Cambial activity in white spruce stems in Alaska was observed from April, 1964 to September, 1965 in over 300 samples taken from 50- to 60-year-old natural stands. Mitotic index was used as a measure of the rate of periclinal division of fusiform cells in the cambial zone. Anticlinal divisions are relatively rare, occurring only about once per 278 periclinal divisions in most stems. Mitotic index, at any given time, is quite uniform throughout the cambial zone of an internode, among internodes of the same tree, and even among trees of a stand growing at markedly different rates. There may be some difference in rate of division across the radial extent of the cambial zone. During dormancy, the radial number of fusiform cells in the cambial zone (NCZ) ranges from two to about eight, the number being related to annual growth rate of xylem and phloem. Two to three overwintering immature (precocious) sieve cells border the cambial zone in each radial file of cells. They are the last phloem...
derivatives of the previous year's cambial activity. They undergo rapid radial enlargement just prior to vernal reactivation of mitosis in the cambial zone, and they become the first new conducting phloem elements of the current year. There are three distinct growing season periods: early period, grand period, and late period. Beginning of early period cambial activity is marked by reactivation of periclinal divisions, first near the apex, then at successively lower stem levels. Each cambial zone fusiform initial divides once before any new xylem or phloem derivatives are produced, thus approximately doubling the NCZ. Mitotic activity varies considerably during the early period, and the time required to double the NCZ varies from year to year depending upon weather conditions. Production of the first new xylem and phloem derivatives marks the beginning of the grand period. Rate of cell production in the cambial zone remains about equal to derivative production for the next 45 to 50 days, when about 80 percent of annual xylem and phloem increment is produced. Rate of division is comparatively uniform in all internodes in all trees. Rate of derivative production is, therefore, dependent on NCZ. There is a rather abrupt drop in NCZ at the beginning of the late period, apparently due to lengthening of the cell division cycle. Rate of derivative production exceeds rate of cambial zone cell production, and NCZ soon drops to about the dormant level. Subsequently, within about ten days, derivative production slows, the
zone of developing tracheids begins to narrow, and radial enlargement of developing tracheids and sieve cells declines. In about two more weeks the zone of developing tracheids is almost eliminated, the most recently produced tracheids show little or no radial enlargement, and there is evidence of partial inception of dormancy in the cambial zone. Complete termination of cambial activity is gradual, extending through late August and, perhaps, into September.
Seasonal Pattern of Secondary Growth in Stems of Northern White Spruce (Picea glauca (Moench) Voss)

by

Robert Aaron Gregory

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SEASONAL PATTERN OF SECONDARY GROWTH IN STEMS OF NORTHERN WHITE SPRUCE
(PICEA GLAUCA (MOENCH) VOSS))

INTRODUCTION

In following the seasonal course of activity associated with annual growth of the secondary vascular system in trees, elements of both time and space must be considered. The latter deserves special emphasis in large trees. Assimilates, water, and nutrients, the raw materials for growth, as well as some growth regulators, are transported long distances in the vascular tissues. They may move one hundred feet or more from points of synthesis or uptake to sites of incorporation or action where cytological events and developing anatomical features are seen. A record of these terminal events and features, with regard both to time and space, is an essential step that must precede a search for the mechanisms promoting them. This has been the objective of the two-year study of the seasonal pattern of cambial activity in white spruce (Picea glauca (Moench) Voss) stems reported here.

Alaskan white spruce is an ideal tree for this purpose. As in most gymnosperms, the secondary vascular tissues are comparatively simple; there are few cell types, and cambial derivatives in both xylem and phloem are arranged in radial files and can be easily traced to their point of differentiation from the cambial zone.
The growing season in Alaska is relatively short and seasonal patterns are accelerated and seem more sharply defined (Gregory and Wilson, 1968). There is little doubt that stems and roots are truly dormant throughout the winter. Vernal reactivation occurs within a short and predictable period. Stem growth begins before the soil has thawed and all the winter's snow has melted. Soil moisture, a troublesome extraneous variable in many field growth studies, is, therefore, unlikely to be limiting at least through June, the month of most vigorous growth. Overall, the native trees of interior Alaska seem well adapted to growing appreciably in a short season and surviving in an inclement environment the remainder of the year, making them well suited for field studies of growth and survival.
MATERIALS AND METHODS

Sample materials were collected from two areas near Fairbanks, Alaska (64°51'N, 147°44'W) each supporting 50- to 60-year-old mixed stands of white spruce and quaking aspen (*Populus tremuloides* Michx.). The first area, located 30 miles west of Fairbanks at about 900 feet elevation, was sampled from April 25, 1964 into January, 1965. The second area, seven miles west of Fairbanks at about 500 feet elevation, was sampled from May 6 through August, 1965. All crown classes of white spruce, dominant, intermediate and suppressed, existed in these stands. Diameters at breast height (b.h.) ranged from two to 14 inches and total stem heights from 15 to 70 feet. Over 300 sample blocks, consisting of bark, cambial zone, and usually all of the previous years xylem, were removed at various stem levels from over 100 trees in the two stands. Size of the blocks, measured on the tangential face (bark surface), was one centimeter by three centimeters.

Beginning April 25, 1964, one sample was taken at b.h. level from each of two dominant, intermediate, and suppressed trees at intervals through that spring and the following summer. During most of this period the sample interval was six days. Different trees were sampled each time. Numerous dormant season samples were taken in November 1964 and January 1965.
In the spring of 1965, from May 6 through June 1, two dominant trees (numbers 2 and 3) were repeatedly sampled at two-day intervals at two stem levels, about b.h. and two-thirds total stem height. The first sample blocks at each stem level were taken from near the base of the internode nearest the respective stem level. Each successive sample was taken a few centimeters above and to the side of the previous one so that the line of the sample sites spiraled completely around the stem within two adjacent internodes. Additional samples were taken from other trees in 1965. Methods involved in taking these will be discussed separately in later sections.

Sample blocks were immediately placed in Navashin's fluid (Jensen, 1962, p. 79) upon removal from the trees, then brought to the laboratory and evacuated. Time between removal of the samples and evacuation was usually about one hour. Subsequent dehydration and paraffin embedding were done as described by Wilson (1964). The blocks were then trimmed (excess bark and wood removed), subdivided, and reembedded in 56°C paraffin.

Serial transverse, radial, and tangential sections were cut on a rotary microtome at 20 μ. Serial tangential sections always included all the conducting phloem and some xylem as well as the cambial zone. In a few samples, some sections were cut in all planes at thicknesses ranging from 5 to 15 μ. These were used for higher resolution study of the cambial zone and conducting phloem.
All serial tangential sections were stained with Heidenhain's iron hematoxylin and some were counterstained with orange G (Jensen, 1962, pp. 91-92). Most of the transverse and radial sections were stained by the method of Cheadle, Gifford, and Esau (1953), but numerous replicates were also made using safranin-fast green (Jensen, 1962, p. 90) or periodic acid-Schiff's reaction (Jensen, 1962, pp. 198-199).

The average number of cells produced in each radial file the previous year and during the current year's growth was determined from the transections. All future references to radial cell numbers for particular samples are based upon the mean values for those samples.

Radial enlargement was used as the basis for distinguishing cambial zone cells from their immediate derivatives. Examination of many transections indicated that this was a suitable criterion. Appreciable radial enlargement, which occurred very rapidly in developing tracheids and sieve cells throughout most of the growing season, proved to be a reliable indicator of the loss of meristematic activity. Either mitotic nuclei or phragmoplasts were often observed in non-radially enlarged cells at the edges of the cambial zone, but never in cells that showed a degree of radial enlargement substantially greater than that observed in cells well within the cambial zone. Apparently all fusiform cells of the cambial zone are continuously
undergoing radial enlargement, but as long as they are still meristematically active they undergo periclinal division before much enlargement takes place.

Methods for determining the mean number of mitoses and mean cambial zone fusiform cell populations per sampling unit, and mitotic indices have been explained by Wilson (1964, 1966). These procedures were followed in this study with two exceptions. First, the sampling unit, a core through the cambial zone made up of successive microscope fields in serial tangential sections, was larger in diameter (1.8 mm vs. 1.6 mm used by Wilson). This provided a considerably larger core volume, making it possible to reduce the number of cores per sample from nine to eight and still retain suitable confidence limits about the mean core values of the measured variable, number of mitoses. In the second exception I departed slightly from Wilson's method of determining mitotic index. In computing this variable, which is simply the percentage of fusiform cambial zone cells in mitosis, he uniformly subtracted a constant number of cells (80) from his estimated total cell population (see Wilson, 1966, p. 369). I did not find a similar basis for lowering the cell population estimates in white spruce.

