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PHYSIOLOGICAL RESPONSES OF TWO PEDUNCULATE OAK (*QUERCUS ROBUR* L.) FAMILIES TO COMBINED STRESS CONDITIONS – DROUGHT AND HERBIVORE ATTACK

FIZIOLOŠKI ODGOVOR DVIJE FAMILIJE HRASTA LUŽNJAKA (*Quercus robur* L.) NA KOMBINACIJU STRESA – SUŠA I DEFOLIJATORI

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SUMMARY

Pedunculate oak (*Quercus robur* L.) is economically and ecologically one of the most important tree species in lowland forests of Southeastern Europe, and it is endangered by numerous biotic and abiotic factors. In this study, we investigated the effect of drought and herbivore attack of gypsy moth (*Lymantria dispar* L.) on two families of young oak seedlings subjected to the following treatments: drought (D); gypsy moth (GM); both drought and gypsy moth (D+GM) and control (Ø) for a period of 15 days followed by a 7-day recovery period. During both treatment and recovery, physiological parameters - net photosynthesis (A), transpiration (E), stomatal conductance (gs), sub-stomatal CO_2 concentration (Ci), water use efficiency (WUE), nitrate reductase activity (NRA) and chlorophyll content (Chl) were measured. Our results showed significant effects of stress factors on physiological processes in oak seedlings which could have potential impact on forest regeneration. Also, differences in the reaction between investigated families indicated the need for breeding and selection of more resistant progenies and provenances of pedunculate oak.

KEY WORDS: pedunculate oak, photosynthesis, WUE, SPAD, nitrate reductase activity

INTRODUCTION
UVOD

As one of a vast number of oak species, pedunculate oak (*Quercus robur* L.) is a species with a wide geographic distribution, starting from western Asia to Europe with reported scattered stands in Mediterranean regions (Gil Sanchez et al., 1994) that grow along the valleys of the major rivers in clear stands or mixed communities with narrow-leaved ash, hornbeam and elms. The pedunculate oak forests in regions of Slavonia and Srem in Western Balkans are well known all over the world for their valued quality wood for different industrial purposes. Nowadays climate change and regulation of watercourses significantly affect the vital-

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ity of oak stands due to limitations in water availability (Stojanović et al., 2014; Stojanović et al., 2015; Kostić et al., 2019). Pedunculate oak is an especially interesting species for breeding and selection programs, taking into consideration its ecophysiology. Dependence of this species on soil water properties plays an uncertain role in its survival and stress response and, therefore, a focus on ecophysiology of this valuable broadleaved species should be a priority in research, breeding and forest management.

Survival and distribution of sessile organisms such as plants depend strongly on their ability to adjust to environmental variation (Beikircher and Mayr, 2009). Water stress, especially drought stress, is the main restriction of plant growth and development (Hu et al., 2004) where competition for water with mature trees may exacerbate drought effects on understory tree seedlings (Aranda et al., 2005). Water shortage and drought periods present limiting factors for forest regeneration in modern silvicultural practice by weakening the seedlings and affecting their performance at several levels. Besides direct effects on whole plant water status, drought causes reduction in stomatal aperture that diminishes CO_2 supply to mesophyll cells, thus causing a reduction in photosynthetic rate (Blödner et al., 2007). However, stomatal limitation is not the only reason for decreased photosynthesis in drought-affected plants. According to Gallé et al. (2007) and the references within, the stomatal limitation predominately affects photosynthesis in plants under moderate drought conditions, whereas in severe drought metabolic limitations become dominant. Various parameters can be assessed in order to be more precise about whether the drought-induced photosynthetic decrease results from stomatal or metabolic limitations, such as chlorophyll a fluorescence (Gallé and Feller, 2007; Arend et al., 2013; Arend et al., 2016; Vastag et al., 2020), chlorophyll content (Gallé et al., 2007; Arend et al., 2012), biochemical markers (Gallé et al., 2007; Stojnić et al.,2019a) or even different non-structural carbohydrates and carbon isotope signatures (Pflug et al., 2018).

Among a vast number of factors, insects present an important biotic stressor that affects plant growth and vitality (Drekić et al., 2019). Outbreaks followed by and combined with climate extremes (e.g. temperature, precipitation, drought) will become more frequent due to the increase of greenhouse gas levels in the atmosphere. Plants, as sedentary organisms, cannot escape from attacks and stress and have to adjust to the surrounding environment and biotic attacks through their life cycle (Niinemets et al., 2013). Decreases in total leaf number and area are not the only response of plants to defoliation (Meyer, 1998; Poljaković-Pajnik et al., 2019), as grazing injury may also include a vast spectrum of metabolic and physical changes in host plants (Oleksyn et al., 1998), including enzymatic activities, gas exchange processes or accumulation of different metabolites like proline. Considering it as a significant determinant of plant growth, yield and fitness (Welter, 1989), plant gas exchange and influence of insects on it, present a significant aspect for further research in selection programs. On the other hand, close correlation of nitrogen metabolism with photosynthesis (Marschner, 1995) highlights nitrate reductase activity (NRA) as a good parameter for investigation since it is the first enzyme in nitrogen metabolism (Kastori and Petrović, 2003). Also, nutrition levels and the presence of allelochemicals in plants determine plant suitability and resistance to insect herbivores (Chen et al., 2011).

