AN ABSTRACT OF THE THESIS OF

ROY HIROFUMI SAIGO for the Ph. D.
(Name of Student) (Degree)

in Botany (Anatomy) presented on November 6, 1968
(Major) (Date)

TITLE: ANATOMICAL CHANGES IN THE SECONDARY PHLOEM OF GRAND FIR (Abies grandis [Dougl.] Lindl.), INDUCED BY THE BALSAM WOOLLY APHID (Adelges piceae Ratz.)

Redacted for Privacy

Abstract approved: Frank H. Smith

The balsam woolly aphid (Adelges piceae Ratz.) feeds by inserting its stylets intra- and intercellularly into the cortex or outer phloem of the true firs. While feeding, the aphid injects into the bark an unknown stimulatory substance which affects the adjacent cortical parenchyma cells, the activity of the vascular cambium and the differentiation of the xylem.

This study examines non-infested, infested, and aphid-abandoned trees of grand fir (Abies grandis [Dougl.] Lindl.) collected through the year by serial cross, radial, and tangential sections of the cortex, secondary phloem, cambium, and a portion of the xylem.

The cortex of grand fir is composed mainly of parenchyma cells filled with resins, tannins, and sometimes crystals. Some of the parenchyma cells differentiate into astrosclereids. After infestation

the cortex is characterized by hypertrophy of the nuclei and cortical parenchyma cells. As these cells enlarge into giant cells, there is an increase in density of the cytoplasm. Later a cork cambium is initiated around the pockets of giant cells.

Reactivation and cessation of the cambium in infested, non-infested, and aphid-abandoned trees do not differ any more than the natural tree-to-tree variations that are due to location and exposure. The dormant cambium of infested trees maintains a wider radial file of cells in the cambial zone than do the cambiums of non-infested and aphid-abandoned trees. The cambium of infested trees generally produces more cells per year by increases in both periclinal and pseudotransverse divisions spread more or less evenly throughout the year. Pseudotransverse divisions in the non-infested and aphid-abandoned trees are generally limited to the latter half of the growing season.

The sieve cells of the non-infested and aphid-abandoned trees are longer than those of the infested trees, but those of the infested trees are larger in radial width. The tangential widths do not vary.

Rays are produced by anticlinal divisions of fusiform initials and by decline of fusiform initials. More declining tiers are noted in infested trees than in non-infested and aphid-abandoned trees. The fusiform initials decline to form one or more ray initials. In the latter case, the segmented initial produces two or more separate

ray initials by unequal periclinal divisions and by maturation of some initials in the strand. The phloem rays usually accumulate a greater abundance of resins than do those of non-infested trees.

The cambium of infested trees produces more tangential bands of phloem parenchyma cells and fiber sclereids than that of non-infested and aphid-abandoned trees. Traumatic resin ducts are initiated in the differentiating xylem, especially in heavily-infested trees. In the material examined, these were initiated in the spring, but they can occur at any time during the growing season. After prolonged presence of feeding aphids, phloem ray cells develop abnormalities such as dumbbell-shaped nuclei or a binucleate condition.

Astrosclereids and resin cells were noted in all categories of trees studied. The astrosclereids were seen in all samples collected, whereas the resin cells appeared only in certain trees of the various categories.

Anatomical Changes in the Secondary Phloem of Grand Fir (*Abies grandis* [Dougl.] Lindl.), Induced by the Balsam Woolly Aphid (*Adelges piceae* Ratz.)

by

Roy Hirofumi Saigo

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1969

APPROVED:

Redacted for Privacy

Professor of Botany
in charge of major

Redacted for Privacy

Chairman of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented November 6, 1968

Typed by Carolyn Irving for Roy H. Saigo

ACKNOWLEDGEMENTS

I dedicate this thesis to my parents, Mr. and Mrs. T. Saigo, for without their guidance, encouragement, and sacrifices I would not have been able to complete my education.

My thanks to the U. S. Forest Service for its financing of this project and to Dr. R. G. Mitchell for his personal concern during this study.

To Dr. Frank H. Smith I extend my gratitude for the initiation of this study, guidance, and critical reading of the manuscript.

I must give special compliments to my wife, Barbara, for her encouragement, reading, and typing of the thesis.

TABLE OF CONTENTS

LIST OF FIGURES

ANATOMICAL CHANGES IN THE SECONDARY PHLOEM OF GRAND FIR (Abies grandis [Dougl.] Lindl.), INDUCED BY THE BALSAM WOOLLY APHID (Adelges piceae Ratz.)

INTRODUCTION

The balsam wooly aphid (Adelges piceae Ratz.) is on the move. From its introduction in Nova Scotia and the Pacific Northwest about 1900, it has spread through the eastern states on balsam fir (A. balsamea [L.] Mill), the southeastern states (Speers, 1958) on Fraser fir (A. fraseri [Pursh] Poir.) and in the Pacific Northwest on three species of Abies. In the Pacific Northwest the aphid has caused major damage to silver fir (A. amabilis [Dougl.] Forbes), sub-alpine fir (A. lasiocarpa [Hook.] Nutt.), and grand fir (A. grandis [Dougl.] Lindl.).

In its probing, the balsam woolly aphid injects a salivary substance into the cortex (Balch, 1952). Kloft (1957), Crystal (1926), and Oechssler (1962) report the close relationship between aphid density and the amount of active protein at the feeding site. Kloft (1957), using mercuric bromphenol blue, also noted an increase of soluble protein elements such as free amino acids and peptides in an area of increased aphid population. This is of special importance to the nutrition of the intercellularly sucking aphids, as only small molecular particles can penetrate the cell membrane. Oechssler (1962) noted that the aphid, through the injection of its saliva,

conditions the food material in the plant tissue to make it available for feeding. She also noticed the solid cell contents, i. e., starch granules, disappear gradually with increased feeding time.

The nature of the growth-stimulating substance in the balsam woolly aphid saliva is still a question. Barnes and Newton (1963) report that tumors caused by leaf-hoppers are induced by an unknown substance in the insect saliva. Plumb (1953) did not detect auxins in extracts of salivary glands of Adelges abietis L. and concluded that auxins are not involved in the production of the Norway Spruce Gall. Oechssler (1962) conjectured that certain constituents of the saliva penetrate into the plant and thereby bring about a cellular change, and at the same time allow the penetration of the cell wall or the plasmalemma by all kinds of unknown substances. This leaves open the question as to whether the expansion of cells is caused by the increase in natural enrichment, or whether material in the saliva stimulates enlargement.

Clark and Bonga (1963) discovered the presence of indole-3-acetic acid and a strong growth inhibitor in the inner bark of balsam fir. Bonga and Clark (1965) identified the growth inhibitor as beta-inhibitor. They found that when applied to cultured balsam fir bark beta-inhibitor caused hypertrophy of phloem parenchyma and ray cells. They likened this swelling to the bark malformation, known as gout disease, caused by the balsam woolly aphid.

Whatever the substance that is injected into the bark by the aphid, it causes an abnormal reaction in the growth of the tree similar to that produced by a hormone (Balch, Clark and Bonga, 1964; Clark and Bonga, 1963). The abnormal xylem produced as a result of aphid feeding is sometimes called "rotholz" (red wood) which in some respects resembles compression wood (Balch, 1952). Analysis of aphid-affected wood of three species of Abies was reported by Doerksen (1964) and Doerksen and Mitchell (1965). They found the cell walls of the infested spring wood to be about 50% thicker than normal, tracheids approximately 40% shorter, fibril angle two to three times greater, and the number of rays per unit nearly doubled. In addition, the rays were also 15% to 35% taller and wider than normal, and the volume of ray tissue in aphid-infested trees was 150% to 180% greater than normal (Mitchell, 1967).

Smith (1967) reports that the vascular cambium reacts to the aphid stimulus by increases in periclinal and anticlinal divisions of fusiform initials and increases in the production of new ray initials from fusiform initials and from anticlinal divisions of existing ray initials.

From these studies arose the question concerning the reaction of the vascular cambium leading to the production of secondary phloem and the inducement of anatomical changes in the phloem itself.

The current study provides a more complete picture of the effects of the aphid on the cambium and phloem of grand fir.

LITERATURE REVIEW

The secondary phloem of conifers has been studied by many workers, including Chang (1954), Grillos and Smith (1959), Esau (1965), and Srivastava (1963).

Chang (1954) described the bark of Abies balsamea (L.) Mill as consisting of three essential zones, the periderm, cortex, and secondary phloem. The periderm consists of the phellogen, a cork layer of varying thickness, and a phelloderm which, if present at all, seems to be restricted to a few layers of cells (Srivastava, 1963). The phelloderm cells contain "resinous" substances, and some cells contain crystals of the same rhombohedral shape as those in the phloem parenchyma but smaller in size (Chang, 1954). According to Grillos and Smith (1959), the cork cambium of Douglas fir (Pseudotsuga menziesii [Mirb.] Franco) becomes active much later in the growing season than the vascular cambium. They also showed that the phellogen may be inactive in some parts of the tree, but very active in other portions. Srivastava (1963) noted that probably all living cells in the bark were capable of giving rise to phellogen cells. With the formation of a new phellogen beneath the old one, the old phellogen layer and all living cells of the bark external to the new phellogen are cut off and die. Many such sequences of cork and dead bark tissue composing the rhytidome may

be seen outside the active phellogen.

The cambium usually is defined as a uniseriate layer of cells producing xylem and phloem derivatives by periclinal divisions (Bannan, 1955; Esau, 1965). Because the initials are difficult to distinguish from their recent derivatives, the term cambium is sometimes used loosely to include the cambial initials as well as the undifferentiated periclinally dividing derivatives. To avoid ambiguity, the terms cambium, cambial initials, cambial zone, and differentiating xylem and phloem as defined by Wilson, Wodzicki and Zahner (1965) will be used.

The width of the dormant cambial zone ranges from one to four cells in each radial row in Thuja (Bannan, 1955) and from four to six cells in Douglas fir (Grillos and Smith, 1959). Bannan (1955) observed that the two internal cells in each tier adjacent to the dormant cambium are generally immature tracheids, and the cells adjacent to the mature phloem are immature sieve cells. Grillos and Smith (1959) confirmed this in Pseudotsuga, and noted that the dormant cambial zone is variable on different sides of the same tree, and in different trees.

Bannan (1955) made a careful study of reactivation of the cambium. He reported that the cambial cells expand radially, the radial walls become thinner, and the cytoplasm takes up a parietal position about a large central vacuole. The first periclinal divisions

in the cambial zone follow closely upon swelling of the cells. The first division takes place in the xylem mother cells adjacent to the late wood. At this time, the nucleus of the cambial initial is still in the resting stage. A week later sporadic divisions can be found in the initial area of the cambial zone. At the height of cambial activity the cambial zone can expand to 100-150 μ. Mitotic activity is greatest in the central portion of that part of the cambial zone consisting of dividing and redividing xylem mother cells. Increase in girth of the cambium is accomplished by an increase in the tangential diameter and in the length and the number of fusiform initials (Bailey, 1920; Sanio, 1873). Increase in number of fusiform initials occurs by pseudotransverse divisions by which an initial divides anticlinally into two shorter ones. Following this anticlinal division, the daughter cells either elongate by apical intrusive growth to regain their earlier initial size, or, by uneven periclinal divisions, progressively shorten and produce a declining tier. In the former case, Bailey (1920), Whalley (1950), and Bannan (1956) noted that fusiform initials elongate by apical intrusive growth, forcing their way between the radial walls of neighboring cells, thereby regaining their normal initial length. Whalley (1950), Bannan (1951, 1953), Barghoorn (1940), and Evert (1960) reported that the non-storied cambium continuously loses some of the fusiform initials during its growth as the shorter initials decline. The cell often goes through several anticlinal

divisions and in the course of this process all or some of the segments may be eliminated. The remaining segments give rise to new rays (Bannan, 1956). Differentiation lags considerably behind cell division, especially in rapidly-growing trees (Grillos and Smith, 1959).

Cambial activity decreases later in the season. When mitosis ceases, the cambial zone of thin-walled cells is usually eight to ten cells wide. Some of the outer cells continue to differentiate through the late fall until the dormant cambium of four to five cells is again established (Grillos and Smith, 1959).

The secondary phloem consists of axial and radial systems. The axial system consists of the sieve cells and phloem parenchyma cells, including fusiform parenchyma cells, phloem parenchyma strands and vertical albuminous cells. The radial system is composed of the ray albuminous cells and ray parenchyma cells (Grillos and Smith, 1959; Esau, 1965; Srivastava, 1963).

In the axial system, bands of sieve cells, usually several cells in radial thickness, alternate with uniseriate bands of phloem parenchyma (Srivastava, 1963). The time of formation and number of tangential bands of phloem parenchyma produced each season can be useful in determining yearly increments (Huber, 1939; Holdheide, 1951). Abbe and Crafts (1939), in their studies of white pine, noted that sieve cells which differentiate during the cooler, moister part

of the year have slightly thinner walls and larger lumina than those found later, and that the most conspicuous zone of parenchyma cells is formed in late spring. Huber (1939) noted that the annual increments in Picea are clearly demarcated by a row of collapsed cells at the boundary between yearly increments. In Chamaecyparis and Thuja Bannan (1955) reported that the first fibers of each season's growth have thicker walls than the later fibers. In other conifer species not having clearly-defined yearly phloem increments, a combination of the relative diameter of sieve cells, the distribution of tangential bands of phloem parenchyma cells, the collapse of erect and vertical albuminous cells, and the appearance and dissolution of callus plugs were found useful in determining yearly increments (Grillos and Smith, 1959).

The sieve cells are elongated cells with sieve areas restricted to the radial walls and the incline of radial faces comprising the end walls (Esau, 1965; Srivastava, 1963; Grillos and Smith, 1959). Grillos and Smith (1959) noted little elongation of sieve cells from the fusiform initials during differentiation. The sieve cells are thought to survive for one or two seasons (Esau, 1950; Grillos and Smith, 1959) after which they are crushed by pressures from the production of new xylem and phloem mother cells, and the enlarging phloem parenchyma cells.

Fusiform paranchyma cells are derived directly from phloem

mother cells without formation of transverse walls. They are filled with resinous materials (Srivastava, 1963) and may accumulate starch. After crystals appear, the cells collapse radially and are crushed between adjacent cells (Grillos and Smith, 1959).

Phloem parenchyma strands usually appear in single layers in more or less continuous tangential bands. The cells in each strand are rectangular in longitudinal sections, except for the end cells, which are tapered (Grillos and Smith, 1959; Srivastava, 1963). The parenchyma cells of the phloem parenchyma strands usually accumulate crystals, tanniniferous materials, resins, and starch. Abbe and Crafts (1939), Grillos and Smith (1959), and Srivastava (1963) noted that crystals may be deposited in some cells close to the cambium, and can be stored in the older phloem parenchyma.

Some phloem parenchyma cells also differentiate into sclereids. Srivastava (1963) found that sclereids generally appear to be restricted to the non-functional phloem. The walls of sclereids are often thick, heavily-lignified, lamellate in structure, and have numerous pit canals. The lumen of the sclereid is usually narrow and is filled with tanniniferous materials but no nuclei are found.

Albuminous cells are found in both the radial and axial systems of the phloem. Albuminous cells differ from phloem parenchyma cells in that they do not accumulate resins or tannins and contain little starch. The adjacent sieve cells develop sieve areas in

conjunction with pits in the albuminous cells, and the albuminous cells collapse after the sieve cells cease to function (Grillos and Smith, 1959). Srivastava (1963) noted that ray albuminous cells and vertical albuminous cells have a more densely-staining granular cytoplasm than the procumbent ray parenchyma cells, which have large vacuoles and lightly-staining cytoplasm. The vertical albuminous cells frequently occur as radial plates of cells (Grillos and Smith, 1959), whereas the ray albuminous cells are located at the margins of the rays, or in the middle of a ray (Srivastava, 1963).

Srivastava (1963) characterized the phloem rays. He noted that phloem rays, except for fusiform rays, are all uniseriate in the Pinaceae. Fusiform rays are conspicuously absent in the phloem of Abies and Cedrus. The procumbent ray cells are generally vacuolate and contain starch. Ray parenchyma cells, like the resin-filled phloem parenchyma cells, enlarge with time.

MATERIALS AND METHODS

Samples including the bark, cambial zone, and a small portion of the adjacent mature or differentiating xylem, were collected near Corvallis, Oregon during 1964, 1965, 1966, and 1968. The age of the trees sampled ranged from three to 100 years but most samples were taken from trees 20 to 30 years old. Collecting was done at monthly intervals during the dormant season, weekly during the early part of the growing season, and at two- to three-week intervals during the latter part of the growing period. At each sampling time, bark was taken from non-infested, infested, and aphid-abandoned trees. Samples were removed with much care to prevent crushing of the tissues, especially during the period of high cambial activity. Each sample was trimmed in the field to approximately 1/2-inch square, and placed immediately into Craf III killing and fixing solution (Sass, 1951). Air was evacuated from the samples at the laboratory. They were then dehydrated and embedded in 60°C. Tissuemat, softened by placing the exposed surface of the block directly into ten percent glycerin and a detergent and sectioned with a rotary microtome to obtain serial cross, radial and tangential sections of the bark, cambium, and adjacent xylem. The sections were cut at 20 μ and stained with safranin and chlorazol black E. Aniline blue was used to test for callose.

In addition to the material collected above, I transferred eggs

from heavily-infested trees to non-infested trees. The eggs became abundant around the first of April, and were collected by shaving off the bark on which they were laid. A thin layer of cotton was spread over the bark of the tree to be infested and the egg-covered bark strips were tied with the eggs facing the cotton. Transfers were made to 40 healthy fir trees, from three to 20 years of age. Transfers were made at five different times at two locations.

