

The Diurnal Time Course of Leaf Gas Exchange Parameters of Pedunculate Oak Seedlings Subjected to Experimental Drought Conditions

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Abstract

The seedlings of pedunculate oak (*Quercus robur* L.) were exposed to different soil water contents (non-limiting and severe drought) for 30 days in a greenhouse in order to examine the effects of prolonged summer drought on diurnal fluctuations of gas exchange parameters.

Results showed that, in control treatment, diurnal course of photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) was bimodal, showing clear midday depression. In stress treatment, the diurnal dynamics of A exhibited an atypical double-peaked pattern, whereas the diurnal change in E and g_s presented a single-peaked curve. A positive and significant correlation between A and g_s was observed in well-watered as well as in drought stressed plants, whereas intercellular CO₂ concentration (C_i) increased with decreasing A rate. Therefore, we might assume that in both treatments the largest part of the reduction in photosynthesis was caused by stomatal closure; however, this finding does not preclude the existence of alternative mechanisms that may also have affected photosynthesis. The diurnal dynamics of instantaneous water use efficiency (WUE) exhibited maximum values in the early morning, afterwards it decreased during late morning and throughout the afternoon with the decline being greater in the stressed than in the control plants. Comparing to the control, the plants subjected to permanent water deficit have increased intrinsic water use efficiency (WUE_i) during the early morning, pointing out on minimized loss of water per fixed CO₂. Leaf temperature (T_l), as an environmental factor, was in a significant negative correlation with WUE, and in a positive one with E, in both treatments. The results of the study point to the adverse effect of prolonged experimental drought on net photosynthesis and other gas exchange parameters in pedunculate oak leaves, allowing them to be used as an important indicator of environmental conditions and trends in climate change.

Keywords: *Quercus robur* L., drought, leaf gas exchange parameters, diurnal variation

Introduction

Global climate changes are leading to increases in temperature and atmospheric CO₂ levels as well as alterations in rainfall patterns (Bhargava and Sawant 2013). Evidence from analyses of long-term data sets show that climatic changes of the past few decades have affected species physiology, the range and distribution of species, the phenology of organisms, and the composition and dynamics of communities (Walther et al. 2002, Parmesan and Yohe 2003).

Climate change is supposed to largely affects plant gas exchange (Song et al. 2016). The results from numerous ma-

nipulative experiments evidenced reduction of photosynthesis rate and related parameters in plants subjected to drought stress. For example, Pšidová et al. (2015) evidenced notable reduction of net photosynthesis and stomatal conductance in beech seedlings exposed to water deficit. Michelozzi et al. (2011) found that stomatal conductance in Aleppo pine seedlings dropped immediately after starting the stress and recovered only slightly after rewatering. Similarly, Gallé and Feller (2007) recorded that with increased water deficit, stomatal conductance and net assimilation rate decreased simultaneously in beech seedlings, and was almost completely inhibited after two weeks without irrigation.

Drought is one of the most important abiotic stress factors that influence the natural distribution of woody plants and limit ecosystem production across the globe (Yang et al. 2010). Drought has been recognized as one of the major problems in forestry that lead to a vitality reduction and dieback of trees. According to the climatic changes models, it is projected that drought will increase as global warming progresses, putting forest health in jeopardy (Rewald 2008). The region of Southern Europe will be particularly threatened due to projected air temperature increase and decline of summer precipitation sums (Lindner et al. 2010). Recent studies demonstrated that drought affects a wide range of plant responses, from changes in plant metabolism to the alteration in leaf and wood anatomical structure (Stojnić et al. 2013, Topić et al. 2013, Borišev et al. 2015, Stojnić et al. 2015). Moreover, severe drought during one growing season may induce physiological responses that are carried over into following years, as well (Irvine et al. 1998).

The effect of water availability on plant photosynthesis is an important area of study due to the fact that photosynthesis, as a sensitive physiological parameter of plant metabolism and development, can rapidly reflect plant response to changes in environmental conditions (Liu et al. 2013). A usual way to get information on specific adaptations of plants to the prevailing climate has been the study of diurnal fluctuations in photosynthetic performance and water relations (Llorens et al. 2003). Photosynthetic rates are often reduced during warm midday hours relative to the cooler morning (Pons and Welschen 2003). According to numerous authors, this midday depression of photosynthesis is caused by excess light, often occurring with high leaf temperature or water deficit (Chaves et al. 1987, Epron et al. 1992). Stomatal closure and photoinhibitory damage are the major physiological factors responsible for the midday depression of assimilation rate (Muraoka et al. 2000). Understanding of midday depression at the scale of the individual leaf is necessary for improving the process-based modeling of ecosystem gas exchange (Koyama and Takemoto 2014). Further, midday depression of photosynthesis, as a regulatory process of the plant itself, is advantageous for the survival of plants under stress conditions (Pessarakli 2001). Midday depression phenomenon of gas exchange has been well documented for different *Quercus* species – e.g. *Q. rubra* and *Q. alba* (Bahari et al. 1985), *Q. suber* (Tenhunen et al. 1984, Faria et al. 1996), *Q. petraea* (Epron et al. 1992), *Q. pubescens*, *Q. ithaburensis* and *Q. frainetto* (Siam et al. 2008).

