The soil hydrogel improved photosynthetic performance of beech seedlings treated under drought

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ABSTRACT

The effect of soil amendment with the STOCKOSORB®500 MICRO hydrophilic polymer on the photosynthetic traits in beech seedlings (*Fagus sylvatica* L.) during 50 days of dehydration was investigated. Dehydration was detected through osmotic potential (Ψ_s) in the assimilatory organs of beech seedlings. The addition of Stockosorb positively affected the CO_2 assimilation rate (A) and instantaneous water use efficiency (A/T), for severely drought-treated seedlings. In comparison with irrigated plants, the values of A of non-irrigated plants with Stockoa sorb substrate decreased by 50%, and in non-irrigated plants with common substrate by 88%. The fast kinetics of chlorophyll *a* fluorescence indicated chronic photoinhibition under drought treatment without Stockosorb, while no significant changes in maximal quantum efficiency (F_v/F_m) were recorded under drought treatment with Stockosorb. The actual quantum efficiency of PSII (Φ_{PSII}) markedly decreased in both treatments – with and without Stockosorb, though significant differences were found only between control treatments and drought treatment without Stockosorb. Moreover, the thermal energy dissipation (NPQ) was strongly limited under severe drought stress. The capacity to down regulate PSII functionality through non-photochemical quenching was maintained under drought treatment with Stockosorb. The results indicate that an amendment with soil conditioner significantly improved the photosynthetic performance of drought-stressed beech seedlings.

Keywords: *Fagus sylvatica* L.; chlorophyll *a* fluorescence; water stress; gas-exchange parameters; stockosorb

Dramatic changes in the water availability in Europe will affect existing tree species and their ability to cope with excess water or the lack thereof. Tree species and provenances that are most capable of tolerating water stress are options for the future of forestation (Bredemeier et al. 2011, Sánchez-Gómez et al. 2013).

Studies concerning the effects of drought on the water regime and photosynthetic activity in beech trees (Bréda et al. 2006, Fotelli et al. 2009) reported that the beech is not a drought-tolerant species, despite built-in mechanisms for controlling water deficit. Water stress strongly affects photosynthetic processes through forcing leaf stomata to close and thereby increasing the diffuse resistance against CO_2 uptake and decreasing CO_2 absorption in the leaves. Closed stomata and reduced mesophyll conductivity decelerate the diffusion of CO_2 from the atmosphere into carboxylation sites in leaves, impeding photosynthetic processes (Grassi and Magnani 2005, Varone et al. 2012). The limited absorption of CO_2 due to stomatal closure might disrupt the equilibrium between the photochemical activity of photosystem II (PSII) and the amount of electrons required for the Calvin cycle, inducing an excess of absorbed excitation energy and

Supported by grants from the Grant Agency APVV No. 0436/10 and by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Science, Grants No. VEGA 2/0006/11 and VEGA 1/0218/12.

causing photo-inhibition damage to the reaction centres of PSII (Foyer and Noctor 2000, Baker and Rosenqvist 2004). The photoinhibition might be reversible, when energy dissipation mechanisms (e.g. non-photochemical quenching of fluorescence) are perfectly efficient, and irreversible with the PSII is completely damaged (Horton et al. 1996).

A potential mechanism for eliminating the effect of drought stress, particularly to young trees and seedlings, could be the application of soil conditioners or hydrogels to improve soil moisture conditions. Hydrogels are water-retaining polymers that absorb and store water at concentrations of approximately 100 and 150 times their weight. A significant fraction of this absorbed water is available to plants and thus acts as an additional water reservoir for the soil-plant-air system (Bhardwaj et al. 2007). The hydrogel Stockosorb (including series: AGRO, MICRO and POWDER) improves plant growth in extremely dry conditions. Water-absorbent STOCKOSORB®500 MICRO is a potassium ammonium polyacrylate/polyacrylamide copolymer. Stockosorb is a cross-linked polymer with the ability to absorb a high volume of water due to network spaces in its cross-linked structure (Chirino et al. 2011). The application of Stockosorb can provide more uniform substrate moisture, prevent leaching of water from soil and increase the water storage capacity of soil substrates to avoid or reduce drought-stress damage (Arbona et al. 2005, Chirino et al. 2011).

