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The influence of powdery mildew on chlorophyll *a* fluorescence and stomatal characteristics of pedunculate oak (*Quercus robur* L.)

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Abstract: Oak powdery mildew (*Erysiphe alphitoides* (Griffon and Maubl.) U. Braun and S. Takam.) is one of the most common foliar pathogenic organism of oaks, exploiting harmful effects, particularly on young seedlings. To assess and evaluate these negative effects, an experiment was conducted under semi-controlled conditions with 20 one-year-old seedlings of *Quercus robur* L. infected with *E. alphitoides*, half of them showing 50% of leaf coverage by epiphytic mycelia and the other half a coverage of 75%. The results of the present study have shown that all observed parameters of chlorophyll *a* fluorescence transient were highly effective in the detection of severe biotic stress. Moreover, the studied parameters of slow fluorescence kinetics were also considerably influenced, with the coefficient of non-photochemical fluorescence quenching and the Stem-Volmer type non-photochemical fluorescence quenching parameters showing the fastest responses. In case of leaf stomatal traits, the decrease of stomata guard cell width coupled with the increase of stomatal density was observed as the protective mechanism of *Q. robur* against the stressor. The overall results showed the adverse effects of powdery mildew infection on the photosynthesis of pedunculate oak seedlings, which progressed in time and depended on the severity of the infection. The importance of the results of the present study lays in evaluation and monitoring of the effects of powdery mildew development on the photosynthetic apparatus of one-year-old *Q. robur* seedlings, which is the most vulnerable stage for the infection by the mentioned pathogen.

Keywords: biotic stress, leaf physiology, chlorophyll *a* fluorescence, pedunculate oak, stomatal traits.

1. Introduction

Pedunculate oak (*Quercus robur* L.) is a widespread tree species in European forests with high economic benefits due to the quality of timber (Heuser and Zimmer 2002; Pietras et al. 2015; Árvai et al. 2018). However, in the last few decades, a diminishing in the vitality of *Q. robur* stands related to biotic and abiotic stress factors, has been observed (Thomas et al. 2002; Sohar et al. 2014; Kostić et al. 2019). In case of biotic stress factors, oak powdery mildew (*Erysiphe alphitoides* (Griffon and Maubl.) U. Braun and S. Takam.) has been recognized as one of the most important and widespread foliar

pathogenic organisms for oaks (Desprez-Loustau et al. 2010; Pap et al. 2014; Copolovici et al. 2014). *E. alphitoides* has particularly harmful effects on young shoots and leaves during the early ontogeny development phase (Pap et al. 2014), although it has been shown that the presence of this obligate pathogen may reduce the vigor of mature trees in case of combined stress conditions, i.e., defoliation caused by insects (Thomas et al. 2002). Likewise, the negative effect of powdery mildew also has a strong correlation with the timing and severity of the infection (Marçais and Desprez-Loustau, 2014).

An infection by powdery mildew occurs as the fungi develop its mycelia on the surface of the host plant's leaf and further differentiates infection structures in the epidermal cells. In addition, the fungi derive the metabolites necessary for its own nutrition through the absorptive organs (i.e. haustoria) (Divon and Fluhr, 2007). As a result of the infection, a large number of conidiophores are produced, which manifest in the form of a silver-white cover on the leaf surface, a typical sign of powdery mildews (Desprez-Loustau et al. 2010). The most common effects of oak powdery mildew are a reduction in carbon assimilation and the translocation of carbohydrates, as the consequence of acquiring nutrients from host plants cells (Hewitt and Ayres, 1976). Indeed, several studies have demonstrated that an infection by *E. alphitoides* may cause either a decline of photosynthesis or stomatal conductance or both physiological processes, in parallel (Brüggemann and Schnitzler, 2001; Hajji et al. 2009; Copolovici et al. 2014).

Upcoming climate changes require more attention to be paid to disease control in nursery production and reforested areas, especially to more harmful diseases, like oak powdery mildew. Namely, the predicted climate change towards warmer winters has been noted to favor the overwintering of *E. alphitoides* in oak buds (Marçais et al. 2017), which contributes to its wider distribution and higher occurrence. As during in the early stage of the oak tree life cycle, powdery mildew was noted to be the most important adverse biotic stress factor (Percival and Fraser, 2002; Marçais and Desprez-Loustau, 2014), so our study investigated the effect of the noted pathogen on one-year-old *Q. robur* seedlings.

