SPIRAL GRAIN FORMATION BY INDUCED REORIENTATION OF THE CONIFER CAMBIUM

by

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Notes to the reader:

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The metafile entitled Series 1 Animation.pdf was not in the thesis. Using the camera lucida drawings in the thesis, it provides a slide-show example of successive changes in serial tangential sections. The animation illustrates abrupt microdomain initiation as it occurred within a singular fusiform cambial cell (compare sections 88 and 89).

On page 1 of the thesis, the following statement occurs:

"Herein the concept of a single layer of initiating cells within the vascular cambial zone is advocated. The cells which comprise that single layer are referred to as "initials" throughout; and the layer which they comprise as the "cambium"."

The author was constrained by precedent (i.e., by speculation of earlier scientists that through promulgation had become dogma) to accept this concept of the cambial initial and cambium when preparing his thesis document. The dogma continues to be promulgated today, although solid scientific evidence in support of it remains to be presented. The author questioned the validity of the concept in a post-thesis publication (Can. J. Bot. **62:** 2872-2879), and as an outcome of his doctoral research he subsequently rejected the concept, as follows:

"*An alternative to the cambial initial concept is that any cell of the cambial zone receiving the necessary balance of physical and chemical factors will develop and function in the manner traditionally ascribed to the hypothetical initial. Whether a cambial cell divides anticlinally or periclinally, becomes incorporated into xylem or phloem, or remains as a cambial cell may be regulated by factors that shift radially across cells of the cambial zone."* (Savidge, RA. 1985. Prospects for manipulating vascular-cambium productivity and xylem-cell differentiation. *In* Attributes of Trees as Crop Plants, edited by M.G. R. Cannell & J.E. Jackson, Institute of Terrestrial Ecology, Abbots Ripton, Huntingdon, U.K., p. 212)

Rodney Arthur Savidge
January 2017

We approve this thesis and affirm that it meets the requirements set down for the Degree of Master of Science in Forestry

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SPIRAL GRAIN FORMATION BY INDUCED REORIENTATION OF THE CONIFER CAMBIUM

ABSTRACT

Processes involved in the reorientation of fusiform cambial initials related to spiral grain formation were investigated. Spiral grain was induced to form in a vigorous, nursery-growing sapling of Pinus strobus L. by constructing narrow diagonal bridges of phloem and cambium across ring girdles of stem internodes in the early spring. After 5 months, the bridges were prepared for light microscopy of transverse, radial and serial tangential sections, and the reorientation process investigated. As a result of this first investigation, further investigations to determine the time required for the cambium to reorient and the effect of wounding upon the reorientation process were begun: in late summer, 18 vigorous, field-growing saplings of Pinus contorta Dougl. were each given two phloem-cambium bridges across ring girdles of two internodes on the same day; the upper of each bridge was oriented diagonally and the lower parallel to the long axis of the tree stem in each case, the lower bridge to serve as a control to the wound response. Periodically, the two bridges of one of each of these 18 trees were then harvested; serial tangential sections of xylem and transverse, radial, and serial tangential sections of the cambial zone were then prepared and investigated.

By tracing radial files of mature xylem tracheids in serial tangential sections through the zone of reorientation, the step-by-step changes which occurred in the cambium as it changed its alignment were deduced for each species and have been illustrated diagrammatically. Relatively small, localized regions of the vascular cambium, termed "microdomains," were found to form the vanguard to the reorientation process, followed by similar reorientation of the intervening regions subsequently. Microdomains were found to begin by one, or sometimes two or more, adjacent fusiform cells successively subdividing to result in a number of shorter fusiform cambial cells. The subdividing divisions were oblique and parallel anticlinal divisions. Accentuation of the reorientation occurred when some of the short fusiform cambial initials failed, permitting adjacent ones to elongate into and occupy the vacated space. In the elongation process, the numerous parallel oblique dividing walls formed a template which guided the extending cells into the new alignment.

Following late-summer girdling, after 50 days reorientation was still in the microdomain stage within the diagonal bridge cambium of Pinus contorta; vertical bridges showed no change in the axial alignment of their fusiform cambial cells throughout the duration of the experiment. Wound responses found to be common to both the diagonal and vertical bridges, as well as to both species, included: swelling of ray parenchyma, true transverse divisions in xylem mother cells, formation of xylary axial parenchyma and traumatic longitudinal resin canals and callus tissue.

It has been concluded that a number of activities are equally necessary within the vascular cambium of Pinus before a group of adjacent fusiform cambial initials become realigned. These include subdivision by anticlinal division of each of the initials into a number of shorter initials; formation of oblique anticlinal dividing walls which are parallel and in the direction of the orientation to be adopted; failure of some of the shortened fusiform initials following division; and occupation of the vacated space by the surviving initials as they are guided by the parallel dividing walls into the new alignment.

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ACKNOWLEDGMENTS

My interest in the phenomenon of spiral grain began as a prospector trying to split "unsplittable" firewood in freezing-cold Yukon weather a number of years before I began my undergraduate University program. In the Faculty of Forestry at the University of Toronto, that interest was rekindled during a lecture given by the late "Mike" Glavicic in a second-year tree-morphology course. Mike encouraged me to try to split open the mystery of spiral grain and introduced me to his "boss", Professor J. L. Farrar, whom, unknown to me at that time, had long been equally as much interested in spiral grain as I. Little more needs to be said. Dr. Farrar deserves special thanks for patiently and tolerantly encouraging and assisting me to think, to persue my own ideas, and to become aware of what previous researchers had found. From the outset he helped me to organize my research thoughts, and his frankness, enthusiasm and wise skepticism and learned biases have given me many new perspectives to the scientific approach. I am especially grateful for the unlimited amount of time and guidance that he has devoted to me. Dr. Farrar has given me the master key to many doors, not without hard work, but it has been a pleasure nevertheless.

Thanks to Mrs. (Toni) Balatinecz for introducing me to the micro-technique for light microscopy, and for her generous help and discussion over the last few years.

Series 1, in the Appendix of this Thesis, is the work of Michelle Stevens, a third-year Faculty of Forestry and Landscape Architecture student during 1976-77 who chose to do her Tree Physiology project on spiral grain. Table 5 (p. 50) is also her work. Both were done under my supervision, and I accept full responsibility should there be any mistakes found; however, in my opinion her work is superior to my own. I thank Michelle for her cooperation and the permission to include her results herein; as well, as my "student" I am sure she has taught me more than I have her about spiral grain.

Many other people have contributed to this endeavour. Thanks very much to Mrs. Jean Bohne, Forestry Librarian, for her kind cooperation and helpfulness; thanks also to her support staff this year: Miss Kitti Hundal and Miss Erika Fetzer, both of whom have assisted me greatly. Thanks to Dr. T. Sawa and Dr. M. C. Heath of the Department of Botany, University of Toronto, for permission to use the scanning electron microscope; and special thanks is due Paul Frame, a Ph. D. candidate in the Botany Department, for showing me how to use the SEM. Robert Sheath, another Ph. D. candidate in the Botany Department, introduced me to darkroom photographic work and first jogged my mind with the idea of the camera lucida, all for which I am very grateful.

Thanks to Dr. N. Dengler in the Botany Department, and to Dr. D. N. Roy, in the Faculty of Forestry and Landscape Architecture, for permission to audit their courses, related to my Thesis topic, this last year, and for their time and discussion on my own research. Thanks to Dr. R. D. Ayling of the Faculty of Forestry and Landscape Architecture for his review of my graduate seminar; and my thanks also to Dr. P. L. Aird within the same Faculty for his own interest in spiral grain and for making me aware of some new references on spiral grain. All of the last four-mentioned people were involved in my oral examination, as well as my supervisor, Dr. Farrar; and I thank each one of them for their reviews and constructive criticism.

Last, but not least, thanks to Mr. and Mrs. Myron Bukojemsky, who have generously fed me, clothed me, and taken care of me in true Ukrainian-Christian spirit during the last four years.

SPIRAL GRAIN FORMATION BY INDUCED REORIENTATION OF THE CONIFER CAMBIUM

INTRODUCTION

General

Spiral grain in conifers consists of fusiform xylary elements which have become inclined relative to the long axis of the tree in the same general direction (Fig. 53). Spiral grain is not only a curious phenomenon; it also often constitutes a serious defect of high economic importance; and as well spiral grain is widespread both in conifers and hardwoods and has been described as the normal growth pattern of the tree (Panshin and de Zeeuw 1970, p. 277).

That spiral grain can be brought about artificially, i.e. induced to form using various spiral girdling techniques, has long been known (Büsgen and Münch 1929, Collins 1930, Fahn 1973, Kirschner et al. 1971, MacDaniels and Curtis 1930, Teodoresco and Popesco 1915, Tupper-Carey 1930, and Sinnott 1960--section on Symmetry). Fahn (1973) and Kirschner et al. (1971) have described experiments on diagonal bridges of phloem and cambium crossing ring-girdled stems of Robinia pseudacacia L. which have shown that auxin brings about this change in polarity of cell orientation. In conifers, Harris (1967, 1969 1973) also credits auxin with the induced reorientation process of the vascular cambium. However, no one has yet shown that diagonal auxin transport constitutes the inducing stimulus in natural spiral grain formation. Indeed, the mechanisms involved in the coordinated change in alignment of fusiform cambial cells remains controversial (Bannan 1964, 1966, Harris 1967, 1969, 1973, and Hejnowicz 1961, 1963, 1967, 1968, 1971) in conifers. Thus this Thesis reports on the mechanism of reorientation of fusiform cambial cells.

Herein the concept of a single layer of initiating cells within the vascular cambial zone is advocated. The cells which comprise that single layer are referred to as "initials" throughout; and the layer which they comprise as the "cambium" (Wilson 1966, Schmid 1976). The other cells within the

vascular cambial zone (i.e. the meristematic zone) are referred to as "phloem mother cells" or "xylem mother cells," both being derived in radial files from the initials. Thus the "cambial zone" is constituted of phloem mother cells, the initials, and xylem mother cells, all of which may divide to produce xylary or phloic derivatives.

Cambial initials originate from the procambium and from interfascicular parenchyma, which themselves are derived from the apical meristems as somewhat elongate and isodiametric cells, respectively (Esau 1960). The apical meristems are "pushed" further from their original points of commencement through a process of division of cells within the apical meristems accompanied by elongation of adjacent, proximal cambial initials. This process of elongation of the vascular cambium initials is in the direction of the shoot or root's long axis. It is later, as the vascular cambium begins forming concentric sheaths of phloem and xylem by periclinal divisions, that the original axial orientation of the cambial initials begins to reorient to form spiral grain. Therefore the approach to explaining the reorientation process in the cambium can be restricted to studies of vascular cambium activity.

Spiral-grain investigators, but few others, have appreciated that the spatial arrangement and configurations of mature xylary derivatives, when serially traced, can be used to deduce the temporal activity and appearance of the vascular cambium at the time it produced those derivatives. Much of the research which has given us the present-day model of the conifer cambium (e.g. see Philipson et al. 1971) rests upon the basic integrity of this fact that any tracheid in the xylem constitutes a record of the appearance of both the fusiform initial and its derivative xylem mother cells at the time that that tracheid was formed (excepting changes in radial dimension, slight elongation of the tips, and secondary-wall formation followed by death). This ability to deduce cambial activity from the xylem will be discussed later.

Concepts which are fundamental to this Thesis include the following:

1. The cambial zone is made up of only four types of potentially meristematic cells:

a. fusiform initials

b. ray initials

c. fusiform phloem mother cells

d. fusiform xylem mother cells.

2. Fusiform initials give rise to fusiform mother cells within a radial file; and these fusiform mother cells give rise to xylary or phloic vascular tissues, also within the same radial file. Thus, all mature vascular tissues within a radial file have a common, and sequentially traceable, ancestry.

3. Vascular cambium initial cells are self-perpetuating; i.e. for every periclinal division in an initial there results two cells: a new initial and another cell (mother cell) destined to become or produce a number of xylary or phloic elements.

4. The length of the periclinal division cycle, including division, synthesis, and enlargement, for any meristematic fusiform cell of the vascular cambial zone averages approximately 10 days (Wilson 1964).

5. During the growing season, approximately one xylem tracheid matures each day (Kennedy and Farrar 1965).

6. Mitosis and cell plate formation occupy approximately one day for periclinal divisions and probably much less for anticlinal divisions, since phragmoplast movement following mitosis in an anticlinal division is considerably less in distance than is the case for fully longitudinal periclinal divisions.

7. Although the periclinal division cycle averages 10 days in time, it is assumed that the multiplicative, or anticlinal, division cycle may

occupy as little as one day and need not be intervened by periclinal divisions (Philipson et al. 1971; Bannan 1950, 1951, 1965a, 1965b, 1968a; Bannan and Bayly 1956). However, no reported research is known which has proven this assumption.

8. Temporary lapses in the 10-day periclinal division cycle of a fusiform cambial initial frequently occur (Bailey 1920; Bannan 1968a).

9. There is an upper limit to the length a fusiform initial can achieve; and once an initial reaches that limit, it divides pseudotransversely The sister initials which result from the pseudotransverse division either elongate again to the maximum size, or they fail (Philipson et al. 1971).

10. Failure of an initial most commonly occurs by gradual shortening through successive periclinal divisions, or through rapid shortening by nearly transverse anticlinal dividing walls multiplying a single fusiform initial into two or more shorter initials (Hejnowicz 1961). Shortened initials commonly fail by giving up the initial role and differentiating into either xylem or phloem elements; frequently fusiform initials also reduce in size to become ray initials (Bannan and Bayly 1956). Adjacent fusiform initials quickly occupy the space vacated by failing initials. This occupation occurs through two processes: intrusive elongation and tangential expansion.

Until 1966, the opinion of most spiral grain researchers (Braun 1854, Hartig 1895, Seifriz 1933, Kohl 1933, Newman 1955, Hejnowicz 1961, 1964, Bannan 1964, Jones 1963) was that spiral grain in conifers resulted by the majority of the pseudotransverse (or "oblique") anticlinal divisions occurring in vascular cambium initials all being oriented in the same direction. Reorientation was said to occur as a result of oriented overlap

of the cell tips during intrusive elongation of the sister initials following the oblique anticlinal divisions. But none of these researchers have ever provided step-by-step evidence of the mechanics of cambial reorientation.

Having done such a step-by-step study, in this Thesis it will be argued that reorientation of conifer cambial initials occurs through the following processes:

1. Long fusiform initials shorten; and this shortening occurs by repetitive unidirectional pseudotransverse anticlinal divisions.

2. The shortened sister initials and their mother cell derivatives then elongate in the direction of their long axis as neighboring initials decline or fail. In the process of elongating, the path of least resistance is taken.

3. In spite of failure of many initials, the numerous parallel oblique dividing walls which precede intrusive elongation act as an overall template to guide the elongation process such that reorientation occurs.

Anticlinal divisions

Very little work has been done on the control of plane of division when a cell divides. Harris (1973) found in radiata pine (Pinus radiata D. Don) that it was impossible to distinguish between a periclinal and an anticlinal division in the early stages of mitosis. But by early metaphase, a distinction usually could be made; chromosomes were observed to either come into line with the longitudinal axis of the cell (prior to anticlinal division), or to lie at right angles to the cell (prior to periclinal division). From his study, Harris concluded that it is the skewing of the poles of the mitotic spindle in the cytoplasm, and the formation of the cell plate between those poles at telophase, that determines the direction of pseudotransverse anticlinal division.

But these findings contrast sharply with the findings of Palevitz and Hepler (1974), who studied the control of the plane of division during stomatal differentiation in Allium. Their observations revealed that the final plane of division is not determined by the orientation of the spindle, but rather is established during late anaphase-telophase as a result of directed reorientation movements of the phragmoplast and associated daughter nuclei. Movement of the phragmoplast was found to be highly directional and to never overshoot the correct alignment. At the ultrastructural level, Palevitz and Hepler (1974) found the position of the preprophase band of microtubules, encircling the nucleus, to always be oriented in the same plane as the final orientation of the cell plate. Thus it was their conclusion that it is the preprophase band, and not the spindle, which correlates with cell plate orientation. It is therefore believed that cells exert a more complex active role in cell plate orientation than simply relying on physical factors.

Two types of anticlinal divisions occur in the cambium of conifers:

1. Pseudotransverse (or "oblique", or "multiplicative") divisions, by which multiplication of fusiform initials occurs or new rays are formed (Bannan 1953, 1957, Hejnowicz 1961).

2. Divisions off the side, which usually also give rise to rays (Bannan 1953, 1957, Hejnowicz 1961).

Bannan (1951) found both types of anticlinal divisions to occur usually near the center of the dividing fusiform initials.

The newly formed partition in pseudotransverse anticlinal division may be short and almost transverse, or it may be as much as one-half the length of the dividing cell (Bannan 1965a, Hejnowicz 1961). Hejnowicz (1961) shows clearly that the divisions are in fact oblique, and not transverse, when they first occur in initials. Bannan (1965a) has reported on many

thousands of measurements on dividing cells; and he states that the length of the partition is generally related to the length of the dividing cell.

Bannan (1957) states that the cambium is made up of a single "tier" (or sheath) of initials, a single tier of phloem mother cells toward the outside of the initiating layer, and one to several tiers of xylem mother cells on the inside. Hejnowicz (1961, p. 739) states that the fusiform initials are in the middle of the cambial zone. Both of these investigators are agreed that all cells within the cambial zone undergo periclinal divisions; and both are agreed that pseudotransverse divisions are largely confined to the initials.

Pseudotransverse divisions sometimes also occur in the derived mother cells, especially on the xylemward side. The frequency of these extra-initial divisions tends to rise with the growth rate (Bannan 1950, 1957, 1967, 1968a). Lateral anticlinal divisions, which yield segments off the side, are infrequent and seem generally to involve only the fusiform initials.

Fewer than 2% of the pseudotransverse divisions were found to be temporary by Bannan (1957) and to involve xylem mother cells rather than initials. The primary criteria used by Bannan was the observation that the pseudotransverse partition appeared in a variable number of successive cells and then disappeared. As well, the pseudotransverse division could be found in the xylem with no counterpart in the phloem; thus it must not have occurred in an initial.

Bannan (1957, p. 883) states: "Despite the fact that the effects of factors such as pressure which influence the frequency of anticlinal division in the cambium must be felt throughout the entire zone of growth, it is usually only the fusiform initials that are responsive." The nature

of the stimulus which causes anticlinal divisions in the cambium is undetermined, but it is clear that the rate of division is not geared solely to an increase in girth (Bannan and Bayly 1956). Although the pseudotransverse anticlinal divisions of fusiform initials do result in accommodation for the increasing girth, they occur at a frequency such that there is an extensive overproduction of new initials. Accordingly, there is also extensive cell loss. Whether a fusiform initial continues or fails after anticlinal division is thought to be related to cell length and extent of ray contacts (Bannan and Bayly 1956).

Bannan (1950, 1957, 1967, 1968a) has shown considerable evidence for the general aestival occurrence of anticlinal divisions within conifer cambia. However, he has also noted many exceptions. Fast-growing trees were often found to show anticlinal divisions occurring throughout the growing season. Under conditions of rapid growth, the proportion of anticlinal divisions occurring outside the initiating layer was also found to increase. Nevertheless, even under conditions of rapid growth, the proportion of anticlinal divisions occurring in xylem mother cells is relatively low (Bannan 1957).

Intrusive growth and failure of fusiform initials

Bannan (1968b) reported both the continuation of the initial function after multiplicative division and the subsequent cell elongation to be under polar influence. He found some species, such as Pinus strobus L., to show a predominant tendency to elongate upwards after multiplicative division. Other species show a downward tendency. However, within a tree, at different heights and at different cardinal directions, acropetal or basipetal elongation may predominate. And reversals in this type of polarity were found to occur after intervals of a few years.

Continuation of an initial's function following its creation by

multiplicative division through a lineal succession (or radial file) of cells also is under polar influence. In both Pinus strobus L. and Pinus contorta Dougl., Bannan (1968b) found that the upper sister series was more prone to survive than the lower, in a ratio of approximately 5:4, following oblique anticlinal division.

Bannan (1968b) notes that there is a correlation here between cell survival and direction (basipetal vs. acropetal) of cell elongation: "In Pinaceae the upper of the two sister fusiform initials formed in pseudotransverse division is more apt to be the forerunner of a continuing cell series than the lower sister, and throughout the cambium acropetal cell elongation surpasses basipetal growth." Bannan considers that certain substances concerned with viability and growth are unequally distributed throughout fusiform initials, with greater concentrations occurring either toward the upper or the lower cell tips. However, there is no direct supporting evidence for this latter statement.

Bannan and Bayly (1956) also found that the longest fusiform initials were most prone to survive and repeat the cycle of elongation and multiplication by pseudotransverse division. In contrast, short fusiform initials were found to decline, passing off into maturation or undergoing further divisions to become ray initials.

The rate of failure of initials tends to increase with increasing frequency of anticlinal division. Bannan (1956) and Hejnowicz (1961) agree that elimination of one or more initials results in strong intrusive growth of neighboring initials into the vacated space. However, Bannan (1956) states that intrusive growth also occurs without failure in the form of early rapid extension, with gradually decreasing rate of growth as initial length increases. Hejnowicz (1961), who studied serial transverse sections of the cambial zone (whereas Bannan studied serial

tangential sections of xylem) disagrees. Hejnowicz found intrusive growth not to be rapid following oblique anticlinal division unless there is also concomitant failure of neighboring cells. However, the age of the stem was found to be important in this respect. Hejnowicz (1961) reports that in older stems, intrusive growth is concentrated at sites of failure of other initials, whereas in young stems the intrusive growth of initials is relatively strong regardless of whether neighboring initials are or are not failing. This difference is apparently associated with the difference in relative rates of girth increase between the different sizes of stems.

Bannan and Bayly (1956) state that a selective mechanism probably operates in cell survival, i.e. the longest starting cells are selected for survival. They argue that otherwise, in instances where the rate of anticlinal division becomes accelerated in the cambium, a continued high level of anticlinal division would result in very short vascular elements. Bannan and Bayly (1956) believe that rays are the source of substances, e.g. carbohydrates, which are required for the sustenance, growth, and division of fusiform initials. They believe that food from rays is specifically involved in processes such as wall construction and maintenance of turgor pressure.

However, Hejnowicz (1961) counters with "selection is not the only motive for elimination of fusiform initials." His results indicate that failure plays a part in accelerating the rate of intrusive growth and of anticlinal divisions! Hejnowicz (1961) concluded that "... the elimination of the fusiform initials constitutes the mechanism accelerating the rates of the changes taking place in the cambium." And, in 1968, Hejnowicz noted that in instances of fast progressing changes in slope of grain, the failing cells occurred in "groups", while other groups of cells were dividing and elongating.

Most failing cambial cells become reduced both in length and in tangential width (Whalley 1950, Bannan and Bayly 1956, Hejnowicz 1961), accompanied by pseudotransverse divisions to the first or farther orders. Hejnowicz (1961) reported that shorter pseudotransverse anticlinal divisions were found to occur in groups of fusiform initials undergoing elimination, whereas longer pseudotransverse anticlinal divisions were found to occur in single initials which would, presumably, survive and repeat the cycle. Bannan (1951) has speculated that failing cells are stimulated to divide because of an altered physiological condition.

Reorientation of the cambium

It is noteworthy that in 1961, Hejnowicz made the following statement (p. 746) based upon a serial transverse sectional study of the vascular cambium of a species of Larix:

> The following hypotheses have been advanced: 1) the one-directional character of pseudotransverse divisions is an important factor of the mechanism controlling the formation of the spiral arrangement of cambium cells and thus also of the spiral grain in wood; and 2) the rate of the structural transformations of the cambium, e.g. the rate at which the spiral arrangement of the cells is formed, is controlled by the intensity of the processes in which the fusiform initials are eliminated.

Later work by Hejnowicz (1964) resulted in the introduction of the term "domain" into the vascular cambium literature. This term was used to designate a region of the cambium where anticlinal divisions, but not necessarily cambial initials (Hejnowicz 1971), were tilted in the same direction. It was shown that the cambial domains differ in areal extent and that the domain pattern changes with time. Limitations on domain size (areal extent) have not been defined, but the implication from the literature is that they are macroscopic regions (Bannan 1966, Hejnowicz 1964, 1971).

In 1966, Bannan summarized existing knowledge on spiral grain formation in conifers, as well as reporting on his own research. Bannan (1966) found there to be a general conformity in the orientation of the partitions in the pseudotransverse division of fusiform initials throughout "sectors" (inferred to mean "domains" of Hejnowicz, 1964) of varying size. The proportion of divisions deviating from the preferred orientation within a sector was usually less than 10%, but this value varied with the tree, the locality, and the species. Bannan (1966) also found that periodic reversals occurred in the orientation of anticlinal divisions, in terms of radial accretion, and that the spacing between reversals, again in terms of radial accretion, was related to the frequency of anticlinal division. These reversals, if balanced, kept the cambium oriented axially, and thus the grain also was axially oriented. If one direction of orientation of anticlinal divisions predominated over the other, spiral grain resulted. Bannan (1966) stated that both orientation of oblique anticlinal divisions and the direction of cell elongation must be under a general polar control, and went on to state the following (p. 1531):

> It may be deduced that the anticipated positive effect of high rates of pseudotransverse division on spirality is partially offset by the associated increase in cambial cell loss. As noted earlier, many of the cell configurations initiated in pseudotransverse division are not maintained. This applies particularly to the overlap between newly formed sister initials. On the other hand, the heightened rate of addition of new initials and loss of others, which accompany high rates of multiplicative division, result in more numerous rearrangements between lineal series which have no recent familial relationship. In these rearrangements the new positions of cell tips tend to conform to the orientation of pseudotransverse divisions in the same sector. Thus counteracting circumstances are operating with reference to the effect on cell alignment. The net result of high rates of pseudotransverse division on slope of grain remains to be determined.

Bannan stated further (1966, p. 1532):

> In the shifting of positions and altering of contacts which accompany the introduction of new cells and loss of others, there is often no perceptible change in basic cell slant with reference to the axis. However, if a substantial net gain of initials were to develop in a cambial sector, with aggravation of space competition, then under such circumstances a general deflection from the vertical might occur.

From the above statements, it is clear that Bannan (1966) did not agree with the second part of the hypothesis of Hejnowicz (1961) as quoted previously (page 11 herein).

Bannan (1966) noted occasions where the rate of unidirectional anticlinal division did not match the deflection in grain. Bannan (1966, p. 1535) therefore stated:

> Not only is there often a lack of agreement between the rate of anticlinal division and alteration in slope of grain, but sometimes the orientation of division and alteration in spirality are not in accord. Such seeming incongruities lead one to believe that the role of multiplicative division and subsequent cell elongation in the development of spiral grain, as proposed by various authors during the past several decades, has been oversimplified. Spiral grain does not appear to be due to a simple cause. Conformity in orientation of anticlinal divisions and direction of cell elongation over a major part of the cambium would doubtless induce the formation of a slanted alignment of vascular elements. A substantial net gain of fusiform initials in cambial sectors, with consequent intensification of spatial competition, might increase the deflection from the vertical. The deflection in such cases would presumably be in the direction of orientation of the partition in pseudotransverse division and resulting cell overlap. However, acting against the development of an excessive spirality are periodic reversals in the orientation of anticlinal division. Another check is provided by the extensive cambial cell loss which is an accompaniment of circumferential expansion. Because of this loss, the cell configurations, including cell overlaps, which are instituted in anticlinal division, are not necessarily maintained.

> Polarity appears to be an important element in cell alignment, having influence on both the orientation of pseudotransverse division and the direction of growth of the cell tips. In most trees a delicate balance apparently exists between events favoring the development of spirality, and controls which serve to restrict the deflection to a low degree. Excessive spirality presumably arises when these controls are rendered inoperative over a major part of the cambium.

Bannan's research was followed by that of Harris (1967, 1969, 1973) and Hejnowicz (1967, 1968, 1971). Harris (1967, 1969) has reported on the results obtained by inducing spiral grain formation in radiata pine. The method used by Harris had been used by numerous other researchers (Büsgen and Münch 1929, Collins 1930, Kirschner et al. 1971, Fahn 1973, MacDaniels and Curtis 1930, Tupper-Carey 1930, Teodoresco and Popesco 1915). Harris removed narrow spiral, or helical, strips of bark and cambium from stem internodes of young radiata pine trees, thereby forcing a spiralling downward movement of photosynthetic products through the phloem tissue. The trees were then left to grow for 8 months without further disturbance other than the occasional removal of callus tissue to prevent the grooves from closing. After 8 months, the bark and cambium between the grooves was peeled away; and it was observed that the grain, or xylem, produced adjacent to and immediately above the grooves had reoriented itself parallel to the spiral grooves. Thus, a marked degree of localized spiral grain had been induced in a very short time.

A microscopic examination of tangential sections of this xylem left Harris (1967, 1969) unconvinced that spiral grain results from unidirectional pseudotransverse anticlinal divisions followed by oriented overlap of the tips during intrusive elongation. Harris reported the occurrence of a nearly 1:1 ratio of divisions slanted to left and slanted to right. (However, Harris (1973, p. 375) states that these results were likely incorrect since the ratio of left to right anticlinal divisions was determined from a

wider radial region than that in which the reorientation occurred).

Harris (1967, 1969) concluded that pseudotransverse divisions were of secondary importance in cambial reorientation, and that they may contribute at most only to the overall pattern of spiral grain formation. The magnitude of grain-angle change, and the small concommitant anatomical changes which Harris observed, led him to state that the cambial layer as a whole must be capable of "radical plastic deformation."

In a preliminary report on spiral grain development in conifers, Hejnowicz (1967) stated that the direction of overlap in elongating cells following pseudotransverse divisions had been found to be non-preferential for either side; nevertheless, the pseudotransverse divisions had been found to be mostly unidirectionally slanted. In 1968, Hejnowicz corrected this statement (p. 363) and stated that both pseudotransverse divisions and overlap were oriented during spiral grain formation in a manner to facilitate the change.

In his 1968 study, Hejnowicz used spiral-grained material (apparently naturally produced) from Pinus silvestris L. trees. One of his samples changed from the axial orientation to more than 50° slanted upward to the left in a radial distance of approximately 3 millimeters (this specimen also changed from zero to 25° during the production of 1 millimeter of xylem). A second sample of Scotch pine changed approximately 20°, to be slanted upward to the right, in approximately 3 millimeters of radial distance. Hejnowicz (1968) has shown clearly that immediately preceding and during the change in grain angle, the pseudotransverse anticlinal divisions show a large increase in frequency; and that all of these divisions are oriented in the direction of the final alignment.

As a result of this research, Hejnowicz (1968, p. 351) stated:

> The events in the cambium underlying the change in the slope of grain in the investigated specimens were: pseudotransverse divisions, intrusive elongation, cell loss and changes of mean cell length. The fusiform rays rotated during the change of grain about their centres, however, the relative position of their centres remained constant. The narrow rays rotated as well keeping relative positions, however, during more abrupt changes of grain or between more distant sections they were split by intrusively growing tips of fusiform initials, or were joined together.

Hejnowicz (1968, p. 354) continues:

> The alteration rate was not linearly proportional to the rate of unidirectional divisions, but increased faster than the latter. Apparently the effect of unidirectional division was augmented by intrusive elongation and cell loss.

On page 356, Hejnowicz (1968) states:

> The rate of elongation varied widely from cell to cell during the formation of a thin layer of wood. Some cells elongated very much while others remained unaffected. Very often the fast-growing cells occurred in clusters.

Finally, Hejnowicz (1968, p. 360) considered the role of failure of fusiform initials:

> Usually the failure of fusiform initial started with a shortening of the initial from one or from both ends. It was observed that in the instances of fast progressing change in the slope of grain the failing cells occurred in groups while other groups of cells were dividing and elongating. ... the total length of cells lost during the production of a mm of xylem was about 200% of the total length of the cells in the given sector of cambium during the fastest changes in the cambium. This made possible a considerable rebuilding of this tissue. No wonder that the grain could then change by about 20°.

Hejnowicz (1971) went on to report on Picea excelsa Link. cambium producing wavy grain. He found that during the production of a particular layer of xylem there were adjacent areas in which cell orientation was changing at a given moment and others in which at the same time no change was noticeable. His studies of serial tangential sections of the xylem showed the anatomical basis for the changing of cell orientation to involve three processes:

1. Oblique anticlinal divisions in fusiform initials;

2. Overlapping of oppositely directed tips of initials, lapping of the tips over the rays, and splitting of the rays, all by intrusive growth of initial tips; and

3. Elimination of certain initials from the cambium following divisions, with intrusive growth of persisting initials.

An important finding of Hejnowicz (1971, p. 509) was stated as follows:

> The similarity of the domain pattern and the pattern of areas of reorientation indicates a causal relationship between the two patterns. However, the domain pattern is known to occur also in cambium which produces straight xylem, i.e. which does not show any reorientation of its cells. The domain structure occurred in the neutral areas of the wavy cambium also but the cambium did not reorient its cells, ...

To explain the lack of reorientation in such neutral, or axially oriented, regions, Hejnowicz (1971) examined frequency of anticlinal divisions, rates of intrusive growth, and the mean lengths of fusiform cells. The wavy cambium was found to differ from the nearby neutral cambium in having lower mean length of fusiform initials and in showing both a high frequency of anticlinal divisions and a high rate of intrusive growth. But though in previous publications (1961, 1968) Hejnowicz has emphasized the role that failure plays in intrusive elongation and reorientation, he reports nothing on the failure rate between the wavy and neutral cambia. It is inferred that his findings of high frequency of anticlinal divisions and high rates of intrusive growth imply that the failure rate was also high.

Harris (1973) has reported on a second spiral-girdling investigation into young radiata pine. This investigation was similar to his previous studies (Harris 1967, 1969) except that trees were sampled three, six and nine weeks after girdling, and finally toward the end of the growing

season. Following these periodic harvests, the cambium was examined under the light and electron microscopes; and serial tangential sections of xylem were prepared to examine the directions and frequency of pseudotransverse anticlinal divisions.

Three weeks after girdling, Harris (1973) found that the grain had changed from 5° upward to the left to 4° upward to the right (girdle orientation was 35° upward to the right) in xylem adjacent to the upper edge of the girdle. Six weeks after girdling, the grain angle in the region immediately above the girdle was 18° to the right. By week 9, the newly-formed xylem immediately above the girdle had oriented 38° to the right. Over the nine-week period, the xylem formed axially immediately below the girdle had changed its orientation very little. Reorientation occurred for the most part only over a distance of 20 millimeters axially above and adjacent to the upper side of the spiral girdle; i.e. on the lower side of the wide phloem-cambium bridge.

Harris (1973, p.376) states that the grain angle was found to be changing most rapidly about 6 weeks after girdling; therefore all of the observations of pseudotransverse divisions and most of the electron microscopy were concentrated on tissue taken from this time of harvesting. It is not clear how he determined this period to be the time when the grain angle was changing most rapidly.

Harris noted that the fusiform initials changed their orientation more quickly than the rays; fusiform rays lagging behind biseriate and uniseriate rays in rate of reorientation. Harris (1973, p. 368-369) therefore states:

> The fact that the two major components of cambium--ray initials and fusiform initials--respond "independently" when grain angle changes is striking evidence of the plasticity of cambium and of its capacity to adapt to major changes without total disruption. Closer examination of the manner in which this adaptation occurs

> provides evidence that fusiform initials are the dynamic component of reorientating cambium, and that they have to overcome considerable inertia on the part of the larger groups of ray initials. ... the complexities of cell shape and orientation made it difficult to assess rate of realignment visually or to obtain clear evidence as to how it came about.

Harris (1973) found that, particularly at week six, there was a tendency for tips of fusiform initials to assume a greater angle to the stem axis than the main body of the initial. He therefore stated (p. 370):

> During the most active period of reorientation a picture is built up of a cell with distinctly sigmoid curvature, caused by cell tips adjusting more rapidly to the new alignment than the central region of the cell. But in week 9, as soon as reorientation is completed and the fusiform initials lie parallel to the girdle, this distinction is lost.

Harris (1973, p. 374) concluded:

> Reorientation is, therefore, envisaged as occurring through differential growth of the cell tips of fusiform initials, with partial adjustments of the cell axis after each periclinal division.

Although Harris (1973) found that pseudotransverse divisions favoring the new orientation predominated over those which did not in a ratio of 4:1, and that anticlinal divisions occurred at the rate of 5.9 per centimeter of radial xylem growth, he states that the observed rate of anticlinal division in the girdled stems could account for a change in grain angle of no more than 0.2° per millimeter of radial growth. (However, Harris overlooks the fact that the initials have shortened in length as a result of the anticlinal divisions in his calculation of the rate of change.) Harris (1973) observed changes as high as 10° per millimeter of radial growth of xylem. As a result, Harris (1973) believes that, though the orientation of pseudotransverse divisions must be in favor of a developing spirality, they are symptomatic of stresses arising within the cell during differential growth of the cell tips, rather

than being a primary cause of realignment.

Although numerous researchers have employed the technique of studying serial tangential sections of xylary elements to deduce cambial activity in spiral grain formation, none of them has presented a diagrammatic series of successive xylary elements within the same radial file to show the reorientation process as they see it under the microscope.

The wound response

Harris (1967, 1969, 1973) has given no report of the effect, if any, that wounding during spiral girdling had upon the reorientation of cambial initials. His control was unwounded cambial tissue.

Wilson (1968) investigated the effect of ring girdling on cambial activity in Pinus strobus L. However, none of the samples which Wilson investigated were closer than 2 centimeters to the wound edge. Nine trees were sampled throughout an extended period; and the effects of girdling at different times of the season were found to be essentially the same. At 2 centimeters above girdles, cell length was found to decrease to about half the original length by pseudotransverse and lateral divisions. At 2 centimeters below the girdle, cell length remained fairly constant (cell division and cell enlargement stopped within a few weeks). Number of cells within the cambial zone, across a radial file, remained nearly constant both 2 centimeters above and below the girdle. However, the rate of mitosis increased sharply 2 centimeters above the girdle and stopped entirely 2 centimeters below the girdle. In the phloem produced after girdling, the tannin-filled parenchyma that normally form bands were found to be scattered throughout the tissue.

Very little further detailed anatomical information on the response of conifers to wounding appears to be in the recent literature. Bannan (1933, 1934) reported two studies on wounding in Tsuga, a genus which

normally does not form resin canals (Panshin and de Zeeuw, 1970). Bannan (1933) states that the tissue in the immediate vicinity of the wound in Tsuga canadensis (L.) Carr was abnormally hypertrophied. Further above and below the wound, the response to wounding was seen as a tangential sheet of parenchymatous tissue within which were traumatic resin "cysts" (or traumatic resin "canals" of Panshin and de Zeeuw, 1970).

In a further study on the same species, Bannan (1934) found that few cysts formed if wounding was done in April or May, and that the greatest response occurred when wounding to stems was done in June and August. Wounding in August was also found to stimulate the cambium to further activity, whereas in unwounded tissue the cambium had ceased activity at that time.

Bannan (1957) states that vertical resin ducts (or canals) apparently originate among the periclinally-dividing xylem mother cells. This was deduced by Bannan from his observation that some of the cross walls in the "septate tracheids ensheathing the ducts" (p. 878) were at the same level in successive elements, whereas other cross walls were at different levels in successive elements.

In concluding this Introduction, it is emphasized that neither of past investigations on natural or induced spiral-grain formation has clearly elucidated the cambial reorientation process. Although the technique of spiral girdling makes possible a rapid and controllable approach to the study of the mechanism of cambial reorientation in conifers, the use of this technique to date has resulted in nothing more than controversial findings when contrasted with other studies of the mechanism of cambial cell realignment in natural spiral-grain formation. The results of the investigations reported on herein provide further insight into the reorientation process.

METHODS AND MATERIALS

Investigation 1 -- Pinus strobus L.

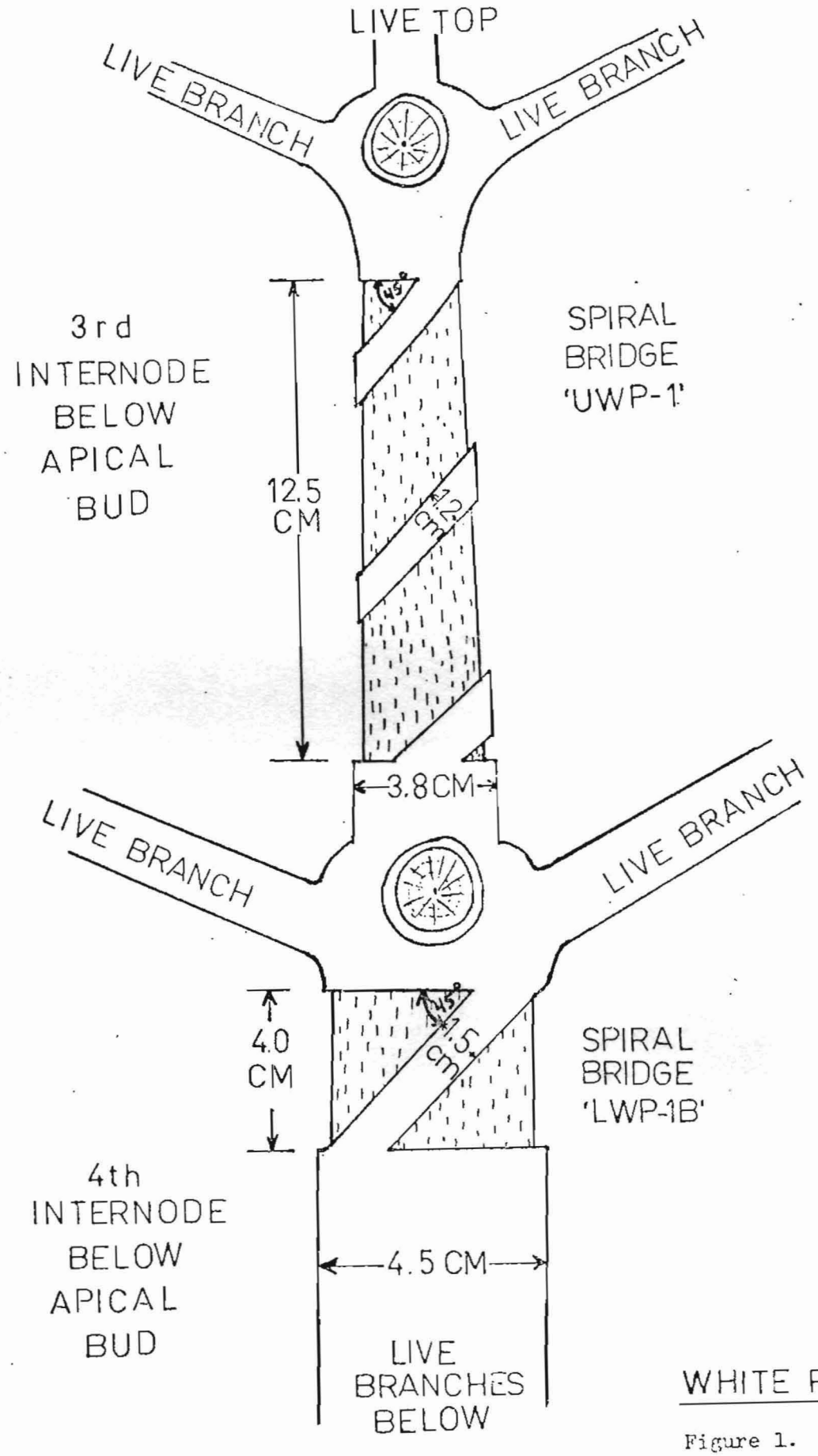
A 9-year-old, 12-foot-high, vigorously growing and regularly watered and fertilized white pine tree was selected for spiral girdling on April 26, 1975. The location of the tree was at the Glendon Hall Research Nursery of the Faculty of Forestry, University of Toronto, within Metropolitan Toronto. This tree is referred to as white pine number one, or tree WP-1.

Tree WP-1 was spirally girdled as shown in Figure 1 on April 26. All the bridges crossing the girdles on tree WP-1 are termed "spiral bridges." The upper spiral bridge, in the third internode from the apex, is termed UWP-1. Two shorter spiral bridges were established in the fourth internode from the apex at the same height in the stem, i.e. 180° to one another; these two spiral bridges are referred to as LWP-1A and LWP-1B. Only LWP-1B, of these two, will be reported on herein.

No further treatment was given tree WP-1 throughout the growing season following the spiral girdling. Measurements were made of the dimensions of the bridges at the time of girdling.

On September 29, 1975, the three spiral bridges of tree WP-1 were remeasured for dimensional changes. Following this, the two internodes containing the bridges were removed from the tree with a handsaw. The upper ends of these internodes were labelled with indelible pencil, and the tissues were immediately fixed in FAA (10% formalin, 50% ethanol, 5% acetic acid, 35% water).

Following two weeks fixation in FAA, the fourth internode, containing spiral bridges LWP-1A and LWP-1B, was split into median longitudinal halves, thus separating the two bridges, and these labelled appropriately.



WHITE PINE

Figure 1.

A small bandsaw was used to subdivide each of the three spiral bridges into pieces suitable for microtoming. The locations of these pieces relative to the overall spiral bridges from which they came are shown in Figures 2 and 3.

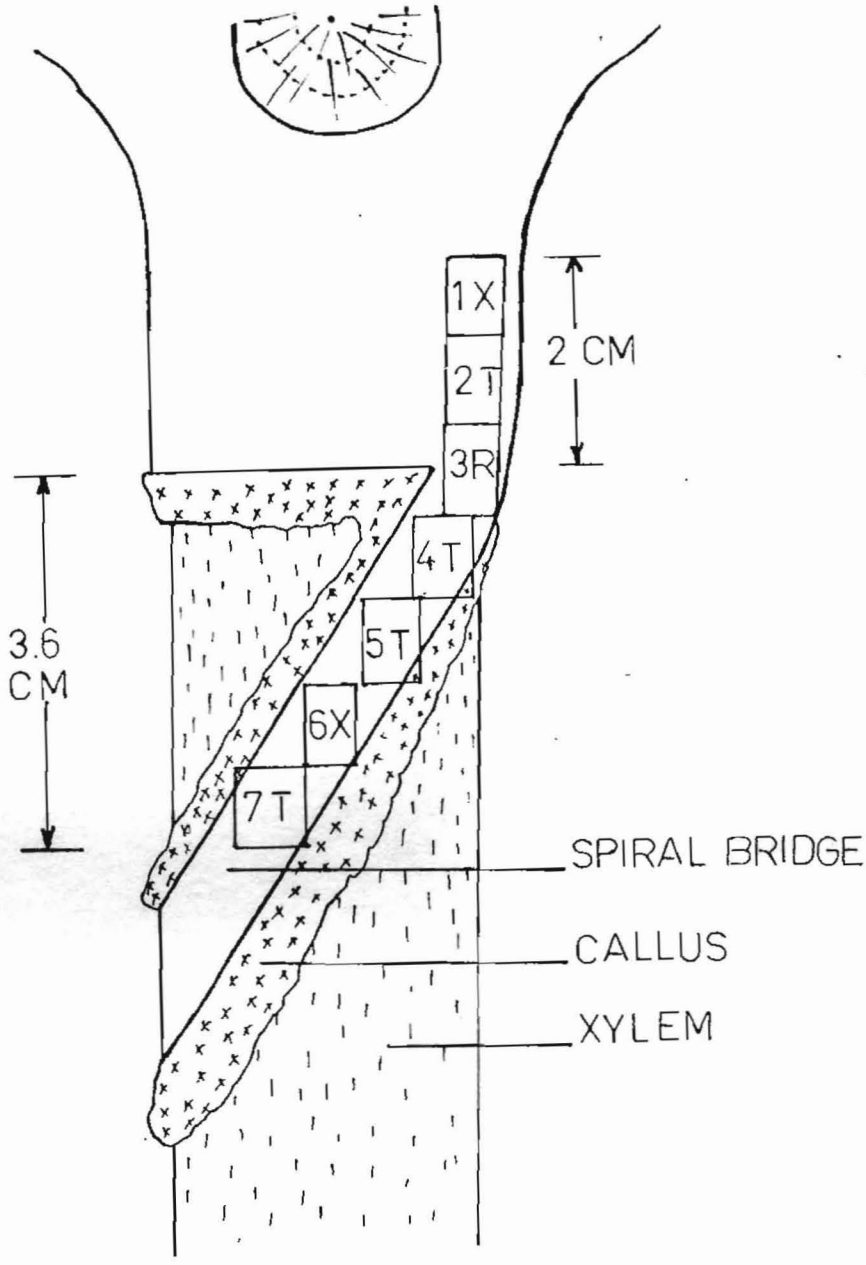
The pieces from each bridge which were selected for microtoming were put through a dehydration and infiltration procedure following the schedule set out in Table 1, and then were embedded into high-purity paraffin (Paraplast®). After embedding, softening of the tissues was done with Mollifex® for a minimum period of two weeks.

Sectioning was done on a rotary microtome using steel knives. Material designed to show radial or transverse faces was sectioned without regard to order; but material designed to show tangential faces was serially sectioned. A section thickness of 20 micrometers was used for all sections, for all investigations.

Sectioned material was transferred to a few drops of 3% formalin in water on labelled, precleaned glass slides; and the glass slides were then warmed on a variable temperature slide warmer to expand the wax and affix the section to the slide. Glass slides with sections were then drained of excess liquid and placed in racks to dry.

