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ORIGINAL PAPER

Physiological response of irrigated and non-irrigated Norway spruce trees as a consequence of drought in field conditions

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Abstract Physiological reactions of 25-year-old Norway spruce (*Picea abies* (L.) Karst.) trees to drought were examined during 2009 vegetation period. During the second half of summer, the decrease in soil water content was observed and irrigation was applied to a group of spruce trees, while the second group was treated under natural soil drought. The response to water deficit was recorded at the level of leaf water potential (Ψ_L). However, it appears that Ψ_L plays minor role in early stomata regulation of Norway spruce as CO_2 assimilation rate (P_N) and stomatal conductance (g_S) were reduced already before water potential decrease. Leaf water potential decreased significantly only in case when soil water content was low in the long run and when transpiration losses were simultaneously relatively high. Almost complete stomatal closure even of the irrigated trees was caused by the increase in the vapour pressure deficit of the air (D) above the value of approximately 1.5 kPa. Low values of D were accompanied by partial stomata opening of drought-treated trees. In non-irrigated spruce trees, the values of P_N decreased by 35–55% in comparison with irrigated trees. No drought-induced significant changes were found either in chlorophyll and carotenoid concentration (chl a + b, car) or in maximal photochemical efficiency of photosystem II (F_v/F_m). High rates of sap flow (F) did not always lead to stomatal closure during midday. It appears that high transpiration rates do not control stomatal response to D.

Keywords *Picea abies* · Water deficit · Leaf water potential · Transpiration · Photosynthesis · Stomatal closure · Vapour pressure deficit

Introduction

Spruce forests decline in Europe

Nowadays, the majority of spruce trees' mortality is caused by bark beetle (Coleoptera, Scolytidae) attacks, honey fungus (*Armillaria* sp.) and mechanical damage by wind (Jakuš 2001). In addition, spruce forest decline phenomenon seems to be strongly associated with global warming (Woo 2009; Hlásny et al. 2011). *Picea abies* is sensitive to soil water supply (Wallin et al. 2002; Schume et al. 2004), as its root system is shallow, since 85% of spruce roots commonly occur only up to the depth of 10 cm (Bjorkhem et al. ex Przybylski 1998). The uppermost soil layers are the most exposed to drought but also to temperature fluctuations and acidification. This has a negative effect on the standing stock of fine roots of spruce, which are mostly

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superficially distributed (Gaul et al. 2008; Konôpka 2009), and consequently on water supply. A common mechanism for plants with isohydric regulation of water status results from avoidance of drought-induced hydraulic failure via stomatal closure, resulting in carbon starvation and a cascade of downstream effects such as reduced resistance to biotic agents (McDowell et al. 2008).

In Slovakia, the impact of low precipitation and high evapotranspiration during the vegetation period will be a particularly significant stress factor in the regions below 1,000 m above sea level (Minďáš et al. 2000). In many regions of Germany and Austria, the growth conditions for Norway spruce are heavily affected by climate change and are already critical (Gartner et al. 2011). Mäkinen et al. (2001) stated that low precipitation can be related to spruce damage and dieback on the sites in southern Finland, which are susceptible to drought. The impact of the expected changes in the amplitude and frequency of extreme climatic events will probably be more significant than the changes at the level of average values of climatic parameters.

Physiological response to low soil moisture and air humidity

Physiological processes are sensitive indicators of stress in plants, especially under extreme environmental conditions. Drought influences plant growth in a number of different ways, that is, through a decrease in water potential in cambial cells, resulting in subsequent inhibition of cell growth, reduction in metabolic activity, and inhibition of stomatal conductance and, consequently, photosynthesis (Centritto et al. 2011a). There are several possible processes of *non-diffusional limitations* of photosynthesis under the water stress: physical limitations, namely mesophyll resistance to CO_2 diffusion in the gas and liquid phases, and biochemical limitations, namely carboxylation rate and efficiency (Centritto et al. 2003). However, stomatal closure is the first response to drought and the dominant limitation to photosynthesis at mild-to-moderate drought events (Cornic 2000; Lawlor and Cornic 2002). Already small reduction in soil water availability leads to fast stomatal closure and osmotic adjustment in order to maintain leaf water potential (Brestič 2002). This contributes to the inhibition of water loss by transpiration, but it is closely related to limitation of CO_2 uptake (Jones 1998).

