst. is considered as synonymous to C. nurvala (Shah, 1978). In Salvadora the petiole vasculature is horse-shoe shaped with discrete strands.

Reorientation of bundles at the petiole base to form the petiole vasculature is straight forward in Crataeva. The arc of bundles exiting the node simply closes, with the outermost bundles forming the ventral chord. This process is in marked contrast to the highly complex mixing of bundles from three traces to produce a series of petiolar bundle tiers as reported in Populus detloides (Isebrands & Larson, 1977a) or formation of rachis vasculature from two traces in Fraxinus pennsylvanica (Larson, 1984b). In Salvadora the two trace strands reversed their orientation during their ascent to the petiole.

4.4 The role of trace strands in the petiole

That part of the node lying between the levels at which leaf traces depart the internodal vascular system and enter the petiole or leaf base is of the extreme vascular diversity (Larson, 1984a). Within this region, the vasculature of many species are reoriented in a multitude of ways by the proliferation of new bundles and frequently by the fusion of bundles both within and among leaf trace derivatives. Consequently, the leaf base has been referred to as the "primary nerving center" (Croizat, 1940), the "leaf basal meristem" (Larson, 1976), and the "lower vascular plexus" (Neubauer, 1972,

1979a,b).

The petiole base in Crataeva is obviously a major centre for strand mixing and proliferating trace strands from the internode. Petiole top, on the contrary, serves primarily as a centre for redistributing or dispersing petiolar strands as laminar strands through the petiolules. Anatomical evidence based on study of serial sections is confirmed by dye suction experiments. Applications of three dye solution through the basal splits of the PL1 system revealed that the dye is distributed over the leaflets situated at the respective feeding sides of the petiole. However, in many instances it was observed that unilaterally applied dye spreads via lateral connections to the other vascular strands in the petiole. This might be because the leaflets obtain their dye solution from the strands on the same side in the petiole but changes in the resistance of the supply routes during transpiration pull might alter this pattern. In the latter case, leaflets might also obtain dye solution via vascular interconnections resulting in to the mixing of dye solution. In Salvadora leaves, the strands in the petiole often continue unaltered throughout its length.

In Crataeva the distal region of the petiole, or petiole top, serves as a secondary centre for strand

mixing. That is, petiolar strands are not only subdivided but also reorganized before being distributed as petiolular strands. However, little vascular orientation occurs at the lamina base. In Salvadora the first secondaries diverged from the petiolar system before the primary vein makes contact with the lamina.

Although anastomoses and approximation of strands are observed to a greater extent in Crataeva than in Salvadora, the identity of principal vascular strands is conspicuous even in mature petioles. In most angiosperms examined, new leaf trace strands originate from parent traces and develop acropetally and continuously toward the site of the primordium they will eventually serve (Larson, 1982). In a trilacunar condition of the node, the primordial site is first served by the median trace and somewhat later by the laterals. In Crataeva, though many trace strands depart the vascular cylinder from a single gap and traverse the petiole, the median strand shows greater individuality, a common feature found in most dicotyledons (Howard, 1979a). In a unilacunar node with two trace strands, as in Salvadora, it is uncertain whether one of these traces is precocious relative to the other as observed in other species (Larson, 1984a). My observations indicate that the two original strands remain as median strands and that bifurcation of strands proceed more or less centrifugally.

The internode-node-leaf continuum (Howard, 1974) is exemplified in Crataeva and Salvadora by the continuity of the vascular tissues in the internode, node, petiole and lamina. Although the network of minor veins feeds into all orders of major veins in about equal frequency, major veins generally feed only progressively larger veins; tertiaries into secondaries, and secondaries into primary vein or mid vein, as countless tributaries feed into the main channel of a complicated river system. It appears that vascularization of leaves in Crataeva and Salvadora 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. The reoriented strands traverse the main part of the petiole with little branching, although adjacent strands merge and anastomose. At the base of the lamina the petiolar strands are distributed as veins.

4.5 Functional significance of vascular strands in the petiole

The complex petiole vasculature in Crataeva and Salvadora consists of many discrete strands. Because each strand is continuous with a specific portion of the lamina, petiolar strands may provide the independent

channels for rapid translocation as envisaged by Bailey (1956). Schmitz (1970) noted that the petiolar bundles of Pelargonium also had phloem subunits connected to specific veins in the lamina. Similarly, Thrower (1962) found in soybean that phloem strands connected to individual leaflets were functionally independent even though they do not appear to be anatomically independent in the petiole.

4.6 Primary vascular differentiation

The procambium is the precursor of the primary vascular system. By demarcating the prospective vascular system in meristematic tissues it determines vascular organization. Vascularization proceeds developmentally from a primary to a secondary vascular system with the primary serving as both a structural and organizational template for the secondary (Larson, 1980a). Primary vascular organization within the growing shoot can be most conveniently analysed in terms of the leaf trace concept (Esau, 1965a). According to this concept, a leaf trace extends from its point of divergence on either a sympodial bundle or a parent trace to a leaf base. The bundles do not, however, terminate at the leaf base. They extend into the node, through the petiole, and finally into the lamina to form the ramifying vein system. The leaf trace system is therefore a functional as well as a developmental

continuum between stem and leaf.