Gypsy moth (*Lymantria dispar* L.) is the most significant pest of hardwood forests in the northern hemisphere (Elkinton and Liebhold, 1990). The species naturally occurs in Eurasia but is also introduced in Northern America. Gypsy moth populations have occasional outbreaks lasting 4 to 5 years (Mihajlović, 2008) when caterpillars cause damage during leaf feeding on almost all forest tree species, except ashes. For its development, gypsy moth mostly prefers pure oak stands, with emphasis on Turkey oak (*Q. cerris* L.) (Milanović, 2006). Defoliation often causes losses in increment and acorn yield, as well as physiological weakening and susceptibility to secondary pests. In the case of consecutive defoliation, increases of increment loss and mortality occur (Mihajlović, 2008), where increment loss may range from 30-70%, according to Mirković and Miščević (1960).

Considering the importance of pedunculate oak for forest biodiversity and ecosystem restoration initiatives, constant efforts for its *ex situ* and *in situ* conservation through research of genetic diversity are of profound importance in order to mitigate climate change (Stojnić et al., 2019b). Apart from these efforts, breeding of more resilient provenances presents one of the efficient ways for future adaptation of forest tree species to climate change, since adaptation enables plants to optimize their life processes in prevailing environmental conditions at an evolutionary scale (Beikircher and Mayr, 2009). In order to achieve these long-term goals of pedunculate oak conservation and selection projects, integrating different research fields such as plant physiology, genetics and entomology presents a holistic, interdisciplinary solution that is appropriate for current and upcoming environmental challenges caused by climate change. Also, success in tree breeding can be facilitated by increased understanding of the physiology of growth and survival during water supply limitations (Wikberg and Ogren, 2007). Therefore, the goal of this research was to determine the simultaneous effect of drought stress and/or attack by caterpillars on the physiological traits of different families of pedunculate oak seedlings, during the stress period and after recovery.

MATERIALS AND METHODS
MATERIJALI I METODE

Acorns of two families of pedunculate oak (*Quercus robur* L.) were collected from two single trees (Nos. 2 and 7) in a seed stand located at the territory of Public Enterprise Vojvodinašume; Forest Estate Sremska Mitrovica; Forest Management Unit Morović; during autumn 2017 and stored in a cooling chamber until March, when seeds were sown in 0.5-liter pots with soil medium consisting of a peat:sand:soil mixture of equal volumes (1:1:1). Prior to sowing, seeds were soaked in water for 24 hours in order to enhance germination. Plants were grown in the laboratory under constant light conditions until the beginning of May and then placed outside in insect cages where they were exposed to treatments. Before the start of the treatments, plants developed 5 to 10 leaves with height ranging from 15 to 35 cm.

The treatments included drought (D), damage caused by gypsy moth (*Lymanthria dispar* L.) (GM), and a combination of both stress factors (D+GM). Drought treatment (D) was preceded by a 15-day consecutive decrease of soil moisture to reach value of 30% of field capacity prior to the beginning of the treatment. Treatment with gypsy moth (GM) included 3 pieces of instar 2 caterpillars which were added to the plants in each pot. Both stress factors (D+GM) included addition of caterpillars to the drought stressed plants, while control treatment used well-watered plants without damages caused by the insects. Treatment lasted for 15 days followed by a 7-day recovery period. During recovery period, drought treated plants were irrigated to obtain optimal field capacity between 70 and 90%, while caterpillars were removed from the affected plants. Measurements of physiological factors were performed at the end of the 15-day treatment period and repeated after the 7-day recovery period. Measured parameters included gas exchange, pigments content and nitrate reductase activity (NRA). Each family had 10 pots per treatment from which four plants were selected for gas exchange measurements. Gas exchange measurements were made with a portable photosynthesis system (LCPro+, ADC Bioscientific, UK) under controlled constant light conditions of 1000 μmol m^{-2} s^{-1} and constant ambient air supply of 100 μmol s^{-1}, with ambient levels of humidity and temperature. Measurements were performed on a fully developed leaf from the first set of leaves. For the gas exchanged measurement on insect damaged plants, selected leaves had a minimum of 50% damage with sufficient leaf blade area remaining for gas exchange measurements. Instantaneous water use efficiency (WUE [μmol $mmol^{-1}$]) was computed as the ratio of net photosynthesis to transpiration (A/E) (Farquhar et al., 1989). Chlorophyll content was determined with the use of a portable chlorophyll meter (Minolta SPAD-502, Tokyo, Japan) on the same leaves where gas exchange measurements were performed. The SPAD values were converted to chlorophyll content (μg cm^{-2}) according to Cerovic et al. (2012): Chl = (99 × SPAD value) $(144 - \text{SPAD value})^{-1}$. The *in vivo* NRA in leaves was assayed using the spectrophotometrical method of Hageman and Reed (1980) and expressed as μmol NO_2^- g^{-1}_{FW} h^{-1}.