Oak species are widespread distributed and display great variations in their abilities to tolerate periods of drought, reflecting adaptation to environments with different water availability (Dickson and Tomlinson 1996). Nevertheless, occurrence of oak dieback has been recorded in many European countries over the last few decades (Thomas et al. 2002). Zadworny et al. (2014) believe that lack of moisture in the soil is one of the most common problems

that affect the growth of *Q. robur*, leading to the gradual mortality of oak trees. According to Čater and Levanič (2015) the result of this declining phenomena is uneven age-class proportions and reduced seedling regeneration, which sometimes might affect even sustainable management of these forests. The management of pedunculate oak forests in Serbia is affected by a number of problems that are reflected in the dieback of individual and groups of trees throughout the area where the pedunculate oak occurs (Stojnić et al. 2014). Moreover, there is a lot of evidence regarding failures in reforestation attempts, especially during dry years. For example, Stojanović et al. (2015) reported that during two extremely dry years in Serbia (2011 and 2012) mortality of one-year-old pedunculate oak seedlings was recorded at almost 80% of regenerated areas. Nevertheless, although numerous studies have been conducted with the aim to divulge the main causes of oak dieback in this region, it still remains the open question within scientific community (Čater and Batič 2006, Dubravac et al. 2011, Stojanović et al. 2013). Finally, it should be highlighted that from the perspective of climate change scenarios for the region of Serbia, pedunculate oak will be the most endangered tree species due to the extreme changes of its habitat (Stojanović et al. 2014).

The studies of the daily patterns of gas exchange activity can provide fundamental information about plant's ability to maintain the photosynthetic apparatus and to respond readily to environmental factors (Geiger and Servaites 1994). In terms of woody plant species, particularly oaks, such studies were often carried out in the field conditions (Damesin and Rambal 1995, Valentini et al. 1995, Raftoyannis and Radoglou 2002). However, very little information on such parameters on oak seedlings growing in semi-controlled conditions (greenhouse) is available. Accordingly, the objective of the study was to examine to which extent, for period of one month, the soil water deficit will affect different physiological processes in pedunculate oak seedlings and to identify those traits that react most sensitively, and thus might be used as early signs of water stress. The results are evaluated in terms of photosynthesis and related character's performance during the diurnal time course, free proline content in leaves and relative growth rate in order to discuss the adaptive responses of this species to an experimental drought conditions. The experimental drought mimicked an extensive summer drought observed in the Balkan Peninsula region on that period (July, 2012). This study will thus allow a detailed insight into variation in some morphological and leaf-scale physiological parameters under extreme drought condition as it might be expected in the course of climate change.

Materials and Methods

Plant material and experimental design

Pedunculate oak (*Quercus robur* L.) seedlings used in the experiment were produced from acorn collected from

natural oak population located in Morović, Serbia (44° 58' 30", 19° 21' 34", altitude: 78 m a.s.l.), during the autumn 2011. Thereafter, the seed was placed in the air chamber for germination (on a 1:1 (v/v) mixture of peat and sand, at a temperature of 25 °C) in order to get seedlings.

The experiment was set at the beginning of growing season 2012 when young pedunculate oak seedlings, approximately 5 cm in height, were re-planted in 5-liter Mitscherlich pots filled with the soil substrate that belongs to the type of undeveloped alluvial soil (fluvisol), sandy loam form, with the dominant fraction of total sand (95.96%) and total clay fraction in a lower concentration (4.04%).

The total number of seedlings was 48 and they were planted randomly in 16 Mitscherlich pots (three seedlings per pot). The plants were grown in a naturally illuminated greenhouse at the Department of Biology and Ecology, University of Novi Sad, under the semi-controlled environment with a day temperature range of 22-35 °C and a night temperature range of 15-23 °C, and the relative humidity range of 55-85% between 19 April and 19 July 2012, by soil culture method.

The seedlings in the pots were divided into two treatments: (1) well-watered plants (control treatment) and (2) drought-stressed plants (stress treatment) for 30 days (from 19 June to 19 July 2012). Seedlings in the control treatment were kept well-watered by maintaining soil in the pots between 70 and 90% of field capacity (FC). To keep soil water content close to the target values, pots were weighed each day and watered as required. Drought stress was induced by withholding water until soil water content fell to 5% of FC, 30 days after the treatment has started.

