Effect of girdling on cambial activity in white pine

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There are no changes in cell dimensions more than 10 cm above a girdle in white pine (*Pinus strobus* L.), but up to 10 cm above, cell radial diameters are less, and at 2 cm cell walls may be thicker. There is an increase in number of cells produced from 2 to 310 cm above girdles, apparently because of a higher mitotic index and longer season for mitotic activity. The radial number of cells in the cambial zone does not change. From 2 to 310 cm below girdles mitotic activity and radial enlargement soon stop, cell wall thickening continues slowly, and there is no change in radial cell number in the cambial zone. These effects above and below girdles appear to be independent of time of girdling. They may be explained by the effect of girdling in blocking both the polarized auxin transport system and the non-polarized phloem transport system.

Introduction

Cambial activity is a general term that covers cell division, enlargement, and maturation (11). There is increasing evidence that these three phases are controlled by growth regulators and carbohydrates produced by the leaves and moving down the stems (3, 4, 5, 6, 8, 12, 13). In this study girdling was used as a technique to modify the phases of cambial activity by blocking the movement of materials down the stem so that activity could be followed above the girdle where the concentration of materials increases and below the girdle where the concentration decreases.

Two different experiments were performed. In the first, the trees were girdled before or during the growing season and cambial activity was studied 2 cm above and below the girdles. This experiment was designed to detect seasonal variations in materials moving down the stem as described in the literature (2, 4, 13). The second experiment followed cambial activity 10, 110, 210, and 310 cm above and below girdles made about 3 weeks after activity had started, to see whether there were gradients in activity with increasing distance from a girdle. Increasing activity with increasing distance below a girdle has been described (9), but Bormann (2) found no gradient in the increase in radial increment up to 5.3 m above a girdle in white pine.

Materials and Methods

The white pine trees (*Pinus strobus* L.) were growing at the Harvard Forest, Petersham, Mass. All girdles were

2 cm wide and filled with petroleum jelly to prevent drying. All samples were taken below the first live branch.

In 1965, nine trees were girdled about 1.5 m above the ground. They were about 25 years old, growing in an old field, and had produced 80–200 cells radially in 1964. These trees were sampled 10 times, 2 cm above and 2 cm below the girdle, at weekly or semiweekly intervals. Two ungirdled control trees were sampled 25 times, at weekly intervals, within one internode.

In 1966, eight trees were selected in a 33-year-old plantation. They had produced from 17 to 56 cells radially in 1965. Four sampling heights were marked on each tree, the lowest at about 1.5 m above the ground and the others 1, 2, and 3 m above the lowest. On May 24, two trees were girdled 10 cm below the lowest sample point (low girdle), two trees were girdled 10 cm above the highest sample point (high girdle), two trees were girdled both low and high (double girdle), and two trees were left ungirdled (control). Six samples were removed from each tree at each sample height, the first just before girdling, the others 1, 3, 5, 7, and 9 weeks after girdling.

All samples were fixed, embedded in paraffin, sectioned tangentially and transversely, and prepared as permanent microslides (10). Mitotic index (percent of cambial zone cells in mitosis) was determined as before (10) for samples taken more than 2 cm from girdles. The high number of pseudotransverse divisions 2 cm above girdles destroyed the radial arrangement of nuclei in radial files, so the number of files had to be estimated. Cell lengths in the cambial zone were calculated using a relationship determined from sections of completed rings (log number of cell tips = 0.6196 − 1.3756 log cell length), and the average number of cell tips from ten 200× microscope fields was determined for each sample. Average cell length multiplied by average tangential cell diameter gave the average tangential cell area which, divided into the area of the 100× microscope field used to count mitoses, gave the number of files per field. Other calculations were as before (10). All cell diameters and cell wall thicknesses are the average of 10 tangentially adjacent cells.

