

Species-specific physiological responses of four sympatric *Quercus* species to water deficit during seedling establishment

Jovana Devetaković^{1,*}, Ljubica Mijatović¹, Janko Ljubičić¹, Branko Kanjevac¹,
Ivona Kerkez Janković¹

¹Faculty of Forestry, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia

Abstract

DEVETAKOVIĆ, J., MIJATOVIĆ, L., LJUBIČIĆ, J., KANJEVAC, B., KERKEZ JANKOVIĆ, I., 2026. Species-specific physiological responses of four sympatric *Quercus* species to water deficit during seedling establishment. *Folia Oecologica*, 53 (3): 264–277.

Climate change projections for Southeast Europe indicate increasing temperatures and more frequent and severe summer droughts, raising concerns about the success of oak regeneration during early developmental stages. This study aimed to compare the drought responses of four sympatric oak species (*Quercus petraea*, *Q. cerris*, *Q. frainetto*, and *Q. pubescens*) during the early developmental stage after seedling establishment under severe water deficit. Seedlings were grown from acorns collected in Serbia and exposed to semi-controlled glasshouse conditions. Water deficit was induced rapidly by withholding irrigation for three weeks without a subsequent recovery period. Physiological responses were monitored through repeated measurements of stomatal conductance – g_{sw} and quantum efficiency in light – Φ_iPS2 , while seedling vitality was evaluated at the end of the experiment. Linear mixed-effects models were used to evaluate the effects of water deficit duration, oak species, treatment, and time of day. The results demonstrated clear interspecific differences in physiological responses to water deficit. Across species, reductions in g_{sw} generally preceded declines in Φ_iPS2 , indicating that stomatal regulation represented an earlier response to decreasing water availability than photochemical efficiency. *Q. cerris* exhibited the earliest physiological response and the greatest decline in vitality under the imposed experimental conditions. In contrast, *Q. frainetto* maintained higher Φ_iPS2 and delayed stress responses throughout the experiment. *Q. petraea* and *Q. pubescens* showed intermediate and similar physiological responses, maintaining relatively stable g_{sw} during the first two weeks before a marked decline. These findings improve our understanding of species-specific drought strategies during the critical establishment phase and provide insights relevant for predicting natural regeneration dynamics of southeastern European oaks under future climate scenarios.

Keywords

oak, drought, physiological performance, regeneration

Introduction

Oaks are widely distributed across the globe, comprising approximately 500 species. They represent one of the most important tree genera in Europe. The genus *Quercus* is complex due to high interspecies hybridization, wide geographical distribution, and great morphological variability, but clearly divided in two subgenera *Quercus* and

Cerris, owing to different evolutionary patterns (DENK et al., 2017). Species from these subgenera differ, not only in morphological traits of leaves, acorns, and cupules, but also in water stress tolerance (DELIGÖZ and BAYAR, 2017; KABAOĞLU et al., 2023), photosynthesis, and biochemical responses (KILLI et al., 2018; KEBERT et al., 2022; SOBRIÑO-PLATA et al., 2024). In Europe, approximately 20 native species are recognized, and many of them are foundational

*Corresponding author:

e-mail: jovana.devetakovic@sfb.bg.ac.rs

© 2026 Authors. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

species, ranking among the most important tree species for biodiversity (LÖF, 2024). Some of them, such as *Q. robur* and *Q. petraea*, are the most important tree species for high-quality timber production (SPIECKER, 2021; NICOLESCU et al., 2025). Others, such as *Q. pubescens*, *Q. cerris*, and *Q. frainetto*, are dominant components of thermophilous deciduous forests in Europe. These thermophilous forests are important mainly for their high biodiversity value and for providing non-timber forestry ecosystem services (EEA, 2006).

Local ecological conditions drive the distribution and mixture patterns of oak species, making them highly susceptible to alterations in air temperature, precipitation, and soil properties. *Quercus petraea* is a mesophilous species sensitive to intense drying trends (EATON et al., 2016), as its distribution is limited by increased drought compared to more xerophilous congeners such as *Q. pubescens*, *Q. cerris*, and *Q. frainetto*. Conversely, the thermophilous and xerophilous *Q. pubescens* occupies warmer, drier sites from coastal to inland continental regions, frequently co-occurring with *Q. frainetto* (GANATSAS and TSAKALDIMI, 2013; PASTA et al., 2016). *Quercus frainetto*, a deciduous meso-xerophilous species of central Balkan and Pannonian forests, occupies the ecotone separating mesophilic and thermophilic forest communities (FEKETE et al., 2016; GANATSAS et al., 2022). Although less drought-tolerant than specific Mediterranean oaks, it outperforms *Q. cerris* in water-stress tolerance (FOTELLI et al., 2000; CHATZIPHILIPPIDIS and SPYROGLOU, 2006), with projections indicating range expansion into Spain, France, and Northern Italy (BASELGA and ARAÚJO, 2009). Finally, *Q. cerris* is a xerophilous species adapted to Mediterranean and sub-Mediterranean climates (DE RIGO et al., 2016), characterized by a moderate drought tolerance that often exceeds that of com-

panion regional oaks (PRACIAK et al., 2013; SAVILL, 2019).

The sympatric distribution of thermophilic *Quercus* species occurs throughout the Mediterranean and sub-Mediterranean zones, with particular prominence on the Apennine and Balkan Peninsulas. On the Balkan Peninsula, the distributions of *Q. petraea*, *Q. pubescens*, *Q. cerris*, and *Q. frainetto* overlap horizontally and, in some cases, vertically (Fig. 1). Therefore, their site- and species-specific responses across different latitudinal and altitudinal microclimates are particularly important, especially within mixed oak forests that occur in hilly and mountainous areas (KANJEVAC et al., 2024).

Projections of regional climate models for South-east Europe indicate a trend of increasing temperatures and decreasing precipitation, with notable local variations (IVETIĆ and DEVETAKOVIĆ, 2016). Analyses suggest an accelerated temperature increase by the end of the 21st century, highlighting the urgent need for mitigation measures. Spatial patterns of warming, intensification of extreme precipitation events, and reduced summer rainfall indicate a shift toward subtropical climate conditions and increased climate-related risks (VUKOVIĆ et al., 2018) with a tendency to show longer and more severe dry periods (FILIPOVIĆ et al., 2025). The frequency and duration of droughts are increasing, particularly between June and September (ŽIVANOVIĆ, 2017). A decline in the distribution of key oak species – *Q. cerris*, *Q. robur*, and *Q. petraea* is also expected (STOJANOVIĆ et al., 2021). In areas where *Q. petraea*, *Q. cerris*, *Q. frainetto*, and *Q. pubescens* naturally coexist, and where a mosaic of forest stands with varying species compositions occurs, it is of crucial importance to thoroughly understand their responses to different abiotic factors, especially drought. This knowledge can be a valuable contribution to oak forest regeneration in the context of climate change. *Q.*

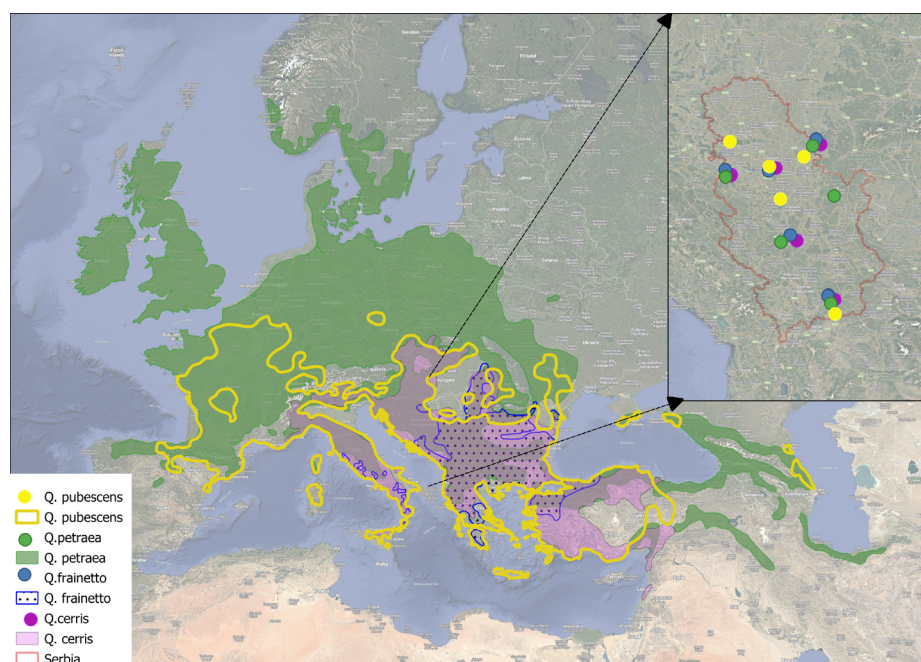


Fig. 1. Distribution map of *Q. petraea*, *Q. pubescens*, *Q. frainetto* and *Q. cerris* (according to EUFORGEN species distribution).

petraea is regarded as a species with moderate sensitivity to drought stress, attributed to its well-developed and deep root system. Owing to these traits, it is considered a potentially adaptable species for regions expected to experience altered precipitation patterns and increasing air temperatures (ČERNÝ et al., 2024). Considering that young seedlings lack a large and well-developed root system, their survival may be called into question if the species does not possess an effective self-protection strategy. Naturally regenerated oak seedlings in field conditions are capable of developing a strong and deep taproot during the first year of growth (TSAKALDIMI et al., 2009). However, the question remains as to how these seedlings survive summer drought prior to the establishment of such a root system. A study on *Q. petraea* conducted in the Czech Republic demonstrated that sprouts exhibit higher survival and growth rates than seedlings, owing to their developed, pre-existing root systems (PIETRAS et al., 2016; STOJANOVIĆ et al., 2017). This may represent a challenge in regions dominated by coppice forests, where forest management strategies are directed towards their conversion, as is the case on the Balkan Peninsula (ZENELI and KOLA, 2017).

