

Carbon assimilation in oak (*Quercus* spp.) populations under acute and chronic high-temperature stress

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Abstract

This study tried to evaluate the influence of high temperatures (HT) on carbon assimilation of two oak species (*Quercus cerris* and *Quercus robur*). The screening of their populations, which are assumed to have a different resistance and acclimatization potential to adverse environmental conditions, can provide essential information for its implementation in reforestation strategies. By employing principal component analysis, the aim was to determine the most variable physiological characteristics of plants exposed to extremely HT during vegetation periods. After 2 d of HT treatment, a significant decline of photosynthetic and transpiration rates, and stomatal conductance were observed in plants of all investigated populations. The decrease of photosynthetic parameters after 2 d of HT treatment was regulated by the stomatal closure which caused the limitation of CO₂ assimilation. Contrary, a chronic HT stress led to an increase in *g_s* and inhibition of photosynthesis at the level of carboxylation.

Additional key words: fluorescence; gas exchange; heat stress; Random Forest analysis; water-use efficiency.

Introduction

Additionally to drought and enhanced CO₂ concentration, another outcome of climate change is average temperature increase (IPCC 2001). An increase in average annual temperature is an abiotic phenomenon which occurs at the same time with (soil and air) drought, all resulting in very serious implications for ecosystems and plant communities. Influences of elevated temperatures on crops have already been studied thoroughly (Prasad *et al.* 2000, Matsui *et al.* 2001, Challinor *et al.* 2005, Wang *et al.* 2010). Due to their great economic significance and importance for the biodiversity maintenance, woody plants and forest populations have been widely investigated with the goal of defining their physiological response and adaptation/acclimatization potential in increasingly frequent stressful environmental conditions (Munné-Bosch *et al.* 2004, Rennenberg *et al.* 2006, Di Filippo *et al.* 2010). Therefore, the observation and determination of specific physiological plant responses to high-temperature (HT) stress is important

in order to define the degree of vulnerability of forest populations, predict effective measures for their conservation, and also to determine resistant genotypes that would maintain stable bioproduction in conditions of rising temperatures followed by drought. For the investigation aimed at detecting the correlation between high-temperature stress and plant physiological response, we chose two oak species, *Quercus cerris* (Turkey oak) and *Quercus robur* (pedunculate oak), which are typical deciduous species on the Balkan Peninsula. Both species have xeromorphic characteristics and economic importance in timber production (Janković 1971). *Q. robur* forests are found mostly on lowland river banks (Pintarić 2002) and they comprise 20.5% of the total forestry in the Republic of Serbia (Banković *et al.* 2009). The *Q. cerris* forests are mostly located on mountain areas (Janković 1970), and they represent about 13% of all forests in Serbia (Banković *et al.* 2009). According to Epron and Dreyer (1993), *Q. robur* has high degree of tolerance to drought, but other authors observed the opposite phenomenon (Dickson and

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Abbreviations: C_i – intercellular CO₂ concentration; DM – dry mass; E – transpiration rate; F₀ – minimal fluorescence yield of the dark-adapted state; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; Gh – glasshouse; g_s – stomatal air conductance; HT – high temperature; MDA – Mean Decrease in Accuracy; MDG – Mean Decrease in Gini; P – population; PCA – principal component analysis; P_N – net photosynthesis; RFA – Random Forest analysis; ROS – reactive oxygen species; WUE – water-use efficiency; WUE_i – intrinsic water-use efficiency.

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Tomlinson 1996). Regarding to some references, well-watered *Q. cerris* had a greater physiological performance than other oaks, but under drought, it had the limiting adaptive potential to water stress (Manes *et al.* 2006). On the other hand, there are references indicating that *Q. cerris* is a drought-tolerant species (Nardini *et al.* 1999).

It is widely known that the increase of photosynthetic CO₂ assimilation by forests will be crucial in the first half of the 21st century (Woodward and Lomas 2004, Schulze *et al.* 2010). Photosynthesis might serve as a reliable physiological parameter for providing relevant information about plant's physiological condition, growth, and development level as well as potential of biomass production. This process is one of the most heat-sensitive in plant metabolism which could be reduced or significantly inhibited by a high temperature (Berry and Björkman 1980, Schrader *et al.* 2004, Camejo *et al.* 2005). A few species, including those of the genus *Quercus* (oak), are characterized with lower transpiration rates. The leaves of those species are particularly resistant to the adverse effects of high temperature on photosynthetic apparatus, since leaf temperature rise cannot be prevented by cooling effect of transpiration (Hanson *et al.* 1999, Singaas *et al.* 1999, Sharkey 2005). Accordingly, high temperature stress in plants should be considered ecologically, as high air/soil temperature as well as physiologically, as variations in leaf tissue temperature which is related to the energy (metabolic) balance of leaves.

Supraoptimal temperatures may not directly and structurally disrupt photosynthetic tissue, but also, their interference in a complex photosynthetic process could be manifested in disturbance of general plant physiological performance, mostly expressed through affecting their carbon balance, nutrient uptake and cycling, and stress compensation mechanisms (Rennenberg *et al.* 2006). Reduction of carbon photosynthetic assimilation is a direct consequence of a reduced production of ATP and NADPH in light reactions, as well as decreased intercellular CO₂ concentration (C_i) under heat stress (Wang *et al.* 2017). Since the PSII is one of the primarily targets of heat stress, measuring its efficiency and determining damage by chlorophyll (Chl) *a* fluorescence might provide a valuable information on its stability and functionality (Jedrowski *et al.* 2015). Thus, the F_v/F_m is considered as a trustworthy parameter for assessment of the level of damage to PSII, provoked by exposure to extremely HT (Brestič and Živčák 2013). The adverse effect of HT on a pigment composition occurs mainly through impairment of their biosynthesis (Wang *et al.* 2009) or oxidative degradation of Chl pigments (Hasanuzzaman *et al.* 2012). Further, prolonged HT might influence the chlorophyllase activity and thus lead to a decrease of photosynthetic pigments, which may ultimately reduce the overall photosynthetic activity (Sharkey and Zhang 2010). Interestingly, thermotolerant genotypes have an increased Chl *a/b* ratio under high temperatures (Camejo *et al.* 2006), indicating that this trait might be related to thermotolerance. According to Selvaraj *et al.* (2011), Chl accumulation might be used for characterization of genetic variability in acquired thermotolerance for many crop species.

Nutrient uptake is mostly dependent on plant water status which implies water-transpiration flow and stomatal opening. Heatwave-driven stomatal closure may trigger photooxidative stress, a set of circumstances when an imbalance between light absorption and energy consumption in photosynthesis causes an increased formation of reactive oxygen species (ROS) (Asada 2006). In adverse environmental conditions, plants are able to accumulate different metabolites, in order to prevent the damage of structural biomolecules. Proline is an amino acid which plays an important role under stress conditions among many plant species. Several authors claimed that the content of proline increases under drought (Anjum *et al.* 2011, Canavar *et al.* 2014), heat (Correia *et al.* 2014), or salt stress conditions (Ben-Rouina *et al.* 2006) in order to sustain cell and tissue activities. Szabados and Savoure (2010) reported that proline is an osmolyte, molecule which controls mitochondrial function, namely, it is a ROS scavenger and it stabilizes protein structure. Proline metabolism leads to increased mitochondrial ROS production *via* the electron transport chain, so proline metabolism impacts cell survival and cell death depending on specific environmental conditions (Liang *et al.* 2013). Moreover, this particular molecule also influences a gene expression in order to prevent cell death.

In order to obtain statistically verified results, which could indicate the influence of high temperatures during the vegetation period on the physiological parameters, we examined two oak species and their four populations that are assumed to have different resistance and acclimatization potential to adverse environmental conditions. We wanted to find out which of the main biochemical and physiological parameters (photosynthesis, transpiration, water-use efficiency, proline content, Chl *a* fluorescence parameters, and pigment contents) are most important for expressing tolerance and acclimatization of selected oak species and populations to high temperatures. Evaluation of specific genetic, biochemical, and physiological tolerance of oak, a tree with primarily great economic value, to unfavorable ecological conditions, could provide essential information for its implementation in reforestation strategies.

