FOLIA OECOLOGICA – vol. 35, no. 2 (2008). ISSN 1336-5266

Variability of chlorophyll and nitrogen content in the leaves of two-year-old seedlings of European chestnut (*Castanea sativa* Mill.) of different origin

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Abstract

Bolvanský, M., Užík, M., Šalgovičová, A., Žofajová, A. 2008. Variability of chlorophyll and nitrogen content in the leaves of two-year-old seedlings of European chestnut (*Castanea sativa* Mill.) of different origin. *Folia oecol*., 35: 9–17.

Two-year-old seedlings of European chestnut derived from twelve 45year-old progenies grown at the permanent experimental plot in Horné Lefantovce were used in the study. The progenies originated from 12 old mother trees grown on four different localities of Slovakia (Jelenec, Horné Lefantovce, Tlstý Vrch and Duchonka). In 2006, at three different dates (June, August, September), indirect measurement of chlorophyll content by a portable device Chlorophyll Content Meter CL-01 (fi. Hansatech) was carried out in 180 seedlings (15 ones in each progeny). In August and September, also direct determination of chlorophyll *a* and *b* content was carried out in 81 seedlings from 9 progenies (after excluding progenies from Duchonka). In the leaves collected in August, nitrogen content was determined with a CNS 2000 Analyzer (LECO corp., USA). Variability of CL-01 values and chlorophyll content values was highly significantly influenced by date of measurement (the lowest CL-01 values in June), individual progenies and provenience (origin) of parental trees. The most distinct differences between the three studied proveniences were observed in chlorophyll *a*+*b* content in the August sampling. Correlation between N content and chlorophyll content was medium strong (r = 0.413 and 0.419).

Key words

chlorophyll *a* and *b*, CL-01 values, nitrogen, progenies, localities

Introduction

Characterisation of genotypes or populations of woody plant species by the chlorophyll content and related bioelements is not very common, but in the last decades many studies occur on indirect chlorophyll content determination in assimilatory organs of crops, grassland and woody plants. In these studies predominantly portable chlorophyll meter SPAD-502 (Minolta, Japan) has been used.

Data obtained by this device were utilized to predict nitrogen content in plants and subsequently to determine an optimal level of nitrogen nutrition in crops and grass (Wood et al., 1993; Gáborčík, 1999; Shapiro,1999). In winter wheat and barley, variability of SPAD values was significantly influenced by cultivars and was in strong correlation with nitrogen content (Užík and Žofajová, 1998; Užík et al., 1999; Užík and Žofajová, 2000a; Užík and Žofajová, 2000b). The authors recommended this method for the selection of genotypes with different N-uptake and N-use efficiency. By SPAD values also Mg content in dry matter of grassland were examined and differences between grass species and cultivars were observed (Míka, 1980). Fanizza et al.(1991) observed that SPAD values measured in apical, fully developed leaves of *Vitis vinifera* are good indicator for screening genotypes on drought tolerance. Hoel (2002) proved, by measurement with a Hydro N-Tester (HNT), a portable chlorophyll meter, differences in leaf chlorophyll content between cultivars of winter wheat.

Studies of chlorophyll content in leaves of woody plant species with the aim to detect genotype differences or origin are very sporadic. Bonneville and Fyles (2005) estimated the relative chlorophyll content and leaf nutrient concentration by the SPAD-502 chlorophyll meter in the leaves of trembling aspen. The best representation of overall leaf chlorophyll was found when six SPAD readings were taken at different locations on each leaf. There was a positive correlation between overall leaf N and estimated chlorophyll content, especially in the top part of the trees. Ichie et al. (2006) reported a significant positive correlation between the actual chlorophylle content and the SPAD-502 readings and less positive correlation between the actual N content and the SPAD-502 readings in leaves of 10 woody plant species. Based on the results of Bauerle et al. (2006), the SPAD meter could be used to provide a rapid estimate of leaf absorbance and transmittance in the 400–700 nm wavelength range in woody plant species.

Within eco-physiological research, our present investigations have been aimed at the measurement of selected physiological characteristics (parameters of chlorophyll *a* fluorescence, chlorophylls *a, b* content) and subsequent evaluation of physiological status of introduced woody species *Quercus rubra* L., *Juglans nigra* L., *Castanea sativa* Mill., growing under different stand conditions at permanent experimental plots (Kmeť and Šalgovičová, 2003, 2004).