The ameliorative effect of hydrogels for drought-stressed seedlings was demonstrated for *Citrus* (Arbona et al. 2005), *Quercus* (Chirino et al. 2011), *Pinus* (Roldan et al. 1996, Hüttermann et al. 1999, Sarvaš et al. 2007), *Fagus* (Beniwal et al. 2011), *Populus* (Shi et al. 2010). However, the data for tree species representing the majority of forests in Central Europe are scarce, particularly in the context of prolonged and repeated drought periods. Moreover, only a few studies concerning a more detailed response to Stockosorb amendment on the level of photosynthesis have been conducted. Hence, the objectives of this study were to investigate the effects of hydrogel through the physiological reactions of 4-year-old European beech seedlings under simulated drought conditions.

MATERIAL AND METHODS

Plant material and experimental design. Four-year-old beech seedlings (*Fagus sylvatica* L.) were planted in April 2011 in plastic pots (7 L). The experiment was performed from 6 July to 24 August (50 days) under greenhouse conditions. The seedlings were arranged in a randomized order in 3 blocks. Plants were divided into four groups of different treatments (45 seedlings each): (i) control under fully irrigated conditions with a common substrate (CC); (ii) control under fully irrigated conditions with hydrogel Stockosorb (CS); (iii) drought-stressed conditions with common substrate (DC); and (iv) drought-stressed conditions with Stockosorb (DS). Control beech trees were irrigated every 2 days; the stressed seedlings were kept without water for 50 days.

The common substrate, containing brown soil, peat and composted bark with N:P:K (3:1:2) fertilizer; 65% maximal moisture content; and a pH value (aqueous extract) of 5.0–6.5. The treatments with Stockosorb contained common substrate with 5 g of hydrogel per 1.9 kg (0.3%) of substrate. Hydrogel STOCKOSORB®500 Micro (Stockhausen GmbH&Co. KG, Germany) comprised the highly cross-linked polyacrylamides (93%) of 0.2–0.8 mm size.

Air temperature, relative humidity (datalogger Minikin TH with built-in sensors; EMS, Brno, Czech Republic), and soil moisture (Datalogger Microlog SP3 with gypsum block soil moisture sensors; EMS, Brno, Czech Republic) were stored every 10 min during the entire experiment. Soil water potential (SWP) was maintained up to –0.05 MPa in control variants, whereas decreased to –0.9 MPa (on Day 50) in drought-stressed plants.

Leaf osmotic potential. Degree of the water stress in assimilatory organs of the seedlings was recorded through the osmotic potential (Ψ_s) from leaf discs (n = 15 per treatment) collected before sunrise, wrapped in aluminum foil and quick-frozen in liquid nitrogen. Subsequently, measurements were done in the laboratory using the psychrometric method with a PSY-PRO (Wescor, USA) via C-52 psychrometric chambers. The measurements of Ψ_s were performed 3-times during the experiment (on Day 1, Day 22 and Day 50).

Gas exchange measurements. Gas exchange was measured at the end of the experiment using a gasometric system Li-6400XT equipped with a 6400-02B LED light source (LI-COR Biosciences; Lincoln, USA). Five values were recorded for each leaf, and at least 2 leaves were measured for each seedling and 15 seedlings were measured for each treatment. The CO_2 concentration was maintained at 385 mol/m, the saturating, photosynthetically active radiation was set to 1200 μmol/m^2/s, and the average air temperature in the chamber was 22 ± 2°C. The values were measured after a brief adaptation period when the A values were stable. The instantaneous water-use efficiency (A/T) was calculated as the carbon uptake per unit of water lost (A) to leaf transpiration (T).

