SEASONAL CHANGES IN THE DEGREE OF SYMPLASMIC CONTINUITY BETWEEN THE CELLS OF CAMBIAL REGION OF *ACER PSEUDOPLATANUS* AND *ULMUS MINOR*

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ABSTRACT

The presence of symplasmic isolation and symplasmic continuity which are functional aspects of cell-to-cell communication, had been studied in cambium of *Acer pseudoplatanus* and *Ulmus minor*, with hope that uniqueness of this meristem, exemplified by its morphology and seasonal variations in its activity is also manifested in differences in the efficiency of communication between cambial cells during the year. The degree of symplasmic continuity was estimated by loading the fluorescent symplasmic tracer to the stem and following its distribution in a population of cambial cells observed on tangential, transverse and radial sections. In active cambium the tracer did not enter the rays. This suggested that the ray and fusiform cells, growing and dividing intensively at different rates were specifically isolated from each other. In the state of dormancy the tracer was present also in the rays implying continuity between the two types of cambial cells. Temporal restriction in tracer spreading from secondary xylem to cambial region was observed on transverse sections in both physiological states of the meristem. Higher degree of symplasmic isolation in active cambium is, most probably, associated with functional distinctiveness of ray and fusiform cells. We hypothesize further that the symplasmic continuity in dormant cambium results from the open conformation states of plasmodesmata, because the energy costs of these states are low. It is reasonable strategy when cambial cells do not divide and maintenance of their functional individuality is not necessary.

KEY WORDS: symplasmic isolation, symplasmic continuity, plasmodesmata, symplasmic tracers, cambium, seasonal changes, *Acer pseudoplatanus*, *Ulmus minor.*

INTRODUCTION

Symplasmic continuity and symplasmic isolation are key features of cell-to-cell communication in plants. Symplasmic continuity is maintained via plasmodesmata (PD; Strasburger 1901). These are highly complex structures forming cytoplasmic intercellular connections across the cell wall that facilitate intercellular transport (Robards 1976). Plasmodesmata have been observed either closed or open or dilated and these conformational states are thought to reflect their transport capabilities (Zambryski and Crawford 2000). When PD are open, cell-to-cell transport of small molecules that are below the size exclusion limit (SEL: for definition see Goodwin 1983) is possible. Macromolecules, such as e. g. nucleic acids and proteins can be transported by dilated PD (Ding et al. 1992; Nakajima et al. 2001). Finally, there is no intercellular symplasmic transport when PD are closed (Rinne et al. 2001). In this way the conformation states of PD contribute to the regulation of intercellular trafficking and determination of symplasmic isolation or symplasmic continuity. Closed PD restrict symplasmic cell-to-cell communication causing isolation of individual cells or groups of cells (Erwee and Goodwin 1985) creating symplasmic fields and domains (Rinne and van der Schoot 1998; Ehlers et al. 1999). Such fields and domains are isolated from their surroundings, but the cells within a field or domain remain in continuity via open PD. Boundaries of symplasmic fields change spatially and temporally resulting in subsequent emergence and disappearance of the field, depending on environmental factors. Boundaries of symplasmic domains are constant and not affected by environmental factors (Gisel et al. 1999).

Symplasmic continuity is observed between mature cells as well as between the cells of undifferentiated tissues and organs. The cells performing different functions or entering different pathways of differentiation are often symplasmically isolated (Ehlers et al. 1999). The phenomenon of symplasmic isolation is universal in plant organisms. It has been detected during development of cotton fibres (Ruan

et. al. 2001; Ruan et al. 2004), and Characeae antheridia (Kwiatkowska and Maszewski 1986), in callus of grasses cultured *in vitro* (Ehlers et al. 1999), in sieve element and companion cell complex (van Bel and Kempers1990; Stadler et al. 2005b), in nodules of legume plants (Complainville et al. 2003) and during embryo development (Kim et al. 2002; Stadler et al. 2005a). Symplasmic isolation occurs in fully differentiated structures – for example between root hair and non-root hair cells in rhizodermis (Duckett et al. 1994) and between guard cells and subsidiary cells (Palevitz and Hepler 1985; Erwee et al.1985), but it is also present in meristematic tissues – in a shoot apical meristem, where it creates temporally and spatially changing symplasmic fields (Gisel et al. 1999; Rinne et al. 2001) and in cambial region, between ray and fusiform initials (van der Schoot and van Bel 1990).

Cambium, a lateral meristem producing secondary phloem and xylem, consists of two different types of cells: ray and fusiform. These cells differ in their morphology and function. In active cambium the ray and fusiform initials divide at different rates and produce derivatives that form a horizontal and vertical system of secondary tissues, respectively (for citations see Romberger et al. 1993, Larson 1994). Cambium shows seasonal changes in its activity; active cambial cells divide intensively, whereas in dormant cambium there are no cell divisions (Farrar and Evert 1997). The most interesting fact is that these two distinct types of cambial cells performing different functions are in contact one with another on the cambial surface, yet the data from literature indicate that they are symplasmically isolated.

The question arises if this isolation is permanent or if it shows the seasonal dynamics similar to that observed by Rinne et al. (2001) in the different zones of shoot apical meristem? We addressed this problem by following the migration of fluorescent tracers in active and dormant cambium.

MATERIALS AND METHODS

Plant material

The presence of symplasmic isolation and symplasmic continuity was investigated in one-to-three year old branches of *Acer pseudoplatanus* L. and *Ulmus minor* Miller. collected from natural stands near Wroclaw, in Poland, during three successive years. The selected species exhibit contrasting patterns of seasonal cycle of cambial activity and dormancy. The activation of the cambial region in *A. pseudoplatanus* starts earlier than in *U. minor*, which allows studying cell-to-cell communication in these species as related to the activity status of the cambium rather than to the environmental conditions. The analysis of active cambium was conducted on the branches (n=7-12) sampled from May to July and kept at room temperature (ca. +22˚C) during experiments. To study the dormant cambia, the branches were collected from December to March and kept at ca. +4˚C during experiments to ensure that dormant state was sustained.

Loading of fluorescent tracers, sectioning and microscopy

To examine symplasmic transport between the cells, aqueous solutions of fluorescent tracers: fluorescein (FA; POCH S.A.) and carboxyfluorescein diacetate (CFDA; Fluka and Riedel-deHaën) were used. These dyes are considered to function as the symplasmic tracers, because they spread from cell to cell mainly in the symplast and their leaking to the apoplast is limited (Oparka 1991). The application methods differed for the two dyes as explained below.

