STUDY OF CONIFEROUS NEEDLES IN RELATION TO ENVIRONMENTAL FACTORS USING APPROACHES OF QUANTITATIVE ANATOMY AND LABORATORY SPECTROSCOPY.

Summary of Ph.D. Thesis

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INTRODUCTION

The essentiality of Earth’s vegetation as the most important primary producer of biomass, the base of the food chain and an important landscape-creating factor is generally acknowledged. In recent decades of constantly increasing CO₂ concentration in the atmosphere, a function of vegetation as a carbon sink is widely discussed. Particularly forests play an important role in the global carbon cycle by sequestering large amounts of atmospheric carbon, and are thought to offer a possible mitigation strategy to reduce global warming. Although in forest ecosystems the great amount of carbon is fixed in non-living biomass of wood, litter, or soil, the green photosynthesizing foliage represents the gate for the carbon, whatever metabolized by the plant itself or fixed into the biomass. As will be mentioned further in more detail, leaf structure is tightly connected with leaf function and vice versa. Not only from the point of view of the plant ecological physiology it is important to understand relationships between leaf structure, functions and environmental factors, to elucidate mechanisms that trigger and modify morphogenetic development and changes at an organ, cell or molecular levels. The knowledge of these mechanisms may help to understand the potential of an individual plant species or the whole ecosystems in carbon sequestration.

The present thesis is focused on conifer leaf anatomy in the context of leaf function under specific environmental conditions. As the main plant material, Norway spruce (Picea abies L. Karst), the most frequently planted tree species in the Czech Republic (53% of the forested area in the Czech Republic consisted of Norway spruce 2006, http://www.uhul.cz/zelenazprava/index.php) and in other countries in the Central and Northern Europe, was selected. In addition, the study was performed on two very common North-American conifers – red spruce (Picea rubens Sarg.) and balsam fir (Abies balsamea L. Mill.) in collaboration with American colleagues from the University of New Hampshire, Durham, U.S.

Norway spruce is a very sensitive species to atmospheric pollution, particularly to deleterious impact of acid rain (e.g. Smith 1990). During the second half of the 20th century loads of SO₂, NOₓ and ozone were extremely high in the Central Europe including the Czech Republic causing tremendous large-scale forest dieback, therefore the forest decline of Norway spruce was in the focus of extensive research in Europe (e.g. Smith 1990, Masuch 1992; Viskari et al. 2000; Soukupová et al. 2001). Alterations in needle anatomy caused by the effect of acid air pollution alone or in combination with harsh climatic conditions have been classified as so called „microscopical markers of forest damage“ and widely used by researchers including the team of Dr. Albrechtová from the Department of Plant Physiology, Faculty of Science, Charles University in Prague and their American collaborators from the University of New Hampshire (Albrechtová et al. 2001; Moss et al. 1998; Soukupová et al. 2001).

Despite that quantitative approach in description of inner leaf structure has quite a long tradition launched by Turrel in the 1930’s (Turrell, 1936), the majority of studies concerning forest decline described the anatomical alterations of leaves only qualitatively, for example, by the loss of integrity of chloroplasts (Moss et al. 1998). However in several cases quantitative evaluation of anatomical changes appeared (e.g. proportion of intercellular spaces in mesophyll (Kukkola et al. 2005). Nowadays, modeling of gas transport (Aalto and Juurola 2002; Juurola et al. 2005), radiative transfer accros the leaf (Ustin et al. 2001) in consequence with photosynthesis and carbon sequestration became the cardinal issue of plant ecophysiology and biophysics. Thus, there is a clear need for efficient and unbiased methods for quantitative description of important leaf anatomical characteristics, such as internal leaf surface area, mean mesophyll cell volume, and cell number in the leaf. Several methods for quantitative description of photosynthetic leaf tissue, the mesophyll, were established: e.g. model-based methods using correction shape factors (Oguchi et al. 2003) or image analysis approach (Niinemets et al. 2007). However, model-based methods for
estimation of geometrical parameters of mesophyll were found to be biased in some cases (Slaton and Smith 2002) and image analysis cannot be always applied. The recently developed methods, based on confocal microscopy and stereology were tested on different kind of plant samples (Kubínová and Janáček 1998; 2001) and appeared to be suitable for use in ecophysiological studies. Feasibility of application of confocal microscopy and stereological methods in purpose to evaluate impact of environmental stress on leaf anatomy was tested on young Norway spruce potted trees treated with simulated acid rain; the first paper included in this thesis is based on the results of this pilot study (Albrechtová et al. 2007, Paper I.). The quantitative studies on leaf internal structure always used sections of fixed and embedded plant material (e.g. Turrell, 1936; Niinemets et al. 2007) but during fixation or embedding the tissue deformation and other artifacts may occur (Uwins et al. 1993; Dorph-Petersen et al. 2001), moreover sample preparation is time consuming and laborious. In Albrechtová et al. (2007) thick sections of fresh needles were used for confocal microscopy. However, fresh material limits the method application in ecophysiological studies, as only few fresh samples can be processed immediately if at all under condition that the experimental plots could not be located too far from the place of analysis. The aim of the second paper included in this thesis is addressed to test feasibility of using frozen stored Norway spruce needles for confocal microscopy and stereological processing and further to deal with another technical problem resulting in biased measurements, caused by the possible distortions of cutting surfaces of transversely cut needle sections (Lhotáková et al. 2008, Paper II.).

