

Gradients of the content of photosynthetic pigments and radiation as manifestations of the health condition of Norway spruce (*Picea abies* [L.] Karst.)

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ABSTRACT: The paper deals with the health condition of sample trees of Norway spruce (*Picea abies* [L.] Karst.) on the basis of the distribution and quantity of chlorophyll *a + b* and carotenoids with respect to the gradient of radiation in the tree crown. The content of photosynthetic pigments (PhP) was determined by spectrophotometric analysis. The sample tree is situated at the Rájec nad Svitavou locality, the Drahaný Upland, altitude 625 to 640 m. Within the gradient of the content of PhP in the vertical and horizontal profile of a tree, it was shown that the inner coordination of the content of PhP in the crown in relation to the age of needles and their insolation was sufficient. Thus, the tree does not show impaired health condition and its growth retardation results from the short crown. The extent of a photosynthetic apparatus and stability of the tree would be increased particularly after elongation of the lower part of a crown, so-called compensating part.

Keywords: chlorophyll; carotenoids; radiation transmittance; health condition; *Picea abies* (L.) Karst.

Methods which can be used for assessing changes of the complex of factors of the environment by means of the response of living organisms (most often plants) are named bioindication methods. Sensitive plant species typical of the given ecosystem are passive bioindicators.

As for forest trees namely conifers, it is Norway spruce (*Picea abies* [L.] Karst.) which is used as a sensitive passive bioindicator in Europe. Based on the rate and character of its response it is possible to assess its health condition. In practice, the fact is monitored by two bioindication methods, viz. terrestrial survey and remote sensing. In terrestrial survey, within non-destructive methods one of the parameters of health conditions is also the colour of foliage.

A spectrophotometric method used for the determination of the content of photosynthetic pigments (PhP) in leaves is an accurate destruction method of terrestrial survey. It refers to a quantitative method of the determination of the content of substances in detached leaves or needles. Absorption curves can be evaluated in detail by the method of derivation spectrophotometry according to FRENCH (1957), MARTIN (1959) and MEISTER (1966) in HASPELOVÁ-HORVATOVIČOVÁ (1981) in Norway spruce and in bristlecone pine according to MARTINKOVÁ et al. (1996) etc.

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in Brno, considerable attention is paid to problems of the dynamics of the concentration of PhP in the vertical and horizontal profile of a Norway spruce crown and other species already for a long time: DOBEŠ (1983), NÁROVEC (1985), JUPA (1987), MARTINKOVÁ (1990, 1992), BARTANUZS (1992), MARTINKOVÁ et al. (1996), STUHLÍK (1997), VARGA (1997), DOBEŠ (2001), BAŘINKA (2002).

An objective of the paper was to determine vitality of the Norway spruce (*Picea abies* [L.] Karst.) sample tree on the basis of distribution and quantity of foliage pigments with respect to the gradient of solar radiation in the tree crown (CURRAN et al. 1990) and by means of the relationships to assess its health condition.

MATERIAL AND METHODS

Characteristics of the locality

Rájec nad Svitavou locality is situated in the Drahaný Upland near Rájec nad Svitavou and NĚMČICE villages (49°26'37"N, 16°42'1"E). It is located on a slight slope (5% inclination) at an altitude of 625 to 640 m. Basic climatic characteristics for the period 1980 to 2001 are as follows: mean annual temperature 6.4°C, mean total of precipitation 647 mm, mean total of precipitation in the growing season 419 mm.

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Material, taking and processing the samples

Material used

A sample tree of Norway spruce (*Picea abies* [L.] Karst.) was selected the crown of which was attainable from a gradient biometeorological tower. The sample tree age was 92 years, total height 25.4 m, diameter at breast height (d.b.h.) 23.7 cm, crown height 19 m, crown length 6.4 m, number of whorls 28. According to the Kraft classification of tree classes (KORPEL et al. 1991) it referred to a partly co-dominant tree achieving the medium stage of maturity from the ontogenetic phase of development.

Taking and processing the samples

Branch sampling from the sample tree was carried out by a single application, namely in spring before budbreak (7 May 1999) under uniform weather and regular cloud cover. Sampling and measurements in the locality were carried out from the gradient biometeorological tower.

