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Apical control of xylem formation in the pine stem. II. Responses of differentiating tracheids

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Abstract

The effect of auxin supplied to the main stem of 5-year-old *Pinus silvestris* trees during various periods after decapitation upon differentiation of the secondary xylem tracheids was investigated. The results revealed the complexity of auxin involvement in the regulatory system of tracheid differentiation of secondary xylem. It is manifested both as the inductive effect to which the cells respond in the meristematic phase and in the continuous control during the consecutive stages of radial growth and maturation. A lack of auxin during the meristematic phase resulted in smaller cell diameters and reduced the daily rate of cell wall deposition even though these cells progressively grew and matured in the presence of auxin. The intensity of these two processes increased and the cells deposited thicker walls when auxin was supplied during all stages of tracheid differentiation even though the period of maturation decreased. Under these conditions tracheids of compression wood type differentiated. Continuous availability of auxin causes earlier termination of tracheid maturation while lack of auxin results in a delay of autolysis of protoplasts. In this case auxin probably functions in a system specifying information concerning the position of the cells in respect to the cambial layer.

INTRODUCTION

Investigation of auxin effect on wood formation in decapitated pine trees indicated that both cambial activity and differentiation of tracheids involving their cell wall thickness, and radial diameter are affected (W o d z i c k i et al. 1982). Various authors studying the effects of photoperiod, gravity and decapitation (W o d z i c k i 1961, K e n n e d y and F a r r a r 1965, N e č e s a n ý 1971, W o d z i c k i and Z a j ą c z k o w s k i 1974) suggested that the morphogenic conditions during the meristema-

tic stage could be decisive for the course of later differentiation of tracheids even if the conditions are no more inductive. In the present investigation the hypothesis was tested that the auxin control of tracheid morphology is, at least partly, independent of the direct effects upon cell differentiation during the postmeristematic stage.

MATERIAL AND METHODS

Apical parts of five-year-old pine trees (*Pinus silvestris* L.) in a forest plantation of the Experimental Forest in Rogów were cut off 18 cm above the third whorl of branches. The decapitated stems were treated at the place of sectioning with 0.5 per cent IAA in aqueous-lanoline paste. The methods of stem decapitation, auxin treatment, collection of the stem segments, stem sectioning and preparation of the sections for the light microscop were as described by W o d z i c k i et al. (1982). All observations were done on transverse stem sections 2 cm below the site of decapitation. The total number of tracheids was determined along two radial files and the cell wall thickness or the tangential diameter of mature tracheids or radial diameter of maturing and mature tracheids were measured along one radial file in the region of the latest annual ring from two sections of each stem segment collected after the end of the experiments.

These measurements served for calculation of the cross-section surface area of the cell wall of the mature cells, the duration of various phases of differentiation and the rate of cell wall deposition according to the procedure described by W o d z i c k i (1971).

EXPERIMENTAL TREATMENTS

Two experiments were performed. The first started on May 21, ended on June 31, 1974 involved 20 decapitated trees in three experiments and control (5 trees each): a) IAA applied 10 days after decapitation for the next 30 days, b) IAA applied 20 days after decapitation for successive 20 days, c) no IAA applied after decapitation. Stem segments for examination were collected from decapitated and IAA-treated trees on the day of decapitation and after the end of the experiment. Untreated decapitated stems were also collected 10 and 20 days after the experiment started. The intact trees were sampled only at the end of the experiment. In the second experiment (May 21 — July 10, 1975) the decapitated stems were treated as specified in Table 1.

Table 1

The experimental series in the experiment started May 21, 1975 concerning the effect of auxin supplied to decapitated stems at various periods of tracheid differentiation upon the radial diameter of tracheids and production of cell wall materials

Date of treatment	Trees decapitated on the start of experiment		Intact trees
	A/II*	B/II*	
May 21	20**	10**	5**
	—	—	
June 3	c		
	+	+	
June 10	c		
	+	—	
June 23	c	c	
	+	+	
July 10	c	c	c

* The markering refer to experimental series presented in Fig. 1 and 3.
** Number of trees in each series before treatment.
+ — auxin applied; - — no auxin; c — collection of 5 trees for examination.

RESULTS

RATE OF TRACHEID DIFFERENTIATION

Decapitation of the tree reduced cambial xylem production in the uppermost part of the stem, but did not arrest the differentiation of the tracheids which had formed from the cambial zone before the experiment was started (Fig. 1). These tracheids consecutively ended differentiation during the period following decapitation, although the process was slower than in the decapitated trees supplied with auxin and, consequently, the cells remained alive longer (Fig. 3).

