AN ABSTRACT OF THE THESIS OF

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Title: Effects of the Balsam Woolly Aphid (Adelges piceae (Ratzburg)) on the Cambial Activity of Grand Fir (Abies grandis (Dougl.) Lindl.) and Subalpine Fir (Abies lasiocarpa (Hook.) Nutt.)

Abstract approved: Redacted for Privacy
Dr. Frank H. Smith

Cambial activity was studied in trees infested by the balsam woolly aphid and in non-infested trees of grand and subalpine fir. Infested and non-infested samples of grand fir were collected near Corvallis during 1968 and 1969. Samples of subalpine fir, non-infested and infested, were collected near Odell Lake, at approximately 5,000 ft. elevation, during 1969. Light infestations of the aphid were observed in both species.

No differences within species between infested and non-infested trees were noted in the number of overwintering immature (precocious) sieve cells or in the dates for reactivation and cessation of mitotic activity in the cambial zone, or in the number of cambial zone cells per radial file. The relative amounts of xylem and

phloem present at any given time during the growing season were the same regardless of the presence or absence of aphids.

Significant differences were noted in the lengths of the fusiform initials in the infested samples when compared to non-infested samples in both species. Xylem production in grand fir, and phloem production in both species was significantly greater in infested samples. A greater number of phloem parenchyma strands were observed in infested samples of grand fir than in non-infested samples.

The rate of cell division, as indicated by the mitotic indices, showed no significant differences between samples of infested and non-infested trees of either species. Differences in xylem and phloem production between infested and non-infested trees could not be attributed to either an increased rate of cell division or an increase in the number of cambial zone cells per radial file in infested samples for either species.

Effects of the Balsam Woolly Aphid (*Adelges piceae* (Ratzburg)) on the Cambial Activity of Grand Fir (*Abies grandis* (Dougl.) Lindl.) and Subalpine Fir (*Abies lasiocarpa* (Hook.) Nutt.)

by

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EFFECTS OF THE BALSAM WOOLLY APHID (ADELGES PICEAE (RATZBURG)) ON THE CAMBIAL ACTIVITY OF GRAND FIR (ABIES GRANDIS (DOUGL.) LINDL.) AND SUB-ALPINE FIR (ABIES LASIOCARPA (HOOK.) NUTT.)

INTRODUCTION

Studies of infestations of the balsam woolly aphid (Adelges piceae (Ratzburg)) on true firs have concentrated on the anatomical changes in the trees brought about by infestations of the aphid as well as studies of the aphid itself. These studies have demonstrated that as the aphid feeds on the tree it injects a salivary substance, the nature of which is unclear (Balch, et al., 1964), which produces abnormal reactions in the cells of the vascular cambium and differentiating tissues of the xylem (Doerksen and Mitchell, 1965) and phloem (Saigo, 1969). The type and amount of damage to the tree caused by the aphid depends upon the size and location of the aphid population on the tree. Attacks of the aphid population on the stem result in the formation of annual rings containing wood which is reddish, hard and resembles compression wood. If the aphid attack is centered on the shoots, they develop "gout" and become swollen and distorted, particularly at the nodes and terminal buds with the result that shoot growth is inhibited or halted entirely (Balch, 1952).

The two species of true firs selected for this study, grand fir (Abies grandis (Dougl.) Lindl.) and subalpine or alpine fir (Abies

lasiocarpa (Hook.) Nutt.) vary in their susceptability to the aphid and their infestation characteristics (Mitchell, 1966). Of the true firs found in the Pacific Northwest, grand fir is the most resistant to the aphid, whereas subalpine fir is the most sensitive. Trees of grand fir have been known to endure heavy infestations for fifteen years and survive while trees of subalpine fir are commonly killed in four to five years following the initial infestation. Infestations in grand fir characteristically start on the lower portion of the main stem, while subalpine fir infestations typically start high on the tree stem. Damage in both species is caused largely by bole infestations, rather than injury stemming from gouting of the shoots.

Investigations of the seasonal activity of the vascular cambium of true firs infested by the balsam woolly aphid have centered mainly on an analysis of its derivatives. No studies, however, have been made on the seasonal course of cambial activity in trees which are infested by the aphid. The objective of this study, therefore, has been to study the seasonal pattern of cambial activity in non-infested and infested trees of two species of true fir, grand and subalpine fir.

MATERIALS AND METHODS

Sample materials of grand fir from non-infested trees and trees infested with the balsam woolly aphid were collected from two locations near Corvallis during 1968, and one location during 1969. Beginning May 2, 1968 samples consisting of bark, cambial zone, and usually all of the previous year's xylem were removed from four non-infested and four infested trees, ranging in diameter at breast height (dbh) from 12 to 32 inches. Subsequent samples were collected from the same trees at weekly intervals through July and at two week intervals during August and September. During 1969 samples were collected from two infested trees of grand fir, both approximately 24 inches (dbh) once a week from the beginning of April through July and twice during August. An estimate of the number of aphids on the boles of the infested trees sampled in 1968 was made by making bi-weekly counts of aphids present in each of six one-inch-square bark plots. A mean number of aphids per square inch was then determined for each sampling date.

Subalpine fir samples were collected during 1969 from trees located near Odell Lake, Oregon at approximately 5, 000 feet in elevation. Seven trees, three non-infested and four with aphids, ranging from 10 to 30 inches (dbh) were sampled at weekly intervals from May through July and twice during August.

The 1968 grand fir samples were collected following the procedure described by Wilson (1966). Each sample consisting of the bark, cambial zone, and in most cases the entire previous year's xylem increment, measured 1.0 cm x 2.5 cm on the tree surface with the greater length being parallel to the longitudinal axis of the stem. The samples removed from the non-infested trees during the growing season were not selected at random but were spaced to obtain an even distribution and to avoid bark irregularities. Samples taken from infested trees were also spaced to obtain an even distribution but in addition samples were removed from areas which contained as many aphids as possible.

Grand fir and subalpine fir samples obtained in 1969 were removed in a different manner. Cores of bark, cambial zone and xylem tissues were obtained by making a circular incision in the bole using a battery powered drill equipped with a 1-1/4 inch hole saw (Carr, 1971). A hole 1/8 inch in diameter was then drilled through the center of the core to facilitate fixation and infiltration. Rectangular pieces of tissue on opposite sides of the circular core were then removed so that a chisel could be hammered behind the core of tissue and the core pried out. As in the case of the 1968 samples, the 1969 samples were spaced to obtain an even distribution on the stem, and in the case of infested trees to include as many aphids as possible.

All samples were fixed in Navashin's III, dehydrated with

tertiary butyl alcohol and paraffin oil, following the procedure described in Johansen (1940), infiltrated with 56°C Tissuemat, with three changes at four hour intervals, and then left overnight. The infiltrated samples were cooled and subdivided, trimmed to remove excess bark, and then reinfiltrated in a vacuum oven under tension at 64°C with two changes of 61°C Tissuemat at 1/2 hour intervals. The infiltrated samples were then embedded for sectioning.

Serial tangential, radial and transverse sections of the cambial zone, bark and at least one annual increment of xylem were made at 20μ on a rotary microtome. Prior to cutting the cross sections, the transverse surface was exposed and soaked overnight in a solution of glycerin and detergent (Alcorn and Ark, 1953). The serial tangential sections were stained with iron hematoxylin and cross sections with iron hematoxylin-safranin using the schedules of Jensen (1962). Radial sections were stained with tannic acid and lacmoid as described by Cheadle, Gifford and Esau (1953).

The average numbers of xylem and phloem cells produced the previous year, and during the year of sampling, were determined from the transactions. Mean values for xylem and phloem increments used in the text were based on the number of xylem or phloem elements observed in ten radial files for each sample. The mean number of cells per radial file in the cambial zone (NCZ), consisting of initial cells and mother cells which are capable of further division

(Wilson, Wodzicki, and Zahner, 1966), was also determined by this method. Lack of radial enlargement was the principle criterion for separating cambial zone cells from their derivatives. No mitotic nuclei or phragmoplasts were observed in the developing tracheids and sieve cells which had undergone appreciable radial enlargement, indicating that radial enlargement is a valid criterion for separating cambial zone cells from their derivatives.

The method used for determining the mean number of cambial zone cells in mitosis per core for each sample was essentially the same as that described by Wilson (1966). Each core consisted of a series of 1.39 mm - diameter (100X) microscope fields located on successive tangential sections through the cambial zone. The total number of periclinally dividing nuclei for nine radially oriented cores through the cambial zone was used to obtain the average number of cambial zone cells in mitosis per core for each sample.

The mean size of the cambial zone fusiform cell population for each of the samples studied was the product of an estimate of the average number of radial files of fusiform cells per core, and the average number of cambial zone cells per radial file (Wilson, 1966). Radial files were counted from tangential sections as in counting mitosis, with only those radial files having one or more nuclei in the area being counted. The mean number of radial files per core for each sample was determined by averaging the number of radial files

observed in six different microscope fields (100X).

The mitotic index, or the percentage of fusiform cells in the cambial zone population in mitosis, was used to make comparisons of the rate of periclinal divisions between trees and over time. From the mitotic index for each of the nine cores, an average mitotic index for each sample was calculated and expressed as the average mitotic index per core.

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 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.

OBSERVATIONS

Aphid Population

Several studies have been made of the seasonal history of the balsam woolly aphid on true firs in the Pacific Northwest. Tunnoch and Rudinsky (1959) studied the life-cycle of aphids on grand fir, Johnson and Wright (1957) on silver fir while Mitchell, et al. (1961) studied the aphid on grand, silver and subalpine fir. In none of these studies was an estimate of the number of aphids typically present in heavy, moderate, and light infestations given. General descriptions of text figures in these papers, however, and consultation with others familiar with aphid infestations indicated that the infested trees sampled in this study were lightly infested. The mean number of aphids, obtained by counting the number of aphids present in each of six one-inch-square bark plots, ranged from 21.3 to 4.1 per square inch on the bole of the most heavily infested and from 5.6 to 0.6 on the least infested grand fir trees sampled in 1968. Visual observation of the 1969 trees of both species indicated that they were also lightly infested, especially those of subalpine fir, having approximately the same number of aphids, or less, than present on the 1968 grand fir trees.

Due to the small number of aphids present on the infested trees

of both species, comparisons made between infested and non-infested samples, in this study, possibly are not as valid as they might be had the trees been more heavily infested.

