VEIN STRUCTURE IN RELATION TO PHLOEM LOADING IN SELECTED RANUNCULACEAE, APOCYNACEAE AND ASCLEPIADACEAE OF THE EASTERN CAPE

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ABSTRACT

The relationship between leaf architecture, vein anatomy and phloem ultrastructure, and that of possible routes from mesophyll cells to phloem and potential phloem loading method was investigated using species adapted to the southern African climate. The research was based on the hypothesis of Gamalei and Van Bel, using nothern hemisphere species only (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996). The thesis commenced with a survey of leaf architecture of the Ranunculaceae, Apocynaceae and Asclepiadaceae. Anatomical and ultrastructural studies followed.

Leaf architecture was described according to Hickey (1973). Within the Ranunculaceae, leaf architecture was found to be marginally actinodromous. Venation pattern consisted of a widely spaced reticulum of delicate veins, especially in *Ranunculus*. Leaf architecture of the Apocynaceae was described as pinnate, camptodromous and brochidodromous. The Asclepiadaceae showed less uniformity in terms of leaf architecture, being pinnate and camptodromous, with mostly brochidodromous and, unexpectedly, eucamptodromous patterns of secondary venation. A predominantly common leaf architecture supported the move to amalgamate the two families. As the less advanced eucamptodromous arrangement could represent a more primitive branch of this huge family, the phylogenetic classification of the new amalgamated family is eagerly awaited for discussion. Allocation of vein order allowed comparisons between species and families to be drawn.

Reticulum density and vein order anatomy was used to indicate potential routes from mesophyll to phloem. A definite contrast was obvious between the loose arrangement of mesophyll and veins in the mesic *Ranunculus*, and the close mesophyll and dense venation of the xeric apocynate and asclepiad species, and was related to habitat.

Ultrastructural characteristics of companion cells, together with plasmodesmatal abundance, were considered especially important for the determination of minor vein configuration. Descriptions of plasmodesmatal distribution did not consider functional status. In this thesis, vein structure and ultrastructure were considered in relation to phloem loading, not as a demonstration thereof.

All three families were designated minor vein configuration type 2a. Two interesting examples that did not adhere to the familial norm, viz. few plasmodesmata and normal companion cells, occured in the Asclepiadaceae. *Secamone alpinii* had abundant aggregated plasmodesmata, forming a potential symplasmic continuum from mesophyll to companion cells. The question of plasmodesmatal functionality remained open. *Ceropegia carnosa* showed folding of the companion cell membrane, but no accompanying wall ingrowths. The folds were suggested to increase surface area for apoplasmic phloem loading in the noted absence of plasmodesmata. Loading routes and methods suggested were based on anatomical and ultrastructural evidence only.

Whilst these results were supported by published data for other species of these families, the prediction of the Gamalei and Van Bel hypothesis did not hold true. The relatively primitive Ranunculaceae were expected to have the least advanced type 1 minor vein configuration, with abundance plasmodesmata providing a symplasmic phloem loading pathway. The relatively advanced Apocynaceae and Asclepiadaceae were predicted to have the most progressive minor vein configuration, type 2b, with specialised transfer cells to maximise apoplasmic uptake. As families with type 2a minor vein configurations, the Ranunculaceae were more advanced than expected and the Apocynaceae and Asclepiadaceae less so.

DECLARATION OF ORIGINALITY

I, Alison Mary Buswell, hereby declare that this thesis represents my own work, except where the assistance or publications of others have been acknowledged.

Alison Mary Buswell
23rd day of January 2001

"All things bright and beautiful,
All creatures great and small,
All things wise and wonderful..."

The only way to eat an elephant is one bite at a time.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Rationale

Veins have three main functions in leaves (Nelson & Dengler 1997). The xylem tissue of the vein brings water into the leaf. Xylem and associated supporting tissues are responsible for support of the wide, flat lamina. Phloem tissue of veins transports dissolved assimilates produced by photosynthesis away from mature leaves. It would seem obvious that, as xylem and phloem occur together as the vascular tissue of veins, the organisation and pattern of venation should determine the efficiency with which these three functions can be carried out (Canny 1990, Roth *et al.* 1995, Nelson & Dengler 1997). It was the association between lamina vein organisation, anatomy and ultrastructure, and the efficiency of phloem loading of assimilates that was of particular interest to me.

A comprehensive theory of phloem loading, encompassing aspects such as loading classification, vein typology, ecophysiological and environmental associations, evolutionary positions of angiosperm families, and plant growth rate and form was hypothesised by Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996). This monumental, holistic treatment examined a very wide range of northern hemisphere angiosperms. The basic premise of the Van Bel and Gamalei theory was that families of primitive evolutionary position possess a less efficient system of phloem loading, with associated anatomical and ultrastructural evidence, than do families of advanced evolutionary position. Furthermore, veins may be classified into four main types, each related to a greater or lesser degree to one of the two main phloem loading routes, viz. symplasmic and apoplasmic.

As certain aspects of the Van Bel and Gamalei theory (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996) are relevant to this thesis, an introduction to their hypothesis and the concept of phloem loading is warranted.

1.2 An introduction to phloem loading

Phloem is a carbohydrate-conducting tissue found in plant vascular tissues (Metcalfe & Chalk 1950, Esau 1972, Raven *et al.* 1982, Bieleski 2000). The function of phloem is to transport the sugars produced during photosynthesis from mature leaves. Phloem loading is the process whereby substances, primarily sugars, are loaded into the sieve tubes at the source, ie: the photosynthesising leaves (Eschrich 1970, Giaquinta 1983, Russin & Evert 1984, McCauley & Evert 1988a & b, Wimmers & Turgeon 1991, Beebe & Evert 1992, Oparka & Van Bel 1992, Komor *et al.* 1996, Turgeon 1996, Van Bel 1996, Sjölund 1997, Turgeon 2000). Sugars are then transported to a sink, such as sites of growth, reproduction or storage (Turgeon 1989, Van Bel 1996).

Phloem loading method is important, affecting efficiency of loading, exudate composition, cambial activity and growth pattern (Terry & Robards 1987, Gamalei 1991, McLean *et al.* 1997, Knoblauch & Van Bel 1998, Turgeon 2000). Many authors have contributed to the finer details of the phloem loading mechanism. However, it was Van Bel and Gamalei who extrapolated the data to include such concepts as evolutionary progression of angiosperm development, selective pressures causing progression, growth form and rate, and adaptations to environment (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996).

As phloem loading occurs predominantly in minor veins of source leaves, a description of venation would be a logical place to begin (Esau 1972, Russin *et al.* 1996, Turgeon 2000). Leaf architecture is a means whereby foliar venation may be described (Hickey 1973). There are different categories of leaf architecture, each with a recognisable pattern of arrangement and complexity. The classification of veins within a leaf is called vein order, and ranges from the largest or lower order veins to the smallest or higher order veins.

Different vein orders exhibit different anatomies, according to size and function of the vein concerned. The degree and form of connectivity within the vascular tissue, and the type of surrounding tissue vary with vein order (Van Bel & Gamalei 1991, Van Bel 1992a, Russin *et al.* 1996). In general, large, lower order veins function for mass translocation of assimilate and are not primarily concerned with loading. Higher order veins, terminating in the photosynthetic

mesophyll, have an important loading function in source sites (Turgeon 1989, Russin *et al.* 1996, Turgeon 2000).

Minor veins are the smallest of veins, forming a network within the lamina mesophyll (Esau 1972, Hickey 1973). During leaf growth, minor veins first function for import of substances required by growing young leaves acting as sinks. Later, minor vein phloem loads and exports photosynthate from leaves acting as sources (Turgeon 1989, Van Bel 1996). In phloem loading, minor vein order is the most important, due to the fact that the majority of photosynthate loading occurs at this level.

In order to appreciate the manner in which the loading process works, it is necessary to examine possible routes of cytoplasmic connectivity, the anatomy of minor veins and the ultrastructure of the cells concerned (Esau 1972). In 1879, Tangl described channels connecting the cytoplasm of adjacent plant cells (Giaquinta 1983, Gunning & Overall 1983, Robards & Lucas 1990, Ding *et al.* 1992, Epel 1994). The term "plasmodesma" was proposed by Strasburger in 1901 to describe these connecting channels. By the time the electron microscope was constructed in the 1960's, botanists had a fair idea of the gross movement of substances in plants via plasmodesmata. Investigations into the actual structure of plasmodesmata continue.

Cytoplasmic connectivity leads to an intercellular continuum called the symplasm, allowing for communication and transport between cells of the mesophyll and phloem (Gunning & Overall 1983, Robards & Lucas 1990, Turgeon & Beebe 1991, Epel 1994, Gamalei 1996, Cook *et al.* 1997, McLean *et al.* 1997, Sjölund 1997, Turgeon 2000, Van Bel & Knoblauch 2000, Botha & Cross 2001). Cytoplasmic connectivity via plasmodesmata, or the lack thereof, suggests potential routes along which photosynthetic assimilates may reach the phloem, viz. phloem loading via symplast or apoplast (Fig. 1.1) (Warmbrodt & Van Der Woude 1990, Beebe & Evert 1992, Evert *et al.* 1996, Gamalei 1996, Komor *et al.* 1996, Russin *et al.* 1996, Kempers *et al.* 1998). However, the presence of plasmodesmata does not necessarily imply functionality and could result in an apoplasmic loading pathway through blockage. The symplasmic route involves passing along the cytoplasmic continuum from cell to cell through functional plasmodesmata (Fig. 1.1a) (Van Bel & Knoblauch 2000, Botha & Cross 2001). As no semi-permeable cell membranes are crossed, this route does not produce selectivity of transported assimilates. The apoplasmic route occurs

via cell walls and intercellular spaces (Fig. 1.1b). This route is more selective, as assimilates will ultimately have to pass through a semi-permeable membrane (Turgeon & Beebe 1991, Oparka & Van Bel 1992, Gamalei 1996, Van Bel 1996).

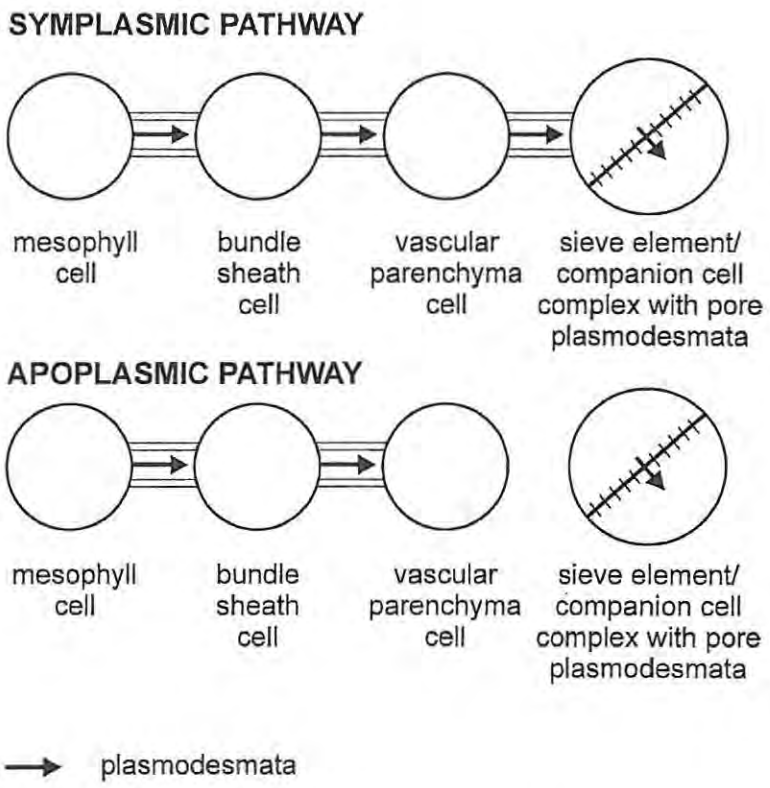

Figure 1.1 Transport from mesophyll to ST/CC complex along (a) symplasmic and (b) apoplasmic routes, redrawn from Van Bel 1992.

A lack of plasmodesmata anywhere along the way from photosynthetic mesophyll cells, to bundle sheath cells, to companion cells implies an apoplasmic pathway (Figure 1.1) (Turgeon & Beebe 1991, Komor *et al.* 1996, Russin *et al.* 1996). Therefore, the frequency and distribution of plasmodesmata along the loading route is crucial to a determination of the phloem loading method from anatomical evidence, although not conclusive (Warmbrodt & Van Der Woude 1990, Gamalei 1996, Russin *et al.* 1996).

An accepted means of illustration became necessary in order to communicate accumulating information. Gamalei (1985a, 1989) at first illustrated his findings as pictograms of the sieve tube/companion cell (ST/CC) complex of minor veins. He then progressed to plasmodesmograms which included mesophyll connections to the ST/CC complex (Van Bel & Gamalei 1991, Van

Bel 1992a). This is a full diagrammatic representation of the pathway of photosynthate from the mesophyll cells to ST/CC complex (Figure 1.2). Plasmodesmata, of great importance in these figures, are usually shown as a relative abundance in pit fields or scattered, as the case may be (Van Bel & Gamalei 1992). Plasmodesmograms make comparisons much simpler, by showing the connections between various cell types and the degree of connectivity, and aid in standardisation of descriptions (Botha & Van Bel 1992, Van Bel 1992a) (Fig. 1.2). Arguments for and against the use of plasmodesmograms continue (Robards & Lucas 1990).

Using plasmodesmograms as a means of illustration, three main types of minor veins were recognised. These were designated type 1, type 2a and 2b (Van Bel & Gamalei 1992) (Fig. 1.2a, b, and c respectively). A further group with an anatomy akin to Kranz structure was subsequently acknowledged as an extreme and called type 2c (Gamalei 1991) (Fig. 1.2d). Types are not rigidly fixed, with combinations and intermediates occurring. Documented combinations include those of type 1-2a, and type 1-2b (Gamalei 1989, Van Bel 1992a). The possibility of other combinations occurring is acknowledged.

Anatomically, a progression of two main features stands out from type 1 through to type 2b, relating to the method of assimilate loading inferred (Table 1.1). Firstly, the abundance of plasmodesmata decreases. In type 1 configurations, there are many plasmodesmata connecting the ST/CC complex with mesophyll cells, suggesting symplasmic transport. In type 2a there are fewer plasmodesmata, and in type 2b almost none, indicating a shift to apoplasmic transport. Secondly, companion cell ultrastructure changes from relatively unspecialized, to highly adapted for apoplasmic transport as intermediary or ultimately transfer cells (Fisher 1986, Schmitz *et al.* 1987, Van Bel *et al.* 1988, Weisberg *et al.* 1988, Turgeon 1989, Warmbrodt & Van Der Woude 1990, Wimmers & Turgeon 1991, Beebe & Turgeon 1992, Oparka & Van Bel 1992, Van Bel 1992a, Flora & Madore 1996, Haritatos *et al.* 1996, Komor *et al.* 1996, Van Bel 1996, Volk *et al.* 1996).

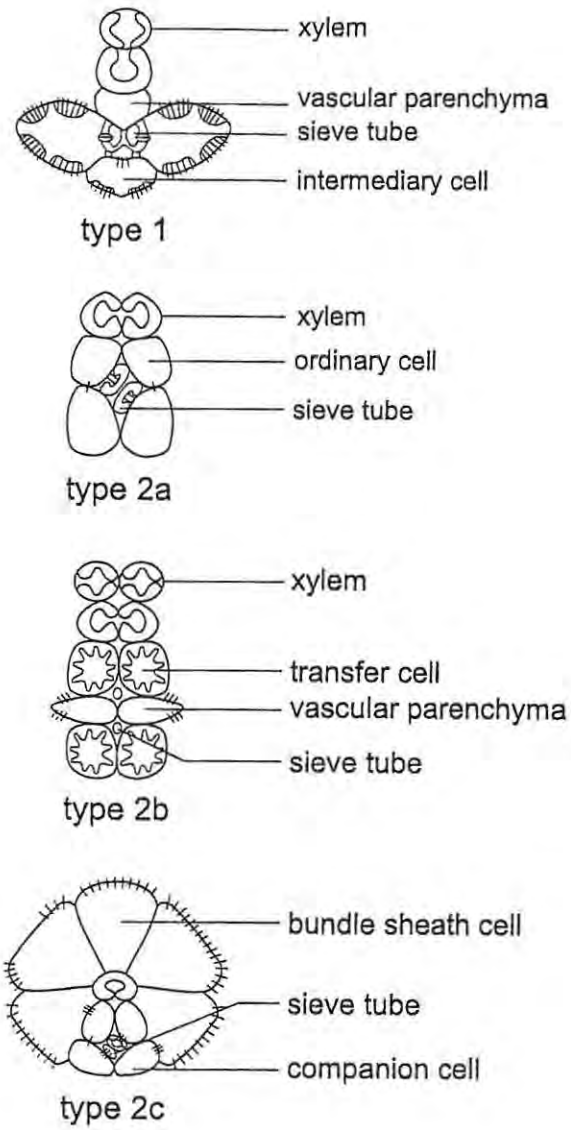

Figure 1.2 Minor vein configurations (a) type 1, (b) type 2a, (c) type 2b and (d) type 2c, redrawn from Van Bel 1992.

The increase in complexity of companion cell ultrastructure seen when comparing types 1 and 2 may be ascribed to increased specialization in terms of selectivity of assimilate transported (Turgeon 1989, Gamalei *et al.* 1992, Van Bel 1992a).

Table 1.1 Minor vein types related to plasmodesmatal abundance, companion cell ultrastructure and phloem loading method (Gamalei 1989)

TYPES	PLASMODESMATAL ABUNDANCE	COMPANION CELL ULTRASTRUCTURE	LOADING METHOD
1	Many plasmodesmata	Intermediary cells	Symplasmic
2a	Few plasmodesmata	Ordinary companion cells with vesicular labyrinths	Apoplasmic
2b	None	Transfer cells with wall ingrowths	Apoplasmic
2c	Many plasmodesmata between mesophyll and bundle sheath cells only	Companion cells	Apoplasmic

Type 2c is represented by Kranz anatomy, and typically occurs in C_4 plants. Kranz anatomy refers to the wreath-like arrangement of the mesophyll and large bundle sheath cells around the vascular tissue of the leaves (Raven, Evert & Curtis 1982). Many plasmodesmata connect the mesophyll and bundle sheath cells (Valle *et al.* 1989).

Therefore, types differ in the organisation of tissues, the cytoplasmic connectivity thereof, and the distances over which solute transport must occur (Gamalei 1991). This in turn affects the efficiency and selectivity of loading (Van Bel 1996).

Vein anatomy, plasmodesmatal frequency, and companion cell ultrastructure have collectively been termed minor vein configuration. Van Bel and Gamalei examined the foliar minor vein structure of a number of angiosperm families, and suggested that plasmodesmatal connectivity, and therefore minor vein configuration, be used to infer phloem loading pathway (Gamalei *et al.* 1992, Van Bel & Gamalei 1992, Flora & Madore 1996).

1.3 Symplasmic versus apoplasmic phloem loading

Two phloem loading pathways are recognised, viz. apoplasmic and symplasmic (Oparka & Van Bel 1992, Van Bel 1996, Turgeon 2000, Botha & Cross 2001). The apoplasmic phloem loading model is an accepted one, but that of symplasmic phloem loading apparently received less support (Giaquinta 1983, Turgeon & Beebe 1991), until evidence from dye-coupling experiments was presented (Van Bel & Gamalei 1992). This involved watching the movement of fluorescent dyes injected intracellularly, demonstrating a potential route of connectivity between mesophyll and ST/CC complex (Madore *et al.* 1986, Meiners *et al.* 1988, Robards & Lucas 1990, Botha 1992).

Proof that the apoplasmic pathway is involved in sugar loading comes from studies using chloromercuribenzenesulfonic acid (PCMBS) which blocks membrane transport (Giaquinta 1979, 1983, M'Batchi & Delrot 1984, M'Batchi *et al.* 1986, Madore & Lucas 1987, Weisberg *et al.* 1988, Bourquin *et al.* 1990, Turgeon & Gowan 1990, Haritatos & Turgeon 1995, Flora & Madore 1996, McLean *et al.* 1997, Turgeon 2000). Sugar transport in supposed symplasmic loaders was unaffected by PCMBS, indicating a lack of apoplasmic transport and the existence of symplasmic transport along a route demonstrated by dye-coupling experiments (Madore *et al.* 1986, Meiners *et al.* 1988, Robards & Lucas 1990, Van Bel & Gamalei 1992, Flora & Madore 1993, 1996).

Symplasmic transfer rate depends on plasmodesmatal abundance (Van Bel *et al.* 1994, Van Bel & Knoblauch 2000). If few plasmodesmata are present, a symplasmic constriction occurs in the pathway and transport becomes apoplasmic (Table 1.2). When apoplasmic transport occurs at any stage along the route from mesophyll to sieve element, transport as a whole is said to be apoplasmic (Oparka 1990, Van Bel 1992a).

Table 1.2 Apoplasmic versus symplasmic transport

SYMPLASMIC/APOPLASMIC LOADING	SYMPLASMIC CONTINUITY	TYPE
Symplasmic	M→M→M→BS→CC→ST	Type 1
Apoplasmic	M→M→M→BS ‖ CC→ST	Types 2a, 2b, 2c

(Key: BS = bundle sheath cells, CC = companion cells, M = mesophyll cells, ST = sieve tubes, → = plasmodesmata, ‖ = no plasmodesmata)

Van Bel (1992a), supported by Gamalei *et al.* (1992, 1994), suggested that the predominant phloem loading process may change within an individual plant as required. Limitations, such as environmental conditions, anatomy and ultrastructure of the minor veins concerned, impose constraints. However, the apparent flexibility of phloem loading route prevents categoric statements that a particular species loads via a symplasmic or apoplasmic pathway only. Therefore, discussion of the predominance of one phloem loading method over another is the accepted terminology used.

Gamalei (1991) suggested ecological and endogenous factors as influencing the symplast/apoplast loading ratio, and postulated possible combinations of these pathways in taxa with symplasmic connections between mesophyll and phloem. Different order veins could load via different routes. At the phloem tissue level, intermediary cells and normal companion cells could load differently within the same vein. Within a single ST/CC complex itself, apoplasmic and/or symplasmic loading could occur at the same time or at different times in response to environmental conditions. Minor vein configuration itself may be facultative and flexible in response to the environment, stage of leaf development and connectivity via plasmodesmata (Gamalei *et al.* 1992, Van Bel 1992a). This flexibility is described as multiprogrammed phloem loading (Turgeon *et al.* 1993, Van Bel *et al.* 1994, Flora & Madore 1996).

Minor veins are not the only sites of photosynthate loading as lower order veins may, to a lesser degree, also be active. The latter are bigger, and more complicated anatomically, than minor veins. Therefore, the method of loading may be different to that of minor veins (Van Bel & Gamalei 1991, Van Bel 1992a). A possible explanation is a combination type 1-2, with the extreme of each route active in different vein orders, further complicating the overall picture (Van Bel *et al.* 1988, 1994, Van Bel 1992a).

1.4 The evolution of vein types and implications thereof

Evolution of the vascular system in early angiosperms as a whole should be considered, as commented on by Gamalei (1989), Bieleski (2000) and Van Bel and Knoblauch (2000). The large size of tropical trees necessitated long-distance transport via the vascular system, in a relatively uniform direction. Such long-distance transport, with associated problems of translocation over long vertical distances, may have imposed constraints on the evolution of phloem and the loading

pathway. The progressive limit under such conditions appears to be epitomised in the development of plasmodesmatal connections (Gamalei 1989, Turgeon 2000).

In contrast, temperate herbaceous plants translocate over shorter distances, but with constantly changing internal source-sink patterns due to seasonality and a deciduous nature. Apoplasmic transport imparts greater selectivity and efficiency due to the types of sugar transported and the resultant osmotic effects thereof. Therefore, the regulation and control of transport was of the utmost importance, rather than initiation and continuity as in trees (Gamalei 1989).

The evolution of veins, at the ultrastructural level, produced two categories of phloem, viz. open and closed (Bourquin *et al.* 1990, Gamalei 1991). The open type (type 1), with a cytoplasmic continuum between cells, is ancestral, occurring in brown algae, gymnosperms, primitive woody angiosperms and more advanced angiosperms of tropical origin (Van Bel & Gamalei 1992, Flora & Madore 1996). The closed condition (type 2) exhibits little to no cytoplasmic continuity. It is derived from the open form by the loss of plasmodesmata between bundle sheath cells and phloem (Gamalei *et al.* 1992), and occurs in advanced angiosperms.

Therefore, with many plasmodesmata forming a symplasmic continuum, type 1 is considered to be the primitive, ancestral minor vein configuration (Fig. 1.3). From this open phloem type, types 2a, 2b and 2c of the closed category arose (Fig. 1.3).

Type 2a retains modified, smooth-walled companion cells, called intermediary cells, but has fewer connecting plasmodesmata (Fig. 1.3). In the more advanced closed type 2b, specialized companion cells with wall ingrowths, called transfer cells occur (Fig. 1.3). These transfer cells are highly specific to the function of apoplasmic loading (Gamalei 1989, Turgeon & Beebe 1991).

A derivative of type 2a is the Kranz-like type 2c (Fig. 1.3). Variations include the presence of many plasmodesmata and transfer cells, while the norm is for few plasmodesmata and smooth walled companion cells. The possibility exists that species with type 2c minor vein configuration form a polyphyletic group, due to the wide variety of exceptions to the basic Kranz type anatomy (Gamalei 1991).

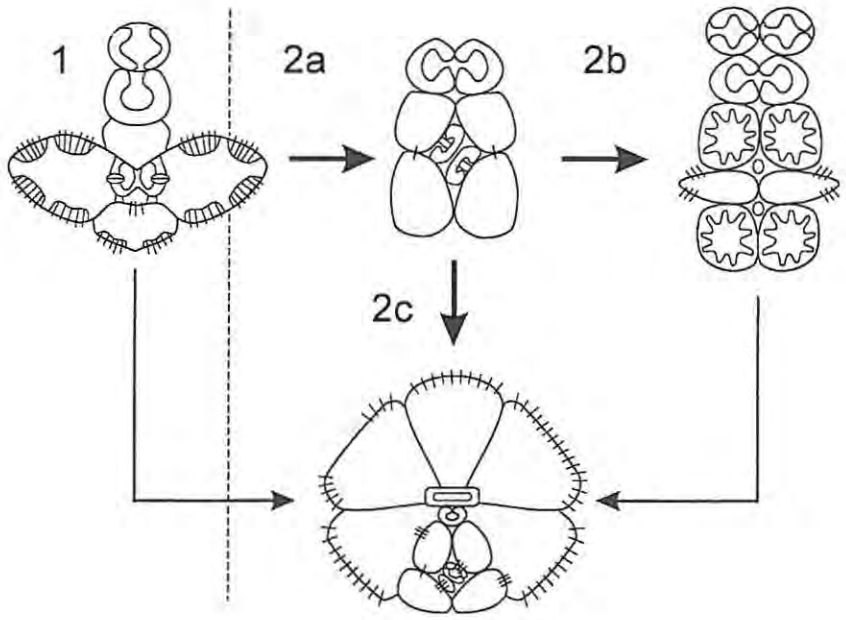

Figure 1.3 Evolutionary relationships between minor vein types, redrawn from Gamalei 1991

Generally, anatomical characteristics tend to be more conservative than plastic, morphological ones (Gamalei 1989). Open minor vein configurations are found among species of tropical origin, which suggests that foliar minor vein types are of a conservative nature. Such correlations could be used in the determination of sites of species origin (Gamalei 1991).

The evolutionary shift from symplasmic to apoplasmic loading was first promoted and later necessitated by various selective pressures. In lush ancestral tropical forests, light would have been a limiting factor for smaller understorey taxa, as it still is today. A selective advantage may have been found in having a more efficient phloem loading system, to be able to compete successfully with those able to reach the canopy. It has been suggested that apoplasmic loading would be an advantage to herbaceous taxa of the forest understorey (Van Bel & Gamalei 1992).

The global climate began to change, causing forests to decrease. This opened up new environments that were unstable and less protected. The suggested selective pressures, faced by taxa evolving in more extreme environments, were low temperatures and water stress (Gamalei *et al.* 1992, 1994, Van Bel 1992a, Van Bel & Gamalei 1992). At temperatures below 10°C plasmodesmata become dysfunctional, impeding symplasmic transport (Minchin *et al.* 1983, Gamalei *et al.* 1992, Van Bel 1992a, Van Bel & Gamalei 1992, Van Dongen & Van Bel 1996,

McLean *et al.* 1997). Symplasmic loaders are therefore highly sensitive to low temperatures, not being able to translocate assimilates under such conditions. Water stress leads to a decrease in leaf cell turgor. Mesophyll cells, the sites of photosynthesis, need a high osmotic potential to maintain water content when leaf water content drops (Van Bel & Gamalei 1992). The result is a strong retention of photosynthate, requiring a highly efficient, strongly competitive phloem loading system for translocation under conditions of water stress. Furthermore, plasmodesmata may close when a difference in turgor pressure is registered across adjacent cells (Gunning & Overall 1983, Côté *et al.* 1987, Epel 1994, McLean *et al.* 1997). The connectivity decreases due to dysfunctional plasmodesmata and the symplasmic pathway is thus blocked (Van Bel & Gamalei 1992). Symplasmic loaders would therefore be drought sensitive and would be disadvantaged in conditions of water stress.

Symplasmic loaders became restricted to the tropics as tall, woody trees as the climate changed (Gamalei 1985b). The smaller, more efficient apoplasmic loaders would have been less inhibited by changing conditions, and were able to colonise new areas of environmental extremes and flourish in the harsher climates (Gamalei *et al.* 1992). The suggestion that low temperatures and seasonality may have driven the evolution of apoplasmic phloem loading is strengthened by the evidence that type 1 and 1-2a symplasmic loaders occur in tropical areas, and that type 2 apoplasmic loaders occur in more temperate and boreal regions (Gamalei *et al.* 1994).

Temperate zones have a shorter growing season than tropical areas, due to seasonality of the climate (Gamalei *et al.* 1992, Van Bel & Gamalei 1992). A plant that could efficiently produce and translocate photosynthate could grow faster in the limited time available. Evidence for this theory of apoplasmic predominance comes from the fact that a number of temperate zone families still have representatives growing in the montane regions of the tropics (Heywood 1978, Thorne 1992, Van Bel & Gamalei 1992). In other words, these montane taxa represent the ancient extremes of a tropical ancestor in which the apoplasmic pathway had grown in importance, making these taxa ideally suited to montane conditions.

Apoplasmic loading is noted as predominating in families adapted to environmental extremes, the so-called cold-resistant taxa (Gamalei *et al.* 1992 & 1994). While predominantly symplasmic loaders may fall back on the apoplasmic pathway in conditions of extreme cold, the pathway is

not sufficiently established to be able to transport the accumulating photosynthate, ultimately inhibiting photosynthesis. Therefore, in the most extreme regions, a predominantly symplasmic pathway would not cope, even with the aid of combined loading (Gamalei *et al.* 1992, Van Dongen & Van Bel 1996). Taxa with true multiprogrammed loading appear to be ideally suited to such extreme conditions, often occurring as endemics of arctic, montane and arid floras (Gamalei *et al.* 1992).

In stable, tropical environments, primitive families of large evergreen trees with slow, steady growth rates and unlimited apical growth were possible (Gamalei 1991, Gamalei *et al.* 1994, Van Bel 1996). Slow growth may be due to the less efficient symplasmic phloem loading method, with open type 1 configurations, predominating. Growth in these groups is of a more cumulative nature (Van Bel 1996).

Advanced, herbaceous forms tend to have higher, but erratic, growth rates due to the more efficient apoplasmic pathway, with closed type 2 configurations, predominating. Taxa tend to be seasonal, with a short life span and high turnover rates of organs (Heywood 1978, Gamalei *et al.* 1992, Thorne 1992, Van Bel & Gamalei 1992). Frequent, successive generations of organs and/or generations are common (Gamalei 1991). Growth form is usually that of a herb with limited apical growth (Gamalei 1991).

The closed type 2a occurs in temperate annuals and perennials, whereas type 2b is found in herbs with short vegetation periods, showing accelerated growth spurts, and growing in arcto-alpine, dry steppe and desert conditions (Table 1.3) (Gamalei 1991, Gamalei *et al.* 1992). Type 2c taxa grow in hot deserts and tend to be restricted to seasonless savannah and desert herbs. Hot deserts epitomise conditions of temperature and water stress, with huge temperature fluctuations, sporadic, low rainfall and the added aggravation of salt stress (Gamalei 1991). Leaves especially experience great fluctuations in temperature due to poor thermoisolation and high transpiration, both of which result in heat and water loss, necessitating a highly adapted, efficient phloem loading system (Van Bel 1996).

Type combinations of semi-open phloem, that utilise both apoplasmic and symplasmic phloem loading, tend to be prevalent in deciduous trees, shrubs and low pillow growths forms. Taxa that

utilise this combined method are able to colonise very large ranges (Gamalei *et al.* 1992).

In a study correlating minor vein configuration with growth form, results indicated exceptions to be approximately 20% (Gamalei 1989). However, all deviations encompassed groups of species, possibly reflecting the evolutionary direction taken by the group as a whole away from the parent stock.

The suggestion has been made that growth form is directly related to minor vein configuration. There is, however, strong evidence to show that growth form is more a result of a combination of factors such as the type of sugar transported, the method and speed of transport, the evolutionary position of the family and the climate in which it grows, than a direct correlation with minor vein configuration (Beslow & Rier 1969, Giaquinta 1983, Gamalei 1989, Gamalei 1991, Komor *et al.* 1996, Van Bel 1996, Kempers *et al.* 1998).

Van Bel (1996) speculated on the links between phloem transport, carbohydrate management and relative growth rate. Symplasmic loaders would require energy to combine sucrose and galactinol units, according to the polymer trap model, in the phloem. Such large sugars possess many carbon atoms per molecule, but a low level of osmotic activity, high viscosity and therefore a slow, low driving force.

Apoplasmic loaders require large amounts of energy to load sucrose against a concentration gradient. Sucrose is a small molecule with fewer carbon atoms per molecule, yet greater osmotic activity resulting in a higher driving force and therefore faster translocation. It is also less viscous and so easier to move. In the long run, this sugar is a more efficient translocate as more carbon atoms are moved per unit time (Gamalei 1991, Van Bel 1996).

Loading method determines phloem sugar composition, as the sugars found in the exudate reflect the selectivity of the loading process (Zimmerman and Ziegler 1975, Giaquinta 1983, Gamalei 1989, Van Bel 1993, Gamalei 1996, Komor 2000). Studies by Gamalei and Van Bel showed that type 1 taxa transport oligosaccharides of the raffinose group via plasmodesmata. Type 2 taxa were found to transport mostly sucrose apoplasmically via the plasmamembrane.

Sugar type has a marked influence on plant growth and development. Sucrose has been found to have a profound effect on cellular activity, especially in terms of cell division in the cambial zone. (Beslow & Rier 1969, Gamalei 1991). Low concentrations of sucrose promote cell division and differentiation, while high concentrations inhibit these growth processes. Auxin has a similar effect on growth. Therefore, obtaining an optimal balance between sucrose and auxin is important to cambial activity, which is subject to regulation in this way.

The selectivity of the apoplasmic pathway produces high concentrations of sucrose in phloem exudate. An extrapolation of this concept is the expected inhibition of cambial activity and the limiting of secondary growth in taxa using apoplasmic routes (Gamalei 1991). If sucrose concentration remained high due to the strong predominance of the apoplasmic pathway under adverse conditions, all cambial and secondary growth would be prevented. As a result, seasonal replacement of aerial plant organs or entire generations would occur. Annual and ephemeral habits are promoted in this manner, and can be regarded as an extreme product of the apoplasmic pathway (Table 1.3) (Gamalei 1991).

Seasonal fluctuations of temperate regions would have favoured a semi-open, multiprogrammed phloem loading organisation, enabling the switch between symplasmic and apoplasmic pathways as required (Gamalei *et al.* 1994). A further consequence would be cyclic fluctuations of high and low sucrose concentrations, resulting in the seasonal bursts of cambial and secondary growth characteristic of temperate regions (Table 1.3).

Table 1.3 A summary of minor vein type associated with growth form and environment

TYPE	GROWTH FORMS	ENVIRONMENT
Type 2a (closed)	Temperate annuals and perennials	Temperate regions
Type 2b (closed)	Herbs with short growing periods	Arcto-alpine, dry steppe and desert regions
Type 2c (closed)	Seasonless herbs	Savannah and hot desert regions

A further consideration is that loading via the apoplast or symplast may affect the rate of mass flow and the Carbon distribution through the plant (Van Bel 1993b). Apoplasmic loading may lead to a higher osmotic gradient in sieve tubes than that for symplasmic loading, because

oligosaccharide solutions become saturated and too viscous before mass flow is maximised. Therefore, apoplasmic loaders are suggested to have higher mass flow rates and pressure gradients (Van Bel 1993b, Gamalei 1996, Turgeon 2000).

However, a low pressure gradient could also result from a high rate of release along the sieve tubes of the stem. This would mean greater investment of daily fixed carbon in stem tissues, characteristic of slow growing symplasmic loaders. At the other extreme, apoplasmic loaders with higher pressure gradients and mass flow rates would loose less assimilate along the stem and show greater investment in terminal sinks (Turgeon 2000). This would result in more rapid growth rates (Van Bel 1993b, Komor *et al.* 1996, Kempers *et al.* 1998).

1.5 Taxonomic stability and occurrence of configurations in current taxa

Having established that there are four main minor vein configurations, and that these configurations relate either to apoplasmic or symplasmic phloem loading, Van Bel and Gamalei set out to determine the stability of this concept within dicotyledonous taxa. It was found to be consistent at the familial and subclass level, for the most part (Gamalei 1985a, 1985b, 1989, Van Bel 1992a, Gamalei *et al.* 1994) (Table 1.4).

Table 1.4 Dicotyledon subclasses related to phloem loading route and type (Radford 1974, Gamalei 1989)

SUBCLASS	PHLOEM LOADING ROUTE	TYPE
Hamamelididae, Lamiidae, most families of Dilleniidae and Rosidae	Symplasmic	Type 1
Ranunculidae (including Ranunculaceae), Caryophyllidae, Asteridae (including Apocynaceae and Asclepiadaceae), and some families of Rosidae and Dilleniidae	Apoplasmic	Types 2a, 2b and 2c

Twelve families did not show consistent configurations, as types ranging from 1 to 2b were observed within each family. The term polytypical was applied to families with multiple

configurations. This includes some 2 332 genera, 51 835 species (Van Bel & Gamalei 1991). Polytypical families encompass more species than any other type and appear to indicate an adaptative advantage in flexibility of phloem loading pathway (Gamalei 1989, Van Bel & Gamalei 1992). Such polytypical families could be exceptions or may indicate that the families concerned are not natural.

Van Bel and Gamalei superimposed the configuration data for families studied on the Takhtajan tree for the evolutionary progression and status of flowering plants (Takhtajan 1969, Radford *et al.* 1974, Gamalei 1991, Van Bel 1992a). The result was a definite progression from the evolutionary more primitive families with type 1 configurations to advanced families with type 2 configurations (Fig. 1.4).

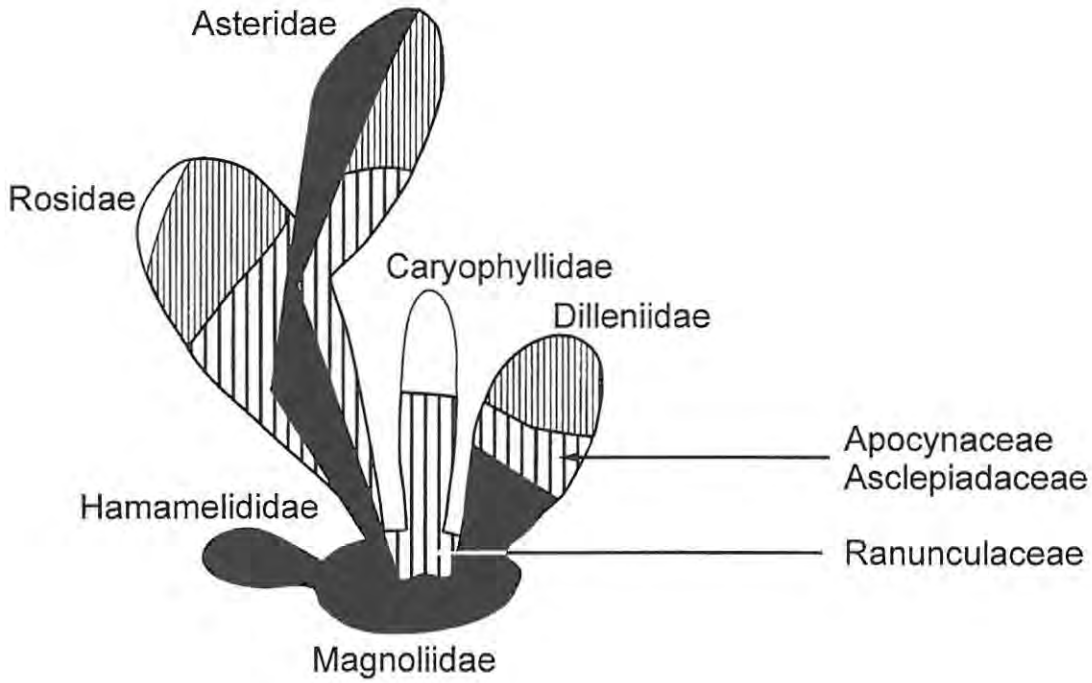

Figure 1.4 Evolutionary relationships amongst dicotyledon families (Takhtajan 1969, Radford 1974) correlated with the concept of minor vein configuration types, redrawn from Gamalei 1989

Primitive families, such as those of the Hamamelididae and Magnoliidae, showed type 1 configurations, and the most advanced type 2c (Fig. 1.4). Type 1, with associated symplasmic phloem loading, is the more ancient ancestral form (Gamalei 1985b, Van Bel 1992a). Type 2, loading apoplasmically, is a more advanced configuration. The specialised type 2c is a

modification of type 2a, occurring in many C_4 and CAM plants, and is considered to be the most advanced (Gamalei 1989, Gamalei *et al.* 1992, Van Bel 1992a) (Fig. 1.4).

1.6 The ecophysiological concept of phloem loading in relation to the current study

The ecophysiological concept of phloem loading therefore encompasses many disciplines, including plant anatomy and ultrastructure, phytogeography and ecology, physiology, and evolutionary biology (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996). The concept is summarised in Table 1.5.

Table 1.5 A summary of the correlations involved in the ecophysiological concept of phloem loading (adapted from Gamalei 1991)

FEATURE	ENVIRONMENTAL CONDITIONS		
	OPTIMAL	AVERAGE	EXTREME
Life form	evergreen	seasonal	ephemeral
Phloem minor veins	open	semi-open	closed
Phloem loading	symplasmic, type 1	mixed, type 1-2a	apoplasmic, type 2b,c
Phloem sap contents	sugar-amino acid complex	alternate	sucrose only
Cambial activity	noninhibited	seasonally inhibited	inhibited constantly

All literature published to date details data accumulated from the study of northern hemisphere taxa. This thesis will be the first attempt to examine southern hemisphere taxa with this concept in mind.

The scope of the Van Bel and Gamalei theory (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996) extends far beyond the realms of this thesis. It was necessary to focus on a particular area, and yet to attempt the incorporation of as many aspects for consideration as possible. The choosing of taxa for study was therefore of the utmost importance.

1.7 Families chosen for the current study

The ecophysiological concept of phloem loading was developed using only northern hemisphere taxa (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b). Whilst it was not expected that taxa from the southern hemisphere would significantly alter the theory proposed, it was decided to extend the study to the southern hemisphere to test it under the rigours of the southern African climate in the hopes of providing a more encompassing picture. Initially the possibility of using families indigenous to southern Africa was suggested. Although this would have produced new and interesting data, due to problems with comparison and validation of results it would not have answered my original question, viz. 'Does the ecophysiological theory of phloem loading hold true in the southern hemisphere?' I therefore decided to use cosmopolitan families with taxa from both hemispheres, with published minor vein configurations for representative taxa. This would provide a framework for comparison within a particular family, and would possibly give a broader understanding of the range within the family. Such data could only expand and consolidate (or refute and undermine!) the theory. At a later stage, small localised indigenous families from either hemisphere could be examined against a more solid, global concept.

Three large families of cosmopolitan distribution were therefore chosen for study. Within each family, one genus (albeit a different species) occurs in both hemispheres and has been described in this context. This has allowed for comparison of results within the families and said taxon globally, and for subsequent comparison with other genera and species studied for the first time from the southern hemisphere.

In the interests of an instudy comparison, an outgroup family of primitive status was chosen as a contrast for two advanced families. It must be remembered that a "primitive" family is a collection of both primitive and advanced features. The designation of primitive status simply reflects the ratio of these features. Therefore, it is reasonable to use a family as a primitive representative for comparison in this study, bearing in mind the possibility that the feature in question may turn out to be more developed and advanced than initially expected.

Cosmopolitan distribution and extreme evolutionary position were considered the most important criteria for selection of families for study in this thesis. It was decided that the Ranunculaceae would be the primitive representatives, and the Apocynaceae and Asclepiadaceae the advanced. From these three families, individual species were chosen to represent taxa with different growth forms and from different habitats.

The order Ranunculales, including the family Ranunculaceae, is closely related to the primitive orders Nymphaeales, Magnoliales and Illiciales (Dahlgren 1975, Heywood 1978, Thorne 1992). These families are considered to have evolved little from a Magnoliales-like ancestor, and as such can be considered to represent early angiosperms (Fig. 1.4, Table 1.4). The Ranunculales are mostly herbaceous, have sealed carpels, tricolporate pollen, many petaloid stamens, and lack ether oil cells which sets the order apart from the Magnoliales (Dahlgren 1975, Heywood 1978, Thorne 1992).

The Apocynaceae and Asclepiadaceae are very closely related, belonging to the order Gentianales (Dahlgren 1975, Chase *et al.* 1993, Sennblad & Bremer 1996). When the research for this thesis began, serious consideration was given to a possible amalgamation and restructuring of the Apocynaceae and Asclepiadaceae. Convincing data has since been published supporting the incorporation of the Asclepiadaceae as a subfamily of the Apocynaceae (Chase 1993, Sennblad & Bremer 1996, Endress & Bruyns 2000). Also included as a subfamily of the Apocynaceae is the closely related family Periplocaceae (Endress & Bruyns 2000). Had the most recent classification been taken into account halfway through the thesis, the new subfamilial groupings would have presented a problem in terms of the genera covered. The treatment would then not have represented the holistic survey of the Apocynaceae and Asclepiadaceae of the eastern Cape, as was originally intended. While the new classification is acknowledged and commented on at every opportunity to offer supporting evidence, a taxonomic review was not the aim of this thesis. For the purposes of this thesis, it was decided to acknowledge the close affiliation, but to leave the two groups as separate entities for discussion. They differ only in certain floral characteristics. The Asclepiadaceae exhibit a highly specialized androecium, with associated pollen transfer method using pollinia, and a gynostegium (Good 1952, Heywood 1978, Sennblad & Bremer 1996, Swarupanandan *et al.* 1996). The sap of both families contains a milky latex, which acts as a deterrent to herbivores (Endress & Bruyns 2000). Both families are regarded as highly advanced within the angiosperm family tree (Dahlgren 1975) (Fig. 1.4, Table 1.4).

As secondary aspects in the selection of families for study, general habitat preference and growth form were considered. A wide range was sought, again for the purposes of comparison. The Ranunculaceae of southern Africa grow mostly in warm, moist temperate areas, and are exclusively herbaceous in southern Africa (Exell & Milne-Redhead 1960, Heywood 1978, Thorne 1992). The Apocynaceae and Asclepiadaceae occur in hot, dry, exposed areas (Good 1952, Codd 1963, Heywood 1978, Thorne 1992, Swarupanandan *et al.* 1996). The Apocynaceae are mostly small trees and shrubs (Codd 1963, Heywood 1978, Kupicha 1982, Leeuwenberg & Kupicha 1985), while the Asclepiadaceae include shrubs, creepers, succulents and herbs (Good 1952, Heywood 1978, Swarupanandan *et al.* 1996).

1.8 The concept behind the hypothesis

The ecophysiological concept of phloem loading has not, to date, been applied to southern hemisphere taxa. This thesis represents the first attempt to test an aspect of the concept using species adapted to the rigours of the southern African climate.

The hypothesis on which the research of this thesis is based is that:

1. The relatively primitive Ranunculaceae show poorly differentiated venation patterns, with inefficient lamina coverage. Concomitant with this, anatomical and ultrastructural evidence should support a more primitive, less selective, symplasmic phloem loading method.

2. The relatively advanced Apocynaceae and Asclepiadaceae show highly organised venation patterns with efficient lamina coverage, where anatomical and ultrastructural evidence should suggest a more advanced, selective, apoplasmic phloem loading method.

1.9 Aims

The specific aims of this thesis are listed as follows. For representative species of the families Ranunculaceae, Apocynaceae and Asclepiadaceae occurring in the eastern Cape:

1. To illustrate leaf venation and describe leaf architecture.
2. To describe leaf and vein anatomy.
3. To examine the phloem ultrastructure, especially that of companion cells.
4. To examine minor vein configuration, based on anatomical and ultrastructural evidence, for certain species within each family.

5. To infer symplasmic or apoplasmic phloem loading for species examined based on anatomical and ultrastructural evidence.
6. To indicate apparent loading efficiency based on aims 4 and 5.

1.10 Outline of thesis

The chapters that follow deal with each of the aims outlined above. Chapter Three is concerned with leaf architecture of the Ranunculaceae, Apocynaceae and Asclepiadaceae. At the light microscopy level, results are presented and discussed. In Chapter Four, I describe the results of foliar anatomical studies, concentrating on vein anatomy for representative species. Chapter Five concentrates on species presented in Chapter Four, dealing with the ultrastructure of phloem in foliar veins, and proposes minor vein configurations for taxa studied.

The distinction is made between quantitative and qualitative data by Lersten (1990), in a literature survey on vein endings. Quantitative data requires many sets of serial sections with many replicas for each taxon studied. This requires a lot of time and repetitive effort to produce a statistically valid scenario as close to the truth as is humanly possible. An approach of this type is well suited to an indepth examination of a restricted taxon. Qualitative data can be gained from a survey of many taxa (Carlquist 1991, Hickey & Taylor 1991, Todzia & Keating 1991). Such a study would be descriptive in nature and would detail features seen in a few replicas. The number of replicas would not necessarily be statistically valid.

Due to the number of species studied and the time constraints of a thesis, the data presented here is qualitative rather than quantitative. A descriptive overview is produced, beginning with leaf architecture, proceeding to leaf and vein anatomy, and culminating with ultrastructural studies of cells along the phloem loading pathway.

In Chapter Six, I have synthesized the information presented in Chapters Three to Five, in an attempt to provide an overall picture in keeping with concepts incorporated in the Van Bel and Gamalei hypothesis (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996). A brief critique of their hypothesis is included, as are prospects for future research based on the findings of the current study.

It is hoped that this step by step description, at each level of leaf structure, will result in a greater understanding of the morphology, anatomy and ultrastructure of the phloem loading route.

CHAPTER TWO: MATERIALS AND METHODS

2.1 Sampling of materials

The material utilized in this study was obtained from the Selmar Schonland Herbarium at the Albany Museum (GRA), which houses a very comprehensive, well-curated collection of Ranunculaceae, Apocynaceae and Asclepiadaceae. The 18 Ranunculaceae, 11 Apocynaceae, and 101 species of Asclepiadaceae (Appendix I), that occur in the Eastern Cape, were sampled for leaf clearing. Mature leaves were chosen from herbarium sheets on the basis of preservation. See Appendix I for full taxonomic names and Appendix II for herbarium sheet data.

For light and transmission electron microscopy, healthy fresh material was required. Representatives from each family were sampled in their natural habitats in the veld.

2.2 Leaf clearing

The clearing technique described by Shobe and Lersten (1967) was followed throughout. Depending on size and shape, excised leaves were placed in vials or jars. Vials were labelled and filled with 10% aqueous Contrad 70 (Merck), and placed on a hot plate at approximately 60 °C for 24 h (Schmid & Turner 1977). Thicker leaves required a change of solution and an additional 24 h on the hot plate. Softened leaves were rinsed in water for 15 min, then gently heated to 60 °C in a 10% aqueous sodium hydroxide (NaOH) (Merck) solution for 24 h. The NaOH solution was changed repeatedly until leaves cleared. The cleared leaves were rinsed in water for 15 min. Persistent dark spots were treated with full strength bleach for 30 min at room temperature, followed by 15 min in distilled water. Finally, leaves were soaked in saturated aqueous chloral hydrate (Unilab product by Saarchem) at room temperature for 2-3 days. The lamina became opaque, whilst veins remained white.

2.3 Staining and mounting of cleared leaves

After clearing, leaves were gently rinsed twice in water, then dehydrated via a graded ethanol series to 100% ethanol at room temperature. The cleared leaves were placed in Safranin O (Merck) in 100% ethanol and left to stain for 24 h. Destaining was done as required in 100% ethanol, using 50:50 100% ethanol and xylene to halt the destaining process. The desired result

was that of an opaque lamina with contrasting dark pink veins. Leaves were soaked in xylene for 3 days to ensure no traces of ethanol or water remained, after which the xylene was drained off. Leaves were mounted between 4mm thick glass plates with DPX mountant (Gainland Chemical Co. UK), a resinous permanent mounting medium. Plates were left flat for 2-3 weeks to allow the DPX to harden.

2.4 Cleared leaf plate photography and analysis

Cleared leaf plates were examined and described according to Hickey's (1973) classification system, using a Wild M400 dissecting microscope with a transmitted light source from beneath the specimen. Photographs were taken with the camera coupled to the dissecting microscope. Mostly, 100 ASA black and white film was used to show veins clearly with good contrast. Illford photographic paper was used. Full leaf photographs of leaves considered typical of each family were taken with 100 ASA Fujicolour film.

Foliar venation characteristics were determined according to Hickey's (1973) terminology and classification system for dicotyledonous leaves. Vein order, areole pattern, shape and size were described. The whole leaf was photographed wherever possible, but some leaves were damaged during clearing. Photographs of the lamina were taken to show areolar pattern and minor vein occurrence, to augment the written description.

2.5 Resin embedding schedule for fresh material

Fresh leaves were cut into small pieces (approx 2mm x 2 mm) in a petri dish containing 6% glutaraldehyde in 0.1M Na-cacodylate buffer (pH 7). The pieces were transferred to vials and fixed in 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer (pH 7) overnight in a refrigerator. Specimens were rinsed twice in cold Na-cacodylate for 15 min and postfixed in 2% osmium tetroxide in 0.05M Na-cacodylate for 90 min. Post-fixed specimens were rinsed in Na-cacodylate for 10 min, then twice in distilled water (10 min x 2). Specimens were stained in 2% aqueous uranyl acetate for 40 min, followed by dehydration in a cold graded ethanol series to 100% ethanol. Specimens were infiltrated with two changes of 100% propylene oxide of 10 min each, then by a 50:50 mixture of propylene oxide and Spurr's (1969) hard resin for 7 h, and finally 100% Spurr's resin overnight. Specimens were arranged in patty pans with fresh 100% Spurr's resin and placed in an oven at 60°C until catalyzed. Embedded specimens were mounted on

blocks. For TEM, blocks were sectioned with a LKB 8800 Ultratome V (Bromma, Sweden) using a diamond knife, whilst for LM an RMC MT-7 Ultratome was used with glass knives. Sections were cut in 3 planes, viz. longitudinal and transverse to the midrib, and paradermally.

2.6 Light microscopy

Monitor sections from blocks embedded for TEM were collected, placed on glass slides and stained with Toluidine blue (Merck). DPX was used to mount slides with coverslips, then left to harden. Slides were examined on an Olympus BX50 light microscope, using bright field optics. Certain sections of leaf and vein anatomy were photographed using black and white 100 ASA film, with the attached Olympus camera and Olympus PM-30 exposure control unit.

2.7 Transmission electron microscopy

Gold-silver sections were cut, floated onto water, and then lifted onto 200-mesh copper grids. Lead citrate was used to post-stain grids for 10 min before viewing on a JEM 1210 Jeol transmission electron microscope (Tokyo, Japan) at 80 and 100kV. EM micrographs were taken to show detail of the vasculature, especially of the relationship between sieve tubes and related vascular parenchyma including companion cells.

CHAPTER THREE: LEAF VENATION

3.1 Introduction

Dicotyledon venation patterns show extreme diversity, yet the functional significance of the diversity remains largely obscure (Nelson & Dengler 1997). Foliar venation patterns can, however, provide useful insight into a number of botanical fields, including palaeobotany and systematics (Takhtajan 1969, Hickey 1973, Carr *et al.* 1986, Spicer 1986, Carlquist 1991), ontogeny (Carr *et al.* 1986), phylogeny (Takhtajan 1969, Doyle & Hickey 1976, Merrill 1978, Franck 1979, Carr et al. 1986, Carlquist 1991, Dickison & Weitzman 1996) and efficiency of phloem loading (McCauley & Evert 1988 a & b, Dannenhoffer *et al.* 1990, Nelson & Dengler 1997).

It is important to begin with descriptions and visual outlines of foliar venation of certain Ranunculaceae, Apocynaceae and Asclepiadaceae taxa. Aspects such as position, size and vein order (ie: leaf architecture) have to be considered. This is necessary before reliable comparisons between taxa could be made and the results discussed.

3.1.1 Leaf architecture

The word "architecture" is defined in Webster's New International Dictionary as "formation or construction, whether as result of conscious act or growth or of a random disposition of parts". Architecture in botanical terminology would therefore be a description of the leaf, in terms of the distribution and pattern of structural components and their relationships to each other, disregarding histology, ontogeny and/or function (Hickey 1973). Leaf architecture is defined precisely by Hickey (1973) as "the placement and form of those elements constituting the outward expression of leaf structure, including venation pattern, marginal configuration, leaf shape, and gland position".

The first comprehensive attempt to describe leaf architecture, in a systematic context, was produced by Constantin von Ettingshausen in 1861. His classification of venation patterns followed a logical progression beginning with the configuration of primary veins and leading to that of areolation. Areolation is the subdivision of the photosynthetic parenchyma into pockets called areoles, bounded by lamina veins. Using von Ettingshausen's classification as a basis,

Hickey broadened and updated the system in 1973. A further classification with a different slant was produced by Melville in 1976, in which ontogeny was considered. Spicer (1986) devised a means of classifying veins without reference to vein thickness, in order to overcome a perceived difficulty in describing fossil leaves where such features become obscured. He based his classification on Hickey's (1973) classification system.

Hickey's interest was to determine phylogenetic trends within dicotyledonous groups using foliar venation patterns. He studied fossil flora from the Early Tertiary through to the present day, producing a comprehensive classification of foliar architecture for dicotyledons. This assisted both botanists and palaeobotanists in precise, efficient cataloguing and description of taxonomically useful features associated with the leaf. Hickey based his classification on the fact that recognisable patterns of architectural organisation tend to be consistent within groups of taxa, especially at the familial level.

3.1.2 Hickey's classification system

Using Hickey's system (1973), venation can be differentiated into a number of size classes, courses and patterns of distribution, and marginal features (Fig. 3.1). A further consideration is the pattern of veins, and their relationship to each other. Descriptions of vein order begin at primary level, viz. the midvein, and progress to secondary veins, tertiary veins, etc (Fig. 3.1). The midvein is taken as a continuation of the vascular bundle from the petiole. Secondary veins are smaller, branching off the midvein. Smaller still, tertiary veins are described as branching off secondaries, and so on.

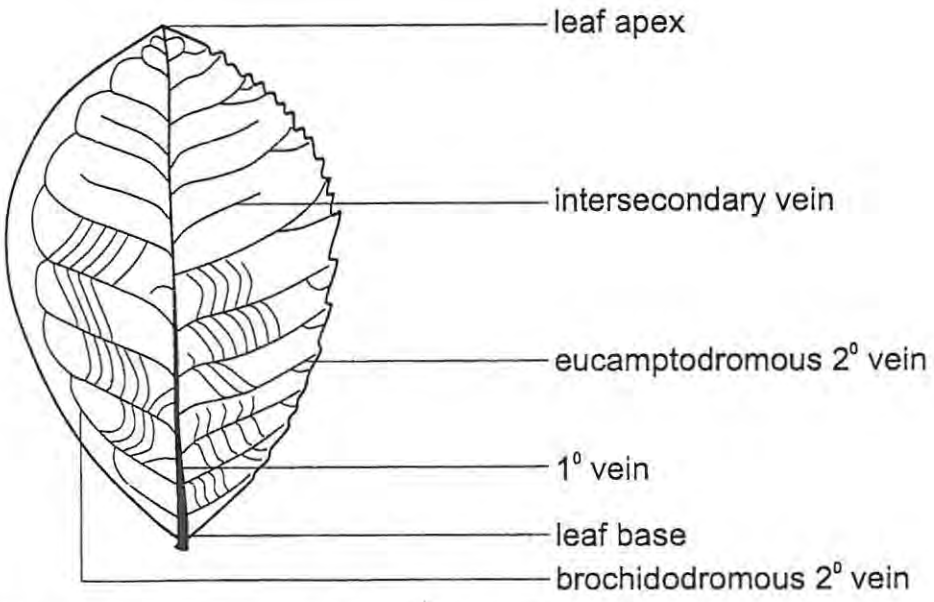

Figure 3.1 Foliar vein order, redrawn from Hickey 1973.

Hickey recognised six basic patterns of organisation in foliar venation, based on the number and pattern of primary and secondary veins (Hickey 1971a & b, 1973). These patterns are pinnate, parallelodromous, campylodromous, acrodromous, actinodromous and palinactinodromous (Fig. 3.2).

Pinnate refers to the presence of a single primary vein, from which all other veins arise (Fig. 3.2a). Parallelodromous describes the situation involving two or more primary veins, diverging at the leaf base, crossing the lamina in parallel and converging at the apex (Fig. 3.2b). Several primary veins arising together from a point, crossing the lamina in strongly recurved arches to converge at the apex form a campylodromous arrangement (Fig. 3.2c). Acrodromous refers to the convergent gentle arches of two or more primaries or secondaries (Fig. 3.2d). Three or more primary veins radiating from a common point is called actinodromous (Fig. 3.2e). Finally, the palinactinodromous pattern exhibits many primary veins originating from one or more subsidiary points above the lowest point of radiation (Fig. 3.2f).

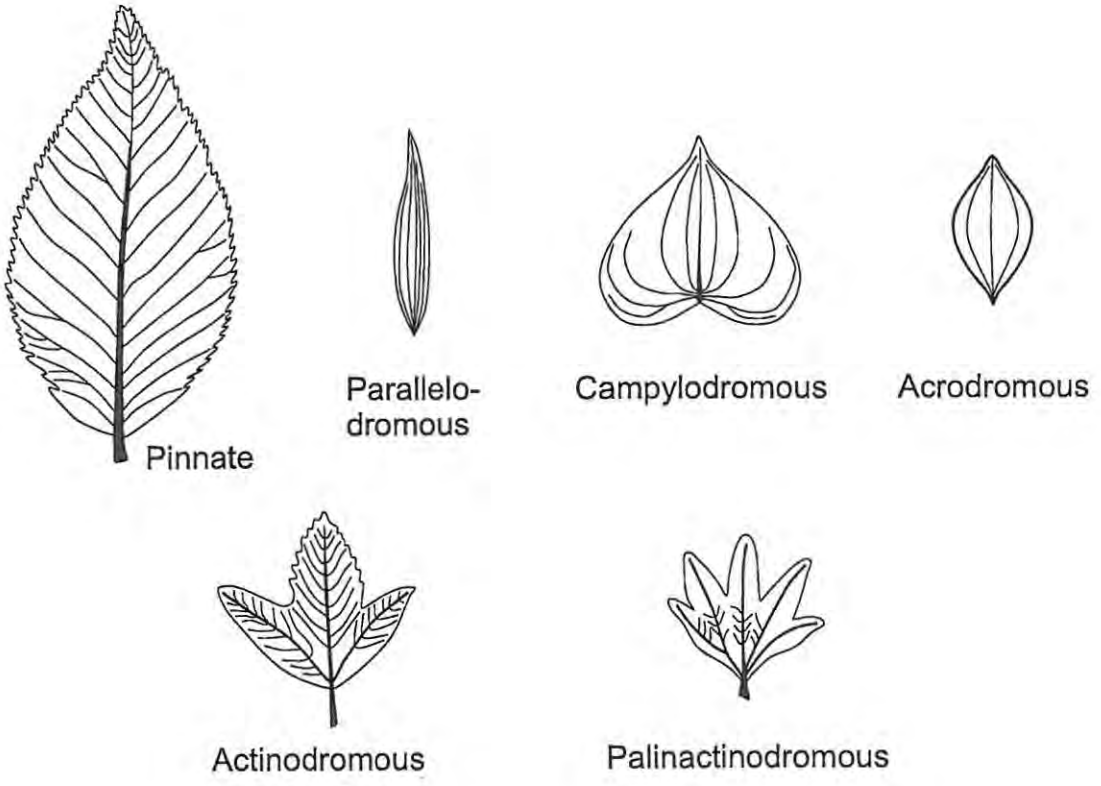

Figure 3.2 Six patterns of foliar venation, redrawn from Hickey 1973.

Hickey proceeds through a step by step description of the primary, secondary, and tertiary veins, the higher order venation of the reticulum and ultimate veinlets, and finally that of the areoles (1973). His descriptions are summarised in Table 3.1.

Table 3.1 Descriptive categories for vein orders according to Hickey (1973)

VEIN ORDER	DESCRIPTIVE CATEGORIES
Primary vein[1]	Dimension of primary vein[1] relative to other veins Course of primary vein from base to apex[2]
Secondary vein[3]	Angle of divergence[4] from primary vein[1] Variation in angle of divergence acropetaly[5] Relative thickness of secondary vein[3] Course[6] of secondary vein from base to apex Behaviour of loop-forming[7] secondary veins[3] Presence and type of intersecondary veins[8]
Tertiary vein[9]	Angle of origin on secondary vein[3] pattern across lamina between adjacent secondaries[3]
Higher order venation[10]	Resolution and determination[11] of higher order venation[10] Quaternary vein[12] characteristics Quinternary vein[13] characteristics Highest vein order[10] present in leaf Highest vein order[10] showing excurrent branching[14] Marginal ultimate venation[15] characteristics
Ultimate veinlets[16]	Presence of ultimate veinlets[16] in areoles[17] Simple or branched ultimate veinlets[18]
Areoles[17]	Development and delimitation[19] of areoles[17] Spatial arrangement and orientation in the lamina Shape of areole[17] Size of areole[17] in millimetres

1

Even though venation characters may overlap for different families or genera, it is the combination of the character set that is important to the description of a particular taxon (Hickey 1973). Certain characters may be important in the data set for one taxon and completely disregarded in another, depending on the integrity of the character and context used. In general,

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1 - the primary vein is the midvein or central vein, 2 - of the leaf, 3 - branches off the primary vein, 4 - the angle between the midvein and the origin of the secondary vein, 5 - towards the leaf apex, 6 - pattern formed, 7 - secondary veins that rejoin adjacent secondary veins to form loops, 8 - veins smaller than secondary veins that branch off the midvein between consecutive secondary veins, 9 - branches off secondary veins, 10 - all vein orders above tertiary level, generally small veins, 11 - whether vein order is obvious or not above tertiary level, 12 - fourth order veins, 13 - fifth order veins, 14 - highest vein order with ultimate veinlets branching off into areoles, 15 - behaviour of ultimate veinlets along margin of leaf, 16 - very small, blind ending veins in areoles, 17 - areas of lamina defined by higher order veins, 18 - whether ultimate veinlets branch in areoles or not, 19 - whether and to what extent areoles are surrounded by veins

areolation, marginal features and marginal tooth types are the most reliable (Hickey 1973, Merrill 1978, Hickey & Taylor 1991).

Subsequent authors publishing in the fields of leaf architecture, anatomy and ontogeny have consistently used Hickey's classification (1973) (Lersten & Carvey 1974, Dengler & MacKay 1975, Fisher & Evert 1982, Mohan & Inamdar 1982, Ramakrishna & Govindappa 1983, Russin & Evert 1984, Fisher 1985, Spicer 1986.Fisher 1990, Lersten 1990). Melville's (1976) system is seldom used, due mostly to the cumbersome terminology and insufficient illustration and explanation. Hickey's (1973) classification and terminology is used throughout this thesis, unless otherwise stated.

3.1.3 Evolutionary and phylogenetic considerations of leaf architecture

Primitive families usually show a lower level of foliar vein organisation and regularity, while advanced families tend to be highly organised and show regular patterning (Hickey 1971a & b, Doyle & Hickey 1976, Merrill 1978, Franck 1979). During a study of the fossil record of angiosperm leaves, Doyle and Hickey (1976) noted an increase leaf architecture regularity as taxa evolved. A regular architecture implies greater order and organisation (i.e. less random), straighter secondary veins and intercostal areas with percurrent tertiary veins (Merrill 1978). Generally, one predominant level of organisation is recognised throughout a family (Hickey 1971a & b).

Some families have been found to exhibit several patterns of leaf architecture (Hickey 1973). Initial explanations for this were that the families were perhaps artificial or paraphyletic, requiring further taxonomic study. It was also noted that variation from the basic familial pattern tended to be restricted to those genera or species adapted to environmental extremes, bordering on the edge of the range to which the parent stock had evolved (Hickey 1973). Some variation could therefore be accounted for by geographic habitat. Leaves of taxa adapted to arctic, alpine and arid environments show a regression in architectural regularity below the level characteristic of the family, tending towards disorganisation (Hickey 1971a & b, 1973, Merrill 1978). Hybrids have also been noted to show lower order vein regularity regression, similar to that seen in xeric leaves (Merrill 1978).

Furthermore, studies comparing juvenile and adult plants indicate different venation patterns at different stages of growth (Carr *et al.* 1986). Retention of the juvenile venation pattern in adult plant leaves could result in two extreme venation patterns, with various intermediates, within a genus or family. This was found to be the case in *Aulomyrcia* (Myrtaceae). The two extreme venation patterns noted in adult leaves were brochidodromous with a paramarginal vein, and acrodromous with intramarginal veins. Intermediates occurred with pseudobrochidodromous venation patterns with secondarily arched intramarginal veins. When the ontogeny of leaves and juvenile leaf venation were examined, it was noted that brochidodromy was the juvenile state. No possible reason for the retention of the juvenile state was discussed in the paper, but the adage `ontogeny recapitulates phylogeny' was implied. Acrodromy would therefore be the more advanced state within the genus (Carr *et al.* 1986). Dickison and Weitzman (1996) noted that the majority of species in the Bonnetiaceae exhibited a combination of pattern, sometimes within a single leaf. Leaves basically showed a pinnate brochidodromous pattern, but some basal secondary veins followed an eucamptodromous pattern with subsequent secondaries reverting to brochidodromy. The functional significance of such a shift is apparently unknown (Dickison & Weitzman 1996), but the epitome noted in the family is almost monocotyledons in appearance with the angle of divergence of secondary veins parallel.

The presence of several patterns of leaf architecture within a genus or family is therefore not necessarily indicative of taxonomic irregularity, although the possibility must not be ruled out initially. Few published studies incorporating the fossil record of families are available. Wilkinson (1989) examined the leaf architecture of the Menispermaceae tribe Tiliacoreae. This family has a long fossil history, dating back 65 million years, and is pantropical occurring in the Americas, Africa, Asia, the Pacific islands and Australia. Leaf architecture was found to show variation around an ancestral pattern. In families that are very big, or very old, or that show wide diversity in habitat and geographic distribution, it is almost to be expected that variations along a progression of patterns will be seen (Wilkinson 1989, Todzia & Keating 1991).

Hickey and Taylor (1991) comment that leaf architecture, a feature that has hitherto been largely ignored in phylogenetic studies, may provide valuable information on phylogenetic relationships.

A number of published reports are available on this theme. Levin (1986a, b & c) compared phylogenies generated using traditional characters and leaf architecture. That produced from leaf architecture was considered superior in certain groups. Todzia and Keating (1991) used foliar features to separate clades in the Chloranthaceae. This technique was most successful where reproductive features had produced ambiguous results. Hershkovitz (1988) considered leaf architecture in the systematics of the Centrospermae. Miller and Nowicke (1990) used leaf architectural characters, in conjunction with a suite of other characters, to show the relationship between two genera of the Boraginaceae.

3.1.4 Aims

The aims of this chapter are as follows:

1. To describe and illustrate the leaf architecture of the Ranunculaceae, Apocynaceae and Asclepiadaceae of the eastern Cape.
2. To compare leaf architecture within and between families.

3.2 Results

3.2.1 Ranunculaceae

Description of venation pattern

In general terms, the description of venation pattern for the Ranunculaceae is marginally actinodromous with three or more primary veins arising from a single point, diverging radially, and ending at the margin (Fig. 3.3 A, B & E). As the primaries spread over the lamina evenly, venation is said to be perfect (Fig. 3.3 A, B & E). The point of radiation of the primaries varies from basal to suprabasal above the leaf base. A confusing aspect is the subdivision of leaves in certain taxa, resulting in leaflets having a pinnate appearance (Fig. 3.3 F & G) or in a much divided lamina as seen in the aquatic *Ranunculus trichophyllus* subsp. *trichophyllus* (Fig. 3.3 C). However, venation is uniform in the family, with the presence of hydathodes in *Ranunculus* the only variation seen (Fig. 3.3 A, B, D & E).

Description of vein order

The course of the midvein(s) is straight and unbranched in all taxa, as seen here in *Clematis brachiata* (Fig. 3.4 G), *C. triloba* (Fig. 3.5 B), *Ranunculus meyeri* (Fig. 3.5 F), *Thalictrum minus* (Fig. 3.6 A & C), *T. rhynchocarpum* (Fig. 3.6 D & E), *Knowltonia filia* subsp. *filia* (Fig. 3.7 A & B), *K. bracteata* (Fig. 3.7 E & F), *K. cordata* (Fig. 3.8 B), *K. vesicatoria* subsp. *grossa* (Fig. 3.8 H), *K. transvaalensis* subsp. *transvaalensis* (Fig. 3.9 B) and *K. brevistylis* (Fig. 3.9 F).

Secondary vein divergence from the primaries (Table 3.1) is narrow (less than 45°) as in *Knowltonia transvaalensis* var. *transvaalensis* (Table 3.1), to moderate (45° to 65°) as in *Ranunculus meyeri* (Fig. 3.5 F), *R. multifidus* (Table 3.3), *Knowltonia cordata* (Fig. 3.8 B), *K. filia* subsp. *scaposa (Table 3.3)*, *K. vesicatoria* subsp. *grossa* (Fig. 3.8 H) and *K. brevistylis* (Fig. 3.9 F), to widely acute (65° to 80°) as in *Thalictrum rhynchocarpum* (Fig. 3.6 D), *Knowltonia capensis* (Table 3.1), *K. filia* subsp. *filia* (Fig. 3.7 A & B) and *K. vesicatoria* subsp. *humilis* (Table 3.1). Present at the tips of primary and secondary veins are hydathodes in *Ranunculus baurii*, *R. multifidus* (Fig. 3.4 B & C), *R. muricatus* (Fig. 3.4 E) and *R. meyeri* (Fig. 3.5 F).

Intersecondary veins, where present, were simple. Examples include *Thalictrum rhynchocarpum* (Fig. 3.6 E), *Knowltonia capensis*, *K. cordata*, *K. filia* subsp. *filia*, *K. vesicatoria* subsp. *grossa* (Fig. 3.8 H) and *Ranunculus meyeri*.

Tertiary veins were uniformly transversely ramified (Table 3.1), as in *Ranunculus multifidus* (Fig. 3.4 A), *R. muricatus* (Fig. 3.4 D), *Clematis triloba* (Fig. 3.5 A), *Thalictrum minus* (Fig. 3.6 A & B), *T. rhynchocarpum* (Fig. 3.6 F), *Knowltonia filia* subsp. *scaposa* (Fig. 3.7 D), *K. vesicatoria* subsp. *humilis* (Fig. 3.8 E & F).

Higher order venation is distinct in resolution and random in orientation. Highest vein order (Table 3.2) ranges from fifth, as in *Thalictrum minus* (Fig. 3.6 C), *T. rhynchocarpum* (Fig. 3.6 F) and *Knowltonia filia* subsp. *filia* (Fig. 3.7 B), to seventh as in *K. capensis* (Fig. 3.8 D), *K. vesicatoria* subsp. *grossa* (Fig. 3.8 G) and *K. brevistylis* (Fig. 3.9 E).

Marginal ultimate venation is uniformly incomplete, as seen in *Clematis brachiata* (Fig. 3.4 F), *C. triloba* (Fig. 3.5 B, C & E), *Thalictrum rhynchocarpum* (Fig. 3.6 E & F), *Knowltonia filia* subsp. *filia* (Fig. 3.7 B), *K. filia* subsp. *scaposa* (Fig. 3.7 C), *K. bracteata* (Fig. 3.7 F), *K. cordata* (Fig. 3.8 A), *K. capensis* (Fig. 3.8 C), *K. vesicatoria* subsp. *grossa* (Fig. 3.8 G), *K. transvaalensis* subsp. *transvaalensis* (Fig. 3.9 C) and *K. brevistylis* (Fig. 3.9 D).

Ultimate veinlets (Table 3.2) range from simple to once-branched as in *Ranunculus baurii* (Table 3.2), *R. meyeri* (Table 3.2), *Clematis triloba* (Fig. 3.5 A to D), *Thalictrum rhynchocarpum* (Fig. 3.6 E & F), *K. filia* subsp. *filia* (Fig. 3.7 A & B), and *Knowltonia capensis* (Fig. 3.8 C & D), to simple to twice-branched as in *Knowltonia bracteata* (Table 3.2), *K. transvaalensis* subsp. *transvaalensis* (Fig. 3.9 A, B & C) and *Knowltonia brevistylis* (Fig. 3.9 E), to once to thrice-branched as in *K. filia* subsp. *scaposa* (Fig. 3.7 D), *K. vesicatoria* subsp. *humilis* (Fig. 3.8 E & F) and *K. vesicatoria* subsp. *grossa* (Fig. 3.8 G).

Areoles are imperfect, with only *Knowltonia filia* subsp. *filia* (Fig. 3.7 A & B) forming incompletely closed meshes. Areoles are random in arrangement and irregular in size, as seen in *Ranunculus muricatus* (Fig. 3.4 E), *Clematis brachiata* (Fig. 3.4 G), *C. triloba* (Fig. 3.5 A, B, C & D), *Thalictrum rhynchocarpum* (Fig. 3.6 F), *Knowltonia filia* subsp. *filia* (Fig. 3.7 B), *K. filia* subsp. *scaposa* (Fig. 3.7 D), *K. cordata* (Fig. 3.8 A & B), *K. capensis* (Fig. 3.8 D), *K. vesicatoria* subsp. *humilis* (Fig. 3.2.8 E & F), *K. vesicatoria* subsp. *grossa* (Fig. 3.8 G), *K. transvaalensis* subsp. *transvaalensis* (Fig. 3.9 A & B) and *K. brevistylis* (Fig. 3.9 E).

Figure 3.3 Cleared leaf examples of the Ranunculaceae showing lower vein order, reticulum organisation and pattern within the family.

A *Ranunculus muricatus* is an annual found in sheltered areas with damp soil. Note the soft, fragile nature of the leaf and the sparse, delicate venation pattern. B *R. multifidus* is a small herbaceous annual found in damp marshy soils. Leaves are delicate and pubescent, with a sparse delicate reticulum. C *R. trichophyllus* subsp. *trichophyllus* is a submerged perennial found in pools. Note the extensive subdivision of the leaves. Venation is poorly developed and fragile. D *R. baurii* occurs in sheltered environments. Leaves are thin and delicate, with a fragile sparse reticulum. E *R. meyeri* is a small perennial, occurring in swamps and shallow pools. Leaves are thin and very fragile, with a widely spaced, delicate reticulum. F *Knowltonia capensis* is a small herb found under dune bush in coastal areas. Leaves are tougher than those of *Ranunculus* species, and the reticulum is more organised and robust. G *Clematis triloba* occurs in grasslands. The leaves show an organised reticulum with good lamina coverage.

