AN ABSTRACT OF THE THESIS OF

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Date thesis is presented August 8, 1966

Title CAMBIAL DIVISIONS IN PSEUDOTSUGA MENZIESII

Abstract approved _____ (Major professor)

Mitotic activity in the vascular cambium was determined from ten samples from a single internode in each of four Douglas-fir [(Pseudotsuga menziesii (Franco.) Mirb.)]trees. Counts of interphase and mitotic nuclei from nine cores in each sample piece were averaged and expressed as the frequency and mitotic index. The sampling error was determined for the average mitotic index per sample and the internode as a whole. Significant differences were found in the frequency of division among samples from each of the four trees, and between mitotic indexes in two of the trees. Significant correlations were established between number of nuclei and frequency of division within the internodes. Variations in the mitotic indexes among samples could not be accounted for by the relationship between nuclei and divisions per core, but may be due to non-homogeneous cambial populations in terms of mitotic activity.

CAMBIAL DIVISIONS IN *PSEUDOTSUGA MENZIESII*

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1967

APPROVED:

Professor of Botany

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Date thesis is presented August 8, 1966

Typed by Joanne Wenstrom

ACKNOWLEDGEMENT

I wish to express my gratitude to Dr. Frank H. Smith, my major professor, and to Robert A. Gregory, research forester, for their advice and assistance during this investigation.

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CAMBIAL DIVISIONS IN PSEUDOTSUGA MENZIESII

INTRODUCTION

Numerous investigations have been made regarding the structure of the vascular cambium and also mitotic activities in the cambium as deduced by analysis of its derivatives. Studies of this nature can determine the types of cell division that occur in the cambium but cannot determine actual mitotic activity. Bailey (1920) described the details of mitosis and cell division and Wilson (1964) estimated the time and rates of the cell division cycle in Pinus strobus L. Bannan (1955), using Thuja occidentalis L., and Grillos and Smith (1959), using Pseudotsuga menzeisii (Franco.) Mirb., demonstrated that the first mitoses in the spring occur within the xylem mother cells. The relative frequency of divisions across the cambium, during the period of most active growth, has been shown for Thuja (Bannan, 1955) and Pinus strobus (Wilson, 1964).

The reliability of the sampling technique used in determining the mitotic index and frequency of cambial divisions is the subject of this paper. In determining mitotic activity of the entire cambium, populations of cambial cells are selected at various internodes, or the intervals between whorls of branches in gymnosperms, from which samples are removed periodically throughout the growing season. Within an internode there are a vast number of

meristematic cells and the validity of the assumption that one sample accurately indicates the mitotic activity in the entire internode is questionable. Bailey (1920) concluded that

> Initials may be dividing actively in one portion of a stem when those in adjacent portions are inactive. Similarly, numerous mitotic figures may be present in a given individual when a neighboring plant appears to be entirely devoid of them.

Wilson (1966) concluded that while the distribution of mitoses within an internode, and even a whole tree, was fairly uniform a single sample might not give a reliable estimate of mitotic activity in the whole internode. The purpose of this study was to determine whether the mitotic index and number of nuclei in mitosis are the same in all areas of the cambial zone of a single internode of Douglas-fir [(Pseudotsuga menziesii (Franco.) Mirb.)] at any one time.

In this paper, the term cambium is used to include all the meristematic cells that produce secondary xylem and phloem, both cambial initials and xylem and phloem mother cells. The mitotic index refers to the percentage of cells in the cambial population that were dividing, while frequency refers to the total number of dividing cells. The mitotic index represents the actual difference in mitotic activity, while the frequency represents the relative difference in mitotic activity within an internode.

MATERIALS AND METHODS

Ten cambium samples were obtained from a single internode for each of four Douglas-fir trees on April 16 and 17, 1966. The trees were growing in MacDonald forest near Corvallis, Oregon. Age and diameter were determined at each sample internode and were as follows: Tree 1, 23 years and 23 cm; Tree 2, 22 years and 16.75 cm; Tree 3, 24 years and 20.0 cm; and Tree 4, 10 years and 14.0 cm.