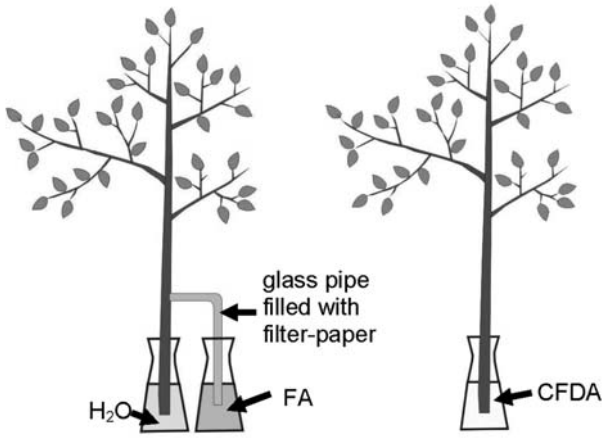


Fig. 1. Scheme of dye application to the branches in the experiments. *On the left*: loading of FA to the exposed tangential surface of secondary phloem; the branch is placed in water. *On the right*: loading of CFDA to the transverse cut surface, through the vascular system; the branch is immersed directly in working solution of the dye.

Fluorescein

0.3 mM FA in 10 mM PBS buffer (pH 6.7) after Gerlach (1972) was applied either through the transversally cut stem or through the exposed tangential surface of the secondary phloem (Fig. 1). In the first case the tracer had been loaded through the vascular system for 5 days. In all other experiments with FA the dye was applied through the exposed surface of the secondary phloem. A thin piece of bark, approximately 1 cm × 1 cm × 2 mm, containing periderm and external layers of the secondary phloem was removed leaving the conductive secondary phloem with at least one layer of phloem fibers intact. The exposed surface was covered by the filter paper dipped in FA solution. To ensure the continuous flow of the dye to the tissues, a glass pipe filled with FA solution was fastened to the application site as shown on Figure 1. Fluorescein was loaded to the branches for 2-4 days during active period and for 3-7 days during dormant period.

Carboxyfluorescein diacetate

An aqueous solution of CFDA was prepared by adding 15 µL of 1% (w/v) CFDA dissolved in acetone to 5 mL 10 mM PBS, pH 6.8 as described by Duckett et al. (1994) and Ruan et al. (2001) with modifications. In the symplast of living cells non-fluorescent and non-polar CFDA is cleaved by cytoplasmic esterases to produce carboxyfluorescein (CF), an impermeant symplasmic fluorescent probe (Goodall and Johnson 1982). The branch was cut and its basal end placed in CFDA solution (Fig. 1). Branches with dormant cambium were placed in CDFA for 7-14 days. Branches, with active cambium, were treated with the dye for 3--4 days, in day-time alternately with tap-water, each change for ca. 30 min, in night-time with tap-water only. Frequent changes of CFDA and water assured loading of small

doses of the tracer to the vascular system (Hukin et al. 2002) and guaranteed long-time dye uptake.

Distribution of the tracers in the stems was analyzed on tangential, transverse and radial hand-sections and on transverse cryo-sections made with a cryomicrotome with selenium rectifier (K TOS – II Medeksport, RSFSR). The sections were mounted in glycerine or in water and observed in epi-fluorescent microscope (Olympus BX50, Olympus Optical Co. Poland) in blue excitation light (470-490 nm). Images were taken with the analog-video-camera (DXC-950P/SONY) and sent directly to the graphic station INDY (Silicon Graphics, Inc.) or were made with digital camera (Camedia C-7070 Wide Zoom, Olympus Optical Co).

Permeability of FA and CFDA through the plasma membranes and their capacity to function as the symplasmic tracers were verified by placing the tangential and transverse sections of the cambial region directly into a specific dye for 5 minutes. After that, sections were rinsed with water and observed in fluorescent microscope. Some of the tangential and transverse sections were boiled for 15 minutes, and then stained with CFDA.

RESULTS

Verification of the symplasmic localization of the tracers

Both tracers when applied directly to tissue sections for 5 minutes were found in the symplast (Fig. 2). In the cambial region, CFDA appeared to be the more selective symplasmic tracer than FA (data not shown). In the phloem, cambial and xylem regions, the tracers were visible in the symplast of live cells (Fig. 2C, E) and they were absent from the apoplast and the lumen of dead cells (Fig. 2C). Control pictures showed only cell wall and chlorophyll autofluorescence (Fig. 2A, D). Sections boiled in water prior to staining with CFDA did not emit fluorescent signal.

Detection of FA staining in the wood forming tissue

Fluorescein entered cambium, both in active and dormant state, only when it was continuously applied to the exposed tangential surface of the secondary phloem (Table 1, Fig. 3A). From the application site the tracer migrated symplasmically but also apoplastically (Fig. 3A, C, D). In the symplast the dye was spreading, at first horizontally, mainly along the phloem and xylem rays and then vertically, mostly upward, up to 15 cm from the application site as schematically shown on Figure 4. Basipetal transport was limited in the cambial and phloem regions, as the dye migrated down only 2 cm below the place of its application. Tracer movement from the rays out was also limited. FA presence in the cell walls of phloem fibers or xylem elements (Fig. 3A, C, D) suggested simultaneous migration of the tracer in apoplast. The movement was generally faster in spring and in the summer than in winter. Because of slower dye spreading in dormant cambium it was necessary to load the branches for seven days to obtain well labelled cambial region