Cambial Zone

The cambial zone of conifers consists of the cambial initials and their immediate derivatives, the meristematically active xylem and phloem mother cells (Wilson, Wodzicki, and Zahner, 1966). These xylem and phloem mother cells may divide one or more times before differentiating into xylem or phloem elements.

Two types of cells are found in the cambial zone, the long radially flattened, tangentially tapered fusiform initials, and the relatively small, almost isodiametric ray initials. The fusiform initials and ray initials divide periclinally to produce radial files of cells making up the secondary xylem and phloem. In addition to the more common periclinal divisions, the fusiform initials occasionally undergo anticlinal divisions which result in an increase in number of fusiform initials. Some smaller cells produced as a result of unequal anticlinal divisions undergo progressive shortening due to further asymmetrical divisions and are lost, by differentiation, while others persist and give rise to new ray initials.

Seasonal changes in the cambial zone of non-infested trees have been described for many species (Bannan, 1955; Grillos and

Smith, 1959; Evert, 1960, 1963; Derr and Evert, 1967) as well as for infested trees of grand fir (Saigo, 1969). The appearance of the fusiform initials in the dormant cambial zone, of both non-infested and infested trees, of grand fir and subalpine fir samples is as described for other species. Nuclei of the dormant fusiform initials are elongated and the cytoplasm appears dense and stains heavily with hematoxylin. In cross section, the radial walls of the fusiform initials, with numerous propits, appear much thicker than they do during the growing season. In contrast, nuclei of the actively dividing fusiform initials appear smaller and more rounded than dormant nuclei, and the cytoplasm stains less intensely.

The dormant cambial zone of non-infested grand fir trees ranged from an average of six to eight cells, while the average number of cambial zone cells of infested grand fir trees ranged from five to eight. The average number of cambial zone cells of subalpine fir ranged from five to six and four to five cells in control and aphid infested trees respectively. The number of cambial zone cells in the dormant cambium is probably related to tree vigor rather than being correlated with aphid infestation (Figure 1). This relationship between the dormant NCZ and growth rate has also been demonstrated by Gregory (1969) in Picea.

In addition to the dormant fusiform initials, one or two "precocious" sieve cells (Grillos and Smith, 1959; Evert, 1960, 1963;

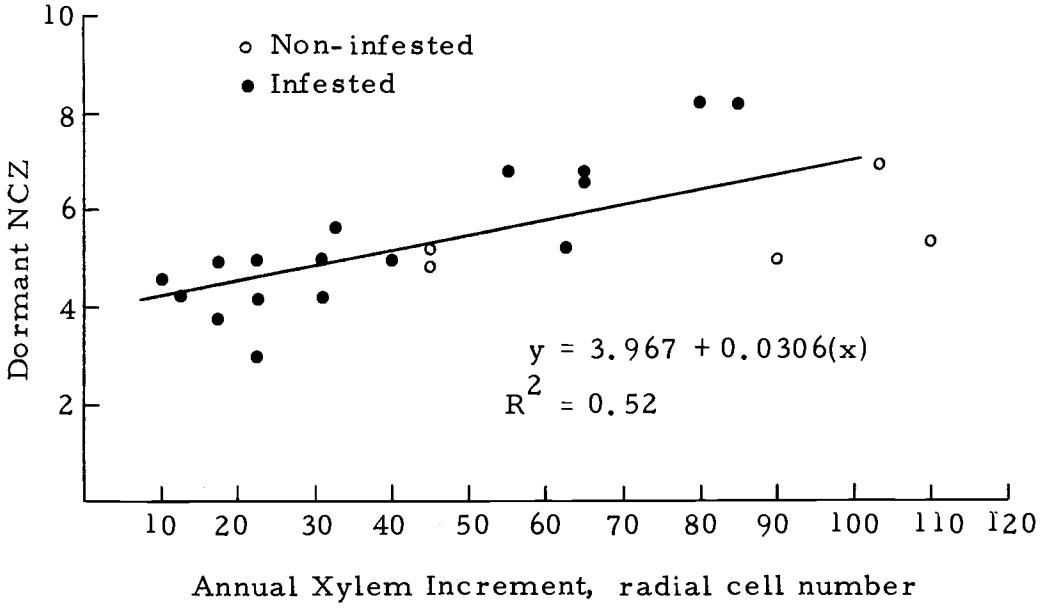


Figure 1. Relationship between the number of dormant cambial zone cells per radial file (NCZ) and the annual xylem increment in infested and non-infested trees of Abies lasiocarpa.

Gregory, 1969) were present in the dormant cambium of grand fir and one in samples of subalpine fir. The number of immature sieve cells appears to be independent of tree vigor and the presence or absence of aphids. These cells, although closely resembling the dormant undifferentiated fusiform cells of the cambial zone, are no longer capable of division. In the spring they undergo appreciable radial enlargement and rapidly mature into conducting phloem elements.

Samples of grand fir, from both non-infested and infested trees, were actively dividing when collected in April and May, with the cambial activity in all grand fir trees remaining at a high level from April through the first part of July. A high rate of cambial activity during this period was also noted by Grillos and Smith (1959) and Saigo (1969) for trees in the Corvallis area. Initiation of mitotic activity for trees in the Corvallis area was found to occur in March (Grillos and Smith, 1959; Saigo, 1969). A decrease in cell division occurred in August with mitotic activity ending first in slow growing trees and then in fast growing trees during September. Cell division in the cambium of subalpine fir trees did not begin until the middle of May when the first mitotic figures were observed in the cambial zone of rapidly growing trees. The most active period of growth for all subalpine fir trees, control and infested, occurred during June and the first half of July. Decreases in cell division were noted in all trees in the latter part of July with cessation of cambial activity in

August. For both species, the onset of cambial activity in the spring and cessation in the fall appeared to be dependent upon tree vigor and independent of whether or not the tree was infested.

The late season decline in cambial activity, in samples from both grand and subalpine fir, was accompanied by a decrease in the number of cambial zone cells per radial file. This decrease in NCZ was also accompanied by continued differentiation of tracheids, indicating that the cambial zone cells were being differentiated faster than they were being produced which would account for the reduction in number of cells per file in the cambial zone. This general pattern of end of the season activity was also noted by Wilson (1966) in Pinus and Gregory (1969)in Picea.

The frequency of anticlinal divisions in the cambial zone and the fate of the declining daughter initials was not investigated in this study, although evidence of anticlinal divisions was abundant in the phloem. Smith (1967) and Saigo (1969) studied these changes in non-infested and infested trees of Abies grandis. Smith studied the frequency of anticlinal divisions and the fate of those daughter initials which decline by an analysis of the xylem produced in an annual ring before infestation and from a ring produced after infestation. He found that growth after infestation was characterized by an increase in frequency of anticlinal division in the fusiform initials. Fifty-eight percent of the initials in the infested condition divided

anticlinally at least once and sometimes two to four times, compared to only 13.3% in the initials before infestation. Carr (1971) found that 17.7% of the fusiform initials in normal trees of Abies lasiocarpa undergo anticlinal division during the growing season, which is similar to the 13.3% found by Smith (1967).

In contrast to the other authors (Bannan, 1950, 1953; Evert, 1961; Srivastava, 1963; Cheadle and Esau, 1964) Smith found no evidence in the xylem that declining fusiform initials are converted into ray initials. The presence of larger rays, as noted by Mitchell (1967) in infested trees, was attributed to fusion of rays due to the decline of intervening fusiform initials, and an increase in transverse divisions in the ray initials. The more numerous rays in the infested grand fir wood, also noted by Mitchell (1967), was attributed to more frequent anticlinal divisions of the fusiform initials to produce new ray initials as described by Barghoorn (1940) and separation of rays by intrusion of rapidly elongating fusiform initials.

Saigo (1969) studied the frequency of anticlinal divisions in the cambial zone and the fate of the declining daughter initials in non-infested and infested grand fir trees through an analysis of cambial derivatives in the phloem. He found that the fusiform initials undergoing pseudotransverse division averaged 11% in non-infested trees and 38% in infested trees. In contrast to the findings of Smith (1967) for xylem, Saigo (1969) found that many of the declining daughter

initials in the phloem in both control and infested trees became ray initials. Both Smith (1967) and Saigo (1969) found that the distribution of anticlinal divisions in infested trees was scattered evenly throughout the growing season rather than being concentrated in the last half or third of the growing season as reported for non-infested trees of grand fir (Smith, 1967) and other species (Bannan, 1950, 1964).

The average length of the fusiform initials is influenced by frequency of anticlinal divisions. Studies of anticlinal divisions in normal growth of conifers show an inverse relationship between the two (Bannan, 1960, 1964). This relationship appears to hold for infested trees of grand fir and subalpine fir. Sieve cells, which closely replicate the length of the fusiform initials, were used to estimate the length of fusiform initials in non-infested and infested trees of both species of Abies. Mean lengths of the fusiform initials were calculated from the length of ten sieve cells in the conducting phloem for each sample. The 30 samples for each species (Tables 1, 2) were selected at random from the non-infested and infested samples collected during the course of the study. Within species the length of the fusiform initials, as reflected in the length of the derived sieve cells, of non-infested trees was significantly higher than the length of the initials in infested trees ($t = 5.42$, $df = 28$ subalpine fir; $t = 5.98$, $df = 28$ grand fir).

A significant relationship was observed between the mean number

Table 1. Mean lengths of fusiform initials in mm for control and infested samples from trees of Abies lasiocarpa. Mean values are based upon 10 randomly selected sieve cells in the conducting phloem of each sample.

	Mean fusiform initial length	
	Control	Infested
	2.81	2.41
	2.84	1.93
	2.53	1.74
	2.62	1.74
	2.26	1.83
	2.33	1.92
	2.24	2.10
	2.42	2.03
	2.20	2.14
	2.35	2.03
	2.17	1.64
		2.32
		2.24
		2.07
		2.15
		2.13
		1.70
		2.02
$\bar{X}$	2.45	1.96

Table 2. Mean lengths of fusiform initials in mm for control and infested samples from trees of Abies grandis. Mean values are based upon 10 randomly selected sieve cells in the conducting phloem of each sample.