All statistical analyses were performed by STATISTICA software, version 13 (TIBCO Software Inc, 2017). Nested analysis of variance (ANOVA) was computed for each trait in order to evaluate relative importance of the following sources of phenotypic variation: date of measurement (the difference between performances of stressed and recovered seedlings), treatment nested within date (treatment effect during stress and recovery), family nested within date*treatment (genetic variation for seedlings response to stress and recovery). Significant differences were determined at $p \leq 0.05$.

RESULTS
REZULTATI

Response of physiological parameters to stress

Induced stress disturbed nitrogen metabolism in both investigated oak families. However, significant decrease of NRA was recorded in family 2, especially during drought treatment. During treatment, in family 2 values decreased by 55.1, 59.2 and 80.5% in plants exposed to GM, D+GM and D, respectively (Chart 1), compared to control. On the other hand, family 7 did not show significant decreases in NRA in all tested treatments although there were even increases in enzymatic activity in treatments D and GM. Net photosynthesis (A) decreased in both families (Chart 1). Significant decrease of A ranging from 42.9 to 61.2% compared to controls was recorded in both families in all treatments. Even though GM treatment had lowest values in both families, it did not significantly decrease compared to other stress treatments. Sub-stomatal concentration of CO_2 (C_i) showed significant increases in GM- and D+GM-treated plants from family 2 (295.4 and 281.1 μmol μmol^{-1}, respectively) and GM and D treated plants from family 7 (340.7 and 315.9 μmol μmol^{-1}, respectively) (Chart 1). Gypsy moth attack decreased transpiration rate of both oak families during the treatment period (Chart 1), regardless if it was sole treatment (GM) or in combination with drought (D+GM). However, the decrease of stomatal conductance of family 2 D+GM plants was not significant (0.092 mol m^{-2} s^{-1}). Within families, the change in WUE values (Chart 1) was not significant between the treatments, although decreases were recorded in plants of family 7 under separate effects of gypsy moth and drought (34.7% and

28.0%, respectively). Gypsy moth and water deficit differentially affected chlorophyll concentration in treated oak families (Chart 1). Values were lower in GM-treated family 2 plants versus the control. However, considerable changes in family 7 were evident only in plants under treatment D.

Recovery of physiological parameters after recovery period

Recovery period of all plants affected by stress factors (GM, D or D+GM) caused an increase in NRA of investigated families, both compared to the control and the measurements performed on stressed plants during the duration of the treatment (Chart 2). When compared with control, the increase was lowest for the GM treatment (49.3% for family 7 and 48.7% for family 2) and highest for the D+GM-treated plants (78.5% for family 7 and 73.3% for family 2) versus the control plants. Due to the increase of A in control plants in families 2 and 7 after recovery (from 6.59 to 9.24 $\mu molCO_2 m^{-1} s^{-1}$ and from 6.44 to 7.35 $\mu molCO_2 m^{-1} s^{-1}$, respectively), significant differences were recorded in treated plants after recovery period (Chart 2). After recovery Ci values were significantly higher in plants of family 7 under all stress treatments D, GM and D+GM (Chart 2), while Ci values in family 2 significantly decreased in drought recovered plants compared to control and treatments GM and D+GM. Stomatal conductance remained significantly decreased in D, GM and D+GM plants of family 2, while there were no significant differences between investigated plants of family 7. On the other hand, the transpiration rate remained decreased in all treatments after the recovery period. After recovery, plants from family 7 did not have significantly-decreased WUE, although values for GM, D and D+GM were lower than in control plants (3.13, 2.91, 3.63 and 4.21 $\mu molCO_2 \mu molH_2O^{-1}$, respectively). Affected plants from family 2 had significantly smaller values of WUE in GM treatment (1.65 $\mu molCO_2 \mu molH_2O^{-1}$) when compared to control plants from the same family (Chart 2). Watering of treated plants substantially affected chlorophyll content of the oak family 2 (Chart 2). Recovered plants under D and GM treatment had significantly lower chlorophyll content when compared to control (26.8, 25.0 and 34.5 $\mu g\ cm^{-2}$, respectively). Chlorophyll content of recovered plants of family 7 did not differ significantly from the control plants.