The procambium is a logical starting point for discussing the vascular differentiation, because it serves not only as a precursor of the cambium but also as a template for all subsequent vascular tissues. Anatomists generally agree that the procambial system progresses acropetally and in continuity with existing procambium in the shoots of most higher plants (Esau, 1965a; Cutter, 1971; Larson, 1982). This acropetally progressing system consists of procambial leaf traces that have united with their respective primordia. The structure of the procambial strands in Crataeva and Salvadora verifies their acropetal and continuous development. New procambial strands that diverge from parent traces in the main axis and develop acropetally into the petiole are both larger and more differentiated at their points of divergence than at their acropetal fronts.

The apical meristem is generally defined as that part of the shoot apex distal to the youngest leaf primordium or the youngest node (Esau, 1965a). Although its form fluctuates during a plastochron, in Crataeva and Salvadora it is dome shaped and to some degree extends beyond the point of attachment of the youngest leaf primordium.

4.6.1 Vascular meristem

The subapical region in which the procambium and/or procambial strands are first evident has been interpreted in different ways. In most apices, there is a ring of meristematic tissue in which procambial strands can first be 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, and residual meristem, and its histogenic significance has varied accordingly (Esau, 1943b). It should be, however, noted that none of these terminologies and concepts are applicable in the petiole development because it develops away from the apex. Procambium develops in the petiole from older procambial strands present below in the axis and it is no way directly related to the apical meristem. Moreover, the development of the procambium and establishment of the primary vascular system in the petiole is independent of the stem-leaf reatlionship. How far the nature of the node and its leaf trace micromorphology affect the procambium and mode of its development and of primary vascular system of the petiole/ leaf base is not fully known.

The meristematic arc occurs in the advancing front of the procambial strands in the developing petioles of

Crataeva and Salvadora appears best to qualify as a vascular meristem. As such it would posses no histological significance as a vascular tissue until the acropetally developing trace strands develop within it. The meristematic region in the path of procambium differentiation may be considered as 'procambial front' or the region of 'procambial fade-out' as proposed by Larson (1975). That part of the vascular meristem not committed to strand development would then become interfascicular vascular meristem as described by Esau (1965a) and Devadas and Beck (1971).

4.6.2 Procambium

Many authors have commented on the difficulty in identifying the first procambial cells (McGahan,1955; Larson 1982). Few details are known of procambial ultrastructure (Catesson, 1974), and no distinguishing characteristics have been observed in advance of morphological differentiation even in culture systems. (Philips, 1976). Moreover, it is seldom possible to recognize anatomically a single procambial cell in either the transverse or longitudinal plane. Only a group or an island of cells develop and a procambial strand can be recognized. However, Mueller (1991) used esterase activity as a histochemical marker to identify and define an early stage of procambium differentiation in primary

roots of Trifolium pratense.

Procambial strands in Crataeva and Salvadora are identified by their greater cell activity and differential staining - probably a result of differential vacuolation. The ground meristem cells show increased vacuolation quite early in development whereas the procambial cells remain densely cytoplasmic. The procambial cells undergo repeated longitudinal divisions but expand transversely to a limited degree. Thus, eventually the procambial cells become distinguishable by their dense narrow cells, elongated parallel with the longitudinal axis of the cell.

Advance of the procambium is an active process (Larson, 1982). In Crataeva and Salvadora the early procambial strand enlargement is by cell divisions within the strand and later by acquisition of cells from the vascular meristem. The first acquired cells are considered as derivatives of vascular meristem (Esau, 1954; Larson, 1975). Later acquired cells divide periclinally and daughter cells enlarge the periphery of the procambial strand. Such an addition first appears more frequent on the cortical side, thus enlarging the phloem region of the strand. The procambial cells elongate by passive accommodative growth during the early elongation of the petiole.

Protophloem and protoxylem differentiation occurs at various times during early procambialization depending on the ontogenetic stage of development of the plant and the species investigated (Larson, 1982). Both in Crataeva and Salvadora protophloem differentiation preceded that of protoxylem.

4.6.3 Metacambium

Esau (1943b, 1965a) has repeatedly emphasized that a distinction must be made between primary and secondary growth. Primary growth begins in the embryo and continues until internodal elongation ceases. The same criterion is used in the present study to distinguish primary and secondary growth in the petiole. Accordingly primary growth in the petiole begins when it starts to differentiate with the leaf primordium and continues till its elongation is ceased, after which the secondary growth may start. During elongation the vascular meristem remains procambial. Nonetheless, vast changes occur both in the procambium and its derivatives during elongation, and these changes have contributed to many contradiction and much confusion regarding the nature and extent of this meristem. Anatomists have recognized and acknowledged changes in the procambial derivatives by distinguishing metaphloem and metaxylem from protophloem and protoxylem,

although not necessarily by universally accepted definitions (Esau, 1965a,b; Fahn, 1982). However, equivalent stages have been neither recognized nor proposed for the lateral meristem.