	Mean fusiform initial length	
	Control	Infested
	2.65	1.98
	3.22	2.32
	2.66	2.02
	3.28	2.02
	2.76	2.04
	3.38	1.97
	2.65	1.90
	2.47	2.03
	2.42	2.10
	2.78	1.77
		1.87
		2.31
		2.12
		2.26
		2.09
		2.24
		2.96
		2.23
		2.57
		2.38
$\bar{X}$	2.85	2.15

of radial files and the mean length of fusiform initials for samples from control and infested trees of grand fir and subalpine fir (Figures 2, 3). The mean number of radial files for each sample was determined from six fields of view (100X) as described by Wilson (1966). All radial files having one or more nuclei in the field of view were counted. Since the nuclei are located near the center of the fusiform cells, files having one half or more of their longitudinal dimension in the field would be included. This relationship between length of initials and number of radial files was also demonstrated by Gregory (1969) in northern white spruce.

Secondary Xylem

Gymnosperm wood is composed primarily of tracheids produced in radial files through periclinal divisions of the initials and xylem mother cells in the cambial zone, with new radial files arising through anticlinal divisions of the fusiform initials. Mature tracheids are somewhat longer than the fusiform initials from which they are derived since both xylem mother cells and tracheids undergo apical intrusive growth (Bailey, 1920). In addition to the tracheids, small amounts of terminal xylem parenchyma strands make up the vertical system.

Infestations by the balsam woolly aphid cause production of compression wood or rotholz (Balch, 1952). Doerksen and Mitchell

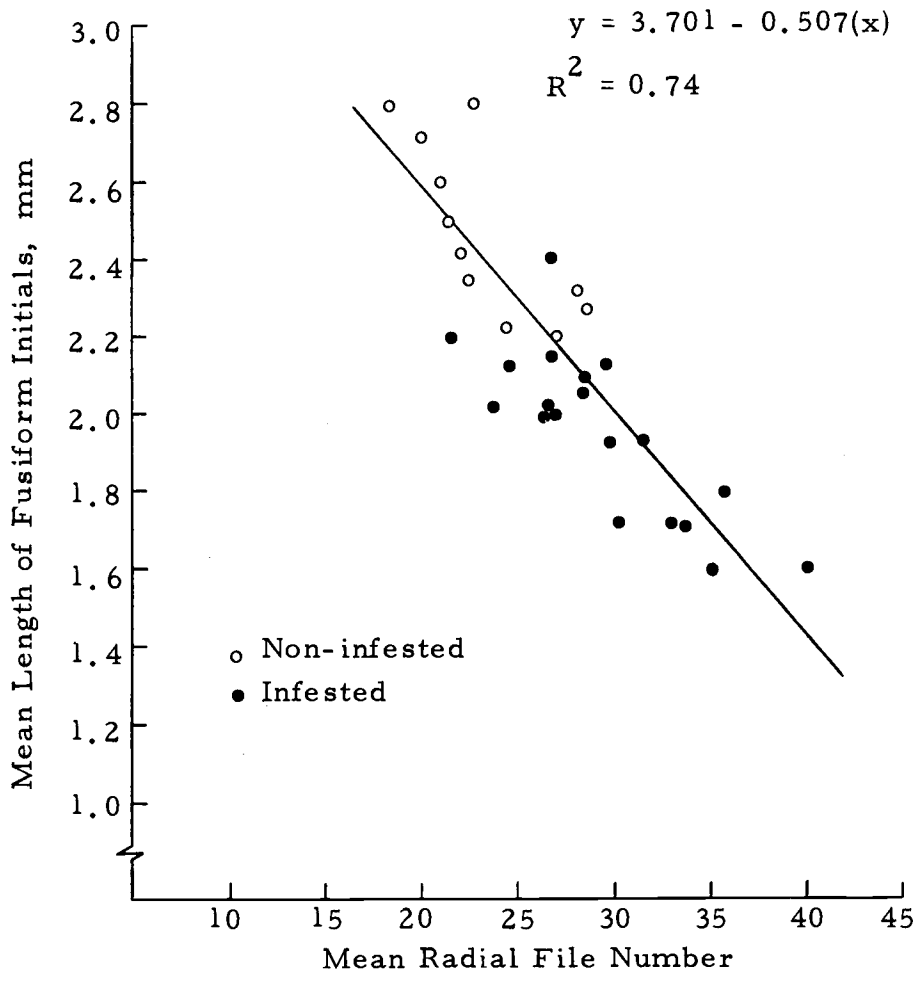


Figure 2. Relationship between the mean number of radial files per tangential field area and mean length of fusiform initials in Abies lasiocarpa. Mean initial length, in each of the 30 samples, is based on the measurement of 10 randomly selected sieve cells in the conducting phloem.

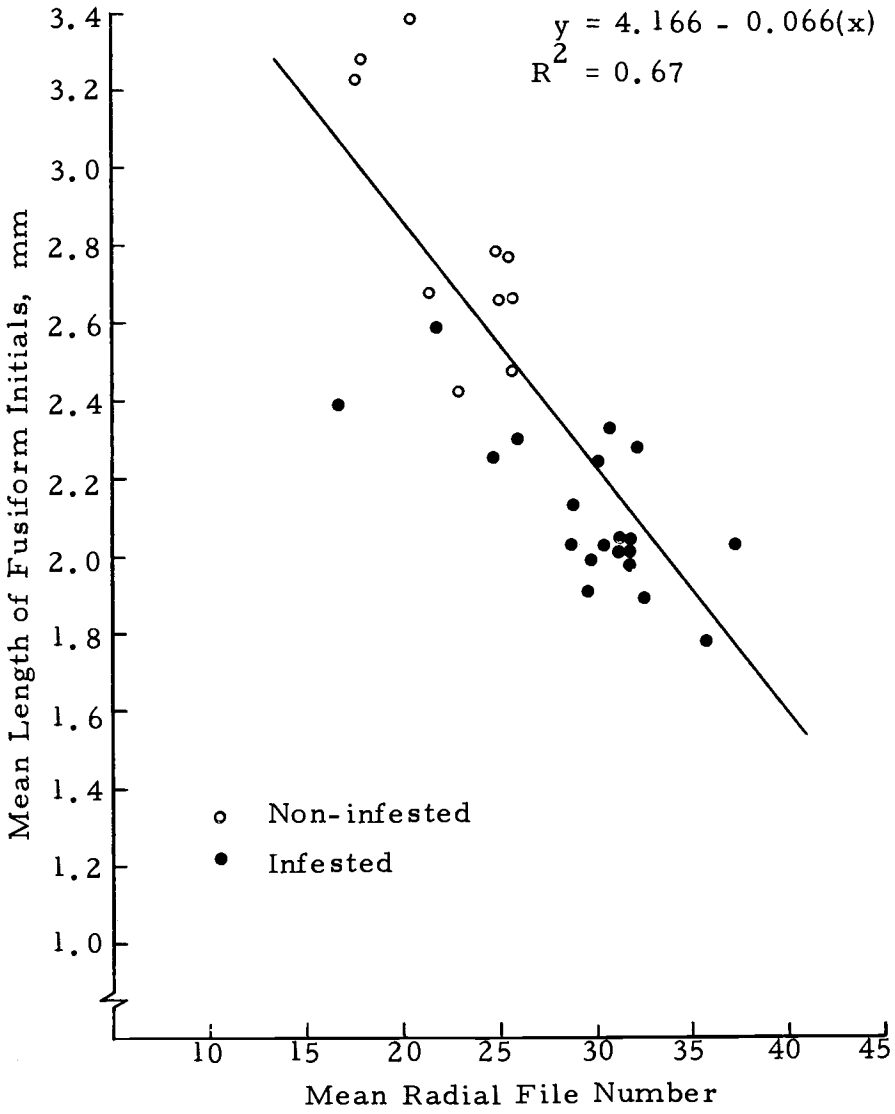


Figure 3. Relationship between the number of radial files per tangential field area and mean length of fusiform initials in Abies grandis. Mean initial length, in each of the 30 samples is based on the measurement of 10 randomly selected sieve cells in the conducting phloem.

(1956) noted that in addition to the greater proportion of summer wood-like tissue, the tracheids had thicker cell walls in the springwood and that the fibril angle of the tracheids was greater in infested wood than in non-infested wood in both A. grandis and A. lasiocarpa. They also found, as might be expected from the discussion of anticlinal divisions, that the average tracheid length of infested wood was 43% and 36% shorter than that of non-infested wood in grand fir and subalpine fir, respectively. Mitchell (1967) in a study of ray tissue, found that the wood from aphid infested trees had approximately double the number of rays of control wood, and that the rays tended to be both higher and wider than normal.

An analysis of xylem production over the growing season was made for non-infested and infested samples of subalpine fir (Figure 4). Unfortunately the entire current year's xylem increment along with the previous year's xylem increment was not available in many of the samples of grand fir so that a similar study could not be made. It appears that at any given date the percentage of xylem elements to be produced during a given year, based upon the prior year's xylem increment, was greater in infested trees than in control trees. This, however, may not be significant as in general the xylem increment for 1969 in infested trees equalled or surpassed that of 1968, while this was not the case in the 1969 increment of non-infested trees.

