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The effect of fertilization on chlorophyll activity, content of photosynthetically active pigments and nutrients in Carpathian birch leaves

Einfluss der Düngung auf die Aktivität des Chlorophylls, Gehalt der photosynthetisch aktiven Pigmente und der Nährstoffe in den Blättern der Karpaten-Birke

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Summary

In many areas industrial air pollutants caused soil acidification and aggravated conditions for forest regeneration, especially on poor mountain soils. Fertilization can contribute to balanced nutrition and support the survival and prosperity of tree species. Methods studying the physiological state of plantations can detect the effect of fertilization before it is observable by measurement of morphological parameters. This study deals with plantati-

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ons of Carpathian birch in the summit part of the Jizerské hory Mts. in the northern mountain range of the Czech Republic. The experiment included a 2-year-old plantation of large-sized plants established next spring after planting with three treatments: control, application of Silvamix Forte fertilizer and application of a combination of Silvamix Forte and Fosmag MG fertilizers; and 17-year-old stand with two treatments: control and application of dolomitic limestone at planting. The parameters of chlorophyll *a* fluorescence, contents of photosynthetically active pigments and macronutrients were analysed. A significant influence of fertilizer application on chlorophyll *a* fluorescence and pigment content was observed for the 2-year-old plantation. An influence of liming was still observed 17 years after planting. The results document that fertilization or liming of plants on poor mountain soils can exert prompt influence and long-term effect on the physiology of Carpathian birch assimilatory tissues.

Zusammenfassung

Als Folge der Industrieimissionen ist es in vielen Gegenden zur Azidifikation der Böden und zur Erschwerung der Bedingungen für die Walderneuerung gekommen, besonders auf armen Böden im Gebirge. Die Düngung kann zum Ausgleich der Ernährung führen und somit die Überlebung und den Wohlstand der Holzarten unterstützen. Die den physiologischen Bestand der Anpflanzungen beobachtenden Methoden können den Effekt der Düngung früher feststellen als er mit den Messungen der morphologischen Merkmale prüfbar ist. Diese Studie befasst sich mit den jungen Beständen der Karpaten-Birke im Gipfelgebiet des Isergebirges in der nördlichen Bergzone der Tschechischen Republik. Es wurde eine zweijährige Anpflanzung der Viertelheister getestet, die nächsten Frühling nach der Anpflanzung in drei Varianten gegründet wurde: die Kontrollvariante; Düngung mit dem Dünger Silvamix Forte und mit der Düngerkombination Silvamix Forte und Fosmag MG; und eine 17jährige Anpflanzung in den Varianten: die Kontrollvariante und Düngung mit dem Dolomit-Kalkstein gleich bei der Anpflanzung. Es wurden die Parameter der Chlorophylfluoreszenz, die Gehalte an photosynthetisch aktiven Pigmenten und an den Grundnährstoffen analysiert. Im zweiten Jahr nach der Applikation der Dünger zu den jungen Setzlingen wurde ein bedeutender Einfluss auf die Chlorophylfluoreszenz und den Gehalt an Pigmenten beobachtet. Im älteren Bestand wurde mit den eingesetzten Methoden der Einfluss der Kalkung noch 17 Jahre nach der Anpflanzung beobachtet. Aus den Ergebnissen ergibt sich, dass die Düngung oder Kalkung der Setzlinge auf armen Böden im Gebirge einen schnellen Effekt und eine langfristige Wirkung auf die Physiologie des Assimilationsapparats der Karpaten-Birke haben kann.

1. Introduction

The area of the Jizerské hory Mts., the northern mountain range in the Czech Republic, was exposed to a strong impact of air pollutants in the seventies and eighties of the 20th century (Vacek et al., 2003) which caused soil acidification (Boruvka et al., 2005) and imbalanced nutrition. Due to adverse soil and climatic conditions forest regeneration is very difficult in these localities.

Liming has become one of the measures taken to support forest tree plantations on clearcuts that originate from salvage felling (Podrázský, 1991; 1999). Alkaline rock powders were often recommended as fertilizers to enhance the vitality of forest tree species and they were used in practice in this way (Nemec, 1956; Materna, 1963). Besides whole-area liming, the dolomitic limestone was applied to plants at planting. The effect of limestone application is a decrease in soil acidity, improvement in the soil sorption complex, reduction in the activity of toxic ions in soil, replenishment of soil calcium and magnesium reserves and humification acceleration (Podrázský, 1991). In general, conditions for plant nutrition may be improved even though risks associated with liming (e.g. accelerated mineralization, nitrate or base cations leaching, stimulation of fine root development in the uppermost soil layers and thus increasing the danger of frost and drought damage) should be taken into account (Kreutzer, 1995; Nilsson et al., 1995; Podrázský and Ulbrichová, 2003).

After the direct effect of air pollutants has abated, modern slow-release fertilizers are tested in mountain locations. Fertilizers on the basis of urea and potassium-manganese phosphates such as Silvamix Forte with a 60% portion of slow-release nitrogen are among them. The application of these fertilizers is usually done subsequent to planting when tablets or granules are applied onto the soil surface or under the upper layer of soil (Kubelka et al., 2010). Fertilization may contribute to balanced nutrition (Dreyer, 1994) and promote the survival and prosperity of tree species mainly in the first years after planting (Óskarsson et al., 2006; Balcar and Kacálek, 2008). There arises the question whether such fertilization has also a long-term effect on further development of plantations.

Changes in nutrition caused by appropriately chosen fertilization lead to an overall improvement in prosperity, i.e. in the physiological state of plantations. It is questionable whether these changes will be expressed directly in the content of photosynthetically active pigments and in the activity of photosynthesizing tissues.

The photosynthetic activity rate is conditioned by the amount of photosynthetically active pigments (Koch, 1976; Richardson et al., 2002). One method to determine the amount of photosyntetically active pigments is spectrophotometric measurement of wavelength absorbance in a mixture of pigments extracted from assimilatory tissues (Lichtenthaler, 1987; Porra et al., 1989). Chlorophyll *a* fluorescence measurement also seems to be a suitable method to evaluate the condition of photosynthesizing tissues as chlorophyll. The analysis of chlorophyll *a* fluorescence provides detailed information about the processes within a photosynthesizing organism (Schreiber, 2004).

