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Review Article

Serbian spruce (*Picea omorika* (Pančić) Purkyné) - endemicity and advantages

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Abstract:

Conifers, as a response to mechanical stress, such as wind and stem lean, form reaction wood called compression wood (CW). CW occurs in a range of gradations from near normal wood (NW) to severe CW (SCW). As the severity of CW affects the mechanical and chemical properties of wood, and as CW has limited value in the forest products industry, it is desirable to be able to measure CW severity. *Picea omorika* belong to slow-growing conifer species in which CW typically occurs in a severe form. We developed different morphometric and non-morphometric methods for estimation of CW severity tested on wood samples of *P. omorika* juvenile trees exposed to long term static bending. This specific review is aimed at presenting *P. omorika* as one of the most adaptable spruces, and as a good model for testing of methods for estimation of compression wood severity. First, we summarize main knowledge about *P. omorika*, features of CW, and methods for assessment of wood quality. Then, we present breifly our recently published methods for estimation of compression wood severity tested on *P. omorika* juvenile wood samples.

Key words:

cell wall, cellulose fibrils, compression wood, fluorescence-detected linear dichroism microscopy, double wall thickness

Apstract:

Omorika (*Picea omorika* (Pančić) Purkiné) - endemičnost i perspektive

Konifere kao odgovor na mehanički stres (vetar, savijanje) formiraju reakciono drvo koje se naziva kompresiono drvo (CW). CW se javlja u nizu gradacija od skoro normalnog drveta (NW) do jako izraženog CW (SCW). S obzirom da stepen izraženosti osobina CW ima značajan uticaj na mehaničke i hemijske osobine drveta i da CW ima ograničenu vrednost za drvnu industriju, poželjno je moći odrediti stepen izraženosti osobina CW u uzorku. *Picea omorika* spada u sporo rastuće četinarske vrste kod kojih se CW tipočno javlja u jako izraženoj formi. Mi smo razvili nekoliko morfometrijskih i ne-morfometrijskih metoda za procenu izraženosti osobina CW u uzorku, testiranih na uzorcima drveta juvenilnih stabala *P. omorika* koja su bila izložena dugotajnom statičkom savijanju. Ovaj revijski rad ima za cilj da predstavi Pančićevu omoriku kao jednu od najadaptabilnijih smrča i kao dobar model za testiranje metoda za procenu izraženosti osobina CW u uzorku. U prvom delu sumiramo znanja o Pančićevoj omorici, osobinama CW i metodama za procenu kvaliteta drveta, a u drugom ukratko predstavljamo naše nedavno objavljene metode za za procenu izraženosti osobina CW u uzorku, testirane na uzorcima drveta juvenilnih stabala *P. omorika*.

Ključne reči:

ćelijski zid, celulozni fibrili, kompresiono drvo, fluorescentna konfokalna mikroskopija, debljina ćelijskog zida

Introduction

Picea omorika (Pančić) Purkyně is a a rare and endangered tertiary relict and endemic species (Jovanović, 1970). Despite its endemism, *P. omorika* is considered as one of the most adaptable spruces (Sevill et al., 2017).

Conifers, as a response to mechanical stress, form reaction wood called compression wood (CW) (Timell, 1986). CW occurs in a range of gradations from near normal wood (NW) to severe CW (SCW). The degree of development of particular features of CW does not necessarily change in parallel to each other, so the severity of a given tracheid is represented as a function of the degrees of development of individual features, mainly lignification, helical cavities and cell wall thickness (Yumoto et al., 1983). As CW has limited value in the forest products industry it is of great importance to be able to measure CW severity (Altaner et al., 2009).

In recent years we worked on the development of different morphometric and non-morphometric methods for distinguishing wood samples on the compression severity scale. They are based on tracheid double wall thickness (Nedzved et al., 2018), cellulose microfibrils order (Savić et al., 2016), or variation in lignin structure (Mitrović et al., 2015). We used confocal fluorescence microscopy and spectroscopy, combined with development of additional equipment, new algorithms and statistical analysis. We tested our methods on stem samples of *P. omorika* juvenile trees exposed to long term static bending.

Picea omorika belongs to slow-growing conifer species, its wood is characterized by small, densely packed tracheids, while CW typically occurs in a severe form (Timell 1986; Donaldson et al., 2004). Juvenile conifer wood is characterized by randomly distributed mild compression wood (MCW), NW often being absent (Donaldson et al., 2004). These are the features that suggest *P. omorika* juvenile wood as a good choice of samples for evaluation of the precision of methods suggested for estimation of compression wood severity.

Our methods for distinguishing wood samples on a compression severity scale provide a fine gradation from NW to the severest form of CW, compression severity scales being partially different. These methods, alone or in combination with each other, could be a useful tool for fine gradation of wood samples on the compression severity scale, either in the estimation of wood quality or environmental influences during growth and developmental process. They confirm juvenile *P. omorika* stem samples as a good choice of samples for evaluation of the methods suggested for compression wood severity estimation.