Stomata generally close as the vapour pressure deficit of the air (D) increases and the response is often depicted as a non-linear decline in g_S with increasing D (Addington et al. 2004). The mechanism of this response is unknown, and there is little agreement regarding the mechanism (or mechanisms) by which stomata sense the perturbations of many aspects of the soil–plant–atmosphere hydraulic continuum (Buckley 2005). Some studies suggest that stomatal closure occurs with increasing D as a feedback response to some aspects of transpiration and water loss from the leaf, rather than as a direct response to humidity (Addington et al. 2004). Some authors incline to the opinion that there exists a mechanism of fast reaction to D increase coupled with ABA (abscisic acid) in leaves likely triggering the response (Tardieu and Davis 1992). ABA is released from mesophyll cells, but its transport through xylem flow from roots is very important—dehydrating roots produce chemical signals already before the leaf water status is changed. As a result, stomatal responses are more closely linked to soil moisture content and air humidity than to leaf water status (Centritto et al. 1999).

To describe the water demand of tree species precisely, it is important to know their response to various water stress levels and characterize symptoms and consequences of the drought effect on forest trees and stands (Centritto et al. 2011a). In spite of the fact that spruce is the most common tree species in Central Europe, there is a shortage of extensive ecophysiological studies oriented at soil and atmospheric conditions performed on high-grown spruces in the conditions of forest stands (e.g. Lu et al. 1995; Lagergren and Lindroth 2002; Čermák et al. 2007; Gömöry et al. 2011). More often, detailed physiological experiments are performed on seedlings growing under controlled or semi-controlled conditions (Ditmarová et al. 2010; Pukacki and Kaminska-Rozek 2005; Wallin et al. 2002; Kronfuss et al. 1998). Therefore, the objective of this study was to investigate the basic physiological traits in high-grown Norway spruce trees under drought conditions. We anticipated that the leaf water potential (Ψ_L), stomatal conductance (g_S) and consequently CO_2 assimilation rate (P_N) would decrease with duration of drought and increasing vapour pressure deficit of the air (D). We discuss the influence of Ψ_L, D and sap flow rate (F) on stomatal closure. We further expected small or no decrease in pigments concentration and maximal photochemical efficiency of photosystem II in non-irrigated trees.

Materials and methods

Site and experiment design description

The experimental site Hriňová is situated in central Slovakia (Central Europe 48°34′24″N, 19°31′22″E), at an elevation of about 670 m above sea level. The locality belongs to the regions with mildly cold to mildly warm mountain climate. Mean annual temperature is 6.5°C, and mean annual precipitation is 740 mm. The experimental plot is located on the south-east hillside (gentle slope of approximately 5%). As in general the rainfall is not evenly distributed throughout the year, in dry periods soil moisture deficit may develop during the growing seasons. Soil is

Table 1 Mean biometrical parameters of irrigated and non-irrigated spruce trees

	Crown projection (m^2)	Diameter at breast height (cm)	Tree height (m)	Height of the crown base (m)	Crown length (m)	Crown width (m)
Irrigated trees	6.3	17.5	17.3	8.3	9.0	2.8
Non-irrigated trees	5.7	17.4	17.1	8.4	8.7	2.7

deep with a low skeleton fraction up to the depth of 65 cm. The bedrock is formed by vulcanites; soil type is Eutric Cambisol (according to WRB; FAO 2006) with dense network of roots to the depth 35 cm. The forest is 25 years old and is dominated by Norway spruce (*P. abies* [L.] Karst), growing there outside the natural occurrence of spruce forests. In summer during the period of soil water deficit, irrigation was applied to a group of 6 spruce trees. From July 16th (when volumetric soil water content decreased to approximately 10% or 13% in a depth of 5–15 cm or 30–40 cm, respectively) till August 28th, 23 m^3 of water was distributed to the irrigated group of 6 trees. Water was slowly outflowing from containers through dripping system in order to minimize surface runoff and to avoid a long-term significant reduction (below 20%) in volumetric soil water content at 30–40 cm. The second group of 6 trees was treated under natural soil drought. There were no significant differences in biometrical parameters between irrigated and non-irrigated group of trees (Table 1). The branches were cut off from the height of about 14 m from the sun-exposed, north-oriented parts of crowns and immediately measured five times during the period of the experiment (see Fig. 1b) always between 9:00 and 14:00. The effect of daytime was minimized by switching between irrigated and non-irrigated spruces.