The main objective of this work is to examine the possibility to exploit data of indirect measurement of chlorophylls *a* and *b* for detection of different origin of European chestnut progenies and also for early test of growth performance and production abilities of these progenies.

Material and methods

Material and sampling

The field part of the study was carried out in the forest nursery within area of village Lovce. This nursery called officially Nursery center Hladomer belongs under the national company Forests of the Slovak Republic, enterprise Semenoles Liptovský Hrádok. It is situated at an altitude of 310 m asl at the foothill of the Tribeč Mountain. Soil in the forest nursery is clayey and on the place with our experimental plot has pH 7.2, content of phosphorus 233 mg, potassium 231 mg and magnesium 295 mg per 1kg of dry soil.

Fruits for growing seedlings were collected from 45year-old progenies derived from 12 old mother trees grown on four different localities of Slovakia, 3 trees from each locality (Jelenec, Horné Lefantovce, Tlstý Vrch and Dolné Príbelce). From each progeny at the time of fruit fall in 2004, about 150 fruits were randomly collected and stored in plastic bags in refrigerator at temperature of about 5 °C. In the mid of April 2005, healthy, germinated or not germinated 90 fruits, were selected from each of 12 samples then divided to three batches of 30 pieces and planted in randomized blocks in three replications.

During the vegetation period 2006, in each of 12 F2 progenies 15 seedlings, 5 in each replication, showing different growth intensity, were selected for investigations. Indirect measurement of chlorophyll content was carried out in all 180 selected seedlings while direct measurement, because of its technical demands and time consuming character, was carried on the reduced number of 81 seedlings in the reduced number of progenies (9 progenies after omitting progenies from Duchonka).

Indirect measurement of chlorophyll content

Indirect measurement was carried out on three successive dates during the vegetation season (June, July, September), each time on 5 leaves selected in acropetal order on the current main shoot or on the most vigorous lateral shoot (in September). At the first sampling date, in June, measurement was done directly on the plants without tearing off leaves. At the second and third dates the leaves were torn off, transferred in the portable cool-box to the laboratory and subjected to the measurement on the same or next day.

Indirect measurement of chlorophyll content was carried out with a portable device Chlorophyll Content Meter CL-01 (fi. Hansatech). Measurements were made either on two spots of leaf – in its middle and on its top or on three leaf spots – on the base, in the middle and on the top, always on the left side of leaf (viewed to the leaf face). Only at one date, CL-01 values were measured on both the left and the right half of the leaf with the aim to assess differences in CL-01 values between the left and right leaf half.

Direct determination of chlorophyll content

Direct determination of chlorophyll content was carried out two times in a season (August, September) immediately after measurement of CL-01 values.

Leaf samples for quantitative analysis of chlorophylls were collected by disc method. Circular discs with diameter of 8.3 mm were cut off with a metal borer on two places – in the middle and on the top of leaf on both right and left half of the leaf. Thus ten discs from the middle and ten discs from the top of leaf obtained per seedling were analyzed separately. Leaf discs were homogenized and processed according to the routine method. Absorbance values of chlophylls *a* and *b* were determined by a spectrophotometer Spekol-211. Calculation of chlorophyll content was done according to Lichtenthaler (1987) applied for weight unit of leaf dry matter (mg g^{-1}).

Determination of nitrogen content

The same leaf samples that were used for indirect and direct chlorophyll content measurements respectively were also used for the analysis of nitrogen content. The leaves were dried up in a drier at 65 °C until their weight remained stabile. The dried leaves were processed in two ways: first they were cut to small pieces and from this sample a batch of 0.2 g was analyzed and then the pieces were grounded with a mixer to powder from which also 0.2 g was analyzed. Nitrogen content was determined on CNS 2000 (LECO corp., USA) Analyzer working on principle of Dumas method (at 1,000 °C). As this method also allows to determine sulfur and carbon simultaneously with nitrogen so these two elements were also determined in our samples.

Data evaluation

Data obtained from indirect measurement of chlorophyll content (values CL-01), direct measurement of chlorophylls *a, b*, determination of N, C and S and morphometric data of the studied seedlings were evaluated by multifactorial analysis of variance – hierarchic model GLM and correlation analysis using statistic program package STATGRAPHIC PLUS 5 for Windows.