Picea omorika: endemicity, natural range, habitat, planting outside its natural range

Picea omorika (Pančić) Purkyně is a slow growing endemic coniferous species and Tertiary relict of the European flora. Its natural habitat is fragmented and reduced to the middle and upper courses of the Drina River, in Western Serbia and Eastern Bosnia and Herzegovina (Jovanović, 1970, Sevill et al., 2017). The species was widespread in Europe and Asia, but after the Pleistocene glaciations, this region represents species long-term, cryptic and last refugium (Aleksić & Geburek, 2014). An Asian origin of Serbian spruce has been recently confirmed (Lockwood et al., 2013), grouping *P. omorika* with the Caucasian *P. orientalis*, and the two Japanese endemics *P. alcoquiana* and *P. maximowiczii*. Serbian spruce ancestors appeared in Asia at the end of the Neogene, but the increasing seasonality and aridity during the late Miocene led to the extinction of *Picea* in the mid latitudes of Eurasia.

Until the middle of the 19th century, the natural range of *P. omorika* was more continuous and less fragmented than it is today (Sevill et al., 2017). It has been legally protected since 1964. Its current distribution is on one side the result of anthropogenic factors such as general forest clearance and harvesting for timber, pastoralism and wildfires (Jovanović, 1986; Sevill et al., 2017). Fire has perhaps been the biggest threat, and logging has been a subsidiary one (Sevill et al., 2017). On the other side the limited natural range of Serbian spruce is the result of the species poor competing ability. It retreats to areas less inhabitable by its competitors, predominantly *Picea abies* and *Fagus orientalis* (Johnson, 1993; Jovanović, 2000). It inhabits open habitats comprising cliffs and forest clearings, characterized by a strong northerly wind, snow, and rockfalls. The climate in its natural range is characterized by very high humidity, high precipitation, regularly distributed over the year, deep snow cover which lasts 4-5 months, and low winter temperatures. *Picea omorika* is drought tolerant, its cold hardiness limit is between -28 °C and -23 °C, it tolerates wide soil pH range and polluted urban conditions (Sevill et al., 2017). In short, it is adapted to extreme environmental conditions.

Planting Serbian spruce outside its natural range has a long tradition in Europe since the late 19th century. In addition to the initial use as ornamental plant species in parks, Serbian spruce has a long tradition of use in forestry (Ivetić & Aleksić, 2016). It is grown to a small extent for Christmas trees, timber and paper production, particularly in northern Europe, although its slow growth makes it less important than Sitka spruce or Norway spruce (Sevill et al., 2017). However, in Britain *P. omorika* and *P. orientalis* are among the alternative species to Sitka spruce and Norway spruces particularly in areas where they might be subject to damage due to drought as the impacts of climate change (Sevill et al., 2017). The great value of this species appears to be successful planting in places where other spruces are susceptible to injury by drought or spring frosts. At present, Serbian spruce is of major importance only as an ornamental tree, mainly in northern Europe and North America. It is regarded as one of the most attractive spruces because of its elegant form and the ability to grow on a wide range of soils (Sevill et al., 2017). Despite its endemism, *P. omorika* is considered as one of the most adaptable spruces.

Wood properties in the forest products industry and methods for estimation of wood quality

Forest products industry is based on wood properties, while wood properties are directly determined by cell arrangement, cell size and shape, and cell wall structure and thickness. In softwood species differences in wood structure result from genetic and abiotic factors: 1) plant age (Zobel & Sprague, 1998; Burdon et al., 2004) - juvenile wood and mature wood; 2) season of maturation within the growth ring (Uggla et al., 2001) – early wood (EW) and late wood (LW); or 3) mechanical stress as a consequence of wind and stem lean (Timell, 1986) - compression wood, opposite wood and normal wood. In the forest products industry, juvenile wood, early wood and compression wood, generally have limited value, and therefore determination of their amounts is of great importance. In this regard, different morphometric and non-morphometric methods were developed for the evaluation of wood quality (**Tab. 1**). The number of developed methods speak in favor of their significance for the forest products industry.

Compression wood - reaction wood in conifers: formation, occurrence, range of gradations

The resistance of trees to mechanical perturbation depends on structural modifications for mechanical strength. The formation of reaction wood in the stem is a reaction of the tree to leaning, as part of the geotropic response. In conifers, reaction wood is known as compression wood (Timell, 1986). Its formation occurs on the lower side of the leaning stem (**Fig. 1**), resulting in eccentric growth (Timell, 1986; Donaldson & Singh, 2013). Inclination at the high angle results in severe compression wood (SCW) formation (Yumoto et al., 1983). CW occurs in a range of gradations from near NW to SCW, mild CW (MCW) forming a continuum between NW and SCW (**Fig. 1**). Wood opposite to the CW in the same growth ring is termed opposite wood (OW) (**Fig. 1**), while wood from growth rings that do not contain any CW is termed normal wood (NW). Also, in the leaning stem, compression wood severity declines from the stem base to the top of the stem (**Fig. 1**).

