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Reconstruction of storeyed cambium in the linden

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Abstract

Cambium and xylem from the stem of an about 100-year-old linden (*Tilia cordata*) was examined. Intrusive growth of cells, leading to forking of cell ends and consequently to a change in the contacts of fusiform cells, occurs in the layer of initial cells. This growth gives two configurations, Z and S. Both appear along the same storey boundary, but they are spatially separated (groups of S and Z endings alternate). A unidirectional lateral shift of the growth activity of the ends was observed. In the examined linden stem a storeyed arrangement of rays was found within the stories of fusiform cells.

INTRODUCTION

In investigations on storeyed cambium reconstruction in *Entandrophragma* (Hejnowicz and Zagórska-Marek 1974, Zagórska-Marek 1975) it was found that the change in cell orientation in the cambium, with preservation of the storeyed arrangement occurs by reciprocal translocation of the fusiform cell ends pointing in opposite directions and belonging to neighbouring storeys. To this leads intrusive growth localised on the lateral edges of cells, causing a transient formation of forked cell ends. Forking may appear at both cell ends. Cells with such ends occur in groups. Forking in the same cell group appears cyclically.

The above described results were obtained in studies in which the history of the cambium was reconstituted by comparing the xylem parenchyma. In the present paper we examined cambium directly, analysing the cells deposited both on the xylem and the phloem sides. The present study was undertaken to check whether the conclusions

reached in investigations of xylem find confirmation in direct investigations of cambium, and particularly whether the forking of cell ends occurs in the layer of initial cells. Cambium from a linden stem was used for the investigations. An additional factor encourageing to these studies was the storeyed arrangement of rays in the wood of the stem chosen for examination, a fact not noted among lindens (K u k a c h k a and R e e s 1943).

MATERIAL AND METHODS

The material for examination was cambium taken from the stem of a solitary linden (*Tilia cordata*) about 100 years old with a diametre of about 70 cm. Cambium was prepared out from the stem at a height of about 1 m after removal of outer bark and unfunctioning phloem on June 28, that is in the period of enhanced seasonal activity. Cambium blocks of several mm^2 surface area were fixed in glutaraldehyde and after dehydration embedded in Epon. The material was cut on an ultramicrotome into 3-μm sections which were stained by the PAS method and with toluidine blue. Series of consecutive cross and tangential sections were studied.

RESULTS

The linden cambium produced xylem the grain of which was of interlocked type with a rather short interlocking cycle about 6 annual growth rings. The last reversion of grain inclination had occurred two years before fixation. The rate of change was about 3 degrees per 1 cm of increment. At the moment of fixation the cambium constituted a layer of 8-12 undifferentiated fusiform cells.

An unusual property of the studied cambium and, consequently of the xylem and phloem, was the storeyed arrangement of the rays (Fig. 1). These rays of height lower than the length of the fusiform cells lie completely within the storeys (Fig. 2). By comparing the xylem in the successive annual rings it was found that the rays inclined with the change of inclination of the fusiform cells.

The storeyed arrangement of the latter cells is shown in Fig. 2. The storeys are deeply indented, their boundaries run horizontally on relatively short segments. Between them the storeys are shifted in relation to one another. A characteristic factor disturbing the regularity of the storeyed structure of the examined cambium fragments is the presence of single long cells usually at the sites of storey shift. They will be