The success of transfers can be rated as fair to good. Some factors appearing to affect the success of transfers are age of the tree, surface texture of the bark, location of the tree in relation to solar exposure, robustness of the tree, and aphid predators.

To further substantiate the effects of the aphid on fir trees, in April and March approximately 30 trees from three to seven years old were collected and potted to be grown under greenhouse conditions. In order to reduce environmental variability and thereby gain a denser population of aphids, I transferred aphid eggs to 20 of these young seedlings in the greenhouse. After two weeks, ten of the infested trees were fumigated with smoldering nicotine powder to kill the aphids. This was done to examine the effects of short-term aphid feeding. Weekly samples were taken from all the artificially infested, non-infested, and fumigated trees during the growing season, and put through the same treatment of killing and fixing, dehydration, sectioning, and staining as described above.

Polaroid photomicrographs of serial tangential sections of phloem were taken to study the radial files of cells produced by individual initials. These photomicrographs covered an area 0.8 mm by 1.0 mm. The serial pictures were oriented by a large ray at one corner. Although this method was adequate for following radial files in a tangential series, there was some misalignment of the pictures because of the change in ray cell number and size, and some crushing with age. Individual serial radial files were followed by the pictures, aided by a hand magnifying glass and frequent examination of the original slides.

OBSERVATIONS

Non-infested Trees

Non-infested older trees were difficult to locate, for most trees eight to ten inches D. B. H. (diameter breast height) have been or are infested by aphids. It appears that in the Willamette Valley most trees over 45 years of age have been infested at one time or another (Mitchell, 1966).

Vascular Cambium

The dormant cambial zone of grand fir consists of from three to six cells in each radial file, with an average of four cells. The cambial zone has one or two xylem mother cells adjacent to the xylem and one and sometimes two phloem mother cells next to the phloem. One can recognize the latter cells in transverse sections because of their wider radial dimensions compared to other cells in the cambial zone (Figures 3, 4). In radial view the xylem mother cells in the dormant cambial zone do not appear to be as wide as the phloem mother cells, although generally they are longer.

An average fusiform initial in the dormant cambium of non-infested trees measured 2400 μ in length by 31.8 μ in tangential width by 4.5 μ in radial width. The nuclei of dormant fusiform initials were relatively long and narrow, 5 μ by 55 μ, in contrast to those of

dividing initials, 16 μ by 36 μ.

Periclinal divisions in the cambial zone were initiated the first week in April in more vigorous trees, but such divisions were delayed in suppressed trees. Many of the trees studied were in an active state of division by the latter part of April, while other trees were still in a dormant state. By the middle of May the cambial cells of most trees were actively dividing. This sequence agrees with that reported by Grillos and Smith (1959) for Douglas fir in the Corvallis, Oregon area.

Cells of the cambial zone divide most actively during May and the first part of June. During the latter part of June the rate of division declines considerably and differentiation progressively reduces the number of cells in the cambial zone.

Differentiation appears to be correlated with the robustness of the tree. In most suppressed trees, differentiation begins earlier in the growing season, while differentiation is initiated a little later in more vigorous trees. In suppressed trees, differentiation begins soon after a few xylem mother cells have been produced. In more robust trees, with a higher population of cells in the cambial zone, differentiation will continue through July and August. By September, most of the differentiation is completed and the cambial zone is back to its dormant state, although some secondary phloem differentiation was noted to continue into October.

Sieve Cells

Approximately four to five sieve cells are produced per year in each radial file of the phloem of grand fir. Sieve cells of non-infested trees average 3003 μ in length, 33 μ in tangential width, and 20 μ in radial width.

All sieve cell measurements were made on ten samples from non-infested trees selected at random through the growing season, and ten measurements per sample were taken in the conducting phloem. Thicknesses of the cell walls were determined by measuring in cross sections the combined thickness of two adjacent tangential cell walls and dividing by two. Cell wall thickness ranged from 0.7 μ to 1.3 μ, with an average of 0.9 μ. There was a gradual increase in cell wall thickness from spring to fall, with the first noticeable increase occurring in mid-July.

The lengths of cells produced in a radial file may remain approximately the same, increase in length, or decrease in length without the occurrence of pseudotransverse divisions. For example, over a two-year period, the first sieve cell produced in one file was 2332 μ in length, while at the end of the same year it measured 2530 μ and by the end of the second year had increased to 2580 μ. Cells in another tier decreased in size as a result of unequal periclinal divisions in the fusiform initial. The initial length at the start of the

first year was 1925 μ, and increased to 2090 μ by the end of the first year. Through the second year, the sieve cells decreased to 1650 μ.

The sieve cells of younger trees, one-half to one inch in diameter at the sampling level, are decidedly smaller. The lengths of the sieve cells range from 1100 μ to 2200 μ and average 1658 μ. The tangential and radial widths average 26 μ and 16 μ, respectively. The latter measurements do not vary as much as the lengths of sieve cells measured.

Phloem Parenchyma Strands

Phloem mother cells destined to differentiate into phloem parenchyma strands are derived from fusiform initials in late May or early June as an initially tangential layer. Resins accumulate very early during the differentiation of these cells. They then expand radially, and the first transverse anticlinal cell divisions occur immediately, near the center of the cells (Figure 33). Resin around the nucleus appears to dissolve and only small drops of resin occur in that general vicinity. Succeeding divisions occur quite regularly, progressively dividing the longer cells in half until the entire strand is sectioned into approximately equal segments. One exception was noted, where a single cell of a phloem parenchyma strand divided longitudinally (Figure 19).

There is generally one tangential layer of phloem parenchyma

strands produced per year, but, depending upon the vigor of the tree, there may be up to three tangential layers per year. Parenchyma strands are produced after two or three sieve cells have been derived. Sometimes a radial file may not have a phloem parenchyma strand, and regularity of the tangential layer will be interrupted (Figure 3). In other instances, a radial file may have two successive phloem parenchyma strands in a yearly increment (Srivastava, 1963). There is some variation in the number of sieve cells that are produced after the phloem parenchyma strand. The number can vary from none to four cells, depending upon the vigor of the tree. The most vigorous trees produce the greatest number of sieve cells.

Sclereids

Sclereids in the bark of grand fir develop from cells of phloem parenchyma strands (Figure 27), and from cortical parenchyma cells. Two types of sclereids may be distinguished, astrosclereids and fiber sclereids.

Astrosclereids originating from cells of the phloem parenchyma strands sometimes are initiated in the three-year-old phloem but the majority differentiate in the five- to six-year-old phloem. Often, continuing sclereid initiation in the older phloem occurs in phloem parenchyma cells adjacent to already-established sclereid groups, although they can develop from any parenchyma cell in the

non-conducting phloem (Figure 28). The former situation results in massive tangential bands of sclereids which crush and distort adjacent phloem cells (Figures 28, 29).

Sclereid differentiation from cells of the phloem parenchyma strands is initiated in early May. The cytoplasm becomes densely granular and projections of the cell walls push outward in all directions. The projections grow intercellularly. This growth and the expansion of developing sclereids crush the surrounding cells. The nucleus persists through the expansion of the thin cell wall, and is still visible during the stages of wall thickening and lignification. Near the end of sclereid differentiation, the deteriorating nucleus can be recognized as a dark mass against the cell wall. Astrosclereid initiation lags behind cambial reactivation. The vascular cambium may produce 20 to 35 tracheids in each radial file before sclereid formation is initiated.

The vascular cambium in suppressed trees is almost dormant by late June and all sclereids are maturing, but in vigorously-growing trees sclereids are immature and the cambium is still active at this time. Thus it appears that sclereid development is progressive but lags behind cambial activity. By the middle of October, however, all sclereids have reached maturity. The cells have greatly-thickened, lignified walls, with some resins still in the lumen, and nuclei have disappeared.

Sometimes a single ray parenchyma cell differentiates into a brachysclereid without any cellular expansion (Esau, 1965). The cell wall thickens and becomes lignified, decreasing the size of the lumen while maintaining the original size and shape. This condition can also be found in the cells of the phloem parenchyma strand where just one cell of a strand matures into a sclereid without any expansion of the cell wall.

The sclereids that develop from cortical parenchyma cells follow the same pattern as described for astrosclereids, although the differentiating cortical sclereids do not elongate or develop as many arms, nor develop as thick a cell wall as the astrosclereids in the secondary phloem.

Fiber sclereids are generally more abundant in more vigorous trees. They are derived directly from phloem mother cells. In contrast to the astrosclereid, the fiber sclereid matures during the current year's growth. They do not elongate or expand as does the astrosclereid, but the cells maintain the approximate size and shape of the phloem mother cell. Differentiation of the fiber sclereid is initiated before phloem parenchyma strand differentiation is completed and before cessation of cambial activity. These sclereids are usually intermingled with the tangential layer of phloem parenchyma strands.

Ray Cells

Procumbent cells. Phloem rays are mainly uniseriate, with the height ranging from one to 20 cells, and averaging eight cells. Fusiform rays with resin canals do not occur in Abies, which was also reported by Srivastava (1963). No resins or tannins occur in the procumbent ray parenchyma cells in either the conducting or non-conducting phloem.

Albuminous cells. Vertical albuminous cells are generally derived from declining fusiform initials which result from unequal pseudotransverse divisions or unequal periclinal divisions. Generally the shorter daughter cell will produce a vertical albuminous strand which may or may not become subdivided. Later, the short initial either becomes a ray initial through uneven periclinal division, or it differentiates and that tier is terminated (Bannan, 1953; Srivastava, 1963). The new ray initial may either initiate a new ray or add to the margins of a pre-existing ray.

The ray albuminous cells are usually radially-expanded and vertically-elongated cells located on the margins of rays. Unequal periclinal divisions in the marginal ray initials may cause an uneven positioning of the marginal ray cells. Some of the ray cells in the erect position are not albuminous cells but will accumulate resins, or form crystals, or both. The ray and vertical albuminous

cells contain cytoplasm denser than other ray cells, contain little stored materials, and share a sieve area with adjacent sieve cells (Grillos and Smith, 1959). Both types of albuminous cells die when the adjacent sieve cells become non-conducting.

Resin Cells

Srivastava (1963) and Holdheide (1951) have described resin "cavities" in the bark of Abies, and Chang (1954) has used the term resin "spaces." I will utilize the term resin cell, as the structure is actually an expanded or enlarged cell. The resin cells differentiate from ray parenchyma cells of the non-conducting phloem, and are initiated from parenchyma cells at the margins of the rays as well as from cells within the rays. The initiation of resin cell development begins with ray parenchyma cells accumulating a fine-textured resinous material (Figure 17). As the cell begins enlargement in all directions, the nucleus seems to disappear. No nuclei were noted in even the earliest developing resin cells (Figures 15, 16).

Typical resin cells measure 40 μ by 80 μ, appear egg-shaped, and develop in tangential bands (Figure 18). In resin cavities described by Srivastava (1963), the resin pulled away from the center of the cell. However, in trees I studied the resin remained distributed throughout the cell in most cases, although in some cells the resinous material did shrink and pull away from the cell wall. This

was possibly due to plasmolysis during dehydration.

Resin cells can differentiate in the phloem as early as the second year, but most arise in the older phloem (Figure 18). The development of the resin cells does not appear to be correlated with the growing season, since resin cells of varying sizes can be found in the same annual increment.

Infested Trees

Vascular Cambium

The dormant cambial zone of infested grand fir trees varies from eight to ten cells in a radial file (Figures 1, 2), as compared to an average of four cells in non-infested trees. Reactivation and cessation of the cambial zone in infested trees did not differ from that of non-infested trees studied in the Corvallis area. Generally the aphid-infested trees produced eight to ten phloem cells per year, while the non-infested trees produced an average of five phloem cells.

Traumatic Resin Ducts

Most infested trees produce vertical traumatic resin ducts in the xylem but not in the phloem, usually in more or less continuous tangential bands (Figure 6). They are often limited to certain sections of the tree, possibly to areas of heavy aphid concentration

(Figure 5).

Traumatic resin ducts can be produced at any time during the growing period but most appear to be initiated in the spring. The earliest differentiation of a resin duct was noted in a sample collected April 17, 1965. Most of the other infested trees exhibited differentiating resin ducts in the latter part of April. The xylem mother cells divide by transverse anticlinal divisions to produce densely-staining epithelial cells which line the canal. Later the differentiating tracheids surrounding the resin ducts mature with thick, lignified walls. The epithelial cell walls, however, do not become lignified and the cells retain their densely-staining cytoplasm. Traumatic resin ducts were not noted in the differentiating xylem of non-infested or aphid-abandoned trees.

Sieve Cells

Sieve cells showed an average length of 1645 μ, tangential width of 32 μ, and radial width of 25 μ. The measurements were taken as described for the normal sieve cells.

Doerksen (1964) and Doerksen and Mitchell (1965) found that the cell walls in aphid-affected spring wood are about 50% thicker than in normal tracheids. Thickness of the tangential walls of sieve cells were measured as described for the non-infested trees. The cell wall thickness ranged from 0.6 μ to 1.2 μ, averaging 0.8 μ.

These measurements were statistically compared with those of non-infested trees but no significant differences were detected.

Phloem Parenchyma Strands

The parenchyma strands are generally derived at approximately the same period of growth as in non-infested trees. One strand per year is typical for non-infested trees. Although the number of tangential layers of phloem parenchyma strands produced in a year varies from tree to tree, generally there are two to three bands produced per year in the infested trees, and these are frequently irregular and discontinuous (Figures 5, 31).

Sclereids

All the major sclereid types found in the bark of non-infested grand fir trees were identified in the infested material. As in phloem of non-infested trees, there is a uniform initiation and differentiation of the sclereids in the infested trees. Fiber sclereids were present in most of the infested trees (Figures 2, 31), but were found only occasionally in non-infested trees that were exceptionally vigorous.

Ray Cells

The rays of infested trees differ from those of non-infested

bark in that biseriate rays are present. Also, some cells have dumbbell-shaped nuclei or are binucleate, and contain much resins and tannins (Figures 24, 25, 26). Phloem rays in the infested bark are mainly uniseriate, but some biseriate rays are present (Figure 20). No triseriate rays were noted as observed in the xylem by Doerksen and Mitchell (1965). The height of rays ranges from one to 34 cells, with an average of 15 cells per ray. This average is considerably more than the average of eight cells for the normal rays. This is in agreement with Mitchell (1967), who found the number of cells in a ray to be greater than that found in the wood from non-infested trees. The phloem rays in a tree abandoned by the aphids average 11 cells in height. It appears that the rays in once-infested trees continue to be more numerous and greater in height than in non-infested trees even after the aphid population abandons the old infestation site (Doerksen and Mitchell, 1965).

No biseriate rays are noted in the phloem of non-infested trees, while some of the infested trees contain biseriate rays and others do not. An average of 12% of the rays are biseriate. It appears that the older, heavily-infested trees have a much higher incidence of taller and biseriate rays. For example, a 12-inch tree D. B. H. had a range of one to 30 ray cells, with an average of 17 cells per ray. Twenty-two percent of the rays were biseriate. This is in agreement with Mitchell (1967) who reports biseriate rays up to

22% of the total rays in aphid-affected wood. This abundance of both tall and biseriate rays could be attributed to long and heavy balsam woolly aphid infestation.

An abnormal nuclear condition of the ray parenchyma cells is found in the older phloem. It is difficult to determine the exact age of the older phloem because of the crushing and distortion that takes place, but one can estimate fairly accurately the age of the phloem up to about five years. Most of the nuclear abnormalities are found in phloem older than five years. The first apparent change in the nucleus is the assumption of an oblong shape. Later the nucleus becomes centrally constricted and apparently divides by a form of amitosis to produce the binucleate condition seen in the older ray cells (Figure 25).

The infested trees have ray parenchyma cells that accumulate resins or tannins close to the cambial zone shortly after their derivation (Figure 26). The number of cells in a ray that accumulate resins will vary from ray to ray even in the same radial section. There are ray cells containing both resins and dumbbell-shaped nuclei. The percentage of resin-filled ray cells in a ray varies from 10% to 95%. The phloem rays in non-infested trees do not accumulate large amounts of resins.

In the conducting phloem, some of the erect ray cells which are not albuminous cells accumulate resins and crystals, as noted

for non-infested trees.

Aphid-abandoned Trees

The balsam woolly aphids feed on a given bark area for variable periods of time, after which they move up the tree. This could be due to diminishment of the food supply, corking over, or both. In most of the aphid-abandoned trees, the rhytidome is extremely thick, which would make it very difficult for the aphid to feed in the secondary phloem. There is much periderm formation under aphid-wounded areas, which also often exude resin. Abundant resin also comes from callus areas on the underside of limbs and where limbs join the main stem (Mitchell, 1967).

Most trees sampled for abandoned bark had aphids up around ten to 15 feet from ground level. The density of aphids at that height ranged from light to heavy. Samples were collected at breast height, so that they were six to 11 feet below active aphid areas. Although the aphids had abandoned the sample area, some of the hormone-like substances in the aphid saliva could conceivably be translocated down to the sampled area.

Vascular Cambium

The dormant cambial zone in an abandoned area averages four cells per radial file. This number is within the range quoted for the

cambial zone in non-infested trees, but contrasts with the eight to ten cell dormant cambial zone in infested trees. Cambial activity of infested and abandoned trees follows approximately the same seasonal changes as that described for the normal tree. The cambium of the abandoned trees produces an average of one phloem parenchyma strand per year in each tier, as does the cambium of most of the non-infested trees. The most vigorous non-infested trees may produce two to three strands in each tier as do the infested trees.

