STRUCTURE AND DEVELOPMENT OF THE BARK OF DOUGLAS-FIR, *PSEUDOTSUGA MENZIESII* (MIRB.) FRANCO.

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1956

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Date thesis is presented: May 2, 1956

Typed by Georgia Grillos

ACKNOWLEDGEMENTS

I wish to express deep appreciation to Dr. Frank H. Smith, my major professor, for suggesting the problem and for guidance throughout this study.

Special thanks are due to Dr. Leo E. Jones for the use of his photographic equipment and his eagerness to offer advice in taking photomicrographs.

To Mr. H. H. Millsap, I am grateful for the innumerable suggestions in developing negatives and the preparation of prints.

I also wish to express my gratitude to the many faculty members and fellow graduate students of the Department of Botany and Plant Pathology for their encouragement offered so willingly during the process of research.

Finally, I wish to express special thanks to the Albertsen's Lumber Company, Philomath, Oregon, and to the School of Forestry, Oregon State College, Corvallis, Oregon, for permission to collect bark samples for this study from Douglas-fir trees under their control.

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INTRODUCTION

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco., also known as Douglas-spruce, Red-fir, Puget Sound-pine, and Douglas-tree (Britton, 12), is the most abundant tree of western North America. It is found growing from British Columbia southward to the mountains of Arizona and into Mexico, eastwardly to Alberta, Colorado, and Texas, at elevations from sea level up to 9,000 feet. Its area of greatest abundance and dimensions is near the coast at low elevations in Oregon and Washington, where it forms pure forests of great extent and reaches its maximum height of 290 feet, with a trunk diameter of approximately 16 feet.

Douglas-fir is considered to be the most important tree species in the Pacific Northwest. Many lumbermen consider Douglas-fir as one of our most important species in the total production of lumber and plywood in the United States. The annual production of lumber in the states of Oregon and Washington alone is somewhere near 10 billion board feet (Kurth, 40).

The lumber industry is responsible for much of the research that has been done on the wood of Douglas-fir. In comparison, however, little has actually been done on the bark. The volume of available bark from Douglas-fir is immense. A study made by Voorhies (49) indicates that roughly 500 pounds of bark, dry weight basis, are obtained at sawmills for every 1,000 board feet of Douglas-fir logs. Because of this enormous volume of bark that is available, the lumber

industry has recognized the importance of this natural resource, and has attempted, in recent years, to support more research projects designed to find more uses for the bark in industry.

At this point there is need for a concise definition of the term bark. Bark, as defined by Esau (24), is applied most commonly to all tissues outside of the vascular cambium of the axis, in either primary or secondary state of growth. In this usage bark includes primary phloem and cortex in axes with primary tissues only, and primary and secondary phloem, various amounts of cortex, and periderm in axes with secondary tissues. For convenience, the bark of older axes is further separated into two categories, the inner bark and the outer bark. The inner bark is that region between the vascular cambium and the last-formed periderm, and is composed of secondary phloem. It usually comprises several seasons' growth. It is often termed "active bark", although some tissues at the outer region of the inner bark are functionless. The outer bark is the region outside the last-formed periderm layer and consists of alternate layers of periderm and dead secondary phloem tissues.

The Douglas-fir bark utilization program had its origin during World War II, when cork was difficult to obtain from the Mediterranean countries. Many research laboratories in this country were then asked to investigate the possibilities of extracting cork from the bark of certain trees growing in the United States. It has been known for a long period of time that the bark of Douglas-fir is extremely rich in cork. Since Douglas-fir has a rich supply of cork, and since it is extremely abundant, investigators began developing methods for removing

the cork.

The best method developed to date for the separation of cork from the bark is a purely mechanical one (Anway, 5). The bark is first washed, chopped, pulverized, and finally screened many times through different sized screens. About 19 per cent is removed from the bark by this method. In additional to the cork, the bark yields about 18 per cent sclereids, and 36 per cent finely powdered, amorphous substance.

This simple method for separation of the various components of the bark paved the way for chemical analysis of the various tissues present. The chemical nature of Douglas-fir bark has been pretty well determined by a number of investigators (4, 17, 27, 28, 33, 35, 36, 37, 38, 39, 40, and 50). The bark contains the following chemical and structural components:

1. Extractives
   a. Approximately 9 per cent tannins
   b. Approximately 7.5 per cent waxes
   c. Approximately 7 per cent dihydroquercetin
   d. Many volatile oils
   e. Organic nitrogen compounds

2. Holocellulose
   a. Cellulose
   b. Hemicellulose

3. "Lignin" - phenolic bark acids - approximately 60 per cent

4. Cork

Many of the above chemical substances have valuable uses in industry. For example, tannins are used in leathery-making and oil-well drilling; "lignin", more correctly a mixture of phenolic acids of low methoxy-group content, is used as a dispersant, binder and de-flocculant in ceramic clays; antioxidant and sequestering agent in

boiler feed water; partial phenol replacement in plywood adhesives and molding compounds; and as a flotation agent in ore beneficiation. These are but a few uses for these chemicals, and many more exist.

Recently the bark of Douglas-fir has been made to yield five valuable products which are marketed under the general trade name of "Silvacon" (4). These five products are groups of chemical and structural components of the bark which have been obtained by treating the bark in five different ways. These products are extremely valuable in industry. They are used in plastic manufacture, as ingredients of magnetsite flooring, in the preparation of absorbent filters for explosives, in tile flooring, buffing and abrasive compounds, casting resin reinforcing agents, hard rubber and asphalt compounds, and as ingredients in fiber paints.

The bark in many cases is cut up into long slabs and used as fuel. Sometimes it is ground up and used as a soil conditioner, as a mulch, and also as a litter in barns and feed lots.

Since most of the uses of Douglas-fir bark are based on chemical derivatives, little attention has been paid to the microscopic structure and development of the bark. This study is intended to meet the need for a fundamental investigation of the structure of the mature bark, and also of the origin and development of the various tissue components that differentiate as the bark matures.

REVIEW OF LITERATURE

Allen (3), investigating the shoot apices of seedlings and older plants, and Crafts (19), investigating mainly the shoot apices of older plants, gave brief descriptions of the origin and differentiation of the primary tissues of the bark of Douglas-fir. Analysis of the shoot apices of older plants was also made by Sterling (46). He confirmed the work of Allen and Crafts, and gave a more detailed description of the sieve fields on the radial walls of the sieve cells, the phloem parenchyma, and the development of resin canals in the cortical region.

Sclereids develop in the cortical region of the bark of Douglas-fir. Sterling (45) gave a concise description of their origin and development. He found that they originate from cortical parenchyma cells which in their development undergo hypertrophic growth, ramification, and finally sclerification. He reported that the mature sclereids are quite irregular in shape, without a living protoplast, have an extremely thick wall with distinct lamellae, and possess an abundance of simple pits which terminate in the cell cavity.

The microscopic structure of mature Douglas-fir bark has been described briefly by several investigators. Hergert and Kurth (28), reporting on the chemical nature of the cork, gave a brief description of the microscopic structure of both the inner and outer bark. The inner bark consists of sieve cells, phloem parenchyma, and sclerenchyma cells that are considered to be bast fibers. The outer bark consists of anastomosing bands of cork in which annual growth layers are usually

visible. Interspersed among these yellow corky bands are areas of red phloem tissues that contain brittle, elongate bast fibers and crushed parenchyma.

Kiefer and Kurth (33), in their investigation of the chemical nature of the bast fibers in the secondary phloem tissues of the inner bark, described these as short, sharply-pointed cells with thick walls that show concentric rings. They also reported the presence of simple pits which terminate in the cell cavity that appeared to contain living protoplasm. The fiber is surrounded by a wall or matrix of reddish-lignified substances that appear to be removed by dilute alkalin solutions.

The most complete description of the structure of Douglas-fir bark was reported by Chang (15). He described the microscopic structure of the inner bark and the outer bark. For the inner bark he recorded the sizes, shapes, abundance, and the arrangement of the following cell types: sieve cells, fibers, parenchyma cells of two types, and the uniseriate and fusiform rays. He also described, in detail, the periderm layers and mentioned the interspersed crushed phloem tissues present in the outer bark.

MATERIALS AND METHODS

Douglas-fir bark collections were made at varying intervals from January 30, 1955 to December 8, 1955. Most of the bark samples used in this study were collected from trees growing in two areas. One area is near Wren, Oregon, at an elevation of approximately 600 feet. The trees in this area are growing in the open. The other area is approximately 7 miles northwest from Corvallis, Oregon, and is located in the MacDonald Forest, at an elevation of about 650 feet where the trees are growing under various degrees of suppression. One collection was made from an old tree found growing approximately 13 miles northwest from Corvallis, Oregon. This tree was estimated to be over 200 years old.

On each collection date samples were taken from several trees of different ages, usually only one sample from each tree. On a few occasions more than one sample was collected from a tree. Wherever possible the samples were taken from trees growing on the south facing slope to eliminate some of the variations due to environmental conditions.

Bark samples were removed from larger trees, at approximately the 4-foot level, by making parallel saw cuts into the wood and chiseling out the blocks. Samples were obtained from smaller trees after cutting them in half. The materials were wrapped in wet paper toweling and placed immediately into pliofilm bags to avoid excessive drying out and oxidation. The following notes were recorded at the time of collecting: the age of the tree; the internal and external coloring of the bark; the appearance of the bark; and the time of bark slippage.

During the time of cambial activity, extreme difficulty was encountered in removing bark samples without damaging the cambium and undifferentiated xylem and phloem tissues.

Some of the bark samples were sectioned and examined in the fresh state. Most of them, however, were immediately cut to the desired size, trimmed to later obtain cross, radial, and tangential sections, and placed into a killing and fixing solution. Two killing and fixing solutions were employed in this study. One was a formalin-acetic acid-alcohol mixture prepared according to Sass (44), and the other was a chrome-acetic-formalin mixture (Randolph's modified Nawaschin's formula) prepared according to Johansen (31). The best results were obtained with the latter killing and fixing solution. All the samples were subjected to aspiration while in the killing and fixing solution. After three days in the fixative, the materials were washed thoroughly in running water and placed in a 15-per cent hydrogen peroxide solution containing approximately 4 drops ammonium hydroxide. Hydrogen peroxide is a bleaching reagent and removes some of the tanniferous substances from the bark. Ammonium hydroxide permits rapid penetration of the bleaching reagent. The samples were then washed in water and dehydrated in 15, 30, 50, and 70 per cent ethyl alcohol. The samples were stored for an indefinite period of time in 70 per cent alcohol.

The following three methods were primarily employed in the preparation of the killed and fixed materials for sectioning:

I. Paraffin Method

The stored bark samples were dehydrated and infiltrated according to Johansen's (31) tertiary butyl alcohol-paraffin oil schedule, and

embedded in 56°-58° C. Fisher Tissuemat. The paraffin-embedded samples were trimmed to expose the surface to be sectioned and soaked for a period of one month at 37° C. in a solution containing glycerol and a detergent (Alcorn and Ark, 2). This soaking solution was then replaced by water and the samples were stored for an indefinite period of time in a refrigerator.

II. Celloidin-Paraffin Method

Many bark samples were first infiltrated and embedded in celloidin and later in paraffin by the double-embedding method of Johansen (31). The samples were gradually brought up to absolute ethyl alcohol, rinsed twice, and transferred to a mixture of equal parts of absolute alcohol and ether. The solution was changed twice over a 24-hour period and the samples were transferred to a wide-mouth jar and covered with 2 per cent celloidin. The jar was then capped and placed in an oven at 53° C. From the 2 per cent celloidin solution, the samples were transferred to a 4 per cent and later to a 6 per cent solution, allowing one to three days in each solution to permit complete penetration of the celloidin into the blocks. The samples were then transferred to chloroform for 24 hours to permit complete hardening of the celloidin in all parts. They were then transferred to tertiary butyl alcohol and later to paraffin; and were finally embedded in 56°-58° C. Fisher Tissuemat. The embedded samples were then trimmed to expose one surface and soaked for a period of one month at 37° C. in a mixture of equal parts of glycerol and 95 per cent ethyl alcohol. After one month of soaking, the samples were then transferred and stored in a fresh mixture of this solution.