Sharp increase of carbon dioxide atmospheric concentration over the last 150 years by app. 1.3 times (AR4 2007), caused mainly by anthropogenic carbon emissions, induces many demands on physiological and developmental changes in plants, which may have strong impact on future crop and wildland productivity, biogeochemical cycling, water resources and other natural processes at global scale. The direct responses of plants to elevated carbon dioxide concentration have been in focus of extensive research at least for two decades and wide spectrum of changes on the physiological level was described (rev. by Urban, 2003). Mostly reported primary physiological responses on photosynthetic level to carbon dioxide enrichment are activation of photosynthetic enzyme Rubisco, decrease of its oxygenase activity followed by the subsequent decrease of photorespiration, stimulation of net photosynthetic rate (Maier et al. 2008) or decreased stomatal conductance (Zhang et al. 2008a). These primary responses lead to the secondary effects like increased content of sucrose and other non-structural carbohydrates (Cabálková et al. 2007), changes in their allocation within the plant or dilution of mineral nutrients, particularly nitrogen, what often leads to acclimation – down-regulation of photosynthesis (Zhang et al. 2008a). However, the plant responses are quite variable depending on a species, age of the plant, mineral nutrition as well as duration and method of CO2 enrichment. The Paper III. included in this thesis focuses on evaluation of changes in photosynthesis (maximum assimilation rate and apparent quantum efficiency) and respiration together with structural acclimation to elevated CO2 in sun and shade needles of Norway spruce, while stereological methods adjusted previously in Papers I. and II. are therein applied in the field study (Lhotáková et al., submitted, Paper III.).

It is not only the elevated carbon dioxide concentration alone, which plants need to evolve acclimations to. Moreover, consequent forcing of atmospheric CO2 enrichment as a temperature rise or changed precipitation patterns and water regimes may fundamentally change species geographical distribution, ecosystems stability or even reduce biodiversity (Kleidon and Mooney 2000). Despite plants are able to develop a broad spectrum of adaptive responses to several environmental factors, they have not experienced such high CO2 concentrations as nowadays for over 20 million years (Pearson and Palmer 2000) and in consequence of rapid rise of CO2 concentration there are uncertainties whether genetic variation is sufficient to manage this shift in the few generations before CO2 concentration doubles or triples (Gutschick et al. 2003). In terrestrial ecosystems, photosynthesis plays a major role of coupling process between
carbon and nitrogen cycles. So does soil organic matter, its production and decomposition by soil microbial and fungal societies, which interconnect demands for nutrition resources by vegetation to pools of those resources available for plants (McDowell, 2003; Pritchard et al. 2008). Particularly in forests, nitrogen often stands as a limiting factor for tree productivity and thus influences the potential of carbon fixation. In several carbon dioxide enrichment experiments nitrogen availability modulated response of trees to elevated CO₂ (Eguchi et al. 2004; Eguchi et al. 2008; Maier et al. 2008), which only emphasizes the importance of elucidating plant–litter–soil interactions. Fluxes of carbon and nitrogen between soil and vegetation are often species-specific, even within one ecosystem or biome and may be influenced by human management (Ollinger et al. 2002). The knowledge of mechanisms, how vegetation and soil processes interconnect carbon and nitrogen cycles, is needed at least at regional scale, which could be further upscaled to continental or global scale. However, it would be extremely time and resource demanding to conduct such an ecosystem field study; therefore robust tools as remote sensing or ecosystem modeling seem to be more suitable.