The methods of chlorophyll *a* fluorescence measurement can provide data on the ability of plants to respond and tolerate environmental stresses and also on the extent of damage to the photosynthetic apparatus caused by these stresses (Maxwell and Johnson, 2000). The use of a plant efficiency analyser (PEA) is the basic approach to chlorophyll *a* fluorescence evaluation, allowing for an insight into the problem of the primary reaction of dark-acclimatized photosynthetically active material to an intensive light impulse (Strasser et al., 2000). The relationship between chlorophyll *a* fluorescence and nutrient status was evaluated in a number of studies on different woody plants, mostly on coniferous tree species (Strand and Lundmark, 1995; Savonen and Sarjala, 1998; Wether and Havranek, 2000 etc.); a study aimed at the Carpathian birch has been missing until now.

The objectives of the present paper were (1) to demonstrate whether there are differences in chlorophyll content, activity of photosynthetically active pigments and foliage chemistry of Carpathian birch detectable **among fertilization treatments for a 2-year-old plantation;** (2) to find whether differences in above mentioned parameters can be detected **17 years after liming at the time of planting;** (3) evaluate **physiological state of the plantations;** and last but not least, (4) to verify the **efficiency** of particular **tested methods.**

2. Methods

2.1 Experimental plantations

Research was conducted in two plantations of Carpathian birch (*Betula carpatica* W. et K.), a species usually growing on avalanche tracks and peaty soils with higher water content – on raised bogs (Úradnícek et al., 2009). The plantations were located on nearby research plots in the summit part of the Jizerské hory Mts. on the northern mountain range in the Czech Republic. Both sites were classified as acid spruce stand (*Piceetum acidophilum*)

according to the Czech forest site classification (Viewegh et al., 2003). Basement rocks are granites and granodiorites, soils are mountain humic podzol (FAO classification) with pH(H_2O) of topsoil ranging in the area from 4.2 to 4.6 (Kuneš et al., 2007).

Table 1: Basic characteristics of experimental treatments.

Tabelle 1: Grundbezeichnung der Versuchsvarianten.

Research plot / Versuchsfläche	Plantation (age from planting) / Bestand (Alter nach der Anpflanzung)	Treatment / Versuchsvariante	Symbol / Bezeichnung	Height (autumn 2009) / Höhe (Herbst 2009)
U Panelové cesty	younger (2 years) / jünger (2 Jahre)	control / Kontrollvariante	Pan_K	132 cm
		Silvamix Forte	Pan_S	106 cm
		Silvamix Forte + Fosmag MG	Pan_S+P	110 cm
Jizerka	older (17 years) / älter (17 Jahre)	control / Kontrollvariante	Jiz_K	330 cm*
		limestone / Kalkstein	Jiz_V	280 cm*
*Source / Quelle: Špulák et al. 2011				

The younger of the analysed plantations is located on research plot called U Panelové cesty in a frost pocket of the Jizerka River valley (50°49'8.1"N 15°21'8.5"E, 870 m a.s.l., SW slope of 3°). The plantation was established in autumn 2008 when 2-year-old large-sized plants (average height 108 cm) that were grown by machine technology of intensive treatments of the root system (Burda and Nárovcová, 2009) were planted. Birches of autochthonous origin were planted in downhill oriented rows ca 2 m apart from each other. Within the rows, the trees were ca 1.5 m from each other. For the plantation three treatments were chosen: a) control (Pan_K), b) Silvamix Forte (Pan_S) and c) Silvamix Forte + Fosmag MG (Pan_S+P). Fertilizers were applied in spring 2009. Three 10 g tablets (= 30 g) of Silvamix Forte fertilizer were distributed regularly around the tree at a distance of 20 to 30 cm from the stem, to a depth of ca. 5 to 10 cm. Of the phosphorus fertilizer Fosmag MG, 30 g were applied onto the soil surface. Fertilizer Silvamix Forte contained 17.5% of N, 17.5% of P, 10.5% of K and 9% of MgO and fertilizer Fosmag MG contained 24% of P and 2% of Mg. Each treatment was established at least in four 60 m long rows, i.e. minimally four replica-

tions. Accidentally, the average height of plants in the control was taller then in other treatments (Table 1). The method of nursery production of all trees was identical, and so samples were taken randomly in all treatments without considering the planting material size.

The second (older) tested plantation of Carpathian birch located ca 700 m northwardly was planted in 1993 on the Jizerka research plot on the central ridge Střední jizerský hreben (50°49'39.6"N 15°21'21.7"E, 970 m a.s.l., SW slope of 2°), situated on an extensive clearcut that originated by salvage felling (Balcar and Podrázský, 1994; Balcar and Kacálek, 2008). The objective of this plantation was to investigate the influence of an extreme fertilizer dose of dolomitic limestone on Carpathian birch. Two year old birches of autochthonous origin were planted in spacing of 1 x 2 m, each treatment on subplots of 10 x 10 m. Two treatments were established in two replications: a) control (Jiz_K), b) limestone (Jiz_V). For the limestone treatment 1 kg of finely ground dolomitic limestone was mixed into a hole during planting (Table 1). Along the contour line, birch subplots alternated with spruce ones, buffer strips of 4 m were left uphill and downhill the subplots. The average height of the control Carpathian birch plantation was 330 cm in 2009 while in the limestone treatment it was 280 cm (Špulák et al., 2011).

2.2 Leaf sampling for analyses

In summer 2010 samples of the assimilatory tissues of randomly selected trees were taken for the analysis of chlorophyll *a* fluorescence on two dates – in high summer (2. 7. 2010) and in late summer (3. 9. 2010). In the older plantation 20 prosperous birches were sampled per treatment, in the younger plantation 15 trees per treatment. Samples were always taken from the upper sunlit part of the crown. The analysis of the content of photosynthetically active pigments and concentration of macronutrients in leaves was done in late summer.

For the chemical analysis in the older plantation samples were taken from 20 trees per treatment and the analysis was done for each tree separately. Due to the limited foliage amount on particular trees in the younger plantation composite samples were made, in total four composite samples in Pan_S and Pan_S+P treatments and eight composite samples in the control.

2.3 Chlorophyll *a* fluorescence

After sampling branch samples in polythene bags were put into a refrigerating box, transported to a laboratory and kept in a refrigerator until the next day. They were analysed in a plant efficiency analyser (PEA, Hansatech

Instruments Ltd., Kings Lynn, England). Before measurement, the samples were adapted to room temperature for half an hour at least. The middle part (without the primary vein) of a randomly chosen leaf without visible damage was used for the analysis. For at least 30 minutes before measurement the analysed leaf area was shaded with an original clip (the diameter of the measured area was 4 mm). The intensity of radiation for tests was set at 50% of maximum (i.e. at 2 100 μmol.m^{-2}.s^{-1}). The measurement of samples from each subject was done in 4–5 replications (leaves).