Gas exchange measurements

Rates of net photosynthesis (A [$\mu\text{mol m}^{-2} \text{s}^{-1}$]), transpiration (E [$\text{mmol m}^{-2} \text{s}^{-1}$]), stomatal conductance (g_s [$\text{mol m}^{-2} \text{s}^{-1}$]) and intercellular CO_2 concentration (C_i [$\mu\text{mol mol}^{-1}$]) were recorded using LCpro+ Portable Photosynthesis System, manufactured by ADC BioScientific Ltd. A temperature sensor in the leaf chamber of the device was used to measure temperature of leaves (T_l) in °C. The measurements were carried out from 6:00 a.m. to 8:00 p.m., with 15 replicates taken each hour. Light conditions inside chamber were set using the LCpro+ light unit, which emitted photosynthetically active radiation (PAR) at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. The air supply unit provided a flow of ambient air to the leaf chamber at a constant rate of $100 \mu\text{mol s}^{-1}$. Humidity was set at 10 mBar of partial water pressure. Temperature and CO_2 concentration were at ambient levels (Topić et al. 2012). Measurements were conducted on five plants from each treatment, selected from different pots, using single mid-stem leaves, in three replications.

Parameters derived from gas exchange measurements

Instantaneous water use efficiency (WUE [$\mu\text{mol mmol}^{-1}$]) was computed as the ratio of net photosynthesis to tran-

spiration (A/E) (Farquhar et al. 1989). Intrinsic water use efficiency (WUE_i [$\mu\text{mol mol}^{-1}$]) was calculated as the ratio between net photosynthesis and stomatal conductance (A/g_s) (Farquhar et al. 1989).

Free proline content

Free proline content was determined using the Bates method (Bates et al. 1973). Plant material (1g), which consisted of young leaves, was ground with 10 ml of 3% sulfosalicylic acid. The homogenate was filtered and 2 ml of glacial acetic acid and 2 ml acid ninhydrin reagent were added to 2 ml of filtrate. Then the mixture was shaken by hand and incubated in boiling water bath for 15 minutes. After that, it was transferred to ice bath and returned to room temperature. Three ml toluene was added to the mixture and the upper toluene layer was measured at 520 nm using UV spectrophotometer. Measurement was made at the end of the experiment on fully expanded leaves and was conducted in three replications on ten plants from each treatment. Contents of proline were expressed as $\mu\text{g g}^{-1}$ fresh weight.

Growth measurements

Plant height was measured with a ruler on June 19 and July 19, respectively, and the relative growth rate was calculated using the formula:

Relative growth rate = $(\ln H_2 - \ln H_1) / (t_2 - t_1)$,
where H_2 , H_1 are the height growth variables at the beginning and the end of the time interval, and $t_2 - t_1$ is the time interval of interest (Chen et al. 1997).

Statistical analysis

All the analyses were performed in Statistica 10 software (StatSoft, Inc. 2011). The data were subjected to the Nested analysis of variance (ANOVA) with the "daytime" set as a fixed factor and factor "treatment" nested within "daytime". Significant differences between treatment means were determined at $p \leq 0.05$. Pearson's correlation analysis was performed using mean values of hourly measurements in order to examine the relationship between traits studied.

Results

The Nested ANOVA procedure showed that differences between daytime measurements as well as differences between control and drought treatments within particular daytime observations were highly significant ($p < 0.001$) for all analyzed traits (Table 1).

Free proline content and relative growth rate

The levels of free proline were significantly increased by drought (Table 2), which was expected and previously confirmed in many studies. Under conditions of optimal soil water content, control plants maintained significantly higher relative height growth rates than stressed plants (Table 3).

Table 1. Results of Nested ANOVA procedure performed individually for each trait

| Effects | df | A | E | g_s | C_i | WUE | WUEi |
|---------------------|----|----------|----------|----------|----------|----------|----------|
| Daytime | 4 | 112.4*** | 265.4*** | 63.5*** | 118.7*** | 341.3*** | 151.6*** |
| Treatment (daytime) | 5 | 927.9*** | 891.4*** | 292.7*** | 274.4*** | 155.6*** | 76.8*** |

*** $p < 0.001$ **Table 2.** Effect of drought stress on leaf proline content ($\mu\text{g g}^{-1}$ FW) of pedunculate oak seedlings

| Treatment | Free prolin content ($\mu\text{g g}^{-1}$ FW) | | |
|-----------|--|--------|-------|
| | Mean | CV (%) | SD |
| Control | 33.98 | 19.74 | 6.71 |
| Drought | 92.58 | 13.99 | 12.95 |
| F value | $F=48.44^{**}$ $p=0.002$ | | |