Differentiation of the first tracheids in all trees of subalpine fir

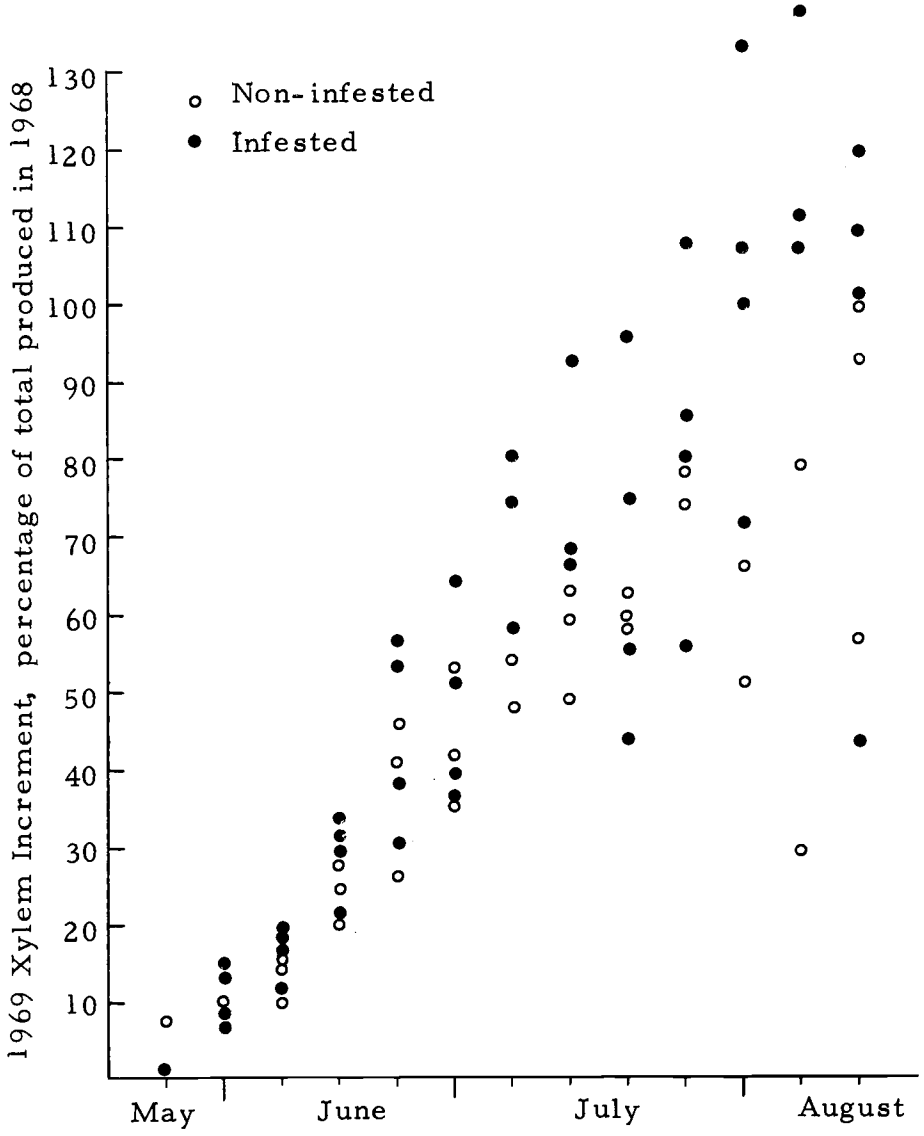


Figure 4. Seasonal course of xylem increment in 1969 for samples from control and infested trees of Abies lasiocarpa. Mean number of 1969 tracheids per file expressed as a percentage of the mean number of 1968 tracheids from the same sample.

regardless of the presence or absence of aphids or difference in growth rates, occurred at the same time, the third week in June. Differentiation of the first tracheids in grand fir occurred in the middle of May in all trees regardless of the presence or absence of aphids.

Production of the annual xylem increment began soon after the first mitosis in the spring. As noted by Wilson (1966), Gregory and Wilson (1968) and Gregory (1969), the amount of radial xylem growth, or tree vigor, appears to be related to the number of cambial zone cells per radial file during the grand period of growth (Figures 5, 6). While a significant increase in the xylem increment was noted in infested samples of grand fir over non-infested samples ($t = 3.53$, $df = 44$), no significant difference was noted in the NCZ between non-infested and infested trees. This suggests that the greater xylem production in infested samples (Figure 6) is due to the effects of the aphid rather than a simple increase in the NCZ. No significant differences in xylem production or in NCZ were evident between infested and control subalpine fir trees, perhaps due to a lower incidence of aphid infestation than in the grand fir trees.

An unusual number of traumatic resin canals are described as being a characteristic feature of wood from infested trees (Balch, 1952; Doerksen and Mitchell, 1965; Saigo, 1969). Resin canals, although not present in the wood of most samples, appeared to be

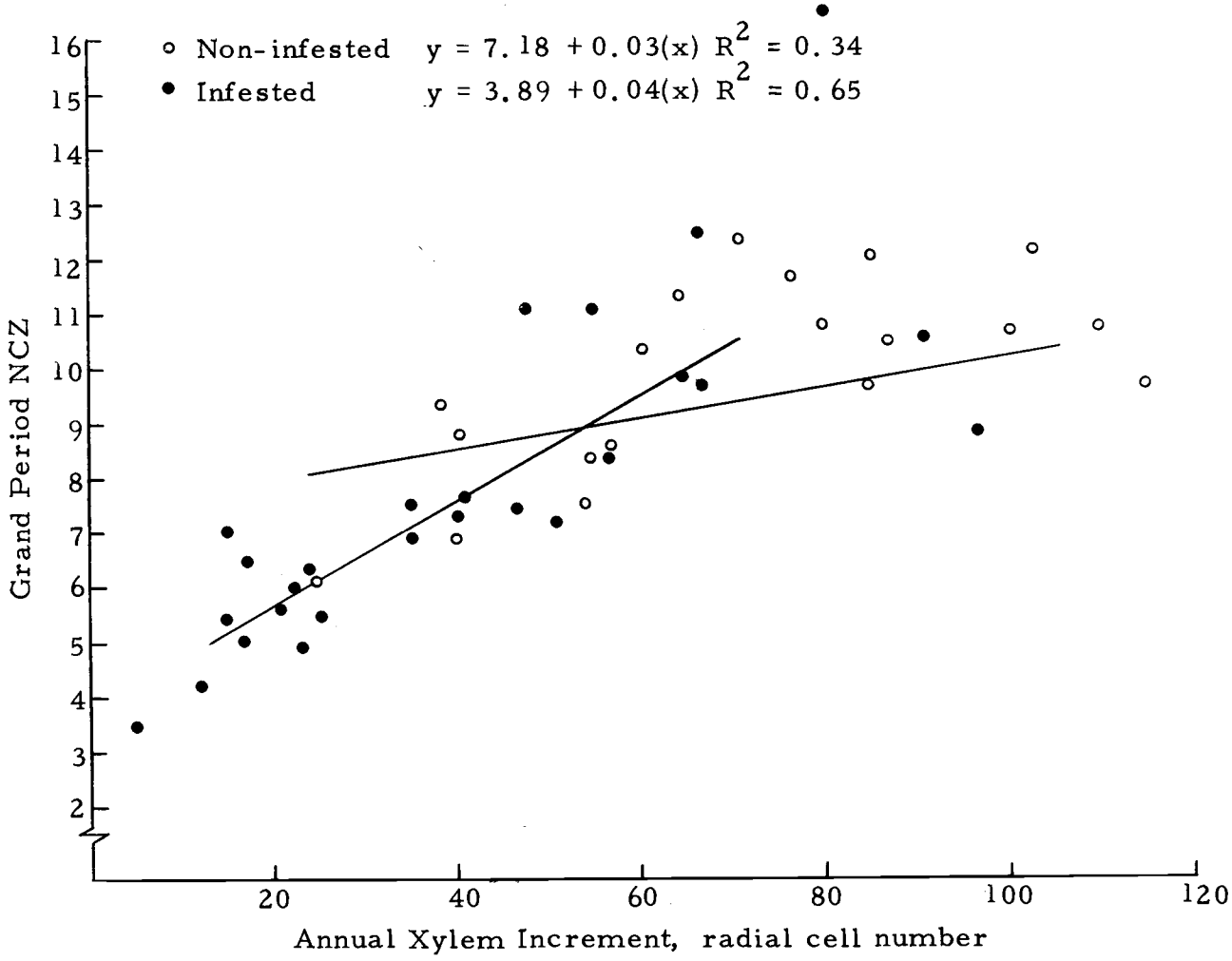


Figure 5. Relationship between the mean number of cambial zone cells per radial file (NCZ) and the mean annual xylem increment for non-infested and infested samples of Abies lasiocarpa.

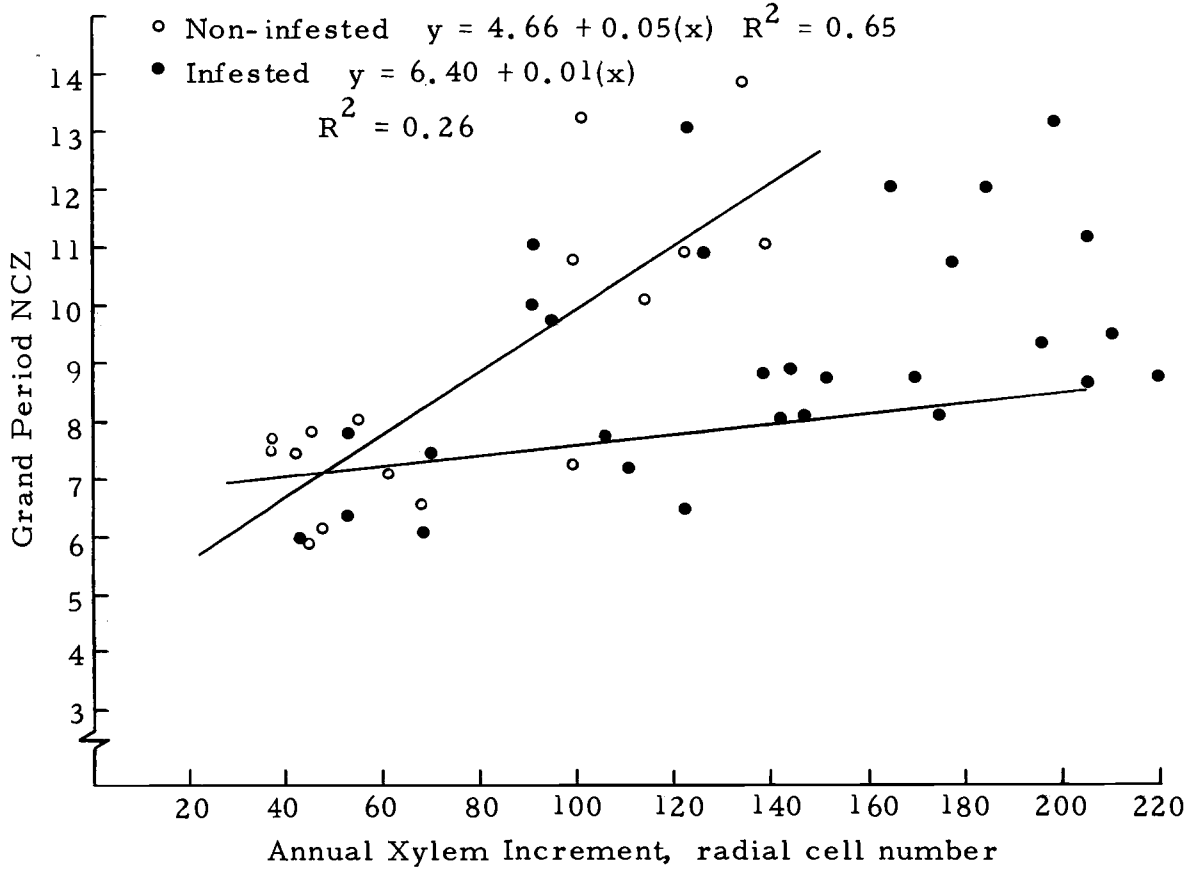


Figure 6. Relationship between the mean number of cambial zone cells per radial file (NCZ) and the mean annual xylem increment for non-infested and infested samples of Abies grandis.

present in relatively the same amounts in wood of both non-infested and infested trees of A. grandis in the 1968, 1969 growth rings. Samples of wood from trees of A. lasiocarpa, for the year of sampling and the prior year, did not have resin canals except for a single sample from an apparently non-infested tree. The presence of resin canals in non-infested trees of both species was probably due to the result of injury (Bannan, 1936). As noted in the literature, the resin canals, when present, appeared in more or less continuous tangential bands and were differentiated at any time from very early in the growing season until just before growth ceased. There did not seem to be any correlation between the appearance of the resin canals and the number of aphids; however, the number of aphids present may not have been sufficient for such relationships to appear. The lack of these traumatic resin canals in samples of infested trees of subalpine fir might also be attributed to comparatively small numbers of aphids, as Doerksen and Mitchell (1956) found an unusual proliferation of traumatic resin canals in the wood of infested trees of both grand and subalpine fir.