The evaluated parameters of chlorophyll *a* fluorescence were (nomenclature according to van Kooten and Snel (1990) and Stirbet and Govindjee (2011)) maximum quantum yield of PSII photochemistry, F_v/F_m ratio in which variable fluorescence in the dark-adapted state F_v is calculated from the equation

$F_v = F_m - F_0$, where F_0 is minimal fluorescence and F_m maximal fluorescence in the dark-adapted state (in arbitrary units).

Another evaluated parameter was the S_m/t_{Fmax} ratio describing the average redox state of Q_A – quantification of the electron transport activity (Strasser et al., 2000). The variable S_m is designated as normalized area growth above the fast fluorescence rise, calculated according to the formula

$$S_m = \frac{Area}{(F_m - F_0)}$$, where

$$Area = \int_0^{t_{F\max}} (F_m - F_t) * dt$$.

The parameter S_m expresses a work-integral; in other words, it is the energy necessary to close all reaction centres and it is proportional to the number of electrons passing through the electron transport chain (Stirbet and Govindjee, 2011). Area is the actual area between the curve and line $F = F_m$, the parameter t_{Fmax} is the time of reaching maximal fluorescence.

Plant vitality was described by the performance index on absorption basis, PI_{abs} (Strasser et al., 2004).

$$PI_{abs} = \frac{1-(F_0/F_m)}{M_0/V_J} * \frac{F_m - F_0}{F_0} * \frac{1-V_J}{V_J}$$, where

$$M_0 = \frac{4*(F_{300\mu s} - F_0)}{F_m - F_0}$$ and

$$V_J = \frac{F_{2ms} - F_0}{F_m - F_0} \quad .$$

Parameters $F_{300\mu s}$ and F_{2ms} mean fluorescence intensity at 300μs and 2ms, respectively. Performance index is a very complex parameter because it is calculated on the basis of characteristics describing the status of practically all main photochemical processes: such as density of reaction centres on chlorophyll basis, flux ratio trapping per dissipation and electron transport further than primary plastoquinone (Q_A) (Strasser et al., 1999; 2004).

The basic parameters were obtained from source data using the WinPEA32 v 1.0 software, parameters S_m, S_m/t_{Fmax} and PI_{abs} were computed in Excel using abovementioned formulas.

2.4 Photosynthetically active pigments

The amounts of chlorophylls and carotenoids were evaluated by spectrophotometric measurement of wavelength absorbance in a mixture of pigments extracted from assimilatory tissues (Lichtenthaler, 1987). Total content of chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and carotenoids (Car_{x+c}) was determined.

Collected samples were kept in a deep-freeze box at a temperature of -80°C and they were gradually analysed. Extraction was done under dim light to suppress pigment photodestruction. From each subject (tree), leaf samples were taken for extraction and weighed at an amount of 0.4–0.5 g of fresh matter in two replications. The leaves were homogenized in mortars containing a pinch of pure sea sand and 80% acetone. The particular samples were transferred into plastic centrifuge tubes of 15 ml in volume. Their processing was performed according to the modified methodology of Kurasová et al. (2002) and Makeen et al. (2007). 80% acetone was added to each prepared sample to the total volume of 15 ml, and the sample was shaken in a laboratory shaker for 20 minutes and put into a refrigerator overnight (4–5°C). On the next day, the sample was shaken again for 20 minutes in a laboratory shaker and put into a centrifuge where it was centrifuged at 4 000 rev.min^{-1} for 4 minutes. After centrifugation, 12–13 ml of clear extract was pipetted to a volumetric 50 ml flask that was stored in a refrigerator at 4–5°C until absorbance measurement. Then again the residues in the tube were washed with 80% acetone, shaken, centrifuged and pipetted. The whole process was repeated third time again. After the third wash with acetone the concentration of pigments in the homogenized leaf tissues was under the detection limit of the method, which was proven, when the method of analyses of the birch leaves was setup.

The obtained extract of pigments in a volumetric flask was filled up to 50 ml with 80% acetone and subsequently it was measured in a Unicam Helios ε spectrophotometer at set wavelengths of 470 nm, 647 nm, 663 nm and 750 nm. Each sample was measured three times and the average of the measured values was used to calculate the content of pigments.

Equations for the calculation of pigment content (Lichtenthaler, 1987) were adjusted according to Makeen et al. (2007) for the reading of absorbance at 750 nm, where neither chlorophylls nor carotenoids absorb any more and the value of absorbance is caused by the dispersion of impurities in the extract:

$$Chl_a = 12.25 * (A_{663} - A_{750}) - 2.79 * (A_{647} - A_{750})$$
$$Chl_b = 21.50 * (A_{647} - A_{750}) - 5.10 * (A_{663} - A_{750})$$
$$Chl_{a+b} = 7.15 * (A_{663} - A_{750}) + 18.71 * (A_{647} - A_{750})$$
$$Car_{x+c} = \frac{1000 * (A_{470} - A_{750}) - 1.82 * Chl_a - 85.02 * Chl_b}{198}$$

The resultant amount of pigments was converted per $mg.g^{-1}$ of fresh matter of leaves.

2.5 Nutrient concentration

In leaf samples collected in both birch stands in late summer the content of macronutrients (N, P, K, Ca, Mg), sulphur and silicon was analysed. Methods described by Zbíral (1994) were used for the analysis. The samples were mineralized, total nitrogen concentration was analysed by the Kjeldahl method, phosphorus was determined colorimetrically, potassium in an atomic absorption spectrophotometer, calcium and magnesium by atomic absorption after the addition of lanthanum, sulphur by the Balks method.

2.6 Statistical evaluation

Based on the methods of exploratory data analysis (EDA) measurements with parameters beyond the range of biophysically justifiable values were excluded and average values for the particular samples were calculated (data on chlorophyll *a* fluorescence and contents of photosynthetically active pigments). In order to improve the normality parameter and the character of variances the data were transformed, if necessary, by the Box-Cox transformation (Fox and Weisberg, 2001). To achieve the set objectives, the influence of fertilization (comparison of treatments within the particular plantations) were determined. Depending on the type of task and data, the

data were statistically evaluated by Student's t-test or by the analysis of variance (and/or by the Kruskal-Wallis non-parametric test) with subsequent comparison by the linear contrast method (Chambers and Hastie, 1992). The tests were run in Unistat 5.601 statistical programme and in R environment (2.10.1, The R Foundation for Statistical Computing 2011). Differences were considered significant if $P < 0.05$.