The number of cambial zone fusiform cells observed microscopically in all eight cores of any sample ranged from about 1000 to 5000 depending upon width of the cambial zone (radial cell number)
and length of the fusiform initials.

The equations for computing the regression curves in Figures 1, 2, 3, 4, 5, 6, 23, 28, and 30 were determined through a polynomial series computer program. The data shown in the scatter diagrams were fitted to first, second, and third degree polynomial equations. The equation giving the "best" fit, that is, the one in which a significantly greater amount of variation could not be accounted for at the probability level $p = 0.05$ by a higher curve form, was selected. The fitted regression equations, and multiple coefficients of determination ($R^2$) which are indices to the amount of variation accounted for by the regressions, are included in most of the above text figures. Confidence limits, the sampling errors for mean values, referred to in the text and figure captions, were calculated at the probability level, $p = 0.05$ unless stated otherwise.
DESCRIPTION OF THE SECONDARY VASCULAR REGIONS

Cambial Zone

Two cell types predominate in the vascular cambium of white spruce: long, radially flattened, tangentially tapered fusiform initials, and relatively small, almost isodiametric ray initials. Both give rise, by periclinal divisions, to radial files of cells comprising the secondary xylem and phloem (Figure 31). Occasionally, a failing fusiform initial undergoes progressive shortening due to asymmetrical periclinal divisions accompanied by reduction in transverse dimensions of the terminal portions of the cells (Bannan, 1953), giving rise to a declining radial file (Figures 35-42).

The immediate derivatives of the cambial initials are meristematically active xylem and phloem mother cells of the cambial zone (Figures 33-34). These usually divide periclinally one or more times before differentiating into xylem and phloem elements. In general, the greater the number of fusiform cells in the cambial zone, the greater the amount of xylem and phloem production (Figures 1, 2, 3), indicating that the mother cells in fast-growing trees undergo more periclinal divisions before differentiation than those in slow-growing trees.

The number of cambial zone cells per radial file (NCZ) not only fluctuates with tree vigor, but also with season of the year
Figures 1, 2. The relationship between number of cambial zone cells per radial file (NCZ) and annual xylem increment.

Figure 3. The relationship between annual phloem increment and annual xylem increment.
Dormant, slow-growing white spruce stems may have as few as two to four fusiform cells per file, while dormant fast-growing stems may have eight or more cells per file. The NCZ is approximately doubled in the spring in the time between the first mitoses and differentiation of the first tracheids, a period that may vary from a few days to two or more weeks depending upon weather conditions. This peak population level (Figure 2) is maintained through the major part of the growing season, declining rather abruptly to about the dormant level after 75 to 80 percent of the year's tracheid increment is produced. These seasonal fluctuations will be dealt with in more detail in a later section.

Dormant and mitotically active cambial zone cells differ considerably in appearance (Bannan, 1955; Grillos and Smith, 1959; Evert, 1960, 1963; Derr and Evert, 1967). Dormant fusiform cells in white spruce have dense, dark-staining cytoplasm; relatively large elongated nuclei; and a very pronounced beaded appearance of the radial walls due to interruption of the thickened walls by primary pit fields (Figures 43, 44). There is little variation in tangential wall thickness or in radial width of the dormant cells as seen in transection (Figure 47). Marked changes from the dormant appearance were not observed in white spruce until shortly after the occurrence of the first periclinal divisions of the growing season when the radial walls of the fusiform cells become thinner and
recently divided nuclei are smaller and more rounded (Figure 45). Soon thereafter the fusiform cells become more variable in radial width, cytoplasm stains less intensely, and all the nuclei appear smaller and more rounded (Figures 33, 34, 46). Toward the end of the growing season, after the cambial zone cell population has declined and mitotic activity is reduced, the cells gradually resume a dormant appearance.

Immature sieve elements in early stages of development, overwintering next to the cambial zone, have been reported in several tree species (Elliot, 1935; Artschwager, 1950; Grillos and Smith, 1959; Evert, 1960, 1963; Wilson, 1966). In white spruce, these so-called "precocious" sieve cells are usually cytologically indistinguishable from the dormant, undifferentiated fusiform cells of the cambial zone (Figure 47). In the spring, shortly before the beginning of mitotic activity in the cambial zone, they undergo appreciable radial enlargement and soon contrast markedly with the first periclinaly dividing cells of the cambial zone (Figures 32, 33, 34). The number of precocious sieve cells per radial file, usually two to three, seems to be independent of tree vigor or NCZ. Although the precocious sieve cells rapidly mature into the first new conducting phloem elements of the growing season, it is important to recognize, as will become apparent in later discussion, that they are not part of the overwintering cambial zone cell population in white
spruce. They are no longer capable of dividing and are, therefore, different from cells of the cambial zone. They are, in terms of growth and differentiation, products of the previous year's cambial activity.

The decline of fusiform initials was not investigated in this study although cambial zone cell alterations associated with multiplication and decline were frequently observed. These changes have been intensively studied (Bailey, 1923; Bannan, 1950, 1951, 1953, 1954, 1957, 1960, 1962a, 1963a, 1963b, 1964; Bannan and Bayly, 1956; Hejnowicz, 1961; Evert, 1960, 1963; Srivastava, 1963; Cheadle and Esau, 1964; Smith, 1967), and it has been well established that the rate of pseudotransverse division of fusiform initials is greater than necessary for circumferential growth. Many of the daughter initials decline and are lost from the cambium or transformed into ray initials. As mentioned previously, the failing initials undergo shortening due to asymmetrical periclinal divisions. Members of declining files are commonly encountered in the cambial zone or conducting phloem of white spruce. The phloem derivatives often appear as segmented parenchyma strands immediately following a pseudotransverse division (Figure 37). Bannan (1953) has observed that the final cells of a radial file in the phloem of Thuja occidentalis are parenchyma strands and that evidence of segmentation often appears in the phloem when none is to be seen in the xylem. A partial
sequence of alterations accompanying decline of a radial file in white spruce is shown in Figures 35-39.

The frequency of pseudotransverse division and survival ratio of the daughter cells influence the average length of fusiform initials. According to Bannan (1960, 1964), both frequency of pseudotransverse division and survival ratios decline with age of an internode, accounting for the frequently observed increase in tracheid length with age (Dinwoodie, 1962).

The length of fusiform initials in white spruce, as reflected by the length of derived sieve cells (Table 1), varies considerably. The 35 samples in Table 1 were selected from over 300 samples taken in this study. They were chosen to uniformly span the range of radial file number per 2 mm-field area. Radial files were counted from tangential sections as described by Wilson (1966, p. 366). All radial files having one or more nuclei in the field area were counted. The nuclei are located near the center of the fusiform cells, so that files having one half or more of their longitudinal dimension in the field would be counted, and those with less than half their longitudinal dimension in the field would be excluded. Since radial file number per unit of sample area is dependent upon fusiform initial length as reflected by sieve cells derived from the initials (Figure 4), the range of mean cell length in the 35 samples, 1.8 to 3.2 mm, should closely approximate the range of mean cell length for all the samples.
Table 1. Mean length of fusiform initials in samples taken at two stem levels: lower (b. h.) and upper (two-thirds of total stem height). The mean values are based upon 20 randomly selected sieve cells in the conducting phloem of each sample.

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<th>Sampling error ¹</th>
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<td></td>
<td>mm</td>
<td>% of mean</td>
<td></td>
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¹ Probability level, p = 0.05
Figure 4. The relationship between number of radial files per two square millimeters of tangential field area and mean length of fusiform initials. Mean initial length, in each of the 35 samples, is based upon measurement of 20 randomly selected sieve cells in the conducting phloem.
Usually, samples from the same internode in a tree (the same tree numbers at the same stem level in Table 1) had similar cell lengths. Because all trees from which the 35 samples were selected were the same age, differences in cell length between trees at the same stem level were probably due to variations in radial growth rate between trees, as explained below. The occurrence of shorter initials at the younger upper-stem levels is in line with what would normally be expected.

Frequency of pseudotransverse division and survival ratio of the daughter cells, both of which influence mean cell length, have been observed to vary with radial growth rate of tree stems. Bannan (1963a), reported that maximum cell length in several species of _Picea_, including white spruce, was associated with an annual ring width of 1 to 2 mm; that cell length declined with increase in ring width, and also decreased slightly when ring width fell below the optimum 1 to 2 mm level. These relationships between ring width and cell length are essentially the same for Alaskan trees (Figure 5).

