CELLULAR KINETICS OF COMPRESSION WOOD FORMATION IN SLASH PINE

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ABSTRACT

The kinetics of auxin-induced compression wood formation in slash pine (*Pinus elliottii* Engelm.) seedlings were investigated with regard to rate and duration of cambial cell division, radial enlargement, and secondary wall formation. Exogenous lateral application of auxin (indole acetic acid) in lanolin paste to the basal portion of the seedling stems markedly increased the rate of cambial cell division and radial enlargement with a nearly proportionate decrease in the duration of both stages. The auxin application had little effect on the rate of secondary wall formation of differentiating tracheids but significantly increased the duration of this stage, resulting in a large increase in tracheid wall thickness. Untreated control seedlings exhibited no evidence of changes in cellular kinetics during the treatment period. Results indicate that the increased wall thickness of compression wood tracheids in slash pine results from an extended duration of secondary wall deposition rather than an increased rate of deposition as previously thought.

Keywords: Southern pine, compression wood, cellular kinetics, tracheid differentiation.

INTRODUCTION

Although the structure and function of compression wood in conifers have been widely explored (Scurfield 1973), very little has been reported on the physiological and cytological events leading to its formation (Westing 1968). For instance, it was not known whether the thick cell walls of compression tracheids result from an enhanced rate or an enhanced duration of secondary wall deposition or from both (Denne 1972). Kennedy and Farrar (1965) tilted potted pine seedlings to induce compression wood formation and examined anatomically the cellular events leading to the differentiation of compression tracheids. These authors assumed that in compression wood formation, the enhanced rate of differentiation of tracheids usually observed (Westing 1968) was accompanied by a similarly enhanced rate of cell-wall deposition, which results in the greatly thickened cell walls characteristic of compression tracheids. However, the micrographs of Kennedy and Farrar (1965) indicated that the zone of secondary cell-wall deposition in their tilted seedlings was greatly extended in width during the formation of compression wood. The authors assumed that the number of tracheids forming secondary wall was the same as before tilting the seedlings and attributed the increase in cell

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number to the greater age of the seedlings as compared to those of an earlier experiment.

Skene (1969) and Wodzicki (1971) demonstrated that the thickening of tracheid walls during latewood formation in several conifers is accompanied by an increased width of the zone of secondary wall deposition resulting from an extended duration of wall deposition. Kennedy and Farrar (1965) did not investigate the kinetics of compression wood tracheid differentiation in their tilted seedlings, but Nix (1974) showed that the increased number of cells undergoing secondary wall deposition during compression wood formation in slash pine was associated with an extended duration of cell-wall deposition. The purpose of this paper is to describe the cellular kinetics of secondary wall deposition during auxin-induced compression wood formation in young slash pine seedlings.

MATERIALS AND METHODS

Two-year-old slash pine (*Pinus elliottii* Engelm.) seedlings growing in a greenhouse under summer conditions were stimulated to form compression wood by application of auxin. Indole acetic acid or IAA (0.1% w/w in a lanolin-water paste) was applied near the base of the seedlings to a 1-cm-wide band of stem cortex tissue exposed by gently scraping away the periderm with a scalpel. At the time the treatment began, the location of the cambium in the seedlings was marked by inserting a small insect-mounting pin into the stem creating a pocket of abnormal wound parenchyma in the cambial zone (Wolter 1968). The treatment paste was removed and reapplied freshly at weekly intervals and at the end of three weeks the application site on the seedlings was reexposed by again gently scraping the treatment site with a scalpel. Each week after the first week, the five seedlings to be collected the following week were pinned a second time at the same height, but 90° adjacent to the first pin in order to temporally mark the progress of wood formation and thus determine the rate of tracheid production during each week.

Five control seedlings were treated and harvested similarly to the IAA-treated seedlings each week but without the IAA in the lanolin paste. Five typical seedlings selected from the group of seedlings were pinned two weeks in advance of the experiment and harvested the day the experiment began to establish the rate of cambial activity and the widths of the zones of tracheid differentiation for the seedlings at the time the treatment began.