Influence of the family, treatment, recovery and their interaction on the investigated physiological parameters

In most of the investigated parameters, origin of the seed (family) showed significant influence on the results. Nested ANOVA results (Table 1) showed significant impact of all calculated factors for NRA: origin ($F=7.76^{***}$), treatment ($F=26.31^{***}$) and recovery ($F=665.0^{***}$). Analysis of variance showed that the effect of treatment was only significant for A ($F=38.50^{***}$ at $p<0.001$), while the recovery period (date) and families showed no significant effect on net photosynthesis ($F=3.51^{ns}$ and $F=1.17^{ns}$, respectively). On the other hand, sub stomatal concentration of CO_2 (C_i) followed a similar pattern as A only for recovery time which had no effect on C_i ($F=0.57^{ns}$), but origin of seedling significantly affected Ci ($F=28.24^{***}$ at $p<0.001$). Transpiration rate (E) and stomatal conductance (g_s) were both affected by the treatment and dependent upon the origin of plants (Table 1), while recovery period did not show an effect on g_s ($F=0.06^{ns}$). Analysis of variance of WUE of treated plants (Table 1) showed significant impact of all three factors: recovery time ($F=16.21^{***}$), treatment ($F=6.30^{***}$) and origin of plants ($F=8.22^{***}$). Results of nested ANOVA (Table 1) showed significant effects, both of treatment ($F=11.44^{***}$) and family ($F=10.04^{***}$), while recovery period did not significantly affect chlorophyll content of the trees.

DISCUSSION
RASPRAVA

Vegetation responses to environmental conditions are mediated by a suite of functional traits affecting water relationships, resource acquisition and other aspects of plant function (Manzoni, 2014). Increase in photosynthetic rate commonly occurs following defoliation events, which is considered a photosynthetic up-regulation caused by the disturbance of a source:sink ratio and increased demand for carbohydrates to rebuild crowns (Pinkard et al., 2007). Various authors found opposite plant photosynthetic responses during defoliation. Peterson et al. (1996) found no alteration of photosynthesis in hardwoods under pest attack, while Turnbull et al. (2007) recorded increased photosynthesis in leaves of eucalyptus (*Eucalyptus globulus* Labill.) under partial defoliation. Decreased photosynthesis was recorded under defoliation treatments in this study.

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

Tablica 1. Rezultati analize varijance po ispitivanim parametrima

Effect	df	A	Chl	E	g_s	C_i	NRA	WUE
Date	1	3.51^{ns}	2.99^{ns}	31.61^{***}	0.06^{ns}	0.57^{ns}	665.0^{***}	16.21^{***}
Treatment (date)	6	38.50^{***}	11.41^{***}	24.13^{***}	14.29^{***}	25.03^{***}	26.31^{***}	6.30^{***}
Family (date*treatment)	8	1.17^{ns}	10.04^{***}	12.34^{***}	4.91^{***}	28.24^{***}	7.76^{***}	8.22^{***}

Legend: ^{ns} non-significant; *** $p<0.001$.