Materials and methods

Plant material and experimental conditions: Four populations of two oak species originated from Serbian forests were used in the experiments. Seeds for experimental cultivation of the most preserved autochthonous monodominant populations of oak species: pedunculate oak (*Quercus robur* L.) and Turkey oak (*Quercus cerris* L.) have been collected from localities which could be distinguished as lowland and mountain by altitude of their natural habitats. Populations have been geographically isolated (specific populations); therefore, we excluded direct pollination and gene transfer between populations and assumed that the seeds/experimental plants had been genetically uniform and specific for population. Seeds for experimental purposes were collected in autumn and kept at dry and dark place before the experiment started. Origin of the populations was as follows:

Species	Population	Origin of population (GPS coordinates)	Habitat altitude [m]
<i>Quercus robur</i> L. (1753)	P1	44.9725707°N 19.2212677°E	86
	P2	45.1552903°N 19.3610001°E	185
<i>Quercus cerris</i> L. (1753)	P3	45.1451209°N 19.5443344°E	365
	P4	45.1468159°N 19.5704269°E	310

In order to stimulate germination, the seeds were exposed to the process of stratification in a cold chamber. After a cold treatment, the seeds were germinated in a growth chamber on sterilized sand, at the air temperature of 25°C and relative sand humidity ranging from 70–80%. Morphologically uniformed seedlings were then planted into soil substrate in *Mitscherlich* pots of 5 L. In general, 30 plants from each population were selected and grown for eight months in a glasshouse I (Gh-I), under semicontrolled conditions with ambient level of illumination. Before the treatments (control and HT) plants of each population were divided in two groups with 15 individuals. During the growing season and experimental evaluation plants were watered daily with tap water [drinking water, pH 7.82, 75–150 mg(CaCO₃) L⁻¹, EC of 425 µS cm⁻¹], to maintain optimal soil water capacity (70–80% of soil relative water capacity). The young experimental plants with four fully expanded leaves were exposed to the acute (2 d of exposure) and chronic (5 d of exposure) HT stress (Nahar *et al.* 2015) in the glasshouse II (Gh-II), where the air temperature ranged between 33–47°C. At the same time, the control plants were kept in a glasshouse Gh-I with optimal air temperature maintained between 25–28°C (Table 1S, *supplement*). Gas-exchange measurements were carried out as follows: on 30 June, after 2 d of exposure to HT, and on 3 July, after 5 d of exposure to HT. Analysis of pigment contents and free proline were taken after 5 d of exposure to HT treatments.

Gas exchange: Rates of photosynthesis (P_N) and transpiration (E), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) were measured using *LC pro+ Portable Photosynthesis System*, manufactured by *ADC BioScientific Ltd.*, Hoddesdon, UK. A temperature sensor in the leaf chamber of the device was used to measure leaf temperature in °C (T_l) (Table 2S, *supplement*). In order to avoid the midday photosynthesis depression (Koyama and Takemoto 2014), measurements were carried out in the morning from 9–12 h, with nine replicates sampled from each population and treatment. Water-use efficiency (WUE) was calculated as the ratio between photosynthetic and transpiration rates ($WUE = P_N/E$). Instantaneous water-use efficiency (WUE_i) was calculated as the ratio between photosynthetic and stomatal conductance ($WUE_i = P_N/g_s$). Light conditions for measurement of photosynthesis were defined using the *LCpro+* light unit, which emitted PAR of 1,000

µmol(photon) m⁻² s⁻¹. The air supply unit provided a flow of ambient air to the leaf chamber at a constant rate of 100 µmol s⁻¹. CO₂ concentration and air humidity were at ambient level.

The differences which are very important in characterization of a specific plant's adaptive potential, referred to the parameter 'intrinsic/instantaneous water-use efficiency (WUE_i)' which is calculated as the ratio of the instantaneous rates of CO₂ assimilation (P_N) and transpiration (E) at the stomata level (g_s). The instantaneous rates of both P_N and E are products of two factors: stomatal conductance (g_s) to either CO₂ (g_c) or water vapor (g_w) and a concentration gradient of either CO₂ ($C_a - C_i$) or water vapor ($w_i - w_a$) between the air outside the leaf (index a) and the air inside the leaf (index i) (Condon *et al.* 2002) and according to Fick's law, $P_N = g_c \times (C_a - C_i)$ and $E = g_w \times (w_i - w_a)$, therefore, $WUE_i = P_N/E$ (at stomatal level) = $[g_c \times (C_a - C_i)]/[g_w \times (w_i - w_a)]$.

Above mentioned equations clearly indicate the significant influence of stomatal conductance as well as boundary layer and mesophyll conductance on CO₂ diffusion into the intracellular spaces/chloroplasts (CO₂ available for carboxylation) (Lambers *et al.* 2008). Therefore, we calculated WUE_i as a ratio between net photosynthetic CO₂ assimilation and stomatal conductance ($WUE_i = P_N/g_s$).

Chl *a* fluorescence was measured using a portable fluorometer (*PSM, BioMonitor, AB*, Sweden) on the middle part of intact leaves (Öquist and Wass 1988). Prior to measuring, the youngest fully developed leaves were kept in dark for 15 min, after which the F_0 (the minimum fluorescence level of the dark-adapted leaves) and F_v/F_m (the maximum quantum efficiency of PSII photochemistry or maximum quantum yield of PSII) fluorescence parameters were determined ($n = 9$).

Biochemical parameters: The analysis of biochemical parameters was done only for a chronic exposure (5 d) of oak populations to HT, in order to keep as much as necessary of intact plants for gas-exchange and fluorescence measurements. Leaves from nine plants were taken and immediately frozen in liquid nitrogen, and afterward kept in a freezer at -84°C.

Chloroplast pigments: Chls and carotenoids (Car) were extracted using 100% acetone. Absorbance of pigment extracts was measured by *Beckman DU-65 UV/Visible spectrophotometer (Beckman Instruments)* at wavelengths of 663 nm (Chl *a*), 646 nm (Chl *b*), and 470 nm (Car). The concentrations of pigments were calculated using molar absorption coefficients following equations determined by Lichtenthaler and Wellburn (1983). Dry mass of oak leaves was determined by oven-drying the plant material at 120°C for 48 h. Afterward, pigment concentrations were calculated using nine replicates and expressed as [mg g⁻¹(DM)].

Proline: Amino acid content was determined using the Bates method (Bates 1973). The samples of fully expanded

oak leaves were used for analysis. Plant material was measured for each of the three replicates (1 g), grinded with addition of 5 ml of 5% sulfosalicylic acid, followed with centrifugation for 12 min at $3,400 \times g$. Afterwards, supernatant was mixed with acid ninhydrin reagent and incubated at 100°C. Prior to absorbance reading by *Beckman DU-65 UV/Visible* spectrophotometer (*Beckman Instruments*) at 520 nm, samples were mixed with toluene and resulting chromophore in upper toluene layer was analyzed.

Data analysis: Proline and pigment content data were analyzed with *Statistica* software (*StatSoft GmbH*, Hamburg, *version 13.2*) by employing two-way factor analyses (*ANOVA*), followed by post-hoc *Fisher's* multiple range test at the level of significance $p < 0.05$. The average values \pm SD are shown in the corresponding table and figure. Different letters following the values indicated statistically significant difference.