At weekly intervals five seedlings, randomly chosen and pinned the week before, were harvested and the treated stem segment (1 cm long) including the pin(s) was collected from each seedling, and killed, fixed, and embedded in paraffin according to Sass (1958). Differentially stained and permanently mounted transections (15–20 μm thick) were prepared from the embedded segments. From a suitable transection of each segment, five radial files of tracheids of maximum radial diameter were chosen, and the number of cells was counted in each of three zones of tracheid differentiation.

The zones of tracheid differentiation were distinguished as described by Skene (1969) and Wodzicki (1971), i.e., the radially enlarging or growing zone extends from the first xylem mother cell derivative exhibiting radial expansion to the first enlarging cell showing maximum radial diameter; the secondary wall deposition

or maturation zone extends from the first cell showing maximum diameter to the last cell having a visible remnant of cytoplasm; the fully differentiated or mature tracheid zone extends from the first tracheid without cytoplasmic residue to the first cell in the annual ring or, as in this study, to the innermost cells of the pin-wound parenchyma pocket marking the location of the cambium at the beginning of each measurement period. The zones may overlap 1–2 cells because of the dynamic nature of the differentiation process, and the exact location of the cambium marked by pin insertion may vary by ±2 cells (Wolter 1968). The radial and tangential tracheid and lumen diameters of the last two fully matured tracheids in each radial file were measured with a projection microscope to the nearest μm at 500×.

Cell number and dimensions were averaged for each seedling. Treatment and control means were calculated for each week of the experimental period (7 weeks). In order to examine rates and duration of secondary wall synthesis, transverse wall area was calculated from tracheid dimensions, assuming a rectangular transverse shape for normal tracheids and an elliptical shape for compression wood tracheids (Nix 1974). Mean cell counts in the zones of differentiation were plotted cumulatively over time (days) on graph paper (20 lines/inch), and the resulting curves were analyzed graphically as described by Skene (1969) and by regression analysis as modified from Denne (1972) to determine the duration (in days) of the phases of tracheid differentiation. The mean daily rate of secondary wall deposition for each week was estimated by dividing the mean transverse wall area (μm^2) of the last two fully matured tracheids by the mean duration of wall deposition (days) for that week.

Transverse cell-wall area is a reasonable indicator of total wall substance deposited, and its rate of increase should reflect the rate of increase in total wall substance (Skene 1969; Wodzicki 1971; Denne 1972). Although pine compression wood tracheids have been found to average about 10% less in length than normal tracheids (Westing 1968), differences in total wall material of the two types of tracheids resulting from this difference in length were assumed negligible because of the blunted, relatively untapered ends of compression wood tracheids, as compared to the tapered ends of normal wood tracheids. Differences in treatment weekly mean values were statistically tested with analysis of variance and the F test for linear contrasts.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate the cellular kinetics of tracheid differentiation in IAA-treated and control seedlings. The three curves of each figure indicate cumulative cell counts in the various stages of tracheid differentiation at weekly intervals during the seven weeks of the experiment. The lowermost curve of Figs. 1 and 2 represents the weekly increase in the number of mature tracheids; the middle curve represents the weekly increase in number of mature and maturing tracheids; and the upper curve shows the weekly increase in the number of mature, maturing, and enlarging tracheids. Dividing cells are not shown. The number of cells vertically between any two curves of the figures represents the number of tracheids undergoing the stage of differentiation indicated during any given week.