The internodes selected for sampling were 1.4 m from the ground and ranged from 0.30 m to 0.58 m in height. The samples were removed from smooth-barked portions of the internode and were spaced sufficiently to avoid traumatic effects upon adjacent samples and to obtain an even distribution over the entire surface of the internode. Each sample removed was 1 cm wide and 2.5 cm in length. They were fixed in Navashin's III, dehydrated with t-butyl alcohol, and placed in TBA and paraffin oil, following the procedure described in Johansen (1940), prior to infiltration. The samples were then placed in molten 56°C Tissuemat with three changes at four hour intervals and left overnight. Following this initial infiltration, the molten paraffin was poured off and the vials, containing the samples, immersed in cold water. After drying, the samples were subdivided into two equal parts, trimmed to

remove excess bark, and then reinfiltrated using a vacuum oven at 60°C and 35 atmospheres with two changes of Tissuemat at half-hour intervals. The thus infiltrated samples were then embedded for sectioning.

Serial tangential sections and transverse sections of the cambial zone of the samples were cut at 20μ on a rotary microtome. Prior to microtoming the cross-sections, the sample was exposed by removing the excess paraffin on the surface to be sectioned 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 safranin-fast green using the schedules in Jensen (1962).

Mitotic indexes and frequencies of division were determined, for each sample, from radially oriented cores through the serial tangential sections of the cambium region. Each core from Tree 1 was 1.25 mm in diameter, and 1.40 mm in diameter for Trees 2-4, as delimited by the microscopic field at 100X magnification. A core was established by locating a prominent ray, or rays, in a section of the phloem and then the ray was followed through successive sections of the cambium. The total number of divisions, both anticlinal and periclinal, and number of nuclei were obtained for each core. Also recorded were the number of nuclei in various stages of mitosis and the number of anticlinal and periclinal

divisions. Nine different cores were counted from each sample and an average mitotic index per core, and the sampling error at the 95% level of probability, were calculated. For each tree the percentage of anticlinal and periclinal divisions, as well as the number of nuclei in the various stages of mitosis were determined. All of the values obtained in the statistical calculations in this paper were compared to the tables in Fisher and Yates (1963).

Mitoses and nuclei were counted at 100X magnification, although higher magnification was frequently required to check various stages of mitosis and groups of nuclei. A cell was considered to be undergoing mitosis from the time that the chromosomes could first be seen in prophase until the two bars of the phragmoplast were distinctly evident. The relatively thick sections lessened the possibility of double-counting nuclei which had been cut in half and appeared in two sections. Nuclei which were degenerating in the differentiating tracheids were hopefully avoided.

An analysis of variance at the 95% level of probability was used to determine whether there was a significant difference in the mitotic indexes and the number of divisions among the ten samples from each tree. Regression constants and correlation coefficients were calculated to determine the relationship between the number of mitoses and the number of nuclei per core in each of the four trees.

Cross-section counts were made to determine the average number of cells in a radial file for the 1966 growing season. In all but two of the samples, one each from Trees 1 and 3, eight radial files were counted and the average number of cells in a radial file for each sample was obtained.

RESULTS

There was no significant difference in the mitotic indexes between samples removed from Tree 2 and Tree 3, but the mitotic indexes between samples for Tree 1 and Tree 4, were significantly different. All four trees showed a significant difference in the number of mitoses, or frequency, between samples from their respective internodes. The sampling errors for the mitotic indexes for most pieces within an internode overlapped the sampling error for the internode as a whole (Figure 1). The chance that a given sample would not be representative of the entire internode was 10-20%.

Differences in the frequency of mitoses in each of the sample pieces reflected differences in number of nuclei, or cells (Bailey, 1920) which were potentially mitotic in the cambial zone. All the trees sampled showed a highly significant correlation between the number of nuclei and number of mitoses per core but the correlations were not high enough to account for the variation in mitotic indexes between pieces within an internode (Table 1, Figures 2 - 5).

