FOLIA OECOLOGICA – vol. 35, no. 1 (2008). ISSN 1336-5266

Analysis of physiological parameters of spruce trees as indicators of spruce dieback in the Spiš region

Ľubica Ditmarová[1], Jaroslav Kmeť[2], Adriana Leštianska[2], Katarína Střelcová[2]

[1]Institute of Forest Ecology of the Slovak Academy of Sciences, Štúrova 2,
960 53 Zvolen, Slovak Republic, E-mail: ditmarova@savzv.sk
[2]Faculty of Forestry, Technical University in Zvolen, T. G. Masaryka 24, 960 53 Zvolen, Slovak Republic

Abstract

Ditmarová, Ľ., Kmeť, J., Leštianska, A., Střelcová, K. 2008. Analysis of physiological parameters of spruce trees as indicators of spruce dieback in the Spiš region. *Folia oecol*., 35: 1–5.

This contribution is dealing with the large-scale dieback of spruce stands in the region Horný Spiš. The physiology of Norway spruce (*Picea abies* L. Karst) in this area is influenced and limited by a range of natural and anthropogenic ecological factors, acting in general in interactive way. The work summarises the results of an eco-physiologically oriented research evaluating physiological and so also health status of the relevant spruce stands. The research was running in year 2004 on two monitoring plots selected at the locality "Hliníky" in the region Horný Spiš. One plot showed symptoms of acute stand decomposition, the other lacked visible damage symptoms. The age of spruce trees on both plots was 80 years (adult trees). The analysis was focussed on appropriate indicators – biomarkers indicating damage to the spruce assimilatory apparatus as a tool for solving the issue of massive dieback of spruce stands. There have been processed the results of measurements of parameters of chlorophyll *a* fluorescence and the values of concentrations of assimilatory pigments.

Key words

eco-physiology, multiple stress, dieback, Norway spruce, Spiš

Introduction

Since 30 years ago, the health state of spruce stands has been recording a dramatic decline over the whole European continent. In Slovakia, there have been damaged extensive areas in the regions Orava, Kysuce, Spiš and in the Tatra Mts.

The physiology of Norway spruce (*Picea abies* L. Karst) is influenced and limited by a range of abiotic and biotic factors, acting in general interactively: drought, wind, snow, frost, acid litter, insects, fungal diseases, management methods and airborne acid pollutants. The rapid dieback of trees and whole stands was also accelerated by extreme weather connected with the global climate change. The result is multiple stress that can cause fading, diseasing and dieback of individual trees up to decomposition of the whole stands. It is high important to seek ways how to indicate stress load to forest woody plants and stands, determine latent diseases and damage because early diagnostic of the disease can prevent from passing to more progressive stages. The goal of eco-physiological research on damage to forest woody plants is to recognise various mechanisms how the stressing factors disturb physiology of trees, shrubs and whole forest ecosystems at a specific site. Bio-indication of stress load is possible at various levels of biological systems and with using a range of physiological and biochemical reactions or parameters – biomarkers. The aim is to obtain reliable diagnostic indicators of the latent damage (Kmeť and Ditmarová, 2003).

Apart from the well recognised and well described types of damage to and decomposition of forest stands, since recently has also been present dieback attacking spruce stands of various age, at various sites. This dieback is not possible to classify to either of the known disturbance patterns according to the symptoms and ecological conditions.

In the frame of solving the APVT project "Analysis of causes and proposal for measures against massive dieback of spruce forest stands in border areas of Northern Slovakia", there has been accomplished a multidisciplinary research on dieback of spruce stands in the regions of Horný Spiš and Kysuce, running under the preliminary working title "Unspecific dieback of spruce stands". It was studied physiological state of spruce trees (transpiration, assimilatory pigments, chlorophyll *a* fluorescence, mineral nutrition, microclimatic characteristics) for different age classes (seedlings, young growth, adult trees). In this contribution we present results of measurements of physiological parameters in adult trees. We are focussing on assimilatory pigments, first of all on measurement of chlorophyll *a* fluorescence – the methods used in bio-indication of stress to forest woody plants.