Larson (1976) attempted to resolve this dilemma in Populus deltoides by subdividing the procambium into procambium and metacambium. Recognition of a metacambium does not contravene the original concept of a procambium - cambium continuum. It simply recognizes an essential transitional stage - procambium - metacambium - cambium continuum. Metacambium is distinguished from procambium both by meristematic cell division planes and by products of these divisions.

Metacambium is identified as an intermediate stage between procambium and cambium in the petiole of Crataeva. Metacambium is preceded by the isolated periclinal divisions in the procambium. As the periclinal divisions increase in frequency a continuous band of metacambium eventually appears across the strands.

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 and they therefore occur in consecutive files of radially arranged cells. It is observed in Crataeva that some metaxylem elements differentiate from procambium before the radially seriated meristem is evident in the strands. As Larson (1982) pointed out the distinction between procambium and metacambium and their derivatives are not precise. As with every attempt to subdivide a continuum, certain arbitrary decisions and definitions must necessarily be employed.

In the petiole of Salvadora the procambium is a homogeneous tissue. During the development procambium imperceptibly transforms into cambium, it was intractable to categorize the intermediate stages based on the characteristics of derivatives because the events rapidly overlap in the successive stages. Hence, the terms early procambium and late procambium are used to categorize the first formed and last formed elements. In the final stages of petiole elongation the metacambium in Crataeva and late procambium in Salvadora show certain features of procambium - cambium transition. However, their derivatives are metaxylem and metaphloem and it must therefore be regarded as part of the primary growth.

According to the suggested usage (Esau,1943b,1965b) the terms primary and secondary growth imply only that one

tissue appears before the other in development of the plant body. The procambium is therefore a primary and the cambium a secondary vascular meristem. I have subdivided the primary meristem to emphasize a distinction between procambium and metacambium in Crataeva on the basis of origin and function of the derivatives.

4.7. Vascular cambium

The vascular cambium in the shoot is the meristematic tissue that contributes to diametrical growth of a tree stem or branch and to some extent petiole. It produces xylem to the inside and phloem to the outside.

The commonly held view concerning the relation of the cambium to the procambium is that the two terms denote two developmental stages of the same vascular meristem (Esau, 1943b, 1965b; Cumbie, 1967; Philipson et al., 1971; Iqbal, 1990). Many criteria have been used in attempts to distinguish cambium from procambium and many of them added much confusion. The observations in the petiole of Crataeva and Salvadora further emphasize the difficulty in separating the procambium and cambium as distinct meristems, the former responsible for primary and the latter for the secondary vascular tissues.

Radial seriation of cells and derivatives of the lateral meristem are the characteristics most commonly associated with cambium by early anatomists. Orderly seriation of vascular elements is suggestive of origin from tangential divisions (Esau,1943b,1965a,b) and this is consistent with the concept of the origin of cells from cambial initials. Of course, as also noted by Esau, radial seriation *per se* does not necessarily distinguish between primary and secondary tissues. In fact, for petioles of *Crataeva* and *Salvadora* procambium as well as vascular cambium have radial seriation. In *Crataeva* radial seriation is evident in early metacambial cells although the protoxylem elements show radial seriation before metacambium differentiation. In *Salvadora* radial seriation is evident during the later stages of procambium differentiation.

Radial seriation during procambium development is common among dicotyledons and its occurrence has been documented in many species (Esau,1943b; Fahn,*et al.*,1972; Soh,1990). Despite its rather common occurrence among many plant species, radial seriation of protoxylem and protophloem elements cannot be considered either as a criterion of or even as an indicator of impending cambial development. Not only do these events occur early during primary elongation but they originate from cells that can

in no way be considered a tangentially oriented meristem.

A characteristic feature of the vascular cambium of most arborescent plants is the existence of two systems of cells, the axially elongated fusiform initials being responsible for the production of the axial elements of the secondary xylem and secondary phloem, and the shorter ray initials being responsible for the production of the horizontal ray system. These characteristics were also not applicable for both the plants tested since rays are absent in mature petioles.

When viewed in the tangential plane the procambial cells appear non-storied in Crataeva and Salvadora. Non-storied procambial cells may differentiate to a storied cambium as in Salvadora. This condition has been reported earlier in the stem of Robinia (Soh,1974b) and Hoheria (Butterfield,1976).