Fast kinetics of chlorophyll *a* fluorescence measurements. A Handy Pea fluorometer (Hansatech Ltd., Kings Lynn, UK) was used to measure the maximal fluorescence yield (F_v/F_m) and photosynthetic performance index (PI) at the end of the experiment. A total of 4 leaves were measured for each seedling, and 15 seedlings were measured for each treatment. After a 30-min dark-adaptation, the samples were irradiated using a saturation pulse with an intensity of 2000 μmol/m^2/s.

F_v/F_m was calculated as the maximal variable fluorescence (F_v) measured on a dark acclimated leaf to maximum fluorescence emission (F_m) measured on a dark acclimated leaf; $F_v = F_m - F_o \times F_o$ means initial fluorescence emission measured on a dark acclimated leaf.

Slow kinetics of chlorophyll *a* fluorescence measurements. A MINI-PAM photosynthesis yield analyzer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany) was used to measure the actual photochemical efficiency of PSII (Φ_{PSII}) and non-photochemical quenching parameter (NPQ) at the end of the experiment. At least 25 leaves per treatment were subjected to non-destructive measurements. The recordings of Φ_{PSII} were made under ambient light, using the fibre optics of the MINI-PAM device conenected to open leaf-clips. The same leaf-clips were thereafter closed for 30 min and follow-up recordings of F_m and F_o were performed after the dark-adapted sample. The efficiency of the thermal dissipation of excessive excitation energy was determined and calculated according to Maxwell and Johnson (2000).

Data analysis. The significance of the differences in photochemical variables was tested using two-way ANOVA with two fixed factors: substrate type (with 2 levels – substrate with hydrogel and common substrate), and soil moisture condition (with 2 levels – watered and drought-stressed). Means were compared using the Tukey-Kramer multiple comparisons test at a significance level of $P < 0.05$. The statistical analysis was performed using Statistica 7 (StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

Water stress. The osmotic potential in the drought-stressed seedlings was reduced to enhance the capacity for water uptake. However, the Ψ_s in plants grown in substrate containing Stockosorb was not reduced to such a large extent as in plants planted without hydrogel (Figure 1). The values obtained for the stressed seedlings without hydrogel were reduced to –2.2 MPa indicated strong drought stress, which caused negative changes in the physiological processes of plants.

Changes in the CO_2 assimilation rate and water use efficiency. Soil moisture and substrate type were both relevant factors affecting the values of A and A/T (Table 1). Also interaction between these two effects was confirmed for instantaneous water use efficiency. We observed a marked decrease in the A for both non-watered treatments (Figure 2a). Moreover, the values for DC were significantly lower compared with the other treatments (including DS). The significant differences were recorded in the instantaneous water use efficiency (A/T) between the DC treatment and the other groups (Figure 2b).

According to our data, the application of hydrogel mediated the efficient use of water. The increased instantaneous water use efficiency confers a fitness advantage under stressful conditions in the field (Heschel et al. 2002) and thus might increase the survival of tree species. The application of hydrogels leads to a significant reduction in the

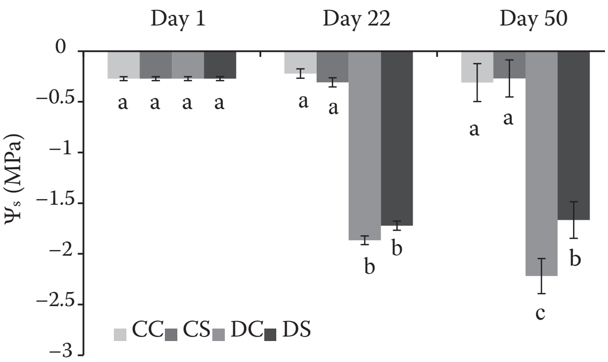


Figure 1. Changes in the osmotic potential (Ψ_s) of beech leaves during dehydration. The data are presented as the means ± SE. The small letters indicate statistically significant differences ($P < 0.05$) among the treatments. CC – control treatment without Stockosorb; CS – control treatment with Stockosorb; DC – drought treatment without Stockosorb; DS – drought treatment with Stockosorb

Table 1. Statistically significant differences

	A	A/T	F_v/F_m	F_v/F_o	Φ_{PSII}	qP	NPQ
Soil moisture	***	***	***	**	***	*	**
Substrate type	***	**	***	***	*	**	***
Soil moisture × substrate type	ns	**	**	ns	ns	ns	***

*P < 0.05; **P < 0.01; ***P < 0.001; ns – non-significant

required irrigation frequency, particularly for coarse-textured soils (Koupai et al. 2008).