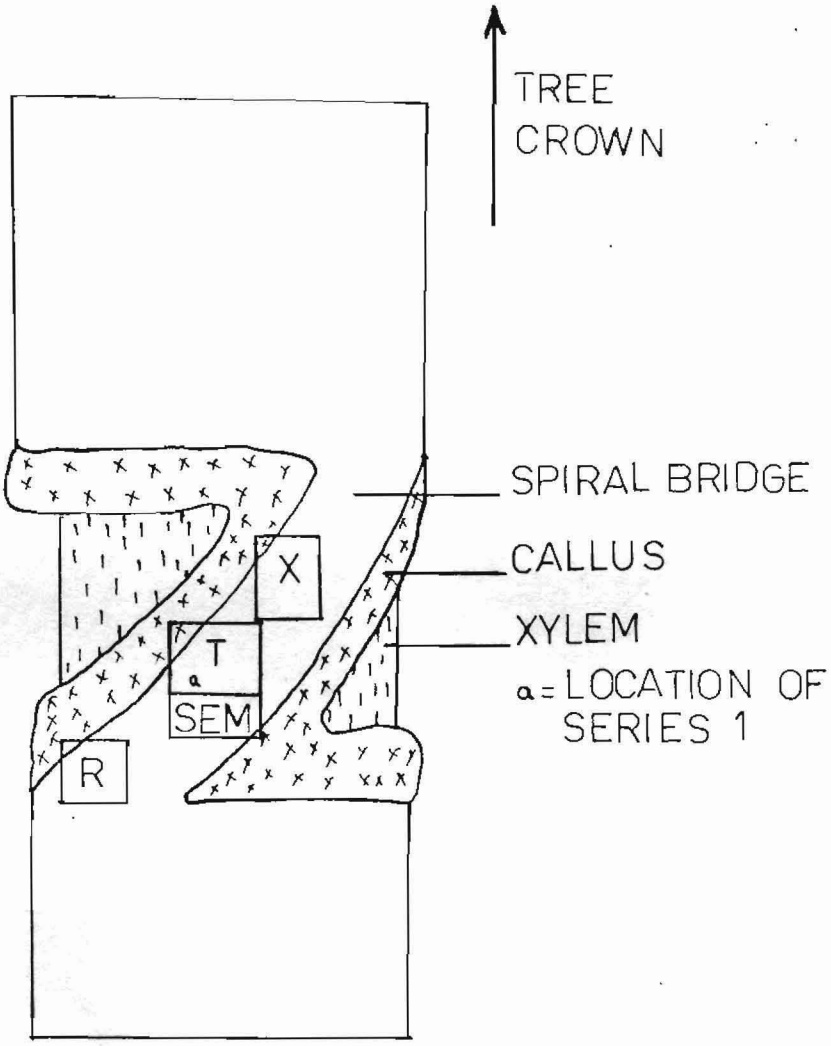
As an added precaution, to ensure that the correct sequence was maintained in the serial tangential sections during the staining process, every tangential section on every slide was tied on with cotton sewing thread. Once tied, the slides were returned to their racks and allowed to air dry for 2 days before staining.

When drying was complete, the tied sections were stained with safranin O and fast green FCF, following the schedule of Table 2. Stained sections were immediately mounted, using Permount®, to make permanent slides. The mounting procedure involved slicing across the thread on



UPPER PORTION OF
SPIRAL BRIDGE UWP-1
SHOWING LOCATIONS OF MICROSCOPIC
EXAMINATIONS ON TRANSVERSE (X),
RADIAL (R), AND TANGENTIAL (T) SECTIONS

Figure 2.



SPIRAL BRIDGE LWP-1B
SHOWING LOCATIONS OF MICROSCOPIC
EXAMINATIONS: TRANSVERSE (X),
TANGENTIAL (T), AND RADIAL (R)
USING THE LIGHT MICROSCOPE;
AND TRANSVERSE AND RADIAL FACES
USING SCANNING ELECTRON MICROSCOPY
(ACTUAL SIZE)

Figure 3.

TABLE 1

DEHYDRATION AND INFILTRATION SCHEDULE PRECEDING PARAFFIN EMBEDDING

	SOLUTION	TIME (hours)
1.	30% water and 50% absolute ethanol and 20% tert-butyl alcohol	12
2.	15% water and 50% ethanol (absolute) and 35% tert-butyl alcohol	1
3.	45% absolute ethanol and 55% tert-butyl alcohol	1
4.	25% absolute ethanol and 75% tert-butyl alcohol	1
5.	100% tert-butyl alcohol	1, 1*, 12*
6.	50% tert-butyl alcohol and 50% paraffin oil	1
7.	12.5% tert-butyl alcohol and 12.5% paraffin oil and 75% parowax (60°C)	1
8.	100% parowax (60°C)	24
9.	100% embedding paraffin (60°C)	24, 24*

* Replicate times

TABLE 2

SAFRANIN O - FAST GREEN FCF STAINING SCHEDULE

	SOLUTION	TIME
1.	Xylene	5 min.
2.	50% ethanol and 50% xylene	5 min.
3.	Ethanol (absolute)	5 min.
4.	85% ethanol and 15% water	5 min.
5.	Safranin (1%) and 70% ethanol	1 h.
6.	85% ethanol and 15% water	dip
7.	85% ethanol and 15% water	dip
8.	0.5% fast green and 95% ethanol	5 sec.
9.	Ethanol (absolute)	dip
10.	Ethanol (absolute)	dip
11.	50% ethanol and 50% xylene	5 min.
12.	Xylene	5 min.
	Ready to mount	

the slide with an industrial razor blade, pulling the cut thread away with the aid of a teasing needle, applying a couple drops of mounting medium, followed by the coverslip. Mounted slides were allowed to dry for a few days under lamplight, and then were ready for microscopic examination.

Investigation 2 -- Ring-girdled Pinus strobus L.

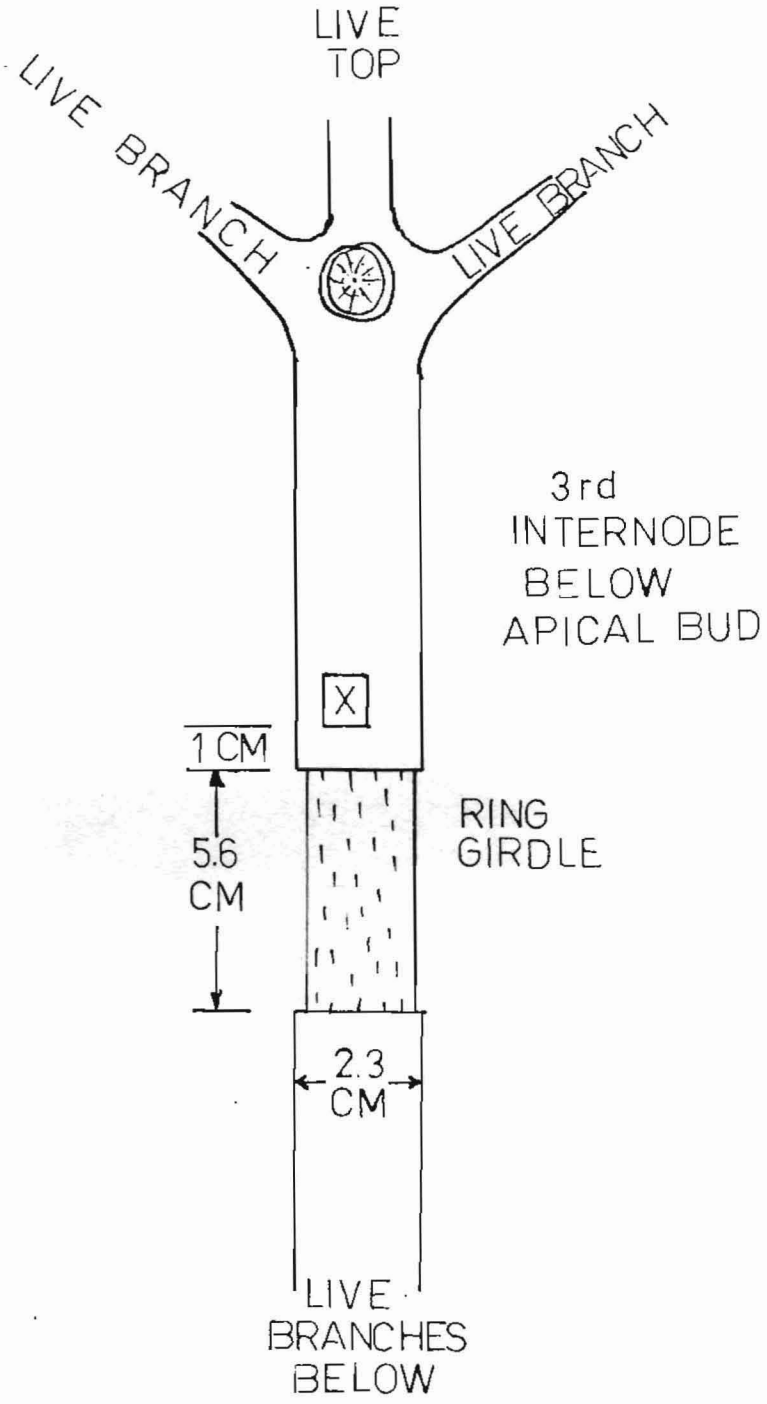
On June 20, 1975, a 7-year-old, 8-foot-tall, vigorously growing and regularly watered and fertilized white pine tree which was growing at the same Toronto site as that described in Investigation 1 was ring girdled in the third internode from the apex, as shown in Figure 4. Nothing further was done to the tree until it was harvested and fixed in FAA on October 5, 1975.

Transverse sections were prepared from a region 1 to 2 cm above the girdle's upper edge, as shown in Figure 4. Preparation of the sections was done by Mr. A. Mosseler (FOR 351, Tree Physiology Report, April 1976; Faculty of Forestry, University of Toronto) and was identical to that described in Investigation 1.

Investigation 3 -- Pinus contorta Dougl.

The author spent the summer of 1976 at Quesnel, British Columbia, and did work on another species of Pinus at that location. On July 8, 1976, at a location 4 miles southwest of the town of Quesnel, 18 open-growing, vigorous, naturally-regenerated lodgepole pine saplings, ranging in age between 10 to 20 years and in height between 4 to 7 meters, were girdled as shown in Figures 5, 6 and 7. The elevation of the site was approximately 760 meters above sea level; soil was moist clay overlying approximately 200 feet of glacial till.

Seventeen of the lodgepole pine trees were girdled in two internodes. The upper internode in every case was given a 45° diagonal bridge traversing the girdle from lower left to upper right. In the next lower internode,



LOCATION OF TRANSVERSE SECTIONS (X) EXAMINED IN RING-GIRDLED WHITE PINE

Figure 4.

DIAGONALLY GIRDLED LODGEPOLE PINE INTERNODE

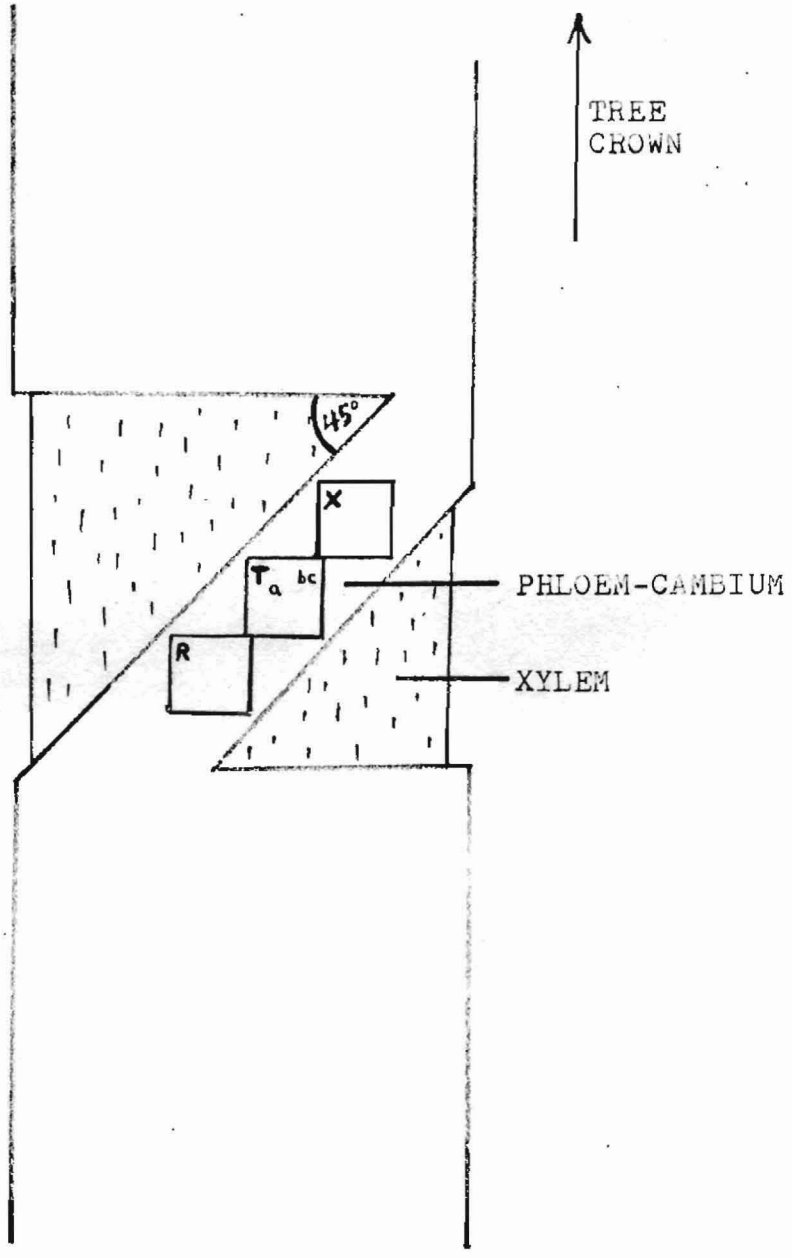


Diagram (X1) of typical lodgepole pine diagonal bridge, showing actual size and location of samples taken from the bridge for microscopic examination. X=transverse sections; T=serial tangential sections; R=radial sections. a, b, and c represent locations of Series 5, 2, and 3, respectively (these Series are in the Appendix of this Thesis), of a bridge harvested 50 days following girdling.

Figure 5.

VERTICALLY BRIDGED LODGEPOLE PINE GIRDLE

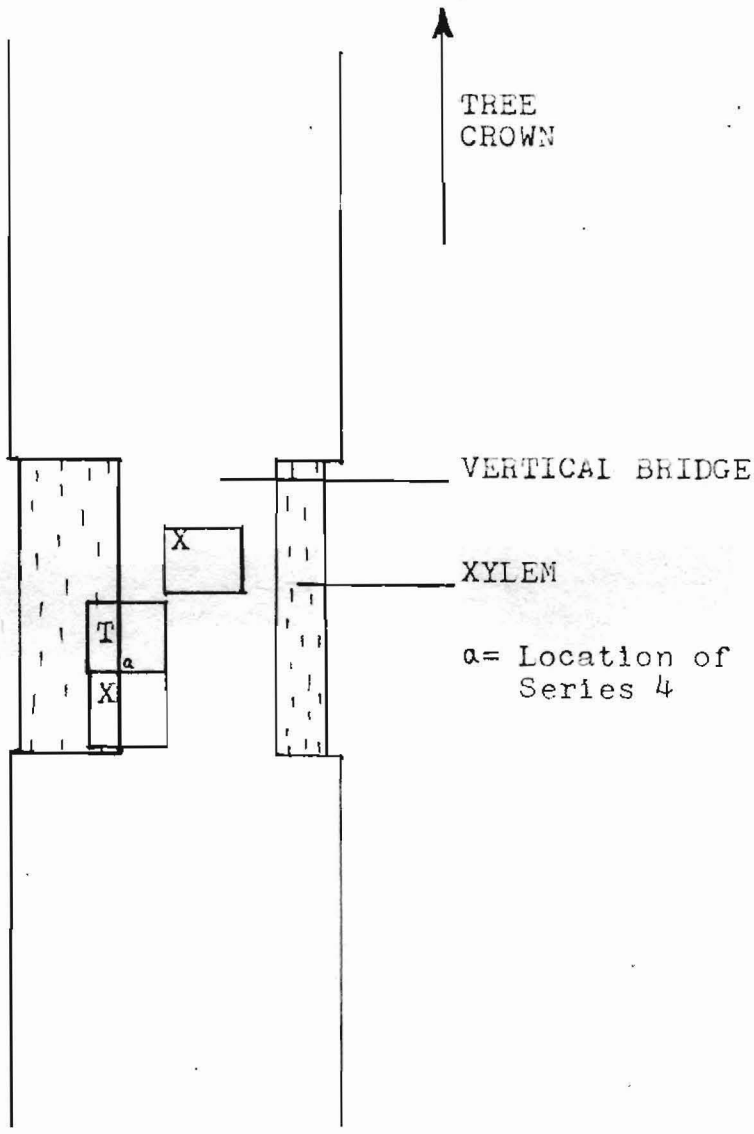


Diagram (X1) of typical lodgepole pine vertical bridge, showing actual size and location of samples taken from the bridge for transverse (X) and serial tangential (T) microscopic examination. Series 4 was traced through a tangential series of a bridge harvested 38 days following girdling.

Figure 6

an axially oriented (i.e. vertical) bridge was established as a control for the wound response (Fig. 7). The eighteenth tree was only diagonally girdled; i.e. no vertical bridge was established in the next lower internode.

(Previous studies on spirally-girdled white pine by the author had indicated that the reorientation process in the cambium was complete after three weeks; but some doubt remained as to the exact time required for reorientation to occur. As well, the white-pine study pointed out the need to separate the wound response from the reorientation response (Savidge 1976--unpublished data). Thus it was decided to do a time study of the reorientation process with controls for the wound response.)

At the time of girdling, the cambial zone of all trees was still moist, but resin secretion in response to the wounding was very slight. The girdling of all 18 trees was completed in two hours' time. Nothing further was done except to harvest the trees.

The first tree was harvested after 2 days, using a handsaw. The girdled diagonal bridge and the vertical bridge were appropriately labelled with indelible pencil on the upper cut surface and then immediately immersed in FAA. The same technique was used to harvest the bridges of all successive trees. Harvesting and fixing dates are set out in Table 4 (p. 35).

Tables 3 and 4 set out the characteristics of the girdled lodgepole pine trees, including (Table 3) the number of days which passed between girdling and harvesting of the girdled internodes, the position and age of the internodes, the length and width of the bridges, and (Table 4) the diameter of the internodes (inside bark) after girdling, the average number of tracheids formed the summer previous to girdling (i.e. during 1975), the average number of cells formed during 1976 up to the time of girdling, and the date and time of harvesting and fixing.

Cell counts per radial file, as shown in Table 4 (see also Table 6, p. 52) represent means of 10 selected files. Dashed areas represent material on

CHARACTERISTICS OF THE GIRDLED LODGEPOLE PINE TREES

No. of Days Between Girdling and Harvesting	Number of Internodes From Top		Age of Bridge Internode		Length of Bridges (cm)		Width of Bridges (cm)	
	45° Diagonal Bridge	Vertical Bridge	45° Diagonal Bridge	Vertical Bridge	45° Diagonal Bridge	Vertical Bridge	45° Diagonal Bridge	Vertical Bridge
2	7	8	7	8	6.0	4.7	1.9	1.9
4	6	7	6	7	8.5	4.2	2.0	1.8
6	5	6	5	6	8.0	6.7	2.0	2.1
8	6	7	6	7	9.0	6.0	2.0	2.1
10	5	6	5	6	8.5	4.5	2.2	2.2
12	4	5	4	5	6.5	5.8	1.9	1.8
14	3	4	3	4	6.0	4.5	2.1	1.9
16	4	5	4	5	7.5	5.0	2.3	2.0
18	3	4	3	4	8.0	4.0	2.2	1.8
20	4	5	4	5	9.0	8.0	2.1	2.1
22	3	4	3	4	7.0	5.0	2.3	2.0
24	4	5	4	5	8.0	4.5	2.3	2.3
26	4	5	4	5	8.0	5.0	2.0	2.1
28	3	4	3	4	11.0	4.5	2.5	2.0
30	3	4	3	4	7.0	5.0	2.0	2.0
32	3	4	3	4	7.0	5.0	2.0	2.4
38	4	5	4	5	7.0	4.0	2.3	2.3
50	8		8		13.0		2.0	

TABLE 3

TABLE 4

CHARACTERISTICS OF THE GIRDLED LODGEPOLE PINE TREES

No. of Days Between Girdling and Harvesting	Diameter of Internodes Inside Bark (cm)		Average Number of Cells in 1975 Radial Files		Average Number of Cells per 1976 Radial File Formed Previous to Girdling		Date and Time of Harvesting and Fixing (Date of Girdling was July 8, 1976, from 4 to 6 p.m.)
	45° Diagonal Bridge	Vertical Bridge	45° Diagonal Bridge	Vertical Bridge	45° Diagonal Bridge	Vertical Bridge	
2	5.0	5.8	117	122	---	---	July 10, 7:00 p.m.
4	5.0	5.6	179	165	---	---	July 12, 8:00 p.m.
6	4.5	4.8	125	120	---	---	July 14, 6:00 p.m.
8	6.2	6.7	158	---	---	---	July 16, 8:00 p.m.
10	7.2	7.6	121	---	---	---	July 18, 7:00 p.m.
12	3.9	4.8	148	114	81	---	July 20, 7:00 p.m.
14	3.6	4.2	111	134	67	80	July 22, 7:00 p.m.
16	4.7	5.6	171	---	138	133	July 24, 7:00 p.m.
18	3.1	3.9	184	148	104	123	July 26, 9:00 p.m.
20	4.2	5.5	---	---	183	221	July 28, 7:00 p.m.
22	4.0	4.8	---	160	158	129	July 30, 6:00 p.m.
24	5.1	6.3	178	---	130	137	Aug. 1, 7:00 p.m.
26	4.8	5.7	127	---	100	98	Aug. 3, 8:00 p.m.
28	3.2	3.9	166	141	103	91	Aug. 5, 7:00 p.m.
30	3.3	3.9	200	178	130	122	Aug. 7, 6:00 p.m.
32	3.2	3.8	189	175	99	117	Aug. 9, 6:00 p.m.
38	4.1	5.3	175	---	106	123	Aug. 15, 6:00 p.m.
50	8.3		184		170		Aug. 29, 7:00 p.m.

which counts could not be made. In the case of Table 4, some counts were not possible because the 1974-75 growth boundary was not present in the transverse section. In Table 6, the wound zone (see Results, p. 42) could not be recognized until 12 days after girdling; therefore the number of tracheids formed after girdling during the first 10 days could not be established. Values which are missing in Table 4, i.e. the average number of cells formed previous to girdling, represent the same thing, since these values were determined by counting up to the beginning of the wound zone.

It will be noted from Tables 3 and 4 that a tree was harvested every second day until 32 days had elapsed and 16 of each of diagonal and vertical bridges had been harvested. During these first 32 days, hand sections were prepared and examined with the microscope in the field. After 32 days, no evidence for recrientation could be found. Therefore the time of the experiment was extended by harvesting the 17th tree on the 38th day after girdling, and the 18th tree on the 50th day after girdling.

All harvested material remained in FAA until September 1976. Samples from each bridge were then prepared for microscopic examination. The identical microtechnique was employed as has been described for Investigation 1. Transverse, radial, and serial tangential sections were prepared from the diagonal bridges. Only transverse and serial tangential sections were prepared from the vertical bridges (see Figures 5 and 6).

Other Methods and Materials

Figure 52 is a transverse section prepared as described in the microtechnique of Investigation 1 from a spirally-girdled stem of Ulmus americana L. growing at the same site as the white pine of Investigation 1. Figures 54, 55 and 56 are sections of xylem prepared using the same microtechnique from the tree (natural spiral grain) shown in Fig. 53.

Specimens from the white-pine spiral bridges described in Investigation 1 herein, and from branch and stem specimens of naturally-formed

spiral-grained wood of Picea glauca (Moench.) Voss from the tree shown in Figure 53 were prepared and examined under the scanning electron microscope. Transverse and radial surfaces were examined in the region where xylem showed rapid change in grain angle.

Preparation entailed air drying, followed by coating with a thin layer of gold transported to the xylem with argon gas (10-minute coating time). Xylem was the only tissue examined.

Transverse surfaces were prepared by slicing them smooth with a sharp razor blade before coating with gold. Slivers of reoriented bridgewood xylem were also partially submerged in 10% and higher concentrations of peracetic acid solution for variable periods of time, up to two weeks. The slivers were then pulled apart by tension using forceps, such that an irregular transverse surface was exposed. After drying, these transverse surfaces were gold coated and examined under the scanning electron microscope.

Radial surfaces were prepared simply by applying pressure with a dull scalpel to a transverse surface until the specimen split along natural radial planes of weakness. The radial surfaces were then gold coated and examined.

The primary objective of the SEM examinations was to determine whether tracheids were interdigitated and interwoven during the reorientation process. No substantial evidence for this was found in any of the samples.

Photographs

Photographs of the microscopic material examined in these investigations are shown in Figures 8 to 57. All photomicrographs shown were taken using Kodak 35 mm Panatomic X® black and white film. A yellow filter was used on a Reichert Zeto® Research Light Microscope. The same film was used on the Model SMSM "Super Mini-SEM"® scanning electron microscope.

Tracing radial files and deducing cambial activity

In Investigations 1 and 3, the activity of the cambial zone during the reorientation process was deduced using serial tangential sections of xylem. These serial tangential sections were followed, section by section, and the elements of interest traced onto paper with the aid of a camera lucida. Cells of interest were carefully centered in the image field in each case. Following completion of the tracing of a series, each tracing was carefully examined and compared once again with the ocular image of the section under high magnification.

The radial files, or series, which were traced are shown in the Appendix. In selecting each series, a xylary tracheid was selected in a section of tissue which had been formed spatially and temporally previous to girdling (i.e. bridge construction). All subsequent cells of each series were then followed from one tangential section to the next. Criteria governing the initial selection of a tracheid for a radial file study included the following:

1. Ease of identification, usually by the manner of association between tracheids and rays; and

2. continuity through all the serial tangential sections.

Lack of continuity of a file occurred frequently in regions of longitudinal traumatic resin canals, primarily as sectioning artifact. In such instances, the radial file being traced was abandoned and a new one selected.

As reorientation of the xylem progressed in the radial file studies of serial tangential sections, more and more tracheids were encountered within a unit area. The single starting tracheid, or tracheids, commonly multiplied to result in numerous shorter derivative tracheids. That is, the cambial cells producing the tracheids frequently multiplied by

lateral or oblique anticlinal divisions; thus, the derivatives of all of these cells had to be traced once encountered in the serial tangential sections.

The criteria used to determine that any one of the shorter tracheids had or had not derived from the same cambial cell derivative as that which had formed the original starting tracheid were purely, but rigorously, of an observational nature. For example, within a single radial file, true transverse divisions were frequently found at different levels in radially successive tracheids. Such activity was deduced to be xylem mother cell activity for two reasons:

1. If a transverse division had occurred in a fusiform initial, all derivatives from the two daughter initials should show the transverse dividing plate between them at the same level, or a gradual transition from that state; but neither of these were observed.

2. Transverse walls between 2 tracheids were found to abruptly cease in serial tangential sections, such that in a single fusiform tracheid similar or identical in all dimensions as one observed in the radial trace prior to encountering the transverse divisions was found.

In general, if in one section of a radial-file series the tracheid of interest was seen as a single, fusiform tracheid, and in the following tangential section there were found two tracheids in the same position, with the same ray contacts, and with the same, or similar, general cell wall outline as that in the previous single tracheid, and also with similar neighboring cells as in the previous tangential section, it would be deduced that a single cell in the cambial zone had divided anticlinally to form two cambial cells. This type of analysis was used for all series traced.

Although considerable time was devoted to accurate tracing and interpretation, admittedly there was an element of subjectivity associated with the tracings. This is true particularly in terms of ancestry decisions for a radial file. However, it will be seen by careful examination of the Series (Appendix) that the changes which occur are rarely so abrupt that there can be any doubt as to the correctness of the ancestry interpretation.

A section thickness of 20 microns was helpful in the interpretive respect; using this thickness, remnants of no more than three tracheids were ever observed in the tangential section width. By focussing up and down under high magnification, it was a fairly simple matter to decide which tracheid had already been seen in the previous tangential serial section, and thus to distinguish any changes which had occurred from one section to the next.

Two features were used to determine the extent of grain angle change from serial tangential section to section:

1. Relative position of ray centers (known from other investigators and from previous research to stay relatively constant); and

2. Ocular protractor measurements, using the section edge as a reference line.

Angle measurements, shown in Table 5, on serial tangential sections were made using an ocular protractor. The section edge was used as the axial reference line for the measurements. Possible measurements range from zero to 360° in the usual clockwise direction. Ray and tracheid angles represent tip to tip measurements. Error involved with the measurements is estimated at $\pm 2^{\circ}$.

RESULTS

Investigations 1 and 2 -- Spirally-girdled and ring-girdled Pinus strobus L.

At the end of the summer, at time of harvesting, the spiral bridges had their elements clearly oriented parallel to the direction of the spiral bridge from one side of the bridge to the other. Those xylary elements on the two edges of the bridge which had derived from callus tissue were also oriented parallel to the long axis of the bridges.

All of the spiral bridges of tree WP-1 (Figures 1, 2 and 3) grew rapidly and without any apparent infection or other problem. Callus tissue quickly healed the wounds along both edges of each spiral bridge. At the time of harvesting, the spiral bridges showed substantially more growth and more callus tissue formation on their lower sides, relative to their upper sides. In the ungirdled internode tissue above the entrance ("entrance" refers to the downward movement of photosynthetic products entering into the acropetal region of the bridge) to each bridge, there was a localized swelling of increased xylem and phloem formation. This localized swelling extended axially up the stem internode until the next branch whorl was intersected, then ceased. In the internode tissue below the exit of each bridge of tree WP-1, there was a lesser ridge of increased growth which extended axially down the tree to the next lower whorl of branches.

Like tree WP-1, the ring-girdled white pine tree (Figure 4) also showed rapid growth above the girdle, but this growth occurred around the entire periphery. There was no apparent infection. As expected, very little growth occurred below the ring girdle.

In microscopic examinations of transverse sections, the time of wounding (i.e. the location of the cambial zone at that time) in the ring-girdled white pine tree (Figure 4) was found to be clearly demarcated by a zone of cells which were heavily lignified, but of narrow radial dimensions.

Following formation of this "wound zone" (this term will be maintained throughout this report), during the remainder of the season's growth, numerous traumatic resin canals were differentiated into the xylem (Figure 8).

In contrast, microscopic examinations of transverse sections of xylem in tree WP-1 which had formed 2 centimeters above the entrance into the spiral bridge showed no wound zone, and traumatic resin canals did not differentiate throughout the season's growth (Figure 9). However, abnormally heavy lignification occurred continually throughout most of the season's growth in this region. It should be remembered in this contrast that tree WP-1 was spirally girdled almost two months before the ring-girdled white pine tree; and that the cambium of the spirally-girdled tree, though moist, had likely not begun producing any xylary elements on their way to maturation at that time. The cambium of the ring-girdled tree was actively producing vascular tissue at the time it was girdled.

Examination of transverse sections of spiral-bridge xylem showed the results observable in Figures 10-12. Within the spiral bridge, the first-formed ray cells, and the ray cells initiated for a considerable period of time thereafter, were found to be swollen and bulging through pits into tracheid lumens. Transverse end walls containing bordered pits were found in the first formed tracheids of the spiral bridge, adjacent to the 1974 latewood, and as far out into the earlywood as 20 tracheids from the 1974 growth boundary. These border-pitted end walls were not continuous within radial files; and they were determined to certainly denote xylem mother cell divisions, as opposed to divisions in fusiform initials.

It is noteworthy that transversely oriented, border-pitted end walls also occurred in response to ring girdling (Figure 8), but that these transverse divisions were much less abundant than in the spirally-girdled

trees. Scanning electron microscopy permitted observation into tracheid lumens and verified that the border-pitted transverse end walls seen in the light microscope were in fact at different levels in adjacent cells within the same radial file (Figure 10).

A second wound response which was common to both ring-girdled and spirally-girdled trees was the presence of swollen and distorted parenchyma cells of rays (Figures 8 and 11).

In the central part of the LWP-1B spiral bridge (Figure 3), reorientation of the xylem was seen to be a gradual process which was not yet complete after approximately 90 tracheids had been cut off (Figure 11A and Series 1 in Appendix). In contrast, near the edges of the bridge, reorientation happened much more quickly. Approximately 25 tracheids were cut off the unwounded spiral-bridge cambial zone of LWP-1B before side walls became evident in transverse section (Figure 12). And in tracheids derived from wound callus, on the edges of the LWP-1B spiral bridge, the first tracheids to differentiate were oriented parallel to the long axis of the spiral bridge (Figures 12, 13 and 14).

Transverse sections of UWP-1 spiral bridge xylem (Figure 2-6X, and Figure 9) showed a marked contrast to transverse sections of LWP-1B spiral bridge xylem (Figure 3-X, Figures 10, 11, 12 and 16; and cf. also Series 1 and Series 6 in Appendix). Reorientation in the UWP-1 spiral bridge occurred very quickly (after production of approximately 20 tracheids), whereas in LWP-1B spiral bridge xylem reorientation took much longer.

The UWP-1 spiral bridge xylem (Figure 2) displayed this rapid reorientation entirely across the bridge (in a horizontal plane, from the left edge to the right edge of the bridge) as well as in the regions of wounding and subsequent callus tissue formation. Reorientation of the cambium in the UWP-1 spiral bridge appeared highly coordinated, i.e. most

cambial cells changed their alignment at the same time, and quite abruptly. (However, some localized, three to four tracheid-cell wide regions were reoriented in the first formed xylem of the spiral bridge; i.e. immediately adjacent to the growth boundary of the previous year.) Reorientation of the cambium in the LWP-1B spiral bridge was not highly coordinated--some regions, or "microdomains" of tracheids showed marked change in alignment much sooner (i.e. radially much closer to the growth boundary of the previous year) than in other regions.

The same results were found in the serial tangential traces of xylem (Appendix). Series 1, from the LWP-1B spiral bridge xylem, shows a gradual reorientation. In contrast, Series 6 of the UWP-1 spiral bridge xylem, shows a rapid change in orientation.

Many dark cells, as seen in Figure 12, were found in the 1974 latewood and in the 1975 earlywood near the bridge's edge in both of the spiral bridges of tree WP-1. Detailed examinations showed these cells to be axially oriented parenchymatous cells, which will hereafter be referred to as "tylosites." The mode of formation of tylosites will be discussed later.

A study of serial tangential sections of LWP-1 spiral bridge xylem (refer to Figure 3) on the acropetal edge of the spiral bridge showed tracheids to differentiate from callus tissue and to elongate parallel to the long axis of the spiral bridge (Table 5 and Figures 12, 13 and 14). Although the tracheids became partially distorted, apparently as a result of overcrowding, their final orientation was parallel to the spiral bridge (Figure 14). Overcrowding was caused by elongation of many more cells than was needed to fill the space beside the wound.

Within the LWP-1B spiral bridge, dorsal to the acropetal edge of the spiral bridge, the reorientation process within the spiral bridge

cambium occurred more slowly (Figure 13B); and different directions of reorientation were found. That is, some fusiform cambial initials were curved such that all or part of them was perpendicular to the long axis of the spiral bridge while others showed a tendency to be curved parallel to the long axis of the spiral bridge.

It should be noted, as is evident in Figures 13A and 13B, that cambial zone cells differentiated into callus after wounding, that the callus cells spread into the unwounded cambial zone in a non-uniform manner, and that the arrangement of the callus tissue showed polarity in that there were found zones of cells, oriented perpendicular to the long axis of the spiral bridge, which stained darker than adjacent zones (Figure 13A).

Although the events which occurred in the formation of wound healing tissues are indicative of strong polarity in the reorientation process, it was found that elongation following transverse, or slightly oblique, divisions in xylem mother cells could not be described as polar (Figure 15).

The great number of transverse, or nearly transverse, divisions which formed in the xylem mother cells of tree WP-1 spiral bridge-xylem during the formation of the first earlywood contrasted sharply with the scarcity of these types of divisions found in the ring-girdled xylem (Figure 4, 8 and 11).

Tylosite formation in the xylem of white pine is exhibited in Figures 16 to 19. Tylosites formed in the 1974 latewood and were a result of dedifferentiation of existing xylary ray parenchyma. The 1974 latewood xylem (formed the year previous to spiral girdling) apparently formed the tylosites in response to wounding. Figures 18B and 19 show how the process occurred. Ray parenchyma bulged through the radial wall pits of adjacent tracheids and then expanded within the tracheid lumens. Mitotic division

followed expansion through the pits, the nucleus having migrated into the tracheid lumen, until the lumen was fully occupied with parenchymatous cells. The time of formation of these axial parenchyma tylosites, with respect to the time of spiral girdling, was not determined.

Tracheids with highly irregular walls, as seen in radial section, were found in the first-formed xylem within the WP-1 spiral bridges (Figure 17).

One of the most significant features of the reorientation process which was found was that of microdomains, as shown in Figure 20. Some regions of the cambial zone reoriented more quickly than did others. Although this frequently involved only fusiform initials, xylem mother cells were also found to participate in microdomain formation. Usually these microdomains were fairly sharply delimited with adjacent cells of the xylem showing strongly contrasting orientation.

Microdomains should not be interpreted to be related to the "domains" of cambium which, by definition, all have their pseudotransverse dividing walls oriented in the same direction (Hejnowicz 1964). Although the two may well be related, microdomain refers to fusiform-shaped tracheids which are aligned at a different angle than adjacent, axially oriented tracheids at the same radial distance from the pith.

Another significant feature of the induced reorientation of the WP-1 spiral bridge cambium is that shown in Figure 21. The number of cambial initials within a unit surface area was multiplied to approximately double its normal number. This multiplication was made possible by each initial expanding little if any tangentially after pseudotransverse anticlinal division.

Without reference to the swelling and distortion of the ray parenchyma cells, which appeared to be strictly a wound response, the number of rays

and the number of parenchyma cells per ray within a unit tangential surface were both found to be increased (Figures 21 and 22, and see Series 1). Quantitative studies were not done on either of these latter two topics; however, the serial tangential traces showed that these increases occurred primarily as a result of the decline of fusiform initials.

Fusiform initials were found to be actively involved in the reorientation process; however, ray initials were passively reoriented and maintained their relative positions (Figure 22).

Both phloem and xylem reoriented in the direction of the long axis of the WP-1 spiral bridges (Figure 23).

Two tangential series of tree WP-1 spiral-bridge xylem (Series 1 and Series 6 in the Appendix) were traced using serial tangential sections. Figure 22 shows the start (closer to the pith) and end of Series 1. Figure 24 illustrates the start and end of Series 6.

The location of Series 1 in respect to the spiral bridge of LWP-1B is shown in Figure 3. Section numbers are to be found at the bottom of each tangential trace of Series 1, in the Appendix.

The reader will note that Figure 22, Series 1 and Table 5 show the reorientation to be upward to the left, whereas the spiral bridge of LWP-1B shown in Figure 3 is oriented upward to the right. However, this is simply because the serial tangential sections were mounted and analyzed proceeding from the pith toward the bark (all other Series reported on herein proceed inward from the bark toward the pith and are therefore in agreement with the orientation of the spiral or diagonal bridges shown in the first six Figures).

Analysis of Series 1 showed the first pseudotransverse divisions which resulted in multiplication of fusiform initials to have occurred at Section 89. Following this first anticlinal division in a fusiform

initial, many more occurred, and each had its dividing wall oriented parallel to the first; i.e. in the direction of alignment which would later be assumed by the initials. In terms of acropetal to basipetal tip orientation relative to the stem axis, these many parallel pseudotransverse divisions were responsible for the first reorientation observed: no other factor was necessary. Elongation of initials following oblique multiplicative division was slow in terms of serial tangential sections. Where elongation did occur (Sections 100-103, Series 1), the tip overlap was usually in the direction needed to facilitate reorientation. Intrusive elongation occasionally occurred extremely rapidly associated with failure of neighboring initials (Sections 101-103, green cells, Series 1).

The first fifty tangential sections of xylem (nearest the 1974 latewood boundary) of the LWP-1B spiral bridge showed little change in orientation. Within the region of Section 100 to Section 112, an abrupt change in alignment occurred (also see Table 5). The primary activities, as deduced from the xylem tracheids, which were associated with that abrupt change included abrupt failure of some fusiform initials and rapid elongation of neighboring fusiform initials into the vacated space. The numerous parallel, oblique dividing walls guided that elongation and thus changed the alignment of the short fusiform initials.

The reorientation of Series 1 was found to be explainable by four processes:

1. Shortening and narrowing of cambial initials by:
2. Parallel oblique anticlinal divisions, followed by:
3. Failure of some initials, and
4. Spurts of intrusive growth in the new direction at the sites of failure.

Accompanying processes included splitting of rays by intrusively

growing fusiform initials, creation of new rays by failing initials, and the passive change in alignment of the rays.

Reorientation of Series 1 is expressed in Table 5 in terms of angle measurements. Reorientation of rays and callus-derived tracheids are included for contrast.

The second Series in tree WP-1 which was traced to show the mechanics of the reorientation process is shown in Series 6. The location of this Series, traced from serial tangential sections of UWP-1 (5T), is shown in Figure 2. Note that it is quite near the bridge's edge. Within this Series, sections 62T1 through 59T1 show alterations (e.g. transverse divisions) which occurred in xylem mother cells following their initiation by the fusiform initials. The first oblique anticlinal division within a fusiform initial occurred in section 58T4. Other parallel pseudo-transverse divisions quickly followed to facilitate the reorientation. A feature of interest, not seen in any of the other Series which were traced, is that shown in section 58T1--the cell on the left appears to have divided to permit the cell on the right to intrude through.

Although there was found to be a marked contrast between rates (per radial millimeter of xylem) of induced cambial reorientation within the long spiral bridge (UWP-1) and the shorter spiral bridge (LWP-1B); i.e. between Series 6 and Series 1, respectively, the cambial activity associated with the reorientation process was found to be the same.

TABLE 5

ANGLE MEASUREMENTS, IN DEGREES, FOR TREE WP-1, SPIRAL BRIDGE LWP-1B

SERIAL TANGENTIAL SECTIONS OF REORIENTATION*

Serial Tangential Section Number	SERIES 1			WOUNDED EDGE OF BRIDGE	
	RAYS			Callus+ or	Tracheids
	Fusiform	Uniseriate	Tracheids	Tracheids	Below Edge
59	1	0	0	0+	0
64	0	0	0	0+	0
69	0	0	0	0+	0
74	0	0	0	0+	0
79	0	0	0	0+	0
84	0	0	0	0+	0
89	0	0	0	320	358
94	0	358	359	320	357
99	0	357	358	315	356
104	356	355	355	310	353
109	353	351	352	310	353
114	350	350	350	/	350
119	350	350	349	/	350
124	348	350	348	/	350
129	344	345	344	/	348
134	345	345	344	/	348
139	343	345	344	315	346
164	343	340	340	315	344
189	340	335	336	315	341
214	334	331	330	320	337
239	334	330	330	320	330

/ =Extreme variability in orientation as a result of overcrowding of elongating fusiform cells; orientations ranged from 270° to 360°.

* See page 40, final paragraph, for further explanation.

\+ Refers to orientation of files of uniformly stained, isodiametric callus cells (e.g. see Fig. 13, p. 81) above the edge of the bridge; tracheids derived from this callus (Sections 89 to 239).

RESULTS

Investigation 3 -- Periodic harvesting of vertical and diagonal bridges in Pinus contorta Dougl.

Analysis of transverse sections of diagonal and vertical bridges of lodgepole pine, such as those bridges shown in Figure 7, allowed calculation of the mean number of tracheids formed by the vascular cambium during 1976 before harvesting (Table 6) and the mean number of tracheids formed up to the time of wounding (bridge construction) (Table 4, p. 35). And, accordingly, the width of the cambial zone at fixation (harvesting) time, and the number of tracheids formed by the cambium since the July 8, 1976, girdling date were determined (Table 6).

Reaction to the girdling was much slower than expected. At two days following girdling there was no evidence of any abnormal activity in either of the diagonal bridge (Figure 25) or the vertical bridge (Figure 26). At 8 days after girdling, numerous radial files were found to be declining toward failure, and anticlinal divisions were common (Figure 27). Similar events were found at 10 days (Figures 28 and 29); although resin canal formation was apparent at 10 days, it was infrequent and appeared to be normal preceding latewood formation. Anticlinal divisions were easily found scattered abundantly throughout the cambial zone, and xylem mother cell anticlinal divisions were also often found (Figure 29).

Anticlinal divisions, resulting in doubling of the number of radial files, were frequently found to first occur one cell proximal to mature phloem (Figure 30B), good evidence that this is the location of the fusiform initial.

At 12 days following girdling, the wound zone (see page 42) first became evident (Figure 30A), as was also previously noted in white pine which was ring girdled. However, rays did not show the irregular

TABLE 6

CHARACTERISTICS OF THE GIRDLED LODGEPOLE PINE TREES AT TIME OF HARVESTING

No. of Days Between Girdling and Harvesting	Mean Number of cells per 1976 radial file of xylem up to cambial zone		Width of cambial zone at time of harvesting		Number of tracheids per radial file formed since the time of girdling	
	45° Diagonal Bridge	Vertical Bridge	45° Diagonal Bridge	Vertical Bridge	45° Diagonal Bridge	Vertical Bridge
2	90	92	11	12	--	--
4	123	126	14	13	--	--
6	80	85	10	11	--	--
8	120	132	20	18	--	--
10	91	92	15	14	--	--
12	92	76	12	10	11	--
14	76	87	11	13	9	7
16	149	150	17	17	11	17
18	119	143	17	17	15	20
20	203	248	17	15	20	27
22	181	152	23	14	23	23
24	152	183	13	14	22	26
26	117	116	13	11	17	18
28	121	118	15	11	18	27
30	155	158	16	16	25	36
32	131	143	15	12	32	26
38	144	143	18	12	38	20
50	248		22		78	

bulging which they did in white pine; rather some were greatly swollen and had become filled with dark-staining ergastic substances (presumably tannin). Other nearby rays were unchanged to all appearances.

An analysis, by transverse sections, of vertical bridge xylem showed the following events to have occurred by 38 days after girdling:

1. Wound zone formation;
2. Callus tissue formation on the bridge edge;
3. Traumatic resin canals were restricted to the region near the bridge edge.

Border-pitted transverse end walls in tracheids formed after wounding, and xylary axial parenchyma were both very scarce; however both were occasionally noted to be present near the wound. Figure 31 illustrates these features.

In transverse sections of diagonal bridges, border-pitted transverse end walls in tracheids were more common, but were not as abundant as in white pine, and they were almost entirely restricted to the wound zone. Traumatic resin canals and axial parenchyma were common entirely across the diagonal bridges (in the horizontal plane) (Figures 32 and 33).

Figure 33 shows an example of a transverse section from the region of growth that occurred within the diagonal bridge during a 50-day period. Figure 34 shows a close up of the cambial zone at 50 days after girdling. Microdomains of reorientation were quite evident within the cambial zone at this stage (see also Figure 47).

Swelling of ray cells was first noted in the cambial zone of tissue fixed six days after girdling (Figure 35). True transverse divisions within xylem mother cells were found in the cambial zone at 8 days after girdling, and thereafter; but none were found in specimens harvested two to six days after girdling. These true transverse divisions appeared scattered irregularly throughout all xylem mother cells of a radial

file at the same time, indicating that there was no ancestral relationship between the transverse divisions. (Occasionally a periclinal division within a xylem mother cell followed the true transverse division; as a result 2 tracheids of a radial file would be found to have transverse, border-pitted end walls at the same level--see Figure 36).

Traumatic resin canals were found to be differentiating within the xylem mother cell zone between 18 and 20 days after girdling (July 8, 1976), as shown in Figure 37.

Four of the 18 girdled trees were found to show atypical wound responses such as that shown in Figures 38 and 39. The primary feature of these atypical wound responses was the presence of many cells: primary-walled, axially oriented, and usually with unstained cytoplasm. These apparently parenchymatous cells were interspersed sporadically between secondary-walled and heavily lignified tracheids within radial files.

The typical wound responses found are shown in Figures 40 and 41, whereas Figure 39 is a radial section of an atypical wound response to contrast with that of the typical in Figure 41. The components of the typical wound response included the following in lodgepole pine girdled in late summer:

1. Temporary cessation of radial enlargement within the xylem mother cell zone and concomitant increased lignin deposition, resulting in wound zone formation, which became visible in the xylem after 12 days;

2. Swelling of some, but not all, ray parenchyma,--visible in the cambial zone after 6 days;

3. True transverse divisions, which occurred in two or more xylem mother cells (also in phloem mother cells) of the same radial file at approximately the same time (8 days after wounding, and thereafter),

resulting in mature, secondary-walled tracheids with border-pitted transverse end walls.

4. Traumatic resin canals differentiated from xylem mother cells of adjacent radial files at 18 to 20 days after wounding.

5. Axial parenchyma differentiated into the xylem from xylem mother cells. Axial parenchyma within the xylem mother cell zone of the cambial zone were first detected at 10 days after wounding.

It should be borne in mind that the control (vertical bridges) did not show the frequency or accentuation of responses 2 to 5 as were found in the diagonal bridges.

Examination of tangential sections of the cambial zone at different times after girdling showed the responses related to reorientation to be relatively slow (Figures 42 and 43). It was only after 30 days that the first hint of microdomain formation was found in serial tangential sections (Figure 43B). (However, evidence for microdomains in the cambium were found in transverse sections of material harvested 16 days after girdling). Figure 44 shows serial tangential sections, spaced 80 micrometers apart, in the cambial zone of a diagonal bridge harvested 50 days after girdling. At this time, as could also be seen in Figure 34, changes are occurring very rapidly in the cambium.

Axial parenchyma formation into the xylem of lodgepole pine was via differentiation of xylem mother cells (Figures 45 and 46). No distinction could be made between these xylary axial parenchyma (which according to Panshin and de Zeeuw (1970) do not normally occur in lodgepole pine) and the usually-formed axial parenchyma of the phloem.