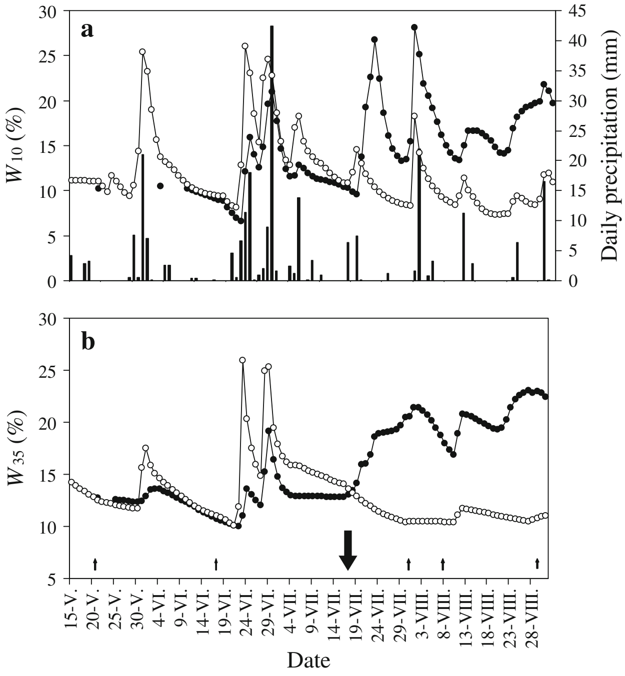


Fig. 1 Volumetric soil water content of the irrigated (*filled circle*) and non-irrigated (*open circle*) plot at a depth of 5–15 cm (W_{10}) and daily precipitation (**a**), volumetric soil water content of irrigated (*filled circle*) and non-irrigated plots (*open circle*) at a depth of 30–40 cm (W_{35}), little arrows indicate the dates of physiological measurements, and the *big arrow* indicates the beginning of irrigation (**b**)

Microclimate measurements and electrical resistivity tomography survey

Air temperature and relative humidity (in degrees Celsius and percentage, respectively; datalogger Minikin TH with in-built sensors, EMS Brno, Czech Republic) and global radiation (in watts per square metre; datalogger Minikin RT with silicone diode sensor) were measured at 5- to 10-min intervals and stored at 30- to 60-min intervals (Table 2, Fig. 1a, b). Precipitation (in millimetres; MetOne 370, USA) was measured continually and stored at 60-min intervals. All mentioned characteristics were measured outside the forest stand at a nearby open space, and vapour pressure deficit of the air (D) was calculated from the saturation vapour pressure (at the air temperature) and the air humidity. Volumetric soil water content (in percentage, Campbell, Campbell Scientific, USA) was measured inside the forest stand, stored at 60-min intervals at two depths and calibrated using the values determined gravimetrically every 2 weeks at depths of 5–15 cm (W_{10}) and 30–40 cm (W_{35}), respectively. Acquired data were processed using Mini32 software (EMS Brno, Czech Republic).

Electrical resistivity tomography survey was implemented for imaging subsurface of the soil. We used the ARES device (GF Instruments, Czech Republic) attached to 24-m cable furnished with active electrodes. Thus, we obtained an electrical resistivity profile for each plot. The results of the electrical resistivity measurements provided us with an estimate of the soil depth, its variability, and large soil skeleton lenses or coverbeds without the need to open soil profiles and disturb adjacent soil environment. Electrical resistivity tomography calibration according to Máliš (2007) was used for the purpose of resistivity data interpretation.

Table 2 Microclimatic data of the days when detailed physiological measurements were taken: daily sum of global radiation (GR), average daily air temperature (T_{air}), average daily relative air humidity (*RH*)

	21.05.09	17.06.09	29.07.09	06.08.09	27.08.09
GR (Wh m^{-2} day^{-1})	6,780	5,690	5,800	3,120	4,520
T_{air} (°C)	16.40	15.18	19.80	18.72	20.56
RH (%)	60.45	51.05	66.00	71.76	62.02

Sap flow and leaf water potential measurement

The sap flow of spruce trees was estimated by direct non-destructive and continuous measurements using tree-trunk heat balance method (THB) with internal heating of xylem tissues and temperature sensing. Sap flow rates (*F*) of particular trees were estimated by applying the tree-trunk heat balance method (Čermák and Kučera 1981, Kučera et al. 1977).

Psychometric method was used for the measurement of water potential (Ψ_L) of one-year-old needles of particular spruce sample trees. The PSY-PRO water potential system with the C-30 sample chambers (Wescor, USA) determines the water potential by measuring the relative humidity in equilibrium with the object. For each measurement date, mean values for each variant (irrigated and non-irrigated) were derived from at least five tree means.

Gas exchange measurements

CO_2 assimilation rate (P_N) and stomatal conductance (g_S) were measured on one-year-old shoots using an open IR system (CIRAS-01, PP Systems, UK). The measurements were taken on the detached shoots. A 10-cm section of a shoot containing approximately 10 cm^2 of leaf area (projected) was enclosed in the chamber (PLC 5 (C) conifer leaf cuvette, PP Systems, UK). CO_2 concentration was kept at 380 ± 5 μmol m^{-1}, saturating photosynthetically active radiation of spruce was set to 1,100 μmol m^{-2} s^{-1} (Šprtová et al. 1999), and temperature control was set to 20°C. Values were measured after short adaptation when P_N values were stable. The vapour pressure deficit in the chamber was calculated from the saturation vapour pressure (at the leaf temperature) and the chamber air vapour pressure. Ten values were recorded for each shoot, and 2–4 shoot values were averaged to obtain the mean for each tree. Six irrigated and six non-irrigated trees were measured on each measurement date.