Most of the materials used in this study were prepared by the paraffin method. The celloidin-paraffin method has certain disadvantages: the preparation of materials for sectioning is too time consuming; it is too costly; and it is very difficult to get good serial sections because the celloidin has a tendency to cause the sections to curl. Materials prepared by both methods were cut at 8 to 18μ with a rotary microtome in which a microtome blade was used. The best sections were obtained when the materials were cooled down with carbon dioxide under pressure just before cutting. Haupt's adhesive (Johansen, 31) was used to mount the sections on slides. The sections were stained progressively in safranin and hematoxylin.

III. Carbowax Method

A few samples were embedded in a water-soluble wax, polyethylene glycol (Carbowax 4,000). This wax was used by Blank and McCarthy (10) in the clinical preparation of animal tissues. Jones (32) has tried this on various plant tissues, and his modifications were followed here. The blocks of bark, previously stored in 70 per cent alcohol, were carried through 50, 30, and 15 per cent alcohol, transferred to a 10 per cent Carbowax solution, and placed in an oven at 37° C. for five days to evaporate the water. Each bark sample was transferred from the concentrated Carbowax to a cooled surface and encased with fresh wax. The encased bark samples were attached to wooden blocks and sectioned at 20 to 30μ with a sliding microtome. To prevent curling of sections obtained by the use of the sliding microtome, the bark samples were covered with a small piece of paper as recommended by Varrelman (48). As the knife passed across the sample, the paper

slid onto the knife together with the section. The section was floated off in water that also served to dissolve the Carbowax.

In addition to the foregoing methods, many freehand sections were cut with a razor blade from fresh bark samples. These fresh sections, and those prepared by the Carbowax method, were observed without staining, or were treated with the following materials that were used to demonstrate specific cell contents: iodine-potassium iodide, ferric chloride, Bismark brown, resorcin blue, and iodine green. The iodine-potassium iodide was used as a test for starch, and the ferric chloride as a test for tannins. The latter three stains were used in combination for the staining of cellulose walls of the sieve cells, callose deposits, and the connecting strands between sieve cells (Esau, 22). Semi-permanent slides were made from the sections cut with the sliding microtome. The sections were mounted in Karo syrup, and diaphane was used to seal the cover glass.

Some bark samples were cut into slivers and treated with Jeffery's macerating solution (equal parts of 10 per cent chromic acid and 10 per cent nitric acid). The bark slivers were kept in this solution for from 1 to 2 days at 37° C., or until macerated. The macerated tissues were mounted, either unstained or stained with 1 per cent safranin, in Karo syrup and sealed with diaphane.

The photomicrographs were taken with a mounted Voigtlander camera on Kodak Panatomic-X film and enlarged for reproduction.

OBSERVATIONS

Gross Features of the Bark

The bark on young Douglas-fir trees, up to about 25 years of age, is rather smooth except for resin blisters, from one-fourth to about three-fourths of an inch thick, somewhat lustrous, and dark grayish-brown (fig. 1). In older trees the bark becomes from three-fourths to 1 inch thick, and sometimes, at the base of these trees, the bark may become as much as 5 to 24 inches thick. The bark on these old trees is divided into oblong plates which are broken into broad, rounded and irregularly connected ridges separating on the surface into small, thick, closely appressed dark reddish-brown scales (fig. 2).

General Histology of the Bark

The inner bark of mature Douglas-fir is composed of secondary phloem tissues, both living and dead. The outer bark is composed of periderm layers and dead phloem tissues.

Present in the inner bark (fig. 3) are sieve cells, both living and non-living, phloem parenchyma, fusiform phloem parenchyma, phloem rays, and sclereids. Sieve cells are arranged in regular rows broken up to some extent by the parenchyma and sclereids. Both types of parenchyma are alined in more or less tangential rows in the innermost part of the inner bark, but these rows are more or less discontinuous in the outer part of the inner bark. The rows of parenchyma are usually a single cell thick, or may be up to 3 cells thick in some parts of the line. Phloem rays are of two sizes, uniseriate, in some

cases biseriate, and fusiform. Sclereids are without definite arrangement, mostly solitary, but frequently do occur in groups of 2 to 6. The mature sclereids are located about 15 to 30 cells away from the cambium.

The outer bark consists of anastomosing layers of periderm in which annual growth layers are visible (fig. 4). Interspersed among these layers of straw-yellow periderms are areas of dark red, dead phloem tissues. The periderm layer is composed of a tier of phellogen, an indefinite number of dead, thin-walled phellem cells that appear collapsed because of the paraffin treatment, and usually 1 to 3 layers of phelloderm cells. Occasionally a few layers of thick-walled phellem cells occur in alternation with the thin-walled phellem cells.

The Vascular Cambium

The dormant cambial region of Douglas-fir appears to be from 4 to 6 cells wide (fig. 5). Approximately 2 to 3 layers of cells nearest to the mature phloem elements are partially differentiated phloem cells that were produced during the latter part of the last growing season but were unable to complete their differentiation. For the most part, these immature phloem elements are sieve cells and partially differentiated sieve fields are evident on their radial walls (fig. 6). One of the remaining layers is the vascular cambium proper, and the other or others are immature xylem cells. These cells could not be identified with certainty at this stage. These immature phloem and xylem cells complete their differentiation at the beginning of the next growing season.

The vascular cambium is of the non-storied type and consists of fusiform and ray initials (fig. 7). Both types of initials are uninucleate, and undergo periclinal divisions forming phloem and xylem derivatives. The fusiform initials give rise to the vertical systems in both xylem and phloem regions. The ray initials give rise to the ray cells, that is, elements of the transverse system of the xylem and the phloem.

The fusiform initials are elongate cells with tapering end walls when seen in a tangential section. Numerous primordial pits appear on their radial walls (fig. 8). The ray initials are small and nearly isodiametric.

In the dormant condition, the fusiform cambial initials are flattened radially (fig. 5) with a mean radial diameter in fixed material of 4 to 6μ. The combined radial walls and middle lamella are appreciably thicker than during the period of active growth, and the cytoplasm is relatively dense. The nuclei are more or less elongate (fig. 9), and usually range from 30μ to 40μ in length.

The fusiform initials differ from the ray initials most notably in length and volume as is usually the case in the cambium of other plants. The ray initials, however, surpass the fusiform initials in their radial dimensions.

Both types of initials show a progressive increase in size with an increase in age of the tree. Bailey (6) observed a similar increase in the size of cambial initials in Pinus strobus L., where the fusiform initials increase from 870μ in a one year old tree to 4,000μ in a 200 year old tree. A point is reached, however, when the initials remain constant in size as the tree ages. In Douglas-fir the length and tangential diameters are most affected by increase in age of the tree (table 1). Since the fusiform initials are longer, the longitudinal elements of secondary xylem and phloem will also be longer in older trees.

Table 1

Comparative Size of Initials in a 1-Year Old and a 200-Year Old Stem of Douglas-fir

	1 year	200 years
Average length of fusiform initials	800 μ	4,500 μ
Average tangential diameter of fusiform initials	14 μ	40 μ
Average tangential diameter of ray initials	12 μ	18 μ

Both initials in Douglas-fir, as in other woody plants, greatly multiply in number as the tree increases in girth. This increase is brought about either by the division of existing initials to produce others of the same type, or by the addition of new initials by division followed by a change in type. The increase in number of fusiform initials is brought about mainly by oblique radial divisions of existing initials, followed closely by apical intrusive growth (Esau, 24). The increase in ray initials usually is not accomplished by the division of existing initials, but by the addition of new ray initials. According to Barghoorn (9), the origin of these initials may be quite complex, and sometimes is very difficult to ascertain. The majority of the new ray initials in Douglas-fir originate by unequal division by which a small cell is cut off from the tip (fig. 8), or from the side (fig. 9) of a fusiform initial. The former method seems to be the most common. These cells then function as ray initials.

The existing rays may undergo many changes in the vascular cambium and because of these changes, they may become reduced in size or may increase in size (Barghoorn, 9). The reduction in size of the ray is brought about by a number of ways. First, there may be a loss of ray initials from the cambium, either from within or from the margins of the ray. Secondly, there can be an actual separation of the ray into two parts which is brought about by the intrusion of a fusiform initial growing at the tips. Rays increase in size by fusion with one another or by radial divisions of ray initials. The fusion of rays is brought about by loss from the cambium of fusiform initials intervening between groups of ray initials. These intervening

fusiform initials are lost from the cambium by differentiating into xylem or phloem elements.

The dormant vascular cambium resumes its activity sometime during the early part of March. Bark samples collected during the latter part of March show that the cambium had been active for a short period of time (fig. 11). During reactivation, the cambial cells expand radially, the radial walls become thinner, and the cytoplasm takes up a parietal position about a large vacuole.

Soon following reactivation, or at the same time, the initials begin to undergo periclinal divisions (fig. 12). The section shown here clearly indicates that it is not only the cambial initials that undergo divisions, but also their immediate derivatives. Figure 13, which is a diagrammatic drawing of a prepared slide from material collected during April, illustrates this better. There is no certain way to distinguish between the cambial layer proper and its derivatives. Bannon (8), from his study of Thuja occidentalis L., concluded that the numerous periclinal divisions in the cambial zone occur mainly in xylem derivatives. He considers this true because the annual xylem increment is greater in width than the annual phloem increment.

In these initials and their immediate derivatives, cell wall formation is a process extended in time and space. The cell plate is initiated between the two new nuclei and then spreads through the entire length of the cell preceded by the phragmoplast fibers (fig. 8, 12, and 13). Eventually the wall separates the original cell into two parts, each with its own nucleus and each a cell in its own right.

The periclinal divisions of the initials and derivatives are relatively slow during early March, but are accelerated during late March and April to form a zone which ranges from 150 μ up to approximately 300 μ (fig. 13). The width of this zone is determined to a great extent by where the tree is growing and the amount of available moisture.

Growth is continuous at a slower rate through May and June, and during July and August only an occasional division can be seen. This seems to indicate, then, that the cambial cells stop dividing sometime in August.

New mature phloem elements begin to show up about early April, and at this time, the phloem varies from 6 to 9 cells. Some of these cells were formed, as previously mentioned, at the end of the last growing season, so no definite line can be established between the cells formed this year and those formed last year. However, there is an exception to this. The sieve cells formed last year develop callose plugs about this time and can be distinguished on this basis. Phloem expansion is continuous at a more or less steady rate through May and June, and appears to slow down sometime during the early part of July. Several layers of phloem cells formed at about this time fail to complete their differentiation.

Rays

There are two types of rays in the secondary phloem, the uniseriate rays, that may become biseriate, and the fusiform rays with radial resin canals (fig. 14). For the most part, the uniseriate rays are only one cell wide and from 1 to 20, sometimes up to 30 cells high. Each ray contains two types of cells, albuminous cells that are in an erect position along the margins (fig. 12), and ray parenchyma cells that are horizontally elongated.

Albuminous cells are rather conspicuous on those rays in the region from the cambium (fig. 12) to about 14 cells away from the cambium. In the older phloem tissues they are completely obliterated (fig. 15). They differ slightly in shape from the ray initials that cut them off (fig. 7). In width they range from 20μ up to 25μ, and in height from 20μ up to 40μ. The nucleus is larger than the nucleus of the ray parenchyma and the protoplasm is extremely dense (fig. 12). The walls of the sieve cells facing the albuminous cells have conspicuous sieve areas and at the time of sieve cell callose formation, large callose deposits appear at this spot. The albuminous cells live as long as the sieve cells remain functional and appear to die when the cytoplasm of the sieve cell disorganizes.

During the height of seasonal activity albuminous cells contain no starch, but starch is formed in these cells during the winter. Chang (15) reported the absence of starch grains in the albuminous cells. Possibly he studied materials collected sometime during the active part of the growing season when starch is absent.

These erect marginal cells were first termed albuminous cells by Strasburger (cited by Esau, 23), because they stain deeply with cytoplasmic stains, particularly near the cambium. Esau (21 and 23) and Chrysler (16) consider the albuminous cells to be analogous to the companion cells in the angiosperms. They base their reasoning on the fact that there is a close physiological association between the albuminous cells and sieve cells. They are connected by cytoplasmic strands transversing the pits and the albuminous cells die and are crushed along with the sieve cells (Strasburger, cited by Esau, 23). This condition is also true for the albuminous cells in Douglas-fir. The albuminous cells do not resemble the companion cells in origin because each one does not differentiate along with a sieve cell from a single fusiform initial.

The ray parenchyma cells first become highly vacuolate as they enlarge rapidly after being produced by the ray initials (fig. 12). The cytoplasm then becomes denser and starch grains accumulate in the cells. In the older outer phloem tissues of the inner bark these cells enlarge (fig. 16), and develop abundant resins and tanniferous substances (fig. 16 and 17).

