CORRELATION OF INTRUSIVE GROWTH OF CAMBIAL INITIALS TO REARRANGEMENT OF RAYS IN THE VASCULAR CAMBIIUM

Anna Wilczek¹, Joanna Jura-Morawiec², Paweł Kojs², Muhammad Iqbal³,⁴ and Wiesław Włoch¹,²*

SUMMARY

It is well documented that apical elongation of fusiform cambial initials through extension of their longitudinal edges, and their intrusion between tangential walls of the neighbouring initials and their closest derivatives cause rearrangement of fusiform cells, without increasing the cambial circumference. However, the concurrent rearrangement of rays is not fully understood. This study deals with *Pinus sylvestris* L., *Tilia cordata* Mill. and *Hippophaë rhamnoides* L., possessing a nonstoreyed, storeyed and double-storeyed type of cambium, respectively, and shows that the mechanism for rearrangement of ray initials is similar to the one proposed for fusiform initials, and includes multiplication of ray initials by anticlinal divisions, intrusive growth of ray initials, elimination of ray initials caused by intrusive growth of neighbouring fusiform initials, and transformation of ray initials into fusiform initials. Intrusive growth of a ray initial does not necessarily lead to the formation of a new fusiform initial, as it is dependent on the extent of the intrusive growth taken place. The extent of rearrangement of cambial cells is determined by the intensity of events occurring among the fusiform as well as ray initials. Intrusive growth of these initials does not influence the size of the cambial circumference.

Key words: Vascular cambium, intrusive growth, ray initials, splitting of rays, fusion of rays.

INTRODUCTION

The vascular cambium is a meristematic tissue placed between xylem and phloem, and generating these tissues. In general, it consists of thin-walled fusiform cells, which are elongated vertically and flattened in radial direction, and the ray cells that are predominantly radially elongate, as viewed in RLS, look almost isodiametric in tangential view (Wodzicki & Brown 1973). Fusiform initials are known to occupy 25–90% of the tangential surface of the cambium. Their proportion is generally greater than that of ray initials through extension of their longitudinal edges, and their intrusion between tangential walls of the neighbouring initials and their closest derivatives cause rearrangement of fusiform cells, without increasing the cambial circumference. However, the concurrent rearrangement of rays is not fully understood. This study deals with *Pinus sylvestris* L., *Tilia cordata* Mill. and *Hippophaë rhamnoides* L., possessing a nonstoreyed, storeyed and double-storeyed type of cambium, respectively, and shows that the mechanism for rearrangement of ray initials is similar to the one proposed for fusiform initials, and includes multiplication of ray initials by anticlinal divisions, intrusive growth of ray initials, elimination of ray initials caused by intrusive growth of neighbouring fusiform initials, and transformation of ray initials into fusiform initials. Intrusive growth of a ray initial does not necessarily lead to the formation of a new fusiform initial, as it is dependent on the extent of the intrusive growth taken place. The extent of rearrangement of cambial cells is determined by the intensity of events occurring among the fusiform as well as ray initials. Intrusive growth of these initials does not influence the size of the cambial circumference.

Key words: Vascular cambium, intrusive growth, ray initials, splitting of rays, fusion of rays.

INTRODUCTION

The vascular cambium is a meristematic tissue placed between xylem and phloem, and generating these tissues. In general, it consists of thin-walled fusiform cells, which are elongated vertically and flattened in radial direction, and the ray cells that are predominantly radially elongate, as viewed in RLS, look almost isodiametric in tangential view (Wodzicki & Brown 1973). Fusiform initials are known to occupy 25–90% of the tangential surface of the cambium. Their proportion is generally greater than that of ray initials through extension of their longitudinal edges, and their intrusion between tangential walls of the neighbouring initials and their closest derivatives cause rearrangement of fusiform cells, without increasing the cambial circumference. However, the concurrent rearrangement of rays is not fully understood. This study deals with *Pinus sylvestris* L., *Tilia cordata* Mill. and *Hippophaë rhamnoides* L., possessing a nonstoreyed, storeyed and double-storeyed type of cambium, respectively, and shows that the mechanism for rearrangement of ray initials is similar to the one proposed for fusiform initials, and includes multiplication of ray initials by anticlinal divisions, intrusive growth of ray initials, elimination of ray initials caused by intrusive growth of neighbouring fusiform initials, and transformation of ray initials into fusiform initials. Intrusive growth of a ray initial does not necessarily lead to the formation of a new fusiform initial, as it is dependent on the extent of the intrusive growth taken place. The extent of rearrangement of cambial cells is determined by the intensity of events occurring among the fusiform as well as ray initials. Intrusive growth of these initials does not influence the size of the cambial circumference.

Key words: Vascular cambium, intrusive growth, ray initials, splitting of rays, fusion of rays.