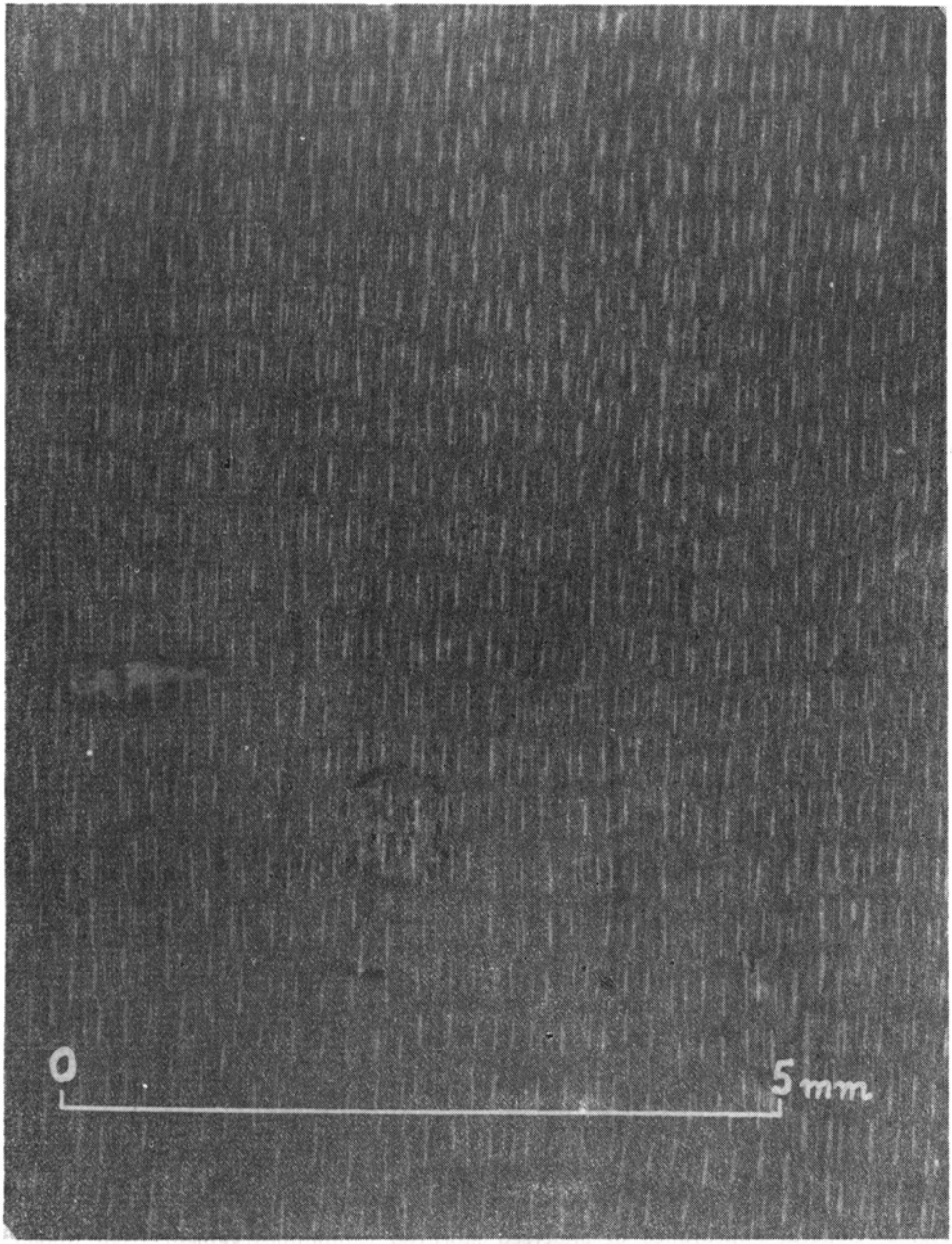

Fig. 1. Tangential sections through wood of linden at the site where cambium was sampled. Storeyed arrangement of rays is visible

further referred to as "nails". It is seen in Fig. 2 that these cells divide anticlinally (pseudotransversely) close to the boundary of the storeys of fusiform cells. Before pseudotransverse division the "nail" cells are the longest fusiform cells.