The fusiform rays are not as abundant as the uniseriate rays. The presence of a resin canal in the fusiform ray makes the ray more than one cell wide except at the upper and lower limits (fig. 14). The ray parenchyma making up these rays develop tannins and resins closer to the cambium than the ray parenchyma of the uniseriate rays. Each resin canal is bordered by thin-walled epithelial cells (fig. 15). The

resin canals enlarge in the outer part of the inner bark by division of these epithelial cells.

The rays remain functional in the older portions of the inner bark until they are cut off by the deep forming periderm layers. The ray parenchyma, along with the phloem parenchyma, help to initiate new deep phellogen layers.

Sieve Cells

Sieve cells are derived from fusiform initials, and undergo very little apical elongation after they are cut off from the vascular cambium. They are cut off from the cambium in radial series (fig. 3) that are maintained in the mature tissues. They are slender, elongate cells (fig. 18) comparable in size to the fusiform initials from which they are derived. Sieve cells overlap each other at their ends, and each is in contact with several rays.

Near the cambium the initials that cut off sieve cells show on their radial walls numerous primordial pits (fig. 8 and 9) that are penetrated by cytoplasmic strands. The walls of young sieve cells are primary in nature and are extremely thin (fig. 18).

Differentiation of the sieve cell begins early after its formation from the cambium. The first indication of growth appears to be the bulging of the primary walls in the areas of the pits (fig. 19). The bulging of the walls indicates early expansion of the sieve cell and has been observed in the phloem tissues of many other conifers (Abbe and Crafts, 1) and is attributed to some type of pressure increase within the cell.

Each immature sieve cell contains a single nucleus that is more or less elongate and occupies the center of the cell between two large vacuoles. There are one to several nucleoli. As the cell matures the nucleus gradually expands and decreases in density (fig. 20), then the chromatin material becomes clumped and the nucleus irregular in outline (fig. 21) before it finally disintegrates and disappears.

The cytoplasm also undergoes a series of changes as the young sieve cell progresses to maturity. In a young sieve cell, the cytoplasm is distinctly broken up into cytoplasmic strands which traverse the lumen of the cell (fig. 21). As the cell matures, the cytoplasm becomes reticulate (fig. 22), and then fibroid, and finally assumes a parietal position and becomes so thin (fig. 23) that it is difficult to detect with any cytoplasmic stains.

Starch grains have been observed in young sieve cells of several conifers (Abbe and Crafts, 1), especially around the nucleus and sometimes near the pits. No starch grains were detected around the nucleus of the sieve cell in Douglas-fir, but there are starch grains scattered around in the cytoplasm (fig. 21 and 22).

During the period of maturation, the walls of the sieve cells undergo a number of changes. The primordial pits differentiate into sieve areas (fig. 23 and 24) on the radial walls of the sieve cells. These sieve areas are mostly oval to elliptical in shape, but sometimes they may be orbicular. They are arranged mostly in a single row, but frequently they do exist in pairs. In diameter, they average 16 μ but the size depends to a great extent on the size of the sieve cell. The pore groups, usually 6 to 15 of them, are quite evident in the sieve areas of the cells near the cambium.

The other parts of the sieve cell walls become thickened by the deposition of secondary walls (fig. 23). The sieve cell walls stain with Bismark brown which indicates that they are cellulose in nature. The sieve cell walls of many other conifers have been observed to develop true secondary layers (Abbe and Crafts, 1).

Along with the development of the sieve areas and secondary walls, callose deposits develop around each of the cytoplasmic strands in the pit areas and form distinct collars (fig. 23 and 24). As the sieve cell develops, the amount of callose in the sieve area is increased. The collar of callose around each strand increases greatly and gradually constricts the connecting strands between cells. When this point is reached, it is extremely difficult to detect the cytoplasmic strands in the sieve areas in radial view. Not only is callose deposited inside of the collar, but it is also deposited over the surface of the entire sieve area. The sieve area then ceases to appear as a depression in the wall, but instead it assumes the appearance of a thickened area, for the callose eventually projects above the walls into the lumen of the cells (fig. 25 and 26). The older sieve cells of the current year's growth in Douglas-fir show heavy deposits of callose as early as early April (fig. 26). These massive callose deposits are termed definitive callose plugs (Esau, 23). In many instances these callose deposits become so massive that they may fuse.

During the growing season, particularly when the callose plugs are prominent, the sieve cell cytoplasm disorganizes and the cytoplasmic strands through the callose cylinders disappear. Also at a progressive rate during the growing season the callose deposits disappear from the sieve areas. The sieve areas, then, devoid of callose represent a thin portion of cellulose wall with numerous open pores. The sieve cells finally collapse.

The sieve cells, in length, range from 800μ to about 4,500μ. In cross section, they range from 20μ to 30μ in tangential dimension.

They are arranged in regular radial rows near the cambium, and are broken up to some extent by the tangential lines of parenchyma. In each row, between the lines of parenchyma, there are from 2 to 8 cells. In the older tissues, the sieve cells are mostly collapsed or obliterated.

Those sieve cells formed last growing season, as previously mentioned, differentiate early and develop callose plugs. These plugs remain on these cells for some time and form a relatively definite dividing line between the phloem formed during the current season and that formed during the previous year. This is extremely valuable in determining the origin of sclereids from the standpoint of their relation to seasonal development of the bark.

Fusiform Phloem Parenchyma

Fusiform phloem parenchyma cells are derived directly from fusiform initial derivatives that usually do not undergo transverse divisions. In many instances, however, some do subdivide, but not as much as in the formation of typical phloem parenchyma.

The first indication of the development of this type of parenchyma cell is the appearance of tannin granules in the vacuole of the derivative (fig. 27). At this stage the nucleus, which was more or less elongate in the derivative, becomes roundish and occupies approximately the center of the cell. The vacuole gradually accumulates large amounts of resin that first appears near the center of the cell and later completely fills the cell. Finally, the cell becomes filled with numerous crystals that are prismatic in shape (fig. 28 and 29).

The mature fusiform phloem parenchyma cell has a primary unlignified wall and contains a living protoplast, that cannot be seen because of the abundance of resins and crystals. It is long-tapered in longitudinal section, with a length ranging from 810 μ to about 4,510 μ. It is more or less rectangular in cross section, with a radial dimension of approximately 10 μ to 15 μ and a tangential dimension of about 60 μ.

These cells appear to develop singly or sometimes 1 to 3 in short radial multiples. They have no definite pattern of arrangement except that they do occur in combination with typical phloem parenchyma.

Those cells formed early in the growing season seem to collapse soon after the crystals appear. By the time the growing season is

over most of the cells are in the collapsed state. These collapsed cells are further crushed in the outer portions of the inner bark by the rapidly expanding phloem parenchyma.

Some derivatives are intermediate between typical fusiform phloem parenchyma and phloem parenchyma. They become subdivided into fewer cells than are formed in a phloem parenchyma strand. Some of these shorter cells of the derivatives develop abundant tannins (fig. 28) and possibly some resins; others develop abundant resins and crystals (fig. 28). During the growing season those cells containing resins and crystals appear to collapse early after their formation. Those cells that contain tannins and resins expand, proliferate, and live for a greater period of time and, in the older phloem tissues, possibly function in the initiation of new deep phellogen layers.

Phloem Parenchyma

Phloem parenchyma strands are derived from fusiform initial derivatives that undergo a series of transverse divisions. During each growing season, 3 to 4 parenchyma zones or tangential bands of parenchyma are formed by the cambium (fig. 3). The derivative from which these parenchyma cells develop undergoes little or no increase in length after it is produced by the cambial initial. There is, however, a slight increase in its radial dimension. The derivative begins to undergo transverse divisions shortly after it is cut off from the cambium. The first division of the centrally located nucleus produces two daughter nuclei separated by a typical cell plate. Subsequent divisions among the daughter nuclei are at random (fig. 30). Transverse walls develop after each division and the derivative becomes subdivided into a series of short cells (fig. 30). The number of cells produced by a single derivative is not consistent, but the usual number is from 7 to 9 cells. The cells occupying the center of the strand are short and have flat end walls, while those formed at the tips have one flat end wall and one tapered end wall (fig. 31).

Early in the differentiation of these parenchyma cells various inclusions develop, especially tannins and resins (fig. 31). Some of these cells develop tannins and resins before mitotic divisions are completed. The majority of the cells become filled with tannins and resins, but in a few cases, some develop numerous prismatic-shaped crystals (fig. 28). These crystals usually develop in those cells that contain resins but no tannins. The sequence for the development of

these inclusions is usually tannins, then resins and crystals. In the older active phloem tissues, the parenchyma cells with tannins develop abundant starch grains that persist during autumn and winter, and gradually decrease as the growing season progresses.

The parenchyma cells are more or less rectangular in cross section near the cambium, but become nearly circular in older phloem tissues (fig. 3), and enlarge to a diameter of approximately 60 μ.

In the older active phloem tissues, the parenchyma cells proliferate (fig. 15) and, with the rays, make up the bulk of the living tissues that function in the initiation of deep phellogen layers.

Sclereids

In the early part of the growing season, approximately mid-May, the sclereids begin to develop from many cells of the phloem parenchyma strands in the previous year's phloem.

The first indication of sclereid development seems to be the gradual enlargement of the cell accompanied by reduction of the cellular inclusions (fig. 32). Most of the tannin deposits disappear from the cell, with the exception of a relatively few tannin granules in the cytoplasm. These tannin granules are retained throughout the growth of the cell (fig. 35, 36, and 37). As the inclusions gradually disappear the nucleus again becomes evident (fig. 33). It is extremely large, centrally located, and occupies a plate of cytoplasm that separates two large vacuoles.

The sclereidal initial, in its early stages of growth, increases in all dimensions (fig. 32). This increase is apparently brought about by symplastic growth of the cell wall. After expanding by symplastic growth for a time, it then begins to lengthen and penetrates into the region of the middle lamella between the adjacent cells (fig. 34, 35, and 36). This lengthening and penetration is brought about by apical intrusive growth.

The growing sclereidal initial does not penetrate the adjacent cells, but buckles and separates these cells by growing along their middle lamellae (fig. 35). The initial is capable of growing in almost any direction if space is available, but the majority of the processes

take a vertical course (fig. 35). This type of growth provides the basis for the extreme irregularities found in the shapes of mature sclereids (fig. 42).

The first secondary wall is laid down immediately after the cell has reached its final stages of growth (fig. 37 and 38). As the growing season progresses additional secondary walls develop and the wall finally appears extremely thick and lamellated (fig. 39 and 41). The nucleus is still present in the cell after the first secondary wall is formed. It apparently disintegrates sometime during the formation of the additional secondary walls. The exact time was not determined. The tannin deposits in the cytoplasm and vacuole increase considerably as the cell wall thickens (fig. 39). The cytoplasm is gradually pushed toward the center of the cell and finally disintegrates. The cell cavity is now occupied by a deeply-staining, amorphous mass (fig. 41) which probably consists of tannins. The cell wall is usually smooth on the surface and extremely thick with distinct lamellae. This wall, according to Kiefer and Kurth (33), is impregnated with lignin that may be removed by dilute alkalin solutions. The wall contains simple pits, occasionally branched, that terminate in the cell cavity.

In longitudinal section, the sclereid may be long-tapered (fig. 43), forked (fig. 39), or highly branched (fig. 42). The cells, or extensions of the cells, may end in sharp projections, or may be blunt, particularly where they have ended against the large surface of a neighboring cell (fig. 39). In length, the sclereids range from 650 μ to about 4,000 μ. In cross section, they are more or less circular (fig. 41), and have a diameter from 50 μ to approximately 92 μ.

The sclereids develop in most cases as isolated cells but frequently may develop in small groups of 2 to 6 (fig. 40). New sclereids develop among the older ones in the previous year's phloem (fig. 38) and also develop in phloem tissues formed about two or three years ago (fig. 40). The number of these sclereids increase each year until the phloem parenchyma cells are killed by periderm formation. Sclereids do not develop in the phloem of trees that are less than 10 years of age.

Structure of the Periderm and Its Development

The first phellogen layer is differentiated completely around the circumference of the stem from the first layer of cortical parenchyma cells just beneath the epidermis. This phellogen layer is probably formed sometime during the second year of growth. The newly-formed phellogen layer undergoes a number of periclinal divisions to form phellem cells to the outside and phelloderm cells to the inside. The phellem cells, which are cut off in radial series, enlarge and finally become suberized. Because of the formation of this superficial periderm, the epidermal cells die and eventually become sloughed off (fig. 44).

