INTRODUCTION

1 INTRODUCTION

Trees have always been of much interest to us. Man has long sought their shelter and protection, utilized their food and fibre and often exploited them to his own detriment. Trees have also the distinction of being the largest and oldest conspicuous living organisms covering the habitable land surface of the earth and offer an amazing diversity of form. We are inseparable from trees probably because we had an arboreal ancestry. Reposing under a tree is like being cuddled by a mother, comfortable and secure. As principal components of forests that form the green mantle over the earth or as individuals, trees play multiple roles in nature. They are unmatched as providers and protectors (Mohan Ram, 1993).

For our ancestors they were a source of fuel and shelter and sometimes an object of worship. To the home owner they are a source of pleasant shade in the summer but in autumn a nuisance which shed leaves on the lawn. To the arborists trees are ornamental objects in the landscape, to foresters a source of timber and pulpwood and to horticulturists, a means of producing fruits. Trees have also been of much interest to the botanists. To physiologists trees are complex biochemical factories which grow from seeds and literally build themselves. Trees are of great interest to molecular biologists because trees perform all the cellular activities. In the recent years molecular biology has made such an unprecedented progress that those who work in this field may seem odd that some biologists still choose to work with whole organisms, especially trees. But where else can one better study the many intriguing problems of water and food transport, dormancy, cambial activity, differentiation of the conducting tissues etc., than in woody plants (Zimmerman and Brown, 1971).

The vascular architecture in a plant body serves as an extensive transport system that permeates almost every organ of the plant. Any blockage or disruption of the vascular system can either kill or debilitate the affected plant part. We are now well informed about the essential processes by which water and minerals are transported from absorbing roots, and assimilates are translocated from photosynthesizing leaves. The specific aspects of vascular structure and function have also been studied quite a lot. But no concerted effort has ever been made either to describe or to comprehend how this system develops and organizes itself to function throughout the development (Larson, 1983).

In recent years a totally new awareness has developed among plant anatomists with regard to the importance of correlating plant structure with function (White and Dickison, 1984; Dengler and Dengler, 1990; Fisher, 1990; Warmbroadt et al., 1990). One of the long standing topics in plant anatomy relates to the development of the leaf at the shoot meristem. The leaf is concerned with one of the most important activities of the green plant, the photosynthesis. A leaf primordium is initially heterotrophic and later becomes an autotrophic organ during its ontogeny. Initial dependence on phloem imported nutrients is lost, as photosynthesizing capacity helps the lamina to satisfy its own carbon requirements and to export excess photosynthates. The potentiality of the leaf to export the assimilates is determined by its vascular organization particularly its phloem. The subject presented throughout the thesis is to view the leaf in a developmental and structural whole.

In this work structure of phloem in the petiole is the central theme. Equal attention has also been given to the study of

(i) internode-node-petiole continuum and petiole vasculature.
(ii) development and structure of procambium and its transformation to cambium as it is related to,
(iii) the development and structure of phloem in the petiole during the life span of the leaf until its senescence.

With a view to understand these aspects in a deciduous and evergreen leaf some parameters have been analyzed in *Gmelina arborea*, a deciduous tree and *Tabebuia rosea*, an evergreen tree and are presented in the thesis.

1.1 Internode-node-petiole continuum

The node is commonly defined as the position on the stem at which leaves occur and the internode as the part of the stem between two successive nodes (Howard, 1974). The leaves are lateral appendages to the stem, dorsiventrally flattened with a restricted terminal growth, with an interpolated petiole and associated primarily with photosynthetic function. But when observed anatomically one is able to observe a continuity between the three portions. Hence for an understanding of the internal structure of any one of them, the information from the adjacent structure is essential. Moreover, the changes taking place during the initiation and ontogeny of the leaves are so intimately bound up with the development of the node and stem that they cannot be considered in isolation. This view has been clearly emphasized by Howard (1979a). In a review of the stem-node-leaf continuum in Dicotyledonae, Howard (1974) wrote "the continuity of vascular tissue from the stem through the petiole to the apex of the leaf is evident, yet the nature of its path and variations in the pattern of bundle arrangement and position along its length have not received much consideration". A similar review ten years later (Larson, 1984a) revealed that our knowledge of the course of vascular bundles through the stem-node-leaf continuum has advanced very little.

