

**THE ANALYSIS OF XYLEM AND PHLOEM CELL NUMBER  
AND ANALYSIS OF THE SAPWOOD AREA PROPORTION  
IN NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.) WITH  
VARIOUS STATE OF HEALTH**

GABRIELA VICHROVÁ, HANUŠ VAVRČÍK

MENDEL UNIVERSITY IN BRNO, FACULTY OF FORESTRY AND WOOD TECHNOLOGY  
DEPARTMENT OF WOOD SCIENCE  
BRNO, CZECH REPUBLIC

TOMÁŠ ŽID

MENDEL UNIVERSITY IN BRNO, FACULTY OF FORESTRY AND WOOD TECHNOLOGY  
DEPARTMENT OF FOREST PROTECTION AND WILDLIFE MANAGEMENT  
BRNO, CZECH REPUBLIC

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**ABSTRACT**

In the project we focused on the evaluation of the health state of Norway spruce (*Picea abies* (L.) Karst.) in the Drahaný highlands. The research was conducted in six research plots. The first indicator of the state of health was the anatomical structure of the last xylem and phloem increment and cambium. The numbers of cells in individual types of tissues was evaluated with the aim to express the current growth potential. Further, the sapwood area proportion at breast height was analysed. This provides the information about the potential physiological activity of the entire stem section. The last type of data was the data from habitual diagnostics of woody plants.

When analysing the sapwood area proportion, we found out that there is no statistically significant difference between means of particular plots. When analysing the numbers of cells in xylem in the latest ring, cambium and phloem, we found statistically significant differences among the research plots. However, these differences were not related to the proportion of sapwood or the data from habitual diagnostics.

Based on the results, the basic hypothesis that the anatomical structure of xylem, cambium, and phloem, and the sapwood proportion can be used as one of the criteria for the evaluation of the state of health of woody plants was not confirmed. However, the contribution of the analysis of the selected indicators can be seen in the fact that the methods used and verified in the forestry practice (habitual diagnostics) were extended by data providing information about the physiological functionality of woody plants in the given conditions.

**KEYWORDS:** Xylem, phloem, *Picea abies* (L.) Karst., sapwood, defoliation, state of health.

## INTRODUCTION

The issue of the state of health of trees and stands is still topical nowadays. The factors influencing tree growth are reflected in the state of health of trees and thus their increment. For example, the dependence between crown thinning and growth of woody species has been proven repeatedly (Solberg and Tveite 2000). However, the terms vitality, or state of health have not been used consistently. Some authors use them for the dynamic ability to grow in current conditions (Gričar et al. 2009). Others define vitality as genetically derived capacity of trees resistant to stress (Shigo 1986). Tree vitality drops with the increasing stress but the optimum vitality is not known; only the minimum vitality can be defined as it is determined by the death of the tree. The term state of health is used for the physiological aspect of the tree existence, sometimes referred to as vitality (Innes 1993). Today, the reasons for tree decline have to be explained based on a complex synthesis of information from related fields, especially plant physiology, biochemistry, bioclimatology, pedology, geochemistry, etc. (Jankovský 2004).

Nowadays, various methods for state of health assessment of woody species are being developed. Often these are based on the characteristics of the crown (Čermák et al. 2005). An extensive monitoring based on crown structure has been in progress in Europe since 1986 in a network of areas "level 1" within the ICP Forests program (the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests). This methodology has been developed by several authors (Lorenz 1995, Lorenz et al. 2001). At the same time, new extension methods are appearing, e.g. the method of monitoring the representation of development directions of buds (Polák et al. 2007). Another option of tree vitality examination is the measurement of the electrical resistance in the cambium (Gehring 2004).

The formation of new xylem cells and phloem is in woody species which exhibit secondary thickening dependent on the cambium activity. Cambium is a secondary meristem whose division activity of cambial initials and phloem mother cells divides the phloem phloem cells centrifugally and xylem cells centripetally (Larson 1994). This leads to an irreversible process of dimension growth referred to as radial growth (Larson 1994, Procházka et al. 1998). Xylem and phloem form vascular bundles. In woody species which exhibit secondary thickening the vascular bundles remain open, i.e. new layers continue growing. Disregarding the mechanical function – this allows trees to grow upright up to a height of several dozens of meters – in a growing tree they perform the conducting function, i.e. they lead water with dissolved minerals to assimilation organs by an 'ascending course'. The phloem part of vascular bundles transfers the products of assimilation (Procházka et al. 1998).