Chlorophyll fluorescence measurements

Chlorophyll *a* fluorescence measurements were taken using a chlorophyll fluorimeter (Plant Efficiency Analyser, Hansatech Ltd, Kings Lynn, UK). The sample was irradiated by one-second-long saturating pulse of white light (approximately 2,500 μmol m^{-2} s^{-1}) following at least 30-min darkness adjustment. The interval was considered sufficient to cease electron transport in thylakoid membranes; after darkness adaptation, the transthylakoid pH gradient fell to a minimum level (Roháček and Barták 1999). The following parameters were determined: F_0 (initial fluorescence—all reaction centres are open), F_m (maximum fluorescence—all reaction centres are closed) and $F_v/F_m = (F_m - F_0)/F_m$ (maximal photochemical efficiency of PSII; Krause and Weiss 1991). Four shoot values were averaged to obtain the mean for each tree. For each measurement date, mean values for each variant (irrigated and non-irrigated) were derived from 6 tree means.

Determination of pigment concentration

The one-year-old needles were homogenized in 80% acetone at room temperature with a small amount of magnesium carbonate. The pigment concentration (chl a + b, car) was determined using spectrophotometry (Cintra, GBS Australia) at 470, 646 and 663 nm and was calculated according to Lichtenthaler (1987). Pigment concentration was calculated on a dry weight basis. For each measurement date, mean values for each variant (irrigated and non-irrigated) were derived from 6 tree means.

Data analysis

The differences in physiological variables were tested using two-way ANOVA. Means were compared using Duncan's multiple-range tests, at a significance level $P < 0.05$. The statistical analysis was performed employing Statistica 7 (StatSoft, USA).

Results and discussion

Soil and meteorological parameters

During the vegetation period 2009, the precipitation total from May to August was only 240 mm at the assessed site. In Central Europe, 300 mm is considered to be the lower

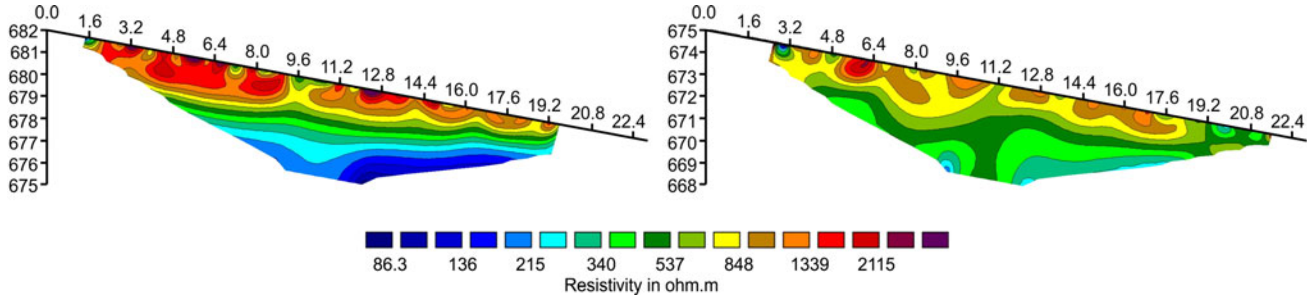


Fig. 2 Soil resistivity on irrigated (*left*) and non-irrigated plot (*right*) with terrain topography: the maximal displayed deep is four metres

limit for spruce growth. In our case, August with its total monthly precipitation of 39 mm was significantly below average. At the assessed site, the plant-available soil water content was already low at the beginning of the vegetation period 2009. During the second half of May, the minimum measured values of W_{10} and W_{35} were 9.4 and 11.8%, respectively (Fig. 1). Generally, the year 2009 can be characterized by dry spring followed by dry and warm summer and autumn.

The profile for resistivity tomography measurements of the irrigated plot (Fig. 2, left) was located on the eastern edge. In the upper profile layers, particularly in the upper part of the profile, we can see the zones with high resistivity (brown to purple colour) typical of coverbeds featuring high amount of coarse fraction, including stones and boulders that are in some places separated by the zones with higher proportion of fine earth with lower skeleton fraction (green colour). The influence of deep water table is indicated by low resistivity (blue colour) on the topsoil, and shallow root system is very unlikely.

The profile of the non-irrigated plot (Fig. 2, right) was also located on its eastern edge. The interpretation of the soil resistivity image indicates a significantly lower proportion of the zones with high resistivity, indicating higher volume of fine earth and better water retention properties in the topsoil, compared to the previous profile with dominant drainage processes. The results of the resistivity tomography show high variability of soil environment even at a microscale and account for the differences in soil water content between the plots prior to irrigation.