At 50 days after girdling, microdomains of reorientation of short cambial fusiform initials were everywhere evident. Although the cambium had shortened its average fusiform initial length by a factor of 3 or 4

through oblique anticlinal divisions, and accordingly multiplied the number of fusiform initials by a factor of approximately 2 (failure was also occurring abundantly), only certain regions became the vanguards of the reorientation process--other adjacent microregions remained axially oriented.

In the vertical bridge harvested after 38 days, shortening of fusiform initials was found to have occurred by a series of parallel oblique anticlinal divisions immediately adjacent to the wound, but no such event was observed in the central region of the vertical bridge (Figure 47).

Four series were traced from the serial tangential sections of diagonal and vertical bridges of lodgepole pine. Three were traced from the diagonal bridge harvested at 50 days after girdling (locations of series are shown in Figure 5), and one was traced from the vertical bridge harvested at 38 days after girdling (location shown in Figure 6). These Series (Series 2, 3, 4 and 5) are in the Appendix, but see also Figures 48, 49, 50 and 51.

Series 2 and Series 3 were adjacent to one another in the xylem which had formed previous to diagonal girdling. After tracing more than 100 serial tangential sections, Series 2 was stopped with a single tracheid, about one third the length of the starting tracheid, remaining as the sole ancestor of that radial file. The first 50 tangential sections of xylem (Sections 144 to 94) showed frequent xylem mother cell divisions to be occurring--either transverse or slightly oblique; however the fusiform initial producing these xylem mother cells remained unchanged other than to shorten slightly and to develop a strong curvature in its basipetal region. The first oblique anticlinal division to occur in

the fusiform initial is seen in section 93 of Series 2. This division was oriented upward to the left, whereas the induced reorientation was to be upward to the right. A second anticlinal division occurred in the fusiform initial at section 85; and this one was oriented to facilitate reorientation. However, the lower sister initial of this second division quickly failed, and this was followed by failure of the upper initial of the first anticlinal initial division and elongation of the middle initial into its place. The final result was little, if any, reorientation (Figure 48).

Series 3, adjacent to Series 2, showed xylem mother cell activity via an intact fusiform initial up until section 109, when the first oblique anticlinal division of the initial occurred. As in Series 2, this first oblique division was oriented in the "wrong" direction. However, by section 93, the overlapping tips of this anticlinal division had rearranged themselves, apparently by slightly off-center periclinal divisions, to be overlapping in such a way as to facilitate reorientation. A second pseudotransverse anticlinal division occurred in the fusiform initial in section 91, this time oriented in the direction necessary to facilitate reorientation. Thus there were at that stage three adjacent fusiform initials. At section 82, two of these three short fusiform initials had produced xylem mother cells which underwent short, oblique anticlinal divisions which were also oriented in the direction to facilitate reorientation. Following this, periclinal divisions of these xylem mother cells resulted in a number of sections (82 to 73) being observed to have more than three derivative tracheids. This was quite a significant finding: the four parallel divisions alone, which resulted in the formation of five related radial files of tracheids in the xylem, had given rise to the first hint of reorientation. This reorientation occurred as a result of the combined action of fusiform initials and

xylem mother cells. Elongation of the resulting five fusiform cells within the xylem mother cell zone (sections 80 to 76) contributed even further to the reorientation. However, this was a short-lived reorientation of xylem mother cells, and to a lesser extent of fusiform initials. By section 72, there were once again only three fusiform initials showing no more change in alignment than when they had first been formed.

One of these three fusiform intials failed (section 56--uppermost). The lower of the three divided obliquely again (section 55) such that there were again three initials; however, the division was opposed to the direction of eventual reorientation of the bridge. The central initial of the original trio then divided obliquely (section 52) in the expected direction. Following this latter division, the lower of the resulting sister initials quickly failed (sections 51 and 50) by moving into line for maturation and being replaced by an adjacent initial (not shown). The upper of the resulting sister initials divided yet again to result in two very short initials (section 50). One of the daughter initials of this latter division was able to elongate intrusively in the direction of its long axis, and thus became reoriented; the other daughter initial failed via maturation. Figure 49 shows the beginning tangential section and a tangential section near the end of Series 3.

Series 4 was traced from xylem adjacent to the left edge of a vertical bridge (see Figure 6) which had been harvested 38 days after girdling. This Series is quite short since very little tracheid production occurred within this vertical bridge (see Table 6, p. 52). Figure 50 shows the start and stop of Series 4. No confident interpretations of initial activity, versus mother cell activity, can be made from such a short

series. However, a number of long oblique divisions (sections 26 to 23) occurred somewhere in the cambial zone, likely in the fusiform initials, and these were oriented parallel to one another, conveying the impression that the intrusive elongation to follow would tend to curve the fusiform cells in a direction approaching perpendicular to the edge of the bridge, such as was found near the edges of spiral bridges in white pine.

Section 23 of Series 4 terminates the series within the cambial zone. Two features of interest in Series 4 were the secondarily thickened, unfinished dividing walls (sections 33, 32, 31, 30 and 29) within the lumens of the tracheids, and the proliferation of parenchymatous cells from existing ray cells (Sections 30 to 23). Although neither of these observations can be given a functional explanation, the unfinished dividing walls would appear to indicate a mitotic phragmoplastic, or nuclear imbalance; and the proliferation of parenchymatous cells appeared related to callus tissue formation.

Series 5, from the same lodgepole pine diagonal bridge serial tangential sections as were Series 2 and 3 (see Figure 5), involves seven starting cells. Because of the obvious complexity in discussing the multiplicative divisions, failures, and interactions between derivatives and initials of those seven, the events will only be presented in general terms here.

Oblique anticlinal divisions did not occur everywhere at the same time; however, when they did occur they were generally oriented in the direction necessary to facilitate overlapping of elongating tips and resulting reorientation. Short oblique anticlinal divisions were found to be commonly occurring in xylem mother cells. And oblique anticlinal divisions in fusiform initials were often found to be oriented

such as to oppose reorientation.

Elongation of sister initials following multiplicative division was at a very slow rate in terms of serial tangential sections of xylem, but occurred quickly where adjacent cells failed. Distortion of some initials occurred as a result of others effectively placing extreme pressure on their flanks.

By section 70 of Series 5, the seven starting initials had multiplied to 14 shorter fusiform initials. However, between sections 92 and 70, many fusiform initials had formed by multiplicative division and had then failed by maturation. The rate of multiplicative division was very rapid, and eventually became coordinated such that all divisions were obliquely sloping in the same direction. As a result, a template was formed, and when one initial began to fail, the neighboring cell immediately elongated and had no alternative but to elongate in the new direction. The controlling factor in the directional realignment of the cells was the template of numerous parallel oblique dividing walls.

Rays were shoved one way and the other, pushed together and pulled apart, and sometimes split. Each ray seemed to maintain its individuality even when pushed against another ray; i.e. it frequently occurred that two rays which had been shoved against one another later separated, and the separation occurred at the same contact point as where they had been forced together.

The beginning and end of Series 5 and shown in Figure 51.

Scanning electron microscopy

No satisfactory evidence was found to indicate that reorientation has as one of its components the interdigitating, or interweaving, of the fusiform cells within the cambial zone. Serial radial photographs from two specimens of WP-1 xylem which showed a narrow radial zone of rapid

reorientation gave no indication, either in the region where alignment first changed, or in following xylem, that cells of one radial file within the cambial zone somehow forced their way between cells of an adjacent radial file other than by the commonly documented and observed process of failure by maturation.

DISCUSSION

From a step-by-step analysis of serial tangential sections of xylem, it has been found that fusiform cambial initials within a spiral bridge effectively change their orientation by undergoing successive parallel oblique anticlinal divisions. It is significant that no other cambial action--e.g. elongation of the tips of the resulting sister initials following anticlinal division, or failure of some of these sister initials--is necessary in order for the first microdomains of reorientation to occur.

Greater changes in alignment follow when neighboring cambial cells, including cells within mother-cell zones, also divide obliquely in the same direction as the oblique divisions which preceded in the fusiform initials. Because the fusiform cambial cells become greatly shortened, very little subsequent intrusive elongation acts to rapidly change the tip-to-tip angle of the fusiform cells relative to the stem axis. The intrusive elongation occurs primarily as a result of failure of adjacent fusiform cells; as fusiform initials elongate into vacated positions very rapid microdomains of reorientation result. In spite of cell failure, the numerous parallel oblique dividing walls function as a template to guide the elongating cells into new alignment. Thus the polar coordination of formation of numerous parallel oblique dividing walls by fusiform cambial cells is seen to be the most essential element in the reorientation process. Nevertheless, following multiplicative division to create shorter fusiform initials, the rate, or rapidity, of reorientation is dependent upon how many and how quickly some fusiform initials give up the space they occupy so that the survivors can use the dividing-wall template to effect the reorientation.

Reorientation is opposed, or resisted, in at least three ways:

1. Sometimes long fusiform initials do not divide into shorter initials (nor do they elongate further). As a result, even though neighboring cells may have divided multiplicatively and may be exerting considerable pressure on the long fusiform cell to change its alignment, it does not cooperate. Such uncooperative individuals often are supported by adjacent fusiform initials, and particularly by numerous ray contacts. Ray cells, by their very presence, act to maintain the original alignment of long fusiform cells. It is only when a fusiform cell shortens that it can rotate past a ray. Not one instance was ever noted where the radial continuity of a ray was broken. Instances were noted where intrusive tip growth of fusiform cells separated the association of one or more ray cells from its companions; nevertheless the radial continuity of the ray was still maintained. Instances were also noted where rays were temporarily, or less often permanently, shoved sideways by the force exerted by elongating fusiform cells; however radial continuity of the rays was always maintained.

2. Even if long fusiform cells do divide into shorter initials, if the oblique dividing walls are slanted against the direction of oblique dividing walls in neighboring cells, the reorientation process will be opposed. That is, the subsequent intrusive elongation which occurs will be guided by the dividing walls. Many examples of this were observed. Very frequently the first oblique anticlinal divisions which occurred in fusiform initials within spiral bridges were slanted in a direction such as to oppose the alignment which would finally be induced. That such alignment of the cell plate following mitosis was a chance accident seems highly unlikely; rather it appears that such action was an expression by some members of the cambial population to maintain their alignment. It is conceivable that the non-dividing fusiform

cambial cell is merely resisting in a different way.

3. Given the situation where numerous fusiform initials become coordinated to all divide with parallel oblique anticlinal divisions, a third mechanism to oppose the reorientation process still exists. If cells refuse to fail, changes in alignment occur very slowly.

Because some cambial cells actively resist changing their alignment, or having their alignment changed for them, while others quickly give in to the stimulus to reorient, microdomains of reorientation precede the overall change in alignment which finally results.

Bannan (1957) has shown that failure can occur in xylem mother cells as well as within initials; and this is in agreement with the finding that microdomains occasionally were formed as a result of xylem mother cell activity. Hejnowicz (1961) has shown that failing cells have shorter oblique anticlinal walls, indicating perhaps a predisposition toward failure. The results of the present investigation agree with that finding. Hejnowicz (1961) also found that failing cells occurred in groups while other groups were dividing and elongation (see also Hejnowicz 1968). Presumably these groups of elongating cells are equivalent to the cells involved in microdomain formation as found in this investigation. It is conceivable that a region of the cambium will not become more or less uniformly aligned in a new direction until microdomains of reorientation overbalance adjacent areas which resist reorientation. That is, microdomains of reorientation may act an an aggressive vanguard to the overall reorientation process.

Whether or not they are aggressive, they certainly constitute a vanguard to reorientation. This applies not only to conifer stems which have been induced to reorient, but also to spirally-girdled hardwood stems (Figure 52) and to natural spiral grain formation in conifers (Figures 53 to 55).

Rather than being aggressors, fusiform cells which results in microdomain formation may represent cells which are unable to offer as much resistance to reorientation as can their neighbors. It is also possible that microdomains represent cambial regions where mitosis followed by anticlinal division occurs more frequently than in neighboring regions due to a higher concentration or a greater sensitivity to some agent which triggers cell division. On the other hand, it may be that although numerous parallel oblique anticlinal divisions do occur in neighboring fusiform initials to form a reorientation template, a more important purpose of the divisions is to initiate failing cells which would then allow actuation of the template mechanism by permitting intrusive elongation.

The results on reorientation by inducement agree strongly with the findings of Hejnowicz (1961, 1964, 1968, 1971) on studies of natural spiral grain formation. Hejnowicz' 1961 statement that "... the elimination of the fusiform initials constitutes the mechanism accelerating the rates of the changes taking place in the cambium" is in complete agreement with the findings of this investigation; and it follows that the hypotheses of Hejnowicz (1961; page 11 herein) have been reinforced by this study.

Although Bannan (1966) seems to have misinterpreted the significance of failure in the reorientation process, the results strongly agree with his 1966 statement that "... the role of multiplicative division and subsequent cell elongation in the development of spiral grain"..."has been oversimplified." No evidence was found that failure of initials acts to prevent fusiform initials from changing their alignment; rather failure seemed to be the rate-determining step which controlled changes in alignment of fusiform intials.

Bannan and Bayly (1956) reported that failing cells undergo tangential

contraction as well as longitudinal shortening. They state that this indicates a reduction in internal pressure and draw a hypothetical correlation between this idea and their belief that the rays are involved in the maintenance of turgor pressure. However, it was commonly found in this study, after successive pseudotransverse anticlinal divisions had occurred, but before some of the resulting initials had declined to give rise to new ray initials, that fusiform initials without any ray contacts whatsoever could rapidly elongate into vacated spaces and seemed to hold their own amongst fusiform initials with numerous ray contacts. Series 5 best shows this feature, but see also Series 3.

Whether fusiform cambial initials in fact compete with one another as Bannan and Bayly (1956) have suggested remains to be shown. Perhaps a better hypothesis would be that cells cooperate with one another and that our concept of "failure" of fusiform initials deserves a less emotional term. That a fusiform initial becomes smaller and eventually either converts into a ray cell initial or matures into functional vascular tissue need not have any competitive connotation.

Sigmoid curvature of fusiform initials, which Harris (1973) has suggested to represent the first stage in fusiform cell realignment, was rarely seen. However, it is plausible, and would appear agreeable with the ideas of Bannan (1968b) that within the individual fusiform initial the cellular components can be unequally distributed. Furthermore, it seems conceivable that the individual fusiform initial may contribute to the reorientation process by expanding tangentially in one of its longitudinal halves while it narrows up in the other half. At the same time adjacent fusiform initials could be doing the reverse (i.e. reverse the halves which expand or narrow). Such a "giving and taking" or "compensatory" contribution to the reorientation

process was not observed, since only mature, dead cells were examined. However, two commonly observed phenomena make this suggestion seem plausible:

1. Tangential expansion of a fusiform initial can be frequently observed as it occupies the space vacated by a failing initial; such expansion can be seen best in transverse section, but as well in serial tangential sections (e.g. see Fig. 27, 3rd and 4th files from left);

2. Multiplicative divisions shorten initials; as a result the length-to-tangential-width ratio of a short initial is much nearer unity than that of a normal-sized fusiform initial. In order for pressure, or growth compensations between adjacent cells to be geometrically conducive to a change in cell alignment, that ratio would have to become nearer unity as is observed in the shortening of cells.

Such an idea seems to be supported by the finding, in the spiral and diagonal bridges examined, that the first-formed xylem after spiral girdling became curved (Figure 56) and distorted (Figure 17) to some extent. However, it is just as plausible that such curvatures and distortions result from many sister xylem mother cells all attempting to elongate after transverse or short oblique anticlinal divisions.

As Figure 21 shows, following reorientation, the short fusiform initials elongate. As a result of the multiplicative divisions which formed the reorientation template, there arose many more fusiform (and ray) initials than normal within a unit tangential surface area of cambium. The realigned and elongating fusiform initials maintain their very narrow tangential diameters from inception of the cells by multiplicative division. It can therefore be deduced that fusiform cell elongation takes priority over tangential expansion and therefore also over failure, since failure of one cell results in tangential expansion of its neighbor.

As callus cells begin to differentiate into xylem tracheids, the cells elongate in the direction of the forced photosynthate movement within a spiral or diagonal bridge. Such polar elongation indicates the inherent tendency of vascular elements to be aligned in the direction of phloem, and perhaps also xylem, transport. It is believed that the diagonal transport of auxin is responsible for the reorientation which occurs within a spiral bridge (Fahn 1973), and Fahn reports good evidence for this. But it is hypothesized that in natural spiral-grain formation, cambial reorientation does not occur as a result of diagonal auxin transport within an otherwise axial vascular system; rather cambial reorientation must occur to bring about diagonal transport of auxin and other leaf products. Thus it is further hypothesized that polarity resides within the cell and that substances such as auxins merely act to stimulate expression of that polarity. Palevitz and Hepler's (1974) correlation of the preprophase band of microtubules with the plane of cell plate formation lends support to this hypothesis in view of the often observed polar nature of the parallel oblique anticlinal dividing walls which occur in neighboring fusiform initials of the vascular cambium.

It may be argued that because callus tissue, as it elongates and differentiates to form tracheids, shows directional growth, cambial initials are also capable of "growing" in the new direction. Although this may well be the case, the research reported on herein has not conclusively shown that; rather, it has shown that the pseudotransverse anticlinal division takes priority over any form of directed elongation in the reorientation process.

Short pseudotransverse anticlinal divisions, as well as transverse divisions, were found to occur frequently in xylem mother cells; however

the subsequent limited tip elongation which followed was not convincingly polar in nature. In the intrusive elongation which followed anticlinal division in the fusiform initials, occasionally, but certainly not always, elongating cells appeared to be under some influence to elongate in a specific direction--that direction being the one which the reoriented cambial cells would finally assume.

Rearrangements of fusiform initial tips so that elongation would proceed in the direction of induced reorientation, rather than in the opposing direction, were occasionally noted as a type of polar response. Such rearrangement seemed to occur by successive periclinal divisions terminating short of the tip such that the length of the cell was shortened. After sufficient shortening, the fusiform tip would then elongate in the correct direction. In Series 6, a transverse division in a fusiform cell which was otherwise impenetrable to an adjacent rapidly elongating fusiform initial, permitted the latter's basipetal tip to elongate through the new "opening" in the correct direction. Although these may be interpreted as polar responses, evidence was also just as often found which indicated that directed tip growth does not occur. Section 103 of Series 1 shows an initial tip (lower orange) which elongated up and around another initial (adjacent to the fusiform ray). In doing so, the tip elongated in the "wrong" direction to facilitate reorientation. A similar example is seen in the lower portions of sections 111 and 112 of Series 1, where the lower tips of two initials (green, and adjacent for most of their lengths) were also elongating in the wrong direction.

The primary factor influencing the direction of fusiform initial elongation, following anticlinal multiplicative division, appeared to be the static orientation of the cell itself. The tips of an initial grew in the same direction as the longitudinal orientation of the initial. Tip

growth of an initial continued in the direction dictated by its longitudinal orientation until a barrier was encountered. Then elongation either ceased or it was diverted around the barrier, in all appearances taking the path of least resistance. As a result, tip growth was frequently seen to be in the wrong direction. In spite of this, in domains (Hejnowicz 1964) where all oblique anticlinal divisions are parallel, the conformity of dividing wall direction acted as an overall template for the reorientation process.

Hejnowicz (1963) and Hejnowicz and Zagorska-Marek (1974) have stated that intrusive growth of cells is strictly limited to the edge of the growing cell end, "at least in the case of ends originating from transverse divisions which had been induced by wounding the stem" (Hejnowicz and Zagorska-Marek, p. 392, 1974). In this study, transverse divisions such as Hejnowicz and Zagorska-Marek (1974, p. 393) show in Larix sp. were found to occur only in xylem mother cells, and not in the fusiform initials. Harris (1973) attempted to show at the ultrastructural level that the tip of the fusiform initial was somehow different than the cytoplasm in the otherwise vacuolate parts of it; however he found no differences. It remains to be shown that tip growth, such as occurs in pollen tubes and fungal hyphae, actually occurs in the cells of the vascular cambium.

The rate of reorientation found in lodgepole pine after late summer girdling contrasts sharply with that found by Harris (1973) on radiata pine girdled in early summer, and by myself on white pine girdled in early summer. Therefore it can be hypothesized that the stimulus which triggers reorientation of the cambium is more abundant in early summer than in late summer. Certainly we know this to be the case for auxin. However, approximately 80 tracheids matured in the 50 days following spiral

girdling in the lodgepole pine; thus it appeared that cell production was increased as a result of wounding in this particular tree. Nevertheless, reorientation was still in the microdomain stage at 50 days. After 42 days, Harris (1973) found strongly reoriented xylem in the radiata pine. Although differences in auxin production are expected to be the causal factor, other factors which deserve consideration are the species difference and the slightly different spiral girdles which were used. Whereas Harris (1973) removed a narrow strip of bark spirally from the tree, the procedure in this investigation was to remove a ring of bark and leave only a narrow strip of bark (phloem and active cambium) running diagonally across the ring girdle.

The localized swellings of increased growth which occurred axially above the entrance into the spiral bridge of white pine tree WP-1, and axially below the exit of the spiral bridge, represent phenomena which deserve further study. Did the swelling which extended in a localized manner vertically upward from the bridge entrance to the next whorl of branches come about by acropetal movement of some substance through the phloem, or is there some sort of communicatory system which informed cells acropetal to the bridge entrance to concentrate photosynthetic products only on one side of the unwounded portion of the bridge internode? Or was water somehow restricted in its upward movement such that it was forced to parallel the spiral bridge in that region, and upon reaching the apex of the bridge commenced to move vertically again? Certainly further work is merited in this regard.

Although there was considerable variability in the productivity of fusiform initials within the same region of the tree, and between internodes of the same tree, and of course between trees of lodgepole pine (see Tables 3, 4 and 6), certain trends in the reorientation and

wound reactions were evident in terms of time.

Although wounding is known to stimulate the cambium to becoming more productive, it appears that wounding of the cambium when it is active initially brings the surrounding cambial zone virtually to a standstill for a brief period. The affected cells are seen in the xylem later as radially narrow, heavily lignfied cells; thus it appears that wall synthesis in radial enlargement was interrupted and lignification was intensified. The formation of the wound zone is believed to be the first physiological response which occurred in the cambium.

Following this, ray parenchyma swelling was evident 6 days after wounding. Sporadic transverse divisions began occurring throughout the xylem mother cell zone at 8 days after wounding. Axial parenchyma began differentiating from xylem mothers cells at 10 days. Between 8 and 10 days, the frequency of anticlinal divisions increased in both initials and in mother cells. The wound zone became visible in the xylem at 12 days. The first microdomains of reorientation were seen in transverse section at 16 days. A wave of traumatic resin canals began differentiating out of the xylem mother cell zone at 18 to 20 days. Strong microdomains of reorientation were evident at 30 days. (The July 8 girdling date should be borne in mind with respect to the usefulness of this data; girdling earlier in the summer may produce much different responses.)

The lack of a wound zone in white pine trees girdled in early spring is not contradictory to the findings in lodgepole pine, since white pine which was ring girdled in mid-summer also showed reaction to the wound by formation of a wound zone. Wounding of the white pine in early spring occurred when the cambial zone was just in the process of increasing its width, before any radial enlargement or vascular tissue maturation processes had begun, in all probability. Since the processes related to wound zone

formation (radial enlargement and lignification) had not yet been activated, they could not be interrupted by the wounding.

Figure 57 shows what has been deduced to be natural wounding; the response as seen in the photograph is in lodgepole pine latewood xylem. It can be seen that the heavily lignified wound zone stands out in spite of the heavy lignification associated with latewood formation. Swelling of ray parenchyma and numerous anticlinal divisions also seem to be generally correlated with wounding; and axial parenchyma also appear to be present.

Swelling of ray parenchyma at, or shortly after wounding, would seem to be correlated with an internal pressure release, or perhaps with increased osmotic pressure. Axial parenchyma formation in pine species, which commonly form bands of phloem axial parenchyma but do not normally form xylary axial parenchyma, indicates that the fine balance which causes cambial zone cells to discriminate in their differentiation to become either xylem or phloem may have been disrupted (however, sieve cells were not found in the xylem). Otherwise, axial parenchyma must have differentiated into the xylem to perform some function yet unknown to us. The fact that mature xylem ray cells, which had formed the year previous to girdling, dedifferentiated into axial parenchyma-like tylosites, which frequently appeared identical to those xylary axial parenchyma produced by the cambium, seems to indicate that their presence could be functional.

In summary, microscopic investigations into induced cambial reorientation within spiral bridges in two pine species have resulted in the following findings:

1. Fusiform initials actively change their alignment, while ray initials are passively reoriented;

2. Reorientation begins in microscopic regions of the cambial zone, termed microdomains, and later spreads to encompass cells between the vanguard of reoriented microdomains;

3. Reorientation occurs by fusiform initials subdividing themselves by successive parallel oblique anticlinal divisions into a number of shorter initials. Some of the shorter fusiform initials fail and permit others to elongate into their vacated space. The numerous parallel oblique dividing walls form the template for reorientation.

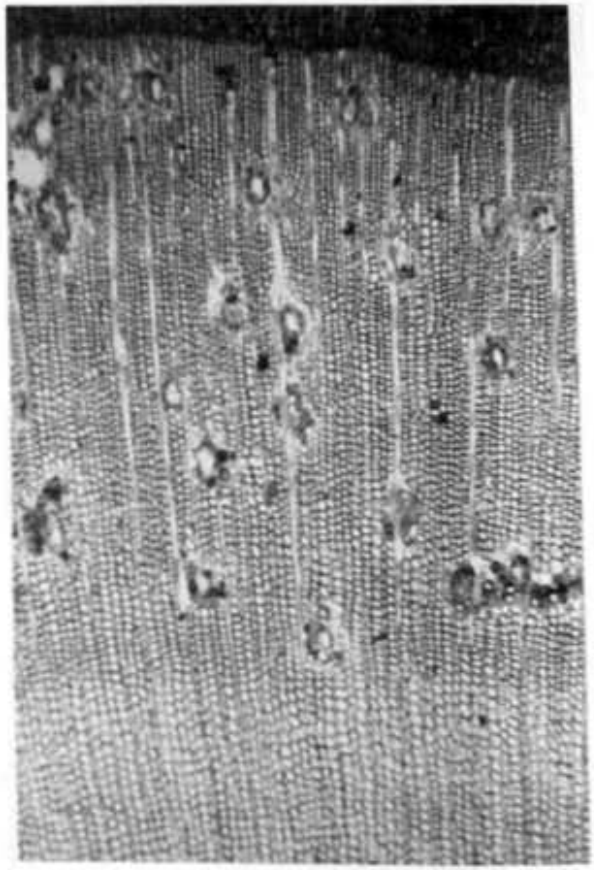
Reorientation of the cambium has been observed to occur quickly and uniformly, and slowly and non-uniformly, within two spiral bridges created at the same time within the same tree. Reorientation occurs more rapidly within spiral bridges constructed in early spring than within those constructed in late summer. It is believed that the diagonal transport of auxin is responsible for the reorientation of the bridge cambium; and it is well known that auxin is also more abundant in early spring than in late summer.

Figures 7 to 57

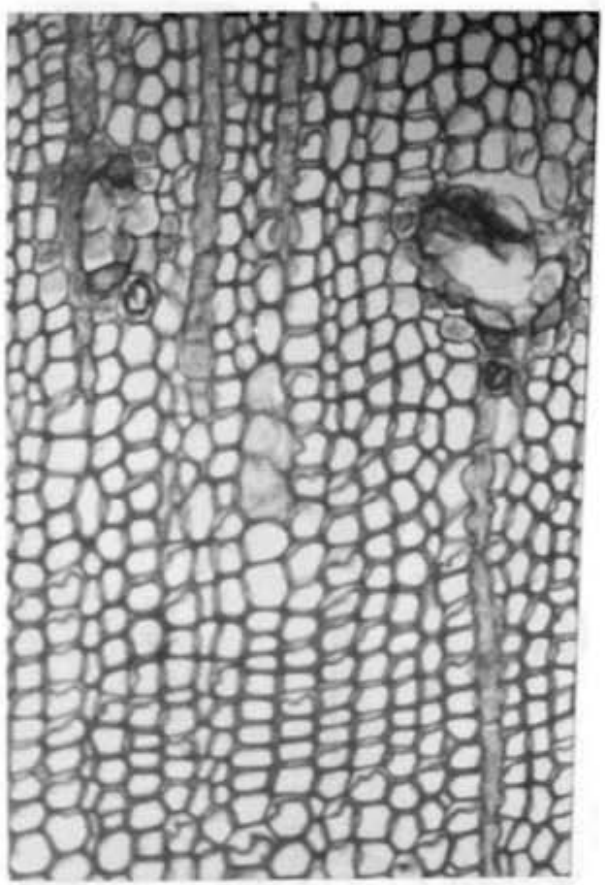
PHOTOGRAPHS

Figure 7

Diagonal and vertical phloem-cambium bridges across girdles in lodgepole pine. Eighteen trees such as this one were all similarly girdled on the same day (July 8, 1976) at this site. Tables 3 and 4 (pages 34 and 35) present pertinent characteristics of these eighteen trees.

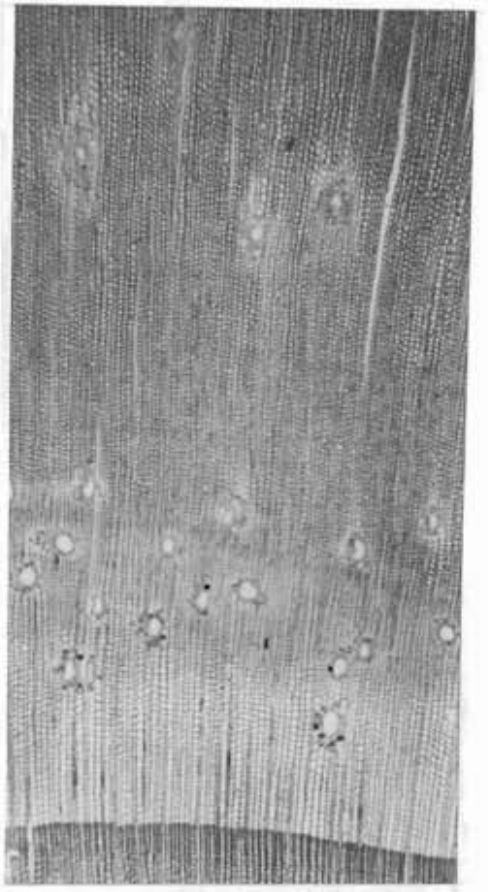


A. Transverse section of ring-girdled white pine (see Figure 4). Girdling was done on June 20, 1975. Time and position of wounding is marked by relatively heavy lignification in radially narrow cells. The phloem is at top. X82

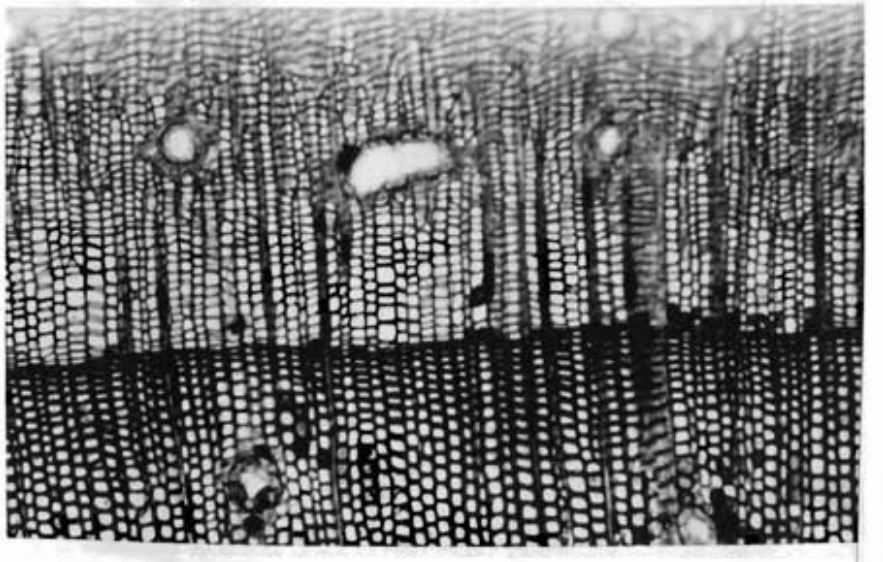


B. Higher magnification of ring-girdled white pine xylem. Note the border-pitted transverse end walls in tracheids, the swollen and distorted ray parenchyma, and the zone of radially narrow, heavily lignified cells which mark the position of the xylem mother cells within the cambial zone at the time of wounding. Partially separated secondary walls are probably a sectioning artifact. X325

Figure 8

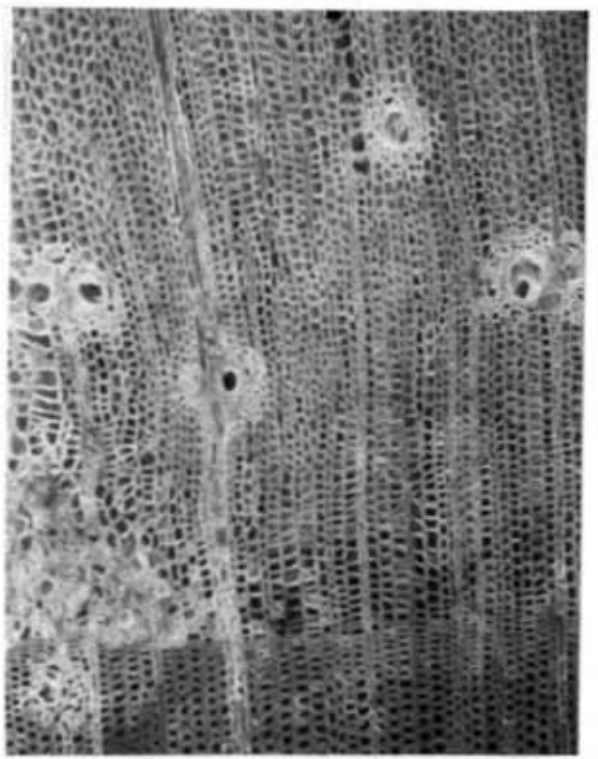


A. Transverse section of UWP-1 white pine xylem (see Figure 2). This is 1975 growth which occurred 2 cm above the entrance into the spiral bridge; 1974 latewood is at the bottom. Note the distribution of the traumatic resin canals (cf. Fig. 8). X51

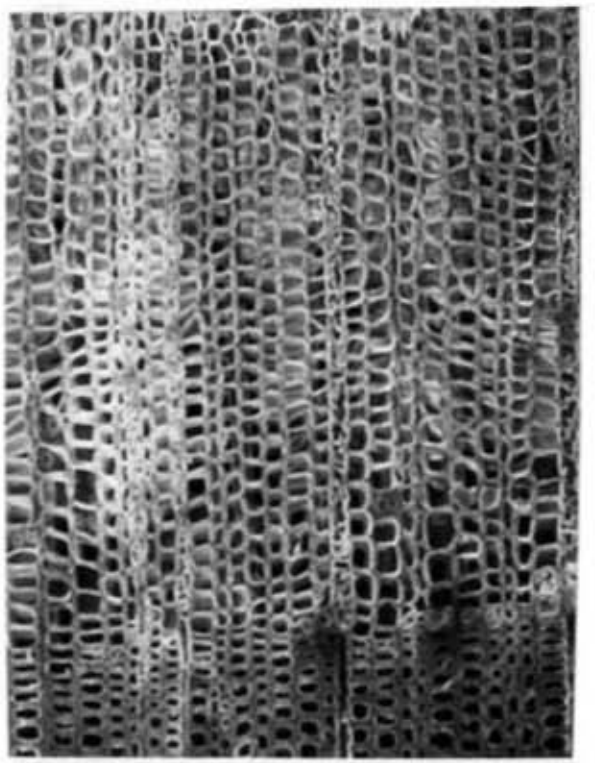


B. Transverse section of UWP-1 white pine xylem formed within the spiral bridge (see Figure 2). The 1974 latewood is below, 1975 bridgewood above. Note the traumatic resin canals and the rapid realignment of the tracheids which has occurred (top of photo). The light area at top was caused by a camera leak. X130

Figure 9

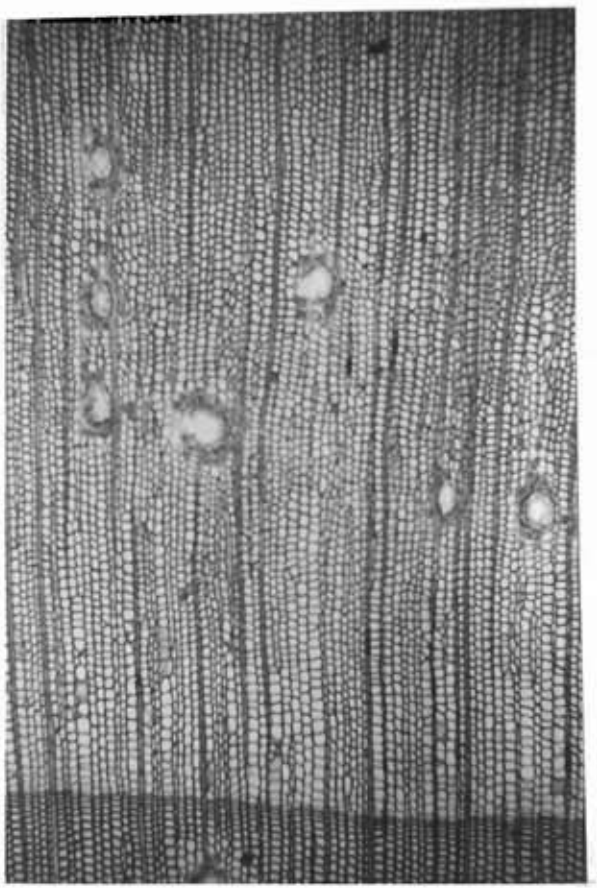


A. Scanning electron micrograph of transverse surface of LWP-1B (see Figure 3). 1975 spiral bridge wood at top; 1974 latewood at bottom. Callus tissue at left denotes the bridge edge. X162

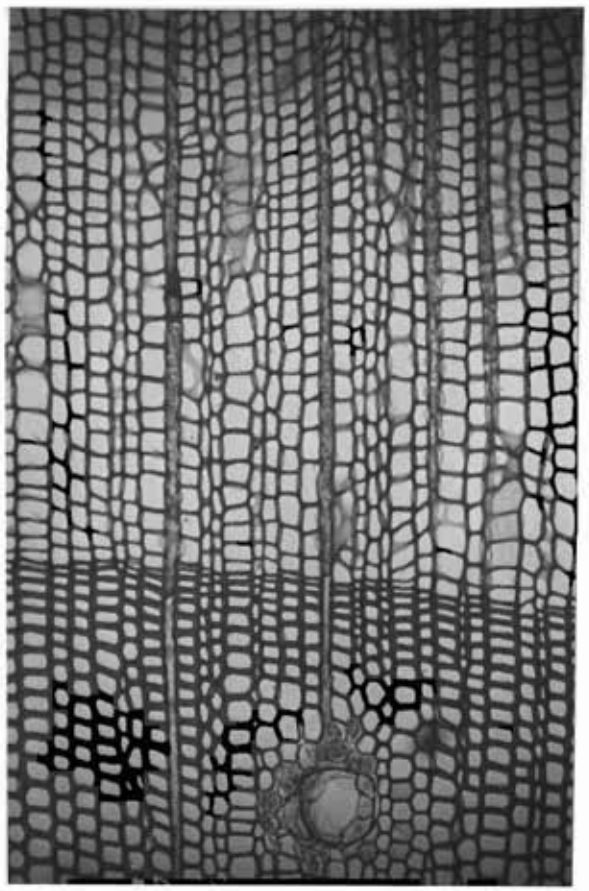


B. Scanning electron micrograph of extreme right portion shown in Fig. 10A. Note border-pitted transverse end walls at different levels within the same radial files. X325

Figure 10



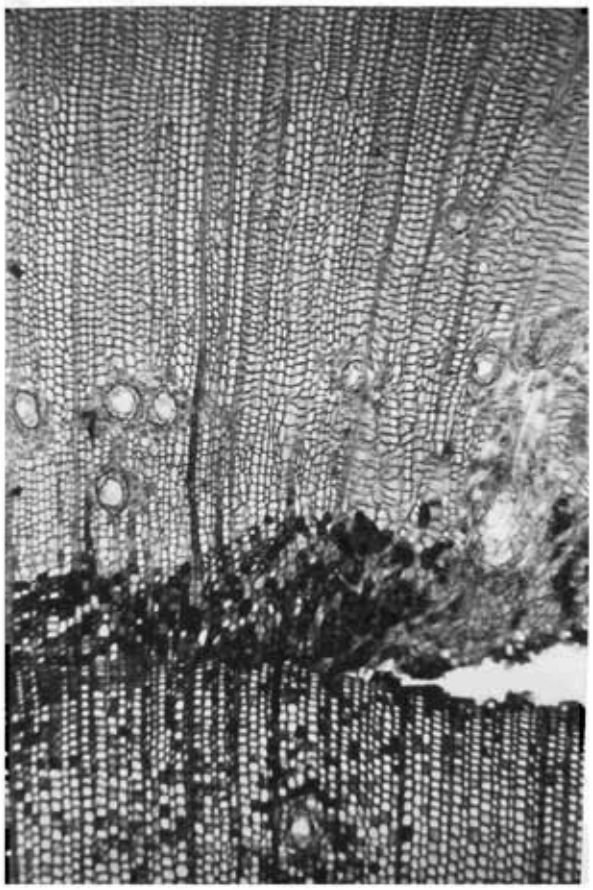
A. Transverse section of white pine LWP-1B spiral bridge earlywood (top) and 1974 latewood (bottom) (refer to Figure 3). Note the maintenance of the alignment of the radial files over the full distance of 1975 bridge growth shown in this photo without marked evidence of reorientation (cf. Figure 9B). X82



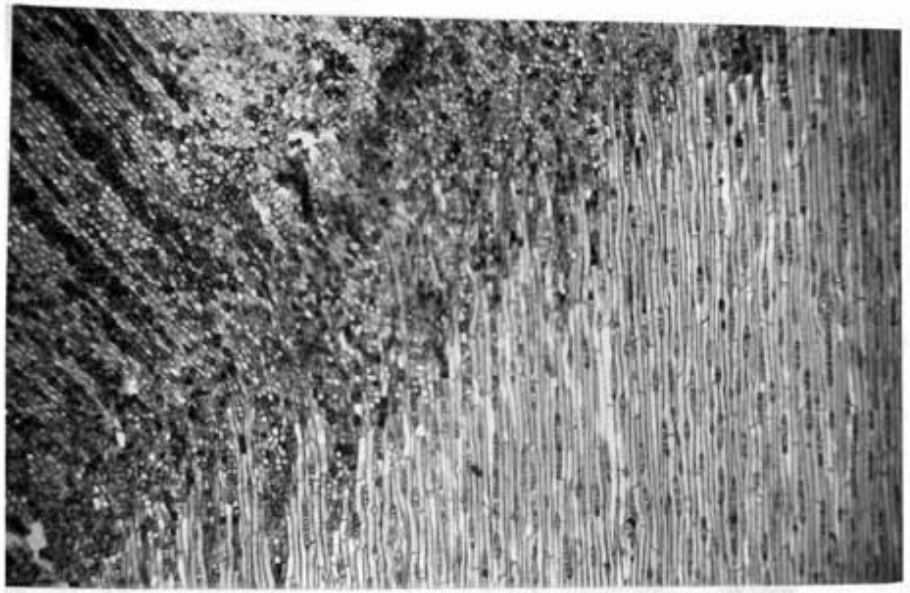
B. A closer look at Fig. 11A. 1974 latewood is at bottom; 1975 spiral-bridge earlywood at top. Note the bulging ray parenchyma and border-pitted end walls of true transverse divisions. X205

Figure 11

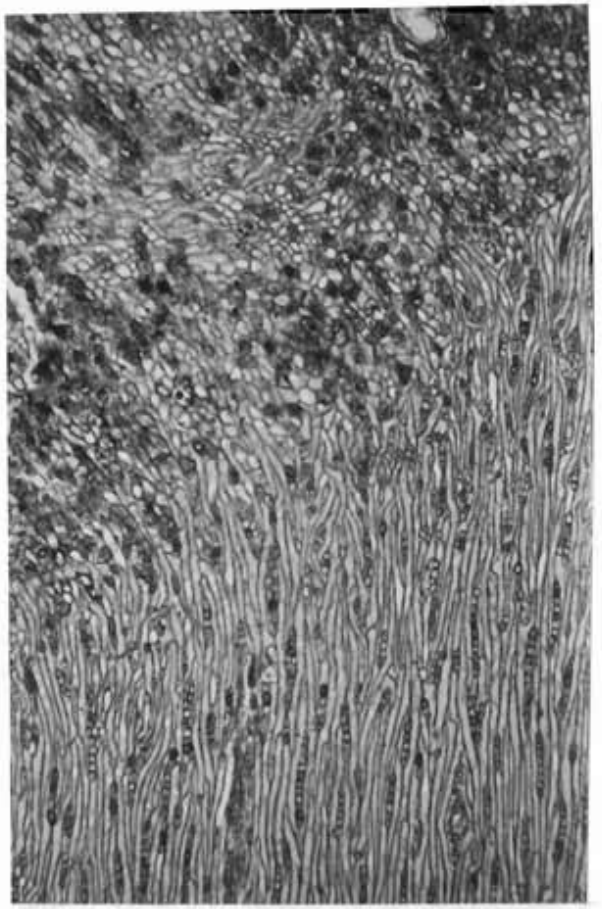
Figure 12



Transverse section of white pine spiral bridge LWP-1B (see Figure 3). 1974 latewood is at the bottom; 1975 spiral-bridge wood is at top. The edge of the spiral bridge is just to the left of the V-shaped open area. The dark mass in lower center is callus tissue. Note latewood tylosites (dark spots) which formed inside of mature tracheids of the previous year. Also note earlywood axial parenchyma at the extreme left. Very sudden reorientation is evident in the tracheids which differentiated from the callus tissue (side walls are evident), but much slower reorientation occurred in the bridge proper. X82

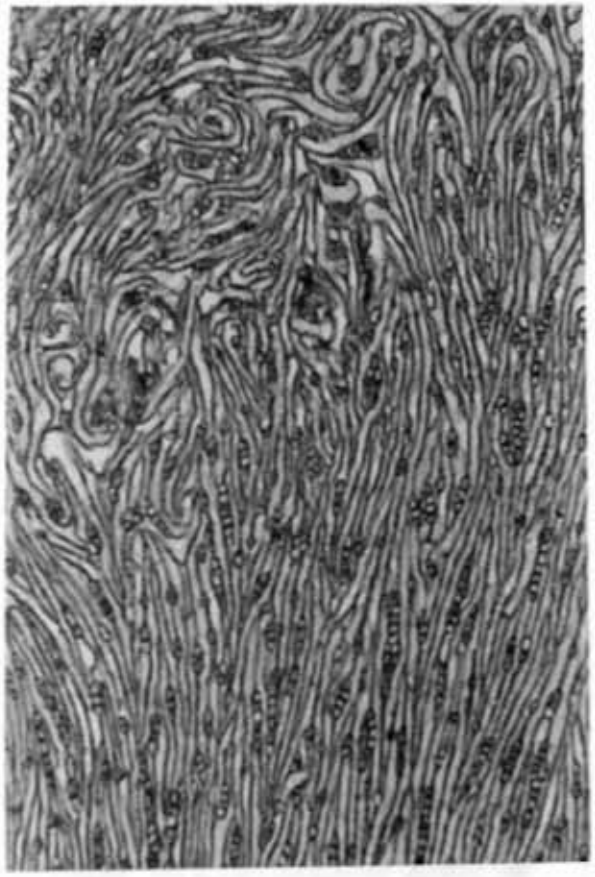


A. Tangential section of white pine LWP-1B spiral bridge xylem (see Fig. 3). The edge of the spiral bridge at approximately 20 days after wounding is evident; the callus tissue which formed over the edge is in upper left. X51

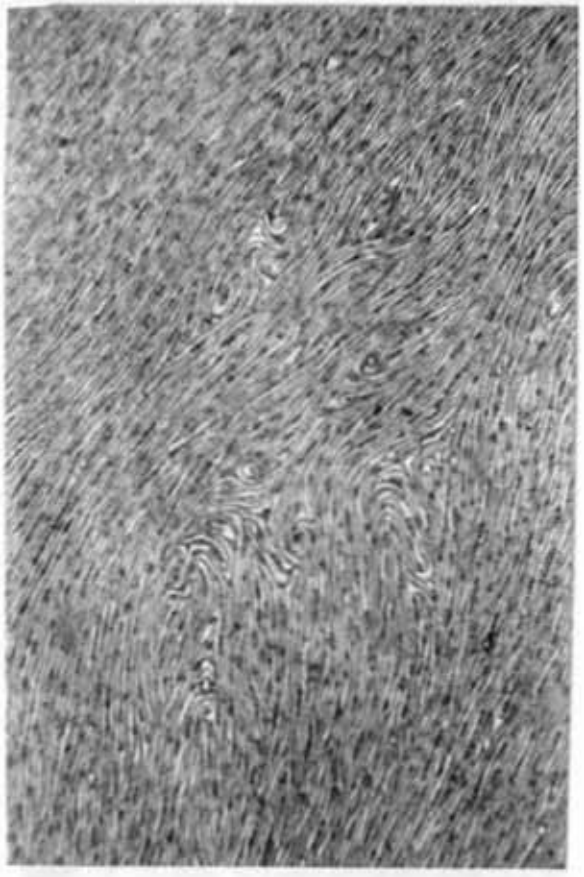


B. Same as Figure 13A, but approximately 40 days after wounding. Callus is differentiating into tracheids which are elongated parallel to the spiral bridge edge. X82