Linkages among foliar chemistry, decomposition, nitrogen cycling, and productivity in forest ecosystems have been documented in literature frequently. Several specific indices and model-based methods derived from foliar reflectance airborne or spaceborne data have been developed and used for foliar chemistry (content of chlorophyll, lignin, nitrogen) estimation at regional scale (Ollinger et al. 2002; Serrano et al. 2002; Ollinger and Smith 2005; Zhang et al. 2008b). Leaf chlorophyll content is the main parameter determining leaf spectral variation in the visible regions, therefore spectral reflectance indices derived from chlorophyll absorbing wavelengths are considered as quite efficient, and sensitive non-destructive method of chlorophyll content assessment. However, those indices are usually developed for an individual species and in case of application on different species or a vegetation type, validation of such an index is needed. Recently, continuous reflectance data with high spectral resolution are used for vegetation monitoring. Similarly, spatial resolution of reflectance data is also very high (Malenovský et al. 2008) and in such a case one tree canopy is represented with several pixels in remote sensing data. The remote sensing approaches developed to estimate the leaf chlorophyll content had been based on the assumption that the leaf optical properties vary little with their location in the canopy. Considering possible physiological and structural variability in Norway spruce needles, a detailed study investigating spatial variability of the leaf optical properties within the tree crowns is required to confirm or reject this assumption. Paper IV. (Lhotáková et al. 2007, Paper IV.) is focused on assessment of variation in selected biochemical, structural and spectral parameters of Norway spruce needles within the upper sun-lit part of the canopy considering orientation of branches in different azimuth directions.

As mentioned above, foliar chemistry, which may be quite easily observable property across broad spatial scales by approach of high spectral resolution remote sensing, could serve as indicator of carbon and nitrogen cycling. The last paper included in this thesis is based on the study conducted on red spruce and balsam fir in New Hampshire focused on linking chlorophyll content in needles and amount of dissolved organic carbon and nitrogen in forest floor with spectral properties of foliage (Albrechtová et al. 2008, Paper V.).
OBJECTIVES AND HYPOTHESES:

1. To develop and adjust an efficient method based on confocal microscopy and stereology for detailed quantitative description of changes in geometrical parameters of coniferous mesophyll.
   Hypotheses:
   • Subtle changes in needle structure, elicited by environmental factors such as simulated acid rain, can be quantitatively characterized using selected sensitive stereological methods: fakir and disector.
   • Hand-cut thick sections of fresh and frozen stored needles are suitable for confocal microscopy and subsequent stereological analysis.
   • Selected geometrical parameters of mesophyll: proportion of intercellular spaces and internal surface density do not suffer from deformation by freezing during needle storage.

2. To characterize changes in Norway spruce mesophyll anatomy under impact of elevated CO₂ concentration, using methods developed and adjusted in Aim 1.
   Hypotheses:
   • Volume proportion of mesophyll in needles increases under impact of elevated CO₂ concentration.
   • Internal needle surface increases under impact of elevated CO₂ concentration.
   • Trees planted under impact of elevated CO₂ concentration will show less pronounced structural differences between sun and shade needle ecotype.

3. To characterize how needle position (azimuth orientation) influences selected biochemical, anatomical and spectral properties of needles.
   Hypothesis:
   • Selected biochemical, anatomical and spectral properties of sun-exposed Norway spruce needles from the upper part of tree crown are different according to branch azimuth orientation.

