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EFFECT OF IMISSION TO XYLEM ANATOMY OF NORWAY SPRUCE

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ABSTRACT

The aim of this work was to analyse the relationship between anatomical parameters of spruce tracheids and climatic factors and air pollution load, in the period before, during and after the maximum air pollution load. In this study we used the method of dividing annual rings into a number of equally wide sectors, for which the average values of the tracheid dimensions, i.e., the lumen area and cell wall width, were determined. This method was compared to the classic approach, which works with the average values of parameters for the entire annual ring, or for earlywood and latewood. The study showed that the trees responded to the increased concentration of pollutants by reducing the widths of the annual rings and the values of the anatomical parameters. The higher resolution of data gives us a better insight on the influence of abiotic factors to the wood structure. The ratio of cell wall thicknesses of earlywood to latewood was also shown as a good indicator of stress.

KEYWORDS: air pollution, quaintitative wood anatomy, cell wall thickness, lumen area, Ore Mountains, *Picea abies* (L.) Karst.

INTRODUCTION

The so-called Black Triangle, which includes Northern Bohemia and the adjacent areas of Germany and Poland, was one of the most affected areas in Europe in the second half of the 20th century due to the enormous burden of fossil fuel emissions (Blažková 1996). In addition, the high level of extreme climate events has also made the situation worse. Cold winters increased the intensity of accumulation of air pollutants, and thereby subsequently in forest stands (Allen et al. 2010). From this entire area, the Ore Mountains were impacted the most (Zimermann et al. 2002) when extensive extinction of spruce stands took place (Kubelka 1992, Materna 1999). The health of the forest deteriorated rapidly in the 1970s and 1980s. With the rapid decline of air pollution in the 1990s, the health of the surviving stands began to improve (Fiala et al. 2002, Hůnová et al. 2004). Specifically, spruces began to regenerate rapidly after deep growth depression (Sander et al. 1995, Kroupová 2002), whereas in the late 1990s the radial growth in fact exceeded the values from the pre-stress period (Rydval and Wilson 2012, Kolář et al. 2015). With the exception of a few studies (Wimmer 2002, Samusevich et al. 2017, Vejpustková et al. 2017), we have very little information about the impact that extreme pollution had on the anatomical structure of wood.

Data obtained via quantification of tracheid dimensions and their statistical processing can provide us with information about the environment in which the growth of the studied vegetation occurred (Wimmer and Grabner 1997, Olano et al. 2012, Ziaco et al. 2014). However, these anatomical characteristics may react to stress differently than the mean ring width (MRW), making them potentially suitable proxies for dendroecological studies (Kozlowski et al. 1991, Schweingruber 1996, Wimmer 2002, Vaganov et al. 2006, Puchi et al. 2020). Anatomical parameters can also be modified by air pollution (Wimmer and Halbwachs 1992, Kurczyńska et al. 1997, Samusevich et al. 2017, Vejpustková et al. 2017). Especially SO_2 load can increase sensitivity to other abiotic factors, such as frost, heat or drought (Keller et al. 1984). Unlike the mere width of the annual ring, data on the dimensions and numbers of tracheids can be investigated with a higher than annual resolution (Fonti et al. 2010), von Arx et al. 2016). One of the approaches to obtaining intra-annual resolution is to divide the annual ring into earlywood (EW) and latewood (LW) (Samusevich et al. 2020). The conditions and factors that influenced the structure and dimensions of tracheids in these two periods of the year may vary (Park and Spiecker 2005, Olano et al. 2012, Ziaco et al. 2014). The issue is that there are a large number of methods for identifying the boundary between EW and LW, such as using the Mork's index (MI) value (Denne 1989), determination via the sum of cells before and after the early and late wood boundary using the MI (Park et al. 2006), or X-ray densitometry (Koubaa et al. 2002). Although this data may provide new results (Samusevich et al. 2017, 2020, Vejpustková et al. 2017), the position of the boundary determined by the individual methods may differ and may not always respect the natural boundary between EW and LW. The primary issue of certain tree species, which also applies to the wood of the investigated spruce, is the gradual transition between EW and LW (Park et al. 2006).

We do not know exactly when a particular cell of a given annual ring is formed, but we do know their sequence. One of the new approaches is to therefore divide each annual ring into a number of equally wide sectors (Carrer et al. 2017, Puchi et al. 2020), in which the average values of the parameters of the anatomical structure – lumen area (LA) and cell wall thickness (CWT) are calculated. The relationship between anatomical parameters and pollution is then analyzed with intra-annual resolution by individual sectors, and it is thereby possible to determine which part of the annual ring was most affected by the investigated factors (Carrer et al. 2017, Pacheco et al. 2017, Castagneri et al. 2018).

The aim of the study was to analyse at the intra-annual level the effect of climate and pollution on the parameters of the anatomical structure of Norway spruce wood in the Klínovec area, i.e., the highest peak of the Ore Mountains. One of the partial goals was to compare the method of analysis of anatomical parameters by sectors with the classic approach, which works with the average values of parameters for the entire annual ring, or for earlywood and latewood. We hypothesized hypothesised that high concentrations of pollutants have a negative effect on the values of the LA and CWT parameters, that pollution changes the nature of the relationship between climate and anatomical parameters, and that dividing the annual ring into smaller parts provides a more detailed insight into xylem growth response to abiotic factors.