Since chlorophyll fluorescence has a direct link to the light stage of photosynthesis, additional valuable information could be provided about the indication of photosynthetic dysfunction (Lucena et al. 2012; Ortoizze, 2016). Chlorophyll *a* fluorescence techniques have been proven to be a reliable tool for the detection of salt stress (Mehta et al. 2010; Lucena et al. 2012), drought stress (Vastag et al. 2019; Vastag et al. 2020), heatwave (Bauweraerts et al. 2014; Guha et al. 2018) and nutrient status identification (Frydenvang et al. 2015; Kalaji et al. 2018). Furthermore, several studies have also demonstrated its effectiveness in the monitoring of various biotic stress conditions (Wang et al. 2014; Dąbrowski et al. 2017; Kaur et al. 2018). However, in terms of powdery mildew, thus far only a small number of studies have applied this method for detection or estimation of its negative effects on photosynthesis (Percival and Fraser, 2002; Dąbrowski et al. 2017). Moreover, the mentioned studies only dealt with a few parameters, insufficient for getting the entire picture about the behavioural patterns of *Q. robur* under biotic stress caused by *E. alphitoides*.

Therefore, having in mind the need for an expansion of the specter of chlorophyll a fluorescence parameters, the present study was meant to investigate the effectiveness of chlorophyll a fluorescence kinetics and chlorophyll *a* fluorescence transient in combination with the stomatal traits for the detection, evaluation and monitoring of severe biotic stress levels caused by powdery mildew fungi on one-year-old *Q. robur* seedlings. Accordingly, the results should provide valuable information about which parameters of chlorophyll a fluorescence and leaf stomatal traits could be used for discovering, evaluating and monitoring powdery mildew infections.

2. Material and methods

2.1. Plant material

Acorns, of a single genotype located along the Vojvode Stepe Boulevard (N 45°25'61.75", E 19°79'73.47", altitude 80 m a.s.l.) in Novi Sad, Serbia, were collected in October 2017. During the winter

months, the collected acorns were stratified in a peat:sand mixture (1:l, v/v, 5 L). Following the stratification, on the 31st of March 2018, a 24h soaking process was conducted in order to eliminate unviable acorns. After the rehydration process, the acorns were sown in PVC pots (20 cm × 16 cm, height × diameter, 3 liters) filled with Stender potting substrate S 200 (organic matter 20%, pH 5.5, electrical conductivity (EC): 670 $\mu\text{S}/\text{cm}$, dry matter 37.1%, fertilization: 1.0 kg NPK 14 + 16 + 28) and maintained in a greenhouse under semi-controlled conditions. The air temperature in the greenhouse ranged between 20°C at night and 30°C during the day, whereas the lightening varied according to outdoor conditions, never exceeding 1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ even under sunny conditions. Throughout this period, the plants were watered every second day to maintain soil water content close to field capacity (-0.03MPa). Tensiometers were placed at a depth of 10 cm for monitoring soil water tension.

The potted seedlings were moved to the outdoors from the 1st of May and kept under a canopy of four mature pedunculate oak trees, located in the forest estate of the Institute of Lowland Forestry and Environment in Novi Sad, Serbia.

2.2. Experiment design and assessment of the severity of the infection

The seedlings kept outdoors were arranged in two groups: one which was covered and protected by cellophane bags, allowing gas exchange and ensuring the prevention of the infection by the studied pathogen; and the second group of seedlings, kept unprotected, which were spontaneously and naturally infected by *E. alphitoides* from the mature oak trees. Prior to the first measurements, ten seedlings were randomly assigned to the control group. A further, ten seedlings, with the coverage of epiphytic mycelia reaching 50%, were selected for the first treatment group. The second treatment group consisted of an equal number of specimens, with the coverage of epiphytic mycelia reaching 75%. The measurements started on the 6th of July 2018 and were repeated, weekly, over the following 5 week period. During this period, the infection advanced according to its natural course, and, by the end, the leaves of the first treatment group had reached a coverage of 75% with epiphytic mycelia, while the second treatment group had 100% leaf infection coverage. The experiment ended when the second treatment group showed symptoms of foliar chlorosis, dead leaves, and branches, considered as signs of plant mortality, according to Barradas et al. (2017).