Results

Variability of CL-01 values

CL-01 values, obtained using the chlorophyllmeter device, were highly significantly influenced by all assumed sources of variance: spacial replication of the trial, date of measurement (June, August, September), provenience of mother trees from which F1 generation was derived (Tlstý Vrch, Horné Lefantovce, Jelenec, Duchonka), progeny of F1 generation within provenience (3 progenies within provenience), leaf position on the annual shoot (5 leaves in acropetal order) (Table 1). Also when CL-01 data were analysed separately on particular sampling dates, significant effect of progenies, leaf position and reading position on the leaf was proved influencing their variation. However, no one of the assumed interactions was proved as significant (Table 2).

In average, the lowest CL-01 values were observed on the first sampling date in June, nearly two-times lower than in August and September dates, respectively (Table 3). Localities were ranked as follows, from the lowest to the highest CL-01 values: Jelenec, Horné Lefantovce, Tlstý Vrch, Duchonka. There were, however, differences between progenies within each origin, and they were very rare ranked into one homogenous group in case when evaluation was done for all dates total. In case of single dates, progenies from one origin occurred more often in one homogenous group (Table 3). For instance, at the first date all progenies of Duchonka and Horné Lefantovce origin and at the second date all progenies of Tlstý Vrch were in a single group. At the third date maximum two progenies of one origin occurred in a single group.

Ranking the progeny means of CL-01 values was similar in all the three sampling dates what also correlation coefficients ($r = 0.61–0.67$) between CL-01 values from particular samplings suggest (Table 4). Some progenies showed at each date either the highest (HL A) or the lowest CL-01 values (DP 3). However, correlation between CL-01 means for seedlings from particular dates was very low ($r = 0.13–0.36$). Individual variation of CL-01 values within particular progeny was rather high in each date and could be affected by different environmental factors.

Table 1. Results of the multiple analysis of variance of CL-01 values obtained from measurement in the leaves of two-year-old seedlings derived from 12 progenies of European chestnut

Source of variation	Df	MS
Spatial replication of trial	2	1,137.84**
Date of measurement	2	15,648.1**
Origin (locality)	3	232.32**
Progeny (within locality. date and replication)	72	139.5**
Date × locality	6	240.02**
Leaf	4	80.25**
Residual	6,656	6.23
Total	6,745	

** $P < 0.01$

Table 2. Results of three multiple analyses of variance of CL-01 values obtained from measurement in the leaves of two-year-old seedlings at three different dates (June, August, September)

Source of variation	Df	MS – June	MS – August	MS – September
Progeny	11	49.1018**	174.955**	175.064**
Leaf	4	136.222**	223.218**	52.2187**
Position on leaf	1	93.5165**	279.682**	253.027**
Progeny x leaf	44	3.7709	9.3846	6.1807
Progeny x position	11	1.2515	1.0263	1.9919
Leaf x position	4	0.6145	1.0438	0.7306
Residual	1,724	2.9847	7.4927	11.9251
Total	1,799			

Table 3. Results of the multiple comparison of LS means of CL-01 values measured in three different dates (June, August, September) in two-year-old seedlings derived from 12 progenies of European chestnut of different origin (TV – Tlstý Vrch; HL – Horné Lefantovce; D – Duchonka; J – Jelenec)

June

Progeny	CL01 Mean	Homogenous groups					
J 11	4.04	×					
J 5	4.50		×				
D 3	4.63		×	×			
D 13	4.67		×	×			
TV 8	4.92			×			
D 5	4.95			×	×		
J 2	5.33				×	×	
TV 2	5.34				×	×	
HL 17	5.44					×	
TV 9	5.47					×	
HL 18	5.68					×	
HLA	6.08						×
Mean	5.09						

August

Progeny	CL01 Mean	Homogenous groups				
D 3	8.52	×				
J 11	8.95	×	×			
D 13	9.00		×			
J 5	9.12		×			
HL 17	9.25		×			
J 2	9.42		×	×		
HL 18	9.86			×		
D 5	10.30				×	
TV 8	10.44				×	
TV 2	10.52				×	×
TV 9	10.68				×	×
HLA	10.90					×
Mean	9.75					

September

Progeny	CL01 Mean	Homogenous groups				
D 3	7.96	×				
J 11	8.71	×				
TV 2	9.84		×			
J 2	9.89		×			
J 5	10.38		×	×		
TV 8	10.49		×	×	×	
HL 17	10.53		×	×	×	
D 13	10.86			×	×	×
HL 18	11.02			×	×	×
TV 9	11.12				×	×
D 5	11.36					×
HLA	11.59					×
Mean	10.32					

Table 4. Correlation coefficients between CL01 obtained in three different dates (June, August, September) based either on seedlings means (n = 180) or on progeny means (n = 12)

	June	August
August	0.3583**	
n = 180	0.6737*	
n = 12		
September		
n = 180	0.1277	0.2946**
n = 12	0.6139*	0.6734*

** P < 0.01

Variability of chlorophylls *a* and *b* content

Content of both chlorophylls in leaves of chestnut seedlings was significantly influenced by origin of progenies and by progenies themselves and seedlings within progeny. Collecting date influenced significantly only content of chlorophyll *b* and replication of trial influenced content of chlorophyll *a* (Table 5).