Wood tracheid cell walls are composed of several layers containing an ordered array of cellulose microfibrils, embedded in a matrix of polysaccharides such as pectin, hemicellulose, and lignin (Harris, 2006). CW is characterized by (**Fig. 2**): increased tracheid wall thickness, reduced lumen diameter, rounder cell cross-sectional profile, presence of intercellular spaces, absence of the S3 cell wall layer and presence of helical cavities in the S2 layer, compared to NW (Donaldson et al., 2004; Donaldson & Singh, 2013). CW is highly lignified, with the changed composition of lignin, increased amounts of *p*-hydroxyphenyl monomers and increased con-

Table 1. Some of the methods for estimation of wood quality

	Methods	Parameter	For estimation of
Morphometric methods	Manual or automated measurements on micrographs (Mork, 1928; Brown et al., 1949; Gofas & Tsoumis, 1975; Klisz, 2009; Selig et al., 2012) Automated measurements from distance maps reconstructed from digital images (Travis et al., 1996; Lorbach et al., 2012; Nedzved et al., 2018)	cell wall area radial cell wall width double wall thickness cell lumen	EW/LW ratio (Mork, 1928) Differences between juvenile and mature wood (Mitchell and Denne, 1997; Loustarinen, 2012) Compression wood severity (Andersson & Walter, 1995; Nyström & Hagman, 1999; Moëll & Fujita, 2004; Duncker & Spiecker, 2009; Nedzved et al., 2018)
Non-morphometric methods	Fluorescence spectroscopy (Donaldson et al., 2010) Chemical analysis (Nanayakkara et al., 2009) Scanning Fourier transform infrared microspectroscopy and immunolabeling (Altaner et al., 2009) Fluorescence-detected linear dichroism (FDLD) microscopy (Savić et al., 2016) Confocal microscopy (Donaldson et al., 2004)	lignin and carbohydrate content/structure cellulose and noncellulosic polysaccharides composition and organization microfibrillar angle	Compression wood severity

densation of monomer units in the polymer (Timell, 1986). Consequently, CW contains less cellulose, with greatly increased amounts of galactan, and slightly lower amounts of mannan and xylan, together with a higher angle of cellulose microfibrils in the S2 layer of the cell wall, compared to NW (Nanayakkara et al., 2009; Donaldson & Knox, 2012; Donaldson & Singh, 2013).

The degree of development of particular features of CW does not necessarily change in parallel to each other, so the severity of a given tracheid is represented as a function of the degrees of development of individual features, mainly lignification, helical cavities and cell wall thickness (Yumoto et al., 1983). Visual detection of compression wood severity, more precisely the determination of MCW, is difficult. As the severity of CW affects mechanical and chemical properties of wood in the forest products industry, it is desirable to be able to measure CW severity (Altaner et al., 2009).

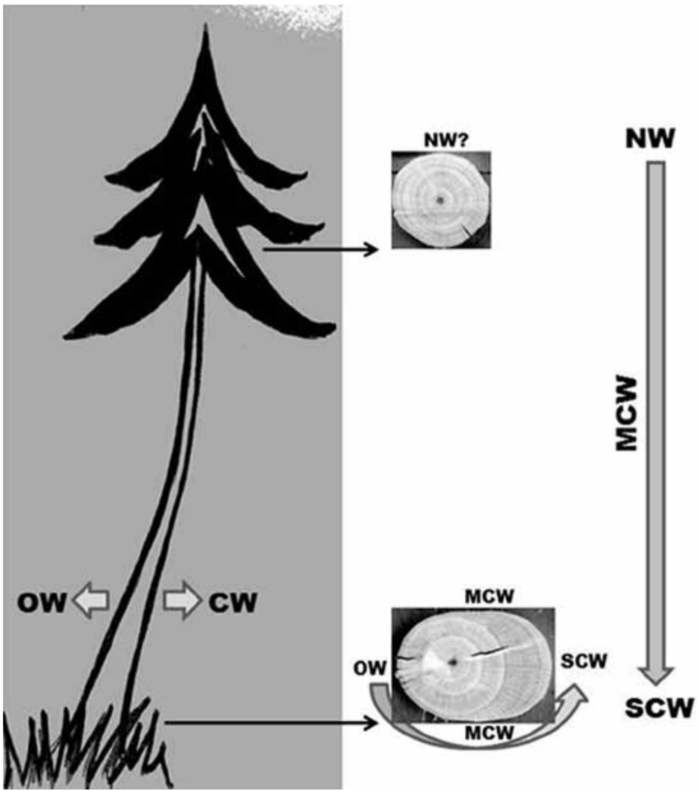

Fig. 1. Scheme of compression wood formation; compression wood occurs in a range of gradations from near normal wood to severe compression wood, mild compression wood forming a continuum between normal wood and severe compression wood; Compression wood severity declines from stem base to the top of the stem; NW – normal wood, CW – compression wood. MCW - mild compression wood, SCW - severe compression wood, OW – opposite wood

