STUDIES ON

THE VASCULAR CAMBIUM

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ABSTRACT

BUTTERFIELD, B.G. 1974. Studies on the Vascular Cambium. Ph.D. thesis. University of Canterbury, Christchurch, New Zealand. The nature of the vascular cambium is discussed and the length of the elements in the secondary xylem related to the division and elongation cycles of the cambial fusiform initials. Particular attention is given to the activities of the storeyed cambium. The ontogeny of the storeyed cambium is described for *Hoheria angustifolia* Raoul. (Malvaceae). The transition from procambium to cambium was found to take place gradually, the meristem acquiring cambial characteristics over a number of internodes, some before and some after internodal elongation had ceased. The cambium is non storeyed at the commencement of secondary growth but later develops a storeyed pattern. Developmental changes in the cambium with radial growth were studied in *Aeschynomene hispida* Willd. (Papilionaceae). Repeated radial longitudinal divisions in the fusiform cambial initials in this plant produce a highly developed storeyed pattern with radial growth. The frequency of these divisions decreases with increasing distance from the stem centre. The mean length of the fusiform initials decreases slightly with radial growth. Variation in the size of the fusiform initials and vessel members was also investigated in *Hoheria angustifolia*. Mean length of the fusiform initials was found to remain constant with increasing distance from the stem centre but a slight decrease was observed with increasing height in the tree. Mean fusiform initial width showed an increase followed by a decrease with increasing height in the tree. The significance of these results is related to the division pattern in the storeyed cambium.

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AUTHORITIES FOR PLANT NAMES

Throughout this thesis, the authority follows the binomial on the first reference to each species.

METRIC UNITS

The metric system of units is used throughout this thesis except for the tree heights given in Section 4. Wood samples for that section were collected in 1965 and the original Imperial measurements have been retained here.

SECTION ONE

THE VASCULAR CAMBIUM AND CELL SIZE IN THE SECONDARY XYLEM: AN INTRIDUCTION AND REVIEW OF LITERATURE

1.1 SUMMARY

The nature of the vascular cambium is discussed. The cambium is defined as a multiseriate cylinder of periclinally dividing cells between the differentiating xylem and phloem. The existance of a single initial in each radial file of cells is recognised and defined as the site of anticlinal division in the cambium.

The relationship in length of the fusiform cambial initials to the xylem elements is also discussed and the literature relating to cell length in the secondary xylem reviewed. Two basic patterns of cell length variation are found: (i) that occurring from the stem centre outwards along a radius and (ii) that occurring up the tree. Variation patterns within the growth ring, within the same species growing on different sites, and between species are also discussed.

Cell length changes in the secondary xylem are attributed to developmental changes occurring in the vascular cambium

with radial growth, the frequency and timing of anticlinal cell division, cell loss and cell elongation in the cambium all contributing to the resultant tracheid and vessel element lengths.

The scope of this thesis is outlined.

1.2 INTRODUCTION

The vascular cambium as a meristem is the source of the greater bulk of plant material, and the tissues produced from it are some of the most complex found in plants. It is from this meristem that wood, one of the world's greatest natural products, is derived. For over a century, various aspects of the action, development and control of the cambium have been studied by a great number of workers. Many of the properties of the meristem remain unknown, however, and there is still room for research within this field. Its complexity and variety of development has induced Lorch (1967) to term it the "elusive cambium".

A number of conflicting views are held on the nature of the cambium. The cambial zone normally consists of an unbroken cylinder of undifferentiated meristematic cells arranged in radial files. These files extend into the mature secondary xylem and secondary phloem where they may become obscured by changes that occur with differentiation. Dividing cells close to the mature phloem form the phloem mother cells, while those adjacent to the xylem are the xylem mother cells. The transitions in cell type and activity through the cambial zone are gradual, especially in the active cambia of dicotyledons, and in practice it is difficult to define the radial extent of the true cambium. Catesson (1964) made a careful attempt to define the limits of the cambium. She concluded that the term was best reserved for a truly meristematic region where mitoses are abundant and where the cells are narrow with thin walls. Such a cambium is separated from mature xylem and phloem tissue by bands of cells in which size changes and other differentiation processes are proceeding.

The concept of a multiseriate cambium may or may not include reference to a single initial within each radial file of cells. One school of thought postulates a multiseriate zone in which all the cells are equivalent. This interpretation proposed by Raatz (1892), has been given support by Catesson (1964). From a meticulous study of active cambium of *Acer pseudoplatinus* L., she found it impossible to distinguish a layer in which divisions were more or less frequent than in adjacent cells. Nor could an initial be distinguished by any other criterion such as histochemical, anatomical or cytological differences. She therefore concluded that the active cambium was formed of several layers of similar cells, each endowed with equal powers of multiplication.

The most widely held interpretation of the cambium, however, postulates the existence of a single initial cell in each radial file of cambial cells from which all the derivatives of the cambium are ultimately derived. Such an initial lies somewhere between the xylem and phloem mother cells. Evidence in favour of this concept has been provided by Bannan (1955, 1968) and Newman (1956), both of whom were able to distinguish single initials between the xylem and phloem in gymnosperms. The continuity of the radial files of cells on either side of the cambium is the most convincing argument in favour of the single initial theory. In conifers, virtually all the anticlinal divisions within the cambium result in doubled files of cells in the tissues to both sides of the meristem. Other features also correspond in the xylem and phloem, including the loss of radial files of axial cells and the replacement of such files by rays. If the initiating layer were more than one cell wide, an exact duplication of all anticlinal divisions, loss or conversion to ray initials would be required in the several initials of the radial file, in order to produce corresponding patterns in the xylem and phloem. Further evidence in support of the existence of a single initial in each radial file of cells in the cambium has been provided by Murmanis (1970). Working on Pinus strobus L., this author detected differences in the thickness of the tangential walls thereby enabling him to group the cells according to the sequence of the previous divisions. This enabled him to deduce the position of the initial. The location of the initial in most species remains, however, difficult if not impossible to determine in light microscope sections.

The small amount of published information on anticlinal divisions in the cambia of dicotyledons shows that they could be restricted to a single layer (Cumbie, 1967a) or be distributed over several layers (Catesson, 1964). This is perhaps not surprising in view of the complexity and variety of activity of the cambia of these plants. The single initial concept, however, is still an attractive one for the same reasons as have been applied to the cambia of conifers. Even where a wide multiseriate zone of periclinally dividing cells occurs, there could still be only one initial in each radial file. Such initials need not be tangentially aligned, that is, the initials may not form a cylinder within the cambium, but may be radially offset according to their immediate state of mitotic activity. While periclinal divisions may continue in the xylem and phloem mother cells, anticlinal (sometimes termed multiplicative) divisions are virtually restricted to the initials (Bannan, 1955).

Despite the difficulty in isolating the actual initials in an actively dividing cambial zone, there is a significant group of anatomists who restrict the use of the word "cambium" to the initials only. By their definition the cambium is a functionally uniseriate layer between actively dividing xylem and phloem. This interpretation is commonly used in the American literature (Wilson et al, 1966; Zimmerman and Brown, 1971).

The present author leans to the interpretation of the cambium as a multiseriate zone of cells lying between the differentiating secondary xylem and phloem but recognizes the existence of a distinct initial lying somewhere within each radial file of cells. This is the definition of the word "cambium" that will be used throughout this thesis. A diagrammatic presentation of the terminology relating to the cambium used here is presented in Fig. 1-1.

Most of our present knowledge on the developmental and size changes that occur in the vascular cambium with radial growth, has been obtained from investigations made on the secondary xylem and phloem and not from direct observations of the cambium itself. For a time there was a tendency to use the phloem derivatives for studying the changes that had occurred in the cambium, the most notable piece of research being that undertaken by Klinken (1914) who, using the phloem derivatives of *Taxus baccata* L., first developed the technique of examining serial tangential sections for studying developmental changes in the cambium. One reason for using the phloem in preference to the xylem was that the phloem elements were thought to change less in size during maturation and hence the tangential sections were expected to give a more accurate picture of the probable lengths of the cambial initials at the time that the phloem cells were formed. Esau and Cheadle (1955), however, have shown that divisions varying in plane from vertical to almost transverse,

SECONDARY PHLOEM		Mature tissue
DIFFERENTIATING PHLOEM		Secondary walls deposited (a) Cells enlarging Limited cell division (b, c)
CAMBIUM	PHLOEM MOTHER CELLS	Periclinal division
	CAMBIAL INITIAL	Periclinal and anticlinal division
	XYLEM MOTHER CELLS	Periclinal division
DIFFERENTIATING XYLEM		Limited cell division (c) Cells enlarging Secondary walls deposited
SECONDARY XYLEM		Mature tissue

Fig.1-1. Terminology used in this thesis for describing the tissues associated with the vascular cambium.

Notes:
(a) Fibres and sclereids only.
(b) Some transverse and longitudinal divisions associated with sieve tube and companion cell development.
(c) Some transverse divisions associated with the development of axial parenchyma strands.

are common within the phloem derivatives of the cambium. Thus although sieve elements may not elongate much during differentiation, they may not bear any relation to the length of the fusiform initials from which they are derived. Furthermore, if the sequence of divisions and size changes is to be traced back over any prolonged period of time, the secondary xylem must be used in preference to the phloem, since distortion and abscission affect the phloem. Since divisions are frequent to the xylem side of the cambium, this tissue gives a more complete picture of the developmental changes that have occurred in the cambium. Bannan (1950) has noted that some anticlinal divisions of the cambium, especially those followed by the loss of certain cells, are not always recorded in the phloem.

Although a record of the principal size changes is preserved in the secondary xylem, this tissue does not give a completely accurate picture of the activities of the meristem. Cell length data, for example, is erroneous to the extent of the elongation of the mature elements. The extent of this elongation and any resulting cell rearrangement varies greatly between cell types and also between cells of the one type occurring in different parts of the growth rings and internodes of any one tree. Since conifer tracheids elongate relatively little during differentiation (Kozlowski, 1971), variations in their lengths are often taken as an approximate indication of the size fluctuations that have occurred in the cambium.

Dicotyledonous vessel members also change very little in length during differentiation. On the other hand, variations in length of dicotyledon fibres are due in part to the intrusive growth that has occurred during differentiation. Studies on plants with storeyed cambia, where the fusiform initials are assumed to remain approximately the same length throughout the growing season, have shown that fibres elongate by varying amounts depending on their position within the growth ring (Chalk *et al*, 1955; Hejnowicz and Hejnowicz, 1959). Factors such as temperature influencing cell wall plasticity may produce changes in the degree of fibre elongation with season. Hence the patterns of size change in the fibres of dicotyledons cannot be regarded as indicating any size changes that have occurred in the cambium with radial growth. While vessel members are generally believed to represent the length of the fusiform initials of the cambium from which they were derived, the method used to measure them would appear to be important. Chalk and Chattaway (1934) concluded that the total member length as measured from tip to tip of the vessel member corresponded more closely in length with the fusiform initial than did the mean body length as measured from the centre of the pores and ignoring any tail or extension.

Section four of this thesis examines the relationship between fusiform cambial initial length and the length of the vessel members in *Hoheria angustifolia* Raoul. It also records data illustrating minor fluctuations in the length of vessel members within the growth ring and relates these to the width of the cell and the angle of the end walls.

1.3 CELL LENGTH VARIATIONS WITHIN THE SECONDARY XYLEM

The general pattern of variation in tracheid length within the secondary xylem of conifers was established by Sanio (1872) for *Pinus sylvestris* L., and has since been observed to occur for other cell types in many other conifers and dicotyledons. The literature dealing with the variation in size of the xylem elements has been reviewed by Spurr and Hyvarinen (1954) and Dinwoodie (1961). Two major patterns of variation occur which may be thought of as (i) horizontal, or occurring across and within the growth rings of the stem from the primary xylem outwards, and (ii) vertical, or the pattern occurring up the tree either within a single growth ring or at a specific radial distance from the stem centre. The literature relating to these two aspects of variation will be considered separately along with the other factors that superimpose minor fluctuations on the basic pattern.

1.3.1 *Variation in cell length at any one level in the tree.*

From his observations on *Pinus sylvestris*, Sanio (1872) concluded that tracheid length at any one level in the tree increased outwards from the pith through a number of annual rings until a certain size was reached and then remained constant through the following rings. While there has since been general agreement that there is an initial increase in cell length over the early part of radial growth until a maximum is reached in many woody plants, investigations beyond this point are somewhat contradictory. While some investigators have recorded results similar to those of Sanio, others have recorded a decrease, an increase, or even considerable fluctuations in cell length after the initial increase.

The tracheid length findings of Sanio for Scot's pine have also been recorded in a number of other studies including those on *Pseudotsuga menziesii* Franco by Mell (1910), *Sequoia sempervirens* (Lamb.) Enal. by Bailey and Faull (1934), *Pinus taeda* L. by Bethel (1941), *Picea abies* Karst. by Nilsson (1943), *Pinus densiflora* Sieb. et Zucc. by Hata (1949), *Pinus radiata* D.Don. by Dadswell (1958) and Chalk and Ortiz (1961), *Pinus sylvestris* by Schultze-Dewitz (1965) and *Pinus kesiya* Royle by Burley (1969). The point at which maximum tracheid length is reached appears to vary both with the species and with the rate of growth, there being considerable variation among the reports listed above.

Not all investigators, however, have agreed with Sanio's concept that a constant cell length is attained after an initial increase. A gradual but progressive increase in tracheid length with radial growth after the initial maximum has been reached, has been recorded for *Pseudotsuga menziesii* by Gerry (1916), for *Pinus caribaea* Morelet and P. by Kramer (1957) for *Pinus taeda* by Jackson and Green (1958) and Kromhout (1963), for *Pinus patula* L. and *Pinus elliotii* Engelm. by Kromhout (1963), for *Larix sibirica* Ledeb. by Bajdalina and Malyseva (1967), for *Thuja plicata* by Wellwood and Jurazs (1969), for *Picea smithiana* Boiss. by Ahmad (1969), for *Abies pindrow* Spach. by Ahmad (1970), and for *Pinus densiflora* by Sudo (1968). In some cases it is doubtful whether the stems examined were of sufficient age for the maximum tracheid length to have been reached.

Other studies have revealed considerable fluctuation in the tracheid length after the initial maximum has been reached. These include studies on *Pinus strobus* L. and *Abies concolor* Lindl. by Shepard and Bailey (1914) and Bailey and Shepard (1915), on *Pinus contorta* Dougl. by Keinholz (1931), on *Larix* by Laing (1948) and on *Picea sitchensis* Carr. by Dinwoodie (1963).

There have been fewer investigations on the pattern of cell length across the stem in dicotyledons. Although Hartig (1885) reported a decrease in fibre length towards the cambium in *Fagus sylvatica* L., the majority of investigators have found that dicotyledon fibres follow essentially the same changes in length with radial growth as do conifer tracheids. Bisset and Dadswell (1949) found that fibre length increased for about the first ten years in *Eucalyptus regnans* F.Muell. and then remained constant. Desch (1932), however, recorded an increase in cell length in *Fagus sylvatica* even after 129 years and on poplar and alder after 100 years although the most rapid increase occurred during the first 5 to 10 years. Rao (1959) found that the most rapid increase occurred in the first 15 rings in *Fagus sylvatica* and in the first 25 rings in *Quercus robur* Liebl. and *Acer pseudoplatanus*, after which only a slight increase in length occurred. Essentially similar results have been recorded for *Tecton~~i~~a grandis* L. by Parameswaran (1964) who found that both septate and non-septate fibres increased in length from the pith to the bark, for *Eucalyptus gomphocephala* A.D.C. by Stern-Cohen and Fahn (1964) and for *Populus x robusta* by Babas (1970).

The fibres in plants with storeyed cambia appear to follow the same general trend of an increase in length with distance from the stem centre followed by a general levelling out of the graph. This type of pattern has been recorded by Chalk *et al.* (1955) for *Pterocarpus angolensis* D.C. and Hejnowicz and Hejnowicz (1959) and Rao (1962) for *Robinia pseudoacacia* L.

Angiosperm vessel members have received less attention than have tracheids and fibres in these attempts to establish the pattern of cell size changes across the stem. Pritchard and Bailey (1916) found no difference in vessel member length in *Carya ovata* K.Koch. with radial distance from the pith. A similar result has been recorded for late wood vessel members in *Fraxinus excelsior* L. by Bosshard (1951). Hejnowicz and Hejnowicz (1958) found that vessel member length doubled between the first and last growth rings in a 31 year old stem of *Populus tremula* L. Ranatunga (1964) also found that vessel member length increased outwards from the pith in *Eucalyptus grandis* Hill, reaching a maximum at about one quarter of the radial distance out in a 9 year old stem. Fabri-Tarchi (1963), however, recorded a decrease in vessel member length with distance from the stem centre in *Prunus persica* Batsch.

The only studies on vessel member length in storeyed woods are those by Chalk *et al*. (1955), who found no change in cell length with distance from the pith in *Pterocarpus angolensis*, *Nesogordonia papavifera* Capuron, and *Aeschynomene elaphroxylon* Taub., Rao (1962) who recorded a similar result for *Robinia pseudoacacia*, and Hejnowicz and Hejnowicz (1959) who recorded a slight increase in vessel length with radial growth also in *Robinia pseudoacacia*.

In both gymnosperms and angiosperms there has been disagreement as to whether or not these size changes are related to growth as measured by growth ring numbers or to linear distance from the stem centre. Anderson (1951) established a relationship between tracheid length and distance from the pith in *Abies concolor*, *Abies procera* Rehder and *Pseudotsuga menziesii*. This relationship could be expressed mathematically for each tree without regard to the number or size of the growth rings. Kuziel (1953) found a similar relationship for fibre length in four species of *Populus* as did Govindarajalu and Swamy (1955) for *Pithecelobium dulce*. Hejnowicz and Hejnowicz (1958) concluded that fibre and vessel member length in *Populus tremula* were related to the total number of cambial generations as expressed by linear distance from the stem centre. This relationship was found to hold for all levels in the tree except the lower 2 m. Stevens (1959) for Grenadier apple trees, Loach (1959) for *Pinus caribaea*, and Saucier and Hamilton (1967) working on *Fraxinus pennsylvanica* Marsh., have also concluded that cell length is related to linear distance rather than to growth ring number. While Hartly (1960) could not find any correlation between tracheid length and distance from the pith in *Pinus radiata*, other workers such as Valentine (1963), who determined fibre length changes in *Populus tremuloides* Michx., have concluded that cell length is more closely related to annual ring number than to distance of the sample from the pith. Similar results have been presented by Brister (1960) for tracheid length in *Pinus patula* and *Pinus radiata*. Buijtenen (1960) believed that fibre length in *Populus tremula* increased with the logarithm of age from the pith.

Still other workers have attempted to show that both age and distance from the pith are important in influencing cell length. Elliott (1960) working with *Picea sitchensis* concluded from a study of correlation coefficients that the effect of age is significant only in the early years, this influence being superseded by ring width later in radial growth. Dinwoodie (1963) also working on *Picea sitchensis* found that the effect of age was most important in the first 15 rings and distance from the pith to be the most important factor from about the 50th ring outwards.

A different type of relationship between cell length and radial growth has been reported by Rumball (1963). This was found in *Elaeocarpus hookerianus* Raoul and *Podocarpus dacrydioides* A.Rich., two trees which pass from juvenile to adult stages marked by a striking change in habit. The rate of increase in cell length outwards along any one radius was found to be rapid during the juvenile phase but when the tree entered its adult phase, the rate of increase declined after a sharp break in the graph corresponding with the time of the habit heteroblastic change from a divaricate shrub to an adult tree. The same effect has been reported by Philipson (1964).

1.3.2 Within ring variation in cell length.

The second basic variation in cell length in the secondary xylem at any one level in the tree, is that found within the individual growth rings. As a general trend, the early wood elements are shorter than those produced later in the same annual ring. This pattern has been recorded for conifer tracheids in *Pseudotsuga menziesii* by Lee and Smith (1916), in *Picea* *sitchensis* by Chalk (1930), in *Abies concolor* and *Abies procera* by Anderson (1951), in *Pinus taeda* by Kramer (1957), in *Picea abies* Karst. by Schultze-Dewitz (1959), in *Pinus radiata* by Chalk and Ortiz (1961), in *Pinus taeda*, *Pinus elliottii* and *Pinus echinata* by Jackson and Morse (1965), and in *Picea sitchensis* by Dinwoodie(1963). It has also been recorded for dicotyledon fibres in *Eucalyptus regnans* by Bissett and Dadswell (1949), in *Populus x robusta* by Liese and Ammer (1958), in *Populus x canadensis* by Scaramuzzi (1959), in *Robinia pseudoacacia* by Hejnowicz and Hejnowicz (1959) and Rao (1962), in *Acer pseudoplatanus* and *Quercus robur* by Rao (1962), in *Tectonia grandis* by Parameswaran (1964), and *Eucalyptus gomphocephala* by Stern-Cohen and Fahn (1964), and also in angiosperm vessel members of *Fraxinus nigra* Marsh. by Chalk (1970) and in a number of other species by Bissett and Dadswell (1950) and Swamy *et al*.(1960).

While some of these authors have taken a few samples from each growth ring of the species under examination, others have sampled more extensively. Where many samples have been taken from each growth ring, the length of the xylem elements has been found to increase from the first formed wood to the last formed latewood with a sharp decrease in cell length near the ring boundary. As might be expected, the percentage difference in length between the earlywood and latewood elements is greater for dicotyledonous fibres than for gymnospermous tracheids (Bisset and Dadswell, 1950). In species lacking distinct growth rings, the length of the xylem elements may change only slightly over radial distances representing several years growth (Kozlowski, 1971).

A number of investigators have examined the length of the tracheids in gymnosperm compression wood and have usually recorded a decrease in the length of the tracheids in such a tissue (Dadswell and Wardrop, 1949; Bisset and Dadswell, 1950).

When a false ring forms, the premature development of latewood often is correlated with an increase in tracheid length (Kozlowski, 1971).

1.3.3 Variation in cell length with height in the tree.

The length of the axial elements of the xylem also vary up the axis of the tree. Within a growth ring, cell length usually increases for some distance up the stem and then decreases progressively towards the top. The xylem elements at the top of a growth ring are usually shorter than those at the base. The point of maximum cell length occurs at progressively higher levels in each successive growth ring. Again this variation pattern has been noted for gymnosperm tracheids in *Pinus palustris* Mill., *Pinus strobus* and *Abies concolor* by Bailey and Shepard (1915), in *Pinus taeda* by Bethel (1941), in *Pinus kesiya* by Burley (1969), in *Picea sitchensis* by Chalk (1930), Elliott (1960) and Dinwoodie

(1963), in *Picea abies* by Schultze-Dewitz (1959), in *Picea smithiana* Boiss. by Ahmad (1969), in *Abies pindrow* by Ahmad (1970); for angiosperm fibres in *Eucalyptus regnans* by Bisset and Dadswell (1949), in *Eucalyptus grandis* by Ranatunga (1964), in *Eucalyptus gomphocephala* by Stern-Cohen and Fahn (1964); and for both fibres and vessel members in *Populus tremula* by Hejnowicz and Hejnowicz (1958), in *Populus x eumericana* by Scaramuzzi (1958) and *Acer pseudeplatanus*, *Quercus robur* and *Robinia pseudoacacia* by Rao (1962).

1.3.4 Other variation patterns.

While most investigations on the variation in length of the xylem elements have concentrated on the horizontal and vertical patterns, there are nevertheless published reports of other size changes within the secondary xylem. Bailey and Tupper (1918) noted that tracheids tended to be shorter at nodes, the junction of the stems and roots or branches and other regions where growth adjustments occur. David *et al.* (1959) noted that tracheids in *Pinus pinaster* Ait. tended to be shorter at the sites of injuries. Liese and Dadswell (1959) examining the influence of solar radiation on tracheid and fibre length in 9 species of gymnosperms and angiosperms, found in all the specimens examined that the cells were shorter on the sun side of the tree.

Differences in cell length also occur between different species and within the same species growing in different provenances. Echols (1958) recorded a significant relationship between length and latitude in *Pinus sylvestris* when plants from different latitudes were grown together. A similar relationship was observed by Dinwoodie (1963) for *Picea sitchensis*. However, the variation in cell length between provenances is small (5-30%) compared with differences in cell length which occur within any one tree (e.g. a 200-500% increase in cell length outwards from the stem centre). Erak (1971) in presenting data on vessel element length in *Fagus* found that the cells were shorter in trees growing at a lower altitude than those growing higher up.

Bannan (1965) found considerable interspecific variation in the earlywood tracheid length of gymnosperm stems of similar size and growth rate. A high correlation was found to exist between potential growth capacity and the size of the cambial derivatives, with gymnosperm trees normally achieving a large size having the longest tracheids. The shortest tracheids were found to occur in low growth gymnosperms growing on poor sites. This correlation between the length of the cambial derivatives and the growth capacity of the species obviously does not hold true for angiosperms where some very tall trees with advanced features have storeyed cambia producing considerably shorter cambial derivatives than many primitive plants of smaller stature.

1.4 THE VASCULAR CAMBIUM AND RADIAL GROWTH.

Most of the variation patterns in the length of the cells in the secondary xylem can be directly attributed to the developmental changes occurring in the vascular cambium with radial growth. Most of the information at present available on cambial growth comes from the detailed analyses of Bailey (1920, 1923), Priestley(1930), Bannan (1950 to 1968) and Cumbie (1963, 1967a, 1969a, 1969b). Bailey (1920, 1923) in an intensive study of the cambia of a large number of gymnosperms and angiosperms, concluded that the increase in the circumference of the cambium with radial growth resulted from pseudotransverse division of the fusiform initials followed by intrusive growth of the new daughter initials. In some of the more highly specialized angiosperms, he observed that the anticlinal division was radial longitudinal, resulting in the grouping of the fusiform initials in neat horizontal rows (storeyed cambia). Priestley (1930) confirmed the account of the anticlinal division sequence proposed by Bailey, and suggested that if rapid radial growth accompanied pseudotransverse division in the cambium, the time lag before these new cells reached mature length could lead to a shorter mean fusiform initial length. The relationship between radial growth and anticlinal division in the cambium, however, is considerably more complex than that proposed by either Bailey or Priestley

(Philipson *et al.*, 1971).

During pseudotransverse division the dividing wall is usually laid down near the centre of the cell, though more rarely it can be nearer one end resulting in daughter cells of unequal size. The length and pitch of the wall are variable, ranging from short and transverse to very oblique and up to half the length of the dividing cell in gymnosperms (Bannan, 1957a) and considerably more in angiosperms (Evert, 1961; Cumbie, 1967a, 1969a). In conifers the length of the wall is related to the length of the dividing cell, long cells tending to have long dividing walls (Bannan, 1964, 1965). In *Leitneria floridana* Chapm. the wall is shorter in older stems than in younger ones (Cumbie, 1967a). The longer the partition wall, the less effect pseudotransverse division has on the mean length of a population of fusiform cambial initials. In the more advanced dicotyledons where the anticlinal division produces a long wall extending from tip to tip of the parent cell, no reduction in cell length results. Repeated radial longitudinal division, where no elongation of the daughter fusiform initials follows, leads to the production of the storeyed or stratified cambium.

In the herbaceous dicotyledon, *Hibiscus lasiocarpus* (T. and G.) Gray, an intermediate state exists where both pseudotransverse and radial longitudinal divisions occur with almost equal frequency (Cumbie, 1963). Following radial longitudinal division, there is little or no elongation of the fusiform initials while elongation following pseudotransverse division is variable.

A surprising feature in the production cycle of new fusiform initials in the cambium is the high rate of loss of new initials. This occurs by a number of different processes as a direct result of over production and follows pseudotransverse division in gymnosperms (Bannan, 1950, 1951, 1953; Scrivastava, 1963a, 1963b; Hejnowicz, 1961; Hejnowicz and Branski, 1966) and in the dicotyledons that have non-storyed cambia (Cumbie, 1963, 1969a; Evert, 1961). The amount of loss of fusiform initials from the cambial cylinder depends on the frequency of anticlinal division, the rate and amount of elongation of the daughter initials and the rate of increase in circumference of the cambium. If the number of initials produced by anticlinal division exceeds that required to meet the expanding cambial circumference, then the excess are lost by transformation into ray initials, progressive decline with periclinal division or by maturing into xylem and phloem elements. Reduction in height of a declining initial is brought about by asymmetric periclinal division in which the new tamgential wall divides the fusiform cell unequally leaving the smaller daughter cell as the functional initial. Repeated asymmetric division leads to a progressive shortening of the initial until its eventual loss. In general, there is a tendency for the longer cells to survive and the shorter to decline (Bannan, 1951, 1957b; Cumbie, 1963, 1969a). Continued selection of the longest initials helps to maintain an adequate or increasing cell length in the cambium and its derivatives.

Although longer cells usually survive and shorter ones decline, the extent of the ray contact may influence survival chances in cells of intermediate length. Bannan and Bayly (1956), Evert (1961), Cheadle and Esau (1964) and Cumbie (1967a) have all shown that daughter cells with higher contact with ray cells have a better chance of survival than those with fewer ray contacts.

There would appear to be an evolutionary trend from the gymnosperms and structurally primitive dicotyledons with non-storeyed cambia and long fusiform initials that undergo pseudotransverse division with subsequent cell elongation and loss, to the structually more advanced dicotyledons with storeyed cambia where short fusiform initials undergo radial longitudinal division with little cell elongation and possibly no loss. Cumbie (1967a) has drawn attention to the progressive series from *Liriodendron tulipifera* L. (Cheadle and Esau, 1964), *Pyrus communis* L. (Evert, 1961) to *Leitneria floridana* and *Hibiscus lasiocarpus*. Although all four species have non-storeyed cambia, fusiform initial length, cell elongation and the proportion of new initials lost from the cambium decrease along the series. Unfortunately there appears to be no recent account of these processes in a plant with a storeyed cambium. Section 3 of this thesis, therefore, attempts to analyse the developmental changes occurring with radial growth in the vascular cambium of *Aeschynomene hispida* Willd., a plant with a storeyed cambium.

Detailed studies of the elongation of fusiform initials following pseudotransverse division have been made by Bannan and Whalley (1950) and Bannan (1956). Such elongation brings about an increase in the number of initials in any given transverse section and therefore increases the circumference of the cambium. Variability resulting from differences in the local environment of the cells often obscures a general trend of early rapid elongation followed by a decline in growth rate as the cell elongates. Loss of neighbouring initials and obstruction by rays both considerably influence the elongation process in individual cells.

The rate and amount of elongation of new fusiform initials is naturally an important factor governing the size of the fusiform cells at any particular time. For example, the rate of fusiform initial elongation following division varies widely with season. Bannan (1951) observed that the rate of elongation of new initials increased during the growing season, reaching a maximum late in the year's growth.

The interplay of these various factors: the frequency and timing of anticlinal cell division, the amount and rate of elongation of the new daughter cells and the preferential loss of the shorter initials influence the mean cell size of the fusiform initials and their derivatives at any given distance from the stem centre. Although detailed investigations on the timing of anticlinal division in the fusiform initials have been made on relatively few species, it would seem in plants with non-storeyed cambia, that this division reaches a peak toward the end of the growing season. This factor, associated with the increasing rate of elongation of new initials during the growing season, result in the recorded increase in cell length within the growth ring now well established for xylem elements.

Pseudotransverse division in the fusiform cambial initials does not appear to be related to the increase in the cambial circumference. Bannan (1960b) recorded a high rate in old stems where circumferential expansion was minimal. On the other hand, most studies indicate an increase in the loss of new fusiform initials with radial growth. During early growth, therefore, as pseudotransverse divisions proceed a relatively high proportion of the new cambial initials survive and continue to increase in length. As the tree ages and the rate of circumferential expansion slows down, fewer of the new cells survive and there is a less pronounced increase in cell length.

Where the anticlinal division is radial longitudinal producing the storeyed type of cambium it is assumed that no cell elongation occurs or the stratification of the initials would be lost. Anticlinal division, less any cell loss (if such occurs) must, therefore, alone supply the needs of the expanding cambial circumference.

The role of cambial behaviour in the relationship of cell length to ring width has been explored by Bannan in a series of papers. A study on white cedar stems (Bannan, 1959) revealed a definite tendency for greater cell length to be associated with decreased ring width. The lack of a relationship between pseudotransverse division and ring width, however, led him to conclude that cell length was due not so much to the frequency of anticlinal division as to inherent determiners, the latter being influenced by growth rates. Studies on *Thuja* (Bannan, 1960b), *Pinus* (Bannan, 1962), *Picea* (Bannan, 1963a) and *Cupressus* (Bannan 1963b) have all illustrated that cell length reaches a maximum in rings approaching 1 mm width in these genera. In very narrow rings, a sharp increase in the frequency ofpseudotransverse division tends to depress cell length.

Bannan (1965) also found that the yearly amount of cambial cell elongation dropped as ring width decreased but not in proportion to the decline in ring width. Thus, the cumulative elongation through a lineal series of cells per centimetre of xylem increment increases as ring width decreases, resulting in the amount of elongation occurring during the production of several narrow rings greatly exceeding that through a single ring of the same width. The higher rate of pseudotransverse division at the end of the growth ring naturally lowers the mean length of the cambial cells at this stage. In an earlier paper (Bannan, 1954) reported that pseudotransverse divisions sometimes occur earlier in the elongation cycle in more vigorously growing trees than in slower growing ones of similar diameter. This would tend to cause a slight decrease in cell length in rapidly growing trees.

In the early part of radial growth where the ratio of anticlinal to periclinal division is high, the actual radial distance from the stem centre appears to be of prime importance in governing the balance between the various factors influencing cell length in the cambium. Further out, however, where the frequency of anticlinal division has diminished slightly and the rate of cell loss is high, secondary factors such as ring width may influence the length of the initials. This would account for the different patterns obtained by the various workers for cell length away from the stem centre. A decrease in mean cell length in this region could be due to the cumulative influence of several wide growth rings during this period. Conversely a number of narrow rings could produce an increase in cell length. The actual radial distance from the stem centre at which this transition in influence occurs will naturally vary from one species to another, its position being influenced by the frequency of anticlinal division, the percentage loss of new fusiform initials and the rates of elongation inherent in that particular species. It is a logical conclusion, therefore, that the diameter of the cambium at its initiation will influence the pattern of variation in cell length outwards from the stem centre. In plants where the cambium develops outside a large core of primary tissue, the increase in cell length may be partially or totally absent. In such cases it must be assumed that the diameter of the cambium at its initiation is such that the initials can divide anticlinally at a rate sufficient to provide an adequate supply of new cells. These new cells meet the needs of the rapidly expanding cambial cylinder and the increase in cell length is partially suppressed.

The rate of height growth of a growing shoot influences the size of the cambial initials originating at any one time. Dinwoodie (1963) noted that rings laid down by the cambium in short internodes had shorter tracheids than did rings laid down in long internodes. Where very little stem elongation has occurred, the cambial initials laid down by the apical meristem are apparently shorter than those laid down during more rapid growth. This relation between the height growth and fusiform initial length has its greatest effect in the first few growth rings. Mell (1910), Lee and Smith (1916) and others also noted that faster growing trees had longer tracheids but did not correlate this with internode length. Stems with a high initial cambial cell length will usually maintain a relatively high length throughout subsequent growth rings (Bisset, Dadswell and Wardrop, 1951). Since many trees pass through a grand period of growth when the rates of height and diameter growth are at a maximum, it could be expected that initials laid down during this period might be appreciably longer than those laid down during periods of slower growth. The general vertical variation in cell length within one growth ring - an increase followed by a decrease - could thus partly be explained in terms of height growth. At the top of the tree, where the stem diameter is very small, the decrease in cell length is partially due, however, to the fact that the horizontal increase in cell length outwards from the stem centre has not yet reached a maximum in this region.

1.5 CONCLUSIONS

The length of the cells in the secondary xylem is governed by the size of the fusiform cambial initials from which they are derived. Tracheids and vessel members are thought to replicate the length of the initials but there are few published results on the relationships between these cell types. Because fibres undergo intrusive growth during their differentiation, they are considerably longer than the fusiform initials.

Although there is a particulary rich literature relating to the size length variation patterns in the secondary xylem, there are, nevertheless, still a number of gaps in our knowledge. The painstaking researches of Bannan, Cumbie and others have added considerably to our understanding of the causes of the cell length variation patterns in plants with non-storeyed cambia. The variables of pseudotransverse division, cell loss and elongation of new initials are now quite well understood in these plants and it is possible to relate these developmental changes occuring in the vascular cambium to features in the secondary xylem.

The biggest gap in our knowledge of the activities of the vascular cambium relates to the more specialized storeyed cambium. There have been few published results of detailed studies on this type of cambium since the pioneering work of Beijer (1927). Investigations on the ontogeny and subsequent developmental changes occuring with radial growth in the storeyed cambium are therefore long overdue. This thesis includes a study on the ontogeny of the storeyed cambium in *Hoheria angustifolia* (Section 2) and a study of the developmental changes occurring in the storeyed cambium with radial growth in *Aeschynomene hispida* Willd. (Section 3). A detailed study is then made on the variation in fusiform cambial initial dimensions with increasing diameter of the cambial cylinder and increasing height in the tree in *Hoheria angustifolia*. The relationship between the length of the cambial fusiform initials and the xylem vessel members is also examined.

SECTION TWO

THE ONTOGENY OF THE VASCULAR CAMBIUM IN HOHERIA ANGUSTIFOLIA RAOUL.

2.1 SUMMARY

The procambium in Hoheria angustifolia (Malvaceae) develops behind the apical meristem as an almost complete cylinder of meristematic cells. Differentiating protophloem strands are separated by narrow interfascicular sectors. These sectors also interrupt the procambial cylinder. The first protoxylem elements differentiate as discrete bundles to the inside of a number of protophloem strands and remain separated from them by an actively dividing procambial cylinder 6 - 8 cells wide. Periclinal divisions also occur in the interfascicular sectors so that by the centre of the first internode below the shoot tip, the procambium forms a complete cylinder. In this way the short cells of the interfascicular sectors become transformed into primordial ray initials. Repeated periclinal division in the procambium leads to the radial seriation of its cells. Subsequent differentiation of the derivatives produces complete cylinders of metaxylem and metaphloem by the third internode.

Internodal elongation ends by the fifth or sixth

internode below the shoot tip after which the transverse ended primordial fusiform cells of the procambium become transformed into fusiform cambial cells with pointed tips. Except during ray formation, transverse division ceases and the non-storeyed cambium gradually acquires a storeyed appearance in tangential view by repeated radial longitudinal divisions accompanying radial growth. The first new rays appear about the seventh internode.

The transition from procambium to cambium in *Hoheria angustifolia* is a gradual one with the meristem acquiring some cambial characteristics before and some after internodal elongation has ceased.

2.2 INTRODUCTION

For convenience, the vascular meristem is divided into a procambium and cambium. The procambium has its origin in the actively dividing and enlarging shoot apex and is the source of the primary vascular tissues. Its cells also form the basis of the vascular cambium.

The precise time of origin of the vascular cambium is difficult to determine both theoretically and practically. Different criteria for defining secondary growth, and considerable variation between the few plants that have been examined in detail, has led to a general vagueness in our understanding of the processes accompanying the transition from procambium to cambium. On the one hand, the cambium may be regarded as the meristem that produces regular files of derivative cells, a definition that if rigorously applied to many plants would include the metaxylem as a product of the cambium. On the other hand, some morphologists would believe that extension growth of the internodes must have ceased before the cambium, as such, can exist.

Primary tissues are usually regarded as products of the apical meristem, but in fact, most of the divisions producing them are located in the axis below the apex. While primary growth is usually associated with extension growth and internodal elongation, lateral growth, on the other hand, is a continuous process from the apex to the mature trunk of the woody plant. While it is useful to separate the early part of this process as primary growth and the later as secondary growth, it is usually impossible to distinguish sharply between primary and secondary tissues. It has long been established that the vascular cambia and vascular tissues of gymnosperms and dicotyledons are normally characterized by the presence of two distinct systems of cells, one composed of axially elongated cells derived from fusiform initials, and the other composed of radially elongated cells derived from ray initials. The procambium, on the other hand, usually has a more homogeneous structure (Esau, 1943a, 1965a).

Similarities and differences between the procambium and cambium in *Canavalia* (Leguminosae) have been noted by Cumbie (1967b). Early in its development the procambium of this plant becomes organized into two systems, one of elongated cells, the other of short cells in axial strands when viewed in tangential section. The elongated cells of the procambium become the fusiform initials of the cambium and the strands of shorter cells are converted to ray initials. In addition, some of the longer procambial cells also form ray initials by subdivision, in a manner similar to the formation of some secondary rays from fusiform cambial initials. There are some features, however, in which the procambium and cambium differ. Most of the end walls of the elongated procambial cells are essentially transverse, whereas most of the fusiform cambial initials have tapering ends. The cells of the procambium elongate during the active growth of the stem, whereas the lengths of the fusiform cambial initials remain relatively constant. Transverse divisions occur in the procambium but were not observed in the cambium. Overall,however, Cumbie concluded that the similarities in structure and development of the two meristems are such that a clear distinction between them is not justified.

The transition from procambium to cambium has been found to be more abrupt in *Acer pseudoplatanus* (Catesson, 1964). The procambium in this species is not differentiated into long and short cells as in Canavalia. Once extension growth of the internodes has ceased the procambial cells of Acer elongate by intrusive growth and develop pointed ends. These cells become fusiform cambial initials. The onset of secondary growth is marked by the conversion of some of these fusiform initials into ray initials by subdivision. Large rays also form in the interfascicular sectors of the stem, becoming dissected into smaller rays by the subsequent intrusion of fusiform initials from the fascicular cambium. Catesson emphasizes that she does not consider the cambium to be merely a residue of the procambium in which the divisions have become regular and concentrated. Instead, she regards it as a distinctive tissue which in the mature state is characterized by a differentiation into fusiform and ray initials with the former showing an increase in length in nonstoreyed cambia when intrusive growth follows anticlinal division.

The procambium of Ginkgo biloba L., like that of Acer,is a rather homogeneous tissue in its early development but a differentiation into two distinct systems of cells occurs late in primary growth by unequal transverse division of the procambial cells (Soh, 1972). In this latter species, some of the procambial cells elongate without transverse division and form a system of axially elongated cells. Frequent transverse divisions in short cells for a time keep pace with the elongation process, then elongate slightly. Ray initials later develop from the short cells after their separation.

The early ontogeny of the vascular cambium has also been studied in *Aucuba japonica* Thunb. and *Weigela coraeensis* Thunb. by Soh (1974). These two angiosperms both have comparatively long fusiform initials in their non-storeyed vascular cambia. The transition from procambium to cambium in *Aucuba* resembles that reported for *Acer* by Catesson (1964). Late in primary growth most of the long procambial cells become transformed into fusiform cambial initials while others undergo segmentation into axial files of short cells and subsequently become transformed into ray initials. The procambium of *Weigela*, however, resembles that found in *Ginkgo*. An uneven distribution of transverse divisions in the long cells results in the production of long and short cells early in the development of the procambium.

The obvious differences between the procambia of *Acer* and *Aucuba* with only one system of cells, *Ginkgo* and *Weigela* with one system in early development and two systems at a later stage, and *Canavalia* with two systems of cells, render the distinction between procambia and cambia on the basis of cell orientation of doubtful value. Such a view has already been expressed by Esau (1943a, 1965a, 1965b) who believes that the procambium and the cambium are best looked upon as two developmental stages of the same meristem. A similar view has been proposed in reviews on the cambium by Philipson and Ward (1965) and Philipson *et al*. (1971).

A number of other criteria for separating the procambium and cambium were investigated by Fahn *et al*. (1972) in *Ricinus communis* L. without success. Wounding experiments showed that there is no special organizing stimulus which is responsible for the differentiation of the cambium as a separate entity from the procambium in this plant.

In many plants, internodes actively increasing in length contain bundles that are expanding rapidly by a meristem that has every appearance of a cambium and is undoubtedly continuous with the cambium of adjacent mature internodes. These meristems are usually regarded as cambial and this is the position held by Sterling (1946) in his study on *Sequoia sempervirens*.

The complete cambial cylinder may develop in a variety of ways. If the metaxylem forms a complete cylinder before secondary growth begins, then the cambium is complete from the time of its inception. More frequently, the primary vascular bundles are separate at the time of cambial inception, in which event regular divisions usually occur earlier in the bundles than in the interfascicular sectors. Additional bundles develop in some plants after cambial activity has commenced. Such bundles are called intermediate bundles by de Bary (1884) and are composed wholly of secondary tissues. Fahn *et al*. (1972), however, have questioned this commonly held concept that the fascicular cambium develops before the interfascicular cambium. Their observations on *Ricinus* suggest that in this plant, at least, the reverse might be true. They suggest that this point should be checked in other plants.

Most studies on the ontogeny of the vascular system in plants have concentrated on either the procambium or the cambium and do not include accounts of the transition from one to the other. The few published studies on the transition by Sterling (1946), Catesson (1964), Cumbie (1967b), Fahn *et al.* (1972), and Soh (1972, 1974), at times present conflicting views. It may well be that the different plants examined in each of these studies have real differences in the sequence in which their lateral meristems acquire cambial characteristics.

Stages in the transition from procambium to cambium must become known in a greater number and variety of plants before many of the fundamental problems associated with the onset of secondary growth can be resolved.

Hoheria angustifolia, a member of the Malvaceae endemic to New Zealand, was chosen for a study on the ontogeny of the vascular cambium for two reasons. Firstly it has an almost complete cylinder of primary xylem before secondary tissues are deposited. Secondly, because its mature cambium is storeyed, it was the plant chosen for a detailed investigation on the cell size changes occurring in the cambium in various parts of the tree for Section 4 of this thesis. It is appropriate, therefore, that the ontogeny of its vascular cambium be investigated.

2.3 MATERIALS AND METHODS

About 50 young seedlings of Hoheria angustifolia were collected from a site in Governors Bay, Banks Peninsula. All were separately potted and glasshouse tendered. Five seedlings of a similar size were selected from these, each with two prominent leaders, transferred in August 1968 to controlled environment growth rooms under conditions of 17°C temperature, 70% humidity and 16 hours daylength. The top leaves of each leader were marked. The plants were left under these conditions for 16 weeks until at least 25 new internodes had developed on each marked shoot. The plants were photographed at 4 week intervals and certain internodes on both leading and lateral shoots of all the plants measured at two day intervals. At the end of the 16 week period, the leading shoots were removed from each plant and the lengths of the top 25 internodes measured on each. Two leading shoots of equal length were removed from one plant (no. 66), and the central leading shoot from each of three other plants (nos. 46, 53 and 58) were removed, cut into internode lengths, fixed in formalin-aceto-alcohol, embedded and sectioned. The internodes of one shoot from plant 66, and the shoots from plants 46 and 58 were sectioned longitudinally, while transverse sections were prepared from the middle of each internode of the other shoot of plant 66 and the shoot from plant 53. The prepared slides were stained in safranin and toluidine-blue and mounted in De Pex for microscope examination.

2.4.1 RESULTS

The transition from primary to secondary growth in the stem is best studied in both transverse and longitudinal sections of internodes. Since any one internode cannot be cut into sections in both planes, the best that can be achieved is to examine internodes from several shoots from the same or similar plants grown under identical conditions. Although 5 young Hoheria angustifolia plants were selected each with two prominent leading shoots, only one plant (no. 66) retained these after 16 weeks growth in the controlled environment growth rooms. The remaining plants all developed large numbers of shoots of similar prominence characteristic of the divaricate juvenile form of this species (see Section 4.2.2).

The growing shoot of Hoheria angustifolia produces petiolate leaves in an alternate manner behind the apex, each separated at maturity by a clearly defined internode (Fig. 2-1). The apical meristem is enclosed by a number of enlarging leaves

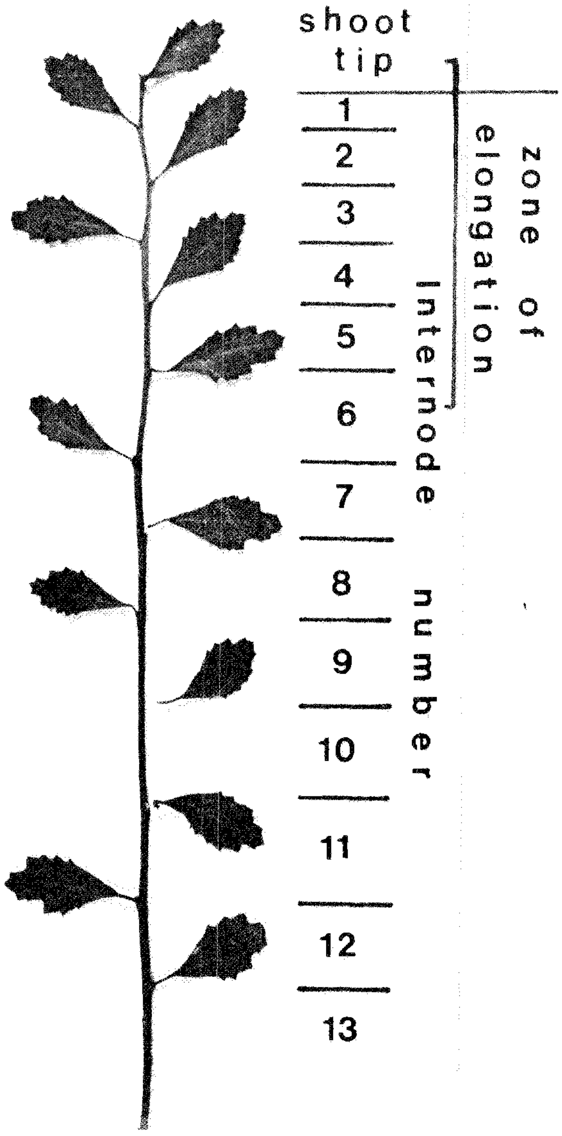


Fig. 2-1. A growing shoot of *Hoheria angustifolia* with the shoot tip and first visible internodes indicated. The shoot tip contains the apical meristem and a further 8 - 11 expanding internodes. X Life size

such that it is impossible to count the primordia and leaves macroscopically in the zone above the uppermost visible internode. This zone is designated the shoot tip for the purposes of this project and number 1 internode as the first visible internode below it. Subsequent examinations of longitudinal sections of the shoot tip indicate that this zone contains the apical meristem and from 8 to 11 primordia and expanding leaves usually enclosed by the lowest two leaves.

From early in their development, the enlarging leaves are densely clothed in stellate hairs (Fig. 2-2). These hairs separate with the expansion of the leaf (Fig. 2-3) and some are subsequently lost. Their presence, however, seriously impedes satisfactory sectioning of the shoot tip.