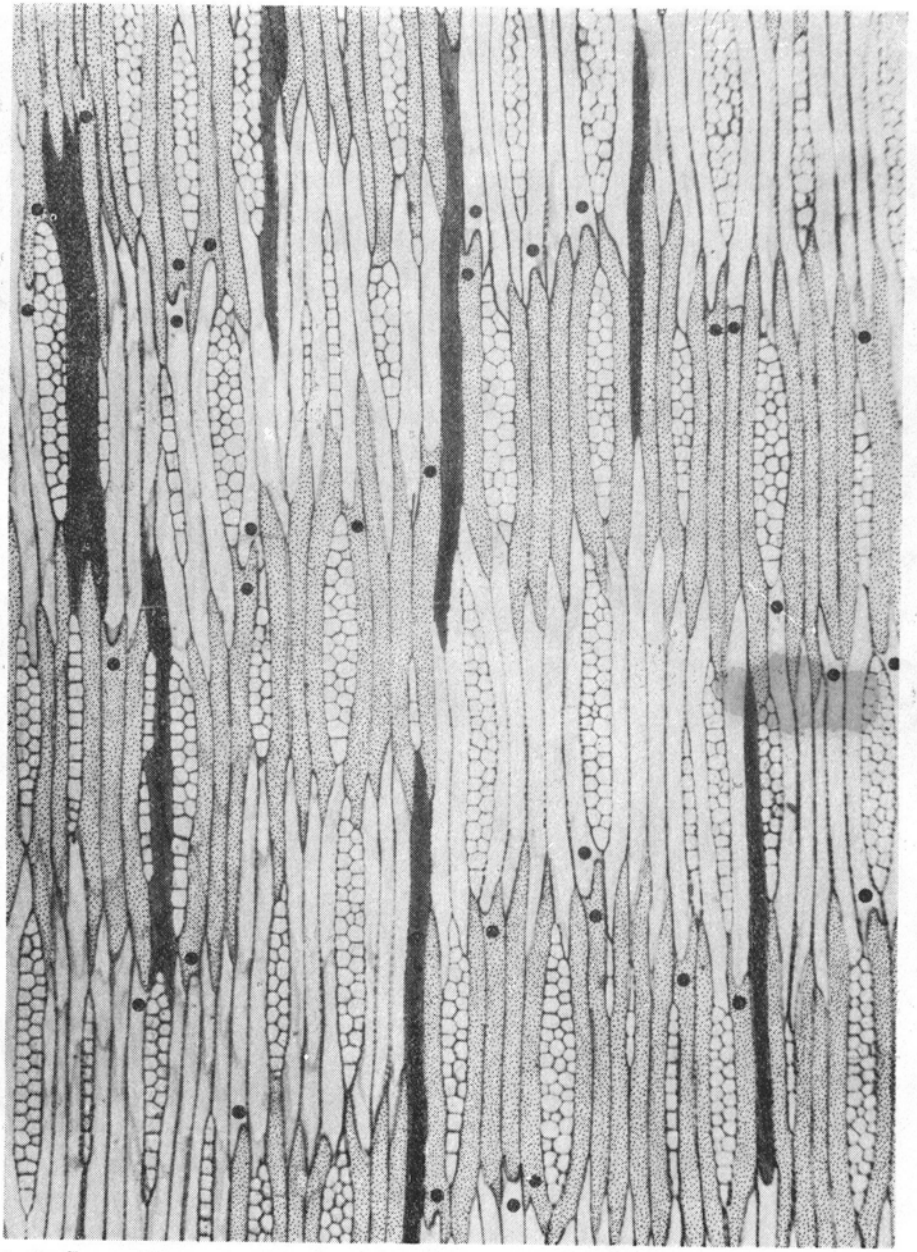

Fig. 2. General appearance of analysed storeyed cambium fragment from linden in tangential section. "Nail" cells are shaded. Forked ends denoted by heavy dots. For clarity every second storey is dotted

In the cambium sample studied formation of partitions in longitudinal anticlinal division was not observed.

The large number of cells with forked ends is noteworthy in the population of fusiform cells. Comparison of successive tangential sections

through the cambium leads to the conclusion that the forked ends of fusiform cells arise owing to intrusive growth of one of the two lateral radial edges (the right or left one) close to the end of the cell, this causing the formation of a new end. In the series of tangential sections in Fig. 3 photos C or D represent the layer of cambial initials while A is on the phloem side and J on the xylem side. The cells in photos A and J arose earliest before those visible in the middle photos. It is seen that the initial in the radial row *a* grew intrusively on the right edge of the lower end and the initial in the row *b* did the same on the left edge of the upper end. This led as consequence to forking of the cell ends.

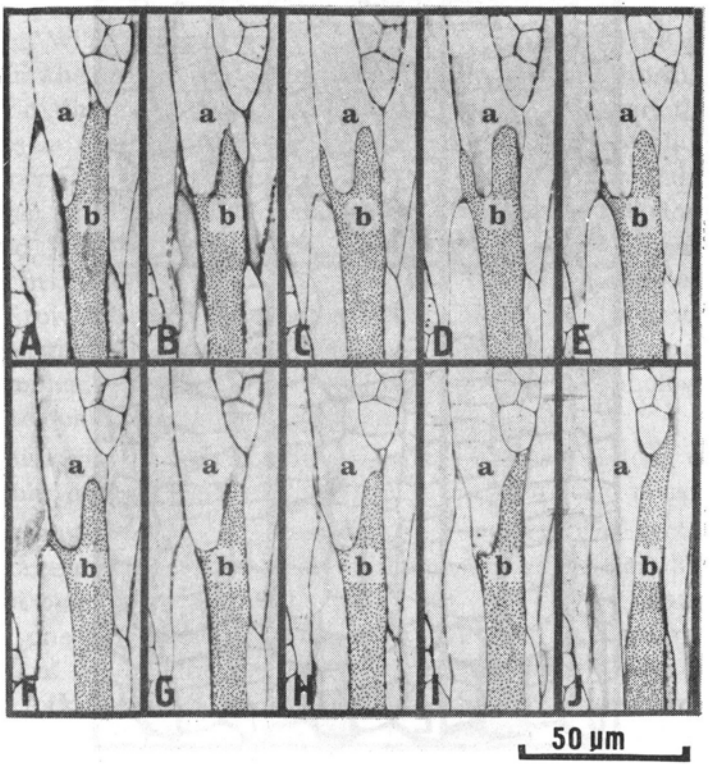

Fig. 3. Series of tangential sections show reconstruction of two forked cell ends in two radial rows of cambium (a, b). Sections 3 µm thick, every second section in series is shown. For clarity the lower cell row is dotted

This forking is transient, the old end in the forking is later eliminated. On a number of serial sections through the cambium which are not shown in the photos, it is seen that the ends are forked in cells

of differentiating xylem and phloem of the same radial row, whereas no forking is visible at the site where we expect to find the layer of initials. The formation of new ends and elimination of the old ones produces a change in the mutual situation of the oppositely oriented cell ends of two storeys.

