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The influence of the method of silver fir growing and nutrition on sprouting and chlorophyll fluorescence during spring

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ABSTRACT: To compare breaking of dormancy in relation with climatic conditions and reaction to late frost fir plants growing in sun, in shade and at magnesium deficiency were evaluated. Sprouting and chlorophyll *a* fluorescence of needles were evaluated from March to May 2011. As a consequence of May frost 100% of plants from sunny treatments and 70% of firs shaded with shade cloth were affected by necrosis of sprouting shoots; fir-trees shaded by the stand remained fully intact. No relationship between the index of shoot sprouting and damage was confirmed. Firs with Mg deficiency had the lowest maximum quantum yield of PSII photochemistry of the dark-adapted tissue (F_V/F_M) and performance index on absorption basis (PI_{abs}) in the long term. Regression models of F_V/F_M and PI_{abs} and selected environmental parameters showed that chlorophyll fluorescence was influenced to the largest extent by minimum temperature of the day of measurement and also by acclimation to the light environment. The process of breaking of dormancy increased fir vulnerability to thermal stresses of the environment – late frosts.

Keywords: *Abies alba*; breaking of dormancy; late frost; damage; sun and shade; magnesium deficiency.

The photosynthetic activity of plants fluctuates in relation to external environmental factors. Dormancy ensuring the ability to overcome the influences of adverse climatic factors of winter is one of the crucial stages of plant development (Bauer et al. 1994). The ecophysiological processes of plants are reduced in an off-season period, and a slow resumption of photosynthetic activity takes place during the breaking of dormancy (Verhoeven et al. 2009). Besides genetically conditioned predispositions dormancy in the given growth conditions is influenced by the physiological state of plants.

In forestry the knowledge of dormancy state or growth activity is of crucial importance particularly for the timing of cultural practices during the production of forest tree species plants in nurseries. It is important mainly in the spring season when nursery operations are followed by the activities associated with forest reproduction because dormant plants are more resistant to adverse influences resulting from plant handling and outplanting (Ritchie 1986; Burr 1990; Landis et al. 2010).

The physiological state of forest tree species plants and morphological parameters are influenced by the method of planting stock production in forest nurseries. The regulation of nutrient availability and light regime are among significant parameters impacting on the quality of produced plants (Kozlowski, Pallardy 2002; Floistad, Kohmann 2004). As for plant nutrition, balanced nutrition is the most desirable (Šrámek et al. 2009).

Woody plants require sufficient light for photosynthesis but they also must be protected from excessive supply of solar energy (Niyogi 1999). A reduced amount of light may result in a reduction in photochemical efficiency of the photosynthetic apparatus. The impact of a restricted amount of solar radiation on morphology and physiology differs among tree species and is related with the degree of their tolerance to shading (Khan et al. 2000).

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With respect to physiological reaction and health status development a reduction in radiation availability during growth may be significant especially in the production of planting stock of shade-tolerant woody plants such as silver fir (Giannini, Paiero 1971; Boni et al. 1978; Khan et al. 2000). Deficiency or excess of elements leading to nutrient imbalance may become a significant stress factor limiting tree species prosperity regardless of the intensity of radiation (Schwab et al. 1994).

The evaluation of chlorophyll *a* fluorescence provides a quantitative assessment of functionality of the photosynthetic apparatus of plants – how it is efficient and how well it works (Ritchie, Landis 2005). It is often used in physiological and ecological research, taking into account the relation of the studied characteristics to the condition of assimilatory organs and photosynthesis (Maxwell, Johnson 2000). The measurement of chlorophyll *a* fluorescence was successfully used to evaluate dormancy e.g. in Douglas fir (Hawkins, Lister 1985), to evaluate cold hardiness (Ritchie, Landis 2005), frost damage (Percival, Henderson 2003); it can also be used for prediction of plant behaviour after outplanting (L'Hirondelle et al. 2007).

The objective of the present study is to compare breaking of dormancy in relation with climatic conditions and reaction to late frost in silver fir plants with different shading and nutrition regime using the method of chlorophyll *a* fluorescence measurement. The hypotheses tested are as follows: (*i*) for fir being a shade-tolerant species, breaking of dormancy is more intensive in the shade; (*ii*) nutrient imbalance slows down breaking of dormancy; and (*iii*) development of the maximum quantum yield of PSII photochemistry as well as performance index on absorption basis are mostly driven by temperature.