Figure 13



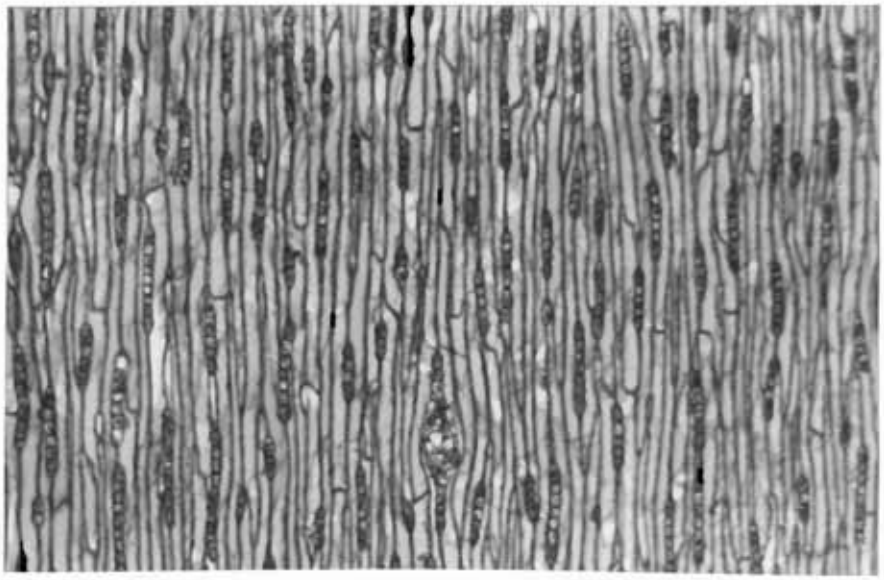
A. Same as Figures 13A and B, but approximately 85 days after wounding. Overcrowded and therefore contorted tracheids have been formed by cambium derived from the callus tissue (upper left) and show a general tendency to be aligned parallel to the long axis of the spiral bridge. X130



B. Same as in previous three Figures, but approximately 140 days after wounding. Tracheids derived from callus (upper left half) have elongated greatly and are mostly parallel to the bridge. The location of the bridge edge can only be guessed at. X51

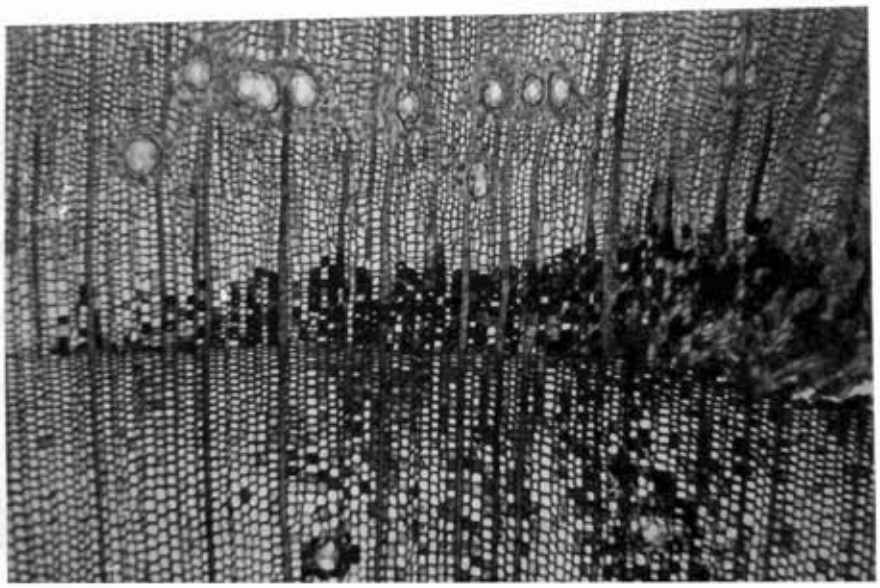
Figure 14

Figure 15



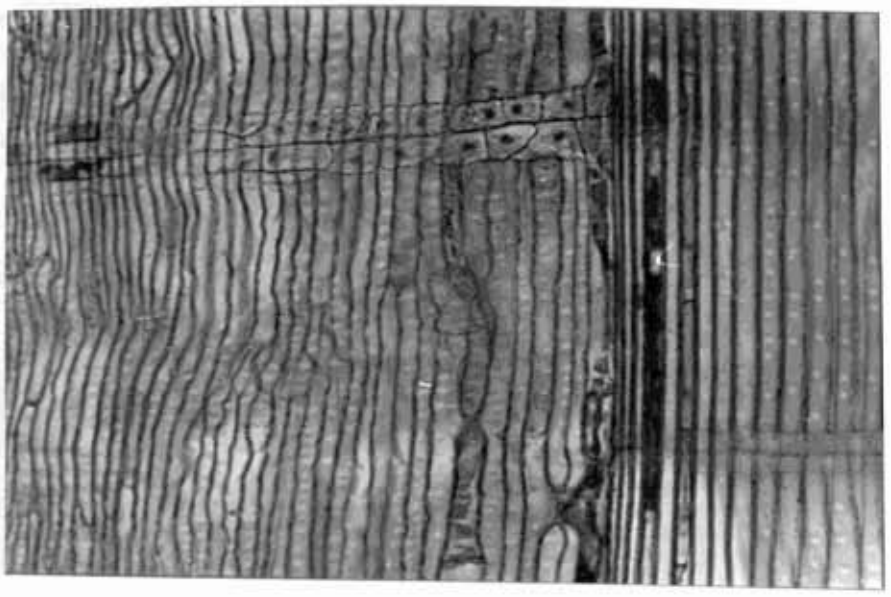
Tangential section of white pine LWP-1B (see Figure 3) showing xylem formed within the spiral bridge which was cut off the cambium at approximately 27 days after wounding. Note that the elongation of tips following transverse, or short oblique, anticlinal divisions in xylem mother cells shows no definite tendency to be polar (final reorientation of the cambium will be upward to the right). X130

Figure 16

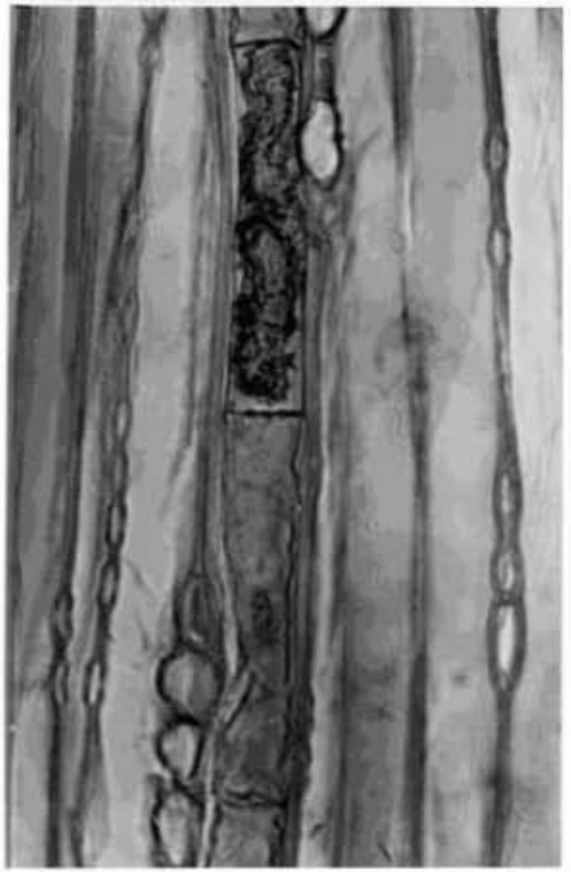


Transverse section of white pine LWP-1B spiral-bridge xylem showing the growth boundary between 1974 and 1975. The tannin-filled tracheids of the 1974 xylem (bottom) contain axial parenchyma cells which formed in 1975 by dedifferentiation of adjacent ray parenchyma. Following penetration into tracheid lumens through cross-wall pits of cytoplasm and nucleus, mitotic divisions occurred to create new parenchyma cells (tylosites) axially oriented within the tracheid lumens. Axial parenchyma also formed in the first part of the 1975 spiral bridge growth, but these were derived directly from the cambium via differentiation of xylem mother cells. The edge of the spiral bridge is at right. X82

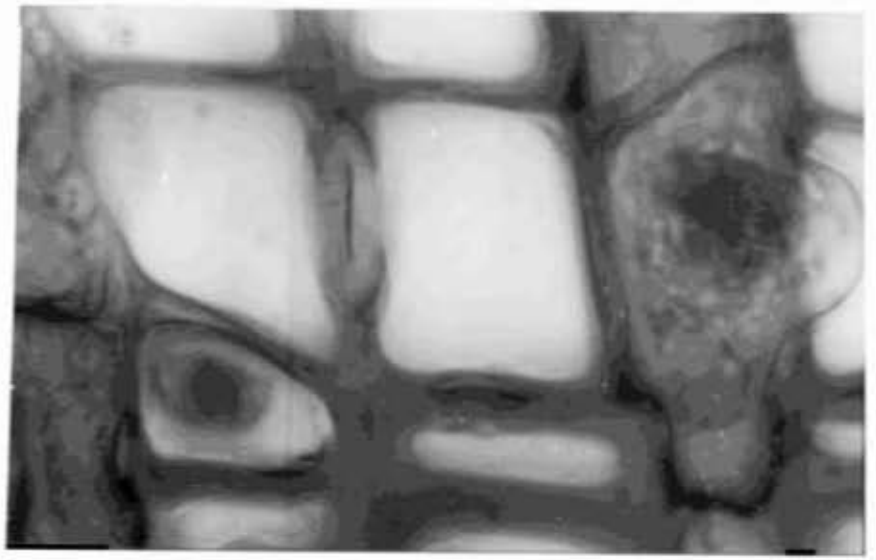
Figure 17



Radial section of white pine xylem. Earlywood formed within the spiral bridge is at left--note the irregular walls and curved nature of the mature tracheids, and the lack of radial elongation of the first formed ray cells. Tannin-filled and more or less empty tylosites are in the 1974 latewood (right). X205



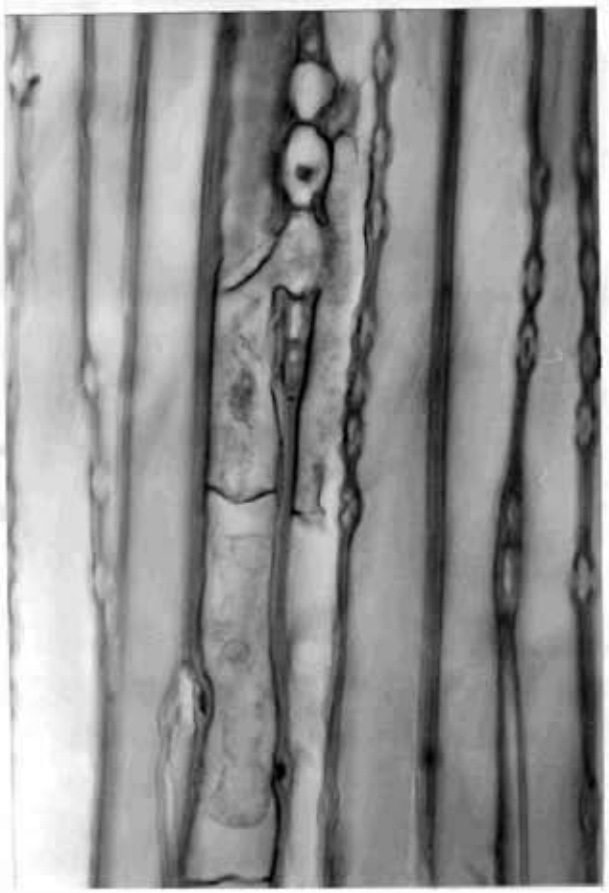
A. Tangential section of latewood axial parenchyma (tylosites) which formed in response to spiral girdling during the next year. Note the nucleus, and the thin walls of the axially-oriented tylosites within the thicker secondary walls of the tracheid. X820



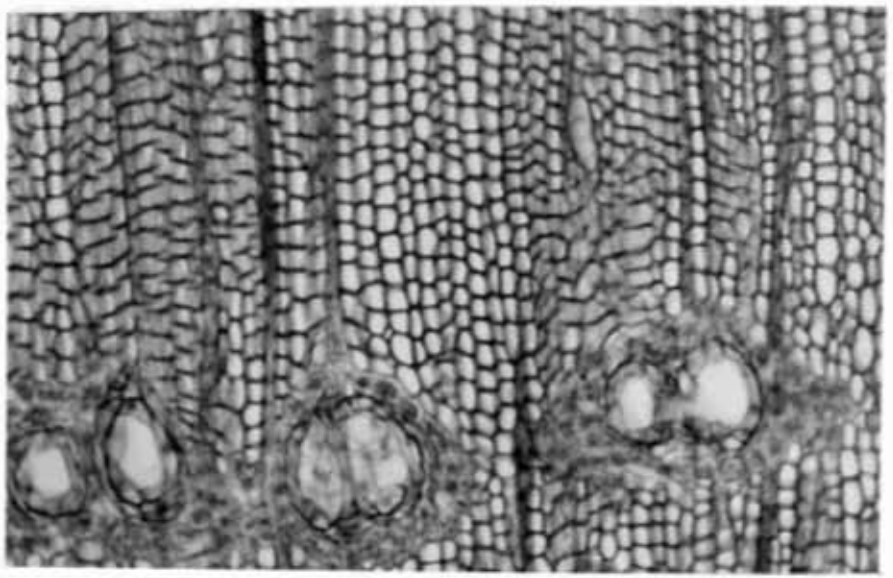
B. Transverse section of 1974-1975 xylem boundary in white pine; latewood is at bottom, earlywood at top. On the left is the nucleus and cytoplasm of a primary-walled parenchyma cell which has bulged into the final latewood tracheid lumen from the adjacent ray. On the right is a ray parenchyma bulging into an earlywood tracheid lumen. X3250

Figure 18

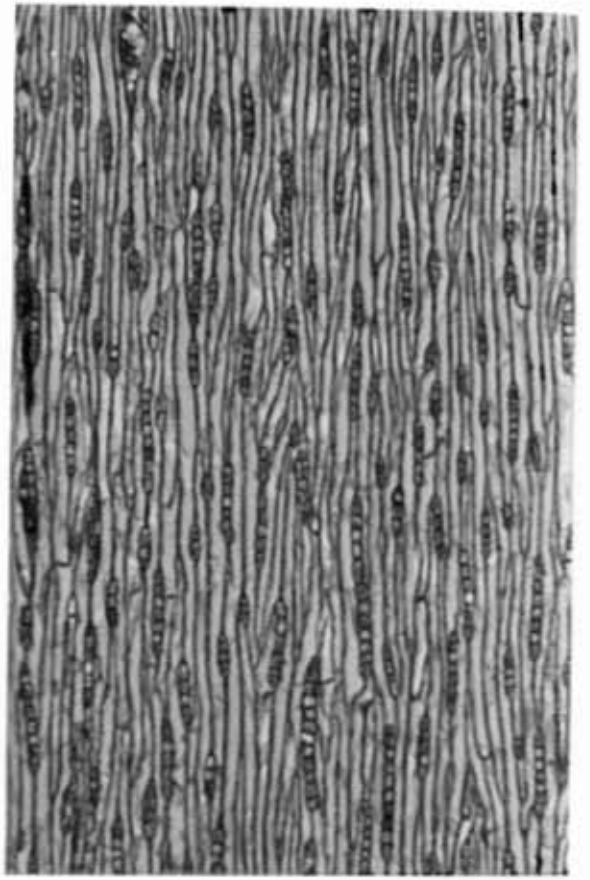
Figure 19



Tangential section of 1974 white pine latewood xylem showing axially oriented tylosite parenchyma formed in the lumens of dead, secondary-walled tracheids as a result of spiral girdling in the spring of 1975. The ray parenchyma expanded through its half-bordered pit into the tracheid lumen, and then divided. Two nuclei are visible. X820

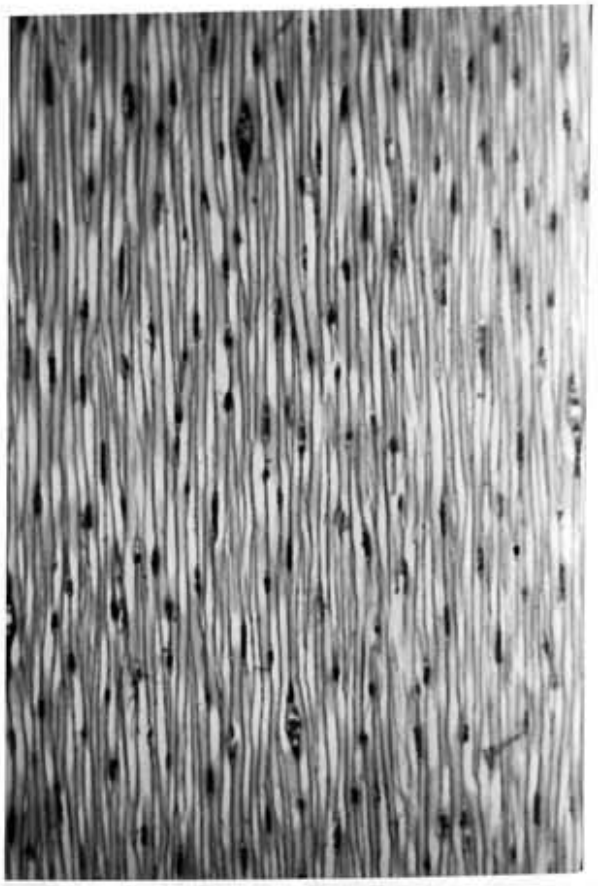


A. Transverse section of white pine spiral-bridge earlywood xylem (pith toward bottom). Microdomains of reorientation began to form at about the same time that traumatic resin canals were differentiated. The side walls of the microdomains indicate that their alignment is different from that of adjacent cells.



B. Tangential section of white pine xylem formed early in a spiral bridge showing the beginnings of a microdomain of reorientation (center). Note the extreme shortness of the strongly reoriented tracheids as a result of successive parallel oblique anticlinal divisions. x 130

Figure 20



A. Tangential section of white pine 1974 latewood xylem of UWP-1 (see Figure 2) which was formed previous to girdling to construct the spiral bridge in the spring of 1975. X82

B. Same magnification, and tangentially in the same location, as Figure 21A. Note the very narrow tangential dimensions of the tracheids which have derived from the reoriented cambium. Portions of a traumatic resin canal are also evident (lower left and center). X82

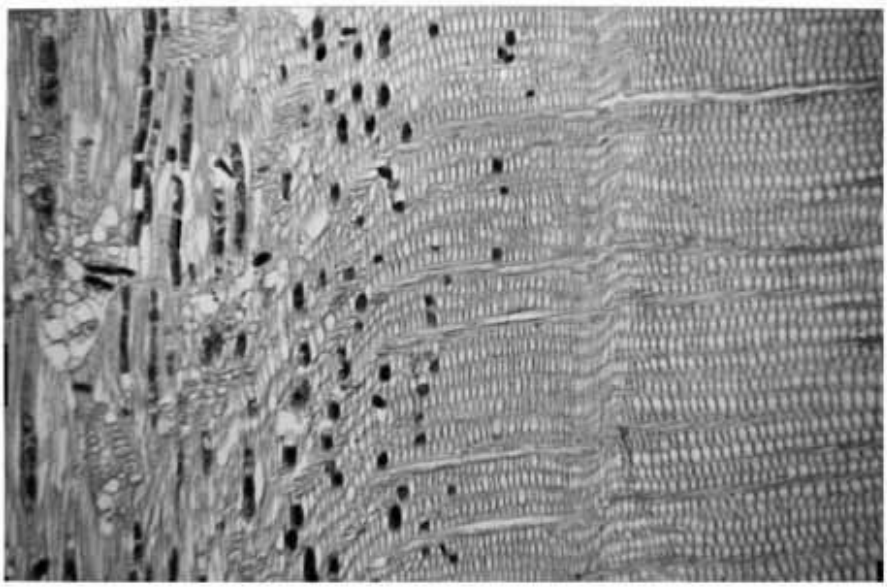
Figure 21

A. Tangential section of white pine LWP-1B xylem (see Figure 3) showing the beginning (most pithward cells) of Series 1 (Appendix). The fusiform ray in the center matches that of the Series tracings. Note the latewood tylosites. X82

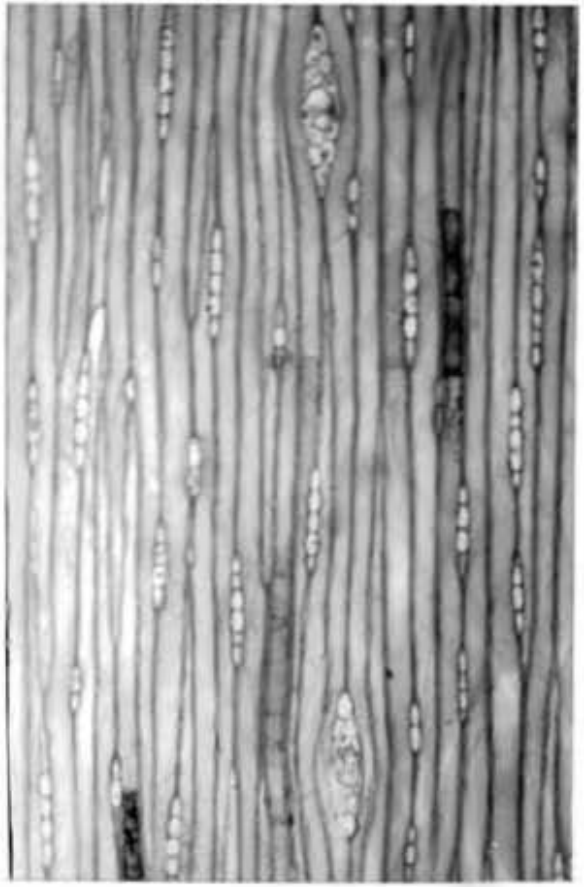
B. Tangential section of white pine LWP-1B xylem at the end of Series 1; i.e. section 118. Note that the three fusiform rays of Figure 22A have been reoriented but have maintained their relative positions. X82

Figure 22

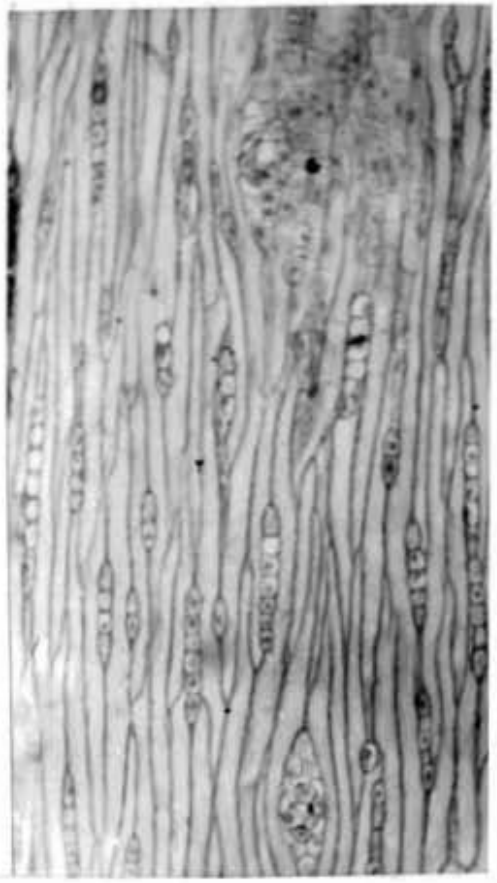
Figure 23



Radial section of white pine spiral-bridge xylem (right), cambial zone (just to the right of center) and phloem (left half). Axially oriented phloem on the extreme left gives an idea of how much the cambium and its derivatives have reoriented. X82

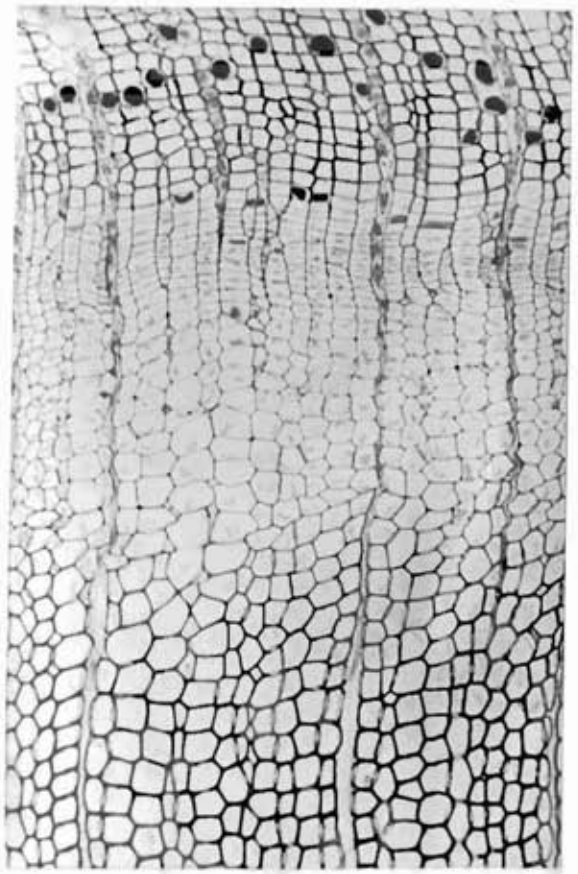


A. Tangential section of 1974 latewood in white pine spiral bridge UWP-1, showing the start (most pithward cells) of Series 6 (Appendix). See Figure 2. X205

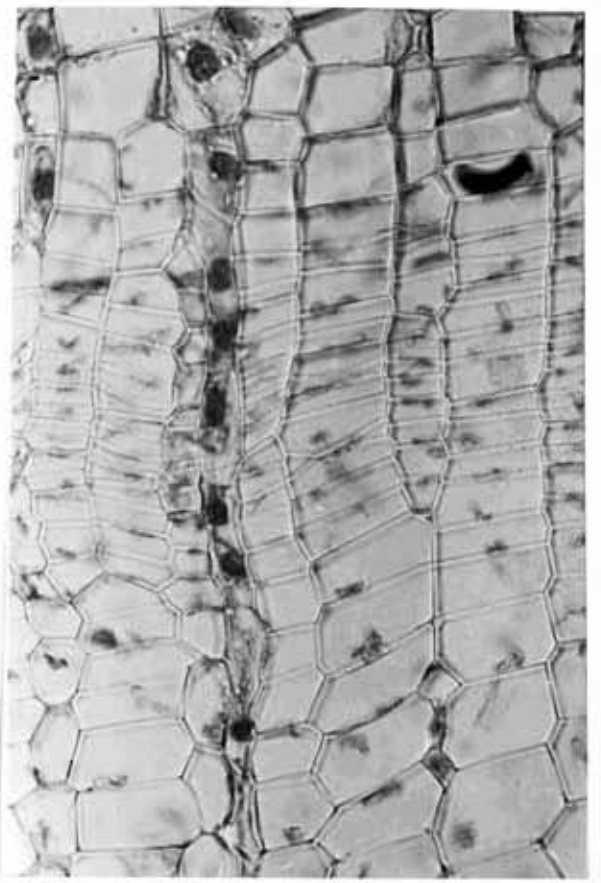


B. Tangential section of 1975 spiral-bridge early wood of white pine UWP-1, showing section 58T1, i.e. the end of Series 6. A traumatic resin canal is beginning to appear in upper center. X205

Figure 24



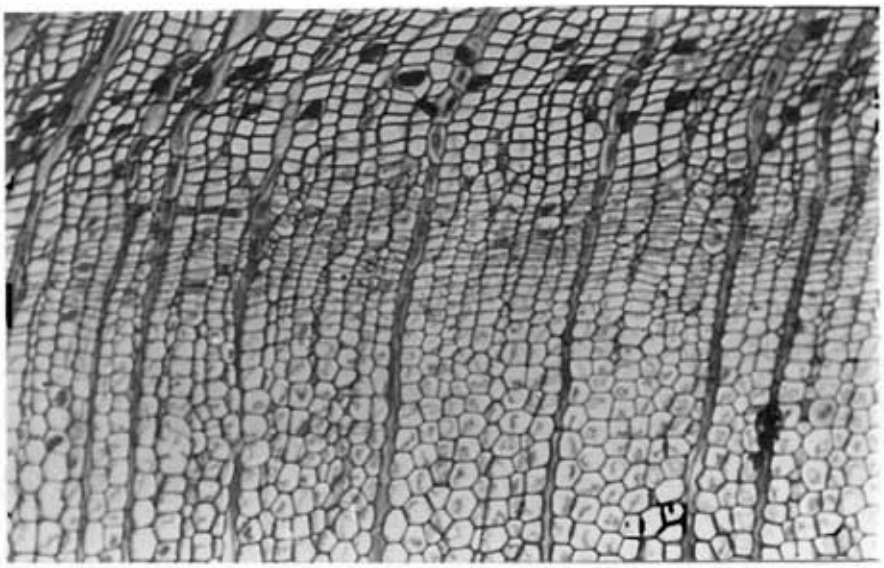
A. Transverse section of the cambial zone (phloem at top) within a lodgepole pine diagonal bridge at 2 days after its construction. Recent intrusive growth and anticlinal divisions are evident as doubling of radial files. X205.



B. Close up of Figure 25A. A pseudotransverse anticlinal division has resulted in 2 files of cells (left) where before there was only one; and intrusive growth is also evident (right) as doubling of files by tangentially narrow tips. A differentiating phloem axial parenchyma is evident in upper right. X820.

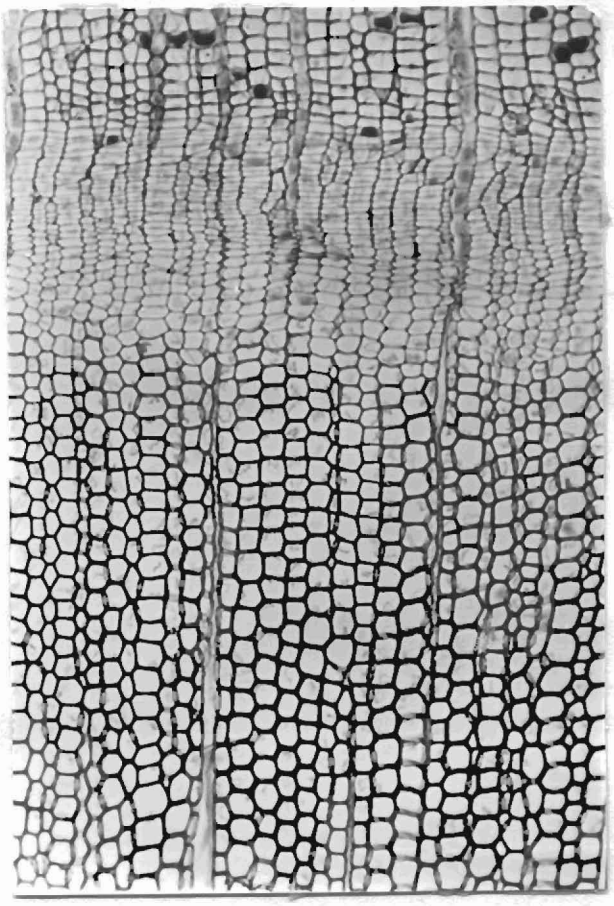
Figure 25

Figure 26

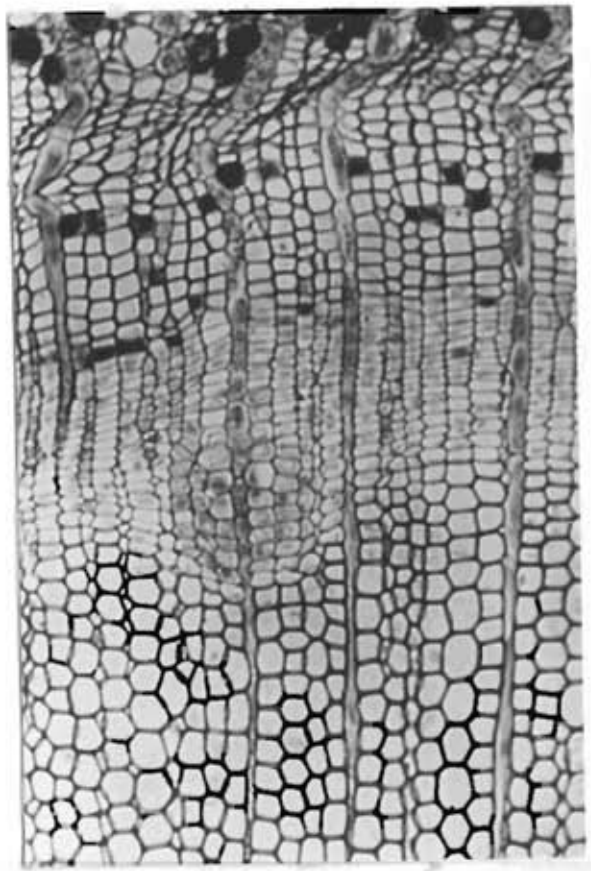


Transverse section of the cambial zone within a lodgepole pine vertical bridge which was harvested and fixed 2 days after the bridge was constructed. The phloem tissue is at top. The edge of the vertical bridge is just off the left side of the photo. The curvature seen is probably a sectioning artifact. X205.

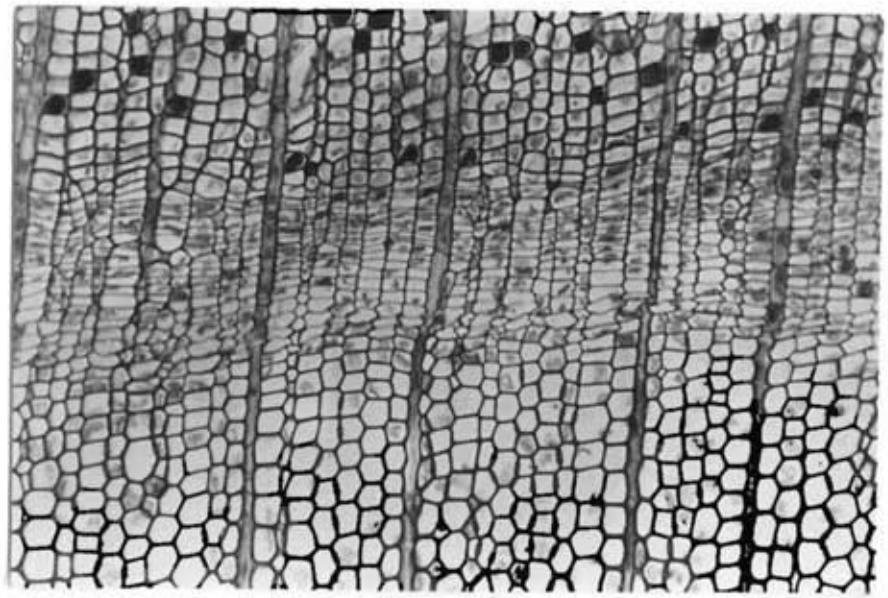
Figure 27



Transverse section of the cambial zone of lodgepole pine within a diagonal bridge which was harvested and fixed at 8 days after bridge construction. Note the decline of 4 files of cells on the left side of the photo, and how adjacent fusiform cells in the cambial zone have expanded tangentially to occupy the vacated space. Three radial files to the right of the center ray within the photograph (this ray is evident in the phloem at top, but not in the mature xylem at bottom), a single anticlinal division in the cambial zone is thought to denote the position of the fusiform initial. X205



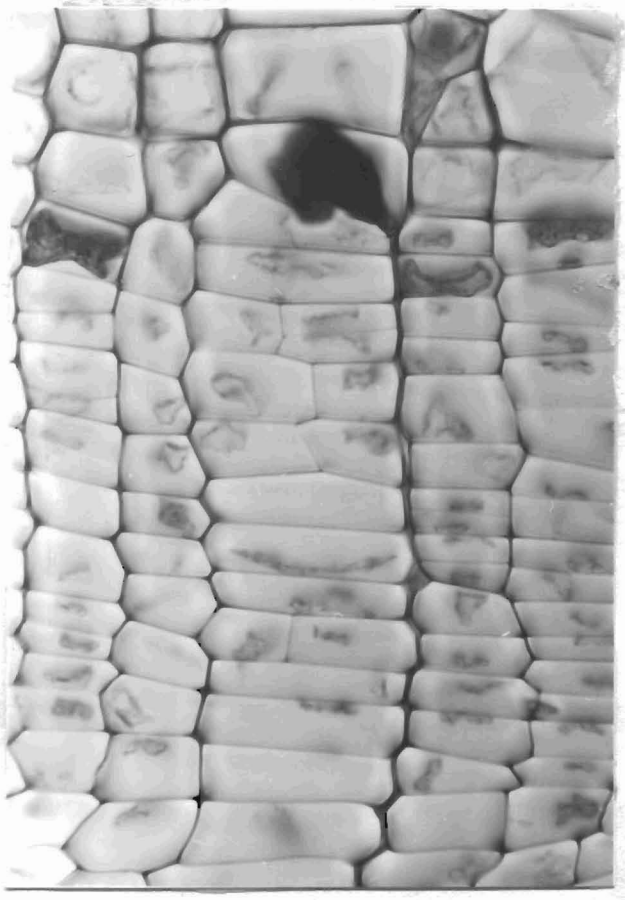
A. Transverse section of the cambial zone within a lodgepole pine diagonal bridge at 10 days after girdling. Note the differentiating resin canal. In the cambial zone on the extreme right side of the photograph, one file has declined and the initial to the left has divided anticlinally, followed by periclinal divisions in both sister initials. X205



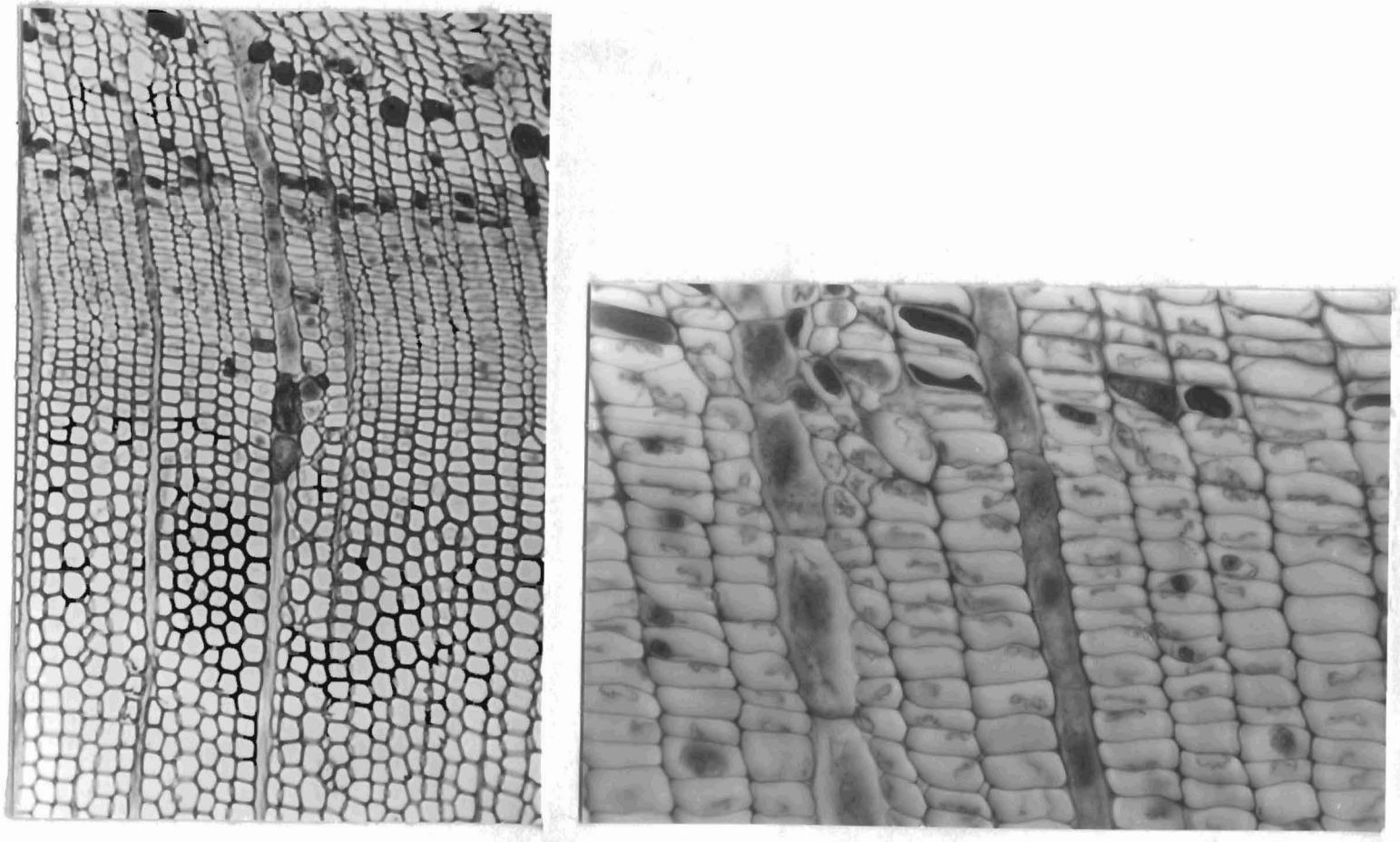
B. Transverse section of the cambial zone within a lodgepole pine vertical bridge at 10 days after girdling. Normal variability in the size of mature tracheids is evident. Note the numerous anticlinal divisions which have recently occurred (doubling of radial files). A sectioning artifact extends across the photograph on the xylem side (lower) adjacent to the cambial zone. X205

Figure 28

Figure 29



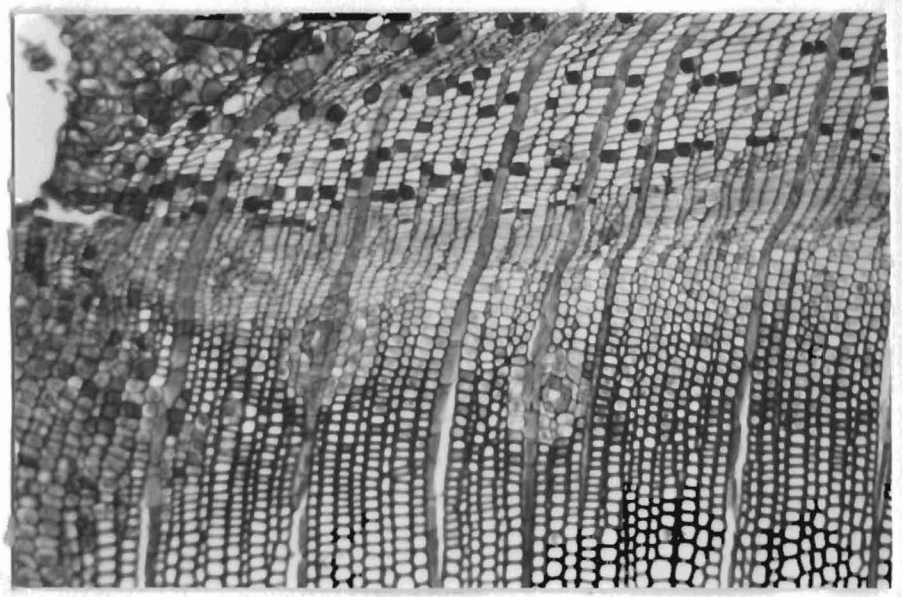
Transverse section of the cambium of a lodgepole pine diagonal bridge which was harvested and fixed 10 days after bridge construction. Note the four anticlinal walls within the single radial file. The phloem is at top. The lower anticlinal division has occurred in a xylem mother cell; whereas the three upper anticlinal walls represent periclinal divisions following an anticlinal division in a fusiform initial. X1300



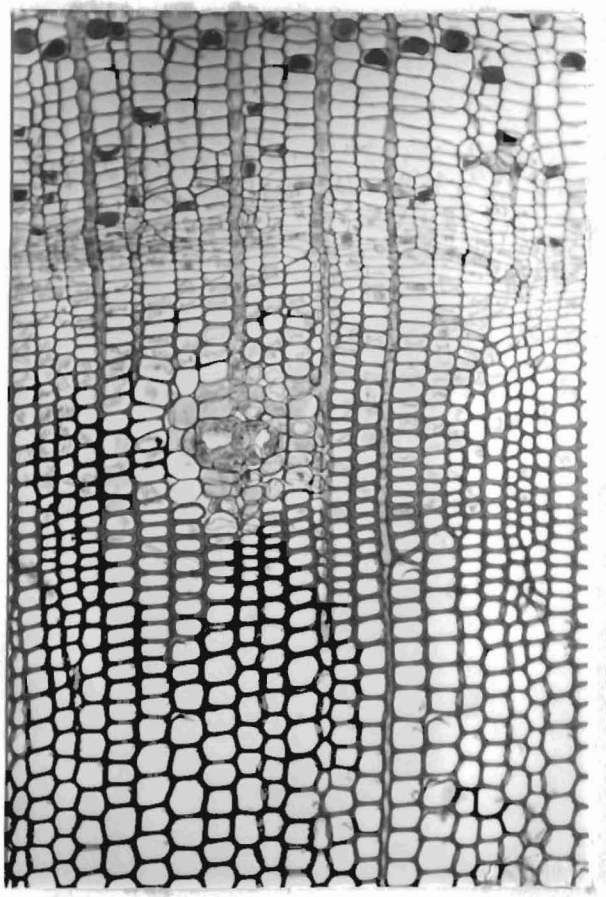
A. Transverse section of the phloem (top), cambial zone, and xylem of a lodgepole pine diagonal bridge which was harvested and fixed at 16 days following bridge construction. Note the swollen, tannin-filled xylem rays; and note also that not all rays are swollen. The cells of narrow radial dimensions which have begun to lignify mark the position of the xylem mother cells within the cambial zone at the time of wounding. X205

B. Transverse section of the cambial zone shown in Figure 30A. The position of four fusiform initials is evident by the four anticlinal divisions. Note that three of them are separated from phloem axial parenchyma by a single phloem mother cell. X820

Figure 30



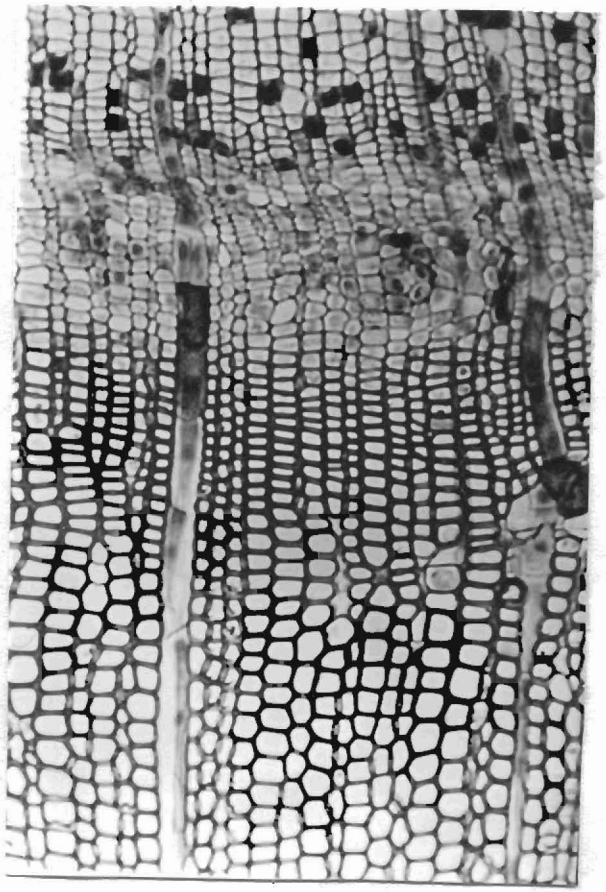
A. Transverse section of the cambial zone (phloem at top) of lodgepole pine at the edge (left side) of a vertical bridge harvested and fixed 38 days after bridge construction. Callus tissue has formed at the left edge. The position of the xylem mother cells at the time of wounding is evident in the radially narrow band of tracheids. Note that transverse end walls in the xylem are very scarce. X130



B. Same as Figure 31A, but near the center of the vertical bridge. The position of xylem mother cells of the cambial zone at the time of wounding is clearly marked in the mature xylem immediately below the resin canal. The radial file to the left of and adjacent to the ray which begins at the resin canal shows an anticlinal division which occurred in a phloem mother cell. X205