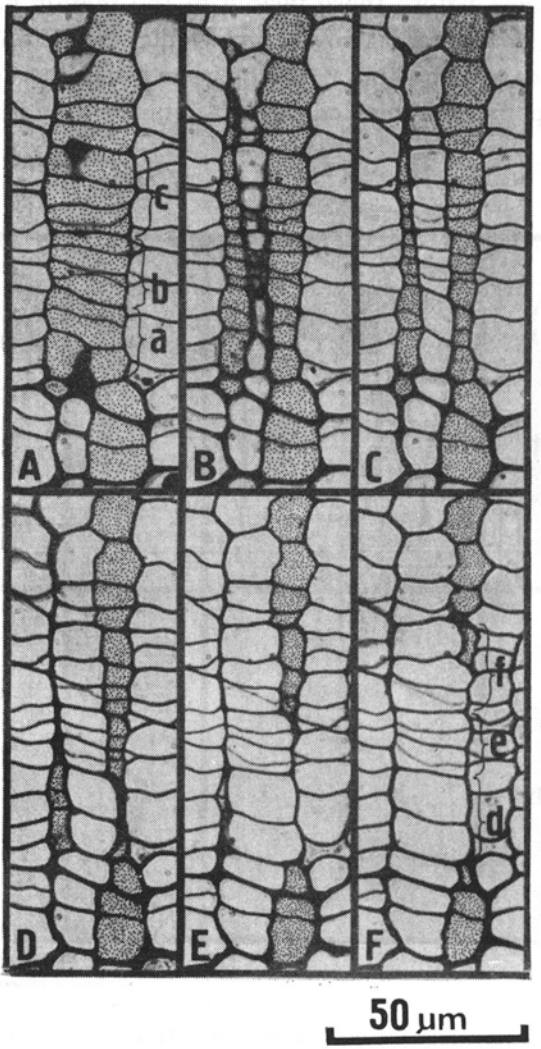

Fig. 4. Series of cross sections shows row of cambium cells (dotted) the lower ends of which are forked. Sections 3 µm thick, every second section in series is shown. In dotted row three cell complexes — *a, b, c* (photo A) — are denoted. In photo F three cell complexes of another row (not dotted) are denoted — *d, e, f*. This row has no cells forked at the ends. Z-type reconstruction of the forked end is visible. The most pronounced change in the vertical position of the forked ends is noted in the complex denoted *a* (compare photos C-E)

Examination of the successive cross sections through the cambium (Figs. 4 and 5) shows that the vertical position of the forked ends is not uniform in cells of the given radial row. It changes discontinuously (Fig. 5). Sister groups appear comprising 2-4 cells in which the position of the forked end is practically the same, but it changes from group to group. The extreme position is in the cell group situated where the initial cell might be expected. It would seem that intrusive growth leading to forking occurs either in the initial cell of the given row or in the xylem or phloem mother cell formed in the division of the initial cell, and the state reached by the cell end is the same in both cells derived from periclinal division. In the cells growing towards the xylem or phloem this state does not change, only in the initial or mother cell intrusive growth continues. As a result of this growth, after several divisions of the mother cell to the xylem or phloem side sister cell groups form with uniform position of the cell ends. If the position of the ends in the group containing the initial cell is uniform, it is impossible to distinguish the latter. In some groups, however, the position of the ends is not identical. Then we consider that the cell with ends most advanced in the group is the initial. This analysis indicates that in the layer of the cambium examined initial cells lie closer to the phloem and frequently these cells have large radial dimensions. The initial cell in Fig. 4 is one of three cells in the group denoted *a* in the dotted cell row (photo A) and one of the two cells in the group denoted *d* in the central cell row without dots (photo F). As seen, to one or two cells determined to phloem there fall six to ten morphologically undifferentiated ones determined to xylem.

The groups on the xylem side are mostly formed of four cells, thus, in the mother cell determined to xylem there occur two more divisions.

From among the 173 cells analysed on the tangential section chosen so as to correspond possibly best to the initial cell layer, 31 per cent had at least one forked end. Cells with both ends forked simultaneously constituted one per cent of the total number studied. The frequency of appearance of forking at the upper or lower ends is not equal. The number of forkings of apical ends exceeds that of forkings of the basal ends in a 1.5:1 ratio. It happens that the apical end of a fusiform cell and the adherent basal end of a cell belonging to the neighbouring storey from above are simultaneously forked. In the layers of xylem and phloem mother cells, that is on tangential sections the percentage of cells with a forked end is similar, with the exception of the initial cell layer.