MATERIAL AND METHODS

Experimental plantations. Research was conducted in the 2011 spring season in an experimental nursery of Opočno Research Station affiliated to Research Institute of Forestry and Game Management. Six-years-old plants of fir ca 45 cm in average height that were set onto seedbeds at a 40 × 60 cm spacing in two unshaded and two shaded treatments were selected for measurements. The plants, 50 individuals per treatment, were grown in these conditions for three years. Unshaded treatments included a control (Sunny-C treatment) and a treatment of plants with symptoms of magnesium deficiency (needle yellowing; Sunny-D). In shaded treatments the light access was reduced either by green shade cloth (ca 70% light transmission; Shaded-F treatment) or by ornamental planting – northern white-cedar (*Thuja occidentalis;* hereinafter Thuja) stand 5 m in height (ca 30% of full light; Shaded-S treatment) (Table 1). Light attenuation was estimated from indicative measurements on sunny and overcast days with a digital lux meter (UNITEST RS-232); spectral measurement was not performed. Magnesium deficiency (magnesium content in dry matter of the first needle age class 0.074%) in Sunny-D treatment was caused by nutrient imbalance due to the base excess in the soil. The content of phosphorus in the needles was also significantly lower (Fig. 1).

Table 1. Experimental treatments of the study of chlorophyll fluorescence in silver fir during spring

Light conditions of seedbed	Treatment	Description
Sunny	Sunny-C	sunny – control
	Sunny-D	sunny – Mg deficiency
Shaded	Shaded-F	shaded by fabric – shade cloth
	Shaded-S	shaded by Thuja stand

There was no fertilization or watering applied during the experiment. The plants were evaluated repeatedly for chlorophyll *a* fluorescence, sprouting onset and damage to sprouted shoots caused by the frost period from May 3 to 6, 2011.

Measurement of climatic conditions. For a description of meteorological conditions data from an automatic meteorological station [NOEL 2000, produced by Ing. Libor Daneš (CR)] located at a distance of about 10 m from the experiment were used. Among the observed parameters these parameters were used for this study: air temperature at a height of 200 and 30 cm above the ground [thermometers of

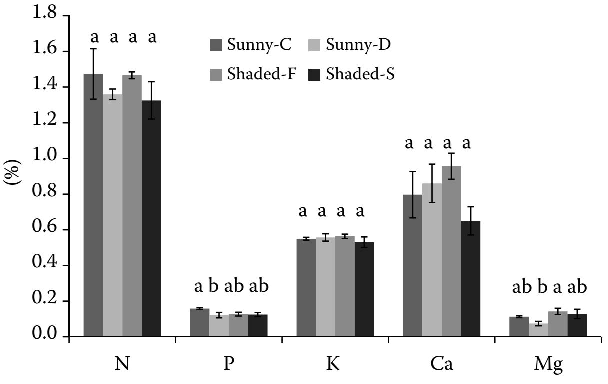

Fig. 1. Proportions of nutrients in the first needle age class of fir treatments mean of three samples and standard deviation

the letters identify statistically homogeneous groups at a 95% significance level (ANOVA and Tukey's post-hoc test), for treatment explanation see Table 1

Pt 100 type, mfd. by Dorfi (CR); °C)], global radiation [SG005 pyranometer, produced by J. Tlusťák (CR); as radiation energy in $W \cdot m^{-2}$] and precipitation (SR1 rain-gauge, produced by J. Tlusťák; mm), all data were observed in one-hour intervals.

Evaluation of sprouting. The progression of shoot sprouting and development was evaluated in approximately one-week intervals from March 14, 2011 to the date of full expansion of new sprouts (the last measurement on May 24, 2011) on the basis of a five-degree empirical scale (Table 2). The tree was attributed the respective index of sprouting if more than a half of buds (new sprouts) complied with the respective criterion.