2.4.2. INTERNODAL ELONGATION

Primary shoot elongation in the seedlings examined occurred in the internodes of the shoot tips and continued in up to five internodes below them. This trend is indicated by the lengths of various internodes at the time of their collection for sectioning (Fig. 2-4) and also by the individual measurements made on selected internodes during their active growth. Considerable variation in the final lengths of mature internodes is also indicated in Figure 2-4, the variation apparently increasing slightly towards the apex, possibly as the plants responded to their new environment in the

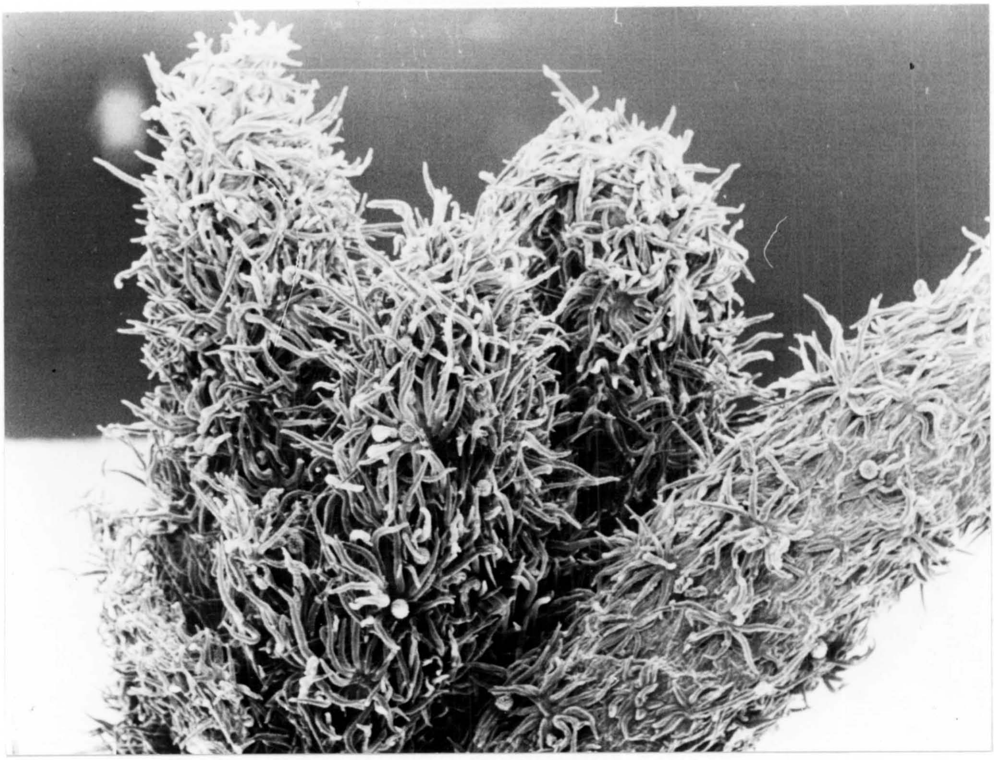


Fig. 2-2. Scanning electron micrograph of a shoot apex showing the expanding leaf primordia densely clothed in stellate hairs. X 65

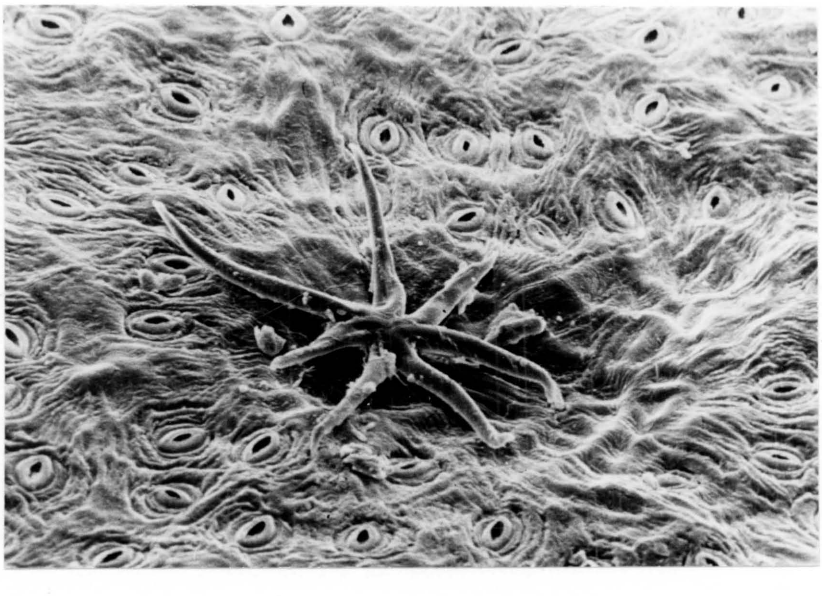


Fig. 2-3. A single stellate hair on the lower surface of a mature leaf. X 600

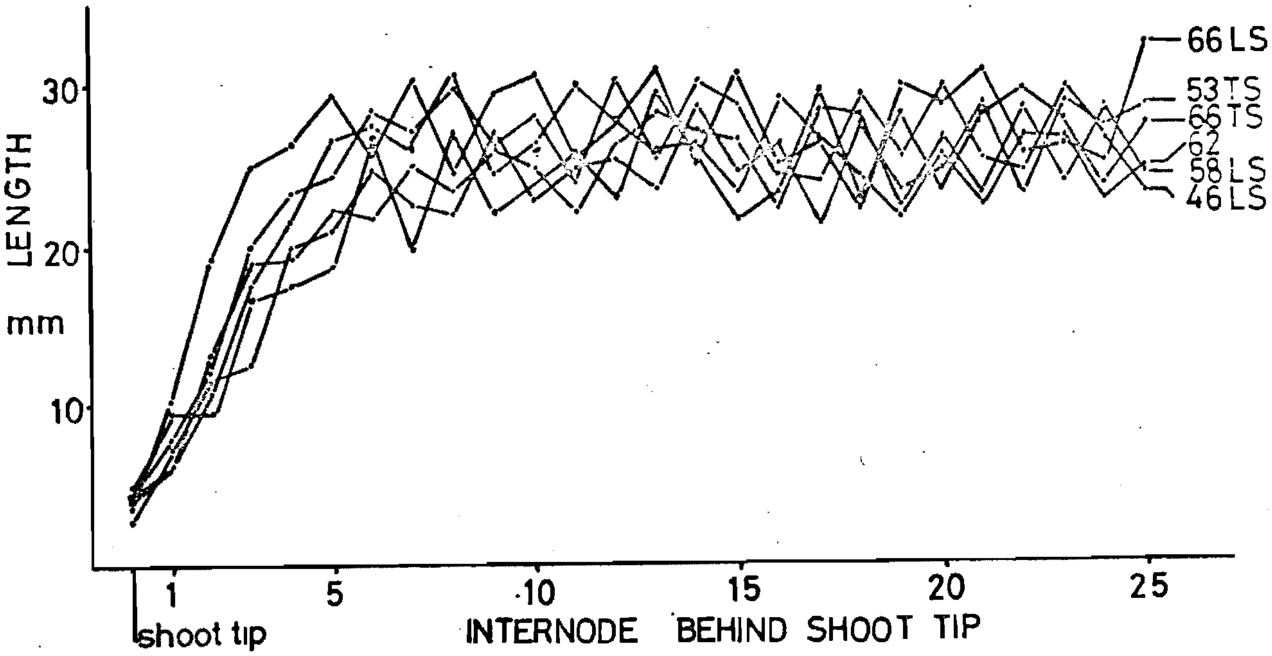


Fig. 2-4. Internode lengths of the two shoots from plant 66 and the leading shoots from plants 46, 52, and 53 measured at the time of their collection for sectioning. Internode lengths of a shoot from plant 62 are also graphed though this shoot was not sectioned. Internodal elongation appears to continue in up to five internodes below the shoot tip.

growth rooms. This fluctuation in the final internode lengths means that it is not possible to say with certainty that elongation has definately ended in the sixth internode below the shoot tip in any one stem collected for examination. In the five plants examined, this zone of elongation represented a mean length of 88.4 mm from the end of the shoot.

2.4.3 DIFFERENTIATION OF THE VASCULAR TISSUES

Transverse sections of the complete shoot tip region, and from the centre of the first 25 internodes of each of the plants examined, provided a complete range of the developmental stages in the differentiation of the vascular tissues.

Shoot tip. This zone is defined as containing the apical meristem and the axis bearing 8-11 primordia and expanding leaves enclosed by the two lowest leaves.

Transverse sections from near the tip of this zone show the procambium as discrete undifferentiated strands forming a ring at about 2/3 of the radial distance from the stem centre. The strands are of a similar size. Each trace is separated by interfascicular sectors one to three cells wide. The term "interfascicular sector" is used here as a matter of convenience. These sectors do not conform to the usual appearance or division cycle of medullary rays and may therefore form an integral part of the procambial cylinder. Differentiation of the protophloem begins almost immediately (Fig. 2-5). The procambial cells are rectangular in transverse view and show a tendency for radial seriation. This pattern is lost in the protophloem. Longitudinal sections of this stage indicate that the procambial cells are elongated in an axial direction, some having transverse and others having oblique end walls. The end walls of the interfascicular cells are normally transverse. It is unfortunate that sectioning difficulties prevented the length of these interfascicular sectors from being studied in longitudinal view.

Differentiation of the protoxylem begins at a point less than 2 mm from the top of the apical meristem (Fig. 2-6). Initially 2 or 3 strands differentiate to the inside of the procambial cylinder. Each strand consists of a number of radial files of vessels separated by thinner walled parenchyma cells (Fig. 2-7) and lies to the inside of a number of the protophloem strands. The protoxylem parenchyma cells are not always in radial continuity with the interfascicular sectors. The number of the protoxylem strands in the stem increases gradually to about 8 with increasing distance from the apical meristem. Each xylem strand remains separated from the protophloem strands by a zone of 5-8 undifferentiated procambial cells.

Towards the base of the shoot tip the undifferentiated

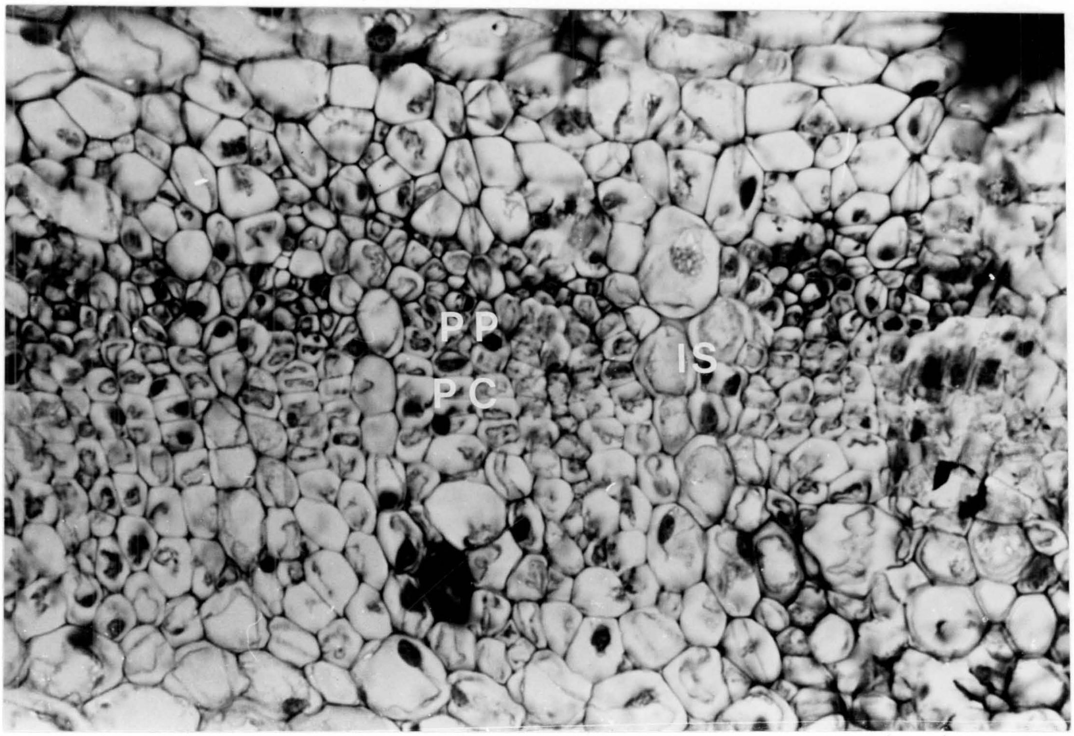


Fig. 2-5. Transverse section of the procambium (PC) in the shoot tip just below the apical meristem. Note the differentiating protophloem traces (PP) and the separating interfascicular sectors (IS). X 360

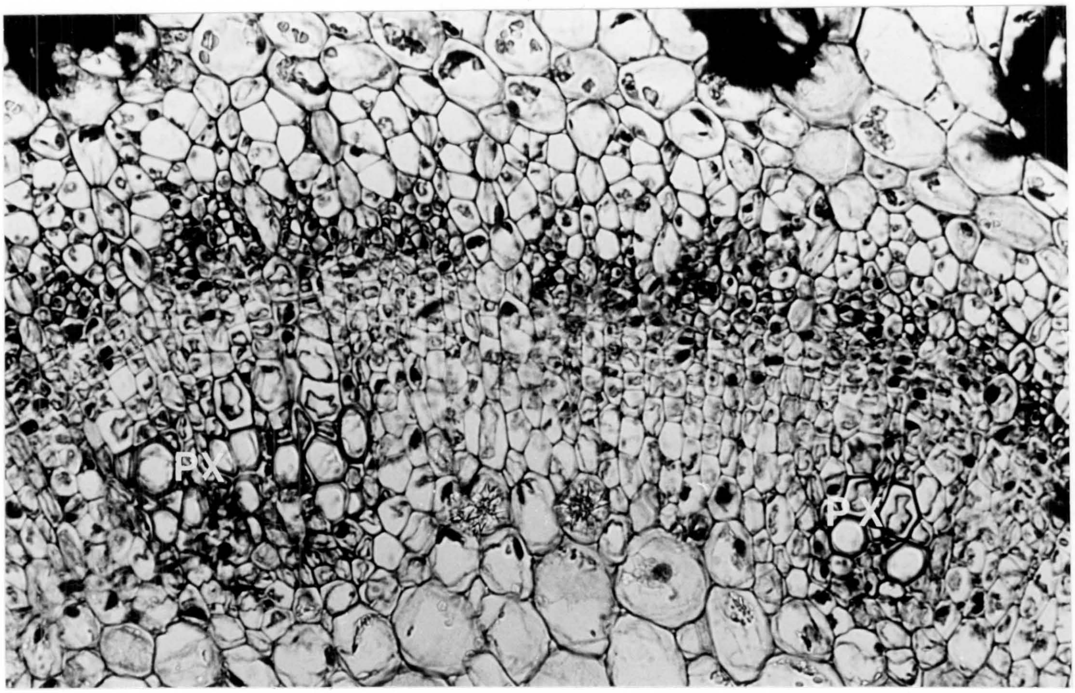


Fig. 2-6. Transverse section from near the base of the shoot tip. Discrete bundles of protoxylem (PX) have now differentiated. Undifferentiated procambial cells exhibit radial seriation. X 360

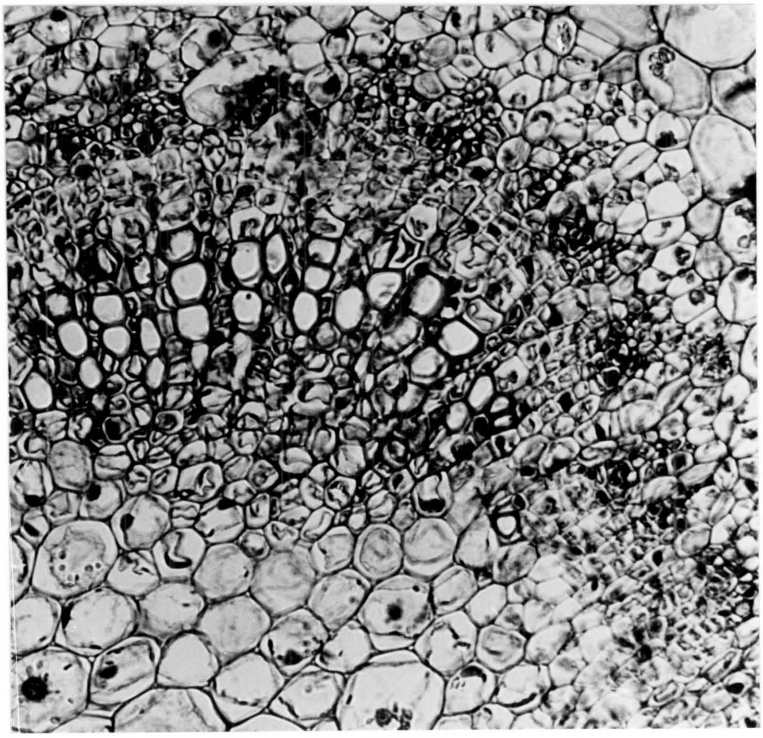


Fig. 2-7. Detail of a vascular bundle from the lower region of the shoot tip. The protoxylem bundles differentiate to the inside of a number of protophloem strands. X 575

procambial cells lie more clearly in radial files, presumably as a result of repeated periclinal division. Between the larger primary bundles a number of protophloem strands continue to survive external to an almost complete cylinder of procambium. Some interfascicular sectors are still visible between the protophloem strands but their course through the procambial cylinder becomes obscured by the periclinal divisions of the latter tissue.

Elongating Internodes. Transverse sections of the internodes below the shoot tip revealed continuous differentiation of the vascular tissues accompanied by a transition from primary to secondary growth. New strands of primary xylem develop from the procambium between the main bundles in the first and second internodes (Fig. 2-8), until a complete cylinder of xylem exists by the third internode (Fig. 2-10). Whether this tissue should be designated protoxylem or metaxylem is debatable. The primary xylem in the larger bundles continues differentiating in a centrifugal direction and new phloem elements are added in a centripetal direction throughout these first three internodes. Periclinal division of the undifferentiated procambial cells continues, approximately keeping pace with this differentiation pattern so that a procambial cylinder of 6-8 cells in thickness remains. In transverse view this repeated periclinal division of uncommitted meristematic cells, accompanied by differentiation into phloem and xylem elements

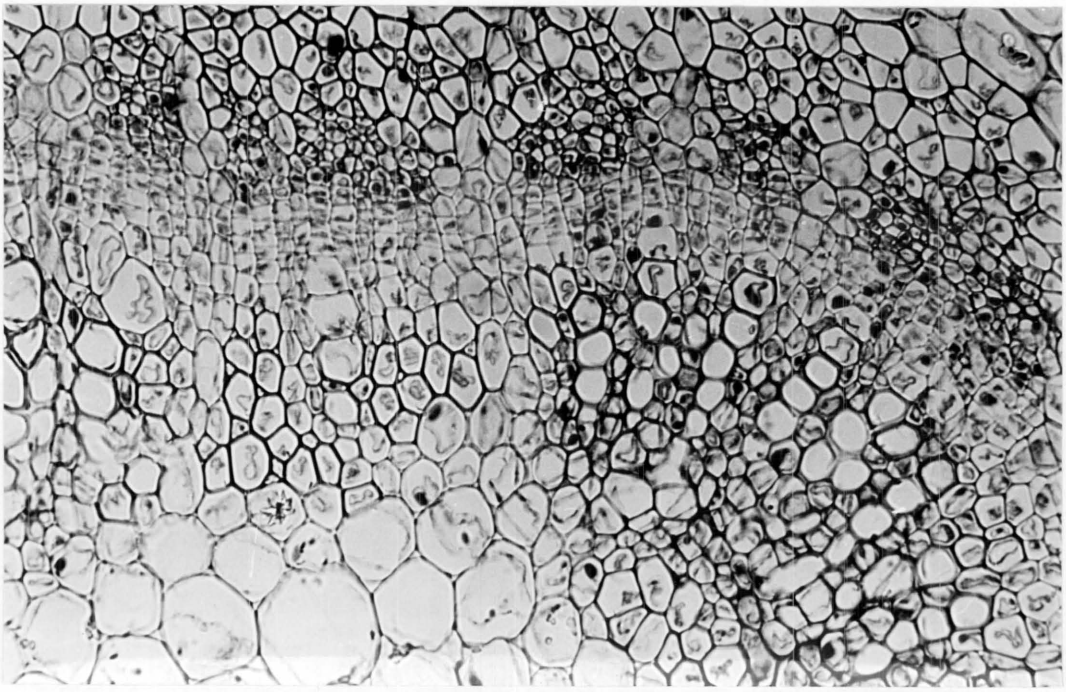


Fig. 2-8. Transverse section of the vascular tissues from the centre of the second internode. The inside derivatives of the procambial cylinder differentiate into an almost complete cylinder of primary xylem. X 360

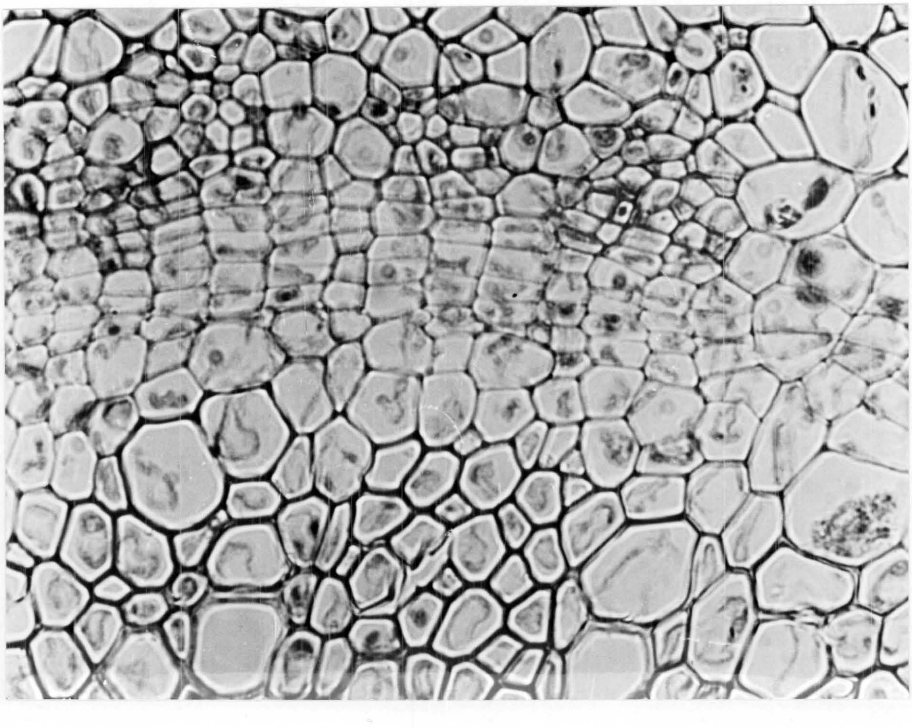


Fig. 2-9. Detail of the procambium and derivative tissues from the second internode. X 575

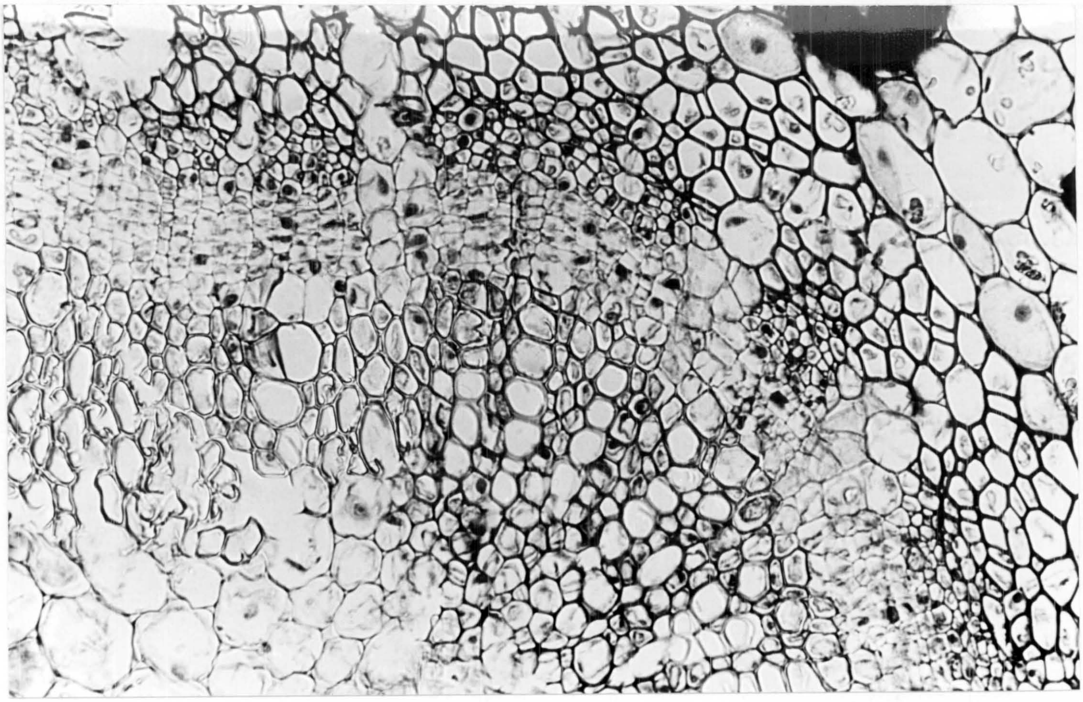


Fig. 2-10. Transverse section from the centre of the third internode. A complete cylinder of primary xylem continues to differentiate to the inside of the procambium and phloem traces. X 360

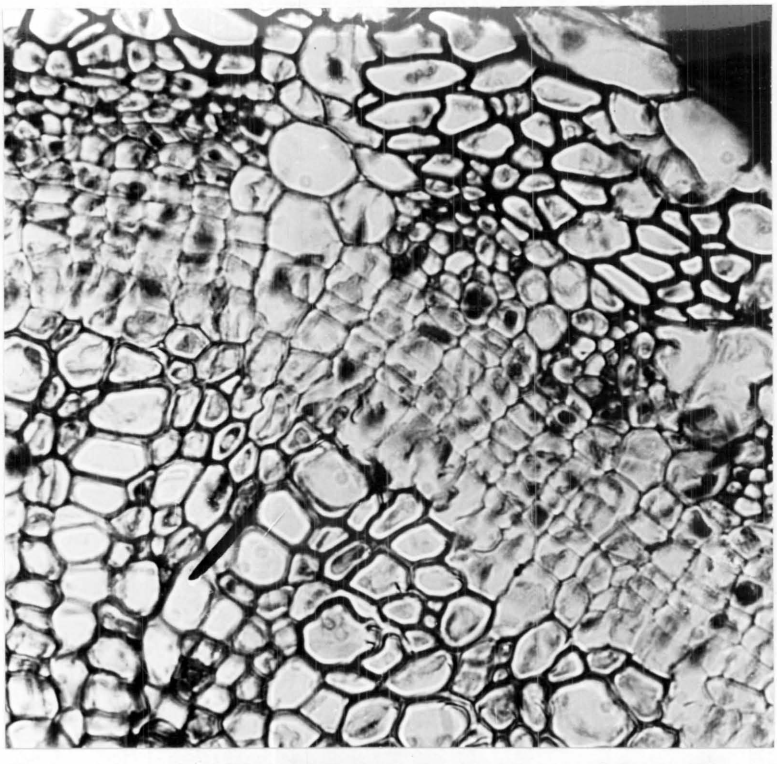


Fig. 2-11. Detail of the procambial zone from the third internode. Note the periclinal divisions in the cells of the interfascicular sectors. X 575

either side of the procambial cylinder, shows a marked similarity to the activity of a vascular cambium (Figs. 2-9, 2-11). A progressive increase in the total thickness of the vascular tissue results. The interfascicular sectors can still be seen between the protophloem bundles but their course through the actively dividing meristematic zone becomes obscured.

By the fourth internode a complete cylinder of xylem is well established (Fig. 2-12). Cell wall thickening becomes marked in the cells to the outside of the phloem, a process which continues until about the ninth internode by which time the bundle cap fibres are fully differentiated. A complete cylinder of phloem is also present by the fourth internode. Anticlinal as well as periclinal divisions can now be detected in the procambial cells (Fig. 2-13).

The cylinder of undifferentiated meristematic cells becomes slowly transformed into a vascular cambium through subsequent internodes (Figs. 2-14, 2-15, 2-16, 2-17). New xylem and phloem elements continue to be laid down by the procambial cylinder which continues active periclinal division.

<u>Internodes beyond the zone of elongation</u>. By the ninth internode (Figs. 2-18, 2-19), a xylem cylinder of considerable thickness exists built up of fibres, vessels and some axial parenchyma cells. The vascular cambium is a cylinder

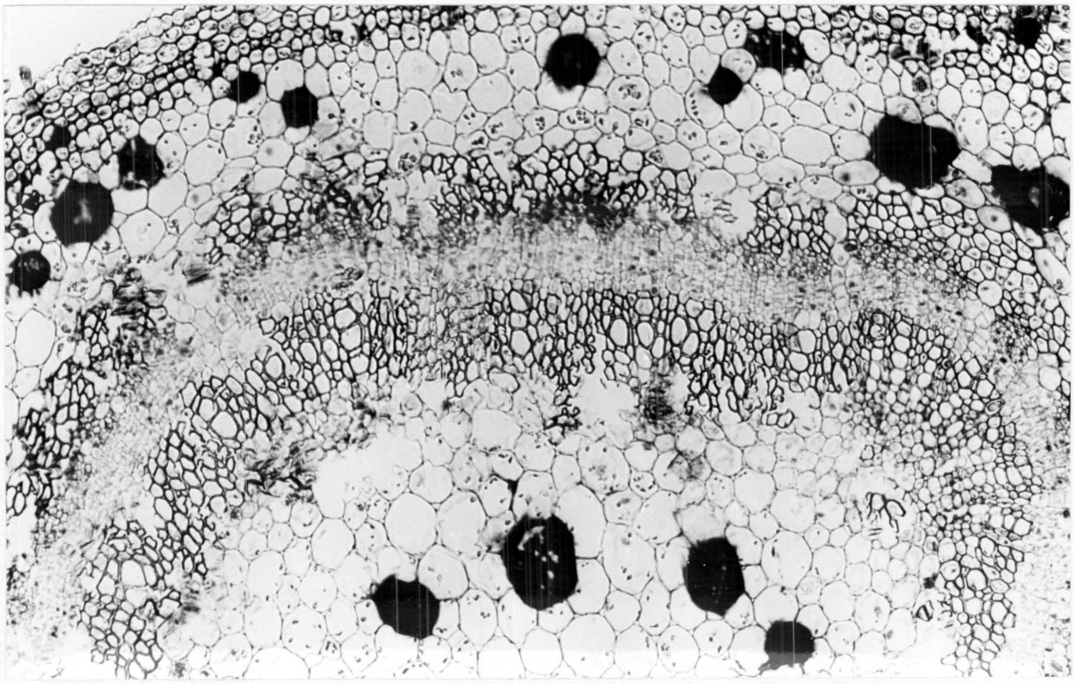


Fig. 2-12. Transverse section through the centre of the fourth internode. A complete cylinder of primary xylem is now well established. X 160

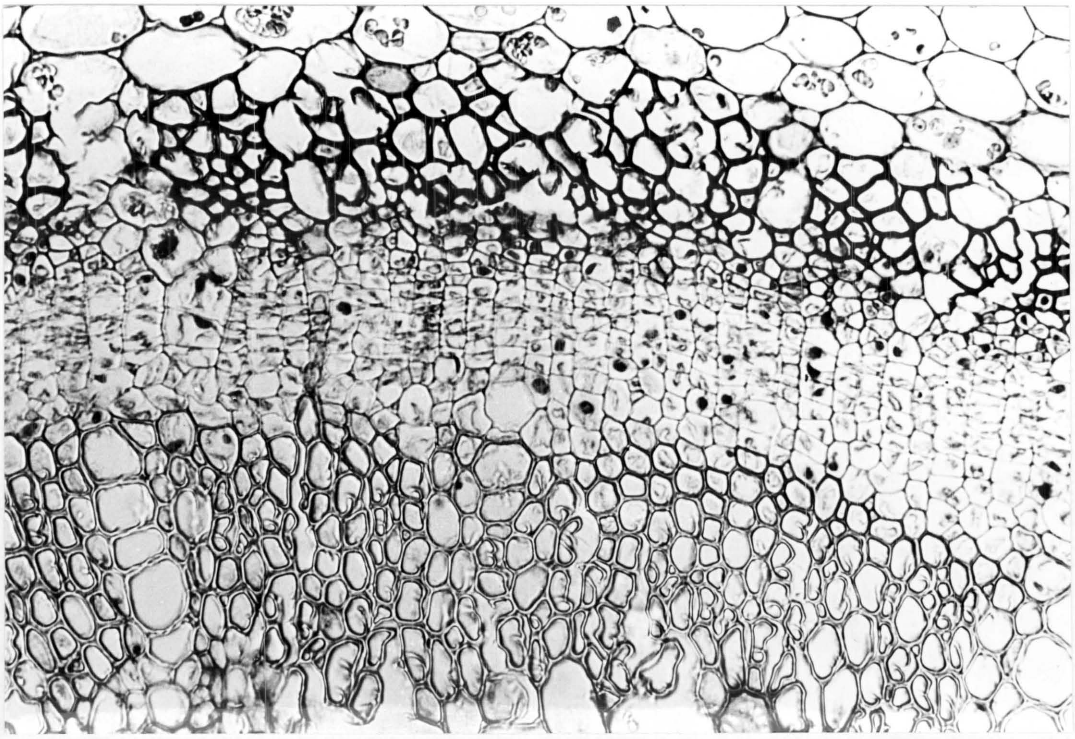


Fig. 2-13. Detail of the primary xylem, procambium and primary phloem from the fourth internode. X 360

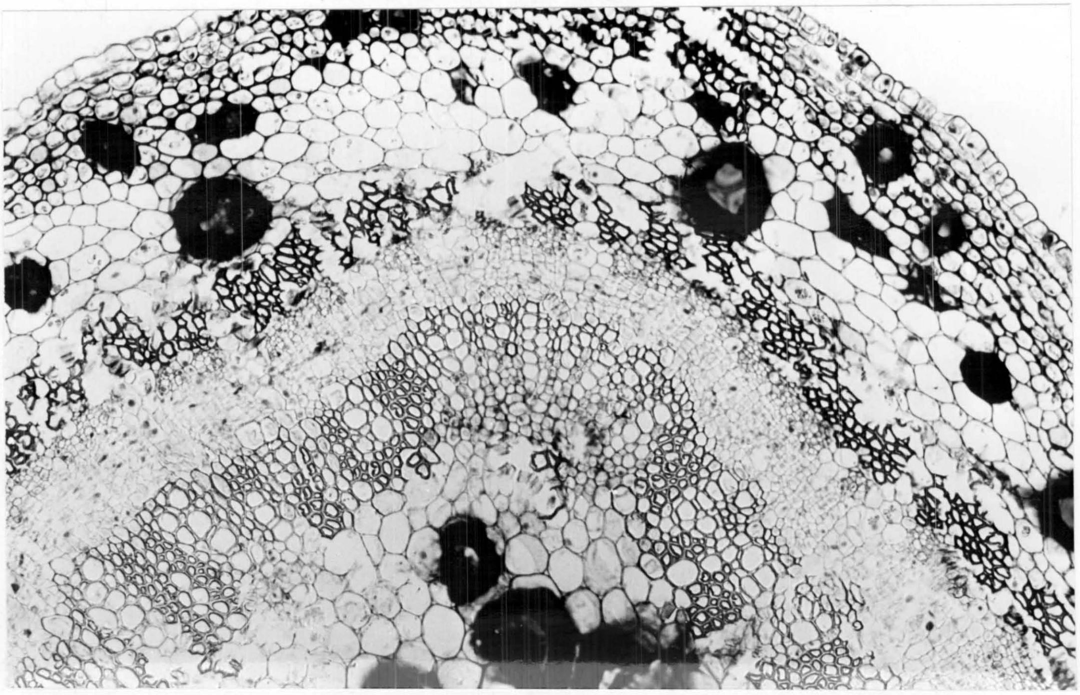


Fig. 2-14. Transverse section from the centre of the fifth internode. X 160

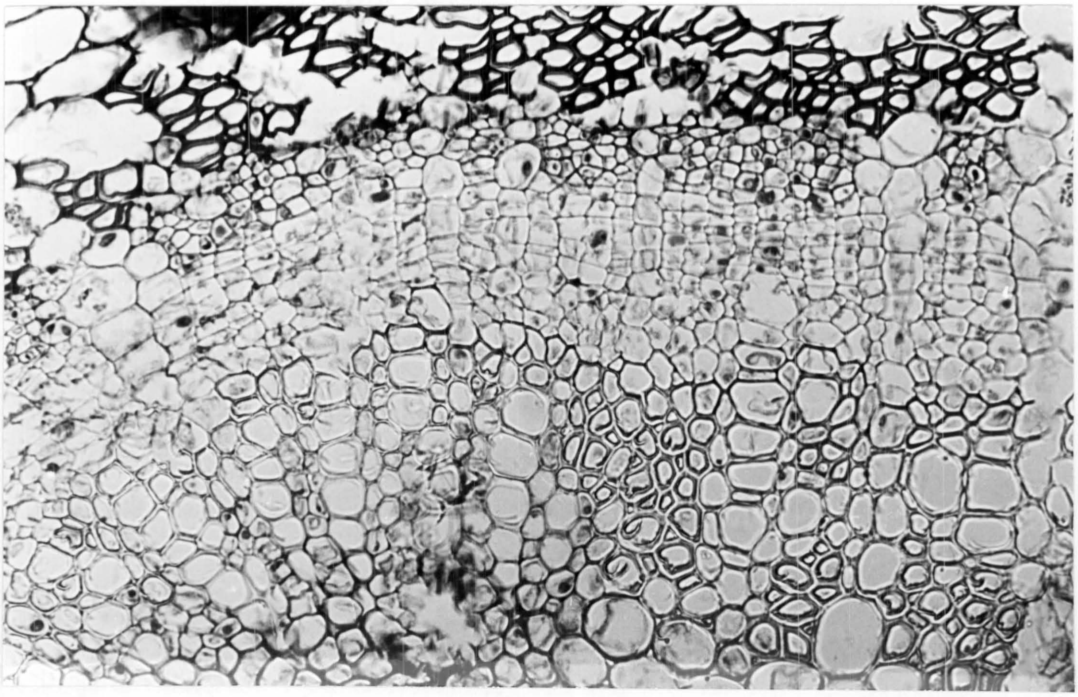


Fig. 2-15. Detail of the vascular tissues of the fifth internode. X 360

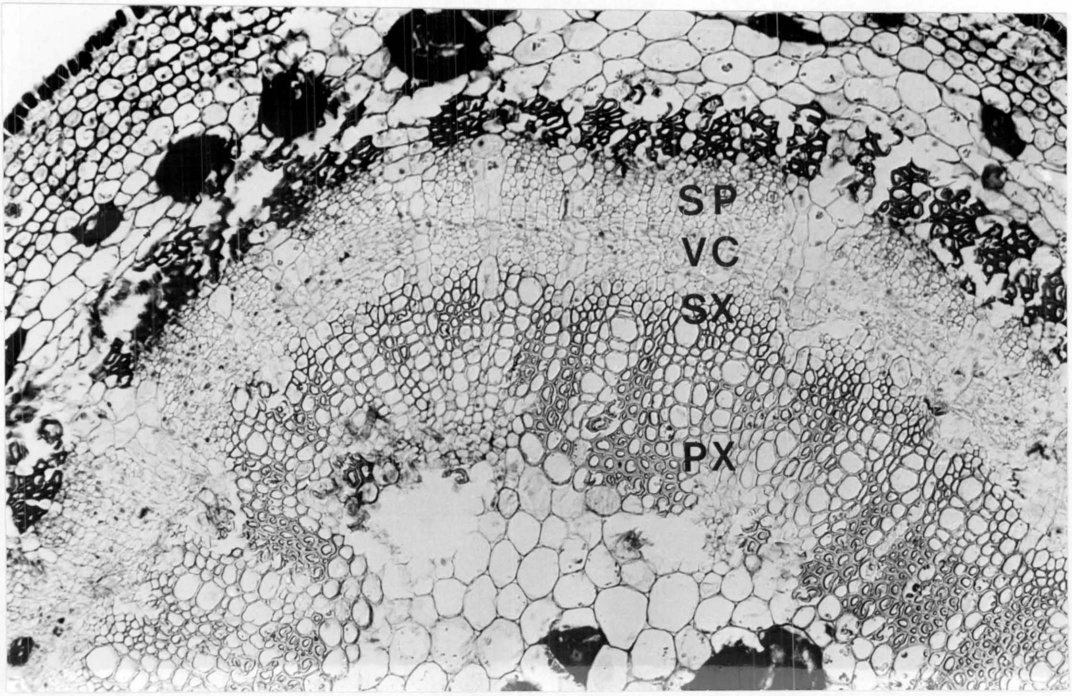


Fig. 2-16. Transverse section from the centre of the seventh internode. Secondary growth is now under way. Primary xylem (PX), secondary xylem (SX), vascular cambium (VC), secondary phloem (SP) are indicated. X 160

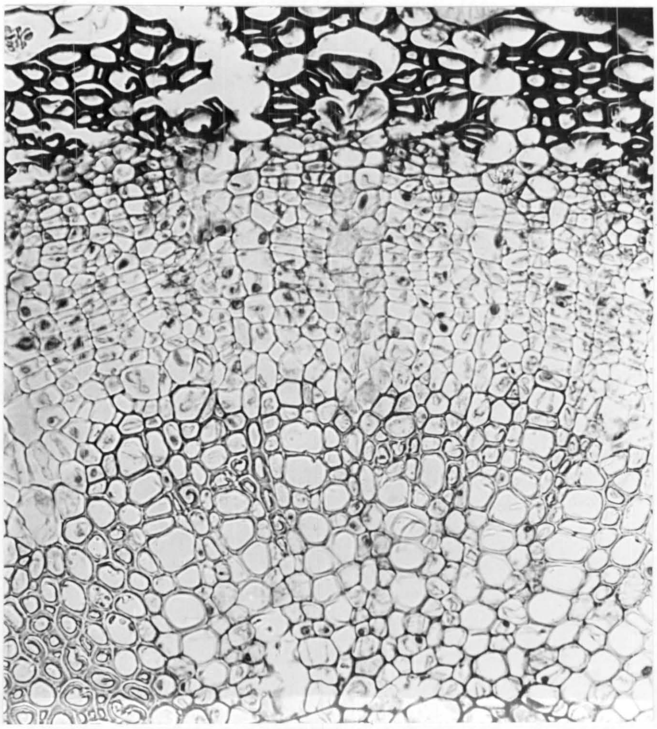


Fig. 2-17. Detail of the vascular tissues form the seventh internode. X 360

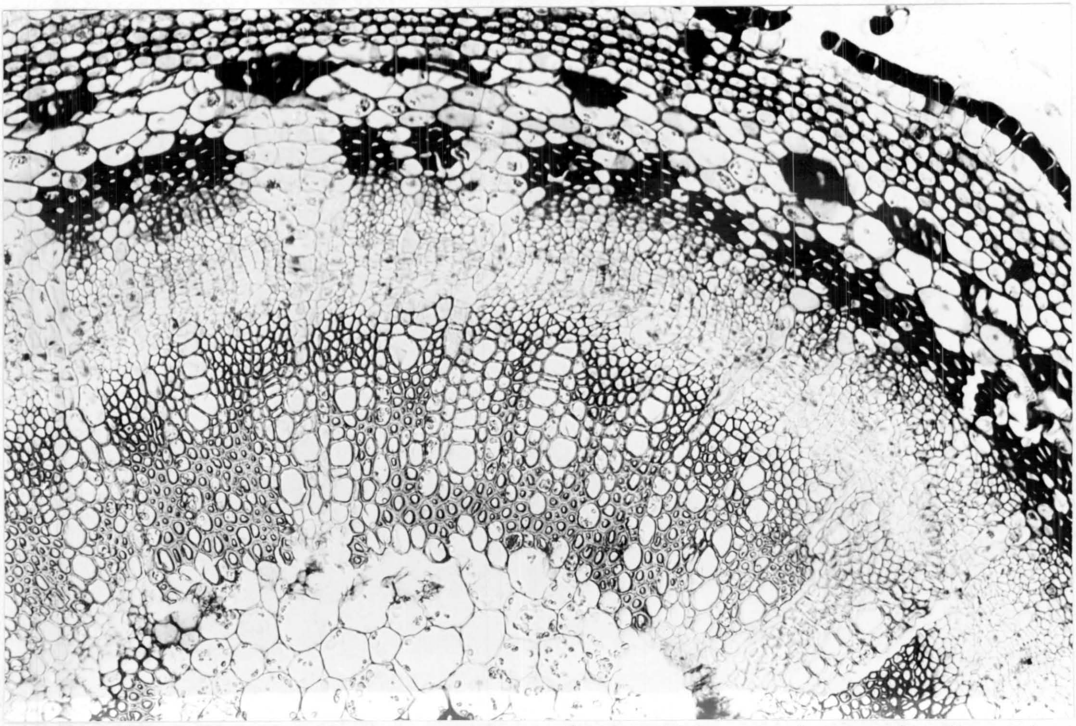


Fig. 2-18. Transverse section from the centre of the ninth internode. X 160

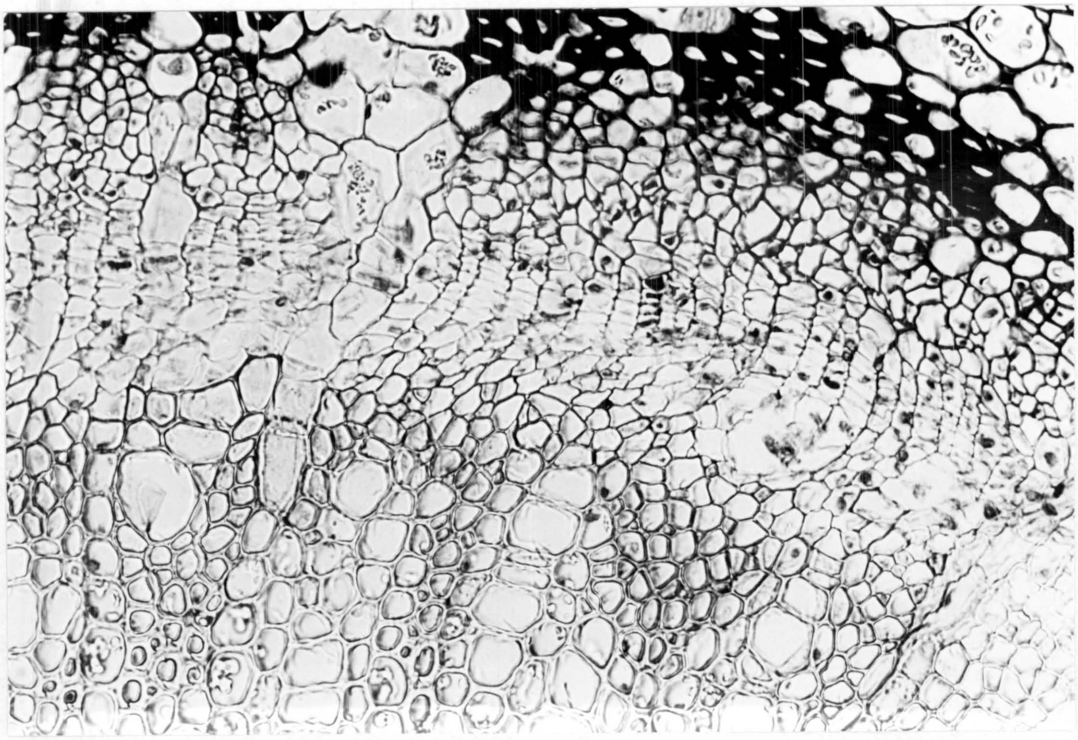


Fig. 2-19. Detail of the vascular tissues from the ninth internode. X 360

of actively dividing cells in radial files 4-8 cells wide lying between the secondary xylem and the secondary phloem. Most of the protophloem elements have collapsed and the entire vascular cylinder now lies inside a broken ring of phloem fibres. To the outside of the cortex a periderm is evident. No new rays could be detected in transverse view, though the former interfascicular sectors continue as rays, sometimes increasing considerably in width towards the cortex. This increase in ray width probably results from the circumferential expansion of the outside tissues with radial growth.

The transition from procambium to cambium as seen in tangential view. The cells of the procambium in the shoot tip give the appearance of being arranged in two systems. Close to the developing leaf primordia the longer cells have transverse to rounded end walls and dense contents. Rows of short rectangular cells form the interfascicular areas.

Towards the base of the shoot tip the long cells have mostly transverse end walls (Fig. 2-20a). Repeated transverse division of the cells within the interfascicular sectors enables them to retain their short length. Radial longitudinal divisions were not observed in either the long or the short cells within the shoot tip though this does not preclude the possibility that such divisions occur. In the shoot tip, however, it is likely that the slight increase in the circumference of the procambial cylinder that occurs with increasing

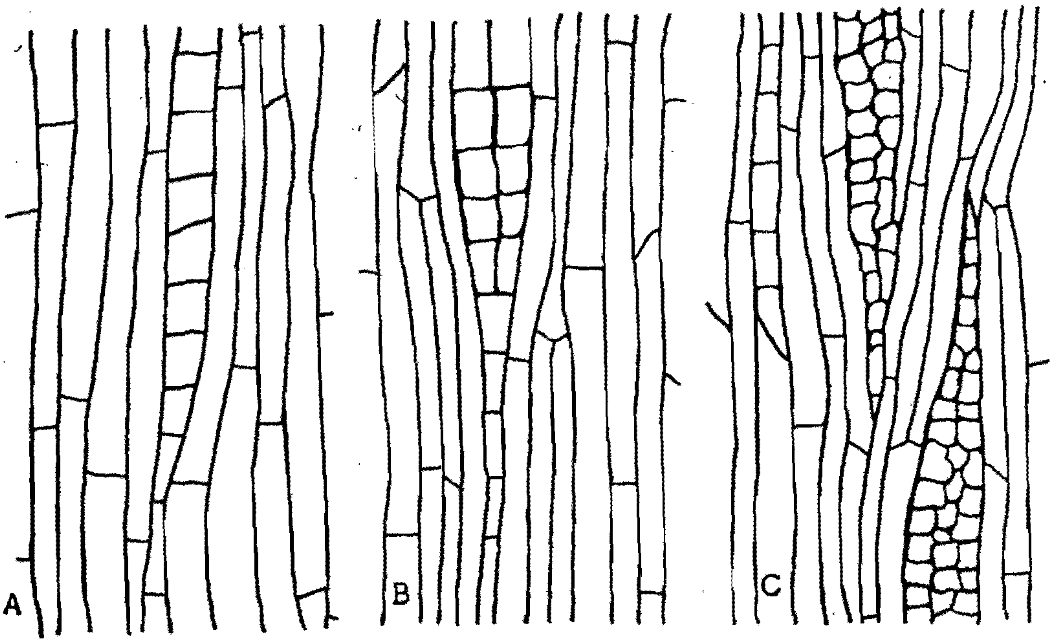


Fig. 2-20. Tangential longitudinal sections of the procambium cut from (A) the base of the shoot tip, (B) the fourth internode and (C) the seventh internode. All X 150

distance from the apical meristem is largely met by the increasing tangential dimensions of the cells.

The long cells of the procambium gradually become transformed into the fusiform initials of the vascular cambium in the internodes below the shoot tip. Radial longitudinal divisions within these cells occur from the first internode downwards, though continuing transverse divisions prevent the development of a storeyed pattern (Fig. 2-20b, 2-20c). This non-storeyed pattern of rectangular cells with transverse end walls is still a feature of the vascular cambium by the seventh internode (Fig. 2-20c). The fusiform initials begin to acquire pointed ends by the ninth internode. Subsequent radial longitudinal divisions are responsible for the development of the storeyed pattern of the fusiform initials in the mature cambium (Fig. 2-21).

Within the elongating internodes, transverse divisions within the interfascicular sectors enable the primordial ray cells to retain their almost square outline when viewed tangentially (Fig. 2-20b). Gradually these cells become more rounded in tangential outline. The cells to the upper and lower limits sometimes divide less frequently, forming uniseriate or biseriate extensions of slightly elongated cells.