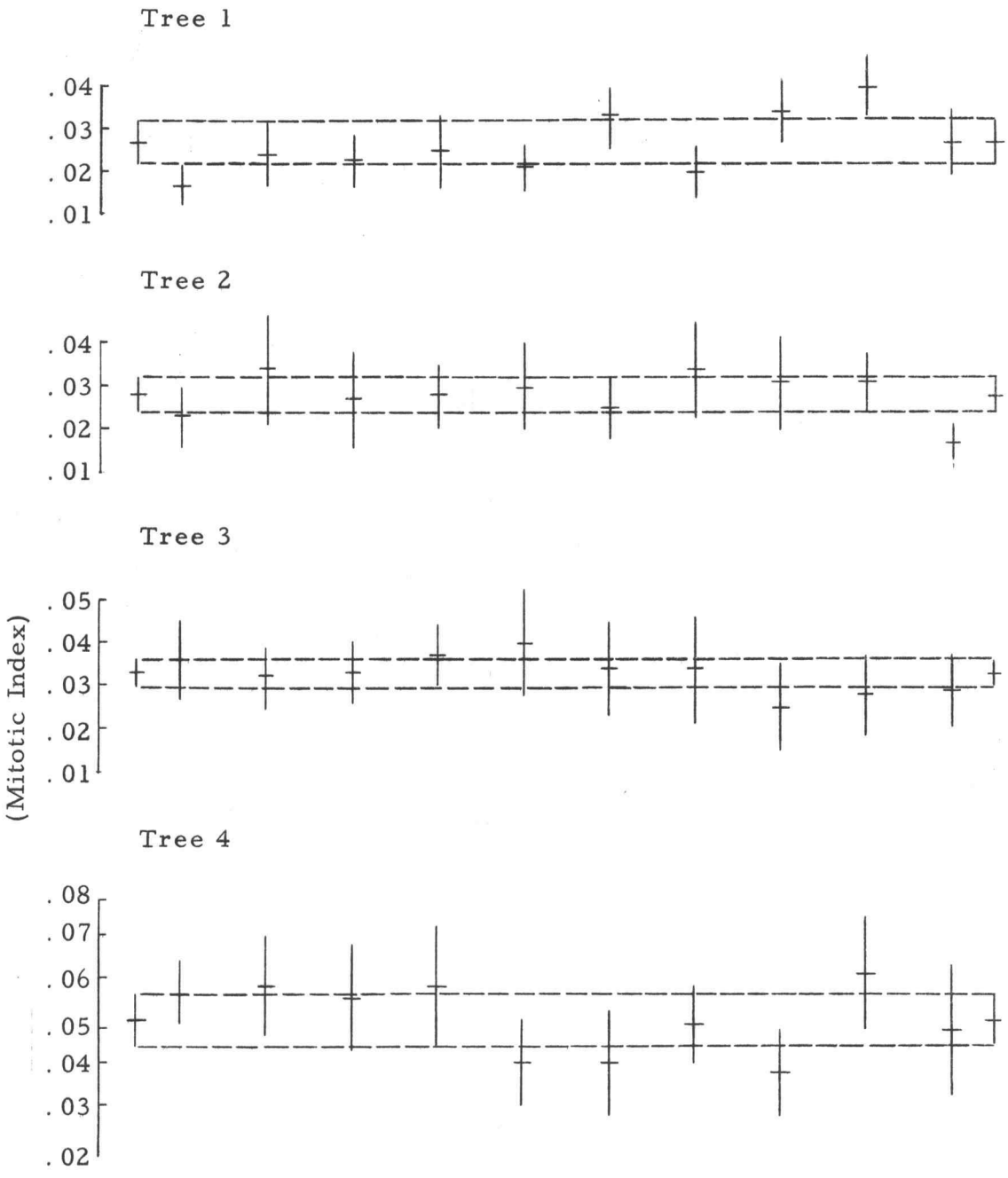

Figure 1. Average mitotic index per core and their sampling errors at p=0.05 for each of the four trees. Values at the extreme right and left are the average for each tree with the sampling error connected by dotted lines.

Table 1. Regression constants and correlation coefficients (r^2) for Trees 1-4

Tree number	Mitoses/core (M_c) on nuclei number per core (N_c)	Correlation Coefficient (r^2)
1	$M_c = 0.42 + 0.025N_c$	0.1225
2	$M_c = 0.16 + 0.029N_c$	0.2809
3	$M_c = -1.87 + 0.041N_c$	0.4356
4	$M_c = -0.62 + 0.056N_c$	0.4356

The slopes of the regression lines (Figures 2-5) were highly significant, that is they departed from the horizontal by an amount that could not be attributed to chance. By multiplying the slope of the regression lines by 100, the variation due to the relationship between nuclei and mitoses per core (r^2) could be eliminated when calculating the average mitotic index per internode. The values thus obtained, 2.4%, 2.8%, 4.1%, 5.6% for Trees 1 through 4, respectively, compares favorably with the 2-3% calculated by Wilson (1964, 1966). None of the cores sampled showed an absence of mitoses.