Secondary Phloem

The general structure of the secondary phloem in conifers has been described in detail in the literature. Srivastava (1963) reviewed much of the past work on the secondary phloem, and reinvestigated

the structure of phloem in the Pinaceae. Grillos and Smith (1959) described the structure and seasonal changes in the phloem of Douglas-fir. Saigo (1969) described the secondary phloem in grand fir and the changes brought about by infestation by the balsam woolly aphid.

The axial system of the functional secondary phloem of Abies consists of radial files of sieve cells interrupted by tangential bands of tannin filled parenchyma strands. Both sieve cells and the parenchyma strands are derived from the fusiform initials and closely resemble the tangential and longitudinal dimensions of cells from which they are derived. The last sieve cells produced during a given growing season are less radially enlarged and are crushed before differentiation of the precocious sieve cells of the following spring. Each year's phloem increment is thus bordered by tangential bands of these crushed cells allowing the limits of a given year's increment to be determined. The phloem increment for at least two years in samples of grand fir and four years in subalpine fir samples could be determined by this means before the tissue became too distorted.

An analysis of the pattern of phloem production, similar to that made for xylem production in subalpine fir, was made for infested and non-infested samples of subalpine and grand fir (Figures 7, 8). To obtain a more representative picture of phloem production, in this analysis, the immature sieve cells which overwinter next to the cambial zone and appear in number independent of tree vigor or the

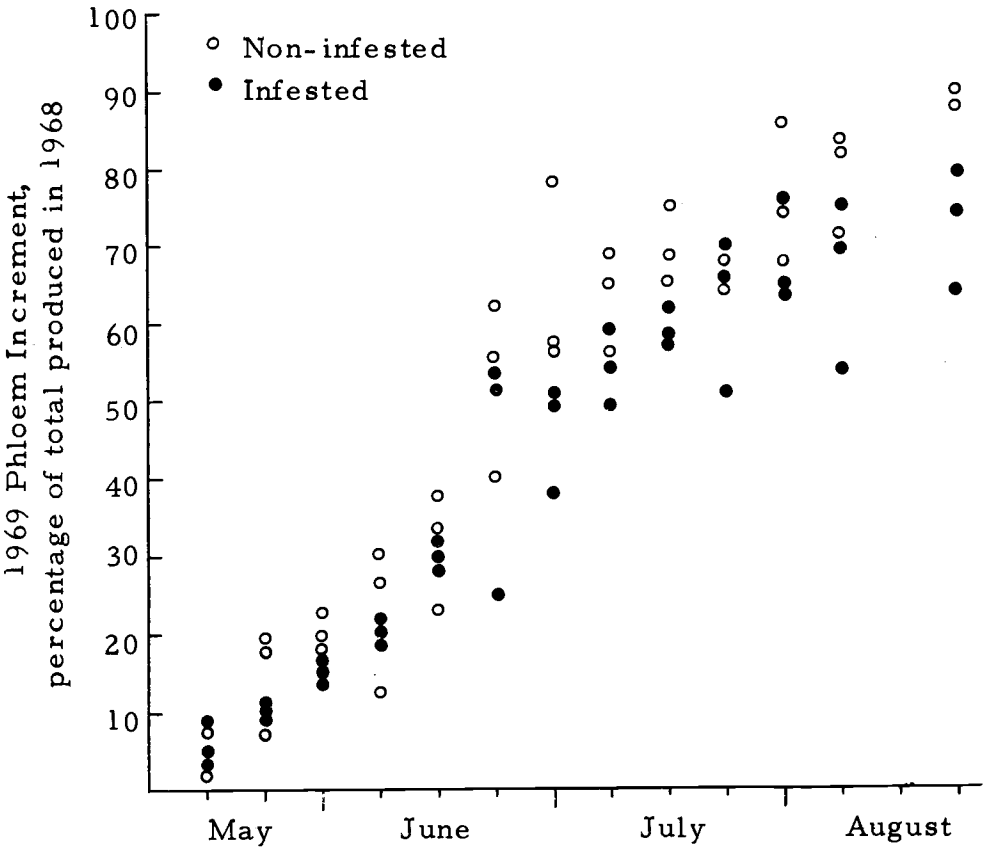


Figure 7. Seasonal development of the phloem increment in 1969 for samples from non-infested and infested trees of Abies lasiocarpa. Mean number of phloem cells per file at the time of sampling expressed as a percentage of the mean number of cells per file produced in the previous year. Precocious sieve cells produced in 1968 were excluded from the 1969 increment.

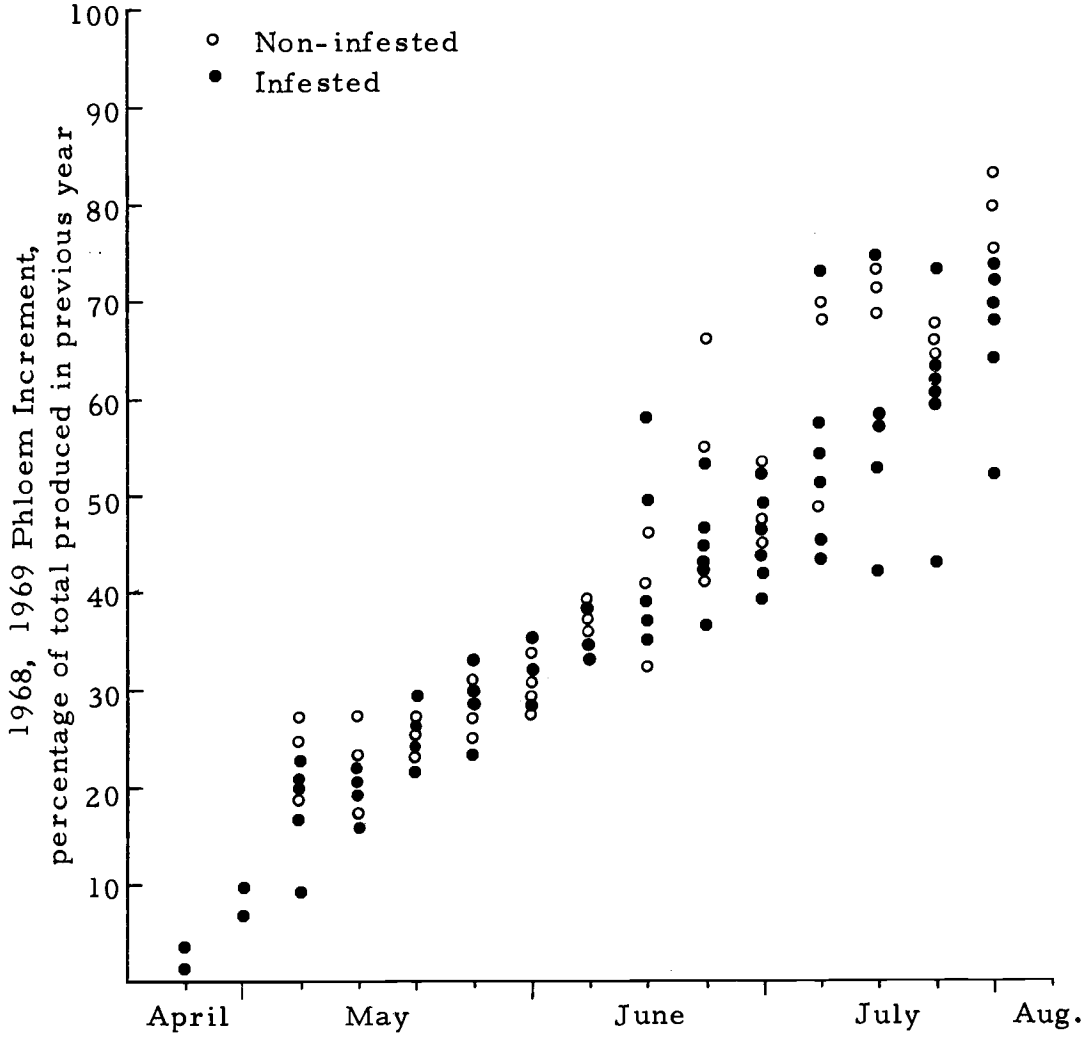
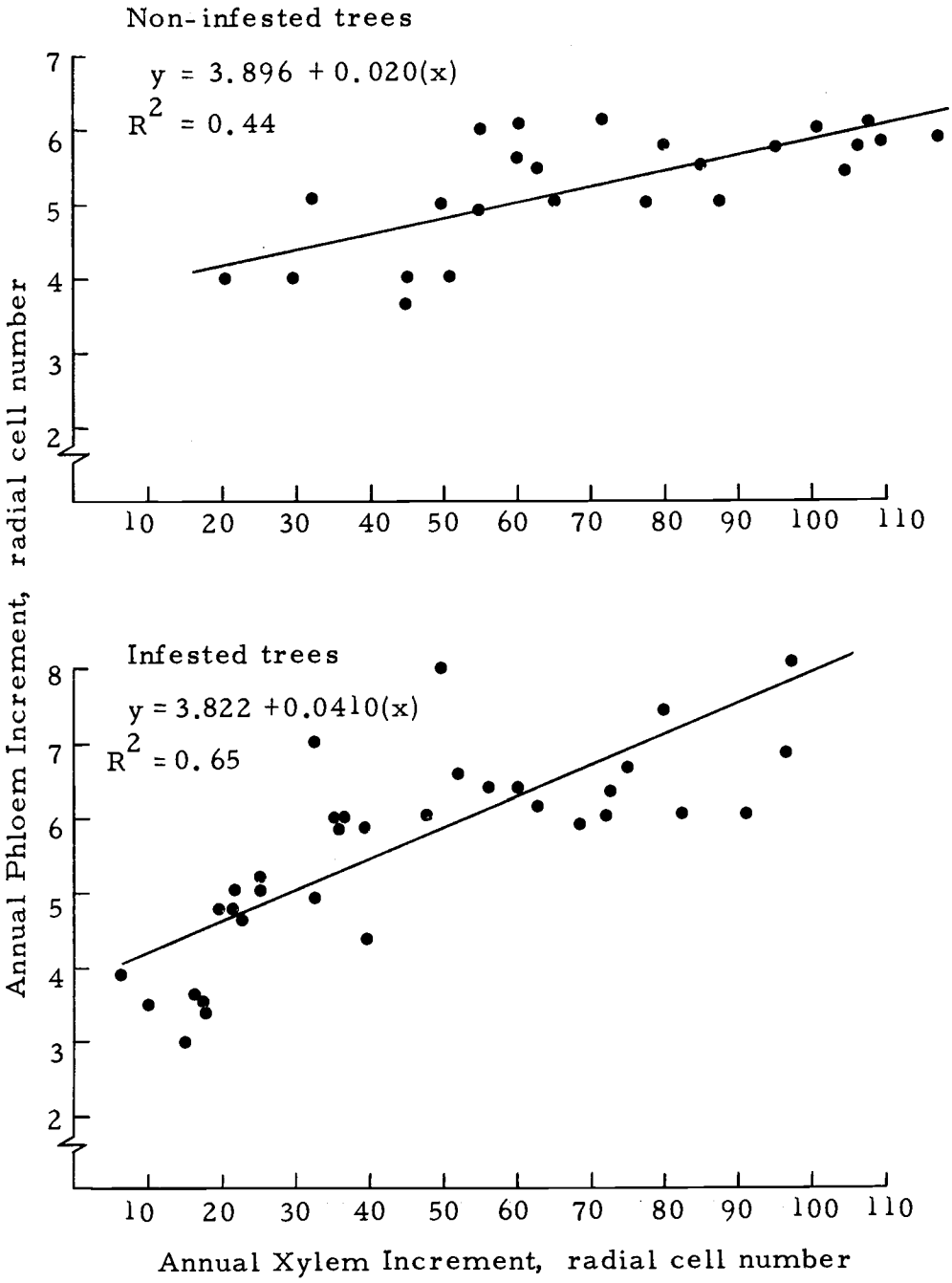