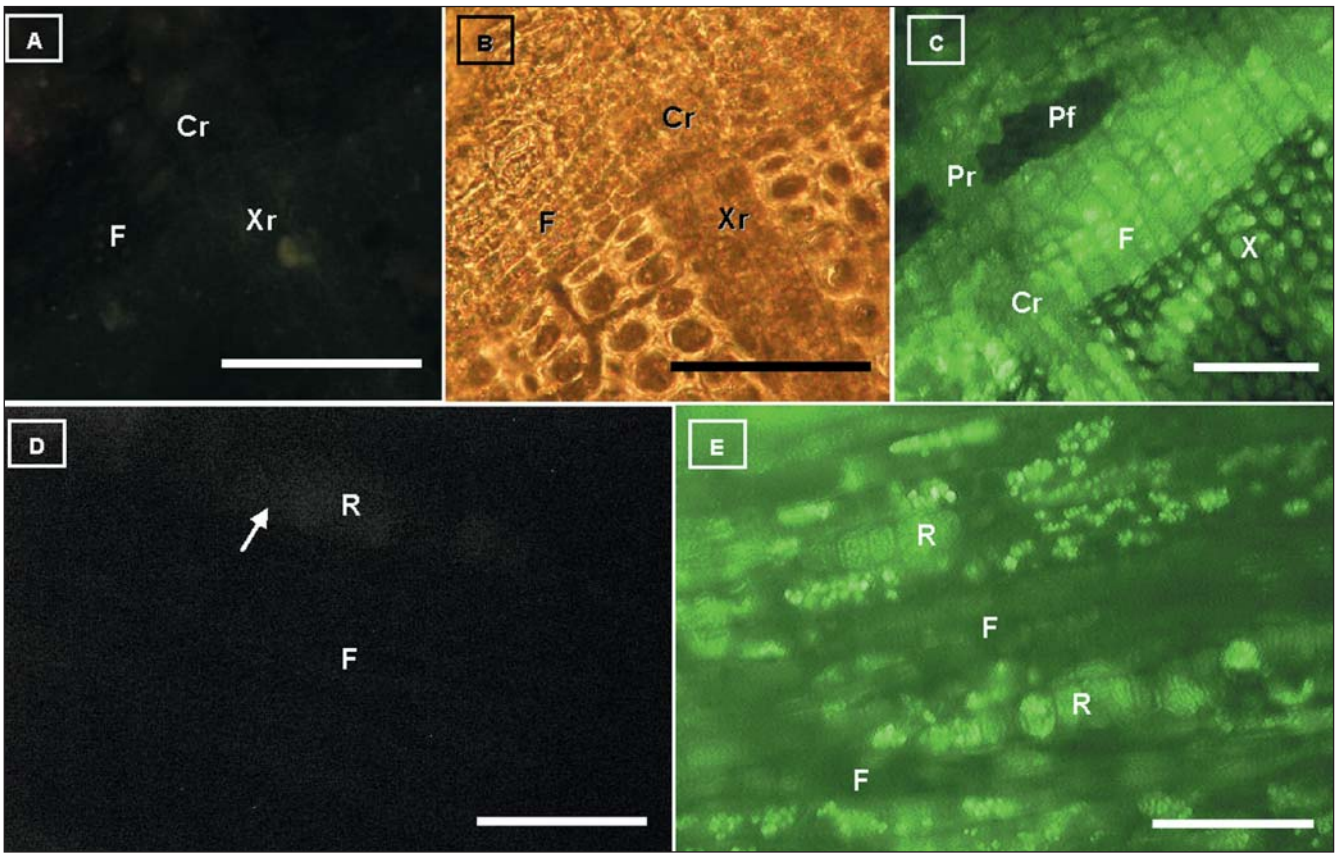


Fig. 2. Control staining showing the capacity of CFDA (A-C) and FA (D, E) to function as the symplasmic tracers in woody stems. Sections of *A. pseudoplatanus* stem were immersed directly in dye solution. (A-C) The fluorescent signal comes from live cells in the regions of phloem, cambium and xylem (C), shown on transverse sections. It is absent in the apoplast and in the lumen of dead cells (exemplified by phloem fibers). (B) The same as (A) showing topology of the region, made in white transmitted light. (D, E) In the cambial region viewed on tangential section the dye is present in the symplast of ray and fusiform cambial cells (E). In controls (A, D) only the autofluorescence of primary and secondary cell walls is visible (arrow). **F** – fusiform cell, **R** – ray cell, **X** – xylem region, **Cr** – cambial ray, **Xr** – xylem ray, **Pr** – phloem ray, **Pf** – phloem fibers. All sections (except B) were excited by a blue light. Scale bar: 50 µm.

TABLE 1. Distribution of FA and CF in the cambial region after application: to the secondary phloem surface (1) or to the secondary xylem surface (2) during the active and dormant stage of growth.

	FA	CF
1	Symplast of the phloem and cambial cells	? (technical problems)
2	No symplasmic staining	Symplast of the xylem, the cambium and the phloem

cells. FA spreading from the secondary phloem to the cambial region was never restricted (Fig. 3A). Attempts to load FA to the symplast through the cut end of the branches were unsuccessful, because they resulted in the fluorescent signal present mainly in the apoplast of xylem elements (Table 1).

Detection of CF signal in the symplast

Carboxyfluorescein diacetate was applied to the branches only through the vascular system. The tracer was also absorbed by the thin layer of secondary phloem, but this route of dye movement was ineffective. In the lower parts of the stem, the dye was transported upward in secondary xylem, both apoplastically in system of cell walls as non-fluorescent form – CFDA and symplasmically in axial parenchyma as visible, fluorescent signal – CF (Fig. 4). Few centimetres above the level of dye application (approximately 3 cm for dormant state, and 6 cm for active one), in addition to the continued acropetal movement, CF started appearing in the xylem rays (Fig. 4, 5A, B, D), and after 2-3 days of tracer loading, it showed up in the cambial region (Fig. 4, 5C, E). Unfortunately application of CFDA for few days caused partial breakdown of the tracer, which means that loaded solution was progressively becoming a mixture of CFDA and CF. Partial decomposition of CFDA caused the presence of fluorescent signal not only in the symplast of living cells, but also in the cell walls of secondary xylem (Fig. 5E). In winter, transport of the dye in the xylem sap was possible, due to the presence of contact cells, known to induce the water movement during dormancy (Sauter et al. 1973)

Because of low stability of CFDA and the necessity of frequent changes of its solution, the tracer was not applied to the exposed tangential surface of the phloem, like FA (Table 1). Carboxyfluorescein could enter the cambial region through the symplast of phloem cells but with the application through the cut branch end was simpler and less invasive. Moreover, loading through the xylem allowed using thinner and younger branches and assured long-distance transport of the tracer.

Temporal isolation of the cambial region from secondary xylem

The route of CF migration from the vascular system to the cambial region was investigated on cross-sections. Initially

