Physiological characteristics and energy accumulation of selected plant species growing in forest ecosystems at different levels of air pollution stress

M. Kuklová, H. Hniličková, F. Hnilička, J. Kukla

Abstract. Physiological characteristics and energy accumulation of plant species growing in the forest phytocoenoses with high gradient of immision load were compared to those with undamaged forest phytocoenoses. Studied localities Žiar n. Hronom and Spiš region (Western Carpathians) belong to the most loaded areas of Slovakia. It has been found out that analysed plant species with their physiological characteristics (rate of photosynthesis, transpiration, stomatal conductance, chlorophyll fluorescence) sensitively reacted on the influence of abiotic factors and anthropogenic pollution of environment. Significant interspecies differences ($p < 0.001$) were found in the measured characteristics and also in their response to negative environmental influences. In the stressed forest stand MP Žiar n. Hronom to anthropogenic impacts of the environment significantly lower value of photosynthesis rate responded Fagus sylvatica species, which is generally considered a sensitive species. On the locality Hliníky (NP Slovenský raj) was the reaction of beech most pronounced in the case of photosynthesis rate and the stomatal conductance. As sensitive in the locality Hliníky seems to be herb understorey, especially with Vaccinium myrtillus and Rubus idaeus species. To the influence of abiotic factors and anthropogenic environmental pollution with significantly lower value of combustion heat in phytomass reacted Carex pilosa species on the stress plot MP Žiar n. Hronom. In the case of the Fagus sylvatica species this difference was not significant. Slightly higher levels of energy showed also the plants growing on the control plot in the locality Hliníky, opposite the stressed plants.

Keywords forest ecosystems, air pollution, photosynthesis, stomatal conductance, chlorophyll fluorescence, ash, energy

Authors. Margita Kuklová (kuklova@savzv.sk), Ján Kukla - Institute of Forest Ecology of the Slovak Academy of Sciences, Department of soil and plant ecology, Zvolen, Slovak Republic, Helena Hniličková, František Hnilička - Czech University of Life Sciences Prague, Department of Botany and Plant Physiology, Kamýcka 129, Prague, Czech Republic.

Manuscript received December 08, 2011; revised August 09, 2012; accepted August 20, 2012; online first October 15, 2013.
Introduction

Vegetation reacts sensitively to environmental pollution by growth slowing, production reducing, morphological leaf changes, mortality of sensitive species and reduction of species diversity. The impact of air pollutants of acid type on forest ecosystems was also manifested by lowered vitality of trees and earlier decline of spruce stands in the Spiš region, East Slovakia (Kukla & Kuklová 2008). Pollutants after release from the source do not remain in the air without changes, physical changes are in progress (movement and distribution in space, turbulent diffusion, changes in the concentration by dilution and other) as well as and chemical changes. Industrial pollution is the most serious threat because industry uses highly toxic contaminants and many of them are released to the atmosphere. The effect of air pollution stress on forests has been well studied by Atkinson & Winner 1990, Fiala et al. 1989, Vacek et al. 1996, 1999, etc. If the file of habitat conditions is not optimal, the photosynthetic efficiency of plants is reduced (Rychnovská et al. 1987). The excessive loss of nutrients occurs especially in soils of forest ecosystems in the long term influenced by the type of acidic air pollutants (Maňkovská 2004, Bučinová & Mihál 2008, Jamnická et al. 2007, Maňkovská & Oszlányi 2009). Contamination of soil by acid rain can affect plants by the disturbance of soil chemistry and this affects plants ability to absorb nutrients and carry out photosynthesis (Bláha & Hnilička 2004, Szalai 2008). In the long-term load by industrial air pollutants, there are changes in the metabolism of plants, especially in photosynthesis and then in dry matter production (Shparyk & Parpan 2004).

Evaluation of growth processes occurring in phytocoenoses is an important source of information about the degree of threat to forest ecosystems, depending on the intensity of the negative external environmental impacts. Transient faults of plant metabolism develop into a chronic injury, the result of which may be substantial changes even initiating extinction of the communities (Kuklová & Kukla 2008). Data of physiological and production characteristics of plant organs are necessary for evaluating the bioproduction process in phytocenoses and for studying the energy and mass exchange in forest biogeocoenoses. Few authors studied the energy balance of the dominant plant species and seasonal variations in the calorific contents (Vookova 1985, Oszlányi 1979, Kováčová & Schieber 2002). Bobkova et al. (2001) found that the heat of plant combustion as a physical parameter is characterized by a relatively high variability, being dependent on plant species, growing conditions, morphological structure, age, period of sampling, and other factors.

In Slovakia, there was found out a high gradient of immission burden of forest ecosystems. The most loaded are the areas of middle Spiš Kremnické vrchy Mts and Slanec surroundings. This part of the territory belongs to the most polluted areas in central Europe known as the „Black Triangle II“ (Maňkovská & Oszlányi 2009, 2010). Compared to data from Austria and Czech Republic, Slovak deposition of elements is 2–3 higher. By exceeding the critical levels of concentrations of air pollution in the atmosphere, autoregeneration and autoregulatory processes are unable to give forest ecosystems to their original state. In the searched area of middle Spiš, climate is influenced mainly by industry processing the wood and raw materials. The main components of the pollutants are sulphur (SO₂), nitrogen (NOₓ), CO emission and also heavy metals (Zn, Hg, Cu, As, Cd a Pb). In Žiar n. Hronom territory occurred in the past a greater environmental pollution mainly caused by the toxic elements (F, Hg and As). After stopping outdated alumina production in furnaces using Söderberg system and by the introduction of new technology in the production of aluminum (1995), although there was a decrease in emissions of fluorine, but also substantially increased emissions of
CO, SO₂, NOₓ.