Gas-exchange parameters, water-use efficiency, and fluorescence measurements were analyzed with two-way *ANOVA* run as a linear model as follows:

$Y \sim \text{Species} * \text{Treatment} + (1|\text{Population})$, where *Y* determines the response variable, *Species* * *Treatment* is an expression determining the factors in model matrix, and the random effects terms (1|Population). All calculation and visualizations were done using the 'nlme' package (Pinheiro *et al.* 2018), 'multcompView' (<https://cran.r-project.org/package=multcompView>), 'lsmeans' (<https://cran.r-project.org/package=lsmeans>), 'rcompanion' (<http://rcompanion.org/handbook/>), 'car' (<https://cran.r-project.org/package=car>), 'ggplot' (<http://ggplot2.tidyverse.org/>), 'ggrepel' (<http://github.com/slowkow/ggrepel>) within the *R Studio* environment. The analysis outputs for gas-exchange, water-use efficiency, and fluorescence measurements are presented in the form of boxplots and can be found in the *supplement*, respectively.

Mixed model was used to test whether there are differences between investigated populations of the two oak species. Normality of distribution was tested by *Shapiro-Wilk's* test (function *shapiro.test*). Modeling was done using 'nlme' package (Pinheiro *et al.* 2018) in *R Studio* environment. Model formula was set as follows:

$Y \sim \text{Stress} + \text{Species} + (1|\text{Treatment:Population}) + (1|\text{Stress:Population}) + (1|\text{Stress:Species})$, where *Y* determines the response variable, *Stress* + *Species* is an expression determining the columns of the fixed-effects model matrix, and the random effects terms, (1|Treatment:Population) + (1|Stress:Population) + (1|Stress:Species), determining the random-effects model matrix.

Besides the chosen model, other models were fitted also and according to the obtained Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC), and log likelihood values the most efficient was selected to analyze the data (Pinheiro and Bates 2000, Spiegelhalter *et al.* 2002, Vaida and Blanchard 2005). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Model comparison was done using the analysis of variance for fitted model objects (*ANOVA* function). Since the applied model for

WUE, WUE_i, C_i, and g_s was characterized with singular fit error, we started dropping terms until model fit became nonsingular as suggested by Barr *et al.* (2013). Post-hoc *Tukey's* HSD test was used to test the fixed effects interactions with 'multcomp' package (Hothorn *et al.* 2008). Random effects estimates were plotted by using the 'sjPlot' package (Lüdtke 2018).

Linear correlations (*r*) were calculated between measured parameters using their average values (Table 3S, *supplement*).

Principal component analysis was conducted and visualized using *R software* packages 'ade4' (<http://pbil.univ-lyon1.fr/ADE-4>), 'vegan' (<https://github.com/vegandevs/vegan>), 'factoextra' (<http://www.sthda.com/english/rpkgs/factoextra>), and 'ggplot2' (<http://ggplot2.tidyverse.org/>) within the *R* environment (*R Core Team* 2016), in order to characterize and visualize patterns of measured photosynthetic parameters by the first two PC vectors. Prior to analysis, dataset was scaled (normalized) making the distributions of variables normalized, with stabilized variance and making tested model multiplicative on the raw scale.

Random Forest analysis (RFA) was employed in order to evaluate the effects of high temperature stress on photosynthetic parameters predicting the most important variable amongst examined ones. Prior to conducting the RFA bootstrap, a sample was selected from the dataset and a classification tree is built (bagging). Each node within the tree is constructed by selecting a random subset of the loaded variables, thus, evaluating the variable which yields the most effective split for maximizing purity in the two resultant groups. Nodes are continuously added to the tree until there is one plot per leaf. The process was repeated until the desired number of trees has been built, which was 500 in this analysis. So, bagging a large number of trees it is no further possible to represent the statistical learning technique using a single tree, thus making it harder to decide which variable is the most important, and improves prediction accuracy at the expense of interpretability (James *et al.* 2013). The importance of each predictor was obtained with Mean Decrease in Accuracy (MDA) and Mean Decrease in Gini (MDG) coefficients. MDA is determined during the bootstrapping phase. The more the accuracy of the Random Forest decreases due to the exclusion of individual variable, the more important is that variable considered. Thus, variables with a large mean decrease in accuracy are more important for classification of the data (Liaw and Wiener 2002). The Mean Decrease in Gini (MDG) coefficient is a value of how each variable contributes to the homogeneity of the nodes and leaves in the resulting Random Forest. Variables that result in nodes with higher purity have a higher decrease in Gini coefficient (Han *et al.* 2016). Programing, analysis, and visualization for RFA was done by Random Forest algorithm (Breiman 2001) using *R software* package 'randomForest' (Liaw and Wiener 2002) within the *R* environment.

Results

Applied acute and chronic stress influenced measured

photosynthetic parameters in a different way, but without significant differences at the species level (*supplement*). For that reason presented random effects variance and estimates might give a closer insight whether there are some differences between selected populations.

Net photosynthetic rate (P_N): As it can be seen from the mixed model output (Table 1), P_N was highly influenced by acute stress. The overall estimate of P_N in control plants was $18.654 \mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$. Chronic exposure to HT led to a certain recovery of P_N resulting in 2.5 fold increase in comparison to the acute stress. *Q. robur* was characterized with slightly higher P_N comparing to the other tested species. When it comes to the random effects, the biggest variance was present in Treatment:Population predictor, which was expected. Species interactions with stress factor had much higher variance in comparison with the Stress:Population. P_N random effects estimates presented in the Fig. 1SA,B,C (*supplement*) are showing that there were differences between tested population, distinguishing slightly *Q. robur* P1 as more efficient under chronic stress.

PSII functionality under acute and chronic HT treatment was investigated by Chl fluorescence measurement (F_v/F_m and F_0) and obtained data are presented in Table 4S and Fig. 8S (*supplement*). No significant differences were observed in case of F_v/F_m . On the contrary, F_0 was significantly higher in *Q. robur* plants. Also, this parameter was significantly different between the control and chronic stress group. *Q. robur* P1 and *Q. cerris* P3 among all tested populations had the lowest F_0 in dark-adapted leaves during chronic exposure to HT stress (Fig. 4S).

Transpiration rate (E) was almost 100% elevated after the chronic exposure of oak plants to HT stress (Table 1; Fig. 6SF, *supplement*). Acute exposure, on the other hand, did not cause disturbances in the activities of this parameter. Similarly to the P_N , the highest variance in case of E was at the Stress:Species level. When it comes to the random effects estimates, P2 seemed to have a slightly higher transpiration intensities during the both acute and chronic stress (Fig. 1SD,E).

Stomatal conductance (g_s): This parameter was significantly altered during the acute exposure to HT (Table 1), leading to almost complete closure of stomata. On the contrary, chronic HT exposure of *Q. cerris* plants seemed to have a stimulative effect on this particular parameter resulting in a 50% higher conductance in comparison with the control group (Fig. 6SH). P3 seemed to be a bit more efficient in case of HT (Fig. 2SC, *supplement*) in comparison to other populations.

Intercellular CO_2 concentration (C_i) significantly increased during both acute and chronic stress (Fig. 6SC,G). Concerning the random effects, the highest variance was at the Treatment:Population level. Stress:Species interactions have a zero variance indicating that there was no excess variability beyond what would be induced by the residual (Table 1). As it can be seen from the Fig. 3SC (*supplement*), C_i varied between populations during

the acute stress. Also, under HT stress, *Q. robur* P1 and *Q. cerris* P3 seemed to have a lower C_i in comparison to the other tested populations (Fig. 3SD).

Water-use efficiency (WUE) in oak plants significantly decreased during both exposures, acute and chronic (Fig. 7SA,B; *supplement*). According to the mixed model analysis output, we did not detect any significant variability at the population level, which is pointing to the fact that this particular parameter was strongly influenced by applied HT (Table 1). Random effects estimates are presented in the Fig. 2SA,B.

Intrinsic water-use efficiency (WUE_i): Contrary to most of the examined gas-exchange parameters, WUE_i severely decreased by chronic exposure to HT (Fig. 7SB,D), in comparison to the acute stress. According to Tukey's HSD post-hoc test, significant difference was not determined between control and acute group (Table 1). Random effects estimates (Fig. 3SA,B) are pointing to the P1 as the most efficient population during the acute exposure to HT. When it comes to chronic exposure to HT, P2 had the highest coefficient among all.

