Morphological and physiological parameters of beech leaves (*Fagus sylvatica* L.) in research demonstration object Poľana

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ABSTRACT: Observations of the beech assimilatory apparatus in different levels of a model tree were realised from 1993 to 1999 in a research demonstration plot situated in Poľana. Different observations of the natural ecosystem condition were carried out. A model beech tree was chosen and marked as No. 228 for the above-mentioned analyses. The different ecophysiological and morphological characteristics of beech leaves were analysed in three (or two) tree crown levels. The results confirmed significant differences in these characteristics in dependence on three crown levels of mature beech tree. The differences in the mentioned characteristics were also shown in the course of particular years of research. In this paper we have presented the results of analysed beech leaves, their average maximum length, width, their average area, thickness, chlorophyll fluorescence during the research period.

Keywords: beech (*Fagus sylvatica* L.); morphological characteristics; assimilatory apparatus; tree crown levels; chlorophyll fluorescence

Beech is an important tree species from the forest management aspect also at the present time. It is a dominant tree species in the forests of Slovakia as well as a tree that can support the ecological stability of forest stands (Čaboun et al. 1997).

European beech (*Fagus sylvatica* L.) belongs to the tree species that are moderately tolerant to air pollution. Different unfavourable abiotic and biotic factors including air pollutants deteriorate the health condition of beech. Light is one of the main factors of environment and the plants respond to changes in the light regime individually.

A model tree growing on a research plot (PRP-0) was chosen and marked as No. 228. It was completely analysed in particular tree crown levels. The research on this tree connected with measurements of physiological and ecophysiological characteristics of beech assimilatory apparatus was also presented by Priwitzer (1999).

We are interested in anatomical-morphological characteristics of beech leaves. It is known that the amount and quality of assimilatory apparatus have an important influence on the tree growth and its production ability (Marek 1992). The assimilatory apparatus is important and can be analysed and characterised from several aspects. The leaf or shoot is a respectable type of structure because it relates between the cell and forest stand levels (Jarvis, Sandford 1986). The information obtained on the leaf level totally explains the processes that take place on the cell level and in an opposite way, the integration of findings on the leaf level enables to deduce the conclusion on the forest stand level (Marek 1992).

The leaf is an essential structural unit for nutrition from the atmosphere (Bobák et al. 1992; Hudák et al. 1991). The complex of morphological-anatomical structures and physiological processes taking place in them create the production activity of

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the assimilation apparatus. They also included interactions of these which cause the production of new biomass and preservation of the existing one (Marek 1992).

As concerns their assimilation apparatus structure, the tree species respond individually to changes in the light intensity regime. These trees have a varied crown according to the influence of different light intensity in these individual tree crown levels. Leaves of these levels differ from each other by their morphological, anatomical, physiological, biochemical or structural parameters (Lichtenthaler 1981).

The variability is apparent not only in the framework of shoots but also at different heights above the ground, where leaves from these three tree crown levels were defined as sunlit, semi-shaded and shaded ones (Čaboun et al. 1997; Priwitzer et al. 1996). Further ecophysiological aspects of the beech assimilatory apparatus were studied by Cicák (1991), Masarovičová and Minarčic (1979, 1985),

Table 1. Fundamental data on research plot

Foundation of RDO	Spring 1991
Region	Zvolen
Cadastral territory	Hriňová
Forest management unit	Poľana
Forest management enterprise	Kriváň
Size of object	37.5 ha
Altitude a.s.l.	800–953 m
Exposition	NE
Inclination	5–35%
Terrain relief	mild slope
Geological substructure	volcanic
Soil type	Cambisol
Average annual temperature	5.5°C
Average annual precipitation	860 mm
Management group of forest types	Fertile *Fagetum* (411) 45%
	Fertile *Abieto-Fagetum* (511) 55%
Age	90–120 years
Composition of tree species	beech, spruce, maple, larch, fir (lime, poplar, ash)
PRP-O (108 × 51 m)	0.55 ha
Group of forest types	*Abieto-Fagetum*
Forest type	nitrogenous *Abieto-Fagetum*
Altitudinal vegetation zone	5.
Altitude a.s.l.	850–860 m
Beech No. 228	Data on model tree
Age	80 (years)
Stem diameter – $d_{1.3}$	50 cm
Height h	35 m
Height of crown setting	16 m
Length of crown	19 m
Width of crown	9.4 m
Crown index	2.02
Slenderness coefficient	71.4
Bush crown index	0.5
Branchiness index	0.5
Crown projection area	69.36 m^2
Crown volume	439.3 m^3

Masarovičová and Štefančík (1990), Masarovičová et al. (1996).