3. Results

3.1 Chlorophyll *a* fluorescence

In the younger stand there were found no significant differences in parameters between the dates of measurement in control. In both fertilization treatments the values of S_m and PI_{abs} increased from high to late summer while in Pan_S+P treatment a decrease in the parameter S_m/t_{Fmax} was also confirmed. In the older plantation a statistically significant decrease in all evaluated parameters of chlorophyll *a* fluorescence was observed between the dates in both control and limed treatment (Table 2A, 2B).

Differences among treatments in the younger plantation were observed only in samples from 1st date (high summer): all studied parameters of chlorophyll fluorescence in Pan_S+P were lower than in Pan_K control (Table 2C). In the older stand in both dates parameter S_m was significantly higher in control than in limed treatment. Also in control treatment, in high summer F_v/F_m was lower while in late summer PI_{abs} was higher (Table 2C).

3.2 Photosynthetically active pigments

In the younger plantation concentrations of chlorophylls and carotenoids (Chl_a, Chl_b, Chl_{a+b} and Car_{x+c}) of control were significantly higher than in the both fertilization treatments (Table 3). No significant difference with control was found only in the Chl_a/Chl_b ratio in Pan_S treatment. The lowest values of the observed parameters were recorded in Pan_S+P treatment (Table 3B).

In the older plantation the values were also significantly higher in control (Jiz_K) than in liming treatment (Jiz_V) with the exception of the Chl_a/Chl_b ratio where no difference was observed (Table 3B). In control treatments of both plantations higher variability in chlorophyll *a* and *b* contents was observed compared to fertilization treatments (Table 3A).

Table 2: Maximum quantum yield of PSII photochemistry (F_v/F_m), normalized area growth above the fast fluorescence rise (S_m), ratio of normalized area growth to the time of reaching maximal fluorescence (S_m/t_{Fmax}) and performance index on absorption basis (PI_{abs}). Mean and standard deviation on both dates of measurement (A), tests of significance of the differences between the dates of measurement (B) and between selected treatments (groups of treatments; C). K-W – Kruskal-Wallis test. P values where p<0.05 are in bold face.

Tabelle 2: Maß für die maximale Quantenausbeute von PSII (F_v/F_m), normalisierte Fläche über der Linie des Anstiegs schneller Fluoreszenz (S_m), Verhältnis der normalisierten Fläche über der Anstiegslinie zur Zeit der Erlangung maximaler Fluoreszenz (S_m/t_{Fmax}) und Performanceindex an Hand von der Absorption (PI_{abs}). Durchschnitt und Standardabweichung in beiden Terminen der Messung (A), Teste der Beweiskraft der Unterschiede zwischen den Terminen der Messung (B) und zwischen gewählten Varianten (Variantengruppen; C). K-W – Kruskal-Wallis-Test. P-Werte wo p<0,05 sind fett gedruckt.

A)

Treatment / Variante	F_v/F_m		S_m		S_m/t_{Fmax}		PI_{abs}	
	mean	sd	mean	sd	mean	sd	mean	sd
1st date – high summer / 1.Termin - Hochsommer								
Pan_K	0.785	0.022	24.2	6.7	0.103	0.013	1.75	0.87
Pan_S	0.776	0.03	20.9	3.6	0.101	0.010	1.37	0.72
Pan_S+P	0.761	0.024	18.1	1.7	0.094	0.005	0.80	0.36
Jiz_K	0.797	0.021	48.7	15.9	0.127	0.016	4.00	1.23
Jiz_V	0.811	0.014	38.1	8.5	0.124	0.009	4.18	1.14
2nd date – late summer / 2.Termin - Spätsommer								
Pan_K	0.767	0.033	24.0	6.2	0.091	0.019	2.05	1.32
Pan_S	0.770	0.039	24.5	5.8	0.092	0.015	2.24	1.46
Pan_S+P	0.750	0.037	21.6	4.1	0.079	0.014	1.23	0.57
Jiz_K	0.745	0.071	31.6	4.1	0.084	0.011	2.80	0.89
Jiz_V	0.705	0.098	28.4	4.3	0.076	0.015	1.94	1.06

B)

Treatment / Variante		F_v/F_m	S_m	S_m/t_{Fmax}	PI_{abs}
	Test	p-value / P-Wert			
Pan_K	t-test	0.320	0.618	0.178	0.203
Pan_S	t-test	0.862	**0.027**	0.133	**0.030**
Pan_S+P	t-test	0.790	**0.005**	**0.009**	**0.008**
Jiz_K	t-test	**0.002**	**<0.001**	**<0.001**	**0.005**
Jiz_V	t-test	**<0.001**	**<0.001**	**<0.001**	**<0.001**

C)

Treatment comparisons / Vergleich der Varianten		Test		Fv/Fm	Sm	Sm/t_{Fmax}	PIabs
				p-value			
Younger plantation / Jüngere Anpflanzungen – RP U Panelové cesty							
Pan_K × Pan_S × Pan_S+P		Anova (*K-W)	1st date	**0.044**	**0.002***	**0.020***	**0.003**
			2nd date	0.202	0.309	0.095	0.096
	Pan_K × Pan_S	linear contrasts / lineare Kontraste	1st d ate	0.655	0.948	0.506	0.616
			2nd date	-	-	-	-
	Pan_K × Pan_S+P		1st date	**0.019**	**0.001**	**0.028**	**0.001**
			2nd date	-	-	-	-
Older plantation / Ältere Anpflanzungen – RP Jizerka							
Jiz_K × Jiz_V		t-test	1st date	**0.021**	**0.005**	0.558	0.570
			2nd date	0.106	**0.024**	0.091	**0.008**
Anmerkungen: mean – Durchschnitt, sd – Standardabweichung; 1st date – 1.Termin, 2nd date – 2.Termin							

Table 3: Contents of photosynthetic pigments (in $mg.g^{-1}$ of fresh matter; mean and standard deviation; A), ratios of photosynthetic pigment groups and tests of significance of the differences between selected treatments (groups of treatments; B). K-W – Kruskal-Wallis test. P values where p<0.05 are in bold face.

Tabelle 3: Gehalte der photosynthetischen Pigmente (in $mg.g^{-1}$) der Grünmasse; Durchschnitt und Standardabweichung; A), Verhältnisse der Gruppen photosynthetischer Pigmente und Teste der Beweiskraft der Unterschiede zwischen den gewählten Varianten (Variantengruppen; B). K-W-Kruskal-Wallis-Test. P-Werte wo p<0,05 sind fett gedruckt.