INTRODUCTION

The vascular cambium is a meristematic tissue placed between xylem and phloem, and generating these tissues. In general, it consists of thin-walled fusiform cells, which are elongated vertically and flattened in radial direction, and the ray cells that are predominantly radially elongate, as viewed in RLS, look almost isodiametric in tangential view (Wodzicki & Brown 1973). Fusiform initials are known to occupy 25–90% of the tangential surface of the cambium. Their proportion is generally greater than that of ray initials through extension of their longitudinal edges, and their intrusion between tangential walls of the neighbouring initials and their closest derivatives cause rearrangement of fusiform cells, without increasing the cambial circumference. However, the concurrent rearrangement of rays is not fully understood. This study deals with *Pinus sylvestris* L., *Tilia cordata* Mill. and *Hippophaë rhamnoides* L., possessing a nonstoreyed, storeyed and double-storeyed type of cambium, respectively, and shows that the mechanism for rearrangement of ray initials is similar to the one proposed for fusiform initials, and includes multiplication of ray initials by anticlinal divisions, intrusive growth of ray initials, elimination of ray initials caused by intrusive growth of neighbouring fusiform initials, and transformation of ray initials into fusiform initials. Intrusive growth of a ray initial does not necessarily lead to the formation of a new fusiform initial, as it is dependent on the extent of the intrusive growth taken place. The extent of rearrangement of cambial cells is determined by the intensity of events occurring among the fusiform as well as ray initials. Intrusive growth of these initials does not influence the size of the cambial circumference.

Key words: Vascular cambium, intrusive growth, ray initials, splitting of rays, fusion of rays.

INTRODUCTION

The vascular cambium is a meristematic tissue placed between xylem and phloem, and generating these tissues. In general, it consists of thin-walled fusiform cells, which are elongated vertically and flattened in radial direction, and the ray cells that are predominantly radially elongate, as viewed in RLS, look almost isodiametric in tangential view (Wodzicki & Brown 1973). Fusiform initials are known to occupy 25–90% of the tangential surface of the cambium. Their proportion is generally greater than that of ray initials through extension of their longitudinal edges, and their intrusion between tangential walls of the neighbouring initials and their closest derivatives cause rearrangement of fusiform cells, without increasing the cambial circumference. However, the concurrent rearrangement of rays is not fully understood. This study deals with *Pinus sylvestris* L., *Tilia cordata* Mill. and *Hippophaë rhamnoides* L., possessing a nonstoreyed, storeyed and double-storeyed type of cambium, respectively, and shows that the mechanism for rearrangement of ray initials is similar to the one proposed for fusiform initials, and includes multiplication of ray initials by anticlinal divisions, intrusive growth of ray initials, elimination of ray initials caused by intrusive growth of neighbouring fusiform initials, and transformation of ray initials into fusiform initials. Intrusive growth of a ray initial does not necessarily lead to the formation of a new fusiform initial, as it is dependent on the extent of the intrusive growth taken place. The extent of rearrangement of cambial cells is determined by the intensity of events occurring among the fusiform as well as ray initials. Intrusive growth of these initials does not influence the size of the cambial circumference.

Key words: Vascular cambium, intrusive growth, ray initials, splitting of rays, fusion of rays.

INTRODUCTION

The vascular cambium is a meristematic tissue placed between xylem and phloem, and generating these tissues. In general, it consists of thin-walled fusiform cells, which are elongated vertically and flattened in radial direction, and the ray cells that are predominantly radially elongate, as viewed in RLS, look almost isodiametric in tangential view (Wodzicki & Brown 1973). Fusiform initials are known to occupy 25–90% of the tangential surface of the cambium. Their proportion is generally greater than that of ray initials through extension of their longitudinal edges, and their intrusion between tangential walls of the neighbouring initials and their closest derivatives cause rearrangement of fusiform cells, without increasing the cambial circumference. However, the concurrent rearrangement of rays is not fully understood. This study deals with *Pinus sylvestris* L., *Tilia cordata* Mill. and *Hippophaë rhamnoides* L., possessing a nonstoreyed, storeyed and double-storeyed type of cambium, respectively, and shows that the mechanism for rearrangement of ray initials is similar to the one proposed for fusiform initials, and includes multiplication of ray initials by anticlinal divisions, intrusive growth of ray initials, elimination of ray initials caused by intrusive growth of neighbouring fusiform initials, and transformation of ray initials into fusiform initials. Intrusive growth of a ray initial does not necessarily lead to the formation of a new fusiform initial, as it is dependent on the extent of the intrusive growth taken place. The extent of rearrangement of cambial cells is determined by the intensity of events occurring among the fusiform as well as ray initials. Intrusive growth of these initials does not influence the size of the cambial circumference.

Key words: Vascular cambium, intrusive growth, ray initials, splitting of rays, fusion of rays.
initials in most species, being 100% in rayless species, like *Alseuosmia* and *Bougainvillaea* (Iqbal & Ghouse 1990; Larson 1994; Iqbal 1995; Lachaud et al. 1999).

Considering the cambium as a three-dimensional tissue, Włoch and Połap (1994) introduced the term ‘initial surface’, which stresses that an initial layer exists only in the ideal sense, but is not a geometric reality (Włoch 1981; Włoch et al. 2001; Kojs et al. 2004a). Cambial initials, dividing predominantly periclinal during the active span of cambium, produce ray cells as well as axially elongated elements of the phloem and xylem mother cells (Schmid 1976; Iqbal & Ghouse 1990; Fahn & Werker 1990; Larson 1994; Lachaud et al. 1999; Barlow 2005); these divisions are located on the initial surface. The intrusive growth of initials occurring on the initial surface results in cambial cell rearrangement (Jura et al. 2006; Karczewska et al. 2009; Włoch et al. 2009).