Pigments: Specific reactions of different populations were observed under HT stress. Both Chl and Car contents in *Q. robur* P1 and *Q. cerris* P4 exposed to elevated temperature overcome obtained pigment concentrations in control plants. On the contrary, the reduced pigment content was detected in *Q. robur* P2 and *Q. cerris* P3 exposed to HT for 5 d (Table 2).

Principal component and Random Forest analysis of photosynthetic traits: The first two principal components (PC) accounted for 73.1% of overall variation in case of acute stress (Fig. 1A–C), and 79.1% in case of chronic stress (Fig. 1D–F). Such high overall variation of first two PC is pointing to the importance of variables which are closely positively correlated with them. Distribution of individuals within applied treatments during acute and chronic stress is presented in Fig. 1A,D. It is clear that after initial exposure to temperatures above 40°C we got two distinct groups of individuals (Fig. 1A). The main factors separating these groups were photosynthetic parameters P_N , g_s , WUE_i , and WUE , which are strongly correlated with the first PC on a biplot. E vector has orthogonal direction which implies that transpiration was insignificant in separation of these groups. Chronic exposure had similar effects on data distribution patterns (Fig. 1D), but in this case, WUE and WUE_i were the most correlated variables with first PC. When plotting the populations as a factor (Fig. 1B,E), clusters were overlapping, without distinct differences. It is worth mentioning that P4 was characterized with the highest variance across the PC1.

Results of conducted RFA in this research were presented for acute (Fig. 2) and chronic (Fig. 3) exposure to HT. Calculated OutOfBag Error was presented in the Fig. 5S (*supplement*), one for every iteration of analysis. MDA and MDG values for examined variables had a very similar output in cases of all tested predictors (treatment,

Table 1. Gas-exchange parameters of oak plants exposed to HT stress. Significance codes: ‘ ’ 0; ‘***’ 0.001; ‘**’ 0.01; ‘*’ 0.05; ‘.’ 0.1. P_N – net photosynthesis; E – transpiration rate; WUE_i – water-use efficiency; WUE_s – intrinsic water-use efficiency; C_i – intercellular CO₂ concentration; g_s – stomatal air conductance. Fixed effects estimates for applied stresses and *Q. robur* plants are presented as deviations from the control group and *Q. cerris*, respectively.

Fixed effects	P_N		E		WUE		WUE _i		C_i		g_s	
	Estimate	Std. Error	Estimate	Std. Error	Estimate	Std. Error	Estimate	Std. Error	Estimate	Std. Error	Estimate	Std. Error
Control group	18.654	1.957	5.5189	0.9741	3.6627	0.2582	37.497	5.913	174.916	11.717	0.5624	0.1401
Stress acute	-15.639	2.304	0.1231	1.1764	-2.8728	0.2995	-8.779	7.007	85.667	13.606	-0.5000	0.1684
Stress chronic	-6.589	2.304	4.8811	1.1764	-2.3207	0.2995	-19.896	7.007	67.222	13.606	0.2667	0.1684
<i>Q. robur</i>	1.901	2.169	0.1865	1.0138	-0.1577	0.2956	-5.075	6.455	-4.444	13.374	0.1970	0.1478
Random effects	Variance	Std. Dev.	Variance	Std. Dev.	Variance	Std. Dev.	Variance	Std. Dev.	Variance	Std. Dev.	Variance	Std. Dev.
Stress:Population	0.3766	0.6137	0.1299	0.3604	0.0000	0.0000	45.41	6.739	33.11	5.754	0.0000	0.0000
Treatment:Population	6.2756	2.5051	0.5015	0.7082	0.1616	0.40200	46.54	6.822	321.81	17.939	0.01438	0.1199
Stress:Species	1.7986	1.3411	1.0351	1.0174	0.0027	0.05196	00.00	0.000	00.00	0.000	0.01823	0.1350
Residual	3.2783	1.8106	0.5965	0.7723	0.1111	0.33339	56.27	7.502	137.99	11.747	0.05258	0.2293
<i>Tukey's HSD post-hoc test</i>												
Species level:	P value	Sign. diff.	P value	Sign. diff.	P value	Sign. diff.	P value	Sign. diff.	P value	Sign. diff.	P value	Sign. diff.
<i>Q. cerris</i>	0.381	a	0.854	a	0.594	a	0.432	a	0.74	a	0.183	a
<i>Q. robur</i>		a		a		a		a		a		a
Stress level:												
Stress acute-Control	<1e-4***	a	0.9940	b	<1e-9***	a	0.4177	a	<1e-5***	c	0.0083**	a
Stress chronic-Control	0.0113*	c	0.0001***	b	<1e-9***	c	0.0123*	ab	<1e-5***	a	0.2516	b
Stress chronic-Acute	<1e-4***	b	<1e-4***	a	1.2e-8***	b	0.0716	b	4.5e-4***	b	<1e-4***	a

Table 2. Photosynthetic pigment contents in oak plants after chronic exposure to HT stress. P1–P4 present the oak population evaluated in this study. Results are presented as average ± SD ($n = 3$). *Different letters* indicate significant differences between populations and treatments according to *Fisher's test* ($p < 0.05$). Chl *a*(*b*) – chlorophyll *a*(*b*); P – population.

Species	Population	Chl <i>a</i>		Chl <i>b</i>		Chl (<i>a+b</i>)		Carotenoids	
		HT stress	Control	HT stress	Control	HT stress	Control	HT stress	Control
<i>Q. robur</i>	P1	12.88 ± 1.22 ^a	9.65 ± 0.31 ^b	3.23 ± 0.14 ^{ab}	3.23 ± 0.14 ^{ab}	12.88 ± 0.44 ^b	12.88 ± 0.44 ^b	18.07 ± 0.79 ^a	2.70 ± 0.02 ^{cd}
	P2	10.93 ± 0.44 ^b	13.49 ± 1.20 ^a	4.59 ± 0.35 ^{abc}	4.59 ± 0.35 ^{abc}	18.08 ± 1.50 ^a	18.08 ± 1.50 ^a	14.84 ± 0.87 ^b	3.50 ± 0.37 ^{abc}
<i>Q. cerris</i>	P3	7.57 ± 0.28 ^c	11.10 ± 0.34 ^b	3.73 ± 0.38 ^{bcd}	3.73 ± 0.38 ^{bcd}	14.83 ± 0.71 ^b	14.83 ± 0.71 ^b	10.09 ± 0.26 ^c	3.01 ± 0.14 ^{bc}
	P4	13.42 ± 0.01 ^a	6.08 ± 0.12 ^c	1.99 ± 0.10 ^c	1.99 ± 0.10 ^c	8.07 ± 0.05 ^c	8.07 ± 0.05 ^c	19.05 ± 1.27 ^a	1.56 ± 0.10 ^e

