INTRODUCTION

1 INTRODUCTION

Trees have the distinction of being the largest and oldest living organisms on earth. Although the herbaceous habit has made unprecedented evolutionary gains since the middle and late Cainozoic, trees still are the most conspicuous plants covering the habitable land surface of the earth. Man has long sought their shelter and protection, utilized their food and fibre, and often exploited them to his own detriment.

Trees have always been of much interest to botanists and many of the early investigations concerning the structure and function of plants were conducted with trees (Zimmermann and Brown, 1971). At the beginning of the present century the use of trees for basic investigations began to decline. The reasons for this are obvious. Investigations of structure and function began to shift from whole organisms to tissues, then to individual cells, and finally to cellular organelles and subcellular particles. Physiological research became increasingly more detailed and complex, requiring more and more precisely controlled laboratory conditions. Hence a relatively small number of herbaceous plants, various unicellular algae, fungi and bacteria have become standard research materials in most laboratories.

Because of the unprecedented progress molecular biology has made in recent years, it may, perhaps, seem odd to those who work at the cellular and molecular levels that some biologists would still choose to work with whole organisms, especially trees! Where else can one better study the many intriguing problems of water and food transport, dormancy, cambial activity etc. than in woody plants (Kozlowski, 1971). Trees are not only an object of interest to plant scientists, but also play an important role in our daily lives. Once we realize how important trees are, then it is obvious that we need to know as much about their structure and function as possible.

Unfortunately, our forestry traditions regarding growing, harvesting and utilizing trees have not left us in such a favourable position to cash in on the green revolution sweeping agriculture. We are still pretty much dependent on the trees that nature gave us. Even today our concept of an ideal tree is essentially an imitation of the ideal tree growing in the natural forest. Because of past traditions of timber abundance, we haven't felt the need to really put our imagination to work to consider seriously how we might alter or restructure a tree to produce more wood. Now that we are beginning to ask these questions we find our background of basic research information in rather short supply (Mohan Ram, 1993).

The main body of most higher plants has an elaborate vascular system that permeates almost every organ of the plant. The vasculature serves as an extensive transport system, and blockage or disruption of this system can either kill or debilitate the affected plant part. Surprisingly, however, we are relatively uninformed about this complex circulatory system when viewed in its entirety. We know much about specific aspects of vascular structure and functions. But no concerted effort has ever been made either to describe or to comprehend how this system develops, how it is organized, or how it functions throughout 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 longstanding topics in plant anatomy relates to the development of the leaf at the shoot meristem. Numerous descriptive studies have indicated considerable variations in patterns among major plant groups (White and Dickison, 1984). The subject present throughout the thesis is to view the leaf in a developmental and structural whole. In this work the structure of the phloem in the petiole is the central theme. Equal attention has also been given to the study of

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

1.1 Internode-node-leaf continuum and petiole vasculature

Although the internode, the node, and the leaf are treated as individual morphological entities, an understanding of the internal structure of anyone of these is associated with the information from the adjacent structure. In a review of the stem-node-leaf vascular continuum in the Dicotyledonae, Howard (1974, p. 134) mentions that "the continuity of the vascular tissue from the stem through the petiole to the apex of the leaf is evident, yet the nature of its path and variation of 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 understanding of the course of vascular bundles through the stem-node-leaf continuum has advanced very little.