MATERIAL AND METHODS

Location

For the purpose of quantitative analysis of anatomical features of wood, five cores were taken from randomly selected Norway spruce (*Picea abies* (L.) Karst) level trees in the autumn of 2015, from each of four stands (Tab. 1) in the vicinity of the highest peak of the Ore Mountains – Klínovec (1,244 m above sea level). Three stands were located at an altitude of approximately 1,000 m and the fourth at an altitude of 1,230 m nearby the hilltop. The reason for choosing this location was mainly the presence of old spruce stands, which survived the air pollution calamity. The climatic conditions are characterized by data from the nearest meteorological station, located on the German side at the top of Fichtelberg. Fig. 1 shows the average annual summer temperature (June– - August) and the annual and summer sum of precipitation (June– - August). Fig. 2 shows the average annual and winter air concentration of SO$_2$ represented by a combined series of measurements from the nearby Blatno and Měděnec stations. The greatest sources of pollutants in the area are the Tušimice and Prunéřov brown coal power plants, which began operating in 1964 and 1967. These power plants were completely desulphurized in the first wave of greening of coal resources from 1996 to 1999. According to a report made in 2010 by ČEZ, the company operating the power plants, the level of SO$_2$ concentration was reduced by 92% compared to the level at the beginning of the 1990s, NO$_X$ by 50%, solid pollutants by 93% and CO by 77%.

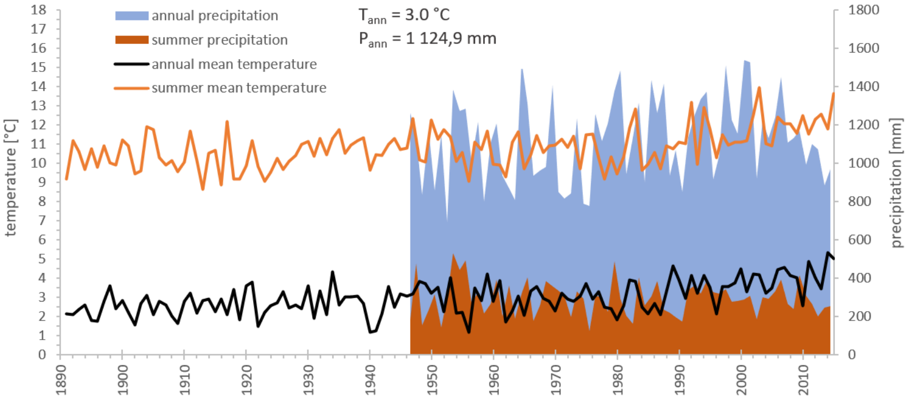

Fig. 1: Average temperatures and precipitation (at meteorological station on the Fichtelberg).

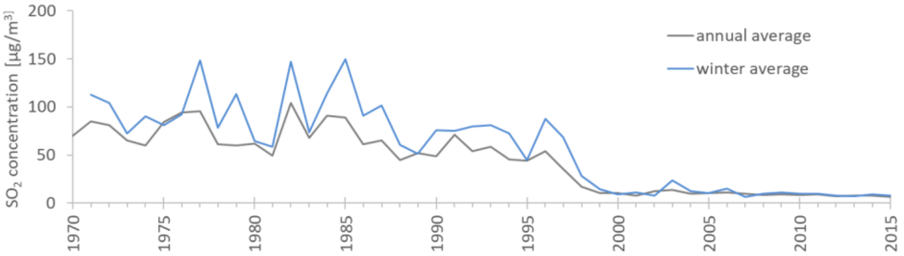

Fig. 2: Average annual and winter SO_2 concentrations (data combined from stations Blatno and Měděnec).

Tab. 1: Description of study plots.

Plot	Coordinates [WGS84]		Altitude	Exposition	Stand age
	latitude	longitude			
Sucha 1	N 50°23‘13“	E 12°59‘07“	1 030	SE	160
Sucha 2	N 50°22‘41“	E 12°57‘51“	1 009	SW	170
Loucna	N50°24‘23“	E 12°58‘14“	1 013	N	180
Klinovec	N 50°23‘40“	E 12°58‘07“	1 230	-	120

Sampling and preparation of samples

A total of 40 cores from 20 trees were collected for the purpose of cross-dating, from each tree on two cores on opposite sides in the direction of the contour line in order to eliminate the occurrence of reaction wood. First, the mean ring width (MRW) was measured using measuring table „TA“ measurement system (Velmex Inc., Bloomfield, NY, USA) with an accuracy of 0.001 mm. Cross-dating was subsequently carried out using PAST 5.1 software. A total of 20 cores, one of which each tree, were then used to create permanent microscopic slides. After dividing the cores into a length that fits on the glass slide (4–5 cm), 12 µm thick cross-cuts were made using a rotary microtome (Leica, Heidelberg, Germany). It is important to ensure that the thickness of the slide is kept constant throughout the length of the cut, as the thickness of the slide can affect the values of the measured parameters, mainly the CWT (von Arx et al. 2016). The specimens were placed on a glass slide, stained with safranin, dehydrated with series of alcohol solutions in ascending concentrating up to 100% and fixed with a synthetic resin (Eukitt, BiOptica, Milan, Italy) (Castagneri et al. 2017). At 100× optical magnification (2.9 µm/px), the specimens were scanned using a Nikon Ni-E motorized microscope. NIS-Elements software was used for capturing the composite image.