(H = hydathode; I = midvein; II = secondary vein; LA = leaf apex)

Bar represents 10mm

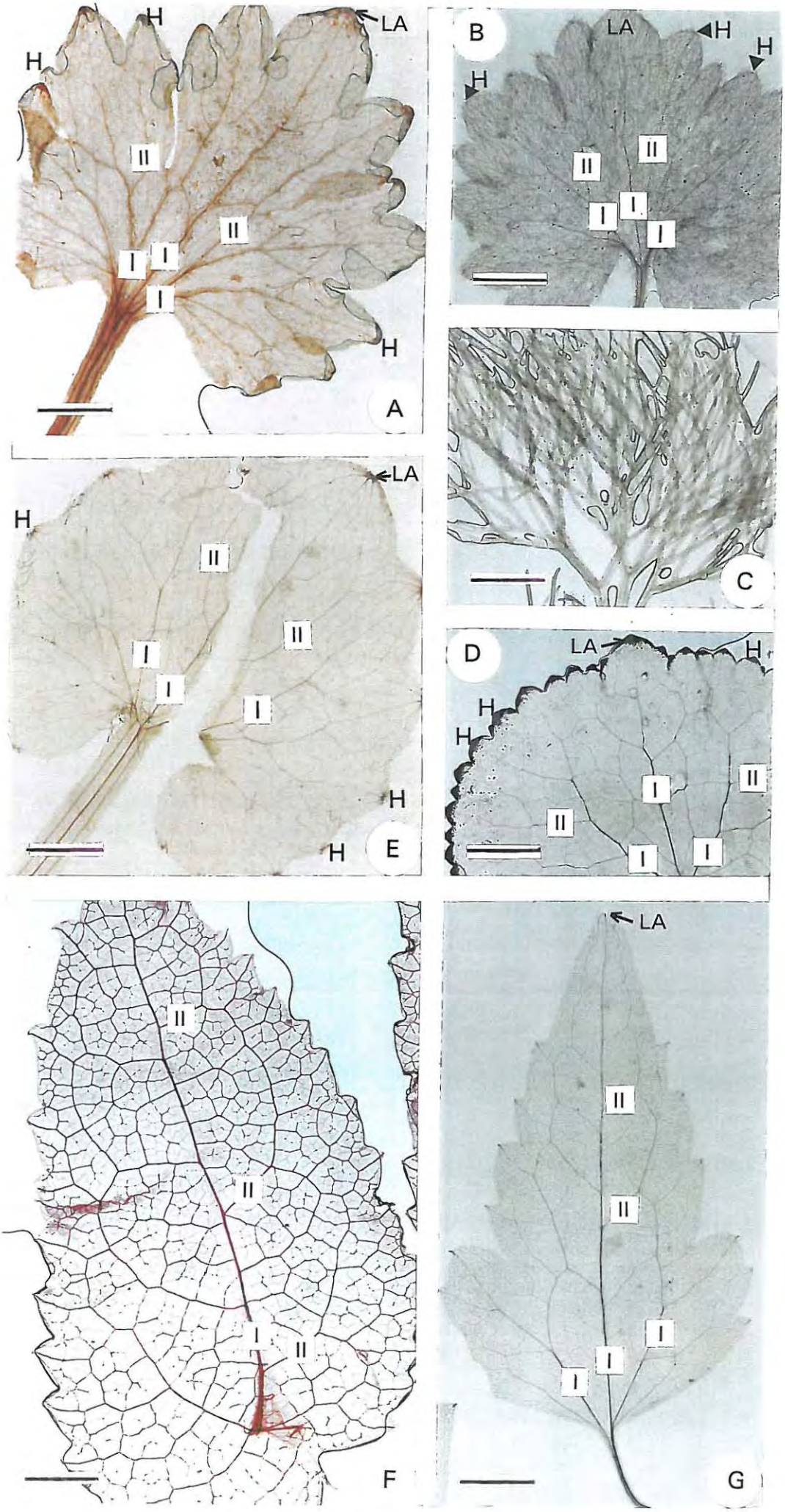
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Figure 3.4 Ranunculaceae: Aspects of cleared leaves of *Ranunculus* and *Clematis*

A *Ranunculus multifidus* showing secondary and tertiary veins with adjacent imperfect areoles in pubescent lamina, B *R. multifidus* with hydathodes at marginal serration apices and incomplete marginal venation in pubescent lamina, C *R. multifidus* showing imperfect areoles, once to twice-branched ultimate veinlets in pubescent lamina and hydathode at leaf apex, D *R. muricatus* showing two primary veins with excurrent secondary and tertiary veins and imperfect areoles, E *R. muricatus* marginal serrations with hydathodes at apices and incomplete marginal venation, F *Clematis brachiata* marginal serration with hydathodes, incomplete marginal venation and imperfect areole pattern, G *C. brachiata* lamina with primary and excurrent secondary vein, imperfect areoles and once to twice-branched ultimate veinlets.

(A = areole; H = hydathode; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm

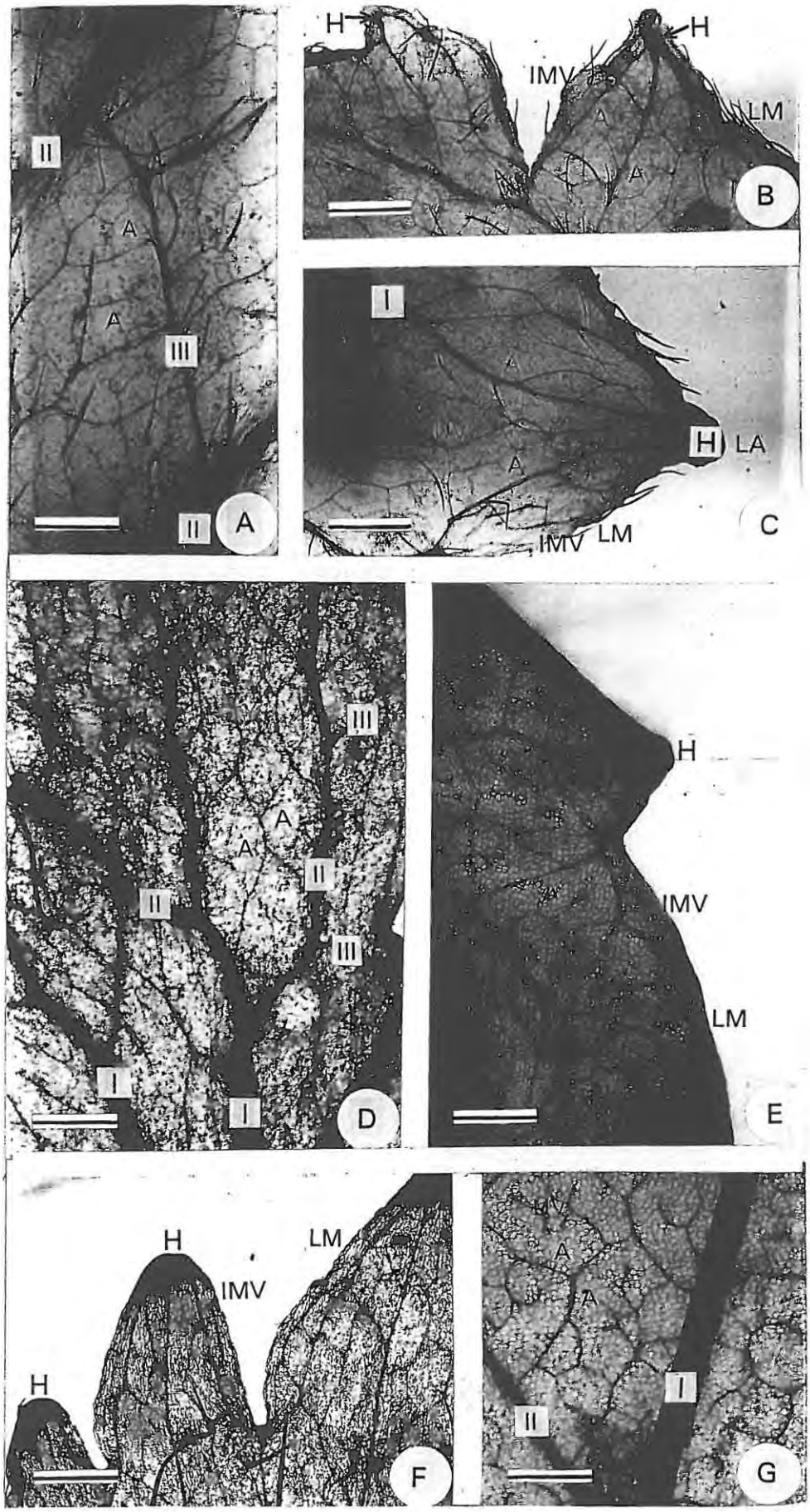
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Figure 3.5 Ranunculaceae: Aspects of cleared leaves of *Clematis* and *Ranunculus*

A *Clematis triloba* lamina with deep marginal serration base, secondary and excurrent tertiary veins, imperfect areoles and simple to once-branched ultimate veinlets, B *C. triloba* leaf apex with midvein and excurrent secondary and tertiary veins, imperfect areoles and incomplete marginal venation, C *C. triloba* marginal serration with blind ending vein at lamina edge, imperfect areoles, simple to once-branched ultimate veinlets and incomplete marginal venation, D *C. triloba* imperfect areole with simple and once-branched ultimate veinlets, E *C. triloba* showing incomplete marginal venation of once and twice-branched ultimate veinlets, F *Ranunculus meyeri* leaf apex with hydathode preceded by anastomosing primary and secondary veins at base

(A = areole; H = hydathode; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A, B, C and F, and 0,05mm for D and E

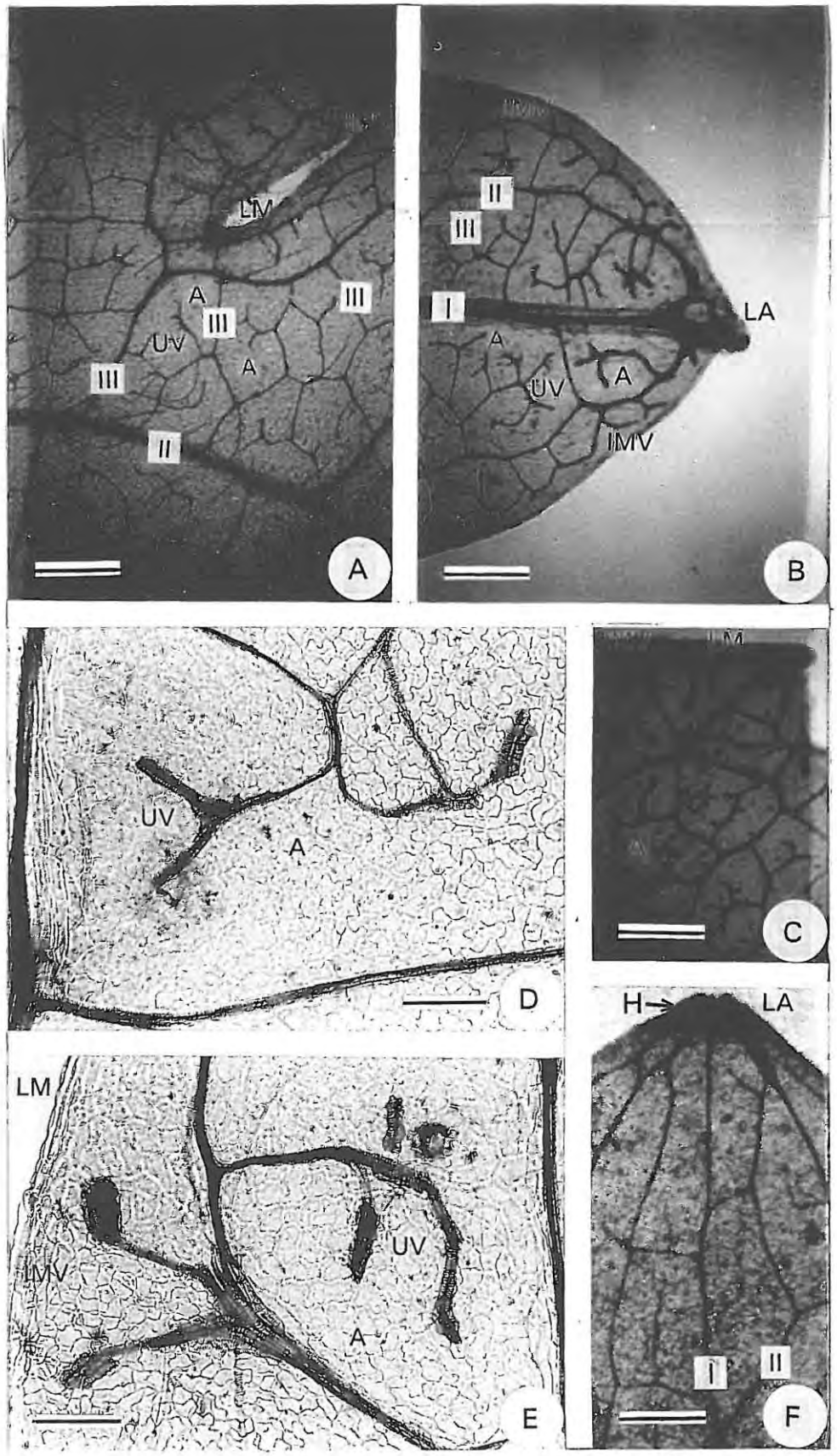
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Figure 3.6 Ranunculaceae: Aspects of cleared leaves of *Thalictrum*

A *Thalictrum minus* showing leaf base with midvein and excurrent secondary, tertiary and quaternary veins, B *T. minus* lamina with midvein and excurrent secondary, tertiary and quaternary veins, C *T. minus* leaf apex with blind ending primary and excurrent secondary and tertiary veins, D *T. rhynchocarpum* midvein with excurrent secondary veins, E *T. rhynchocarpum* leaf apex showing midvein and excurrent secondary and tertiary veins with adjacent imperfect areoles and incomplete marginal venation at lamina edge, F *T. rhynchocarpum* lamina with central midvein, secondary vein with excurrent tertiary vein, imperfect areoles and incomplete marginal venation at lamina edge

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin)

Bar represents 0,25mm

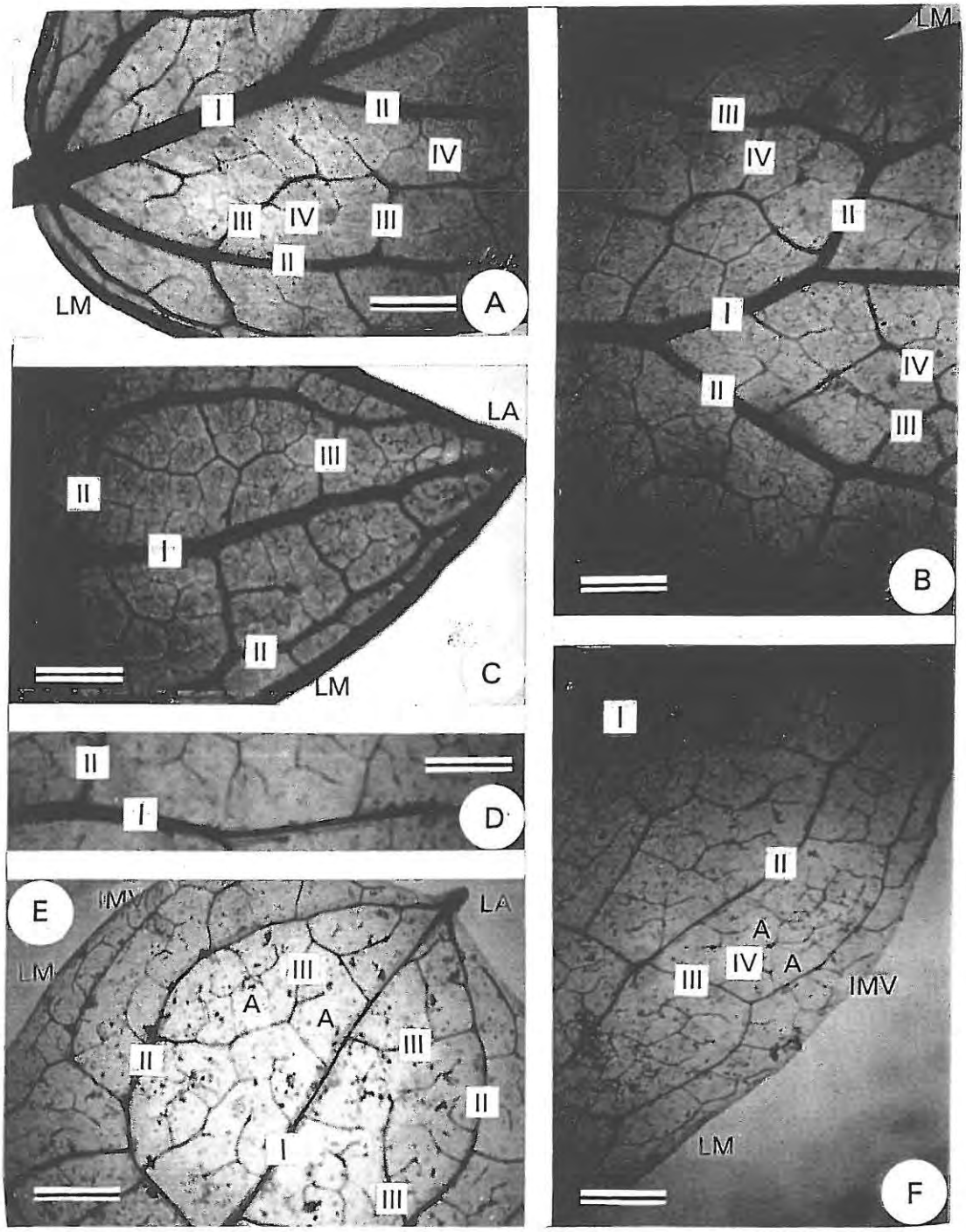
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Figure 3.7 Ranunculaceae: Aspects of cleared leaves of *Knowltonia*

A *Knowltonia filia* subsp. *filia* leaf showing midvein and excurrent secondary, tertiary and quaternary veins, incompletely closed areoles and incomplete marginal venation, B *K. filia* subsp. *filia* lamina showing midvein and excurrent secondary, tertiary, quaternary and fifth order veins, blind ending secondary vein at serration apex, incompletely closed areoles and incomplete marginal venation, C *K. filia* subsp. *scaposa* showing marginal serrations with blind ending minor veins and incomplete marginal venation of simple to once-branched ultimate veinlets, D *K. filia* subsp. *scaposa* lamina with secondary vein and excurrent tertiary, quaternary and fifth order veins, imperfect areoles and simple to twice-branched ultimate veinlets, E *K. bracteata* pubescent lamina showing midvein with excurrent secondary and tertiary veins and imperfect areoles, F *K. bracteata* marginal serration showing blind ending vein at edge of pubescent lamina and incomplete marginal venation

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LM = leaf margin; V = fifth order vein)

Bar represents 0,25mm

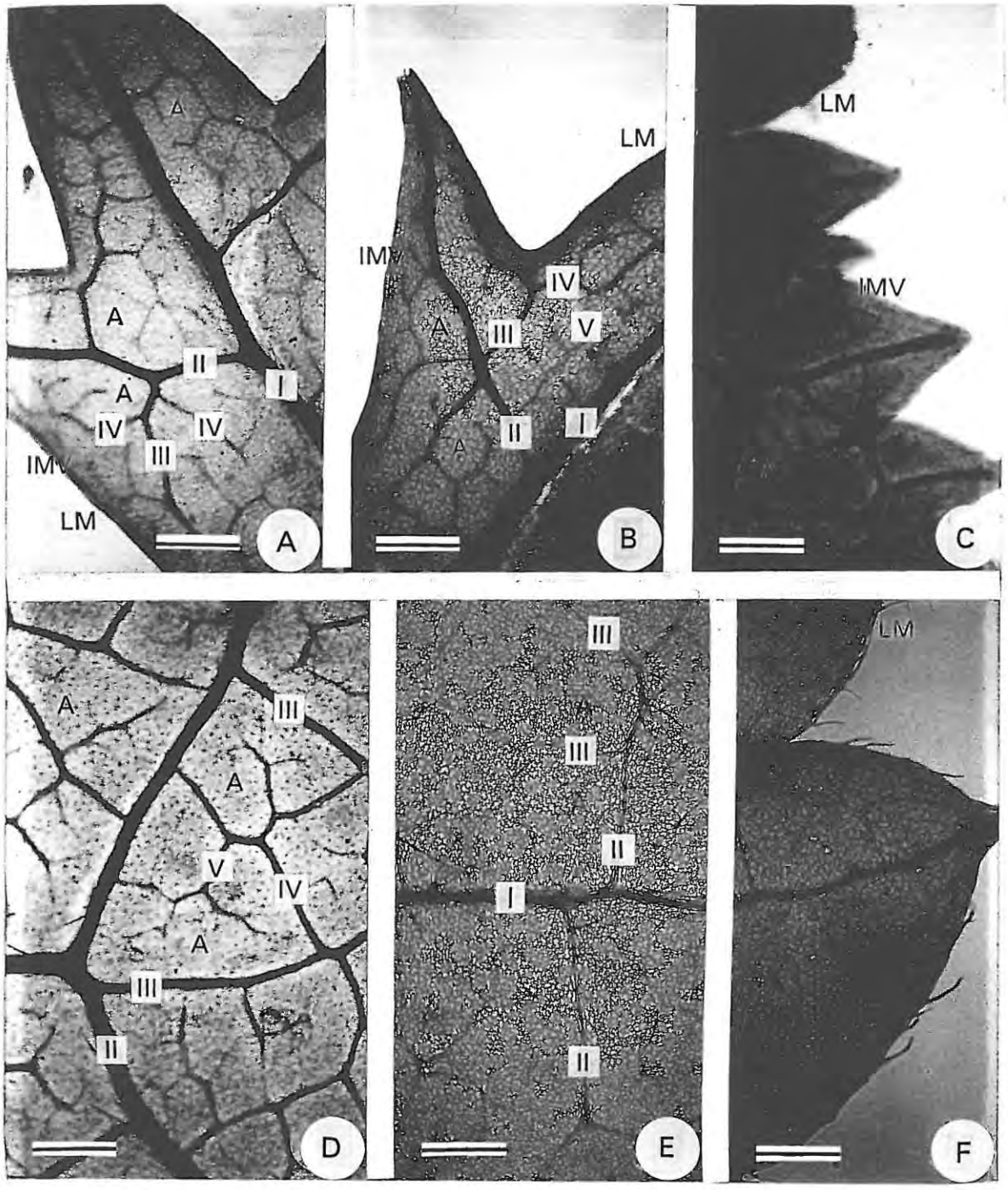
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Figure 3.8 Ranunculaceae: Aspects of cleared leaves of *Knowltonia*

A *Knowltonia cordata* marginal serration showing imperfect areoles, once to twice-branched ultimate veinlets and incomplete marginal venation, B *K. cordata* showing midvein with excurrent secondary, tertiary, quaternary and fifth order veins, imperfect areoles and once to twice-branched ultimate veinlets, C *K. capensis* marginal serration showing blind ending vein at apex and incomplete marginal venation of once to twice-branched ultimate veinlets, D *K. capensis* showing secondary vein with excurrent tertiary, quaternary and fifth order veins, imperfect areoles and once-branched ultimate veinlets, E *K. vesicatoria* subsp. *humilis* showing secondary vein with excurrent tertiary, quaternary and fifth order veins, imperfect areoles and simple to twice-branched ultimate veinlets, F *K. vesicatoria* subsp. *humilis* showing secondary vein near margin with excurrent tertiary, quaternary and fifth order veins, imperfect areoles, and simple to twice-branched ultimate veinlets, G *K. vesicatoria* subsp. *grossa* lamina margin with incomplete marginal venation of simple to once-branched ultimate veinlets and imperfect areoles with simple to twice-branched ultimate veinlets, H *K. vesicatoria* subsp. *grossa* showing midvein with excurrent secondary veins

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm

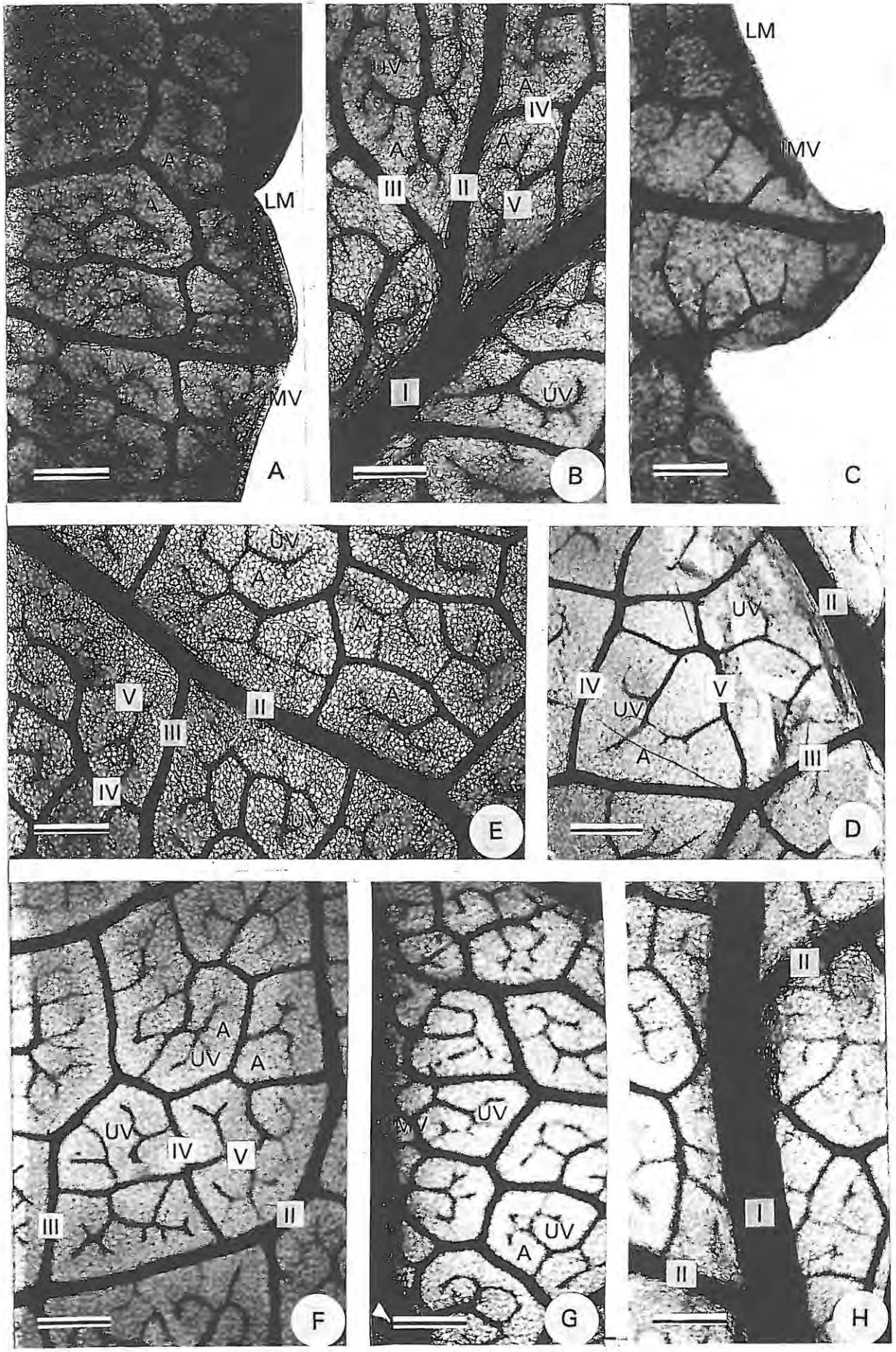
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Figure 3.9 Ranunculaceae: Aspects of cleared leaves of *Knowltonia*

A *Knowltonia transvaalensis* var. *transvaalensis* lamina showing secondary vein with excurrent tertiary and quaternary veins, imperfect areoles, simple to twice-branched ultimate veinlets and incomplete marginal venation, B *K. transvaalensis* var. *transvaalensis* leaf apex showing primary vein with excurrent secondary vein and incomplete marginal venation, C *K. transvaalensis* var. *transvaalensis* marginal serration with incomplete marginal venation of simple to once-branched ultimate veinlets and imperfect areoles, D *K. brevistylis* leaf serration showing blind ending vein at apex, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, E *K. brevistylis* lamina showing secondary vein with excurrent tertiary and quaternary veins, imperfect areoles and simple to once-branched ultimate veinlets, F *K. brevistylis* midvein with excurrent secondary, tertiary and quaternary veins and imperfect areoles

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm

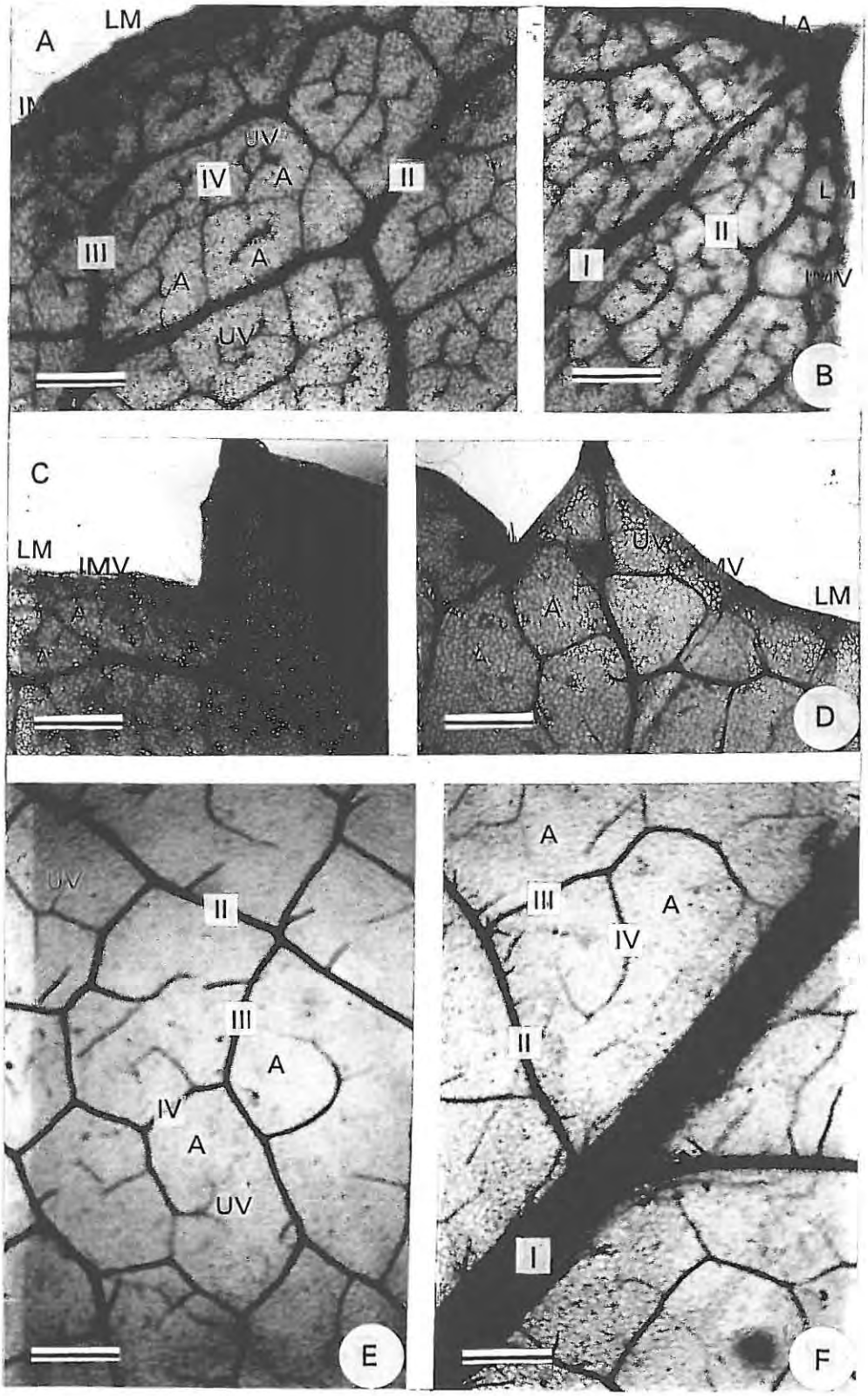
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Table 3.2 Summary of the angles of divergence of secondary veins and tertiary vein organisation in leaves of the Ranunculaceae

SPECIES	SECONDARY VEIN DIVERGENCE	TERTIARY VEIN PATTERN
Ranunculus baurii	Moderately acute	Transversely ramified
Ranunculus multifidus (Fig. 3.4 A, B & C)	Moderately acute	Transversely ramified
Ranunculus muricatus (Fig. 3.4 D & E)	Moderately acute	Transversely ramified
Clematis brachiata (Fig. 3.4 F & G)	Moderately acute	Transversely ramified
Clematis triloba (Fig. 3.5 A to E)	Moderately acute	Transversely ramified
Ranunculus meyeri (Fig. 3.5 F)	Moderately acute	Transversely ramified
Thalictrum minus (Fig. 3.6 A, B & C)	Widely acute	Transversely ramified
Thalictrum rhynchocarpum (Fig. 3.6 D, E & F)	Widely acute	Transversely ramified
Knowltonia filia subsp. *filia* (Fig. 3.7 A & B)	Widely acute	Transversely ramified
Knowltonia filia subsp. *scaposa* (Fig. 3.7 C & D)	Moderately acute	Transversely ramified
Knowltonia bracteata (Fig. 3.7 E & F)	Moderately acute	Transversely ramified
Knowltonia cordata (Fig. 3.8 A & B)	Moderately to widely acute	Transversely ramified
Knowltonia capensis (Fig. 3.8 C & D)	Widely acute to perpendicular	Transversely ramified
Knowltonia vesicatoria subsp. *humilis* (Fig. 3.8 E & F)	Widely acute	Transversely ramified
Knowltonia vesicatoria subsp. *grossa* (Fig. 3.8 G & H)	Moderately acute	Transversely ramified
Knowltonia transvaalensis var. *transvaalensis* (Fig. 3.9 A, B & C)	Narrowly acute	Transversely ramified
Knowltonia brevistylis (Fig. 3.9 D, E & F)	Moderately acute	Transversely ramified

Table 3.3 Summary of the highest vein order counted and ultimate veinlet condition in leaves of the Ranunculaceae

SPECIES	HIGHEST VEIN ORDER	ULTIMATE VEINLET CONDITION
Ranunculus baurii	Sixth	Simple to once-branched
Ranunculus multifidus (Fig. 3.4 A, B & C)	Sixth	Simple to once-branched
Ranunculus muricatus (Fig. 3.4 D & E)	Sixth	Simple to once-branched
Clematis brachiata (Fig. 3.4 F & G)	Sixth	Simple to once-branched
Clematis triloba (Fig. 3.5 A to E)	Sixth	Simple to once-branched
Ranunculus meyeri (Fig. 3.5 F)	Sixth	Simple to once-branched
Thalictrum minus (Fig. 3.6 A, B & C)	Fifth	Simple to once-branched
Thalictrum rhynchocarpum (Fig. 3.6 D, E & F)	Fifth	Simple to once-branched
Knowltonia filia subsp. *filia* (Fig. 3.7 A & B)	Seventh	Simple to once-branched
Knowltonia filia subsp. *scaposa* (Fig. 3.7 C & D)	Sixth	Once, twice and thrice-branched
Knowltonia bracteata (Fig. 3.7 E & F)	Sixth	Simple, once and twice-branched
Knowltonia vesicatoria subsp. *grossa* (Fig. 3.7 G & H)	Sixth	Once, twice and thrice-branched
Knowltonia cordata (Fig. 3.8 A & B)	Sixth	Once to twice-branched
Knowltonia capensis (Fig. 3.8 C & D)	Seventh	Simple to once-branched
Knowltonia vesicatoria subsp. *humilis* (Fig. 3.8 E & F)	Sixth	Once, twice and thrice-branched
Knowltonia transvaalensis var. *transvaalensis* (Fig. 3.9 A, B & C)	Sixth	Simple, once and twice-branched
Knowltonia brevistylis (Fig. 3.9 D, E & F)	Seventh	Simple, once and twice-branched

3.2.2 Apocynaceae

Description of venation pattern

Venation pattern of the Apocynaceae is pinnate with a single midvein giving rise to secondary veins, camptodromous as the secondary veins do not terminate at the margin, and brochidodromous in that distinct arches are formed by anastomosing secondary veins at the margins (Hickey 1973). All taxa examined in this family adhered to this description with no deviations (Fig. 3.10 A to E).

Description of vein order

In all instances, the course of the midvein is straight and unbranched to the apex (Fig. 3.10 A to E). This can be seen in *Gonioma kamassi* (Fig. 3.11 D), *Carissa bispinosa* subsp. *acuminata* (Fig. 3.12 A & C), *C. macrocarpa* (Fig. 3.13 C) and *Landolphia capensis* (Fig. 3.13 E).

Secondary veins arise from the midvein at an angle of divergence ranging from narrowly acute (less than 45°) (Table 3.3) as in *Carissa wyliei* and *Landolphia capensis* (Fig. 3.13 E), to widely acute (between 65° and 80°) as in *L. kirkii* (Table 3.3). Secondary veins closer to the apex tend to be more obtuse (greater than 100°) than the lower (Fig. 3.11 C, D & E) (Table 3.3). A range of angles of divergence occurred both within and between taxa (Table 3.3).

The brochidodromous pattern is produced by curvature of the secondary veins apically to join supra-adjacent secondary veins (Fig. 3.10 A to F). Curvature ranged from uniform and smooth as in *Acokanthera oblongifolia*, *A. oppositifolia* and *C. macrocarpa* (Fig. 3.13 B), to abruptly distinct as in *Landolphia capensis* (Fig. 3.13 E), *L. kirkii* and *Gonioma kamassi* (Fig. 3.11 C).

Two types of intersecondary veins of intermediate thickness were noted. *Pachypodium bispinosum* had simple intersecondary veins consisting of a single vein segment arising from the midvein. In *Landolphia kirkii*, composite intersecondary veins made up of anastomosing tertiary vein segments were seen.

Tertiary venation within the family was mostly transversely ramified, with tertiaries constantly rebranching into smaller veins and forming a reticulum across the intercostal area (Table 3.3), as seen in *Acokanthera oppositifolia* (Fig. 3.11 B), *Gonioma kamassi* (Fig. 3.11 C), *Carissa wyliei* (Fig. 3.11 G), *C. bispinosa* subsp. *acuminata* (Fig. 3.12 D), *C. macrocarpa* (Fig. 3.13 B) and *Landolphia capensis* (Fig. 3.13 E).

Whilst most of the Apocynaceae examined showed a range of uniform tertiary vein patterns across the lamina (Table 3.3), *Carissa bispinosa* var. *acuminata* (Fig. 3.12 A to D) exhibited three recognisable patterns across the leaf blade. Near the margin the pattern was transversely ramified (Fig. 3.12 B), while near the midvein it was random reticulate (Fig. 3.12 C) and in between the secondary veins simple percurrent (Fig. 3.12 D). *Landolphia kirkii (Table 3.3)* showed an almost orthogonal reticulate pattern between secondary veins, but mostly transversely ramified. In *Acokanthera oppositifolia* (Table 3.3), reticulate venation was mostly transversely ramified, but weakly percurrent near secondary vein bases (Fig. 3.11 B).

Higher order venation, viz. fourth and fifth order veins, was distinct with random orientation. Higher vein orders noted (Table 3.4) ranged from fifth, as in *Acokanthera oblongifolia* (Table 3.4), *A. oppositifolia* (Fig. 3.11 A & B) and *Pachypodium bispinosum* (Table 3.4), to sixth as in *Gonioma kamassi* (Fig. 3.11 C, D & E), *Carissa wyliei* (Fig. 3.11 F & G), *C. bispinosa* subsp. *acuminata* (Fig. 3.12 B, C & D), *C. macrocarpa* (Fig. 3.2.13 A & B), *C. haematocarpa* (Table 3.4), *Landolphia kirkii* (Table 3.4) and *L. capensis* (Fig. 3.13 F & G).

Marginal ultimate venation was uniformly incomplete in the family, with free ending veins occurring at the margins, as seen in *Acokanthera oppositifolia* (Fig. 3.11 A), *Gonioma kamassi* (Fig. 3.11 C & E), *Carissa wyliei* (Fig. 3.11 F), *C. bispinosa* subsp. *acuminata* (Fig. 3.12 A & B), *C. macrocarpa* (Fig. 3.13 A & C), *C. haematocarpa* (Fig. 3.13 D) and *Landolphia capensis* (Fig. 3.13 E & G).

Areoles were bound by large, incompletely enclosed meshes of irregular shape, as seen in *Gonioma kamassi* (Fig. 3.11 C to E), *Carissa wyliei* (Fig. 3.11 F & G) and *C. bispinosa* var. *acuminata* (Fig. 3.12 A to D). One or more sides of the areole were unbound by a vein. *Landolphia capensis* (Fig. 3.13 F & G) and *L. kirkii* showed the highest level of areole organisation in the family. In general, areole arrangement was random showing no distinct orientation (Fig. 3.10 A to E).

Branching of ultimate veinlets within areoles ranged from simple and unbranched to once and/or twice-branched (Table 3.4). *Gonioma kamassi* (Fig. 3.11 C, D & E) ultimate veinlets were simple and once-branched. In *Acokanthera oblongifolia* (Table 3.4), *A. oppositifolia* (Fig. 3.11 A & B) and *Pachypodium bispinosum* (Table 3.4), ultimate veinlets were consistently once-branched. *Landolphia capensis* exhibited simple, once and twice-branched ultimate veinlets (Fig. 3.13 F & G).

Figure 3.10 Cleared leaf examples of the Apocynaceae showing lower vein order, reticulum organisation and pattern within the family

A *Carissa macrocarpa* is a tall shrub found in dune vegetation. Leaves are large, waxy and tough. The reticulum is widely spaced, compared to other members of the family. B *Acokanthera oppositifolia* is a shrubby tree occurring in shady riverine vegetation. Venation is robust and dense with good lamina coverage. C *Gonioma kamassi* occurs in coastal forest as a shrubby tree. Leaves are very tough with a very dense reticulum. D *Carissa bispinosa* var. *acuminata* is a tall shrub found in forests both inland and coastal. The reticulum is very dense and veins are robust.

(I = midvein; II = secondary vein; III = tertiary vein; LA = leaf apex)

Bar represents 10mm

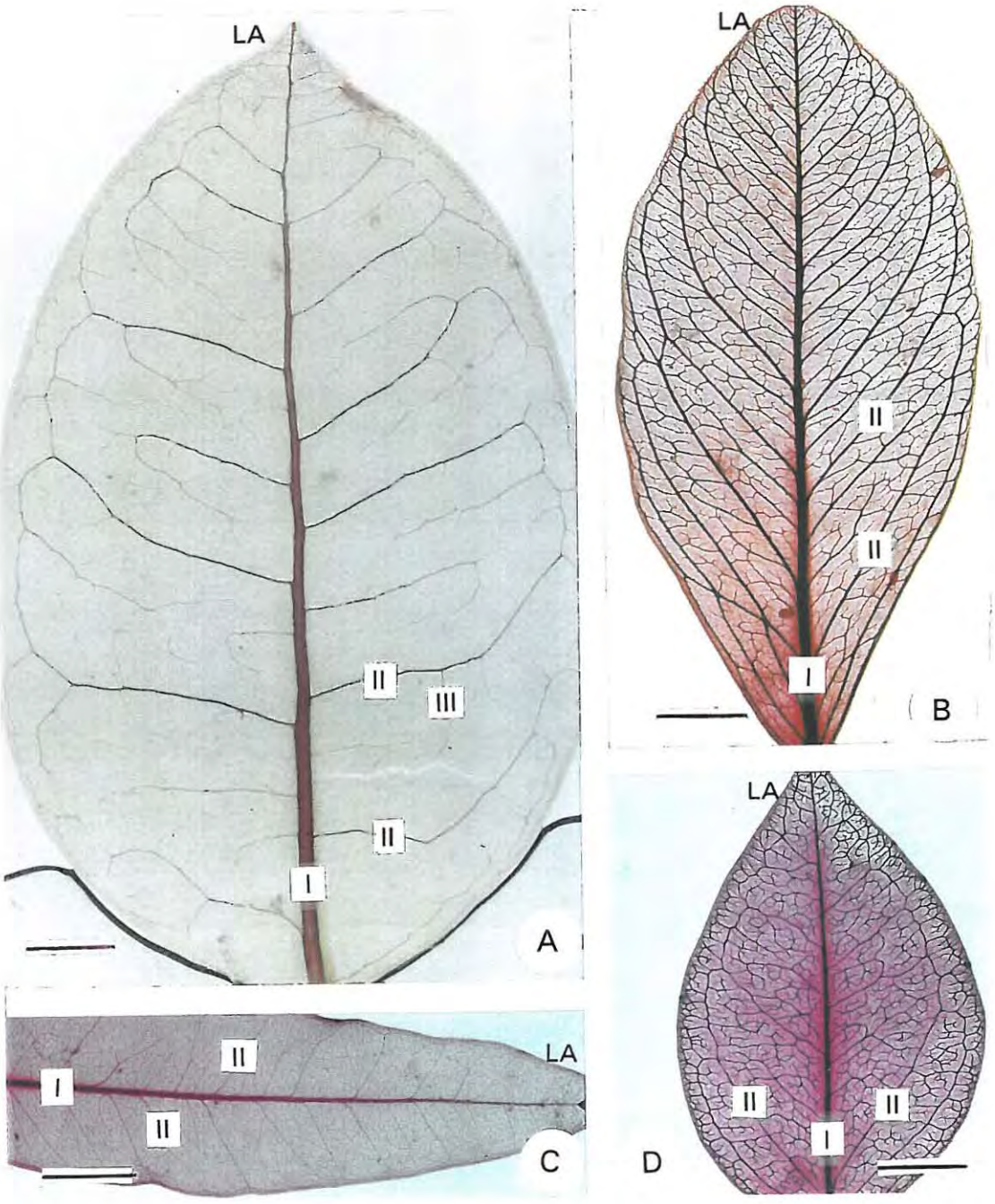
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Figure 3.11 Apocynaceae: Aspects of cleared leaves of *Acokanthera*, *Gonioma* and *Carissa*

A *Acokanthera oppositifolia* leaf margin showing brochidodromous secondary vein with excurrent tertiary and quaternary veins, and incomplete marginal venation of simple to twice-branched ultimate veinlets, B *A. oppositifolia* lamina showing secondary veins with excurrent tertiary, quaternary and fifth order veins, and incompletely closed areoles, C *Gonioma kamassi* leaf apex showing primary vein with excurrent brochidodromous secondary, and tertiary and quaternary veins, D *G. kamassi* lamina showing primary and excurrent secondary, tertiary and quaternary veins and incompletely closed areoles, E *G. kamassi* lamina showing tertiary vein with excurrent quaternary and fifth order veins, incomplete marginal venation and incompletely closed areoles with simple to once-branched ultimate veinlets, F *Carissa wyliei* leaf margin showing incomplete marginal venation and incompletely closed areoles with simple to once-branched ultimate veinlets, G *C. wyliei* lamina showing brochidodromous secondary vein with excurrent tertiary, quaternary and fifth order veins, incompletely closed areoles with simple to once-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm

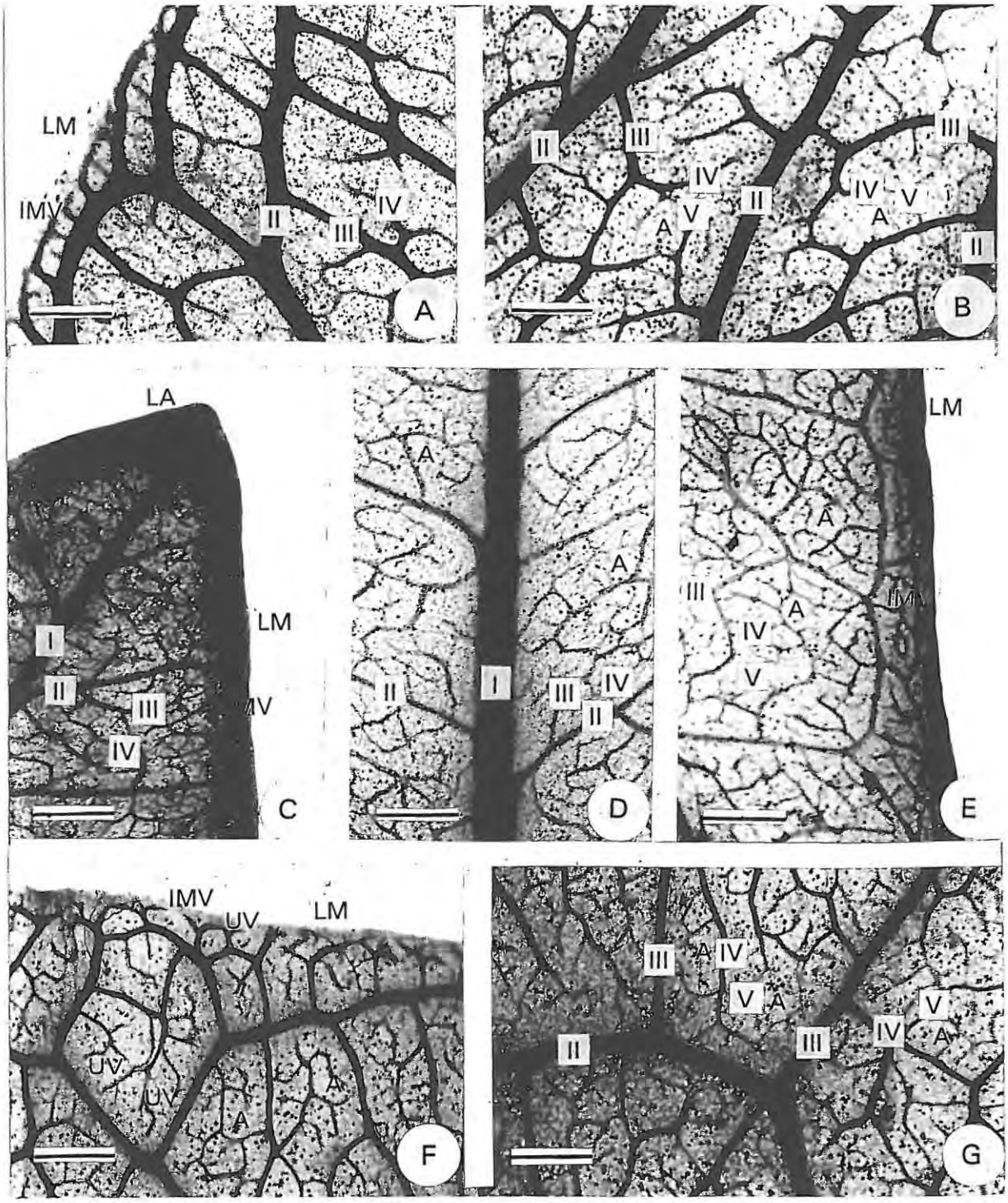
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Figure 3.12 Apocynaceae: Aspects of cleared leaves of *Carissa*

A *Carissa bispinosa* var. *acuminata* leaf tip showing primary vein with excurrent brochidodromous secondary veins and tertiary and quaternary veins, incompletely closed areoles and incomplete marginal venation with once to twice-branched ultimate veinlets, B *C. bispinosa* var. *acuminata* margin showing brochidodromous secondary vein with excurrent tertiary vein, incompletely closed areoles and once to twice-branched ultimate veinlets, C *C. bispinosa* var. *acuminata* lamina showing primary vein with excurrent secondary, tertiary and quaternary veins, incompletely closed areoles and once to twice-branched ultimate veinlets, D *C. bispinosa* var. *acuminata* lamina with brochidodromous secondary vein and excurrent tertiary and quaternary veins, incompletely closed areoles and once to twice-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm

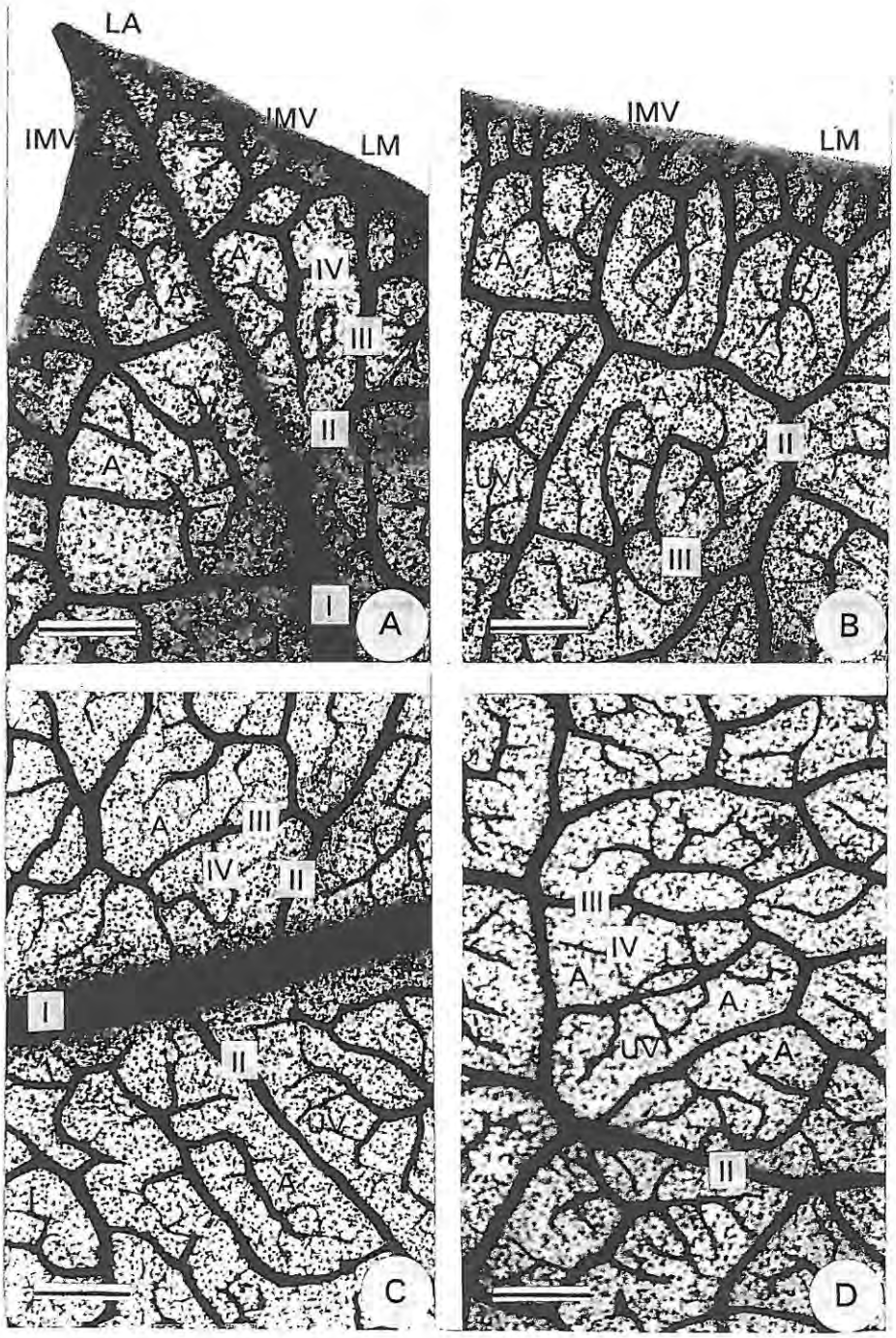
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Figure 3.13 Apocynaceae: Aspects of cleared leaves of *Carissa* and *Landolphia*

A *Carissa macrocarpa* leaf edge showing brochidodromous secondary vein with excurrent tertiary vein, and incomplete marginal venation of simple ultimate veinlets, B *C. macrocarpa* lamina with brochidodromous secondary veins and excurrent tertiary, quaternary and fifth order veins, C *C. macrocarpa* leaf apex showing blind ending primary vein with excurrent brochidodromous secondary veins, and incomplete marginal venation of simple ultimate veinlets, D *Carissa haematocarpa* leaf edge showing incomplete marginal venation of simple to once-branched ultimate veinlets, E *Landolphia capensis* lamina with brochidodromous secondary veins arising from a central primary vein, transversely ramified tertiary veins and incomplete marginal venation, F *L. capensis* lamina showing incompletely closed areoles with simple to twice-branched ultimate veinlets, G *L. capensis* leaf edge showing incompletely closed areoles with simple to twice-branched ultimate veinlets and incomplete marginal venation of simple to once-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm for B, C and D, and 0,05mm for A, E, F and G

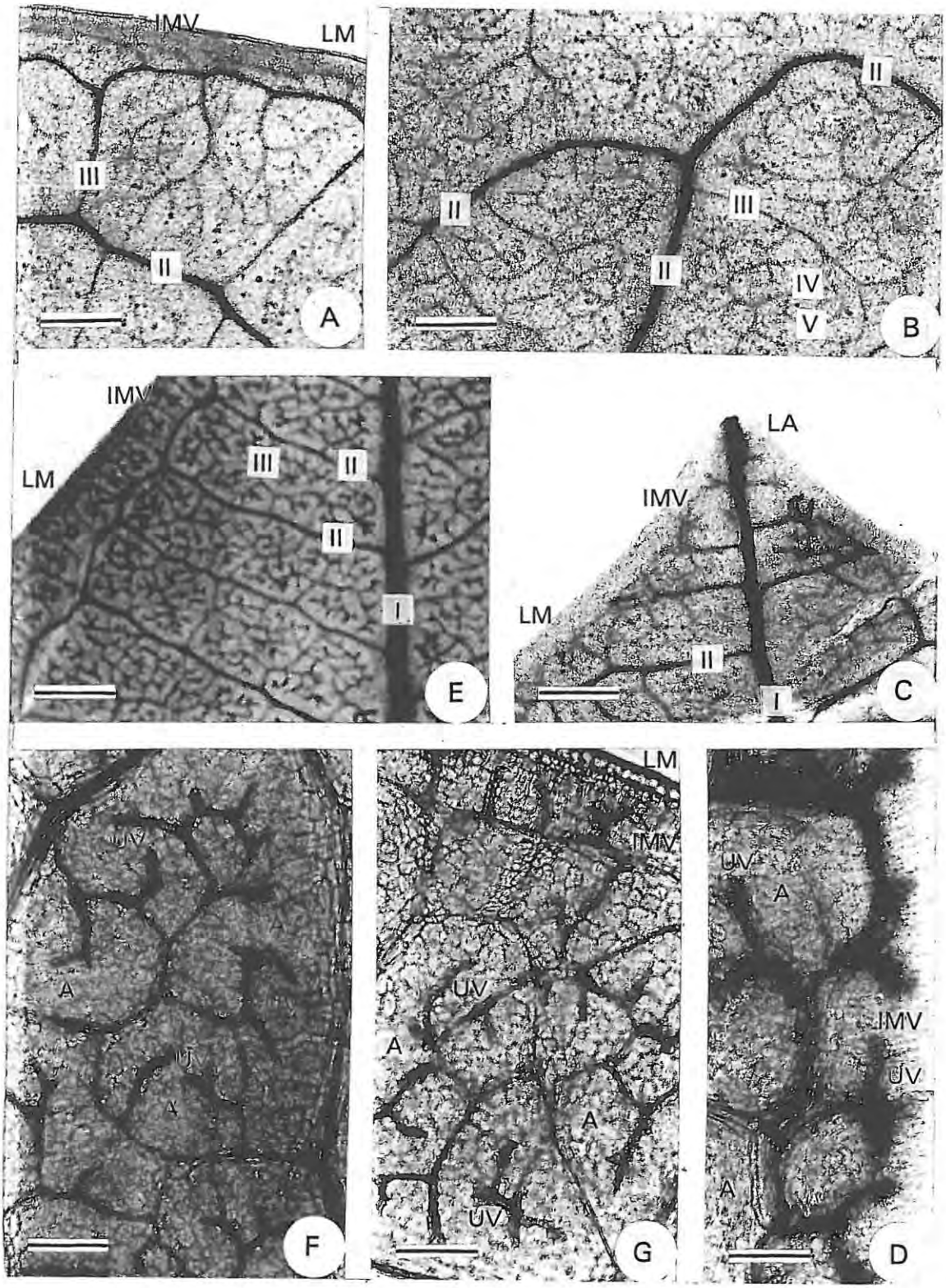
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Table 3.4 Summary of the angles of divergence of secondary veins and tertiary vein organisation in leaves of the Apocynaceae

SPECIES	SECONDARY VEIN DIVERGENCE	TERTIARY VEIN PATTERN
Acokanthera oblongifolia	Widely acute	Transversely ramified
Acokanthera oppositifolia (Fig. 3.11 A & B)	Moderately acute	Mostly transversely ramified, with weakly percurrent near secondary vein bases
Gonioma kamassi (Fig. 3.11 C, D & E)	Moderately acute	Transversely ramified
Carissa wyliei (Fig. 3.11 F & G)	Narrowly acute	Transversely ramified
Carissa bispinosa (Fig. 3.12 A to D)	Moderately acute	Transversely ramified near margin, random reticulate in lamina, simple percurrent near secondary veins
Carissa macrocarpa (Fig. 3.13 A, B & C)	Widely acute	Transversely ramified
Carissa haematocarpa (Fig. 3.13 D)	Moderately acute	Transversely ramified
Landolphia capensis (Fig. 3.13 E, F & G)	Widely acute	Mostly transversely ramified with simple percurrent
Landolphia kirkii	Widely acute	Almost orthogonal reticulate, mostly transversely ramified
Pachypodium bispinosum	Moderately acute	Transversely ramified

Table 3.5 Summary of the highest vein order counted and ultimate veinlet condition in leaves of the Apocynaceae

SPECIES	HIGHEST VEIN ORDER	ULTIMATE VEINLET CONDITION
Acokanthera oblongifolia	Fifth	Once-branched
Acokanthera oppositifolia (Fig. 3.11 A & B)	Fifth	Once-branched
Gonioma kamassi (Fig. 3.11 C, D & E)	Sixth	Simple to once-branched
Carissa wyliei (Fig. 3.11 F & G)	Sixth	Simple to once-branched
Carissa bispinosa (Fig. 3.12 A to D)	Sixth	Once to twice-branched
Carissa macrocarpa (Fig. 3.13 A, B & C)	Sixth	Mostly simple, also once-branched
Carissa haematocarpa (Fig. 3.13 D)	Fifth	Mostly simple, also once-branched
Landolphia capensis (Fig. 3.13 E, F & G)	Sixth	Simple, once and twice-branched
Landolphia kirkii	Sixth	Some simple, mostly once and twice-branched
Pachypodium bispinosum	Fifth	Once-branched

3.2.3 Asclepiadaceae

Description of venation pattern

In general terms, the description of venation pattern for the Asclepiadaceae is pinnate with one unbranched midvein, camptodromous, as the secondary veins do not terminate at the margin, and mostly brochidodromous with distinctly arching secondary veins (Fig. 3.14). Eucamptodromous species were noted in the genus *Aspidoglossum*, in which apically directed, gradually diminishing secondary veins were connected to adjacent secondaries by smaller cross veins, without forming prominent arches (Fig. 3.28 D, E, F & G).

Description of vein order

In all instances, the course of the midvein is straight and unbranched, as seen in *Asclepias dregeana* (Fig. 3.15 A), *A. crinita* (Fig. 3.15 E), *A. aurea* (Fig. 3.15 J), *A. expansa* (Fig. 3.15 M), *A. burchelli* (Fig. 3.2.16 D), *A. physocarpa* (Fig. 3.2.16 F), *Brachystelma meyeranum* (Fig. 3.18 A & B), *B. schizoglossoides* (Fig. 3.18 E), *Xysmalobium prunelloides* (Fig. 3.19 A & C), *X. pearsonii* (Fig. 3.20 B), *X. involucratum* (Fig. 3.20 C), *Ceropegia distincta* subsp. *haygarthii* (Fig. 3.20 A & B), *C. radicans* subsp. *radicans* (Fig. 3.20 G), *C. linearis* (Fig. 3.22 E), *C. carnosa* (Fig. 3.22 F), *Tenaris rubella* (Fig. 3.24 D & E), *Sisyranthus compactus* (Fig. 3.24 F & G), *Pachycarpus inconstans* (Fig. 3.25 E), *Schizoglossum cordifolium* (Fig. 3.27 A), *S. aschersonianum* (Fig. 3.27 F), *Pentarrhinum insipidum* (Fig. 3.27 H), *Riocreuxia torulosa* (Fig. 3.28 A), *Astephanus triflorus* (Fig. 3.28 H), *Tylophora lycioides* (Fig. 3.29 A), *T. umbellata* (Fig. 3.29 H), *Microloma sagittatum* (Fig. 3.30 A), *M. massonii* (Fig. 3.30 C) and *Oncinema lineare* (Fig. 3.30 E).

Divergence of secondary veins from the midvein varies from narrowly acute (less than 45°) (Table 3.6) as in *Brachystelma cathcartense* (Fig. 3.17 F), *Xysmalobium involucratum* (Fig. 3.20 C), *Sisyranthus compactus* (Fig. 3.24 F & G) and *Aspidoglossum biflorum* (Fig. 3.28 G), to perpendicular (Table 3.6) as in *Asclepias navicularis* (Fig. 3.15 A), *Brachystelma meyeranum* (Fig. 3.18 A) and *Ceropegia carnosa* (Fig. 3.22 F), to obtuse (greater than 100°) (Table 3.6) in the apical secondary veins of *Asclepias dregeana* (Fig. 3.15 A), *Tenaris rubella* (Fig. 3.24 D), *Schizoglossum aschersonianum* (Fig. 3.27 F), *Pentarrhinum insipidum* (Fig. 3.27 H), *Tylophora lycioides* (Fig. 3.29 A & B), *T. umbellata* (Fig. 3.29 F & H) and *Oncinema lineare* (Fig. 3.30 E).

In a number of taxa, secondary veins do not bend to join supra-adjacent secondary veins. Instead, the veins diminish apically inside the margin. This pattern is known as eucamptodromous and can be seen in *Aspidoglossum biflorum* (Fig. 3.28 D & G), *A. carinatum*, *A. virgatum*, *A. ovalifolium* (Fig. 3.28 E) and *A. heterophyllum* (Fig. 3.28 F).

Intersecondary veins are simple where present, and are often not found between each pair of secondary veins. Examples include *A. dregeana* (Fig. 3.15 A), *Asclepias aurea* (Fig. 3.15 J), *A. physocarpa* (Fig. 3.16 F), *Brachystelma meyeranum* (Fig. 3.18 A & B), *Xysmalobium pearsonii* (Fig. 3.20 B), *Ceropegia distincta* subsp. *haygarthii* (Fig. 3.21 B), *Tenaris rubella* (Fig. 3.24 E), *Sisyranthus compactus* (Fig. 3.24 F & G), *Schizoglossum aschersonianum* (Fig. 3.27 F), *Pentarrhinum insipidum* (Fig. 3.27 H), *Astephanus triflorus* (Fig. 3.28 H), *Fockea cylindrica* (Fig. 3.28 I), *Tylophora lycioides* (Fig. 3.29 B) and *Microloma massonii* (Fig. 3.30 C). For further examples of taxa with intersecondary veins see Table 3.5.

Table 3.6 Asclepiadaceae taxa with intersecondary veins

TAXA WITH INTERSECONDARY VEINS	
Astephanus marginatus	*Woodia mucronata*
Microloma sagittatum	*Asclepias expansa*
Xysmalobium confusum	*A. navicularis*
Aspidoglossum carinatum	*A. gibba*
A. ovalifolium	*A. crinita*
Schizoglossum cordifolium	*Oncinema lineare*
S. bidens	*Sisyranthus imberbis*
Pachycarpus rigidus	*Brachystelma cathcartense*
P. vexillaris	*Ceropegia radicans* subsp. *radicans*
Riocreuxia torulosa	*Ceropegia radicans* subsp. *smithii*
Tylophora umbellata	
Marsdenia floribunda	
Telosoma africana	

Tertiary veins produce a mostly transversely ramified pattern (Table 3.6), as in *Asclepias dregeana* (Fig. 3.15 A & B), *A. aurea* (Fig. 3.15 I & J), *A. gibba* (Fig. 3.15 L), *A. burchelli* (Fig. 3.16 D), *Brachystelma cathcartense* (Fig. 3.17 F), *B. schizoglossoides* (Fig. 3.18 E), *Xysmalobium pearsonii* (Fig. 3.20 A & B), *X. involucratum* (Fig. 3.20 C), *Telsoma africana* (Fig. 3.20 F),

Ceropegia distincta subsp. *haygarthii* (Fig. 3.21 B), *C. radicans* subsp. *radicans* (Fig. 3.21 E & F), *C. linearis* (Fig. 3.22 E), *C. carnosa* (Fig. 3.22 F), *Cynanchum obtusifolium* (Fig. 3.23 A), *Woodia marginata* (Fig. 3.23 D & E), *Secamone alpinii* (Fig. 3.24 A & B), *Tenaris rubella* (Fig. 3.24 D & E), *Sisyranthus compactus* (Fig. 3.24 F & G), *Schizoglossum cordifolium* (Fig. 3.27 C), *S. atropurpureum* subsp. *tridentatum* (Fig. 3.27 D), *S. hamatum* (Fig. 3.27 E), *S. aschersonianum* (Fig. 3.27 F), *Fockea cylindrica (Fig. 3.28 I)*, *Tylophora lycioides* (Fig. 3.29 A & B), *T. umbellata* (Fig. 3.29 F & G) and *Marsdenia floribunda* (Fig. 3.30 F). Examples with simple percurrent reticulate venation show unbranched tertiary veins from adjacent secondary veins joining (Table 3.6), as in *Xysmalobium undulatum* (Fig. 3.20 D & E), *Woodia mucronata* (Fig. 3.23 G, H & I), *Pachycarpus natalensis* (Fig. 3.25 A & B), *P. inconstans* (Fig. 3.25 D & E) and *P. reflectens* (Fig. 3.26 D). Random reticulate patterns were seen, where tertiary veins from adjacent secondary veins anastomose at random angles (Table 3.6), as in *Xysmalobium confusum* (Fig. 3.19 E), *X. orbiculare* (Fig. 3.19 F) and *X. prunelloides* (Fig. 3.19 A & B).

In some instances, combinations of patterns were noted, usually a combination of simple percurrent and transversely ramified (Table 3.6), as in *Brachystelma meyeranum* (Fig. 3.18 A & B), *B. elongatum*, *Pentarrhinum insipidum* (Fig. 3.27 G & H), *Pachycarpus vexillaris*, *P. dealbatus*, *P. grandiflorus*, *Asclepias fructicosa*, *A. physocarpa* and *A. crinita*.

Higher order venation was consistently distinct in resolution and random in orientation (Table 3.7). The highest vein order noted was eighth as in *Xysmalobium undulatum* (Fig. 3.20 D & E), *X. orbiculare* (Fig. 3.19 F), *Schizoglossum cordifolium* (Fig. 3.27 A & C), *Pachycarpus reflectens* (Fig. 3.26 D), *Asclepias dregeana* (Fig. 3.15 A & B), *Pentarrhinum insipidum* (Fig. 3.27 G & H), *Brachystelma cathcartense* (Fig. 3.17 F) and *Tylophora umbellata* (Fig. 3.29 F & G), and the lowest *Asclepias navicularis* (Fig. 3.15 G) with fourth. However, most taxa extended only to sixth order (Table 3.7).

Marginal ultimate venation was consistently incomplete as seen in *Asclepias dregeana* (Fig. 3.15 C), *A. aurea* (Fig. 3.15 I), *A. gibba* (Fig. 3.15 L), *A. fructicosa* (Fig. 3.16 A), *Brachystelma decipiens* (Fig. 3.17 A), *B. huttonii* (Fig. 3.17 C), *B. cathcartense* (Fig. 3.17 G), *B. meyeranum* (Fig. 3.18 A, B & D), *B. schizoglossoides* (Fig. 3.18 E & G), *Xysmalobium prunelloides* (Fig. 3.19 B), *X. confusum* (Fig. 3.19 D), *X. involucratum* (Fig. 3.20 C), *Telosoma africana* (Fig. 3.20

F), *Ceropegia distincta* subsp. *haygarthii* (Fig. 3.21 C), *C. radicans* subsp. *radicans* (Fig. 3.21 F & G), *C. carnosa* (Fig. 3.22 F & G), *Cynanchum capense* (Fig. 3.23 C), *Secamone alpinii* (Fig. 3.24 B), *Tenaris rubella* (Fig. 3.24 D & E), *Sisyranthus compactus* (Fig. 3.24 F & G), *Pachycarpus inconstans* (Fig. 3.25 D & E), *P. rigidus* (Fig. 3.25 G), *Schizoglossum aschersonianum* (Fig. 3.27 F), *Pentarrhinum insipidum* (Fig. 3.27 G & H), *Riocreuxia torulosa* (Fig. 3.28 B), *Tylophora lycioides* (Fig. 3.29 A, B & C), *T. umbellata* (Fig. 3.29 G & H), *Microloma tenuifolium* (Fig. 3.30 B), *Oncinema lineare* (Fig. 3.30 D) and *Marsdenia floribunda* (Fig. 3.30 F & H).

Areoles were mostly imperfect with meshes of irregular shape and size, and random arrangement. This can be seen clearly in *Asclepias dregeana* (Fig. 3.15 A to D), *A. crispa* (Fig. 3.15 F), *A. navicularis* (Fig. 3.15 G), *A. aurea* (Fig. 3.15 H, I & J), *A. meyeriana* (Fig. 3.15 K), *A. fructicosa* (Fig. 3.16 A & B), *A. erinens* (Fig. 3.16 C), *A. physocarpa* (Fig. 3.16 E), *Brachystelma decipiens* (Fig. 3.17 A & B), *B. elongatum* (Fig. 3.17 D & E), *B. cathcartense* (Fig. 3.17 F & G), *B. meyeranum* (Fig. 3.18 C), *Xysmalobium pearsonii* (Fig. 3.20 A), *X. involucratum* (Fig. 3.20 C), *X. undulatum* (Fig. 3.20 D), *Telosoma africana* (Fig. 3.20 F), *Ceropegia distincta* subsp. *haygarthii* (Fig. 3.21 A to D), *Cynanchum obtusifolium* (Fig. 3.23 A), *C. capense* (Fig. 3.23 B), *Woodia marginata* (Fig. 3.23 F), *W. mucronata* (Fig. 3.23 H), *Secamone alpinii* (Fig. 3.24 A & B), *Sisyranthus compactus* (Fig. 3.24 F & G), *Pachycarpus natalensis* (Fig. 3.25 C), *P. inconstans* (Fig. 3.25 D), *P. grandiflorus* (Fig. 3.25 F), *Schizoglossum cordifolium* (Fig. 3.27 A & C), *Pentarrhinum insipidum* (Fig. 3.27 I), *Riocreuxia flanaganii* (Fig. 3.28 C), *Tylophora lycioides* (Fig. 3.29 A, B & D) and *Oncinema lineare* (Fig. 3.30 D). Certain taxa exhibited areoles that formed incompletely enclosed meshes, such as *Brachystelma schizoglossoides* (Fig. 3.18 E & F), *Ceropegia radicans* subsp. *radicans* (Fig. 3.21 E & F) and *smithii* (Fig. 3.22 A), *Ceropegia carnosa* (Fig. 3.22 F), *Tenaris rubella* (Fig. 3.24 D & E), *Aspidoglossum biflorum* (Fig. 3.28 G), *A. carinatum*, *A. virgatum*, *A. heterophyllum*, *Fockea cylindrica* (Fig. 3.28 J), *F. multiflora*, *Tylophora umbellata* (Fig. 3.29 E, F & G) and *Marsdenia floribunda* (Fig. 3.30 F & G).

Ultimate veinlets ranged from just simple as in *Fanninia caloglossa* (Table 3.7), *Asclepias erinens* (Fig. 3.16 C) and *Brachystelma huttonii* (Fig. 3.17 C), to just once-branched as in *Xysmalobium involucratum* (Fig. 3.20 C). In most cases, a combination of simple and once-branched occurred (Table 3.7). Other combinations included simple to twice-branched as in

Telsoma africana (Fig. 3.20 F & G), *Secamone alpinii* (Fig. 3.24 A & B), *Pachycarpus natalensis* (Table 3.7), *P. inconstans* (Fig. 3.25 D & E), *Tylophora umbellata* (Fig. 3.29 E to H) and *Marsdenia floribunda* (Fig. 3.30 F, G & H), once to twice-branched as in *Fockea multiflora* and *Astephanus triflorus* (Table 3.7), and once to thrice-branched as in *Fockea cylindrica* (Fig. 3.28 I & J).

Figure 3.14 Cleared leaf examples of the Asclepiadaceae showing lower vein order, reticulum organisation and pattern within the family

A *Ceropegia meyeri* has soft fleshy leaves, with a sparse reticulum. B *Ceropegia dubia* leaves have a dense reticulum with brochidodromous secondary veins. C *Ceropegia radicans* subsp. *smithii* leaves are very similar to those of *C. dubia*, but are bigger. D *Pentarrhinum insipidum* grows as a slender twining herb from a woody perennial rootstock in grasslands. The leaves are tough, non succulent and have a dense reticulum of robust veins. E *Riocreuxia torulosa* leaves are small and tough, with a dense reticulum of robust veins. F *Astephanus marginatus* is a slender twining plant found on secondary dunes. Leaves are small and tough with a dense reticulum. G *Tylophora lycioides* leaves are small, showing brochidodromous secondary veins in a dense reticulum. H *Asclepias fructicosa* grows as a soft shrub in disturbed areas. Leaves are thin but tough. Brochidodromous secondary veins arise from a central primary vein. The reticulum is delicate but dense.