The first comprehensive survey of petiole anatomy was that published by De Candolle in 1879. He described open system, closed system and intermediate system. In the open system the bundles, as seen in a cross section, are arranged in an arc. In the closed system the bundles formed a circle comparable to that of the stem. The bundles could be free or united. Among dicotyledons with helical phyllotaxy, the open system is by far the most common; closed vascular system occurs in both woody and herbaceous species. The system that is partly open is designated intermediate system. An extensive survey of vascular structure of the petiole and leaf has been made by Watari for the Fabaceae (1934), Acer (1936) and the Saxifragaceae (1939). Watari (1934) considered the entire length of the vascular tissue from the node through the petiole and lamina. His detailed work (1936) concerned the many branchings of the traces and their inter-connection and for each group Watari (1939) proposed a classification based on the petiole structure. Hare (1943) proposed a simple classification of the vascular structure of the petioles in transverse section as U-shaped, I-shaped or O-shaped, the latter being the hollow cylinder of the petiole stele. The vascular pattern of the petiole is of value as a taxonomic character useful in the identification of taxa. The systematic level of the taxonomic value does vary from one taxon to another. In some cases families can be recognized; in other cases genera, species or varieties can be distinguished on the basis of petiole vascular patterns. Several investigations have been done in this area (Zubkova, 1965; Sokolova, 1968, 1989; Pimenov, 1970;

Dehgran, 1982; Petrova, 1983; Semichishina et al., 1985; Zakorko, 1985; Dickison, 1987; Gendels, 1989; Khare and Ramashankar, 1989; Wilkinson, 1989). Although many of these workers indicate that there is a continuity of the vascular supply from the stem to the apex of the leaf through the interpolated petiole, the nature of its variation, as revealed in transverse section within its length, is presented in the existing literature in a very few papers (Schofield, 1968; Sugiyama, 1972; Howard, 1979b; Larson, 1984a).

1.2 Primary vascular system

At the shoot apex the primordium of a leaf is distinguishable in a definite relationship to other primordia, which is expressed eventually as the phyllotaxy of leaves on a stem. This arrangement may be established as alternate, opposite or whorled. As the leaf primordium develops into a recognizable leaf form, its vascular system is differentiated in stages, first as procambium and subsequently as xylem and phloem. The differentiation of the procambium is generally described as acropetal, taking place while the primordium is still undergoing cell divisions and even elongation (Esau, 1965a). Later, differentiation of the xylem begins at the base of the leaf primordium and develops acropetally into the leaf and basipetally into the stem (Dormer, 1972). The continuity of conducting tissue from the stem into the leaf is thus established.

Some of the many vascular patterns in the stem of angiosperms were known in the nineteenth century, but it has been only within the last three or four decades that the remarkable diversity of vascular architecture in different organs among angiosperms has become clearly apparent (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, number of axial bundles between orthostichies, the open or closed nature of the system, the presence or absence of cortical and medullary bundles etc.

Two contrasting interpretations of the primary vascular system of dicotyledons are current in botanical literature (Philipson and Balfour, 1963). One of these holds that in most dicotyledons the primary system forms a cylinder which is perforated by leaf and branch gaps. The other interpretation considers that even in woody plants the vascular tissues arise as a system of discrete bundles. The general development and micromorphology of the primary vascular tissues of seed plants have been studied in detail by Esau (1943b, 1965a). Devadas and Beck (1971, 1972) attempted to determine the relationship between the development of primary vascular bundles and their morphology and the variation in morphology among vascular bundles of different types in the stem. Studies of Ezelarab and Dormer (1963) in Ranunculaceae and Weidlich (1976a,b) in Nymphaeaceae offers several characters of the primary vascular system which are important in taxonomy.

I have studied the development of the primary vascular system of the petiole as it originates from the leaf trace. The concept of leaf trace and leaf gap is not involved in the establishment of this vascular system. The establishment of procambium and/or its precursors starts with the leaf trace meristem associated with the young leaf primordium. Thus the developmental situation controlling the establishment of the procambial pattern at the base of the leaf primordium - future petiolar region in petiolate leaves - is altogether different from that prevailing at the shoot apex where the primary vascular meristem pattern of the stem axis is founded.

1.3 Leaf vasculature

Leaves are usually described as lateral appendages to the stem, dorsiventrally flattened, with a restricted terminal growth, an interpolated petiole and having primarily a photosynthetic function. There are many variations to all of these morphologic phases. The term leaf architecture denotes the position and form of the elements contributing the outward expression of leaf structure. Architecture is the aspect of morphology which applies to the special configuration and co-ordination of those elements making up part of a plant without regard to histology, function, origin or homology (Hickey, 1979).

