

**Can the structure of dormant cambium and the widths of phloem and xylem increments be used as indicators for tree vitality?**

Jožica GRIČAR<sup>1\*</sup>, Špela JAGODIČ<sup>1</sup>, Bogoslav ŠEFC<sup>2</sup>, Jelena TRAJKOVIĆ<sup>2</sup>, Klemen ELER<sup>3</sup>

<sup>1</sup>Slovenian Forestry Institute, Department of Yield and Silviculture, Vecna pot 2, SI-1000 Ljubljana, Slovenia

<sup>2</sup>Faculty of Forestry, University of Zagreb, Svetosimunska 25, 10002 Zagreb, Croatia

<sup>3</sup>Biotechnical Faculty, Department of Agronomy, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

\*Corresponding author:

E-mail: [jozica.gricar@gozdis.si](mailto:jozica.gricar@gozdis.si)

Phone: [+386-1-200-78-53](tel:+38612007853)

Fax: [+386-1-257-35-89](tel:+38612573589)

For submission to: European Journal of Forest Research

*Word count of the abstract: 213*

*Total word count of the main body of the text: 5379*

*Number of references: 45*

*Number of tables: 5*

*Number of figures: 7*

**This is an Authors's Accepted manuscript of an article published in [Gričar j. et al. 2014. Can the structure of dormant cambium and the widths of phloem and xylem increments be used as indicators for tree vitality? European journal of forest research, 133, 3: 551-562, DOI 10.1007/s10342-014-0784-8]**

## **Abstract**

We investigated the structure and width of the dormant cambium and of the increments of phloem and xylem of *Quercus robur* to estimate their potential as indicators for tree vitality. The samples were taken from three woodlands, two in Slovenia (KRA and MUS) and one in Croatia (KOB), with reported tree decline. The number of dormant cells seems to reflect the initial capacity of the cambium to accomplish cell division. With the exception of two trees at KRA, cell production was always higher on the xylem side than on the phloem side. The annual phloem increments were narrower, less variable among trees and with clear lower and upper limits. With increased cambial cell productivity, the share of the xylem in the total annual radial increment increased following a curvilinear function. In trees with an annual radial increment  $> 3.5$  mm, the xylem size represented more than 90 % of the total radial growth. The anatomical variables analyzed show that the most limiting environmental conditions seem to prevail at KRA, whereas the conditions at MUS seem to be most favorable in terms of radial growth. Analysis of the width and structure of xylem and phloem increments, the number of dormant cambial cells and their inter-relationships can provide additional information on the vitality of oaks.

**Keywords** European oak, Lowland forests, Tree decline, Radial growth, Light microscopy

## Introduction

Anticipated environmental changes are threatening the vitality of economically important tree species. Therefore, knowledge of the growth characteristics of a species and the effects that climatic variables and silvicultural management decisions have on tree growth are a key issue for assessing and preserving the sustainability of forests (UN-ECE 2008).

Tree vitality is the dynamic ability of a tree to grow under the actually prevailing conditions. The hypothetical optimal tree vitality is not known; only the minimal vitality (i.e., tree death) can be identified (Dobbertin 2005). Among the commonly adopted indicators for assessing tree vitality, radial growth is of particular value (Beck 2009). Decreasing growth curves are among the most obvious growth-related characteristics of dying trees and are both species-specific and site-specific (Bigler and Bugmann 2004). The early detection of trees with a high mortality risk may help evaluate and control future mortality in forest stands affected by dieback (Bigler et al. 2004).

The annual wood production of a tree can be understood as a suitable indicator of tree vitality, since wood formation is the final result of a tree's complete metabolic balance (Kozlowsky and Pallardy 1997); what remains after foliage production and consumption by respiration and all higher priority allocations to other tree organs (e.g., roots and fruits). This remaining share refers directly to the reserve pool (Beck 2009). The annual layer of wood is the final product of cambial cell division and differentiation of the newly formed derivatives (Plomion et al. 2001). However, the cambium is a bi-facial lateral meristem producing two complex tissues: secondary xylem (wood) in a centripetal direction and secondary phloem (bast) in a centrifugal direction (Larson 1994). The divisional activity of the cambium is normally more intense on the xylem side than on the phloem side and, consequently, xylem accumulates faster than phloem in a tree stem (Panshin and de Zeeuw 1980; Larson 1994). In contrast to xylem formation, the formation of phloem each year is crucial for tree survival; the translocation pathways for photosynthates and biomolecules must be maintained because sieve cells function for only 1–2 growing seasons (e.g., Evert 2006; Taiz and Zeiger 2006). During unfavorable growth conditions, therefore, when radial growth is reduced, the priority of the tree is phloem formation and, consequently, the ratio between xylem and phloem becomes progressively smaller (Bauch 1986).

In Slovenia and Croatia, oaks (*Quercus robur* L. and *Q. sessiliflora* Salisb.) are economically and ecologically very important tree species, representing 7 and 22 %, respectively, of the entire wood stock (Gozdnogospodarski etc. 2006; avlovi 2010). As in many European countries (e.g., Klimo and Hager 2001), a

trend of decreasing vitality of *Q. robur* in the form of defoliation and reduced height and girth increment has been observed at most sites in recent decades (Čater et al. 2001; Tikvič et al. 2009; Levanič et al. 2011). Possible reasons may be decreasing ground water levels due to changes in climatic conditions and unsuitable conversion of land for agricultural purposes (Čater et al. 2001). In addition to ecological issues, tree vitality affects wood quality and thus its properties, causing a lower value of wood products and financial losses (Tikvič et al. 2009).

The main goal of the research was therefore to investigate the relationship between the widths of the xylem and phloem increments and of the dormant cambium in *Q. robur* from three different locations, two in Slovenia and one in Croatia. A decrease in the vitality of *Q. robur* has been reported at all sites (Čater et al. 2001; Pavlovič 2010). In particular, the objectives of the study were to (1) analyze the anatomical structure of the xylem and phloem growth rings in relation to their width; (2) compare the relationship between the widths of xylem and phloem and the number of cells in the dormant cambium at different locations; and (3) estimate the ability to assess the vitality of trees in general.

## **Method and material**

### Study sites

This study was performed in three representative lowland forest stands of pedunculate oak (*Quercus robur* L.) of significant economic and ecological impact within their region: two in Slovenia (Krakovo forest – KRA and Murska Suma – MUS) and one in Croatia (Kobiljak – KOB) (Table 1).

### [Insert Table 1.](#)

The appearance of the specific forest association is mostly determined by microtopography and soil properties, which greatly influence the runoff, distribution and movement of rainwater in the soil. At KRA, water temporarily stagnates on the surface and slowly evaporates or is absorbed into the soil during rainy periods. However, due to the low infiltration capacity of this soil type, most of the rainwater on slightly inclined slopes or somewhat higher elevations can runoff before the soil absorbs it (Čater et al. 2001). In addition to the groundwater level and the rainwater, frequent autumn and spring flooding of the Krka River, the main river in this area, significantly affect the hydrologic conditions. Similarly at MUS, the hydrologic conditions depend on

rainwater, groundwater level and regular flooding by the Mura and Ledava Rivers in spring and autumn (Urban *et al.* 2000; Frantar 2005). At KOB, soil moisture is affected by the groundwater level and amount of precipitation. No floods occur at the KOB site.

#### Climate conditions

At all three sites, the strong effect of the sub-pannonian continental climate is reflected, with the coldest temperatures being recorded in January and the highest in July. Approximately 70 % of all precipitation falls during the growing season (March to October) and a very small amount in winter (Table 2). For KRA, the climate dataset was obtained from the nearby Novo mesto climate station, located approximately 20 km from the forest site (45°48'N, 15°11'E; altitude 220 m). For MUS, the climate dataset was obtained from the nearby Murska Sobota climate station, located approximately 30 km from the forest site (46°39'N, 16° 11'E; altitude 188 m). Both stations belong to the Environmental Agency of the Republic of Slovenia. For KOB, the climate dataset was obtained from the nearby Zagreb-Gri climate station of the Meteorological and Hydrological Service of the Republic of Croatia. This station is located approximately 20 km from the forest site (45°49'N, 15° 59'E; altitude 157 m).

