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SEASONAL ULTRASTRUCTURAL CHANGES IN THE CAMBIAL ZONE OF BEECH (*FAGUS SYLVATICA*) GROWN AT TWO DIFFERENT ALTITUDES*

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SUMMARY

Seasonal structural changes of cambial cells in mature beech (*Fagus sylvatica* L.) trees growing at elevations of 400 m a.s.l. (lowland) and 1200 m a.s.l. (mountains) are presented on the basis of light (LM) and electron microscopy (TEM). For LM, samples from trees were collected at weekly intervals and for TEM at two-month intervals from March till September, 2008. LM enabled us to follow the production of new xylem and phloem cells that lasted for 16.5 ± 3.7 weeks at the lowland site and for 10.7 ± 1.3 weeks in the mountains. TEM revealed differences in ultrastructure of cambial cells in the phases of dormancy, reactivation, activity and transition to dormancy. The seasonal patterns of ultrastructural changes in cambial cells were similar at both sites but their timing was different. TEM revealed changes in the fine structure of cambial cells, indicating their activation in spring and the earliest stages of cell divisions and development of new cell walls. When using LM, the onset of cambial activity could be observed one month later, compared with TEM. LM therefore enabled us to follow cambial productivity but not the activity and related cytoplasmic modifications during reactivation.

Key words: *Fagus sylvatica*, cambium, seasonal changes, light microscopy, transmission electron microscopy.

INTRODUCTION

The vascular cambium, as a secondary meristem, produces xylem and phloem and is responsible for self-maintenance and signal transfer via translocation of growth regulators (Larson 1994; Chaffey 1999; Rensing & Samuels 2004). Cambial activity depends on a complex of interactions among intrinsic and extrinsic factors, such as

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*) Dedicated to Prof. Dr. Josef Bauch on the occasion of his 75th birthday.

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phytohormones, DNA binding proteins, monolignols, nutrients, minerals, pressure, temperature, water potential gradients, photoperiod (Savidge 1996).

Cambial activity is seasonal in trees of temperate climatic regions (Evert 2006). Detailed studies of ultrastructural and biochemical changes in cambium related to its active or dormant state have been carried out in various tree species of the temperate zone, where divisions generally start in spring and continue until late summer or early autumn (Farrar & Evert 1997a, b; Rensing & Samuels 2004; Samuels *et al*. 2006).

In general, first divisions in spring occur at the end of a 1–4-week period characterised by changes in the vacuolar system following the resumption of cyclosis, *i.e*., elongation of the small vacuoles and their progressive fusion into one or two large vacuoles (Lachaud *et al*. 1999). Dividing cambial cells contain, among other elements, large vacuoles, rough ER (endoplasmic reticulum), numerous dictyosomes that produce vesicles and lack storage products, such as lipid droplets. The transition from activity to dormancy involves processes whereby large vacuoles fragment into a number of smaller ones, which intersperse throughout the cytoplasm. Rough ER is replaced by smooth ER, and an accumulation of storage products takes place, the nature of which depends on the species. Dictyosomes become fewer and mainly inactive. The main characteristic of dormant cambium is lack of cell divisions but metabolic activity may still continue if the weather conditions are favourable (Farrar & Evert 1997a, b; Lachaud *et al*. 1999; Rensing & Samuels 2004). However, studies have shown that changes in the ultrastructure are characteristic of the cambium zone in the temperate region. It has also been shown that the timing of ultrastructural changes differs among trees and growth sites.

In deciduous tree species, such studies have been performed on black locust (*Robinia pseudoacacia* L.) (Farrar & Evert 1997a, b), horse chestnut (*Aesculus hippocastanum* L.) (Barnett 1992) and willow (*Salix fragilis* L.) (Robards & Kidwai 1969). They have also been studied in beech (*Fagus sylvatica* L.), in which the ultrastructure of dormant cambium in young trees was mostly characterised by a smooth endoplasmic reticulum, lipid droplets, protein bodies and numerous small vacuoles (Kidwai & Robards 1969).

In recent years, studies of cambial dynamics have often been linked to wood formation studies (Schmitt *et al*. 2004; van der Werf *et al*. 2007; Seo *et al*. 2008; Gričar & Čufar 2008; Rossi *et al*. 2008; Makinen *et al*. 2008; Deslauriers *et al*. 2009; Oberhuber & Gruber 2010; Moser *et al*. 2010; Camarero *et al*. 2010; Rathgeber *et al*. 2010). In such cases, the morphology of cambial cells and newly formed xylem and phloem has mainly been observed by means of light microscopy (LM) for which several techniques and protocols have been developed (Chaffey 2002). Using LM, it has been agreed that reactivation is indicated by the initial appearance of newly formed xylem or phloem cells (*e.g*. Antonova & Stasova 1997; Deslauriers *et al*. 2003; Rossi *et al*. 2006b; Gričar 2007).

Due to different methodological approaches to observe the tissues, it has been emphasised that we need agreement for determining cambial activity and that the appearance of the first formed xylem cells is not optimal for determining the onset of cambial activity (Frankenstein *et al*. 2005). Frankenstein *et al*. (2005) confirmed that the most appropriate criterion for cambial reactivation is the onset of cell divisions, as already suggested by Farrar and Evert (1997b). However, even the definition of cell divisions

might vary if different preparation and observation techniques are used, such as light microscopy or transmission electron microscopy. Furthermore, the findings of wood formation in beech from various locations in Europe have shown that the dynamics of cambium in beech may vary with latitude and altitude (Schmitt *et al.* 2000; van der Werf *et al.* 2007; Čufar *et al.* 2008a).