(I = midvein; II = secondary vein; III = tertiary vein; LA = leaf apex)

Bar represents 10mm

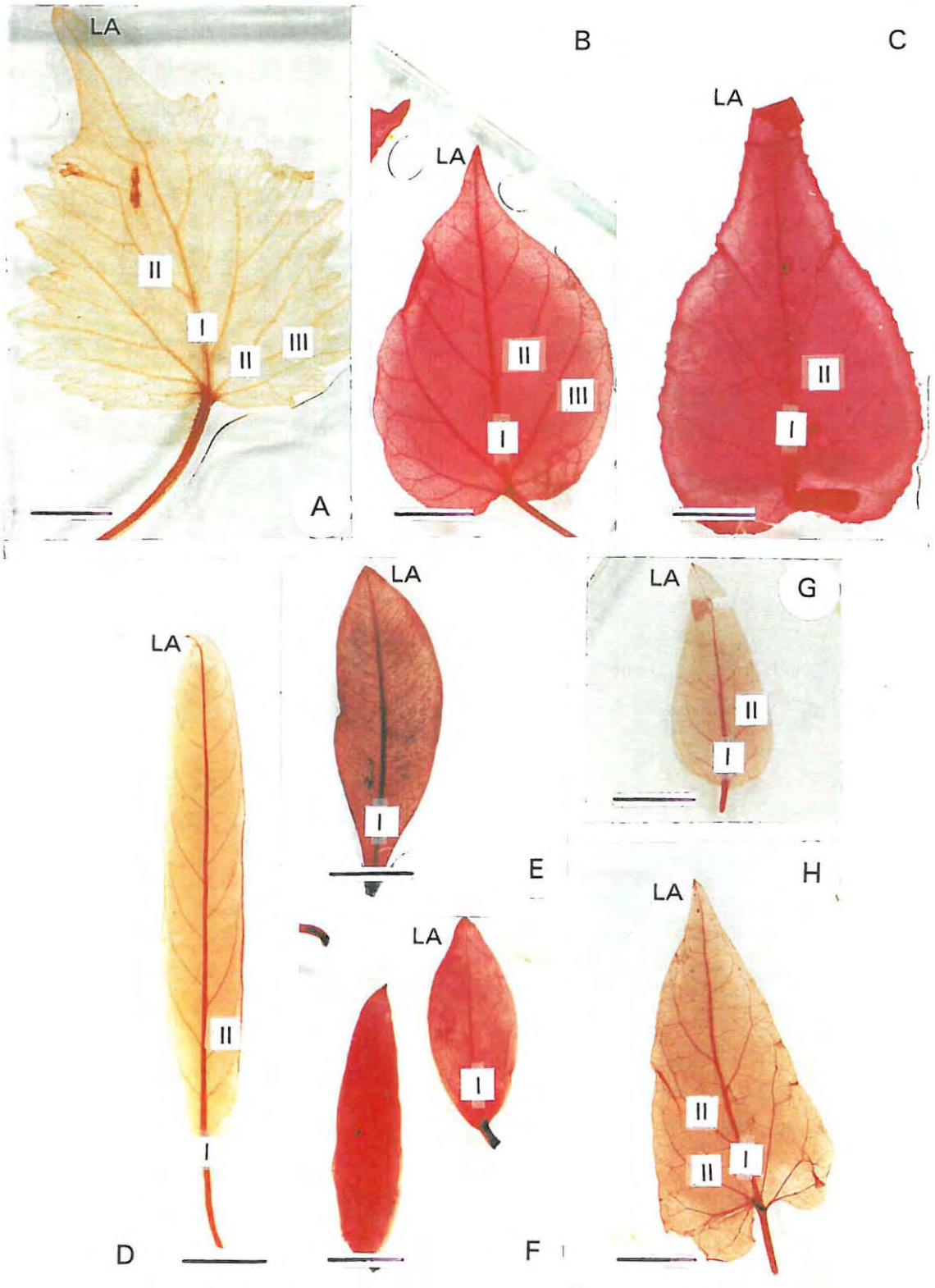
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Figure 3.15 Asclepiadaceae: Aspects of cleared leaves of *Asclepias*

A *Asclepias dregeana* leaf apex showing central primary vein with excurrent brochidodromous secondary veins and tertiary veins, imperfect areoles and incomplete marginal venation with simple to once-branched ultimate veinlets, B *A. dregeana* margin with brochidodromous secondary vein and excurrent tertiary and quaternary veins, imperfect areoles with simple to once-branched ultimate veinlets, C *A. dregeana* margin showing incomplete marginal venation and imperfect areoles with simple to once-branched ultimate veinlets, D *A. dregeana* lamina with imperfect areoles and simple to once-branched ultimate veinlets, E *A. crinita* leaf apex showing primary and excurrent secondary veins, F *A. crispa* lamina showing trichome, imperfect areoles and simple ultimate veinlets, G *A. navicularis* lamina showing primary vein with excurrent secondary and tertiary veins, and imperfect areoles with simple and once-branched ultimate veinlets, H *A. aurea* pubescent lamina with imperfect areoles and simple to once-branched ultimate veinlets near a secondary vein, I *A. aurea* leaf margin showing primary vein with excurrent brochidodromous secondary veins and tertiary veins, imperfect areoles and incomplete marginal venation with simple to once-branched ultimate veinlets, J *A. aurea* showing primary vein with excurrent brochidodromous secondary veins and tertiary veins, with imperfect areoles and simple to once-branched ultimate veinlets, K *A. meyeriana* lamina with imperfect areoles and simple to once-branched ultimate veinlets, L *A. gibba* lamina with central primary vein and excurrent brochidodromous secondary veins and incomplete marginal venation of simple ultimate veinlets, M *A. expansa* leaf apex showing central primary and excurrent secondary veins in narrow lamina

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A, B, E, I, J, L and M, and 0,05mm for C, D, F, G, H and K

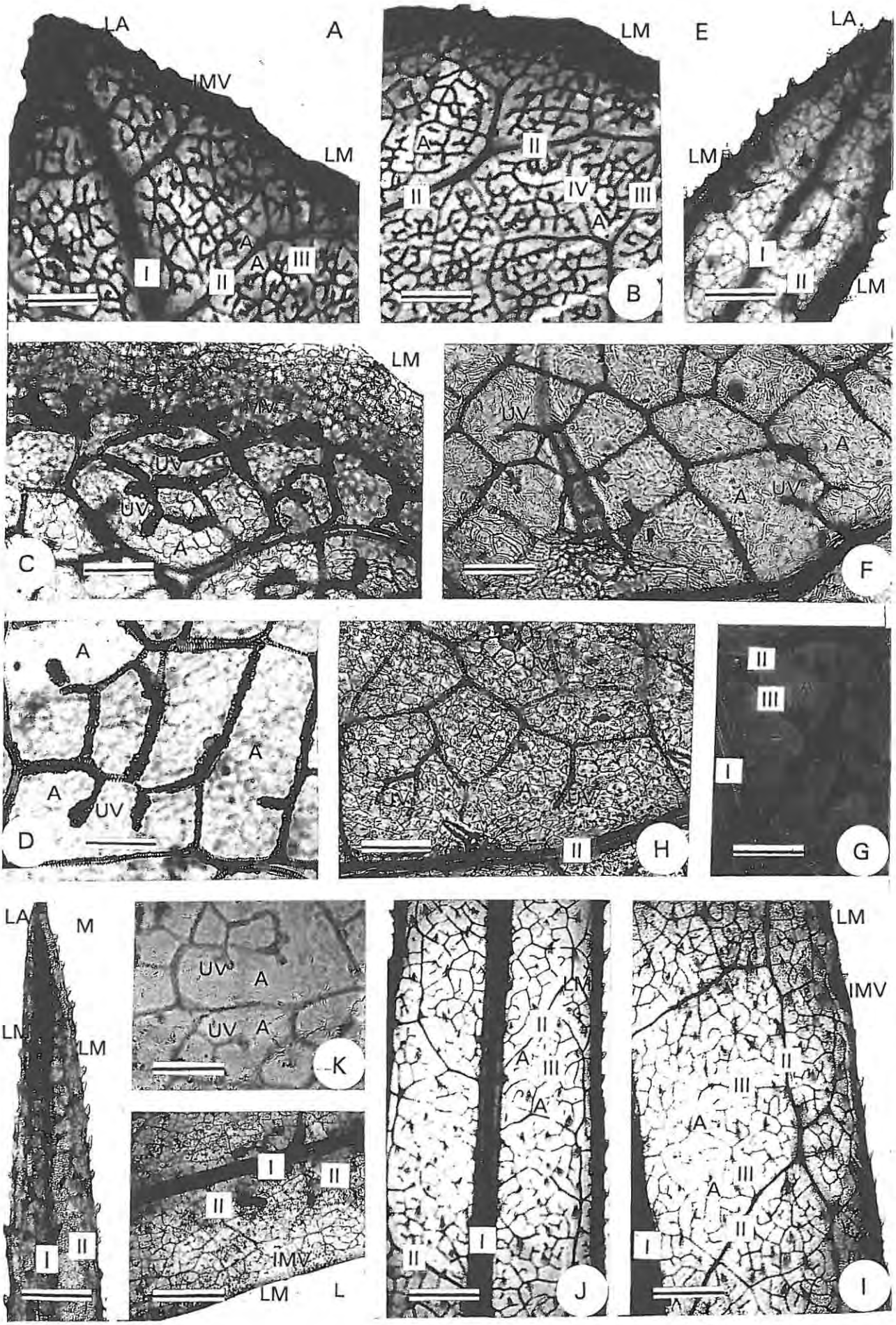
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Figure 3.16 Asclepiadaceae: Aspects of cleared leaves of *Asclepias*

A *Asclepias fructicosa* margin showing brochidodromous secondary vein with excurrent tertiary, quaternary and fifth order veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, B *A. fructicosa* lamina with imperfect areoles and simple to once-branched ultimate veinlets, C *A. erinens* lamina showing imperfect areoles and simple ultimate veinlets, D *A. burchelli* lamina with raised midvein and excurrent secondary and tertiary veins, E *A. physocarpa* lamina showing brochidodromous secondary vein with excurrent tertiary and quaternary veins, imperfect areoles and incomplete marginal venation with simple to once-branched ultimate veinlets, F *A. physocarpa* lamina with central primary vein and excurrent secondary and tertiary veins

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm for A, D, E and F, and 0,05mm for B and C

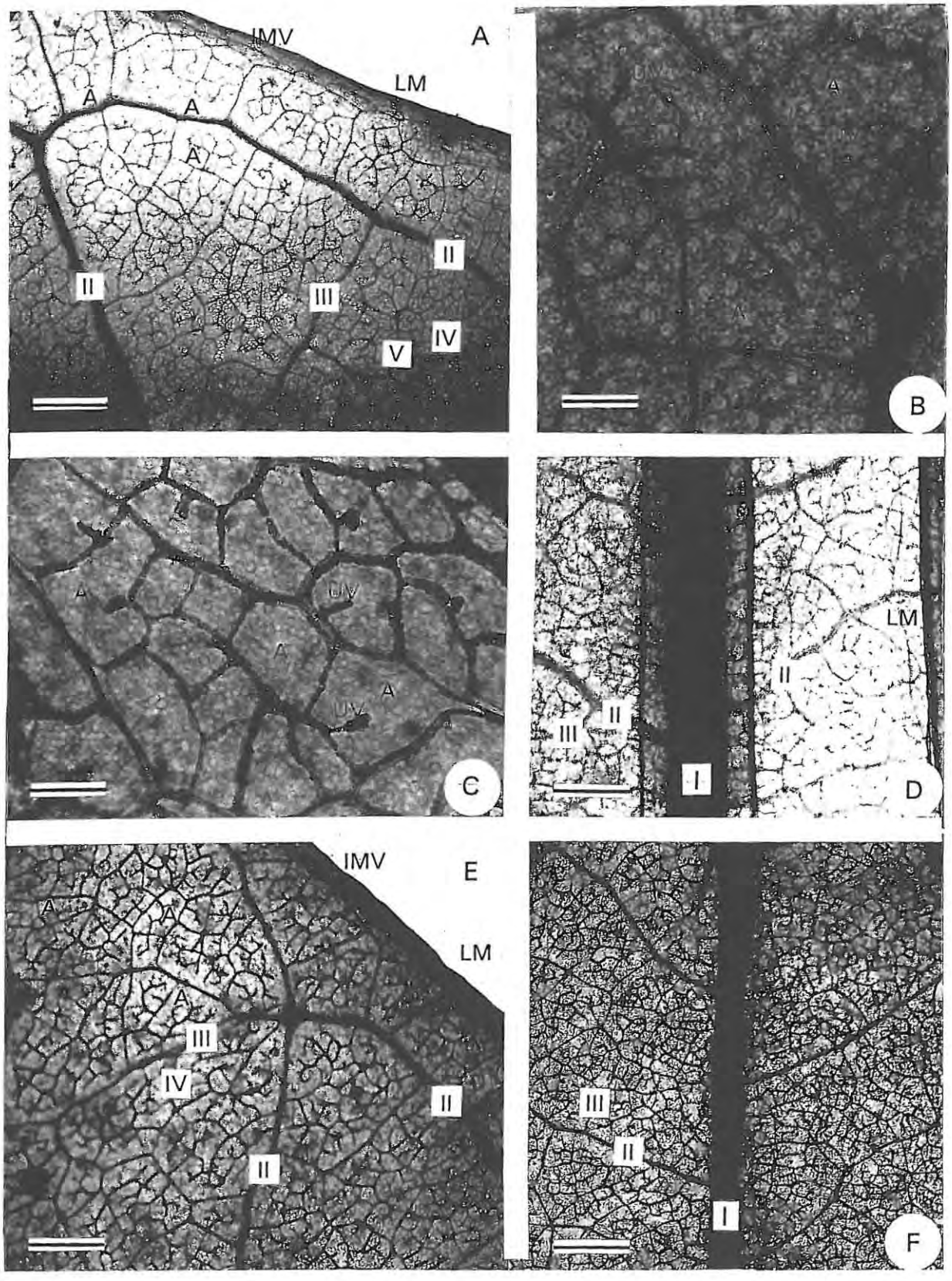
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Figure 3.17 Asclepiadaceae: Aspects of cleared leaves of *Brachystelma*

A *Brachystelma decipiens* leaf margin with imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, B *B. decipiens* lamina with imperfect areoles and simple to once-branched ultimate veinlets, C *B. huttonii* leaf margin showing incomplete marginal venation of simple ultimate veinlets and imperfect areoles, D *B. elongatum* lamina showing imperfect areoles with simple to once-branched ultimate veinlets, E *B. elongatum* lamina showing imperfect areoles with simple ultimate veinlets, F *B. cathcartense* lamina showing primary vein with excurrent secondary, tertiary and quaternary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, G *B. cathcartense* leaf margin with incomplete marginal venation of simple ultimate veinlets, and imperfect areoles

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A and F, and 0,05mm for B, C, D, E and G

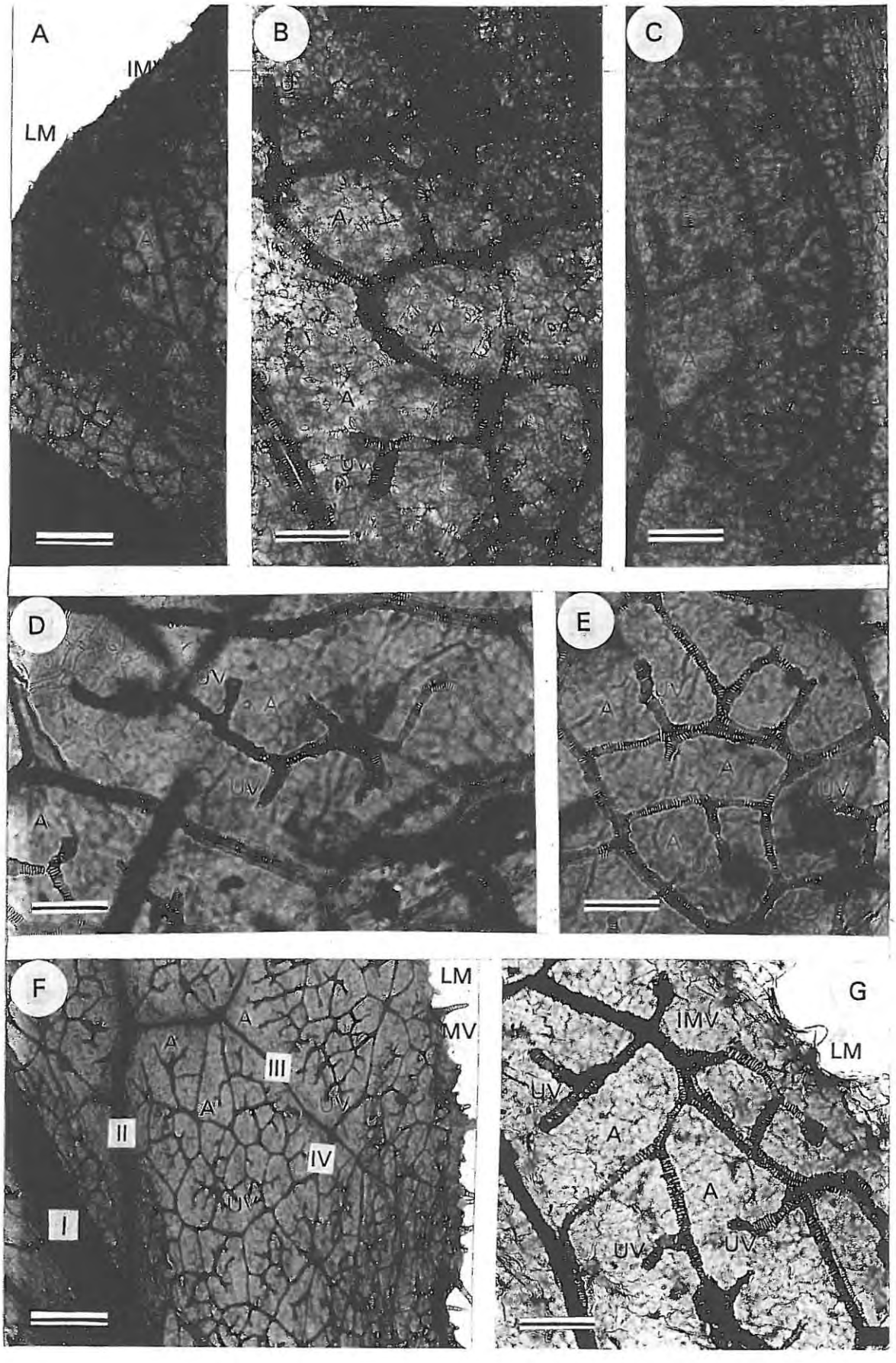
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Figure 3.18 Asclepiadaceae: Aspects of cleared leaves of *Brachystelma*

A *Brachystelma meyeranum* leaf apex showing central primary vein with excurrent brochidodromous secondary veins, and incomplete marginal venation of simple to once-branched ultimate veinlets, B *B. meyeranum* lamina showing central primary vein with excurrent brochidodromous secondary vein, and incomplete marginal venation of simple to once-branched ultimate veinlets, C *B. meyeranum* lamina showing imperfect areoles with simple to once-branched ultimate veinlets, D *B. meyeranum* leaf margin showing imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, E *B. schizoglossoides* lamina showing raised midvein with excurrent secondary and tertiary veins, incompletely closed areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, F *B. schizoglossoides* lamina with incompletely closed areole and simple to once-branched ultimate veinlets, G *B. schizoglossoides* leaf margin showing incompletely closed areoles and incomplete marginal venation of simple to once-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A, B and E, and 0,05mm for C, D, F and G

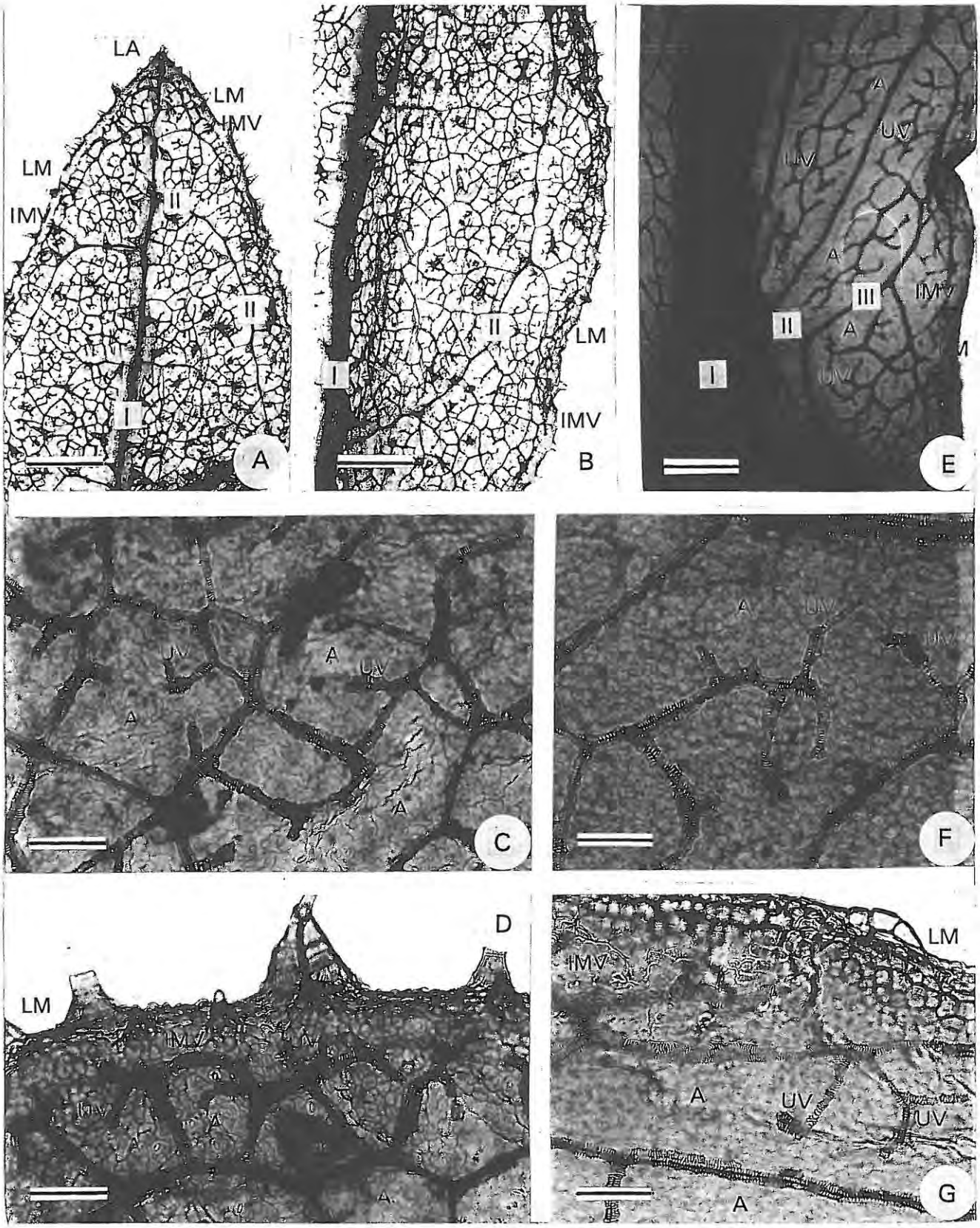
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Figure 3.19 Asclepiadaceae: Aspects of cleared leaves of *Xysmalobium*

A *Xysmalobium prunelloides* lamina with very robust, pubescent midvein and excurrent secondary vein, B *X. prunelloides* pubescent leaf margin showing brochidodromous secondary vein with excurrent tertiary and quaternary veins, C *X. prunelloides* leaf apex showing blind ending primary vein with excurrent secondary vein, D *X. confusum* leaf margin showing incomplete marginal venation with simple to once-branched ultimate veinlets, E *X. confusum* lamina showing secondary veins with excurrent, random reticulate tertiary veins and quaternary veins, F *X. orbiculare* leaf margin showing brochidodromous secondary veins and excurrent tertiary and quaternary veins

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IV = fourth order vein; LA = leaf apex; LM = leaf margin)

Bar represents 0,25mm

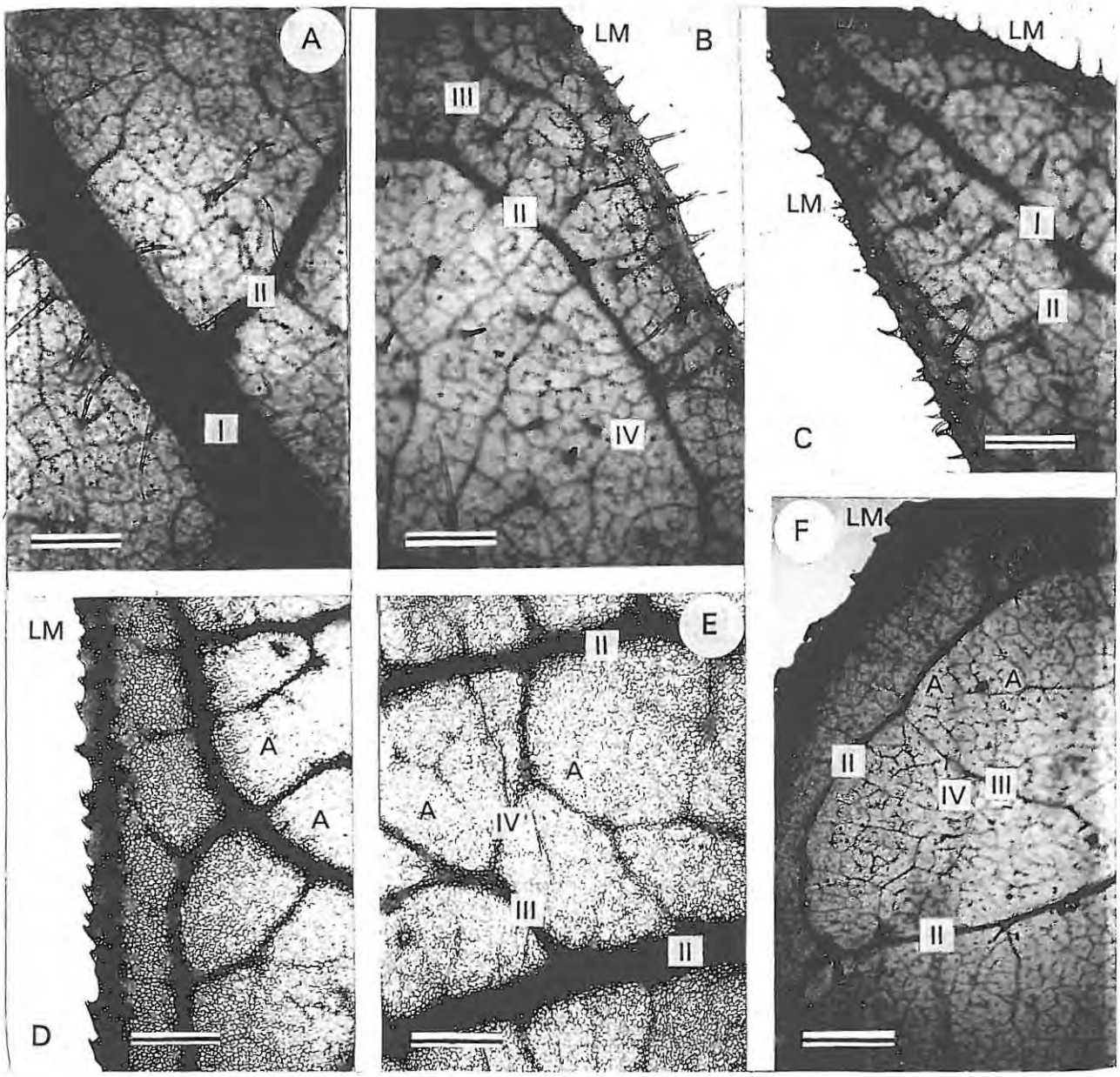
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Figure 3.20 Asclepiadaceae: Aspects of cleared leaves of *Xysmalobium* and *Telosoma*

A *Xysmalobium pearsonii* lamina showing secondary vein with excurrent tertiary, quaternary and fifth order veins, imperfect areoles with simple to once-branched ultimate veinlets, B *X. pearsonii* lamina with raised midvein and excurrent secondary and tertiary veins, C *X. involucratum* lamina showing central primary vein with excurrent secondary and tertiary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, D *X. undulatum* lamina showing consecutive secondary veins with excurrent, simple percurrent tertiary veins and quaternary veins, imperfect areoles with simple to once-branched ultimate veinlets, E *X. undulatum* leaf margin with brochidodromous secondary veins and excurrent tertiary and quaternary veins, F *Telosoma africana* leaf margin showing brochidodromous secondary veins with excurrent tertiary and quaternary veins, imperfect areoles and incomplete marginal venation of simple to twice-branched ultimate veinlets, G *T. africana* lamina with imperfect areoles and simple to twice-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm for A to F, and 0,05mm for G

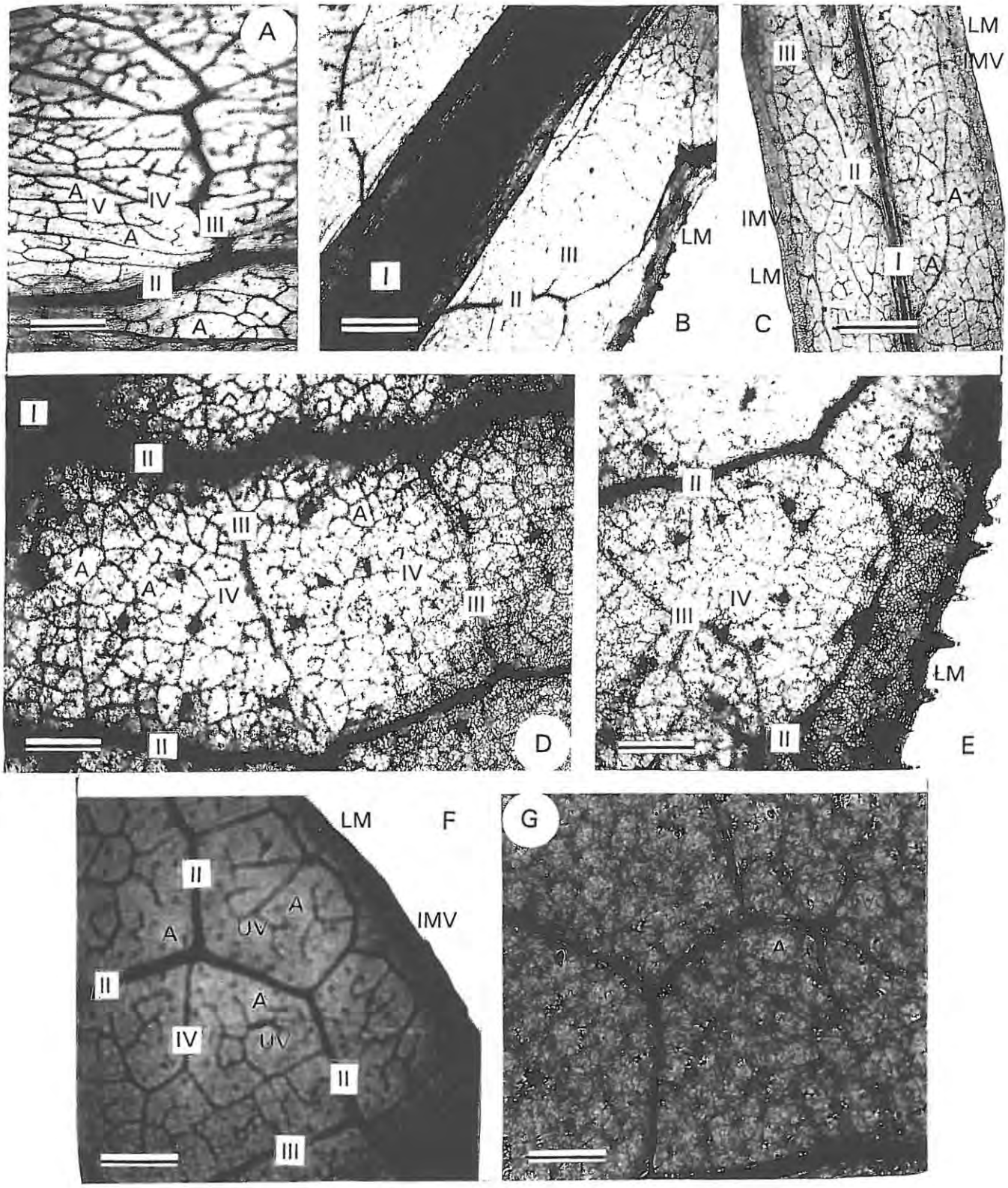
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Figure 3.21 Asclepiadaceae: Aspects of cleared leaves of *Ceropegia*

A *Ceropegia distincta* subsp. *haygarthii* leaf apex with central primary vein, secondary with excurrent tertiary veins, imperfect areoles and incomplete marginal venation with simple to once-branched ultimate veinlets, B *C. distincta* subsp. *haygarthii* lamina showing primary vein with excurrent secondary and tertiary veins, imperfect areoles and simple to once-branched ultimate veinlets, C *C. distincta* subsp. *haygarthii* leaf margin showing imperfect areoles and incomplete marginal venation with simple to once-branched ultimate veinlets, D *C. distincta* subsp. *haygarthii* lamina showing imperfect areole with simple and once-branched ultimate veinlets, E *C. radicans* subsp. *radicans* lamina with incompletely closed areoles and simple to once-branched ultimate veinlets, F *C. radicans* subsp. *radicans* leaf margin with imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, G *C. radicans* subsp. *radicans* leaf apex showing blind ending primary vein, incompletely closed areoles and incomplete marginal venation of simple to once-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A, B, C, E, F and G, and 0,05mm for D

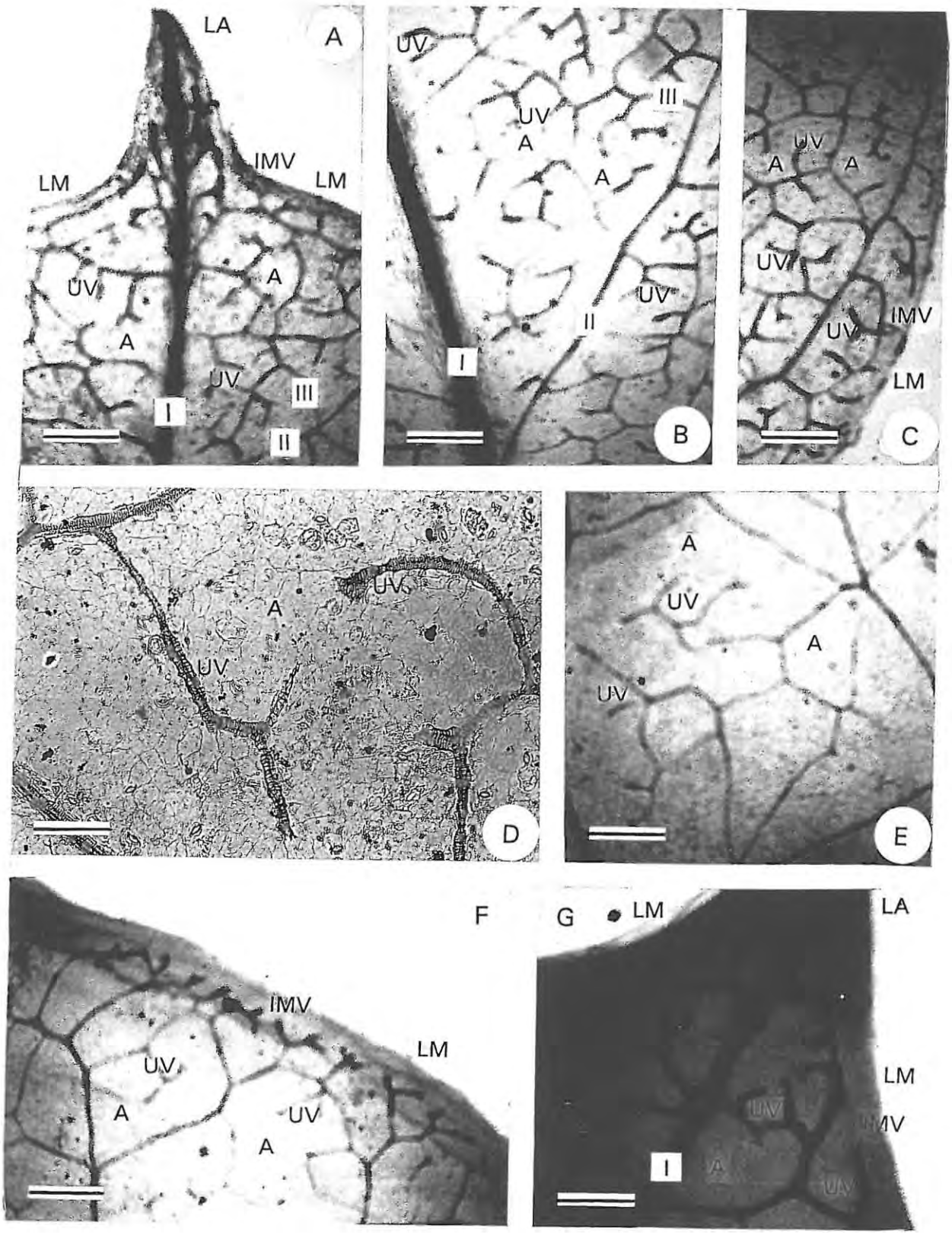
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Figure 3.22 Asclepiadaceae: Aspects of cleared leaves of *Ceropegia*

A *Ceropegia radicans* subsp. *smithii* lamina with incompletely closed areoles and simple to once-branched ultimate veinlets, B *C. radicans* subsp. *smithii* lamina with simple and once-branched ultimate veinlets in incompletely closed areole, C *C. radicans* subsp. *smithii* lamina with simple ultimate veinlet in incompletely closed areole, D *C. meyeri* leaf margin showing pubescence and deep serrations, E *C. linearis* lamina showing central primary vein with excurrent brochidodromous secondary veins and tertiary vein, F *C. carnosa* lamina showing central primary vein with excurrent brochidodromous secondary veins and tertiary and quaternary veins, incompletely closed areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, G *C. carnosa* leaf margin showing incomplete marginal venation with once-branched ultimate veinlet

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A, D, E and F, and 0,05mm for B, C and G

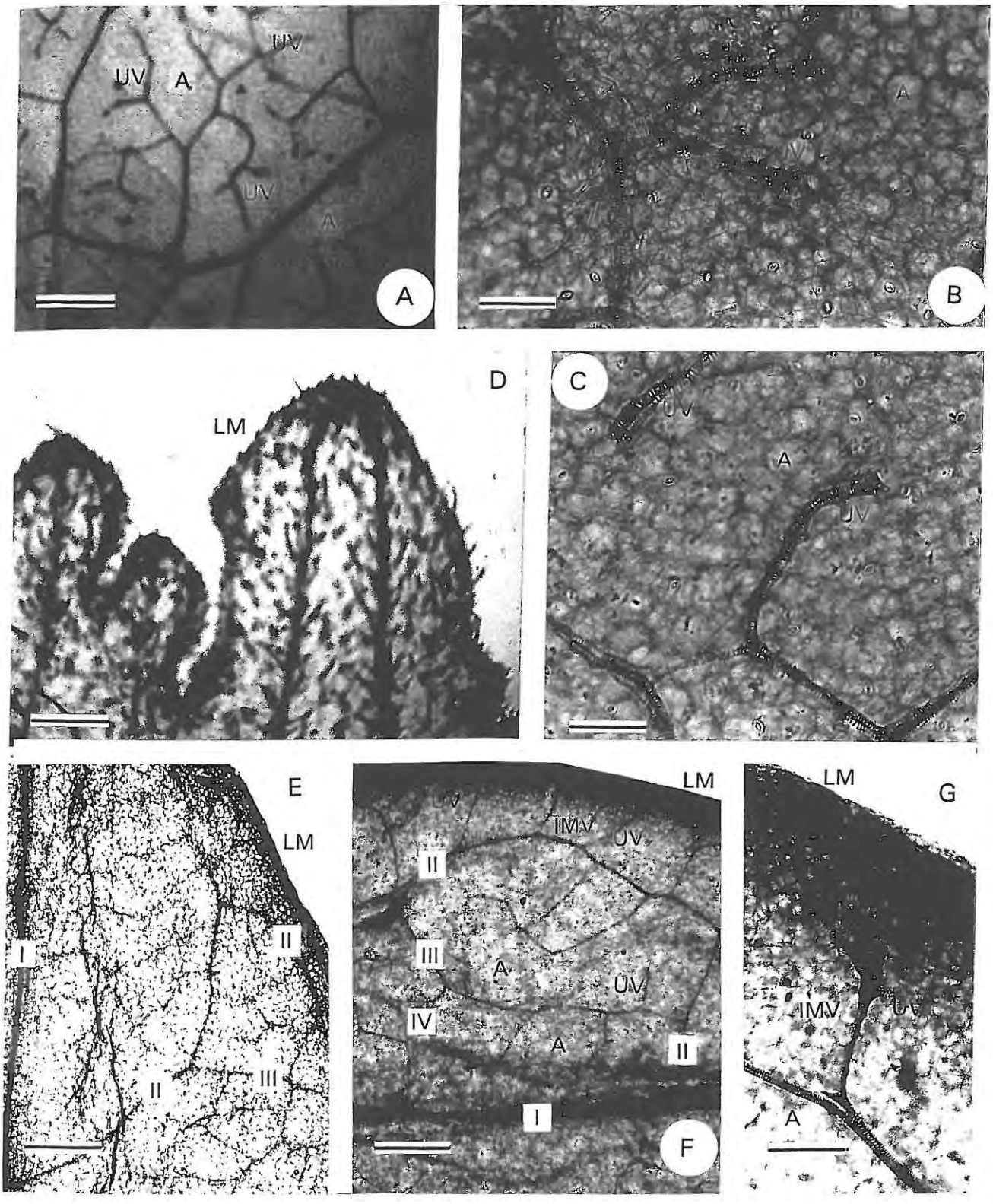
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Figure 3.23 Asclepiadaceae: Aspects of cleared leaves of *Cynanchum* and *Woodia*

A *Cynanchum obtusifolium* lamina showing imperfect areoles with both simple and branched ultimate veinlets, B *C. capense* lamina showing imperfect areoles and simple ultimate veinlets, C *C. capense* leaf margin with incomplete marginal venation of simple ultimate veinlets, D *Woodia marginata* lamina showing primary vein with excurrent secondary and tertiary veins, and imperfect areoles with simple to once-branched ultimate veinlets, E *W. marginata* leaf margin showing brochidodromous secondary veins with excurrent tertiary veins, F *W. marginata* lamina with imperfect areoles and simple to once-branched ultimate veinlets, G *W. mucronata* leaf margin with brochidodromous secondary vein and excurrent tertiary vein, H *W. mucronata* lamina showing consecutive secondary veins with excurrent, simple percurrent tertiary veins and quaternary and fifth order veins, and imperfect areoles with simple to once-branched ultimate veinlets, I *W. mucronata* leaf apex showing blind ending primary vein with excurrent secondary vein

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm for D, E, F, G, H and I, and 0,05mm for B, C and F

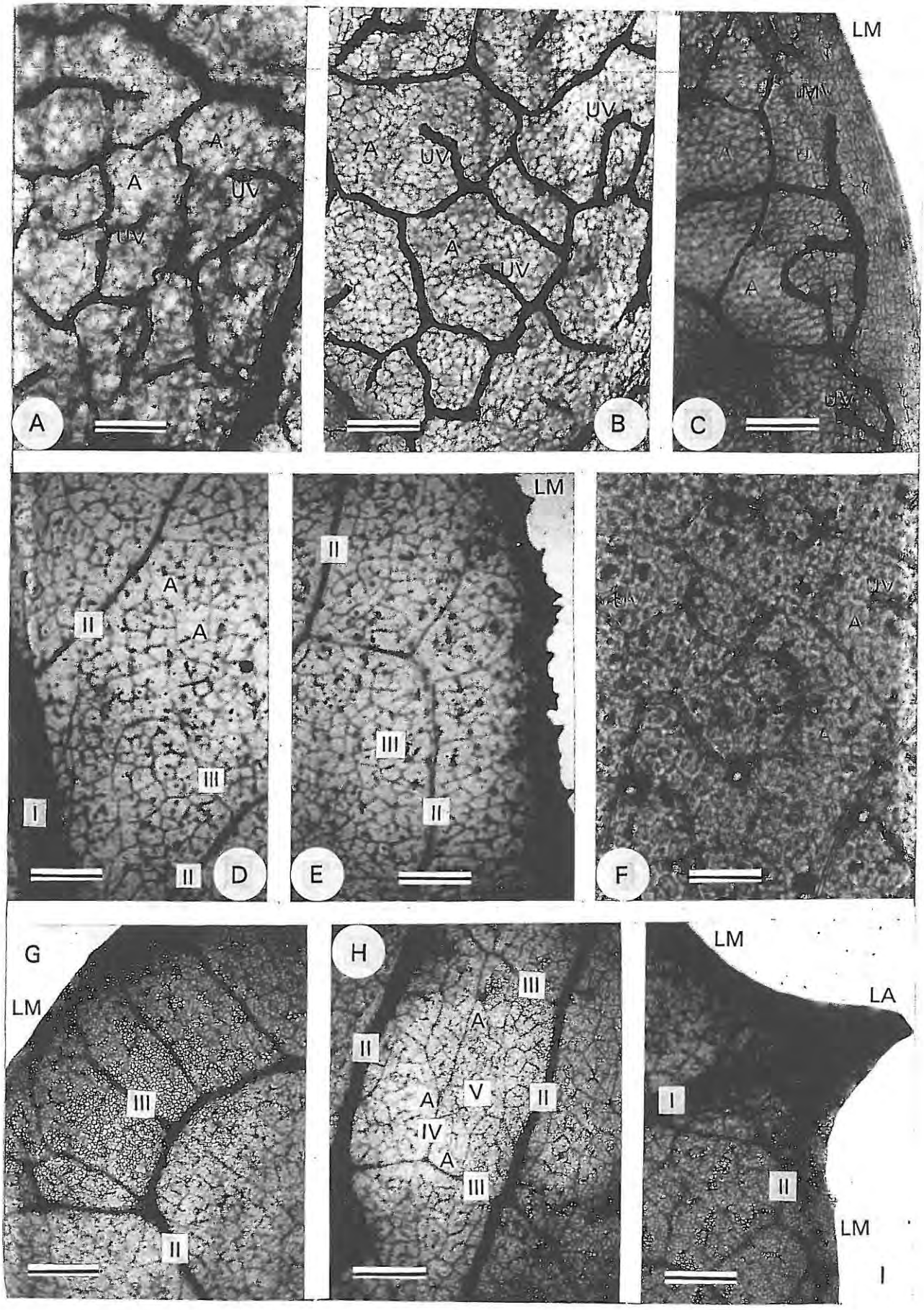
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Figure 3.24 Asclepiadaceae: Aspects of cleared leaves of *Secamone*, *Tenaris* and *Sisyranthus*

A *Secamone alpinii* lamina showing consecutive secondary veins with excurrent, transversely ramified tertiary veins and quaternary and fifth order veins, imperfect areoles with simple to twice-branched ultimate veinlets, B *S. alpinii* leaf margin showing brochidodromous secondary veins with excurrent tertiary, quaternary and fifth order veins, imperfect areoles and incomplete marginal venation of simple to twice-branched ultimate veinlets, C *S. alpinii* lamina showing simple to once-branched ultimate veinlets, D *Tenaris rubella* lamina with raised midvein and incomplete marginal venation of simple to once-branched ultimate veinlets, E *T. rubella* leaf apex showing blind ending primary vein with brochidodromous secondary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, F *Sisyranthus compactus* leaf apex showing central primary vein with excurrent secondary veins and incomplete marginal venation of simple to once-branched ultimate veinlets, G *S. compactus* lamina showing raised midvein with excurrent secondary and tertiary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm for A, B, D, E, F and G, and 0,05mm for C

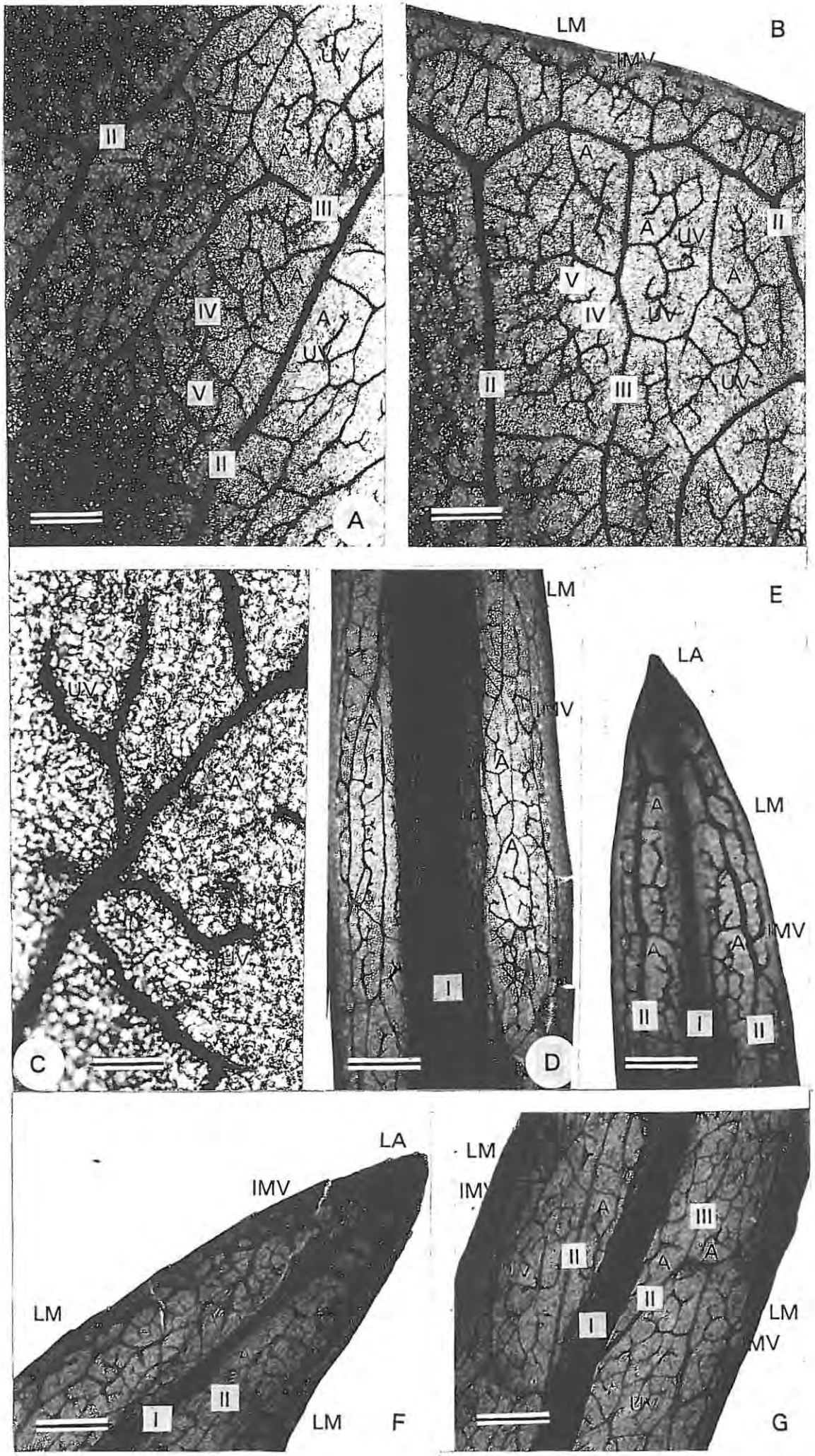
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Figure 3.25 Asclepiadaceae: Aspects of cleared leaves of *Pachycarpus*

A *Pachycarpus natalensis* lamina showing raised midvein with consecutive excurrent secondary veins, B *P. natalensis* lamina showing consecutive secondary veins with excurrent, simple percurrent tertiary veins, C *P. natalensis* leaf margin showing brochidodromous secondary veins, D *P. inconstans* leaf margin with brochidodromous secondary vein with excurrent, simple percurrent tertiary veins and quaternary veins, imperfect areoles and incomplete marginal venation of simple to twice-branched ultimate veinlets, E *P. inconstans* leaf apex showing blind ending primary vein with excurrent brochidodromous secondary veins, imperfect areoles and incomplete marginal venation of simple to twice-branched ultimate veinlets, F *P. grandiflorus* lamina showing raised midvein and excurrent consecutive secondary veins, G *P. rigidus* leaf margin showing imperfect areoles with simple ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A to G

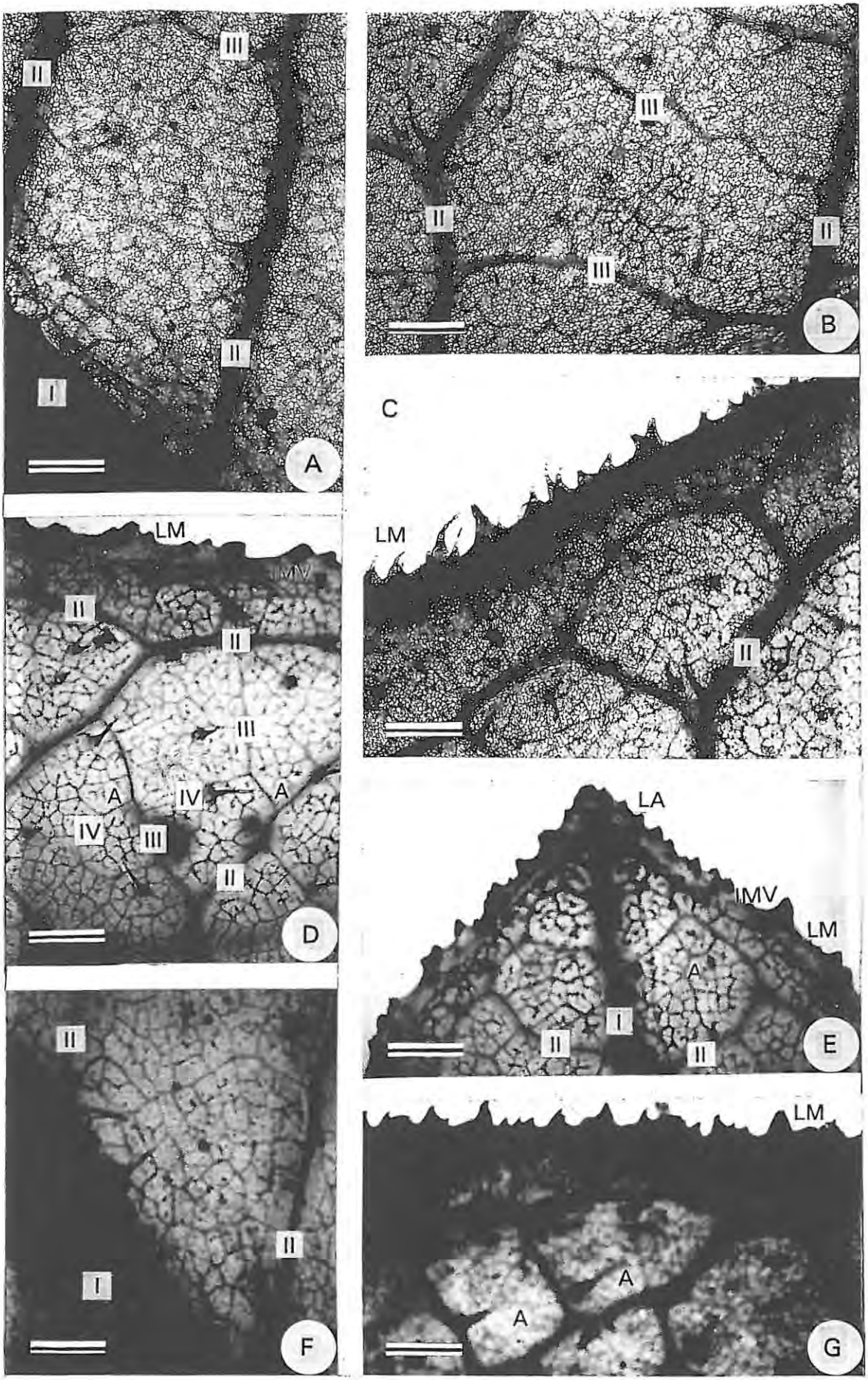
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Figure 3.26 Asclepiadaceae: Aspects of cleared leaves of *Pachycarpus*

A *Pachycarpus dealbatus* leaf margin with brochidodromous secondary vein and excurrent tertiary vein, B *P. dealbatus* lamina showing raised pubescent midrib with excurrent consecutive secondary veins and tertiary and quaternary veins, C *P. reflectens* leaf margin with brochidodromous secondary vein and excurrent tertiary veins, D *P. reflectens* lamina showing consecutive secondary and excurrent simple percurrent tertiary veins and quaternary veins, E *P. vexillaris* leaf margin showing trichomes, F *P. vexillaris* pubescent lamina showing consecutive secondary veins with excurrent tertiary and quaternary veins

(I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LM = leaf margin)

Bar represents 0,25mm for A to F

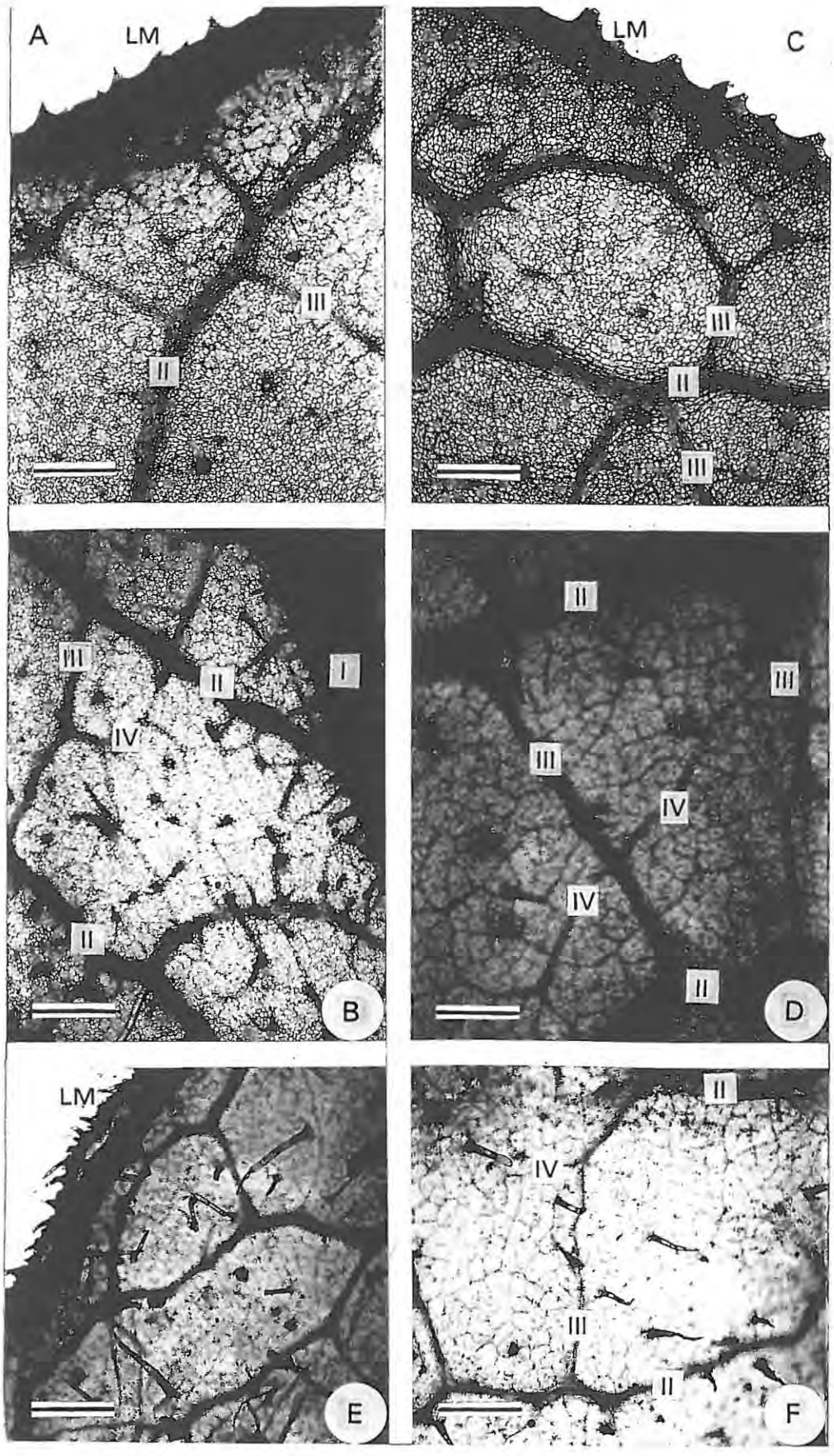
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Figure 3.27 Asclepiadaceae: Aspects of cleared leaves of *Schizoglossum* and *Pentarrhinum*

A *Schizoglossum cordifolium* leaf apex showing trichomes and blind ending primary vein with excurrent secondary veins, B *S. cordifolium* lamina showing pubescent midrib with excurrent secondary and tertiary veins, imperfect areoles with simple to once-branched ultimate veinlets, C *S. cordifolium* pubescent lamina showing secondary vein with excurrent tertiary vein, imperfect areoles with simple to once-branched ultimate veinlets, D *S. atropurpureum* subsp. *tridentatum* pubescent leaf margin showing brochidodromous secondary veins with excurrent tertiary vein, incomplete marginal venation of simple to once-branched ultimate veinlets, E *S. hamatum* lamina with consecutive secondary veins and excurrent tertiary and quaternary veins, F *S. aschersonianum* leaf apex showing central blind ending primary vein with excurrent secondary veins, incomplete marginal venation of simple to once-branched ultimate veinlets, G *Pentarrhinum insipidum* leaf margin showing brochidodromous secondary vein with excurrent tertiary and quaternary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, H *P. insipidum* leaf apex showing blind ending primary vein with excurrent brochidodromous secondary veins and tertiary vein, incomplete marginal venation of simple to once-branched ultimate veinlets, I *P. insipidum* lamina showing imperfect areoles with simple to once-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A to H, and 0,05mm for I

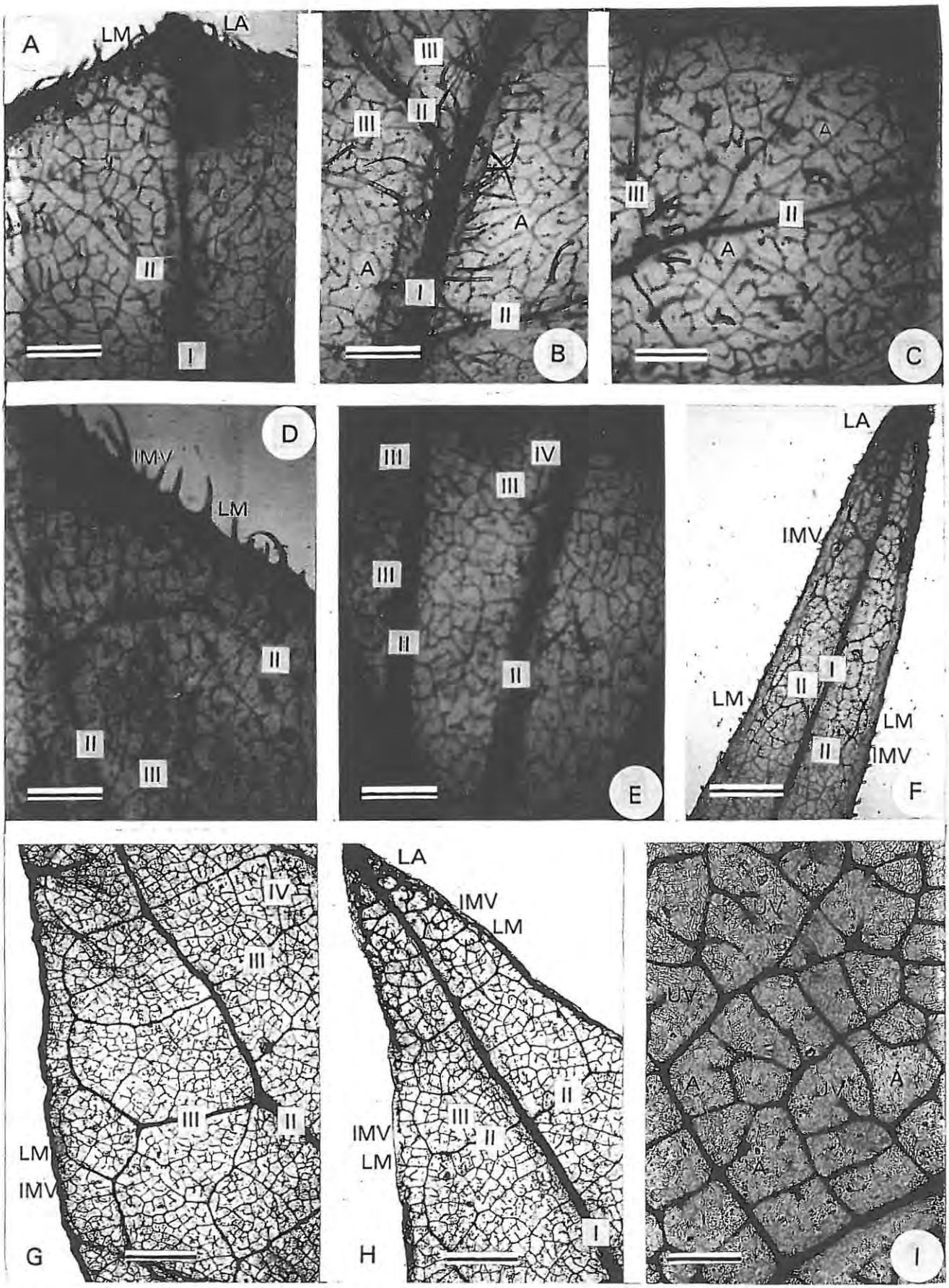
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Figure 3.28 Asclepiadaceae: Aspects of cleared leaves of *Riocreuxia*, *Aspidoglossum*, *Astephanus* and *Fockea*

A *Riocreuxia torulosa* pubescent leaf apex showing blind ending primary vein with excurrent secondary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, B *R. torulosa* leaf margin showing trichomes, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, C *R. flanaganii* lamina showing imperfect areoles with simple ultimate veinlets, D *Aspidoglossum biflorum* lamina showing central primary vein with excurrent eucamptodromous secondary and tertiary veins, E *A. ovalifolium* pubescent lamina with consecutive eucamptodromous secondary veins, F *A. heterophyllum* pubescent lamina showing consecutive eucamptodromous secondary veins, G *A. biflorum* lamina showing central primary vein with excurrent eucamptodromous secondary veins, and tertiary and quaternary veins, incompletely closed areoles with simple to once-branched ultimate veinlets, H *Astephanus triflorus* lamina showing central primary vein with excurrent secondary veins, I *Fockea cylindrica* lamina showing central primary vein with excurrent brochidodromous secondary veins and tertiary veins, incompletely closed areoles with once to thrice-branched ultimate veinlets, J *F. cylindrica* lamina showing incompletely closed areole with thrice-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A, D, E, F, G, H and I, and 0,05mm for B, C and J

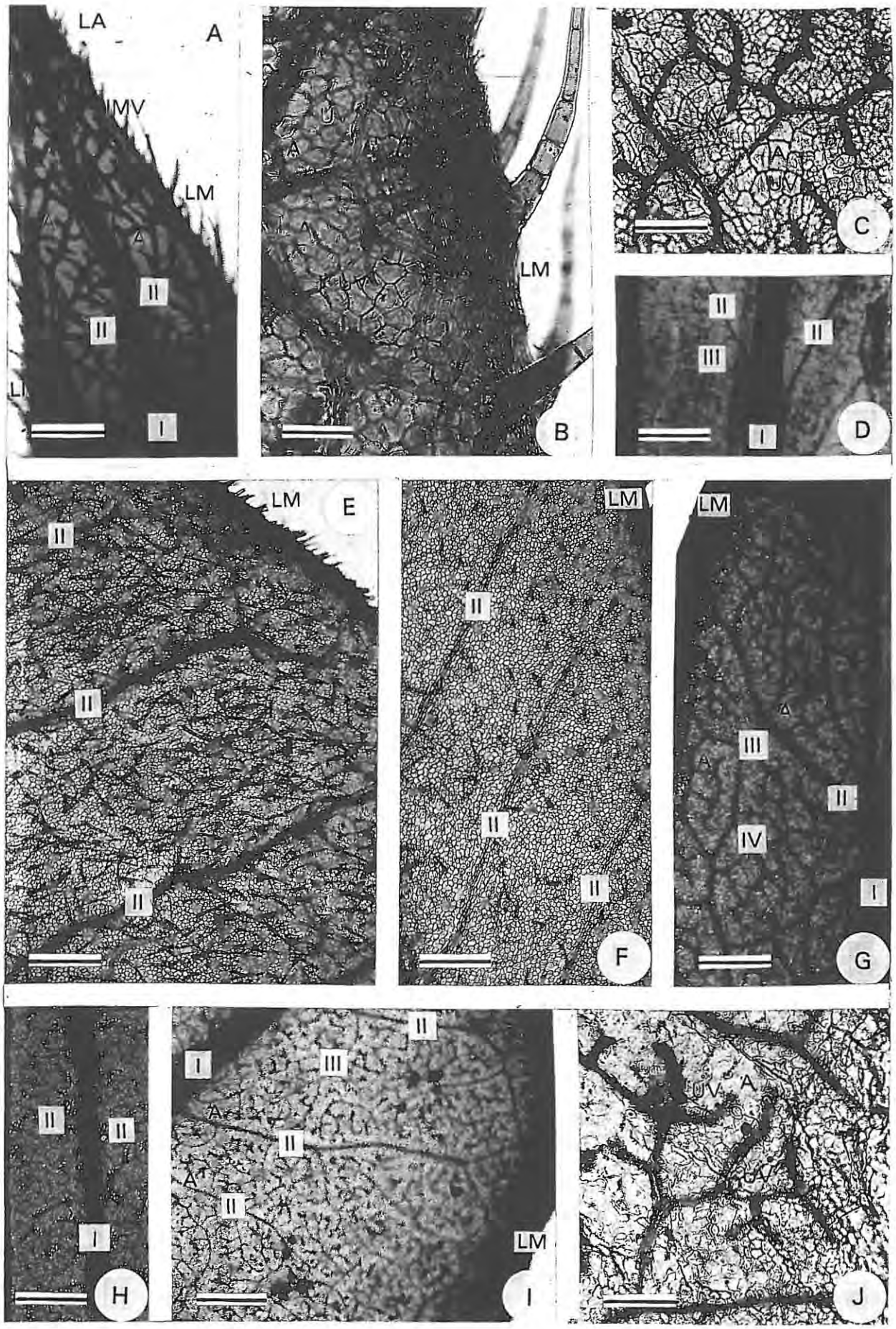
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Figure 3.29 Asclepiadaceae: Aspects of cleared leaves of *Tylophora*

A *Tylophora lycioides* leaf apex showing blind ending primary vein with excurrent secondary, tertiary and quaternary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, B *T. lycioides* lamina showing central primary vein with excurrent secondary and tertiary veins, imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, C *T. lycioides* leaf margin showing incomplete marginal venation of simple to once-branched ultimate veinlets, D *T. lycioides* lamina with imperfect areoles and simple ultimate veinlets, E *T. umbellata* lamina showing once-branched ultimate veinlet in incompletely closed areole, F *T. umbellata* lamina showing central primary vein with excurrent secondary and tertiary veins, incompletely closed areoles with once and twice-branched ultimate veinlets, G *T. umbellata* leaf margin with brochidodromous secondary vein and excurrent tertiary and quaternary veins, incompletely closed areoles and incomplete marginal venation of once to twice-branched ultimate veinlets, H *T. umbellata* leaf apex showing blind ending primary vein and excurrent secondary vein, incompletely closed areoles and incomplete marginal venation of once to twice-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets)

Bar represents 0,25mm for A, B, F, G and H, and 0,05mm for C, D and E

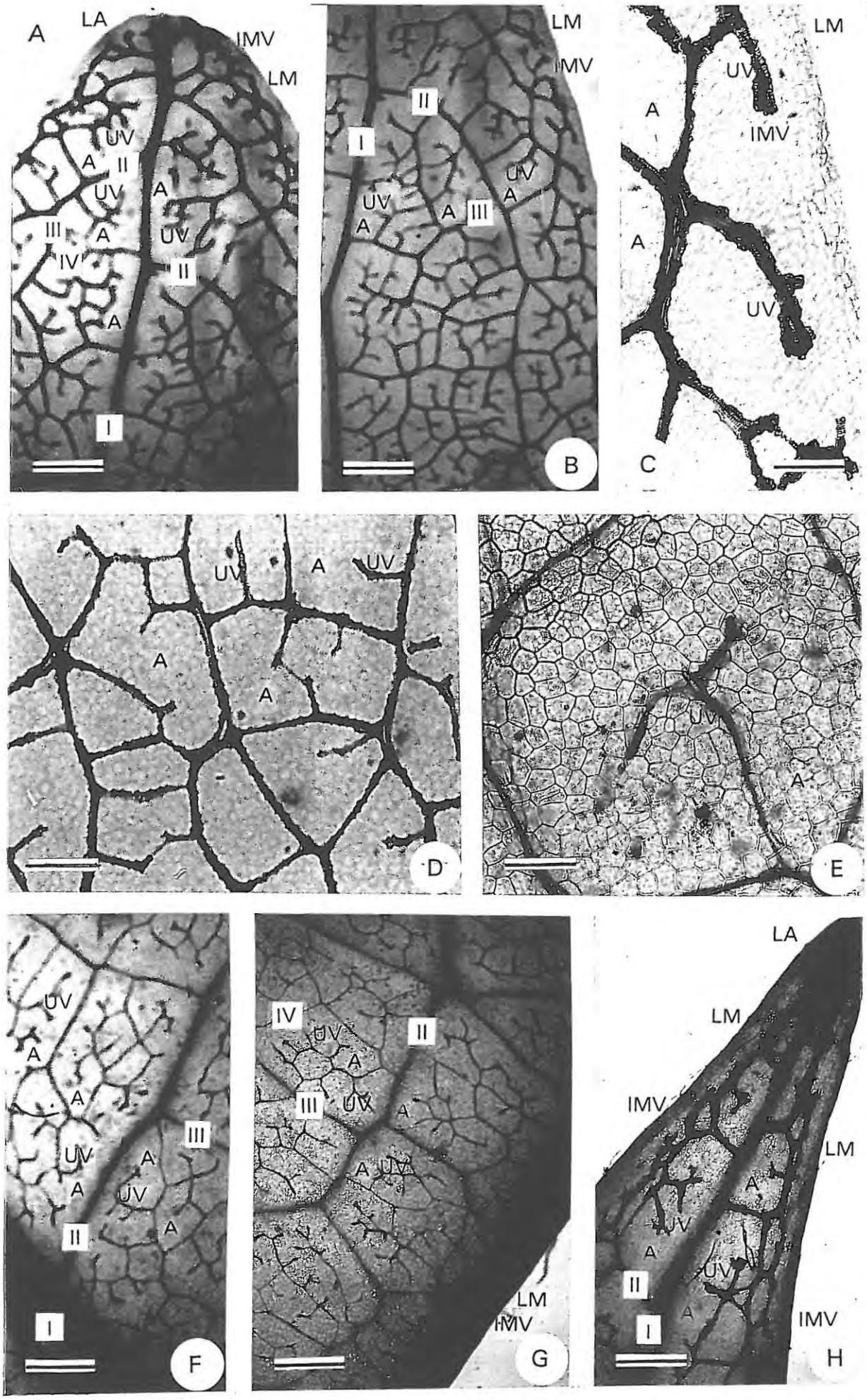
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Figure 3.30 Asclepiadaceae: Aspects of cleared leaves of *Microloma*, *Oncinema* and *Marsdenia*

A *Microloma sagittatum* leaf showing central primary vein with excurrent secondary veins, B *M. tenuifolium* leaf margin showing incomplete marginal venation of simple ultimate veinlets, C *M. massonii* lamina showing central primary vein with excurrent secondary veins, D *Oncinema lineare* lamina showing imperfect areoles and incomplete marginal venation of simple to once-branched ultimate veinlets, E *O. lineare* leaf apex showing blind ending primary vein with excurrent secondary vein, incomplete marginal venation of simple to once-branched ultimate veinlets, F *Marsdenia floribunda* lamina showing secondary vein with excurrent tertiary, quaternary and fifth order veins, incompletely closed areoles and incomplete marginal venation of simple to twice-branched ultimate veinlets, G *M. floribunda* lamina showing incompletely closed areoles with simple and once-branched ultimate veinlets, H *M. floribunda* leaf margin with incomplete marginal venation of simple and once-branched ultimate veinlets

(A = areole; I = midvein; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; IV = fourth order vein; LA = leaf apex; LM = leaf margin; UV = ultimate veinlets; V = fifth order vein)

Bar represents 0,25mm for A, C, E and F, and 0,05mm for B, D, G and H

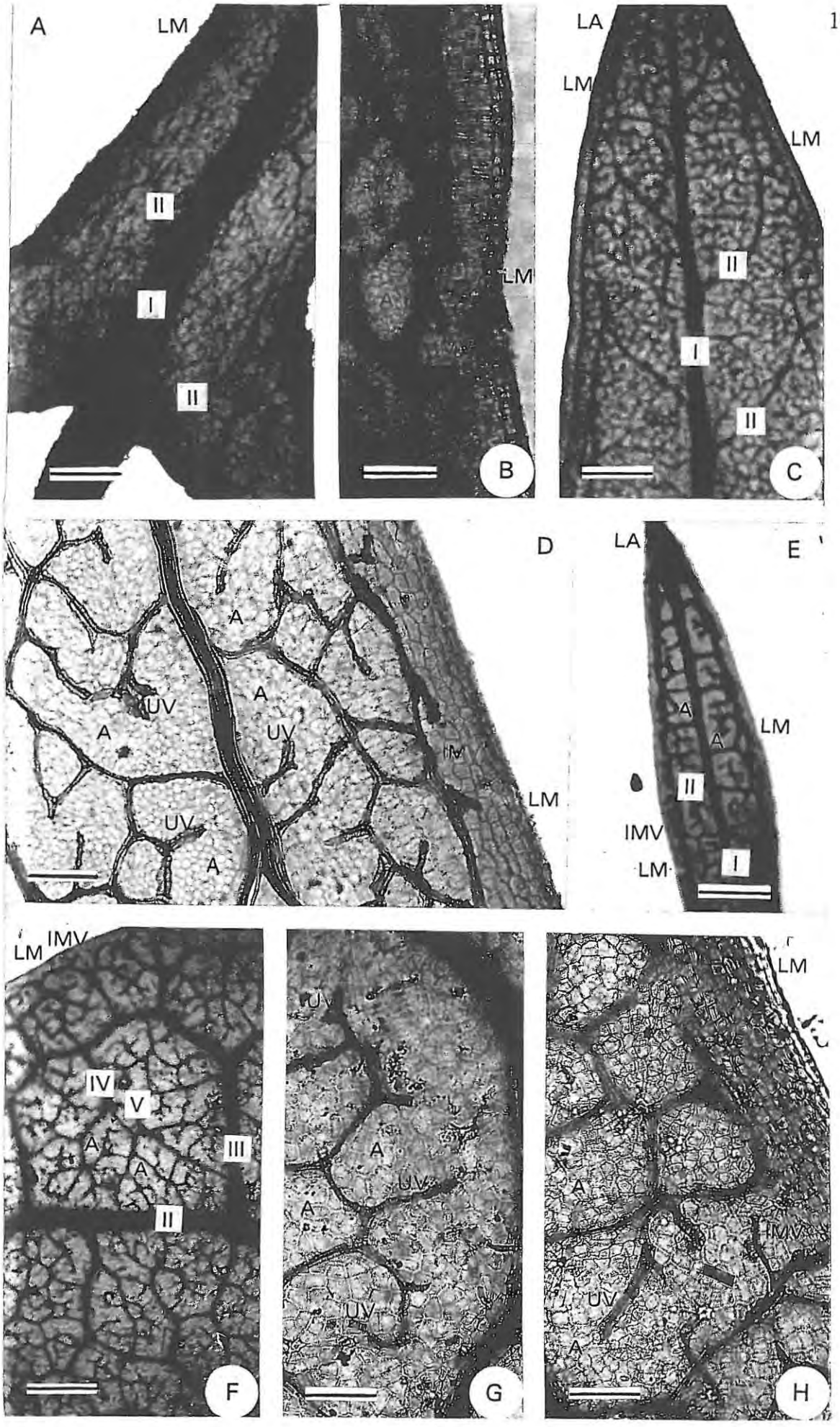
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Table 3.7 Summary of the angles of divergence of secondary veins and tertiary vein organisation in leaves of the Asclepiadaceae

SPECIES	SECONDARY VEIN DIVERGENCE	TERTIARY VEIN PATTERN
Asclepias dregeana (Fig. 3.15 A to D)	Moderately to widely acute	Transversely ramified
Asclepias crinita (Fig. 3.15 E)	Moderately acute	Simple percurrent to transversely ramified
Asclepias crispa (Fig. 3.15 F)	Widely acute	Transversely ramified
Asclepias navicularis (Fig. 3.15 G)	Perpendicular	Transversely ramified
Asclepias aurea (Fig. 3.15 H, I & J)	Widely acute	Transversely ramified
Asclepias meyeriana (Fig. 3.15 K)	Perpendicular	Transversely ramified
Asclepias gibba (Fig. 3.15 L)	Narrowly acute to perpendicular	Transversely ramified
Asclepias expansa (Fig. 3.15 M)	Widely acute	Transversely ramified
Asclepias fructicosa (Fig. 3.16 A & B)	Widely acute	Simple percurrent to transversely ramified
Asclepias erinens (Fig. 3.16 C)	Moderately acute	Transversely ramified
Asclepias burchelli (Fig. 3.16 D)	Widely acute	Transversely ramified
Asclepias physocarpa (Fig. 3.16 E & F)	Widely acute	Simple percurrent to transversely ramified
Asclepias stellifera	Moderately acute	Transversely ramified
Brachystelma decipiens (Fig. 3.17 A & B)	Narrowly acute	Transversely ramified
Brachystelma huttonii (Fig. 3.17 C)	Moderately acute	Transversely ramified
Brachystelma elongatum (Fig. 3.17 D & E)	Narrowly acute	Simple percurrent to transversely ramified
Brachystelma cathcartense (Fig. 3.17 F & G)	Narrowly acute	Transversely ramified
Brachystelma meyeranum (Fig. 3.18 A to D)	Perpendicular	Simple percurrent to transversely ramified
Brachystelma schizoglossoides (Fig. 3.18 E, F & G)	Narrowly acute	Transversely ramified
Xysmalobium prunelloides (Fig. 3.19 A, B & C)	Widely acute	Random reticulate
Xysmalobium confusum (Fig. 3.19 D & E)	Widely acute	Random reticulate
Xysmalobium orbiculare (Fig. 3.19 F)	Moderately acute	Random reticulate
Xysmalobium pearsonii (Fig. 3.20 A & B)	Moderately acute	Transversely ramified
Xysmalobium involucratum (Fig. 3.20 C)	Narrowly acute	Transversely ramified
Xysmalobium undulatum (Fig. 3.20 D & E)	Widely acute	Simple percurrent
Xysmalobium zeyheri	Narrowly acute	Transversely ramified
Telsoma africana (Fig. 3.20 F & G)	Perpendicular	Transversely ramified

Ceropegia distincta subsp. *haygarthii* (Fig. 3.21 A to D)	Moderately acute	Transversely ramified
Ceropegia radicans subsp. *radicans* (Fig. 3.21 E, F & G)	Widely acute	Transversely ramified
Ceropegia radicans subsp. *smithii* (Fig. 3.22 A, B & C)	Widely acute	Transversely ramified
Ceropegia linearis (Fig. 3.22 E)	Widely acute	Transversely ramified
Ceropegia carnosa (Fig. 3.22 F & G)	Perpendicular	Transversely ramified
Ceropegia dubia	Widely acute	Transversely ramified
Woodia marginata (Fig. 3.23 D, E & F)	Moderately to narrowly acute	Transversely ramified
Woodia mucronata (Fig. 3.23 G, H & I)	Moderately acute	Simple percurrent
Secamone alpinii (Fig. 3.24 A, B & C)	Moderately acute	Transversely ramified
Tenaris rubella (Fig. 3.24 D & E)	Narrowly to widely acute	Transversely ramified
Sisyranthus compactus (Fig. 3.24 F & G)	Narrowly acute	Transversely ramified
Sisyranthus imberbis	Moderately acute	Transversely ramified
Pachycarpus natalensis (Fig. 3.25 A, B & C)	Widely acute	Simple percurrent
Pachycarpus inconstans (Fig. 3.25 D & E)	Widely acute	Simple percurrent
Pachycarpus grandiflorus (Fig. 3.25 F)	Moderately acute	Simple percurrent to transversely ramified
Pachycarpus rigidus (Fig. 3.25 G)	Widely acute	-
Pachycarpus dealbatus (Fig. 3.26 A & B)	Moderately acute	Simple percurrent to transversely ramified
Pachycarpus reflectens (Fig. 3.26 C & D)	Widely acute	Simple percurrent
Pachycarpus vexillaris (Fig. 3.26 E & F)	Moderately acute	Simple percurrent to transversely ramified
Pachycarpus linearis	Narrowly to moderately acute	-
Schizoglossum cordifolium (Fig. 3.27 A to C)	Moderately acute	Transversely ramified
Schizoglossum atropurpureum subsp. *tridentatum* (Fig. 3.27 D)	Moderately acute	Transversely ramified
Schizoglossum hamatum (Fig. 3.27 E)	Narrowly acute	Transversely ramified
Schizoglossum aschersonianum (Fig. 3.27 F)	Narrowly to moderately acute	Transversely ramified
Schizoglossum bidens subsp. *bidens*	Moderately acute	Transversely ramified
Pentarrhinum insipidum (Fig. 3.27 G, H & I)	Moderately to widely acute	Simple percurrent to transversely ramified
Riocreuxia torulosa (Fig. 3.28 A & B)	Moderately acute	Transversely ramified
Riocreuxia flanaganii (Fig. 3.28 C)	Moderately acute	Transversely ramified
Aspidoglossum biflorum (Fig. 3.28 D & G)	Narrowly acute	-
Aspidoglossum ovalifolium (Fig. 3.28 E)	Moderately acute	Transversely ramified
Aspidoglossum heterophyllum (Fig. 3.28 F)	Narrowly acute	Transversely ramified

Aspidoglossum carinatum	Perpendicular	Transversely ramified
Aspidoglossum virgatum	Widely acute	Transversely ramified
Astephanus triforus (Fig. 3.28 H)	Moderately acute	Transversely ramified
Astephanus marginatus	Moderately acute	Transversely ramified
Fockea cylindrica (Fig. 3.28 I & J)	Widely acute	Transversely ramified
Fockea multiflora	Widely acute	Transversely ramified
Tylophora lycioides (Fig. 3.28 A to D)	Widely acute to obtuse	Transversely ramified
Tylophora umbellata (Fig. 3.29 E to H)	Moderately to widely acute	Transversely ramified
Microloma sagittatum (Fig. 3.30 A)	Moderately to widely acute, lower more acute than upper	Transversely ramified
Microloma massonii (Fig. 3.30 C)	Moderately acute	Transversely ramified
Microloma incanum	Widely acute	Transversely ramified
Oncinema lineare (Fig. 3.30 E)	Widely acute to perpendicular	Transversely ramified
Marsdenia floribunda (Fig. 3.30 F, G & H)	Moderately acute	Transversely ramified
Parapodium crispum	Moderately acute	-
Fanninia caloglossa	Moderately acute	-
Cynanchum africanum	Moderately acute	Transversely ramified

Table 3.8 Summary of the highest vein order counted and ultimate veinlet condition in leaves of the Asclepiadaceae

SPECIES	HIGHEST VEIN ORDER	ULTIMATE VEINLET CONDITION
Asclepias dregeana (Fig. 3.15 A to D)	Eighth	Simple to once-branched
Asclepias crinita (Fig. 3.15 E)	Seventh	Simple to once-branched
Asclepias crispa (Fig. 3.15 F)	Seventh	Simple to once-branched
Asclepias navicularis (Fig. 3.15 G)	Fourth	Simple to once-branched
Asclepias aurea (Fig. 3.15 H, I & J)	Sixth	Simple to once-branched
Asclepias meyeriana (Fig. 3.15 K)	Fifth	Simple to once-branched
Asclepias gibba (Fig. 3.15 L)	Fifth	Simple
Asclepias expansa (Fig. 3.15 M)	Sixth	Simple to once-branched
Asclepias fructicosa (Fig. 3.16 A & B)	Seventh	Simple to once-branched
Asclepias erinens (Fig. 3.16 C)	Sixth	Simple
Asclepias burchelli (Fig. 3.16 D)	Sixth	Simple to once-branched
Asclepias physocarpa (Fig. 3.16 E & F)	Seventh	Simple to once-branched
Asclepias stellifera	Sixth	Simple to once-branched
Brachystelma decipiens (Fig. 3.17 A & B)	Sixth	Simple to once-branched
Brachystelma huttonii (Fig. 3.17 C)	Seventh	Simple
Brachystelma elongatum (Fig. 3.17 D & E)	Seventh	Simple to once-branched
Brachystelma cathcartense Fig. 3.17 F & G)	Eighth	Simple to once-branched
Brachystelma meyeranum Fig. 3.18 A to D)	Sixth	Simple to once-branched
Brachystelma schizoglossoides (Fig. 3.18 E, F & G)	Seventh	Simple to once-branched
Xysmalobium prunelloides (Fig. 3.19 A, B & C)	Seventh	Simple to once-branched
Xysmalobium confusum (Fig. 3.19 D & E)	Eighth	Simple to once-branched
Xysmalobium orbiculare (Fig. 3.19 F)	Eighth	Simple to once-branched
Xysmalobium pearsonii (Fig. 3.20 A & B)	Seventh	Simple to once-branched
Xysmalobium involucratum (Fig. 3.20 C)	Sixth	Once-branched
Xysmalobium undulatum (Fig. 3.20 D & E)	Eighth	Simple to once-branched
Xysmalobium zeyheri	Sixth	Once-branched
Telsoma africana (Fig. 3.20 F & G)	Seventh	Simple, once and twice-branched
Ceropegia distincta subsp. *haygarthii* (Fig. 3.21 A to D)	Sixth	Simple to once-branched
Ceropegia radicans subsp. *radicans* (Fig. 3.21 E, F & G)	Sixth	Simple to once-branched
Ceropegia radicans subsp. *smithii* (Fig. 3.22 A, B & C)	Sixth	Simple to once-branched
Ceropegia linearis (Fig. 3.22 E)	Sixth	Simple to once-branched

Ceropegia carnosa (Fig. 3.22 F & G)	Sixth	Simple to once-branched
Ceropegia dubia	Sixth	Simple to once-branched
Woodia marginata (Fig. 3.23 D, E & F)	Seventh	Simple to once-branched
Woodia mucronata (Fig. 3.23 G, H & I)	Seventh	Simple to once-branched
Secamone alpinii (Fig. 3.24 A, B & C)	Sixth	Simple, once and twice-branched
Tenaris rubella (Fig. 3.24 D & E)	Sixth	Simple to once-branched
Sisyranthus compactus (Fig. 3.24 F & G)	Sixth	Simple to once-branched
Sisyranthus imberbis	Sixth	Simple to once-branched
Pachycarpus natalensis (Fig. 3.25 A, B & C)	Seventh	Simple, once and twice-branched
Pachycarpus inconstans (Fig. 3.25 D & E)	Seventh	Simple, once and twice-branched
Pachycarpus grandiflorus (Fig. 3.25 F)	Seventh	Simple, once and twice-branched
Pachycarpus rigidus (Fig. 3.25 G)	Seventh	Simple, once and twice-branched
Pachycarpus dealbatus (Fig. 3.26 A & B)	Seventh	Simple, once and twice-branched
Pachycarpus reflectens (Fig. 3.26 C & D)	Eighth	Simple, once and twice-branched
Pachycarpus vexillaris (Fig. 3.26 E & F)	Seventh	Simple, once and twice-branched
Pachycarpus linearis	Seventh	Simple, once and twice-branched
Schizoglossum cordifolium (Fig. 3.27 A to C)	Eighth	Simple to once-branched
Schizoglossum atropurpureum subsp. *tridentatum* (Fig. 3.27 D)	Seventh	Simple to once-branched
Schizoglossum hamatum (Fig. 3.27 E)	Seventh	Simple to once-branched
Schizoglossum aschersonianum (Fig. 3.27 F)	Sixth	Simple to once-branched
Schizoglossum bidens subsp. *bidens*	Sixth	Simple to once-branched
Pentarrhinum insipidum (Fig. 3.27 G, H & I)	Eighth	Simple to once-branched
Riocreuxia torulosa (Fig. 3.28 A & B)	Sixth	Simple to once-branched
Riocreuxia flanaganii (Fig. 3.28 C)	Sixth	Simple to once-branched
Aspidoglossum biflorum (Fig. 3.28 D & G)	Sixth	Simple to once-branched
Aspidoglossum ovalifolium (Fig. 3.28 E)	Sixth	Simple to once-branched
Aspidoglossum heterophyllum (Fig. 3.28 F)	Sixth	Simple to once-branched
Aspidoglossum carinatum	Sixth	Simple to once-branched
Aspidoglossum virgatum	Sixth	Simple to once-branched
Astephanus triforus (Fig. 3.28 H)	Seventh	Once to twice-branched
Astephanus marginatus	Seventh	Once to twice-branched
Fockea cylindrica (Fig. 3.28 I & J)	Seventh	Once, twice and thrice-branched
Fockea multiflora	Sixth	Once to twice-branched
Tylophora lycioides (Fig. 3.29 A to D)	Seventh	Simple to once-branched
Tylophora umbellata (Fig. 3.29 E to H)	Eighth	Once to twice-branched

Microloma sagittatum (Fig. 3.30 A)	Seventh	-
Microloma massonii (Fig. 3.30 C)	Seventh	Simple to once-branched
Microloma incanum	Seventh	-
Oncinema lineare (Fig. 3.30 E)	Sixth	Simple to once-branched
Marsdenia floribunda (Fig. 3.30 F, G & H)	Seventh	Simple, once and twice-branched
Parapodium crispum	Sixth	-
Fanninia caloglossa	Sixth	Simple
Cynanchum africanum	Seventh	Simple to once-branched

3.3 Discussion

3.3.1 Character consistency in families

Gross vegetative plant features are notoriously plastic, and have been documented as varying with environmental conditions (Carlquist 1991). Leaf size, abundance and pubescence especially are highly changeable amongst plants of different areas. However, leaf architecture, being strictly conservative (Gamalei 1989, Hickey 1973, Merrill 1978), would not be expected to change with ecotypes of environmental extremes. The surface area covered by venation may vary with leaf size, yet the actual pattern of venation will remain the same (Merrill 1978). Arroyo (1986) noted the usefulness of the conservative nature of leaf venation in the taxonomy of the Tecophilaeaceae, where pattern was found to be diagnostic at family level. Conover (1991) employed the concept when studying the Liliiflorae to separate species with reticulate venation for taxonomic grouping. Leaf architecture thus provides a rare foliar taxonomic feature, useful in the field identification and systematics of both fossilized and living specimens (Dickison & Weitzman 1996). In addition, there is a vast literature on fossil leaf venation.

