



Drought as stress factor and its role in spruce (*Picea abies* /L./ Karst) dieback

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In this paper we present the results of ecophysiological monitoring (chlorophyll *a* fluorescence measurements, assimilatory pigments concentration, needles water potential) of spruce forest stands health state in the Kysucké Beskydy Mts. The focus has been set on evaluation of the role of drought acting as one of numerous factors causing dieback of this tree species. Our research was a part of an extensive project aimed at providing sufficient analytic data on spruce dieback at all levels: beginning with individual trees, through forest stands, to large-sized areas. The next step is to synthesise these data and recognise how the dieback process works, which will eventually enable us to design more suitable models and to propose management directions for the endangered forests in north-west and north part of Slovakia.

Keywords: *Picea abies*, drought, decline, ecophysiology, Kysucké Beskydy

Introduction

Physiology of Norway spruce (*Picea abies* /L./ Karst) growing in mountain areas is influenced and limited by numerous ecological factors, both natural and anthropogenic, acting generally in interactions. The result is multiple stress that may entail weakening, diseases and dieback of individual trees, followed by gradual stand decomposition. At the same time, the spectre of stress factors and their effectiveness is very variable in space and time. In conditions of mountain spruce forests, the today's situation is governed by climatic and meteorological factors, high ground-level ozone concentrations and excessive solar radiation. Simultaneous presence of these factors, together with their interactions, enhances the oxidation stress. Towards the end of winter and in early spring is common occurrence of so called water stress caused by so called winter desiccation. At present, the effect of air pollution in mountain conditions is mostly indirect – it acts through wet and hidden deposition. Input of acid substances causes acidic stress to the rhizosphere which may, apart from damaged needle surface and nutrient leaching, result in enhanced nutrition stress (Kmeť et al. 2004).

As water stress sets basic limits for plants' physiological activities, the study of spruce's response to various levels of water supply – monitored through suitably selected physiological markers, enables us to identify certain symptoms and effects of drought impact on spruce stands. Physiological processes are a very sensitive indicator of stress in plants, primarily under extreme environmental conditions.

In this contribution we present results of an ecophysiological research on spruce dieback in the Kysucké Beskydy Mts pursued in frame of the extensive project entitled „Restoration and conversion of allochthonous forest communities endangered by changing natural conditions (primarily climate) to ecologically more stable ecosystems“.

The research aims for the year 2007 issued from the main goals of the exploration phase. In our case was the primary goal set at dissemination of scientific information on influence of changing environment, global climate change included, on dying of spruce forest stands, contribution to understanding the dieback process and providing more precise diagnostic tools for prediction of development of these ecosystems in conditions of climate change.

An important partial task is to assess the role of tree's physiological state in context of its dieback and dying and analyse the relation „tree physiology–environmental parameters“, focussing on role of drought as a stress factor.

Water stress (drought stress) is one of the most frequent ecological limits determining realisation of the plant's production potential. It is one of the accompanying phenomena of the running climatic changes. Today, the problem is getting global, because the predicted ecological problems connected with increasing greenhouse effect, global warming and with more frequently occurring periodical drought periods are turning to reality.

Drought influences woody plants and stands either directly or as a primary stressor lowering the resistance of forest stands and initiating activation of other harmful factors. The problem is most conspicuous in spruce stands, primarily in lower or medium situated areas, often in spruce as an introduced species. In context of evaluation of dry periods, their duration, intensity and prognoses of occurrence, we are facing a serious problem connected with definition of drought from the viewpoint of ecological demands of the given wood plant as well as the problem of involving all relevant factors influencing the water regimen of forest stands.

The hitherto obtained knowledge on water demands of Norway spruce is mostly general. Apart from characterising the spruce as a woody plant belonging to cold and moist climate, there are mostly utilised values of precipitation totals at limits of the distribution area of the species and the values obtained at its growth optimum. A comprehensive summary of these values measured by several authors can be found in the book Schmidt–Vogt (1987). In Central-European conditions, the lower limit for spruce cultivation has been set as a cumulative value of 300 mm precipitation over the period May–August.