#### [Insert Table 2.](#)

#### Sample collection and preparation

At each location, we sampled approximately 40 randomly selected dominant and co-dominant trees with diameter at breast height of 60-80 cm, height of 25-30 m and an age of approximately 100 years. In December 2009, we took two micro-cores (2.4 x 2.4 x 20 mm<sup>3</sup>) from each tree containing inner phloem, cambium and outer xylem, at 1.3 m above the ground. The material was immediately fixed in formalin-ethanol-acetic acid solution (FEA), dehydrated in a graded series of ethanol solutions (30, 50 and 70 %) after one week and finally infiltrated with D-limonene (Bio Clear, Bio Optica, Milano, Italy) and paraffin (Paraplast plus, ROTH, Karlsruhe, Germany) (Griar 2007). Permanent cross-sections of 10-13 µm thickness were prepared for light microscopy on a Leica RM 2245 rotary microtome (Leica Microsystems, Wetzlar, Germany), using disposable Leica blades. The sections were transferred to object glass and stained with a water mixture of safranin (Merck, Darmstadt,

Germany) (0.04%) and astra blue (Sigma-Aldrich, Steinheim, Germany) (0.15 %) (Werf van der et al. 2007) and embedded in Euparal (Waldeck, Münster, Germany). An Olympus BX51 light microscope (Tokyo, Japan) and the Nikon NIS-Elements Basis Research v.2.3 image analysis system (Tokyo, Japan) were used for observations and evaluation of the tissue.

#### Anatomical observations of the cambium and of the phloem and xylem increments

We analyzed the structure of the dormant cambium and of the adjacent youngest xylem and phloem growth rings (2009) for three radial files in each histological section, including (1) number of cambial cells, (2) widths of the xylem increments, (3) widths of the earlywood, (4) widths of the latewood, (5) widths of the phloem increments, (6) widths of the early phloem and (7) widths of the late phloem. In addition, we measured the xylem ring widths of the two previous years (i.e., 2007 and 2008) better to estimate the site-specific xylem growth trend of the trees. The measurements were performed on both micro-cores and then averaged. We calculated the ratios between the xylem and phloem increments in order to estimate and compare the cambial cell production on the xylem and phloem sides.

We adopted the multi-seriate concept of cambium structure, which states that the vascular cambium comprises both cambial initial cells and xylem and phloem mother cells (Plomion et al. 2001). The tissues were defined as follows: (1) Dormant cambial cells were identified by their narrow radial lumen and thin, unlignified primary cell walls that stained blue. (2) Phloem sieve tubes were defined by thin, unlignified, blue-stained walls and a round to irregular shape. The dimensions of the early sieve tubes were greater than in the late phloem. (3) Phloem fibers with thick, lignified walls that stained red are typical for late phloem. The fiber walls exhibited birefringence under polarized light. (4) The axial parenchyma were distinguished from the sieve tubes by their smaller dimensions and bluish content in the lumen. (5) The xylem of ring-porous oak is composed of vessels, ground tissue of vasicentric tracheids and libriform fibers, and axial and ray parenchyma cells. The walls of the vessels and fibers are thick, multi-layered and lignified. They are red-stained and exhibit birefringence under polarized light. We distinguished early- and latewood. Most of the transverse earlywood area is occupied by vessels that can be seen with the naked eye (diameters > 200  $\mu\text{m}$ ), whereas the vessels in latewood are much smaller (diameter approximately 50  $\mu\text{m}$ ). Latewood vessels are distributed singly or in wide growth rings as radially oriented groups, alternating with groups of thick-walled libriform fibers (Grosser 1977).

## Data processing

Means for all xylem/phloem parameters means were first calculated for each tree (using two replicates). Each parameter was then subjected to univariate analysis of variance with site as the categorical factor. Before ANOVA, a Levene test checked the homogeneity of variances; in cases with a significant test, robust ANOVA on ranks (Kruskal-Wallis test) was used. The significance of differences between pairs of sites was ascertained using a Duncan post-hoc test in cases when ANOVA was used or multiple comparisons following Siegel and Castellan (1988) when a Kruskal-Wallis test was used. For the analysis of relations between pairs of parameters, a simple Pearson correlation was applied. Box-Cox transformation was used when necessary to linearize the relationships. Additionally, general linear modelling was performed to test whether general relations between the selected pairs of parameters exist, irrespective of sites. If such generalities do occur, one would expect similar regression slopes for individual pairs of parameters among sites. All tests were performed at a 0.05 significance level. Analyses were performed in R 2.15.2 (R Core Team 2012).

## Results

### Xylem rings

The oaks at MUS had significantly wider xylem increment in 2009 than those at KRA and KOB (Table 3). Similarly, the xylem increment in 2007 and 2008 were also widest at MUS and narrowest at KOB (Table 4). Classification of the trees according to their xylem ring widths shows that the majority of trees (82 %) with a xylem increment narrower than 0.50 mm were at KRA, whereas only two oaks (5 %) from MUS had xylem increments narrower than 1.0 mm (Fig. 1). Two thirds of the trees had xylem rings between 1.0 and 3.0 mm; i.e., at KRA = 63 %, at KOB = 76 % and at MUS = 56 %. In addition, oaks with rings wider than 3.5 mm were detected only at MUS (26 %).

[Insert Table 3.](#)

[Insert Table 4.](#)

[Insert Figure 1.](#)

Wood anatomical structure and xylem ring width are related to each other, as is typical for ring-porous species (Fig. 2). At all three sites, latewood width was strongly positively correlated with the xylem increment and was significantly wider at MUS than at KRA and at KOB (Table 3; Fig. 3b). Unlike the linear relationship between the widths of latewood and xylem increment, earlywood showed a curvilinear relationship with xylem increment (Fig. 3a). Here, the narrowest earlywood was at KRA and the widest at MUS (Table 3). In xylem rings narrower than 1.0 mm, earlywood width tended to increase rapidly with wider xylem rings until its width was approximately 0.50 mm. Thereafter, earlywood increased slowly with wider xylem rings and, even with the widest xylem rings, it did not exceed 1.5 mm. Due to this non-linear relationship between earlywood and xylem increment and wider xylem rings at MUS, the relation between earlywood and xylem increment differed highly significantly at this location; however, if xylem ring values wider than 3.5 mm were not considered in the analysis, there were no significant differences among the sites (Fig. 3b). For xylem rings wider than 3.0 mm, the earlywood width was more or less stable, between 0.90 and 1.3 mm. For xylem rings narrower than 1.1 mm, the proportion of earlywood was greater than the proportion of latewood, whereas for a xylem increment between 1.1 and 2.1 mm, either earlywood or latewood prevailed (approximately 50% each); for xylem rings wider than 2.1 mm, latewood predominated.