Table 2. Empirical scale of fir sprouting

State	Index
Not sprouting	0
Enlarged buds	1
Green needles show in buds	2
Confined bundles of needles growing from buds	3
Open bundles of needles, beginning extension growth	4
Extension growth of new sprouts	5

Measurement of chlorophyll fluorescence. Chlorophyll *a* fluorescence of one-year-old needles was evaluated with a Plant Efficiency Analyser (PEA, Hansatech Instruments, UK) on the same dates as the sprouting index. The measurement with PEA is the basic approach to the evaluation of chlorophyll *a* fluorescence, allowing a prompt insight into the primary reaction of photosynthetically active dark-acclimated material to an intensive light impulse (Strasser et al. 2000). Chlorophyll fluorescence measurements were always done between 11 and 13 h CET on one-year needles from the upper side of lateral annual shoots in the upper part of the crown (except the leader). Before measurement the samples were shaded with original clips for 30 min at least. The intensity of radiation was set at 50% ($2.1\ mmol \cdot m^{-2} \cdot s^{-1}$). Ten plants per treatment were measured; each sample tree was measured on three randomly chosen shoots, i.e. in three replications. The assimilatory tissues were evaluated on the basis of selected complex parameters of chlorophyll *a* fluorescence. The maximum quantum yield of photosystem II photochemistry of dark-adapted tissue (F_V/F_M) is calculated from the values of maximal fluorescence (F_M) and minimal fluorescence (fluorescence intensity in 50 µs; F_0) while their difference is designated as variable fluorescence (F_V). The value F_V/F_M was calculated by the service software (WinPEA32 Version v 1.00) according to the relation $F_V/F_M = (F_M - F_0)/F_M$.

Subsequently, the performance index on absorption basis (PI_{abs}) was computed; it is a performance index for energy conservation from photons absorbed by PSII antennae to the reduction of plastoquinone (Q_B) (Stirbet, Govindjee 2011). Performance index is a very complex parameter because it is calculated on the basis of characteristics describing the state of practically all major photochemical processes: such as density of reaction centres on chlorophyll basis, flux ratio trapping per dissipation and electron transport further than to primary plastoquinone (Q_A) (Strasser et al. 1999, 2004).

Performance index on absorption basis was computed as follows (Strasser et al. 2004):

$$PI_{abs} = \frac{1-(F_0/F_M)}{M_0/V_J} \times \frac{F_M - F_0}{F_0} \times \frac{1-V_J}{V_J} \qquad (1)$$

where:

F_0 – minimal fluorescence,

F_M – maximal fluorescence,

M_0 – initial slope of fluorescence kinetics is computed as follows: $M_0 = 4 \times (F_{300\mu s} - F_0)/(F_M - F_0)$, where $F_{300\mu s}$ is fluorescence intensity in 300 µs,

V_J – relative variable fluorescence in 2 ms computed as: $V_J = (F_J - F_0)/(F_M - F_0)$, where F_J is fluorescence intensity in 2 ms.

Statistical evaluation. After removal of measurement outliers the data were transformed by the Box-Cox transformation to stabilize variance and to ensure normality (Fox, Weisberg 2011). The data were compared in R statistical environment (3.0.1, The R Foundation for Statistical Computing 2013) using the analysis of variance (ANOVA) with subsequent Tukey's test; if deviations from normality persisted, the Kruskal-Wallis test was used with subsequent comparison by the kruskalmc function of the pgirmess package using a Siegel and Castellan (1988) algorithm.

The method of multivariate linear regression was applied to evaluate the influence of environmental factors on the average values of parameters Fv/Fm and PI_{abs} on the particular dates of measurement. Explanatory variables tested in several cycles were as follows: minimum temperature at a height of 30 cm above the ground from the day of measurement ($minT_0$) and from two preceding days ($minT_{-1}$, $minT_{-2}$), maximum temperature of two preceding days at a height of 30 cm ($maxT_{-1}$, $maxT_{-2}$), the sum of global radiation of preceding day (Sun, in $kW \cdot m^{-2}$), mean temperature of preceding day at a height of 200 cm above the ground ($meanT_{-1}$), actual temperature on the date of measurement (T), interaction between minimum temperature and the sum of global radiation ($minT_0$:Sun) and the ordinal number of the day (Day) as a measure of spring onset. $P < 0.05$ was used as a significance level for the applied tests.