By comparing the successive tangential sections from the layer comprising differentiating phloem and the inner part of the cambium zone (beginning with sections lying closest to the phloem) we followed

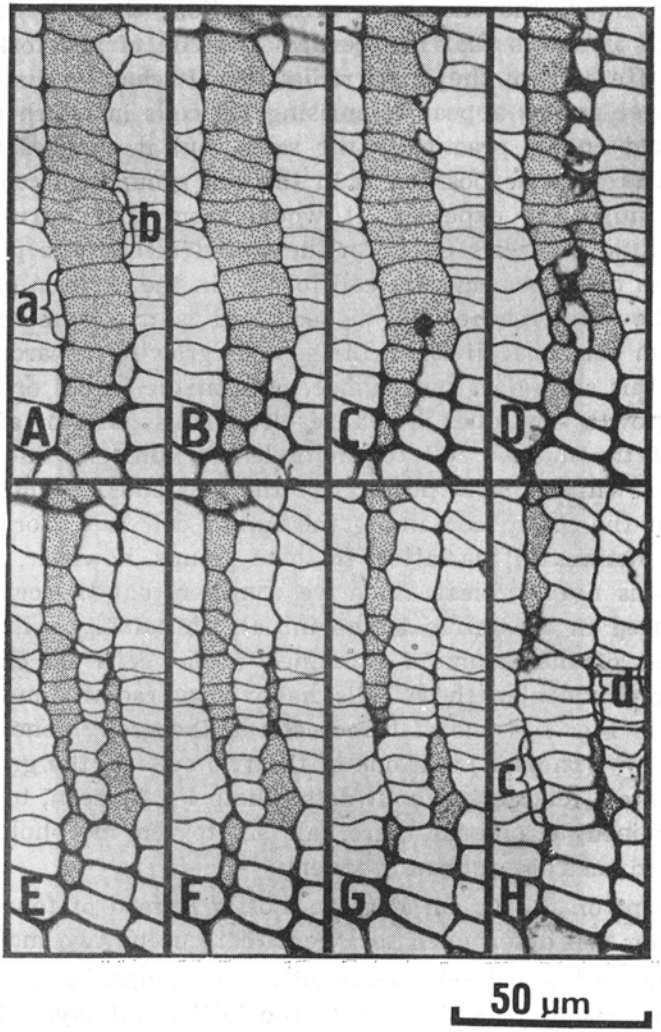

Fig. 5. Series of cross sections showing row of cambium cells the lower ends of which are forked (dotted). Sections 3 µm thick, every second section of series is shown. The widest changes of vertical position of forked end is seen in cell complex denoted *a* (see photos G, H). S-type reconstruction of forked end is visible. The middle cell row in F-H is not forked at upper ends, complexes *c*, *d*, on photo H can be distinguished in it

the sequence of appearance, the spatial distribution and the configuration of the events leading to a change in the position of the ends of the initials, which occurred in the cambium in the period preceding directly the moment of material sampling for study.

We analysed a total of 282 cell ends (apical and basal) at five levels of the examined cambium surface (five storey boundaries). For each level we plotted a diagram showing schematically the sequence and