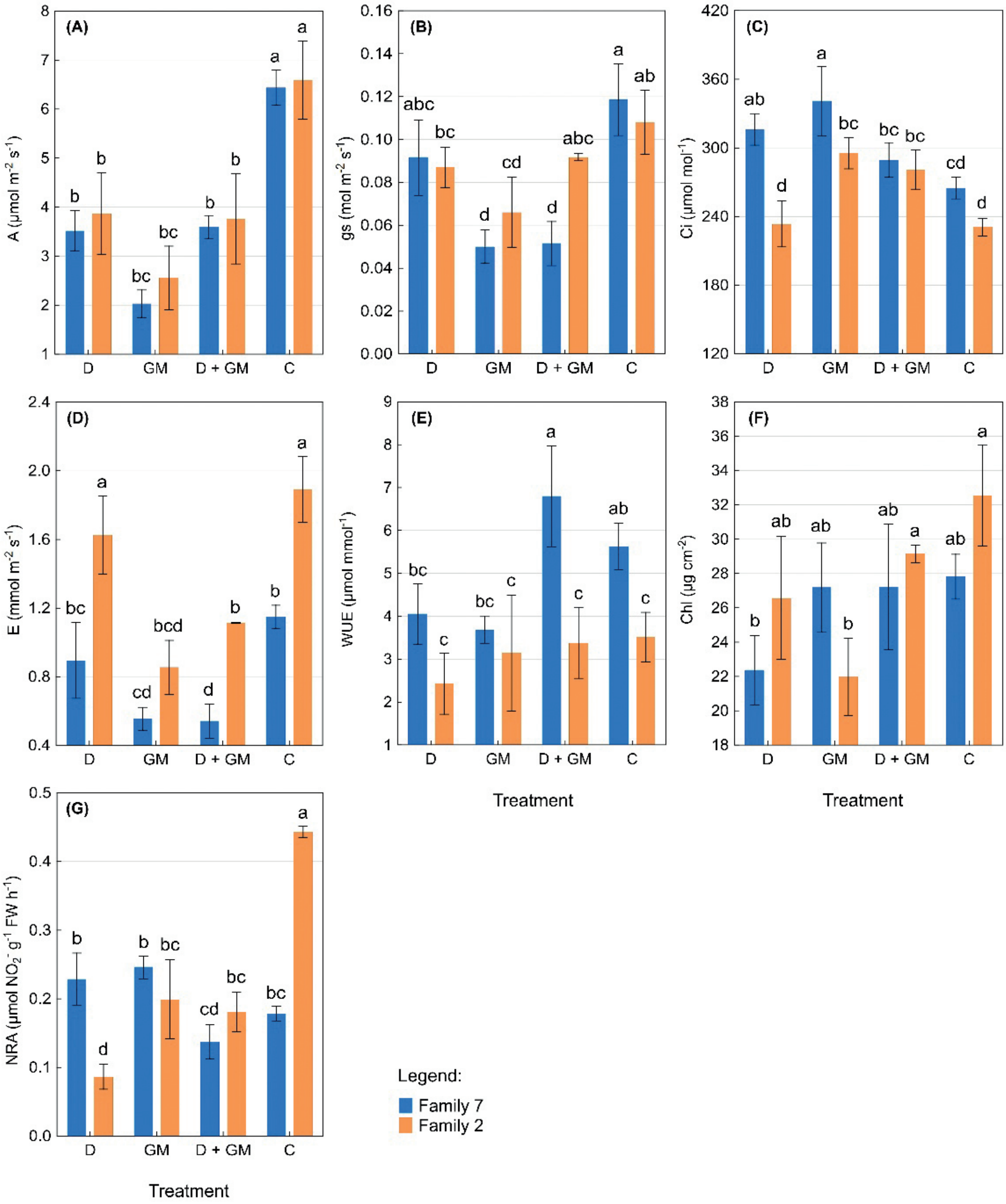

Chart 1. Results of measurement of physiological parameters of investigated families during the induced stress. Net photosynthesis (A), chart A; stomatal conductance (gs), chart B; sub-stomatal CO2 concentration (Ci), chart C; transpiration rate (E), chart D; water use efficiency (WUE), chart E; chlorophyll content (Chl), chart F; nitrate reductase activity (NRA), chart G. (vertical bars with the same letter did not differ at $p=0.05$; vertical bars include standard deviations)

Graf 1. Rezultati mjerenja fizioloških parametara dviju istraživanih familija hrasta lužnjaka izloženih različitim tretmanima na vrhuncu stresa. Stopa neto fotosinteze (A), (prikaz A); stopa provodljivosti puči (gs), (Prikaz B); intercelularna koncentracija CO2 (Ci), (prikaz C); stopa transpiracije (E), (Prikaz D); učinak iskorištenja vode (WUE)(prikaz E); sadržaj klorofila (Chl), (Prikaz F);, aktivnost nitrat reduktaze (NRA), (prikaz G);, (Vrijednosti s istim slovima se nisu signifikantno razlikovale pri $p=0{,}05$, vertikane linije predstavljaju standardnu devijaciju)

Our results are in concordance with Aldea et al. (2006) who recorded a decline in photosynthesis of remaining leaf tissue in understory oak saplings, while Schaffer et al. (1997) recorded significant correlation between the damage intensity and net photosynthesis of citrus attacked by leaf miners. Besides a direct reduction of photosynthesis due to less leaf mass, defoliation indirectly reduces photosynthesis and transpiration of leaves by causing damage to vasculatures that supply leaf tissues (Nabity et al., 2009). Decrease of both photosynthesis and stomatal conductance in our study supported the above-mentioned hypothesis. On the other hand, Meyer and Whitlow (1992) found no alteration in photosynthesis and stomatal conductance in goldenrod under attack of leaf beetle, although significant increases in sub-stomatal CO_2 concentration suggested a possible decrease of photosynthetic rate. The decrease in photosynthesis and stomatal conductance in our study was also followed by decreases of Ci under defoliation. Following analogy of the abiotic stress impact (i.e. drought) on the limitation of photosynthesis recorded in various papers (Gallé et al., 2007; Gallé and Feller, 2007; Haldimann et al., 2008; Arend et al., 2013; Arend et al., 2016), increases in Ci could be defined as a metabolic limitation of photosynthetic process of gypsy moth attacked leaves.

The decrease in photosynthesis of young oak seedlings under drought treatment is difficult to interpret and generalize because, besides the factor of treatment and date, the origin of seedlings plays a significant role given genetic effect on the physiological performance of the plants. Correlation between the genetic background and leaf gas exchange parameters is very well documented (Orlović et al., 1998), which often causes different physiological responses of the individuals within the same species to unfavorable conditions (Pilipović et al., 2014; Pointeau and Guy, 2014; Bojović et al., 2017; Vastag et al., 2019). Alterations of photosynthetic parameters differed between the families, where decreases in A in family 2 were not followed by increases in Ci like in family 7. Such results indicate possible higher drought sensitivity of family 7, compared to family 2. Similar results were observed by Haldimann et al. (2008) in *Quercus pubescens* L. who hypothesized that drought-induced metabolic limitations and drought-dependent increases in mesophyll resistance to the diffusion of CO_2 were present. As mentioned previously, sub-stomatal concentrations of carbon dioxide (C_i) together with stomatal conductance (g_s) indicate mechanisms and severity of photosynthetic inhibition where decreased C_i is related with stomata and increased C_i is described as a metabolic limitation of photosynthesis. Different studies (Lawlor, 1995; Gallé et al., 2007; Mathobo et al., 2017) confirmed that an intense drought leads to an increase in the values of C_i, while the conditions of a moderate drought result in reduction of C_i. Considering the results of this study, drought treatment affected C_i values in different manners, emphasizing the importance of genetic background in understanding drought responses of tested families. Opposite to photosynthesis, drought did not affect transpiration of investigated oak families. Bréda et al. (1993) concluded that oaks were drought-tolerant species due to their ability to maintain significant transpiration intensity under reduced water availability in the soil. This corroborates results obtained in this study (i.e., transpiration did not decrease under drought treatment).