Sieve Cells

The sieve cells measured an average of 2466 μ in length, 33 μ in tangential width, and 22 μ in radial width. These measurements were determined as described in the study of the phloem of non-infested trees.

The average length of sieve cells of aphid-abandoned trees was greater than that of infested trees (1645 μ), but not as great as in non-infested trees (3003 μ). The tangential width of sieve cells in all the categories did not vary appreciably, with an average of 33μ for non-infested and 32 μ for infested trees. There was some variation in the averages of radial dimension, with sieve cells of the aphid-abandoned trees being intermediate to those of the non-infested trees (20 μ) and infested trees (25 μ).

Fiber Sclereids

Fiber sclereids are found in the phloem of very fast and vigorously growing non-infested trees, but are mostly lacking in the phloem of slower growing non-infested trees and the recent phloem of abandoned trees. Therefore one could postulate that the appearance of fiber sclereids is related to the rapid rate of growth in a tree. It appears that the cambium of an aphid-infested tree is stimulated to produce more cells in a year and can be considered as vigorous; consequently fiber sclereids are prevalent in most aphid-infested trees studied, and in the older phloem of aphid-abandoned trees.

Ray Cells

The ray cells in the recent to intermediate age phloem are not binucleate and do not contain resins, as do the ray cells in the older phloem. Since resin in aphid-infested trees accumulates in the ray parenchyma adjacent to the cambium, one could assume that resin accumulation in the ray cells of the abandoned material was initiated by prior aphid infestation sometime in the history of the tree. Although the majority of ray cells that contained abnormal nuclei showed dumbbell-shaped nuclei, there were a few which were binucleate. The latter situation is not found in great abundance

as in the infested trees.

Cambial Activity

Non-infested Trees

I started this study by analysis of individual tiers for pseudo-transverse divisions and cell types produced. After many hours of work, I found that this method was too slow and tedious. Some factors contributing to this difficulty were 1) the lack of a marker for orientation in the phloem of grand fir, i.e., a fusiform ray canal, 2) the distortion and crushing in the secondary phloem, and 3) the changes in position of the marker rays and their cell number. Thus, the use of polaroid photomicrographs was employed. I found this method to be satisfactory for this study.

Cross sections were taken of the same samples studied by serial tangential sections in order to approximately correlate the cell types produced to a particular period of the growing season.

In the first study, samples of trees used for analysis of individual tiers were selected randomly through the growing season and the tiers studied were randomly selected in the tangential sections. Activity of the initials as reflected by the cells produced was followed for one year.

Forty-one tiers were analyzed, and in seven (17%) of these

pseudotransverse divisions occurred. There were four (10%) pseudotransverse divisions where both daughter initials survived and three (7%) where one daughter cell persisted and the other declined. No ray initials were derived from the declining tiers, and in no case did both daughter cells decline after an anticlinal division. In summary, after seven pseudotransverse divisions, following which three declining tiers were terminated, there was a net gain of four new tiers.

Analysis of tiers by photomicrographs of serial tangential sections was done on samples collected at breast height in February and May from non-infested trees. The February samples were analyzed for one year. Fifty-four fusiform initials were in the study area at the start of the year's growth. There was a total of four (7%) pseudotransverse divisions for the year. Three pseudotransverse divisions occurred where both daughter initials persisted. One tier (Figure 34, tier 2) declined by unequal periclinal division, producing a vertical abluminous strand. After a pseudotransverse division, both resulting initials became segmented. A number of cells in the segmented initials were terminated, forming separate ray initials of four, two, and two cells from one daughter cell and one, two, and one cells from the other. Finally at the end of the year all these ray initials were terminated. No consecutive divisions of an initial in a radial file were noted, nor were there

pseudotransverse divisions of phloem mother cells. Therefore, with a total of four pseudotransverse divisions and the loss of one tier, there was a net gain of three tiers.

The third sample was collected in May and was also studied by photomicrographs. Forty-three tiers of fusiform cells were included in the first section of the study area. This study area covered 300 μ in a radial direction, figured by the total number of sections cut at 20 μ from the cambium into the secondary phloem. This distance included four years of phloem. Although the yearly increments are not readily distinct, by using the combination of characteristics listed previously I was able to approximately separate the secondary phloem into yearly increments. Most of the yearly phloem increments included one tangential row of resin-filled phloem parenchyma strands. Although most of the increments are composed of approximately five phloem cells per year, with time the increments are crushed radially. Due to this compression the number of cells included within a given radial distance will increase with age.

In the four years of phloem studied, there was a total of 17 (39%) anticlinal divisions. The distribution of the divisions was 5%, 9%, 14%, and 11% during 1961, 1962, 1963, and 1964, respectively. Following six (19%) of the pseudotransverse divisions, both daughter cells survived the four-year period. Five tiers (Figure 35, tiers

3, 5, 6, 8, 9) divided twice over the four years, of which tiers five, eight, and nine divided transversely for their second division. Generally, such transverse anticlinal divisions occurred at the tips of fusiform initials, with the resulting short initial becoming a ray initial. Only tier three divided twice in one year; the others varied their second pseudotransverse division over a period of two to three years.

Over the four years studied, no tiers were noted where immediately following the first pseudotransverse division in a tier, one of the daughter cells survived and the other declined. However, declining initials do occur following subsequent anticlinal divisions in the same sequence after one or two years. In three tiers (Figure 35, tiers 6, 8, 9) after the second division one daughter initial persisted and the other declined. All three divisions were transverse anticlinal divisions, where each initial divided approximately one-quarter of its length from the tip. The longer initial regained its normal status while the shorter initial divided by unequal periclinal divisions producing vertical albuminuous strands and eventually declined to form a ray initial. In tiers eight and nine each of the short initials was transformed into a ray initial, but in tier six the declining initial became segmented, dividing by unequal periclinal divisions, and some of the center cells were terminated, resulting in two separate ray initials.

Only one case was noted where a phloem mother cell divided pseudotransversely to form two sieve cells (Figure 35, tier 2). This initial later declined by unequal periclinal divisions to form a ray initial after two years, and the ray was terminated during the fourth year of growth.

Forty-three tiers were studied over a four-year period. There was a total of 14 (32%) pseudotransverse divisions and three (7%) transverse anticlinal divisions. At the end of the fourth year there was a loss of one tier, a decline of five initials to ray initials, and a net gain of 11 tiers for a total of 54 fusiform initials.

Infested Trees

Two samples from infested trees were analyzed by means of photomicrographs. One sample was from an infested tree that was collected May 2, 1965, and measured ten inches, D. B. H. The secondary phloem of 1964 and approximately half that of 1965 were studied. There were 56 initials in the first section of the 1964 phloem. The sections were cut at 20 μ, covering a radial distance of 220 μ.

There was a total of 19 (33%) anticlinal divisions during 1964 and six (10%) during the first part of 1965. The distribution of anticlinal divisions during 1964 by thirds was four (7%), six (10%), and nine (16%). No pseudotransverse divisions were noted in phloem

mother cells.

In 1964, only one tier (Figure 36, tier 27) had two successive pseudotransverse divisions. One of the daughter cells of the first division declined by unequal periclinal divisions, giving rise to a strand of four ray initials. The other daughter cell divided anticlinally and formed two new fusiform initials, both of which survived.

There were pseudotransverse divisions in seven (12%) tiers where both daughter cells persisted. Four tiers (7%) were noted to divide pseudotransversely where one of the daughter cells survived and one other declined (Figure 36, tiers 2, 15, 16, 22). In one tier the declining daughter cell divided by a transverse anticlinal division and the two new short initials were soon terminated (Figure 36, tier 15). In tiers 16 and 22 one daughter cell declined and the tier was terminated while the other daughter cell persisted. In tier two, the declining daughter cell produced vertical albuminous strands. Eventually, transverse segmentation of the declining initial was followed by a loss of some segments through maturation and by shortening of others through successive unequal periclinal divisions. This left three ray initials, of which two were terminated, leaving one ray initial that persisted to produce a ray.

Four tiers (7%) were noted where after a pseudotransverse division both daughter cells declined (Figure 36, tiers 3, 6, 12, 25). In tiers 3 and 25, both daughter cells declined and the tiers were

terminated. In tiers six and 12 one tier was terminated while in the other the initial declined by unequal periclinal divisions to produce a ray initial which joined together adjacent rays to form one high ray (Figures 7-10).

There were six tiers (10%) that declined without the occurrence of pseudotransverse divisions (Figure 36, tiers 1, 10, 11, 17, 19, 26). Tiers 1, 17, and 26 declined and were terminated, while the initials producing tiers 10, 11, and 19 declined through unequal periclinal divisions to become ray initials. Tier ten declined to form vertical albuminous strands. Later cells in the segmented initial were separated by unequal periclinal divisions and maturation of some of the initials, resulting in three separate short ray initial strands and one ray initial. The one ray initial was lost but the remaining short ray initial strands declined and three separate ray initials were produced. Tier 11 declined by unequal periclinal divisions to form one new ray, while tier 19 declined in the same manner, resulting in separation of the segmented initial to form two separate rays (Figures 11, 12).

Only one tier was noted where an unequal transverse anticlinal division resulted in the short initial declining by unequal periclinal division to become a new ray initial, while the larger daughter cell persisted (Figure 36, tier 8).

Since only part of the season's growth for 1965 was available,

only six (10%) pseudotransverse divisions were observed and both daughter cells persisted in each case. This is not significant, however, because only the early growth was available.

In 1964, of 56 initials, 19 (33%) divided anticlinally, ten new tiers were terminated, and ten new ray initials were produced. The number of initials remained the same, at 56.

The second infested tree analyzed was sampled April 17, 1965, and was 5-1/2 inches D. B. H. The serial tangential sections were cut at 20 μ, and the 1964 increment covered a distance of 180 μ. There were 54 initials in the study area at the start of the growing season.

There were 24 (44%) anticlinal divisions. The distribution of the divisions in 1964 by thirds was six (11%), 15 (28%), and three (5%), with five in the developing phloem of 1965. No pseudotransverse divisions were noted in the phloem mother cells.

Only one tier showed two successive pseudotransverse divisions in one series (Figure 37, tier 23). After the first pseudotransverse division, one of the daughter cells declined by unequal periclinal divisions to form vertical albuminous strands. Later, the initial became segmented and central cells were terminated by maturation. The upper and lower cells of the segmented initial were reduced by unequal periclinal divisions to two separate ray initials, which each gave rise to a ray. One of the rays was terminated and

the other persisted, adding to the height of an established ray. After the second anticlinal division of the other daughter cell, one of the resulting tiers was terminated while the other persisted.

Six tiers (11%) were noted where both daughter cells persisted (Figure 37, tiers 1, 7, 9, 11, 16, 19). Tiers one and 19 showed at the end of the year that one of the daughter cells had started to decline by unequal periclinal divisions and was producing short vertical albuminous strands.

Five tiers (9%) showed a decline of one daughter cell after the pseudotransverse division (Figure 37, tiers 4, 13, 18, 21, 25). In tiers 4, 13, 21, and 25, one daughter cell declined and the tier was terminated. Tier 18 had one daughter cell producing vertical albuminous strands. The initial became segmented and the central cells were lost by maturation. The remaining initials declined by unequal periclinal divisions to become ray initials. Then one of the rays was terminated and the other persisted.

Two tiers were noted where after a pseudotransverse division one of the daughter cells underwent a transverse anticlinal division (Figure 37, tiers 8, 12), with the resultant shorter initial declining and the longer cell regaining its normal length and persisting. In tier eight the shorter initial formed a ray by unequal periclinal divisions, while the short initial in tier 12 declined and was terminated.

Seven tiers (13%) were noted where both daughter cells declined after a pseudotransverse division (Figure 37, tiers 3, 5, 6, 10, 15, 20, 26). In tiers 3, 5, 10, 20, and 26 both daughter cells declined by unequal periclinal divisions and the tiers were terminated. In tier six, one daughter cell matured and the tier was terminated, while the other initial declined and was transformed by unequal periclinal division into a new ray initial. In tier 15, both daughter cells declined by unequal periclinal division forming new ray initials.

Three tiers declined without the occurrence of a pseudotransverse division (Figure 37, tiers 2, 14, 17). The initial of tier two was transformed into a new ray initial and the initial of tier 17 declined to form vertical albuminous strands. Later, the initial became segmented and there was a loss of some segments by maturation and unequal periclinal divisions, resulting in three separate ray initials. Only tier 14 was terminated completely.

For 54 initials during 1964, there were 24 (44%) anticlinal divisions, with 17 tiers terminated, nine new rays and the number of initials remained the same at 54.

One sample from an infested tree was collected April 25, 1968. The tree was moderately infested and measured seven inches, D. B. H. The 1966 and 1967 xylem both showed the presence of traumatic resin ducts, and it appeared that a new row of resin ducts was being

initiated in the new growth of 1968.

Thirty tiers selected at random from the 1967 phloem were analyzed. Sections were cut at 20μ and covered a radial distance of 280 μ. The last 80 μ included the cambial zone and the differentiating traumatic resin ducts of the 1968 xylem. There was a total of ten (33%) pseudotransverse divisions. The distribution of the divisions by thirds of the annual increment was two (7%), three (10%), and five (16%). No pseudotransverse divisions of phloem mother cells were noted. Seven tiers showed pseudotransverse divisions where both the daughter cells persisted. In two tiers, one daughter cell declined and the other survived (Figure 38, tiers 6, 7). The declining daughter cells produced vertical albuminous strands and were shortly terminated. Only tier five showed both daughter cells declining to produce vertical albuminous strands and finally disappearing by maturation at the end of the year. There was a net gain of six tiers for the year.

Aphid-Abandoned Trees

A single sample of bark from an area that had been abandoned by the aphids was analyzed by using photomicrographs. The sample was collected April 22, 1965, from a seven-inch tree, D. B. H. The serial tangential sections analyzed included two years' growth, 1963 and 1964. Forty-eight initials were present in the study area

in the first section of the spring growth of 1963. There were 14 anticlinal divisions during the two years, with ten (20%) occurring in 1963 and four (7%) in 1964. No pseudotransverse divisions were noted in phloem mother cells.

In 1963, eight initials (17%) divided pseudotransversely and both daughter cells survived (Figure 39, tiers 1, 2, 3, 9, 11, 12, 15, 19) although in two cases a daughter cell declined and was terminated by maturation in the latter part of the following year (Figure 39, tiers 9, 11). In the same year two divisions (4%) were noted where one of the daughter cells declined and the other survived (Figure 39, tiers 7, 22). Tier seven divided by a transverse anticlinal division near the tip of the initial to form a ray initial. After a pseudotransverse division in tier 22, one of the daughter cells declined by unequal periclinal divisions and was terminated abruptly by maturation. Two fusiform cambial initials (4%) divided by unequal periclinal divisions and were terminated by maturation during this year.

In 1964, there were four (7%) pseudotransverse divisions with three of them occurring during the last half of the growing season. Three initials divided pseudotransversely and both daughter cells survived. In tier 14 (Figure 39), after a pseudotransverse division one daughter initial declined by unequal periclinal divisions and the tier was terminated. Seven initials were noted to decline during the

year without pseudotransverse divisions. Tiers 8, 10, 16, 18, and 21 were terminated abruptly, but the fusiform initials of tiers 13 and 17 were transformed to ray initials before they disappeared by maturation.

Seedling Infestation Under Controlled Conditions

Approximately 30 trees from three to seven years of age were transplanted to #10 cans in the greenhouse. Ten of the trees were transplanted September 10, 1965, and the remaining trees March 24, 1966. April 29, 1966, 20 of the trees were moved to a room of the greenhouse, and aphid eggs were transferred to them from naturally infested trees in the forest. A thin layer of cotton was spread around the trunk of the tree to be infested and a small piece of bark carrying aphid eggs was then tied to the tree, with the egg-bearing side facing the cotton. The remaining ten trees were held in a separate room as controls.

Within three days most of the eggs had hatched. Most of the crawlers (first instar) remained localized under the cotton, although some were seen moving along the tree. Soon after hatching the aphids initiated production of wool and appeared to increase in size. By May 14, 1966, almost all trees were heavily infested.

On May 18, 1966, ten of the 20 infested trees were fumigated to kill the aphids. For this process, the trees were placed

individually into an airtight box with smoldering nicotine powder for five minutes. The fumigated trees were then placed in a separate room of the greenhouse. Samples were taken from non-infested, infested, and fumigated seedlings at approximately two-week intervals. Each sample consisted of a 3/4 to one inch long piece of the main stem taken at the level to which the eggs were transferred, or at a level corresponding to this in the non-infested seedlings.

Infested and Non-infested Seedlings

Cortex. The first samples were collected two weeks after eggs were placed on the trees. The degree of infestation was from moderate to heavy but there were no external changes in appearance of the stem as a result of the infestation. However, internal changes are occurring by this time.

The nuclei of the cortical cells in the feeding area appear to be the first structures affected, and the first response is enlargement. Two weeks after eggs were transferred, the nuclei range in size from 13.0 μ to 20.8 μ, with an average of 15.3 μ. In contrast, the nuclei of cortical cells in non-infested trees range in size from 7.8 μ to 13.0 μ, and average 10.2 μ. The cortical cells are also undergoing enlargement, thus initiating giant cell development. At this time they range from 52.0 μ to 109.0 μ, with an average diameter of 82.7 μ. The cortical cells of non-infested trees range from

21.0 μ to 91.0 μ, and average 62.0 μ in diameter.