Data processing

The composite images were analyzed using software ROXAS v 3.0.1 (von Arx and Carrer 2014). For measuring parameters we used the same configuration file as Castagneri et al. (2015). The MRW, lumen area (LA) and cell wall thickness in the radial direction (CWT) were measured. For each measured cell, its relative distance from the beginning of the annual ring was recorded.

In order to find one boundary between EW and LW, each annual ring was divided into one hundred equally wide sectors, and the average value of the Mork’s index (MI) was calculated for each of them. This value is calculated as a ratio of four times the radial thickness of the cell wall and the radial diameter of the lumen (Denne 1989). The moving average of the MI with a length of nine sectors was then calculated, and it was further ensured that this value only increased, so that if the moving average value was lower than the previous value, the previous value was recorded instead. All sectors for which the calculated MI was less than or equal to 1 were considered earlywood. In addition to earlywood and latewood, 10 equally wide sectors numbered along the direction of the wood growth were defined within each annual ring based on the relative distance of the cells from the beginning of the annual ring (Fig. 3) (Carrer et al. 2017).

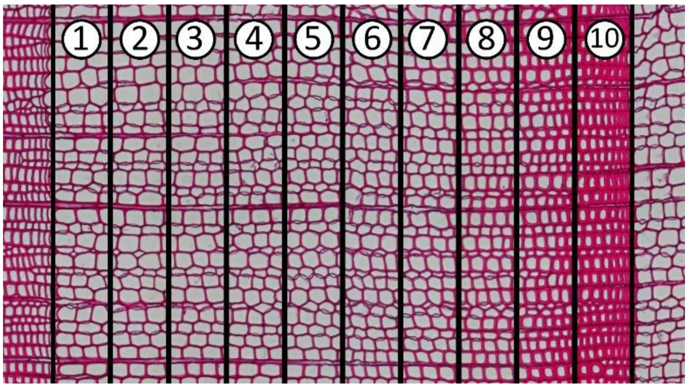

Fig. 3: Defining of ten sectors within the annual ring.

The mean value of the LA and CWT was calculated for each annual ring (RW), its earlywood and latewood, and for all ten sectors of each tree. The parameter data from the individual series were detrended using a 100 years spline, and then the average standard chronologies of all 20 trees were calculated using ARSTAN software. Furthermore, a sensitivity parameter was calculated for each chronology (Carrer and Urbinati 2006), characterizing the fluctuation of the data series, as the average absolute percentage change in the value of the relevant parameter for the annual ring compared to the value for the previous annual ring. In order to show the differences in the long-term CWT trend of earlywood and latewood, the course of CWT chronologies was approximated via a polynomial function of the sixth degree and shown in the graph.

Using XLSTAT software, a Principal Component Analysis (PCA) was carried out for all chronologies of parameters covering the common period from 1870 to 2015. The obtained chronologies were further correlated with temperature, precipitation and the SO_2 air concentrations. For the temperature, a time series with monthly averages from 1891 was available from the Fichtelberg station, whilst for precipitation the station data was from 1947, the CRU TS 4.04 (Climatic Research Unit, 2021) grid data was from 1901, and daily concentrations from 1970 were available for SO_2, which were obtained by combining data from the Blatno and Mědĕnec stations. The chronologies of the parameters were correlated with the monthly values of climatic parameters and the SO_2 series for the longest possible time period. In order to verify the stability of the climate–growth relationship over time, running correlations were calculated with a thirty-year window in five-year steps.

RESULTS

The strongest reaction to the air pollution stress in the 1980s is evident for MRW, where we can observe a rapid decline in values since the late 1960s, as well as increased variability (Fig. 4). The values of LA and CWT show an incomparably smaller reaction to such stress. There is only a slight decrease in the LA values.

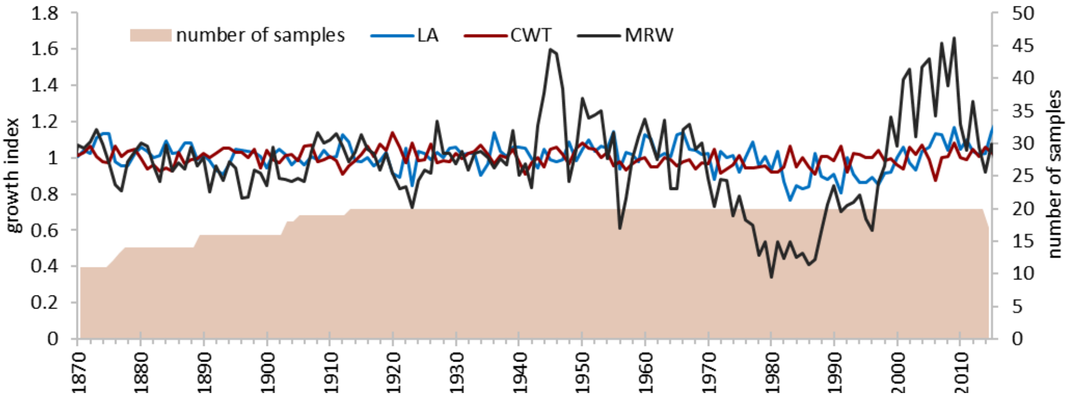

Fig. 4: Comparison of annual mean chronologies of studied parameters.