The structure of angiospermous leaves has long been an object of intensive investigation by plant biologists (Esau, 1965b, 1977; Colbert and Evert, 1982; Fahn, 1982; Fisher and Evert, 1982a; Russin and Evert, 1984; Russel and Evert, 1985). The angiospermous leaf functions as a highly efficient organ as it manufactures photosynthates and exports them to other parts of the plant. The capacity of a 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. Thus, mapping the details of the vasculature of a mature leaf is an indispensable prerequisite for understanding the functional aspects of assimilate transport (McCauley and Evert, 1988). A series of investigation in this area have been conducted by Evert and his team at the University of Wisconsin (Evert et al., 1978; Evert, 1980; Colbert and Evert,1982; Fisher and Evert, 1982 Russin and Evert, 1984; Russel and Evert, 1985). The interrelation between the vasculature of a leaf and the pattern of assimilate transport from the leaf has been demonstrated for several woody species also (Larson and Dickison,1986). In cottonwood, the structural pathways revealed by anatomical analysis (Isebrands and Larson, 1977a, 1980) were demonstrated to be functional pathways by ^{14}C-translocation experiments (Isebrands et al., 1976; Vogelmann et al.,1982). Similar results were obtained from anatomical and experimental studies on honey locust and green ash leaves (Larson, 1985; Larson and Dickison, 1986), Paliwal et al. (1975b) investigated the leaf development in Crataeva adansonii with special reference to the gradual expansion of the epidermal cells, increase in the areole size and growth of the lamina.

1.4 Procambium and cambium

In general, developmental studies have been concerned with leaf primordia and very immature leaves. With few exceptions, no literature seems to concern the development of the many varied and complex patterns of arrangement of mature vascular tissues in the petiole of a mature leaf or how such complexities are established in the interpolated organ (Howard, 1979b). Petioles of leaves more than one year old usually show secondary growth with some evidence of annual increments (Barykina and Chubatova,1980; Steingraeber and Fisher, 1986).

It is obvious that the vascular system is essential to the development and functioning of leaves. It is perhaps less obvious that prior knowledge of stem vasculature is essential to understanding leaf vasculature. A consideration of the procambial system that serves both stem and leaf is therefore germane to an inquiry into leaf growth and function (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; Larson, 1983). However, in general, they deal with questions of procambial differentiation and the formation of primary conducting elements. There is much less data available concerning the presence of a cambium, its origin from procambium and 'secondary' growth of the vascular system in the leaf. Although a number of authors note the presence of cambium in the leaves of woody dicotyledons, they do not give any detailed description of the meristematic activity through the life span of the leaf. (Napp-Zinn, 1966; Howard, 1974; Samantarai and Kabi, 1974).

Vascular development in the leaves of evergreen and deciduous trees has hitherto been neglected and survey of the literature reveals that attention paid to these species has been divided between their structure in the stem and its seasonal activity (Paliwal and Prasad, 1970, 1971; Ghouse and Yunus, 1973, 1974; Paliwal *et al*.,1975a, 1976; Dave and Rao, 1981,1982; Rao and Dave, 1981,1983,1985; Khan *et al*.,1988; Venugopal and Krishnamurthy, 1989). Elliot (1937) has studied the development of vascular system in the evergreen leaves of angiosperms which are more than one year old. In almost every species he examined, differentiation of the cambium was more prominent and more advanced in the petiole than in other parts of the leaf. Shtromberg (1959) reported the absence of cambium in deciduous trees investigated. On the contrary, the leaves of all evergreen species studied were characterized by considerable cambial activity. In *Pinus longaeva*, Ewers (1982) reported the presence of a unidirectional phloem producing cambium.