[Insert Figure 2.](#)

[Insert Figure 3.](#)

#### Phloem rings

At all sites, the phloem rings were on average 0.30 mm wide, with the highest mean values at KOB and the lowest at KRA (Table 3). The narrowest phloem ring widths, approximately 0.15 mm, were recorded at KRA and KOB, whereas the minimum values were always greater than 0.20 mm at MUS. In contrast, at all three sites the maximum phloem ring widths were less than 0.50 mm. As shown in Fig. 4, the majority of oaks from all three locations had phloem ring widths of 0.20-0.40 mm (KRA = 89 %, KOB = 74% and MUS = 83 %). Nearly three-quarters of the phloem increments narrower than 0.20 mm were from oaks at KRA and the remainder from

oaks at KOB. Moreover, the widths of phloem rings in only four trees at KRA exceeded 0.40 mm (versus 11 trees from KOB and 7 trees at MUS). There were significant differences in early phloem widths between KRA and MUS, while late phloem widths were greatest at KOB (Table 3). Early- and late phloem widths were both larger with larger phloem ring width. Although the relationship between early phloem and the phloem ring did not differ among the sites, the relationship between late phloem and phloem increment at MUS differed significantly from the other two sites if all trees were considered in the analysis; however, if xylem rings wider than 3.5 mm were not taken into account, there were no significant differences among the sites (Fig. 3c, d). Irrespective of phloem ring width, late phloem was generally wider than early phloem, which occupied more than half of phloem ring in only 22 % of the oaks. Early phloem predominated in 37 % of the oaks at MUS, 19 % at KRA and 12 % at KOB.

[Insert Figure 4.](#)

[Insert Figure 5.](#)

#### Xylem ring vs. phloem ring

The widths of xylem and phloem rings were positively correlated (Fig. 3e). At all three locations, the variability in the widths of the annual increments was higher in the xylem (0.135–5.327 mm) than in the phloem (0.146–0.499 mm). For example, if xylem and phloem rings are expressed in percentages, then the narrowest xylem ring represented only 2.5 % of the widest xylem ring, whereas the narrowest phloem ring represented approximately 30 % of the widest phloem ring. The relation between phloem and xylem rings at MUS differed significantly from the other two sites (Fig. 3e) if all trees were included in the analysis but if xylem rings wider than 3.5 mm were not included in the analysis, there were no significant differences among the sites (Fig. 3e). With the exception of two oaks at KRA with very narrow xylem rings (0.135 and 0.267 mm), xylem increments were always wider than phloem ones and represented more than 50 % of the annual radial increment. As the radial increment increased, both xylem and phloem rings increased; however, because the relative increase of xylem increment was higher, its contribution to the total annual radial increment increased more rapidly (Fig. 5). The xylem increment therefore had a positive curvilinear relation with radial increment ( $r = 0.91$ ), and phloem increment had a negative relation ( $r = -0.37$ ). Thus, more than 60 % of the yearly cambial cell production

resulted on the xylem side if xylem rings were 0.30-0.70 mm wide, and more than 75 % if xylem rings were 0.75-1.5 mm wide (Fig. 5). Furthermore, xylem rings that were 1.5-2.5 mm wide represented more than 85 % of the radial increment, and xylem rings wider than 2.5 mm represented 90 % or more. With the exception of two trees at KRA, all of the other 12 trees with xylem rings wider than 3.0 mm were at MUS.

Insert Figure 6.

Insert Figure 7.

Xylem vs. phloem vs. dormant cambium

There was no significant difference in the number of dormant cambial cells among the locations. The dormant cambium of the oaks contained 4-7 cell layers on average, but it varied from 2 to 8 (Fig. 6). At KRA, the cambium of 71 % of the trees was 4-7 cell layers wide, at KOB 84 % and at MUS 76 %. The greatest range in the number of cambial cells was found at KRA (2-8) and the smallest at MUS (3-7).

Insert Figure 8.

At all three sites, the average number of cambial cells was positively correlated with the widths of xylem and phloem rings (Fig. 7). The correlation was generally higher between the number of cambial cells and xylem ring widths at MUS than at the other two sites if all trees were included. However, if xylem rings wider than 3.5 mm were removed, there were no significant differences among the sites. When xylem rings were separated into earlywood and latewood, latewood showed a stronger positive correlation with the number of cambial cells, and the  $R^2$  values were comparable to the correlation between the number of cambial cells and xylem ring widths, whereas the correlation between the number of cambial cells and earlywood was generally weaker and more variable among sites (Table 5). The correlation between the number of cambial cells and phloem ring widths was weaker than that of the number of cambial cells and xylem ring widths but did not differ among the locations. As with xylem, late phloem showed a stronger correlation with the number of cambial cells than early phloem, and the  $R^2$  values were comparable to the correlation between the number of cambial cells and phloem ring widths (Table 5). The dispersion of the points describing the correlation between the number of cambial cells and xylem

ring widths (and, to some extent, the correlation between the number of cambial cells and phloem ring widths) was greater for cambium with more than 6 cell layers.

[Insert Figure 9.](#)

[Insert Table 5.](#)

## **Discussion**

### Anatomical characteristics of xylem and phloem rings

*Q. robur* has been characterized as a sensitive woodland tree species, based on a strong response of its wood anatomical characteristics to environmental changes (Levani et al. 2011). In recent decades, high rates of decline and dieback of this tree species have been reported regularly for all three study sites (Kater et al. 2001; Tikvi et al. 2009). The environmental (climatic and/or hydrological) conditions at KRA seem to be most limiting for the annual wood production, since the ring widths were in some cases less than 0.2 mm. At MUS, in contrast, the annual wood increments in some trees even exceeded 5 mm. The widths of the latewood and of the xylem ring are linearly correlated in oaks (Rao et al. 1997), whereas a curvilinear relation between the widths of the xylem ring and the earlywood was found. The width of earlywood did not exceed 1.5 mm and was essentially stable in rings wider than 3 mm, which is in accordance with observations by Zhang (1997) in *Q. sessiliflora* and *Q. robur*. The structural differences between early- and latewood are typical for ring-porous species because they are destined for different functions in the tree. The dominant function of earlywood, composed of large vessels, is conduction, whereas latewood, consisting of small vessels and libriform fibers, is primarily for mechanical support (e.g., Tyree and Zimmermann 2010). Any changes in the morphological characteristics of the cells or the proportion between these two parts are therefore very likely to modify the hydraulic and mechanical properties of the wood and thus affect the survival and efficiency of the living tree (e.g., Rao et al. 1997).

In contrast, the annual phloem increments were narrower (0.20-0.40 mm) and less variable among trees, as was reflected in approximately three-quarter of the trees. In addition, clear lower (0.145 mm) and upper limits (0.5 mm) of the increment widths were evident. As with xylem, early and late phloem are also structurally different, resulting from their different roles in a tree. In the first part of the growing season, the main function of

phloem is conduction (early phloem), whereas in the second part of the season, the function is storage (late phloem) (Taiz and Zeiger 2006). There was not a clear connection between the phloem ring width and the proportion of early and late phloem in *Q. robur*. The widths of both parts increased with increasing width of the phloem increment; however, the proportion of late phloem was generally greater in most of the trees. We found that most of the trees with a higher proportion of early phloem were located at MUS, suggesting that this relation also depends to some degree on local environmental conditions. Although the narrowest phloem increments at KOB and KRA were approximately 0.15 mm, this value at MUS was never less than 0.20 mm. Our observations partially refute the speculation that phloem formation and structure are relatively stable (e.g., Larson 1994). This statement could be true if only trees of a similar age, position in stand, vigor, vitality and growing in similar environments are observed; otherwise, the phloem structure could vary, thus providing valuable information on the physiological condition of the tree or stand.

#### Relation between the number of dormant cambial cells and widths of xylem and phloem increments

Total annual xylem cell production depends not only on the number of cells in the cambium (Gregory and Wilson 1968; Skene 1972; Griar et al. 2009), but also on the rate of cambial cell production of xylem cells during a growing season (Gregory and Wilson 1968). Vaganov et al. (2006) reported that the formation of the widest rings is more strongly associated with an increasing growth rate of cambial cells than with increasing numbers of cells in the cambium (Vaganov et al. 2006). In *Q. robur*, we also observed that in trees with cambium wider than 6 cell layers, the xylem increment widths can vary from 1 to 5.3 mm. However, the relationship between the rate of production of xylem cells and the size of the cambium also depends on site conditions, tree vigor, tree age, social position and position in a stem (Wilson 1966; Gregory and Wilson 1968; Gregory 1971). In *A. alba*, for example, the dormant cambium of most productive trees could be up to 11 cell layers wide (Griar et al. 2009), whereas the upper limit was 8 cell layers in the case of *Q. robur*. The narrowest cambium widths, composed of only 2-3 cell layers, were detected at KRA and KOB.