The course of the LA earlywood and latewood chronologies is very similar, but the LA latewood chronology shows higher variability (Fig. 5). The sensitivity for EW is 4.1%, and for LW 9.4%. A separate representation of the LA chronologies shows a decrease in values during the stress period.

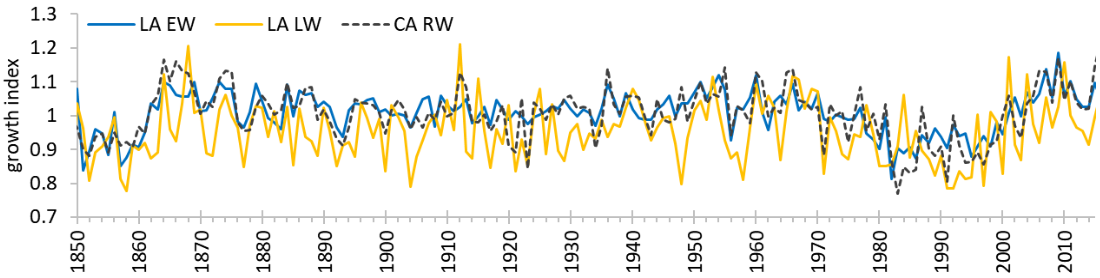

Fig. 5: Comparison of lumen area chronologies for earlywood, latewood and whole ring.

The CWT chronologies indicate similar fluctuations, but they differ in their course. Although no decrease in CWT is evident during the stress period, the course of the chronology of CWT for EW practically never exceeds the course of the chronology of the CWT for LW except for the stress period (Fig. 6). For a better representation, both chronologies in Fig. 7 are approximated by a polynomial function of the sixth degree. Herein we can better see the intersection of the CWT chronologies for early and late wood from the early 1960s to approximately the end of the millennium.

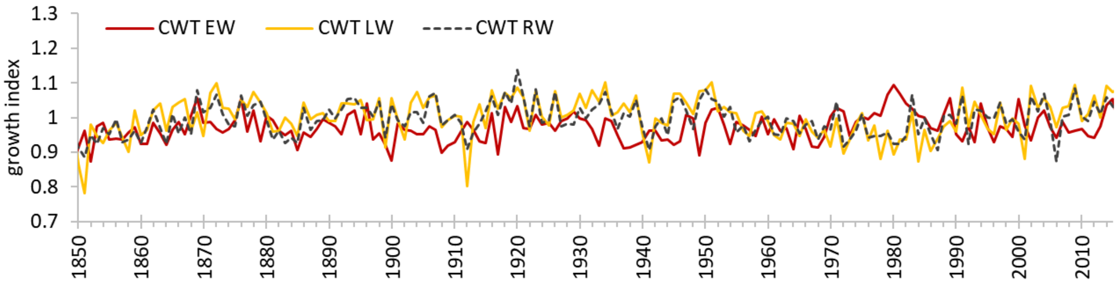

Fig. 6: Comparison of cell wall thickness chronologies for earlywood, latewood and whole ring.

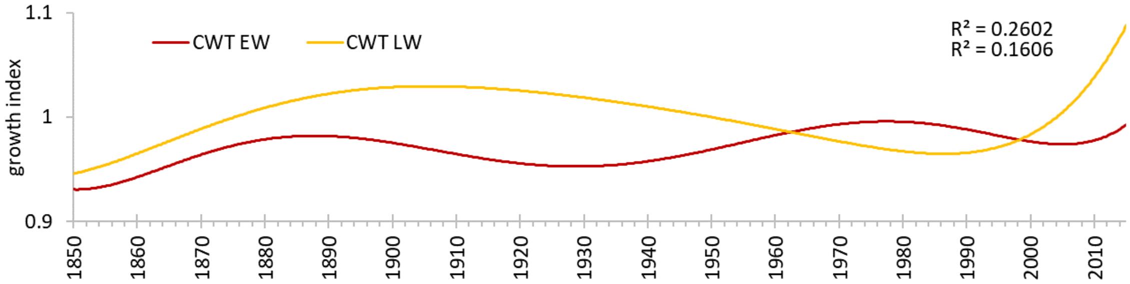

Fig. 7: Approximation of the long – term trend of cell wall thickness values for earlywood and latewood.

The LA chronology for individual sectors is shown in Fig. 8. The growth index is offset by a value of 0.5 for each subsequent chronology. It is clear that their volatility is gradually increasing. The first sector, corresponding to the beginning of xylem growth (LA1), has a sensitivity parameter of 4.8%, whereas the last (LA10) has a sensitivity parameter of 24.0%. The decrease in values during the stress period is also most evident in the last sectors.