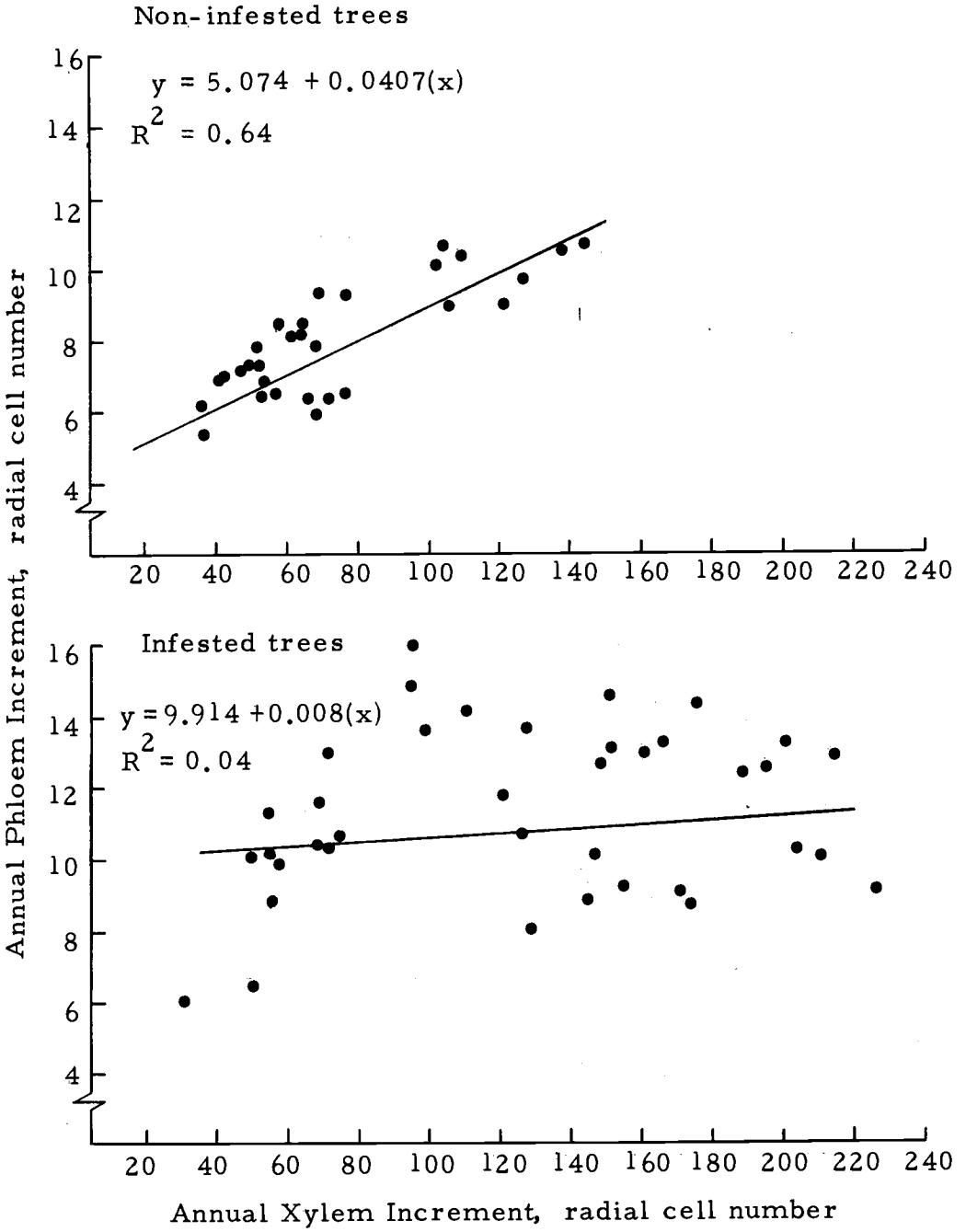
Figure 8. Seasonal development of phloem increment in 1968, 1969 for samples from non-infested and infested trees of Abies grandis. Mean number of phloem cells per file at the time of sampling expressed as a percentage of the mean number of cells per file produced in the previous year. Precocious sieve cells were excluded from the data.

presence or absence of aphids, were not counted as derivatives of the current year's cambial activity as they were in fact derived from the previous year's activity. An estimate of phloem production resulting solely from 1968 cambial activity in grand fir and 1969 activity for subalpine fir was made by arbitrarily subtracting the average of two precocious sieve cells per file observed in samples of the former and one precocious sieve cell in samples of the latter from the radial cell number in the phloem of the current year's sample. Use of these adjusted values indicate that the pattern of phloem production in both species closely resembles that of the xylem, with no suggestion, however, that the percentage of phloem produced at a given time was greater in infested trees than in non-infested trees.

Gregory (1969) determined that there was a significant relationship between the annual phloem increment and the annual xylem increment in northern white spruce. This would indicate that the greater number of phloem cells in the annual increment of infested trees of both species is correlated with greater xylem production. The relationship between annual phloem increment and xylem increment (Figures 9-12) was significant for both infested and non-infested subalpine fir and for control trees of grand fir. This relationship, however, was not observed in infested grand fir (Figure 12), suggesting that the greater number of phloem cells in infested trees of grand fir was a result of the aphid infestation and not solely the result of increased



Figures 9, 10. Relationship between the annual phloem increment and annual xylem increment in infested and non-infested trees of Abies lasiocarpa. Figure 9 - Samples from non-infested trees. Figure 10 - Samples from infested trees.

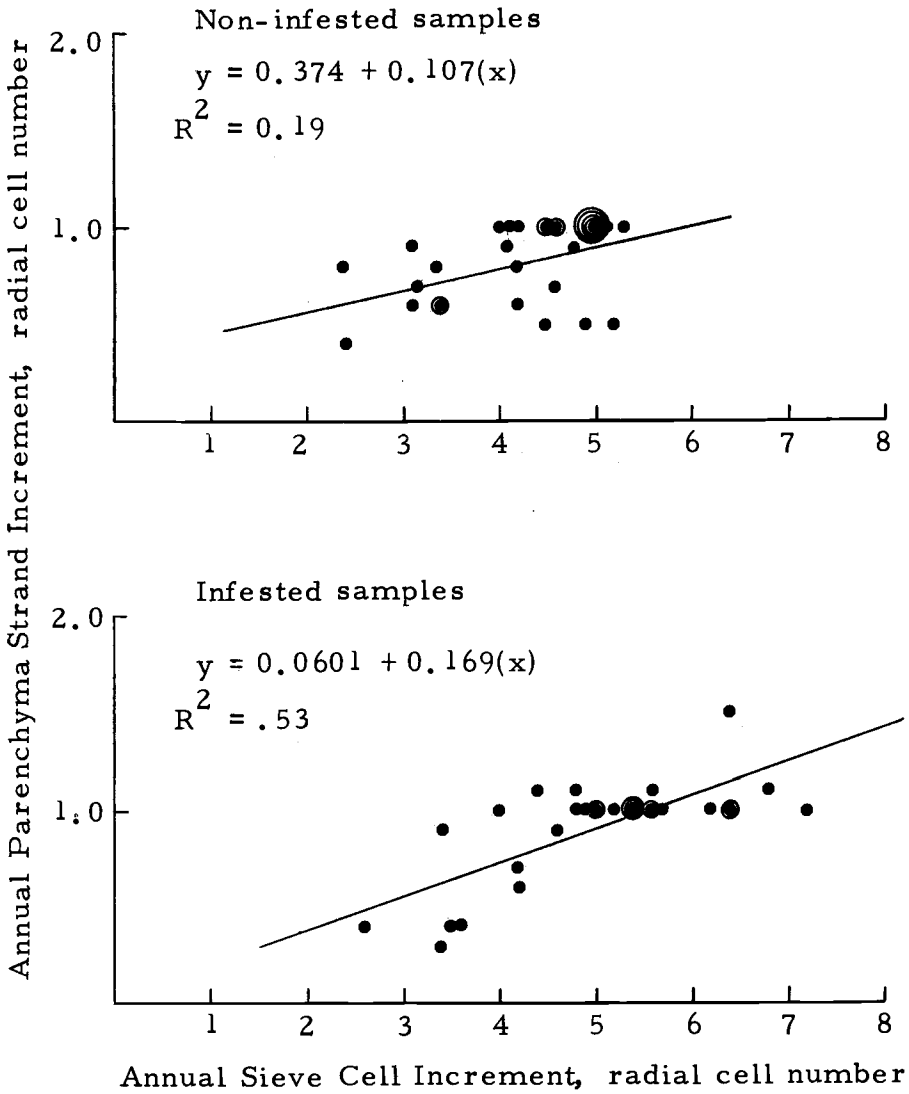


Figures 11, 12. Relationship between the annual phloem increment and annual xylem increment in infested and non-infested trees of Abies grandis.
Figure 11 - Samples from non-infested trees.
Figure 12 - Samples from infested trees