Auxin applied to decapitated trees stimulated formation of new cells from the cambial zone (Fig. 1). The stimulation increased in the successive periods of treatment (Fig. 1/II: A/II). Auxin also prevented reduction of the rate of completion of radial growth by cells which at the time of its application continued this phase of differentiation, regardless of whether auxin (natural or synthetic) was present during their meristematic stage (Fig. 1). In cells which at the time of auxin application were maturing the autolytic destruction of protoplasts increased but only when their maturation started before the date of decapitation (Fig. 1/II in both series A/II and B/II — cells with numbers 33-44, period 13th to 20th day after decapitation) or when IAA was applied during

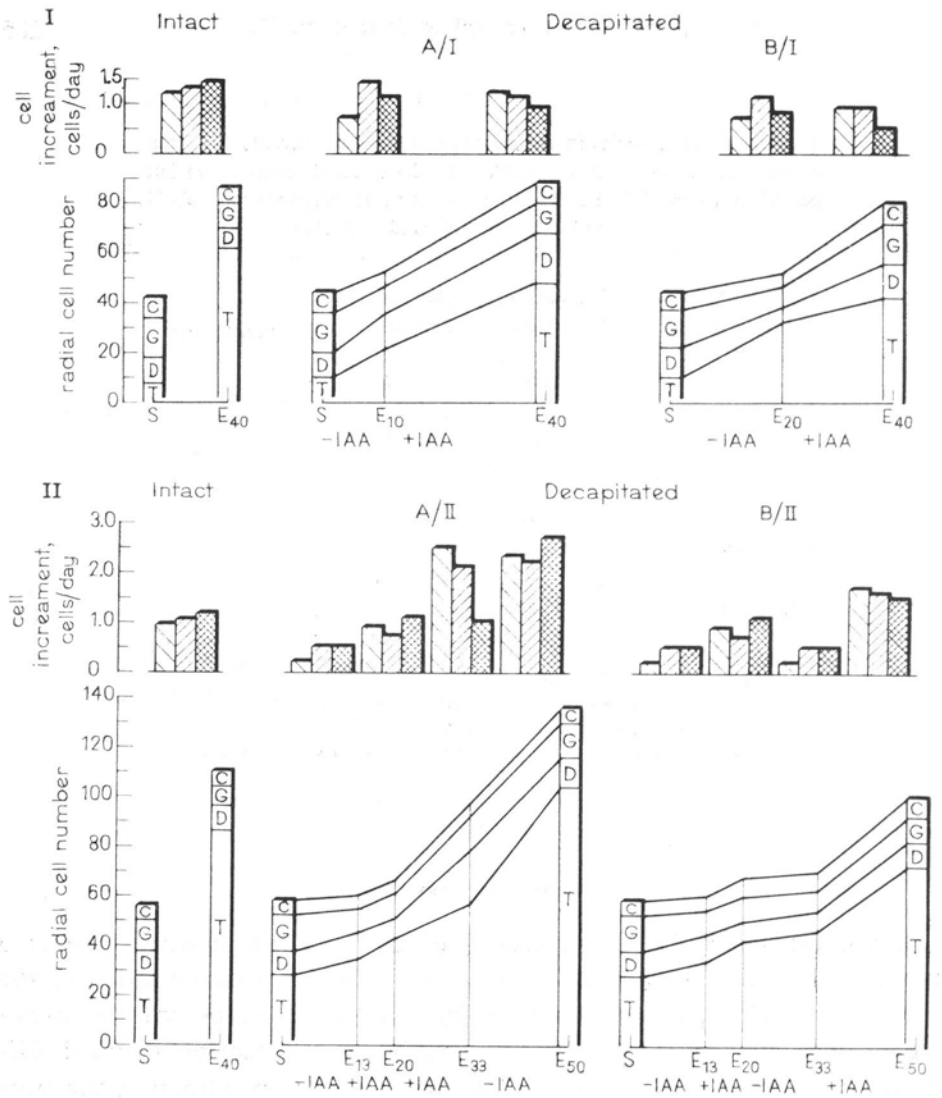


Fig. 1. Total number of tracheids in each of the zones of differentiating xylem; cambial zone — C, zone of radial growth — G, zone of maturation — D, and zone of mature tracheids — T, determined before the experiment started (S) or after the end of experimental treatments (E). Numbers given with the symbol E indicate days from the date when the experiment started. Vertical bars indicate daily rate (the number of cells per day) at ending the successive stages of differentiation in intact and in decapitated trees during the successive periods of auxin and no-auxin treatments. S-dashed bars — cell increment per day into the zone of radial growth. Z-dashed bars — cell increment per day into the zone of maturing tracheids. Double dashed bars — cell increment per day into the zone of mature tracheids. I — experiment performed in 1974, II — experiment performed in 1975. A/I — auxin supplied 10 days after decapitation for 30 consecutive days; B/I — auxin supplied 20 days after decapitation for 20 consecutive days; A/II — auxin supplied 13 days after decapitation for 37 consecutive days; B/II — auxin supplied during two periods; first — 13th to 20th day after decapitation and second — 33rd to 50th day after decapitation