Nodal anatomy has been an active area of interest and investigation because it is widely regarded as a reliable indicator of natural relationship (Howard, 1974; Dickison, 1975; Esau, 1977). Based on the characters like the gap and its number and number of traces, different nodal types have been proposed by many authors (Sinnott, 1914; Marsden and Bailey, 1955; Pant and Mehra, 1964). Howard (1979a) classified nodal structures from the level of gaps to the vascular pattern occurring in sections through the lower pulvinus and at successive higher levels in the petiole and into the midrib of the lamina. This classification has been applied successfully to a majority of vascular system patterns found in the dicots as a whole. It does not, however, cover all the possible combinations of node-petiole vascular structure with other useful characters.

Stems where a cortical vascular system is present, which may or may not contribute to the vascular supply of the leaf have been reported by many (Fahn and Bailey, 1957; Balfour and Philipson, 1962). But the developmental study of cortical vascular system in the stem of Chimonanthus by Balfour and Philipson (1962) is noteworthy.

1.2 Petiole vasculature

Although the pattern of petiole vasculature is often a species characteristic and can be used for taxonomic purposes (Hare, 1943; Howard, 1963), there is much variability among petioles on a single plant depending upon their stages of development and positions sampled. The first comprehensive survey of petiole anatomy was that published by De Candolle in 1879. He proposed the terminology of an open system versus a closed system. In the open system the strands are arranged in an arc. In the closed system the strands form a circle comparable to that of the stem. He also recognised a principal system and an accessory system. Only a few studies have described the entire leaf in detail, describing the course of the vascular bundles beginning with the leaf traces through the petiole and midrib to their points of departure as secondary veins of the lamina (Watari, 1934, 1936, 1939; Isebrands and Larson, 1977a,b; Howard, 1979b; Fisher and Evert, 1982a; Russin and Evert, 1984; Larson, 1986a,b; McCauley and Evert, 1988). Hare (1943) classified the vascular structure of the petiole on the basis of the arrangement of the vascular strands. Metcalfe and Chalk (1950) devised a classification in which nine types of steles are recognized varying from the arc to the open or closed ring of xylem and phloem. The concept of stele is mostly derived from the vascular pattern of the stem based on its vascular relationship with the leaf. No such relationship exists in the petiole and lamina of a simple leaf. Howard (1963) in his more comprehensive system of classification of petiole types recognized some 40 categories grouped according to the nodal structure which pertains to the point of attachment of petiole to the stem and the subsequent fate of a trace. Gendels (1989) studied the structure of the conductive system of the node and petiole with respect to the morphogenesis of woody dicotyledonous leaves.

The arrangement of vascular strands in the petiole and the midrib of angiospermous leaves has been studied primarily with regard to its taxonomic value (Zubkova, 1965; Pimenov, 1970; Dehgran, 1982; Petrova, 1983; Khare and Shankar, 1989). In some cases families can be recognized, in other cases genera, species or varieties can be distinguished on the basis of petiole vascular patterns (Semichishina _et al_, 1985; Zakorko, 1985; Dickison, 1987; Sokolova, 1989; Wilkinson, 1989). Though some of these workers indicate a continuity of the vascular supply from the stem to the apex of the leaf through the petiole, the nature of its variations in its entirety is presented only in a very few papers (Schofield, 1968; Sugiayama, 1972,1976; Howard, 1979a; Larson, 1984b).

1.3 Primary vascular system

At the shoot apex a leaf primordium is distinguishable in a definite relationship with other primordia, which is expressed eventually as the phyllotaxy of leaves on a stem. This arrangement may be established as alternate, opposite or whorled. The leaf primordium developing on the flanking slope of the shoot apex develops into a recognizable leaf form. The vascular system in it is differentiated in stages first as procambium and subsequently as phloem and xylem, thus consequently establishing the continuity of conducting tissue from the stem into the leaf and is recognized as the primary vascular system (Howard, 1974). The primary vascular system may be viewed as a template upon which the secondary system is superimposed as it develops. Thus, to understand how the secondary vascular system is organized it is first necessary to possess a thorough knowledge of how the primary vascular system achieves its organization.

A remarkable diversity of vascular architecture among the angiosperms has been noticed (Beck et al., 1982). This diversity lies not in the fundamental nature of the vascular patterns but rather in such details as direction of trace divergence, direction of the ontogenetic spiral, number of traces per leaf, origin of traces to a single leaf, nature of leaf insertion, the open or closed nature of the system and the presence or absence of cortical and medullary bundles.