In the current study, we analysed the various phases of activity or inactivity of the cambium zone in beech during the year. We used light microscopy and transmission electron microscopy with different fixation and embedding techniques, in order to determine their comparability and suitability for studying cambium phenology. Mature beech trees from sites with different climatic regimes were included in the study. The objectives were:

- to investigate seasonal changes in the cambial zone of beech (*Fagus sylvatica*) at the tissue and cellular levels in samples prepared by the common procedure for wood formation studies (fixation in ethanol-formalin-acetic acid, embedding in paraffin) and observation with a light microscope, and samples prepared for ultrastructural observations with transmission electron microscope (fixation with glutaraldehyde, paraformaldehyde, and osmium tetroxide and embedding in Spurr's epoxy resin), and
- to compare the results obtained at a low and a high elevation site with different climatic regimes.

MATERIALS AND METHODS

Study sites

We selected two forest sites in Slovenia with *Blechno fagetum* forest associations. The low elevation site, Panška reka (46° 00' N, 14° 40' W, 400 m a.s.l.), is located near Ljubljana. In 2008 it had a 1490-mm annual sum of precipitation and 11.6 °C average temperature. The high elevation site, Menina planina (46° 16' N, 14° 48' E, 1200 m a.s.l.), is located in the Alps. For 2008 the nearest climate station of the Environmental Agency of the Republic of Slovenia, Krvavec (46° 17' N, 14° 32' E, 1740 m a.s.l.), recorded a 1542-mm total precipitation and 3.9 °C mean temperature.

Plant material and sample collection

In each of the forest sites, six dominant and co-dominant, healthy beech trees aged over 100 years were selected. Tissue samples for light microscopy (LM) were collected with a Trephor tool (Rossi *et al.* 2006a). Microcores containing phloem, cambial zone, and outer xylem were collected at weekly intervals from March until October 2008. On five dates (8 and 17 March, 5 May, 7 July and 8 September 2008) in addition to microcores also larger samples were taken with the intact tissue sampling method, using a chisel and knife (Gričar 2007), for transmission electron microscopy (TEM). All samples were collected from stems at approx. 1.3 m above ground. They were taken 10 cm apart from each other, following a spiral up the stem to avoid wound effects.

Light microscopy (LM)

Microcores were first fixed in FEA (formalin-ethanol-acetic acid solution), dehydrated in a graded series of ethanol and D-limonene and infiltrated, as well as embedded

in paraffin using a Leica TP 1020-1 tissue processor (Rossi *et al*. 2006a). Cross sections (8–10 μm thick) were prepared with a Leica RM 2245 rotary microtome, stained with safranin and astra blue, embedded in Euparal and examined with a Nikon Eclipse E800 microscope (*e.g*. Schmitt *et al*. 2003; Gričar 2007). LM was used to determine onset and cessation of divisions in the cambial zone, as well as to follow the dynamics of cambial activity throughout the growth season.

Transmission electron microscopy (TEM)

After removal from the stem, the samples were fixed for one day in a mixture of 5 % glutaraldehyde, 8 % paraformaldehyde and 0.3 M cacodylate buffer. They were then washed in 0.1 M cacodylate buffer (pH 7.3) and postfixed for one additional day in a 2 % aqueous osmium tetroxide solution. They were then again washed in 0.1 M cacodylate buffer (pH 7.3), dehydrated through a graded series of acetone and embedded in Spurr's (1969) epoxy resin. Semi-thin sections (1 μm thick) stained with toluidine blue were first prepared for pre-examination under LM. Afterwards, ultrathin transverse sections of the cambial zone were prepared and stained with uranyl acetate and lead citrate, and examined with a Philips CM12 TEM at an accelerating voltage of 80 kV.

RESULTS

Lowland site Panška reka

Light microscopy

Examination of cross sections by light microscopy revealed that, on 17 March 2008 (day of the year (DOY) 77), the cambial zone of beech at the low elevation site Panška reka was on average four cell layers wide (Fig. 1, 2a). Based on the increased number of cells in the following weeks and their thin cell walls, we determined the onset of cell

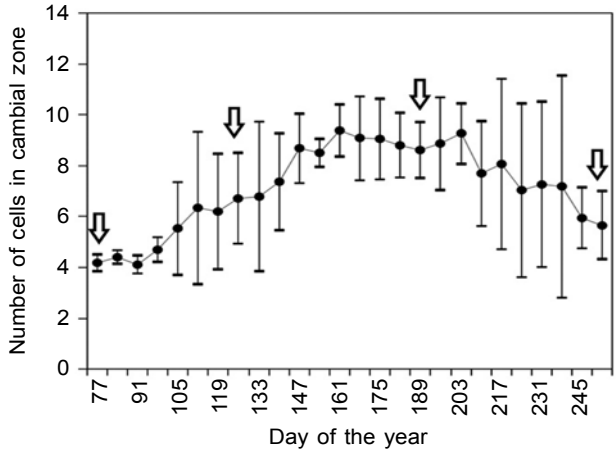


Figure 1. Variability in the number of cells in the cambial zone of beech at the lowland site Panška reka (400 m a.s.l.) in the growth season 2008, with 95 % confidence intervals as observed by light microscopy. The arrows indicate sampling days for transmission electron microscopy.