Esau (1943b, 1965a,b) has asserted that the classification into primary and secondary growth has a broader basis than the single characteristic of cell orientation. She believed that the procambium and cambium may be looked upon as two developmental stages of the same meristem. Philipson and Ward (1965) have recognized that primary growth of the vascular system merges gradually into secondary growth and the two phases cannot be distinguished sharply. However, Philipson *et al*. (1971) have pointed out that the fundamental problem can be resolved only when the early ontogeny of the cambium becomes known in many plants and they suggested that great diversity will be revealed. A definition of the transition from procambium to cambium was established for *Populus deltoides* by Larson and Isebrands (1974) based on birefringent properties of the fibre wall. They suggested cessation of either internode elongation or lamina expansion as an acceptable criterion for certain developmental features.

Early ontogeny of the cambium in the stem and root has been studied in a number of plants by several workers (Fahn *et al.*, 1972; Soh, 1972, 1974a,b, Soh *et al.*, 1988; Catesson, 1974; Butterfield, 1976; Larson, 1982; Cumbie, 1984). The procambium and cambium are both meristematic tissues which give rise to xylem and phloem. At least part of the cambium is normally formed from the procambium, and the problem dealt with in the present work is characteristics of the transition leading to a typical cambium. It is hoped that knowledge concerning this transition would lead to a better understanding of the relationship of these meristems in the three organs of a plant.

Cambial structure, development and activity were extensively reviewed some years ago in the first published comprehensive account on the vascular cambium (Philipson et al.,1971) and more recently by Iqbal (1990).

1.5 Phloem in the leaves

Although it is now more than 150 years since Hartig (1837) first discovered sieve tubes and associated solute transport with sieve elements, the structure of functioning sieve elements and the mechanism of phloem transport are still controversial issues. The extreme sensitivity of sieve elements to injury has made physiological and structural investigations of elements very difficult (Parthasarathy, 1975). But in recent years there has been an increasing realization that the structure of phloem is closely related to its function in translocation (Behnke and Sjolund,1990). Refined techniques of electron microscopy have produced substantial progress in our understanding of phloem cell differentiation and maturation.

Studies conducted on phloem in leaves were mostly centered on the minor veins because of their important role in translocation. There are only few reports of the structure of phloem in the petiole. Most of these studies have been conducted in herbaceous annuals (Mehta and Spanner, 1962; Mehta, 1964; Shah and Jacob,1969; Shah and

James, 1969; Thorsch and Esau, 1988). Esau (1943a) reported the vascular differentiation in the vegetative shoot of Linum perenne and gave detailed description of the early differentiation of vascular elements. The structure, development and seasonal variations of phloem is presented by Esau (1969) in her monumental work on phloem. She studied (Esau,1967) the minor venation of sugar beet, a plant that transports large amount of sugar from the leaves to the sugar-storing fleshy beet root. The nucleate parenchyma cells in the phloem is assumed to be equipped to carry on the enzymatic activities necessary for the transportation of sugar moving from the mesophyll into the phloem and for their secretion into the sieve elements. She also reported (Esau, 1973) usual dicotyledonous type of sieve element in the leaf and petiole of Mimosa pudica and studied certain developmental features of the primary phloem in Phaseolus vulgaris (Esau, 1978).

Several papers on the anatomy of leaf have attempted to describe the constituents of minor veins and their role in translocation studies (Walsh, 1974; Fisher and Evert, 1982b; Russin and Evert, 1985). Assimilate export from photosynthesizing leaves begins with the transport of photosynthates from the mesophyll cells to the sieve tubes of minor veins. Because of their numerous connections with sieve tube member 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; Evert and Mierzwa,1986). The phloem in 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 of 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.

Young leaves are typically strong sinks for photosynthates whereas mature leaves are usually strong sources. However, during maturation a leaf passes through a transition period during which it may act as both sink and a source. This period of simultaneous import and export is a result of the sequence in which tissues mature within the leaf. The vasculature in the mid vein and petiole somehow accommodate these concurrent export and import functions. Vogelmann *et al.*(1982) have conducted detail studies on this aspect and reported bidirectional transport in *Populus deltoides*.