In August, content of both chlorophylls was the lowest in seedlings of Jelenec origin, and the highest in seedlings of Tlstý Vrch origin (Table 6). On the contrary, in September, seedlings of Jelenec origin exhibited on average the highest while seedlings of Tlstý Vrch origin the lowest content of both chlorophylls. On both dates, content of chlorophyll *a* was highly significantly influenced by trial replications – the highest chlorophyll *a* content was observed in the third replication and the lowest one in the first replication.

Variability of characteristics related to the chlorophyll content

Nitrogen concentration in the leaves of 81 seedlings was not significantly affected by any of the assumed sources of variation (Table 7). Carbon and sulphur concentration in the same seedlings was highly significant affected by the progeny origin but only in the variant of analysis based on the grounded leaf matter. Concentration of both elements was higher in the progenies of Tlstý Vrch origin (Table 8).

Weight of leaf dry matter was statistically significant influenced by replication of experiment and progeny origin. It was significantly higher in seedlings of Horné Lefantovce origin than in the remaining seedlings.

Height and diameter of seedlings were significantly influenced by replications. The highest seedlings were in the first replication and the lowest ones in the third. Seedling height varied also by progeny origin.

Table 5. Results of the analyses of variance of chlorophyll *a*, chlorophyll *b* and chlorophyll *a*+*b* data obtained in two sampling dates (August. September) in leaves of two-year-old seedlings of European chestnut derived from 9 progenies of three different origins

Source of variation	Df	MS Chlorophyll *a*	MS Chlorophyll *b*	MS Chlorophyll *a*+*b*
Origin	2	1.3245**	0.3567**	3.0419**
Progeny (within origin)	6	0.5452**	0.0591*	0.8568*
Seedling (within progeny)	34	0.3059*	0.0402*	0.5587*
Date of sampling	1	0.2255	0.5803**	1.5305*
Position on leaf	1	0.0861	0.0313	0.2214
Replication of trial	2	0.7557*	0.0524	1.2060*
Date × position	1	0.0082	0.0004	0.0052
Date × seedling	34	0.1936	0.0220	0.3339
Position × seedling	34	0.0455	0.0055	0.0805
Residual	208	0.1782	0.0237	0.3216
Total	323			

* $P < 0.05$, ** $P < 0.01$

Table 6. Mean values of chlorophyll *a* and chlorophyll *b* content (in mg g^{-1} of dry matter) in the leaves of two-year-old seed lings derived from 12 progenies of European chestnut

	Chlorophyll *a*		Chlorophyll *b*	
Progeny	August	September	August	September
HL 17	1.58	1.68	0.56	0.51
HL 18	1.97	1.58	0.72	0.49
HL A	1.70	1.75	0.66	0.50
Mean	**1.75**	**1.67**	**0.64**	**0.50**
J 2	1.66	1.67	0.43	0.52
J 5	1.48	1.80	0.52	0.56
J 11	1.27	1.83	0.47	0.58
Mean	**1.47**	**1.76**	**0.47**	**0.55**
TV 2	1.62	1.59	0.63	0.53
TV 8	2.40	1.56	0.88	0.51
TV 9	2.10	1.58	0.70	0.49
Mean	**2.04**	**1.57**	**0.73**	**0.51**
Mean	**1.75**	**1.67**	**0.61**	**0.52**

Table 7. Results of the analyses of variance (GLM model) of 14 characteristic values measured in 81 two-year-old seedlings of European chestnut derived from 9 progenies of different origin. All data except those for height and diameter of seedlings obtained from one sampling in August 2006