Material and methods

The assessment of changes to physiology and also to the health status of spruce trees in the studied territory at the locality "Hliníky" in the region Spiš was made on 10 adult sample trees. Five trees were selected from the border of the stand with evident signs of decomposition; five trees were selected from the same plot, inside the stand without visible damage symptoms. The stand age is approximately 80 years.

The locality "Hliníky" Spiš is situated in the Slovenské rudohorie Mts unit Volovské vrchy. The altitude is 950 m asl, exposition S-SW, slope inclination 10%. The parent rock consists of agglomerates. The most frequent soil type at the site is podzolic cambisol. The climate is moderate cold; with a mean annual temperature of 6.8 °C and mean annual precipitation total of 700 mm. The locality belongs to the 5-th forest vegetation tier, group of forest types Abieto-Fagetum. The proportion of spruce is 100%.

Improper species composition of the stand, secondarily altered in profit for spruce and the fact that the spruce is the woody plant least resistant against effects of airborne pollutants are resulting in gradual decomposition of the ecosystems at this locality.

Analyses of chlorophyll a fluorescence

In 2004 we measured parameters of both phases – fast and slow of fluorescence of chlorophyll *a* in one-year old spruce needles (year 2003), on September 27.

Analysis of chlorophyll fluorescence represents a non-destructive and fast method enabling us to gain timely information about physiological activity of leaves and whole plants and about internal structure of their assimilatory organs.

The method is working based on the fact that the light energy exceeding the needs of photosynthetic process is spread either in form of fluorescence or in form of heat. The changes in rate of the photosynthetic process or in the dispersed heat cause total changes in the fluorescence emission. If all the reaction spots of the photosystem II (RC PS II) are closed, 95–97% of the absorbed anergy is converted to heat and 2.5–5% to fluorescence (Bolhar-Nordenkampf and Öquist, 1993). If the assimilatory organ is suddenly irradiated (after having been adapted to the dark), the studied fluorescence induction line is dependent on time (more in Kmeť, 1999). We were focussing on the baseline parameters of the fluorescence phenomenon (F_0, F_m, F_v, F_v/F_m T_m and *Area*) determined with a transportable apparatus – fluorimeter Plant Efficiency Analyser (PEA, Hansatech Ltd, Kings Lynn, UK). The measurements were made after a 30-min adapting period, at a 50% level of intensity of light incidence (2100 μmol m^{-2} s^{-1}) and an interval of 1 second of data recording.

Analyses of assimilatory pigments

In fresh spruce needles (delivered to the laboratory in a portable cold box) we determined concentrations of photosynthetic pigments (chlorophylls *a, b*, total content of carotenoids and their mutual ratios). The chlorophylls and carotenoids were analysed from 80% water solution of acetone with spruce needles homogenised in a homogeniser. The absorbance values were measured spectre-photometrically (apparatus Cintra 6.5, GBS, Australia), the concentrations of photosynthetic pigments were determined with adjusted equations according to Lichtenhalter (1987). Concentrations of chlorophylls *a, b, a* + *b* and carotenoids *x* + *c* are given per a unit dry mass amount (mg g^{-1}).

Statistical analyses

The results were processed statistically, with variance analysis, software STATISTICA 5.5. The individual components of the variance corresponding to the known factors were compared with the residual variance, by means of the F-test. The analysis was focussed on the influence of the specified factor – experimental plot on values of parameters of fluorescence of chlorophyll *a* (*Area*, F_v/F_m), on the determined concentrations of the other chlorophylls (chl *a*+*b*, car *x*+*c*) and their mutual ratios (chl *a*/*b*, chl *a*+*b*/car *x*+*c*).

Results and discussion

Over the year 2004, we examined contents of chlorophyll *a*, chlorophyll *b* and the total amount of chlorophyll *a*+*b* in assimilatory organs of adult spruce trees

on the control plot without visible damage symptoms and on the plot with remarkable signs of stand decomposition (Table 1).