Under normal conditions, cambium produces more xylem than phloem cells (Panshin and de Zeeuw 1980; Larson 1994); however, in trees with diminished vitality, xylem production is reduced and, consequently, the ratio between xylem and phloem becomes progressively smaller (Bauch 1986). Most previous studies have focused on the cambium-xylem relationship, whereas cambium-phloem has been largely overlooked, although annual production capacity cannot be investigated excluding phloem. Within certain limits, phloem production

remains approximately the same irrespective of the rate of growth, its width is less variable and, consequently, changes in the xylem-phloem ratio depend very much on differences in the amount of xylem (Larson 1994). Fromm (2013) reported that most tree species have a xylem to phloem ratio between 4:1 and 10:1; however, the ratio can vary from 20:1 to 0.75:1, as demonstrated for *A. alba* trees of different vitalities (Griar et al. 2009). Furthermore, the uniform increment of phloem during seasonal growth combined with the great variability in xylem production, suggest that phloem formation appears to have priority over xylem formation (Evert 2006) and is presumed to be predominantly endogenously controlled (e.g., Alfieri and Evert 1968; Griar and Ufuar 2008). Missing or discontinuous rings in xylem have been regularly reported, particularly in trees from extreme environments or in trees with reduced vitality. In contrast, phloem is always formed, even if very narrow. Since phloem sieve elements function for only one to two growing seasons, tree survival depends on the yearly formation of new phloem to maintain and extend the translocation pathways for photosynthates and biomolecules (Evert 2006; Taiz and Zeiger 2006). In *A. alba*, for instance, the narrowest phloem increments consisted of at least three cell layers (Griar et al. 2009). Similarly, clear lower and upper limits of increment widths were evident in *Q. robur*.

We observed a positive relationship between the annual phloem cell number increment and the average cell number of the dormant cambium. The separation of both xylem and phloem growth rings into early and late components showed that the relation between the number of cambial cells and earlywood or early phloem was generally weaker and more variable among the sites than that with latewood or late phloem. These results support the statements of Gregory and Wilson (1968) that tissues formed in the first part of the growing season depend more on the rate of production of cambial cells than of their number. In addition, the weaker relation between the number of cambial cells and earlywood/early phloem also demonstrates the greater importance of early growth components for a tree in terms of their functions.

In general, phloem comprises approximately 10-20 % of the annual cell production in dicotyledons and 20-30 % in conifers (Larson 1994). In contrast, our observations demonstrated that this ratio is highly dependent on the width of the annual radial increment. With the exception of two trees at KRA, the cambial cell production was always higher on the xylem side, thus representing more than 50 % of the annual radial growth (excluding secondary changes in older bark tissue or cork cambium production). With increased cambial cell productivity, an increase in the share of the xylem increment in the total annual radial increment follows a curvilinear pattern. In trees of *Q. robur* with a total annual radial increment wider than 3.5 mm, the xylem proportion therefore represents more than 90 % of the yearly radial growth. Knowing that the relationship between the widths of

phloem and xylem in trees with different productive cambium is not linear could be particularly useful for improving data accuracy and the interpretation of studies monitoring stem radius changes with automated dendrometers (e.g., Zweifel et al. 2010). Moreover, the extent of the contribution of each cambial product to the radial increments in *Q. robur* in this study demonstrates that relationships among processes and tissues that affect the annual increase in tree girth are not simple and can be influenced by numerous factors, with the radial growth rate being only one of them. As previously mentioned, other secondary changes occurring in older bark (i.e., the collapse of sieve elements, the inflation of parenchyma cells and the formation of dilatation tissue and sclereids), as well as the activity of cork cambiums, which largely depends on tree age and tree species (e.g., Evert 2006), were not considered in this study. However, since no such data so far are available, it would be very interesting to investigate this aspect in future research because these basic anatomical studies could very well complement our recent results by bringing new insight into the complete annual radial growth of trees.

Could the structures of xylem, phloem and dormant cambium and their relationships be used as sensitive indicators for tree vitality?

Because vital forest resources are the basis of sustainable forest management and the production of quality timber, the early identification of trees with an increased risk of dying could help assess and manage the vitality of forest stands in the future (Bigler et al. 2004; Dobbertin 2005). Tree decline is characterized by reduced cambial cell production, especially on the side towards the xylem, shorter cambial activity and crown damage, including needle loss and yellowing foliage (e.g., Bauch 1986; Fink 1986; Innes 1993; Schmitt et al. 2003; Schweingruber 1986). Reduced wood formation often occurs prior to visual symptoms of crown decline (Torelli et al. 1999), which highlights the usefulness of assessing the current mortality risk of a tree based on radial growth patterns (Bigler et al. 2004). Sampling performed at the base of mature trees seems to be appropriate for investigations of radial growth, since xylem formation at this position is not a priority for tree survival, and thus, its yearly increment is directly related to the state of the reserve pool (Beck 2009). The phloem translocation pathway, in contrast, is important because it links the source of metabolic energy in the foliage to tissues that require photosynthates for respiration and biosynthesis (Oparka and Robert 1999). Finally, the number of dormant cells seems to reflect the initial capacity of the cambium to accomplish division. The number of dormant cambial cells thus increases with increasing annual radial growth, which indicates better tree vitality. From this perspective, the width and structure of the increments of xylem and phloem, as well as the number of

cells in the dormant cambium, can provide additional information on physiological condition in *Q. robur*, which is in line with our previous observations in *A. alba* (Griar et al. 2009). Based on cross-sections containing dormant cambium and the youngest annual phloem and xylem increments of 81 *A. alba* trees of different vitalities that were archived for 20-years, we were able to group the trees into three vitality classes, according to the structural characteristics and widths of their tissues. A recent visit to the site and an inspection of the health of the sampled trees revealed that two-thirds of them with a ratio between phloem and xylem increments less than 4:1 died in the years following the sampling, implying a strong link between the measured anatomical variables and the health of *A. alba* (Griar et al. 2009). In addition, samples taken from a tree are very small and therefore do not affect the long-term survival of the tree.

The comparison of the number of dormant cambial cells, the anatomical structure of the increments and ratios of xylem and phloem shows that among the selected three locations, KRA seems to have the most limiting environmental (climatic and/or hydrologic) conditions and MUS more favorable conditions in terms of the radial growth of *Q. robur*. Nevertheless, decreasing growth curves are among the most obvious growth-related characteristics of dying trees, which is both species-specific and site-specific (Bigler et al. 2004). Similar as in *A. alba*, analysis of the width and structure of xylem and phloem increments, the number of dormant cambial cells and their inter-relationship can provide additional information on tree vitality and, consequently, can be a useful tool for forest management. Since diminished radial growth in ring-porous tree species results in an increase in the percentage of less dense earlywood (Griar et al. 2013), tree vitality has a major effect on wood quality and properties (e.g., Rao et al. 1994); the relationships among all these parameters deserve more in depth investigation. The economic consequences of oak decline are reflected in the lower value of wood products. The timely selection of damaged trees for felling is thus a key factor that ensures the quality of the wood in disturbed and unstable forest ecosystems (Tikvi et al. 2009).

## **Acknowledgements**

The authors would like to thank the anonymous reviewers for their valuable comments and suggestions to improve the quality of the paper. The work was supported by the Slovenian Research Agency, program P4-0107 and project Z4-9662 and by EUFORINNO (RegPot No. 315982) of the FP7 Infrastructures programme. The cooperation among the international partners was supported by the COST Action FP1106, STREeSS.