4. To examine potential links among selected biochemical and spectral properties of needles and water extractable dissolved organic C and N in forest floor of mixed coniferous forest in New Hampshire.
   Hypotheses:
   • There are links between foliage biochemistry and possibly chlorophyll content in Red spruce and Balsam fir needles and soil DOC and DON concentration.
   • These links could be detected by chlorophyll spectral reflectance indices.
RESULTS AND DISCUSSION

1 Geometrical parameters of Norway spruce mesophyll – quantitative assessment using confocal microscopy and stereology

Fresh sections of Norway spruce needles proved to be very suitable for confocal laser scanning microscopy even without any special staining - due to the autofluorescence of chloroplasts containing photosynthetic pigments and also phenolic compounds localized either in cell walls or in vacuoles (Albrechtová et al. 2008, Paper I.). For estimation of internal surface of mesophyll we used fakir method (Kubínová and Janáček 1998), which in contrary to other methods, does not require randomization of sectioning, because the method is based on application of digitally designed virtual 3D probes with random orientation (Figure 1). The sensitivity of selected geometrical parameters of mesophyll to the impact of environmental factors was tested using simulated acid rain, which is known to cause damage in the mesophyll with already described symptoms, such as a decrease in needle length and volume or an increase of the volume of the intercellular spaces (Masuch et al. 1992). We obtained quite consistent results, e.g. increase in the volume of intercellular spaces per mesophyll cell volume, significant decrease of needle volume, needle internal surface area, total number of mesophyll cells, and number of mesophyll cells per unit volume of a needle (Albrechtová et al. 2008, Paper I.).

Figure 1: Measurement of the internal surface area of Norway spruce needle mesophyll.

a) Fakir probe intersecting an isolated mesophyll cell. The red balls denote the intersection points between the test lines and the cell surface.

b) Individual sampling window in Ellipse software with fakir probes generated in random orientation. The procedure is shown in three subsequent optical sections. The first optical section is shown in c), the second one in d) and the third one in e). The test lines of the fakir probe piercing the mesophyll cells are white at the beginning of the measurement c). The centres of white squares denote the intersection points between the test lines and the current section. As soon as such a point comes into contact with the cell wall during focusing through (d), it is marked by a mouse and the square is filled with cyan (d); the part of the test line above this point also becomes cyan (d,e). One intersection point is marked here.
1.1 Frozen-stored needles can be used for stereological measurements – method adjustment

Using fresh material brings further constraints to application of proposed method in extensive ecophysiological field studies, because large number of samples is usually analysed and only few fresh needles can be processed simultaneously. The effect of freezing on selected geometrical parameters (volume of intercellular spaces in mesophyll and internal surface density) was negligible and therefore the possibility to use frozen-stored needle material proves to be very promising for further research involving field studies (Lhotáková et al. 2008, Paper II.).

The structural arrangement of coniferous mesophyll makes the avoidance of tearing marginal mesophyll regions during hand sectioning to be quite difficult if not impossible, which could bias stereological measurements. As the proportion of intercellular spaces in mesophyll may be overestimated in the marginal optical sections due to this effect, mesophyll geometrical parameters should be measured only in the middle region of optical section series of frozen-stored Norway spruce needles (Figure 2, Lhotáková et al. 2008, Paper II.).

Figure 2:
1.2 Importance of the systematic uniform random sampling in needle anatomy

The principle of histo-physiological gradients (Prat 1948) emphasizes importance of heterogeneity of anatomical parameters within plant or even individual organs, which is very important for sampling designs of anatomical experiments. A gradient in the cross-sectional area along the Norway spruce needle axis were shown in our study (Figure 3, Lhotáková et al. 2007, paper IV.), when parameters of cross-sections sampled in the middle part of the needle differed from those sampled at the tip and base, however area proportions of individual needle tissues remained constant. Similarly neither the proportion of intercellular spaces in mesophyll nor the internal surface density differed along the needle axis (Lhotáková et al., 2008, Paper II.). These findings are in agreement with measurement of Zwieniecki et al. (2006), who observed other needle parameters (needle circumference, stomatal density, ratio of xylem area to tracheid dimension) to be constant along 80% of the needle length when studying three pine species. The principle of systematic uniform random sampling of sections along the needle axis allows the inclusion of a potential gradient in anatomical characteristics within the needle.