After three weeks, the developing giant cells have enlarged more and approximately five weeks after infestation they have increased to an average of 102 μ in diameter. With the increase in over-all cell size at this time, the giant cells also develop prominent protrusions on the cell walls, giving the cells an irregular shape (Figure 23). The cell wall areas forming the protrusions are much thinner than non-protruded areas. By nine weeks after infestation the area infested by the aphids is greatly puffed up and distorted, and the final giant cell measurements ranged from 130 μ to 169 μ, for an average diameter of 143 μ. The expansion of this abnormal cortical tissue increases with density of the aphid population and the development of the insect through the first stages of its life cycle.

The nuclei also continued to hypertrophy to a 22 μ average diameter three weeks after transfer of the aphid eggs. There is a slight change up to the final measurement taken nine weeks after infestation when the nuclear diameters ranged from 26 μ to 42 μ, averaging 34 μ, in the now fully-developed giant cells.

In addition to hypertrophy of the cells and nuclei, there is also a gradual change in cell contents over the period studied. After three weeks of moderate to heavy infestation, the resins and tannins in the giant cells gradually dissolve and there is an increase in denser, more darkly-staining cytoplasm (Figure 21). In the feeding

area, some of the cortical cells appear to be dead or dying, as they are devoid of cell contents and the walls stain a dull red. These changes are probably due directly to the feeding aphids. Approximately five weeks after infestation most of the cells in the pockets of giant cells have dense cytoplasm although a few still have resins and tannins in various stages of dissolution. After seven weeks most of the cortical parenchyma cells surrounding the giant cell pockets are dead and devoid of contents. By nine weeks after infestation the cortex acquires an over-all dead appearance, the majority of cells empty with walls stained a dull red color. The giant cells, however, somehow appear to resist this change as the cytoplasm is dense and the nuclei still distinct.

Phellogen formation first appears approximately seven weeks after infestation around groups of giant cells. At this time most of these cork cambial cells also appear to be dead, apparently due to aphid feeding. Therefore, initiation of the phellogen must have occurred between five and seven weeks after infestation, before the demise of adjacent cortical cells.

Stylet Tracks. The aphid stylets are directed through the periderm and into the cortex but do not appear to extend into the functional phloem. The paths of these stylets are very irregular and often make sharp right-angle turns. The stylet tracks are mostly

intercellular but are also often seen taking an intracellular path. Where the stylets probe intercellularly the cell walls adjacent to the stylets do not appear to react by becoming thicker as reported by various workers (Crystal, 1926; Varty, 1956; Oechssler, 1962; Balch, Clark and Bonga, 1964); nor do the nuclei appear to be moving toward the cell walls adjacent to the stylets as reported by Balch (1952).

Ray Cells. The measurements of the rays and number of cells per ray seen in tangential section did not appear to differ greatly through the season, although there was a slight increase in the average number of cells per ray in the infested trees later in the season. The average number of cells per ray was five, with an average height of 114.6 μ per ray.

There appears to be one good indication of aphid feeding, and that is the production of resins in the ray cells. Although only a few rays with resins were noted in the sample collected on May 14, 1966, two weeks after infestation, most ray cells had accumulated resins after three weeks. All samples collected after this period showed ray cells containing resins (Figure 26).

Most infested trees contained only uniseriate rays, but one tree also contained some biseriate rays as early as three weeks after infestation.

Fumigated Seedlings

Cortex. Cortical reaction to 19 days of aphid infestation in the fumigated trees did not differ greatly from that of the infested trees discussed previously. Data to compare average cortical cell and nuclear measurements are given briefly below. Since different trees were involved at each time of sampling, seeming discrepancies in growth trends may be due to individual differences among the trees. Generally, however, it appears that the trees released from aphid feeding pressure by fumigation experienced a rapid period of cell enlargement, followed by a decline. In comparison to this reaction in the fumigated trees, those with continuous aphid feeding appear to have undergone a more gradual enlargement, and the giant cells may have continued to expand beyond the nine weeks studied.

Infested Trees			Fumigated Trees		
Time Elapsed	Ave. Cell Diameter	Ave. Nuclear Diameter	Time Elapsed	Ave. Cell Diameter	Ave. Nuclear Diameter
3 weeks	102 μ	22 μ	4 weeks	119 μ	26 μ
5 weeks	87 μ	23 μ	5-1/2 weeks	146 μ	18 μ
7 weeks	97 μ	20 μ	7-1/2 weeks	132 μ	23 μ
9 weeks	143 μ	34 μ	---	---	---

Traumatic Resin Ducts. Traumatic resin duct formation was initiated after fumigation. No ducts were initiated in any samples studied for non-infested, infested, and the early samples of fumigated trees. These structures appeared only in those samples of fumigated trees collected in the latter part of the study (Figure 26).

DISCUSSION

The balsam woolly aphid feeds by inserting its stylets into the cortex. The stylets are usually inserted intercellularly, following the borders of the cortical parenchyma cells, but often probe intracellularly as well. Plumb (1953), in his studies of the Norway Spruce Gall, also observed the passage of the stylets to be both inter- and intracellular. Varty (1956) described stylet penetration as usually being between the walls of neighboring cells, although in one section the stylets were found to pursue an apparently intracellular path. Other researchers have reported only intercellular probing and feeding of the balsam woolly aphid on true fir (Abies) trees (Balch, 1952; Balch, et al., 1964; Crystal, 1926; Kloft, 1957).

Although I did not do extensive stylet penetration studies, I noted a twisted appearance of the inserted aphid stylets (Figure 22). This twisted appearance could possibly indicate a screwing-in action by the aphid. Balch (1952) reported seeing a partial rotation of the aphid body during stylet insertion. Oechssler (1962) described the method by which the aphid pushes the upper and then the lower part of the stylets alternately, thereby gaining entrance into the feeding area. The stylets are not inserted in a straight line, but often follow an irregular pathway, at times even bending in a backwards direction.

After the aphid stylets have been inserted, the cells adjacent to the stylets react to form giant cells. These cells respond to the salivary secretions and react within a period of two weeks. The cells increase in all dimensions with small bulges occurring in the cell wall (Figure 23). I did not observe any ridges on the inner surfaces of the giant cells as reported by Balch (1952). Also it was reported by Varty (1956), Crystal (1926), Balch, et al. (1964), and Oechssler (1962) that the cell walls adjacent to the stylets thicken; however, I did not observe this in the trees studied. Neither were the nuclei found close to the cell wall adjacent to the probing stylets as described by Balch (1952). Although Balch (1952) noted the sequential reaction of the cortical parenchyma as enlargement of cells, followed by enlargement of the nuclei, I noted the nuclei enlarging at a greater rate than the cells. Crystal (1926) and Balch (1952) report that giant cells enlarge greatly and have a dense, deeply-staining cytoplasm, but Oechssler (1962), studying the effects of Drefusia nusslini and D. piceae on grand fir, noted little change in cell size and the cell content differed only slightly from the surrounding cells. Crystal (1926) infested grand fir trees with D. nusslini, and found that the cortical cells were markedly affected, becoming hypertrophied, but did not always exhibit dense cytoplasm or enlarged nuclei.

I observed as did Balch, et al. (1964), that with increased feeding time, the cortex initiates periderm formation under the giant

cell pockets. Also the cells in the feeding area became empty with the remains lining the cell walls, thus giving the cortex a dead appearance. Although the surrounding cells died, the last cells to break down are the giant cells. This longevity could be attributed to the accumulations of food material and increased abilities for metabolic activity derived from a very large size, dense cytoplasm, and hypertrophied nucleus.

Cambium

The cambium, as well as the cortex, is affected by aphid infestation. The radial width of the dormant cambial zone of infested trees (Figure 1) is much greater than in normal trees (Figure 3). The cambium of aphid-abandoned trees resembles that of non-infested trees. The cambial zone of dormant infested trees numbers eight to ten cells in a radial file, compared to an average of four cells in the dormant cambial zones of normal and abandoned trees.

Reactivation and cessation of cambial activity of infested and non-infested grand fir trees are initiated at approximately the same time near Corvallis, Oregon. Although there are many variations on reactivation of the cambial zone, this is often a function of the location at which the tree is growing. Most trees begin cambial activity around the first of April and are fully active by mid- and late April. This is in agreement with Grillos and Smith (1959) in their

study of Douglas fir in the same area near Corvallis. During the latter part of June the rate of division declines and differentiation reduces the number of cells in the cambial zone. Differentiation varies in suppressed and robust trees. In the former differentiation appears to occur soon after a few xylem mother cells are produced while in the latter, with a higher population of cambial zone cells differentiation will continue through July and August. Most differentiation is completed by September, but some divisions were noted occurring in the phloem parenchyma strands into October.

The time interval from aphid infestation to cambial reaction can be learned from the controlled greenhouse experiments. Aphid eggs were placed on the study trees and the collection of samples started two weeks later. The exact date of aphid infestation must be approximated to within three days, for the eggs appeared to hatch at different times. This of course could be due to differences in the maturity of the eggs and conditions at the various collection areas.

Concurrent with the cortical reaction to aphid feeding, the secondary phloem reacted noticeably to aphid presence. One of the earliest effects is the accumulation of resins in the ray cells (Figure 26). Later, approximately three weeks after aphid infestation, some biseriate rays were produced and the phloem ray cells continued to accumulate resins until the end of the growing season.

The cambial reaction to aphid abandonment was studied by

fumigating aphid populations on potted trees, since in nature aphids leave a feeding area only after they have exhausted the food supply. In the study of aphid-abandoned (fumigated) trees the cambium appears to remain under the initial influence of the aphid infestation. All rays studied showed that most if not all of their cells contained resins.

In the photographed study area measuring 0.8 mm by one mm, there were 43 and 54 initials for non-infested, 56 and 54 for the infested, and 48 and 48 for the abandoned samples. The 54 initials counted in one of the non-infested samples is unusual. One explanation could be that this tree was younger than the other trees, although I made a special effort to control this possibility by trying to select similar age trees, as indicated by diameter. Smith (1967) controlled this by using the same tree before and after infestation. This is an effective method to use with wood, but is difficult to apply to the phloem. In the phloem there is too much crushing and distortion resulting from the increasing expansion of phloem parenchyma cells, ray parenchyma cells, differentiating sclereids, and resin cells. Also the older bark is sloughed off with the formation of the periderm.

The average percentages of pseudotransverse divisions in non-infested, infested, and abandoned samples were 11, 38, and 14, respectively. Smith (1967) reported 15% pseudotransverse divisions in his sample before infestation. This percentage is lower than other authors, i.e., Srivastava (1963), who studied whole individual

initials, while Smith (1967) and I counted initials cut off by the limits of the photographs as whole initials. We were thus unable to follow divisions that took place in the unobserved parts of the initials outside the photographed area. This higher percentage is noted in the study of normal cambial activity by following whole initials. The percentage of pseudotransverse divisions noted in the study of whole initials was 17% as compared to 8% for the photographed study.

Infested trees showed an average of 38% anticlinal divisions, which is lower than the 58% reported by Smith (1967). Although Smith (1967) did not note any pseudotransverse divisions in the xylem mother cells, he did report strictly transverse divisions occurring in 12% of the tiers. These usually resulted in two or three square-ended tracheids in each tier. Bannan (1964) reported 6% of the xylem mother cells dividing pseudotransversely in Pseudotsuga menziesii. I found only one such division in a phloem mother cell of the non-infested tree, and none was noted in the infested or abandoned trees.

The percentages of all anticlinal divisions were determined for non-infested, infested, and aphid-abandoned trees with regard to 1) tiers with anticlinal divisions where both daughter cells survived, 2) tiers with anticlinal divisions where one daughter cell persisted and the other declined, 3) tiers with anticlinal divisions where both daughter cells declined, 4) tiers without anticlinal divisions which declined by unequal periclinal divisions.

Infested trees had the highest percentage where both daughter cells survived after an anticlinal division (15.3%), with the aphid-abandoned tree next (11.5%), and the non-infested trees last (7.6%). The percentages for the survival of only one daughter cell after a division are 7.6% for infested, 2.6% for non-infested and 2.5% for aphid-abandoned trees. In all cases, the percentage of both daughter cells surviving after an anticlinal division was much higher than that of one daughter surviving and the other declining. The percentage of tiers where both daughter cells declined after a pseudotransverse division fell drastically in non-infested trees (0.16%), none were noted in the aphid-abandoned tree, but in infested trees the percentage remained high (7.6%).

Declining tiers following unequal periclinal divisions were unusually abundant (14%) in the aphid-abandoned tree, with 5.3% in the infested, and none in the non-infested trees.

Declining tiers after anticlinal divisions for the three non-infested trees numbered five, two, and zero. The former produced five rays (100%) from the declining tiers (one declining tier formed two separate rays, and one declining tier was terminated). The second and third trees did not produce any rays in this manner. The declining tiers for the three infested trees numbered 20, 26, and 4. The declining tiers in the first tree produced nine rays (45%), the second nine rays (34%) with one declining tier forming three separate

rays, and the third formed no rays. The aphid-abandoned tree had four declining tiers for the first year, with one ray formed (25%) and ten declining tiers the second year, with no rays formed.

As noted from the discussion above, there is much variation in the three trees sampled in the non-infested, infested, and aphid-abandoned trees. The percentage of rays initiated from declining tiers in one tree of the non-infested as compared to another varies from 100% to none and with the three infested trees studied 45%, 34%, and 0%. In the aphid-abandoned trees, during the first year 25% produced rays and the second year there were none. This variation appears to differ from tree to tree in infested and non-infested material and in different years in the aphid-abandoned trees. Although Smith (1967) did not find any rays formed from declining tiers, this can possibly be explained by his limited sampling in the wood of grand fir. Another explanation might be that the sequence of decline was much shorter in the xylem than in the phloem (Srivastava, 1963). Srivastava noted that once an initial starts to decline it produces derivatives mainly on the phloem side. Chrysler (1913), Barghoorn (1940), and Grillos and Smith (1959) noted a similar one-sided production of vascular derivatives in connection with what they describe as radial plates of cells (Figures 13, 14). This one-sided production of derivatives may explain the lack of rays formed from declining initials in the xylem sampled by Smith (1967).

Most of the pseudotransverse divisions in the non-infested trees during the growing season were limited to the latter half or third of the yearly growth increment. This is similar to the findings reported by Bannan (1950, 1964) and others. This situation was also found for the multiplicative anticlinal divisions noted in the aphid-abandoned trees. In contrast, the infested trees show a more general spread throughout the growing season. To demonstrate this, I divided the year's increment into thirds. The distribution of anticlinal divisions in the first infested tree sample was four, six, nine; and in the second sample 6, 15, 3. This is similar to the distribution of anticlinal divisions as reported by Smith (1967).

Traumatic Resin Ducts

Traumatic resin ducts in association with aphid infestation were described by Balch (1952). Doerksen (1964) and Doerksen and Mitchell (1965) also noted their presence in infested grand fir trees. Traumatic resin ducts were observed in many samples of infested trees, especially in trees that were heavily infested. The resin ducts are longitudinally oriented and usually produced in tangential bands. The tangential band is usually limited to a certain perimeter of the tree (Figure 6), possibly an area of heavy aphid concentration.

In the aphid-abandoned (fumigated) material the last two trees collected exhibited the production of traumatic resin ducts in the

differentiating xylem. Since traumatic resin ducts may be produced in response to a variety of kinds of shocks to the tree, this could be a reaction of the tree to a high density of feeding aphids. Many of the infested trees collected bearing heavy aphid populations were noted to produce traumatic resin ducts annually, although the formation of the ducts is not circular but is limited to certain areas of the tree. This limited area of resin duct formation on a tree could indicate a site of high density of feeding aphids.

Sieve Cells

The sieve cells of infested trees were 45% as long as those of non-infested trees. Doerksen (1964) and Doerksen and Mitchell (1965) reported that aphid-affected tracheids are some 60% as long as normal tracheids in three species of Abies. Smith (1967) related this decrease in length to increase in pseudotransverse divisions. Accompanying this shortened tracheid length is a decrease in tangential diameter, which results in more tiers per unit area.

The latter was exhibited by the photomicrographs taken in the study of the cambial activity by analysis of the secondary phloem, but was not the case in the measurements of sieve cells taken at random. A possible explanation could be that most infested trees studied were often older and larger in diameter; therefore, the initials would be generally larger in proportion. Most of the

non-infested trees were usually three to six inches D. B. H., since these were trees with a smoother surface which did not attract aphids. The sieve cells of aphid-abandoned trees appeared to be longer than those of infested trees but shorter than those in non-infested trees. Some of the aphid-abandoned trees still had aphids present up around 15 feet above the ground. It is conceivable that some influence of the feeding aphids was transported to the area studied.

No significant difference was noted in the thickness of the sieve cell walls of infested and non-infested trees, although there was a seasonal change in thickness. The cell wall increased in thickness from spring to summer, when the walls were the thickest.

Generally there are more sieve cells produced in a radial file per year in infested trees than in non-infested trees, although this can differ according to aphid density, tree vigor, and microenvironmental differences at different tree locations (Balch, 1952).

Phloem Parenchyma Strands

The cambium of non-infested trees produces in a radial file approximately five phloem cells per year, including one phloem parenchyma strand and four sieve cells. The phloem parenchyma strands are derived from phloem mother cells (Figure 33) which are produced after two or three sieve cells have been derived. Grillos and Smith (1959) found that in slow-growing Douglas fir trees one

tangential layer of phloem parenchyma cells was produced at the very last of the season's growth, but in rapidly-growing trees two bands were usually produced, one early and the other late in the season. Almost immediately after derivation, and before the parenchyma cells have started to expand radially, a thin layer of resin is accumulated. With time and radial expansion, the density and volume of resin increases. Soon after radial expansion, cell division takes place. This begins at the center of the cell, and continued divisions divide the resulting cells, until a fairly uniform strand of parenchyma cells is produced. These cell divisions continue into late October.