The rate of tracheid entry into and exit from a specific differentiation stage

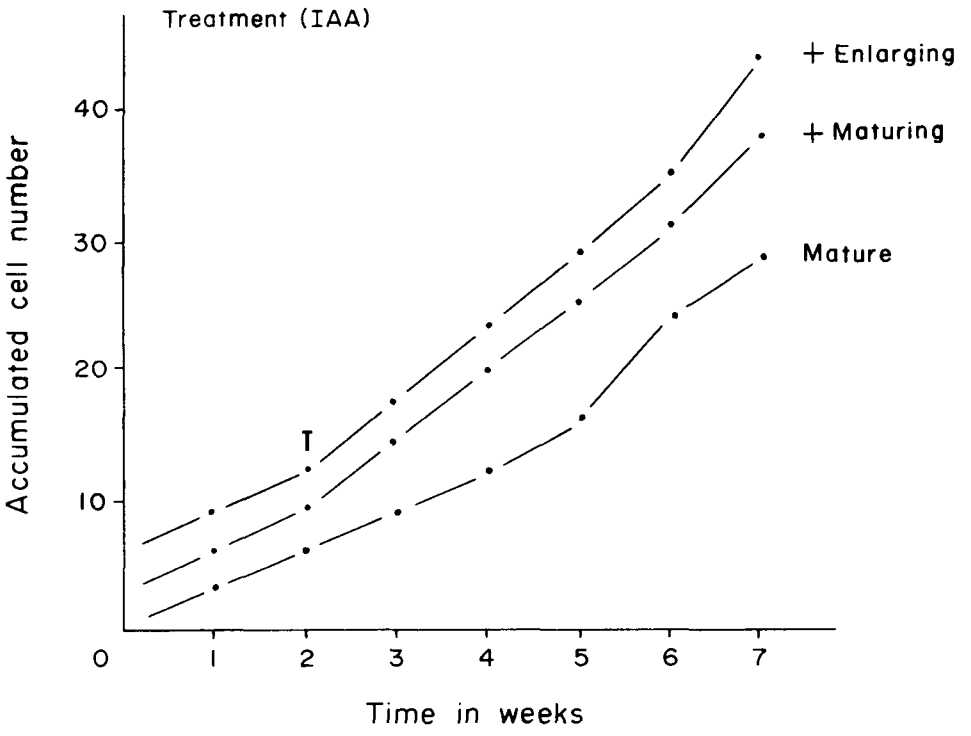

FIG. 1. The kinetics of compression wood tracheid differentiation during the period of stem application of auxin to slash pine seedlings. Various zones of differentiation are indicated (enlarging, maturing, and mature). The letter T indicates beginning of treatment.

during a week is shown by the slope of the line connecting the weekly cell counts of the curves. The rate of exit from a differentiation zone is equal to the rate of tracheid entry into the next lower (or histologically innermost) stage. The duration of a differentiation phase during any week shown in Figs. 1 and 2 can be determined graphically by placing a ruler horizontally (parallel to the X axis) across the two curves delineating the phase of interest and measuring the distance (horizontal) between the curves. This horizontal measurement can be readily converted to units of time (Skene 1969).

In the IAA-treated seedlings (Fig. 1), the rate of cell division increased rapidly during the first week of treatment as indicated by the increased rate of accumulation of total cells, i.e., the increased slope of the upper curve. The rate of exit of differentiating tracheids from the enlarging stage also increased during this period resulting in no increase in the number of cells in the enlarging stage. The increased rate of cell entry and exit from the enlargement zone reflects the decrease in the duration of radial enlargement. However, measurements of tracheid dimensions showed no reduction in radial or tangential diameter, therefore, the rate of radial enlargement must have increased in proportion to the reduction in duration of enlargement.

The rate of entry of differentiating tracheids into the maturing stage increased after IAA treatment began, but the rate of exit from this stage remained constant