More exact description of water demands of spruce trees requires identification of their response to varying water supply and characterisation of symptoms and effects of drought on spruce stands. Several authors point out direct bonds between the drought presence and physiological processes running in plants, for example transpiration (Matejka et al. 2002), photosynthesis, assimilatory pigments (Brestič, Olšovská 2001), fluorescence of chlorophyll *a* (Kmeť et al. 2006), mineral nutrition (Grabařová, Martinková 2001, Wallin et al. 2002), changes in stem diameter (Tardif et al. 2001, Sevanto 2003) (etc).

Material and Methods

The research subject is situated in area of the Kysucké Beskydy Mts. (FE Čadca, FD Stará

Bystrica, locality Šadibolovci) in stands No 5226 and 5225, at an altitude of 950 m asl. Meteorological records obtained in the stands in years 1951–1980 resulted in a mean annual value of 1230 mm and a mean annual temperature of 4.5 °C. The stands belong to the category of commercial ones. The major part represents fir–beech forest vegetation zone. The slopes are moderately steep to steep. The soils are medium deep loamy cambisols, mesotrophic, skeletal, slightly humic. Parent rock material consists of sandstone and schists. The locality belongs to the edaphic–trophic nutritive order B, group of forest types *Abieto–Fagetum*.

In winter (2006) and in early spring (2007) were the plots damaged by wind calamity, and most of the studied adult sample trees (except of three ones inside the stand) were destroyed. Consequently, the continuity of measurements from 2006 had been interrupted (Kmeť et al. 2007), and in 2007 we had to select new sample trees. The sample set has been enhanced ($n = 18$). The trees were tested in groups of three: one situated in precipitation shadow, another supposed to be watered in precipitation-critical periods, and control. Each of the three variants was established at a south-oriented stand edge and inside the stand. The last group of three trees was selected at the north stand edge.

In 2007, we three times (May, July, October) sampled branches from these 18 sample trees. The assimilatory organs were collected manually, from the upper thirds of enlightened crown parts, following the international methods ICP Forests.

Two branches from each adult sample tree were used for measuring parameters of fast kinetics of chlorophyll *a* fluorescence. These parameters reflect the state and functionality of photosynthetic apparatus – one of the most important physiological systems of the tree. The method is based on high sensitivity of the photosynthetic chain to different stress factors.

We measured background parameters of the fast phase of fluorescence phenomenon (F_0 – minimal fluorescence, F_v – variable fluorescence, F_m – maximal fluorescence, F_v/F_m – maximal photochemical activity of the photosystem II, T_m – time (ms) of reaching maximum fluorescence, Area – area above the fluorescence induction curve). The parameters were measured in one-year-old spruce needles (2006) on individual spruce sample trees „in situ“. We used a transportable equipment – fluorimeter *Plant Efficiency Analyser* (PEA, Hansatech Ltd., Kings Lynn, UK).

The measurements were conducted after 30min dark adaptation, at 50 % intensity level of saturation light ($2100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and recorded at intervals of one second. There exist no stand-

ard units for fluorescence calibration; the fluorescence signal is quantified through relative response in bits with the detector of the absorbed re-emitted radiation (Kmet 1999).

In the same sampled needles were also determined assimilatory pigments' concentrations. Analysis of chlorophylls and carotenoids was carried out from 80 % water solution of acetone, after having homogenised the needle samples in a homogeniser. The absorbance values were measured spectroscopically (equipment Cintra 6.5, GBS, Australia), and the concentration of assimilatory pigments was calculated with using the relations proposed by Lichtenthaler (1987). The values of concentrations of chlorophylls *a*, *b*, *a+b* and carotenoids *x+c* are given per a dry weight unit ($\text{mg}\cdot\text{g}^{-1}$).

Selected spruce sample trees were once in summer and once in autumn examined to measure water potential of their needles (needle year 2006), using the equipment PSY PRO (WESCOR, USA). The measured values have been expressed directly as water potential values in MPa. The values of water potential provide us with information whether or not suffers the plant from drought stress (water potential has an important role – being a driving force of the water transport); consequently, monitoring and evaluation of this parameter can contribute significantly to make things clear, namely in case of experiment with “simulated” drought stress.

Providing with the test Mann-Whitney U – we tested significance of differences mean values of chlorophyll concentrations and water potential of needles in selected groups of sample trees.