During later stages of petiole elongation, the procambium in Salvadora and metacambium in Crataeva assume characteristics more closely resembling those of the procambium-cambium transition. The width of periclinally dividing cells increases radially. At this stage the vascular strands possess both the general appearance and many of the structural characteristics attributed to

cambium when viewed in the transverse plane. In the longitudinal plane, the procambial cells in Salvadora and metacambial cells in Crataeva appear elongated with tapering end walls suggestive of apical intrusive growth. The intrusive growth of the elongated procambial cells (the incipient fusiform initials) occurs during the final stages of petiole elongation. Nonetheless, neither the meristematic tissues nor their derivatives assume the true characteristics attributed to cambium until petiole elongation has ceased. They are, therefore, part of the primary body and they should be considered as transitional or incipient stages of the vascular cambium.

Events leading to the identification of cambium occur gradually. The lateral meristem cannot stabilize until petiole elongation has ceased. Several investigators described events that occurred during the final stages of primary growth in the internode and attempted to define cambium in terms of these events (Catesson,1964; Cumbie, 1967; Enright and Cumbie,1973; Butterfield,1976). Fahn et al.(1972) also described these events in detail but they did not relate them to primary growth. None of the attempts to distinguish cambium from procambium was successful when based exclusively on either the appearance or structure of the meristematic cells.

One of the most definitive criteria of 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 that this decrease might be used to distinguish the two growth regions. Cumbie (1963) confirmed this abrupt decrease in Hibiscus, but not in Canavalia (Cumbie,1967). Both in Crataeva and Salvadora this feature was evident. Secondary xylem elements are shorter than last formed metaxylem vessel elements.

At the termination of petiole elongation, vascular tissues of the primary body give way to those of the secondary body. Although overlapping occurs during the transition secondary tissues maintain developmental continuity with those of the primary tissues. For example, metaxylem is not 'transformed' to secondary xylem. Rather, metaxylem vessels anastomose with secondary xylem vessels during transition and thus functional continuity is also maintained. This feature has been reported earlier in Populus seedlings (Larson,1980b).

Larson and Isebrands (1974) used these events to identify the primary secondary transition in Populus. Although secondary elements may be observed differentiating during final stages of internodal

elongation they do not mature until internodal elongation has ceased. When these elements attain their final length, their walls begin to lignify. Lignified elements can be readily detected by their birefringent walls in prolarized light. Secondary vessels are sometimes difficult to distinguish from late metaxylem vessels in transverse sections, but xylem fibres and lignification of xylem parenchyma are easily observed. Because fibres are associated with secondary vessels but not with metaxylem vessels, the primary - secondary transition was judged to occur when fibres with birefringent walls were first detected within the strands forming the vascular cylinder. This definition was more applicable in Crataeva than in Salvadora. In Salvadora, the cambium during the secondary growth shows anomaly hence not all the secondary regions have secondary xylem vessels. Rather, in this case, lignification of protophloem fibres and development of included phloem were found to be more useful criteria.

This foregoing identification based on the birefringence applies to the derivatives of cambium and not to the cambium per se. Though arbitrary it has been found to be remarkably consistent and reproducible as well as simple to use and evaluate. Other elements and tissues also attain secondary characteristics at the termination of petiole elongation. Primary phloem fibres differentiate contiguous to the obliterated protophloem.

The observations of this study support the view that the procambium and cambium are best regarded as two developmental stages of the same meristem. Beginning with the earliest detectable procambium in the vascular meristem, the procambium develops imperceptibly through a series of stages. Although differences can be noted when discrete parts of this continuum are compared, but the transitional regions between the parts nonetheless presents a continuum. Because of the gradual changes that occur with the procambium-cambium continuum and the fact that it has not been possible to precisely distinguish vascular cambium from procambium, most workers agree that procambium and cambium are sequential stages of the same meristem (Esau,1943b; Philipson et al.,1971; Fahn et al., 1972).

4.8 Secondary growth in the petioles

During the post elongation period, the cambium in the petiole of both the plants produces considerable amount of secondary tissues. In contrast to the middle region, distal and proximal regions of petioles in both the plants have no or very little secondary tissues. No birefringent xylem fibres or lignified xylary parenchyma and protophloem fibres were present. This suggests that secondary growth is not uniform. Howard (1974) suggested

that cambium develops first near the middle of the petiole and then extends bidirectionally without developing in the pulvinus. However, present study shows that cambium is observed in the basal and distal regions but very little or no secondary tissues are produced by the cambium at these regions.

Information on the secondary growth of leaves is meagre. Elliot (1937) found that in most evergreen 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 occurred. She observed more pronounced activity of cambium in the petiole, less so in the mid vein, but was still noticeable in the lateral veins of first and second order. Both secondary phloem and secondary xylem production are noticed in Crataeva and Salvadora. In both these plants secondary xylem production was more when compared to the secondary phloem. In Pinus longaeva, Ewers (1982) found that uniseriate cambium is unidirectional in function. Only secondary phloem was noticed throughout the life span of the needles. Although Samantarai and Kabi (1974) noted secondary growth in detached leaves, their data are not conclusive. Apart

from this limited research which has been carried out earlier, no detailed study has been undertaken to study the role of leaf vascular cambium, its development and activity in the deciduous and evergreen subtropical dicotyledons.