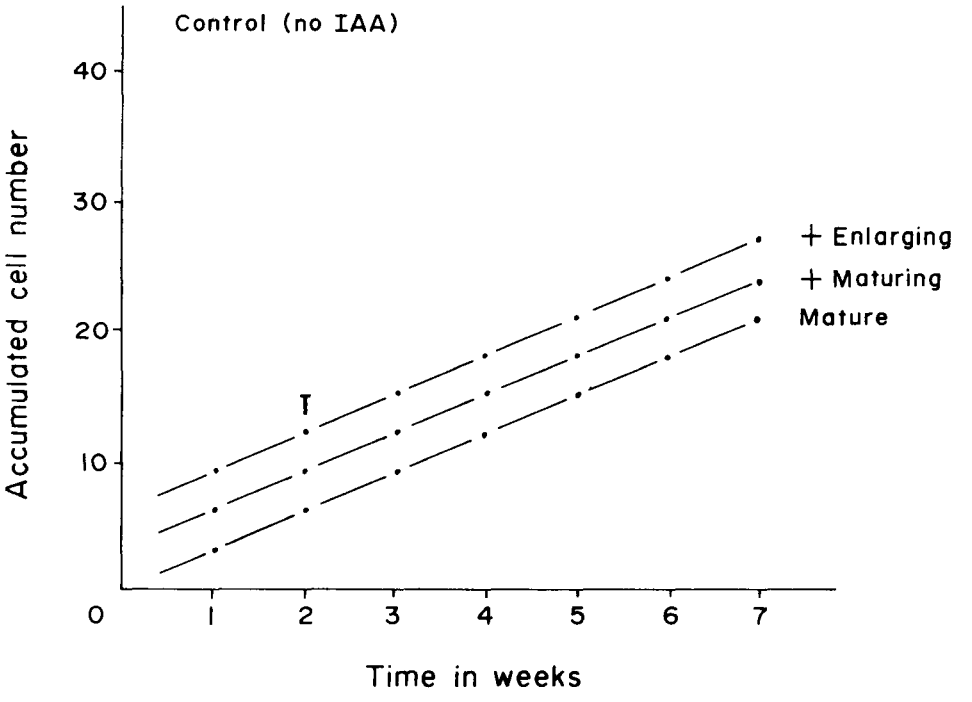

FIG. 2. The kinetics of normal tracheid differentiation during the period of stem application of lanolin and water alone to slash pine seedlings. Various zones of differentiation are indicated (enlarging, maturing, and mature). The letter T indicates beginning of treatment.

until the fifth week of the experiment, resulting in an increasing number of tracheids in the maturing stage and reflecting a corresponding increased duration of this stage. The increased width of the maturing zone in an IAA-treated seedling can be seen in Fig. 3, a photomicrograph of a transection through the differentiating stem tissues of a seedling after three weeks of IAA treatment. In contrast, the control seedlings exhibited essentially no change in any parameters of differentiation kinetics during the experiment (Fig. 2).

Rates of entry and exit, the number of cells, and the duration of the maturing stage of tracheid differentiation for each week of treatment are shown for both IAA-treated and control seedlings in Table 1. Since the data for the first two weeks of the experiment were taken from the same seedlings (untreated) to establish the initial cell differentiation kinetics, only the weeks of the actual treatment are shown in Table 1. These data were obtained by graphic analysis from the unrounded mean weekly cell counts of Figs. 1 and 2. The data from Table 1 show that the tracheid differentiation kinetics of the control seedlings remained relatively constant, whereas those of the IAA-treated seedlings were highly variable. For example, during the fourth week of treatment, the rate of exit of tracheids from the maturation zone increased substantially in IAA-treated seedlings, while the rate of entry slightly decreased, resulting in a pronounced decrease in both the number cells in, and the duration of, the maturation zone (Table 1).