the meristematic stage (Fig. 1/II: A/II cells with numbers 57-136, period 33rd to 50th day after decapitation). This rate did not increase (or only very little) if the cells divided and just started radial growth in the period following decapitation but previous to auxin treatment (Fig. 1/I: A/I — cells nos. 20-48, period 10th to 40th day after decapitation; B/I — cells nos. 32-42, period 20th to 40th day after decapitation; Fig. 1/II: A/II — cells nos. 43-56, period 20th to 33rd day after decapitation). This resulted in extension of the differentiation period of these cells as compared with the cells which were meristematic on the days of auxin treatment (Fig. 3 A/II cells with numbers 43-56 and higher).

RADIAL GROWTH

Tracheids which on the day of tree decapitation formed the cambial zone differentiated during the following periods with reduced diameters, irrespective of auxin supply to the stem (Fig. 2 A/I cells nos. 38-45, Fig. 2 B/I cells nos. 38-42, Fig. 3 B/II cells nos. 53-72). Cells in the radial growth stage of differentiation also formed later with smaller diameters (Fig. 2 A/I cells nos. 22-37, Fig. 3 B/II cells nos. 38-52) althought it was not possible to demonstrate this effect in all cases (Fig. 2 B/I cells nos. 22-37, Fig. 3 A/II cells nos. 39-53), probably owing to the considerable difficulty in identifying the boundaries between the growing and maturing cells in some trees. Only the cells originating from cambium during the period of auxin supply had greater radial diameters, provided, however, that during further stages of differentiation the auxin treatment was not interrupted (Fig. 2 A/I cells nos. 46-48, Fig. 3 B/II cells nos. 73-80).

CELL WALL FORMATION

Tracheids which matured in the absence of auxin during a short period after decapitation (Fig. 2 A/I cells nos. 10-21, Fig. 2 B/I cells nos. 10-21, Fig. 3 A/I cells nos. 29-34, Fig. 3 B/II cells nos. 28-40) formed unchanged or slightly reduced cell wall deposits in spite of extension of their maturation period resulting from the delay in autolysis of protoplasts (see Fig. 1). Thus, reduction of the rate of cell wall formation occurred. This is seen especially in the cells which on the day of decapitation belonged to the zones of radially enlarging xylem or were just starting maturation (Fig. 2 A/I cells nos. 22-37, Fig. 2 B/I cells nos. 22-37), Even though these cells progressively matured during the following period when auxin was already supplied to the stem and a gradual increase of the daily rate of cell wall formation occurred, their cell wall thickness remained unchanged or increased shightly because