Variable	Mean squares (MS) for sources of variation				
	Replication of trial	Origin of progenies	Progeny within origin	Tree within progeny	Residual
Chlorophyll *a*	0.4088	2.0518**	0.6594**	0.1540	0.1955
Chlorophyll *b*	0.0239	0.4151**	0.0622*	0.180	0.2778
Chlorophyll *a* + *b*	0.630	4.2662**	1.0390*	0.2706	0.3597
CL01	29.919**	6.170	6.390	3.8760	4.0761
N[1]	0.0747	0.0314	0.0407	0.0445	0.0828
C[1]	0.5030	65.6188**	11.0921	4.1249	5.8407
S[1]	0.0839	0.8704**	0.0887	0.0623	0.0571
N_1[2]	0.080	0.0067	0.0734	0.0491	0.0950
C_1[2]	0.0203	1.0348	1.8502	1.3081	1.3382
S_1[2]	0.0000	0.0929	0.0612	0.0513	0.0643
Dry matter	1.4073*	1.4469*	0.3941	0.3168	0.3648
Leaf disk	0.2048	0.1604	0.0757	0.0880	0.1290
Stem height	7,036.67**	1,066.54*	198.67	269.58	209.05
Stem diameter	90.263**	9.871	10.234	9.600	9.458

Df for individual sources of variations are as follows: replication – 2, origin – 2, progeny within origin – 6, tree within progeny – 34, residual – 36. Df total (corrected) – 80
* $P < 0.05$, ** $P < 0.01$
[1]Data obtained from cut leaf samples, [2]Data obtained from grounded leaf samples

Relationship between characteristics related to the chlorophyll content

For correlation analysis between characteristics related to the chlorophyll, data from August sampling served as a basis (Table 9). At this date, medium strong positive correlation (r = 0.61 and 0.58) was calculated between values of CL-01 and chlorophylls *a* and *b* observed in particular seedlings. On the contrary, on the September date, very low correlation was between the mentioned two values (r = 0.22 and 0.17). Between nitrogen content analyzed from grounded leaves (N1) and content of directly measured chlorophylls *a* and *b* as well as CL-01 values, medium strong correlation was observed. Morphological characteristics of seedlings (leaf dry matter, stem height, stem diameter) did not correlate with chlorophyll, CL-01 and N data.

Discussion

Chlorophyll content in leaves may vary depending on both internal and external factors. The leaves grown in shade have higher chlorophyll content per weight unit compared with the leaves exposed to the sunshine (Masarovičová and Štefančík, 1990; Gratani and Foti, 1998). The higher content of the chlorophylls *a* and *b* was observed also in the leaves of black walnut, red oak and European chestnut, grown in the shade of canopy (Kmeť et al., 2001; Kmeť and Šalgovičová, 2003). It is also known seasonal fluctuation of chlorophylls *a* and *b*, which may display a various course (Duda and Masarovičová, 1976). The ratio between chlorophyll *a* and chlorophyll *b* is not fixed. Usually the content of chlorophyll *a* is three times higher than the content of chlorophyll *b* (ratio 3 : 1). Our results have proved this ratio and also seasonal fluctuation of chlorophyll content. In addition to that, also significant effect of provenience of parental trees on chlorophyll content in seedlings was observed.

In winter wheat, SPAD values (values obtained from indirect measurement of chlorophyll content, similar to CL-01 values) strongly correlated with N content in soil and with the wheat genotype and varied according to the reading position on the leaf (Užík and Žofajová 2000; Užík and Žofajová, 2003). The lowest SPAD value was read on the top of leaf and the highest one in the middle of the leaf what is in accord with our results.

Correlation between CL-01 values and content of chlorophyll *a* and *b* varied according to the date of measurement. While in the measurement at the beginning of August was this correlation medium strong, in the measurement in mid September it was low and not significant. Increasing or decreasing CL-01 values as well as chlorophyll content values from August

Table 8. Least square means and standard errors (in parenthesis) of physiological and morphological character values measured in 81 two-year-old seedlings of European chestnut derived from 9 progenies of different origin (locality). All data except height and diameter of seedlings obtained from the sampling in August 2006. Total means in single rows labeled with different letters are significantly different at $p < 0.05$ according to LSD test