In total, four branches of the 1st order with NW orientation were taken from the crown vertical profile: from the 7th whorl – numbered from the tree apex (i.e. 14.0% crown depth), from the 11th whorl (i.e. 28.1% crown depth), from the 17th whorl (i.e. 50.0% crown depth) and from the 25th whorl (i.e. 86.7% crown depth).

In the locality, branches were roughly washed by distilled water, placed into PE bags and transported to a laboratory. In the laboratory, samples of needles were taken from the sample tree branches from selected annual growth modules to determine the content of photosynthetic pigments (PhP) and dry matter (DM). The area of an average leaf was determined using the method of computer image analysis in the Lucia G system (Laboratory Imaging Ltd., Prague).

Before the branch sampling, the vertical permeability of radiation was measured within the sample tree (by means of two ALAI-2V radiometers). During the measurement, the amount of incident and diffused radiation was recorded by the first radiometer on the level of particular storeys of the gradient biometeorological tower (the distance of particular storeys was 3 m). In the same time intervals, the second radiometer measured the amount of incident and diffused radiation at the tower foot. The measurement occurred during midday hours of summer time. Reference measurements of an unshaded area was carried out at a height of 3 m above the stand (LARCHER 1995). Based on measured data, relative insolation was determined in particular places of the crown where samples of shoots were taken.

Methods of the determination of PhP

The content of chlorophyll *a*, *b* and of the total amount of carotenoids was determined using a threewave spectrophotometric method (ŠESTÁK et al. 1971) and the second derivation of absorption curves – automatic output from a HP 8453 spectrophotometer (DOBEŠ 1983; MARTINKOVÁ et al. 1996). Conversion equations were used according to LICHTENTHALER and WELLBURN (1983). Always two

series of fresh needles were taken from space- and age-defined shoots. Both series were saturated with water (in a refrigerator for a period of 24 hours). One series was used to determine the content of chlorophylls and carotenoids, the second one became a basis for the determination of water content under fully saturated condition and DM.

The content of PhP (chlorophyll *a*, *b*, *a + b* and carotenoids) was expressed per the weight unit of leaves of each of the samples in mg/g, per an average shoot in mg, per an average leave in µg and per the leaf area unit in µg/cm².

Methods of the statistical evaluation of data

A relation between the content of PhP in needles or shoots and insolation in the given space of a crown was determined using correlation analysis in the Microsoft Excel program. The correlation coefficient was interpreted as follows:

$r = 0$ expresses independence;

$0 < r < 0.30$ expresses low degree of dependence;

$0.31 < r < 0.50$ expresses medium degree of dependence;

$0.51 < r < 0.70$ expresses marked degree of dependence;

$0.71 < r < 0.90$ expresses high degree of dependence;

$0.91 < r < 1$ expresses very high degree of dependence and

$r = 1$ expresses strong dependence.

Positive values of the correlation coefficient express direct dependence, negative values express indirect dependence (MELOUN, MILITKÝ 1994).

RESULTS AND DISCUSSION

The range of values of the content of PhP in the sample tree crown is described in Table 1.

Expression of the content of PhP per DM unit of needles

Mean values of the content of chlorophyll *a + b* in the first three year-classes in needles from the apical and basal part of the crown amounted to 1.85 and 2.13 mg. In relation to the apical part of the crown increase by 15% occurred in its basal part. MARTINKOVÁ (1992) mentions (for the same locality) increase in the content of chlorophyll *a + b* with respect to the apical part of the crown in a subdominant tree by 9% (apical part of the crown 1.71 mg, basal part of the crown 1.87 mg) and in a dominant tree by 29% (apical part of the crown 1.24 mg, basal part of the crown 1.61 mg). In a 75-year-old sample tree, KOCH (1976) mentions values of the range of chlorophyll *a + b* content from 1.9 mg in the youngest sun needles to 4.7 mg for 4 to 5-year-old shade needles. Older shade needles contained by 247% more chlorophyll *a + b* than the youngest sun needles. In case of the sample tree studied by our team the difference amounted to 359%. Thus, the gradient of the chlorophyll *a + b* content was even steeper.