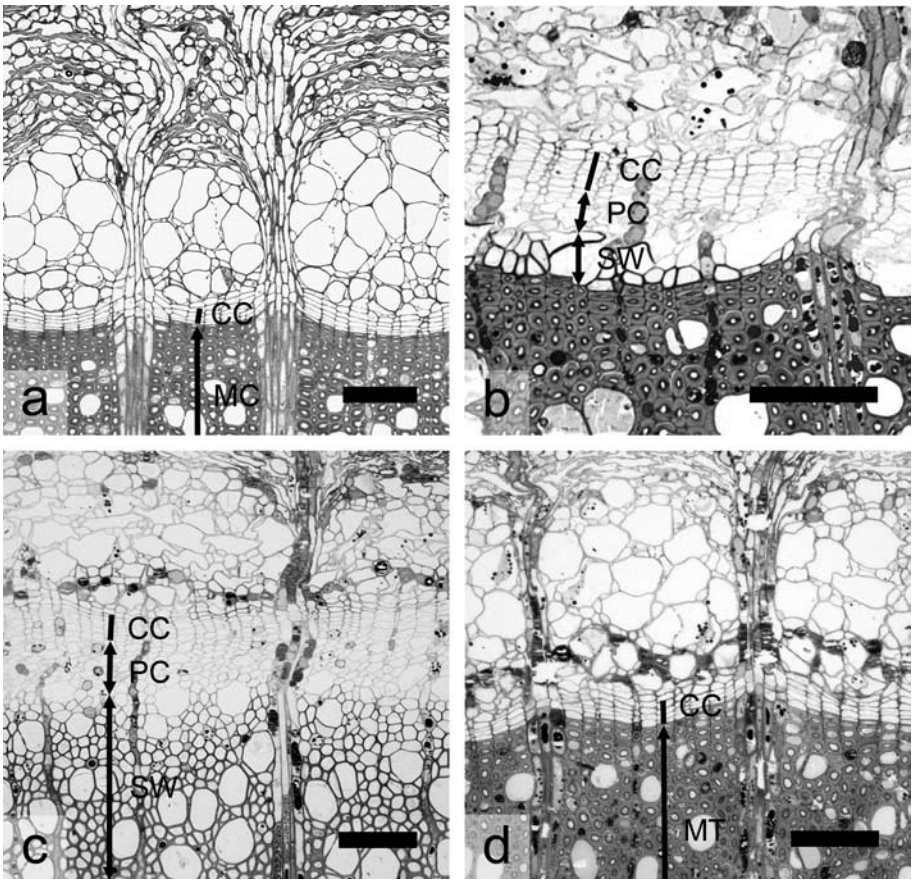


Figure 2. Light micrographs of cambial zone (CC) in beech from the lowland site at different phases of activity throughout the 2008 season, stained with toluidine blue. Situation on (a) 17 March (DOY 77) with dormant CC containing on average four layers of cells; (b) 5 May (DOY 126) with active CC consisting of about seven cell layers; newly formed xylem below the CC consists of enlarging cells (PC) and cells in the phase of secondary wall formation (SW); (c) 7 July (DOY 189), the rate of cell divisions reached its maximum and the CC is on average nine cell layers wide. Xylem cells of the current growth ring are in all developmental stages (PC, SW and mature cells (MT)); (d) 9 September (DOY 252), the CC is five cell layers wide and the xylem cells formed in 2008 are fully developed (MT). — Scale bars 100 μm.

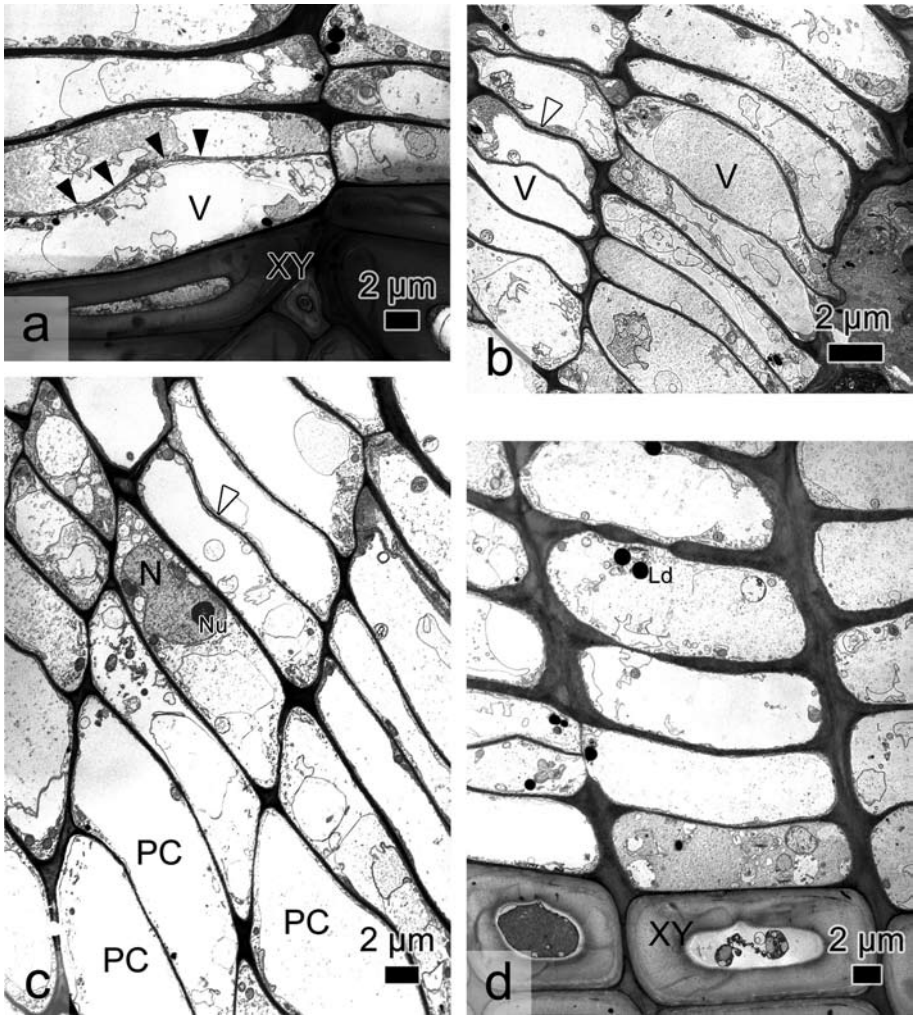
division between 14 April and 12 May (DOY 105 and 133) as recorded by analysis of the corresponding microcores. Onset varied among trees and occurred earliest in the week between 14 and 21 April (DOY 105 and 112) in trees 4 and 6, between 21 and 28 April (DOY 112 and 119) in trees 1 and 3, and between 5 and 12 May (DOY 126 and 133) in trees 2 and 5 (Fig. 2b).