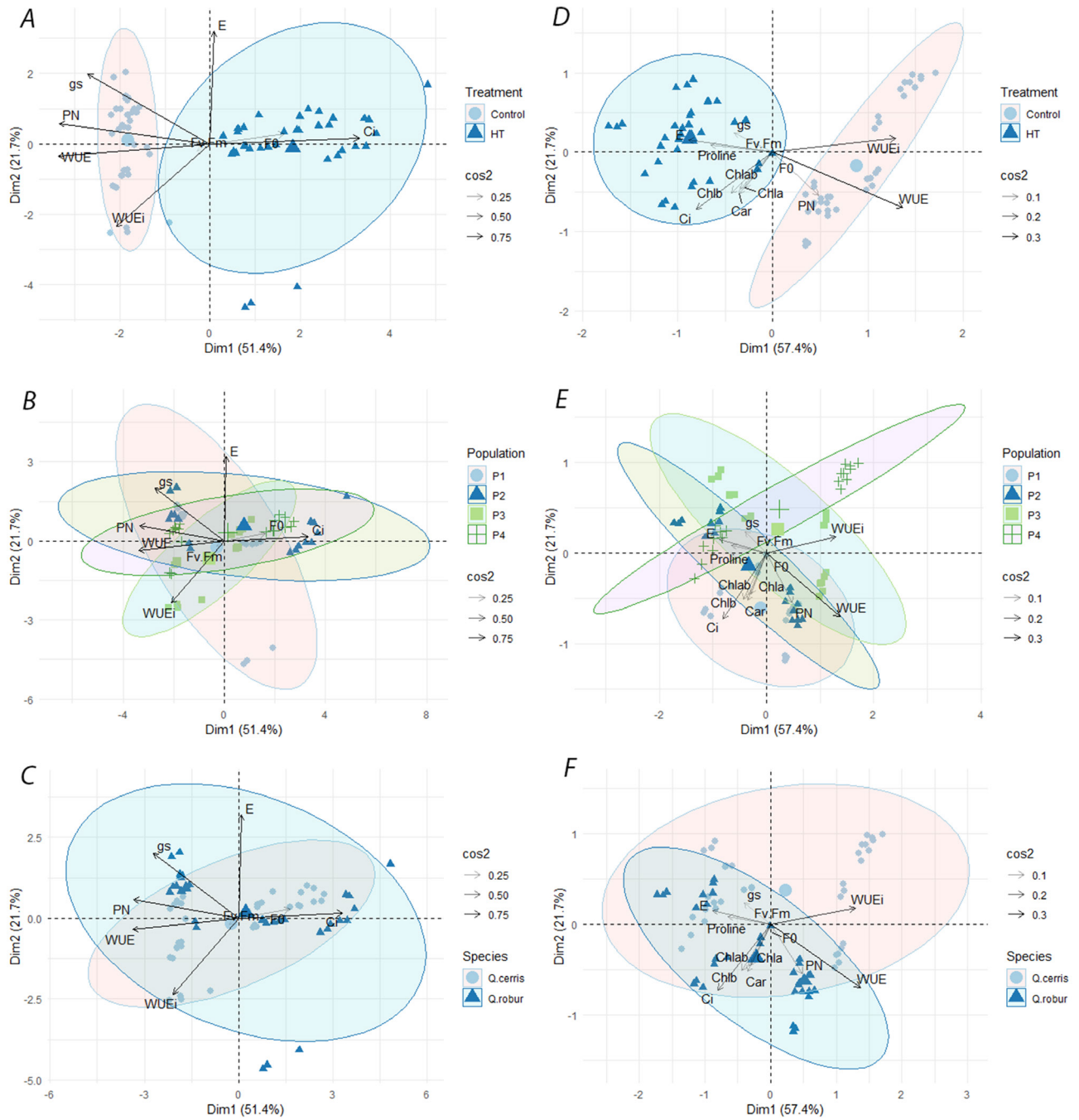


Fig. 1. Principal component analysis (PCA) of investigated physiological parameters. Acute stress examined parameters: (A–C), chronic stress examined parameters: (D–F). Biplots are presented with first and second principal components (PC1, PC2). Biplots (A) and (D) are presenting the distribution pattern of applied treatments; (B) and (E) the distribution pattern of investigated oak populations (P1–P4); (C) and (F) the distribution pattern of investigated oak species. Ellipses on biplot assume multivariate normal distribution. Biplot axes for acute stress are 2-fold higher than that of chronic stress, attention needed when interpreting them. Chl *a*(*b*) – chlorophyll *a*(*b*); *C_i* – intercellular CO₂ concentration; *E* – transpiration rate; *F*₀ – minimal fluorescence yield of the dark-adapted state; *F_v/F_m* – maximal quantum yield of PSII photochemistry; *g_s* – stomatal conductance; HT – high temperature; *P_N* – net photosynthesis; WUE – water-use efficiency; WUE_{*i*} – intrinsic water-use efficiency.

population, and species). The MDG slightly changed from acute to chronic exposure to HT. Acute stress defined *C_i*, WUE, and *P_N* as the most important variables, when treatment was a predictor in analysis. Chronic exposure to HT, in case of choosing treatments as a predictor in

analysis, defined WUE, *E*, and proline content as the most valuable variables (Fig. 3). After analyzing populations as a predictor, *P_N* was computed as the most important one after the initial exposure to HT. Over time, variable importance changed in favor of proline and *C_i* at chronic

