Czech University of Life Sciences
Faculty of Forestry and Wood Sciences
Department of Wood Processing and Biomaterials

PhD Thesis

Quantitative wood anatomy as a stress indicator at *Picea abies* stands in the Ore Mountains

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Ph.D. THESIS ASSIGNMENT

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Thesis title

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Objectives of thesis

The forests in the Czech Republic could serve as a good example for Central European areas with a long-term history of air pollution during the 20th century. The most significant influence was detected at high mountain regions covered with Norway spruce (Picea abies L. Karst), one of the most common and economically valid tree species in the country. The aims of our research thus will be:
1) to evaluate the impact of extreme stress events on wood anatomy parameters of
spruce trees at different damage levels;
2) to estimate the tree recovery stage on basis of quantitative wood anatomy analysis;
3) to evaluate dendroclimatic potential of wood anatomy parameters as stress indicators including their potential for long tree-ring series.

Methodology

The research will be carried out in the Ore Mountains, where permanent plots along the main ridge were established. For wood anatomy analyses samples from sites with different damage level will be gathered. Microslides will be prepared and analysed with the necessary digital equipment and software for image analysis. Using microslide analyses, quantitative wood anatomy parameters such as, for example, proportion of LW, cell-wall thickness, number of tracheids, and statistical analysis, the direct influence of abiotic stress factors on cell anatomy will be determined.

The proposed extent of the thesis

120-140

Keywords

wood anatomy, cell wall thickness, radial cell diameter, lumen width, latewood proportion, air pollution, Ore Mountains

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Recommended information sources

COOK, E. (1985): A time series analysis approach to tree ring standardization. Ph.D. Dissertation, The University of Arizona, Tucson, 171 pp.

FONTI, P., BRYUKHANOVA, M.V., MYGLAN, V.S., KIRDYANOV, A.V., NAUMOVA, O.V., VAGANOV, E.A. (2013): Temperature-induced responses of xylem structure of Larix sibirica (Pinaceae) from the Russian Altay. American Journal of Botany, 100/7, 1332 – 1343.

FRITTS, H. C. (1976): Tree Rings and Climate. Academic Press, London, New York, San Francisco, 567 pp.

GARTNER, B., ALONI, R., FUNADA, R., LICHTFUSS-GAUTIER, A.N., ROIQ, F.A. (2002): Clues for dendrochronology from studies of wood structure and function. Dendrochronologia, 20/1 – 2, 53 – 61.

GÄRTNER, H., LUCCHINETTI, S., SCHWEINGRUBER, F.H. (2014): New perspectives for wood anatomical analysis in dendrosciences: The GSL-1 microtome. Dendrochronologia, 32, 47 – 51.

GINDL, W., GRABNER, M. (2000): Characteristics of Spruce (Picea abies [L.] Karst.) latewood formation under abnormally low temperatures. Holzforschung, 54, 9 – 11.

GUERRIERO, G., SERGEANT, K., HAUSMAN, J-F (2014): Wood biosynthesis and typologies: a molecular rhapsody. Tree Physiology, 34, 839–855.

IPCC (2007): Climatic Change 2007. The physical scientific basis. Cambridge University Press. Cambridge.

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Prague on 14. 06. 2020

I declare that I worked out the presented thesis "Quantitative wood anatomy as a stress indicator at *Picea abies* stands in the Ore Mountains" independently and I quoted all used sources of information in accordance with Methodical instructions about ethical principles for writing academic thesis.

Prague, June 24th, 2020

Author's signature

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Abstract

The aim of the research was to evaluate the potential of wood anatomy parameters as stress indicators on base of changing cell characteristics and the proportion of earlywood/latewood in spruce trees damaged by pollution influence. The research was carried out in the Ore Mountains (Czech Republic). Sites were located along the gradient of forest damage after 1995/1996 cold winter (heavy, medium, and slightly damaged sites). All the stands were the pure spruce stands with 50-60-year-old trees. The research also took the advantage of the annually measured data from 1996 – 2013 on tree vitality, tree nutrition and air pollution. Analyses showed the effect of stress on lumen area and the amount of tracheids in the tree ring. The difference in reaction dynamics between individual parameters was recorded. The length of stands regeneration was shown to be from 1 to 3 years, depending on the pollution rate. At heavily polluted sites 1-year lag in growth reaction to stress was observed. Fluorine content in 2nd needle age class (F2) was highly significant parameter explaining the highest proportion of data variability and could be considered as a reliable stress indicator for young spruce stands in the Ore Mountains.

Different methods for earlywood/latewood demarcation (fixed and flexible thresholds) were tested to identify the most precise thresholds suitable for *Picea abies (L.) Karst.* growing in the analysed environment. T-test analyses revealed the significant correspondence between visual identification of earlywood/latewood transition and demarcation based on standard Mork's index and flexible density threshold derived as 80% of the maximum latewood density.

The calibration of Mork's index for Norway spruce was done as well. Index value equal or higher than 0.83 was shown to be enough to determine latewood cells in Norway spruce trees growing in temperate conditions.

Keywords: wood anatomy, cell wall thickness, radial cell diameter, lumen width, latewood proportion, air pollution, Ore Mountains, *Picea abies (L.) Karst.*

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1 Introduction into problematics

Radial growth of the tree (tree-ring width) is a result of a combined influence of tree genetics and environmental factors (Fritts 1976). Cook (1985) defines radial growth as a function of climate, disturbances (external and internal) and biological variability. This model was primarily constructed by Cook (1985) for ring-width characteristics and is applicable for cell growth analysis as well, as changes in tree growth are determined by modifications in cell structure (Wimmer 2002, Vaganov et al. 2006). The research of changing wood anatomy parameters under the environmental influence is usually defined as "ecological wood anatomy" (Wimmer 2002). Ecological wood anatomy focuses on two main points: 1) the description of direct changes in wood structure as a response to one or several stress factors and 2) the evaluation of adaptation strategies of the tree on base of correlation values between environmental events and individual cell parameters (Wimmer 2002).

Research in the wood anatomy field started in the beginning of the 20th century. Its potential for ecology was evident from the beginning, however, there were a lot of questions on how to obtain correct ecological evidence from the data, how to quantify and analyse it, how to link it with plant structure and adaptation strategies of the plants, which parameters to measure, how to evaluate the significance of data etc. (Carlquist 1980).

With time and practice the knowledge in this field expended a lot. Different cell features (cell diameter, tissue proportions, wall thickness, lumen area, latewood proportion, resin ducts, density fluctuations etc.) became a useful source of ecological information. All these factors react on stress events in a different way, which makes them a good proxy for dendroecological studies (Wimmer and Grabner 1997, Wimmer 2002, Olano et al. 2012, Ziaco et al. 2014).

High attention was paid especially to the trees at sites with severe climates (alpine and northern forests). Attempts were made to understand the link between the genetics of the tree and environmental signal in anatomical features (Gartner et al. 2002). Wimmer and Grabner (1997) showed, for example, that the amount of ethylene in trees increases with the influence of exogenous disturbances or stresses. Following this problem Guerriero et al. (2014) in their work summarised the knowledge about the influence of genes and hormones on wood formation to make this issue better understood. A lot of research during the past years were dedicated to the influence of droughts on wood anatomy. As water transport within the conifer trees depends greatly on tracheid size, even small changes in tracheid diameter can cause big implications in hydraulic conductivity and tree resistance to cavitation (Hacke and Jansen 2009, Ziaco et al. 2014). This problem was studied from different perspectives. For example, Martin-St Paul et al. (2014) focused on different methods used for evaluation of xylem vulnerability to cavitation. Oddo et al. (2014) in his research paid attention to the link between drought stress and plant-hydraulic conductance, which is dependent on the size of the conductive elements and modulated by potassium concentration in sap-solute content.

The application of wood anatomy is growing fast, and its potential is used in many areas (Choi and Kwon 2019, Cuny et al. 2019, González-Cásares et al. 2019, Olaoye et al. 2019 Babushkina et al. 2020).

Recent research showed that anatomical parameters can be useful not only for a short timescale analyses but can be applied for the evaluation of long-term changes in growth as well. For example, Fonti et al. (2013) in their research in the Russian Altay investigated the influence of summer temperatures on tracheid anatomy on a base of 312-year long chronology.

The combination of short-term intensive monitoring with long-term dendroanatomy allowed a better insight into tree growth by helping create the models of their responses to climate variability. It can help in reconstruction of past xylogenetic phases and improve the construction of vegetation models (Carrer et al. 2017).

1.1 Reaction of individual cell parameters to different types of stressors

A great amount of research focused on the description of individual stress environmental factors and their influence on cell structure can be found in the literature for both coniferous (Olano et al. 2012, Bryukhanova and Fonti 2013) and deciduous trees (Perez-de-Lis et al. 2016, Castagneri et al. 2017).

A number of researchers showed the importance of temperatures, especially during the beginning of the growing season, on tree growth (Rossi et al. 2008, Körner 2012). There is a lot of evidence that temperature around 0°C limit the formation of plant tissues (Körner and Paulsen 2004). Growth remains slow in cambial and apical meristems both above and under the ground between 0°C - 5°C (Rossi et al. 2008). Low temperatures can inhibit resource distribution in plant (Rossi et al. 2008). In wood anatomy it can be reflected by different ways. Pritzkow et al. (2014) showed the negative correlation of the minimum lumen area with summer temperatures and precipitation. Other cell parameters, as number of cells and lumen length, on the opposite showed significantly positive correlation with summer temperatures. Significant influence of preceding autumn was also pronounced. Mean lumen area and maximum lumen length showed a strong precipitation signal. Mean lumen area, for example, reflected the importance of precipitation during

July – September period. Strong correlation between cell number and summer temperatures showed also Carrer et al. 2017. Ziaco et al. (2014) found out significantly high correlations between spring temperatures and earlywood anatomy. Olano et al. (2012) pointed out that tracheid size at the beginning of xylogenesis highly depend on the winter conditions, with a larger tracheid diameter after warm February. Park and Spiecker (2005) showed that trees growing at colder site have larger lumen and thinner cell walls, which lead to a higher proportion of earlywood. Another research came to a different result and supposed that smaller lumen areas in trees at cold climates can be the adaptation mechanism to withstand freezing-induced embolism (Pittermann and Sperry 2003).

Frost events cause the changes in cell lignification thus leading to the appearance of less lignified terminal tracheids (Gindl and Grabner 2000).

Interesting research was conducted by Pacheco et al. (2015). They compared the growth of Aleppo pine and Spanish juniper growing in the Mediterranean area, where soil water availability is the main driver of wood formation. It was shown that wet conditions during the early growing season in the spring or before the winter result in numerous tracheids with relatively large lumens in case of pine, while lumens are smaller in juniper. This makes juniper species less vulnerable to cavitation as compared to pine species (Willson et al. 2008), which means less vulnerable to drought-induced die offs (McDowell et al. 2008). In conditions of enough precipitations pines showed better growth rates in comparison with junipers. Wet and cool May conditions enhanced lumen in both species. Similar climatic signal was observed for the tree ring widths (Pasho et al. 2012). The difference in the root system between two species also led to the variations in wood anatomy structure. Junipers having shallower roots than pines are more dependent on summer and autumn precipitations and they are therefore more prone to formation of latewood intraannul density fluctuations, IADFs, (Camarero et al. 2010). Warm winter conditions led to thicker cell wall for the whole tree ring at both pine and juniper trees. Warm growing season and autumn conditions preceded by lower soil moisture enhanced cell wall thickness in Spanish juniper while at Aleppo pine the same effect was caused by wet summer (Pacheco et al. 2015).

To summarise the above-mentioned examples, we can conclude that the tree growth is a complex and complicated process dependent on tree species and specific environment. It is sensitive to any changes in the surroundings, which is reflected not only in its radial and height increment but also in its anatomical structure.

Besides the influence of water and temperature conditions, there are a lot of other environmental factors that can be reflected in the structure of tree rings. For example, influence of lightening on tree growth can cause callus formation, traumatic resin ducts formation, cell collapse as it was described by Schweingruber (2007). One of the most well-known reactions of the wood on mechanical stress is the formation of reaction wood, which is the result of non-optimal orientation of the stem caused by gravity (wind, snow, slope etc.) (Du and Yamamoto 2007). Reaction wood is divided into two types: compression wood in coniferous trees and tension wood in deciduous trees. Reaction wood is characterised by round tracheids with thicker cell walls (latewood like) caused by changes in its submicroscopic structure. Spaces in-between tracheids are observed as well (Gandelová et al. 2002).

Wood anatomy changes caused by insect gradation can be reflected in the formation of false rings, light rings, and pith flecks (Timell 1986, Filion and Cournoyer 1995).

1.2 Influence of anthropogenic stress on wood anatomy

One of the most typical anthropogenic stressors on tree growth is pollution, which can influence the tree growth in different ways (Schweingruber 2007). On the basis of a wide literature research Schweingruber (2007) showed that very often it is difficult to distinguish the influence of air pollution in cell structure from other environmental factors, as its signal can be covered by stronger stresses.

Sulphur oxides can influence the tree growth by limiting the availability of basic elements, such as magnesium (Lomský et al. 2013). The damage will be observed first on the needles (or leaves) of the trees (defoliation, yellow colour, Lomský et al. 2012). Strong influence of sulphur oxides will be reflected in the wood structure as well. On the basis of the research in the Norilsk area (Sibirean larch, Siberian spruce), where forests were highly damaged by sulphur dioxide emissions, the variability in latewood cell wall growth with the process of tree dying was shown at larch stands (Schweingruber 2007). It was pointed out that cell elongation was not affected by pollution and that tracheids size remained the same at spruce stands. Schweingruber (2007) also observed that the tree-ring boundary at larch species was usually marked by flattened cells with thin walls. At Baikal in Siberian fir samples the changes in the anatomy were observed gradually as the tree was dying and latewood was rudimental (only one row or discontinuous row of flattened latewood cells).

The influence of oxides of nitrogen can be negative as well. Though nitrogen directly influences the increment of tree biomass, large amounts of it can slow down the growth of the tree and increase the acidification of soil (Norhstedt 2001).

It is assumed that conifers are more susceptible to pollution influence than deciduous trees (Vacek et al. 2013).

Wimmer (2002) carried out his research study in the German part of the Ore Mountains. His study focused on the influence of SO_2 emissions on the growth of *Picea abies* forests at stands with different damage level. The following anatomic parameters were analysed: latewood proportion, maximum latewood density, wood ray height, amount of resin ducts and microfibril angle.

One of the latest research of the pollution influence on Norway spruce growth in the mountains of Central Europe (the Sudetes) was done by Myśkow et al. (2019). Their research group showed that the main changes in anatomical structure were observed in the earlywood part of the tree ring, where the decrease in number of tracheids and lumen width was observed. On the opposite the latewood tracheids characteristics stayed homogenious. The increased number of resin ducts was observed as well (Myśkow et al. 2019).

1.3 Earlywood and latewood problematics

Earlywood (EW) and latewood (LW) properties and structure has been studied since the 1960s. A lot of attention was paid to how their characteristics influence mechanical, physical, and chemical properties of the wood (Stamm 1973, Warren 1979, Taylor and Moore 1981). The first application of EW and LW research in the field of forestry and ecology appeared (Smith et al. 1966, Doley 1974). A more intensive and productive period in earlywood and latewood research in ecology began after 1990. The investigations were made for both coniferous and deciduous trees, with the prevailing of the latter (Schweingruber 2007). Vaganov et al. (2006) showed that wood anatomy parameters are mainly determined by genetics of the tree, but a large part of intra and inter annual variability is a result of adjustments of their xylem structure to the climatic and environmental conditions trees are growing in (Fonti and Jansen 2012, Wimmer 2002). Anatomical features, especially those related to hydraulic efficiency, contain climatic and environmental information, and can enhance traditional dendroclimatic studies (Olano et al. 2012).

Earlywood and latewood characteristics as well as their anatomy can be strongly influenced by different abiotic and biotic factors (Schweingruber 2007). Moreover, their reaction differs according to the type of environmental or anthropogenic stress (Park and Spiecker 2005, Schweingruber 2007, Olano et al. 2012, Ziaco et al. 2014). Lebourgeois et al. (2010) showed that EW and LW width can provide more detailed information about site conditions than the total tree ring width.

Among the most widely studied wood anatomy parameters besides already mentioned number of tracheids, lumen area, radial lumen width, radial diameter of the cell, and cell wall thickness, is also the proportion of LW. For example, Rigling et al. (2002) showed lower latewood proportion in the tree rings of conifers at dry sites in comparison with mesic ones. Axelson et al. (2014) have found a decrease of LW proportion in tree rings of Douglas fir after the outburst of Southern spruce budworm. Ziaco et al. (2014) showed significant influence of spring temperatures on earlywood anatomy. Park and Spiecker (2005) pointed out that trees growing at colder sites have larger lumen and thinner cell walls, which lead to a higher proportion of earlywood. Mild winters, high spring and low June temperatures were shown to increase the LW/EW ratio in Norway spruce (Miina 2000). Domec and Gartner (2002) highlighted that latewood is more vulnerable to embolism than earlywood. In general, earlywood characteristics usually reflect the conditions of the early season or the growing season of the previous year, while latewood is changing under the influence of the current growing season (Martin-Benito et al. 2013).

Air pollution can influence EW and LW in different ways as well. On the basis of the research at Norilsk area Schweingruber (2007) showed that the tree ring boundary at larch species was usually marked by flattened cells with thin walls. Sites with higher defoliation level, caused by air pollution, were characterised by a smaller proportion of LW in the years following the stress event due to the pollution influence on the cambial activity through changing the differentiation of cambial derivatives in xylem side (Kurczyńska et al. 1997). The same impact of pollution on conifer growth was also observed by Wimmer (2002) in Norway spruce. Myśkow et al. (2019) on the opposite showed the main changes in the size and number of EW tracheids which supported the increase in latewood density.

To make the correct estimation of EW/LW properties (their density, proportion, characteristics of cell walls and lumen), however, is important to choose the suitable demarcation method for EW/LW zones. EW/LW characteristics can directly influence the mechanical and physical properties of the wood. There are different methods of defining the EW/LW border, such as Mork's index, the threshold density method, the inflection point method (Samusevich et al. 2020). Mork's index is one of the most popular methods. It is based on the cell wall/lumen relation within the cell (Mork 1928). According to Mork's index latewood cell is defined as a cell where double cell wall thickness/lumen width ratio exceeds "1". This principle is widely used and easily applicable for the trees in theory. In practice however, there are usually substantial variations in the structure of the tree ring, especially for trees growing in the extreme environments. Among the most known of such variations are light tree rings (Liang et al. 1997), dark rings (Novak et. al., 2016) and IADFs (Campelo et al. 2007, De Micco et al. 2007, Vieira et al. 2009). Another problem in EW/LW demarcation can be represented by trees with a gradual transition from earlywood to latewood as for example in *Picea abies.* In such cases a modification of Mork's index should be made (Park et al. 2006). The age of the tree can also influence the demarcation results and must be taken into consideration (Antony et al. 2012).

The X-ray densitometry method (Cown and Clement 1983) is also widely used for the determination of the EW/LW border, as it allows to proceed with a continuous measurement and to determine the minimum and maximum densities for both EW and LW, EW and LW proportions, average density for the ring, etc. This method however also faces the problem of transition zones (Cown and Ball 2001). A more detailed insight into these problematics was given by Samusevich et al. (2020) and will be presented in the results.

2 Aims of the research

The main aims of the research were shaped based on the gaps in wood anatomy studies and potential of the Ore Mountains area as described in chapters 1 and 3.
The main aims are:

1) to evaluate the impact of extreme stress events on wood anatomy parameters of Norway spruce trees at sites with different damage level;
2) to estimate the tree recovery stage based on quantitative wood anatomy;
3) to evaluate the dendroclimatic potential of wood anatomy parameters as stress indicators, including their potential for long tree-ring series;
4) to improve the methodology for analysis of EW/LW anatomy parameters based on *Picea abies* trees growing in the Ore Mountains.

3 Research in the Ore Mountains

3.1 *Picea abies* potential for study of pollution influence tree growth

Picea abies is one of the most important commercial tree species in the northern hemisphere. Its timber production started in the beginning of the 20th century. Since then it is widely studied from the point of view of taxonomy, morphology, genetics, wood chemistry, forestry, wood anatomy, wood technology etc. (Lagercrantz and Ryman 1990, Goncharenko et al. 1995, Anttonen et al. 2002, Koprowski and Zielski 2006, Rybníček et al. 2012). An interest in the research of

Norway spruce forests grew immensely in connection with the long-term acidic air pollution, which was typical for the second half of the 20th century in Europe (Stern 2005).

Economic growth after World War II led to a rapid increase in global emissions, mainly sulfur dioxide and nitrogen oxides (Grübler 2002, Smith et al. 2011). The highest loads were observed in the so called black triangle (Grübler 2002), an area with numerous coal power plants (Kopáček and Veselý 2005) described as "ecological disaster zone" (Grübler 2002). One of the first areas where the decline of spruce forests in connection with the black triangle attracted attention was Bavarian West Germany, where in the late '70s the wide yellowing and loss of needles was observed. In '80s around 25% of *Picea abies* stands in the area were classified as moderately damaged from unknown factors. Few hypotheses were formulated: from natural climatic variations, through fungal infection, to gaseous pollutants (NO_x, ozone and SO_2) and emissions of SO_2 from burning coal. Cation leaching and acidification of soil with Al as well as nutrient deficiency were named as the main reasons. On the territory of the Czech Republic an increased level of pollutants was observed between 1950 and 1980 (Kopáček and Veselý 2005). Forests in the Czech Republic could thus serve as a good example for Central European areas with a long-term history of air pollution as well.

High and long-term sulfur dioxide pollution in the Czech Republic led to extensive forest decline (Vávrová et al. 2009). High-elevation conifer ecosystems were among the most damaged areas (Vacek et al. 2013). The greatest decline of growth was observed between 1979 and 1982. Based on observations in 1980, 99% of trees reacted negatively to air pollution. Other declines were also observed in 1974 and 1996. The most pronounced tree injuries were typically observed during winter periods due to temperature inversions and high SO_2 concentration (Lomský et al. 2012), which for example happened in the Ore Mountains (Krušné hory) in the winter of 1977/1978. Drops in temperature can cause buds damage and change in phytohormone content. When touching the plant, pollutants causes chlorophyll damage and withering of the needles (Hruška et al. 2009). Moreover, the tree is losing adaptability to emissions. The same way forest health stay in the Czech Republic was interrupted by extreme winter in 1995/96. Stress years 1995/1996 are characterised by a sudden temperature decrease in November 1995, which was followed by heavy frosts and long-term inversion in the Ore Mountains, resulted in extreme frost deposits and high winter transpiration in February and March 1996. It also created good conditions for air pollutants accumulation (SO_2 and F). The most affected area within the Czech Republic was the eastern part of the Ore Mountains, where the air pollution load was the highest. Here about 12 500 ha of spruce stands were heavily damaged, 1 300 of which has completely died.

Pollution stress caused missing tree rings and increasing defoliation as well (Vacek and Matějka 2010). The decline in forest stands, poor crown condition, and yellowing of the needles were recorded by numerous researchers (Akselsson et al. 2004, Rydval and Wilson 2012, Vacek et al. 2013).

Anthropogenic emissions dropped considerably in the early 1990s due to political and economic change. According to the Gothenburg Protocol, the Czech Republic achieved its targets in the reduction of air pollution already in 2007, when SO_2 emissions had decreased by 88% and NO_x emissions by 62% (Helliwell et al. 2014). The decrease of the air pollution load led to an improvement of the forest condition in Central European countries, especially in formerly heavily polluted areas. Despite occasional declines in growth after 1990 due to meteorological conditions, a tree growth recovery was observed (Treml et al. 2012). As soon as the influence of pollution dropped, May–July temperatures and March precipitation effects on tree growth intensified again, as Norway spruce is extremely sensitive to temperature regime and precipitations (Mäkinen et al. 2000, Savva et al. 2006).

The situation in the forests of the Czech Republic is observed and monitored by Forests of the Czech Republic (a state-run enterprise) and Ministry of Agriculture of the Czech Republic. Their common interest resulted in a project, Forestry Management in the Air Pollution Area of the Krušné Hory Mountains, financed by the Grant Agency of Forests of the Czech Republic. Different other accompanying projects launched by Ministry of Agriculture and universities study the condition of forest stands and game management in the Ore Mountains. The availability of long-term series of measurements, allowing the detailed analysis of forest stands during the second half of the 20th century creates a good background for different types of research in this area.

3.2 Research area

The Ore Mountains are situated on the border between the Czech Republic and German Saxony. It is a typical mountain region with an area of 180 015 ha. It is a long mountain range (around 130 km on the Czech side) stretched in NE-SW direction. The width of the range is only 6-19 km (Lomský et al. 2013).

Mother rock is formed by gneiss, granite, phyllite and mica schist. The climate of the Ore Mts. is moderately cold with mean July temperatures around 12 – 15°C and mean annual temperature 5,4°C. On the higher parts of the range mean July temperatures can drop below 10°C. Border parts of the mountains are warmer with higher amount of precipitations. The average precipitation rate equals to 750 mm, with around 450 mm during the vegetation period that lasts for 112 days.

The most common soil type (around 43.7%) is formed by Podzols, followed by Cambisols (39.8%) (Kulhavý et al. 2008).

For our research purposes 9 permanent plots along the main ridge were established. Plots were located along the gradient of forest damage after the winter 1995/1996 in similar site conditions, to exclude the microsite influence. All the stands were chosen to be pure spruce stands. The sites were divided into three groups according to the defoliation rate: slight damage (defoliation rate under 40%), medium damage (40 – 60%) and heavy damage (above 60%) (Fig. 1, Tab. 1). The analysed period was 1991 – 2001. The plots have an area of 25x25 m. The defoliation of trees and their annual increment was assessed.

Fig. 1 Map of the sampled plots

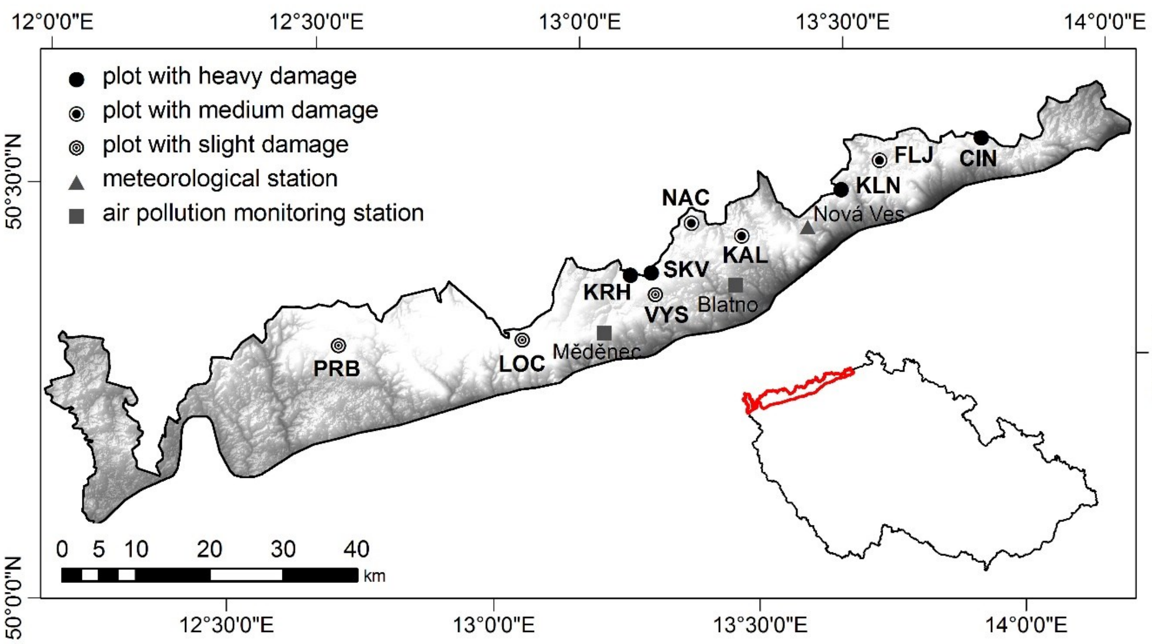

Table 1 Characteristics of the research plots in the Ore Mountains

Plot	Coordinates JTSK		Altitude, m a.s.l.	Exposition	Age class*	Forest type	Damage level 1995/1996
Cínovec (CIN)	777321,9	965822,9	820	NW	6	Acidic Beech–Spruce	heavy damage
Fláje (FLJ)	791175,4	968867,6	750	SW	6	Acidic Beech–Spruce	medium damage

Klíny (KLN)	796352	972834,5	800	0	6	Acidic Beech–Spruce	heavy damage
Kálek (KAL)	809840,9	979101,5	815	SW	6	Acidic Beech–Spruce	medium damage
Kryštofovy Hamry (KRH)	816671,8	977359,6	900	W	6	Acidic Beech–Spruce	heavy damage
Skelný vrch (SKV)	822108,5	984114,8	875	NE	5	Acidic Beech–Spruce	heavy damage
Výsluní (VYS)	821557,6	987059,2	810	SW	6	Acidic Spruce–Beech	slight damage
Loučná (LOC)	5587800	3356300	990	NW	5	Acidic Beech–Spruce	slight damage
Přebuz (PRB)	864532,6	993937,8	885	0	5	Nutrient-poor Beech–Spruce	slight damage

*Age 5: 40–50 years old, age class 6: 50–60 years old

3.3 Methodology

3.3.1 Collecting samples for dendrochronological analysis. Creating ring-width chronologies

Dendrochronology and quantitative wood anatomy are based on extracting information from stems' structure, shoots, branches, roots etc. The sample from the tree is usually taken with the help of an increment borer, which for wood anatomy purposes should have a well sharpened cutting edge to avoid any micro-cracks that can influence the further processing. It is important to core in exact radial position from bark to pith and keep the borer in a fixed position during the drilling. The use of a pusher is recommended, but not necessary. Cores of bigger diameters (10–12 mm) are preferred to minimise the risk of fractures and twisting. Depending on the purpose of the research and tree species the diameter of the borer can be smaller. Samples can be also extracted from stem discs; in branches and smaller plant stems and/or root collars the entire samples can be used (von Arx et al 2016). Detailed description of collection procedures and further storage and labelling of the samples is given in Gärtner and Schweingruber (2013).

Dendrochronology analysis was made to better understand the area, detect tree growth patterns, and estimate the factors influencing the tree growth. Increment cores from at least 20 dominant or co-dominant trees were sampled from each plot in 2014. Two cores per tree were taken at breast height.

Ring-width chronologies were developed using the standard dendrochronological methods (Cook and Kairiukstis 1990). Ring widths were measured with the accuracy of 0.01 mm, using

TimeTable 2, and were cross-dated and statistically verified using the COFECHA programme (Holmes 1983). Mean sensitivity, the average correlation with master chronology and the first-order autocorrelation in the series were computed for tree-ring chronologies.

The ring-width series were standardised using the ARSTAN programme (Cook and Holmes 1996). The trend was approximated by the Hugershoff function (Warren 1980). The remaining autocorrelation was eliminated by autoregressive modelling. Stand-level chronologies were created using bi-weight robust mean (Vejpustkova et al. 2017).

3.3.2 Collecting samples for wood anatomy analysis. Creating microsections

For wood anatomy analyses three trees per site were chosen and sampled: always two cores per tree. The 1991 – 2001 period was analysed for anatomical parameters.

In general, microsections can be prepared with a sledge or a rotary microtome. Their ideal thickness is 10–20 μm, though 30 μm is also acceptable. The thinner the microsection is, the more precise measurements of the cell elements (lumen length and cell wall thickness) are possible to achieve. On the other hand, the microsection should be thick enough for distinguishing between the cell parts. The cut microsections are usually stained with safranin and astrablue to create contrast in an anatomical slide (Gärtner and Schweingruber 2013). Boiling, soaking the samples in water, embedding in paraffin, or using corn starch solution can help avoid damages in cell structure while cutting (Schneider and Gärtner 2013). Depending on the tree species, other methods can be used for getting quality microsections. For example, if samples have narrow cell lumen, rice starch is advised instead of corn starch. While analysing samples of ring-porous species it is efficient to smooth the wood surface by sanding, then remove sawdust and tyloses using high-pressure air or water blast, and use the chalk to increase the contrast between cells (Gärtner and Schweingruber 2013). All the microsections within the same research should be cut with the same thickness to avoid deviations in the measurements (von Arx et al. 2017).

Microtome blades must be sharp and without defects to avoid damage of the cell structure. It is therefore important to change the blade/use unused part of the blade after few cuts. According to von Arx et al. (2016) good results in preparation of microsections were achieved by using Leica DB80 LX and Leica 819 low-profile blades (Leica Biosystems, Wetzlar, Germany), and Feather N35HR and N35 blades (Feather Safety Razor Co., Ltd., Osaka, Japan, Prislan et al. 2013, Gričar et al. 2014, Pacheco et al. 2015, Pellizzari et al. 2016). To choose the right blade one should experiment a little and take into consideration the type of the microtome, tree species and purpose of the research.

Wood samples should be cut perpendicular to the axially oriented xylem cells to avoid over- and underestimation of the measured anatomical features. For longitudinal sections wood samples should be cut parallel to the axially oriented xylem cells (von Arx et al. 2016).

Our microsections were prepared according to the following steps. In the laboratory the cores were segmented into 2 – 3 cm sections. The segments were cut using the GSL-1 microtome (Gärtner et al. 2014). The microsections were sliced into 15 µm and non-Newtonian fluid was used to preserve the integrity of the cells (Schneider and Gärtner 2013). They were stained using Safranin, then dehydrated with alcohol, embedded in Canada balsam and dried (50-60 °C, 24 h) (Gärtner and Schweingruber 2013).

3.3.3 Making the images of microsections

High-resolution digital images of anatomical sections are usually obtained with a camera mounted on an optical microscope. To observe and analyse conifers 10x objectives are usually recommended, while for angiosperms the 4x objectives are usually sufficient (von Arx et al. 2017).

Once the image is produced, different image-analysis tools are used to quantify the anatomical features. The most common ones are ImageJ (Rasband 1997–2016), ROXAS (von Arx and Carrer 2014, www.wsl.ch/roxas), WinCELL (Guay 2013) and NIS Elements software (Nikon Instruments). The tools differ in functioning and their choice usually depends on the needs of the research. For longer wood anatomy series ROXAS proved to be the most convenient and fast tool for measurements of the cell parameters. NIS Elements can be quite a good substitute for it. However, the good quality of images is essential for the usage of any of the above mentioned image analysis software.