Figure 2: Optical sections of Norway spruce needle acquired by confocal microscopy. Autofluorescence of phenolic acids bond in the cell walls in green, autofluorescence of chlorophyll in red. (A) Transverse section of Norway spruce needle. Mesophyll tissue highlighted, IC = intercellular spaces, white arrows show the cell surface exposed to intercellular spaces which was measured as internal needle surface. (B) Longitudinal needle section. (C) Detail of the mesophyll cell in the transverse section. Note the irregularity of the cell shape. (D) Detail of the mesophyll cell in the longitudinal section. E) Proportion of intercellular spaces in mesophyll measured in optical sections at different depths of the confocal stack. Numbers on x-axis correspond to the order of the optical section within the 40-µm-thick stack of optical sections. Bars refer to standard deviations, different letters show significant differences based on one-way ANOVA. There is no significant difference between fresh and frozen needles, based on paired t-test, α = 0.05. (Lhotáková et al. 2008, Paper II.)
2 Mesophyll structure of Norway spruce needles under long-term impact of elevated CO₂ concentration

Internal leaf structure, especially geometrical parameters of mesophyll and chloroplast arrangement, influences the interception of radiation and diffusion of carbon dioxide within the leaf, which are both essential for photosynthesis process (Pandey and Kushwaha 2005). Several environmental factors such as irradiance, surrounding carbon dioxide concentration, temperature, and water availability or air pollution are known to affect both, leaf structure and function. It is important to keep in mind that environmental factors usually interact between each other and foliage is often exposed to simultaneous light, heat and water stresses (Niinemets, 2007).

Recent studies show that relationships between leaf anatomical parameters and photosynthesis are important in leaf acclimation to high or low irradiances (Pandey and Kushwaha 2005; Oguchi et al. 2005) or elevated CO₂ concentrations (Eguchi et al. 2004). We recorded the increase of net CO₂ assimilation rates for Norway spruce even after 8 years of CO₂ enrichment (Lhotáková et al. submitted, Paper III.) and this phenomenon was confirmed also in the long-term FACE studies with Loblolly pine (Crous et al. 2008; Maier et al. 2008).

Findings of Eguchi et al. (2004) suggest, that nitrogen supply modulates mesophyll cell response to elevated [CO₂] in the sense of different impact on cell division and cell expansion. The character of changes in Aₘₑₙ/A in Japanese larch (Larix kaempferi Carr.) needles under the impact of elevated [CO₂] have been observed to be strikingly different (opposite) depending on nitrogen supply (Eguchi et al. 2004). The authors attributed changes in Aₘₑₙ/A to changes in mesophyll cell size and mesophyll cell number: The cell size increased but mesophyll cell number decreased in high nitrogen supply, while under low nitrogen supply a higher number of smaller cells was observed.

In our study on Norway spruce, the trees were not suffering from nitrogen or any nutrient shortage; therefore we rather expected internal needle surface to increase. Nevertheless, we found all selected structural parameters (needle volume, internal needle surface, internal needle surface density and volume ratios of individual tissues) unchanged under elevated CO₂ (Figure 4, Lhotáková et al. submitted, Paper III.).

Previous investigations of photosynthetic characteristics (Hrstka et al. 2005; Marek et al. 2002) conducted on the same trees as those involved in the present study, revealed that the shade needles displayed a stronger stimulation of assimilatory activity than sun needles. This finding led us to the hypothesis that shade needles could benefit more from increased [CO₂] and that their anatomical structure might be changed more than that of sun needles. However, all measured anatomical parameters displayed the similar differences between sun and shade needles, irrespective of [CO₂]. Therefore we suggested that irradiance is the more important factor, which drives structure differentiation in contrast to enriched CO₂ concentration in the atmosphere (Lhotáková et al. submitted, Paper III.).

Figure 3: Sampling design of needle specimen preparation. (A) Systematic uniform random sampling of transverse free-hand sections: \( z = \) random position of the first section within \( (0; T_f) \), where \( T = 2 \text{ mm} \). Positions of transverse sections along the needle longitudinal axis are denoted by a, b, c, d, e, f. (B) 2-mm-thick needle segment. (C) 80-µm-thick free-hand section from which the 40µm thick stack of optical sections was acquired. (D) Stack of 40 optical sections 1µm apart, lines refer to optical sections 5µm apart, where proportion of intercellular spaces in mesophyll was measured. (Lhotáková et al. 2008, Paper II.)
Figure 4: Comparison of anatomical parameters of sun and shade Norway spruce needles in 2004. In the left part of the figure data are sorted according to treatment. In the right part of the figure, the data are sorted according to treatment and needle ecotype. a, b) Internal surface density of mesophyll; c, d) Needle volume; e, f) Internal surface area of mesophyll. The measurements were done on the sun and shade needles of the trees cultivated at the control open-air site (Control) and inside the glass domes with ambient (Ambient; 365 µmol mol⁻¹) and elevated (Elevated; 700 µmol mol⁻¹) CO₂ concentrations. Mean values (columns) and standard errors (bars) are presented, (n = 5). Different letters above columns show significant differences, p<0.05. Lhotáková et al. submitted, Paper III.
3  **Heterogeneity in leaf biochemistry and related optical properties within the tree crown**