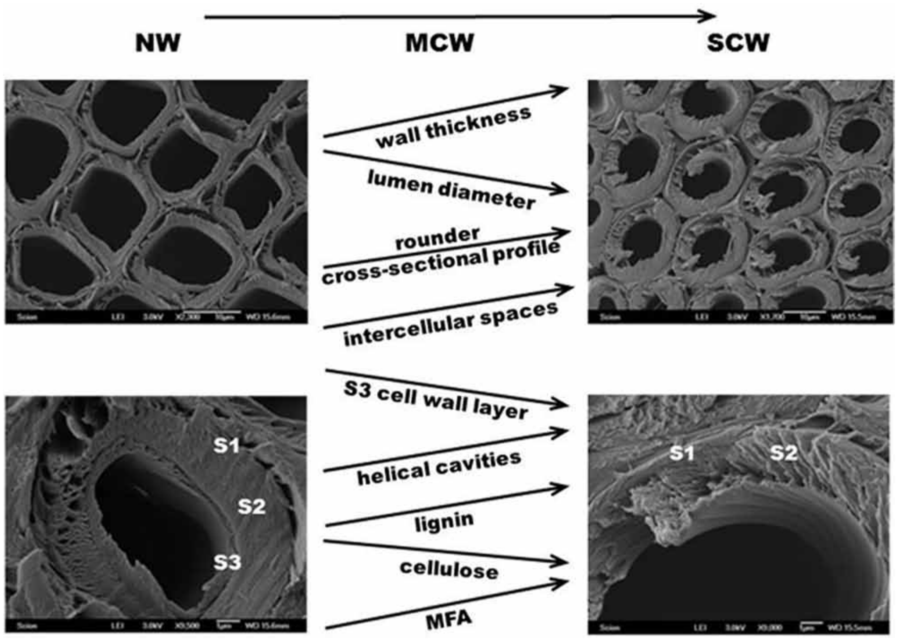

Fig. 2. Scheme for changes in degree of development of tracheid cell wall features characterizing the transition from normal wood (NW) to severe compression wood (SCW), mild compression wood (MCW) forming a continuum between NW and SCW; on the left – field emission scanning electron microscopy (FESEM) images of NW, on the right – FESEM images of SCW; NW – normal wood, MCW - mild compression wood, SCW - severe compression wood, S1, S2, S3 – layers of secondary cell wall