Bannan (1963a) measured the length of mature tracheids. Both xylem mother cells and tracheids undergo apical intrusive growth, resulting in a five to 10 percent increase in length (Bailey, 1920). In this study, sieve cells, which closely replicate the length of the cambial initials, were used. Thus mean cell lengths reported by
Figure 5. The relationship between radial file number or cell length and annual tracheid increment or ring width. The transformed $x$ and $y$ variables at the upper and right hand side of the figure, respectively, were calculated from the regression equations in Figures 4 and 6.
Bannan should be slightly longer, but otherwise comparable in all cases to mean cell lengths reported in this paper.

Maximum cell length, according to Bannan, appears to be associated with the particular ring width which marks the lowest point to which radial growth may drop without bringing about an increase in frequency of pseudotransverse divisions. He found that pseudotransverse divisions take place at a mean rate of slightly less than one per cm of radial xylem increment when the annual rings are 2 to 9 mm wide, but that the rate increases sharply as ring width falls below 2 mm. It has already been noted that an increase in the rate of pseudotransverse divisions will cause a corresponding decrease in mean cell length, so that one would expect a decrease at ring widths less than 2 mm providing that survival of daughter initials remains about the same. The decline in cell length above the 2 mm ring width, where rate of division remains constant, is apparently due to a higher rate of daughter cell survival resulting in less intrusive growth of the initials.

When considering the similarity in the regressions of cell length on ring width between Alaskan white spruce and those sampled by Bannan (1963a), it seems reasonable to assume that the rate of pseudotransverse division in the Alaskan trees is similar to that reported by Bannan for white spruce elsewhere; that is, one division per cm of radial xylem increment when ring width is 2 mm or
greater. The regression coefficient in Figure 6 indicates that there are 24.4 tracheids per radial mm or 244 per cm in Alaskan white spruce. For every 244 tracheids produced in the xylem there would be 34 fusiform cells produced in the phloem (Figure 3). In other words, there would be, on the average, about 278 periclinal divisions in the cambial zone for every pseudotransverse anticlinal division in a fusiform initial when xylem ring width is 2 mm or greater. The ratio for stems with ring widths less than 2 mm would be somewhat lower. Even allowing for occasional pseudotransverse divisions in xylem and phloem mother cells, it is obvious that the great majority of mitoses in cambial zone fusiform cells are associated with periclinal division.

Functional Xylem

The structure of most gymnosperm woods is comparatively simple and well known (see Panshin and De Zeeuw, 1964; and Esau, 1965 for detailed anatomy). Tracheids, which account for over 90 percent of xylem volume in white spruce, are uniformly arranged in radial files emanating from the fusiform initials of the cambial zone (Figures 74, 75). Mature tracheids are somewhat longer than the fusiform initials from which they were derived since the xylem mother cells and developing tracheids undergo apical intrusive growth (Bailey, 1920). Radial xylem growth is controlled by
Figure 6. The relationship between annual tracheid increment and annual xylem ring width.

\[ y = 10.07 + 24.41x \]

\[ R^2 = 0.98 \]
periclinal division of the initials and xylem mother cells of the
cambial zone, size of the cambial zone cell population, and radial
enlargement of the developing tracheids. Circumferential growth
is accomplished by pseudotransverse anticlinal division of the fusi-
form initials (Bailey, 1920; Bannan, 1951) resulting in the multipli-
cation of radial files and addition of new rays to the cambial zone
and derived tissues. Vertical resin canals occur sporadically in
white spruce, being relatively abundant in some growth rings and
absent in others. Most rays are uniseriate but some are fusiform
and enclose a horizontal resin canal. Ray tracheids are usually
limited to the upper and lower marginal cells of all rays. Fusiform
rays, with their associated horizontal resin canals, are randomly
scattered but uniformly abundant; one or more are usually found in
any 100 X microscope field when the xylem is viewed tangentially.

Conducting Phloem

Srivastava (1963) has reviewed past work on the secondary
phloem in Pinaceae and reinvestigated in detail the structure of the
phloem and ontogeny of cell types in 19 species and six genera of
this family. Although there is much similarity in the conducting
phloem among members of the Pinaceae, appreciable differences
do occur both in cell types and overall structure.

In white spruce, most of the volume in the conducting phloem
consists, as in other Pinaceae, of radial files of sieve cells inter-
rupted by tangential bands of tannin-filled parenchyma strands (Fig-
ures 31, 40). Both these cell types belong to the axial system and
are derived from the same fusiform initials as the tracheids on
the xylem side of the cambial zone. Sieve cells and tannin-filled
parenchyma strands in the conducting phloem closely replicate the
tangential and longitudinal dimensions of the initials from which they
were derived (Figures 31, 49), enlarging only in the radial direction
during development. Later, in the nonconducting phloem, segments
of the strands continue to undergo additional enlargement in all direc-
tions of the transverse plane (Figure 31), occasionally dedifferenti-
ating and redeveloping into sclerids (see Srivastava, 1963, p. 17).

The only other members of the axial system observed in the
conducting phloem of white spruce were derivatives of declining
fusiform initials. The phloem products of these initials typically
fail to develop into sieve cells or tannin-filled phloem parenchyma
strands. Instead, nontanniferous parenchyma strands (Figure 37)
appearing as plates of parenchyma cells in radial view (Figures
40-42) occur on the phloem side of the declining initial. Along with
continued reduction of the file due to asymmetrical periclinal divi-
sions in the initial, parts of the initial are lost ("breakage" as de-
scribed by Srivastava, 1963, p. 58), and the segmented phloem de-
rivatives are thus continually shortened. This reduction sequence
in white spruce is recorded in a radial view of a declining file (Figure 42). Both Bannan (1953) and Srivastava (1963) have illustrated in more detail the sequence of events leading eventually to loss of the initials from the cambium, or survival of one or more of the reduced segments as new ray initials. Members of a declining file of cells on the phloem side of the cambial zone, sometimes referred to as radial plates, have been observed by others (Chrysler, 1913; Barghoorn, 1940; Grillos and Smith, 1959) in several gymnosperms.

Two cell types occur in the ray system of conducting white spruce phloem: marginal upright parenchyma, and submarginal procumbent parenchyma. The former elongate longitudinally during their development, while the latter elongate radially. As in the xylem, most rays are uniseriate (Figure 43) but some are fusiform, enclosing a horizontal resin canal (Figures 44, 45).

Both the axial parenchyma from declining fusiform initials and the upright marginal ray cells differ from other phloem parenchyma in two respects. They die and collapse at the same time that the sieve cells with which they are associated become nonfunctional, and they commonly have connections with sieve cells by means of well developed sieve areas on the sieve cell side associated with what appear to be simple pits on the parenchyma cell side (Figures 50, 51). Thus they fit the description of ray and vertical albuminous cells repeatedly noted by others (Strasburger,
1891; Hill, 1901; Chrysler, 1913; Barghoorn, 1940; Grillos and Smith, 1959; Srivastava, 1963; Srivastava and O'Brien, 1966; Evert, 1965; Alfieri and Evert, 1968) in numerous gymnosperms. According to Alfieri and Evert (1968) the only reliable criterion for identification of albuminous cells is their conspicuous connection with sieve cells. They found, in several species of Pinus, that presence or absence of starch containing plastids, size of nuclei, and density of cytoplasm were not suitable criteria for distinguishing albuminous cells from other phloem parenchyma. In the white spruce samples of this study, prominent sieve cell connections were observed in the majority of upright marginal ray parenchyma and axial parenchyma from declining initials. Whether any individuals of these two cell types ever lacked sieve cell connections was not determined; none were positively observed.

There are clearly marked growth rings in white spruce phloem. The last few noticeably developed sieve cells of the growing season are less radially enlarged and have thinner secondary walls than other sieve cells (Figures 32-34, 47, 52, 57-59, 72, 73). The radial walls of these mature, late-year cells collapse before subsequent development of the last differentiated sieve cells (precocious sieve cells) the following spring. As noted by Huber (1939) in Picea excelsa, each year's phloem increment is bordered by tangential bands of these radially narrow, collapsed, thin-walled cells (Figures 31, 72, 73). In the older nonconductive phloem, however, this pattern is lost due to massive crushing and distortion of the tissue.