As in previous studies the radial number of cells in the cambial zone (NCZ) was determined by counting the radial number of thin-walled, unenlarged cells between the xylem and the phloem (10, 11). It would be desirable to count only those cells which are actually dividing;

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however it is not possible to determine if a non-mitotic cell is in some preparative phase of cell division. Mitoses are not frequent enough (usually less than 3% of NCZ) to count from serial tangential sections the radial number of cells in the band that includes mitotic cells. Thus, counts of NCZ are all overestimates of the number of dividing cells. Below girdles, where all mitotic activity may stop (Fig. 1), NCZ may not include any cells that under current conditions are dividing. It is not known if, were these conditions changed, as when the girdle is bridged by callus, the cells counted in NCZ would be dividing cells or not.
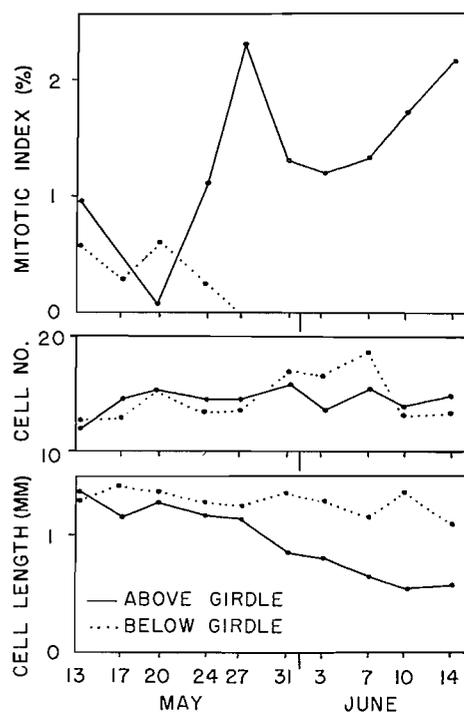

FIG. 1. An example of changes in mitotic index, number of cells in the cambial zone, and cell length, 2 cm above and below a girdle. Tree girdled May 13, 1965. Much of the variability is due to variability among samples taken at different points around the circumference of the stem.

Results

Above Girdles

Changes in the dimensions of cells maturing after girdling were observed only at 2 and 10 cm above girdles (Tables I, II). The effects of girdling at different times of the season were essentially the same (Table I). More than 10 cm above the girdles there were either no significant differences from dimensions before girdling, or the changes were not greater than those that occurred in control trees (Table II). The changes in dimensions were as follows: at 2 and 10 cm

above girdles cell diameter decreased; cell wall thickness was generally unchanged after girdling, but 2 cm above girdles there was a significant increase in wall thickness in two of nine trees (Table I); cell length decreased to about half the original length, because of pseudotransverse and lateral divisions, at 2 cm above girdles (Fig. 1), but was unchanged at all other sampling heights.

Although cell dimensions were modified only up to 10 cm above girdles, tracheid production was increased at all sample points above girdles (Table III). At 2 cm, six trees showed increased cell number, one showed no change, and two trees showed a decrease (Table I). Samples taken farther above the girdles in trees with decreased ring width at 2 cm showed that, at 10 cm, ring width was greater in the year of girdling than the year before. In samples from 10 to 310 cm above girdles the percentage increase in cell production was comparable at all heights (Table III).

The production of phloem also increased 2 cm above girdles. The rate of production increased from about 0.11 cell per day in controls and treated trees before girdling, to 0.21–0.27 cell per day. The percentage increase in phloem production was generally greater than the increase in xylem production. In the phloem produced after girdling the tannin-filled parenchyma that form bands under normal conditions were scattered throughout the tissue (Fig. 2). No change in phloem production was detected at 10–310 cm above girdles, but these trees were growing relatively slowly and only one or two phloem cells were produced after girdling in any of the trees.