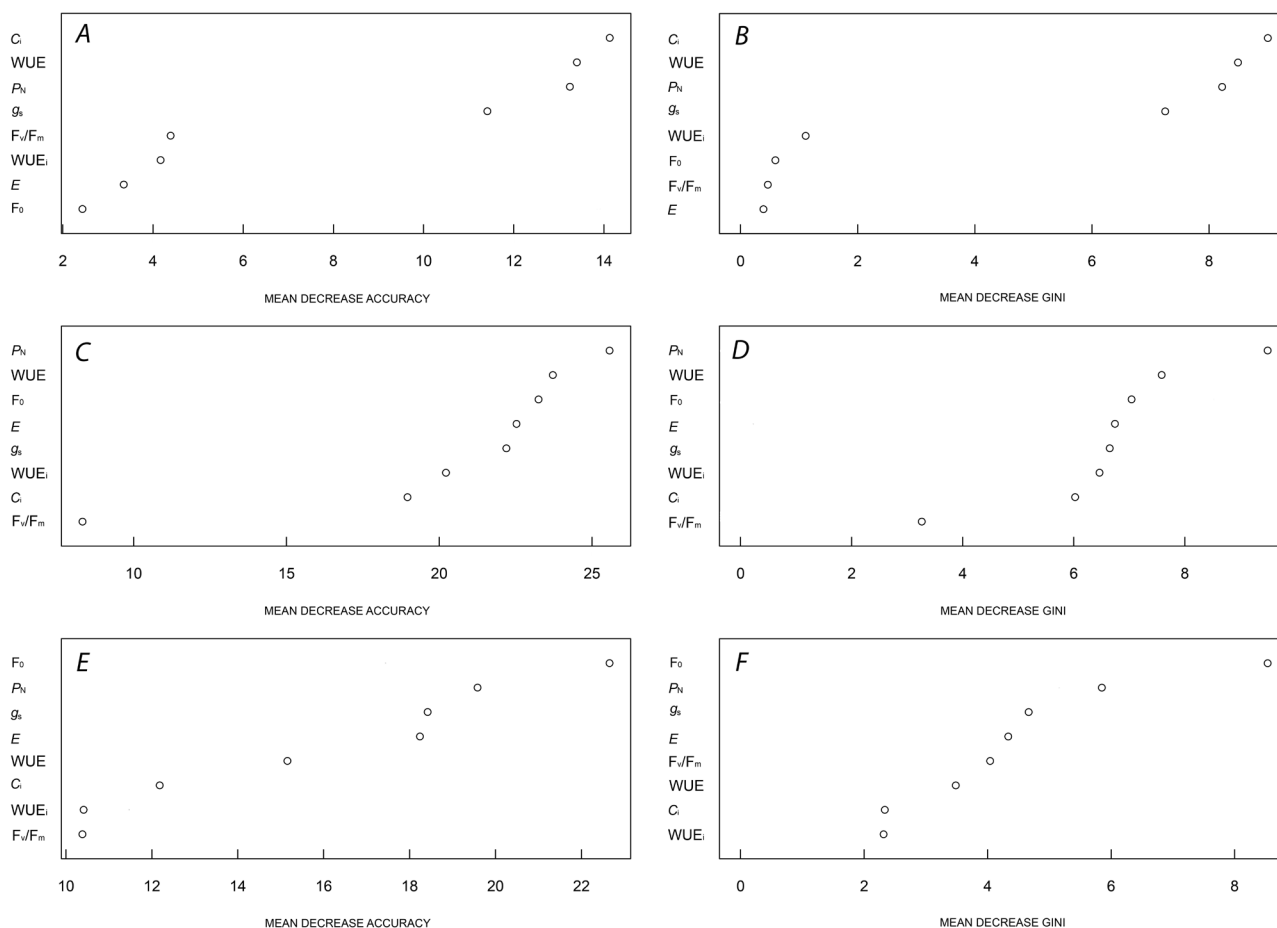


Fig. 2. Variable importance based on Mean Decrease in Accuracy and Gini calculation for acute stress. Graphs were plotted for different predictors in analysis such as treatment (A,B), population (C,D), and species (E,F). Mean Decrease in Accuracy – the more the accuracy of the Random Forest decreases due to the exclusion of individual variable, the more important that variable is considered. Mean Decrease in Gini – the mean decrease in Gini is a value of how each variable contributes to the homogeneity of the nodes and leaves in the resulting Random Forest. Chl *a(b)* – chlorophyll *a(b)*; C_i – intercellular CO_2 concentration; E – transpiration rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; HT – high temperature; P_N – net photosynthesis; WUE – water-use efficiency; WUE_i – intrinsic water-use efficiency.

stress. When it comes to species level, the most important variable during the acute stress was F_0 , but in case of chronic exposure to HT, the most important variables were represented by C_i and WUE_i .

Proline: Five days after treatment implementation, the concentration of proline was significantly higher in the oak seedlings exposed to elevated temperature (Fig. 4). The highest values were registered in P2 population, in case of both treatments applied. The smallest increase of accumulated proline between treatments was evident in P4 population.

Discussion

Comprehensive plant response to high temperature is a result of different abiotic as well as biotic parameters including intensity/temperature of heat waves, exposure time, and especially plant species (Wang *et al.* 2017). The main focus in our research was the evaluation of

physiological, primarily photosynthetic response of oak populations originating from geographically different habitats (populations) to high temperature stress. Investigated oak populations differed in their specific response to elevated temperature even though their habitats might be nearby. At the species level, we did not observed significant differences in this study, as reported by the applied mixed model (Table 1).

The sensitivity of photosynthetic parameters of different plant species to stressful abiotic conditions in microhabitats has been confirmed in numerous studies (Valentini *et al.* 1995, Pajević *et al.* 2010, Horak *et al.* 2014). According to Stojnić *et al.* (2016), physiological parameters are influenced by environmental conditions of sites and genetic constitution of proveniences or populations. Altitudinal effects on photosynthesis of different proveniences or populations were elucidated by Chai *et al.* (2015), Benavides *et al.* (2016), Ran *et al.* (2013), Pšidová *et al.* (2017). Effects of slope are poorly investigated as far as we know. Borišev *et al.* (2015) have investigated effects

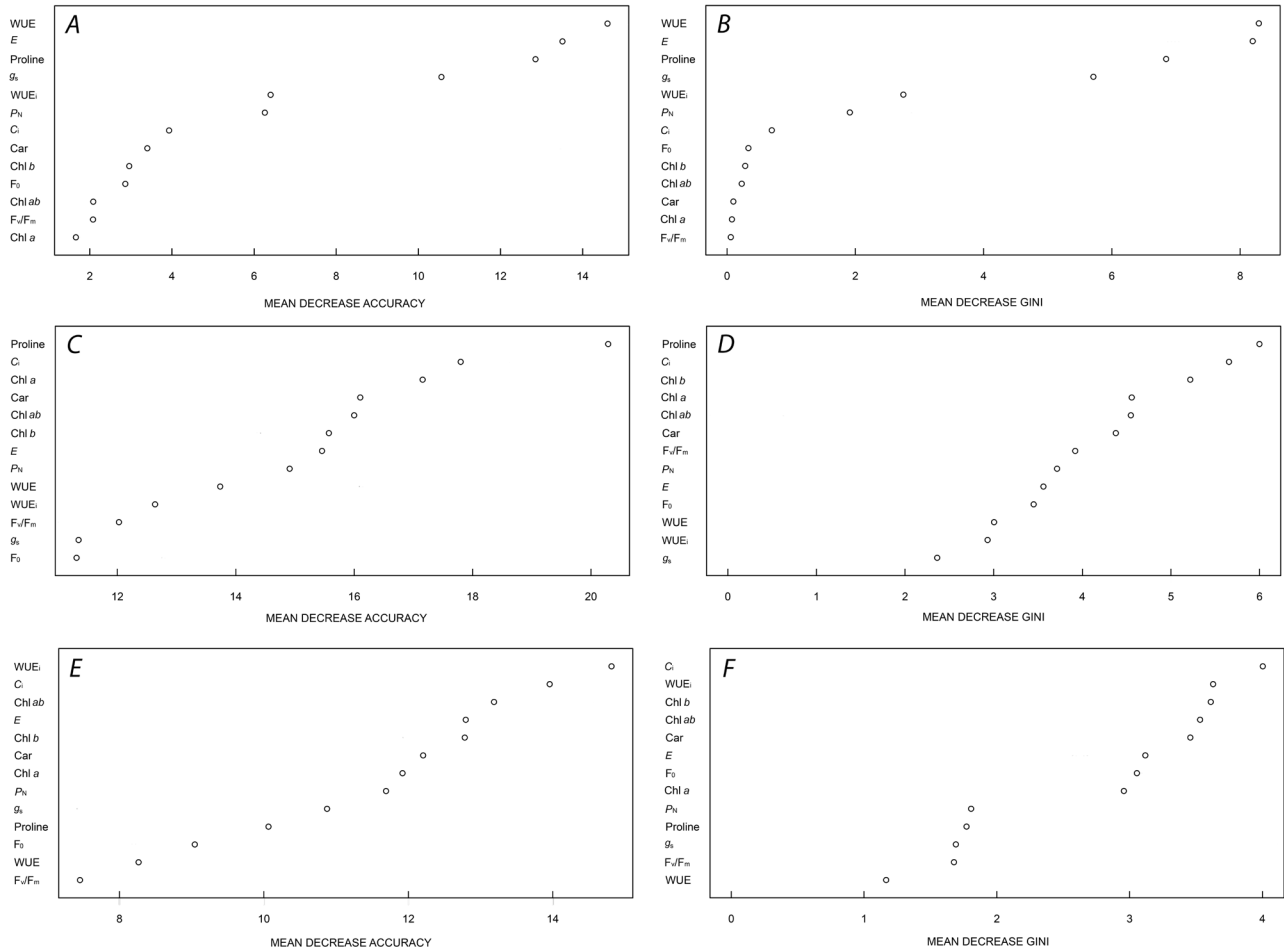


Fig. 3. Variable importance based on Mean Decrease in Accuracy and Gini calculation for chronic stress. Graphs were plotted for different predictors in analysis such as treatment (A,B), population (C,D), and species (E,F). Mean Decrease in Accuracy – the more the accuracy of the Random Forest decreases due to the exclusion of individual variable, the more important that variable is considered. Mean Decrease in Gini – the mean decrease in Gini is a value of how each variable contributes to the homogeneity of the nodes and leaves in the resulting Random Forest. Chl a(b) – chlorophyll a(b); C_i – intercellular CO₂ concentration; E – transpiration rate; F₀ – minimal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; HT – high temperature; P_N – net photosynthesis; WUE – water-use efficiency; WUE_i – intrinsic water-use efficiency.