Dormer (1945) classified the vascular systems as 'open' or 'closed'. The open system is characterized by bundles which branch but only exceptionally rejoin. The closed system is essentially a reticulum or network of bundles which branch or anastomose. He could also recognize intermediates, or both types could occurring in the same vascular system. The primary vascular system in some species has been studied with regard to its taxonomic importance (Ezelarab and Dormer, 1963; Philipson and Balfour, 1963; Weidlich, 1976a,b). Devadas and Beck (1971, 1972) studied two species of Leguminosae and five species of Rosaceae in an attempt to determine the relationship between the development of primary vascular strands and their morphology and the variations in morphology among vascular strands of different types in the stem.

The development of primary vascular system in the petiole of Gmelina and Tabebuia has been studied as it originates from leaf trace and described in the thesis.

1.4 Leaf vasculature

The structure of angiospermous leaves has long been an object of intensive investigation by plant biologists (Esau, 1965b, 1977; Colbert and Evert, 1982; Fisher and Evert, 1982a; Russin and Evert, 1984; Russel and Evert, 1985). Most of these investigations on the leaf, however, have emphasized particular aspects of leaf structure such as vascularization of the petiole or lamina or features related to ecological adaptations, environmental factors or photosynthetic pathways. The capacity of the leaf to export assimilates is determined in part by the extent and arrangement of its vascular system, moreover the pathway followed by the photosynthates out of a leaf is determined in large part by the canalizing effect of the vascular organization of the leaf. A thorough knowledge of the vasculature of a mature leaf is also an indispensable prerequisite for understanding the functional aspects of assimilate transport over both short and long distance (McCauley and Evert, 1988). A series of investigation in this area have been conducted (Isebrands *et al*., 1976; Isebrands and Larson, 1977a,b, 1980; Evert *et al*., 1978; Evert, 1980; Fisher and Evert, 1982b;

Colbert and Evert, 1982; Vogelmann et al., 1982; Russin and Evert, 1984; Larson and Dickison, 1986; McCauley and Evert, 1988).

Leaf architecture is yet another aspect which remains as a virtually unexploited area. Leaf architecture denotes the placement and form of those elements constituting the outward expression of leaf structure including the venation pattern, marginal configuration and leaf shape. Architecture is the aspect of morphology which applies to the spatial configuration and co-ordination of those elements making up part of a plant with regard to histology, function, origin or homology (Hickey, 1979).

1.5 Procambium and cambium

Procambium demarcates the prospective vascular system in the meristematic tissue, determining the vascular organization. By serving as a template for primary vascular differentiation, it directs the vascular function. Development and organization of the procambium are, therefore, fundamental to development and organization of the mature vascular system (Larson, 1983). A considerable number of investigations have been devoted to the ontogenesis of the vascular system of the leaf (Esau, 1942; Devadas and Beck, 1971, 1972; Isebrands and Larson, 1980). However, in general, they deal with questions of procambial differentiation and the formation of primary conducting elements. There is insufficient information concerning the presence of cambium and of secondary growth of the vascular system in the leaf. Although a number of authors have noted the presence of cambium in the leaves of woody dicotyledons (Elliot, 1937; Esau, 1953; Samantarai and Kabi, 1953, 1974; Shtromberg, 1959), they have not given any detailed descriptions of its meristematic activity.

Esau (1943a, 1965 a, b) has emphasized that the classification into primary and secondary growth should have a broader basis rather than the single characteristic of cell orientation. Procambium and cambium responsible for primary and secondary growth are looked upon as two developmental stages of the same meristem and there are no clear cut criteria by which they can be separated since all the differences between the two tissues appear gradually at different stages. Philipson and Ward (1965) have also suggested that the primary growth of the vascular system merges gradually into secondary growth and the two phases cannot be distinguished sharply.

The development of procambium and cambium in the leaves of evergreen and deciduous trees has been neglected and the literature reveals that most of the attention has been paid on their development and structure in the stems and roots (Esau, 1942; Paliwal and Prasad, 1970, 1971; Fahn et al., 1972; Soh, 1972, 1974a,b; Ghouse and Yunus, 1973, 1974; Catesson, 1974; Paliwal et al., 1975, 1976; Butterfield, 1976; Dave and Rao, 1981, 1982; Rao and Dave, 1981, 1983, 1985; Soh et al., 1988, 1991;

Venugopal and Krishnamurthy, 1989; Hong and Soh, 1993). Very few studies have been conducted to trace the development of cambium in the petiole of evergreen and deciduous dicot leaves. Elliot (1937) studied the development of vascular system in some species of the evergreen dicotyledonous and coniferous leaves which were more than one year old. He also showed that differentiation of cambium was more evident in the petiole than in other parts of the leaf. In the leaves of the five species of evergreen dicotyledonous plants studied by Shtromberg (1959), cambial activity was more clearly expressed in the petiole than in other parts of the leaf. Ewers (1982) reported a unidirectional phloem producing cambium in the leaves of *Pinus longaeva*. The characteristic changes during the transformation of the procambium into the secondary vascular meristem in the petiole of the selected plants have been studied and presented in the thesis.