New rays first appear about the seventh internode, each formed by the segmentation of a fusiform cambial initial

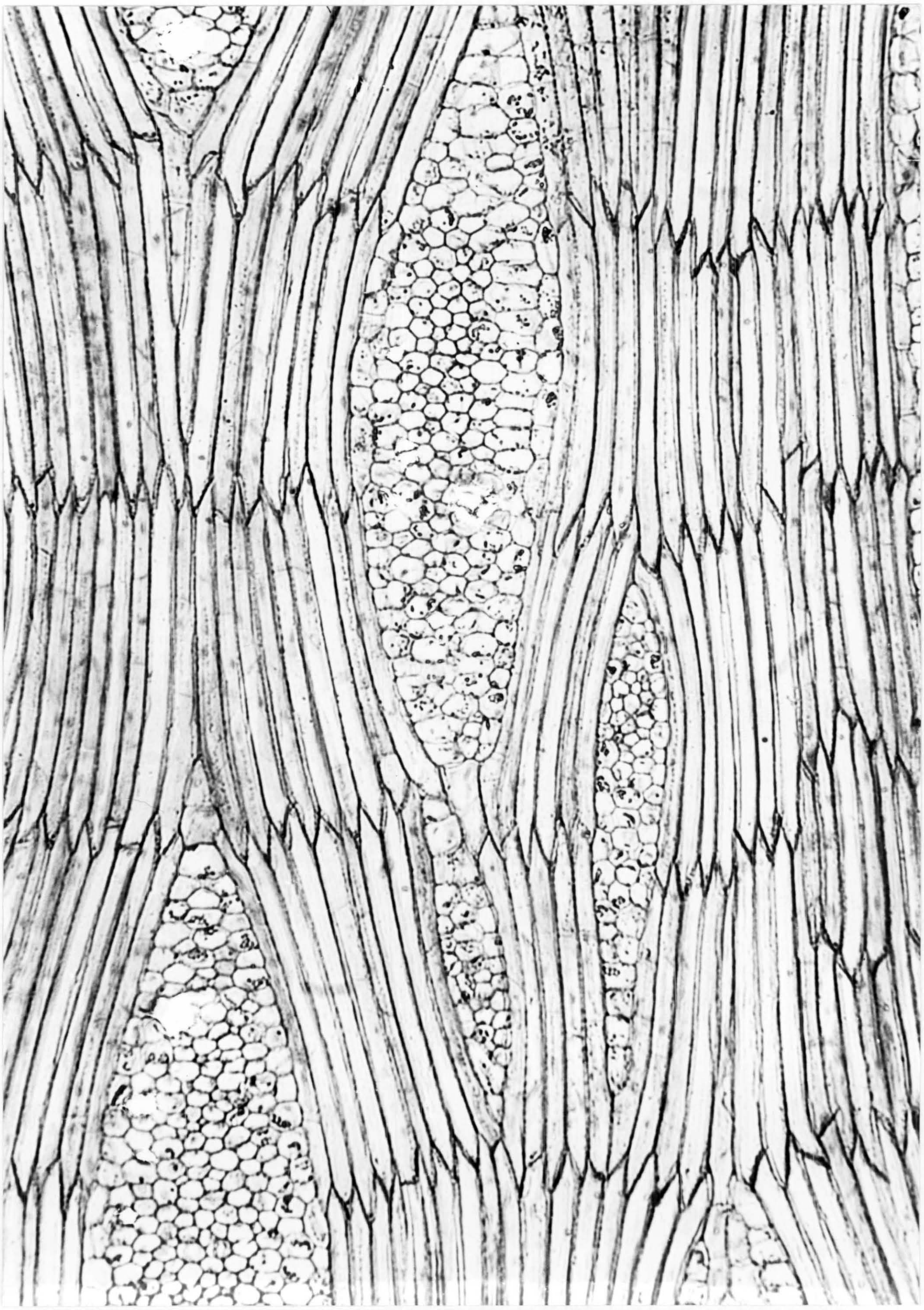


Fig. 2-21. Tangential longitudinal section of the mature vascular cambium of Hoheria angustifolia. The short fusiform initials are in distinct horizontal tiers. This section was cut from a stem with a cambial radius of approximately 6 cm. X 180

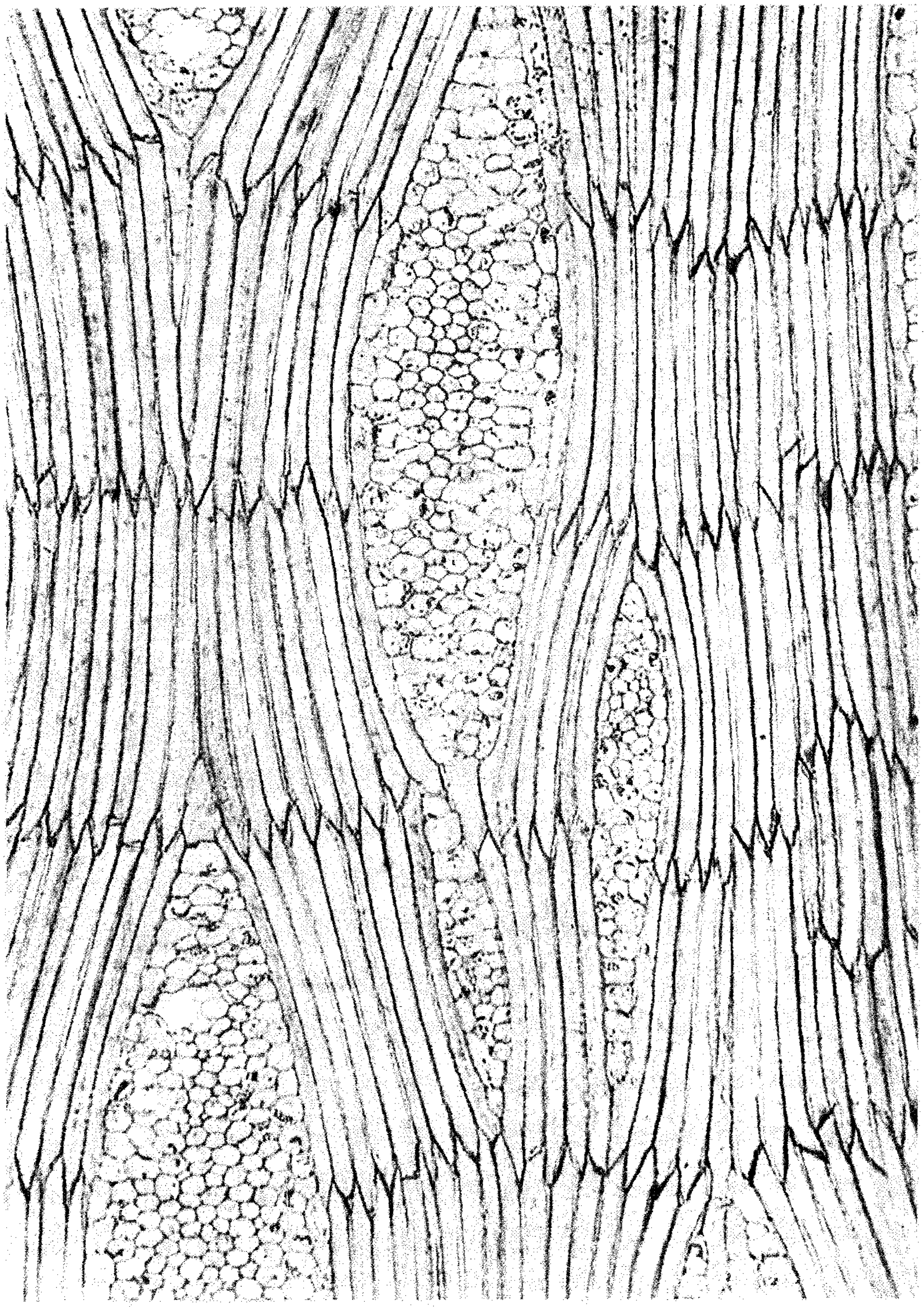


Fig. 2-21. Tangential longitudinal section of the mature vascular cambium of Hoheria angustifolia. The short fusiform initials are in distinct horizontal tiers. This section was cut from a stem with a cambial radius of approximately 6 cm. X 180

by a series of transverse divisions (Fig. 2-20c). This method continues to provide a source of new uniseriate rays as radial growth proceeds, though divisions off the ends or sides of individual fusiform initials are a less common source of new rays in larger stems. Longitudinal divisions of the individual ray initials in uniseriate rays produce new multiseriate rays. These in turn increase in size by further division of their initials or by the addition of new cells from an adjoining fusiform initial that has undergone segmentation.

2.5 DISCUSSION

The observations of this study on Hoheria angustifolia further emphasize the difficulty in separating the procambium and the cambium as distinct meristems, with the former responsible for the primary and the latter for the secondary vascular tissues of the stem.

Although internodal elongation ceased by about the fifth or sixth internode behind the shoot tip in these plants grown under controlled growth conditions, some of the characters associated with a vascular cambium were evident within the internodes that were still elongating. Other characters were first evident in older internodes below the zone of elongation.

Radial seriation of the procambial cells resulting from repeated early periclinal division develops near the top of the shoot tip, at a distance of less than 2 mm from the apical meristem. As a consequence the vessels and parenchyma cells of the primary xylem and their mother cells also develop in radial files. The occurrence of repeated periclinal division in the procambium has already been reported for a number of plants by Esau (1936, 1938, 1942, 1943b), Sterling (1946, 1947), Parke (1963), Thompson and Heimsch (1964), Soh (1972, 1974) and Fahn *et al*. (1972). The cells of the protophloem in *Hoheria angustifolia*, however, do not lie in radial files. A cylinder of 6-8 periclinally dividing procambial cells is well established by the base of the shoot tip and continues in a similar form right through into secondary growth. In this respect the procambium closely resembles the vascular cambium in appearance and division cycle.

It is common for the procambium in gymnosperms and dicotyledons to first appear as a system of discrete strands separated by interfascicular areas. The linking of the fascicular meristems in each strand usually occurs following the onset of secondary growth by the development of sectors of interfascicular cambium from the parenchyma cells between each primary vascular bundle. There are, however, a number of plants where the procambium forms an almost complete cylinder from its earliest appearance. In such cases the primary phloem usually differentiates as a complete cylinder also (Thompson and Heimsch, 1964; Cumbie, 1967b; Soh, 1974). Although the procambium forms an almost complete cylinder from a point very close behind the apical meristem in *Hoheria angustifolia*, the protophloem in this plant first appears in discrete strands separated by interfascicular sectors.

Differentiation of the primary xylem in *Hoheria angustifolia* occurs in two stages. Initially a number of protoxylem bundles develop to the inside of the procambial cylinder. These are gradually linked into a complete cylinder of primary xylem by the differentiation of the derivatives of the actively dividing procambium.

Soh (1972) noted that the radial dimensions of the fusiform cambial initials were slightly less than those of the procambial cells due to the increased frequency of periclinal division in the former. If such size changes occur in *Hoheria angustifolia* they were not evident in the material examined.

Perhaps the most striking similarity between the procambium and the cambium resulting from this repeated periclinal division, is the progressive differentiation of new vascular tissues to the inside and outside of the actively dividing cylinder. From its inception in the shoot tip, to the centre of the sixth internode (by which time it may be assumed that internodal elongation has ceased), the procambial cylinder and its primary phloem and primary xylem derivatives have increased in radial extent by about 6 times.

A characteristic of the vascular cambium of most arborescent plants is the existence of two systems of cells, the axially elongated fusiform initials being responsible for the production of the axial elements of the secondary xylem and secondary phloem, and the shorter ray initials being responsible for the production of the horizontal ray system.

On the other hand, the procambium has traditionally been thought of as a more homogeneous tissue (Esau, 1943b, 1965a; Catesson, 1964). The observations of Cumbie (1967b) and Soh (1972, 1974), however, suggest that this may not always be the case. Whether Hoheria angustifolia has one or two systems of cells in the procambium depends on the interpretation of the narrow interfascicular sectors. These sectors are present from the very earliest appearance of the procambium at the top of the shoot tip (Fig. 2-5) and pass from the pith into the cortex dividing the protophloem into strands. Periclinal division within these interfascicular sectors begins by the first internode below the shoot tip so that a complete cylinder of dividing procambium exists from a very early stage.

One interpretation of these sectors is that they are true interfascicular medullary rays across which the procambium links to form a complete cylinder at a very early stage of primary growth. If this is the case, then the procambium in Hoheria angustifolia develops from fascicular and interfascicular sectors in much the same way as the vascular cambium develops in plants that retain discrete vascular bundles throughout their primary growth. Alternatively, the interfascicular sectors can be interpreted as systems of short cells, similar in form and activity to medullary rays but forming an integral part of the procambial cylinder. The presence of two systems of cells has already been reported at a very early stage of development in the procambium of Canavalia (Cumbie, 1967b) and at a later stage in the procambium of Ginkgo (Soh, 1972) and Weigela (Soh, 1974). Anatomists who believe that the procambium at its inception must form a system of strands to the developing leaf primordia will prefer the first interpretation. If this is the case, then they will need to concede that the procambium links across the interfascicular sectors at a very early stage in development. On the other hand, if the procambium is thought of as a complete cylinder from its inception, then in Hoheria angustifolia it must contain two systems of cells - long primordial fusiform cells and groups of short primordial ray cells. It is unfortunate that sectioning difficulties made observations of the lengths of the interfascicular sectors difficult in the region of the shoot tip. By the time that they could be clearly seen (in the longitudinal sections of the first internode) they certainly did not extend the full length of the internode as might be expected of the medullary rays. It is possible, however, that they could undergo some separation during internodal elongation. The most likely interpretation is that the procambium in *Hoheria angustifolia* first develops as a series of discrete strands with protophloem elements differentiating in the exarch position of each strand (Fig. 2-5). The first protoxylem strands differentiate to the inside of a number of protophloem strands and their interfascicular sectors. Periclinal division in the procambial cells begins at a very early stage in both the fascicular and the interfascicular sectors so that a complete cylinder of procambial cells exists from the base of the shoot tip. This cylinder continues periclinal division and derivatives from it differentiate into complete cylinders of metaxylem and metaphloem. The cells of the fascicular sectors are long and consitute primordial fusiform initials. The cells of the interfascicular sectors form groups of primordial ray initials.

Separation of the procambial cells into two systems is maintained throughout internodal elongation by a higher frequency of transverse division in the short cells of the interfascicular sectors than in the longer procambial cells. It would seem likely that a separation of the procambium into short and long cells might well be a feature of other plants where the primary xylem forms a complete cylinder prior to the onset of secondary growth.

Although most longitudinal divisions in the long primordial fusiform procambial cells of *Hoheria angustifolia* are truly radial longitudinal, the frequent yet irregular occurrence of transverse division prevents the early establishment of a storeyed pattern. This storeyed pattern does not become evident in *Hoheria angustifolia* until considerable radial growth has occurred and is likely to be more closely related to the radial distance from the stem centre than to the internodal number behind the apex.

Cumbie (1967b) distinguished between the long cells of the procambium and the fusiform initials of the cambium on the basis of differences in the shape in their end walls. In *Canavalia* he found that most of the long cells of the procambium had transverse end walls, whereas the cambial fusiform initials had abruptly tapering pointed tips. In *Hoheria angustifolia* the end walls of the fusiform initials are not markedly pointed until at least the ninth internode. Pointed ends are gradually acquired following the cessation of transverse division in the long cells at the end of internodal elongation, followed by repeated periclinal and occasional anticlinal division. In this respect *Hoheria angustifolia* resembles *Nicotiana* (Esau, 1938), *Canavalia* (Cumbie, 1967b) and *Phaseolus* (Enright and Cumbie, 1973).

New ray initials in the vascular cambium of *Hoheria angustifolia*, resulting from the segmentation of fusiform initials, first appear about the seventh internode. The subsequent development of new rays is necessary to maintain the fusiform to ray initial balance as the cambial circumference expands with radial growth. The appearance of new rays in the cambium, however, is not necessarily a reliable indication that secondary growth has commenced. New rays are known to appear in the procambia of *Canavalia* (Cumbie, 1967b), *Ginkgo* (Soh, 1972) and *Phaseolus* (Enright and Cumbie, 1973) by the segmentation of long procambial cells before these cells become transformed into fusiform cambial initials.

Like *Canavalia* (Cumbie, 1967b), *Hoheria angustifolia* is a plant in which the procambium and vascular cambium are similar in many respects. Vascular tissues differentiate from the products of repeated periclinal division in both meristems. Both meristems are also organized into two systems of cells. The longer transverse ended cells of the procambium gradually become transformed into fusiform cambial initials while the shorter cells become transformed into cambial ray initials. Together these cells form a complete cylinder of meristematic tissue separating the xylem and phloem from a very early stage of shoot development. Anticlinal division occurs in both the procambium and cambium by means of radial longitudinal divisions producing new daughter cells side by side in tangential view. This storeyed pattern is not retained in the procambium due to the subsequent transverse divisions that accompany internodal elongation. The development of a storeyed pattern in the fusiform initials of the vascular cambium, however, is not dependent on a storeyed pattern within the axially elongated cells of the procambium.

Some differences between the procambium and cambium in Hoheria angustifolia are evident. Because the procambium is active at a time when the internodes are elongating, its cells maintain their length by transverse division. Although the nature of the secondary xylem of Hoheria angustifolia prevents the sequence of such divisions from being observed in the derivatives of the vascular cambium, measurements of the mean lengths of vessel members (Section 4 of this thesis) indicate that the transverse division of fusiform cambial initials is likely to be a very rare event. The axially elongated cells of the two systems also differ in the shape of the end walls. The transverse ends of the long procambial cells again are a consequence of the transverse divisions occurring within these cells. The pointed ends of the fusiform cambial initials in Hoheria angustifolia are acquired gradually and do not appear immediately on the cessation of internodal elongation but rather as a product of radial growth.

The observations of this study support the view that the procambium and cambium are best regarded as two developmental stages of the same meristem. Although some differences are evident between the mature storeyed cambium and the non-storeyed procambium, these differences are directly attributable to the primary elongation of the internodes behind the apical meristem. The transition from procambium to cambium is a gradual one with the meristem taking on the characteristics of the latter over a number of internodes, some before and some after internodal elongation has ceased.

SECTION THREE

DEVELOPMENTAL CHANGES IN THE VASCULAR CAMBIUM OF *AESCHYNOMENE HISPIDA* WILLD. WITH RADIAL GROWTH.

3.1 SUMMARY

Developmental changes in the vascular cambium of *Aeschynomene hispida* Willd. were determined from an analysis of transverse and tangential longitudinal sections of the secondary xylem. Increase in the number of fusiform cambial initials in this plant is brought about by two types of anticlinal division. The majority of these divisions are radial longitudinal contributing to the number of fusiform cells in the tangential plane. Occasional transverse division of fusiform initials can add cells in the vertical plane. Apart from a slight rearrangement of the cell tips, there is no intrusive growth of the daughter cells following either type of division. Repeated radial longitudinal divisions produce a highly developed storeyed pattern of the fusiform initials when viewed in tangential section, following radial growth. The frequency of radial longitudinal division, recorded as a percentage of the fusiform initials that divide for each millimetre of radial growth, declines with radial distance from the stem centre. The mean length of the fusiform initials remains

fairly constant with radial growth but the tangential width of these cells increases rapidly over the first 10 mm and then remains constant. An apparent deficiency in the frequency of anticlinal division over the first few millimetres of radial growth is compensated for by an increase in the tangential dimension of the fusiform initials. No loss of fusiform initials occurs other than that resulting from their transformation into ray initials. Both uniseriate and multiseriate ray initials generally occupy similar tangential dimensions to neighbouring fusiform initials. New rays develop by the subdivision of individual fusiform initials. The secondary xylem produced by the cambium is unusual in that the axial parenchyma cells, vessel members and fibres all remain largely within the confines of the storeys.

3.2 INTRODUCTION

The production of secondary xylem by periclinal divisions in the vascular cambium results in a progressive increase in the circumference of the cambial cylinder. To keep pace with this expansion, the fusiform initials of the cambium undergo occasional anticlinal division. In the gymnosperms and in many of the dicotyledonous plants, this division involves the development of an oblique cell plate which divides the parent fusiform initial into two shorter daughter cells. Following this pseudotransverse division, the two daughter cells elongate by intrusive growth thereby expanding the cambial circumference (Bailey, 1923). This process of pseudotransverse division followed by intrusive growth produces the non-storeyed type of cambium. The developmental changes associated with this process in the gymnosperms, including the division cycle, the elongation of the daughter cells and the loss of some of these from the cambium has been intensively studied in recent years (Bannan, 1950, 1957a, 1957b, 1968; Whalley, 1950; Hejnowicz, 1961,1964; Scrivastava, 1963b). There have also been a number of studies of the developmental changes in the vascular cambium in dicotyledons. These include investigation on *Pyrus communis* (Evert, 1961), *Liriodendron tulipifera* (Cheadle and Esau, 1964), *Leitneria floridana* (Cumbie, 1967a), *Hibiscus lasiocarpus* (Cumbie, 1963; Walker and Cumbie, 1968), *Polygonum lapathifolium* L. (Cumbie, 1969a) and *Apocynum sibiricum* L. (Cumbie, 1969b). While the nature of the anticlinal division and the amount of cell loss following division varies between these plants, each of them essentially has a non-storeyed cambium. A number of the more highly advanced dicotyledons possess vascular cambia where the fusiform initials are in definite storeys. In this type of cambium, anticlinal division takes the form of a more truly radial longitudinal division and there is no intrusive growth of the daughter cells following division.

Compared with the investigations that have been undertaken on the non-storeyed cambium, the storeyed cambium has been somewhat neglected. The only comprehensive account is that of Beijer (1927).

The increasing circumference of the cambium also requires that the number of ray cells be continually increased if the relationship between the axial and radial elements is to be maintained. The origin of new rays in gymnosperms has been studied in detail by Bannan (1934, 1951, 1953 and 1956), Barghoorn (1940a) and Scrivastava (1963a, 1963b) and in angiosperms by Barghoorn (1940b, 1941a, 1941b), Evert (1959, 1961), Cumbie (1963) and Cheadle and Esau (1964).

In many species, a new secondary ray arises from a segment cut off one side of the parent fusiform initial, or it may arise from the end of the fusiform by a transverse division. The ray then increases in height by transverse divisions, and in width by radial divisions. Occasionally new cells are added to the side of a ray from a neighbouring fusiform initial. New rays may also develop by the partial or total segmentation of a fusiform initial or from the surviving cell of a declining fusiform initial.

The relative frequency of the various types of ray origin varies considerably in both gymnosperms and angiosperms. The only trend that has emerged from the literature is the increasing tendency for ray origin to occur through segmentation with increasing structural specialization, shortening of initials and the trend towards decreasing loss of fusiform initials (Philipson *et al*., 1971). In the primitive dicotyledon *Liriodendron* (Cheadle and Esau, 1964) the segments of the subdivided fusiform initials undergo reduction in height with the loss of some segments. In *Leitneria* (Cumbie, 1967a) long initials may decrease in height with some loss of segments but the shorter initials undergo segmentation without reduction in height. In *Hibiscus* and *Polygonum* (Cumbie, 1963, 1969a) there is little or no reduction in height and no loss of segments.

While segmentation of a fusiform initial to form a uniserate ray represents a loss in multiplicative dividing power for the cambium, the ray initials themselves can make a significant contribution to the expanding cambial circumference by themselves dividing radially to form a multiseriate ray.

This section of the thesis describes the developmental changes occurring within the vascular cambium of *Aeschynomene hispida* (Papilionaceae). This plant is unusual in that none of the xylary derivatives of the vascular cambium - vessel elements, fibres or parenchyma strands, undergo any appreciable intrusive growth during differentiation. The cells of the wood replicate the pattern of the cambial initials, remaining in neat horizontal rows when viewed in tangential longitudinal section. It also has the advantage of having narrow rays whose tangential dimensions do not exceed those of the fusiform initials. Its wood, therefore, is a particularly ideal tissue in which to study the frequency and nature of the anticlinal divisions in the fusiform initials, and the subsequent cell adjustments that lead to the production of a storeyed cambium.

3.3 MATERIALS AND METHODS

Material of Aeschynomene hispida, collected from the British Honduras, was kindly sent to this Department by Dr C.R.Metcalfe of the Royal Botanical Gardens, Kew. The date of collection is not known. A block of stem material 48 mm in diameter was used as the basis for this study. From this, a rectangular block 12 mm wide by 6 mm deep was cut to include the pith and the wood on both sides of the stem. This was further divided into eight blocks as indicated in Fig. 3-1. These blocks were prepared for sectioning using standard paraffin methods. A small number of transverse sections were first cut off each block and then after remounting on the microtome, each block was completely cut into serial tangential sections of 12 μm thickness. These sections (approximately 4,000 in number), after mounting, were stained according to standard safranin - fast green procedures and mounted in DePex.

In order to study the developmental changes that had

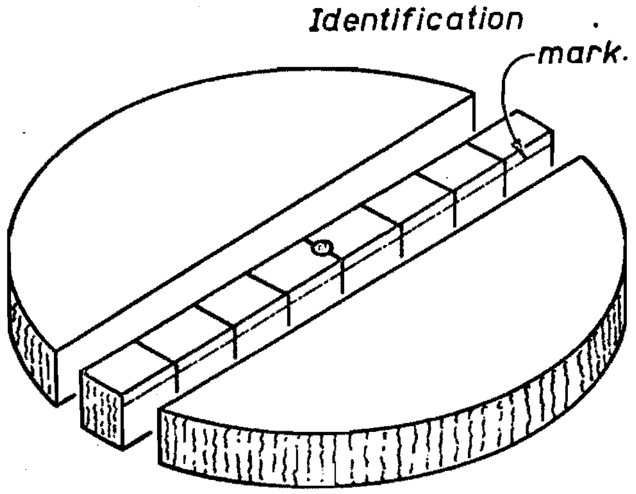


Fig. 3-1. A diagram to illustrate the way in which the stem material of Aeschynomene hispida was divided into eight blocks for sectioning. Transverse sections were first cut off the blocks and then after remounting, each block was cut into serial tangential sections. A small line cut along the side of the blocks assisted orientation under the microscope.

occurred in the cambium as it progressed outwards in the stem, a number of techniques were employed. The transverse sections were placed in an overhead projection arrangement and photographic prints made directly from the image of the slides. About twelve photographs were needed to cover each section and these were assembled to form a photograph mosaic of the stem measuring approximately 4 metres by 76 cm. By tracing the files of cells in these photographs, the positions of the anticlinal divisions and any subsequent cell loss could be detected (Fig. 3-2).

The serial tangential sections were also examined using the overhead projector. The developmental changes in the cambium were studied, in this case, by following the radially aligned derivatives in successive tangential sections. Two methods were employed. For the sections cut from one side of the stem, approximately every sixth section was photographed and files of cells studied using a cardboard template over the photographs. In the serial tangential sections prepared from the other side of the stem, one storey of cells was selected and drawings made of these cells in sections in which changes in the cell number or arrangement could be detected.

Cell length and width measurements were made using the overhead projection system, the image being projected onto a calibrated scale. Fifty cells were measured in each sample. Two arrows mounted on the projection screen enabled two cells

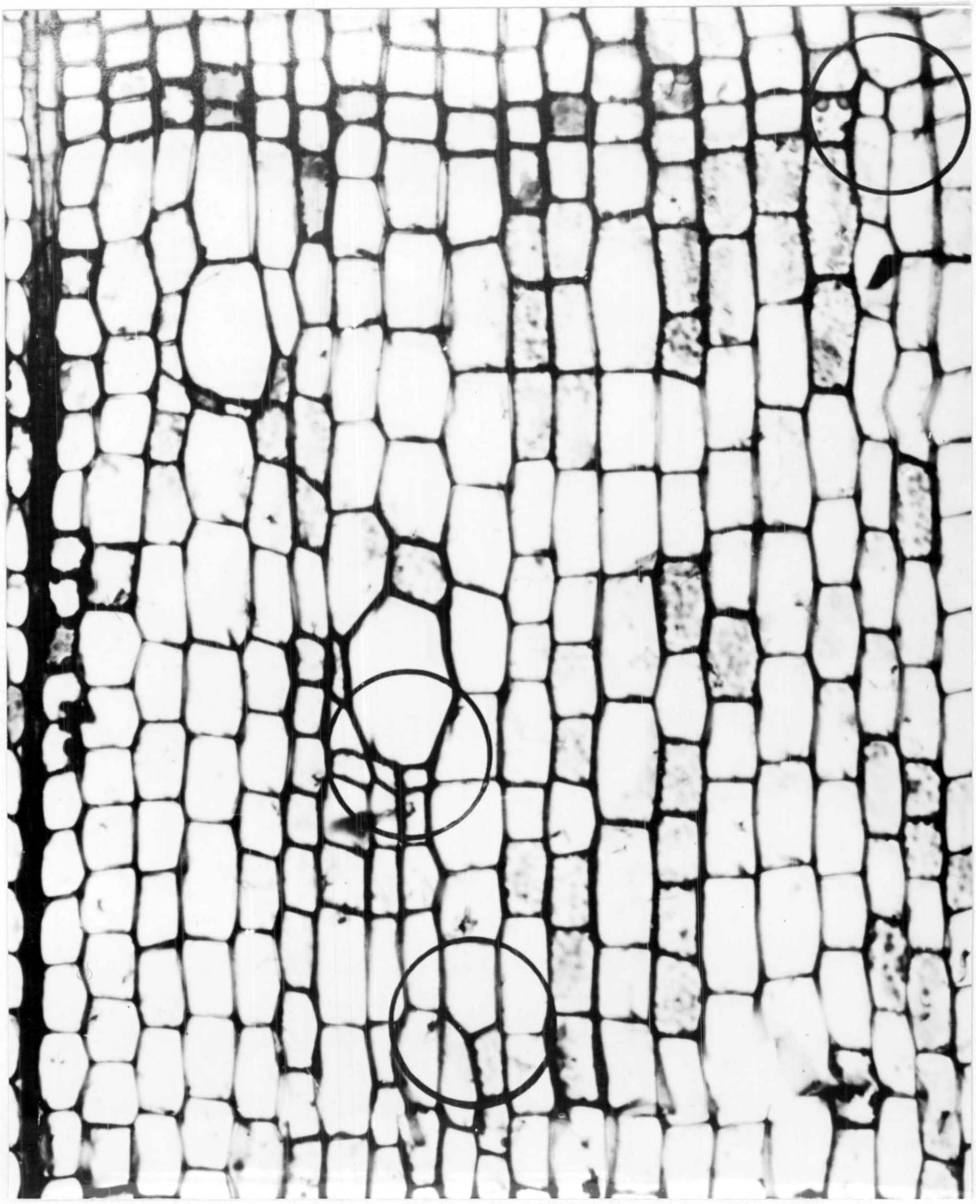


Fig. 3-2. A transverse section of the secondary xylem of Aeschynomene hispida. Because the majority of the anticlinal divisions in the cambium are radial longitudinal, the derivatives of the fusiform initials remain in neat radial files. Three radial longitudinal divisions are indicated by the circles drawn on the photograph. X 190

to be selected at random for length and width measurement from the tangential longitudinal sections. The image was then moved vertically through twenty-five storeys to obtain the full data.

3.4 OBSERVATIONS

The secondary xylem of Aeschynomene is very light, being made up largely of thin walled parenchyma cells. No growth rings were observed in the examined material. The parenchyma tissue is built up of vertically elongated cells, fusiform in outline, with hexangular tips (Figs. 3-3 and 3-4). In tangential section the cells reflect the storeyed pattern of the vascular cambium, the cell tips of one storey only slightly overlapping with the cells of vertically adjacent storeys. Interparenchyma pit-pairs are of the simple type. Some of the parenchyma cells, especially those close to vessels, show subdivision into two (Fig. 3-3), or occasionally more cells per strand. They may also contain crystals, the number of crystals per strand being usually between 8 and 11 (Fig. 3-5).

The vessels are solitary (Fig. 3-2), or very occasionally, in small radial multiples. The individual vessel elements remain within the storey and have simple perforation plates. Vessel to vessel pit-pairs are of the bordered type.

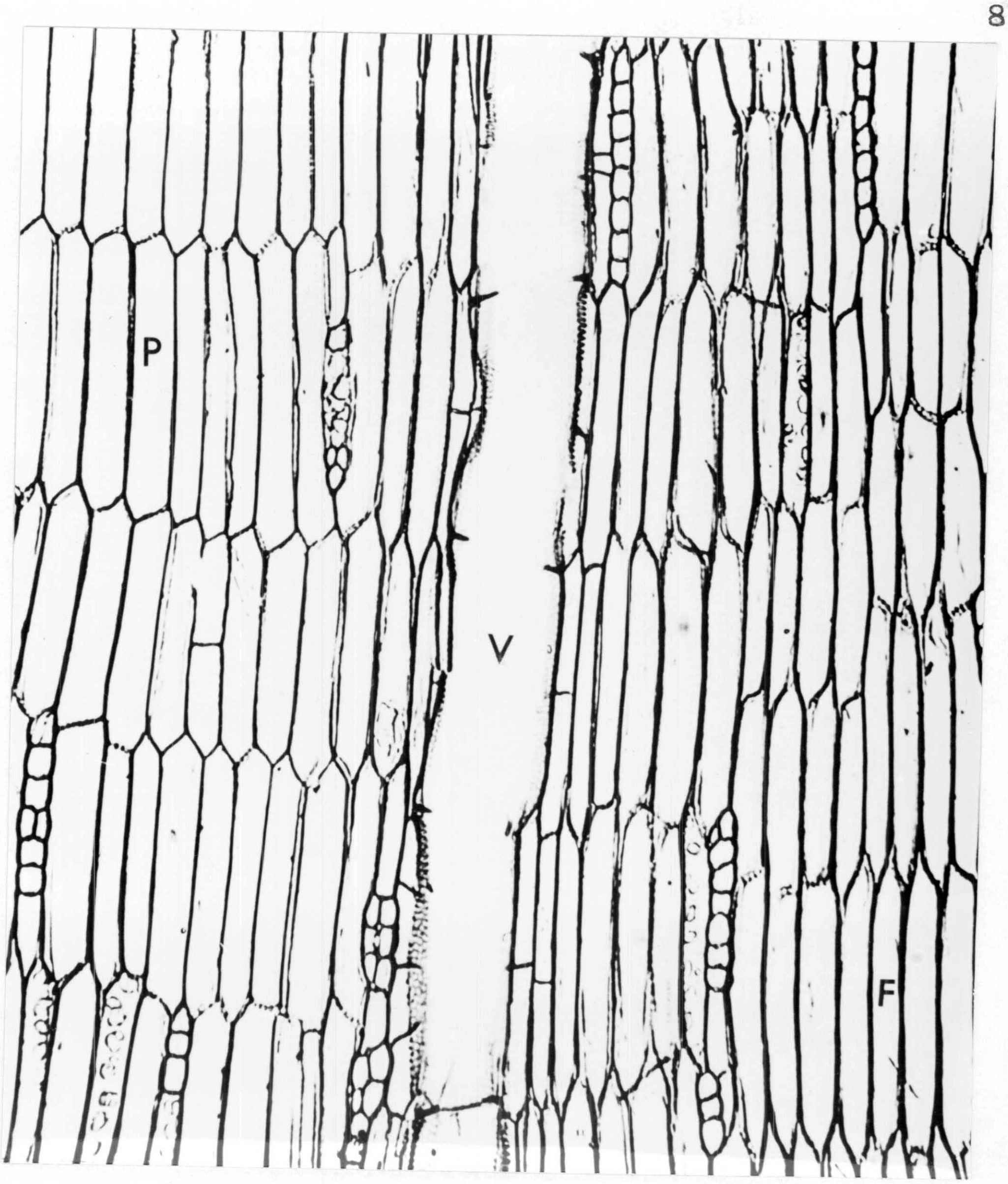


Fig. 3-3. Tangential longitudinal section through the secondary xylem. The axial parenchyma cells (P) that form the bulk of the wood are fusiform in outline with abruptly tapering ends. Subdivision of these cells into axial strands occurs only rarely and then mainly in those cells close to a vessel. The vessel members (V) and the main body of the fibres (F) also remain within the confines of the storeys. X 250

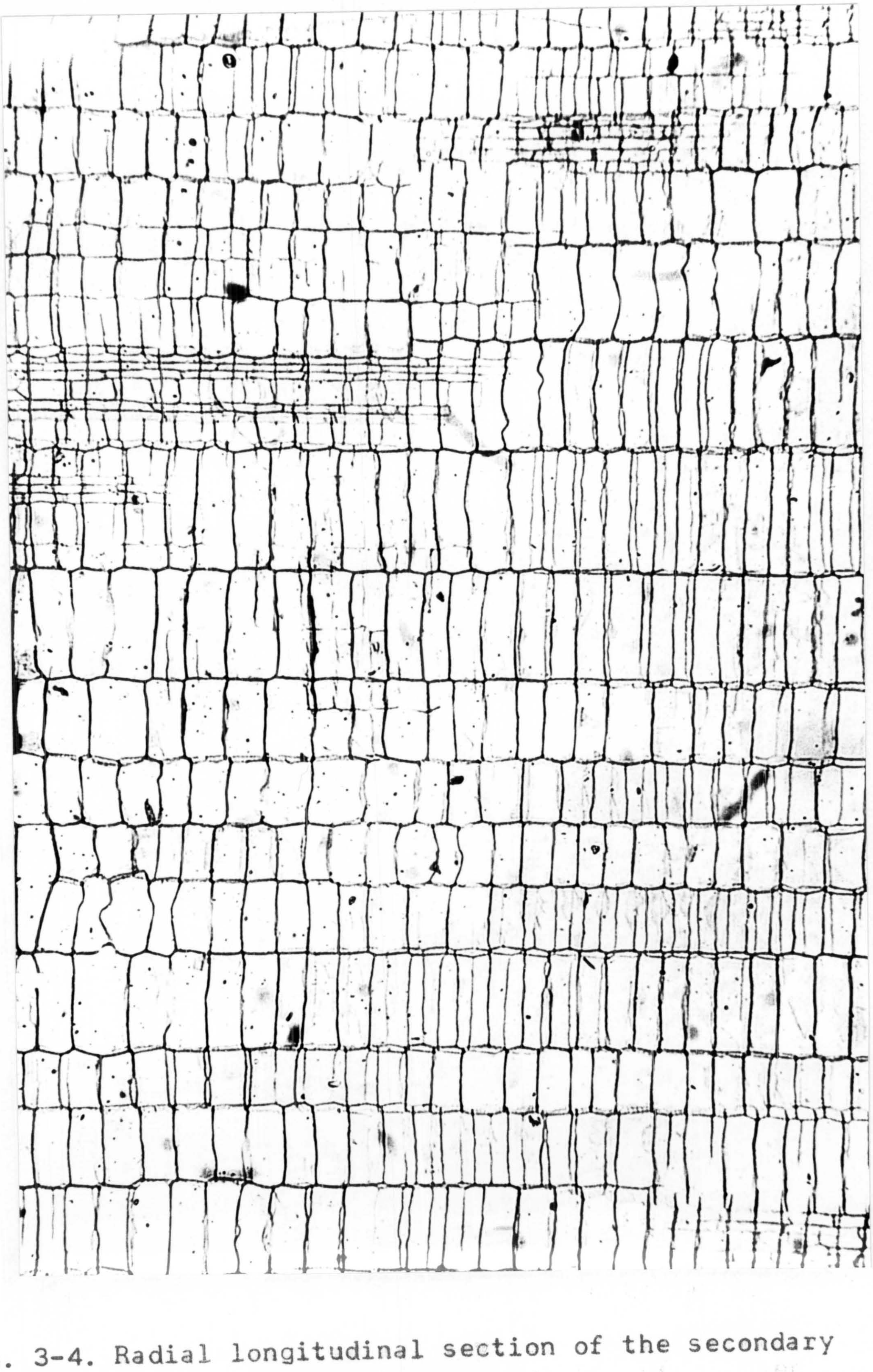


Fig. 3-4. Radial longitudinal section of the secondary xylem through parenchyma. X 77

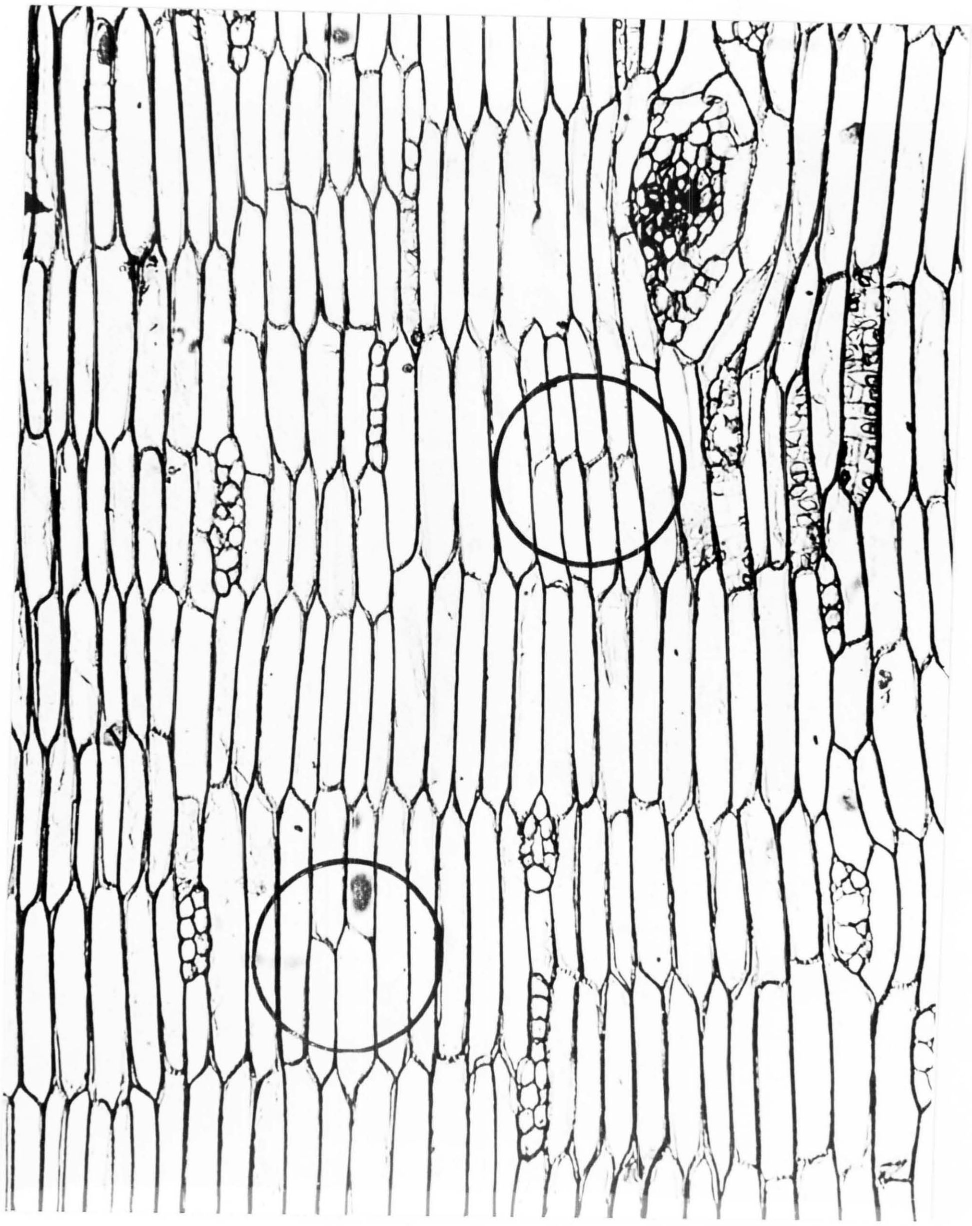


Fig. 3-5. The majority of the rays, both uniseriate and multiseriate, remain generally within the storeys and have similar tangential dimensions to the adjacent fusiform initials. A few large multiseriate rays with horizontal tracheary elements occur. The circles marked on the photograph indicate short storeys that have resulted from radial longitudinal divisions following a transverse anticlinal division. A number of parenchyma strands showing subdivision and containing crystals can also be seen in this photograph. X 170

The wood is unusual in that the fibres undergo comparatively little intrusive growth during differentiation. They are distributed around the vessels in an aliform to banded distribution when viewed in transverse section. In longitudinal section they can be distinguished from the parenchyma cells by their thicker, more easily stained walls and their more pointed ends (Fig. 3-3). Occasionally the tips of fibres intrude between the cells of the storeys immediately above and below. When this happens the main body of the cell still remains within the confines of the storey.

Both multiseriate and uniseriate rays are present, but the majority are uniseriate and confined to one storey or less in height. Some rays are multiseriate for part of their height only. Even when multiseriate, the rays rarely exceed the tangential width of the adjacent parenchyma cells. A few larger multiseriate rays also occur and these frequently possess horizontal tracheary elements (Fig. 3-5).

The pattern of anticlinal cell division in the cambium was clearly replicated in the cells of the secondary xylem examined. Except where large vessels obscured the pattern, the files of cells cut off the fusiform and ray initials could be readily traced in the tangential longitudinal sections. The examination of these sections indicated that in *Aeschynomene hispida*, anticlinal division in the cambium almost always takes the form of a radial longitudinal division extending the full length of the fusiform initial (Fig. 3-6b). This results in the two daughter cells lying side by side in tangential view after division and produces the storeyed cell arrangement of the initials. As a consequence of this repeated radial longitudinal division, the number of cells per storey shows an increase with radial distance from the stem centre (Fig. 3-7). More rarely a transverse division was observed, the new wall dividing the fusiform initial approximately into two. Generally such a division is the first stage in the development of a new ray, but it can also lead to the development of two storeys each about half the height of the adjacent storeys, if radial longitudinal division follows (Fig. 3-5). Although transverse divisions were not observed to have occurred simultaneously in two or more adjacent fusiform initials, this does not preclude such a possibility.

Ray counts made on the tangential sections indicated that the ray initials constituted only a small proportion of the cambial circumference at any given distance from the stem centre (Fig. 3-8), the percentage of the cambial circumference occupied by ray initials showing a decrease over the first few millimeters from the stem centre, and then remaining fairly constant at about 5%.

New ray initials are continually being added to the cambium as radial growth proceeds, in order to maintain this balance of ray to fusiform initials. In the majority

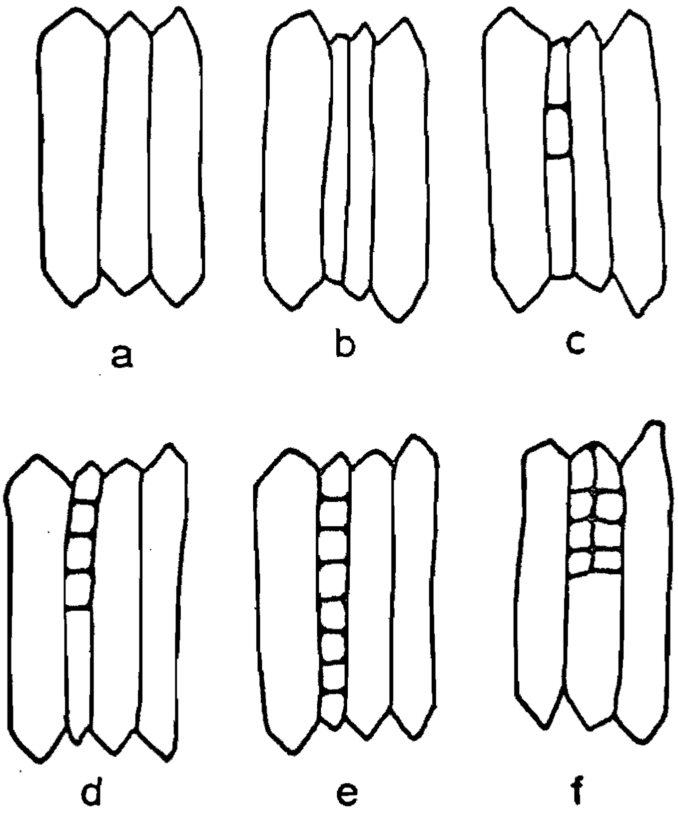


Fig. 3-6. (a) and (b): Stages in radial longitudinal division within the cambium as observed from the parenchyma of the secondary xylem. (c), (d) and (e): Stages in ray development. Following subdivision of the fusiform initial, the upper or the lower daughter cell undergoes further subdivision into two or four cells before the other daughter cell subdivides. (f): Multiseriate rays can occupy either half the height of the storey or by subdivision of the other daughter cell they may extend the full height of the storey. (Drawn from tangential longitudinal sections)

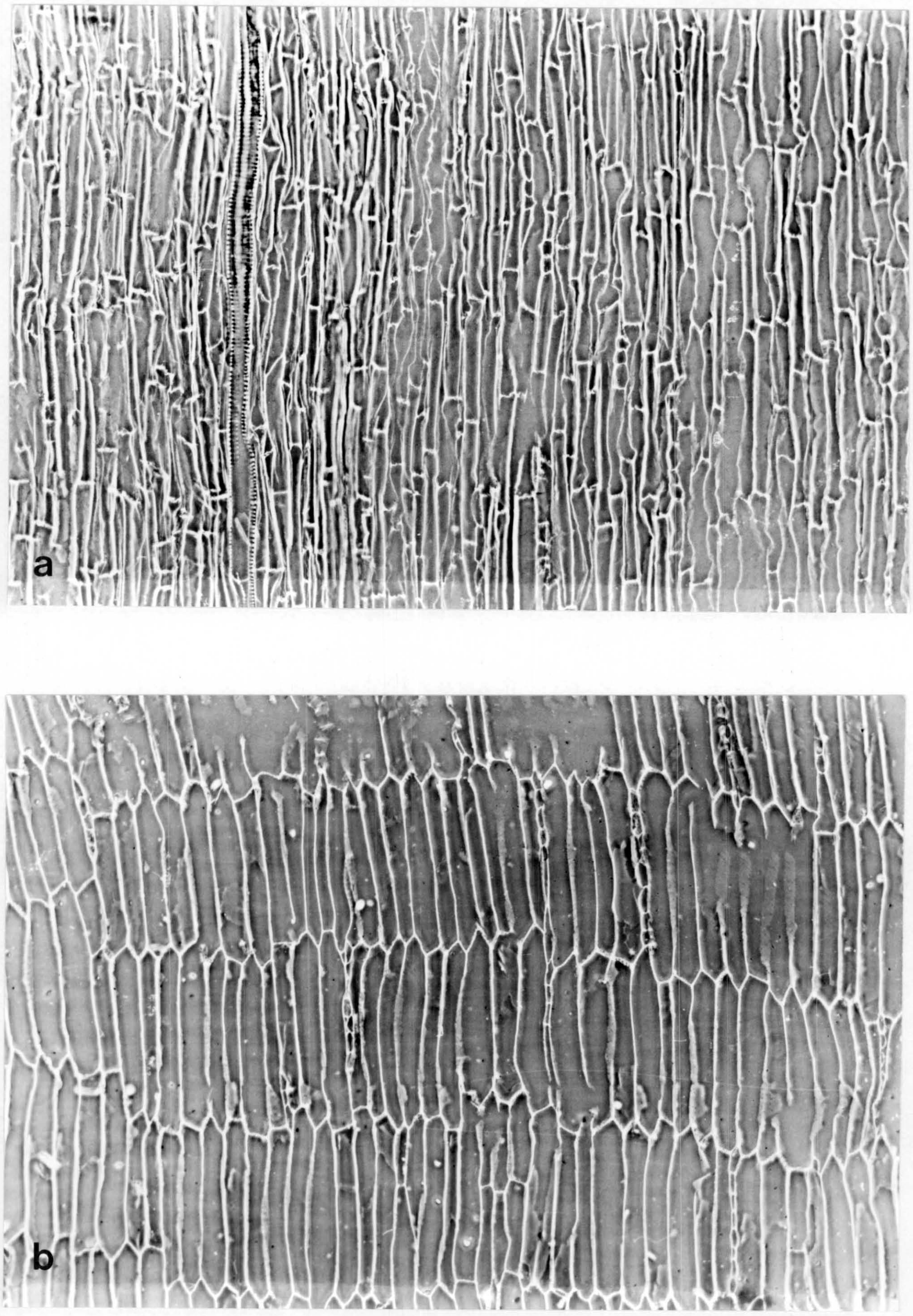


Fig. 3-7. Tangential longitudinal sections of Aeschynomene hispida: (a) at 2 mm radius and (b) at 23 mm radius. Both X 130

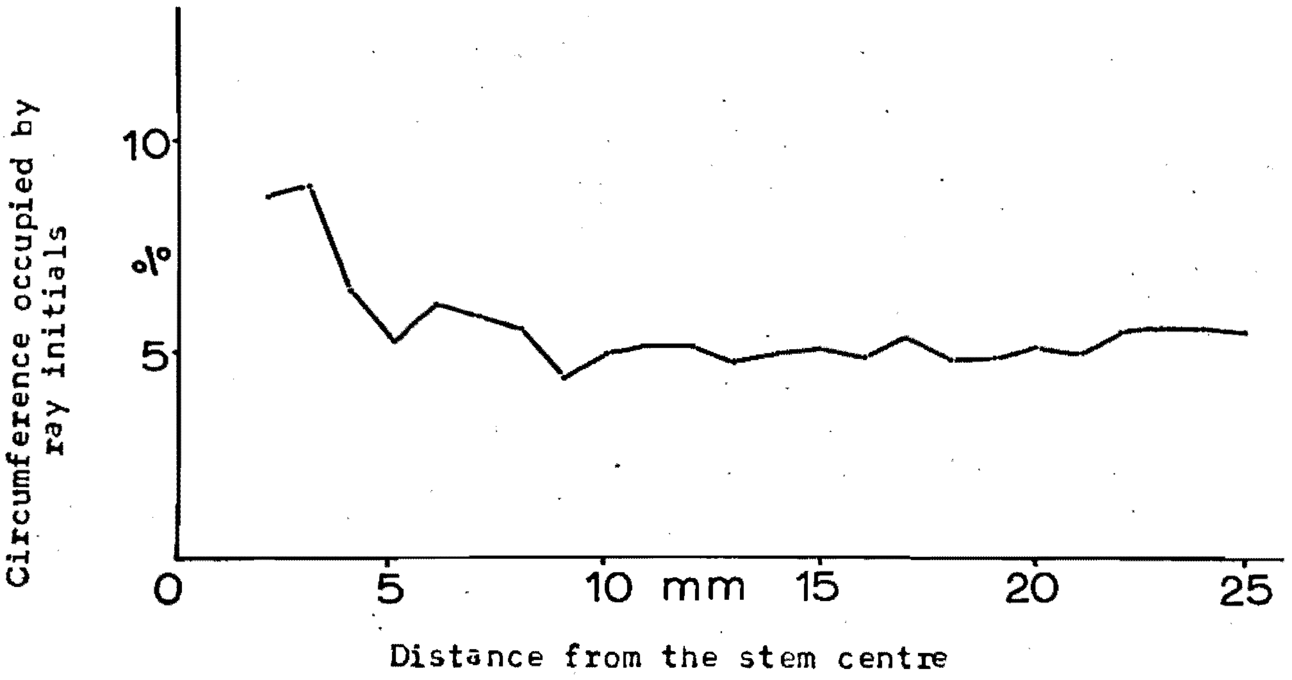


Fig. 3-8. The percentage of the cambial circumference occupied by ray initials plotted against radial distance from the stem centre.

of cases a new ray develops by the transverse division of a fusiform initial, dividing the parent cell approximately into two. The upper or the lower of the two daughter initials then further subdivides by transverse division, usually into two or four cells (Fig. 3-6 c and d). The ray produced may continue through a considerable number of periclinal divisions remaining, in effect, only half a storey in height. These ray initials may then divide longitudinally to give a multiseriate ray or the shorter fusiform initial below may subdivide to produce a ray equal in height to the adjacent fusiform initials (Figs. 3-3, 3-5, 3-6a and f). Ray initials, either uniseriate or biseriate, normally form a ray with similar tangential dimensions to neighbouring fusiform initials.