During the fifth week of treatment in the IAA-treated seedlings, the rate of

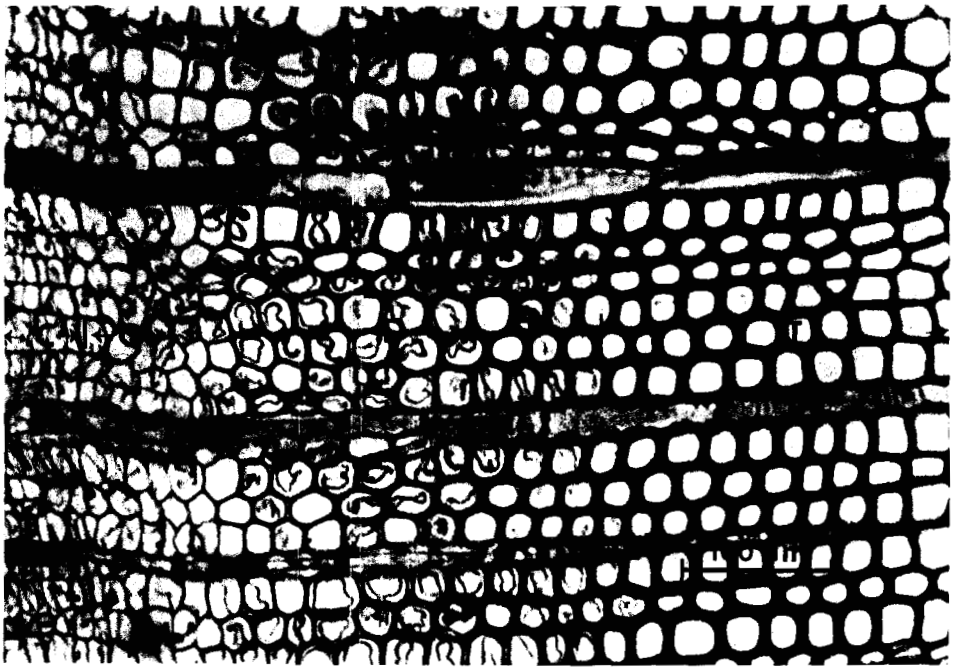

FIG. 3. Transection through differentiating stem tissues of a slash pine seedling after 3 weeks of stem application of auxin. Histological zones are as indicated: C—cambial cells; G—enlarging tracheids; D—maturing tracheids; T—mature tracheids.

entry of tracheids into the maturation zone increased while the rate of exit decreased, resulting in an increased number of cells in, and an increased duration of, the maturing stage. The fifth week treatment effects indicate a possible cyclic pattern in the response of the seedlings to supra-optimum levels of IAA, but the experiment was not continued long enough to prove or disprove this possibility. The rate of cell division and enlargement remained essentially unchanged during the period of the change in the maturation response; thus it is difficult to interpret the reduced response as a decrease in available IAA.

The results indicate that there was an apparent difference of response in the enlarging and maturing zones simultaneously. It is generally thought that radial enlargement and secondary wall deposition are under separate physiological control during tracheid differentiation (Brown 1970). Thus, it may be possible that the level of IAA in the dividing and enlarging zones was high enough to maintain the high rate of activity there during the fourth week, but at the same time the level of IAA in the maturing zone was not high enough to maintain the increased duration of tracheid life in the secondary wall deposition stage.

Table 2 shows the amount of wall material, expressed as transverse wall area, deposited in the last matured tracheid by the end of each week of treatment and the corresponding rate and duration of wall deposition for that tracheid. Transverse tracheid wall area of the treated seedlings was maximum at the end of the second week of IAA treatment, decreased during the third and fourth weeks and increased slightly during the fifth week. Tracheid differentiation in the control

TABLE 1. *Maturation zone cellular kinetics in IAA-treated and control slash pine seedlings.*[1]

Week	Rate of entry (no. cells/week)	Rate of exit (no. cells/week)	Number of cells in the zone	Duration of maturation (days)
Control (No IAA)				
1	2.8 a	3.0 a	2.9 a	7.5 a
2	2.6 a	2.9 a	2.7 a	7.0 a
3	3.2 a	2.6 a	3.2 a	7.0 a
4	2.8 a	3.0 a	3.0 a	7.5 a
5	3.0 a	3.0 a	3.0 a	7.0 a
Treatment (+IAA)				
1	4.6 b	2.7 a	5.0 b	8.2 b
2	4.8 b	3.1 a	6.7 c	10.0 c
3	6.5 d	4.6 b	8.6 d	10.0 c
4	5.6 c	7.3 c	6.9 c	8.5 b
5	7.4 e	4.2 b	9.1 d	9.0 b

[1] Mean values for five seedlings collected each week from the two groups. Values in the same column followed by the same letter are not significantly different at the 0.01 level.

seedlings remained essentially unchanged during the treatment period and the duration of maturation and transverse wall area were significantly less than those of the IAA-treated seedlings.

In the control seedlings, tracheid wall area and duration of wall deposition changed very little but rate of deposition varied considerably (Table 2). The duration of deposition and transverse wall area of the IAA-treated seedlings are considerably greater than those of the control seedlings during every week of the treatment, but the weekly rates of wall deposition in the two groups are not so easily contrasted. Whereas mean weekly values for wall area and duration are significantly different between the two groups, mean weekly rates of deposition are not significantly different (at the 0.01 level by analysis of variance).

The amount of wall material deposited during tracheid differentiation is dependent upon the rate and duration of wall deposition during the maturation stage (Wodzicki 1971). Increased wall deposition during latewood formation in several *Pinus* species with distinct earlywood–latewood transition has been shown to be associated with an extended duration of wall deposition and a reduced rate of deposition (Skene 1969; Wodzicki 1971). The relative importance of the duration and rate of wall deposition in the increased wall thickness of compression wood tracheids in the present study can be assessed with correlation analysis, which was performed on the combined data from Table 2. The relationship between transverse wall area and duration of wall deposition was strong as indicated by a correlation coefficient, $r = +0.9446$, which was significant at the 0.01 level. In addition, 89% ($r^2 = 0.8924$) of the variation in wall area was associated with variation in duration. The correlation coefficient ($r = +0.7157$) of the relationship between transverse wall area and rate of deposition was not significant at the 0.01 level and only 51% ($r^2 = 0.5123$) of the variation in wall area was associated with the variation in rate of wall deposition.