Taxonomically, character consistency in families is an interesting concept (Dickison & Weitzman 1996). It was expected that leaf architecture would be consistent within a particular family, again in view of the conservative nature of the feature (Gamalei 1989, Hickey 1971 & 1973). In the current study, this proved true for two groups, viz. the Ranunculaceae and Apocynaceae.

Ranunculaceae

In 1960 Foster and Arnott published their work on *Kingdonia uniflora*, a herbaceous perennial endemic to China. This paper is of particular interest here due to two venation characteristics mentioned.

Firstly, *K. uniflora* leaf margins are described as being serrated, with each marginal tooth containing a single ultimate veinlet. In the current study, the presence of marginal serrations with a single ultimate veinlet is a noted characteristic of the Ranunculaceae, especially in *Knowltonia* species (Fig.s 3.7 B, C & F, 3.8 A & C and 3.9 B-D).

Secondly, Foster and Arnott (1960) describe the lamina venation of *K. uniflora* as showing the distinctly primitive feature of being openly dichotomously veined. Anastomoses are rare, with a maximum of one per leaf noted. In a study by Mortlock (1952), the aquatic *Ranunculus fluitans* was also reported to have dichotomously dividing veins, with no connecting laminar webbing inbetween. The aquatic *R. trichophyllus* subsp. *trichophyllus*, seen here, did show dichotomously branching venation and webbing resulting in a much divided leaf, with no anastomosing veins. Whilst the venation of even the most delicate of the terrestrial Ranunculaceae from the eastern Cape, viz. *Ranunculus*, tends to be sparse and fragile, it does form a closed though widely spaced reticulum of anastomosing veins (Fig. 3.1 C). The presence of a reticulum indicates advancement from the open dichotomous type of venation (Ramji 1967), and has been suggested to increase efficiency of transport within the leaf (McCauley & Evert 1988 a & b).

The occurrence of hydathodes in *Ranunculus* species has been known for over a century (Schenck 1886-7 in Mortlock 1952). There is, however, a paucity of published anatomical data and of group surveys for the cosmopolitan genus *Ranunculus*. Hydathode structure is well described for species *R. reptans* (De Kock & Rutherford 1971) and *R. fluitans* (Mortlock 1952), both European species. The results presented here comprise the first documented account of hydathodes in southern African species of *Ranunculus*.

It is interesting to note that of all the Ranunculacean taxa examined here only the genus *Ranunculus* exhibits fully developed cup-shaped hydathodes (Fig.s 3.4 B, C & E and 3.5 F). These structures can be seen at the leaf apices and apices of marginal serrations. In other Ranunculacean taxa, such as *Clematis* (Fig. 3.5 B & C), *Thalictrum* (Fig. 3.6 C & E) and *Knowltonia* (Fig.s 3.7 B, C & F, 3.8 A & C, and 3.9 B, C & D), the primary and secondary veins terminate blindly at the apices of leaves and marginal serrations. Occasionally veins may anastomose with these lower order veins, but the structure does not form a typical cup-shaped hydathode. This can be seen in *Clematis brachiata* (Fig. 3.4 F).

The presence of hydathodes in marginal teeth is a characteristic ancestral character (Merrill 1978). The cup-shaped hydathodes of *Ranunculus*, seen here, would therefore represent a more ancient feature. Simple vein endings in marginal teeth, such as those of *Clematis*, *Thalictrum* and *Knowltonia*, could represent a more advanced stage through the loss of cup-shaped hydathodes.

A possible reason for the retention of hydathodes in *Ranunculus* and not other taxa could be the disparity in their respective habitats. *Ranunculus* prefers a moist to almost aquatic environment, whilst *Clematis*, *Thalictrum* and *Knowltonia* occur in drier grassland regions (Appendix III) and are less likely to guttate.

Apocynaceae and Asclepiadaceae

All examples of leaf architecture in the Apocynaceae showed a pinnate, camptodromous, brochidodromous arrangement (Hickey 1973). This is in agreement with reports published on lthe venation of the Apocynaceae by Sharma *et al.* (1970), Chandra *et al.* (1969, 1972), and Mohan and Inamdar (1982).There were no exceptions (Fig. 3.14 A to E). This is not surprising as the family is homogenous in terms of habitat and habit.

The Asclepiadaceae, encompassing taxa more diverse than those of the Apocynaceae, showed less uniformity in terms of leaf architecture. All taxa examined had a pinnate, camptodromous arrangement, yet both brochidodromous and eucamptodromous patterns of secondary venation were noted (Fig.s 3.14 F and 3.28 E, F & G). Although considering different taxa of Indian origin, the results for the family presented here agree with those of Ramakrishna and Govindappa (1983) and Mohan and Inamdar (1984). Eucamptodromous species all occurred in the genus *Aspidoglossum*, in which apically tapering secondary veins do not form the arches so characteristic of the brochidodromous pattern (Fig. 3.28 E, F & G). It is unfortunate that only species occurring in the eastern Cape were examined, as it would have been interesting to see the consistency of this feature in the genus *Aspidoglossum* as a whole, and possibly within other genera of the Asclepiadaceae in the rest of southern Africa.

Hickey (1973) noted that some families contained several leaf architecture patterns. One explanation proposed was that the family was artificial or paraphyletic. Another possibility was that the deviant genus had adaptated to an environment other than that to which the family as a whole had progressed, resulting in an architectural shift (Hickey 1973). Whilst a third possibility has received more attention in the literature in recent years, these former possibilities should not be ruled out until carefully considered.

A third explanation for the occurrence of more than one architectural pattern within a family has

since been proposed in the literature (Lorence 1985, Wilkinson 1989, Hickey & Taylor 1991, Todzia & Keating 1991, Nelson & Dengler 1997). It is a seemingly simple explanation where one architectural pattern is proposed to have evolved from another. In order to pronounce one architecture ancestral to another, ontogenic studies and extensive architectural surveys of extinct and extant genera of a family would be required (Wilkinson 1989, Todzia & Keating 1991). Extremely few studies of this nature and magnitude are available for discussion in the literature. Consequently, there is little understanding of what would be considered ancestral or advanced (Lorence 1985, Todzia & Keating 1991). Furthermore, even amongst extant angiosperm genera there is little in the way of detailed familial surveys of leaf architecture.

Todzia and Keating (1991) published a very interesting analysis of leaf architecture within the Chloranthaceae. They included data from the fossil record. This is a very old family first appearing in the Early Cretaceous Period. They note the occurrence of both eucamptodromous and brochidodromous architectural pattern within the family, from Early Cretaceous to present times, and postulate the derivation of eucamptodromy from an ancestral brochidodromous pattern. McCauley and Evert (1988a) described the venation of *Solanum tuberosum* as being eucamptodromous, or intermediate between brochidodromous and eucamptodromous. Lorence (1985) noted brochidodromous, semicraspedodromous and craspedodromous patterns within the Monimiaceae (Laurales). From the literature, it is interesting to note how often brochidodromy and eucamptodromy coexist within taxonomic groupings.

The Asclepiadaceae are cosmopolitan, occurring in mesic to xeric environments (Heywood 1978, Thorne 1992). Worldwide, the family consists of about 250 genera and 2 000 species, encompassing growth forms from herbs to creepers to small trees (Heywood 1978, Thorne 1992). The taxonomy of the apocynate and asclepiad complex is still open to discussion and fine tuning (Chase *et al.* 1993, Sennblad & Bremer 1996, Endress & Bruyns 2000). Once the subfamilial and tribal relationships have been addressed, an interesting study would be the determination of leaf architecture progression as related to hierarchical position.

Taking current thinking into account, viz. that a progression of leaf architectural patterns may be seen within large, old, diverse families (Todzia & Keating 1991, Nelson & Dengler 1997), a problem with Hickey's (1973) classification system is noticed in this thesis. The system only

considers the final resultant pattern and does not take into account how the pattern arose, as ontogeny and phylogenetic hierarchy are ignored. This means that while similar patterns may result in adult leaves, these patterns could have arisen along entirely different routes, via different ontogeny and/or phylogeny. The whole developmental history is obscured (Carr *et al.* 1986). Definitive comments on the relatedness of families, based on leaf architecture, are therefore problematic, as the similarity could be a result of convergent evolution. This emphasises the necessity for studies involving leaf ontogeny, the examination of fossil leaves from the family and phylogenetic studies to determine the historic progression of the resultant leaf architecture within a family.

Both the Apocynaceae and Asclepiadaceae, with the exception of *Aspidoglossum*, show the same brochidodromous leaf architecture. With the recent move to place the two as subfamilies of the family Apocynaceae (Liede 1997, Chase *et al.* 1993, Struwe *et al.* 1994, Sennblad & Bremer 1996, Endress & Bruyns 2000), the results presented in this thesis are reassuring. Character sets for venation and leaf features cannot be taken as conclusive diagnostic data for families, especially in light of the previous discussion, as no ontogenetic or phylogenetic studies on leaf architecture have been undertaken. Furthermore, substantial overlap and plasticity occurs in the case of leaf features. It is, however, encouraging to find the same data set consistently within the majority of the family. As a wide variety of taxonomic and systematic methods have been employed in the decision to amalgamate the Asclepiadaceae and Apocynaceae, the results of the present research support the placement of the Asclepiadaceae as a subfamily of the Apocynaceae.

3.3.2 Minor vein distribution within areoles

Pray (1954) stated that "one of the most neglected aspects of foliar venation is the nature and structure of the freely-terminated vein endings". A number of authors, including Fisher have examined and compared a few examples from a scattering of families, but seldom all taxa of a family for an intrafamilial survey. The emphasis is usually on gathering new character variations, such as in the work of Melville (1976) and Hickey (1973).

Apparently, one of the most extensive studies of vein endings in dicots was done by Fischer in 1885 (as reported in Pray 1954). Fischer was particularly interested in the nature of phloem in vein endings, and established two categories to that effect. The first group he called "Hauptenden"

or principal endings. These were usually branched and always had phloem tissue present. This grouping would fit in with the primary, secondary and tertiary veins of Hickey's (1973) classification system. His second group called "Nebeneden" were secondary or minor endings, and were characterised as being short with a single tracheid and no phloem. These would correspond with quaternary and higher order vein endings.

Minor veins comprise the majority of leaf vasculature (Lersten 1990). Fisher (1985 & 1990) calculated higher order veins to total between 86% and 99% for various taxa (see Table 3.8). Russin and Evert (1984) mention an average of 96% for woody dicots in general.

Table 3.8 Summary of minor vein percentages of total foliar venation as determined by various authors

AUTHOR	SPECIES AND FAMILY	% TOTAL FOLIAR VENATION
Fisher 1985	*Coleus blumei* (Lamiaceae)	86%
Fisher & Evert 1982	*Amaranthus retroflexus* (Amaranthaceae)	95%
Fisher 1990	*Cananga odorata* (Annonaceae)	99%
Russin and Evert 1984	*Populus deltoides* (Salicaceae)	96%

Haberlandt (1914) put forward the theory of expeditious translocation in which he says "plants could be expected to have evolved tissue arrangements for the most direct and efficient removal of photosynthates from the cells in which they are produced" (in Russin & Evert, 1984). Rapid and continual removal of photosynthate from mesophyll cells promotes further synthesis, an obvious advantage especially in adverse conditions (Franceschi & Giaquinta 1983 a & b).

Minor vein distribution and coverage in areoles of the lamina is therefore important in terms of phloem loading (Haberlandt 1924, Wylie 1939, Esau 1972, Fisher & Evert 1982, Giaquinta 1983, Russin & Evert 1984, McCauley & Evert 1988a, Dannenhoffer *et al.* 1990, Fisher 1990, Russin *et al.* 1996, Nelson & Dengler 1997). The closer the individual chlorophyllous mesophyll cells to transporting phloem, the shorter and more expedient the loading route. Conversely, the less

abundant minor veins are in the lamina, the longer the loading route, with a corresponding decrease in efficiency.

Taxa of the Apocynaceae and Asclepiadaceae, being highly adapted and advanced, would be expected to show a dense arrangement of minor veins ramifying through mesophyll tissue. The opposite condition would be expected in the relatively primitive Ranunculaceae (Hickey 1971a & b, Doyle & Hickey 1976, Merrill 1978, Franck 1979). When the photographs from the current study are examined, this does appear to be the case. It is interesting to note that the venation results for *Beta vulgaris* (Chenopodiaceae) describe a sparse, delicate reticulum (Evert & Mierzwa 1986). Both the Chenopodiaceae and Ranuculaceae fall into the same minor vein type, 2a, in Gamalei's system (1989), which tempts the author to suggest similarities in loading method corresponding to a similar distribution of minor veins.

3.3.3 Venation in relation to habitat of taxon

It would be a logical step to assume that the spatial arrangement of the vascular system of a terrestrial plant would affect its ability to transport water and dissolved organic photosynthates around efficiently (Haberlandt 1914, Wylie 1939, Fisher & Evert 1982, Russin & Evert 1984, Fisher 1990). In other words, aquatic or semi-aquatic plants often have poorly developed vascular tissues, coupled with a paucity of lamina and stem venation (Schuster 1908, Wylie 1939). These plants grow in mesic environments where water is abundant in the external medium, and an efficient internal transport system is not required. Plants from xeric environments need effective, efficient internal transport systems as water availability is a problem (Tucker 1964). Such plants usually show highly developed spatial arrangement of vascular tissue in stems and leaves, to cope with the replacement of water.

This follows the reasoning of the theory presented by Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996), in that plants adapting to harsher environments would need a more efficient phloem loading system (Table 1.3). Coupled

with this would be better organisation and spatial coverage of the lamina by veins. Their data are mostly on a microscopic and ultrastructural level. It seemed an intriguing idea then to consider the venation, at a gross morphological level, of taxa examined here in terms of their habitat to see if there was any correlation.

Species adapted to environmental extremes such as alpine or xeric conditions generally show reduced leaves, lacking features normally used to delimit species of the same genus from less harsh environments (Tucker 1964, Hickey 1971 & 1973). Xerophytes may also have succulent leaves containing a tangled three dimensional venation pattern that does not follow any structured order (Rost 1969).

In all three families, species adapted to environmental extremes, other than those to which the family as a whole has become adapted, provide exceptions. For example in the Ranunculaceae, *Ranunculus multifidus* (Fig. 3.4 A, B & C), *R. muricatus* (Fig. 3.4 D & E) and *R. meyeri* (Fig. 3.5 F) occur in marshy areas (see Appendix III) (Exell & Milne-Redhead 1960, Gledhill 1969, Bond & Goldblatt 1984). Their lamina venation is sparse and delicate. Taxa from slightly drier areas, such as *Knowltonia capensis*, *K. filia* subspecies and *K. transvaalensis* subspecies (see Appendix III), show a spatially more dense, organised, robust venation (Fig.s 3.8 C & D, 3.7 A to D and 3.9 A, B & C respectively). Areoles are better defined and are more consistently traversed by minor veins. These features are consistent with a more xeric habitat, providing better lamina coverage and therefore transport.

The Apocynaceae do not show such a wide range of habitat. Plants occur in coastal to inland forests and scrub vegetation (see Appendix IV) (Codd 1963, Leeuwenberg & Kupicha 1985). Foliar venation is correspondingly more uniform (Fig.s 3.11 to 3.13).

The Asclepiadaceae is a very large family, and is well represented in the Eastern Cape (Gledhill 1969, Bond & Goldblatt 1984, Van Wyk & Malan 1988). Specific data on habitat for taxa was difficult to obtain due to the lack of a comprehensive Flora on this family. It would seem however that asclepiads occur in a wide range of habitats (see Appendix V), from karoo, coastal and

riverine scrub, to dunes and grassland (Gledhill 1969, Bond & Goldblatt 1984, Van Wyk & Malan 1988). Many asclepiads were excluded from this study as leaves are absent or vestigial. Such taxa are succulent xerophytes from extremely arid areas. The stem has taken over the role of photosynthesis and also serves to store water.

Generally speaking, the foliar venation is spatially well organised and dense (Fig.s 3.15 to 3.30). Taxa from grasslands (see Appendix V) generally display small, neat areoles with ultimate veinlets. Spatial arrangement is dense and close. For the most part, venation appeared robust. The same is true for taxa of dunes and coastal scrub (see Appendix V).

There are no representatives of mesic or aquatic environments in taxa of the Asclepiadaceae studied here. This is unfortunate as there is therefore no means of comparison within the family. When compared with the Ranunculaceae, however, the differences between the spatial arrangement of lamina venation from aquatic, mesic and xeric examples become obvious. When viewed in this light, the dense, efficient spatial coverage of the veins of taxa from arid areas are distinct from the poorly organised, spare arrangement seen in mesic and aquatic species.

3.3.4 Venation in relation to habit

The Van Bel and Gamalei theory (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996) also correlates phloem loading method with growth form (Table 1.4), again on the assumption that perennial trees will have a less efficient phloem loading system than annuals, climbers and slow growing herbs. A brief examination of gross venation features in terms of habit seemed prudent, although there is not a great variety of habits as represented by taxa studied here.

Clematis brachiata, of the Ranunculaceae, is a climber over scrub bush (see Appendix III) (Exell & Milne-Redhead 1960, Gledhill 1969, Bond & Goldblatt 1984). This species is the only climber amongst the Ranunculaceae of the Eastern Cape, the rest being small to tall herbs. The venation is well organised and ultimate vein endings are evenly distributed within areoles, though veins are not robust in dimension (Fig. 3.4 F & G). Venation is very similar to that of other taxa in the

Ranunculaceae from drier areas, such as *Knowltonia capensis* (Fig. 3.8 C & D), *K. filia* subspecies (Fig. 3.7 A to D) and *K. transvaalensis* (Fig. 3.9 A, B & C). Most of the Ranunculaceae of the eastern Cape are small herbs. There are no tree growth forms represented here.

The general Apocynaceae growth form, based on taxa included in this study, is that of a shrub or small tree (see Appendix IV) (Codd 1963, Leeuwenberg & Kupicha 1985). One exception is *Landolphia kirkii* which may grow up to 30m as a liana, though venation pattern and distribution remains consistent with that of the family (Tables 3.3 & 3.4). The other exception is that of *Pachypodium bispinosum*, a succulent shrublet with a tuberous underground stem. Lamina venation is consistent with that described for the family, but the midvein is thick and raised. Leaves are small and very tough (Tables 3.3 & 3.4).

The Asclepiadaceae incorporate a wide diversity of growth forms (see Appendix V), also encompassing habitat extremes (Gledhill 1969, Bond & Goldblatt 1984, Van Wyk & Malan 1988). Of the taxa examined here, habit is either that of a climber or a herb. The climbers with delicate, sparse venation included *Oncinema lineare* (Fig. 3.30 D), *Ceropegia carnosa* (Fig. 3.22 F & G) and *Cynanchum obtusifolium* (Fig. 3.23 A). Other climbers such as *Astephanus triflorus* (Fig. 3.28 H) and *Pentarrhinum insipidum* (Fig. 3.27 G, H & I) showed delicate, yet dense spatial arrangement of higher order veins. There was, however, no noticeable difference between these latter climbers and the herbaceous forms (see Appendix V).

On the basis of these observations, it does not appear that habit, as such, has much bearing on leaf architecture and higher order venation pattern. It would seem that habitat is of much greater importance.

3.3.5 Venation in relation to evolutionary position of family

Still in keeping with the theory of Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996), it was expected that the sophistication of venation development would be greater in more evolutionarily advanced families, and conversely less so in more primitive families (Hickey 1971a & b, Doyle & Hickey 1976, Merrill 1978, Franck

1979). This hypothesis arises simply from the fact that advanced taxa would have had to face harsher conditions in the colonization of non-tropical areas. A shorter, more sophisticated transport system to carry photosynthate away would have been a distinct advantage (Franceschi & Giaquinta 1983).

In an overview of the data presented here, this hypothesis does appear to hold true. The venation of the relatively primitive Ranunculaceae is generally more sparse and delicate than that of the other families. There are few ultimate vein endings in poorly defined areoles, especially in taxa from more mesic environments. In contrast, the venation of the tougher, more advanced Apocynaceae and Asclepiadaceae is considerably more robust, closely packed, and better distributed and organised, suggesting a more efficient transport system.

Lersten (1990) commented that surveys combining leaf clearings with selected sections would be the most efficient method of obtaining quantitative data on minor veins. Chapter Three dealt with leaf clearings for the Ranunculaceae, Apocynaceae and Asclepiadaceae. With a clearer understanding of vein patterns, the following two chapters deal with the anatomy and ultrastructure of representatives from each family.

CHAPTER FOUR: LEAF ANATOMY

4.1 Introduction

Essential to any study on venation and phloem loading is an examination of foliar anatomy as a whole and, in particular, the anatomy and ultrastructure of veins. The omission of this step in available treatments is widely lamented in the literature (Gambles & Dengler 1974, Evert *et al.* 1978, Fisher & Evert 1982, Fisher 1985, 1990, Lersten 1990). Fisher (1990), especially, noted the lack of comprehensive structural studies, going on to say that those published detail either foliar and vein anatomy as a whole for one species only, or else concentrate purely on the paraveinal mesophyll, bundle sheath features or vein anatomy in the assimilate loading pathway. He criticises these studies as being limited in scope, ignoring primitive groups of flowering plants, as being inconsiderate of growth form and habitat, and as not being representative of plants in general. As Van Bel and Gamalei's theory (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996) incorporates all these aspects, it is hoped that the current study will provide a holistic approach to the study of phloem loading for the three families of flowering plants chosen.

4.1.1 Leaf anatomy

Ranunculaceae

The Ranunculaceae produce delicate, herbaceous leaves (Heywood 1978). While certain taxa are found in arid, hot areas of the Eastern Cape, plants usually occur in protected enclaves of rocks and larger plants, and seldom in the open (Gledhill 1969, Moriarty 1982). Strasburger (1891) and later De Kock and Rutherford (1971) examined the leaves of *Ranunculus repens* and *R. reptans* respectively. Working on foliar veins of *R. repens*, Strasburger (1891) noted that vein tips were swollen and lacked phloem sieve tubes. *R. reptans* is a rare boreal plant that grows along pond margins in Northern and Central Europe (De Kock & Rutherford 1971). Hydathode-like structures were reportedly present at the tips of the leaves. Hydathodes have not previously been reported for any southern African species of *Ranunculus*. Indeed, practically nothing has been published on the leaf anatomy of the southern African Ranunculaceae.

Apocynaceae and Asclepiadaceae

Many Asclepiadaceae have leaves reduced to scales in response to life in harsh arid environments (Heywood 1978, Swarupanandan *et al.* 1996). As already noted, only taxa with suitable leaves have been included in this study (Appendix I). The Apocynaceae posses tough, waxy, elliptical leaves, preferring hot climates with a good water supply (Heywood 1978).

4.1.2 Laticifers

An extensive review on laticifers was compiled by Mahlberg (1993). The Asclepiadaceae and Apocynaceae are known to have laticifers with a sticky milky latex (Dahlgren 1975, Heywood 1978, Munday 1988, Mahlberg 1993, Struwe *et al.* 1994, Liede 1997, Endress & Bruyns 2000). The laticifers follow the vascular bundles in both leaves and stems. According to a classification by Esau (in Mahlberg 1993), the Apocynaceae and Asclepiadaceae possess nonarticulated, unbranched and branched laticifers.

In some instances the latex is toxic, containing cardiac glycosides and skin irritants. This is especially true of the Apocynaceae (Heywood 1978, Munday 1988). The milky latex forms an effective antiherbivory defence.

4.1.3 Bicollateral bundles

Bicollateral bundles are reported from the Asclepiadaceae and Apocynaceae (Metcalf & Chalk 1950). This double layer of phloem is referred to as abaxial (lower) and adaxial (upper) phloem in lamina bundles. Abaxial phloem is formed first, and therefore older, and is slower to develop. Adaxial phloem develops afterwards and much faster (Metcalf & Chalk 1950, Turgeon & Webb 1976). Bicollateral bundles are usually only noted in laminar midvein and stem vascular bundles, less often in higher order veins.

Having noted the occurrence of bicollateral phloem in *Solanum tuberosum*, McCauley and Evert (1988b) considered how this extra phloem layer might affect loading and transport. They determined separate roles for ad- and abaxial phloem. The same conclusion was drawn by other researchers. Schmitz *et al.* (1987), studying *Cucumis melo* minor veins, noted that the bicollateral arrangement extended to ultimate veinlets. Adaxial phloem terminated before the ultimate veinlet tip, while abaxial phloem extended all the way. This was suggested to indicate a primary function

of loading for abaxial phloem, and of transport for adaxial phloem. Bonnemain (1969a) noted that adaxial phloem functions for import, abaxial phloem for export of assimilates in developing tomato leaves. Ho and Shaw (1977) obtained a similar result in young tomato leaves. Using ^{14}C labelling experiments, Zamski and Tsivion (1977) reported a greater concentration of ^{14}C in the abaxial phloem of the midvein, than in the adaxial phloem. By watching the movement of dye in petioles of *Ecballium elaterium*, Peterson and Currier (1969) noted that dye moved into the leaf in the adaxial phloem and out in the abaxial phloem. Pristupa (1983) reported that only the abaxial phloem of *Cucurbita pepo* was involved in loading and export of assimilates from photosynthetic tissues.

The presence of both ad- and abaxial phloem obviously contributes to the complexity of the phloem loading pathway. This concept has not, to my knowledge, been incorporated into the theory proposed by Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996). It is considered beyond the scope of the current thesis, but is worth mentioning in view of future studies.

4.1.4 Anatomy and phloem loading

Anatomy may reflect the efficiency of photosynthate transport out of the leaf and water into the leaf, in response to the less equable conditions of harsher environments (Gambles & Dengler 1974, Carlquist 1991). This would be visible as a well organised system of minor veins, ramifying in close contact with photosynthetic mesophyll cells. Such close contact results in a short transport route from mesophyll to transporting phloem, and thus increased efficiency (Tucker 1964, Fisher & Evert 1982, Giaquinta 1983). This is the basis for the "principle of expeditious translocation" proposed by Haberlandt (1914). He suggested that plants will have evolved tissue organizations for rapid and efficient removal of photosynthetic products. This can be taken one step further by adding that such organizations should be more obvious in plants adapted to more rigorous environments (Tucker 1964).

The related concept of interveinal distance was proposed by Wylie (1939 & 1946), in an attempt to relate leaf structure to photosynthate transfer efficiency. His basic premise was that the distance between foliar minor veins is determined by relative amounts of nonvascular tissues. More

palisade mesophyll forces veins closer together, while more spongy mesophyll pushes veins further apart. Fisher (1990) considered this observation valid, but noted the possibility of many exceptions and the fact that it holds true only for veins with sieve tubes. In order to calculate interveinal distances then, one would first have to ascertain which vein orders posses sieve tubes.

4.1.5 Anatomy of vein orders

Fisher (1990), in a study of vein order anatomy in *Cananga odorata* leaves, noted that different vein orders have different anatomies. The misconception that all veins in a leaf have the same anatomy is rife in many published phloem loading studies. The anatomy of one vein is described, with no mention of it's order, and the arrangement seen then extrapolated to all veins of the leaf. As a change in anatomy, as related to differing vein order, could well reflect a change in primary function, the results would be incorrect for the majority of veins and correct only for the order described.

There is, however, no sharp distinction in anatomy between consecutive vein orders. The change is gradual and is related to the size of the vein and the change in surrounding tissues (Fisher & Evert 1982, McCauley & Evert 1988b, Russin *et al.* 1996). The midvein and secondary veins, termed major veins, are usually embedded in nonphotosynthetic, supportive tissues. The phloem of these veins is therefore distanced from the photosynthetic mesophyll tissues, indicating a primary function of mass translocation (Fisher & Evert 1982, Russin & Evert 1984, McCauley & Evert 1988b, Russin *et al.* 1996). The midvein is generally considered to be an extension of the petiole/stem vascular tissue. It usually contains both primary and secondary vascular tissues (Nelson & Dengler 1997). As the stem and petiole phloem is exclusively transporting in function, it can therefore be expected that the primary function of the midvein will be transportation too, and that the anatomies and ultrastructure of all three will be similar.

Minor veins are embedded in photosynthetic mesophyll cells (Esau 1972). The close proximity of the phloem to the site of photosynthesis indicates a primary loading function (Fisher & Evert 1982, Russin & Evert 1984, McCauley & Evert 1988b, Fisher 1990, Russin *et al.* 1996). McCauley and Evert (1988b) did note a correlation between foliar venation and the gross route of photosynthate translocation from leaves for a number of woody species. Foliar anatomy can be used, therefore, to infer the efficiency and possible route of phloem loading.

4.1.6 Bundle sheath cells

Of great importance to the loading route followed by photosynthates into sieve tubes, are bundle sheath cells. These cells form the bridge between photosynthetic mesophyll and transporting sieve tubes (McCauley & Evert 1988b).

There appears to be some confusion in the literature surrounding the correct terminology for cells found between the sieve tubes and mesophyll. Where clear distinction between companion cells, bundle sheath cells and mesophyll cells can be seen, there is no problem designating classification. Where the distinction between such cells is unclear, problems with terminology arise. Haberlandt (1914) noted large, dense cells between mesophyll cells and sieve tubes, and introduced the term "intermediary" to classify such cells. In retrospect it appears he was discussing companion cells. Fisher (1967) talked of "paraveinal mesophyll", with the same loose regard, to describe cells between mesophyll and sieve tubes. Franceschi and Giaquinta (1983b) proceeded to suggest the incorporation of specialized bundle sheath cells into the "paraveinal mesophyll" concept, in instances where bundle sheath and mesophyll cells are similar in appearance and possibly function, so becoming part of the same layer. Companion cells were excluded, being classed as separate entities. This view was supported by Everard *et al.* (1990).

The presence of radiating extensions from bundle sheath cells of minor veins has been documented (Dengler & MacKay 1975, Franceschi & Giaquinta 1983, Russin & Evert 1984, Dannenhoffer *et al.* 1990, Fisher 1990). It has been suggested that this allows the phloem loading route to become more direct, and therefore more efficient, as these extensions increase contact with photosynthetic mesophyll cells.

Bundle sheath cell type changes with vein order, further augmenting the argument for a change in function with decreasing vein order. In *Fagus grandifolia*, a sclerenchymatous bundle sheath is noted for lower order veins, changing to a parenchymatous one in higher order veins (Dengler & MacKay 1975). The same situation was described for *Populus deltoides* (Russin & Evert 1984).

In some instances, bundle sheath cells appear indistinct from surrounding mesophyll cells, in terms of size, shape and chloroplast content (Morretes 1962, Fisher & Evert 1982, Fisher 1985). It would not be unreasonable then to assume a similar function for the two cell types, and their description as paraveinal mesophyll (Franceschi & Giaquinta 1983a). Paraveinal mesophyll is a distinct layer of cells, noted particularly in the legume family. Bundle sheath cells are generally smaller than paraveinal mesophyll, but have similar features. Paraveinal mesophyll is usually almost twice the size of regular spongy mesophyll, has a large nucleus and a very large vacuole, but fewer chloroplasts. Paraveinal mesophyll is thought to be a highly specialized cell type in the pathway of photosynthates from mesophyll to phloem (Everard *et al.* 1990).

4.1.7 Vein endings and phloem cytology

In the developing vascular system of a leaf primordium, vein endings are the last to come into being (Lersten 1990), and for the most part, it would appear that phloem tissue is not present in ultimate vein endings in areoles (Morretes 1962, Lersten & Carvey 1974, Lersten 1990, Fisher 1990), although this does vary (Morretes 1962, Lersten 1990).

The cytology of phloem at vein endings, and in minor veins surrounding areoles, deserves special attention in view of role played by these veins in loading (Morretes 1962). Reports concentrate on the presence and abundance of sieve tubes as the main transporting cells, and to a lesser extent on companion cells (Morretes 1962, Lersten 1990, Fisher 1990). Fisher (1990) produced an interesting account of the minor veins and vein endings in *Cananga odorata*. He categorised these veins into four types based on their cytology, number and characteristics of cell types, and the size of the vein. He concluded that due to a paucity of sieve tubes in a significant percentage of minor veins, alternative phloem loading pathways may have to be considered in which longitudinal transport along bundle sheath cells regularly occurs. This supports the intermediary cell/paraveinal mesophyll layer concept, as a means of photosynthate shunting bridge to the nearest sieve tube.

A further consideration is the activity of the phloem tissue. Lersten and Carvey (1974) noted darkly-stained sieve tube walls in the phloem of *Fouquieria splendens*. Morretes (1962) reported darkly-stained, granular cytoplasm in minor veins of *Phaseolus vulgaris* and *Capsicum annuum*. Due to the similarity of this result with that obtained from metabolically active secretory cells,

the conclusion was that the phloem of these species must have a high metabolic activity too. This would be in keeping with the energy requirement of rapid, active transport.

4.1.8 Applications of leaf anatomy within the ecophysiological concept of phloem loading

In chapter three, the gross spatial arrangement of veins within the lamina was considered. Minor vein distribution, areole development and coverage of areoles by ultimate veinlets was related to apparent phloem loading efficiency. This concept can now be examined at the anatomical level. The proximity of mesophyll cells to bundle sheath cells, and then to phloem, can be described for certain species from the three families chosen for this study.

In terms of Van Bel and Gamalei's theory (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996), the Apocynaceae and Asclepiadaceae as evolutionarily advanced families, would be expected to show a close affiliation between mesophyll, bundle sheath cells and phloem. Conversely, a less advanced family like Ranunculaceae would not be expected to show such intense spatial organisation.

4.1.9 Aims

For certain species of the Ranunculaceae, Apocynaceae and Asclepiadaceae, the aims of this chapter are as follows:

1. To illustrate leaf anatomy.
2. To determine the proximity of photosynthetic mesophyll to phloem tissue.
3. To describe the anatomy of lower and higher order veins.
4. To describe the hydathodes of *Ranunculus*.

4.2 Results

4.2.1 Ranunculaceae: *Ranunculus multifidus*

Leaf anatomy

The adaxial epidermis consists of a single layer of large, rounded cells with an overlying cuticle (Fig. 4.1 B). No stomata were noted in the adaxial epidermis. The abaxial epidermis contains stomata amongst unevenly sized epidermal cells.

Leaves are unifacial, with a single layer of elongated palisade mesophyll under the adaxial epidermis (Fig. 4.1 B, C & E). The spongy mesophyll is multilayered, but not compactly arranged. Mesophyll cells are irregular in shape (Fig. 4.1 B, E & H). Air spaces are large and many. No tanniniferous cells, laticifers, crystals or any other defensive structures were noted (Fig. 4.1 A to I).

Veins ramify at the palisade/mesophyll interface. Bundle sheath cells are elongated parallel to the vein, forming an enclosing sheath. The bundle sheath contains few chloroplasts when compared with the mesophyll cells. Chloroplasts are clustered on the peripheral side of the cell away from the vascular tissues (Fig. 4.1 D, G, H & I). Bundle sheath extensions were noted, but were few and far between (Fig. 4.1 D, G, H & I). Photosynthetic mesophyll and bundle sheath cells could not be described as being densely associated, but did show many connections between cells (Fig. 4.1 E, G, H & I).

Hydathode structure

Hydathodes are cup-shaped, epithem hydathodes (Fig. 4.1 F). Each hydathode is supplied by at least one vein, with short xylem vessels forming the base of the supplying cup. Epithem cells occur just above the xylem vessels. These cells are very small and fairly tightly packed (Fig. 4.1 F). Surrounding the epithem is a sheath of cells with chloroplasts, which forms from the extension of the vascular bundle sheath. In the overlying epidermis of the hydathode are enlarged, modified stomata called water pores (Fig. 4.1 F).

Vein anatomy

The midvein is supported on all sides by collenchyma cells, distancing phloem from mesophyll, but mostly below (Fig. 4.1 A). Secondary veins do not have supporting tissue (Fig. 4.1 B). A unicellular, thin-walled bundle sheath surrounds the xylem and phloem. Bundle sheath cells of secondary veins have chloroplasts and show connections with mesophyll cells (Fig. 4.1 G & I). There is no adaxial phloem (Fig. 4.1 A).

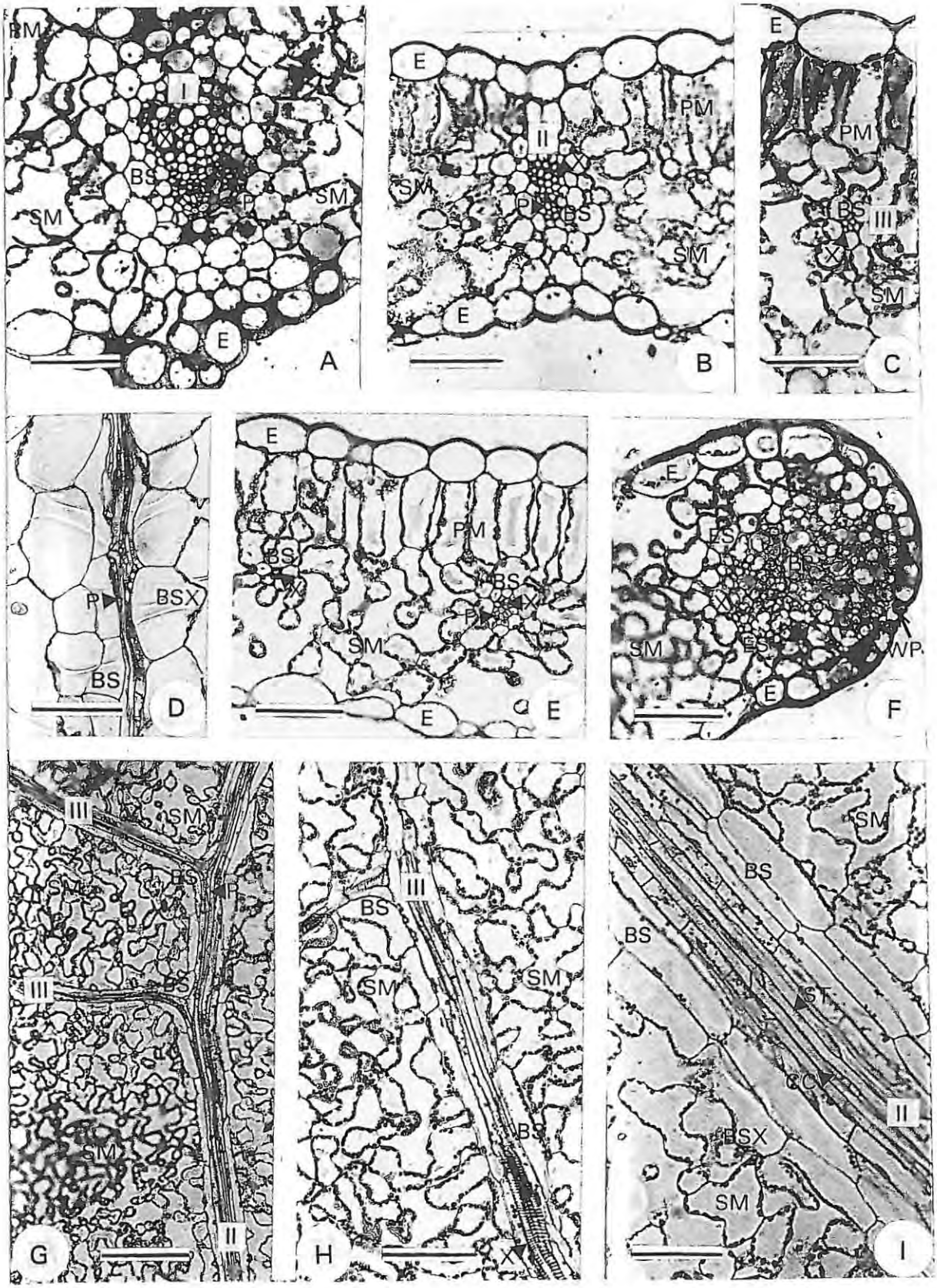
Higher order veins are bounded by a thin-walled, chlorophyllous bundle sheath (Fig. 4.1 C, D, E, G, H & I). The higher the vein order the fewer xylem and phloem elements there are, to just a single vessel element and sieve tube (Fig. 4.1 C, D, E & G). Phloem tissue does not stain well (Fig. 4.1 G, H & I), except in minor veins (Fig. 4.1 D). Sieve tubes and companion cells can be seen in longitudinal section (Fig. 4.1 I). There does not appear to be much phloem parenchyma present (Fig. 4.1 D, G, H & I). Sieve tubes are present in minor veins (Fig. 4.1 D).

Figure 4.1 Ranunculaceae: Aspects of the leaf anatomy of *Ranunculus multifidus*

A Transverse section of *Ranunculus multifidus* midvein with supporting collenchyma and adjacent mesophyll layers, B Transverse section of *R. multifidus* lamina showing secondary vein with bundle sheath, spongy mesophyll and palisade mesophyll, C Transverse section of *R. multifidus* lamina showing quaternary vein with bundle sheath surrounding xylem vessels, D Longitudinal section of minor vein in *R. multifidus* lamina showing phloem and bundle sheath cells with extensions to adjacent mesophyll cells, E Transverse section of *R. multifidus* lamina showing minor vein with xylem vessels only and tertiary vein with both xylem and phloem at palisade/spongy mesophyll interface, F Transverse section of hydathode at leaf apex of *R. multifidus* showing epithem cells surrounded by epithem sheath as an extension of the subtending bundle sheath and water pores in the overlying epidermis, G Paradermal section of *R. multifidus* lamina showing darkly-stained phloem of secondary vein and excurrent tertiary veins, bounded by elongated bundle sheath cells in spongy mesophyll, H Paradermal section of tertiary vein in *R. multifidus* lamina showing xylem and phloem in longitudinal section, bounded by elongated bundle sheath cells in spongy mesophyll, I Paradermal section of secondary vein of *R. multifidus* showing companion cells and sieve tubes of phloem in longitudinal section and elongated bundle sheath cells with bundles sheath extensions to adjacent spongy mesophyll cells

(BS = bundle sheath; CC = companion cell; E = epidermis; EP = epithem cells; ES = epithem sheath; I = midvein; II = secondary vein; III = tertiary vein; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; ST = sieve tube; WP = hydathode pore; X = xylem)

Bar represents 25μm for A to F, H and I, and 50μm for G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4.2.2 Apocynaceae: *Acokanthera* and *Carissa*

Leaf anatomy

In *Acokanthera oppositifolia*, *Carissa bispinosa* and *C. haematocarpa* leaves are unifacial. The adaxial epidermis consists of regular, box-shaped cells with a very thick cuticle. There are no stomata in the adaxial epidermis (Fig.s 4.3 A & C and 4.9 A, B & D). Cells of the abaxial epidermis are smaller than those of the adaxial epidermis, but the cuticle is just as thick (Fig. 4.9 A & D). Stomata are sunken below projections of the cuticle in *A. oppositifolia* (Fig. 4.3 E).

Below the epidermis is a multilayered palisade mesophyll (Fig.s 4.3 A, D & E, 4.5 D & E and 4.9 A, B & D). Cells are slender, long, closely packed and have many chloroplasts. The innermost layer of palisade of *C. haematocarpa* consisted of a row of shorter cells (Fig. 4.9 D). The uppermost layer of palisade mesophyll of *Carissa bispinosa* stained very darkly due to the presence of tannins (Fig. 4.5 D & E). Tanniniferous cells were abundant in all lamina tissues, except the epidermis, of *C. bispinosa* (Fig.s 4.5 A to H, 4.6, 4.7 and 4.8). At the palisade/ spongy mesophyll interface veins are present (Fig.s 4.3 A, C & D and 4.5 D & E).

Spongy mesophyll cells are large and rounded (Fig. 4.3 A, C & D), with many tanniniferous cells interspersed in *C. bispinosa* (Fig. 4.5 D, E, F & G) and *C. haematocarpa* (Fig. 4.9 B & D). Air spaces are connected to stomata in the abaxial epidermis (Fig. 4.3 E). In *C. bispinosa*, intercellular air spaces are very wide (Fig. 4.5 D to H). Two layers of spongy cells just above the abaxial epidermis are brick-shaped and densely packed in *A. oppositifolia* (Fig. 4.3 A & E) and *C. haematocarpa* (Fig. 4.9 D). These cells do not have the same chloroplast content, size, shape or close packing as palisade tissue, and so are designated spongy tissue. Abaxial spongy mesophyll cells of *C. bispinosa* are tanniniferous. Crystals were noted in both *A. oppositifolia* (Fig. 4.3 A, D & E) and *C. bispinosa* (Fig.s 4.5 D, E & H, 4.7 and 4.8) mesophyll tissues.

In paradermal section, veins appear robust and densely distributed (Fig.s 4.2, 4.4, 4.5 G & H, 4.7 and 4.8). Higher order veins show close association with photosynthetic mesophyll, although this

was more prevalent in *A. oppositifolia* (Fig.s 4.2 C & D and 4.4) than in *C. bispinosa* (Fig.s 4.7 and 4.8). Bundle sheath cells of all species are small and rounded, contain many chloroplasts and have small, pointed extensions (Fig.s 4.2 B & D, 4.4, 4.5 G & H, 4.7, 4.8 and 4.9 E & F). Air spaces amongst mesophyll cells were larger in *C. bispinosa*.

Vein anatomy

In both *Acokanthera oppositifolia* and *Carissa bispinosa*, the midvein is supported above and below by strengthening collenchyma tissue, but is surrounded by a chlorenchymatous bundle sheath on the sides (Fig.s 4.5 A and 4.6). Neither of these features was seen in the midvein of *C. haematocarpa* (Fig. 4.9 A). Fig. 4.6 shows a paradermal section through the midvein of *C. bispinosa*, in which laticifers, tanniniferous, parenchyma and chlorophyllous bundle sheath cells can be seen. Bundle sheath cells connected to mesophyll cells can be seen.

A wide band of cambium is present in both primary and secondary veins, especially so in the midvein of *C. haematocarpa* (Fig. 4.9 A) and the secondary vein of *A. oppositifolia* (Fig. 4.3 C). There is a thick layer of xylem. Primary abaxial phloem occurred in a C-shape around the cambium and xylem. Adaxial phloem was present as isolated strands in both primary and secondary veins (Fig.s 4.3 C, 4.5 A and 4.9 A). Phloem tissue stained very darkly in *A. oppositifolia* (Fig. 4.3 F & G) and *C. bispinosa* (Fig.s 4.5 H, 4.6, 4.7 and 4.8).

There is a definite chlorenchymatous bundle sheath in *A. oppositifolia* and *C. bispinosa* (Fig.s 4.3 B & F, 4.5 B, C, D & G, 4.7 and 4.8). Tanniniferous bundle sheath cells were seen occasionally in *C. bispinosa* (Fig. 4.5 G & H, 4.7 and 4.8) and *C. haematocarpa* (Fig. 4.9 B, D, E & F).

Xylem forms the bulk of each minor vein, though narrow threads of phloem tissue can be seen following the xylem (Fig.s 4.3 B & F, 4.4, 4.5 E to H, 4.7, 4.8 and 4.9 E & F). Ultimate veinlets consist of xylem only (Fig.s 4.2 C & D, 4.4, 4.5 G, 4.7 and 4.9 E & F). In *A. oppositifolia* especially, ultimate veinlets ended as bundles of splayed xylem vessels, with no phloem tissue present. Sieve tubes are present in minor veins surrounding areoles (Fig.s 4.4, 4.7 and 4.9 E).

Marginal venation contains both xylem and phloem (Fig. 4.3 F & G). The ultimate veinlets forming the incomplete marginal venation pattern appear to be bundles of xylem vessels only (Fig. 4.3 G).

Laticifers were seen near all vein orders of *A. oppositifolia* and *C. bispinosa* (Fig.s 4.4, and 4.5 A, C & D, 4.6 and 4.8 respectively).

A. oppositifolia (Fig. 4.3 C), *C. bispinosa* (Fig. 4.5 A) and *C. haematocarpa* (Fig. 4.9 A) primary and secondary veins showed isolated patches of adaxial phloem, forming bicollateral bundles. Adaxial phloem was also noted in the tertiary veins of *C. bispinosa* (Fig. 4.5 B). This was not seen in higher order veins, as no adaxial phloem was noted.

Figure 4.2 Apocynaceae: Aspects of the leaf anatomy of *Acokanthera oppositifolia*

A Paradermal section of tertiary vein in *Acokanthera oppositifolia* lamina showing rounded chlorophyllous bundle sheath cells surrounding darkly-stained phloem, B Paradermal section of minor vein in *A. oppositifolia* lamina showing darkly-stained phloem surrounded by rounded chlorophyllous bundle sheath cells, embedded in spongy mesophyll tissue interspersed with crystals, C Paradermal section of lamina of *A. oppositifolia* with incompletely closed areole with once-branched ultimate veinlet, showing overlying chlorophyllous bundle sheath cells and xylem in spongy mesophyll, D Composite of paradermal section of *A. oppositifolia* lamina showing minor veins surrounding incompletely closed areoles with simple and once-branched ultimate veinlets, darkly-stain phloem and xylem in longitudinal section bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells

(A = areole; BS = bundle sheath; BSX = bundle sheath extensions; C = crystal; CC = companion cell; III = tertiary vein; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; UV = ultimate veinlets; X = xylem)

Bar represents 12.5μm for A, 25μm for B, and 50μm for C and D

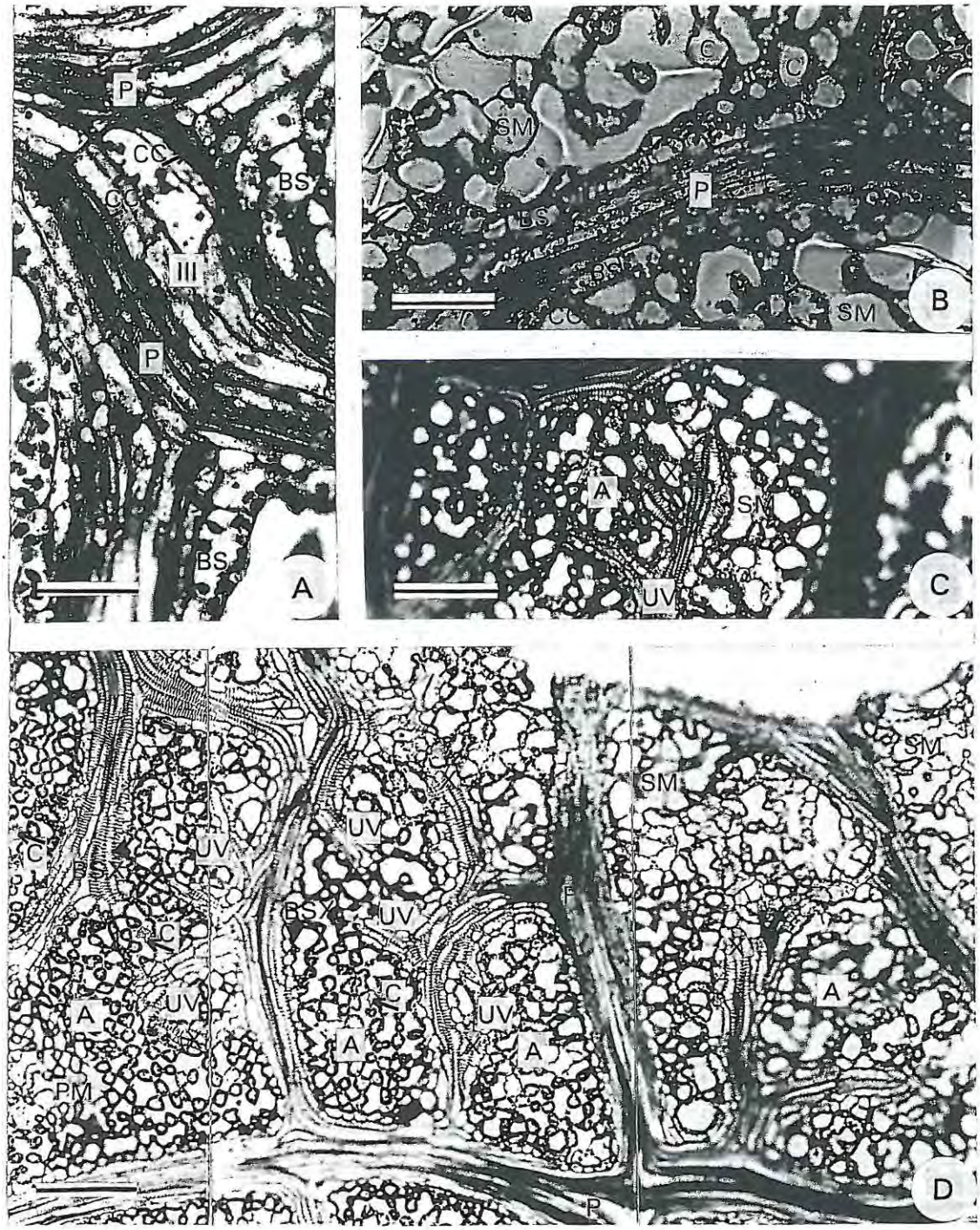
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Figure 4.3 Apocynaceae: Aspects of the leaf anatomy of *Acokanthera oppositifolia*

A Transverse section of *Acokanthera oppositifolia* lamina showing xylem and phloem surrounded by chlorophyllous bundle sheath cells at the spongy/palisade mesophyll interface with crystals occurring in the wide spongy mesophyll layer, B Transverse section of minor vein in *A. oppositifolia* lamina showing xylem and phloem surrounded by chlorophyllous bundle sheath cells at the spongy/palisade mesophyll interface, C Transverse section of secondary vein in *A. oppositifolia* lamina showing xylem with both abaxial and adaxial phloem, and excurrent tertiary vein with xylem and abaxial phloem, both bounded by chlorophyllous bundle sheath cells at the spongy/palisade mesophyll interface, D Transverse section of *A. oppositifolia* lamina with longitudinal section of minor vein showing xylem and abaxial phloem bounded by chlorophyllous bundle sheath cells, spongy mesophyll layer is interspersed with crystals, E Transverse section of *A. oppositifolia* lamina showing lower epidermis with thick cuticle and sunken stomata, and spongy mesophyll layer interspersed with crystals, F Paradermal section of marginal vein of *A. oppositifolia* lamina showing darkly-stain phloem and xylem bounded by chlorophyllous bundle sheath cells, G Paradermal section of *A. oppositifolia* lamina with incomplete marginal venation of once-branched ultimate veinlets showing darkly-stained phloem and xylem bounded by chlorophyllous bundle sheath cells

(E = epidermis; BS = bundle sheath; C = crystal; II = secondary vein; III = tertiary vein; IMV = incomplete marginal venation; P = phloem; PM = palisade mesophyll; S = stoma; SM = spongy mesophyll; UV = ultimate veinlets; X = xylem)

Bar represents 50μm for A, C and D, 25μm for E, F and G, and 12.5μm for B

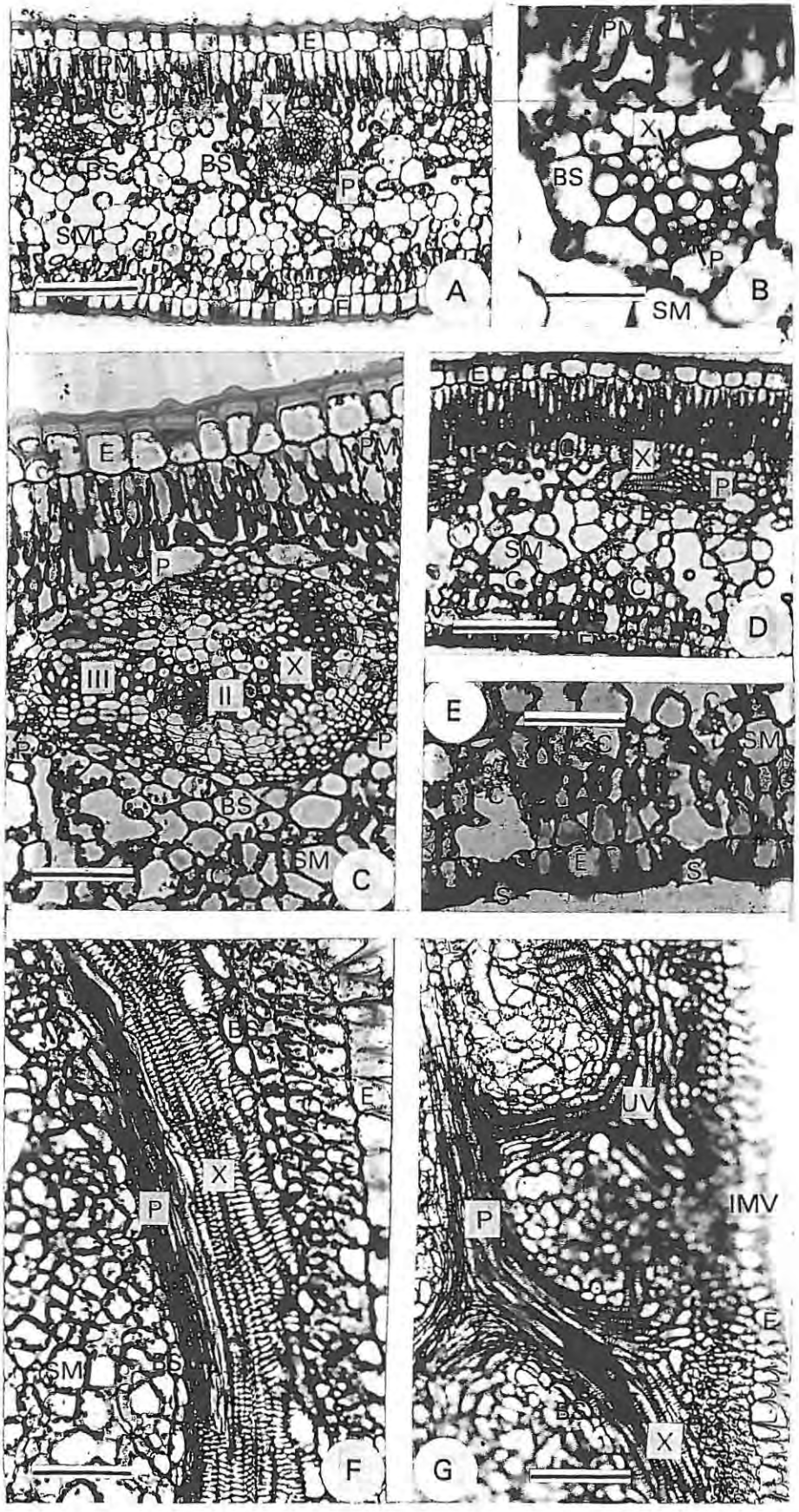
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Figure 4.4 Apocynaceae: Aspects of the leaf anatomy of *Acokanthera oppositifolia*

Composite plate of paradermal section of *Acokanthera oppositifolia* lamina with secondary vein, adjacent incompletely closed areoles and once-branched fifth order ultimate veinlets showing xylem and darkly-stained phloem bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, laticifers follow all vein orders

(A = areole; BS = bundle sheath; BSX = bundle sheath extensions; II = secondary vein; L = laticifer; P = phloem; SM = spongy mesophyll; UV = ultimate veinlets; X = xylem)

Bar represents 50μm

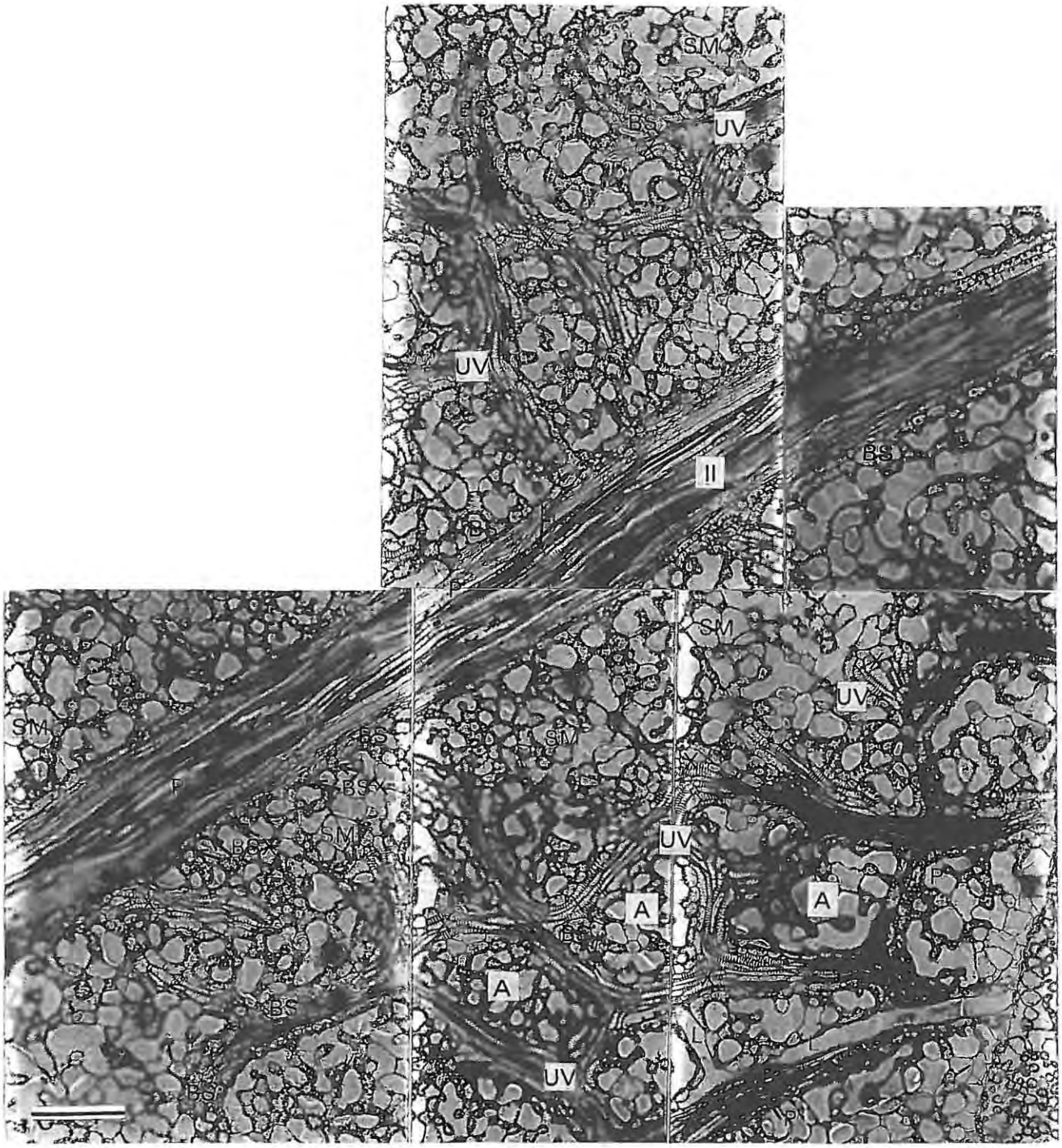
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Figure 4.5 Apocynaceae: Aspects of the leaf anatomy of *Carissa bispinosa*

A Transverse section of *Carissa bispinosa* midvein showing cambium, xylem and bicollateral phloem in small pockets, tanniniferous cells and laticifers occur in the region of the midvein, B Transverse section of *C. bispinosa* lamina with tertiary vein showing bicollateral phloem and xylem bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, C Transverse section of *C. bispinosa* lamina with quaternary vein showing abaxial phloem and xylem bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, D Transverse section of *C. bispinosa* lamina showing a quaternary vein containing xylem and abaxial phloem and a sixth order vein of xylem vessels only, both bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, tanniniferous cells and crystals occur interspersed amongst spongy mesophyll cells, the upper layer of palisade mesophyll is tanniniferous, E Transverse section of *C. bispinosa* lamina with longitudinal section of fifth order vein showing xylem and abaxial phloem bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, F Transverse section of *C. bispinosa* lamina with sixth order ultimate veinlet showing xylem only surrounded by chlorophyllous bundle sheath cells with extensions to spongy mesophyll cells, tanniniferous cells and crystals present, G Paradermal section of *C. bispinosa* with incompletely closed areoles with once-branched sixth order ultimate veinlets showing xylem and darkly-stained phloem bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, H Paradermal section of tertiary vein of *C. bispinosa* showing xylem and darkly-stained phloem bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, tanniniferous cells and crystals present in widely spaced spongy mesophyll layer

(A = areole; BS = bundle sheath; BSX = bundle sheath extensions; C = crystal; CA = cambium; I = midvein; III = tertiary vein; IV = quaternary vein; L = laticifer; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; T = tanniniferous cell; UV = ultimate veinlets; X = xylem)

Bar represents 25μm for A, D, E, F and H, 12.5μm for B and C, and 50μm for G

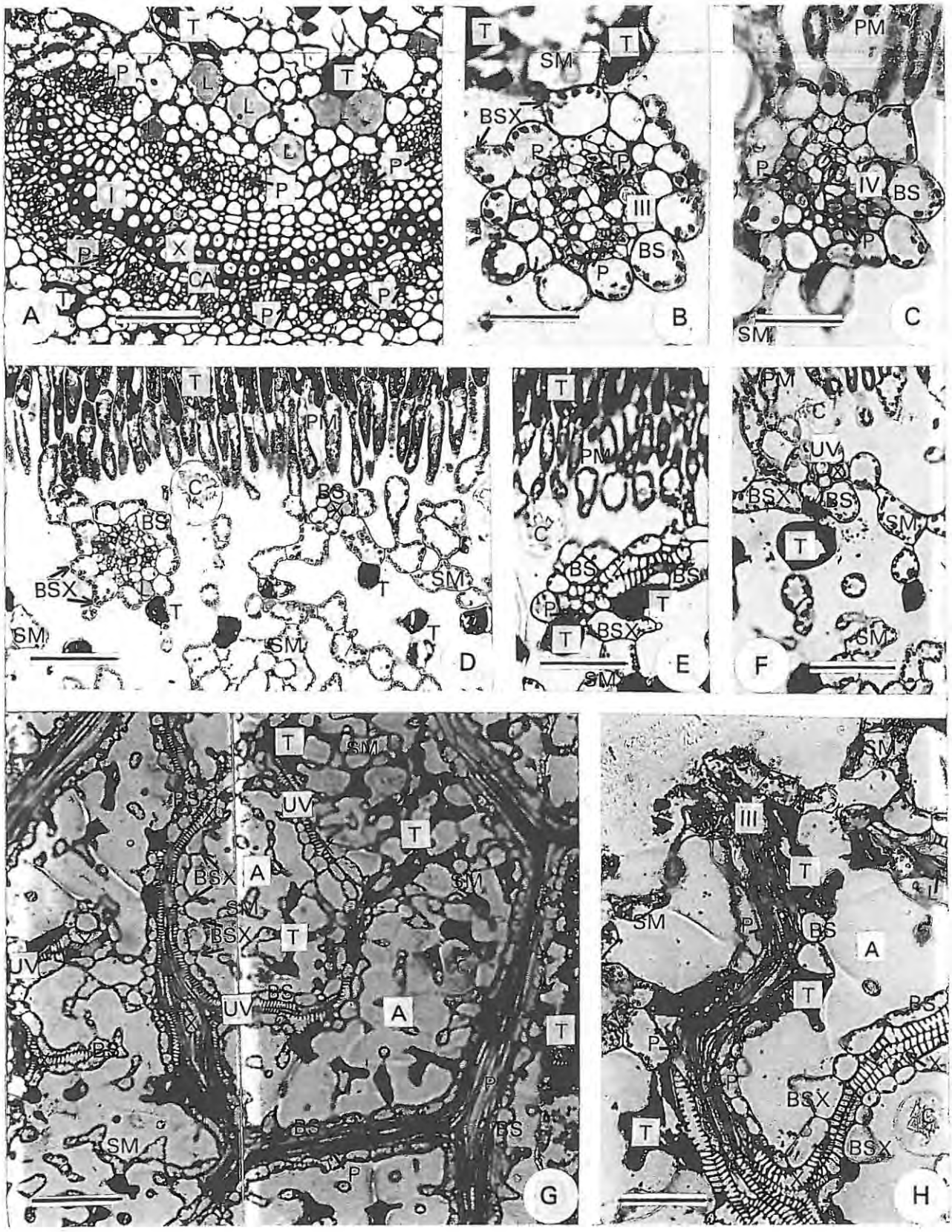
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Figure 4.6 Apocynaceae: Aspects of the leaf anatomy of *Carissa bispinosa*

Composite plate of paradermal section of *Carissa bispinosa* midvein showing darkly-stained phloem interspersed with parenchyma cells, tanniniferous cells and laticifers, with excurrent secondary vein showing xylem, both veins bounded by chlorophyllous bundle sheath cells and embedded in spongy mesophyll with interspersed tanniniferous cells

(BS = bundle sheath; I = midvein; II = secondary vein; L = laticifer; P = phloem; SM = spongy mesophyll; T = tanniniferous cell; X = xylem)

Bar represents 50μm

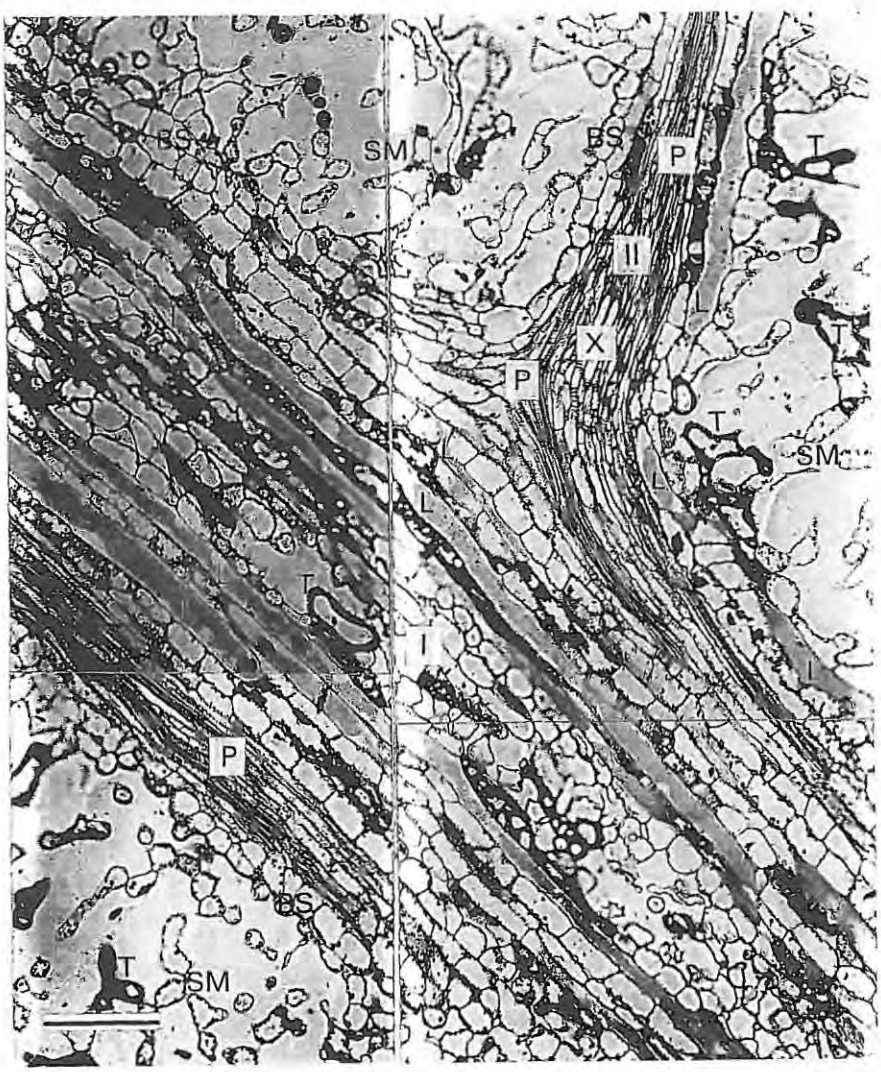
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Figure 4.7 Apocynaceae: Aspects of the leaf anatomy of *Carissa bispinosa*

Composite plate of paradermal section of incompletely closed areoles of *Carissa bispinosa* with once and twice-branched ultimate veinlets, showing xylem and darkly-stained phloem bounded by chlorophyllous bundle sheath cells with extensions to widely spaced spongy mesophyll cells, tanniniferous cells and crystals present amongst spongy mesophyll cells

(A = areole; BS = bundle sheath; BSX = bundle sheath extensions; C = crystal; P = phloem; SM = spongy mesophyll; T = tanniniferous cell; UV = ultimate veinlet; X = xylem)

Bar represents 50μm

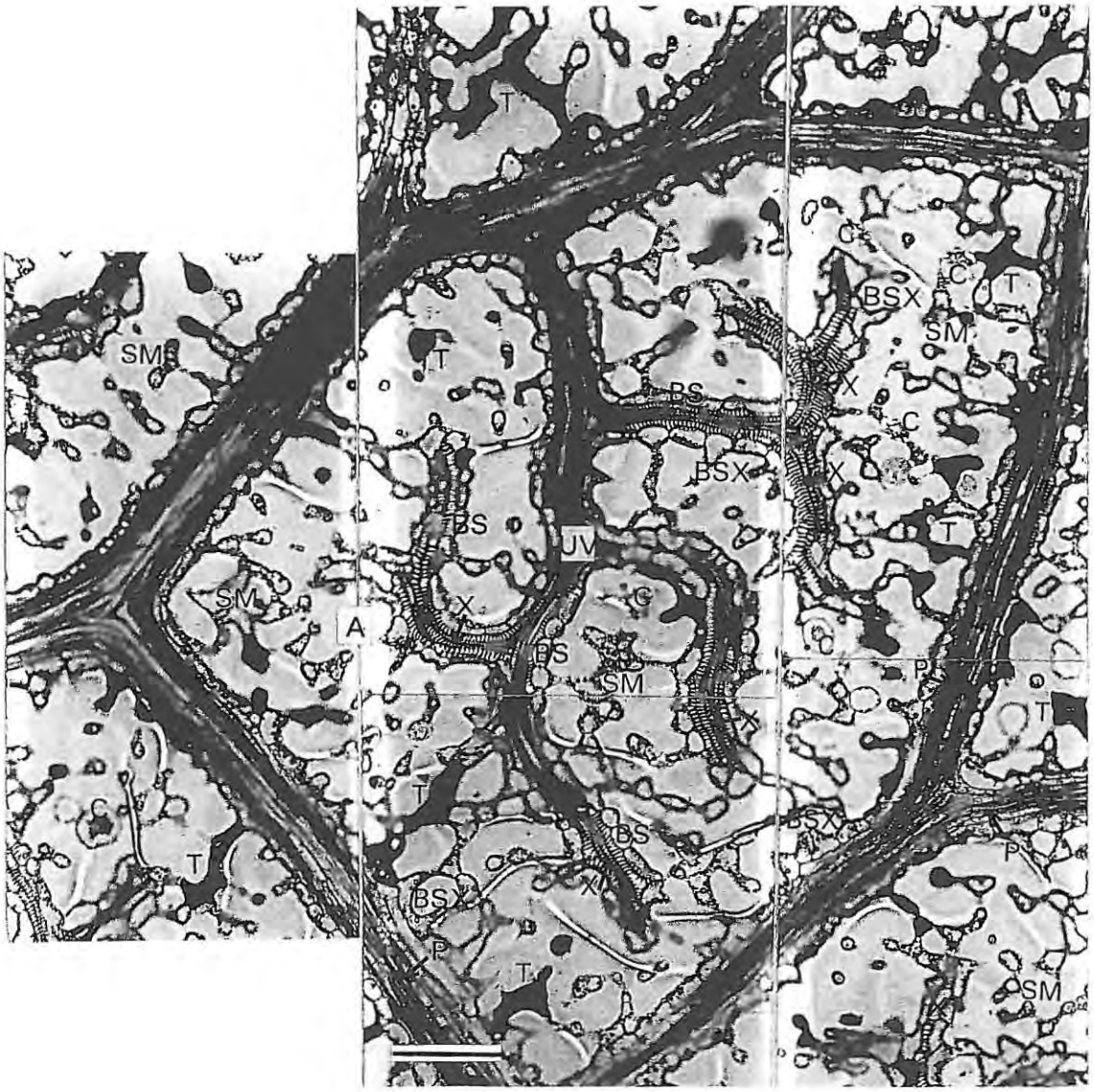
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Figure 4.8 Apocynaceae: Aspects of the leaf anatomy of *Carissa bispinosa*

Composite plate of paradermal section of incompletely closed areoles of *Carissa bispinosa* near quaternary vein, showing xylem and darkly-stained phloem bounded by chlorophyllous bundle sheath cells with extensions to widely spaced spongy mesophyll cells, tanniniferous cells and crystals present amongst spongy mesophyll cells

(A = areole; BS = bundle sheath; C = crystal; IV = quaternary vein; L = laticifer; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; T = tanniniferous cell; UV = ultimate veinlet; X = xylem)

Bar represents 50μm

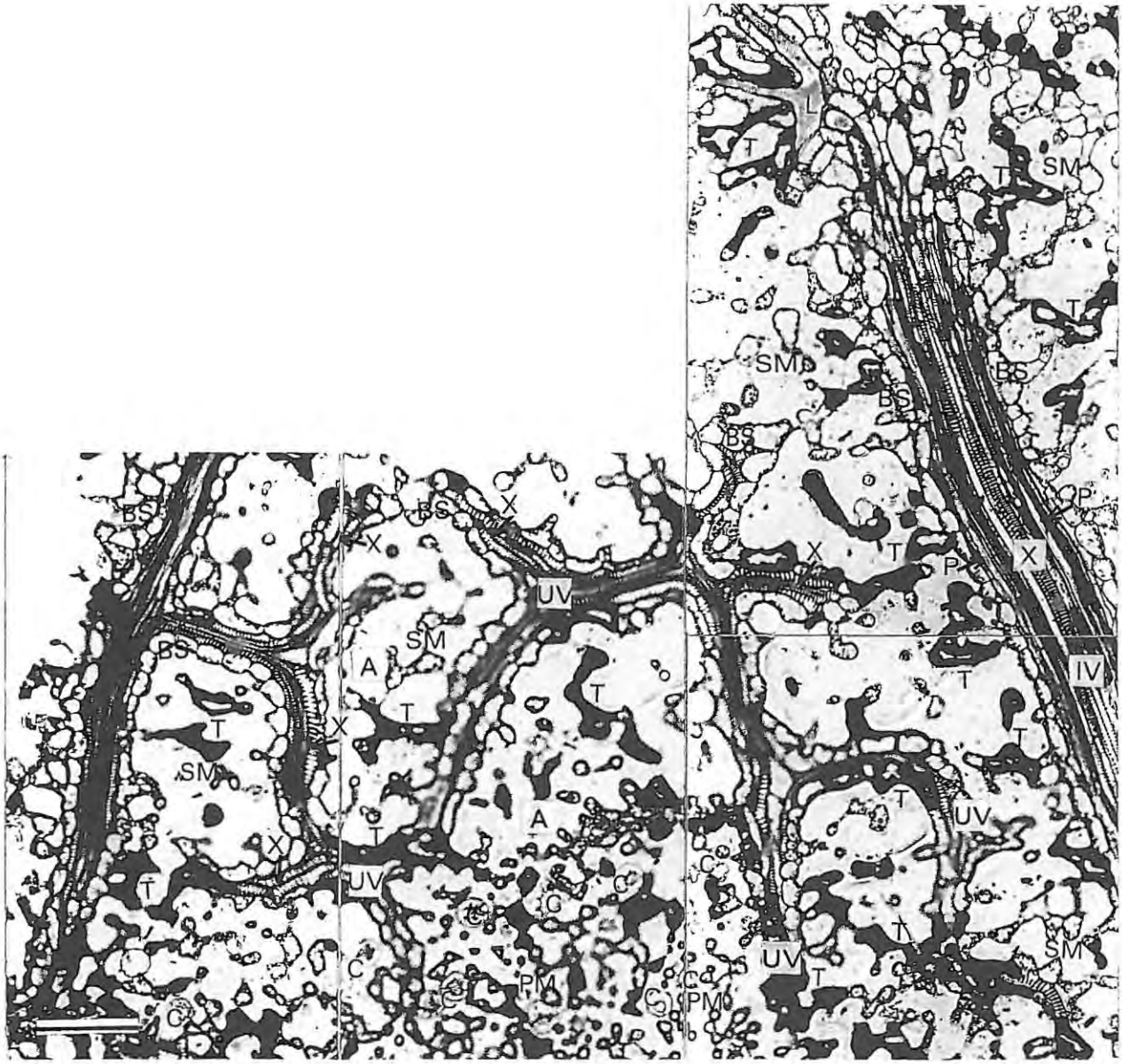
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Figure 4.9 Apocynaceae: Aspects of the leaf anatomy of *Carissa haematocarpa*

A Transverse section of *Carissa haematocarpa* midvein showing xylem, cambium and bicollateral phloem supported by tanniniferous parenchyma cells and overlaid by palisade mesophyll cells, B Transverse section of *C. haematocarpa* lamina with fourth minor vein showing xylem and abaxial phloem surrounded by chlorophyllous and tanniniferous bundle sheath cells, tanniniferous cells abundant in both spongy and palisade mesophyll layers, C Transverse section of *C. haematocarpa* fifth order minor vein showing xylem only in chlorophyllous bundle sheath, D Transverse section of *C. haematocarpa* lamina showing abundant tanniniferous cells in both spongy and palisade mesophyll layers, E Paradermal section of *C. haematocarpa* lamina showing incompletely closed areoles with simple and once-branched ultimate veinlets showing xylem and darkly-stained phloem bounded by chlorophyllous and tanniniferous bundle sheath cells with extensions to spongy mesophyll cells, F Paradermal section of *C. haematocarpa* lamina showing incompletely closed areoles with once-branched ultimate veinlets showing xylem and darkly-stained phloem bounded by chlorophyllous and tanniniferous bundle sheath cells with extensions to spongy mesophyll cells

(A = areole; BS = bundle sheath; BSX = bundle sheath extensions; E = epidermis; I = midvein; L = laticifer; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; T = tanniniferous cell; UV = ultimate veinlet; X = xylem)

Bar represents 50μm for A, D, E and F, 25μm for B, and 12.5μm for C


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4.2.3 Asclepiadaceae

Secamone, *Asclepias*, *Cynanchum* and *Ceropegia* represented the Asclepiadaceae in anatomical and ultrastructural studies.