Table 3. Effect of drought stress on relative height growth rates ($\text{cm cm}^{-1} \text{day}^{-1}$) of pedunculate oak seedlings

| Treatment | Relative height growth rates ($\text{cm cm}^{-1} \text{day}^{-1}$) | | |
|-----------|--|--------|---------|
| | Mean | CV (%) | SD |
| Control | 0.01324 | 75.72 | 0.01003 |
| Drought | 0.00027 | 172.29 | 0.00046 |
| F value | $F=11.59^{**}$ $p=0.005$ | | |

The rate of photosynthesis

In both groups of plants, the maximum values of A were observed in the morning hours and thereafter they decreased (Figure 1A). A typical double-peaked pattern with a midday (2 p.m.) depression in the diurnal change of A was exhibited by well-watered plants. In drought stressed plants, diurnal course of A showed an atypical double peaked pattern, clearly different from that of well-watered plants, with midday inhibition occurred earlier, at 10 a.m.

The rate of transpiration

Daily dynamics of E in examined groups of plants showed different changing trends (Figure 1B). In control group (well-watered plants), the pattern was bimodal, and the first peak occurred at 10 a.m., while the second one appeared at 3 p.m. The highest values of E were reached between 2 p.m. and 5 p.m., coinciding with the highest values of Tl, after which E decreased. On the other hand, diurnal variation of E in drought stressed plants was more stable throughout the day, presented a unimodal pattern where the peak value was reached at noon.

Stomatal conductance

Diurnal variation of g_s in examined groups of plants showed different changing trends also (Figure 1C). In control plants, g_s was low in the early morning, increasing with time and reaching a maximum ($0.8 \text{ mol m}^{-2} \text{ s}^{-1}$) at 11 a.m. Thereafter, g_s decreased, and its midday minimum (at 2 p.m.) was 50% of the morning maximum. The second peak for g_s appeared at 3 p.m., although its value ($0.5 \text{ mol m}^{-2} \text{ s}^{-1}$) was significantly lower than the first peak. After this time, g_s decreased gradually without any substantial rise for the rest of the day. In drought stressed leaves, g_s remained low

throughout the day, with slight decrease at 10 p.m., and increase at noon.

Intercellular CO_2 concentration

C_i was higher in drought stressed than in well-watered plants during the whole day of measurement (Figure 1D). In both treatments, recorded C_i values showed opposite daily dynamics to A. Similar behaviour was also observed between C_i and g_s in stress treatment. Further, in both groups of plants, the lowest photosynthetic rates were accompanied by the highest values of intercellular CO_2 concentration.

Instantaneous water use efficiency (WUE)

The maximal values of WUE were observed in the early morning, and for well-watered and drought stressed plants they were $11.0 \mu\text{mol mmol}^{-1}$ and $8.2 \mu\text{mol mmol}^{-1}$, respectively (Figure 1E). Greater WUE in the morning than in the rest of the day was related to larger increase of A than E. Beyond 7 a.m. in control treatment, and 6 a.m. in stress treatment, WUE decreased gradually due to the higher temperatures which increased evaporation needs. Furthermore, WUE declines sharply since net photosynthetic rates were reduced nearly to zero, at 10 a.m., and from 3 p.m. to the rest of the day, in drought stressed plants.

Intrinsic water use efficiency (WUEi)

During the early morning (until 9 a.m.), the values of WUEi were higher in stress treatment compared to the control, pointing out on minored loss of water per fixed CO_2 in stressed plants (Figure 1F). Thereafter, WUEi pattern in drought stressed plants was very similar to the daily dynamics of A. On the other hand, the diurnal change in WUEi in well-watered plants exhibited a "high-low-high" pattern. Namely, WUEi decreased towards midday and then slightly increased and remained relatively constant until 5 p.m. after which it increased.

Leaf temperature

In both treatments, Tl showed bimodal diurnal pattern (Figure 1G). Tl was low in the early morning, increasing with time and reaching a first peak (38°C) at 10 a.m. Thereafter, Tl decreased at noon. The second peak for Tl appeared at 3 p.m., and its value (39°C) was significantly higher than the first peak. Thus, diurnal changes in Tl showed similar daily dynamics to that of E recorded on control treatment.