On the basis of many facts the aim of this paper was:
- to analyse measured data from individual tree crown levels,
- to elaborate and determine the characteristics of assimilatory apparatus: length, width, area, thickness, chlorophyll fluorescence of beech leaves in the period of observations.

MATERIAL AND METHODS

The research demonstration object (RDO) Poľana-Hukavský grúň, which involves Permanent Research Plot (PRP-0), is situated in Protected Landscape Area (PLA) Poľana, which was proclaimed in 1981. The territory is a part of the subprovince West Carpathians in the Slovak Middle Mts., it was included in the international net of Biosphere Reservations – UNESCO in 1991. The Poľana Mts. belong to the extinguished volcanoes in Europe. The Biosphere Reserve Poľana is placed among 300 world territories that should provide fundamental research knowledge and practical experience in connection with their conservation and economic development and that should serve to long-term research and monitoring (Čaboun et al. 1993). Data on the research plot are presented in Table 1. The above-mentioned locality is of natural origin, it has been not devastated by pollutant impacts and there were not done any silvicultural measures.

The individual mentioned characteristics of model tree and of its research plot were elaborated according to these methods: Réh (1997), Priwitzer (1999).

Methodical practices

Beech No. 228, which was examined and analysed, was situated on PRP-0. The access to the crown levels was possible from the northern side of an experimental tower 35 m (+10 m) high that was built and used for many other ecophysiological experiments (Minďáš 1999; Priwitzer 1999). General research was conducted from 1993 to 1999. The tree crown levels were defined at these heights: upper level = 32 m, middle one = 29 m, lower one = 19 m, according to light conditions in this beech tree crown. The values are represented by the lower limits of these crown levels (Fig. 5).

The analysed branches (twigs) were marked, the representative leaves were selected (Cicák 1991) and the characteristics of assimilation apparatus: maximum length, maximum width, area and thickness of leaves, chlorophyll fluorescence were measured on them. The method of representative leaves was used by finding an average leaf on the shoot and it was determined as the second leaf from the shoot base. There were mainly 4 leaves on shoots, 7 to 9 leaves on terminal ones.

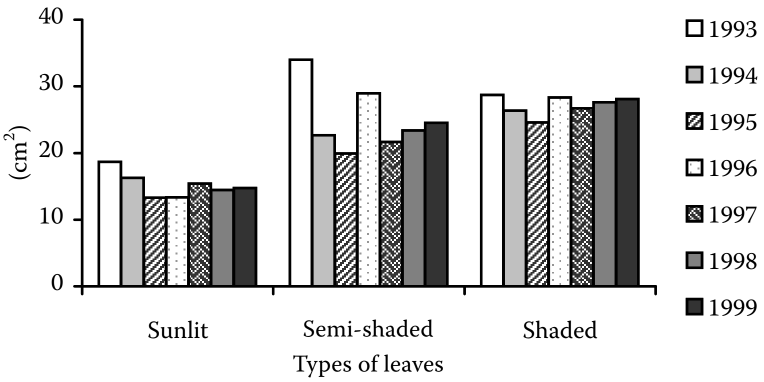

Fig. 1. Average values of beech leaves area in Poľana during research period

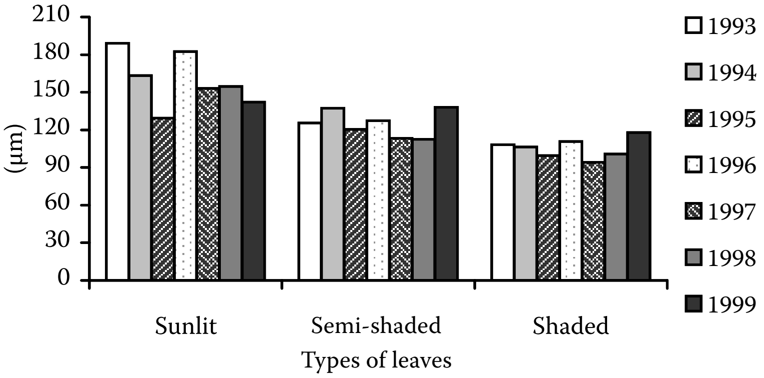

Fig. 2. Average values of beech leaves thickness in Poľana during research period

From 1993 to 1997 the leaf area was measured with digital analyser Area-meter LI-3000 A on 20 leaves destructively and on 30 leaves non-destructively, at each tree crown level at three replications. The analyser also recorded the length and width of leaves (maximum values).