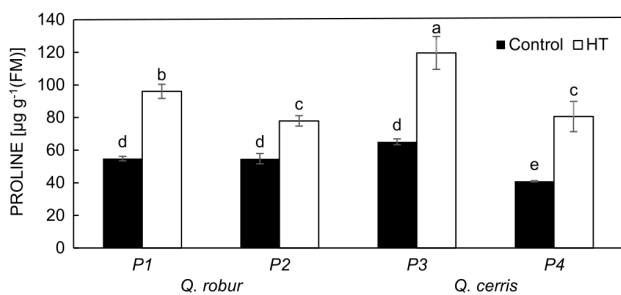


Fig. 4. Proline contents in leaves of oak plants after chronic exposure to HT stress. P1–P4 present the oak population evaluated in this study. Results are presented as average ± SD (n = 3). Different letters indicate significant differences between populations and treatments according to Fisher's test (p < 0.05).

of drought and summer heat waves on beech population at Fruška Gora Mountain. Observed population was located at the top of the ridge where drought conditions and

heat waves are very prominent during summer months, precipitation is poorly held by the soil, since surface runoff is high. Such adverse microhabitat conditions significantly determined photosynthesis and other physiological parameters in forest populations.

High temperature (HT) stress and photosynthesis: Generally, the decrease of photosynthesis was one of the main physiological signs of HT stress. Following the acute as well as chronic exposure to HT stress, photosynthetic rates were significantly lower among the oak population, in comparison to control group of plants. Scafaro *et al.* (2010) suggested that intensity of photosynthesis is one of the parameters which can indicate the heat tolerance of plants. Among all oak populations investigated, physiological reaction to HT in populations of P1 (*Q. robur*) and P3 (*Q. cerris*) might imply a beneficial acclimatization to adverse conditions. We assume that the decrease of photosynthesis after 2-d treatment was regulated by stomatal

closure which caused the diffusional limitation of CO₂ assimilation. A significant positive correlation was detected between stomatal conductance and the rate of photosynthesis (Table 3S). On the other hand, stomatal limitations might provoke the impairment in PSII photochemistry and thus lead to the decline of measured gas-exchange parameters as reported by Pšidová *et al.* (2017). Our results indicated that after acute exposure to HT, g_s decrease was observed at investigated oak population. However, after prolonged exposure to HT, the contrary phenomenon was detected, namely the significant increase of g_s . This acclimation of plant response was possible in relation to unlimited water supply provided by well-watered growth conditions. Therefore, the decrease in photosynthesis after prolonged HT stress, which was not very pronounced, is the consequence of its nonstomatal limitation. Similar results were obtained by Camejo *et al.* (2005) on tomato plants under HT stress: nonstomatal limitation and decrease in Rubisco enzyme capacity decreased the photosynthesis, when g_s and C_i were enhanced. C_i was significantly higher among the experimental populations in comparison to the control groups even following the second and fifth days of HT, which was due to the decrease of photosynthesis and consequential low utilization of CO₂. The phenomenon can also be related to the stomata openness (after 5 d) and undisturbed gas-exchange processes. Considering these results, it can be concluded that the decrease of photosynthesis was not a consequence of the low CO₂ availability, which means that nonstomatal regulation occurred. These results are in accordance with the research of Zhang *et al.* (2001), namely in case of *Quercus liaotungensis* at the temperature of 40°C, inactivation of mesophyll carboxylation along with photorespiration resulted in C_i rise, even if g_s and E were low. In contrary to our results, C_i decrease was detected due to heat stress, according to Wang *et al.* (2010) and Hassan (2006), in case of *Triticum aestivum* plants and in case of *Populus simonii* plants (Song *et al.* 2014). Regarding the present research, negative significant correlation between C_i and photosynthesis, as well as C_i and WUE were observed (Table 3S). It could be assumed that an increase in CO₂ occurred in the intercellular space as a consequence of photosynthesis decrease, since there was not reduction by CO₂ assimilation. Similarly, the correlation between C_i and WUE follows the same logic of explanation.

High temperatures during growing seasons, often combined with a rise in atmospheric CO₂ and humidity deficit, may limit carbon assimilation because of stomatal limitation on photosynthesis as well as photoinhibition of the photosynthetic apparatus (Battipaglia *et al.* 2014). The rise in atmospheric CO₂ concentration as well as severe water shortage affect plants, which respond directly by increasing net carbon assimilation and reducing stomatal conductance (Knauer *et al.* 2017). Since the stomata tend to close under increase of atmospheric CO₂ (Vitale *et al.* 2008), the main mechanism for saving water is an enhancement of WUE_i. In order to define regulatory mechanisms involved in stomatal response towards disturbed abiotic factors as high temperature and variable CO₂ concentration on carbon assimilation (Arena *et al.*

2008, Vitale *et al.* 2012), we calculated intrinsic water-use efficiency as the relationship between net photosynthetic assimilation and stomatal conductance (Gilbert *et al.* 2011). Feng (1999) reported that WUE_i of the most investigated trees increased with increasing atmospheric CO₂ concentrations which was caused by an increase of carbon assimilation rate and/or decrease in g_s . When a decline in stomatal conductance is accompanied with an increase in carbon assimilation, gradient between CO₂ of surrounding atmosphere and that of internal leaf (C_i) correspondingly changes and determines photosynthesis (Huang *et al.* 2017). Further, HT depending decrease of PSII photochemistry was less noticeable than that of net photosynthesis, which indicates that electrons might flow to the alternative sinks and photorespiration could take place. These findings are in agreement with several other studies (Crafts-Brandner and Salvucci 2000, 2002). Tremendous damage to photosystems usually occurs at very high temperatures. A stability of thylakoid membrane of PSII reaction centers remains preserved at temperature up to 45–47°C, as evidenced for *Q. pubescens*, *Q. cerris*, and *Q. canariensis* (Haldimann and Feller 2004, Daas *et al.* 2008).

We obtained a reduction of a net photosynthesis ranged from 40 to 90% in stressed plants after acute HT exposure. Acclimatization of examined *Q. robur* and *Q. cerris* populations was evident after chronic exposure, minimizing the shifting of net photosynthesis to 20–50% between treatments. These findings are in accordance with the results of Daas *et al.* (2008) who have reported such acclimation of photosynthetic apparatus in different oak species. They have found that critical temperature might be highly plastic, depending on initial ambient temperature before the experiments have started. However, it is well-known that heat positively affects photosynthesis to a certain extent. Regarding bean, for instance, increase of photosynthesis was registered up to 35°C (Pastenes and Horton 1996a). In accordance, the research data of Avola *et al.* (2008) indicated that if water is not a limiting factor, photosynthesis increases simultaneously with temperature increase within natural temperature limits (27–32°C). Results obtained in our research point to some level of acclimatization in the oak populations after chronic exposure. However, photosynthetic rates measured on stressed plants were significantly lower comparing to the respective controls, indicating that maximal air temperature of 47°C is too high even under sufficient water supply.

Photosynthetic pigments: The results of the experiments indicated significant changes in photosynthetic pigments regarding HT treatments in oak populations. Regarding investigated populations, P1 (*Q. robur*) and P4 (*Q. cerris*), a significant pigment concentration rise along with the rise in temperature was detected. These results showed that Chl biosynthesis was affected by heat to some extent. Dhyani *et al.* (2013) examined the reactions of *Triticum aestivum* genotypes under terminal heat stress and concluded that changes in concentration of Chl are very important for marking and defining heat-tolerant genotypes. Camejo *et al.* (2006) observed stable Chl concentration on

Nagcarlang genotypes of tomato under heat stress as well as the significant increase in Car. Regarding our data, the increase in Car concentration was also observed. Contrary to these results, Chl and Car concentration decrease in wheat (*Triticum aestivum*) was observed by Balouchi (2010), Wang *et al.* (2010). Similarly, Jin *et al.* (2016) also recorded a heat stress caused significant reduction of Chl content in *Portulaca oleracea*. According to their results, high temperature inhibits the Chl biosynthesis. The main effects which contribute to the decreased Chl content is destruction and inhibition of several enzymes essential in Chl biosynthesis, such as protochlorophyllide reductase and magnesium-chelatase subunit (Farhad *et al.* 2011). The accumulation of Chl is one of the parameters which can be used to determine the thermotolerance between genotypes (Selvaraj *et al.* 2011).