Character (unit)	Locality/progeny											
	Tlstý Vrch				Horné Lefantovce				Jelenec			
	2	8	9	**Total**	A	17	18	**Total**	2	5	11	**Total**
Chlorophyll *a* [mg g^{-1}]	1.5913 (0.1617)	2.4147 (0.1617)	2.1948 (0.1617)	**2.0669 a (0.0933)**	1.6443 (0.1617)	1.5826 (0.1497)	1.9491 (0.1534)	**1.7253 b (0.0895)**	1.6592 (0.1630)	1.4661 (0.1616)	1.2609 (0.1617)	**1.4621 b (0.0936)**
Chlorophyll *b* [mg g^{-1}]	0.6230 (0.0609)	0.8785 (0.0609)	0.7317 (0.0609)	**0.7442 a (0.0352)**	0.6428 (0.0609)	0.5645 (0.0564)	0.7180 (0.0578)	**0.6417 a (0.0337)**	0.4330 (0.0614)	0.5176 (0.0609)	0.4719 (0.0609)	**0.4742 b (0.0353)**
Chlor. *a* + *b* [mg g^{-1}]	2.2217 (0.2233)	3.306 (0.2233)	2.9342 (0.2233)	**2.8206 a (0.1289)**	2.2667 (0.2233)	2.1471 (0.207)	2.6541 (0.2118)	**2.356 b (0.1237)**	2.0828 (0.2250)	1.9635 (0.2233)	1.746 (0.207)	**1.9308 c (0.1293)**
CL01 [no unit]	10.0527 (0.8514)	11.1835 (0.8514)	10.2687 (0.8514)	**10.5016 a (0.4915)**	11.4012 (0.8514)	8.7795 (0.7892)	9.8379 (0.8077)	**10.0062 a (0.4915)**	9.1293 (0.858)	9.6469 (0.8514)	9.2108 (0.8514)	**9.329 a (0.4928)**
N [mg g^{-1}]	1.9402 (0.1052)	1.9846 (0.1052)	1.9129 (0.1052)	**1.9459 a (0.0607)**	1.9375 (0.1052)	1.9140 (0.0974)	1.8096 (0.0998)	**1.8870 a (0.0582)**	1.9802 (0.1061)	1.8698 (0.1052)	1.7798 (0.1052)	**1.8766 a (0.0610)**
C [mg g^{-1}]	43.949 (0.8836)	44.185 (0.8836)	46.194 (0.8836)	**44.776 a (0.5102)**	43.485 (0.8836)	44.1 (0.8181)	41.13 (0.8384)	**42.905 b (0.4893)**	41.001 (0.8911)	41.801 (0.8836)	41.240 (0.8836)	**41.350 b (0.5120)**
S [mg g^{-1}]	1.0944 (0.0873)	0.8369 (0.0873)	0.8866 (0.0873)	**0.9393 a (0.0504)**	0.8070 (0.0874)	0.7593 (0.0809)	0.5869 (0.0829)	**0.7177 b (0.0484)**	0.6011 (0.0881)	0.5229 (0.0873)	0.5118 (0.0873)	**0.5453 c (0.0506)**
Leaf dry matter [g]	2.1847 (0.2208)	1.9398 (0.2208)	1.9975 (0.2208)	**2.0407 ac (0.1275)**	2.5971 (0.2208)	2.3698 (0.2446)	2.0927 (0.2095)	**2.3532 b (0.1223)**	1.6803 (0.2227)	2.1761 (0.2208)	1.7322 (0.2208)	**1.8629 ac (0.1280)**
Leaf disc [mg]	84.578 (4.211)	76.163 (4.211)	77.313 (4.211)	**79.351 a (2.431)**	74.27 (4.211)	74.029 (3.903)	73.01 (3.994)	**73.77 a (2.331)**	72.196 (4.243)	76.57 (4.21)	78.55 (4.21)	**75.772 a (2.437)**
Height of stem [cm]	47.7 (8.705)	46.567 (8.705)	45.667 (8.705)	**46.644 a (5.026)**	69.0 (8.705)	51.229 (8.070)	60.3 (8.259)	**60.176 b (4.820)**	43.833 (8.773)	51.967 (8.705)	46.05 (8.705)	**47.28 a (5.039)**
Stem diam. [mm]	14.148 (1.352)	11.699 (1.352)	12.523 (1.352)	**12.79 a (0.781)**	15.791 (1.352)	12.868 (1.253)	13.738 (1.283)	**14.132 a (0.749)**	13.198 (1.363)	14.306 (1.352)	14.606 (1.352)	**14.037 a (0.783)**

to September sampling was not regular in individual seedlings but certain regularity occurred in mean values for individual progenies especially for CL-01 values. Mean chlorophyll values varied mostly irregularly – for instance in seedlings of Tlstý Vrch and Horné Lefantovce origin decreased but in seedlings of Jelenec origin increased from August to September. Explanation of these results needs apparently a new experiment and especially new analysis of chlorophyll content on a new device. Namely the old device for determination of chlorophyll content might be the reason of low correlation between values of direct and indirect chlorophyll measurements observed in our work. Literature data point mostly at strong correlations between chlorophyll content and SPAD values (Castelli et al., 1996; Yamameto et al., 2002; Ichie et al., 2006)

Acknowledgement

The work was supported by the Slovak Grant Agency VEGA through grants for projects 2/4157/04 and 2/7165/27.