Our permanent slides were photographed at a magnification of 20x using NIS Elements software. Eleven annual tree-rings were measured along 3 to 5 files depending on the ring width. The following anatomical parameters were measured: number of cells, radial lumen width, cell-wall thickness, and radial cell diameter (Samusevich et al. 2017, Vejpustkova et al. 2017). The same parameters were measured for EW and LW part of the tree ring.

3.3.4 Densitometry analysis

Density of the samples was defined using air-dry method. Sample moisture content was determined before density measurements. The cores were cut transversely into 1.2-mm strips with the DENDROCUT twin-bladed saw (WALESCH Electronic GmbH, Effretikon, Switzerland;

www.walesch.ch) and measured with the QTRS-01X Data Analyzer and Scanner (QMS, Knoxville, TN, USA; www.qms-density.com). The highest (i.e. 0.02 mm) linear resolution step was used for the measurement. Densitometry analysis was used to improve EW/LW demarcation methodology in Norway spruce trees growing in the temperate climate (Samusevich et al. 2020).

Methods used for EW/LW demarcation are listed in the Table 2.

Table 2 Density thresholds and corresponding latewood percentages calculated based on wood anatomy measurements and densitometry results (Samusevich et al. 2020).

Type of density threshold (DT)	Definition	Latewood percentage (LW%) based on given DT	Data source
DTvisual	DT calculated from LW width determined by visual evaluation applied to the results of densitometry. The DT was calculated by averaging density value corresponding to visual LW width and four density values measured at the nearest side of this point.	LW%visual	Densitometry/ visual analysis
DTmork	DT calculated from LW width determined by Mork´s criterion applied to the results of densitometry. The DT was calculated by averaging density value corresponding to LW width and four density values measured at the nearest side of this point.	LW%MI	Densitometry/ wood anatomy analysis
DT450	Fixed density threshold of 450 kg/m^3.	LW%450	Densitometry
DTqm	Quadratic mean of measured densities within the entire tree ring.	LW%qm	Densitometry
DTavg	Average of measured densities within the entire tree ring.	LW%avg	Densitometry

DT50 DT60 DT70 DT80	DT calculated as specified percentage of the maximum LW density.	LW%50 LW%60 LW%70 LW%80	Densitometry
DTmaxmin	Average of maximum and minimum density within a ring (according to Nicholl and Brown (1971)	LW%maxmin	Densitometry
DT2/3	DT2/3=2/3*(density max – density min)	LW%2/3	Densitometry
DT2/3+	DT2/3=[2/3*(density max – density min)]+density min (according to Hylen, 1999)	LW%2/3+	Densitometry

3.3.5 Statistical analysis of data

Statistical evaluation of data and extraction of the relevant information from numbers of rows is an important part of any research. It requires the knowledge of statistics and good understanding of data. The methodology often changes with time following new technologies and findings in the field. Different statistical characteristics are used for different purposes. Already van Vliet (1979) in his research showed which information can be obtained with the help of various statistics, from numerical ranges for particular features, to means, medians and extreme values.

Doing the analysis of wood anatomy data, the understanding of ecological regimes and extremes responsible for plant structure rather than model ecological situation is important: rainfall and water regime in the soil, temperatures, humidity, shading and sun exposure, anthropogenic factors, extremes and others. The complex measurement of site conditions is necessary to get quality results (Carlquist 1980). Statistical methods always differ based on the research, from simple descriptive ones to complex statistical modelling. The methods that have been used within this research are detailly described in the attached articles (Samusevich et al. 2017, Vejpustková et al. 2017, Lexa et al. 2018, Samusevich et al. 2020).

4 Publications

During the given research project, the following articles were published:

1. SAMUSEVICH, A., LEXA, M., VEJPUSTKOVÁ, M., ALTMAN, J., ZEIDLER, A. (2020): Comparison of different methods for demarcation between earlywood and latewood parts in tree rings of young Norway spruce trees. Dendrochronologia 20, 1 - 6.

2. SAMUSEVICH, A., ZEIDLER, A., VEJPUSTKOVÁ, M (2017): Influence of Air Pollution and Extreme Frost on Wood Cell Parameters at Mountain Sprce Stands (Picea Abies (L.) Karst.) in the Ore Mountains. Wood Research, 62(1).

3. VEJPUSTKOVÁ, M., ČIHÁK, T., SAMUSEVICH, A., ZEIDLER, A., NOVOTNÝ, R., ŠRÁMEK, V. (2017): Interactive effect of extreme climatic event and pollution load on growth and wood anatomy of spruce. Trees, 31 (2)

4. LEXA, M., VEJPUSTKOVÁ, M., SAMUSEVICH, A., ZEIDLER, A. (2018): Stopa Imisní Kalamity v Anatomických Znacích Dřeva Smrku (Picea Abies (L.) karst) v oblasti Klínovce (Krušné Hory). Zprávy Lesnického Výzkumu.

4.1 Comparison of methods for the demarcation between earlywood and latewood in tree rings of Norway spruce

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Highlights

- The methodology of calibration of criteria for EW-LW demarcation under specific conditions is tested and proposed.
- Density threshold expressed as 80% of the maximum LW density is a reliable method for EW/LW demarcation in Norway spruce.
- Modified Mork's index of 0.83 is appropriate for demarcation between EW and LW in tree rings of Norway spruce.
- Developed discriminant function can be used for EW/LW cells classification as an alternative to the Mork's index method.

Abstract

The precise demarcation between earlywood and latewood is important for the detailed analysis of intra-annual tree ring features. Different techniques based on visual assessment, wood anatomy analysis and X-ray densitometry have been developed and are currently used for this purpose. Depending on the chosen method, tree species and environmental conditions, the results can significantly vary. Thus, it is important to determine the technique optimal for a particular research. Here, we investigated Norway spruce (*Picea abies)* tree rings to examine the agreement among the following demarcation methods: (1) direct visual assessment, (2) Mork's index (anatomical definition of the transition from earlywood to latewood based on cell wall-lumen ratio) and (3) fixed and floating density thresholds applied to intra-ring density profiles. The aim was to modify both the Mork's criterion and density thresholds on the basis of reference values given by visual identification of earlywood/latewood transition. A total of 231 tree rings were analysed by all methods. Our results showed that the usage of floating threshold (defined for each ring separately based on density profiles) is more reliable in comparison with fixed threshold (the same threshold value used for all tree rings and samples). Statistical analysis revealed the best correspondence between visual identification of earlywood/latewood transition and demarcation based on the standard Mork's index and the floating density threshold derived as 80% of maximum latewood density. In terms of Mork's index calibration, the results showed that to determine latewood cells in Norway spruce trees growing in temperate conditions, it is sufficient to use an index value equal to 0.83. The results are applicable for the studied spruce population growing in a temperate climate. The methodology itself, however, is universal and can help to calibrate criteria for earlywood-latewood demarcation under specific conditions.

Keywords: wood anatomy, Mork's index, density threshold, X-ray densitometry, *Picea abies*

4.1.1 Introduction

Intra tree-ring density variation is caused by changes in the anatomy of tracheids and the transition from earlywood (EW)[1] to latewood (LW) (Rathgeber et al., 2006, Björklund et al., 2017). It provides information on wood formation and physiological processes (Cuny et al., 2014, Carteni et al., 2018); valuable climate signals can be extracted from EW and LW features (Wood and Smith, 2015, Arsalani et al., 2018, Acosta-Hernandez et al., 2019). The precise estimation of the EW/LW transition point is crucial; however, up to now, a generally applicable methodology of EW/LW demarcation, which takes into consideration a tree-species and specific environmental conditions, is missing.

The classic way to distinguish between EW and LW is by using the Mork's index (Mork, 1928, Denne, 1988). This method is based on wood anatomy measurements and requires the precise and consistent measurements of lumen width and cell wall thickness in radial direction, obtained from tree ring micro sections, representing a thorough, but labour-intense method.

Another commonly used method of determining the EW/LW transition point is X-ray densitometry (Polge, 1963, Schweingruber et al., 1978), which is based on the density measurements within the tree ring where the tree-ring part of densities exceeding a certain threshold is considered the LW (Mothe, 1998, Antony et al., 2012). A threshold value varies from species to species and is usually set manually. Densitometric analysis provides valuable information on maximum and minimum densities for EW and LW, their widths, percentages, and other factors. Information on maximum LW densities can also be determined by blue intensity measurements, reliable, inexpensive and accessible alternative to X-ray densitometry (Campbell et al., 2007).

Recently, Koubaa et al. (2002) have used a maximum derivative method, where maximum represents an inflexion point in the intra-ring wood density profile and could be determined

[1] Abbreviations: **CD** - cell diameter, **CO** – cell order within the tree ring, **CWT** - cell wall thickness, **DT** – density threshold, **DTvisual** – DT calculated from LW width determined by visual evaluation applied to the results of densitometry, **DTmork** - DT calculated from LW width determined by Mork's criterion applied to the results of densitometry, **DT450** - fixed density threshold of 450 kg/m^3, **DTqm** - quadratic mean of measured densities within the entire tree ring, **DTavg** - average of measured densities within the entire tree ring, **DT50/DT60/DT70/DT80** – DT calculated as 50%/60%/70%/80% of maximum latewood density, **DTmaxmin** - average of maximum and minimum density within a ring, **DT2/3** – 2/3*(density max – density min), **DT2/3+** - [2/3*(density max – density min)] + density min, **EW**- earlywood, **LD** - lumen diameter, **LW** – latewood, **LW%** - latewood proportion, **LW%visual** – latewood proportion corresponding to DTvisual, **LW%MI** – latewood proportion corresponding to DTmork, **LW%450** - latewood proportion corresponding to DT450, **LW%qm** - latewood proportion corresponding to DTqm, **LW%avg** - latewood proportion corresponding to DTavg, **LW%50/60/70/80** - latewood proportion corresponding to DT50/60/70/80 respectively, **LW%maxmin** - latewood proportion corresponding to DTmaxmin, **LW%2/3** - latewood proportion corresponding to DT2/3, **LW%2/3+** - latewood proportion corresponding to 2/3+, **MI** – Mork's index.

mathematically. Park et al. (2006) have suggested an error zone analysis method, which is based on the fact that cells are produced in a sequence from EW to LW. It aims to divide a tree ring into E and L zones by classifying cells in a radial cell file into only one EW and one LW zone (Park et al., 2006).

Both Mork's index and densitometric analysis classically apply a fixed threshold for each tree ring in a sample to distinguish EW/LW boundary. Fixed thresholds have certain limitations in the correct determination of EW/LW transition if large variations in wood density between individual tree rings, even within trees, are considered (Björklund et al., 2017). The demarcation of the EW/LW boundary is difficult especially when the EW/LW transition is gradual or anomalies such as intra-annual density fluctuations (IADFs) (Campelo et al., 2007, De Micco et al., 2007, Vieira et al., 2009, de Luis et al., 2011) or light rings (Liang et al., 1997) or dark rings occur (Novak et. al., 2016). Therefore, the possibilities of modifying Mork's index or fixed density thresholds have been explored for the precise estimation of the EW/LW border. Nicholl and Brown (1971) have proposed a floating density threshold, derived as the average of the minimum and maximum density in a tree ring. Hylen (1999) has suggested a method of two thirds of the difference between the minimum and maximum density greater than minimum density.

In this study, we focused on Norway spruce trees growing in a temperate climate. When analysing LW width, we applied the various approaches based on wood anatomy analysis (Mork's criterion) and X-ray densitometry (fixed threshold, same for all tree-rings, and different floating thresholds, varying among individual tree rings). The objectives of the study were (1) to examine the agreement among the methods (visual assessment, Mork's index, fixed and floating density thresholds) for demarcation of the border between EW and LW parts in the tree-rings and (2) to modify both Mork's criterion and the density threshold on the basis of reference values obtained by the visual identification of the EW/LW transition.

We assumed that Mork's index and the fixed density threshold are rigid criteria that are not able to properly reflect inter- and intra-ring density variation. Therefore, we expected a better performance of demarcation methods based on floating density thresholds.

4.1.2 Material and methods

Study sites and data collection

We used samples from our previous study in the Ore Mountains, Czech Republic (for details, see Samusevich et al., 2017). In total, 11 spruce trees (231 tree rings) were chosen for the methodological study presented here. We always used two pairs of cores extracted from the opposite sides of the stem. Tree age at the beginning of the analysed time period 1991-2001 ranged from 30 to 40 years. We worked with young spruce trees with relatively wide rings: mean ring width was 3.74 mm +/- 1.67 mm.

To examine the width and proportion of LW, cores were subjected to wood anatomy analyses and X-ray densitometry. The following methods of EW/LW demarcation were compared: Mork‘s index based on wood anatomy results, visual assessment of EW/LW transition point and fixed and floating thresholds applied to the intra-ring density profile.

Wood anatomy analysis

For wood anatomy analysis, cores were cut using the GSL-1 microtome (Gärtner et al., 2014). The microsections were cut into 15-µm slices, and non-Newtonian fluid was used to avoid cell wall breakage (Schneider and Gärtner, 2013). The microsections were double stained using Astrablue and Safranin, dehydrated with alcohol and embedded in Canada balsam (Gärtner and Schweingruber, 2013). The permanent slides were then photographed at a magnification of 20x with a Nicon Eclipse 80i microscope.

The microscopic images were analysed using the NIS-Elements software (version AR 4.11.00). As conifers have a relatively homogeneous structure, within each tree ring, three to five files were measured, depending on the ring width (Axelson et al., 2014, DeSoto et al., 2011). For the purpose of this research, radial cell diameter (CD), lumen diameter (LD) and cell wall thickness (CWT) were measured for each cell. A detailed description is given in Samusevich et al. (2017). The EW/LW transition was identified based on the standard Mork's index (MI, Mork, 1928), while the LW tracheid was defined as one in which the double cell wall width between two adjacent tracheids was equal to or greater than the radial width of the lumen (Fig. 1). Subsequently, LW width was calculated as the sum of radial cell diameters within identified LW part, and the LW percentage was expressed (LW%MI).

The same samples were used for visual assessment (later taken as reference). The border between the EW and LW was distinguished subjectively by naked eye, and the LW percentage (LW%visual) was calculated on the basis of this assessment. The MI corresponding to the visually determined EW/LW transition (MIvisual) was expressed as the average MI of five cells surrounding the visual border. Subsequently modified MI was expressed as the median of MIvisual calculated for all tree rings analysed. This modified MI is suggested for accurate demarcation of LW for spruce trees.

Densitometric analysis

Densitometric analysis was applied to all 22 samples, and density profiles were obtained for each of them. Density of the sample was defined using air-dry method. Sample moisture content was determined before density measurements. The cores were cut transversely into 1.2-mm strips with the DENDROCUT twin-bladed saw (WALESCH Electronic GmbH, Effretikon, Switzerland; www.walesch.ch) and measured with the QTRS-01X Data Analyzer and Scanner (QMS, Knoxville, TN, USA; www.qms-density.com). The highest (i.e. 0.02 mm) linear resolution step was used for the measurement. Depending on the species, a wood density from 400 to 550 kg/m^3 is usually chosen to differentiate between EW and LW (Koubaa et al., 2002). In our study, the transition point was determined by a threshold density of 450 kg/m^3. The density data were further used for density threshold (DT) calculations.

Altogether, 12 DTs were computed (10 DTs coming from density profiles and 2 DTs calculated on the basis of MI and visual assessment evaluation). The LW percentage (LW%) was estimated on base of all DTs. The full list of calculated DTs and corresponding LW% values is shown in Table 1. The differences between individual DTs are illustrated in Figure 2.

Table 1 Density thresholds and corresponding latewood percentages calculated on the basis of wood anatomy measurements and densitometry results.

Type of density threshold (DT)	Definition	Latewood percentage (LW%) based on given DT	Data source
DTvisual	DT calculated from LW width determined by visual evaluation applied to the results of densitometry. The DT was calculated by averaging density value corresponding to visual LW width and four density values measured at the nearest side of this point.	LW%visual	Densitometry /visual analysis

DTmork	DT calculated from LW width determined by Mork´s criterion applied to the results of densitometry. The DT was calculated by averaging density value corresponding to LW width and four density values measured at the nearest side of this point.	LW%MI	Densitometry /wood anatomy analysis
DT450	Fixed density threshold of 450 kg/m^3.	LW%450	Densitometry
DTqm	Quadratic mean of measured densities within the entire tree ring.	LW%qm	Densitometry
DTavg	Average of measured densities within the entire tree ring.	LW%avg	Densitometry
DT50 DT60 DT70 DT80	DT calculated as specified percentage of the maximum LW density.	LW%50 LW%60 LW%70 LW%80	Densitometry
DTmaxmin	Average of maximum and minimum density within a ring (according to Nicholl and Brown (1971)	LW%maxmin	Densitometry
DT2/3	DT2/3=2/3*(density max – density min)	LW%2/3	Densitometry
DT2/3+	DT2/3=[2/3*(density max – density min)]+density min (according to Hylen, 1999)	LW%2/3+	Densitometry

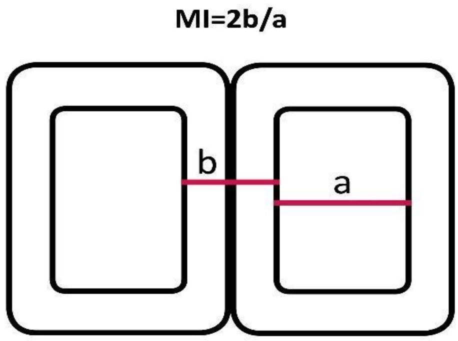

Fig. 1: Definition of Mork's index according to Mork (1928), where *a* is the radial width of the lumen and *b* is the double cell wall thickness between two adjacent tracheids.

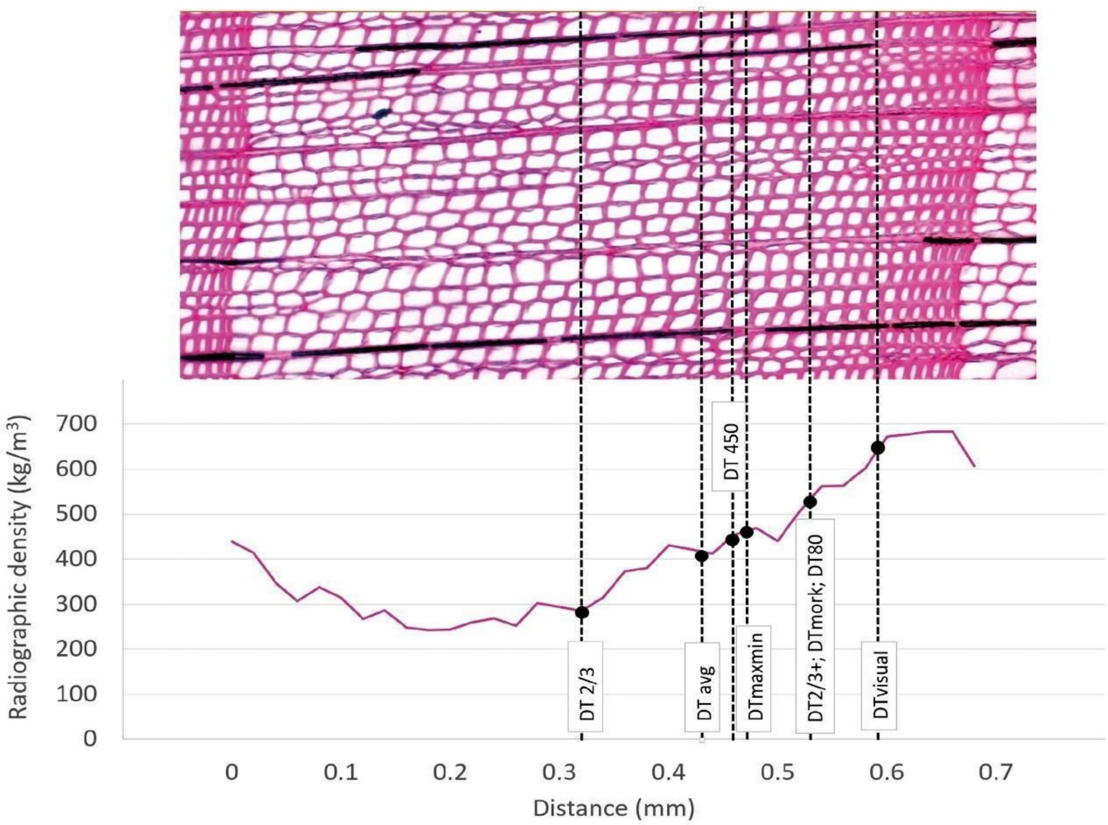

Fig. 2: Microscopic image of cross section and density profile of the tree ring of 1996 with marked individual density thresholds (for explanations, see Table 1).

Statistical analysis

We used a simple t test and hierarchical cluster analysis (Blashfield, 1980) to explore the similarity between different threshold densities calculated for each analysed ring using 12 different methods (Table 1). Ward's multivariate technique (Euclidean distance) was employed to define the clusters. The same method was applied to test the match between LW% values calculated using 12 different DTs. The threshold density, which determined the LW% that was closest to the reference values obtained by visual evaluation (LW%visual), was selected as the most appropriate and recommended for future use.

Linear discriminant analysis (Lachenbruch and Goldstein, 1979) was applied to classify the wood cells into EW and LW groups, using the measured anatomical parameters CWT, LD and CD together with "cell order within the tree ring" (CO) as the predictor variables. The visual assignment of cells into EW or LW part was used as the grouping variable. The value of a partial Wilks' lambda was computed to test the impact of each of the independent variables on the discriminant analysis. A higher lambda value indicates a lower predictive power of the variable and vice versa. The F-ratio was used to test the significance of Wilks' lambda. All statistical analyses were performed in Statistica v. 12 (StatSoft 2013).

4.1.3 Results

Density thresholds and the resulting LW% values varied distinctly depending on the chosen method (Fig. 3).

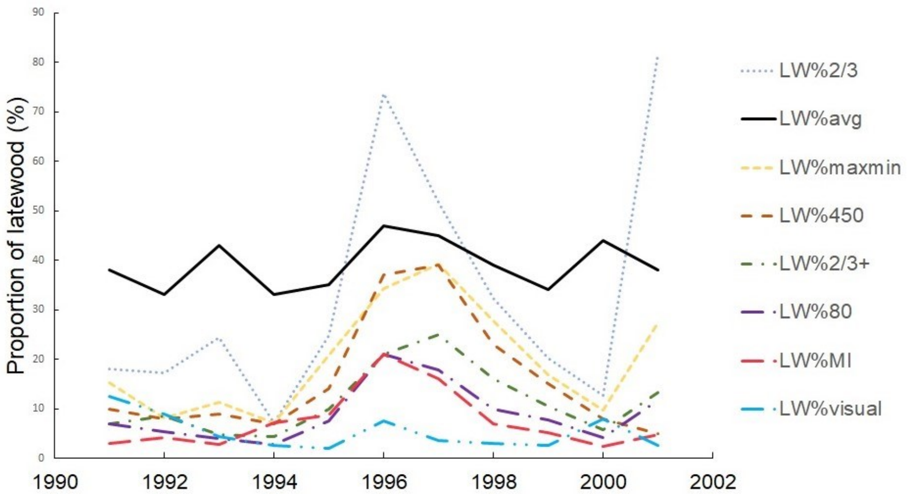

Fig. 3: Comparison of latewood percentages determined by different methods for one sample (for explanations, see Table 1).

Densities corresponding to the visually delimited EW/LW border had a large variability, ranging from 303 to 970 kg/m^3, with a median value of 646 kg/m^3 (average value 643 kg/m^3), which is significantly higher than the used fixed threshold of 450 kg/m^3 (Fig. 4a). The variability of DTmork values showed a similar pattern to DTvisual and ranged from 424 to 970 kg/m^3, with a median value of 647 kg/m^3 (average value 651 kg/m^3). On the other hand, a low variability was recorded for DTqm, DTavg and DT50 with minimum values of 267, 254 and 243 kg/m^3, respectively, and maximum values of 664, 617 and 581 kg/m^3, respectively. The median values were 430 kg/m^3 (average 432 kg/m^3), 395 kg/m^3 (average 401 kg/m^3) and 403 kg/m^3 (average 398 kg/m^3), respectively. These values are significantly lower than the reference values of DTvisual.

The t test revealed no significant differences between DTvisual and DT80 and between DTvisual and DTmork. Cluster analyses assorted the threshold densities into two distinct groups (Fig. 5a). In the first group, the median of threshold densities was around 600 kg/m^3, comprising DTvisual, DTmork, DT2/3+, DT70 and DT80. The second group consisted of the remaining DTs, with median values varying between 400 and 500 kg/m^3.

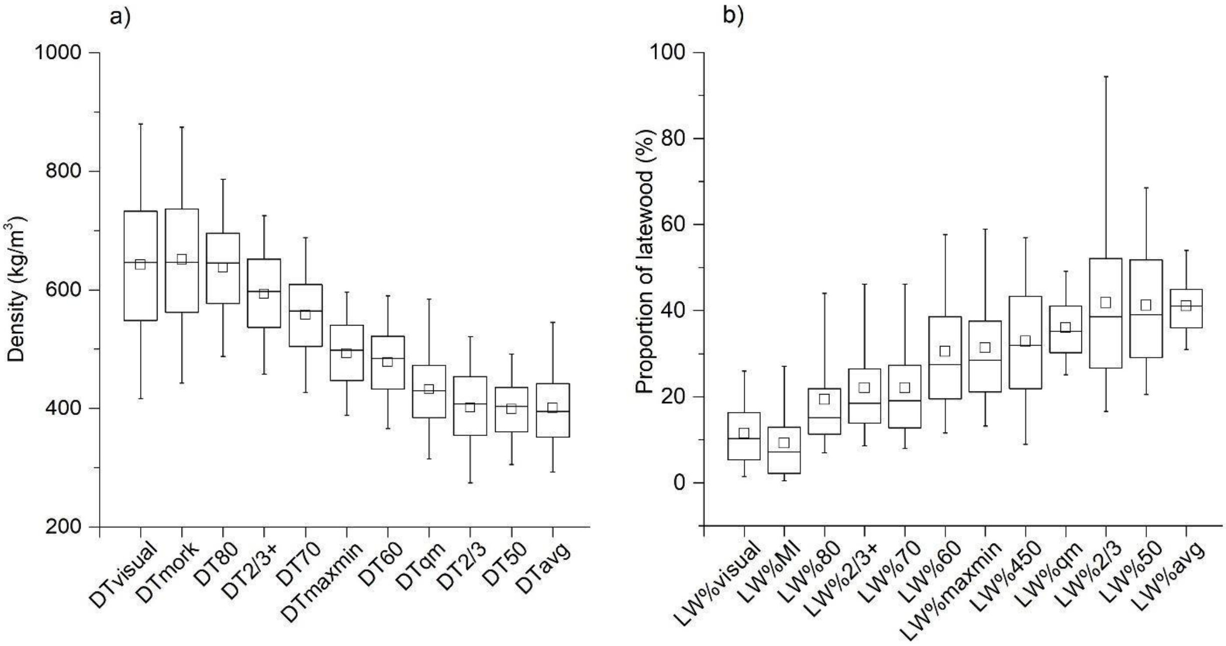

Fig. 4: Box plot of a) density threshold values and b) latewood percentages determined by 12 different methods for all 231 analysed tree rings (for explanations, see Table 1). Box depicts upper and lower quartiles, line represents the median, square the arithmetic mean and the whiskers the 5th and 95th percentiles.

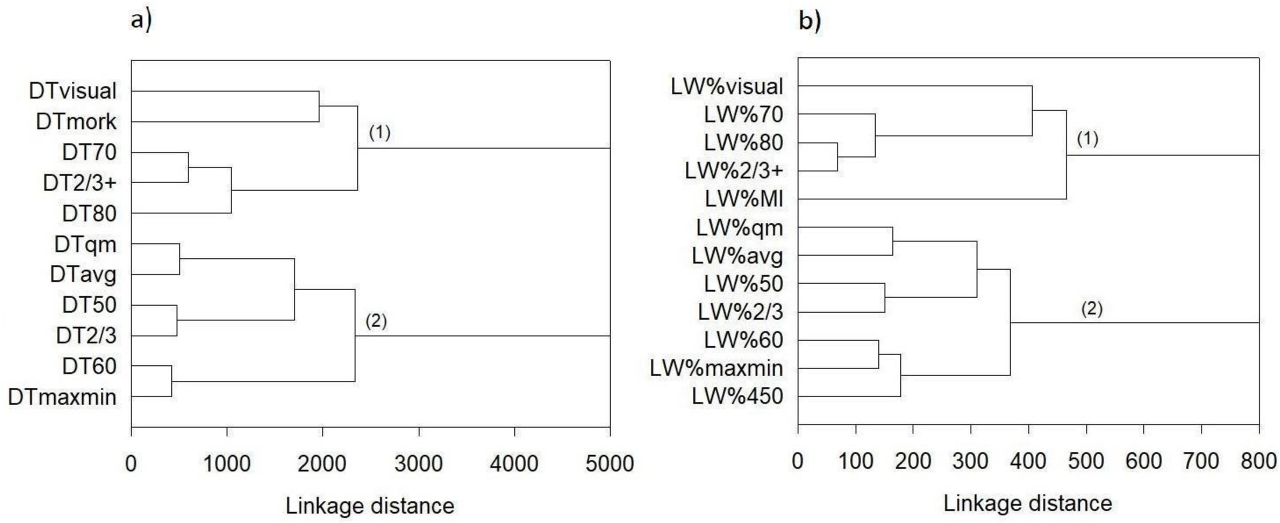

Fig. 5: Dendrogram of a hierarchical cluster analysis (Ward's method, Euclidean distances) of a) density thresholds and b) latewood percentages determined by 12 different methods for all 231 analysed tree rings (for explanations, see Table 1).

The largest variability of LW% data was identified for LW%50 and LW%2/3 (Fig. 4b). The minimum LW% values for these parameters were 10 and 7%, with a maximum of 100% for both of them and a median of 39% (average 41%) and 38% (average 41%), respectively. These values are close to the LW% determined by the fixed DT 450 kg/m^3 (minimum value 3%, maximum value 100%, median 32%, average 33%), but differ significantly from the reference LW% values obtained by visual determination (LW%visual), where minimum LW% was 0%, maximum was 39%, median was 10% and the average was 12%. The LW%visual and LW%MI were similar in their mean values and in their variability, which was the lowest compared to the LW% values determined by other methods (Fig. 4b).

The cluster analysis partitioned the LW% into two groups (Fig. 5b). The first group with a median of around 13% comprised LW%visual, LW%MI, LW%70, LW%80 and LW%⅔+. The second group consisted of the remaining LW% values, with a median of around 31%.

By using the values of MIvisual and calculating its median for all analysed tree rings, we calibrated the standard Mork's index (MI = 1) criterion accordingly. The result shows that to determine LW cells in Norway spruce trees, it is sufficient to use a modified MI value equal to 0.83.

Results of the discriminant analyses with the four independent variables CO, LD, CWT and CD revealed that the discriminant function (Table 2) is able to successfully classify 96% of EW cells and 64% of LW cells within the sample (Table 2a). The overall success of the classification was 90.6%. However, the high value of partial Wilks' lambda for LD points to the low contribution of this variable to overall discrimination. Therefore, in the following step, we excluded LD from the set of discriminators and calculated the discriminant functions based on the three variables CO, CWT and CD only (Table 2b). The success of discrimination for both EW and LW cells was almost the same as in the case of discriminant functions with four variables. For the purpose of comparison, we calculated the success of classification based on the standard Mork's index (MI = 1) only. In that case, 96.7% of EW cells and 53.6% of LW cells were differentiated properly, with an overall success of 89.4%.

Table 2 Results of the linear discriminant analysis a) with four independent variables (CO, LD, CWT and CD) and b) with three independent variables (CO, CWT and CD).

a) Independent variables CO, LD, CWT and CD

Variable influence				*Linear discriminant functions*			*Classification count table for EW, LW*				
	Wilks' λ	F-Value	F-Prob		EW	LW		EW predicted	LW predicted	Total	Success of discrimin. (%)
CO	0.955	1,281.389	0.000	Constant	-14.280	-14.977	EW actual	21,572	922	22,494	95.9
LD	0.999	23.102	0.000	OC	0.041	0.056	LW actual	1,618	2,902	4,520	64.2
CWT	0.947	1,507.368	0.000	LD	-0.017	0.008	Total	23,190	3,824	27,014	90.6
CD	0.828	5,617.149	0.000	CWT	1.283	1.715					
				CD	0.600	0.422					

b) Independent variables CO, CWT and CD

Variable influence				*Linear discriminant functions*			*Classification count table for EW, LW*				
	Wilks' λ	F-Value	F-Prob		EW	LW		EW predicted	LW predicte d	Total	Success of discrim. (%)
CO	0.955	1,258.579	0.000	Constant	-14.277	-14.977	EW actual	21,566	928	22,494	95.9
CWT	0.924	2,215.223	0.000	CO	0.041	0.056	LW actual	1,634	2,886	4,520	63.8
CD	0.829	5,589.918	0.000	CWT	1.266	1.723	Total	23,200	3,814	27,014	90.5
				CD	0.600	0.422					

4.1.4 Discussion

The demarcation of EW and LW, as well as their transition, is important for the accurate estimation of EW and LW parts and their density and proportion, characteristics of cell walls and lumens. Based on the chosen method of EW/LW demarcation, the results can vary significantly. Even though they can show a similar general trend, the absolute values of calculated tree ring features will be different (Koubaa et al., 2002). In our study, we used 12 different methods of EW/LW demarcation, including the classic one based on wood anatomy measurements (Mork's index) and floating and fixed density thresholds derived from density profiles to define the most suitable method.

Substantial differences were identified in the time series of the obtained LW% values. To verify the results, we set up the visual assessment of the EW/LW border as a reference. Although Mork's index is commonly used as the standard by which other methods are judged, Larson (1969) clearly states that Mork's definition fails in juvenile wood and in growth rings with diffuse transition zones. Because we worked with young spruce trees with relatively wide rings and gradual changes between EW and LW cells, we preferred the visual assessment over Mork's index reference. Nevertheless, the final comparison of visual and Mork´s index demarcation revealed similar results. This finding might justify the use of visual identification of the EW/LW border for the first quick calibration of the density threshold values.

We demonstrated that the usage of floating thresholds in densitometric analyses is a reliable method to correctly distinguish EW/LW parts in the tree rings. Two floating density thresholds, DT80 and DT2/3+, provided the best outcome, showing that the density value around 600 kg/m^3 is appropriate for distinguishing between EW and LW. Both these DTs can be suggested for the evaluation of densitometric profiles of Norway spruce trees growing in a temperate climate. A similar density threshold was also suitable for EW/LW demarcation for black spruce by Koubaa et al. (2002).