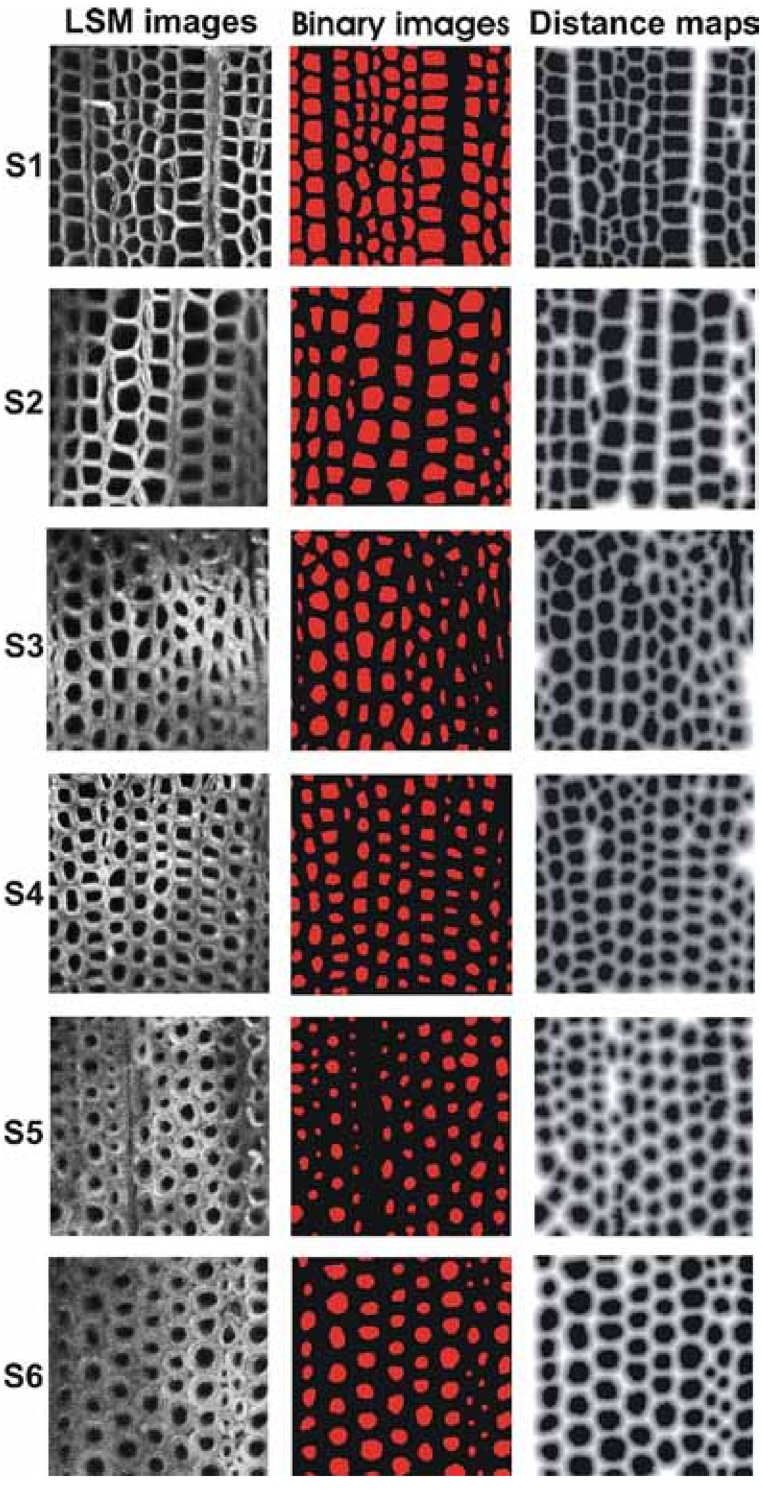

Fig. 3. Reprinted by permission from: Springer Nature, Trees 32, 1347–1356; Nedzved et al. (2018) Automatic image processing morphometric method for the analysis of tracheid double wall thickness tested on juvenile *Picea omorika* trees exposed to static bending. Trees 32, 1347–1356.: The CLSM images (first column), corresponding binary images (second column) and distance maps (third column) of *P. omorika* stem samples; S1 and S2 – NW samples; S3 and S4 - MCW samples; S5 and S6 – SCW samples

Our morphometric and non-morphometric methods for distinguishing conifer wood samples on a compression severity scale

In recent years we worked on the development of different morphometric and non-morphometric methods for distinguishing wood samples on a compression severity scale. The first method is based on tracheid double wall thickness analysis (Nedzved et al., 2018). The second method is based on cellulose microfibrils order (distribution and alignment of cellulose microfibrils) analysis in tracheid (double) walls (Savić et al., 2016). The third one we are still developing, based on the analysis of structural modifications of lignin, and it is related to our results published a few years ago (Mitrović et al., 2015).

These 3 methods cover the main features of CW, related to changes in tracheid cell wall shape, lignin and cellulose organization. Hence, alone or in combination with each other, they could be suggested for use in fine gradation of wood samples on the compression severity scale, either in the estimation of wood quality or in the estimation of environmental influences during growth and developmental process.

We present here, in more detail, 2 methods, one morphometric (Nedzved et al., 2018) and one non-morphometric (Savić et al., 2016), for distinguishing wood samples on a compression severity scale. In **Tab. 2**, the main characteristics, similarities, differences, and advantages of these methods are summarized.

It is known that radial and tangential walls can vary significantly regarding different features of wood cell walls. So far, most investigations, regarding cellulose microfibrils (Donaldson, 2008), or other features of wood cell walls such as cell wall thickness (Mork, 1928), have been carried out on radial cell walls. Accordingly, our methods confirm the selection of radial walls for the analysis as an excellent choice of tracheid cell wall region for the determination of MCW.