In the non-infested trees only one tangential band of phloem parenchyma strands is usually produced per year, but in more vigorous trees two and sometimes three are produced. Generally, infested trees produce two or three layers of phloem parenchyma strands per year (Figures 5, 31). Possibly one could attribute the increased production of tangential layers of phloem parenchyma strands to the vigor of the trees. This increased production is seen in the normal robust trees, and in most aphid-infested trees, since the latter can also be considered robust in growth due to the aphid stimulation.

Sclereids

Astrosclereids

Astrosclereids develop from phloem parenchyma cells. Generally their initiation can be found in any tangential band of phloem parenchyma strands in the older phloem. The earliest appearance of astrosclereids is in the three-year-old phloem, as Grillos and Smith (1959) reported for Pseudotsuga menziesii. However, the sclereids in Douglas fir phloem resemble fiber sclereids more than they do astrosclereids.

In the younger non-conducting phloem, some sclereids can develop from individual cells within the phloem parenchyma strand. However, it usually appears that when astrosclereid formation occurs it involves entire strands of a particular tangential layer of phloem parenchyma strands (Figure 27). This is in accordance with Srivastava (1963), who noted this type of clump sclereid formation in Abies concolor, A. magnifica, Cedrus deodera, and Picea pungens. Sclereids are often initiated adjacent to already matured sclereid clusters (Figure 28), thus increasing the density of their concentration (Sterling, 1946).

Astrosclereid development normally takes place later than the activation of the cambium, i.e., middle of May. This is in agreement with Grillos and Smith's (1959) discussion of Douglas fir

sclereid development. The differentiating sclereids mature fairly uniformly through the growing season and most sclereids have completed their development by autumn.

Fiber Sclereids

Fiber sclereids are noted in the more vigorous and fast-growing non-infested grand fir trees. These sclereids differentiate from phloem mother cells during the same time of the year the phloem parenchyma strands are produced. Differentiation of the fiber sclereids is rapid and they are fully matured just a few cells away from the cambium.

Fiber sclereids are prevalent in the infested tree samples, and some are found in the robust non-infested trees. Therefore, the production of fiber sclereids appears to be a function of vigor, which is imparted to most aphid-infested trees by the stimulus of the feeding aphid. The fiber sclereids expand in radial diameter but do not expand tangentially.

Resin Cells

Little work has been done on resin cells. Srivastava (1963) and Holdheide (1951) briefly describe resin "cavities" in the bark of Abies and Chang (1954) used the term resin "spaces." Since these structures do develop from a cell, they will be referred to here as

resin "cells."

There appears to be some difference in the origin of resin cells. Srivastava (1963) reported that resin cavities appear to develop from a phloem parenchyma strand, whereas Chang (1954) described resin spaces as forming from marginal ray cells. Possibly Chang was referring to albuminous cells, or cells at the margin of the rays, as his usage is not clearly defined. I noted resin cells in the intermediate as well as the older bark as did Srivastava (1963).

Resin cells are found in non-infested, infested, and abandoned trees but are not found in all samples of any one of the categories. Also, the development of resin cells does not appear to be correlated with the seasonal cycle of growth.

I described the initiation and development of the resin cells (Figures 15-18) but did not notice any breakdown or degeneration. Chang (1954) describes the breakdown of a resin space, resulting in the formation of a large resin passage. He noted that, unlike resin canals, this passage is formed without border cells. I did not find any such resin passages.

Ray Cells

The ray parenchyma cells of infested bark samples differ from those of the non-infested bark by having dumbbell-shaped nuclei which apparently undergo amitosis, resulting in a binucleate cell (Figure

24). They also accumulate large amounts of resins or tannins (Figures 25, 26).

Binucleate ray cells were not observed in the non-infested trees. Balch, Clark and Bonga (1964) found more than one nucleus in cortical parenchyma cells of Abies balsamea trees infested with the balsam woolly aphid. They found binucleate cortical parenchyma cells adjacent to the stylets of the probing aphid, but I did not find this in my study. Dumbbell-shaped nuclei were also noted in the older phloem of the aphid-abandoned trees, but only a few binucleate cells were observed. None of these nuclear abnormalities were present in greenhouse infested trees. This seems to indicate that the abnormal nuclear condition results only from prolonged aphid influence.

No resins accumulated in the ray cells of the conducting and non-conducting phloem of the non-infested trees, whereas in the ray cells of infested trees, resins accumulated almost immediately after derivation of the ray cells from the cambium (Figure 26). In a single ray, the number of cells that accumulate resin varies from 10% to 95%. Ray cells in the bark of aphid-abandoned trees were noted to have some accumulation of resins in older phloem but not in phloem of the most recent increments. The presence of resins in the older bark may be an indication of an earlier aphid history.

Biseriate rays are found in the phloem of infested trees

(Figure 20), and none are noted in the phloem of non-infested trees. This is in agreement with Doerksen (1964) and Doerksen and Mitchell (1965), although no triseriate rays were noted in this study as reported by Doerksen and Mitchell (1965).

The infested trees had an average of 12% biseriate rays in the phloem. Mitchell (1967) found that up to 22% of the rays in aphid-affected wood are biseriate. Smith (1967) reported that the biseriate rays result from vertical anticlinal divisions in the ray initials and not from declining initials in contact with the sides of rays. Biseriate rays were not produced in the non-infested trees, but were present in the aphid-infested trees after approximately three weeks of infestation.

SUMMARY

The balsam woolly aphid feeds by inserting its stylets intra- and intercellularly into the cortex. The cortical parenchyma cells adjacent to the stylets react to the aphid feeding with an increase in dense cytoplasm and hypertrophy of the cell and nucleus. Later, with increased feeding time, the cortex initiates periderm formation under the giant cell pockets. Also the cells in the feeding area become empty with the remains lining the cell wall.

The cambium reacts soon after aphid infestation. The first visible reaction is noted in the stimulated production of aphid-affected tracheids and some biseriate rays. Later, most of the phloem ray cells accumulate resins and other materials. The reactivation and cessation of cambial activity for all categories of trees does not appear to differ in timing. Although variation does occur among trees in the same category and in different categories, this is apparently determined primarily by geographical location of the trees.

Over a longer period of infestation, the cambium reacts by maintaining a wider radial file of cells in the cambial zone during the dormant period, and producing more cells during the growing season than the non-infested and aphid-abandoned trees. The latter is accomplished by an increase in both periclinal and pseudotransverse divisions, with the pseudotransverse divisions spread almost

equally through the year's growth. In the non-infested and aphid-abandoned trees fewer cells are produced per year with the majority of pseudotransverse divisions limited to the latter half of the growing season.

Rays are initiated by anticlinal divisions of fusiform initials and from declining fusiform initials. Infested trees have more declining tiers than non-infested or aphid-abandoned trees. These declining tiers are formed with or without benefit of anticlinal division resulting in ray initials or termination by maturation. Generally the first indication of a declining tier is the production of vertical albuminuous strands. The initial declines to form a ray initial, ray initial strand, or two or more separate ray initials. In the latter case rays are formed by unequal periclinal divisions and maturation of cells in the initial strand to form two or more ray initials. New ray initials are also added to margins of pre-existing rays or join adjacent rays to establish one high ray. After prolonged presence of the aphids, phloem ray cells develop nuclear abnormalities. Most of the cells develop dumbbell-shaped nuclei and many become binucleate.

The cambium of infested trees produces more tangential bands of phloem parenchyma strands and also more fiber sclereids. Traumatic resin ducts are produced in most heavily-infested trees. The majority of the resin ducts are initiated in the spring, although

they may be initiated at any time during the growing season. Apparently the influence of continuous feeding by a high population of aphids initiates traumatic resin duct formation.

The sieve cells of trees in the three categories appear to differ in dimension. The length of sieve cells of non-infested trees is greatest, with aphid-abandoned trees intermediate and infested trees the shortest. The tangential width for all three does not differ greatly, whereas in radial width the sieve cells of infested trees are largest, followed by aphid-abandoned trees and then the non-infested trees.

Astrosclereids and resin cells are found in infested, non-infested, and aphid-abandoned trees. Astrosclereids are present in all trees studied, but the resin cells are present only in certain trees of each category. Therefore one cannot attribute formation of these structures to the presence or absence of feeding aphids.

Figures 1-6. Cross sections through the cambial zone and adjacent vascular tissues of infested and non-infested trees. (X = xylem, CZ = cambial zone, RC = resin cell)

Figure 1. Infested tree. The dormant cambial zone and radially expanded immature sieve cells (arrow). Sample collected from 10-1/2 inch (D. B. H.) tree February 15, 1966. X 450.

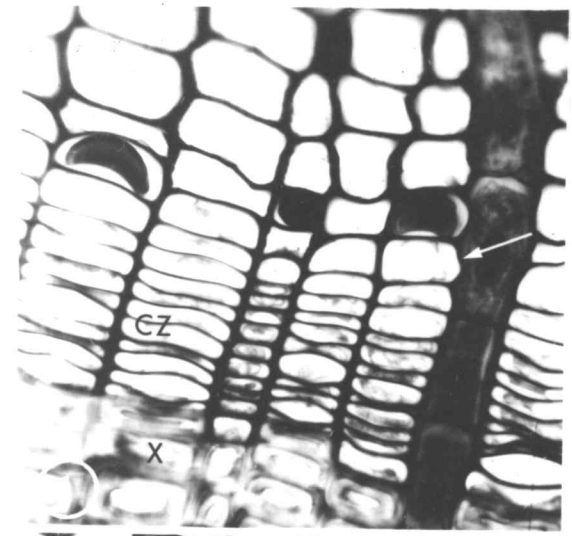
Figure 2. Infested tree. Same section as Figure 1, showing a year's phloem increment with approximately three tangential bands of resin-filled phloem parenchyma strands. A phloem fiber (arrow) is intermingled with these strands. X 100.

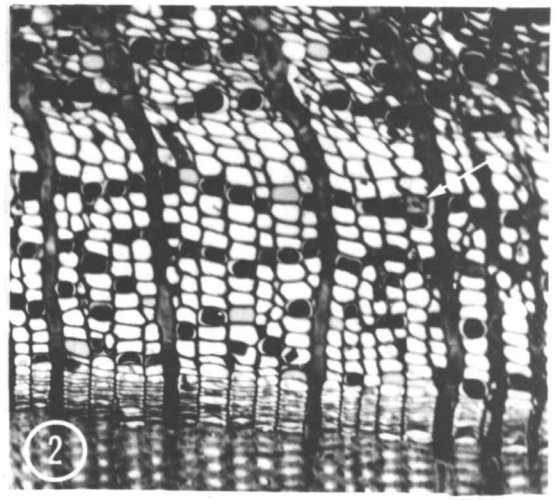
Figure 3. Non-infested tree. The dormant cambial zone, and radially expanded immature sieve cells (arrow), including one year's phloem increment with one tangential layer of phloem parenchyma strands (pps). Sample collected from one inch (D. B. H.) tree January 16, 1965. X 450.

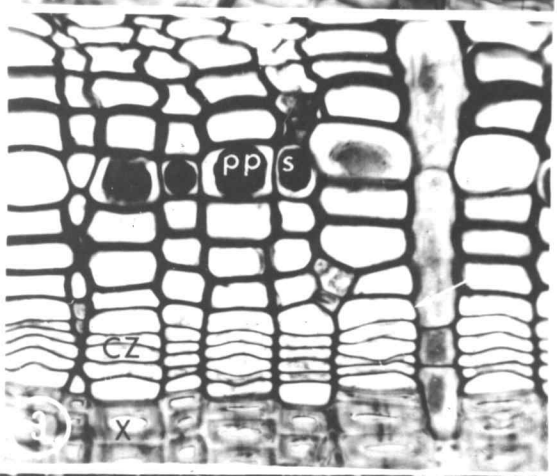
Figure 4. Non-infested tree. Part of the 1965 xylem, dormant cambial zone and several years of phloem increment, each with one tangential band of resin-filled phloem parenchyma strands. There are also resin cells in the non-conducting phloem. Sample collected 3-1/2 inch (D. B. H.) tree February 15, 1966. X 100.

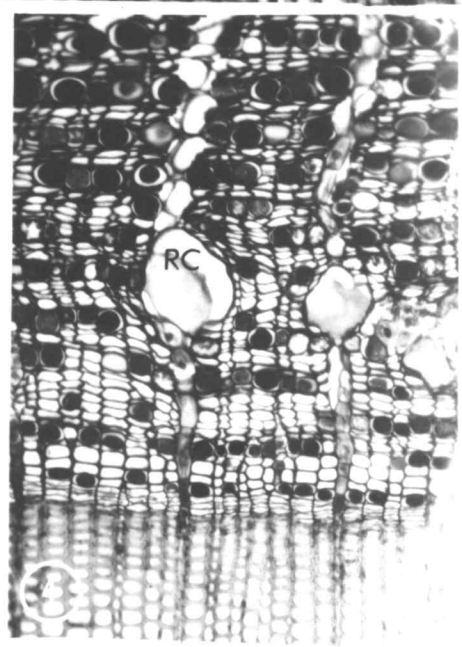
Figure 5. Infested tree. 1964 phloem and initiated 1965 phloem (bracket), actively dividing cambial zone and differentiating xylem. Note the formation of a traumatic resin duct (arrow) in the differentiating xylem. Sample collected from 10 inch (D. B. H.) tree May 2, 1965. X 100.

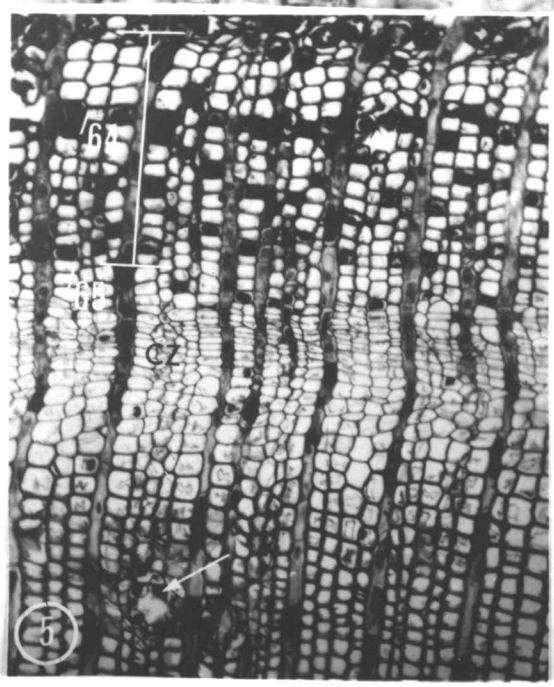
Figure 6. Infested tree. Secondary phloem with discontinuous bands of resin-filled phloem parenchyma strands, cambial zone, and differentiated tangential band of traumatic resin ducts (arrows). Sample collected from 10 inch (D. B. H.) tree April 23, 1965. X 100.

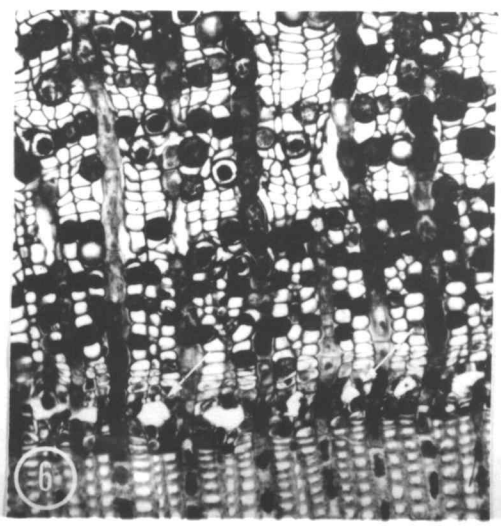

CZ
X




PP
S
CZ
X


RC


CZ



Figures 7-10. Infested tree. Decline of a fusiform initial recorded from 20 μ serial tangential sections from the conducting phloem to the middle of the cambial zone. X 100.

Figure 7. Declining tier, approximately 60 μ from the cambial zone, showing vertical albuminous strand (arrow).

Figure 8. Declining tier approximately 40 μ from the cambial zone (arrow).

Figure 9. Declining tier approximately 20 μ from the cambial zone. The vertical albuminous cells join together adjacent rays to form one long ray (arrow).

Figure 10. Adjacent to the cambial zone. Note long ray (arrow) resulting from the declining tier.

Figures 11-12. Non-infested tree. Decline of a fusiform initial recorded in 20 μ serial tangential sections from the conducting phloem to the cambial zone. X 100.

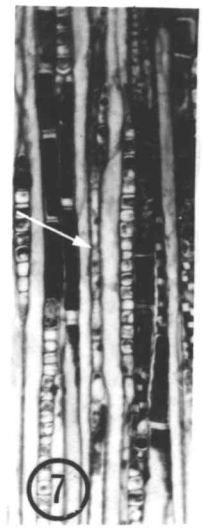
Figure 11. Sections at 60, 40, and 20 μ from the cambial zone show vertical albuminous strands (arrow). This section is 20 μ from the cambial zone.

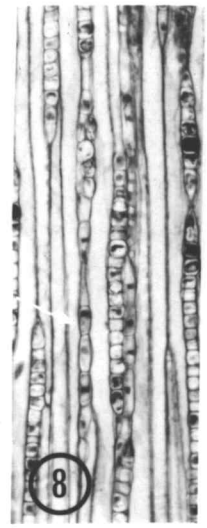
Figure 12. Cambial zone adjacent to the xylem shows separation of a segmented initial into two separate groups of ray initials. (Arrow indicates loss of segment by maturation.)