The primary aim of this study was to compare the responses of *Q. petraea*, *Q. cerris*, *Q. frainetto*, and *Q. pubescens* to severe water deficit during early developmental stages after germination. This investigation was prompted by the current lack of clear evidence in the literature regarding interspecific differences at juvenile stages, which represent the period of greatest vulnerability and a critical window for future growth and development.

Materials and methods

Plant material

Seedlings were produced from acorns collected for each species separately in October and November 2024 at five locations in Serbia (Fig. 1). Seeds collection was performed in the natural forest of each species from the minimum of ten trees per population. The collected seeds were stored briefly in plastic zip bags in a refrigerator at 4 ± 2 °C for 2–7 weeks. Seeding was conducted on Novem-

ber 15, 2024, when the seeds were sown into containers (polyethylene Hiko V265 trays, consisting of 28 cells (each with a volume of 265 cm³), filled with substrate composed of peat and perlite (a volume ratio of 2:1)). Seeds from different provenances for each oak species were mixed during sowing, (14 seeds per 5 populations, and total of 70 seeds per species), so provenance was not considered as a determining factor in subsequent analyses. In one container, sowing was carried out so that each oak species was represented in seven cells (one row per container with an alternating sequence of species across rows within the container), and a total of 10 containers were sown. The sown containers were stored outdoors, where germination began at the end of April and continued for the next 4 weeks. Young seedlings were regularly watered and fertilized (two times per week with combined NPK fertilizer 16-69-16 GroGreen GL STARTER® Lima Europe NV) from mid-May to the end of June. At the end of June, a sample of eight containers with high germination percentage and well-developed (more than three leaves) healthy young seedlings were selected and placed in a glasshouse for the experiment, where it was possible to control irrigation and monitor environmental conditions. The first week in the glasshouse was considered an acclimatization period for the seedlings during which regular watering was provided. The experiment commenced on July 1st, after which four containers were not irrigated (water deficit treatment), while other containers served as the control, receiving regular irrigation at two- to three-day intervals. A total of 196 oak seedlings were included in the experiment, with a minor disproportion between species (*Q. petraea* – 50 plants, *Q. frainetto* – 49 plants, *Q. cerris* – 49 plants, and *Q. pubescens* – 48 plants) and treatment (112) and control (84) groups. The experiment was conducted in the nursery at Faculty of Forestry University of Belgrade, Serbia (44°46'58.2" N 20°25'31.3" E).

Site conditions and experimental design

During the experimental period air temperature and relative air humidity were measured using LOG210 data logger (DOSTMANN electronic GmbH) (Fig. 2). Volumetric moisture content into the substrate was followed using a

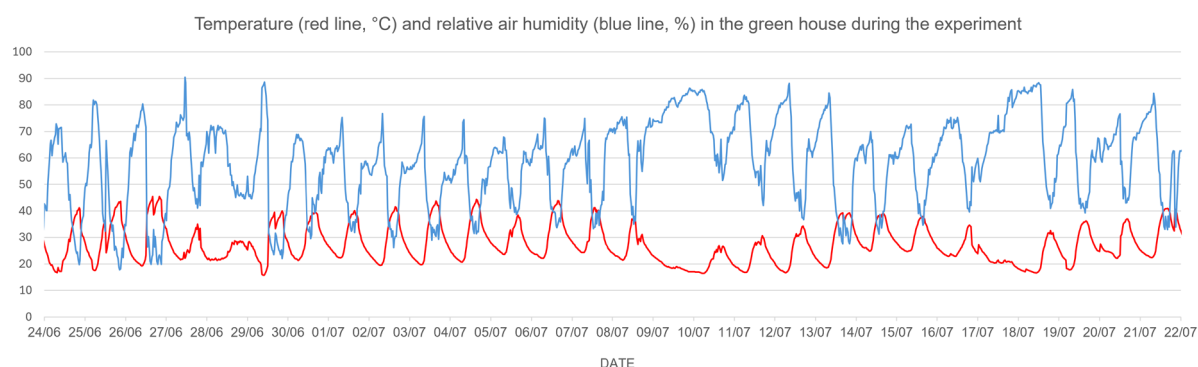


Fig. 2. Temperature (red line, °C) and relative air humidity (blue line, %) in the greenhouse during the experiment.

Wet 150 Kit (Delta-T Devices) in control and treatment containers during conducting each physiological measurement (Fig. 3).

At the beginning of the experiment, all seedlings were measured for ground level diameter (d – mm) and height (h – cm) using digital caliper and ruler, respectively. At the end of the experiment, the vitality of the seedlings was evaluated as follows: 0 – without leaves; 1 – dry yellow leaves; 2 – dry green leaves; 3 – most of the leaves are damaged or dry; 4 – healthy seedling with at most one damaged leaf; 5 – completely healthy and vigorous seedling.

Physiological measurements started on July 1st, and were repeated twice per week for the next three weeks. On each measurement day, the procedure was repeated four times: in the morning (between 8.00 and 9.00 h), at noon (between 11.00 and 12.00 h), in the afternoon- mid-day (between 15.00 and 16.00 h), and in the evening (between 18.00 and 19.00 h). Physiological measurements were always conducted on one leaf per seedling, which was well developed and appeared to be the healthiest at the time of measurement (often it was same leaves for

all measurements). Quantum efficiency in light (Φ_{PSII} ; Φ_{PSII}) and stomatal conductance (g_{sw}) were measured using LI-600 leaf porometer/fluorometer (LI-COR Biosciences, Lincoln, NE, USA) on one leaf per plant. The maximum quantum efficiency of photosystem II – PSII (Φ_{PSII}/Φ_{PSII}) was calculated using the formula $\Phi_{PSII} = (F_m' - F_s)/F_m'$ at the current light intensity, where F_m' is maximum fluorescence in light and F_s is minimum fluorescence in light. Calculation of stomatal conductance g_{sw} ($\text{mol m}^{-2} \text{s}^{-1}$) = $1/((1/g_{tw}) - (1/g_{blw}))$, where g_{tw} ($\text{mol m}^{-2} \text{s}^{-1}$) is total conductance to water vapor and g_{blw} ($\text{mol m}^{-2} \text{s}^{-1}$) is boundary layer conductance (data provided by LI-COR). The device was on auto mode setup with measurement settings recommended by the producer.

Seven measurements were conducted over a period of three weeks, when seedlings without leaves became evident in some cells of the unirrigated containers. A severe water deficit was recorded in the treatment group and evaluated using the relative change in substrate moisture of unirrigated seedlings over time in comparison to irrigated cells ($\% = ((\text{moisture of treatment} - \text{moisture of control}) / \text{moisture of control}) \times 100$), (Fig. 3).

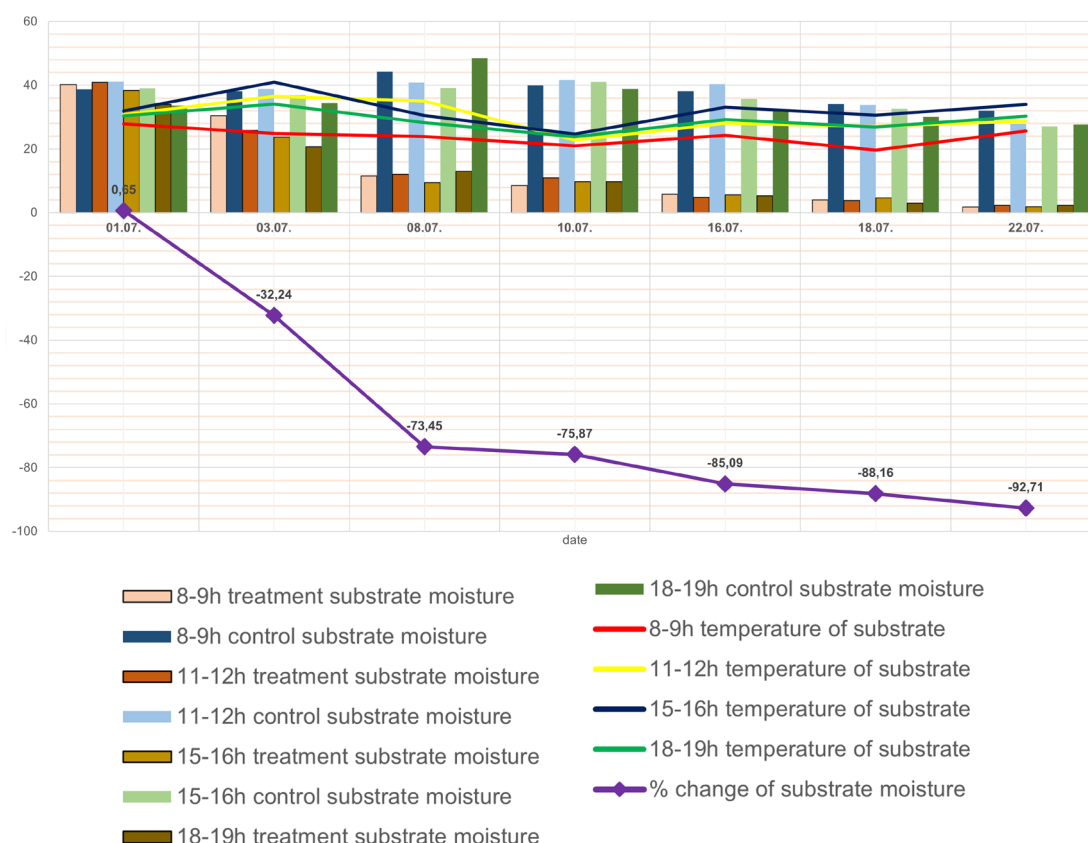


Fig. 3. Volumetric moisture content and temperature of the substrate – control and unirrigated cells, and substrate temperature during the experimental period, the lower panel shows the percentage change in substrate moisture content.

