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Fibrillation of events in the cambial domains of *Tilia cordata* Mill.

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Abstract

The cambium and 9 annual growths from a linden stem were studied. Some of the fusiform cell ends changed their contacts — which is shown by the comparison of the cambium layer and terminal parenchyma of the phloem. This means that these ends were active in the period the layers under comparison were formed. The active ends were found in groups numbering a few to ten-odd ends alternating with groups of inactive ends. About 70% of the ends retained their activity for only one year. In the studied areas, in a given year, one direction of migration of the active ends dominated. As a rule, this configuration changed every two years. Along with the change in the configuration of the active ends, the position of the active areas also changed in such a way that after the change of configuration, the areas previously inactive became active. No migration of activity along the borders of the storeys was observed, but only its appearance and disappearance, which was called fibrillation of activity. Fibrillation of activity becomes noticeable when there is a low intensity of events. Because the active areas at a given moment are characterized by one type of configuration of events, the whole studied surface can be seen as a single domain. Fibrillation gives the impression of frequent changes of domain type, these changes, however, are of a different nature than the movement of domain borders found in cambium characterized by a high intensity of events.

Key words: *storeyed cambium, interlocked grain, fibrillation of activity*

INTRODUCTION

In storeyed cambium, a reconstruction of the arrangement of fusiform cells occurs, in which the cell contacts at the borders of the storeys change. The change of contact takes place in the following way: the lateral radial edge at the cell end grows intrusively causing the forking

of the end and the establishment of a new contact, after which the old end is eliminated, causing the disappearance of the old contact (Hejnowicz and Zagórska-Marek 1974, Zagórska-Marek 1975, 1984, Włoch and Zagórska-Marek 1982, Włoch 1985). This splitting of the end is the equivalent of the passage of the cell from one stable position to another at the border of the storey. The end at this stage is called active.

The distribution of active ends within the cambium is not accidental. They are grouped together, thus there are active and nonactive areas (Zagórska-Marek 1975, Włoch and Zagórska-Marek 1982). The same area changes its state in time (Zagórska-Marek 1975, Włoch 1985). In the last cited paper, the state of the cambium area was determined on the basis of the occurrence of uniting and splitting rays.

Studies on *Entandrophragma* sp. (Zagórska-Marek 1975) and linden (Włoch and Zagórska-Marek 1982) indicate the cross-wise migration of growth activity along the cell storeys. Migration of growth activity along storeys takes place when activity disappears from one end of an active area and appears at a cell end previously inactive. In this way, the cross-wise migration of activity would constitute one of the possible mechanisms of the change in the state of the cambium. Further studies have shown that this is not the only mechanism of such a change. Disappearance and appearance of activity in cells also takes place without the migration of this activity on the surface of the cambium. This phenomenon and its relationship to the direction of the reconstruction of cell ends, that is, to the configuration of events, which can be "right" (Z) or "left" (S), is explained in this paper.

MATERIAL AND METHODS

Fragments of cambium and phloem sampled from an approx. fifty year-old linden (*Tilia cordata* Mill.) were studied. The samples were taken on July 9, 1980 from a live tree, during the period of the greatest activity of the cambium, on the level of the breast hight diameter. In the phloem, on the tangential surface, a waving of the grain was noticeable. The amplitude of the change of the angle of inclination was about 3°, the length of the section over which one cycle of the change in orientation of cells took place (wave length) was about 1 meter. Unfortunately, the thickness of the phloem layer equivalent to one cycle is unknown. A single sample encompassed about 2 mm^2 of the tangential area by 1 mm of the radial dimension containing 8–9 annual growths of the phloem along with cambium. Three samples taken at 1 cm intervals from each other along the trunk axis were chosen for detailed analysis

of events. In the case of the first sample, marked "0", only the cambium was analysed (Fig. 1). In the second sample (marked "A" in this paper), the cambium and 8 yearly phloem growths, while in the third (marked "B") the cambium and 9 yearly growths of phloem were analysed. The cambium layer in the studied samples had a thickness of about, 50 μm, encompassing on average 12 fusiform cells in the radial file.

The samples were fixed in glutaraldehyde, dehydrated in an acetone gradient, embedded in epon and sectioned with an ultramicrotome so as to give semi-thin sections which were stained using the PAS method. The sections were embedded in euparal and photographed under a microscope. A series of photographs taken of a sequence of fragments of tangential sections through the cambium and phloem was the object of detailed analysis.

RESULTS

From the 218 examined initial cell ends in sample "O", 44 (20%) were active. The arrangement of cells in this sample is shown in Fig. 1. It is possible to see that the active ends are close to each other in such a way that active and inactive areas are formed. The active area has its active ends grouped either along only one side of the storey border or on both sides. In the sample being presently described, upper end activity prevailed. Comparison of the state of forking of the ends in a sequence of slides showed that 43 forkings led to the reconstruction of the contacts "to the left", that is, that they were of type "S", and only one "to the right", that is, was of type "Z". In the studied area we therefore are dealing with an "S" type reconstruction.