Effects of Stockosorb amendment on chlorophyll *a* fluorescence. The chlorophyll fluorescence associated with parameters of fast and slow kinetics was markedly affected by drought and substrate type at the end of experiment (Table 1).

The F_v/F_m was clearly reduced under DC treatment (Figure 3a). The differences in the F_v/F_m values among the treatments with Stockosorb (CS and DS) ranged about 9%. However, the percentage differences among the treatments without conditioner (CC and DC) ranged about 28%.

The values of Φ_{PSII} for DC were significantly lowered (0.07), indicating that the proportion of light absorbed by chlorophyll was markedly reduced (Figure 3b). Effective quantum yield under drought treatment with Stockosorb also decreased (0.12), but statistically significant differences between control treatments and DS were not confirmed.

The maximal quantum efficiency of PSII was less sensitive to changes induced under different water storage conditions compared with the actual efficiency of PSII. The differences between DC and DS in the F_v/F_m were approximately 9%, while the differences in the Φ_{PSII} were over 40%.

Although there was some reduction in the open reaction centers of photosystem II under DS, these changes did not severe. On the other hand, chronic photoinhibition by water stress under DC treatment supported not only reduction of Φ_{PSII}, but mainly a reduction in F_v/F_m (Baquedano and Castillo 2006, Silva et al. 2010). Moreover, the maximum rate of photosynthesis and the actual quantum efficiency of PSII were simultaneously reduced under DC treatment, indicating that photoinhibition is chronic (Osmond 1994, Santos et al. 2013).

The NPQ was reduced under DC treatment (0.4–0.6) compared with the DS treatment (0.9–1.1) (Figure 3c). Non-photochemical quenching typically increased in moderately stressed plants (DS) to protect the leaves from damage (Maxwell and Johnson 2000, Li et al. 2013). Stress conditions (DC) lowered the capacity to down regulate PSII functionality through thermal energy dissipation. Colom and Vazzana (2003) suggested that under severe drought stress, the thermal energy dissipation (linearly related to NPQ) is highly limited.

Also photosynthetic performance index showed the considerable positive effect of hydrogel (Figure 4). PI values indicated high photosynthetic vitality of seedlings in treatments with Stockosorb.