Statistical analysis

Statistical analyses were conducted in R version 4.6.0 (R CORE TEAM, 2026). The packages “dplyr” (WICKHAM et al., 2023a), “tidyr” (WICKHAM and VAUGHAN, 2023), “readr” (WICKHAM et al., 2023b), “stringr” (WICKHAM, 2019), and

“writexl” (WALKER, 2024) were used for data manipulation, descriptive statistics, and post-hoc analyses (Wilcoxon rank-sum tests and pairwise t-tests). Variable distributions were assessed using the Anderson–Darling normality test implemented in the “nortest” package (GROSS and LIGGES, 2015).

Linear mixed-effects models (LMMs) were fitted us-

ing the “lme4” package (BATES et al., 2015), with Satterthwaite approximations for degrees of freedom and p-values obtained via “lmerTest” (KUZNETSOVA et al., 2017). The model structure was:

$$\log(\text{response}) \sim \text{Date_num} * \text{Species} * \text{Type} + \text{Part_of_day} + (1|\text{Tree_ID}),$$

where Date_num represents the continuous temporal variable (day of month), Species is a four-level factor representing *Quercus* species, Type is a two-level treatment factor (Control vs. Water Deficit), Part_of_day is a four-level covariate representing measurement time, and Tree_ID was included as a random intercept to account for repeated measurements of individual seedlings.

The response variables (g_{sw} and PhiPS2) were log-transformed prior to analysis to normalize the right-skewed distributions. Marginal and conditional R^2 values (R_m^2 , R_c^2) were calculated following NAKAGAWA and SCHIELZETH (2013) using the “MuMIn” package BARTON, 2023). Compact letter displays were generated us-

ing the “multcompView” package (GRAVES et al., 2019). Figures were produced using the “ggplot2” package (WICKHAM, 2016).

Results

Experimental conditions

During the experimental period daily patterns of temperature were similar in both treatments, with small and consistent differences between treatment and control across times of day. As expected, mid-day was the hottest part of the day with an average temperature of $\approx 33^\circ\text{C}$, while morning was the coolest ($\approx 23.5^\circ\text{C}$), (Fig. 3). Volumetric moisture content expressed a huge difference between treatment and control, with a sharp drop throughout the experimental period (Fig 4). Air temperature differed significantly among morning, noon, midday, and evening

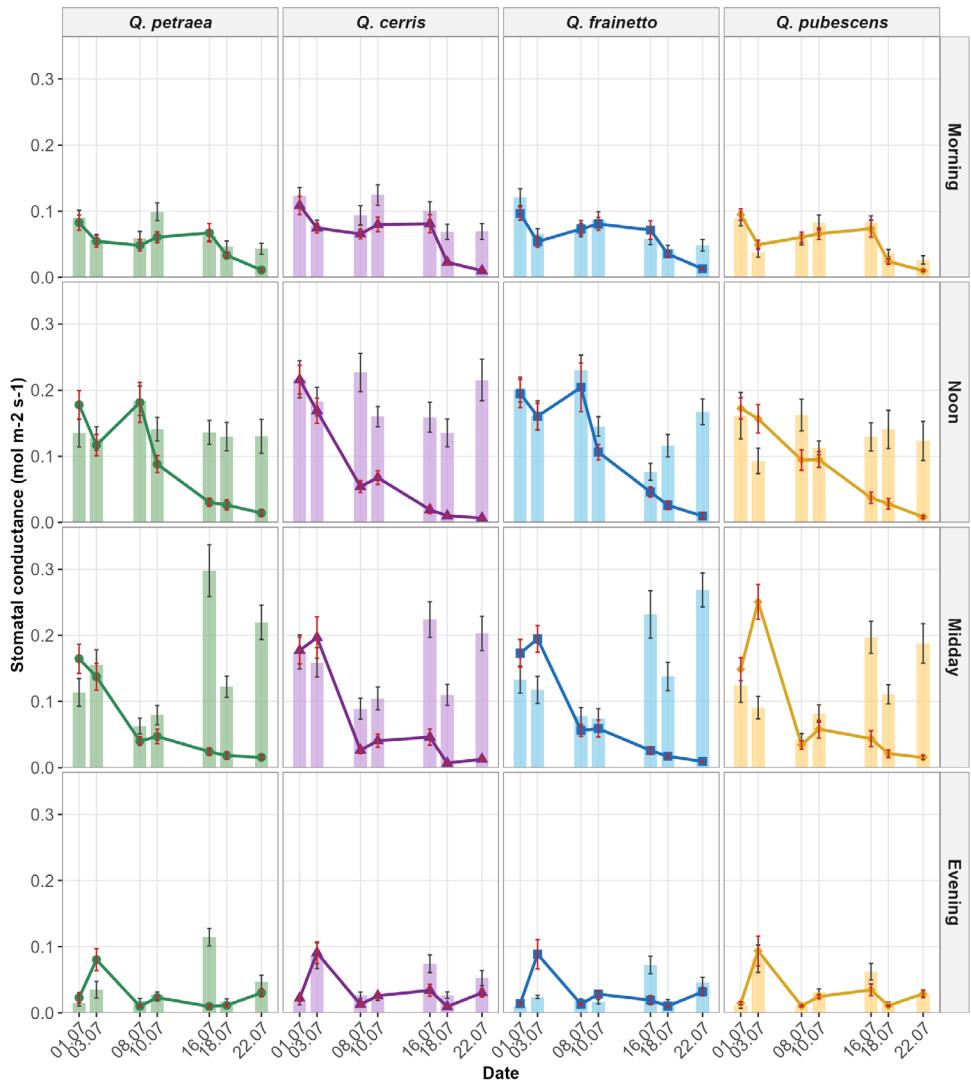


Fig. 4. Stomatal conductance (g_{sw} – mean values $\pm$ SE) for *Q. cerris*, *Q. frainetto*, *Q. petraea* and *Q. pubescens* seedlings from control (bars) and treatment (lines) in the morning, noon, midday and evening (from top to bottom, respectively) on different dates.

Table 1. Ground-level diameter (mm), height (cm) and vitality of oak seedlings by species and treatment (Wilco.= Wilcoxon)

Species	Treatment	Diameter mean±SD	Diameter <i>p</i> value	Height mean±SD	Height <i>p</i> value	Vitality mean±SD	Vitality range
<i>Q. cerris</i>	Control	2.84 ± 0.80	t-test 0.719	8.95 ± 2.98	Wilco. 0.599	4.95 ± 0.22	4–5
	Unirrigated	2.92 ± 0.64	–	9.88 ± 4.12	–	1.36 ± 0.49	1–2
<i>Q. petraea</i>	Control	2.28 ± 0.69	t-test 0.350	6.35 ± 2.18	t-test 0.444	5.00 ± 0.00	5–5
	Unirrigated	2.45 ± 0.56	–	6.84 ± 2.13	–	2.23 ± 0.97	0–4
<i>Q. pubescens</i>	Control	2.15 ± 0.72	Wilco. 0.942	7.12 ± 4.15	Wilco. 0.220	4.86 ± 0.65	2–5
	Unirrigated	2.16 ± 0.78	–	7.77 ± 3.53	–	2.74 ± 1.58	1–5
<i>Q. frainetto</i>	Control	2.45 ± 0.69	t-test 0.334	5.82 ± 1.41	Wilco. 0.627	4.86 ± 0.65	2–5
	Unirrigated	2.27 ± 0.58	–	5.90 ± 2.34	–	2.54 ± 1.04	1–5
Total		2.44 ± 0.72	–	7.37 ± 3.27	–	–	–

Notes: Ground-level diameter and height were measured at the start of the experiment (July 1st), before treatments diverged; vitality was assessed at the end on an ordinal 0–5 scale. Unirrigated denotes the water-deficit treatment.