The percentages of the prophase nuclei and various types of division occurring within each of the four internodes sampled are given in Table 2.

The number of cells in a radial file of the growth for 1966, for each of the four trees sampled had the following range and averages: 42.0 - 54.0, 46.41; 21.4 - 37.40, 28.36; 26.30 - 43.40,

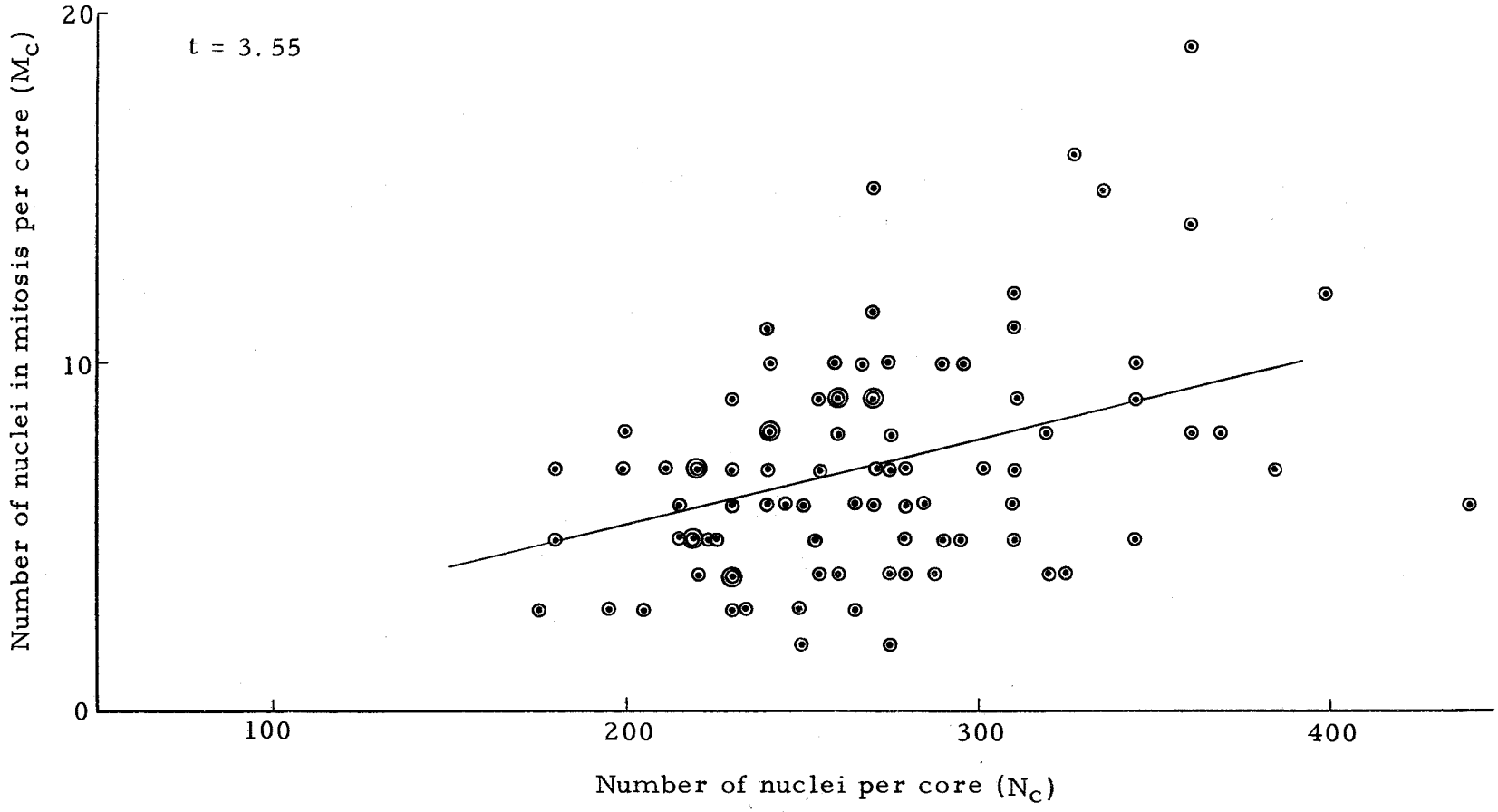

Figure 2. Relationship between the number of mitoses per core and the number of cells per core for Tree 1. Regression constants and correlation coefficients are shown in Table 1.