No detailed study was made on the length of time that this first periderm functions, but it apparently may persist for as long as the cortex remains on the trees. In most cases, the cortex is sloughed off of the trees when they reach approximately 25 to 35 years of age.

The first-formed periderm is replaced by the first deep periderm layer that arises deeper into the cortical region (fig. 45) and the first-formed periderm layer and outer cortical cells are killed and eventually sloughed off. Other phellogen layers develop deeper in the cortex and finally from among the outermost, proliferated parenchyma cells of the secondary phloem (fig. 16, 17, 46 and 47). The early stages of the differentiation of the new phellogen layers in the deeper cortical and phloem layers were not available for study. Their position, however, seems to indicate that they are initiated from cortical parenchyma in the cortex and from the proliferated phloem parenchyma

and phloem ray parenchyma in the secondary phloem.

Each deeper periderm layers extends for only a limited distance and the central portions of such layers are more or less parallel to the outer surface of the bark, but their edges curve outward and intersect or abut upon the older, outer phellem layers. Thus a lenticular scale of cortical or phloem tissue is cut off (fig. 4).

As the tree increases in girth, more periderm layers are formed deeper in the secondary phloem. The bark of an older tree is differentiated into two distinct layers, the inner bark, that consists mainly of living tissues (fig. 3), and the outer bark or rhytidome (fig. 4), that consists of anastomosing phellem layers with interspersed crushed phloem elements. The increase in girth of the tree brings about the separation of outer cells of the rhytidome, and the external surface of the bark becomes deeply cracked (fig. 2). Plates of phellem cells and dead phloem are gradually sloughed off.

The last-formed phellogens in older trees become active sometime during the latter half of May, early in June, or in some cases as late as early August. Some, however, do not become active at all. The phellogen cells during dormancy contain abundant starch and tannin deposits (fig. 16 and 17). Hergert and Kurth (28) treated the phellogen cells with a fat stain, Sudan III, and found wax to be present. The cells in this layer, during early activity, gradually lose their cellular inclusions and begin dividing periclinally to form radial series of phellem and phelloderm cells (fig. 46, 47, and 50). The number of phellem layers formed each year is not consistent but usually ranges from 6 to about 15 layers.

The new phellem cells (fig. 46 and 50) are comparatively thin and rectangular in cross section, somewhat flattened radially at first, but later expand because of the growth of their radial walls. In radial view (fig. 47), they are more or less rectangular, but occasionally deviate from this. In tangential view (fig. 48), they appear approximately pentagonal in shape and are arranged compactly without any intercellular spaces.

Suberization of the phellem cells begin before they attain their full size. After they are fully differentiated, they usually become filled with resins and tannins (fig. 49), and finally become devoid of protoplasm. Frequently the older phellem cells collapse slightly because of growth pressures in the stem. Materials prepared by the paraffin technique show these cells to be extremely collapsed (fig. 46 and 47). This is not the condition of these cells in fresh materials.

Some periderm cells develop thick walls (fig. 45) and appear to alternate with the thin-walled phellem cells. This is sometime termed woody cork. These thick-walled cells occur in various proportions in the phellem. The walls are thick, lignified, contain simple pits and are lamellated (fig. 51). The cell cavities are occupied by deeply-staining, amorphous substances which are probably tannin in nature. They are more or less square in cross section and are arranged in short radial rows. In tangential dimension, they range from 30 μ to 50 μ and in radial dimension, from 50 μ to 90 μ.

The phelloderm is composed of 1 to 3 layers of cells, that are arranged in short radial rows (fig. 46 and 47). These cells contain

large nuclei, have comparatively thick walls, and contain abundant tannins and resins. Their shape is similar to that of the phellogen cells. They are distinguished from cortical and phloem parenchyma by their radial arrangement.

The deeper phellogen layers may remain active for many years to produce phellem that shows distinct annual growth layers (fig. 4). This is especially true of the phellogen layers present at the base of old trees where the periderm layers sometime get to be from one-fourth to about three-fourths of an inch wide. There are some indications that the outer (first) phellogen divides more slowly and perhaps is not as active as the successively deeper ones in secondary phloem.

DISCUSSION

The present study confirms and further explains the observations (Chang, 15; Hergert and Kurth, 28; Kiefer and Kurth, 33) of the structure of the bark of Douglas-fir in its entirety, or with respect to some of its tissues. The literature indicates that little was actually known about the structure of the bark up until the time of Chang's comprehensive work. Prior to this, the description of the bark was very inadequate. For example, Hergert and Kurth gave a very brief descriptive analysis of the bark giving only a few of the cell types present. In the inner bark they mentioned the sieve cells, phloem parenchyma cells, and sclerenchyma cells that they considered to be fibers. Nothing was reported concerning the phloem rays, or the types of parenchyma cells including their variations. In the outer bark they reported the anastomosing bands of cork and the interspersed dead phloem tissues. Kiefer and Kurth also described the presence of bast fibers in the secondary phloem tissues of the bark.

Since Chang published the most complete descriptive analysis of the structure of the bark of Douglas-fir, his work should undoubtably be compared with what was gathered in this study. Most all of the cell types, their arrangement, as recorded by Chang were also found to be present in the material studied here. There is one exception to this, however, and this is in connection with the presence of phloem fibers in the bark. Chang, as well as the other above workers, reported that the sclerified elements in the secondary phloem

are phloem fibers. Phloem fibers, however, are sclerified elements that are derived directly from fusiform initials. No true fibers develop from fusiform initials in the phloem of Douglas-fir. The thick-walled cells present in the phloem tissues develop from phloem parenchyma cells which undergo expansion, ramification, and finally sclerification, and should be classed as sclereids.

The development of the secondary phloem tissues of Douglas-fir follows a somewhat similar pattern to that recorded in the literature for other conifers (Abbe and Crafts, 1; Strasburger, 47). The secondary phloem tissues function for only one growing season, and some of the cambial derivatives, mostly those on the phloem side, formed toward the end of the growing season pass through the winter in a partially differentiated state. These partially differentiated elements in Douglas-fir are mostly sieve cells.

It is well-known that the secondary phloem tissues of most woody plants show no distinct annual rings. It is easy, however, to distinguish between the current season's phloem and that formed the previous year in Douglas-fir. Those partially differentiated sieve cells formed toward the end of one growing season differentiate early in the next growing season and develop callose plugs early and these remain for an indefinite period of time and provide a relatively definite line for the separation of these two growth layers.

One of the most interesting aspects of this problem is the intergradation that can be found between the typical phloem parenchyma and the fusiform phloem parenchyma. Both types of cells develop from the same type of initial, the fusiform initial, but they undergo different

developmental changes, and are physiologically differentiated. The typical phloem parenchyma cells originate from fusiform initial derivatives that undergo a series of transverse divisions. The segments of the strands usually develop tannins, resins, and finally starch grains, and live for an indefinite period of time in the older phloem tissues. These cells are the most active in the initiation of deep phellogens that appear in the secondary phloem of older trees. The fusiform phloem parenchyma cells also originate from fusiform initials, but they usually do not undergo transverse divisions. In the course of their development they accumulate abundant resins and prismatic-shaped crystals. They do not live as long as the typical phloem parenchyma and collapse early after their formation. Hence, they neither store food nor function in phellogen initiation.

As mentioned above, in the secondary phloem tissues of Douglas-fir can be found parenchyma cells that intergrade between the typical phloem parenchyma and the fusiform phloem parenchyma. The fusiform derivative becomes subdivided, but there are fewer cell divisions than occur in the formation of typical phloem parenchyma. Hence, some of the cells produced are similar in size to typical phloem parenchyma, and some are larger. The shorter elements tend to accumulate tannins, resins and starch grains similarly to phloem parenchyma. The longer elements of the strand tend to develop resins and crystals as do the typical fusiform phloem parenchyma cells.

No where in the literature, as far as the writer knows, is there any report making a comparison of the time at which the vascular

cambium and phellogen layer become active during the growing season. Normally you would expect these two cambia to begin their activity at approximately the same time. In Douglas-fir this is not the case. The vascular cambium begins its activity approximately two months earlier than the phellogen layer.

Another interesting point that should be brought out is the difference in rate of activity between the two types of phellogen layers. As was pointed out earlier there is a difference in rate of activity between the deep phellogen layers and the first phellogen that differentiates in the cortex. The last-formed phellogens resume divisions earlier and function for a longer period of time to produce a greater number of phellem cells each season than does the first phellogen layer. The first-formed or superficial phellogen of the stem is retained for many years, but produces only a relatively small number of phellem cells as compared to the deeper phellogen layers. Some of the superficial cork is sloughed off each year, of course, but there are not as many cork cells produced each year by the first phellogen as are produced by the deep phellogens in an older tree. Only the deep phellogens produce enough cork to show distinct annual rings.

SUMMARY

The structure and development of the bark of Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco., was studied through bark samples collected principally in two locations in Oregon at regular intervals during 1955.

Young Douglas-fir bark is smooth except for resin blisters, thin, rather lustrous, and dark grayish-brown. In old trees, the bark is thick, and divided into oblong plates which are broken into broad, irregularly connected ridges that separate on the surface into small, thick, closely appressed dark reddish-brown scales.

The inner bark is light in color, relatively thin, and is composed of secondary phloem tissues, both living and dead. The outer bark (rhytidome) is composed of anastomosing periderm layers with interspersed areas of dead phloem tissues.

The dormant vascular cambial region appears to be from 4 to 6 cells wide. There are 2 to 3 layers of partially differentiated sieve cells. These cells produced during the latter part of the growing season are unable to complete their differentiation. One of the remaining layers is the vascular cambium proper and the other or others are immature xylem cells. These partially differentiated and immature phloem and xylem cells complete their differentiation at the beginning of the next growing season.

The vascular cambium resumes its activity sometime during the early part of March. The initials undergo periclinal divisions to form radial series of derivatives. Many of these derivatives also

undergo periclinal divisions. Cambial activity is relatively sluggish during early March, but is accelerated during late March and April to form a broad cambial zone. Growth is continuous at a steady rate through May and June, and falls at a diminishing rate during July and August. Divisions completely stop sometime in August.

New phloem elements begin to mature about early April. Some of these cells were formed at the end of the last growing season and differentiated quite early. Phloem expansion is continuous at a more or less steady rate through May and June and appears to slow down sometime during the early part of July.

There are two types of initials in the vascular cambium, ray initials that are nearly isodiametric and give rise to the rays, and fusiform initials that are elongate cells with tapered end walls and give rise to the sieve cells, phloem parenchyma, and fusiform phloem parenchyma. The cambium is of the non-storied type.

Uniseriate and fusiform rays are present in the secondary phloem. Two types of parenchyma cells are present in the uniseriate rays. In addition to the albuminous cells that are in an erect position along the margins of each ray, there are typical ray parenchyma cells that are horizontally elongated. The albuminous cells have large nuclei, dense protoplasm, and store starch during dormancy. They die and are crushed along with the sieve cells. The ray parenchyma cells are highly vacuolate during the early stages of differentiation. They then develop a dense cytoplasm and finally become filled with starch. In the older phloem tissues of the inner bark the ray parenchyma enlarge, and develop abundant resins and tanniferous substances. The fusiform

rays are similar to the uniseriate rays except that they contain resin canals that are bordered by epithelial cells and that they seem to develop tannins and resins sooner than the uniseriate ray cells. The resin canals enlarge in the outer part of the inner bark by division of the epithelial cells.

Sieve cells originate from fusiform initials. These initials have primordial pits on their radial walls that give rise to sieve areas in mature sieve cells. There are from 6 to 15 pore groups present in each sieve area. During early differentiation of a sieve cell the primary walls bulge in the areas of the pits; the nucleus expands, becomes less dense, and finally disintegrates. The cytoplasm, which at first traverses the cell lumen as strands, becomes reticulate and then fibroid and finally assumes a parietal position. Also during this period the walls undergo a series of changes. Portions of the walls become thickened by the deposition of secondary walls, and the sieve areas begin to show callose formations. Callose is deposited, at first, around the cytoplasmic strands in the pit areas to form definite collars. Later callose is deposited over the entire surface of the sieve area to form massive plugs. The cytoplasm of the sieve cells disappears sometime during the formation of these massive callose deposits. The callose deposits also disappear progressively from the sieve areas during the growing season. The sieve cells finally collapse. T he partially differentiated sieve cells formed at the beginning of last growing season differentiate early in the next growing season and develop callose plugs that remain for sometime and form a relatively definite dividing line between the phloem formed

during current season and that produced the previous year.