With this background, the present study was undertaken with the objective to compare changes in physiological characteristics (rate of photosynthesis, transpiration, stomatal conductance, chlorophyll fluorescence) and energy accumulation of assimilatory organs of plants (Fagus sylvatica L., Dryopteris flix-mas (L.) Schott, Vaccinium myrtillus L., Carex pilosa Scop., Rubus idaeus L.) in forest ecosystems disturbed by aggressive action of acid type of air pollution and toxic elements (stress forest stands) to the plant species from undamaged forest phytocenoses (control forest stands).

Materials and methods

The research was performed in 2012 on 2 monitoring plots situated at different distances from the emission source – aluminium works in Žiar nad Hronom (MP Žiar n. Hronom in Štiavnické vrchy Mts is located at a distance of 1.5 km from the emission source – stress stand; Ecological Experimental stationary (EES) Kremnicky vrchy Mts, 18 km from the emission source – control stand). Other 2 stands are situated in the locality Hliníky (stress stand – with lower stand density and canopy compactness due to tree decline and control stand – undamaged) in the buffer zone of the National Park Slovenský raj.

The main woody species on monitoring plots in Kremnicky and Štiavnické vrchy Mts is Fagus sylvatica, in the buffer zone of the NP Slovenský raj Fagus sylvatica L. and Abies alba Mill.. Studied geobiocenoses differ from each other not only in their localization but also in their altitude, geological characteristics of the locality, soil subtype, but also in other factors, such as age, stocking and canopy of stands, air pollution (Table 1).

MP Žiar n. Hronom and EES Kremnicky vrchy Mts are situated in the temperate climate region, with the mean temperature in July, 17-18 °C. The mean annual temperature ranges

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Ecological characteristics of studied forest ecosystems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orographic unit</td>
<td>Štiavnické vrchy Mts</td>
</tr>
<tr>
<td>Locality</td>
<td>Žiar nad Hronom</td>
</tr>
<tr>
<td>Forest stand</td>
<td>stress</td>
</tr>
<tr>
<td>Altitude[m]</td>
<td>450</td>
</tr>
<tr>
<td>Geographical coordinates</td>
<td>48°32’N</td>
</tr>
<tr>
<td>Exposure</td>
<td>NNW</td>
</tr>
<tr>
<td>Slope [°]</td>
<td>5-10</td>
</tr>
<tr>
<td>Stand age</td>
<td>upper storey</td>
</tr>
<tr>
<td></td>
<td>lower storey</td>
</tr>
<tr>
<td>Stocking</td>
<td>upper storey</td>
</tr>
<tr>
<td></td>
<td>lower storey</td>
</tr>
<tr>
<td>Canopy</td>
<td>upper storey</td>
</tr>
<tr>
<td></td>
<td>lower storey</td>
</tr>
<tr>
<td>Vegetation unit</td>
<td>Fagetum pauper superiora</td>
</tr>
<tr>
<td>Parent rock</td>
<td>rhyolitic tuff, tuffite</td>
</tr>
<tr>
<td>Soil subtype</td>
<td>Stagnic Cambisol</td>
</tr>
<tr>
<td>pH₄H₂O in 0-5 cm</td>
<td>4.58–4.70</td>
</tr>
<tr>
<td>pH_KCl in 0-5 cm</td>
<td>3.38–3.55</td>
</tr>
<tr>
<td>C/N in 0-5 cm</td>
<td>14–19</td>
</tr>
</tbody>
</table>
from 6–7 °C and mean annual precipitation reaches 700–800 mm. The locality Hliníky is situated in the cool climatic region, with the mean temperature in July, 12–16 °C. The mean annual temperature is 4–5 °C, and average annual precipitation reaches 700-800 mm (Miklós et al. 2002).

The soils were classified according to World Reference Base for Soil Resources 1994 (Bednára et al. 2000). Values of soil reaction were determined potentiometrically – using a digital pH meter Inolab pH 720. Total content of N and C was determined by NCS analyzer type FLASH 1112.

Soil reaction of Cambisols located in forest stands of Kremnické and Štiavnické vrchy Mts is acid to slightly acid (Table 1) and with growing distance from the emission source increases (from pH$_{H_2O}$ values 4.6–4.7 to 5.8-6.3). Higher pH values were only found in organomineral layer of Andic Cambisol on EES Kremnické vrchy Mts where are increasing above the value 6, i.e. within the reaction characteristic for heminitrophic trophic range (Kukla 1993). In mineral rich soil horizons on EES Kremnické vrchy Mts the values of C/N ratio (9–15) markedly lower and closer to the source of emissions (stressed stand MP Žiar n. Hronom) they increase to the value 14–19.

Soil reaction of Dystric Cambisol on the control (pH$_{H_2O}$ 5.1) and damaged stand (pH$_{H_2O}$ 4.2) in the locality Hliníky is very strongly acid that is characteristic for these soils. Values of C/N ratio in the upper layers of soils (0-5 cm) both on damaged and control stand reach the value 10.3.