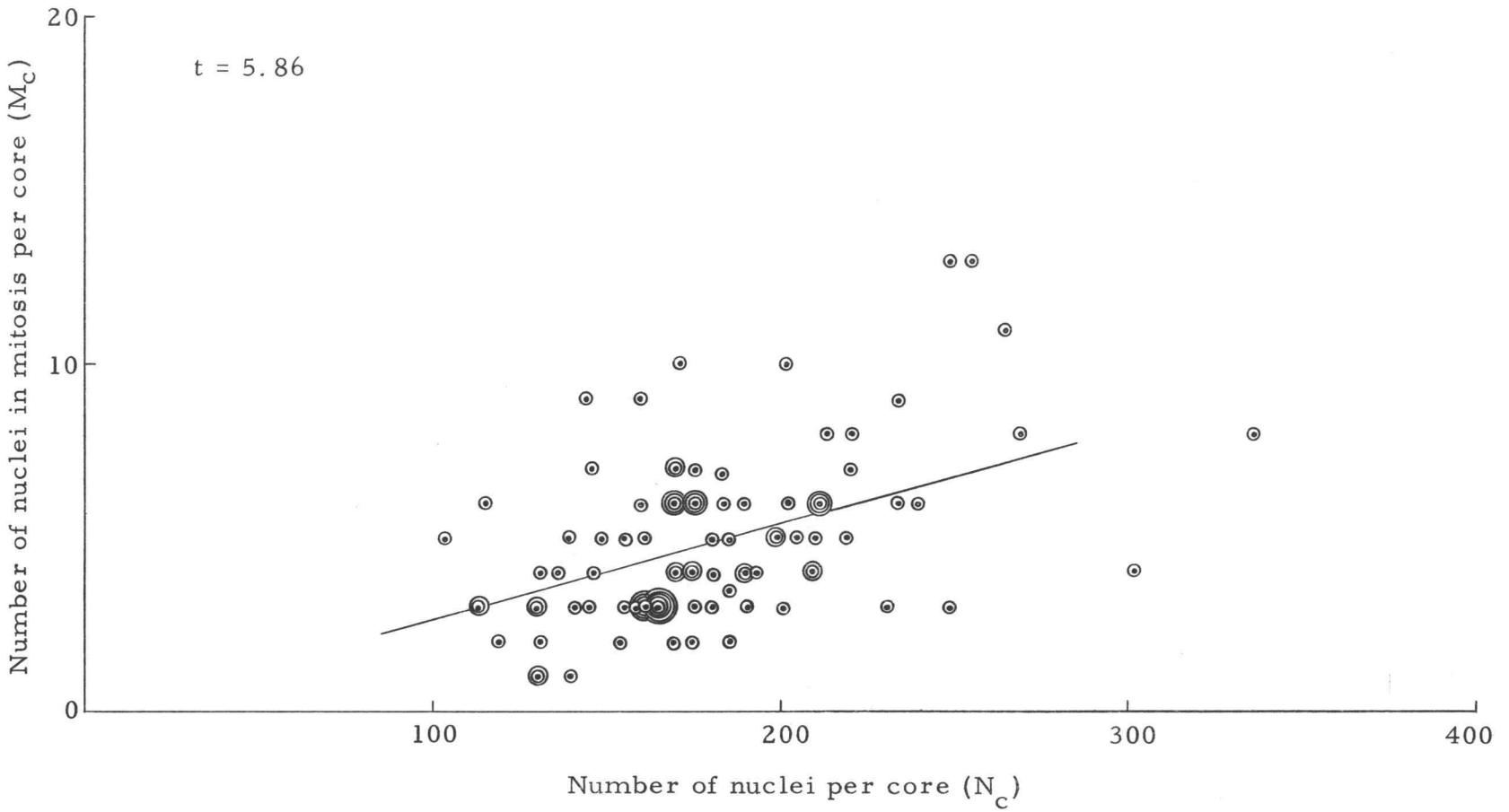

Figure 3. Relationship between the number of mitoses per core and the number of cells per core for Tree 2. Regression constants and correlation coefficients are shown in Table 1.