From an operational point of view, the use of X-ray densitometry and the application of floating density thresholds is rapid, consistent and easy to be integrated into X-ray densitometry computational programs, making this method more suitable when compared to the maximum derivative method (Koubaa et al., 2002, Antony et al. 2012). At the same time, the method is less time-consuming than the wood anatomical measurements and the application of Mork's index. Different floating thresholds have also been used in other studies (Sauter et al., 1999, Björklund et al. 2017). The use of six-order polynomials, despite the good results and proven accuracy (Kharrat et al, 2019), is a more complicated method and requires a deeper understanding of the process itself. Besides the compared methods, there are other techniques used for EW/LW demarcation. For example, Maxwell et al. (2011) used an image analysis and changes in light intensity as one of the ways to distinguish between EW and LW.

Even though the demarcation using the standard Mork's index (MI = 1) shows a good correspondence with the visual identification of the EW/LW border, the further adjustment made to the standard value of this criterion allows an increased accuracy of EW/LW determination for particular tree species growing in different site conditions. We concluded that for spruce trees in our sites, the modified MI value of 0.83 is appropriate to distinguish between EW and LW.

Developed discriminant functions were more successful in the classification of cells into EW/LW part than the classification based on the standard Mork's index only. Thus, the use of these functions might substitute the classic Mork's index approach. For further use, we can recommend a discriminant function based on CO, CWT and CD variables.

The results of our study are applicable for studied population of Norway spruce growing in a temperate climate. The methodology itself, however, is universal and can help researchers to calibrate criteria for EW/LW demarcation (MI and DTs) under specific conditions. Compared to fixed DT, floating DTs, especially DT80 and DT2/3+, were more accurate in distinguishing intra-annual parts of the tree ring. When applying Mork's index, its modified value equal to 0.83 was suitable to distinguish between EW and LW within the tree rings of Norway spruce.

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Footnotes

[1] – Abbreviations used in the text

References

Acosta-Hernandez, A., Camarero, J., Pompa-Garcia, M., 2019. Seasonal growth responses to climate in wet and dry conifer forests. IAWA Journal 40 (2), 311–S1.

Antony, F., Schimleck, L.R., Daniels, R.F., 2012. A comparison of earlywood-latewood demarcation methods – a case study in loblolly pine. IAWA Journal 33 (2), 187–195.

Arsalani, M., Bräuning, A., Pourtahmasi, K., Azizi, G., Mohammadi, H., 2018. Multiple tree-ring parameters of *Quercus brantii* Lindel in SW Iran show a strong potential for intra-annual climate reconstruction. Trees 32 (6), 1531–1546.

Axelson, JN., Bast, A., Alfaro, R., Smith, DJ., Gärtner, H., 2014. Variation in wood anatomical structure of Douglas-fir defoliated by the western spruce budworm: a case study in the coastal-transitional zone of British Columbia, Canada. Trees 28, 1837–1846.

Björklund, J., Seftigen, K., Schweingruber, F., Fonti, P., von Arx, G., Bryukhanova, M. V., Cuny, H. E., Carrer, M., Castagneri, D., Frank, D. C., 2017. Cell size and wall dimensions drive distinct variability of earlywood and latewood density in Northern Hemisphere conifers. New Phytologist 216, 728–740.

Blashfield, R. K., 1980. The growth of cluster analysis: Tryon, Ward, and Johnson. Multivariate Behavioral Research 13, 439–458.

Campbell, R., McCarroll, D., Loader, N. J., Grudd, H., Robertson, I., Jalkanen, R., 2007. Blue intensity in Pinus sylvestris tree-rings: developing a new palaeoclimate proxy. The Holocene 17/6, 821 - 828.

Campelo, F., Nabais, C., Freitas, H., Gutierrez, E., 2007. Climatic significance of tree-ring width and intra-annual density fluctuations in *Pinus pinea* from a dry Mediterranean area in Portugal. Annals of Forest Science 64/2, 229–238.

Cartenì, F., Deslauriers, A., Rossi, S., Morin, H., De Micco, V., Mazzoleni, S., Giannino, F., 2018. The physiological mechanisms behind the earlywood-to-latewood transition: A process-based modeling approach. Frontiers in Plant Science 9, 1053.

Cuny, H.E., Rathgeber, C., Frank, D., Fonti, P., Fournier, M., 2014. Kinetics of tracheid development explain conifer tree-ring structure. New Phytology 203, 1231–1241.

De Luis, M., Novak, K., Raventós, J., Gričar, J., Prislan, P., Čufar, K., 2011. Climate factors promoting intra-annual density fluctuations in Aleppo pine (*Pinus halepensis*) from semiarid sites. Dendrochronologia 29, 163–169.

De Micco, V., Saurer, M., Aronne, G., Tognetti, R., Cherubini, P., 2007. Variations of wood anatomy and δ13C within tree rings of coastal *Pinus pinaster* Ait. showing intra-annual density fluctuations. IAWA Journal 28, 61–74.

Denne, M.P., 1988. Definition of latewood according to Mork. IAWA Bull. 10, 59–62.

DeSoto, L., De la Cruz, M., Fonti, P., 2011. Intra-annual patterns of tracheid size in the Mediterranean tree *Juniperus thurifera* as an indicator of seasonal water stress. Canadian Journal Forest Research 41, 1280–1294.

Gärtner, H., Schweingruber, F. H., 2013. Microscopic preparation techniques for plant stem analysis. Remagen-Oberwinter: Verlag Dr. Kessel., 78 pp.

Gärtner, H., Lucchinetti, S., Schweingruber, F.H., 2014. New perspectives for wood anatomical analysis in dendrosciences: The GSL-1 microtome. Dendrochronologia 32, 47–51.

Hylen G., 1999. Age trends in genetic parameters of wood density in young Norway spruce. Canadian Journal of Forest Research 29(1), 135–143.

Kharrat, W., Koubaa, A., Khlif, M., Bradai, Ch., 2019. Intra-ring wood density and dynamic modulus of elasticity profiles for black spruce and Jack pine from X-ray densitometry and ultrasonic wave velocity measurement. Forests 10 (7), 569.

Koubaa, A., Zhang, S.Y., Makni, S., 2002. Defining the transition from earlywood to latewood in black spruce based on intra-ring wood density profiles from X-ray densitometry. Annals of Forest Science 59, 511–518.

Lachenbruch, P. A., Goldstein, M., 1979. Discriminant analysis. Biometrics 35 (1), 69–85.

Larson, P., 1969. Wood formation and the concept of wood quality. Yale University, School of Forestry, Bulletin No. 74, Yale University, New Haven, 54 pp.

Liang, C., Filion, L., Cournoyer, L., 1997. Wood structure of biotically and climatically induced light rings in eastern larch (*Larix laricina*). Canadian Journal of Forest Research 27 (10), 1538–1547.

Maxwell, R. S.,Wixom, J. A., Hessl, A. E., 2011. A comparison of two techniques for measuring and crossdating tree rings. Dendrochronologia 29, 237–243.

Mork, E., 1928. Die Qualität des Fichtenholzes unter besonderer Rücksichtnahme auf Schleif- und Papierholz. Der Papier-Fabrikant 48, 741–747.

Mothe, F., Sciama, D., Leban, JM., Nepveu, G., 1998. Localisation de la transition bois initial-bois final dans un cerne de chêne par analyse microdensitométrique. Annals of Forest Science 55, 437–449.

Nicholls, J. W., Brown, A. G., 1971. The ortet-ramet relationship in wood characteristics of *Pinus radiata*. Appita Journal 25, 200–209.

Novak, K., de Luis, M., Gričar, J., Prislan, P., Merela, M., Smith, K. T., Čufar, K., 2016. Missing and dark rings associated with drought in *Pinus halepensis*. IAWA Journal 37, 260–274.

Park, IY., Dallaire, G., Morin, H., 2006. A method for multiple intra-ring demarcation of coniferous trees. Annals of Forest Science 63(1), 9–14.

Polge, H., 1963. L´analyse densitometrique de cliches radiographiques. Une nouvelle methode de determination de la texture du bois. Ann. ecole nat. eaux et for. et de la stat. rech, et exper. 20, 531–581.

Rathgeber, C., Decoux, V., Leban, J-M., 2006. Linking intra-tree-ring wood density variations and tracheid anatomical characteristics in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Annals of Forest Science 63 (7), 699–706.

Samusevich, A., Zeidler, A., Vejpustková, M., 2017. Influence of air pollution and extreme frost on wood cell parameters at Mountain Spruce Stands (*Picea abies* (L.) Karst.) in the Ore Mountains. Wood Research 62(1), 79–90.

Sauter, U. H., Mutz, R., Munro, B. D., 1999. Determining juvenile-mature wood transition in Scots pine using latewood density. Wood and Fiber Sciences 31 (4), 416–425.

Schneider, L., Gärtner, H., 2013. The advantage of using a starch based non-Newtonian fluid to prepare micro sections. Dendrochronologia 31 (3), 175–178.

StatSoft, Inc. (2013): STATISTICA (data analysis software system), version 12. www.statsoft.com.

Schweingruber, F.H., Fritts, H.C., Braeker, O.U., Drew, L.G., Schaer, E., 1978. The X-ray technique as applied to dendroclimatology. Tree-Ring Bulettin 38, 61–91.

Vieira, J., Campelo, F., Nabais, C., 2009. Age-dependent responses of tree-ring growth and intra-annual density fluctuations of *Pinus pinaster* to Mediterranean climate. Trees 23, 257–265.

Wood, L., Smith, D., 2015. Intra-annual dendroclimatic reconstruction for northern British Columbia, Canada, using wood properties. Trees 29 (2), 461–474.

4.2 Stopa imisní kalamity v anatomických znacích dřeva smrku (*Picea abies* (L.) Karst.) v oblasti Klínovce (Krušné hory)

Trace of air pollution disaster in the xylem traits of Norway spruce (*Picea abies* (L.) KARST) in the Klínovec area (Ore Mountains, czech republic)

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Abstract

Anatomical parameters can be considered as a sensitive indicator of environmental changes on the intra-annual level. Methods of quantitative wood anatomy were used to study the dynamics of xylem traits for spruce growing in the so called „Black Triangle“ region where the fossil fuel emissions caused one of the globally highest pollutant depositions (SO_2, NO_x, F) in the 1970s and 1980s. The Klínovec study area was selected as a model area considering the combination of harsh climate and long-term air pollution load. The series of anatomical features such as number of tracheids, lumen area and cell wall thickness together with ring widths were examined for the entire tree life span. In the result we obtained a unique, more than hundred years long sequence of anatomical parameters covering the period before, during and after the extreme air pollution load. Number of cells and tree-ring widths were detected as the most responsive features, while lumen area and cell-wall thickness were only slightly affected during the peak time of pollution concentration. For the future research, we suggest studying the anatomical features of early wood and late wood cells separately to verify the differences in their response.

Klíčová slova: Černý trojúhelník, kvantitativní anatomie dřeva, SO_2, NO_x, růstový trend, plocha lumenu, tloušťka buněčné stěny

Key words: Black Triangle, quantitative wood anatomy, SO_2, NO_x, growth trend, lumen area, cell wall thickness

4.2.1 Úvod

Radiální růst stromů spolu se sezónností klimatu vedou u dřevin v mírném podnebném pásu k tvorbě letokruhů. Jejich utváření je však komplikovaný proces, který je determinován jak environmentálními podmínkami, tak geneticky podmíněnými vlastnostmi dané dřeviny (Fritts 1976). Cook (1985) definuje radiální přírůst jako funkci klimatu, disturbancí a biologické variability. Wimmer (2002) a Vaganov et al. (2006) zjistili, že Cookův model je aplikovatelný nejen pro radiální přírůst, ale rovněž pro změny anatomické stavby dřeva, neboť změny v tloušťkovém růstu jsou primárně determinovány modifikací buněčné struktury. Anatomické parametry tracheid, jako jsou například radiální rozměr tracheidy, tloušťka buněčné stěny, plocha lumenu, podíl letního dřeva, výskyt pryskyřičných kanálků či fluktuace hustoty uvnitř letokruhu nám mohou poskytnout užitečné informace o prostředí, ve kterém růst probíhal (Wimmer, Grabner 1997; Wimmer 2002; Olano et al. 2012; Ziaco et al. 2014). Anatomické charakteristiky mohou reagovat na stres rozdílným způsobem, což z nich činí potenciálně vhodné proxy pro dendroekologické studie (Kozlowski et al. 1991; Schweingruber 1996; Gindl et al. 2000; Wimmer 2002; Vaganov et al. 2006).

Jak prokázali Wimmer, Halbwachs (1992), Kurczyńska et al. (1997), Samusevich et al. (2017) a Vejpustková et al. (2017), k modifikaci anatomických parametrů může docházet i vlivem imisní zátěže. Dle studie Kurczyńska et al. (1997) produkovali jedinci borovice lesní na znečištěných stanovištích menší počet xylémových a floémových buněk než na stanovištích bez znečištění. Docházelo také k narušení diferenciace tracheid. Dle této studie za těmito efekty nestojí nedostatek živin, ale pravděpodobně toxický efekt polutantů, zejména síry a těžkých kovů. Vlivem toxicity dochází ke snížení fotosyntetické kapacity stromů, což negativně ovlivňuje dostupnost a ukládání živin, a tedy i produkci růstových regulátorů, kterým je například auxin. Změny v polárním transportu auxinu v kambiální zóně pak negativně ovlivňují kambiální aktivitu (Kurczyńska et al. 1997). Vejpustková et al. (2017) uvádí, že v reakci na klimatický extrém spojený s vysokou imisní zátěží byla krom poklesu šířek letokruhů pozorována odezva v počtu tracheid v letokruzích a šířkách lumenů, nejvíce v jarní části letokruhu.

Ve 2. pol. 20. století patřila oblast tzv. Černého trojúhelníku, zahrnující Severní Čechy a přilehlé oblasti Saska a Polska k nejvíce znečištěným v rámci celé Evropy (Blažková 1996). V 70. a 80. letech, kdy znečištění kulminovalo, došlo k prudkému zhoršení zdravotního stavu lesa (Fiala et al. 2002). Nejvíce postižena byla východní část Krušných hor (Zimmermann et al. 2002), kde došlo k rozsáhlému odumírání smrkových porostů (Kubelka et al. 1992; Materna 1999). S rychlým poklesem imisní zátěže v 90. letech (Hůnová et al. 2004) se začal zlepšovat i zdravotní stav přežívajících porostů (Fiala et al. 2002). Dendrochronologické studie realizované v oblasti Černého trojúhelníku ukázaly, že smrk po hluboké přírůstové depresi na přelomu 70. a 80. let začal rychle regenerovat (Sander et al. 1995; Kroupová 2002) a na konci 90. let tloušťkový přírůst dokonce převýšil hodnoty z období před extrémním znečištěním (Rydval et al. 2012; Kolář et al. 2015). Velmi málo je však známo o tom, jakým způsobem extrémní míra znečištění ovlivnila mikroskopickou stavbu dřeva smrku. Výjimkou je práce Wimmera (2002) ze saské strany Krušných hor a dále studie Samusevich et al. (2017), která se však zabývá pouze epizodou akutního poškození smrkových porostů v Krušných horách po zimě 1995/96.

Cílem předkládané práce je retrospektivně analyzovat vliv imisní zátěže v 70. a 80. letech 20. století na mikroskopickou stavbu dřeva smrku a určit, který anatomický parametr je nejvhodnějším proxy pro studium vlivu znečištění. Vzhledem k tomu, že změny v radiálním růstu jsou podmíněny změnami buněčné struktury, předpokládáme, že znečištění významně ovlivnilo anatomické charakteristiky tracheid, kterými jsou plocha lumenu (MCA), počet buněk v letokruhu v radiálním směru (Nmm) a tloušťka buněčných stěn v radiálním směru (CWTrad), podobně jako šířku letokruhu (MRW).

4.2.2 Materiál a metodika

Lokalita

Jako studijní lokalita byl zvolen nejvyšší vrchol Krušných hor – Klínovec (1244 m n. m.) – obr. 1. Důvodem je jednak existence starých smrkových porostů, které přežily imisní kalamitu, jednak možnost na relativně malém území studovat porosty s různou expozicí vůči největším zdrojům polutantů v oblasti. Klimatické poměry lokality charakterizuje klimadiagram z nejbližší meteorologické stanice na Fichtelbergu (obr. 2).

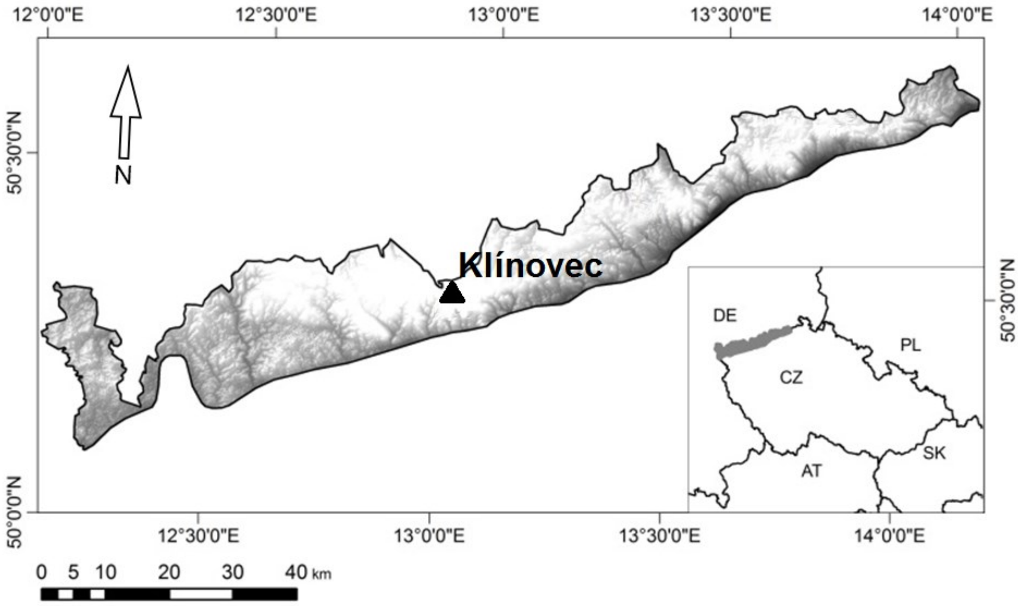
Na svazích Klínovce byly vybrány tři plochy v nadmořské výšce okolo 1000 m, čtvrtá, srovnávací plocha se nacházela v blízkosti samotného vrcholu Klínovce v nadmořské výšce 1230 m (tab. 1). První tři zmíněné plochy byly zejména v zimním období často ovlivněny vrstvou inverze, v níž se v období vysoké imisní zátěže kumulovaly znečišťující látky. Inverzní situace jsou v zimním období v Krušných horách velmi časté a mohou přetrvávat až několik týdnů. Plocha na vrcholu Klínovce je pak specifická tím, že se často nachází nad touto vrstvou, a proto v našem výběru paradoxně patří k plochám s nižší imisní zátěží. U plochy Suchá 1 na JV svahu Klínovce předpokládáme přímou exponovanost k oběma největším zdrojům polutantů v oblasti – tepelným elektrárnám Prunéřov a Tušimice. Plochy Suchá 2 (JZ expozice) a Loučná (S expozice) byly exponované k těmto zdrojům méně.

Obr. 1.

Poloha zájmové oblasti

Fig. 1.

Area of interest



Tab. 1.

Popis zájmových ploch

Areas of interest – description

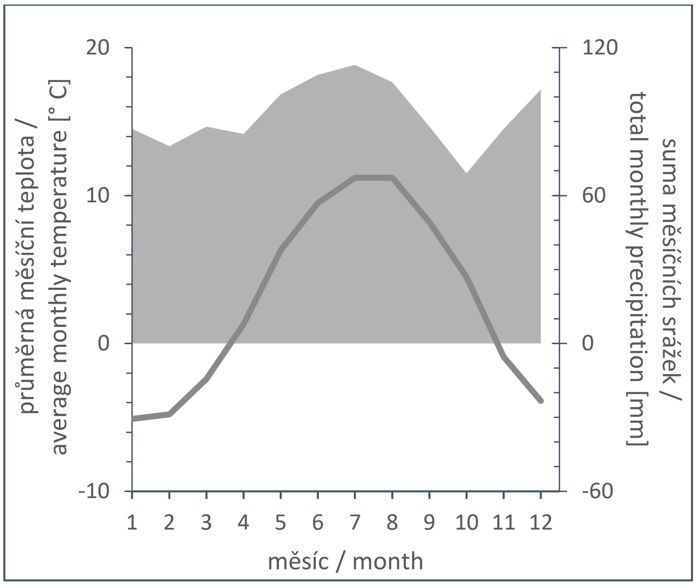
Plocha/Plot	Souřadnice/Coordinates [WGS84]		Nadmořská výška/Altitude	Expozice/Exposition	Věk porostu/Stand age	Číslo porostu/Stand number
	zem. šířka/latitude	zem. délka/longitude				
Suchá 1	N 50°23‘13“	E 12°59‘07“	1 030	JV	160	273C16/2/1p
Suchá 2	N 50°22‘41“	E 12°57‘51“	1 009	JZ	170	37D16
Loučná	N50°24‘23“	E 12°58‘14“	1 013	S	180	267A17/1p
Klínovec	N 50°23‘40“	E 12°58‘07“	1 230	-	120	36B16/2c

Obr. 2.

Klimadiagram z Fichtelbergu – nejbližší meteorologické stanice (nadmořská výška 1213 m n. m., průměrná roční teplota 2,9 °C, průměrný roční úhrn srážek 1121 mm)

Fig. 2.

Climadiagram from the nearest weather station (Fichtelberg) – 1213 m a. s. l., average annual temperature 2.9°C, annual precipitation 1121 mm



Odběr a příprava vzorků

Pro křížové datování bylo přírůstovým nebozezem v prsní výšce (130 cm) odebráno celkem 23 vývrtů ze 14 stromů. Bylo dbáno na to, aby byly vzorkovány úrovňové stromy. Na ploše na vrcholu Klínovce bylo odebráno pět stromů, přičemž z každého jedince byl odebrán jeden vývrt. Na ostatních třech plochách byli vzorkováni vždy tři jedinci, z každého byly odebrány dva vývrty na protilehlých stranách kmene ve směru po vrstevnici, abychom se vyhnuli případnému výskytu tlakového dřeva. Šířky letokruhů byly změřeny na měřicím stole "TA" measurement system (Velmex Inc., Bloomfield, NY, USA) s přesností na 0,001 mm. Jednotlivé vývrty byly srovnány navzájem metodou křížového datování v programu PAST 5.1. Tímto postupem byly konkrétním letokruhům přiřazeny odpovídající kalendářní roky. Pro následnou tvorbu trvalých mikroskopických preparátů byl použit jeden vývrt z každého stromu, celkově tedy 14 vývrtů. Vývrty byly nejprve rozděleny na části dlouhé 4–5 cm tak, aby je bylo možné umístit na podložní sklo. Rotačním mikrotomem (Leica, Heidelberg, Germany) byly vytvořeny příčné řezy o tloušťce 12 μm, které byly umístěny na podložní sklo, obarveny směsí safraninu a astra blue, dehydrovány alkoholem a zafixovány na podložní sklo montovacím médiem (Eukitt, BiOptica, Milan, Italy) (Castagneri et al. 2017). Bylo důležité dbát na to, aby tloušťka všech preparátů byla stejnoměrná, neboť může ovlivňovat hodnoty naměřených parametrů, zejména pak hodnoty tloušťky buněčných stěn (Arx et al. 2016). Následně byly preparáty nasnímány pomocí motorizovaného mikroskopu Nikon Ni-E při stonásobném optickém zvětšení. Pro snímání byl použit program NISS-Elements. Pro získání ostřejších snímků bylo využito modulu EDF, pomocí kterého lze kombinovat snímky pořízené v různých polohách osy Z. Každý snímek byl zachycen v minimálně pěti různých polohách. Byl nasnímán vždy co nejlépe zaostřený snímek a poté dva snímky s vyšší a dva s nižší ohniskovou vzdáleností objektivu, ta se vždy měnila o 7,5 μm. Z těchto snímků byl vytvořen kompozitní snímek, který obsahoval pouze nejostřejší oblasti ze všech snímků. Tento postup pomáhá eliminovat rozostření způsobené mírným zvlněním preparátu na podložním skle. Šířka snímků byla přibližně 3600 pixelů, což odpovídá 1,25 mm na preparátu.

Mikroskopické snímky byly analyzovány pomocí programu ROXAS v3.0.1 (Arx et al. 2014), pro nastavení programu pro smrk byl použit stejný soubor, jaký používá Castagneri et al. (2015). Byly měřeny a analyzovány tyto parametry: šířka letokruhu (MRW), plocha lumenu tracheid (MCA) a průměrná tloušťka buněčné stěny v radiálním směru (CWTrad). Parametr počet tracheid v letokruhu v pásu širokém 1 mm s osou v radiálním směru (Nmm) byl vypočtený z parametru „počet tracheid na mm^2“, který byl násoben šířkou letokruhu v milimetrech. Jednalo se vždy o průměrnou šířku letokruhu pro celý snímek. Měření nebylo omezeno pouze na určité časové období či na určitou sekvenci letokruhů, ale byly měřeny celé letokruhové série. Celkově byly analyzovány anatomické parametry v 1961 letokruzích.

4 Zpracování dat

Letokruhy měřené na mikroskopických preparátech byly přiřazeny k jednotlivým kalendářním rokům na základě srovnání s letokruhovými sériemi daných vývrtů měřených na měřicím stole. Prostým zprůměrováním byly pro každou plochu vypočteny průměrné chronologie anatomických parametrů a byl vytvořen jejich krabicový diagram. Zároveň byly z dat ze všech ploch vypočteny pro každý parametr průměrné hodnoty a hodnoty popisující variabilitu parametrů v desetiletých periodách od roku 1930. Dále byly určeny negativní a pozitivní významné roky, tzv. „pointer years“. Jedná se o roky, kdy alespoň v polovině všech sérií daný letokruh vykazoval minimálně o 30 % rozdílné hodnoty oproti průměrné hodnotě za poslední tři roky. Byla použita metodika dle Schweingrubera (1990), kritéria však byla zmírněna. Při identifikaci významných roků v chronologiích šířek letokruhů a počtu tracheid bylo potřeba zmírnit kritické hodnoty pro označení letokruhu významným z doporučovaných 60 % rozdílu oproti průměrné hodnotě z předchozích tří let (Schweingruber et al. 1990) na 30 %, protože změny v sériích jsou spíše pozvolné. Data shromážděná ze všech ploch byla využita pro analýzu charakteru věkového trendu v časových řadách anatomických parametrů. Trend v časových řadách byl aproximován pomocí vhodných přírůstových funkcí. Následně byly vypočteny hodnoty indexů jako poměr naměřené a aproximované hodnoty. Pro jednotlivé plochy byly vypočteny průměrné hodnoty indexů buněčných charakteristik pro období před hlavním stresovým obdobím (1921–1970), během něj (1971–1990) a po něm (1991–2015) a jejich procentuální změna.

Pomocí jednofaktorové Anovy bylo testováno, zda mezi plochami s různou expozicí existují významné rozdíly v anatomických parametrech ve stresovém období. Pro analýzu byly použity průměrné řady indexů anatomických parametrů pro danou plochu v období 1971–1990. Pokud byl výsledek Anovy signifikantní, byl následně aplikován Tukeyho test mnohonásobných srovnání k detekci významných rozdílů mezi jednotlivými plochami.

4.2.3 Výsledky

Šířka letokruhů

Průměrná šířka ročních přírůstů dřeva byla nejvyšší na ploše Suchá 2 (1,33 mm). Nejnižší průměrné přírůsty (1,22 mm) se vyskytovaly na ploše Klínovec, která se nachází v nejvyšší nadmořské výšce. Nejstarší vzorkovaný jedinec měl první datovaný letokruh v roce 1834, nejstarší datovaný letokruh použitý pro měření anatomických parametrů je z roku 1839 z plochy Suchá 1. Nejvyššího průměrného stáří dosahovali zkoumaní jedinci na ploše Loučná (171 let), naopak průměrně nejmladší jedinci se nacházeli na ploše poblíž vrcholu Klínovce (113 let), stáří nejmladšího jedince z této lokality a zároveň i z celého souboru je 103 let.

Na všech plochách jsou patrná lokální minima MRW v letech 1948 a 1956 (obr. 3a). Dále je zjevný pokles hodnot MRW od druhé poloviny 60. let. Následný strmý vzestup hodnot koncem 80. let je na třech plochách přerušen lokálním minimem v roce 1996, na ploše Suchá 2 však tento pokles zaznamenán nebyl. Průměrné šířky letokruhů pro jednotlivé plochy se mezi sebou statisticky významně liší ($p < 0{,}05$). Z krabicového diagramu (obr. 3b) je patrné, že nejvíce se odlišuje plocha Suchá 2, která má největší rozptyl hodnot. Od 80. let můžeme v řadách pozorovat zvýšenou variabilitu mezi plochami (obr. 4). Negativní významné roky byly zaznamenány v letech 1948, 1956 a 1978, pozitivní významné roky pak v letech 1943, 1945, 1959, 1988, 1989, 1990, 1998, 1999 a 2002.

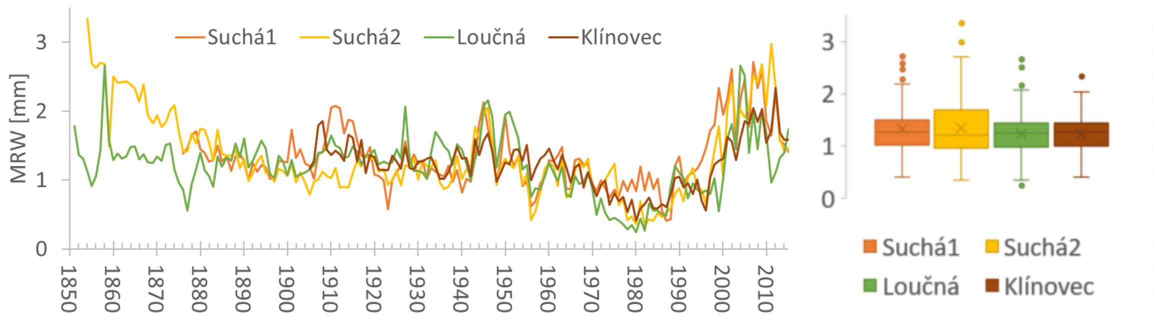
Obr. 3.

Průběh průměrných letokruhových chronologií (MRW) pro jednotlivé plochy (a) a krabicové grafy přírůstu pro jednotlivé chronologie (b)

Fig. 3.

Mean tree-ring width chronologies (MRW) of individual series (a) and box plots of increment of individual chronologies (b)

(a) *(b)*

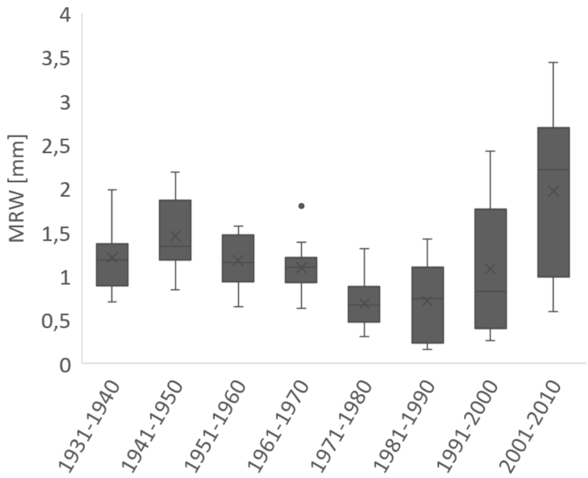


Obr. 4.

Variabilita šířek letokruhů (data ze všech ploch) v desetiletých periodách v období 1930–2010

Fig. 4.

Variability of tree ring widths (data from all plots) in ten-year periods in 1930–2010



Anatomické parametry

Průměrné chronologie plochy lumen vykazují na všech plochách vzestupný trend se stoupajícím věkem (obr. 5a). Plocha Suchá1 se vyznačuje nižší průměrnou hodnotou MCA než ostatní plochy (obr. 5b). Průměrná MCA pro jednotlivé plochy se však mezi sebou statisticky významně neliší ($p < 0,05$). Na některých plochách je patrný vzestup hodnot od konce 90. let, zejména pak na ploše Klínovec. V časových řadách nebyly identifikovány žádné významné roky.

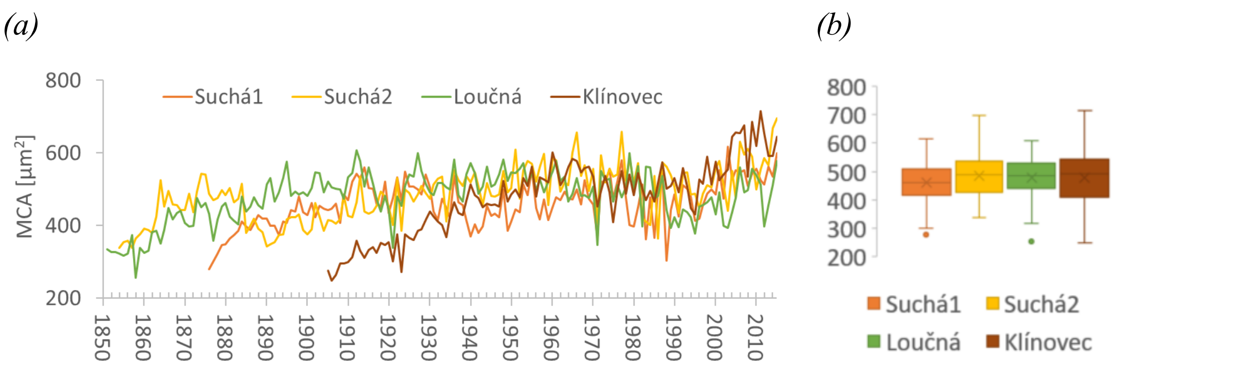
V řadách je možné pozorovat v prvních několika dekádách graduální vzestup hodnot, jejich mírný pokles v 70. letech a výrazný vzestup v poslední dekádě (obr. 6).

Obr. 5.

Průběh průměrných chronologií plochy lumen (MCA) pro jednotlivé plochy (a) a krabicové grafy MCA pro jednotlivé chronologie (b)

Fig. 5.