Biochemical composition of leaves, e.g. concentration of photosynthetic pigments (Tzvetkova et al. 2006) and leaf structure (Nakatani et al. 2004; Albrechtová et al. 2007) are often used as indicators of the actual plant physiological status, therefore the plant optical properties, particularly reflectance, were recognized as suitable non-invasive technique for monitoring of plant stress occurrence (Horler et al. 1980). For monitoring physiological status of vegetation, particularly of forest stands, hyperspectral data from air-borne sensors provide high spatial resolution (1m, Moorthy et al. 2008), or less (Malenovský et al. 2008). Then an individual tree crown is represented with several pixels of spectral image and a potential heterogeneity in leaf optical properties should be taken into account for proper interpretation and verification of remote sensing data.

In evergreen species, particularly conifers, the influence of leaf age on biochemical composition, internal structure and related optical properties or reflectance spectra derived indices are known (e.g. Rock et al. 1988, 1994; Soukupová et al. 2001). In our study relationships between optical spectral indices, computed from shoot reflectance, and needle chlorophyll content stronger correlation was shown when analyzing younger needles (youngest three age classes against two oldest) (Albrechtová et. al. 2008, Paper V.). Contribution of bottom shaded parts of the tree crown to the top-of-canopy reflectance is usually negligible, but the information about potential horizontal variability of leaf optical properties within the sun-lit crown part is crucial for correct interpretation of remote sensing data using very high spatial resolution. We tested the hypothesis that branch azimuth orientation influence selected biochemical, structural and spectral properties of Norway spruce needles within the sun-lit the upper crown part and no such a heterogeneity was revealed (Lhotáková et al. 2007, Paper IV.). Similarly Zhang et al. (unpublished) observed only insignificant difference in chlorophyll content of black spruce needles from differently oriented branches. These findings support the assumption that information obtained from the sunlit upper part of a coniferous tree crown can be used as ground truth or calibration input in remote sensing techniques designed for forest monitoring.

4  **Relationships between forest floor chemistry, needle chlorophyll content and needle spectral properties**

Dissolved organic matter particularly carbon and nitrogen (DOC and DON) play central role in nitrogen and carbon cycles in forest ecosystem (McDowell, 2003) and also in ecosystem processes such as pedogenesis (Dawson et al. 1978) or microbial metabolism (Yano et al. 1998). Previous studies have shown that forest floor DOC production is related to forest floor C:N ratio (e.g. Aitkenhead et al. 2000), which could be predicted from foliar chemistry, particularly lignin:N ratio (Ollinger et al. 2002; Aitkenhead-Peterson et al. 2006). Much less is known about forest floor DON and it seems that DON production is in many ways decoupled from DOC production (e.g. (McDowell et al. 2004). We focused on relationships between forest floor water-extractable DOC and DON and needle chlorophyll content of two common Northern American coniferous species (red spruce and balsam fir) (Albrechtová et al. 2008, Paper V.). Foliar chlorophyll content is closely related to leaf nitrogen content, it represents well established parameter reflecting current physiological status of a plant (e.g. Larcher 2003) and it is often used as indicator of forest health (e.g. Abrechrová et al. 2001; Petkovsek et al. 2008). As chlorophyll could be monitored using reflectance-based remote sensing techniques (Huber et al. 2008; Moorthy et al 2008; Zhang et al. 2008b). At the lever of laboratory reflectance measurements we revealed correlations between forest floor DOC and DON and selected chlorophyll-related optical indices (TCARI/OSAVI, ANMB650–725 and Chl NDI) for balsam fir and red spruce foliage (Figure 5 and 6, Albrechtová et al. 2008, Paper V.). However, it would be necessary to verify potential of those relationships in large scale monitoring of DOC and DON dynamics needs to be verified as well for other forest tree species and air-born spectral data.
Figure 5: Relationships between mean forest floor water-extractable dissolved organic carbon WEDOC (mg C/g soil) and mean chlorophyll content for balsam fir (open diamonds) and red spruce (filled diamonds) needles of different age. Data points represent one site. In case of (a) a mean value for the three youngest needle age classes was processed per one sampled tree, while in (b) a mean value for the two oldest needles age classes was processed. a, b: n=6 trees per site. Regression equation is given, relationships are significant at *p<0.05, **p<0.01 and or otherwise non-significant (Albrechtová et al. 2008, Paper V.).