A)

Treatment / Variante	Chl_a		Chl_b		Chl_{a+b}		Car_{x+c}		$Chl_{a+b}+Car_{x+c}$		Chl_a/Chl_b		Chl_{a+b}/Car_{x+c}	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Pan_K	1.276	0.084	0.381	0.013	1.657	0.092	0.404	0.009	2.061	0.098	3.35	0.18	4.11	0.19
Pan_S	1.207	0.033	0.371	0.009	1.578	0.033	0.406	0.007	1.984	0.036	3.26	0.13	3.89	0.08
Pan_S+P	0.824	0.014	0.268	0.009	1.093	0.010	0.330	0.005	1.422	0.012	3.08	0.15	3.31	0.06
Jiz_K	1.623	0.061	0.481	0.025	2.104	0.084	0.483	0.011	2.587	0.088	3.38	0.09	4.36	0.17
Jiz_V	1.441	0.040	0.426	0.023	1.867	0.061	0.464	0.008	2.331	0.066	3.39	0.11	4.02	0.12

B)

Treatment comparisons/ Vergleich der Varianten		Test	Chl_a	Chl_b	Chl_{a+b}	Car_{x+c}	$Chl_{a+b}+Car_{x+c}$	Chl_a/Chl_b	Chl_{a+b}/Car_{x+c}
			p-value / P-Werte						
Younger plantation / Jüngere Anpflanzungen – RP U Panelové cesty									
Pan_K × Pan_S × Pan_S+P		K-W	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Pan_K × Pan_S	linear contrasts / lineare Kontraste	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	0.529	**0.007**
	Pan_K × Pan_S+P		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Older plantation / Ältere Anpflanzungen – RP Jizerka									
Jiz_K × Jiz_V		t-test	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.7967	**<0.001**
Anmerkungen: mean – Durchschnitt, sd – Standardabweichung									

3.3 Foliage chemistry

In the younger plantation the particular fertilization treatments were different only in P content – the treatment with application of phosphorus fertilizer (Pan_S+P) had significantly higher phosphorus content in leaves (Table 4). In the older stand the leaves of the limestone treatment had higher contents of P, Ca, Mg and also S compared to the control (Table 4).

Table 4: Nutrient concentrations in the leaves of analysed treatments of Carpathian birch (%), sampling in high summer 2010 (mean and standard deviation; A) and tests of significance of the differences between selected treatments (groups of treatments; B). P values where p<0.05 are in bold face.

Tabelle 4: Nährstoffgehalte in den Blättern der analysierten Varianten der Karpaten-Birke (%), Entnahme Hochsommer 2010 (Durchschnitt und Standardabweichung; A) und Teste der Beweiskraft zwischen den gewählten Varianten (Variantengruppen; B). K-W-Kruskal-Wallis-Test. P-Werte wo p<0,05 sind fett gedruckt.

A)

Treatment / Variante	N		P		K		Ca		Mg		S	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Pan_K	1.522	0.206	0.133	0.043	0.490	0.031	0.689	0.076	0.363	0.045	0.113	0.028
Pan_S	1.609	0.192	0.178	0.042	0.525	0.038	0.645	0.054	0.375	0.021	0.104	0.012
Pan_S+P	1.557	0.159	0.235	0.064	0.525	0.029	0.773	0.041	0.393	0.020	0.103	0.015
Jiz_K	2.543	0.210	0.164	0.015	0.632	0.073	0.426	0.076	0.240	0.020	0.187	0.028
Jiz_V	2.551	0.261	0.186	0.026	0.648	0.069	0.684	0.112	0.297	0.035	0.214	0.047

B)

Treatment comparisons / Vergleich der Varianten		Test	N	P	K	Ca	Mg	S
			p-value / P-Wert					
Younger plantation / Jüngere Anpflanzungen – RP U Panelové cesty								
Pan_K × Pan_S × Pan_S+P		Anova	0.815	**0.012**	0.194	0.062	0.394	0.793
	Pan_K × Pan_S	linear contrasts /	-	0.861	-	-	-	-
	Pan_K × Pan_S+P	lineare Kontraste	-	**0.021**	-	-	-	-
Older plantation / Ältere Anpflanzungen – RP Jizerka								
Jiz_K × Jiz_V		t-test	0.856	**0.003**	0.474	**<0.001**	**<0.001**	**0.037**
Anmerkungen: mean – Durchschnitt, sd – Standardabweichung								

4. Discussion

4.1 Chlorophyll *a* fluorescence

With regard to chlorophyll *a* fluorescence measurement the quantum yield of photochemistry PSII (F_v/F_m) is the most frequently evaluated parameter. In normally developing leaves the value of F_v/F_m ranges from 0.74 to 0.85 (Lichtenthaler et al., 2005) and the typical average value of the properly functioning photosynthetic apparatus in tree species is 0.832 (Mohamed et al., 1995). The parameter decreases in the presence of stress factors. Bolhar-Nordenkampf and Götzl (1992) considered 0.72 as the threshold value of reversible disorders of the assimilatory tissues. In our study the F_v/F_m of limed birches dropped below this threshold in late summer date (Table 2A). Percival and Galloway (1999) assumed in Betula pendula seedlings the beginning of stress at the values lower than 0.8. It would suggest a stress presence on both research plots in our study.

In our study, the only variable with significant differences among treatments on both dates was parameter S_m (Table 2C) expressing the energy necessary for the closure of all reaction centres of photosystem II (Stirbet and Govindjee, 2011). The S_m value of older plantations was distinctly higher on both dates than in younger plantations, which indicates a increased reduction of Q_A (Strasser et al., 2000). Performance index (PI_{abs}), describing the overall photosynthetic performance, was low in all treatments of the younger plantation on the first date of measurement (Table 2). The values correspond to data acquired in maple leaves at senescence phase (Lepeduš et al., 2010). The lower photosynthetic performance may be caused by the influence of persisting post-planting shock that could also decelerate budbreak and accelerate leaf aging (Cabral and O'Reilly, 2008; Beniwal et al., 2011).

The results confirmed an assumption that in the evaluation of silvicultural measures on the basis of chlorophyll *a* fluorescence measurement it is possible to compare only the values determined on the same date. Higher sensitivity of the method was found in high summer.