The average number of cambial cells increased from 7 April (DOY 98) until 9 June (DOY 161), when the cambial zone reached its maximum width of approximately ten

cells per radial row (Fig. 1, 2c). This width of cambial zone was maintained for the next six weeks, *i.e.*, until 21 July (DOY 203), and thereafter decreased slowly until 8 September (DOY 252), when the cambium was built of only four or five cell layers (Fig. 1, 2d). Cessation of cambial activity was observed between 28 July (DOY 210) and 8 September (DOY 252) and varied among the trees. In trees 1 and 5, it was observed between 28 July and 4 August (DOY 210–217), in trees 2 and 3 between 18 and 25 August (DOY 231–238), in tree 4 between 1 and 8 September (DOY 245–252) and, finally, in tree 6 between 8 and 15 September (DOY 252–259).

Transmission electron microscopy

Ultrastructural observations of cytoplasmic changes in the cambial zone revealed that, on the day of the first sampling (17 March – DOY 77), fusiform cambial cell were



already highly vacuolated, which indicated early stages of activation (Fig. 3a). Their cell walls were still thicker than in active state; however, newly forming cell walls in the middle of some cambial cells were already observed (Fig. 3a). Active dictyosomes with visible secretory vesicles were present; mitochondria were numerous and mostly had a spherical or oval shape, as seen in transverse sections. The endoplasmatic reticulum was rough (rarely smooth) and cisternal in form. Numerous lipid droplets and peroxisomes were also present. Plastids or amyloplasts were rare.

At the beginning of May (DOY 126), the cambial zone of the investigated trees contained on average six layers of cells with large central vacuoles, whereas the other cell organelles aggregated in narrow cytoplasmic strands attached to the cell wall. The walls of these cells were significantly thinner than in the cambial cells of March samples (Fig. 3b). Dictyosomes were numerous and had a typical cis-trans orientation. They were surrounded by several vesicles, indicating high secretory activity. Plastids containing starch grains were present in both ray and fusiform cells; however, they were more numerous in ray cells. In addition, we noticed phragmoplasts in some of the cells (Fig. 4b).

One month later, on 7 July (DOY 189), the number of cells in the cambial zone increased to eight layers; however, we could not observe ultrastructural changes compared to the previous collection. Cambial cells were highly vacuolated, with well visible nuclei (N) containing dark stained nucleoli (Nu) (Fig. 4c). Adjacent xylem and phloem cells were in the phase of postcambial growth (PC) (Fig. 3c).

Cambial cell division in the investigated trees at Panška reka ceased before 8 September. At that time, the number of layers decreased to about four cells and the latest formed xylem cells adjacent to the cambial zone were still undergoing secondary wall formation and lignification (Fig. 3d). Cells in the cambial zone had thick radial and tangential cell walls and contained lipid droplets; plastids containing starch were again rare; their cytoplasm became denser, and the vacuoles were smaller and more frequent (Fig. 3d).

High elevation site Menina planina

Light microscopy

At the higher altitude Menina planina, we first sampled on 8 March (DOY 68) and continued with weekly sampling from 18 April 2008 (DOY 109) on. On 18 April, the cambial zone was still dormant and contained four layers of cells, as similarly recorded

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Figure 3. Transmission electron micrographs of the cambial zone (CC) of beech from the lowland site throughout 2008. (a) On 17 March (DOY 77), fusiform cells with thick cell walls contain large vacuoles (V). Division is indicated by newly formed thin cell wall (black arrowheads). Below the CC are mature xylem cells (XY) of the previous year's growth ring. (b) On 5 May and (c) 7 July (DOY 126 and 189), the walls of the cells in the active CC are thin, vacuoles occupy most of the lumina and a thin parietal layer of cytoplasm is present (white arrowhead). Xylem postcambial cells (PC) have larger radial dimensions; nucleus (N), nucleolus (Nu). (d) On 8 September (DOY 252), the cells in the dormant CC have thick cell walls. Lipid droplets (Ld) are numerous and the cytoplasm is denser.