Leaf water potential and its influence on stomatal conductance

The measured values of leaf water potential (Ψ_L) in May and June fluctuated between −0.15 and −0.3 MPa (Fig. 3a), which are the values of healthy plants with sufficient moisture. In the second half of the experiment, Ψ_L of non-irrigated individuals significantly decreased—the lowest average value was approximately −1.4 MPa. These values indicate moderate drought stress. The decrease in Ψ_L in spruce due to drought is not as pronounced as in sclerophyllous tree species, for example in Scots pine (*Pinus sylvestris*) or Holm oak (*Quercus ilex*) (Cregg and Zhang 2001; Pesoli et al. 2003). Neither Ditmarová et al. (2010) nor Pukacki and Kaminska-Rozek (2005) observed the decrease in water potential in highly stressed spruce seedlings below the value −2.5 MPa. Galle and Feller (2007) measured similar values of Ψ_L in European beech (*Fagus sylvatica*). In spite of rainless period, we did not detect such low values in non-irrigated spruces. In mature trees, the degree of rehydration in the morning and at night fluctuates significantly not only depending on soil water availability but also due to the amount of water stored in a stem (Borchert 1994), which reduces the impact of shorter drought periods. From this point of view, trees have a considerable advantage over agricultural plants or seedlings. Nevertheless, the decrease in water stored in a stem accounts for the susceptibility of tree species to bark beetles.

According to some authors, plant water potential is a basic factor, which regulates the width of stomata openings. Larcher (1988) presents that in evergreen coniferous, the closing of stomata begins after reaching the values of −0.5 to −1 MPa, and the stomatal closure is completed when the values between −1 and −2 MPa are reached. However, as we can see in Fig. 3b, c, in spite of optimum values of Ψ_L, the values of stomatal conductance (g_S) and CO_2 assimilation rate (P_N) were lower at the first two measurements than at other dates, when we observed long-term soil water deficit and pronounced decrease in Ψ_L. Therefore, the decrease in Ψ_L seems to be the simple result of reduced water availability rather than the reason of stomatal closure. Leaf water potential decreased more significantly only when soil water content was low in the long run and when transpiration losses were simultaneously relatively high (during summer months). Apparently, for spruce protection against excessive water loss, fast chemical signalling of drought in substrate is more important than Ψ_L. Stomata can also respond to local variations in epidermal turgor—epidermal water relations may act as a modulator of the responses of stomata to ABA (Tardieu and Davis 1992).

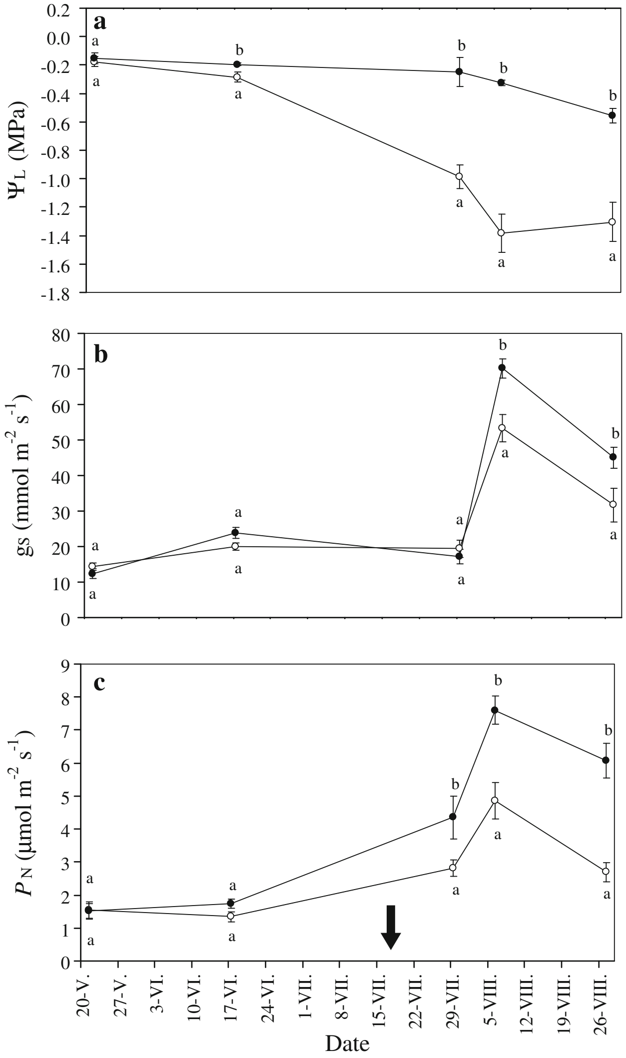


Fig. 3 Mean leaf water potential (Ψ_L, a), stomatal conductance (g_S, b) and CO_2 assimilation rate measured under light-saturated conditions (P_N, c) in irrigated (*filled circle*) and non-irrigated (*open circle*) spruces over the course of the experiment. The *arrow* indicates the beginning of irrigation, and *letters* refer to significant differences ($P \leq 0.05$) between groups (ANOVA, Duncan's test). Each data point is mean ± standard error of 5–6 trees; on each tree, 1–4 shoots were measured