Mean lumen area chronologies (MCA) of individual series (a) and box plots of MCA of individual chronologies (b)



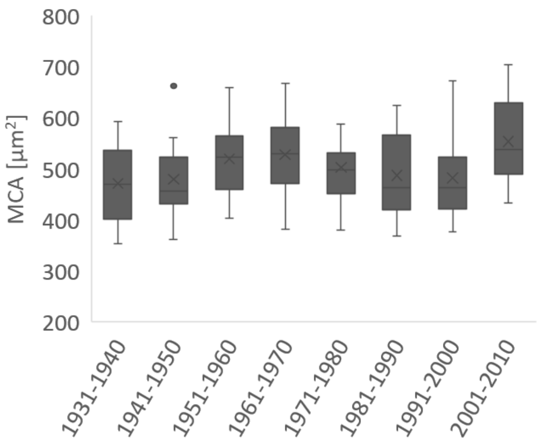
Průběh chronologií počtu buněk v radiálním směru je velmi podobný průběhu letokruhových chronologií (obr. 7a). Nejvyšší variabilita Nmm byla zaznamenána u plochy Suchá 2 (obr. 7b). Průměrná hodnota Nmm pro jednotlivé plochy se mezi sebou statisticky významně neliší ($p < 0,05$). V časových řadách Nmm je možné pozorovat pokles hodnot v 70. a 80. letech a jejich prudký nárůst v 90. letech. Rovněž lze pozorovat zvýšenou variabilitu v posledních třech dekádách (obr. 8). Negativní významné roky však byly zaznamenány ve více případech než u prostých šířek letokruhů. Jedná se o roky 1948, 1956, 1978, 1980 a 1996. Pozitivní významné roky byly zaznamenány naopak v menším počtu případů, konkrétně v letech 1943, 1988, 1989, 1990 a 2001.

Obr. 6.

Variabilita plochy lumen (data ze všech ploch) v desetiletých periodách v období 1930–2010

Fig. 6.

Variability of lumen area (data from all plots) in ten-year periods in 1930–2010



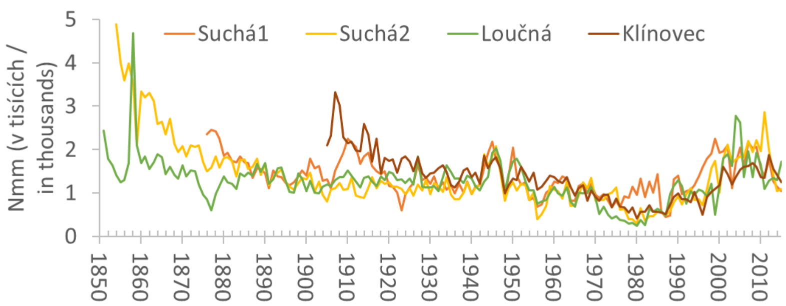
Obr. 7.

Průběh průměrných chronologií počtu tracheid v radiálním směru (Nmm) pro jednotlivé plochy (a) a krabicové grafy Nmm pro jednotlivé chronologie (b)

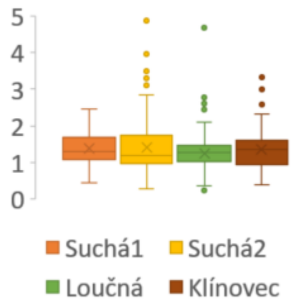
Fig. 7.

Mean chronologies of number of tracheids (Nmm) of individual series (a) and box plots of Nmm of individual chronologies (b)

(a)



(b)

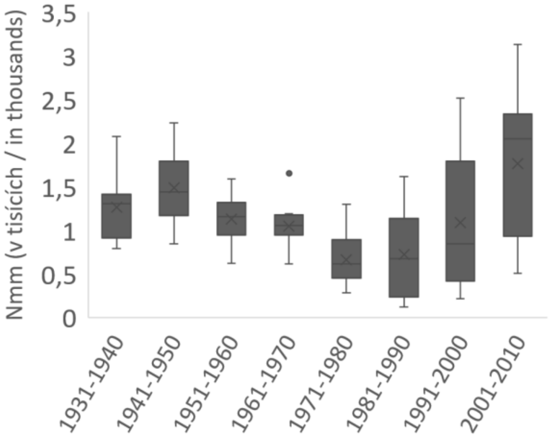


Obr. 8.

Variabilita počtu tracheid (data ze všech ploch) v desetiletých periodách v období 1930–2010

Fig. 8.

Variability of number of tracheids (data from all plots) in ten-year periods in 1930–2010



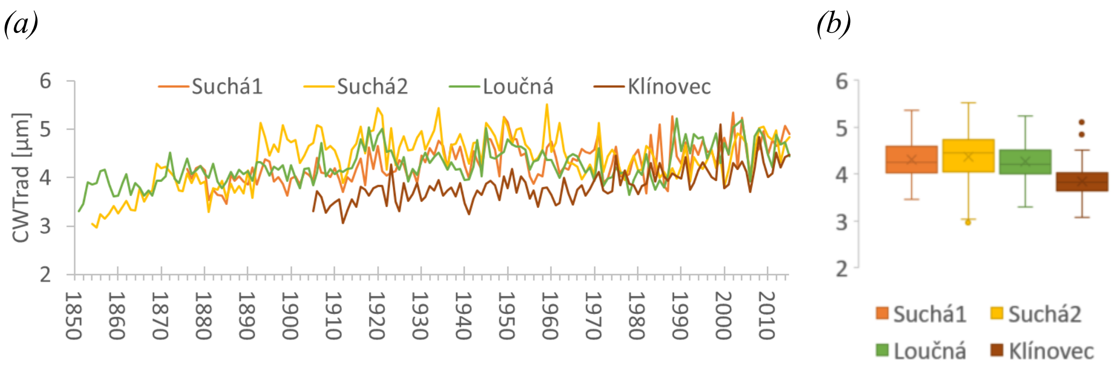
Podobně jako MCA také CWTrad vykazuje na všech plochách vzestupný trend se stoupajícím věkem. Imisní zátěž se v časových řadách CWT projevila pouze mírným poklesem v 70. letech (obr. 9a). Z obr. 9b je vidět značný rozdíl v tloušťkách buněčných stěn mezi plochami. Tyto rozdíly jsou statisticky signifikantní ($p < 0{,}001$). Svým průměrem i variabilitou se nejvíce odlišuje plocha Klínovec. Na obr. 10 je zjevné snížení průměrných hodnot CWTrad, avšak i zvýšení variability mezi jednotlivými sériemi v 70. letech. Zajímavý je vzestup hodnot v poslední hodnocené dekádě (obr. 10). V časových řadách nebyly odhaleny žádné významné roky.

Obr. 9.

Průběh průměrných chronologií tloušťky buněčné stěny (CWTrad) pro jednotlivé plochy (a) a krabicové grafy CWTrad pro jednotlivé chronologie (b)

Fig. 9.

Mean radial cell wall thickness chronologies (Nmm) of individual series (a) and box plots of CWTrad of individual chronologies (b)

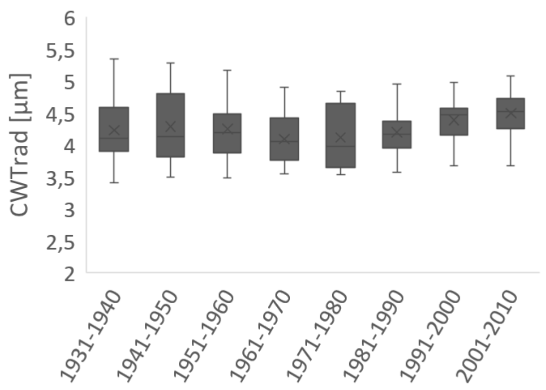


Obr. 10.

Variabilita tloušťky buněčné stěny (data ze všech ploch) v desetiletých periodách v období 1930–2010

Fig. 10.

Variability of radial cell wall thickness (data from all plots) in ten-year periods in 1930–2010



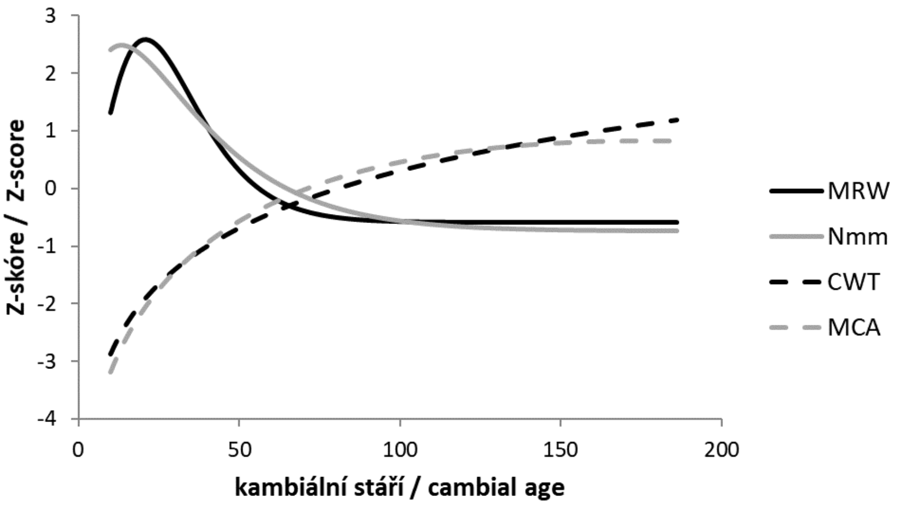
U zkoumaných parametrů byly zaznamenány dva typy věkového trendu (obr. 11). U parametrů MRW a Nmm se jedná o klesající trend, který nejlépe aproximuje Hugershoffova funkce. Naproti tomu MCA a CWTrad s věkem stoupají a tento trend nejlépe popisuje Chapman-Richardsova funkce.

Obr. 11.

Srovnání průběhu věkového trendu pro jednotlivé parametry; pro lepší porovnatelnost byly hodnoty všech parametrů vyjádřeny formou Z-skóre

Fig. 11.

Comparison of age trends of individual parameters; the values are expressed in standard Z-score to ensure better comparability



Největší relativní pokles hodnot měřených parametrů ve stresovém období byl zaznamenán u MRW a Nmm, a to na všech plochách (tab. 2). V období silného znečištění byl přírůst nejvíce redukován na lokalitě Loučná. Mírným poklesem v období 1971–1990 reagovaly na znečištění také MCA a CWTrad s výjimkou lokality Klínovec. V období po ukončení stresového období je u parametrů MRW a Nmm patrný výrazný nárůst, a to dokonce i nad hodnoty před rokem 1970.

Tab. 2.

Průměrné standardizované hodnoty MRW, MCA, Nmm a CWTrad pro jednotlivé plochy před hlavním stresovým obdobím, během něj a po něm a jejich procentuální změna vzhledem k období před hlavním stresovým obdobím (1921–1970)

Mean values of standardized MRW, MCA, Nmm a CWTrad for individual plots before, during and after the main stressed period and their percentage change in relation to the period 1921–1970

	Suchá 1			Suchá 2			Loučná			Klínovec		
Parametr	1921 - 1970	1971 - 1990	1991 - 2015	1921 - 1970	1971 - 1990	1991 - 2015	1921 - 1970	1971 - 1990	1991 - 2015	1921 - 1970	1971 - 1990	1991 - 2015

MRW	**0,95**	**0,58**	**1,26**	**1,05**	**0,63**	**1,51**	**1,10**	**0,49**	**1,16**	**0,99**	**0,62**	**1,27**
MRW [%]	*100,0*	*60,5*	*132,0*	*100,0*	*60,3*	*144,4*	*100,0*	*44,9*	*106,1*	*100,0*	*63,2*	*128,0*
MCA	**0,95**	**0,96**	**1,09**	**1,02**	**0,99**	**1,01**	**1,00**	**0,98**	**0,99**	**1,01**	**0,92**	**1,02**
MCA [%]	*100,0*	*100,9*	*114,3*	*100,0*	*96,5*	*98,7*	*100,0*	*97,8*	*99,2*	*100,0*	*91,0*	*100,2*
Nmm	**1,03**	**0,65**	**1,14**	**1,01**	**0,57**	**1,27**	**1,07**	**0,49**	**1,13**	**1,02**	**0,65**	**1,24**
Nmm [%]	*100,0*	*62,9*	*110,6*	*100,0*	*56,2*	*126,1*	*100,0*	*45,6*	*106,1*	*100,0*	*63,8*	*120,7*
CWTrad	**0,99**	**0,97**	**1,04**	**1,01**	**0,95**	**1,03**	**0,99**	**0,93**	**1,05**	**0,97**	**0,99**	**1,05**
CWTrad [%]	*100,0*	*97,5*	*104,5*	*100,0*	*93,8*	*101,8*	*100,0*	*93,4*	*105,1*	*100,0*	*102,1*	*107,7*

Analýza variance ukázala, že ve stresovém období nejsou mezi plochami statisticky významné rozdíly ($p < 0,05$) v anatomických parametrech MRW, MCA a CWTrad. Signifikantní rozdíl mezi plochami je pouze u parametru Nmm. Tukeyho test mnohonásobných srovnání indikuje, že se od sebe významně liší plochy Loučná a Suchá1 a dále Loučná a Klínovec.

4.2.4 Diskuze

Graduální vzestup hodnot plochy lumen na počátku růstu je důsledkem věkového trendu, který souvisí s výškou stromu a projevuje se u většiny cévnatých rostlin. Pokud by byl průměr vodivých elementů stejný v celé výšce jedince, hydrodynamický odpor by vzrůstal od kořene k vrcholu (Ryan et al. 1997). Rozšiřováním průměru vodivých elementů xylému, tedy i zvětšováním plochy lumen tracheid, od vrcholu směrem dolů, cévnaté rostliny eliminují tento nepříznivý efekt související s růstem do výšky (Carrer et al. 2015).

Pokles hodnot měřených charakteristik v období od konce 60. let do poloviny 80. let je nejvíce patrný u šířek letokruhů a počtu tracheid. Tento pokles byl způsoben chladnějším obdobím s častějším výskytem extrémních zim v kombinaci se silnou imisní zátěží (Kroupová 2002). Znečištění má vliv na kambium a diferenciaci jeho derivátů (Kurczyńska et al. 1997), poškození jehlic či sekundárních kořenů (Vacek et al. 2015). Poškození během zimního období může u stromů prodloužit dobu regenerace, odsunout počátek aktivace kambia a zkrátit vegetační sezonu (Kurczyńska et al. 1997). U stromů rostoucích ve znečištěných oblastech ve srovnání se stromy rostoucími v normálních podmínkách obvykle dochází k opoždění iniciace kambiální aktivity (Rajput et al. 2008). Dünisch et al. (1996) a Rajput et al. (2008) pozorovali rozdíl ve struktuře a rozložení derivátů mezi postiženými oblastmi a oblastmi bez stresové zátěže. Došlo ke snížení počtu tracheid v letokruhu, ke zmenšení radiálního rozměru tracheid a tloušťky buněčných stěn. V naší studii se potvrdilo významné snížení počtu tracheid, naproti tomu plocha lumenu a tloušťka buněčné stěny reagovaly na znečištění jen málo. To pravděpodobně souvisí s negativním vlivem znečištění na činnost kambia, která primárně determinuje počet vyprodukovaných buněk (Kurczyńska et al. 1997). Počet tracheid v letokruhu a šířka letokruhu jsou silně korelované parametry, proto je průběh jejich časových řad velmi podobný (Xu et al. 2014).

Po ukončení hlavní stresové periody dochází k vzestupu hodnot všech měřených charakteristik, který je nejvíce patrný u počtu buněk a šířek letokruhů, u ostatních parametrů je vzestup jen malý, kromě vzestupu plochy lumen na ploše Klínovec. Pozitivní vliv na růst těchto hodnot může mít zejména snížení množství polutantů v ovzduší a prostředí (Lomský, Šrámek 2004), zvýšení teplot v letním období (Vacek et al. 2015), spady dusíku (Spiecker 1996), či tzv. uvolnění růstu, které nastává důsledkem omezení kompetice a zvýšením množství dostupných růstových zdrojů pro přeživší jedince po stresové události (Wimmer 2002; Tsvetanov et al. 2011). Po ukončení stresového období si můžeme také povšimnout zvýšené variability v hodnotách počtů buněk a šířek letokruhů, což je pravděpodobně způsobeno rozdílnou reakcí jedinců na stres a jejich odlišnou regenerační schopností.

Vliv znečištění je detekovatelný pouze v parametrech MRW a Nmm. U parametru MRW však nebyl zaznamenán významný rozdíl mezi zkoumanými plochami v hlavním stresovém období. Předpoklad o vyšší zátěži znečišťujícími látkami u plochy Suchá1 v důsledku přímé exponovanosti k lokálním zdrojům znečištění se tedy neprokázal. Signifikantní rozdíly mezi plochami byly zaznamenány pouze u parametru Nmm, nicméně se jedná o rozdíly na hranici významnosti. Významně se liší plocha Loučná od plochy Suchá1 a Klínovec. U plochy Loučná došlo ve stresovém období k nejvýraznějšímu poklesu Nmm v porovnání s předchozím obdobím, což může být způsobeno vlivem znečištění ze saské strany (nejbližší elektrárna Chemnitz se nachází ve vzdálenosti cca 50 km), roli však může hrát i to, že stáří stromů na této ploše je nejvyšší.

Negativní významné roky byly zaznamenány pouze u MRW a Nmm. Rok 1948 je možné vysvětlit jako odezvu na sucho v předchozím roce. V roce 1956 udeřily během února silné mrazy kombinované s vysokou imisní zátěží (Kroupová 2002; Kolář et al. 2015). Výskyt snížených přírůstů byl v letech 1948 a 1956 pozorován také v Beskydech a Orlických horách (Kroupová 2002). Léto roku 1978 bylo jedním z nejvlhčích a nejchladnějších, avšak svou roli sehrál také negativní vliv imisí (Kroupová 2002). Celá 80. léta, do nichž patří i rok 1980, jsou charakterizována vysokými koncentracemi SO_2 v ovzduší (Kolář et al. 2015). V roce 1980 bylo navíc vůbec nejstudenější léto za celou dobu sledování (Kroupová 2002). Významný pokles v roce 1996, který je patrný pouze u parametru Nmm, způsobil extrémní průběh klimatických faktorů v zimě 1995/1996 v kombinaci s vysokými koncentracemi SO_2 (Vejpustková et al. 2017).

V 70. a 80. letech byly zaznamenány opakované epizody akutního poškození smrkových porostů vlivem SO_2, které se projevily červenáním jehličí a jeho následným opadem (Zimmermann et al. 2002). Jak prokázali Samusevich et al. (2017) na epizodě imisního poškození ze zimy 1995/96, změny v anatomických parametrech byly intenzivnější v rámci jarního dřeva, což pravděpodobně souvisí se zmíněnou výraznou redukcí asimilačního aparátu v průběhu jarních měsíců. V průběhu vegetační sezony je pak ztráta jehličí kompenzována tvorbou nových letorostů. Proto v dalším výzkumu plánujeme studovat zvlášť anatomické znaky jarního a letního dřeva, neboť z nich mohou být extrahovány další environmentální informace (Axelson et al. 2014).

4.2.5 Zěvčr

Výzkum byl proveden na smrkových porostech v okolí Klínovce, nejvyššího vrcholu Krušných hor, které byly během druhé poloviny 20. století silně postiženy zátěží polutantů zejména z tepelných elektráren. Pozornost byla zaměřena na srovnání vlivu znečištění na anatomické charakteristiky mikroskopické stavby dřeva v době nejvyšší zátěže v 70. a 80. letech s obdobím před zátěží a po ní. Studie ukázala, že dobrými identifikátory stresu ze znečištění jsou počty tracheid v letokruhu a šířka letokruhu. Hodnoty těchto parametrů významně poklesly ve stresovém období, přičemž po ukončení stresového období vystoupaly nad původní hodnoty. Variabilita těchto parametrů mezi sériemi se během periody s vysokou imisní zátěží zvýšila, přičemž vysoká variabilita přetrvala i po ukončení stresového období. Parametry plocha lumen a tloušťka buněčných stěn na stres reagovaly jen málo. Rozdílné výsledky však může ukázat další výzkum, který se zaměří na srovnání daných parametrů zvlášť pro jarní a letní dřevo.

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Literatura

Arx G. von, Carrer, M. (2014): ROXAS – A new tool to build centuries-long tracheid-lumen chronologies in conifers. Dendrochronologia 32 (3), 290–293. DOI: 10.1016/j.dendro.2013.12.001

Arx G. von, Crivellaro, A., Prendin, A. L., Čufar, K., Carrer, M. (2016): Quantitative wood anatomy – practical guidelines. Frontiers in Plant Science 7, 781. DOI: 10.3389/fpls.2016.00781

Axelson, J.N., Bast, A., Alfaro, R., Smith, D.J., Gärtner, H. (2014): Variation in wood anatomical structure of Douglas-fir defoliated by the western spruce budworm: a case study in the coastal-transitional zone of British Columbia, Canada. Trees 28 (6), 1837–1846.

Blažková, M. (1996): Black Triangle — the most polluted part of Central Europe. In: Rijtema, P.E., Eliáš, V. (eds.): Regional approaches to water pollution in the environment. Proceedings of the NATO advanced research workshop. Liblice, Czech Republic, September 5–10, 1995. ASI Series, Springer Netherlands: 227–249. NATO ASI Series, Series 2: Environment, 20.

Carrer, M., Arx G. von, Castagneri, D., Petit, G. (2015): Distilling allometric and environmental information from time series of conduit size: the standardization issue and its relationship to tree hydraulic architecture. Tree Physiology 35, 27–33.

Castagneri, D., Petit, G., Carrer, M. (2015): Divergent climate response on hydraulic-related xylem anatomical traits of *Picea abies* along a 900-m altitudinal gradient. Tree Physiology, 35 (12): 1378–1387. DOI: 10.1093/treephys/tpv085

Castagneri, D., Regev, L., Boaretto, E., Carrer, M. (2017): Xylem anatomical traits reveal different strategies of two Mediterranean oaks to cope with drought and warming. Environmental and Experimental Botany 133, 128–138.

Cook, E.R. (1985): A time series analysis approach to tree ring standardization. Thesis. Tucson, University of Arizona: 171 s. Dostupné na/Available on: https://ltrr.arizona.edu/sites/ltrr.arizona.edu/files/bibliodocs/CookER-Dissertation.pdf

Dünisch, O., Bauch, J., Puls, J., Müller, M. (1996): Biological and chemical wood properties of long-term polluted spruce (*Picea abies* [L.] Karst.) at high-altitude stands of the Erzgebirge. Holzforschung, 50 (6): 497–506. DOI: 10.1515/hfsg.1996.50.6.497

Fiala, J., Bretschneider, B., Materna, J. (2002): Development of the atmospheric pollution and forest decline in the Czech part of the Ore Mts. In: Lomský B. et al. (eds): SO2-pollution and forests decline in the Ore Mountains. Jíloviště-Strnady Forestry and Game Management Research Institute: 50–85.

Fritts, H.C. (1976): Tree rings and climate. London, Academic Press: 567 s.

Gindl, W., Grabner, M. (2000): Characteristics of spruce [*Picea abies* (L.) Karst] latewood formed under abnormally low temperatures. Holzforschung 54 (1), 9–11. DOI: 10.1515/HF.2000.002

Hůnová, I., Šantroch, J., Ostatnická, J. (2004): Ambient air quality and deposition trends at rural stations in the Czech Republic during 1993–2001. Atmospheric Environment 38 (6), 887–898.

Kolář, T., Čermák, P., Oulehle, F., Trnka, M., Štěpánek, P., Cudlín, P., Hruška, J., Büntgen U., Rybníček M. (2015): Pollution control enhanced spruce growth in the 'Black Triangle' near the Czech-Polish border. The Science of the Total Environment 538, 703–711.

Kozlowski, T. T., Kramer, P. J., Pallardy, S. G. (1991): The physiological ecology of woody plants. San Diego, Academic Press: 657 s.

Kroupová, M. (2002): Dendroecological study of spruce growth in regions under long-term air pollution load. Journal of Forest Science 48 (12), 536–548.

Kubelka, L. Karásek, A., Rybář, A., Badalík, V., Slodičák, M. (1992): Obnova lesa v imisemi poškozované oblasti severovýchodního Krušnohoří. [Restoration of the imission endangered forest in the northeast Krusne hory Mts.] Praha, Arospoj: 133 s.

Kurczyńska, E. U., Dmuchowski, W., Włoch, W., Bytnerowicz, A. (1997): The influence of air pollutants on needles and stems of scots pine (*Pinus Sylvestris* L.) trees. Environmental Pollution 98 (3), 325–334.

Lomský, B., Šrámek, V. (2004): Different types of damage in mountain forest stands of the Czech Republic. Journal of Forest Science 50, 533–537.

Materna, J. (1999): Development and causes of forest damage in the Ore Mts. Journal of Forest Science 45, 147–152.

Olano, J.M., Eugenio, M., García-Cervigón, A.I., Folch, M., Rozas, V. (2012): Quantitative tracheid anatomy reveals a complex environmental control of wood structure in continental Mediterranean climate. International Journal of Plant Sciences 173 (2), 137–149. DOI: 10.1086/663165

Rajput, K.S., Rao, K.S., Kim, Y.S. (2008): Cambial activity and wood anatomy in *Prosopis Spicigera* (Mimosaceae) affected by combined air pollutants. IAWA Journal 29 (2), 209–219.

Ryan, M.G., Yoder, B.J. (1997): Hydraulic limits to tree height and tree growth: what keeps trees from growing beyond a certain height? BioScience 47 (4), 235–242. DOI: 10.2307/1313077

Rydval, M., Wilson, R. (2012): The impact of industrial SO2 pollution on north Bohemia conifers. Water, Air, & Soil Pollution 223 (9), 5727–5744.

Samusevich A., Zeidler A., Vejpustková M. (2017): Influence of air pollution and extreme frost on wood cell parameters at mountain spruce stands (*Picea abies* (L.) Karst) in the Ore mountains. Wood Research 62, 79–90.

Sander C., Eckstein D., Kyncl J., Dobrý J. (1995): The growth of spruce (*Picea abies* (L) Karst) in the Krkonoše-(Giant) Mountains as indicated by ring width and wood density. Annales des Sciences Forestières 52 (5), 401–410.

Schweingruber, F.H., Eckstein, D., Serre-Bachet, F., Bräker, O. (1990): Identification, presentation and interpretation of event years and pointer years in dendrochronology. Dendrochronologia 8, 9–38.

Schweingruber, F.H. (1996): Tree rings and environment dendroecology. Berne, Haupt: 609 s.

Spiecker, H. (1996): Growth trends in European forests: studies from 12 countries. Berlin, Springer-Verlag: 372 s.

Tsvetanov, N., Nikolova, N., Panayotov, M. (2011): Trees reaction after windthrow recorded in tree rings of pristine *Picea abies* forest ”Parangalitsa”. In: Maaten-Theunissen M. et al. (eds.): TRACE - Tree Rings in Archaeology, Climatology and Ecology. Vol. 9. GFZ German Research Centre for Geosciences: 89–96. Scientific Technical Report 11/07

Vacek S., Hůnová I., Vacek Z., Hejcmanová P., Podrázský V., Král J., Putalová T., Moser W.K. (2015): Effects of air pollution and climatic factors on Norway spruce forests in the Orlicke hory Mts. (Czech Republic), 1979–2014. European Journal of Forest Research 134, 1127–1142. DOI: 10.1007/s10342-015-0915-x

Vaganov, E.A., Hughes, M.K., Shashkin, A.V. (2006): Growth dynamics of conifer tree rings: images of past and future environments. Berlin, Springer: 354 s.

Vejpustková, M., Čihák, T., Semusevich, A., Zeidler, A., Novotný, R., Šrámek, V. (2017): Interactive effect of extreme climatic event and pollution load on growth and wood anatomy of spruce. Trees 31 (2), 575–586.

Wimmer, R., Halbwachs, G. (1992): Holzbiologische Untersuchungen an fluorgeschädigten Kiefern. Holz als Roh und Werkstoff 50 (7–8), 261–267.

Wimmer, R., Grabner, M. (1997): Effects of climate on vertical resin duct density and radial growth of Norway spruce [*Picea abies* (L.) Karst.]. Trees 11 (5), 271–276.

Wimmer, R. (2002): Wood anatomical features in tree-rings as indicators of environmental change. Dendrochronologia 20 (1–2), 21–36.

Xu, J., Lu, J., Evans, R., Downes, G.M. (2014): Relationship between ring width and tracheid characteristics in *Picea crassifolia*: Implication in dendroclimatology. BioResources 9 (2), 2203–2213.

Ziaco, E., Biondi, F., Rossi, S., Deslauriers, A. (2014): Climatic influences on wood anatomy and tree-ring features of Great Basin conifers at a new mountain observatory. Applications in Plant Sciences 2 (10), 1400054. DOI: 10.3732/apps.1400054

Zimmermann, F., Lux, H., Reuter, F., Wienhaus, O. (2002): SO_2 pollution and forest decline in the Ore Mountains - historical aspects, scientific analysis, future developments. In: Lomský B. et al. (eds): SO_2-pollution and forests decline in the Ore Mountains. Jíloviště-Strnady, Forestry and Game Management Research Institute, 86–116.

Trace of air pollution disaster in the xylem traits of Norway spruce (*Picea abies* (L.) KARST.) in the Klínovec area (Ore Mountains, czech republic)

Summary

Tree-ring growth can be defined as a function of climate, biological activity and disturbances. Radial growth is mainly determined by cell structure modification. Anatomical parameters as diameter of tracheids, cell wall thickness, lumen area, proportion of latewood, presence of raisin ducts or wood density fluctuation can give us useful information about environment and its changes. They can react on stress in different ways, which makes them potentially applicable proxy for dendroecological studies. Pollution load is one of the types of abiotic stress that may have significant impact on microscopic structure of wood. Toxicity influences photosynthetic capacity of trees, food storage and production of auxin. Changes in polar transport of auxin than negatively influences cambial activity.

So called “Black Triangle” is a border region covering parts of Northern Bohemia (Czechia), Saxony (Germany) and Poland, and it is characterised by extremely high levels of pollution in the second half of the 20th century. Eastern part of the Ore Mountains was the most affected area with massive dieback of spruce stands. Dendrochronological studies show that deep growth depression in the 1970s and ´80s was followed by sudden regeneration of survived individuals, which furthermore exceeded former values of increment. The aim of this research is to retrospectively analyze the influence of pollution on microscopic structure of wood and to find appropriate pollution stress indicators in wood anatomy.

As a model area, Klínovec hilltop was chosen (Fig. 1). It is characterised by the presence of old spruce stands, and occurrence of extreme pollution levels. The total number of 23 cores has been extracted from 14 trees growing on 4 plots (Tab. 1) with different exposition to the main pollutant sources. Two cores from the opposite sides of tree stem were sampled from 3 trees at each plot, and used for crossdating. Only one core per tree was measured for cell characteristics. As a comparative site, 5 cores were extracted from trees at Klínovec hilltop plot. Safranin-Astra blue stained permanent slides of 12 μm thickness were prepared using rotary microtome and Eukitt mounting medium. Slides were captured with motorised microscope using EDF function that produces fully focused composite images using different values of Z-axis to capture one shot. Parameters of tree ring width (MRW), lumen area (MCA), radial cell wall thickness (CWTrad) and number of cells in 1 mm wide band in one ring (Nmm) were measured or calculated using ROXAS software. Average values of parameters for individual years were calculated for each series. Chronologies were calculated by series averaging. Mean values and variability of

parameters between the plots were computed for every decade since 1930. Years showing the abrupt change higher than 30 % compared to average value of the last three years in more than 50 % of examined series were determined as positive or negative pointer years. Age trend was approximated using appropriate growth functions. Average values of indexed cell parameters were compared for pre-stress, stress and post-stress period. Statistical differences between the plots during the stress period were examined by one-way ANOVA using detrended values of parameters.

Abrupt growth decreases in MRW in 1948 and 1956 are evident (Fig. 3a). The drop of MRW since the second part of the 1960s followed by the steep increase of those values since the 1980s is common for all ring-width series. In the boxplot we can see Suchá 2 as a plot with highest variance (Fig. 3b). Increased variability between average plot values can be observed since the 1980s (Fig. 4). Negative pointer years were determined in 1948, 1956 and 1978, positive years in 1943, 1945, 1959, 1988, 1989, 1990, 1998, 1999 and 2002. Rising tendency of growth trend in all series of MCA is apparent (Fig. 5a). Steep increase of MCA values is evident in the last decade (Fig. 6). No pointer years have been identified in MCA. Course of Nmm curve is very similar to MRW (Fig. 7a). We can also observe higher variability in last three decades (Fig. 8). Negative pointer years were determined in 1948, 1956, 1978, 1980 and 1996, which is more than in MRW, positive pointer years were determined in 1943, 1988, 1989, 1990 and 2001. No decline in 1970s and 1980s is apparent in CWTrad time series, however, an increase in the last valuated decade was detected (Fig. 9a). Significant difference in CWTrad average values between individual plots is obvious (Fig 9b). No pointer years have been identified. Increased variability in the values of individual series in 1970s can be observed (Fig. 10). Negative pointer year 1948 was probably caused by drought in previous year, 1956 is a result of heavy frost in February of that year together with pollution load. Summer 1978 was one of the coldest one. Year 1980 is characterised by high SO_2 concentration and cold summer. In 1996, extreme course of climatic factors during the winter together with pollution accumulation in the inversion layer were the main causes for growth decline.

Age trend was approximated using Hugershoff function for MRW and Nmm and Chapman-Richards function for CWTrad and MCA (Fig. 11). During the most extreme period of pollution load the highest decrease of values was recorded for MRW and Nmm (Tab. 2). Contrary to our expectations, Loučná was the plot with the most reduced values of Nmm and MRW. That can be caused by the impact of pollution from Saxonian side or by the highest age of the stand on this plot. The values of MRW and Nmm in the post-stress period even exceeded the values of pre-stress period.

There is no significant difference ($p > 0.05$) between the plots for MRW, MCA and CWTrad parameters in the stress period. Loučná plot significantly differs ($p < 0.05$) from the plots Suchá1 and Klínovec at Nmm parameter. The study shows Nmm and MRW as useful indicators of pollution stress. Values of those parameters declined during the stress period and rapidly increased afterwards. Variability increased during the stress period, and persisted even after its ending. Parameters as MCA and CWTrad poorly reacted to stress. For future studies the anatomical parameters should be studied separately for the early- and latewood to reveal if different response occurred.

4.3 Influence of air pollution and extreme frost on wood cell parameters at mountain spruce stands (*Picea abies* (L.) KARST.) in the Ore Mountains

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Abstract

The aim of the research was to evaluate the potential of wood anatomy parameters as stress indicators on base of changing cell characteristics and proportion of latewood in *Picea abies* stands damaged by extreme climatic conditions in combination with high air pollution load during the winter 1995/96.

The research was carried out in the Ore Mountains (Czech Republic), where sites were located along the gradient of forest damage.

Preliminary analyses showed the decrease of lumen width, cell wall thickness and the number of tracheid in the tree rings of spruce at heavily damaged site. Significant difference was shown between sites with different damage level. Moreover the difference in reaction dynamics of earlywood and latewood parameters was recorded. The length of stands regeneration was shown to be around 3 years depending on the assessed parameter and the damage rate.