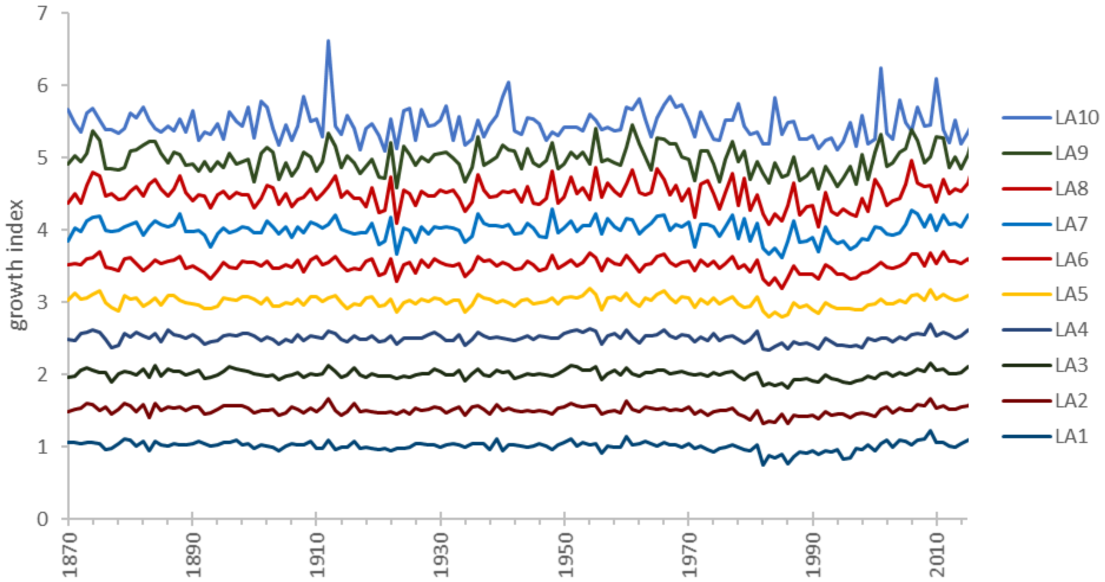

Fig. 8: Mean lumen area chronology for each of the ten sectors.

In CWT, on the other hand, the increase in sensitivity from the first to the last sectors is not as large. The first sector (LA1) has a sensitivity parameter of 2.8% and the sector with the highest value sensitivity parameter (LA8) is 9.2%. This parameter rises sharply between the sixth (LA6) and seventh (LA7) sectors from 4.7% to 8.3%. In the last sectors, there is only a slight decrease in the values of the growth index during the stress period. The growth index is offset by 0.25 for each subsequent chronology.

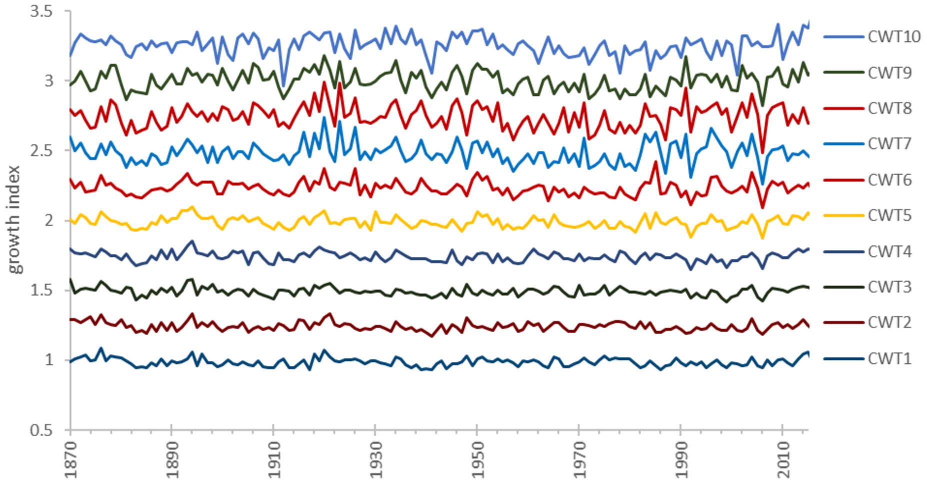

Fig. 9: Mean cell wall thickness chronology for each of the ten sectors.

The PCA shows that with regard to the LA, the adjacent sectors show a high degree of similarity (Fig. 10). With regard to CWT, the sector vectors are arranged more randomly and, unlike the LA, there is a higher similarity between more distant sectors. The MRW parameter correlates well with the first LA sectors. The first four components of the PCA analysis explain 81.2% of the data variability - the first component explains 35.8%, the second 24.7%, the third 12.1% and the fourth 8.5%.

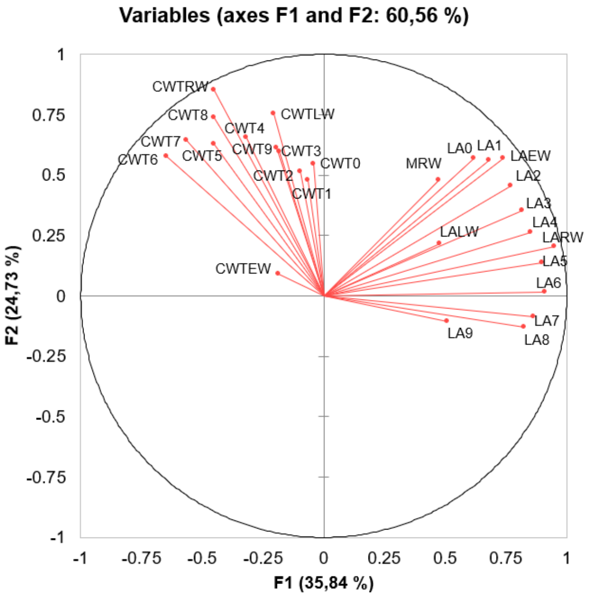

Fig. 10: Loading plot of first two components of PCA analysis.