The question arises if it is possible to decipher the events which had taken place over a lengthy period of time on the basis of analysis of successive phloem layers. Such a possibility extists due to the presence in the annual ring of a layer of terminal parenchyma of the phloem in which the arrangement of initial cells is preserved (Fig. 2). Because the annual growth of the phloem did not exceed 200 μm (Fig. 3), the only way of isolating these layers was by making a series of semi-thin tangential sections (approx. 4 μm thick) from the phloem layer encompassing several annual rings. It can be seen from Fig. 3 that in 1980, the year in which the cambium was sampled, a yearly increment of phloem was formed with fibers still in the process of differentiation with the yet unlignified secondary wall. The annual phloem ring from the preceding year was poorly developed; the place from which the section was taken was almost completely devoid of fibers. The annual ring in 1974 was similarly poorly

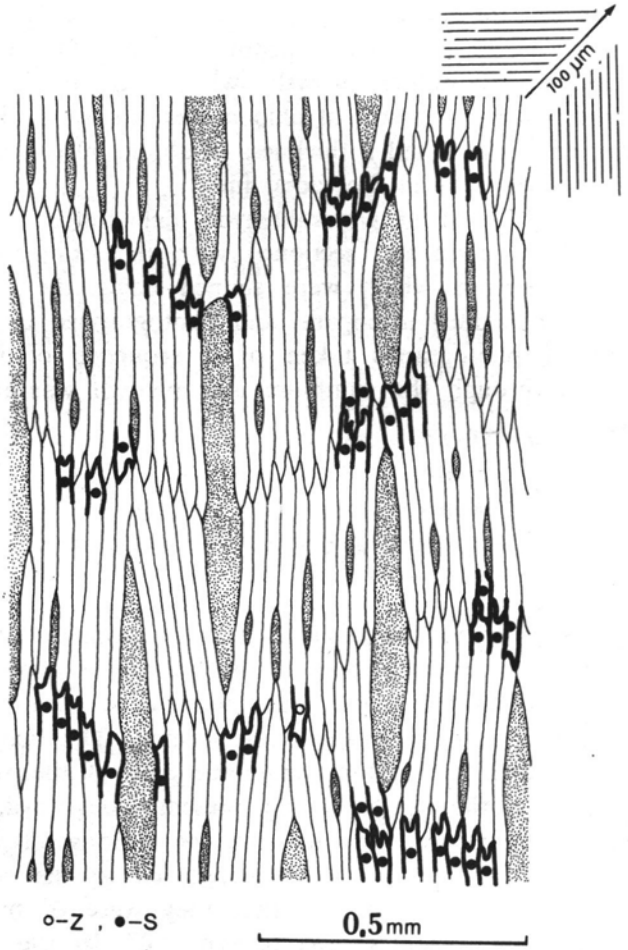


Fig. 1. Cambium surface. The cell ends which exhibited migration are marked with a dark line. The direction of migration is reconstructed on the basis of comparison of 21 consecutive sections forming a layer 100 µm thick and is marked with a circle. Black circles — direction of reconstruction to the left, that is, to S. White circles — direction of reconstruction to the right, that is, to Z. Rays are dotted

developed. Figs. 4 and 5 show both series A and B for which events were deciphered on the basis of comparison of layers of parenchyma cells in a series of semi-thin sections. On these figures, the arrangement of cells in the cambium in successive layers of phloem parenchyma is shown without the transverse septums being marked, that is, as if they were systems of initials.

In this way, the arrangement of initial cells has been reconstructed as it was eight years before in sample A and nine years previously in sample B. The size of the analysed tangential surface was in both

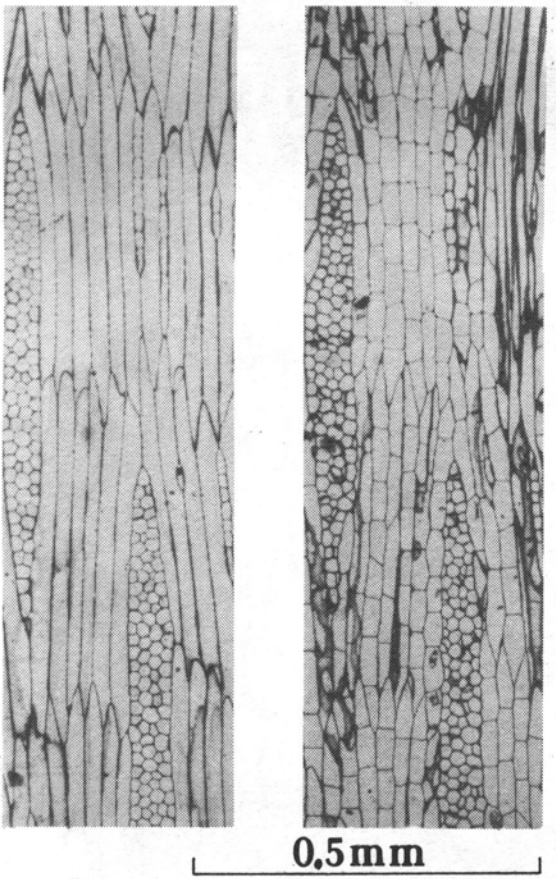


Fig. 2. Tangent sections through cambium and terminal parenchyma of the phloem chosen from a series of sections made for the same fragment of the tangential surface

cases 1.5 mm^2 (1.5 mm long and 1 mm wide). The condition of the end and direction of the change in cell contacts was taken into account. It can be seen in sample A (Fig. 4) that in 1974, 8 ends were changed into Z and 28 into S. In the following years this ratio was: 1975 — 25 Z: 11 S, 1976 — 31 Z: 5 S, 1977 — 17 Z: 21 S, 1978 — 65 Z: 0 S, 1979 — 50 Z: 12 S, 1980 — 10 Z: 36 S. The configuration of events is the same in sample B (Fig. 5) as in sample A with the exception of 1974 and 1976.