RESULTS

Climatic conditions, sprouting and frost damage

In the studied spring season (March to May 2011) there was a gradual increase in the sunshine intensity (solar irradiance), and concomitantly in average daily temperatures. The distribution of precipitation higher than 10 mm per day was even and so sufficient moisture was provided (Fig. 2). The daily temperature amplitude at a height of 30 cm above the ground reached up to 22.7°C. Night freezing temperatures occurred frequently until the end of March while the last episode of late frost was registered on May 3 to 6, when the ground temperature dropped to –3.1°C (May 5, 2011).

Fir shoots started sprouting between April 14 and 27, 2011, while Shaded-F treatment showed the earliest sprouting and the sprouting of Shaded-S and Sunny-D treatments was delayed. On May 5 (in the late frost period) the average index of sprouting was comparable in Sunny-D, Sunny-C and Shaded-F treatments (Fig. 3). Visual symptoms of frost damage to sprouting shoots appeared within 2–3 days following the frost episode. Necrosis of sprouting shoots was observed in all plants from treatments grown in full sunshine, irrespective of the sprouting index. In Shaded-F treatment 70% of the plants were damaged by necrosis, the remaining trees suffered from chlorotic symptoms, without any relation with the sprouting index. Fir-trees shaded by the Thuja stand were absolutely without damage (Shaded-S).

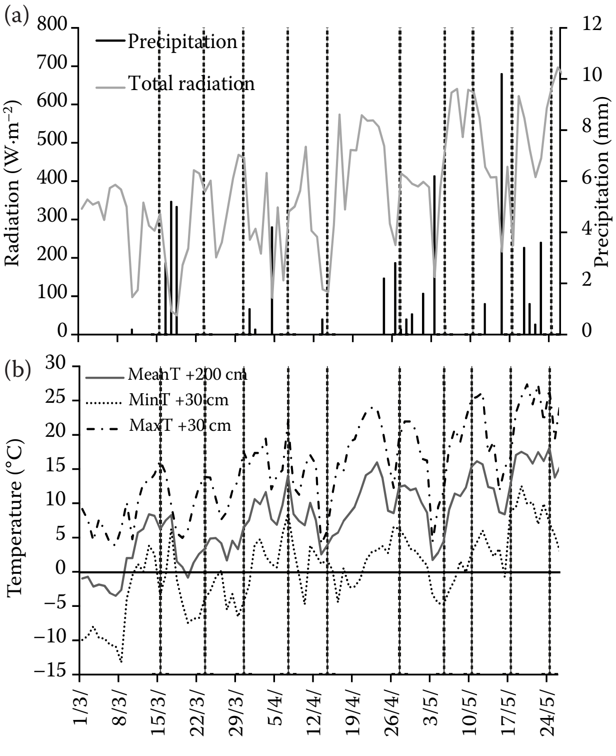

Fig. 2. Daily sums of radiation and precipitation (upper part) and average daily temperature at a height of 200 cm above the ground (MeanT +200 cm), and daily minimum (MinT +30 cm) and maximum (MaxT +30 cm) temperatures at 30 cm above the ground (lower part) on the particular days of the studied period. Vertical dotted lines represent the dates of chlorophyll fluorescence measurement

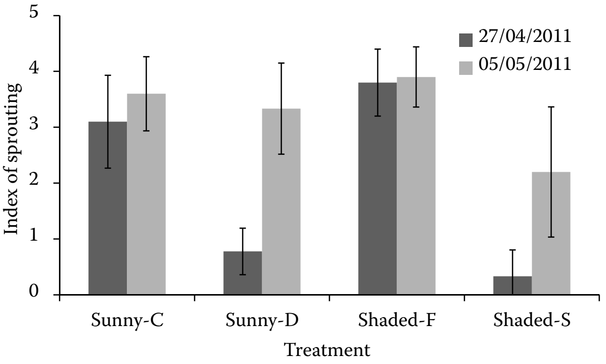

Fig. 3. Average index of sprouting in the studied fir treatments (20 plants per treatment) on April 27 and May 5, 2011. Mean and standard deviation. For treatment explanation see Table 1

Chlorophyll a fluorescence

At the beginning of the period of observation (March 14, 2011) significant differences in the values of maximum quantum yield of PSII photochemistry (F_V/F_M) were found out between the experimental treatments, these differences were maintained practically for the entire period (Fig. 4a). In Shaded-S treatment the value of F_V/F_M was stabilized for the entire period, being above 0.8 without great fluctuations. In the other treatments an increase in the values was observed, with a temporary fall after the frost episode around May 5, 2011. The fall was steepest in treatments grown in the sun (Sunny-C and Sunny-D). Freezing temperatures before the beginning of May, i.e. before the onset of sprouting, had a minimum influence on the development of F_V/F_M – an insignificant fall in the values after the frost episode at the end of March occurred only in treatments grown in the sun.