In Fig. 11 we can see the correlations of all chronologies with SO_2 air concentrations in individual months for the period from 1970 to 2015. For comparison, the MRW chronology is plotted for both parameters, which shows high correlation with all monthly and the annual and winter average of these concentrations. We can see that the LA chronologies correlate best in the first sectors, which is also reflected in the EW and RW chronologies, and thereafter the correlation gradually weakens and no longer correlates in the final sector. We find the exact opposite with regard to correlations with the chronologies of the CWT parameter - the last sector correlates best, which is also reflected in the LW chronology.

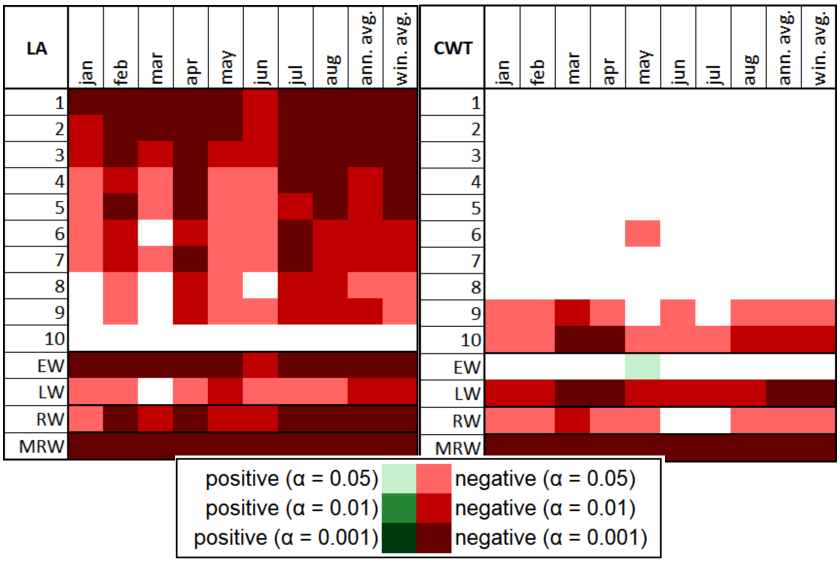

Fig. 11: Correlation of anatomical parameters chronologies with monthly, annual and winter SO_2 air concentration for the period 1970 – 2015.

Running correlations of anatomical parameters with precipitation did not show any valuable relationship. A strong negative correlation during the stress period appeared in the last few CWT sectors for LW in July, and a positive correlation during the same month with the LA in the second half of the annual ring. The running correlation of the temperature from the Fichtelberg station with the chronologies of the parameters is shown in Fig. 12. The top line always shows the middle year of the thirty-year window. For comparison, the correlation with MRW is also shown in all correlation matrices. With the exception of the post-stress period, the LA negatively correlates in the latter months (July – August) with the second half of the sectors in almost all windows. This correlation is evident at the sector level. It is also noticable in LW and RW, but not in all windows. During the stress period (1960-1990), the LA in the middle part of the annual ring correlates positively with the June temperature. For the post-stress period (1995-2015), there is only a positive correlation between the LA and the temperature in April, but in almost all of the sectors except the last, which can be seen both at the level of EW and at the level of the RW. The correlation of the CWT with the temperature in the summer months (July – August) during the stress period is also interesting. In the sectors in the first half of the annual ring the CWT correlates negatively with the temperature, whilst a positive correlation was demonstrated for the CWT in the second half of the annual ring. The second mentioned correlation is clearly evident at the level of LW and RW, but the first correlation has not reflected into these levels. Furthermore, we can see that the CWT in the second half of the sectors correlated positively with the temperature in the first half of the last century (most strongly in the middle sectors), but in the subsequent period this relationship weakens and there is a positive correlation between temperature and the CWT in May in the last three sectors, with the strongest being in the 9th and 10th sectors. The correlation of temperatures with MRW is in most cases weaker than the correlation with anatomical parameters. However, none of the correlations of any of the parameters are stable throughout the observed period.