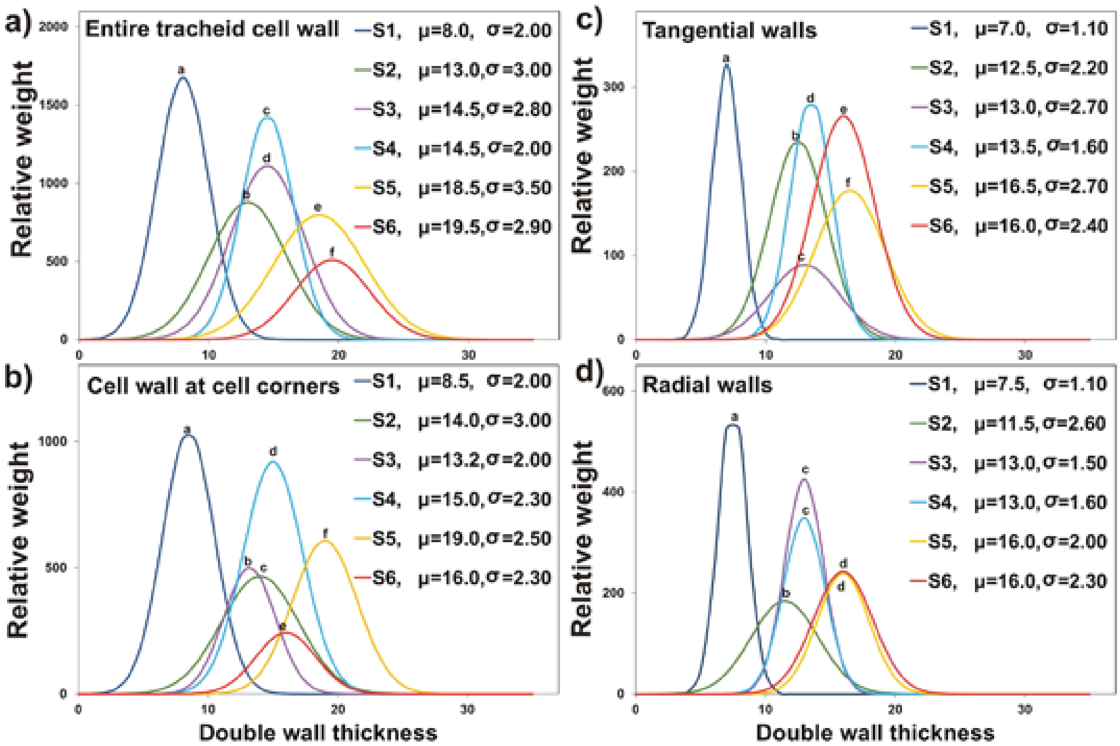

Fig. 4. Reprinted by permission from: Springer Nature, Trees 32, 1347–1356; Nedzved et al. (2018) Automatic image processing morphometric method for the analysis of tracheid double wall thickness tested on juvenile *Picea omorika* trees exposed to static bending. Trees 32, 1347–1356.: Distribution of double wall thickness of a) entire tracheid walls, b) tracheid at cell corners, c) tangential walls and d) radial walls, determined by morphological processing of structural elements with different orientation from distance maps obtained from the CLSM images of (S1 – S6) samples (Fig. 3); a, b, c, d, e, f - significant difference at 5% level of significance of the maxima positions of Gaussian curves between samples S1 – S6; μ - mean values, σ – standard deviation

Compression wood – reaction wood in conifers: formation, occurrence, range of gradations

Measurements of various anatomical characteristics of wood cells are of great importance in the research of wood structure. Tracheid double wall thickness, as an important wood anatomical feature, besides being known as an indicator of transition from EW to LW within an annual ring in gymnosperms (the ratio between radial double wall thickness and cell lumen diameter, Mork, 1928), also characterizes the differences between juvenile and mature wood (Mitchell & Denne, 1997; Loustarinen, 2012), as well as between normal and compression wood (Timell, 1986; Plomion et al., 2001). Hence, the determination of tracheid double wall thickness is of great importance in estimation of wood quality.

Image-processing techniques are used for estimation of the EW and LW ratio as a significant feature for forest products industry (Mork, 1928; Jagels & Dyer, 1983; Diao et al., 1999). However, such techniques for estimation of compression wood severity were more (Duncker & Spiecker, 2009) or less (Andersson & Walter, 1995; Nyström & Hagman, 1999; Moëll & Fujita, 2004) successful in recognizing MCW.

For testing of our automatic image processing morphometric method for the analysis of tracheid double wall thickness (Nedzved et al., 2018) we used confocal laser scanning microscopy (CLSM) images of stem cross sections of juvenile *P. omorika* trees exposed to static bending. Our algorithm (Nedzved et al., 2018) was developed using Imagewarp A&B Software company (USA) and software for image analysis QTIP developed in United Institute of Informatics Problems (UIIP), National Academy of Sciences (Belarus). It consists of the extraction of cell patterns from the original micrograph via binarization using Otsu's threshold method (Otsu, 1979) and reconstruction of the distance maps (Kimmel et al., 1996).

The use of Euclidian distance maps for calculating the thickness of the wood cell wall was suggested earlier in estimations of pulp or paper quality (Travis et al., 1996; Koskenhely & Paulapuro, 2005; Selig et al., 2012; Lorbach et al., 2012). The novelty of our method is the use of morphological image processing of structural elements with different orientation (Dougherty, 1992) on Euclidian distance maps reconstructed from microscopic images, allowing determination of the distribution of tracheid double wall thickness separately for tangential walls, radial walls, and cell corners (it yields valuable information on circularity/rectangularity of cross-sectional profile of tracheid cell wall).

We applied IBM SPSS software, Nonlinear Curve Fit function, for fitting the Gaussian curves on the data (distribution of double wall thickness in pixels) to show the overall variation in tracheid double wall thickness in a response to mechanical stress. For comparison of the maxima positions of Gaussian curves between samples, one-way ANOVA and Duncan test were used. As a result, our nonmorphometric image processing method provides a fine gradation of *P. omorika* juvenile wood samples on the compression severity scale from NW to SCW (**Fig. 4 a-d**), suggesting that it could be used as a tool for estimation of compression wood severity.