4.2 Photosynthetically active pigments

The concentration of chlorophylls and/or carotenoids and particularly the Chl_a/Chl_b ratio are often used to find out how the plant responds to abiotic factors (Peñuelas and Filella, 1998; Carter and Knapp, 2001; Kwak et al., 2001). In all our treatments the Chl_a/Chl_b ratio was in the range of 3:1 to 4:1 (Tab. 3A) that is reported in plants growing in conditions with minimum environmental stress (Lichtenthaler et al., 2007; Cha-um et al., 2009; Kwak et al., 2011). Lower values can already indicate the negative influence of stress factors on growth. The observed lower ratio in Pan_S+P compared to Pan_K indicate the presence of a stressor, what is supported by the overall low content of photosynthetically active pigments ($Chl_{a+b}+Car_{x+c}$, Table 3A). According to various studies (Rabe and Kreeb, 1980; Kwak et al., 2001; Liu et al., 2011) the change in the Chl_a/Chl_b ratio needs not be markedly expressed on tested plants after stress in certain cases, but the amount of chlorophyll *a* and *b* in assimilatory tissues decreases in the majority of stress situations. As reported in literature (e.g. Demmig-Adams et al., 1996), carotenoids in the plant have not only the light-gathering function (as supplementary pigments) but also the photo-protective function. The lowest content of Car_{x+c} was observed in Pan_S+P treatment of the younger plantation, where also Chl_{a+b} was the lowest (Table 3A). It reflected in the low Chl_{a+b}/Car_{x+c} ratio that was beyond the range of normal values (3.8–4.4) reported for sunlit leaves by Lichtenthaler et al. (2007).

4.3 Relations with nutrition

A relationship was observed between fluorescence parameters and nutrient content. Strand and Lundmark (1995) reported lower values of F_v/F_m in fertilized spruce seedlings as compared to control in the course of spring. In Scotch pine seedlings, differences of F_v/F_m in treatments of potassium application and control were revealed during the growth period (Savonen and Sarjala, 1998). The influence of the date of evaluation in the growth period was proved also in our study, but differences in F_v/F_m between fertilization and control treatments were found only on the high summer date. Unlike the cited papers, fertilization treatments of younger plantation had a lower maximum quantum yield compared to the control. The response of photosynthetically active tissues to fertilization is related not only to the overall nutritional status but also to the tree species.

Dreyer et al. (1994) investigated the influence of application of lime-magnesium fertilizer on improvement in the condition of ten-year-old Norway spruce plantation with distinct symptoms of magnesium deficiency. After 18 months the better nutrition of fertilized trees was reflected both in colour changes of assimilatory tissues and in the content of elements and chlorophyll in leaves but no changes either in photosynthetic activity or in growth were observed. On the contrary, in our study nutrient concentration in Carpathian birch leaves seemed to be a less reliable indicator of a change in nutrient content in soil. With the exception of P it indicated only differences in chemistry caused by the application of an extreme dolomitic limestone dose (Table 4). On the other hand, the method of measuring the chlorophyll *a* fluorescence of samples from high summer was able to differentiate practically all studied treatments, except Pan_S treatment and Pan_K control.

Generally, phosphorus is considered as a limiting factor in the upper parts of the Jizerské hory Mts. (e.g. Slodicák et al., 2005). Its supply, significantly expressed in foliage chemistry, should have quite a favourable effect on the plantation. However, in Pan_S+P treatment our evaluated parameters indicate rather a worse physiological state compared to the other treatments (Table 2 and 3).

Carpathian birch is a species usually growing on acid soils – avalanche tracks and peaty soils (Úradnícek et al., 2009). Especially the liming created conditions not natural for the species concerned. It can explain the long-term lower height increment in birches with limestone application (Jiz_V) (Balcar and Kacálek, 2001). However, the mortality of limed birches was lower by 11% compared to the control (Špulák et al., 2011). In our study the influence of liming was significantly observable in all the studied groups of parameters even after 17 years.

In our experiment lower chlorophyll contents than in the respective control were found in fertilization treatments of the younger and older plantation. This fact is in contradiction e.g. with results of the investigation of the influence of nitrogen application on the relative concentration of chlorophylls in *Acer rubrum* and *Betula papyrifera* (Gloser et al., 2008) as well as with the studies of Minotta and Pinzauti (1996), who described the rising chlorophyll concentration in beech leaves with the increasing nutrient reserve in soil. On the other hand, Merilo et al. (2006) did not observe any significant difference in the total content of chlorophylls in sunlit leaves of fertilized (NPK) and unfertilized willows.

Comparing the used methods, it is to state that differences between fertilization treatments of plantations were expressed in the most pronounced

way by results of the analysis of photosynthetically active pigment content. As it is the manual processing of samples, it is the time-consuming and costly method. Potential of a quick evaluation of photosynthetically active pigment content by indirect, approximation methods based on chlorophyll fluorescence ratio F_{735}/F_{700} should be verified. To some extent, when samples were taken in high summer, the analysis of chlorophyll *a* fluorescence was similarly efficient in distinguishing Carpathian birch treatments. The finding that the foliage chemistry responds to changes in nutrition more distinctly only at the application of extreme dose of limestone corresponds e.g. to the results of the study conducted by Crous et al. (2011) in *Pinus patula*. It is to assume that in bare-rooted plants the influence of nutrition in a forest nursery on foliage chemistry should not be obvious in two years after planting any more. Here is a further space for research on chemistry development with plantation age.

5. Conclusions

The evaluation of the condition of Carpathian birch assimilatory tissues on two research plots in the Jizerské hory Mts. provided this knowledge:

- Already in the second year after the application of Silvamix Forte and Fosmag MG fertilizers to young plants their influence on chlorophyll *a* fluorescence and pigment content was significant. Chlorophyll *a* fluorescence was influenced particularly by the combination of fertilizers, which resulted in higher phosphorus concentration in the leaves of plants of this treatment. In both fertilization treatments lower concentrations of chlorophylls and carotenoids in leaves were observed compared to the control treatment without fertilization.
- Using the selected methods, the influence of liming was still observed 17 years after planting in the older birch stand. The evaluation of chlorophyll *a* fluorescence showed significant differences in parameter S_m on high and late summer dates while the most frequently evaluated parameter F_v/F_m appeared less sensitive. The foliage of limed treatment had higher concentration of P, Ca and Mg.
- In the younger plantation the post-planting shock was confirmed, which was the most important in the treatment with combination of both fertilizers.
- Limed treatment of 17-year-old plantation despite higher content of P, Ca and Mg in leaves has lowered physiological performance as compared to control.
- Among the studied characteristics, the analysis of the content of photosynthetically active pigments had the highest capacity of distinguishing the particular treatments of the experiment. In high summer similar in-

formation was provided by parameter S_m from chlorophyll *a* fluorescence measurement. The concentration of macroelements in leaves would probably require taking a higher amount of samples to ensure the significance of results.

The results document that fertilization or liming of plants on poor mountain soils may exert prompt influence and long-term effect on the physiology of assimilatory tissues in Carpathian birch. The study of physiological characteristics can indicate the presence of improvement or stress that need not always be revealed by the evaluation of nutrient concentration or tree species morphology.