The values of performance index PI_{abs} were the lowest in Sunny-D treatment in the entire period. The highest values were reached by fir-trees belonging to shade-grown treatments (Fig. 4b), from the statistical aspect the values of these two treatments were identical. The index PI_{abs} reacted to the specific weather development by the fluctuation of its values more than did the values F_V/F_M. The period of freezing temperatures was always followed by a decrease in the performance index PI_{abs}.

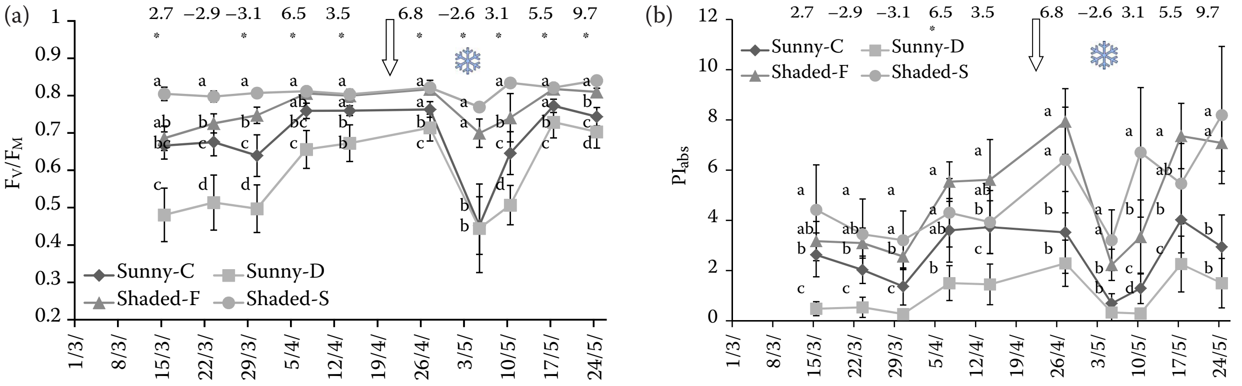

Fig. 4. Maximum quantum yield of PSII photochemistry on the dates of measurement (a), performance index on absorption basis PI_{abs} (b) average minimum temperatures for 3 days including the day of measurement are shown in the upper part; arrow indicates the period of the beginning of fir sprouting; snowflake shows the period with late frost; 10 plants per treatment were evaluated; for treatment explanation see Table 1; letters identify statistically homogeneous groups at a 95% significance level; *Kruskal-Wallis test; ANOVA in the other cases

Linear regression

The results of evaluation of linear regression between climatic conditions and chlorophyll *a* fluorescence have shown that among the tested parameters minimum temperature of the day of measurement ($minT_0$) was the only significant explanatory variable of resultant models for parameter F_V/F_M in all treatments. The resultant models described 42 to 76% variability (Table 3), the lower closeness of the model was observed in Sunny-C and Shaded-S treatments.

Table 3. Resultant models of linear regression of parameters F_V/F_M and PI_{abs}

Treatment	Parameters	Estimate	Std.	Pr(>|t|)	Adj. R^2
F_V/F_M					
Sunny-C	(intercept)	0.639	0.026	<0.001	0.518
	$minT_0$	0.013	0.004	0.011	
Sunny-D	(intercept)	0.526	0.021	<0.001	0.762
	$minT_0$	0.018	0.003	<0.001	
Shaded-F	(intercept)	0.736	0.010	<0.001	0.742
	$minT_0$	0.008	0.002	<0.001	
Shaded-S	(intercept)	0.802	0.006	<0.001	0.420
	$minT_0$	0.003	0.001	0.025	
PI_{abs}					
Sunny-C	(intercept)	3.197	0.478	<0.001	0.745
	$minT_0$	0.154	0.036	0.004	
	Sun	–3.209	1.123	0.024	
Sunny-D	(intercept)	0.647	0.179	0.007	0.666
	$minT_0$	0.122	0.028	0.002	
Shaded-F	(intercept)	3.533	0.414	0.000	0.718
	$minT_0$	0.346	0.065	0.001	
Shaded-S	(intercept)	2.099	0.457	0.003	0.890
	$minT_{-1}$	0.275	0.036	<0.001	
	Sun	1.077	5.752	0.001	