In addition, as different regions of *P. omorika* tracheid cell wall show somewhat different cell wall thickening in response to mechanical stress (**Fig. 4**), compression severity scales based on double wall thickness distribution of entire tracheids, tracheids at cell corners, tangential and radial walls, differ to some degree. Radial walls- double wall thickness distribution (**Fig. 4d**) shows the additional advantage and specificity over a number of methods for estimation of compression wood severity: it groups, and consequently sharply distinguishes MCW samples from SCW samples.

Our non-morphometric method for estimation of compression wood severity based on cellulose microfibrils order (Savić et al., 2016) - short report

The distribution and orientation of cellulose microfibrils (MFs) in wood cell walls is determined by both, genetic and abiotic factors. Genetic factors include: cell wall layer (primary wall, S1, S2, S3 layer of secondary cell wall), position (radial or tangential cell wall), plant age (juvenile or mature wood), season of maturation within the growth ring (early and late wood), while abiotic factors include wind and stem lean (compression wood, normal wood).

Since cellulose fibrils, as reinforcing material in conifer wood cell walls, determine tracheid cell wall properties and wood quality, one of the most frequently measured ultrastructural variables in wood cell wall is microfibrilar angle (MFA) (Donaldson, 2008).

Various microscopic techniques have been used to study the orientation of cellulose microfibrils in the wood cell wall, using variations in polarised light techniques or directly visualizing orientation of the microfibrils (Donaldson, 2008). In our method for estimation of compression wood severity (Savić et al., 2016) we observed the relative order of cellulose fibrils in cell walls. Fluorescence detected linear dichroism (FDLD) imaging was performed using confocal laser scanning microscope (CLSM) additionally equipped with constructed differential polarization extension (DPLSM). FDLD microscopy exploits fluorescence originating from cellulose fibrils stained specifically by Congo red, enabling screening of cellulose microfibrils order in the X–Y plane of the cross section, which means - separately in tangential and radial walls. The method was tested on stem cross sections of juvenile *P. omorika* trees exposed to static bending.

Blue colour represents dipoles (cellulose fibers) predominantly parallel with X-axis (tangential walls), yellow colour indicates dipoles (cellulose fibers) oriented predominantly parallel with the Y-axis (radial walls) (**Fig. 5**), while the grey colour represents fibers orientated at about 45°.

Image processing was performed using ImageJ program with macros developed for this analysis. FDLD images were quantified and presented as histograms (**Fig. 5**). The decrease in cellulose fibrils order from CW to NW samples is obvious, in both radial and tangential tracheid walls (**Fig. 5**). This is in line with Xu et al. (2011) work; they showed that the characteristics of cellulose fibrils reinforcements in S2 layer of severe compression juvenile wood include lower number, abundant dislocation segments and shorter length of cellulose MF, compared to NW.

We additionally showed (Savić et al., 2016) that radial and tangential tracheid cell walls in *P. omorika* juvenile wood differ considerably regarding cellulose fibril order, and that FDLD of radial walls (**Fig. 5 u**), showing fine gradation from CW to NW, could be suggested as an easily applicable technique for estimation of CW severity.

Picea omorika juvenile wood samples – a model for testing of methods for estimation of compression wood severity.

We tested our methods on stem samples of *P. omorika* juvenile trees exposed to long term static bending (bending procedure described in detail in Mitrović et al., 2015).

P. omorika belong to slow-growing conifer species in which CW typically occurs in a severe form (SCW) (Donaldson et al., 2004). Juvenile conifer wood is characterized by randomly distributed MCW, NW often being absent (Donaldson et al., 2004). These are the features that suggest *P. omorika* juvenile wood a good choice of samples for evalua-