4.9 Function of leaf vascular cambium

From a functional point of view, it is not surprising that the leaves possess a vascular cambium that remains active throughout the post elongation life of the leaf. It is likely that older leaves contribute significant amounts of photosynthate 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 transport of photosynthate and other substances. The xylem in the petiole, of course, is non-living at functional maturity and apparently remains functional in water transport throughout the life of the leaf. Thus, the vascular cambium provides both secondary phloem and xylem required for the maintenance of the leaf.

4.10 Wound cambium in Crataeva

Vascular cambium in the petiole of Crataeva consist of only fusiform initials. But on wounding, it is

observed that rays are present among the population of fusiform initials. The enlargement and initiation of vascular rays following wounding mainly reflects wound effects rather than enhanced cambial activity. A major question concerning the ray formation is the question of ray initiation. Initiation of new rays starts with shortening or subdivision of fusiform cambial initials (Philipson et al.,1971). The unsolved question is, what is the signal for these changes in the fusiform initials. Since ethylene is the signal for transformation of fusiform initials to ray initials (Yadun and Aloni,1992), it may also be the signal for initiation of new rays. Formation of new rays followed by wounding in the stems is reported earlier (Tippet and Shigo, 1981; Lowerts et al.,1986). Moreover, wounding is known to cause shortening of fusiform cambial initials (Rier and Shigo,1972; Kuroda and Shimaji,1985). When fusiform cambial initials, not associated with rays, are exposed to higher levels of ethylene which is produced after wounding, become shorter and divided to form ray initials.

The development of fusiform and ray initials in normal development merely follows a blueprint laid down in the procambium which can be recognizable but not understandable. However, the plant can cope quite readily

with a disturbance of this blueprint, as witnessed by regeneration after wounding (D.D.Sabnis, personal communication) in Crataeva.

4.11 Differentiation of phloem in the petiole

The establishment of procambium in the leaf primordium is followed by the differentiation of most of its cells into phloem and xylem elements. The maturation of the first vascular elements in the procambial strand occurs while the procambium is still actively dividing and the procambial strands clearly show the outline and the internal pattern of the future vascular system. The relatively early delimitation of the procambial system is characteristic in the primary vascular differentiation of many vascular plants (Esau,1943b,1965a,b; Wetmore,1943; Steeves and Sussex,1972).

4.11.1 Protophloem

The procambium is a homogeneous tissue in respect to its cellular composition and does not give apparently any evidence of reflecting its potentiality of differentiating into numerous cell types such as tracheary elements, fibres, sieve tube elements, companion cells or parenchyma. The procambial strands

delimited in the vascular meristem of the petiole, in their meristematic state expand and elongate with the petiole, and this growth overlaps with the differentiation of procambial cells and maturation of vascular elements. Both in Crataeva and Salvadora the differentiation of the first phloem elements occurs before a given procambial strand attains its final size and form.

The protophloem and protoxylem initiate vascular differentiation, they occur in peripheral position with regard to subsequently developing vascular cells. In a given procambial strand the recognition of these elements is facilitated by their thicker and more deeply staining walls than those of the associated procambial cells, and by the scarcity of stainable contents in their lumina. The light staining of contents often makes the sieve elements particularly conspicuous among the adjacent protophloem parenchyma cells still possessing dense protoplasts.

In Crataeva and Salvadora the differentiation of the first phloem cells in the petiole occurs in centripetal direction. They differentiate towards the outer periphery of the procambial strands, that is, they are separated from the cortex by at least a few procambial cells. It is

also reported earlier by Esau (1943a) and McGahan (1955) that in the stem first phloem elements differentiated in the outer periphery of the procambial strands leaving a layer of procambial cells which later transforms into phloem fibres.

Differentiation of protophloem always precedes that of the protoxylem. The phloem elements in the petiole generally develop acropetally, although occasional reports of basipetal development are present (Larson,1984a). In both Crataeva and Salvadora the phloem elements are differentiated acropetally. This aspect is illustrated by the appearance of mature sieve elements in a given vascular strand in the basal region of the petiole before such elements differentiate at higher levels and by the increase in number of sieve elements towards the downward direction.

The primary phloem in the petioles of Crataeva and Salvadora consists of three different types of cells, viz. sieve elements, companion cells and phloem parenchyma. The sieve plates are mostly localized at the transverse end walls. As the procambial cells in the petiole have transverse end wall this condition was rather expected as reported in other plants (Esau,1938).

As discussed previously, the first cells to mature in a petiolar strand are sieve elements of the phloem and the maturation progresses acropetally. Initially only a single file of cells in the outer region of the strand differentiate. Subsequently other files of procambial cells located successively deeper in the strand undergo final differentiation and maturation. Thus, in addition to the acropetal wave of maturation as seen longitudinally, there is a centripetal progression as viewed transversely at any level in the strand and the primary phloem forms a continuous system throughout its development.