In both samples A and B, distant from each other by about 1 cm, changes in the configuration of cell ends came about more or less simultaneously (Fig. 6). It seems then, that the change in configuration took place over an area whose length was greater than the distance between the studied samples. Fig. 6 also shows that the change in the

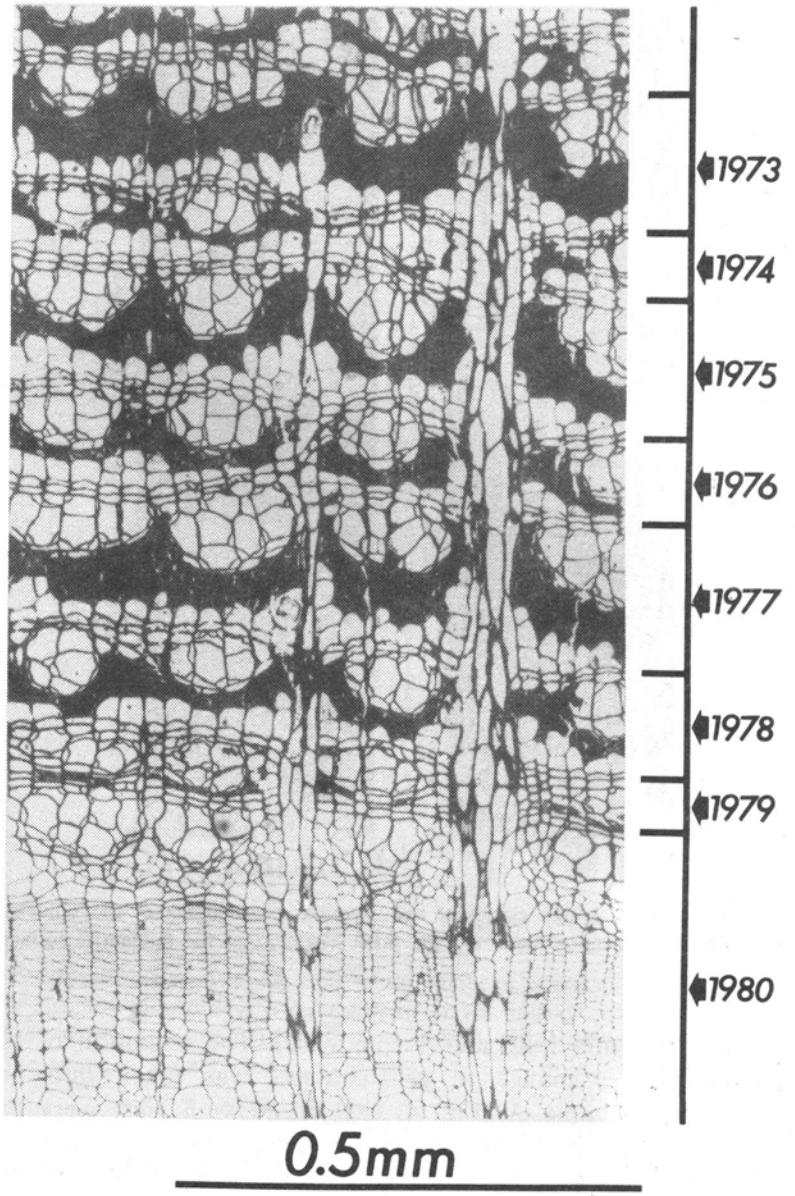


Fig. 3. A cross section through the analysed cambium and phloem. The consecutive yearly growths are marked. The growth for 1980 encompasses phloem, cambium and the cells of the differentiating wood

dominating configuration took place, on the whole, every two years. A clearly intense change in configuration from S to Z is seen in 1977 and 1978, which corresponds to an approximately 200 μm thick layer of phloem. It can also be seen from Fig. 1 that during the period

Table 1

The activity and configuration of initials during their 8 year period of development

	Year	Number of studied ends	Number of active ends	% Active ends	Active basal ends %	Active apical ends %	No. cell-end configurations		Number of cell ends active for 2 years	
							S	Z	no.	% in relation to active ends
Sample A	1974	241	36	14.9	25.0	75.0	28	8	–	–
	1975	237	36	15.2	63.9	36.1	11	25	6	16.7
	1976	237	36	15.2	8.4	91.6	5	31	5	13.9
	1977	237	38	16.0	73.7	26.3	21	17	6	15.8
	1978	241	65	27.0	32.3	67.7	0	65	10	15.4
	1979	247	62	25.1	41.9	58.1	12	50	18	29.0
	1980	251	46	18.3	36.9	63.1	36	10	12	26.1
Sample B	1973	270	35	13.0	62.9	37.1	18	17	–	–
	1974	272	43	15.8	32.6	67.4	10	33	4	9.3
	1975	274	53	19.3	49.1	50.9	12	41	9	17.0
	1976	272	42	15.5	31.0	69.0	30	12	13	31.0
	1977	284	64	22.5	54.7	45.3	57	7	12	18.8
	1978	286	47	16.4	32.0	68.0	5	42	16	34.0
	1979	292	84	28.8	40.5	59.5	11	73	15	17.9
	1980	295	52	17.6	38.5	61.5	34	18	13	25.0