Changes in total cell production above a girdle may be determined by changes in the length of season, mitotic index, or the radial number of dividing cells in the cambial zone. In trees sampled 2 cm above girdles, the tree girdled August 18 still had a mitotic index of 0.3 on October 9, 2 weeks after all mitotic activity had stopped in control trees; the tree girdled July 15 and sampled at weekly intervals had a mitotic index of 1.8 on September 16, when the mitotic index of controls had decreased to less than 0.2. The cells produced during the extended season for cambial activity above girdles were narrow latewood cells (Fig. 3). The other seven trees, sampled the next winter, all had 10–20 latewood cells, compared to two to four latewood cells in
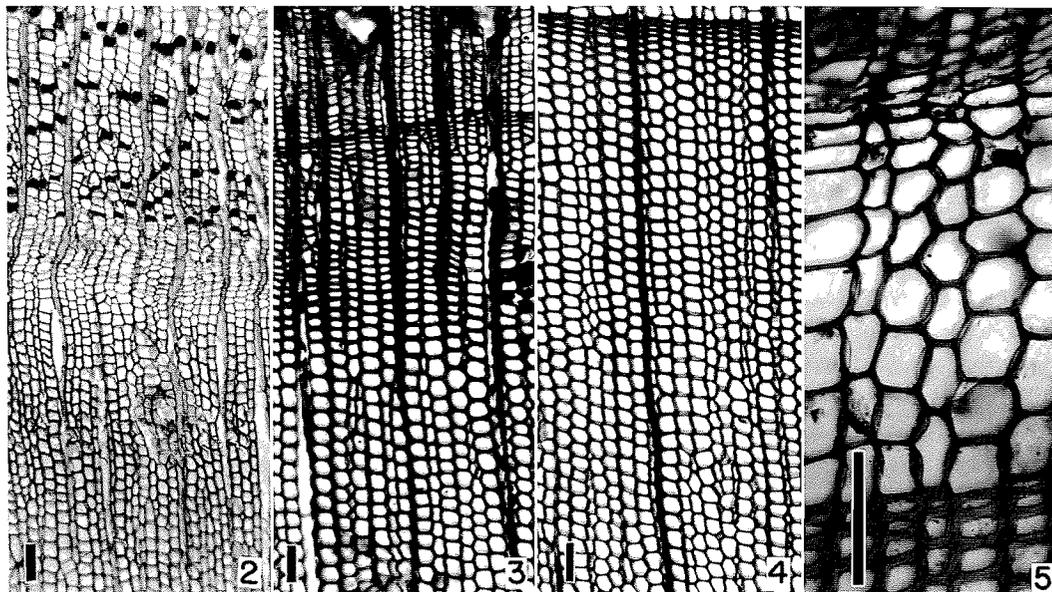
PLATE I

FIG. 2. Transverse section 2 cm above a girdle 9 weeks after girdling (black bar = 0.1 mm).
FIG. 3. Transverse section showing latewood produced 2 cm above a girdle made August 15 (black bar = 0.1 mm).
FIG. 4. Transverse section showing normal latewood (black bar = 0.1 mm).
FIG. 5. Transverse section sample taken after the growing season 110 cm below a girdle made May 24 (black bar = 0.1 mm).

TABLE I

Effect of girdling during 1965 on cambial activity 2 cm above girdles. Changes in radial cell diameter and cell wall thickness are given only if they are significant at .05 or better using Student's t test, ns = not significant. Average cell dimensions in 1964 were radial diameter = 37 μ, cell wall thickness = 3.2 μ

Date girdled	Tree No.	1965 dimension/1964 dimension, %			Mitotic index, $\frac{\text{before girdling}}{\text{after girdling}}$, %
		Radial diameter	Wall thickness	Total cell no.	
April 1	9	56	ns	136	*
	2	69	ns	129	*
May 12	5	60	ns	84	200
	3	64	ns	156	104
June 16	7	71	124	171	91
	8	54	ns	129	222
July 15	6	66	145	216	200
	4	83	ns	80	170
Aug. 18	1	*	*	98	150
Control	A	ns	ns	116	*
	B	ns	ns	95	*

*Cannot be computed for this tree.

TABLE II

Effect of low girdling on cell dimensions above the girdle for trees girdled May 24, 1966. A = last cell in latewood, B = 10 cells nearer the pith than A. Only differences significant at .05 or better using Student's t test are given, ns = not significant. Average 1965 dimensions were, for cell diameter, A = 16 μ, B = 36 μ; for cell wall thickness, both A and B = 3.8 μ

		1966 dimension/1965 dimension, %							
		Radial cell diameter				Cell wall diameter			
		Control		Low girdle		Control		Low girdle	
		Tree No.							
		2	7	4	8	2	7	4	8
310 cm above girdle point	A	127	ns	ns	ns	126	ns	ns	ns
	B	84	82	88	ns	111	ns	110	140
210 cm ” ” ”	A	127	ns	ns	ns	ns	ns	ns	120
	B	128	133	ns	ns	82	81	ns	120
110 cm ” ” ”	A	152	ns	ns	ns	ns	115	ns	ns
	B	ns	127	ns	ns	ns	87	ns	128
10 cm ” ” ”	A	138	ns	ns	57	79	ns	ns	83
	B	120	88	63	73	76	ns	ns	ns