Leaf anatomy

The adaxial epidermis of *Secamone alpinii* consisted of tightly-packed cells, covered by a thick cuticle (Fig. 4.10 C). Cells of the abaxial epidermis were much smaller (Fig. 4.10 C). Adaxial epidermal cells of *Asclepias fructicosa* were large, irregularly shaped, and interspersed with very large tannin cells (Fig. 4.10 D & E). Cells were more uniform in the abaxial epidermis (Fig. 4.10 D, E & F). A very thick cuticle was noted in *A. physocarpa*, with adaxial epidermal cells rounded and irregular (Fig. 4.12 A, B & D). Abaxial cells were smaller (Fig. 4.12 D). *Cynanchum obtusifolium* had a thinner cuticle than other species, overlying small rounded adaxial epidermal cells (Fig. 4.13 A, B & C) and very small abaxial cells (Fig. 4.13 B). *C. carnosa* adaxial epidermal cells are irregular in size with a thin cuticle (Fig. 4.14 A). The abaxial layer contained smaller cells (Fig. 4.14 A & B). In all taxa, stomata were noted in the abaxial epidermis only.

The leaves of all Asclepiadaceae sectioned were unifacial. The palisade mesophyll consisted of one layer of cells in *S. alpinii* (Fig. 4.10 B & C), *A. fructicosa* (Fig. 4.10 D & E) and *A. physocarpa* (Fig. 4.12 B & D). *C. obtusifolium* (Fig. 4.13 A to D) and *C. carnosa* (Fig. 4.14 A & C) showed a bilayer of palisade mesophyll cells with the occasional long cell traversing both layers. Only *S. alpinii*, *A. physocarpa* and *C. carnosa* did not have any tanniniferous cells present. The tannin cells of *A. fructicosa* were very much bigger than all other cells (Fig. 4.10 D & E).

In most species, the spongy mesophyll consisted of rounded, irregular cells. The only exception was *A. physocarpa* in which the cells were elongated with the long axis perpendicular to the epidermis (Fig. 4.12 E & F). *S. alpinii* (Fig. 4.10 C), *C. carnosa* (Fig. 4.14 A to D) and *C. woodii* (Fig. 4.14 E to H) did not have any tannin cells in the spongy mesophyll. In *A. physocarpa* (Fig. 4.12 E & F) and *C. obtusifolium* (Fig. 4.13 B, C & F) tannin cells were few and dispersed amongst spongy mesophyll cells. Tannin cells were abundant in *A. fructicosa* (Fig.s 4.10 D & E and 4.11). Crystals were noted in tissues of *A. fructicosa* (Fig.s 4.10 D & E and 4.11) and *C. obtusifolium* (Fig. 4.13 F).

The association of mesophyll cells with veins was determined as being close for the most part, but especially so in *A. fructicosa* (Fig.s 4.10 G & H and 4.11), *A. physocarpa* (Fig. 4.12 B, D, E & F), *C. obtusifolium* (Fig. 4.13 C, E & F) and *C. woodii* (Fig. 4.14 E). Air spaces were small and cells closely packed in these taxa.

Vein anatomy

A chlorophyllous bundle sheath surrounded vascular tissues in all species. Bundle sheath cells were elongated and box-like in *A. fructicosa* (Fig.s 4.10 G & H and 4.11), and rounded in all others. The bundle sheath cells of *C. woodii* were poorly defined and looked like normal mesophyll cells (Fig. 4.14 E to H). A few bundle sheath cells with extensions were seen in *A. physocarpa* (Fig. 4.12 C, E & F), *C. obtusifolium* (Fig. 4.13 F) and *C. carnosa* (Fig. 4.14 B & C). Occasionally in paradermal sections, it was possible to cut through the bundle sheath cells overlying a vein. In these instances a view of the surface of the sheath could be ascertained (Fig.s 4.12 F and 4.13 F).

The midvein or primary vein can be described as being robust in *S. alpinii* (Fig. 4.10 A), *A. physocarpa* (Fig. 4.12 A) and *C. obtusifolium* (Fig. 4.13 A). The midvein in both *A. fructicosa* (Fig. 4.10 F) and *C. carnosa* (Fig. 4.14 A) was small and delicate by comparison. Bicollateral phloem was noted in all species except *A. fructicosa* (Fig. 4.10 F). The *S. alpinii* midvein was supported by collenchyma on the abaxial side. A thin cambial layer was present. Xylem was arranged in neat rows, with small pockets of phloem (Fig. 4.10 A). The cambial layer of *A. fructicosa* was very narrow and difficult to define, with little xylem or phloem tissue present (Fig. 4.10 F). Large laticifers followed the veins. *A. physocarpa* had an obvious cambial layer in the midvein, which was supported on the abaxial side by collenchyma (Fig. 4.12 A). The cambial layer of the *C. obtusifolium* midvein was wide, with neat rows of radiating xylem vessels. The midvein was supported by collenchyma on both sides (Fig. 4.13 A). The midvein of *C. carnosa* was very small, with little xylem tissue present and few pockets of phloem (Fig. 4.14 A). The cambial layer was narrow and poorly defined. No supporting collenchyma was noted.

Bicollateral phloem was noted in the secondary veins of *A. physocarpa* (Fig. 4.12 B), *C. obtusifolium* (Fig. 4.13 B) and *C. woodii* (Fig. 4.14 F). That of *C. ellpiticum* was supported by collenchyma (Fig. 4.13 B).

In all instances, phloem was seen in the minor veins surrounding the areoles (Fig.s 4.10 B, G & H, 4.11, 4.12 C, D & E, 4.13 F and 4.14 B, D & H). Phloem stained darkly in *A. fructicosa* (Fig.s 4.10 G, H & I and 4.11), *A. physocarpa* (Fig. 4.12 E), *C. obtusifolium* (Fig. 4.13 E & F) and *C. woodii* (Fig. 4.14 E). All the ultimate veinlets examined contained only xylem (Fig. 4.11). Laticifers were seen to follow minor veins in *A. physocarpa* (Fig. 4.12 F).

Figure 4.10 Asclepiadaceae: Aspects of the leaf anatomy of *Secamone alpinii* and *Asclepias fructicosa*

A Transverse section of *Secamone alpinii* midvein showing small pockets of bicollateral phloem, xylem, supporting collenchyma and overlying palisade mesophyll, B Transverse section of *S. alpinii* lamina with fifth order vein showing xylem and abaxial phloem bounded by chlorophyllous bundle sheath cells at the spongy/palisade mesophyll interface, C Transverse section of *S. alpinii* lamina showing fifth order ultimate veinlets with xylem and phloem, and widely spaced spongy mesophyll overlaid by palisade mesophyll, D Transverse section of *Asclepias fructicosa* lamina showing seventh order ultimate veinlets of xylem surrounded by chlorophyllous bundle sheath cells at spongy/palisade mesophyll interface, very large tanniniferous cells interspersed amongst mesophyll layers, E Transverse section of *A. fructicosa* lamina with fifth/sixth order vein showing xylem and abaxial phloem bounded by a chlorophyllous bundle sheath at spongy/palisade mesophyll interface, tanniniferous cells interspersed amongst mesophyll layers, F Transverse section of delicate midvein of *A. fructicosa* showing little xylem or abaxial phloem, G Paradermal section of *A. fructicosa* lamina with fourth and excurrent fifth order veins forming imperfect areoles, showing xylem and phloem bounded by chlorophyllous bundle sheath cells amongst spongy mesophyll cells, some of which are tanniniferous, H Paradermal section of *A. fructicosa* lamina with tertiary and quaternary veins forming imperfect areoles, showing xylem and darkly-stained phloem in longitudinal section bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, some of which are tanniniferous, I Paradermal section of *A. fructicosa* lamina with secondary vein showing darkly-stained phloem bounded by chlorophyllous bundle sheath cells, embedded in spongy mesophyll interspersed with tanniniferous cells

(A = areole; BS = bundle sheath; BSX = bundle sheath extension; C = crystal; E = epidermis; I = midvein; II = secondary vein; III = tertiary vein; IV = quaternary vein; P = phloem; PM = palisade mesophyll; S = stoma; SM = spongy mesophyll; T = tanniniferous cell; UV = ultimate veinlet; V = fifth order vein; X = xylem)

Bar represents 50μm for A, C and F, 25μm for B, D, E, G and H, and 12.5μm for I

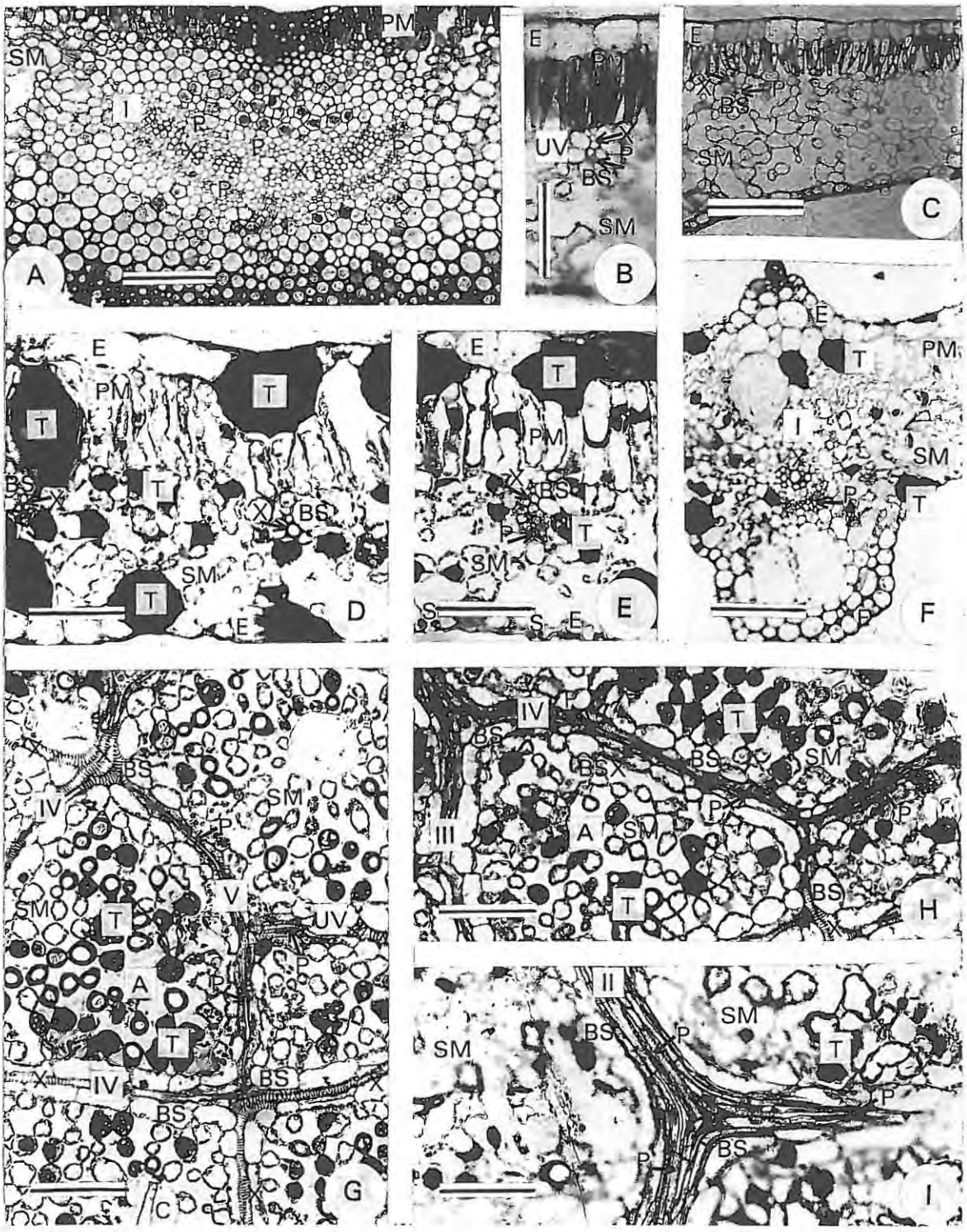
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Figure 4.11 Asclepiadaceae: Aspects of the leaf anatomy of *Asclepias fructicosa*

Composite plate of paradermal section of *Asclepias fructicosa* lamina with imperfect areoles and simple to once-branched, sixth to seventh order ultimate veinlets, showing xylem and darkly-stained phloem bounded by chlorophyllous bundle sheath cells, embedded in spongy mesophyll to the left of the plate and palisade mesophyll to the right, mesophyll layers interspersed with crystals and tanniniferous cells

(A = areole; BS = bundle sheath; C = crystal; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; T = tanniniferous cell; UV = ultimate veinlet; X = xylem)

Bar represents 50μm

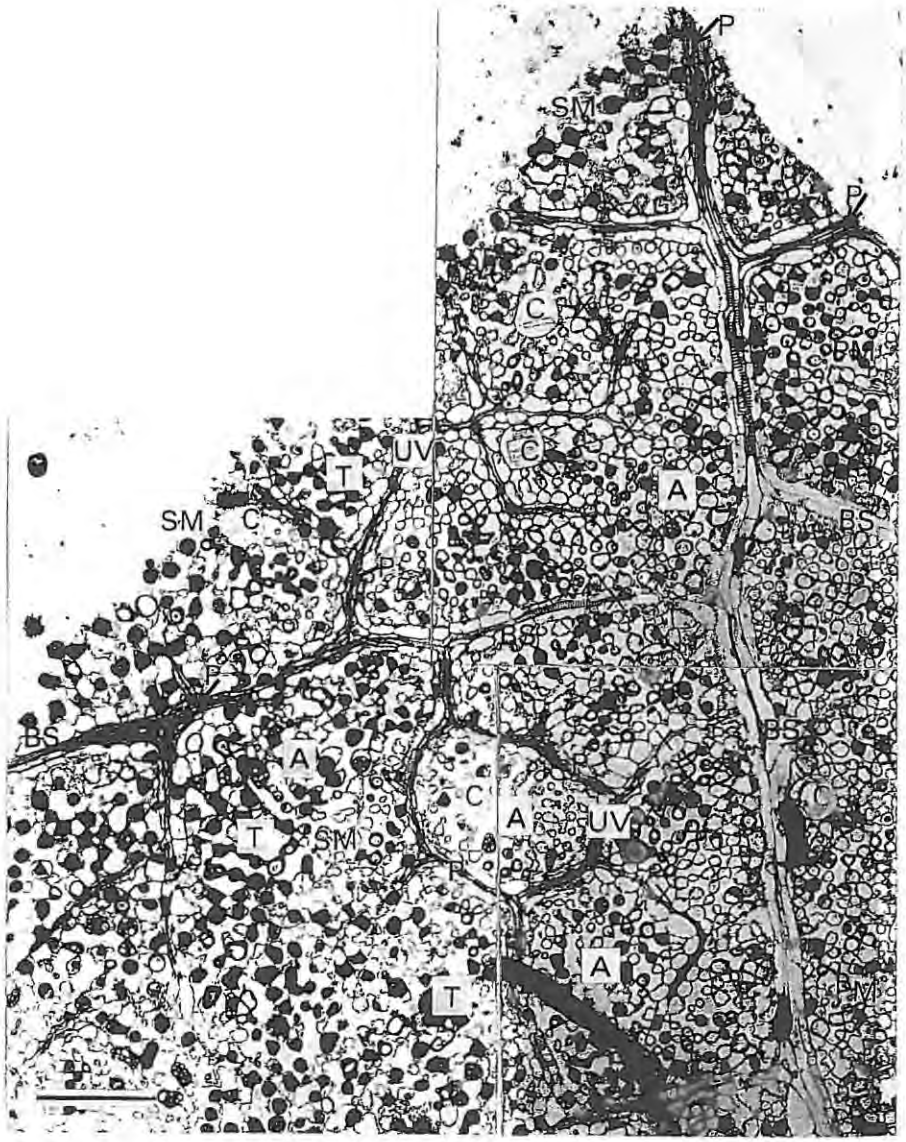
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Figure 4.12 Asclepiadaceae: Aspects of the leaf anatomy of *Asclepias physocarpa*

A Transverse section of *Asclepias physocarpa* midvein showing xylem, cambium, and pockets of bicollateral phloem, supported by collenchyma with laticifers, spongy and palisade mesophyll layers begin on either side of the midvein, B Transverse section of *A. physocarpa* secondary vein showing xylem and bicollateral phloem bounded by chlorophyllous bundle sheath cells at the spongy/palisade mesophyll interface, C Transverse section of *A. physocarpa* fifth to sixth order vein in lamina showing xylem and abaxial phloem bounded by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, D Transverse section of *A. physocarpa* lamina showing minor veins ranging from fourth to sixth order, with xylem and abaxial phloem bounded by chlorophyllous bundle sheath cells at the spongy/palisade mesophyll interface, E Paradermal section of *A. physocarpa* lamina with imperfect areoles and simple to once-branched ultimate veinlets of sixth to seventh order, showing xylem and darkly-stained phloem in longitudinal section bounded by chlorophyllous bundle sheath cells, embedded in spongy mesophyll, F Paradermal section of *A. physocarpa* lamina showing laticifers and chlorophyllous bundle sheath cells overlying minor veins embedded in spongy mesophyll

(A = areole; BS = bundle sheath; CA = cambium; E = epidermis; I = midvein; II = secondary vein; L = laticifer; P = phloem; PM = palisade mesophyll; S = stoma; SM = spongy mesophyll; UV = ultimate veinlet; X = xylem)

Bar represents 25μm for A, D and F, 12.5μm for B and C, and 50μm for E

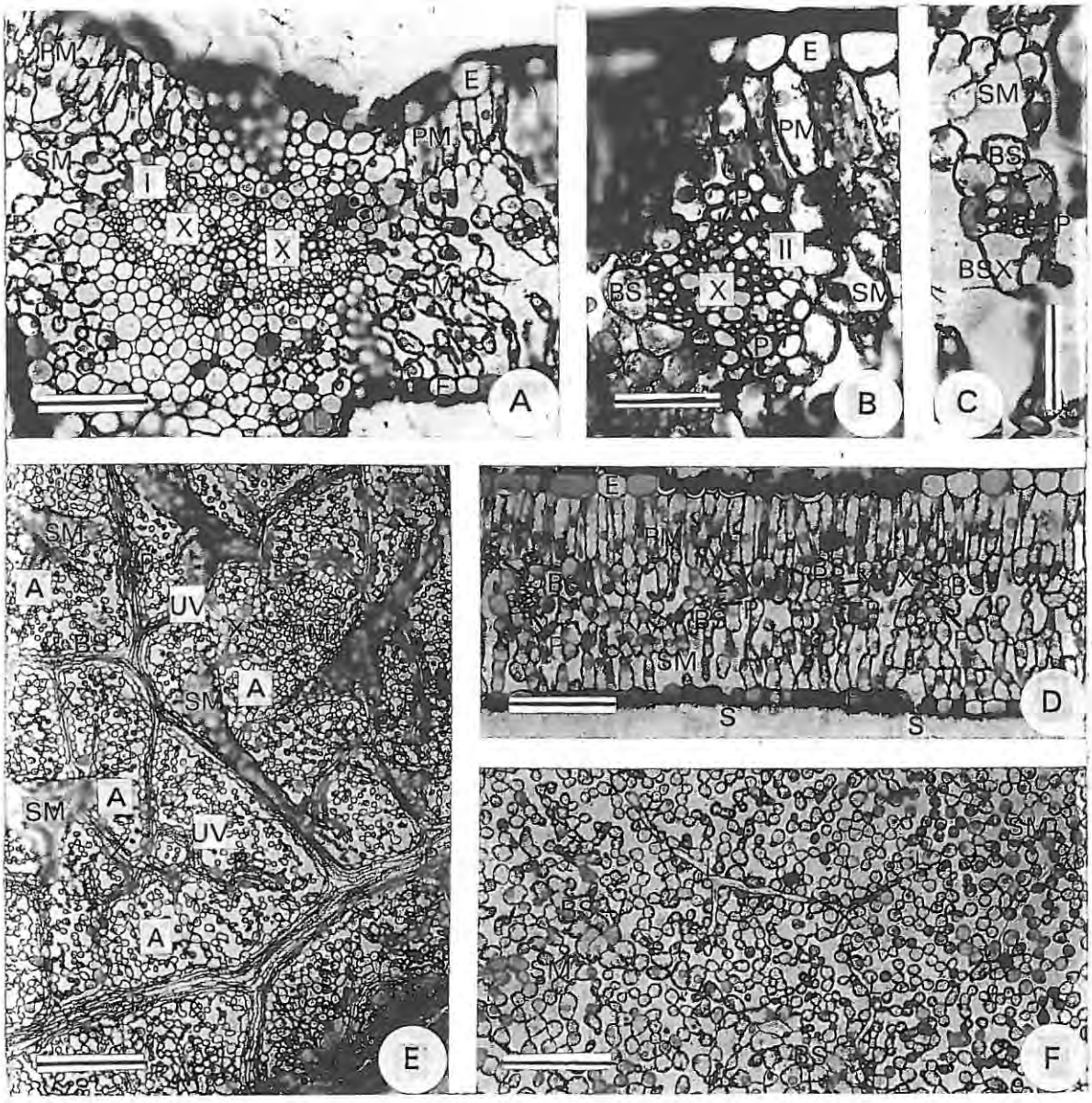
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Figure 4.13 Asclepiadaceae: Aspects of the leaf anatomy of *Cynanchum obtusifolium*

A Transverse section of *Cynanchum obtusifolium* midvein showing xylem, a wide cambial layer, small pockets of bicollateral phloem, with supporting collenchyma both above and below, B Transverse section of *C. obtusifolium* lamina with secondary vein at centre showing xylem and small pockets of bicollateral phloem, sixth order ultimate veinlet at left showing xylem and abaxial phloem, and tertiary to quaternary vein at right showing xylem and abaxial phloem, vascular tissues bounded by a chlorophyllous bundle sheath, embedded at the spongy/palisade mesophyll interface, mesophyll layers interspersed with tanniniferous cells, C Transverse section of *C. obtusifolium* lamina with sixth order vein at left and quaternary to fifth order vein at left vein, both showing xylem and abaxial phloem bounded by a chlorophyllous bundle sheath, embedded at the spongy/palisade mesophyll interface, D Transverse section of *C. obtusifolium* fifth order vein showing xylem and abaxial phloem bounded by a chlorophyllous bundle sheath, embedded at the spongy/palisade mesophyll interface with large tanniniferous cells amongst palisade mesophyll cells, E Paradermal section of *C. obtusifolium* lamina with tertiary vein, imperfect areoles and simple to once-branched, fifth to seventh order ultimate veinlets, showing darkly-stained phloem bordered by chlorophyllous bundle sheath cells in spongy mesophyll, F Composite of paradermal section of lamina of *C. obtusifolium* with imperfect areoles and once-branched ultimate veinlets showing darkly-stained phloem bordered by chlorophyllous bundle sheath cells in spongy mesophyll interspersed by tanniniferous cells and crystals

(A = areole; BS = bundle sheath; C = crystal; CA = cambium; E = epidermis; I = midvein; II = secondary vein; III = tertiary vein; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; T = tanniniferous cell; UV = ultimate veinlet; X = xylem)

Bar represents 50μm for A, B and F, 25μm for C, 12.5μm for D, and 12.5μm for E

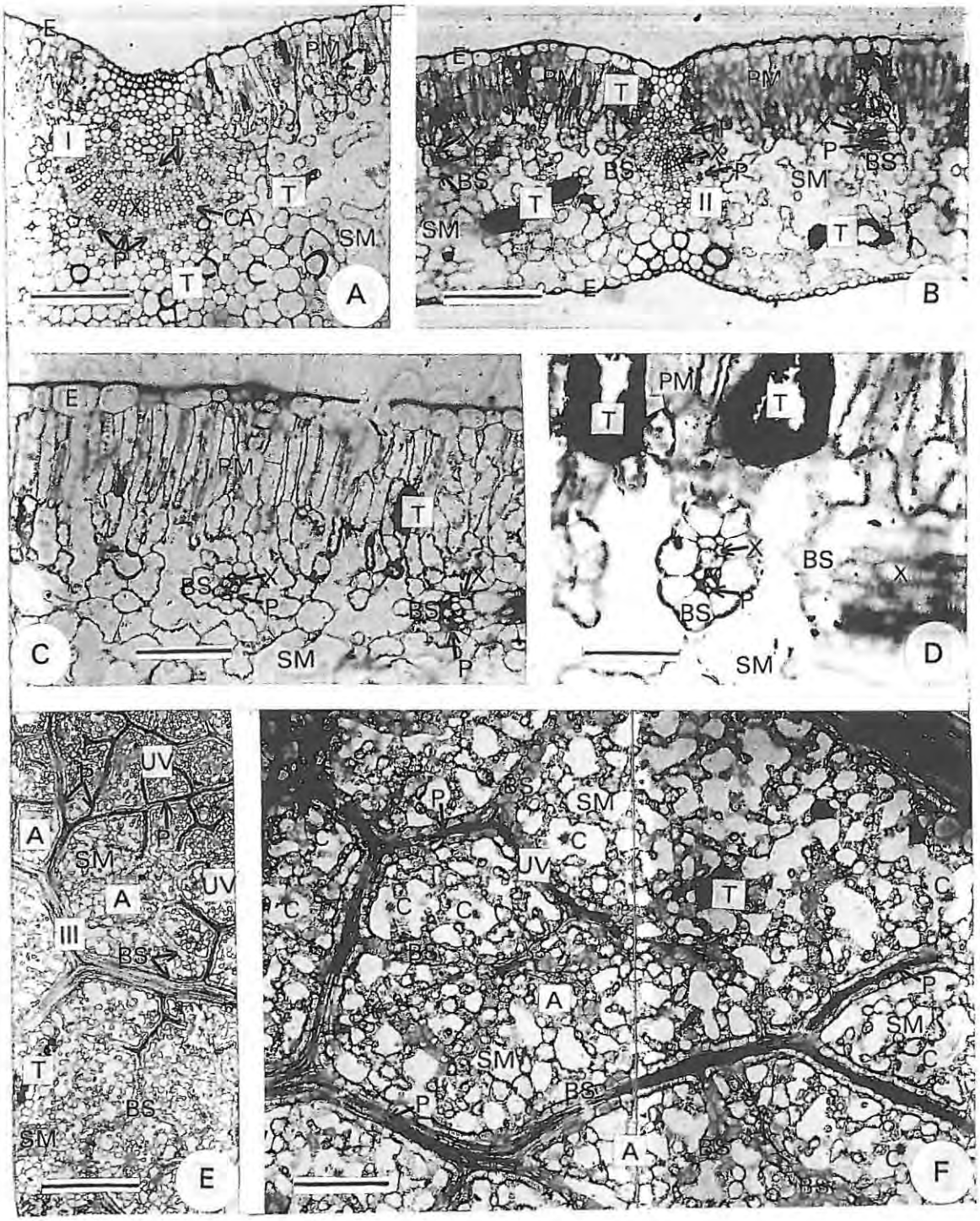
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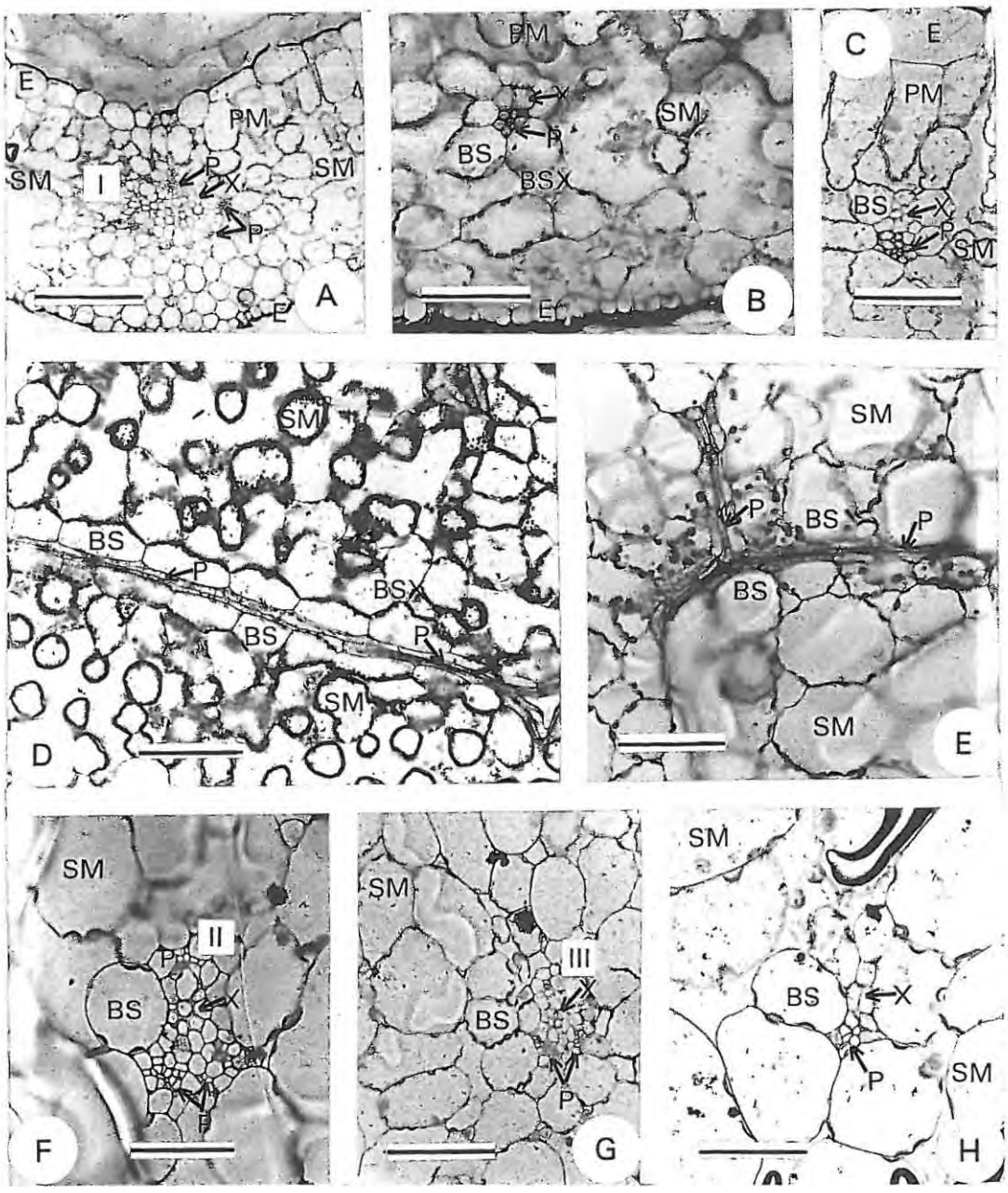
Figure 4.14 Asclepiadaceae: Aspects of the leaf anatomy of *Ceropegia carnosa* and *C. woodii*

A Transverse section of delicate midvein of *Ceropegia carnosa* showing little xylem and few, small pockets of bicollateral phloem, B Transverse section of *C. carnosa* fifth order vein in lamina showing xylem and abaxial phloem bounded by chlorophyllous bundle sheath cells with extensions to spongy mesophyll cells, C Transverse section of *C. carnosa* tertiary vein in lamina showing xylem and abaxial phloem bounded by chlorophyllous bundle sheath cells at spongy/palisade mesophyll interface, D Paradermal section of *C. carnosa* lamina with fourth order vein showing darkly-stained phloem in longitudinal section bordered by chlorophyllous bundle sheath cells with extensions to surrounding spongy mesophyll cells, E Paradermal section of *C. woodii* lamina with fifth order vein showing darkly-stained phloem in longitudinal section bordered by poorly defined chlorophyllous bundle sheath cells embedded in spongy mesophyll, F Transverse section of *C. woodii* secondary vein in lamina showing xylem and small pockets of bicollateral phloem bounded by poorly defined chlorophyllous bundle sheath cells embedded in spongy mesophyll, G Transverse section of *C. woodii* tertiary vein in lamina showing xylem and abaxial phloem bounded by poorly defined chlorophyllous bundle sheath cells embedded in spongy mesophyll, H Transverse section of *C. woodii* fifth order vein in lamina showing xylem and abaxial phloem bounded by poorly defined chlorophyllous bundle sheath cells embedded in spongy mesophyll

(BS = bundle sheath; BSX = bundle sheath extension; E = epidermis; I = midvein; II = secondary vein; III = tertiary vein; P = phloem; PM = palisade mesophyll; SM = spongy mesophyll; X = xylem)

Bar represents 50μm for A, 25μm for B, C, D, E and G, and 12.5μm for F and H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4.3 Discussion

4.3.1 Qualitative data

As in the case of the paper by Lersten (1990), similar aspects arise in that due to the constraints of time and the number of taxa involved, the data presented here are purely qualitative. Descriptions of features seen are detailed and illustrated for certain taxa. What began at the level of gross venation in Chapter Three proceeds to the anatomical level now in Chapter Four.

4.3.2 The relationship between photosynthetic mesophyll and phloem

As has already been noted, the proximity of photosynthetic mesophyll to phloem is of great importance to loading efficiency (Haberlandt 1914, Wylie 1939, Tucker 1964, Esau 1972, Fisher & Evert 1982, Giaquinta 1983, Fisher 1990, Nelson & Dengler 1997). There are a number of factors that need to be considered in this regard.

Firstly, the presence of sieve tubes in minor veins is essential for loading to occur in that particular location. As described for all three families, sieve tubes were not seen in free vein endings in areoles (Fig.s 4.2 C & D, 4.4, 4.5 G, 4.7 and 4.9 E & F), nor in the marginal ultimate veinlets of the apocynate *Acokanthera oppositifolia* (Fig. 4.3 G). It would appear then, that ultimate veinlets are not involved in loading of assimilates as such. Minor veins binding areoles, thereby delimiting areas of photosynthetic mesophyll, contained sieve tubes indicating a potential for loading. This is supported by the fact that in most cases, the phloem of minor veins stained darkly. Such a reaction has been interpreted in the literature as being indicative of intense metabolic activity, as would be expected in the event of active loading of photosynthate (Morretes 1962, Lersten & Carvey 1974).

Secondly, bundle sheath cell features and type are important, as these cells connect mesophyll to phloem in the loading pathway. When viewed in conjunction with the surrounding tissues, the primary function of a particular vein order can be inferred (Fisher & Evert 1982, Russin & Evert 1984, Fisher 1990). The midvein of most species studied here was embedded in collenchyma (Fig.s 4.1 A, 4.5 A, 4.6, 4.10 A, 4.12 A and 4.13 A). This supporting tissue distanced the phloem from the photosynthetic mesophyll, making a loading route into such a vein inefficient and therefore unlikely. The replacement of a chlorophyllous bundle sheath with achlorophyllous

supporting tissues indicates a function primarily concerned with mass translocation (Fisher & Evert 1982, Russin & Evert 1984, Fisher 1990).

Higher order veins showed chlorophyllous, thin-walled bundle sheath cells, often with bundle sheath extensions (Dannenhoffer *et al.* 1990). The fact that these cells have thin walls and chloroplasts makes them very similar to mesophyll cells in appearance and therefore possibly in function. In such instances the bundle sheath cells become part of the paraveinal mesophyll, aiding in the photosynthetic effort, and forming a direct link to phloem sieve tubes.

Thirdly, the spatial organization and density of minor veins across the lamina is of importance. Obviously, the less the density and organization, the slower and more inefficient the loading route, and vice versa. *Ranunculus multifidus*, a mesic member of the Ranunculaceae, did not show efficient lamina coverage, with wide interveinal distances (Fig.s 3.1 B, 3.2 A, B & C and 4.1 E & G). Bundle sheath cells of this species had few chloroplasts compared with mesophyll cells. Large air spaces separated individual mesophyll cells, and veins and mesophyll (Fig. 4.1 A to I). Insufficient replicas are presented here to discuss in depth the concept of interveinal distances, as proposed by Wylie (1939 & 1946). However, the impression obtained when considering the data for this family, is that of a widely spaced network of relatively few, long, winding routes from mesophyll to phloem sieve tubes (Wylie 1939 & 1946).

Densely-clustered mesophyll cells occurred in close association with phloem in species of Apocynaceae and Asclepiadaceae sectioned. This is not surprising, as these taxa are all from more xeric environments (Appendix IV & V respectively), than *R. multifidus* (Appendix III). In conjunction with bundle sheath cells being very similar in appearance to mesophyll cells, bundle sheath extensions to the surrounding mesophyll cells were seen. This qualifies the bundle sheath cells to be classified as paraveinal mesophyll, and forms a direct link in the phloem loading route (Franceschi & Giaquinta 1983a). This can be seen clearly in *C. woodii*, in which bundle sheath cells of minor veins are indistinguishable from mesophyll cells (Fig. 4.14 E to H). This dense association of many mesophyll cells, bundles sheath cells and sieve tubes in the wide robust veins characteristic of these two families facilitate many potential loading routes, allowing for rapid, efficient removal of photosynthate.

An interesting anatomical extension to Van Bel and Gamalei's theory (Gamalei 1985a, b, 1989, 1991,Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996) would be to consider a possible correlation between symplasmic and apoplasmic phloem loading and bundle sheath features. Is there a consistent progression from elongated, bundle sheath cells with few chloroplasts and extensions, apparently characteristic of primitive symplasmic loaders, to rounded, paraveinal mesophyll/bundle sheath cells, characteristic of apoplasmic loaders? This distinction is noticed in the current study, although the assumption is made that the Ranunculaceae will be symplasmic loaders and the Apocynaceae and Asclepiadaceae will be apoplasmic loaders. More examples are needed from all three families, and other families of dicotyledonous plants, before any serious postulations can be made.

4.3.3 Leaf anatomy related to vein order

The only real distinction in anatomy of differing vein orders of the Ranunculaceae, is the cell type constituting the bundle sheath. Characteristically, the midvein exhibited a collenchymatous bundle sheath (Fig. 4.1 A), whilst higher order veins tended towards a chlorophyllous, thin-walled bundle sheath (Fig. 4.1 B to I). From midvein to minor veins, a progressive decrease in xylem and phloem tissues was apparent, yet no clear distinction was ascertained in anatomy of orders. Vein endings consisted of xylem only, with no phloem discernable. Therefore on an anatomical basis, only the midvein and free ending ultimate veinlets can be separated out from other vein orders in *R. multifidus*.

The Apocynaceae also showed a clearly distinct midvein with supporting tissue (Fig.s 4.5 A and 4.6). Secondary veins appeared as smaller versions of the midvein, lacking supporting tissue (Fig. 4.3 C). Cambium and bicollateral phloem were present in both midvein and secondary veins of species examined. From tertiary to minor veins it becomes increasingly difficult to determine vein order using anatomy. Bundle sheath cells are all chlorophyllous.The only possible hint as to vein order is the gradual decrease in xylem and phloem tissues. Phloem is present in minor veins around areoles (Fig. 4.4, 4.7 and 4.9 E), but is absent from the free-ending ultimate veinlets inside areoles (Fig.s 4.2 D, 4.4, 4.7 and 4.8) and from marginal ultimate veinlets (Fig. 4.3 G). Again, this anatomical feature sets the ultimate veinlets apart from minor veins delimiting areoles. Laticifers followed veins of all orders. The only useful observation in this regard being that the number of

laticifers present decreased numerically with vein order, down to just one in minor veins.

Therefore, the midvein can be distinguished by its size, and the presence of a cambial layer, bicollateral phloem, supporting tissue and many laticifers. Secondary veins are smaller versions of the midvein, with less supporting tissue. Minor veins have no supporting tissue, bicollateral phloem or cambium, are progressively smaller with fewer laticifers, and have a chlorophyllous bundle sheath. Minor veins have both phloem and xylem, a chlorophyllous bundle sheath, and usually only one laticifer. Ultimate veinlets lack phloem.

Taxa of the Apocynaceae sectioned had similar anatomies, probably due to the uniformity in habitat and habit exhibited by the taxa examined (Appendix IV).

The Asclepiadaceae showed a distinct similarity to the Apocynaceae in terms of the above description of vein anatomy. Bicollateral phloem was present in the midvein of most species sectioned, and in the secondary veins of *A. physocarpa* (Fig. 4.12 B), *C. obtusifolium* (Fig. 4.13 B) and *C. woodii* (Fig. 4.14 F). Bicollateral phloem was not noted in all taxa sectioned, being absent from the small, delicate midvein of *A. fructicosa* (Fig. 4.10 F). Bicollateral phloem can therefore only be used to separate lowest order veins from other orders. Schmitz *et al.* (1987) noted bicollateral bundles all the way to higher order veins in *Cucumis melo*, but that the adaxial phloem ended before the tips of ultimate veinlets while abaxial phloem extended all the way. Bundle sheath features could be used to discern the midvein from other orders. The midvein was, most often, supported by collenchyma, lacking a chlorophyllous bundle sheath (Fig.s 4.10 A, 4.12 A and 4.13 A). Secondary to minor veins around areoles exhibited progressively more mesophyll-like bundle sheath cells. Concurrent with this trend was a gradual decrease in amounts of xylem and phloem tissues present. Minor veins could be separated from ultimate veinlets due to a lack of phloem in the latter (Fig. 4.11). Again, only bundle sheath cell type, the presence of bicollateral phloem and phloem in general may be used to infer vein order from anatomical data.

The wider anatomical variation seen in veins of the same order most probably reflects the greater variety in habit and habitat present in this very large family (Appendix V) (Carlquist 1991). There are no strikingly aberrant deviations, just differing amounts of the same tissue and the loss of superfluous ones.

4.3.4 Anatomy related to taxonomy

As with the data presented in Chapter Three, nothing has been noted to hinder the placement of the Asclepiadaceae in the Apocynaceae. Anatomically, species sectioned from both families appeared very similar. Bicollateral bundles, crystals and laticifers were noted from both. Conversely, it must be stated that no single feature could be used to support this amalgamation at an anatomical level due to the generality of the data presented.

4.3.5 Hydathodes of *Ranunculus*

Ranunculus is a cosmopolitan genus (Heywood 1978). Foliar hydathodes are described for certain taxa in Metcalfe Chalk 1950, yet there is little to no published data on southern African taxa. All species of *Ranunculus* examined here were noted to have epithem hydathodes at leaf and serration apices. Whether these hydathodes are functional or not was not determined and is a moot point. On an anatomical basis, these structures can be defined as hydathodes, even though functionality has not been demonstrated (Metcalfe & Chalk 1950). The presence of an epithem and water pores is sufficient for them to be designated hydathodes. In order to be retained, even though anatomical features are conservative, the hydathodes would have to confer an advantage of some sort on the plant as a whole. Hydathodes are not uncommon in mesic taxa, providing an outlet for excess water. The fact that they are absent from grassland taxa of the Ranunculaceae studied here is interesting (Appendix III).

4.3.6 From leaf clearings and anatomy, to ultrastructure

The morphology and anatomy of leaves of selected Ranunculaceae, Apocynaceae and Asclepiadaceae have been described fully. This should provide adequate background to the ultrastructural aspects of phloem discussed in the following chapter. Although much has been gleaned from the leaf clearings and anatomical data, it is only at the ultrastructural level that the ultimate aim of this thesis may be considered. Does the ultrastructure suggest symplasmic or apoplasmic phloem loading in the species studied? Will the minor vein configurations of these families fulfil the hypothesis set out? Chapter Five presents ultrastructural studies for consideration in this regard.

CHAPTER FIVE: PHLOEM ULTRASTRUCTURE

5.1 Introduction

Bieleski (2000) rates the development of phloem transport as an extremely important evolutionary event in the dawning of land plants. With increasing size and exploitation of two media, viz. soil and air, specialized plant tissues and organs were required, further necessitating a system for the passage of water and nutrients upwards, and photosynthates downwards. In order to understand the complex functioning of higher, land plant phloem, a study of lower plant phloem might provide insight into less advanced phloem function (Van Bel & Knoblauch 2000). General trends in phloem evolution include increased elongation, interconnectivity and wall width of sieve elements to produce long, narrow tubes, almost empty except for a peripheral layer of cytoplasm. With the loss of the sieve element nucleus, making room for longitudinal flow, neighbouring parenchyma cells took on a specialized dependence, with increased cytoplasmic connectivity to adjacent sieve elements. These became the companion cells and controlled the physiology of sieve elements (Van Bel & Knoblauch 2000).

Cook *et al.* (1997) examined plasmodesmata of Charophycean algae and bryophytes in order to better understand plasmodesmata of higher land plants. The plasmodesmata of these primitive plants displayed the same internal basic pattern as those of higher plants. Specialization and an increase in complexity occur in higher plants, as is to be expected after many millions of years of evolution (Cook *et al.* 1997).

The embryos of higher plants apparently show initial symplasmic continuity between all neighbouring cells. With tissue differentiation and the loss or occlusion of plasmodesmata, symplasmic isolation of cells or tissues occurs. This allows for separate functional compartments within the plant body. McLean *et al.* (1997) describes mature flowering plants as "mosaics of symplasmic domains." Sjölund (1997) takes this concept one step further by describing the phloem sieve tube continuum as a syncytium, i.e. an isolated symplasmic domain bounded by a continuous plasma membrane. Transport via the phloem compartment therefore occurs within cells (Gunning & Overall 1983), requiring integration of cellular function within and amongst phloem cells (Van Bel 1996). The phloem compartment, or symplasmic domain, extends throughout the plant body, being essential for nutrient flow and communication (Gunning &

Overall 1983, Epel 1994, Cook *et al.* 1997, McLean *et al.* 1997, Sjölund 1997, Van der Schoot & Rinne 1999, Turgeon 2000, Botha & Cross 2001).

Oparka and Van Bel (1992) divide the phloem domain into three interleading compartments, viz. the production, collection and export compartments. The concept of phloem loading is expressed as a collaboration of the production and collection compartments. In a later paper, Van Bel (1996) revisits this idea, envisaging the phloem continuum as three successive sections, viz. the collection, transport and release phloem. Each section has a certain function to perform, albeit in opposite directions for collection and release phloem. Transport phloem is regarded as having a dual function. The first involves providing food to stem and lateral meristem tissues through leakage, whilst the second is long distance transport, by maintenance of pressure flow, of food to terminal sinks.

The focus of this thesis is the collection phloem, with a brief mention of transport phloem as it pertains to major veins in the leaf. It must also be remembered that all cell types along the loading pathway, from production site to destination in veins, show important ultrastructural features with regard to phloem loading method and therefore deserve mention.

5.1.1 Ultrastructure of the loading pathway

The phloem loading pathway begins in the chlorophyllous mesophyll, as this is the site of photosynthate production (Russin & Evert 1985, McCauley & Evert 1989). The transfer step from mesophyll domain to collection phloem in the sieve tube/companion cell (ST/CC) complex is crucial to phloem loading (Oparka & Van Bel 1992, Van Bel 1996). Although the majority of taxa examined in the literature show symplasmic connectivity though the mesophyll to the minor vein interface, the range of plasmodesmatal frequency is great (Erwee *et al.* 1985, Fisher 1986, Fisher 1988, Van Bel et al. 1988, Robinson-Beers & Evert 1991a, Turgeon and Beebe 1991, Beebe & Evert 1992).

On examining the mesophyll cells of spinach leaves, Warmbrodt and Van Der Woude (1990) noticed an interesting feature of the plasma membrane in regions of chloroplasts. The same phenomenon was seen in *Beta vulgaris* leaf mesophyll by Evert and Mierzwa (1986). The plasma membrane showed a great increase in surface area due to the presence of invaginations. Outer

chloroplast envelopes showed corresponding evaginations in the same region. This was suggested to greatly increase the symplast-apoplast interface and to offer structural evidence of an apoplasmic step in the export of assimilates into the free space (Evert & Mierzwa 1986, Warmbrodt & Van Der Woude 1990). The presence of starch in mesophyll cells can also be taken as an indication of symplasmic phloem loading. Apoplasmic loaders have been found to have very little in the way of starch deposits (Oparka & Van Bel 1992, Volk *et al.* 1996).

Surrounding the vascular tissues, bundle sheath cells lie directly in the path of assimilates en route to the collection phloem (Oparka & Van Bel 1992). Bundle sheath cells may or may not be chlorophyllous themselves (Giaquinta 1983, Russin & Evert 1985, Beebe & Evert 1992), but are nevertheless regarded as being part of the production compartment in the apoplasmic model of phloem loading. Assimilates are supposedly released from the production compartment into the free space or apoplast, and then loaded apoplasmically into the ST/CC complexes (Oparka & Van Bel 1992).

Bundle sheath cells may have suberized walls, forcing a symplasmic route into the vascular tissue (Evert & Mierzwa 1986, Robinson-Beers & Evert 1991a). Evert *et al.* (1978), studying *Zea mays*, noted the occurrence of suberin in the bundle sheath walls near plasmodesmata. Suberin lamellae may form localized barriers around bundle sheath cells, such as Casparian strips in roots, creating an apoplasmic step across the cell membrane and protoplast (Fisher 1986).

Esau (1973) comments that vascular parenchyma and companion cells have much in common, sharing similar origin, cytology and relation to the sieve element. The vascular parenchyma cells of minor veins are described as being large with many organelles (Esau 1972, 1973). Esau and Cronshaw (1968) describe plastids of vascular parenchyma as showing the greatest degree of internal development amongst phloem cell types. Such plastids are recognisable as chloroplasts with grana and starch grains.

In *Pisum sativum*, Wimmers and Turgeon (1991) note that vascular parenchyma cells are often adjacent to bundle sheath and transfer cells, and therefore are possibly involved in the loading route. Plasmodesmata were seen to be most abundant between vascular parenchyma cells themselves, and less so between vascular parenchyma and other cells types of the phloem. Whilst

vascular parenchyma cells of *Mimosa pudica* were noted to have plasmodesmatal connections with sieve elements, these were not as numerous as between companion cells and sieve elements. Vascular parenchyma cells are described as being infrequent in small and intermediate bundles of sugarcane, but where present are abundantly connected to bundle sheath cells by plasmodesmata (Robinson-Beers & Evert 1991a). Russin *et al.* (1996) noted that vascular parenchyma cells occupy most of the interface between bundle sheath cells and sieve tubes in minor veins of a maize mutant. However, aberrant plasmodesmata at the bundle sheath/vascular parenchyma interface forces an apoplasmic step, preventing sucrose loading and transport. The few companion cells present apparently cannot load fast enough to bypass this block.

Companion cells have traditionally been regarded as the site of sugar uptake for subsequent delivery into sieve elements (Warmbrodt & Van Der Woude 1990, Van Bel & Knoblauch 2000). Typical features of companion cells include a dense cytoplasm rich in organelles, and many pore-plasmodesmatal connections with associated sieve elements (Barnabas 1983, Turgeon 1989, Warmbrodt & Van Der Woude 1990). Pore-plasmodesmata are usually branched on the companion cell side (Van Bel & Knoblauch 2000). Esau and Cronshaw (1968) describe the plastids of companion cells as containing a dense matrix with few underdeveloped thylakoids.

Specialized forms of companion cells exist in dicotyledonous plants, specifically correlated with phloem loading method. Apart from normal companion cells, modifications called transfer and intermediary cells are described in the literature (Gunning *et al.* 1968, Pate & Gunning 1969, Van Bel *et al.* 1988, Turgeon 1989, Warmbrodt & Van Der Woude 1990, Flora & Madore 1996, Komor *et al.* 1996, Van Bel 1996, Gamalei *et al.* 2000, Turgeon 2000).

Transfer cells are symplasmically isolated, possessing wall ingrowths with increased surface area aiding apoplasmic phloem loading (Pate & Gunning 1969, Turgeon 1989, Wimmers & Turgeon 1991, Oparka & Van Bel 1992, Van Bel 1996, Gamalei *et al.* 2000). Wall ingrowths are noted to vary with the rate of assimilate transit (Wimmers & Turgeon 1991, Oparka & Van Bel 1992, Van Bel 1996). It has been noted in developmental studies that wall ingrowths are produced only when export of photosynthate begins in source leaves (Wimmers & Turgeon 1991). On examining solute flux rates and anatomies of plants grown in high-light and low-light, Wimmers and Turgeon (1991) concluded that the only obvious anatomical difference between the minor

veins of the two groups was the increase in surface area of the sieve element/transfer cell interface in high-light plants. This was correlated with increased net solute flux. It was therefore postulated that wall ingrowths allow for greater assimilate uptake by increasing surface area for apoplasmic phloem loading.

Symplasmic loaders possess companion cells termed intermediary cells (Fritz *et al.* 1983, Weisberg *et al.* 1988, Holthaus & Schmitz 1991, Beebe & Turgeon 1992, Turgeon 2000). Intermediary cells are connected with the mesophyll via numerous plasmodesmata (Fisher 1986, Schmitz *et al.* 1987, Weisberg *et al.* 1988, Turgeon 1989, Beebe & Turgeon 1992, Oparka & Van Bel 1992, Haritatos *et al.* 1996, Van Bel 1996, Turgeon 2000). A distinct feature of these cells is vesicular labyrinths, the volume of which appears to fluctuate with the rate of photosynthate transit (Schmitz *et al.* 1987, Oparka & Van Bel 1992, Van Bel 1996). Plastids without granal stacks or starch deposits were noted in intermediary cells of *Cucumis melo* and *Cucurbita pepo* (Volk *et al.* 1996). While the number of plastids did not increase as cells matured, mitochondrial number increased significantly. Volk *et al.* (1996) propose that the abundance of mitochondria is related to the energy required to synthesize oligosaccharides in intermediary cells, according to the polymer trapping model of symplasmic transport.

The phloem of *Coleus blumei* contained both large, laterally-positioned intermediary cells and smaller normal companion cells (Fisher 1986, Weisberg *et al.* 1988). Histochemical and microautoradiographic evidence apparently indicates that intermediary cells accumulate considerable quantities of assimilates, more so than the normal companion cells. A similar situation was seen in *Cucumis melo* (Schmitz *et al.* 1987), in which companion cells and intermediary cells were seen in the same bundle, usually in vein orders four to seven. Schmitz *et al.* (1987) also comment that vein orders one to three did not have any intermediary cells. These orders were determined to be involved in long distance transport, so it would be logical to assume from this data that intermediary cells are specialized for loading of assimilates.

The sieve element/companion cell complex so characteristic of phloem develops from the division of a phloem mother cell (Turgeon 1989, Sjölund 1997, Van Bel & Knoblauch 2000). The sole purpose of sieve tubes is conventionally seen as the transport of assimilates (Van Bel & Kempers 1990). Mature sieve elements do not have nuclei, vacuoles, Golgi bodies or ribosomes

(Sjölund 1997). These organelles are removed and thus allow for uninterrupted assimilate flow through the cell. Mature sieve elements show a clear protoplast with peripherally located cytoplasm containing ER, mitochondria and P-plastids, and fully developed sieve pores (Warmbrodt & Van Der Woude 1990, Knoblauch & Van Bel 1998. Van Bel & Knoblauch 2000).

Sieve element walls are modified to prevent them from bursting due to the high pressures generated inside (Van Bel & Knoblauch 2000). The cellulose microfibrils in the walls are orientated circularly around the sieve element. This decreases bulging and strain on the walls (Sjölund 1997).

Plasmodesmata in the walls between adjacent sieve elements enlarge to form pore-plasmodesmata to allow for large volumes of assimilate traffic. Pore-plasmodesmata range from 200 to 400nm in diameter and are generally branched on the companion cell side (Sjölund 1997, Van Bel & Knoblauch 2000).

Whilst companion cells have traditionally been regarded as the sites of sugar uptake, evidence from recent observations has suggested the plasma membrane of the sieve elements themselves may be capable of loading sugars from the apoplast (Warmbrodt & Van Der Woude 1990, Sjölund 1997). A localized protein, similar to a previously isolated sucrose binding protein, was discovered to occur on the plasma membrane of sieve elements of spinach leaf phloem. This protein did not occur in any other foliar cell types (Warmbrodt & Van Der Woude 1990).

The occurrence in the phloem of thick- and thin-walled sieve elements is well documented in the literature (Evert *et al.* 1978, Barnabas 1983, Fritz *et al.* 1983, Russin & Evert 1984, Fisher 1986, Van Bel *et al.* 1988, Van Kesteren *et al.* 1988, Evert & Mierzwa 1989, Dannenhoffer *et al.* 1990, Botha 1992). Thick-walled sieve tubes are metaphloem components (Barnabas 1983, Van Bel *et al.* 1988). Russin and Evert (1985) note that the thick-walled sieve elements of maize lack companion cells, but have numerous connections to vascular parenchyma cells. In studies of *Zea mays* leaf strips fed C^{14} sucrose, the sugar was taken up by the vascular parenchyma of the phloem and loaded directly into thick-walled sieve tubes (Fritz *et al.* 1983). Vascular parenchyma and thick-walled sieve tubes were interconnected by numerous plasmodesmata. The conclusions were that vascular parenchyma cells were able to retrieve labelled sucrose from the apoplast, that thick-

walled sieve tubes were not involved in long distance transport of the sucrose, transferring it immediately to thin-walled sieve tubes, and that thin-walled sieve tubes were also capable of sucrose retrieval, without mediation from companion or intermediary cells. Therefore, thick and thin-walled sieve tubes appeared to have different functions in phloem (Fritz *et al.* 1983).

The interaction of sieve element and companion cell is of the utmost importance to the functioning of the phloem (Van Bel 1996, Van Bel & Knoblauch 2000). The relative size of sieve element to companion cell offers hints as to the function of the companion cell in the different compartments of the phloem. Generally, companion cells are big in comparison with sieve element in collection phloem, whilst in release phloem the companion cells are very small and may even not be present. This suggests an important role for companion cells in the sugar uptake process (Van Bel 1996, Van Bel & Knoblauch 2000).

5.1.2 Plasmodesmatal structure and function in the symplasmic continuum

Plasmodesmata are defined as being structurally complex channels that cross the cell wall, connecting the protoplasts of adjacent plant cells and thereby allowing for intercellular communication (Olesen 1979, Goodwin 1983, Gunning & Overall 1983, Meiners *et al.* 1988, Van Bel 1988, Robards & Lucas 1990, Cook *et al.* 1997, McLean *et al.* 1997, Van der Schoot & Rinne 1999, Botha & Cross 2001). Structure, frequency and distribution, and plasmodesmatal function between all cell types must be considered in order to elucidate the potential phloem loading pathway (Madore et al. 1986, Warmbrodt & Van Der Woude 1990, Beebe & Evert 1992, Gamalei 1996, Russin *et al.* 1996, Van Bel & Knoblauch 2000).

Plasmodesmatal structure has been a subject of intense debate. Thomson and Platt-Aloia (1985) commented that the bulk of knowledge on plasmodesmata, at that stage, was based on ultrastructural studies. Such descriptive interpretations did not contemplate the chemical nature of the density patterns seen in electron micrographs. Restrictions imposed in terms of section thickness compared to the plasmodesmatal size obscured finer details. Furthermore, chemical fixation may produce artifacts as a wounding response to gluteraldehyde, resulting in the loss of detail and an altered plasmodesmatal state (Hughes & Gunning 1980, Turgeon & Beebe 1991, Ding *et al.* 1992). However, Botha et al. (1993) compared their results of chemically fixed plasmodesmata with those of rapid, high-pressure freezing techniques and found them to be of

an equally high standard in terms of detail and clarity.

Studies on the diffusion of lipids along the ER connected to the desmotubule show the ER of one cell, the connecting desmotubule and the ER of the adjacent cell to all belong to one continuous membrane system. This provides a membrane transport pathway (Overall *et al.* 1982, Goodwin 1983, Meiners *et al.* 1988, Gamalei 1996, McLean *et al.* 1997). Hepler (1982) confirmed the involvement of ER in cell plate formation during cytokinesis. The vesicles that form the cell plate must be held in certain positions, a role fulfilled by the ER. The ER network is proposed to regulate ionic conditions that must be set in order for vesicle fusion to occur normally. The trapped tubule of ER becomes tightly curled on itself, eliminating the lumen, and ends up as the desmotubule (Overall *et al.* 1982, Hepler 1982, Thomson & Platt-Aloia 1985, Cook *et al.* 1997). Thomson and Platt-Aloia (1985) prefer the term axial component over desmotubule. They argue that there is no tubule as the membrane bilayer has no lumen, due to extremely tight curling, and so cannot function as a tube as the name desmotubule implies. This concept appears to be supported in later discussions on desmotubule structure (Turgeon & Beebe 1991), although the term desmotubule is retained in the literature by some.

Transmission electron micrographs of plasmodesmata show two concentric cylinders, viz. the outer plasma membrane and the inner desmotubule (Robinson-Beers & Evert 1991b). The cytoplasm inbetween the two cylinders is known as the cytoplasmic annulus (Olesen 1979, Overall *et al.* 1982, Goodwin 1983, Terry & Robards 1987), or more recently the cytoplasmic sleeve (Robinson-Beers & Evert 1991b, Turgeon & Beebe 1991, Ding *et al.* 1992, Oparka & Van Bel 1992, White *et al.* 1994, McLean *et al.* 1997, Botha & Cross 2000). The plasma membrane, cytoplasm of the cytoplasmic sleeve and the desmotubular ER of plasmodesmata are continuous with like components of adjacent cells (Robinson-Beers & Evert 1991b, Cook *et al.* 1997). The cytoplasmic sleeve is believed to be the main pathway for transport via plasmodesmata (Goodwin 1983, Terry & Robards 1987, Meiners *et al.* 1988, Robinson-Beers & Evert 1991b, Turgeon & Beebe 1991, Oparka & Van Bel 1992).

Plasmodesmata can be described in terms of their morphology as simple/unbranched and branched, or else as a product of development being either primary or secondary (McLean *et al.* 1997). Simple plasmodesmata are formed in the cell plate during cytokinesis and arise as

continuous channels connecting adjacent cells from the beginning (Kollmann & Glockmann 1991, Monzer 1991, Wolf & Lucas 1994, Ehlers & Kollmann 1996, Cook *et al.* 1997, McLean *et al.* 1997, Botha & Cross 2001). Ehlers and Kollmann (1996) present a hypothesis for branched primary plasmodesmata. As the cell wall thickens, plasmodesmata are required to lengthen correspondingly. The desmotubule may be associated branched ER cisternae. Branching plasmodesmata will occur as these cisternae become enclosed in wall material. If the cisternae show no branching, the plasmodesma will develop straight and unbranched. Primary plasmodesmata may undergo drastic structural changes, until they can no longer be separated from secondary plasmodesmata. The evidence presented for this hypothesis comes, firstly, from the absence of branched plasmodesmata in thin, young cell walls. Branched plasmodesmata occur with greater frequency as walls age and thicken. Secondly, branches always occur in younger layers of the cell wall, with the oldest part in the centre being unbranched. Thirdly, intermediate stages have been observed in studies (Ehlers & Kollmann 1996).

Secondary plasmodesmata are thought to be quite common (Monzer 1991), arising in cell walls secondarily, i.e. between cells that did not share a cell plate during development (Kollmann & Glockmann 1991, Wolf & Lucas 1994, Ehlers & Kollmann 1996, Volk *et al.* 1996, McLean *et al.* 1997). Secondary plasmodesmata are usually, but not always, branched (Wolf & Lucas 1994). Branched plasmodesmata comprise many cytoplasmic channels, joined at the middle lamella by a median cavity (Kollmann & Glockmann 1991, McLean *et al.* 1997, Van Bel & Knoblauch 2000). Apart from occurring in areas of wall thinning, secondary plasmodesmatal formation shows many similarities with that of primary ones (Kollmann & Glockmann 1991, Monzer 1991). Strands of cytoplasm containing ER cisternae become trapped by surrounding Golgi vesicles packing wall materials. The vesicles fuse to form another layer of wall material and the strands of cytoplasm become half plasmodesmata which later fuse (Kollmann & Glockmann 1991, Monzer 1991). A typical feature of branched plasmodesmata are median cavities, called central cavities by Van Bel & Knoblauch (2000), which arise in the region of half plasmodesmatal fusion (Kollmann & Glockmann 1991), although there are reports of median cavities being absent (Volk *et al.* 1996). The function of central/median cavities remains obscure (Van Bel & Knoblauch 2000).

Using purely structural data, it is not always possible to distinguish between primary and secondary plasmodesmata (Wolf & Lucas 1994, Ehlers & Kollmann 1996). Ehlers and Kollmann (1996) comment that it is only acceptable to use the terms 'primary' and 'secondary' once ontogeny is determined. General terms such as 'single', 'branched' and 'unbranched' should rather be used to describe plasmodesmata if in doubt.

Gating of plasmodesmata has been shown to be influenced by various developmental and environmental signals, and would be important in maintaining cellular integrity and protection against damage (Goodwin 1983, Turgeon & Hepler 1989, Epel 1994, Gamalei *et al.* 1994, McLean *et al.* 1997, Van der Schoot & Rinne 1999, Botha & Cross 2000, Turgeon 2000, Botha & Cross 2001). Plasmodesmatal function corresponds directly to abscisic acid concentration, important for plant development (Botha & Cross 2000). Conversely tissue development is influenced by closure of plasmodesmata (Meiners *et al.* 1988, Van der Schoot & Rinne 1999).

There are two suggested sites and mechanisms for plasmodesmatal gating, viz. the neck region by sphincters and the cytoplasmic sleeve by actin/myosin interaction (Hepler 1982, Robinson-Beers & Evert 1991b, Badelt *et al.* 1994, White *et al.* 1994, McLean *et al.* 1997).

The neck region of the cytoplasmic sleeve is usually narrower than the main plasmodesmatal corridor (Olesen 1979, Overall *et al.* 1982, Thomson & Platt-Aloia 1985, Badelt *et al.* 1994, White et al. 1994). The occurrence of sphincters in the neck region of plasmodesmata has been involved in the gating or regulation of intercellular traffic (Thomson & Platt-Aloia 1985, Terry & Robards 1987, Robinson-Beers & Evert 1991b, Ding *et al.* 1992, Oparka & Van Bel 1992, Badelt *et al.* 1994, White *et al.* 1994, Botha & Cross 2000, Botha & Cross 2001). Sphincters are apparently composed of circularly spaced particles (Oparka & Van Bel 1992), that might simply be an extension of the actin/myosin arrangement seen in the cytoplasmic sleeve (White *et al.* 1994). The rapid, reversible deposition of callose in the neck region has also been associated with closure of plasmodesmata (Hughes & Gunning 1980, White *et al.* 1994, Botha & Cross 2000).

Particles previously noted in the cytoplasmic sleeve (Robinson-Beers & Evert 1991b, Turgeon & Beebe 1991), are now suggested to be actin, as this contractile protein has been localized at the neck and along the cytoplasmic sleeve of the plasmodesma (White *et al.* 1994). Actin is known

to be a component of the plant cytoskeleton and also a contractile protein (White *et al.* 1994). It was therefore hypothesised that actin/myosin interaction brings about constriction of the cytoplasmic sleeve and thus closure of the plasmodesma, and also allows for expansion to admit particles above the size exclusion limit (McLean *et al.* 1997). Proteinaceous particles noted in the cytoplasmic sleeve are suggested to be actin, while myosin is thought to be connected to the plasma membrane and attached to the actin particles (McLean *et al.* 1997). The interplay of these cytoskeletal contractile proteins apparently allows the cytoplasmic sleeve to widen or contract, thereby effectively gating the plasmodesma (Ding *et al.* 1992, White *et al.* 1994, McLean *et al.* 1997). Evidence suggests a spiral arrangement of actin particles, creating microchannels in the cytoplasmic sleeve (Ding *et al.* 1992).

There are many descriptions of plasmodesmatal frequencies in the literature, covering a wide range of species and showing great variation (Fisher 1986, Warmbrodt & Van Der Woude 1990). These include spinach (Warmbrodt & Van Der Woude 1990), *Thalassodendron ciliatum* (Barnabas 1983), potato (McCauley & Evert 1989), *Pisum sativum* (Wimmers & Turgeon 1991), *Cucurbita pepo* (Haritatos & Turgeon 1995), *Abutilon striatum* (Terry & Robards 1987), sugarcane (Robinson-Beers & Evert 1991a), *Cucumis melo* (Volk *et al.* 1996), *Cucurbita pepo* (Turgeon & Hepler 1989, Volk *et al.* 1996), *Coleus blumei* (Fisher 1986, 1988), *Moricandia arvensis* (Beebe & Evert 1992), *Themeda triandra*, *Panicum maximum*, *Eragrostis plana* and *Bromus unioloides* (Botha 1992), *Zea mays* (Evert *et al.* 1978), *Beta vulgaris* (Maynard & Lucas 1982, Gamalei & Pakhomova 1983a, 1983b, Evert & Mierzwa 1986), *Ricinus communis* and *Salix alba* (Van Bel & Kempers 1990), *Commelina benghalensis* (Van Kesteren *et al.* 1988), and *Ipomea tricolour* (Madore *et al.* 1986). Plasmodesmatal frequency is of the utmost interest as it denotes the degree of symplasmic transport possible (Russin & Evert 1985, Van Bel *et al.* 1988, McCauley & Evert 1989, Gamalei 1996). Whilst plasmodesmatal density may be used to infer the possible intensity of exchange between cells (Russin & Evert 1985, Van Bel *et al.* 1988, McCauley & Evert 1989, Van Bel & Kempers 1990, Kempers *et al.* 1998), caution is expressed against using plasmodesmatal frequency alone to determine apoplasmic or symplasmic phloem loading (Madore *et al.* 1986, Van Kesteren *et al.* 1988, Turgeon 1989, Van Bel & Kempers 1990, Warmbrodt & Van Der Woude 1990). Questions arise as to the minimal frequency needed to signify symplasmic loading. Kempers *et al.* (1998) ask if a paucity of plasmodesmata represents a physiological bottleneck in the loading pathway of assimilate. Plasmodesmata may be few but

loading at extremely high rates, or may be many and occluded or nonfunctional (Hughes & Gunning 1980, Van Bel *et al.* 1988, McCauley & Evert 1989, Turgeon 1989).

Functionality of plasmodesmata has to be considered before symplasmic or apoplasmic pathways can be confidently determined (Terry & Robards 1987, Van Bel *et al.* 1988, McCauley & Evert 1989, Warmbrodt & Van Der Woude 1990, Russin *et al.* 1996). Typical characteristics of functional plasmodesmata include visibility of an intact desmotubule and its connection to ER in adjacent cells, and nontruncated end regions (Fisher 1986).

A further consideration is the molecular size exclusion limit and the gating status of plasmodesmata (Haritatos *et al.* 1996, Russin *et al.* 1996, Kempers *et al.* 1998, Turgeon 2000). Recently, much attention has been focused on viral infection of plant tissues (McLean *et al.* 1997). The discovery that viruses use plasmodesmata to traffic their genomes between cells provided evidence for the theory that plasmodesmata are dynamic structures, capable of upgrading to allow passage of large molecules (Ding *et al.* 1992, McLean *et al.* 1997).

All plasmodesmata across the plant are not likely to be identical in terms of structure and, therefore, function (Robinson-Beers & Evert 1991b, Oparka and Van Bel 1992, Epel 1994, Russin *et al.* 1996, McLean et al. 1997). Furthermore, plasmodesmata are dynamic structures with the ability to up- or downregulate gating (McLean *et al.* 1997, Botha & Cross 2000). In doing so, the size exclusion limit may be changed (McLean *et al.* 1997, Turgeon 2000). Turgeon (2000) was particularly interested in the cell-cell interactions of sieve elements and companion cells, indicating that certain proteins and nucleic acids could travel between them, and could be transported over long distances in phloem. He noted an increase in the size exclusion limit of plasmodesmata between sieve elements and companion cells of the stem, and went on to ask three important questions. "What happens to the small molecules in companion cells? Do they continually leak into the rushing current that flows through the sieve element? How can companion cells maintain their metabolic integrity in the face of constant efflux of intermediates?" (Turgeon 2000). He noted that there was a net directional flux of solute transfer to minor veins from mesophyll. Two proposals by which metabolic integrity could be maintained in companion cells were the limiting of flux between sieve element and companion cell, and/or by photosynthates entering sieve elements directly, bypassing companion cells.

5.1.3 Phloem loading

Loomis (1955) first defined the term "loading" to denote the active placement of sugar in minor vein phloem tissue. He specifically described "loading" as an active pumping action across the mesophyll/phloem interface (in Turgeon 2000). The terminology then progressed to "vein loading" in a review paper by Eschrich (1970). This was later changed to phloem loading, as xylem was seen to have a separate function. Phloem loading is currently defined as the transfer of photosynthates from the site of production, via the mesophyll symplast, to the sieve tubes (Wimmers & Turgeon 1991, Oparka & Van Bel 1992, Komor *et al.* 1996, Van Bel 1996, Sjölund 1997, Turgeon 2000).

Two methods of phloem loading are generally recognised, viz. apoplasmic and symplasmic phloem loading (Oparka & Van Bel 1992, Gamalei 1996, Van Bel 1996, Gamalei *et al.* 2000, Voitsekhovskaja *et al.* 2000). Apoplasmic phloem loading involves the symplasmic transport of sucrose across the mesophyll until the phloem is reached. There sucrose passes into the apoplast, or free extracellular space, and is actively loaded into ST/CC complexes apoplasmically (Turgeon & Beebe 1991, Beebe & Evert 1992, Russin *et al.* 1996). Symplasmic loading involves the movement of assimilate into the sieve element/companion cell complex via numerous plasmodesmata, without active transfer across a membrane (Oparka & Van Bel 1992, Gamalei 1996, Van Bel 1996). However, Turgeon (2000) pointed out that the term "loading" was originally used to denote active pumping of assimilates into phloem to create an increase in solute concentration, and that on these grounds, the symplasmic pathway could not be included in phloem loading, as it does not involve active concentration.