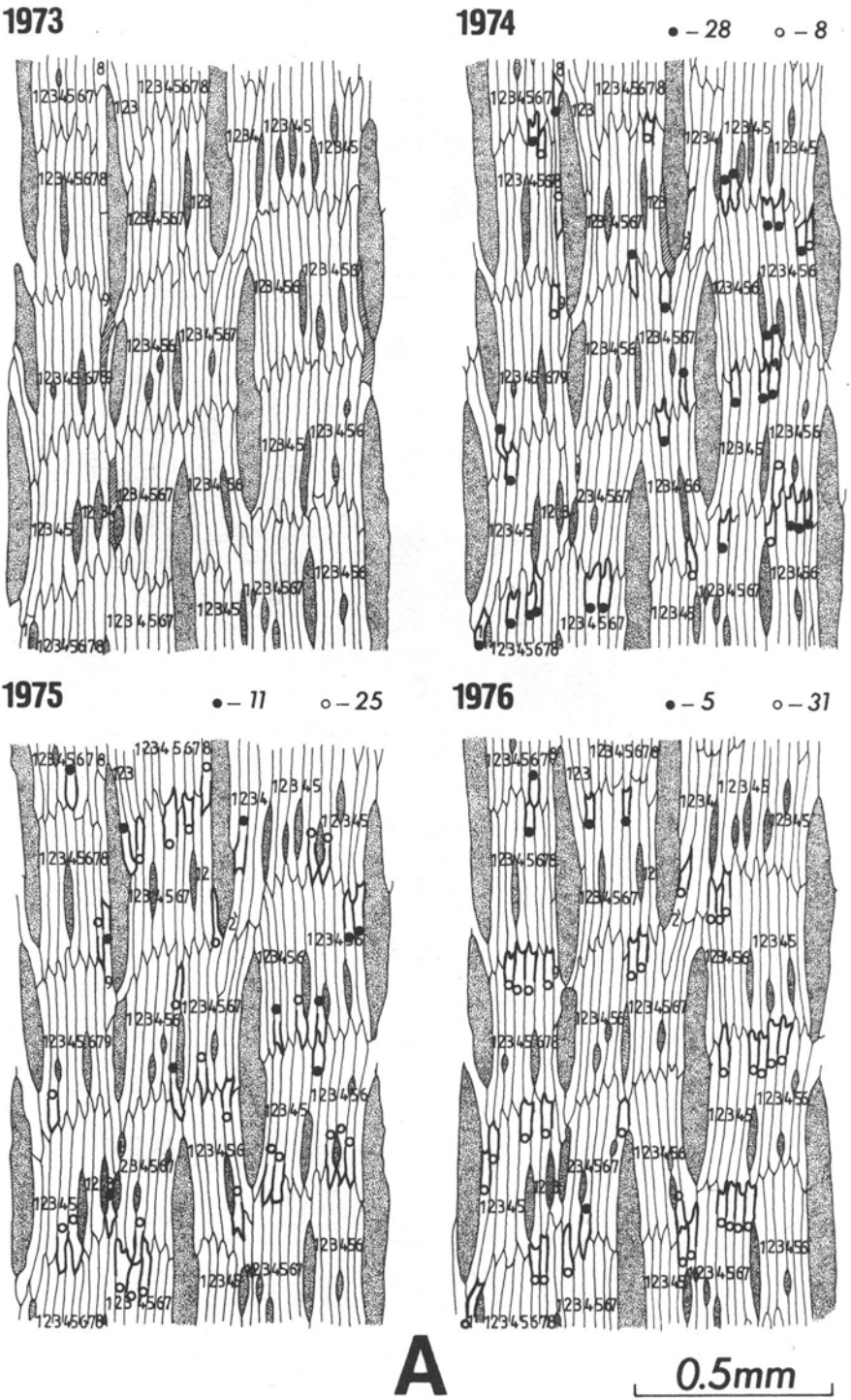
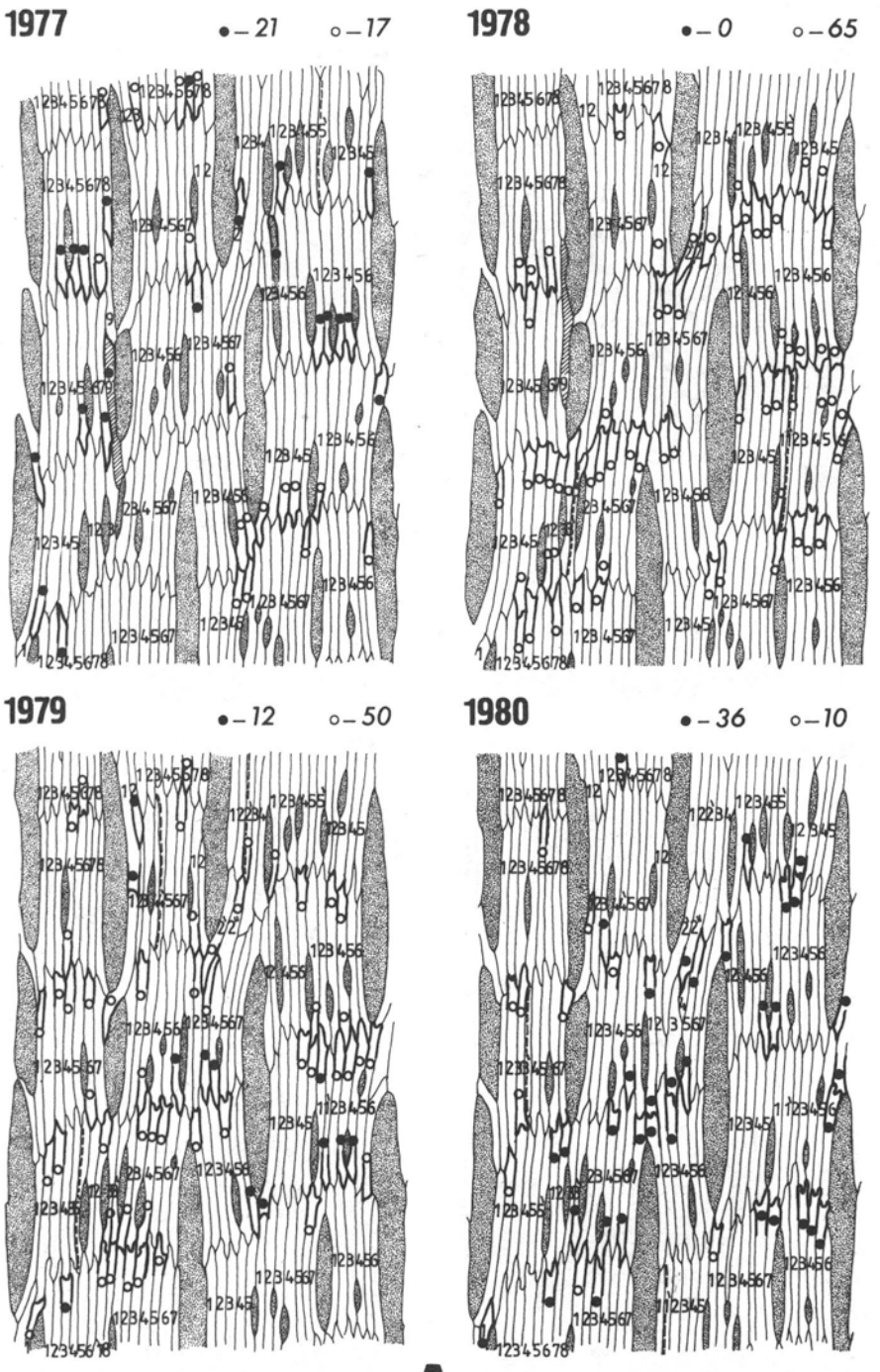