## References

- Alfieri FJ, Evert RF (1968) Seasonal development of the secondary phloem in *Pinus*. *American Journal of Botany* 55:518-528
- Bauch J (1986) Characteristics and response of wood in declining trees from forests affected by pollution. *IAWA Bulletin ns 7* (4):269-276
- Beck W (2009) Growth patterns of forest stands - The response towards pollutants and climatic impact. *iForest – Journal of Biogeosciences and Forestry* 2:4-6
- Bigler C, Bugmann H (2004) Assessing the performance of theoretical and empirical tree mortality models using tree-ring series of Norway spruce. *Ecological Modelling* 174:225-239
- Bigler C, Griar J, Bugmann H, Ufar K (2004) Growth patterns as indicators of impending tree death in silver fir. *Forest Ecology and Management* 199 (2-3):183-190
- Bjater M, Kutnar L, Accetto M (2001) Slovenian lowland and floodplain forests In: Klimo E, Hager H (eds) *The floodplain forests in Europe: current situation and perspectives*. Leiden, Boston, Köln: Brill, pp 233-248
- Čavlović J (2010) *Prva nacionalna inventura šuma u Republici Hrvatskoj*. Zagreb
- Dobbertin M (2005) Tree growth as indicator of tree vitality and tree reaction to environmental stress: a review. *European Journal of Forest Research* 124 (4):319-333
- Evert RF (2006) *Esau's plant anatomy meristems, cells, and tissues of the plant body: their structure, function, and development*. John Wiley & Sons, Inc., Hoboken, New Jersey
- Fink S (1986) Microscopical investigations on wood formation and function in diseased trees. *IAWA Bulletin ns 7* (4):351-355
- Frantar P (2005) Pretoni režimi v Sloveniji med letoma 1971 in 2000. *Geografski vestnik* 77 (2):115-127
- Fromm J (2013) Xylem development in trees: from cambial divisions to mature wood cells. In: Fromm J (ed) *Cellular aspects of wood formation*. Springer, Berlin Heidelberg, pp 3-39
- Gozdnogospodarski načrt gozdnogospodarske enote Krakovo 2006 - 2015 (Forest management plan for Forest Management Unit Krakovo 2006 -2015). Brežice
- Gregory RA (1971) Cambial activity in Alaskan white spruce. *American Journal of Botany* 58 (2):160-171
- Gregory RA, Wilson BF (1968) A comparison of cambial activity of white spruce in Alaska and New England. *Canadian Journal of Botany* 46:733-734
- Griar J (2007) Xylo- and phloemogenesis in silver fir (*Abies alba* Mill.) and Norway spruce (*Picea abies* (L.) Karst.), vol 132. *Studia Forestalia Slovenica*, Slovenian Forestry Institute, Ljubljana
- Griar J, Ufar K (2008) Seasonal dynamics of phloem and xylem formation in silver fir and Norway spruce as affected by drought. *Russian Journal of Plant Physiology* 55 (4):538-543
- Griar J, Krže L, Ufar K (2009) Relationship among number of cells in xylem, phloem and dormant cambium in silver fir (*Abies alba* Mill.) trees of different vitality. *IAWA Journal* 30 (2):121-133
- Griar J, de Luis M, Hafner P, Levanić T (2013) Anatomical characteristics and hydrologic signals in tree-rings of oaks (*Quercus robur* L.). *Trees – Structure and Function* 27 (6):1669-1680
- Grosser D (1977) *Die Hölzer Mitteleuropas*. Springer-Verlag Berlin Heidelberg New York
- Innes JL (1993) Methods to estimate forest health. *Silva Fennica* 27 (2):145-157
- Klimo E, Hager H (2001) Executive summary. In: Klimo E, Hager H (eds) *The floodplain forests in Europe: current situation and perspectives*. Brill, Leiden Boston Köln, p vii
- Kozłowsky TT, Pallardy SG (1997) *Growth control in woody plants*. Academic Press, Inc, San Diego, California
- Larson PR (1994) *The vascular cambium: development and structure*. Springer-Verlag, Berlin-Heidelberg-New York
- Levanić T, Bjater M, McDowell NG (2011) Associations between growth, wood anatomy, carbon isotope discrimination and mortality in a *Quercus robur* forest. *Tree Physiology* 31:298-308
- Oparka KJ, Robert T (1999) Sieve elements and companion cells—traffic control centers of the phloem. *The Plant Cell* 11 (4):739-750
- Panshin AJ, de Zeeuw C (1980) *Textbook of wood technology*. Fourth edn. McGraw-Hill, New York
- Plomion C, Leprovost G, Stokes A (2001) Wood formation in trees. *Plant Physiology* 127 (4):1513-1523
- R Core Team (2012) *R: A language and environment for statistical computing*
- Rao RV, Aebischer DP, Denne MP (1997) Latewood density in relation to wood fibre diameter, wall thickness, and fibre and vessel percentages in *Quercus robur* L. *IAWA Journal* 18:127-138
- Schmitt U, Grünwald C, Griar J, Koch G, Ufar K (2003) Wall structure of terminal latewood tracheids of healthy and declining silver fir trees in the Dinaric region, Slovenia. *IAWA Journal* 24 (1):41-51
- Schweingruber FH (1986) Abrupt growth changes in conifers. *IAWA Bulletin ns 7* (4):277-283
- Siegel S, Castellan NJ (1988) *Non parametric statistics for the behavioural sciences*. 2nd edn. MacGraw Hill Int., New York

- Skene DS (1972) The kinetics of tracheid development in *Tsuga canadensis* Carr. and its relation to tree vigour. *Annals of Botany* 36:179-187
- Taiz L, Zeiger E (2006) *Plant physiology*. Fourth edn. Sinauer Associates, Sunderland, Massachusetts
- Tikvi I, Zei Ž, Ugarkovi D, Posari D (2009) Damage of forest trees and quality of timber assortments of pedunculate oak on Spa va Area. *Šumarski list* 5–6:237-248
- Torelli N, Shortle WC, Šušar K, Ferlin F, Smith KT (1999) Detecting changes in tree health and productivity of silver fir in Slovenia. *European Journal of Forest Pathology* 29 (3):187–197
- Tyree MT, Zimmermann MH (2010) *Xylem structure and the ascent of sap*. Second edition. Springer-Verlag, Berlin Heidelberg New York
- UN-ECE (2008) *The condition of forests in Europe: 2006 executive report*. Institute for World Forestry, Hamburg, Germany
- Urban I M, Simon I P, Smolej I (2000) Pedunculate oak stands in lowland regions of Slovenia - soil water conditions. *Glasnik za šumske pokuse* 37:215-227
- Vaganov EA, Hughes MK, Shashkin AV (2006) Seasonal cambium activity and production of new xylem cells, vol Ecological Studies. Growth dynamics of conifer tree rings. Images of past and future environments, vol 183. Springer Berlin Heidelberg. doi:10.1007/3-540-31298-6\_3
- Werf van der GW, Sass-Klaassen U, Mohren GMJ (2007) The impact of the 2003 summer drought on the intra-annual growth pattern of beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) on a dry site in the Netherlands. *Dendrochronologia* 25:103-112
- Wilson BF (1966) Mitotic activity in the cambial zone of *Pinus strobus*. *American Journal of Botany* 53:364-372
- Zhang SY (1997) Variations and correlations of various ring width and ring density features in European oak: implications in dendroclimatology. *Wood Science and Technology* 31 (1):63-72
- Zweifel R, Eugster W, Etzold S, Dobbertin M, Buchmann N, Häsler R (2010) Link between continuous stem radius changes and net ecosystem productivity of a subalpine Norway spruce forest in the Swiss Alps. *New Phytologist* 187:819-830

## **Table captions**

**Table 1** Location and characteristics of the study sites and descriptions of the sampled trees.

**Table 2** Climate data: mean monthly temperatures (T) (minimum, mean and maximum), monthly total precipitation (P) and periods covered.