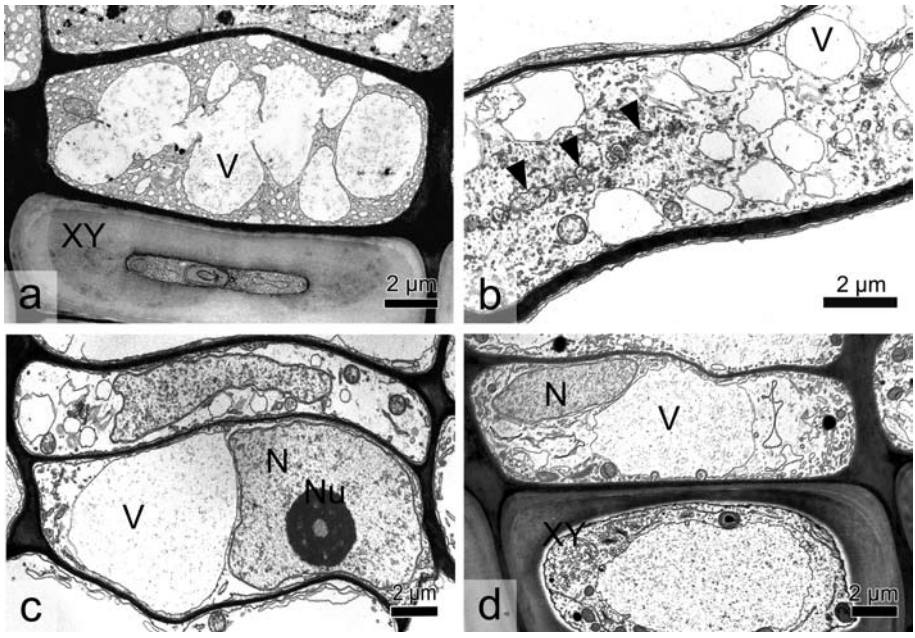

Figure 4. Ultrastructure of cambial zone of beech. (a) Dormant cambial cell (8 March 2008 – DOY 68) with thick cell walls, few larger vacuoles (V) and numerous smaller vacuoles; xylem (XY). (b & c) In the middle of the vegetation period (7 July 2008 – DOY 189); formation of new (thinner) cell plate (black arrowheads) (b) and newly formed cell with thin cell wall and large vacuole (V), nucleus (N) and nucleolus (Nu) (c). (d) At the end of cell division (8 September 2008 – DOY 252), cambium cells still have large vacuoles; the differentiating xylem cell (XY) below the cambium still contains cytoplasm.

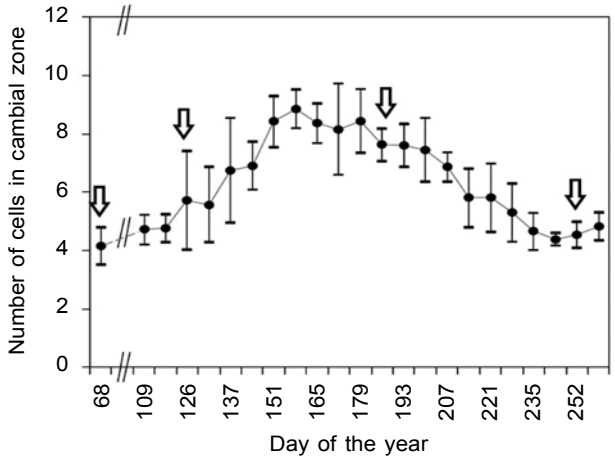

Figure 5. Variability in the number of cells in the cambial zone of beech at the higher altitude site Menina planina (1200 m a.s.l.) in the growth season 2008, with 95% confidence intervals as observed by light microscopy. The arrows indicate sampling days for transmission electron microscopy.

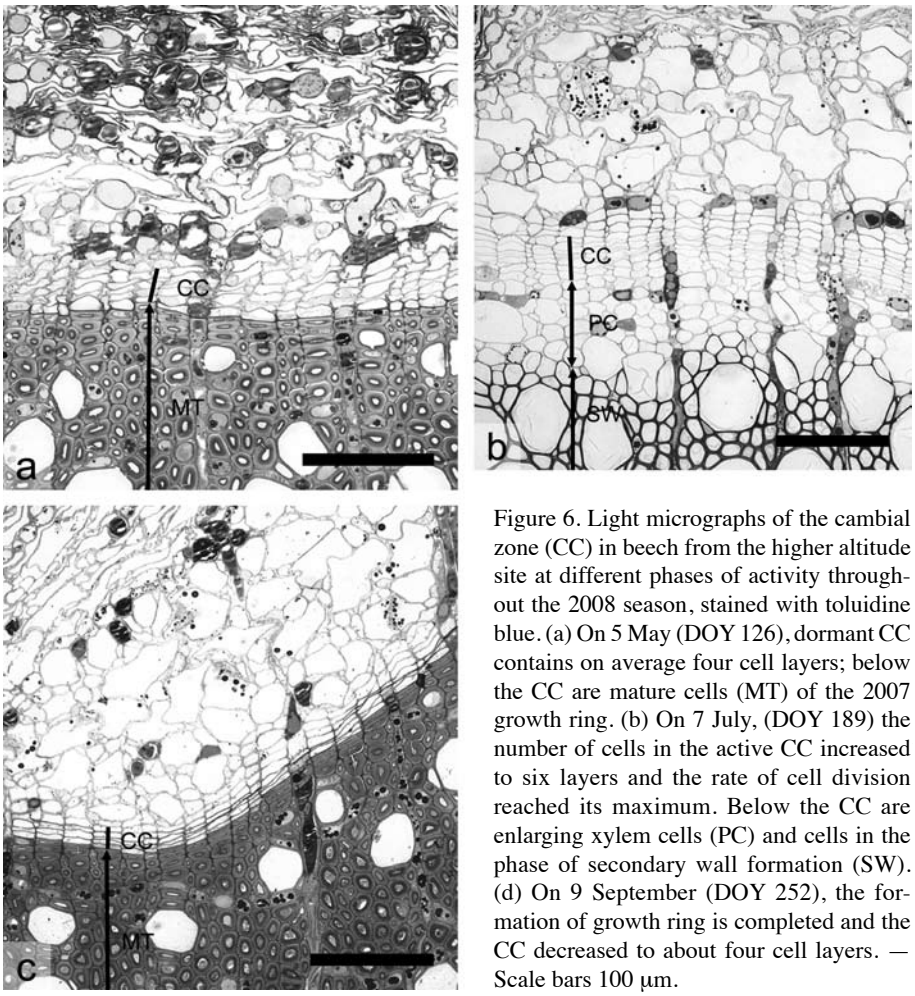

Figure 6. Light micrographs of the cambial zone (CC) in beech from the higher altitude site at different phases of activity throughout the 2008 season, stained with toluidine blue. (a) On 5 May (DOY 126), dormant CC contains on average four cell layers; below the CC are mature cells (MT) of the 2007 growth ring. (b) On 7 July, (DOY 189) the number of cells in the active CC increased to six layers and the rate of cell division reached its maximum. Below the CC are enlarging xylem cells (PC) and cells in the phase of secondary wall formation (SW). (d) On 9 September (DOY 252), the formation of growth ring is completed and the CC decreased to about four cell layers. — Scale bars 100 μm.