Fusiform phloem parenchyma cells differentiate directly from fusiform initial derivatives that usually do not undergo transverse divisions. Some occasionally subdivide, but not as much as in the formation of typical phloem parenchyma. Tannin granules first are deposited in these cells during the early stages of differentiation, followed by heavy deposits of resinous substances, and finally the formation of crystals having prismatic shapes. These fusiform phloem parenchyma cells occur in combination with the typical phloem parenchyma but less frequently. Those cells formed early in the growing season seem to collapse soon after their formation and in the older phloem tissues they are further crushed by the rapidly expanding phloem parenchyma. Some derivatives subdivide a variable number of times and the shorter cells may develop abundant tannins with some resins or with both resins and crystals. Those containing resins and crystals collapse early, and those having tannins and resins live for a greater period of time, expand and proliferate in the older phloem tissues.

Phloem parenchyma strands are derived from fusiform initial derivatives by a series of transverse divisions. After the first mitotic division of the derivative's nucleus the daughter nuclei divide at irregular intervals a variable number of times. The number of cells in a single strand varies from 7 to 9. Most of these cells develop tannins, resin, and finally starch grains; others develop resins and prismatic crystals. The parenchyma cells are arranged in more or less tangential rows near the cambium, but in the older phloem tissues,

these lines are discontinuous. The cells containing the tannins, resins and starch grains enlarge and proliferate in the older phloem tissues.

Sclereids develop from phloem parenchyma cells that were produced the previous year. They differentiate by progressive enlargement, ramification, and finally sclerification. The phloem parenchyma cell first loses its cellular inclusions, expands, and finally penetrates the middle lamellae region between the adjacent cells. Sclerification of the wall takes place as soon as the cell completes its growth. The nucleus disappears from the cell sometime during the formation of the secondary walls. The mature sclereid is without a protoplast, the cell cavity is occupied by a deeply-staining, amorphous mass which probably consists of tannins, and the walls are extremely thick, lamellated and with simple pits. The sclereids are usually quite irregular in shape and develop either singly or in groups of 2 to 6 throughout the older secondary phloem. Sclereids differentiate first in one-year old phloem but the number of sclereids may be increased each year until the phloem parenchyma cells are killed by periderm formation.

The first-formed phellogen layer is differentiated completely around the circumference of the stem from the first layer of cortical parenchyma cells just beneath the epidermis. This is formed sometime during the second year of growth and functions for 25 to 35 years. This is soon replaced by the first deep phellogen layer that develops deeper in the cortical region. Finally successive phellogen layers differentiate from among the outermost, proliferated parenchyma cells

of the secondary phloem. Each new deep phellogen layer extends for only a limited distance with the center part more or less parallel with the surface of the bark while the edges curve outward. The last-formed phellogen layers become active sometime during the latter part of May, early in June, or in some cases as late as early August. In some cases, they do not become active at all during the growing season. During dormancy the phellogen cells contain abundant starch and tannin deposits. During early activity the cells in this layer gradually lose their cellular inclusions and begin dividing periclinally to form radial series of phellem cells and phelloderm cells. After the phellem cells are formed, they expand radially, become suberized, develop abundant resins and tannins and finally become devoid of protoplasm. Sometimes in alternation with the thin-walled cells, are cells with thick, lignified, lamellated walls that have abundant simple pits. This is sometimes termed woody cork. The cell cavity is occupied by a deeply-staining, amorphous mass that is probably tannin in nature. The phelloderm is composed of 1 to 3 layers of cells that are arranged in short radial series. The cells have large nuclei, and contain abundant tannins and resins at maturity.

BIBLIOGRAPHY

1. Abbe, L. B. and A. S. Crafts. Phloem of white pine and other coniferous species. Botanical gazette 100:695-722. 1939.

2. Alcorn, Stanley M. and Peter A. Ark. Softening of paraffin-embedded plant tissues. Stain technology 28:55-56. 1953.

3. Allen, G. Embryogeny and the development of the apical meristems of Pseudotsuga. III. Development of the apical meristems. American journal of botany 34:204-211. 1947b.

4. Anonymous. Fir bark. Made to yield five valuable new products. Timberman 48:86-88, 90, 92. 1947.

5. Anway, Herman W. Method of treating bark. U. S. Patent 2,437,672. March 16, 1948. (Abstracted in Biological abstracts 23 (4), No. 16761. 1949.)

6. Bailey, I. W. The cambium and its derivative tissues. III. A reconnaissance of cytological phenomena in the cambium. American journal of botany 7:417-434. 1920b.

7. Bannan, M. W. Comparison of the distribution of albuminous and tracheary ray in the gymnosperm. American journal of botany 23:36-40. 1936.

8. _____. The vascular cambium and radial growth in Thuja occidentalis L. Canadian journal of botany 33:113-138. 1950.

9. Barghoorn, E. S., Jr. Origin and development of the uniseriate ray in the coniferae. Torrey botanical club bulletin 67:303-328. 1940a.

10. Blank, Harvey and Philip L. McCarthy. A general method for preparing histologic sections with a water-soluble wax. The journal of laboratory and clinical medicine 36:776-781. 1950.

11. Block, R. Differentiation and pattern in Monstera deliciosa. The idioblastic development of the trichosclereids in the air root. American journal of botany 33:544-551. 1946.

12. Britton, Nathaniel L. North American trees. New York, Henry Holt and Co., 1908. 894 p.

13. Brown, H. P. Growth studies in forest trees. 2. Pinus strobus L. Botanical gazette 59:197-241. 1915.

14. Chang, Ying-Pe. Anatomy of common North American pulpwood barks. Tappi monograph series - No. 14, 1954. 249 p.

15. _____. Bark structure of North American conifers. Washington, U. S. Government printing office, 1954. 86 p. (U. S. Dept. of agriculture. Bulletin no. 1095)

16. Chrysler, M. A. The origin of the erect cells in the phloem of the Abietineae. Botanical gazette 56:36-50. 1913.

17. Cooke, G. B. Cork and cork products. Economic botany 2:393-402. 1948.

18. Crafts, A. S. The relation between structure and function of the phloem. American journal of botany 26:172-177. 1939a.

19. _____. Vascular differentiation in the shoot apices of ten coniferous species. American journal of botany 30:382-393. 1943b.

20. DeBary, A. Comparative anatomy of the vegetative organs of the phanerogams and ferns. Oxford, Clarendon Press, 1884. 659 p.

21. Esau, Katherine. Development and structure of the phloem tissue. Botanical review 5:373-432. 1939.

22. _____. Phloem structure in the grapevine, and its seasonal changes. Hilgardia 18:217-296. 1948.

23. _____. Development and structure of the phloem tissue. II. Botanical review 16:67-114. 1950.

24. _____. Plant anatomy. New York, McGraw-Hill, 1953. 735 p.

25. Foster, A. S. Structure and development of sclereids in the petiole of Camellia japonica L. Torrey botanical club bulletin 71:302-326. 1944.

26. _____. Origin and development of sclereids in the foliage leaf of Trochodendron aralioides Sieb. & Zucc. American journal of botany 32:456-468. 1945b.

27. Friedman, Leo and A. I. Ezell. Cork from Douglas-fir bark. The timberman 43:26-27. 1942.

28. Hergert, Herbert L. and E. F. Kurth. The chemical nature of the cork from Douglas-fir. Tappi 35:59-66. 1952.

29. Hill, A. W. The histology of the sieve tubes of Pinus. Annals of botany 15:575-611. 1901.

30. Isenberg, I. H. The anatomy of redwood bark. Madrono 7:85-91. 1943.

31. Johansen, D. A. Plant microtechnique. New York, McGraw-Hill, 1940. 623 p.

32. Jones, Leo E. Polyethylene glycol as a supporting matrix for sectioning plant tissues. Paper read before the Oregon Academy of Science meetings, University of Portland, Portland, Oregon, February 25, 1956.

33. Kiefer, Harry J. and E. F. Kurth. The chemical composition of the bast fibers of Douglas-fir bark. Tappi 36:14-19. 1953.

34. Knudson, L. Observations on the inception, season and duration of the cambium development in the American larch (Larix laricina (du Roi) Koch.) Torrey botanical club bulletin 40:271-293. 1913.

35. Kurth, E. F., Kiefer, Harry J. and J. K. Hubbard. Utilization of Douglas-fir bark. The timberman 49:130-131. 1948.

36. Kurth, E. F. The chemical analysis of western woods. Part II. Douglas-fir bark analysis. Tappi 36:175-176. 1949.

37. Kurth, E. F. and H. J. Kiefer. Wax from Douglas-fir bark. Tappi 33:183-187. 1950.

38. Kurth, E. F. and C. V. S. Ratnam. Products obtained by destructive distillation of Douglas-fir bark. Tappi 33:517-519. 1950.

39. Kurth, E. F. Chemicals from Douglas-fir bark. Tappi 36:112-119. 1953.

40. _____. Utilization of barks. Corvallis, Oregon state college, 1954. 10p. (a talk presented before the Pacific Northwest industrial waste conference.)

41. Lewis, F. T. The shape of cork cells: A simple demonstration that they are tetrakaidecahedral. Science 68:625-626. 1928.

42. Milanez, F. R. Crystalliferous strands. Chronica botanica VII. 2:68-69. 1942.

43. Priestley, J. H. Radial growth and extension growth in the tree. Forestry 9:84-95. 1935.

44. Sass, J. E. Elements of botanical microtechnique. New York, McGraw-Hill, 1940. 222 p.

45. Sterling, C. Sclereid formation in the shoot of Pseudotsuga taxifolia. American journal of botany 34:45-52. 1947.

46. _____. Organization of the shoot of Pseudotsuga taxifolia (Lamb.) Britt. II. Vascularization. American journal of botany 34:272-280. 1947b.

47. Strasburger, E. Über den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. Histologische Beiträge. Jena, Gustav Fischer. 1891.

48. Varrelman, F. A. Paper apron to prevent curling of microtome sections. Science 74:20. 1931.

49. Voorhies, Glenn. An inventory of sawmill waste in Oregon. Corvallis, Oregon state college, 1942. (Oregon state engineering station bulletin series no. 17)

50. Weiser, M. Proximate analysis of Douglas-fir bark. Master's thesis. Corvallis, Oregon state college, 1947. 70 numbered leaves.

FIGURES AND DESCRIPTIONS

PLATES I-VIII

Explanation of symbols used in labeling photomicrographs:

a -- albuminous cell

c -- cortex

d -- definitive callose plug

dp -- dead phloem tissues

e -- epidermis

fi -- fusiform initial

fpp -- fusiform phloem parenchyma

p -- phragmoplast

phd -- phelloderm

phg -- phellogen

phl -- phellem cells

pp -- phloem parenchyma

r -- phloem ray

rc -- resin canal

ri -- ray initial

rp -- ray parenchyma

s -- sclereid

sc -- sieve cell

sp -- sclerified phellem cells

vc -- vascular cambium

x -- xylem

PLATE I

Figures 1-6

Figure 1. Bark of a young Douglas-fir tree.

Figure 2. Bark of an old Douglas-fir tree.

Figure 3. Cross section of the inner bark and cambial zone. 108X.

Figure 4. Cross section of the outer bark (rhytidome). Note the annual growth layers in each band of phellem. IX.

Figure 5. Cross section of dormant cambium region. 460X.

Figure 6. Radial section of dormant cambium region. Note the partially differentiated sieve fields on the radial walls of the sieve cells. 460X.