The variability of the anatomic structure and the size of increment of phloem in relation to growth conditions in fir were described by Holdheide (1951). His work shows that trees growing in adverse conditions are characterized by disconnected or even missing tangential bands of axial parenchyma and a reduced number of sieve cells in the late phloem.

It was found out that the phloem/xylem cell number ratio in the latest layer decreased with the decreasing tree vitality in most affected trees (4 % of the studied set), i.e. more phloem cells than xylem cells were formed (Gričar et al. 2009). The phloem formation is probably controlled by more endogenous factors than xylem formation (Gričar and Čufar 2008). The width and the structure of phloem and the ratio among the numbers of cells of phloem, xylem and cambium provide data which could subsequently represent a useful tool in forest economy.

The dynamics of the growth of new xylem and phloem cells is different. While the formation of phloem in a specific growing season is unconditional and its amount is generally stable even in various conditions, the situation of xylem is completely different. During the growing season

the air temperature is a limiting factor for the synthesis of the secondary cell wall of tracheids and its lignification (Horáček et al. 1999). In a healthy tree more cells of xylem than of phloem are formed (Požgaj et al. 1993). However, if the tree starts to be affected by stress, the number of newly formed xylem cells decreases. In extreme cases, the tree ring is not formed at all and this does not mean death of the tree because the cells of previous rings remain active. This principle is used in the studies assessing vitality of trees based on the ratio of xylem and phloem cells formed during one growing season (Gričar et al. 2009).

Another indicator of vitality is the area of sapwood showing the amount of the physiologically active part of stem. Procházka et al. (1998) use the theory of that the number of needle-years and the sapwood area are in close correlation. The created assimilates are directed from each needle-year towards a specific tree ring which was formed in the same year as the needles. Similar situation occurs with nutrients conducted by xylem cells of individual tree rings. These conduct the nutrients to needle-years of the same age. Thus the number of needle-years determines to a degree the number of active cells in the stem, i.e. the sapwood area, and vice versa. The relation between defoliation (or transparency) and growth (ring width, production of sapwood) has been confirmed by e.g. Waring et al. (1981), Solberg (1999), Eckmüllner and Sterby (2000) or Dobbertin (2005).

The objective of the research was to analyse the indicators of the state of health of Norway spruce. As one of the characteristics, the number of phloem and xylem cells formed during the last growing season (2009) was analysed, further, the number of cells of inactive cambium in the stem of Norway spruce (*Picea abies* (L.) Karst.). The number of xylem and phloem cells formed during one growing season provides information about the growth potential of an individual. Therefore, the essential objective is to verify the hypothesis that the number of xylem and phloem cells formed during one growing season can be used as an indicator of the state of health of an individual.

Another explored property was the amount of sapwood at the breast height. This data provides information about the physiological functionality of the entire stem diameter. The data on the habitual diagnostics of trees, such as the degree of crown defoliation and the percentage of secondary shoots, had been established during the previous studies (Cudlín et al. 2001).

The project was a continuation of previous research conducted in experimental plots in the Drahaný highlands. Here the monitoring of crown defoliation ICP Forests was carried out (Čermák et al. 2005) with a slight extension (ICP Forest 2007). Further, the tree ring analysis and the analysis of the wood basic density were carried out in chosen plots (Rybníček et al. 2010). Six experimental plots with twenty trees each were selected for this research. Age of trees varied from 86 to 119 years. The average age was 102.3 year. Diameter at breast height varied from 28 to 61 cm (average 44.2 cm).

## MATERIAL AND METHODS

### Samples for xylem and phloem cell number analysis

For the purposes of the analysis of the number of xylem and phloem cells formed in the latest growing season (2009), the samples were taken in the period of dormancy, i.e. in winter. In this period, the latest tree ring has been fully formed and the individual anatomical elements have been fully differentiated. No morphological changes of xylem or phloem cells occur on the level of cells in the period of dormancy.