**Table 3** Descriptive statistics and the significance of differences ( $\alpha=0.05$ ) among the sites for the parameters of xylem and phloem in 2009. Different letters (Hom.group after Duncan) explain significant differences between sites for a given parameter (row-wise). SD - standard deviation

**Table 4** Descriptive statistics (mean, standard deviation, minimum and maximum) of widths of xylem rings, earlywood and latewood at the study sites in 2007 and 2008.

**Table 5** Relation ( $R^2$ ) between the numbers of cambial cells and the widths of the xylem and phloem increments at the study sites.

## Figure captions

**Fig. 1** Classification of the oaks from each location according to their xylem ring widths.

**Fig. 2** Structure of xylem and phloem rings of different widths in *Q. robur*; scale bars = 500  $\mu\text{m}$

**Fig. 3** Relationship between (a) widths of xylem ring and earlywood (KRA:  $r = 0.92$ , KOB:  $r = 0.86$ , and MUS:  $r = 0.81$ ), (b) widths of xylem ring and latewood (KRA:  $r = 0.98$ , KOB:  $r = 0.97$ , and MUS:  $r = 0.99$ ), (c) widths of phloem ring and early phloem (KRA:  $r = 0.78$ , KOB:  $r = 0.73$ , and MUS:  $r = 0.91$ ), (d) widths of phloem ring and late phloem (KRA:  $r = 0.88$ , KOB:  $r = 0.88$ , and MUS:  $r = 0.87$ ) and (e) widths of phloem and xylem rings (KRA:  $r = 0.79$ , KOB:  $r = 0.60$ , and MUS:  $r = 0.71$ ); black dots = MUS, grey dots = KRA, white dots = KOB, smaller black dots indicate oaks with xylem rings  $> 3500 \mu\text{m}$

**Fig. 4** Classification of the oaks from each location according to their phloem ring widths.

**Fig. 5** Proportion of (a) xylem ring width of annual radial increment width versus xylem ring width and (b) phloem ring width of annual radial increment width versus phloem ring width.

**Fig. 6** Classification of the oaks from each location according to the number of dormant cambial cells.

**Fig. 7** Relationship between (a) xylem ring width and the number of dormant cambial cells ( $r = 0.79$ ) and (b) phloem ring width and the number of dormant cambial cells ( $r = 0.68$ ).

## Tables

**Table 1** Location and characteristics of the study sites and descriptions of the sampled trees.

ID	Site	Country	Latitude	Longitude	Altitude (m a.s.l.)	Forest association	Soil type
KRA	Krakovo forest	Slovenia	45°54'N	15°25'E	150-170	<i>Pseudostellario- Carpinetum roboris</i>	Pseudogleys and amphigleys on pleistocene clays and loams
MUS	Murska suma	Slovenia	46°29'N	16°31'E	150-155	<i>Genisto elatae- Quercetum roboris</i>	Hydromorphic soils (eutric gleysoils and fluvisols) on holocene and pleistocene gravel and sand deposits
KOB	Kobiljak	Croatia	45°82'N	16°03'E	120-148	<i>Epimedio-Carpinetum betuli</i>	Stagnosols and gleysoils on pleistocene loamy deposits

**Table 2** Climate data: mean monthly temperatures (T) (minimum, mean and maximum), monthly total precipitation (P) and periods covered.

<b>ID</b>	<b>Mean annual T [°C]</b>	<b>T range [°C]</b>	<b>T<sub>Jan</sub> [°C]</b>	<b>T<sub>Jul</sub> [°C]</b>	<b>Mean annual amount of P [mm]</b>	<b>P range [mm]</b>	<b>P<sub>Jan</sub> [mm]</b>	<b>P<sub>Aug</sub> [mm]</b>	<b>Climate dataset period</b>
KR A	10.1	8.6-12.0	-0.1	20.1	1149	827-1405	51.3	126.9	1970-2009
MU S	9.6	8.0-12.0	-1.6	19.8	806	500-1100	32.9	100.3	1961-2009
KO B	11.5	9.7-13.8	-0.4	19.7	886	581-1387	50.5	84.8	1862-2009

**Table 3** Descriptive statistics and the significance of the differences ( $\alpha=0.05$ ) among the sites for the parameters of the xylem and phloem in 2009. Different letters (Hom.group after Duncan) explain significant differences between sites for a given parameter (row-wise). SD - standard deviation

	KRA			KOB			MUS		
	Mean	SD	Hom. group	Mean	SD	Hom. group	Mean	SD	Hom. group
	[ $\mu\text{m}$ ]	[ $\mu\text{m}$ ]		[ $\mu\text{m}$ ]	[ $\mu\text{m}$ ]		[ $\mu\text{m}$ ]	[ $\mu\text{m}$ ]	
Xylem ring	1660.4	999.9	b	1498.3	684.4	b	2672.6	1292.0	a
Latewood	930.9	698.1	b	702.5	494.5	b	1806.3	1114.4	a
Earlywood	697.1	323.7	b	795.8	238.1	ab	866.4	229.0	a
Phloem ring	294.4	83.4	b	336.6	84.6	a	326.1	78.1	ab
Early phloem	131.1	43.7	b	140.8	41.7	ab	157.5	48.1	a
Late phloem	163.3	56.4	b	195.7	61.4	a	168.6	39.3	b

**Table 4** Descriptive statistics (mean, standard deviation, minimum and maximum) of widths of xylem rings, earlywood and latewood at the study sites in 2007 and 2008. SD - standard deviation

		KRA		KOB		MUS	
		Mean	SD	Mean	SD	Mean	SD
		[ $\mu\text{m}$ ]					
Xylem ring	2007	1580.5	920.2	1149.8	463.4	1643.0	563.4
	2008	1757.4	1143.7	1536.8	789.9	2375.7	1139.1
Latewood	2007	886.5	686.5	542.6	323.2	997.4	454.7
	2008	1037.5	914.0	761.0	609.0	1531.4	977.0
Earlywood	2007	712.9	283.0	607.2	180.8	645.6	154.4
	2008	719.9	322.9	775.8	248.6	844.3	249.8

**Table 5** Relation ( $R^2$ ) between the numbers of cambial cells and the widths of the xylem and phloem increments at the study sites.

	KRA	KOB	MUS
Xylem ring	0.862	0.740	0.686
Earlywood	0.839	0.625	0.508
Latewood	0.821	0.724	0.690
Phloem ring	0.682	0.621	0.669
Early phloem	0.440	0.315	0.539
Late phloem	0.667	0.642	0.670

Figures

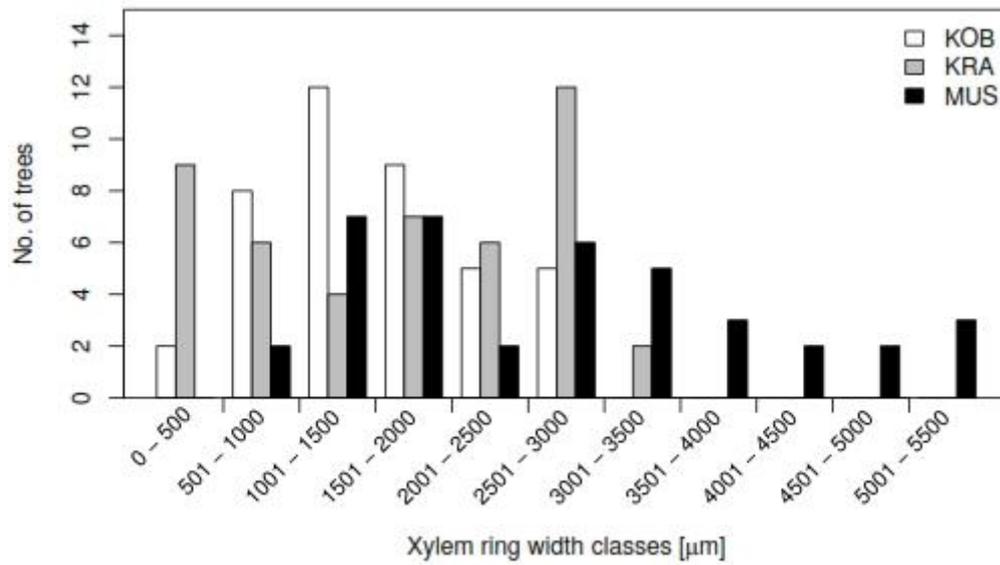


Fig. 1 Classification of the oaks from each location according to their xylem ring widths.