Thus, in regard to duration of secondary wall deposition, compression wood formation in seedlings is similar to latewood formation as described in mature *Pinus* by Skene (1969) and Wodzicki (1971). However, there are many dissimi-

TABLE 2. *Rate, duration, and amount of secondary wall thickening in IAA-treated and control slash pine seedlings.*[1]

Week	Duration (days)	Transverse wall area (μm^2)	Rate of wall deposition (μm^2 day^{-1})
Control (No IAA)			
1	7.5 a	241.0 a	32.1 a
2	7.0 a	259.0 a	37.0 a
3	7.0 a	258.0 a	36.9 a
4	7.5 a	251.0 a	33.5 a
5	7.0 a	253.0 a	36.1 a
Treatment (+IAA)			
1	8.2 b	314.0 b	38.3 a
2	10.0 c	407.0 d	40.7 a
3	10.0 c	358.0 c	35.8 a
4	8.5 b	328.0 b	38.6 a
5	9.0 b	331.0 b	36.8 a

[1] Mean values for five seedlings collected each week from the two groups. Values in the same column followed by the same letter are not significantly different at the 0.01 level.

larities in the differentiation of the two types of wood. During latewood differentiation, the rate of cell division decreases, tracheid radial enlargement decreases, rate of wall deposition decreases, and total time required for tracheid differentiation greatly increases (Skene 1969; Wodzicki 1971); during compression wood differentiation rate of cell division increases, tracheid radial enlargement is unchanged, rate of wall deposition is essentially unchanged, and total time required for tracheid differentiation decreases.

The similarities and dissimilarities in compression and latewood tracheid differentiation are quite difficult to interpret with regard to a universal mechanism controlling secondary wall thickening. It is most interesting that high levels of IAA can decrease the duration of one phase of tracheid differentiation (radial enlargement) and at the same time increase the duration of another phase (maturation). The findings of Morey and Cronshaw (1968) that low concentrations of auxin exogenously applied to the stems of *Acer* seedlings induce the formation of tension wood, normally a result of auxin deficit, while at the same time stimulating cambial activity (cell division) led them to conclude that there are sharp radial gradients of biochemical and physiological conditions across the differentiating cambial tissues of trees. These authors concluded that the high rate of cambial activity caused by the exogenously applied auxin depleted endogenous auxin supplies, resulting in an auxin deficit in the region of maturation and the subsequent formation of tension wood.

Morey and Cronshaw (1968) also noted that high levels of exogenous auxin, though increasing cell division, effectively prevented the differentiation of tension wood fibers in tilted *Acer* seedlings and concluded that at higher levels of application the endogenous auxin-depleting system is saturated, leaving adequate residual auxin to stimulate cell division and to permit normal fiber differentiation. In the present experiment concerning pine seedlings, the high level of applied auxin (1,000 ppm) probably saturated the differentiating cambial tissues of the seedlings, resulting in an increased rate of cell division and enlargement and an

increased duration of the maturation stage. It is probable that the endogenous levels of growth regulators (such as auxin) in the cambial tissues of trees are delicately balanced between production and destruction, with seasonal changes in these levels being quite small but engendering pronounced changes in the differentiation processes.

The suspected separation of the physiological controls of tracheid enlargement and maturation (Brown 1970; Wodzicki 1971) and the existence of sharp physiological gradients radially across the cambial tissues (Morey and Cronshaw 1968) may be the keys to solving the riddle of the mechanisms controlling cell-wall formation in trees. There have been very few attempts to investigate the physiology of wood formation in the separate histological zones of differentiating wood tissues, perhaps because of the difficulty in separating these tissues for study. The results of the present study indicate that there is much yet to be discovered before a complete understanding of the biology of wood formation in trees is achieved.

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