The forest geobiocoenoses were determined according to Zlatník (1976) and the names of plant taxa were given according to Marhold & Hindák (1998). The sampling of 30–60 shoots from an area of 400 m² was randomized. There were collected assimilatory organs of Fagus sylvatica L. (leaves from the bottom third of the tree crown), Dryopteris fílix mas (leaves), Vaccinium myrtillus (green twigs), Rubus idaeus (shoots growing out from creeping stems) and Carex pilosa (shoot – rosette of leaves). All plant samples were dried at 80°C for 48 hours and homogenised with a Fritsch planetary micro mill (<0.001 mm).

The energy content of phytomass (J.g⁻¹ of dry matter) was determined using an adiabatic calorimeter IKA C-4000 (software C-402). The samples weighing 0.7-1 g and homogenised were pressed into a form of briquette, dried up to a constant weight at 105 °C and burnt in pure oxygen under a pressure of 3.04 MPa (DIN 51900). The ash content was determined gravimetrically, by total oxidation of specimens in a muffle furnace at 500 °C (Javorský et al. 1987).

The gas exchange – rate of photosynthesis ($P_N$), rate of transpiration ($E$) and stomatal conductance ($g_s$) and the intercellular CO₂ concentration ($c_i$) were measured on the upper surface of leaves (the middle part of the leaf blade) in situ using the portable gas exchange system LCpro+ (ADC BioScientific Ltd., Hoddesdon, Great Britain). This instrument measures the gas exchange based on method of an open system (Šesták et al. 1966, Holá et al. 2010). Measurement time was set according to the work by Tucci et al. (2010). These physiological characteristics were measured under adjusted light and temperature conditions, the irradiance was 650 μmol m⁻² s⁻¹ of photosynthetically active radiation, the temperature in the measurement chamber was 25°C, the CO₂ concentration was 420 ± 35 vpm (μmol mol⁻¹), the air flow rate was 205 ± 30 μmol s⁻¹ and the duration of the measurement of each sample was 20 min after the establishment of steady-state conditions inside the measurement chamber. The value of VSD (vapour pressure deficit) was 0.85 ± 0.15 kPa.

Measured parameters of gas exchange rates were calculated using the following formulas:

Rate of photosynthesis ($P_N$) [μmol CO₂ m⁻² s⁻¹]

$$P_N = u_s \cdot \Delta c$$
where Δc is the difference in concentration of CO₂ on input and output of the chamber [μmol mol⁻¹],
where uₘ is concentration – air flow per the m² leaf area [mol m⁻²s⁻¹].

Rate of transpiration (E) [mmol H₂O m⁻²s⁻¹]
E = uₘ · ΔW

where ΔW is the difference in concentration water vapor [mol mol⁻¹],
us is concentration – air flow per the m² leaf area [mol m⁻²s⁻¹].

Stomatal conductance (gₛ) [mol m⁻² s⁻¹]
gₛ = 1 / rₛ

where rₛ are stomatal resistens.

The minimum Chl fluorescence (F₀) and the maximum Chl fluorescence (Fₘ) were measured also in situ with the portable 1 Chl fluorometer ADC:OSI 1 FL (ADC BioScientific Ltd., Hoddesdon, Great Britain) with 1 s excitation pulse (660 nm) and saturation intensity 3000 μmol m⁻² s⁻¹ after 20 min dark adaptation of the leaves. The maximum quantum efficiency of Photosystem (PS) II was calculated as Fv/Fm (Fv = Fm−F₀). That ratio indicates the potential photochemical efficiency of electron transport in photosystem II. In the case of C₃ plants of photosynthesis cycle as a “normal” value appears to be 0.830. It can be assumed that the higher this value is the better efficiency of electron transport. Values around 0.250 usually indicate stress response.

Statistical analyses were made with the use of a statistics program Statistica 9 software and the variability in mean physiological and energy characteristics of selected plant species growing in forest ecosystems at different levels of air pollution stress was tested by ANOVA model. The significance of differences was verified by Fisher-LSD test (p <0.05, p <0.01). The measured parameters included rate of photosynthesis, rate of transpiration, stomatal conductance, chlorophyll fluorescence, energy and ash accumulation. Measured average value (average ± standard deviation) for the physiological characteristics was one plant species while maintaining the 4 replicates. For each plant species were made three measurements of energy value (average ± standard deviation).

Results

Rate of photosynthesis (Pₚₙ)

On the control plot EES Kremnické vrchy Mts and in stress stand MP Žiar n. Hronom was measured the rate of photosynthesis in the case of beech and sedge depending on source of environmental pollution (aluminium works in Žiar nad Hronom). The measured values of photosynthetic rate of dominant plant species are recorded in Fig 1. The figure shows a statistically significant effect of plant species on the rate of photosynthesis as the lowest rate of photosynthesis was found in sedge (5.23 μmol CO₂.m⁻².s⁻¹) while the highest in the assimilatory organs of beech (10.84 μmol CO₂.m⁻².s⁻¹).