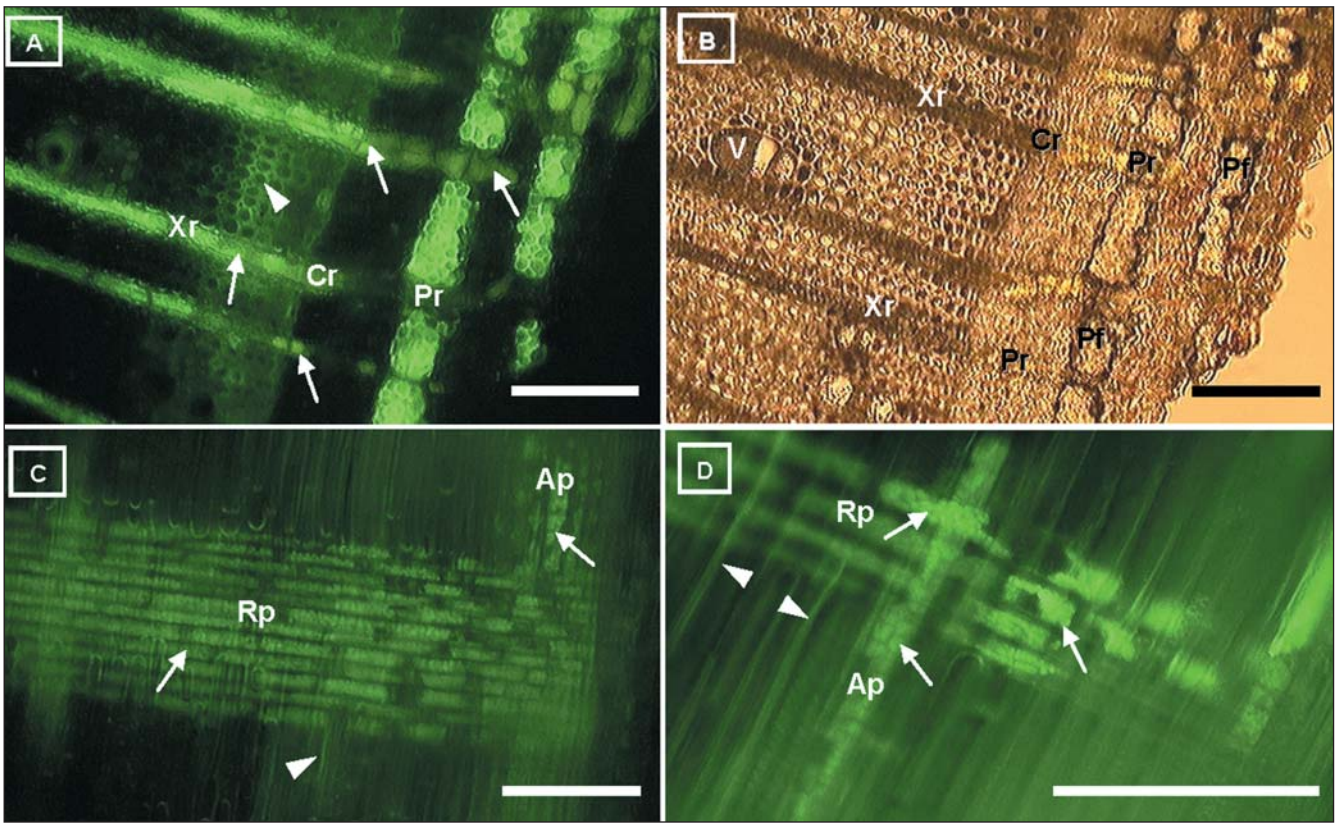


Fig. 3. Distribution of FA throughout the dormant stem of *A. pseudoplatanus* as seen on transverse (A-B) and radial (C-D) handsections made at the site of dye application to the phloem. Fluorescein is present mainly in the symplast (arrows), but also in the apoplast (arrowheads). (A) Strong fluorescent signal in ray cells is continuous through the phloem, cambium and xylem suggesting a quick movement of the tracer in the symplast of rays. In the apoplast an intensity of the signal is gradually decreasing – it is strong in the cell walls of phloem fibers – close to the site of dye application and much weaker in the walls of secondary xylem elements. (B) The same fragment of the stem (as in A) shown in white transmitted light. Details of the bark and xylem region morphology can be seen. (C) Transport of FA in radial and axial wood parenchyma system. Cell walls of the xylem elements show weak fluorescence. (D) Magnification of (C) showing details of stained symplast of axial and radial parenchyma cells and fluorescence in the cell wall. **Pr** – phloem ray, **Cr** – cambial ray, **Xr** – xylem ray, **Pf** – phloem fibers, **Ap** – axial parenchyma, **Rp** – radial parenchyma, **V** – vessel. All sections (except B) were excited by a blue light. Scale bar: 100 µm.

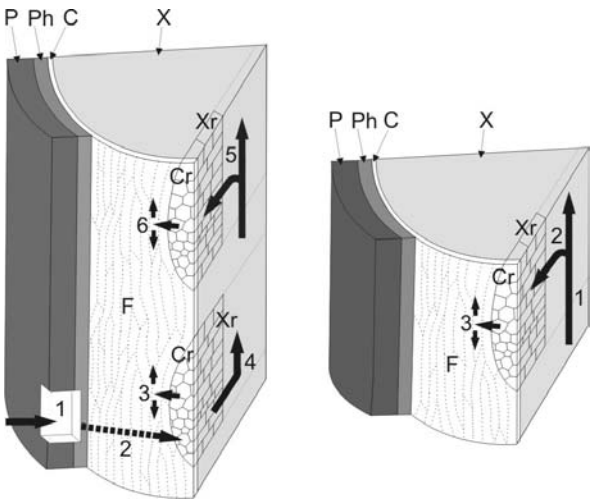


Fig. 4. Dye movement in tissues of woody stems of tree branches used in the experiments. *On the left*: application of the dye (FA) to the exposed tangential surface of secondary phloem. After removing the thin piece of the bark, the empty space (1) is filled with filter paper absorbing FA (for details see M&M). The tracer transported both symplasmically – from phloem rays to cambial rays and further to xylem rays – and apoplastically – in the system of phloem, cambial and xylem cell walls – (2) is laterally spreading in a cambial region from cambial ray cells to the adjoining fusiform cells and then, axially in the symplast of fusiform cells (3). From symplast of xylem rays the dye migrates to axial parenchyma of secondary xylem, and then upward in the transpiration stream (4). From the parenchyma the tracer keeps entering xylem rays where it moves radially backward to the cambial zone (5). Here (6) it migrates further as in (3). *On the right*: application of the dye (CF) to the exposed bottom surface of the branch cut transversally. The tracer transported acropetally within the symplast of axial parenchyma xylem cells as CF, and within the apoplast of tracheary elements as CFDA (1) enters the xylem rays (2) and from there it migrates horizontally to cambial zone. From cambial ray cells it is spreading to fusiform cells and then in axial direction (3). **P** – periderm, **Ph** – secondary phloem, **C** – cambial region, **X** – secondary xylem, **Xr** – xylem ray, **F** – fusiform cell, **Cr** – cambial ray.

the dye did not enter cambium from xylem rays showing clearly that these tissues were isolated from each other (Fig. 5A, B, D). To check out whether the observed barrier was permanent, we prolonged loading the tracer for 3-4 days in actively growing and for 7-14 days in dormant branches. This showed that the isolation was temporal. After long-time loading the signal appeared in cambium (Fig. 5C, E). Initially CF showed up in cambial rays, and from there it migrated symplasmically both in radial direction to the phloem ray cells and in tangential direction to fusiform cambial cells. Within the system of neighbouring fusiform cells, the dye was transported axially (Fig. 4). In the cambial region it was remarkably long-distance transport reaching even 80 cm above the place of dye application in active state.