The frequency of radial longitudinal division in the cambium was determined from the photographs of the transverse sections. A sector subtending an angle of 24^{o} was marked out and counts made of the radial longitudinal division that had occurred within each millimetre of radial growth. The percentage of the fusiform initials that had divided during each millimetre of radial growth was calculated, after subtracting the rays from the original cell count on the basis that these are not adding anything additional to the new cambial circumference. A summation of the cell division for each five millimetres of radial growth is shown in Table 3-1, and the percentage of fusiform initials that had

Millimetres distance from stem centre	1	6	11	16	21	26
Total cell count within 24^{o} segment	30	87	139	218	287	399
Total number of rays	2	5	7	10	14	21
New rays	-	3	2	3	4	7
Total fusiform initials	28	82	132	208	273	378
New fusiform initials	-	54	50	76	65	105
Radial longitudinal division per fusiform initial	1.25	0.50	0.48	0.32	0.31	

Table 3-1. A summary of the number of fusiform and ray initials at 5 mm intervals from the stem centre.

undergone radial longitudinal division for each millimetre of growth is graphed in Fig. 3-11 line B. No loss of fusiform initials following radial longitudinal division was observed,

The mean length of parenchyma cells showed a slight increase followed by a slight decrease (Fig. 3-9) with increasing radial distance from the stem centre. These data are based on the tip to tip measurement of fifty parenchyma cells selected at random in the tangential longitudinal sections. The mean width of these cells showed an increase over the first ten millimetres from the stem centre and then decreased slightly outwards to the cambium (Fig. 3-10).

3.5 DISCUSSION

Most of our present information on changes occurring in the vascular cambium of seed plants has been obtained from investigations made on the secondary xylem, and not from direct examinations of the cambium itself. Since the cambial initials leave a record of their activities in these derivatives, the general sequence of changes in cell size and arrangement, that occur in the cambium with radial growth, can be determined by an examination of the secondary xylem. However, since the xylary derivatives of the cambium undergo elongation during their differentiation, data thus obtained are of necessity erroneous to the extent of any elongation or cell

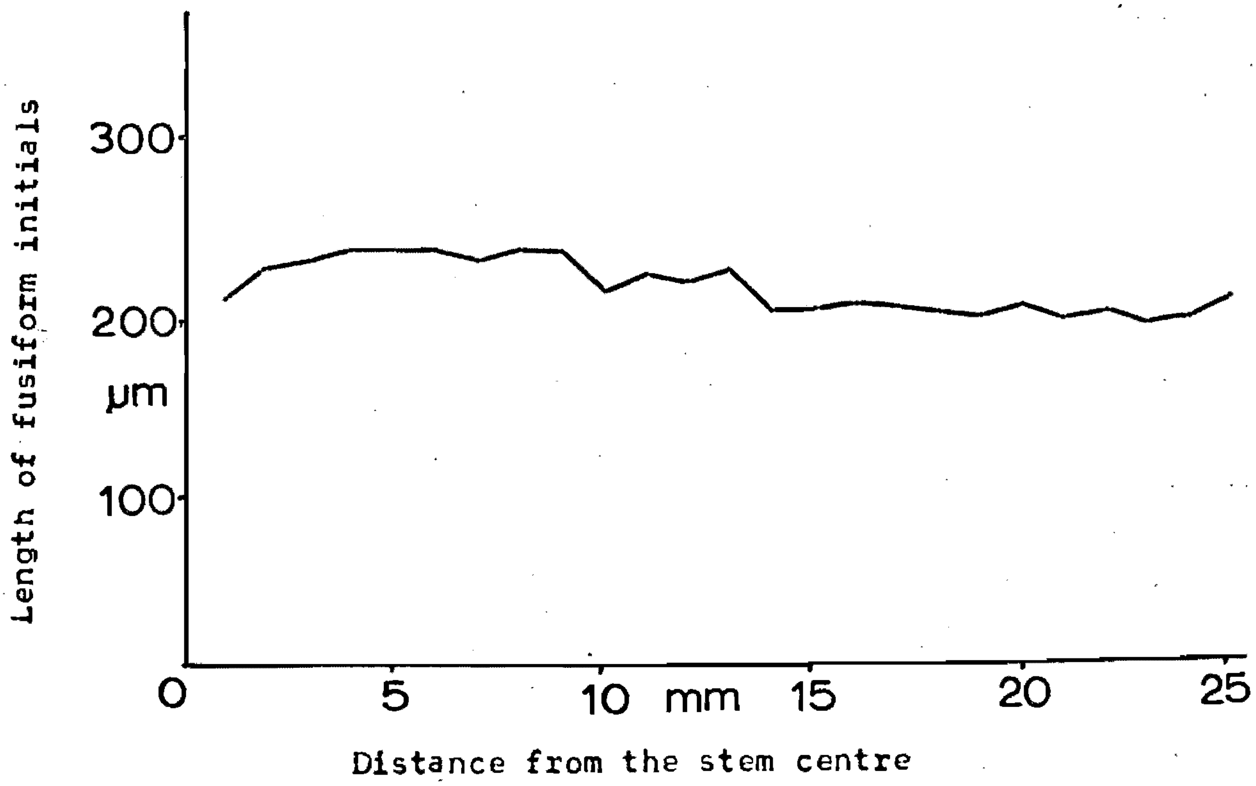


Fig. 3-9. Mean length of fusiform initials plotted against radial distance from the stem centre.

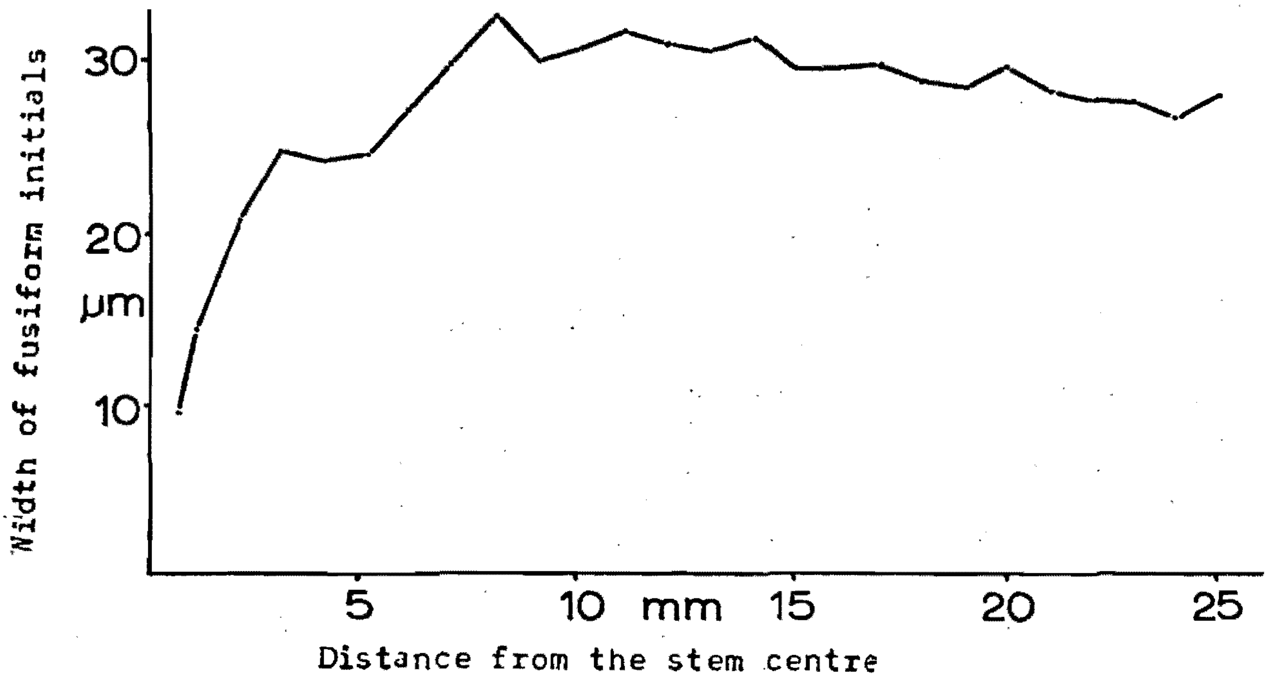


Fig. 3-10. Mean width of fusiform initials plotted against radial distance from the stem centre.

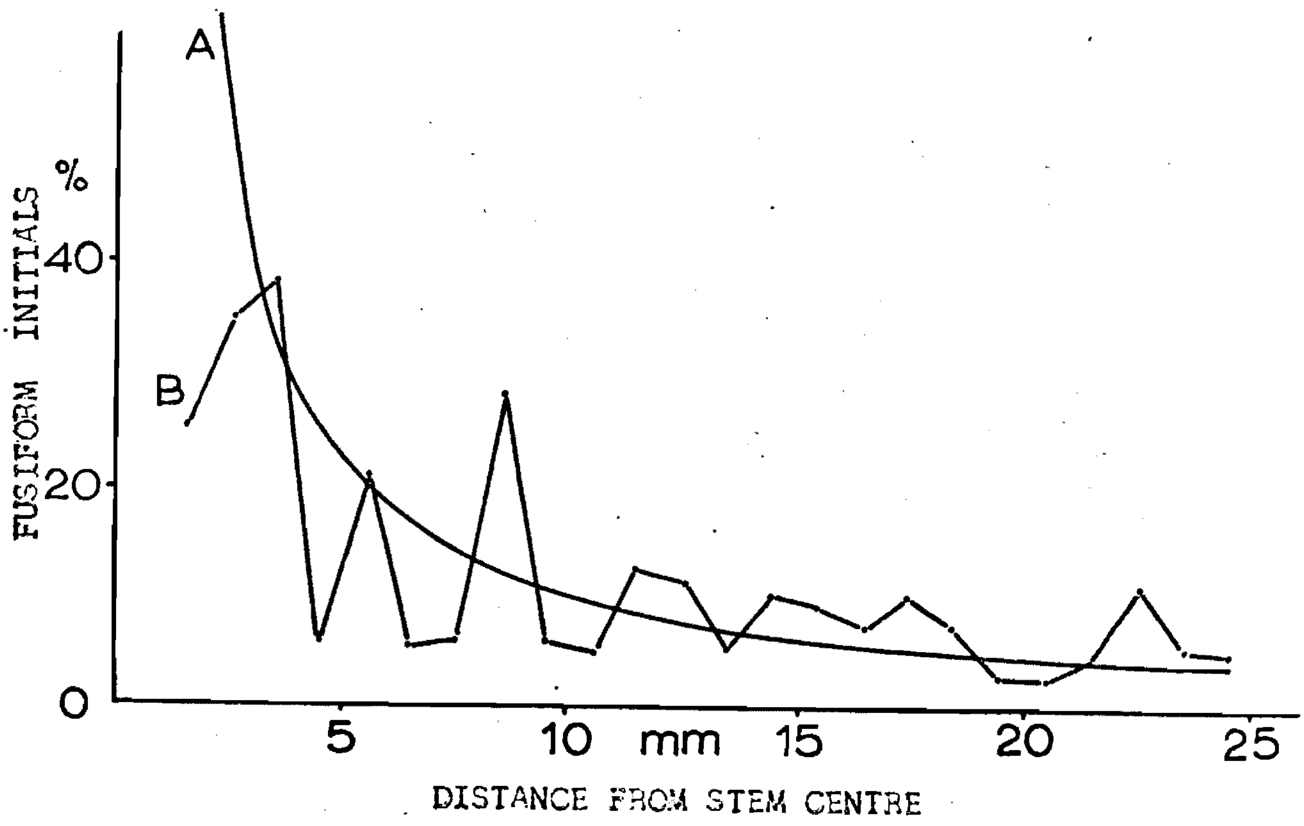


Fig. 3-11. Line A: The theoretical percentage of the fusiform cambial initials that need to divide anticlinally per millimetre of radial growth in order to supply the needs of the expanding cambial circumference. Line B: The measured percentage of the fusiform initials in <u>Aeschynomene hispida</u> that divided radial longitudinally per millimetre of radial growth.

rearrangement that has occurred after the derivatives have been cut off the cambium.

The bulk of the wood of *Aeschynomene hispida* is built up of axial parenchyma cells and vessel members which do not undergo any appreciable elongation with differentiation. These cells, therefore, replicate closely the basic cell pattern found in the vascular cambium of this species. Even the fibres do not show any appreciable elongation. Where their tips do intrude between the cells in adjacent storeys, the main part of the cell remains generally fusiform in outline within the confines of the original storey. It is possible, therefore, to study the developmental changes occurring in the vascular cambium relatively easily in this plant by examining transverse and tangential longitudinal sections of the xylem and cambial regions.

When a stem expands radially, the number of cells in the cambium is continually increasing to keep pace with the increasing circumference of the stem. In the gymnosperms and dicotyledonous plants with the non-storeyed arrangement of fusiform initials in the cambium, the processes responsible for the circumferential expansion of the cambium are complex because they involve the interplay of a number of factors; the frequency of pseudotransverse division, the amount and timing of the elongation of the new daughter cells to reach their final size, and the preferential loss of some of these new cells (Philipson *et al.*, 1971). *Aeschynomene hispida* is an interesting plant in which to study the frequency of anticlinal division in the cambium because the elongation of daughter fusiform initials, and the preferential loss of some of these, are eliminated by virtue of the nature of the division pattern within the storeyed cambium.

In *Aeschynomene hispida* anticlinal division in the fusiform initials of the cambia, in most cases, takes the form of a radial longitudinal division running the full length of the parent cell. Such divisions are easily detected in transverse sections of the secondary xylem as a doubling in the radial files of cells (Fig. 3-2). The occasional transverse division in the cambium does not add any new cells in the tangential plane of the expanding cambium, but does result in the production of a number of short storeys after further radial longitudinal division. Although such transverse divisions were only occasionally observed, they are probably responsible for the slight decrease in the mean cell length recorded with increasing distance from the stem centre (Fig. 3-9). No oblique anticlinal divisions were recorded. Ray development in *Aeschynomene hispida* occurs as the result of partial or total segmentation of a fusiform initial. Where only half of a fusiform initial undergoes segmentation, the undivided half survives but does not undergo any further anticlinal division (though it also may undergo segmentation at a later stage). Radial division within the segments of a subdivided fusiform initial occasionally produces a multiseriate ray. Such division may occur in all the segments of the ray, in only half of them or be confined to a single cell only. Both the uniseriate and the multiseriate rays occupy similar tangential dimensions as the fusiform initials from which they are derived. They represent, however, a loss in multiplicative dividing power to the cambium since neither type of ray undergoes any further radial division. The measured frequency of radial longitudinal division in the cambium of *Aeschynomene hispida*, as determined from observations on transverse sections of the secondary xylem, therefore accounts for all the anticlinal divisions that have in fact added in any way to the expanding circumference of the cambium.

Over the first few centimetres of radial growth the perimeter of the stem is increasing very rapidly in relation to the radius and a much higher rate of anticlinal division is required to provide new initials for the cambium than is required later in radial growth (Philipson and Butterfield, 1967). For a one millimetre increment of radial growth occurring from one to two millimetres from the stem centre, for example, the cambial circumference will have doubled. In a plant with a storeyed cambium, where anticlinal division is of the radial longitudinal type, in theory every fusiform initial will have divided at least once. As radial growth proceeds, however, the percentage of such cells that need to divide decreases. For one millimetre of radial growth between twenty and twenty-one millimetres from the stem centre, only one cell in every twenty fusiform initials in a storeyed cambium will need to divide. The theoretical frequency of anticlinal division is plotted in Fig. 3-11, line A. It is clear, therefore, that a higher frequency of anticlinal division in the cambium is required close to the stem centre than is required later in radial growth when the cambial cylinder has attained a greater diameter. This phenomenon has been recorded for a number of plants with non-storeyed cambia (Bannan, 1950, 1960a; Scrivastava, 1963b; Cumbie, 1967a) but has not previously been shown for a plant with a storeyed cambium. Its significance in the overall division cycle of the storeyed cambium has never been recorded.

The recorded results for anticlinal division in the cambium of *Aeschynomene hispida*, graphed as a percentage of fusiform initials undergoing division, are presented in Fig. 3-11, line B. This pattern corresponds closely with the theoretical curve on the same figure. Over the early part of radial growth there is an apparent deficiency in the production of new fusiform initials to meet the demands of the rapidly expanding cambial circumference. This would appear to be compensated for by the increase in the tangential dimensions of the cells over this period of radial growth (Fig. 3-10). Throughout the later part of radial growth, the frequency of anticlinal division in the fusiform initials is slightly higher than the theoretical figure because of the percentage of the cambial cylinder that is occupied by the ray initials. In *Aeschynomene hispida* these initials do not add to the cambial circumference by anticlinal division. Even when the rays become multiseriate they rarely exceed the tangential dimensions of the fusiform cells. The presence of these groups of ray initials partly explains the discrepancy between the observed and the theoretical frequencies of anticlinal division in Fig. 3-11.

The irregularity in the line for the recorded percentage of the fusiform cambial initials undergoing division is the result of a number of factors including the cyclic production of new rays (see Fig. 3-8), and a natural irregularity in the frequency of radial longitudinal division in the fusiform initials compensated for by slight changes in the mean tangential widths of the cells (Fig. 3-10). An apparently perfect balance between the supply and demand of new fusiform initials is thus maintained in this species and no loss of cells from the cambial radius occurs.

It is clear from this study that in plants with storeyed cambia the diameter of the cambium at the time of its initiation will have a direct bearing on the subsequent arrangement of the fusiform initials. The smaller the diameter of the cambium at its initiation, the higher will be the rate of anticlinal division required during the early stage of secondary growth. Such stems tend to have a more highly developed storeyed pattern at any given radial distance from the stem centre, in contrast to stems in which the cambium, as such, begins its activities outside a considerable thickness of pith and primary xylem tissues.

SECTION FOUR

VARIATION IN THE SIZE OF FUSIFORM CAMBIAL INITIALS AND VESSEL MEMBERS IN HOHERIA ANGUSTIFOLIA RAOUL.

4.1 SUMMARY

The lengths and widths of fusiform cambial initials and vessel members are examined in *Hoheria angustifolia* Raoul, a habit-heteroblastic tree with a storeyed cambium.

A significant correlation is shown to exist between the lengths of the fusiform cambial initials and the vessel members in the last formed xylem in a number of trees sampled at various heights.

No change in mean vessel member length could be detected with increasing distance from the stem centre in nine discs, though in one stem with a markedly eccentric growth, cell length decreased slightly with distance from the stem centre. Within the individual growth rings, the mean length of the vessel members tends to increase towards the late wood. This change is brought about by an increasing angle of the end wall in the late wood vessel members and is not a result of developmental changes occurring in the vascular cambium. It is, therefore, concluded that the fusiform cambial initials

in this species normally proceed without change in length from the onset of secondary growth.

Although individual trees showed a variety of cell length sequences, the means of fusiform initial lengths for fixed heights in a number of trees showed a slight decrease with increasing height in the tree. The analyses of variance indicated a significant difference between samples at various heights but not between trees, and showed that the linear decrease in cell length up the tree was responsible for most of the differences among the height samples.

Mean fusiform initial width showed an increase followed by a decrease with increasing height up the tree. Except at the lower levels in the tree, this appears to be related to the cambial radius.

4.2.1 INTRODUCTION

The literature relating to cell size variation in the secondary xylem of woody plants is vast (see reviews by Spurr and Hyvarinen, 1954; Dinwoodie, 1961; and Philipson *et al.*, 1971). In view of the importance of wood as a raw material this is not surprising. Factors such as cell length, width, and wall thickness have been examined in many tree species with respect to position in the tree, the age of the plant, genetic constitution of the species, and environmental factors.

The overall picture, nevertheless, is complex and the various findings are at times conflicting.

Since the time of Sanio (1872), whose findings for *Pinus sylvestris* are now regarded as "Sanio's Laws", wood anatomists have attempted to establish patterns of cell length variation within the growth ring, across the stem from pith to bark and up the tree. Attempts have been made to relate these findings to the rates of radial and terminal growth of the shoots. Seedlings have been subjected to various day length and temperature regimes in attempts to establish the causes of these variation patterns. Cell length, in particular tracheid and fibre length variation, has been the subject of most of the studies. By comparison, cell width and wall thickness variation patterns have received less attention. Because of their greater potential economic importance, coniferous tree species have been studied more intensively than have angiosperm species.

The length of the axial cells in the xylem is dependent on two factors, namely the length of the fusiform initials from which they are derived by periclinal division, and the amount of intrusive growth which they undergo during their differentiation. Unfortunately, it is not possible to separate these factors in an examination of the mature secondary xylem of most woods. Tracheids and fibres undergo quite appreciable amounts of intrusive tip growth during their differentiation, the actual amount varying with the season during which these cells are laid down. Vessel members and strands of axial parenchyma cells undergo fewer changes in length with differentiation and are, therefore, most likely to represent the sequence of size changes that have occurred within the vascular cambium. Few studies, however, have been made on the size variations of these cells or their relationship to the size of the fusiform cambial initials.

In addition to the size changes that result from varying amounts of intrusive tip growth of the xylary derivatives during their differentiation, differences in the length of these cells with position in the tree are also the result of changes that have occurred within the vascular cambium with radial growth. Once a population of cambial initials has become established at the end of primary growth, it will move horizontally outwards, normally dividing off new xylem cells to the inside and new phloem cells to the outside. This radial growth is accompanied by circumferential expansion of the cambium, a process which involves an increase in the number of fusiform initials. In plants with a non-storeyed cambium, this occurs by means of a pseudotransverse wall being laid down usually near the centre of the dividing cell. The two daughter fusiform initials so formed then elongate by the intrusive growth of their cell tips. The resultant size of the fusiform initials in the non-storeyed cambium is governed, therefore, by the frequency of pseudotransverse division and the rate and amount of elongation of the new initials. The preferential loss of short fusiform initials following pseudotransverse division adds a further complication.

The nature of the division cycle with circumferential expansion in plants with a storeyed cambium is such that one would expect to find little change in size of the fusiform initials with radial growth (Section 3). In these species, radial longitudinal divisions divide the parent fusiform cambial initials from tip to tip (Philipson *et al.*, 1971). Repeated anticlinal division of this type results in the fusiform initials lying in neat rows or storeys when viewed tangentially. Apart from the minor rearrangement of the cell tips (Beijer, 1927), daughter cells show little or no intrusive growth following such division. Indeed, if newly formed fusiform initials were to crowd past one another, the storeyed or stratified arrangement would rapidly be lost. Once laid down at the onset of secondary growth a population of fusiform cambial initials, therefore, should maintain a more or less constant cell length with radial growth in plants possessing storeyed cambia.

The object of this study is to examine the effect of radial growth on the apparent length of the fusiform initials using the vessel members as an indication of fusiform initial length, to relate the length of the vessel members in the last formed xylem to the length of the fusiform initials and to establish any patterns of variation in length and width of fusiform initials with position in a tree with a storeyed cambium.

4.2.2

The New Zealand flora has a number of woody plants where the vascular cambium is storeyed. The cells of the xylem, however, are rarely storeyed in these genera with the exception of the vessel members and axial parenchyma strands.

The genus *Hoheria* (adapted from the Maori name houhere), a member of the Malvaceae, is endemic to New Zealand. All its five recognized species have storeyed cambia. (A sixth possible species grows in the Tararuas but is as yet unnamed). It is an interesting genus in that its species illustrate a range of habit forms. The two mountain species, *Hoheria lyalli* Hook.f. and *Hoheria glabrata* Sprague et Summerhayes are small deciduous trees that do not show any marked change in habit with age. Two other species, however, *Hoheria sexstylosa* Col. and *Hoheria angustifolia* Raoul., both have a markedly shrubby phase with heteroblastic leaf development.

The species *Hoheria angustifolia* (narrow leaved lacebark) was chosen for the major portion of this study on variation in cell size in the cambium and its derivatives because of its storeyed cambium, its unusual growth froms and the ready availability of mature trees within a reasonable distance of the University of Canterbury.

The juvenile shrub of Hoheria angustifolia consists of a number of rather strong upright shoots bearing long whip like lateral branches, usually deflexed, which become closely interwoven (Fig. 4-1a). In this form the plant bears small leaves (Fig. 4-2). When the shrub attains a height of about 3 metres, the laterals become arched and the leaves show a transition toward the adult form. In this way a small fastigiate tree is formed with one or sometimes several trunks still clothed in persistant interlaced laterals, many still bearing the small leaves characteristic of the juvenile form. The fully mature tree looses its fastigiate habit, having a spreading crown of several strong branches (Fig. 4-1b). Its leaves are larger and more strongly toothed than the juvenile and intermediate ones. Fully grown it can reach 10 metres and prefers the lowland or swamp forest where it grows naturally from Hawke's Bay to Foveaux Strait.

4.3 MATERIALS AND METHODS

Fifteen Hoheria angustifolia trees growing on Banks Peninsula, New Zealand, were used in this study. All were growing in open situations in full sunlight. As this species

Fig. 4-1. (a) Juvenile and (b) adult forms of *Hoheria angustifolia* growing in Prices Valley, Banks Peninsula, New Zealand. Altitude 80 m.

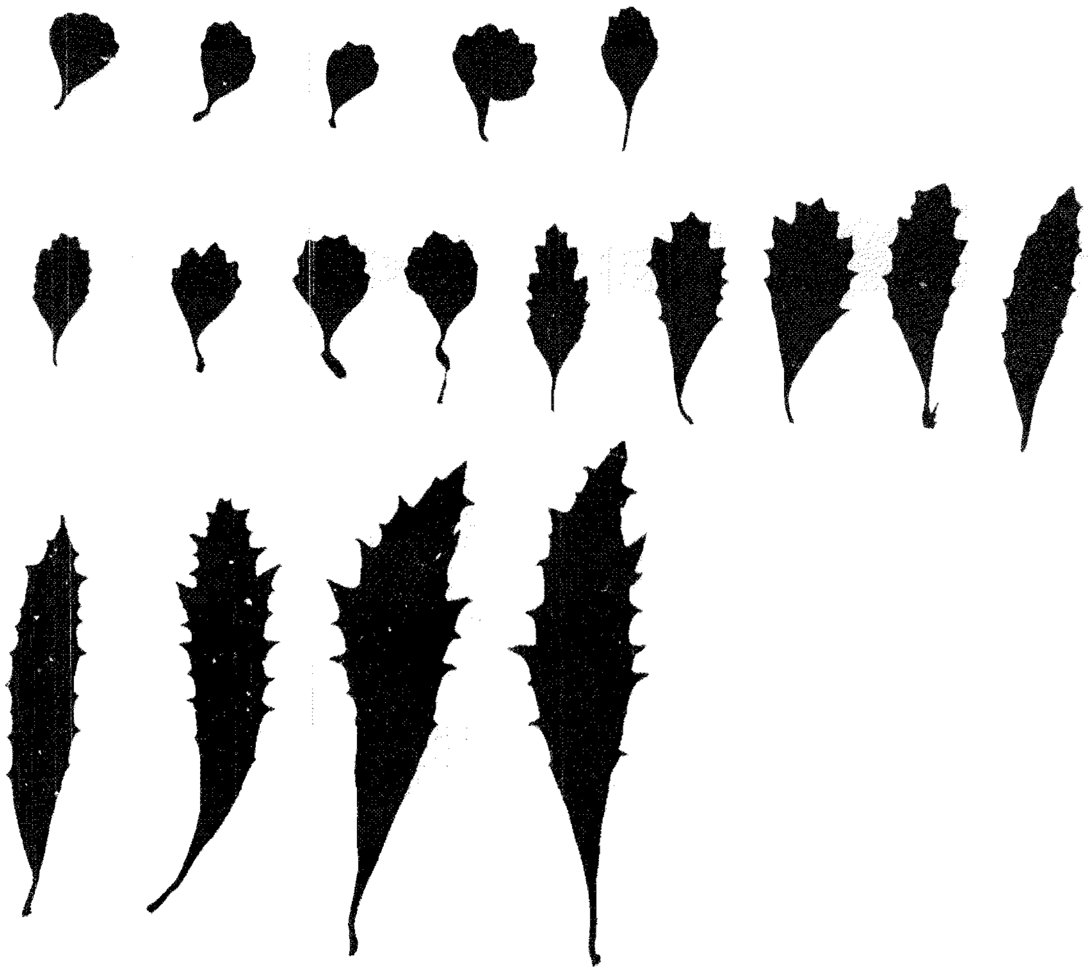


Fig. 4-2. A range of leaves from juvenile (top row), intermediate (middle row) and adult (bottom row) trees showing the transition in leaf size and form that accompanies the habit heteroblastic development in *Hoheria angustifolia*. X Life size.

illustrates leaf and habit heteroblastism (Philipson, 1963), specimens were selected so that five showed mature adult form with adult foliage, five were intermediate in form and leaf shape, and five were juvenile in form and bore small juvenile leaves. These trees were designated A1-5, I1-5, J1-5 respectively. Discs of wood were cut from the leading stem or trunk of each tree at 3 foot intervals beginning at the 2 foot level. The stem material of small diameter was fixed immediately in formalin-aceto-alcohol. The larger discs of wood were brought back to the laboratory and transferred to formalin-aceto-alcohol. The entire stem of the tree I5 was cut into 4 inch lengths and preserved in formalin-aceto-alcohol.

Samples from the outside of other mature trees were also collected for the examination of the wood using scanning electron microscopy. These were also preserved in formalin-aceto-alcohol and small cubes of about 4 mm per side were cut from them, dehydrated and mounted by the technique outlined by Exley, Butterfield, and Meylan (1974). They were then coated with 20 nm of carbon followed by about 20 nm of 60:40 gold-palladium and examined in the vacuum dry state in the column of a Cambridge Series IIA scanning electron microscope.

Cubes of wood including the cambium were cut from each disc of wood of trees A1-5, I1-4 and J1-5 and from every inch of the main stem of tree I5. All these wood and cambium specimens were dehydrated in tertiary butyl alcohol and embedded in paraffin wax. Tangential longitudinal sections of 10 – 12 μm thick of the cambium and xylem were cut using a rotary microtome. The sections were stained according to standard safranin fast-green procedures and mounted in De Pex. Macerations of the secondary xylem were made using 50/50 nitrochromic acid mixture and mounted in glycerine jelly.

Cell lengths and widths were measured using an overhead projection system, the image of each slide being projected on to a calibrated scale. Usually 25 or 50 cells were measured in each sample. A string bearing two arrows lying over the projected image enabled cells to be randomly selected for measurement. A measuremental error of approximately 3% was incurred by this technique. Unless otherwise stated, all measurements of vessel members and fusiform cambial initials were made from tip to tip of the cell. Cell widths were measured at the mid-point of the cell. Growth ring measurements were carried out directly on the stem blocks after the cut surfaces had been sanded.

Cell length and width measurements were transferred to computer data cards and processed by the IBM 360/44 computer at the University of Canterbury. Computation of the basic statistics, analyses of variance, regression equations and product-moment correlations were carried out by programs based on those published by Sokal and Rohlf (1969) and designated A3.1, A3.4, A3.12 and A3.13 by these authors.

In the course of this investigation 2,540 microscope slides were prepared and more than 50,000 cell measurements made.

4.4.1 BASIC STRUCTURE OF THE SECONDARY XYLEM.

The wood of Hoheria angustifolia is relatively light. Although growth rings are readily visible when cut stems are viewed macroscopically, they are more difficult to detect under the microscope. Each growth ring is built up of several concentric bands of fibres alternating with vessels and axial parenchyma (Fig. 4-3).

The vessels are distributed in multiples of up to 10 pores and are separated into tangential groups by broad heterocellular multiseriate rays. The individual vessel members are interconnected by exclusively simple perforation plates (Fig. 4-4), the angle of the end wall varying from transverse to oblique. The secondary walls of the adjoining vessel members form a small rim around the vessel wall at the perforation and rarely form the overarching borders characteristic of the vessels of many species with simple perforation plates (Butterfield and Meylan, 1971; Meylan and Butterfield, 1972). The walls of the vessel members are overlaid by prominent helical thickenings (Fig. 4-5) that occasionally show branching. The intervessel pit-pairs are bordered with

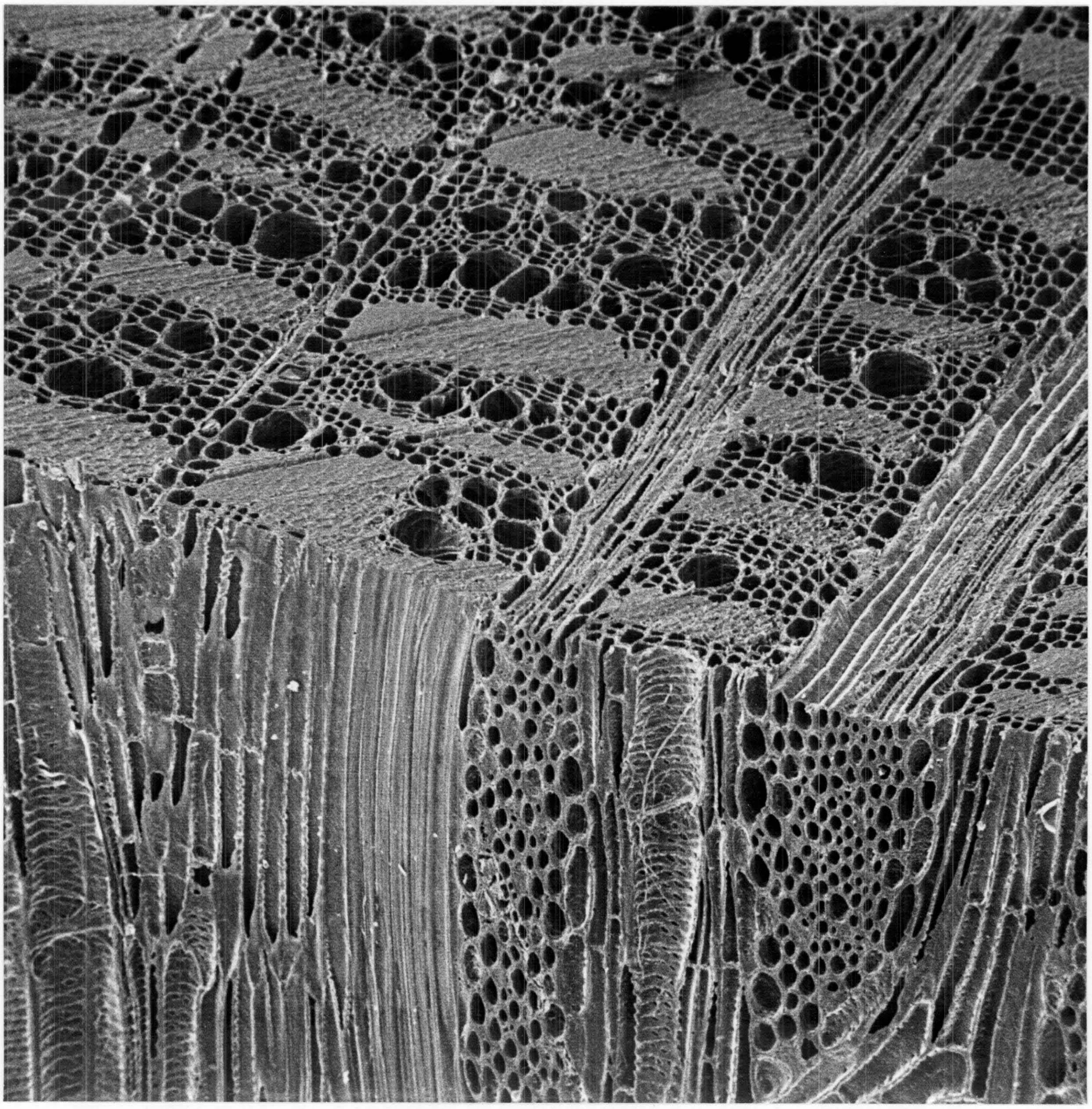


Fig. 4-3. Transverse and tangential longitudinal faces of a cube of Hoheria angustifolia wood. Concentric bands of fibres alternate with groups of vessels and axial parenchyma cells within each growth ring. The larger multiseriate rays are conspicuous on both faces. X 180

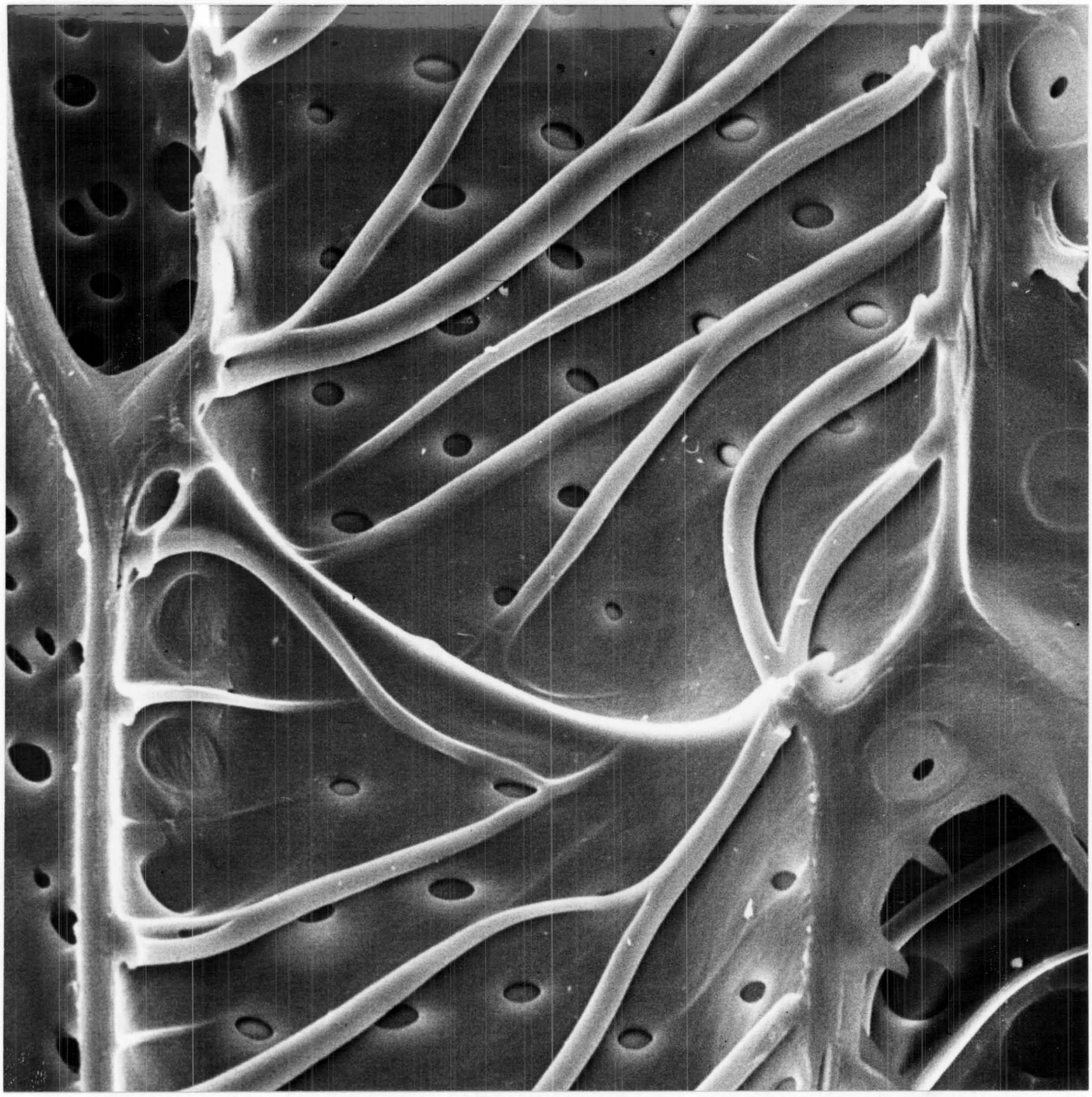


Fig. 4-4. A simple perforation plate between two adjoining vessel members. X 2,100

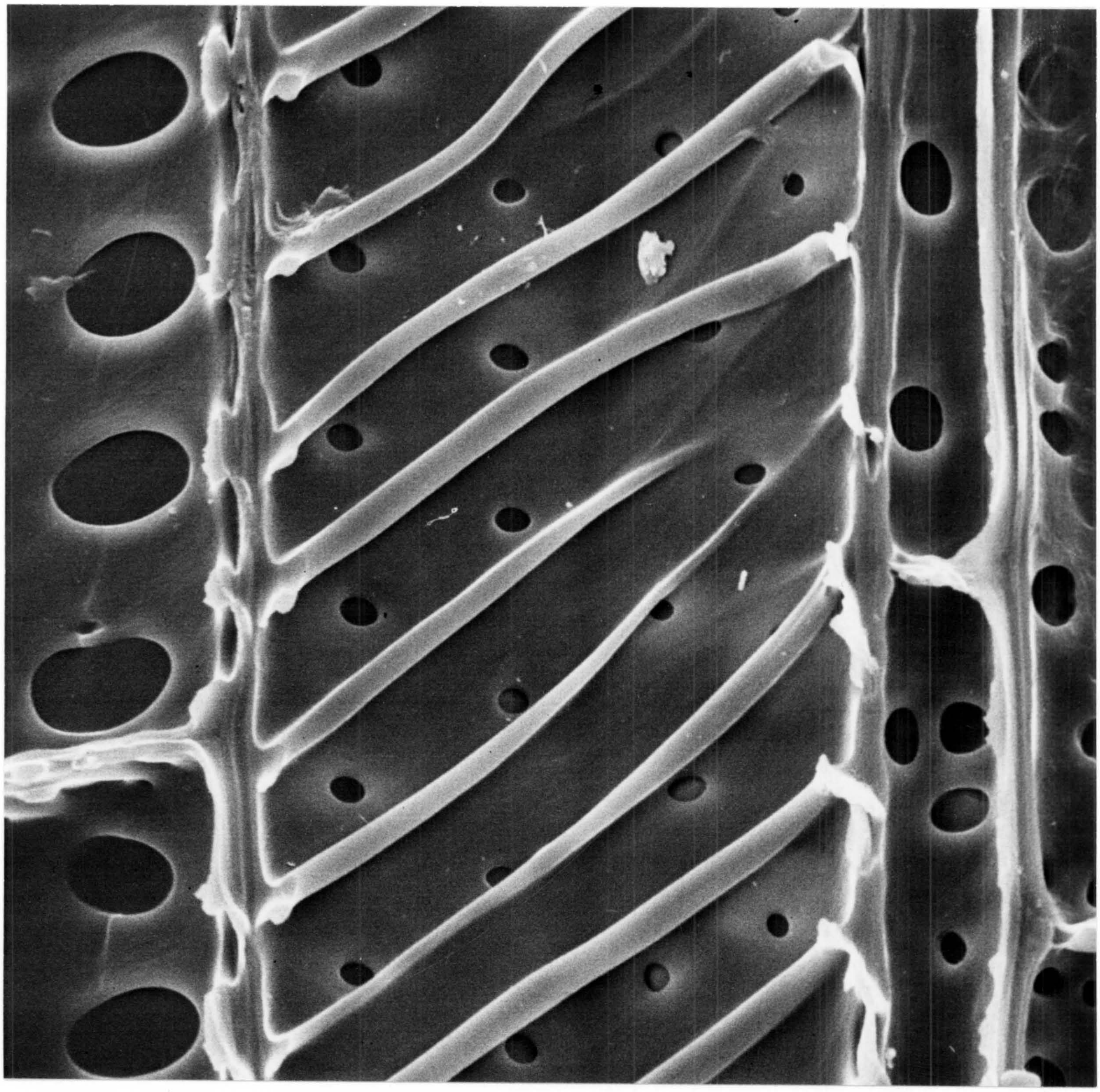


Fig. 4-5. Detail of a vessel wall showing helical thickening and intervessel pit apertures. Axial parenchyma cells can be seen on either side of the vessel. X 2,850

circular to slightly oval pit apertures. Vessel to ray pits are larger and more oval in outline than the vessel to vessel pits.

In transverse view the libriform fibres are angular in outline and very thick walled (Fig. 4-6). They are arranged in concentric bands. The pits are generally simple with slit shaped apertures aligned at a low angle to the axis of the cell.

Axial parenchyma cells are grouped adjacent to the vessels, though occasional isolated or groups of cells may occur among the fibres. In the axial direction they are grouped into strands of two or four cells, the end walls of the strands forming a point while dividing walls are transverse (Fig. 4-7).

Both uniseriate and multiseriate rays occur. The multiseriate rays are wide and conspicuous in both the transverse and tangential longitudinal views. The cells to the centre of the larger rays are usually thicker walled and of smaller tangential dimensions than the cells to the periphery Crystals and other inclusions occur in many ray cells.

Both the vessel members and axial parenchyma strands retain the storeyed pattern characteristic of the vascular cambium of this species (Fig. 2-21).