According to Esau (1943b) interfascicular cambium is a product of residual meristem or procambium, while some others suggest its origin from the interfascicular parenchyma (Catesson, 1964, 1974; Devadas and Beck, 1971; Phillips, 1976; Swamy and Krishnamurthy, 1980). These authors have mainly studied the development of the interfascicular cambium in the shoot. The nature of the interfascicular tissue in the vascular system during the different developmental stages of the petiole in the selected evergreen and deciduous dicot leaves has also been investigated during this study.

1.6 Phloem

The leaf of angiosperms is an elegantly integrated structure associated with a number of complex physiological processes. As the organ specialized for photosynthesis, it is the principal source of assimilates in the plant (Russin and Evert, 1984). The petiole is directly concerned with the transport of photosynthates more than any other region in a plant and the phloem tissue within it is the principal food conducting tissue of the vascular plants. Phloem has received much attention after the discovery of sieve elements in the bark by Hartig (1837). This event has further led to the exploration of physiological, cytological and ontogenetic aspects of phloem and the pertinent literature has been reviewed by many (Esau, 1969; Parthasarathy, 1975; Evert, 1977; Cronshaw, 1981; Behnke and Sjolund, 1990).

The structure of phloem in the petiole especially in deciduous and evergreen types of leaves of the tropical and subtropical trees has received scant attention. (Ewers, 1982; Ewers and Aloni, 1987). The structural and functional significance of the primary and secondary phloem of the petiole in translocation, in the evergreen and deciduous leaves through their various developmental stages and survival is not fully investigated.

Studies conducted on phloem in leaves were mostly centered on the minor veins because of their important role in translocation (Esau, 1967; Fisher and Evert, 1982b; Evert, 1984; Russin and Evert, 1985; Gamalei, 1988a,b). Very few studies reveal the structure of phloem in the petiole, and most of these studies have been conducted in the herbaceous annuals (Esau, 1938, 1970, 1973, 1975; Mehta and Spanner, 1962; Mehta, 1964; Shah and Jacob, 1969; Shah and James, 1969; Esau, 1978).

Assimilate export from photosynthesizing leaves begins with the transport of photosynthates from the mesophyll cells to the sieve tubes of the minor veins. Because of their numerous connections with the sieve tube members and their general ultrastructural resemblance to secretory cells, companion cells have long been regarded as intermediary cells specialized for the uptake of sugar and its delivery into the sieve tube (Esau, 1977; Giaquinta, 1983). The phloem in the minor veins of dicot leaves is characterized by the presence of diminutive sieve tube members and large companion cells and other parenchymatous cells (Cronshaw, 1981). This size relationship and the high solute concentration in the companion cells, equal to or greater than those of the sieve tube members, are generally regarded as further evidence of the role of companion cells in phloem loading in minor veins. The present thesis describes the development and structure of phloem in young, mature and senescent petioles.

Many workers (Esau and Cheadle, 1955; Cheadle and Esau, 1958; Evert, 1960, 1963) have attempted to study the various planes of divisions occurring in the cambial derivatives (phloem initials) to know the interrelationship among various cells of the secondary phloem. In the present work also a similar attempt has been made to investigate the pattern of differentiation of phloem initials and the ontogenetic relationship of various secondary phloem cells.

Some of the main aspects of controversy over the structure of a sieve element are the nature and behaviour of its contents and their location in a mature uninjured sieve element (Evert *et al.*, 1973; Wergin *et al.*, 1975; Behnke and Sjolund, 1990). Depending upon the state of the injured sieve tube element the distance from which the material is sectioned after the first injury is done, the sieve tube element contents are aggregated at or near the sieve plate of the sieve tube element giving distinct and varied morphological configurations. The study of these various configurations may, perhaps, give an indication of the nature of the contents of sieve tube element and its functioning state. I have studied these configurations at the light microscopic level.