The mean rate of photosynthesis of stressed plants of both plant species was 7.98 ± 0.27 μmol CO₂.m⁻².s⁻¹, whereas in control plants 8.61 ± 0.25 μmol CO₂.m⁻².s⁻¹. Within both studied species is observed decline of rate of photosynthesis on the stress plot MP Žiar n. Hronom, thus in the locality with markedly anthropogenic damage, compared to the undamaged locality. Fig. 1 also shows different reaction of the studied plant species on effecting anthropogenic damage, as less sensitive to the stress responsed sedge compared with beech. The difference in photosynthetic rate between stressed and control variation within of sedge was 0.21 μmol CO₂.m⁻².s⁻¹. Statistical evaluation of results shows that between the two variations there is not statistically significant difference. Within Fagus sylvatica species was found significant difference between the rate of photosynthesis of control and stressed plants.
where a higher rate of photosynthesis was observed in assimilatory organs of control plants compared with the stressed plants (10.34 μmol CO₂.m⁻².s⁻¹). The difference between both habitats was 1.79 μmol CO₂.m⁻².s⁻¹, Fig. 1.

In the locality Hliníky (NP Slovenský raj) was measured the rate of photosynthesis on two plots that included damaged (stressed) and undamaged (control) forest stand. In this locality was the rate of photosynthesis measured in case of the beech, male fern, blueberry and raspberry. The obtained results (Fig. 1) show that the rate of photosynthesis is influenced not only by plant species, but also by the plot. From the studied plant species, assimilatory organs of male fern showed the lowest rate of photosynthesis and on the contrary the maximum photosynthesis was recorded in case of beech leaves. It can be said that also in this locality herb species show again lower photosynthesis compared to assimilatory organs of tree species. Rate of photosynthesis of plants in the damaged stand was in the interval from 4.51 μmol CO₂.m⁻².s⁻¹ (Dryopteris filix mas) to 13.29 μmol CO₂.m⁻².s⁻¹ (Fagus sylvatica) and in case of control plants was photosynthesis the lowest in ferns (5.74 μmol CO₂.m⁻².s⁻¹) and the highest in assimilation organs of the beech (16.31 μmol CO₂.m⁻².s⁻¹). Statistical evaluation of results shows that between control and damaged forest stand there are significant differences (ANOVA: F(3, 57) = 58.931, p = 0.0000). The highest significant difference in the rate of photosynthesis between the two variations showed the assimilatory organs of beech. In this plant, the difference between the two variants was 3.01 μmol CO₂.m⁻².s⁻¹. On the contrary, as tolerant to the anthropogenic damage seems to be Rubus idaeus species, because the difference in photosynthesis rate between the variants was 0.88 μmol CO₂.m⁻².s⁻¹ (Fig. 1).

The results of regression analysis showed moderate positive relationships between Pₙ vs gs and Pₙ vs ci for all the tested plant species and localities (F-ratio = 124.83, p < 0.05, R-squared = 0.438, R = 0.504). The correlation between the intercellular CO₂ concentration (ci) and stomatal conductance (gs) was strong (F-ratio = 183.16, p < 0.05, R-squared = 0.675, R = 0.834).

**Rate of transpiration (E)**

Similarly, as in the case of photosynthetic rate were found between the observed plant species growing on the control plot EES Kremnické vrchy Mts and in the stress stand MP Žiar n. Hronom significant differences in the rate of transpiration (ANOVA: F(3, 233) = 204.55, p = 0.0000). The measured values show that lower mean transpiration has sedge compared to beech. Fig. 2 also shows an increase in rate of transpiration in plants growing on plot situ-
ated closest to the sources of environmental pollution (0.27 mmol H₂O.m⁻².s⁻¹) in comparison with the plants from the control plot EES Kremnické vrchy Mts (0.16 mmol H₂O.m⁻².s⁻¹).

Transpiration rate was significantly affected by plant species also in the investigated locality Hliníky (ANOVA: F(2, 204) = 17.415, p = 0.0000, Fig. 2). The lowest transpiration rate was observed in case of the Vaccinium myrtillus species (0.55 mmol H₂O.m⁻².s⁻¹) while the highest in the beech (2.10 mmol H₂O.m⁻².s⁻¹). Fig. 2 also shows insignificant increase of transpiration rate between the control and the stressed stand in the locality Hliníky, where slightly higher average transpiration show plants in damaged stand (1.33 mmol H₂O.m⁻².s⁻¹) in comparison with the plants from undamaged forest stand (1.25 mmol H₂O.m⁻².s⁻¹). Insignificant differences were found out also between assimilatory organs of beech, blueberries and raspberries growing in the control and the stressed stand. Transpiration rate of the plants in the locality Hliníky was the highest on the control plot in case of beech (2.10 mmol H₂O.m⁻².s⁻¹) while the lowest in male fern (0.49 mmol H₂O.m⁻².s⁻¹). In the plants growing on the damage plot transpiration rate ranged from 2.11 mmol H₂O.m⁻².s⁻¹ (Fagus sylvatica) to 0.56 mmol H₂O.m⁻².s⁻¹ (Vaccinium myrtillus). Studied plant species responded differently to stress conditions, as documents Fig. 2. The figure shows that only raspberries transpiration rate was insignificantly higher in the control plants (1.65 mmol H₂O.m⁻².s⁻¹) compared to the plants growing in the damaged stand (1.53 mmol H₂O.m⁻².s⁻¹). For other plant species was observed opposite trend, and the lowest difference between the control and the stressed plants was found in the case of blueberries, the difference between the two habitats was 0.01 mmol H₂O.m⁻².s⁻¹. On the contrary, for assimilatory organs of male fern was the difference in transpiration between both stands the highest (0.78 mmol H₂O.m⁻².s⁻¹), Fig. 2.