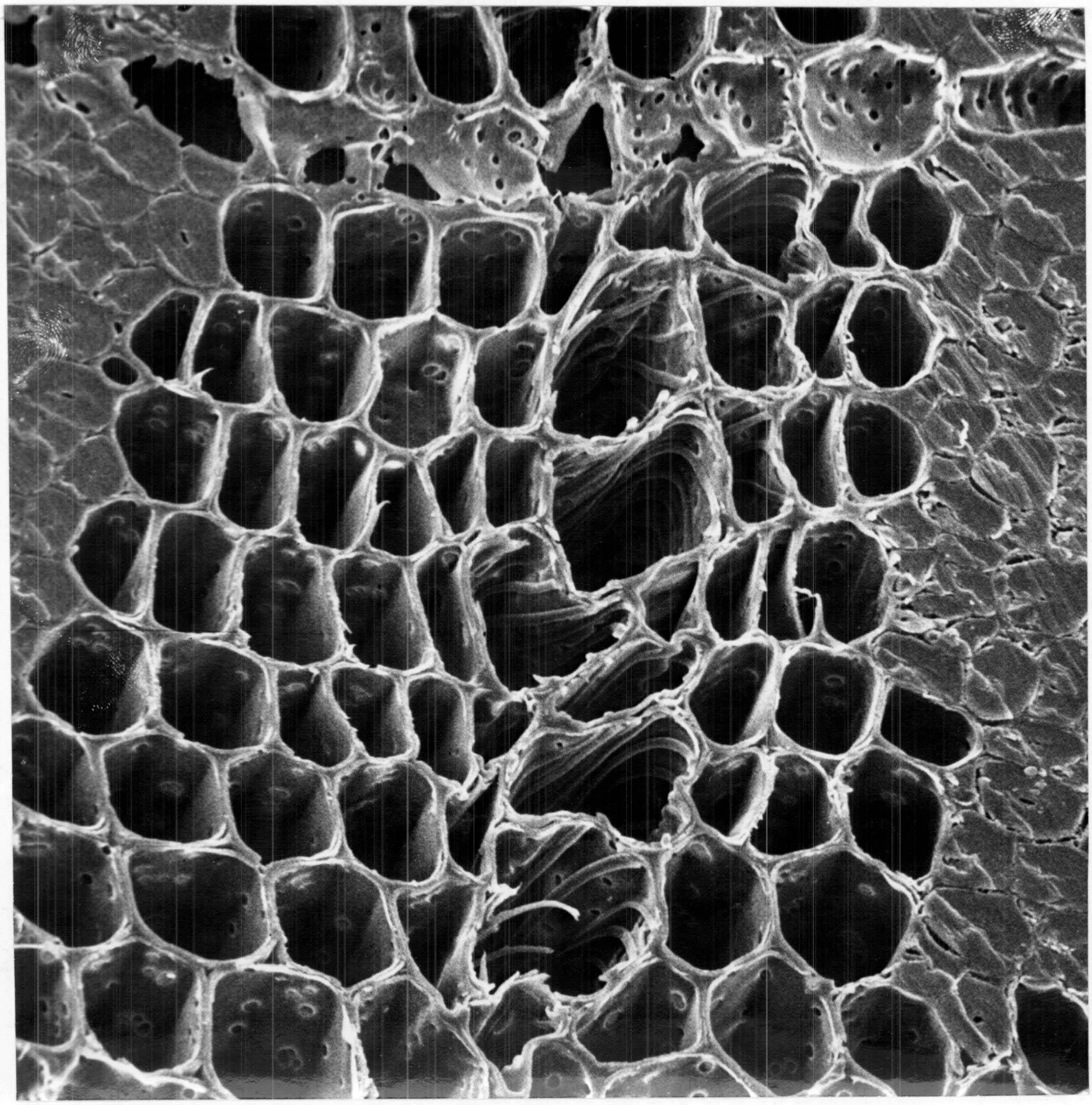


Fig. 4-6. Fibres, vessels and axial parenchyma cells in transverse view. The vessels can be distinguished by their helical thickenings. The fibres are very thick walled and angular in outline. X 825

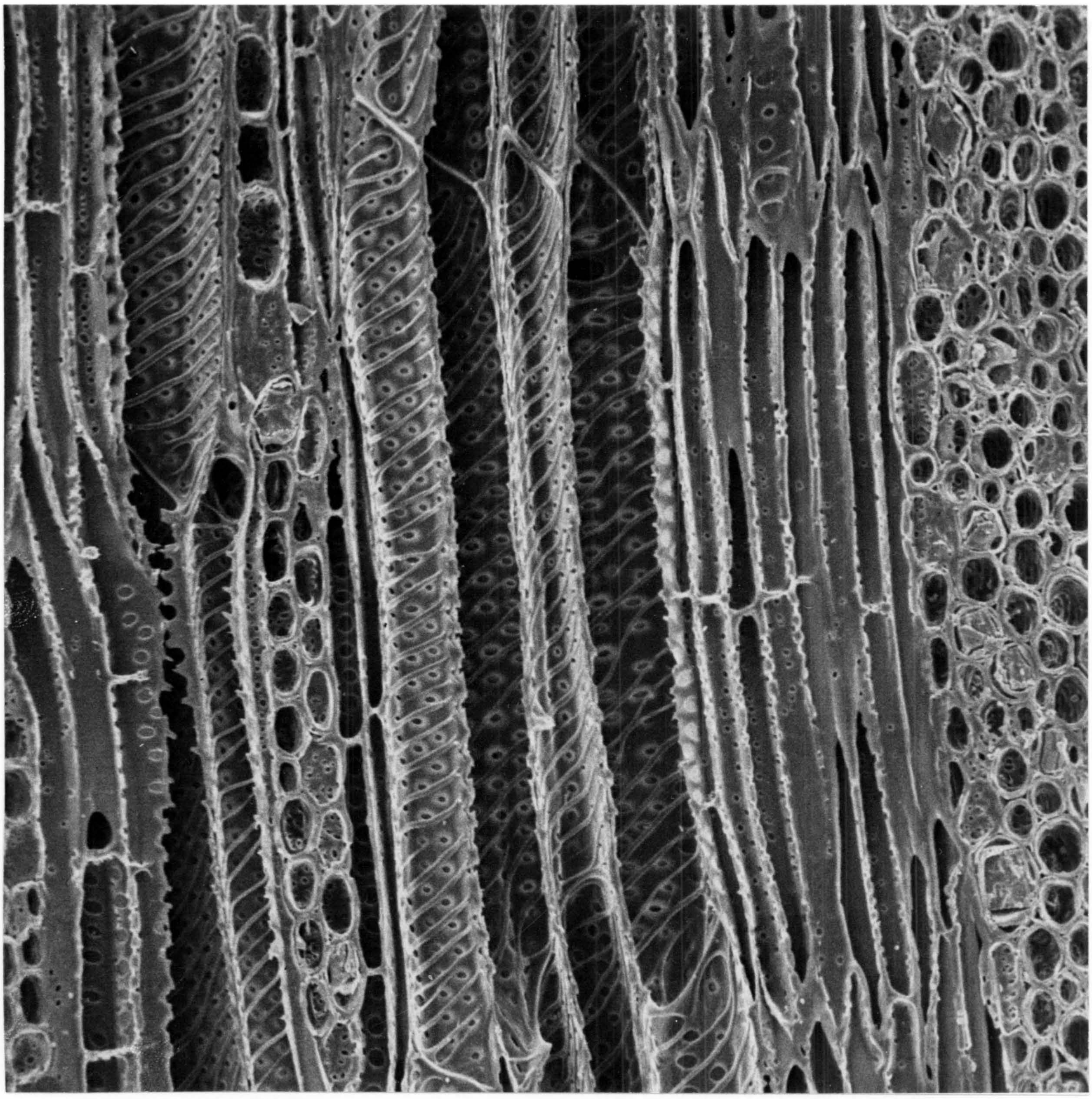


Fig. 4-7. Vessels, rays and axial parenchyma strands in tangential longitudinal view. Each parenchyma strand is subdivided into two or four cells. X 365

4.5.1 VARIATION IN VESSEL MEMBER LENGTH AT ANY ONE LEVEL IN THE TREE.

From his observations on _Pinus sylvestris_, Sanio (1872) concluded that tracheid length at any one level in the tree increased outwards from the pith through a number of annual rings until a certain size was reached, and then remained constant throughout the following rings. There has since been general agreement that there is an initial increase in tracheid and fibre length over the early part of radial growth in both gymnosperms and angiosperms but investigations beyond this point are somewhat contradictory. While some investigators have recorded results similar to those of Sanio, others have recorded a decrease, an increase, or considerable fluctuation in cell length after the initial increase (see Section 1.3.1). Since vessel members are generally assumed to approximate the length of the fusiform cambial initials from which they are derived, size change sequences in the secondary xylem of these cells should reflect the developmental changes that have occurred in the cambium with radial growth.

Most woods also show a within-ring variation pattern, the cells of the early wood generally being shorter than those produced later in the same annual ring. This variation pattern has been recorded for the vessel members of some non-storeyed woods (Chalk and Chattaway, 1935; Bisset and Dadswell, 1950; Bosshard, 1951; Swamy _et al_., 1960) as well for tracheids and fibres in many species (Dinwoodie, 1961). Such size changes have been recorded for fibres in woods with storeyed cambia (Chalk *et al*., 1955) but not for vessel members.

4.5.2 METHOD

Rectangular blocks of wood were cut from 10 *Hoheria angustifolia* stem discs. The sample numbers are indicated in Table 4-1. All except sample 10 came from the adult trees A1-5 listed earlier. Sample 10, taken from an inclined branch of another adult tree, included reaction wood. Each block was cut so as to pass from the stem centre out along a radius to the cambium, measuring 6 mm wide on the transverse face and 12 mm deep on the radial face. After staining the sanded transverse surfaces with a methylene-blue/ malachite-green solution, the positions of the growth ring boundaries were noted.

Each sample was then cut into 1 mm wide slices, an oblique cut first being made along the block in order to assist sample orientation (Fig. 4-8). After gentle maceration, each slice was washed in distilled water and a scraping of the separated cells taken from its outer face. Where possible the lengths of 25 vessel members were measured in each preparation. The widths of the same 25 vessel members were also recorded for rings 6 to 9 in the A1, 2 foot sequence and correlation coefficients calculated.

Sequence	Tree number	Height (feet)	Radius (mm)	Number of growth rings	Number of measurements	Mean vessel member length (μm)	Regression equation	95% confidence limits of slope		Mean length early wood vessel members (μm)	Mean length late wood vessel members (μm)
1	A1	2	81	17	1987	252.0	$y = 246 + 0.13x$	-0.01	+0.28	243.8	257.0
2	A1	5	63	13	1486	257.3	$y = 249 + 0.17x$	-0.05	+0.40	246.2	263.9
3	A2	2	46	12	1147	293.1	$y = 311 - 0.49x$	-1.02	+0.04	289.6	298.7
4	A2	5	37	8	917	267.3	$y = 259 + 0.43x$	-0.85	+1.01	251.2	277.6
5	A3	2	57	16	1469	260.9	$y = 260 + 0.01x$	-0.11	+0.14	256.7	262.3
6	A4	2	52	11	986	264.6	$y = 254 + 0.47x$	-0.97	+0.04	259.5	268.3
7	A4	5	33	8	796	272.8	$y = 279 - 0.37x$	-0.83	+0.10	262.6	277.8
8	A5	2	63	16	1439	298.6	$y = 302 - 0.10x$	-0.24	+0.05	290.3	301.1
9	A5	5	46	12	1109	271.5	$y = 264 + 0.33x$	-0.17	+0.83	266.5	278.5
10	Reaction wood sample		37(8)	10(4)	375	261.8	$y = 274 - 0.56x$	-1.07	-0.05		

Table 4-1. The tree numbers and heights from which the 10 discs were examined to determine the changes in vessel member length with distance from the stem centre, the number of vessel members measured for each disc, their mean length and the regression equations for each. With the exception of sample 10 the 95% confidence limits of the slope lie on either side of zero. There is no evidence, therefore, to suggest either an increase or a decrease in vessel member length with increasing distance from the stem centre. Sample 10 shows a slight decrease in vessel member length with distance from the stem centre. The mean lengths of the vessel members from the first and the last mm sample of each growth ring are shown in the last two columns.

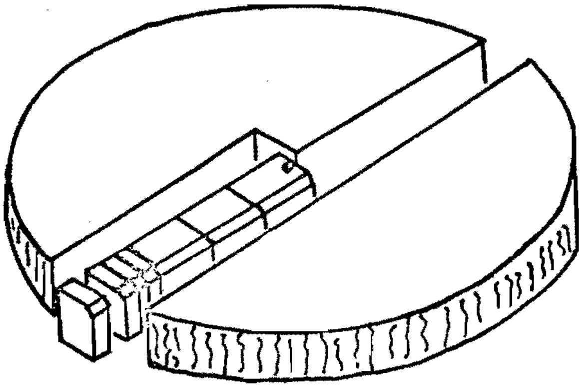


Fig. 4-8. A diagram to illustrate the way in which the 1 mm thick wood samples were removed from along the radius of each stem disc studied. Scrapings of macerated wood were prepared from the outside of each slice.

4.5.3 RESULTS

The vessel member lengths for each millimetre of wood from the stem centre to the cambium for three of the discs, trees A1, A2, and A4 (all at the 2-foot level) are presented in Fig. 4-9 (a)-(c). The positions of the growth ring boundaries to the nearest millimetre are also indicated. Except where indicated, the Kolmogorov-Smirnov stastic d max for each sample is below 0.316, the critical value for samples where n=25. It is, therefore, assumed that there is no evidence to suggest anything other than a normal distribution within most samples. The 95% confidence limits (mean ± 2 standard errors) are indicated by bar marks. The results for six of the other seven discs are not graphed, but they showed essentially the same pattern.

The results of these measurements indicate that there is no significant change in vessel member length with radial distance from the stem centre in Hoheria angustifolia. The absence of any trend in vessel member length upwards or downwards with radial distance from the stem centre, is confirmed by the fact that the 95 percent confidence limits of the slope of the regression equations for each sample lie on either side of zero (Table 4-1).

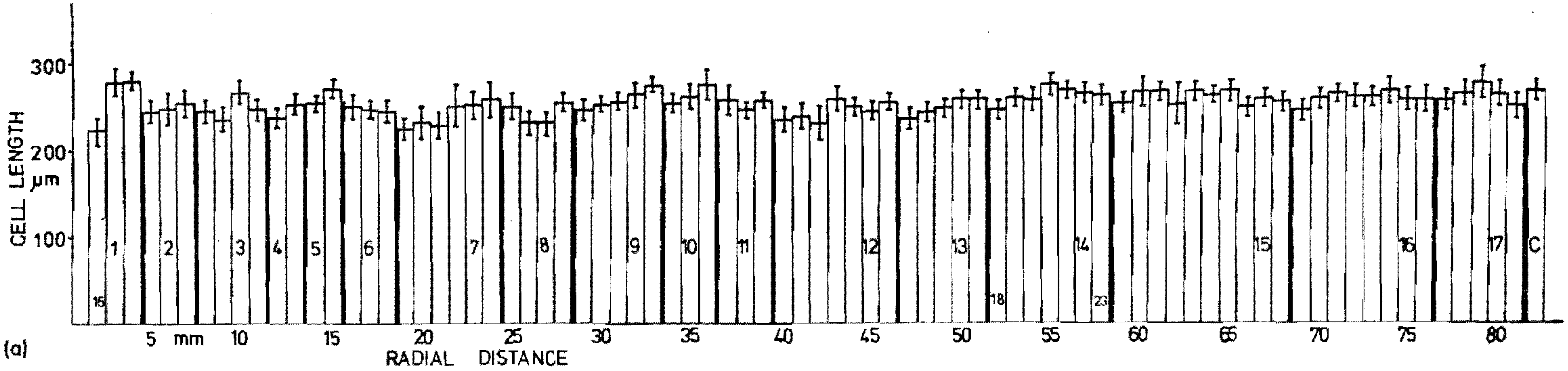
The tenth disc examined showed an eccentricity due to the presence of tension wood on one side of the stem. The vessel member lengths for each mm of wood on either side of the stem are presented in Fig. 4-9d. The confidence limits for the slope of the regression equation for vessel member length in this sample indicates a slight decrease in length with radial distance from the stem centre in the tension wood (Table 4-1).

It is evident from Fig. 4-9 that there is a tendency for the late wood vessel members to be slightly longer than the early wood cells in many of the growth rings, but in most cases the 95% confidence limits overlap. Mean vessel member lengths from the first and last mm samples in each growth ring are tabulated in the last two columns of Table 4-1. Without exception these means indicate that the late wood vessel members are very slightly longer than those found in the early wood.

The lengths and widths of the vessel members from rings 6 - 9 inclusive in tree A1 (2 foot level) show a negative correlation significant to the 99% level (Table 4-2A). An analysis of the angle of the end walls of these cells indicates that the wider, shorter cells tend to have nearly transverse perforation plates at both ends, but the narrower vessel members have one or both end walls at an angle and are probably slightly longer as a consequence (Table 4-2B). The slight changes in mean vessel member length within the growth rings indicated by Fig. 4-9 and Table 4-1, therefore, do not result from changes that have occurred in the length of the fusiform

On the following pages:

Fig. 4-9. The relation of mean vessel member length to radial distance from the stem centre in four discs of *Hoheria angustifolia* examined. (a) tree A1, 2 foot, (b) tree A2, 2 foot, (c) tree A4, 2 foot, (d) reaction wood disc. The bar marks indicate the 95% confidence limits of the mean (± 2 standard errors). Occasional figures along the x - axis indicate the number of vessel members where fewer than 25 were measured. The Kolmogorov-Smirnov statistic d max indicated a non - normal distribution for the sample marked with an asterisk. The position of the growth ring boundaries to the nearest millimetre are indicated by the heavy lines and the rings numbered. The letter C marks the position of the cambium.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C
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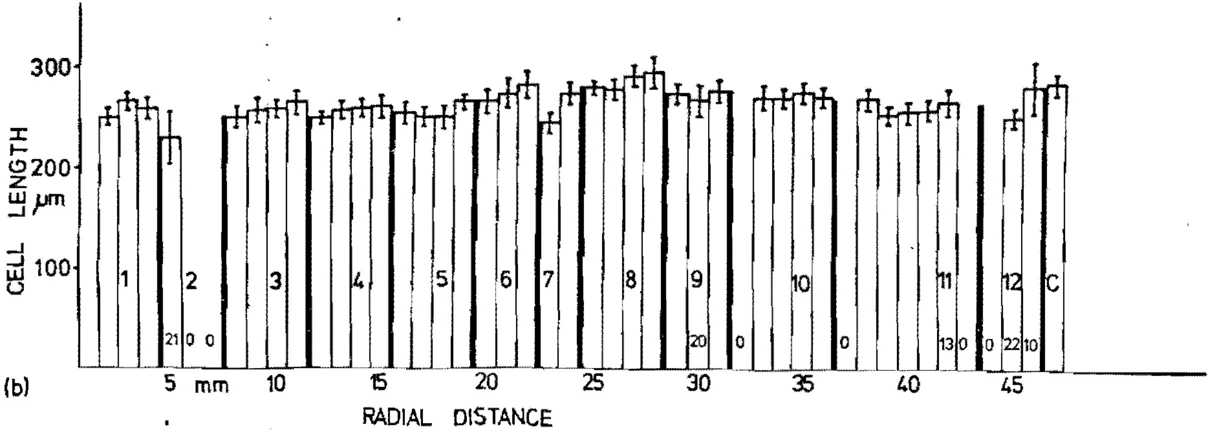
75
80
RADIAL DISTANCE

(a)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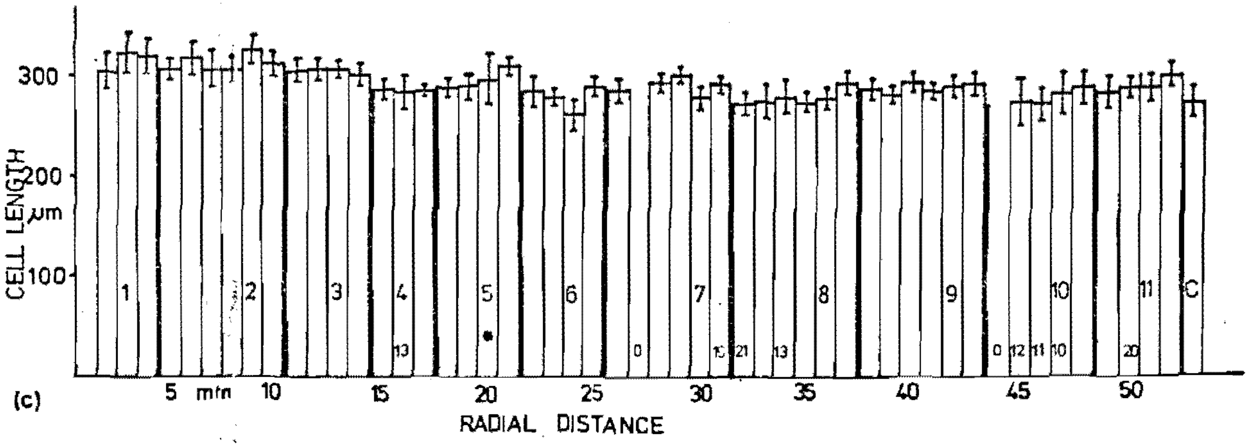
RADIAL DISTANCE

(b)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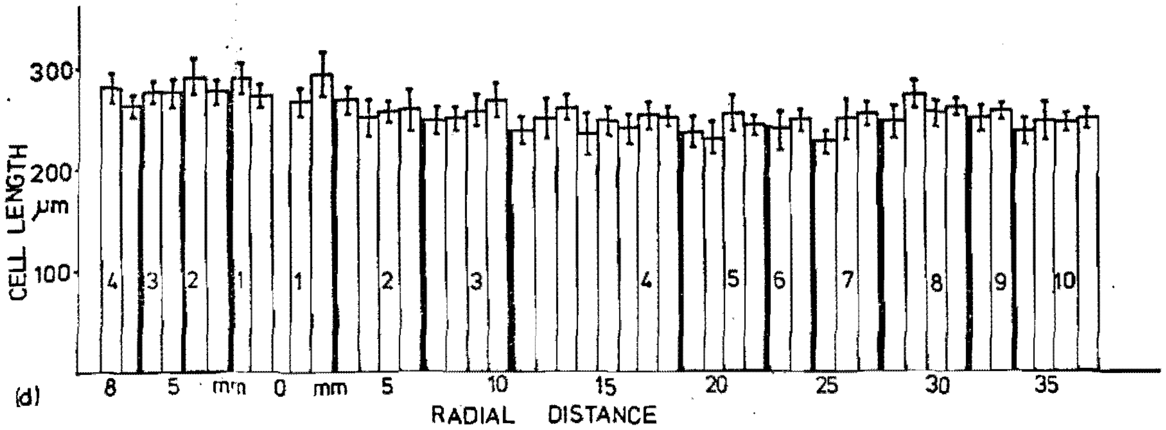
RADIAL DISTANCE


(d)
CELL LENGTH µm
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mm
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mm
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25
30
35
RADIAL DISTANCE

A. Number of vessel members measured	Mean length (µm)	S.E.	Mean width (µm)	S.E.	Correlation coefficient
450	266.5	1.4	54.7	1.1	-0.186**

Regression equation: Length = 454 - 3.4 width

B.	Mean length (µm)	S.E.	Mean width (µm)	S.E.
Both end walls transverse	251.9	2.1	62.3	1.6
One end wall oblique	289.4	4.0	46.3	3.6
Both end walls oblique	296.1	1.9	42.9	1.6

Table 4-2. A. The length to width relationship of the vessel members from the rings 6-9 inclusive of tree A1 (2 foot level).
B. The relationship of vessel member length and width to the angle of the end wall.

cambial initials with season but result from a tendency for the late wood derivatives of the cambium to differentiate into narrower vessel members with more oblique end walls than those of the early wood.

4.6.1 THE RELATIONSHIP OF FUSIFORM CAMBIAL INITIAL LENGTH TO VESSEL MEMBER LENGTH

There are few published results on the relation in size between the fusiform cambial cells and their derived elements. Bailey (1920), from a comparative study of the size variation of fusiform cambial initials and their derivatives in Ginkgo and the Coniferae, concluded that the lengths of the tracheids of the last formed xylem closely resembled the lengths of the fusiform initials from which they were derived, but were slightly longer. This relationship has been generally thought to apply to most conifers and a great number of studies on the changes in cell length in the conifer cambium have now been based on this assumption. In dicotyledons, Bailey found that the initials were much shorter than the fibres but were approximately the same length as the vessel members. Chattaway (1936) found that the fibres in dicotyledonous woods can be 1.1 to 9.5 times lomger than the cambial initials, though elongation to several times the initial length only occurs when the initials are very short.

As most of our present information on developmental and size changes occurring in the vascular cambium of plants has been obtained from investigations on the secondary xylem, and not from direct examinations of the cambium itself, it is surprising that more attempts have not been made to establish the size relationship between fusiform initials and their derivatives.

4.6.2 METHOD

Twenty-five samples including wood and bark from 8 trees A1-5, I1, 2 and 4 were examined. The trees and heights from which the samples were removed are listed in Table 4-3. Serial tangential longitudinal sections of the cambium were cut from each (wood) sample by the standard paraffin technique. Twenty-five fusiform initials selected at random were measured from each sample. Small cubes of wood from behind the cambium were isolated and macerations prepared from the outside face of each block. Every effort was made to ensure that these preparations included fully differentiated vessel members from wood as close to the cambium as possible. Twenty-five cells were measured in each of these macerations.

4.6.3 RESULTS

The mean lengths of the fusiform cambial initials and vessel members and their standard deviations, the confidence limits at the 95 per cent level, and the Kolmogorov-Smirnov statistic d max for each sample are presented in Table 4-3. In all 50 samples the d max values were below 0.316, the critical value for samples of 25. There is, therefore, no evidence to suggest anything other than normal distribution. A number of samples were also frequency checked on probability graph paper where straight line graphs confirmed normal distributions in each case. Transformation of the data was therefore considered unnessary.

The t - values for the comparisons between the pairs of means are also shown in Table 4-3. In only four of the twenty-five pairs of samples are significant differences between the mean fusiform initial length and the corresponding mean vessel member length detected: samples 11 (tree A3, 8 feet), 13 (tree A4, 2 feet), 15 (tree A4, 8 feet) and 24 (tree 12, 5 feet). When all twenty-five pairs of samples are compared the product-moment correlation coefficient is significant to the 99% level, indicating a real and positive correlation between the pairs of samples (Table 4-4). A better correlation, significant to the 99.9% level is obtained when samples 11, 13, 15 and 24 are ignored. The correlation is graphed in Fig. 4-10 showing the principal axis and the 99% confidence ellipse for the grand mean.

A positive relationship between fusiform cambial initial length and the length of the vessel members derived from them is therefore established, the vessel members having a similar

Sample	Tree	Height	$\bar{Y}_f$	S.E.F.	Confidence Limits (95 per cent)	KSD_{max}	$\bar{Y}_v$	S.E.V.	Confidence Limits (95 per cent)	KSd_{max}	$t_{f,v}$
1	A1	2	263.6	5.1	253.4 - 273.8	0.12	246.3	6.2	233.9 - 258.7	0.12	2.00
2	A1	5	268.6	5.4	257.8 - 279.4	0.11	283.2	5.2	272.8 - 293.6	0.10	1.96
3	A1	8	252.6	7.7	237.2 - 268.0	0.12	267.8	4.6	258.5 - 277.0	0.16	1.68
4	A1	11	232.6	7.2	218.2 - 247.0	0.10	237.2	4.2	228.8 - 245.6	0.17	0.53
5	A2	2	286.6	3.2	280.2 - 293.0	0.08	277.1	3.8	269.5 - 284.7	0.13	1.27
6	A2	5	273.5	6.1	261.3 - 285.7	0.10	259.1	7.1	244.9 - 273.3	0.18	1.54
7	A2	8	272.3	5.4	261.5 - 283.1	0.06	270.9	3.4	264.1 - 277.7	0.17	0.21
8	A2	11	259.5	6.9	245.7 - 273.3	0.12	270.3	4.6	261.1 - 279.5	0.08	1.29
9	A3	2	269.3	4.7	259.9 - 278.7	0.09	281.2	4.3	272.6 - 289.8	0.14	1.85
10	A3	5	254.9	4.0	246.9 - 262.9	0.08	261.3	4.7	251.9 - 270.7	0.11	1.10
11	A3	8	251.2	3.2	244.8 - 257.6	0.14	269.2	3.2	262.8 - 275.6	0.16	3.09 **
12	A3	11	252.0	2.8	246.4 - 257.6	0.14	266.7	3.6	259.5 - 273.9	0.09	2.03
13	A4	2	268.2	5.8	256.6 - 279.8	0.10	298.1	5.2	287.7 - 308.5	0.11	3.83 ***
14	A4	5	280.2	6.9	266.4 - 294.0	0.10	283.9	4.9	274.1 - 293.7	0.12	0.94
15	A4	8	263.2	4.9	253.4 - 273.0	0.06	310.2	6.2	297.8 - 322.6	0.10	5.92 ***
16	A4	11	265.0	6.4	252.2 - 277.8	0.09	270.2	6.0	258.2 - 282.2	0.11	1.45
17	A5	2	313.3	6.4	300.5 - 326.1	0.11	292.8	5.5	281.8 - 303.8	0.13	1.92
18	A5	5	277.1	6.9	263.3 - 290.9	0.09	281.6	4.1	273.4 - 289.8	0.12	0.56
19	A5	8	270.4	6.3	257.8 - 283.0	0.13	270.3	3.8	262.7 - 277.9	0.12	0.01
20	A5	11	252.7	8.8	235.1 - 270.3	0.10	277.3	5.4	266.5 - 288.1	0.10	1.77
21	A5	14	256.8	8.1	240.6 - 273.0	0.13	260.0	4.5	251.0 - 269.0	0.12	0.73
22	I1	2	269.3	3.5	263.2 - 276.3	0.06	274.7	3.9	266.9 - 282.5	0.11	1.37
23	I2	2	288.7	6.0	276.7 - 300.7	0.09	283.9	4.1	275.7 - 292.1	0.11	0.65
24	I2	5	269.4	5.1	259.2 - 279.6	0.12	250.5	4.2	242.1 - 258.9	0.14	2.65 *
25	I4	2	280.1	5.7	268.7 - 291.5	0.16	282.1	4.8	272.5 - 291.7	0.15	0.25

Table4-3 Correlation of mean fusiform initial length ($\bar{Y}_f$) with mean vessel member length ($\bar{Y}_v$) from the last formed xylem and their standard errors (S.E.F. and S.F.V.) confidence limits and the kolmogorov-smirnov statistic Dmax resulting from a comparison of the observed sample with a normal distribution based on the sample mean and variance. The value of t for a comparison of the means is shown in the last column. (For 48 degrees of freedom the critical values of t are $t_{0.05} = 2.01$, $t_{0.01} = 2.68$,

n	$\bar{\bar{Y}}_f$	SE_f	$\bar{\bar{Y}}_v$	SE_v	Correlation coefficient	Equation of principal axis
25	268.9	3.4	272.6	3.3	0.496**	$Y_f = -20.5 + 1.06\ Y_v$
21	269.9	3.8	269.9	3.0	0.673***	$Y_f = -98.9 + 1.36\ Y_v$

Table 4-4 The means of the mean lengths for fusiform cambial initials and vessel members and their standard errors, the product-moment correlation coefficients and the equations of the principal axes for all twenty-five pairs of samples and for twenty-one pairs of samples (samples 11, 13, 15 and 24 omitted).

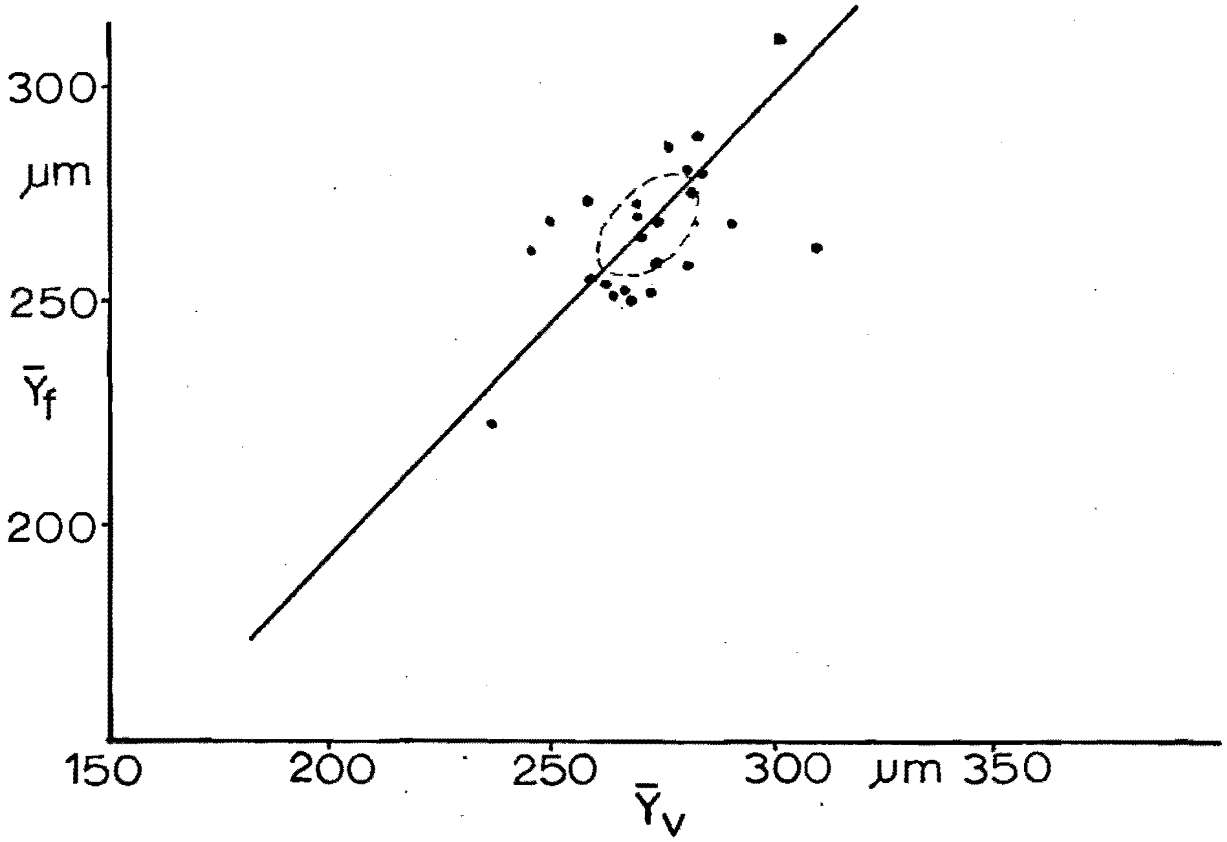


Fig. 4-10. The relationship between the mean lengths of the fusiform cambial initials ($\bar{Y}_f$) and the vessel elememts from the last formed xylem ($\bar{Y}_v$). The straight line indicates the principal axis and the ellipse the 99% confidence level for the grand means for all 25 samples.

length to the fusiform initials.

4.7.1. VARIATION IN FUSIFORM CAMBIAL INITIAL LENGTH WITH HEIGHT IN THE TREE.

In many plants with non-storeyed cambia, there is a gradient in tracheid and fibre length along the axis of the tree. When the pattern of cell length has been followed upwards in the same growth ring, the average length has been found to increase from the base upwards to reach a maximum and then to decrease gradually towards the top of the stem (Sanio, 1872; Dinwoodie, 1961,1963). The point of maximum cell length occurs at approximately one third of the tree height and therefore occurs at progressively higher levels in successive annual rings.

While this pattern of variation with height in the tree is well known for fibres and tracheids, there have been few studies on vessel members. Hejnowicz and Hejnowicz (1958) have recorded an increase followed by a decrease in vessel member length with height in the tree in *Populus tremula*, as has Rao (1962) for vessel members in *Acer pseudoplatanus*, *Quercus robusta* and *Robinia pseudoacacia*. The last named of these three trees has a storeyed cambium. The study of vessel member length in *Robinia pseudoacacia* by Hejnowicz and Hejnowicz (1959) did not reveal sufficient information for this trend to be confirmed.

4.7.2 METHOD

Small cubes including wood and bark were removed from the outside of the stem discs taken from the trees A1-5, I1-4, and J1-5. These discs had been cut at 3 foot intervals from the trunks of each tree. Similar samples were removed at 1 inch intervals from tree I5. Serial tangential longitudinal sections were prepared from each sample using the standard paraffin technique.

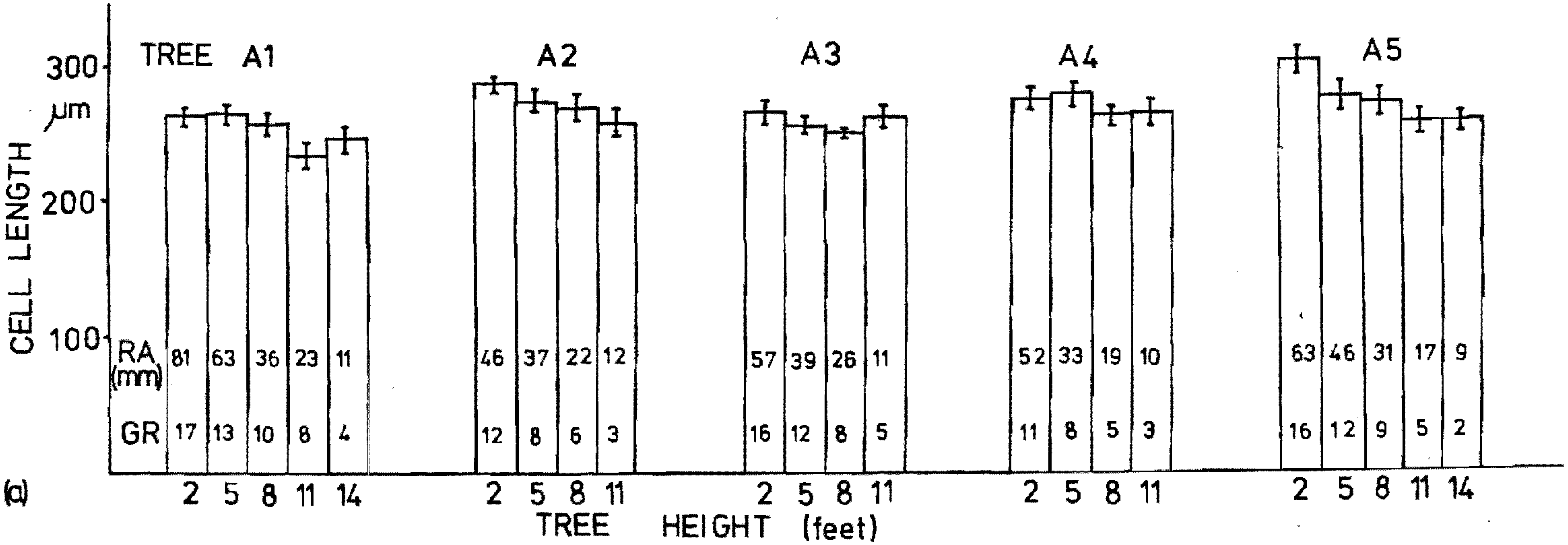
Sections containing predominantly cambial cells were selected for measurement and the lengths and widths of 50 randomly selected fusiform cambial initials were measured using an overhead projection system. These measurements were transferred to computer cards and the basic statistics computed.

4.7.3 RESULTS

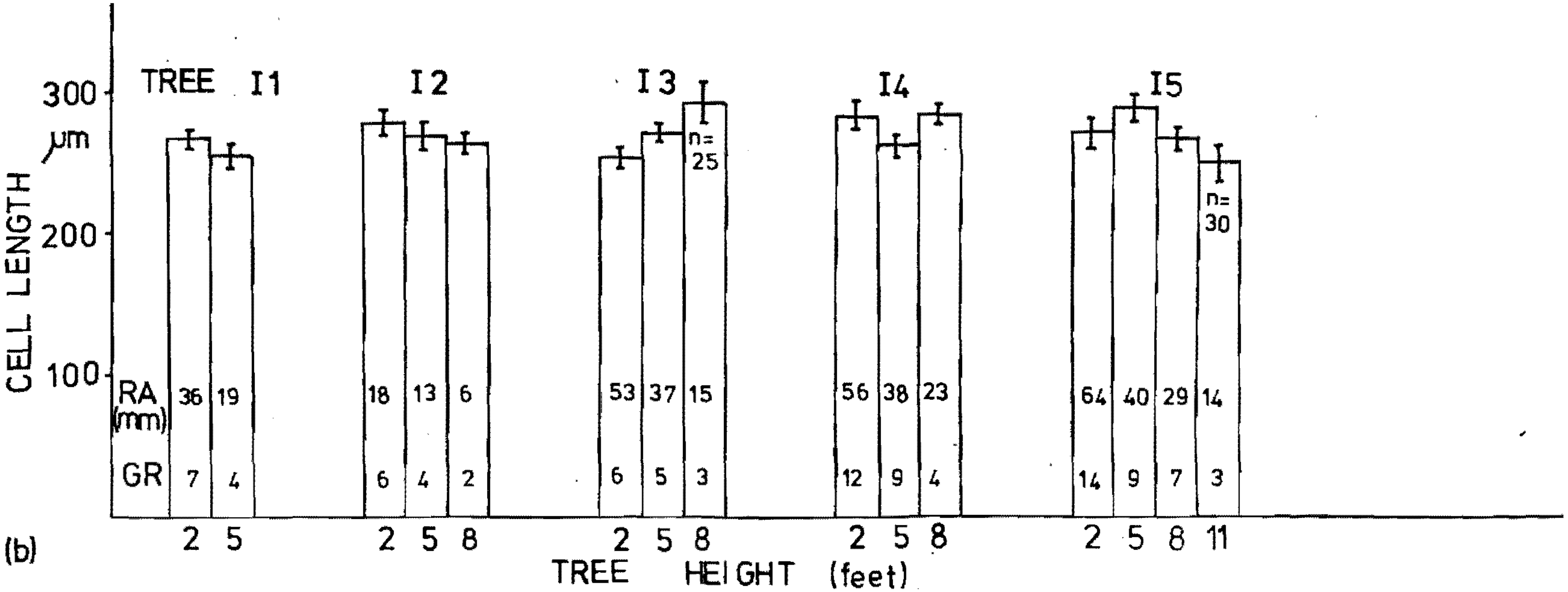
Figure 4-11 presents means and their 95% confidence limits for fusiform initial length at selected heights for trees A1-5 and I1-5. Due to the small diameter of the J1-5 stem discs, tangential longitudinal sections cut from them contained insufficient areas of cambium for 50 fusiform initials to be measured by the technique used in the study. The means for fusiform initial length for each inch of tree I5 from ground level to 11ft 8ins are presented in Fig. 4-12.

On the following page:

Fig. 4-11. Changes in mean fusiform cambial initial length with height in the tree for (a) 5 adult trees and (b) 5 intermediate trees. The bar marks indicate the 95% confidence limits of the mean (± 2 standard errors). Where the number of fusiform initials measured was less than 50, this is indicated near the top of each bar. The radius of the stem within the cambium and the number of growth rings in the secondary xylem are also indicated for each sample.


TREE A1
A2
A3
A4
A5
300
200
100
µm
CELL LENGTH
RA (mm)
GR
81 63 36 23 11
17 13 10 8 4
46 37 22 12
12 8 6 3
57 39 26 11
16 12 8 5
52 33 19 10
11 8 5 3
63 46 31 17 9
16 12 9 5 2
2 5 8 11 14
2 5 8 11
2 5 8 11
2 5 8 11
2 5 8 11 14
TREE HEIGHT (feet)

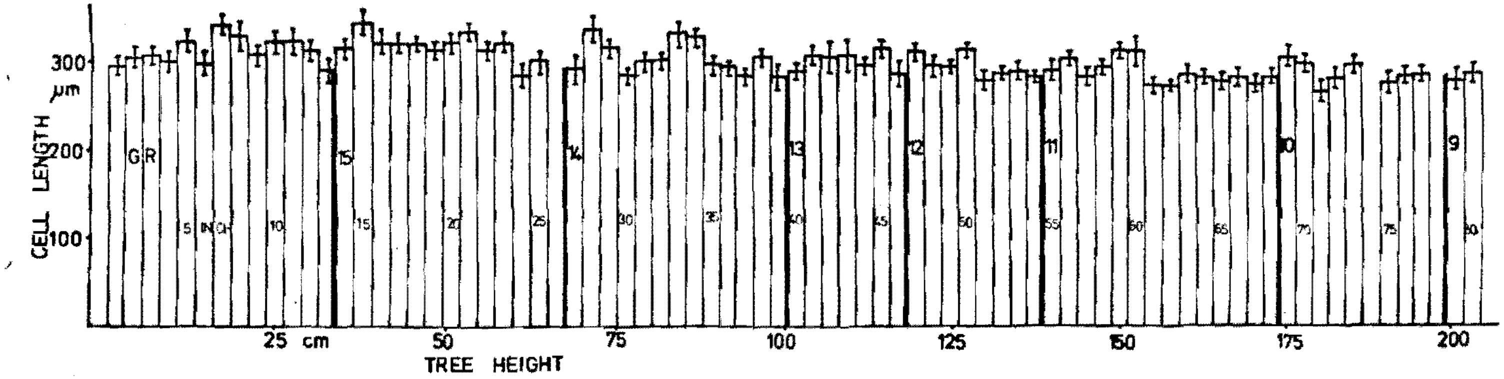
(a)

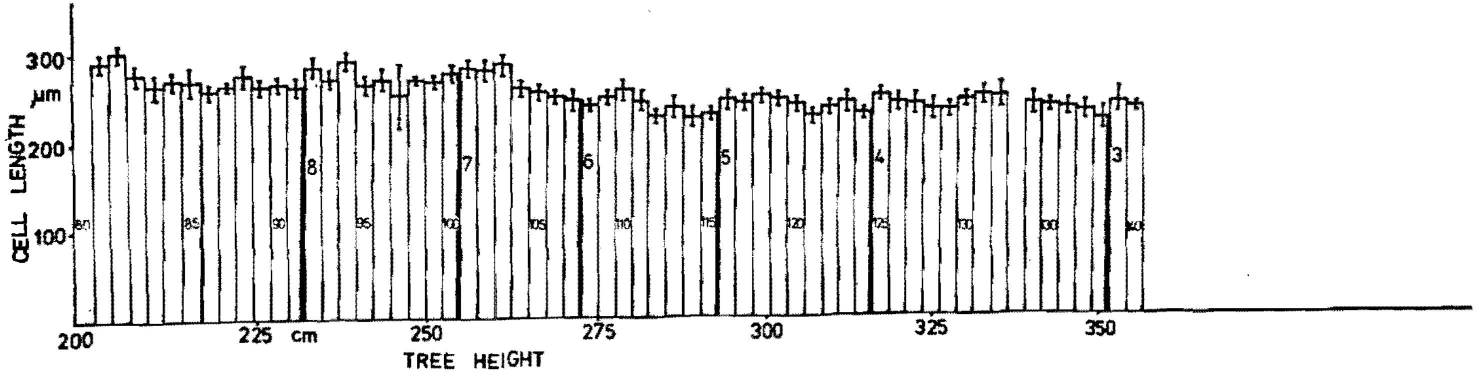

TREE I1
I2
I3
I4
I5
300
200
100
µm
CELL LENGTH
n= 25
n= 30
RA (mm)
GR
36 19
7 4
18 13 6
6 4 2
53 37 15
6 5 3
56 38 23
12 9 4
64 40 29 14
14 9 7 3
2 5
2 5 8
2 5 8
2 5 8
2 5 8 11
TREE HEIGHT (feet)

(b)

On the following page:

Fig. 4-12. Changes in the mean fusiform cambial initial length with height in the tree for tree I5. The bar marks indicate the 95% confidence limits of the mean (± 2 standard errors). The approximate limits of each seasons height growth are also indicated.


300
µm
200
100
CELL LENGTH
GR
15
14
13
12
11
10
9
5 INCH
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
25 cm
50
75
100
125
150
175
200
TREE HEIGHT


300
µm
200
100
CELL LENGTH
8
7
6
5
4
3
80
85
90
95
100
105
110
115
120
125
130
130
140
200
225 cm
250
275
300
325
350
TREE HEIGHT

Although no trend in fusiform initial length with height in the trees is evident from most of the sequences illustrated in Fig. 4-11 and 4-12, the means for each height of the 10 trees A1-5 and I1-5 indicate a slight downwards trend with height when the trees are taken collectively (Fig. 4-13). The analysis of variance based on trees (Table 4-5A) shows that there is no significant difference in the fusiform cambial initial lengths among the trees, but there are significant differences among the various height samples among the trees. Further anaylsis of variance indicates that the among heights difference is caused mostly by the linear decrease in cell length with increasing height in the tree (Table 4-5B).

Data on the widths of the fusiform cambial initials are presented in Fig. 4-14 and the analysis of variance in Table 4-6. In the five larger trees, fusiform initial width increases, and then decreases, with increasing height in the tree. This pattern is followed for the mean initial width when all the 10 trees are taken collectively (Fig. 4-15). As the radial distance of the cambium from the stem centre decreases with increasing height in the tree, mean fusiform initial width is likely to be related either to this factor (Fig. 4-16) or to a related one, the number of growth rings behind the cambium. In fact, cell width was found to be more closely related to the cambial radius than to the number of growth rings behind the cambium. Except at the lower levels of the larger trees,

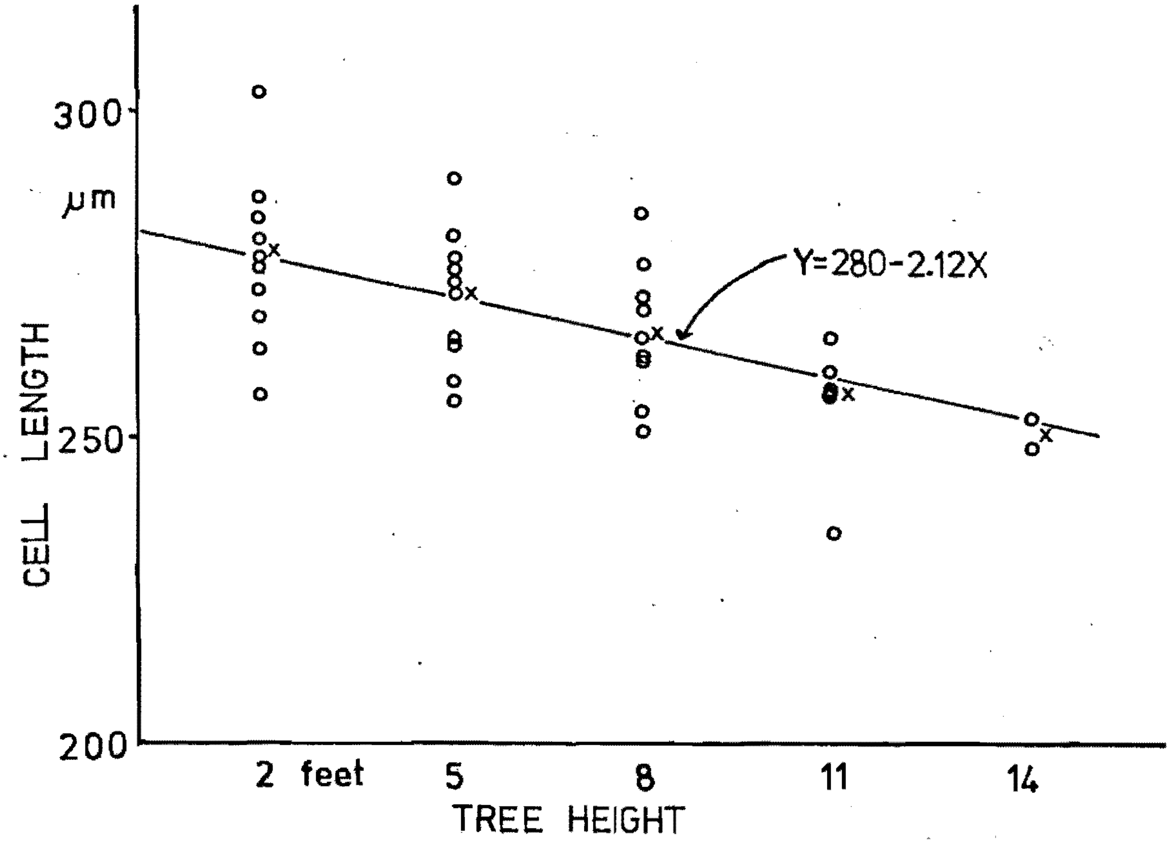


Fig. 4-13. Mean fusiform cambial initial lengths for the individual trees plotted against height in the tree. The mean for the trees at each height (indicated by an x offset slightly to the right in each case), and the line of the regression equation are also indicated.