Pearson's correlation

In both treatments, photosynthesis presented a significant positive correlation with the g_s and a negative correlation with C_i , which was significant only in stress treatment (Figures 2A, B). A decrease in g_s in stress treatment, affected C_i , which was demonstrated by significant negative correlation between the two parameters (Figure 2C). Transpiration rate showed significant positive correlation with the Tl in both

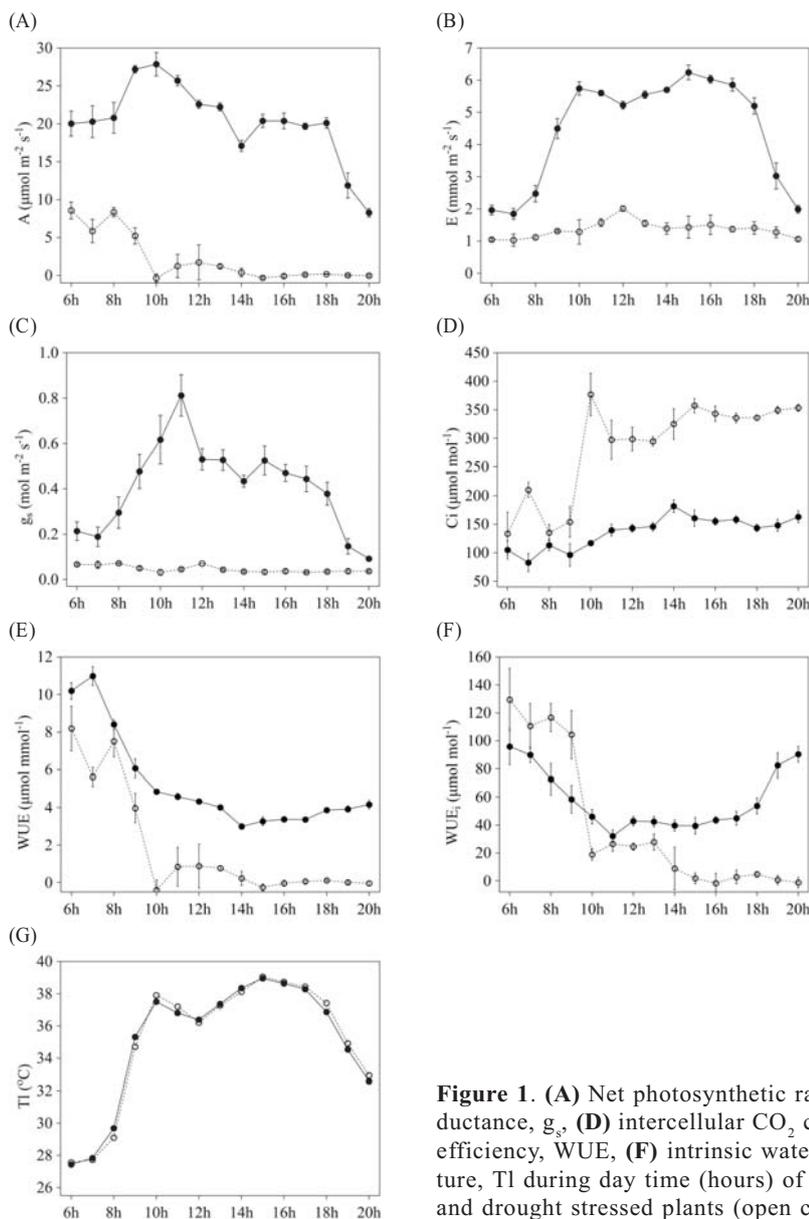


Figure 1. (A) Net photosynthetic rate, A, (B) transpiration, E, (C) stomatal conductance, g_s , (D) intercellular CO_2 concentration, C_i , (E) instantaneous water-use efficiency, WUE, (F) intrinsic water use efficiency, WUE_i and (G) leaf temperature, T_l during day time (hours) of well-watered plants (filled circles, solid line) and drought stressed plants (open circles, dash line). Bars represent means \pm SD

treatments, and with g_s in well-watered plants (Figures 2D, E). Stomatal conductance (g_s) presented a significant negative correlation with the T_l in drought stressed plants (Figure 2F). The instantaneous water use efficiency (WUE) had a strong negative correlation with T_l and E in both treatments (Figures 2G, H) and a positive with photosynthesis, which was significant only in stress treatment (Figure 2I).

Discussion

In the territory of Serbia, 2012, ranked as the second warmest year since the records began in 1951, with a mean maximum air temperature of 17.7 °C. The summer of 2012 was the warmest summer on record at 19 out of the 28 main mete-

orological stations in Serbia. In Novi Sad, the mean maximum air temperature in July was 25.2 °C, while the monthly rainfall was 47.7 mm (<http://www.hidmet.gov.rs/podaci/meteorologija/eng/2012.pdf>, Republic Hydro meteorological Service of Serbia). Considering all above, this study can help in answering how seedlings of *Q. robur* response to extreme water deficit carried out in experimental conditions that mimicked drought stress which is expected to be more frequent event in the following years.

The results revealed that drought significantly affects relative growth rate in pedunculate oak seedlings. It was expected due to the reduction in shoot height has been described as one of the early avoidance mechanisms in plants exposed to drought stress (Lei et al. 2006).