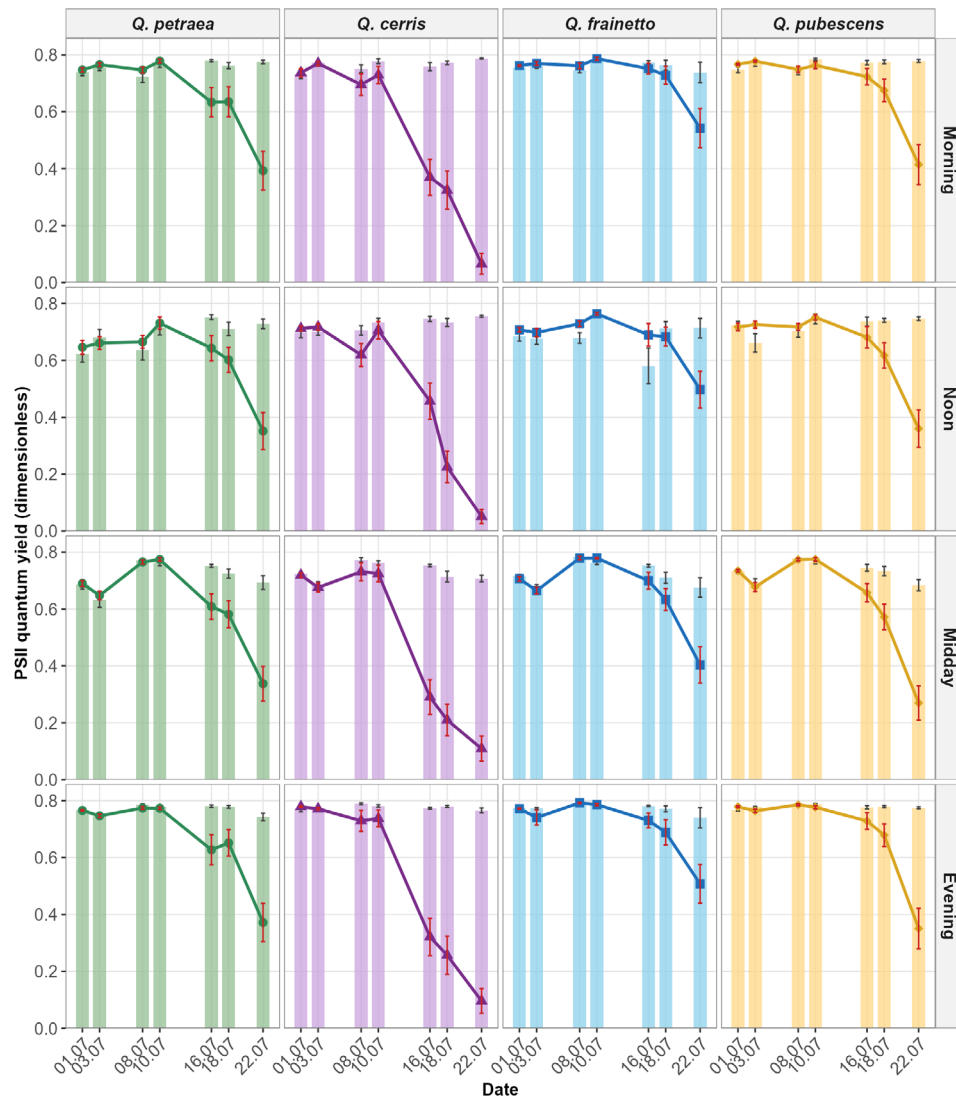


Fig. 5. Quantum efficiency in light (PhiPS2 – mean values reported with SE) for *Q. petraea*, *Q. cerris*, *Q. frainetto* and *Q. pubescens* seedlings from control (bars) and treatment (lines) in the morning, noon, midday and evening (from top to bottom, respectively) on different dates.

($F = 16.03$, $p < 0.001$), but not between treatment and control ($F = 0.366$, $p = 0.778$). Volumetric moisture content in substrate was not affected by the time of day ($F = 16.03$, $p < 0.001$), but was strongly significant by treatment ($F = 151.115$, $p < 0.001$), (Table S1).

Morphological plant characteristics

The mean seedling ground level diameter was 2.44 ± 0.72 mm, while the mean seedling height was 7.37 ± 3.27 cm (Table 1). Among all four species, *Q. cerris* had the tallest seedlings with the largest stem diameters in both the control and treatment groups prior to treatment. Other species showed smaller values for both morphological parameters. No statistically significant initial morphological differences were detected between control and water-deficit treatment seedlings within species.

At the end of the experiment, seedlings were evaluated for vitality and ranged from 0–5, with the highest mean values for *Q. petraea* and *Q. cerris* in the control group (5.00 ± 0.00 and 4.95 ± 0.22 , respectively), while the lowest grade was observed in the *Q. cerris* treatment group (1.36 ± 0.49). Seedlings from the control were mostly evaluated as healthy and vigorous seedlings, while seedlings from the treatment were evaluated as seedlings with mostly dry and damaged leaves (Table 1).

Physiological plant characteristics of irrigated (control) seedlings

Daily oscillations of g_{sw} followed a similar pattern across all oak species (Fig. 4), with values increasing from morning to noon and declining again in the evening. Morning and evening g_{sw} values were generally below $0.150 \text{ mol m}^{-2} \text{ s}^{-1}$, while noon values mostly ranged between 0.1 and $0.2 \text{ mol m}^{-2} \text{ s}^{-1}$. The highest g_{sw} was recorded in *Q. cerris* and *Q. frainetto*, with a maximum midday value of $0.298 \text{ mol m}^{-2} \text{ s}^{-1}$ observed in *Q. petraea* seedlings during the third week of the experiment. Midday g_{sw} was lower during the second week, coinciding with lower air temperatures and higher relative humidity.

PhiPS2 levels also showed consistent diurnal oscillations, with the highest values recorded in the evening (Fig. 5). Morning values ranged from 0.723 in *Q. petraea* to 0.787 in *Q. cerris*, decreased at noon, and partially recovered during midday and evening measurements. Overall, PhiPS2 levels remained relatively stable in control seedlings throughout the experimental period.

Physiological plant characteristics of unirrigated (water deficit treatment) seedlings

The effects of water deficit on g_{sw} and PhiPS2 became distinct after the first week of treatment, with reductions being less immediate and less pronounced in PhiPS2 than in g_{sw} .

During the second week, *Q. cerris* exhibited a rapid decline in g_{sw} ($< 0.1 \text{ mol m}^{-2} \text{ s}^{-1}$), which remained low thereafter. Conversely, *Q. frainetto*, *Q. petraea*, and *Q.*

pubescens showed only slight reductions during this period, maintaining midday values between 0.1 and $0.2 \text{ mol m}^{-2} \text{ s}^{-1}$, comparable to that of the control. However, by the third week, midday g_{sw} in these three species dropped below $0.1 \text{ mol m}^{-2} \text{ s}^{-1}$, leading to a clear divergence from that of the control (Fig. 4). Relative changes indicated that the strongest reductions occurred at noon and midday, whereas differences in the morning and evening were less pronounced. Notably, *Q. frainetto* and *Q. pubescens* exhibited transient positive responses in evening and midday measurements at the onset of the experiment before progressive stomatal closure occurred.

Progressive reductions in PhiPS2 emerged after two weeks of treatment, which were characterized by strong species-specific dynamics. *Q. cerris* showed the most severe decline, with daytime PhiPS2 falling below 0.455 and reaching an approximate 90% reduction relative to the control by the end of the experiment (Fig. 6). *Q. petraea* experienced moderate reductions (0.581 – 0.652) before dropping below 0.400 in the final period (Fig. 5). *Q. pubescens* maintained higher values (>0.700) until day 17, after which the values declined below 0.400 across most daytime periods. In contrast, *Q. frainetto* demonstrated the highest short-term stability, maintaining elevated PhiPS2 for longer than the other species and occasionally exhibiting higher PhiPS2 values than control seedlings. Overall, reductions in PhiPS2 were less immediate and less pronounced than reductions in g_{sw} , suggesting that stomatal regulation responded earlier to drought stress than to photochemical efficiency.

Linear mixed-effects models revealed that both g_{sw} and PhiPS2 were strongly influenced by the experimental duration and treatment (Table 2). For g_{sw} , the most important fixed effects were measurement date ($F = 518.84$, $p < 0.001$), part of the day ($F = 338.41$, $p < 0.001$), and the interaction between the date and treatment ($F = 571.65$, $p < 0.001$). These factors explained 8.46%, 11.98%, and 6.75% of the variance, respectively, indicating that g_{sw} was primarily driven by the temporal progression of drought stress and daily environmental fluctuations. The treatment effect was highly significant ($F = 26.33$, $p < 0.001$), whereas species differences were comparatively weak, although still significant ($p = 0.0205$). Non-significant interactions involving species indicated that all species exhibited a generally similar temporal pattern of stomatal responses to water deficit.

For PhiPS2, the strongest effects were also associated with measurement date ($F = 879.27$, $p < 0.001$) and the interaction between date and treatment ($F = 839.08$, $p < 0.001$), explaining 12.82% and 8.99% of the variance, respectively. In contrast to g_{sw} , significant interactions between date and species ($F = 71.84$, $p < 0.001$), and date, species, and treatment ($F = 85.80$, $p < 0.001$) demonstrated species-specific temporal responses of PhiPS2 efficiency under water deficit. Although the main effect of species was not significant, species differed in the dynamics and magnitude of PhiPS2 decline over time, consistent with the observed stronger sensitivity of *Q. cerris* and greater stability of *Q. frainetto*. The effect of part of the day on PhiPS2 was statistically significant but explained only a small proportion of variance (0.13%), suggesting lower diurnal sensitivity of