Several authors (eg Matyssek et al., 1993) suggest that the declining physiological state of spruce needles is connected with decline in chlorophyll content and with decreasing rate of photosynthesis, whereas the values of ratio chl *a*/chl *b* are increasing. On the other hand, the ratio chl *a*+*b*/car *x*+*c* shows a decreasing trend. According to Lichtenhalter (1985), the values of ratio of chlorophylls to carotenoids (*a*+*b*/*x*+*c*) in healthy trees (primarily spruces and firs) range within 5–8. If the trees are influenced by stress factors, then the values of this ratio can be within 3–5, whereas the needles can keep their green colouring. In yellow-green needles, these values sink below 3, frequently to 1–2. Consequently, it is possible to provide with the pigments ratios as another biomarker of damage to trees indicating whether the photosynthetic apparatus being under the influence of stress has already been severely damaged or not yet.

In our case, the *a*+*b*/*x*+*c* values are within 3–7, equally in trees growing on both plots: without and with decomposition symptoms. The results manifest a worse performance of the assimilatory apparatus in the examined sample trees on both plots.

From the F-test (variance analysis) performed on the photosynthetic pigments, it is obvious that there is not any significant difference between the two plots at the significance level of 95%.

Consequently, our results show that the different contents of chlorophylls, carotenoids and their mutual ratio are not unequivocal indicators of better physiological status of spruce trees on the control plot in comparison with the trees on the plot with advanced decomposition symptoms. It is important to point out that both plots are situated in the same forest stand, so the damage to their physiological and health status is present on both.

It is well-known that the dynamics of photosynthetic pigment contents in tree assimilatory organs is influenced by a range of environmental factors (natural seasonal changes, differences between the sun and the shade needles, airborne pollutants, drought, extreme temperatures, mineral nutrition and others) (Amundson et al., 1993; Mikkelsen et al., 1996).

The results of many research works confirm that the measurement of chlorophyll *a* fluorescence is the method enabling rapid diagnostic of the tree physiological state. This knowledge provides with a background of correlation between photochemical capacity and some parameters of the induction curve of chlorophyll *a* fluorescence, and, on the other hand, with a high sensitivity of the photosynthetic chain for a number of stress factors.

The parameters F_v/F_m and *Area* have namely the highest description value and reflect most appropriately the state and functioning of the assimilatory apparatus. F_v/F_m corresponds to the maximum quantum yield of the primary photochemical reactions of the Photo-system II (PSII). Its value is lower than 0.725, and it indicates the starting of certain physiological disturbances. The parameter *Area* is a measure of the area above the induction curve between the basal (F_0) and the maximum (F_m) fluorescence. It provides sufficient information about the capacity of the transport chain of electrons in primary photosynthetic processes, and it is close correlated with the contents of assimilatory pigments. It is well-known that the values of these parameters are in general higher in assimilatory organs that are not subjected to negative influence.

Bolhar-Nordenkampf and Götzl (1992) having accomplished a great number of measurements, report the following threshold values of the parameter F_v/F_m: 0.85 – normal, 0.72 – threshold values for disturbances, 0.60 – area of severe but reversible damage, 0.30 – area of severe structural damage.

The evaluation of the baseline parameter F_v/F_m shows that its course follows the pattern recognised in

Table 1. Mean values of concentrations of plastid pigments measured in adult spruce trees on plot with symptoms of acute stand decomposition and without visible symptoms of stand decomposition. Date: 27 September 2004

Plot		Chl *a* [mg g^{-1}]	Chl *b* [mg g^{-1}]	Chl *a*/*b* [mg g^{-1}]	Chl *a*+*b* [mg g^{-1}]	Car *x*+*c* [mg g^{-1}]	Chl *a*+*b*/car *x*+*c* [mg g^{-1}]
With vds	average	1.65	0.57	2.91	2.22	0.44	5.01
	STD	0.28	0.11	0.25	0.39	0.07	0.25
	sx	0.13	0.05	0.11	0.17	0.03	0.11
Without vds	average	1.36	0.51	2.89	1.87	0.37	5.01
	STD	0.39	0.25	0.57	0.62	0.06	1.12
	sx	0.18	0.11	0.26	0.28	0.03	0.50