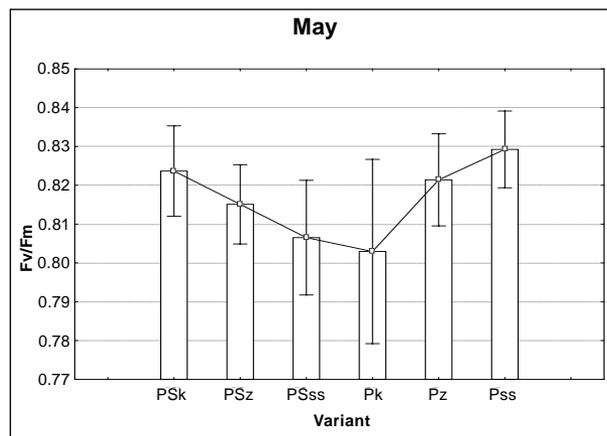
The programme STATISTICS was our tool for evaluation of the mean values of the core parameter of fast phase of chlorophyll *a* fluorescence – ratio F_v/F_m in frame of the individual variants (three-trees groups) for the corresponding measurement date.

Results

The parameters of chlorophyll *a* fluorescence measured towards the end of May 2007 revealed that the core parameter had not decreased under the physiological limit of disturbances (0.725) even in a single case (spruce sample tree). Also other parameters of fast phase of chlorophyll *a* fluorescence indicate that primary photosynthetic processes in needles of adult spruce trees were running without disturbances.

Neither the same parameters measured in July 2007 indicated, in spite of dry and very hot weather, worsened performance of needles in primary photosynthetic processes. Only in one case (sample tree 24), namely in the control stand, the ratio of variable to maximal fluorescence had sunk below the limit of physiological disturbances ($F_v/F_m = 0.695$). It is interesting that neither plastic foils (so called roofs) provoked effects of drought stress identifiable at level of physiological parameters of the needles. The values of soil moisture content did not required using the planned experiment with watering of adult trees – which is highly indicative in context of weather in summer 2007 (extremely high temperatures, minimal precipitation).

The results measured in October 2007 copy the results obtained in October 2006. Also in this case, dominant was influence of low temperatures in this period (first night frosts in the preceding days). It is, however, interesting that the much more sensitive response was observed in assimilatory organs of spruce trees growing at open stand edge where many parameters are deep below the limit of physiological damage compared to the trees growing inside the stand. In this case, the coupled effect of low temperatures and intensive solar radiation influenced negatively the course of primary photosynthetic processes (possible result of photo-inhibition).



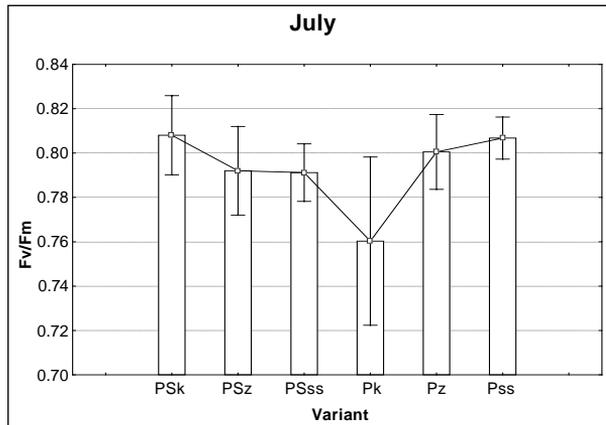


Fig. 1: Mean values of parameter F_v/F_m in May and July 2007 (average \pm 0.95 confidence interval)

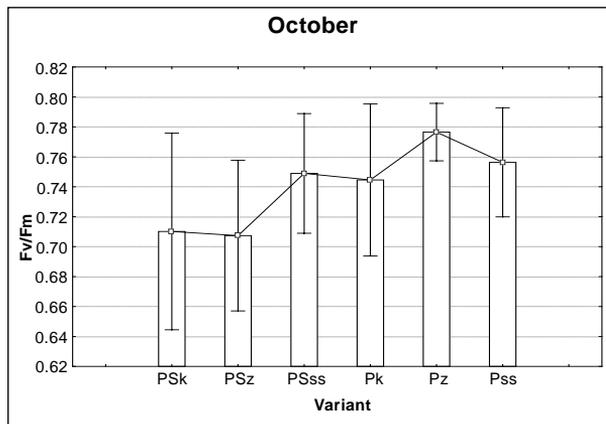


Fig. 2: Mean values of parameter F_v/F_m in October 2007 (average \pm 0.95 confidence interval)