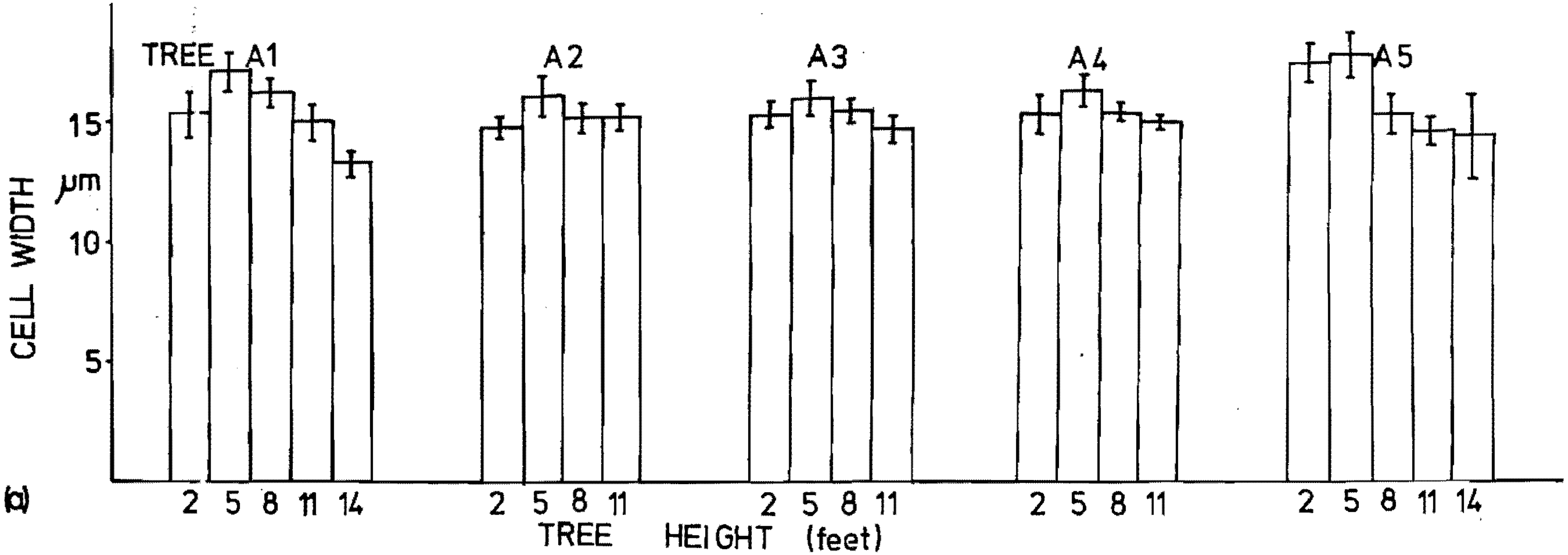
ANAYLSES OF VARIANCE

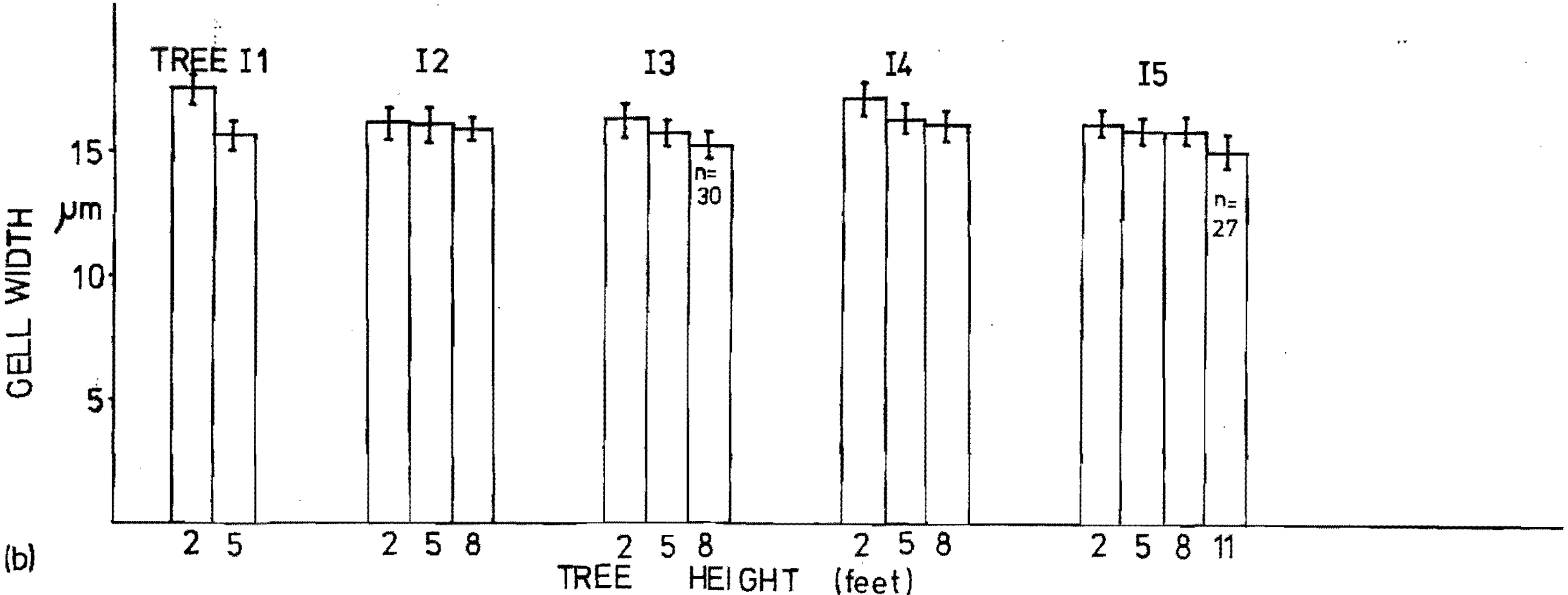
		Degrees of freedom	Mean squares	Variance ratio	Percent variance components
A.	Among trees	9	14760	1.70ns	3.3
	Among heights and trees	27	8542	10.18***	15.5
	Within samples	1751	839		81.2
B.	Among heights-linear components	1	2153	41.40**	
	Among heights-remainder	3	53	0.39ns	
	Within heights	32	132		

Table 4-5. Analyses of variance for fusiform cambial initial length based on A, trees and B, heights. There is no significant cell length difference among different trees, but there is a highly significant difference in cell length among heights among the trees. When based on the individual heights, this significant difference is caused mostly by the linear decrease in cell length with height in the tree.

On the following page:

Fig. 4-14. Mean tangential width of fusiform cambial initials at selected heights in the tree in (a) 5 adult trees and (b) 5 intermediate trees and their 95% confidence limits. Figures for cambial radius and the number of growth rings behind the cambium are the same as in figure 4-11.


(a)
CELL WIDTH µm
15
10
5
TREE A1
A2
A3
A4
A5
2 5 8 11 14
2 5 8 11
2 5 8 11
2 5 8 11
2 5 8 11 14
TREE HEIGHT (feet)


(b)
CELL WIDTH µm
15
10
5
TREE I1
I2
I3
I4
I5
n= 30
n= 27
2 5
2 5 8
2 5 8
2 5 8
2 5 8 11
TREE HEIGHT (feet)

ANALYSIS OF VARIANCE

	Degrees of freedom	Mean squares	Variance ratio	Percent variance components
Among trees	9	54.9	1.27ns	1.5
Among heights and trees	24	43.1	11.42***	17.0
Within samples	1666	3.7		81.5

Table 4-6. Analysis of variance for fusiform cambial initial width based on trees. There is no significant difference in widths among trees but there is a highly significant difference in cell width among heights among the trees.

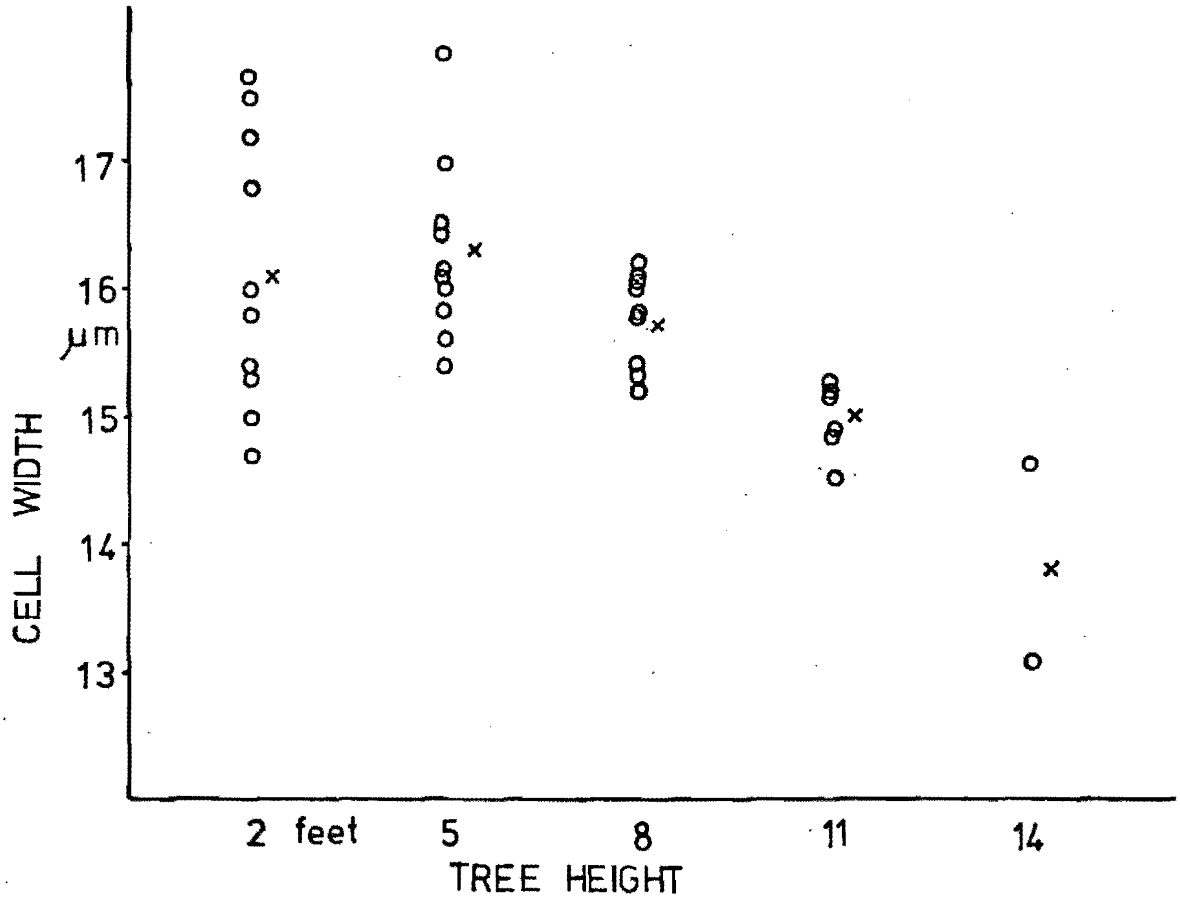


Fig. 4-15. Mean fusiform cambial initial widths for the individual trees plotted against tree height. The mean for the trees at each height is also indicated (the x's are slightly offset to the right in each case).

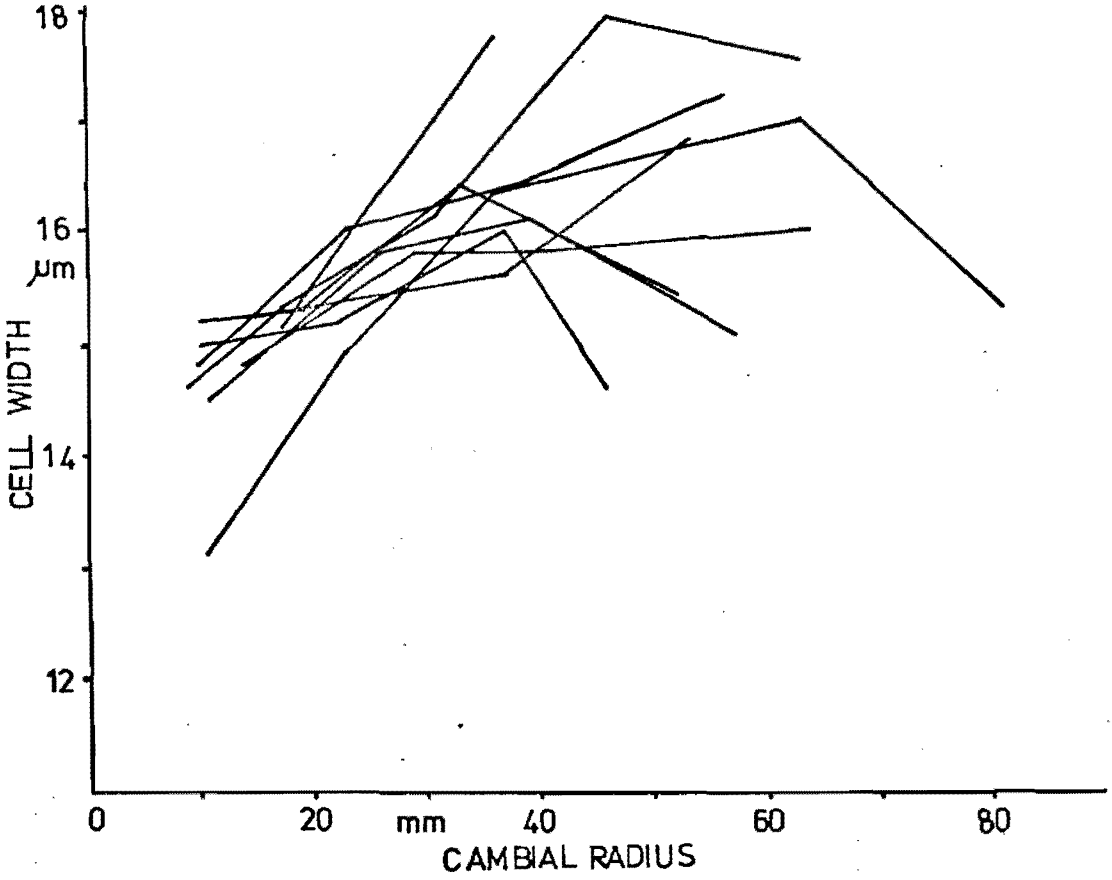


Fig. 4-16. Mean fusiform cambial initial width plotted against cambial radius for the ten trees.

fusiform initial width would appear to be related to the cambial radius.

4.8 DISCUSSION

This investigation has shown that there is no change in the length of the fusiform cambial initials with radial growth in *Hoheria angustifolia*. The minor fluctuations that occur in the length of vessel members within the growth rings are caused by minor changes in the angles of the end walls of these cells. This is a significant result because it indicates that the mean length of a population of fusiform cambial initials, in this species, can remain unchanged from the onset of secondary growth and probably reflects the length of the procambial cells. Such a relationship is not possible in plants with a non-storeyed cambium where circumferential expansion of the cambium is brought about by pseudotransverse anticlinal divisions of the fusiform initials, followed by intrusive growth of the daughter cells, and the preferential loss of some of these. Even in a wood possessing a storeyed cambium, slight increases or decreases in mean fusiform initial length with radial growth can result from the preferential loss of the shorter or longer fusiform initials or the conversion of these into new ray initials. A slight decrease in the length of parenchyma cells in *Aeschynomene hispida*, another plant with a storeyed cambium, has been recorded in Section 3 of this thesis. In this species, however, occasional transverse divisions of the fusiform initials were responsible for the decrease in cell length with secondary growth. Unfortunately, because of the large amount of cell readjustment that occurs with differentiation in *Hoheria angustifolia*, it was not possible to detect whether any such transverse divisions had occured in the fusiform initials of this species.

A positive relationship between fusiform cambial initial length and the length of vessel members in the wood behind the cambium has also been established for *Hoheria angustifolia*. This confirms the general relationship between the length of these cell types proposed by Bailey (1920). The lengths of the vessel members and fusiform cambial initials are similar, indicating that no transverse divisions occur in the xylem mother cells prior to the differentiation of the vessel members. Some rearrangement of the end walls must occur as the fusiform initials have pointed hexagonal tips (Fig. 2-21), and the mature vessel members have slightly oblique to transverse end walls. The extent of this rearrangement would appear to vary between the early wood and late wood cells within each growth ring. As a general trend, the early wood vessel members are slightly wider and shorter than the late wood vessel members and have transverse end walls, while the latter have oblique end walls. It must be pointed out that the position of the growth ring boundaries in Fig. 4-9 are to the nearest millimetre so that some experimental error is involved in the summation of cell dimensions from the first and last millimetre samples from each growth ring.

Since the wood collections were all made on the same day, (18th February, 1965), the slight within ring variations in vessel member length are not responsible for the poor correlation in four of the twenty-five pairs of samples where vessel member and fusiform initials lengths were compared. It is obvious from the measurements of fusiform initial lengths with height in the tree (Figs. 4-11 and 4-12), that some quite considerable variations in length occur with position in the tree. It is of considerable importance, therefore, that samples of vessel members and cambial initials in this species come from exactly adjacent positions in the stem.

The within ring variations in the dimensions of the vessel members are in themselves interesting though their measurement was not intended to be a major part of this study. The variation in tracheid and vessel member length within the growth ring of most woods is generally attributed to seasonal differences in the frequency of pseudotransverse divisions in the cambium (Bannan, 1950), differing rates of elongation of the new daughter initials and varying proportions of cell loss. The extent of the elongation of the derivative cells after they have been cut off from the cambium is, therefore, difficult to assess in different parts of the growth ring. It must be assumed, however, that no such cyclic changes in length occur in the fusiform initials of a storeyed cambium such as Hoheria angustifolia. Any pseudotransverse division late in the season would be perpetuated throughout subsequent radial growth, lowering the mean fusiform initial length. As this does not happen, the slight within ring fluctuations in vessel member length must be attributed to adjustments to the end walls during differentiation.

A decrease in the mean length of the fusiform cambial initials with height in the tree occurs in Hoheria angustifolia, although considerable fluctuations in cell length are apparent in individual trees. This pattern differs from that established for most trees with non-storeyed cambia where cell length increases and then decreases with increasing height in the tree. In these species at least part of the decrease in cell length up the tree must be attributed to the decreasing cambial radius, since this is a factor governing the length of the fusiform initials at any one height (Hejnowicz and Hejnowicz, 1958). Since fusiform initial length is not dependent on the radial distance of the cambium from the stem centre in Hoheria angustifolia, it implies that the decrease in fusiform initial length up the tree occurs with the ageing of the leading shoot. Such a result is not unexpected as it confirms the views of Dinwoodie (1963) and others, that the activity of the terminal meristem could influence the length of the fusiform initials in the cambium and therefore the length of their derivatives throughout the tree.

The pattern of the fusiform initial length within the internodes of a stem has never been adequately investigated. Bailey and Tupper (1918) noted that tracheids tended to be shorter at nodes and other regions where growth adjustments occur. It is possible that internodal variation in the length of the procambial initials in *Hoheria angustifolia*, perpetuated in the cambium even after radial growth, may be the cause of the considerable differences in the cell length sequences in the individual trees studied (Fig. 4-11). Although no obvious pattern emerges from the cell length measurements made at every inch on tree I5 (Fig. 4-12), a summation of the mean fusiform initial lengths from near the base, the middle and the top of each year's height growth (Fig. 4-17), tends to indicate that longer fusiform initials are laid down during the "height" of the year's growth than later in the season. As with the within-ring summation of mean lengths, difficulty in determining the exact resting point of the apex each winter on ring counts alone may well account for the inconsistency of some of the results. The relation of these inch sample points to the internodes is also unknown.

The mean width of the fusiform initials in *Hoheria*

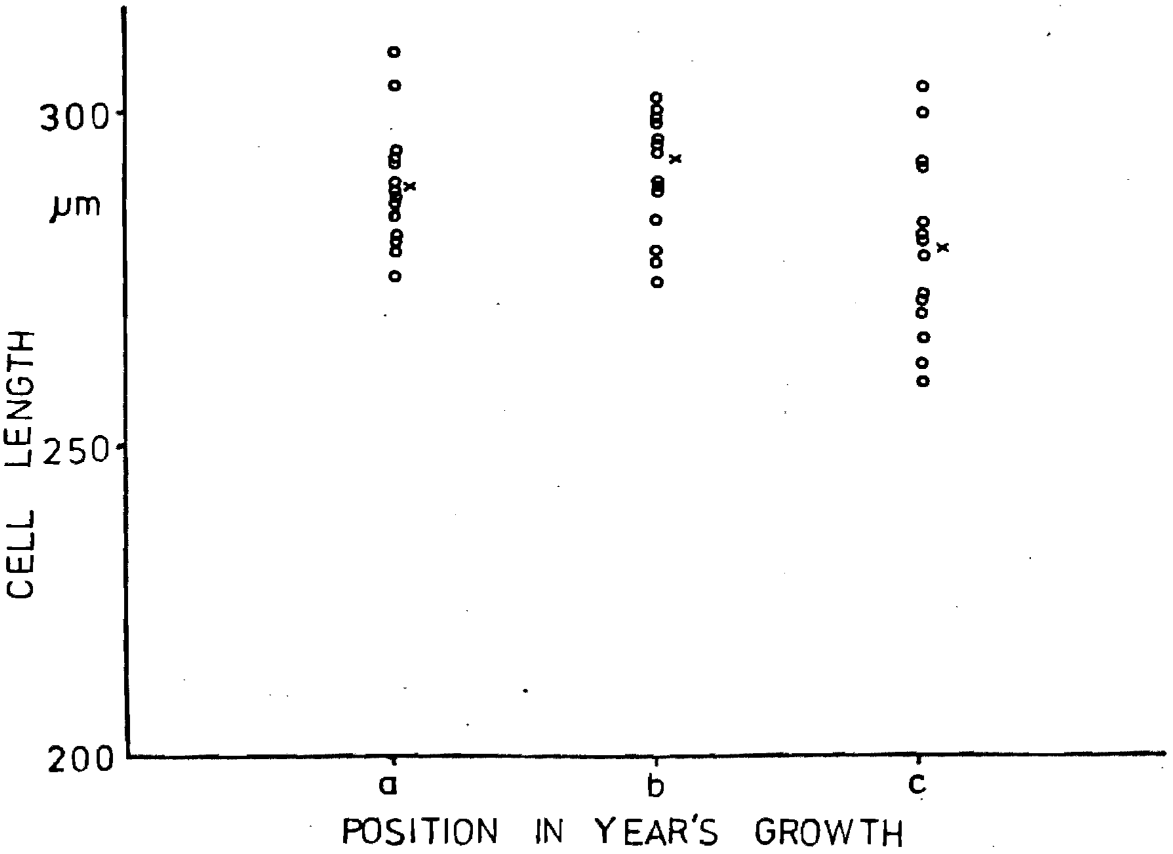


Fig.4-17. Mean fusiform initial length for (a) the lower third, (b) the middle third and (c) the upper third of each year's height growth for the tree I5. The means of the means are marked with x's offset slightly to the right in each case. Different amounts of shoot growth are involved for each year (see Fig. 4-12).

angustifolia increases and then decrease with increasing height in the tree. Since stems taper with increasing height, fusiform initial width would appear to be governed by the radial distance of the cambium from the stem centre. A similar result has been recorded for *Aeschynomene hispida*, but in this case cambial derivatives were used to determine cell widths from a single stem disc. The circumferential expansion that accompanies radial growth would appear to be met in a storeyed wood by an increase in the tangential widths of the fusiform initials, as well as by anticlinal divisions in the radial longitudinal plane. The variation in the mean fusiform initial widths in *Hoheria angustifolia* stems greater than 25 mm radius remains unexplained.

SECTION FIVE

THE VASCULAR CAMBIUM AND CELL SIZE IN THE SECONDARY XYLEM - GENERAL CONCLUSIONS

5.1

The lateral meristem known as the vascular cambium is the source of the greater bulk of plant material, and the tissues it produces are among the most complex found in plants. It is from the cambium that wood (secondary xylem) is derived. For over a century now, scientists have attempted to relate the structure and arrangement of the cells in the secondary xylem to the development and activity of the vascular cambium.

Probably because of the importance of cell length as an index of the physical and paper making qualities of timber, an unusually extensive literature has accumulated on the variation in size of the xylem cambial derivatives. A number of investigations have also been made on the development, action and control of the vascular cambium and some reports have attempted to relate these activities to the cell length variation patterns within secondary xylem of certain trees. Because of their economic importance, interest has been centred mainly on the gymnosperms and those dicotyledonous trees whose timbers are regarded as usable. This has meant that the majority of studies on the vascular cambium and its derivatives have been made on plants with the non-storeyed type of cambium.

By comparison the storeyed cambium has been somewhat neglected. It is hoped that the observations recorded in this thesis will fill some of the gaps in our present understanding on the activities of this meristem and its influence on the size of certain xylem derivatives.

The association of storeyed cambia in woods with other highly specialized features has led to the conclusion that storeyed structure is an advanced character. Storeyed cambia occur in many systematically widely different dicotyledonous families, sometimes in all the members of a family and sometimes only in a few. This irregular taxonomic distribution has led to the belief that this character has arisen independently in many lines (Philipson *et al.*, 1971).

The observations on the ontogeny of the storeyed cambium in *Hoheria angustifolia* (Section 2) have revealed some interesting differences between the early development of the cambium in this species and those already investigated by other anatomists. The study has also confirmed the present author's belief that the procambium and cambium are best regarded as different stages of the same lateral meristem. Perhaps the most useful observation, however, is that the interfascicular sectors in *Hoheria angustifolia* are converted to systems of primordial ray initials at a very early stage of development so that a complete cylinder of cambium exists long before internodal elongation has ceased. This meristematic cylinder remains non-storeyed until elongation has ceased. The development of the storeyed pattern is therefore a consequence of radial growth in the absence of internodal elongation. If the meristem had any tendency to storeyedness as a result of radial longitudinal anticlinal division in the procambial cells, it would be lost as a result of the transverse divisions necessary to maintain optimum cell length in the elongating internodes.

The development of the storeyed pattern with radial growth has been described for _Aeschynomene hispida_ (Section 3). It would have been interesting to have followed this process in _Hoheria angustifolia_ but the nature of the secondary xylem in this species is such that the division pattern of the cambium cannot be traced in the cambial derivatives. It is clear that with the evolution of the radial longitudinal division, the cambium has also acquired the ability to balance the frequency of anticlinal division exactly with needs of the expanding cambial circumference.

Finally the lengths and widths of the fusiform cambial initials in _Hoheria angustifolia_ have been examined with reference to position in the tree and their relation to the lengths and widths of the vessel members in the secondary xylem (Section 4). The absence of cell elongation after anticlinal division in the fusiform cambial initials of this plant has the effect of eliminating one of the causes of the cell length variation patterns that are found in plants with non-storeyed cambia. The recorded result of a decrease in cell length with increasing height in the tree is therefore of particular interest since it reflects a decrease in the size of the primordial fusiform initials produced by the apex as the tip of the leading shoot ages. The apparent differences in fusiform cambial initial length between sampling points in any one tree and the variation in vessel member length with position in the growth ring, emphasize the importance of adequate sampling methods and the value of full statistical treatments of the data in any cell dimensions project. It is likely that some of the results recorded by other anatomists on cell length patterns within the tree could be regarded as suspect on these grounds. This may in part explain the apparent differences in the cells size sequences reported by various workers in this field.

This thesis adds but a little to our knowledge of the cell size variation patterns in trees. Written by a botanist with an interest in the anatomy of plants rather than by a timber technologist, the thesis is intended to present some new information on the activities of the vascular cambium and the influence that these activities have on the size of the axial elements in the secondary xylem in certain species. If nothing else, it has added considerably to the author's understanding of the complexity of the processess that accompany radial growth in woody plants.

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PUBLISHED PAPERS RELEVANT TO THIS THESIS

PAPER ONE

A THEORY OF THE CAUSES OF SIZE VARIATION IN WOOD ELEMENTS

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and
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A THEORY ON THE CAUSES OF SIZE VARIATION IN WOOD ELEMENTS

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General patterns of variation in the size of wood elements in the trees of both softwoods and hardwoods have long been recognized (Sanio, 1872; Spurr & Hyvarinen, 1954; Dinwoodie, 1961) but no explanation of them has been proposed. We believe that these patterns result from a few principles underlying the development of the cambium. The relationships have been neglected because more attention has been given to variation displayed by the cambial derivatives, in particular the xylem elements, than by the cambial initials themselves. In the cambial derivatives the variation patterns are complicated by the introduction of modifying factors. These take effect during the elongation of elements, particularly hardwood fibres, during differentiation. Consideration is given here only to variation imposed on the xylem elements by reason of variation in the size of the initials from which they have been derived. Such variation is preserved approximately unchanged in the tracheids of softwoods and the vessel elements of hardwoods (Bailey, 1920).

Two major patterns of variation occur, each with its own underlying causes. They may be thought of as (a) horizontal, or occurring across the growth rings from the primary xylem outwards; and (b) vertical, or occurring up the tree within any given growth ring. These two aspects of variation within a tree will now be considered separately.

Horizontal Variation

The average length of a population of cambial initials does not normally remain constant: as the population moves outwards with increasing girth of the stem, average length is usually found to increase over the first few centimeters. After this early steady increase in average length the behaviour is less regular. The average length may remain virtually constant over a long period, it may gradually increase or decline or it may fluctuate. The general pattern of horizontal variation is represented in Fig. 1 (curve A).

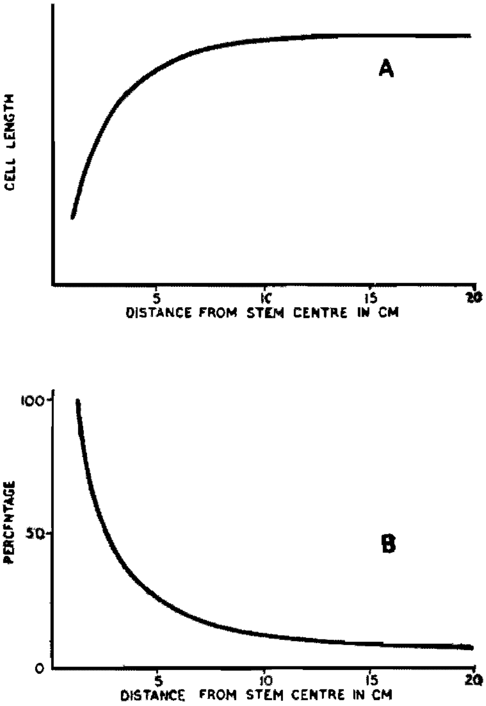

Fig. 1 — Curve **A**. Generalized pattern of increase in cell length with radial distance from the stem centre common to most woody plants with non-storeyed cambia. Curve **B**. Decrease in percentage of the total cells of the cambial cylinder which must divide anticlinally for every centimeter of radial growth (average cell size remaining constant).

The factor which 'permits' this variation is the method of anticlinal cell division found in most dicotyledons and all gymnosperms, namely the pseudotransverse division and the subsequent recovery of size of the cells by intrusive growth. That this is so becomes apparent when a comparison is made with a storeyed cambium, in which anticlinal divisions are radial-longitudinal with little or no subsequent increment in length. In trees with this type of cambium, horizontal variation of vessels is lacking (Bailey, 1923). While pseudo-transverse division allows variation to appear, the occurrence and extent of this variation is dependent on changes in the balance between several factors. It is because the balance of these factors changes in a regular way as growth of the tree progresses, that the variation follows the particular pattern of variation shown in Fig. 1A, which is so general in trees. These factors are: (a) the frequency of the pseudo-transverse divisions; (b) the relative size of the parent and daughter cells; and (c) the preferential loss of short initials (by no means all initials survive, and it is usually the shorter which are lost; Bannan & Bayly, 1956).

The new cells produced by the pseudotransverse divisions provide for the increase in circumference of the cambium as the girth of the trunk increases. Over the first few centimeters of radial growth the perimeter of the stem is increasing very rapidly and a much higher rate of anticlinal division is required to provide new initials for the cambium than is required later in radial growth. Consider a hypothetical case where the cambium is thought of as a cylinder of ever increasing diameter. Since the circumference of this cambium is always proportional to its radius ($C=2\pi r$), for a one centimeter increment of radial growth occurring from one to two centimeters radius, the cylinder will have doubled its circumference, i.e. during this one centimeter of radial growth every fusiform initial of the cambium will have on the average divided anticlinally at least once. This is assuming that the daughter initials elongate to the length of the parent initial and retain the same tangential dimension. However, further out at say twenty centimeters from the stem centre, for an equal increment of radial growth, the circumference will increase by only one-twentieth, i.e. during this one centimeter of growth from 20 to 21 centimeters radius only one cell in every twenty will need to divide anticlinally. The " required rate " of anticlinal division per centimeter of radial growth which is necessary to maintain the cambium is illustrated graphically in Fig. 1 (curve B).

It is evident, therefore, that a lower frequency of anticlinal division in the cells of the cambial cylinder is required when it has attained a greater diameter than during its early growth. In fact, over the outer portion of radial stem growth the rate of anticlinal division is higher than is required to supply the needs of the expanding cambium and a high loss of initials occurs. Over the first few centimeters distance from the stem centre, although the rate of anticlinal division is relatively high (Bannan, 1950) and the loss of initials somewhat lower than further out, the resultant effective supply of new fusiform initials does not alone supply the needs of the rapidly expanding perimeter of the cambium. During this period, the " deficiency " of initials is compensated for by an increase in the dimensions of the daughter initials beyond those of their parents. While some increase in the tangential dimension of initials occurs, this is of less importance than their elongation well beyond the length of the former initials. That is to say, a steady increase in cell length occurs outwards during the early growth of a stem. This increase in length makes an important contribution to the circumferential increase of the cambium (Bailey, 1923), because the number of cells at any level becomes increased by intrusion of adjacent cells. These considerations explain the nature of the curve shown in Fig. 1A which is typical of most trees with a non-storeyed cambium.

The high relative increase in circumference in an axis with a small diameter is suggested as the cause of the initial elongation typical of a cambium which increases by pseudotransverse division.

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The diameter of the cambium, at the time primary growth ceases and secondary activity begins, will have a direct bearing on the subsequent amount of elongation of its cells and consequently on the amount of core wood developed.

In concluding this account of horizontal variation it is necessary to add that the basic pattern may be modified. For example, most woods show seasonal fluctuations in the dimensions of their elements, but these do not affect the overall trend in the average over a number of years. Also, it is found that the average length of elements decreases when a tree is growing in the girth rapidly. These fluctuations are due to shifts in the balance between the three factors considered above. Since they are here regarded as secondary modifications of the basic pattern, they will not be considered further.

Vertical Variation

In attempting to understand the causes underlying the variation in element size throughout the length of a trunk, it is useful first to consider a hypothetical tree in which the trunk tapers evenly, the growth rings being parallel and equal in breadth. It might be expected that the horizontal variation previously considered would be repeated at all levels up the trunk. If that were so there would be no vertical variation, and the trunk would consist of an elongated cone containing a true cylinder of " core " wood. " Core " wood is the wood formed in stems while the length of the initials is increasing. At the top of this hypothetical cone the immature " core " would project as the tapering apex of the tree. Such a uniform trunk has never been reported: the pattern of variation most generally found is for the size of elements to increase gradually from the base of the tree, to reach and maintain a more or less steady state, and then to decline again in the upper part of the trunk. To understand this pattern it must be recognized that it is made up of two components. The first is due to variation in the average size of the initials at the time they originate at the end of primary growth. The second consists of modifications of the horizontal variation. Since these modifications are found to be correlated with height from the ground, they impart vertical variation patterns on the ultimate size of the elements of the mature wood of the trunk. These two components will be considered separately.

THE LENGTH OF THE CAMBIAL INITIALS AT THE TIME OF THEIR ORIGIN — This is found to increase gradually from the base of the tree to a more or less steady figure, and then declines again. This variation is interesting in itself, but its chief importance lies in the influence it has on the size of subsequent xylem elements produced at that level. There is evidence that, in non-storeyed woods, where the initials are long at their inception the mature wood contains longer elements (Bisset *et al.*, 1951). In storeyed cambia the original initial length is of even greater significance as the length remains constant throughout the life of the tree. In *Hoheria* very local variations due originally to position relative to the nodes are continued into the mature wood (unpublished information). In non-storeyed cambia, on the other hand, the much more fluid conditions, with an initial very rapid increase in length, probably obliterates these immature irregularities and results in a more homogeneous mature size distribution, although the over-all vertical variation pattern is retained.

The following hypothesis is presented to explain the pattern of variation in the length of cambial initials at the time of their origin. There is some evidence that the original size of the initials is correlated with the rate of height growth (Dinwoodie, 1963; Bannan, 1966). The pattern of variation in the original size of the initials can be explained, therefore, by changes in the rate of height growth during the development of the tree. The slow beginning, followed by a grand period of growth and a subsequent decline (Husch, 1963) correspond with the observed pattern of fusiform initial length.

It is frequently reported that the height at which maximum length of elements is attained increases in successive growth rings. If the measurements are related to distance from the pith, rather than the number of growth rings, it is found that cell length is the same at equal distances from the pith (Hejnowicz & Hejnowicz, 1958). The apparent delay in attaining full length in the former example is due to the increase in breadth of growth rings as ground-level is approached, which is very general in trees (Husch, 1963).

This gradual increase in the original length of the initials followed by a decline is a simplification of the variation patterns actually present in trunks. These may show fluctuations in average initial size with quite minor increments in height. These modifications of the overall trend could be due to fluctuations in the rate of height growth, whether seasonal or intermittent; to position in the internode; or to change in the diameter of the cambium at its initiation. This last factor can become significant in trees which, like *Paulownia*, have very strong primary growth in spring.

MODIFICATIONS OF HORIZONTAL VARIATION CORRELATED WITH HEIGHT — In the account of horizontal variation, it was noted that a number of factors, not all well understood, can affect the balance between the three factors governing population size in the cambium at any one level in the trunk. This may lead to such modifications of curve A as the steepness of the initial rise, the mature length attained, the radius of the " core ", and the shape of the later part of the curve. These effects may occur in a random manner, due to the exigencies of the environment. However, in addition to any random changes, there may be characteristic modifications of this kind which are common to most trees or to a particular class of tree. Unfortunately, published information on these effects is fragmentary. To illustrate their possible nature the typical canopy tree with boughs forming a rounded crown will be considered. The trunk of such a tree is columnar in form, without taper. This is due to the late formed growth rings becoming broader in their upper part as the crown develops. As rapid increase in diameter is known to be associated with a decrease in the length of elements, it is to be expected that trees of this kind would display a vertical modification of the normal horizontal variation: the later part of curve A would be depressed in the upper part of the trunk. It is also to be expected that the thicker basal part of trunks would show curves with a falling average length of mature elements. In this type of trunk, therefore, modification of horizontal variation would augment the variation at the top and base of the tree attributable to the smaller size of the initials.

It may be concluded that the familiar distribution pattern of variation in the size of wood-elements within a tree can be theoretically explained in terms of factors influencing the development of the cambium. Some of the explanations submitted here lack a firm evidential foundation. To supply this, data relating to the development of the cambium and its derivatives are being sought.

Summary

Variations in the size of wood elements within most trees conform to a pattern which has long been recognized. Some features of this pattern are explained in terms of factors affecting the development of the cambium. The rate of increase of the circumference of the cambium per unit increase in radius is much higher in thin than in thick stems: this fact underlies the typical increase in element-length near the pith. A theory is proposed that variation at different heights from the ground is due, in part, to different rates of growth of the axis at the time the cambial initials were formed.

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PAPER TWO

THE VASCULAR CAMBIUM: ITS DEVELOPMENT AND ACTIVITY

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Relevent chapters that were written by the present author.

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Variations in the size of fusiform cambial initials

Most of our present information on changes occurring in the vascular cambium of plants has been obtained from investigations made on the secondary xylem and secondary phloem, and not from direct examinations of the cambium itself. Since the fusiform initials leave a record of their activities in both the secondary xylem and secondary phloem, either tissue can be used to study the sequence of changes in cell size and arrangement in the meristem. For a time there was a tendency to use the phloem derivatives for studying changes that had occurred in the cambium, the most notable piece of research being that by Klinken (1914), who, using the phloem derivatives of *Taxus baccata*, first developed the technique of examining serial tangential sections for studying cell changes in the cambium. One reason for using the phloem in preference to the xylem is that the phloem elements change less in size during maturation, and hence the tangential sections were thought to give a more accurate picture of the probable lengths of the cambial initials at the time these cells were formed. However, Esau and Cheadle (1955) have shown that divisions, varying in plane from vertical to almost transverse, are common within the phloem derivatives of the vascular cambium. The derivatives of a division that is nearly transverse will obviously be shorter than the fusiform initial from which they are indirectly derived. Since some of these derivatives will differentiate into sieve elements which do not elongate further before maturation, the length of the sieve elements may have no relation to the length of the fusiform initials. Secondly, as other recent investigators have pointed out, if the sequence of divisions and size changes in the cambium is to be followed over any prolonged period of time the secondary xylem must be used, since distortion and abscission affect the phloem. Furthermore, since divisions are more frequent to the xylem side of the cambium, this tissue gives a more complete picture of events. Bannan (1950) points out that some anticlinal divisions in the cambium, especially those followed by the loss of certain cells, are not recorded in the phloem.

Data on changes in the cambium obtained from investigations on the xylem and phloem and not on the cambium itself, however, do not give us a complete or entirely accurate picture of the activities of this meristem. Although a record of the principal size changes and divisions in the cambium is preserved in its derivative tissues, data obtained from derivative elements of necessity are erroneous to the extent of the elongation of the matured elements. Regrettably few results have been published on the relation in size between the cambial cells and their derived elements. Bailey (1920), from a comparative study of the size variations of cambial initials and their derivatives, concluded that in *Ginkgo* and the Coniferae the length of the tracheids of the last-formed xylem closely resembled that of the fusiform initials from which they were derived but were slightly longer. He concluded that the elongation occurring during differentiation was of the order of 5–10%. In dicotyledons he found that the initials were much shorter than the fibres but were approximately the same length as the vessel members. Chattaway (1936) found that the fibres in dicotyledonous woods were 1·1–9·5 times longer than the cambial initials, though elongation to several times the original length occurs only when the initials are very short.

The assumption that the patterns of variation in length of xylem elements reflect the principal size changes in the cambial initials can hold therefore for only two cell types. Since conifer tracheids elongate relatively little during differentiation, variations in their length may be taken as an approximate indication of the size fluctuations in the cambium. Dicotyledonous vessel members also change very little in length during differentiation. On the other hand, variations in the length of dicotyledon fibres are due in part to intrusive growth during differentiation. Studies in plants with storeyed cambia, where the fusiform initials remain approximately the same length throughout the growing season, have shown that fibres elongate by varying amounts, depending on their position within the growth ring. Factors such as temperature influencing cell-wall plasticity may produce changes in the degree of fibre elongation. Hence the patterns of size changes in wood fibres cannot be regarded as indicating the size changes that have occurred in the cambium. The method of measuring the vessel elements is important if these cells are to be used as a measure of the length of the fusiform initials at the time they were cut off from the cambium. Chalk and Chattaway (1934) concluded that the 'total member length' as measured from tip to tip of the cell corresponded more closely in length with the fusiform initial than did 'mean body length' as measured from the centre of the pores and ignoring any tail or extension. Philipson and Butterfield (unpublished data) found in the wood of *Hoheria* *angustifolia* Raoul, a plant with a storeyed cambium, that the mean body length of the vessel members of the last-formed xylem corresponded more closely with the mean storey height of the fusiform initials than did the total vessel-member length. Minor fluctuations in the total vessel-member length were found to exist within the growth ring, although there are no comparable changes in the size of the fusiform initials in a storeyed cambium. These fluctuations are due to the broad vessel elements of the early wood having almost transverse end walls compared with the more oblique end walls of the narrow elements formed in the late wood.

Size variations within the one tree

The general pattern of variation in tracheid size within the secondary xylem of conifers was established by Sanio (1872) for *Pinus sylvestris*, and has since been observed in many other softwoods and hardwoods. The literature dealing with the variation in size of the xylem elements has been reviewed by Spurr and Hyvärinen (1954) and Dinwoodie (1961). Two major patterns of variation occur which may be thought of as: (1) horizontal, or occurring across the growth rings from the primary xylem outwards, and (2) vertical, or the pattern occurring up the tree within any given growth ring. These two aspects of variation in initial length will be considered separately along with the other factors that superimpose minor fluctuations on the basic patterns.

Changes in the fusiform cambial cell length at any one level within the tree

From his observations on *Pinus sylvestris*, Sanio concluded that tracheid length at any one level in the tree increased outwards from the pith through a number of annual rings until a definite size was reached, which then remained constant through the following rings (Fig. 4.1). While there has since been

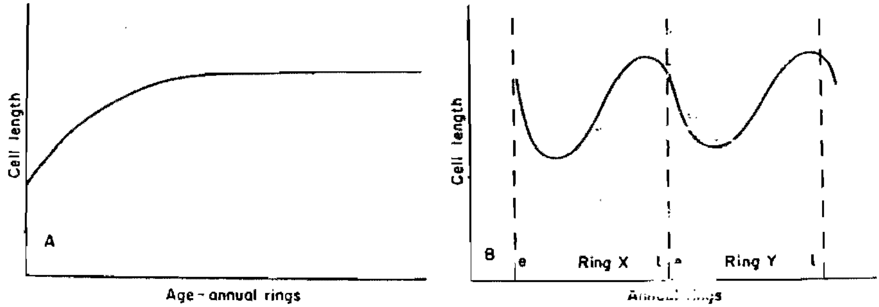

Fig. 4.1 Graph A illustrates the generalized pattern of increase in the average length of derivatives in passing from the innermost to the outermost secondary xylem of the stem. Graph B illustrates this variation within individual growth rings, (e) early wood and (l) late wood of two adjacent growth rings.

general agreement (Lee and Smith, 1916; Anderson, 1951; Hejnowicz and Hejnowicz, 1956) that there is an initial increase in tracheid length over the first growth rings until a maximum is reached, investigations beyond this point have been rather contradictory. Some investigators have recorded results similar to those of Sanio, while others record a further slight increase or even a decrease in mean cell length. Most investigators have observed minor fluctuations in the mean cell length in the outer growth rings, but these are mainly due to secondary influences which will be discussed later.

Once a group of cambial initials has been laid down at the end of primary growth it will move horizontally outwards as the stem grows radially. Radial growth is accompanied by circumferential expansion of the cambium which involves an increase in the number of fusiform cells in the cambium (Chapter 2). This occurs in the non-storeyed cambium by means of a pseudotransverse wall being laid down usually near the centre of the dividing cell. The two daughter initials so formed then elongate by the intrusive growth of their cell tips. The resultant size of the fusiform initials in the cambium is governed by changes in the frequency of pseudotransverse division and the rate and amount of elongation of new initials.

(a) *Frequency of pseudotransverse division.* The length of the cambial fusiform initials is related in part to the frequency of the anticlinal divisions in the cambium owing to the semi-transverse nature of these divisions. A high frequency of pseudotransverse division naturally tends to depress cell length, whereas a low frequency is usually associated with a high mean cell length.

Although high frequencies of pseudotransverse division in the cambium tend to depress cell length, the resultant reduction is not as great as might be expected, since high rates of anticlinal division are usually accompanied by the loss of initials and more rapid elongation of the surviving daughter initials.

(b) *Elongation of new cambial initials.* The rate and amount of elongation of new fusiform cambial initials is naturally an important factor governing the size of the cells at any particular time. For example, the rate of fusiform cell elongation following division varies widely according to the time of year. Bannan (1951b) found that in both *Chamaecyparis* and *Thuja* the rate of elongation of sister fusiform initials was slow during the first quarter of the year's growth, tending to increase during the second and third quarters of the year and reaching a maximum during the last quarter. Evert (1961), however, found in *Pyrus communis* that most elongation occurred before the cambium had resumed activity each year. Local factors often operate to produce irregularities in the rate of cell elongation. For example, after the loss of an initial a neighbouring initial may elongate rapidly into its place. Since this loss of initials is highest towards the end of the year's growth, it no doubt plays an important part in the increase in cell elongation during this period. This results in a high average cambial cell length towards the end of the growth ring. There is considerable variation in the rate and amount of cell elongation from cell to cell in the cambium. One fusiform initial may elongate a great deal and another only slightly. The presence of obstructing rays may reduce the possible elongation of new initials.

(c) *Preferential loss of new fusiform initials.* A third factor that plays a part in the maintenance of a high mean cell length is the preferential loss of short initials after pseudotransverse division. Loss of initials occurs in various ways in all parts of the tree (Bannan, 1951a). The pitch and length of the anticlinal wall at the time of pseudotransverse division fluctuate widely. This can result in two long daughter initials lying almost side by side where the anticlinal wall is very oblique, or in the case where the division is almost transverse the new cells are comparatively short. Often an uneven division results in the loss of the shorter daughter initial. Progressive shortening occurs, resulting in a contraction in size of the radial file of derivatives produced by a disappearing initial. After the pseudotransverse division the initial usually undergoes sporadic periclinal divisions, progressively shortening, and then ceases division altogether. Initials which are lost from the cambium lose their capacity for periclinal division and pass off into maturation, becoming malformed xylem or phloem elements or undergoing reduction to become ray initials. The continuation of the longest cells and the elimination of the shortest is a factor in the maintenance of an efficient cell length (Bannan, 1957b).

The interplay of these factors, (*i*) the frequency of the pseudotransverse divisions, (*ii*) the amount and rate of elongation of the new cells, and (*iii*) the preferential loss of the shorter daughter initials, influences the mean cell size at any given distance from the centre of the stem. Over the first few centimetres of radial growth the perimeter of the stem is increasing very rapidly, and a much higher rate of anticlinal division is required to provide new initials for the cambium than is required later in radial growth. Consider a hypothetical case where the cambium is thought of as a cylinder of ever-increasing diameter. Since the circumference of this cambium is always proportional to its radius ($C = 2\pi r$), for a 1 cm increment of radial growth occurring from 1 to 2 cm radius, the cylinder will have doubled its circumference, i.e. during this 1 cm of radial growth every fusiform initial of the cambium will have on the average divided anticlinally at least once. This is assuming that the daughter initials elongate to the length of the parent initial and retain the same tangential dimension. However, farther out, at, say, 20 cm from the stem centre, for an equal increment of radial growth, the circumference will increase by only one twentieth, i.e. during this 1 cm of radial growth from 20 to 21 cm radius only one cell in every twenty will need to divide anticlinally. The 'required rate' of anticlinal division per centimetre of radial growth which is necessary to maintain the cambium is illustrated graphically in Fig. 4.2, curve B.

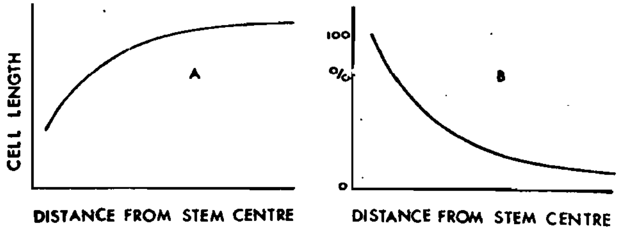

Fig. 4.2 Curve A illustrates the generalized pattern of increase in cell length with radial distance from the stem centre common to most plants with non-storeyed cambia. Curve B illustrates the decrease in the percentage of the cells of the cambial cylinder which must divide anticlinally for every increment of radial growth.

It is evident, therefore, that a lower frequency of anticlinal division in the cells of the cambial cylinder is required when it has attained a greater diameter than during its early growth. In fact, over the outer portion of radial stem growth the rate of anticlinal division is higher than is required to supply the needs of the expanding cambium, and a high loss of initials occurs. Over the first few centimetres distance from the stem centre, although the rate of anticlinal division is relatively high (Bannan, 1950) and the loss of initials somewhat lower than farther out, the resultant effective supply of new fusiform initials does not alone supply the needs of the rapidly expanding perimeter of the cambium. During this period the 'deficiency' of initials is compensated for by an increase in the dimensions of the daughter initials beyond those of their parents. While some increase in the tangential dimension of initials occurs, this is of less importance than their elongation well beyond the length of the former initials. That is to say, a steady increase in cell length occurs outwards during the early growth of a stem. This increase in length makes an important contribution to the circumferential increase of the cambium (Bailey, 1923), because the number of cells at any level becomes increased by intrusion of adjacent cells. These considerations explain the nature of the curve shown in Fig. 4.2A, which is typical of most trees with a non-storeyed cambium.

In the above hypothetical case the rate at which new daughter initials reach or exceed the length of their former parent cell was not considered. The very high rate at which the daughter cells attain their full size during the first few centimetres of radial growth further adds to the maintenance of the cambium during this period of rapid expansion of the stem. It must be emphasized, however, that all these processes are complementary, and one is not necessarily the cause of the other. In plants with non-storeyed cambia, where no elongation of new initials occurs after pseudotransverse division, there is necessarily a decrease in the mean cell length outwards from the centre of the stem. An interesting example where this type of behaviour occurs is found in *Hibiscus lasiocarpus.* In this species Cumbie (1963) found that the anticlinal divisions occurred in many planes, varying from pseudotransverse to truly radial longitudinal. This partly compensates for the lack of elongation in new cells. In the storeyed cambium, where all the anticlinal divisions are in the radial longitudinal plane, the percentage rate of anticlinal divisions per centimetre of radial growth is sufficient to meet the requirements of the expanding cambium, except possibly in the first few millimetres of radial growth. In this region, adjacent to the primary xylem, many woods that otherwise possess a typically storeyed nature in the outer regions of the stem show a somewhat non-storeyed appearance.

Secondary influences

While the decreasing rate of necessary anticlinal division in the cambium is the primary cause of the increase in mean fusiform initial length for a distance outwards from the centre of the stem, secondary influences may impart fluctuations to the basic pattern or changes in the maximum cell length attained or the rate at which it is attained. The most important of these factors is the actual rate of radial growth with time as expressed by growth-ring width. There are numerous reports on the relation of tracheid length to ring width (see Dinwoodie, 1963). Although some authors have claimed that wide rings induce the formation of long cells, the converse is generally the case. Bisset, Dadswell, and Wardrop (1951) showed that, in general, wide rings correspond with short tracheid length in *Pinus radiata.* Other authors have been unable to find any relationship.

The problem of the relationship between ring width and cambial behaviour with reference to cell length was explored by Bannan (1959) by examining white cedar stems of the following types: (1) stems of similar size but with rings of diverse widths; (2) stems showing a transition in their peripheral growth from wide rings to narrow rings; (3) stems exhibiting an alteration from narrow to wide rings, in their final growth; and (4) stems with rings of varying widths in different sectors. In the first type much variation occurred from tree to tree, with no consistent relationship between the frequency of pseudotransverse division in the cambium and ring width. However, mean cell length at pseudotransverse division showed an increase with decrease in ring width. In the second type the transition was usually accompanied by a slight increase in cell length and the third by a small decrease in cell length. All comparisons revealed a definite tendency for greater cell length to be associated with decreased ring width. The lack of a relationship between the rate of multiplicative division and ring width, however, led Bannan to conclude that cell size is due not so much to the frequency of pseudotransverse division as to inherent determiners, the latter being influenced to some extent by growth rates.