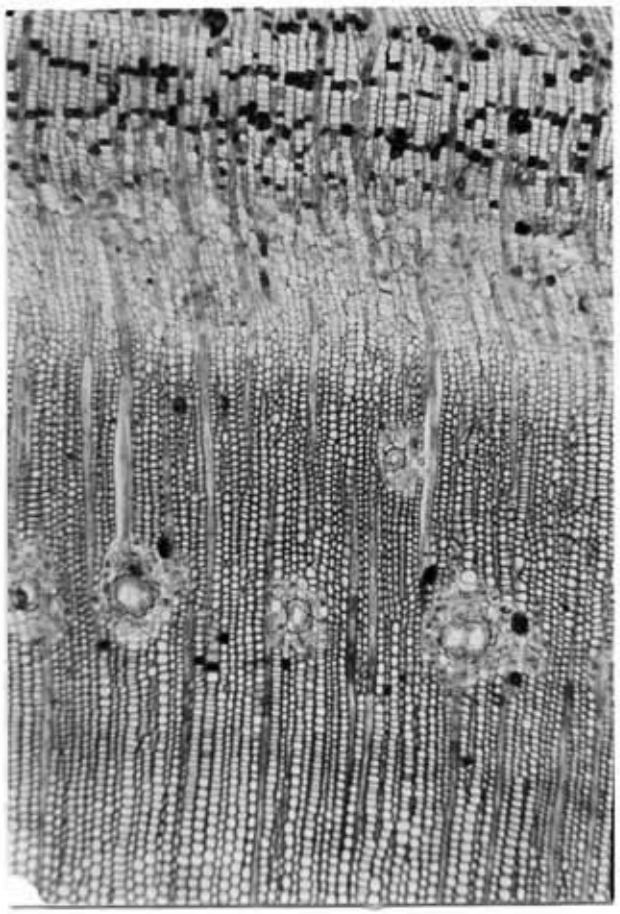
Figure 31

Figure 32

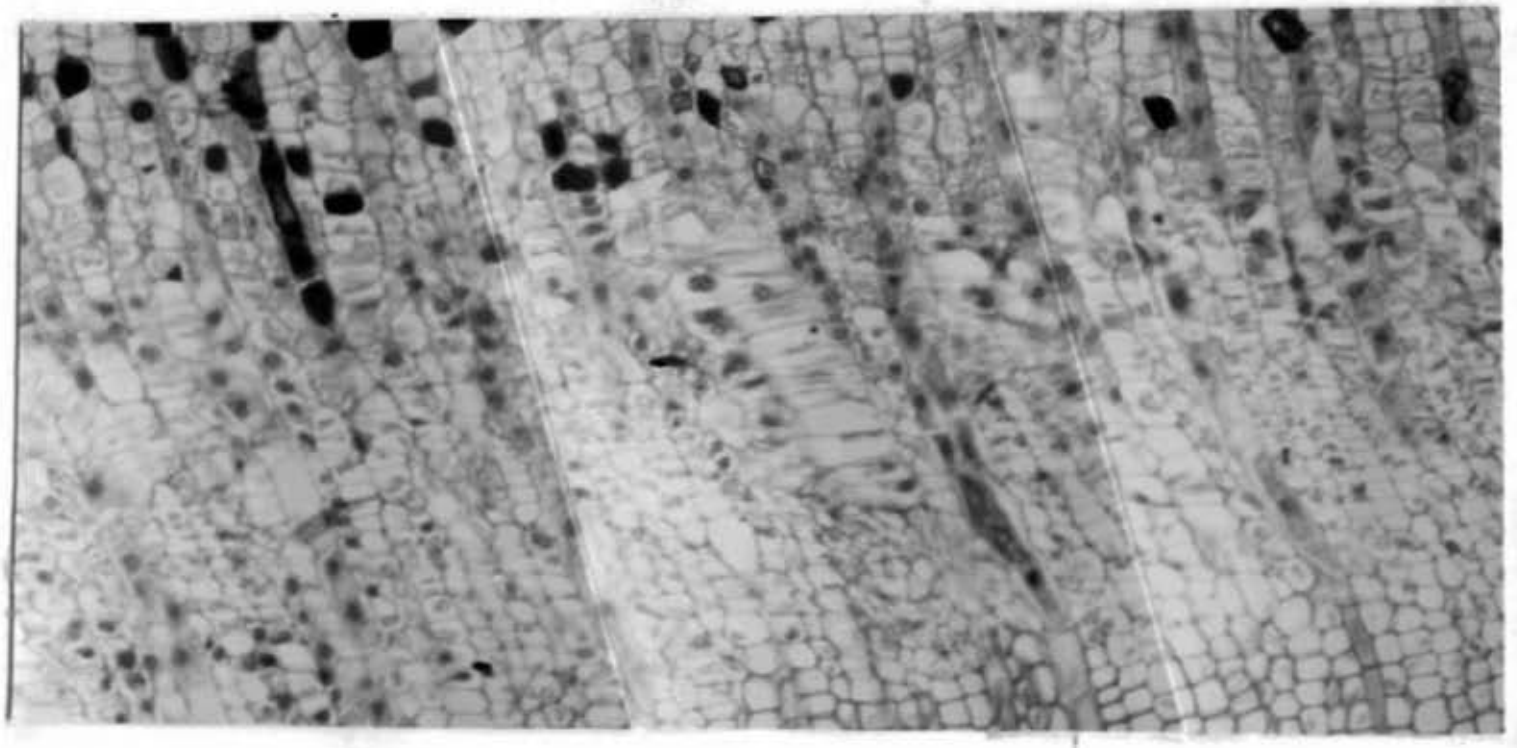


Transverse section of the cambial zone within a lodgepole pine diagonal bridge which was harvested and fixed at 26 days after bridge construction. Note the heavily lignified and radially narrow zone of mature tracheids which mark the position of the xylem mother cells within the cambial zone at the time of wounding. One border-pitted transverse end wall is barely discernible within this zone. Two traumatic resin canals are differentiating within xylem mother cells of the cambial zone (phloem at top). Note the swollen rays with abnormal ergastic contents, and the anticlinal division which occurred in a ray cell. X205

Figure 33



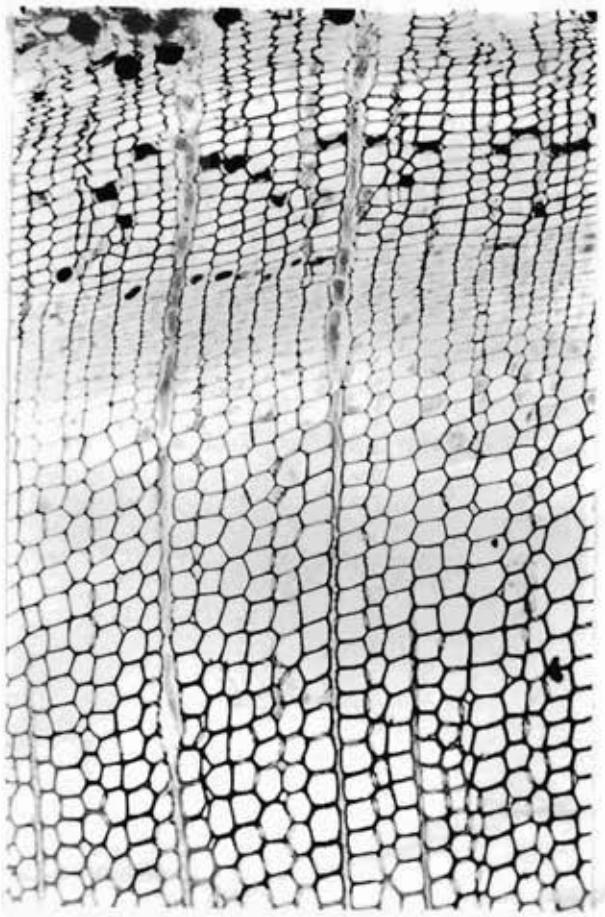
Transverse section of xylem, cambium and phloem (top) of lodgepole pine diagonal bridge which was harvested at 50 days after bridge construction. The position of the xylem mother cells of the cambial zone at the time of girdling is evident near the bottom of the photograph. Note that the radial files are continuous on the xylem side up to the cambial zone; and that they decline or double by the usual events of failure, intrusive growth and anticlinal divisions. The blurred regions in the present cambial zone which extend from the phloem downward across the cambial zone to the xylem are microdomains of reorientation. Note that adjacent regions show no definite reorientation. Note that there is no similar wound zone in the phloem as occurs in the xylem. The slight curvature of the files in the region of the cambium is thought to be a sectioning artifact. X82



Transverse section of the cambial zone (phloem at top) showing a higher magnification of the microdomains of reorientation, as shown in Figure 33. Note that the radial files are continuous across the cambial zone from xylem to phloem except in regions where microdomains have formed. Reorientation within the central microdomain occurred as a result of failure of at least three adjacent fusiform initials, as is evident in the interruption of the radial files in that region. All fusiform cambial cells have relatively small tangential dimensions as a result of numerous long oblique anticlinal divisions multiplying long fusiform initials into many shorter ones. Note the abundance of nuclei within the cambial zone--an indication of much activity. To right of center there appears to be a microdomain within the phloem but not in the cambium or xylem. Other microdomains appear to just be forming in the cambial zone. X325

Figure 34

Figure 35

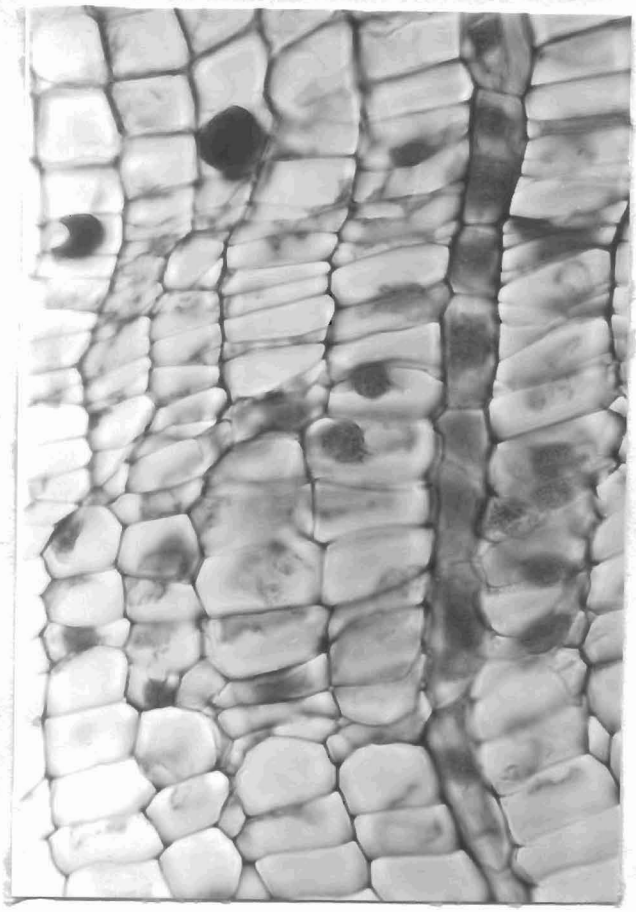


Transverse section of the cambial zone (phloem at top) within a lodgepole pine diagonal bridge which was constructed 6 days previous to harvesting. The first wound reaction was apparent in the swelling of some, but not all, ray parenchyma near the cambial zone (cf. Figures 8 and 11B). X205

Figure 36

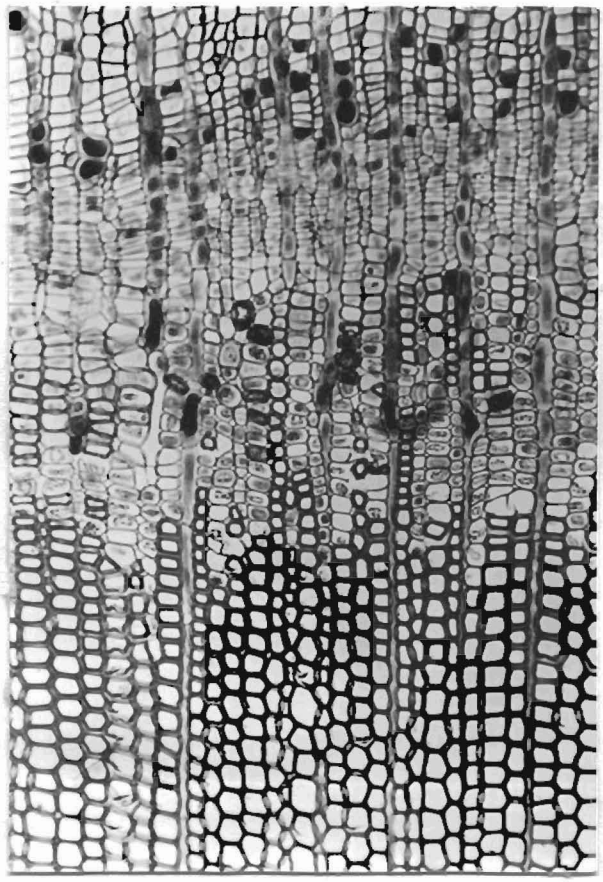
Radial section of the cambium within a lodgepole pine diagonal bridge which was constructed 10 days previous to harvesting and fixing. The cambial initial is one of the cells on the left side of the photograph; xylem mother cells are on the right side. The transverse divisions as seen here occurred more or less simultaneously in already existing xylem and phloem mother cells; and were first detected in material harvested and fixed 8 days after girdling, scattered throughout those tissues in each radial file. Note that periclinal divisions occasionally follow the transverse divisions; thus the productivity of a xylem mother cell in terms of periclinal divisions can be determined. As a result of this observation, it was determined that most xylem mother cells differentiate directly into tracheids; occasionally they divide periclinally to result in two tracheids; and very rarely three or four tracheids result from a single xylem mother cell. X820

Figure 37



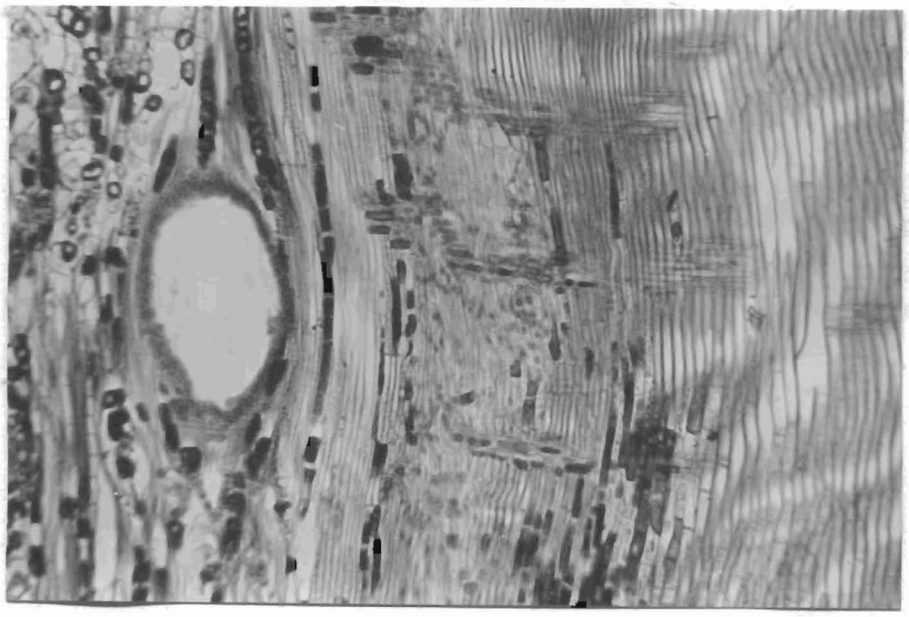
Transverse section of the cambium within a lodgepole pine vertical bridge which was harvested and fixed 10 days after bridge construction. (cf. Figure 29). The phloem at top is distinguishable by its axial parenchyma. A traumatic resin canal is differentiating out of the xylem mother cells of the cambial zone at bottom. The center of the large resin canal is characterized by the four relatively large central cells (on the left side of the ray). X820

Figure 38



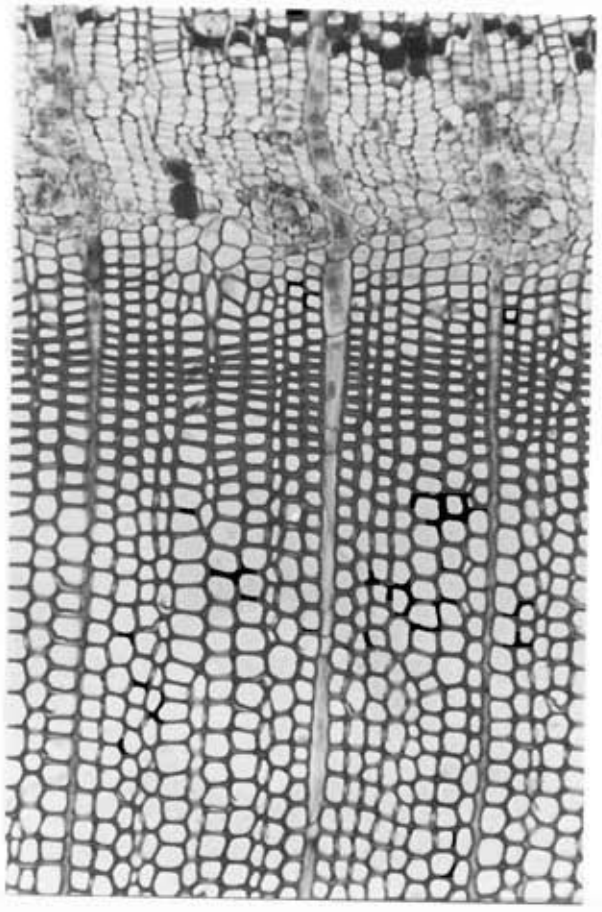
Transverse section of the cambial zone (phloem at top) within a lodgepole pine diagonal bridge constructed 24 days before harvesting and fixing. Mature, heavily lignified tracheids are interrupted within the radial files by cells which appear to have only primary walls and no lignification. This type of wound response was atypical, and occurred in only four of the 18 trees examined. Some of the cells are filled with dark-staining ergastic contents interpreted to be tannins. X205

Figure 39

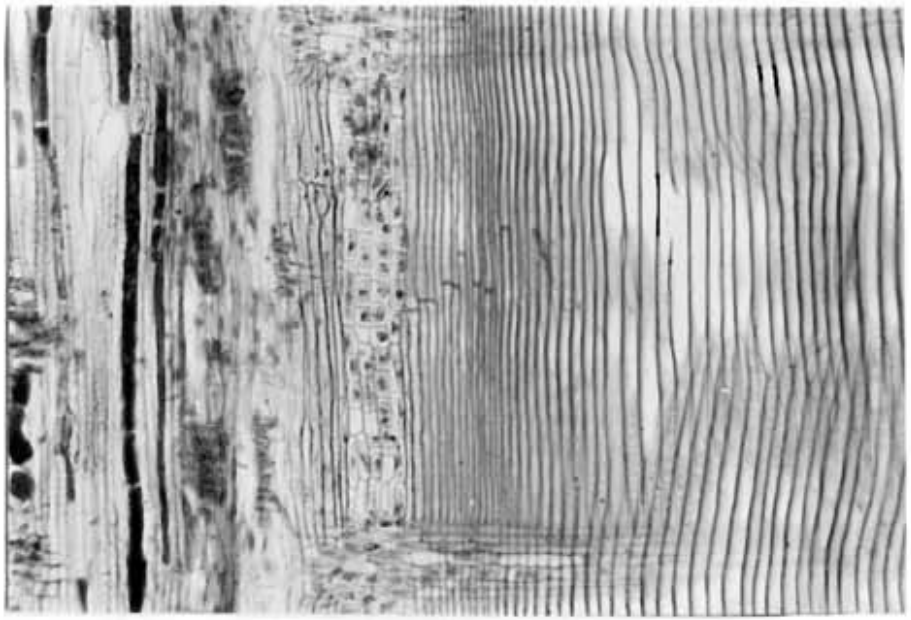


Radial section of phloem (left), cambial zone (center) and xylem (right) of a lodgepole pine diagonal bridge which was constructed 30 days previous to fixation. The nuclei of the cambial zone distinguish it. The large resin cyst in the phloem is normal; Chafe (1969) states that these are responsible for the xylem dimples which are commonly found in lodgepole pine wood. Note the scattered transverse divisions in tracheids and the numerous tannin-filled axial parenchyma in the xylem. X82.

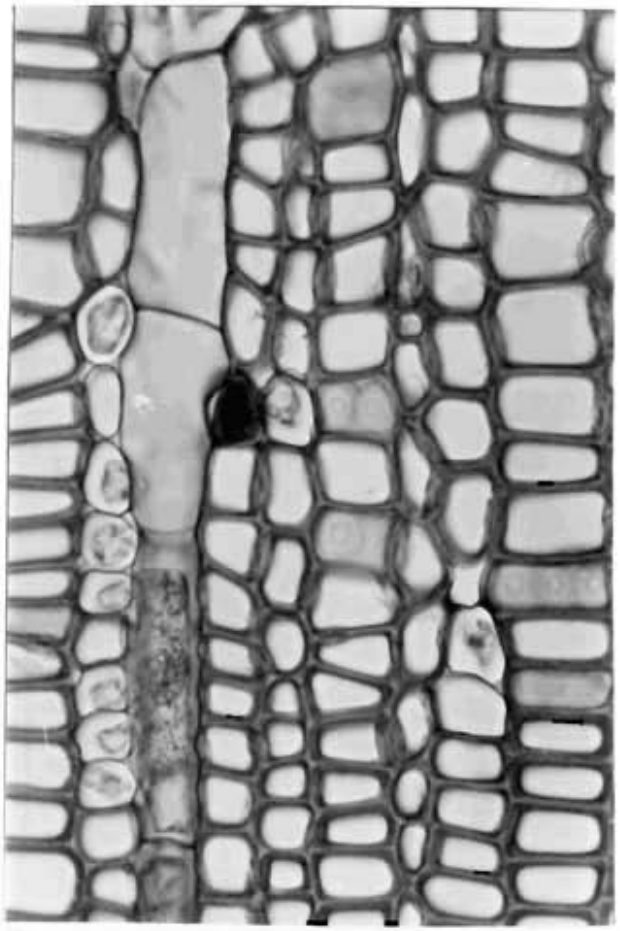
Figure 40



Transverse section of phloem (top), cambium and xylem of lodgepole pine diagonal bridge that was constructed 28 days previous to harvesting and fixation. The position of the xylem mother cells within the cambial zone at the time of wounding is demarcated by the zone of cells which show very slight radial enlargement but relatively heavy lignification. Note the swollen ray parenchyma, the two axial parenchyma adjacent to the cambial zone within the xylem, and the three traumatic resin canals. X205.



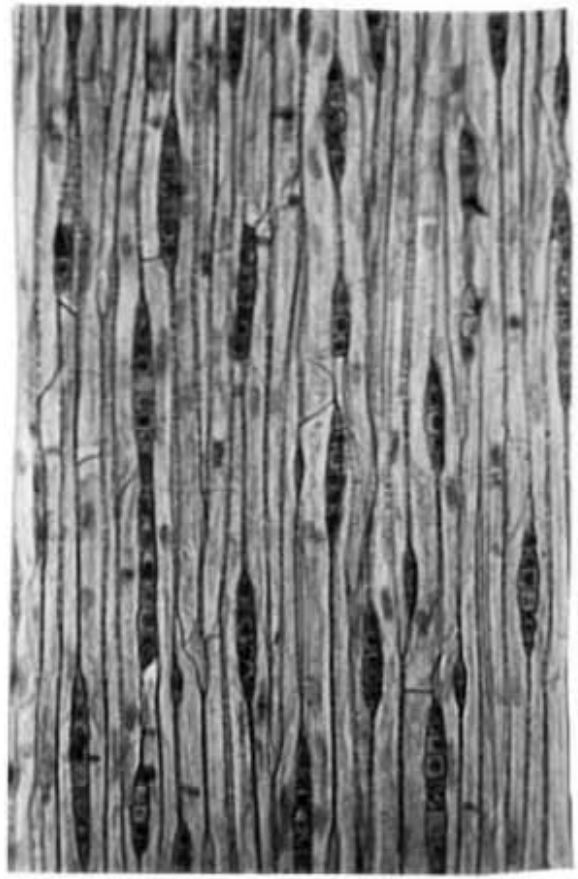
A. Radial section of phloem (left), cambial zone and xylem of a lodgepole pine diagonal bridge which was constructed 32 days previous to harvesting and fixation. The position of the cambial zone at the time of girdling is demarcated by the narrow diameter, heavily lignified cells; and by the first transverse divisions. A traumatic resin canal extends from top to bottom across the photo. Note the variability in size and position of the dividing walls (transverse) of the parenchyma of the resin canal. X130



B. Transverse section of same material as that shown in Figure 41A, showing the position of the cambial zone at the time of girdling, and border-pitted transverse end walls, swollen rays, and primary-walled axial parenchyma which differentiated from xylem mother cells. X820

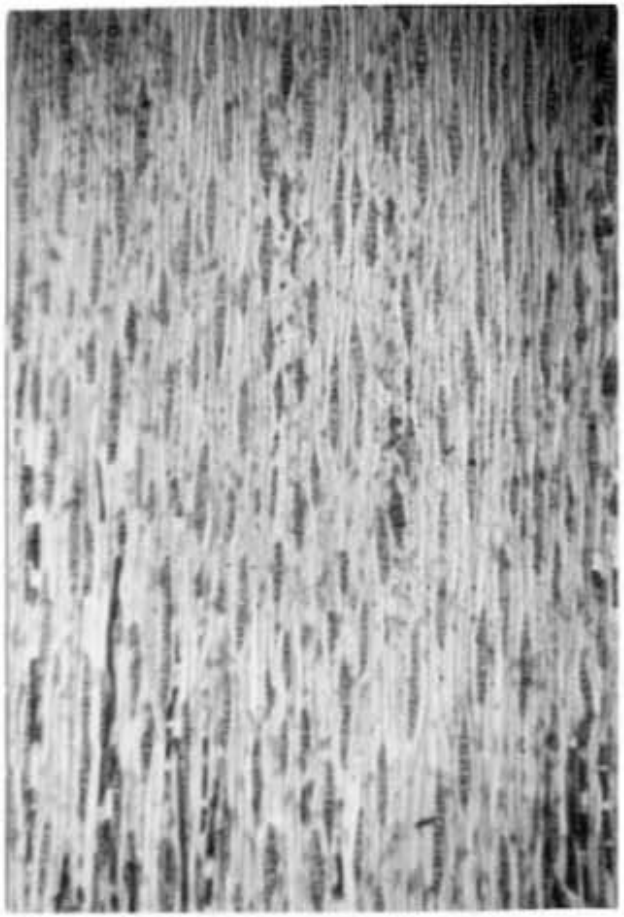
Figure 41

A. Tangential section of lodgepole pine phloem mother cells within a diagonal bridge which was constructed 6 days previous to harvesting and fixation. X82

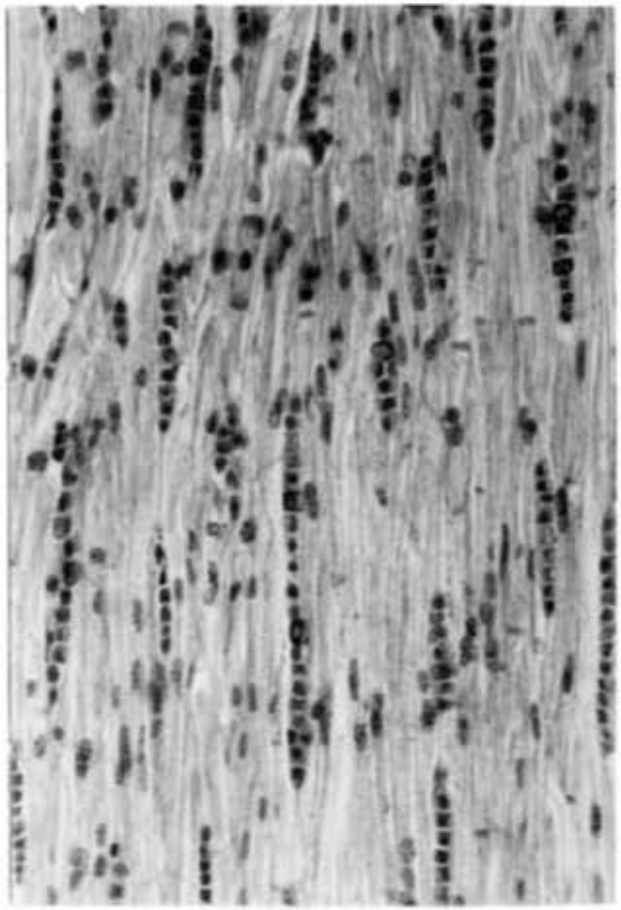


B. Tangential section of lodgepole pine xylem mother cells within a diagonal bridge which was constructed 12 days previous to harvesting and fixation. Tip elongation of the xylem mother cells following transverse, or slightly oblique division is evident. X20?

Figure 42

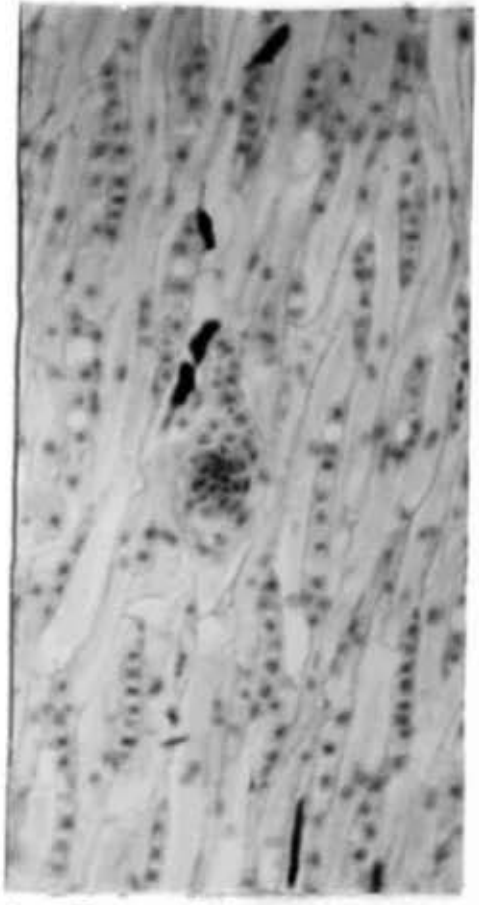


A. Tangential section showing lodgepole pine phloem mother cells (bottom), initials, and xylem mother cells (top) within a bridge constructed 18 days prior to harvesting and fixation. The bridge was oriented at 45° upward to the right. X82

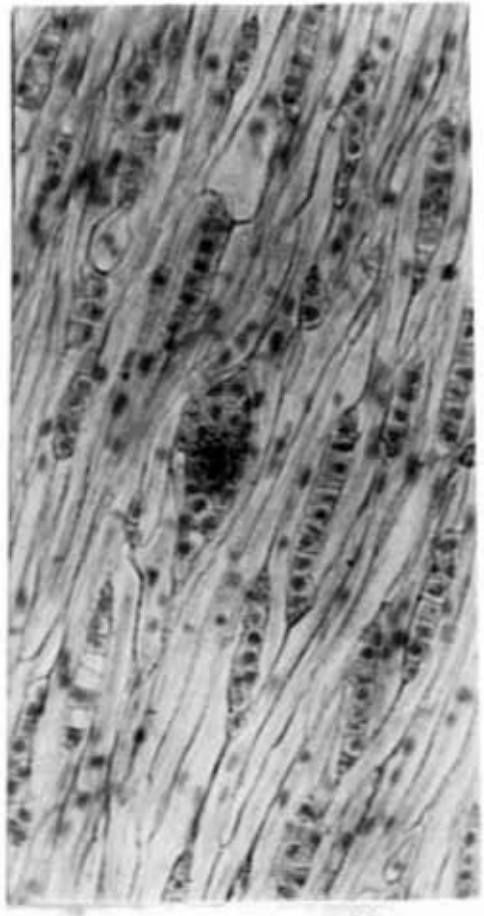


B. Tangential section showing lodgepole pine cambium within a diagonal bridge which was constructed 30 days before it was harvested and fixed. The first localized reorientation is evident in the upper left corner. X205

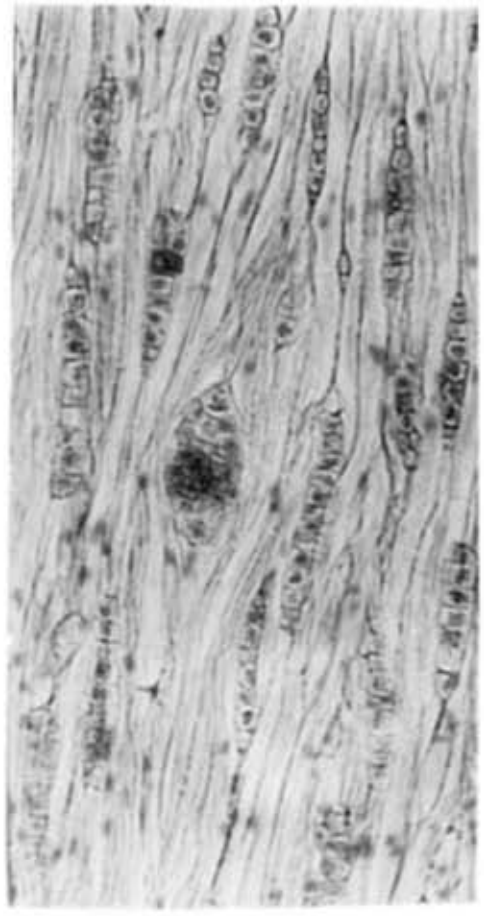
Figure 43



A. Tangential section of lodgepole pine phloem mother cells in a diagonal bridge havested and fixed at 50 days after bridge construction. X205



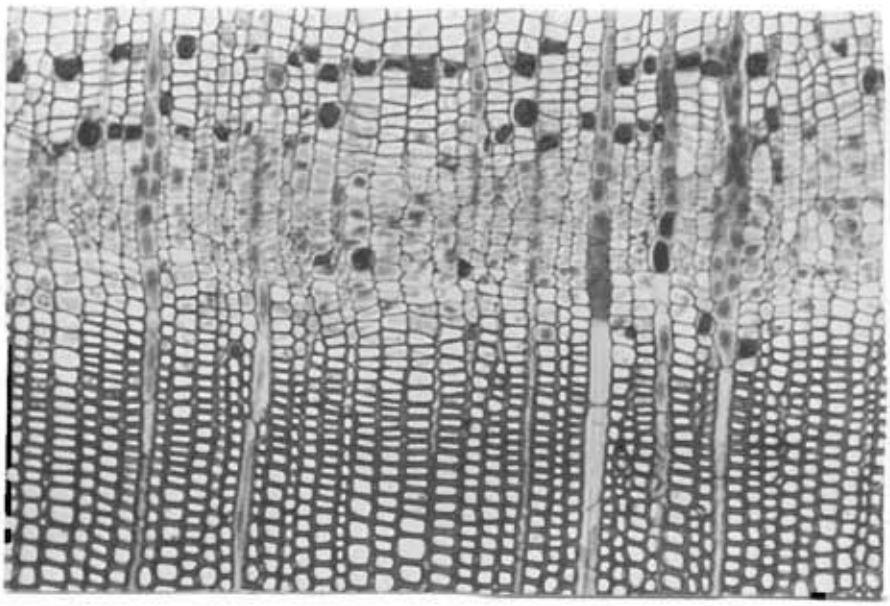
B. Same as Figure 44A, but 80 micrometers closer to the pith into the cambial zone; showing the same fusiform resin canal at 50 days after bridge construction. X205



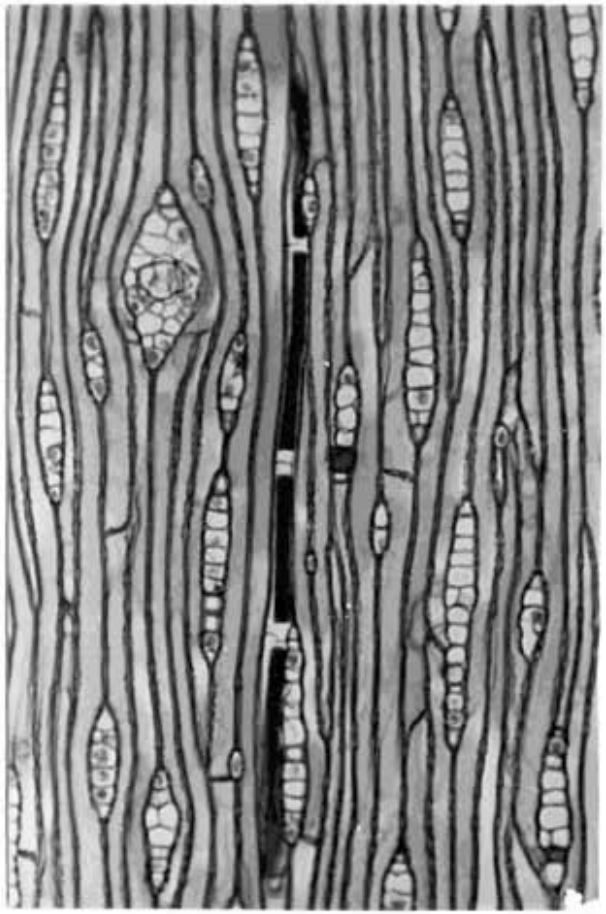
C. Same as Figures 44A and B but 160 micrometers closer to the pith into the cambial zone than Figure 44A; showing the same fusiform resin canal at 50 days after bridge construction. X205

Figure 44

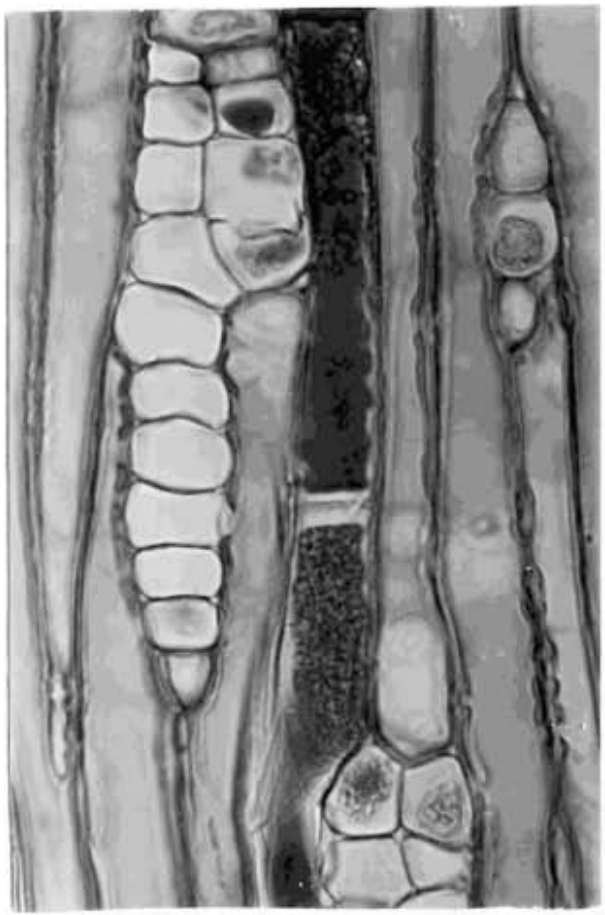
Figure 45



Transverse section of phloem (top), cambial zone and mature xylem of lodgepole pine diagonal bridge which was girdled 24 days previous to harvesting and fixing. At least three axial parenchyma are at the edge of the xylem mother cell zone (dark-stained cells in center) and many more have moved into the mature xylem. Note the similarity of the axial parenchyma in the xylem to those in the phloem. X205



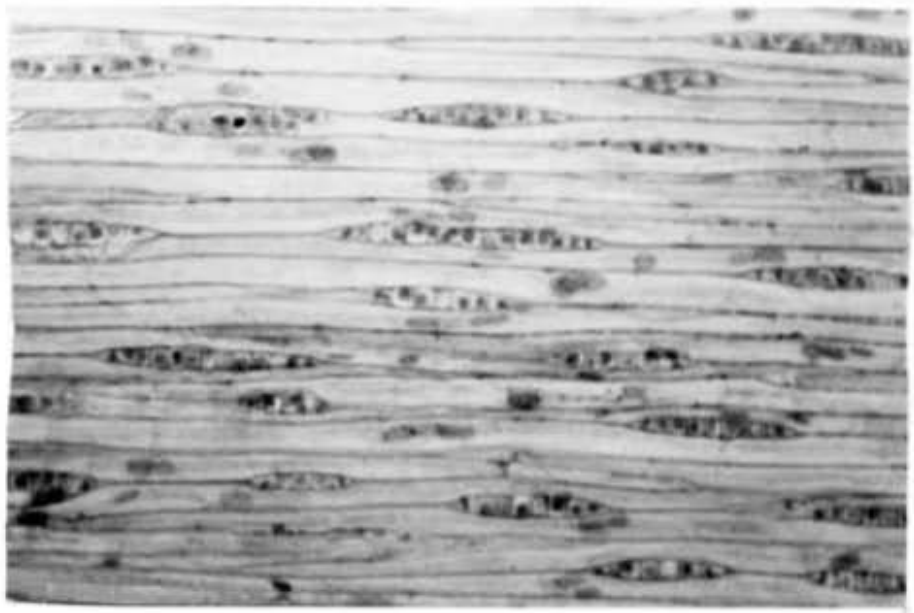
A. Tangential section of xylem formed from the cambium after a lodgepole pine tree was diagonally girdled showing four axial parenchyma. Serial tangential sections showed this to be a single, isolated strand of axial parenchymatous cells formed in the central region of the spiral bridge. X205



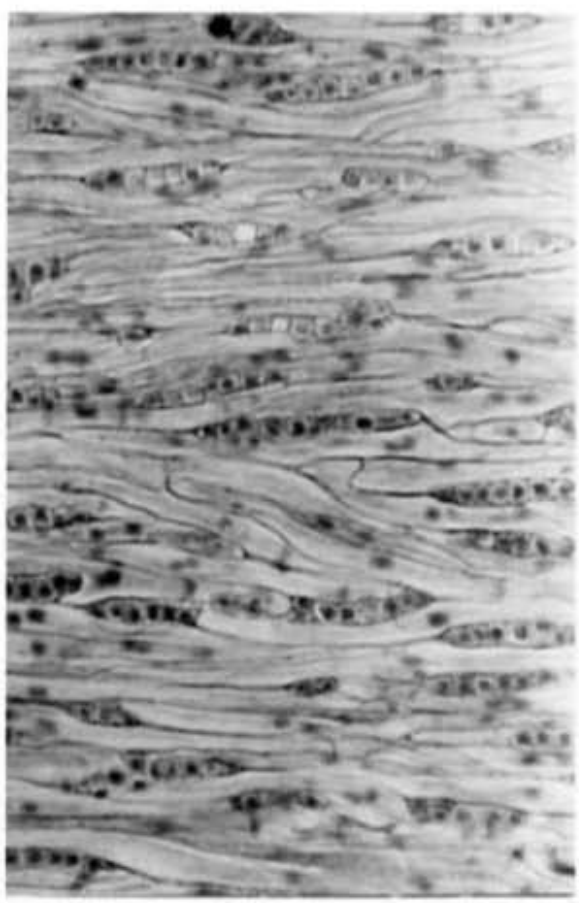
B. Tangential section of xylem formed from the cambium after a lodgepole pine tree was diagonally girdled, showing axial parenchyma and calcium oxalate crystals (bottom) normally found only in the phloem. X820

Figure 46

Figure 47



B. Tangential section of the cambial region within a vertical bridge of lodgepole pine at 38 days after girdling and bridge construction. X205



A. Tangential section of the cambial region within a diagonal bridge of lodgepole pine at 50 days after girdling. Note the pronounced microdomain of reorientation (cf. Figure 34). X325

A. Tangential section showing the beginning of lodgepole pine diagonal-bridge Series 2 (see Figure 5 and the Appendix). Counting rightward from the bottom of the fusiform ray, the file which was traced is represented by the 8th tracheid. X130

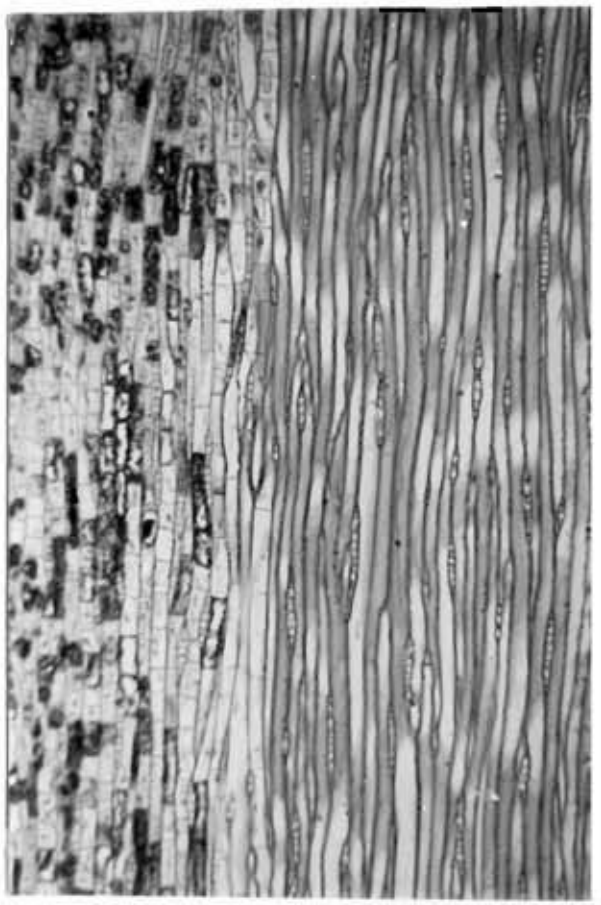
B. Tangential section showing the end of Series 2 (i.e. closest to the cambium; Figure 48A is closest to the pith). Very slight local reorientation has occurred. X130

Figure 48

A. Tangential section showing the beginning (nearest the Pith) of lodgepole pine diagonal-bridge Series 3 (see Figure 5 and Appendix). Counting rightward from the lower tip of the fusiform ray, the file which was traced is represented by the 7th tracheid. X130

B. Tangential section showing the end (furthest from the pith) of Series 3. X130

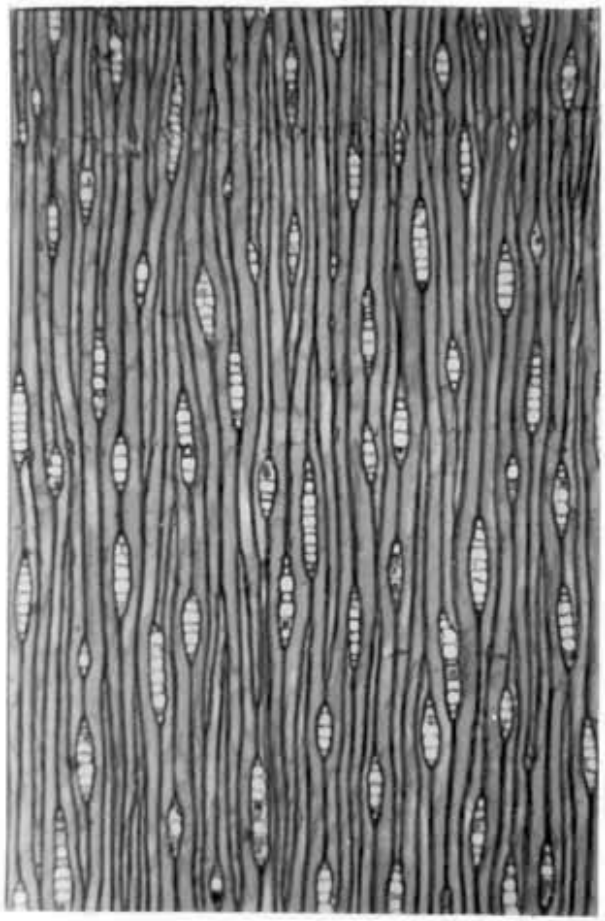
Figure 49



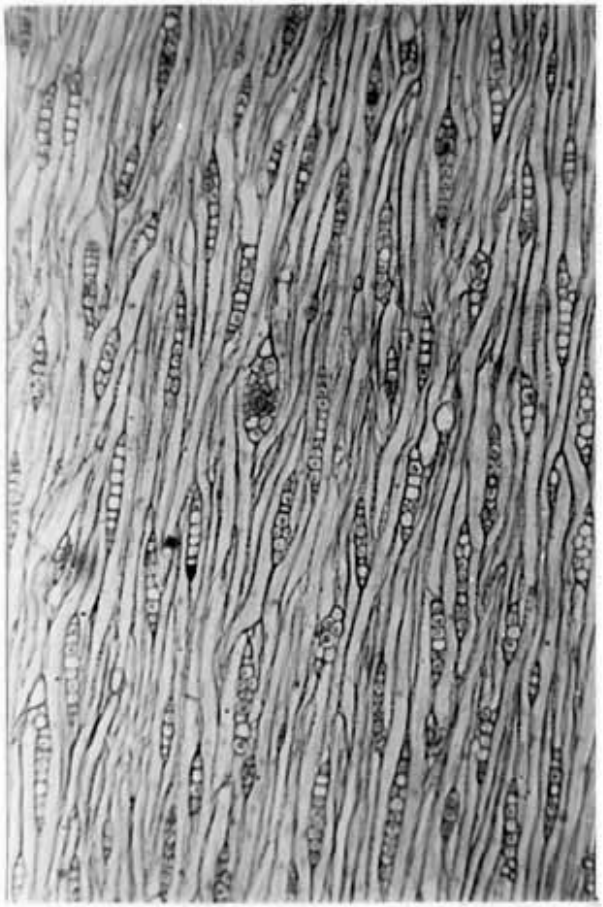
A. Tangential section showing the beginning (closest to the pith) of lodgepole pine vertical-bridge Series 4 (see Figure 6 and the Appendix) adjacent to the bridge edge. Counting leftward from the center of the photograph's right side, the file traced is represented by the 21st tracheid encountered. X130

B. Tangential section showing the end (closest to the cambium) of Series 4, at a slightly lower magnification. X82

Figure 50



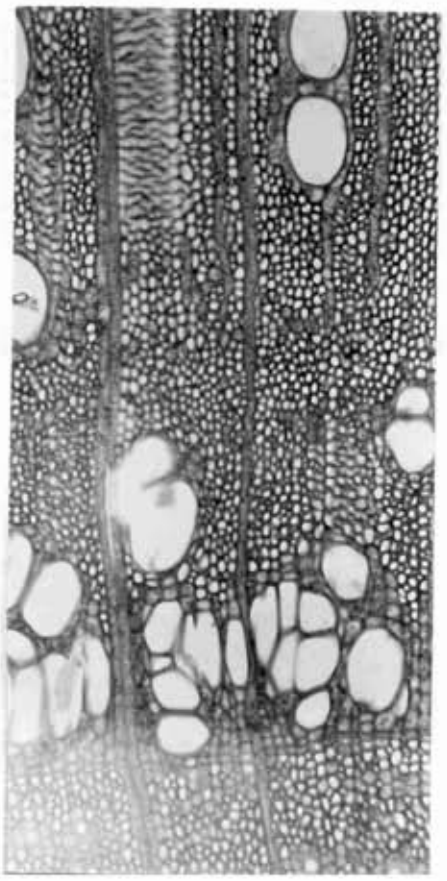
A. Tangential section showing the beginning (closest to the pith) of lodgepole pine diagonal-bridge Series 5, in the center of the photograph. X130



B. Tangential section showing the end (closest to the cambium) of Series 5. Note that the fusiform ray in the center of this photograph has arisen from an uniseriate ray, as shown in Figure 51A. See Figure 5 and the Appendix. X130.