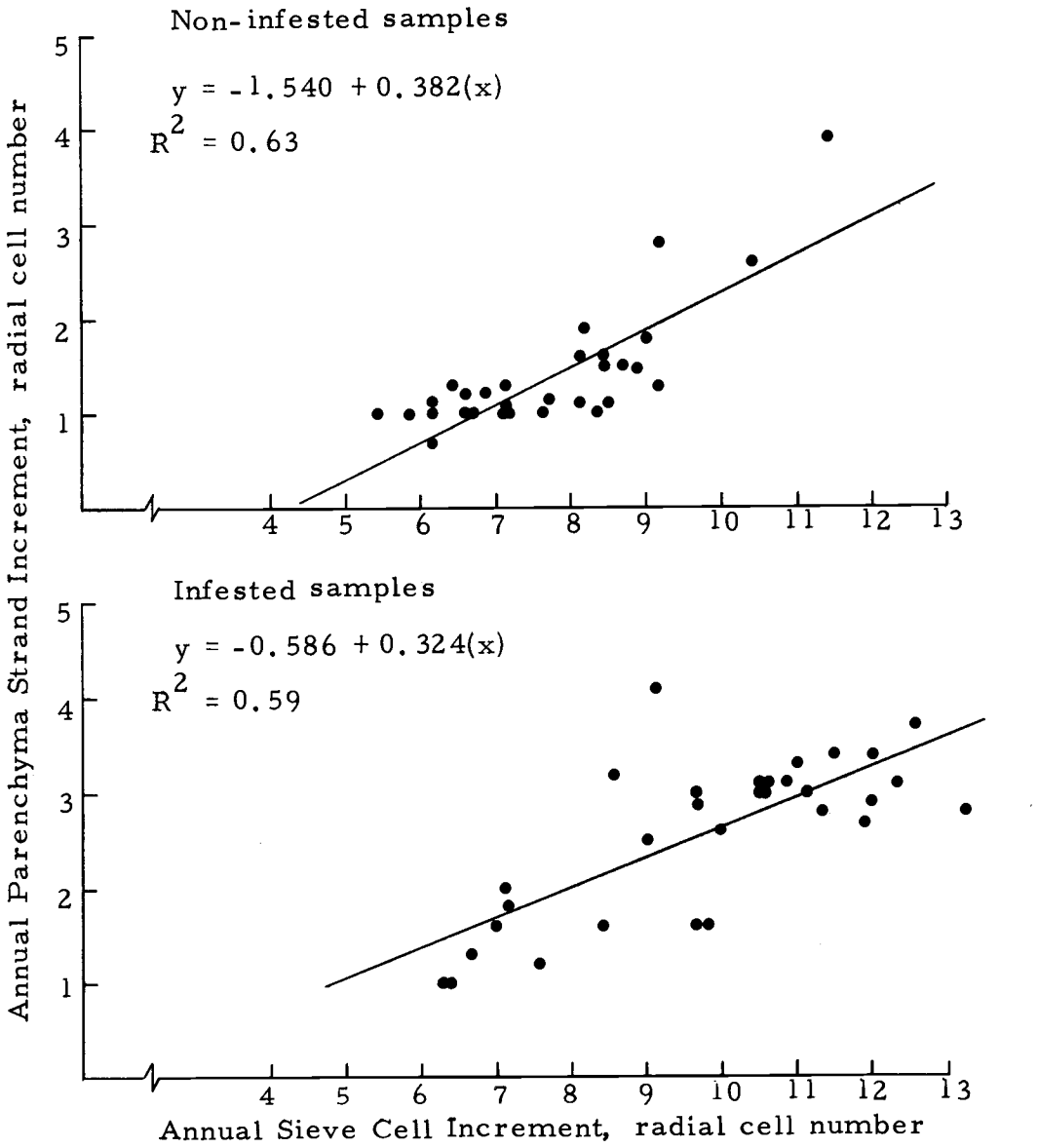
xylem production.

Phloem parenchyma strands were differentiated shortly after differentiation of the first tracheids, the third week in June in A. lasiocarpa and middle of May in A. grandis, when at least three functional sieve cells were visible in all samples. The development of the strands from the phloem mother cells followed the same pattern for all samples regardless of the presence or absence of aphids. The mother cells from which the phloem parenchyma strands are to be derived, accumulated tannins and resins before transverse divisions take place. Soon after the accumulation of the tannins and resins, numerous transverse divisions occurred to divide the cell into approximately equal segments and required two to three weeks to complete. The parenchyma cells of the strands undergo relatively little radial enlargement, in the functional phloem, and are easily identified in transverse section by their cell contents.

In general there was one tangential band of phloem parenchyma produced in an annual increment of phloem in samples from subalpine fir while three to four bands were common in samples of grand fir. The relationship between the annual increment of phloem parenchyma strands and the annual sieve cell increment in samples from both species of trees is shown in Figures 13-16. Significant relationships were observed in all cases except for that of non-infested samples of subalpine fir (Figure 13). This, however, may be due to the small



Figures 13, 14. Relationship between annual increment of phloem parenchyma strands and annual increment of sieve cells, in infested and non-infested samples of Abies lasiocarpa. Values for each of the 30 samples, for non-infested and infested trees, are based on counts of 10 radial files in the 1968 growth rings. Figure 13 - Non-infested samples. Figure 14 - Infested samples.



Figures 15, 16. Relationship between annual increment of phloem parenchyma strands and annual increment of sieve cells, in infested and non-infested samples of Abies grandis. Values for each of the 30 samples, for non-infested trees and infested trees, are based on counts of 10 radial files in the 1968 growth rings. Figure 15 - Non-infested samples. Figure 16 - Infested samples.

number of sieve cells produced rather than to a difference between control and infested samples. The data indicate that the control samples had between four and five sieve cells for at least one phloem parenchyma strand.

The ratio of phloem parenchyma strands to sieve cells is significantly higher in infested samples of grand fir than in control samples. This suggests that not only was the annual phloem increment greater in infested samples of grand fir, but, that also there was a greater number of phloem parenchyma strands produced than one can account for by an increase in the number of sieve cells. This relationship was not apparent in subalpine fir, perhaps because of the lack of a sufficient aphid population, or the comparatively small number of sieve cells produced in infested trees of this species.

Phloem parenchyma strands in both species, and independently of the presence of aphids, developed in three ways as described by Srivastava (1963): (1) accumulation of crystals; (2) differentiation into sclerids; (3) or radial enlargement and being sloughed off by the active phellogen. Crystals appeared occasionally in the first year phloem, but more commonly in the older phloem in samples of subalpine fir. Sclerids were noted only in three to four year old phloem in subalpine fir. In samples of grand fir, crystal cells and developing sclerids were noted in the first year phloem, with large sclerids present in the second year phloem.

MITOTIC INDEX AND THE PRODUCTION OF SECONDARY TISSUES

The rate of periclinal division of the fusiform initials in the cambial zone was measured by determining the mitotic index or the percentage of fusiform cells in the cambial zone undergoing mitosis, for different parts of the growing season. Using the method described by Wilson (1964, 1966), mitosis was considered to have started when the chromosomes first became visible in early prophase and finished when the cell plate ceased to be circular in tangential view and the two bars of the phragmoplasts were distinct. Although the rate of anticlinal division was found to be higher in infested trees than in non-infested trees (Smith, 1967; Saigo, 1969), all fusiform cells in the cambial zone that were in mitosis were assumed to be dividing periclinally, regardless of the condition of the tree.

Sources of Variation in Measuring the Mitotic Index

The question of whether the mitotic activity of a single sample removed from an internode is representative of the mitotic activity of the internode as a whole has been studied by several authors. Wilson (1966) working with Pinus strobus in New England, found that the number of mitoses among numerous samples from a single internode were similar at any one time, and that the mitotic indices were similar in all levels of the stem. This author (Sisson, 1968) compared

the mitotic indices within single internodes and among trees of Pseudotsuga menziesii near Corvallis. While significant differences were noted in the mitotic index in internodes of three of the four trees, the magnitude of the differences were small since in no case did the confidence limits for a given sample not overlap those for the internode as a whole. The differences in the mitotic indices among trees were not significant even though the four trees varied considerably in their rates of growth. Gregory (1969), from samples of an internode of Picea glauca in Alaska, confirmed these findings. These studies indicated that the mitotic index for a single sample, at least in normal trees, is representative of the mitotic activity within that tree. The presence of aphids should not have altered this relationship as the aphids were more or less evenly distributed around the boles of the trees sampled.

Diurnal variations in the mitotic activity should not have influenced measurements of the seasonal trend of mitotic activity in the trees sampled. Wilson (1966) observed that afternoon peaks in mitotic activity may occasionally occur in Pinus strobus. Gregory (1969) found in Picea glauca that there were significant variations in the diurnal pattern of mitotic activity within an internode. Peak rates of cell division occurred around noon and minimum rates around midnight. Differences in the daytime rate of cell division were not significant, although they were slightly below the daytime peaks.