In dormant cambium the strong signal was present in almost all cambial and phloem rays, viewed on cross-sections (Fig. 5C). In active cambium in most of its rays the CF signal was absent, or very weak. At the same time the strong signal occurred in the xylem and, sometimes, in the phloem rays (Fig. 5E).

The fusiform cells, longitudinally elongated (Larson 1994), were always cut open in transverse cryo-sections, both in active and dormant cambium. This is why they were depleted of the fluorescent signal (Fig. 3A, 5A, C, E). The ray cells with their short vertical dimensions (Wodzicki and Brown 1973; Larson 1994) remained mostly intact on relatively thick transverse sections (Fig. 3A, 5A, C, E).

Symplasmic grades of the rays – evaluation of symplasmic isolation/continuity

The presence of symplasmic continuity or symplasmic isolation of the cambial rays was examined based on the presence of fluorescent tracer in the ray cells of the cambium as seen on the tangential sections. Only these rays that were entirely surrounded by fusiform cells filled with the fluorescent dye were analyzed (which guaranteed that the cells were intact) and the percentage of the ray area showing a fluorescent signal was determined. Each cambial ray was classified into one of the eight arbitrarily established symplasmic grades (A-H; Table 2). The symplasmic grades of more than 800 and almost 150 rays in active and dormant cambium respectively of *A. pseudoplatanus* and about 200 rays in each cambium state of *U. minor* were analysed on tangential sections. The region of cambium was recognized by the uniform shape of the fusiform cells (initials and their closest undifferentiated derivatives), particularly, by the presence of the primary pit fields in radial walls (Romberger et al. 1993).

In the active cambium, the spreading of fluorescent tracer to the cambial rays was restricted. On the tangential sections the dye was almost always present in the fusiform cells whereas it occurred rarely in the rays (Fig. 6A-D, 7). Frequently either the entire ray was depleted of the signal (Fig. 6A) or the signal was present in a few cells (Fig. 6C, 7). The C, D and E symplasmic grades (each present in about 20-30% of all analyzed rays in *Ulmus* and in 15-20% in *Acer*) occurred most frequently (Fig. 7).

In the dormant cambium, the fluorescent tracer was present both in the rays and in fusiform cells suggesting unrestricted migration of the dye between both types of cambial cells (Fig 6E, F). Quantitatively the high level of symplasmic continuity was indicated by the presence of F, G and H symplasmic grades in both species (Fig. 7). A and B types of the symplasmic grades were never observed in dormant cambium (Fig. 7).

Seasonal changes in radial dimensions of cambial cells

Radial dimensions of the ray and fusiform cells in the cambial region were changing significantly throughout the year (*t*-test, 0.05 significance level). Especially, there was a striking difference between active and dormant cambium in radial dimensions of both the ray cells (*t*-test 9.77) and fusiform cells (*t*-test 3.77). In active state ray cells were almost twice as long as in dormant state (Table 3). Such an increase of radial dimension was also observed in fusiform cells, but in this case it was less pronounced. For technical reasons sections obtained from dormant cambium were always thinner, having about 30-40 μm, than those prepared from the active one (average 40-50 μm). These facts together suggest that even in the thinnest tangential hand-sections of both active and dormant cambium, the absence of the fluorescent signal in the ray cells is not an artefact, resulting from the cell rupture and escape of the tracer due to sectioning.

DISCUSSION

The presence of symplasmic continuity and symplasmic isolation has been studied intensively in many plant tissues and plant species (Erwee and Goodwin 1985; Duckett et al.

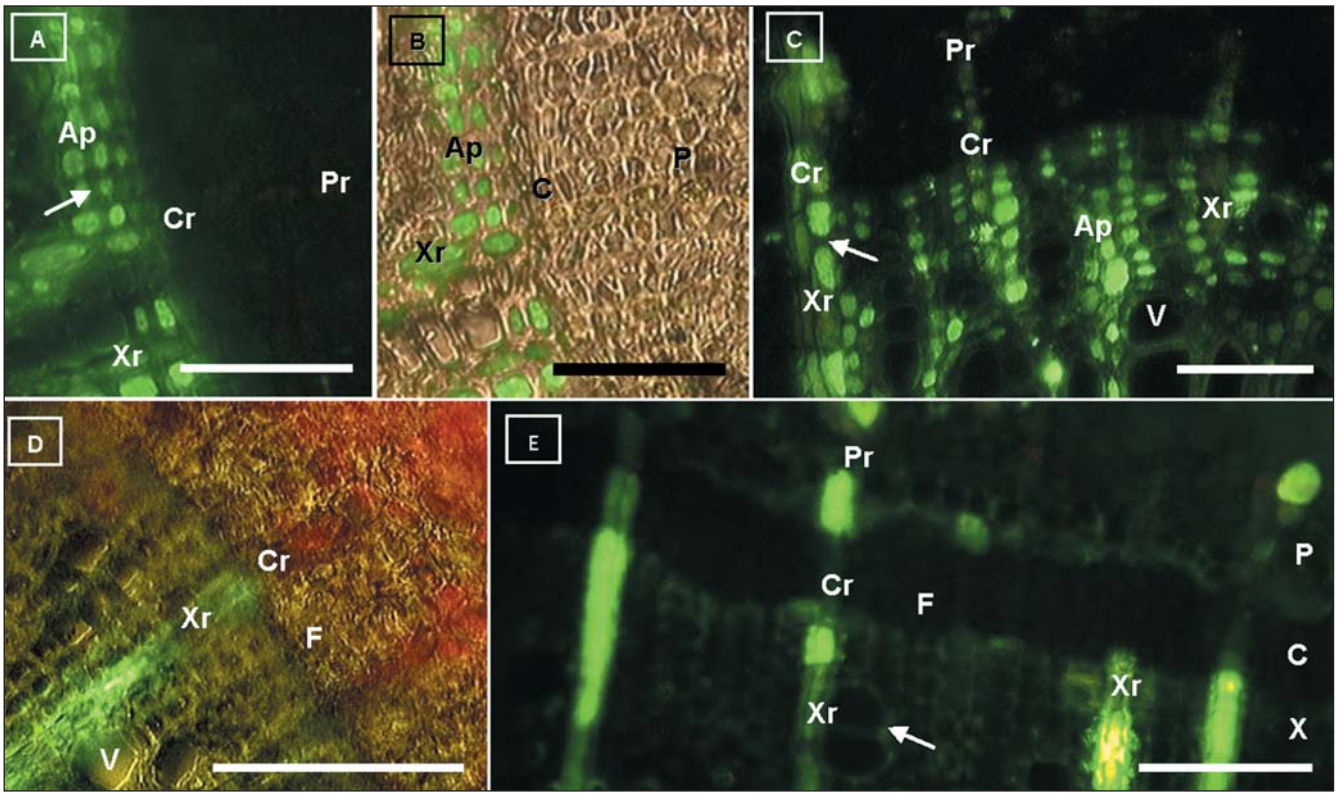
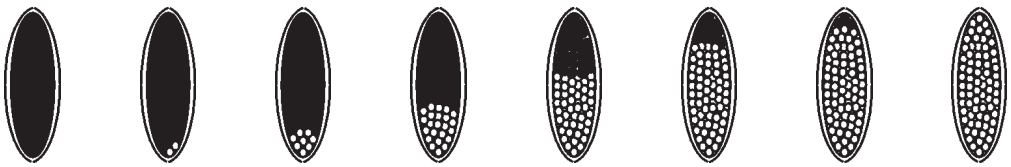