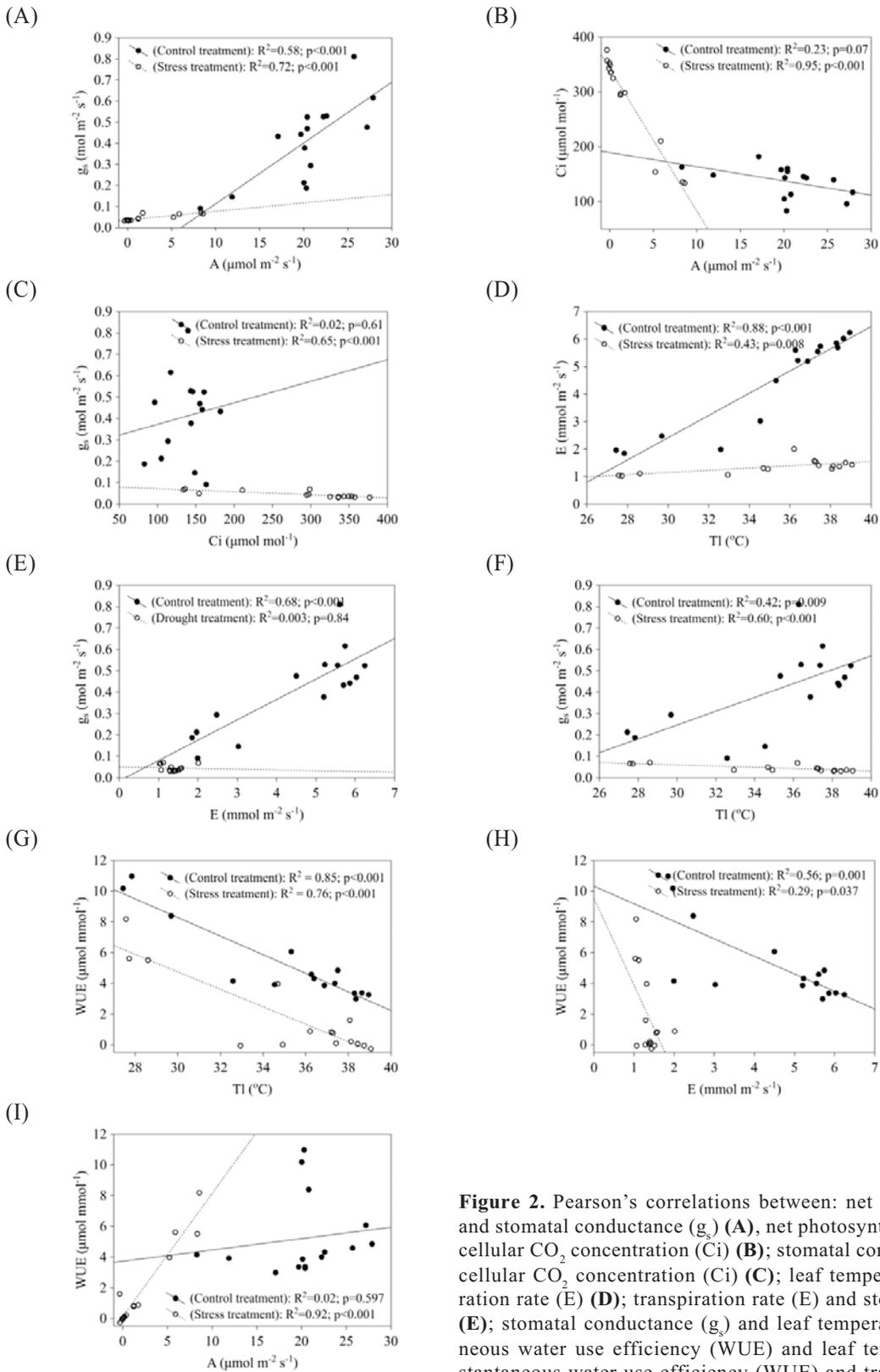


Figure 2. Pearson's correlations between: net photosynthetic rate (A) and stomatal conductance (g_s) (A); net photosynthetic rate (A) and intercellular CO₂ concentration (Ci) (B); stomatal conductance (g_s) and intercellular CO₂ concentration (Ci) (C); leaf temperature (TI) and transpiration rate (E) (D); transpiration rate (E) and stomatal conductance (g_s) (E); stomatal conductance (g_s) and leaf temperature (TI) (F); instantaneous water use efficiency (WUE) and leaf temperature (TI) (G); instantaneous water use efficiency (WUE) and transpiration rate (E) (H) and instantaneous water use efficiency (WUE) and net photosynthetic rate (A) (I), in well-watered plants (filled circles, solid line) and drought stressed plants (open circles, dash line)