References

Bauerle, W.L., Weston, D.J., Bowden, J.D., Dudley, J.B., Toler, J.E. 2006. Leaf absorbance of photosynthetically active radiation in relation to the chlorophyll meter estimates among woody plant species. *Sci. Hort.,* 101: 169–178.

Bonneville, M.C., Fyles, J.W. 2005. Assessing variations in SPAD-502 chlorophyllmeter measurements and their relationships with nutrient content of trembling aspen foliage. *Comm. Soil Sci. Pl. Anal.*, 37: 525–539.

Bolvanský, M., Šalgovičová, A., Užík , M. 2005. Vplyv lokality a genotypu na variabilitu chlorofylu v listoch gaštana jedlého. In Zima M., Boleček, P., Omelka, R.. *Progres v biológii: 4. Biologické dni: zborník referátov z medzinárodnej konferencie, 8.–9. september 2005, Nitra.* Nitra: Univerzita Konštantína Filozofa, p. 114–116.

Duda, M., Masarovičová, E. 1976. Príspevok k ekofyziologickému štúdiu asimilačných pigmentov dominantných drevín dubovo-hrabového lesa v Bábe. *Acta Mus. Siles., Ser. C, Dendrol.*, 25: 35–44.

Fanizza, G., Ricciardi, L., Bagnulo, C. 1991. Leaf greenness measurements to evaluate water stressed genotypes in Vitis vinifera. *Euphytica*, 55: 27–31.

Gáborčík, N. 1999. Detekcia koncentrácie horčíka v listoch rastlín prenosným chlorofylmetrom. *Poľnohosp. Výr. Skúšob.*, 7: 28–30.

Gratani, L., Foti, I. 1998. Estimating forest structure and shade tolerance of the species in a mixed deciduous - broad leaved forest in Abruzzo, Italy. *Ann. Bot. Fenn.,* 35: 75–83.

Hoel, B.O. 2002. Chlorophyll meter readings in winter wheat: cultivar differences and prediction of grain protein content. *Acta Agric. scand.*, *Sect. B, Soil and Pl. Sci*., 52: 147–157.

Ichie, T., Kitahashi, Y., Matsuki, S., Maruyama, Y., Koike, T. 2006. The use of a portable non-destructive type nitrogen meter for leaves of woody plants in field studies. *Photosynthetica*, 40: 289–292.

Kmeť, J., Šalgovičová, A. 2003. Ecophysiological aspects of growth of European chestnut (Castanea sativa Mill.) in Slovakia. *Folia oecol.*, 30: 141–147.

Table 9. Correlation matrix (Pearson correlation coefficents) for physiological and morphological characteristics based on data obtained from 81 two-year-old seedlings of European chestnut derived from 9 progenies of different origin

Characteristics	Chl_*a*	Chl_*b*	Chl_*a*+*b*	CL01	N	N1	C	S	Dry matter	Height
Chl_*b*	**0.906****									
Chl_*a* + *b*	**0.993****	**0.949****								
CL01	**0.607****	**0.584****	**0.612****							
N	0.206	0.118	0.186	**0.302****						
N1	**0.357****	**0.278***	**0.342****	**0.413****	**0.701****					
C	0.132	0.136	0.136	0.089	**0.336****	–0.049				
S	0.040	0.094	0.055	–0.086	0.200	0.092	0.252			
Dry matter	–0.112	–0.065	–0.102	0.000	0.273	0.220	0.201	0.106		
Height	–0.134	–0.036	–0.110	–0.084	–0.021	0.097	–0.108	–0.036	**0.554****	
Diameter	–0.252*	–0.239*	–0.253*	–0.013	0.037	0.087	–0.069	–0.067	**0.484****	**0.736****

* $P < 0.05$, ** $P < 0.01$

Kmeť, J., Šalgovičová, A., Konôpková, J. 2001. Vybrané fyziologicko-biochemické charakteristiky asimilačného aparátu duba červeného (Quercus rubra L.) a orecha čierneho (Juglans nigra L.) rastúcich v rozdielnych stanovištných podmienkach. *Folia oecol.,* 28: 67–80.

Lichtenthaler, H.K. 1987. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Meth. Enzymol.*, 148: 350–382.