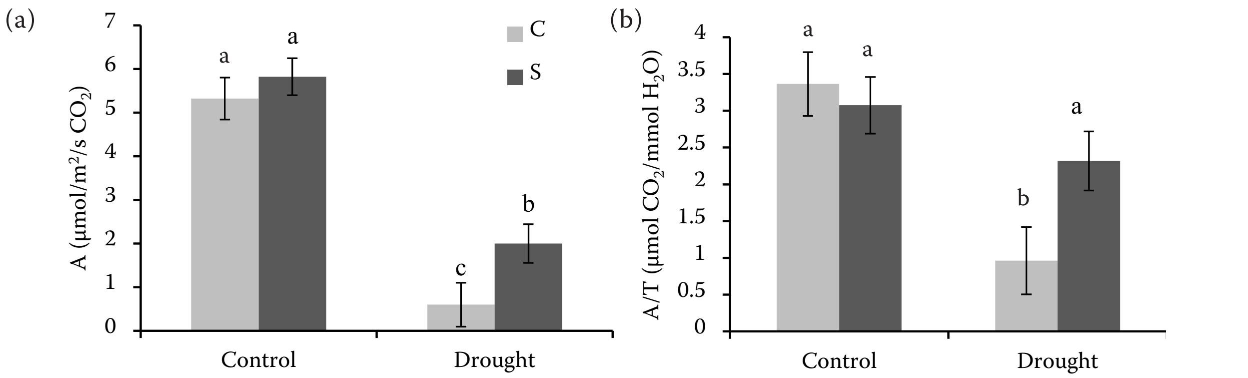
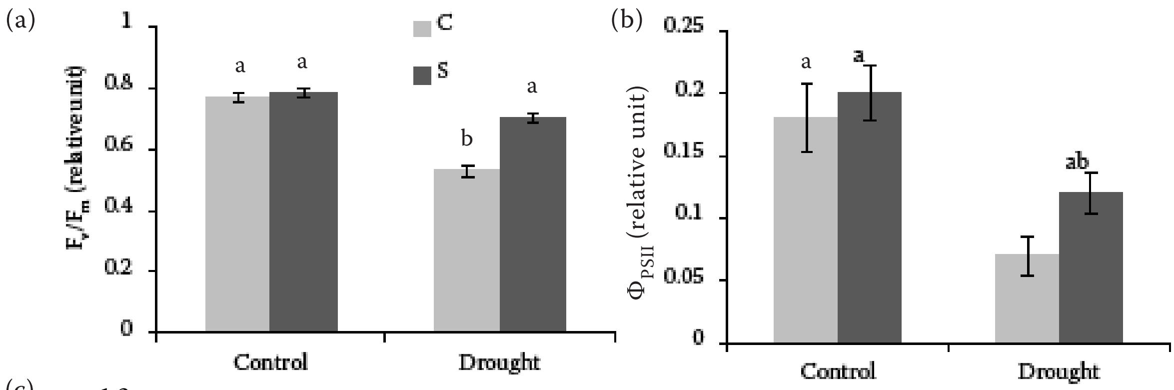


Figure 2. Effects of Stockosorb addition on (a) the CO_2 assimilation rate (A) and (b) instantaneous water use efficiency (A/T) of beech seedlings under different soil moisture (control – full-watered; drought – non-watered). C – common substrate; S – substrate with Stockosorb. The data are presented as the means ± SE. The small letters indicate statistically significant differences (P < 0.05) among the treatments



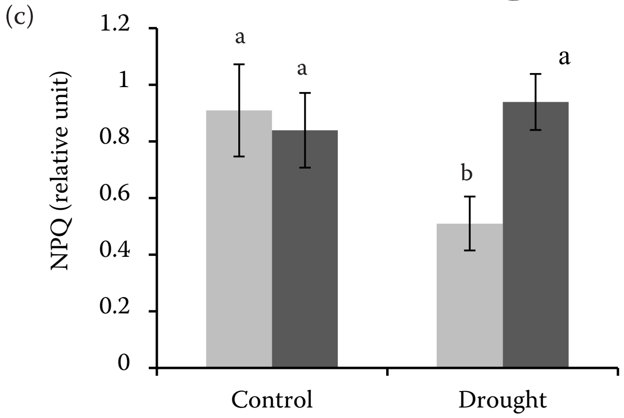


Figure 3. Effects of Stockosorb addition on chlorophyll *a* fluorescence parameters: (a) the F_v/F_m; (b) Φ_{PSII} and (c) NPQ parameter under different soil moisture (control – full-watered; drought – non-watered). C – common substrate; S – substrate with Stockosorb. The data are presented as the means ± SE. The small letters indicate statistically significant differences ($P < 0.05$) among the treatments

There are following mechanisms for the explanations: (1) roots took up the retained water from hydrogel during water deficiency (higher rate of water uptake); (2) the addition of Stockosorb positively affected the CO_2 assimilation rate, instantaneous water use efficiency drought-treated plants and consequently significantly influencing plant growth and biomass accumulation.

Moreover, there exists a strong relationship between the fine root biomass and the above-ground biomass particularly in young trees because of high and non-linear changing root/shoot ratio. The observations showed that even relatively light drought decreased total fine root biomass (Konôpka et al. 2010, Konôpka and Lukac 2013).