Cambial cells grow both symplastically and intrusively. Symplastic growth that occurs in a continuous and highly coordinated way and keeps the contacts between adjacent cells intact, is common to all growing tissues (Butterfield 1972, 1973; Cumbie 1984; Erickson 1986; Jura et al. 2006; Karczewska et al. 2009; Włoch et al. 2009). Intrusive growth, associated mainly with the cambium (Sinnott & Bloch 1941; Majumdar 1941; Schoch–Bodmer & Huber 1952; Ghouse & Yunus 1975; Jura et al. 2006; Włoch et al. 2009) and with its closest derivatives such as elongating fibres and enlarging vessel elements (Ghouse & Yunus 1975; Ghouse & Iqbal 1979; Larson 1994; Iqbal 1995; Evert 2006), allows for developing new contacts between adjacent cells. It is suggested that intrusive growth of initials occurs in areas where curvature of cell wall is maximal, and stress owing to turgor pressure is minimal (Hejnowicz 1980), a feature distinguishing intrusive growth from symplastic growth. Schoch-Bodmer and Huber (1952) explained that the growing cell dissolves the middle lamellae of the neighbouring cells and grows into the resulting space. Formation of those spaces may involve unequal symplastic growth in radial direction of cells in neighbouring radial files (Jura et al. 2006). It has been shown that intrusive growth and elimination of the cambial initials, widely described in the literature as two separate events (Bannan 1950, 1953; Bannan & Bailey 1956; Hejnowicz 1961, 1967, 1980; Hejnowicz & Brański 1966; Cumbie 1967; Hejnowicz & Krawczyszyn 1969; Rao & Dave 1985), are actually different manifestations of the same process (Kojs et al. 2004 a,b; Jura et al. 2006; Karczewska et al. 2009; Włoch et al. 2009).

Symplastic growth of the cambial initials occurs in both radial and circumferential directions. The growth in the radial direction causes thickening of tree trunk and its branches, whereas the growth in the circumferential direction leads to expansion of the cambial circumference. Symplastic growth in circumferential direction was described first for the fusiform initials of storeyed cambium (Butterfield 1972; Cumbie 1984), and later in the nonstoreyed cambium also (Karczewska et al. 2009). Symplastic growth is believed to occur also in the ray initials and contribute to the increase of the cambial circumference (Bailey 1923).

Symplastic growth of the cambial cells does not occur in axial direction. However, the axially oriented individual initials grow apically in axial direction by intrusive growth, which is clearly visible in the nonstoreyed cambia (Barghoorn 1940b; Włoch et al. 2001, 2002, 2009; Jura et al. 2006). Intrusive growth in the developing cam-
bium normally does not occur in radial direction (Jura et al. 2006; Karczewska et al. 2009). Both the symplastic and intrusive growths are involved in the circumferential cell expansion; the former leads to expansion of the cambial circumference, whereas the latter is solely related to the cambial cell rearrangement (Karczewska et al. 2009; Włoch et al. 2009). Directed intrusive growth leads to changes in the degree of inclination and often also in the orientation of the initials, especially in trees having wood with interlocked grain (Krawczyszyn 1971; Harris 1989; Romberger et al. 1993; Kojs et al. 2004b). In view of the above, intrusive growth may likely manifest an entirely different mechanism (Lev-Yadun 2001) although apparently it looks to be confluent and have much in common with symplastic growth.

Ray initials grow in the radial direction symplastically, in consonance with the rate of radial growth of neighbouring fusiform cells, and also divide periclinal with a frequency similar to that of fusiform initials (Wodzicki & Brown 1973). The derivatives of fusiform initials frequently undergo periclinal divisions, whereas those of ray initials usually do not. Consequently, the fusiform cells form 4–8-celled packets on the xylem side and 2-celled ones on the phloem side (Mahmood 1968), while the ray cells elongate in radial direction. During the peak activity, 15–24 layers of fusiform cells in the cambial zone of Tectona grandis occupied a radial distance that was covered only by 2–4 ray cells (Rao 1988). In most of the arborescent species with cylindrical cambium, the length of ray cells usually corresponds to the radial dimension of fusiform-cell packets (Mahmood 1968; Timell 1980; Włoch & Szendera 1989; Włoch & Polap 1994). In general, the ray mother cells rarely divide periclinal. However, in some species of the Malvales, where rays contain tile cells and procumbent cells (Chattaway 1933a, b), the derivatives of tile-cells undergo frequent periclinal divisions, keeping their radial dimension similar to that of the fusiform initials.

Ray initials divide periclinal in such a way that their newly formed tangential walls lie close to those of fusiform initials (Wodzicki & Brown 1973; Imagawa 1984). This unequal periclinal division of a radially-extended ray initial gives rise to (a) a new ray-initial cell with smaller radial dimension, which stays on the initial surface of the cambium together with the fusiform initials, and (b) a ray cell with larger radial dimension located in the zone of derivative cells either on the phloem or on the xylem side. Periclinal divisions of individual ray initials within a ray are not synchronized and may occur at different times in cells situated closely together (Catesson 1964). The planes of additive periclinal divisions of neighbouring ray initials are slightly shifted in radial direction. When the ray cells grow symplastically in the radial direction, the periclinal walls of the neighbouring initials can move away from each other. Consequently, beyond the cambium the ray structure exhibits more or less a chequered cell arrangement, as seen in radial plane (Wodzicki & Brown 1973). This arrangement of ray cells may suggest that the rays possibly pose a mechanical barrier to the intrusive growth of neighbouring fusiform initials, taking place between periclinal walls. If so, the mechanism of rearrangement of ray cells should differ from the one described for fusiform initials.