As expected, the presence of stress factors affected metabolic processes in both investigated oak families. On the other hand, expected synergistic effects of both drought and defoliation were not observed in this study, probably due to the various expressions of simultaneously occurring stressors. According to Copolovici et al. (2014), one type of stress could weaken or enhance the effects of another simultaneous stress factor by direct physiological cumulative or interactive effects. In contrast, La Spina et al. (2010) emphasized a lack of expected parabolic response of herbivore performance to tree water status.

Despite chlorophyll a and b being highly sensitive to decreased soil moisture (Farooq et al., 2009) and that drought-induced reductions in pigment contents were previously found in many woody plant species (Lei et al., 2006; Gallé and Feller, 2007; Guerfel et al., 2009, Arend et al., 2013), chlorophyll contents of tested families did not respond similarly. In family 7 D treatment decreased chlorophyll content, while a significant decrease of pigments in family 2 was recorded only under gypsy moth defoliation (GM). Similar observation also was noted during summer drought in *Q. robur* and *Q. petraea* (Epron and Dreyer, 1993). According to Rahdari and Hoseini (2012), an increase in chlorophyll levels under conditions of environmental stress is one of the resistant symbols in plants that are proportional to stress. Since family 2 had a higher content of pigments under drought treatment than family 7, the results indicated that those plants may have had a higher drought tolerance and provided a stronger photoprotective system against drought stress than plants from family 7.

It is very well documented that NRA generally decreases in leaves of plants subjected to water stress (Kaiser and Förster, 1989; Foyer et al., 1998; Garg et al., 2001). During water deficit, NRA decreases more rapidly than most enzymes (Huffaker et al.,1970) and often presents more sensitive physiological indicators of water stress than either stomatal closure or photosynthesis (Bardzik et al., 1971; Hsiao et al., 1976). The negative effect of water deficit on the activity of this enzyme may result from decreased nitrate reductase protein or decreased activation of the existing protein (Correia et al., 2005). In our experiment, compared to the control, NRA appears to vary significantly in the leaves of family 2 in response to drought treatment. On the other hand, family 7 did not express decrease in any of the treatments. One of the reasons for this may be the fact that compatible solutes may contribute to the maintenance of enzyme activity. According to Smirnoff et al. (1985), accumulation of proline may facilitate the continued synthesis of nitrogenous compatible solutes using excess photochemical energy available when stomata are closed, as recorded in family 7. In addition to carbon metabolism, nitrogen metabolism is also affected by drought stress.

Recovery period still showed a significant decrease of net photosynthesis and transpiration in both investigated fa-