As for absolute values, values measured by our team did not reach those which were found by NÁROVEC (1985) at the same locality from a comparable sample tree in 1982

Table 1. The range of values of the content of PhP in the tree crown

Method of expression of the content of PhP	Apex		Basis	
	value/needle-year		value/needle-year	
	chlorophyll <i>a + b</i>	carotenoids	chlorophyll <i>a + b</i>	carotenoids
Per 1 g DM (mg)	1.00/1	0.28/1	3.59/7	0.61/7
Per cm ² of needle (µg)	–	8.58/2	19.09/3–52.17/7	4.27/3
Per an average needle (µg)	16.01/6	2.50/6	2.38/1	0.50/1
Per an average shoot (mg)	51.76/1	12.68/1	0.44/9	0.08/9

– chlorophyll *a + b* content: apex 2.2–2.4 mg/g, basis 3.8–4.0 mg/g DM (dry matter). It means that in the course of about 17 years, a marked decrease occurred in the potential of accumulation of PhP. Even higher values were determined under optimum conditions for the growth of spruce in the Beskids in 1999. In the first needle year-class, the range of the chlorophyll *a + b* content amounted to 1.7–2.35 mg/g. The highest values were measured in the 4th needle year-class (3.1–4.77 mg/g DM). The lowest values in the vertical profile of the tree crown were found in the region of Bílý Kříž (the Beskids) in 1990, i.e. in the period of negative effects of air pollution. In this year, the content of chlorophyll *a + b* ranged from 0.79 to 3.00 mg/g DM.

In the horizontal profile (basal part of the tree crown), an increasing trend was noticed from the youngest to older needle year-classes, in needle generations older than 9 years, gradual decrease occurred in the chlorophyll *a + b* content (Fig. 1). Differences in the chlorophyll *a + b* content between particular needle year-classes in the horizontal level (within a branch) were greatest in the apical part of the crown and towards the tree base they decreased. In the basal part of the tree crown, MARTINKOVÁ (1992) noticed decreasing trend in the chlorophyll *a + b* content already from the 5th needle-year.

In the basal part of the crown, the trend of changes showed more markedly (e.g. decrease in the chlorophyll

a + b content already in 3-year-old needles) in needle generations from the peripheral (distal) part of the crown than in those from the inner (proximal) part of the crown.

In the apical part of the crown, increase in the content of carotenoids was noticed from the youngest to older needle generations, in the central and basal part of the crown, however, rather equalization or slight decrease in the content of carotenoids occurred during ageing. The fact corresponds to trends determined already previously (KRPEŠ 2002).

Other trends in the content of carotenoids in the sample tree crown can be characterized similarly as in the content of chlorophyll *a + b*.

Expression of the content of PhP per cm² of needle area

Within the vertical profile of the crown, decrease in the content of chlorophyll *a + b* occurred from the apical to the central part of the tree crown; in the basal part of the crown, the decrease was stopped. In the horizontal profile, an increasing trend was noticed from the youngest to older needle generations.

In carotenoids, it is possible to characterize possible trends in their content in the vertical and horizontal profile of the crown similarly as in chlorophyll *a + b*.

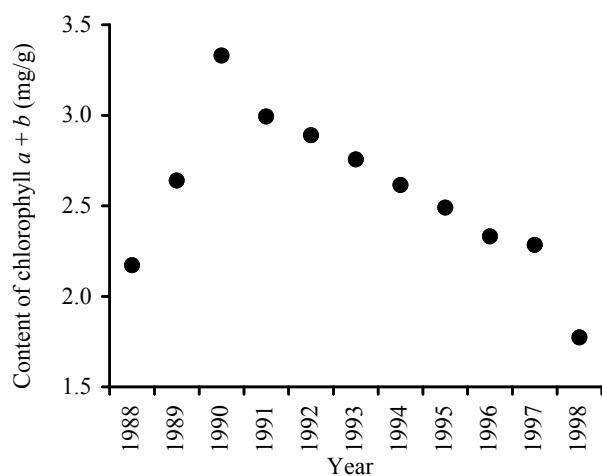


Fig. 1. The content of chlorophyll *a + b* (per needle dry matter) within particular needle year-classes in the basal part of the sample tree crown