Further information on the relation of cell length to growth-ring width has been revealed by Bannan in a more recent series of papers. It was shown for *Thuja* (Bannan, 1960) that, among trees of similar diameter, the maximum cell lengths occurred in growth rings less than 1 mm wide, while trees with wider growth rings tended to have shorter cells. However, the rate of pseudotransverse division was uniform in both fast-grown trees (i.e. those with wider growth rings) and slow-grown trees (i.e. those with narrower growth rings). In *Pinus* (Bannan, 1962) maximum cell lengths were also associated with growth rings less than 1 mm wide, but the frequency of pseudotransverse division increased sharply as ring width decreased below 1·3 mm. Results similar to those for *Pinus* were found in *Picea* (Bannan, 1963a) and *Cupressus* (Bannan, 1963b). This increase in the rate of pseudotransverse divisions tends to decrease the cambial cell length in trees with very narrow growth rings. Hence, although there is, in general, an inverse relationship between ring width and cambial cell length, the sharp increase in the rate of pseudotransverse division in very narrow rings can counteract this effect by tending to depress cell length. Although this inverse relationship holds, Bannan (1965) found that the yearly amount of cambial cell elongation dropped as ring width decreased, but not in proportion to the decline in the ring width. Thus, the cumulative elongation through a lineal series of cells per centimetre of xylem increment increases as ring width decreases, resulting in the amount of elongation occurring during the production of several narrow rings greatly exceeding that through a single ring having the same width. The higher rate of pseudotransverse division towards the end of the growth ring naturally lowers the mean length of the cambial cells at this stage. Bannan (1954) reported that the pseudotransverse divisions sometimes occur earlier in the elongation cycle in more vigorously growing trees than in slower-growing ones of similar diameter. This may cause a slight decrease in cell length in rapidly growing trees.

Chalk and Ortiz (1961) have suggested that the decrease in mean cell length in wide rings in *Pinus radiata* is due to an increase in the number of pseudotransverse divisions occurring in the xylem mother cells. Bannan (1957a), however, has shown that extra-initial pseudotransverse divisions are very few and normally constitute only about 2% of this type of division, though in a very fast grown specimen of *Cedrus deodora* (Roxb.) Lovd. a figure of 20% was recorded. Chalk and Ortiz believe that this increase in the number of pseudotransverse divisions in the xylem mother cells in the early wood is a major factor in lowering the length of the derivatives in this part of the growth ring.

Although the basic pattern for increase in cell length outwards from the centre of the stem, at any one level, has been established by numerous workers for equally numerous trees, remarkably few investigations have been made of the relative effects of age and distance from the pith on tracheid length. Anderson (1951) in a study of a number of conifers, found that cell length at a given distance from the pith was the same throughout the trunk, irrespective of the number of rings involved. Hejnowicz and Hejnowicz (1958), from measurements made on a 50-yr-old tree of *Populus tremula* L., obtained results similar to those reported by Anderson for conifers; the length of elements at a given distance from the pith was the same throughout the trunk irrespective of the number of rings involved, except in this instance for the lower part of the trunk up to 2 m above ground. At the same time they concluded that the distance from the pith was a comparative measure of the number of cambial generations involved in wood formation, i.e. a measure of the relative age of the cambium. This meant that elements situated at the same distance from the pith at various levels were formed by the cambium at the same relative age. Thus the length of the vessel elements, and by inference the fusiform initial length, is more closely related to the relative age of the cambium than to the absolute age of the cambium as measured in terms of growth rings.

It would appear that in the early part of radial growth, where the ratio of anticlinal to periclinal divisions in the cambium is very high, the actual radial distance from the stem centre is of prime importance in governing the balance between the various factors influencing cell size in the cambium. In the region more distant from the stem centre, however, where the frequency of anticlinal divisions has diminished and loss of initials is high, secondary factors, such as growth-ring width, may influence the size of the initials. This would account for the different patterns obtained by various workers for cell length in the later part of the curve, i.e. in the wood farther removed from the stem centre. For example, a decrease in cell length in this later region, as recorded by many workers, could be due to the cumulative influence of several wider growth rings during this period of growth. Conversely, a number of narrow rings could cause a slight increase in the mean cell length. The actual radial distance from the centre of the stem at which this transition in influence occurs will naturally vary from one species to another, its position being influenced by the actual rates of anticlinal division and the percentage loss of initials, these factors being inherent in a particular species. It is a logical conclusion, therefore, that the diameter of the cambium at its initiation will influence the pattern of variation in cell length outwards from the centre of the stem. In plants where the cambium develops outside a [illegible] primary tissue the characteristic increase in cell length may be partially or totally absent. In such cases it must be assumed that the diameter at its initiation is such that the cambium is able to divide anticlinally at a rate sufficient to provide an adequate supply of new cells. The new cells meet the needs of the rapidly expanding cambial cylinder, and the increase in length of cells is suppressed.

Variations in length of fusiform initials within the growth ring

There are no published results of direct cambial measurements taken at intervals during the growing season, so most of our knowledge on within-ring variation comes from the many studies on the size of the cambial derivatives. Early wood elements are shorter than those produced later in the same annual ring (Fig. 4.1B). This refers to fibres, tracheids (Lee and Smith, 1916; Kribs, 1928; Bisset, Dadswell, and Amos, 1950; Bosshard, 1951; Dinwoodie, 1963; and others) and vessel members in non-storeyed woods (Chalk and Chattaway, 1935; Bisset and Dadswell, 1950; Bosshard, 1951).

Anticlinal divisions in the fusiform initials occur predominantly at the end of the growing season (Bannan, 1950) and are partly responsible for the cyclic changes in the lengths of their derivatives. Elongation of daughter fusiform initials is also uneven, elongation reaching a maximum in the last quarter of the growing season. Part of this variation within the ring, however, is due to the elongation of the derivative cells after they have been cut off from the cambium. This is particularly so in the case of wood fibres and fibre tracheids. Some light has been thrown on the degree and timing of this intrusive growth of derivatives by studies on plants with storeyed cambia (Chapter 5). In these plants anticlinal divisions in the cambium are all in the radial longitudinal plane, and no increase in length of the cambial cells occurs outwards from the pith. Within the growth ring, fibre length rises to a maximum in the middle of the ring and then drops abruptly to the ring boundary (Chalk, Marstrand, and Walsh, 1955). Vessel element and parenchyma strand lengths remain constant throughout the growth ring.

Changes in the fusiform cambial initial length in relation to height in the tree

There is a gradient in cell length along the axis of the tree. When the pattern of cell length is followed upwards in the same growth ring the average length increases from the base upwards, reaching a maximum and then decreasing towards the top (Sanio, 1872; Pritchard and Bailey, 1916; Bailey and Shepard, 1915; Lee and Smith, 1916; Chalk, 1930; Bethel, 1941; Bisset and Dadswell, 1949), the longest cells occurring at a higher level in successive annual rings (Bailey and Shepard, 1915; Bethel, 1941).

With increasing height up the stem, within any one growth ring, the cell length increases from the base to about one-third of the stem height and then decreases. Thus the point of maximum length is located at progressively higher levels as successive annual rings are formed (Fig. 4.3A). Chalk (1930) pointed out that the different patterns of variation in cell size within the tree could be different aspects of the same phenomenon. If this is so, the upward gradient is a result of the fact that the radial distance of the cambium from the centre of the stem decreases with the tapering of the tree. While this is in part true, its influence is most pronounced towards the very top of the trunk, where the stem diameter is relatively small. The changing radial distance of the cambium from the stem centre cannot explain the increase in cell length over the first third of the total stem height. Hejnowicz and Hejnowicz (1958) explained the pattern in this region of the stem as influenced by its proximity to the root.

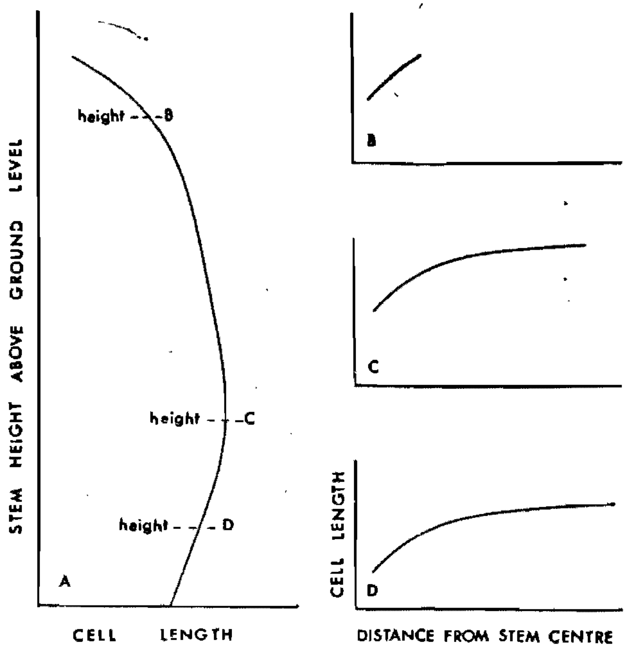

Fig. 4.3 Graph A illustrates the typical pattern of change in cell length with increasing height up the stem. Graphs B, C, and D show the increase in cell length with radial distance from the stem centre at the corresponding heights B, C, and D indicated on graph A.

Another approach to the problem of variation in cell length with tree height comes from our knowledge that trees pass through a grand period of growth before entering old age. It would appear certain that the rate of height growth at a particular time has an influence on the size of the cambial initials originating during that period. Dinwoodie (1963) found evidence that rings laid down by the cambium in short internodes possessed shorter tracheids than did rings laid down in long internodes. Where very little stem elongation has occurred, the cambial initials laid down by the apical meristem are apparently shorter than those occurring as a result of normal growth. This relation between amount of height growth and initial length has its greatest effect in the first growth rings. Mell (1910), Lee and Smith (1916), and others also noted that faster-growing trees possess longer tracheids, but did not correlate this with internode length. Trees with this high initial cambial cell length will usually maintain the relatively high length throughout subsequent growth rings (Bisset, Dadswell, and Wardrop, 1951). Many trees pass through a grand period of growth when the actual rates of height and diameter growth are at a maximum. It would be expected therefore that initials laid down during this period might be appreciably longer than those laid down at periods of slower growth. The general pattern of increase then decrease in cell length when followed in any one growth ring up the tree could thus be partly explained in terms of height growth (Fig. 4.3B, C, D). Towards the top, however, when the stem diameter becomes relatively small, the decrease in cell size is due to the 'core' effect, i.e. the increase in cell length outwards from the stem centre has not reached its maximum in this region (Fig. 4.3B).

Secondary influences

Just as secondary influences impart fluctuations to the basic pattern of cell size outwards from the stem centre at any one level, so, too, do secondary influences cause fluctuations to the normal pattern of cell size with tree height. The most prominent of these is the rate of height growth. If, for any environmental or other reason, the rate of height growth is suppressed in any one year the mean cell size of the new cambial initials laid down during this period will be shorter. Even after radial growth of several years these initials will be shorter than those in neighbouring internodes laid down during more favourable conditions of height growth.

Other causes of variation in cell size

Kaeiser (1964) has shown in Eastern cottonwood that the fusiform cambial initials show an increase in length on both the upper and lower sides of a leaning trunk. Generally, however, compression wood is characterized by shorter tracheids than the corresponding normal wood (Dadswell and Wardrop, 1949; Bisset and Dadswell, 1950). Some workers have shown that tension-wood fibres are longer than those in normal wood. It is not clear if these size changes in fact occur in the cambium, or are the result of reduced or increased amounts of intrusive elongation of the derivatives.

The pattern of size changes within the internode has not as yet been adequately investigated. Bailey and Tupper (1918) noted that tracheids tended to be shorter at nodes, the junction of stems and roots or branches, and other regions where growth adjustments occur. Initials also tend to be shorter at the sites of injuries.

Another pattern of variation which departs from the general pattern for trees has been recorded by Rumball (1963). This is found in trees which pass through a juvenile stage marked by a striking change in habit to that of the adult tree. The rate of increase in cell length outwards along any one radius was found to be very rapid during the juvenile phase, but when the tree entered the adult phase the rate of increase declined after a sharp break in the graph representing cell length against distance from the pith. This break in the curve corresponds with the time of the habit change from a divaricate shrub to a normal tree.

Size variation between different trees

Differences in cell length also occur between specimens of the same species with different provenances. Echols (1958) found a significant relationship between tracheid length and latitude in *Pinus sylvestris* when plants from different latitudes were grown together. Cell length showed a decrease with increasing latitude. A similar relationship was observed by Dinwoodie (1963) for Sitka spruce. However, the variation in cell length between provenances is small (5–30%) compared with the differences in cell length which occur within any one tree (e.g. a 200–400% increase in cell length outwards from the pith).

Harlow (1927), working with *Thuja occidentalis*, concluded that the differences in site were negligible in view of the fluctuations among trees growing on the same site. Bannan (1959) examined nearly 100 trees from diverse habitats and corroborated Harlow's findings. Site would seem important only in as much as it affects growth generally. Bannan (1963b) studied the interaction of environmental and inherent factors on cambial cell size in *Cupressus*. In general, he found the species from arid regions had smaller cells than those from the damper regions. The effects of temperature and day length on tracheid size have been studied by other workers, but since no measurements have ever been made directly on the cambial initials, it is not clear how much these factors influence the size of the initials or how much they alter the rate and amount of elongation of the derivatives by changing the cell-wall plasticity or otherwise affecting differentiation.

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5

The storeyed cambium

When viewed in tangential section the cells of the vascular cambium and its derivatives show one of two basic patterns. In gymnosperms and in many dicotyledonous plants the cambial initials are arranged in a more or less irregular pattern with the ends of adjoining cells overlapping. In some of the more highly specialized dicotyledonous plants, however, the cells appear to be in definite horizontal rows or tiers (Fig. 5.1). The cells in such an arrangement are said to be storeyed or stratified.

The storeyed arrangement of the cambial initials may result in a similar stratified condition being present in the secondary xylem and secondary phloem, though in these derivatives the pattern is frequently obscured by the changes that occur during their respective differentiation. This is particularly evident in the case of the xylem, where intrusive growth of the fibres well beyond the limits of the cambial tier may obscure the formerly storeyed pattern.

The fusiform initials in the non-storeyed cambium are vertically elongated cells with tapering ends which overlap with those of vertically adjacent cells (Fig. 1.2). By comparison, the initials of the storeyed cambium are roughly hexangular in outline, with long parallel sides and abruptly tapering ends. The ends of cells in two adjacent storeys do not overlap to any great extent. The fusiform initials of a storeyed cambium are also considerably shorter than those of a non-storeyed cambium. For example, in *Robinia*, a plant possessing a storeyed cambium, the fusiform initials are approximately 175–200 μm long, as compared to those in a conifer, which may have its non-storeyed fusiform initials up to 4,000 μm in length. In an extreme case, such as *Sequoia*, the initials have been known to attain a length of 9,000 μm (Bailey, 1923).

The stratified arrangement of the cells in the storeyed cambium is produced as a direct consequence of the nature of the anticlinal division and the absence of intrusive growth of the daughter cells following such a division. Other factors which influence the resulting nature of the cambium include the size of the cambial radius at its initiation and the amount of primary longitudinal growth which continues after radial growth has commenced.

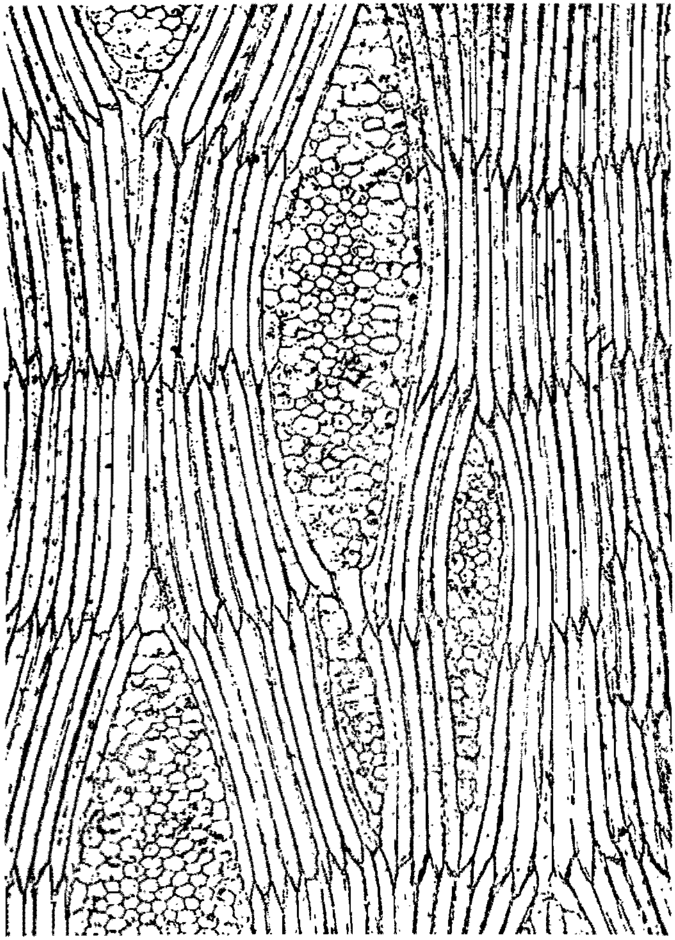

Fig. 5.1 Tangential longitudinal section of the vascular cambium of *Hoheria angustifolia*. The short fusiform initials are in horizontal tiers, with their abruptly tapering tips at approximately the same level. (× 135.)

When a stem grows radially the number of cells in the cambium is constantly increasing, thus keeping pace with the increase in the circumference of the stem. In non-storeyed woods this occurs by the pseudotransverse anticlinal division of the fusiform cambial initial followed by the intrusive elongation of the subsequent daughter initials (Chapter 2). Bailey (1923) pointed out, however, that by its very nature, increase in girth of the storeyed cambium cannot involve the elongation of daughter cells to any great extent, or the tier-like nature of the cambium would be lost. In the storeyed cambium the fusiform initials divide anticlinally by the formation of the new cell wall in the radial longitudinal plane (Fig. 2.2). This results in the two daughter initials lying side by side after division, and the absence of any subsequent elongation of these initials permits the development of the characteristic storeyed pattern. As a consequence of this method of division, the number of cells in each storey is continually increasing with increasing radial distance from the stem centre. It is this fundamental difference in the orientation of the new cell wall at anticlinal division, combined with a total absence of intrusive growth of the daughter cells, which separates the storeyed from the non-storeyed cambium.

Compared with the investigations that have been undertaken on non-storeyed cambia, the storeyed cambium has been somewhat neglected. The most comprehensive account published is that of Beijer (1927), who made a meticulous study of cell division in the root cambium of *Aeschynomene elaphroxylon* Taub. (*Herminiera elaphroxylon* Guill. and Perr.). Using the method devised by Klinken (1914), he traced the pattern of cell division through serial tangential sections. One aspect of his investigation involved a study of the readjustments which occur at the cell ends after the anticlinal division. Beijer concluded that the new cell wall may intersect the existing cell wall at any one of a number of positions, but this is always followed by growth adjustments in the form of slight intrusive growth so as to restore the pointed ends to both daughter cells. This limited intrusive growth ensures that the cell walls comply with what he termed 'the law of minimal area'.

The arrangement of the cells at the time of initiation of the cambium in plants later possessing a storeyed cambium has been little studied, possibly due to the difficulty of obtaining good sections of the procambium. Von Hohnel (1884) was of the opinion that the storeyed arrangement of the cells was already present in the procambium and that the development of the storeyed cambium was simply a consequence of this stratification in the procambium. Klinken (1914), however, pointed out that this was not a necessary prerequisite for a storeyed cambium. According to Klinken, the young cambial cylinder expands so rapidly in a tangential direction at the beginning of secondary growth that a distinct storeyed pattern is possible at a very early stage simply by means of the radial longitudinal divisions occurring within the cambial cylinder. Simple calculations on roots of *Aeschynomene* by Beijer (1927) have shown that this is indeed the case. In *Aeschynomene* the cells of the procambium are non-storeyed and the expansion of the initially small cambial cylinder by radial longitudinal divisions results in the development of tiers of cells.

Although it is only in stems of considerable thickness that the tiers of cells in a storeyed cambium assume a marked regularity, the diameter which a stem must attain before the storeyed pattern becomes pronounced is subject to wide variations. Record (1919) reported that, in certain stems of the Leguminosae and Zygophyllaceae, the initials are found to be in regular tiers in what was apparently only the second growth ring and less than $\frac{1}{8}$ in from the pith. In general, however, it is not until stems are considerably thicker that the storeyed pattern is marked.

The apparent variation between species was known to be influenced by growth rates, but it was Beijer (1927) who first realized that the radius of the cambium at the time of its initiation could have an important bearing on the subsequent development of storeys. By contrasting the average number of cells per storey at a given radius in stems and roots where the cambial radius differs at initiation, he concluded that the smaller the radius of the cambium at its initiation, the more cells there would be present in any storey at any given distance from the centre of the stem. Thus, in stems with a large core of pith and primary xylem, the storeyed arrangement of the cells in the cambium will not become apparent until the stem has acquired a considerable thickness, whereas in stems with a small core of primary tissue the tier-like nature of the cambium will become apparent at a much smaller thickness.

Although some morphologists do not recognize the existence of a distinct 'cambium' until primary growth has ended, it is nevertheless true that in some plants radial growth has commenced some time before primary longitudinal extension has ceased. Before a cambium can become storeyed this primary growth must have ended. It is likely that the occurrence of the non-storeyed state near the centre of some stems which later possess a truly storeyed cambium is due, in part, to the occurrence of transverse and pseudotransverse divisions in a meristem that is still being subject to primary extension.

Derivatives of the storeyed cambium remain grouped in horizontal tiers unless elongation of the derivatives modifies this pattern. Usually the storeyed pattern does not remain in all elements. In the extreme case, as in some members of the Zygophyllaceae, the Bignoniaceae and the Leguminosae, all the rays, vessel members, tracheids, fibres, and wood parenchyma strands remain grouped in horizontal tiers. However, this is not the case in most plants with a storeyed cambium. Vessel elements are commonly storeyed and correspond approximately in length to the height of each storey, though occasionally short segments are found that are subdivisions of a storey produced by a transverse division of a mother cell. The parenchyma is also usually storeyed. Cells may be one storey high or form a vertical strand of two, four, or more parenchyma cells within the storey (Fig. 5.2). Record

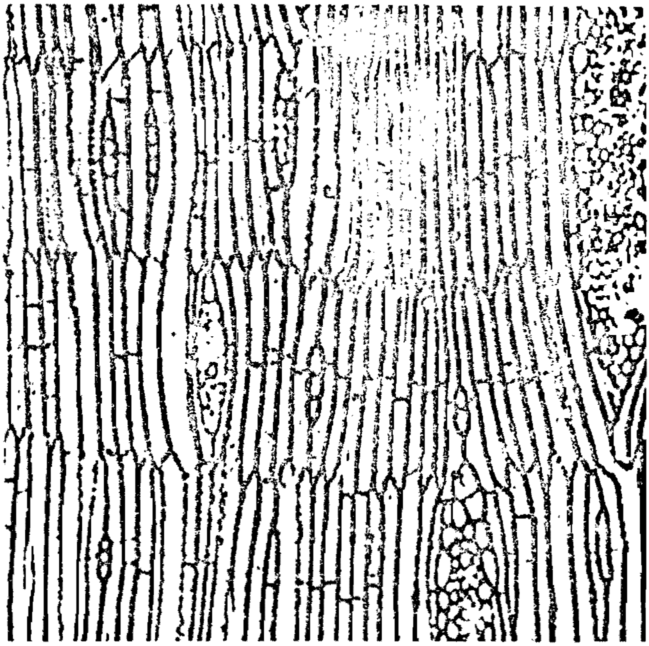

Fig. 5.2 Tangential longitudinal section through the secondary xylem of *Hoheria angustifolia*. The strands of axial parenchyma have either two or four parenchyma cells within the storey, giving the impression of secondary stratifications. (× 150.)

(1919) suggests that it is not uncommon to find metatracheal strands composed of two cells and paratracheal strands of four cells. Where these dividing walls are regularly disposed, they may appear as secondary striations within the storey. Record cited *Bombax*, *Ceiba*, and *Heliocarpus* as examples having four parenchyma cells per strand, *Charpentiera*, *Diaphysia*, and *Lonchocarpus* as having two cells per strand, and *Gossypium* and *Pterocymbium* as having two cells per strand in the metatracheal and four cells per strand in the paratracheal parenchyma.

The wood fibres and fibre tracheids more rarely show a storeyed nature, since they seldom remain the same length as the cambial initials from which they are derived. Sometimes the elongated fibres have a widened middle portion corresponding in length to that of the original cambial initial. Where the fibres have undergone no elongation, as in *Triplochiton*, *Scleroxylon*, and *Aeschynomene*, the cells remain storeyed. In *Aeschynomene* the fibres are similar in size to the parenchyma cells but have thicker walls and more pointed ends (Fig. 5.3).

Rays may be of only one storey in height, e.g. *Daniella thurifera* Bennett, or they may extend through several storeys yet still retain a storeyed pattern.

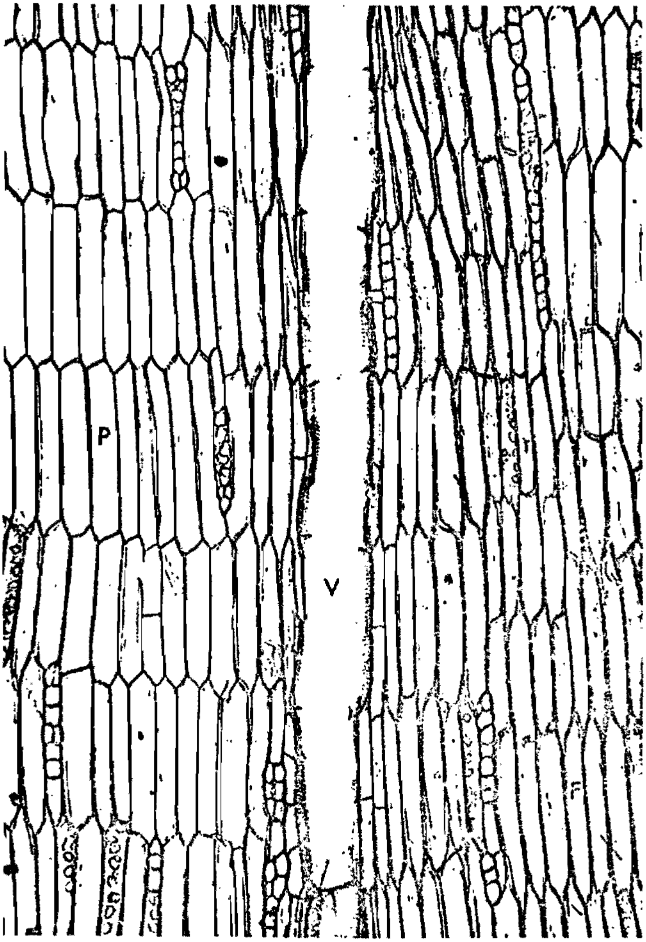

Fig. 5.3 Tangential longitudinal section through the secondary xylem of *Aeschynomene hispida* Willd., a plant where all the derivatives of the storeyed cambium remain in distinct tiers. (P) undivided parenchyma cells which form the bulk of the 'wood', (V) vessel members, and (F) the fibres with thicker walls and slightly more pointed cell tips than the parenchyma cells. (× 100.)

Such an arrangement can give the wood a distinctive pattern of bands or striations when examined macroscopically, termed *ripple marks*. Where the rays are all storeyed they generally occupy the median portion of each tier, the height of which is usually greater than that of the rays. In some storeyed woods the rays may be of two types, the smaller only being storeyed, while the larger are more irregularly dispersed. This is especially common in the Bombacaceae, Malvaceae, and the Sterculiaceae (Record, 1919). There is frequently an aggregation of pits on the fibre end walls in storeyed woods, where the lumina tend to constrict, and this often makes the lines between the storeys more distinct. Solerader (1908) figures a species of *Aeschynomene* with the parenchyma cells pitted prominently at the ends.

Two factors therefore influence the retention of the storeyed pattern in the derivates of a storeyed cambium. These factors are: (*a*) the distribution and size of the ray initials in the cambium, and (*b*) the amount of intrusive growth occurring in the derivatives of fusiform initials. For example, although the fusiform initials might be in clear storeys, the storeyed arrangement is not noticeable because of the presence of many rays of unequal size. Conversely, the storeyed pattern may be visible in the derivatives due to the fact that the rays are nearly all of an even size and lying at the same height, even if the storeyed structure has been lost in the mass of cells due to intrusive growth.

Due to their relative unimportance to the pulp and paper industry, little research has been carried out on the size variations in the cambial derivatives of storeyed hardwoods. Hejnowicz and Hejnowicz (1959) made measurements of fibres and vessel members of *Robinia pseudoacacia* L. They report a distinct change in fibre length within the growth ring, fibre length increasing from the first formed early wood to the late wood, with an abrupt drop in length at the ring boundary. The amplitude of fibre-length variation increased from the pith outwards. They also report that the fibres of the first-formed early wood showed no great tendency to increase from the pith outwards, but the fibres in other parts of the wood showed this tendency as clearly as the fibres in a non-storeyed wood. In contrast to this, Chalk, Marstrand, and Walsh (1955) reported that, in *Pterocarpus angolensis* DC., *Nesogordonia papavifera* Capuron and *Aeschynomene elaphroxylon*, neither the fibres nor the parenchyma strands showed any general tendency to increase in length from the pith outward. Within the growth ring the fibre length rose to a maximum in the middle of the ring and dropped abruptly at the ring boundary, but the length of the parenchyma strands remained constant. Philipson and Butterfield (unpublished data) have shown in *Hoheria angustifolia* that the vessel members within a growth ring display a slight increase in length towards the end of the ring. From associated studies, however, it has been shown that this is due to the angle of inclination of the end wall, the vessel members towards the end of the growth ring being narrower and with more tapering ends compared with the wider transverse-ended vessel members of the early wood. Over seventeen growth rings no increase in the mean length of the vessel members was observed. The possibility that the length of the derivative was dependent upon the plane of the anticlinal division was investigated by Esau and Cheadle (1955), who examined ninety-one species of seventy-one genera representing plants with both storeyed and non-storeyed cambia. They showed in *Asimina triloba* Dunal. (storeyed cambium) that the phloem elements derived from the cambial initials had divided along various planes, resulting in the sieve elements being shorter than the cambial initials from which they were derived. The vessel elements, however, corresponded in length to that of the initials.

Beijer (1927) and others have shown that storeyed structure is related to length of cambial initials, storeyed structure being associated with short initials. It is worth pointing out that some authors have inferred that the change in angle of the anticlinal wall from the pseudotransverse position to the radial longitudinal position has been made possible by the short length of the cambial initials. However, while it is true that storeyed arrangement is associated with short fusiform initials, one is not necessarily the cause of the other. It should be remembered that divisions in the periclinal plane usually occur from tip to tip of the initial irrespective of the length of the cell.

Bailey (1920, 1923) noted from his studies on the length of fusiform initials in various plants that as the initials become shorter, the ends of the more oblique partitions tend to approach the extremities of the cell. Thus certain of the more highly differentiated dicotyledonous plants will tend to show transitional types of meristems with incipient stages of stratifications. Furthermore, he postulated that the variation in size of adjacent fusiform initials in storeyed cambia may be due at least in part to some of the anticlinal divisions being somewhat oblique. The gradation from non-storeyed to storeyed arrangement of cells in some plants has also been noted by Chalk (1937).

The association of storeyed cambia in woods with short vessel elements and other highly specialized features has led to the conclusion that storeyed structure is an advanced character. Storeyed cambia occur in very many systematically widely different dicotyledonous families, perhaps only in some species, or in almost all the members of the family (e.g. the Papilionaceae). This irregular taxonomic distribution of storeyed wood has led to the belief that this character has arisen independently in many lines. In any case, since the development of storeys is dependent on several factors, a simple phylogenetic relationship seems unlikely. Record in a series of papers (1911, 1912, 1919, 1927a, 1927b, 1936, and 1943) has listed families and genera with storeyed wood, giving extensive lists of storey heights and noting which elements are storeyed in a particular wood. Metcalfe and Chalk (1950) also list families where some genera possess storeyed elements. The association of storeyed structure with other advanced features has led taxonomists to use it as a criterion for suggesting that a particular plant is advanced. Metcalfe 1945) concluded that where storeyed structure is confined to certain genera a family it must be presumed that these genera are more advanced than the ainder.

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PAPER THREE

DEVELOPMENTAL CHANGES IN THE VASCULAR CAMBIUM OF *AESCHYNOMENE HISPIDA* WILLD.

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Developmental Changes in the Vascular Cambium of Aeschynomene hispida Willd.

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(Received 20 October 1971)

SUMMARY

Developmental changes in the vascular cambium of *Aeschynomene hispida* Willd. were determined from an analysis of transverse and tangential longitudinal sections of the secondary xylem. Increase in the number of fusiform cambial initials in this plant is brought about by two types of anticlinal division. The majority of these divisions are radial longitudinal, contributing to the number of fusiform cells in the tangential plane. Occasional transverse division of fusiform initials can add cells in the vertical plane. Apart from a slight rearrangement of the cell tips, there is no intrusive growth of the daughter cells following either type of division. Repeated radial longitudinal divisions produce a highly developed storeyed pattern of the fusiform initials when viewed in tangential section, following radial growth. The frequency of radial longitudinal division, recorded as a percentage of the fusiform initials that divide for each millimetre of radial growth, declines with radial distance from the stem centre. The mean length of the fusiform initials remains fairly constant with radial growth, but the tangential width of these cells increases rapidly over the first 10 mm and then remains constant. An apparent deficiency in the frequency of anticlinal division over the first few millimetres of radial growth is compensated for by an increase in the tangential dimension of the fusiform initials. No loss of fusiform initials occurs other than that resulting from their transformation into ray initials. Both uniseriate and multiseriate ray initials generally occupy similar tangential dimensions to neighbouring fusiform initials. New rays develop by the subdivision of individual fusiform initials. The secondary xylem produced by the cambium is unusual in that the axial parenchyma cells, vessel members, and fibres all remain largely within the confines of the storeys.

INTRODUCTION

The production of secondary xylem, by periclinal divisions in the vascular cambium, results in a progressive increase in the circumference of the cambial cylinder. To keep pace with this expansion, the fusiform initials of the cambium undergo occasional anticlinal division. In the gymnosperms and in many of the dicotyledonous plants, this division involves the development of an oblique cell plate which divides the parent fusiform initial into two shorter daughter cells. Following this pseudotransverse division, the two daughter cells elongate by intrusive growth thereby expanding the cambial circumference (Bailey, 1923). This process of pseudotransverse division followed by intrusive growth produces the non-storeyed type of cambium. The developmental changes associated with this process in the gymnosperms, including the division cycle, the elongation of the daughter cells, and the loss of some of these from the cambium, has been intensively studied in recent years (Bannan, 1950, 1957a, 1957b, 1968; Whalley, 1950; Hejnowicz, 1961, 1964; Srivastava, 1963). There have also been a number of studies of the developmental changes in the vascular cambium in dicotyledons. These include investigations on *Pyrus* (Evert, 1961), *Liriodendron* (Cheadle and Esau, 1964), *Leitneria* (Cumbie, 1967), *Hibiscus* (Cumbie, 1963; Walker and Cumbie, 1968), *Polygonum* (Cumbie, 1969a), and *Apocynum* (Cumbie, 1969b). While the nature of the anticlinal division and the amount of cell loss following division varies between these plants, each of them essentially has a non-storeyed cambium. A number of the more highly advanced dicotyledons possess vascular cambia where the fusiform initials are in definite storeys. In this type of cambium, anticlinal division takes the form of a more truly radial longitudinal division and there is no intrusive growth of the daughter cells following division. Compared with the investigations that have been undertaken on the non-storeyed cambium, the storeyed cambium has been somewhat neglected. The only comprehensive account is that of Beijer (1927).

This paper describes the developmental changes occuring within the vascular cambium of *Aeschynomene hispida* Willd. This plant is unusual in that none of the xylary derivatives of the vascular cambium—vessel elements, fibres, or parenchyma strands, undergo any appreciable intrusive growth during differentiation. The cells of the wood replicate the pattern of the cambial initials, remaining in neat horizontal rows when viewed in tangential longitudinal section.

MATERIALS AND METHODS

Material of *Aeschynomene hispida*, collected from the British Honduras, was sent to this department by Dr C. R. Metcalfe of the Royal Botanic Gardens, Kew. The date of collection is not known. A block of stem material 48 mm in diameter was used as the basis for this study. From this, a rectangular block 12 mm wide by 6 mm deep was cut to include the pith and the wood on both sides of the stem. This was further divided into eight blocks as indicated in Fig. 1. These blocks were prepared for sectioning using standard paraffin methods. A small number of transverse sections were cut off each block and then after remounting on the microtome, each block was completely cut into serial tangential sections of 12 μm thickness. These sections (approximately 4,000), after mounting, were stained according to standard safranin-fast green procedures and mounted in DePex.

To study the developmental changes that had occurred in the cambium as it progressed outwards in the stem, a number of techniques were

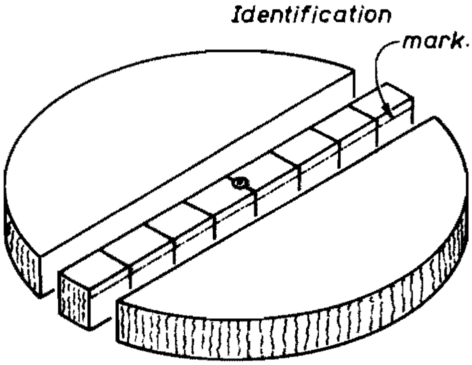


FIG. 1—A diagram to illustrate the way in which the stem material of *Aeschynomene hispida* was divided into eight blocks for sectioning. Transverse sections were cut off the blocks, then after remounting, each block was cut into serial tangential sections. A small line was cut along the side of the blocks to assist orientation under the microscope.

employed. The transverse sections were placed in an overhead projection arrangement and photographic prints made directly from the image of the slides. About 12 photographs were needed to cover each section and these were assembled to form a photograph mosaic of the stem measuring approximately 4 m by 76 cm. By tracing the files of cells in these photographs, the positions of the anticlinal divisions and any subsequent cell loss could be detected (Fig. 2).

The serial tangential sections were also examined using the overhead projector. Developmental changes in the cambium were studied, in this case, by following the radially aligned derivatives in successive tangential sections. Two methods were employed. For the sections cut from one side of the stem, approximately every sixth section was photographed and files of cells studied using a cardboard template over the photographs. In the serial tangential sections prepared from the other side of the stem, one storey of cells was selected and drawings made of these cells, in sections in which changes in cell number or arrangement could be detected.

Cell length and width measurements were made using the overhead projection system, the image being projected onto a calibrated scale. Fifty cells were measured in each sample. Two arrows mounted on the projection screen enabled two cells to be selected at random for length and width measurement from the tangential longitudinal sections. The image was then moved vertically through 25 storeys to obtain the full data.

OBSERVATIONS

The wood of *Aeschynomene* is very light, being made up largely of thin-walled parenchyma cells. No growth rings were observed in the material examined. The parenchyma tissue is built up of vertically elongated cells, fusiform in outline, with hexangular tips (Figs. 3, 4). In tangential section the cells reflect the storeyed pattern of the vascular cambium, the cell tips of one storey only slightly overlapping the cells of vertically adjacent storeys. Interparenchyma pit-pairs are of the simple type. Some of the parenchyma cells, especially those close to vessels, show subdivision into two (Fig. 3), or occasionally more cells per strand. They may also contain crystals, the number of crystals per strand being usually between 8 and 11 (*see* Fig. 5).

The vessels are solitary (*see* Fig. 2), or very occasionally, in small radial multiples. The individual vessel elements remain within the storey and have simple perforation plates. Vessel to vessel pit-pairs are of the bordered type.

The wood is unusual in that the fibres undergo comparatively little intrusive growth during differentiation. They are distributed around the vessels in an aliform to banded distribution when viewed in transverse section. In longitudinal section they can be distinguished from the parenchyma cells by their thicker, more easily stained walls and their more pointed ends (Fig. 3). Occasionally the tips of fibres intrude between the cells of the storeys immediately above and below. When this happens the main body of the cell still remains within the confines of the storey.

Both multiseriate and uniseriate rays are present, but the majority are uniseriate and confined to one storey or less in height. Some rays are multiseriate for part of their height only. Even when multiseriate, the rays rarely exceed the tangential width of the adjacent parenchyma cells. A few larger multiseriate rays also occur and these frequently possess horizontal tracheary elements (Fig. 5).

The pattern of anticlinal cell division in the cambium was clearly replicated in the cells of the secondary xylem examined. Except where large vessels obscured the pattern, the files of cells cut off from the fusiform and ray initials could be readily traced in the tangential longitudinal sections. The examination of these sections indicated that in *Aeschynomene hispida*, anticlinal division in the cambium almost always takes the form of a radial longitudinal division extending the full length of the fusiform initial (Fig. 6b). This results in the two daughter cells lying side by side in tangential view after division and produces the storeyed cell arrangement of the initials. As a consequence of this repeated radial longitudinal division, the number of cells per storey shows an increase with radial distance from the stem centre. More rarely a transverse division was observed, the new wall dividing the fusiform initial approximately into two. Generally such a division is the first stage in the development of a new ray, but it can also lead to the development of two storeys each about half the height of the adjacent storeys, if radial

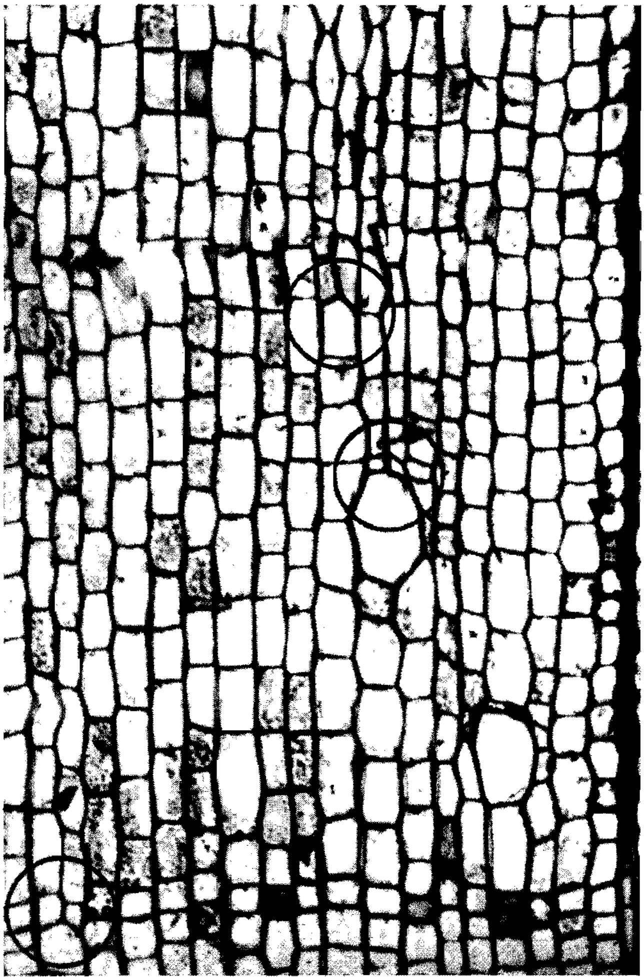


FIG. 2—A transverse section of the secondary xylem of *Aeschynomene hispida*. Because the majority of the anticlinal divisions in the cambium are radial longitudinal, the derivatives of the fusiform initials remain in neat radial files. Three radial longitudinal divisions are indicated by the circles drawn on the photograph. (× 165)

longitudinal division follows (*see* Fig. 5). Although transverse divisions were not observed to have occurred simultaneously in two or more adjacent fusiform initials, this does not preclude such a possibility.

Ray counts made on the tangential sections indicated that the ray initials constituted only a small proportion of the cambial circumference

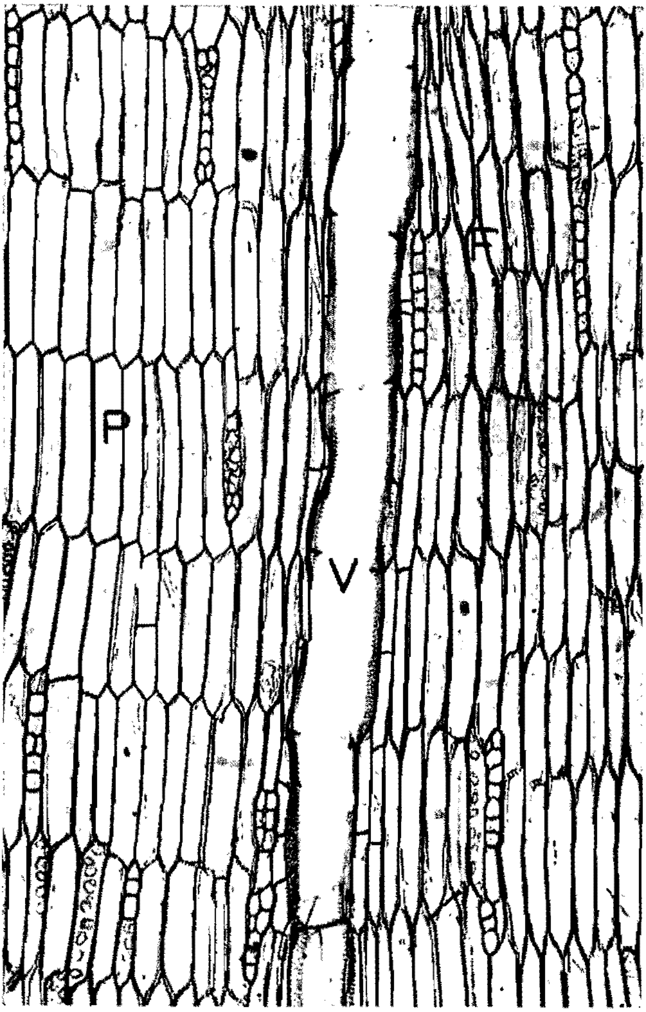


FIG. 3—Tangential longitudinal section through the secondary xylem. The axial parenchyma cells (P) that form the bulk of the wood are fusiform in outline with abruptly tapering ends. Subdivision of these cells into axial strands occurs only rarely, and then mainly in those cells close to vessels. The vessel members (V) and the main body of the fibres (F) also remain within the confines of the storeys. (× 165)

at any given distance from the stem centre (Fig. 7), the percentage of the cambial circumference occupied by ray initials showing a decrease over the first few millimetres from the stem centre, and then remaining fairly constant at about 5%.

FIG. 4—Radial longitudinal section of the secondary xylem through parenchyma. (× 44)

New ray initials are continually being added to the cambium as radial growth proceeds, in order to maintain this balance of ray to fusiform initials. In the majority of cases a new ray develops by the transverse division of a fusiform initial, dividing the parent cell approximately into two. The upper or the lower of the two daughter initials then further subdivides by transverse division, usually into two or four cells (Fig. 6c, d). The ray produced may continue through a considerable number of periclinal divisions remaining, in effect, only half a storey in

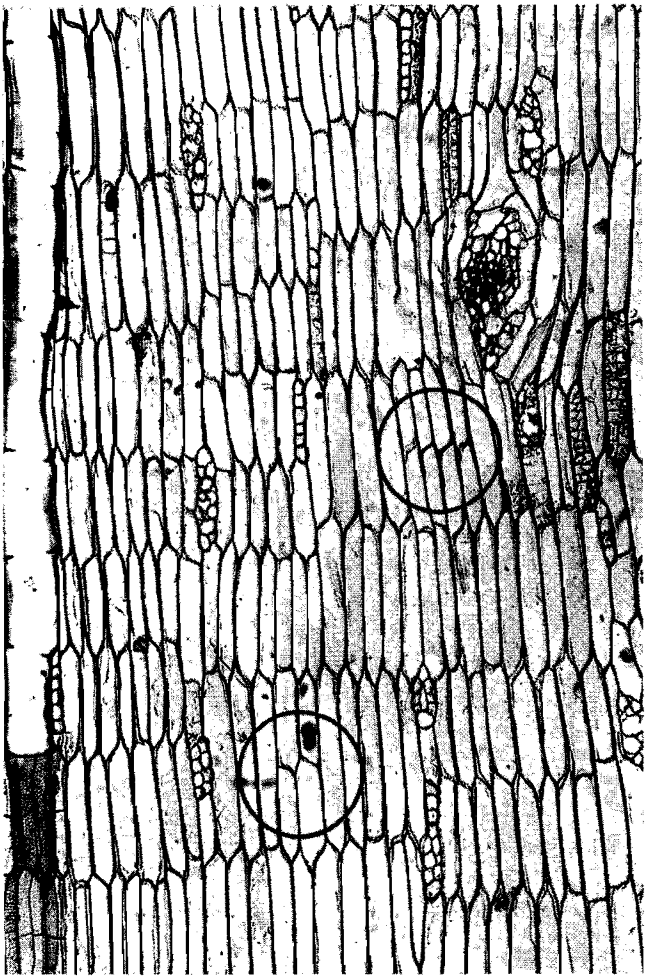
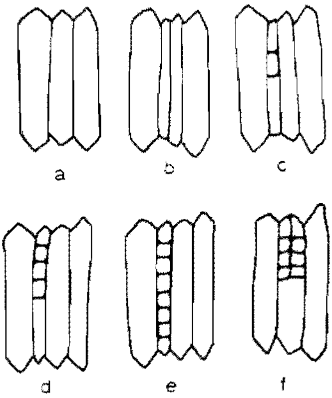


FIG. 5—The majority of the rays, both uniseriate and multiseriate, remain generally within the storeys and have similar tangential dimensions to the adjacent fusiform initials. A few large multiseriate rays with horizontal tracheary elements occur. The circles marked on the photograph indicate short storeys that have resulted from radial longitudinal divisions, following a transverse division. A number of parenchyma cells, showing subdivision and containing crystals, can also be seen in this photograph. (× 110)

height. These ray initials may then divide longitudinally to give a multiseriate ray, or the shorter fusiform initial below may subdivide to produce a ray equal in height to the adjacent fusiform initials (*see* Figs. 3, 5, 6e, and f). Ray initials, either uniseriate or biseriate, normally form a ray with similar tangential dimensions to neighbouring fusiform initials.

FIG. 6—a, b—Stages in radial longitudinal division within the cambium, as observed from the parenchyma of the secondary xylem. c, d, e—Stages in ray development. Following subdivision of the fusiform initial, the upper or the lower daughter cell undergoes further subdivision into two or four cells before the other daughter cell subdivides. f—Multiseriate rays can occupy either half the height of the storey, or, by subdivision of the other daughter cell, they may extend the full height of the story. (Drawn from tangential longitudinal sections).



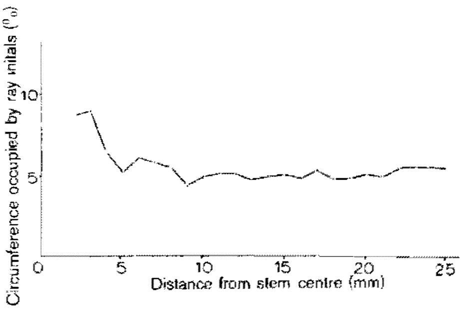


FIG. 7—The percentage of the cambial circumference occupied by ray initials, plotted against radial distance from the stem centre.

TABLE 1—A summary of the number of fusiform and ray initials at 5 mm intervals from the stem centre.