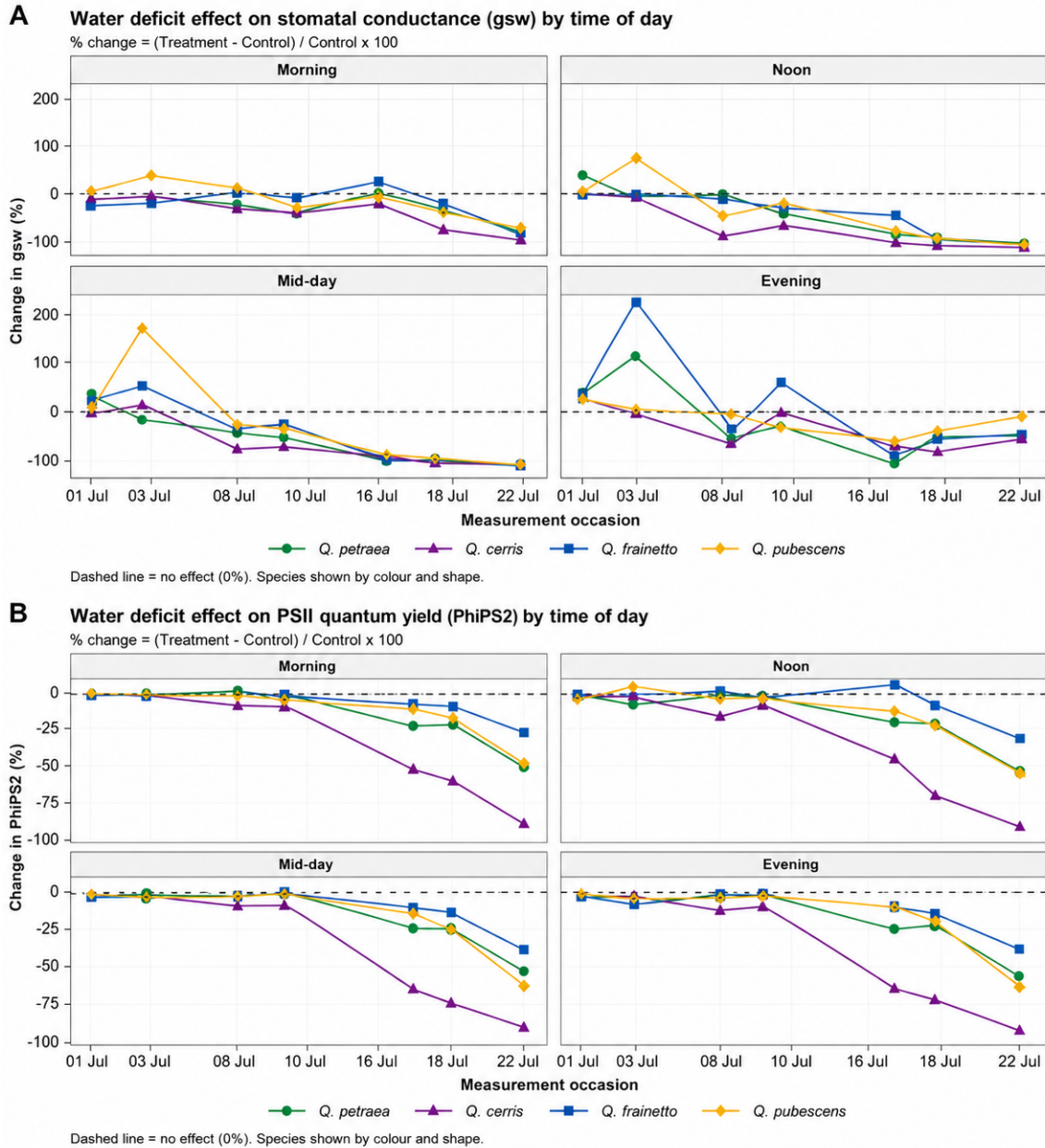


Fig. 6. Reduction in percent of g_{sw} (A) and PhiPS2 (B) for *Q. petraea*, *Q. cerris*, *Q. frainetto* and *Q. pubescens* seedlings in treatment over the period.

PSII efficiency compared with stomatal conductance.

Random effects associated with individual seedlings accounted for 6.16% of the variance in g_{sw} and 11.71% in PhiPS2, indicating greater between-seedling variability in photochemical responses than in stomatal regulation. The residual variance remained relatively high for both variables, particularly for g_{sw} , reflecting substantial unexplained physiological variability under fluctuating environmental conditions.

Model performance statistics indicated moderate explanatory power. Marginal R^2 values showed that fixed effects explained 32.9% of the variation in g_{sw} and 36.5% in PhiPS2, while conditional R^2 values increased to 38.8% and 47.0%, respectively, after the inclusion of random ef-

fects. Overall, the model performance indicated moderate explanatory power, with slightly better fit for PhiPS2 than for g_{sw} .

Discussion

Morphological attributes strongly influence hydraulic architecture and long-term drought survival, whereas seedling height is a general indicator of photosynthetic and transpiration capacity (GROSSNICKLE, 2012). These morphological constraints likely explain why *Q. cerris* seedlings in the present study suffered more severe damage than the other species. The larger initial size of *Q. cerris*

Table 2. Linear mixed model results for a) stomatal conductance (g_{sw}) and b) the effective quantum yield of PSII ($\Phi PSII$): effects of date, species, treatment and part of day, and model fit

a) Stomatal conductance (g_{sw})

Effect	Num DF	Den DF	F value	p value	Variance (%)
Date_num	1	5,292	518.84	<0.001	8.46
Species	3	521.1	3.29	0.021	0.06
Type	1	521.1	26.33	<0.001	1.88
Part_of_day	3	5,292	338.41	<0.001	11.98
Date_num:Species	3	5,292	2.01	0.110	0.05
Date_num:Type	1	5,292	571.65	<0.001	6.75
Species:Type	3	521.1	1.59	0.190	0.12
Date_num:Species:Type	3	5,292	0.20	0.895	0.01
Random: Tree_ID	—	—	—	—	6.16
Residual	—	—	—	—	64.52

Model fit: $R^2_{\text{marginal}} = 0.329$; $R^2_{\text{conditional}} = 0.388$; RMSE (log scale) = 1.197.

b) Effective quantum yield of PSII ($\Phi PSII$)

Effect	Num DF	Den DF	F value	p value	Variance (%)
Date_num	1	5,292	879.27	<0.001	12.82
Species	3	359.9	2.05	0.106	0.51
Type	1	359.9	45.97	<0.001	0.95
Part_of_day	3	5,292	4.19	0.006	0.13
Date_num:Species	3	5,292	71.84	<0.001	3.10
Date_num:Type	1	5,292	839.08	<0.001	8.99
Species:Type	3	359.9	2.41	0.066	0.45
Date_num:Species:Type	3	359.9	85.80	<0.001	2.76
Random: Tree_ID	—	—	—	—	11.71
Residual	—	—	—	—	58.57

Model fit: $R^2_{\text{marginal}} = 0.365$; $R^2_{\text{conditional}} = 0.470$; RMSE (log scale) = 1.130.

Notes: Fixed effects were tested with Satterthwaite-approximated degrees of freedom (*lmerTest*). p-values below 0.001 are reported as <0.001. Variance (%) is the proportion of total variance attributed to each term. Random and residual rows report variance components only. Type, treatment (Control vs. Water Deficit/Unirrigated); Part_of_day, time of measurement (four levels); Tree_ID, individual seedling (random intercept). R^2_{marginal} , variance explained by fixed effects; $R^2_{\text{conditional}}$, variance explained by fixed and random effects.

seedlings may have contributed to their faster depletion of available soil water because larger seedlings generally have greater transpiring leaf area and higher water demand. These morphological constraints likely explain why *Q. cerris* seedlings in the present study suffered more severe damage than the other species. Previous studies have shown that *Q. cerris* reduces growth under drought conditions (DELIGÖZ and BAYAR, 2018) but can recover morphological performance after rewatering (MONDANELLI et al. 2026). In this study, the recovery period was not included, and the seedlings were exposed to a strong water deficit for 21 days, with substrate moisture declining by more than 70% relative to the control after the first week of treatment (Fig. 3). Water deficit developed rapidly due to the small

container volumes, supporting observations that small pots are not recommended for drought studies (TURNER, 2018). While the ideal alignment of plant size and pot volume was satisfied as most important in this study (POORTER et al., 2012), this setup realistically simulated rapid summer droughts in Serbia, where intense summer dry spells frequently follow wet springs (FILIPOVIĆ et al., 2025). Therefore, the stronger physiological response observed in *Q. cerris* seedlings in this study may reflect both species-specific drought sensitivity and differences in plant size. The severe damage of *Q. cerris* seedlings in this study contrasts with the high field survival reported by MIJATOVIĆ et al. (2026). One possible explanation is root restriction. *Q. cerris* allocates resources to rapid shoot growth, requiring

an expansive root system. In small pots, its high shoot-to-root ratio and transpiration demand trigger rapid substrate dehydration and hydraulic failure. Conversely, in open forest soils, unrestricted root elongation allows *Q. cerris* to avoid upper-soil drought. Thus, while field conditions filter survival via microclimatic gradients (MIJATOVIĆ et al., 2026), our container setup selectively tested immediate tissue tolerance under acute, unavoidable water deficit. This controlled-to-field discrepancy aligns with LEVERKUS et al. (2026), who emphasized that specific habitat conditions act as severe filters shaping initial oak seedlings.

The diurnal patterns of g_{sw} and PhiPS2 observed in irrigated seedlings are consistent with the reported trends for various oak species, including *Q. pubescens* (DAMESIN and RAMBAL, 1995), *Q. ilex* (OGAYA and PEÑUELAS, 2003), *Q. robur*, and *Q. petraea* (GROSS et al., 1996). The rapid decline of g_{sw} within eight days of the onset of the experiment in our study closely mirrors the responses observed in two-year-old *Q. ilex* seedlings (FILELLA et al., 1998). A study conducted on *Q. robur*, *Q. petraea*, and *Q. pubescens* reported a pattern similar to that observed in this study, with a delayed and less pronounced response of PhiPS2 compared to g_{sw} and PhiPS2 values below 0.7 during drought (AREND et al., 2013). Stomatal regulation operates as the primary line of defense under early or moderate drought, whereas PhiPS2 remains relatively insensitive until stress intensifies (KALAJI et al., 2017). This high initial photochemical stability may also be attributed to the substantial plasticity of the oak photosynthetic apparatus under fluctuating temperatures (DAAS et al., 2008), which can explain the absence of significant temperature influence during the first two weeks of the experiment (Fig. 2). This temporal decoupling between rapid stomatal closure and delayed photochemical decline is further supported by MIJATOVIĆ et al. (2026). Their survival models proved that while both parameters predict first-year establishment, surviving oak seedlings consistently maintain a PhiPS2 above 0.65, whereas non-survivors exhibit a steep reduction under advanced atmospheric and soil water stress.

Drought responses in oaks are highly divergent and cannot be simply categorized along a strict isohydric-anisohydric spectrum (MARTÍNEZ-VILALTA and GARCIA-FORNER, 2016; KAPROTH et al., 2023). In this study, *Q. cerris* exhibited the earliest response, characterized by coordinated reductions in g_{sw} followed by a sharp drop in PhiPS2. *Q. cerris* showed a reaction similar to that of an isohydric plant, responding with decreasing stomatal conductance under conditions of low water availability, with expressed senescence and a strong tendency to abscise leaves as a mechanism for survival (MCDOWELL et al., 2008; WU et al., 2022). This contrasts with field studies on mature *Q. cerris* trees, which typically exhibit less conservative, anisohydric water-use strategies compared to *Q. pubescens* (TOGNETTI et al., 2007). This discrepancy suggests that the combination of seedling growth stage and the high evaporative demand of the glasshouse environment may amplify stress, forcing an anisohydric species into a conservative avoidance strategy.