$minT_0$ – minimum temperature of the day of measurement (°C); $minT_{-1}$ – minimum temperature of the day preceding measurement (°C); Sun – the sum of global radiation of preceding day ($kW\cdot m^{-2}$), for treatment explanation see Table 1

The significance of variables explaining the value of PI_{abs} was different among the treatments. While in Sunny-D and Shaded-F treatments the resultant model was based on variable $minT_0$ again, in Sunny-C treatment the parameter Sun is added and in Shaded-S the model is composed of $minT_{-1}$ and Sun. The closeness of fitting of resultant models was higher than in F_V/F_M (66 to 89%).

DISCUSSION

In spring breaking of plant dormancy and resumption of the activity of physiological processes connected with respiration and metabolism take place. Vulnerability of plants to negative environmental influences is increased at that time (Burr 1990). In our study it was confirmed in fir during the frost episode at the beginning of May that had a more distinct impact on a decrease in the studied fluorescence parameters than a similar situation at the end of March.

Yatsko et al. (2011) reported the winter (December to March) values of maximum quantum yield of photochemistry (F_V/F_M) in *Abies sibirica* to amount to 0.45–0.50, and they increased to 0.72–0.77 until May. Maximum values were observed in June to August (0.82–0.85). The winter and spring values of F_V/F_M are very similar to the values we observed in *Abies alba* on unshaded seedbeds. Robakowski (2005b) also reported the steepest fall of F_V/F_M values in February in silver fir plants grown in a plastic house, but with higher minimum values (0.71). These values are close to our findings in fir plants growing under the protection of shade cloth or tree stand. The above-mentioned authors found out relatively high values of F_V/F_M in fir compared to the other conifer species.

The growing of silver fir plants in shaded treatments influenced sprouting, evaluated parameters of chlorophyll *a* fluorescence and extent of damage by late frosts. Under the shelter of the Thuja stand (Shaded-S treatment) the fir was growing for a long time in shaded, more stable microclimatic conditions that had a positive influence on its prosperity. The positive influence was reflected in high and relatively stable values of F_V/F_M and PI_{abs} from the beginning of the studied period. Sprouting was somewhat delayed, but the firs were not damaged in the May frost period at all. A delay in sprouting in shaded conditions was confirmed in a number of other tree species (Caffarra, Donnelly 2011).

The influence of the shade cloth on the growth environment of Shaded-F treatment was less intensive. Fir sprouting had a similar pattern like in firs from the sunny control, but the extent of frost damage was smaller. Besides the partial alleviation of frost by the fabric, the reasons may be differences in the intensity of tissue irradiation after the frost period (Strand, Öquist 1985), and also different structure of shaded and sunlit tissues (Robakowski et al. 2003; Sariyildiz, Anderson 2003; Robakowski 2006; Lichtenthaler et al. 2013) which can also influence vulnerability to frost. If late frosts last for a longer time, the influence of the shade cloth on a reduction in temperature extremes will probably be minimal. Nevertheless, a decrease in the parameters F_V/F_M and PI_{abs} was not so distinct after frost in either of the shaded treatments as in the treatments grown in full sun. It may be connected with a reaction to subsequent irradiation – with photoinhibition. Comparing photoinhibition caused by a low temperature in dwarf pine, Norway spruce and fir, Robakowski (2005a,b) evaluated the fir as a tree species with the smallest inclination to photoinhibition. On the contrary, the fir belongs among tree species vulnerable to so called freeze desiccation. This phenomenon is usually observed in spring when there is little snow, the soil is frozen and days are sunny and warm (Krasowski 1996).