The hitherto held common view that the fusiform initials grow intrusively between the radial walls of the neighbouring initials, thus increasing the cambial circumfer-
ence (Esau 1965; Larson 1994; Steeves & Sussex 1996; Dickinson 2000; Evert 2006), suggests that the increase in the cambial circumference involves contribution from both fusiform and ray initials. The concept that the arrangement of rays in the active cambium changes due to intrusive growth of elongating cells and elimination of their neighbouring cells, involves not only the fusiform initials but also the ray initials (Iqbal & Ghouse 1985, 1987; Rao 1988). It is believed that intrusive growth of ray initials can transform them into fusiform initials thus leading to ray splitting (Barghoorn 1940b, 1941a; Braun 1955; Cumbie 1963; Krawczyszn 1971, 1972; Rao 1988; Romberger et al. 1993; Larson 1994). Elimination of ray cells due to intrusion of adjacent fusiform initials into a ray is another cause of the splitting of rays (Barghoorn 1940b, Hejnowicz & Krawczyszn 1969; Krawczyszn 1971; Ghouse & Hashmi 1980; Iqbal & Ghouse 1987; Włoch & Szendera 1989). Ray height may be reduced by elimination of marginal cells of the ray (Evert 1961; Srivastava 1963). Moreover, vertically adjacent rays may fuse by partial elimination of intervening fusiform initials (Hejnowicz & Krawczyszn 1969; Krawczyszn 1971; Ghouse & Hashmi 1980; Rao 1988; Włoch & Szendera 1989) and/or by the transverse segmentation of these fusiform initials (Barghoorn 1940b; Krawczyszn 1971; Iqbal & Ghouse 1987).

Recent observations suggest that intrusive growth of fusiform initials involves elongation of longitudinal edges of cell ends between tangential walls of their neighbours, causing rearrangement of cells and making no contribution to the circumferential growth of the cambium (Włoch & Połap 1994; Włoch et al. 2001, 2002; Kojs et al. 2004a, b; Jura et al. 2006, Karczewska et al. 2009; Włoch et al. 2009), and not between the radial walls, as speculated earlier (Hejnowicz 1961, 1967; Hejnowicz & Brański 1966; Larson 1994; Steeves & Sussex 1996; Evert 2006). If so, the ray initials, which elongate in the radial direction, must cause a barrier to the localized intercellular tangential intrusion of the growing initials. The present study, therefore, investigates whether the rearrangement of ray initials is connected, like that of fusiform initials, to their intrusive growth between the periclinal walls of the neighbouring initials and their closest derivatives, and whether the intrusive growth of ray initials has any bearing on the size of the circumference of the cambial cylinder.

MATERIALS AND METHODS

The cambial tissue was collected from the main trunks of *Pinus sylvestris* L. and *Tilia cordata* Mill., and from young branches of *Hippophaë rhamnoides* L., growing in the temperate zone in Poland. Sampling was done early in October (end of active phase of the cambium) for *P. sylvestris* and early in July (peak time for cambial activity) for *T. cordata* and *H. rhamnoides*. These species, representing the nonstoreyed, storeyed and double-storeyed cambium, respectively, were selected to confirm whether the described mechanism was present in the cambia of different structural types. The collected samples were fixed in 2.5% glutaraldehyde and embedded in Epon (Meek 1976). Long series of tangential and transverse sections of about 3 μm thickness were prepared with an ultramicrotome, as described earlier (Włoch et al. 2001). The sections were glued to glass with Haupt adhesive (1% gelatine in water with 2% phenol crystals and 15%
glycerine), stained with periodic acid Schiff’s reagent (Schiff reagent – $C_{19}H_{18}N_3Cl + H_2SO_3$) and toluidine blue ($C_{15}H_{16}N_3SCl$), and mounted in Euparal (Włoch & Połap 1994). On the basis of a series of transverse sections of the *Pinus sylvestris* cambium, reconstructions were made to depict the arrangement of cambial cells in tangential and radial planes. For *Hippophaë rhamnoides* and *Tilia cordata*, reconstructions showing the expected position of cambial cell walls in transverse plane were developed on the basis of tangential sections. Reconstructions made from long series of thin transverse sections provide a very precise method, like tomography, for examining the cell arrangement and revealing the actual positions of cells (Kojs et al. 2004a, b; Jura et al. 2006).

The double storeyedness of the cambium of *Hippophaë rhamnoides* was determined by the percentage of rays located within the storeys, as calculated in two different areas for each sample, during the first fifteen years of cambial activity (Fig. 4).