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References:

Balcar, V., Kacálek, D., 2001. Vývoj výsadeb brízy belokoré a brízy karpatské v hrebenové partii Jizerských hor. [Development of European birch and Carpathian birch plantations on the ridge part of the Jizerské hory Mts]. In: 50 let pestebního výzkumu v Opocne. Sborník z celostátní konference konané ve dnech 12. 9. - 13. 9. 2001 v Opocne. Jílovište-Strnady, Výzkumný ústav lesního hospodárství a myslivosti 2001, 193 - 200, 223pp. ISBN 80-86461-11-4

Balcar, V., Kacálek, D., 2008. Growth and health state of silver fir (*Abies alba* Mill.) in the ridge area of the Jizerské hory Mts. Journal of Forest Science 54 (11): 509 – 518

Balcar, V., Podrázský, V., 1994. Založení výsadbového pokusu v hrebenové partii Jizerských hor. [Establishment of a tree planting experiment in the ridge part of the Jizerské hory Mts]. Zprávy lesnického výzkumu 39 (2): 1 - 7

Rajender, B.S., Hooda, M.S., Polle A., 2011. Amelioration of planting stress by soil amendment with a hydrogel – mycorrhiza mixture for early establishment of beech (*Fagus sylvatica* L.) seedlings. Annals of Forest Science 68: 803 – 810

Bolhar-Nordenkampf, H.R., Götzl, M., 1992. Chlorophyllfluoreszenz als Indikator der mit der Seehöhe zunehmenden Streßbelastung von Fichtennadeln.- FBVA-Berichte (Federal Forest Research Centre, ed.) 67: 119-132

Boruvka, L., Podrázský, V., Mládková, L., Kuneš, I., Drábek, O., 2005. Some approaches to the research of forest soils affected by acidification in the Czech Republic. Soil Science and Plant Nutrition 51, 745-749

Burda, P., Nárovcová, J., 2009. Overování technologie pestování poloodrostku a odrostku v lesních školkách. [Testing of the technology for production of large-sized planting stock in forest nurseries]. Zprávy lesnického výzkumu 54 (2): 92-98

Cabral, R., O'Reilly, C., 2008. Physiological and field growth responses of oak seedlings to warm storage. New Forests 36: 159–170

Carter, G.A., Knapp, A.K., 2001. Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. Am J Bot 88 (4): 677-684

Crous, J.W., Morris, A.R., Scholes M.C., 2011. Changes in topsoil, standing litter and tree nutrient content of a *Pinus patula* plantation after phosphorus and potassium fertilization. Eur J Forest Res 130: 277–292

Demmig-Adams, B., Gilmore, A.M., Adams III, W.W., 1996. In vivo functions of carotenoids in higher plants. FASEB J. 10 (4): 403-412

Dreyer, E., Fichter, J., Bonneau, M., 1994. Nutrient content and photosynthesis of young yellowing Norway spruce trees (*Picea abies* L. Karst.) following calcium and magnesium fertilisation. Plant and Soil 160 (1): 67-78

Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression, Second Edition, Sage. 472 pp. ISBN 978-1412975148

Gloser, V., Libera, K., Orians, C.M., 2008. Contrasting below - and aboveground responses of two deciduous trees to patchy nitrate availability. Tree Physiology 28 (1). 37-44

Chambers, J.M., Hastie, T.J., 1992. Statistical models. Chapter 2 of Statistical Models in S. Eds. Chambers, J.M., Hastie T.J., Wadsworth & Brooks/Cole. 624 pp. ISBN 978-0412830402

Cha-um, S., Boriboonkaset, T., Pichakum, A., Kirdmanee, Ch., 2009. Multivariate physiological indices for salt tolerance classification in indica rice (*Oryza sativa* L. spp. *indica*). General and Applied Plant Physiology 35 (1-2): 75-87

Rehiman, K.S., Rose, R., Haase, D.L., Sabin, T.E., 2000. Effects of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence of four Pacific Northwest conifer species. New Forests 19: 171-186

Koch, W., 1976. Blattfarbstoff von Fichte (*Picea abies* (L.) Karst.) in Abhänkeit von Jahresgang, Blattalter und -typ. Photosynthetica 10: 280-290

Kooten, O. van, Snel, J.F.H., 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynthesis Research 25: 147-150

Kreutzer, K., 1995. Effects of forest liming on soil processes. Plant and Soil 168–169: 447-470

Kubelka, L., Kubícek, J., Soukup, J., Capek, J., 2010. Aplikace speciálních tabletovaných a práškových hnojiv Silvamix ®. [Application of special tabletted and pulverized fertilizers Silvamix ®.]. Online: http://www.silvamix.com/index.php?menu=10, cit: 10-08-10.

Kurasová, I., Cajánek, M., Kalina, J., Urban, O., Špunda, V., 2002. Characterization of acclimation of Hordeum vulgare to high irradiation based on

different responses of photosynthetic activity and pigment composition. Photosynthesis Research 72: 71-83

Kwak, M.J., Lee, S.H., Woo, S.Y., 2011. Physiological and biochemical trails of different water and light intensities on cork oak (*Quercus suber* L.) seedlings. African Journal of Biotechnology 10 (68): 15305-15319

Kuneš, I., Balcar, V., Zahradník, D., 2007. Influence of a planting hole application of dolomitic limestone powder and basalt grit on the growth of Carpathian birch (*Betula carpatica* W. et K.) and soil chemistry in the air-polluted Jizerské hory Mts. Journal of Forest Science 53: 505–515.

Lepeduš, H., Jurkovic V., Štolfa, I., Curkovic-Perica, M., Fulgosi, H., Cesarb, V., 2010. Changes in Photosystem II Photochemistry in Senescing Maple Leaves. Croat. Chem. Acta 83 (4): 379-386

Lichtenthaler, H.K., 1987. Chlorophyls and carotenoids: Pigments of photo synthetic biomembranes. Mcth. Enzymol. 148: 350-382

Lichtenthaler, H.K., Buschmann, C., Knapp, M., 2005. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. Photosynthetica 43 (3): 379-393

Lichtenthaler, H.K., Ac, A., Marek, M.V., Kalina, J., Urban, O., 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiology and Biochemistry 45: 577-588

Liu, Ch., Liu, Y., Guo, K., Fan, D., Li, G., Zheng, Y., Yu, L., Yang, R., 2011. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. Environmental and Experimental Botany 71: 174-183