Keywords: radial lumen width, latewood proportion, cell-wall thickness, air pollution, *Picea abies*, Ore Mountains.

4.3.1 Introduction

Radial growth of the tree is a result of a combined influence of tree genetics and environmental factors (Fritts 1976). Cook (1985) defines radial growth as a function of climate, disturbances (external and internal) and biological variability. Though this model was primarily constructed by Cook (1985) for ring-width characteristic, it is applicable for cell growth analysis as well, as changes in tree growth are determined by modifications in cell structure (Vaganov et al. 2006, Wimmer 2002). The study of the environmental influence on wood anatomy is usually defined as "ecological wood anatomy" (Wimmer 2002). Ecological wood anatomy is focused on two main points: 1) the description of direct changes in wood structure as a response to one or several stress factors and 2) the evaluation of adaptation strategies of the tree on base of correlation values between environmental events and individual cell parameters (Wimmer 2002). Different cell features (both continuous and discontinuous) such as cell diameter, tissue proportions, wall thickness, lumen area, latewood proportion, resin ducts, density fluctuations etc. can provide useful information about the growth environment (Ziaco et al. 2014, Olano et al. 2012, Wimmer 2002, Wimmer and Grabner 1997). All these factors react on stress events in a different way thus make them a good proxy for dendroecological studies.

In Central Europe one of the most significant abiotic stress factors influencing forest growth during the last 150 years was continuous transboundary air pollution. One of the most polluted areas in Europe is considered to be known as "Black triangle" – the area that covers northern Bohemia, southern Saxony and part of lower Silesia. This area received its name due to the high emissions of sulphur dioxide, nitrogen oxides and dust, coming from anthropogenic activities (mainly power plants burning brown coal, petrochemical and heavy industry, Renner 2002, Blažková 1996). Emission of main pollutants SO_2, NOx culminated in 1980´s. While in the beginning of 90's situation has significantly changed with the changing of the political and economic situation in Europe.

Generally it is assumed, that conifer trees are more susceptible to pollution load than deciduous trees (Vacek et al. 2013). In Europe the most significant influence was detected at high mountain regions covered with Norway spruce (*Picea abies* L. Karst) (Godek et al. 2015, Staszewski et al. 2012, Fleischer et al. 2005, Herman et al. 2001, Bytnerowicz et al. 2003), one of the most common tree species in Europe with a high economic value. Among the Central European countries the forests in Czech Republic are considered to be the most influenced ones (Lomský et al. 2012, Šrámek et al. 2008, Bridgman et al. 2002, Blažková 1996).

In this case study we will present the first results of air pollution influence on the wood anatomy structure of spruce stands in the Ore Mountains, where the positive development of forest health stay after 1990 was interrupted by extreme winter 1995/96 (Lomský et al. 2013, Lomský and Šrámek 2004). Extreme winter 1995/1996 is characterised by a sudden temperature decrease in November 1995, which was followed by heavy frosts and long-term inversion, resulted in extreme frosts deposits. It also created very good conditions for air pollutants accumulation (SO2 and F) (Lomský et al. 2012, Bridgman et al. 2002). The most affected area within the Czech Republic was an eastern part of the Ore Mountains where air pollution load was the highest. Here about 12 500 ha of spruce stands were heavily damaged, 1 300 ha of them have completely died (Lomský et al. 2013, Lomský and Šrámek 2004). The rest gradually regenerated in the following years.

Our aim was to see: 1) how pollution and climatic conditions during the winter of 1995/1996 influenced the main cell anatomy parameters (radial lumen width, cell wall thickness, proportion of latewood and tracheid number); 2) if there is a difference in stress reaction between the earlywood and latewood and 3) if there is a lag in tree growth reaction on stress event.

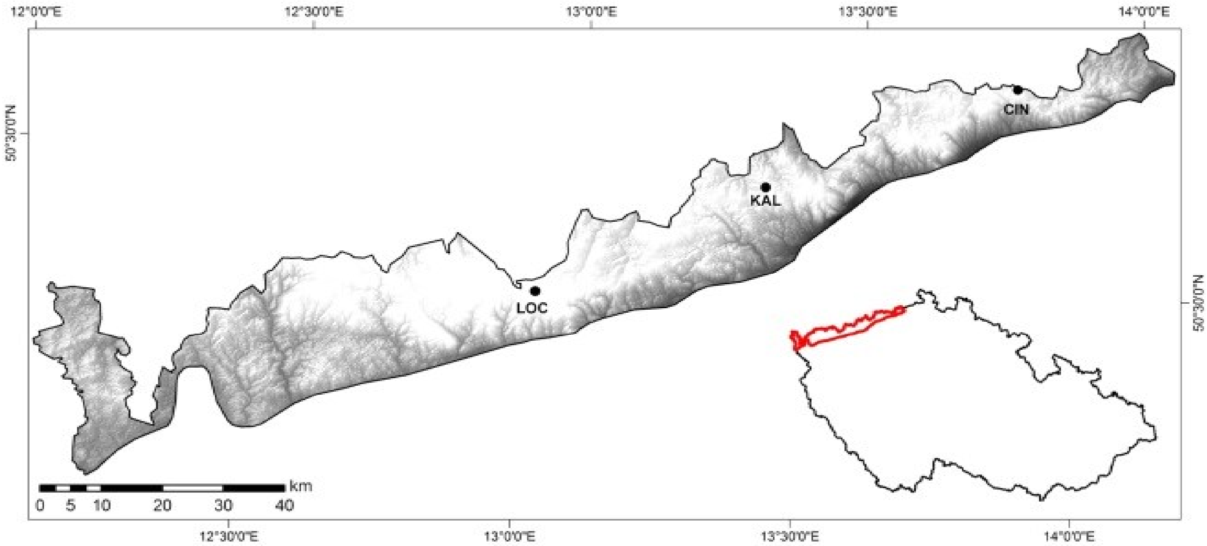
4.3.2 Material and method

Study sites

The Ore Mountains are situated on the border between Czech Republic and German Saxony. It is a typical mountain region with the area of 114 357 ha. Mother rock is formed by gneiss, granite, phyllite and mica schist. Climate of the Ore Mts. is moderately cold with mean July temperatures around 12 – 15°C and mean annual temperature 5,4°C. On top parts of the range mean July temperatures can decrease under 10°C. Border parts of the mountains are warmer with higher amount of precipitations. Average precipitation rate is equals to 750 mm, with around 450 mm during the vegetation period that lasts for 112 days.

For our research purposes 7 permanent plots along the main ridge were established. Plots were located along the gradient of forest damage after the winter 1995/1996 in similar site conditions to exclude the microsite influence. All the stands were chosen to be pure spruce stands with 50-60 year old trees. The sites were divided into three groups according to the defoliation rate: slight damage (defoliation rate under 40%), medium damage (40 – 60%) and heavy damage (above 60%). In this preliminary paper the results from 3 sites, Cínovec (CIN, heavy damaged site), Kálek (KAL, medium damage site) and Loučna (LOC, slight damage site), will be presented (Fig. 1, Tab. 1). While plot CIN in the eastern part of the Ore Mountains represents the most damaged stands with the mean defoliation rate of 77.4%, plot KAL in the middle part of the mountain ridge is moderately damaged with mean defoliation of 46%. Plot LOC (mean defoliation rate of 33.3%) was selected in less affected stands in the western part of the Ore Mountains.

Fig. 1: Map of sampled sites



Tab. 1: Description of studied sites

Plot	Coordinates JTSK X	Coordinates JTSK Y	Altitude, m a.s.l.	Exposition	Age class*	Forest type	Demage level 1995/96
LOC: Loučná	993239,23	839595,89	990	NW	5	Acidic Beech–Spruce	slight
KAL: Kálek	979101,47	809840,91	815	SW	6	Acidic Beech–Spruce	medium
CIN: Cínovec	777321,85	965822,91	820	NW	6	Acidic Beech–Spruce	heavy

*Age class 5 - 40 – 50 years old stands, age class 6: 50 – 60 years old stands)

Dendrochronology

For tree-ring analysis the increment cores were sampled from at least 20 dominant or co-dominant trees per each plot in spring 2014. Two cores per tree were taken at breast height (1.3 m above ground). Ring widths were measured to an accuracy of 0.01 mm, using TimeTable 2, and were subsequently visually cross-dated and statistically verified using the COFECHA programme (Holmes 1983). The basic statistics of ring-width chronologies such as the mean sensitivity (a measure of the annual variability in tree rings), the average correlation with master chronology and the first-order autocorrelation in the series (a measure of the association between growth in the previous year and that in the current year) were computed.

The ring-width series were standardised to eliminate the age trend using the ARSTAN programme (Cook and Holmes 1996). The trend was approximated by the Hugershoff function (Warren 1980), which effectively reflects the exponential decrease of annual increments in young trees. The resulting chronologies were aggregated in stand-level chronology by calculating bi-weight robust mean.

Wood anatomy

For wood anatomy analyses were sampled 3 trees per site, always two cores per tree. The 1991 – 2001 period was analysed. Cores were cut using the GSL-1 microtome (Gärtner et al. 2014). The microsections were sliced into 15 μm and non-Newtonian fluid was used to avoid the breakage of cell walls (Schneider and Gärtner 2013). The microsections were double-stained using Astrablue and Safranin, dehydrated with alcohol and embedded in Canada balsam (Gärtner and Schweingruber 2013). The permanent slides were then photographed at a magnification of 20x with Nicon Eclipse 80i microscope.

The microscopic images were analysed using NIS-Elements software. Within the each tree ring from 3 to 5 files were measured depending on the ring width. It was assumed that wood anatomy characteristics will change differently for earlywood (EW) and latewood (LW) part of the tree. Thus chosen anatomical parameters were measured separately for both EW and LW: tracheid number (NT), radial lumen width (RLW), cell-wall thickness (CWT) and proportion of LW (PrLW). EW and LW were distinguished on base of the Mork index (Mork 1928, Denne 1988). A latewood tracheid was defined as one in which the twice cell wall width between two adjacent tracheids was equal to or greater than the radial width of the lumen. The non-parametric Kruskal-Wallis test (Quinn and Keough 2002) was employed to test the differences in anatomical properties of wood during the period prior to stressful winter 1995/1996 (1993 – 1995), three years after the stress event (1996 – 1998) and the period of recovered growth (1999 – 2001).

4.3.3 Results and discussion

Dendrochronology

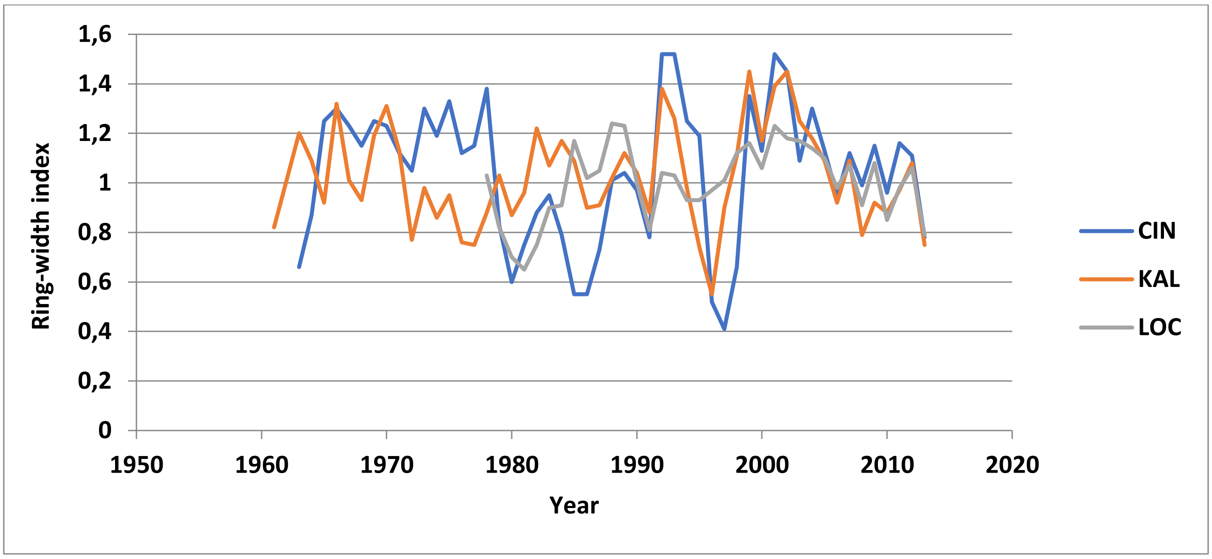
The lowest average ring width was recorded for the most damaged plot CIN. In the same time the highest annual variability in tree-ring widths expressed in the mean sensitivity value was observed for this plot (Tab. 2). Wider tree rings but lower annual variability was proved for tree-ring series of less affected trees at the plots KAL and LOC. The growth in the previous year strongly affected the growth in the current year primarily on slightly damaged plot LOC.

Tab. 2: Properties of ring-width chronologies

Plot	Number of radii	Master chronology	Missing rings (%)	Avg ring width (mm)	Std dev	Mean sensitivity	Corr with master (r)	Autocorr of 1st order
CIN Cínovec	42	1963-2013	0.7	2.21	1.038	0.319	0.652	0.640
KAL Kálek	42	1961-2013	-	2.91	1.046	0.250	0.641	0.627
LOC Loučná	42	1978-2013	-	3.63	1.048	0.167	0.582	0.708

An abrupt growth decrease in 1996 was observed at plots CIN and KAL, but no growth reduction was obvious for LOC (Fig. 2). There was a difference in growth regeneration between CIN and KAL. While at the first plot the growth declined even in 1997 and growth depression lasted till 1999, at the second plot the ring widths had increased sharply already in 1997 and growth rate fully recovered in 1998.

Fig. 2 Standard ring-width chronologies



Radial growth showed a clear reaction to the stress event in the winter of 1995/96, which manifested as an abrupt decrease in the values recorded. The intensity of growth reduction was dependent on forest damage. On the heavily damaged plots CIN the mean defoliation in 1996 and 1997 was 77.4% and 60% respectively. In 1996 most of the first and the second year needles reddened and subsequently fell as a consequence of direct impact of SO_2 (Fabiánek 1997). The increment responds sensitively as the youngest, most photosynthetically active needle sets are impaired (Straw et al. 2002). In tree-ring series of heavily damaged trees the missing rings were detected in the period 1996-1998 with maximum in 1997. In the case of foliage loss over 60% the radial growth is significantly reduced not only in the year of stress event but also in 2 or 3 years following it (Armour et al. 2003, Kurkela et al. 2005). It also coincides with the results of Lomský et al. 2013 when the recovery of tree growth after 1995/1996 stress years continued for around 3 years.

Wood anatomy

Altogether for wood anatomy purposes 18 cores from 9 trees sampled at 3 sites were analysed. According to the aims of the research we conducted more detailed analyses of the period prior to stress event (1993-1995) and two periods after the stress event (1996 – 1998 and 1999 - 2001). The main descriptive characteristics for all three periods are introduced in Tab. 3. At CIN and LOC sites was observed the decrease in mean values of all wood anatomy characteristics after the stress event. Similar trend was shown at KAL site with the exception of CWT parameter. All characteristics returned to their near pre-stress values or even exceeded it in a period 1999 - 2001.

Tab. 3: Mean values of radial lumen width (RLW), radial cell diameter RCD), cell wall thickness (CWT) and tracheid number (NT) at three chosen sites before stress event and after it.

Plot	RLW (µm)			RCD (µm)			CWT (µm)			NT		
	1993 – 1995	1996 – 1998	1999 – 2001	1993 – 1995	1996 – 1998	1999 – 2001	1993 – 1995	1996 – 1998	1999 – 2001	1993 – 1995	1996 – 1998	1999 – 2001
CIN	18,42	15,88	19,65	24,81	21,09	26,18	3,2	2,71	3,27	39,98	16,84	32,61
KAL	18,03	17,95	18,94	24,87	23,88	25,74	3,43	3,72	3,41	51,6	45,7	64
LOC	20,67	20,06	20,56	27,14	26,1	27,18	3,25	3,04	3,32	67,51	60,39	59,53

The initiation of cambial activity in trees growing in the polluted areas is usually delayed in comparison with trees of normal conditions. The cease of vegetation period at polluted sites also starts earlier than at unstressed sites. The difference is shown in structure and arrangement of xylem derivatives between affected and unstressed sites as well, which leads to the decrease of radial cell diameter and number of tracheids (Rajput et al. 2008).

At CIN site the missing ring of 1997 year was registered in 2 cores. No missing rings were found at KAL and LOC sites. The lowest values for lumen width, cell wall thickness, proportion of LW and number of tracheids at CIN site were reached in 1997 year. The growth at LOC site as the least stressed one showed the slight decrease of cell wall thickness, proportion of LW and tracheid number in 1996, the significant decrease in tracheid number at KAL site was also registered in 1996 (Fig. 3, 4, 5).

Figure 3: Radial lumen width of cells for earlywood and latewood at three-level damaged sites (a – Cínovec, b – Loučna, c – Kálek)

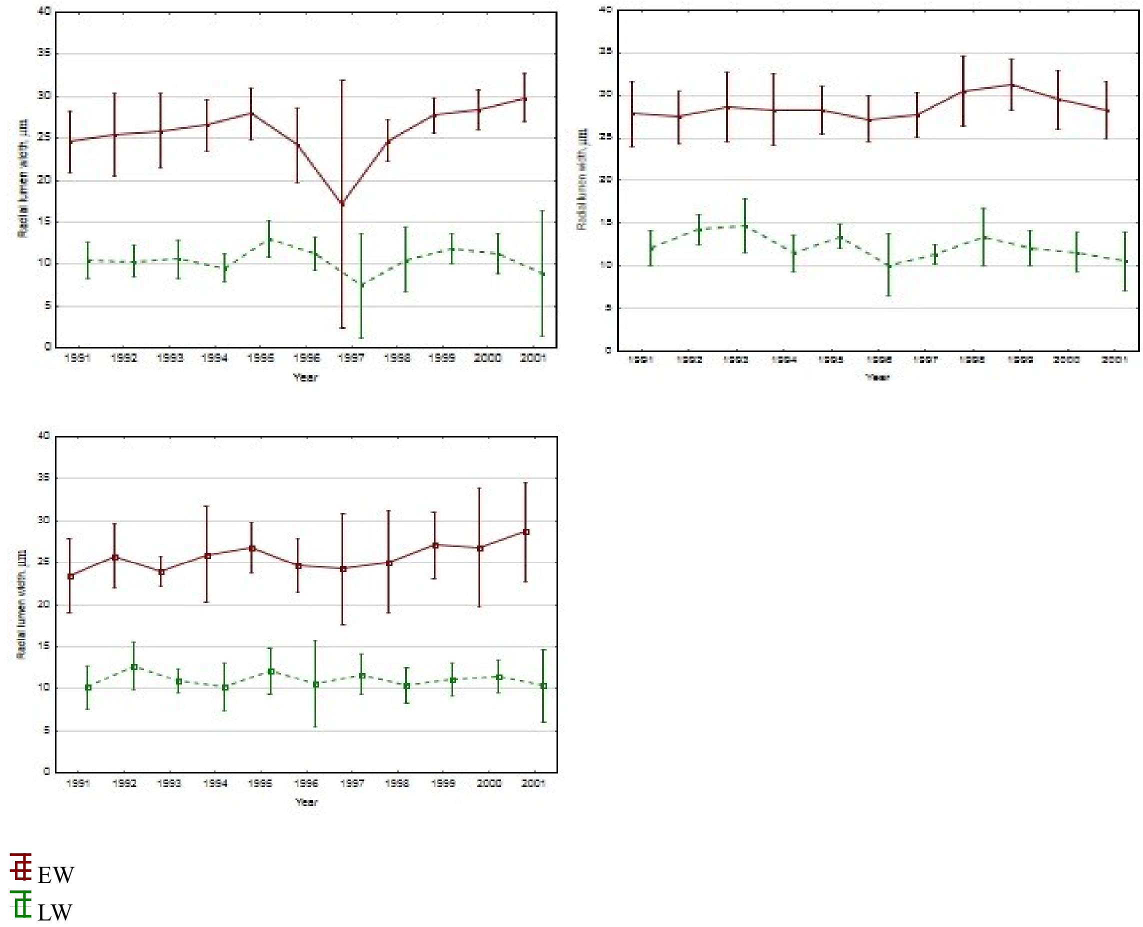
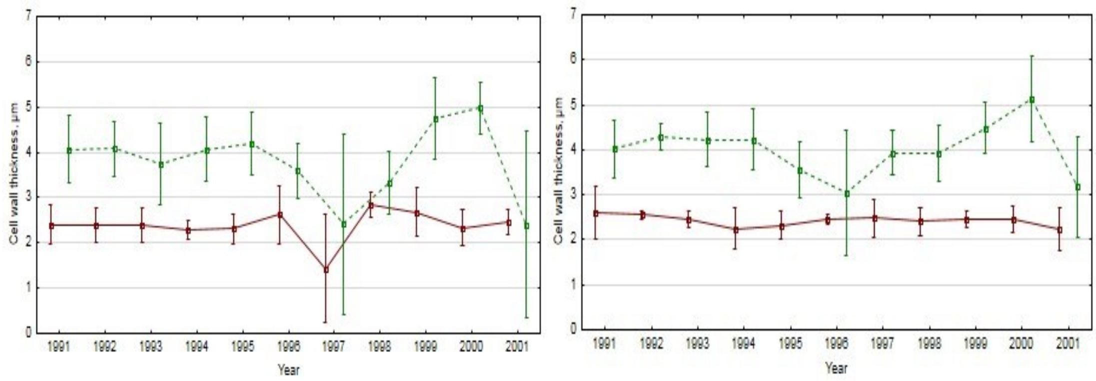
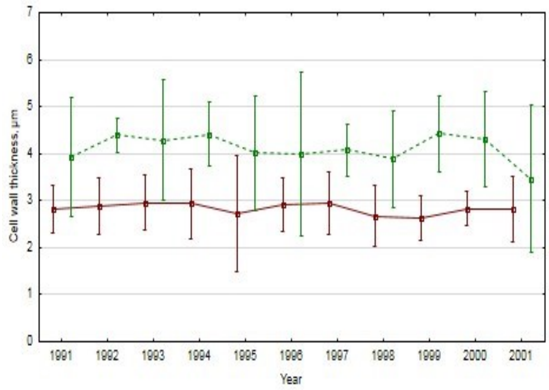


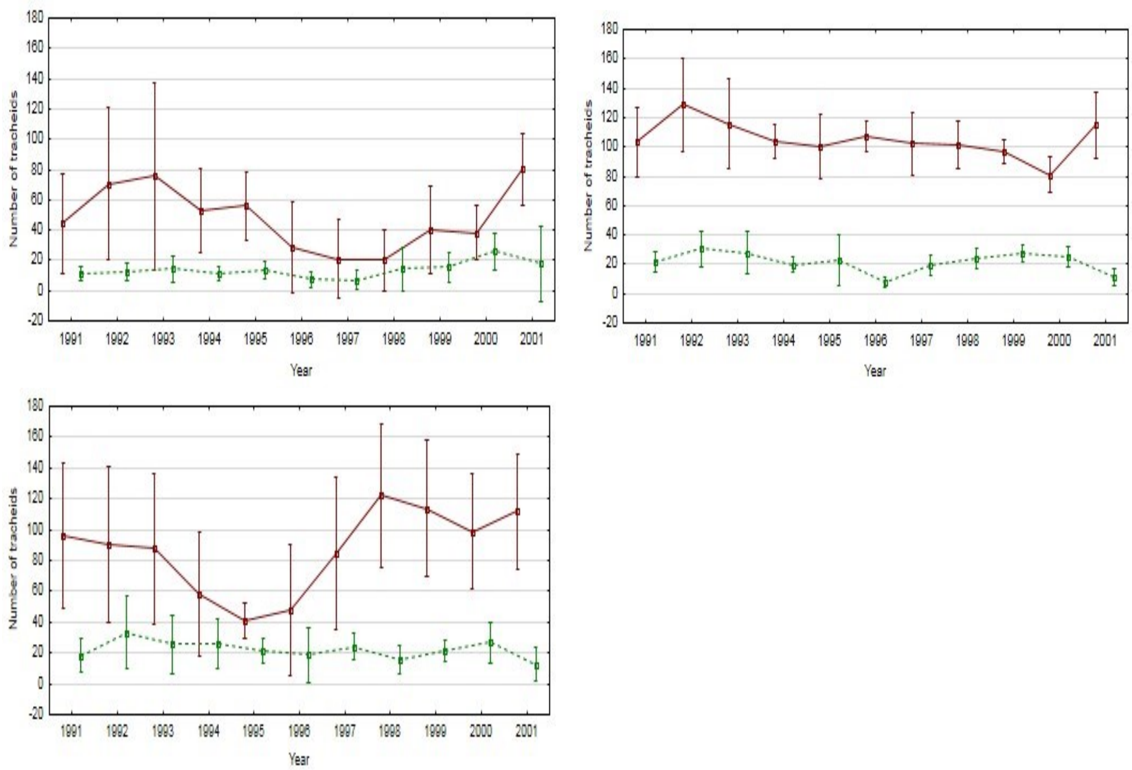
Figure 4: Cell wall thickness of cells for earlywood and latewood at three-level damaged sites (a – Cínovec, b – Loučna, c – Kálek)





EW
LW

Figure 5: Number of tracheids in tree rings for earlywood and latewood at three-level damaged sites (a – Cínovec, b – Loučna, c – Kálek)



At CIN site the period of decreased lumen width and tracheid number lasted from 1996 to 1998, from 1999 was observed the recovery of growth (Fig. 3, 5). For cell wall thickness the period of decline lasted from 1997 to 1998, thus this parameter reacted on stress event with the one year lag (Fig. 4). At less damaged KAL and LOC sites the decline period of tracheid number lasted from 1996 to 1997, thus the recovery hear started earlier than at heavy damaged CIN site (Fig. 5).

The time-lag of the growth reaction to environmental stress was also observed by Axelson et al. (2014). In our study, however, EW part reacted more intensively to the stress event which is in contrast to the results of Axelson et al (2014). This is however directly connected to the type of stress event supressing the tree growth. In the Ore Mts. the stress event occurred during the winter time causing serious damage to the foliage and disrupting the EW formation. Strong air pollution influences the cambium and differentiation of cambial derivatives, thus influencing the time of recovery. Its influence on xylogenesis however need further investigations (Kurczynska et al. 1997).

At CIN site the decrease of all cell parameters in after-stress period was characterised for EW, for LW the decrease of only cell wall thickness was registered. At KAL and LOC site the results were not so conclusive. The decrease of tracheid number has been clearly seen only at earlywood zone at KAL site (Fig. 3, 4, 5).

The analyses implied the different sensitivity of EW and LW of different parameters to stress event. The difference between pre-stress and after-stress periods at KAL and LOC sites were not significant. At CIN site the decrease of lumen width and tracheid number for EW and cell wall thickness for LW was found to be significant in comparison to prior to stress period. The different sensitivity of EW and LW parameters was also shown by the other research (Ziaco et al. 2014, Olano et al. 2012, Park and Spiecker 2005). Wimmer and Halbwachs (1992) observed the changing wood-anatomical features of Norway spruce under the pollution on German side of the Ore Mountains. They pointed out the decrease thickness of the latewood tracheids and decrease of tracheid length. A reduction of earlywood tracheid cross-area was also observed.

The results of wood anatomy analyses correspond well with the dendrochronology results. Our analyses showed, that the most responsive anatomy parameter to pollution was the number of tracheids. Minimum values of tracheid number were reached in 1996 or 1997 depending on the pollution load at the site. Both EW and LW amount of cells within the tree ring decreased in the years following the stress event.

Sites with higher defoliation level were also characterised by smaller proportion of LW in the years following the stress event due to the pollution influence on the cambial activity through changing the differentiation of cambial derivatives in xylem side (Kurczyńska et al. 1997). The same impact of pollution on conifer growth was also observed by Kurczyńska et al. (1997) in Scots pine and by Wimmer (2002) in Norway spruce. Other anatomical parameters as radial lumen width and cell wall thickness showed clear response to the pollution stress in decreasing values only at heavy damaged site. Similar impact of pollution was observed by Maranho et al. (2009) on the example of *Podocarpus lambertii* Klotzsch ex Endl. (*Podocarpaceae*) in Sourthern Brazil. They showed that the trees that were exposed to petroleum pollution had smaller cell diameter and cell wall thickness.

In comparison to heavy polluted CIN site, only a slight decrease of anatomy values was observed at control sites KAL and LOC which highlight the role of pollution intensity in activating the changes in wood anatomy. Genetics, age of the stand (younger forest stands at LOC site) and local site specifics can further modify the reaction of tree growth on pollution stress. Wimmer (2002) highlights that different wood anatomy parameters in the tree are under the genetic control to some degree. The genetic influence can change with time thus causing the changing of environmental signal strength in wood anatomy parameters. Lomský and Šrámek (1999) and Sperry et al. (2006) pointed out, that younger trees are more sustained to pollution and are capable of faster regeneration.

4.3.4 Conclusion

The research was conducted on the Norway spruce stands in the Ore Mountains that were intensively suffered from industrial pollution during the 20th century. A special focus was put on extremely harsh winter of 1995/1996, when the fog and atmospheric inversion created favourable conditions for drastic emission accumulation. Our preliminary research showed that pollution caused the abrupt growth reduction in 1996-1997 in affected sites. The decrease in values of the main cell anatomy parameters as radial lumen width, cell wall thickness, number of tracheids and the proportion of LW was identified. Number of tracheids was defined as the most responsive parameter to the stress event, which was registered at all sites with different damage level. The difference in sensitivity of LW/EW cells was shown. Further research in this field however is needed to identify which EW and LW parameters are more sensitive to stress and how fast they are able to recover after it. In general, longer regeneration period was observed at heavily polluted site in comparison to control site.

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Reference

Armour, H., Straw, N., Day, K. (2003): Interactions between growth, herbivory and long-term foliar dynamics of Scots pine, Trees - Structure and Function 17, 70–80.

Axelson, J.N., Bast, A., Alfaro, R., Smith, D.J., Gärtner, H. (2014): Variation in wood anatomical structure of Douglas-fir defoliated by the western spruce budworm: a case study in the coastal-transitional zone of British Columbia, Canada, Trees 28, 1837-1846.

Blažková, M. (1996): Black Triangle – Most Polluted Part of Central Europe. Regional Approaches to Water Pollution in the Environment. NATO ASI Series, 2. Environment – Vol.20. Kluwer Academic Publishers, Netherland. 227–249 pp.

Bridgman, H.A., Davies, T.D., Jickells, T., Hunova, I., Tovey, K., Bridges, K., Surapipith, V. (2002): Air pollution in the Krusne Hory region, Czech Republic during the 1990s, Atmospheric Environment 36 (21), 3375 – 3389.

Bytnerowicz, A., Badea, O., Barbu, I., Fleischer, P., Fraczek, W., Gancz, V., Godzik, B., Grodzinska, K., Grodzki, W., Karnosky, D., Koren, M., Krywult, M., Krzan, Z., Longauer, R., Mankovska, B., Manning, W.J.,McManus, M., Musselman, R.C., Novotny, J., Popescu, F., Postelnicu, D., Prus-Glowacki, W., Skawinski, P., Skiba, S., Szaro, R., Tamas, S., Vasile, C. (2003): New international long-term ecological research on air pollution effects on the Carpathian Mountain forests, Central Europe, Environment International 29 (2-3), 367 – 676.

Cook, E., Holmes, R.L. (1996): Users Manual for Program ARSTAN. Laboratory of Tree-Ring Research. University of Arizona, Tucson, USA.

Cook, E. (1985): A time series analysis approach to tree ring standardization. Ph.D. Dissertation, The University of Arizona, Tucson, 171 pp.

Denne, M.P. (1988): Definition of latewood according to Mork (1928), IAWA Bulletin 10, 59-62.

Gärtner, H., Lucchinetti, S., Schweingruber, F.H. (2014): New perspectives for wood anatomical analysis in dendrosciences: The GSL-1 microtome, Dendrochronologia 32, 47 – 51.

Gärtner, H., Schweingruber, F.H. (2013): Microscopic preparation techniques for plant stem analysis. Kessel Publishing House, Remagen.

Godek, M., Sobik, M., Blas, M., Polkowska, Z., Owczarek, Z., Bokwa, A. (2015): Tree rings as an indicator of atmospheric pollutant deposition to subalpine spruce forests in the Sudetes (Southern Poland), Atmospheric Research 151, 258 – 269.

Fabiánek, P. (ed), 1997: Ore Mountains – winter 1995/96. FGMRI, Prague.

Fleischer, P., Godzik, B., Bicarova, S., Bytnerowicz, A. (2005): Effects of air pollution and climate change on forests of the Tatra Mountains, Central Europe, Plant Responses to Air Pollution and Global Change 111 – 121.

Fritts, H. C. (1976): Tree Rings and Climate. Academic Press, London, New York, San Francisco, 567 pp.

Herman, F., Smidt, S., Huber, S., Englisch, M., Knoflacher, M. (2001): Evaluation of pollution-related stress factors for forest ecosystems in central Europe, Environmental Science and Pollution Research 8 (4), 231 – 242.

Holmes, R. (1983): Computer-assisted quality control in tree-ring dating and measurement, Tree-Ring Bulletin 39, 77-82.

Kurczynska, E., Dmuchowski, W., Wloch, W., Bytnerowicz, A. (1997): The influence of air pollutants on needles and stems of Scots pine (Pinus sylvestris L.) trees, Environmental Pollution 98 (3), 325 – 334.

Kurkela, T., Aalto, T., Varama, M., Jalkanen, R. (2005): Defoliation by the common pine sawfly (Diprion pini) and subsequent growth reduction in Scots pine: a retrospective approach, Silva Fennica 39, 467–480.

Lomsky, B., Sramek, V., Novotny, R. (2013): The health and nutritional status of Norway spruce stands in the Krušné hory mts. 15 years subsequent to the extreme winter of 1995/96, Journal of Forest Science 59, 359-369.

Lomsky, B., Sramek, V., Novotny, R. (2012): Changes in the air pollution load in the Jizera Mts.: effects on the health status and mineral nutrition of the young Norway spruce stands, European Journal of Forest Research 131 (3), 757 – 771.

Lomsky, B., Sramek, V. (2004): Different types of damage in mountain forest stands of the Czech Republic, Journal of Forest Science 50 (11), 533 – 537.

Lomsky, B., Srámek,V. (1999): Damage of the forest stands in the Ore Mts. during period 1995-1997, Journal of Forest Sciences 45, 169-180.