**RESULTS**

*Intrusive growth of ray initials in nonstoreyed cambium*

Intrusive growth of ray initials was studied in the cambium of a 50-year-old *Pinus sylvestris* tree, having spiral-grained wood. Figure 1 displays a series of selected transverse sections of the cambium, showing ray cells sandwiched between two rows of fusiform initials, and also the reconstructions made from these sections to exhibit the cambial–cell arrangement in tangential and radial planes. The samples represent the cambial state late in the active phase, and the last changes in the arrangement of the initials are displayed in the few successive layers of derivatives. Observations revealed that a ray initial could increase its tangential dimension without causing any change in the position of neighbouring cells. This can be explained only by considering the intrusive growth of the ray initial occurring along the tangential walls of the neighbouring fusiform cells. The reconstructions [Fig. 1(ii)], made by the superimposition of layers XL+IL and IL+PhL as marked on Fig. 1(i), reveal a dynamic rearrangement of the ray initials and their neighbouring fusiform initials. The upper initial of the two-celled ray (dark grey and dashed) as well as two fusiform initials (bright grey and dashed), one above the ray and the other left to the ray, undergo intrusive growth. In the tangential plane, the position of other neighbouring fusiform cells remains unchanged. This means that expansion of ray cells has no effect on the tangential dimension of the examined sector of the cambium. The radial reconstruction [Fig. 1(iii)] shows that both fusiform and ray initials are vertically adjacent and maintain their contacts.

*Intrusive growth of ray initials in storeyed cambium*

Figure 2 shows the process of ray fusion in the cambium of *Tilia cordata*. Details can be seen in the series of tangential sections, from the phloem [Fig 2(i)a] to the cambial initials [Fig. 2(i)f], and in the drawings [Fig. 2(ii)]. Two cells located at the end of two (upper triseriate and lower multiseriate) rays grow intrusively along tangential plane, and then divide anticlinally [Fig. 2(i)d,e], thus fusing two separate rays into a higher one. At the same time, lateral protrusions of the neighbouring fusiform initials, separating the two vertically adjacent rays, were eliminated [Fig. 2(ii)a+f, c+f].
The transverse section in Figure 2(iii) shows a biseriate ray (from a different area of cambium, because it is not possible to obtain tangential as well as transverse sections exactly at the same place), and the diagram 2(iv) drawn on the basis of this section illustrates the transverse dimensions of cambial cells at radial distances between tangential sections (a–f) shown in Figure 2(i). It shows the probable location of cambial cells in the transverse plane at a position indicated in Figure 2(i) with the horizontal line x. The location of the fusiform and ray initials, in the transverse section and the drawing, are marked as FI and RI respectively. It is obvious that the mutual contacts of fusiform cells are disrupted by intrusively growing ray initials, visible in Figure 2(iv) as a new radial file of ray cells. This event, occurring on the ‘initial surface’ of the cambium, was recorded both on the phloem side and the xylem side. Due to vast changes in cell arrangement during differentiation of vascular tissues, the best record of cambial events in derivative layers may be seen either in close vicinity of the initial surface of the cambium or in the terminal parenchyma of the wood, which is formed once in a year (usually one layer of parenchyma per 2–3 mm of radial growth) and reflects the arrangement of the cambial initials at that point of time when these layers were generated by the cambium. Radial growth occurs on the phloem and the xylem sides with different intensity during the growth season: in most species cells are deposited first on the phloem side and then on the xylem side. Also, the xylem mother cells divide periclinaly more intensively than the phloem mother cells. At the time of sampling (early July), accumulation of cambial derivatives was remarkably larger on the xylem side than on the phloem side. It may be understood with reference to Figure 2(iv) that the cell events recorded in two phloem mother cells is likely to figure on the wood side quite distant from the cambium, where reconstruction of cellular events may not be possible due to intensive changes in cell arrangement (mostly of vessel elements and wood fibres) during differentiation. Therefore, the record of events visible in the phloem in Figure 2(iv) is not shown in the xylem, as the number of cell layers added to xylem side was significantly greater than the number of layers deposited on phloem side during the active cambial phase, and the matching events on the xylem side were considerably obscured by the differentiation of vessels and fibres.

**Intrusive growth of fusiform initials between ray initials in double-storeyed cambium**

A long-term study of the double-storeyed cambium of *Hippophaë rhamnoides* revealed that the cambial cell arrangement was clearly nonstoreyed in the one-year-old stem, showing a random distribution of both the fusiform and ray initials as seen in the tangential view (Fig. 3A). In the two-year-old cambium, a storeyed pattern of fusiform initials began to appear, but the arrangement of rays was still nonstoreyed (Fig. 3B). In the fourth year, the fusiform initials formed regular storeys with most (about 70%) of the rays confined within the height of individual storeys, thus forming the double-storeyed pattern (Fig. 3C). The regular double-storeyed pattern, showing 100% of rays located within the confines of individual storeys, was finally established in 10–11-year-old cambium (Fig. 4).
Figure 1. Intrusive growth of ray initials between tangential walls of neighbouring fusiform cells in the nonstoreyed cambium of *Pinus sylvestris* L.

(i) Examples chosen from the series of transverse sections of cambium (a–j): Horizontal lines XL, IL and PhL indicate the position where reconstructions in tangential plane have been made (ii). The lines XL, IL and PhL represent the layer of xylem mother cells, the most probable location of the initial cell layer, and the layer of phloem mother cells respectively. The vertical line in a–j indicates the site where reconstruction of cells in the radial plane (iii) was made.

(ii) Tangential reconstructions made on the basis of transverse-section series (a–j) superimposed in pairs (XL+IL; IL+PhL; XL+PhL). Type of lines corresponds with lines on figure (i): thick - XL, dashed - IL, thin - PhL. The areas covered by oblique lines indicate the change in location of the initials caused by intrusive growth between tangential walls. Fusiform initials growing intrusively are marked with a bright grey shade (XL+IL; IL+PhL), whereas the ray cells are with a dark grey shade. Horizontal arrows (a–j) correspond to the locations of the respective transverse sections.