Fig. 4. Series "A" drawings from the successive surfaces of phloem terminal parenchyma and cambium from 1973 to 1980. The ends of the cells which underwent transposition are marked with a thick line (active ends). The direction of migration of the ends is marked with a circle. Black circles — direction of reconstruction to the left, that is, to S. White



circles — direction of reconstruction to the right, that is, to Z. Rays are dotted. The year the parenchyma was formed is marked on the upper left corner. The number of active ends is marked on the upper right corner. The eliminated cells are crossed out. Anticlinal divisions are marked with a continuous and broken line

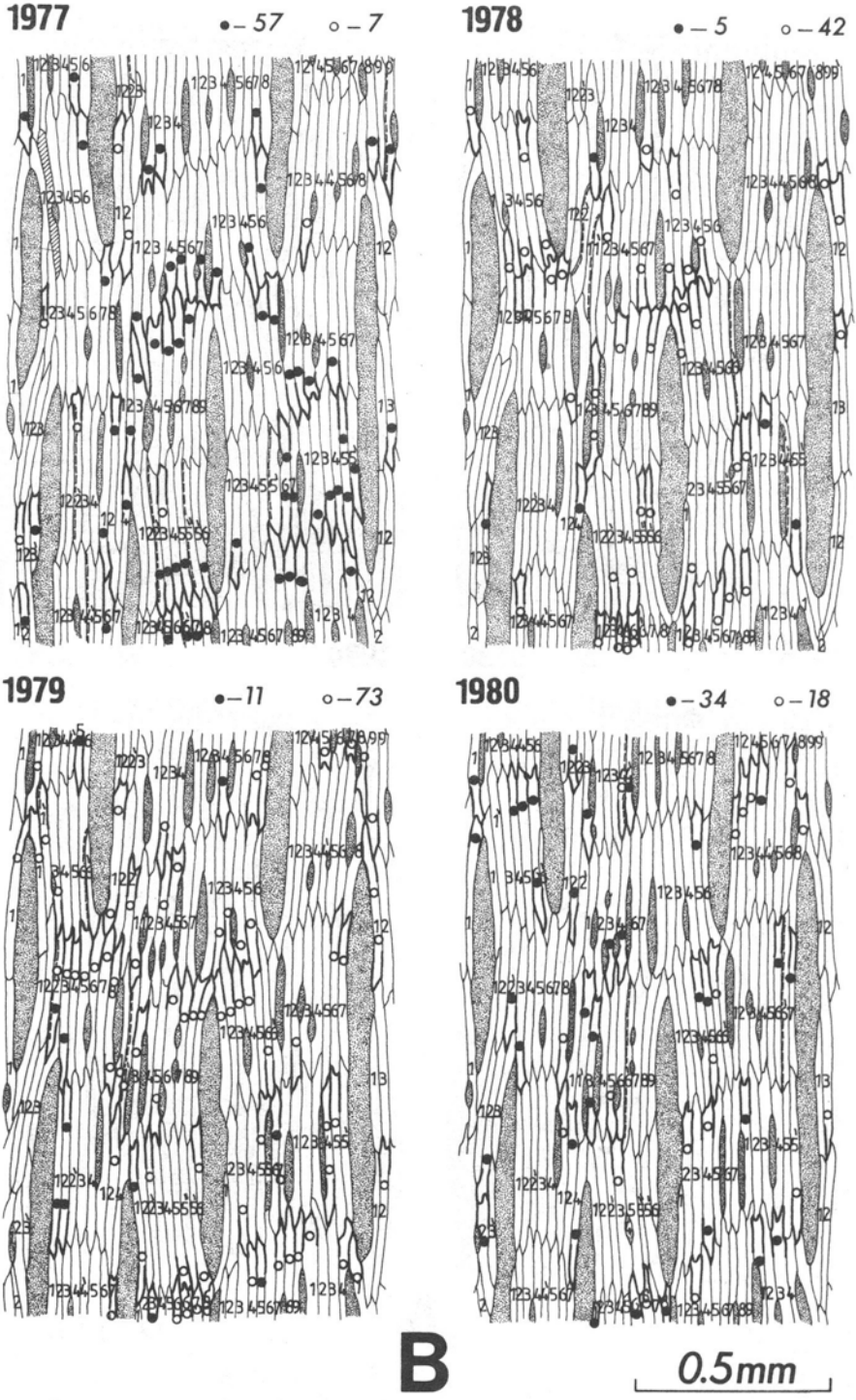
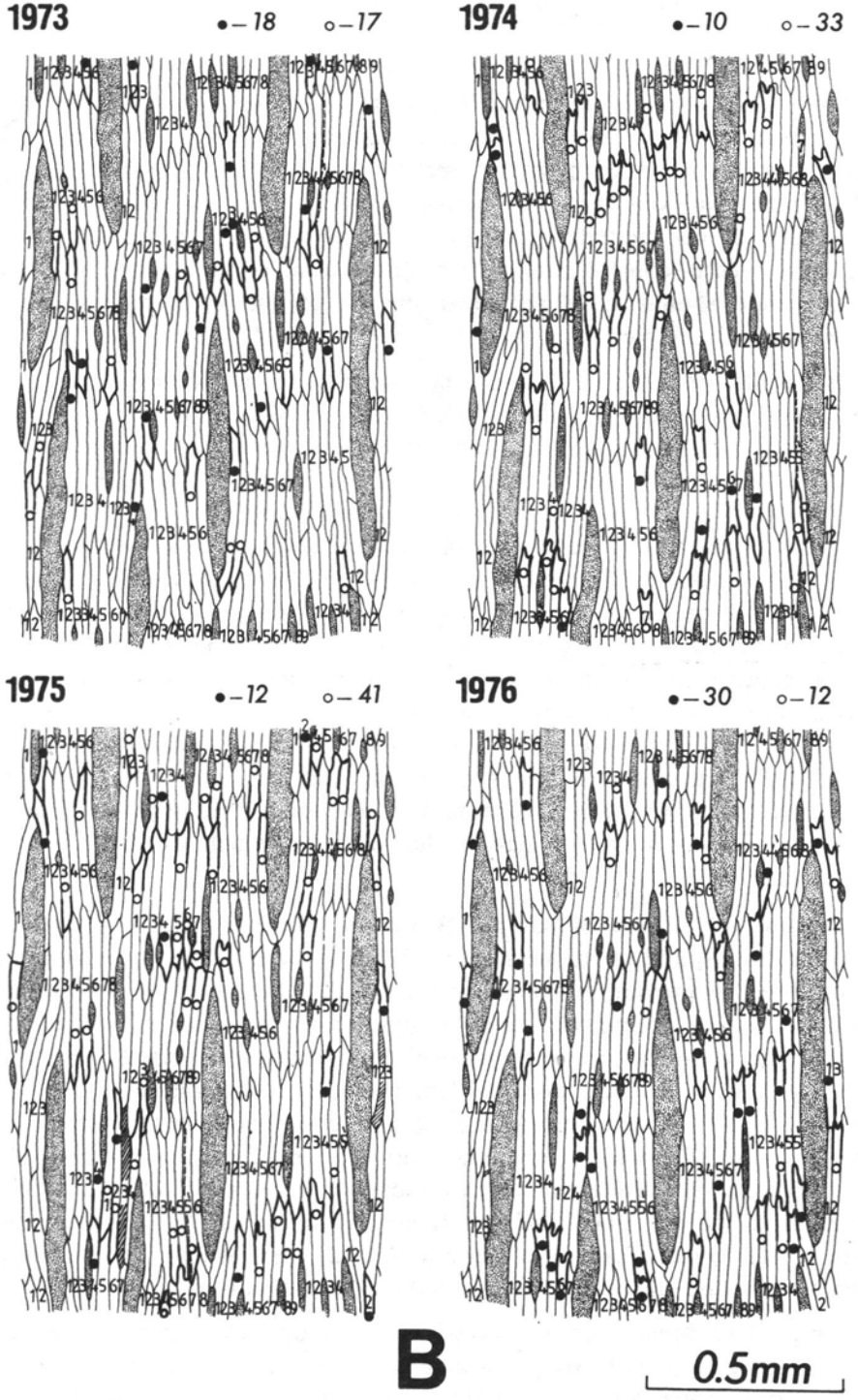


Fig. 5. Series "B" drawings of successive surfaces of terminal phloem parenchyma and



cambium from 1973 to 1980. The fragment of tissue from which this series is taken was located about 1 cm above the tissue used for series "A". Other explanations as above

in which the 100 μm thick layer had been formed, the active areas had not changed their position. It can therefore be presumed that during the interval between both successive samples of terminal parenchyma, the borders of areas of a given type of dominant configuration migrated many times quicker than the activity of cell ends. In addition, if the movement of cell activity took place along with the movement of the dominant type of configuration, a permanent trace of the change of position of the ends should have remained on all of the cells on the surface of the studied sample.

It results from Table 1 that the number of active ends in relation to all of the ends on the studied area oscillated from 13 to 29% (18.7% on average), that is, that there was an average of 5 ends to every 1 active end. At the same time we observed that 70% of the ends that had been active in a given year became inactive the next year. The change in the configuration of events took place in such a way that the active areas with a given configuration disappeared and new areas with an opposite configuration appeared over a large area of the cambium. There were areas in which the cells remained inactive for several years in spite of the general configuration over the studied area having changed twice. It was also observed that some of the ends of the initials retained their activity even up to three years while changing their contact at least once each year. Comparison of the state of the cells in successive years shows then, that what is taking place is not as much the shifting of groups of active cells, as it is a change in their activity, which we call fibrillation of activity.