Fig. 5. Distribution of CF throughout the stems of *A. pseudoplatanus* shown on transverse sections made from upper parts of the branches (approximately from 10 to 20 cm above the place of dye application) with dormant (A-C) and active (D, E) cambium. Symplasmic isolation between cambial and xylem regions present within a short-time period of CFDA loading (A, B, D), becomes broken after 2-3 days of dye application (C, E) to the bottom transverse surface of the branch. (A, B, D) The tracer is present only in the symplast of axial and/or radial parenchyma cells and does not enter the cambial region where only autofluorescence is visible. (C, E) The dye enters dormant (C) and active (E) cambium from xylem rays. In dormant branches CF is present in the whole xylem and in the symplast of cambial and phloem rays. In active state intensive fluorescent signal occurs in the symplast of rays of secondary xylem and phloem, it is clearly weaker in cambial rays. Some cambial rays do not even show the presence of the tracer. Dyes were excited by blue light. In B and D, the white transmitted light was used simultaneously with the blue epifluorescence to show the non-fluorescent part of the section. Fluorescent signal in the apoplast (E) results from partial decomposition of the tracer. **X** – secondary xylem, **C** – cambial region, **P** – secondary phloem, **Xr** – xylem ray, **Cr** – cambial ray, **Pr** – phloem ray, **F** – fusiform cells, **Ap** – axial parenchyma, **V** – vessel, **arrow** – cell wall. Scale bar – 100 μm (C, F).

TABLE 2. Classification of the symplasmic grades of the rays in cambium. Schematic drawings of the rays, classified into eight symplasmic grades (A-H), depending on the size of the ray area filled with the dye (expressed in percents below each drawing). The absence of the fluorescent tracer in the ray cell is shown with black, its presence with white. Symplasmic isolation is represented by A, B and C symplasmic grades, and symplasmic continuity by F, G, H grades. D and E grades represent the state between the symplasmic isolation and continuity.

	A	B	C	D	E	F	G	H
Types of the symplasmic grades								
Ray area showing dye's presence	0	10	20	40	60	80	90	100

1994; van Bel and Kempers 1990; Rinne and van der Shoot 1998; Kim et al. 2002; Complainville et al. 2003; Ruan et al. 2004; Stadler et al. 2005b). However, these phenomena are poorly known and understood in cambium. In one of the first attempts van der Schoot and van Bel (1990) have performed iontophoretic injection of the dye to the cambial cells, but they went across a lot of technical problems with tracer loading. Additionally their results have not clearly explained the problem of symplasmic communication in cambium. The cambial region poses a true challenge for the symplasmic dye application, because it is an internal tissue compressed between secondary xylem and phloem. Perhaps this is why the problem of symplasmic continuity and symplasmic isolation in the cambium has not been elucidated.

Our analysis of fluorescent signal spreading in cambial region revealed seasonal changes in the degree of symplasmic continuity between two types of cambial cells (Fig. 6). In dormant cambium, the fluorescent symplasmic tracers were present in both ray and fusiform cells, suggesting