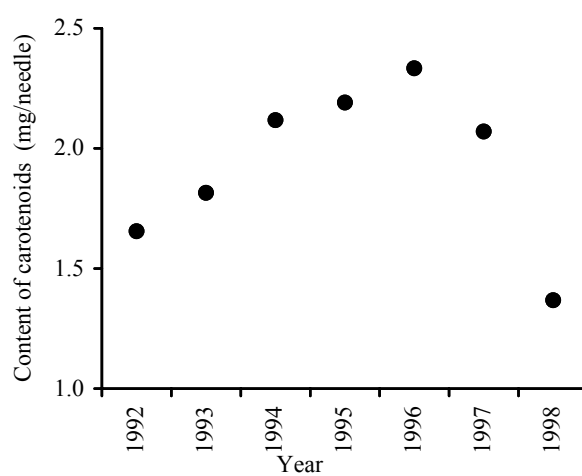


Fig. 2. The content of caretonoids (per an average needle) within particular needle year-classes in the apical part of the sample tree crown

Expression of the content of PhP per an average needle

Within the crown vertical profile, the chlorophyll *a + b* content decreased from the apical to the basal part of the crown as expressed per an average needle, i.e. the trend was similar as in expression per leaf area unit. In the apical part of the sample tree crown, the decrease was more marked. In the horizontal profile, an increasing trend was noticed from the youngest to older needle year-classes, in needle generations older than 9 years (i.e. from 1990), gradual decrease in its content occurred. Differences in the content of chlorophyll *a + b* between particular needle generations were more marked in the distal part of the crown.

In carotenoids, decrease in the content occurred within the vertical profile of the crown from the apical part to the central part of the crown; in the basal part of the crown, the changes were not marked and the content of carotenoids was balanced. In the horizontal profile, increase in the content of carotenoids was noticed in the distal part of the crown from the youngest to older needle generations up to three-year-old needles (i.e. differentiated in 1996); then, a gradual decrease followed (Fig. 2). Towards the proximal part of the crown, a slight increase in the content of carotenoids occurred in younger needle generations whereas in older needle year-classes, equalization or slight decrease in the content occurred.

Other trends in the content of carotenoids in the sample tree crown can be characterized similarly as in chlorophyll *a + b*.

Expression of the content of PhP per an average shoot

In the horizontal profile, decreasing trend was noticed in chlorophyll *a + b* from the youngest to older needle-years.

In carotenoids, trends in their content in the horizontal profile of the crown can be characterized similarly as in chlorophyll *a + b*.

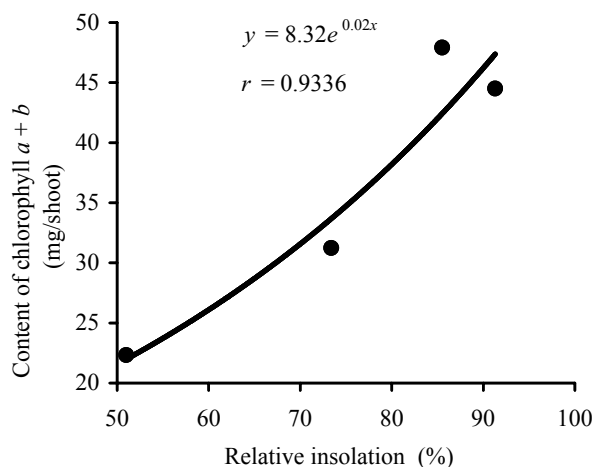


Fig. 3. Dependence of the chlorophyll *a + b* content (per an average shoot) in relative insolation

Relationships between the content of PhP and relative insolation

Between the content of PhP and relative insolation in the vertical profile of the crown, interrelationships were found described for example by LARCHER (1995). The degree of the interrelationships differed in relation to the expression of PhP. Very high degree of the relationship was obtained in expressing the PhP content per an average shoot, namely both in chlorophyll *a + b* ($r = 0.93$) and carotenoids ($r = 0.91$), it referred to direct dependence (Fig. 3). High degree of the relationship was achieved in expressing the PhP content per an average needle (Fig. 4); it referred to direct dependence and the degree of the relationship was comparable both for chlorophyll *a + b* ($r = 0.84$) and for carotenoids ($r = 0.81$).