The state of the assimilatory tissues of forest tree species plants is influenced by available and balanced nutrition. Strand and Lundmark (1995) described that during the development of fertilized and unfertilized spruce seedlings fertilized seedlings had higher values of F_V/F_M in spring while the largest difference was observed just before the termination of the winter inhibition period. When Boyce (2007) compared the treatments additionally fertilized with calcium and control plantings of *Abies balsamea* and *Picea rubens*, he observed differences in F_V/F_M as late as in September, no differences were observed in spring (March). On the contrary, in our experiment the parameters F_V/F_M and PI_{abs} of fir plants with magnesium deficiency were the lowest in the long term and the health status impairment was visible on changes in needle colour and delayed sprouting. Colour changes are caused by a chlorophyll loss due to nutrient deficiency (Kölling et al. 1997; Solberg et al. 1998). However, the intensity of colour changes in local populations can be specific. E.g. Robakowski (2006) suggested that firs originating from the Krkonoše Mts. were probably adapted to growth in soils poor in Ca^{2+} and Mg^{2+} ions that were formed on the acid granite parent rock because at a low concentration of macroelements in needles neither needle discoloration nor longer-time decrease in F_V/F_M was observed that could prove permanent nutrient deficiency.

Unlike these studies, an increased availability of bases in the soil led to nutritional problems and to the occurrence of a stressor in our case. The importance of a stressor at a decrease in the values of F_V/F_M and PI_{abs} was confirmed in a number of tree species (Cregg et al. 2004; Volgusheva et al. 2011; Koller et al. 2013). The resumption of photosynthetic activity and growth after adjustment of nutrient balance in the soil can be a long-term process (Dreyer et al. 1994). In spite of nutrient imbalance and high percent damage to new shoots by frost the firs of Sunny-D treatment survived in our experiment and their growth was not restricted to a greater extent.

The evaluation of regression relations between the parameters F_V/F_M and PI_{abs} and selected environmental parameters indicated that temperature is the main factor influencing chlorophyll *a* fluorescence and/or photosynthetic rate during spring. The significance of minimum temperature on the day of measurement in relation to F_V/F_M was demonstrated in all studied treatments of fir growing. The index PI_{abs} reacted to weather conditions in a more distinct way. The resultant models of PI_{abs} for Sunny-C and Shaded-S treatments contained also the sum of global radiation of the preceding day besides the value of minimum temperature. However, its significance was opposite: while in Sunny-C treatment the resultant index of the model was negative, it was positive in firs grown in the shade of the Thuja stand. The significant influence of minimum temperature of the day of preceding measurement in Shaded-S treatment, instead of temperature of the day of measurement significant in the other variants, will probably stem from more balanced conditions of the shade microclimate. A close correlation between chlorophyll *a* fluorescence and light and thermal conditions in the period preceding measurements was observed also in other tree species (Bolhár-Nordenkampf, Lechner 1988; Bergh, Linder 1999).

Plant responses to light depend not only on light quantity (photosynthetic photon flux density), but also on the share of light wavelengths in the light spectrum illuminating leaves (e.g. Sellin et al. 2011). The tree canopy mostly reduces blue light (< 490 nm; Giuliani et al. 2000). However, the proportions of light spectra of the treatments were not evaluated in this study. Their impact on breaking of dormancy can be the subject of subsequent research.

CONCLUSIONS

The observations of breaking of dormancy and reaction to late frost in silver fir plants showed that from the aspect of chlorophyll fluorescence parameters the prosperity of shaded treatments was higher during the entire studied period. The highest photosynthetic activity evaluated by this method was found out in fir plants shaded by the stand, when their sprouting was delayed but the reaction to late frost was minimal. Firs with magnesium deficiency had the lowest values of F_V/F_M and PI_{abs} in the long term. Regression models of relations between parameters F_V/F_M and PI_{abs} and selected environmental parameters showed that minimum temperature of the day of measurement had the most pronounced influence on chlorophyll *a* fluorescence.

The process of breaking of dormancy increased fir vulnerability to environmental thermal stresses – late frosts. Frost damage to sprouting shoots was markedly restricted or fully eliminated by growing the plants in the shade. The shading of firs during spring is crucial for an improvement in the physiological state of plants and minimization of risks connected with potential late frosts.

Sprouting of fir treatments did not confirm the hypothesis of more intensive breaking of dormancy in the shade. On the other hand, hypotheses of the delay of breaking of dormancy in nutrient imbalance of firs and of the controlling effect of temperature on the development of maximum quantum yield of PSII photochemistry as well as performance index on absorption basis were confirmed.

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