Since the samples taken in this study were removed at approximately the same time at each sampling date, little variation due to diurnal variation might be expected.

Mitotic Index During the Grand Period of Growth

In both species of firs, an increase in the number of cambial zone cells marked the beginning of the grand period of growth (Figures 17, 18). The grand period, when 70 to 80 percent of the annual radial xylem increment was produced, extended from late May through the first two weeks in July in subalpine fir and April through July in grand fir.

The rate of cell division during the grand period of growth, as indicated by the indices, showed no significant difference between samples of infested and non-infested trees of either species. Thus the greater annual xylem production observed in infested trees of grand fir and the greater phloem increment for infested samples of both species does not appear to be related to an increased rate of cell division in infested trees during the grand period of growth.

The increase in number of cambial zone cells at the beginning of the grand period of growth, accompanied by a higher rate of mitotic activity, corresponds to the findings of Wilson (1966) and Gregory (1969). The gradual decline in the number of cambial zone cells as the growing season progressed (Figures 17, 18) was also observed

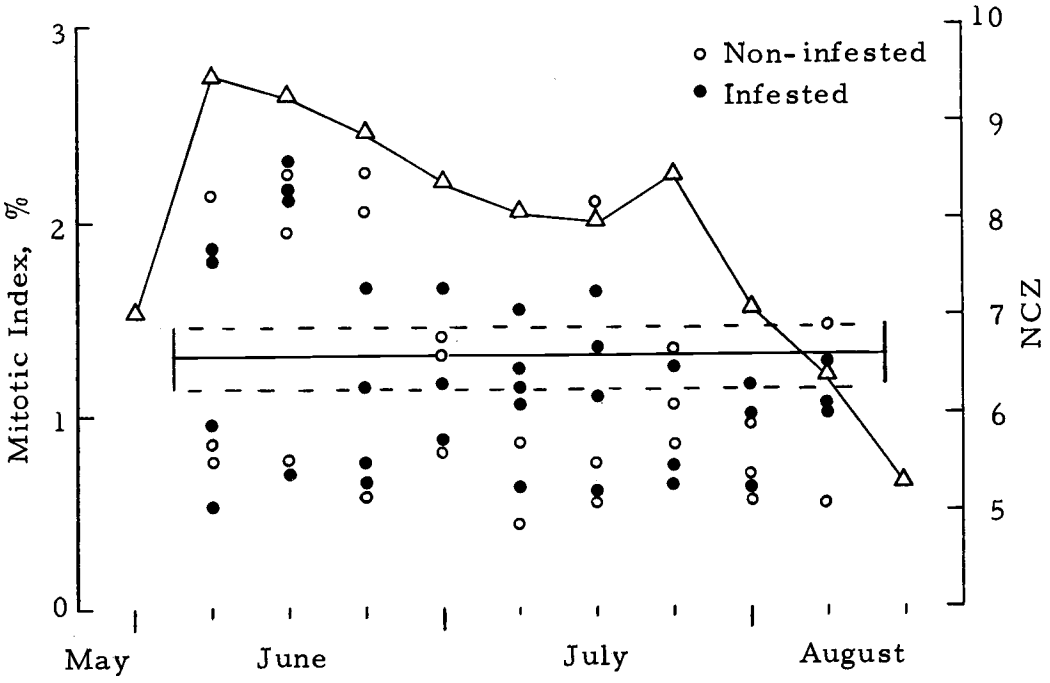


Figure 17. Mean core mitotic indices and mean radial cell numbers in the cambial zone (open triangles connected by solid line) of non-infested and infested samples of Abies lasiocarpa. Values at the extreme right and left represent the mean mitotic index for all samples with confidence limits connected by dash lines.

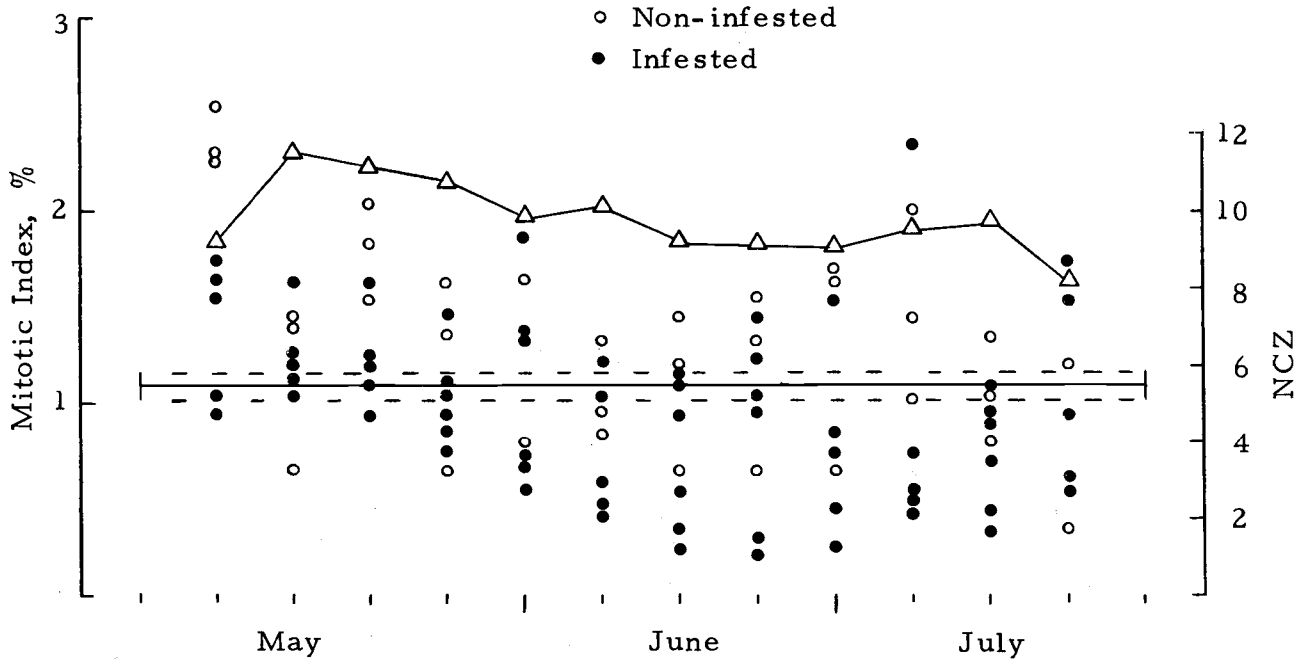


Figure 18. Mean core mitotic indices and mean radial cell numbers in the cambial zone (open triangles connected by solid line) of non-infested and infested samples of Abies grandis. Values at the extreme right and left represent the mean mitotic index for all samples with confidence limits connected by dash lines.

by Wilson (1966). Along with the gradual decline in NCZ, it appears that there was a slight decrease in mitotic activity in samples of both species, with the mitotic index remaining at this new level for the remainder of the grand period of growth.

SUMMARY

Some general features of the cambial zone in conifers as described in the literature appear to be consistent in Abies grandis and Abies lasiocarpa, regardless of whether or not the trees were infested by the balsam woolly aphid. The appearance of the fusiform initials in the dormant and actively dividing condition were as described for conifers in all samples. Undifferentiated (precocious) sieve cells, found in many conifers, were present in the dormant cambial zone of both species of Abies in numbers, within species, independent of tree vigor and the presence or absence of aphids. Dates for the reactivation and cessation of mitotic activity in the cambial zone between the two species depended upon location, and differences within species depended upon tree vigor but not the presence or absence of aphids.

Significant decreases in the length of the fusiform initials, due to an increase in the frequency of anticlinal divisions as reported by Smith (1967) and Saigo (1969), led to an increase in the number of radial files in the cambial zone of infested samples in both grand and subalpine fir. Peak numbers of cambial zone cells per radial file for all samples occurred early in the growing season, with a gradual decline in number as the growing season progressed, as reported by Wilson (1966) and Gregory (1969).

Differentiation of xylem and phloem elements in trees of both subalpine and grand fir followed the same pattern regardless of tree vigor or the presence of aphids. A similar pattern for both xylem and phloem was noted for Picea by Gregory (1969). While the pattern of development was similar in all samples significant differences between infested and non-infested samples were noted, although the differences were probably not as great as might have been observed had the infested trees been more heavily infested. The annual xylem increment in grand fir, and the phloem increment of both species, were significantly greater in infested trees when compared to non-infested trees. Infested samples of grand fir also had a greater ratio of phloem parenchyma strands to sieve cells than that found in non-infested samples.

Increased xylem production in infested trees of grand fir and phloem production in infested trees of both species could not be accounted for by an increased rate of cell division in infested trees. The rate of cell division, as indicated by the mitotic indices, showed no significant difference between samples of infested and non-infested trees of either species (Figures 17, 18). Other studies on the relationship of the rate of cell division and radial growth of tree stems (Wilson, 1966; Gregory, 1969) tend to support this observation that the amount of radial growth is independent of the rate of division in the cambial zone cells.

Significant differences were not observed in the number of cambial zone cells per radial file between infested and non-infested trees of either species during the grand period of growth. Significant differences in the NCZ could have accounted for differences in the xylem production in grand fir and phloem production in both species between infested and non-infested trees. Evidence presented in other studies, however, by Wilson (1966), Gregory and Wilson (1968), Sisson (1968), and Gregory (1969) have shown consistent relationships between the number of cambial zone cells per radial file and radial growth rates in tree stems. These studies indicate that had the infested trees of both species been more heavily infested, increased xylem production, normally associated with aphid activity, would have been accompanied by an increase in the NCZ.

The salivary substances secreted by the balsam wooly aphid are still unknown, although evidence of their effects are suggested in this paper and others cited in this study. It appears that the aphid saliva does not affect the rate or the process of cell division but alters the type of cell division which occurs in the cambium as shown by the greater frequency of anticlinal division (Smith, 1967; Saigo, 1969) in infested trees. The seasonal pattern of development also does not appear to be altered by the aphid secretions; however, noticeable changes are brought about in the cells produced (Doerksen and Mitchell, 1965; Mitchell, 1966; Smith, 1967; Saigo, 1969). These

characteristics suggest that influence of the aphid is directed at both cambial activity and cellular differentiation. The physiological and chemical nature of such reactions are unclear although they may be related to disturbances in the normal balance of growth-regulating substances in the tree as suggested by Balch, et al. (1964).

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