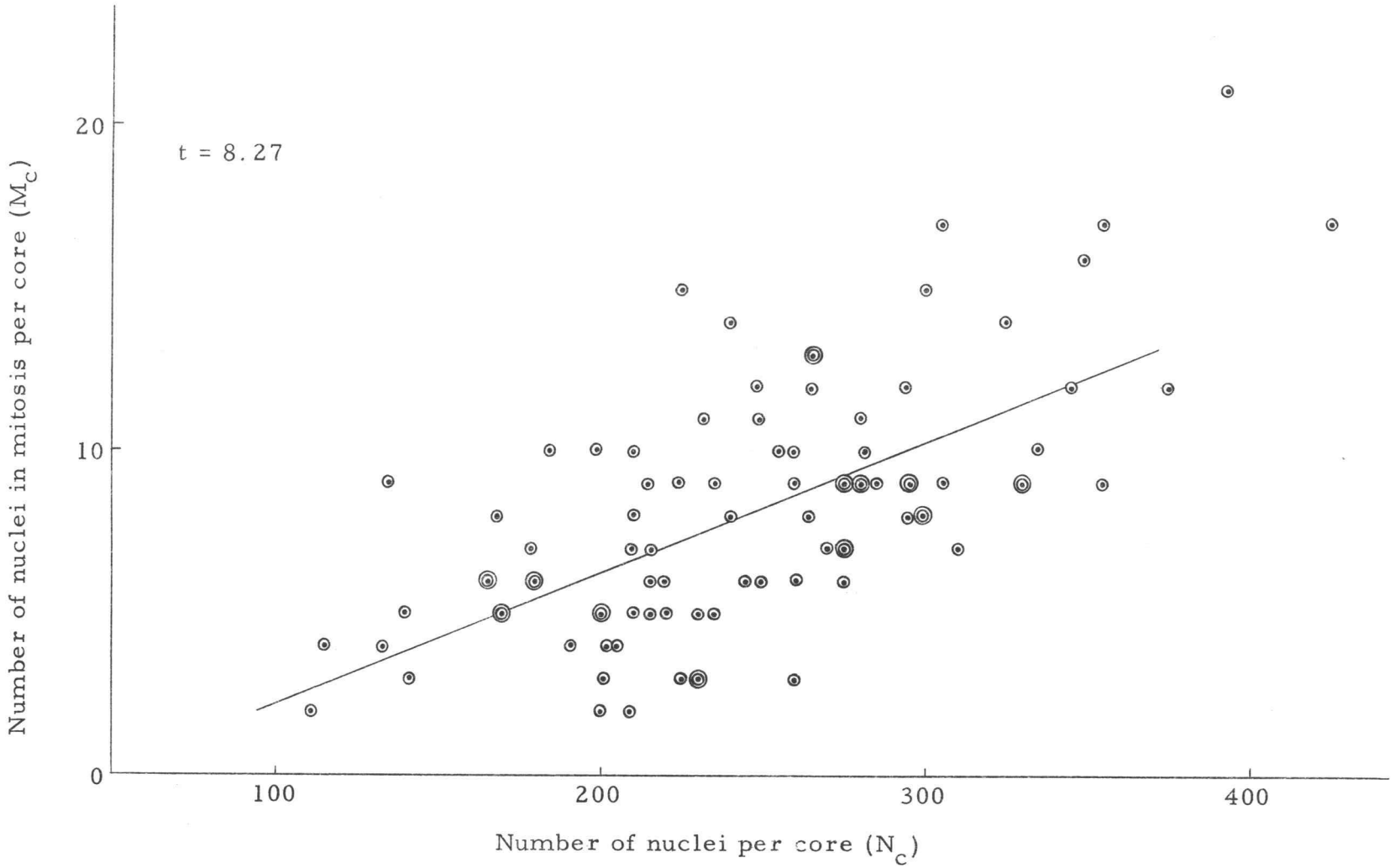

Figure 4. Relationship between the number of mitoses per core and the number of cells per core for Tree 3. Regression constants and correlation coefficients are shown in Table 1.