For an estimation of the percentage of the leaf area occupied with epiphytic mycelia of *E. alphitoides* the upper side of ten leaves per treatment (30 leaves in total) was chosen at the beginning and at the end of the measurements. After the collected leaves were photographed, the obtained images were processed by ImageJ freeware software (Schneider et al. 2017) for image analysis.

2.3. Measurements of chlorophyll a fluorescence transient

Fast chlorophyll fluorescence induction curves (OJIP) were recorded with a PAM-2500 portable chlorophyll fluorometer (Walz, Germany). All measurements were conducted on the 3rd fully expanded leaf, aged 20 days or more, between 9:00 AM and 11:00 AM due to midday depression of photosynthesis. During this period, ten seedlings from each treatment group were recorded for fluorescence transient as well as slow kinetics. Prior to measurements, the leaves were dark-adapted for 30 min with light exclusion clips. The OJIP transient was induced by strong light pulses of 3000 photons $\mu\text{mol m}^{-2}\text{s}^{-1}$ and recorded between 10 μs and 320 ms (Kautsky curve). The obtained data were analyzed using OJIP test protocol of Strasser et al. (2000) (Table 1).

2.4. Measurements of slow chlorophyll fluorescence kinetics

Slow chlorophyll fluorescence kinetics were assessed using the automatic rapid light curve function of the PAM-2500 portable chlorophyll fluorometer (Walz, Germany), between 9:00 AM and 11:00 AM. Measurements included seven periods of actinic illumination, ranging from 144 to 2443

$\mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$. The illumination periods lasted for 20 s each and were divided by a saturating flash of $\sim 3000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ lasting for 0.8 s. Relevant fluorescence parameters (Table 2) were derived by using the recorded data.

Table 1. Parameters deduced by the OJIP test analysis of chlorophyll *a* fluorescence transient.

Abbreviation and formula	Basic physiological interpretation
Basic fluorescence parameters of OJIP-transient	
F_j (2 ms)	Fluorescence value at the J-step (2 ms) of OJIP
F_i (30 ms)	Fluorescence value at the I-step (30 ms) of OJIP
Derived fluorescence parameters of OJIP-transient	
$F_v = F_m - F_0$	Maximum variable fluorescence from dark adapted leaf ¹
$\Phi_{Po} = F_v/F_m = 1 - (F_0/F_m)$	Maximum quantum yield of primary PSII photochemistry ¹
$F_v/F_0 = (F_m - F_0)/F_0$	Efficiency of the water-splitting complex on the donor side of PSII ¹
$PI_{ABS} = (RC/ABS) [\Phi_{Po}/(1 - \Phi_{Po})] * [\psi_{ET0}/(1 - \psi_{ET0})]$	Performance index on absorption basis ¹

¹(Strasser et al. 2000)

Table 2. Derived parameters of slow chlorophyll fluorescence kinetics.

Abbreviation	Basic physiological interpretation
Derived fluorescence parameters	
$Y(NO) = F/F_m$	Quantum yield of non-regulated heat dissipation and fluorescence emission (Genty et al. 1996)
$NPQ = F_m/F_m' - 1$	Stem-Volmer type non-photochemical fluorescence quenching (Schreiber et al. 1986, as formulated by van Kooten and Snel, 1990)
$ETR = PAR * 0.84 * 0.5 * Y(II)$	Relative electron transport rate
$qN = 1 - ((F_m' - F_0')/(F_m - F_0))$	Coefficient of non-photochemical fluorescence quenching (Schreiber et al. 1986, as formulated by van Kooten and Snel, 1990)
$qP = (F_m' - F)/(F_m' - F_0')$	Coefficient of photochemical fluorescence quenching (Schreiber et al. 1986, as formulated by van Kooten and Snel, 1990)

2.5. Measurements of stomatal characteristics

Stomatal imprints were made using the collodion method according to the described protocol by Stojnić et al. (2015). Stomatal imprints were analyzed using an *Olimpus* BX 53F microscope in order to assess the following primary stomatal characteristics: stomatal density per mm^2 (SD), stomata guard cell length (L_A [μm]) and width (W_B [μm]), and stomatal aperture length (L_a [μm]) and width (W_b [μm]). SD was determined at five randomly chosen visual areas using the freeware software *tpsDIG2* (Rohlf FJ, 2017). *QuickPhoto Camera 3.2*. software was used to measure stomata guard cell and stoma aperture size, on a sample of five stomata per five randomly chosen fields of view.