With vds – the stand with visible damage symptoms
Without vds – the stand without visible damage symptoms

the assimilatory pigments. It points out gradually worsening physiological state of assimilatory apparatus in the selected adult sample trees (decreasing F_v/F_m), both on the plot without and with symptoms of acute stand decomposition. These results confirm that physiological damage to the needles on both plots is followed by rapid defoliation and that the residual assimilatory apparatus is not able to supply the given tree with necessary organic materials.

Fig 1 illustrates the course of parameters F_v/F_m and *Area* measured on plots with different decomposition grade. From the comparisons between F_v/F_m values performed by means of variance analysis it follows that the differences of the values of this parameter between the plots with different decomposition stage are not statistically significant ($p > 0.05$). A statistically insignificant difference between the plots was also recorded for the parameter *Area*.

The results of a lot of research works as well as our results confirm that the measurements of physiological parameters (assimilatory pigments concentrations, chlorophyll *a* fluorescence) enable us to analyse more rapidly the physiological and health state of trees.

Křístek (1996) suggests that the dieback of forest woody plants will be caused by their weakening and physiological disturbances. This statement is not only crucial for orientation of further research on dieback of woody plants, but it also presents another question – which are the actual causes of the physiological disturbances.

Conclusions

The worsening health state of forest woody plants and of their dieback is still a topical issue, unfortunately. The results presented in this contribution are a part of more comprehensive multidisciplinary research on dieback of spruce forest stands in the Horný Spiš region. The research was oriented at study of physiological state of spruce trees in all age categories (seedlings, young growth, mature stand), in connection with site conditions. The results of measurements of physiological parameters in adult spruce trees manifest a lower level of photosynthetic process in the assimilatory apparatus and insufficient nutrient supply to the examined spruce sample trees growing on both plots – without and with visible symptoms of acute stand decomposition.

Acknowledgement

The research was supported by the grants APVT No. 51-019302 research-oriented No. 2/4159/24 GA SAV and VEGA 1/3524/06.

References

Amundson, R. G., Hadley, J. L., Fincher, J. F., Fellows, S., Alscher, R.G. 1993. Comparison of seasonal changes in photosynthetic capacity, pigments and carbohydrates of healthy sapling and mature red spruce and of declining and healthy red spruce. *Can. J. Forest Res*., 22: 1605−1616.

Bolhar-Nordenkampf, H. R., Götzl, M. 1992. Chlorophyll-fluoreszenz als Indikator der mit der Seehöhe zunehmenden Stressbelastung von Fichtennadeln. *FBVA- Berichte*, 67: 119−131.

Bolhar-Nordenkampf, H. R., Öquist, G. O. 1993. Chlorophyll fluorescence as a tool in photosynthesis research. In Hall, D. O., Scurlock, J. M. O., Bolhar-Nordenkampf, H. R., Leegood, R. C.,