Masarovičová, E., Štefančík, L. 1990. Some ecophysiological features in sun and shade leaves of tall beech trees. *Biol. Plant.,* 32: 374–387.

Shapiro, CH.A. 1999. Using a chlorophyll meter to manage nitrogen applications to corn with high nitrate irrigation water. *Commun. Soil Sci. Pl. Anal*, 30: 1037–1049.

Šalgovičová, A., Kmeť, J. 2004. Influence of site conditions on physiological status of black walnut (*Juglans nigra* L.) stands. *Folia oecol.,* 31: 100–110.

Míka, V. 1980. *Minerální látky v trávach*. Praha: Academia. 105 p.

Upov 1989. *Guidelines for the conduct of tests for distinctness, homogeneity and stability. Chestnut (Castanea sativa Mill.).* TG/124/3 (Paris). 23 p.

Užík, M., Žofajová, A. 1998. Vplyv genotypu a výživy na obsah chlorofylu a N v listoch ozimnej pšenice. In *Nové poznatky z genetiky a šľachtenia poľnohospodárskych rastlín: zborník zo 4. odborného seminára Piešťany 26. November 1998.* Piešťany: Výskumný ústav rastlinnej výroby, p. 92–98.

Užík, M., Žofajová, A., Bieliková, M. 1999. Variabilita obsahu chlorofylu (SPAD) a dusíka v listoch odrôd jarného jačmeňa. In *Ved. práce Výsk. Úst. rast. Výr. Piešťany*, 29: 79–88.

Užík, M., Žofajová, A. 2000a. Vplyv odrôd a výživy na obsah chlorofylu (SPAD) a N v listoch jarného jačmeňa. *Poľnohospodárstvo,* 46: 587–604.

Užík, M., Žofajová, A. 2000b. Chlorophyll and nitrogen content in leaves of winter wheat at different genotypes and fertilization. *Rostl. Výr.,* 46: 237–244.

Variabilita obsahu chlorofylu a obsahu dusíka v listoch dvojročných semenáčikov gaštana jedlého rôzneho pôvodu

Súhrn

Ako pokusný materiál boli v práci použité dvojročné semenáčiky gaštana jedlého vypestované v lesnej škôlke Hladomer pri obci Lovce z plodov zozbieraných v roku 2004 na trvalej experimentálnej ploche v Horných Lefantovciach z 12 rôznych potomstiev vo veku 45 rokov. Tieto potomstvá pochádzali z 12 starých stromov zo štyroch lokalít Slovenska, tri stromy z každej lokality (Jelenec, Horné Lefantovce, Tlstý Vrch a Dolné Príbelce). V roku 2006 v troch rôznych termínoch (jún, august, september) sa robilo nepriame meranie obsahu chlorofylu v listoch semenáčikov pomocou prenosného prístroja – chlorofylmetra Chlorophyll Content Meter CL-01 (fi. Hansatech). V auguste a septembri následne po meraní chlorofylmetrom sa robilo aj priame stanovenie chlorofylu deštrukčnou metódou pri 81 semenáčikoch z 9 potomstiev (nehodnotili sa potomstvá z lokality Duchonka). Pri listoch zobraných v auguste sa zisťoval aj obsah dusíka na CNS 2000 analyzátore. Variabilita hodnôt nepriameho stanovenia chlorofylu (CL-01 hodnoty) ako aj priameho stanovenia chlorofylu bola štatisticky významne ovplyvnená dátumom odberu vzoriek, pôvodom – lokalitou materských stromov ako aj jednotlivými potomstvami v rámci jedného pôvodu. Hodnoty CL-01 boli najnižšie v júnových a najvyššie v septembrových vzorkách a hodnoty priameho stanovenia chlorofylu boli vyššie v auguste ako v septembri, no štatisticky významne iba pri chlorofyle *b*. Pri augustových vzorkách boli pozorované aj štatisticky významné rozdiely v celkovom obsahu chlorofylov (*a*+*b*) medzi semenáčikmi rôzneho pôvodu. Najvyšší obsah bol pri semenáčikoch pôvodu Tlstý Vrch, nižší pri pôvode Horné Lefantovce a najnižší pri pôvode Jelenec. V obsahu dusíka však neboli zaznamenané rozdiely medzi jednotlivými pôvodmi. Korelácia medzi obsahom chlorofylu a obsahom dusíka pri 81 sledovaných semenáčikoch bola len stredne silná (r = 0,413 a 0,419).

Received June 6, 2008
Accepted August 5, 2008