4.11.2 Metaphloem

With regard to the composition of primary phloem, Esau (1943b,1965a) stresses the need of a distinction between the earlier and later parts of the tissue, the protophloem and metaphloem respectively. In Crataeva and Salvadora the two tissues usually merge gradually and their delimitation is exclusively based on a developmental study.

The subdivision of the primary phloem into protophloem and metaphloem in Crataeva and Salvadora is rather arbitrary. In Crataeva the distinction of

metacambium made it possible to demarcate protophloem from metaphloem, while in Salvadora much overlap occurs during the primary development, only early formed and late formed elements are conclusively designated as protophloem and metaphloem respectively. Hence, the classification into protophloem, metaphloem and secondary phloem conveniently designate the successive stages in the development of the phloem (Esau,1943b,1965a) and are used in this sense in the present work.

The metaphloem which appears inward from the protophloem consists of groups of sieve tube elements, companion cells and phloem parenchyma cells. Since it arises in the petiole which shows comparatively decreasing rate of elongation, metaphloem is more persistent than the protophloem. The distribution of various cells in the metaphloem shows no distinctive pattern. It differentiates from a more highly vacuolated procambium than does the protophloem. At the time of metaphloem differentiation the procambium shows a radial seriation of cells. In Crataeva this is clearly evident in the early stage of metaphloem differentiation whereas in Salvadora radial seriation of the procambial cells is noticed at the final stages of elongation. This radial pattern of arrangement is evident in later stages of petiole development as well although eventually the

orderly cell arrangement in the phloem is obscured by the variously oriented divisions concerned with the formation of sieve elements.

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 (Esau,1969; Evert,1977,1990; Cronshaw,1981). The highest level of specialization is found between the sieve tube member and companion cell. Not only are these two cells derived from the same mother cell but they cease to function at the same time.

Metaphloem sieve elements in Crataeva and Salvadora are usually associated with one companion cell, although more than one companion cells are occasionally observed in Crataeva. As is characteristic of phloem processed for paraffin embedding (Esau,1969,1970) the sieve elements in both the plants are deficient in stainable contents, except Rprotein plugs, and contrast sharply with the dense companion cells. In Crataeva these sieve tube elements are comparatively shorter than metacambial cells from which they arose because of transverse divisions in the phloem mother cells. In Salvadora these divisions were not observed.

4.11.3 Secondary phloem

The petioles of Crataeva and Salvadora which ceased to elongate produce secondary phloem. The secondary phloem is derived from the vascular cambium which is composed only of fusiform initials. The secondary phloem reflects this structure of the cambium as it is composed only of the axial system. The production of secondary phloem occurs by tangential divisions of the cambial cells so that the derivatives occur in radial files. Although there is general resemblance in arrangements of the cells of cambium and the resulting secondary phloem, the differentiation of cambial derivatives into phloem elements may 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 toward the phloem usually do not directly differentiate in to 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). The analysis of radial files of cells to determine the ontogenetic relationship of the derivatives was carried out in Crataeva, where the secondary phloem

elements showed a perfect radial arrangement. Although the radial alignment of secondary phloem elements was noticed in Salvadora petiole too, certain growth adjustments hindered their lineages.

In Crataeva, the mother cell producing a sieve tube element, a companion cell and a phloem parenchyma cell commonly undergoes a periclinal and an anticlinal divisions. The anticlinal partition wall generally extends from one end to the other which means that the sieve element and its companion cell are mostly of equal length and the latter has its wall surface in contact with the radial walls of the sieve element. Evert (1963) also 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.

Sieve elements may or may not have an ontogenetically related parenchyma cell, but they generally are associated with a companion cell. The wall between the short companion cell and the sieve element is curved. This feature was more evident in Crataeva than in Salvadora and is already reported in the phloem of Vitis vinifera (Esau,1965c). In the region of interspersed

parenchyma cells they are also contiguous with them. Since the secondary phloem of the stem shows companion cells contiguous to the ray cells (Evert,1963; Cheadle and Esau,1964), it might be that interspersed parenchyma cells also play the role of ray cells during translocation.

The differentiation of the phloem cell as a specific element of the tissue begins after the various divisions are completed. These cells may increase in size, but the sieve elements and the parenchyma cells undergo mainly lateral expansion. The secondary phloem elements in both the plants generally agree in length with the fusiform initials and they are less commonly ontogenetically shortened by anticlinal divisions.

4.12 Anomalous behaviour in Salvadora

In the common pattern of phloem differentiation only the derivatives produced by the cambium in the abaxial direction become part of the phloem tissue. The deviation from this pattern is usually referred to as anomalous (Esau,1969). Metcalfe and Chalk (1950) classified the anomalous structure in to three categories: (1) divided or compound xylem (ii) interxylary or included phloem (iii) secondary thickening from successive cambia. The

anomalous secondary growth in the petiole of Salvadora fits in the second category. Although the cambium is in the normal position, in addition to producing xylem inwards and phloem outwards it forms some increments towards inside so that phloem becomes embedded in the xylem.