The average value of g_S increase was observed in non-irrigated trees even after the decrease in Ψ_L on the fourth measuring day. Increase in g_S was most probably due to optimal conditions (as the day was cloudy, air humidity was relatively high and D relatively low), though still significantly lower than in irrigated trees. Bigras (2005) recorded high variability of g_S until the Ψ_L decreased below critical value −2 MPa for white spruce (*P. glauca*). Lu et al. (1995) observed close correlation between pre-dawn leaf water potential and g_S in *P. abies*, which is not in accordance with our observations. Duan et al. (2007) investigated the effects of exogenous ABA on the acclimation of *Picea asperata* to water deficit in two populations originating from wet and dry climate. ABA application significantly decreased the g_S and transpiration under water-deficit conditions in the dry climate population, whereas ABA application did not significantly affect these parameters in the wet climate population. Variations in physiological mechanisms rather than different rates of ABA absorption explain the population differentiation in the sensitivity to exogenous ABA. The results provide the evidence for adaptive differentiation between populations, which can also explain the above-mentioned contrary results.

Photosynthetic response to drought

Both P_N and g_S values were within the range found for young needles of maturing and mature *P. abies* trees (Ewers et al. 2001; Ward et al. 2008). On the first two dates, P_N and g_S were very low in spite of optimum values of Ψ_L (Fig. 3b, c). The stomatal conductance response to drought can be often observed before the changes in relative water content and leaf water potential. Therefore, Medrano et al. (2002) considered stomatal conductance as a parameter suitably and complexly reflecting plant drought stress. Species with high g_S assign higher sensitivity of this parameter to environmental changes in optimum conditions (Ewers et al. 2001). The authors compare the values of g_S between the species *P. abies* and *P. tadea*, which showed to have approximately half values of spruce and hence lower sensitivity of stomata reaction to drought. Individuals, species and stands with high stomatal conductance show even greater sensitivity to D, as required by the role of stomata in regulating leaf water potential (Oren et al. 1999).

Statistically significant differences in g_S were detected on the last two measurement dates, and significant differences in P_N were found on all summer measurement dates (after the beginning of irrigation). In non-irrigated spruce trees, the values of P_N decreased by 35–55% in comparison with irrigated trees.

To test whether the conductance differences are sufficient to account for the differences in assimilation, assimilation rate was plotted against conductance (Urban et al. 2007). The correlation between P_N and g_s was closer in case of irrigated individuals ($R^2 = 0.71$) than for non-irrigated spruce trees ($R^2 = 0.58$) (Fig. 4a). This indicates the limitation of CO_2 assimilation rate by the mechanism other than closure of stomata (Centritto et al. 2003; Brestič et al. 1995) in case of non-irrigated trees.

The performed measurements did not reveal any drought-induced significant changes in the concentration of carotenoids and chlorophylls or changes in the maximal photochemical efficiency of photosystem II (F_v/F_m). PS2

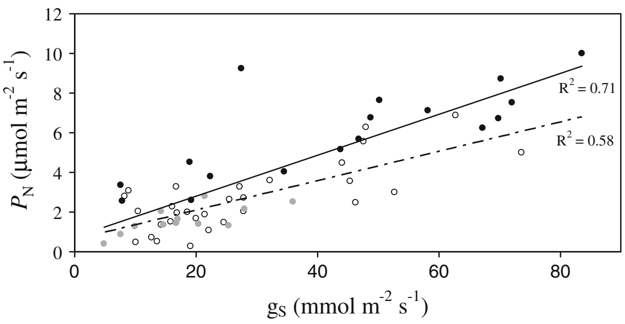


Fig. 4 CO_2 assimilation rate (P_N) as a function of stomatal conductance (g_S) measured under light-saturated conditions in irrigated (*grey filled circle* before first irrigation, *black filled circle* after first irrigation, *solid line*) and non-irrigated (*open circle*, *dashed line*) spruces. Each data point ($n = 30$ for both variants) represents an individual tree; on each tree, 2–4 shoots were measured. *Fitted curves*: irrigated trees: $y = 0.1029x + 0.7461$, non-irrigated trees: $y = 0.0741x + 0.6147$

energy conversion is not so sensitive to drought and continues even after CO_2 uptake is limited (Brestič et al. 1995). Particularly in Norway spruce, photosystem II is very efficiently protected by violaxanthin deepoxidation that leads to the dissipation of thermal energy (Štroch et al. 2010). Differences in chl a + b and car were relatively high even before the first irrigation, and they were not the result of drought impact (Tables 3, 4). The values were within the range found for a mature spruce (Wallin et al. 2002; Bolhar-Nordenkampf et al. 1992). In accordance with several studies, we found that the given parameters are relatively resistant to dehydration and that the decrease is caused only by severe drought stress (Ditmarová et al. 2010; Manes et al. 2001; Duan et al. 2005).