In 1998, maximum length and width were measured and analysed on 25 representative leaves using the scale ruler. We measured to the nearest 1 mm. The change of the method of leaf measurements (width, length) in 1998 was due to the problem with area meter. It was not available in that year.

Beech leaves are ellipse shaped, therefore we can obtain their area also from the values of maximum length and width of measured leaves. This model formula for ellipse was used also by Tichá (1982):

leaf area = leaf width × leaf length × 0.785.

The leaf thickness was measured in 20 leaves taken from each crown level, from 20 cross-sections at magnification 400 × by using an Olympus microscope. Leaves were stored in a fridge and they were processed within two days. The maximum length and width were measured also with a scale ruler in 1998 and 1999 to compare the values measured with area meter at individual tree crown levels.

Chlorophyll *a* fluorescence was measured with a portable fluorimeter PEA (Plant Efficiency Analyser). These measurements were performed from 1997 to 1998 on three branches which were selected from individual levels of crown (sunlit, semi-shaded and shaded branches). We measured 10 leaves on each branch from their upper and lower sides. The capacity of the mentioned instrument did not enable to measure a higher number of leaves. The procedure of measurement was already published in detail in a previous paper (Čaňová 1999).

From the individual parameters *Fv/Fm* is the most important. *Fv/Fm ratio (Fv/Fm = Fm – Fo/Fm, Fv* – variable fluorescence, *Fm* – maximum fluorescence, *Fo* – initial fluorescence) is proportional to the photosynthetic quantum yield and it is highly correlated with the quantum yield of net photosynthesis. The values below 0.725 indicate a decrease in photochemical capacity of PS II (reaction centre 2) and they are below the limiting value of failures (Bolhar-Nordenkampf et al. 1989). Photosystem II decomposes the molecule H_2O into H_2 and electrons are moving from photosystem II to photosystem I. The long-wave forms of chlorophyll a predominate in photosystem I and the short-wave forms of chlorophyll *a* and additional pigments are present in photosystem II (Haspelová-Horvatovičová 1981).

All measurements were done on physiologically mature leaves (July–August). All results were processed by statistical software Excel. The other microclimatic data obtained by measuring on the observation tower were recorded with digital station analyser Delta-T, they are presented in papers or theses (Čaboun et al. 1997; Minďáš 1999; Priwitzer, 1999, etc.).

RESULTS

The measured characteristics of beech leaves, their area and thickness were analysed during the research period 1993–1999 in different tree crown levels. Distribution of average values is presented in Figs. 1 and 2. The leaf area values are presented in Fig. 1. The differences are significant with 95% reliability by Tukey's test with regard to determined tree crown levels and also in individual years of research.

Table 2. Statistical overview of morphological characteristics for sunlit and shaded beech leaves in 1998

Poľana	1998	Leaf area (cm^2)	Leaf length (cm)	Leaf width (cm)
	Average	15.723	5.58	3.785
	Median	15.21	5.55	3.7
Sunlit	Standard deviation	4.2	0.713	0.474
	VAR	17.603	0.508	0.22
	VARP	16.722	0.483	0.21
	Average	21.648	5.67	5.12
	Median	21.05	5.8	4.75
Shaded	Standard deviation	4.646	1.083	1.044
	VAR	34.578	1.876	1.569
	VARP	32.85	1.782	1.782
F-test		**	–	*
T-test		**	–	*

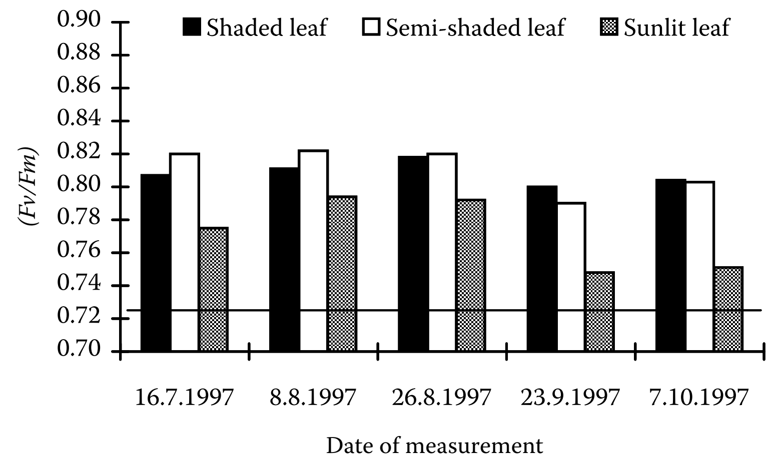

Fig. 3. Parameter *Fv/Fm* measured on the upper (adaxial) side of beech leaves in Poľana in 1997