The rate of transpiration (E) was directly correlated with stomatal conductance (gs). The statistical evaluation of the results confirms strong positive linear relationship between the rate of transpiration and stomatal conductance of the plant species growing in the studied forest ecosystems (F-ratio = 263.06, p < 0.05, R-squared = 0.703, R = 0.841).

**Stomatal conductance (gs)**

From the studied plant species showed assimilatory organs of beech lower average stomatal conductance, compared to the sedge leaves, see Fig. 3. That difference is not statistically significant in the case of beech, but in the case of sedge was found significant difference in stomatal conductance between localities (ANOVA: F(2, 204) = 17.415, p = 0.0000, Table 4). In both investigated species was observed an increased stomatal conductance in plants from...
contaminated locality (MP Žiar n. Hronom) compared to the control locality (EES Kremnické vrchy). However, the difference between the localities within studied plant species is not the same, as higher contrast showed assimilation organs of sedge (0.26 mol.m⁻².s⁻¹) and lower of beech (0.15 mol.m⁻².s⁻¹).

Measured values of stomatal conductance of selected plant species in studied stands in the locality Hliníky is shown in Fig 3. There were found out significant differences between the studied plant species growing in the control and damaged stand. The lowest mean stomatal conductance exhibit assimilatory organs of fern (0.19 mol.m⁻².s⁻¹), while the highest raspberry shoots (0.57 mol.m⁻².s⁻¹), Fig. 3. The slightly higher average values of stomatal conductance show plants in damaged stand (0.37 mol.m⁻².s⁻¹) compared to the plants sampling from the control stand (0.33 mol.m⁻².s⁻¹). From Fig. 3 it is evident that the highest significant difference between the variants was found out in the case of blueberries. In the case of this plant, the difference in stomatal openness between control and damaged stand was 0.21 mol.m⁻².s⁻¹. On the other hand, the lowest difference between variants was found in ferns, where it was 0.11 mol.m⁻².s⁻¹.

The rate of gas exchange can be limited not only by stomatal conductance (gs), but also by substomatal CO₂ (ci). The values of substomatal CO₂ varied from 490 vpm to 610 vpm. The results of regression analysis confirm a positive linear relationship between gs and ci for the tested plant species and localities (F-ratio = 45.524, p < 0.01, R-squared = 0.153, R = 0.364).

Chlorophyll fluorescence

On the plots of EES Kremnické vrchy Mts (control stand) and MP Žiar n. Hronom (stress stand) was measured chlorophyll fluorescence in leaves of beech and sedge in dependence to anthropogenic pollution of environment. Measured values of potential photochemical efficiency of electron transport in photosystem II of predominating plant species are recorded in Fig. 4. From the calculated values of the ratio Fv/Fm is obvious that this characteristic is influenced by plant species. From Fig. 4 it is evident that the lowest value of the ratio has assimilatory organs of beech (0.55), while the highest has sedge (0.66). From the measured values is evident that plants responded to anthropogenic pollution by reducing the potential photochemical efficiency, because an average value of the ratio Fv/Fm was in the plants from MP Žiar n. Hronom 0.552 and in control plants 0.655. More sensitively to anthropogenic pollution reacted sedge where the electron transport decreased in comparison with the control environment (by 0.163).

In the locality Hliníky was the fluorescence investigated in the case of three plant species.
— beech, blueberry and raspberry. Among individual plant species were found out differences in the fluorescence of photosystem II, as the lowest fluorescence showed assimilatory organs of beech (0.686) and the highest raspberry (0.807). From Fig. 4 can be seen that on the damaged plot was average fluorescence value lower (0.732) in comparison with the control, undamaged stand (0.789). The highest difference was found out between the leaves of beech in control conditions (0.801) and the stressed environment (0.570). At least responded to anthropogenic pollution raspberry shoots, because the difference between the variants was the lowest and reached the value 0.012 in favor of the control plants.

The statistical evaluation of the results confirms moderate positive linear relationship between the rate of photosynthesis ($P_N$) and $F_v/F_m$ for the tested plant species and localities ($F$-ratio = 43.296, $p < 0.01$, $R$-squared = 0.405, $R = 0.598$).

**Energy and ash accumulation**

The mean values of combustion heat in the dry matter of analyzed plant species are shown in Fig. 5. The observed values of combustion heat on the control plot EES Kremnické vrchy Mts and MP Žiar n. Hronom (stress stand) showed that this characteristics is influenced by plant species because there were found out significant interspecies differences (ANOVA: $F_{(3,8)}$ =32.946, $p <0.001$). Mean value of combustion heat in case of plants growing on MP Žiar n. Hronom was 18730 ± 1442 J.g⁻¹, in case of plants from control conditions it was 18977 ± 955 J.g⁻¹. In the dry matter of leaves of *Fagus sylvatica* species on EES Kremnické vrchy Mts and MP Žiar n. Hronom were observed similar energy values (19 653, respectively 19 750 J.g⁻¹). The lowest values was found in the assimilatory organs of sedges growing on MP Žiar n. Hronom (17 711 J.g⁻¹) and approximately 3.3% higher value was found in control stand on EES Kremnické vrchy Mts (18 302 J.g⁻¹). This difference was statistically significant on the level $\alpha =0.05$. From the measured energy values it is clear that the *Carex pilosa* species growing on MP Žiar n. Hronom reacted more sensitively on the influence of abiotic factors and anthropogenic pollution of environment by lower value of combustion heat. In case of *Fagus sylvatica* species the difference was insignificant.