Callose deposition in the sieve areas was helpful in estimating
the radial extent of the conducting phloem. Callose was present in
the sieve area pores of all mature sieve cells in the conducting
phloem of white spruce at all seasons of the year. Some of the
calloose observed was due to disturbance in sampling as indicated
by a few samples that were rapidly killed in July. To prepare these,
small pieces of bark were quickly removed from trees growing next
to the laboratory and "quick-killed" with Navashin's fluid in the man-
ner described by Evert (1964). Callose was much reduced in these
samples but still present in the sieve area pores of mature elements.
Some quick-killed samples were also taken in late November and
December when temperatures were -30° to -40°F. Sample blocks
were removed from the tree stems and brought to the laboratory
without subjecting them to higher temperatures. Before bringing
the samples inside, thin (about 1 mm) radial slices of bark were
cut from the frozen blocks, placed in Navashin's fluid, and then
promptly brought inside and evacuated in a vacuum chamber. Pres-
umably, there was little chance for alteration of callose deposits
before the tissue was killed. Callose deposition was similar in all
the quick-killed winter samples. Sieve cells produced in the early
part of the previous growing season had massive (definitive) callose
deposits on the sieve areas while the younger sieve cells did not,
although sieve area pores of the latter were occluded with much
heavier deposits (Figure 55) than present in quick-killed summer
samples. In the spring, upon reactivation of the cambium, massive callose persisted in the older sieve cells of the previous year, and those cells soon collapsed. The younger sieve cells of the previous year appeared to remain functional for varying periods of time during the current year. The oldest of these cells acquired definitive callose as new sieve cells were added to the radial files during the growing season until, at the end of the season, all the previous year's sieve cells were nonfunctional and most were collapsed (Figure 53). It appears that part of each year's sieve cell increment is reactivated the following spring, and that all sieve cells remain functional for a period equivalent to about one summer season. A similar functional period was described by Huber (1939) for *Picea excelsa*.

One striking characteristic, an apparent artifact, was frequently noted in the phloem of samples taken during the growing season. Contents of the sieve cells were often clustered at the ends of the cells and against the sieve areas (Figures 48, 49) suggesting that the conducting elements of the phloem were under pressure when disturbed. Evert (1965), in a study of sieve cell ontogeny, observed similar disruptions due to tissue manipulations. He stated that there was aggregation of sieve cell contents into variously shaped bodies and stringy masses, and amorphous accumulations at the ends of the cells reminiscent of the slime plugs of sieve tube members. In white spruce, the disruptions often appeared massive.
(Figure 49). The extent of alteration is unknown and creates a serious obstacle to investigation of natural protoplasmic structure in samples removed from trees during the period of assimilate movement.
SOURCES OF VARIATION IN MEASURING RATE OF CELL DIVISION IN THE CAMBIAL ZONE

Mitotic index (percentage of fusiform cells in the cambial zone with mitotic nuclei) was used in this study as a measure of the rate of periclinal division of fusiform cells in the cambial zone. As defined by Wilson (1966), the beginning of mitosis was considered to be the point when chromosomes first became visible in early prophase and the end as the point when the cell plate ceased to be circular in tangential view and the two bars of the phragmoplast were distinct. All fusiform cells in the cambial zone that were in mitosis, as defined above, were considered to be dividing periclinally. Undoubtedly, an occasional anticlinal pseudotransverse division was counted as a periclinal division, but as shown previously, the ratio of periclinal to anticlinal divisions is about 278 to 1 so that this source of error is of little consequence. There are, however, several sources of variation relating to rate of periclinal division that must be considered in evaluating subsequent sample data.

Place-to-Place Variation in Mitotic Activity

The method of obtaining samples for estimates of mitotic activity was destructive so that comparisons of activity from time-to-time throughout the growing seasons were based upon samples taken at
different places within an internode or from different trees at the same stem level. Hence the question: did place-to-place variation mask changes that occurred with time? It is necessary, therefore, to know how much variation in mitotic activity may occur at any given time within an internode and between trees at the same stem level.

Wilson (1966), working with Pinus strobus in New England, found that the number of mitoses among numerous samples from an internode was similar at any one time, but that the number was higher within the crown than below the crown. The differences between stem levels were due, however, to differences in size of the cambial zone cell populations; mitotic indices were similar (2 to 3 percent) at all stem levels. Wilson concluded that if samples are taken only from the center of the internode, avoiding grooves and bark distortions, single samples will provide acceptable estimates of activity for the whole internode. Confidence limits (sampling errors at the probability level, $p = 0.05$) associated with each sample estimate of number of mitoses were usually less than 33 percent of the mean.

Sisson (1968) compared mitotic index within internodes and among trees at the same stem level in Pseudotsuga menziesii. He found significant differences in mitotic index in three of four trees sampled. The magnitude of the differences were, however, relatively small; the overall mean mitotic index of the four internodes
ranged from 2.1 to 2.8 percent, and in no case did the confidence limits for a particular sample not overlap those for the internode as a whole. The differences in mitotic index among trees were not significant even though the four trees varied considerably in rate of growth.

In this study, a b.h. internode of a dominant 50-year-old white spruce stem was sampled for a test of internodal variation on July 7, 1965. Ten samples were removed at systematic intervals around the internode between 11:00 and 11:30 a.m. Mitotic indices and their respective sampling errors are shown for each sample and for the composite of all samples in Figure 7. Mean mitotic index ranged from 1.6 to 2.7 percent for the individual samples. The mean for the entire internode was 2.1 percent. Sampling errors for the individual samples averaged 31 percent of the means. An analysis of variance showed that there was no significant difference between the samples at the probability level, $p = 0.05$.

All the tests of variation summarized above were made during that part of the growing season when, as demonstrated in a later section, mitotic activity and rate of xylem production are at the peak seasonal level and comparatively constant for an extended period of time. The samples were also taken in late morning or early afternoon when mitotic activity could be expected to be near the maximum daily level. These tests indicate that mitotic index
Figure 7. Mean core mitotic indices with confidence limits for each of ten samples taken from a single internode between 11:00 a.m. and 11:30 a.m., July 7, 1965. Circles represent mean values; vertical lines show extent of the confidence limits. Circles at the extreme right and left represent the mean mitotic index for all samples of the internode with confidence limits connected by dash lines.
during these periods is quite consistent within an internode, uniformly falling within the narrow limits of two to three percent, and that a single sample from an internode will be reasonably representative of that internode providing one accepts confidence limits equivalent to about 33 percent of the mean. Furthermore, mitotic indices seemed to be quite constant between internodes of a tree, and even between trees growing at markedly different rates. Other analyses, provided later, strengthen these impressions.

**Diurnal Variation in Mitotic Index**

Another possible source of variation that might have masked the seasonal trend of mitotic index was diurnal variation. Wilson (1966) observed that afternoon peaks in mitotic activity may occasionally occur in *Pinus strobus*. Samples used in this study for day-to-day comparison were taken between the hours of 10:00 a.m. and 12:00 noon. If appreciable diurnal fluctuations in rate of cell division exist, and if the daytime peak were sharp and of short duration, a comparison of the seasonal trend of mitotic index would be hindered by an additional source of variation.

To determine if diurnal variation did exist, and if so the magnitude of this variation, a vigorous 50-year-old dominant white spruce was sampled at four hour intervals June 22-24, 1965, at two stem levels: b.h. and approximately two-thirds total stem height.
Mitotic indices with confidence limits are shown in Figure 8. There appeared to be diurnal fluctuations in rate of cell division at both stem levels with maximum rates between noon and 4:00 p.m., and minimum rates between midnight and 4:00 a.m. Differences between the daily maxima and minima are highly significant (probability level, \( p = 0.01 \)), but differences between 8:00 a.m. and noon are not significant (probability level, \( p = 0.05 \)). The time interval, 10:00 a.m. to noon, when other samples were taken for tracing the seasonal trend of mitotic activity, does not seem to be a period subject to much variation in rate of cell division, although activity during this period may be slightly below the daytime peak.

**Variation in Mitotic Index Across the Cambial Zone**

All fusiform cells in the cambial zone may not be dividing periclinally at the same rate. Bannan (1955, 1962b) observed that there is a peak in the frequency of dividing cells in the central part of the cambial zone in *Thuja occidentalis*. Wilson (1964) also found the highest frequency in the middle of the cambial zone in *Pinus strobus* with a slight drop on the phloem side, and a large drop, almost to zero, in the quarter of the cambial zone nearest the xylem. These differences in mitotic frequency suggest that the rate of cell division across the cambial zone varies in a like manner. Wilson suggests, however, that the lower frequencies toward the edges of the cambial
Figure 8. Mean core mitotic indices and confidence limits at lower (b. h.) and upper (two-thirds of total stem height) stem levels in the same tree (no. 6) at four-hour intervals for a 48 hour period.
zone may not be due to a difference in rate of division but, rather, to variability in radial width of the cambial zone; because there are fewer cells at the extreme widths of the cambial zone, the frequency of mitosis drops. The greater mixture of differentiating cells on the xylem side of the cambial zone would result in a sharper drop in the frequency of division next to the xylem. For the purpose of estimating length of the cell division cycle, Wilson assumed the working hypothesis that frequency of cell division is the same throughout the radial population of the cambial zone.