The chosen tree species was Norway spruce (*Picea abies* (L.) Karst.). Samples were taken at breast height – 1.3 m – in the amount of 3 pieces from each tree for possible storage. They were extracted by means of a specially designed increment puncher Trepfor (Rossi et al. 2006), which took cylindrically shaped microcores, about 15 mm in length and 1.8 mm in diameter.

Microcores were paraffin embedded and sectioned according to standard methodology (Gričar et al. 2007, Gryc et al. 2012) to make permanent microslide preparations of transversal sections. These were analysed using the Leica DM LS light microscope. The analysis included counting of randomly chosen five radial rows of cells in each sample, during which the following types of tissues were distinguished: xylem, cambium, early and late phloem sieve cells and axial parenchyma (Fig. 1).

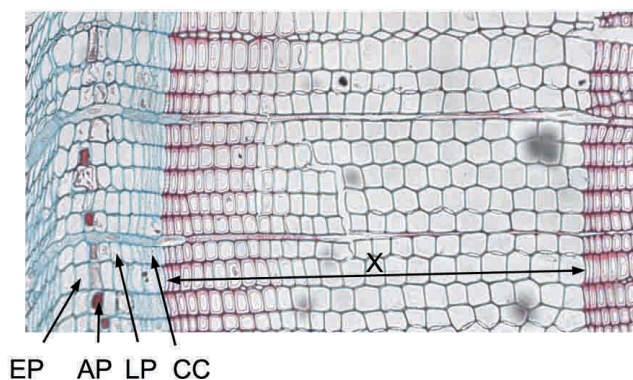


Fig. 1: Transversal section of xylem and phloem. EP – early phloem, AP – axial parenchyma, LP – late phloem, CC – cambial cells, X – xylem.

### Samples for sapwood area proportion analysis

The samples were taken from the same trees as for the analysis of xylem and phloem proportions. They were taken by means of Pressler increment borer and glued to wooden slats. Then the cores were treated with Lugol's solution ( $H_2O$  – 40 ml, I – 2 g, KI – 4 g), which caused darkening of the surface. However, the solution evaporated from ripewood (light heartwood) overnight and only sapwood retained the dark brown colour (Fig. 2). The staining is based on the principle of molecule binding of Lugol's solution to starch grains in sapwood. This results in a colour differentiation of ripewood and sapwood. The sapwood was measured with 1 mm accuracy and this data was used to calculate the proportion of sapwood for particular trees.



Fig 2: A part of a core with dark coloured sapwood after treatment by Lugol's solution.

## Habitual diagnostics

In the representative number of trees basic habitual characteristics were according to Cudlín et al. (2001). First, the growth habit of a tree was described, namely, social position, type of branching, type of the tree top, crown form, the presence of stem, crown and top breaks. Crowns were visually divided to three parts: upper juvenile part, central production part and lower saturation part. In the juvenile part, its form was evaluated (according to the modified method of Lesinski and Landman 1995), in the production part, total defoliation, defoliation of the primary structure, the percentage of secondary shoots and types of damage (Cudlín et al. 2001).

## RESULTS

The results of the habitual diagnostics are presented in Tab. 1. The table shows the average values of all basic characteristics of habitual diagnostics for individual plots and the mean value for the entire examined area. The total defoliation in the area ranged only slightly above the average of the Czech Republic and the mean is 36.47 %. The values of the defoliation of the primary structure and the percentage of secondary shoots were average.

Tab. 1: The results of habitual diagnostics.

Plots		Total defoliation		Defoliation of the primary structure		Secondary shoots (%)		Degree of transformation	
No.	Stand	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
5 D16	184 A12	32.75	5.80	60.75	9.78	42.25	14.79	1.30	0.46
7 D18	178 A10	29.75	7.33	72.25	11.12	60.25	16.47	1.80	0.68
8 D19	197 A9	30.00	7.75	51.00	9.82	32.50	9.94	1.00	0.00
9 D20	184 D11	30.75	9.39	54.75	13.92	37.25	17.14	1.20	0.60
10 D21	184 C8	34.25	9.65	79.50	10.59	69.75	14.27	2.10	0.62
13 D23	196 C9	33.50	5.27	85.50	8.79	78.50	15.50	2.40	0.66

The trees in the research plots were classified into categories according to their stress response on the basis of their habitual diagnostics (Fig. 3). Nearly two thirds were classified as resistant, i.e. the internal tolerance of the tree had not been exceeded. Over a third of the trees were classified as damaged, slightly transformed, where the internal tolerance had been exceeded but the trees had not started to respond by the formation of a new assimilation apparatus. Four percent of the trees were damaged and heavily transformed. There were no resilient trees.