(iii) Radial reconstruction of cell-end location prepared on the basis of the transverse-section series: the dashed area indicates the probable location of the fusiform and ray initials. Horizontal arrows (a–j) correspond to sections a–j, whereas vertical arrows XL, IL, PhL on the upper side of this sketch correspond to lines XL, IL, PhL drawn on transverse sections a–j.
Figure 2. Intrusive growth of ray initials causing fusion of two rays in the storeyed cambium of *Tilia cordata* Mill.

(i) Series of selected tangential sections from phloem (a) to initial surface (f), in the stem. Line x indicates the location of the transverse reconstruction (iv).

(ii) Drawings made by superimposing three tangential sections [(i) a, c, f] in pairs (a+f) and (c+f). Continuous lines indicate the position of initial cells [(i)f], whereas dashed lines indicate the position of phloem cells (a, c). Two ray cells in the phloem zone are shaded with a slanted line (a). Intrusive growth of two ray initial cells preceded their anticlinal divisions, forming four ray initials (shaded) (f). Two new ray initials derived from the growing ray initial on the upper...
end of the lower ray are shaded in dark grey, while two new ray initials derived from the growing ray initial on the lower end of the upper ray are shaded in bright grey. New anticlinal wall formed due to division of fusiform initial located at the left side of rays is marked with a double line (continuous and dashed).

(iii) A transverse section of cambium, showing with horizontal arrows the probable location of tangential sections of the same sample, as shown in (i) a–f. The probable position of the initial surface [(i) f] is marked with FI (fusiform initials) and RI (ray initials).

(iv) Reconstruction of a transverse view of the cambium including two radial rows of fusiform cells and a uniseriate ray from the plane marked with horizontal line x in figure (i). Arrows (a–f) correspond to the possible locations of tangential sections a–f. Dimensions of cambial cells are comparable with cells presented in (iii).

Figure 3. Tangential longitudinal sections of cambium in the stem of *Hippophaë rhamnoides* L. – A: 1 year old. – B: 2 years old. – C: 3 years old.

Figure 4. Correlation between the storeyedness of rays and the age of the cambium in *Hippophaë rhamnoides* L.
During the development of the double-storeyed pattern, rays underwent prominent rearrangement, showing (i) a change in their location in relation to storeys of fusiform cells as a result of anticlinal divisions of ray initials at one end of the ray and elimination of initials at the other, thus leading to a vertical shift of rays with respect to storey borders; and (ii) splitting and fusion of rays on borders of storeys, thus causing significant changes in the height and location of the rays concerned.

The process of ray splitting was studied in a series of tangential longitudinal sections obtained from one-year-old cambium of *Hippophaë rhamnoides* [Fig. 5(i)a–j]. The selected sections illustrate the arrangement of cambial cells from phloem mother cells (a), through the cambial initials (f), to xylem mother cells (j). Figure 5(ii) superimposing sections ‘a’ and ‘f’ from series (i)a–j, shows the intrusive growth of two fusiform initials, contiguous to the ray. Arrows indicate the size and direction of cell-tip extension and intrusion. The fusiform initial marked with letters FI in Figure 5(i)f has ‘replaced’ a ray initial (# 2) in the layer of initials, as shown in Figure 5(ii). However, these events of cell growth did not push the neighbouring fusiform cells apart in tangential direction, nor did the total tangential width of the selected cell group increase. This means that the intrusive growth of one initial is balanced by a partial or complete elimination of the neighbouring initials. Figure 5(iv) was drawn based on a transverse section of cambium [Fig. 5(iii)] showing a uniseriate ray. Dimensions of cells in this diagram correspond to the change in the location of walls of the growing cells [Fig. 5(i)], which would be visible if the same fragment of cambium is sectioned transversely instead of tangentially. Cells displaying the maximum positional change were identified on Figure 5 as the initials and marked as FI. The extent of growth of one initial, marked...
as FI₁, was significantly larger than the other, marked as FI. These cells on transverse reconstruction [Fig. 5(iv)] are situated between slanting walls. The initial, while growing intrusively, records these changes in successive layers of phloem and xylem, which can be seen in transverse sections of the cambial zone as the slanting walls, whose orientation is opposite on the xylem and phloem sides. Because the number of cells deposited on the phloem side is significantly less than that on the xylem side, the slanting cell walls on the phloem side are more inclined. Comparison of locations of tangential sections a–j revealed that only one cell on the phloem side, the closest derivative of FI₁, exhibits such slanted walls [Fig. 5 iv (c, d)]. At the same time, these walls are less inclined and appear in 7 cells on the xylem side.