Maranho, L.T., Dziedzic, M., Muniz, G.I.B., Kuniyoshi, Y.S., Galvao, F. (2009): Effects of the pollution by petroleum on the tracheids along the stem of Podocarpus lambertii Klotzsch ex Endl., Podocarpaceae, Brazilian Journal of Biology 69 (2), 263 – 269.

Mork, E. (1928): Die Qualität des Fichtenholzes unter besonderer Rücksichtnahme auf Schleif- und Papierholz, Der Papier-Fabrikant 48, 741-747.

Olano, J.M., Eugenio, M., Garcia-Cervigon, A.I., Folch, M., Rozas, V. (2012): Quantitative tracheid anatomy reveals a complex environmental control of wood structure in continental Mediterranean climate, International Journal of Plant Sciences 173, 137 – 149.

Park, D., Spiecker, H. (2005): Variations in the tree-ring structure of Norway spruce (Picea abies) under contrasting climates, Dendrochronologia 23 (2), 93 – 104.

Quinn, G., Keough, M. (2002): Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

Rajput, K.S., Rao, K.S., Kim, Y.S. (2008): Cambial activity and wood anatomy in Prosopis spicigera (Mimosaceae) affected by combined air pollutants, Iawa Journal 29 (2), 209 – 219.

Renner, E. (2002): The Black Triangle area - Fit for Europe? Numerical air quality studies for the Black Triangle area, Ambio 31 (3), 231 – 235.

Schneider, L., Gärtner, H. (2013): The advantage of using non-Newtonian fluids to prepare micro sections, Dendrochronologia 31, 175–178.

Sperry, J., Hacke, U., Pittermann, J. (2006): Size and function in conifer tracheids and angiosperm vessels, American Journal of Botany 93 (10), 1490 – 1500.

Šramek, V., Lomsky, B., Slodicak, M., Balcar, V., Kulhavy, J., Hadas, P., Pulkrab, K., Sisak, L., Penicka, L., Sloup, M. (2008): The Ore Mountains: Will Successive Recovery of Forests from Lethal Disease Be Successful? Mountain Research and Development 28 (3–4), 216 – 221.

Staszewski, T., Kubiesa, P., Likasik, W. (2012): Response of spruce stands in national parks of southern Poland to air pollution in 1998-2005, European Journal of Forest Research 131 (4), 1163 – 1173.

Straw, N.A., Fielding, N.J., Green, G., Price, J. (2002): The impact of green spruce aphid, Elatobium abietinum (Walker), on the growth of young Sitka spruce in Hafren Forest, Wales: delayed effects on needle size limit wood production, Forest Ecology and Management 157, 267–283.

Vacek, S., Bílek, L., Schwarz, O., Hejcmanová, P., Mikeska, M. (2013): Effect of air pollution on the health status of spruce stands, Mountain Research and Development 33, 40-50.

Vaganov, E.A., Hughes, M.K., Shashkin, A.V. (2006): Growth dynamics of conifer tree rings: images of past and future environments. Springer, Berlin, Heidelberg, 354 pp.

Warren, W.G. (1980): On removing the growth trend from dendrochronological data. Tree-Ring Bulletin 40, 35–44.

Wimmer, R. (2002): Wood anatomical features in tree-rings as indicators of environmental change, Dendrochronologia 20(1 – 2), 21 – 36.

Wimmer, R., Grabner, M. (1997): Effects of climate on vertical resin duct density and radial growth of Norway spruce [Picea abies (L.) Karst.], Trees 11, 271 – 276.

Wimmer, R., Halbwachs, G. (1992): Wood biological studies on Scotch pine trees damaged by fluorides, Holz als Roh-Und Werkstoff 50 (7-8), 261 – 267.

Ziaco, E., Biondi, F., Rossi, S., Deslauriers, A. (2014): Climatic influences on wood anatomy and treering features of Great Basin conifers at a new mountain observatory, Application in Plants Sciences 2(10), 1 – 13.

4.4. Interactive effect of extreme climatic event and pollution load on growth and wood anatomy of spruce

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Author contribution statement:

MV performed the data analysis and wrote the manuscript,

TČ carried out ring-width measurements and analysed the data,

AS carried out wood anatomy measurements in the laboratory and analysed the data,

AZ supervised the wood anatomy analysis,

RN performed the statistical analysis,

VŠ provided data from long-term assessment in monitoring plots.

Conflict of interest statement: The authors declare that they have no conflict of interest.

Key message: Air pollution intensified the effect of climatic extreme and left a detectable mark in growth and wood anatomy parameters over three to five years following the stress event.

Abstract

The forests in the region of so-called “Black Triangle” suffered from heavy air pollution load until the end of 1980s. Acid deposition reduction in 1990s led to an improvement of forest condition. However, the positive development was interrupted by an extreme climatic and pollution stress during the winter of 1995/96. It resulted in an acute damage of spruce stands, manifested by drying and falling of needles and significant decrease of bud vitality.
The methods of tree-ring and quantitative wood-anatomy analysis were employed to study the impact of this event on growth and wood anatomy of Norway spruce (*Picea abies* (L.) Karst.). Reexamining the tree response remains challenging because the knowledge about the impact of acute pollution stress on xylem traits is still lacking.

The annual radial growth showed a clear reaction to the stress event, manifesting as a strong growth reduction in 1996-1998. Height growth was affected in similar manner, but only recovered in 2001. Anatomical features of both earlywood (EW) and latewood (LW) were affected by the stress, however, with the maximal effect occurring with a lag of one year from the time of the event. The EW part was more responsive and showed a higher variability of parameters than the LW part. During the 1996–2000 stress period, tree growth was driven by climatic factors and by the air-pollution load, later the impact of air pollution was no longer pronounced and tree growth was correlated to the content of nutrients.

Keywords: air pollution, climatic extreme, spruce, tree-rings, wood anatomy

4.4.1 Introduction

The region of the Ore Mountains (Erzgebirge) in Northern Bohemia is an example of a Central European area with a long-standing history of air pollution load. It is a part of the so-called "Black Triangle", the area covering Northern Bohemia, Southern Saxony and part of lower Silesia, where emission of pollutants such as sulphur dioxide, fluorine and nitrogen oxides have significantly increased since 1950 and culminated in the 1980s (Kopáček and Veselý 2005). The main source of pollution were primarily the power stations burning locally mined brown coal with a high sulphur and fluoride content and the chemical industry constructed in North-west Bohemia and Southern Saxony. The Eastern part of the Ore Mts. suffered much more from air pollution than the Western part (Zimmermann et al. 2002). Here, tremendous dieback of spruce forest took place in the 1970s and 1980s (Materna 1999).

The decrease of industrial production and the implementation of new technologies during the 1990s led to a rapid decrease of the air pollution load (Šrámek 1998, Hůnová et al. 2004) and to an improvement of the forest condition (Fiala et al. 2002). Several dendrochronological studies proved a recovery of spruce growth in different regions of the Black Triangle since the end of the 1980s (e.g. Kolář et al. 2015, Rydval and Wilson 2012, Treml et al. 2012, Danek 2007). However, only few studies are available from the Ore Mts. (Wimmer 2002, Kroupová 2002).

The positive development of forest health in the Ore Mts. was interrupted by an extreme stress event that occurred during the winter of 1995/96 (Bridgman et al. 2002). It was characterised by a sudden temperature decrease in November 1995 that was followed by a period of heavy frosts and a long-term inversion situation associated with the accumulation of air pollutants (SO_2, F) in the inversion layer. Tree crowns were subsequently covered by extreme hard rime with high concentrations of pollutants, lasting continuously since the end of November until mid-February (Lomský and Šrámek 1999). Combination of these factors resulted in an acute damage of spruce stands, manifested by drying and falling of needles and significant decrease of bud vitality. The area most affected was the Eastern part of the Ore Mountains with highest air pollution levels (Lomský and Šrámek 2004). In reaction to this event, the network of permanent research plots was established in the spruce stands for the long-term monitoring of forest health. Continual assessment of crown condition, tree nutrition and pollution load documented spruce gradual regeneration in the years following the stress event (Lomský et al. 2013). However, the effect on radial growth and wood anatomy has not been examined yet, most likely because the knowledge about the impact of acute pollution stress on xylem traits is lacking.

Here, we evaluate the impact of extreme climatic and pollution stress during the winter 1995/96 on radial and height increment and wood anatomy of Norway spruce (*Picea abies* (L.) Karst.) in the Ore Mountains. We assess also the relationship of growth and xylem traits to the air pollution load, climatic factors and tree nutrition. We expected a strong growth reduction in reaction to the stress event and corresponding changes in wood anatomy in both EW and LW parts of tree rings. We assumed slow recovery of ring widths as the stem growth has lower priority compared to foliage growth or bud growth.

4.4.2 Material and methods

Study sites

The transect of 20 permanent research plots located along the main ridge of the Ore Mountains was established in 1995 within the young spruce (*Picea abies* (L.) Karst) stands to monitor forest health, nutrition and air pollution load. Ten plots covering the gradient of forest damage after the winter of 1995/96 were selected for this study (Fig. 1, Table 1). Figure 2 gives the quantitative details about the intensity of climatic and air pollution stress during the winter 1995/96 (Fig. 2). The result of defoliation assessment in 1996 (Lomský et al. 2013) was the main criterion for the selection of plots and for their classification in three damage classes: slight damage (mean defoliation up to 40%), medium damage (40-60%) and heavy damage (above 60%). All study plots were located in similar site conditions on the plateau between the altitudes of 795 and 990 m asl. The plateau of the Ore Mts. is a climatically humid and cool region characterised by a mean annual temperature of 5.4°C, a mean temperature during the vegetation season of 11.5°C and by a vegetation period of 112 days (Plíva and Žlábek 1986). The annual and the vegetation period sums of precipitation amount to 840 and 440 mm, respectively. Winter inversions accompanied by the occurrence of rime and icing are typical for this region. Rime is very frequent in the altitude above 650 m asl, where it is formed by freezing of warm air saturated with water vapour and smoke (Lange et al. 2003).

Fig. 1 Location of sample plots (circles), meteorological station (triangle) and air pollution monitoring stations (squares) against the background of a shaded relief image of the Ore Mts.

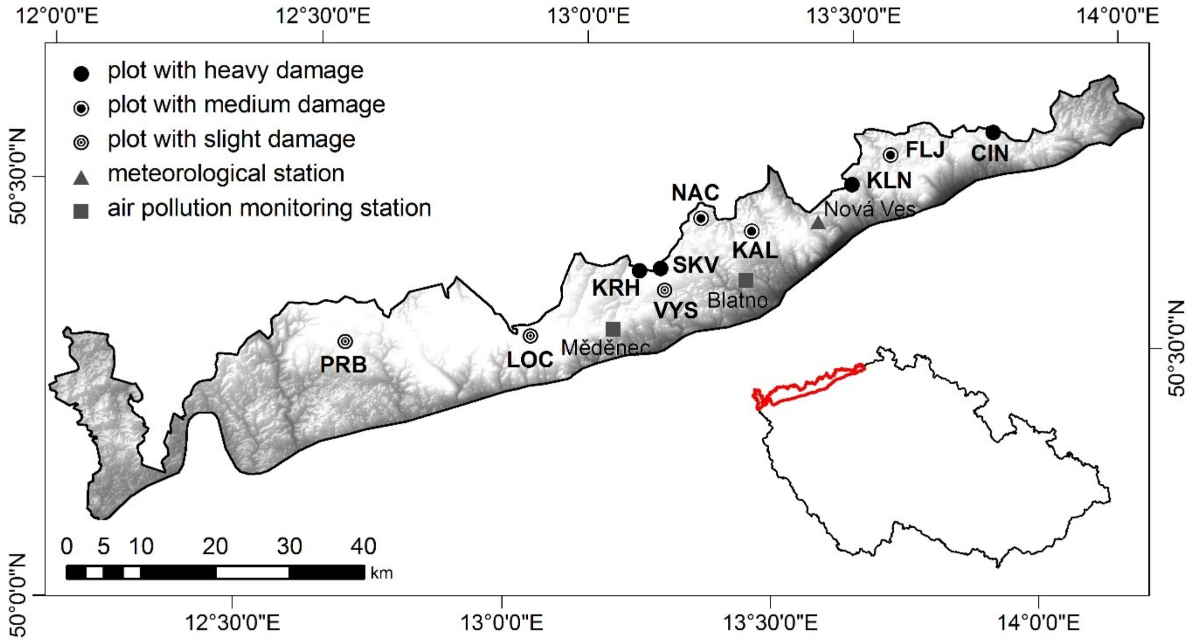
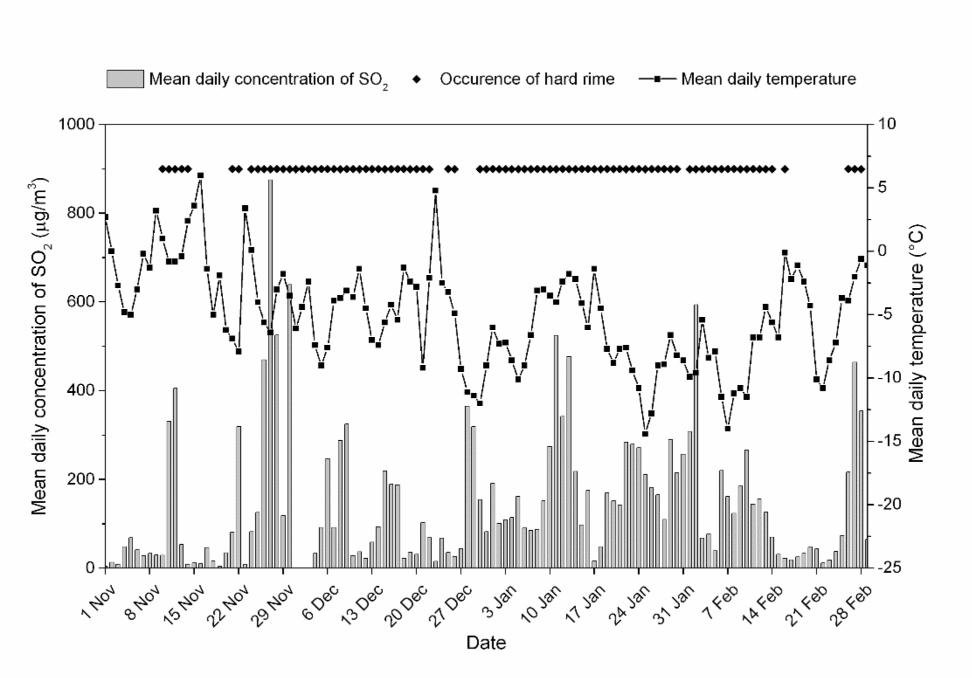


Table 1 Characteristics of study sites located in the Ore Mts., Czech Republic (50°21’-50°43’N, 12°37’-13°47’E)

Plot	Altitude	Exposition	Age class*	Forest type	Soil	Level of damage 1995/96
PRB: Přebuz	885	-	6	Nutrient-poor Beech-Spruce	Haplic Podzols	slight
LOC: Loučná	990	NW	5	Acidic Beech–Spruce	Entic Podzols	slight
VYS: Výsluní	810	SW	5	Acidic Spruce-Beech	Entic Podzols	slight
NAC: Načetín	795	-	6	Acidic Beech–Spruce	Haplic Podzols	medium
KAL: Kálek	815	SW	6	Acidic Beech–Spruce	Haplic Podzols	medium
FLJ: Fláje	750	SW	6	Acidic Beech–Spruce	Entic Podzols	medium
KRH: Kryštofovy Hamry	900	W	6	Acidic Beech–Spruce	Haplic Podzols	heavy
SKV: Skelný vrch	875	SE	5	Acidic Beech–Spruce	Haplic Podzols	heavy
KLN: Klíny	800	-	6	Acidic Beech–Spruce	Haplic Podzols	heavy
CIN: Cínovec	820	NW	6	Acidic Beech–Spruce	Haplic Podzols	heavy

Fig. 2 Daily variation of pollution load, air temperature and occurrence of hard rime during the winter 1995/96 (depicted period: 1.11.1995 – 29.2.1996)



Dendrochronology

Increment cores were sampled in spring 2014, 18 years after the stress event. At least 20 dominant or co-dominant trees were selected per plot. Two cores per tree were taken at breast height (1.3 m above ground).

Ring widths were measured to an accuracy of 0.01 mm, using TimeTable 2, and were subsequently visually cross-dated and statistically verified using the COFECHA programme (Holmes, 1983). The basic statistics of ring-width chronologies such as the mean sensitivity (a measure of the annual variability in tree rings), the average correlation with master chronology and the first-order autocorrelation in the series (a measure of the association between growth in the previous year and that in the current year) were computed.

Ring-width chronologies were developed using the standard dendrochronological methods (Cook and Kairiukstis 1990). The trend was approximated by the Hugershoff function (Warren 1980), which effectively reflects the exponential decrease of annual increments in young trees. The remaining autocorrelation was eliminated by autoregressive modelling. The resulting residual chronologies were aggregated in stand-level chronologies by calculating bi-weight robust mean.

Wood anatomy

For wood anatomy analyses samples from the heavily damaged site Klíny and the slightly damaged site Přebuz were used. Three trees per site were randomly chosen and sampled; always two cores per tree. The 1991-2001 period was analysed for anatomical parameters. In the laboratory, the cores were segmented into 2-3 cm sections for preparing microslides. The segments were cut using the GSL-1 microtome (Gärtner et al. 2014). The microsections were sliced into 15 μm and non-Newtonian fluid was used to preserve the integrity of the cells (Schneider and Gärtner 2013). The microsections were double-stained using Astrablue and Safranin, then dehydrated with alcohol and embedded in Canada balsam (Gärtner and Schweingruber 2013). The permanent slides were then photographed at a magnification of 20x.

The microscopic images were analysed using NIS-Elements software. Eleven annual tree-rings were measured along three to five files depending on ring width. The following anatomical parameters were measured separately for both EW and LW: number of cells, lumen width and cell-wall thickness. Proportion of LW was also taken into consideration. Separation between the EW and LW was made based on the Mork index (Mork 1928, Denne 1988). A latewood tracheid was defined as one in which the width of the common cell wall between two adjacent tracheids, multiplied by two, was equal to or greater than the width of the lumen.

Forest monitoring and climatic data

The study took advantage of a complete dataset from the ongoing annual assessment implemented on permanent research plots between 1995 and 2013. For further analyses, we used the following data: defoliation, height increment - i.e. the annually measured length of terminal shoots, content of nutrients N, P, 0K, Ca, Mg and of load elements S and F in current year needles. The used methods of plot assessment and datasets are described in detail in Lomský et al. (2013). The climatic conditions were characterised by mean monthly temperatures and monthly sums of precipitation, measured at the meteorological station Nová Ves v Horách, located directly on the plateau of the Ore Mts. at an altitude of 726 m asl (Fig. 1). Monthly SO_2 concentrations from the stations Blatno (650 m asl) and Mědĕnec (827 m asl) (Fig. 1) describe air pollution load in the region.

Statistical analysis

The data standardization had preceded the multivariate analysis. The data were standardised by subtracting their means and dividing by their standard deviations. Hence the differences in variables scale were removed.

Ward's multivariate technique (Euclidian distance) was applied to cluster the plots into individual stress level groups using the standardised values of the stand-level data on radial and height growth, defoliation and content of load elements S and F in needles during the 1995-2013 period. The Kryštofovy Hamry plot was not included in this analysis because data are not available for the entire period evaluated.

In our study, principal component analysis (PCA) (Jolliffe, 2002) was preferred to regression analysis to avoid the problems with data normality and with multicollinearity between the variables. We applied PCA to the dataset comprising available stand-level data together with mean monthly temperatures, sum of monthly precipitation and mean monthly SO_2 concentrations in the air. Monthly variables entered the PCA in sequence from January of the previous year to September of the current year, i.e. observation year for growth parameters. In a first step, we repeatedly run PCA to search for a pattern within the dataset and to reduce the number of variables to include only those with a high significance. Thereby, the final dataset for PCA comprises aggregated climatic and pollution variables (Table 2) complemented by the content of the nutrients N, P, K, Ca and Mg and the load elements S and F in the current year needles and by the growth variables – radial (RWI) and height (HI) increment. In a second step, the structure and the linkages between the variables were evaluated for the stress period between 1996 and 2000, when the trees were gradually regenerating after the stress event, and also during the post-stress period between 2001 and 2013. The significant principal components were selected in accordance with Kaiser's rule (eigenvalue>1) (Kaiser 1992).

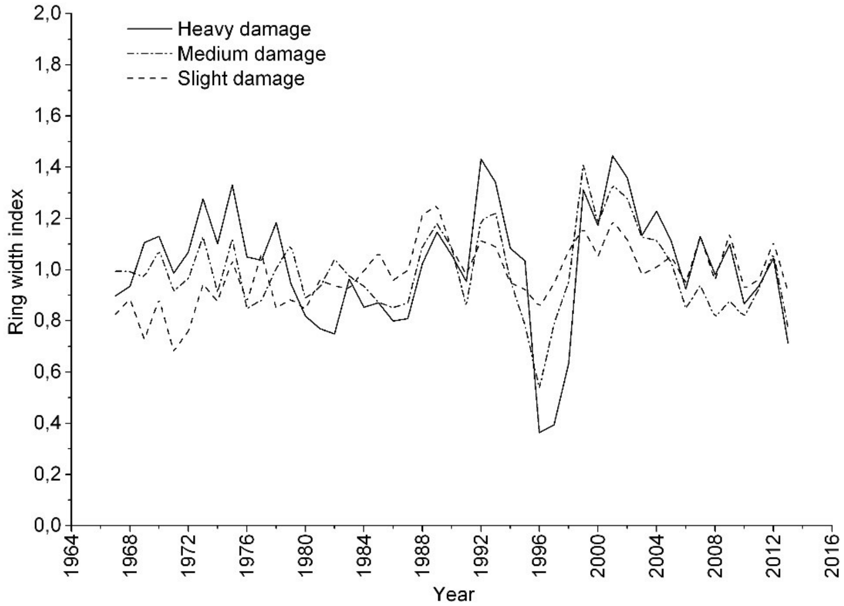
Table 2 Aggregated variables for PCA analysis

Aggregated variable for PCA	Abbreviation
Mean temperature of current vegetation season *(from May to September of current year)*	**Temp**
Sum of precipitation for previous vegetation season *(from May to September of previous year)*	**PRV**
Sum of precipitation for dormancy period prior to growth period *(from October of previous year to April of current year)*	**PRD**
Mean SO_2 concentration for previous vegetation season *(from May to September of previous year)*	**SO_2V**
Mean SO_2 concentration for dormancy period *(from October of previous year to April of current year)*	**SO_2D**

4.4.3 Results

Mean ring-width chronologies (Fig. 3) show the high agreement in inter-annual fluctuations between the plots of different damage level. Long-term growth pattern reveals that the stress event in 1995/96 was exceptional, no comparable abrupt growth decrease had been recorded before.

Fig. 3 Mean ring-width chronologies of the plots with different damage levels



Tree-ring analysis detected failures in tree-ring formation in four plots (Table 3). We found the highest proportion of missing rings in the heavily damaged plots. Mean sensitivity values indicated an increasing annual variability in tree rings with increasing level of damage. Simultaneously, damaged plots showed a higher inter-correlation with master chronology than the plots with only slight or medium damage. The growth in the previous year strongly affected the growth in the current year primarily in slightly damaged plots.

Table 3 Properties of ring-width chronologies from ten research plots that were used for assessing the growth reaction of spruce to the stress event during the winter of 1995/96

Level of damage 1995/96	Plot	Number of radii	Master chronology	Missing rings (%)	Avg ring width (mm)	Std dev	Mean sensitivity	Corr with master (r)	Autocorr of 1st order
slight	**PRB** Přebuz	48	1965-2013	-	3.07	1.399	0.191	0.488	0.805
	LOC Loučná	42	1978-2013	-	3.63	1.048	0.167	0.582	0.708
	VYS Výsluní	44	1982-2013	-	4.24	1.378	0.231	0.524	0.558
medium	**NAC** Načetín	44	1961-2013	0.2	3.18	1.098	0.225	0.589	0.631
	KAL Kálek	42	1961-2013	-	2.91	1.046	0.250	0.641	0.627
	FLJ Fláje	42	1967-2013	-	3.39	1.191	0.244	0.597	0.625
heavy	**KRH** Kryštofovy Hamry	40	1958-2013	0.6	2.14	0.945	0.245	0.632	0.756
	SKV Skelný vrch	42	1979-2013	-	3.76	1.356	0.270	0.776	0.563

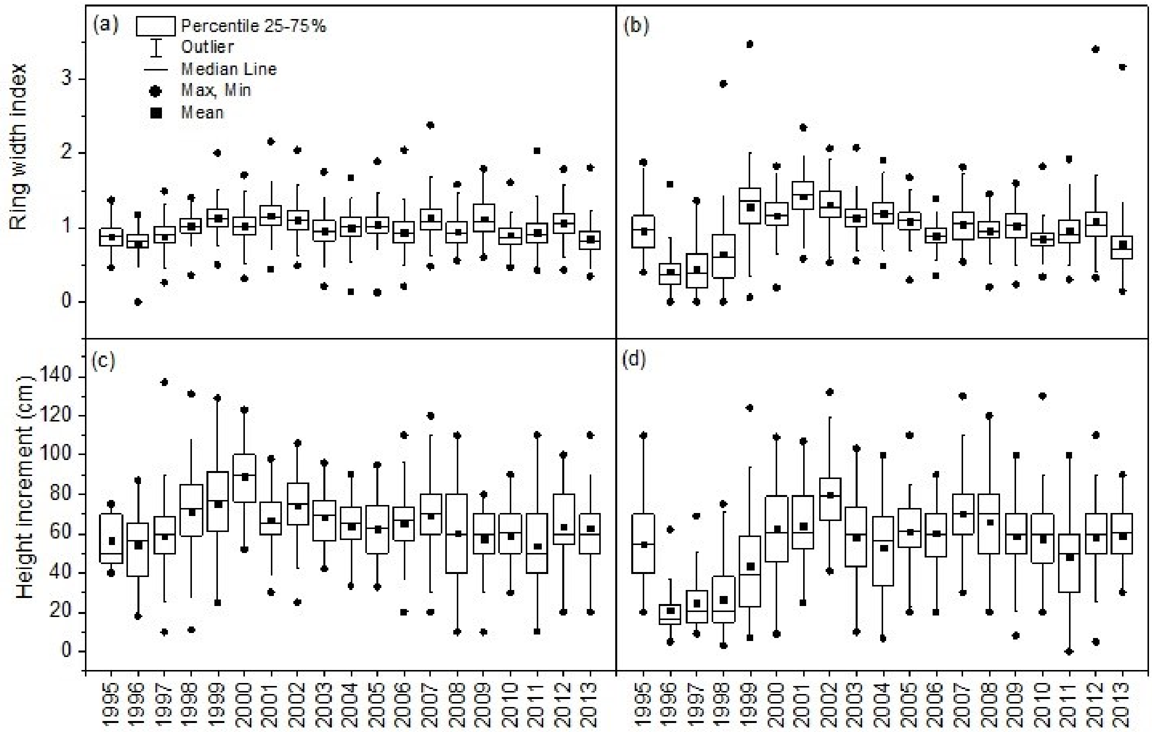
KLN Klíny	44	1966-2013	0.7	2.84	1.132	0.309	0.683	0.583
CIN Cínovec	42	1963-2013	0.7	2.21	1.038	0.319	0.652	0.640

Ring-width data demonstrate a sensitive response to the stress event that occurred during the winter of 1995/96. While a minor growth reduction and no changes in inter-tree variability were detected in tree-ring series from slightly damaged plots (Fig. 4a), the abrupt drop of increments in 1996 is evident for heavily damaged stands (Fig. 4b). The increments in the heavily affected plots stagnated until 1998, followed by a sharp increase in 1999. Inter-tree variability in terms of radial growth increased significantly during the phase of tree regeneration after the stress event. Missing rings were detected during the 1996-1998 period with utmost occurrence in 1997. In that year, tree-ring formation failed in 2.7% of all radii that were analysed.

Height growth in slightly damaged plots increased steadily from 1995 till 2000 without any sign of a stress event effect (Fig. 4c), whereas in heavily damaged plots, the annual height increment was strongly reduced between 1996 and 2000 (Fig. 4d). In contrary to ring widths, the variability of height increments in heavily damaged plots decreased sharply in 1996-1998.
After the stress event, the growth parameters indicated a regeneration phase of differing lengths, ranging between three to five years. According to their radial growth, the trees had already recovered in 1999, when the mean ring widths exceeded those of the slightly damaged plots. According to height growth the regeneration period lasted until 2001.

Cluster analysis assorted the plots into three distinct groups with different stress levels: heavy (CIN, KLN, SKV), medium (FLJ, NAC, KAL) and slight (LOC, PRB, VYS) (Fig. 6). The dendrogram clearly shows the initial split between the heavy stress and the medium/slight stress plots. The medium and the slight stress plots split from the same branch of the hierarchical tree, indicating that the spruce stands from these two groups have more common variability in terms of their growth pattern than the heavily stressed stands.

Fig. 4 Box plot of **(a)** ring-width indices in slightly damaged plots, **(b)** ring-width indices in heavily damaged plots, **(c)** height increments in slightly damaged plots and **(d)** height increments in heavily damaged plots. All box plots are based on data covering the 1995-2013 period.



At the heavily damaged site Klíny, all anatomical parameters responded to the stress event; the lowest values for the lumen width, cell wall thickness and number of tracheids were observed in 1997 (Fig. 5). EW was more responsive with higher variability in number of tracheids and lumen width than LW. On the other hand, the effect of stress on cell wall thickness was more pronounced in LW than in EW. At the slightly damaged Přebuz site, only cell-wall thickness of LW showed a clear decrease in the stress year. Its lowest values were observed in 1996. Proportion of LW showed a clear increase in stressful years only at the Klíny site. The highest proportion of LW was observed in 1997. Analyses of variance at Klíny site showed that the most responsive anatomical parameter is the number of tracheids.

Cluster analysis assorted the plots into three distinct groups with different stress levels: heavy (CIN, KLN, SKV), medium (FLJ, NAC, KAL) and slight (LOC, PRB, VYS) (Fig. 6). The dendrogram clearly shows the initial split between the heavy stress and the medium/slight stress plots. The medium and the slight stress plots split from the same branch of the hierarchical tree, indicating that the spruce stands from these two groups have more common variability in terms of their growth pattern than the heavily stressed stands.