Influence of vapour pressure deficit and sap flow rate on stomatal closure

The lowest values of g_S were measured on the first two measurement dates, when D values were the highest (as marked in Fig. 5). On the contrary, the highest average values of gs were recorded on 4th date, when both average D and daily sum of global radiation (GR) were the lowest and relative air humidity was the highest (Table 2). Stomatal conductance of irrigated individuals correlated with D more tightly ($R^2 = 0.55$) than in case of non-irrigated individuals ($R^2 = 0.36$). Almost complete stomatal closure of the irrigated trees was caused by the increase in D above the value of approximately 1.5 kPa. The assessment of irrigated spruces in the period of good water supply (first and second dates excluded) revealed close linear correlation between D and g_S ($R^2 = 0.76$). This suggests that the regulation of stomata opening of the spruces that are well supplied with water was mainly influenced by the actual status of atmosphere. Lendzion and Leuschner (2008) observed strong growth and development dependence of beech samplings not only on soil moisture but also on D level. The possible reason of weak correlation between D and g_S of non-irrigated trees is washing out of ABA from roots as a reaction to soil drought. Stomata of drought-stressed individuals stay often closed even under optimum atmospheric conditions for photosynthesis. Tardieu and Davis (1992, 1993) proposed that mild stresses induce small hydraulic changes that can directly sensitize guard cells to ABA so that stomata close and maintain leaf water status (Wilkinson and Davies 2002).

In the variant of irrigated spruce trees, sap flow (F) was already lower prior to the beginning of the irrigation (Fig. 6a), which is most probably caused by higher skeleton fraction (Fig. 2) and hence lower soil water content at both measured depths (W_{10} and W_{35}, Fig. 1). In non-irrigated individuals, we can see the peak of F at 11:00 AM followed by its decrease—trees show the noon depression of transpiration, which continues in the afternoon (Fig. 6a, b).

Increased transpiration of non-irrigated individuals observed at the beginning of August (Fig. 6c) resulted from the precipitation from the previous week, which was 28 mm in total. Even this heaviest rain in August did not cause the change in W_{35}, and several days after precipitation, W_{10} was again lower by 10%. Therefore, the transpiration of non-irrigated spruce trees was still lower than that of irrigated trees, and it seems that the soil layer in the depth of 15–30 cm had the decisive impact on water supply in this period. The decrease in F in the afternoon (in both groups of trees) was not caused by afternoon stomatal closure, but by the reduction in GR and D. Matyssek et al. (2009) stated that the daily crown transpiration of individual trees is driven by the daily light sum. Both the quantity and quality of light play an important role in the reopening of closed stomata during the day (Zweifel et al. 2002).

Table 3 Statistical significance—asterisks indicate significant effect of time, treatment and their interaction: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

	Ψ_L	g_S	P_N	Chl a + b	Car	F_v/F_m
Time	***	***	***	ns	*	***
Treatment	***	***	***	***	**	ns
Time × treatment	***	***	***	ns	ns	ns

Table 4 Mean (and standard error) chlorophyll and carotenoid concentration (in milligrams per gram dry weight) and the maximal photochemical efficiency of photosystem II (F_v/F_m) of irrigated and non-irrigated spruces

	May 21	June 17	July 29	August 27
Chl a + b (mg g^{-1})				
Non-irrigated	3.45 ± 0.13	3.30 ± 0.80	3.38 ± 0.15	3.38 ± 0.14
Irrigated	4.08 ± 0.22	3.71 ± 0.18	3.97 ± 0.25	3.87 ± 0.23
Car (mg g^{-1})				
Non-irrigated	0.64 ± 0.02	0.68 ± 0.02	0.68 ± 0.02	0.58 ± 0.02
Irrigated	0.74 ± 0.04	0.78 ± 0.05	0.74 ± 0.03	0.68 ± 0.06
F_v/F_m				
Non-irrigated	0.846 ± 0.001	0.843 ± 0.003	0.842 ± 0.003	0.846 ± 0.002
Irrigated	0.843 ± 0.002	0.843 ± 0.003	0.844 ± 0.003	0.845 ± 0.002