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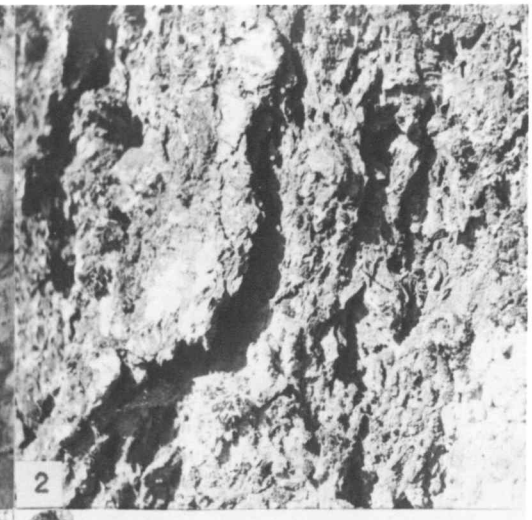
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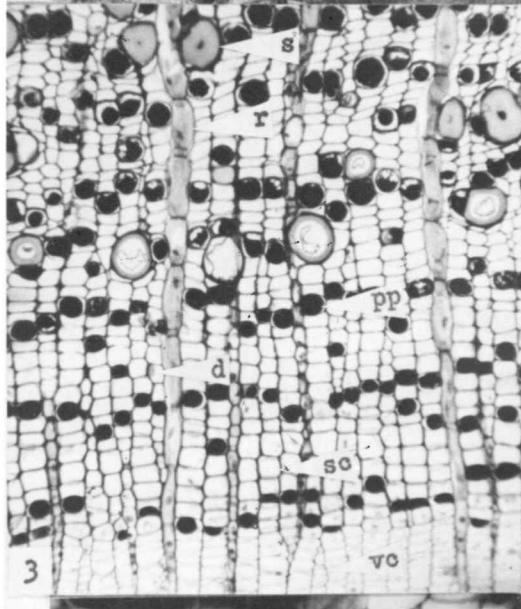

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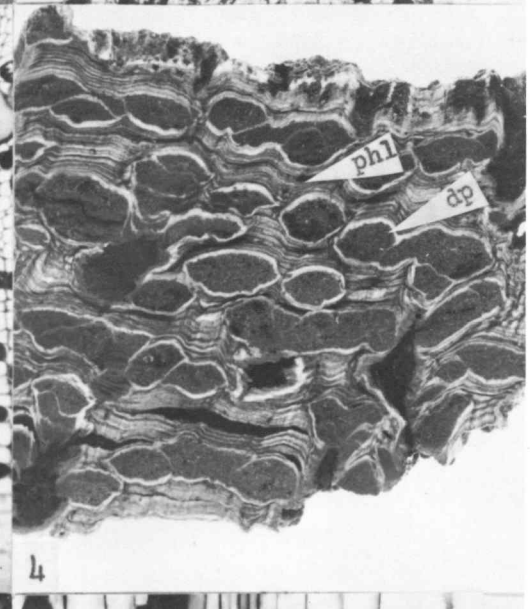

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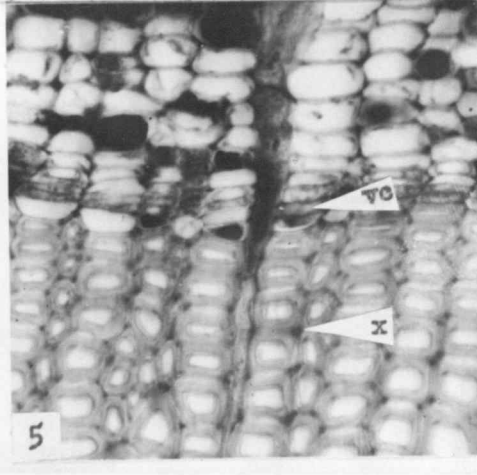

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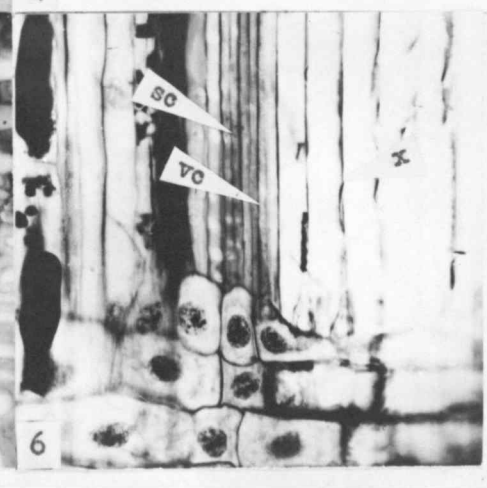

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PLATE II

Figures 7-14

Figure 7. Tangential section of active vascular cambium region. 108X.

Figure 8. Tangential section of active vascular cambium region. Note the phragmoplasts at each end of the mitotic nucleus, also a newly-formed ray initial cut off from the tip of a fusiform initial, and the well-developed primordial pits on the radial walls of the fusiform initials. 460X.

Figure 9. Tangential section through vascular cambium region showing the elongate nuclei of the fusiform initials, and a newly-formed ray initial cut off from the side of a fusiform initial. 460X.

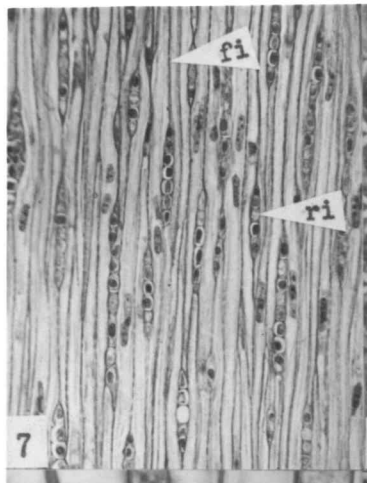
Figure 10. Tangential section of active vascular cambium region. 460X.

Figure 11. Cross section of active vascular cambium region collected March 28, 1955. 460X.

Figure 12. Radial section of active vascular cambium region collected March 28, 1955. Cambial initials and their derivatives are undergoing periclinal divisions. 460X.

Figure 13. A diagrammatic illustration of the broad vascular cambium region during April.

Figure 14. Tangential section of the previous year's phloem. 108X.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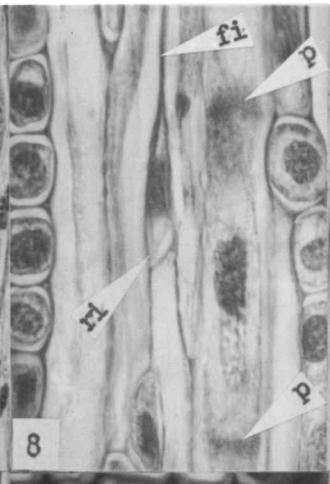
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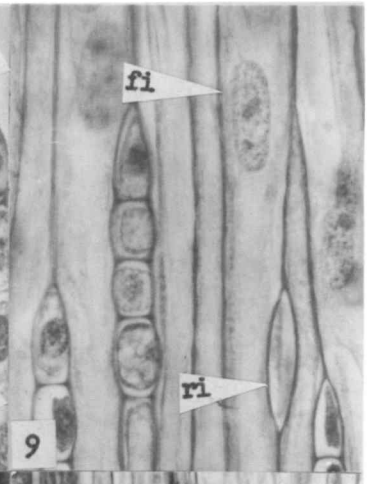
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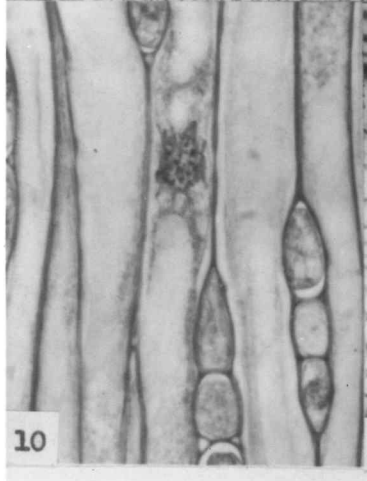
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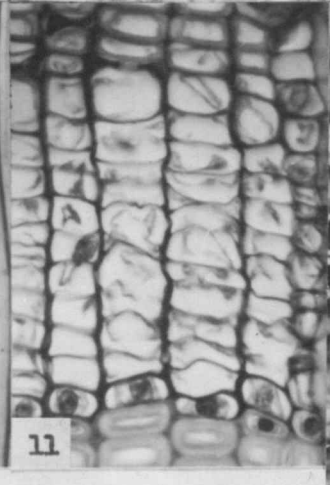
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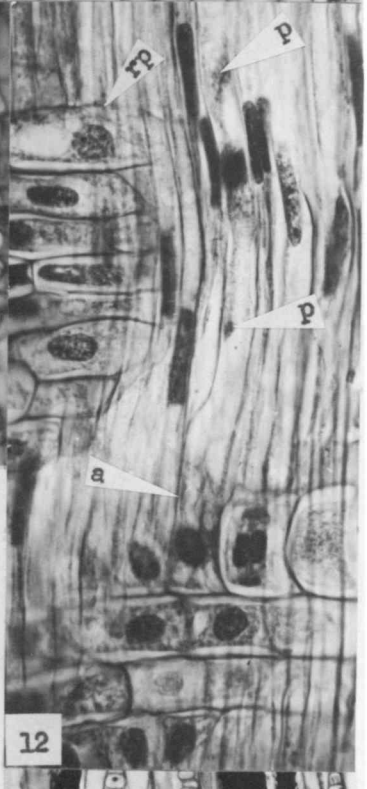
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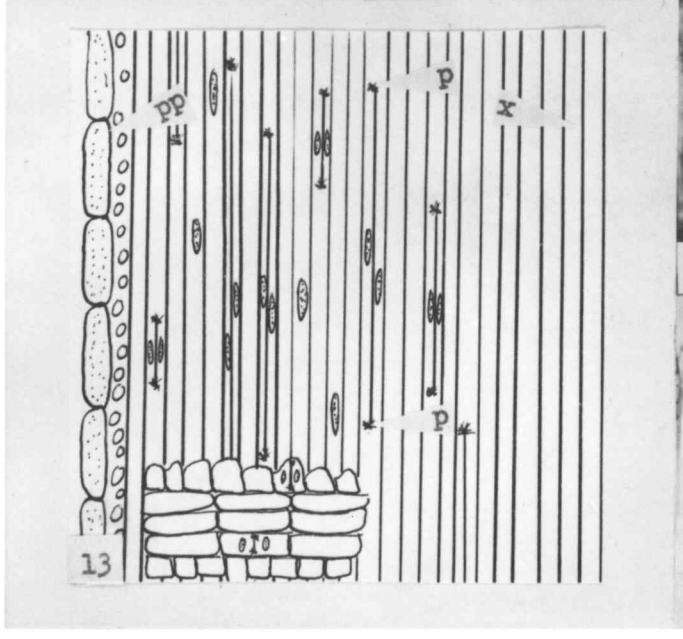
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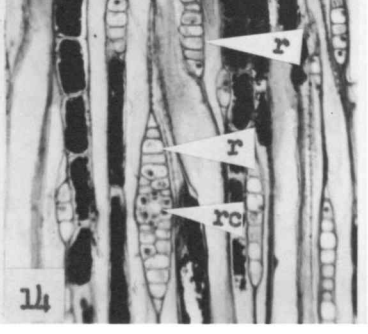
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PLATE III

Figures 15-23

Figure 15. Tangential section of older phloem tissues showing expanded resin canal and proliferated phloem parenchyma. This sample was collected April 2, 1955. 108X.

Figure 16. Cross section of last-formed periderm layer and adjacent phloem. 108X.

Figure 17. Radial section of the last-formed periderm layer and adjacent phloem. 108X.

Figure 18. Tangential section of current season's phloem. 108X.

Figure 19. Tangential section of a young sieve cell showing part of the bulging wall. 460X.

Figure 20. Tangential section of young sieve cell with its centrally located nucleus. 460X.

Figure 21. Tangential section of sieve cell and degenerating nucleus. 460X.

Figure 22. Tangential section of sieve cell showing reticulate cytoplasm. 460X.

Figure 23. Tangential section of sieve cell showing parietal cytoplasm, secondary wall thickening, and callose cylinders through the sieve areas. 1100X.