In expressing PhP per cm^2 of the needle area and DM unit, high degree of the relation was demonstrated ($r = 0.82$) but only for the youngest needle year-classes (1998, 1997); older needle generations provided only low degree of the relation ($r = 0.21$). In expressing PhP per the needle area unit, it referred to direct dependence; in expressing PhP per DM unit it referred to indirect dependence.

CONCLUSION

In expressing PhP per the area unit, average needle and average shoot decrease in their content occurred from the apical to the basal part of the sample tree crown. In expressing PhP per DM unit, the situation was converse.

In the vertical profile of the tree crown, an increase in the content of pigments occurred from the youngest to the oldest needle year-classes in expressing PhP per DM unit, per area unit and per an average needle. In expressing PhP per an average shoot the situation was converse.

The data obtained and given trends correspond to literature data and prove that the methodical procedure was correct. Comparisons of data on the content of PhP from various papers are difficult with respect to the develop-

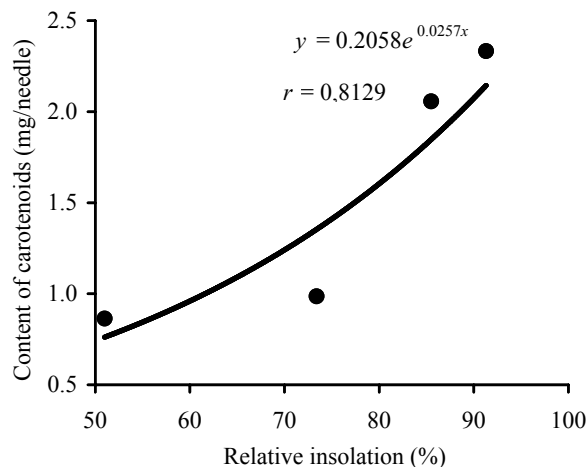


Fig. 4. Dependence of the content of carotenoids (per an average needle) on relative insolation

ment of a new instrumentation. If we use relative values comparisons of particular sample trees are possible.

In relation of the distribution of PhP (expressed per an average shoot) to the availability of solar radiation within the vertical light gradient of the crown a very high degree of dependence was achieved ($r = 0.93$ in chlorophyll $a + b$, $r = 0.91$ in carotenoids).

Trends in the distribution of PhP in the crown and trends in the content of PhP with respect to the availability of radiation showed in the distal part of the apical part of the sample tree crown, i.e. in part which is youngest within the sample tree crown, relatively most healthy and best insulated.

Considering the social position of the sample tree in a stand (partly a co-dominant tree), a lower gradient of the content of PhP was determined in the tree crown in comparison with data of MARTINKOVÁ (1992) from the same locality which is rather characteristic of subdominant trees. It also corresponds to the measured length of the sample tree crown. On the other hand, as compared with the tree of a lower age class the gradient of the content of PhP was steeper in a tree studied by our team.

As compared with optimum values determined in the Beskids (DOBEŠ 2001), the sample tree in the given locality achieved 90% of the PhP content in the crown apex and only 50% at the crown base. As compared with a spruce tree analyzed at Bílý Kříž in the Beskids in 1990–1991 (BARTANUZS 1992) which was negatively affected by heavy air pollution stress, the sample tree studied by our team showed higher pool of pigments (at the crown apex by 26%, at the crown base by 19%). The studied sample tree did not show optimum pigment pool but, on the other hand, it was not markedly damaged as it was in pollution-damaged areas in the 90s.

As for the ontogenetic stage, d.b.h. and length of the tree crown correspond to a subdominant tree which (with respect to its height) does not reach average diameter dimensions (difference 4 cm). Within the gradient of the content of PhP in the vertical or horizontal profile of the tree crown it was shown that the inner co-ordination of the content of PhP in the crown related to the age of needles and their insolation was sufficient. Therefore, the tree does not show impaired health condition but its growth retardation results from its short crown (or short basal part of the crown) and thus from the decreased extent of the photosynthetic apparatus which should be larger.