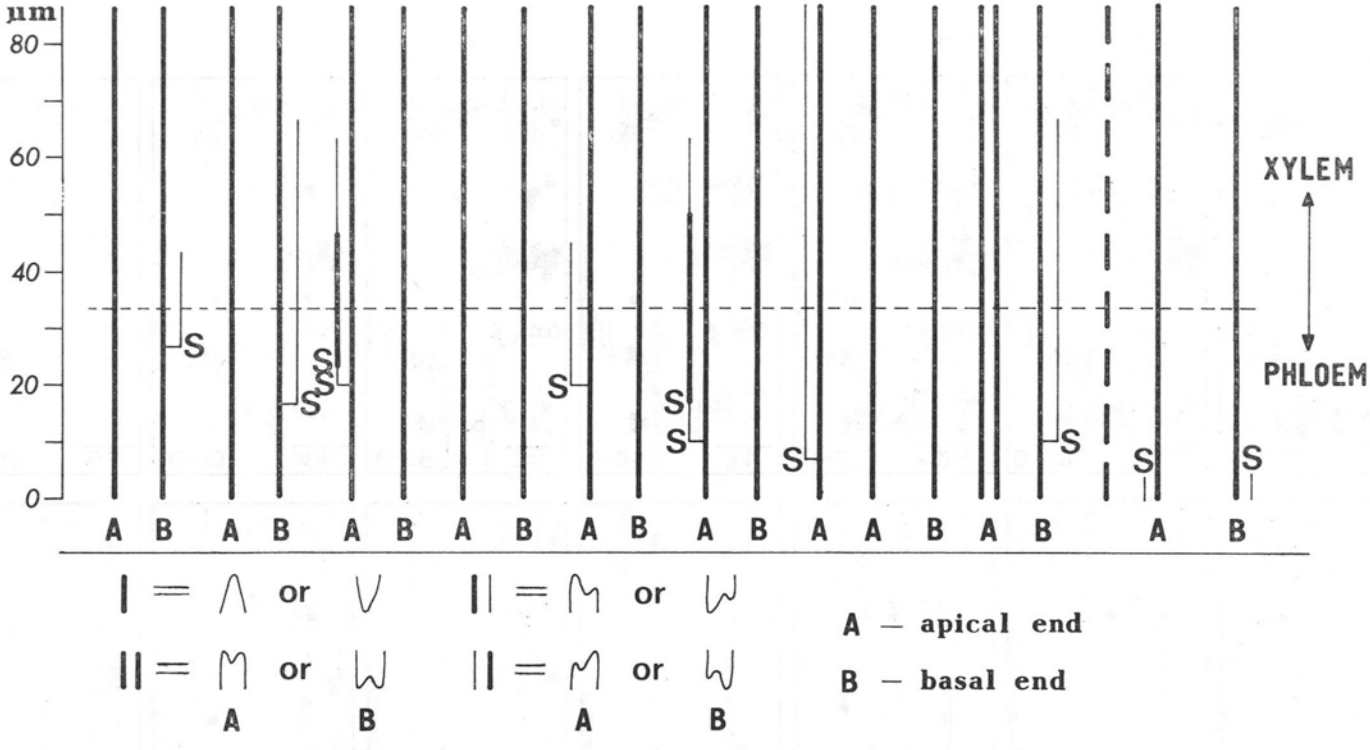

Fig. 6. Diagram illustrating the process of formation and disappearance of forkings at ends pointing in opposite directions (apical — A, and basal — B). The diagram is prepared on the basis of a series of tangential sections 3 µm thick. Scale on left refers to thickness of examined cambium zone. Vertical dashed line denotes radial row of "nail" cells. Each vertical continuous line refers to one end of the radial cell row. Double continuous line denotes forking of ends in given row, the shorter end is denoted by a thinner line. The dashed horizontal line denotes the position of the initial layer

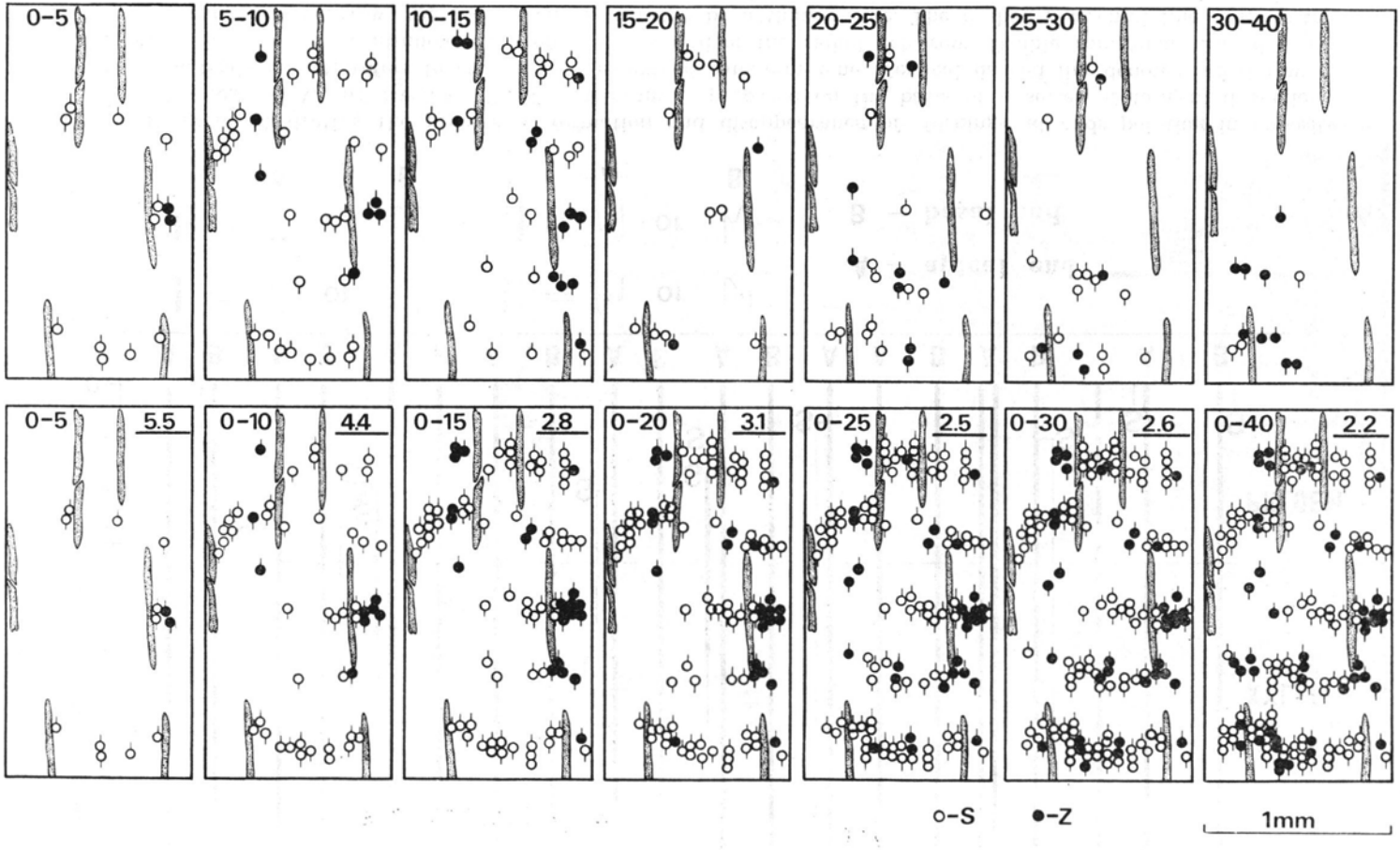
0-5
5-10
10-15
15-20
20-25
25-30
30-40
0-5 5.5
0-10 4.4
0-15 2.8
0-20 3.1
0-25 2.5
0-30 2.6
0-40 2.2
○-S
●-Z
1mm