Fig. 5 Mean chronologies of anatomical parameters for the period 1991-2001 for (a) Klíny and (b) Přebuz. Vertical bars indicate ± 0.95 confidence interval

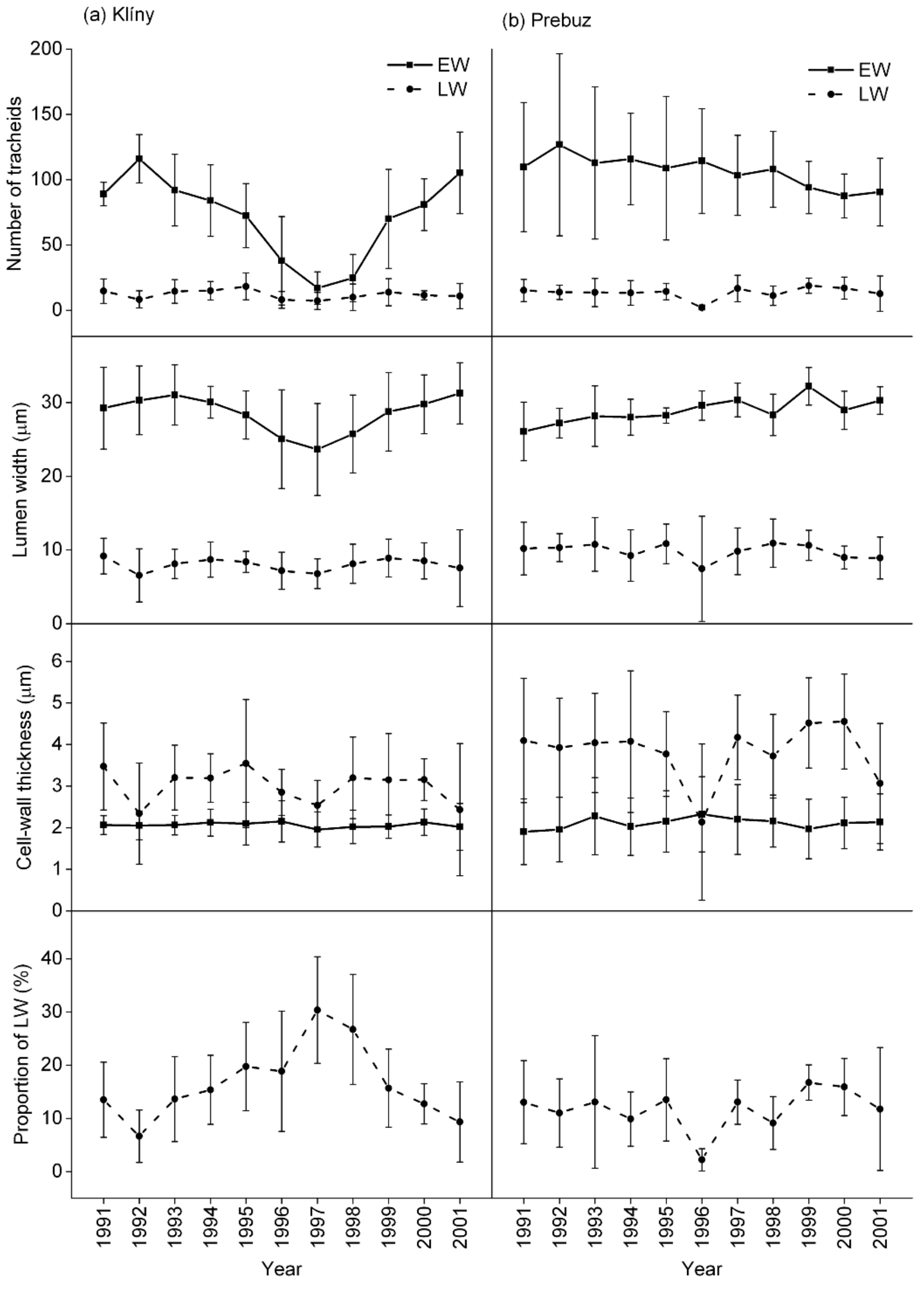
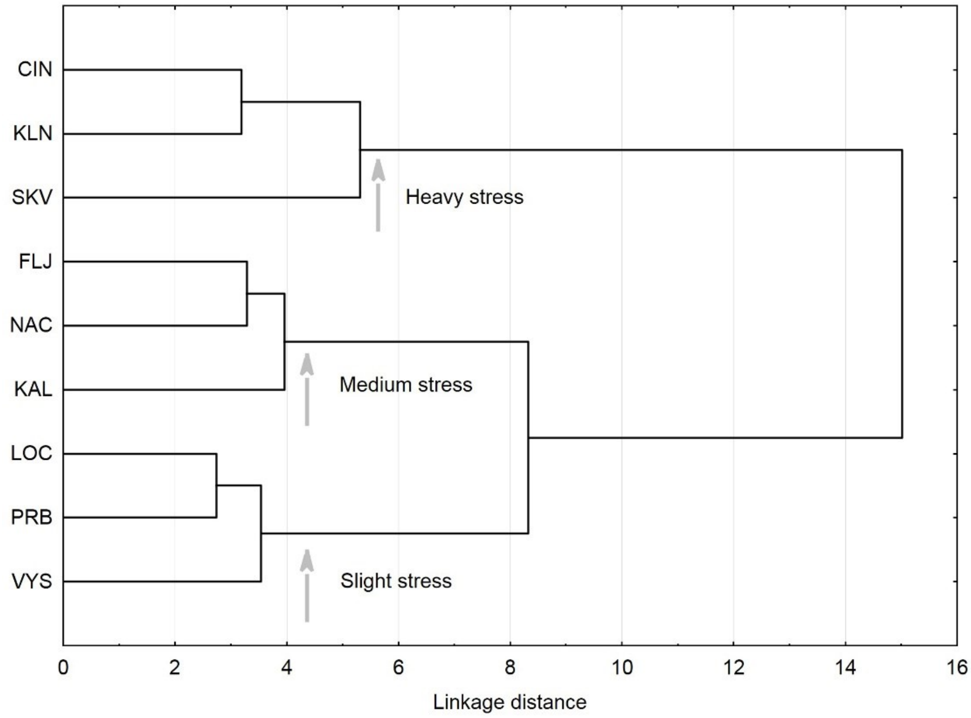


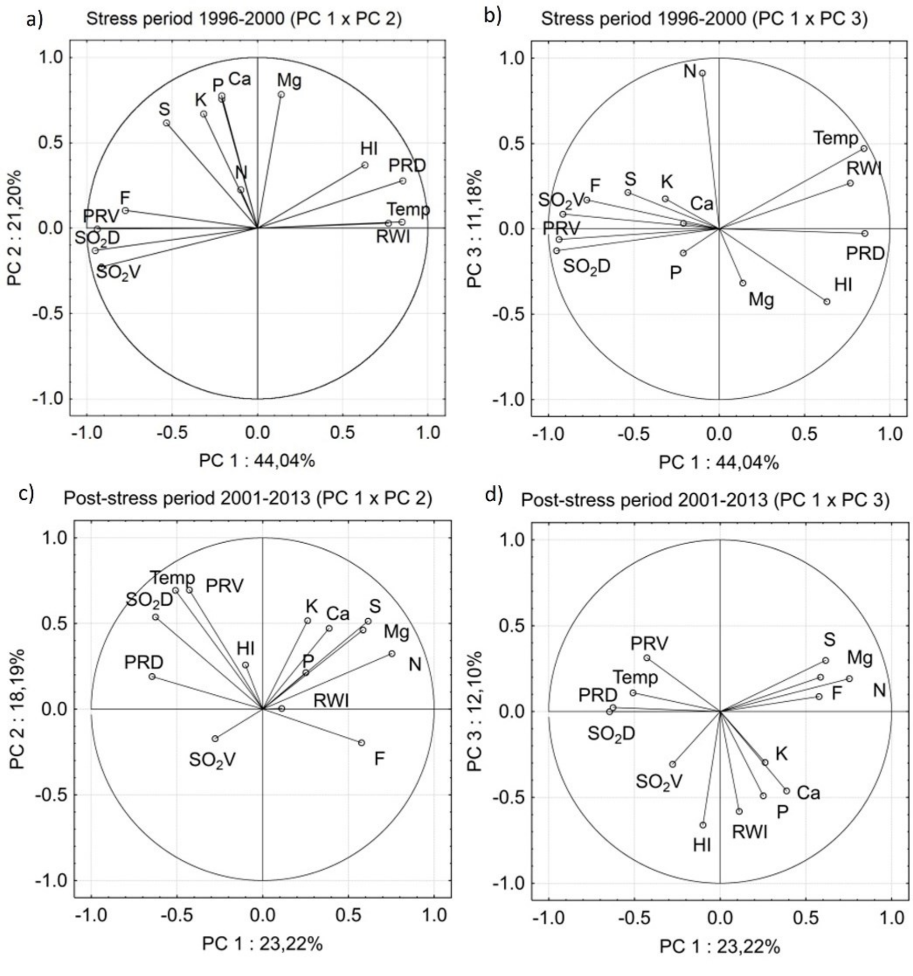
Fig. 6 Dendrogram of a hierarchical cluster analysis (Ward's method, Euclidean distances). Plots were clustered based on stand-level data on radial and height increments, defoliation and content of load elements (S,F) in needles during the 1995-2013 period



For the stress period 1996-2000, PCA identified three significant principal components. The loading plot PC1xPC2 revealed three distinct clusters of variables determining tree growth, air-pollution load and tree nutrition (Fig. 7a). The first two principal components retained 65.2% of the total variability. PC1 accounted for 44% of variance and was determined by growth parameters, temperature in vegetation season and precipitation in dormancy period, all of these with positive loadings. Tree growth was negatively correlated with air pollution load represented by SO_2 concentration in the air and the fluorine content in needles. Those parameters, together with precipitation in the previous vegetation season, showed high negative loadings on PC1. PC2 represented mainly the nutritional status of trees. The basic nutrients P, K, Ca and Mg achieved the highest positive loadings. Nitrogen participated more in the description of PC3 (Fig. 7b). By adding PC3, the variance explained increased to 76.4%.

A different picture was obtained for the 2001-2013 post-stress period (Fig. 7c). PCA identified five principal components with eigenvalues $\lambda>1$ explaining 70% of the common variability. The first principal component explained 23.2% of the variation and was related mainly to tree nutrition with high positive loadings represented by N, S and Mg. PC2 retained 18.2% of the variation and represented the climatic factors temperature and precipitation in the vegetation season. Both the diameter and height growth loaded strongly upon PC3, which explained 12.1% of the common variance (Fig. 7d). Tree growth appeared positively related to the content of the nutrients P, Ca and K.

Fig. 7 PCA loading plots **(a)** PC1 x PC2 for the stress period 1996-2000, **(b)** PC1 x PC3 for the stress period 1996-2000, **(c)** PC1 x PC2 for the post-stress period 2001-2013 and **(d)** PC1 x PC3 for the post-stress period 2001-2013



4.4.4 Discussion

Unfavourable weather conditions during the winter of 1995/96 affected forests in many regions of the Czech Republic. In spring 1996, mechanical damage to trees and the reddening of needles were observed frequently at altitudes of over 700 m asl. The area most affected was the Eastern part of the Ore Mountains, where about 12,500 ha of spruce stands were heavily damaged and 1,300 ha died completely (Lomský and Šrámek 2004). Tree mortality was recorded also at our heavily damaged plots Cínovec, Klíny and Skelný vrch. While in 1996 the mortality rate was 15, 35 and 40% respectively, in 1997 it varied between 5-10%. In 1998 no newly dead trees were recorded at these plots. Hence certain limitations exist when studying retrospectively growth response of trees to the stress event; only the radial growth of survivors is examined, and the history of the trees studied (their origin and competitive status) and the potential effect of forest management activities have to be considered.

In the Eastern part of the Ore Mountains, the impact of the climate extreme was intensified by air pollution load. From November 1995 to February 1996, the maximum half-hour concentration of SO_2 exceeded 2,000 µg m^{-3}, and the mean monthly concentration of SO_2 ranged between 122 and 193 µg m^{-3}, with the maximum recorded in January 1996 (Lomský and Šrámek 1999). The forest damage occurred in the form of an irregular mosaic reflecting the exposure of specific sites to the main pollution sources – coal power plants situated in the foothills of the Ore Mountains. Therefore, both slightly and heavily damaged plots were close to each other. The effect of air pollution was obvious at heavily damaged plots. The increased pollution load was confirmed by the high concentrations of load elements (S>2,000 mg kg^{-1}, F>10 µg g^{-1}) found in the needle samples in 1996 (Lomský and Šrámek 1999).

Substantial difference between the plots with varying damage levels was manifested also by the basic properties of tree-ring series. Increased mean sensitivity and higher inter-correlation with master chronology in heavily damaged plots indicate that tree growth has been modified by a single common factor that has suppressed the influence of individual differences between trees given by genetic predisposition and microsite conditions.

In 1996 at heavily damaged plots, most of the first and the second year needles reddened and subsequently fell as a consequence of direct impact of SO_2 (Fabiánek 1997). If the youngest, most photosynthetically active needle sets, are impaired, the increment responds sensitively (Straw et al. 2002). In heavily damaged plots in the Ore Mts. the mean defoliation in 1996 and 1997 was 77 and 66%, respectively. In case of foliage loss over 60%, radial growth is significantly reduced not only in the year of the stress event, but also in the subsequent two or three years (Armour et al. 2003, Kurkela et al. 2005). This corresponds with the observed three-year period of growth regeneration at our heavily and medium damaged plots.

Wimmer and Halbwachs (1992) observed the changes of wood-anatomical features of Scots pine under the pollution load in Austria. They pointed out the reduction of cell-wall thickness of the LW tracheids and decrease of tracheid length. A reduction of EW tracheid's cross-area was observed as well. Wimmer (2002), during research of polluted areas in the Saxony part of the Ore Mts., showed changes in other wood anatomy parameters, such as microfibril angel and wood ray height. On the basis of our results, it is possible to categorise the cellular characteristics in accordance with their sensitivity to the stress event starting with the most sensitive ones: number of tracheids, lumen widths, cell-wall thickness and proportion of LW. The result of LW proportion has to be interpreted cautiously, however. During the analysis, we discovered that the Mork index (Denne, 1988) has a limited applicability when working with young spruce trees. The occurrence of density fluctuations or of relatively thin-walled cells in LW caused the demarcation of the transition from EW to LW to fail. Similarly, Antony et al. (2012) demonstrated that the Mork index consistently overestimated the amount of EW in annual rings, especially in the juvenile wood zone.

The lowest values for all anatomical parameters, particularly for EW, at heavily damaged Klíny site were observed in 1997, hence we assume a time lag of one year in reaction to the stress. The time-lag of the growth reaction to environmental stress was also described by Axelson et al. (2014). In our study, however, the EW part reacted more sensitively to the stress event, which is in contrast to the results of Axelson et al (2014). In the Ore Mts., the stress event occurred during winter and caused serious damage to the foliage, thereby disrupting the EW formation. The recovery from the stress impact at the slightly damaged site Přebuz lasted around one year, while the heavily damaged Klíny site regenerated during the three years after the event. This can be explained by a strong influence of air pollution on the cambium and differentiation of cambial derivatives (Kurczynska et al. 1997). In comparison to the heavily polluted Klíny site, only a slight decrease of anatomy values was observed at the slightly damaged Přebuz site, which highlights the role of pollution intensity in activating the changes in wood anatomy.

Three distinct stress-level groups were identified by means of cluster analysis. This result is consistent with the original plot classification into different damage classes based on the levels of defoliation in 1996. It confirms that the most damaged plots in 1996 belong amongst the heavily stressed plots in the long-term perspective. Another important finding from the cluster analysis is that the signal change between the plots is related to the stress level and not to the distances between the sites. For example, the spatial proximity of the Skelný vrch (SKV) and Výsluní (VYS) sites is not reflected in the clusters.

For the stress-period (1996-2000), PCA distinctly separated growth parameters, air-pollution load and nutritional status of trees. As expected, tree growth was positively correlated with temperature since we were working in a mountainous area in which mainly summer temperatures restrict tree growth (Sander et al. 1995, Treml et al. 2012). We found a negative relationship between tree growth and air-pollution load represented by SO_2 concentration in the air and fluorine content in needles. Despite the decrease of emitted SO_2, the proportion of fluorine in the Ore Mts. started to rise. It was emitted by the coal power plants and also the glass and china factories located in the region (Lomský and Pasuthová 1996, Sedláček et al. 2001). Interestingly, the main nutrients did not significantly affect tree growth. In the stress-period, the air pollution load was probably a dominant factor that superimposed the effect of nutrition. Sulphur content in needles stands between the clusters of nutrients and pollution load. This reflects its role as an important nutrient on the one hand and as a load element on the other hand. For spruce, a sulphur content ranging from 1,000 to 1,100 mg kg^{-1} is considered as optimal, while a content above 1,500 mg kg^{-1}indicates an increased load (Šrámek et al. 2009). In the Ore Mts., sulphur content exceeded 1,500 mg kg^{-1} until 1997 and then decreased below this limit (Lomský et al. 2013).

During the post-stress period (2001-2013), the effect of pollutants on tree growth decreased significantly while, on the other hand, the importance of the nutrient content increased. Tree growth was dependent on the availability of P, Ca and K. This corresponds with the recent findings of Lomský et al. (2013) that reveal the long-term tendency towards a decrease in the content of these elements in spruce needles in the Ore Mts. At the same time, the nitrogen content rises (Lomský et al. 2013), hence the trees are endangered by a risk of nutritional imbalance (De Vries et al. 2014). Since 1999, the mean annual concentration of SO_2 in the air has remained below the limit of 20 µg m^{-3}, and annual sulphur deposition has stayed below 10 kg ha^{-1} year^{-1} (Šrámek et al. 2008). During this period, the sulphur content in needles ranged between 1,000-1,500 mg kg^{-1} (Lomský et al. 2013), which is below the limit of increased load.

4.4.5 Conclusions

Climatic extreme, intensified by the impact of air pollution load, left a detectable mark in growth and wood anatomy parameters over three to five years following the stress event. Both radial and height increments are reliable stress indicators, and we detected an immediate reaction to the stress event in the winter of 1995/96. The number of tracheids and lumen widths followed the response pattern of radial growth, with EW being more responsive to the stress event. However, a time-lag in the reaction of wood anatomy parameters has to be considered. The significance of nutrition level in regard to tree growth increased with the decreasing level of pollution load. During the post-stress period, tree growth was mainly governed by the availability of the macronutrients P, K and Ca. The micronutrients were not covered by this study but for the future research we suggest paying attention also to the microelements which are essential for tree growth and health.

Since the level of emissions has decreased significantly, the recurrence of similar stress events is unlikely. However, the negative effect of acid deposition that contributes to the degradation of forest soils remains an important stressor for forest ecosystems in this region.

Acknowledgments

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References

Antony, F., Schimleck, LR., Daniels, RF. (2012): A comparison of the earlywood-latewood demarcation methods—a case study in loblolly pine. IAWA J 33, 187–195. doi: 10.1163/22941932-90000088

Armour, H., Straw, N., Day, K. (2003): Interactions between growth, herbivory and long-term foliar dynamics of Scots pine. Trees Struct Funct 17, 70–80. doi:10.1007/s00468-002-0212-4

Axelson, JN., Bast, A., Alfaro, R., Smith, DJ., Gärtner, H. (2014): Variation in wood anatomical structure of Douglas-fir defoliated by the western spruce budworm: a case study in the coastal-transitional zone of British Columbia, Canada. Trees Struct Funct 28, 1837-1846. doi:10.1007/s00468-014-1091-1

Bridgman, HA., Davies, TD., Jickells, T., Hůnova, I., Tovey, K., Bridges, K., Surapipith, V. (2002): Air pollution in the Krusne Hory region, Czech Republic during the 1990s. Atmos Environ 36, 3375-3389. doi:10.1016/s1352-2310(02)00317-5

Cook, ER., Kairiukstis, LA. (eds) (1990): Methods of dendrochronology: applications in the environmental science. Kluwer Academic Publishers, Dordrecht-Boston-London.

Danek, M (2007): The influence of industry on Scots pine stands in the south-eastern part of the Silesia-Krakow Upland (Poland) on the basis of dendrochronological analysis. Water Air Soil Pollut 185, 265–277. doi:10.1007/s11270-007-9448-3

De Vries, W., Dobbertin, MH., Solberg, S., Van Dobben, HF., Schaub, M. (2014): Impacts of acid deposition, ozone exposure and weather conditions on forest ecosystems in Europe: an overview. Plant Soil 380, 1-45. doi:10.1007/s11104-014-2056-2

Denne, MP. (1988): Definition of latewood according to Mork (1928) IAWA Bull 10, 59-62. doi:10.1163/22941932-90001112

Fabiánek, P. (ed) (1997): Ore Mountains – winter 1995/96. FGMRI, Prague.

Fiala, J., Bretschneider. B., Materna. J. (2002): Development of the atmospheric pollution and forest decline in the Czech part of the Ore Mts. In: Lomský B, Materna J, Pfanz H (eds) SO2-pollution and forests decline in the Ore Mountains, Forestry and Game Management Research Institute, Jíloviště-Strnady, CZ, pp 50-85.

Gärtner. H., Schweingruber. FH. (2013): Microscopic preparation techniques for plant stem analysis. Kessel Publishing House, Remagen.

Gärtner, H., Lucchinetti, S., Schweingruber, FH. (2014): New perspectives for wood anatomical analysis in dendrosciences: the GSL1- microtome. Dendrochronologia 32, 47–51. doi:10.1016/j.dendro.2013.07.002

Holmes, R. (1983): Computer-assisted quality control in tree-ring dating and measurement. Tree-Ring Bull 39, 77-82.

Hůnová, I., Šantroch, J., Ostatnická, J. (2004): Ambient Air Quality and Deposition Trends at Rural Stations in the Czech Republic during 1993–2001. Atmos Environ 38, 887–898. doi:10.1016/j.atmosenv.2003.10.032

Jolliffe, IT. (2002): Principal component analysis, 2nd ed. Springer.

Kaiser, HF. (1992): On Cliff's formula, the Kaiser–Guttman rule, and the number of factors. Percept Motor Skill 74, 595–598. doi:10.2466/pms.1992.74.2.595

Kolář, T., Čermák, P., Oulehle, F., Trnka, M., Štěpánek, P., Cudlín, P., Hruška, J., Büntgen, U., Rybníček, M. (2015): Pollution control enhanced spruce growth in the „Black Triangle" near the Czech-Polish border. Sci Total Environ 538, 703–711. doi:10.1016/j.scitotenv.2015.08.105

Kopáček, J., Veselý, J. (2005): Sulfur and nitrogen emissions in the Czech Republic and Slovakia from 1850 till 2000. Atmos Environ 39, 2179–2188. doi:10.1016/j.atmosenv.2005.01.002

Kroupová, M. (2002): Dendroecological study of spruce growth in regions under long-term air pollution load. J For Sci 48, 536–548.

Kurczynska, E., Dmuchowski, W., Wloch, W., Bytnerowicz, A. (1997): The influence of air pollutants on needles and stems of Scots pine (Pinus sylvestris L.) trees. Environ Pollut 98, 325 – 334. doi:10.1016/s0269-7491(97)00141-3

Kurkela, T., Aalto, T., Varama, M., Jalkanen, R. (2005): Defoliation by the common pine sawfly (Diprion pini) and subsequent growth reduction in Scots pine: a retrospective approach. Silva Fenn 39, 467–480. doi:10.14214/sf.360

Lange, CA., Matschullat, J., Zimmerman, F., Sterzig, G., Wienhaus, O. (2003): Fog frequency and chemical composition of fog water-a relevant contribution to atmospheric deposition in the eastern Erzgebirge, Germany. Atmos Environ 37, 3731-3739. doi:10.1016/S1352-2310(03)00350-9

Lomský, B., Pasuthová, J. (1996): Imisní zatížení a defoliace mladých smrkových porostů [Pollutant load of young spruce stands]. Lesnictví – Forestry 42, 449-459

Lomský, B., Šrámek, V. (1999): Damage of the forest stands in the Ore Mts. during the period 1995 – 1997. J For Sci 45, 169 – 180.

Lomský, B., Šrámek, V. (2004): Different types of damage in mountain forest stands of the Czech Republic. J For Sci 50:533 – 537.

Lomský, B., Šrámek, V., Novotný, R. (2013): The health and nutritional status of Norway spruce stands in the Krušné hory Mts. 15 years subsequent to the extreme winter of 1995/96. J For Sci 59, 359 – 369.

Materna, J. (1999): Development and causes of forest damage in the Ore Mts. J For Sci 45, 147-152.

Mork, E. (1928): Die Qualität des Fichtenholzes unter besonderer Rücksichtnahme auf Schleif- und Papierholz. Der Papier-Fabrikant 48, 741-747.
Plíva, K., Žlábek, I. (1986): Přírodní lesní oblasti ČSR [Natural forest regions of the Czech Republic]. Státní zemědělské nakladatelství, Praha.

Rydval, M., Wilson, R. (2012): The impact of industrial SO_2 pollution on North Bohemia conifers. Water Air Soil Pollut 223, 5727–5744. doi:10.1007/s11270-012-1310-6

Sander, C., Eckstein, D., Kyncl, J., Dobrý, J. (1995): The growth of spruce (Picea abies (L.) Karst.) in the Krkonoše Mountains as indicated by ring width and wood density. *Ann Sci For* 52, 401–410. doi:10.1051/forest:19950501

Schneider, L., Gärtner, H. (2013): The advantage of using non-Newtonian fluids to prepare micro sections. Dendrochronologia 31, 175–178. doi:10.1016/j.dendro.2013.04.002

Sedláček, F., Zemek, F., Heřman, M., Kierdorf, H., Kierdorf, U. (2001): Fluoride load on ecosystems in western part of Krusne hory Mts determined by bioindication. Ekológia 20, 70-79

Straw, NA., Fielding, NJ., Green, G., Price, J. (2002): The impact of green spruce aphid, *Elatobium abietinum* (Walker), on the growth of young Sitka spruce in Hafren Forest, Wales: delayed effects on needle size limit wood production. Forest Ecol Manag 157, 267–283. doi:10.1016/s0378-1127(00)00656-3

Šrámek, V. (1998): SO2 air pollution and forest health status in northwestern Czech Republic. Chemosphere 36, 1067-1072. doi:10.1016/s0045-6535(97)10173-4

Šrámek, V., Hadaš, P., Lachmanová, Z., Fadrhonsová, V., Vortelová, L., Lomský, B., Kulhavý, J. (2008): Air pollution in the Krušné hory Mts. In Slodičák et al. (eds) Forestry management in the Krušné hory Mts, LČR, Hradec Králové, VÚLHM, Strnady, CZ, pp 45-70.

Šrámek, V., Lomský, B., Novotný, R. (2009): Hodnocení obsahu a zásoby živin v lesních porostech – literární přehled [Content of nutrients in forest stands - review]. Zprávy lesnického výzkumu 54, 307-315.

Treml, V., Ponocná, T., Büntgen, U. (2012): Growth trends and temperature responses of treeline Norway spruce in the Czech-Polish Sudetes Mountains. Climate Res 55, 91-103. doi :10.3354/cr01122

Warren, WG. (1980): On removing the growth trend from dendrochronological data. Tree-Ring Bull 40, 35–44..

Wimmer, R., Halbwachs, G. (1992): Holzbiologische Untersuchungen an fluorgeschädigten Kiefern. Holz Roh Werkst 50, 261 – 267.

Wimmer, R. (2002): Wood anatomical feature in tree-rings as indicators of environmental change. Dendrochronologia 20, 21 – 36. doi:10.1078/1125-7865-00005.

Zimmermann, F., Lux, H., Reuter, F., Wienhaus, O. (2002): SO2 pollutionand forest decline in the Ore Mountains – historical aspects, scientific analysis, future developments. In Lomský B, Materna J, Pfanz H (eds) SO2-pollution and forests decline in the Ore Mountains, Forestry and Game Management Research Institute, Jíloviště-Strnady, CZ, pp 86 – 116.

5 Summary of the results

The results will be discussed according to the aims of the research, as stated in Chapter 2.

5.1 The impact of extreme stress events on wood anatomy parameters of Norway spruce trees growing in the Ore mountains

The impact of stressful conditions on the growth of Norway spruce in the Ore Mountains is described in detail in Samusevich et al. (2017), Vejpustková et al. (2017) and Lexa et al. (2018). For better understanding of the growth trends the dendrochronological analysis and height increment analyses were evaluated. Rich data about forest condition (defoliation, height increment, content of nutrients and load elements of S and F in current year needles) gathered between 1996 and 2013 was used as well.

The radial increment of the trees after the stressful winter 1995/1996 showed a significant decrease at all sites, regardless of damage level. Missing tree rings were detected mainly at heavily polluted sites. Mean sensitivity values showed an increase in the annual variability in tree rings with increasing site damage. The opposite situation was observed for height increment, where the variability of values was less pronounced in stress years.

In terms of wood anatomy characteristics, a decrease of tracheids number and lumen width was observed at heavily polluted sites, with minimum values reached in 1997. Analysis of variance showed that the number of tracheids is the most responsive parameter to pollution stress, as its decrease was significant at all sites. The changes in individual wood anatomy parameters for all sites are shown at Fig. 2,3, and 4. The most prominent decrease was observed for EW tracheids, which led to increased LW density. The higher sensitivity of EW cells to pollution was detected by Myśkow et al. (2019) as well. On the contrary higher responsiveness of LW to pollution load was noticed by Wimmer (2002). The variability of lumen width values and number of tracheids in EW was shown to be higher than for the same parameters in LW.

The higher response rate from EW tracheids can be explained by the timing of the stress event. As the stressful pollutation load occurred in winter, it delayed the initiation of the cambial activity, influenced the structure and arrangement of xylem derivatives, and caused a decrease in lumen size and number of tracheids (Rajput et al. 2008).

Fig. 2 Change in the tracheid number from 1991 – 2001

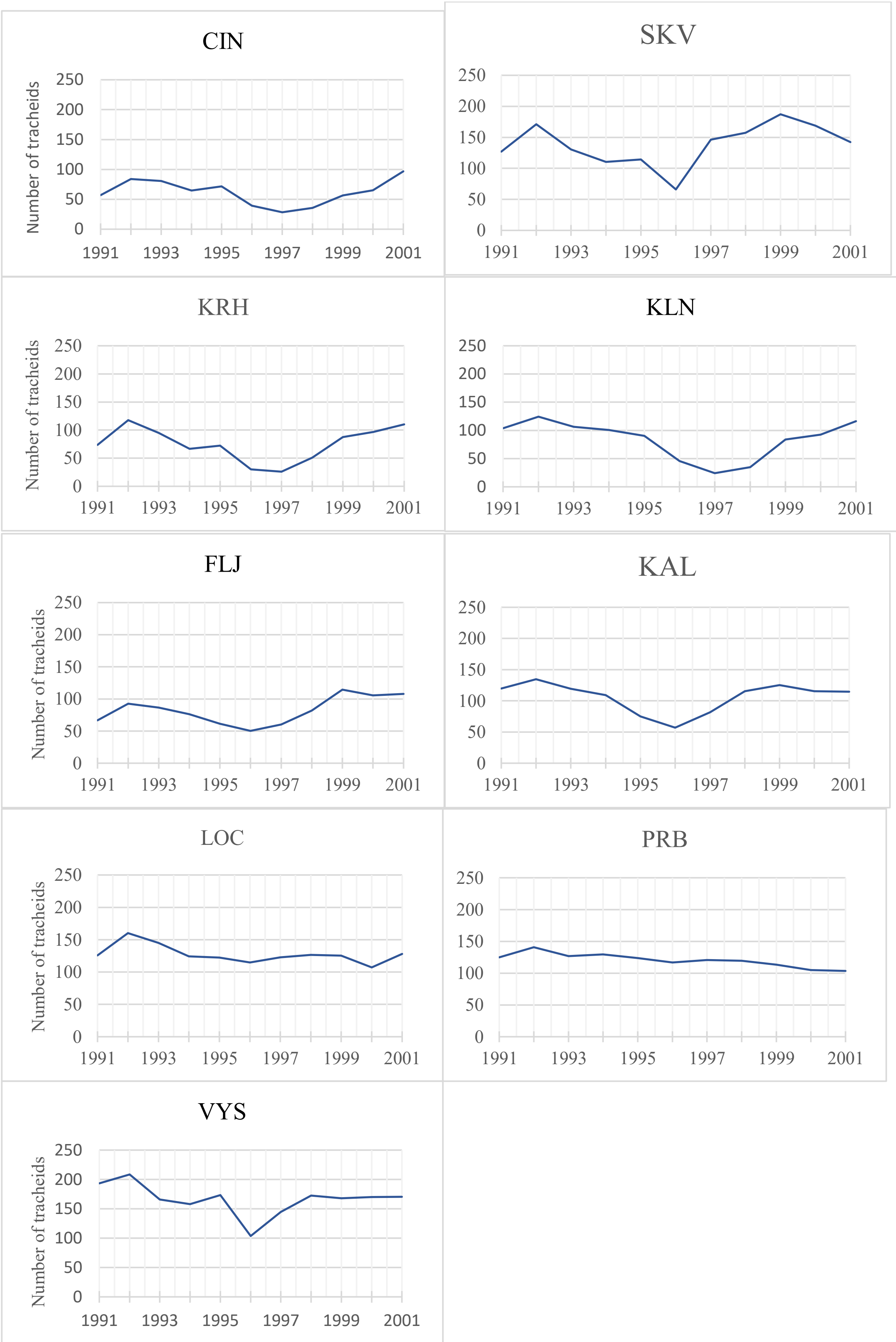


Fig. 3 Changes in lumen width from 1991 – 2001

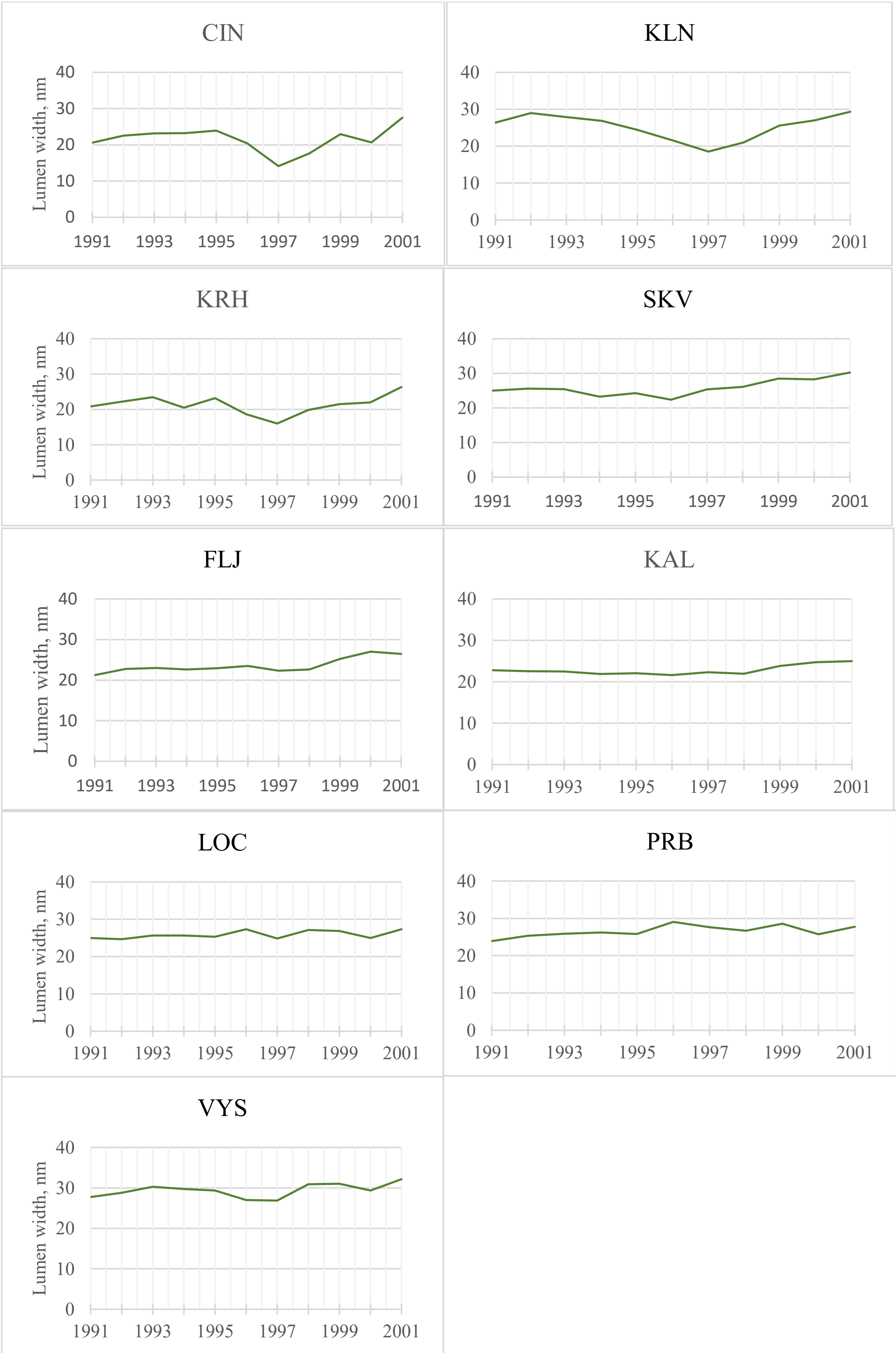
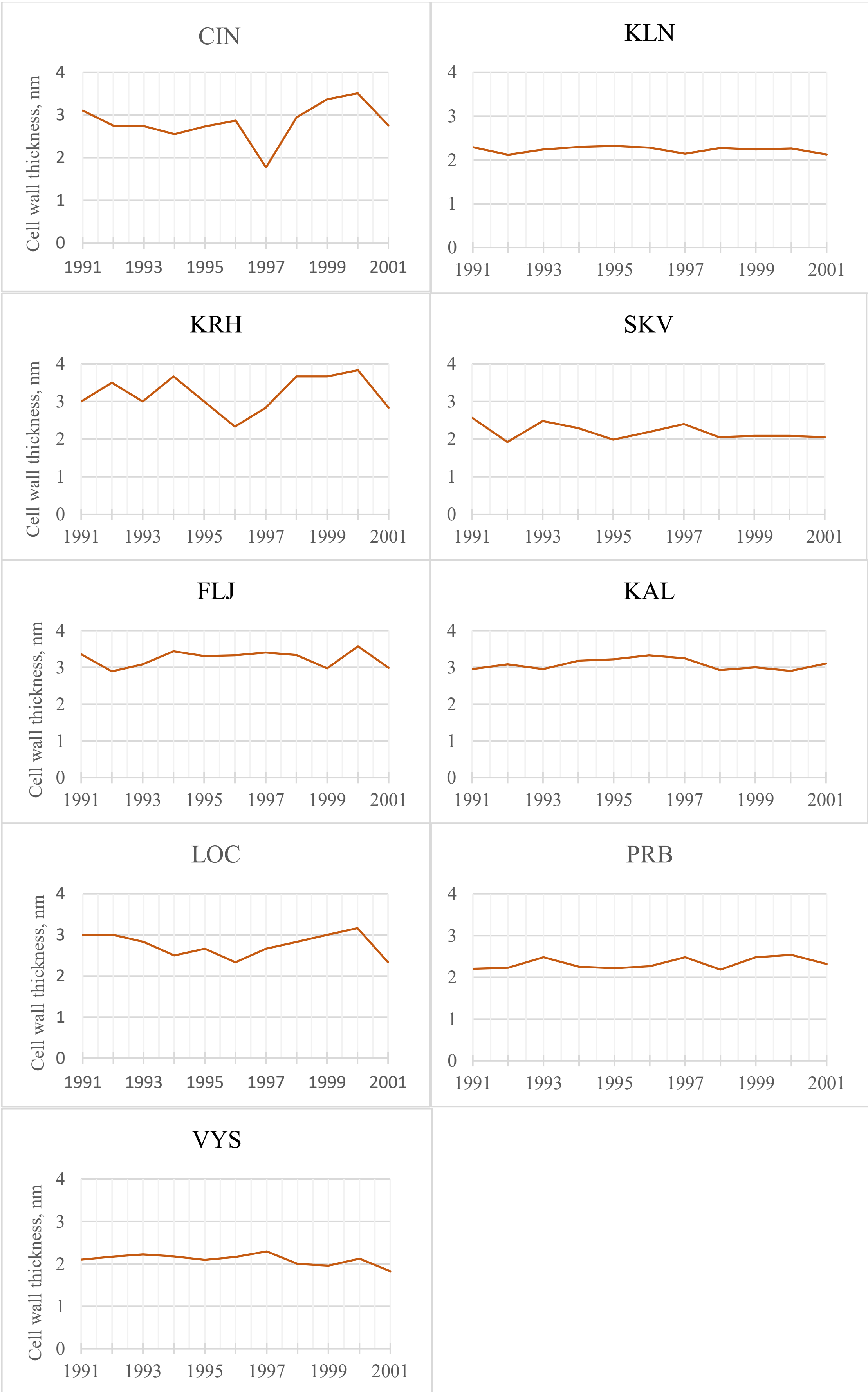


Fig. 4 Changes in cell wall thickness from 1991 – 2001



5.2 Estimation of the tree growth recovery after the stress event

Tree growth recovery after 1995/1996 winter stress event varies in relationship to the rate of each site's damage. The minimum anatomical values in the analysed period were observed in 1996 at slightly and medium polluted sites, and already next year they started to get back to normal. The comparison of the main anatomical parameters' mean values in pre-stress, stress and after-stress period is given in Samusevich et al. (2017). Trees at heavily polluted sites showed different results. There we observed a one-year lag in reaching the minimum values. The recovery stage lasted longer as well. It took trees around 3 years to come back to their pre-stress anatomical characteristics (Samusevich et al. 2017, Vejpustková et al. 2017). The lag in the reaction of trees to stress was also observed by Axelson et al. (2014).