in samples with dormant cambium from the lower altitude Panška reka area (Fig. 5, 6a). We determined the onset of cell division in the period between 16 and 23 May (DOY 137 and 144), which is about four weeks later than at Panška reka. Only in tree 4 reactivation occurred one week earlier, *i.e.*, from 10 until 17 May (DOY 130–137). At the height of division activity, from 30 May until 27 June (DOY 151–179), the cambial zone was on average eight cell layers wide (Fig. 5). At that time, newly formed xylem cells at different stages of cell development (PC, SW) were observed near the cambial zone (Fig. 6b). After that date, the number of cambial cells started to decrease slowly. In trees 1, 5 and 6, cambial activity ceased between 25 July and 1 August (DOY 207–214), in trees 2 and 3 between 1 and 8 August (DOY 214–221), and in tree 4 between 8 and 15 August (DOY 221–228). The period of cell division was shorter and the variability in the number of cambial cells among the trees was generally smaller at Menina planina than at Panška reka (Fig. 1 and 5). During the period of most intense

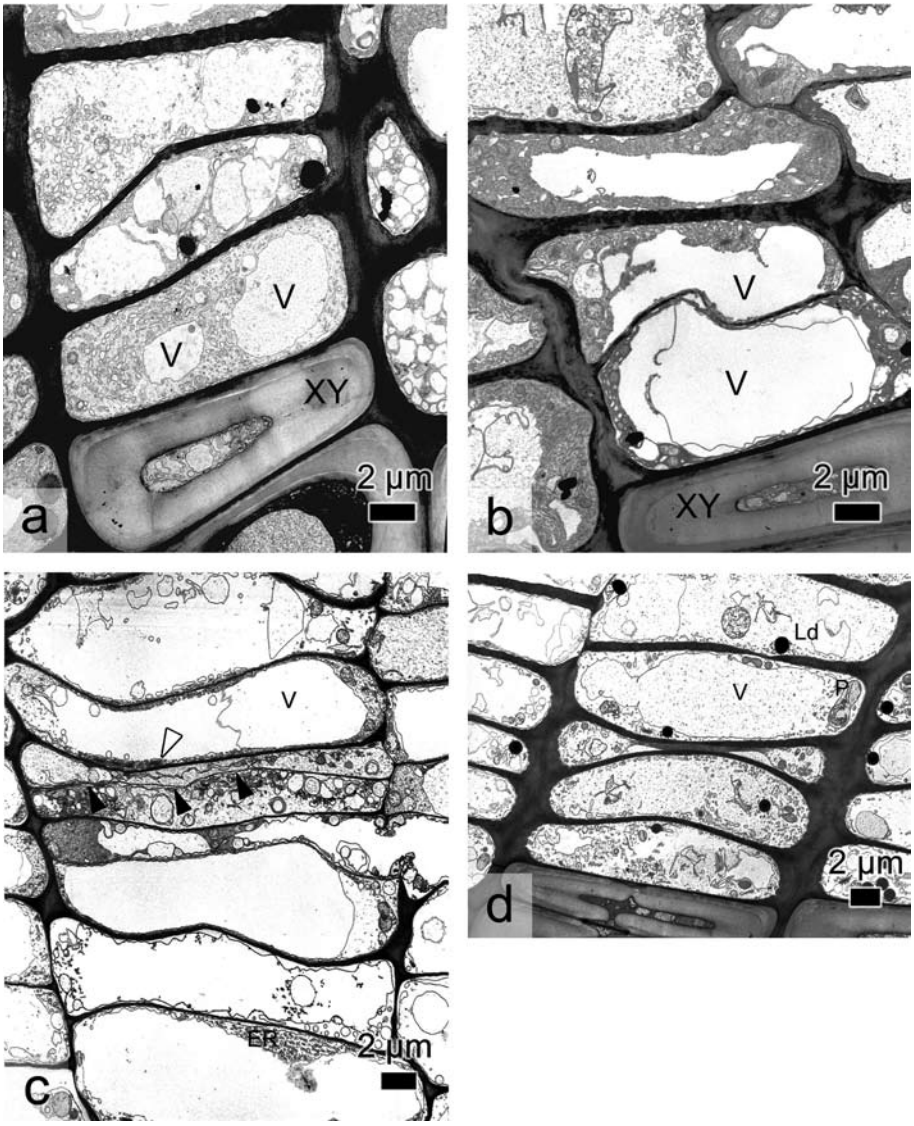

Figure 7. Transmission electron micrographs of cambial zone of beech at the higher altitude site throughout 2008. (a) On 8 March (DOY 68), thick-walled fusiform cells contain few large vacuoles surrounded by a dense cytoplasm with numerous small vacuoles. Below the CC are mature xylem cells (XY) of the previous year's growth ring. (b) On 5 May (DOY 126), the CC contains thick-walled fusiform cells with one large central vacuole per cell, surrounded by dense cytoplasm. (c) On 7 July (DOY 189), CC active with thin cell walls (black arrowheads), large vacuoles that occupy almost the entire lumina and parietal layer of cytoplasm (white arrowhead). (d) On 8 September (DOY 251), CC dormant with thick-walled cells, numerous lipid droplets and denser cytoplasm.

cell production, the number of cells in the cambial zone was lower at Menina planina (eight layers) than at Panška reka (ten layers) but the dormant cambial zone consisted of four to five cell layers at both sites (Fig. 6c).