TABLE III

Effect of low girdling on cell production above girdles. Average 1965 cell numbers at all heights for trees 2, 7, 4, and 8 were respectively 44, 32, 17, and 47

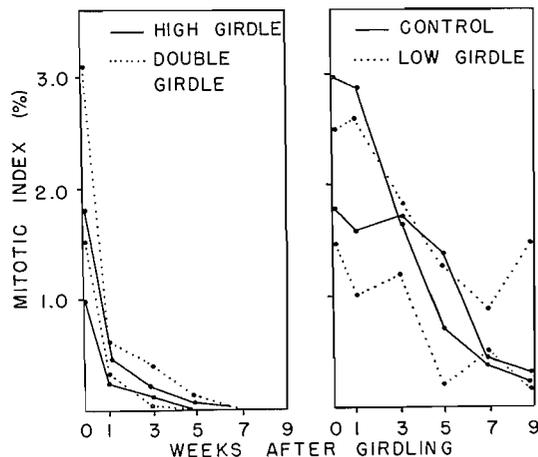|	1966 cell no./1965 cell no., %			
	Control		Low girdle	
	Tree no.			
	2	7	4	8
310 cm above girdle point	79	125	105	138
210 cm ” ” ”	91	73	107	123
110 cm ” ” ”	98	59	108	103
10 cm ” ” ”	68	51	175	129

FIG. 6.   Change in mitotic index after 1966 girdles. Data from all four sampling heights are averaged for each point.

control trees (Fig. 4); thus it may be assumed that the season for cambial activity was extended in all trees 2 cm above the girdle. In the trees sampled from 10 to 310 cm above a girdle the mitotic indexes of samples taken from a tree at a given time were generally comparable at the four-sample height. The sampling error for number of mitoses per core (10) overlapped between samples except for a few cases, and the exceptions followed no pattern. Therefore, the data for mitotic index are given as averages of all heights sampled (Fig. 6). The seasonal

decrease of mitotic index appeared to be slower in low-girdled than in control trees. Thus, the season for mitotic activity may also have been extended up to 310 cm above girdles; however, no extra latewood cells were produced from 10 to 310 cm above girdles in low-girdled trees.

Determination of the effect of girdling on mitotic index was complicated by variability between samples taken at different points around the circumference of the stem and under different weather conditions. The extent of this variability is suggested by the mitotic index of the control trees throughout the season (Fig. 7). In five trees girdled after April 1 and sampled 2 cm above girdles, the mitotic index increased up to threefold (Fig. 1); in two trees there was no change in mitotic index or even a slight decrease. No increase in mitotic index was detected at 10–310 cm above girdles (Fig. 6).

The radial number of dividing cells in the cambial zone (NCZ) did not change above girdles. The NCZ 2 cm above a girdle was not higher than the NCZ below the girdle (Fig. 1), even though the mitotic index had increased above and stopped below. In the example shown in Fig. 1 there is a gradual rise in NCZ, but this rise was not observed in all trees. As in the determination of mitotic index variability around the circumference of the stem undoubtedly contributes to much of the variation between successive samples. In trees sampled at different
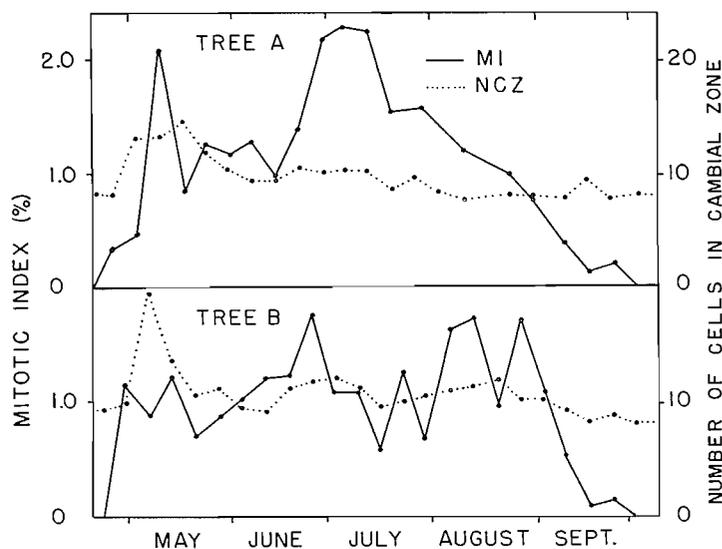

FIG. 7.   Seasonal change in mitotic index (MI) and radial number of cells in the cambial zone (NCZ) of two control trees in 1965.

heights, the seasonal change in NCZ appeared to be related to the NCZ at the time of girdling rather than to the girdling treatment, so that high- and double-girdled trees had the same patterns as low girdled and control trees.