In contrast to *Q. cerris*, *Q. frainetto* showed the slow-

est physiological response to water deficit among the studied species, maintaining relatively stable g_{sw} and PhiPS2 values during the first half of experiment period. Similar findings were reported by MANES et al., 2006, who described *Q. frainetto* as physiologically intermediate between *Q. cerris* and the evergreen Mediterranean species *Q. ilex*. Although *Q. ilex* is generally considered as anisohydric, previous studies also documented conservative stomatal sensitivity and osmotic adjustment under drought conditions (MEDIIVILLA and ESCUDERO, 2003; LIMOUSIN et al., 2022; MORENO et al., 2025), highlighting the complexity of drought-response strategies in oaks. Some studies identified *Q. frainetto* as relatively drought-sensitive compared with *Q. ilex*, *Q. pubescens*, *Q. macrolepis*, and *Q. ithaburensis* (FOTELLI et al., 2000; SIAM et al., 2009; BANTIS et al., 2019), which contrasts with the comparatively stable short-term physiological performance observed in this study.

Q. pubescens and *Q. petraea* displayed similar physiological responses to water deficit, with declines in stomatal conductance and PhiPS2 becoming pronounced mainly during the third week of the experiment. The relatively stable PhiPS2 values observed in *Q. pubescens* may be associated with photoprotective mechanisms previously described for this species, including reversible down-regulation of photochemistry and efficient dissipation of excess energy (GALLÉ et al., 2007). In addition, FOTELLI et al. (2000) reported that *Q. pubescens* seedlings can regulate stomatal conductance relatively independently of instantaneous plant water status, which may explain the irregular midday and noon fluctuations observed in this study (Fig. 4). Some deciduous Mediterranean oaks such as *Q. pubescens* and *Q. faginea* exhibit more conservative, water saving behavior consistent with isohydric like or drought avoiding strategies (ALONSO-FORN et al., 2021). Similar physiological behavior of *Q. pubescens* and *Q. petraea* may indicate shared drought-response strategies during early seedling establishment, when oak seedlings often exhibit convergent physiological adaptations under competitive and environmentally variable conditions (MEDIIVILLA and ESCUDERO, 2004).

Dendrochronological studies conducted on mature oak trees support several of our findings regarding interspecific responses. POPA and POPA (2026) identified *Q. frainetto* as one of the most stable oak species under drought conditions, whereas *Q. cerris* exhibited the lowest long-term resistance and the weakest recovery following intense summer droughts in Central and Eastern Europe. Conversely, when compared across a broader range of deciduous species in Central Europe, *Q. cerris* and *Q. pubescens* are still recognized as solid foundations for future forest stands, even under drier climatic conditions (MÓRICZ et al., 2025). Finally, spatial modeling of forest ecosystem vulnerability in Serbia predicts that thermophilous species like *Q. pubescens* will exhibit range expansion and higher stability, showing greater potential in higher-elevation habitats under projected warming. However, high-emission scenarios by the end of the 21st century likely portend an overall reduction in low-elevation reserves for these species, alongside a severe decline for *Q. petraea* (STOJANOVIĆ et al., 2026).

Conclusions

This research revealed distinct responses to substrate water deficit among four oak species during the early developmental stage, which represents a critical period for seedling establishment success. The experiment was conducted under semi-controlled glasshouse conditions, where substrate water deficit was induced rapidly and followed by no recovery period (re-watering). Therefore, the observed responses should primarily be interpreted as indicators of drought resistance rather than drought resilience.

Across species, changes in g_{sw} generally preceded declines in Φ_iPS2 , indicating that stomatal regulation represented an earlier physiological response to decreasing soil water availability than impairment of photosynthetic function. However, the timing of measurements during the day may also have influenced the magnitude of physiological responses due to daily variation in evaporative demand and stomatal behavior.

Among the studied species, under the experimental conditions, *Q. cerris* exhibited the earliest and most severe response to water deficit, characterized by rapid stomatal closure, subsequent reductions in photochemical efficiency, and a pronounced decline in seedling vitality. In contrast, *Q. frainetto* maintained higher Φ_iPS2 and delayed stress responses throughout the experimental period. *Q. petraea* and *Q. pubescens* exhibited intermediate and similar responses, maintaining relatively stable g_{sw} during the first two weeks before a significant decline, suggesting comparable Φ_iPS2 during early developmental stages after seedling establishment.

This study further demonstrates that drought responses observed at the seedling stage may differ substantially from patterns reported for mature trees. The observed interspecific differences indicate that drought response strategies during seedling establishment vary considerably among southeastern European oaks and should be considered when predicting regeneration under increasingly frequent summer droughts. Consequently, future research should evaluate oaks responses under repeated drought cycles, include post-drought recovery periods to assess resilience capacity, and compare responses across developmental stages.

Acknowledgement

This research was supported by the Science Fund of the Republic of Serbia under Grant No. 11096, “Physiological and morphological responses of oaks seedlings to drought stress - PRO_DROAKS”.

References

ALONSO-FORN, D., PEGUERO-PINA, J.J., FERRIO, J.P., MENCUCINI, M., MENDOZA-HERRER, Ó., SANCHO-KNAPIK, D., GIL-PELEGRÍN, E., 2021. Contrasting functional strategies following severe drought in two Mediterranean

oaks with different leaf habit: *Quercus faginea* and *Quercus ilex* subsp. *rotundifolia*. *Tree Physiology*, 41 (3): 371–387. <https://doi.org/10.1093/treephys/tpaa135>

AREND, M., BREM, A., KUSTER, T.M., GÜNTHARDT-GOERG, M. S., 2013. Seasonal photosynthetic responses of European oaks to drought and elevated daytime temperature. *Plant Biology*, 15: 169–176. <https://doi.org/10.1111/j.1438-8677.2012.00625.x>

BANTIS, F., RADOGLU, K., BRÜGGEMANN, W., 2019. Differential ecophysiological responses to seasonal drought of three co-existing oak species in northern Greece. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 153 (3): 378–384. <https://doi.org/10.1080/11263504.2018.1492990>

BARTON, K., 2023. *MuMIn: Multi-Model Inference*. R package version 1.48.19. [cit.2026-04-17]. <https://CRAN.R-project.org/package=MumIn>

BASELGA, A., ARAÚJO, M.B., 2009. Individualistic vs community modelling of species distributions under climate change. *Ecography*, 32 (1): 55–65. <https://doi.org/10.1111/j.1600-0587.2009.05856.x>

BATES, D., MAECHLER, M., BOLKER, B., WALKER, S., 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67 (1): 1–48. <https://doi.org/10.18637/jss.v067.i01>

ČERNÝ, J., ŠPULÁK, O., KOMÁNEK, M., ŽIŽKOVÁ, E., SÝKORA, P., 2024. Sessile oak (*Quercus petraea* [Matt.] Liebl.) and its adaptation strategies in the context of global climate change: a review. *Central European Forestry Journal*, 70 (2): 77–94. <https://doi.org/10.2478/forj-2024-0012>

CHATZIPHILIPPIDIS, G., SPYROGLOU, G., 2006. Modelling the growth of *Quercus frainetto* in Greece. In *Sustainable forest management: growth models for Europe*. Berlin, Heidelberg: Springer, p. 373–395. https://doi.org/10.1007/3-540-31304-4_21

DAAS, C., MONTPIED, P., HANCHI, B., DREYER, E., 2008. Responses of photosynthesis to high temperatures in oak saplings assessed by chlorophyll-a fluorescence: interspecific diversity and temperature-induced plasticity. *Annals of Forest Science*, 65 (3): 305. <https://doi.org/10.1051/forest:2008002>

DAMESIN, C., RAMBAL, S., 1995. Field study of leaf photosynthetic performance by a Mediterranean deciduous oak tree (*Quercus pubescens*) during a severe summer drought. *New Phytologist*, 131 (2): 159–167. <https://doi.org/10.1111/j.1469-8137.1995.tb05717.x>

DE RIGO, D., ENESCU, C.M., HOUSTON DURRANT, T., CAUDULLO, G., 2016. *Quercus cerris* in Europe: distribution, habitat, usage and threats. In SAN-MIGUEL-AYANZ, J., DE RIGO, D., CAUDULLO, G., HOUSTON DURRANT, T., MAURI, A. (eds). *European atlas of forest tree species*. Luxembourg: Publication Office of the European Communities, p. 148–149.

DELIGÖZ, A., BAYAR, E., 2017. Variations in physiological and biochemical traits of drought-stressed *Quercus cerris* seedlings. *Turkish Journal of Forestry*, 18 (4): 269–74. <https://doi.org/10.18182/tjf.318318>

DELIGÖZ, A., BAYAR, E., 2018. Drought stress responses of seedlings of two oak species (*Quercus cerris* and *Quercus robur*). *Turkish Journal of Agriculture and Forestry*, 42 (2): 114–123. <https://doi.org/10.3906/tar-1709-29>

DENK, T., GRIMM, G.W., MANOS, P.S., DENG, M., HIPPI, A.L., 2017. An updated infrageneric classification of the oaks: review of previous taxonomic schemes and synthesis