On the 1977 map of sample B we see areas which were active in configuration S, and in the next year, 1978, new active areas in configuration Z appeared in previously inactive sites, so that the situation was reversed both in respect to activity and configuration. It is as if a chessboard of inactive white squares and active dark squares reversed the "color" of the squares and simultaneously changed the configuration of the active squares. It seems that the square of the chessboard, which at a given time is an area in which only one configuration of events is taking place, is a unit domain while the entire studied area is a set of two types of domains, although only one of them is active at a given moment. This would mean that in a certain period they would be undergoing events from domian S, at other periods, from domain Z.

The active area often contained only groups of upper (lower) ends. The presence of activity only (or mainly) on the ends of a given type may characterize larger areas of cambium, as can be seen by comparing the maps of both studied samples for the same year. It was also observed that the predominance of activity on one type of ends may appear

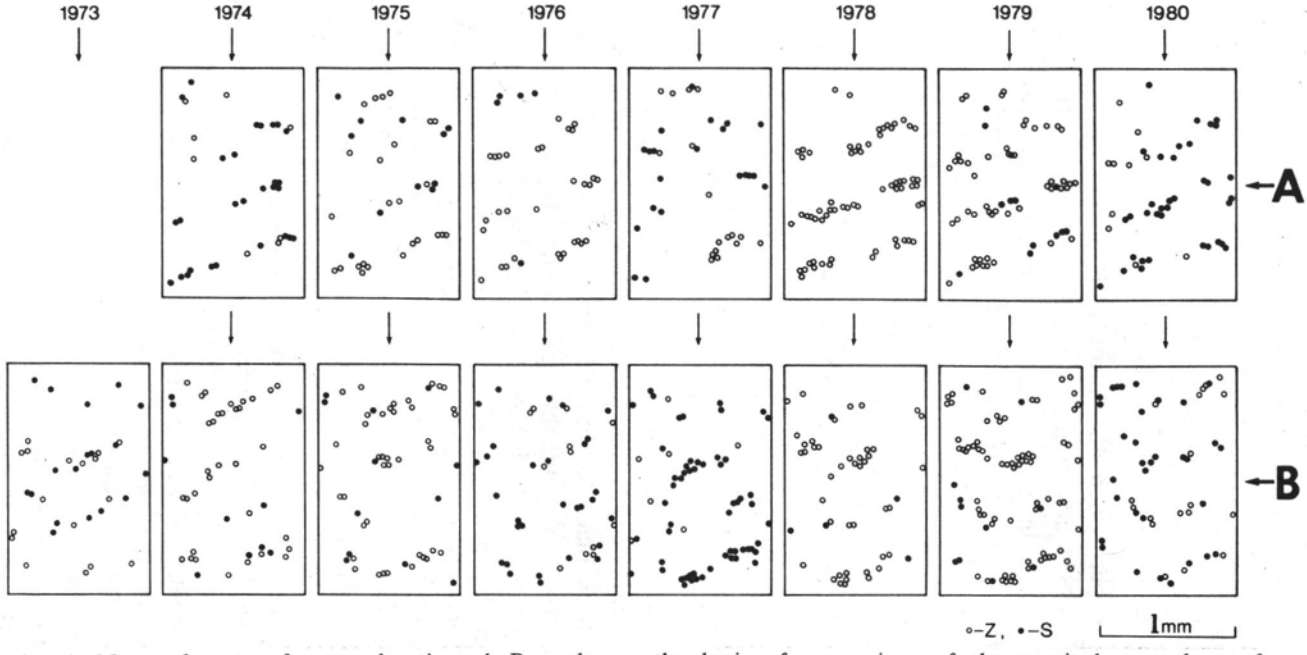


Fig. 6. Maps of events for samples A and B made on the basis of comparison of the terminal parenchyma from consecutive years (1973–1980). The direction of migration of active ends is marked on the map. Black circles — reconstruction to the left, that is, to S. White circles — reconstruction to the right, that is, to Z. The succesive maps for samples "A" and "B" correspond to each other in respect to years (arrows)

alternately, e.g. almost all of the active ends in sample A in 1976 were upper (91.6%), then in the following year 1977, the lower ends were active (73.7%).

DISCUSSION

In a previous paper which also dealt with linden, but with a different specimen (Włoch 1985), active and inactive areas were also distinguished on the basis of events in rays (splitting and uniting). The active areas were of one type in respect to configuration. At a certain moment the dominant type of events changed and a change in the state of activity of the area also took place. The areas of one type before the change did not correspond to the areas having a changed type. On the basis of these observations, it was concluded that the entire area under study then (1.3 cm high and 0.5 cm wide) was not a fragment of a domain, in spite of the fact that at a given time, one type of configuration dominated over it. It was said to be a set of small domains from among which at a given period, Z type domains were filled, at other times, S type domains.

On the entire surface examined in the previous study, one type of event dominated for several years, after which a long period of domination of the opposite type took place. It may be that in this case, the change in the dominant configuration took place through a shift in the borders between areas of different configurations, which would correspond to the migration of the domain pattern in the cambium of trees from wavy or interlocked wood (Hejnowicz and Romberger 1973, 1979, Krawczyszyn 1972). The area of a given configuration would not, however, be a proper domain, but an area in which only domains of one type are active. The activation of the domains of one type and inactivation of those of the other would manifest itself as fibrillation.