High temperature (HT) stress and plant water management: The different rates of transpiration were obtained between populations following acute HT stress. General physiological response of populations P3 and P4 (*Q. cerris*) to temperature rise was an increase of transpiration, while in case of P1 and P2 populations (*Q. robur*), lower transpiration was detected as a consequence of stomatal closure. Song *et al.* (2014) claimed that following HT stress, significant transpiration decrease was observed in a case of well-watered (relative humidity set to 50±1%) poplar plants. These results are in accordance with our experiment, mainly in the case of both *Q. robur* populations. High transpiration rates of *Q. cerris* populations indicated their faster adaptive metabolic response to heat stress.

However, after 5 d under HT treatments, each investigated populations showed a significant increase in transpiration, which was related to an adequate water supply of plants. The atmospheric vapor pressure deficit is the evaporative driving force for water transport and associated CO₂ photosynthetic consumption. Therefore, stomatal response to vapor pressure deficit significantly altered plant water and general physiological status.

Intensive transpiration, which lowered the temperature of the leaves, indicated undisturbed physiological processes of gas exchange, *i.e.*, there was no disruption in the cell membrane structure. The same results were observed in the article of Wang *et al.* (2010), when heat stress resulted in opening stomata and increasing transpiration of *Triticum aestivum*, *Phaseolus vulgaris* (Pastenes and Horton 1996b), *Zea mays* (Crafts-Brandner and Salvucci 2002), *Lycopersicon esculentum* (Camejo *et al.* 2006), and *Picea abies* (Filella *et al.* 2007).

A significant WUE decrease in plants from all populations, related to adequate water supply, intensive transpiration, and reduced photosynthesis, was detected for HT treatments. The highest values of WUE were registered in a *Q. robur* P1 population, which indicates that this population is effective in overcoming heat stress. Craufurd *et al.* (1999) has also observed WUE decrease in case of HT stress on *Arachis hypogaea* when water was not limited. Similarly, Cui *et al.* (2006) in their research on two *Festuca arundinacea* cultivars obtained reduction of WUE after 10 and 20 d of heat treatment. Our findings indicate

that WUE positively correlates with photosynthesis (Table 3S), so the increase of photosynthesis contributed to the increase of WUE after 5-d exposure to HT. Song *et al.* (2014) came to the opposite results by experimenting with poplars. Applying HT stress at 50 ± 1% of relative humidity, a significant rise of WUE was detected over 3, 6, 12, and 24 h, which can be explained by the decreased stomatal conductance and transpiration. Explanation for such inconsistency might be due to the phenomenon of transpiration stimulation by regulated stomata opening.

Proline content significantly increased due to heat stress. As known, proline is an osmoprotectant that is produced under heat stress following the expression of certain genes with the important role of protecting the cells from damage (Hasanuzzaman *et al.* 2013). Our obtained results confirmed this fact. Correia *et al.* (2014) also detected high content of proline at *Quercus suber* grown at high temperature between 45–55°C, which lowered (photo) damages in the thylakoid membranes by scavenging and/or reducing the production of superoxide free radical. The increased proline concentration under heat stress can be explained not only by the increased proline synthesis, but also by the decrease of proline degradation. Jansen *et al.* (2014) analyzed changes in growing rates and metabolite profiles of the Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] from different proveniences due to the increased temperature and atmospheric drought. They observed the influence of heat on the growth rate and concluded that heat waves induced a switch in stress-protecting compounds from proline to polar low molecular metabolites – polyols, pinitol, and ononitol, which also act as ROS scavengers.

Multivariate interactions of gas-exchange parameters: Interpretation of data from univariate analysis has some limitations, regarding the lack of interaction analyses between measured variables. Principal component analysis (PCA) allows researchers to identify and explain variation among groups in a large dataset through simultaneous consideration of multiple parameters (Polutchko *et al.* 2018). By employing PCA in order to distinguish functional relations between investigated parameters, we tried to define which population/species would be the most suitable for tolerating more and more frequent hot summer temperatures.

Exposing plants to elevated temperature led to a separation of experimental groups. Segregation of experimental groups after initial exposure to HT should be primarily attributed to changes in stomatal conductance, and subsequently decrease in net photosynthetic rate and WUE parameter. Contrary, 5 d of exposure to elevated temperature changed roles of main factors which are influencing the segregation, defining WUE and WUE_i as major influencers. When it comes to plotting the populations as factor setup, it could be emphasized that *Q. cerris* P3 and P4, both from Fruška Gora Mountain, have much smaller variance within the groups. Such characteristics might be very valuable since these populations are reacting to adverse environmental conditions with much more consistency, regarding measured photosynthetic parameters. Also, it

is interesting that P1 was clustered oppositely to other three investigated populations. Its specific segregation might be a result of emergence and occurrence in a very specific habitat, with lowest altitude and periodic flooding. This might be a reason for much efficient water-use strategy, when exposed to HT, in comparison to other tested populations. Besides PCA, Random Forest analysis was utilized to screen the variables since it is easily applicable method for classification and regression problems. RFA is an appropriate statistical tool due to its high classification accuracy, the ability to characterize complexity of interactions between variables, and furthermore to determine variable importance (Breiman *et al.* 1984). By defining the most important variables, we tried to determine which parameter should be considered as the most valuable primarily for the population selection. Even though soil water content could be low during hot summers, we tried to establish an unfavorable environment condition only with extremely high air temperature in order to evaluate the effects of this particular factor on selected oak populations. Analysis output showed a different importance based on the fact what level was chosen as factor (treatment/population/species), which was expected especially in case of population and species. If we are concerning populations as a specific group of individuals from the same species, differences in response of individual populations to changed environmental conditions may occur. According to the results of Random Forest algorithm, at the level of population, the most important traits which are defining those populations were P_N , WUE, and F_0 for acute exposure to HT, and proline together with C_i for chronic conditions. Other gas-exchange parameters were also significant, but their importance for population selection was a bit lower. When it comes to the species level, C_i , WUE, and F_0 would be the most important parameters in oak species-based selection for habitats with elevated temperature.

Conclusions and perspectives: Investigated oak species did not differ significantly in specific response to elevated temperature. On the contrary, population reactions seem to be very distinct, including *Q. robur* P1 (44.9725707°N, 19.2212677°E; altitude of 86 m) from the rest as a very efficient population, with the highest P_N and WUE under harsh conditions. The high environmental temperature induced a reduction of a net photosynthesis, ranging from 40 to 90% in 8-month-old plants after 2 d of exposure (acute), while an acclimatization of examined *Q. robur* and *Q. cerris* populations were evident after 5 d of exposure, minimizing the shifting of net photosynthesis to 20–50% between treatments. Increase in the net photosynthetic rate after prolonged (chronic) temperature stress applied, indicated preserved functional stability of thylakoid membranes and PSII reaction centers, even when leaf temperature continued to rise. Results pointed out that an acute HT led to stomatal closure and subsequent inhibition of photosynthesis, while a chronic HT stress led to an increase in stomatal conductivity and inhibition of photosynthesis at the level of carboxylation.

Regarding results of Random Forest algorithm, the

most important adaptive metabolic response to supra-optimal ambient temperatures, which separate populations according to their tolerance specificity, were P_N and proline content. Other gas-exchange parameters have also some significance, but their importance was much lower. The most important physiological parameter, which should be considered in selection for genotypes tolerant and adaptive to habitats with elevated temperatures, was transpiration. Concerning all investigated variables, functional relations and analysis factor setup, it can be concluded that specific oak population reaction to adverse temperatures can provide essential information for future reforestation strategies. Since populations originated from specific habitats, they already have the adaptive potential, allowing them to adapt/acclimatize faster to different stress factors in changing environment. Future research should be focused on associated high temperature and drought related stresses, since they very often act simultaneously.

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