- of evolutionary patterns. In GIL-PELEGRÍN, E., PEGUERO-PINA, J., SANCHO-KNAPIK, D. (eds). *Oaks physiological ecology. Exploring the functional diversity of genus Quercus L.* Tree Physiology, vol 7. Cham: Springer, p. 13–38. https://doi.org/10.1007/978-3-319-69099-5_2
- EATON, E.G.S.D.J., CAUDULLO, G., OLIVEIRA, S., DE RIGO, D., 2016. *Quercus robur* and *Quercus petraea* in Europe: distribution, habitat, usage and threats. In SAN-MIGUEL-AYANZ, J., DE RIGO, D., CAUDULLO, G., HOUSTON DURANT, T., MAURI, A. (eds). *European atlas of forest tree species*. Luxembourg: Publication Office of the European Communities, p. 160–163.
- EUROPEAN ENVIRONMENT AGENCY, 2006. *EEA environmental statement 2006*. Luxembourg: Office for Official Publications of the European Communities.
- FEKETE, G., KIRÁLY, G., MOLNÁR, Zs., 2016. Delineation of the Pannonian vegetation region. *Community Ecology*, 17(1): 114–124. <https://doi.org/10.1556/168.2016.17.1.14>
- FILELLA, I., LLUSIA, J., PIÑOL, J., PEÑUELAS, J., 1998. Leaf gas exchange and fluorescence of *Phillyrea latifolia*, *Pistacia lentiscus* and *Quercus ilex* saplings in severe drought and high temperature conditions. *Environmental and Experimental Botany*, 39 (3): 213–220. [https://doi.org/10.1016/S0098-8472\(97\)00045-2](https://doi.org/10.1016/S0098-8472(97)00045-2)
- FILIPOVIĆ, L., PUTNIKVIĆ, S., STOSIC, B., STOSIC, T., DJURDEVIĆ, V., TOŠIĆ, I., 2025. Analysis of spatio-temporal characteristics of drought in Serbia from 1961 to 2020 using SPI and SPEI. *International Journal of Climatology*, 45 (7): e8803. <https://doi.org/10.1002/joc.8803>
- FOTELLI, M.N., RADOGLU, K.M., CONSTANTINIDOU, H.I., 2000. Water stress responses of seedlings of four Mediterranean oak species. *Tree Physiology*, 20 (16): 1065–1075. <https://doi.org/10.1093/treephys/20.16.1065>
- GALLÉ, A., HALDIMANN, P., FELLER, U., 2007. Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. *New Phytologist*, 174 (4): 799–810. <https://doi.org/10.1111/j.1469-8137.2007.02047.x>
- GANATSAS, P., TSAKALIMI, M., 2013. A comparative study of desiccation responses of seeds of three drought-resistant Mediterranean oaks. *Forest Ecology and Management*, 305: 189–194. <https://doi.org/10.1016/j.foreco.2013.05.042>
- GANATSAS, P., TSAKALIMI, M., KARYDOPOULOS, T., PETALOUDI, L.M., PAPAEMMANOUIL, A., PAPADOPOULOS, S., GEROCHESTOU, S., 2022. Carbon pools in a 77 year-old oak forest under conversion from coppice to high forest. *Sustainability*, 14(21): 13764. <https://doi.org/10.3390/su142113764>
- GRAVES, S., PIEPHO, H.-P., SELZER, L., DORAI-RAJ, S., 2019. *multcompView: visualizations of paired comparisons*. R package version 0.1.11. [cit. 2026-05-04]. <https://CRAN.R-project.org/package=multcompView>
- GROSS, K., HOMLICHER, A., WEINREICH, A., WAGNER, E., 1996. Effect of shade on stomatal conductance, net photosynthesis, photochemical efficiency and growth of oak saplings. *Annales des Sciences Forestières*, 53 (2-3): 279–290. [cit. 2026-08-02]. <https://hal.science/hal-00883051v1>
- GROSS, J., LIGGES, U., 2015. *nortest: Tests for Normality*. R package version 1.0-4. [cit. 2026-05-04]. <https://CRAN.R-project.org/package=nortest>
- GROSSNICKLE, S.C., 2012. Why seedlings survive: influence of plant attributes. *New Forests*, 43 (5): 711–738. <https://doi.org/10.1007/s11056-012-9336-6>
- IVETIĆ, V., DEVETAKOVIĆ, J., 2016. Reforestation challenges in Southeast Europe facing climate change. *Reforesta*, 1 (1): 178–220. <https://doi.org/10.21750/10.21750/REFOR.1.10.10>
- KABAOĞLU, A., KULAÇ, S., BAYSAL, İ., ÖZBAYRAM, A.K., AKBULUT, S., ÖZTÜRK, N., 2023. Some physiological and biochemical changes in oak trees after fire. *Cerne*, 29: e-103199. <https://doi.org/10.1590/01047760202329013199>
- KALAJI, H.M., SCHANSKER, G., BRESTIC, M., BUSSOTTI, F., CALATAYUD, A., FERRONI, L., GOLTSEV, V., GUIDI, L., JAJOO, A., LI, P., LOSCIALE, P., MISHRA, V.K., MISRA, A.N., NEBAUER, S.G., PANCALDI, S., PENELLA, C., POLLASTRINI, M., SURESH, K., TAMBUSSI, E., YANNICCARI, M., ZIVCAK, M., CETNER, M.D., SAMBORSKA, I.A., STIRBET, A., OLSOVSKA, K., KUNDERLIKOVÁ, K., SHELONZEK, H., RUSINOWSKI, S., BAĞA, W., 2017. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynthesis Research*, 132 (1): 13–66. <https://doi.org/10.1007/s1120-016-0318-y>
- KANJEVAC, B., LJUBIČIĆ, J., KERKEZ JANKOVIĆ, I., MIJATOVIĆ, LJ., DEVETAKOVIĆ, J., 2024. Regeneration of hilly-mountainous oak forests in Serbia – past experiences and future perspectives. *Reforesta*, 18: 34–58. <https://doi.org/10.21750/REFOR.18.03.119>
- KAPROTH, M.A., FREDERICKSEN, B.W., GONZÁLEZ-RODRÍGUEZ, A., HIPPEL, A.L., CAVENDER-BARES, J., 2023. Drought response strategies are coupled with leaf habit in 35 evergreen and deciduous oak (*Quercus*) species across a climatic gradient in the Americas. *New Phytologist*, 239 (3): 888–904. <https://doi.org/10.1111/nph.19019>
- KEBERT, M., VUKSANOVIĆ, V., STEFELS, J., BOJOVIĆ, M., HORÁK, R., KOSTIĆ, S., KOVAČEVIĆ, B., ORLOVIĆ, S., NERI, L., MAGLI, M., RAPPARINI, F., 2022. Species-level differences in osmoprotectants and antioxidants contribute to stress tolerance of *Quercus robur* L., and *Q. cerris* L. seedlings under water deficit and high temperatures. *Plants*, 11 (13): 1744. <https://doi.org/10.3390/plants11131744>
- KILLI, D., BUSSOTTI, F., GOTTARDINI, E., POLLASTRINI, M., MORI, J., TANI, C., PAPINI, C., FERRINI, F., FINI, A., 2018. Photosynthetic and morphological responses of oak species to temperature and [CO₂] increased to levels predicted for 2050. *Urban Forestry & Urban Greening*, 31: 26–37. <https://doi.org/10.1016/j.ufug.2018.01.012>
- KUZNETSOVA, A., BROCKHOFF, P.B., CHRISTENSEN, R.H.B., 2017 lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, 82 (13): 1–26. <https://doi.org/10.18637/jss.v082.i13>
- LEVERKUS, A.B., MEDINA, M., LÁZARO-GONZÁLEZ, A., LEVY, L., LORENTE-CASALINI, O., MARTÍN, M.P.R., VENDIÑA, V., 2026. Drivers of seedling emergence and early growth of 12 European oak species: results from a cross-continental experiment. *Forest Ecology and Management*, 599: 123223. <https://doi.org/10.1016/j.foreco.2025.123223>
- LIMOUSIN, J.M., ROUSSEL, A., RODRÍGUEZ-CALCERRADA, J., TORRES-RUIZ, J.M., MORENO, M., GARCÍA DE JALÓN, L., OURCIVAL, J.M., SIMIONI, G., COCHARD, H., MARTIN-ST PAUL, N., 2022. Drought acclimation of *Quercus ilex* leaves improves tolerance to moderate drought but not resistance to severe water stress. *Plant, Cell & Environment*, 45 (7): 1967–1984. <https://doi.org/10.1111/pce.14326>
- LÖF, M., 2024. Challenges for management of rich *Quercus* forests in Europe. In MARTENS, C., CLARK, S., SCHWEITZER, C. (eds). *The international oak symposium: science-based management for dynamic oak forests*. Gene-