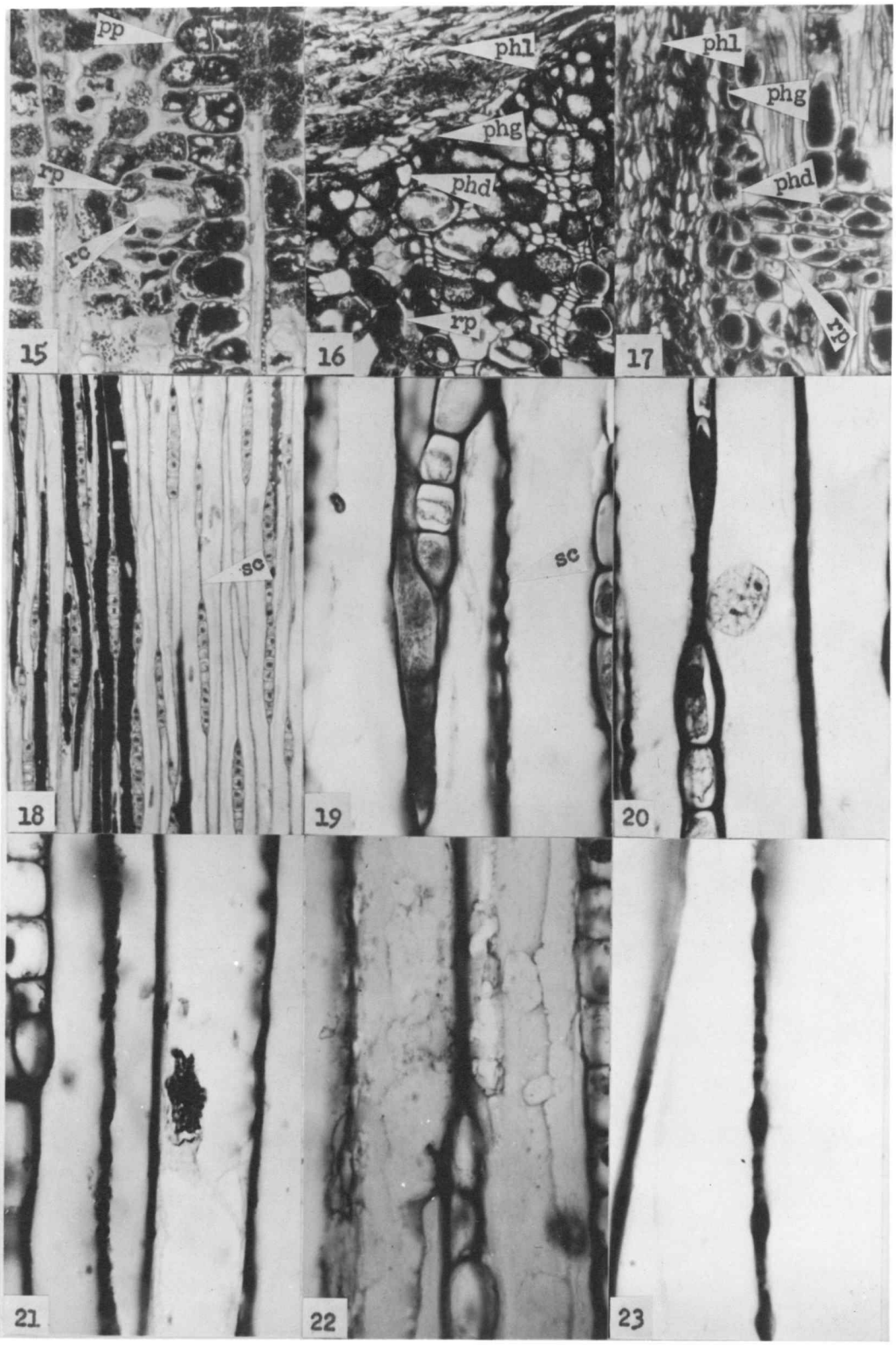
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PLATE IV

Figures 24-32

Figure 24. Mature sieve areas on the radial walls of the sieve cells near the cambium. Note the well-developed callose cylinders in the pores. 1100X.

Figure 25. Tangential section of sieve cells with well-developed definitive callose plugs. 1100X.

Figure 26. Cross section of sieve cells with massive callose formations. These plugs are on the sieve cells formed early in the growing season. 1100X.

Figure 27. Young fusiform phloem parenchyma cell showing tannin granules that are mostly in the cytoplasm. 460X.

Figure 28. Tangential section of current season's phloem. Most of the prismatic-shaped crystals are in the fusiform phloem parenchyma, although a few phloem parenchyma contain these crystals. 108X.

Figure 29. A fusiform phloem parenchyma cell with prismatic-shaped crystals. 460X.

Figure 30. Radial section near the cambium. Note the mitosis in the derivative that is dividing to form a phloem parenchyma strand. 460X.

Figure 31. Tangential section of current season's phloem. Tannins are present in the phloem parenchyma. 108X.

Figure 32. Cross section of previous year's phloem showing an immature sclereid. 460X.

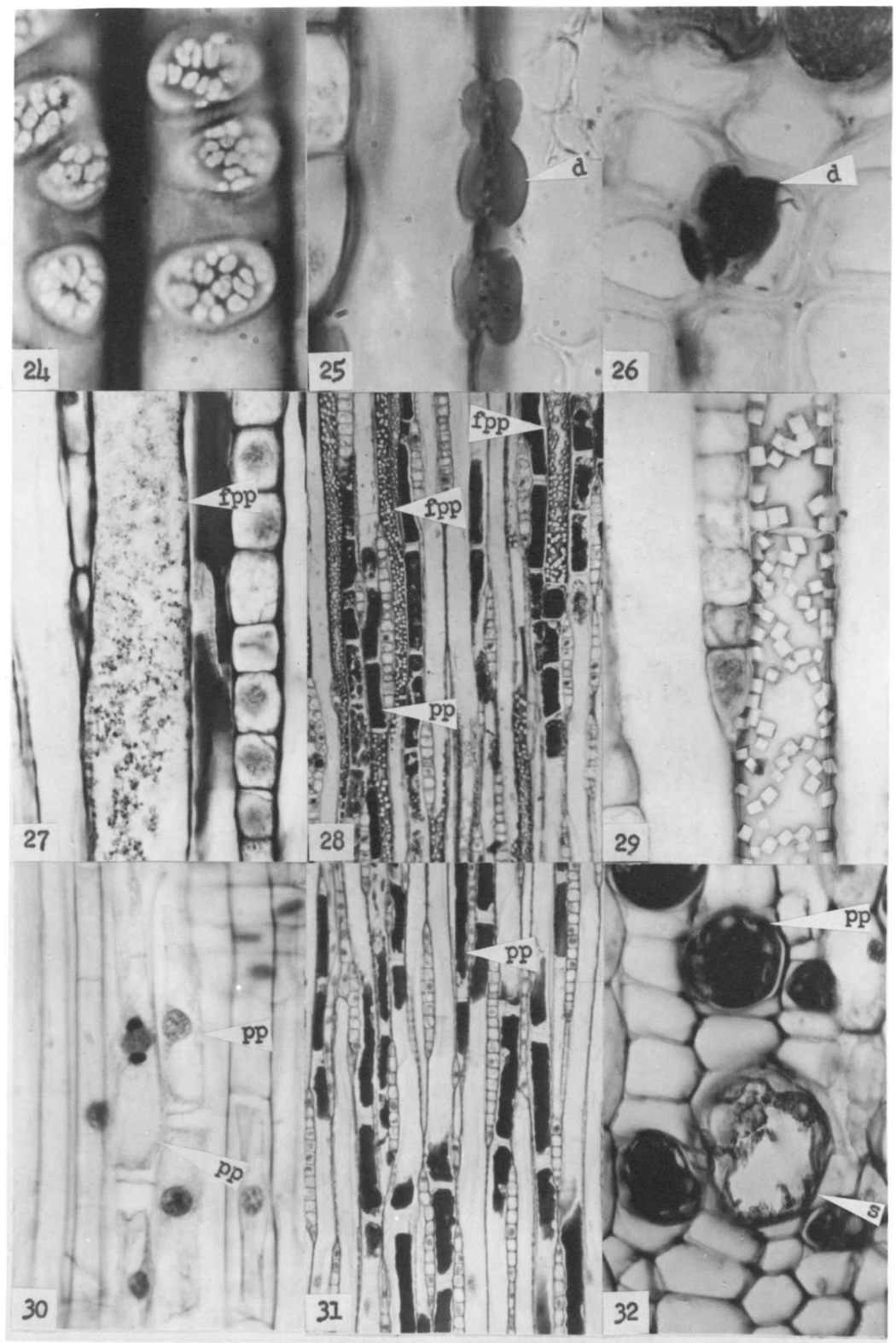
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PLATE V

Figures 33-37

Figure 33. Tangential section of an immature sclereid with a large, centrally located nucleus. 460X.

Figure 34. Radial section of an immature sclereid derived from the terminal cell of the parenchyma strand. 108X.

Figure 35. Radial section of enlarged sclereid. Note the separation of adjacent cells by the growth of the tip of the sclereid along the middle lamellae. 108X.

Figure 36. Fully enlarged sclereids prior to deposition of secondary walls. 108X.

Figure 37. Sclereids with the first lignified secondary wall. Note that the tannin granules have increased in the cytoplasm. 108X.

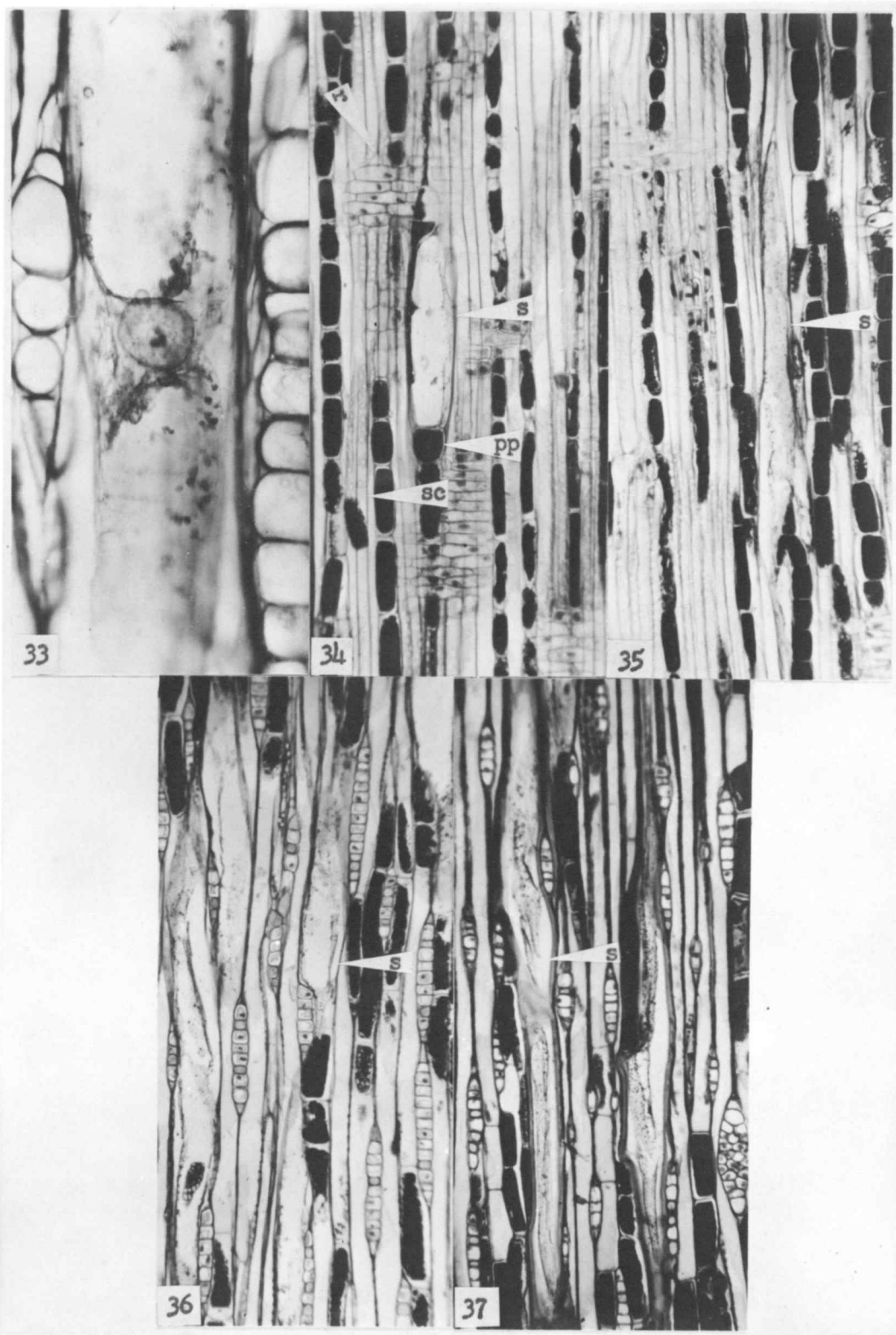
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PLATE VI

Figures 38-43

Figure 38. Cross section of previous year's phloem with developmental stage of sclereids. 108X.

Figure 39. Sclereids with three to four lignified secondary walls. The tannins have increased considerably in the center of the cell cavity. 108X.

Figure 40. Cross section of two and three year old phloem. Note the groups of sclereids developing in last year's phloem, and a number of solitary sclereids developing in phloem tissues formed about two years ago. 108X.