Further, in response to drought conditions, the active accumulation of solutes, such as proline, allows plants to maintain positive turgor pressure, a requirement for maintaining stomata aperture and gas exchange (White et al. 2000). Proline is very often considered to be a compound used as general indicator of stress at cellular level (Zarafshar et al. 2014). In this study, the concentration of free proline was significantly increased in the plants grown under the drought stress conditions compared to well-watered controls. Similarly, studies by Topić (2015) and Stojnić et al. (2016) showed significant reduction of free proline content in different oak species subjected to water deficit. According to many authors, it has been interpreted as a mechanism to lower the osmotic potential and contribute to osmotic adjustment (Lei et al. 2006, Yang and Miao 2010).

Modifications of the diurnal patterns of photosynthesis and related characters may be considered as a part of the whole plant acclimation process to water deficit (Vadell et al. 1995). Additionally, maintenance of the capacity for gas exchange under drought conditions may favour both survival and plant productivity (Ni and Pallardy 1991).

As it has been reported for temperate-zone plants (Pathre et al. 1998, Pallardy 2008), a double-peaked (bimodal) diurnal pattern of A in both treatments was observed, suggesting that midday inhibition of photosynthesis occurred in well-watered as well as in drought stressed plants. Furthermore, in both treatments the maximum values of A were observed in the morning hours. It corresponds to the results of Raftoyannis and Radoglou (2002) who reported that photosynthesis in sessile oak trees was greatest in the morning and did not recover following the midday depression during measurements in July, August and September.

Midday depression of photosynthesis can be partitioned between stomatal and non-stomatal effects (Pons and Welschen 2003). Several studies demonstrated that, for many C3 species, stomatal limitations account largely for a reduction of CO_2 assimilation during mild to moderate drought stress, whereas non-stomatal limitations may become dominant under severe drought (Tezara and Lawlor 1995, Flexas et al. 2004, 2006). In our study, a significant positive correlation between A and g_s was observed in both treatments, suggesting that stomatal closure was the main factor affecting CO_2 assimilation. Similar results were reported for other tree species as well (Tognetti et al. 1999, Romero and Botía 2006, Borišev et al. 2015). Likewise, a direction of C_i change is also very important criterion for analysis of the stomatal limitation of photosynthesis (Pessarakli 2001). Xu (1997) emphasized that a necessary criterion of predominantly stomatal limitation is a decreased C_i rather than a positive correlation between leaf photosynthetic rate and stomatal conductance, while a reliable criterion of predominantly non-stomatal limitation is an increased rather than a constant C_i .

The results of our study showed a significant negative correlation between A and C_i in stress treatment indicating

that reduction in A was not accompanied by a reduction in C_i . Similar results were reported by dos Santos et al. (2013) for *J. curcas* plants subjected to water stress. The same authors reported that a significant negative correlation between A and C_i suggests that the internal concentration of CO_2 was not limiting for photosynthesis. Further, our results showed that the C_i was higher in drought stressed than in well-watered plants during the whole day of measurement. According to Epron and Dreyer (1993), the maintenance of high C_i in oak trees submitted to a long period of drought under natural conditions leads to the assumption that they could suffer from non-stomatal inhibition of photosynthesis, as well. Likewise, in drought stressed plants, a decrease in g_s was associated with increases in C_i , which was demonstrated by a significant negative correlation between g_s and C_i , indicating that mesophyll factors may be involved in photosynthetic limitations (Pons and Welschen 2003).

In control treatment, recorded C_i values also showed opposite daily dynamics to A which was demonstrated by negative, but not significant, correlation between these two parameters. Therefore, we might assume that in mentioned treatment, midday reduction in A was partly accompanied by increased of C_i . However, strong relationship between A and g_s in control treatment suggests that stomatal closure was the driving factor that affects CO_2 assimilation in plants growing under optimal conditions. Considering all the facts, we might assume that in both treatments the largest part of the reduction in photosynthesis was caused by stomatal closure; however, this finding does not preclude the existence of alternative mechanisms that may also have affected photosynthesis, especially during severe drought conditions.

Diurnal variation of E in both treatments were consistent with the results reported by Infante et al. (2003) who evidenced that E in *Q. ilex* during the spring-summer period increased in the morning, decreased at midday and rose to a second peak in the afternoon, while during the summer, as drought was prolonged, the morning E peak disappeared and lower maximum was reached at midday. The midday depression of transpiration was determined for other tree species, as well (Schulze et al. 1972, Tenhunen et al. 1984, Küppers et al. 1986, Pathre et al. 1998). However, Robinson and Bower (1998) reported that under summer stress conditions, the tendency for E was to peak at 11 a.m. in banana leaves. Our findings are similar, to some extent, to the previously mentioned results since we observed a peak for E at noon in drought stressed oak leaves.