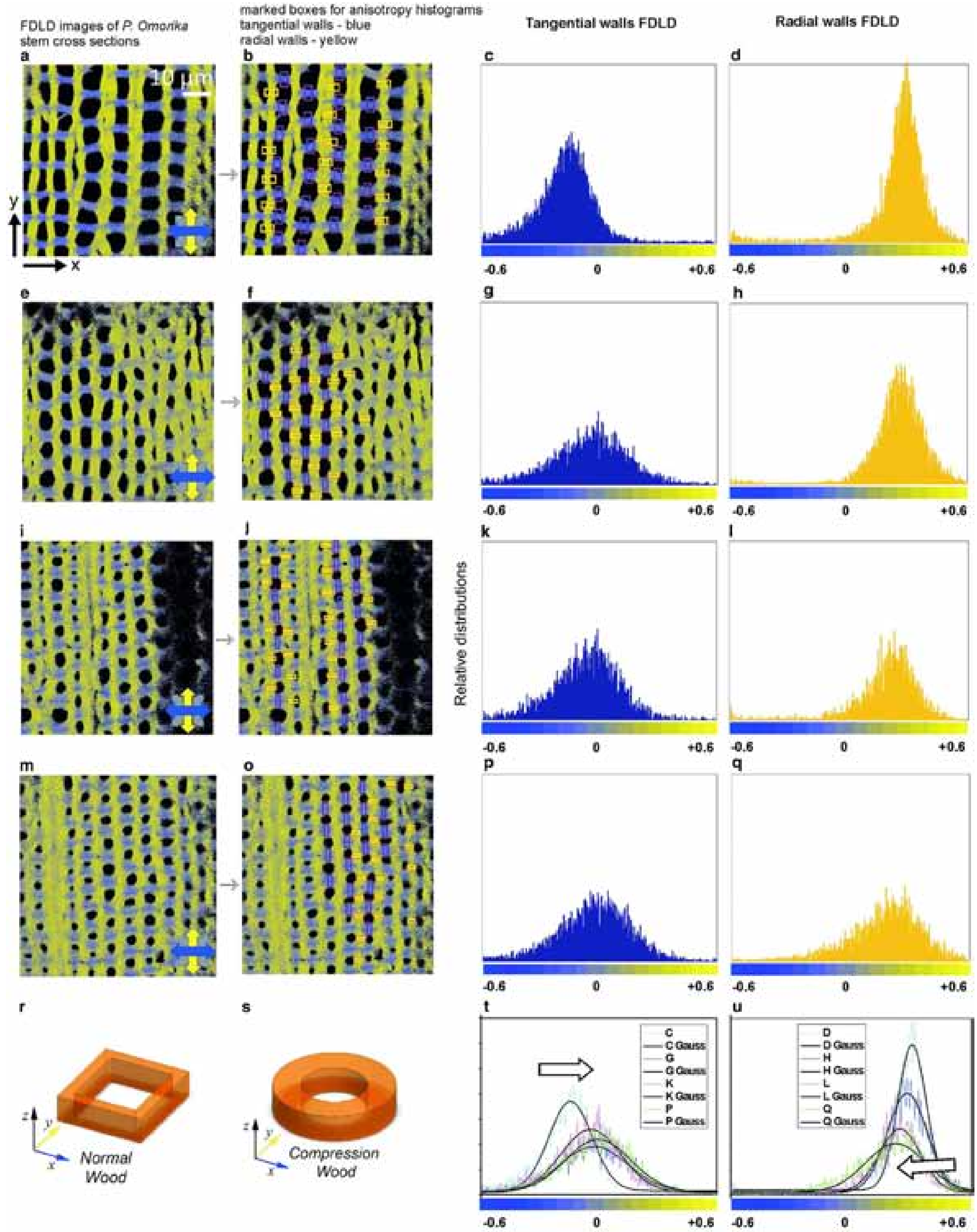

Fig. 5. Reprinted by permission from: Cambridge University Press, Microscopy and Microanalysis 22, 361–367.; Savić et al. (2016) Fluorescence-detected linear dichroism of wood cell walls in juvenile Serbian spruce: estimation of compression wood severity. Microscopy and Microanalysis 22, 361–367.: Fluorescence-detected linear dichroism (FDLD) images of *P. omorika* sections and corresponding anisotropy histograms. a: Normal wood (NW) samples; (e) mild compression wood (MCW) samples; (i,m) severe compression wood (SCW) samples; (b,f,j,o) pixel values were collected in the marked areas, in tangential walls (blue boxes) and in radial walls (yellow boxes), and used to obtain anisotropy distributions; (c,g,k,p) tangential walls FDLD distributions; (d,h,l,q) radial walls FDLD distributions; (t) overlaid distributions (c,g,k,p) with black lines representing corresponding Gaussian fits (white arrow represents gradual shifts toward gray—increasing number of disorientated fibrils); (u) overlaid distributions (d,h,l,q) with black lines representing corresponding Gaussian fits (white arrows represent gradual shifts toward gray—increasing number of disorientated fibrils); (r,s) schemes of NW and CW tracheid sections, respectively. Excitation at 488 nm, emission above 560 nm; image size is 64 × 64 µm

tion of the precision of methods suggested for estimation of compression wood severity.

After testing of our methods (Savić et al., 2016; Nedzved et al., 2018) on stem cross sections of juvenile *P. omorika* trees exposed to long term static bending, we can confirm *P. omorika* juvenile wood samples as a good model for testing of methods suggested for estimation of compression wood severity.

Conclusion

Picea omorika, despite its endemism, and thanks to her adaptability and long tradition of planting, nowadays is present widely outside its natural range.

We confirmed *Picea omorika* juvenile wood samples as a good model for testing of methods suggested for estimation of compression wood severity.

Our methods, based on the analysis of tracheid double wall thickness, cellulose fibril order and structural modifications of lignin, using confocal fluorescence microscopy and spectroscopy, cover all the main features of CW. Therefore, alone or in combination with each other, they could be a useful tool for fine gradation of wood samples on compression severity scale, as a valuable advantage over many other methods for the estimation of compression wood severity, as the determination of mild compression wood is difficult. Hence they can be of great benefit either for estimation of wood quality in forest products industry, or for estimation of environmental influences during growth and developmental process in tree physiology.

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