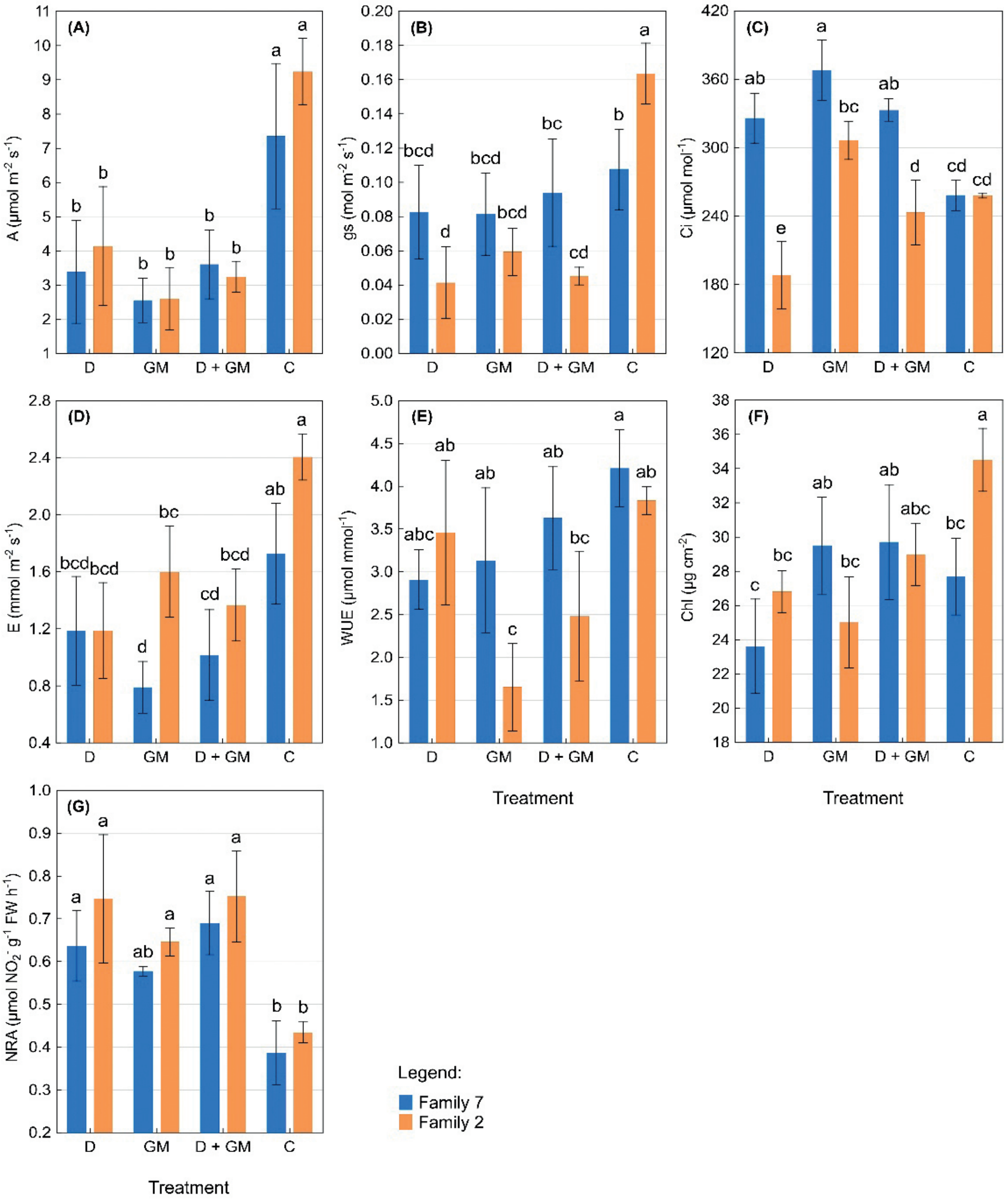

Chart 2. **Results of measurement of physiological parameters of investigated families during after the 7-day recovery period. Net photosynthesis (A), chart A; stomatal conductance (g_s), chart B; sub-stomatal CO_2 concentration (C_i), chart C; transpiration rate (E), chart D; water use efficiency (WUE), chart E; chlorophyll content (Chl), chart F; nitrate reductase activity (NRA), chart G. (vertical bars with the same letter did not differ at p=0.05; vertical bars include standard deviations)**

Graf 2. Rezultati mjerenja fizioloških parametara dviju istraživanih familija hrasta lužnjaka izloženih različitim tretmanima nakon 7-dnevnog oporavka. Stopa neto fotosinteze (A), (prikaz A); stopa provodljivosti puči (gs), (Prikaz B); intercelularna koncentracija CO2 (Ci), (prikaz C); stopa transpiracije (E), (Prikaz D); učinak iskorištenja vode (WUE)(prikaz E); sadržaj klorofila (Chl), (Prikaz F);, aktivnost nitrat reduktaze (NRA), (prikaz G);, (Vrijednosti s istim slovima se nisu signifikantno razlikovale pri p=0,05, vertikane linije predstavljaju standardnu devijaciju)

milies. However, differences between stomatal conductance and intercellular CO_2 concentrations among families were evident, indicating genetic background of their recovery. Compared to control, recovered plants of family 2 showed no increase of Ci, while stomatal conductance remained low, indicating cessation of metabolic limitation of photosynthesis. Decreases of stomatal conductance recorded in family 2 can be attributed to the inability of stomata to completely re-open (Kozlowski, 1982) or to the structural changes in stomata (Gallé and Feller, 2007). The increase of NRA after the 7-day period indicated an ongoing recovery process in the stressed plants, which is consistent with previous studies (Bardzik et al., 1971; Ferrario-Méry et al., 1998; Foyer et al., 1998; Correia et al., 2005).

Although at first glance various research (Gallé et al., 2007; Gallé and Feller, 2007; Haldimann et al., 2008; Arend et al., 2013; Arend et al., 2016) indicated slightly opposite results on the recovery of the forest tree species after stress, deeper analysis of the both obtained and quoted results show similarities and concordance. Arend et al. (2013) showed that the drought affected the physiological processes and their recovery in *Q. robur* to a greater extent than in *Q.petreae* and *Q. pubescens*. Net photosynthesis and chlorophyll content were strongly affected by drought, and the recovery period was prolonged. Comparing our results with aforementioned research, it can be suggested that the 7-day recovery period was not sufficient for oaks to restore their physiological processes to optimum. In most cases, recovery period can vary upon the origin of the seedlings where provenances from xeric sites had shorter recovery period (Arend et al., 2016), or in some cases, atmospheric conditions such as elevated CO_2 can accelerate recovery of the photosynthesis (Gallé et al., 2007). Sometimes even when the net photosynthesis recovers fully, the recovery of the entire photosynthetic process can be prolonged due to the disturbance in stomatal performance (Gallé and Feller, 2007).