Minor vein phloem that loads apoplasmically is characterised by sieve element-companion cell complexes that are symplastically isolated. The companion cells may be ordinary or with wall ingrowths and termed transfer cells (Gamalei 1989, Wimmers & Turgeon 1991, Kempers *et al.* 1998). Transfer cells tend to be large, surrounding much smaller sieve elements (Van Bel 1993b). Characteristic features of cells engaged in highly active metabolism are displayed by transfer cells. These include many mitochondria and ribosomes which provide the necessary energy and

enzymes, respectively, for such intense activity (Wark 1965, McCauley & Evert 1989). ATPase activity has also been noted on the plasma membrane of transfer cells (Warmbrodt *et al.* 1989, Wimmers & Turgeon 1991, Bouché-Pillon *et al.* 1994). Transfer cells of minor veins of *Vicia faba* were found to have a greater concentration of ATPase on cell membranes of walls facing the incoming route of assimilates, viz. less on the wall facing the sieve tube. Therefore, the membrane infoldings of transfer cells possess the ATPase for uptake of photosynthates from the apoplast and for efficient retrieval (Bouché-Pillon *et al.* 1994).

Transfer cells possess wall invaginations. Consequently, the plasma membrane is greatly increased. This provides a larger surface area over which apoplasmic loading can occur. Virtually absent from transfer cell walls are plasmodesmata (Pate & Gunning 1969, Evert *et al.* 1978, Fisher 1986, Gamalei 1989, Evert *et al.* 1996, Kempers *et al.* 1998, Gamalei *et al.* 2000). Although symplasmic loading cannot be ruled out on structural evidence alone, it is unlikely to be the main loading pathway with a paucity of plasmodesmata (Maynard & Lucas 1982, Gamalei and Pakhomova 1983a, 1983b, Evert & Mierzwa 1986, Botha & Evert 1988, Van Bel & Kempers 1990).

Symplasmic phloem loading implies a direct symplasmic continuum from mesophyll to sieve element (Madore *et al.* 1986, Turgeon & Beebe 1991, Beebe & Evert 1992, Turgeon *et al.* 1993, Haritatos *et al.* 1996, Van Bel & Knoblauch 2000). However, in supposed symplasmic loaders, it is possible that apoplasmic loading may be occurring in conjunction with symplasmic loading. There is no evidence that this is not possible (Flora & Madore 1996).

Symplasmic loaders typically have intermediary cells, connected at the mesophyll interface by abundant plasmodesmata (Evert *et al.* 1996, Turgeon *et al.* 1993, Flora & Madora 1996, Volk *et al.* 1996). Plasmodesmata are usually branched on the intermediary cell side (Flora & Madora 1996, Volk *et al.* 1996). Wall ingrowths are absent. Intermediary cell protoplasts contain extensive vesicular networks (Gamalei 1989, Turgeon *et al.* 1993, Kempers *et al.* 1998).

The thermodynamics of symplasmic phloem loading has been problematic since the concept was first suggested (Turgeon & Beebe 1991, Voitsekhovskaja *et al.* 2000). The solute concentration of the phloem cells is much higher than that of the surrounding cells (Turgeon *et al.* 1975, Fisher

1986, Turgeon & Beebe 1991, Gamalei 1996, Van Bel 1996, Turgeon 2000, Voitsekhovskaja *et al.* 2000), as is the internal hydrostatic pressure (Sjölund 1997, Turgeon 2000). In an effort to explain how symplasmic loading may occur against a concentration and pressure gradient, three models have been put forward (Van Bel 1996).

A polymer-trapping model has been proposed, in which small molecule sugars formed in the mesophyll would be able to cross the plasmodesmata into the intermediary cells (Holthaus & Schmitz 1991, Turgeon & Beebe 1991, Beebe & Turgeon 1992, Oparka & Van Bel 1992, Flora & Madore 1993, Haritatos & Turgeon 1995, Haritatos *et al.* 1996, Sjölund 1997, Turgeon 2000). There these sugars would be used to synthesise larger molecules, such as stachyose and raffinose. These larger sugars would be able to traverse the relatively wide sieve pores into the sieve tubes, but would be too big to flow back out of the intermediary cell plasmodesmata. This would cause the solute concentration of the phloem cells to increase (Turgeon & Beebe 1991, Haritatos & Turgeon 1995, Haritatos *et al.* 1996).

The other suggestion to explain symplasmic loading is that oligosaccharides are produced in the mesophyll and then package delivered to the sieve tubes. These large sugars are supposedly carried in an intercellular membrane system that begins in the mesophyll cells, passes though all plasmodesmata en route and terminates in the actual sieve elements (Oparka & Van Bel 1992, Van Bel 1996). This would apparently bypass any barriers provided by the size exclusion limits of plasmodesmata.

A further suggested mechanism to prevent backflow during symplasmic transport is that the plasmodesmata may act as pressure valves, allowing transport in one direction only. This theory does not appear to have received much support in the literature. Evidence for this concept apparently comes from the presence of a high pressure differential between the ST/CC complex and the surrounding photosynthetic cells (Van Bel & Kempers 1990, Flora & Madore 1996).

Experimentally, abundant plasmodesmatal connectivity and insensitivity to PCMBS have regularly been used to demonstrate symplasmic loading (Madore & Lucas 1987, Weisberg *et al.* 1988, Bourquin *et al.* 1990, Turgeon & Gowan 1990, Turgeon *et al.* 1993, Haritatos & Turgeon 1995, Flora & Madore 1996, McLean *et al.* 1997). Microinjection of membrane impermeable

dyes demonstrate the interconnectivity of cells via plasmodesmata (Madore *et al.* 1986, Meiners *et al.* 1988). Confocal laser scanning microscopy is the latest method for watching dye transport/movement in intact plants (Van Bel & Knoblauch 2000). Intercellular transport of such dyes does not occur between cells not joined by plasmodesmata. Transport rates of substances between cells appears to be proportional to plasmodesmatal frequency (Meiners *et al.* 1988, Botha 1992). Immunolocalization of raffinose oligosaccharides in intermediary cells was used to support the polymer-trapping model (Holthaus & Schmitz 1991, Beebe & Turgeon 1992, Flora & Madore 1993, Haritatos & Turgeon 1995).

^{14}C-labelled sucrose applied to leaf discs took longer to arrive at ST/CC complexes in symplasmic loaders than in apoplasmic loaders. This was due to an inability of ST/CC complexes to load directly from the free space, requiring absorbed sucrose to travel through the mesophyll first. Mesophyll cells of symplasmic loaders generally store starch (Oparka & Van Bel 1992, Russin *et al.* 1996, Van Bel 1996), probably in response to slower export rates. In an attempt to lower the sugar concentration gradient between companion cells and other cells, sugar is deposited as insoluble starch, so further loading can occur against less of a gradient (Komor 2000).

Turgeon and Beebe (1991) caution the interpretation of experimental results, saying that no single experimental methodology published to date is able to distinguish unequivocally between apoplasmic and symplasmic phloem loading. Plasmodesmatal distribution and frequency can infer loading method and route, but data on functionality and transport rates are required for validation. Free space studies present a whole range of problems in terms of technique, identification of mobile compounds, quantification and sites of leakage. Exogenously supplied sugars may not follow *in situ* transport pathways (Madore & Webb 1981, Fritz *et al.* 1983, Madore & Lucas 1987, Turgeon & Beebe 1991).

Oparka *et al.* (1991) modified the pressure probe used for studying water relations of cells to incorporate both types of microinjection: i.e. iontophoretic and pressure injection. This technique allowed for the recording of cell turgor pressure, the injection of fluorescent probes, minimal cellular disruption, and identification of the impaled intracellular compartment. However, following the pathway of injected dyes does not necessarily mean that one is following the pathway of photosynthate as one is simply following an available symplasmic route (Erwee *et al.*

1985, Madore *et al.* 1986, Fisher 1988, Van Kesteren *et al.* 1988, Turgeon & Beebe 1991). Furthermore, sieve elements are extremely sensitive to experimentation (Knoblauch & Van Bel 1998).

Komor *et al.* (1996) attempted to unify the concepts of apoplasmic and symplasmic loading by focusing on a distinct similarity. Leakage from sieve tubes, and the transport stream in general, requires an active loading step at either the companion cell or sieve element membrane, or both, regardless of how phloem loading occurred in the first place (Komor *et al.* 1996, Kempers *et al.* 1998). From an evolutionary perspective, the more efficient the active retrieval system and the greater the blockage of plasmodesmata connecting other cells to the ST/CC complex, the faster and more efficient the transport rate (Komor *et al.* 1996). Symplasmic loading would therefore be the older, less efficient system, suitable only for stable, equable regions. Apoplasmic loading would have arisen in response to more stressful conditions, where the plant could not afford to loose assimilates along the pathway and thereby favouring a more efficient loading, retrieval and transport system. Plasmodesmata between the ST/CC complexes and other cells became occluded or lost and transmembrane loading became more efficient, leading to the advanced apoplasmic loading pathway (Komor *et al.* 1996, Van Bel 1996).

5.1.4 Loading versus transporting phloem

A pressure differential in the phloem is created by active loading in source leaves and the resultant drain by unloading in sinks (Van Bel 1993b, Haritatos & Turgeon 1995, Sjölund 1997). The anatomy and physiology of phloem from loading, transport and unloading regions may reasonably be expected to reflect function, especially with regard to the changes in pressure and solute movement along the pathway (Fritz *et al.* 1989, Van Bel 1993b, Van Bel 1996, Kempers *et al.* 1996, Sjölund 1997). Pertinent to this thesis is the comparison between loading and transport phloem, with the exclusion of unloading phloem.

Characteristic ultrastructure of loading phloem has already been discussed (see 5.1.1). Features of transport phloem sieve elements include the presence of P-proteins and P-plastids, a few, underdeveloped organelles, and no nucleus or tonoplast. Companion cells typically show a large nucleus, a dense cytoplasm with many ribosomes, many mitochondria and a small vacuole; all features indicative of high metabolic activity (Van Bel 1993b). Schmitz *et al.* (1987) noted in

lower order veins of *Cucumis melo* the occurrence of intermediary cells associated with long distance transport. Intermediary cells were not seen on loading veins of higher orders. Companion cells occurred in all vein orders, making the disappearance of intermediary cells from higher order veins much more noticeable.

On the surface, there does not appear to be much ultrastructural difference between loading and transport phloem. However, when structure and spatial arrangement of cells is considered, the differences become apparent (Van Bel 1993b, Van Bel 1996, Van Bel & Knoblauch 2000). Sieve elements of loading minor veins tend to be smaller in diameter than their associated companion cells. In transport phloem the opposite occurs, where sieve elements are much bigger than companion cells (Van Bel & Knoblauch 2000). This probably reflects the intensity of the loading process in companion cells and the importance of the sieve elements in transport (Van Bel 1993b, Kempers *et al.* 1998).

It must be remembered that the phloem in foliar minor veins is primary tissue, while that of major veins and petiole may be secondary, and that of the stem most likely secondary (Van Bel 1996). Furthermore, foliar minor veins have characteristic anatomy and ultrastructure according to their minor vein configuration and loading method, i.e. symplasmic or apoplasmic (Van Bel 1996).

In woody plants, studies indicate that the transport phloem is a slightly downscaled reflection of the collection phloem configuration (Van Bel 1996, Kempers *et al.* 1998). In particular, the companion cells of transport phloem show ultrastructural adaptations with regard to the symplasmic or apoplasmic phloem loading method of the collection phloem. In plants with minor veins possessing symplasmic configurations and phloem loading, companion cells of the transport phloem contain many vesicles. In those of apoplasmic species, companion cell walls posses invaginations, although to a lesser degree than seen in the collection phloem (Van Bel 1996, Kempers *et al.* 1998).

Van Bel (1996) concludes that the potential exists for two structurally and physiologically distinct phloem transport systems, within the confines of the ST/CC complex, based on the configuration of the collection phloem. However, he also comments on the symplasmic constriction seen generally in transport phloem at the ST/CC and vascular parenchyma interface. Evidence for such

conjecture comes from studies involving ultrastructure, microinjection of fluorescent probes, and studies on electrical conductance and membrane potentials (Robinson-Beers & Evert 1991a, Van Bel 1996). The few plasmodesmata noted at the ST/CC complex and vascular parenchyma interface would most likely be blocked under normal conditions. This would result in symplasmic isolation of the ST/CC complexes and would produce a faster route for long distance transport (Van Bel 1996, Kempers *et al.* 1998).

5.1.5 Aims

The aims of this chapter are as follows. For certain taxa of the Ranunculaceae, Apocynaceae and Asclepiadaceae:

1. To describe and illustrate the cell types which occur along the loading pathway
2. To determine minor vein type categories
3. To suggest symplasmic versus apoplasmic transport, based on ultrastructural evidence

5.2 Results

5.2.1 Ranunculaceae: *Ranunculus multifidus*

Cellular ultrastructure

Mesophyll cells show starch deposits in chloroplasts. Cell walls are thin. Mesophyll cells are abundantly interconnected by branched plasmodesmata.

Starch deposits in chloroplasts of bundle sheath cells were noted. Bundle sheath cells are abundantly interconnected to other bundle sheath cells, especially in free ending ultimate veinlets in the mesophyll. Again, branched plasmodesmata were seen (Fig. 5.1 A, B & C).

Vascular parenchyma cells had a denser cytoplasm than that seen in bundle sheath cells (Fig. 5.1 A & B). Vacuoles were present, as was a large nucleus. Plastids with small starch granules, osmiophilic inclusions and some granal development were present (Fig. 5.1 A & B). Plasmodesmatal connections to bundle sheath cells were noted, though very seldom (Fig. 5.1 A, B & C).

Companion cells showed a dense cytoplasm with many ribosomes and mitochondria. A large nucleus and small vacuoles were present (Fig. 5.1 A, B & D). No wall ingrowths or plastids were seen. Companion cells were connected to sieve tubes by pore-plasmodesmata, and, to a much lesser extent, to vascular parenchyma and bundle sheath cells by very few plasmodesmata (Fig. 5.1 B & D).

Sieve tubes do not show uneven distribution of wall thickness, except in the region of pore-plasmodesmata (Fig. 5.1 A, B & D). Pore-plasmodesmata connected sieve tubes to companion cells, but to no other cells. Sieve tubes appeared empty, except for some peripheral cytoplasm.

Plasmodesmata

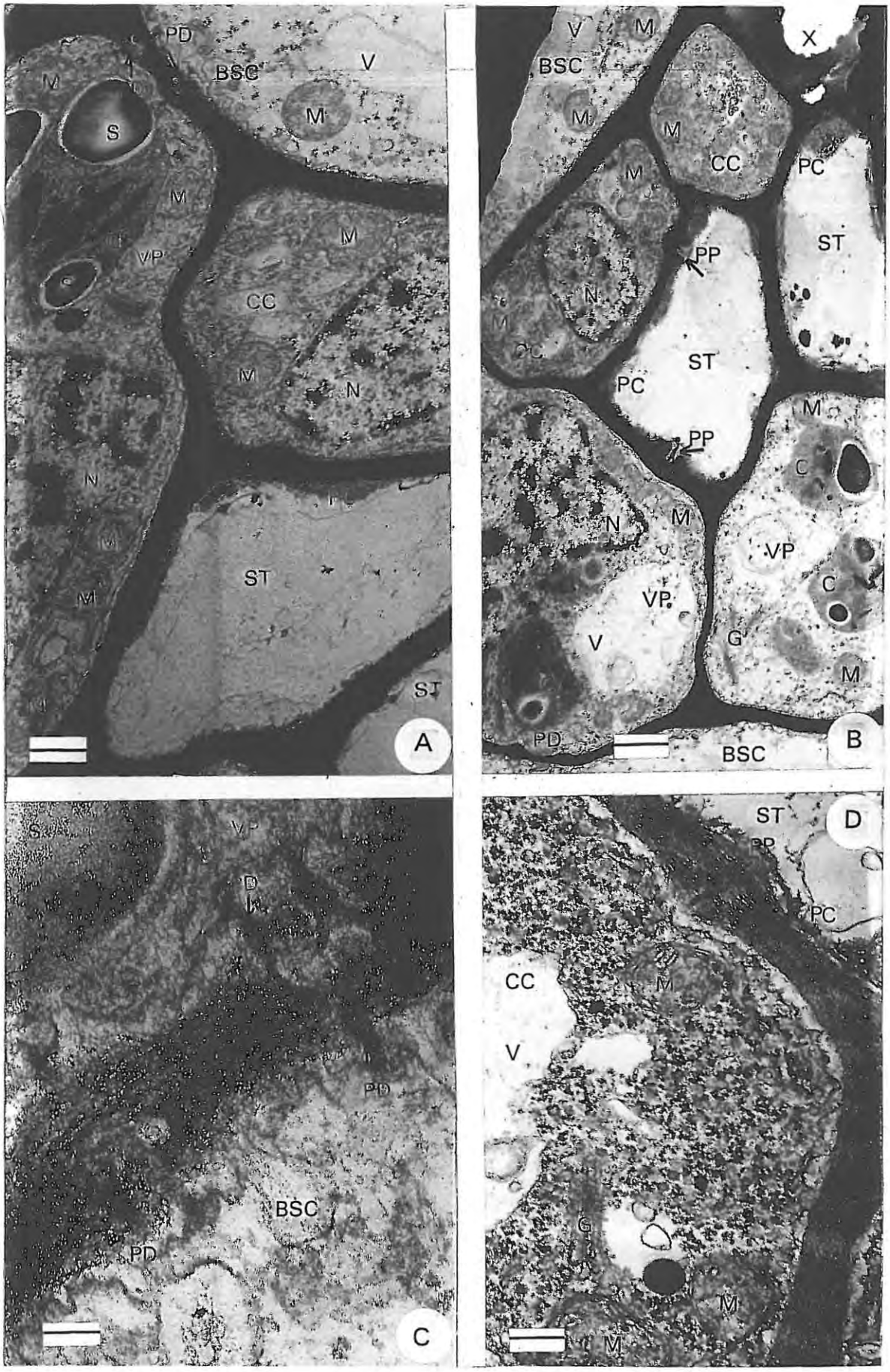
Plasmodesmata were noted between all cell types, but less abundantly so between vascular parenchyma and companion cells. Generally, plasmodesmata appeared to be branched. The micrographs suggest the possibility of a median cavity (Fig. 5.1 C). However, further studies would be needed to confirm this observation.

Figure 5.1 Ranunculaceae: Aspects of phloem ultrastructure of *Ranunculus multifidus*

A Transverse section of *Ranunculus multifidus* secondary vein phloem near bundle sheath cell showing branched plasmodesmata connecting vacuolate bundle sheath cell to vascular parenchyma cell, vascular parenchyma cell contains cytoplasm rich in ribosomes and mitochondria, and chloroplasts with starch granules, companion cell with dense cytoplasm rich in ribosomes and mitochondria, small vacuoles present, sieve tube with parietal cytoplasm, B Transverse section of *R. multifidus* secondary vein phloem showing plasmodesmata connecting bundle sheath cell to vascular parenchyma cell, and pore-plasmodesmata from vascular parenchyma and companion cells to sieve tubes, vascular parenchyma cells with large vacuoles and chloroplasts containing starch granules, companion cells with large central nucleus, small vacuoles, no chloroplasts, cytoplasm rich in ribosomes and mitochondria, sieve tubes with parietal cytoplasm, C Transverse section of secondary vein phloem of *R. multifidus* showing wall separating bundle sheath and vascular parenchyma cells with branched plasmodesmata and possible median cavity, D Transverse section of *R. multifidus* companion cell showing cytoplasm rich in ribosomes and mitochondria, with small vacuoles present, and adjacent sieve tube with parietal cytoplasm and pore-plasmodesmata connecting companion cell to sieve tube

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; CW = cell wall; G = Golgi body; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; S = starch granule; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; X = xylem; PD = plasmodesma; PP = pore plasmodesma)

Bar represents 0.05μm for A, 1μm for B, 0.1μm for C and 0.2μm for D.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5.2.2 Apocynaceae: *Acokanthera* and *Carissa*

Cellular ultrastructure

Mesophyll cells contained starch deposits in the abundant chloroplasts of *A. oppositifolia* and, especially, *C. bispinosa* (Fig. 5.2 F). In *C. bispinosa*, some mesophyll cell vacuoles were filled with tanniniferous compounds (Fig. 5.2 F). Cell walls were noted to be thin, with numerous plasmodesmatal interconnections. Considering the size of mesophyll cells, relatively few mitochondria were seen.

Bundle sheath cells were smaller than mesophyll cells. Starch deposits in plastids were noted, but both in much less abundance than those of mesophyll cells. The vacuole was large, filling most of the cell (Fig. 5.2 C). Plasmodesmatal connections with other bundle sheath cells and with mesophyll cells were seen in both species (Fig. 5.4 D).

Vascular parenchyma cells appeared bigger than companion cells and smaller than bundle sheath cells. The cytoplasm was clear compared with that of companion cells (Fig. 5.2 C). No plastids were seen in *C. bispinosa* (Fig.s 5.2 B & C, 5.3), while in *A. oppositifolia* underdeveloped plastids with starch granules were noted (Fig. 5.4 C). Large nuclei were seen. Small vacuoles were found in *A. oppositifolia* only (Fig. 5.4 C). Plasmodesmatal connections to other vascular parenchyma cells, bundle sheath cells and companion cells occurred in both species (Fig.s 5.2 B, 5.3). *C. bispinosa* vascular parenchyma cells were seen to be connected to sieve tubes on a number of occasions (Fig. 5.2 C).

Companion cells generally contain a dense cytoplasm with abundant ribosomes, mitochondria and ER. Small vacuoles and large nuclei were seen. No wall ingrowths could be detected (Fig.s 5.2 B & C, 5.3, 5.4 A & C). *A. oppositifolia* did show chloroplast development with grana (Fig. 5.4 A & C), but no starch was seen in companion cells of either species. Companion cells were connected to sieve tubes by pore-plasmodesmata, branched on the companion cell side (Fig.s 5.2 E, 5.4 D). Plasmodesmatal connections to vascular parenchyma cells were noted to be very few in *C. bispinosa*, and very sparse in *A. oppositifolia* in general.

Sieve tubes uniformly possessed thickened nacreous walls and parietal cytoplasm. Companion cells were notably bigger than sieve tubes in both species (Fig.s 5.2 B, C & D, 5.3, 5.4 C).

Plasmodesmata

In both species of Apocynaceae examined, plasmodesmatal connections were few between all cell types along the loading route. This is especially true of the ST/CC complex and surrounding cell types. Within the few plasmodesmata observed, branched examples did occur (Fig.s 5.3, 5.4 B & E).

Figure 5.2 Apocynaceae: Aspects of phloem ultrastructure of *Carissa bispinosa*

A Transverse section through midvein of *Carissa bispinosa* with supporting collenchyma, cambium, xylem and abaxial phloem showing darkly-stained companion cells with adjacent sieve tubes and larger vascular parenchyma cells, B Transverse section through midvein of *C. bispinosa* with compacted protophloem and abaxial metaphloem showing large vacuolate vascular parenchyma cells, smaller companion cells with dense cytoplasm and sieve tubes with parietal cytoplasm and nacreous walls, C Transverse section through fourth to fifth order vein of *C. bispinosa* showing large vacuolate bundle sheath cells, vascular parenchyma cells with large central nucleus and vacuole, smaller companion cells with cytoplasm rich in ribosomes and mitochondria, small vacuoles present, sieve tubes with parietal cytoplasm, D Transverse section through midvein of *C. bispinosa* showing adaxial phloem in pocket above xylem with large laticifer embedded in supporting collenchyma, E Transverse section through wall separating companion cell and sieve tube of *C. bispinosa* phloem showing branched pore-plasmodesmata, F Longitudinal section of palisade mesophyll cells of *C. bispinosa* showing abundant starch granules in peripheral chloroplasts, central vacuole filled with cell sap in above cell and tannin in lower cell, plasmodesmata in wall connect cells

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; CO = collenchyma; CW = cell wall; L = laticifer; M = mitochondrion; MP = metaphloem; N = nucleus; P = phloem; PC = parietal cytoplasm; PM = palisade mesophyll; PPM = protophloem; S = starch granule; ST = sieve tube; T = tannin; V = vacuole; VP = vascular parenchyma cell; X = xylem; PD = plasmodesma; PP = pore plasmodesma)

Bar represents 20μm for A and D, 4μm for B and F, 2μm for C and 0.1μm for E

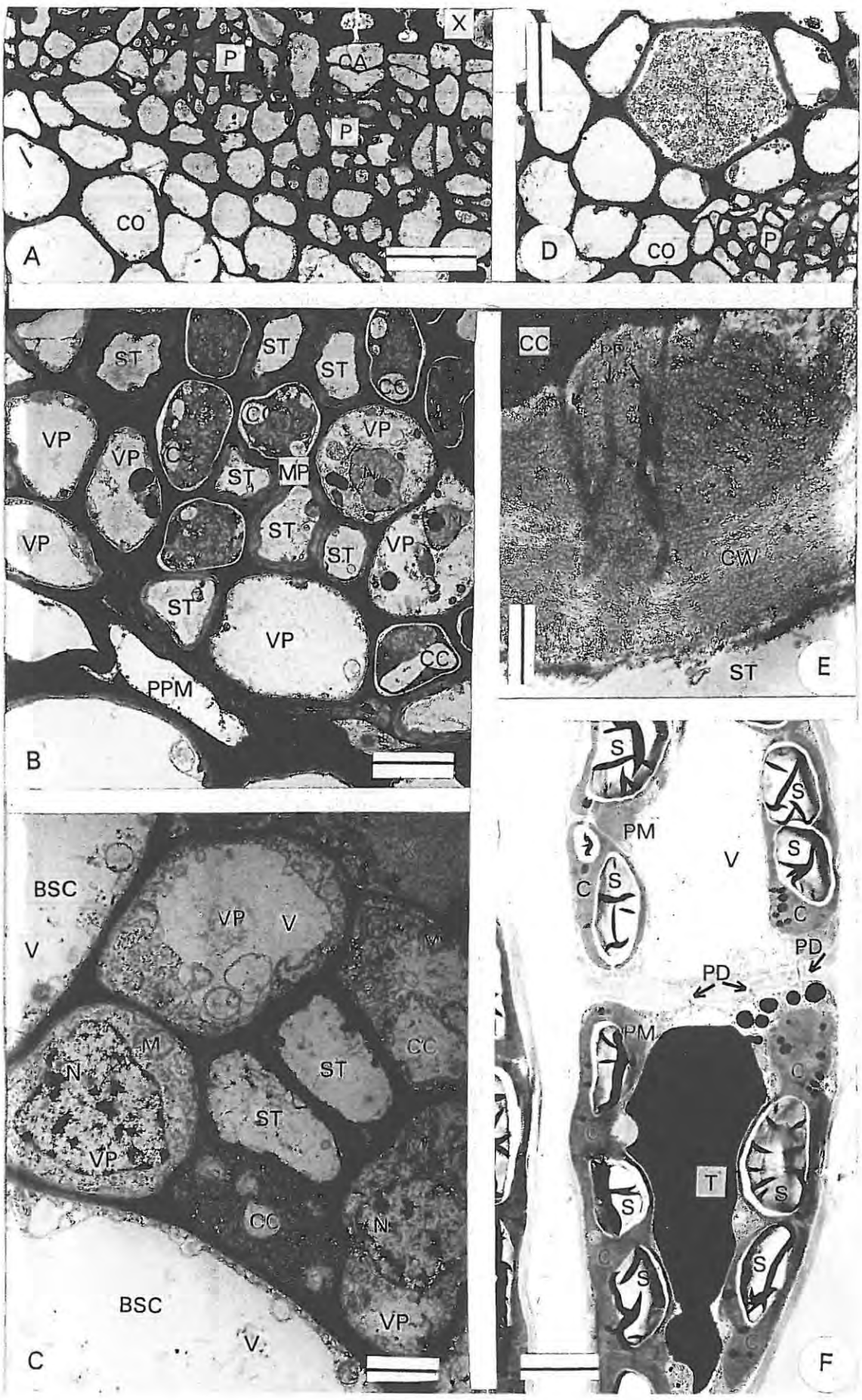
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Figure 5.3 Apocynaceae: Aspects of phloem ultrastructure of *Carissa bispinosa*

Transverse section of abaxial metaphloem of midvein of *C. bispinosa* showing large vascular parenchyma cells with central nucleus, mitochondria, ER and ribosomes in cytoplasm, branched plasmodesmata interconnect vascular parenchyma cells, smaller companion cells with denser cytoplasm rich in ribosomes and ER, small vacuoles present, sieve tubes with nacreous thickened walls and parietal cytoplasm

(CC = companion cell; ER = endoplasmic reticulum; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; PD = plasmodesma)

Bar represents 1μm

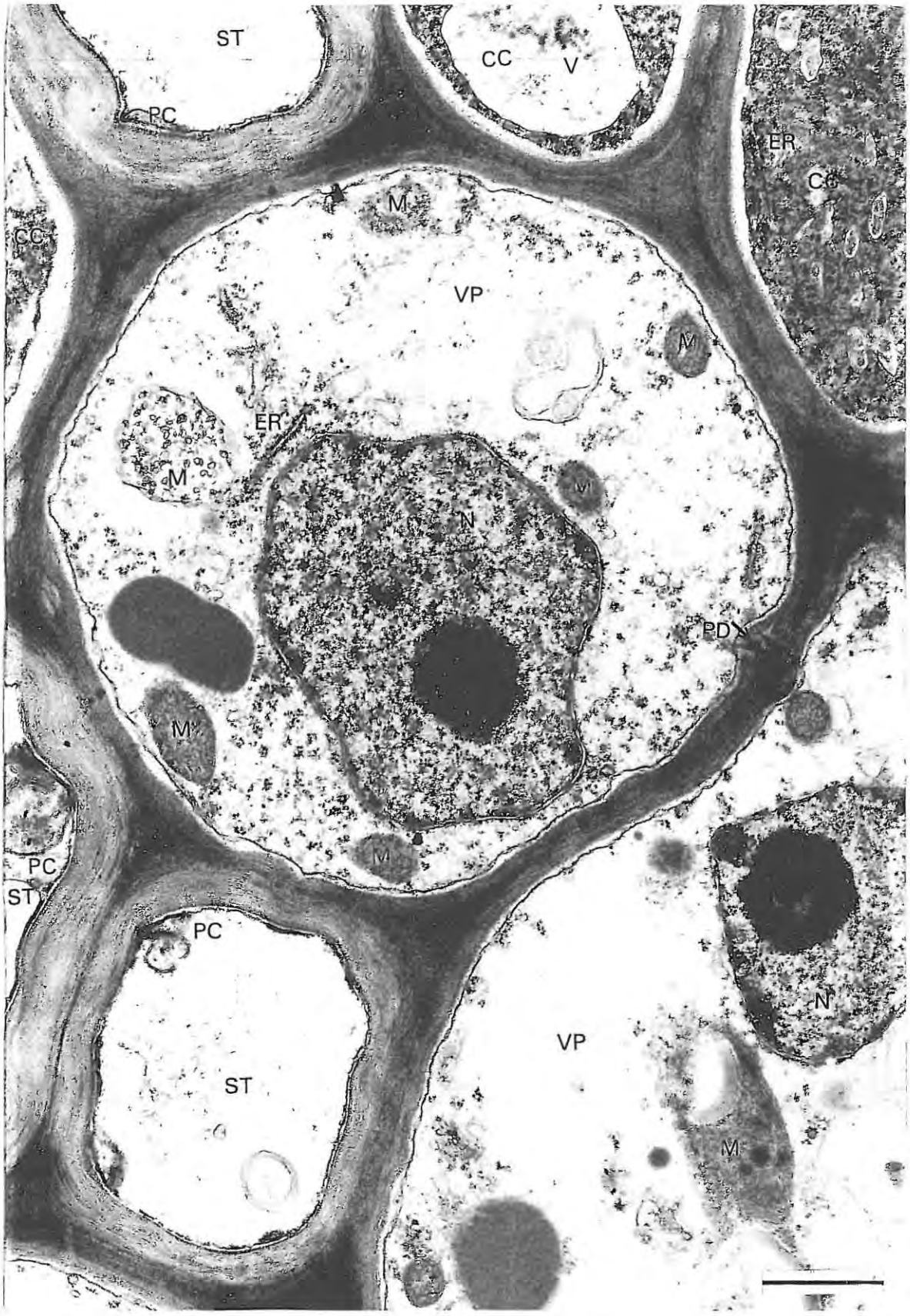
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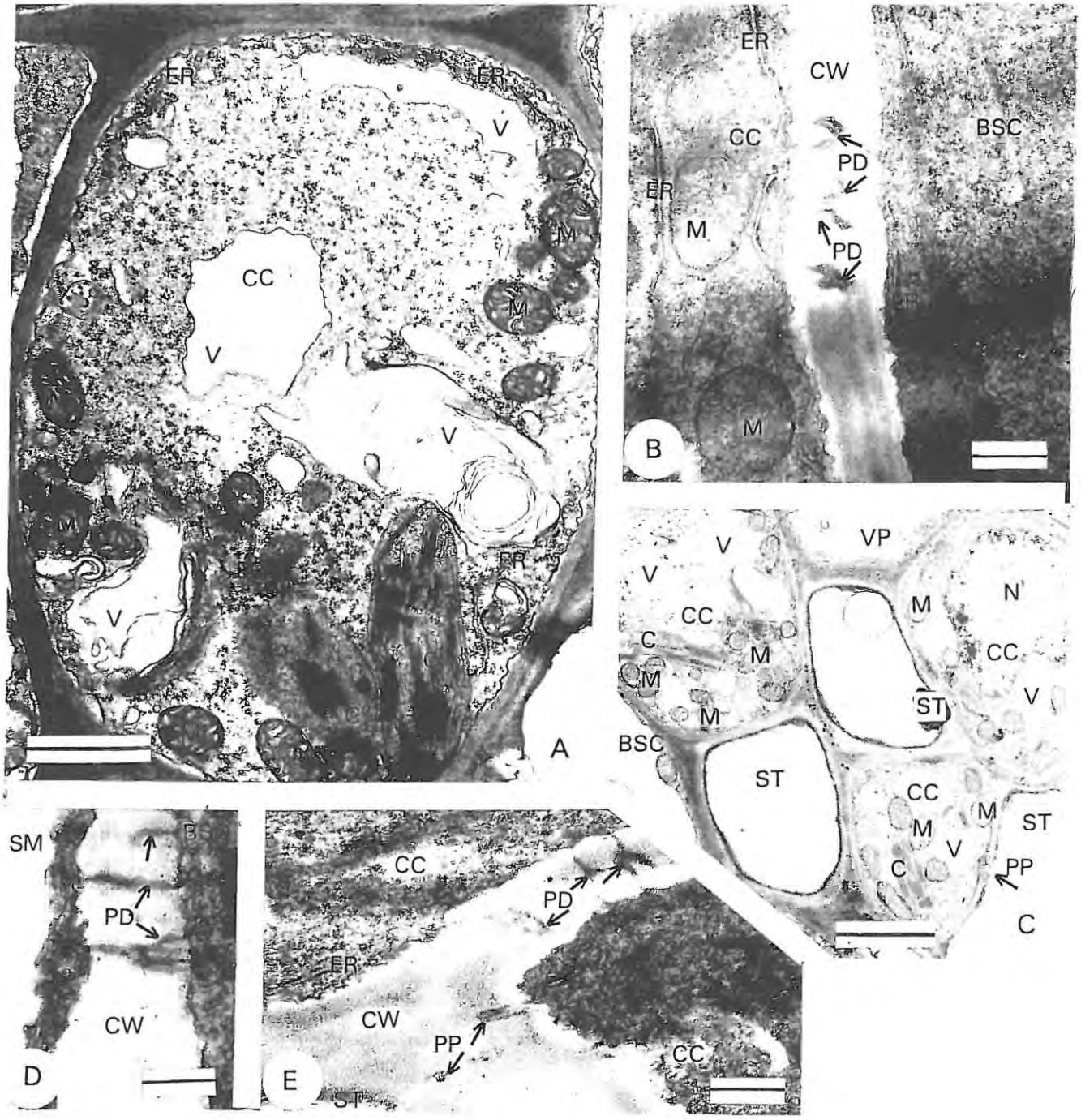
Figure 5.4 Apocynaceae: Aspects of phloem ultrastructure of *Acokanthera oppositifolia*

A Transverse section through minor vein of *Acokanthera oppositifolia* showing companion cell with small vacuoles, abundant mitochondria, ER and ribosomes in cytoplasm, and chloroplasts without starch granules, B Transverse section through wall separating bundle sheath and companion cells of *A. oppositifolia* minor vein showing branched plasmodesmata in wall, C Transverse section through quaternary vein of *A. oppositifolia* showing vacuolate bundle sheath cell, vascular parenchyma cell with granular cytoplasm and large vacuole, companion cells with cytoplasm rich in ER and ribosomes, abundant mitochondria, chloroplasts without starch granules, and small vacuoles present, sieve tubes with pore-plasmodesmata to companion cells and parietal cytoplasm, D Transverse section through wall separating bundle sheath and spongy mesophyll cells of *A. oppositifolia* minor vein showing branched plasmodesmata, E Transverse section of minor vein of *A. oppositifolia* through wall separating companion cells and sieve tube with pore-plasmodesmata and branched plasmodesmata

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; CW = cell wall; ER = endoplasmic reticulum; M = mitochondrion; N = nucleus; ST = sieve tube; SM = spongy mesophyll; V = vacuole; VP = vascular parenchyma cell; PD = plasmodesma; PP = pore plasmodesma)

Bar represents 1μm for A, 2μm for C, and 0.4μm for B, D and E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5.2.3 Asclepiadaceae: *Secamone*, *Asclepias*, *Cynanchum* and *Ceropegia*

Cellular ultrastructure

Mesophyll cells generally had thin walls, especially in *Secamone alpinii* and *Ceropegia woodii*. Mitochondria and many chloroplasts with starch granules were noted in *S. alpinii*, *Cynanchum obtusifolium*, *Ceropegia carnosa*, *Ceropegia distincta* and *Asclepias physocarpa* (Fig. 5.8 B). Exceptionally large starch granules were noted in *Asclepias physocarpa* (Fig. 5.8 B). No starch granules were seen in mesophyll chloroplasts of *C. woodii*. A large central vacuole filled most of the cell in all species studied. Abundant plasmodesmata were seen in *S. alpinii*, *A. physocarpa*, *C. carnosa* and *C. woodii* between mesophyll and bundle sheath cells.

Bundle sheath cells of all species studied here typically contained a large central vacuole and many mitochondria (Fig.s 5.5, 5.6 A - C, 5.8 C, 5.11 B). *C. carnosa* appeared to have fewer mitochondria in the bundle sheath cells than in the mesophyll cells.

Fully developed chloroplasts were seen in bundle sheath cells of most species, but were much smaller and fewer than in mesophyll cells (Fig.s 5.8 B & C, 5.9 A, 5.14, 5.15 A). *S. alpinii* and *C. woodii* bundle sheath cells had few chloroplasts and no starch granules (Fig.s 5.5, 5.6 A - C and Fig.s 5.14, 5.15 A respectively). In *A. physocarpa*, chloroplasts were orientated parallel to the vascular tissue, on the far side of the cell (Fig. 5.9 A). Chloroplasts contained many large starch granules. Chloroplasts of *C. obtusifolium* did show starch deposits (Fig. 5.11 B).

From my observations it would appear that many plasmodesmatal connections to other cell types occur in *S. alpinii* (Fig.s 5.5, 5.6 A & B, 5.7 B). In *A. physocarpa*, plasmodesmata were seen to connect adjacent bundle sheath cells. Occasional plasmodesmata connecting vascular parenchyma cells to bundle sheath cells were seen in *C. carnosa* and *C. woodii*, but very seldom. No plasmodesmatal connections were seen between bundle sheath cells and other cells in *C. distincta*.

Vascular parenchyma cells were usually distinguished from bundle sheath cells by being much smaller with a denser cytoplasm, and from companion cells by being bigger and having a less dense cytoplasm (Fig.s 5.5, 5.6 A - D, 5.8 A - C, 5.10 A - C, 5.11 A - D, 5.12, 5.13, 5.15 D, 5.16).

The cytoplasm of *S. alpinii* was, however, just as dense as that of the companion cells (Fig.s 5.5, 5.6 A - D). Many ribosomes, ER and mitochondria were noted generally in all species. A large central vacuole filled the centre of the cell in most species.

Chloroplasts were generally small and very few, and contained no starch granules in *S. alpinii* and *C. woodii* (Fig.s 5.5, 5.6 A - D and Fig. 15 D respectively). In *A. physocarpa* (Fig.s 5.8 B & C, 5.9 B), *A. fructicosa* (Fig. 10 A), *C. obtusifolium* (Fig.s 5.11 A & B, 5.12, 5.13), *C. carnosa* (Fig. 5.16) and *C. distincta*, starch granules were present in chloroplasts.

Many plasmodesmata connected vascular parenchyma cells to other cell types in *S. alpinii* (Fig.s 5.5, 5.6 B & C, 5.7 A - C). In *C. distincta*, many plasmodesmata were noted at the vascular parenchyma/companion cell interface, although such abundance was not noticed at any other interface along the loading route. Plasmodesmatal connections between vascular parenchyma cells and other cell types were not common in *A. physocarpa* (Fig.s 5.8 B & C), *A. fructicosa*, *C. obtusifolium (Fig.s 5.11 A, 5.12, 5.13)*, *C. carnosa* (Fig. 5.16) and *C. woodii*. Plasmodesmata occasionally connected vascular parenchyma and companion cells in *A. fructicosa*, but were not seen at other interfaces.

Companion cells were discernible by their dense cytoplasm, with abundant ER, ribosomes and mitochondria. The nuclei were generally large and central, and vacuoles small and fragmented (Fig.s 5.6 A, B & D, 5.10 A - C, 5.11 A - D, 5.12, 5.15 D). In *A. physocarpa* and *C. carnosa* (Fig.s 5.8 A - C, 5.9 B & C and Fig. 5.16 respectively), the vacuole appeared to be single and larger than that seen in other species.

In *S. alpinii*, very few underdeveloped chloroplasts were seen (Fig. 5.5). Chloroplasts of *A. physocarpa* (Fig.s 5.8 B & C), *C. obtusifolium* (Fig.s 5.11 A & B, 5.12) and *C.woodii* (Fig. 5.15 D) were fully developed, but few and very small without starch granules. Chloroplasts of companion cells of *C. carnosa* did have starch granules present (Fig. 5.16).

Pore-plasmodesmata connected companion cells with sieve tubes in all species (Fig.s 5.6 A - C, 5.8 A, 5.10 B, 5.11 A, C & D, 5.12, 5.14, 5.15 A - C, 5.16). Companion cells of *S. alpinii* were connected to all other cell types by abundant plasmodesmata (Fig.s 5.5, 5.6 A - D, 5.7 A & C).

Extremely few plasmodesmatal connections were noted between companion cells and other surrounding cell types in *A. physocarpa* (Fig.s 5.8 A - C, 5.9 A - C), *A. fructicosa* (Fig. 5.10 A & B), *C. carnosa* (Fig. 5.16), *C. distincta* and *C. woodii* (Fig. 5.15 D).

The plasma membrane of the *C. carnosa* companion cells showed extensive foldings (Fig. 5.16). The membrane foldings were not consistent with that seen in plasmolysed cells, being quite distinct and characteristic. No accompanying wall ingrowths were seen in this species, nor were wall ingrowths noted for any other species examined.

Sieve tubes contain peripheral cytoplasm with plastids and parietal ER (Fig.s 5.5, 5.6 A - D, 5.8 A - C, 5.9 B, 5.10 A & B, 5.11 A - D, 5.12, 5.14, 5.15 D, 5.16). Walls were of even thickness within species. In *A. physocarpa* (Fig.s 5.8 A - C, 5.9 B), *A. fructicosa* (Fig. 5.10 A & B) and *C. distincta*, sieve tube walls were relatively thin.

Sieve tubes were connected to companion cells by pore-plasmodesmata in all species studied (Fig.s 5.5, 5.6 A - D, 5.8 A -C, 5.9 B, 5.10 A & B, 5.11 A - D, 5.12, 5.14, 5.15 D, 5.16). Occasionally, plasmodesmata were seen connecting sieve tubes to vascular parenchyma, as in *S. alpinii* (Fig.s 5.5, 5.6 B).

Plasmodesmata

S. alpinii was unusual compared to other Asclepiadaceae studied, in that plasmodesmata were abundant along the phloem loading route (Fig.s 5.5, 5.6 A - D). Plasmodesmata occurred in aggregates in localized areas of wall thickening. Both branched and unbranched plasmodesmata were seen (Fig.s 5.5, 5.6 A - D, 5.7 A - C).

In the other species, plasmodesmata were rare and seemed much smaller than those of *S. alpinii*. Branched and unbranched plasmodesmata were noted. Where present in *A. fructicosa* and *C. obtusifolium* (Fig.s 5.11 A & B, 5.12, 5.13), few plasmodesmata were aggregated in wall thickenings. In most instances, plasmodesmatal frequency was seen to diminish towards the sieve element/companion complex.

Figure 5.5 Asclepiadaceae: Aspects of phloem ultrastructure of *Secamone alpinii*

Composite plate of transverse section through third order vein in lamina of *Secamone alpinii* showing large bundle sheath cells with central vacuole and peripheral cytoplasm, smaller vascular parenchyma cells with central vacuole and abundant mitochondria, companion cells with granular cytoplasm rich in ribosomes, ER and mitochondria, small vacuoles present, sieve tubes with parietal cytoplasm, plasmodesmata aggregated in raised areas of cell wall common, pore-plasmodesmata between companion cells and sieve tubes

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; M = mitochondrion; PC = parietal cytoplasm; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; PD = plasmodesma; PP = pore plasmodesma)

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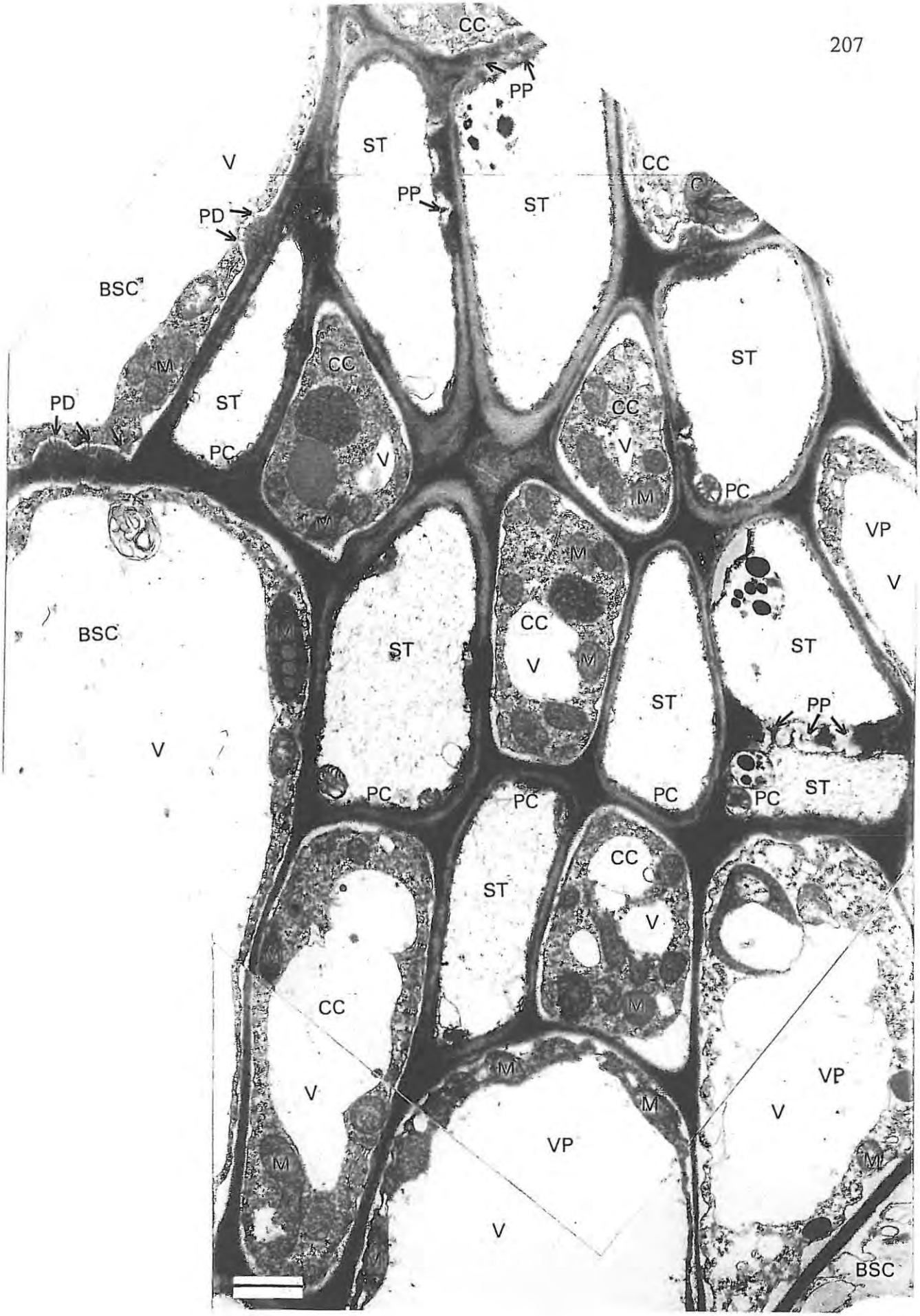
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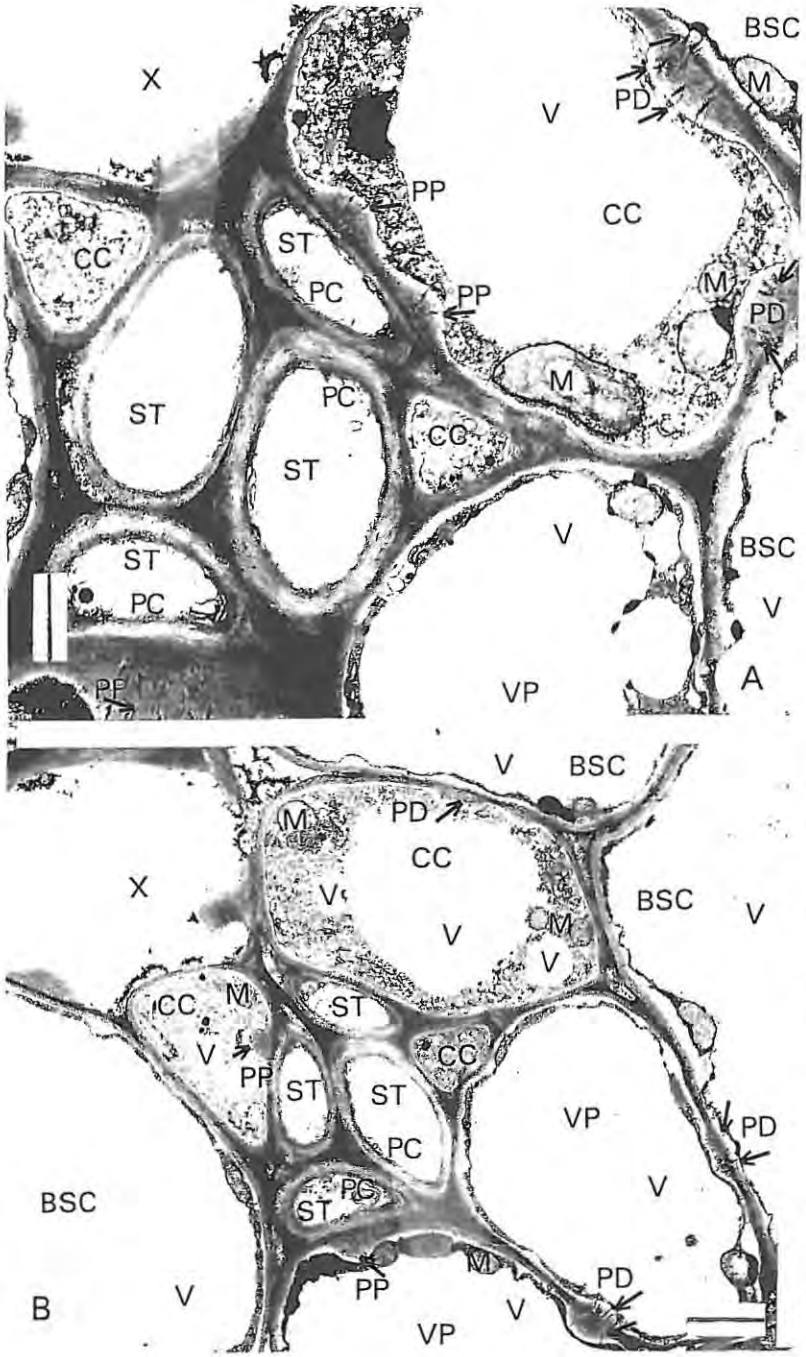
Figure 5.6 Asclepiadaceae: Aspects of phloem ultrastructure of *Secamone alpinii*

A Transverse section through fifth order vein of *Secamone alpinii* showing xylem and abaxial phloem, vacuolate bundle sheath and vascular parenchyma cells, companion cells with cytoplasm rich in ribosomes, ER and mitochondria, sieve tubes with parietal cytoplasm, abundant aggregates of plasmodesmata in raised areas of cell wall, pore-plasmodesmata connect companion cell to sieve tube, B Serial section of fifth order vein of *S. alpinii* shown in A, further along showing xylem and abaxial phloem, vacuolate bundle sheath and vascular parenchyma cells, companion cells with cytoplasm rich in ribosomes, ER and mitochondria, sieve tubes with parietal cytoplasm, abundant aggregates of plasmodesmata in raised areas of cell wall, pore-plasmodesmata connect companion and vascular parenchyma cells to sieve tubes, C Transverse section of sixth order vein of *S. alpinii* vacuolate bundle sheath cells with peripheral cytoplasm and mitochondria, companion cells with cytoplasm rich in ribosomes, ER and mitochondria, underdeveloped chloroplasts present, sieve tubes with parietal cytoplasm, abundant aggregates of plasmodesmata in raised areas of cell wall, pore-plasmodesmata connect companion cells to sieve tubes, D Transverse section of sixth order vein of *S. alpinii* showing vascular parenchyma cells with large central vacuole and peripheral cytoplasm with chloroplasts and mitochondria, companion cells with cytoplasm rich in ribosomes, ER and mitochondria, sieve tubes with parietal cytoplasm

(BSC = bundle sheath cell; CC = companion cell; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; X = xylem; PD = plasmodesma; PP = pore plasmodesma)

Bar represents 1μm for A, B and C, and 2μm for D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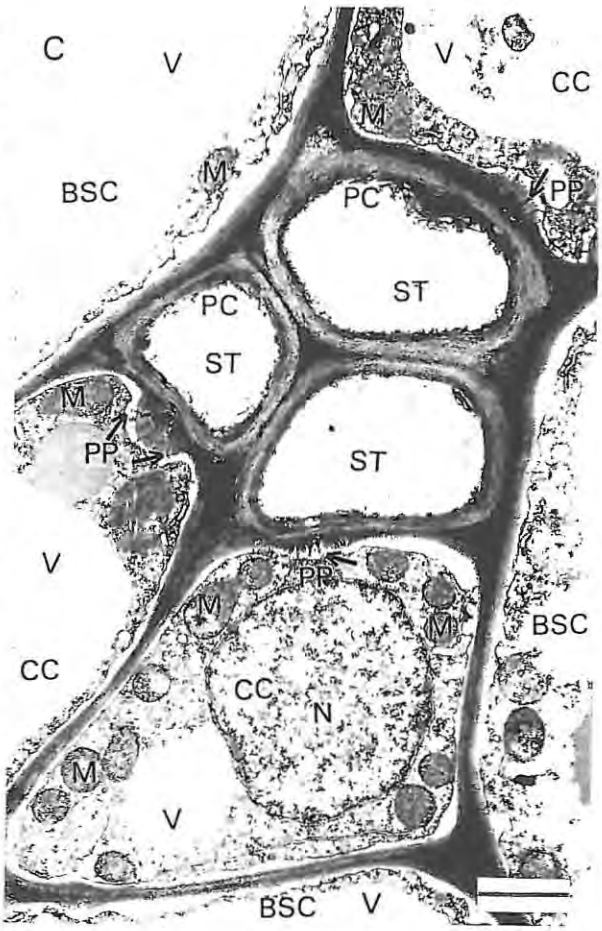
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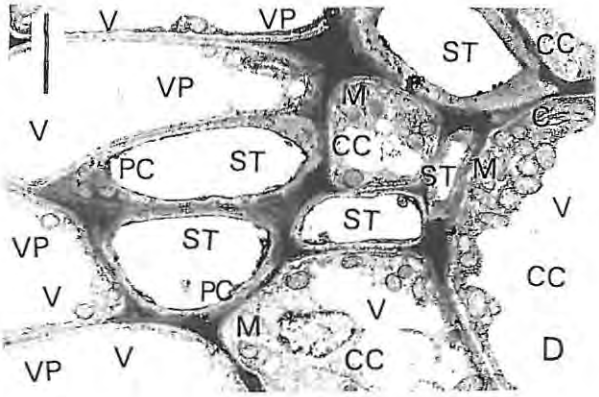
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Figure 5.7 Asclepiadaceae: Aspects of phloem ultrastructure of *Secamone alpinii*

A Section through wall separating vascular parenchyma and companion cells showing abundant branched plasmodesmata aggregated in areas of thickened wall in minor vein of *Secamone alpinii*, B Section through wall separating vascular parenchyma and bundle sheath cells with abundant plasmodesmata aggregated in thickened wall region in minor vein of *S. alpinii*, C Section through wall separating vascular parenchyma and companion cells with abundant plasmodesmata aggregated in thickened cell wall region in minor vein of *S. alpinii*

(BSC = bundle sheath cell; CC = companion cell; CW = cell wall; VP = vascular parenchyma cell; PD = plasmodesma)

Bar represents 0.1 μm

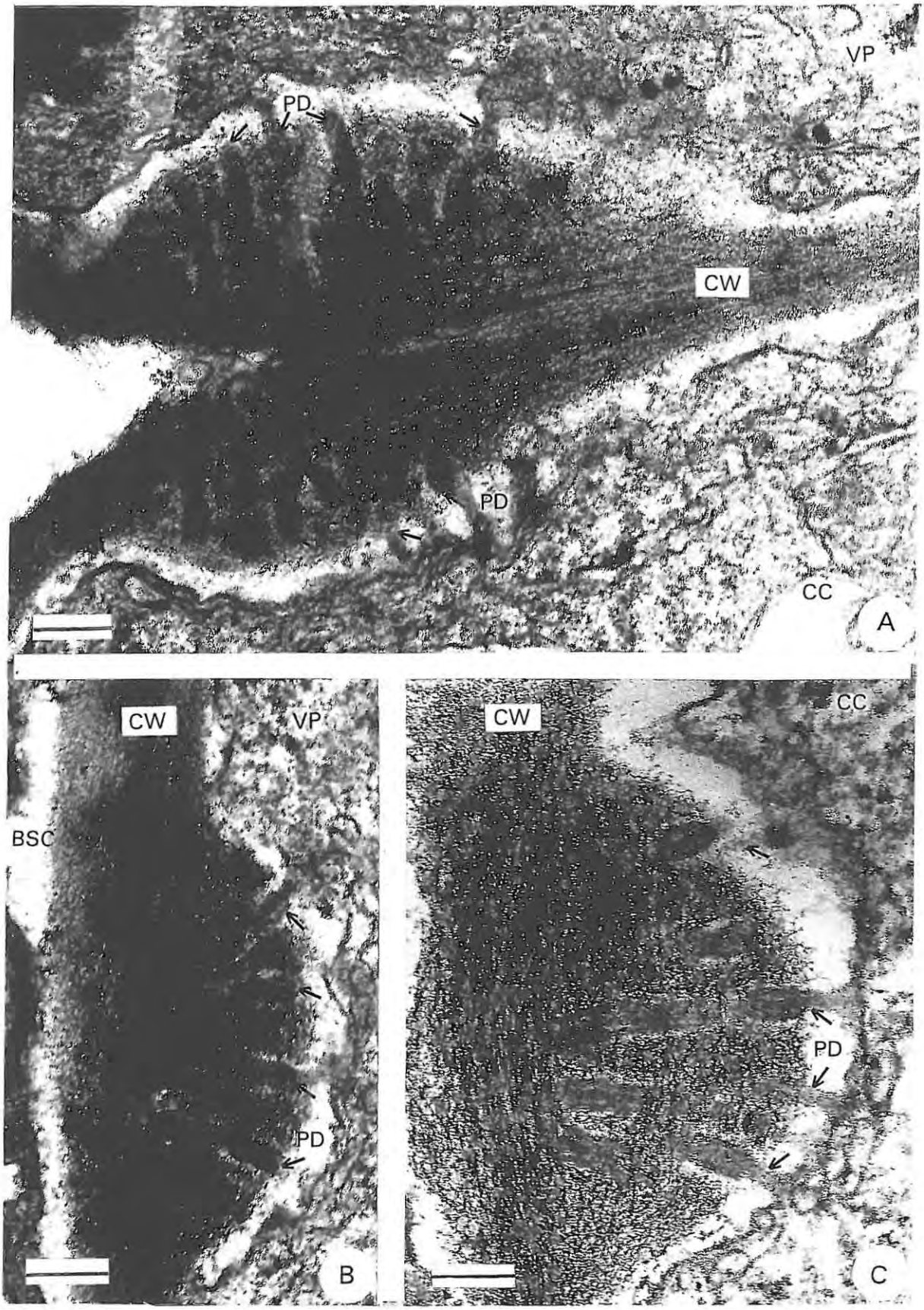
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Figure 5.8 Asclepiadaceae: Aspects of phloem ultrastructure of *Asclepias physocarpa*

A Composite plate of companion cells and adjacent sieve tubes of secondary vein abaxial phloem of *Asclepias physocarpa* showing companion cells with abundant mitochondria and ribosomes, ER and vacuoles present, pore-plasmodesmata branched on companion cell side connect cell to sieve tube, sieve tubes with parietal cytoplasm, B Transverse section of minor vein of *A. physocarpa* showing vascular parenchyma cells with large central vacuole, chloroplasts with starch granules, peripheral nucleus and mitochondria, companion cells with abundant mitochondria and ribosomes in cytoplasm, large nucleus, chloroplasts without starch granules and small vacuoles, sieve tubes with parietal cytoplasm, no plasmodesmata or pore-plasmodesmata noted, C Composite plate of abaxial phloem of secondary vein of *A. physocarpa* showing large vacuolate bundle sheath cells with chloroplasts and starch granules, peripheral nucleus and cytoplasm, smaller vascular parenchyma cells with large central vacuole and chloroplasts without starch granules, companion cell with mitochondria, chloroplasts without starch and small vacuoles in cytoplasm rich in ribosomes, sieve tubes with parietal cytoplasm, no plasmodesmata or pore-plasmodesmata seen

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; ER = endoplasmic reticulum; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; S = starch granule; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; X = xylem; PD = plasmodesma)

Bar represents 0.2μm for A, 2μm for B and 2μm for C

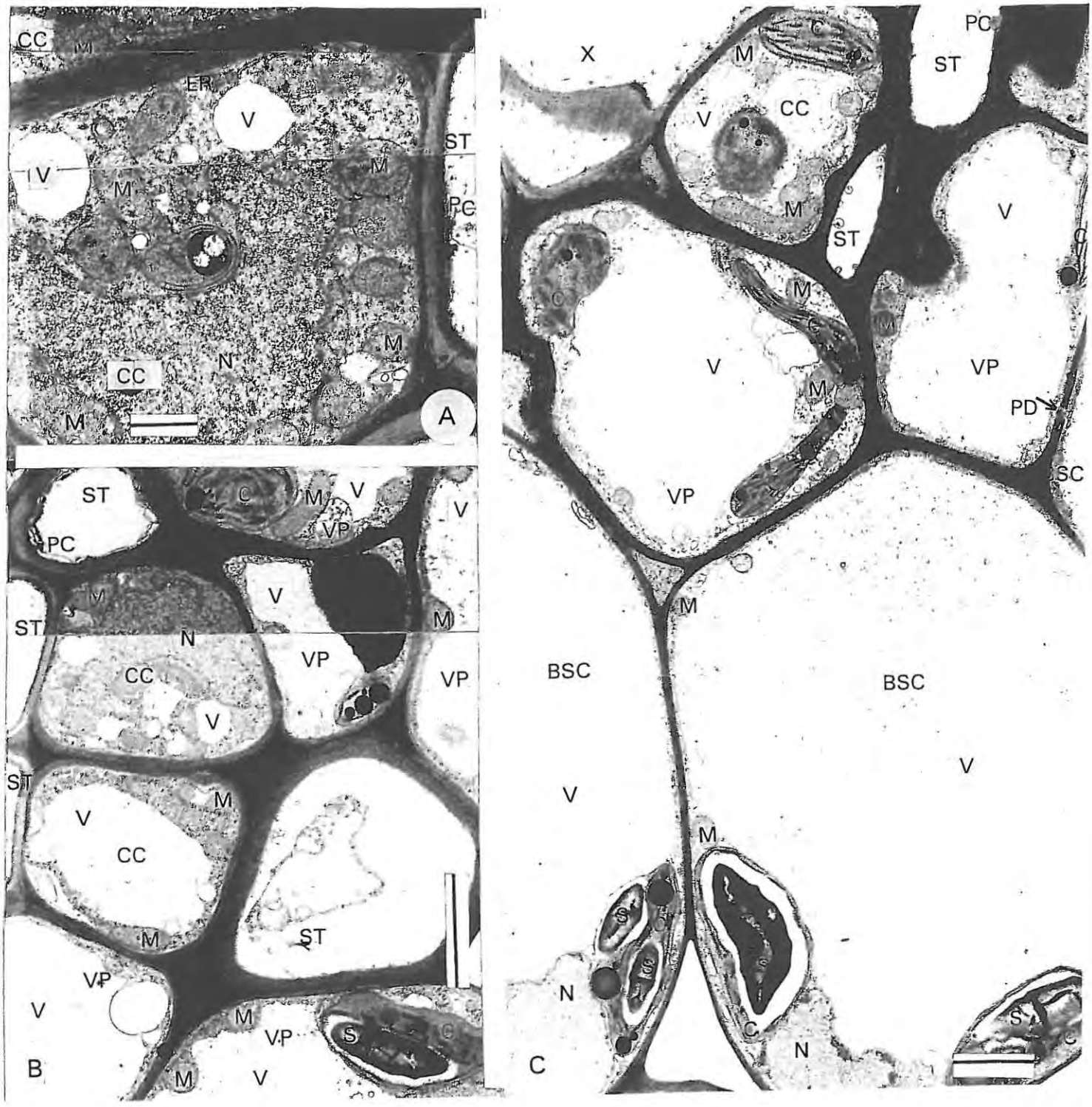
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Figure 5.9 Asclepiadaceae: Aspects of phloem ultrastructure of *Asclepias physocarpa*

A Longitudinal section of minor vein of *Asclepias physocarpa* showing bundle sheath cells with chloroplasts containing starch granules aggregated on wall away from vascular tissue, large central vacuole with peripheral cytoplasm and mitochondria, companion cells with small vacuoles, mitochondria, central nucleus and chloroplasts without starch granules, vascular parenchyma cell with large central vacuole, chloroplasts and mitochondria, sieve tube with parietal cytoplasm, no plasmodesmata or pore-plasmodesmata noted, B Transverse section of minor vein of *A. physocarpa* showing companion cells with dense granular cytoplasm, chloroplasts without starch granules, many mitochondria and ribosomes, and a central nucleus, sieve tubes with parietal cytoplasm, no plasmodesmata or pore-plasmodesmata seen, C Longitudinal section of minor vein companion cells of *A. physocarpa* showing large central nucleus, cytoplasm rich in ribosomes and mitochondria, small vacuoles, vascular parenchyma cell with central vacuole and peripheral cytoplasm, sieve tube with parietal cytoplasm

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; S = starch granule; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell)

Bar represents 2μm for A, 1μm for B, and 1μm for C

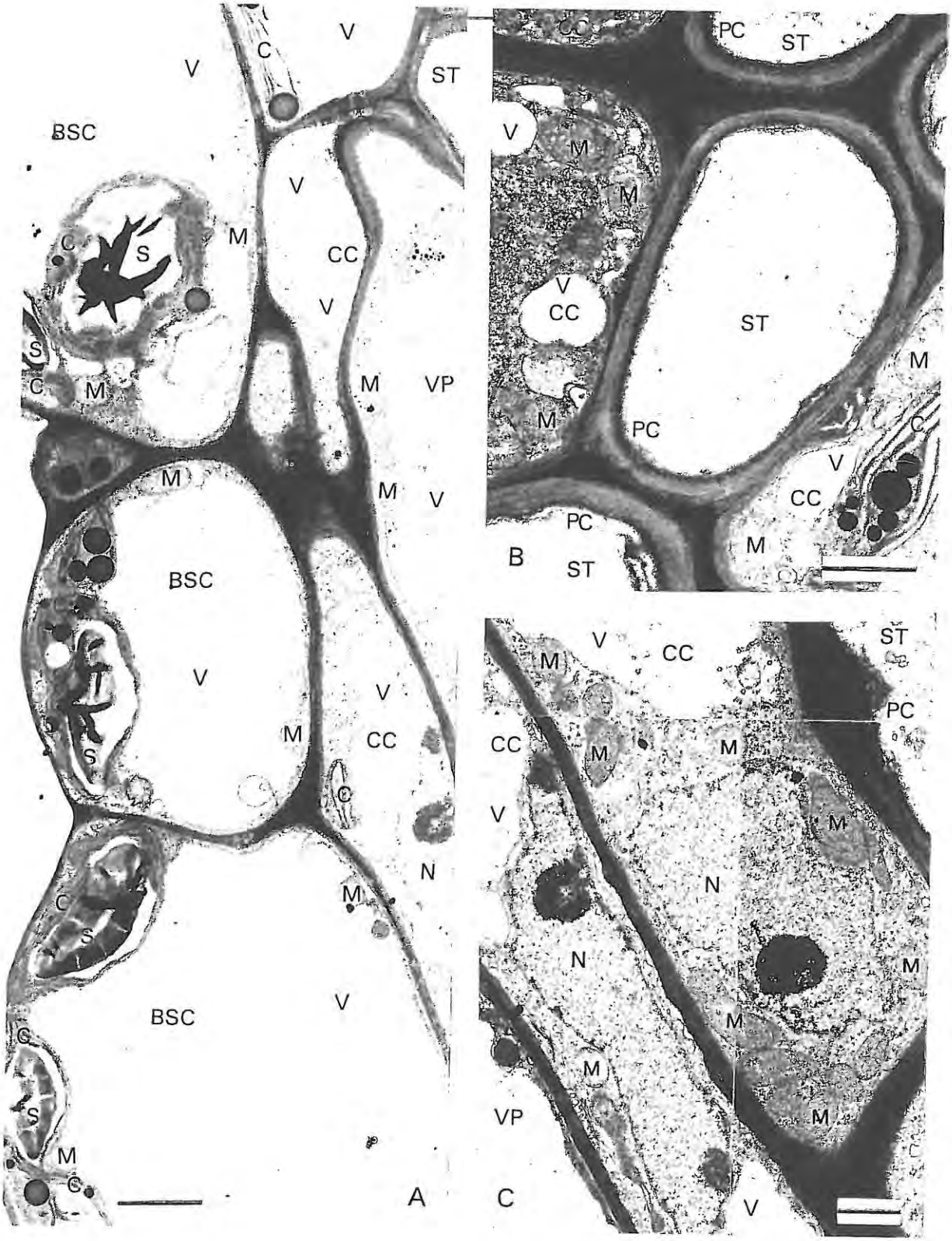
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Figure 5.10 Asclepiadaceae: Aspects of phloem ultrastructure of *Asclepias fructicosa*

A Transverse section through phloem of fourth to fifth order vein of *Asclepias fructicosa* showing vascular parenchyma cells with large central vacuole, peripheral cytoplasm with mitochondria and chloroplasts with starch granules, companion cells with large central nucleus, abundant mitochondria, chloroplasts without starch granules and small vacuoles, sieve tubes with parietal cytoplasm and pore-plasmodesmata to companion cells, no plasmodesmata noted, B Transverse section through sixth to seventh order vein of *A. fructicosa* showing bundle sheath cell containing large central vacuole with peripheral cytoplasm and nucleus, vascular parenchyma cell with large central nucleus and peripheral cytoplasm with mitochondria and chloroplasts with starch granules, companion cells with vacuoles and abundant mitochondria in a cytoplasm rich in ribosomes, sieve tubes with parietal cytoplasm and pore-plasmodesmata to companion cell, no plasmodesmata noted, C Transverse section through companion and vascular parenchyma cells of sixth to seventh order vein of *A. fructicosa* showing companion cell with vacuoles, chloroplasts without starch granules, abundant mitochondria in granular cytoplasm rich in ribosomes and ER, vascular parenchyma cell with large vacuole and peripheral cytoplasm with chloroplasts, mitochondria and Golgi body

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; G = Golgi body; ER = endoplasmic reticulum; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; S = starch granule; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; X = xylem; PP = pore plasmodesma)

Bar represents 2μm for A and B, and 1μm for C

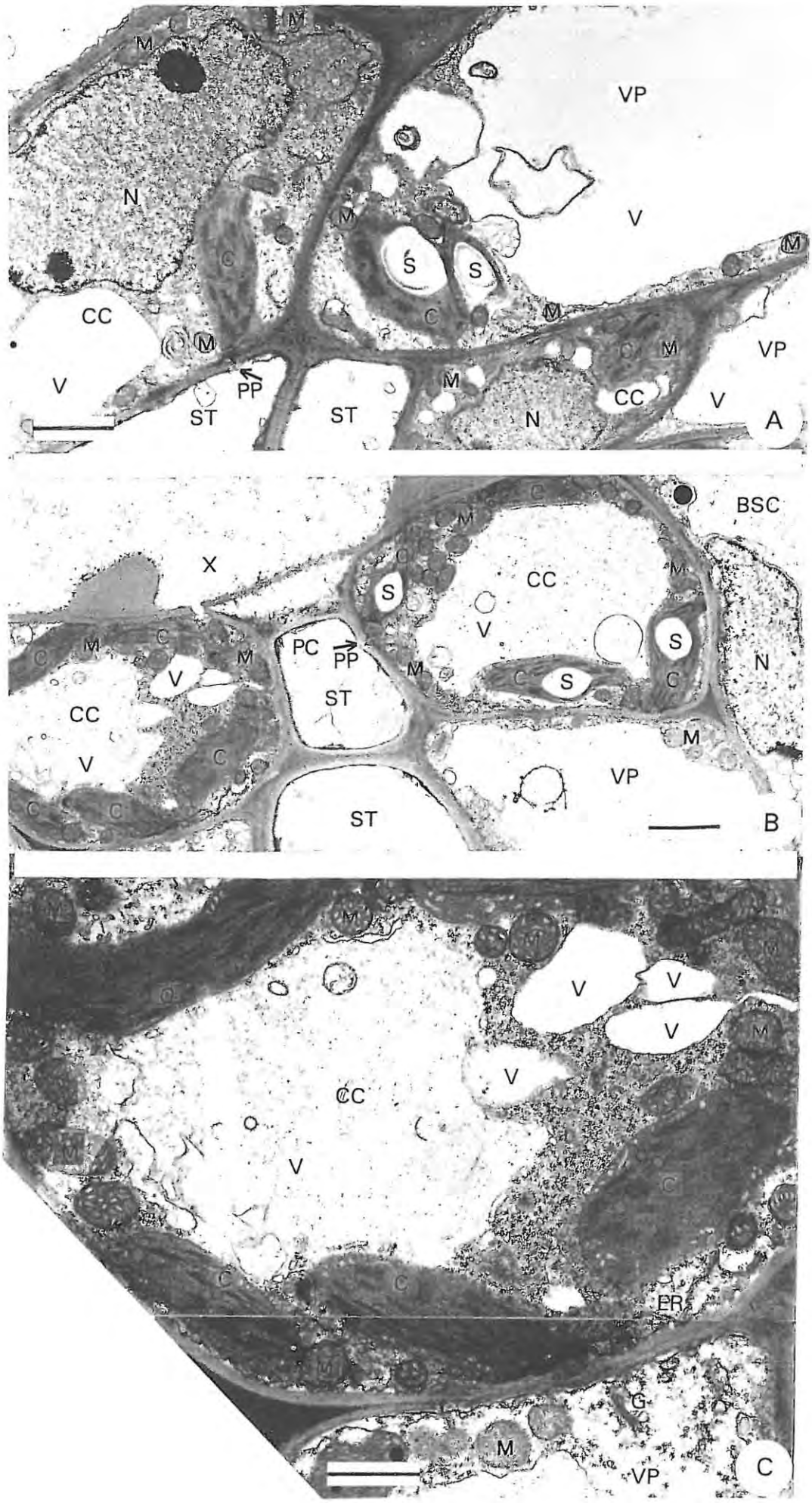
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Figure 5.11 Asclepiadaceae: Aspects of phloem ultrastructure of *Cynanchum obtusifolium*

A Transverse section through adaxial phloem of secondary vein of *Cynanchum obtusifolium* showing vascular parenchyma cells with large central vacuole, chloroplasts with starch granules, mitochondria and plasmodesmata to adjacent vascular parenchyma and companion cells, companion cells with small vacuoles, abundant mitochondria and chloroplasts without starch granules, pore-plasmodesmata to sieve tubes, sieve tubes with parietal cytoplasm, B Transverse section through fourth to fifth order vein of *C. obtusifolium* showing bundle sheath cells with large central vacuole and peripheral cytoplasm with chloroplasts and mitochondria, vascular parenchyma cells with large central vacuole, chloroplasts with starch granules, nucleus, mitochondria and plasmodesmata to adjacent vascular parenchyma cell, companion cell with nucleus, small vacuole, mitochondria and chloroplast without starch granules, C Transverse section through abaxial phloem of secondary vein of *C. obtusifolium* showing vascular parenchyma cells with large central vacuole, nucleus, chloroplast with starch granules, mitochondria, companion cells with nucleus, vacuoles and abundant mitochondria, sieve tubes with parietal cytoplasm, pore-plasmodesmata to companion and vascular parenchyma cells, D Transverse section through sixth to seventh order vein phloem of *C. obtusifolium* showing vacuolate bundle sheath cells with peripheral cytoplasm, vascular parenchyma cell containing vacuole and peripheral cytoplasm, companion cells with vacuoles, granular cytoplasm with abundant mitochondria, sieve tubes with parietal cytoplasm and pore-plasmodesmata to companion cell

(BSC = bundle sheath cell; C = chloroplast; CC = companion cell; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; S = starch granule; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; X = xylem; PD = plasmodesma; PP = pore plasmodesma)

Bar represents 2μm for A and B, and 1μm for C and D

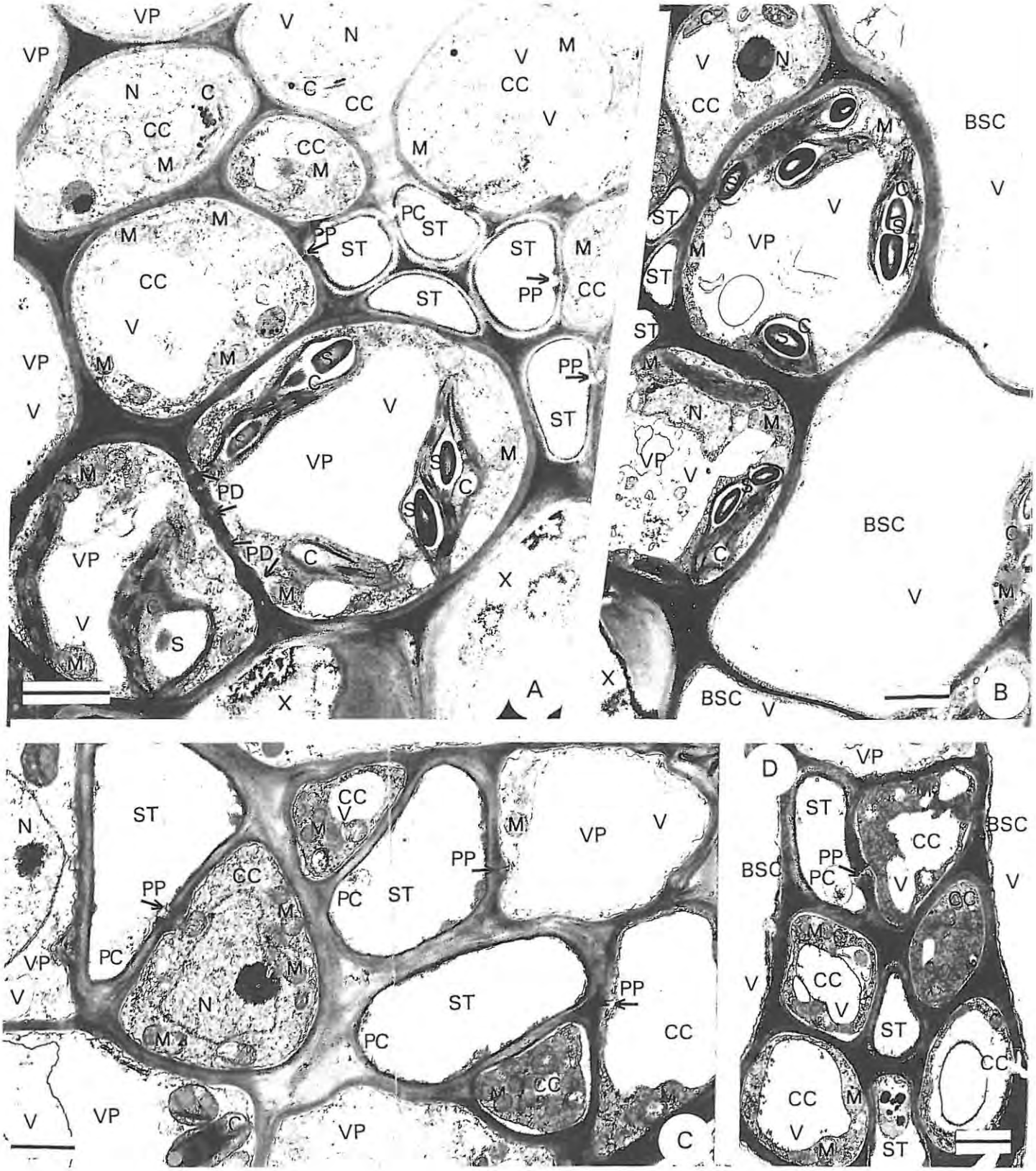
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Figure 5.12 Asclepiadaceae: Aspects of phloem ultrastructure of *Cynanchum obtusifolium*