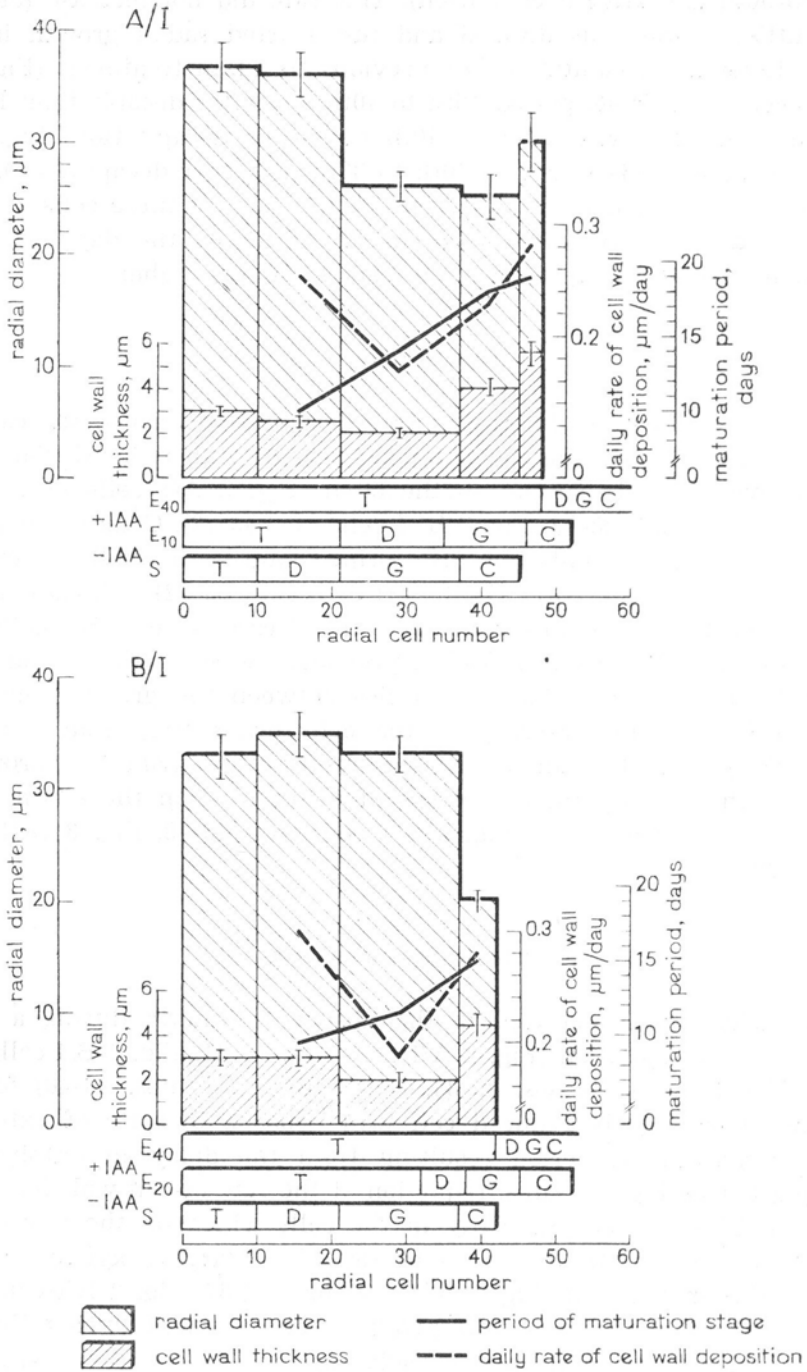

A/I
B/I
radial diameter, µm
cell wall thickness, µm
daily rate of cell wall deposition, µm/day
maturation period, days
radial cell number
+IAA
−IAA
E40
E10
E20
S
T
D
G
C
radial diameter
cell wall thickness
period of maturation stage
daily rate of cell wall deposition

Fig. 2. Effect of presence of auxin applied after stem decapitation upon radial diameter, cell wall thickness, period of maturation and daily rate of cell wall deposition of tracheids, corresponding to each of the successive zones of differentiating and mature xylem, observed before the start of the experiment. Averages from four successive cells from each zone. S — start of experiment, E — collection of stem segments for anatomical investigation. Numbers given with the symbol E indicate days from the date the experiment was started; C — cambial zone, G — zone of enlarging xylem, D — zone of maturing xylem, T — zone of mature xylem. Experiment performed in 1974. A/I — auxin applied 10 days after decapitation for 30 consecutive days, B/I — auxin applied 20 days after decapitation for 20 consecutive days

the period of maturation decreased (Fig. 3 A/II cells nos. 35-48, Fig. 3 B/II cells nos. 34-42). Tracheids differentiating somewhat later formed thicker walls owing to the significant increase of the daily rate of cell wall deposition, provided auxin was continuously supplied (Fig. 3 A/II cells nos. 44-50). However, if the auxin supply was interrupted during the maturation phase of these cells, a second period of extension of this phase occurred and the cell wall thickness increased again, although the daily rate of wall deposition remained reduced (Fig. 3 B/II cells nos. 43-46).