CONCLUSION
ZAKLJUČAK

Obtained results showed significant decrease of net photosynthesis of investigated oak families under the simultaneous effect of drought stress and/or attack by caterpillars. However, NRA did not decrease under stress in both families. Recovery period did not increase net photosynthesis in plants but showed differences in limitation (stomatal/metabolic) between families. On the other hand, nitrate reductase activity was increased in stressed plants of both oak families indicating beginning of recovery. Based on comparison of the recovery measurements data and existing literature it can be concluded that the 7-day recovery was insufficient for oak seedlings to fully restore their optimal physiological processes. Analysis of data also showed that there is a significant genetic effect in relation to the families' response to induced stress and recovery, indicating the possibility to use physiological parameters in the selection of stress-tolerant oak progenies and provenances.

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SAŽETAK

Hrast lužnjak (*Quercus robur* L.) predstavlja jednu od ekonomski i ekološki najznačajnijih drvenastih vrsta u jugoistočnoj Europi, čiji je opstanak znakovito ugrožen promjenama okoliša koje se najčešće očituju u poremećaju vodnog režima i napadima kukaca. Imajući u vidu činjenicu da oba negativna čimbenika dovode do poremećaja fizioloških procesa u biljkama i da često utječu na biljke zajedno, cilj ovoga pokusa bio je ispitati utjecaj pojedinačnog i zajedničkog djelovanja suše i napada kukaca na fiziologiju hrasta lužnjaka i mogućnost različite reakcije uvjetovane porijeklom sadnica.

U ovom smo radu ispitivali utjecaj suše i napada gubara (*Lymantria dispar*) na klijance hrasta lužnjaka porijeklom iz dvije familije polusrodnika koji su bili naklijani u kontroliranim uvjetima i potom izloženi sljedećim tretmanima: suša (D); gubar (GM); suša i gubar (D+GM) te kontrola (Ø) u trajanju od 15 dana, nakon čega je slijedio oporavak biljaka u trajanju od 7 dana. Krajem tretmana i nakon oporavka, izvršena su mjerenja fizioloških parametara – stopa neto fotosinteze (A), stopa transpiracije (E), stopa provodljivosti puči (g_s), intercelularna koncentracija CO_2 (C_i), učinkovitosti korištenja vode (WUE), aktivnosti nitrat-reduktaze (NRA) i sadržaja klorofila.

Rezultati mjerenja fizioločkih parametara prilikom induciranog Kod tretiranih klijanaca A opala je za 42-61% u odnosu na kontrolni tretman (Graf 1) a u isto vrijeme, zabilježen je porast C_i u svim tretmanima, što ukazuje na metaboličku limitiranost fotosinteze, osobito ako se uzme u obzir da nije zabilježeno signifikantno smanjenje provodljivosti puči u svim tretmanima. Inducirani stres je različito utjecao na aktivnost nitrat-reduktaze koja je bila smanjena samo kod familije 2 u svim tretmanima. Period oporavka biljaka (Graf 2) nije doveo do povećanja intenziteta fotosinteze kod ispitivanih sijanaca hrastova u obje familije, ali je zabilježena razlika u pogledu razine njene limitiranosti. Kod familije 2 limitiranost je bila uvjetovana zatvorenošću puči (smanjena vrijednost g_s), dok je kod familije 7 ona i dalje bila metaboličke prirode (visoke vrijednosti C_i). Oporavak je uvjetovao povećanje razine aktivnosti nitrat-reduktaze, što ukazuje na postepeni oporavak biljaka.

Analiza varijance utjecaja tretmana, familije i perioda oporavka (Tablica 1) pokazala je znakoviti utjecaj porijekla sjemena na sve ispitivane parametre osim intenziteta neto fotosinteze ($p>0.001$), dok je utjecaj tretmana bio signifikantan kod svih ispitivanih parametara ($p>0.001$) Period oporavka je imao signifikantan utjecaj ($p<0.001$) samo za E, NRA i WUE.

Dobiveni su rezultati pokazali značajan utjecaj stresa na fiziološke procese u klijancima hrasta lužnjaka, posebice na intenzitet neto fotosinteze, dok je oporavak pokazao različitu reakciju ispitivanih familija unatoč i dalje smanjenom intenzitetu neto fotosinteze. Dobijeni rezultati ukazuju i na značaj porijekla sjemena, odnosno utjecaj njegove genetske pozadine na rekaciju ispitivanih familija glede njihove reakcije i oporavka od stresa. To ukazuje na mogućnost korištenja fizioloških parametara u procesu selekcije reporduktivnog materijala hrasta lužnjaka na otpornost prema stresnim čimbenicima poput suše ili napada defolijatora.

KLJUČNE RIJEČI: hrast lužnjak, fotosinteza, WUE, SPAD, aktivnost nitrat-reduktaze