In the locality Hliníky the content of combustion heat was studied in case of four plant species (beech, blueberry, raspberry and male fern). There were found out significant interspecies differences as well as differences between plots (ANOVA: $F_{(7,16)}$ =24.230, $p <0.001$). On studied plots the highest values of combustion heat showed the dry matter of assimilation organs of beech (19 989, respectively 19 984 J) and of blueberries (20 482, respectively 20 551 J). The lowest values were
observed in the raspberry shoots (18 562 J.g⁻¹, respectively 18 511 J.g⁻¹) and in fern leaves (18 562, respectively 19 049 J.g⁻¹, Fig. 5). In the damaged stand was mean energy value of the studied plants lower (19 399 ± 987 J.g⁻¹) compared to the control stand (19 524 ± 916 J.g⁻¹). From the Fig. 5 we can see the different reaction of studied plants on the impact of environmental conditions. In the control stand had slightly higher energy value the Dryopteris filix mas and Vaccinium myrtillus species.

From the measured values of combustion heat of plant species is evident not only effect of habitat, but also the differences between plant genotype. On locality Hliníky the highest significant differences were found in the case of blueberries opposite assimilation organs of raspberries and ferns and among Fagus sylvatica species opposite raspberry and fern leaves.

Mean value of ash in case of plants growing in stress stand MP Žiar n. Hronom was 76.2 ± 31.9 mg.g⁻¹ and in case of plants from control conditions 64.7 ± 17.4 mg.g⁻¹. A higher value of ash was found in sedge assimilation organs, compared to the ash content in beech leaves (Fig. 6). In the locality Hliníky was ash content studied in case of three plant species (blueberry, raspberry, male fern). Mean value of ash in case of plants from damaged plot (stress stand) was 43.8 ± 4.3 mg.g⁻¹, in case of plants from control conditions 43.7 ± 10.1 mg.g⁻¹. On the studied plots, only slightly higher values of ash showed the dry matter of assimilation organs of raspberry (46.4, resp. 46.1 mg.g⁻¹) and fern (48.4, resp. 55.9 mg.g⁻¹), lower values were recorded in blueberry shoots.

**Discussions**

The rate of photosynthesis of control and stressed plants of Fagus sylvatica species on EES Kremnické vrchy Mts and MP Žiar n. Hronom showed that there are interspecies differences in the response to the effect of abiotic stressor. According to the results of Woo (2009), beech belongs to the sensitive woody species on air pollution which is also reflected in the change of rate of photosynthesis in the studied plots. Interspecies differences in the rate of photosynthesis and growth of biomass within plants of peat ecosystems are mentioned by Kool & Heijmans (2009) and within boreal forests are confirmed by Carew et al. (2003) and Kulmala et al. (2009). For all studied plant species in locality Hliníky (NP Slovenský raj) was recorded decrease in the rate of photosynthesis in plants growing in damaged forest stand, compared to the plants from the control plot. Decrease in the rate of photosynthesis between forest ecosystems affected by anthropogenic pollution and from the localities undamaged by air pollutants states Ammann et al. (1999). Similar results mentions e.g. for Lactuca sativa species also Pavlík et al. (2012).

The results of regression analysis showed moderate positive relationships between P_N vs
gs and $P_N$ vs $ci$ for all the tested plant species and localities. Similar results were found in the work by Kumar et al. (2013), who observed effect of bacterial blight on gas exchange in rice. According to several authors there exists linear dependence between $P_N$ and gs, e.g. *Ficus carica* species (González-Rodríguez & Peters 2010), *Bactris gasipaes* species (Tucci et al. 2010) and *Pueraria lobata* species (Ding et al. 2011). Similar conclusions were confirmed for the plant species on the monitoring plots, too. Genhe et al. (2011) found out, that for dependence $P_N$ on intercellular CO$_2$ concentration ($ci$) of oil tea varieties there were the regression relationships uncorrelated. This conclusion was not confirmed for the plant species of beech ecosystems, because the results of regression analysis showed moderate positive relationships between $P_N$ and $ci$. The results indicate that among the factors affecting photosynthetic properties, some of them can be grouped as stomatal limitations while others are non-stomatal limitations (e.g. temperature, humidity, nutrition, PAR etc.).

The measured values of transpiration rate on EES Kremnické vrchy Mts (control stand) and MP Žiar n. Hronom (stress stand) show that lower mean transpiration has sedge compared to beech. Based on these results it can be stated that the rate of transpiration is significantly affected by the plant species. These conclusions are confirmed in the work of Wang et al. (2011), who investigated the six trees in the polluted air of Beijing and also Dang et al (1997), who have studied the gas exchange in three coniferous trees of boreal forest of Canada. Higher difference between the stressed and the control stand was recorded in the case of beech. In the case of sedge was transpiration rate between the two variants relatively smoother, compared with beech. According to Farner (1993), airborne dust in the case of forest ecosystems but also in heathlands influences transpiration rate. This finding was confirmed also in our experiments. According to Ali et al. (2011), e.g. aluminum supplied to hydroponics in barley plants causes a decrease in transpiration depending on changes in pH. Transpiration rate was significantly affected by plant species also in the investigated locality Hliníky. Interspecies differences in the rate of transpiration confirmed in six grass species Li et al. (2007) and in the herb layer of northern deciduous forest Diekmann (1999). Interspecies differences of five plants in the response to air pollution are stated in the work of e.g. Enete et al. (2012).