Variability of the measured values of the ratio F_v/F_m at level of individual trees had increasing trend from May to October. In most branches sampled in October, we observed conspicuously enhanced variability. The trend of the core parameter of fast kinetics of chlorophyll *a* fluorescence (F_v/F_m) was found decreasing over the whole period, which indicates reduced performance of photosynthetic processes and higher measure of ineffective re-radiation of energy into the environment. In May and July sampling, one single spruce (number 24 – control tree growing inside the stand) manifested F_v/F_m values lower than 0.725. It is interesting that, in spite of increased fluorescence dissipation observed almost in all trees, only the tree No 24 manifested marked improving when the average of all measured values converged to the global average obtained for all the measured trees. In October sampling, however, the variability of parameter F_v/F_m in this individual tree was found considerably higher (0.569–0.853).

In the same way, variability of values measured in frame of variants increases with decreasing parameter F_v/F_m .

The most conspicuous changes over the year were recorded in sample trees at the stand edge (except from individuals covered with foils – PSss, where arises a question whether or not this shelters inhibited evaporation) which manifests increasing rate of worsening health state of these trees. Negative influence of the used foils has not been found evident, and because there were not recorded any marked changes in water potential of these sample trees; it is disputable whether or not our experiment with precipitation shadowing has resulted in the supposed effect.

Based on comparison of parameters of photosynthetic pigments in selected three-tree-groups in the stand (stand – control, stand – drought stress) we can conclude that there has not been observed hypothesised decrease in concentrations of chlorophylls in the group subjected to the simulated drought stress. Somewhat lower

values of the ratio between chlorophylls and carotenoids (chl/car) found in the group situated inside the stand and subjected to simulated drought stress manifest somewhat lower photo-protective performance of the photosynthetic apparatus.

Comparing the observed values of assimilatory pigment concentrations and their mutual ratios in the selected three-tree-groups at stand edge (PS control, PS drought stress), we meet distinctly different situation as in the former case. The three-tree-groups growing at the stand edge under influence of drought stress manifest, in almost all examined parameters, lower values compared to the control unstressed groups. It is not

possible to conclude unambiguously about the cause of this state – if it is the simulated drought or generally weakened vitality of trees growing at the stand edge, caused by a range of factors (apart from others – position most exposed to radiation). Also the random sampling may have resulted in choosing weakened individuals.

In frame of statistical analysis we tested significance of differences in mean chlorophyll values between the three-tree-groups. There were found significant differences – primarily between the mean chlorophyll contents at the stand edge and the values obtained inside the stand. The differences were found more conspicuous in summer and autumn sampling (Tab. 1).

Tab. 1: Chlorophylls – mean values, locality Šadibolovci, the Kysucké Beskydy Mts, year 2007 – Mann-Whitney U – test, * – significant difference is defined as $P < 0.05$.

	28. 5. 2007						31. 7. 2007					
	PSk	PSss	Pk	Pss	Pz	PSz	PSk	PSss	Pk	Pss	Pz	PSz
PSk												
PSss	0.83						0.51					
Pk	0.13	0.049*					0.38	0.27				
Pss	0.27	0.51	0.51				0.049*	0.049*	0.049*			
Pz	0.13	0.049*	0.27	0.83			0.049*	0.049*	0.049*	0.51		
PSz	0.83	0.51	0.27	0.27	0.13		0.51	0.83	0.13	0.049*	0.049*	
16. 10. 2007												
	PSk	PSss	Pk	Pss	Pz	PSz						
PSk												
PSss	0.049*											
Pk	0.049*	0.83										
Pss	0.049*	0.13	0.049*									
Pz	0.049*	0.27	0.049*	0.13								
PSz	0.38	0.83	0.83	0.049*	0.13							

Stand edge control, PSk: sample trees No 8,9,10

Stand edge stressed PSss: sample trees No 12,13,14

Stand control, Pk: sample trees No 22,23,24

Stand – provided with shelters, Pss: sample trees No 31,33,35

Stand – planned watering, Pz: sample trees No 37,40, 41

Stand edge – planned watering, PSz: sample trees No 43, 44, 45

In conditions of experiment with drought stress at the locality Šadibolovci (Kysucké Beskydy), there was not recorded drought effect, as no value of water potential had decreased under – 0.6 MPa. The water potential of spruce sample trees growing at the stand edge manifested the trend moderately decreasing to this value.