Figure 41. Cross section of mature sclereids with lamellate walls, simple pits, and cell cavity with deeply-staining, amorphous substance that is probably tannin in nature. 460X.

Figure 42. Mature sclereids from macerated phloem 108X.

Figure 43. Tangential section of mature sclereids. 108X.

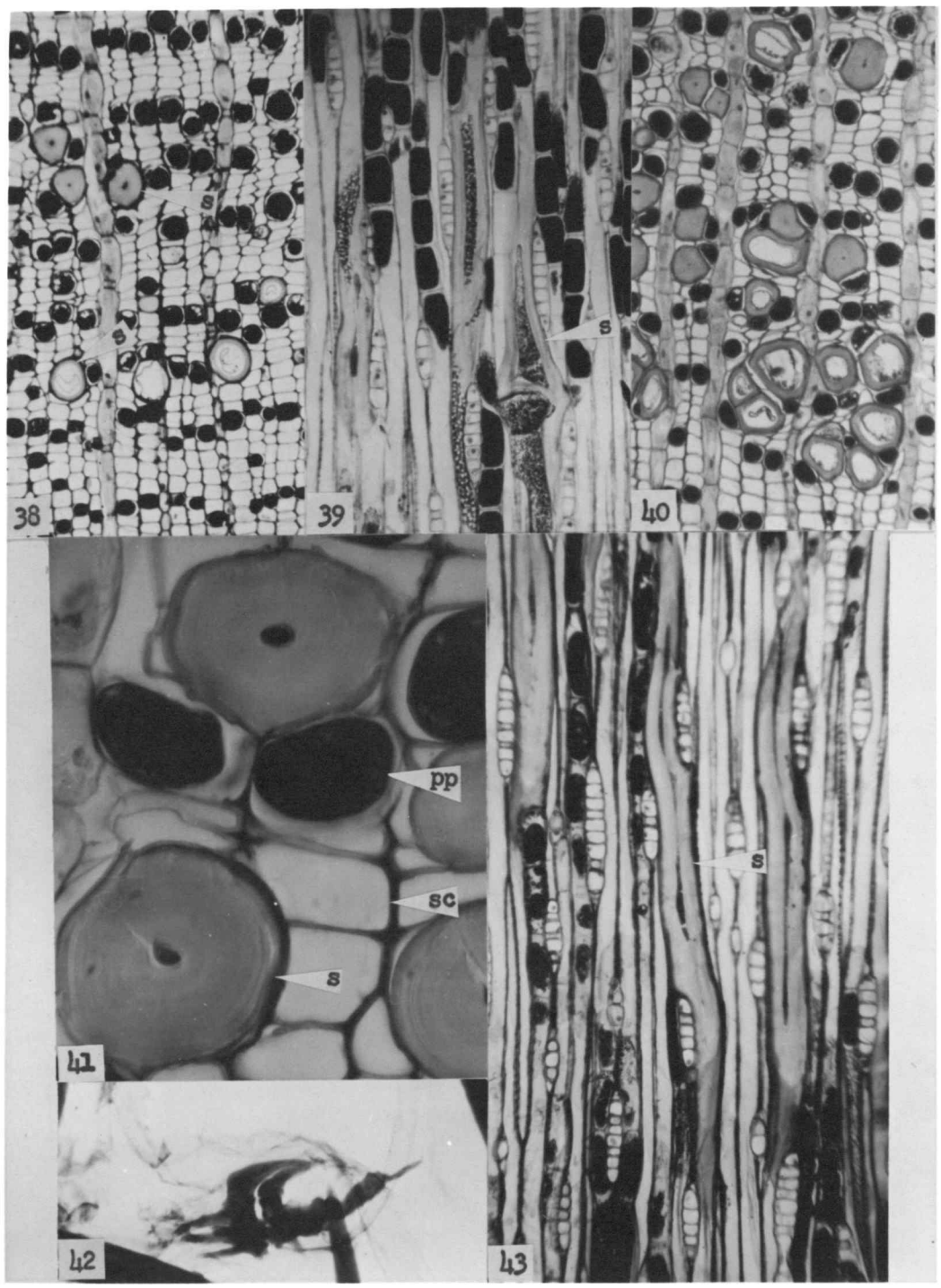
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PLATE VII

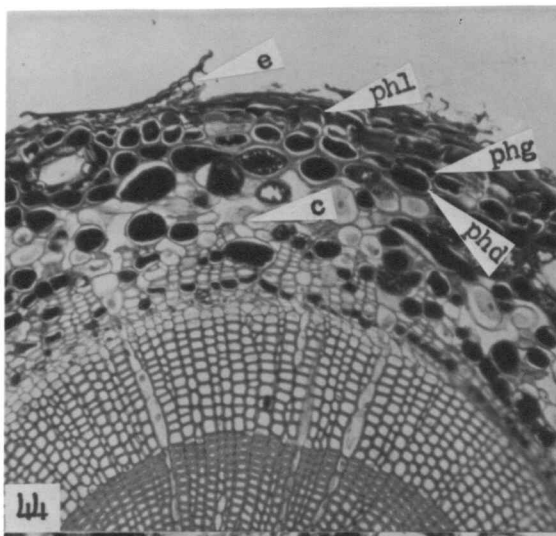
Figures 44-47

Figure 44. Cross section of a two and one-half year old stem. 108X.

Figure 45. Cross section of first-formed deep periderm layer and cortical region. 108X.

Figure 46. Cross section of active phellogen layer and adjacent phloem tissues. 108X.

Figure 47. Radial section of active phellogen layer and adjacent phloem tissues. 108X.



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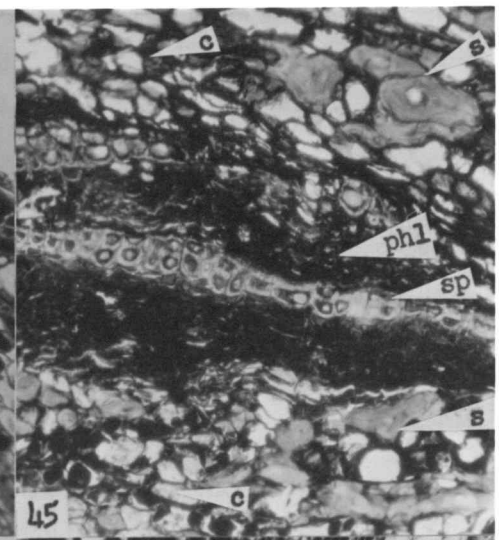

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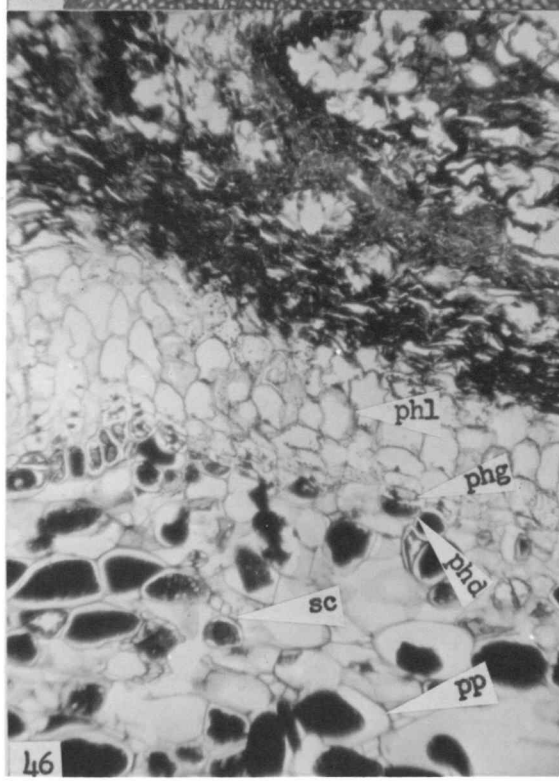

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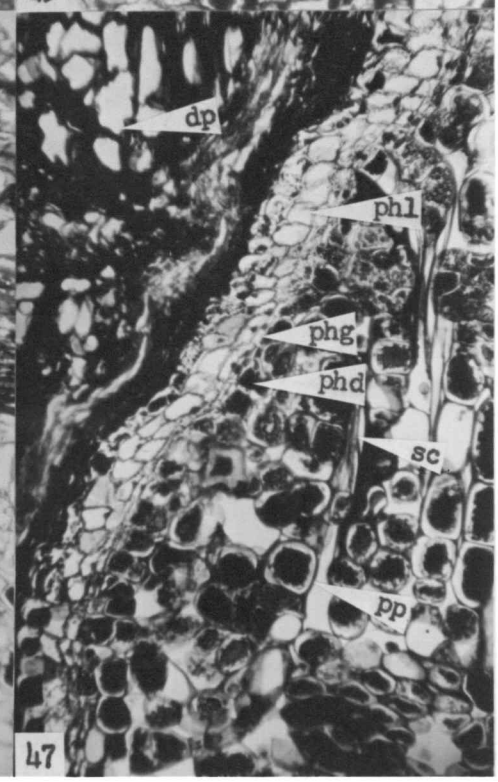

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PLATE VIII

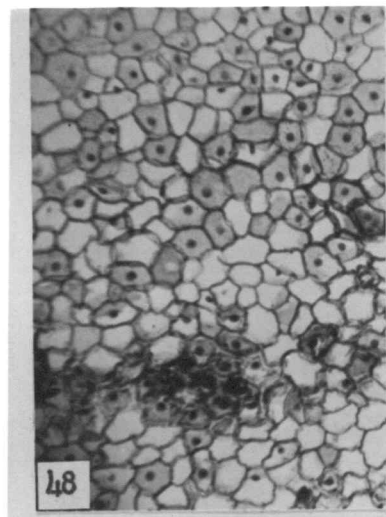
Figures 48-51

Figure 48. Tangential section of newly-formed phellem cells. 108X.

Figure 49. Tangential section of phellem cells with abundant tannins and resins. 108X.

Figure 50. Cross section through active phellogen layer. 460X.

Figure 51. Cross section of a portion of the periderm. 460X.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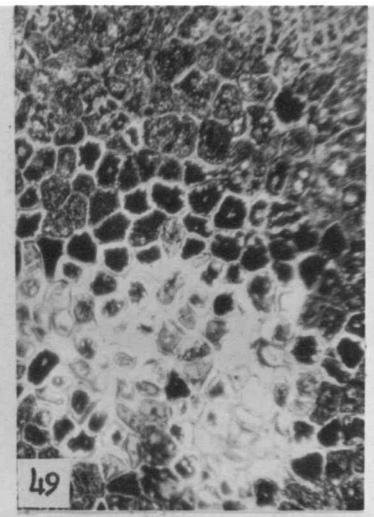
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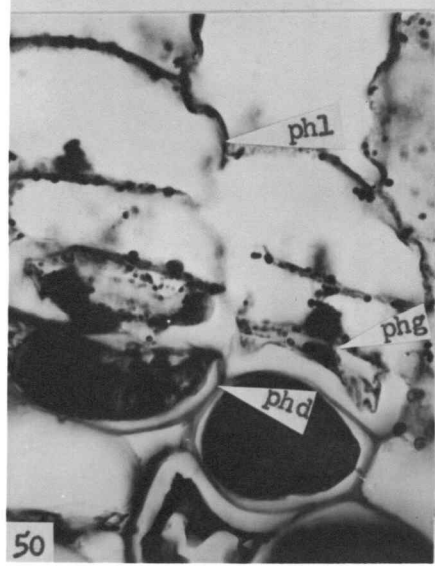

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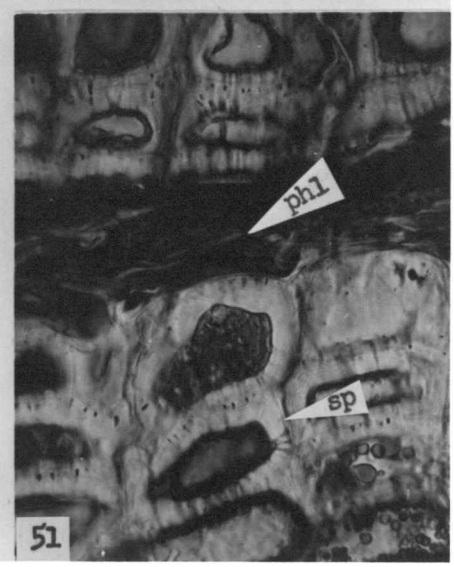

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