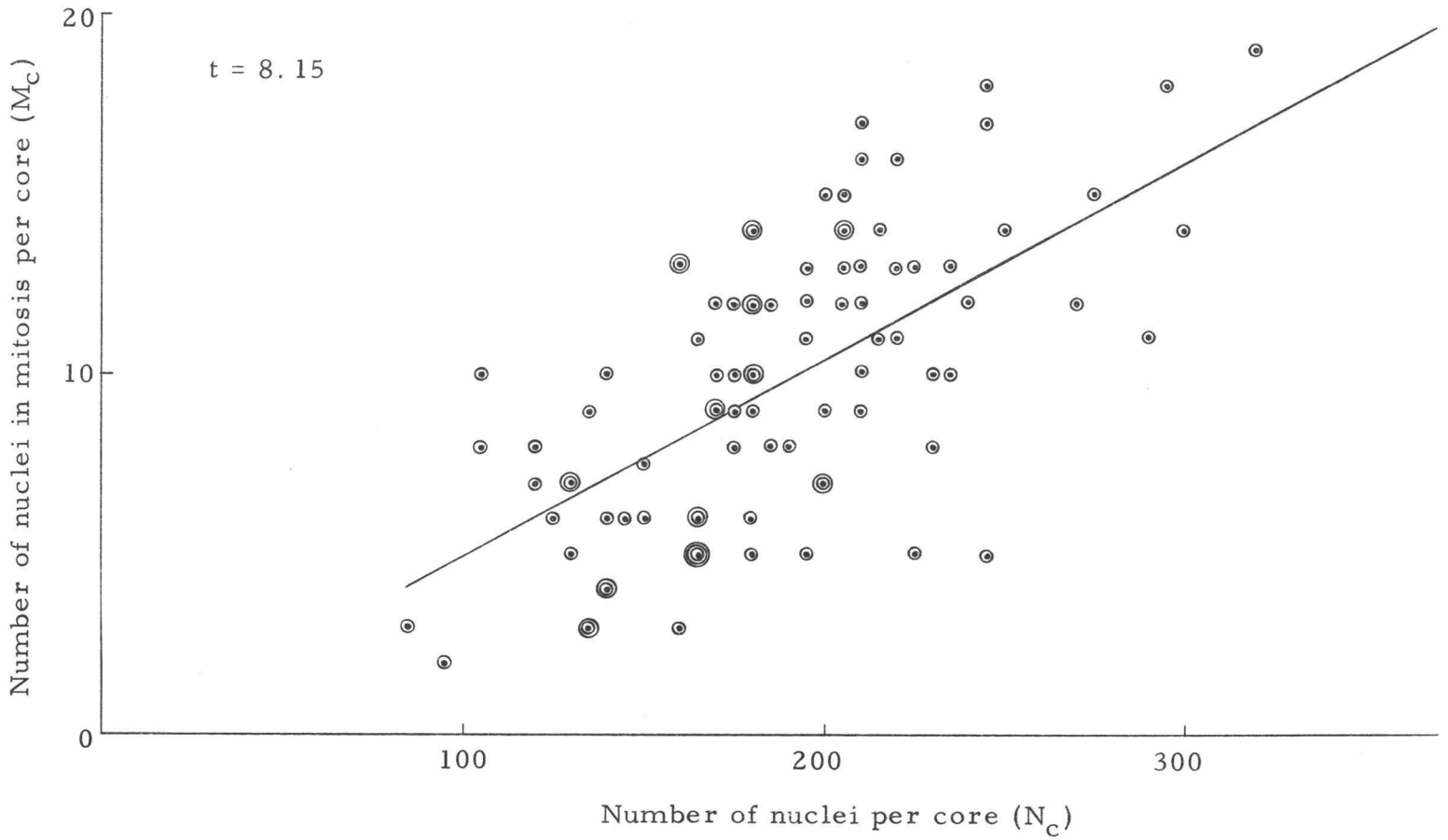

Figure 5. Relationship between the number of mitoses per core and the number of cells per core for Tree 4. Regression constants and correlation coefficients are shown in Table 1.

Table 2. Mitotic Frequency and Plane of Cell Division for Each Tree. Plane of Division of Cells in Prophase Cound Not be Determined.