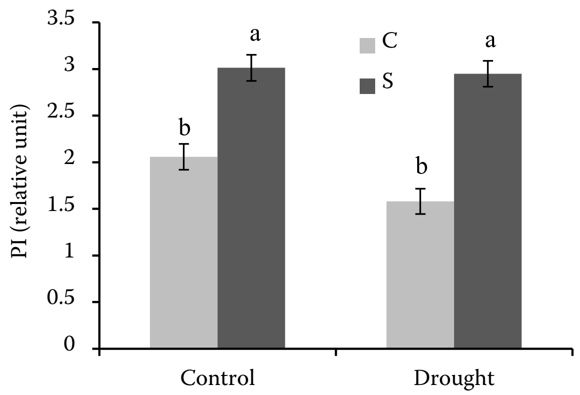


Figure 4. Changes in photosynthetic vitality (PI) of beech seedlings. Control – full-watered; drought – non-watered; C – common substrate; S – substrate with Stockosorb. The data are presented as the means ± SE. The small letters indicate statistically significant differences ($P < 0.05$) among the treatments

These results show that soil amendment with Stockosorb significantly improves the photosynthetic effectiveness of beech seedlings under drought stress. Stockosorb using in forestry practises may affect the survival of young beech trees during longer drought period. However, the optimum application doses and types of hydrogel for the individual tree species and specific soil conditions are unknown and remain a challenge for future research.

REFERENCES

Arbona V., Iglesias D.J., Jacas J., Primo-Millo E., Talon M., Gomez-Cadenas A. (2005): Hydrogel substrate amendment alleviates drought effects on young citrus plants. Plant and Soil, *270*: 73–82.

Baker N.R., Rosenquist E. (2004): Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. Journal of Experimental Botany, *55*: 1607–1621.

Baquedano F.J., Castillo F.J. (2006): Comparative ecophysiological effects of drought on seedlings of the Mediterranean water-saver *Pinus halepensis* and water-spenders *Quercus coccifera* and *Quercus ilex*. Trees, *20*: 689–700.

Beniwal R.S., Hooda M.S., Polle A. (2011): Amelioration of planting stress by soil amendment with a hydrogel-mycorrhiza mixture for early establishment of beech (*Fagus sylvatica* L.) seedlings. Annals of Forest Science, *68*: 803–810.

Bhardwaj A.K., Shainberg I., Goldstein D., Warrington D.N., Levy G.J. (2007): Water retention and hydraulic conductivity of cross-linked polyacrylamides in sandy soils. Soil Science Society of America Journal, *71*: 406–412.

Breda N., Huc R., Granier A., Dreyer E. (2006): Temperate forest trees and stands under severe drought: A review of ecophysiological responses, adaptation processes and long-term consequences. Annals of Forest Science, *63*: 625–644.

Bredemeier M., Cohen S., Godbold D.L., Lode E., Pichler V., Schleppi P. (eds.) (2011): Forest Management and the Water Cycle. An Ecosystem-Based Approach. Ecological Studies 212, Springer, Dordrecht.

Chirino E., Vilagrosa A., Vallejo V.R. (2011): Using hydrogel and clay to improve the water status of seedlings for dryland restoration. Plant and Soil, *344*: 99–110.

Colom M.R., Vazzana C. (2003): Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. Environmental and Experimental Botany, *49*: 135–144.

Fotelli M.N., Nahm M., Radoglou K., Rennenberg H., Halyvopoulus G., Matzarakis A. (2009): Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe. Forest Ecology and Management, *257*: 1157–1164.

Foyer C.H., Noctor G. (2000): Oxygen processing in photosynthesis: Regulation and signaling. New Phytologist, *146*: 359–388.

Grassi G., Magnani F. (2005): Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant, Cell and Environment, *28*: 834–849.

Heschel M.S., Donohue K., Hausmann N., Schmitt J. (2002): Population differentiation and natural selection for water-use efficiency in *Impatiens capensis* (Balsaminaceae). International Journal of Plant Science, *163*: 907–912.