Figure 51

Figure 52

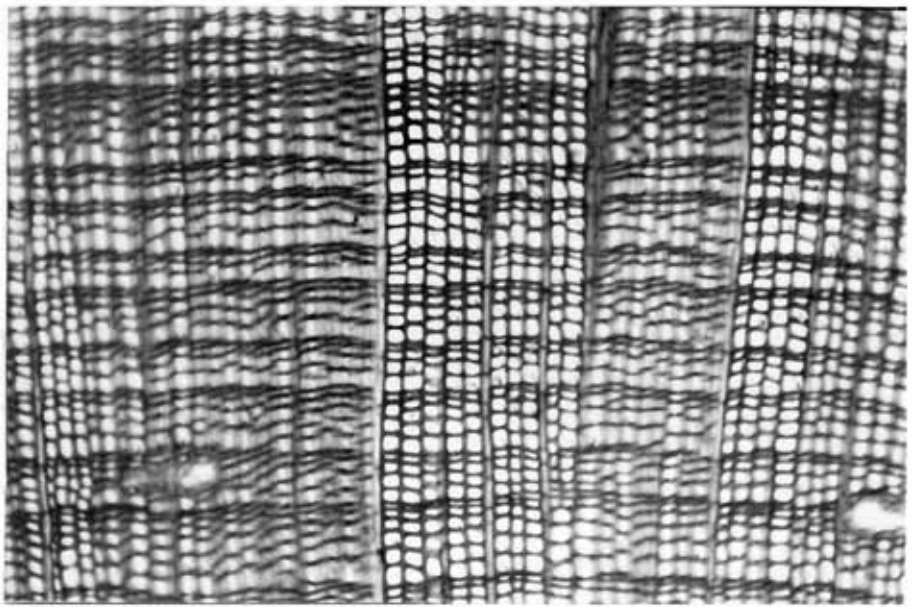


Transverse section of white ash (Fraxinus americana L.) xylem which formed within a spiral bridge similar to those shown for tree WP-1 (Figure 1). Latewood of the previous year is at the bottom; girdling was done in early spring before the cambium had activated. Note the distinct microdomains of reorientation in upper left. X205

Figure 53

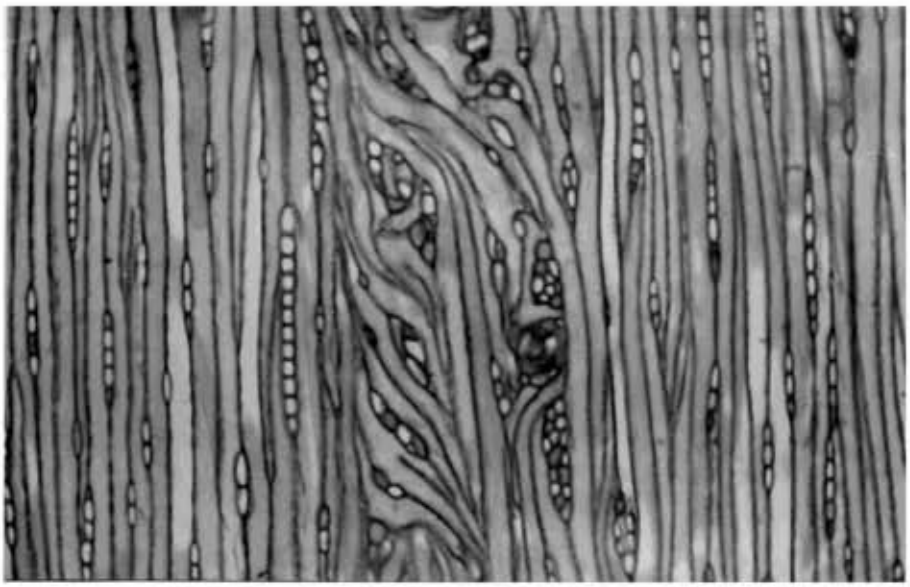
An example of extreme natural spiral grain formation. This tree, a white spruce (Picea glauca (Moench.) Voss) in the central Yukon Territory, lived for more than 600 years and began spiral grain formation at approximately 100 years of age. The tree is 7 meters high.

Figure 54



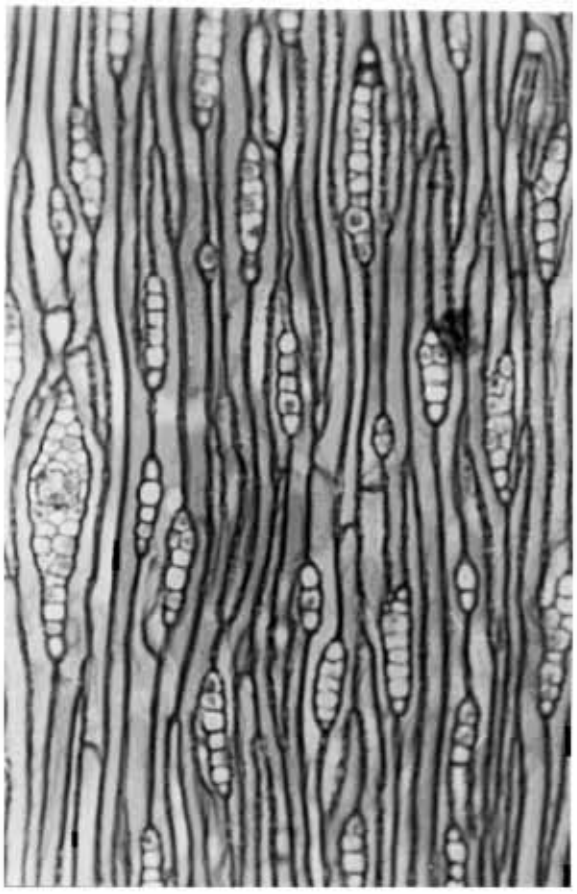
Transverse section of xylem from the stem of the white spruce tree shown in Figure 53 in the region where reorientation first began. Note that only a few tracheids were produced each growing season. Note the very distinct microdomains of reorientation. X205

Figure 55



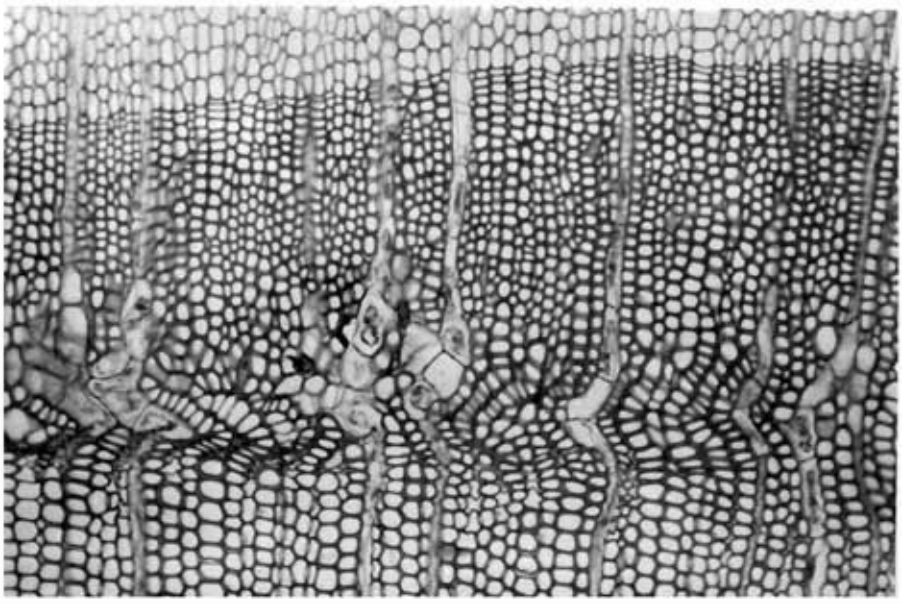
A tangential section of xylem from the white spruce shown in Figure 53. Note the distinct microdomain of reorientation. X205

Figure 56



A tangential section of xylem formed within a lodgepole pine diagonal bridge to show the sinuous nature of the tracheids formed shortly after diagonal girdling (cf. Figure 46A). This sinuous feature is best seen by looking at the photograph such that the eye is almost in the plane of the photograph. X205

Figure 57



Transverse section of latewood of lodgepole pine which was formed in 1974 (bottom). Earlywood of 1975 is at the top. Both growth layers were formed long before girdling in 1976. The distorted region was highly localized and is believed to be the site of a pinpoint wound. Pressure was likely applied in the central region of the distortion, as shown in this photograph, upon the cambial zone. The response was similar to that seen in the girdled bridges: cessation of radial enlargement of xylem mother cells with accompanying heavy lignification; swollen ray parenchyma; and narrowing of the tangential dimensions of the cells, presumably by oblique anticlinal divisions. Note that there are no traumatic resin canals, possibly due to the fact that the cambial zone was well on its way into winter rest. X205

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APPENDIX

TRACES OF SERIAL TANGENTIAL SECTIONS OF XYLEM

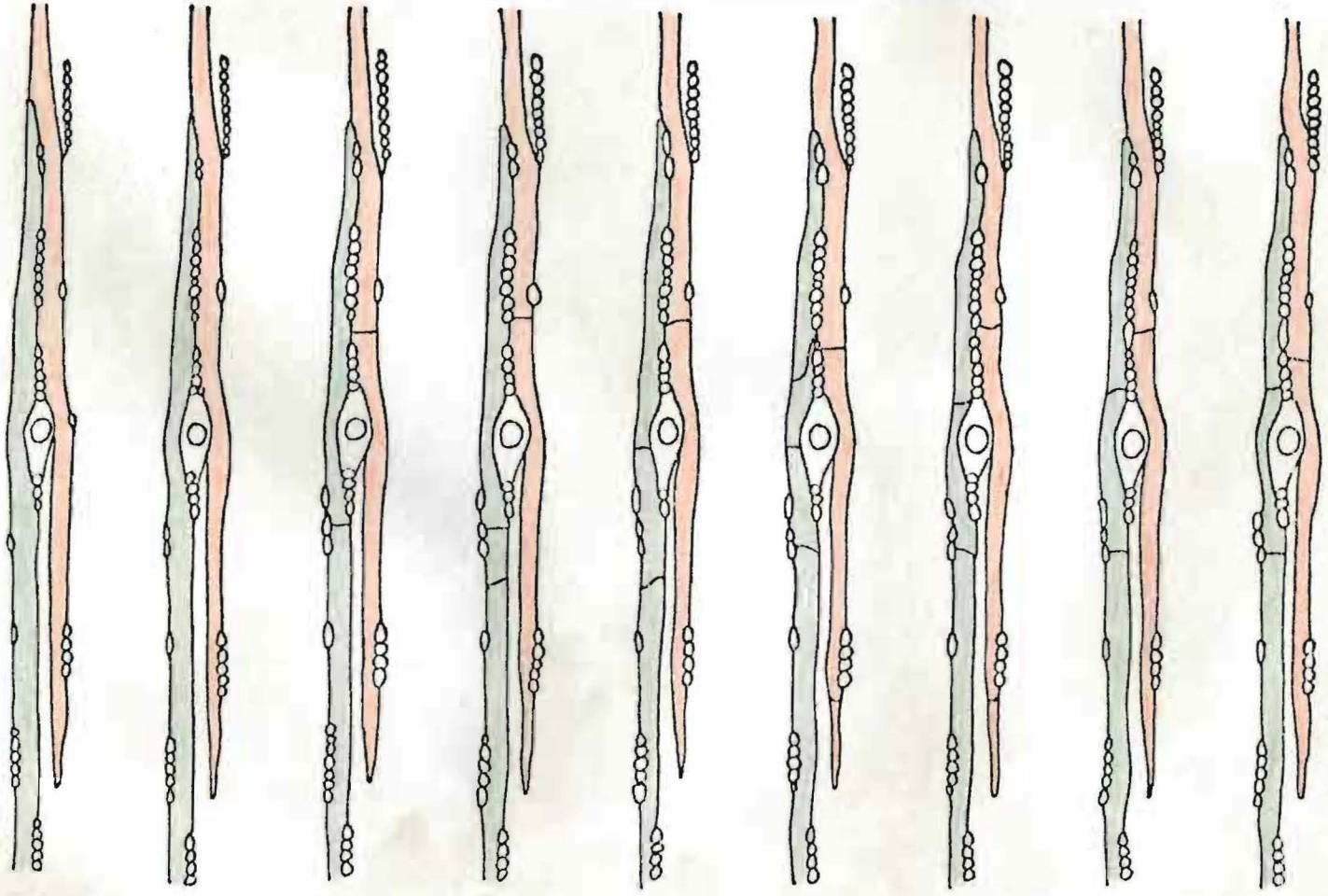
SERIES 1

White Pine

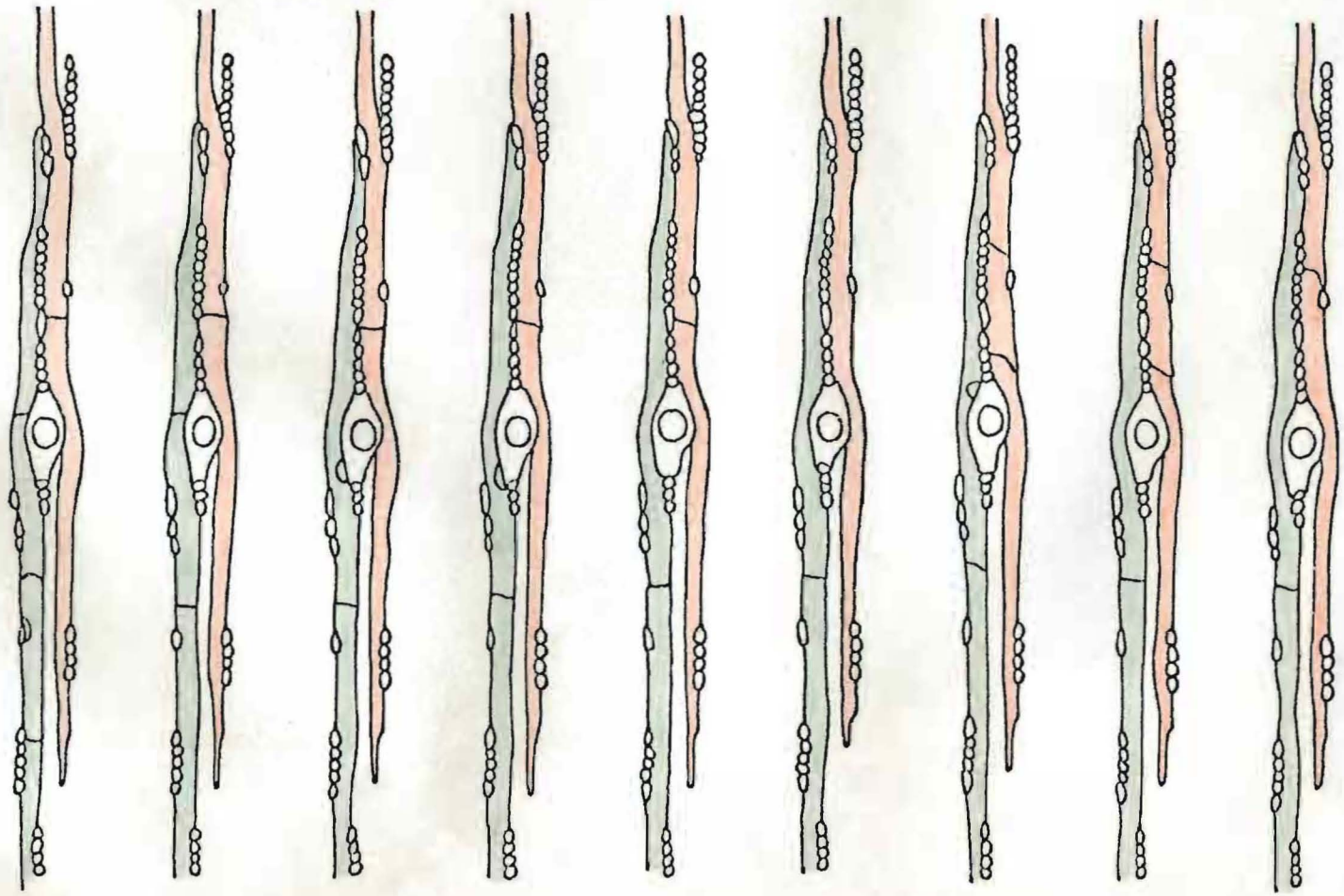
Section 59 is nearest the pith; Section 118 is furthest from the pith. This Series should be viewed as if the observor were looking from the pith toward the bark. That is, the spiral bridge (Figure 3) was oriented upward to the right whereas the reorientation occurs upward to the left in this series.

Serial tangential section numbers are at the bottom of each sheet.

SERIES 1

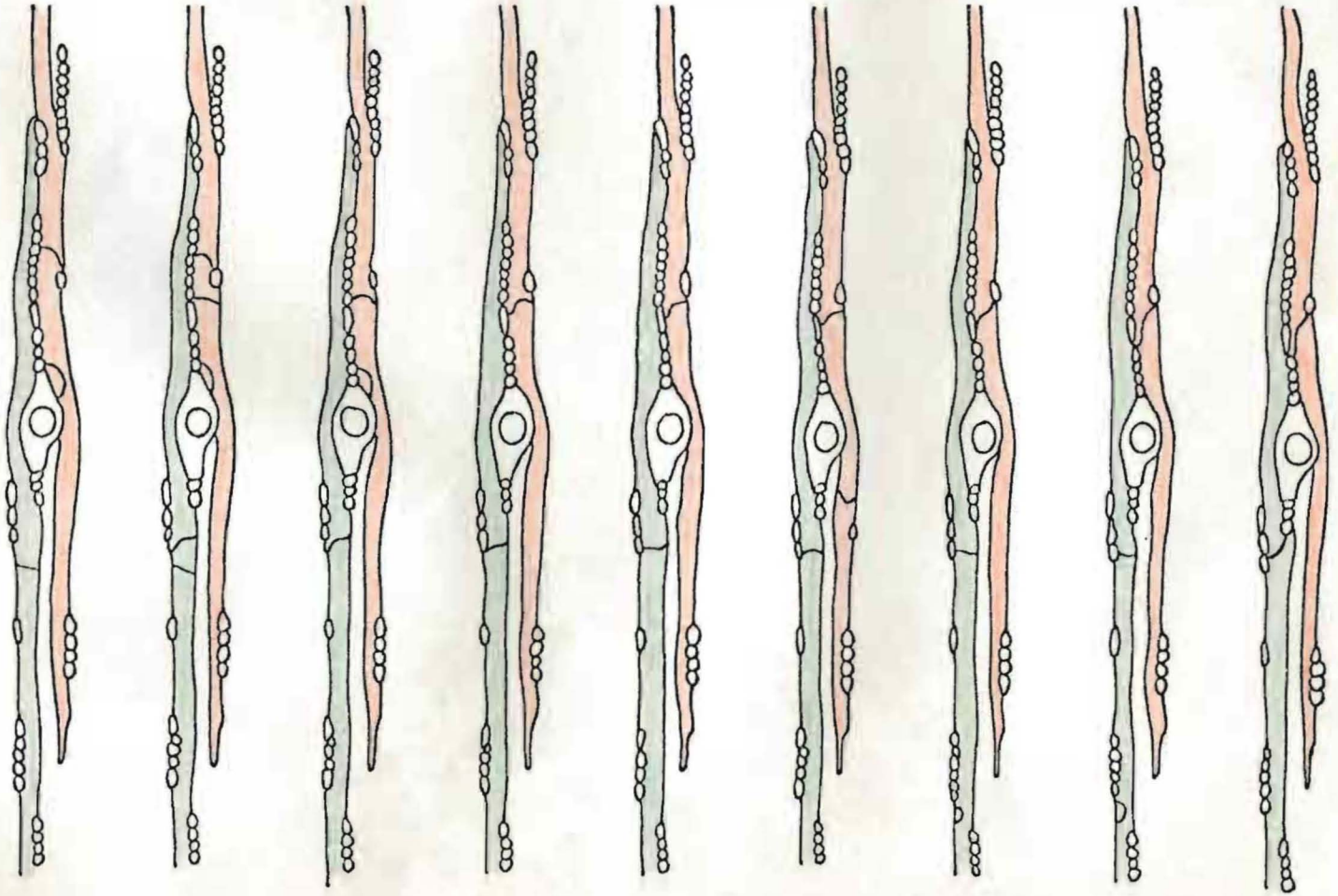


SERIES 1



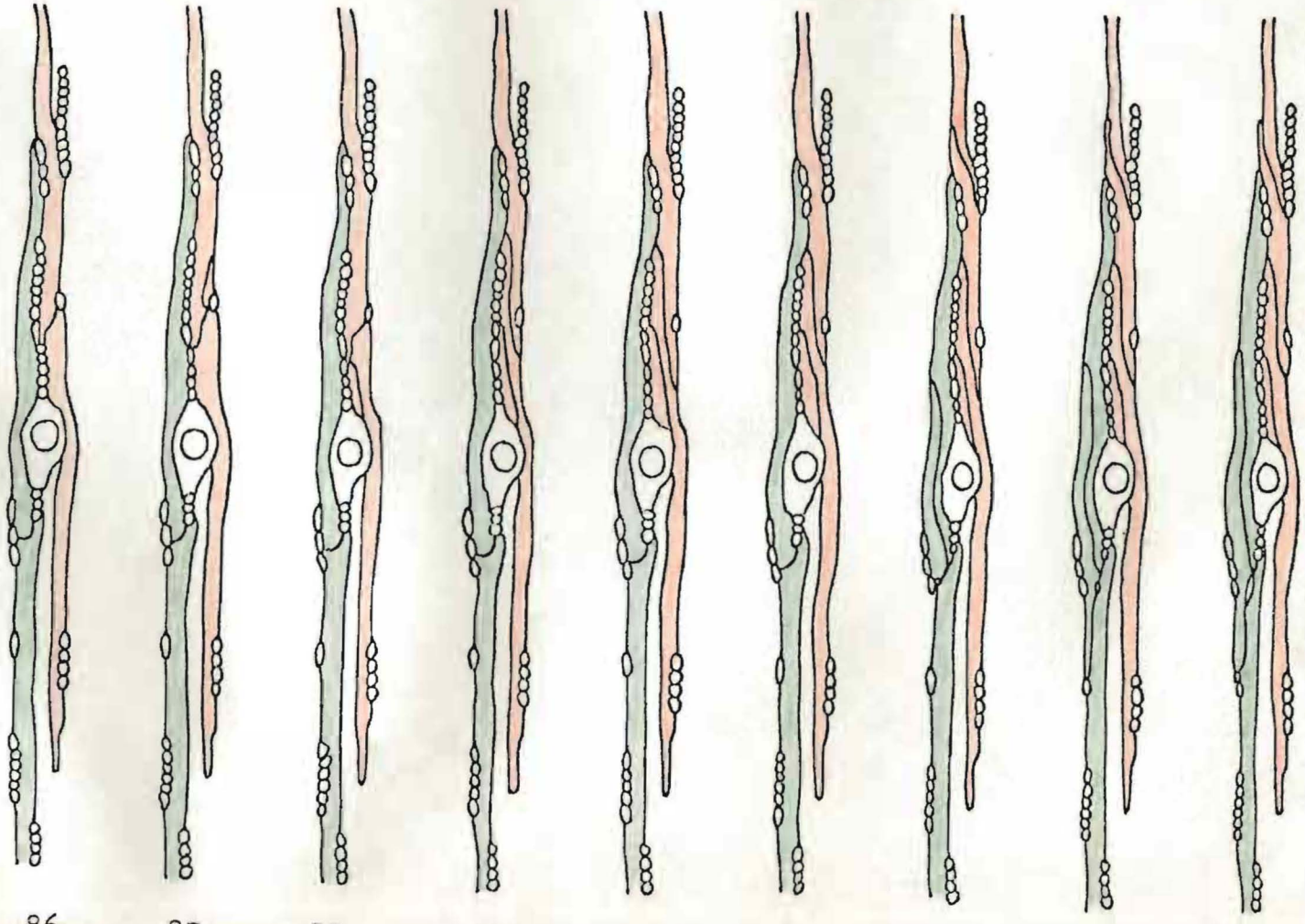
68 69 70 71 72 73 74 75 76

SERIES 1



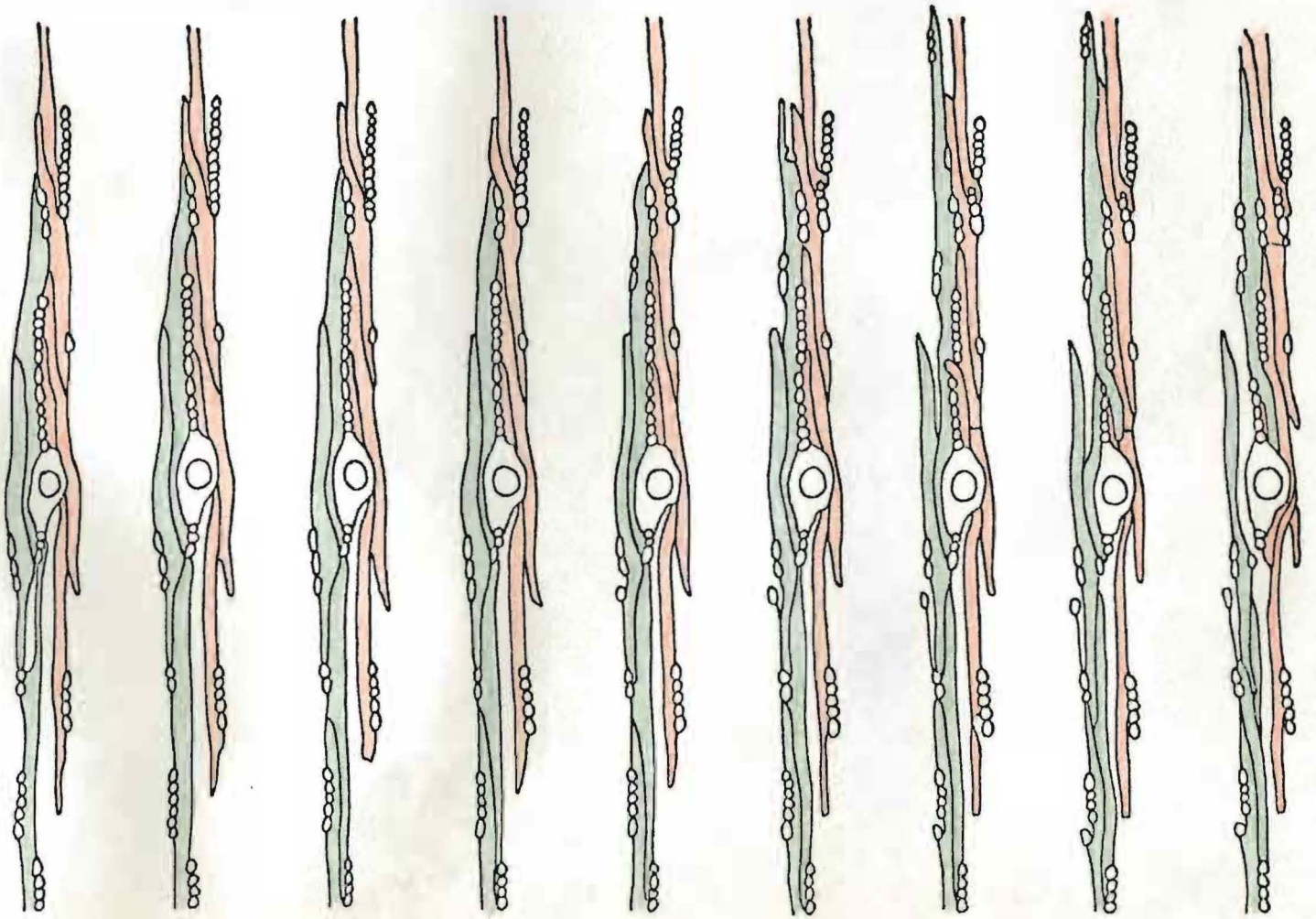
77 78 79 80 81 82 83 84 85

SERIES 1



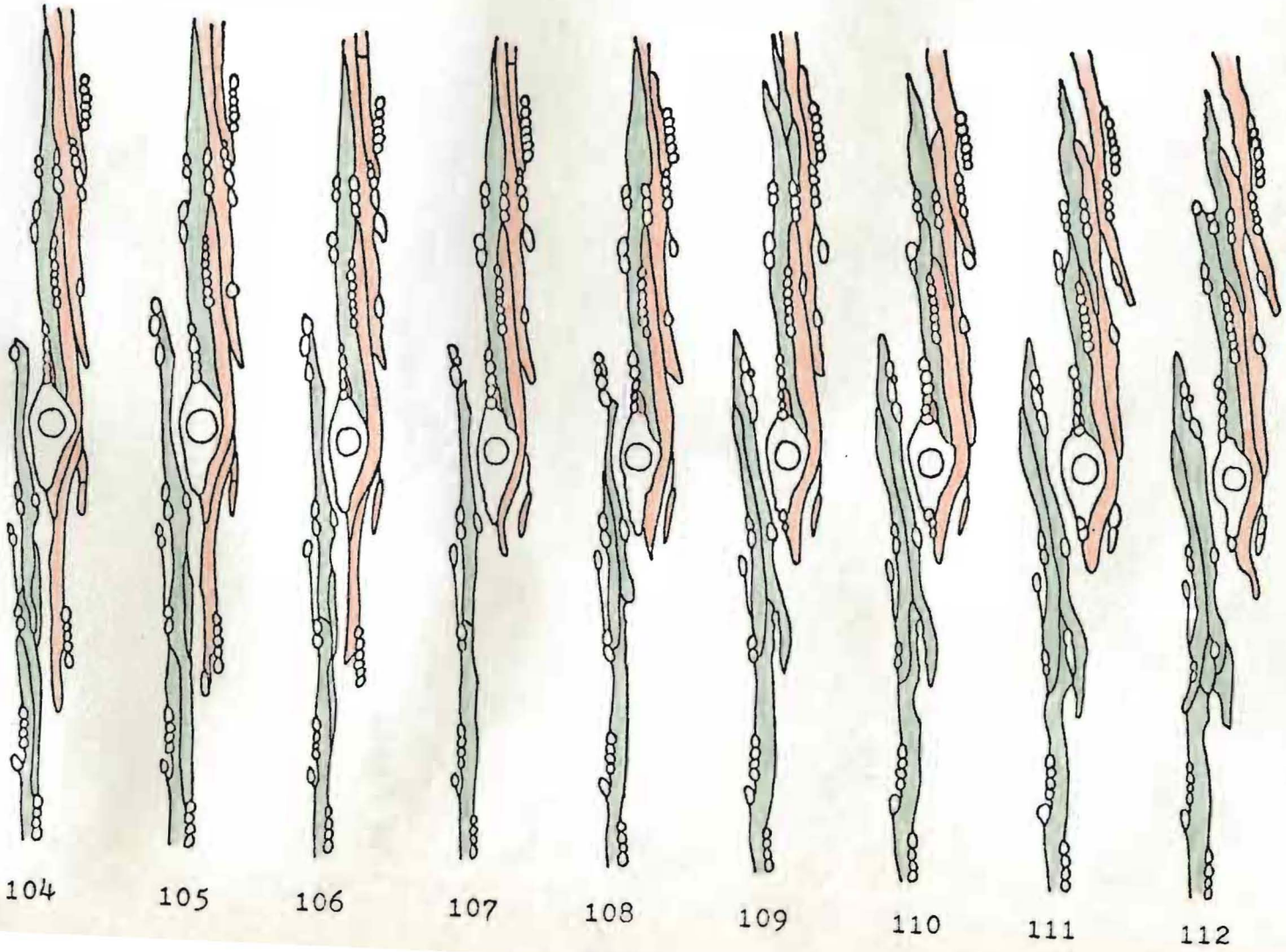
86 87 88 89 90 91 92 93 94

SERIES 1

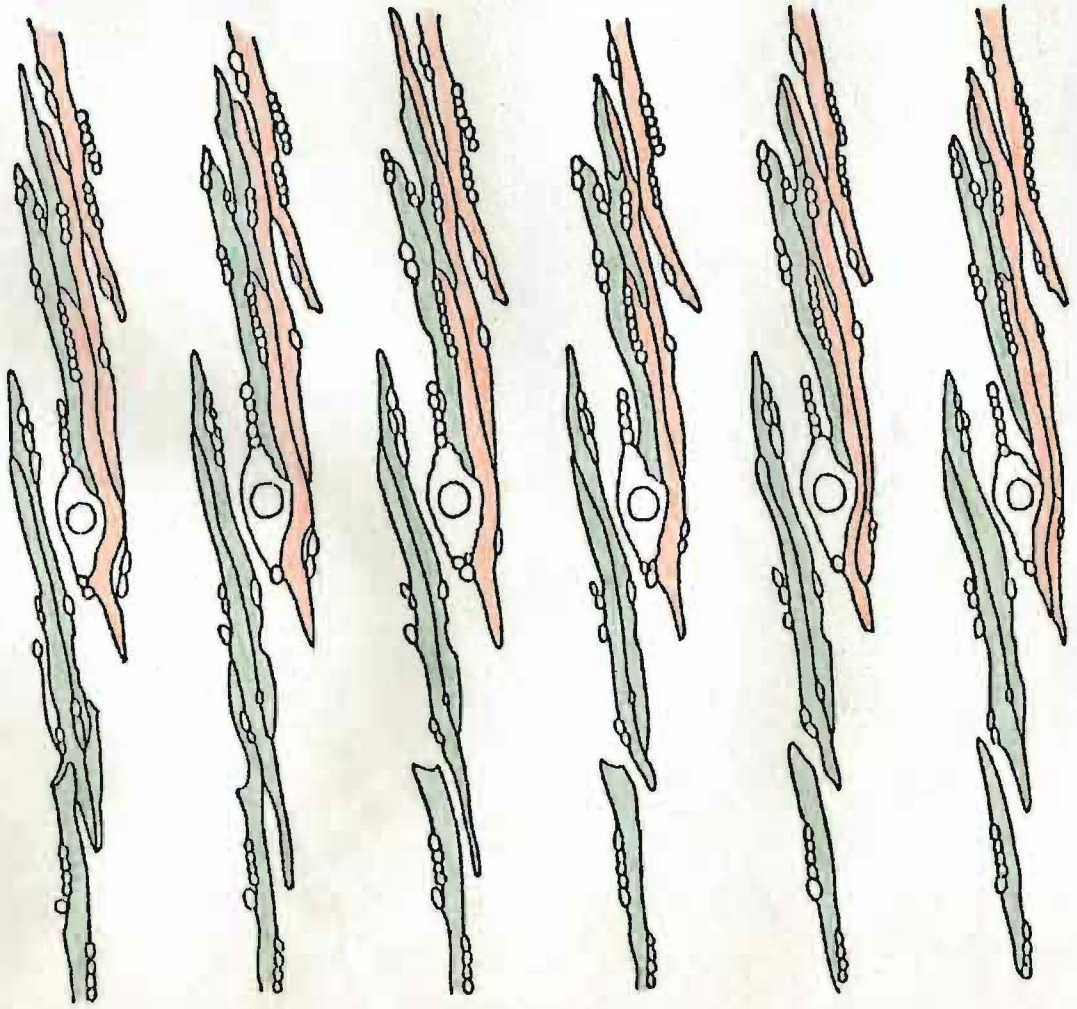


95 96 97 98 99 100 101 102 103

SERIES 1



SERIES 1



113 114 115 116 117 118

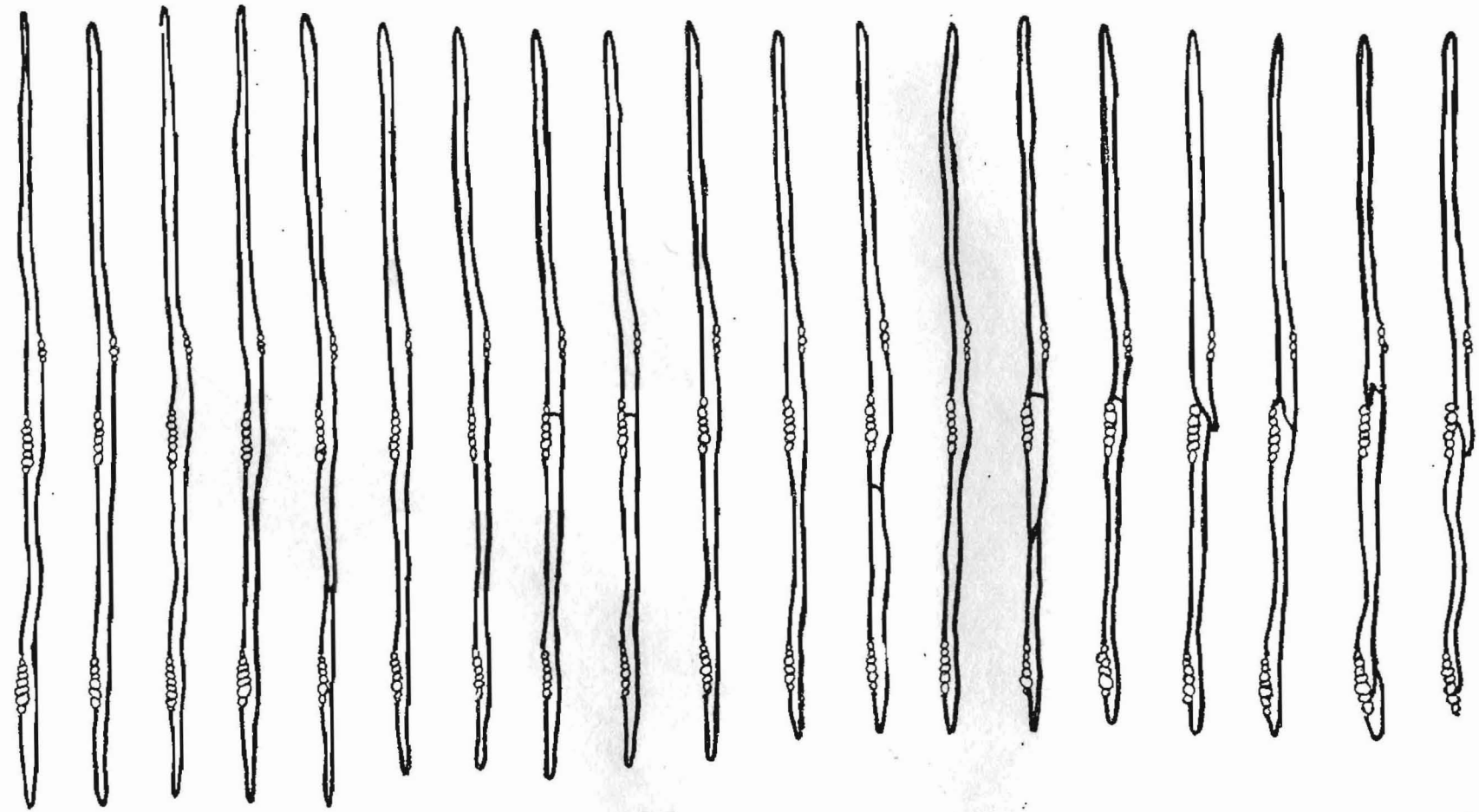
SERIES 2

Lodgepole Pine

Diagonal Bridge

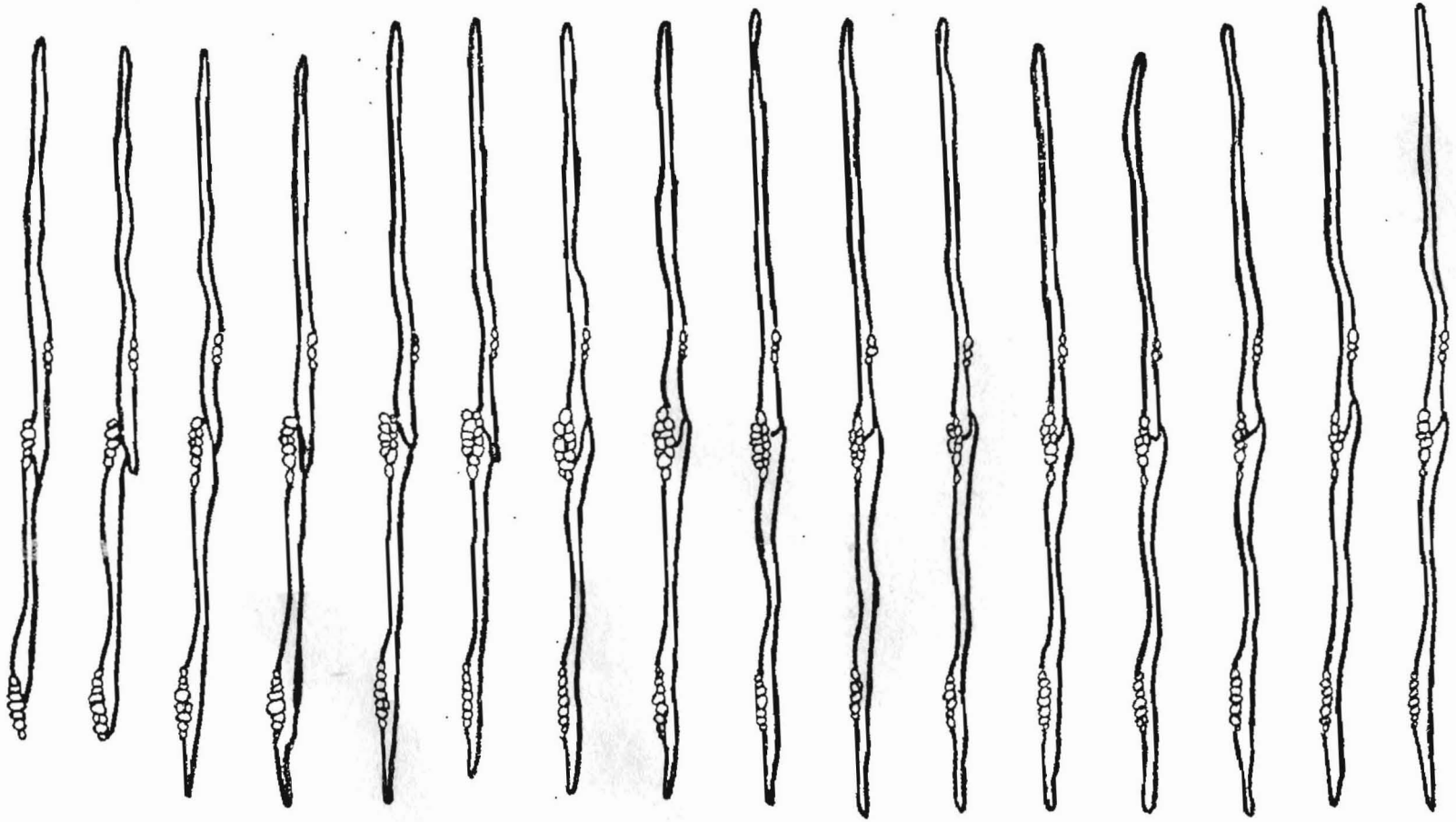
Section 144 is closest to the pith. Section 38 is furthest from the pith (closest to the cambium). This series should be viewed as if the observor were close to the pith and moving backwards toward the bark. Figure 5 shows the location of Series 2 relative to the diagonal bridge. This Series was traced from xylem which had formed over a period of 50 days following the construction of the diagonal bridge.

SERIES 2



Section No.

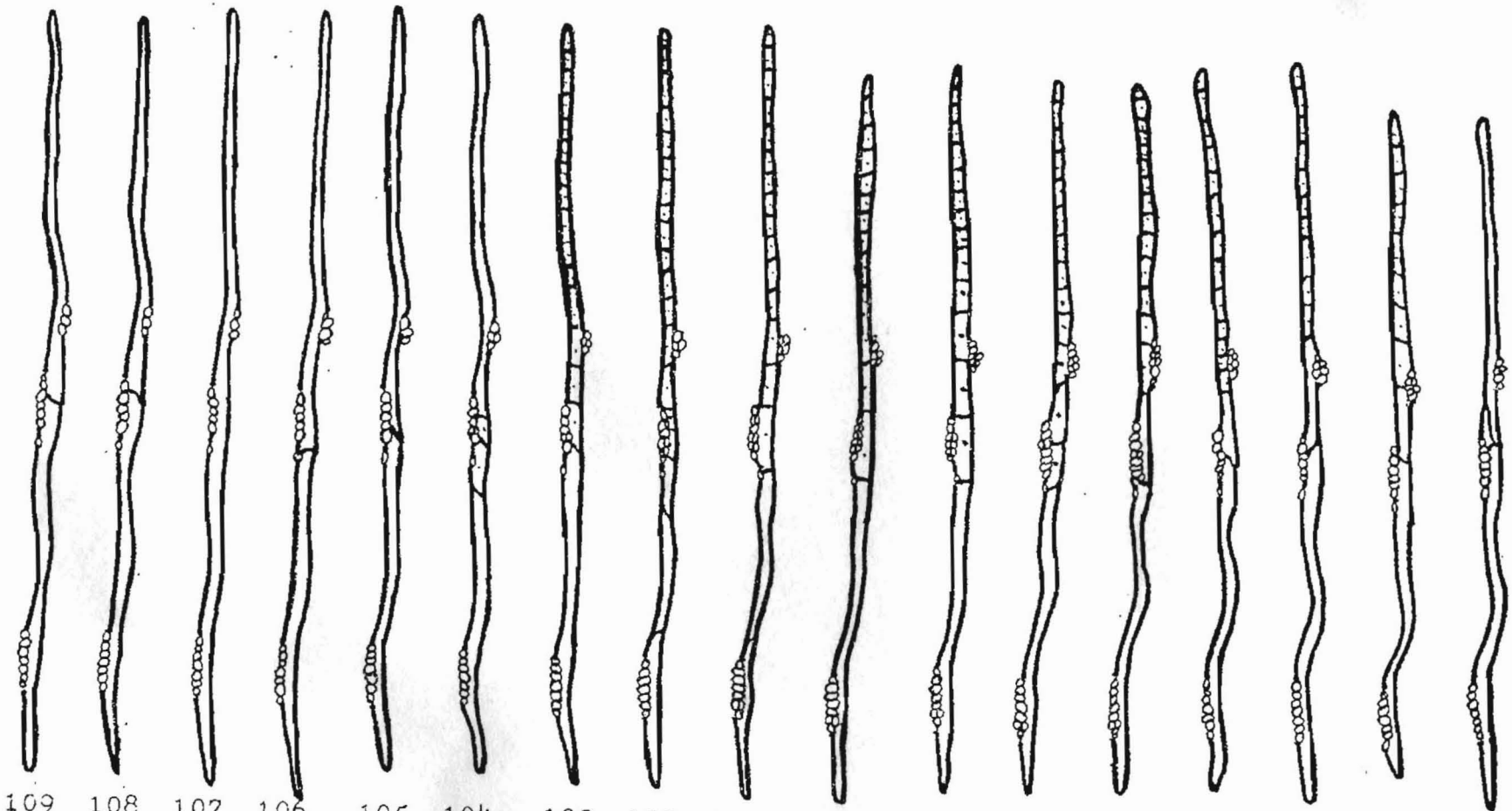
SERIES 2



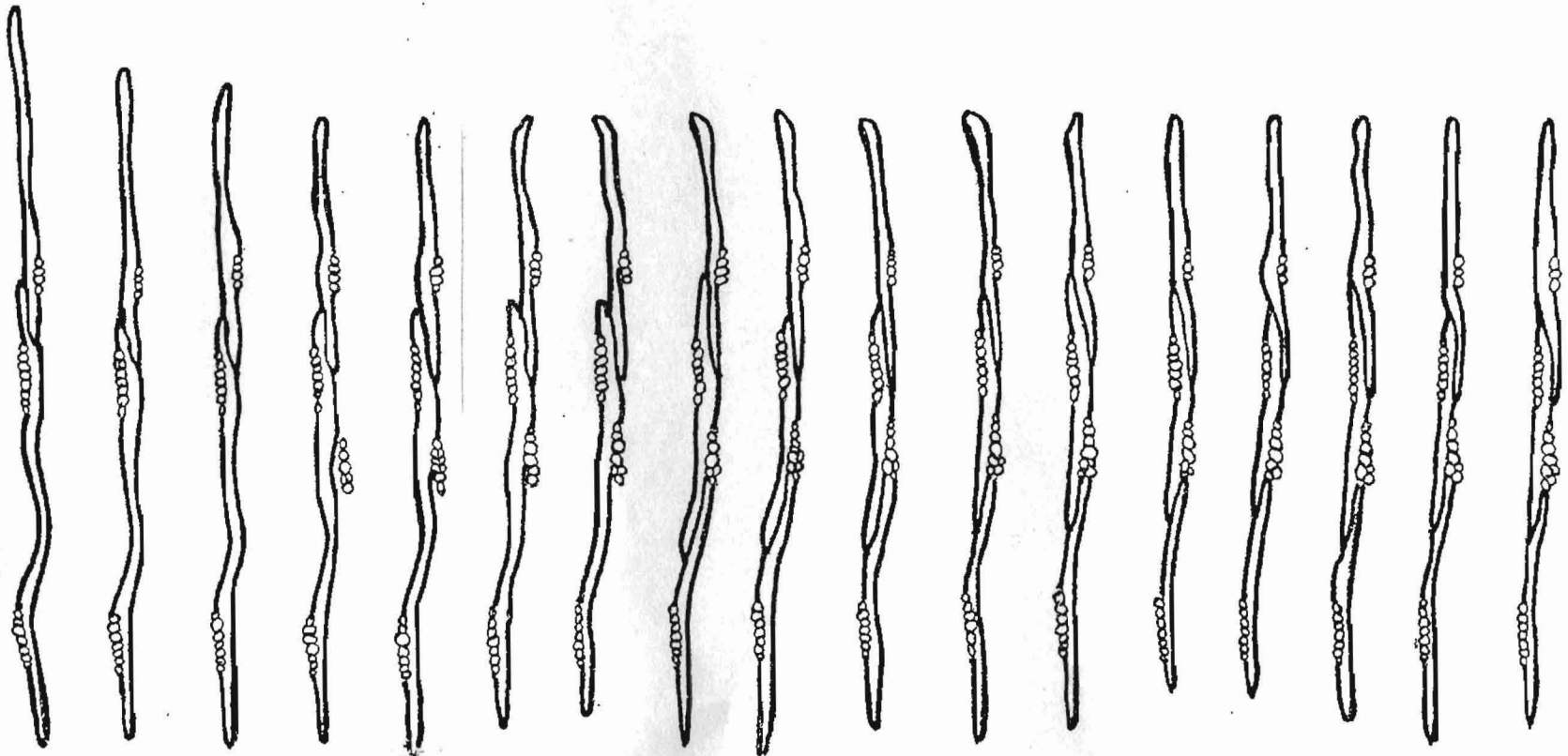
125 124 123 122 121 120 119 118 117 116 115 114 113 112 111 110

Section No.