In the case of dependance the rate of transpiration (E) on a stomatal conductance (gs) of plant species growing in studied beech ecosystems there was found out strong positive linear relationship. This result is consistent with the conclusions by Bsoul & Hilaire (2006), who investigated the effect of drought on assimilatory organs of maples.

Stomatal conductance is one of the important factors affecting the metabolism of plants and it is also influenced by external factors, such as illustrated by e.g. Agbaire (2009). The present
results on the control plot EES Kremnické vrchy Mts and in the stress stand MP Žiar n. Hronom indicated that stomatal conductance is affected by plant species but also by distance of the locality from the emission source. Interspecies differences in stomatal conductance confirm Tomasevic et al. (2005), who observed the influence of pollutants on the characteristics of hazel and horse chestnut. Differences in stomatal conductance depending on the locality are also confirmed by Nizzetto et al. (2012). According to Dohmen et al. (1990) and Leuzinger & Körner (2007), stomatal conductance usually due to air pollution and the increasing concentration of CO₂ decreases, but the reaction in plants is not always reflected. From measured values of stomatal conductance of selected plant species in the locality Hliníky is evident not only effect of habitat, but also the differences between plant genotype. The slightly higher average values of stomatal conductance show plants in damaged stand compared to the plants sampling from the control stand. These results do not correspond to the conclusions of Dohmen et al. (1990), Davies & Unam (1999), as due to air pollution and an increase in the concentration of CO₂ typically occurs the reduction of stomatal conductance, but such response is influenced by genotype of plants and concentration of pollutants. Studied plants reacted differently to anthropogenic pollution of environment, because in assimilatory organs of beech and blueberry stomatal conductance was higher on the control plot in the locality Hliníky, whereas in the case of raspberry and fern it was on the damaged plot. The obtained results in the case of beech and blueberries correspond to the work of Prasad (1995), who states that the plants from the control conditions have higher stomatal conductance in comparison with the plants from the experimental conditions. Interspecies differences in stomatal conductance and response to environmental pollution in the case of corn and soya are stated in the work of Bernacchi et al. (2007).

In the study forest ecosystems there was also found evident decrease of net photosynthetic rate (Pₜ), transpiration rate (E) and stomatal conductance (gs) of plant species with the increase of anthropogenic pollution. However the difference in genotype showed resistance to anthropogenic pollution between the studied species. Decrease in physiological properties of plants, reflecting strong salinity is dealt in the work of Qin et al. (2010).

An important physiological indicator is also chlorophyll fluorescence, which appears to be a suitable indicator of plant reaction to stress (Maxvell & Johnson 2000). Negative impact of stressors on chlorophyll fluorescence was studied by Gamon & Surfus (1999), Takayama & Nishina (2009). These authors state that not always, these changes are accompanied by visible signs of damage leaves (Boyer et al. 1986). Interspecies differences in chlorophyll fluorescence are stated e.g. by DeEll & Toivonen (2003). Changes in the values of the fluorescence, depending on the activity of stress factors studied e.g. by Panicucci et al. (1998) and Omasa & Takayama (2002). On the studied plots we can see different response of observed plants to anthropogenic pollution. At least responded to anthropogenic pollution raspberry shoots growing in locality Hliníky because the difference between the variants was the lowest. Similarly, responded to air pollution Taraxacum species and it is illustrated in the works of Lanaras et al. (1994) and Meinander et al. (1996) within pine tree.

A moderate positive linear relationship was found out between the rate of photosynthesis (Pₜ) and Fₚ/F₇₄ for the tested plant species and studied localities. The result corresponds with the findings in work by Bredahl et al. (2004) and Yang et al. (2009). The authors found out positive correlations between Fv/Fm and Pn for Salix arctica, Vaccinium uliginosum and Brassica napus species.

The obtained results show that especially the influence of imissions and ecological conditions of environment significantly influenced content of energy in assimilatory organs of plants. Lin & Cao (2008) state that plants
growing in different environment and at different stages of development prefer different strategies of energy accumulation. Several authors reported higher levels of energy and biomass parameters of plants growing in stand gaps and unclosed stands (Gazda et al. 2007, Pancer-Koteja et al. 1998). Kováčová & Schieber (2002, 2003), Schieber & Kováčová (1999, 2000, 2002) e.g. in the beech stand with stocking 0.9 in Kremnické vrchy Mts found out energy content accumulated in beech herbaceous species (Carex pilosa, Dentaria bulbifera, Galium odoratum, Fragaria vesca) ranging from 15 295 to 18 639 J.g⁻¹ and only slightly higher energy values observed for Veronica officinalis species (19 453 J.g⁻¹) growing on parallel, anthropogenic influenced plot (clear cut area). Negative effects of pollutants on Polish and Slovak side of Bielovodská dolina valley in the High Tatras, in herb understory by air pollutants and bark beetles damaged stands and on parallel undamaged spruce stands were observed by Kukla et al. (2002). In case of blueberry the authors found higher mean energy value in undamaged stands (21031 ± 177 J.g⁻¹) opposite species growing in stressed conditions (20947 ± 145 J.g⁻¹) and compared to our results found out in the control and stressed stand in the locality Hliníky the difference was about 1.8-2.6 %. The caloric values of different plant species growing in forest ecosystems of the taiga zone studied Bobkova et al. (2001). The energy values of plants in the lower layers of forest communities varied from 17.44 to 19.76 kJ.g⁻¹, in the case of bilberry it was 18.50 ± 0.13 kJ.g⁻¹, that is approximately 10% lower value than are our findings in the locality Hliníky.