Makeen, K., Suresh Babu, G., Lavanya, G.R., Grard, A., 2007. Studies of chlorophyll content by different methods in black gram (*Vigna mungo* L.). Int. J. Agric. Res. 2: 651-654. DOI: 10.3923/ijar.2007.651.654

Materna, J. 1963. Výživa a hnojení lesních porostu. [Nutrition and fertilization of forest stands]. Praha, Státní zemedelské nakladatelství, 227 pp

Maxwell, K., Johnson, G. J., 2000. Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany 51 (345): 659-668

Merilo, E., Heinsoo, K., Kull, O., Söderbergh, I., Lundmark, T., Koppel, A., 2006. Leaf photosynthetic properties in a willow (*Salix viminali*s and *Salix dasyclados*) plantation in response to fertilization. Eur J Forest Res 125: 93-100

Minotta, G., Pinzauti, S., 1996. Effects of light and soil fertility on growth, leaf chlorophyll content and nutrient use efficiency of beech (*Fagus sylvatica* L.) seedlings. Forest Ecology and Management 86 (1/3): 61-71

Mohammed, G. H., Binder, W. D., Gillies, S. L., 1995. Chlorophyll fluorescence: a review of its practical forestry applications and instrumentation. Scandinavian Journal of Forest Research, 10 (4): 383-410

Nemec, A., 1956. Meliorace degradovaných lesních pud. [Amelioration of de-

graded forest soils]. Praha, Státní zemedelské nakladatelství, 291 pp

Nilsson, L.O., Hüttl, R.F., Johansson, U.T., Jochheim, H., 1995. Nutrient uptake and cycling in forest ecosystems - present status and future research directions. Plant and Soil, 168-169: 5-13

Óskarsson, H., Sigurgeirsson, A., Raulund-Rasmussen, K., 2006: Survival, growth, and nutrition of tree seedlings fertilized at planting on Andisol soils in Iceland: Six-year results. Forest Ecology and Management, 229: 88–97

Peñuelas, J., Filella, I., 1998. Visible and near-infrared reflectance techniques for diagnosing plant physiological status. Trends in Plant Science 3:151-156

Percival, G. C., Galloway, A., 1999. The potential of chlorophyll fluorescence measurements to detect salt and waterlogging stress in urban trees. Acta Horticulturae 496: 253-259

Podrázský, V., 1991. Krátkodobé úcinky vápnení v extrémních imisne ekologických podmínkách Orlických hor. [Short-term effect of liming in the extremely polluted ecological conditions of the Orlické hory Mts]. Lesnictví 37 (12): 1009-1023

Podrázský, V., 1999: Význam vápnení a prirozené sukcese pro obnovu Jizerskohorských lesu. In: Obnova a stabilizace horských lesu. [Purpose of liming and natural succession for regeneration of the Jizerské hory Mts.]. Sborník z celostátní konference s mezinárodní úcastí. Bedrichov v Jizerských horách, 12.-13. 10. 1999. M. Slodicák (Ed.). Jílovište-Strnady, VÚLHM 1999: 39-45. ISBN 80-902615-4-X

Podrázský, V., Ulbrichová, I., 2003. Surface liming of immission clear-cuts: benefits and risks. Ekológia 22 (1): 277-283

Porra, R.J., Thompson, W.A., Kreidemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectrometry. Biochim Biophys Acta 975: 384-394

Rabe, R., Kreeb, K.H., 1980. Bioimdication of air pollution by chlorophyll destruction in plant leaves. OIKOS 34: 163-167

Richardson, A.D., Duigan, S.P., Berlyn, G.P., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153: 185-194

Savonen, E.M., Sarjala, T., 1998. Effect of potassium availability on in vivo chlorophyll fluorescence and polyamines of Scots pine seedlings. Aquilo. Ser. Botanica, 37: 7-14

Schreiber, U., 2004. Pulse-Amplitude-Modulation (PAM) Fluorometry and Saturation Pulse metod: An Overview. In: Chorophyll a fluorescence: a signature of photosynthesis. Ed. Papageorgiou, G.C. Dordrecht, Springer: 279-319

Slodicák, M. et al., 2005. Lesnické hospodarení v Jizerských horách. [Forestry

management in the Jizerské hory Mts]. Hradec Králové, Lesy Ceské republiky; Jílovište-Strnady, VÚLHM, 232 pp. ISBN 80-86945-00-6 (LCR); ISBN 80-86461-51-3 (VÚLHM)

Stirbet, A., Govindjee, 2011. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient. J. Photochem. Photobiol. B: Biol. 104 (1-2): 236-257. doi: 10.1016/j.jphotobiol.2010.12.010

Strand, M., Lundmark, T., 1995. Recovery of photosynthesis in 1-year-old needles of unfertilized and fertilized Norway spruce (*Picea abies* (L.) Karst.) during spring. Tree Physiology, 15 (3): 151-158

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 1999. Screening the vitality and photo-synthetic activity of plants by fluorescent transient. Behl, R.K., Punia, M.S., Lather, B.P.S., (Eds.): Crop Improvement for Food Security. SSARM, Hisar, India: 72-115

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. Yunus, M., Pathre, U., Mohanty, P., (Eds.): Probing Photosynthesis: Mechanism, Regulation and Adaptation., Taylor and Francis, UK, Chapter 25: 445-483

Strasser, R. J., Tsimilli- Michael, M., Srivastava, A., 2004. Analysis of the Chlorophyll a Fluorescence Transient. G.C., Papageorgiou, Govindjee, (Eds.): Chlorophyll a fluorescence: a signature of photosynthesis. Advances in Photosynthesis and Respiratio 19: 321-362

Špulák, O., Balcar, V., Kacálek, D., 2011. Vliv mletého vápence v jamce na prosperitu výsadeb, fluorescenci chlorofylu a obsah živin v listech brízy karpatské a buku lesního. [Effect of ground limestone in planting hole on plantation prosperity, chlorophyll fluorescence and foliar nutrients of Carpathian birch and European beech]. Zprávy lesnického výzkumu 56 (Special): 57-64

Úradnícek, L., Madera, P, Tichá, S., Koblížek, J., 2009. Dreviny Ceské republiky. 2. prepracované vydání. [Wood plants of the Czech Republic. 2nd edition.]. Lesnická práce. Kostelec nad Cernými lesy. 366 pp

Vacek, S. et al., 2003. Mountain forests of the Czech Republic. [Prague], Ministry of agriculture of the Czech Republic. 311 pp. ISBN 80-7084-240-7

Zbíral, J., 1994. Analýza rostlinného materiálu. Jednotné pracovní postupy. [Analysis of plant material. Unified methods of operation.]. Brno, SKZUZ: separate pagination.