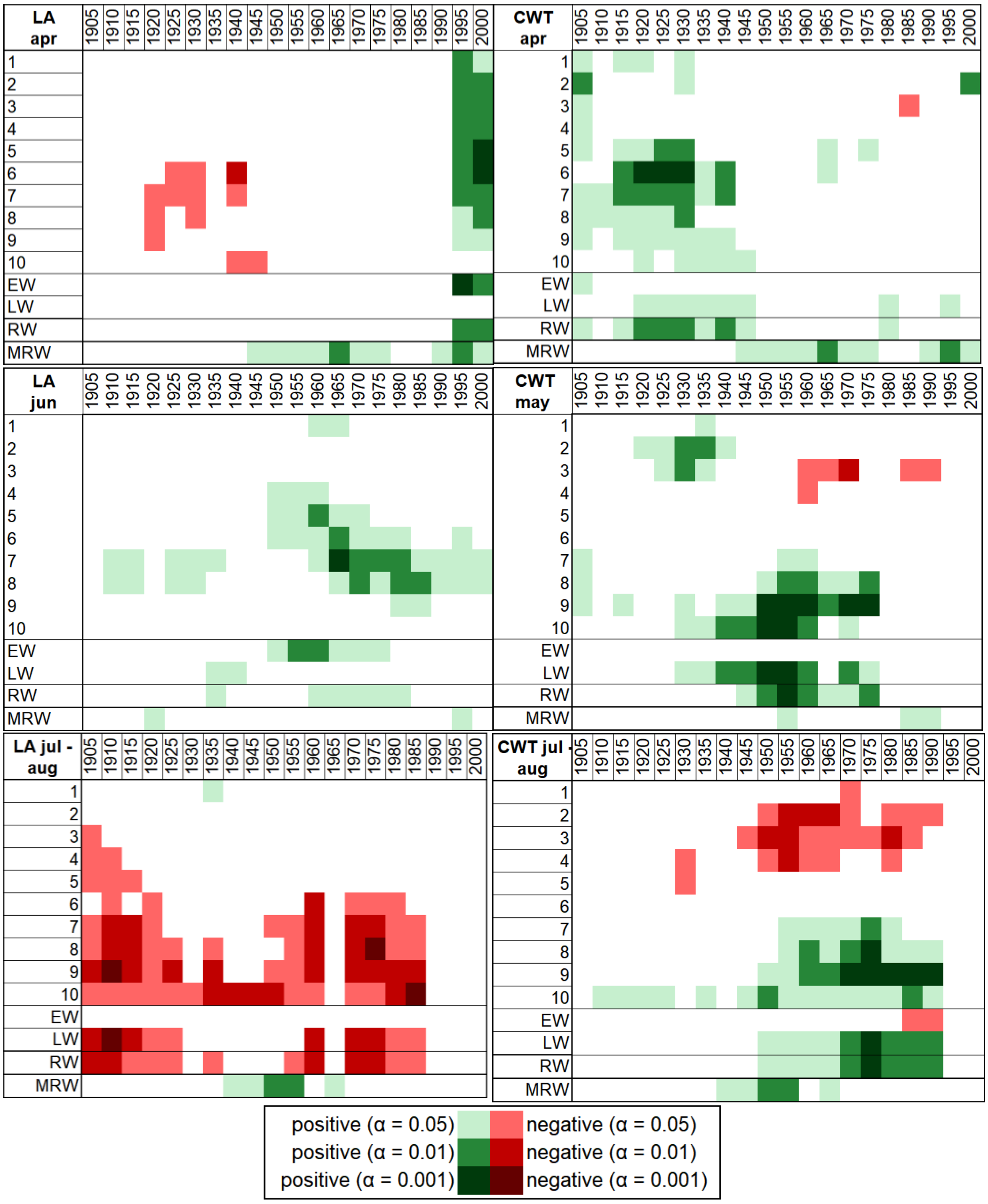

Fig. 12: Running correlation of anatomical parameters chronologies with monthly mean temperatures for the period 1891 – 2015.

Fig. 13 shows the running correlation of the LA with July and August temperatures. The sectors in the middle part of the annual ring correlate with the July temperature, whereas only the last sectors correlate with the August temperature.

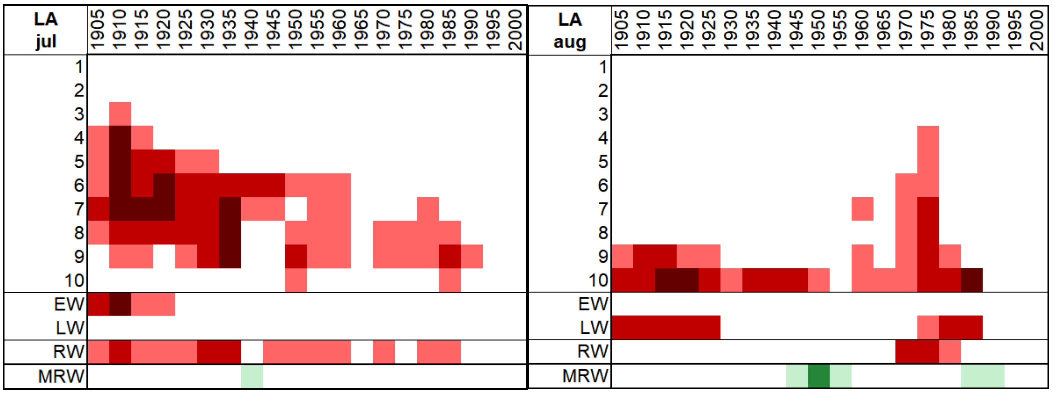

Fig. 13: Comparison of the correlation of lumen area with the mean temperature of two consecutive months.