Transmission electron microscopy

On 8 March 2008 (DOY 67), the cambial zone at Menina planina contained on average four cell layers. The thick-walled cells had only a few larger vacuoles that were surrounded by a dense cytoplasm and numerous small vacuoles (Fig. 4a, 7a). Some cells contained only large vacuoles and also numerous lipid droplets (Fig. 7a), which were larger than those in active cambial cells at Panška reka at that date. Plastids lacking starch grains were present. Mitochondria were less frequent than in Panška reka samples. On 5 May (DOY 126), the cambial zone was still only four cell layers wide (Fig. 7b). However, we noticed numerous changes at the ultrastructural level. Fusiform cells mainly contained a large central vacuole and smaller vacuoles in the narrow cytoplasm, which was closely appressed to the wall (Fig. 7b). Bigger vacuoles observed during the previous sampling were now merged into one large central vacuole. The distribution of other organelles was similar as in March, except that the number of dictyosomes had increased significantly. These were flattened and only partly cis-trans orientated. In some cells, newly formed thin cell walls were observed, indicating the beginning of cell divisions around this date.

On 7 July (DOY 189), the cambial zone reached its maximum thickness with an average of eight cells in a radial file. Actively dividing fusiform cells contained large central vacuoles and a thin parietal layer of cytoplasm (Fig. 7c). At that time, the ultrastructure of the cambial cells was similar at the two sites (Fig. 4b,c, 7c).

Cell divisions at Menina planina stopped between 25 July and 15 August (DOY 207–228). On 8 September (DOY 252), the cambial zone contained only five cells per radial file (Fig. 7d). All these cells lacked newly formed cell walls. Fusiform cells were again thick-walled and contained numerous lipid droplets, as similarly observed before their reactivation in March and May. However, they still contained large vacuoles.

DISCUSSION

Our study of mature beech trees from sites with different climatic regimes have shown that processes which occur in cambial cells during various phases were similar in the two environments but their timing was different (Fig. 8). The width of the dormant cambium was comparable for the beech trees from the lower and higher altitude sites. However, the active cambium of trees from the lower Panška reka site consisted of more cell layers (9.0 ± 0.2 cell layers) than that of trees from the higher Menina planina site (8.3 ± 0.4 cell layers). At Panška reka, the cambial activity lasted for 16.5 ± 3.7 weeks; cambium reactivated earlier and ended its activity later than at Menina planina, where the activity lasted for 10.7 ± 1.3 weeks. The shorter duration of cambial activity at the higher Menina planina could be ascribed to the generally lower air and soil temperatures, and a long period of snow cover, since such conditions are limiting for physiological processes, particularly at the beginning of the vegetation period, as reported for

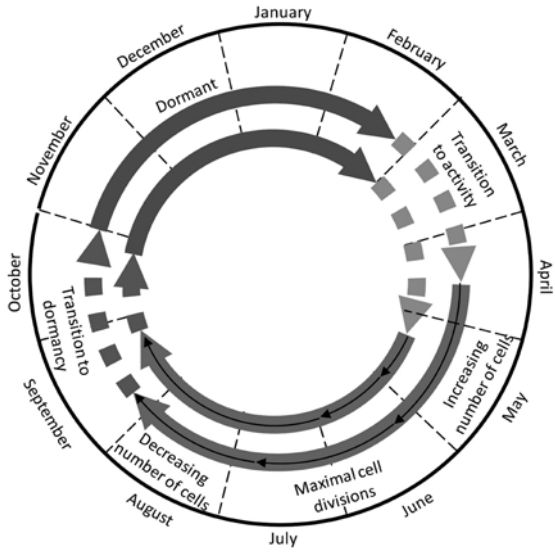

Figure 8. Comparison of cambial activity at the growth sites Panška reka at 400 m a.s.l. (outer circle) and Menina planina at 1200 m a.s.l. (inner circle) in the vegetation period 2008. Transition to activity/dormancy is the time at the beginning/end of the growth season, when the number of cell layers in the cambial zone does not change but we can observe ultrastructural changes in cambial cells.

instance by Kirdyanov *et al*. (2003). This is also consistent with the findings of Rossi *et al*. (2008), who reported that the beginning of wood formation in conifers from colder areas of the northern hemisphere occurs when spring minimum, average and maximum air temperatures exceed 4–5, 8–9 and 13–14 °C, respectively. Our previous wood formation study in beech at Panška reka in 2006 showed that cell divisions started in the second half of April, which coincided with the development of the first leaves (Čufar *et al*. 2008a). In addition, dendroecological analyses at the same site showed that it is fairly representative of lowland beech sites in Slovenia, at altitudes of 300 to 1000 m a.s.l., where dry and hot summer periods (particularly in June) reduce the intensity of wood formation (Čufar *et al*. 2008b). Furthermore, tree-ring analyses have shown that wood production in beech at Menina planina is typical of high elevation (above 1200 m a.s.l.) alpine sites (Prislan *et al*. 2010), at which wood formation is mainly favoured by warmer summer temperatures (Di Filippo *et al*. 2007; Čufar *et al*. 2008b).

As already reported by several authors, dormant cambium is characterised by small vacuoles surrounded by dense cytoplasm, while active cambium contains large central vacuoles, which confine the cytoplasm to narrow peripheral layers (Rao & Catesson 1987; Rensing & Samuels 2004). Although the duration of cambial activity was shorter at the higher altitude site, the structural patterns of cambial cells during transition from dormancy to activity were comparable at the two sites. In this phase, small vacuoles coalesced to form fewer larger vacuoles devoid of proteinaceuos material (Farrar & Evert 1997b; Rensing & Samuels 2004; Frankenstein *et al*. 2005). Dictyosomes began to form vesicles. In most other studied tree species, dictyosome activity proved to follow the same pattern; they were inactive in dormant cambium, reactivated and were active in the period of cell division and again became inactive during transition to dormancy (Farrar & Evert 1997b). However, Barnett (1992) reported that dictyosomes in *Aesculus*

hippocastanum were also active during the dormant period and similar observations have been made in *Pinus strobus* (Srivastava & O'Brien 1966). In these studies, the dictyosomes were probably still active because of favourable climatic conditions during winter (Farrar & Evert 1997b).