Composite plate of adaxial phloem of secondary vein of *Cynanchum obtusifolium* showing vascular parenchyma cells with large central vacuole, chloroplasts with starch granules, abundant mitochondria and ER, and plasmodesmata to adjacent vascular parenchyma cell, companion cells with abundant mitochondria, small vacuoles, granular cytoplasm and chloroplasts without starch granules, sieve tubes with parietal cytoplasm and pore-plasmodesmata to companion and vascular parenchyma cells

(C = chloroplast; CC = companion cell; ER = endoplasmic reticulum; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; S = starch granule; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; X = xylem; PD = plasmodesma; PP = pore plasmodesma)

Bar represents 1μm

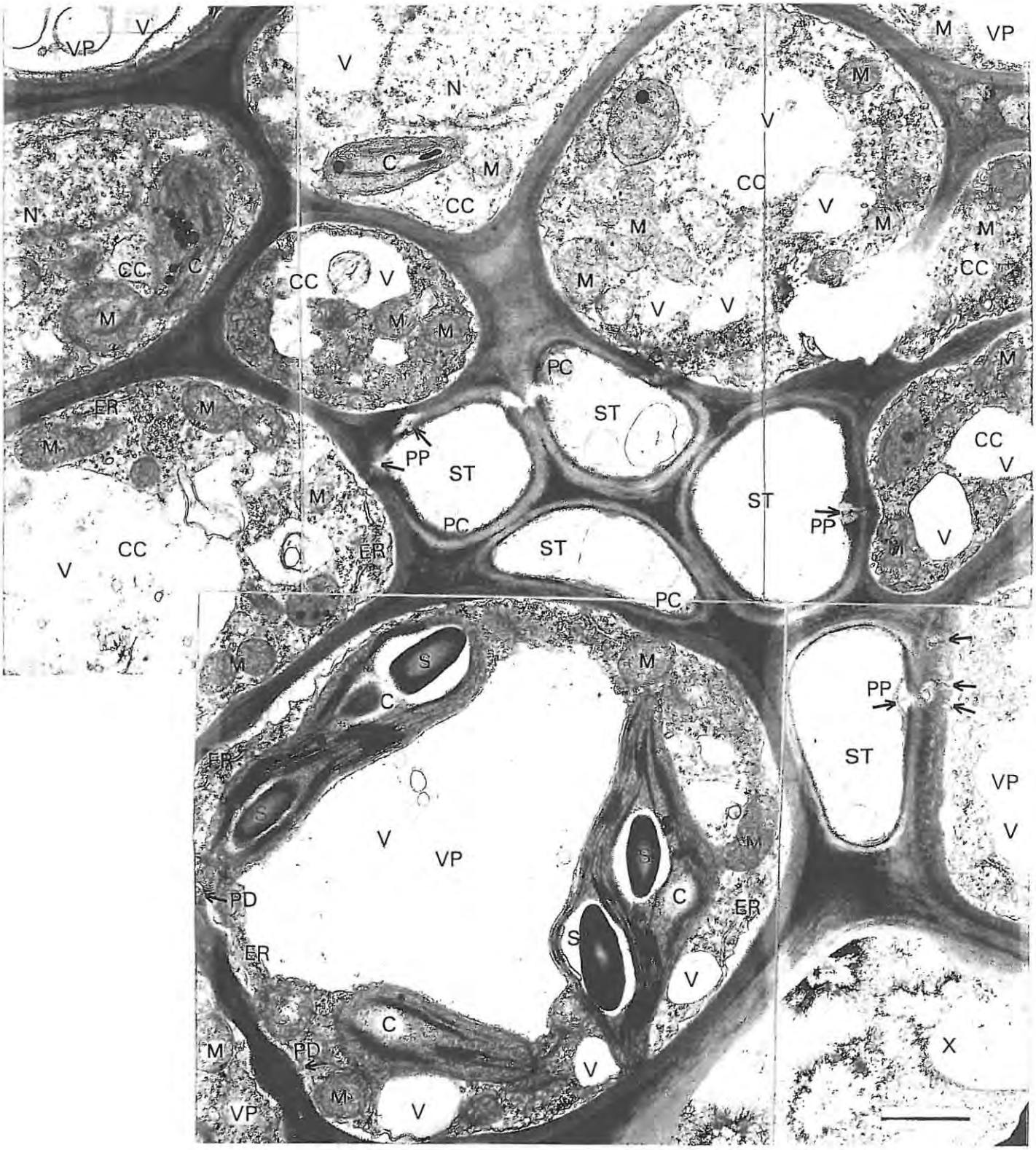
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Figure 5.13 Asclepiadaceae: Aspects of phloem ultrastructure of *Cynanchum obtusifolium*

Composite plate of vascular parenchyma and companion cells of adaxial phloem of secondary vein of *Cynanchum obtusifolium* showing vascular parenchyma cells with large central vacuole, chloroplasts with starch granules, mitochondria, granular cytoplasm with ribosomes and ER, and plasmodesmata to adjacent vascular parenchyma and companion cells, companion cell with abundant mitochondria, small vacuoles and cytoplasm rich in ribosomes and ER

(C = chloroplast; CC = companion cell; ER = endoplasmic reticulum; M = mitochondrion; S = starch granule; V = vacuole; VP = vascular parenchyma cell; X = xylem; PD = plasmodesma)

Bar represents 1μm

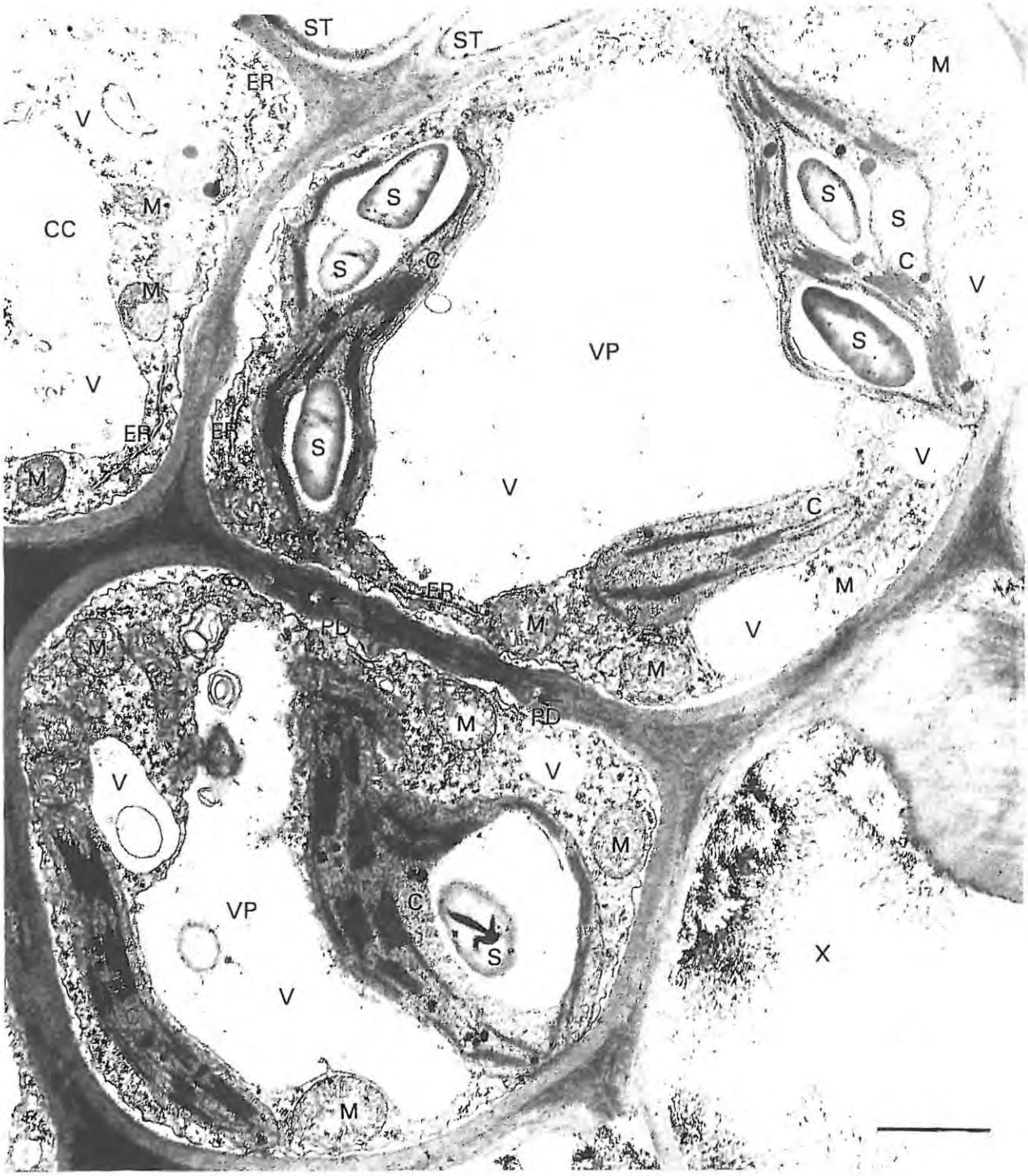
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Figure 5.14 Asclepiadaceae: Aspects of phloem ultrastructure of *Ceropegia woodii*

Composite plate of tangential longitudinal section of minor vein of *Ceropegia woodii*, showing sieve tubes with parietal cytoplasm and clusters of pore-plasmodesmata, companion cells with vacuoles, mitochondria and many chloroplasts without starch granules in a granular cytoplasm

(C = chloroplast; CC = companion cell; CW = cell wall; M = mitochondrion; ST = sieve tube; V = vacuole; PP = pore plasmodesma)

Bar represents 2μm

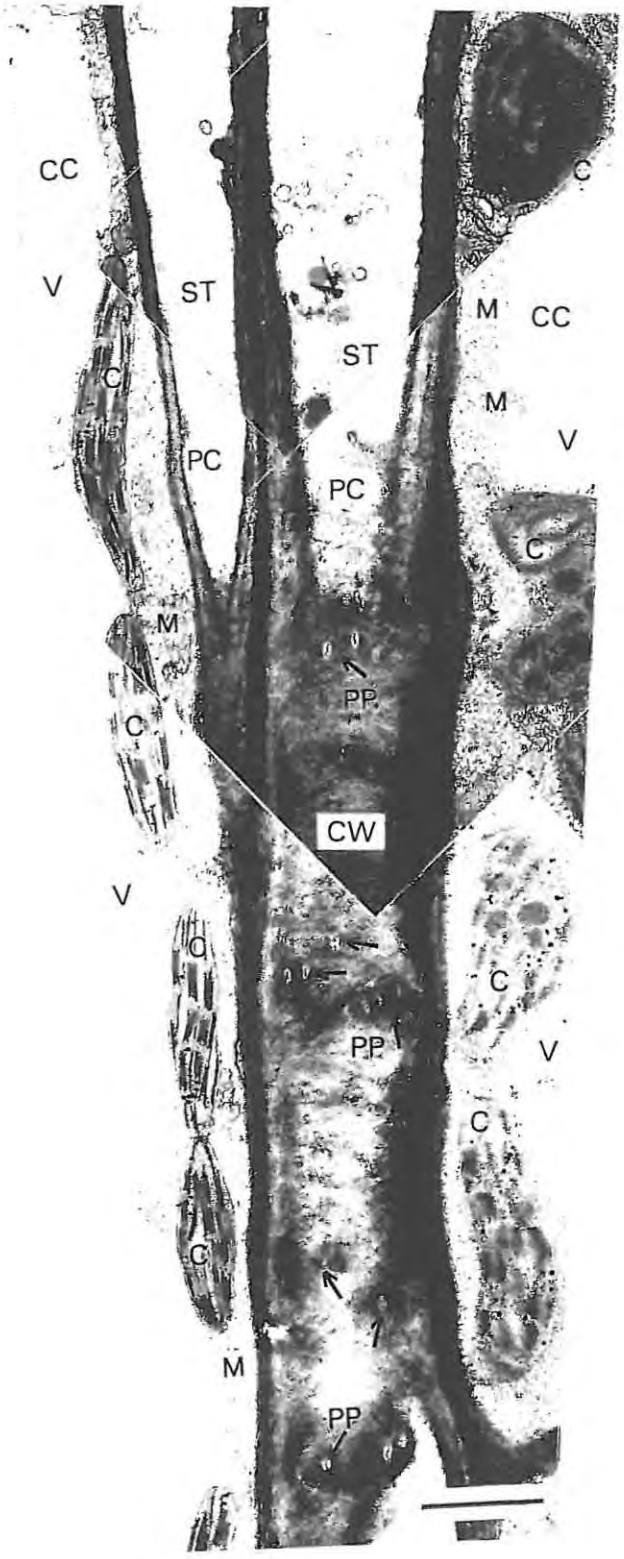
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Figure 5.15 Asclepiadaceae: Aspects of phloem ultrastructure of *Ceropegia woodii*

A Longitudinal section of minor vein sieve tube wall of *Ceropegia woodii* showing pore-plasmodesmata in clusters and parietal cytoplasm, adjacent companion cells with chloroplasts without starch granules, mitochondria and vacuoles in granular cytoplasm, B Transverse section of clustered pore-plasmodesmata in sieve tube wall of *C. woodii* as shown in A, C Transverse section of clustered and branched pore-plasmodesmata in sieve tube wall of *C. woodii* as shown in A, D Transverse section through midvein of *C. woodii* showing vascular parenchyma cells containing large central vacuole and peripheral cytoplasm with chloroplast lacking starch granules, companion cell with granular cytoplasm rich in ribosomes, chloroplasts without starch granules, and abundant mitochondria, and sieve tubes with parietal cytoplasm

(C = chloroplast; CC = companion cell; CW = cell wall; M = mitochondrion; PC = parietal cytoplasm; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; PP = pore plasmodesma)

Bar represents 1μm for A, 0.2μm for B and C, and 2μm for D

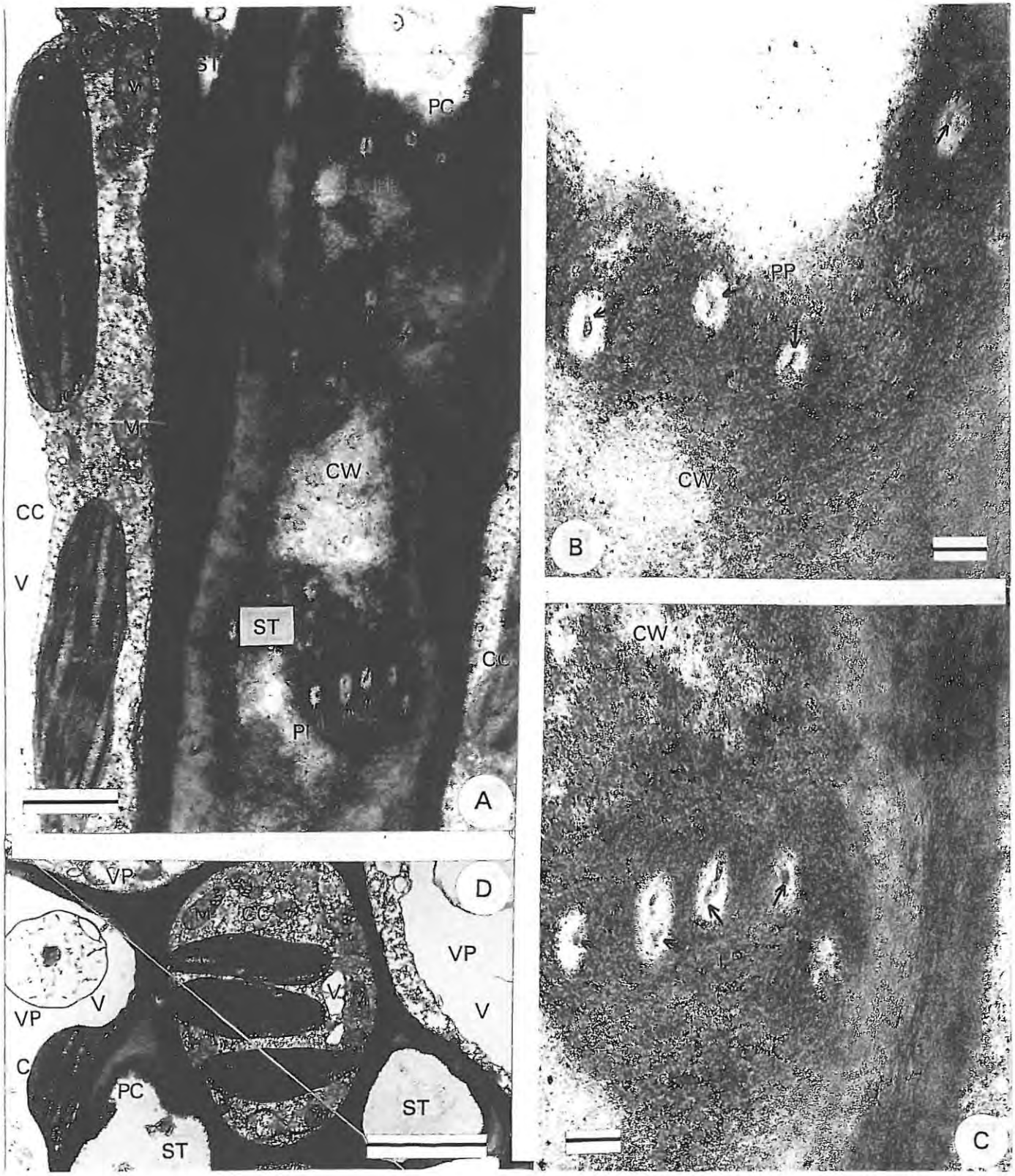
PC
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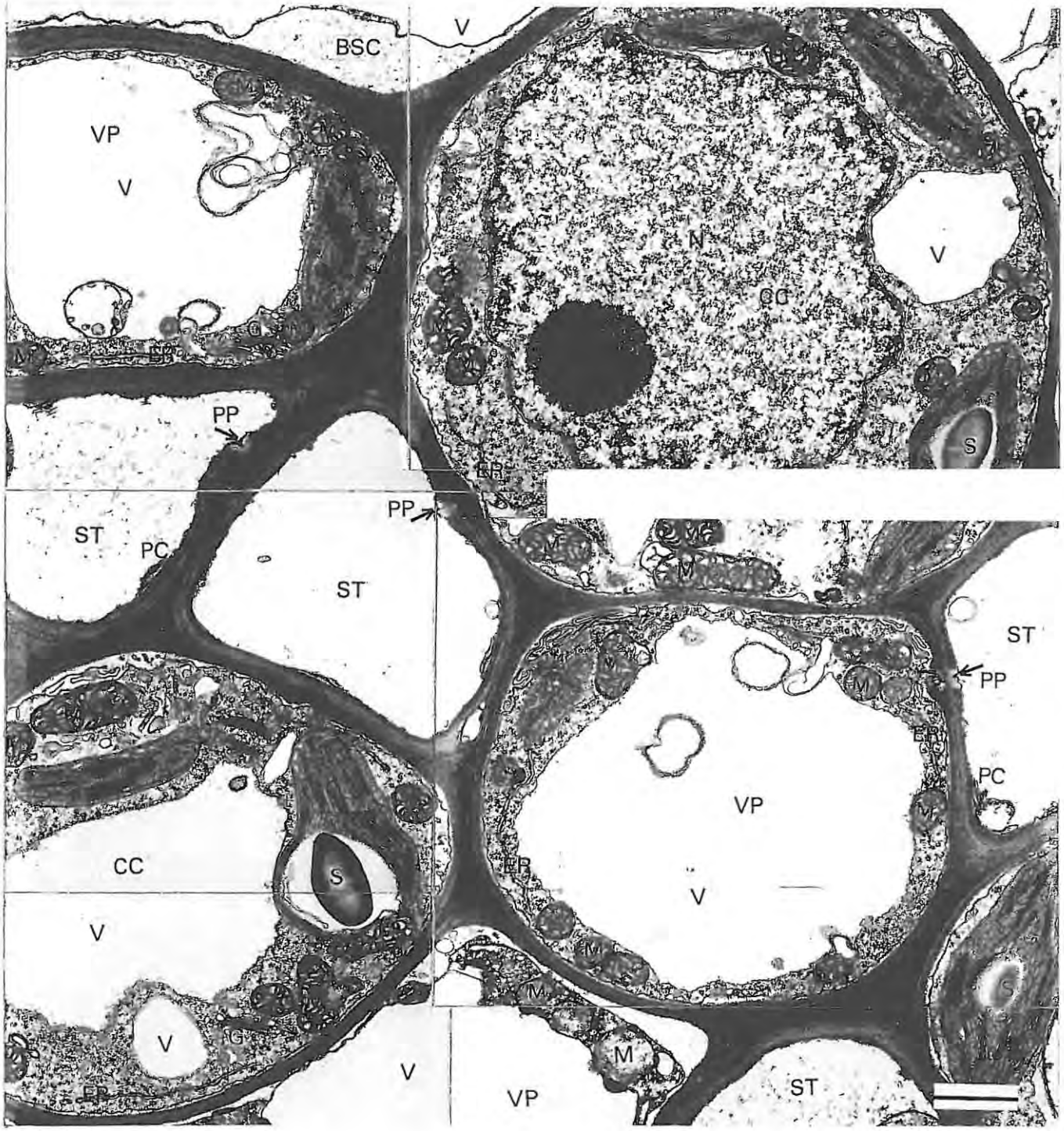
Figure 5.16 Asclepiadaceae: Aspects of phloem ultrastructure of *Ceropegia carnosa*

Composite plate of abaxial phloem of secondary vein of *Ceropegia carnosa* showing vascular parenchyma cells with large central vacuole and peripheral cytoplasm containing ER and Golgi bodies, mitochondria and chloroplasts without starch granules, companion cells with small vacuoles, chloroplasts with starch granules, abundant mitochondria, ER and Golgi bodies in cytoplasm rich in ribosomes, companion cell membrane shows extensive folding, sieve tubes with parietal cytoplasm and pore-plasmodesmata to companion and vascular parenchyma cells

(C = chloroplast; CC = companion cell; G = Golgi body; ER = endoplasmic reticulum; M = mitochondrion; N = nucleus; PC = parietal cytoplasm; S = starch granule; ST = sieve tube; V = vacuole; VP = vascular parenchyma cell; PP = pore plasmodesma)

Bar represents 1μm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5.3 Discussion

Cellular ultrastructure of the loading route must first be considered. This information should then be applied to type categories in order to determine minor vein configuration, and consequently to infer phloem loading method.

5.3.1 Ultrastructure of the loading pathway supposedly followed by assimilates

Mesophyll cells of all three families displayed expected features such as thin walls, a central vacuole with a peripheral cytoplasm, and many chloroplasts. No cell membrane foldings were noticed in the chloroplast vicinity in any of the species studied (Evert & Mierzwa 1986, Warmbrodt & Van Der Woude 1990).

Symplasmic connectivity generally characterises the production compartment, viz. mesophyll and bundle sheath cells (Erwee *et al.* 1985, Fisher 1986, Fisher 1988, Van Bel et al. 1988, Robinson-Beers & Evert 1991a, Turgeon and Beebe 1991, Beebe & Evert 1992). In the Ranunculaceae, bundle sheath cells bound veins of all orders, extending to the tips of free ending ultimate veinlets in areoles. Particularly abundant plasmodesmata interconnected bundle sheath cells of ultimate veinlets. As phloem is absent in this region, it is interesting to speculate on the significance of such symplasmic connectivity. Bundle sheath cells are considered to be part of the production compartment (Oparka & Van Bel 1992), but could also represent an important collection point in the loading pathway from mesophyll cells in areole centres to surrounding minor vein phloem. Future loading studies based on such conjecture could provide interesting results.

Vascular parenchyma cells were present in all vein orders. These cells are easily discernible, being smaller with a denser cytoplasm than bundle sheath cells, and larger with a less dense cytoplasm than companion cells. A medium sized central vacuole generally occurred, in a cytoplasm rich in ribosomes, ER and mitochondria.

Companion cells were usually smaller than vascular parenchyma cells, but bigger than sieve elements, in all families. The size ratio of companion cell to sieve element noted in foliar veins is consistent for with that of collection phloem (Van Bel 1993b, Van Bel 1996, Kempers *et al.*

1998, Van Bel & Knoblauch 2000). No distinct difference in size ratio was noted between minor veins and primaries for any of the species studied, emphasizing the collective role of phloem in mature leaves.

Companion cell cytoplasm was characteristically dense with many ribosomes, ER and very many mitochondria. Such ultrastructural features are indicative of the high metabolic activity that would be expected in actively collecting and transporting tissue (Warmbrodt & Van Der Woude 1990).

As can be seen from Table 5.1, there is a trend towards the loss of plastids and starch granules from mesophyll to companion cell. The deposition of starch in chloroplasts is a feature associated with symplasmic loaders (Oparka & Van Bel 1992, Volk *et al.* 1996). *S. alpinii* was considered to be a possible symplasmic loader due to an abundance of plasmodesmata all along the loading route, yet lacks starch accumulations in phloem tissue. The mesophyll cells of *S. alpinii* and *C. woodii* (Asclepiadaceae) did, however, store starch.

Callose deposition was noted occluding pore-plasmodesmata between adjacent sieve elements of *Carissa bispinosa*, Apocynaceae. This has been discussed previously in the literature by Hughes and Gunning (1980), in which callose deposition was seen in the same position in nectaries of *Abutilon*. Their paper was aimed at elucidating the deposition of callose in response to wounding by glutaraldehyde. No mention was made of the possible significance of callose blocking pore-plasmodesmata in the paper. In light of the current study, it is suggested that the blockage of interconnecting pore-plasmodesmata between adjacent sieve tubes in *Carissa bispinosa* might be significant in terms of preventing lateral leakage of assimilates and therefore aiding in a faster export system. However, it must be noted that the possibility of osmotic shock during fixation could well have stimulated its formation.

None of the species studied showed ingrowths of the companion cell walls, eliminating the possibility of transfer cells. This result is supported by the results of Batashev (1996), in which minor vein configurations of northern hemisphere Apocynaceae were examined. He concluded that transfer cells, and therefore type 2b, were absent from the family Apocynaceae (in the old sense, viz. excluding Asclepiadaceae).

Table 5.1 Starch accumulation and plastids occurrence from mesophyll to companion cell for selected species of the Ranunculaceae, Apocynaceae and Asclepiadaceae

SPECIES (FAMILY)	MESOPHYLL CELLS	BUNDLE SHEATH CELLS	VASCULAR PARENCHYMA	COMPANION CELLS
R. multifidus (Ranunculaceae)	starch granules	starch granules	starch granules present but small	no plastids or starch granules
C. bispinosa (Apocynaceae)	starch granules	starch granules	no plastids noted in current study	plastids present but no starch
A. oppositifolia (Apocynaceae)	starch granules	starch granules	poorly developed plastids with starch	no starch
S. alpinii (Asclepiadaceae)	starch granules	no starch	no starch	poorly developed plastids without starch
A. physocarpa (Asclepiadaceae)	very large starch granules	very large starch granules	starch granules	no starch granules
A. fructicosa (Asclepiadaceae)	starch granules	starch granules	starch granules	starch granules
Cynanchum obtusifolium (Asclepiadaceae)	starch granules	starch granules	starch granules	no starch
Ceropegia carnosa (Asclepiadaceae)	starch granules	starch granules	starch granules	starch granules
C. woodii (Asclepiadaceae)	no starch granules	no starch	no starch	no starch
C. distincta (Asclepiadaceae)	starch granules	starch granules	starch granules	no starch granules

Vacuoles were typically small and fragmented in companion cells of most species, with the exception of *A. physocarpa* and *C. carnosa* in which vacuoles appeared single and large. Fragmented vacuoles are a distinct feature of intermediary cells (Oparka & Van Bel 1992, Van Bel 1996). Companion cells could, therefore, be classified as intermediary cells. However, bearing in mind that the main feature of intermediary cells is abundant plasmodesmata, the designation of ordinary companion cells is preferred in this case. The companion cell membrane of *C. carnosa* (Asclepiadaceae) did show extensive foldings, but no corresponding growth of the cell wall. This may indicate ultrastructural evidence for a tendency towards apoplasmic loading. However, phloem loading studies *per se* would be required to verify such speculation.

Turgeon (2000) speculated on the requirement and presence of plasmodesmata in minor veins of species that load apoplasmically. Plasmodesmata could allow for exchange of certain sugars or molecules functioning for communication. Plasmodesmata could also be needed to allow electrical conduction, maintaining an even charge across cells. As retaining plasmodesmata puts the cells, and plant body as a whole, at risk from viral infection, it would be logical to assume that these channels must still be of use to the plant somehow (Turgeon 2000).

5.3.2 Suggested type categories and inferred phloem loading method

The anatomical and ultrastructural evidence presented in this thesis is used to determine minor vein type, which in turn may be used to infer the associated phloem loading method (Gamalei & Pakhomova 1983a & b, Evert & Mierzwa 1986, McCauley & Evert 1989, Gamalei *et al.* 1992, Van Bel & Gamalei 1992, Flora & Madore 1996). It must, however, be stressed that such evidence may be used to *suggest* method of phloem loading only, not to demonstrate it. For each species presented here, further studies determining functionality of plasmodesmata, active uptake cross companion cell membranes, and type of sugar translocated would need to be undertaken in order to confidently propose the method of phloem loading employed.

The hypothesis of Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel

1992a-c, 1993a, b) was discussed in detail in chapter one. The basis of the hypothesis is minor vein configuration, of which four categories were recognised. Type 1 configuration depends on an abundance of plasmodesmata connecting companion cells to other cell types, implying predominantly symplasmic phloem loading via functional plasmodesmata. Type 2a shows a paucity of plasmodesmata at this interface, with companion cells, termed ordinary, lacking wall ingrowths. Loading would therefore be predominantly apoplasmic across the companion cell membrane. Type 2b also lacks plasmodesmatal connectivity, but shows extensive wall ingrowths in modified companion cells, called transfer cells, to increase surface area to maximise apoplasmic uptake (Wimmers and Turgeon 1991). Type 2c displays the Kranz anatomy typical of C_4 plants. Many plasmodesmata connect mesophyll to bundle sheath cells, yet loading would be predominantly apoplasmic as few plasmodesmata are present at the companion cell/bundle sheath interface.

Minor vein category may therefore be determined by anatomical and ultrastructural evidence, in particular that of abundance and position of plasmodesmata, and ultrastructure of companion cells.

Ranunculaceae

R. multifidus showed abundant plasmodesmatal connectivity between mesophyll cells, and between mesophyll and bundle sheath cells. However, the closer to the companion cell/sieve element complex, the fewer the plasmodesmatal connections seen. Very few plasmodesmata were noted between companion cells and other surrounding cell types. Companion cells ultrastructure did not reveal wall ingrowths. On the basis of this evidence, *R. multifidus* is proposed to display type 2a minor vein configuration with ordinary companion cells. This result agrees with that proposed by Gamalei (1989, 1991) for the Ranunculaceae.

The predominant phloem loading method suggested to accompany a type 2a configuration is apoplasmic (Gamalei 1989, Gamalei *et al.* 1992, Van Bel 1992, Van Bel & Gamalei 1992, Flora & Madore 1996). However, certain reservations are expressed over the determination of phloem loading processes in the current study, as loading tests were not conducted. Furthermore type 2a does not represent an extreme type in which loading could only be one method or the other. The potential for

a certain amount of symplasmic loading may not be excluded until further studies rule out the possibility. At this stage, a very tentative proposal for a predominantly apoplasmic phloem loading method in *R. multifidus* is presented, and is supported by the anatomical and ultrastructural evidence presented here and by the literature (Gamalei 1989, Gamalei *et al.* 1992, Van Bel 1992, Van Bel & Gamalei 1992, Flora & Madore 1996).

Apocynaceae and Asclepiadaceae

The Apocynaceae and Asclepiadaceae are considered together due to their close taxonomic relationship (Dahlgren 1975, Heywood 1978, Thorne 1992, Chase *et al.* 1993, Struwe *et al.* 1994, Sennblad & Bremer 1996, Swarupanandan *et al.* 1996, Liede 1997, Endress & Bruyns 2000), and the fact that at the ultrastructural level no differences are apparent.

Despite the lack of statistical evidence, plasmodesmatal frequency appeared to diminish along the route from mesophyll to companion cell/sieve element complex. Companion cell/sieve element complexes were virtually isolated by a paucity of plasmodesmatal connections to other surrounding cell types. The only exception to this trend was *S. alpinii* of the Asclepiadaceae. Abundant plasmodesmata were noted between all cell types, especially between companion cells and other cell types.

Companion cell ultrastructure did not reveal transfer cells in any of the taxa examined from the Apocynaceae nor Asclepiadaceae, in agreement with the results of Batashev (1996). An anomaly was noted in the companion cells of *C. carnosa* of the Asclepiadaceae. The companion cell membrane showed extensive foldings, but no corresponding wall ingrowths.

Using these anatomical observations, it is suggested that the Apocynaceae and Asclepiadaceae taxa examined here generally show type 2a configurations (Gamalei 1989, Gamalei *et al.* 1992, Van Bel 1992, Van Bel & Gamalei 1992, Flora & Madore 1996). *S. alpinii* tended more towards a type 1 configuration due to the abundance of plasmodesmata connecting companion cells to other surrounding cell types. Studies to determine phloem loading method may modify this categorization

to that of a combination of type 1 and 2a, if both symplasmic and apoplasmic loading is demonstrated. *C. carnosa* may be closer to that of type 2b. This suggestion would be based on the presence of companion cell membrane folds, that would supposedly increase surface area over which apoplasmic uptake could occur (Wimmers & Turgeon 1991). Again, phloem loading studies evidencing apoplasmic uptake are required to support this conjecture.

Batashev (1996) found most types of minor vein configuration in the Apocynaceae from the northern hemisphere. Type 1 with intermediary cells included *Rauvolfia*, *Plumeria*, *Pachypodium* and *Voacanga*. Type 2a with symplasmically isolated companion cells and no wall ingrowths were found in *Thevetia* and *Allamanda*. What he describes as subtype 1-2a with intermediary cells was seen in *Amsonia*, *Wrightia*, *Acokanthera* and *Trachelospermum*, and subtype 2a-2b without transfer cells in *Melodinus*. Type 2b with transfer cells was not seen in the family. Of these taxa, *Pachypodium* (Table 3.3 & 3.4) and *Acokanthera* (Fig.s 3.10 B & 3.11 A & B) occur in the eastern Cape. These species were cleared and the results can be seen in Chapter Three, but only *Acokanthera* represented the Apocynaceae, in Chapters Four on anatomy and Five on ultrastructure, in this thesis. The results presented here agree with Batashev (1996), although while he mentioned genera, he did not list the species. Therefore direct comparisons cannot be made.

The Apocynaceae and Asclepiadaceae represent a very large, diverse, cosmopolitan group and it should not be surprising to find slight deviations around a familial norm, suggested here to be type 2a. This result agrees with that published by Gamalei (1989, 1991) and Batashev (1996) on Apocynaceae and Asclepiadaceae of the northern hemisphere, in which the familial norms are described as a mostly type 1 to 1-2a and a combination of 1-2a respectively.

The same reservations, discussed above for the Ranunculaceae, are held in suggesting apoplasmic loading as the predominant phloem loading method for this group.

The present result supports the contention that *Ranunculus* shows a more advanced minor vein configuration than expected, and that that of the Apocynaceae and Asclepiadaceae is generally more

primitive than expected, casts doubt on the simplicity of the hypothesis first proposed by Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b). It is acknowledged that few species from three very large, diverse, cosmopolitan families have been studied in this thesis, and that ultrastructural studies of many more taxa are required. However, the minor vein configuration within families is not expected to be greatly variable. Also, extremes of the familial norm are expected to occur in environmental extremes, and therefore to be, to a certain extent, predictable. Nevertheless, on closer examination the overall hypothesis of Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996), stunningly fascinating in its entirety, is perhaps lacking when widespread, highly diverse families are considered. Smaller families, more homogenous in terms of structure, habit and habitat diversity, might be expected to hold true to the hypothesis to a greater degree.

Furthermore, the concept of primitive versus advanced families is a debatable issue, as each family is a composite of both primitive and advanced features, expressed to differing degrees within each species of the family. An overall consensus for the primitiveness of a family still allows for advanced features in a particular aspect of the plant. Here, the trend may be for a move away from symplasmic phloem loading, suggested to be the more primitive method, but it does not necessarily hold that all primitive families must show type 1 configuration with the associated symplasmic loading. This aspect may have developed to a more advanced level in an otherwise primitively-denoted family. The converse holds true for advanced families. In terms of flower structure, the Apocynaceae and Asclepiadaceae show highly advanced features, yet minor vein configuration tends towards the primitive side of the scale. The Van Bel and Gamalei hypothesis (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996) appears to consider the overall concept of familial evolutionary development too superficially.

5.3.3 A summary of companion cell ultrastructure as related to minor vein configuration and phloem loading method.

Many aspects are involved in determining minor vein configuration, as can be summarised in Table 5.2. Of the results reported in this chapter, some fit the overall concept, but some do not. *S. alpinii* does not contain the starch accumulations of a symplasmic loader, even though minor vein configuration is that of 1-2a due to abundant plasmodesmatal connections (Table 5.2). *C. distincta* and *S. alpinii* have abundant plasmodesmatal connection with adjacent cell types, even though the familial type is 2a (Table 5.2). *C. carnosa* is designated 2a, but has foldings of the companion cell membrane to increase surface area for apoplasmic uptake (Table 5.2). *A. physocarpa* and *C. carnosa* do not have the small, fragmented vacuoles characteristic of intermediary cells (Table 5.2).

Table 5.2 Companion cell ultrastructure as related to minor vein configuration and phloem loading method

SPECIES	PLASTIDS/ STARCH	VACUOLE	SURFACE AREA OF MEMBRANE	PLASMODESMATA	COMPANION CELL TYPE	TYPE	PREDOMINANT LOADING METHOD
R.multifidus Ranunculaceae	-/-	small and fragmented	-	very few	ordinary	2a	apoplasmic
C.bispinosa Apocynaceae	-/-	small and fragmented	-	very few	ordinary	2a	apoplasmic
A.oppositifolia Apocynaceae	√/-	small and fragmented	-	very few	ordinary	2a	apoplasmic
S.alpinii Asclepiadaceae	rare/-	small and fragmented	-	abundant	ordinary	1-2a	symplasmic/apoplasmic
A.physocarpa Asclepiadaceae	√/-	large and single	-	very few	ordinary	2a	apoplasmic
A.fructicosa Asclepiadaceae	√/√	small and fragmented	-	very few	ordinary	2a	apoplasmic
Cynanchum obtusifolium Asclepiadaceae	rare/-	small and fragmented	-	very few	ordinary	2a	apoplasmic
Ceropegia carnosa Asclepiadaceae	√/√	large and single	cell membrane folded	very few	ordinary	2a-2b	apoplasmic
C.woodii Asclepiadaceae	√/-	small and fragmented	-	very few	ordinary	2a	apoplasmic
C.distincta Asclepiadaceae	-/-	small and fragmented	-	abundant	ordinary	1-2a	symplasmic/apoplasmic

Whilst the overall concept of Van Bel and Gamalei is good for making general predictions based on certain criteria, at the level of species it does not allow for ecotypic variation either between or within species (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996). However, it would make a very interesting study to consider a species with a wide array of environmental ecotypes to determine the amount of variation. The particular characters that show variation amongst ecotypes could provide further insights into the relationship between environmental extremes and minor vein configuration with the associated method of phloem loading.

CHAPTER SIX: DISCUSSION AND CONCLUSION

6.1. General discussion

The concept behind this thesis was to investigate the relationship between leaf architecture, vein anatomy and phloem ultrastructure, and that of possible photoassimilate loading pathways from the mesophyll cells to phloem, the efficiency thereof, and potential phloem loading pathways into companion cells of minor veins, for species adapted to the southern African climate.

The hypothesis was divided into two parts, the one expected to lead on from the other. In the first part, leaf architecture was considered in relation to familial evolutionary position. The second step involved consideration of minor vein configuration and associated phloem loading pathways, in relation to the evolutionary position of the family. Vein anatomy was included as an extension of leaf architecture to discuss photosynthate transport routes and efficiency, and as a prelude to a discussion on phloem position and ultrastructure to determine minor vein configuration.

A superficial progression of features, varying from primitive to advanced, was noted in leaf architectural descriptions. Differences between the relative evolutionary positions of the Ranunculaceae and the Apocynaceae and Asclepiadaceae were distinguishable, in that the relatively less advanced Ranunculaceae showed primitive features such as sparse, poorly differentiated venation, dichotomous dividing of veins and hydathodes. These features were especially visible in *Ranunculus* species, which occur in aquatic, semi-aquatic and mesic areas (Fig. 6.1) (Appendix VI). *Ranunculus* therefore probably represents the less advanced end of the Ranunculaceae family, having changed little from ancestral stock due to a lack of selective pressure from environmental extremes. *Thalictrum*, *Clematis* and *Knowltonia* could represent a slightly more advanced branch of the Ranunculaceae, in which venation was denser and more organised with better lamina coverage. A much greater proportion of anastomosing veins were observed. These genera occur in grasslands. Whilst in relatively mesic, shaded habitats, these species are nevertheless more exposed to dry conditions than their *Ranunculus* counterparts. Environmental extremes most probably produced the selective pressure required to develop a more organised and efficient lamina venation to cope with

greater water loss and the necessarily rapid removal of photosynthate during fluctuating conditions to storage organs. It is important that photosynthates be removed rapidly, as an accumulation of soluble sugars inhibits photosynthesis. Therefore the shift seen in the structure/function relationship of leaf venation is possibly due to changing habitat within the Ranunculaceae.

In contrast, venation within the Apocynaceae and Asclepiadaceae was very dense with individual veins apparently wider and thicker. Lamina coverage was very efficient and showed a greater degree of organisation than that seen in the Ranunculaceae (Fig.s 6.2 , and 6.3 & 6.4 respectively). Taxa with succulent leaves showed a more three-dimensional arrangement of veins. The Apocynaceae and Asclepiadaceae of the eastern Cape occur in very hot, dry, coastal areas. Living under such harsh environmental conditions, a highly organised, efficient system of venation would be a necessity for a number of reasons, as water must reach mesophyll cells and photosynthate must be removed to storage organs. Robust veins offer mechanical strength, helping to prevent leaves from wilting during the extremely hot, dry conditions experienced by this habitat. In terms of this thesis, a dense reticulum would mean a shorter, more efficient route for photosynthates to reach veins.

The anatomical survey was used to determine the proximity of photosynthetic mesophyll to phloem, as this is of great importance to phloem loading efficiency (Haberlandt 1914, Wylie 1939, Tucker 1964, Esau 1972, Fisher & Evert 1982, Giaquinta 1983, Fisher 1990, Nelson & Dengler 1997). *R. multifidus* of the Ranunculaceae had wide interveinal distances and large intercellular air spaces between mesophyll cells, producing a very loose arrangement. Bundle sheath extensions were seen, providing bridges of contact for photosynthates to reach phloem tissue, albeit rather circuitous.

The Apocynaceae and Asclepiadaceae sectioned had densely-clustered mesophyll cells separated by small intercellular air spaces and had bundle sheath extensions. Such close mesophyll arrangement, coupled with small interveinal distances, would offer two main advantages to xeric taxa. Firstly, less exposed surface area for evaporation inside the leaf means less water loss, and secondly, a shorter, more direct route from mesophyll cells to phloem would mean efficient, rapid transport for subsequent export. Furthermore, the wide robust veins of these families could probably cope with

greater volume than that carried by the delicate veins of the Ranunculaceae. Greater and faster export volume would mean more of a concentration gradient, allowing for more photosynthesis. The overall result would be more photosynthate available for growth and storage to cope with adverse conditions, allowing these families to successfully compete and increase biomass in environmental extremes.

Phloem was absent from ultimate veinlets in areoles, leaving three potential pathways available for photosynthates to reach minor vein phloem. Sugars could pass into bundle sheath cells of ultimate veinlets and then to phloem of minor veins bordering areoles. It was interesting to note that bundle sheath cells of ultimate veinlets of *R. multifidus* possessed abundant plasmodesmata connecting adjacent cells. Another route would be through adjacent mesophyll cells to minor veins bordering areoles. Mesophyll cells were seen to be interconnected by numerous plasmodesmata in most species studied, providing a symplasmic route for photosynthates within the mesophyll. The third possible pathway could be via the apoplast, outside cell membranes and then through cell walls and free space. At this stage, such discussion is purely speculation as no studies to determine the exact route were carried out in this thesis. The determination of photosynthate route to phloem of minor veins would be an interesting project, incorporating ultrastructural and iontophoretic studies.

Leaf architecture and anatomy are conservative features. As seen in this thesis, anatomy did not seem to vary much in that all leaves sectioned had the same basic arrangement, with only the volume and proximity of adjacent tissues changing. Variation appeared to be mostly a reflection on habitat, with more efficient organisation of cells and tissues occurring in xeric families. Leaf architecture, in contrast to anatomy, was found to be more a result of familial evolutionary status and habitat combined. The results of leaf architecture and anatomical studies for the Ranunculaceae, Apocynaceae and Asclepiadaceae therefore fulfilled the expectations set out in the first part of the hypothesis mentioned earlier.

The two most pertinent aspects of phloem ultrastructure in the discussion of photosynthate loading were companion cell features and plasmodesmatal frequency and distribution, as these determined

minor vein configuration and potential phloem loading pathways. No transfer cells were seen in the current study, as none of the companion cells had wall ingrowths. Companion cells uniformly showed a large nucleus, many small vacuoles and a dense cytoplasm rich in ribosomes, ER and mitochondria (Table 5.2). For the most part, companion cells did not store starch, probably due to rapid transfer to sieve tubes for export (Table 5.1). The darkly-stained phloem was taken as being indicative of intense metabolic activity, to be expected in actively loading and transporting phloem of minor veins (Warmbrodt & Van Der Woude 1990).

Based on the description of the companion cells, type 2b with transfer cells did not occur in any of the families studied and reported on here. Type 2c was ruled out due to the absence of Kranz anatomy. The only remaining options were type 1 or 2a minor vein configurations.

Plasmodesmatal frequency and distribution diminished drastically in all three families en route from mesophyll to companion cells. Due to a relative paucity of plasmodesmata, type 1 was no longer a possibility, as this type required extensive plasmodesmatal interconnection for symplasmic transport. The Ranunculaceae, Apocynaceae and Asclepiadaceae were therefore designated type 2a based on anatomical and ultrastructural evidence (Fig.s 6.1- 4) (Table 6.1) (Appendix VI).

The ten species studied at the anatomical and ultrastructural levels were discussed and compared in the relevant chapters. This information, together with the leaf architecture information, was then used to create a thorough description of each species on all levels, including habit and habitat information, and offering a description of photosynthate pathway from mesophyll to phloem and phloem loading method. These descriptions can be found in Appendix VI for comparison. It was felt that this exercise would produce a good overview for each species studied. A brief summary of vein typology and associated phloem loading method can be found for comparative purposes in Table 6.1. To illustrate the data in Appendix VI, Fig.s 6.1 - 4 were drawn up. The Ranunculaceae were represented by *Ranunculus multifidus* in Fig. 6.1, the Apocynaceae by *Carissa bispinosa* in Fig. 6.2, and the Asclepiadaceae by *Secamone alpinii* and *Ceropegia carnosa* in Fig.s 6.3 and 6.4 respectively. The latter two species were chosen for illustration as they were the most interesting of the Asclepiadaceae studied, representing variations on the familial norm.

Table 6.1 Minor vein configuration of the Ranunculaceae, Apocynaceae and Asclepiadaceae studied

SPECIES	TYPE	PHLOEM LOADING METHOD
Ranunculus multifidus (Ranunculaceae)	2a	Apoplasmic
Acokanthera oppositifolia (Apocynaceae)	2a	Apoplasmic
Carissa bispinosa (Apocynaceae)	2a	Apoplasmic
Asclepias physocarpa (Asclepiadaceae)	2a	Apoplasmic
Asclepias fructicosa (Asclepiadaceae)	2a	Apoplasmic
Cynanchum obtusifolium (Asclepiadaceae)	2a	Apoplasmic
Ceropegia carnosa (Asclepiadaceae)	2a - 2b	Apoplasmic
Ceropegia woodii (Asclepiadaceae)	2a	Apoplasmic
Secamone alpinii (Asclepiadaceae)	1 - 2a	Symplasmic/apoplasmic
Ceropegia distincta (Asclepiadaceae)	2a	Symplasmic/apoplasmic

Secamone alpinii had abundant plasmodesmata at every interface en route from mesophyll to companion cells. This species was designated type 1-2a due to the symplasmic interconnectivity seen (Fig.6.3) (Table 6.1) (Appendix VI). It was therefore possible to suggest the potential for a symplasmic phloem loading pathway, without totally excluding the likelihood of a concurrent apoplasmic one. Phloem loading studies *per se* would be required to demonstrate the predominant pathway and to validate the symplasmic option in an otherwise apparently apoplasmic family.

The other anomaly was *Ceropegia carnosa*, in which companion cell membranes had extensive foldings. No accompanying wall ingrowths were seen. Due to a lack of ultrastructural evidence to the contrary, such as abundant plasmodesmata or wall ingrowths, the type designation remained that of the family, type 2a (Fig. 6.4) (Table 6.1) (Appendix VI). It was suggested that folding increases surface area for increased apoplasmic uptake. The investigation suggested that symplasmic phloem loading was improbable due to a paucity of plasmodesmata on nearing the companion cell/sieve tube complex, although the possibility could not be ruled out until a thorough investigation demonstrating phloem loading method is undertaken.

It is difficult to speculate why *S. alpinii* and *C. carnosa* deviated from the familial norm, as insufficient data was presented for such discussion. These plants are creepers that occur in dry coastal and riverine scrub respectively, as are all other asclepiad taxa studied here, yet the minor vein configuration was quite different from that of other family members. Interesting projects to allow debate on this issue include the determination of the phylogenetic position of *S. alpinii* and *C. carnosa* within the Asclepiadaceae. *S. alpinii* could represent a less advanced example within the family, retaining the ancestral symplasmic pathway. Examination of other members of the tribe, or even subtribe, would enable the consistency of type 1-2a within the subgrouping to be established.

Although the other species of *Ceropegia* studied did not have folding of the companion cell membrane, three species by no means delimit the genus, never mind the family. However, the temptation to speculate remains. *C. carnosa* could be a more advanced example within the family, showing a possible line of evolution to the more efficient apoplasmic pathway.

It is unfortunate that the long-awaited conspectus of the Asclepiadaceae remains incomplete. Given the lack of comparative information available in publications and the potential variation in habitat and structure within the family, it would be foolhardy to offer reasons for the deviation seen in an already small sample of a huge family.

Surprisingly, familial evolutionary status does not appear to be a strong influencing factor on phloem loading method or minor vein configuration in the Ranunculaceae, Apocynaceae or Asclepiadaceae, thus not fitting the expected second hypothesis component. The supposedly primitive *Ranunculus* had the more advanced minor vein configuration of type 2a, and therefore the potential for the more efficient apoplasmic phloem loading method, than the expected type 1 for the familial status (Fig.s 1.4 & 6.1) (Tables 1.4, 6.1 & 6.2). Furthermore, the relatively advanced Apocynaceae and Asclepiadaceae had a more primitive configuration, type 2a, than the type 2b originally predicted in the hypothesis (Fig.s 1.4 & 6.2-4, Tables 1.4, 6.1 & 6.2). Although the advanced apoplasmic loading method was suggested to predominate in all three families, no transfer cells were present to epitomise and maximise apoplasmic loading at the companion cell interface.

It is important to stress that the terms "primitive" and "advanced" are based on many characters, both vegetative and reproductive. Families are ranked according to the ratio of advanced to primitive characters displayed. Each family is therefore a conglomerate of both primitive and advanced features, depending on which areas of plant structure and function have been required to adapt and change, and which have remained static. It is therefore quite possible for an otherwise advanced family to show some primitive features and vice versa. The trick remains in determining why certain features advanced and others did not within a given family. Venation and anatomical features related strongly to habitat. It should not be surprising, therefore, to find an advanced phloem loading method in the Ranunculaceae of more mesic areas. An interesting project would be to determine minor vein configuration for the aquatic taxa for comparison.

Whilst such conjecture seems logical for the Ranunculaceae, it does not explain the result seen in the Apocynaceae and Asclepiadaceae. For plants from very harsh environments, the most rapid and advanced minor vein configuration and phloem loading method was expected. From the variation seen in this small sample of asclepiads alone, it would appear that the situation is not so simple. It could well be that a different picture may emerge when more species are examined, although the results presented here are supported by other published accounts. Transfer cells were found to be absent in species studied by Batashev (1996). It is a pity that information on habitat and phylogenetic position were excluded from this paper. Until a comprehensive study of the family, in the new sense, is undertaken, including aspects such as habitat and phylogenetic position, the resultant description of minor vein configuration will remain unclear.

From the results presented in this thesis, it did appear that habitat and habit affected leaf architecture and foliar anatomy, and therefore phloem loading efficiency (Table 6.2). At the ultrastructural level, habitat could still affect method of phloem loading as plasmodesmata cannot function under conditions of temperature and/or water stress (Gunning & Overall 1983, Minchin *et al.* 1983, Côté *et al.* 1987, Gamalei *et al.* 1992, Van Bel 1992a, Van Bel & Gamalei 1992, Epel 1994, Gamalei *et al.* 1994, Van Dongen & Van Bel 1996, McLean *et al.* 1997). Species from the Eastern Cape would be exposed to both, so from this point of view, it should not be surprising that most taxa studied fell into the type 2a category with few plasmodesmata.

Table 6.2 Summary of key features of Ranunculaceae, Apocynaceae and Asclepiadaceae as seen in this thesis

FAMILY	HABITAT	VENATION	ANATOMY	MINOR VEIN CONFIGURATION
Ranunculaceae	Aquatic, semi-aquatic to mesic	Poorly organised, widely spaced, delicate veins	Large air spaces in mesophyll with wide interveinal distances, bundle sheath extensions to mesophyll cells	Type 2a
Apocynaceae and Asclepiadaceae	Xeric	Close, dense reticulum of robust veins	Mesophyll cells in close association with bundle sheath cells of veins, bundle sheath extensions present	Type 2a

If one follows the dogma of Van Bel and Gamalei (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988,Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996), then habit would be more a result of phloem loading typology rather than an influence on it, with symplasmic loaders losing more assimilate to lateral sinks than apoplasmic loaders, and therefore investing more in woody stem tissues than apoplasmic loaders (Beslow & Rier 1969, Giaquinta 1983, Gamalei 1989, Gamalei 1991, Van Bel 1993b, Komor *et al.* 1996, Van Bel 1996, Kempers *et al.* 1998, Turgeon 2000). In the families studied in this thesis, this trend was not observed, as the supposed apoplasmic loaders ranged from small trees to vines to small herbs. However, determination of the type of sugar transported may shed more light on this aspect, as sugar type and concentration has been reported to have a marked effect on cambial activity, and therefore on growth rate and form.

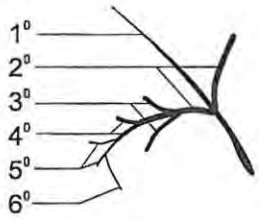
Komor *et al.* (1996) cautioned against transferring the results gained from one species to another. Variation was noted in the small sample of Asclepiadaceae presented in this thesis, albeit around a central norm, supporting the need for such warning. Extreme care must be exercised in extrapolating data to include all taxa of very large, diverse families, in which groups may have diverged from the familial norm whilst adapting to extreme conditions. If criticism of the Van Bel and Gamalei

hypothesis (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996) must be made, it is that they are guilty of this. As a suggestion for further studies of this ilk, the phylogeny, hierarchy and local distribution of the family concerned should be considered. Variation between offshoot groups and the familial norm are to be expected and should provide interesting information on deviations in relation to the environment. A further cause for concern when considering the Van Bel and Gamalei hypothesis (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996), is the unavailability of herbarium sheet data for the specimens on which the hypothesis was originally based. Families are discussed and categorised with very few example species listed by name. It must be stressed, however, that the hypothesis of Van Bel and Gamalei has provided a useful framework in which to explore, has generated immense discussion and has incorporated diverse aspects of plant structure and function into a holistic picture of assimilate loading and transport by phloem in relation to the environment.

Once the morphology, leaf architecture, anatomy and ultrastructure of minor veins had been considered, the resultant information allowed for a more detailed discussion of a phloem loading method specific to a particular species (Evert & Mierzwa 1986, Komor *et al.* 1996, McLean *et al.* 1997, Turgeon 2000). Summaries of the relationship between leaf architecture, vein anatomy with phloem ultrastructure, and that of possible photosynthate pathways from mesophyll cells to phloem and potential phloem loading pathways into companion cells of minor veins were presented for *Ranunculus multifidus* (Ranunculaceae) (Fig. 6.1), *Carissa bispinosa* (Apocynaceae) (Fig. 6.2), and *Secamone alpinii* (Fig. 6.3) and *Ceropegia carnosa* (Asclepiadaceae) (Fig. 6.4). However, it must be remembered that different solutes may load along different routes (Evert & Mierzwa 1986). Komor *et al.* (1996) stress the importance of discussing physiological data with anatomical data to obtain a true reflection of phloem loading for a particular species. The data presented here was purely anatomical and it is acknowledged that studies to physically demonstrate phloem loading method, and determination of sugar transported, may possibly change the conclusions drawn.

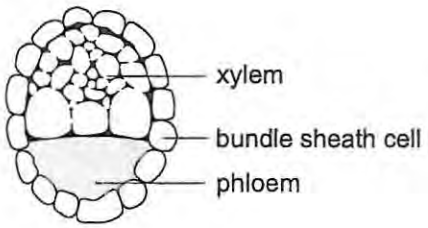
Ranunculus multifidus
Ranunculaceae

- soft leaves on small, herbaceous plant
- moist, sheltered habitat
- primitive family status



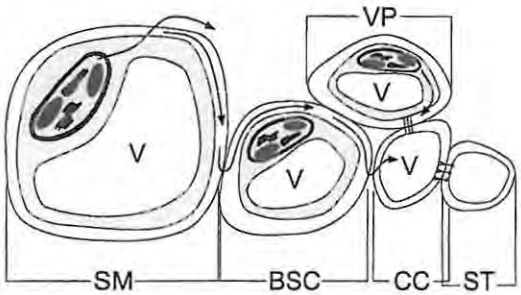
VENATION

- widely spaced reticulum
- up to sixth order
- marginally actinodromous
- areoles imperfect
- low level of organisation and



VEIN ANATOMY

- all vein orders with same anatomy, except for veinlets of xylem only
- bundle sheath cell with extensions to mesophyll cells
- very small veins
- no cambium or supporting tissues



MINOR VEIN CONFIGURATION

- type 2a
- little starch accumulation
- normal companion cell
- few plasmodesmata

CONCLUSION

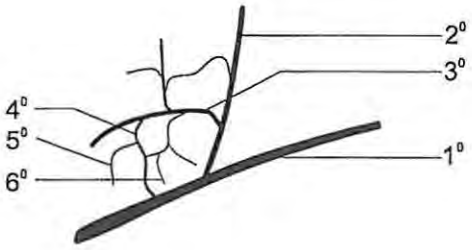
Apoplasmic loader based on anatomical evidence, sugar suggested to travel through free space in the cell walls until companion cells, then crosses cell membrane to load sieve tube via pore plasmodesmata.

KEY			
1°	Primary vein	BSC	Bundle sheath cell
2°	Secondary vein	VP	Vascular parenchyma cell
3°	Tertiary vein	CC	Companion cell
4°	Quaternary vein	ST	Sieve tube
5°	Fifth order vein	C	Chloroplast
6°	Sixth order vein	S	Starch granule
V	Vacuole	⫴	Plasmodesmata
P	Phloem	→	Pore plasmodesmata

Figure 6.1 Summary of leaf architectural, anatomical and ultrastructural data to illustrate vein structure/function relationship with respect to phloem loading in *R. multifidus* (Ranunculaceae)

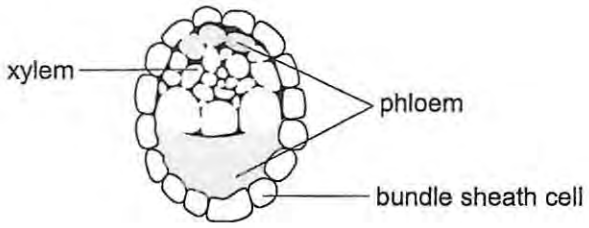
Carissa bispinosa
Apocynaceae

- ◇ small woody tree
- ◇ leaves tough and waxy
- ◇ advanced family status
- ◇ coastal forest margins



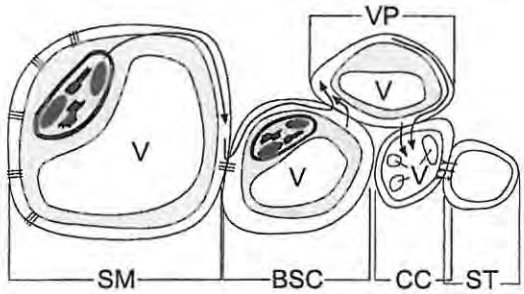
VENATION

- ◇ pinnate, camptodromous and brochidodromous
- ◇ dense reticulum of robust veins
- ◇ areoles incompletely closed



VEIN ANATOMY

- ◇ bicolateral phloem from 1° to 3° order veins, 4° to 6° order veins abaxial phloem only and veinlets of xylem only
- ◇ bundle sheath extensions to mesophyll cells
- ◇ close association between bundle sheath cells and mesophyll cells



MINOR VEIN CONFIGURATION

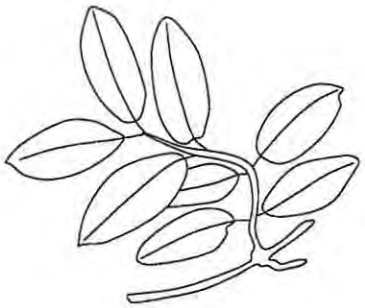
- ◇ type 2a
- ◇ plasmodesmatal abundance decreases towards companion cell / sieve tube complex
- ◇ normal companion cells
- ◇ starch accumulation decreases towards companion cell / sieve tube complex

CONCLUSION

Possible initial symplasmic transport with apoplasmic loading at companion cell / sieve tube interface via pore plasmodesmata from companion cell to sieve tube.

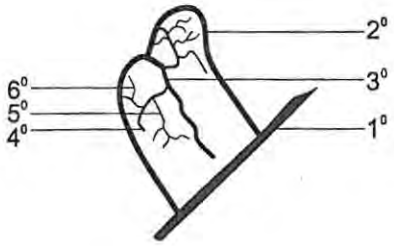
KEY			
1°	Primary vein	BSC	Bundle sheath cell
2°	Secondary vein	VP	Vascular parenchyma cell
3°	Tertiary vein	CC	Companion cell
4°	Quaternary vein	ST	Sieve tube
5°	Fifth order vein	C	Chloroplast
6°	Sixth order vein	S	Starch granule
V	Vacuole	≡	Plasmodesmata
P	Phloem	⋕	Pore plasmodesmata
		→	Potential pathway for sugar transport

Figure 6.2 Summary of leaf architectural, anatomical and ultrastructural data to illustrate vein structure/function relationship with respect to phloem loading in *C. bispinosa* (Apocynaceae)



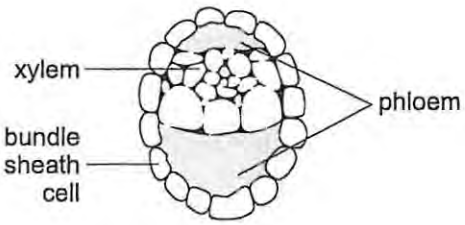
Secamone alpinii
Asclepiadaceae

- ◇ creeper over other plants
- ◇ in dry coastal scrub
- ◇ advanced family status
- ◇ waxy leaves



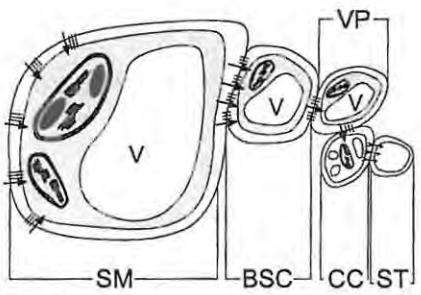
VENATION

- ◇ pinnate, camptodronous and brochidodromous
- ◇ dense reticulum of robust veins
- ◇ areoles imperfect



VEIN ANATOMY

- ◇ bicolateral phloem in midrib only, abaxial phloem and xylem in 2^0 to 5^0 order veins, xylem only in veinlets
- ◇ no close association between bundle sheath cells and mesophyll cells noted



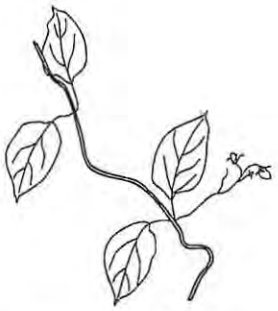
MINOR VEIN CONFIGURATION

- ◇ type 1 -2a
- ◇ normal companion cells
- ◇ abundant plasmodesmata *en route* to companion cell / sieve tube complex
- ◇ very little starch accumulation in mesophyll cells only when present

Symplasmic transport and loading suggested to predominate but no evidence to eliminate apoplasmic route.

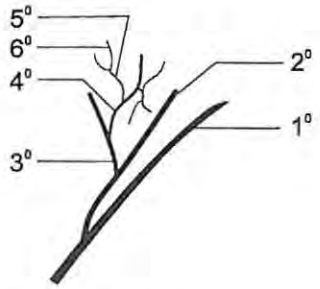
KEY			
1^0	Primary vein	BSC	Bundle sheath cell
2^0	Secondary vein	VP	Vascular parenchyma cell
3^0	Tertiary vein	CC	Companion cell
4^0	Quaternary vein	ST	Sieve tube
5^0	Fifth order vein	C	Chloroplast
6^0	Sixth order vein	S	Starch granule
V	Vacuole	≡	Plasmodesmata
P	Phloem	⋕	Pore plasmodesmata
		⟶	Potential pathway for sugar transport

Figure 6.3 Summary of leaf architectural, anatomical and ultrastructural data to illustrate vein structure/function relationship with respect to phloem loading in *S. alpinii* (Asclepiadaceae)



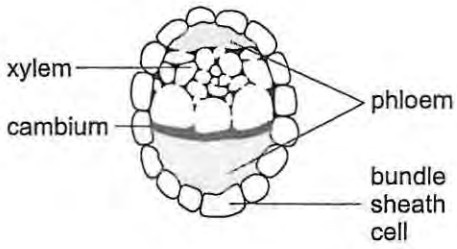
Ceropegia carnosa
Asclepiadaceae

- ◇ vine over other plants
- ◇ fleshy leaves
- ◇ dry, riverine scrub
- ◇ advanced family status



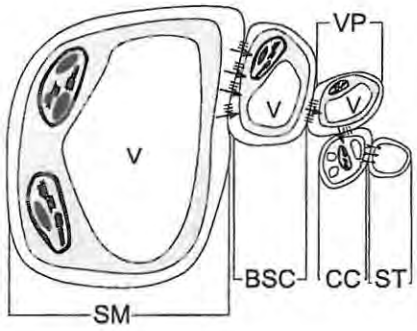
VENATION

- ◇ pinnate,camptodromous, brochidodromous
- ◇ widely spaced, three dimensional reticulum of delicate veins
- ◇ areoles imperfect



VEIN ANATOMY

- ◇ bicolateral phloem in midrib only, 2° to 6° order veins abaxial phloem and xylem, veinlets of xylem only
- ◇ close association with mesophyll cells, bundle sheath extensions to mesophyll cells



MINOR VEIN CONFIGURATION

- ◇ type 2a - 2b
- ◇ companion cells with extensive folding of cell membrane to increase surface area, otherwise normal
- ◇ starch accumulation in all cell types
- ◇ plasmodesmatal frequency decreased towards companion cell / sieve tube complex

Possible initial symplasmic and apoplasmic transport, but companion cell loaded mostly apoplasmically as suggested by increased surface area produced by extensive cell membrane folding.

KEY			
1°	Primary vein	BSC	Bundle sheath cell
2°	Secondary vein	VP	Vascular parenchyma cell
3°	Tertiary vein	CC	Companion cell
4°	Quaternary vein	ST	Sieve tube
5°	Fifth order vein	C	Chloroplast
6°	Sixth order vein	S	Starch granule
V	Vacuole	≡	Plasmodesmata
P	Phloem	⧧	Pore plasmodesmata
		⟶	Potential pathway for sugar transport

Figure 6.4 Summary of leaf architectural, anatomical and ultrastructural data to illustrate vein structure/function relationship with respect to phloem loading in *C. carnosa* (Asclepiadaceae)

6.1.4 The value of hindsight

Whilst it may be argued that no statistical analysis was carried out, it must be remembered that this thesis was presented in the form of a survey to gain general understanding. Furthermore, when the time constraints of the thesis were considered, it was necessary to define and restrict the survey to taxa of the Eastern Cape, and to sample only a fraction of those species for anatomical and ultrastructural studies. The Ranunculaceae, including *Ranunculus multifidus,* were chosen as a primitive outgroup for comparative purposes. *R. multifidus* is a soft herb and completely different to anything sampled from the Apocynaceae or Asclepiadaceae in terms of habit, habitat and evolutionary status. *Acokanthera oppositifolia* and *Carissa bispinosa* of the Apocynaceae were chosen as tree species from very dry regions, possessing tough, waxy leaves and an advanced familial status. Of the Asclepiadaceae, *Ceropegia, Asclepias, Cynanchum* and *Secamone* were chosen as creepers from hot, dry areas. A greater range of growth forms within a particular family would probably have been a wiser choice in retrospect, adding a new dimension for comparison.

6.1.5 Future prospects based on this thesis

I hope that this thesis will provide a framework for related projects. The current leaf architecture data should be useful if used in conjunction with anatomical and ultrastructural studies for other species of the Ranunculaceae, Apocynaceae and Asclepiadaceae, to describe more fully the relationship between vein structure and phloem loading in these families. Extension of the leaf architecture analysis to include all southern African species of these families, with studies on leaf development and ontogeny, would produce a useful database for future phylogenetic studies.

The most obvious use of the current study would be as a starting point for experimentation on phloem loading pathways. PCMBS and dye injection studies could be carried out for the ten taxa fully described at leaf architectural, anatomical and ultrastructural levels (Appendix VI). *Secamone alpinii*, with abundant plasmodesmata, is the obvious choice for dye injection studies to demonstrate symplasmic transport. *Ceropegia carnosa*, with the companion cell membrane foldings, would be a good candidate for PCMBS experimentation. Determination of sugar transported would be a most useful supplement to such investigations.

Secamone alpinii would be a good choice for studies on plasmodesmatal structure, size exclusion limits and responses of plasmodesmata to external and internal influences. The plasmodesmata in this species are plentiful, easy to find and appear to be relatively large.

A further project for consideration would be an indepth study of a family indigenous to southern Africa, examining leaf architecture, anatomy and ultrastructure in relation to phloem loading. As the ecophysiological concept of phloem loading is refined and altered to incorporate new information on isolated indigenous families, a more comprehensive understanding should be attained (Gamalei 1985a, b, 1989, 1991, Van Bel *et al.* 1988, Van Bel 1992, 1994, 1996, Van Bel & Gamalei 1991, 1992, Gamalei *et al.* 1992, 1994, 1996, Van Bel 1992a-c, 1993a, b, 1996).

6.2 Conclusion

I have attempted to answer the aims of this thesis in the preceding chapters. I believe that the hypothesis for the research held true in part. The relatively primitive Ranunculaceae showed poorly differentiated venation patterns with inefficient lamina coverage by veins and wide interveinal distances, and vice verse for the relatively advanced Apocynaceae and Asclepiadaceae (Table 6.2). The hypothesis broke down on the second step, as ultrastructural evidence did not support the prediction of the primitive type 1 minor vein configuration with associated symplasmic phloem loading for the Ranunculaceae, nor the most advanced type 2b minor vein configuration with associated apoplasmic phloem loading for the Apocynaceae or Asclepiadaceae (Appendix VI). Instead, all three families displayed the middle minor vein configuration, type 2a (Table 6.1), with supposed predominant apoplasmic loading, but not completely excluding the possibility of concurrent symplasmic loading. In this aspect, the Ranunculaceae were therefore more advanced than expected, and the Apocynaceae and Asclepiadaceae less so.

APPENDIX I Full taxonomic names of all species sampled

Ranunculaceae L.

Knowltonia capensis (L.) Huth.
Knowltonia filia (L.f.) Dur. & Sch. subsp. *scaposa* Rasm.
Knowltonia filia (L.f.) Dur. & Sch. subsp. *filia*
Knowltonia vesicatoria (L.f.) Sims subsp. *grossa*
Knowltonia vesicatoria (L.f.) Sims subsp. *humilis* H.Rasm.
Knowltonia brevistylis Szysz.
Knowltonia bracteata Harvey ex Zahlbruehner
Knowltonia transvaalensis Szysz. var. *transvaalensis*
Knowltonia cordata H.Rasm.
Ranunculus baurii MacOwan
Ranunculus meyeri Harv.
Ranunculus multifidus Forsk.
Ranunculus muricatus L.
Ranunculus trichophyllus Chaix subsp. *trichophyllus*
Clematis brachiata Thunb.
Clematis triloba Thunb.
Thalictrum rhynchocarpum Dill & Rich.
Thalictrum minus L.

Apocynaceae

Acokanthera oblongifolia (Hochst.) L.E.Codd
Acokanthera oppositifolia (Lam.) L.E.Codd
Landolphia capensis Oliv.
Landolphia kirkii Dyer
Gonioma kamassi E.Mey.
Pachypodium bispinosum (L.f.) A.D.C.
Carissa macrocarpa (Eckl.) A.D.C.
Carissa haematocarpa (Eckl.) A.D.C.
Carissa bispinosa (L.f.) Desf. ex Brenan var. *bispinosa*
Carissa bispinosa (L.f.) Desf. ex Brenan var. *acuminata* (E.Mey.) L.E.Codd
Carissa wyliei N.E.Br.

Asclepiadaceae R.Br.

Microloma tenuifolium K.Schum.
Microloma sagittatum R.Br.
Microloma incanum Decne.
Microloma massonii (Schultes) Schltr.
Xysmalobium involucratum (E.Mey.) Decne.
Xysmalobium zeyheri N.E.Br.
Xysmalobium undulatum (L.) Ait.f.
Xysmalobium confusum Scott Elliott
Xysmalobium orbiculare (E.Mey.) D.Dietr.
Xysmalobium prunelloides Turcz
Xysmalobium pearsonii L.Bolus
Schizoglossum hamatum E.Mey.
Astephanus marginatus Decne.
Astephanus triflorus (L.f.) Schultes
Parapodium crispum N.E.Br.
Microglossum anomalum (N.E.Br.) Kupicha
Aspidoglossum biflorum E.Mey.
Aspidoglossum carinatum (Schultr.) Kupicha
Aspidoglossum virgatum (E.Mey.) Kupicha
Aspidoglossum flanaganii (Schltr.) Kupicha
Aspidoglossum heterophyllum E.Mey.
Aspidoglossum fasciculare E.Mey.
Aspidoglossum ovalifolium (Schltr.) Kupicha
Fanninia caloglossa Harv.

Schizoglossum atropurpureum E.Mey. subsp. *tridentatum* (Schltr.) Kupicha
Schizoglossum cordifolium E.Mey.
Schizoglossum bidens E.Mey. subsp. *bidens*
Schizoglossum bidens E.Mey. subsp. *gracile* Kupicha
Schizoglossum aschersonianum Schltr.
Woodia marginata Schltr.
Woodia mucronata N.E.Br.
Asclepias expansa (E.Mey.) Schltr.
Asclepias stellifera Schltr.
Asclepias meyeriana (Schltr.) Schltr.
Asclepias navicularis (E.Mey.) Schltr.
Asclepias gibba (E.Mey.) Schltr.
Asclepias erinens (Harv.) Schltr.
Oncinema lineare (L.f.) Bullock
Asclepias aurea (Schltr.) Schltr.
Asclepias burchelli Schltr.
Asclepias fructicosa L.
Asclepias physocarpa (E.Mey.) Schltr.
Asclepias crinita Berg.
Asclepias dregeana Schltr.
Asclepias albens (E.Mey.) Schltr.
Asclepias hastata (E.Mey.) Schltr.
Asclepias crispa Berg.
Ceropegia distincta N.E.Br. subsp. *haygarthii* (Schltr.) Huber
Ceropegia radicans Schltr. subsp. *radicans*
Ceropegia radicans Schltr. subsp. *smithii* (Henderson) R.A.Dyer
Ceropegia carnosa E.Mey.
Ceropegia dubia R.A.Dyer
Ceropegia africana R.Br.
Ceropegia occulta R.A.Dyer
Ceropegia woodii Schlechter
Ceropegia meyeri Decne.
Pachycarpus rigidus E.Mey.
Pachycarpus reflectens E.Mey.
Pachycarpus inconstans N.E.Br.
Pachycarpus natalensis N.E.Br.
Pachycarpus vexillaris E.Mey.
Pachycarpus dealbatus E.Mey.
Pachycarpus grandiflorus E.Mey.
Pachycarpus linearis (E.Mey.) N.E.Br.
Pentarrhinum insipidum E.Mey.
Cynanchum africanum R.Br.
Cynanchum capense Thunb.
Cynanchum obtusifolium L.f.
Secamone alpinii Schult.
Sisyranthus compactus N.E.Br.
Sisyranthus imberbis Harv.
Anistoma cordifolia Fenzl.
Brachystelma tuberosum R.Br.
Brachystelma decipiens N.E.Br.
Brachystelma meyeranum Schltr.
Brachystelma cathcartense R.A.Dyer
Brachystelma huttonii (Harv.) N.E.Br.
Brachystelma elongatum (Schltr.) N.E.Br.
Brachystelma comptum N.E.Br.
Brachystelma schizoglossoides (Schltr.) N.E.Br.
Riocreuxia torulosa Decne.
Riocreuxia flanaganii Schltr.
Tylophora cordata (Thunb.) Druce
Tylophora lycioides Decne.
Tylophora umbellata Schltr.
Tenaris rubella E.Mey.

Ceropegia linearis E.Mey.

Ceropegia cancellata Reichb.

Marsdenia floribunda E.Mey.

Telosoma africana (N.E.Br.) N.E.Br.

Fockea cylindrica R.A.Dyer

Fockea multiflora K.Schum.