2.6. Statistical analysis

The differences between the control and the two treatment groups were analyzed by one-way Analysis of Variance (ANOVA). When values from ANOVA were shown to be statistically significant,

a comparison of means was performed by applying the Tukey test, so as to determine the level of significance. For better visualization, the results were shown in forms of linear and bar graphs. Statistical analyses was performed using the R 3.3.2. software (R Core Team, 2017) for Windows. A significance level of 0.05 was considered for all analyses.

3. Results

3.1. Meteorological characteristics

The meteorological data was obtained from the nearby hydrometeorological station Rimski Šančevi (N 45°20', E19°51', altitude 84 m a.s.l.) (Republic Hydrometeorological Service of Serbia, 2018). In accordance with the obtained data (Figure 1), the period of the experiment can be characterised as wet and mildly warm. The mean air temperatures were mostly in the range of 22°C and 24°C, never exceeding 28°C. Regarding the daily sums of precipitation, an abundance was observed, including one extreme weather event on the 30th of June, with a recorded 116.6 mm for that day. The described climatic data provided favorable weather conditions for the spontaneous infection of the studied one-year old oak seedlings and the further fungal development of powdery mildew.

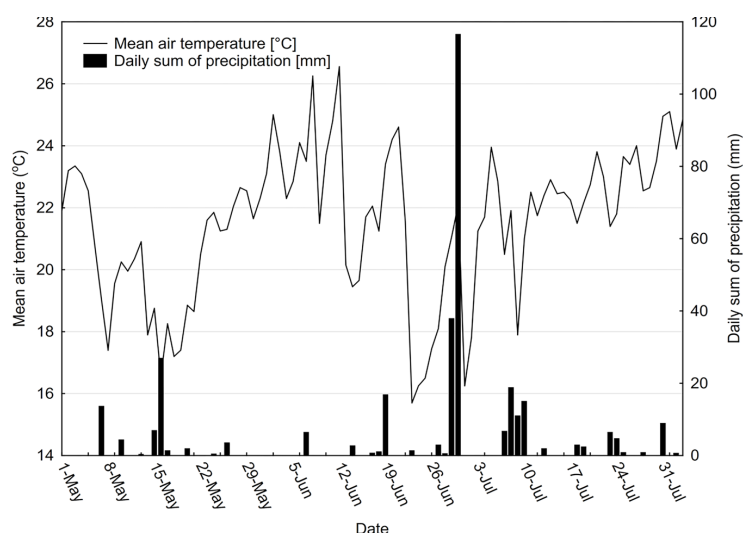


Figure 1. Mean daily air temperature (°C) and daily total precipitation (mm) during the period from 1th of May to 3rd of August 2018.

3.2. Chlorophyll *a* fluorescence transient

All of the observed parameters of chlorophyll *a* fluorescence transient showed to be significantly influenced by the mentioned obligate leaf parasite, in both of the studied treatment groups, detected during the first round of conducted measurements (Figure 2). The studied biotic stress significantly inhibited fluorescence transients across the basic observed parameters of the OJIP curve, causing a reduction of 36.4% and 45.35% in case of F_j , 28.9% and 49.8% for F_i , within leaves covered with 50% or 75% *E. alphitoides* mycelia in the first round of measurements. The decline in the observed primary parameters progressed with time and the severity of the infection, reaching 66.0% and 78.4% for F_j and 60.0% and 81.1% for F_i by the end of the experiment, for leaves occupied 75% or 100% by the studied pathogen (Figure 2a and 2b).

In case of the F_v parameter, the control group remained within 0.87-0.77 throughout the whole experiment, whereas in the two treatment groups the mentioned parameter showed a gradual decrease with increasing levels of stress, reaching a reduction of 17.38% or 49.33% for leaves infected 75% and 100%, respectively (Figure 2c).

Furthermore, a significant reduction of the Φ_{P_0} parameter was detected, compared to the control plants, in both of the treatment groups. While the values of the control group were between 0.75 and 0.85, a range documented for healthy plants (Bolh ar-Nordenkampf and Orquist, 1993), the first treatment group exhibited a decline of 41.9% and the second a 53.5% decline during the first measurements. At the end of the experiment, the reduction reached 61.2% and 77.1% for *Q. robur* leaves infected 75% and 100% with *E. alphitoides* respectively (Figure 2d).