Fig. 7. Maps showing spatial distribution of events in successive tangential layers corresponding to successive steps of *Tilia* cambium reconstruction. The "nail" cells are marked (dotted). Empty circles denote S events, full ones Z events. The line at the circle pointing upwards denotes the apical end. Joined circles denote events occurring successively in time at the same cell end. In the upper row on each map only those events are shown which occur in the given layer. The position of the latter is given (in μm) in relation to the first layer at the boundary of mature phloem (0-5, 5-10, 10-15... in the left upper corner of each map). In lower row on each successive map all events are shown occurring in the cambium from the beginning of the studied period of reconstruction (0-5, 0-10, 0-15...). The figures in the right upper corner of the map give for each step of reconstruction the ratio of the number of S events to the number of Z ones (all which have so far appeared in the cambium). The last map of the upper row shows the distribution of events in the initial layer, the last map in the lower row presents the distribution of all events which took place in the cambium in the studied period of reconstruction

configuration of the events appearing on oppositely oriented (apical and basal) ends of cells forming the given storey boundary. It results for instance from the diagram shown in Fig. 6 that the appearance of forkings (in the given case they all appeared in the S configuration) in the group of neighbouring ends is not simultaneous: on the right side the forkings earlier produced disappear, while on the left they are just appearing in the successive cells (from right to left) at later and later times. It results from that there is a shift in time here in the formation of forkings in the direction from right to left. The smaller the group of cells in the radial row in which forking of the given ends occurs the later the latter appeared in the initial producing the given radial row of mother cells.

On the basis of the diagrams we then elaborated maps of the events in order to follow the changes in the distribution of Z and S events. A typical case is shown in Fig. 7. The spatial ordering of S and Z events in the cambium is visible (see map 0-40 in lower row). Along the storey boundary these events occur in alternating S- and Z-groups and spatial time-connected changes in the frequency of the events are visible.

The conclusions resulting from analysis of the diagrams and maps are as follows:

1. In the investigated period of reconstruction — from differentiating phloem to the initial layer — events Z and S occurred on the analysed cambium surface.

2. In the cell layer in differentiating phloem (beginning of series of tangential sections), representing the state of initials several score days ago, S events prevailed (S:Z ratio 11:2). In the successive cell layers situated increasingly closer to the initial layer the S:Z ratio decreased and in the initial layer Z events prevailed (S:Z=3:9) (Fig. 7, upper row of drawings).

3. Events Z in the initial layer appeared at the cell ends situated between the groups of ends on which previously S-events occurred. In the cambium S- and Z-events along the given storey boundary are, therefore, spatially separated: groups of S ends (on which S events appeared) and groups of Z ends alternated. Consequently, there arises a spatial pattern of Z and S events (Fig. 7).

4. The events evoking a change in the situation of cell ends may occur in successive cells along the storey not simultaneously but with a certain delay (shift in time). This may be an indication of transverse unidirectional movement of the stimulus releasing an oriented change in the position of fusiform cell ends.

So far we have been dealing with intrusive growth associated with reconstruction of the system of cambium cells. At the boundary of the cambium zone and maturating xylem or phloem intrusive growth occurs with differentiation of fibre cells. This growth has no definite configuration, conforming to the current direction of cambium reconstruction. For instance in the cells of rows *a* and *b* in Fig. 3 intrusive growth of the differentiating fibres (photos I, J) occurs on the radial edges opposite (direction Z) to those where intrusive growth connected with forking (direction S) takes place (photos B-H).