An attempt was made in this study to determine whether peri-clinal divisions occurred at the same or different frequencies across the cambial zone of white spruce. In this species it is not possible to obtain reliable estimates of these data from tangential or radial sections. Tangential and radial undulations occur in nearly all sample material so that it is impossible to get a perfect tangential or radial view and to make anything better than a very rough estimate of the radial position of a dividing cell within the cambial zone. Only transverse sections could be relied upon to provide accurate data of this nature. Numerous transverse sections were available from samples taken from tree number 4 early in the growing season just before production of the first new xylem and phloem, and from tree number 6 in the middle of the grand period of growth. Peri-clinal divisions, as indicated by either mitotic nuclei or
phragmoplasts, were recorded by radial position; that is, number of cells inward from the previous year's phloem (Figures 9 and 10).

To determine the uniformity in division rate across the cambial zone it is necessary to relate the number of dividing cells at each radial position to the number of meristematic cells at that same position. There is considerable mixing of meristematic and differentiated cells from file to file at radial positions near the boundaries of the cambial zone, especially on the xylem side. This situation exists because, at the moment of sampling, some files have a wider cambial zone than others, and because there is not perfect tangential alignment of the cambial zone or of individual cells in neighboring radial files. In order to obtain a relative estimate of the number of meristematic cells by radial position, the boundaries of the cambial zone were observed in 110 to 120 files in each of the two trees. The status of the cells in each file, meristematic and thus within the cambial zone or differentiated from the cambial zone, was then recorded by radial position, the number of cells inward from the previous year's phloem (Figures 9 and 10). For example, in tree 4 (Figure 9), the first two cells inward from the previous year's phloem (positions 1 and 2) were, on the basis of radial enlargement, outside the cambial zone in all files. At position 3 there were meristematic cells in 28 percent of the files. At positions 4 through 12 there were meristematic cells in 100 percent of the files. Inward
Figures 9, 10. The number of periclinaly dividing cells (solid circles) and the percentage of radial files in the cambial zone (open circles) by radial position. --Figure 9. Tree number 4, May 10 and 20, 1965. --Figure 10. Tree number 6, June 22-24, 1965.
from position 12 the percentage of files having meristematic cells declined progressively until, at position 20, none of the files had cambial zone cells.

As expected, the number of divisions increased abruptly on the phloem side of the cambial zone, reached a maximum plateau in the middle of the zone, and declined over a considerable radial distance on the xylem side. The percentage of files with cambial zone cells showed a similar pattern. The decline of the former, however, is not in proportion to the latter; frequency of division declines more rapidly suggesting that the division rate is less toward the cambial zone margins. On the other hand, it is possible that some cells, counted as meristematic solely on the basis of not having enlarged radially, may have differentiated from the cambial zone and been in early stages of development. For the present, the mitotic indices presented in this paper should be considered as estimates of the average rate of division; some cells, namely those near the middle of the cambial zone, may be dividing at a faster rate than others.
SEASONAL PATTERN OF CAMBIAL ACTIVITY

Early Season Period

In the first part of the growing season there was a period of increase in the cambial zone cell population (NCZ), marked at the beginning by the first mitoses of the season, and at the end by radial enlargement of the first new xylem and phloem elements to be produced from the reactivated cambial zone. Each of the previously dormant cambial zone fusiform cells divided periclinally, usually just once during this period, thus approximately doubling the NCZ.

There is a striking difference in length of the early season period in the two study years. May temperatures in 1964 (Figure 15) were abnormally low and no mitosis were observed until May 28, shortly after the appearance of a warm, static, high pressure area which triggered a burst of activity; mitotic indices were unusually high (Figure 16) and within one week the first newly produced and radially enlarging tracheids and sieve cells were observed (Figures 17, 20). In contrast, the 1965 spring warming trend began much earlier (about May 2, Figure 11) and was gradual, extending the early season period to about 16 days. In tree 2, the first periclinal divisions, both in fusiform and ray cells, occurred about May 6 at the upper stem level and about May 10-12 at b.h. (Figure 12). Radially
Figure 11. Daily maximum and minimum temperatures during May, 1965. Readings were taken at the University Agricultural Experiment Station, College, Alaska, located about two miles from the 1965 sample area.

Figure 12. Mean core mitotic indices and confidence limits for samples taken at lower (b. h.) and upper (two-thirds of total stem height) stem levels of tree number 2.

Figure 13. Mean radial cell number in the cambial zone (NCZ), 1965 xylem, and 1965 phloem (precocious sieve cells included) at upper and lower stem levels of tree number 2.
enlarging tracheids first appeared about May 22 at both stem levels (Figures 13, 14).

Periclinal divisions during the first week of mitotic activity in 1965 did not appear to be more prevalent in one part of the cambial zone than another. From a few transections available from samples taken during the first week from trees 2 and 3, the number of periclinal divisions, as indicated by mitotic nuclei or phragmoplasts, were observed and recorded by radial distance from the previous year's phloem (Tables 2 and 3). The first two to three cells next to the fully developed cells of the previous year's phloem were nonmeristematic, radially enlarged precocious sieve cells (Figures 32, 57-59). On the xylem side of the cambial zone, meristematic cells bordered the latewood tracheids of the previous year as indicated by divisions (Figures 57-59) and the usual appearance of newly formed, thin tangential walls dividing most of these cells after a week or two of activity (Figures 33, 34). There is a slight suggestion from the data in Tables 2 and 3 that the very first divisions of the season, those occurring within a day or two of the first mitoses, may take place in cells nearest the xylem and phloem. On May 6 at the upper stem level in trees 2 and 3, and on May 10 at b.h. in tree 3, the few divisions observed were toward the margins of the cambial zone, none in the middle. For the remainder of the first week, dividing cells seemed randomly distributed. The frequency of
Figure 14. Mean radial cell number in the cambial zone (NCZ), 1965 xylem, and 1965 phloem (precocious sieve cells included) at upper and lower stem levels of tree number 3.
Table 2. Frequency of periclinal divisions across the cambial zone at upper stem level during the first week of mitotic activity

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<td>5.3</td>
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Table 3. Frequency of periclinal divisions across the cambial zone at b.h. during the first week of mitotic activity

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<th>Tree no.</th>
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<th>Mitotic index, %</th>
<th>Radial distance from the previous year's phloem, no. of cells</th>
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divisions across the cambial zone after about ten days of mitotic activity (May 20 at b.h.) is shown in Figure 9 and the appearance of the cambial zone at that time in Figures 33 and 34.

Whether divisions begin on the xylem or phloem side of the cambial zone seems immaterial with regard to differentiation of new tissue since the differentiation of new xylem and phloem cells did not begin until all the fusiform cells present in the dormant cambial zone had undergone one periclinal division. Figures 33 and 34 show the cambial zone two days prior to the time that the first radial enlargement of differentiating tracheids was observed. The thin tangential walls that regularly alternate with the thicker tangential walls convincingly illustrates doubling of the NCZ during the early season period.

There was an appreciable amount of variation in mitotic index in the early season period with both unusually high (greater than 5 percent) and low (less than 1 percent) values. Daily minimum temperatures between May 6 and May 19, 1965 (Figure 11) were consistently at or below the freezing level contributing, perhaps, to sporadic bursts and decline of activity. Doubling of the NCZ seems to have occurred around May 20 to 24, 1965, coincident with the first appearance of newly produced tracheids and sieve cells. In trees 2 and 3, the NCZ and mitotic index then tended to level off and remain fairly uniform, indicating that rate of periclinal division and
derivative production were in equilibrium.

**Grand Period**

In 1964, rate of cell division was fairly uniform as indicated by mitotic indices (Figure 16) ranging mostly between one and three percent for a 40- to-45 day grand period of growth when about 80 percent of the annual radial xylem increment was produced (Figures 17 and 18). Cumulative rate of xylem production also remained quite constant and relatively the same for all crown classes during this period both in 1964 (Figures 17 and 18) and 1965 (Figures 24 and 25).

Doubling of the NCZ and production of the first derivatives marked the beginning of the grand period at about June 3, 1964 and May 22, 1965. A rather abrupt lengthening of the cell division cycle initiated a sequence of events, discussed in the next section, marking the end of the grand period in 1964. Growing season samples were not taken after July 7, 1965 so that the terminal date of the grand period was not determined that year.

The actual rate of derivative production during the grand period was related to NCZ but not to rate of cell division. The more vigorous the tree in terms of annual xylem increment, the larger the NCZ (Figure 2) and rate of tracheid production (Figures 23, 29). Tree vigor, on the other hand, was unrelated to mitotic index (Figures...
Figure 15. Daily maximum (open circles) and minimum (closed circles) temperatures May through August 1964. Readings taken at the University Agricultural Experiment Station, College, Alaska, located 25 miles east of and 400 feet lower than the 1964 sample area. Temperatures in the 1964 sample area, measured only intermittently, were consistently lower than at the Experiment Station.