Figure 6: Relationships between TCARI/OSAVI chlorophyll index and (a, b) chlorophyll (mg/g d.w.) and (c, d) mean forest floor water-extractable dissolved organic carbon WEDOC (mg C/g soil) for Balsam fir (open diamonds) and red spruce (filled diamonds) needles of different age. Data points represent one site. In case of (a, c) a mean value for the three youngest needle age classes was processed per one sampled tree, (b, d) a mean value for two oldest needles age classes was processed. n=6 trees per site, regression equation is given, relationships are significant at *p<0.05, **p<0.01 and or otherwise non-significant. (Albrechtová et al. 2008, Paper V.).
SYNTHESIS – CONCLUSIONS:

This thesis combines two methodical approaches at different hierarchical levels of plant ecophysiology, which both are applied in field research and bring new valuable information for comprehension of several aspects of carbon cycle in forest ecosystem, particularly coniferous.

The quantitative anatomy represents the first approach. At the leaf level application of confocal microscopy on fresh and frozen-stored needles and subsequent stereological estimation of mesophyll geometrical parameters proved to be suitable and sensitive enough for estimation of changes in mesophyll after simulated acid rain or differentiation of sun and shade needle ecotype. Application of systematic uniform random sampling of sections along the needle axis revealed insight to heterogeneity of mesophyll geometrical parameters within the needle and confirmed representativeness of the sampling from the middle region of the spruce needles.

The second approach, laboratory measurement of shoot reflectance, represents important step in verification of relationships between leaf biochemical and optical properties, which can be subsequently used for monitoring of forest health, foliar or soil chemistry using remote sensing techniques. At the level of Norway spruce crown, samples of even-aged needles from the upper sunlit crown part proved to be representative irrespective of branch azimuth orientation regarding biochemical, structural and spectral parameters. And selected chlorophyll content related spectral indices revealed to be potential indicators of forest floor dissolved organic carbon and nitrogen pools in mixed coniferous forest.

According to the determined aims and tested hypotheses, the following conclusions were drawn:

1. Thick free-hand sections of fresh and frozen needles proved to be suitable for estimation of important mesophyll photosynthesis-related geometrical parameters using contemporary unbiased stereological methods (fakir, dissector) in combination with confocal microscopy image acquisition.
   - Selected stereological methods, fakir and dissector, revealed to be sensitive for quantitative description of changes in Norway spruce mesophyll structure induced with simulated acid rain treatment.
   - Selected geometrical parameters of mesophyll: proportion of intercellular spaces and internal surface density did not suffer from deformation by freezing during needle storage. However, stereological measurements should avoid regions of the physical sections near to cut planes.
2. The detailed study of mesophyll parameters with unbiased stereological estimators proved that an observed increase in assimilation rates in both sun and shade Norway spruce needles after eight-year exposure to elevated CO₂ concentration was not connected with structural changes.

- Neither volume proportions of individual needle tissues nor internal surface of mesophyll changed under impact of elevated CO₂ concentration.
- Since sun and shade needle ecotypes exhibited the same pattern of differences in needle anatomy irrespective of the CO₂ enrichment, it appears that irradiance might be a higher priority environmental factor for leaf structure differentiation than an enriched CO₂ concentration in the atmosphere.

3. Selected biochemical, anatomical and spectral properties of Norway spruce needles revealed to be independent from branch azimuth orientation.