Distance from stem centre (mm)	1	6	11	16	21	26
Total cell count within 24° segment	30	87	139	218	287	399
Total number of rays	2	5	7	10	14	21
New rays	—	3	2	3	4	7
Total fusiform initials	28	82	132	208	273	378
New fusiform intials	—	54	50	76	65	105
Radial longitudinal division per fusiform initial		1.25	0.50	0.48	0.32	0.31

The frequency of radial longitudinal division in the cambium was determined from the photographs of the transverse sections. A sector subtending an angle of 24° was marked out, and counts made of the radial longitudinal division that had occurred within each millimetre of radial growth. The percentage of the fusiform initials that had divided during each millimetre of radial growth was calculated, after subtracting the rays from the original cell count because these do not add to the new cambial circumference. A summation of the cell division for each 5 mm of radial growth is shown in Table 1, and the percentage of fusiform initials that had undergone radial longitudinal division for each millimetre of growth is graphed in Fig. 10, line B. No loss of fusiform initials following radial longitudinal division was observed.

The mean length of parenchyma cells showed a slight increase followed by a slight decrease (Fig. 8) with increasing radial distance from the stem centre. These data are based on the tip to tip measurement of 50 parenchyma cells selected at random in the tangential longitudinal sections. The mean width of these cells showed an increase over the first 10 mm from the stem centre and then decreased slightly outwards to the cambium (Fig. 9).

DISCUSSION

Most of our present information on changes occurring in the vascular cambium of seed plants has been obtained from investigations made on the secondary xylem, and not from direct examinations of the cambium itself. Since the cambial initials leave a record of their activities in these derivatives, the general sequence of cell size changes and changes in cell arrangement, that occur in the cambium with radial growth, can be determined by an examination of the secondary xylem. However, since the xylary derivatives of the cambium undergo elongation during their differentiation, data thus obtained are of necessity erroneous to the extent of any elongation or cell rearrangement that has occurred after the derivatives have been cut off the cambium. The extent of this cell elongation and subsequent cell rearrangement is difficult to assess in most plants, as it varies with several factors, including position within the growth ring. Bailey (1920) concluded that the tracheids in *Ginkgo* and most members of the Coniferae were slightly longer than the fusiform cambial initials from which they were derived. This relationship was generally thought to apply to most conifers and a great number of studies on the changes in cell length in the conifer cambium were based on this assumption. Chattaway (1936) found that the fibres of dicotyledonous woods were 1.1 to 9.5 times longer than the fusiform initials from which they were derived. The only cambial derivatives that show little elongation with differentiation are vessel members and strands of axial parenchyma cells.

The bulk of the wood of *Aeschynomene hispida* is built up of axial parenchyma cells and vessel members which do not undergo any elonga-

FIG. 8 — Mean length of fusiform initials, plotted against radial distance from the stem centre.

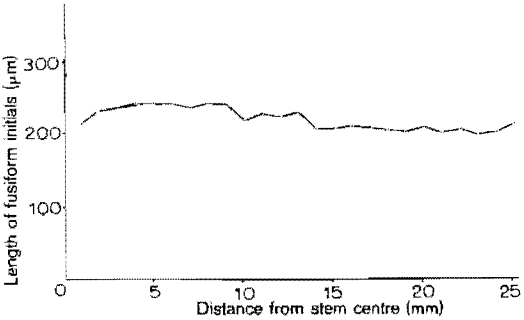
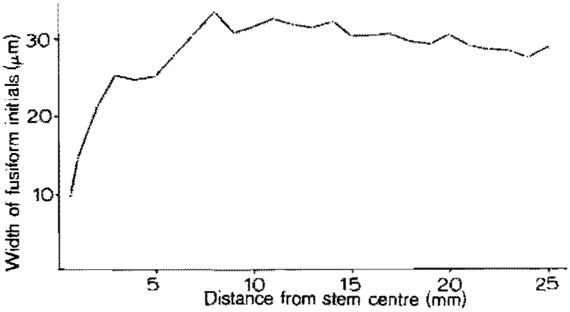


FIG. 9 — Mean width of fusiform initials, plotted against radial distance from the stem centre.



tion with differentiation. These cells, therefore, replicate closely the basic cell pattern found in the vascular cambium of this species. Even the fibres do not show any appreciable elongation. Where their tips do intrude between the cells in adjacent storeys, the main part of the cell remains generally fusiform in outline within the confines of the original storey. It was possible, therefore, to study the developmental changes occurring in the vascular cambium relatively easily in this plant, by examining transverse and tangential longitudinal sections of the xylem and cambial regions.

When a stem expands radially, the number of cells in the cambium is continually increasing to keep pace with the increasing circumference of the stem. In the gymnosperms and dicotyledonous plants with the non-storeyed arrangement of fusiform initials in the cambium, the processes responsible for the circumferential expansion of the cambium are complex because they involve the interplay of a number of factors; the frequency of pseudotransverse division, the amount and timing of the elongation of the new daughter cells to reach their final size, and the preferential loss of some of these new cells (Philipson *et al.*, 1971). *Aeschynomene hispida* is an interesting plant in which to study the frequency of anitclinal division in the cambium because the elongation of daughter fusiform initials, and the preferential loss of some of these, is eliminated by the nature of the division pattern within the storeyed cambium.

In *Aeschynomene hispida* anticlinal division in the fusiform initials of the cambium, in most cases, takes the form of a radial longitudinal division running the full length of the parent cell. Such divisions are easily detected in transverse sections of the secondary xylem as a doubling in the radial files of cells (*see* Fig. 2). The occasional transverse division in the cambium does not add any new cells in the tangential plane of the expanding cambium, but does result in the production of a number of short storeys after further radial longitudinal division. Although such transverse divisions were only occasionally observed, they are probably responsible for the slight decrease in the mean cell length recorded with increasing distance from the stem centre (*see* Fig. 8). No oblique anticlinal divisions were recorded. The measured frequency of radial longitudinal division in the cambium of *Aeschynomene hispida*, determined from observations on transverse sections of the secondary xylem, therefore accounts for all the anticlinal divisions that have added in any way to the expanding circumference of the cambium.

Over the first few centimetres of radial growth the perimeter of the stem is increasing very rapidly, and a much higher rate of anticlinal division is required to provide new initials for the cambium than is required later in radial growth (Philipson and Butterfield, 1967). Since the circumference of the expanding cambium is proportional to its radius ($C = 2\pi r$), for a 1 mm increment of radial growth occurring from 1–2 mm from the stem centre, for example, the cambial circumference will have doubled. In a plant with a storeyed cambium, where anticlinal division is of the radial longitudinal type, in theory every fusiform initials will have divided at least once. As radial growth proceeds, however, the percentage of such cells that need to divide decreases. For 1 mm of radial growth between 20 and 21 mm from the stem centre, only 1 cell in every 20 fusiform initials in a storeyed cambium will need to divide. The theoretical frequency of anticlinal division is graphed in Fig. 10, line A. It is clear, therefore, that a higher frequency of anticlinal division in the cambium is required close to the stem centre than is required later in radial growth when the cambial cylinder has attained a greater diameter. This phenomenon has been recorded for a number of plants with non-storeyed cambia (Bannan, 1950, 1960; Srivastava, 1963; Cumbie, 1967), but has not previously been graphed for a plant with a storeyed cambium. Its significance in the overall division cycle of the storeyed cambium, therefore, has never been recorded.

The recorded results for anticlinal division in the cambium of *Aeschynomene hispida*, graphed as a percentage of fusiform initials undergoing division, are presented in Fig. 10, line B. This pattern corresponds closely with the theoretical curve on the same figure. Over the early part of radial growth there is an apparent deficiency in the production of new fusiform initials to meet the demands of the rapidly expanding cambial circumference. This appears to be compensated for by the increase in the tangential dimensions of the cells over this period of radial growth (*see* Fig. 9). Throughout the later part of radial growth, the frequency of anticlinal division in the fusiform initials is slightly

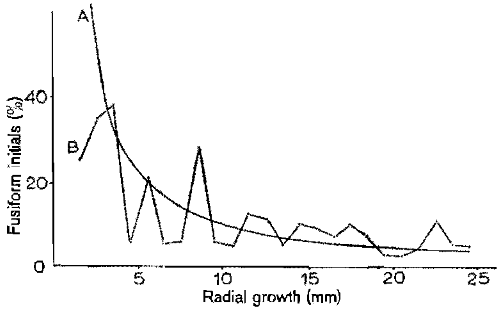


FIG. 10—Line A: The theoretical percentage of the fusiform cambial initials that need to divide anticlinally per millimetre of radial growth, in order to supply the needs of the expanding cambial circumference. Line B: The measured percentage of the fusiform initials of *Aeschynomene hispida* that divided radial longitudinally per millimetre of radial growth.

higher than the theoretical figure because of the percentage of the cambial cylinder occupied by the ray initials. In *Aeschynomene hispida* these initials do not add greatly to the cambial circumference by anticlinal division. Even when the rays become multiseriate they rarely exceed the tangential dimensions of the fusiform cells. The presence of these groups of ray initials partly explains the discrepancy between observed and theoretical frequencies of anticlinal division in Fig. 10.

It is clear from this study that in plants with storeyed cambia, the diameter of the cambium at the time of its initiation will have a direct bearing on the subsequent arrangement of the fusiform initials. The smaller the diameter of the cambium at its initiation, the higher will be the rate of anticlinal division required during the early stage of secondary growth. Such stems tend to have a more highly developed storeyed pattern at any given radial distance, than stems in which the cambium, as such, begins its activities outside a considerable thickness of pith and primary xylem tissues.

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PAPER FOUR

VARIATION IN THE SIZE OF FUSOFORM CAMBIAL INITIALS AND VESSEL MEMBERS IN *HOHERIA ANGUSTIFOLIA* RAOUL.

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1973

Variation in the Size of Fusiform Cambial Initials and Vessel Members in *Hoheria angustifolia* Raoul.

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ABSTRACT

The lengths and widths of fusiform cambial initials and vessel members are examined in *Hoheria angustifolia* Raoul, a habit-heteroblastic tree with a storeyed cambium.

A significant correlation is shown to exist between the lengths of the fusiform cambial initials and the vessel members in the last formed xylem in a number of trees sampled at various heights.

No change in mean vessel member length could be detected with increasing distance from the stem centre in nine discs, though in one stem with a markedly eccentric growth, cell length decreased slightly with distance from the stem centre. Within the individual growth rings, the mean length of the vessel members tends to increase towards the late wood. This change is brought about by an increasing angle of the end wall in the late wood vessel members, and is not a result of developmental changes occurring in the vascular cambium. It is, therefore, concluded that the fusiform cambial initials in this species normally proceed without change in length from the onset of secondary growth.

Although individual trees showed a variety of cell length sequences, the means of fusiform initial lengths for fixed heights in a number of trees showed a slight decrease with increasing height in the tree. The analyses of variance indicated a significant difference between samples at various heights, but not between trees, and showed that the linear decrease in cell length up the tree was responsible for most of the differences among the height samples.

Mean fusiform initial width showed an increase followed by a decrease with increasing height up the tree. Except at the lower levels in the tree, this appears to be related to the cambial radius.

INTRODUCTION

The literature relating to cell size variation in the secondary xylem of woody plants is vast (see reviews by Spurr & Hyvärinen 1954, Dinwoodie 1961, and Philipson *et al.* 1971). In view of the importance of wood as a raw material this is not surprising. Factors such as cell length,

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width, and wall thickness have been examined in many tree species with respect to position in the tree, the age of the plant, genetic constitution of the species, and environmental factors. The overall picture, nevertheless, is complex and the various findings are at times conflicting.

Since the time of Sanio (1872), whose findings for *Pinus sylvestris* are now regarded as "Sanio's Laws", wood anatomists have attempted to establish patterns of cell length variation within the growth ring, across the stem from pith to bark, and up the tree. Attempts have been made to relate these findings to the rates of radial and terminal growth of the shoots. Seedlings have been subjected to various day length and temperature regimes in attempts to establish the causes of these variation patterns. Cell length, in particular tracheid and fibre length variation, has been the subject of most of the studies. By comparison, cell width and wall thickness variation patterns have received less attention. Because of their greater potential economic importance, coniferous tree species have been studied more intensively than have angiosperm species.

The length of the axial cells in the xylem is dependent on two factors, namely the length of the fusiform initials from which they are derived by periclinal division, and the amount of intrusive growth which they undergo during their differentiation. Unfortunately, it is not possible to separate these factors in an examination of the mature secondary xylem of most woods. Tracheids and fibres undergo quite appreciable amounts of intrusive tip growth during their differentiation, the actual amount varying with the season during which these cells are laid down. Vessel members and strands of axial parenchyma cells undergo fewer changes in length with differentiation and are, therefore, most likely to represent the sequence of size changes that have occurred within the vascular cambium. Few studies, however, have been made on the size variations of these cells or their relationship to the size of the fusiform cambial initials.

In addition to the size changes that result from varying amounts of intrusive tip growth of the xylary derivatives during their differentiation, differences in the length of these cells with position in the tree are also the result of changes that have occurred within the vascular cambium with radial growth. Once a population of cambial initials has become established at the end of primary growth, it will move horizontally outwards, normally dividing off new xylem cells to the inside and new phloem cells to the outside. This radial growth is accompanied by circumferential expansion of the cambium, a process which involves an increase in the number of fusiform initials. In plants with a non-storeyed cambium, this occurs by means of a pseudotransverse wall being laid down usually near the centre of the dividing cell. The two daughter fusiform initials so formed then elongate by the intrusive growth of their cell tips. The resultant size of the fusiform initials in the non-storeyed cambium is governed, therefore, by the frequency of pseudotransverse division and the rate and amount of elongation of the new initials. The preferential loss of short fusiform initials following pseudotransverse division adds a further complication.

The nature of the division cycle with circumferential expansion in plants with a storeyed cambium is such that one would expect to find little change in size of the fusiform initials with radial growth. In these species, radial longitudinal divisions divide the parent fusiform cambial initials from tip to tip (Philipson *et al.* 1971). Repeated anticlinal division of this type results in the fusiform initials lying in neat rows or storeys when viewed tangentially. Apart from the minor rearrangement of the cell tips (Beijer 1927) daughter cells show little or no intrusive growth following such division. Indeed, if newly formed fusiform initials were to crowd past one another, the storeyed or stratified arrangement would rapidly be lost. Once laid down at the onset of secondary growth a population of fusiform cambial initials, therefore, should maintain a more or less constant cell length with radial growth in plants possessing storeyed cambia.

The object of this study is to examine the effect of radial growth on the apparent length of the fusiform initials, using the vessel members as an indication of fusiform initial length, to relate the length of the vessel members in the last formed xylem to the length of the fusiform initials, and to establish any patterns of variation in length and width of fusiform initials with position in the tree for *Hoheria angustifolia* Raoul, a plant with a storeyed cambium.

MATERIALS AND METHODS

Fifteen *Hoheria angustifolia* trees growing on Banks Peninsula, New Zealand, were used in this study. All were growing in open situations in full sunlight. As this species illustrates leaf and habit heteroblastism (Philipson 1963), specimens were selected so that five showed mature adult form with adult foliage, five were intermediate in form and leaf shape, and five were juvenile in form and bore small juvenile leaves. These trees were designated A1-5, I1-5, J1-5 respectively. Discs of wood were cut from the leading stem or trunk of each tree at 3-ft intervals beginning at the 2-ft level. The stem material of small diameter was fixed immediately in formalin-aceto-alcohol. The larger discs of wood were taken to the laboratory and transferred to FAA.

Cubes of wood including the cambium were cut from each disc of wood for trees A1-5, I1-5, and J1-5. All these specimens were dehydrated in t-butyl alcohol and embedded in paraffin wax. Tangential longitudinal sections about 10-12 μm thick of the cambium and xylem were cut using a rotary microtome. The sections were stained according to standard safranin fast-green procedures and mounted in De Pex. Macerations of the secondary xylem were made using 50/50 nitrochromic acid mixture and mounted in glycerine jelly.

Cell lengths and widths were measured using an overhead projection system, the image of each slide being projected on to a calibrated scale. Usually, 25 or 50 cells were measured in each sample. A string bearing two arrows lying over the projected image enabled cells to be randomly selected for measurement. A measuremental error of approximately 3% was incurred by this technique. Unless otherwise stated, all measurements of vessel members and fusiform cambial initials were made from tip to tip of the cell. Cell widths were measured at the mid-point of the cell. Growth ring measurements were carried out directly on the stem blocks after the cut surfaces had been sanded.

Cell length and width measurements were transferred to computer data cards and processed by the IBM 360/44 computer at the University of Canterbury. Computation of the basic statistics, analyses of variance, regression equations, and product-moment correlations were carried out by programs based on those published by Sokal & Rohlf (1969) and designated A3.1, A3.4, A3.12, and A3.13 by these authors.

In the course of this investigation 2540 microscope slides were prepared and more than 50 000 cell measurements made.

VARIATION IN VESSEL MEMBER LENGTH AT ANY ONE LEVEL IN THE TREE

From his observations on *Pinus sylvestris*, Sanio (1872) concluded that tracheid length at any one level in the tree increased outwards from the pith through a number of annual rings until a certain size was reached, and then remained constant throughout the following rings. There has since been general agreement that there is an initial increase in tracheid and fibre length over the early part of radial growth in both gymnosperms and angiosperms, but investigations beyond this point are somewhat contradictory. While some investigators have recorded results similar to those of Sanio, others have recorded a decrease, an increase, or considerable fluctuation in cell length after the initial increase (Dinwoodie 1961).

Vessel members have received considerably less attention than have tracheids and fibres in these attempts to establish the pattern of cell size changes across the stem. Pritchard & Bailey (1916) found no difference in vessel member length in *Carya ovata* with radial distance from the pith. A similar result has been recorded for late wood vessel members in *Fraxinus excelsior* by Bosshard (1951). Hejnowicz & Hejnowicz (1958) found that vessel member length doubled between the first and last growth rings in a 31-year-old stem of *Populus tremula*. Fabri Tarchi (1963), however, recorded a decrease in vessel member length with distance from the stem centre in *Prunus persica*. Assuming that vessel members approximate the length of the fusiform cambial initials, these sequences probably result from the developmental changes that have occurred in the non-storeyed cambia of these species with radial growth.

The only studies on vessel member length in storeyed woods are those by Chalk *et al.* (1955), who found no change in cell length with distance from the pith in *Pterocarpus angolensis, Nesogordonia papaverifera, and Aeschynomene elaphroxylon,* Rao (1962), who recorded a

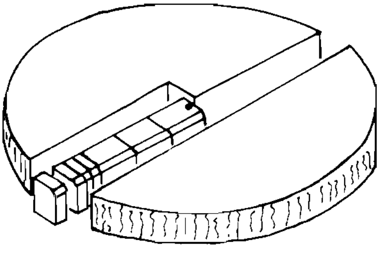

FIG. 1—The way in which the 1 mm thick wood samples were removed from along the radius of each stem disc studied. Scrapings of macerated wood were prepared from the outside of each slice.

similar result for *Robinia pseudoacacia,* and Hejnowicz & Hejnowicz (1959), who recorded a slight increase in vessel length with radial growth also in *Robinia pseudoacacia.*

Most woods show a within-ring variation pattern, the cells of the early wood generally being shorter than those produced later in the same annual ring. This variation pattern has been recorded for the vessel members of some non-storeyed woods (Chalk & Chattaway 1935, Bissett & Dadswell 1950, Bosshard 1951, Swamy *et al.* 1960) as well as for tracheids and fibres in many species (Dinwoodie 1961). Such size changes have been recorded for fibres in woods with storeyed cambia (Chalk *et al.* 1955), but not for vessel members.

METHOD

Rectangular blocks of wood were cut from 10 *Hoheria angustifolia* stem discs. The sample numbers are indicated in Table 1. All except sample 10 came from the adult trees A1-5 listed earlier. Sample 10, taken from an inclined branch of another adult tree, included reaction wood. Each block was cut so as to pass from the stem centre out along a radius to the cambium, measuring 6 mm wide on the transverse face and 12 mm deep on the radial face. After staining the sanded transverse surfaces with a methylene-blue/malachite-green solution, the positions of the growth ring boundaries were noted.

Each sample was then cut into 1 mm wide slices, an oblique cut first being made along the block in order to assist sample orientation (Fig. 1). After gentle maceration, each slice was washed in distilled water and a scraping of the separated cells taken from its outer face. Where possible the lengths of 25 vessel members were measured in each preparation. The widths of the same 25 vessel members were also recorded for rings 6 to 9 in the A1, 2-ft sequence and correlation coefficients calculated.

RESULTS

The vessel member lengths for each millimetre of wood from the stem centre to the cambium for three of the discs, trees A1, A2, and A4 (all at the 2-ft level), are presented in Fig. 2 (a)–(c). The positions

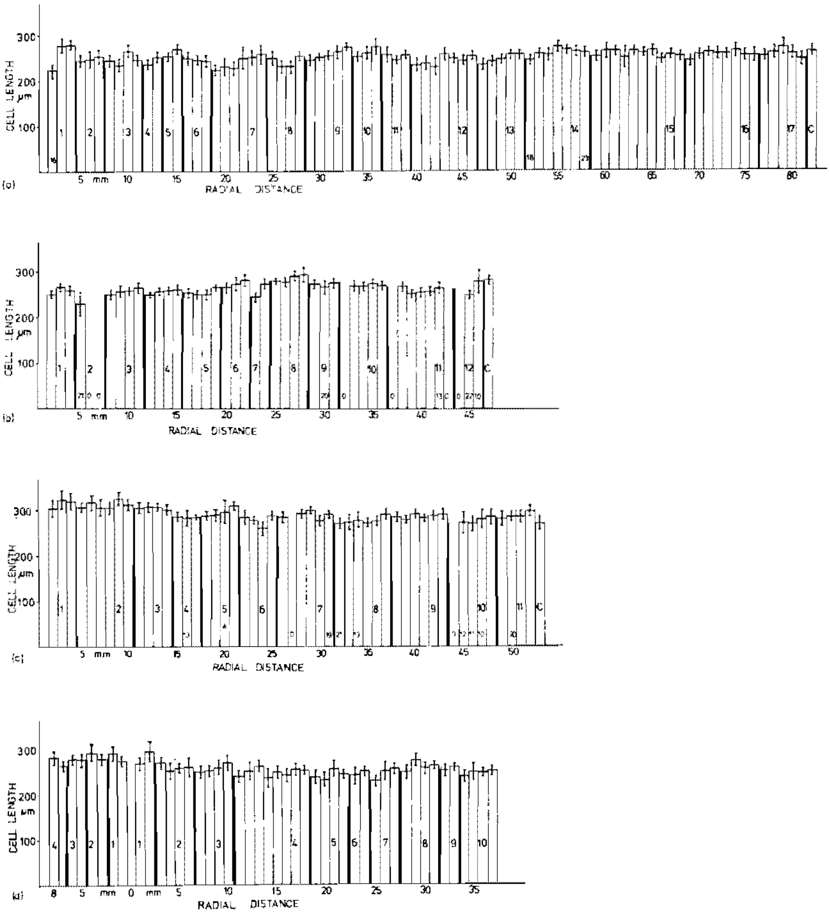

FIG. 2—The relation of mean vessel member length to radial distance from the stem centre in four of the discs of *Hoheria angustifolia* examined. (a) tree A1, 2 ft, (b) tree A2, 2 ft, (c) tree A4, 2 ft, (d) reaction wood disc. The bar marks indicate the 95% confidence limits of the mean (± 2 standard errors). Occasional figures along the x-axis indicate the number of vessel members where fewer than 25 were measured. The Kolmogrov–Smirnov statistic ^{d}max indicated a non-normal distribution for the sample marked with an asterisk. The positions of the growth ring boundaries to the nearest millimetre are indicated by the heavy lines and the rings numbered. C indicates the cambium.

of the growth ring boundaries to the nearest millimetre are also indicated. Except where indicated, the Kolmogorov–Smirnov statistic ^{d}max for each sample is below 0.316, the critical value for samples where $n = 25$. It is, therefore, assumed that there is no evidence to suggest anything other than a normal distribution within most samples. The 95% confidence limits (mean ± 2 standard errors) are indicated by bar marks. The results for six of the other seven discs are not graphed, but they showed essentially the same pattern.

The results of these measurements indicate that there is no significant change in vessel member length with radial distance from the stem centre in *Hoheria angustifolia.* The absence of any trend in vessel member length upwards or downwards with radial distance from the stem centre is confirmed by the fact that the 95% confidence limits of the slope of the regression equations for each sample lie on either side of zero (Table 1).

The tenth disc examined showed an eccentricity due to the presence of tension wood on one side of the stem. The vessel member lengths for each mm of wood on either side of the stem are presented in Fig. 2 (d). The confidence limits for the slope of the regression equation for vessel member length in this sample indicate a slight decrease in length with radial distance from the stem centre in the tension wood (Table 1).

It is evident from Fig. 2 that there is a tendency for the late wood vessel members to be slightly longer than the early wood cells in many of the growth rings, but in most cases the 95% confidence limits overlap. Mean vessel member lengths from the first and last mm samples in each growth ring are tabulated in the last two columns of Table 1. Without exception these means indicate that the late wood vessel members are very slightly longer than those found in the early wood.

The lengths and widths of the vessel members from rings 6–9 inclusive in tree A1 (2-ft level) show a negative correlation significant to the 99% level (Table 2A). An analysis of the angle of the end walls of these cells indicates that the wider, shorter cells tend to have nearly transverse perforation plates at both ends, but the narrower vessel members have one or both end walls at an angle and are probably slightly longer as a consequence (Table 2B). The slight changes in mean vessel member length within the growth rings indicated by Fig. 2 and Table 1, therefore, do not result from changes that have occurred in the length of the fusiform cambial initials with season, but result from a tendency for the late wood derivatives of the cambium to differentiate into narrower vessel members with more oblique end walls than those of the early wood.

THE RELATIONSHIP OF FUSIFORM CAMBIAL INITIAL LENGTH TO VESSEL MEMBER LENGTH

There are few published results on the relation in size between the fusiform cambial cells and their derived elements. Bailey (1920), from

TABLE 1—Tree numbers and heights from which the 10 discs were examined to determine the changes in vessel member length with distance from the stem centre, the number of vessel members measured for each disc, their mean length, and the regression equations for each. The mean lengths of the vessel members from the first and the last mm sample of each growth ring are shown in the last two columns.

Sequence	Tree no.	Height (ft)	Cambial radius (mm)	No. of growth rings	No. of measurements	Mean vessel member length (μm)	Regression equation (95% confidence limits of slope) y =	Mean length early wood vessel members (μm)	Mean length late wood vessel members (μm)
1	A1	2	81	17	1987	252.0	246 + 0.13x (− 0.01 + 0.28)	243.8	257.0
2	A1	5	63	13	1486	257.3	249 + 0.17x (− 0.05 + 0.40)	246.2	263.9
3	A2	2	46	12	1147	293.1	311 − 0.49x (− 1.02 + 0.04)	289.6	298.7
4	A2	5	37	8	917	267.3	259 + 0.43x (− 0.85 + 1.01)	251.2	277.6
5	A3	2	57	16	1469	260.9	260 + 0.01x (− 0.11 + 0.14)	256.7	262.3
6	A4	2	52	11	986	264.6	254 + 0.47x (− 0.97 + 0.04)	259.5	268.3
7	A4	5	33	8	796	272.8	279 − 0.37x (− 0.83 + 0.10)	262.6	277.8
8	A5	2	63	16	1439	298.6	302 − 0.10x (− 0.24 + 0.05)	290.3	301.1
9	A5	5	46	12	1109	271.5	264 + 0.33x (− 0.17 + 0.83)	266.5	278.5
10	Reaction wood sample		37(8)	10(4)	375	261.8	274 — 0.56x (− 1.07 − 0.05)		

a comparative study of the size variation of fusiform cambial initials and their derivatives in *Ginkgo* and the Coniferae, concluded that the lengths of the tracheids of the last formed xylem closely resembled the lengths of the fusiform initials from which they were derived, but were slightly longer. This relationship has been generally thought to apply to most conifers and a great number of studies on the changes in cell length in the conifer cambium have now been based on this assumption. In dicotyledons, Bailey found that the initials were much shorter than the fibres but were approximately the same length as the vessel members. Chattaway (1936) found that the fibres in dicotyledonous woods can be 1.1 to 9.5 times longer than the cambial initials, though elongation to several times the initial length only occurs when the initials are very short.

As most of our present information on developmental and size changes occurring in the vascular cambium of plants has been obtained from investigations on the secondary xylem, and not from direct examinations of the cambium itself, it is surprising that more attempts have not been made to establish the size relationship between fusiform initials and their derivatives.

METHOD

Twenty-five samples including wood and bark from 8 trees (A1-5, I1, 2, and 4) were examined. the trees and heights from which the samples were removed are listed in Table 3. Serial tangential longitudinal sections of the cambium were cut from each (wood) sample by the standard paraffin technique. Twenty-five fusiform initials selected at random were measured from each sample. Small cubes of wood from behind the cambium were isolated and macerations prepared from the

TABLE 2—A: the length to width relationship of the vessel members from the rings 6–9 inclusive of tree A1 (2-ft level).
B: the relationship of vessel member length and width to the angle of the end wall.

A. No. of vessel members measured	Mean length (μm)	S.E.	Mean width (μm)	S.E.	Correlation coefficient
450	266.5	1.4	54.7	1.1	—0.186**

Regression equation: Length = 454 – 3.4 width

B.	Mean length (μm)	S.E.	Mean width (μm)	S.E.
Both end walls transverse	251.9	2.1	62.3	1.6
One end wall oblique	289.4	4.0	46.3	3.6
Both end walls oblique	296.1	1.9	42.9	1.6

TABLE 3—Correlation of mean fusiform initial length ($\bar{Y}_f$) with mean vessel member length (Y_v) from the last formed xylem, and their 95% confidence limits, and the Kolmogorov–Smirnov statistic dmax, resulting from a comparison of the observed sample with a normal distribution based on the sample mean and variance. The value of t for a comparison of the means is shown in the last column. (For 48 degrees of freedom, the critical values of t are t 0.05 = 2.01, t 0.01 = 2.68, t 0.01 = 3.49.)

Sample	Tree	Height (ft)	$\bar{Y}_f$ (95% confidence limits) (μm)	dmax	$\bar{Y}_v$ (95% confidence limits) (μm)	dmax	$t_{f,v}$
1	A1	2	263.6 (253.4–273.8)	0.12	246.3 (233.9–258.7)	0.12	2.00
2	A1	5	268.6 (257.8–279.4)	0.11	283.2 (272.8–293.6)	0.10	1.96
3	A1	8	252.6 (237.2–268.0)	0.12	267.8 (258.5–277.0)	0.16	1.68
4	A1	11	232.6 (218.2–247.0)	0.10	237.2 (228.8–245.6)	0.17	0.53
5	A2	2	286.6 (280.2–293.0)	0.08	277.1 (269.5–284.7)	0.13	1.27
6	A2	5	273.5 (261.3–285.7)	0.10	259.1 (244.9–273.3)	0.18	1.54
7	A2	8	272.3 (261.5–283.1)	0.06	270.9 (264.1–277.7)	0.17	0.21
8	A2	11	259.5 (245.7–273.3)	0.12	270.3 (261.1–279.5)	0.08	1.29
9	A3	2	269.3 (259.9–278.7)	0.09	281.2 (272.6–289.8)	0.14	1.85
10	A3	5	254.9 (246.9–262.9)	0.08	261.3 (251.9–270.7)	0.11	1.10
11	A3	8	251.2 (244.8–257.6)	0.14	269.2 (262.8–275.6)	0.16	3.09**
12	A3	11	252.0 (246.4–257.6)	0.14	266.7 (259.5–273.9)	0.09	2.03
13	A4	2	268.2 (256 6–279.8)	0.10	298.1 (287.7–308.5)	0.11	3.83***
14	A4	5	280.2 (266.4–294.0)	0.10	283.9 (274.1–293.7)	0.12	0.94
15	A4	8	263.2 (253.4–273.0)	0.06	310.2 (297.8–322.6)	0.10	5.92***
16	A4	11	265.0 (252.2–277.8)	0.09	270.2 (258.2–282.2)	0.11	1.45
17	A5	2	313.3 (300.5–326.1)	0.11	292.8 (281.8–303.8)	0.13	1.92
18	A5	5	277.1 (263.3–290.9)	0.09	281.6 (273.4–289.8)	0.12	0.56
19	A5	8	270.4 (257.8–283.0)	0.13	270.3 (262.7–277.9)	0.12	0.01
20	A5	11	252.7 (235.1–270.3)	0.10	277.3 (266.5–288.1)	0.10	1.77
21	A5	14	256.8 (240.6–273.0)	0.13	260.0 (251.0–269.0)	0.12	0.73
22	I1	2	269.3 (263.2–276.3)	0.06	274.7 (266.9–282.5)	0.11	1.37
23	I2	2	288.7 (276.7–300.7)	0.09	283.9 (275.7–292.1)	0.11	0.65
24	I2	5	269.4 (259.2–279.6)	0.12	250.5 (242.1–258.9)	0.14	2.65*
25	I4	2	280.1 (268.7–291.5)	0.16	282.1 (272.5–291.7)	0.15	0.25

outside face of each block. Every effort was made to ensure that these preparations included fully differentiated vessel members from wood as close to the cambium as possible. Twenty-five cells were measured in each of these macerations.

RESULTS

The mean lengths of the fusiform cambial initials and vessel members and their standard deviations, the confidence limits at the 95% level, and the Kolmogorov–Smirnov statistic ^{d}max for each sample are presented in Table 3. In all 50 samples the ^{d}max values were below 0.316, the critical value for samples of 25. There is no evidence, therefore, to suggest anything other than a normal distribution. A number of samples were also frequency checked on probability graph paper and straight-line graphs confirmed normal distributions in each case. Transformation of the data was therefore considered unnecessary.

The t-values for the comparisons between the pairs of means are also shown in Table 3. In only four of the 25 pairs of samples are significant differences between the mean fusiform initial length and the corresponding mean vessel member length detected: samples 11 (tree A3, 8 ft), 13 (tree A4, 2 ft), 15 (tree A4, 8 ft), and 24 (tree 12, 5 ft). When all 25 pairs of samples are compared the product-moment correlation coefficient is significant to the 99% level, indicating a real and positive correlation between the pairs of samples (Table 4). A better correlation, significant to the 99.9% level, is obtained when samples 11, 13, 15, and 24 are ignored. The correlation is graphed in Fig. 3 showing the principal axis and the 99% confidence ellipse for the grand mean.

A positive relationship between fusiform cambial initial length and the length of the vessel members derived from them is therefore established, the vessel members having a similar length to the fusiform initials.

VARIATION IN FUSIFORM CAMBIAL INITIAL LENGTH WITH HEIGHT IN THE TREE

In many plants with non-storeyed cambia, there is a gradient in tracheid and fibre length along the axis of the tree. When the pattern

TABLE 4—The means of the mean lengths for fusiform cambial initials and vessel members and their standard errors, the product-moment correlation coefficients, and the equations of the principal axes for all 25 pairs of samples and for 21 pairs of samples (samples 11, 13, 15, and 24 omitted).

n	$\bar{\bar{Y}}_f$	SE_f	$\bar{\bar{Y}}_v$	SE_v	Correlation coefficient	Equation of principal axis
25	268.9	3.4	272.6	3.3	0.496**	$Y_f = -20.5 + 1.06Y_v$
21	269.9	3.8	269.9	3.0	0.673***	$Y_f = -98.9 + 1.36Y_v$

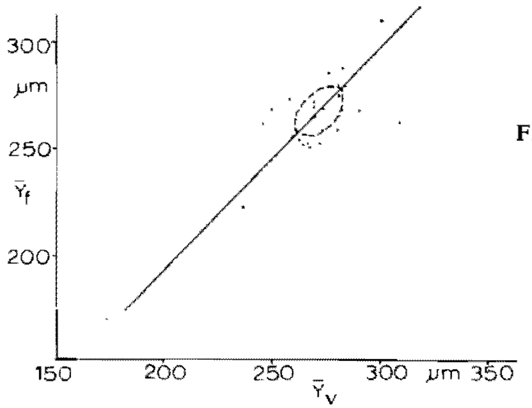

FIG. 3—The relationship between mean lengths of fusiform cambial initials (Y_f) and vessel elements from the last formed xylem (Y_v). The straight line indicates the principal axis and the ellipse the 99% confidence level for the grand means for all 25 samples.

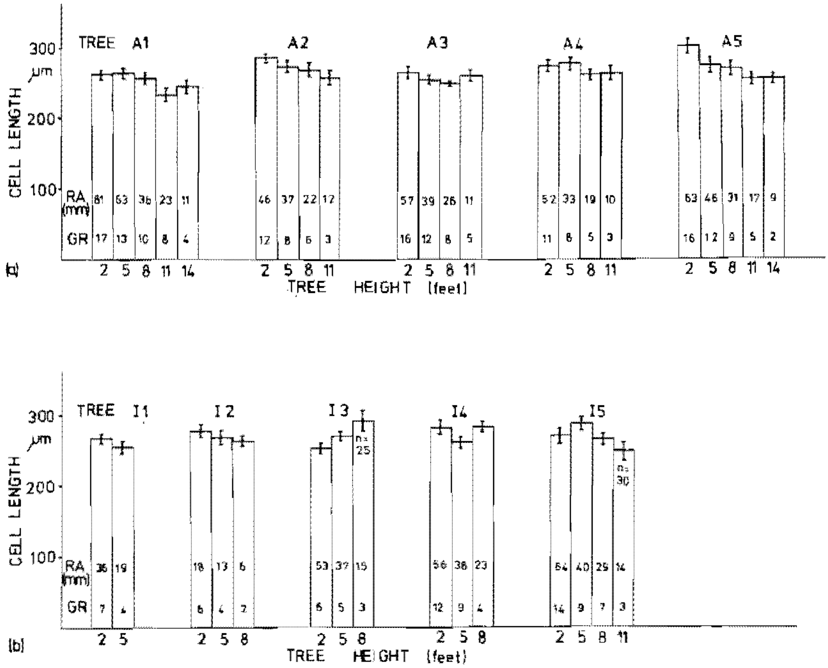

FIG. 4—Changes in mean fusiform cambial initial length with height in the tree in (a) 5 adult trees and (b) 5 intermediate trees. The bar marks indicate the 95% confidence limits of the mean (± 2 standard errors). Where the number of fusiform initials measured was less than 50, this is indicated near the top of each bar. The radius of the stem disc within the cambium and the number of growth rings in the secondary xylem are also indicated for each sample.

of cell length has been followed upwards in the same growth ring, the average length has been found to increase from the base upwards to reach a maximum and then to decrease gradually towards the top of the stem (Sanio 1872; Dinwoodie 1961, 1963). The point of maximum cell length occurs at approximately one-third of the tree height and therefore occurs at progressively higher levels in successive annual rings.

While this pattern of variation with height in the tree is well known for fibres and tracheids, there have been few studies on vessel members. Hejnowicz & Hejnowicz (1958) have recorded an increase, followed by a decrease, in vessel member length with height in the tree in *Populus tremula*, as has Rao (1962) for vessel members in *Acer pseudoplatanus, Quercus robusta,* and *Robinia pseudoacacia.* The last named of these three trees has a storeyed cambium. The study of vessel member length in *Robinia pseudoacacia* by Hejnowicz & Hejnowicz (1959) did not reveal sufficient information for this trend to be confirmed.

METHOD

Small cubes including wood and bark were removed from the outside of the stem discs taken from the trees A1-5, I1-5, and J1-5. These discs had been cut at 3-ft intervals from the trunks of each tree. Serial tangential longitudinal sections were prepared from each sample using the standard paraffin technique.

Sections containing predominantly cambial cells were selected for measurement and the lengths and widths of 50 randomly selected fusiform cambial initials were measured using an overhead projection system. These measurements were transferred to computer cards and the basic statistics computed.

RESULTS

Fig. 4 presents means and their 95% confidence limits for fusiform initial length at selected heights for trees A1-5 and I1-5. Due to the small diameter of the J1-5 stems discs, tangential longitudinal sections cut from them contained insufficient areas of cambium for 50 fusiform initials to be measured by the technique used in the study.

Although no trend in fusiform initial length with height in the trees is evident from most of the sequences illustrated in Fig. 4, the means for each height do indicate a slight downwards trend with height when the trees are taken collectively (Fig. 5). The analysis of variance based on trees (Table 5A) shows that there is no significant difference in the fusiform cambial initial lengths among the trees, but there are significant differences among the various height samples among the trees. Further analysis of variance indicates that the among-heights difference is caused mostly by the linear decrease in cell length with increasing height in the tree (Table 5B).

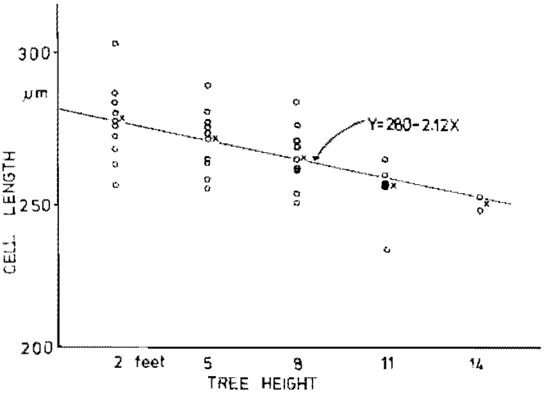

FIG. 5—Mean fusiform cambial initial lengths for the individual trees plotted against height in the tree. The mean for the trees at each height (indicated by an x offset slightly to the right in each case) and the line of the regression equation are also indicated.

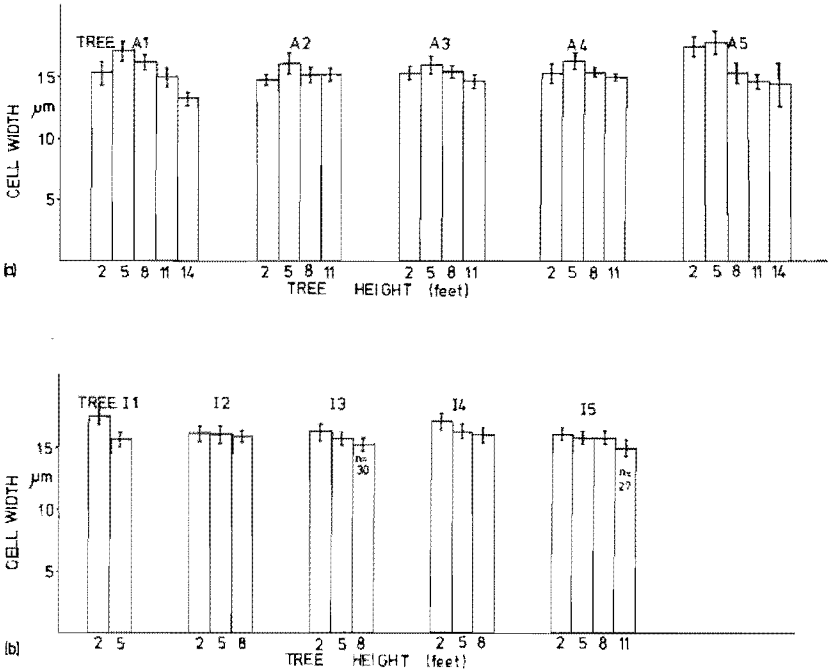

FIG. 6—Mean tangential width of fusiform cambial initials at selected heights in the tree in (a) 5 adult trees and (b) 5 intermediate trees, and their 95% confidence limits. Figures for cambial radius and the number of growth rings within the cambium are the same as in Fig. 4.

TABLE 5—Analyses of variance for fusiform cambial initial length based on A, trees and B, heights. There is no significant cell length difference among different trees, but there is a highly significant difference in cell length among heights among the trees. When based on the individual heights, this significant difference is caused mostly by the linear decrease in cell length with height in the tree.

	Degrees of freedom	Mean squares	Variance ratio	% variance components
A. Among trees	9	14760	1.70ns	3.3
Among heights and trees	27	8542	10.18***	15.5
Within samples	1751	839		81.2
B. Among heights—linear component	1	2153	41.40**	
Among heights—remainder	3	53	0.39ns	
Within heights	32	132		

Data on the widths of the fusiform cambial initials are presented in Fig. 6 and the analysis of variance in Table 6. In the five larger trees, fusiform initial width increases, and then decreases, with increasing height in the tree. This pattern is followed for the mean initial width when all the 10 trees are taken collectively (Fig. 7). As the radial distance of the cambium from the stem centre decreases with increasing height in the tree, mean fusiform initial width is likely to be related either to this factor (Fig. 8) or to a related one, the number of growth rings behind the cambium. In fact, cell width was found to be more closely related to the cambial radius than to the number of growth rings behind the cambium. Except at the lower levels of the larger trees, fusiform initial width would appear to be related to the cambial radius.

DISCUSSION

This investigation has shown that there is no change in the length of the fusiform cambial initials with radial growth in *Hoheria angustifolia*. The minor fluctuations that occur in the length of vessel members within the growth rings are caused by minor changes in the angles of the end

TABLE 6—Analysis of variance for fusiform cambial initial width based on trees. There is no significant difference in widths among trees but there is a highly significant difference in cell width among heights among the trees.

	Degrees of freedom	Mean squares	Variance ratio	% variance components
Among trees	9	54.9	1.27ns	1.5
Among heights and trees	24	43.1	11.42***	17.0
Within samples	1666	3.7		81.5

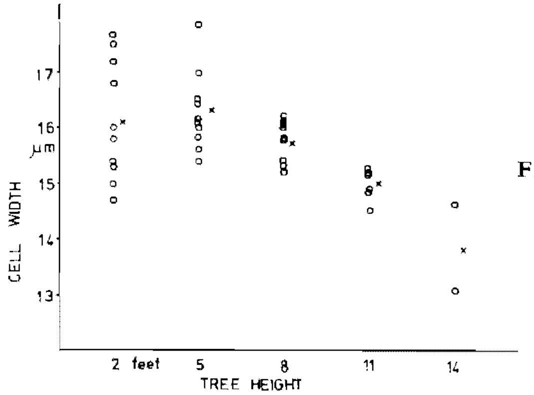

FIG. 7—Mean fusiform cambial initial widths for the individual trees plotted against tree height. The mean for the trees at each height is also indicated (the x's are offset slightly to the right in each case).

walls of these cells. This is a significant result because it indicates that the mean length of a population of fusiform cambial initials, in this species, can remain unchanged from the onset of secondary growth, and probably reflects the length of the procambial cells. Such a relationship is not possible in plants with a non-storeyed cambium where circumferential expansion of the cambium is brought about by pseudotransverse anticlinal divisions of the fusiform initials, followed by intrusive growth of the daughter cells, and the preferential loss of some of these. Even in a wood possessing a storeyed cambium, slight increases or decreases in mean fusiform initial length with radial growth can result from the preferential loss of the shorter or longer fusiform initials, or the conversion of these into new ray initials. A slight decrease in the length of parenchyma cells in *Aeschynomene hispida* Willd., another plant with a storeyed cambium, has been recorded by Butterfield (1972). In this species, however, occasional transverse divisions of the fusiform initials were responsible for the decrease in cell length with secondary growth. Unfortunately, because of the large amount of cell readjustment that occurs with differentiation in *Hoheria angustifolia*, it was not possible to detect whether any such transverse divisions had occurred in the fusiform initials of this species.

A positive relationship between fusiform cambial initial length and the length of vessel members in the wood behind the cambium has also been established for *Hoheria angustifolia*. This confirms the general relationship between the length of these cell types proposed by Bailey (1920). The lengths of the vessel members and fusiform cambial initials are similar, indicating that no transverse divisions occur in the xylem mother cells before the differentiation of the vessel members. Some rearrangement of the end walls must occur, as the fusiform initials have pointed hexagonal tips and the mature vessel members have simple slightly oblique to transverse end walls. The extent of this rearrangement would appear to vary between the early wood and late wood cells within each growth ring. As a general trend, the early wood vessel members are slightly wider and shorter than the late wood vessel members and

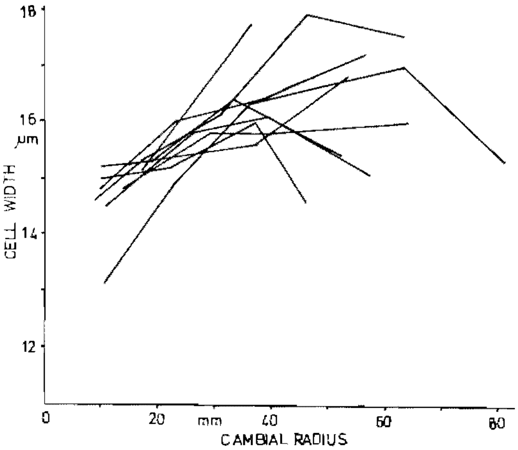

FIG. 8—Mean fusiform cambial initial width plotted against cambial radius for the 10 trees.

have transverse end walls, and the latter have oblique end walls. The positions of the growth ring boundaries in Fig. 2 are to the nearest millimetre, so that some experimental error is involved in the summation of cell dimensions from the first and last millimetre samples from each growth ring.

As the wood collections were all made on the same day (18 February 1965), the slight within-ring variations in vessel member length are not responsible for the poor correlation in 4 of the 25 pairs of samples where vessel member and fusiform initial lengths were compared, It is obvious from the measurements of fusiform initial lengths with height in the tree (Fig. 4) that some quite considerable variations in length occur with position in the tree. It is of considerable importance, therefore, that samples of vessel members and cambial initials in this species come from exactly adjacent positions in the stem.

The within-ring variations in the dimensions of the vessel members are in themselves interesting though their measurement was not intended to be a major part of this study. The variation in tracheid and vessel member length within the growth ring of most woods is generally attributed to seasonal differences in the frequency of pseudotransverse divisions in the cambium (Bannan 1950), differing rates of elongation of the new daughter initials, and varying proportions of cell loss. The extent of the elongation of the derivative cells after they have been cut off from the cambium is, therefore, difficult to assess in different parts of the growth ring. It must be assumed, however, that no such cyclic changes in length occur in the fusiform initials of a storeyed cambium such as *Hoheria*. Any pseudotransverse division late in the season would be perpetuated throughout subsequent radial growth, lowering the mean fusiform initial length. As this does not happen, the slight within-ring fluctuations in vessel member length must be attributed to adjustments to the end walls during differentiation.

A decrease in the mean length of the fusiform cambial initials with height in the tree occurs in *Hoheria angustifolia*, although considerable fluctuations in cell length are apparent in individual trees. This pattern differs from that established for most trees with non-storeyed cambia, where cell length increases and then decreases with increasing height in the tree. In these species at least part of the decrease in cell length up the tree must be attributed to the decreasing cambial radius, as this is a factor governing the length of the fusiform initials at any one height (Hejnowicz & Hejnowicz 1958). As fusiform initial length is not dependent on the radial distance of the cambium from the stem centre in *Hoheria*, it implies that the decrease in fusiform initial length up the tree occurs with the ageing of the leading shoot. Such a result is not unexpected, as it confirms the views of Dinwoodie (1963) and others that the activity of the terminal meristem could influence the length of the fusiform initials in the cambium and, therefore, the length of their derivatives throughout the tree.

The pattern of the fusiform initial length within the internodes of a stem has never been adequately investigated. Bailey & Tupper (1918) noted that tracheids tended to be shorter at nodes and other regions where growth adjustments occur. It is possible that internodal variation in the length of the procambial initials in *Hoheria*, perpetuated in the cambium even after radial growth, may be the cause of the considerable differences in the cell length sequences in the individual trees studied (Fig. 4).

The mean width of the fusiform initials in *Hoheria angustifolia* increases and then decreases with increasing height in the tree. As stems taper with increasing height, fusiform initial width would appear to be governed by the radial distance of the cambium from the stem centre. A similar result has been recorded for *Aeschynomene hispida* (Butterfield 1972), but in this case cambial derivatives were used to determine cell widths from a single stem disc. The circumferential expansion that accompanies radial growth would appear to be met in a storeyed wood by an increase in the tangential widths of the fusiform initials, as well as by anticlinal divisions in the radial longitudinal plane. The variation in the mean fusiform initial widths in *Hoheria angustifolia* stems greater than 25 mm radius remains unexplained.

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