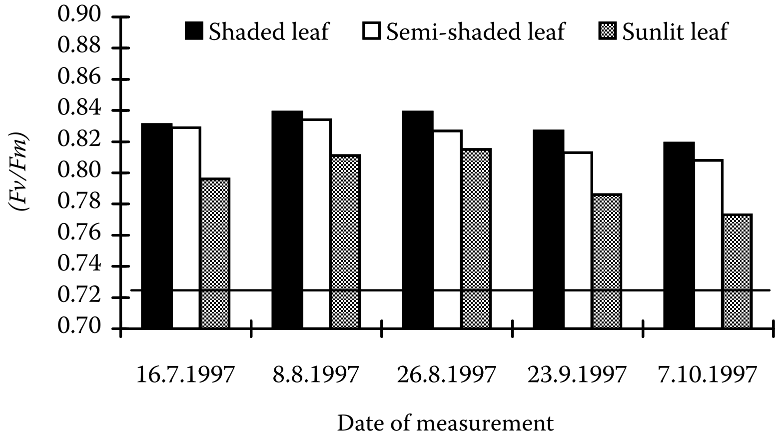

Fig. 4. Parameter *Fv/Fm* measured on the lower (abaxial) side of beech leaves in Poľana in 1997

The lowest values of all leaf area data were analysed in 1995 and the highest values in 1993. The sunlit leaves at the upper level attained leaf area values about 14–19 cm^2, semi-shaded leaves at the middle level 20–34 cm^2 and shaded leaves at the level 24 to 29 cm^2. The highest variability of area values during the research period was observed in semi-shaded leaves. The average area of semi-shaded leaves has attained the extremely high value in 1993. The measurement results of area and thickness of leaves from previous years are published in papers (Hladká 1999, 2004).

The beech leaf thickness is presented in Fig. 2. Significant differences in leaf thickness values with reliability 99% were determined by Tukey's test for individual tree crown levels. The sunlit leaves are mostly the thickest. They have 2 rows of palisade parenchyma and they contain 9 cell rows in their cross-sections (Hladká 1999). The thickness of sunlit leaves ranged from 130 to 190 μm in individual years of research period (1993–1999). The shaded leaves are the thinnest with one layer of palisade parenchyma. They contain 7 rows of cells, and their thickness was between 106 and 119 μm. The sunlit leaves were the thickest in 1993 or 1996 and the thinnest in 1995. The shaded leaves were the thickest in 1999 and the thinnest in 1997. The values of semi-shaded leaves thickness were between those of sunlit and shaded leaves. They have 1 row of palisade parenchyma, 7–8 rows of cells and they formed a transient type. The semi-shaded leaves thickness ranged from 110 to 140 μm.

The statistical analysis of area, length and width in beech leaves for sunlit and shaded leaves from 1998 is presented in Table 2. The average values of leaf length are 4–6.8 cm for sunlit leaves and 2.9–8.1 cm for shaded leaves. The average values of leaf width are 2.7–4.7 cm for sunlit leaves and 3.2–5.8 cm for shaded leaves.

After the multiplication of the values leaf thickness × leaf area = leaf volume it is possible to obtain another characteristic (leaf volume). It could be used for observations of productive processes and measuring of photosynthesis or respiration. For sunlit leaves the value of leaf volume can be 345–355 mm^3 from our data and for shaded leaves 305–315 mm^3. The mentioned parameters (leaf area and thickness) were measured in the previous period (1993–1997) and their statistical analysis is presented in the thesis (Hladká 2004). The variation of these parameters in 1993–1997 was a consequence of various climatic factors (precipi-

Fig. 5. Access to the beech crown using the monitoring tower in RDO Poľana

tation, temperature and radiation). These factors were measured continually and they are presented in the thesis (MINĎÁŠ 1999).

Further observed parameters were the characteristics of chlorophyll fluorescence that were measured in 1997–1998 and that are presented in Table 3 and Figs. 3 and 4.