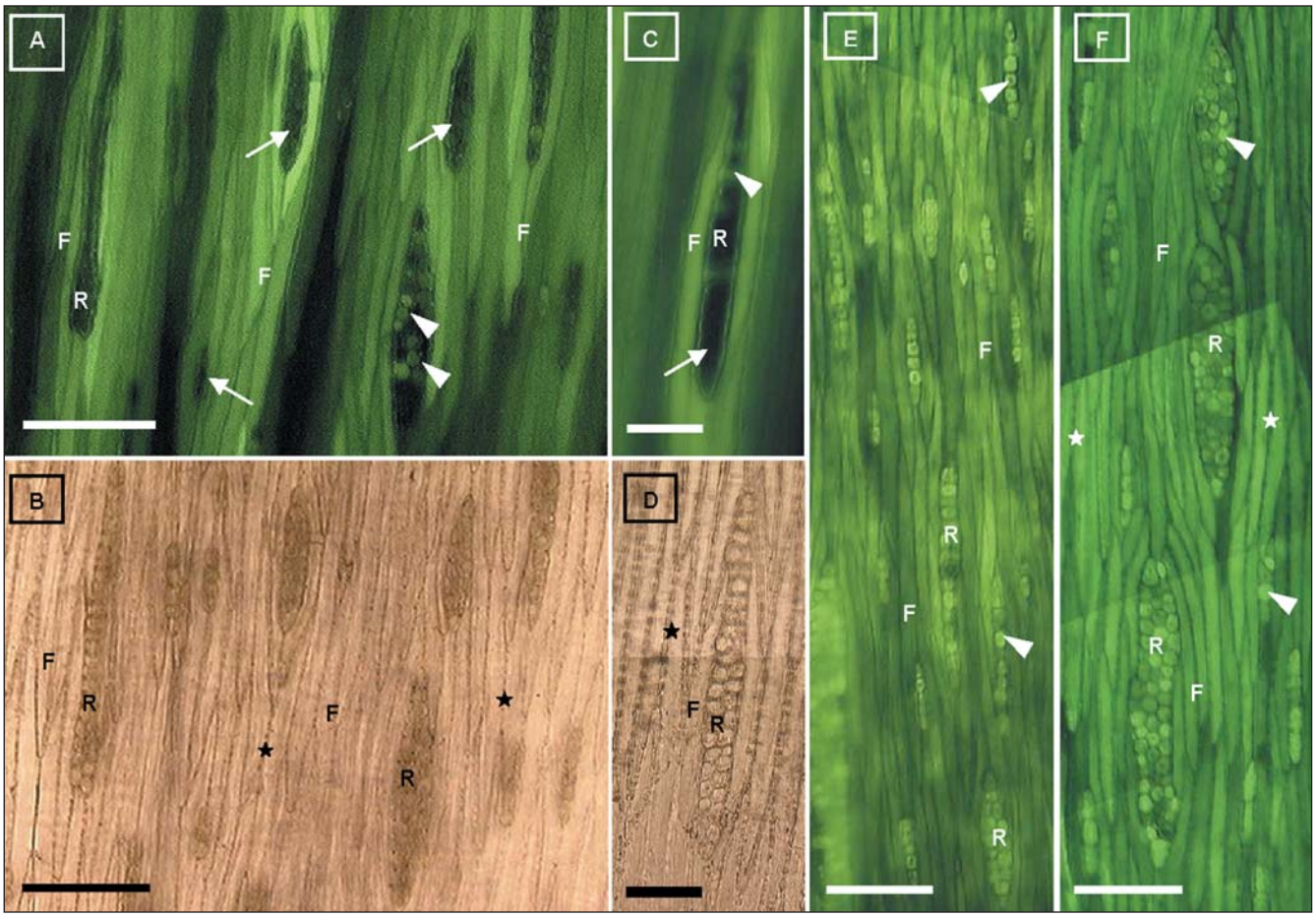


Fig. 6. Seasonal changes in the degree of symplasmic continuity between ray and fusiform cells in cambial region. *On the left*: symplasmic isolation of the rays in active cambium of *A. pseudoplatanus* (A-D). Two fragments (A, B and C, D) of the cambium surface viewed in blue light showing fluorescence of FA (A, C) and in white transmitted light (B, D). Spreading of the symplasmic tracer between fusiform and ray cells is restricted. *On the right*: symplasmic continuity in dormant cambium of *A. pseudoplatanus* (E) and *U.minor* (F). The fluorescent signal of FA (observed in blue light) is present both in the ray and in the fusiform cells. **R** – cambial ray cell **F** – fusiform cell, **arrow** – ray cell without the tracer, **arrowhead** – ray cell containing the dye, **asterisks** – primary pit fields. Scale bar – 100 µm (A-B; E-F), 50 µm (C-D).

the presence of symplasmic continuity between them. In active cambium the tracer migration to the rays was strongly restricted. There are two possible explanations of this phenomenon. The first one suggests unidirectional symplasmic transport between cambial cells as a potential source of symplasmic isolation. The other one stresses the role of symplasmic isolation in maintenance of the functional distinctiveness of ray and fusiform cells in active cambium.

Asymmetry of radial and tangential symplasmic transport within the cambial region

After short-time loading of CFDA to the cut stem, the fluorescent signal occurred only in the symplast of secondary xylem, and was never observed in cambial cells (Fig. 5A, B, D). The absence of the tracer in the cambial region when loaded through the xylem system was previously observed by Gisel et al. (1999), Pradel et al. (1999) and Marek (2005, not published). However, which was never reported before, breaking of the cambial-xylem isolation was possible after long-time loading of CFDA (Fig. 5C, E). Surprisingly, such a delay in radial transport has not been observed when the tracer was loaded through the surface of secondary phloem and was migrating centripetally from phloem rays to the cambial and xylem rays (Fig. 3A). Similar experiments analysing the routes of symplasmic tracers spreading throughout the stem were described previously by van der Schoot and van Bel (1990). They found that the primary xylem tissues were symplasmically isolated from the enclosed pith cells. Thus, centrifugal symplasmic transport to the xylem region was restricted. Radial transport from the bark, through the cambial region to the xylem rays was possible, although in most cases spreading of CF or Lucifer Yellow (LYCH) was halted at the border of cambial region, where the tracers were rapidly transferred particularly in axial direction (van der Schoot and van Bel 1990).

Our findings (Fig. 4) are in agreement with these results. Temporal isolation of cambial and xylem regions clearly depended on the direction of the tracer's horizontal movement in the stem. **Centripetal transport from the phloem and cambial rays to the xylem region proceeded along favourable route of radial transfer and consequently, dye spreading from the phloem side was not limited** (Fig. 3A). Conversely, **migrating of CF from the xylem to the cambial region occurred in centrifugal, unfavourable direction, causing temporal restriction of this transport** (Fig. 5A, B, D).

It is possible that also the presence of symplasmic isolation in active cambium is connected with the asymmetry of radial symplasmic transport through the xylem, cambium and phloem. Chaffey and Barlow (2001) visualized the three-dimensional symplasmic pathway in the stem, consisting of the

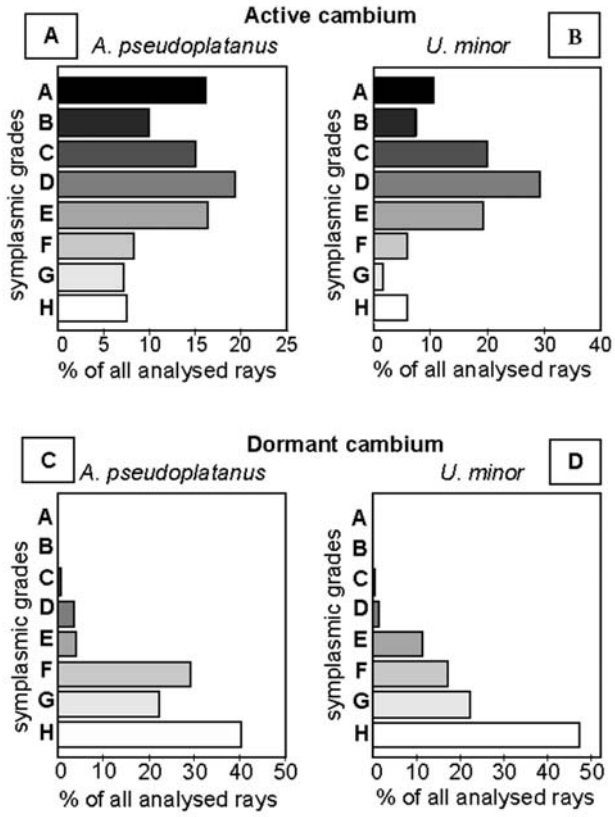


Fig. 7. Comparison of symplasmic continuity and symplasmic isolation in active and dormant cambium of *A. pseudoplatanus* and *U. minor*. The percentage of analysed rays, belonging to different categories of symplasmic grades are shown in the diagrams. The gradient of shades (black and dark grey referring to symplasmic isolation, white and light grey to symplasmic continuity) illustrates seasonal changes in the degree of symplasmic continuity between cambial cells. **A-B**: Symplasmic isolation in active cambium of *A. pseudoplatanus* (A) and *U. minor* (B). **C-D**: Symplasmic continuity in dormant cambium of *A. pseudoplatanus* (C) and *U. minor* (D).