- ral Technical Report SRS-278. Asheville: U.S. Department of Agriculture, Forest Service, Southern Research Station, p. 9–10.
- MANES, F., VITALE, M., DONATO, E., GIANNINI, M., PUPPI, G., 2006. Different ability of three Mediterranean oak species to tolerate progressive water stress. *Photosynthetica*, 44 (3): 387–393. <https://doi.org/10.1007/s11099-006-0040-7>
- MARTÍNEZ-VILALTA, J., GARCIA-FORNER, N., 2016. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. *Plant, Cell & Environment*, 40 (6): 962–976. <https://doi.org/10.1111/pce.12846>
- MCDOWELL, N., POCKMAN, W.T., ALLEN, C.D., BRESHEARS, D.D., COBB, N., KOLB, T., PLAUT, J., SPERRY, J., WEST, A., WILLIAMS, D.G., YEPEZ, E.A., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist*, 178 (4): 719–739. <https://doi.org/10.1111/j.1469-8137.2008.02436.x>
- MEDIAVILLA, S., ESCUDERO, A., 2003. Stomatal responses to drought at a Mediterranean site: a comparative study of co-occurring woody species differing in leaf longevity. *Tree Physiology*, 23 (14): 987–996. <https://doi.org/10.1093/treephys/23.14.987>
- MEDIAVILLA, S., ESCUDERO, A., 2004. Stomatal responses to drought of mature trees and seedlings of two co-occurring Mediterranean oaks. *Forest Ecology and Management*, 187 (2-3): 281–294. <https://doi.org/10.1016/j.foreco.2003.07.006>
- MIJATOVIĆ, L.J., KANJEVAC, B., LJUBIČIĆ, J., KERKEZ JANKOVIĆ, I., DEVETAKOVIĆ, J., 2026. Early post-germination physiological traits of oak species under various environmental conditions in oak forests. *Forests*, 17 (3): 1–26. <https://doi.org/10.3390/f17010003>
- MONDANELLI, L., MARIOTTI, B., MALTONI, A., SALBITANO, F., TRAVERSI, M.L., GIOVANNELLI, A., MASERTI, B.E., COCOZZA, C., 2026. First-year seedlings respond to a 21-day summer water deficit: morphological and physiological insights of two populations of five Mediterranean Quercus species. *iForest*, 19: 149–156. DOI: 10.3832/for4928-019
- MORENO, M., SIMIONI, G., COCHARD, H., DOUSSAN, C., GUILLEMOY, J., DECARSIN, R., FERNANDEZ-CONRADI, P., DUPUY, J.L., TRUEBA, S., PIMONT, F., RUFFAULT, J., JEAN, F., MÓRICZ, N., MÉSZÁROS, I., KERN, Z., ILLÉS, G.Z., GARMSZEGI, B., EÖTVÖS, C. B., ..., NÉMETH, T.M., 2025. Evaluating the drought tolerance of five native broadleaf tree species using dendroecological analysis in East Central Europe. *Frontiers in Forests and Global Change*, 8: 625371. DOI: 10.3389/ffgc.2025.1625371
- NAKAGAWA, S., SCHIELZETH, H., 2013. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4 (2): 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- NICOLESCU, V.N., VOR, T., BRUS, R., DODAN, M., PERIĆ, S., PODRÁZSKÝ, V., ... BARTLETT, D. 2025. Management of sessile oak (*Quercus petraea* (Matt.) Liebl.), a major forest species in Europe. *Journal of Forestry Research*, 36 (1): 78.
- OGAYA, R., PEÑUELAS, J., 2003. Comparative seasonal gas exchange and chlorophyll fluorescence of two dominant woody species in a Holm Oak Forest. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 198 (2): 132–141. <https://doi.org/10.1078/0367-2530-00085>
- PASTA, S., DE RIGO, D., CAUDULLO, G., 2016. *Quercus pubescens* in Europe: distribution, habitat, usage and threats. In SAN-MIGUEL-AYANZ, J., DE RIGO, D., CAUDULLO, G., HOUSTON DURRANT, T., MAURI, A. (eds). *European atlas of forest tree species*. Luxembourg: Publication Office of the European Communities, p. 156–157.
- PIETRAS, J., STOJANOVIĆ, M., KNOTT, R., POKORNÝ, R., 2016. *Oak sprouts grow better than seedlings under drought stress*. *iForest-Biogeosciences and Forestry*, 9 (4): 529. <https://doi.org/10.3832/for1823-009>
- POORTER, H., BÜHLER, J., VAN DUSSCHOTEN, D., CLIMENT, J., POSTMA, J. A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, 39 (11): 839–850. <https://doi.org/10.1071/FP12049>
- POPA, I., POPA, A., 2026. Finding the winner: resilience to drought of six oak species in a forest-steppe long-term multi-species trial. *Forest Ecology and Management*, 602: 123319.
- PRACIAK, A., PASIECZNIK, N., SHEIL, D., VAN HEIST, M., SASSEN, M., CORREIA, C.S., DIXON, C., FYSON, G., RUSHFORTH, K., TEELING, C., 2013. *The CABI encyclopedia of forest trees*. Wallingford, Oxfordshire, UK: CABI. 536 p.
- R CORE TEAM, 2026. *R. A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. [cit.2026-05-18]. <https://www.R-project.org/>
- SAVILL, P.S., 2019. *The silviculture of trees used in British forestry*. Wallingford, Oxfordshire, UK: CABI. 424 p.
- SIAM, A.M., RADOGLU, K.M., NOITSAKIS, B., SMIRIS, P., 2009. Differences in ecophysiological responses to summer drought between seedlings of three deciduous oak species. *Forest Ecology and Management*, 258 (1): 35–42. <https://doi.org/10.1016/j.foreco.2009.03.048>
- SOBRINO-PLATA, J., CANO, F.J., ARANDA, I., FERNÁNDEZ DE SIMÓN, M.B., RODRÍGUEZ-CALCERRADA, J., 2024. The impact of drought on plant metabolism in Quercus species—from initial response to recovery. In ANTÓNIO, C. (ed.). *Monitoring forest damage with metabolomics methods*. Hoboken, New Jersey, USA: John Wiley and Sons, p. 267–313. <https://doi.org/10.1002/9781119868750.ch10>
- SPIECKER H., 2021. Production of valuable oak wood in Europe. *Annals of Forest Research*, 64 (1): 5–12. <https://doi.org/10.15287/afr.2021.2207>
- STOJANOVIĆ, D., ORLOVIĆ, S., ZLATKOVIĆ, M., KOSTIĆ, S., VASIĆ, V., MILETIĆ, B., KESIĆ, L., MATOVIĆ, B., BOŽANIĆ, D., PAVLOVIĆ, L., MILOVIĆ, M., PEKEČ, S., ĐURĐEVIĆ, V., 2021. Climate change within Serbian forests: current state and future perspectives. *Topola*, 208: 39–56. <https://doi.org/10.5937/topola2108039S>
- STOJANOVIĆ, D.B., STOJSAVLJEVIĆ, R., PAVKOV, S.D., TENJI, D., DJALOVIĆ, I., VIDOVIĆ, D., ... VIŠACKI, V., 2026. Impact of climate change on tree species distribution and vulnerability in key protected forest ecosystems in Serbia. *Forests*, 17 (4): 469. <https://doi.org/10.3390/f17040469>
- STOJANOVIĆ, M., SZATNIEWSKA, J., KYSELOVÁ, I., POKORNÝ, R., ČATER, M., 2017. Transpiration and water potential of young *Quercus petraea* (M.) Liebl. coppice sprouts and seedlings during favourable and drought conditions. *Journal of Forest Science*, 63 (7): 613–623. <https://doi.org/10.17221/36/2017-JFS>
- TOGNETTI, R., CHERUBINI, P., MARCHI, S., RASCHI, A., 2007.

- Leaf traits and tree rings suggest different water-use and carbon assimilation strategies by two co-occurring *Quercus* species in a Mediterranean mixed-forest stand in Tuscany, Italy. *Tree Physiology*, 27 (12): 1741–1751. <https://doi.org/10.1093/treephys/27.12.1741>
- TSAKALDIMI, M., TSITSONI, T., GANATSAS, P., ZAGAS, T., 2009. A comparison of root architecture and shoot morphology between naturally regenerated and container-grown seedlings of *Quercus ilex*. *Plant and Soil*, 324 (1): 103–113.
- TURNER, N.C., 2018. Imposing and maintaining soil water deficits in drought studies in pots. *Plant and Soil*, 439 (1): 45–55. <https://doi.org/10.1007/s11104-018-3893-1>
- VUKOVIĆ, A.J., VUJADINOVIĆ, M.P., RENDULIĆ, S.M., ĐURĐEVIĆ, V.S., RUMML, M.M., BABIĆ, V.P., POPOVIĆ, D.P., 2018. Global warming impact on climate change in Serbia for the period 1961–2100. *Thermal Science*, 22 (6 Part A): 2267–2280. <https://doi.org/10.2298/TSCI180411168V>
- WALKER, A., 2024. *writexl: Export Data Frames to Excel 'xlsx' Format*. [cit.2026-04-30]. <https://CRAN.R-project.org/package=writexl>
- WICKHAM, H., 2019. *stringr: Simple, Consistent Wrappers for Common String Operations*. [cit.2026-04-30]. <https://CRAN.R-project.org/package=stringr>
- WICKHAM, H., FRANÇOIS, R., HENRY, L., MÜLLER, K., 2023a. *dplyr: A Grammar of Data Manipulation*. [cit.2026-04-30]. <https://CRAN.R-project.org/package=dplyr>
- WICKHAM, H., HESTER, J., BRYAN, J., 2023b. *readr: Read Rectangular Text Data*. [cit.2026-06-01]. <https://CRAN.R-project.org/package=readr>
- WICKHAM, H., VAUGHAN, D., 2023. *tidyr: Tidy Messy Data*. [cit.2026-04-30]. <https://CRAN.R-project.org/package=tidyr>
- WU, J., WANG, J., HUI, W., ZHAO, F., WANG, P., SU, C., GONG, W., 2022. Physiology of plant responses to water stress and related genes: a review. *Forests*, 13 (2): 324. <https://doi.org/10.3390/f13020324>
- ZENELI, G., KOLA, H., 2017. Coppice forests: can traditional coppice forest management help the Western Balkan region? *Forest Research*, 6 (3). <https://doi.org/10.4172/2168-9776.1000212>
- ŽIVANOVIĆ, S., 2017. Impact of drought in Serbia on fire vulnerability of forests. *International Journal Bioautomation*, 21 (2): 217. https://biomed.bas.bg/bioautomation/2017/vol_21.2/files/21.2_06.pdf

Received June 30, 2026

Accepted August 24, 2026

Supplementary material

Table S1. Two-way ANOVA (Type III sums of squares, Satterthwaite correction) for a) substrate temperature and b) substrate volumetric moisture content: effects of treatment, part of day and their interaction

a) Substrate temperature

Effect	Sum Sq	Mean Sq	Num DF	Den DF	F value	p value
Type	0.02701	0.02701	1	24	1.1439	0.2955
Part_of_day	1.13563	0.37854	3	18	16.0300	<0.001
Type: Part_of_day	0.03885	0.01295	3	24	0.5484	0.6540

b) Substrate volumetric moisture content

Effect	Sum Sq	Mean Sq	Num DF	Den DF	F value	p value
Type	7169.2	7169.2	1	42	151.1145	<0.001
Part_of_day	52.1	17.4	3	42	0.3664	0.7776
Type: Part_of_day	2.1	0.7	3	42	0.0148	0.9975

Notes: Sum Sq, sum of squares (Type III); Mean Sq, mean square; Num DF/Den DF, numerator and denominator degrees of freedom (Satterthwaite approximation). p values below 0.001 are reported as <0.001. Type, treatment (Control vs. Water Deficit/Irrigated); Part_of_day, time of measurement (four levels); Type: Part_of_day, their interaction.