The average values of the *Fv/Fm* ratio for ten leaves ranged from 0.75 to 0.85 in the assimilatory apparatus in this period. The chosen beech model had the healthy assimilatory apparatus within the whole crown during the observation period. We found out differences between the individual types of leaves in mutual comparison of sunlit, semi-

Table 3. Average values of the parameter *Fv/Fm* measured on beech branches in Poľana during 1998

Date	Side	Branch	Leaf									
			1	2	3	4	5	6	7	8	9	10
17. 6. 1998	upper	shaded	0.792	0.805	0.823	0.797	0.786	0.793	0.80	0.79	0.804	0.792
		semi-shaded	0.815	0.813	0.841	0.808	0.697	0.823	0.812	0.815	0.815	0.807
		sunlit	0.797	0.782	0.79	0.775	0.794	0.79	0.814	0.807	0.794	0.801
	lower	shaded	0.825	0.794	0.829	0.828	0.813	0.807	0.805	0.801	0.817	0.833
		semi-shaded	0.834	0.833	0.844	0.835	0.832	0.841	0.837	0.83	0.83	0.827
		sunlit	0.803	0.803	0.789	0.809	0.812	0.786	0.783	0.80	0.804	0.794
22. 9. 1998	upper	shaded	0.805	0.82	0.823	0.815	0.792	0.813	0.818	0.81	0.806	0.807
		semi-shaded	0.803	0.81	0.825	0.801	0.797	0.811	0.801	0.803	0.817	0.774
		sunlit	0.791	0.758	0.771	0.769	0.777	0.783	0.81	0.788	0.789	0.787
	lower	shaded	0.833	0.838	0.821	0.818	0.829	0.825	0.84	0.807	0.836	0.826
		semi-shaded	0.822	0.833	0.817	0.833	0.837	0.838	0.832	0.829	0.83	0.814
		sunlit	0.817	0.789	0.81	0.783	0.797	0.783	0.81	0.821	0.821	0.819

shaded and shaded leaves, with 95% reliability by *F*-test.

The lowest values of the *Fv/Fm* ratio were measured on sunlit leaves, the highest ones were measured on shaded leaves (abaxial side of leaves) or on semi-shaded ones (adaxial side). The values of the *Fv/Fm* ratio in semi-shaded leaves were found to be between the values of sunlit and shaded leaves. These values on the adaxial side of leaves showed the highest variability. The parameter of the *Fv/Fm* ratio, measured on the abaxial side of leaves, reached higher values of the ratio in comparison with the adaxial leaf side with reliability of 95% by *F*-test. The largest differences between the adaxial and abaxial side of leaves were ascertained in sunlit leaves, the lowest ones were found in shaded leaves. The decrease in the *Fv/Fm* ratio values was visible in all three types of leaves and also on the adaxial and abaxial side of leaves towards the end of vegetation period. Similar results were presented by Lichtenthaler (1986), who found out that the shaded leaves had higher fluorescence intensity than the sunlit ones. The reciprocal function between chlorophyll fluorescence intensity and intensity of photosynthesis was also confirmed. The differences between the individual types of leaves are caused by different organisation of photosynthetic apparatus and by different anatomical and morphological composition according to different authors.

DISCUSSION

In spite of that, the growth of leaves and their development are also determined by genetic factors. The leaf area (A), specific leaf area (SLA) or specific leaf mass (SLM) could be recognised as suitable and sensitive physiological characteristics for adaptation to environmental conditions (Masarovičová, Štefančík 1990). These authors also found out significant differences in A, SLA and SLM between sunlit and shaded leaves. Masarovičová et al. (1996) presented similar values. They reported that shaded leaves had the larger leaf area than sunlit leaves.

Certainly, the values of leaf area and thickness also depend on leaf ontogenesis (mature leaves were measured only), on the age of tree species, their shape (habit), their heights, diameter or their localisation in forest stand. The microclimatic conditions (air humidity and temperature, precipitation, radiation, mineral nutrition, pH of soil, soil humidity, etc.) also influence the leaf area and thickness in the same way.

Lichtenthaler (1984) reported differences in morphology and chemical composition of leaves that were growing under varied light intensities. According to the cited author, growth and development of plants, especially of leaves, was strongly influenced by light, which was the most important environmental factor influencing many organisms and their structure as well as the processes taking place in them.

The light absorbed by the photosynthetic pigments will be used for photosynthesis or dissipated as heat or as red chlorophyll fluorescence. Chlorophyll fluorescence is inversely related to the rate of photosynthesis (Lichtenthaler 1986) and therefore in the last years some authors investigated the fast kinetics of chlorophyll fluorescence. We determined a decrease in the values of the *Fv/Fm* ratio in all three types of leaves and also on the adaxial and abaxial side of leaves towards the end of vegetation period. Similar results were presented by Lichtenthaler (1986), who found out that the shaded leaves had higher fluorescence intensity than the sunlit ones. The reciprocal function between chlorophyll fluorescence intensity and intensity of photosynthesis was also confirmed.