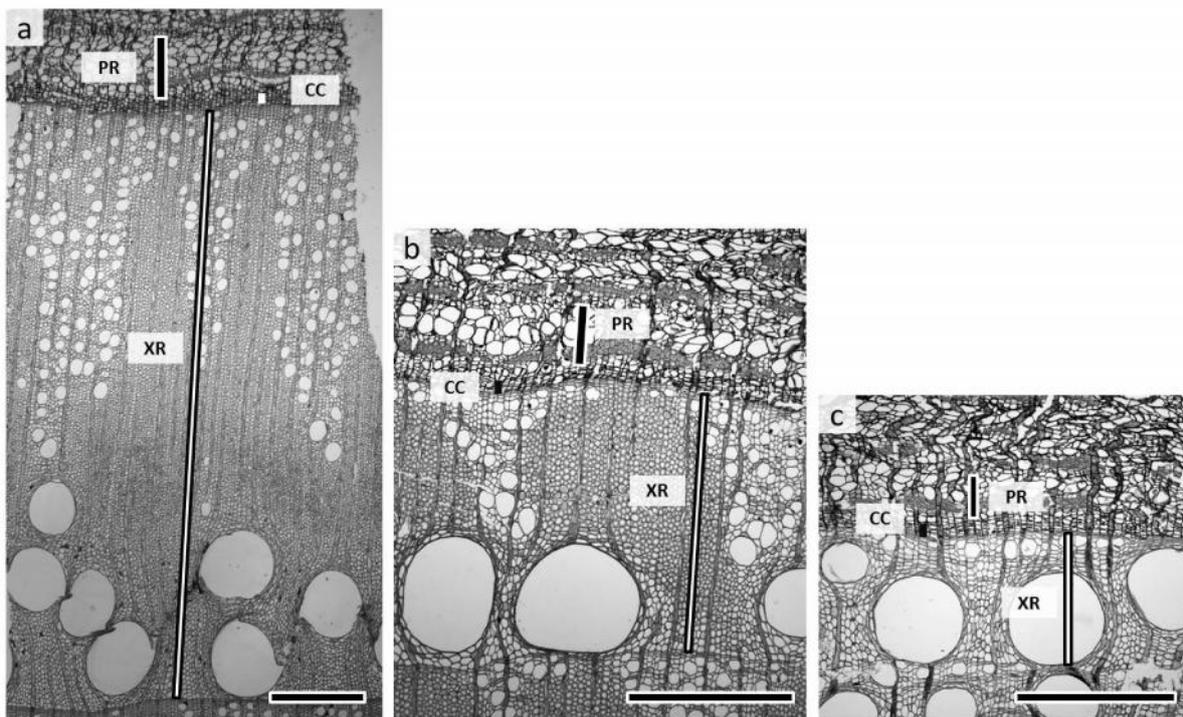
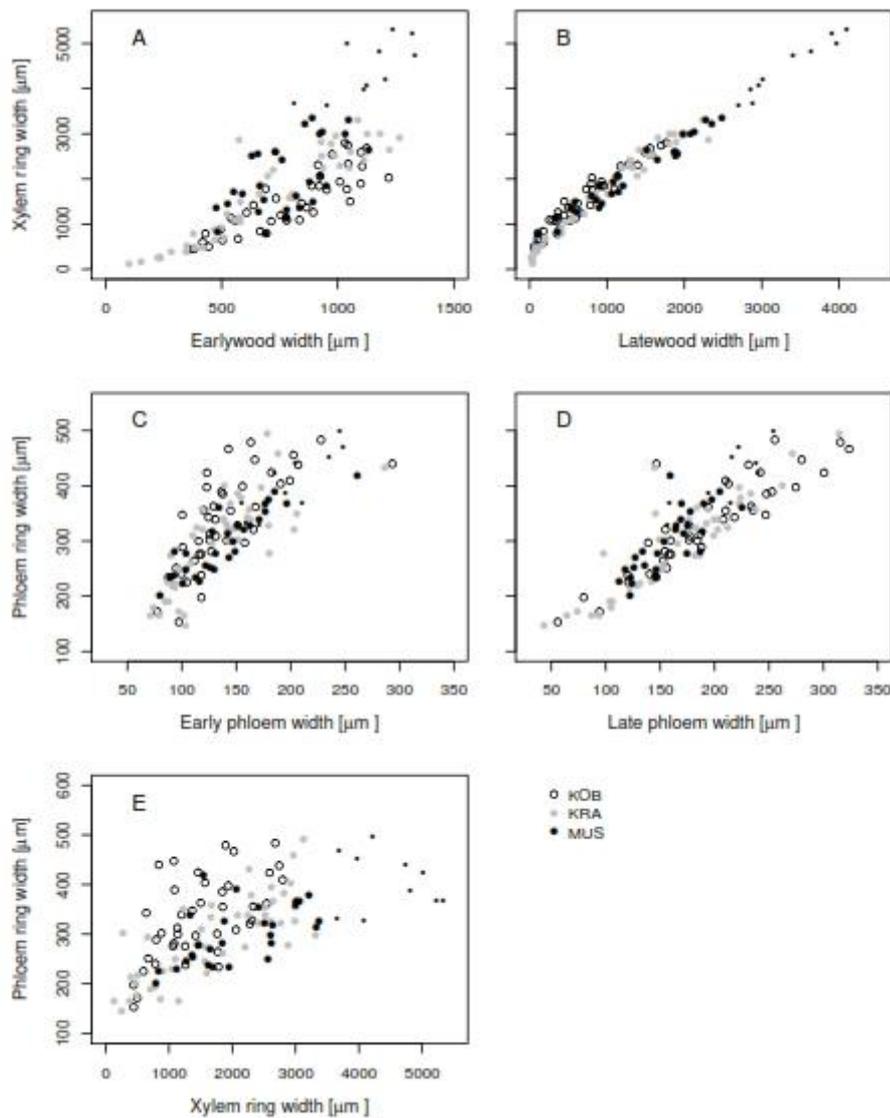
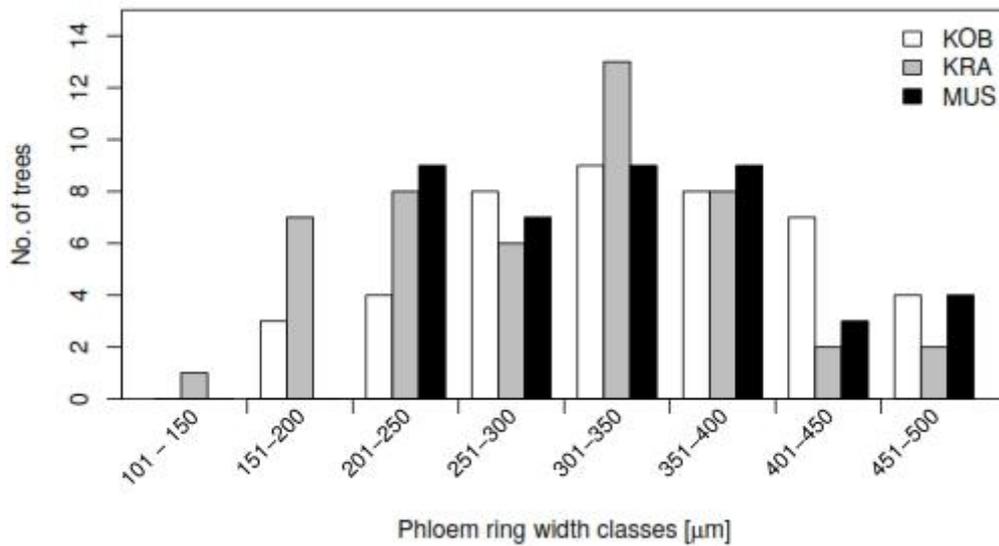