- Non-varying structural parameters, content of photosynthetic pigments and soluble phenolic compounds and selected optical vegetation indices derived from even-aged needles from branches of the four azimuth orientations suggest that the entire upper sunlit production part of the crown of Norway spruce canopy is representative in support of remote sensing studies.

4. Relationships between chlorophyll content in needles of red spruce and balsam fir and forest floor water-extractable dissolved organic C and N were revealed, which may be monitored by remote sensing techniques via selected chlorophyll-related optical indices.

- The mechanisms underlying negative correlations between needle chlorophyll content and forest floor water extractable DOC and DON concentration remain uncertain. However, the relationships were stronger for young and more physiologically active foliage rather than older senescent needles, which could indicate that the direction in the response–effect relationships is from soil to plant.
- Selected chlorophyll-related optical indices (ChlNDI, TCARI/OSAVI and ANMB650–725) showed potential for monitoring forest floor water-extractable DOC and DON via those relationships with chlorophyll content in needles. From the standpoint of remote sensing studies, stronger relationships between forest floor water-extractable DOC and DON and chlorophyll content in younger needles are encouraging, because young physiologically active foliage occupies the upper crowns of coniferous trees, which contributes as the main source to canopy reflectance signal.
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Affiliation and Address:
Department of Plant Physiology
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Education:
1991 – 1998 Grammar school Ústavní, Prague
1998 Graduation

1998 – 2004 Charles University in Prague, Faculty of Science, Department of Plant Physiology
2004 Master degree: Mgr.
Master thesis: „Heterogeneity of selected physiological and structural parameters of Norway spruce needles within the tree crown and application of these parameters on the physiological tree status assessment.“

2004 - Ph.D. study, Charles University in Prague, Faculty of Science, Department of Plant Physiology
Ph.D. thesis: „Study Of Coniferous Needles In Relation To Environmental Factors Using Approaches Of Quantitative Anatomy And Laboratory Spectroscopy“

Work experience:
2001 - team of Dr. Albrechtová (The Laboratory of Ecophysiological and Developmental Anatomy), field research in mountain coniferous forest ecosystems, biochemical, anatomical and spectral analyses of coniferous foliage
2005 one-month research stay at the University of New Hampshire, Department of Natural Resources in laboratory of W.H. McDowell
2005 - employment as researcher at the Department of Plant Physiology, Faculty of Science, Charles University in Prague
2005 - employment as researcher at the Department of Biomathematics, Institute of Physiology, Academy of Sciences of the Czech Republic

Teaching:
2005 – 2008 Practical training to the course Plant Anatomy and Morphology
2005 – 2007 Practical training of stereological methods in the course „Image acquisition and processing in microscopy“ organized by the Czechoslovakian Microscopy Society
2007 Practical training to the course Quantitative Plant Anatomy
2007 – 2008 Lectures for high school students and teachers for GLOBE pilot schools

Supervision:
Other scientific activities:

2007 – 2008   Member of International Society for Stereology

Additional scientific qualification:
12. – 16. 11. 2007   Image acquisition and processing in microscopy

Participation in grant projects:

2003 – 2006   Kontakt: ME 658 / NSF: DEB 108385, Czech American project titled "Linking Stream Chemistry to Soils and Foliar Chemistry Along a Pollution Gradient in the Czech Republic". The project was funded in 2003-2006 by NSF (National Science Foundation) on American part and by the Ministry of Education, Youth and Sports of the Czech Republic on the Czech part.


PUBLICATIONS

Papers in journals with impact factor:


Paper in web journal without impact factor:


http://las.physik.uni-oldenburg.de/eProceedings/vol05_2/05_2_malenovsky1.html

* Publication is included in PhD Thesis
Oral presentations:


Lhotáková Z. 2008 Detection of changes in mesophyll structure using confocal microscopy and stereology: application on Norway spruce needles treated with elevated CO2. Conference of PhD students Fresh Insights in Plant Affairs, Nové Hrady, Czech Republic. Award for the best presentation of young scientists.

Selected poster presentations:


Lhotáková Z, Albrechtová J, Kubínová L. 2006 Anatomical study of Norway spruce needles exposed to ambient and elevated CO2 – introduction of confocal stereology. 15 years of EU supported ecophysiological research in the Czech Republic, Olomouc, Czech Republic.