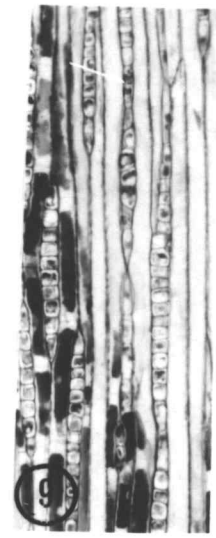
Figures 13-14. Radial plates of cells from non-infested trees. X 450.

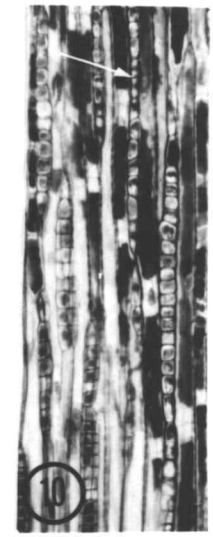
Figure 13. Declining tiers of vertical albuminous cells (ac) extending from the phloem through the cambial zone to the xylem (X). A crystal cell (cc) is located in the upper portion of the radial plate.

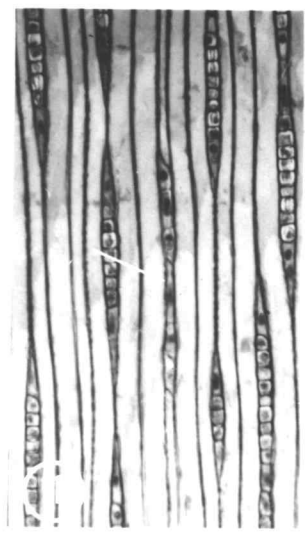
Figure 14. Declining tier with numerous vertical albuminous cells resulting in a ray initial (arrow) adjacent to the xylem (X).

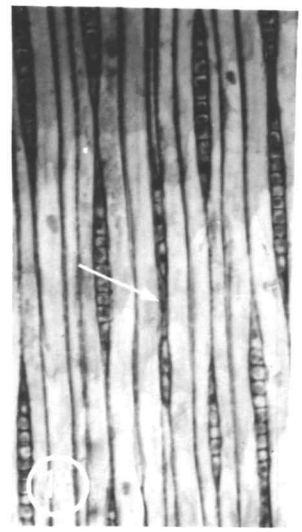

7

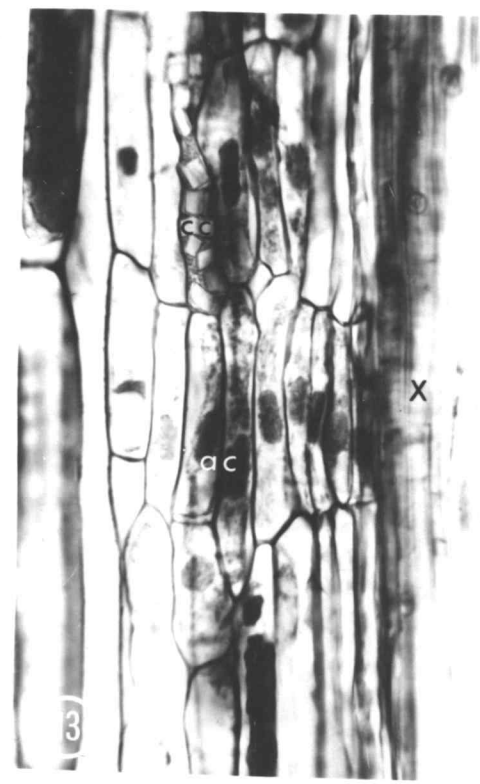

8


9


10






ca
ac
X
3


X
14

Figures 15-18. Cross, radial, and tangential sections of the secondary phloem of a non-infested tree. (RC = resin cell, CZ = cambial zone)

Figure 15. Radial section showing the initiation of a resin cell in the middle of a ray. X 450.

Figure 16. Tangential section of the non-conducting phloem showing various stages of resin cell development. X 100.

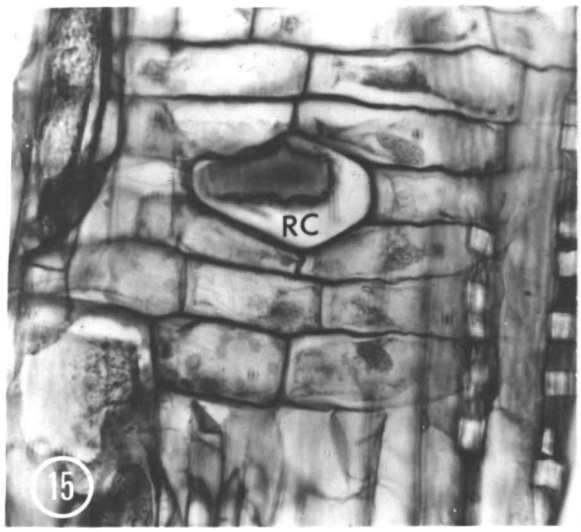
Figure 17. Tangential section of the non-conducting phloem showing initiation of a resin cell (arrow). X 450.

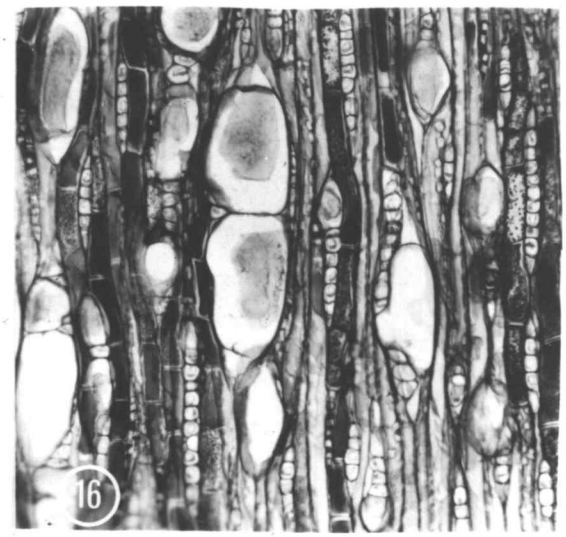
Figure 18. Cross section showing resin cells developing in the non-conducting phloem. Cambial zone (CZ) is at right. X 100.

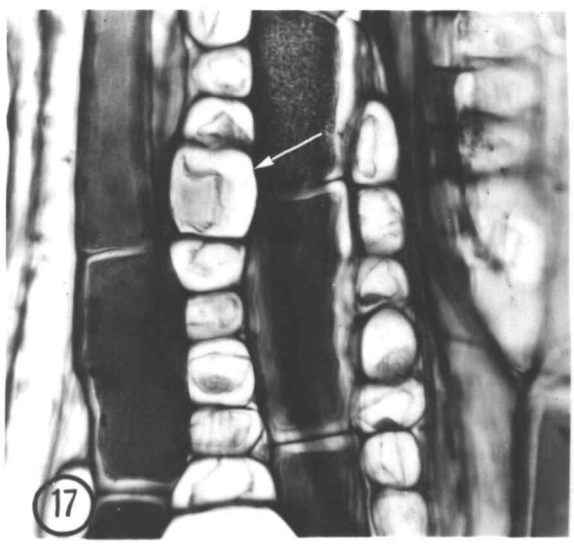
Figures 19-20. Tangential sections of the secondary phloem of infested and non-infested trees. X 100.

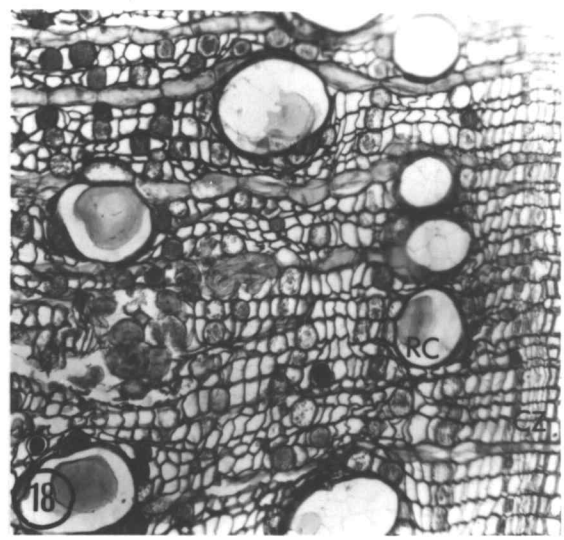
Figure 19. Non-infested tree, with tangential bands of resin-filled phloem parenchyma strands. Note longitudinal anticlinal division of phloem parenchyma cell (arrow).

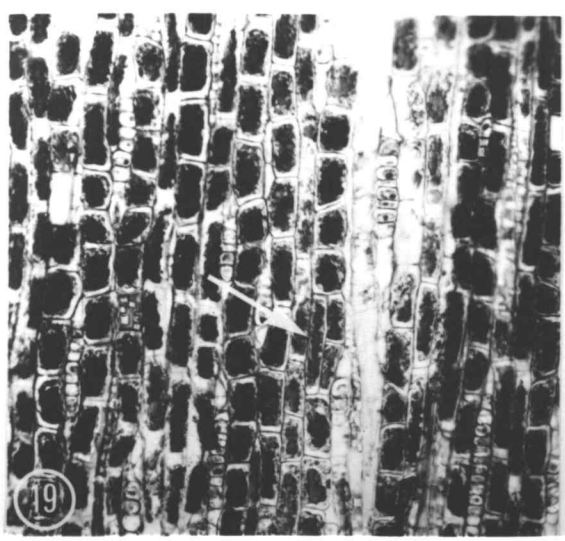
Figure 20. Infested tree with biseriate ray in the conducting phloem (arrow).


RC
15


16


17


RC
18


19


20

Figures 21-26. Tissue reactions to aphid infestation.

Figure 21. Tangential section of cortex showing giant cells with hypertrophied nuclei and densely-staining cytoplasm. X 100.

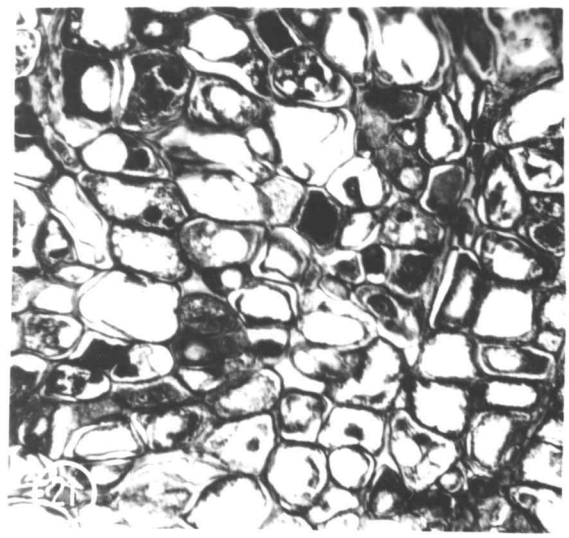
Figure 22. Cross section through a hypertrophied cortical cell showing intracellular path of aphid stylets. X 970.

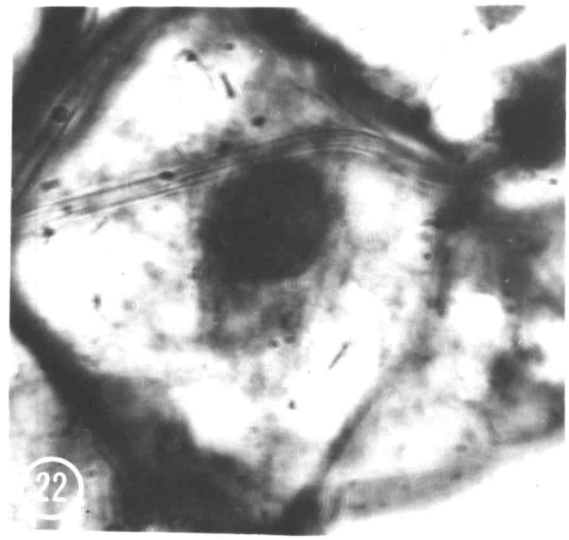
Figure 23. Cross section of cortex showing protrusion of giant cell wall. X 970.

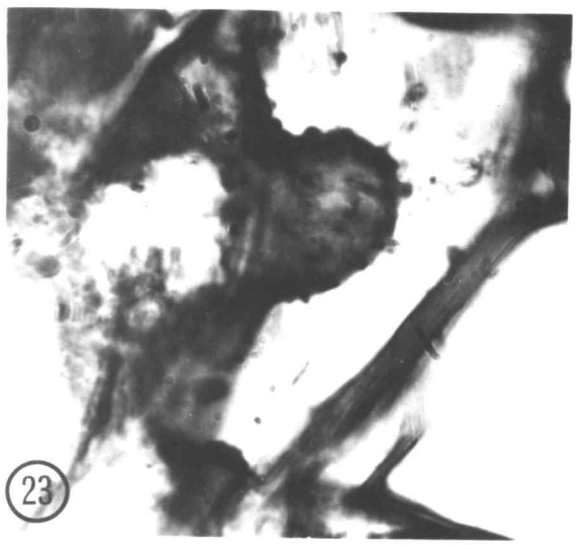
Figure 24. Radial section of non-conducting phloem showing a ray with resin-filled cells and a binucleate cell. X 970.

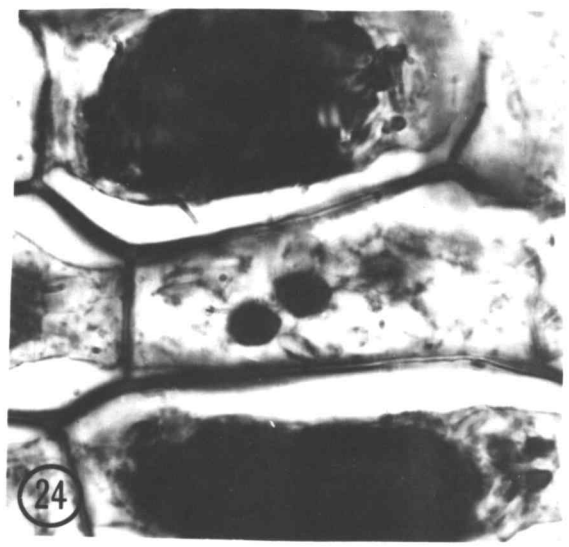
Figure 25. Radial section showing resin-filled ray cells, and ray cells with double and dumbbell-shaped nuclei. X 450.

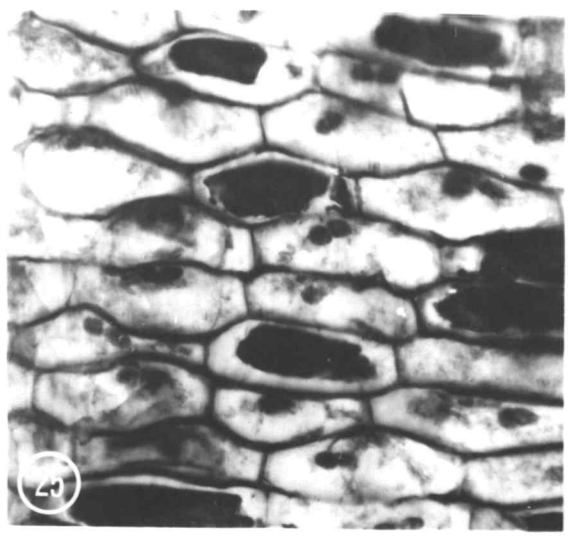
Figure 26. Radial section of the cambial zone, conducting phloem with resin-filled ray cells, and a traumatic resin duct (RD) in the differentiating xylem (X). X 100.

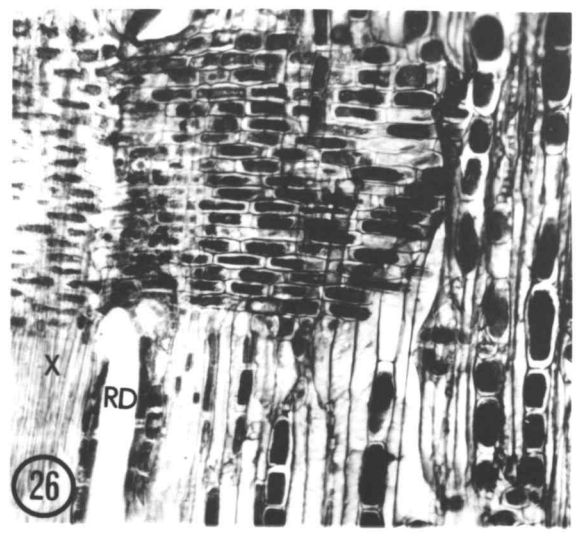



22


23


24




X
RD
26

Figures 27-30. Astrosclereid development in the non-conducting phloem of non-infested trees.

Figure 27. Tangential view of differentiating sclereid group from a tangential band of phloem parenchyma strands. X 100.

Figure 28. Cross section showing initiation of a new astrosclereid group (arrow) adjacent to an already established astrosclereid group (S). X 100.

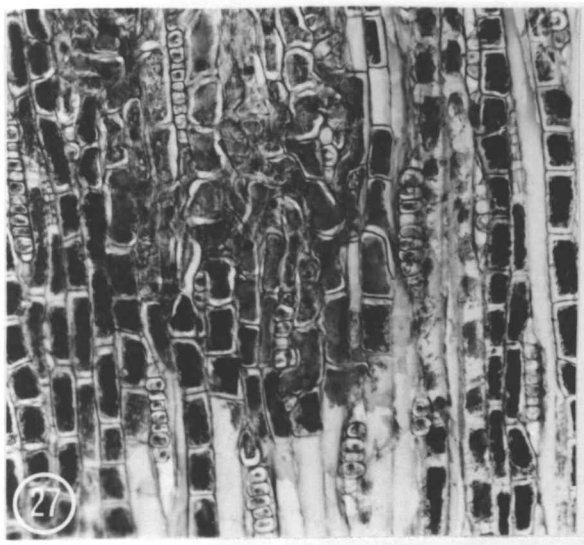
Figure 29. Cross section showing an almost fully-mature astrosclereid group. Note thickened walls and presence of nuclei. X 100.