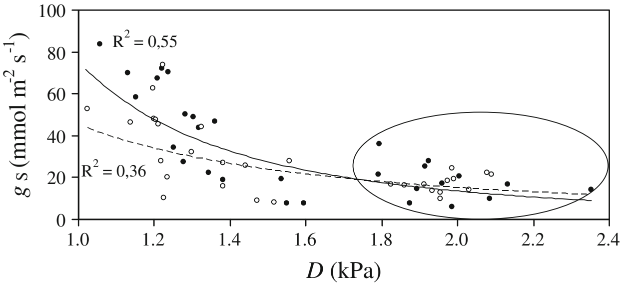


Fig. 5 Leaf stomatal conductance (g_S) as a function of leaf-to-air vapour pressure deficit (D) in irrigated (*filled circle*, *solid line*) and non-irrigated (*open circle*, *dashed line*) spruces. The *encircled* values were measured on the first 2 days (before first irrigation). Each data point ($n = 30$ for both variants) represents an individual tree; on each tree, 2–4 shoots were measured. *Fitted curves*: irrigated trees: $y = 74.94x^{-2.465}$, non-irrigated trees: $y = 45.216x^{-1.5795}$

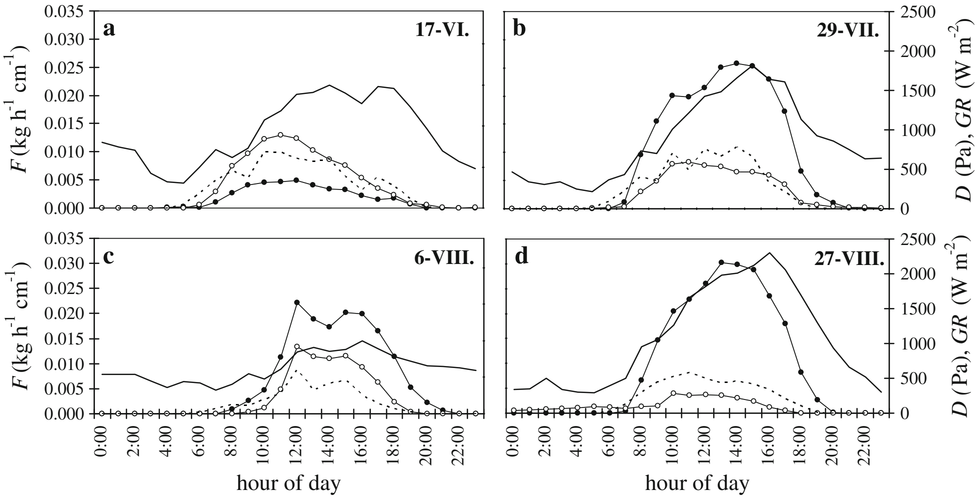
On the last measurement date, we recorded the decrease in F by 86% in non-irrigated trees, the value approaching cuticular transpiration. Transpiration is an efficient way to reduce plant temperature. However, if it is permanently minimal during the clear days with extremely high temperatures, stress from leaf overheating can additionally occur. Low water availability, especially when coupled with rising temperatures, represents the main environmental constraint for plant growth and productivity (Centritto et al. 2011b).

The diurnal course of F in the individuals supplied with water copied the changes in D and GR well. In irrigated individuals, we did not record the noon depression of transpiration in spite of the reduction in Ψ_L to −0.56 MPa on the last measurement day (Fig. 6d). Zweifel et al. (2002) observed the diurnal dynamics of mature spruce trees and found that high rates of sap flow in twigs did not always lead to stomatal closure and therefore could not explain the phenomenon. It seems that high transpiration rates did not control stomatal response to D (as assumed by Monteith 1995).

Conclusions

The decrease in Ψ_L in non-irrigated Norway spruces below −1.5 MPa was not observed despite the long-term drought. This indicates relatively efficient protection of spruce

Fig. 6 Daily course of sap flow rate (F) in irrigated (*filled circle*) and non-irrigated (*open circle*) spruces before the irrigation (**a**) and after the beginning of irritation (**b**–**d**), vapour pressure deficit (D—*solid line*) and global radiation (GR—*dashed line*). Each data point is mean of at least five trees



against the water loss. In addition, during shorter drought periods, stems of mature trees act as water storage. The stomatal closure was controlled by atmospheric conditions, not by Ψ_L. The stomata of non-irrigated individuals were not completely closed when D values were low; on the contrary, even the stomata of the trees well supplied with water were closed when D value was high. The response of stomata to changes in D appears to be the fastest and most efficient protection against excessive water loss. Our results did not prove that the transpiration controls stomatal response to D. Hlásny et al. (2011) state that in Central Europe, spruce growth will be probably enhanced in its upper distribution ranges, while drought-induced production decline is projected at receding edges. Our study confirmed significant differences in P_N between irrigated and non-irrigated trees. In spite of relatively efficient protection of the adult trees, the occurrence of long-term drought periods and high D may cause heavy loss of productivity and protection potential against fungal and bark beetle attacks, particularly of trees growing outside their natural occurrence.

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Conflict of interest The authors declare that they have no conflict of interest.

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