Bolhar-Nordenkampf and Öquist (1993) determined the values of chlorophyll fluorescence for healthy plants to be higher than 0.725, which was also consistent with our results.

The photomorphogenesis of plants as well as leaf development depend on the amount and quality of light. Therefore plants under higher light intensity have the typical sunlit structure and those growing under low light intensity are determined as the shaded ones. This was also confirmed by our results. These two different types of leaves depend on light intensity, which also demonstrated plant ability to adapt to the light conditions.

CONCLUSION

We found out significant differences between the individual types of leaves mostly in dependence on radiation and light penetration. The sunlit leaves were the smallest, thickest and the values of the *Fv/Fm* ratio were the lowest. The shaded leaves were the largest, thinnest and the *Fv/Fm* ratio was the highest. Substantial differences in individual years of research were also determined.

It can be summarised that the above-mentioned research and individual biochemical and anatomical-morphological analyses of the assimilation apparatus of model tree are of great importance. The other possibilities of observations can be open for giving the detailed observation of primary photosynthetic processes in the assimilation apparatus of important tree species.

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Morfologické a fyziologické charakteristiky listov buka (*Fagus sylvatica* L.) na výskumno-demonštračnom objekte Poľana

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ABSTRAKT: Výskum asimilačného aparátu buka v jednotlivých úrovniach modelovej dreviny prebiehal v rokoch 1993–1999 na výskumnej ploche Poľana, kde sme sledovali stav pôvodného ekosystému. Modelovú drevinu buk č. 228 sme vybrali a označili ako vzorník pre uvedené analýzy. Rôzne ekofyziologické a morfologické charakteristiky listov buka sme sledovali v troch (resp. dvoch) úrovniach. Výsledky potvrdili významné rozdiely meraných charakteristík v závislosti od troch úrovní koruny dospelého buka. Rozdiely v uvedených charakteristikách sme však zistili aj v priebehu jednotlivých rokov výskumu. V práci uvádzame výsledky analyzovaných listov, ich dĺžku, šírku, plochu, hrúbku listov, fluorescenciu chlorofylu, ktoré sme zistili počas výskumu.

Kľúčové slová: buk (*Fagus sylvatica* L.); morfologické parametre; asimilačný aparát; úroveň koruny stromu; fluorescencia chlorofylu

Práca prináša experimentálne výsledky meraní morfologických a ekofyziologických charakteristík asimilačného aparátu modelovej dreviny (buk č. 228) na trvalej výskumnej ploche TVP-0. Táto plocha bola súčasťou Výskumno-demonštračného objektu (VDO-Hukavský grúň), nachádzajúceho sa na území CHKO Poľana. Výskum prebiehal v období rokov 1993–1999.

Monitorovacia veža (postavená v roku 1992) umožňovala prístup do koruny modelovej dreviny zo severnej strany a uvedené charakteristiky boli merané a analyzované v jednotlivých korunových úrovniach. Obr. 1 uvádza priemerné hodnoty listovej plochy, obr. 2 priemerné hodnoty listovej hrúbky. V tab. 2 sa nachádzajú štatisticky spracované hodnoty priemernej plochy, dĺžky a šírky z vybraných 20 listov (z troch konárov) z hornej a dolnej úrovne v roku 1998. Obr. 3 a 4 udávajú parametre fluorescencie chlorofylu *Fv/Fm* v jednotlivých úrovniach na vrchnej a spodnej strane listov.

Boli zistené štatisticky významné rozdiely v uvedených charakteristikách v rámci jednotlivých úrovní, z tohoto dôvodu boli listy označené ako slnné, polotienne a tienne.

Na základe týchto poznatkov môžeme skonštatovať, že štruktúra asimilačného aparátu modelovej dreviny je ovplyvnená množstvom prenikajúceho žiarenia do koruny. Obr. 5 nám znázorňuje výškovú diferenciáciu a pohľad do koruny modelovej dreviny spolu s monitorovacou vežou.

Všetky uvedené výsledky participujú na globálnej analýze asimilačného aparátu modelovej dreviny na špeciálnej výskumnej ploche Lesníckeho výskumného ústavu (LVÚ) vo Zvolene a boli súčasťou riešených výskumných projektov.

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