DISCUSSION

Forking of ends of cambium cells is evidence that the conclusions drawn on the basis of wood examination, concerning the mechanism of changes in storeyed cambium cell arrangement are correct (Hejnowicz and Zagórska-Marek 1974). The forking represents the intermediate state between two successive positions of the end at the boundary of the storeys and they are the result of intrusive growth of the lateral radial edge of the fusiform cell end.

Forkings appear in the *Tilia cordata* cambium according to a polar pattern. The ratio of apical end forkings to basal ones is, however, smaller than in the storeyed cambium of *Entandrophragma* (Zagórska-Marek 1975). In general there are more forked ends in *Tilia* so that sometimes the basal and apical ends of cells of two neighbouring storeys are doubly indented into one another (Fig. 3). The polarity concerning intrusive growth on fusiform cell ends directed oppositely in the cambium is a commonly known finding (Bannan 1966, 1968, Cheadle and Esau 1964, Brański 1970).

The presence in the cambium examined of the long "nail" cells probably indicates the occurrence of prolonged intrusive growth and oblique anticlinal divisions — developmental events occurring in unstoreyed cambium ontogenesis. It is in general considered that such features do not occur in storeyed cambium when its development is

normal and undisturbed. In experiments in which cambium was incised, a prolonged intrusive growth and oblique divisions were observed, this, however, leading to the disappearance of the storeyed arrangement of the fusiform cells (Hejnowicz and Zagórska-Marek 1974). It is possible that the fragment here examined represents a case of storeyed cambium structure dynamically maintained: intrusive growth typical for unstoreyed cambium ontogenesis leads to "nail" formation and, on the other hand, oblique division in the "nails" occurs at a level where the continuity of the storey boundary can be reconstructed.

In the examined cambium fragment walls derived from anticlinal longitudinal divisions were not noted in the fusiform cells, although oblique divisions occurred. The so far accepted view that the development of a storeyed structure of cambium is conditioned by longitudinal anticlinal division may be supplemented by the observation that in some cases the prolonged intrusive growth and oblique anticlinal division may function in such a way as to preserve the storeyed arrangement of the fusiform cell system. It was believed so far that the definite structural patterns of the cambium (storeyed or unstoreyed) are the result of a characteristic quality of elementary events: the storeyed arrangement — of the lack of intrusive growth, and longitudinal anticlinal divisions; the unstoreyed arrangement — of intrusive growth and oblique anticlinal divisions (Zagórska-Marek 1981). It would seem, however, at present that the developmental events, notwithstanding their quality, are subordinated to the existing structural pattern in the tissue.

Analysis of xylem formed in the period preceding cambium sampling indicates that in the course of the latest years the cell pattern in this cambium had been reconstructed to the left. The present Z and S events may, therefore, be interpreted as a phenomenon resulting from the currently occurring change in the direction of cell reconstruction in the cambium, that is change of type of domain from the S one to the Z one. Such an interpretation is supported by the prevalence of Z events in the initial layer, whereas in the earlier formed layers of differentiating phloem S events dominate. This may mean that so far the initial cells were reconstructed to the left, while at present they change the direction of reconstruction from left to right. It is interesting that in the spatial ordering of S and Z events within the given boundaries of cambium storeys in *Tilia*, groups of cell ends S occur alternately with groups of ends Z. A similar situation prevails in the storeys constituting the boundary between regions differing by the direction of cell reconstruction in the cambium of *Entandrophragma* (Zagórska-Marek 1977).

In the latter plant transverse shifting of growth activity of the cell ends along the storey was observed (Zagórska-Marek 1975). A similar happening is found in the analysed *Tilia* cambium. It would

seem, therefore, that the problem of a transversely shifting spatial pattern of Z and S growth activity of cell ends in storeyed cambium is noteworthy and requires further study. The observation is also interesting that intrusive growth on the lateral edges of the radial ends of fusiform cells which is decisive for storeyed cambium reconstruction, occurs only in initial cells and their closest derivatives, thus the mechanism of storeyed cambium reconstruction is localised probably only in the layer of initials and their direct derivatives.

Acknowledgments

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Przebudowa kambium piętrowego lipy

Streszczenie

Przebadano próbki kambium i drewna z pnia około 100-letniej lipy (*Tilia cordata*). Wzrost intruzywny komórek doprowadzający do rozwidlenia końców i w konsekwencji do zmiany kontaktów komórek wrzecionowatych występuje w warstwie komórek inicjalnych w dwu konfiguracjach Z i S. Obie konfiguracje występują wzdłuż tej samej granicy pięter, są jednak przestrzennie rozdzielone (na przemian występują grupy końców S i grupy końców Z). Zaobserwowano boczne jednokierunkowe przesuwanie się aktywności wzrostowej końców. W badanym pniu lipy stwierdzono piętrowe ułożenie promieni w obrębie pięter komórek wrzecionowatych.