According to Mullenders (1947) in Thunbergia mysorensis and T. grandiflora the included phloem is produced on the inner phase of the cambium directly from its derivatives cut toward the xylem. In Leptadenia reticulata and L.spartium, Singh (1943) found that the included phloem differentiated within blocks of parenchyma intermittently formed by the cambium towards the xylem. He (Singh,1944) further made a comparative study of the development of included phloem in the stem of Salvadora persica and other plants reported earlier.

4.13 Contents in the sieve elements

Because of the difficulties of preparing mature sieve tube elements that have a high hydrostatic pressure for electron microscopic examination, the precise distribution of contents in these cells remains controversial. Some workers have described a parietal distribution where it is closely appressed to the plasma

membrane of mature sieve elements (Esau,1969,1978; Evert et al.,1973; Walsh,1980). This type of distribution has been described for several dicotyledonous species, while other accounts (Jarvis et al.,1973; De Maria and Thaine,1974; Thaine et al.,1975) describe the contents having more or less even distribution throughout the lumen of the mature seive tube elements.

The present study has convincingly shown that the contents of a mature sieve tube element at least during a certain stage of development lie at the periphery in Crataeva. The peripheral contents show thin strands or string like extensions. The presence of central cavities is evident in plug formation. The different morphological appearances of the peripheral contents are presumably directly related to the peripheral slime which has undergone varying degrees of disturbances. If the observations of Evert (1977, 1982, 1984) and Evert et al. (1973) is taken into consideration one can visualize that the peripheral cytoplasm and slime may also be bound by a similar membrane. The present study, however, has no evidences for the presence of any disturbed stages of tonoplast in mature sieve tube elements. According to Cronshaw and Sabnis (1990) the relationship between morphologically distinct protein subunits fractionated by gel electrophoresis causes problems 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-protein. In the present work the term slime is being used to mean the P-protein and other contents collected at the sieve plate in the mature sieve elements.

It has been shown by mercuric bromophenol blue that the slime plugs in mature sieve tube elements are proteinaceous in nature in Crataeva and Salvadora. Although oval shaped P-protein bodies and senescent nuclei have been observed in the mature sieve elements of the bark in Crataeva, no such characteristics are observed in the sieve elements of the petiole.

4.14 Companion cells

Many researchers on phloem indicated the presence of P-proteins in the companion cells and phloem parenchyma cells (Esau,1969,1975,1978; Deshpande,1974; Cronshaw, 1975; Wergin et al.,1975). The mature companion cells in the petiole of Crataeva show darkly stained cytoplasmic

contents which may be considered as a substance similar to the slime since it shows a positive reaction with mercuric bromophenol blue. These contents of varying densities are more or less uniformly dispersed. Contrary to the behaviour of slime in the sieve elements, these densely stained contents do not form any strands or plugs, but it always takes a deeper staining compared to slime of the associated sieve element.

Companion cell is the most intimately related parenchymatic element of the sieve tube element. Not only is it derived from the same mother cell as its associated sieve tube element, it remains alive as long as its sieve tube element does. The presence of slime in it is another indication of its close morphological and physiological association with the sieve element.

4.15 Comparison between phloem in the stem and petiole

Studies on the structure and differentiation of the secondary phloem have dealt almost entirely with stems. Occasional references to roots and petioles are made in a comparative way (Mehta and Spanner,1962; Mehta, 1964; Shah and Jacob,1969; Shah and James,1969, Shah and Daniel,1970; Eleftheriou and Tsekos,1982). The observations on the phloem in the petiole and stem of Crataeva and Salvadora reveal more dissimilarities than

similarities. One of the consistent differences in the structure of this tissue in the two parts of the tree, apart from dimensions, is the larger ratio of living to nonliving cells in the stem. Sclereids, secondary phloem fibres and periderm are completely absent in the petiole of both the plants investigated. Another noteworthy feature which is discussed earlier is the absence of rays in the petiole.

4.16 Nonconducting phloem

The term nonconducting denotes the part of the phloem in which the sieve elements have ceased to conduct (Esau,1969). The break down or degeneration of sieve elements in the older phloem is known as obliteration. Although the degeneration of the sieve elements itself and of the closely associated cells appears to be the primary cause of the obliteration, the actual crushing results from growth adjustments within the tissue.

As is characteristic of many dicotyledons the protophloem sieve elements in the petiole of Crataeva and Salvadora differentiate in the outer layers of the procambium while the surrounding cells are still meristematic. They apparently function for a brief period only. During early rapid elongation of the petiole they are destroyed, soon after maturation,by the effects of

elongation of the surrounding cells. It might be that being enucleate cells these sieve elements are unable to keep pace with the growth by active elongation and are passively stretched and become functionless. The various signs of the inactive state of the sieve elements are readily detected. The sieve plates and sieve areas are covered with a mass of definitive callose. No dormancy callose was observed in Crataeva and Salvadora during the study period. The determination of the nonfunctioning state of the phloem is certain because the sieve elements are more or less collapsed.