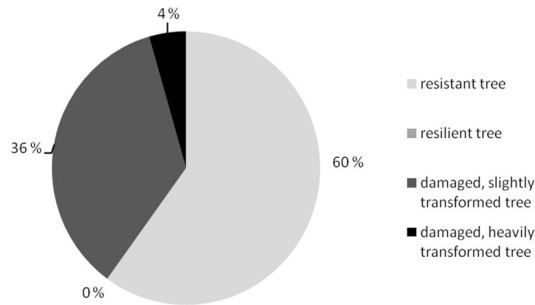


Fig. 3: The distribution of categories of tree stress response in the explored area.

### Sapwood area proportion

The values of the sapwood area proportion in the cross section at breast height gained from all sample trees in all research plots showed a normal distribution. The mean value of all 108 sample trees was 36.1 % of the cross section area. The minimum value was 4.7 %, the maximum 66.8 %.

One sample t-test proved that 95 % confidence interval for the true mean was from 33.89 % to 38.38 %. In other words, sapwood proportion for all Norway spruce in the Drahany highlands should be within the interval from which data do not deviate significantly. One-way analysis of variance of sapwood area proportion proved that there is no significant difference ( $p < 0.05$ ) in means between locations, which is also obvious in Fig. 4. The highest value of the arithmetic mean (42.7 %) was found in research plot D23; by contrast, the lowest mean value (33.3 %) was found in research plot D19. The highest span of non-outliers was found in research plot D21.

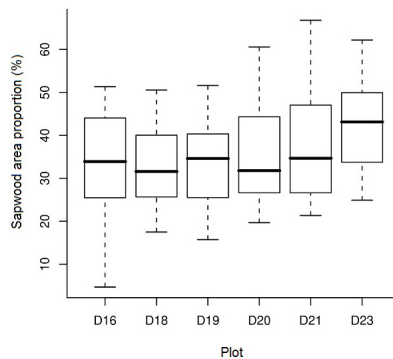


Fig. 4: The sapwood area proportion in the cross section at breast height for individual research plots.

Correlation analyses did not find any clear dependence between sapwood area proportion and the other examined parameters, which were the number of wood and phloem cells, the degree of crown defoliation and the percentage of secondary shoots.

### Xylem and phloem cell number analysis

The average number of dormant cambium ranged between 2 and 9 per a radial row.

In phloem, first the numbers of cells of early phloem and late phloem were counted separately. The number of cells in early phloem and late phloem was 0–5 and 1.5–8.5, respectively. With respect to the small range of the examined average number of cells from the five randomly chosen radial rows, for the further expression of potential growth capacities of phloem the total number of cells without any distinction between the early and the late layer was used.

Fig. 5 shows that the lowest mean number of phloem cells (5.2) was found in research plot D23, whereas the highest mean number was found in research plot D19 (6.6). At the same time, plot D19 also had the highest variability of the phloem cell number (28.7 %). The lowest variability was found in research plot D16 (13.9 %).

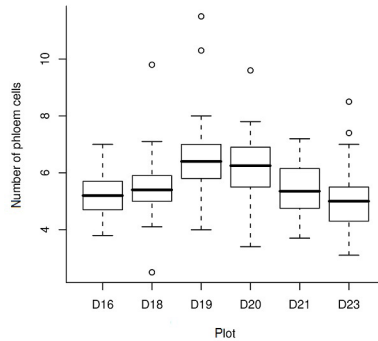


Fig. 5: Phloem cell number variability.