The endoplasmic reticulum (ER) was smooth in dormant cambial cells but later became rough-surfaced and cisternal. In most of the studies ER was described as smooth during dormancy and rough or ribosome-studded during active state (Catesson 1994). The appearance different types of ER (tubular, vesicular or cisternal) in the cambium can differ between active and dormant state (*e.g.* Robards & Kidwai 1969; Rao & Dave 1983; Farrar & Evert 1997b) but also among species (Robards & Kidwai 1969; Kidwai & Robards 1969; Srivastava 1966; Srivastava & O'Brien 1966; Barnett 1992).

We observed that the size of lipid droplets gradually decreased during reactivation of the cambium. Most of them disappeared by the end of the reactivation, as already stated by Farrar and Evert (1997b). However, at the end of our sampling period, *i.e.*, at the beginning of September, lipid droplets were again present in cambial cells at both sites. The presence of lipid droplets in dormant cambial cells and their paucity in active cambial cells have been reported in various tree species, such as *Robinia pseudoacacia* (Farrar & Evert 1997b), *Salix fragilis* (Robards & Kidwai 1969), *Aesculus hippocastanum* (Barnett 1992) and *Tectona grandis* (Rao & Dave 1983). O'Brien (1967) even suggested that lipids may locally inhibit wall synthesis. The seasonal cycle of lipid droplets was similar to that of proteinaceous material.

On the sampling date of 5 May 2008, we detected starch containing plastids in the cambial zone of beech at both elevations and they were more numerous in ray cambial cells, whereas in samples from previous sampling dates, starch containing plastids were rare. Farrar and Evert (1997b) similarly found starch grains containing plastids in active cambium in ray cells of *Robinia pseudoacacia*, whereas plastoglobuli containing plastids were present in dormant cambium. However, Kidwai and Robards (1969) observed plastids with large starch grains in dormant ray cambial cells of *Fagus sylvatica*, similar as Frankenstein *et al.* (2005) in *Acer platanoides* and *Fraxinus excelsior* and Srivastava (1966) in the 'quiescent cambium' of *Fraxinus americana*.

According to the presented ultrastructural analyses, lipid droplets and amyloplasts presumably have opposite cycles. Lipid droplets have been described as being present during dormancy, whereas starch containing plastids could be seen in the cells of the active cambial zone (Farrar & Evert 1997b).

Large differences in the presence/absence of amyloplasts during dormancy have been recorded among different tree species, which is probably due to different climate conditions and different physiologies. Some authors have suggested that the hydrolysis of starch could be a protection against frost (Essiamah & Eschrich 1985; Kuroda & Sagisaka 1993, 2005). However, Pomeroy and Siminovitch (1971) stated that, in addition to starch, sugar transformations, augmentation of total protoplasm, extending to organelles, surface membranes and lipid transformations also participate in frost resistance in plant cells. Another reason why Kidwai and Robards (1969), in contrast to our findings, observed starch grains in dormant cambium might be the different age of trees (young trees vs. mature trees), different sampling dates and different latitudes.

Comparison of results obtained from samples prepared for wood formation studies with LM (fixation in FEA, embedding in paraffin) and TEM (fixation with glutaraldehyde, paraformaldehyde, and osmium tetroxide and embedding in Spurr's epoxy resin) showed that the onset of cambial activity was determined much earlier (approx. one month earlier) when using TEM. The results were affected by the media used for tissue fixation and embedding, but also by the thickness of sections and microscope magnification. Namely, the fixative (FEA) used for our LM preparation is well suited to revealing cell wall development but it does not fully preserve the cytoplasm in living cambial cells and it is therefore not possible to observe changes associated with different phases of their activity. Furthermore, examination of thicker sections (8–10 μm) and the use of lower magnification do not allow the detection of the first stages of development of new forming cell walls, while they are still very thin (for instance cell plate cannot be recognized under LM with the above mentioned sample preparation procedure).

When studying wood formation on samples fixed with FEA under LM, the reactivation of the cambium is histologically defined by an increased number of cambial cells and the presence of newly formed xylem and phloem cells in early developmental stages. We can therefore clearly follow the productivity of the cambium (*i.e.* number of newly formed cells in a certain time interval) and differentiation of xylem and phloem cells. The results obtained with two different techniques helped us to get detailed information on the fine structure of cambial cells, dynamics of cambial activity and production of new xylem cells in beech at two sites with different climatic regimes (Fig. 8). Observations with TEM have been particularly important in the phase of transition of cambial cells to activity and dormancy, when the number of cell layers in the cambial zone did not change but ultrastructural changes already occurred in the cambial cells.

In conclusion, when studying the dynamics of cambial activity, it is important to better understand the physiology of trees growing under varying conditions. The cambium is mainly susceptible to environmental signals during the active period and archives them in the wood and bark structure (*e.g.* Frankenstein *et al.* 2005; Gričar & Čufar 2008). Our study shows that when different fixation, embedding and microscopy techniques and criteria (cellular morphology vs. ultrastructure) are used, the established dates of cambial reactivation can vary but combination of the techniques enables us to follow the activity of cambium and wood formation. This is in agreement with the proposal of Frankenstein *et al.* (2005) that identification of the onset of cambium activity (and other processes) needs to be standardised. Since preparation techniques for TEM are very demanding and time consuming, it is usually difficult to use them when working with large sample series taken at short time intervals as in wood formation studies.

However, the results of the present work using both LM and TEM will help in future studies of cambial activity, particularly when the results of different studies using different techniques have to be compared. The results should also contribute to a better understanding of relations between physiological processes in the cambium and climate with regard to predictions of tree responses to the anticipated climate change.

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