Ash contents found out in stress stand on MP Žiar n. Hronom and EES Kremnické vrchy Mts (control stand) are compared to the limit values according to Larcher (1988) characteristic for plants growing on soils with an average content of available nutrients. Ash values in plant organs in the locality Hliníky are according to Larcher (1988) typical for the plants growing on acidic soils, poor in nutrients.

**Conclusions**

Within the study we found out significant differences in physiological characteristics (rate of photosynthesis, transpiration, stomatal conductance, chlorophyll fluorescence) and energy accumulation of plant species (Fagus sylvatica L., Dryopteris filix-mas (L.) Schott, Vaccinium myrtillus L., Carex pilosa Scop., Rubus idaeus L.) growing in the forest ecosystems disturbed by aggressive action of air pollution to the plant species from undamaged forest phytocoenoses.

On MP Žiar n. Hronom (stress forest stand) very sensitively reacted on anthropogenic environment impacts significantly lower value of photosynthetic rate Fagus sylvatica species, which is generally regarded as a sensitive species. Fagus sylvatica species reacted very sensitively significantly lower values in measured physiological characteristics (rate of photosynthesis and the stomatal conductance) also in stress stand on the locality Hliníky (NP Slovenský raj). As sensitive in the locality Hliníky seems to be herbaceous understory and especially raspberry and blueberry (lower average values of measured physiological characteristics show plants in damaged stand compared to the plants sampling from the control stand). In general, we can say that to the anthropogenic stress reacted more sensitively assimilation organs of beech compared to herb organs, although there are differences in the localities.

Accumulated energy was influenced especially by plant species and environment conditions because among studied species significant differences were found out. From the data on EES Kremnické vrchy Mts (control stand) and MP Žiar n. Hronom (stress stand) is clear that species Carex pilosa growing close to the pollution source reacted more sensitively on impact of abiotic factors and anthropogenic
pollution by significantly lower value of combustion heat. In the case of the *Fagus sylvatica* species this difference was not significant. In the analyzed stands a higher amount of ash was determined in assimilation organs of sedges, compared with ash content in leaves of beech. The higher amounts of ash accumulated in the assimilatory organs of plants growing on MP Žiar n. Hronom and EES Kremnické vrchy Mts pointed out to the quality of soils with an average content of available nutrients.

The different reaction of plants on the influence of environmental conditions was also confirmed in the locality Hliníky (NP Slovenský raj). Slightly higher values of energy showed leaves of male fern and blueberries on the control plot, compared to the stressed plants. The significantly highest accumulation of energy was found out in *Vaccinium myrtillus* and *Fagus sylvatica* species growing in both stands. In the studied stands only slightly higher levels of ash showed the dry matter of assimilation organs of raspberry and fern compared to the values observed in the blueberry. The lower average amounts of ash accumulated in the assimilatory organs of plants growing on the plots of the locality Hliníky pointed out to the presence of acid soils poor in nutrients.

The obtained data can be useful as a reference level for comparison with the future measurements of plant species growing in forest ecosystems at different levels of air pollution stress. There will be assessed data on the physiological status of selected plant species focusing on the photosynthetic apparatus, water relations, damage to cell membrane and information about the processes of accumulation and spatial distribution of toxic elements in soils and plant species.

**Acknowledgements**

This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences (project no. 2/0027/13), project no. SK-CZ-0213-11, project no. 7AMB12SK017 of Ministry of education, youth and sports CR and by the project implementation: Extension of the centre of Excellence „Adaptive Forest Ecosystems“, ITMS: 2622012049, supported by the Research & Development Operational Programme funded by the ERDF.

**References**


Kuklova et al.

Kuklova et al.


of visual estimates of tree defoliation. Lesnictvi-Fores-
try 42: 49-53.
Species of Forest Ecosystems of the Male Karpaty
Mountains. Ekologia (CSSR) 4: 33-42.
Y., 2011. Water, heat and airborne pollutants effects on
transpiration of urban trees. Environmental Pollution
159: 2127-2137.
Woo S.Y., 2009. Forest decline of the world: A linkage
with air pollution and global warming. African Journal
of Biotechnology 8: 7409-7414.
Yang G., Zuo Q., Tang Y., Shi J., Leng S., 2009. Diffe-
rences of Photosynthetic Characteristics in Rapeseed
(Brassica napus.L) at Seedling Stage with Different
Nitrogen Utilization Efficiency for Grain Production.
Zlatnik A., 1976. The survey of groups of types of geobi-
ocoens primarily forest and shrubby in the C.S.S.R..
News of Geographic Institute Brno 13: 55-64.