Figure 16. Mean core mitotic indices (closed circles) and mean radial cell numbers in the cambial zone (open circles connected by solid line) of dominant trees sampled during the 1964 growing season.
Figures 17, 18. The seasonal course of xylem increment in 1964. Mean number of 1964 tracheids per file at the time of sampling expressed as a percentage of the mean number of 1963 tracheids per file. — Figure 17. Individual samples by crown class. — Figure 18. Mean increment and confidence limits for each sample date through July 14, and for 28 dominant tree samples (August 30) taken after the end of the 1964 growing season.
Thus size of the cambial zone cell population, not length of the cell division cycle, was the major factor affecting differences in growth rate.

Production of new xylem and phloem derivatives began at about the same time in 1964, but cumulative production in the phloem, unlike the xylem, did not appear relatively the same for all crown classes (Figure 19). In slow growing suppressed trees, all the cells that would be completely developed in 1964 had been produced by the end of June, while in vigorous dominant trees obvious production of new phloem tissue continued into August. Noticeably developed cells do not, however, represent all of the year's increment. It was mentioned previously that partially developed, immature sieve cells overwinter next to the cambial zone and that the radial number of these cells seemed to be two to three in all trees regardless of vigor. This observation is supported by the "a" constant in Figure 3: if the curve is extended to zero on the X axis, radial cell number in the phloem is 2.5, the number of cells that overwinter in an incipient stage of development. Including these precocious sieve cells, derived from the previous year's cambial activity, with the current year's derivative production gives a distorted picture of derivative production from the current year's cambial activity. For example, in a very slow-growing tree annually producing four phloem cells per radial file, only two of the cells will be completely developed in
Figures 19-21. The seasonal course of phloem increment in 1964. Mean number of 1964 phloem cells per file at the time of sampling expressed as a percentage of the mean number of 1963 cells per file. --Figure 19. Individual unadjusted values by crown class. --Figure 20. Individual adjusted values by crown class. Precocious sieve cells differentiated in 1963 excluded from 1964 increment. --Figure 21. Mean increment and confidence limits for each sample date through July 14 from adjusted values as in Figure 20.
1964 Phloem Increment, percentage of total produced in 1963

- Dominant trees
- Intermediate trees
- Suppressed trees

June July August
Figures 22, 23. The relationship between cambial activity and annual xylem increment, a measure of tree vigor, for samples collected during the 1964 grand period, June 1-July 14. --Figure 22. Mitotic index was not related to tree vigor. Solid line depicts mean mitotic index (2.28) for all samples during the grand period; dashed lines represent confidence limits. --Figure 23. Rate of tracheid production was related to tree vigor as shown.
Figures 24, 25.

The seasonal course of xylem increment in 1965.

- Figure 24. Mean increment and confidence limits for each sample date.
- Figure 25. Mean number of 1965 tracheids per file at the time of sampling expressed as a percentage of the mean number of 1964 tracheids per file. All trees dominant.

1965 Xylem Increment, percentage of total produced in 1964.
Figures 26, 27. The seasonal course of phloem increment in 1965. Mean adjusted number of 1965 phloem cells per file at the time of sampling expressed as a percentage of the mean number of 1964 cells per file. All trees dominant. --Figure 26. Individual samples. --Figure 27. Mean increment and confidence limits for each sample date.
Figures 28, 29. The relationship between cambial activity and annual xylem increment, a measure of tree vigor, for samples collected during the 1965 grand period, May 22-July 7. --Figure 28. Mitotic index was not related to tree vigor. Solid line depicts mean mitotic index (2.52) for all samples during the grand period; dashed lines represent confidence limits.--Figure 29. Rate of tracheid production was related to tree vigor as shown.

\[ y = 0.015x \]
the year they are derived from the cambial zone, while the other two cells overwinter in a partially developed state next to the cambial zone. Therefore, at the beginning of the growing season, 50 percent of the phloem cells that will become fully developed that year are already present. On the other hand, in a fast-growing tree annually producing 14 phloem cells per radial file, only 14 percent of the cells that will become fully developed that year are present at the beginning of the year. An estimate of phloem production resulting solely from 1964 cambial activity was obtained by assuming that there are two precocious sieve cells per file overwintering in all trees, and arbitrarily subtracting these two cells from the radial cell number in the phloem of the 1964 samples. Use of the adjusted values (Figure 20) indicated that all trees had completed about the same relative amount of the total yearly phloem increment at any time of the growing season in contrast to Figure 19 which gives the impression that slow-growing trees completed the year's increment much earlier than fast-growing trees.

The regularity in appearance of the tangential bands of tannin-filled phloem parenchyma strands is a striking feature in the development of new phloem tissue. They appeared early in the grand period, shortly after differentiation of the first tracheids, and were first identifiable by their contents (Figures 60, 61) rather than radial enlargement. In contrast to sieve cells, they underwent relatively
little radial enlargement during development, and lacked secondary cell walls when mature (Figure 71). In both radial and tangential view, numerous transverse divisions were seen in the developing strands next to the cambial zone (Figures 64, 68). At this early stage structure of the cell contents was quite variable ranging from small globular or granular inclusions to large amorphous bodies (Figures 60-68). Within ten days, transverse divisions were completed in the oldest of the developing stands, and all members of these older strands were filled with a dark brown, amorphous or finely granular substance presumed to be mostly condensed tannins (Figures 69, 70). In most intermediate and dominant trees of the two sampled stands, the entire annual production of parenchyma strands was included in one tangential band (Figure 31). In vigorous, open-grown dominants, additional parenchyma strands were scattered randomly in radial files after production of the tangential band (Figure 72), while in slow-growing, suppressed trees the tangential bands were only produced in alternate years (Figure 73).

Regardless of growth rate, however, the ratio of parenchyma strands to sieve cells produced by the cambium was, on the average 13.4:1 (Figure 30). Furthermore, the bands of parenchyma strands always differentiated at about the same time in all trees at any given stem level; at b.h.: June 9 in 1964 and May 30, 1965, about eight days after appearance of the first differentiated tracheids. At the upper
Figure 30. The relationship between annual increment of phloem parenchyma strands and annual increment of sieve cells. Values for each of the 14 samples are based upon counts in two adjacent growth rings in each of 15 radial files.
stem level in trees 2 and 3 the parenchyma strands appeared four to six days earlier than at b.h. The stimulus, whatever it may be, that is responsible for development of phloem parenchyma strands rather than sieve cells moved basipetally from the apex of the stem.

**Late Season Period**

The limited number of samples from the late season period does not permit as detailed an analysis as for the early season period. However, the few samples from 1964, taken after July 14, clearly indicate that there was an extended period of relatively low cambial activity conspicuously marked at the beginning by a decline in NCZ (Figure 16).

Lengthening of the cell division cycle seemed to initiate the sequence of events associated with the late season period. Rate of cell production in the cambial zone must have dropped below the grand period level after July 14 to bring about the noticeable decline in NCZ on July 22. There was a coincident decline in mitotic index (Figure 16) further implicating lengthening of the cell division cycle. Rate of tracheid production in the latter part of July was still at about the grand period level (Figure 17), indicating that cambial zone cells were being differentiated faster than they were being produced, accounting for the decline in NCZ. By the end of July, NCZ was at the dormant level and rate of tracheid production also
had begun to decline as indicated by narrowing of the zone of developing tracheids.

Cambial divisions continued through August, 1964 but the level of activity was much reduced from that observed earlier in the season. By August 14, the zone of developing tracheids was almost eliminated. The cambial zone had begun to acquire a dormant appearance. Growth for the season was essentially over. At most, two or three latewood tracheids per file may have been produced in the last half of August, adding very little to the radial width of the xylem. Surprisingly, there were still a very few periclinal divisions on August 31 (less than one division per three thousand fusiform cells of the cambial zone), but from all other appearances derivative production had ceased, and nearly all cells of the cambial zone were dormant (Figure 44).

Figures 17 and 20 indicate that xylem and phloem production ended at about the same time. At least it seems safe to say that there was not much difference in the terminal dates of derivative production on the two sides of the cambial zone. The appearance of large and elongated nuclei, typical of dormant cells, in mid August suggests that some cambial zone cells may stop dividing before others. Whether these were first confined to one side of the cambial zone could not be determined because of the meager amount of sample material and low level of activity toward the end
of the season.