In both the plants the phenomenon of obliteration is pronounced in the early formed phloem. The later formed metaphloem shows slow and partial obliteration. Similar observations on obliteration are reported by Esau (1938). The metaphloem undergoes obliteration after secondary growth is initiated. The obliteration of cells frequently seems to progress more slowly than that of the protophloem. In both the cases obliteration proceeds from the periphery to the centre.

After the obliteration of the early formed sieve elements the parenchyma cells surrounding them rapidly enlarge. They gradually elongate alongwith the petiole.

Both in Crataeva and Salvadora in the middle region of the petiole these cells develop wall thickening and lignification during secondary growth, while in the distal and proximal regions they remain comparatively thin walled. These fibres are all procambial in origin.

4.17 Secondary phloem production and longevity of sieve elements in the petiole.

In Crataeva and Salvadora with advancing leaf age the radial length of the secondary phloem remains fairly constant or shows gradual increase despite monthly production of new phloem cells along the entire length of the vascular strands. The comparatively low rate of leaf phloem production per month might explain why the leaf vascular cambium always remains narrow at two to four cells layers. It may also be that newly formed phloem cells continuously replace senescent phloem cells in position and, presumably in function. Similar results have been reported by Ewers (1982) in Pinus longaeva and Ewers and Aloni (1987) in Pinus strobus and Pinus brutia needles.

Secondary growth in the petiole of both the plants is much more localized than Elliot (1937) described for leaves of Araucaria imbricata, a confier with leaves

living upto 30 years. In this species the transfusion mesophyll tissues proliferates, the leaf epidermis splits and cork forms underneath the epidermis. In contrast, the present work shows, except for phloem, little histological changes with advancing leafage. He also found for thirteen species of evergreen dicotyledons that leaves had secondary xylem with annual increments but no secondary phloem. He also claimed that in leaves of many evergreen dicotyledons neither secondary xylem nor secondary phloem was produced. In both Crataeva and Salvadora secondary xylem production was more than that of the secondary phloem.

The behaviour of sieve tube elements and contiguous cells during the transition of the phloem from a functional to a non-functional condition has long been recognized (Esau,1969). Commonly the earliest sign of initiation of cessation of function is the appearance of definitive callose at the sieve plates and lateral sieve areas of the sieve tube elements. Unlike the stem, sieve tube elements of the petiole have only a limited span of life. Undoubtedly the shortest lived sieve elements are those of the protophloem which are soon replaced by those of the metaphloem and later by the secondary phloem.

4.18 Wound phloem

In intact plants, the causal relations of phloem development and function are difficult to investigate, since initiation and differentiation of sieve elements occur continuously and asynchronously in adjacent (pro-)cambial cell files. Phloem research has, therefore, increasingly focussed on conditions where new elements are experimentally induced (Schulz, 1990). Severing the vascular strand leads to the development of wound phloem and xylem elements which bridge the vascular discontinuities and reconnect the interrupted strands within few days. The idea of inducing wound phloem in Crataeva, however, was to compare the structure of sieve tube elements in both intact as well as wounded system. Compared to the regular sieve elements the shape of wound sieve elements is very variable and it is mostly determined by the outline of the original cell and the angle by which this cell is subdivided during the initial meristematic divisions. The aniline blue induced fluorescence of sieve plates and sieve areas helped to demarcate them in their early stage of differentiation.

The restitution of an experimentally wounded petiole is dependent on rapid recovery of the interrupted transport paths. Thus severance of pre-existing phloem in

the strands induces the regenerating tissue to form sieve elements as quickly as possible. In Crataeva wound sieve tubes were differentiated within 8 - 10 days.

4.19 Colouration by thiazolyl blue and physiology of the phloem

Thiazolyl blue staining, taken as an indicator for metabolic activity of phloem and contiguous tissues in the petiole, indicated that contact parenchyma cells of the xylem and companion cells of the phloem were very active. The sieve tubes contain less cytoplasm and mitochondria than companion cells. Companion cells in general have a very dense cytoplasm crowded with ribosomes and mitochondria (Behnke,1975). The mitochondria in the companion cells are very active, indicating a high level of metabolic activity. The metabolic activity of the companion cells and xylem contact parenchyma cells indicates cytological convergencies like mitochondria and little or no storage material (Czaninski,1977). Consequently, there might be a functional analogy between the companion cells (Sauter et al.,1976; Czaninski,1977) and the xylem contact parenchyma cells (Sauter,1972) in controlling the solute content of the translocation channels.

In Salvadora, the included phloem elements show deep staining than outer secondary phloem elements. It may be

that the included phloem acts as a major route for translocation than outer secondary phloem which is comparatively less in area than that of included phloem.