TABLE 3. Seasonal changes of average radial dimensions [µm] of ray and fusiform cells in investigated species examined on the cross-sections.

	Active cambium		Dormant cambium	
	Fusiform cells	Ray cells	Fusiform cells	Ray cells
Acer pseudoplatanus	5.96±1.48	19.1±4.8	3.66±0.88	9.76±3.55
Ulmus minor	6.96±1.49	23.56±5.8	4.09±1.04	11.66±4.1

The mean thickness of the cells is given, ± standard deviation

long-lived rays and axial parenchyma cells, extending from the secondary phloem through the cambial region to the secondary xylem. Xylem rays transfer water and mineral nutrients to the actively dividing cambial cells, whereas phloem cells deliver photosynthates from the phloem (Ziegler 1964; van der Schoot and van Bel 1989; van Bel 1990; Barnett 2006). The high PD density in tangential cell walls between ray cells (Goosen-de Roo 1981; Farrar 1995, unpublished), radial arrangement of microtubules and microfilaments in ray cells (Chaffey and Barlow 2001), radial translocation rate of sugar in poplar (Sauter and Kloth 1986) and results obtained from dye-coupling of the cells in tomato stem (van der Schoot and van Bel 1990) support the idea that the main symplasmic transport occurs through the ray cells in radial direction (Sauter and Kloth 1986). Medium density of PD on radial walls between ray/fusiform cell, fusiform/fusiform cell and as well as between ray/ray cell (Farrar 1995, unpublished) suggests, that the substances from ray cells may move toward to the fusiform initials, but this is not a favourable direction of the transport.

Similarly it can be expected that the symplasmic tracers and another substances, with similar effective Stokes radius, are transported along the rays from xylem and phloem region to the cambium and, there tangentially, from ray to fusiform cells (Fig. 4). Results obtained from transverse sections of active cambium, where strong fluorescent signal was present in xylem and phloem rays while disappearing in cambial ray cells (Fig. 5E), supports this proposition. Possibly, **the isolation of cambial cells observed on tangential surface of active cambium results from rapid radial transport of the tracer between the ray cells and one-way transport from ray to fusiform cells** (Fig. 4). Tangential unidirectional symplasmic transport is not unusual in plants. It has been documented in tomato wood, where the dye moved from xylem rays to the adjacent fibres (van der Schoot and van Bel 1990) and in root apical meristem (Tirlapur and König 1999). If indeed, as we postulate, the transport in active cambium is unidirectional, then, fluorescent tracers transported along the rays to cambium and unloaded there to the fusiform cells migrate only between fusiform cells because they can not move back to the ray cells. This produces the effect of symplasmic isolation of ray cells from fusiform cells. Nevertheless, in some cases, ray isolation in active cambium may be result of rapid radial transport between cambial cells. In the situation when the tracer is still present at least in some ray cells, the ray viewed on tangential surface is partially filled with the dye. When the tracer has already been unloaded from all ray cells to the fusiform cells, on tangential view the ray lacks of the fluorescent signal and seems to be symplasmically isolated from fusiform cells.

In dormant cambium, the tracer is spreading freely, penetrating slowly all cambial cells, suggesting symplasmic continuity between them. Possibly, in this case, the symplasmic transport occurs in both directions.

Symplasmic isolation assures functional distinctiveness of ray and fusiform cells in active cambium

In active state, when the cambial cells proliferate intensively, two types of the cells: ray and fusiform seem to be symplasmically isolated from each other. It is possible that this isolation is required for maintenance of functional identity by each of the cambial cells. Functional isolation allows them to grow and divide at different rates, leading in consequence to the formation of distinct radial and axial systems of the secondary tissues. In dormant cambium, ray and fusiform initials do not divide, and functional distinction between them is not necessary. This is probably why all cambial cells may be symplasmically connected. Similar dynamically changing symplasmic isolation was shown previously for the shoot apical meristem (Rinne and van der Schoot 1998; Gisel et al. 1999). It was suggested that symplasmic isolation guaranteed in this case the mainte-

nance of different functions by neighbouring cells which belong to distinct symplasmic fields, resulting in production of various parts of the plant body (van der Shoot and Rinne 1999).

Process of changing the PD conformation states requires energy. The state of closed PD is the most energy-consuming, while the open state is less demanding (Cleland et al. 1994). If in dormant cambium the symplasmic isolation is not necessary and if the open conformation state needs lower input of energy, than the closed state, then it is logical to assume that the behaviour of the cambium is opportunistic: PD are open resulting in symplasmic continuity between ray and fusiform cells. In active cambium symplasmic isolation between ray and fusiform cells, dividing and growing at different rates (e.g. ray cells are longer than fusiform cells in radial direction), is a necessity, regardless the energy costs.

Final conclusions

Seasonal changes in the degree of symplasmic continuity in the cambial region represent an attractive example of possible regulation of the cellular processes in the woody plants. In active cambium rays are symplasmically isolated from the fusiform initials, whereas in dormant state all cambial initials are in symplasmic continuity. We are conscious that transport of the tracers occurs, partially, along the apoplastic route. But it does not make our most important finding invalid. The differences between active and dormant cambia are not artefact, because both the chemical properties of the dyes and the methodology of their application were always the same, regardless the cambial state. We discussed here two hypothetical explanations of this phenomenon. The first one suggests that horizontal symplasmic spreading through the rays as well as tangential unidirectional transport from rays to fusiform cells is responsible for the emergence of symplasmic isolation. The second one proposes that it is the functional distinctiveness of ray and fusiform initials in active cambium, which is a reflected in differences in dye spreading. In both hypotheses the crucial role perform PD. The conformation states of PD in the radial walls between ray and fusiform cells decide about the presence or absence of symplasmic isolation in active cambium. One-way transport, from ray to fusiform cells, occurring in active state of cambium, assures transfer of water, minerals and photosynthates from xylem and phloem tissues to actively dividing cambial cells. The restriction of the transport in opposite direction seems to guarantee the maintenance of functional distinctiveness of cambial cells.

Until now little is known about the efficiency of the symplasmic isolation in active cambium and its possible dynamics. The developed method of calculating the symplasmic grades allows us to address this problem in near future.

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