The cells which during the meristematic phase or during transition from this phase to the phase of radial growth were under no-auxin conditions formed thicker cell walls during later periods of auxin supply to the stem (Fig. 2 A/I cells nos. 38-45, Fig. 2 B/II cells nos. 38-42, Fig. 3 A/II cells nos. 54-58). This growth resulted from an increase of the daily rate of cell wall formation and further extension of the maturation phase. An additional increase of the rate of cell wall deposition is evident especially in the cells which, in all phases of differentiation, remained under conditions of auxin supply to the stem (Fig. 2 A/I cells nos. 46-48, Fig. 3 A/II cells nos. 60 and next, Fig. 3 B/II cells nos. 55-72).

DISCUSSION

The results revealed the complexity of auxin involvement in the regulatory system of tracheid differentiation of secondary xylem. They generally confirmed the hypothesis proposed earlier (W o d z i c k i and Z a j ą c z k o w s k i 1974), that auxin controls radial growth and maturation of tracheids already during the meristematic stage of differentiation. A lack of auxin during this stage results later in smaller cell diameters, reduced daily rate of wall deposition and also to some extent reduced responsiveness of cells to the mechanism controlling the destruction of protoplasts in the final stage od differentiation. Cells

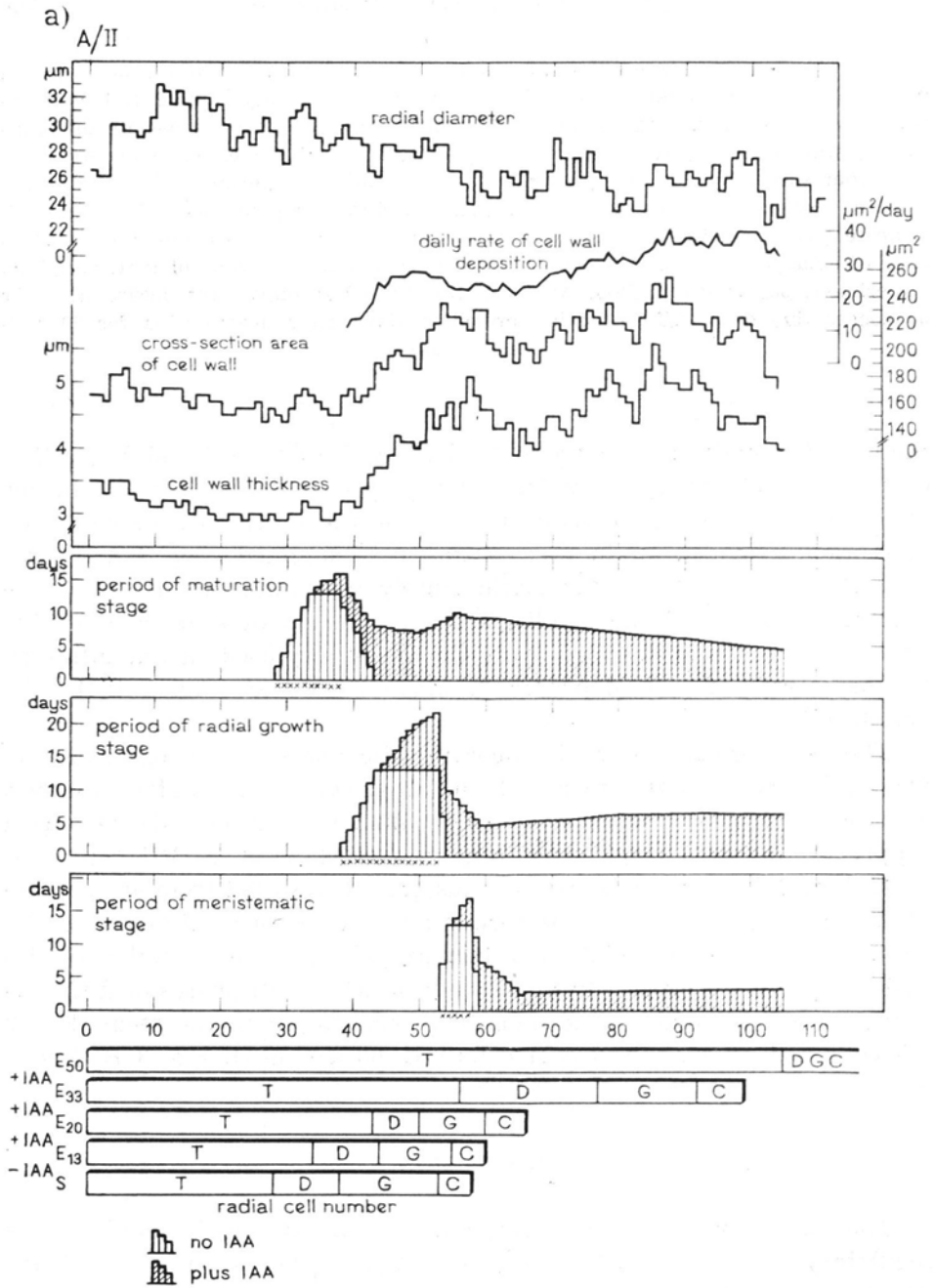
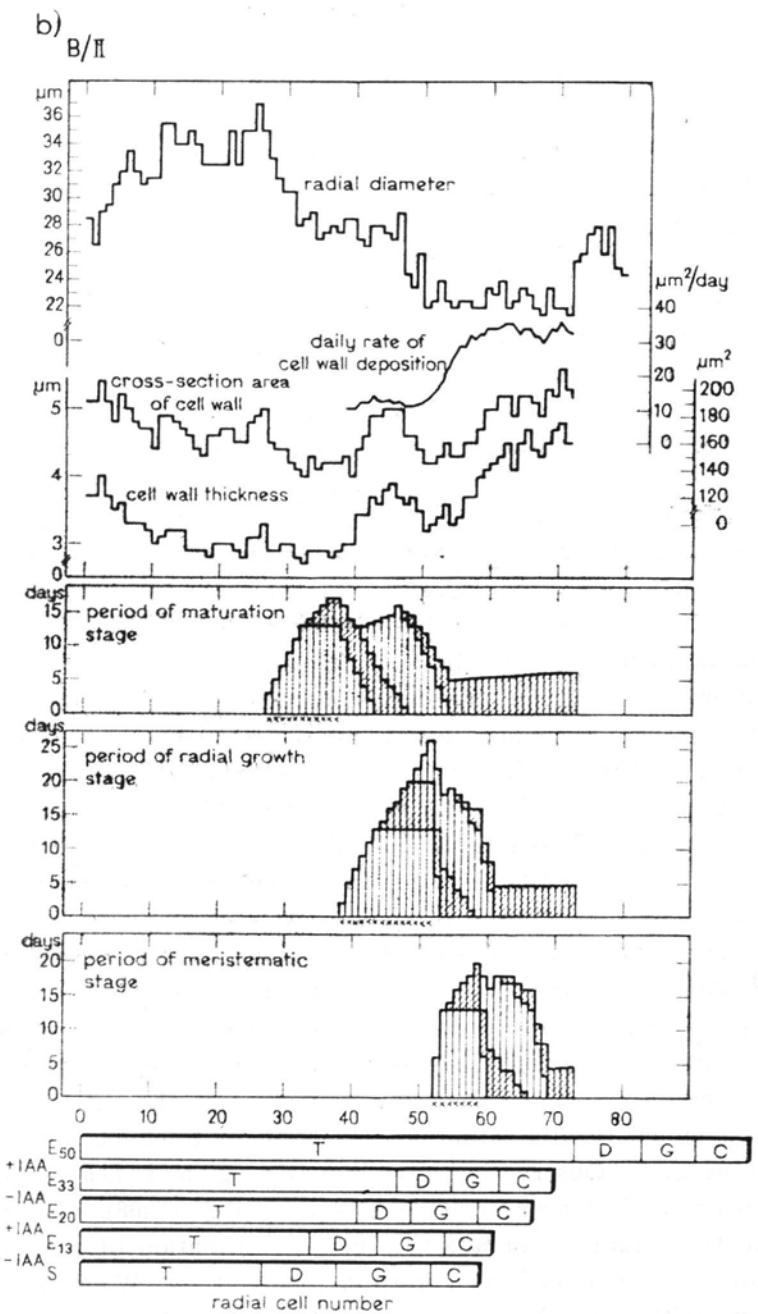