DISCUSSION

The pattern of rearrangement of cambial initials is recorded in the successive layers of secondary phloem and xylem in woody plant species. This record can be traced easily in mature softwood as the arrangement of tracheids reflects the original arrangement of the cambial initials (Bannan 1950, 1953; Bannan & Bailey 1956). In hardwoods this is impossible due to considerable changes in cell arrangement taking place during tissue differentiation (mainly during formation of fibres and vessel elements). Therefore, most studies tracing the patterns of cell rearrangement in the mature xylem focus on parenchyma cells, which do not change their dimensions during maturation and hence reflect the arrangement of the cambial initials at the moment that parenchyma strands were formed (Hejnowicz & Krawczyszyn 1969; Włoch 1985, 1987; Włoch & Bileczewska 1987; Włoch & Połap 1994). In some species a layer of parenchyma is formed once a year, at the end of the growth season, and termed ‘terminal parenchyma’ (Carlquist 2001). In some other species, especially in tropical trees like Terminalia tomentosa and Gmelina spp., this parenchyma layer is called ‘initial parenchyma’ as it is formed at the beginning of the growth season rather than at the end (Chowdhury 1934, 1936, 1953). Yet other species, like Robinia pseudoacacia, form both terminal and initial parenchyma (Carlquist 1980). The term ‘marginal parenchyma’ can apply alike to ‘terminal’ and ‘initial’ parenchyma (Carlquist 1980, 2001; IAWA Committee 1989).

Intrusive growth of cambial fusiform initials is considered to be a major cause for cell rearrangement (Bannan 1950, 1953; Bannan & Bailey 1956). Recent investigations have revealed that the fusiform initial of a radial file grows on its longitudinal edge between the periclinal walls of an initial cell and its closest derivative located in the neighbouring radial file. The intrusive growth of the para-apical edge of the cell leads to a rapid change in the initial’s orientation and its degree of inclination. The occurrence of intrusive growth is counterbalanced with cell elimination and hence does not change the circumferential dimension of the cambial cylinder (Kojs et al. 2004a, b; Karczewska et al. 2009; Włoch et al. 2009). Mathematical models of cambial growth (Barlow et al. 2002; Forest et al. 2004) do not consider the intrusive growth and still explain the increase of cambial circumference.

The groups of fusiform initials in a given area of the tangential cambial surface (cambial domain) grow intrusively in the same, either a Z (right) or S (left), orientation.
Changes in the orientation and the degree of inclination of the initials in a given cambial domain are recorded in successive layers of secondary xylem as the wood grain. One cycle of change in cell orientation usually lasts for at least a few years (Krawczyszyn 1971, 1972; Hejnowicz 1973, 1975; Hejnowicz & Romberger 1973, 1979; Krawczyszyn & Romberger 1980), which can be possible only when these changes occur on the cambial initial surface (Włoch et al. 2002; Kojs et al. 2004a, b). Changes occurring with rays, like fusion and splitting, also follow the domain pattern, and are recorded in successive layers of secondary xylem and phloem (Krawczyszyn 1971, 1972, 1973, 1977; Włoch 1985, 1987; Włoch & Szendera 1989, 1992; Włoch & Wawrzyniak 1990; Harris 1989; Włoch et al. 1992). Rearrangements of ray initials and fusiform initials have been described, unrealistically, as separate events. Moreover, occurrence of intrusive growth in ray initials was regarded as having been restricted to such occasions when a ray initial supposedly transforms to a fusiform initial (Barghoorn 1940a, 1941b). However, recent observations as described above, suggest that the mechanism of cell rearrangement of ray initials is the same as the one described for fusiform initials. The intrusive growth of ray initials also brings change in their contacts with neighbouring cells and increases their tangential dimension, which is followed by anticlinal divisions, as described in this paper with reference to ray fusion in the cambium of _Tilia cordata_. Rao (1988) also pointed to the rearrangement of ray initials through intrusive growth, irrespective of their transformation into fusiform cells.

According to Evert (1961), ray splitting by intrusively growing fusiform initials in the cambium of _Pyrus communis_ is always preceded by elimination of ray initials from the area covered by the apical intrusive growth of the fusiform initials. As a result, the neighbouring ray initials do not change their location, and therefore the ray-cell location may be considered as a location marker. The fusiform initial that grows intrusively between ray cells, reduces the width and/or height of a multiseriate ray (Srivastava 1963; Esau 1965; Krawczyszyn 1971; Süss & Müller-Stoll 1973). Barghoorn (1940b) presented an example of ray splitting by apical intrusive growth of fusiform initial between ray cells in his successive drawings of cambial cells in tangential sections. We modified his diagram, using ray cells as markers of location, to find out some more details of cell rearrangement at the time of splitting (Fig. 6). Superimposition of drawings before and after the intrusive growth has taken place (A+C) reveals that around the area of fusiform initial’s intrusive growth the radial walls of the neighbouring ray cells did not change their location. Some partially eliminated ray initials can be seen on the pathway of intrusively growing fusiform initials, which could be described as the ray cells with decreased tangential surface. We realized that besides the fully eliminated ray initial, which was shaded by Barghoorn, another ray initial, marked in Figure 6 of this paper with a star, was also eliminated. The ray initials did not change their location, although it would have been unavoidable if the intrusive growth had taken place between the radial walls. The actual situation presented here can be described as a replacement of two whole ray initials and fragments of many other ray initials, with the intrusively growing tip of the fusiform initial. This is possible only when the elongation of the fusiform initial takes place along the tangential surface. From this we can also conclude that intrusive growth and elimination of the initials,
two sides of the same process (Włoch et al. 2002; Kojs et al. 2004a), are associated with both ray initials and fusiform initials. Elimination of initial cells also results from successive unequal periclinal divisions and is recorded in the corresponding layers of the derivative cells. Significance of (a) intrusive growth between tangential walls of neighbouring cells and (b) unequal periclinal divisions, in relation to elimination of the initials, has been discussed by Kojs et al. (2004a, b), Jura et al. (2006), Włoch et al. (2009) and Karczewska et al. (2009).