SERIES 2

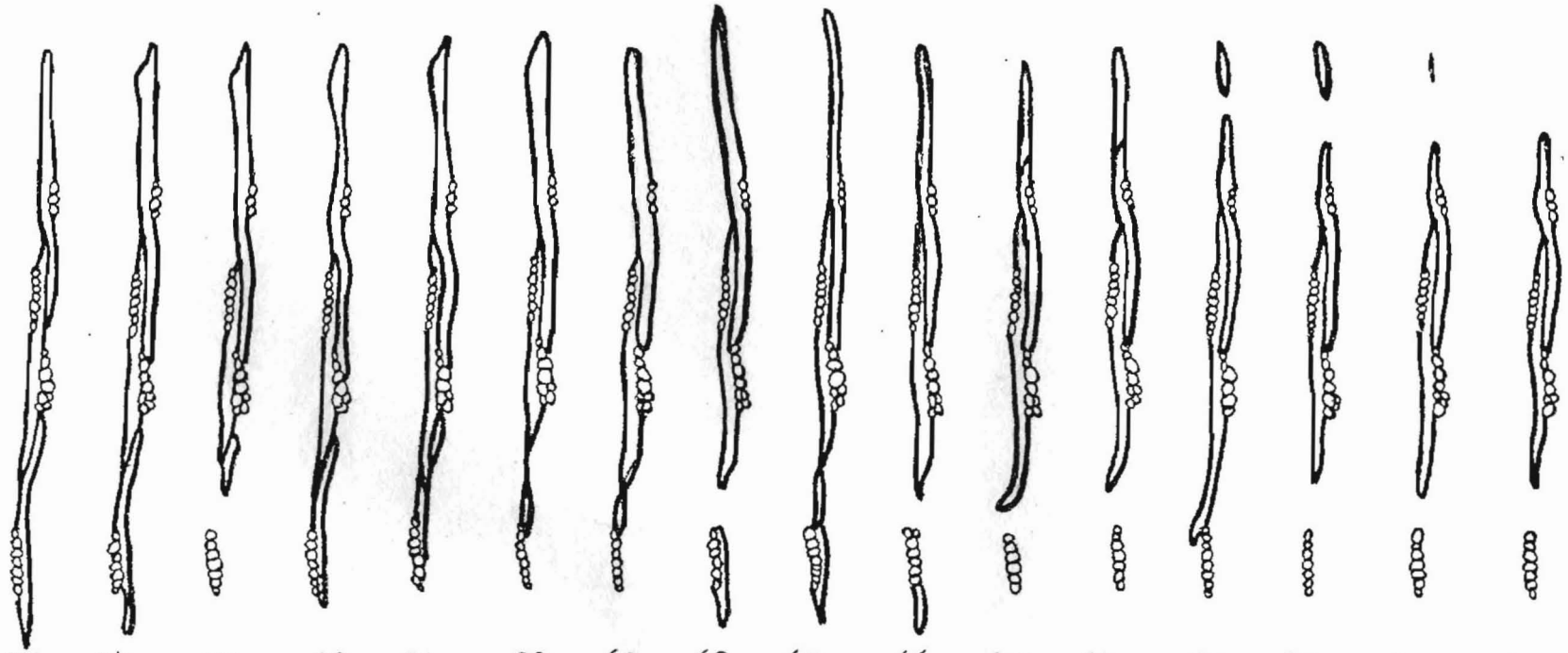

109
108
107
106
105
104
103
102
101
100
99
98
97
96
95
94
93
Section No.

SERIES 2

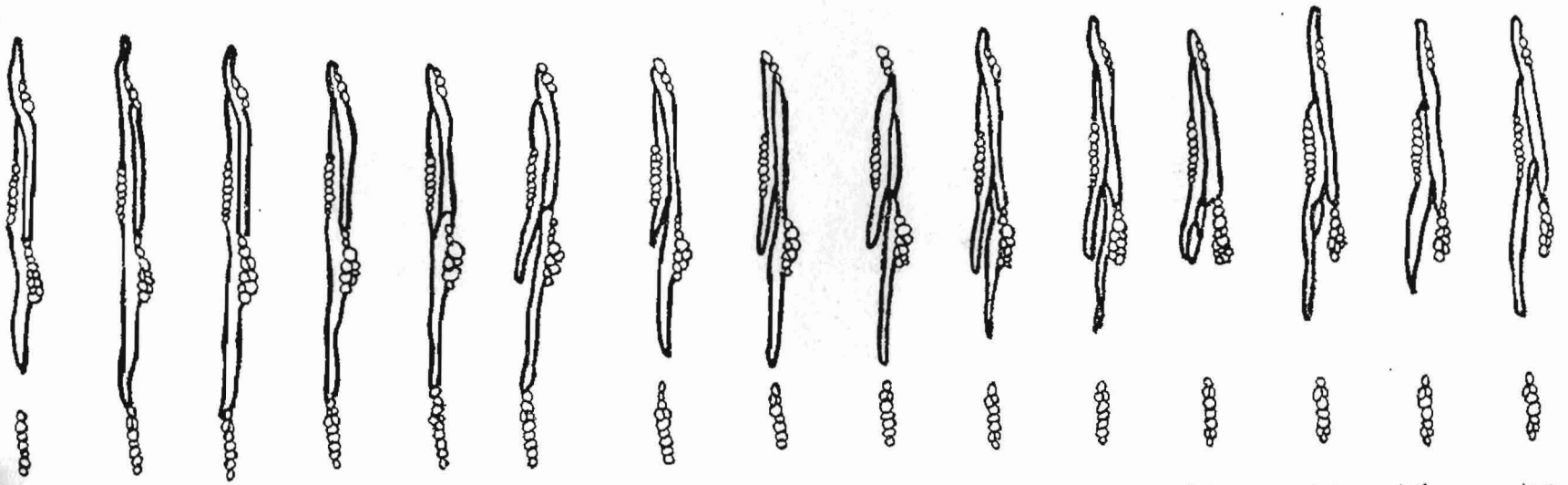


Section No.

SERIES 2

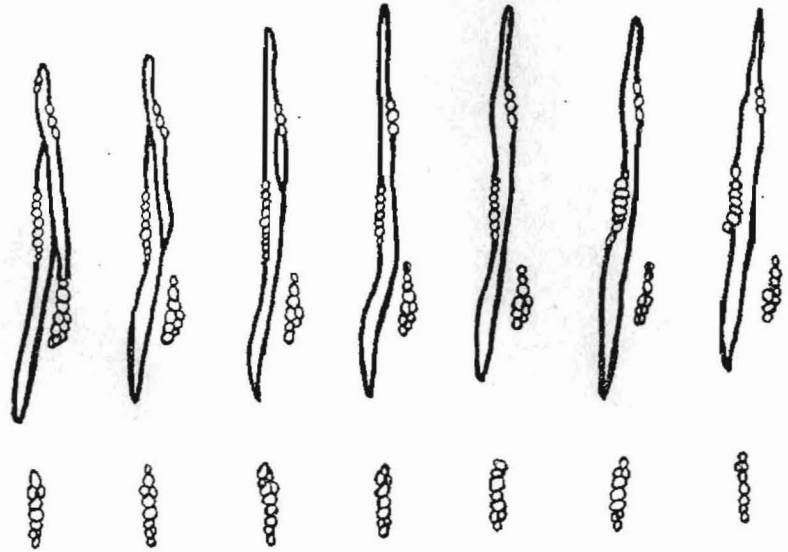


SERIES 2



Section No.

SERIES 2



Section No.

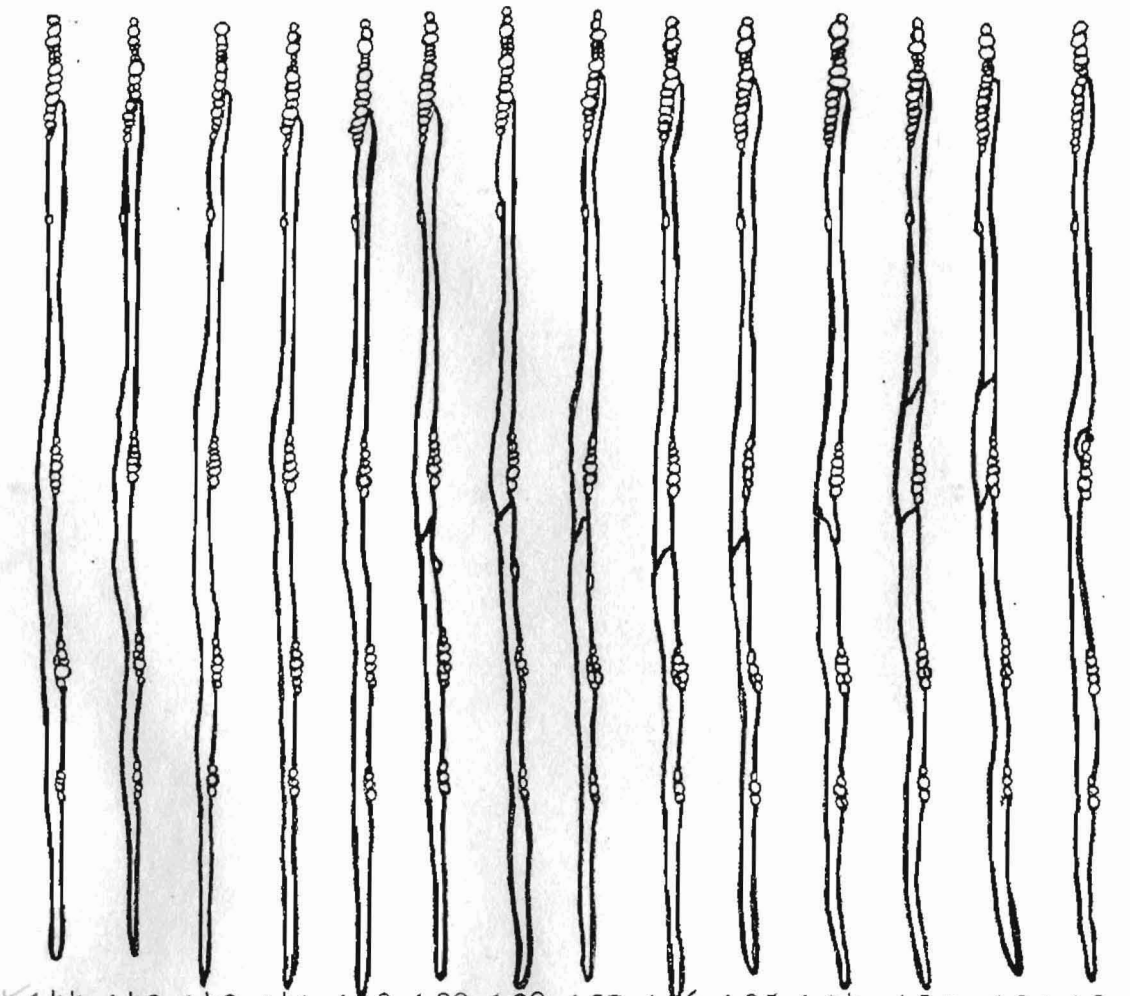
SERIES 3

Lodgepole Pine

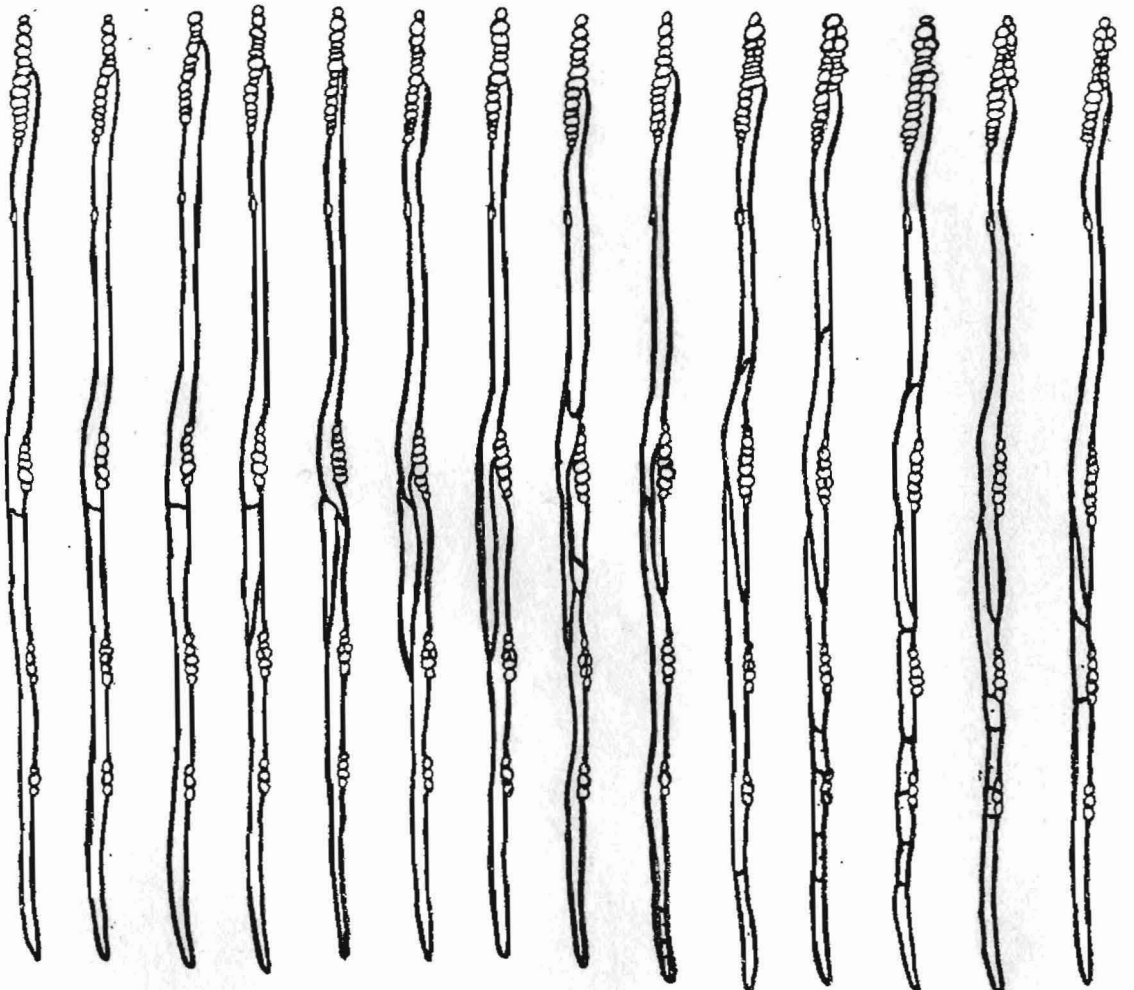
Diagonal Bridge

Section 144 is closest to the pith. Section 36 is furthest from the pith (closest to the cambium). This series should be viewed as if the observor were close to the pith and moving backwards toward the bark. Figure 5 shows the location of Series 3 relative to the diagonal bridge. This series was traced from xylem which had formed over a period of 50 days following the construction of the diagonal bridge.

SERIES 3

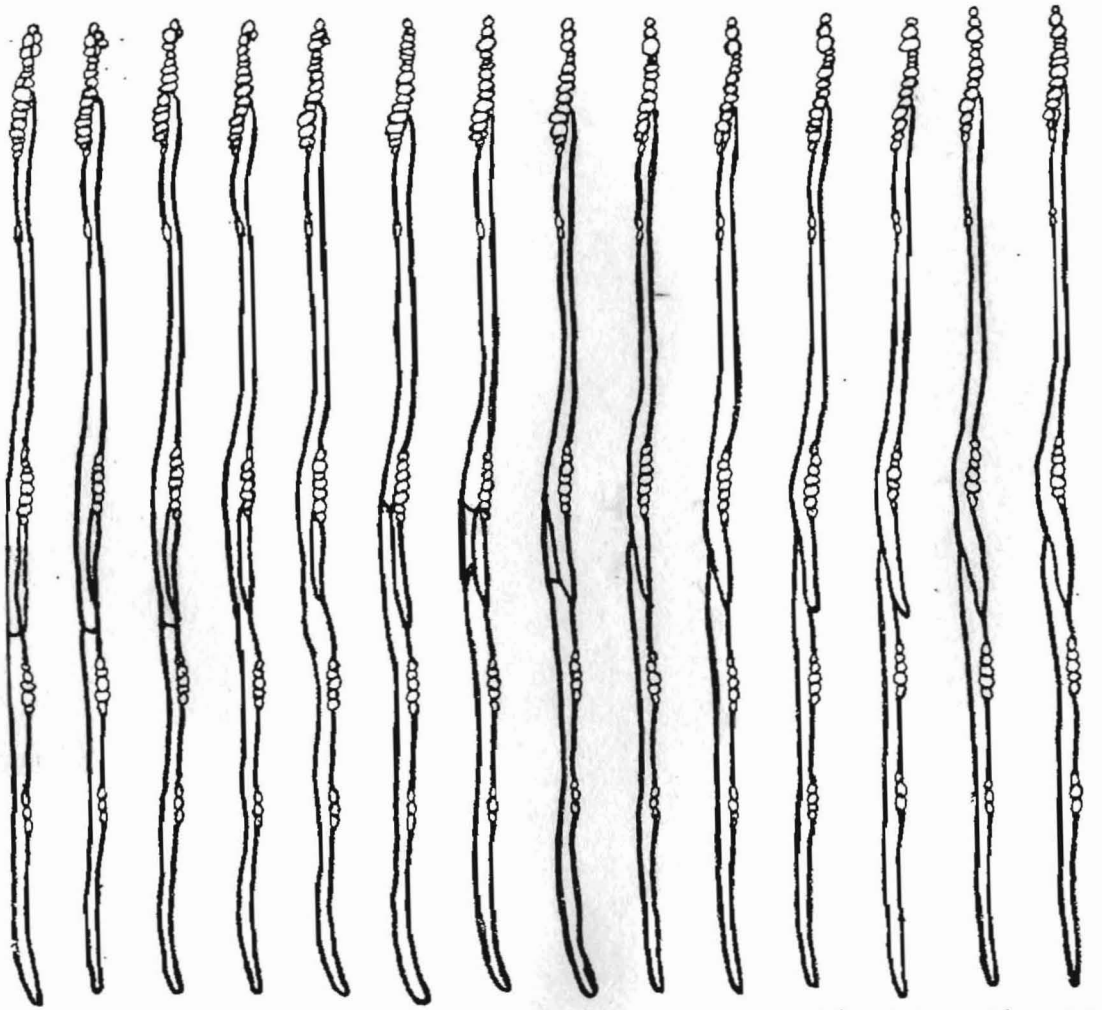

144 143 142 141 140 139 138 137 136 135 134 133 132 131
Section No.

SERIES 3



Section No.

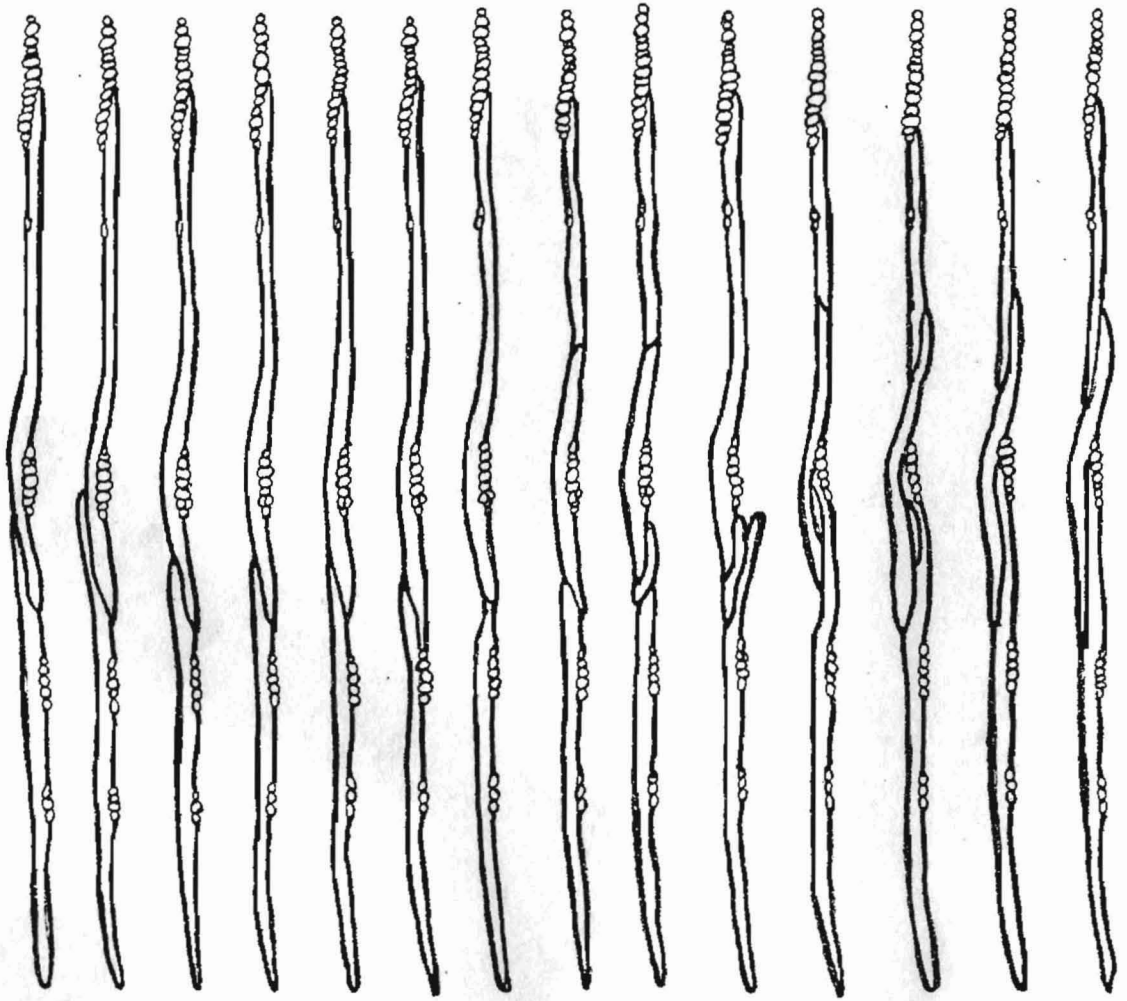
SERIES 3



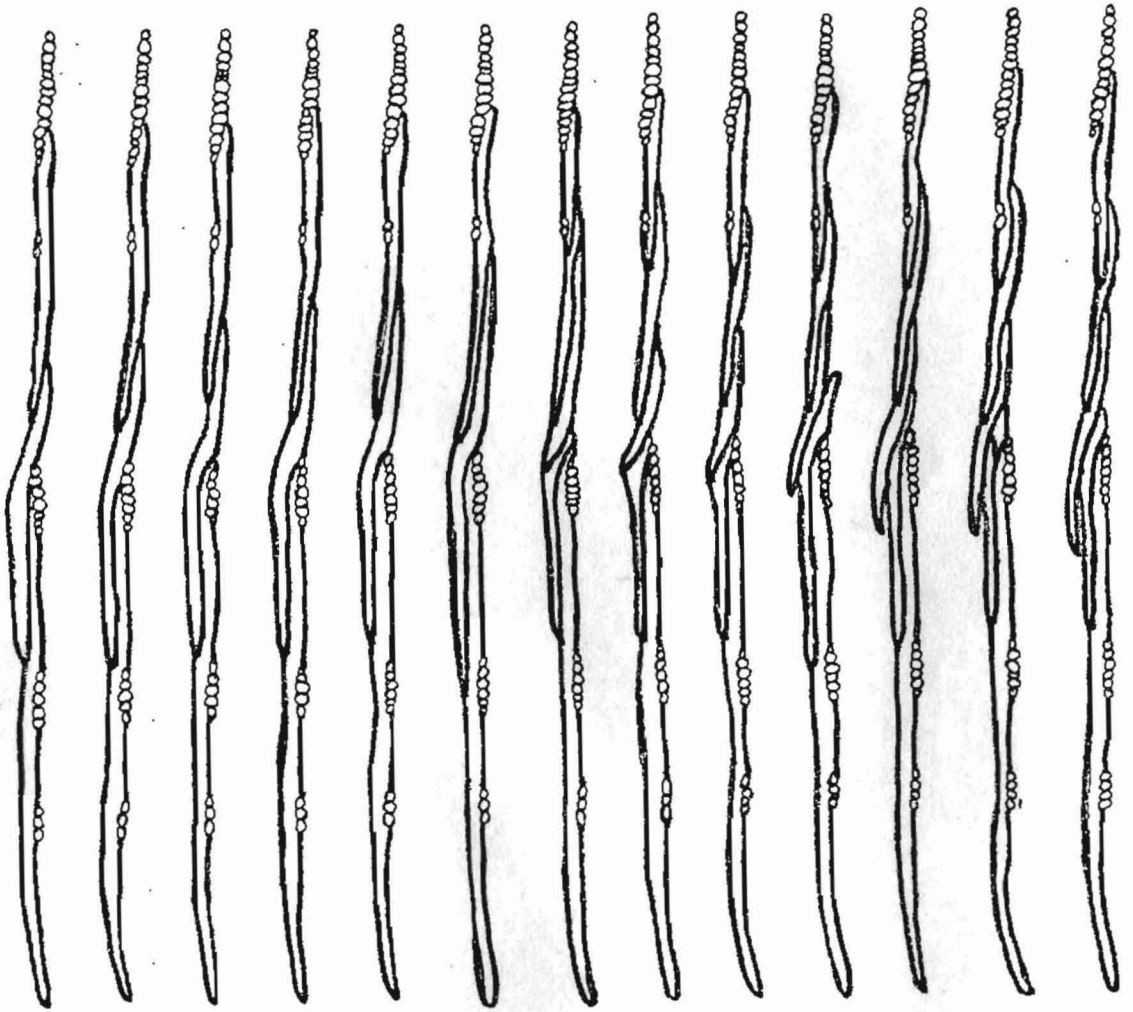
116 115 114 113 112 111 110 109 108 107 106 105 104 103

Section No.

SERIES 3

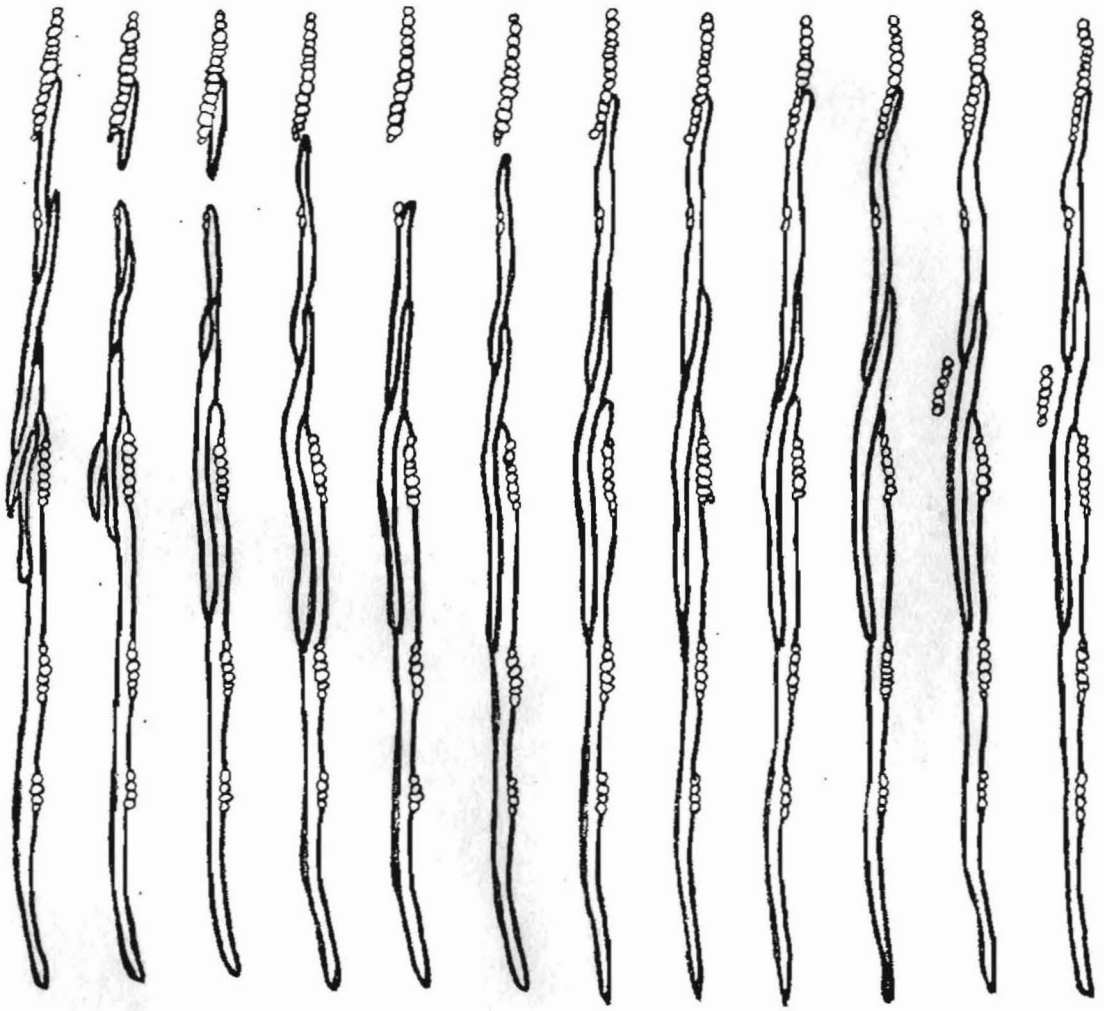


SERIES 3



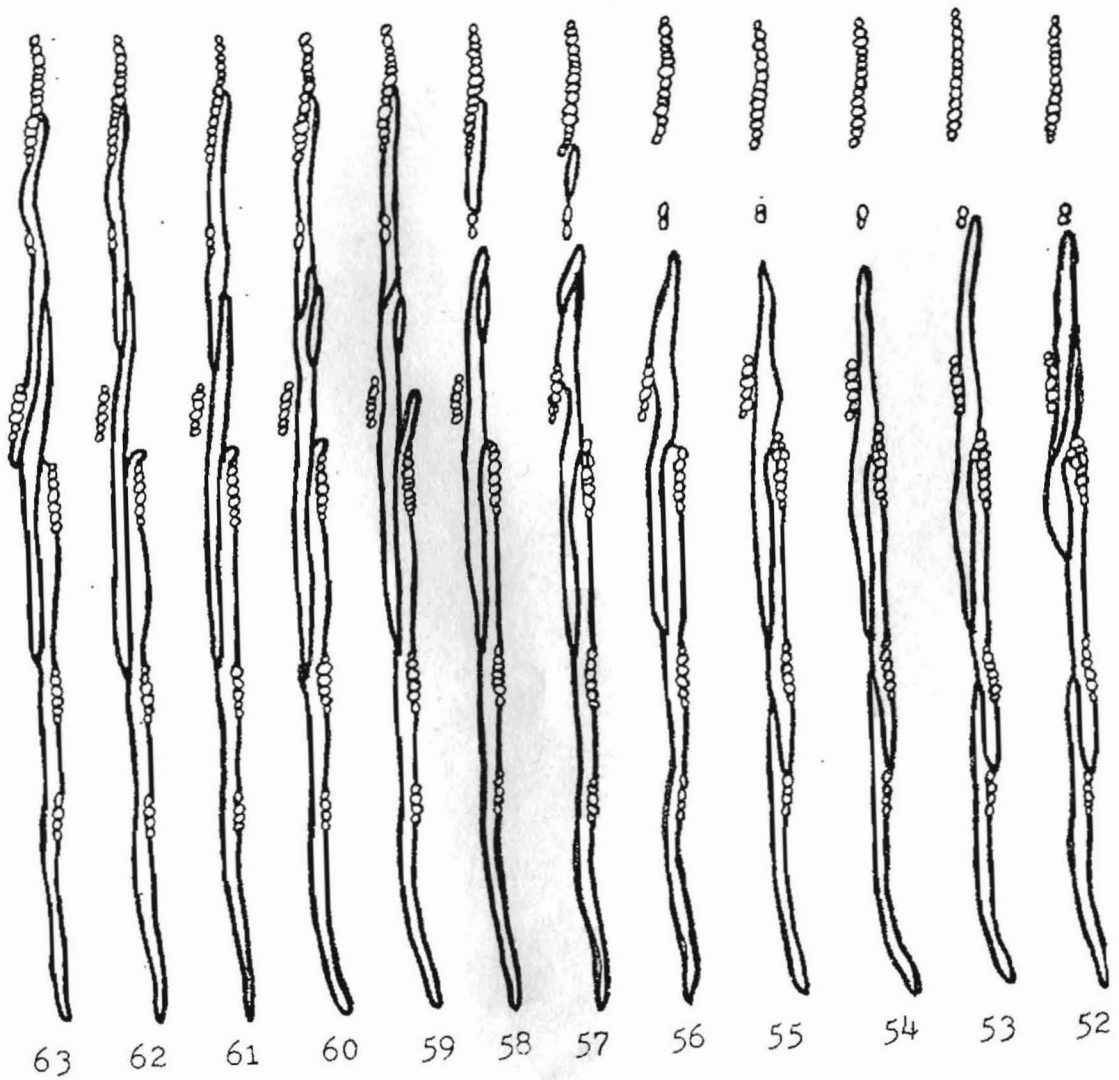
Section No.

SERIES 3

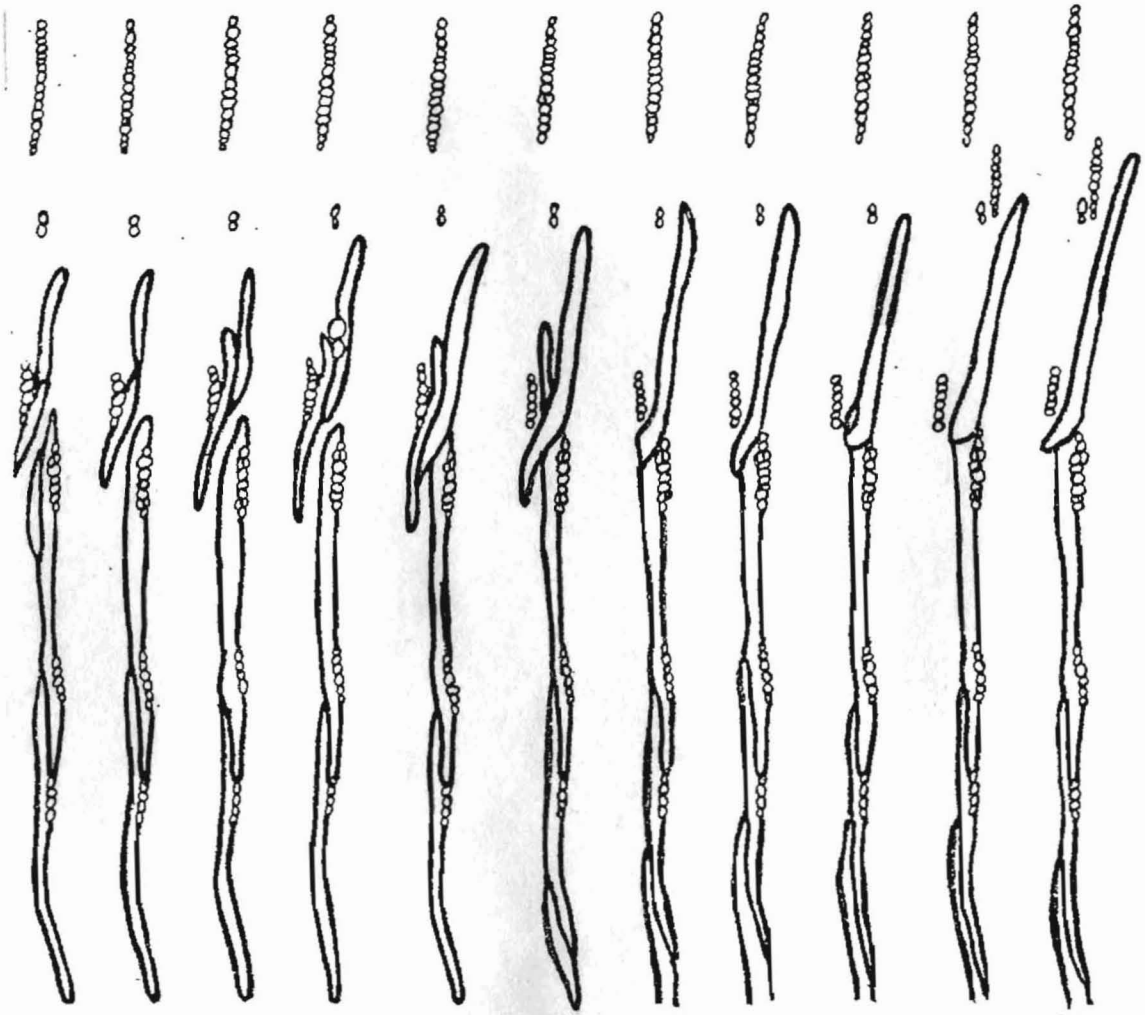


Section No.

SERIES 3

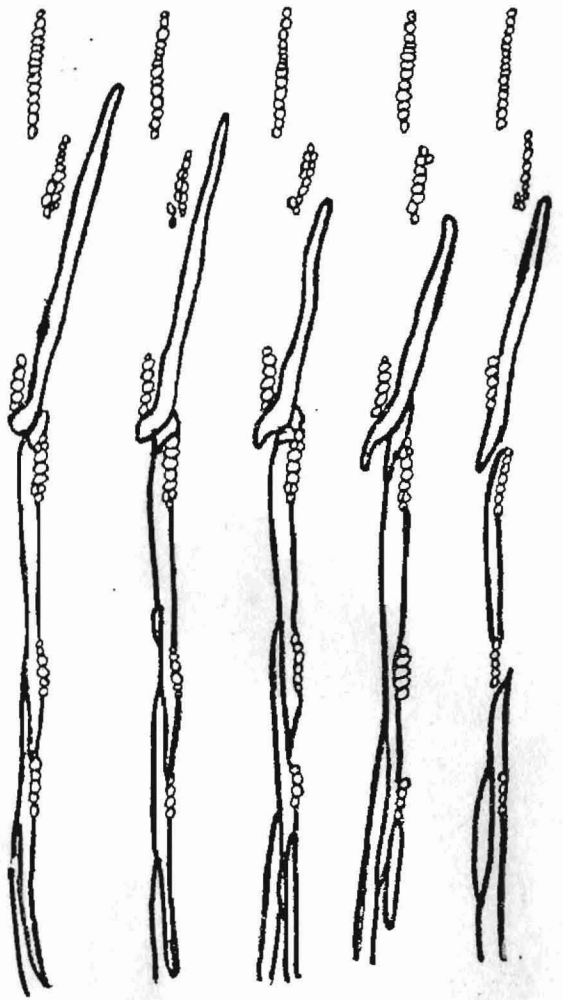


SERIES 3



Section No.

SERIES 3



40 39 38 37 36

Section No.

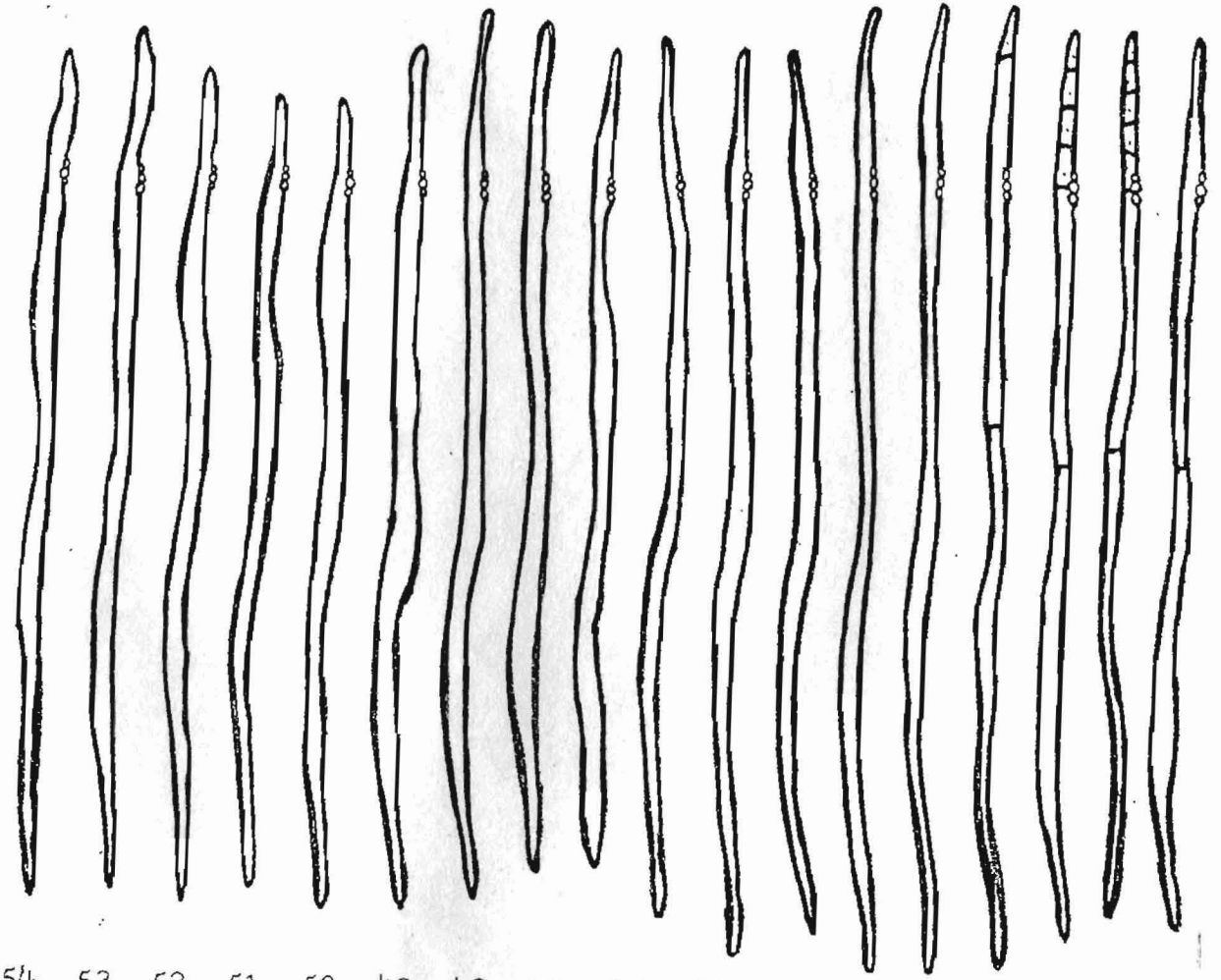
SERIES 4

Lodgepole Pine

Vertical Bridge

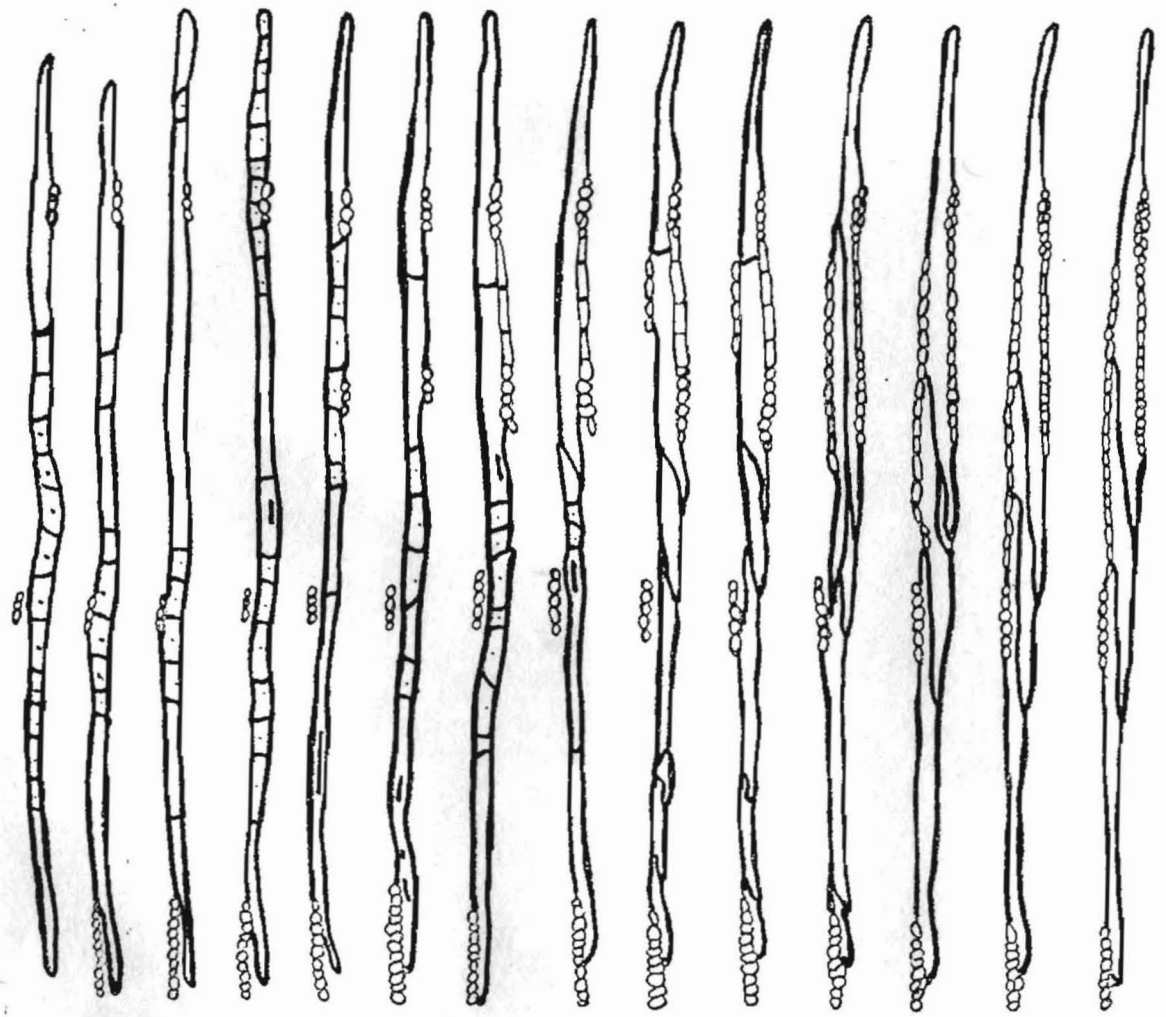
Section 54 is closest to the pith. Section 23 is in the cambial zone. This series should be viewed as if the observor were close to the pith and moving backwards toward the bark. Figure 6 shows the location of Series 4 relative to the vertical bridge. This series was traced from xylem which had formed over a period of 38 days following construction of the vertical bridge.