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Gradientsy obsahu fotosynteticky aktivních barviv a záření jako projevy zdravotního stavu smrku ztepilého (*Picea abies* [L.] Karst.)

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ABSTRAKT: Příspěvek se zabývá zdravotním stavem vzorníku smrku ztepilého (*Picea abies* [L.] Karst.) na základě distribuce a kvantity chlorofylu *a + b* a karotenoidů vzhledem ke gradientu záření v koruně. Obsah fotosynteticky aktivních (FA) barviv byl stanoven spektrofotometrickou analýzou. Vzorník se nachází na lokalitě Rájec nad Svitavou na Dražanské vrchovině v nadmořské výšce 625–640 m. V rámci gradientu obsahu FA barviv ve vertikálním a horizontálním profilu koruny se ukázalo, že je vnitřní koordinace obsahu FA barviv v koruně v závislosti na věku jehličí i v závislosti na jejich ozáření dostatečná. Strom tedy nemá snížený zdravotní stav, jeho růstová retardace vyplývá z krátkosti koruny. Rozsah fotosyntetického aparátu a stabilita stromu by byly zvýšeny zejména při prodloužení dolní části koruny, tzv. vyrovnávací části.

Klíčová slova: chlorofyl; karotenoidy; propustnost záření; zdravotní stav; *Picea abies* (L.) Karst.

Cílem práce bylo zjistit vitalitu vzorníku smrku ztepilého (*Picea abies* [L.] Karst.) na základě distribuce a kvantity listových pigmentů vzhledem ke gradientu záření v koruně a pomocí těsnosti vztahu konfrontovat jeho zdravotní stav.

Na lokalitě Rájec nad Svitavou na Dražanské vrchovině (49°26'37" s.š., 16°42'1" v.d.) v nadmořské výšce 625–640 m byl v rámci smrkového porostu vybrán vzorník s korunou v dosahu z gradientové věže. Vzorník dosáhl věku 92 let, jeho celková výška činila 25,40 m, výčetní tloušťka 23,7 cm. Podle Kraftovy klasifikace stromových tříd (KORPEL et al. 1991) se jednalo o strom částečně úroveň, z hlediska ontogenického vývinu dosáhl střední fáze dospělosti.

Před odběrem větví byla v rámci vzorníku měřena vertikální propustnost záření (pomocí dvou radiometrů ALAI-2V). Odběry větví ze vzorníku se uskutečnily jednorázově – na jaře před vyrašením pupenů (7. 5. 1999). Celkem byly z vertikálního profilu koruny odebrány čtyři větve 1. řádu se severozápadní orientací.

Obsah chlorofylu *a*, *b* a celkového množství karotenoidů byl stanoven třívlňovou spektrofotometrickou metodou (ŠESTÁK et al. 1971) a druhou derivací absorpčních křivek – automatický výstup ze spektrofotometru HP 8453 (DOBEŠ 1983; MARTINKOVÁ et al. 1996). Přepočtové rovnice byly použity podle LICHTENTHALERA a WELLBURNA (1983).

Průměrné hodnoty obsahu chlorofylu *a + b* vyjádřené na 1 g sušiny u prvních tří ročníků v jehlicích z apikální a bazální části koruny činily 1,85 mg až 2,13 mg. Vzhledem k apikální části koruny došlo v její bazální části ke zvýšení o 15 %. MARTINKOVÁ (1992) uvádí ze stejné lokality navýšení obsahu chlorofylu *a + b* vzhledem k apikální části koruny u podúrovňového jedince o 9 % (apikální část koruny 1,71 mg, bazální část koruny 1,87 mg) a u nadúrovňového jedince o 29 % (apikální část koru-

ny 1,24 mg, bazální část koruny 1,61 mg). KOCH (1976) uvádí u 75letého vzorníku hodnoty rozpětí obsahu chlorofylu *a + b* od 1,9 mg u nejmladších slunných jehlic až po 4,7 mg pro čtyřleté až pětileté stinné jehlice. Starší stinné jehlice obsahovaly o 247 % chlorofylu *a + b* více než jehlice nejmladší slunné. V případě námi studovaného vzorníku tento rozdíl činil 359 %. Gradient obsahu chlorofylu *a + b* byl tedy ještě strmější.