Horton P., Ruban A.V., Walters R.G. (1994): Regulation of light harvesting in green plants. Plant Physiology, *106*: 415–420.

Hüttermann A., Zommorodi M., Reise K. (1999): Addition of hydrogels to soil for prolonging the survival of *Pinus halepensis* seedlings subjected to drought. Soil and Tillage Research, *50*: 295–304.

Konôpka B., Pajtík J., Moravčík M., Lukac M. (2010): Biomass partitioning and growth efficiency in four naturally regenerated forest tree species. Basic and Applied Ecology, *11*: 394–405.

Konôpka B., Lukac M. (2013): Moderate drought alters biomass and depth distribution of fine roots in Norway spruce. Forest Pathology, *43*: 115–123.

Koupai J.A., Eslamian S.S., Kazemi J.A. (2008): Enhancing the available water content in unsaturated soil zone using hydrogel, to improve plant growth indices. Ecohydrology and Hydrobiology, *8*: 67–75.

Li G.L., Wu H.X., Sun Y.Q., Zhang S.Y. (2013): Response of chlorophyll fluorescence parameters to drought stress in sugar beet seedlings. Russian Journal of Plant Physiology, *60*: 337–342.

Maxwell K., Johnson G.N. (2000): Chlorophyll fluorescence – a practical guide. Journal of Experimental Botany, *51*: 659–668.

Santos C.M., Verissimo V., Wanderley Filho H.C. de L., Ferreira V.M., Cavalcante P.G. da S., Rolim E.V., Endres L. (2013): Seasonal variations of photosynthesis, gas exchange, quantum efficiency of photosystem II and biochemical responses of *Jatropha curcas* L. grown in semi-humid and semi-arid areas subject to water stress. Industrial Crops and Products, *41*: 203–213.

Osmond C.B. (1994): What is photoinhibition? Some insights from comparisons of shade and sun plants. In: Baker N.R., Bowyer J.R. (eds.): Photoinhibition of Photosynthesis. BIOS Scientific, Oxford, 1–24.

Roldan A., Querejeta I., Albaladejo J., Castillo V. (1996): Survival and growth of *Pinus halepensis* Miller seedlings in a semi-arid environment after forest soil transfer, terracing and organic amendments. Annals of Forest Science, *53*: 1099–1112.

Sánchez-Gómez D., Robson T.M., Gascó A., Gil-Pelegrín E., Aranda I. (2013): Differences in the leaf functional traits of six beech (*Fagus sylvatica* L.) populations are reflected in their response to water limitation. Environmental and Experimental Botany, *87*: 110–119.

Sarvaš M., Pavlenda P., Takáčová E. (2007): Effect of hydrogel application on survival and growth of pine seedlings in reclamations. Journal of Forest Science, *53*: 204–209.

Shi Y., Li J., Shao J., Deng S., Wang R., Li N., Sun J., Zhang H., Zhu H., Zhang Y., Zheng X., Zhou D., Hüttermann A., Chen S. (2010): Effects of Stockosorb and Luquasorb polymers on salt and drought tolerance of *Populus popularis*. Scientia Horticulturae, *124*: 268–273.

Silva E.V., Fereira-Silva S.L., Fontenele A.V., Ribeiro R.V., Viégas R.A., Silveira A.G. (2010): Photosynthetic changes and protective mechanisms against oxidative damage subjected to isolated and combined drought and heat stresses in *Jatropha curcas* plants. Journal of Plant Physiology, *167*: 1157–1164.

Varone L., Ribas-Carbo M., Cardona C., Gallé A., Medrano H., Gratani L., Flexas J. (2012): Stomatal and non-stomatal limitations to photosynthesis in seedlings and saplings of Mediterranean species pre-conditioned and aged in nurseries: Different response to water stress. Environmental and Experimental Botany, *75*: 235–247.

Received on March 11, 2013
Accepted on September 10, 2013

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