SERIES 4



Section No. 54 53 52 51 50 49 48 47 46 45 44 43 42 41 40 39 38 37

SERIES 4



Section No. 36 35 34 33 32 31 30 29 28 27 26 25 24 23

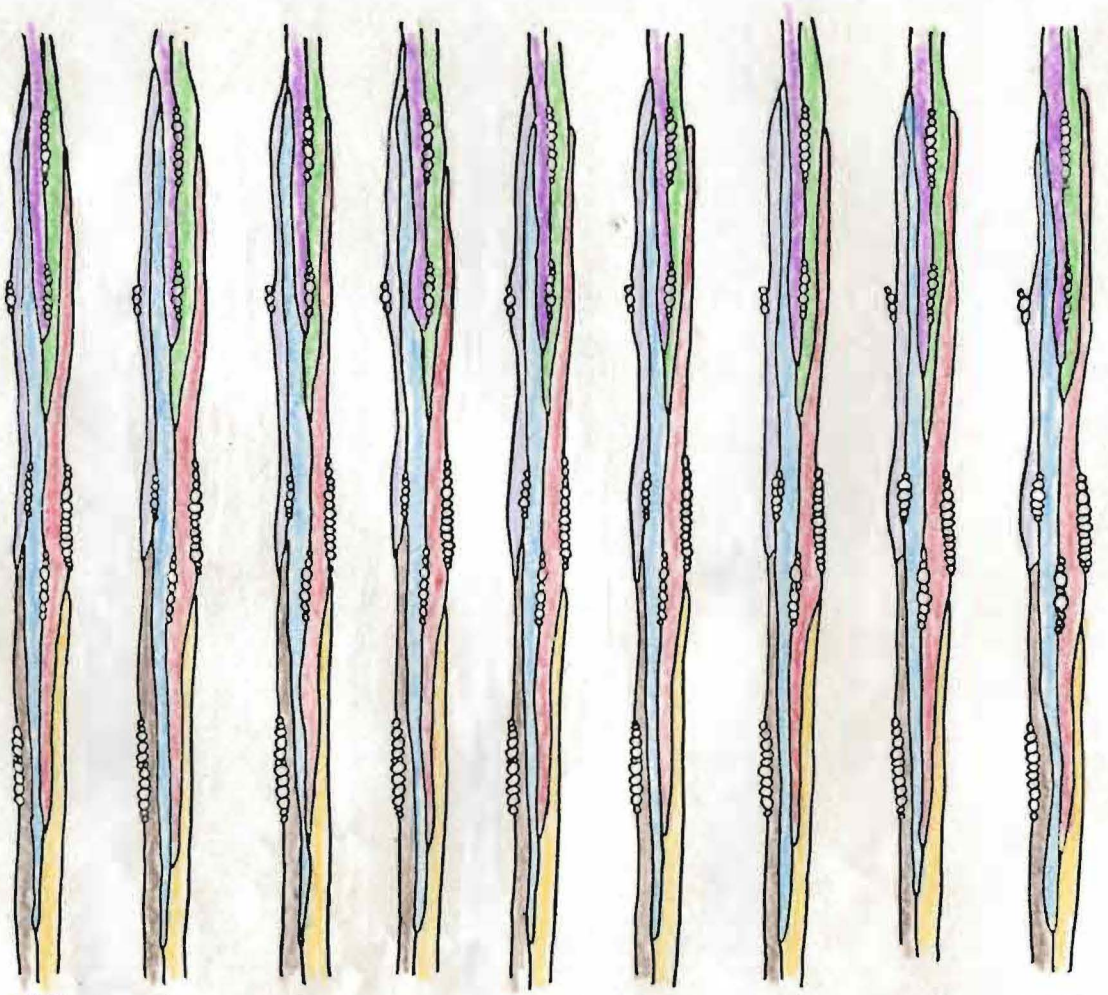
SERIES 5

Lodgepole Pine

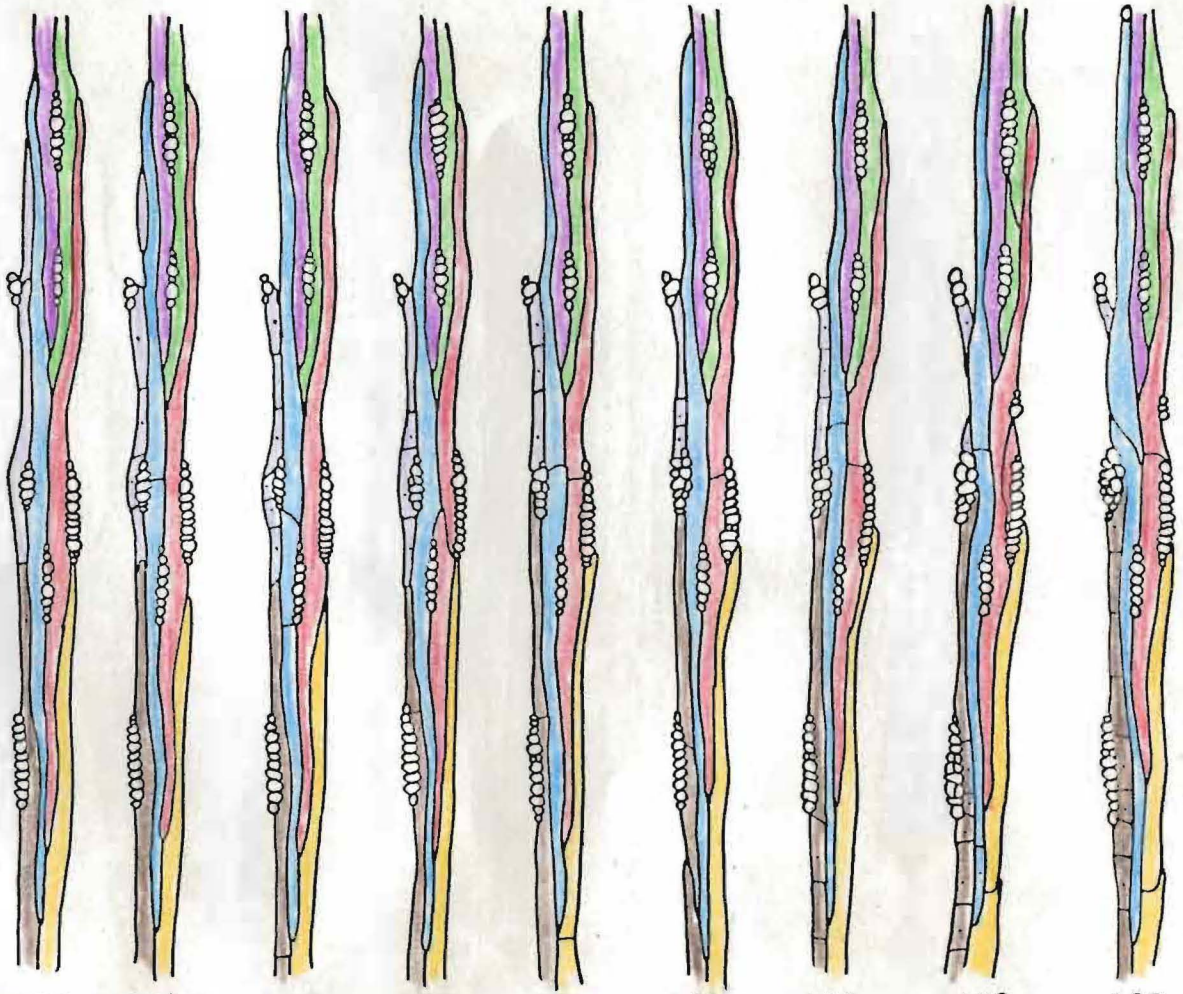
Diagonal Bridge

Section 144 is closest to the pith. Section 41 is furthest from the pith (closest to the cambium). This Series should be viewed as if the observor were close to the pith and moving backwards toward the bark. Figure 5 shows the location of the series relative to the diagonal bridge. This series was traced from xylem which had formed over a period of 50 days following the construction of the diagonal bridge.

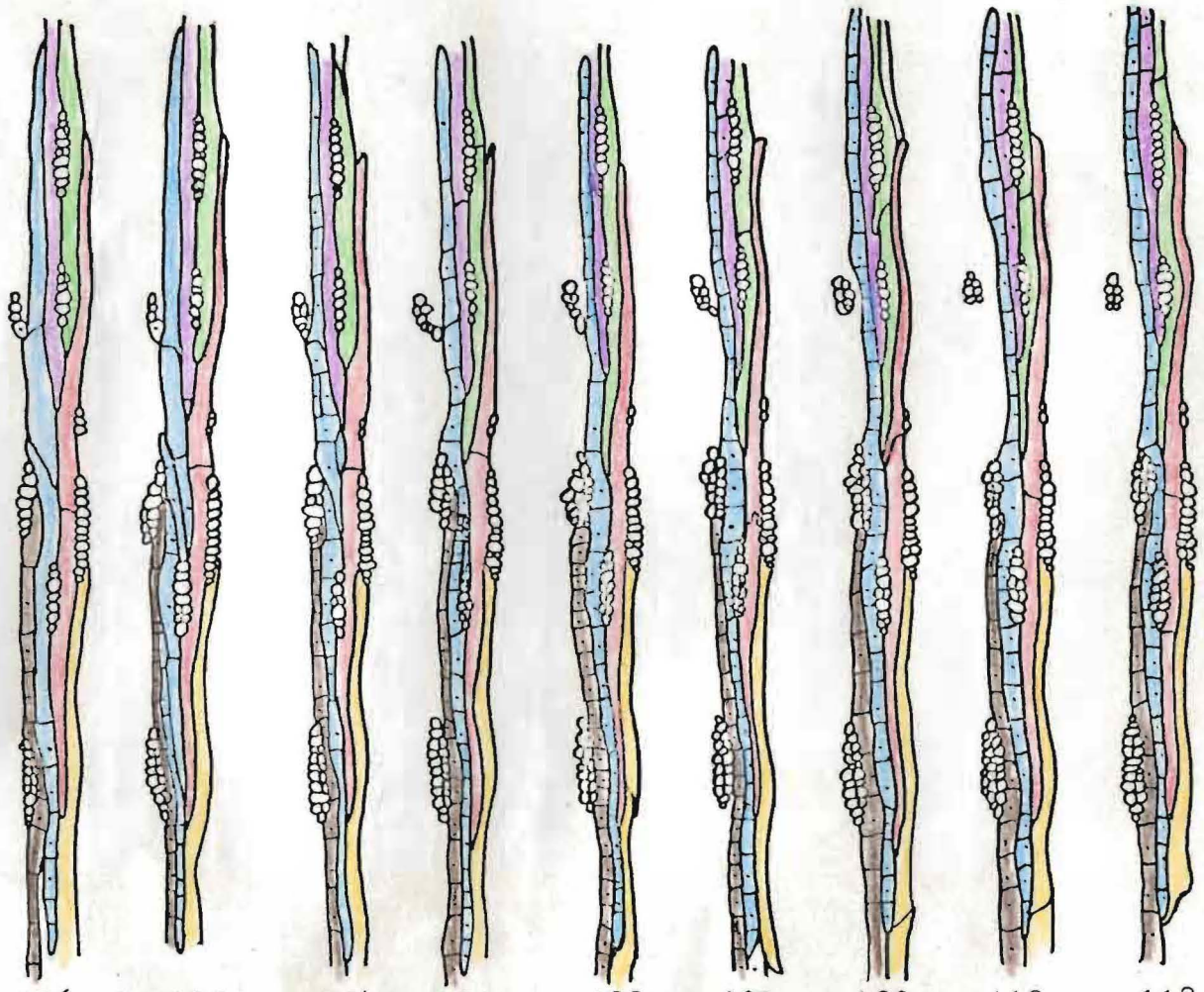
SERIES 5



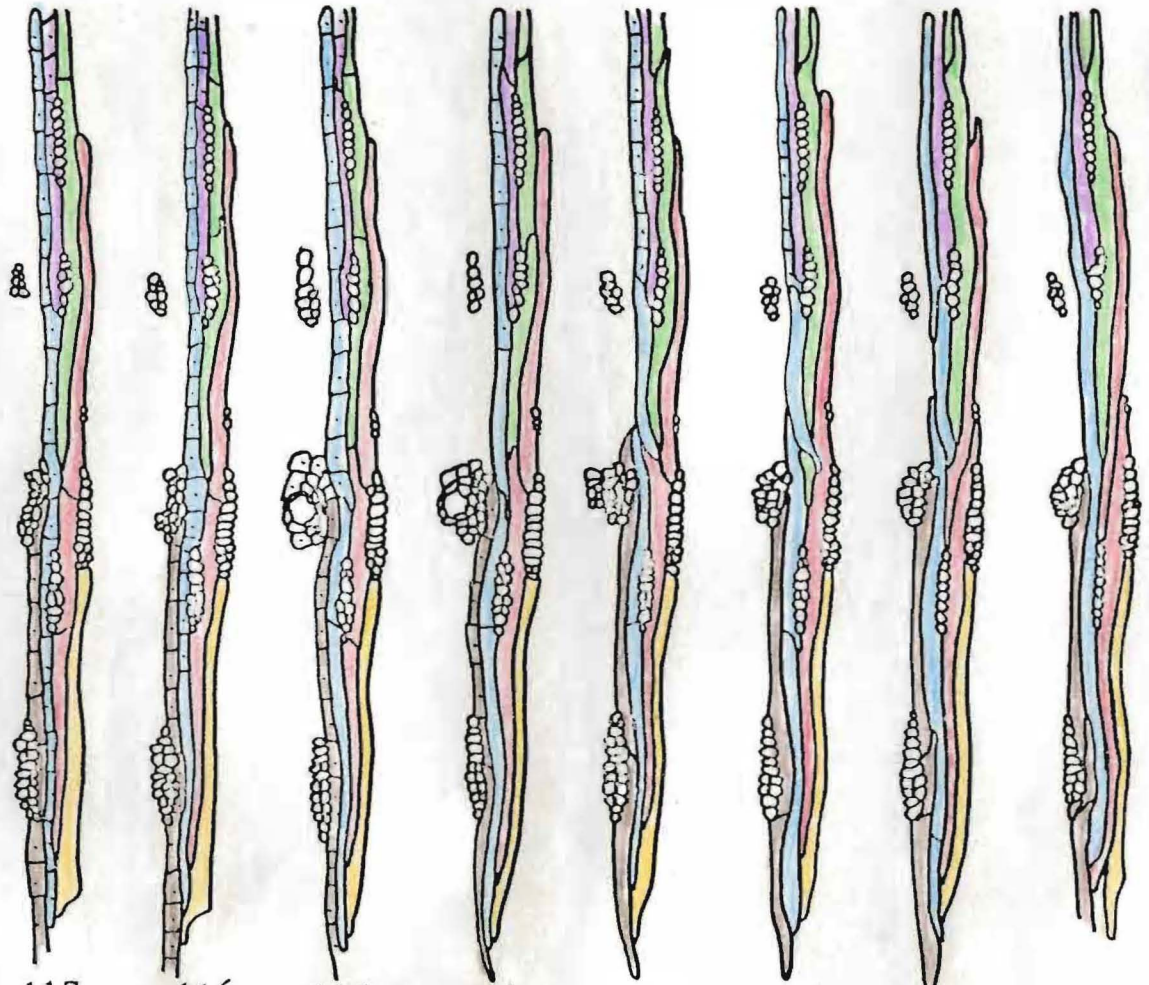
SERIES 5



SERIES 5



SERIES 5

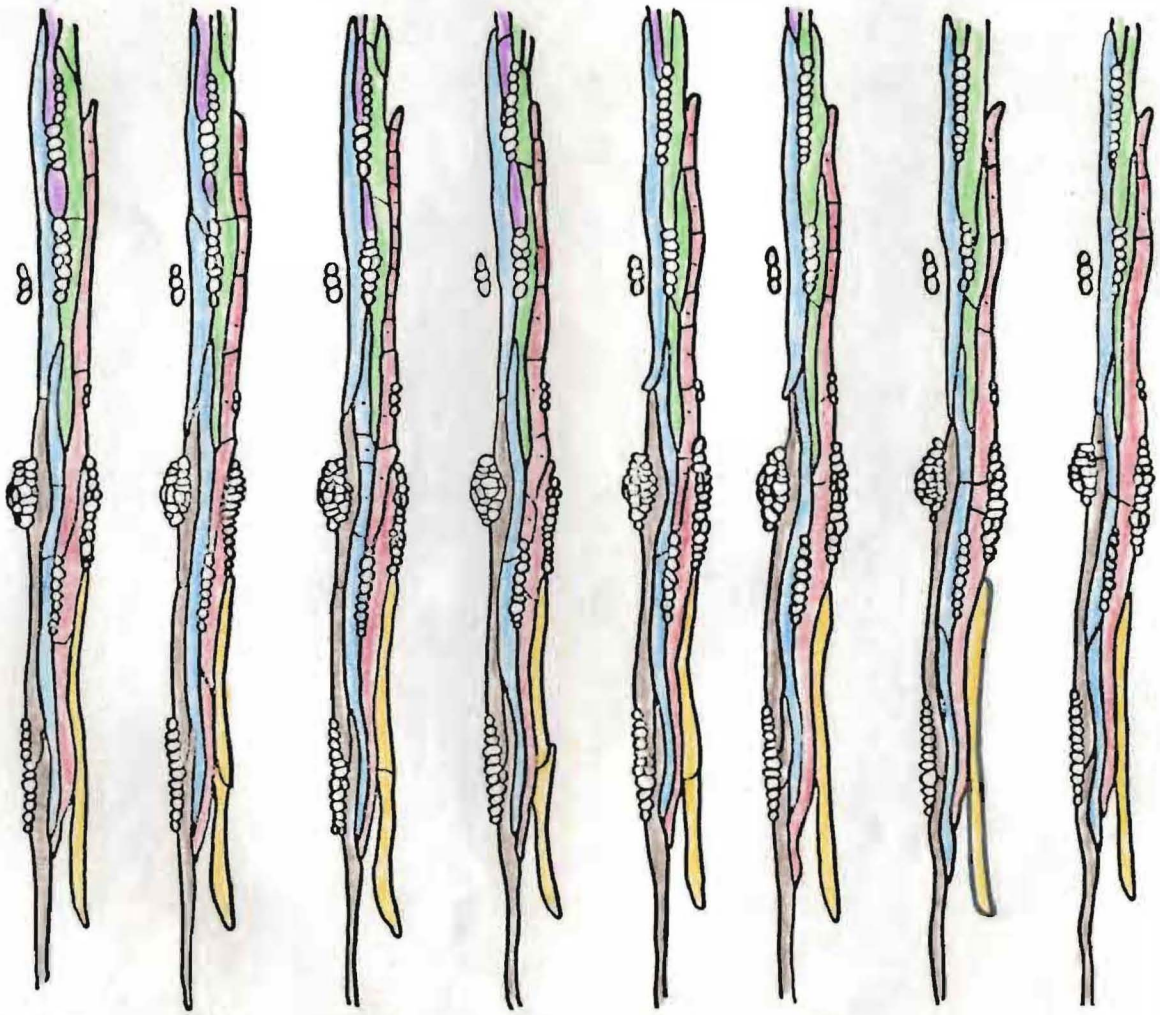


Section No.

SERIES 5

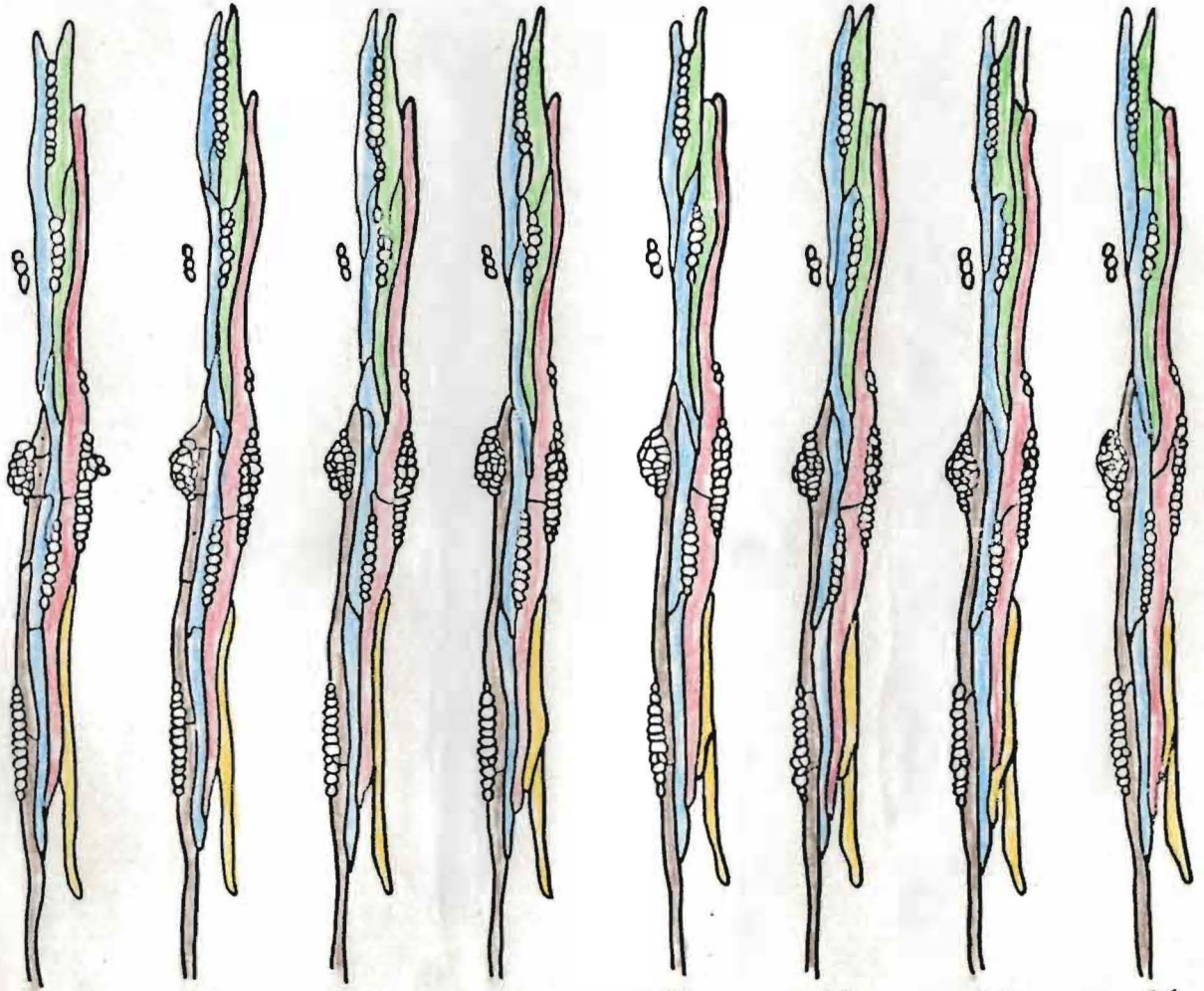
Section No.

SERIES 5



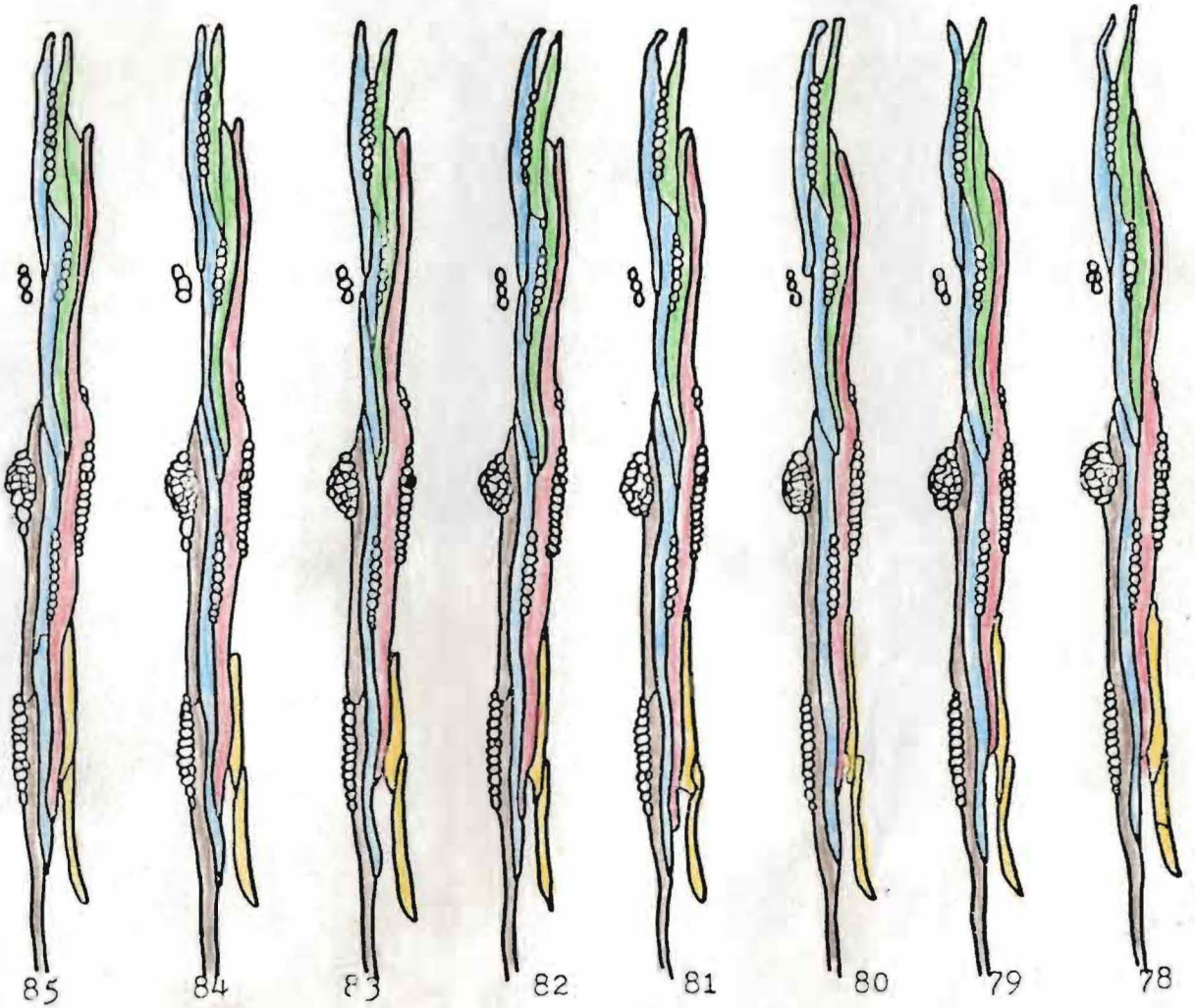
101 100 99 98 97 96 95 94

SERIES 5



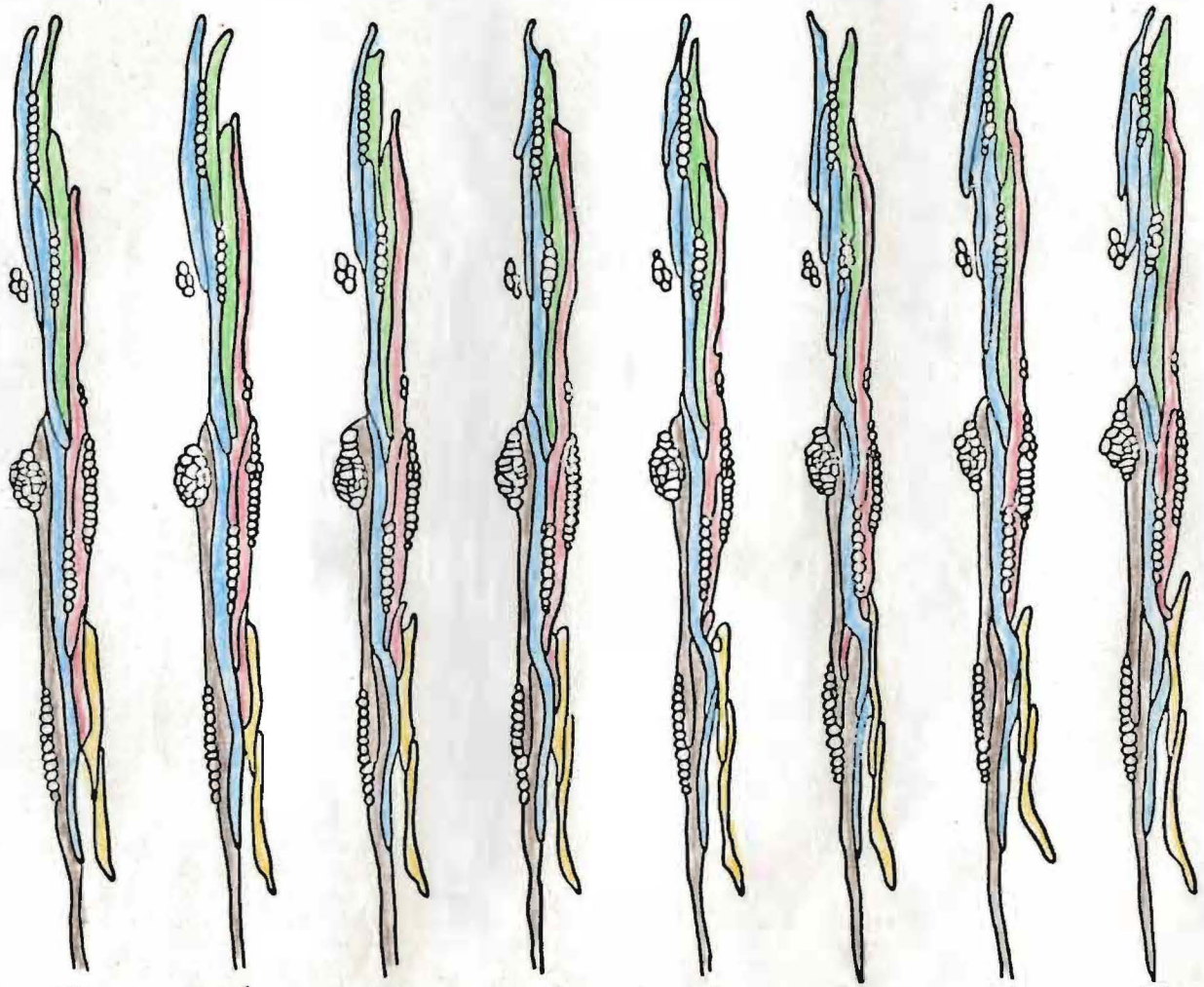
Section No.

SERIES 5



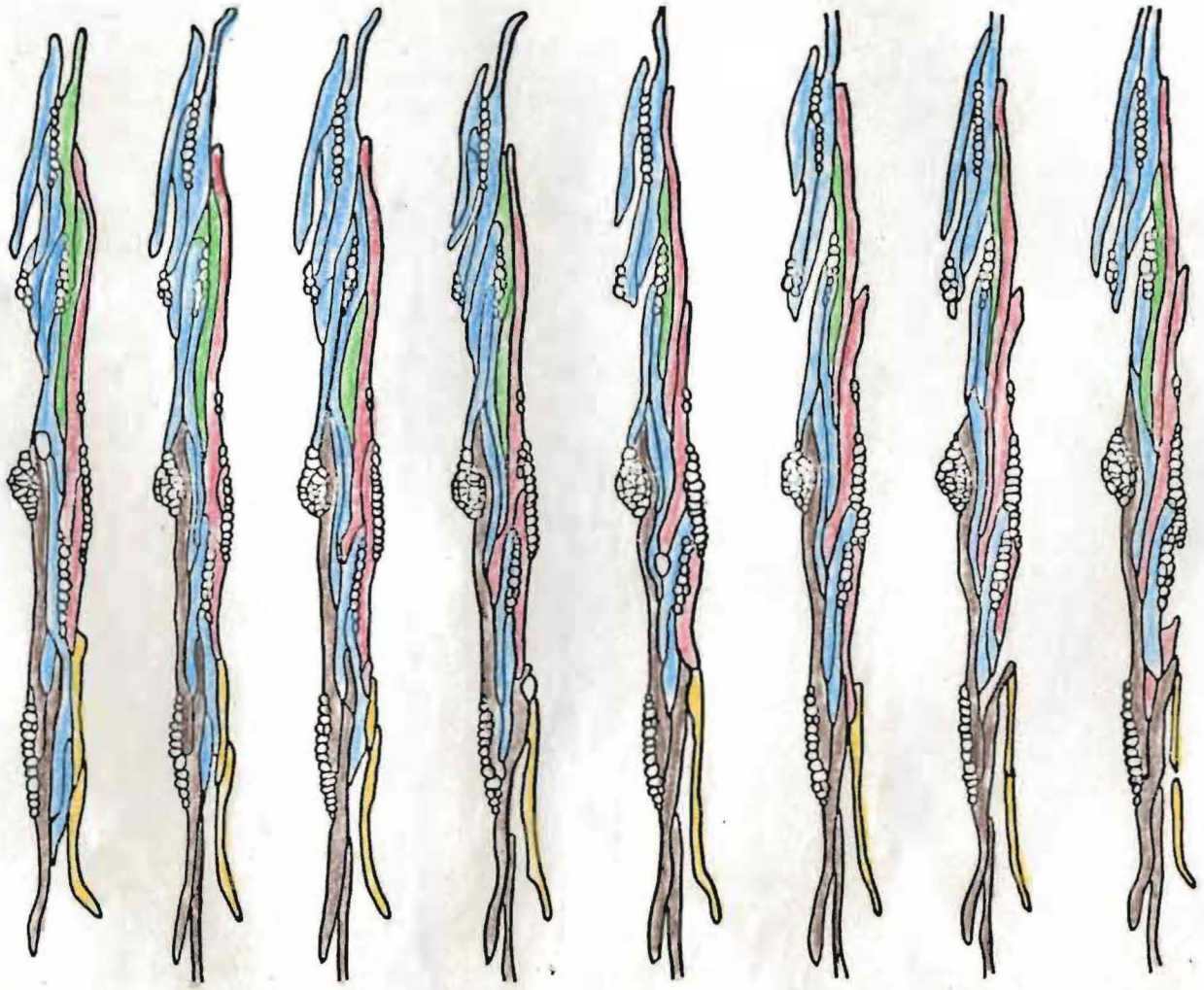
Section No.

SERIES 5



Section No.

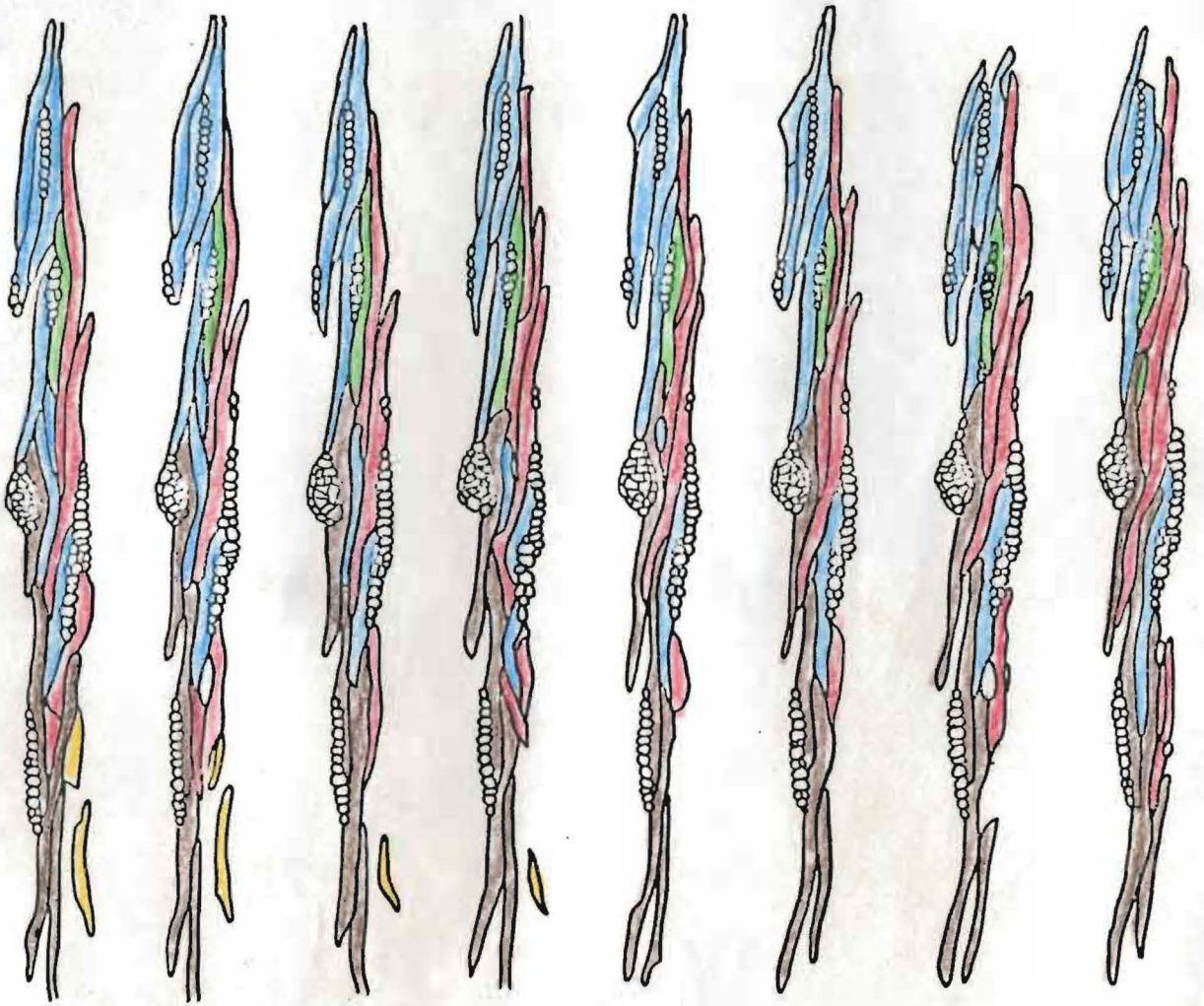
SERIES 5



69 68 67 66 65 64 63 62

Section No.

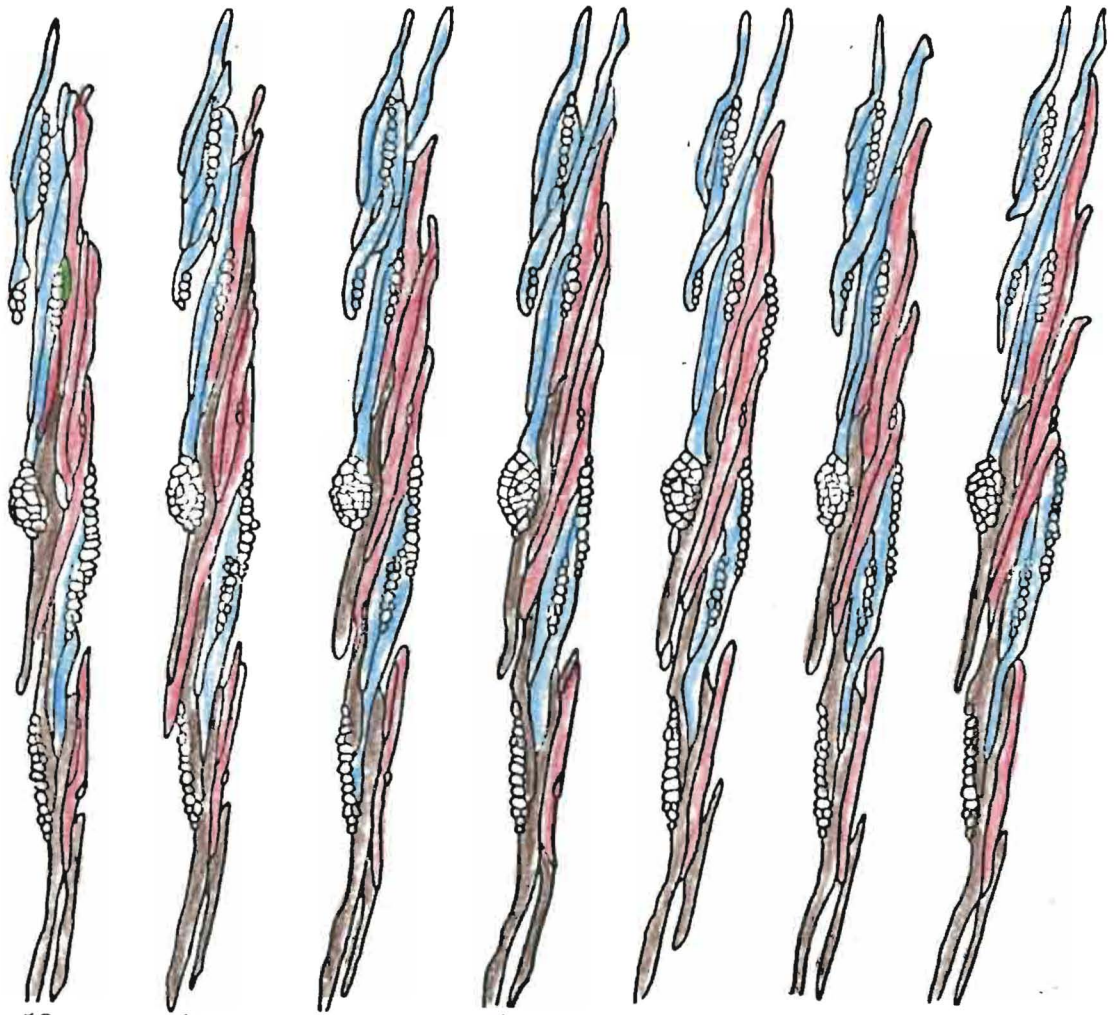
SERIES 5



60 59 58 57 56 55 54 53

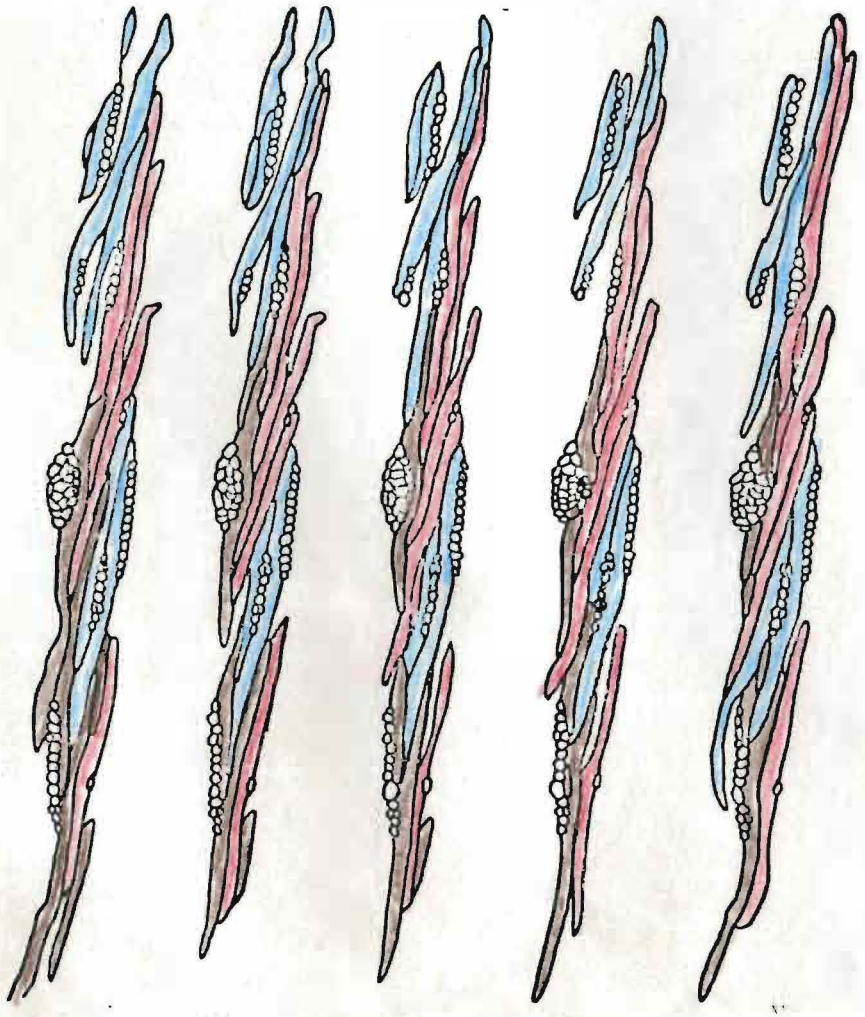
Section No.

SERIES 5



Section No.

SERIES 5



45 44 43 42 41

Section No.

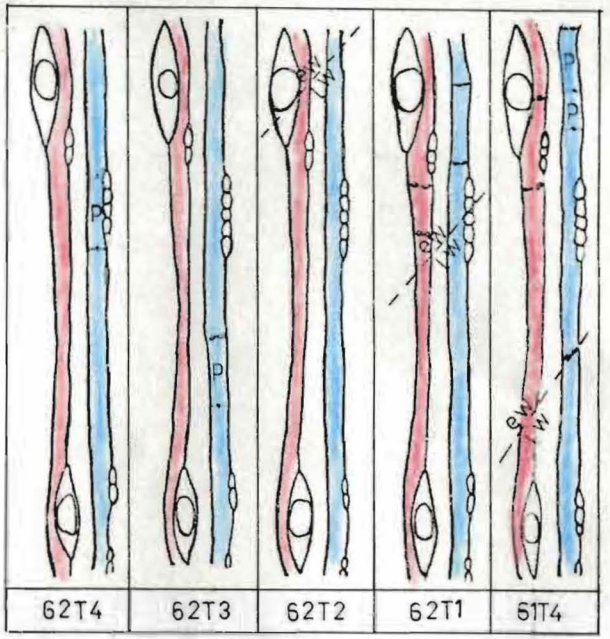
SERIES 6

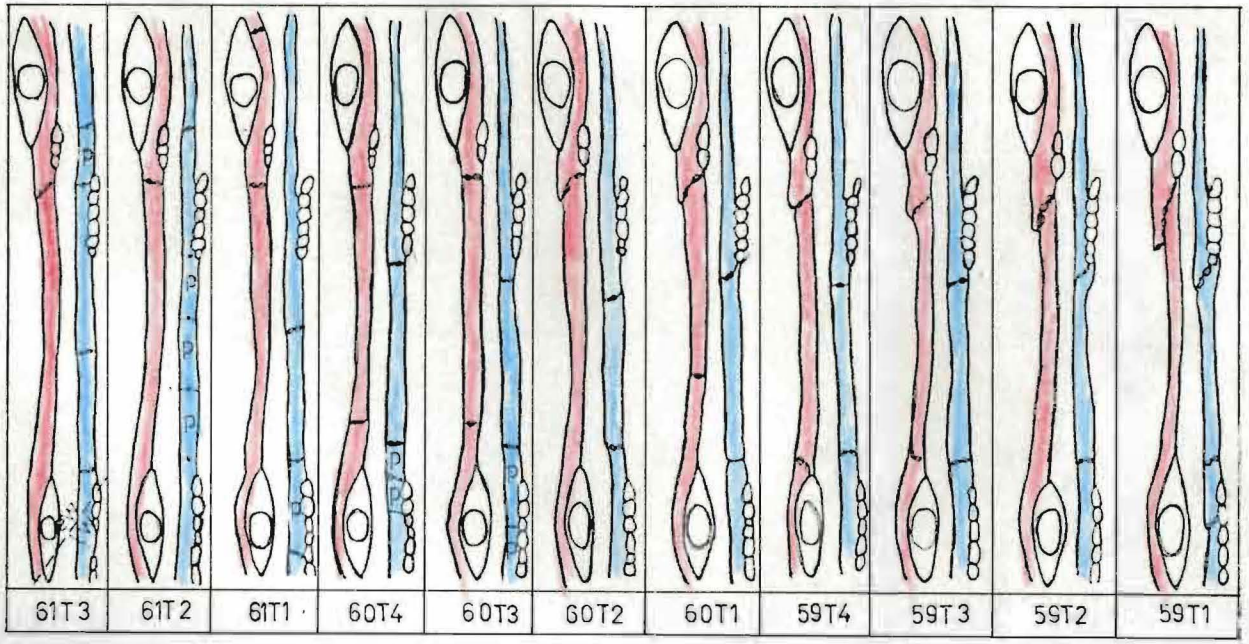
SERIAL TANGENTIAL SECTIONS, REPRODUCED BY TRACING OF PHOTOMICROGRAPHS, OF Pinus strobus SHOWING A 1-mm IN-LENGTH VIEW OF XYLARY ELEMENTS AT SEQUENTIAL STAGES WHICH RESULT IN A CHANGE IN ORIENTATION FROM THE VERTICAL TO THE BEGINNING OF AN UPWARD-TO-THE-RIGHT SLANT IN THE EARLYWOOD OF A PHLOEM BRIDGE ORIENTED 45° UPWARD TO THE RIGHT

SPECIMEN UWP 1
DISK NO 5(T)
} (SEE FIGURE 2)

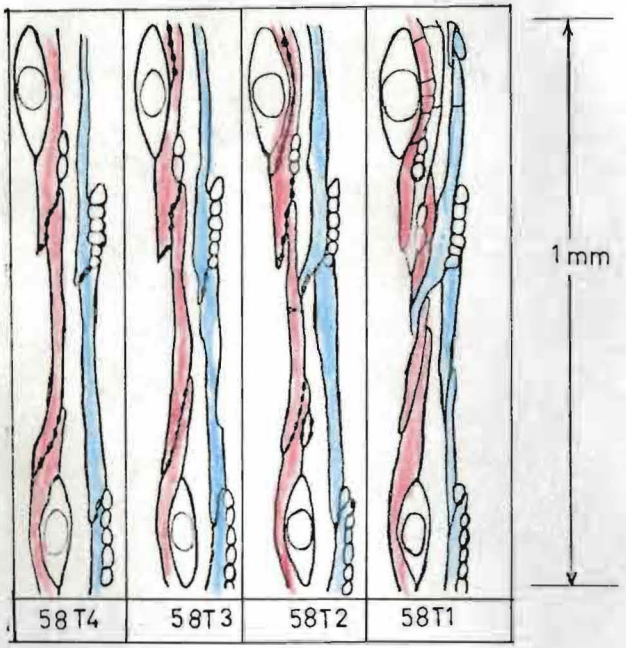
SECTION THICKNESS: 20 μ

p - AXIAL PARENCHYMA
⊷ - BORDER-PITTED END WALL
⊸ - SIMPLE-PITTED END WALL
— - UNPITTED END WALL
EW / LW - EARLYWOOD-LATEWOOD BOUNDARY.





TANGENTIAL SECTION NUMBER


1 mm
58T4
58T3
58T2
58T1