The present paper is based on events studied much more precisely than in the previous one since it deals with all of the fusiform cells on the surface of the studied area (1.5 mm × 1 mm). The sample studied in this paper is smaller than the active (inactive) area from the earlier paper. Fibrillation in the previous paper dealt with areas significantly larger than in this study. Both areas as small as those determined in this paper and as large as those in the previous paper can undergo fibrillation. The mechanism would still be similar — fibrillation would depend on the change of the type of configuration of events and the simultaneous change of activity of small areas. In the areas studied in this paper, the intensity of events was small enough so that not even one ray was splitting as well as not even one case of uniting rays had place.

Therefore, according to the criteria of the previous paper, the areas studied here were inactive. The difference then between the cambiums studied in both papers lies not only in the scale of size but also in the scale of intensity of events.

Can the effect of fibrillation of events be also found in previous papers on linden cambium? It seems that it can. We had described (Włoch and Zagórska-Marek 1982) migration of cambium cell activity along the border of storeys, but it was measured over a very small area; for 10 cells per storey and an increment of 80 μm in thickness. Samples of wood taken from the same tree 5 cm further along the axis of the trunk were studied in a different paper (Zagórska-Marek 1984) covering periods of 13 and 16 years. The configuration of events changed there in various periods every few years. That study did not take under consideration the running wave of grain, but on the basis of the size of the studied area it can be concluded that the rate of movement of the wave of the grain was many times greater than that of the areas of activity of cell ends. If in this study the duration of the grain change cycle had been known (unfortunately it was only possible to determine the length of the grain wave), it could have been compared to the duration of the dominant configuration in the process of fibrillation. Although the duration of the cycle is unknown, it can be presumed on the basis of the general knowledge of the wave grain, that it is greater than very two years, thus fibrillation is not related in a simple manner to the running wave grain. It should be expected that in the changing of an area from one type of configuration to an area with the other type, in connection with the wave of the grain, small active areas with both types of configurations will be found next to each other. Such situations are in fact found in the 1982 paper of Włoch and Zagórska-Marek on Fig. 7, in the paper by Zagórska-Marek from 1984 on Fig. 2 and also in this paper.

On the basis of the studies done until now, it is known that in linden cambium there is a great differentiation in the positioning and amounts of cellular events, and because of this, in the intensity of the reconstruction of the arrangement of cells. The cambiums samples from the trunks of different trees of the same species can also differ greatly. The differences deal with the presence or lack of a storeyed arrangement of rays, the presence or absence of oblique anticlinal divisions alongside radial divisions, and the presence or absence of long fusiform cells (nails) (Włoch and Zagórska-Marek 1982, Zagórska-Marek 1984, Włoch 1985).

Along with such a high degree of variability in linden cambiums, we also observe significant variability in the wood grain, which can be obliquely wavy or interlocked, where the cells can change their inclination

in different periods of time. If there is a long wave of grain in the wood, then it is always accompanied by a small, more or less visible wave up to a few mm in length, which moves very slowly in the successive rings along the rays, so that it is almost transverse. However, it still has not been possible to determine the connection between the high differentiation of wood grain and the properties of the cambium, excluding the relationship between the wavy grain and the high intensity of reconstruction. Even so, this paper points to the possibility of a connection between the size of the areas characterized by a predominance of one type of event, that is, "domains" and the intensity (frequency) of these events: the smaller the intensity, the smaller the size. The smallest domains correspond to very low intensity. At the same time, small areas do not as much migrate but fibrillate. It seems that migration is a property of relatively large domains.

In her paper from 1975, Zagórska-Marek called attention to the activity of upper and lower cell ends. She reported in the paper on *Entandrophragma* sp. that cells with forked ends made up 18% of the total amount of cambium cells, and 16.8% of those cells had their upper ends forked with only 1.2% having forked lower ends. However, she notes, citing Bannan (1956) that sectors can exist on the surface of cambium in which intrusive growth dominates on the basal ends in some of them, in others, on their apical ends. In this study, it was possible to see a periodical dominance of apical ends alternating with that of basal ends.

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Migotanie zdarzeń w domenach kambium Tilia cordata Mill.

Streszczenie

Zbadano kambium i 9 przyrostów rocznych łyka z pnia lipy. Część końców komórek wrzecionowatych zmieniała kontakty komórkowe — co wynika z porównania warstwy kambium i miękiszu terminalnego łyka — czyli była aktywna w okresie wytwarzania porównywanych warstw. Końce aktywne występują grupami po kilka do kilkunastu na przemian z grupami końców nieaktywnych. Około 70% końców zachowywało swoją aktywność tylko jeden rok. Na badanych obszarach w danym roku występuje przewaga jednego kierunku konfiguracji przemieszczania się końców aktywnych. Konfiguracja zmieniała się w zasadzie co dwa lata. Wraz ze zmianą konfiguracji końców aktywnych zmieniało się położenie obszarów aktywnych, tak, że po zmianie konfiguracji aktywne były obszary poprzednio nieaktywne. Nie zaobserwowano przy tym przesuwania się aktywności wzdłuż granic pięter, lecz jej pojawienie się i znikanie, co oznaczono jako migotanie aktywności. Migotanie aktywności ujawnia się przy słabej intensywności zdarzeń. Ze względu na to, że obszary aktywne w danym momencie charakteryzują się jednym typem konfiguracji zdarzeń, cała badana powierzchnia może przedstawiać się jak pojedyncza domena. Migotanie daje wrażenie częstej zmiany typu domeny, zmiana ta jednak ma inny charakter niż przesuwanie się granic domen występujące w kambium charakteryzującym się dużą intensywnością zdarzeń.