This corresponds with Armour et al. (2003) and Kurkela et al. (2005). Due to the loss of approximately 60% of the foliage, radial growth is significantly reduced not only the year following the stress, but also 2-3 years afterwards, which causes the observed longer time for regeneration.

The slowest rate of recovery was shown for height increment of the trees. A decrease in height increment after stress was observed only at heavily polluted sites, and it took almost 5 years for the trees to recover from it.

5.3 Evaluation of dendroclimatic potential of wood anatomy parameters for long tree-ring series

The attempt to evaluate the potential of wood anatomy for long tree-ring series was made in Lexa et al. (2018). A period from 1850 was analysed.

Tracheid number chronologies showed a similar trend as mean tree-ring chronologies. More negative pointer years were observed here in comparison with tree-ring width series (for example, in 1948, 1956, 1978, 1980 a 1996). Observed pointer years were related not only to pollution stress, but also temperatures decrease, precipitation availability and frosts, especially prior to the start or at the beginning of the growing season. Lumen area and cell-wall chronologies did not show significant changes during the stress events and reflected only a slight decrease in the '70s.

An increase in all measured parameters, especially visible at tree-ring widths and number of tracheids, was observed after 1990. The decrease in air pollution (Lomský and Šrámek 2004), increase in summer temperatures (Vacek et al. 2015) and availability of nutrients in the results of reduced competition (Wimmer 2002) all have a positive influence on that.

Wood anatomy data can be a valuable source of environmental information. As we have shown on the example of changes in tracheids numbers, this anatomical parameter is more sensitive to changes in the environment than the classic tree-ring width, thus giving us more information about individual stress events. Higher sensitivity is related to the negative influence of stress on cambium, which primarily determines the number of formed cells (Kurczyńska et al. 1997).

Long series of anatomical data, especially from the past events, are rare, which makes them especially valuable for the research.

5.4 Methodology improvement for EW/LW demarcation and its analysis from the anatomical point of view

It was already discussed (Vejpustková et al. 2017) that the evaluation of LW proportion is a very delicate topic as it depends a lot on the chosen methodology, especially for trees with the occurrence of irregular IADF, light and dark rings or gradual transition zones between EW and LW.

We have tested 12 methods in total based on visual estimation of LW, Mork's index and densitometry analysis. The detailed description is given in Samusevich et al. (2020). Fixed density thresholds were compared with flexible density thresholds to identify the most suitable approach for Norway spruce growing in temperate climate. For verification of the results we used visual assessment of EW/LW boundary as a reference.

In Samusevich et al. (2020) we demonstrated that the usage of flexible density thresholds gives us reliable results for distinguishing between EW/LW in tree rings of Norway spruce growing in the temperate climate. The two best thresholds were proved to be DT80 and DT2/3+. Wood density around 600 kg/m^3 corresponds to these thresholds. A similar density threshold was stated by Koubaa et al. (2002) in his research of black spruce.

The usage of X-ray densitometry and floating thresholds is also faster and easier from an operational point of view in comparison with microsection preparation, Mork's index calculation or other alternative methods, such as the maximum derivative method (Koubaa et al. 2002, Antony et al. 2012). The effectiveness of floating density thresholds was shown in other studies (Sauter et al. 1999, Björklund et al. 2017) as well.

Based on our results we have conducted the adjustment of Mork's index (MI=1) to make it more suitable for a specific tree species growing in different conditions. We concluded that for Norway spruce trees at our sites MI value of 0,83 or higher is enough to distinguish between the EW and LW parts of the tree ring.

The developed discriminant function was shown to be successful in EW/LW classification as well and can be a substitute to Mork's index (Samusevich et al. 2020). Despite our results can be applied primarily to Norway spruce growing in temperate climate, the methodology itself is universal and can be adjusted to other species and environments.

6 Outcome of the research and practical application of the results

The research proved the high potential of wood anatomy for studying the changes in the growing environment and the reaction of trees to stress events. The results are applicable to Norway spruce growing in the temperate climate. The main results are summarised below:

1) a number of tracheids was proved to be the most sensitive anatomical parameter to air pollution at tree rings of *Picea abies* growing in the Ore mountains. The decrease in the number of cells was shown at sites with different pollution load. The changes in lumen width and cell wall thickness were less pronounced and mostly observed only at heavily polluted sites (Lexa et al. 2018, Samusevich et al. 2017).
2) the recovery rate of wood anatomy parameters took from 1 to 3 years, depending on the pollution load. The same trend was observed for tree ring width and height increment. Among all the parameters height increment took more time to achieve pre-stress values after the growth interruption.
3) the lag in wood anatomy reaction was observed at heavily polluted sites, where minimum values for all three parameters (number of tracheids, radial lumen width and cell wall thickness) were observed 1 year after the stress event.
4) different sensitiveness of EW and LW cells to pollution was shown, with the higher sensitivity of EW tracheids to stress. More research will be done in this direction to better understand the reaction of EW and LW parts of the tree ring to pollution load and other types of stress.
5) a thorough analysis of different EW/LW demarcation methods was done to identify a more precise methodology suitable for *Picea abies* trees growing in the Ore Mountains. The flexible threshold of 80% of the maximum tree ring density was shown to give the best

results. Modification of classic Mork's index was done as well. It was shown that for distinguishing EW from LW cell it is enough to have Mork's index equal or higher than 0,83.

Better understanding of wood anatomy of the trees is essential. Rendle (1932) called wood anatomy a link between botany and forestry. It helps to model the tree growth and understand the formation of its woody tissue, according to the environment and stress factors (Ezquerra and Gil 2001). The understanding of the links between the structure and the physical properties of wood helps to assess the technical value of wood and its quality, which is especially important with changing climate and anthropogenic pressure on the forests (Rendle 1932, Beery et al. 1982, Jozsa and Middleton 1994, Butterfield 2003).

7 List of abbreviations

CD - cell diameter

CO – cell order within the tree ring

CWT - cell wall thickness

DT – density threshold

DTvisual – DT calculated from LW width determined by visual evaluation applied to the results of densitometry

DTmork - DT calculated from LW width determined by Mork's criterion applied to the results of densitometry

DT450 - fixed density threshold of 450 kg/m^3

DTqm - quadratic mean of measured densities within the entire tree ring,

DTavg - average of measured densities within the entire tree ring,

DT50/DT60/DT70/DT80 – DT calculated as 50%/60%/70%/80% of maximum latewood density

DTmaxmin - average of maximum and minimum density within a ring

DT2/3 – 2/3*(density max – density min)

DT2/3+ - [2/3*(density max – density min)] + density min

EW- earlywood,

IADF – intra-annual density fluctuations

LD - lumen diameter

LW – latewood

LW% - latewood proportion,

LW%visual – latewood proportion corresponding to DTvisual

LW%MI – latewood proportion corresponding to DTmork

LW%450 - latewood proportion corresponding to DT450

LW%qm - latewood proportion corresponding to DTqm

LW%avg - latewood proportion corresponding to DTavg

LW%50/60/70/80 - latewood proportion corresponding to DT50/60/70/80 respectively

LW%maxmin - latewood proportion corresponding to DTmaxmin

LW%2/3 - latewood proportion corresponding to DT2/3

LW%2/3+ - latewood proportion corresponding to 2/3+

MI – Mork's index

CIN – Cínovec

FLJ - Fláje

KAL - Kálek

KLN - Klíny

KRH – Kryštofovy Hamry

LOC - Loučná

PRB - Přebuz

SKV – Skelný Vrch

VYS - Výsluní

References

Antony, F., Schimleck, L.R., Daniels, R.F. (2012): A comparison of earlywood-latewood demarcation methods – a case study in loblolly pine. IAWA J. 33 (2), 187–195.

Anttonen, S., Manninen, A.M., Saranpää, P., Kainulainen, P., Linder, S., Vapaavuori, E. (2002): Effects of long-term nutrient optimisation on stem wood chemistry in Picea abies. Trees 16, 386–394.

Armour, H., Straw, N., Day, K. (2003): Interactions between growth, herbivor, and long-term foliar dynamics of Scots pine. Trees 17, 70–80.

Axelson, J.N., Bast, A., Alfaro, R., Smith, D.J., Gärtner, H. (2014): Variation in wood anatomical structure of Douglas-fir defoliated by the western spruce budworm: a case study in the coastal-transitional zone of British Columbia. Canada. Trees 28, 1837–1846.

Babushkina, E., Zhirnova, D., Belokopytova, L., Vaganov, E. (2020): Warming induced changes in wood matter accumulation in tracheid walls of spruce. Journal of Mountain Science 17 (1), 16 – 30.

Beery, W.H., Ifu, G., Mclain, T.E. (1982): Quantitative wood anatomy-relating anatomy to transverse tensile strength. Wood and Fiber Science 15 (4), 395 – 407.

Björklund, J., Seftigen, K., Schweingruber, F., Fonti, P., Von Arx, G., Bryukhanova, M.V., Cuny, H.E., Carrer, M., Castagneri, D., Frank, D.C. (2017): Cell size and wall dimensions drive distinct variability of earlywood and latewood density in Northern Hemisphere conifers. New Phytologist 216, 728–740.

Bryukhanova, M., Fonti, P. (2013): Xylem plasticity allows rapid hydraulic adjustment to annual climatic variability. Trees 27 (3), 485-496.

Butterfield, B.G. (2003): Wood anatomy in relation to wood quality. In: J.R. Barnett & G. Jeronimidis (eds.), Wood quality and its biological basis: 30–52. Oxford, UK.

Camarero, JJ., Olano, JM., Parras, A. (2010): Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. New PhytolOGIST 185, 471–480.

Campelo, F., Nabais, C., Freitas, H., Gutierrez, E. (2007): Climatic significance of tree-ring width and intra-annual density fluctuations in Pinus pinea from a dry Mediterranean area in Portugal. Ann. For. Sci. 64 (2), 229–238.

Carlquist, S. (1980): Further concepts in ecological wood anatomy, with comments on recent work in wood anatomy and evolution. A Journal of Systematic and Evolutionary Botany 9 (4), 499 - 553 pp.

Carrer, M., Castagneri, D., Prendin, A., Petit, G., Von Arx, G. (2017): Retrospective Analysis of Wood Anatomical Traits Reveals a Recent Extension in Tree Cambial Activity in Two High-Elevation Conifers. Frontiers in Plant Science 8, 1 – 13.

Castagneri, D., Regev, L., Boaretto, E., Carrer, M. (2017): Xylem anatomical traits reveal different strategies of two Mediterranean oaks to cope with drought and warming. Environmental and Experimental Botany 133, 128–138.

Choi, HK., Kwon, J. (2019): Modeling and control of cell wall thickness in batch delignification. Computers and Chemical Engineering 128, 512 – 523.

Cook, E. (1985): A time series analysis approach to tree ring standardization. Ph.D. Dissertation, The University of Arizona, Tucson, 171 pp.

Cook, ER., Kairiukstis, LA. (eds) (1990): Methods of dendrochronology: applications in the environmental science. Kluwer Academic Publishers, Dordrecht.

Cook, E., Holmes, R.L. (1996): Users Manual for Program ARSTAN. Laboratory of Tree-Ring Research. University of Arizona, Tucson, USA.

Cown, DJ., Ball, RD. (2001): Wood densitometry of 10 Pinus radiata families at seven contrasting sites: Influence of tree age, site, and genotype. New Zealand Journal of Forestry Science, 31/1, 88-100.

Cown, DJ., Clement, BC. (1983): A wood densitometer using direct scanning with X-rays. Wood Science and Technology, 17/2, 91 – 99.

Cuny, H.E., Fonti, P., Rathgeber, C., Von Arx, G., Peters, R.L., Frank, D.C. (2019): Couplings in cell differentiation kinetics mitigate air temperature influence on conifer wood anatomy. Plant, Cell and Environment 42 (4), 1222-1232.

De Micco, V., Saurer, M., Aronne, G., Tognetti, R., Cherubini, P. (2007): Variations of wood anatomy and δ13C within tree rings of coastal Pinus pinaster Ait. showing intra-annual density fluctuations. IAWA J. 28, 61–74.

Doley, D. (1974): Alternatives to assessment of earlywood and latewood in dicotyledonous trees – study of structural variation in growth rings of apple (Malus-Pumila Mill). New Phytologist, 73/1, 157 – 171.

Domec, J-CH., Gartner, B. (2002): How do water transport and water storage differ in coniferous earlywood and latewood? Journal of Experimental Botany, 53/379, 2369 – 2379.

Du, S., Yamamoto, F. (2007): An Overview of the Biology of Reaction Wood Formation. Journal of Integratice Plant Biology 49 (2), 131 – 143.

Ezquerra, F.J., Gil, L.A. (2001): Wood anatomy and stress distribution in the stem of Pinus pinaster Ait. Invest. Agr.: Sist. Recur. For. 10 (1), 165 – 177.

Filion, L., Cournoyer, L. (1995): Variation in wood structure of eastern larch defoliated by the larch sawfly in subarctic Quebec, Canada. Canadian Journal of Forest Research 25 (8), 1263-1268.

Fonti, P., Bryukhanova, M.V., Myglan, V.S., Kirdyanov, A.V., Naumova, O.V., Vaganov, E.A. (2013): Temperature-induced responses of xylem structure of *Larix sibirica* (Pinaceae) from the Russian Altay. American Journal of Botany, 100/7, 1332 – 1343.

Fritts, H. C. (1976): Tree Rings and Climate. Academic Press, London, New York, San Francisco, 567 pp.

Gandelová, L., Horáček, P., Šlezingerová, J. (2002): Nauka o dřevě. Mendelova zemědělská a lesnická univerzita v Brně, 176 pp.

Gartner, B., Aloni, R., Funada, R., Lichtfuss-Gautier, A.N., Roiq, F.A. (2002): Clues for dendrochronology from studies of wood structure and function. Dendrochronologia, 20/1 – 2, 53 – 61.

Gärtner, H., Schweingruber, F. (2013): Microscopic Preparation Techniques for Plant Stem Analysis. Verlag Dr. Kessel, Remagen, 22 pp.

Gärtner, H., Lucchinetti, S., Schweingruber, F.H. (2014): New perspectives for wood anatomical analysis in dendrosciences: The GSL-1 microtome. Dendrochronologia, 32, 47 – 51.

Gindl, W., Grabner, M. (2000): Characteristics of Spruce (Picea abies [L.] Karst.) latewood formation under abnormally low temperatures. Holzforschung, 54, 9 – 11.

González-Cásares, M., Camarero, J., Colangelo, M., Rita, A., Pompa-García, M. (2019): High responsiveness of wood anatomy to water availability and drought near the equatorial rear edge of Douglas-fir. Canadian Journal of Forest Research 49 (9), 1114 – 1123.

Goncharenko, G., Zadeika, I., Birgelis, J. (1995): Genetic structure, diversity and differentiation of Norway spruce (*Picea abies* (L.) Karst.) in natural populations of Latvia. Forest Ecology and Management 72 (1), 31 – 38.

Gričar, J., Prislan, P., Gryc, V., Vavrčík, H., De Luis, M., Čufar, K. (2014): Plastic and locally adapted phenology in cambial seasonality and production of xylem and phloem cells in *Picea abies* from temperate environments. Tree Physiology 34 (8), 869 – 881.

Grubler, A. (2002): Trends in global emissions: carbon, sulphur, and nitrogen. In: Douglas, I. (Ed.) Encyclopedia of Global Environmental Change volume 3, pp. 35–53.

Guay, R. (2013): WinCELL. For Word cell analysis. Regent Instruments Canada Inc.
Guerriero, G., Sergeant, K., Hausman, J-F (2014): Wood biosynthesis and typologies: a molecular rhapsody. Tree Physiology, 34, 839–855.

Hacke, U.W., Jansen, S. (2009): Embolism resistance of three boreal conifer species varies with pit structure. New Phytologist, 182/3, 675 – 686.

Helliwell, R.C., Wright, R.F., Jackson-Blake, L.A., Ferrier, R.C., Aherne, J., Cosby, B.J., Evans, C.D., Forsius, M., Hruska, J., Jenkins, A., Kram, P., Kopáček, J., Majer, V., Moldan, F., Posch, M., Potts, J.M., Rogora, M., Schöpp, W. (2014): Assessing recovery fromacidification of european surface waters in the year 2010: evaluation of projections made with the MAGIC model in 1995. Environ. Sci. Technol. 48 (22), 13280–13288.

Holmes, R. (1983): Computer-assisted quality control in tree-ring dating and measurement. Tree-Ring Bull 39, 77–82.

Hruška, J., Oulehle, F., Krám, P., Skořepová, I. (2009). Účinky kyselého deště na lesní a vodní ekosystémy 2. Vliv depozic síry a dusíku na půdy a lesy (Acid Rain Effects on Forest and Water Ecosystems 2. The Effect of Sulfur and Nitrogen Deposition on Soil and Forests). Živa 3, 141–144.

Jozsa, L.A., Middleton, G.R. (1994): Discussion of wood quality attributes and their practical impli-cations. Forintek Canada Special Publication No. SP-34.

Kopáček, J., Veselý, J. (2005): Sulfur and nitrogen emissions in the Czech Republic and Slovakia from 1850 till 2000. Atmos. Environ. 39 (12), 2179–2188.

Koprowski, M., Zielski, A. (2006): Dendrochronology of Norway spruce (Picea abies (L.) Karst.) from two range centres in lowland Poland. Trees 20, 383–390.

Koubaa, A., Zhang, S.Y., Makni, S. (2002). Defining the transition from earlywood to latewood in black spruce based on intra-ring wood density profiles from X-ray densitometry. Ann. For. Sci. 59, 511–518.

Körner, CH. (2012): Treelines will be understood once the functional difference between a tree and a shrub is. Ambio, 41 (3), 197 – 206.

Körner, CH., Paulsen, J. (2004): A world-wide study of high altitude treeline temperatures. Journal of Biogeography, 31, s. 713 – 732.

Kulhavý J., Šrámek V., Lomský B., Fiala P., Matějka K., Borůvka L., Menšík L. (2008): Stav lesních půd v zájmové oblasti. [Condition of forest soils in the Krušné hory Mts.] In: Slodičák M., Balcar V., Novák J., Šrámek V. (eds): Lesnické hospodaření v Krušných horách. [Forestry Management in the Krušné hory Mts.] Jíloviště-Strnady, VÚLHM, 71–98.

Kurczynska, E., Dmuchowski, W., Wloch, W., Bytnerowicz, A. (1997): The influence of air pollutants on needles and stems of Scots pine (Pinus sylvestris L.) trees. Environmental Pollution, 98 (3), 325 – 334.

Kurkela, T., Aalto, T., Varama, M., Jalkanen, R. (2005): Defoliation by the common pine sawfly (Diprion pini) and subsequent growth reduction in Scots pine: a retrospective approach. Silva Fennica 39, 467–480.

Lagercrantz, U., Ryman, N. (1990): Genetic structure of Norway spruce (Picea abies): concordance of morphological and allozymic variation. Evolution 44 (1), 38 – 53.

Lebourgeois, F., Rathgeber, C.B., Ulrich, E. (2010): Sensitivity of French temperate coniferous forests to climate variability and extreme events (Abies alba, Picea abies and Pinus sylvestris). Journal of Vegetation Science, 21/2, 364 – 376.

Lexa, M., Vejpustková, M., Samusevich, A., Zeidler, A. (2018): Stopa imisní kalamity v anatomických znacích dřeva smrku (*Picea abies (L.) Karst.*) v oblasti Klínovce (Krušné hory). Zprávy lesnického výzkumu 63, 136 – 146.

Liang, C., Filion, L., Cournoyer, L. (1997): Wood structure of biotically and climatically induced light rings in eastern larch (Larix laricina). Canadian Journal of Forest Research 27 (10), 1538–1547.

Lomský, B., Šrámek, V. (2004): Different types of damage in mountain forest stands of the Czech Republic. Journal of Forest Science, 50, 533–537.

Lomský, B., Šrámek, V., Novotný, R. (2012): Changes in the air pollution load in the Jizera Mts.: Effects on the health status and mineral nutrition of the young Norway spruce stands. European Journal of Forest Research, 131, 757-771.

Lomský, B., Šrámek, V., Novotný, R. (2013): The health and nutritional status of Norway spruce stands in the Krušné hory mts. 15 years subsequent to the extreme winter of 1995/96. Journal of Forest Science, 59, 359-369.

Martin-Benito, D., Beeckman, H., Canellas, I. (2013): Influence of drought on tree rings and tracheid features of Pinus nigra and Pinus sylvestris in a mesic Mediterranean forest. European Journal of Forest Research, 132 (1), 33 – 45.

Martin-StPaul, MK., Longepierre, D., Huc, R., Delzon, S., Burlett, R., Joffre, R., Rambal, S., Cochard, H. (2014): How reliable are methods to assess xylem vulnerability to cavitation? The issue of 'open vessel' artifact in oaks. Tree Physiology, 34, 894–905.

Mäkinen, H., Nöjd, P., Mielikäinen, K. (2000): Climatic signal in annualgrowth variation of Norway spruce (Picea abies) along a transectfrom central Finland to the Arctic timberline. Canadian Journal od Forest Research 30, 769–777.

McDowell, N., Pockman, WT., Allen, CD., Breshears, D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D., Yepez, E. (2008): Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist 178, 719–739.

Miina, J. (2000): Dependence of tree-ring, earlywood and latewood indices of Scots pine and Norway spruce on climatic factors in eastern Finland. Ecological modelling, 132, 259 – 273.

Mork, E. (1928): Die Qualität des Fichtenholzes unter besonderer Rücksichtnahme auf Schleif- und Papierholz. Der Papier-Fabrikant 48, 741–747.

Myśkow, E., Blaś, M., Sobik, M., Godek, M., Owczarek, P. (2019): The effect of pollutant fog deposition on the wood anatomy of subalpine Norway spruce. European Journal of Forest Research 138, 187–201.

Novak, K., De Luis, M., Gričar, J., Prislan, P., Merela, M., Smith, K.T., Čufar, K. (2016): Missing and dark rings associated with drought in Pinus halepensis. IAWA J. 37, 260–274.

Nohrstedt, H.Ö. (2001): Response of coniferous forest ecosystems on mineral soils to nutrient additions: A review of Swedish experiences. Scandinavian Journal of Forest Research, 16, 555-573.

Oddo, E., Inzerillo, S., Grisafi, F., Sajeva, M., Salleo, A. (2014): Does short-term potassium fertilization improve recovery from drought stress in laurel? Tree Physiology, 34, 906–913.

Olano, J.M., Eugenio, M., Garcia-Cervigon, A.I., Folch, M., Rozas, V. (2012): Quantitative tracheid anatomy reveals a complex environmental control of wood structure in continental Mediterranean climate. International journal of plant sciences, 173, 137 – 149.

Olaoye, K.O., Oluwadare, A.O., Ariwoola, O.S. (2019): Relationship between Fibre Characteristics and Physico-Mechanical Properties of Aningeria robusta (A.CHEV) Wood. International Journal of Environment, Agriculture and Biotechnology (IJEAB) 4(1), 116 – 120.

Pacheco, A., Camarero, J.J., Carrer, M. (2015): Linking wood anatomy and xylogenesis allows pinpointing of climate and drought influences on growth of coexisting conifers in continental Mediterranean climate. Tree Physiology 36, 502 – 512.

Park, D., Spiecker, H. (2005): Variations in the tree-ring structure of Norway spruce (Picea abies) under contrasting climates. Dendrochronologia, 23/2, 93 – 104.

Park, I.Y., Dallaire, G., Morin, H. (2006): A method for multiple intra-ring demarcation of coniferous trees. Ann. For. Sci. 63 (1), 9–14.

Pasho, E., Camarero, JJ., De Luis, M., Vincento-Serrano, SM. (2012): Factors driving growth responses to drought in Mediterranean forests. European Journal of Forest Research 131, 1797–1807.

Pellizzari, E., Camarero, J., Gazol, A., Sangüesa-Barreda, G., Carrer, M. (2016): Wood anatomy and carbon-isotope discriminationsupport long-term hydraulic deterioration as a majorcause of drought-induced dieback. Global Change Biology 22, 2125–2137.

Pérez-De-Lis, G., Olano, JM., Rozas V., Rossi, S., Vázquez-Ruiz, RA., García-González, I. (2016): Environmental conditions and vascular cambium regulate carbon allocation to xylem growth in deciduous oaks. Functional Ecology 31 (3), 592 – 603.

Pittermann, J., Sperry, J. (2003): Tracheid diameter is the key trait determining the extent of freezinginduced embolism in conifers. Tree Physiology, 23/13, 907/914.

Prislan, P., Gričar, J., De Luis, M., Smith, K., Čufar, K. (2013): Phenological variation in xylem and phloem formation in *Fagus sylvatica* from two contrasting sites Author links open overlay panel. Agricultural and Forest Meteorology Volume 180, 142-151.

Pritzkow, C., Heinrich, I., Grudd, H., Helle, G. (2014): Relationship between wood anatomy, tree-ring widths and wood density of Pinus sylvestris L. and climate at high latitudes in northern Sweden. Dendrochronologia, 32/4, 295 – 302.

Rasband, W.S. (1997-2016). Image J.U.S. National Institute of Health, Bethesda, Maryland, USA.

Rendle, BJ. (1932): Wood Anatomy as a Link between Botany and Forestry. Nature 130, 834 – 836.

Rigling, A., Braker., O., Schneiter, G., Schweingruber, F. (2002): Intra-annual tree-ring parameters indicating differences in drought stress of Pinus sylvestris forests within the Erico-Pinion in the Valais (Switzerland). Plant Ecology, 163/1, 105 – 121

Rossi, S., Deslauriers, A., Gričar, J., Seo, JW, Rathgeber, C., Anfodillo, T., Morin, H., Levanic, H., Oven, P., Jalkanen, R. (2008): Critical temperatures for xylogenesis in conifers of cold climates. Global Ecology and Biogeograhy, 17, 696 – 707.

Rybníček, M., Kolář, T., Čermák, P., Žid, T., Hadaš, P. (2012): Dendrochronological analysis and habitual stress diagnostic assessment of Norway spruce (Picea abies) stands in the Drahany highlands. Wood Research 57 (2), 189 – 206.

Rydval, M., Wilson, R. (2012): The impact of industrial SO2 pollution on north Bohemia conifers. Water, Air, & Soil Pollution, 223 (9), 5727–5744.

Samusevich, A., Lexa, M., Vejpustková, M., Altman, J., Zeidler, A. (2020): Comparison of methods for the demarcation between earlywood and latewood in tree rings of Norway spruce. Dendrochronologia 20, 1 – 6.

Samusevich, A., Zeidler, A., Vejpustková, M. (2017): Influence of air pollution and extreme frost on wood cell parameters at Mountain Spruce Stands (Picea abies (L.) Karst.) in the Ore Mountains. Wood Res. 62 (1), 79–90.

Sauter, U.H., Mutz, R., Munro, B.D. (1999): Determining juvenile-mature wood transition in Scots pine using latewood density. Wood Fiber Sci. 31 (4), 416–425.

Savva, Y., Oleksyn, J., Reich, P., Tjoelker, M., Vaganov, E., Modrzynski, J. (2006): Interannual growth response of Norway spruce to climate alongan altitudinal gradient in the Tatra Mountains, Poland. Trees 20, 735 – 746.

Schneider, L., Gärtner, H. (2013): The advantage of using non-Newtonian fluids to prepare micro sections. Dendrochronologia 31, 175–178.

Schweingruber, FH. (2007): Wood structure and environment. Springer-Verlag Berlin Heidelberg, pp. 279.

Smith, J.H. (1966): Patterns in growth of Earlywood latewood and percentage latewood determined by complete analysis of 18 Douglas-fir trees. Canadian Journal of Botany, 44 (4), 453 - &.

Smith, S.J., Van Aardenne, J., Klimont, Z., Andres, R.J., Volke, A., Delgado Arias, S. (2011): Anthropogenic sulfur dioxide emissions: 1850–2005. Atmos. Chem. Phys. 11 (3), 1101–1116.

Sperry, J., Hacke, U., Pittermann, J. (2006): Size and function in conifer tracheids and angiosperm vessels. American Journal of Botany, 93/10, 1490 – 1500.

Stamm, A. (1973): Maximum effective lumen and pit pore sizes of earlywood and latewood of never dried loblolly-pine sapwood. Wood Science and Technology, 7 (3), 212 - 217.

Stern, D.I. (2005): Global sulphur emissions from1850 to 2000. Chemosphere 58, 163–175.

Taylor, F., Moore, J. (1981): A comparison of earlywood and latewood tracheid lengths of loblolly-pine. Wood and Fiber, 13 (3), 159 - 165.

Timell (1986): Compression wood in gymnosperm. Springer, Berlin, Heidelberg, New York, Tokyo, volume 1, 2150 pp.

Treml, V, Ponocná, T., Büntgen, U. (2012): Growth trends and temperature responses of treeline Norway spruce in the Czech-Polish Sudetes Mountains. Climate Res 55, 91-103.

Vacek, S., Bílek, L., Schwarz, O., Hejcmanová, P., Mikeska, M. (2013): Effect of air pollution on the health status of spruce stands. Mountain Research and Development, 33, 40-50.

Vacek, S., Hůnová, I., Vacek, Z., Hejcmanová, P., Podrázský, V., Král, J., Putalová, T., Moser, W.K. (2015): Effects of air pollution and climatic factors on Norway spruce forests in the Orlicke hory Mts. (Czech Republic), 1979–2014. European Journal of Forest Research, 134, 1127–1142.

Vaganov, E.A., Hughes, M.K., Shashkin, A.V. (2006): Growth dynamics of conifer tree rings: images of past and future environments. Springer, Berlin, Heidelberg, 354 pp.

Van Vliet, G.J.C.M. (1979): Wood anatomy of the Combretaceae. Blumea 25:141-223

Vávrová, E., Cudlín, O., Vavříček, D., Cudlín, P. (2009): Ground vegetation dynamics in mountain spruce (Picea abies (L.) Karsten) forests recovering after air pollution stress impact. Plant Ecol. 205, 305–321.

Vejpustková, M., Čihák, T., Samusevich, A., Zeidler, A., Novotný, R., Šrámek, V. (2017): Interactive effect of extreme climatic event and pollution load on growth and wood anatomy of spruce. Trees 31, 575–586.

Vieira, J., Campelo, F., Nabais, C. (2009): Age-dependent responses of tree-ring growth and intra-annual density fluctuations of Pinus pinaster to Mediterranean climate. Trees 23, 257–265.

Von Arx, G., Carrer, M. (2014): ROXAS–Anewtooltobuildcenturies-longtracheid-lumenchronologiesinconifers. Dendrochronologia 32, 290 – 293.

Von Arx, G., Crivellaro, A., Prendin, A., Čufar, K., Carrer, M. (2016): QuantitativeWoodAnatomy—PracticalGuidelines. Frontiers in Plant Science 7, 1 – 13.

Warren, W. (1979): Contribution of earlywood and latewood specific gravities to overal wood specific gravity. Wood and Fiber, 11/2, 127 - 135.

Warren, W. (1980): On removing the growth trend from dendrochronological data. Tree-Ring Bulletin 40, 35–44.

Willson, CJ., Manos, PS., Jackson, RB. (2008): Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus Juniperus (Cupressaceae). American Journal of Botany 95 (3), 299–314.

Wimmer, R. (2002): Wood anatomical features in tree-rings as indicators of environmental change. Dendrochronologia, 20/1 – 2, 21 – 36.

Wimmer, R., Grabner, M. (1997): Effects of climate on vertical resin duct density and radial growth of Norway spruce [Picea abies (L.) Karst.]. Trees, 11, 271 – 276.

Ziaco, E., Biondi, F., Rossi, S., Deslauriers, A. (2014): Climatic influences on wood anatomy and treering features of Great Basin conifers at a new mountain observatory. Application in Plants Sciences, 2/10, 1 – 13.