About 20 percent of the annual xylem increment, in terms of radial cell number, was produced during the late season period. It was mentioned previously, however, that radial xylem growth is controlled by radial enlargement of developing tracheids as well as rate of tracheid production. In the late season period, both controlling factors decline; fewer cells are produced and width of these cells becomes progressively less, so that in terms of actual radial width, late period increment is appreciably less than 20 percent of total annual xylem increment (Figures 74, 75).
DISCUSSION

Some general features of cambial activity in trees, repeatedly observed in independent studies, seem to be consistent phenomena. For example, the vernal reactivation of the cambial zone first near the apex of stems and twigs and then successively in a basipetal direction; variation in radial width of the cambial zone from one time of the year to another; and the vernal surge of activity and mid- to late-summer decline are well substantiated. Overwintering precocious sieve cells, the last growing season products of phloem mother cells, have been observed in many conifers and a few dicotyledons. Pseudotransverse anticlinal divisions described by Bailey (1923) have been verified in cambia of numerous tree species. Bannan (1950, 1951, 1953, 1957), Hejnowicz (1961) and others (Evert, 1960, 1963; Srivastava, 1963; Cheadle and Esau, 1964; Derr and Evert, 1967; Smith, 1967) have shown that the frequency of pseudotransverse divisions exceeds the needs of circumferentially expanding cambia, that many of the daughter initials decline and drop out of the cambium or are transformed into ray initials, that maximum ray contact is favorable to survival of the fusiform initials, and that pseudotransverse divisions occur more frequently toward the end of the growing season. Bannan (1954, 1960, 1962a, 1963a, 1963b, 1964) has repeatedly noted a relationship between xylem ring
width, tracheid length, and frequency of pseudotransverse divisions in several conifers. The relationship that he observed between cell length and ring width in white spruce was substantiated in this study and interpreted with regard to the ratio of periclinal to anticlinal divisions. Bannan (1957) has also provided convincing evidence supporting Sanio's original, and now generally accepted, concept of a single cambial initial in each radial file by observing that new files of cells originating from a pseudotransverse division of a fusiform initial extend radially in both directions—toward the xylem and toward the phloem—while occasional pseudotransverse divisions in mother cells cause only a temporary doubling of the original file in one direction.

Two interesting features of the seasonal pattern of cambial activity in Alaskan white spruce, corresponding to observations made by Wilson (1966) with *Pinus strobus* in New England, were the buildup of the NCZ during the early season period, and maintenance of this population in equilibrium with derivative production for an extended period of relatively constant mitotic activity. Wilson observed that the early season buildup of NCZ was followed by a period during which the number of cambial zone cells and rate of derivative production remained nearly in balance, with the former declining gradually as the season progressed. With Alaskan white spruce, however, there was not a detectable decline in NCZ or mitotic
activity for a 45- to 50-day grand period, although a slight decline could have been masked by sampling errors associated with estimates of mean cell population and mitotic activity. Nonetheless, there does seem to be an extended period between the early season buildup of NCZ and the midsummer decline in radial growth, when both rate of cell division and rate of derivative production remain nearly constant.

There is now considerable confirmation of the theory presented earlier by Wilson (1964) that radial growth rate of tree stems is independent of the rate of division of cambial zone cells. The amount of radial xylem growth, and probably the amount of radial phloem growth also, is primarily dependent upon NCZ. In this study and other recent studies by Wilson (1966), Gregory and Wilson (1968), and Sisson (1968) consistent relationships have been observed between NCZ and tree vigor. During any period of cambial activity there appears to be little difference in length of the division cycle within an internode, at different stem levels, or even between trees of a stand having vastly different growth rates. It seems, instead, that rate of derivative production is controlled chiefly by size of the cambial zone cell population. Indeed, there is a significant relationship in white spruce between NCZ at any time of the year, during the dormant season as well as the growing season, and annual radial increment in both the xylem and phloem.
Perhaps a certain amount of reservation is justified regarding this place-to-place uniformity in length of the division cycle. A small but real difference within and between trees of a stand is still a possibility. An appreciable difference in mitotic index was noted in white spruce growing at two extremes of its range (Gregory and Wilson, 1968) due, apparently, to long term inherent adjustments to different environmental conditions. Similar but smaller differences, not detectable due to the magnitude of sampling errors involved in estimating mitotic index, may exist within a region, between trees of a stand, or even within individual trees.

Whether reactivation of cambial activity in the spring occurs first in one particular area of the cambial zone is questionable. Bannan (1955a, 1962b), Grillos and Smith (1959), and Tepper and Hollis (1967) working with Thuja occidentalis, Pseudotsuga menzeisii, and Fraxinus americana respectively, report that the first divisions occur most frequently in the xylem mother cells next to the previous year's latewood. However, Evert (1963) and Derr and Evert (1967) working with Pyrus malus and Robinia pseudoacacia respectively, state that the first divisions were evenly distributed throughout the cambial zone. The contradiction may simply be due to different behavior in different species or, perhaps, to observation at different times relative to the actual beginning of mitotic activity. The question has significance because as Bannan (1955) and Romberger (1963)
point out the initiation of activity in the xylem mother cells may relate to supply of water, nutrients, and growth regulators. In this study, the few divisions observed within a day or two of the first mitoses were toward the margins of the cambial zone on both the xylem and phloem sides, but these data were too few to substantiate even a highly qualified statement about the actual site of the first divisions. During the remainder of the first week, however, numerous dividing cells were randomly scattered across the cambial zone. In the above references, estimates of the time of observation relative to the first mitoses and quantitative data supporting the observations were not given. Bannan did state that 70 percent of the divisions noted on the dates when mitoses were first observed were in the cells nearest the latewood tracheids. Data taken from white spruce samples collected at relatively long intervals, such as a week, do not allow one to accurately locate the site of the first mitoses, for within three to four days of the first activity periclinal divisions are scattered across the cambial zone. Intense, short-interval sampling would be necessary to answer the question, at least for white spruce.

There is also uncertainty whether all cambial zone cells in a radial file divide at the same rate. It has been shown by Bannan (1955), Wilson (1964), and in this study that the curve form of division frequency over radial distance from the phloem is usually bell-shaped and skewed toward the xylem. At first glance, the frequency
of mitosis curves suggest that the cell division rate is highest in the middle region of the cambial zone. As Wilson (1964) pointed out, however, the mixture of differentiating cells near the margins of the cambial zone could be entirely responsible for the reduced frequency of division near the margins. This is at least partly true for white spruce. The problem is likely to be solved only if meristematic cells can be accurately distinguished from similar appearing cells in the initial stage of differentiation.

Inception of dormancy in the cambial zone is gradual, extending over a period of three or more weeks in Alaskan white spruce. The decline in mitotic index at the beginning of the late season period (mid July) is probably due simply to a lengthening of the cell division cycle; all cells in the cambial zone had every appearance of being mitotically active during at least the first week or two of the late season period. By the middle of August some cells appeared typically dormant and mitotic activity was very low, suggesting that some of the cambial zone cells become dormant before others. It is not known if the cells that first appear dormant during the late season period are associated with a particular region of the cambial zone or are simply randomly scattered. By the end of August mitotic activity is virtually nonexistent and all cells of the cambial zone typically dormant.

Cambial activity and radial growth of white spruce
in the sub-arctic region of interior Alaska differ in at least two respects from other trees of this species at more southern latitudes. As reported recently (Gregory and Wilson, 1968), white spruce producing annually the same number of tracheids had a much shorter season for cambial activity in Alaska (65°N) than in New England, and rate of tracheid production was higher in Alaskan trees due to their higher rate of cell division.

Another interesting observation in the Alaskan trees was the diurnal variation in mitotic index at the beginning of summer when there is almost continuous daylight. Daylength, the period between sunrise and sunset, is over 22 hours at 65° north latitude on June 22, with sunset at approximately 11:00 p.m. and sunrise at 1:00 a.m. The interval between sunset and sunrise is a period of twilight rather than complete darkness; sampling at midnight was accomplished without any need for artificial light, even beneath the forest canopy. There was, though, a considerable diurnal range in temperature with the maximum in the sample area on June 22 at 68°F, and the minimum that night at 38°F. The next day's maximum was 69°F, followed by a nighttime low of 45°F. The observed diurnal variation in mitotic index on these dates may have been influenced more by diurnal temperature patterns than by light intensity.

It has been shown, here and elsewhere, that rate of tree growth is largely dependent upon growth rate of the secondary vascular
cambium. The cambium, as it grows circumferentially, gives rise to all secondary xylem and phloem in continuously increasing quantities. Rate of circumferential growth of the cambium is, in turn, dependent upon survival rate of the daughter initials following pseudo-transverse anticlinal division of the fusiform initials. It has been shown that daughter initial survival increases relative to increasing rate of radial derivative production by cells of the cambial zone, and that derivative production is dependent upon NCZ, not duration of the cell division cycle. Thus both rate of circumferential growth and radial production of wood and bark are dependent upon NCZ. An important question, then, is what controls NCZ? Or, more specifically, what controls the length of time that immediate derivatives of the fusiform initials, the xylem and phloem mother cells, remain meristematically active?
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1957. The relative frequency of the different types of anticlinal division in conifer cambium. Canadian Journal of Botany 35:875-884.