Among the parameters affecting the capacity of a leaf to collect and export assimilates are the extent of its vascular system and the amount of phloem, or more importantly the amount of functional sieve tubes in its vascular bundles. The quantitative studies on the leaf of *Populus deltoides* (Russin and Evert, 1985) have revealed that with decreasing vein size, a reduction takes place both in total number and total cross-sectional area of each cell type in all three regions of the lamina examined. Similar trends were found in the leaf of *Amaranthus retroflexus* (Fisher and Evert, 1982b).

The structural and functional evolution of leaf minor veins and their taxonomic distribution in 750 species from 140 families of flowering plants has been well documented by Gamalei (1988a,b). In another review (Gamalei, 1991) he analysed taxonomic groups in relation to plant evolutionary, ecological and growth form schemes. A reductive evolution from evergreen trees to annual herbs is accompanied by gradually increasing symplastic isolation of the mesophyll and the phloem. He suggested that the phloem loading for assimilate transport is double routed. The symplastic route is considered more ancient and economical for loading. The apoplastic pathway becomes the main or the only route under favourable conditions. Behnke and Sjolund (1990) reviewed the structure of the conducting elements of the major plant groups, from algae to the flowering plants from extant and extinct groups, revealing both common features and divergent solutions to the problems of nutrient transport.

The need for a flexible approach to categorizing tissues, cells and cell components has been repeatedly indicated by Esau (1969). Such approach is especially important in dealing with ontogenetic phenomena. A set of criteria established for classifying developmental stages of a tissue in one group of plants may not be serviceable for another group; and even in the same plant, the stages in different organs may have their peculiarities which only a broad classification can encompass.

Protophloem and protoxylem are simply the initial vascular tissues of a system whose character they define by their position (Esau, 1965b). The distinction between the earlier and later primary vascular tissues is usually not sharp. But in ontogenetic studies it is convenient to use separate terms for them, protoxylem and protophloem for earlier, metaxylem and metaphloem for the later vascular tissues. These terms are most useful if they are applied chiefly to indicate the temporal and positional characteristics of the two parts of the vascular tissues.

The delimitation between protophloem and metaphloem is sometimes rather clear, as, for example, in the aerial parts of monocotyledons having only sieve tubes in the protophloem and distinct companion cells associated with the sieve tubes in the metaphloem (Esau, 1965b). In dicotyledons the two tissues usually merge gradually, and their limitation must be based on a developmental study. In plants having secondary phloem the distinction between the tissue and the metaphloem may be quite uncertain. The delimitation of the two tissues is particularly difficult if radial seriation of cells occurs in both tissues. In general, the developmental relations between the two parts of the phloem have not been sufficiently investigated (Esau, 1969).

In *Populus deltoides* seedlings, Larson (1976) has studied the development of protoxylem and metaxylem from procambium and metacambium respectively. He observed a short functional lifetime for protoxylem because internodal stretching obliterates the first formed elements. This functional loss is subsequently compensated in part by late formed protoxylem files and in part by early metaxylem development which is so timed that it either slightly overlaps or quickly succeeds that of late protoxylem. The protoxylem to metaxylem anastomoses would provide for continuity of the xylem translocation system during the early stages of primordium development. Based on this observation Larson (1976) presumptively visualized an analogous phloem-metaphloem-protophloem spatially paralleling but temporally lagging. He, however, cautioned that the continuum can only be observed by following serial sections of an ontogenetic series of leaf traces throughout its entire length.

1.6 Outline of the investigation

Although an important area in plant anatomy, the structure of the phloem in the petiole of tropical trees is not well understood. Moreover, little is known about the development and activity of the vascular cambium in the petiole of evergreen and deciduous leaves.

The present study describes the anatomical and experimental studies on the vascular cambium and phloem in the petiole of leaves. The internode-node-petiole continuum and the petiole vasculature have been investigated with special reference to the cambium and phloem.

1.7 Terminology

The terms defined in the present anatomical study conform with definitions in general use (Esau, 1965b; Fahn, 1982).