The average number of xylem cells in the latest formed tree ring ranged 8.8–148.8 in one radial row for different plots. The lowest average number of xylem cells was found in research plot D18 (29.8) and the highest in plot D23 (60.8), as is shown in Fig. 6. The lowest variability was found in plot D21 (32.9 %), the highest in plot D23 (50.9 %). The values measured within particular research plots and between sites did not manifest any trend.

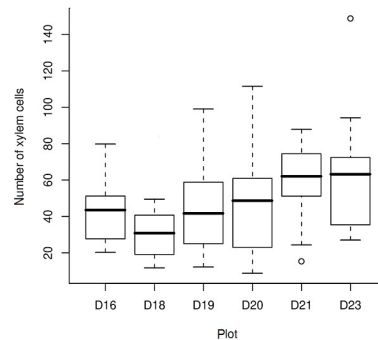


Fig. 6: Xylem cell number variability.

No clear correlation dependence among the monitored parameters (sapwood area proportion, crown defoliation, percentage of secondary shoot) was revealed.

For the indicator of the growth potential on the level of cells we chose the ratio between the number of phloem cells and xylem cells, which ranged from 1.5 to 30. The average values of this

ratio for particular research plots ranged between 5.7 and 12.6. The variability of the values of the xylem to phloem cell number ratio can be seen in Fig. 7.

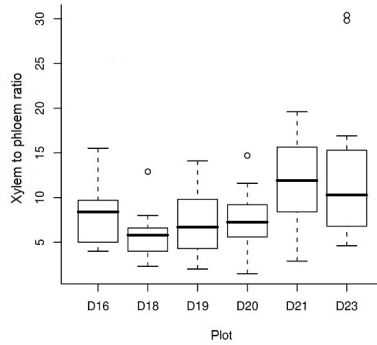


Fig. 7: The xylem to phloem cell number ratio for particular research plots.

The analysis of the dependence between the number of xylem and phloem cells and the sapwood area proportion (Fig. 8) revealed no significant dependence ( $R^2 = 0.08$ ). Neither the dependence between the mentioned xylem to phloem cell number ratio and the crown defoliation was found ( $R^2 = 0.02$ ) as seen in Fig. 9.

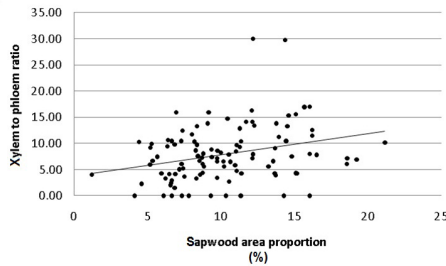


Fig. 8: The dependence between the number of xylem and phloem cells and the sapwood area proportion.

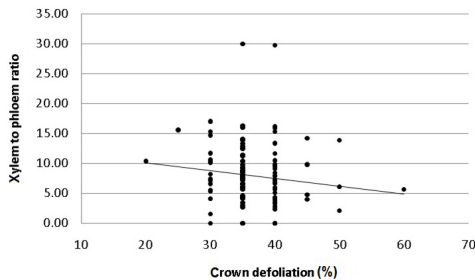


Fig. 9: The dependence between the xylem/phloem cell number ratio and the crown defoliation.



## DISCUSSION

### Sapwood area proportion

The analysis of the sapwood area proportion in the cross section at breast height for particular trees did not reveal any significant deviations, either within particular research plots or among research plots. No clear trend was found.

Sapwood is the physiologically active part of a growing tree stem, whereas ripewood (light-coloured heartwood) is physiologically dead (Panshin and de Zeeuw 1998). The assumption was that the decrease in sapwood area will result in decrease in sapflow and that the supply of water with dissolved minerals to the assimilation apparatus will decrease as well (Procházka et al. 1998), which could have an effect on the crown foliage. However, the dependence between the sapwood area and the morphological factors (the degree of defoliation, secondary shoots, etc.) was not confirmed. Maguire and Kanaskie (2002) stated that there is a relationship between live crown lengths and sapwood area. However, they analysed the live crown length and not the degree of defoliation. The defoliation is a quantity which is affected by a number of external and internal factors that enter the sapwood-foliage relationship and can have a synergic effect. Therefore, it is difficult to identify the effect of a single external factor – sapwood area.