DISCUSSION

As a traditional, quickly and easily measurable parameter, MRW is a very good indicator of stress. This is evident both from the course of the average chronology of this parameter (Fig. 4) and from the correlations with SO_2 (Fig. 11). The sharp increase of MRW values after the end of the stress impact can be due to both the effect of release (Kolář et al. 2015) and high nitrogen depositions (Lomský et al. 2013), or because of the rising temperatures in recent years due to global warming, which prolongs the growth season (Linderholm 2006, Puchi et al. 2020), or it could be a combination of these factors. This effect is also evident to a slightly lesser extent in anatomical parameters.

For the CWT values for EW and LW, approximated by the polynomial function in Fig. 4, we can see their intersection, which defines the stress period relatively accurately timewise. Likely due to the delayed initiation of cambial activity during the stress period (Rajput et al. 2008, Samusevich et al. 2017), cells with as thin cell wall as under normal circumstances are no longer formed at the beginning of the growth season, and therefore their average thickness in EW increases, and the thickness of the cell walls of the LW thin out rapidly, because during the stress period the tree does not have enough resources for the cell walls in the LW to be able to mature to full dimensions. This may be due to the fact that increased SO_2 concentrations damage the assimilation apparatus of trees, thereby reducing photosynthetic activity, which directly affects nutrient availability and the production of growth regulators (Fritts 1976, Kurczyńska et al. 1997). The same trends in the LA and CWT were also confirmed in the sectors approach (Figs. 9 and 10), where we see how their course gradually changes from the beginning to the end of the annual ring. Samusevich et al. (2017) demonstrated that, similarly to the entire MRW, the LA decreases during the stress period. Moreover, this parameter in the EW and the first sectors strongly positively correlates with the MRW, as can be seen in the results of the PCA analysis in Fig. 10.

With regard to the effects of SO_2 (Fig. 11), the LA of EW is the most affected parameter, which is also related to the fact that this parameter strongly correlates with MRW. There is also an indication that CWT is affected only in the last two sectors, i.e., in the LW, which confirms that the maturation of the cell wall of LW cells is disrupted during the stress period. Compared to the study by Vejpustková et al. (2017), in which the EW parameters were mainly sensitive to pollution, and the study by Axelson et al. (2014), where primarily the LW parameters were sensitive, our study demonstrates that EW was more sensitive in the LA, and LW in CWT.

Due to their distribution, running correlations with precipitation are likely only anticorrelations to temperature. Precipitation is usually not a limiting factor at higher altitudes, and plant phenology is mainly controlled by temperature (Kramer et al. 2000). Interestingly, apart from the significant positive correlation of the LA with the temperature in April, no significant relationship between climatic factors and the investigated parameters was recorded in the last 2-3 correlation windows. This probably means that if a tree is not limited by precipitation or pollution and has enough nutrients and the growing season lengthens with increasing temperature (Linderholm 2006, Puchi et al. 2020), wider annual rings are formed, whose tracheids have lumens with a larger area. Adversely, during the stress and pre-stress period, the high temperature in summer months had a negative effect on the size of the LA, most likely due to the limited availability of water. During the stress period, temperature only had a positive effect on the lumen size of the middle sectors in June. Summer temperatures (July – August) also affected CWT during the stress period. They correlate positively with CWT in last sectors, which what probably means that if the temperature was higher in August, the growing season lengthened and the cells were able to mature to a greater extent. In the warmer years during the pollution period, the nature of EW cells was generally more standard – EW cells have a thinner cell wall during these years, whilst in the period of pollution load, the differences between EW and LW decrease. In this case, the increased sensitivity of the anatomical parameters with regard to temperature in the period of high SO2 concentrations was confirmed (Keller et al. 1984).

The correlation of CWT with the temperature in July to August over individual sectors shows both a positive and a negative relationship within one annual ring (Fig. 12). However, the negative correlation of CWT in the first four sectors was not reflected in the CWT relationship within the entire annual ring, nor within EW. We can therefore examine this relationship only while using the sector method, wherein the annual ring is divided into smaller parts than EW and LW. A comparison of the relationship between the LA and the temperatures of July and August once again shows the benefit of dividing the annual ring into smaller parts, because we are able to examine in detail which part of the annual ring is affected during a specific period (Puchi et al. 2020). Specifically, we can find that the cells of the last sectors were formed mainly in August over the course of the majority of the studied period.

CONCLUSIONS

Mean ring width is very sensitive to pollution. However, this study showed that the anatomical parameters were also affected by the pollution, which is evident from the significant correlations with the pollution, and from the change in the reaction to the climate during the stress and post-stress period. The relationship between earlywood and latewood cell wall thickness was shown as a sensitive indicator. The contradictory trends of these parameters in the culminating pollution period indicates the effect of the stress factor. The presence of pollutants, in particular SO_2, also affects the sensitivity of anatomical parameters to temperature. The greatest disturbance of the xylem formation occurred primarily in years with the combination of high concentrations of SO_2 with low temperature during the growing season. LW cells have thinner cell walls and the difference between CWT for EW and LW is decreasing, and during these years the LA is decreasing throughout the annual ring. Dividing of the annual ring into earlywood and latewood and into smaller sectors gives us a more detailed insight into the relationships between wood production and environmental factors with a higher than annual resolution. Although a number of relationships are already apparent from the average values of the parameters for the entire annual ring, some relationships may remain hidden without this dividing approach.

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