As for the comparison between the mean values of water potential, in summer were identified

significant differences between the three-tree groups under simulated drought stress inside the stand and the groups planned for watering – both in the stand and at the stand edge. In autumn, there were observed most significant differences in mean values primarily between the groups inside the stand and the groups at the stand edge (Tab. 2).

Tab. 2: Water potential of needles – mean values, locality Šadibolovci, the Kysucké Beskydy Mts, year 2007 – Mann-Whitney U – test, * – significant difference is defined as $P < 0.05$.

	31. 7. 2007						16. 10. 2007					
	PSk	PSss	Pk	Pss	Pz	PSz	PSk	PSss	Pk	Pss	Pz	PSz
PSk		0.83	0.27	0.65	0.08	0.51		0.049*	0.049*	0.27	0.049*	0.83
PSss			0.51						0.049*			
Pk												
Pss		0.51	0.51					0.27	0.049*			
Pz		0.13	0.51	0.046*				0.18	-0.51	0.13		
PSz		0.82	0.51	0.043*	0.046*			0.13	0.049*	-0.27	0.049*	

Discussion and conclusion

Similar results with measuring parameters of chlorophyll *a* fluorescence also report Lechner and Bolhar-Nordenkampf (1989), who studied influence of different combinations of stressors determined by the altitude at which the trees were growing. Considerable drop of parameter F_v/F_m at higher altitudes was also associated with the temperature course. Especially temperature stress resulting from low night temperatures causes direct reduction of photosynthetic capacity, which is consequently reflected on the tree health state. Graded water stress together with low and high temperatures cause changes in course of fluorescence curves. Different from the temperature stress, the quantum photochemical yield (F_v/F_m) is, up to certain level of relative moisture content (RWC) in the plant assimilatory organs, only slightly sensitive to the water stress (Fracheboud, Leipner 2003). In this case is suitable, if possible, to provide also with other parameters of slow phase of chlorophyll *a* fluorescence (F_s , $\Phi PSII$, ETR).

The obtained results manifest a trend similar to that one recorded in the preceding season 2006. All the followed parameters show natural seasonal evolution (seasonal dynamics of assimilatory pigments). Also in this case, it is necessary to point out unfavourable values of the ratio between chlorophylls and carotenoids (chl/car) reflecting lowered photo-protective performance of the photosynthetic apparatus. Exactly carotenoids with their xanthophyll cycle represent an important mechanism protecting from excessive radiation that is especially demanded in case of water deficit. Lichtenthaler (1985) reports for healthy trees (primarily spruce and fir) the values of ratio between chlorophylls and carotenoids (Chl a+b/Car x+c) ranging 5–8. If the trees are under influence of stress factors, these values can sink to 3–5, with the needles still keeping

their green colour. In case of yellow-green coloured needles, the values are below 3, frequently between 1–2. That means that these trees suffer from increased photo-instability of assimilatory pigments in their needles, both green and yellow-green. The ratios between pigments are possible to use as an important bio-marker of damage, indicating whether or not the photosynthetic apparatus has already been severely damaged by the stress.

Water potential in healthy, appropriately watered plants ranges from -0.2 to -0.6 MPa, the plants suffering from drought have ψ from -1.5 up to -5 MPa. Because water uptake and transport is a passive process, the plants are able to uptake water only in case when water potential of their roots is lower than water potential of the soil. With decreasing water potential of soil also water potential of plants will decrease. A drop in water potential is most sensitively reflected by the plant's growth, processes occurring in its cell walls and synthesis of proteins. Less sensitive is response of biochemical and photochemical processes associated with photosynthesis. Water potential of plant assimilatory organs having decreased to the interval (-1; -2) MPa results in serious metabolic changes. In our experiments the values from this range have not been recorded.

The choice of spruce sample trees and of the working methods was made in accordance with hypothesis on spruce dieback in territory of the Kysucké Beskydy (locality Šadibolovci), according to which the drought stress is sharing on worsened health state of the studied spruce stands.

The presented results of our research on physiological and biochemical parameters of assimilatory organs of adult spruce trees allow us to declare with high probability that the influence of water deficit on general dieback of spruce forests in this area is „weak“.

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