Fig. 3. Effect of application of IAA to decapitated main stem of 5-year-old pine trees at various periods of tracheid differentiation upon radial diameter, cell wall thickness, cross-section area of cell wall tracheids, daily rate of cell wall deposition and duration of particular stages of tracheid differentiation of consecutive cells formed in the current annual ring. Experiment started on May 21 ended on July 10, 1975. Averages for 5 trees. Horizontal bars at the bottom indicate radial pattern of cell distribution in various periods of the experiment in the zones of differentiating and mature xylem: C — cambial zone, G — enlarging xylem, D — matu-



ring xylem, T — mature xylem in current annual ring. S — start of experiment, E — collection of stem segments for anatomical investigation. Numbers given with the symbol E indicate days from the date the experiment was started. A/II — auxin supplied 13 days after decapitation for 37 consecutive days; B/II — auxin supplied during two periods: first — 13th to 20th day after decapitation and second — 33rd to 50th day after decapitation. xxx — cells which entered particular zones of tracheid differentiation before the experiment started. The total duration of the given stage of differentiation of these cells is not known

formed from the cambial zone under conditions of auxin availability may grow larger and produce cell walls with a higher daily rate of increment. In respect to growth and maturation this meristematic control by auxin may be considered as induction or activation of the enzymatic potential associated with production of the specialized endomembranous structures involved in cell wall formation. The present experiments also confirmed the continuous involvement of auxin in the processes of tracheid differentiation. The presence of auxin during radial growth and maturation makes possible the expression of the cellular potential in respect to the rate of wall formation. This may be connected with the attraction by auxin of assimilates described by Seth and Wareing (1967) necessary as substrates for cell wall synthesis.

Another effect connected with auxin continuous control of tracheid differentiation was manifested in the termination of the maturation phase. Reduction of auxin supply resulted in a delay of autolysis of protoplasts, while continuous availability of auxin caused earlier termination of differentiation. In this case auxin specifies the positional information essential for autolytic processes starting probably with breakdown of the vacuolar membrans as described by Wodzicki and Brown (1973). This type of information transfer by auxin has been proposed by Zajączkowski and Wodzicki (1978) and Wodzicki and Wodzicki (1980), who suggested that autolytic destruction of protoplasts of maturing tracheids is determined by the vectorial field resulting from the polar transport of auxin in association with the oscillatory phenomena in the cambial zone. Our results provide further support to this hypothesis. The mechanism described above could be significant in the regulation of early-to-late-wood transition under natural conditions. It would be independent of the increased cell wall thickening associated with auxin control of the rate of cell wall deposition which results rather in formation of tracheids with features of reaction wood. Observations by Wardrop and Davies (1964), Kennedy and Farrar (1965), Westing (1968) who described the inductive character of auxin effects in formation of reaction wood in Conifers as well as earlier results obtained by Wershing and Bailey (1942) or Nečesaný (1958) seem to agree with such a supposition.

Acknowledgments

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Wpływ wierzchołka pędu na różnicowanie drewna sosny. II. Reakcja różnicujących się cewek

Streszczenie

Badano wpływ auksyny, dostarczonej w poszczególnych fazach różnicowania cewek drewna wtórnego, na średnicę promieniową i wielkość ściany komórkowej różnicujących się cewek w pędzie głównym dekapitowanych 5-letnich drzew *Pinus silvestris* L. Stwierdzono, że udział auksyny w procesie regulacji różnicowania cewek jest złożony; przejawia się poprzez wpływ następczy reakcji komórek w fazie merystematycznej oraz oddziaływanie w czasie dalszych stadiów wzrostu i dojrzewania. Cewki, które w okresie merystematycznym znajdowały się w pędzie de-

kapitowanym, bez auksyny, charakteryzowały się mniejszą intensywnością wzrostu i tworzenia ściany wtórnej nawet w późniejszym okresie różnicowania przy egzogennie dostarczonej auksynie. Cewki tworzące się w warunkach obecności auksyny we wszystkich fazach różnicowania charakteryzują się większą intensywnością tych procesów i wytwarzają grubszą ścianę chociaż równocześnie skraca się czas fazy dojrzewania, kiedy to odkłada się ściana wtórna. W tych warunkach ściana komórkowa ma cechy cewek drewna reakcyjnego. Działanie auksyny na długość okresu dojrzewania cewek przejawia się w końcowej fazie różnicowania. Stały dopływ auksyny powoduje wcześniejsze kończenie różnicowania cewek, natomiast ograniczenie tego dopływu (nawet w fazie merystematycznej), a szczególnie w okresie dalszych stadiów różnicowania, opóźnia autolizę protoplastu. W tym przypadku auksyna prawdopodobnie funkcjonuje w układzie spełniającym rolę informacji pozycyjnej o położeniu komórek względem kambium.