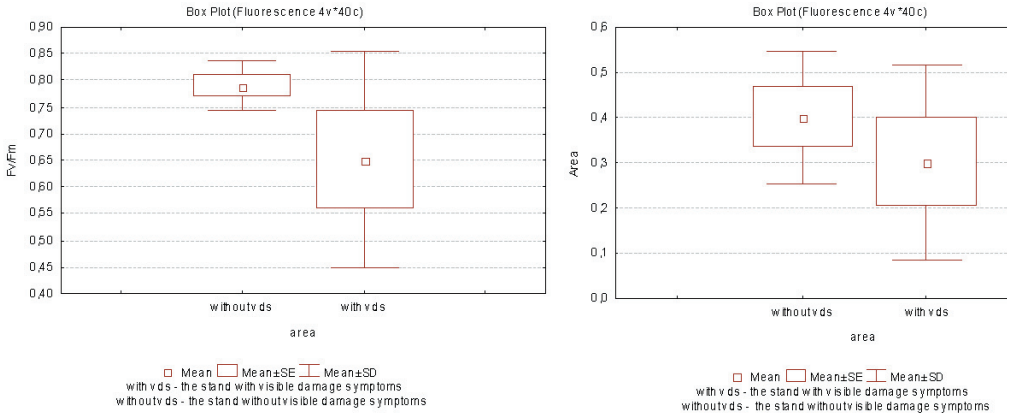

Fig 1. Course of values of parameters of chlorophyll *a* fluorescence (F_v/F_m, Area) measured on plots with different grade of stand decomposition

Long, S. P. (eds). *Photosynthesis and Production in a Changing Environment: a field and laboratory manual*. London: Chapmann & Hall, p. 193–206.
Kmeť, J. 1999. *Fluorescencia chlorofylu ako indikátor stresového zaťaženia drevín a jej aplikácia v lesníctve* [Chlorophyll fluorescence as an indicator of stress load to forest woody plants and its application in forestry]. Vedecké štúdie, 3/1999/A. Zvolen: Technická univerzita vo Zvolene. 67 p.
Kmeť, J., Ditmarová, Ľ. 2003. *Ekofyziologický výskum bukových porastov v regióne Zvolen – Žiar nad Hronom* [Ecophysiological research on beech forest stands in the region Zvolen – Žiar nad Hronom]. Vedecké štúdie, 5/2003/A. Zvolen: Technická univerzita vo Zvolene. 47 p.
Křístek, J. 1996. Chřadnutí lesu [Withering forests]. *Lesn. Práce*, 75 (5): 166–167.
Lichtenthaler, H. K. 1985. Stand der Ursachenforschung des Waldsterbens aus der Sicht des Pflanzenphysiologen. In *Baden-Württembergischer Forstverein. Berichte der Hauptversammlung*, 22: 51–65.
Lichtenthaler, H. K. 1987. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Meth. Enzymol*., 148: 350−382.
Matyssek, R., Keller, T., Koike, T. 1993. Branch growth and leaf gas exchange of Populus tremula exposed to low ozone concentrations through growing season. *Envir. Poll*. 79: 1–7.
Mikkelsen, N. T., Heide-Jorgensen, H. S. 1996. Acceleration of leaf senescence in Fagus sylvatica L. by low levels of tropospheric ozone demonstrated by leaf colour, chlorophyll fluorescence and chloroplast ultrastructure. *Trees,* 10: 145−156.

Analýza fyziologických parametrov ako ukazovateľov odumierania smrečín v oblasti Horného Spiša

Súhrn

V danom príspevku je riešená problematika multifaktoriálneho odumierania smrekových porastov v oblasti Horného Spiša. Štruktúra škodlivých činiteľov v tejto oblasti je veľmi pestrá, čo sťažuje objasňovanie kauzality poškodzovania porastov.

V práci uvádzame výsledky ekofyziologického výskumu uskutočnenom v priebehu roku 2004, v rámci ktorého bol hodnotený fyziologický a následne zdravotný stav dospelých jedincov smrek. V príspevku sú zhodnotené výsledky merania parametrov fluorescencie chlorofylu *a*, koncentrácie asimilačných pigmentov (chlorofyl *a, b*, karotenoidy v mg g^{-1} sušiny) a následne vyhodnotený zdravotný stav smrekových porastov.

Výsledky získané analýzou fyziologických parametrov na úrovni dospelých jedincov smreka, svedčia o nižšej úrovni fotosyntetického procesu asimilačného aparátu u jedincov, ktoré sa nachádzali na ploche s akútnymi príznakmi rozpadu porastu, ako aj na ploche bez výrazne viditeľných príznakov rozpadu.

Received June 6, 2007
Accepted August 30, 2007