V horizontálním profilu (bazální část koruny) byl při vyjádření na 1 g sušiny zaznamenán stoupající trend od nejmladších ke starším ročníkům jehličí, u ročníků starších než devět let pak docházelo k postupnému snižování obsahu chlorofylu *a + b*. Rozdíly v obsahu chlorofylu *a + b* mezi jednotlivými ročníky v horizontální rovině (v rámci větve) byly největší v apikální části koruny, směrem k bázi se snižovaly. MARTINKOVÁ (1992) zaznamenala v bazální části koruny klesající trend v obsahu chlorofylu *a + b* již od 5. ročníku.

V bazální části koruny se trend změnil při vyjádření na 1 g sušiny projevilo u ročníků z její okrajové (distální) části výrazněji (např. snížení obsahu chlorofylu *a + b* již u tříletých jehlic), než tomu bylo u ročníků z vnitřní (proximální) části koruny. V apikální části koruny bylo zaznamenáno navýšení obsahu karotenoidů od nejmladších ke starším ročníkům jehlic, ve středové a bazální části koruny docházelo během stárnutí jehlic spíše k vyrovnání nebo k mírnému poklesu obsahu karotenoidů. To odpovídá již dříve nalezeným trendům (KRPEŠ 2002). Další trendy v obsahu karotenoidů v koruně vzorníku je možné charakterizovat obdobným způsobem jako u chlorofylu *a + b*.

Mezi obsahem FA barviv a relativní ozářeností ve vertikálním profilu koruny byl nalezen vzájemný vztah, jehož těsnost se lišila v závislosti na vyjádření FA barviv. Velmi vysoké těsnosti vztahu bylo dosaženo při vyjádření FA barviv na průměrný prýt, a to jak u chlorofylu

$a + b$ ($r = 0,93$), tak u karotenoidů ($r = 0,91$); jednalo se o závislost přímou.

Vzhledem k sociálnímu postavení vzorníku v porostu (strom částečně úrovňový) byl – v porovnání s údaji MARTINKOVÉ (1992) ze stejné lokality – zjištěn nižší gradient obsahu FA barviv v koruně, charakteristický spíše pro jedince podúrovňové. To koresponduje i se změřenou délkou koruny vzorníku. Na druhé straně ve srovnání s jedincem nižší věkové třídy byl gradient obsahu FA barviv u námi studovaného jedince příkřejší.

Vzorník na dané lokalitě svým pigmentovým fondem, ve srovnání s optimálními hodnotami zjištěnými v Beskydech (DOBEŠ 2001), dosáhl 90 % na apexu koruny a pouze 50 % na bázi koruny. Ve srovnání se smrkem analyzovaným v letech 1990–1991 na Bílém Kříži v Beskydech (BARTANUZS 1992), který byl negativně ovlivněn silným imisním spadem, měl však námi studovaný vzorník vyšší pigmentový fond (na apexu koruny

o 26 %, na bázi koruny však jen o 19 %). Sledovaný vzorník sice nevykázal optimální pigmentový fond, ale na druhé straně nebyl tak výrazně poškozen, jako tomu bylo ještě v devadesátých letech na imisně poškozených územích.

Co se týká ontogenické fáze, výčetní tloušťka i délka koruny stromu odpovídá spíše podúrovňovému jedinci, který vzhledem ke své výšce nedosahuje průměrné tloušťkové dimenze (rozdíl 4 cm). V rámci gradientu obsahu FA barviv ve vertikálním nebo horizontálním profilu koruny se ukázalo, že je vnitřní koordinace obsahu FA barviv v koruně v závislosti na věku jehličí i v závislosti na jejich ozáření dostatečná. Strom tedy nemá snížený zdravotní stav, ale jeho růstová retardace vyplývá z krátkosti koruny (resp. z krátkosti bazální části koruny). Pokud by pěstební opatření vedla k prodloužení korun, byl by fotosyntetický aparát rozsáhlejší a stromy by byly stabilnější vzhledem k eventuálním stresorům.

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