APPENDIX
Figures 31-34. Transections through the cambial zone and adjacent vascular tissues.

Figure 31. Part of the 1965 xylem (x), the dormant cambial zone (cz), and several years of phloem increment each with one tangential band of "tannin-filled" phloem parenchyma strands. Sample taken August 31, 1965. X100.

Figure 32. May 10, 1965, one to two days after the first mitoses in the cambial zone. Two to three precocious sieve cells (arrows) in each radial file show appreciable radial enlargement, variation in radial width of cambial zone fusiform cells is more noticeable than in the dormant state, and radial walls of the last developed 1964 sieve cells are collapsed. X250.

Figures 33, 34. May 20, 1965, eight to ten days after the beginning of mitotic activity, and about two days before appearance of the first radially enlarging tracheids. Precocious sieve cells (arrows) fully enlarged radially. Alternating thick and thin tangential walls indicate that most cambial zone cells had divided once. X400.

cz, cambial zone; p, phloem; r, ray, rc, resin canal; x, xylem.
Figures 35-39. Multiplication and decline of a fusiform initial recorded in 20 µ thick serial tangential sections from the middle of the conducting phloem (Figure 35) to the edge of the cambial zone (Figure 39). X100.

Figure 35. 80-100 µ from the cambial zone.

Figure 36. 60-80 µ from the cambial zone. Appearance of a pseudotransverse wall (arrow) indicating that the fusiform initial of that radial file had divided anticlinally.

Figure 37. 40-60 µ from the cambial zone. First products of the daughter initials appeared as vertical strands of phloem parenchyma (arrows). Most of the strand elements have connections with bordering sieve cells (see Figures 50, 51).

Figure 38. 20-40 µ from the cambial zone. Narrowing and shortening of subsequent derivatives indicating decline of both daughter initials. Vacated space has been filled by apical intrusive growth of adjacent initials.

Figure 39. 0-20 µ from the cambial zone. Absence of derivatives below the pseudotransverse partition indicating loss of the lower initial from the cambium.

Figures 40-42. Radial sections showing declining radial files (radial plates) similar to the one shown in Figures 35-39.

Figure 40. A radial plate of cells extending from within the cambial zone outward into the mature, conducting phloem. Asymmetric cambial zone cells, the surviving remnants of the declining file, can be seen in the lower half of the figure. X100.

Figure 41. Lower portion of the declining file shown in Figure 40. X250.

Figure 42. Remnants of a declining file in the phloem (left), cambial zone (cz) and xylem. X250.

cz, cambial zone; df, declining radial file; pps, phloem parenchyma strand
Figures 43-46. Tangential sections showing the appearance of the cambial zone at different times of the year. X100.

Figure 43. May 1, 1965. Dormant appearance: large elongate nuclei; beaded radial cell walls; dense, dark-staining cytoplasm.

Figure 44. August 31, 1965. Late summer dormant-appearance.

Figure 45. May 10, 1965. Appearance at the time of vernal reactivation showing two dividing cells, one with a mitotic nucleus, the other with small, rounded daughter nuclei separated by an extending, periclinally oriented cell plate.

Figure 46. May 20, 1965. Appearance at the end of the early season period. Highly active cambial zone with several periclinally dividing cells.

fr, fusiform ray; mn, mitotic nucleus; nu, nucleus; ph, phragmoplast.
Figure 47. Transection through dormant cambial zone and neighboring vascular tissues. There are five to eight cytoplasm-containing fusiform cells per radial file in the vicinity of the cambial zone; the outer two to three are precocious sieve cells, and the inner two to six are cambial zone cells. Innermost fully-developed sieve cells of the previous year (lsc) are collapsed and the outer tangential walls of the outermost precocious sieve cells have bulged into the collapsed areas. X404.

Figures 48, 49. Radial sections in the conducting phloem of samples taken July 22, 1965 and treated with periodic acid-Schiff's reagent. (Free hydroxyl groups of polysaccharide units remaining after fixation and dehydration were oxidized with periodic acid; resulting aldehydes stained sharply when complexed with N-sulfinic acid). Insoluble carbohydrates (cb) clustered at ends of the sieve cells indicate massive disruption of cell contents probably due to release of pressure in the conducting elements when the samples were removed from the trees. Radial file of cells in Figure 49 illustrates absence of apical growth in developing sieve cells. -- Figure 48, X400. -- Figure 49, X250.

cb, carbohydrate bodies; cz, cambial zone; lsc, late season sieve cell; lt, latewood tracheids; sc, sieve cell.
Figures 50, 51. Tangential sections from conducting phloem showing vertical albuminous elements (va) in strands of phloem parenchyma derived from declining fusiform initials as in Figures 37-39. X1000.

Figure 52. Transection from phloem of a dormant season sample. Thin-walled, late season sieve cells (lsc) mark the boundaries of annual rings. Other sieve cells are wider radially and have thicker secondary walls. X250.

Figure 53. Radial section from a sample taken in the late season period (July 31, 1964). Conducting phloem (cp) comprises all the 1964 increment. All 1963 sieve cells nonfunctional with massive definitive callose (dca) in those that had most recently become nonfunctional. X100.

Figures 54-56. Radial sections showing sieve areas. X1000.

Figure 54. Empty pores in the sieve areas of a nonfunctional sieve cell.

Figure 55. Callose-plugged sieve area pores in a quick-killed winter sample.

Figure 56. Sieve area pores plugged with wound callose in a normally treated (see methods) growing season sample.

cp, conducting phloem; cz, cambial zone; dca, definitive callose; lsc, late season sieve cell; ncp, nonconducting phloem; sa, sieve area; sc, sieve cell; sw, secondary wall; va, vertical albuminous element.
Figures 57-59. Transections showing dividing cambial zone cells (arrows) in samples taken during the first week of mitotic activity in 1965. X400.


Figure 60. May 22. Tangential view of developing phloem at the edge of the cambial zone. Occasional developing strands (pp) with scattered, relatively large inclusions (arrows) first appeared this date in samples taken at two-day intervals. X250.

Figure 61. May 22. Radial view of developing strand at the edge of the cambial zone. X250.

Figure 62. May 24. Tangential view showing developing strands, now fairly abundant, two to three days after their first appearance. X100.

Figure 63. Same section as Figure 62 showing appearance of finely granular substance in the older developing strands in addition to the first observed, larger cell inclusions. X250.

cp, cell plate; cz, cambial zone; ph, phragmoplast; pp, developing phloem parenchyma strand.

Figure 64. May 24. Telophase nucleus in a transversely dividing strand (td) in tangential view. X250.

Figure 65. May 26. Tangential view of the abundant strands in various stages of development four to five days after their first appearance. Elements of older strands separated by transverse walls, and filled with a very fine granular or amorphous substance. X100.

Figure 66. May 30. Tangential view of developing strands eight to nine days after their first appearance. Most strands well advanced; the more recently produced strands are in early stages of development. X100.

Figure 67. Same section as Figure 66 showing nature of cell contents and transverse walls (arrows) separating elements in older strands. X250.

Figure 68. Same section as Figures 66 and 67 showing metaphase nucleus in a transversely dividing strand. X250.

Figure 69. June 1. Tangential view of strands 10 to 11 days after their first appearance. Oldest strands appear fully developed. X100.

td, transverse division.
Figure 70. June 22. Tangential view of well developed phloem parenchyma strands one month after their first appearance. Strands from the same sample are shown in transection in Figures 71 and 72. X100.

Figure 71. Transection through recently produced phloem illuminated with polarized light showing sieve cells with highly birefringent secondary walls in contrast to non- or very-weakly birefringent walls of cambial zone cells and phloem parenchyma strands. X400.

Figure 72. Transection from the phloem of a fast-growing dominant stem sampled June 22, 1965, showing a tangential band of tannin-filled phloem parenchyma strands plus additional scattered strands in each annual ring. X100.

Figure 73. Transection from a slow-growing suppressed stem sampled January, 1965 showing the occurrence of tangential bands of tannin-filled phloem parenchyma strands only in alternate years. X100.

cz, cambial zone.
Figures 74, 75. Transections through 1964 xylem from two dormant stems, illustrating a comparison between radial tracheid number and radial xylem width for the grand and late season periods.
Late season period
18 tracheids
20% of radial cell no.
13% of radial width

Grand period
73 tracheids
80% of radial cell no.
87% of radial width

Late season period
14 tracheids
20% of radial cell no.
16% of radial width

Grand period
57 tracheids
80% of radial cell no.
84% of radial width