APPENDIX II Herbarium data sheets

Ranunculaceae

SPECIES	COLLECTOR	DATE	NO.	LOCALITY
Knowltonia capensis	J.R. & B.R.	28/9/31	144	Howisons Poort, GHT
Knowltonia capensis	T.Dold	7/8/94	1026	3325CD, Vanstadensberg Trig. Beacon, Witteklip Mt.
Knowltonia cordata	L.Prosser	7/1967		Lovemore Park, PE
Knowltonia vesicatoria	E.E.A.Archibald	9/1951	3823	Bushmans River Mouth, Alexandria
Knowltonia filia subsp. *filia*	F.V.Paterson	1/1916	1215	George
Knowltonia filia subsp. *scaposa*	K.A.Dahlstrand	30/12/68	1695	3323, Storms River Forest Res., Willowmore
Knowltonia vesicatoria subsp.*humilis*	H.H.Burrows	3/6/90	3312	3326DA, Boknesstrand, Alexandria
Knowltonia vesicatoria subsp.*grossa*	H.G.Forcade	3/1921	1204	Eerste River
Knowltonia brevistylis	E.E.Galpin	12/1896	3428	West Gate, Port St.John
Knowltonia bracteata	S. Idirnlaur	1894	852	Perie Curk, Albany
Knowltonia transvaalensis var. *transvaalensis*	E.E.Galpin	11/1889	460	Barberton
Clematis brachiata	D.M.Comins	19/5/56	1538	Cove Rock Beach, EL
Clematis brachiata	J.L.Gordon-Gray	25/7/66	574	The Haven. Elliotdale
Clematis triloba	E.Esterhuizen	3/1939	771	Hongerdoorn
Clematis triloba	D.M.Comins	15/5/55	1050	Bonza Bay, EL
Ranunculus aquatilis	Gibbs Russell, Robinson & Herman	16/4/78	448	3222BD, Mountain View Farm, Beaufort West
Ranunculus baurii	E.E.Galpin	11/3/1904	6567	Witteberg
Ranunculus meyeri	MacOwan	10/1904	1555	Boschberg
Ranunculus multifidus	L.Britten	11/10/25	5241	Kowie, Bathurst
Ranunculus multifidus	M.J.Wells	13/9/61	2802	Belmont Valley, Albany
Ranunculus muricatus	G.Rattray	1/1920	1294	East London
Ranunculus trichophyllus subsp. *trichophyllus*	Linder	14/1/90	5085	3225AA, Cradock, Nardouwsberg
Thalictrum minus	MacOwan	10/1904	1720	Little Fish River, Somerset East
Thalictrum rhynchocarpum	E.Archibald	2/1942	26	Hogsback

Apocynaceae

SPECIES	COLLECTOR	DATE	NO.	LOCALITY
Acokanthera oblongifolia	J.Cameron	29/11/84		3326BC, 1820 Settlers Wild Flower Garden, GHT
Acokanthera oblongifolia	H.H.Burrows	12/2/89		3326DA, Boknesstrand, Alexandria
Acokanthera oppositifolia	M.van Niekerk	31/12/69		3326, Farm Handsworth, GHT
Acokanthera oppositifolia	B.Osborne	7/7/65		3322DC, Wilderness Heights, Oudtshoorn
Carissa macrocarpa	A.Booi	12/1/83	162	Albany Museum, GHT
Carissa macrocarpa	A.J.Mullins	29/11/84		3326BD, Martindale, GHT
Carissa haematocarpa	G.La Cock	-		3225BB, Commando Duff Nat. Res.
Carissa haematocarpa	A.Booi	4/8/76		Nature Res. GHT
Carissa bispinosa var. *bispinosa*	J.E.Reed	20/10/68	31	3326BC, Howesons Poort, GHT
Carissa bispinosa var. *bispinosa*	H.Deacon	16/10/69	P78	3325, Amanzi, PE
Carissa bispinosa var. *acuminata*	M.J.Wells	22/10/65	3223	Cata Forest, Keiskammahoek
Carissa bispinosa var. *acuminata*	J.L.Gordon Gray	22/7/66	558	The Haven, Elliotdale
Carissa wyliei	S.Schonland	01/1921	4210	Port St. Johns
Carissa wyliei	M.J.Wells	15/2/66	3416	Needles Hotel, Port St.Johns
Landolphia capensis	G.Lishman	15/9/92	68	Kloofendal Nat. Res., Roodepoort
Landolphia capensis	J.M.van Staden	16/4/93	1682	2528CB, Silverton
Landolphia kirkii	S.Hobson	25/6/89	76	Lake St.Lucia
Gonioma kamassi	M.J.Wells	1/12/65	3349	Van Stadens Nat. Res., PE
Gonioma kamassi	T.T.Hoole	5/7/75		Beggars Bush, Albany
Pachypodium bispinosum	A.Jacot-Guillarmod	10/10/88	10732	3326AA, Carlisle Bridge, GHT
Pachypodium bispinosum	J.Chan	6/3/92	32	3326BA, Ecca Res., GHT

Asclepiadaceae

SPECIES	COLLECTOR	DATE	NO.	LOCALITY
Microloma tenuifolium	M.C.Oliver	8/1970	439	3325, Kabega Park, PE
Microloma tenuifolium	R.M.Cowling	29/8/79	806	3324DD, Gamtoos Valley, Humansdorp
Microloma sagittatum	H.H.Burrows	17/5/89	2959	2917DB, Springbok
Microloma incanum	H.H.W.Pearson	26/1/09	4549	Raman's Drift
Microloma massonii	M.T.Hoffman	23/4/85	714	3329AB, Teasdale, Klipplaat
Astephanus marginatus	T.Dold	23/9/94	956	3326CD, Alexandria Coast
Astephanus triflorus	E.E.Esterhuysen	14/6/46	12835	Strathmore Rd., Camps Bay
Parapodium crispum	R.D.A.Bayliss	2/3/78	8474	3325BA, PE
Xysmalobium involucratum	T.Dold	17/1/94	901	3326BC, Mountain Drive, GHT
Xysmalobium zeyheri	H.J.Van Der Plank	12/1991		3325DC, Kunene Park, PE
Xysmalobium undulatum	T.Dold	17/12/93		3326BC, GHT
Xysmalobium confusum	D.Comins	16/1/60	1903	Mount Currie, Swartberg
Xysmalobium orbiculare	H.G.Flanagan	1/1892	758	Komgha
Xysmalobium prunelloides	E.E.Galpin	25/12/11	8375	3226DA, Katberg
Xysmalobium pearsonii	H.H.W.Pearson	1/1910	6560	Khamiesberg Plateau
Microglossum anomalum	H.G.Flanagan	11/1889	396	Kei River
Aspidoglossum biflorum	J.L.Drege	12/11/04	132	PE
Aspidoglossum carinatum	F.A.Rogers	11/1908	3999	Stones Hill, GHT
Aspidoglossum virgatum	H.G.Flanagan	2/1892	1045	Komgha
Aspidoglossum fasciculare	E.Brandert	18/11/20	65	Cedarville
Aspidoglossum heterophyllum	J.R. & B.R.	20/11/32	569	Featherstone Kloof, GHT
Aspidoglossum ovalifolium	H.G.Flanagan	11/1893	1307	Komgha
Aspidoglossum flanaganii	H.G.Flanagan	1/1892	1044	Kei Mouth
Aspidoglossum gracile	J.Wood	5/11/1898	3125	EL
Schizoglossum hamatum	M.Bowker	1904		Transkei
Schizoglossum atropurpureum subsp. *tridentatum*	E.E.Galpin			GHT
Schizoglossum atropurpureum subsp. *vireus*	R.Story	17/8/53	4174	Umtentu Mouth
Schizoglossum cordifolium	W.G.Bennie	10/1891	169	GHT
Schizoglossum bidens subsp. *bidens*	H.Bolus	1904	1620	Graaf Reynet
Schizoglossum bidens subsp. *gracile*	G.Hilner	26/12/21	427	Iora River Mouth, Willowvale
Schizoglossum aschersonianum	P.MacOwan	1904	906	GHT
Schizoglossum filiforme	T.Dold	12/11/92	212	Mahlasela Park, GHT

Fanninia caloglossa	G.E.Gibbs Russel	25/1/77	3484A	Keiskammahoek, Stutterheim
Periglossum angustifolium	H.G.Flanagan	2/1891	590	Komgha
Pachycarpus rigidus	H.Bolus	11/1903	10496	Elands Hoek, Aliwal North
Pachycarpus reflectens	H.G.Flanagan	11/1889	16	Kei River
Pachycarpus inconstans	S.Schonland	1/1921	4204A	East Gate, Port St. Johns
Pachycarpus natalensis	R.Schonland	1/1923	82	Fort Cuynghame, Sutterheim
Pachycarpus vexillaris	J.P.H.Acocks	24/1/56	18680	Penhoek Pass, Wodehouse
Pachycarpus dealbatus	E.E.A.Archibald	12/2/53	5028	Bushman's River Mouth, Alexandria
Pachycarpus concolor	H.G.Flanagan	1/1891	760	Komgha
Pachycarpus grandiflorus	J.L.Drege	4/1913		PE
Pachycarpus linearis	L.Britten	29/1/23	4626	Maclear
Woodia marginata	K.Wosmald	1/1908	22	Cambridge, EL
Woodia mucronata	G.Rattray	2/1908	168	EL
Asclepias expansa	H.G.Flanagan	12/1890	398	Komgha
Asclepias peltigera	W.Bennie	12/1892	419	Nqama River, Transkei
Asclepias stellifera	E.Bandert	18/11/20	9	Mvenyani
Asclepias meyeriana	W.S.Stritton	21/1/87	313	Buffelsfontein
Asclepias navicularis	D.M.Comins	11/11/55	1355	EL
Asclepias gibba	T.R.Simm	11/1892	1307	King William's Town
Asclepias erinens	E.E.A.Archibald	1/1946	683	Mount Austein
Asclepias aurea	T.Dold	12/11/94	1216	Willowvale
Asclepias burchellii	Gibbs Russel	13/4/78	303	3222A, Beaufort West
Asclepias fructicosa	C.Youthed	22/2/94	15	GHT
Asclepias physocarpa	A.Jacot-Guillarmod	29/11/87	10079	3226BC, GHT
Asclepias crinita	E.Cloete	3/4/94	2617	Kap River Res.
Asclepias dregeana	A.Jacot-Guillarmod	15/11/80	8375	3326BC, GHT
Asclepias hastata	H.G.Flanagan	1893	391	Komgha
Asclepias crispa	H.H.Burrows	26/11/89	3152	3326DA, Alexandria
Asclepias albens	J.R. & B.R.	4/12/32	533	Featherstone Kloof, GHT
Pentarrhinum insipidum	H.Bolus	10/1904		Graaf Reinet
Cynanchum africanum	H.Van Der Plank	7/1990		3325DC, PE
Cynanchum capense		4/1969		Thomas Baines Nat. Res., GHT
Cynanchum natalitum	D.J.Brothers	16/5/65	73A	Port Alfred
Cynanchum obtusifolium	H.H.Burrows	18/4/86	2573	3326DA, GHT
Oncinema lineare	W.Dix	1/1935	173	PE
Secamone alpinii	E.E.A.Archibald	28/7/54	5529	Alexandria
Secamone alpinii	E.E.A.Archibald	29/7/52	4359	Alexandria

Secamone filiformis	M.J.Wells	4/4/62	2654	Alexandria
Sisyranthus compactus	E.E.Galpin	11/1895	3032	Port Alfred
Sisyranthus imberbis	P.MacOwan		664	GHT
Anisotoma cordifolia	T.Dold	27/11/93	662	Amatole Mts, Hogsback
Brachystelma tuberosum	E.E.A.Archibald	3/11/52	4834	Alexandria
Brachystelma decipiens	A.Jacot-Guillarmod	15/11/80	8382	3326AD, GHT
Brachystelma meyeranum	R.A.Dyer	12/1925	379	King William's Town
Brachystelma cathcartense	P.F.Du Toit	11/1970	3	3227AC, Cathcart
Brachystelma huttonii	R.D.A.Bayliss	4/1/69	4404	3326, GHT
Brachystelma elongatum	L.Britten	12/11/06		GHT
Brachystelma comptum	R.A.D.Bayliss	23/11/78	8163	3326AC, GHT
Brachystelma schizoglossoides	A.Jacot-Guillarmod	29/11/80	8362	3326AD, GHT
Ceropegia distincta subsp. *haygarthii*	R.S.Guest	3/5/30		E.Cape
Ceropegia radicans subsp. *radicans*	H.G.Flanagan	1891	384	Kei River, Komgha
Ceropegia radicans subsp. *smithii*	A.Hechter	3/1968	7	Kirstenbosch
Ceropegia crassifolia	Schlechter	9/3/08	1691	King William's Town
Ceropegia carnosa	J.O.Wirminghaus	5/5/85	233	3326BD, GHT
Ceropegia dubia	R.A.Bayliss	19/2/76	7280	3325DB, Uitenhage
Ceropegia africana	Taylor & Edwards	11/1974	8798	3326BA, Ecca Pass
Ceropegia occulta	P.A.B.van Breda	4/6/56	85	Worcester
Ceropegia cancellata	T.Dold	27/11/93	1234	3326BC, GHT
Ceropegia woodii	F.H.Holland	20/12/43		Bushman's River Mouth, Alexandria
Ceropegia linearis	T.Dold	29/12/95	1260	Willowvale
Ceropegia meyeri	M.J.Wells	20/2/66	3519	Elliotdale
Riocreuxia torulosa	K.A.Dahlstrand	14/1/71	2646	3226, Fort Beaufort
Riocreuxia flanaganii	K.Wormald	1/1908	42	EL
Tylophora cordata	T.Dold & A.Booi	5/11/93	498	3327AC, Peddie
Tylophora lycioides	L.Britten	5/4/26	5262	Bathurst
Tylophora umbellata	G.Rattray	11/1908	246	EL
Marsdenia floribunda	M.H.Griffen	15/10/42	1497	3226DD, Fort Beaufort
Telosoma africana	M.J.Wells	15/2/69	4315	3129, Port St.Johns
Tenaris rubella	J.R. & B.R.	4/12/32	541	GHT
Fockea cylindrica	E.E.A.Archibald	24/8/53	5985	Alexandria
Fockea edulis	M.T.Hoffman	27/8/85	1023	3325DC, Kirkwood
Fockea multiflora	A.Jacot-Guillarmod	9/10/88	10125	3326AB, GHT

APPENDIX III Habit and habitat of Ranunculaceae taxa in the Eastern Cape

SPECIES	HABIT	HABITAT	REFERENCE
Knowltonia capensis	small herb	under dune bush in coastal areas	Gledhill 1969
K.cordata	perennial up to 700mm	rocky lower slopes	Bond and Goldblatt 1984
K.filia subsp.*filia*	small herb	forest margins and grassland	Exell & Milne-Redhead 1960
K.vesicatoria subsp. *humilis*	small herb	grassland	Exell & Milne-Redhead 1960
K.vesicatoria subsp. *grossa*	small to medium sized herb	shady bush and coastal scrub	Exell & Milne-Redhead 1960
K.transvaalensis	perennial herb with rhizome	damp grassland, often among bracken	Exell & Milne-Redhead 1960
Clematis brachiata	climber over scrub	scrub and edges of forests	Gledhill 1969
Ranunculus meyeri	small, perennial	swamps and shallow pools	Exell & Milne-Redhead 1960
R.multifidus	small herb	damp, marshy grassland	Gledhill 1969
R.muricatus	annual up to 400mm	damp areas	Bond & Goldblatt 1984
R.trichophyllus subsp.*trichophyllus*	submerged perennial	pools	Bond & Goldblatt 1984
Thalictrum rhynchocarpum	tall perennial herb	montane forest edges in undergrowth	Exell & Milne-Redhead 1960

APPENDIX IV Habit and habitat of Apocynaceae of the Eastern Cape

SPECIES	HABIT	HABITAT	REFERENCE
Acokanthera oblongifolia	shrub/small tree	in sclerophyllous maquis on littoral dunes	Codd 1963/ Leeuwenberg & Kupicha 1985
A.oppositifolia	shrub/tree	in riverine vegetation in shade of smaller trees	Codd 1963/ Leeuwenberg & Kupicha 1985
Carissa macrocarpa	1-3m shrub	coastal dunes and sand dunes	Codd 1963/ Leeuwenberg & Kupicha 1985
C.haematocarpa	3m shrub	succulent scrub	Codd 1963/ Leeuwenberg & Kupicha 1985
C.bispinosa var.*bispinosa*	3m shrub	coastal forest margins	Codd 1963/ Leeuwenberg & Kupicha 1985
C.bispinosa var. *acuminata*	5m shrub	dense woodland, forest margin, scrub forest	Codd 1963/ Leeuwenberg & Kupicha 1985
C.wyliei	scandent shrub	forest undershrub	Codd 1963/ Leeuwenberg & Kupicha 1985
Landolphia capensis	scandent shrub, rambling, up to 5m high	dry bush covered rocky areas	Codd 1963/ Leeuwenberg & Kupicha 1985
L.kirkii	straggling shrub or liana up to 30m	in woodland, deciduous, riverine and evergreen forests	Codd 1963/ Leeuwenberg & Kupicha 1985
Gonioma kamassi	shrub/tree up to 6m	coastal forest	Codd 1963/ Leeuwenberg & Kupicha 1985
Pachypodium bispinosum	succulent shrublet, underground tuberous stem	dry rocky situations, succulent scrub vegetation	Codd 1963/ Leeuwenberg & Kupicha 1985

APPENDIX V Habit and habitat of Asclepiadaceae of the Eastern Cape

SPECIES	HABIT	HABITAT	REFERENCE
Microloma tenuifolium	fleshy rooted perennial climbing herb	climbing on *Passerina* and *Restio* species	Gledhill 1969
M.sagittatum	dainty climber	succulent karoo scrub	Gledhill 1969
Astephanus marginatus	slender twining plant	on secondary dunes	Gledhill 1969
A.triflorus	slender vine	coastal to inland scrub	Bond & Goldblatt 1984
Xysmalobium undulatum	robust, erect herb up to 1,5m	moist grassland, especially vleis	Van Wyk & Malan 1988
Schizoglossum cordifolium	perennial herb up to 450mm	-	Bond & Goldblatt 1984
S.aschersonianum	perennial herb up to 250mm	flats and slopes	Bond & Goldblatt 1984
Pachycarpus dealbatus	stout, erect perennial up to 450mm	lower grassy slopes	Bond & Goldblatt 1984
P.grandiflorus	decumbent perennial herb with rhizome	grassland	Gledhill 1969
Woodia mucronata	erect perennial with tuberous rootstock	grassland	Bond & Goldblatt 1984
Asclepias expansa	delicate perennial herb	mixed grassland	Gledhill 1969
A.stellifera	herb with erect to spreading branches from perennial rootstock	grassland	Van Wyk & Malan 1988
A.gibba	perennial herb with tuberous roots	grassland	Van Wyk & Malan 1988
A.aurea	very slender herb with perennial rootstock	grassland	Van Wyk & Malan 1988
A.burchelli	erect perennial shrub up to 1m	grassland and bushveld, along roadsides	Van Wyk & Malan 1988
A.fructicosa	soft shrub 1-3m	disturbed areas	Bond & Goldblatt 1984
A.physocarpa	herbaceous shrub up to 2m	roadsides and disturbed ground	Gledhill 1969
A.dregeana	small perennial	grassland	Bond & Goldblatt 1984
A.crispa	small perennial herb	flats and slopes	Bond & Goldblatt 1984
Pentarrhinum insipidum	slender twining herb from woody perennial rootstock	grassland and clumps of bush, along fences	Van Wyk & Malan 1988
Cynanchum africanum	succulent, perennial climber	sandy soils	Bond & Goldblatt 1984
C.obtusifolium	tall climber	coastal bush	Bond & Goldblatt 1984
Oncinema lineare	slender vine	riverbanks and forests	Bond & Goldblatt 1984
Brachystelma elongatum	tuberous rooted perennial herb	in mixed grassland on northern slopes	Gledhill 1969
Ceropegia carnosa	fleshy leafed vine	dry riverine scrub	Bond & Goldblatt 1984
C.africana	tuberous, fleshy leafed vine	karoo	Bond & Goldblatt 1984
C.occulta	perennial with prostrate to straggling stems up to 300mm	dry rocky slopes	Bond & Goldblatt 1984

APPENDIX VI Vein structure in relation to phloem loading in certain species of Ranunculaceae, Apocynaceae and Asclepiadaceae of the Eastern Cape

An overview of vein structure and phloem loading pathway may be described for certain species of Ranunculaceae, Apocynaceae and Asclepiadaceae, based on the results presented in this thesis.

Ranunculaceae

Familial evolutionary position: The Ranunculaceae are generally considered to be a relatively primitive family in the angiosperm hierarchy (Takhtajan 1969, Heywood 1978, Thorne 1992).

Ranunculus multifidus (Illustrated in Fig.6.1)

Habit: *R.multifidus* is a small, delicate herbaceous plant with soft leaves (Appendix III). The cuticle is thin and there is very little in the way of secondary thickening.

Habitat: Usually found in damp, marshy ground (Appendix III).

Leaf architecture: The overall description was marginally actinodromous, and perfect with origin of primary veins basal to suprabasal (Hickey 1973) (Fig.s 3.3 B & 3.4 A-C) (Tables 3.1 & 3.2). Venation was sparse and delicate. The primitive condition of dichotomous branching of veins could be seen, especially in species of *Ranunculus*, yet veins did anastomose forming a widely spaced reticulum across the lamina. Areolar arrangement was imperfect and poorly differentiated, consisting of large, irregular, incompletely enclosed meshes.

Anatomy: Leaves were unifacial with large air spaces between the widely spaced spongy mesophyll cells (Fig. 4.1). Interveinal distances appeared large. There was very little in the way of supporting tissues, except for collenchyma surrounding the midvein. Ultimate veinlets lacked phloem, although phloem of minor veins bordering areoles stained darkly indicating intense activity. Chlorophyllous bundle sheath cells were elongated along vein axes, with few bundle sheath extensions seen.

Ultrastructure: Bundle sheath cells were vacuolate and contained many peripheral chloroplasts (Fig. 5.1). An increase in cytoplasmic density was seen from bundle sheath through vascular parenchyma to companion cells, as was a decrease in starch accumulation and plastid occurrence. Companion cells had many ribosomes,

mitochondria and small vacuoles, but lacked wall ingrowths and plastids.

Plasmodesmata: Branched plasmodesmata were noted (Fig. 5.1). Bundle sheath cells in particular were abundantly interconnected by plasmodesmata, especially those of ultimate veinlets. Companion cells were occasionally connected to vascular parenchyma and bundle sheath cells by plasmodesmata, but this was more the exception than the rule. Sieve tube walls were thin and of uniform thickness. Pore-plasmodesmata joined companion cells with adjacent sieve tubes.

Minor vein configuration: Type 2a

Description of phloem loading pathway: An illustrated summary of the potential photosynthate pathway and phloem loading method is presented in Figure 6.1. Leaf architecture and spatial arrangement are not conducive to efficient transport of assimilate to loading sites. Veins were delicate and poorly interconnected. The sparse, widely spaced reticulum and large air spaces would present a rambling, tortuous, inefficient route for assimilates moving from mesophyll to veins. Bundle sheath extensions were few, again preventing a direct route for assimilates to reach veins. Ultimate veinlets did not have phloem. The phloem of minor veins bordering areoles were therefore suggested to be the main loading sites. The dark staining reaction of phloem supports this suggestion as it is indicative of intense activity. Bundle sheath cells of ultimate veinlets did, however, show abundant plasmodesmatal interconnection. Perhaps such interconnectivity facilitates assimilate collection in areole centres, for transport through bundle sheath cells to those of minor veins and ultimately phloem. This could perhaps compensate for the poor, sparse lamina venation coverage observed. Due to the lack of plasmodesmata connecting companion cells to other cell types, minor vein configuration was designated type 2a. Assimilate would therefore most likely be loaded apoplasmically. Up to the companion cell interface, assimilate transport could well be symplasmic as branched plasmodesmata were seen.

Apocynaceae

Familial evolutionary position: The Apocynaceae are generally considered to be a one of the most advanced families in the angiosperm hierarchy, and very closely related to the Asclepiadaceae (Takhtajan 1969, Heywood 1978, Thorne 1992).

Acokanthera oppositifolia

Habit: *A.oppositifolia* grows as a small tree or shrub. The stem is strong and woody. Leaves are tough, with

a thick waxy cuticle and strengthening tissues (Appendix IV).

Habitat: Usually found in riverine vegetation in the shade of small trees (Appendix IV).

Leaf architecture: Leaves were described as pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig.s 3.10 B, & 3.11 A & B) (Tables 3.3 & 3.4). The reticulum was relatively dense and veins were robust in appearance. Areoles consisted of incompletely enclosed meshes of irregular shape with no distinct orientation.

Anatomy: Leaves were unifacial (Fig.s 4.2, 4.3 & 4.4). Abaxial stomata were sunken below projections of a very thick cuticle. Palisade mesophyll was multilayered, closely packed and had many chloroplasts. Crystals were present. The midvein was supported by collenchyma. Laticifers were present. Secondary thickening occurred in both primary and secondary veins. Paradermal sections showed a reticulum of robust veins with relatively small interveinal distances. Higher order veins occurred in close association with mesophyll, joined directly by bundle sheath extensions. Phloem of minor veins stained darkly indicating an active metabolism. Ultimate veinlets consisted of splayed xylem vessels and bundle sheath cells only.

Ultrastructure: Vacuolate bundle sheath cells contained many peripheral chloroplasts (Fig. 5.4). Companion cells contained a dense cytoplasm with abundant ribosomes, mitochondria, ER and small vacuoles. Chloroplasts were present with grana but no starch granules. Sieve tube walls were of uniform thickness.

Plasmodesmata: Branched plasmodesmata were seen (Fig. 5.4). Plasmodesmata were not particularly abundant, but were present all along the route from mesophyll to companion cell. Wide pore-plasmodesmata connected companion cells to sieve tubes.

Minor vein configuration: Type 2a

Description of phloem loading pathway: Leaf architecture showed efficient lamina coverage for assimilate loading into veins. Veins were robust, well interconnected, close, and bundle sheath extensions connected mesophyll to higher order veins providing an efficient, direct route for assimilates to reach phloem. Ultimate veinlets lacked phloem, so minor veins bordering areoles would be the main loading sites. Minor vein phloem

stained darkly, indicating the intense metabolic activity required for active loading of assimilates. Type 2a was designated due to lack of wall ingrowths in companion cells and relatively few plasmodesmata. As plasmodesmata were seen all the way to the companion cell interface, symplasmic transport could occur up to the companion cell/sieve complex. However, insufficient plasmodesmata were noted at the companion cell interface for symplasmic loading to be predominant. Phloem loading is therefore suggested to be apoplasmic.

Carissa bispinosa (Illustrated in Fig. 6.2)

Habit: *C.bispinosa* occurs as a large shrub or small tree (Appendix IV).

Habitat: Usually found in coastal forest margins (appendix IV).

Leaf architecture: Leaves were described as pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig.s 3.10 D & 3.12 [as var. *acuminata*]) (Tables 3.3 & 3.4). The reticulum was relatively dense and veins were robust in appearance. Areoles consisted of incompletely enclosed meshes of irregular shape with no distinct orientation.

Anatomy: Leaves were unifacial, with an adaxial tanniniferous palisade mesophyll layer overlying tightly packed palisade and spongy mesophyll layers (Fig.s 4.5, 4.6, 4.7 & 4.8). Spongy mesophyll cells closest to the abaxial epidermis were also tanniniferous. Crystals were present. Chlorophyllous bundle sheath cells were small and rounded, with extensions connecting to adjacent mesophyll cells. The broad midvein contained laticifers, tanniniferous cells and parenchyma cells, and was supported by collenchyma. Secondary thickening was noted in primary and secondary veins. Primary, secondary and tertiary veins had bicollateral phloem, but higher vein orders did not. No phloem was present in ultimate veinlets, but phloem of minor veins bordering areoles stained darkly, indicating an active metabolism.

Ultrastructure: Mesophyll cells contained extensive starch accumulations in chloroplasts, while bundle sheath cells had fewer and vascular parenchyma cells had none (Fig.s 5.2 & 5.3). Companion cells showed a dense cytoplasm with small vacuoles. No plastids or starch granules were seen. Sieve tube walls were thickened in some instances in the midvein, but were uniformly thinner in minor veins.

Plasmodesmata: Plasmodesmata, having been plentiful amongst mesophyll cells, decreased in abundance on nearing the companion cell/sieve element complex (Fig.s 5.2 & 5.3). Branched pore-plasmodesmata

connected companion cells with adjacent sieve elements, with branching occurring on the companion cell side only. Pore-plasmodesmata between adjacent sieve elements were blocked with callose. Occasionally, plasmodesmata were seen connecting vascular parenchyma cells with sieve tubes.

Minor vein configuration: Type 2a

Description of phloem loading pathway: Figure 6.2 illustrates a summary of aspects related to potential phloem loading pathways and methods. A dense reticulum of robust veins and intense lamina coverage indicated short, efficient routes for photosynthates through mesophyll to minor veins. Furthermore, bundle sheath extensions in direct contact with mesophyll cells provided abundant access to minor veins. These two features allowed for rapid removal of assimilates to phloem for export from the leaf. Ultimate veinlets lacked phloem. In minor veins around areoles, darkly-stained phloem was indicative of cells actively engaged in the loading and transporting of assimilates. No starch accumulation was noted in phloem cell types, indicating rapid export of assimilates. Minor vein configuration was designated type 2a due to companion cells containing many small vacuoles, no wall ingrowths and very few plasmodesmata. Assimilates probably followed a symplasmic pathway, as evidenced by abundant plasmodesmata, until the companion cell interface was reached. There, assimilates would be loaded apoplasmically. Callose deposition in pore-plasmodesmata of adjacent sieve elements would aid in decreasing lateral leakage of assimilates, and therefore increase export efficiency.

Asclepiadaceae

Familial evolutionary position: The Asclepiadaceae are generally considered to be a one of the most advanced families in the angiosperm hierarchy, and very closely related to the Apocynaceae (Takhtajan 1969, Heywood 1978, Thorne 1992).

Secamone alpinii (Illustrated in Fig. 6.3)

Habit: *S.alpinii* is a creeper over other plants (Appendix II).

Habitat: Usually found in dry coastal scrub (Appendix II).

Leaf architecture: Leaves were pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig. 3.24 A - C) (Tables 3.6 & 3.7). Veins were robust and the reticulum was dense. Areoles were imperfect, with

meshes of random orientation and irregular shape and size.

Anatomy: Leaves were unifacial with a thick cuticle (Fig. 4.10 A - C). There was one layer of palisade mesophyll, with widely spaced, rounded, irregular spongy mesophyll underneath. No tanniniferous cells were seen. The association of mesophyll and bundle sheath cells was not particularly close. A chlorophyllous bundle sheath of rounded cells occurred around veins. The midvein was supported by collenchyma and exhibited bicollateral phloem. Phloem was present in higher orders bordering areoles, but absent from ultimate veinlets.

Ultrastructure: Mesophyll cells contained many chloroplasts but few starch granules, while bundle sheath cells had few chloroplasts and no starch granules (Fig.s 5.5 & 5.6). Vascular parenchyma cells contained a very dense cytoplasm with many ribosomes, mitochondria and ER. Small vacuoles were present, as well as very few chloroplasts. Companion cells appeared very similar to vascular parenchyma cells. Chloroplasts were few and poorly developed. Sieve elements walls were uniformly thin compared to other species.

Plasmodesmata: Plasmodesmata were abundant between all cell types from mesophyll to companion cell (Fig. 5.7). Branched and unbranched plasmodesmata were seen. Plasmodesmata occurred in localised raised aggregations.

Minor vein configuration: Type 1 - 2a

Description of phloem loading pathway: Figure 6.3 illustrates a summary of aspects related to potential phloem loading pathways and methods. Venation was robust and the reticulum dense across the lamina. Small interveinal distances resulted in fewer mesophyll cells for assimilates to traverse en route to veins. Bundle sheath and mesophyll cells were not noted to be in especially close contact. Phloem was absent from ultimate veinlets, but present in minor veins bordering areoles, presumably the sites of assimilate loading. Abundant plasmodesmatal connectivity was noted all the way from mesophyll to companion cell. Plasmodesmata occurred in raised aggregations between all living cell types of phloem, bundle sheath and mesophyll. Based on companion cell ultrastructure, a combination type 1-2a was designated. Assimilates probably followed a symplasmic route and also loaded symplasmically. There was, however, no evidence that apoplasmic loading could not be occurring concurrently.

Asclepias physocarpa

Habit: *A.physocarpa* is a herbaceous shrub up to 2m tall, with tough, waxy leaves (Appendix V).

Habitat: Usually found along roadsides or on disturbed ground, and in dry scrub (Appendix V).

Leaf architecture: Leaves were pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig. 3.16 E & F) (Table 3.6 & 3.7). Simple intersecondary veins occurred. Veins were robust and the reticulum was particularly dense. Areoles were small with imperfect meshes of irregular size and shape, and random arrangement.

Anatomy: Leaves were unifacial with a very thick cuticle (Fig. 4.12). There was one layer of palisade cells. Spongy mesophyll cells were elongated and box-like, with a few interspersed tanniniferous cells. Air spaces were small, as were interveinal distances. Mesophyll was in close association with veins, being directly connected by bundle sheath extensions. The midvein was broad, with large laticifers and a wide cambial layer. Both primary and secondary veins had bicollateral phloem. Phloem extended to minor veins bordering areoles, but not to ultimate veinlets. Phloem stained darkly indicating an active metabolism. Laticifers were seen to follow minor veins as well.

Ultrastructure: Mesophyll and bundle sheath cells possessed many chloroplasts with very large starch granules (Fig.s 5.8, 5.9, 5.10 & 5.11). The cytoplasm was surprisingly rich in ER and ribosomes. Vascular parenchyma cells contained many mitochondria, chloroplasts with starch granules and a large central vacuole. Companion cell cytoplasm was denser still, with a relatively large central vacuole. Chloroplasts were fully developed but small, and lacked starch granules. Sieve element walls were of uniform thickness.

Plasmodesmata: Mesophyll cells were abundantly interconnected by plasmodesmata (Fig.s 5.8, 5.9, 5.10 & 5.11). Plasmodesmatal frequency was seen to diminish on nearing the companion cell/sieve tube complex, with plasmodesmata between vascular parenchyma and companion cells being extremely rare. Pore-plasmodesmata connected companion cells with adjacent sieve tubes.

Minor vein configuration: Type 2a

Description of phloem loading pathway: Veins were robust, occurring as a particularly dense reticulum.

This, together with bundle sheath extensions to the mesophyll, provided a short, direct, efficient pathway for assimilates to follow en route to the phloem for export. Phloem extended as far as minor veins bordering areoles, but not to ultimate veinlets. The dark staining reaction of phloem indicated an active metabolism, probably due to active loading of assimilates. Companion cells contained a dense cytoplasm. Many mitochondria occurred in both companion and vascular parenchyma cells, again indicating a high degree of metabolic activity. Minor vein configuration was designated as type 2a due to a lack of wall ingrowths, very few plasmodesmata and many small vacuoles in companion cells. Abundant plasmodesmata between mesophyll cells suggested initial symplasmic transport, changing to apoplasmic transport at the companion cell interface.

Asclepias fructicosa

Habit: *A.fructicosa* is a soft, scandescent shrub of 1-3m (Appendix V).

Habitat: Usually occurs in disturbed areas of dry scrub (Appendix V).

Leaf architecture: Leaves were pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig.s 3.14 H & 3.16 A & B) (Tables 3.6 & 3.7). The reticulum was very dense, although veins did not appear robust. Areoles were imperfect with meshes of irregular size and shape, and random arrangement.

Anatomy: Leaves were unifacial with tanniniferous cells interspersed amongst large, irregular adaxial epidermal cells (Fig.s 4.10 D - I & 4.11). There was one layer of palisade mesophyll, including some very large tanniniferous cells. Spongy mesophyll cells were round and irregular in shape. Many tanniniferous cells and crystals occurred in this tissue. Air spaces were small. Mesophyll cells occurred in close association with veins and interveinal distances were relatively small. A chlorophyllous bundle sheath of box-like cells enclosed vascular tissue. The midvein was small and delicate, lacking bicollateral phloem. The cambial layer was narrow and poorly defined. Large laticifers followed all veins. Minor veins bordering areoles contained darkly-stained phloem, indicating an active metabolism. Phloem was absent from ultimate veinlets.

Ultrastructure: Vascular parenchyma contained a relatively clear cytoplasm, with a large central vacuole and many mitochondria (Fig. 5.12). Chloroplasts included starch granules. Companion cells had small fragmented vacuoles in a dense cytoplasm, with many ribosomes, ER and mitochondria. Sieve element walls were of uniform thickness.

Plasmodesmata: A distinct paucity of plasmodesmata was noted between all cell types, even between mesophyll cells (Fig. 5.12). Very narrow branched and unbranched plasmodesmata were occasionally seen. Pore-plasmodesmata were branched on the companion cell side.

Minor vein configuration: Type 2a

Description of phloem loading pathway: A dense reticulum of delicate veins covered the lamina, creating small interveinal distances. The route for photosynthates to veins for export would therefore be short, direct and efficient. Air spaces in spongy mesophyll were small and mesophyll cells were noted to be in close association with veins. No phloem occurred in ultimate veinlets. Phloem was present in minor veins bordering areoles and stained darkly. Minor vein configuration was designated type 2a as companion cells contained many small vacuoles, very few plasmodesmata and no wall ingrowths. Very few plasmodesmata were noted generally. Assimilates probably moved apoplasmically, and to a limited degree symplasmically, until the companion cell/ sieve element complex was reached. Assimilate loading for export was indicated to be apoplasmic at this interface.

Cynanchum obtusifolium

Habit: *C.obtusifolium* is a tall climbing creeper with tough leaves (Appendix V).

Habitat: Usually in coastal bush (Appendix V).

Leaf architecture: Leaves were pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig. 3.23 A) (Tables 3.6 & 3.7). Areoles were imperfect with meshes of irregular size and shape, and random arrangement.

Anatomy: Leaves were unifacial with a thin cuticle (Fig.4.13). A bilayer of palisade mesophyll, with a few interspersed tanniniferous cells, overlay spongy mesophyll cells, with scattered tanniniferous cells and crystals. A chlorophyllous bundle sheath occurred with extensions in direct contact with mesophyll cells, forming a close association between these two cell types. The midvein was robust with a thin cambial layer. Both primary and secondary veins possessed bicollateral phloem and supporting collenchyma. Phloem of minor veins bordering areoles stained darkly, but was absent from ultimate veinlets.

Ultrastructure: Mesophyll cells contained chloroplasts with starch accumulations, as did bundle sheath and

vascular parenchyma cells (Fig.s 5.13, 5.14 & 5.15). Vascular parenchyma cell cytoplasm was rich in ribosomes, ER and mitochondria. A large central vacuole was present. Companion cell cytoplasm was only slightly denser than that of vascular parenchyma. The occasional plastid was seen, though very rare, and did not hold any starch granules. Sieve tube walls were of even thickness.

Plasmodesmata: Plasmodesmata were noted between all cell types up to the companion cells, but were very rare (Fig.s 5.13, 5.14 & 5.15). Where present, plasmodesmata occurred in localised aggregations. Pore-plasmodesmata were branched on the companion cell side.

Minor vein configuration: Type 2a

Description of phloem loading pathway: Bundle sheath extensions in direct contact with mesophyll cells provided an efficient pathway for assimilates to reach the phloem. Mesophyll was therefore in close association with veins. Ultimate veinlets lacked phloem. In minor veins bordering areoles, phloem stained darkly indicating intense metabolic activity: important as this was the proposed loading site of assimilates for export. Companion cells indicated minor vein configuration type 2a. No wall ingrowths were noted, plasmodesmata were few and numerous small vacuoles occurred. Few plasmodesmata were seen generally, indicating a predominantly apoplasmic pathway all the way from mesophyll to companion cell, although limited symplasmic transport could occur.

Ceropegia carnosa (Illustrated in Fig. 6.4)
Habit: *C.carnosa* is a fleshy leaved vine (Appendix V).

Habitat: Usually found in dry riverine scrub (Appendix V).

Leaf architecture: Leaves were pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig. 3.22 F & G) (Tables 3.6 & 3.7). Veins were delicate and the reticulum widely spaced. Areoles were imperfect with incompletely enclosed meshes of irregular shape and size, and random orientation.

Anatomy: Leaves were unifacial with a thin cuticle (Fig. 4.14 A - D). A bilayer of poorly defined palisade mesophyll overlay spongy mesophyll. No tanniniferous cells were present. The bundle sheath was chlorophyllous with extensions to adjacent mesophyll cells. The midvein was small and delicate, without any

supporting tissue. Bicollateral phloem was present as was a poorly defined cambial layer. Phloem was absent from ultimate veinlets.

Ultrastructure: Mesophyll cells contained many chloroplasts with abundant starch accumulations (Fig. 5.18). Bundle sheath cells appeared to have fewer chloroplasts than mesophyll cells. Vascular parenchyma cells had a large central vacuole, dense cytoplasm, many mitochondria and chloroplasts with grana and starch granules. Companion cell cytoplasm was dense with many ribosomes, ER, Golgi bodies and mitochondria. Chloroplasts with starch granules were present as was a fairly large vacuole. The cell membrane showed extensive foldings to increase surface area, but no wall ingrowths. Sieve tube walls were of even thickness, except where thickened in regions of pore-plasmodesmata.

Plasmodesmata: Plasmodesmatal abundance decreased on nearing the companion cell/sieve element complex (Fig. 5.18). Where present, plasmodesmata occurred in raised aggregations. Pore-plasmodesmata were branched on the companion cell side.

Minor vein configuration: Type 2a - 2b

Description of phloem loading pathway : An illustrated summary of the potential photosynthate pathway and phloem loading method is presented in Figure 6.4. The reticulum was widely spaced, veins were delicate and mesophyll layers were poorly defined: features common to succulent leaves. Bundle sheath extensions provided direct access for assimilates to reach veins, although numerous mesophyll cells would have to be crossed due to wide interveinal distances. Starch accumulations in all cell types indicated a slower route. The companion cell membrane was extensively folded, but no corresponding wall ingrowths were seen. The suggested function of the folds was to increase surface area over which apoplasmic loading of assimilates could occur. This feature, together with the distinct paucity of plasmodesmata, indicated a definite predominance of apoplasmic loading at the companion cell interface. Symplasmic transport could occur up to that point, as evidenced by interconnectivity of cells by plasmodesmata. Minor vein configuration was designated combination type 2a-2b accordingly.

Ceropegia woodii

Habit: *C.woodii* is a creeper over other plants (Appendix II).

Habitat: Usually in dry riverine scrub (Appendix II).

Leaf architecture: Leaves were pinnate, camptodromous and brochidodromous (Hickey 1973). Areoles were imperfect with incompletely enclosed meshes of irregular shape and size, and random orientation.

Anatomy: Leaves were unifacial (Fig. 4.14 E - H). Mesophyll cells were rounded and closely packed, with no clear distinction between palisade and spongy mesophyll. A chlorophyllous bundle sheath of rounded cells enclosed vascular tissues. Bicollateral phloem occurred in primary and secondary veins. There were no supporting tissues around major veins. Phloem of minor veins bordering areoles stained darkly. Ultimate veinlets consisted of xylem and bundle sheath cells only.

Ultrastructure: Mesophyll cells contained chloroplasts, but no starch accumulations (Fig. 5.16 & 5.17). Bundle sheath cells had large central vacuoles, few chloroplasts, no starch granules and a clear cytoplasm. Vascular parenchyma cells showed chloroplasts with grana, but no starch granules. The cytoplasm was dense with many ribosomes, ER and mitochondria, and a large vacuole. Companion cells possessed a very dense cytoplasm. Chloroplasts were present but very few and lack starch. Sieve element walls were of uniform thickness.

Plasmodesmata: Very few plasmodesmata were seen (Fig. 5.16 & 5.17). Pore-plasmodesmata were branched on the companion cell side.

Minor vein configuration: Type 2a

Description of phloem loading pathway: The mesophyll did not show a clear distinction of layers and was not in particularly close contact with veins, creating an inefficient route for assimilate transport. Ultimate veinlets lacked phloem. Minor veins bordering areoles contained darkly-stained phloem, indicating intense metabolic activity in these veins as the sites of assimilate loading for export. Companion cells had a very dense cytoplasm, no wall ingrowths and very few plasmodesmata. Minor vein configuration was therefore designated type 2a. Assimilate loading into the companion cell/ sieve element complex was suggested to be predominantly apoplasmic, based on anatomical and ultrastructural evidence.

Ceropegia distincta

Habit: *C.distincta* is a vine/creeper over other plants (Appendix II).

Habitat: Usually dry scrub (Appendix II).

Leaf architecture: Leaves were pinnate, camptodromous and brochidodromous (Hickey 1973) (Fig. 3.21 A - D) (Tables 3.6 & 3.7). The reticulum was dense with delicate venation. Areoles were imperfect with incompletely enclosed meshes of irregular shape and size, and random orientation.

Anatomy: Leaves were unifacial. Tanniniferous cells were absent. A chlorophyllous bundle sheath enclosed vascular tissues. Veins were delicate with little xylem or phloem.

Ultrastructure: Chloroplasts of mesophyll cells included starch accumulations. Bundle sheath cells appeared to have fewer chloroplasts than mesophyll cells. Vascular parenchyma cells possessed a large vacuole, many mitochondria, fully developed chloroplasts with starch granules and many plasmodesmata between vascular parenchyma and companion cells. Companion cells showed a dense cytoplasm with many mitochondria and small vacuoles. Sieve element walls were uniformly thin and even.

Plasmodesmata: Very few plasmodesmata were seen between cell types, except where vascular parenchyma and companion cells interfaced. Pore-plasmodesmata were branched on the companion cell side.

Minor vein configuration: Type 2a

Description of phloem loading pathway: A dense reticulum of delicate veins covered the lamina, resulting in small interveinal distances and a close association of mesophyll and veins. This would provide a short, efficient route for assimilates to reach veins for export. Very few plasmodesmata were seen generally. Companion cells lacked plasmodesmatal interconnectivity with other cell types and wall ingrowths. Minor vein configuration was designated type 2a, in which assimilates would be loaded apoplasmically at the companion cell interface.

REFERENCES

Arroyo, S. (1986) Leaf anatomy in the Tecophilaeaceae. *Bot. J. Linn. Soc.* 93: 323.

Badelt, K., White, R.G., Overall, R.L., Vesk, M. (1994) Ultrastructural specialization of the cell wall sleeve around plasmodesmata. *Am. J. Bot.* 81: 1422-1427.

Barnabas, A.D. (1983) Composition and fine structural features of longitudinal veins in leaves of *Thalassodendron ciliatum*. *S. Afr. J. Bot.* 2: 316-325.

Batashev, D.R. (1996) Taxonomic variety of minor vein ultrastructure and phloem loading types in Apocynaceae. *J. Exp. Bot.* 47: 1301.

Beebe, D.U., Evert, R.F. (1992) Photosynthate pathway(s) and phloem loading in the leaf of *Moricandia arvensis* (L.) DC. (Brassicaceae). *Int. J. Plant Sci.* 153: 61-77.

Beebe, D.U., Turgeon, R. (1992) Localization of galactinol, raffinose, and stachyose synthesis in *Curcubita pepo* leaves. *Planta* 188: 354-361.

Beslow, D.T., Rier, J.P. (1969) Sucrose concentration and xylem regeneration in *Coleus* internodes in vitro. *Plant Cell Physiol.* 10: 69-77.

Bieleski, R.L. (2000) The bigger picture - phloem seen through horticultural eyes. *Aust. J. Plant Physiol.* 27: 615-624.

Bond, P., Goldblatt, P. (1984) Plants of the Cape flora - a descriptive catalogue, pp. 145, 146 and 379. National Botanic Gardens, SA

Bonnemain, J.-L. (1969a) Transport du ^{14}C assimilé à partir des feuilles de tomate en voie de croissance et vers celles-ci. *C.R. Acad. Sci.* 269D: 1660-1663. [UNSEEN]

Botha, C.E.J. (1992) Plasmodesmatal distribution, structure and frequency in relation to assimilation in C_3 and C_4 grasses in southern Africa. *Planta* 187: 348-358.

Botha, C.E.J., Cross, R.H.M. (2000) Towards reconciliation of structure with function in plasmodesmata - who is the gatekeeper? *Micron* 31: 713-721.

Botha, C.E.J., Cross, R.H.M. (2001) Regulation within the supracellular highway - plasmodesmata are the key. *South African Journal of Botany* 67: 1-9.

Botha, C.E.J., Evert, R.F. (1988) Plasmodesmatal distribution and frequency in vascular bundles and contiguous tissues of the leaf of *Themeda triandra*. *Planta* 173: 433-441.

Botha, C.E.J., Hartley, B.T., Cross, R.H.M. (1993) The ultrastructure and computer-enhanced digital image analysis of plasmodesmata at the Kranz mesophyll-bundle sheath interface of *Themeda triandra* var. *imberbis* (Retz) A.Camus in conventionally fixed leaf blades. *Ann. Bot.* 72: 255-261.

Botha, C.E.J., Van Bel, A.J.E. (1992) Quantification of symplasmic continuity as visualised by plasmodesmograms: diagnostic value for phloem-loading pathways. *Planta* 187: 359-366.

Bouché-Pillon, S., Fleurat-Lessard, P., Fromont, J-C., Serrano, R., Bonnemain, J-L. (1994) Immunolocalization of the plasma membrane H^+-ATPase in minor veins of *Vicia faba* in relation to phloem loading. *Plant Physiol.* 105: 691-697.

Bourquin, S., Bonnemain, J.L., Delrot, S. (1990) Inhibition of loading of C^{14} assimilates by p-cloromercuribenzenesulfonic acid. *Plant Physiol.* 92: 97-102.

Canny, M.J. (1990) What becomes of the transpiration stream? *New Phytol.* 114: 341-368.

Carlquist, S. (1991) Leaf anatomy of Bruniaceae: ecological, systematic and phylogenetic aspects. *Bot. J. Linn. Soc.* 107: 1-34.

Carr, D.J., Carr, S.G.M., Lenz J.R. (1986) Leaf venation in *Eucalyptus* and other genera of Myrtaceae: implications for systems of classification of venation. *Aust. J. Bot.* 34: 53-62.

Chase, M.W., Soltis, D.E., Olmstead, R.G. and 41 others (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Ann. Missouri Bot. Gard.* 80: 528-580.

Chandra, V., Mitra, R., Kapoor, S.L., Kapoor, L.D. (1969) Epidermal and venation studies in Apocynaceae I. *Bull. Bot. Surv. India* 11: 286-289.

Chandra, V., Mitra, R., Kapoor, S.L., Kapoor, L.D. (1972) Epidermal and venation studies in Apocynaceae IV. *Bull. Bot. Surv. India* 14: 76-82.

Codd, L.E. (1963) Apocynaceae. *Flora of S.A.* 26: 244-297.

Conover, M.V. (1991) Epidermal patterns of the reticulate-veined Liliiflorae and their parallel-veined allies. *Bot. J. Linn. Soc.* 107: 295-312.

Cook, M.E., Graham, L.E., Botha, C.E.J., Levin, C.A. (1997) Comparative ultrastructure of plasmodesmata of *Chara* and selected bryophytes: towards an elucidation of the evolutionary origin of plant plasmodesmata. *Am. J. Bot.* 84: 1196-1178.

Côté, R., Thain, J.F., Fensom, D.S (1987) Increase in electrical resistance of plasmodesmata of *Chara* induced by an applied pressure gradient across nodes. *Can. J. Bot.* 65: 509-511.

Dahlgren, R. (1975) A system of classification of the angiosperms to be used to demonstrate the distribution of characters. *Bot. Notiser* 128: 119-147.

Dannenhoffer, J.M., Ebert, W.Jr., Evert, R.F. (1990) Leaf vasculature in barley, *Hordeum vulgare* (Poaceae). *Amer. J. Bot.* 77: 636-652.

De Kock, P.C., Rutherford, M. (1971) The leaf tip of *Ranunculus reptans* L. *Ann. Bot.* 35: 1191-1195.

Dengler, N.G., MacKay, L.B. (1975) The leaf anatomy of beech, *Fagus grandifolia*. *Can. J. Bot.* 53: 2202-2211.

Dickison, W.C., Weitzman, A.L. (1996) Comparative anatomy of the young stem, node and leaf of Bonnetiaceae, including observations on a foliar endodermis. *Amer. J. Bot.* 83: 405-418.

Ding, B., Haudenshield, J.S., Hull, R.J., Wolf, S., Beachy, R.N., Lucas, W.J. (1992) Secondary plasmodesmata are specific sites of localization of the tobacco mosaic virus movement protein in transgenic tobacco plants. *Pl. Cell* 4: 915-928.

Ding, B., Turgeon, R., Parthasarathy, M.V. (1992) Substructure of freeze-substituted plasmodesmata. *Protoplasma* 169: 28-41.

Doyle, J.A., Hickey, L.J. (1976) Pollen and leaves from the mid-Cretaceous Potomac Group and their bearing on early angiosperm evolution. In: Origin and evolution of angiosperms. Columbia University Press, pp 139-206. [UNSEEN]

Ehlers, K. Kollman, R. (1996) Formation of branched plasmodesmata in regenerating *Solanum nigrum* protoplasts. *Planta* 199: 126-138.

Endress, M.E., Bruyns, P.V. (2000) A revised classification of the Apocynaceae s.l. *Bot. Rev.* 66: 2-56.

Epel, B.L. (1994) Plasmodesmata: composition, structure and trafficking. *Plant Molecular Biology* 26: 1343-1356.

Erwee, M.G., Goodwin, P.B., Van Bel, A.J.E. (1985) Cell-cell communication in the leaves of *Commelina cyanea* and other plants. *Plant, Cell and Environment* 8: 173-178.

Esau, K. (1972) Cytology of sieve elements in minor veins of sugar beet leaves. *New Phytol.* 71: 161-168.

Esau, K. (1973) Comparative structure of companion cells and phloem parenchyma cells in *Mimosa pudica* L. *Ann. Bot.* 37: 625-632.

Esau, K., Cronshaw, J. (1968) Plastids and mitochondria in the phloem of *Cucurbita*. *Can. J. Bot.* 46: 877-887.

Eschrich, W. (1970) Biochemistry and fine structure of phloem in relation to transport. *Ann. Rev. Pl. Physiol.* 21: 193-214.

Ettingshausen, C. von (1861) Die Blattskelete des Dicotyledonen. K.K. Hof. Staatsdrucken, Vienna. [UNSEEN]

Evert, R.F., Eschrich, W., Heyser, W. (1978) Leaf structure in relation to solute transport and phloem loading in *Zea mays* L. *Planta* 138: 279-294.

Evert, R.F., Mierzwa, R.J. (1986) Pathway(s) of assimilate movement from mesophyll cells to sieve tubes in the Beta vulgaris leaf. *In*: Phloem Transport, pp. 419-432.

Evert, R.F., Mierzwa, R.J. (1989) The cell wall-plasmalemma interface in sieve tubes of barley. *Planta* 177: 24-34.

Evert, R.F., Russin, W.A., Botha, C.E.J. (1996) Distribution and frequency in relation to photosynthate pathways and phloem loading in the barley leaf. *Planta* 198: 572-579.

Exell, A.W., Milne-Redhead, E. (1960) Ranunculaceae. *Flora Zambesica* 1: 89-101.

Fischer, A. (1885) Studien über die Siebröhren der Dicotylenblätter. *Ber. Sächs. Ges. Wiss. Leipzig Math. Phy. Cl.* 37: 245-290. [UNSEEN]

Fisher, D.B. (1967) An unusual layer of cells in the mesophyll of the soyabean leaf. *Bot. Gaz.* 128: 205-218.

Fisher, D.G. (1985) Morphology and anatomy of the leaf of *Coleus blumei* (Lamiaceae). *Amer. J. Bot.* 72: 392-406.

Fisher, D.G. (1986) Ultrastructure, plasmodesmatal frequency, and solute concentration in green areas of variegated *Coleus blumei* Benth. leaves. *Planta* 169: 141-152.

Fisher, D.G. (1988) Movement of lucifer yellow in leaves of *Coleus blumei* Benth. *Plant, Cell and Environment* 11: 639-644.

Fisher, D.G. (1990) Leaf structure of *Cananga odorata* (Annonaceae) in relation to the collection of photosynthate and phloem loading: morphology and anatomy. *Amer. J. Bot.* 68: 354-363.

Fisher, D.G., Evert, R.F. (1982) Studies on the leaf of *Amaranthus retroflexus* (Amaranthaceae): Morphology and anatomy. *Amer. J. Bot.* 69: 1133-1147.

Flora, L.L., Madore, M.A. (1993) Stachyose and mannitol transport in olive (*Olea europaea* L.). *Planta* 189: 484-490.

Flora, L.L., Madore, M.A. (1996) Significance of minor-vein anatomy to carbohydrate transport. *Planta* 198: 171-178.

Foster, A.S., Arnott, H.J. (1960) Morphology and dichotomous vasculature of the leaf of *Kingdonia uniflora*. *Am. J. Bot.* 47: 684-698.

Franceschi, V.R., Giaquinta, R.T. (1983a) The paraveinal mesophyll of soyabean leaves in relation to assimilate transfer and compartmentation. I Ultrastructure and histochemistry during vegetative development. *Planta* 157: 411-421.

Franceschi, V.R., Giaquinta, R.T. (1983b) Specialized cellular arrangements in legume leaves in relation to assimilate transport and compartmentation: comparison of the paraveinal mesophyll. *Planta* 159: 415-422.

Franck, D.H. (1979) Development of vein pattern in leaves of *Ostrya virginiana* (Betulaceae). *Bot. Gaz.* 140: 77-83.

Fritz, E., Evert, R.F., Heyser, W. (1983) Microautoradiographic studies of phloem loading and transport in the leaf of *Zea mays* L. *Planta* 159: 193-206.

Fritz, E., Evert, R.F., Nasse, H. (1989) Loading and transport of assimilates in different maize leaf bundles. *Planta* 178: 1-9.

Gamalei, Y.V. (1985a) Plasmodesmata: intercellular communication in plants. *Soviet Plant Physiol.* 82: 134-148.

Gamalei, Y.V. (1985b) Characteristics of phloem loading in woody and herbaceous plants. *Soviet Plant Physiology* 32: 656-665.

Gamalei, Y.V. (1989) Structure and function of leaf minor veins in trees and herbs. A taxonomic review. *Trees* 3: 96-110.

Gamalei, Y.V. (1991) Phloem loading and its development related to plant evolution from trees to herbs. *Trees* 5: 50-64.

Gamalei, Y.V. (1996) Membrane compartmentation and cell-to-cell transport of photosynthates. *J. Exp. Bot.* 47: 1297.

Gamalei, Y.V., Pakhomova, M.V. (1983a) Minor veins of dicotyledonous leaves. I. Structure and typology. *Bot. Zh.* 68: 287-301. [UNSEEN]

Gamalei, Y.V., Pakhomova, M.V. (1983b) Minor veins of dicotyledonous leaves. II. Taxonomic distribution of main types. *Bot. Zh.* 68: 428-438. [UNSEEN]

Gamalei, Y.V., Pakhomova, M.V., Syutkina, A.V. (1992) Ecological aspects of assimilate outflow. I. Temperature. *Soviet Plant Physiology* 39: 705-712.

Gamalei, Y.V., Pakhomova, M.V., Sjutkina, A.V. (1996) Regulation of assimilate translocation by plasmodesmata: effect of temperature and water stress. *In*: Workshop on Basic and Applied Research in Plasmodesmatal Biology (Israel) pp. 132-135.

Gamalei, Y.V., Pakhomova, M.V., Syutkina, A.V., Voitsekhovskaja, O.V. (2000) Compartmentation of assimilate fluxes in leaves. II Ultrastructural responses of mesophyll and companion cells to the alternation of assimilate export. *Plant Biol.* 2: 98-106.

Gamalei, Y.V., Van Bel, A.J.E., Pakhomova, M.V., Sjutkina, A.V. (1994) Effects of temperature on the conformation of the endoplasmic reticulum and on starch accumulation in leaves with the symplasmic minor-vein configuration. *Planta* 194: 443-453.

Gambles, R.L., Dengler, N.G. (1974) The leaf anatomy of hemlock, *Tsuga canadensis*. *Can. J. bot.* 52: 1049-1056.

Giaquinta, R.T. (1979) Phloem loading of sucrose. *Plant Physiol.* 63: 744-748.

Giaquinta, R.T. (1983) Phloem loading of sucrose. *Ann. Rev. Plant Physiol.* 34: 347-387.

Gledhill, E. (1969) Eastern Cape veld flowers, The Department of Nature Conservation, CT, SA

Good, R. (1952) An atlas of the Asclepiadaceae. *New Phytologist* 51: 198-209.

Goodwin, P.B. (1983) Molecular size limit for movement in the symplast of the *Elodea* leaf. *Planta* 157: 124-130.

Gunning, B.E.S., Overall, R.L. (1983) Plasmodesmata and cell-to-cell transport in plants. *Bioscience* 33: 260-265.

Gunning, B.E.S., Pate, J.S., Briarty, L.G. (1968) Specialized "transfer cells" in minor veins of leaves and their possible significance in phloem translocation. *J. Cell Biol.* 37: C7-C12.

Haberlandt, G. (1914) Physiological plant anatomy (translated by M. Drummond) Macmillan & Co., Ltd, London.

Haritatos, E., Keller, F., Turgeon, R. (1996) Raffinose oligosaccharide concentration measured in individual cell and tissue types in *Cucumis melo* L. leaves: implications for phloem loading. *Planta* 198: 614-622.

Haritatos, E., Turgeon, R. (1995) Symplasmic phloem loading by polymer trapping. *Sucrose Metabolism, Biochemistry, Physiology and Molecular Biology* 14: 216-224.

Hepler, P.K. (1982) Endoplasmic reticulum in the formation of the cell plate and plasmodesmata. *Protoplasma* 111: 121-133.

Hershkovitz, M.A. (1988) Leaf architectural studies in Centrospermae. *Amer. J. Bot.* 72: 179.

Heywood, V.H. (1978) Flowering Plants of the World. Oxford University Press, Oxford, U.K.

Hickey, L.J. (1971a) Leaf architectural classification of the angiosperms (abstr.). *Amer. J. Bot.* 58: 450.

Hickey, L.J. (1971b) Evolutionary significance of leaf architectural features in the woody dicots (abstr.). *Amer. J. Bot.* 58: 469.

Hickey, L.J. (1973) Classification of the architecture of dicotyledonous leaves. *Amer. J. Bot.* 60: 17-33.

Hickey, L.J., Taylor, D.W. (1991) The leaf architecture of *Ticodendron* and the application of foliar characters in discerning its' relationships. *Ann. Miss. Bot. Gard.* 78: 105-130.

Ho, C., Shaw, A.F. (1977) Carbon economy and translocation of ^{14}C in leaflets of the seventh leaf of tomato during leaf expansion. *Ann. Bot.* 41: 833-848.

Holthaus, U., Schmitz, K. (1991) Distribution and immunolocalization of stachyose synthase in *Cucumis melo* L. *Planta* 185: 479-486.

Hughes, J.E., Gunning, B.E.S. (1980) Glutaraldehyde-induced deposition of callose. *Can. J. Bot.* 58: 250-258.

Kempers, R., Ammerlaan, A., Van Bel, A.J.E. (1998) Symplasmic constriction and ultrastructural features of the sieve element/ companion cell complex in the transport phloem of apoplasmically and symplasmically phloem-loading species. *Plant Physiol.* 116: 271-278.

Knoblauch, M., Van Bel, A.J.E. (1998) Sieve tubes in action. *Plant Cell* 10: 35-50.

Kollmann, R., Glockmann, C. (1991) Studies on graft unions III. On the mechanism of secondary formation of plasmodesmata at the graft interface. *Protoplasma* 165: 71-85.

Komor, E. (2000) Source physiology and assimilate transport: the interaction of sucrose metabolism, starch storage and phloem export in source leaves and the effects on sugar status in phloem. *Aust. J.Plant Physiol.* 27: 497-505.

Komor, E., Orlich, G., Weig, A., Köckenberger, W. (1996) Phloem loading - not metaphysical, only complex: towards a unified model of phloem loading. *J. Exp. Bot.* 47: 1155-1164.

Kupicha, F.K. (1982) Studies on African Apocynaceae: the genus *Acokanthera*. *Kew Bulletin* 37: 41-67.

Leeuwenberg, A.J.M., Kupicha, F.K. (1985) Apocynaceae. *Flora Zambesica* 7: 395-465.

Lersten N.R. (1990) Sieve tubes in foliar vein endings: Review and quantitative survey of *Rudbeckia laciniata* (Astersceae). *Amer. J. Bot.* 77: 1132-1141.

Lersten, N.R., Carvey, K.A. (1974) Leaf anatomy of ocotilla (*Fouquieria splendens*; Fouquieriaceae), especially vein endings and associated veinlet elements. *Can. J. Bot.* 52: 2017-2021.

Levin, G. (1986a) Systematic foliar morphology of Phylanthoideae (Euphorbiaceae) I. Conspectus. *Ann. Miss. Bot. Gard.* 73: 29-85.

Levin, G. (1986b) Systematic foliar morphology of Phylanthoideae (Euphorbiaceae) II. Phenetic analysis. *Ann. Miss. Bot. Gard.* 73: 86-98.

Levin, G. (1986c) Systematic foliar morphology of Phylanthoideae (Euphorbiaceae) III. Cladistic analysis. *Syst. Bot.* 11: 515-530.

Liede, S. (1997) Subtribes of the tribe Asclepiadeae (Apocynaceae, Asclepiadaceae) - a synopsis. *Taxon* 46: 233.

Loomis, W.E. (1955) Resistance of plants to herbicides. Origin of resistance to toxic agents. Eds M.G.Sevag, R.D.Reid and O.E.Reynolds Academic Press, New York pp.99-121 [UNSEEN].

Lorence, D.H. (1985) A monograph of the Monimiaceae (Laurales) in the Malagasy region (Southwest Indian Ocean). *Ann. Missouri Bot. Gard.* 72: 1-165.

M'Batchi, B., Delrot, S. (1984) Parachloromercuribenzenesulfonic acid - A potential tool for differential labelling of the sucrose transporter. *Plant Physiol.* 75: 154-160.

M'Batchi, B., El Ayadi, R., Delrot, S., Bonnemain, J.L. (1986) Direct versus indirect effects of *p*-chloromercuribenzenesulphonic acid on sucrose uptake by plant tissues: The electrophysiological evidence. *Physiol. Plantarum* 68: 391-395.

Madore, M.A., Lucas, W.J. (1987) Control of photosynthate movement in source-leaf tissues of *Ipomoea tricolor* Cav. *Planta* 171: 197-204.

Madore, M.A., Oross, J.W., Lucas, W.J. (1986) Symplasmic transportation in *Ipomea tricolor* source leaves. *Pl. Physiol.* 82: 432-442.

Madore, M.A., Webb, J.A. (1981) Leaf free space analysis and vein loading in *Cucurbita pepo*. *Can. J. Bot.* 59: 2550-2557.

Mahlberg, P.G. (1993) Laticifers: an historical perspective. *Bot. Rev.* 59: 1-23.

Maynard, J.W., Lucas, W.J. (1982) A reanalysis of the two-component phloem loading system in *Beta vulgaris*. *Plant Physiol.* 69: 734-739.

McCauley, M.M., Evert, R.F. (1988a) Morphology and vasculature of the leaf of potato (*Solanum tuberosum*). *Am. J. Bot.* 75: 377-390.

McCauley, M.M., Evert, R.F. (1988b) The anatomy of the leaf of potato, *Solanum tuberosum* L. 'Russet Burbank'. *Bot. Gaz.* 149: 179-195.

McCauley, M.M., Evert, R.F. (1989) Minor veins of the potato (*Solanum tuberosum* L.) leaf: Ultrastructure and plasmodesmatal frequency. *Bot. Gaz.* 150: 351-368.

McLean, B.G., Hempel, F.D., Zambryski, P.C. (1997) Plant intercellular communication via plasmodesmata. *Plant Cell* 9: 1043-1054.

Meiners, S., Baron-Epel, O., Schindler, M. (1988) Intercellular communication - Filling in the gaps. *Plant Physiol.* 88: 791-793.

Melville, R. (1976) The terminology of leaf architecture. *Taxon* 25: 549-561.

Merrill, E.K. (1978) Comparison of mature leaf architecture of three types in *Sorbus* L. (Rosaceae). *Bot. Gaz.* 139: 447-453.

Metcalfe, C.R., Chalk, L. (1950) Anatomy of the dicotyledons. Clarendon Oxford.

Miller, J.S., Nowicke, J.W. (1990) Dioecy and a re-evaluation of Lepidocaria and Antrophora (Boraginaceae: Ehretioidea). *Amer. J. Bot.* 77: 543-551.

Minchin, P.E.H., Lang, A., Thorpe, M.R. (1983) Dynamics of cold induced inhibition of phloem transport. *J. of Exp. Bot.* 34: 156-162.

Mohan, J.S.S., Inamdar, J.A. (1982) Leaf architecture of Apocynaceae. *Proc. Indian Acad. Sci.* 91: 189-200.

Mohan, J.S.S., Inamdar, J.A. (1984) Leaf venation studies in some Asclepiadaceae. *Phytomorphology* 34: 36-45.

Monzer, J. (1991) Ultrastructure of secondary plasmodesmata formation in regenerating *Solanum nigrum*-protoplast cultures. *Protoplasma* 165: 86-95.

Moriarty, A. (1982) Outeniqua, Tsitsikamma and eastern Little Karoo (Botanical Society of SA) pp. 100, 148 & 152.

Morretes, B.L.D. (1962) Terminal phloem in vascular bundles of leaves of *Capsicum annuum* and *Phaseolus vulgaris*. *Amer. J. Bot.* 49: 560-566.

Mortlock, C. (1952) The structure and development of the hydathodes of *Ranunculus fluitans* Lam. *New Phytol.* 51: 129.

Munday, J. (1988) Poisonous plants in South African gardens and parks (Delta Books, SA) pp. 21 and 31.

Nelson, T., Dengler, N. (1997) Leaf vascular pattern formation *Plant Cell* 9: 1121-1135.

Olesen, P. (1979) The neck constriction in plasmodesmata: evidence for a peripheral sphincter-like structure revealed by fixation with tannic acid. *Planta* 144: 349-358.

Oparka, K.J. (1990) What is phloem unloading? *Plant Physiol.* 94: 393-396.

Oparka, K.J., Murphy, R., Derrick, P.M., Prior, D.A.M., Smith, J.A.C. (1991) Modification of the pressure-probe technique permits controlled intracellular microinjection of fluorescent probes. *J. of Cell Science* 98: 539-544.

Oparka, K.J., Van Bel, A.J.E. (1992) Pathways of phloem loading and unloading: a plea for uniform terminology. *In*: Carbon Partitioning Within and Between Organisms (Farrar, J.F., Gordon, A.J., Pollock, C.J. eds) Bios Oxford, pp. 249-254.

Overall, R.L., Wolfe, J., Gunning, B.E.S. (1982) Intercellular communication in *Azolla* roots: I. Ultrastructure of plasmodesmata. *Protoplasma* 111: 134-150.

Pate, J.S., Gunning, B.E.S. (1969) Vascular transfer cells in angiosperm leaves: A taxonomic and morphological survey. *Protoplasma* 68: 135-156.

Peterson, C.A., Currier, H.B. (1969) An investigation of bidirectional translocation in the phloem. *Physiol. Plant.* 22: 1238-1250.

Pray, T.R. (1954) Foliar venation of angiosperms. I. Mature venation of *Liriodendron. Amer. J. Bot.* 41: 663-670.

Pristupa, N.A. (1983) Distribution of ketosugars among cells of conducting bundles of the *Cucurbita pepo* leaf. *Sov. Plant Physiol.* 30: 372-378. [UNSEEN]

Radford. A.E., Dickison, W.C., Massey, J.R., Bell, C.R. (1974) Vascular plant systematics. Harper and Row Publishers, U.S.A

Ramakrishna T.M., Govindappa D.A. (1983) Studies of areoles in Asclepiadaceae and Periplocaceae. *Current Science* 52: 1145.

Ramji, M.V. (1967) Morphology and ontogeny of the foliar venation of *Calophyllum inophyllum* L. *Aust. J. Bot.* 15: 437-443.

Raven, P.H., Evert, R.F., Curtis, H. (1982) Biology of plants. (Worth Publishers, U.S.A) pp.109.

Robards A.W., Lucas, W.J. (1990) Plasmodesmata. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41: 369-419.

Robinson-Beers, K., Evert, R.F. (1991a) Ultrastructure of and plasmodesmatal frequency in mature leaves of sugarcane. *Planta* 184: 291-306.

Robinson-Beers, K., Evert, R.F. (1991b) Fine structure of plasmodesmata in mature leaves of sugarcane. *Planta* 184: 307-318.

Rost, T.L. (1969) Vascular patterns and hydathodes in leaves of *Crassula argentea* (Crassulaceae). *Bot. Gaz.* 130: 267-270.

Roth, A. Mosbrugger, V., Belz, G. Neugebauer, H.J. (1995) Hydrodynamic modelling study of angiosperm leaf venation types. *Bot. Acta* 108: 121-126.

Russin, W.A., Evert, R.F. (1984) Studies on the leaf of *Populus deltoides* (Salicaceae): Morphology and anatomy. *Amer. J. Bot.* 71: 1398-1415.

Russin, W.A., Evert, R.F., Vanderveer, P.J., Sharkey, T.D., Briggs, S.P. (1996) Modification of a specific class of plasmodesmata and loss of sucrose export ability in the *sucrose export defective1* maize mutant. *The Pl. Cell* 8: 645-658.

Schmid, R., Turner, M.D. (1977) Contrad 70, an effective softener of herbarium material for anatomical study. *Taxon* 26: 551-552.

Schmitz, K., Cuypers, B., Moll, M. (1987) Pathway of assimilate transfer between mesophyll cells and minor veins in leaves of *Cucumis melo* L. *Planta* 171: 19-29.

Schuster, W. (1908) Die Blattaderung des Dicotylenblattes und ihre Abbägigkeit von äusseren Einflüssen. *Ber. Deutsch. Bot. Ges.* 26: 194-237. [UNSEEN]

Sennblad, B., Bremer, B. (1996) The familial and subfamilial relationships of *Apocynaceae* and *Asclepiadaceae* evaluated with *rbc*L data. *Pl. Syst. Evol.* 202: 153-175.

Sharma P.C., Chandra, V., Kapoor, L.D. (1970) Epidermal and venation studies in Apocynaceae III. *Bull. Bot. Surv. India* 12: 92-96.

Shobe, W.R., Lersten, N.R. (1967) A technique for clearing and staining gymnosperm leaves. *Bot. Gaz.* 128: 150-152.

Sjölund, R.D. (1997) The phloem sieve element: a river runs through it. *Plant Cell* 9: 1137-1146.

Spicer, R.A. (1986) Pectinal veins: a new concept in terminology for the description of dicotyledonous leaf venation patterns. *Bot. J. Lin. Soc.* 93: 379-388.

Spurr, D. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruc. Res.* 26: 31.

Strasburger, E. (1891) Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. *Histologische Beitrage* 3, Gustav Fisher, Jena. [UNSEEN]

Strasburger, E. (1901) *Jahrb. Wiss. Bot.* 36: 493. [UNSEEN]

Struwe, L., Albert, V.A., Bremer, B. (1994) Cladistics and family level classification of the Gentianales. *Cladistics* 10: 175-206.

Swarupanandan, K., Mangaly, J.K., Sonny, T.K., Kishorekumar, K., Chand Basha, S. (1996) The subfamilial and tribal classification of the family Asclepiadaceae. *Bot. J. Linn. Soc.* 120: 327-369.

Takhtajan, A. (1969) Flowering plants: origin and dispersal. (Edinburgh)

Tangl, E. (1879) Ueber offene Communicationen zwischen den Zellen des Endosperms einiger *Samen Jahrb. Wiss. Bot.* 12: 170. [UNSEEN]

Terry B.R., Robards, A.W. (1987) Hydrodynamic radius alone governs the mobility of molecules through plasmodesmata. *Planta* 171: 145-157.

Thompson, W.W., Platt-Aloia, K. (1985) The ultrastructure of the plasmodesmata of the salt glands of *Tamarix* as revealed by transmission and freeze-fracture electron microscopy. *Protoplasma* 125: 13-23.

Thorne, R.F. (1992) Classification and geography of the flowering plants. *Bot. Rev.* 58: 225-348.

Todzia, C.A., Keating, R.C. (1991) Leaf architecture of the Chloranthaceae. *Ann. Miss. Bot. Gard.* 78: 476-496.

Tucker, S.C. (1964) The terminal idioblasts in magnoliaceous leaves. *Amer. J Bot.* 51: 1051-1062.

Turgeon, R. (1989) The sink-source transition in leaves. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 40: 119-138.

Turgeon, R. (1996) Plasmodesmata and the evolution of phloem loading. *In*: Third International Workshop on Basic and Applied Research in Plasmodesmatal Biology (Israel) pp. 114-116.

Turgeon R. (2000) Plasmodesmata and solute exchange in the phloem. *Aust. J. Plant Physiol.* 27: 521-529.

Turgeon, R., Beebe, D.U. (1991) The evidence for symplasmic loading. *Plant Physiol.* 96: 349-354.

Turgeon, R., Beebe, D.U., Gowan, E. (1993) The intermediary cell: Minor-vein anatomy and raffinose oligosaccharide synthesis in the Scrophulariaceae. *Planta* 191: 446-456.

Turgeon, R., Gowan, E. (1990) Phloem loading in *Coleus blumei* in the absence of carrier-mediated uptake of export sugar from the apoplast. *Plant Physiol.* 94: 1244-1249.

Turgeon, R., Hepler, P.K. (1989) Symplasmic continuity between mesophyll and companion cells in minor veins of mature *Cucurbita pepo* L. leaves. *Planta* 179: 24-31.

Turgeon, R., Webb, J.A. (1976) Leaf development and phloem transport on *Cucurbita pepo*: Maturation of minor veins. *Planta* 129: 265-269.

Turgeon, R., Webb, J.A., Evert, R.F. (1975) Ultrastructure of minor veins in *Cucurbita pepo* leaves. *Protoplasma* 83: 217-232.

Valle, E.M., Craig, S., Hatch, M.D., Heldt, H.W. (1989) Permeability and ultrastructure of bundle sheath cells isolated from C_4 plants: structure-function studies and the role of plasmodesmata. *Botanica Acta* 102: 276-282.

Van Bel, A.J.E. (1992a) Different phloem-loading machineries correlated with the climate. *Acta. Bot. Neerl.* 41: 121-141.

Van Bel, A.J.E. (1992b) Pathways and mechanisms of phloem loading. *In*: Carbon Partitioning Within and Between Organisms (Farrar, J.F., Gordon, A.J., Pollock, C.J. eds) Bios Oxford, pp. 53-70.

Van Bel, A.J.E. (1992c) Mechanisms of sugar translocation. *In*: Crop Photosynthesis: Spatial and Temporal Determinants (Baker, N.R., Thomas, H. eds) Chapter 8.

Van Bel, A.J.E. (1993a) Strategies of phloem loading. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44: 253-281.

Van Bel, A.J.E. (1993b) II. The transport phloem. Specifics of its functioning. *Progress in Botany* 54: 134-150.

Van Bel, A.J.E. (1996) Interaction between sieve element and companion cell and the consequences for photosynthate distribution. Two structural hardware frames with associated physiological software packages in dicotyledons? *Journal of Experimental Botany* 47: 1129-1140.

Van Bel, A.J.E. (1996) VIII. Carbohydrate processing in the mesophyll trajectory in symplasmic and apoplasmic phloem loading. *Progress in Botany* 57: 140-167.

Van Bel, A.J.E., Ammerlaan, A., Van Dijk, A.A. (1994) A three-step screening procedure to identify the mode of phloem loading in intact leaves. *Planta* 192: 31-39.

Van Bel, A.J.E., Gamalei, Y.V. (1991) Multiprogrammed phloem loading. *In*: Recent Advances in Phloem Transport and Assimilate Compartmentation (Bonneman, J.L., Delrot, S., Lucas, W.J., Dainty, J. eds) Quest Editions, Nantes, pp. 128-139.

Van Bel, A.J.E., Gamalei, Y.V. (1992) Ecophysiology of phloem loading in source leaves. *Plant Cell Environment* 15: 265-270.

Van Bel, A.J.E., Kempers, R. (1990) Symplasmic isolation of the sieve element-companion cell complex in the phloem of *Ricinus communis* and *Salix alba* stems. *Planta* 183: 69-76.

Van Bel, A.J.E., Kesteren, W.J.P., Papenhuijzen, C. (1988) Ultrastructural indications for coexistence of symplasmic and apoplasmic phloem loading in *Commelina benghalensis* leaves. *Planta* 176: 159-172.

Van Bel, A.J.E., Knoblauch, M. (2000) Sieve element and companion cell: the story of the comatose patient and the hyperactive nurse. *Aust. J.Plant Physiol.* 27: 477-487.

Van Der Schoot, C., Rinne, P. (1999) Networks for shoot design. *Trends in Plant Science* 4: 31-37.

Van Dongen, J.Th., Van Bel, A.J.E. (1996) Only symplasmic phloem loading suffers from low temperatures. *J. Exp. Bot.* 47: 1299.

Van Kesteren, W.P.J., Van Der Schoot, C., Van Bel, A.J.E. (1988) Symplasmic transfer of fluorescent dyes from mesophyll to sieve tube in stripped leaf tissue and partly isolated minor veins of *Commelina benghalensis*. *Plant Physiol.* 88: 667-670.

Van Wyk, B., Malan, S. (1988) Field guide to the wild flowers of the Witwatersrand and Pretoria region (Struik Publishers, SA).

Voitsekhovskaja O.V., Pakhomova, M.V., Syutkina, A.V., Gamalei, Y.V., Heber, U. (2000) Compartmentation of assimilate fluxes in leaves: II Apoplastic sugar levels in leaves of plants with different companion cell types. *Plant Biol.* 2: 107-112.

Volk, G.M., Turgeon, R., Beebe, D.U. (1996) Secondary plasmodesmata formation in the minor-vein phloem of *Cucumis melo* L. and *Cucurbita pepo* L. *Planta* 199: 425-432.

Wark, M.C. (1965) Fine structure of the phloem of *Pisum sativum*. II. The companion cell and phloem parenchyma. *Aust. J. Bot.* 13: 185-193.

Warmbrodt, R.D., Van Der Woude, W.J. (1990) Leaf of *Spinacia oleracea* (spinach): ultrastructure and plasmodesmatal distribution and frequency, in relation to sieve-tube loading. *Amer. J. Bot.* 77: 1361-1377.

Warmbrodt, R.D., Bubkhout, T.J., Hitz, W.D. (1989) Localization of a protein, immunologically similar to a sucrose-binding protein from developing soybean cotyledons, on the plasma membrane of sieve-tube members of spinach leaves. *Planta* 180: 105-115.

Weisberg, L.A., Wimmers, L.E., Turgeon, R. (1988) Photosynthate - transport characteristics of nonchlorophyllous and green tissue in variegated leaves of *Coleus blumei* Benth. *Planta* 175: 1-8.

White, R.G., Badelt, K., Overall, R.L., Vesk, M. (1994) Actin associated with plasmodesmata. *Protoplasma* 180: 169-184.

Wilkinson, H.P. (1989) Leaf anatomy of the Menispermaceae tribe Tiliacoreae Miers. *Bot.J.Linn.Soc.* 99: 125-174.

Wimmers, L.E., Turgeon, R. (1991) Transfer cells and solute uptake in minor veins of *Pisum sativum* leaves. *Planta* 186: 2-12

Wolf, S., Lucas, W.J. (1994) Virus movement proteins and other molecular probes of plasmodesmatal function. *Plant, Cell and Environment* 17: 573-585.

Wylie, R.B. (1939) Relations between tissue organisation and vein distribution in dicotyledon leaves. *Amer. J. Bot.* 26: 219-225.

Wylie, R.B. (1946) Relations between tissue organization and vascularization in leaves of certain tropical and subtropical dicotyledons. *Amer. J. Bot.* 33: 721-726.

Zamski, E., Tsivion, Y. (1977) Translocation in plants possessing supernumerary phloem. I ^{14}C-assimilates and auxin in the internal phloem of tobacco (*Nicotiana tabacum* L.). *J. Exp. Bot.* 28: 117-126.

Zimmerman, M.H., Ziegler, H. (1975) List of sugars and sugar alcohols in sieve-tube exudates. *In*: Phloem transport encyclopedia of Plant Physiology (Zimmerman, M.H., Milburn, J.A. eds) Springer Verlag, Heidelberg, 1: 480-503.