Tree Number	Total Dividing Nuclei	Periclinal Divisions	% Periclinal Divisions	Pseudo-transverse Divisions	% Pseudo--transverse Divisions	Prophase Nuclei	% Prophase Nuclei
1	644	237	36.80	39	6.06	368	57.14
2	456	186	40.78	38	8.33	232	50.87
3	738	275	37.26	38	5.14	425	57.58
4	890	290	32.58	98	11.01	500	56.18

36.77; 30.0 - 39.4, 35.31 in Trees 1 to 4, respectively. None of the tracheids were completely differentiated. Only for the internodes from Trees 3 and 4 did the regression lines vary enough from the horizontal to show a significant correlation between the average number of mitoses and average number of cells in a radial file for each sample.

Examination of the cross-sections showed that the number of tracheids produced during the 1965 growing season in Trees 1 and 4 were at least three times greater than those produced during the same period of time in Trees 2 and 3.

DISCUSSION

The results of this study indicated a greater uniformity in mitotic activity within an internode than suggested by Bailey (1920), but more variation than found by Wilson (1966). In contrast to Bailey's (1920) observation, that fusiform initials may be dividing in one area of the stem and not in others, the results of this study showed that in no sample was there an absence of mitoses. The significant differences in frequencies and mitotic indices, as noted in the results, would suggest a greater variation in mitotic activity within an internode than the uniform distribution of mitoses noted in internodes, and trees, of Pinus by Wilson (1966).

Differences in the mitotic indexes, and deviations from the average mitotic index for the internode, may be due to areas of fusiform initials with differences in the length of their cell division cycles as well as their capability for division. Wilson (1964, 1966) and Robert A. Gregory[1] have suggested from their work on cambial divisions, that there is a constant lengthening of the cell cycle during the growing season which accompanies the gradual decline in mitotic activity toward the end of the growing season. As well as

[1] Personal correspondence

seasonal changes in the cell division cycle, there may also be differences in the length of the cell cycle between areas within an internode. While there was a difference in relative numbers of mitoses, or frequency, between samples within an internode there was also an actual difference in mitotic indexes among samples from Tree 1 and Tree 4 which could not be accounted for by the variation due to the relationship between the number of cells and divisions per core (r^2). This indicates that the variations in mitotic activity are, in at least some cases, too great for one to assume a homogeneous cambial population in terms of mitotic activity at any one time. As mentioned previously, relative differences, or frequency differences, among tree samples reflected the number of cells, as determined by the number of nuclei, present in the cambial zone (Table 1, Figures 2 - 5). This observation confirms the findings of Wilson (1966).

Diurnal fluctuations, comparable to those found by Wilson (1966) in the crown of Pinus strobus and Jensen and Kavaljian (1958) in the root apices of Allium, may also be responsible for the variation in mitotic indexes between samples in Trees 1 and 4. It is also possible that the time required to remove all the samples from an internode, about one hour, contributed to some of the differences in the number of mitoses counted in the samples.

Rate of growth in trees may affect the relationship in mitotic indexes between internodal samples. When the 1965 growth rings were compared, Trees 1 and 4 were relatively faster growing than Trees 2 and 3. As noted in the results, Trees 1 and 4 showed a significant difference in the mitotic indexes among pieces removed from a single internode, while there was no significant difference noted in samples from Trees 2 or 3.

Correlations of ring width and frequency of division are not reliable as a measure of the actual mitotic activity within an internode at any given time. Differences in cell size (Dinwoodie, 1961) and the total time during which the cambium is active must be taken into consideration when determining the true relationship between mitotic activity and ring width. Ring widths, however, are of value in making rough estimates of variations in mitotic activity between pieces taken simultaneously from an internode.

Until more is known regarding the capability of a given cambial cell to divide, and thus whether it should be counted as a part of the cambial zone, determinations of actual mitotic activity made from any one sample may not be reliable.

In terms of sampling techniques, the question of whether the place to place differences in actual mitotic activity within internodes, as suggested by Trees 1 and 4, mask trends in mitotic activity during a season's sampling must be answered. From work

done on seasonal mitotic activity [(Gregory[2], Wilson (1966)], it would appear that actual differences at any one time are not important but relative differences between samples over a period of time are. In this respect, a reasonably true picture of actual mitotic activity, as shown by Wilson (1966) and illustrated by Trees 2 and 3, are obtained by the present method of sampling.

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[2]Personal correspondence

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