Below Girdles

The effect of girdling on the phases of cambial activity were essentially the same from 2 to 310 cm below girdles in single- and double-girdled trees. These effects may be summed up as follows: cell division and cell enlargement stopped within a few weeks (Figs. 1, 6); cell wall thickening continued at a much reduced rate; and the final wall thickness of about 1.1 μ was less than one-third the wall thickness of the controls (Fig. 5). In general, all aspects of cambial activity were stopped by girdling on April 1, before activity had started, although some mother cells formed septa and filled with tannin-like materials. Trees girdled after cambial activity had started continued activity for a short time, with the exception of wall thickening, which continued for several months at a reduced rate.

Discussion

There seem to be both local and long distance effects on cambial activity above girdles in white pine. There is a relatively local effect from 2 to 10 cm above where cell length and cell diameter decrease, wall thickness may increase, and the rate of phloem production is doubled. The long distance effect of increased production of xylem cells was observed up to 310 cm above girdles in this study, and more than 5 m above a girdle by Bormann (2). These two effects may result because girdling interferes with both the auxin and the phloem transport systems. Auxin transport is polarized so that it moves only basipetally in woody stems (1); thus, auxin presumably accumulates directly above girdles. This high auxin concentration, interacting with any substances produced by the wound tissue at the very edge of the girdle, produces a type of wood that in the decreased radial diameter of tracheids and thicker walls resembles compression wood (7). The phloem transport system, however, is not polarized and does not have to move basipetally. A girdle cuts off the sink represented by the root system and can cause the concentration of phloem exudate to increase in a column more than 5 m above the girdle (14), thus producing the long distance effect. Because the carbohydrates tend to "pile up" for long distances above the girdle there is no gradient in cell production up to 310 cm above the girdles.

No local effect was observed below girdles. The cessation of mitotic activity and cell enlargement, and the reduced wall thickening, were comparable in samples from 2 to 310 cm below girdles. These effects suggest that the transport systems were essentially inoperative in the 3 m below the girdles. No materials were added to the systems from the leaves, so available carbohydrates and growth-regulating substances were used up locally and did not drain out toward the root system. As wall thickening continued at the expense of stored carbohydrates, wall thickening may be somewhat independent of growth-regulating substances from the leaves.

Tracheid dimensions are largely determined by growth-regulating substances moving from the leaves (5, 13). In white pine there seems to be a major change in the nature of these substances about the middle of August, about the time when the needles stop elongating. Up to the middle of August thin-walled tracheids of large diameter are produced (they are slightly narrower 2 cm above girdles), but after the middle of August thick-walled tracheids of narrow diameter are produced. In control trees cell production stops soon after the middle of August so that only two to four latewood cells are formed, but girdling increases the number of cells produced after August 15 by extending the season for cambial activity, so that 10–20 latewood tracheids are produced. Apparently materials promoting cambial activity continue to move down the stem for some time after activity normally stops.

The increase in cell production above girdles may have been due to an increased mitotic index, an increased season for mitotic activity, or both, but it was not due to an increase in the radial number of dividing cells in the cambial zone (NCZ). The data on mitotic index are too variable for one to draw definite conclusions, but the data on NCZ show clearly that NCZ is insensitive to the rate of cell production. In control tree A (Fig. 7), the mitotic index more than doubled for a few weeks with no change in NCZ and, in those trees in which the rate of cell production increased above a girdle, the mitotic index increased with no change in NCZ. Below girdles the NCZ stayed unchanged even though

mitotic activity stopped. The NCZ is determined by a balance between the production of new cambial zone cells by cell division and the loss of cambial zone cells by their entry into the phase of enlargement. The NCZ appears to undergo a consistent seasonal pattern of change which is relatively independent of other aspects of cambial activity. The factors determining this balance between the phases of enlargement and division are not known.

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