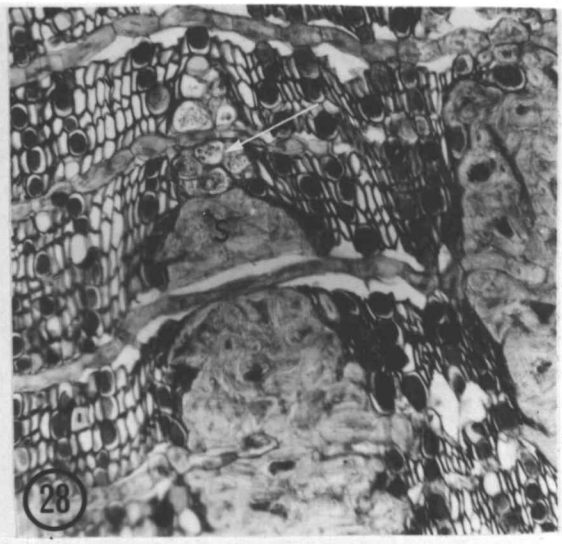
Figure 30. Astrosclereid group from macerated bark. Note radiating and intertwining spicules of the sclereids. X 100.

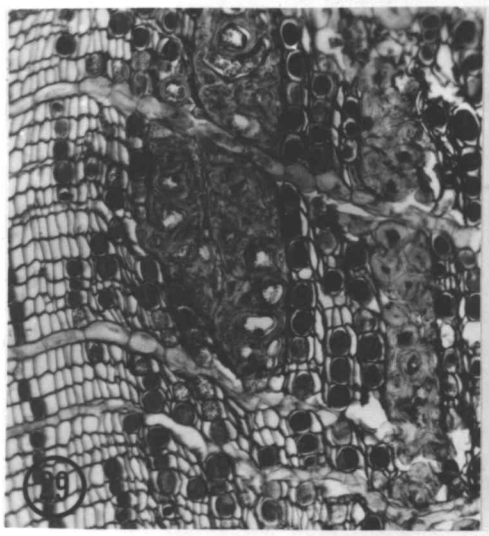
Figure 31. Infested tree. 1964 phloem and initiated 1965 phloem (brackets), actively dividing cambial zone (CZ) and differentiating xylem (X). Note fiber sclereids interrupting tangential band of phloem parenchyma strands. X 100.

Figure 32. Cross section of wood showing tracheids before and after infestation. Note thick, rounded walls of aphid-affected xylem cells to the right of the line. X 450.

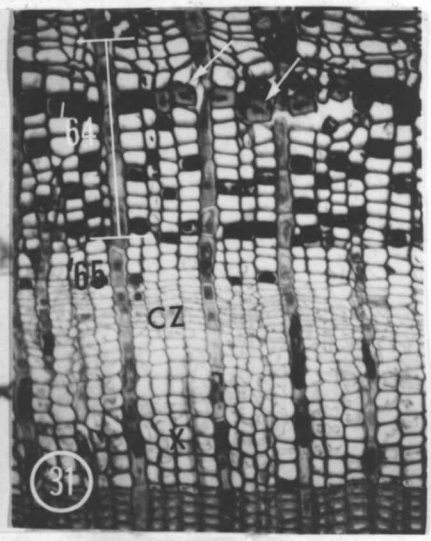
Figure 33. Tangential section of conducting phloem of a non-infested tree showing developmental stages of a phloem parenchyma strand. Arrow indicates transverse division in telophase stage. Phloem mother cell (PMC) accumulates resins prior to transverse divisions to produce phloem parenchyma strand. X 450.

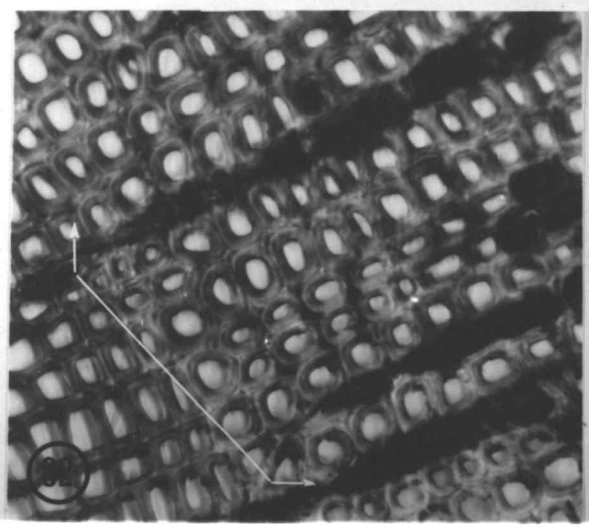

27

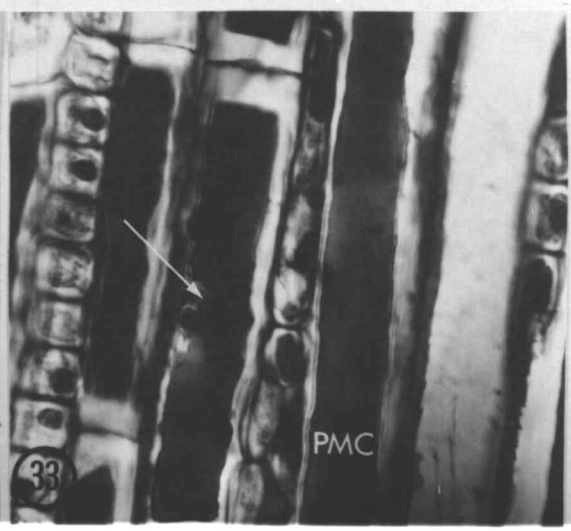

S
28


29


30


64
65
CZ
X
31


32


PMC
33

LEGEND FOR FIGURES 34-39

VAS = vertical albuminous strand

AC = albuminous cell

R = ray

I = intruded initial

CZ = cambial zone

X = xylem

= initial declined and was terminated at that point

= division of phloem mother cell

The vertical lines divide the annual increment into thirds.

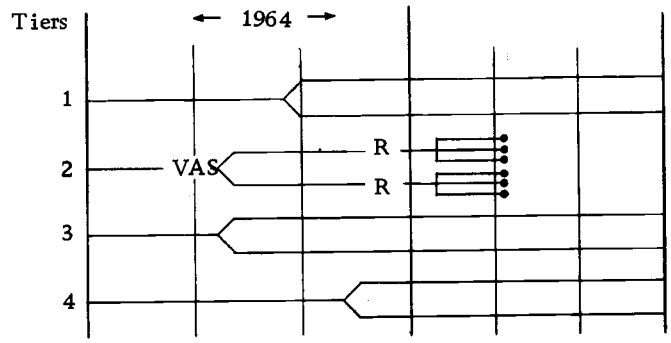


Figure 34. Cambial activity of non-infested tree.

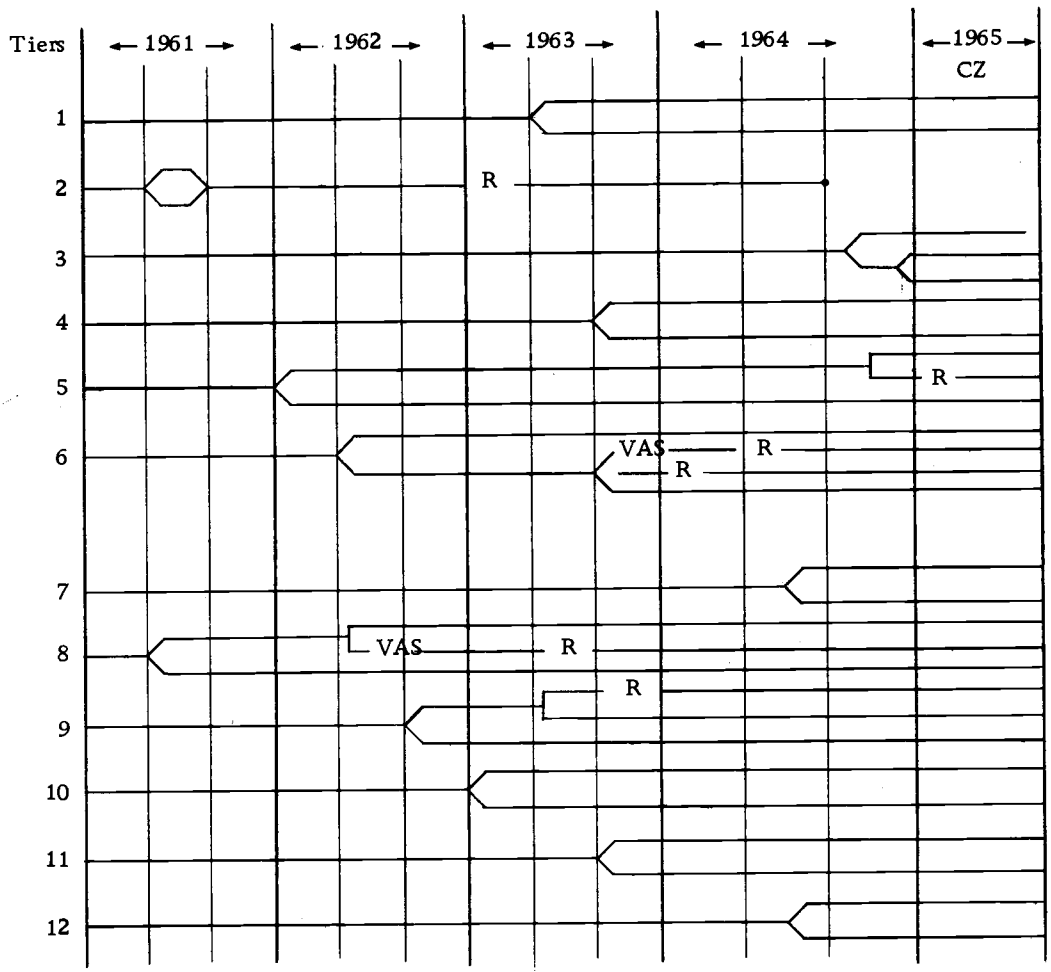


Figure 35. Cambial activity of non-infested tree.

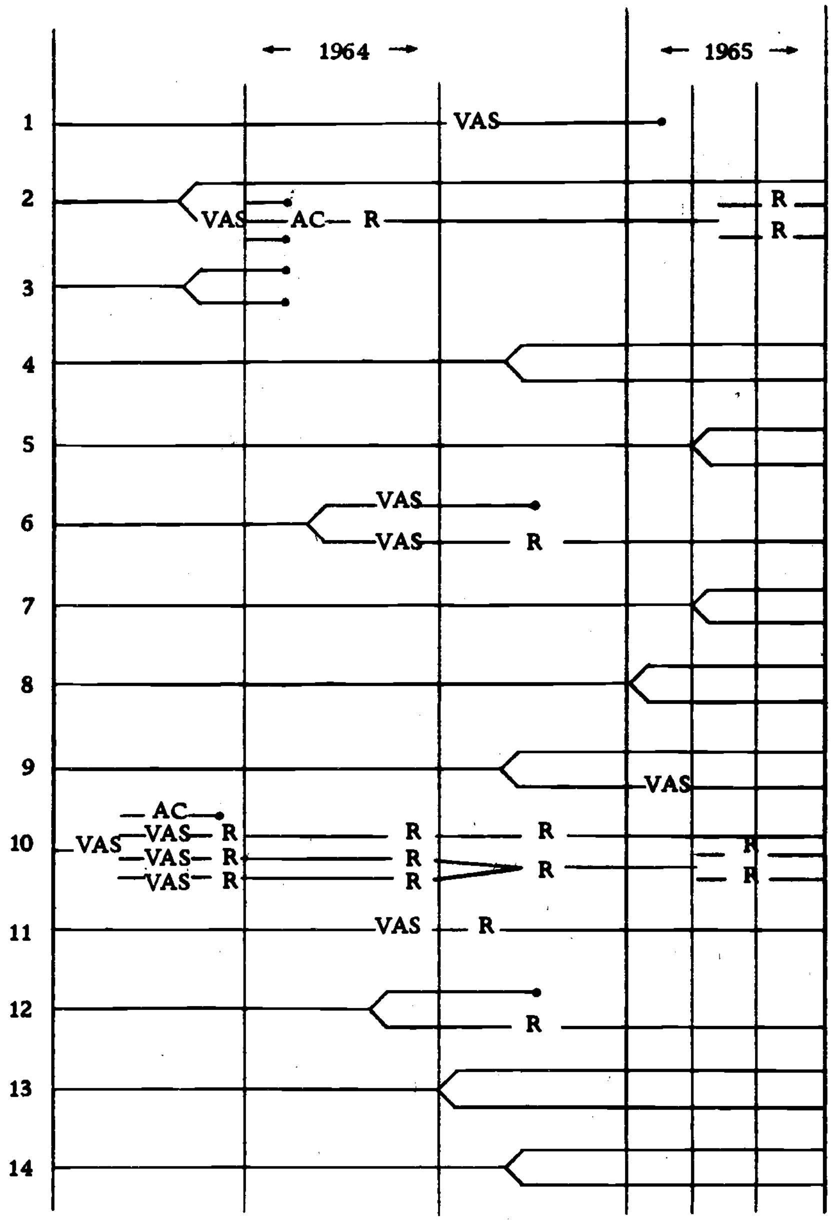


Figure 36. Cambial activity of infested tree.

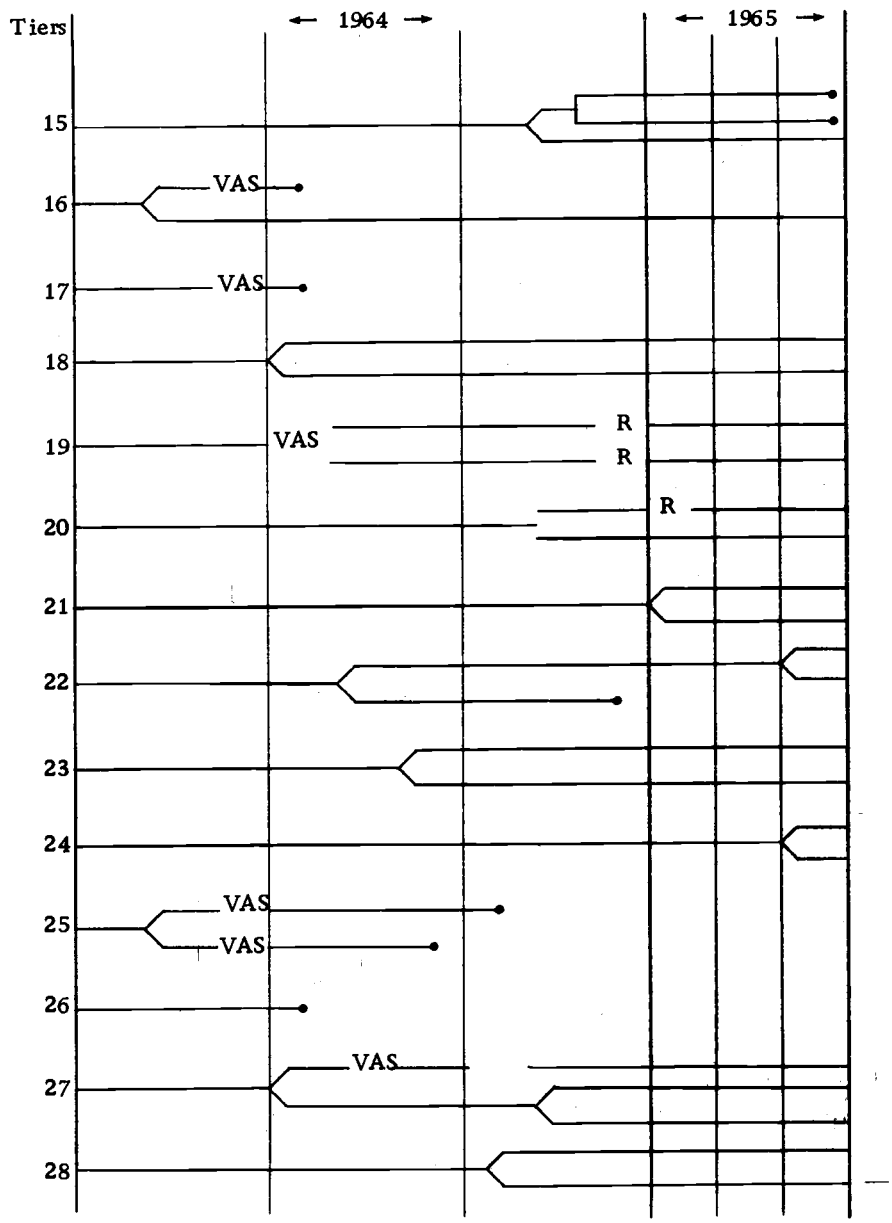


Figure 36. Continued.