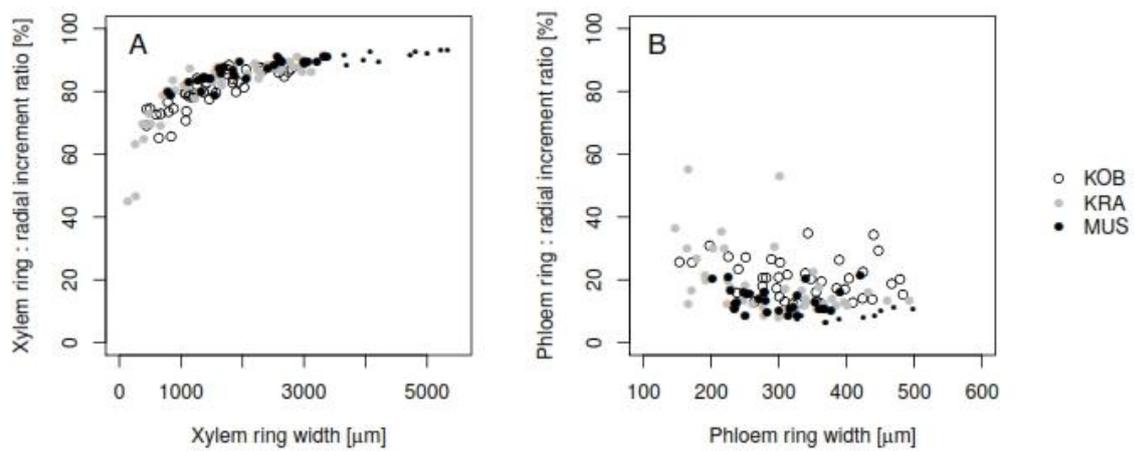
Fig. 2 Structure of xylem and phloem rings of different widths in *Q. robur*; scale bars = 500 μm



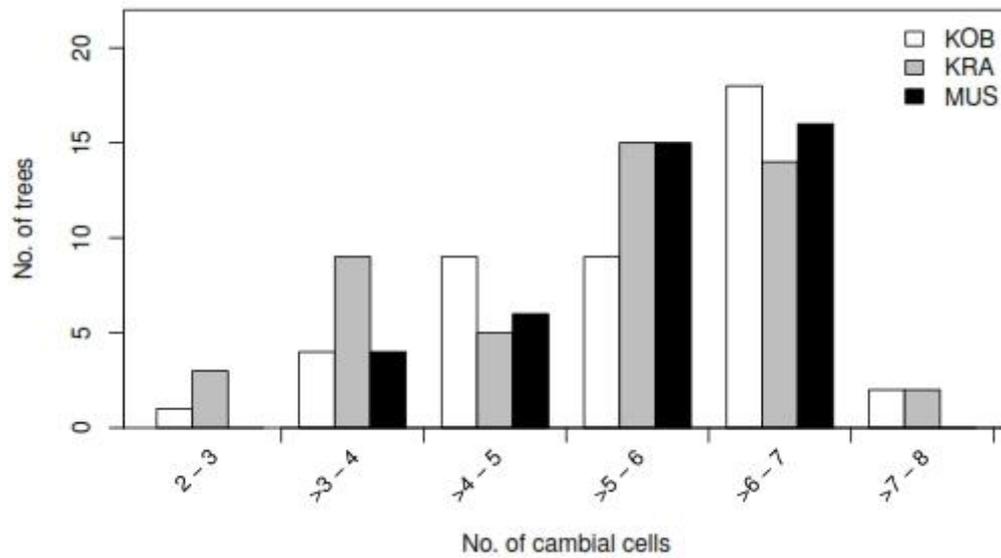
**Fig. 3** Relationship between (a) widths of xylem ring and earlywood (KRA:  $r = 0.92$ , KOB:  $r = 0.86$ , and MUS:  $r = 0.81$ ), (b) widths of xylem ring and latewood (KRA:  $r = 0.98$ , KOB:  $r = 0.97$ , and MUS:  $r = 0.99$ ), (c) widths of phloem ring and early phloem (KRA:  $r = 0.78$ , KOB:  $r = 0.73$ , and MUS:  $r = 0.91$ ), (d) widths of phloem ring and late phloem (KRA:  $r = 0.88$ , KOB:  $r = 0.88$ , and MUS:  $r = 0.87$ ) and (e) widths of phloem and xylem rings (KRA:  $r = 0.79$ , KOB:  $r = 0.60$ , and MUS:  $r = 0.71$ ); black dots = MUS, grey dots = KRA, white dots = KOB, smaller black dots indicate oaks with xylem rings > 3500 µm



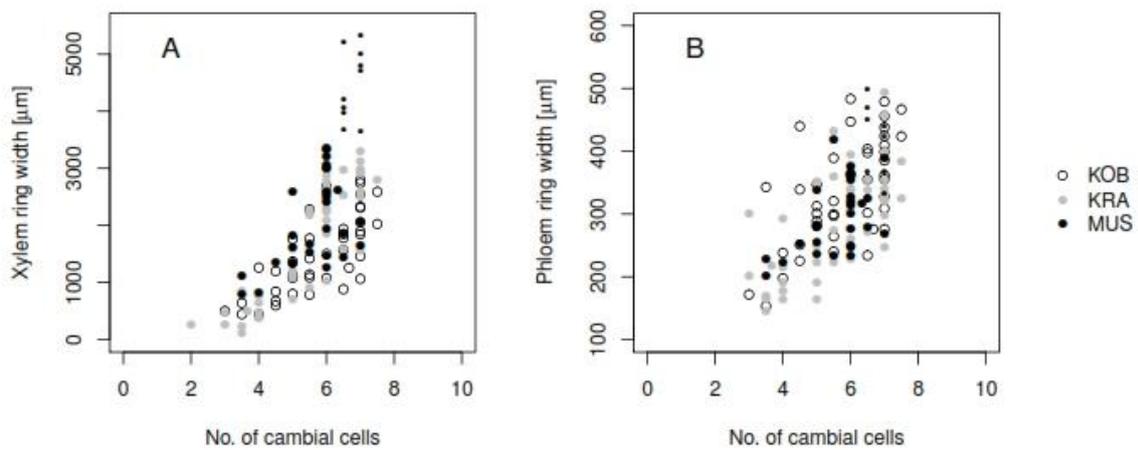
**Fig. 4** Classification of the oaks from each location according to their phloem ring widths.



**Fig. 5** Proportion of (a) xylem ring width of annual radial increment width versus xylem ring width and (b) phloem ring width of annual radial increment width versus phloem ring width.



**Fig. 6** Classification of the oaks from each location according to the number of dormant cambial cells.



**Fig. 7** Relationship between (a) xylem ring width and the number of dormant cambial cells ( $r = 0.79$ ) and (b) phloem ring width and the number of dormant cambial cells ( $r = 0.68$ ).