### Xylem and phloem cell number analysis

The correlation between data such as the number of formed cells and the degree of crown defoliation or the percentage of secondary shoots was not revealed either. The initial hypothesis that the sapwood proportion and the ratio of the formed amount of cells can be used as an indicator of the state of health could not be confirmed. The revealed dependences between the number of formed cells and the amount of sapwood and the results of habitual diagnostics were not significant.

Similar research into the European silver fir (*Abies alba* Mill.) was conducted in Slovenia (Gričar et al. 2009) in 1999–2003. The research was focused on analysis of the numbers of cells of xylem, phloem and cambial zone in stands of various vitalities. The health state of the trees was assessed on the basis of the crown surface index (CSI) based on a progressive loss of the assimilation apparatus (a modified method according to Bosshard 1986) and the cambium electrical resistance – CER (Torelli et al. 1999). Trees were divided into three groups according to the ratio of phloem and xylem cell number. These three groups of data corresponded to a changing crown status index (CSI), which was statistically significant. Early phloem showed a relatively stable number of cells with a changing width of the total phloem increment but the late phloem representation was highly variable. The numbers of formed cells of phloem, xylem and the number of cambium cells correlate positively. In comparison to the Slovenian classification in the above mentioned study (Gričar et al. 2009), all the examined trees fall within one group only. There are three possible explanations:

#### 1) Less differentiated stands

It is supposed that considerably less differentiated stands, as regards the state of health, were chosen for our research. Although some differences between the stands were found during the habitual diagnostics (Tab. 1), these differences were not significant enough to get reflected in the stem anatomic structure.

#### 2) Delayed stem growth reaction

Tree vitality cannot be measured directly. Indicators, such as tree growth or crown transparency, may instead be used. Different indicators have different delay between stress factor occurrence and tree reaction. For example, defoliation by insects becomes first visible in crown

transparency while stem growth reaction occurs with delay. On the other hand, extreme summer drought as observed in large parts of Europe in 2003 affects stem growth almost immediately, while foliage reduction becomes only visible months later (Dobbertin 2005). It is supposed that that in our case stems growth reaction was delayed or stress factors were not so relevant to induce growth reaction.

### 3) Influence of stand structure

Matovič (1985) found out that dominant trees start with cambial activity sooner than co-dominant and subdominant trees. That is why a higher number of cells are formed in the dominant trees. However, we could not confirm this hypothesis as the research presented in this paper explored stands undifferentiated in height, which did not manifest any considerable deviation when examining differences at the measured heights. Based on this fact, a new hypothesis was formed: the anatomical structure of phloem, or the last ring of xylem, and the amount of sapwood may reflect the structure of the stand rather than its health condition. This could even reach to the issue of competition relationships.

## CONCLUSION

No dependence between the analysed parameters (numbers of cells, sapwood area, crown defoliation, percentage of secondary shoots) was revealed.

The suitability of the use of the cellular analysis of phloem and the xylem of the last ring and the analysis of sapwood proportion in the stem of Norway spruce for the indication of the state of health of woody plants was not confirmed in the study. However, the significance of the analysis of the xylem and phloem cell number is considered auxiliary for the data about the state of health, which had already been evaluated by methods used and proved in the forestry practice (habitual diagnostics). The results presented in this research provide information about the structure of anatomical elements in the mentioned conditions, which can lead to the deduction of physiological functionality of the woody plants in the mentioned conditions.

## ACKNOWLEDGMENT

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GABRIELA VICHROVÁ, HANUŠ VAVRČÍK  
MENDEL UNIVERSITY IN BRNO  
FACULTY OF FORESTRY AND WOOD TECHNOLOGY  
DEPARTMENT OF WOOD SCIENCE  
ZEMĚDĚLSKÁ 3, 613 00 BRNO  
CZECH REPUBLIC  
PHONE: +420545134313  
Corresponding author: xvichrov@node.mendelu.cz

TOMÁŠ ŽID  
MENDEL UNIVERSITY IN BRNO  
FACULTY OF FORESTRY AND WOOD TECHNOLOGY  
DEPARTMENT OF FOREST PROTECTION AND WILDLIFE MANAGEMENT  
ZEMĚDĚLSKÁ 3, 613 00 BRNO  
CZECH REPUBLIC