Besides water stress, E was also driven by other factors as well. We observed significant positive correlation between E and T_l in both treatments, which was expected as it is well known that T_l drives E (Archontoulis et al. 2006). In control treatment, transpiration was the greatest at the time when T_l was maximal (about 39°C). Similar results were reported by Álvarez et al. (2011) who found that transpiration was greater at the time of higher water demand (higher tem-

perature and illumination conditions) in *Callistemon* plants subjected to no water restriction. Furthermore, the negative correlation between g_s and TI, reported in drought stressed plants, indicates that lower stomatal conductance limits evaporative cooling, leading to higher leaf temperatures, particularly during periods of high irradiance (Matsumoto et al. 2000, Gomez et al. 2005). Likewise, a significant positive correlation between E and g_s , observed in well-watered plants, indicates that transpiration was greatly influenced by stomatal regulation (dos Santos et al. 2013). Similar effects have been also demonstrated in drought stressed plants grown in a greenhouse (Pompelli et al. 2010) and the field condition (Endres 2007, Borišev et al. 2015).

Permanent drought decreased significantly instantaneous WUE in the stress treatment compared to the control. This is in agreement with studies on other tree species and shrubs that reported decreases in WUE as water deficit intensified (Klein et al. 2001, Llorens et al. 2003, Liang et al. 2006). The diurnal dynamics of WUE followed the pattern similar to that of A, especially in drought stress treatment (significant positive correlation was observed between the two parameters in stress treatment). Similar findings have been reported by Liu et al. (2013) for *Robinia pseudoacacia* seedlings and Moriana et al. (2002) for olive trees. Further, according to Larcher (1995), sometimes when stomata close, WUE falls sharply since net photosynthetic rates are reduced drastically, but transpiration continues because of the high evaporative demand. This is probably in line with our observation for drought stressed plants, since we showed that WUE falls sharply when net photosynthetic rates were reduced nearly to zero, at 10 a.m., and from 3 p.m. to the rest of the day. Likewise, lower values of WUE that were found after early morning can be explained by the higher leaf temperatures (Archontoulis et al. 2006). In both treatments, WUE decreased with the increase in TI (observed negative and significant correlation between WUE and TI), which was consistent with the results obtained for *Q. ilex* during morning hours (10:30–12:30) (Gratani et al. 2000). Also, the same authors stated that a reduction of WUE during summer was due to the combined effects of decreased assimilation and increased water loss. In this study, E was negatively correlated with WUE in both treatments, and similar behavior has also been observed in other plant species, grown in semi-humid and semi-arid areas (Endres, 2007, dos Santos et al. 2012).

According to Álvarez et al. (2011), most woody species increase their WUEi as an additional drought acclimation mechanism. Our results showed that compared to the control treatment, the plants exposed to permanent water deficit increased WUEi in the early morning (until 10 a.m.). Previous studies also showed that plants in drought treatment had higher WUEi, which was caused by large decreases in g_s relative to A (Rouhi et al. 2007, Wu et al. 2008, Edwards et al. 2012). These results indicate that the loss of water from

transpiration during water deficit was a key regulator of drought response (Chaves 1991), suggesting also a predominant stomatal control over photosynthesis (Gulías et al. 2009). According to Hessini et al. (2008), an increase in WUEi found in the water-stressed plants, could contribute to the maintenance of leaf water status under drought conditions. Further, we evidenced that maximum values of WUEi in control treatment occurred in the early morning and late afternoon, which goes along with findings of Romero and Botía (2006), who evidence similar daily pattern of WUEi for almond trees under semiarid conditions during May (well-irrigated conditions).

Conclusions

The present paper has demonstrated that prolonged drought had significant effect on the diurnal dynamics of leaf gas exchange parameters in pedunculate oak leaves. Our measurements document significantly lower values in the majority of these parameters in drought stressed plants compared to well-watered ones. As pedunculate oak forests are considered to one of the most valuable forests in Serbia, monitoring of physiological processes under climate change, particularly under prolonged drought conditions, will contribute to better understanding of drought responses in one-year old seedlings. Our results confirmed the idea that proline is known to accumulate in large quantities in higher plants in response to environmental stresses, such as drought. Likewise, we have demonstrated that young seedlings of pedunculate oak were able to cope with experimental drought conditions predominantly by stomatal closure. However, in order to get more precise information about adaptive responses of pedunculate oak seedlings to extensive experimental drought it is necessary to analyze additional parameters, such as parameters of chlorophyll a fluorescence, to validate the stomatal or non-stomatal limitation of photosynthesis, as well as vulnerability to cavitation due to the fact that cavitation resistance is an important trait that defines drought tolerance.

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