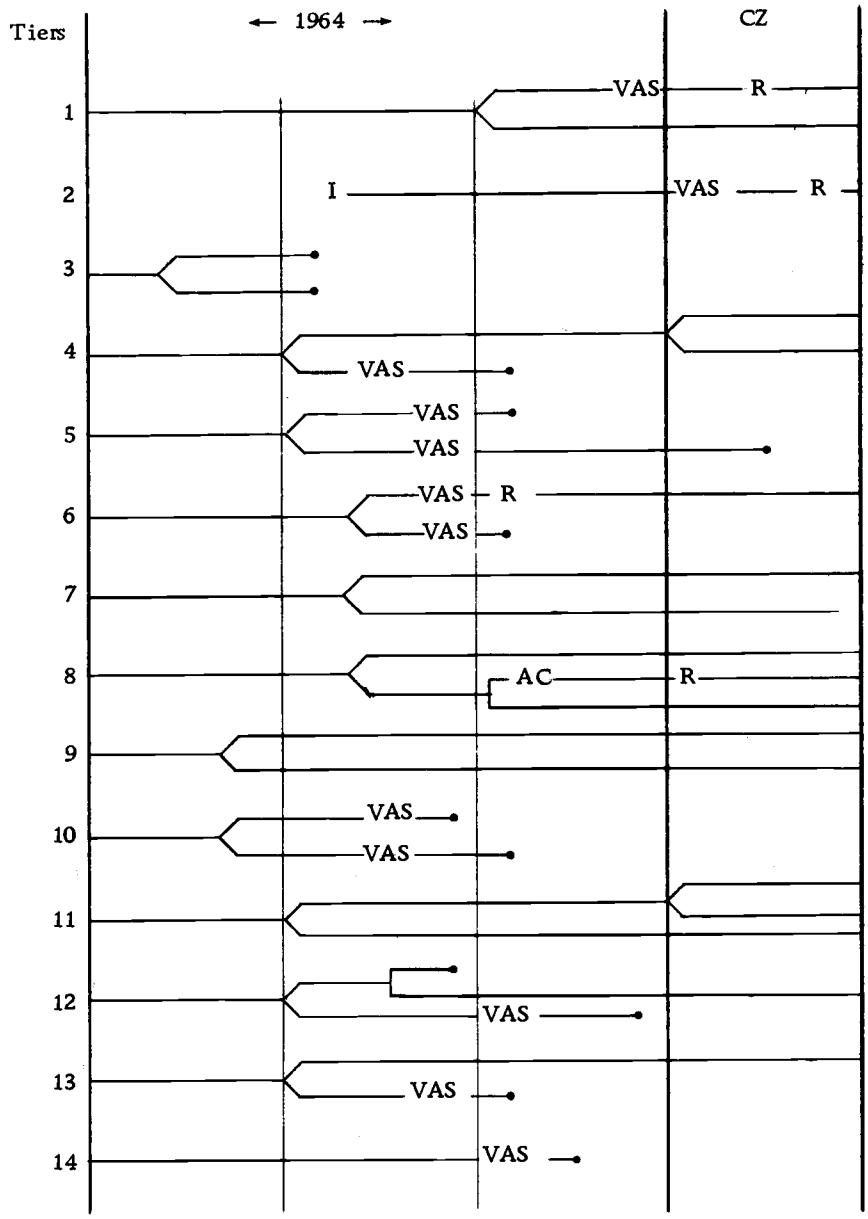


Figure 37. Cambial activity of infested tree.

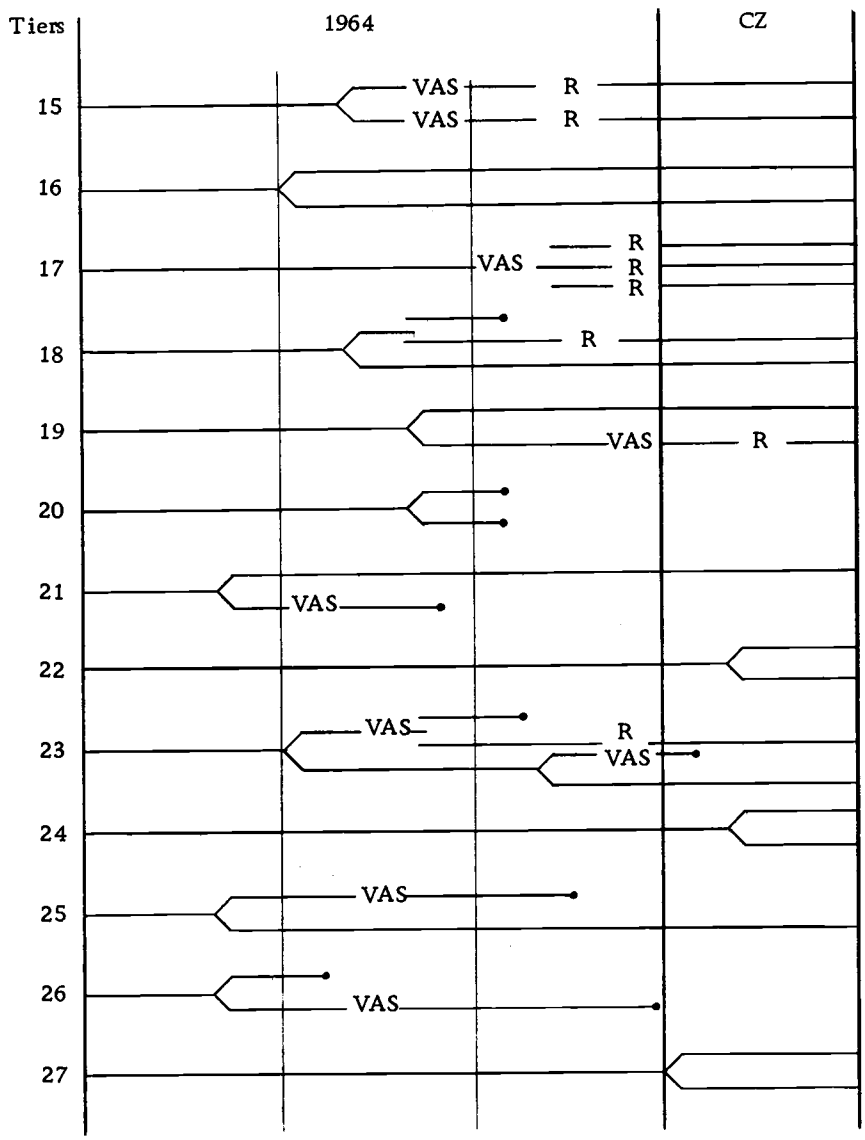


Figure 37. Continued.

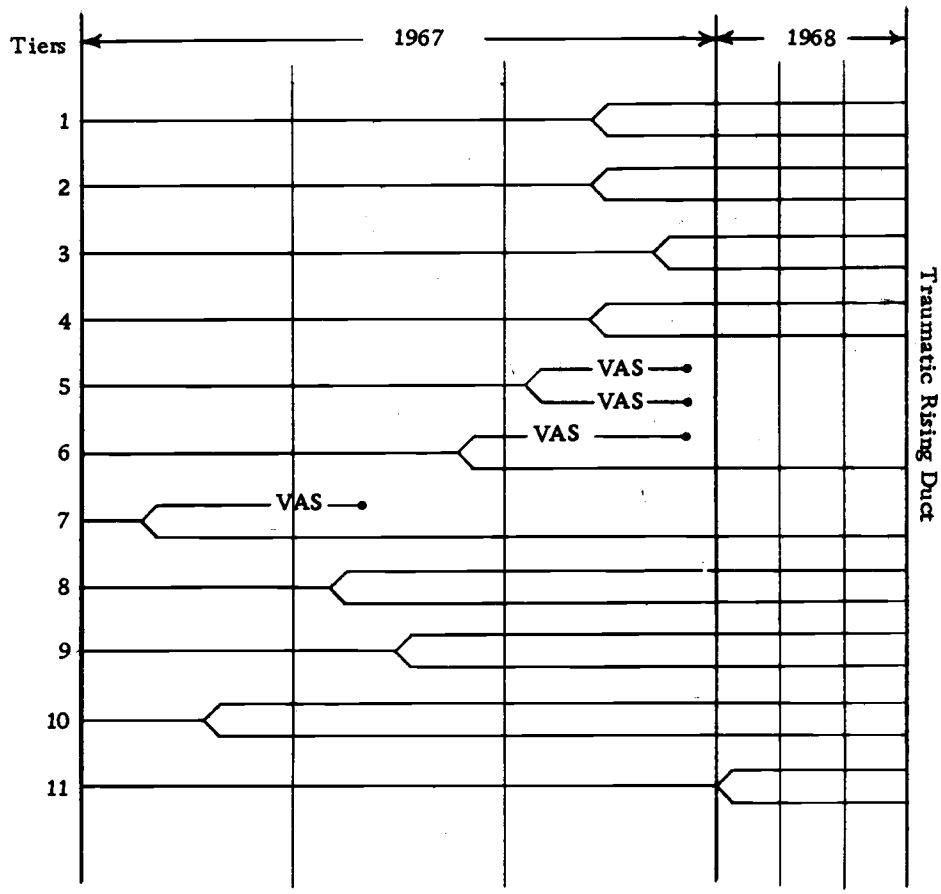


Figure 38. Cambial activity of infested tree.

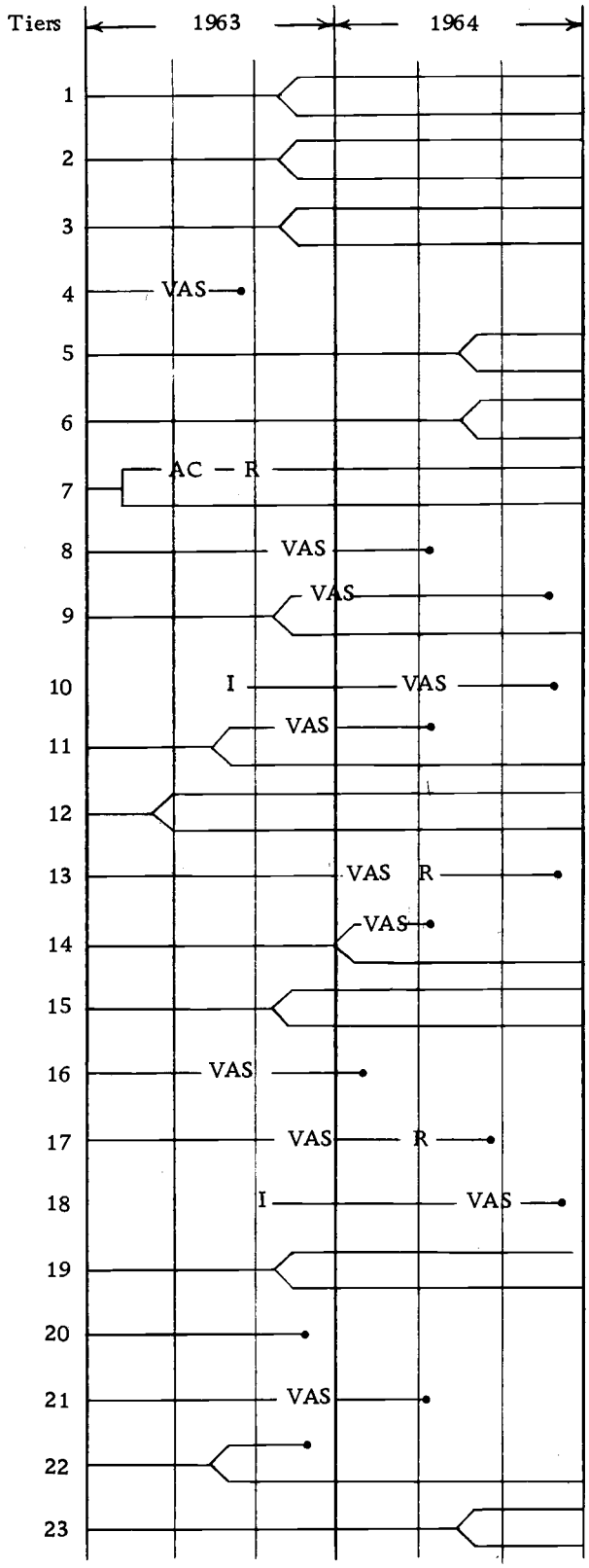


Figure 39. Cambial activity of aphid-abandoned tree.

BIBLIOGRAPHY

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

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

Balch, R. E. 1952. Studies of the balsam woolly aphid, Adelges piceae (Ratz.) and its effects on balsam fir, Abies balsamea (L.) Mill. Ottawa. 76 p. (Canada. Department of Agriculture. Publication no. 867)

Balch, R. E., J. Clark and J. M. Bonga. 1964. Hormonal action in production of tumors and compression wood by an aphid. Nature 202:721-722.

Bannan, M. W. 1950. The frequency of anticlinal divisions in fusiform cambial cells of Chamaecyparis. American Journal of Botany 37:511-519.

_____ 1951. The annual cycle of size changes in the fusiform cambial cells of Chamaecyparis and Thuja. Canadian Journal of Botany 29:421-437.

_____ 1953. Further observations on the reduction of fusiform cambial cells in Thuja occidentalis L. Canadian Journal of Botany 31:63-74.

_____ 1955. The vascular cambium and radial growth in Thuja occidentalis L. Canadian Journal of Botany 33:113-138.

_____ 1956. Some aspects of the elongation of fusiform cambial cells in Thuja occidentalis L. Canadian Journal of Botany 34:175-196.

_____ 1964. Tracheid size and anticlinal divisions in the cambium of Pseudotsuga. Canadian Journal of Botany 42:603-631.

Bannan, M. W. and I. L. Bayly. 1956. Cell size and survival in conifer cambium. Canadian Journal of Botany 34:769-776.

Barghoorn, E. S., Jr. 1940. Origin and development of the uniseriate ray in the Coniferae. Bulletin of the Torrey Botanical Club 67:303-328.

Barnes, D. K. and R. C. Newton. 1963. Amorphous tumors induced in alfalfa by potato leaf hoppers. Nature 199(1):95.

Bonga, J. M. and J. Clark. 1965. The effect of B-inhibitor and histogenesis of balsam fir bark cultured in vitro. Forest Science 11:271-278.

Chang, Ying-Pe. 1954. Bark structure in North American conifers. Washington, D. C. 86 p. (U. S. Department of Agriculture. Technical Bulletin no. 1095)

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

Clark, J. and J. M. Bonga. 1963. Evidence for indole-3-acetic acid in balsam fir, Abies balsamea (L.) Mill. Canadian Journal of Botany 41:165-173.

Crystal, R. N. 1926. The genus Dreyfusia (Order Hemiptera, Family Chermesidae) in Britain, and its relation to the silver fir. Philosophical Transactions of the Royal Society of London, ser. B, 214:29-61.

Doerksen, A. H. 1964. The effects of balsam woolly aphid infestations on wood anatomy of true firs. Master's thesis. Corvallis, Oregon State University. 36 numb. leaves.

Doerksen, A. H. and R. G. Mitchell. 1965. Effects of the balsam woolly aphid upon wood anatomy of some western true firs. Forest Science 11:181-188.

Esau, K. 1950. Development and structure of the phloem tissue. II. Botanical Review 16:67-114.

_____ 1965. Plant Anatomy. 2d ed. New York, John Wiley and Sons. 767 p.

Evert, R. F. 1960. Phloem structure in Pyrus communis L. and its seasonal changes. University of California Publications in Botany 32:127-194.

Grillos, S. J. and F. H. Smith. 1959. The secondary phloem of Douglas fir. Forest Science 5:377-388.

Hejnowicz, Z. 1961. Anticlinal division, intrusive growth, and loss of fusiform initials in nonstoried cambium. Acta Societatis Botanicorum Poloniae 30:729-748.

Holdheide, W. 1951. Anatomie mitteleuropäischer Gehölzrinden. Handbuch der Mikroskopie in der Technik 5(1):193-367.

Huber, B. 1939. Das Siebröhrensystem unserer Bäume und seine jahreszeitlichen Veränderungen. Jahrbuch für Wissenschaftliche Botanik 88:176-242.

Kloft, W. 1957. Further investigations concerning the interrelationship between bark condition of Abies alba and infestation by Adelges piceae typica and A. nusslini schneideri. Zeitschrift für Angewandte Entomologie 41:438-442.

Mitchell, R. G. 1966. Infestation characteristics of the balsam woolly aphid in the Pacific Northwest. Washington, D. C. 18 p. (U. S. Forest Service. Research Paper no. PNW-35)

_____ 1967. Abnormal ray tissue in three true firs infested by the balsam woolly aphid. Forest Science 13(3): 327-332.

Oechssler, G. von. 1962. Studien über die Saugschäden mitteleuropäischer Tannenläuse im Gewebe einheimischer und ausländischer Tannen. Zeitschrift für Angewandte Entomologie 50:408-454.

Plumb, G. H. 1953. The formation and development of the Norway spruce gall caused by Adelges abietis L. New Haven. 77 p. (Connecticut. Agriculture Experiment Station. Bulletin no. 566)

Sanio, K. 1873. Anatomie der gemeinen kiefer (Pinus silvestris L.). Jahrbuch für Wissenschaftliche Botanik 9:50-126.

Sass, J. E. 1951. Botanical Microtechnique. 2d ed. Ames, Iowa State College Press. 228 p.

Smith, F. H. 1967. Effects of balsam woolly aphid (Adelges piceae) infestation on cambial activity in Abies grandis. American

Journal of Botany 54(10):1215-1223.

Speers, C. F. 1958. The balsam woolly aphid in the Southwest. Journal of Forestry 56:515-516.

Srivastava, L. M. 1963. Secondary phloem in the Pinaceae. University of California Publications in Botany 36:1-142.

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

Varty, I. W. 1956. Adelges insects of silver firs. London. 75 p. (Great Britain. Forestry Commission. Bulletin no. 26)

Whalley, B. E. 1950. Increase in girth of the cambium in Thuja occidentalis L. Canadian Journal of Research, sec. C, 28:331-340.

Wilson, B. F., T. J. Wodzicki and R. Zahner. 1966. Differentiation of cambial derivatives. Proposed Terminology. Forest Science 12:438-440.