Rearrangement of rays due to the intrusion of fusiform initials into ray bodies has been observed by several authors (Braun 1955; Evert 1961; Krawczyszn 1971; Savidge & Farrar 1984; Iqbal & Ghouse 1985, 1987, 1990; Rao 1988; Otegui 1994), but none of them located the growth on any specific wall of the growing initials, and consequently did not specify whether the growing cell tip intrudes between the radial or the tangential walls of the neighbouring cells. In the past, intrusive growth has been considered to occur between radial walls (Hejnowicz 1961, 1967), leading to the concept that the nonstoreyed cambium increases its circumference by means of intrusive growth and anticlinal divisions of the initials. Later, it was suggested that the elongating fusiform initial splits the ray by its intrusion between tangential walls of the ray cells (Myśkow & Zagórska-Marek 2004), but the mechanism of this event was not explained.

In the storeyed cambium, rearrangement of initials is related to a cyclic change in the orientation of fusiform initials, which does not affect the constant storey height and hence the overall storeyed pattern (Hejnowicz 1973; Hejnowicz & Zagórski-Marek 1974; Krawczyszyn & Romberger 1979; Włoch & Zagórska-Marek 1982; Włoch 1989). In fact, parallel to fusiform initials, the ray initials also undergo readjustment (Krawczyszyn & Romberger 1979), but this correlation often went unnoticed. The only assertion was that the rays change their inclination together with fusiform initials, and

![Figure 6](image-url)
often unite or split on storey borders (Włoch 1987; Włoch & Szendera 1989). In fact, the rearrangement of cambial initials, including the fusion and splitting of rays, comes in place of intrusion of fusiform initials between tangential walls of the neighbouring initial and its closest derivative. This occurs without any increase in the cambial surface, as the growing initial does not push the neighbouring initials aside.

In species with storeyed cambium there is a strong correlation between the ray width and the tendency for double-storeyed pattern. Some species of Sterculiaceae and Fabaceae are known to have fusiform initials and low and narrow rays arranged in storeys, while the large wide rays, also occurring in these woods, are nonstoreyed. *Tilia cordata* has also diverse types of ray, ranging from uniseriate to multiseriate (more than 10 cells in width), and hence the tendency for double-storeyed pattern is weak. A distinct double-storeyed pattern is often observed in species with narrow, mostly biseriate and rarely triseriate or tetraseriate rays (Włoch & Szendera 1989).

In the cambium with a regular double-storeyed pattern, rearrangement of rays occurs in accordance with the change in the fusiform-initial inclination (Hejnowicz & Zagórska-Marek 1974; Włoch 1987; Włoch & Szendera 1989; Włoch & Wawrzyniak 1990). Formation of the double-storeyed pattern is related to the axial shift of rays, which may involve loss of the ‘initial’ status of a ray cell located at one end of the ray (elimination of cell caused by intrusively-growing fusiform initial) and addition of a new ray initial, as a result of anticlinal division, at the opposite end of the ray (Kojs et al. 2004b; Myśkow & Zagórska-Marek 2004). Ray height in double-storeyed cambium usually does not exceed the height of storeys; these rays are mostly uniseriate, as in *Dalbergia melanoxylon* (Ghouse & Yunus 1975), *Pterocarpus soyauxii* (unpublished data) and other species of tropical rainforests, to tri- and tetraseriate, as in *Millettia laurentii* (Wagenführ & Scheiber 1974), *Dalbergia lanceolaria* (Ghouse & Yunus 1975), *Nesogordonia papaverifera* (Carlquist 1988) and *Lonchocarpus sericeus* (Kojs et al. 2004; Jura-Morawiec et al. 2008).

The origin of new rays in double-storeyed cambium is regarded to be the result of highly synchronized transverse anticlinal divisions of fusiform initials; the first anticlinal division occurs in the central part of the fusiform initial, resulting in two equal ‘sister initials’ (Barghoorn 1940a, b; Philipson et al. 1971), which keep dividing similarly, forming a ray composed of 4–8 ray initials. Another way of ray origination was described in *Aeschynomene virginica* (Cumbee 1984), where one of the sister cells, after the first transverse anticlinal division, maintains the status of the fusiform initial, while the other further divides anticlinally transversely, giving rise to a ray.

Ray initials divide periclinally near fusiform initials (Wodzicki & Brown 1973), and the new tangential walls are accessible for intrusive growth between these walls. The examples described above clearly indicate that rays are not an obstacle for intrusively growing fusiform initials. This is evident from the unique ray anatomy near the cambial initial surface, where the tangential walls of neighbouring ray initials are close to each other. The mechanism of cambial-initial rearrangement is the same both in fusiform and ray initials; *i. e.* through the occurrence of intrusive growth of the cambial initials between the tangential walls of the adjacent fusiform/ray initials and their closest derivatives.
REFERENCES


Barghoorn Jr, E.S. 1940b. The ontogenetic development and phylogenetic specialization of rays in the xylem of dicotyledons. I. The primitive ray structure. Amer. J. Bot. 27: 918–928.