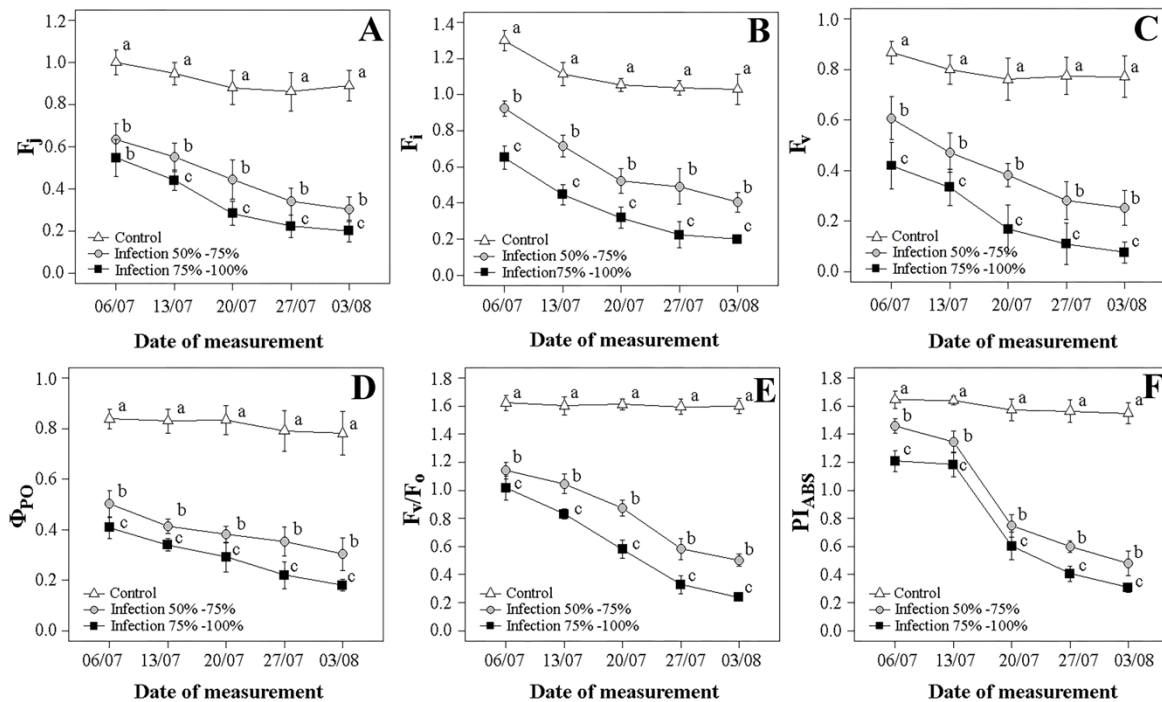


Figure 2. Progress curves of chlorophyll *a* fluorescence transient parameters measured on leaves of *Q. robur* seedlings. **A** - Fluorescence value at the J-step (F_j [relative units]); **B** - Fluorescence value at the I-step (F_i [relative units]); **C** - Maximum variable fluorescence from dark adapted leaf (F_v [relative units]); **D** - Maximum quantum yield of primary PSII photochemistry (Φ_{P_0} [relative units]); **E** - Efficiency of the water-splitting complex on the donor side of PSII (F_v/F_0 [relative units]); **F** - Performance index on absorption basis (PI_{ABS} [relative units]). All values are presented as means \pm standard errors ($n = 10$). The different small letters next to error bars indicate significant differences between the values (Tukey's honestly significant difference (HSD) test; $p \leq 0.05$).

3.3. Slow fluorescence kinetics

Concerning the parameters of slow fluorescence kinetics, $Y(NO)$ showed an increasing trend, meanwhile NPQ, ETR, qN and qP were decreased under severe biotic stress caused by *E. alphitoides* (Figure 3). From all of the obtained parameters, only $Y(NO)$ was shown to be mildly sensitive in the detection of the differences between the control and the infected plants, showing statistically significant differences during the second round of measurements. At the end of the experiment, the values of $Y(NO)$ increased by 15.2% and 21.2% in leaves occupied 75% and 100% by the studied pathogen (Figure 3a).

On the other hand, statistically highly significant differences between the two treatment groups, as well as between the healthy and the infected plants, were observed for NPQ and qN parameters (Figure 3b and 3d), discovered as early the first round of measurements, indicating a high effectiveness in the detection of biotic stress.

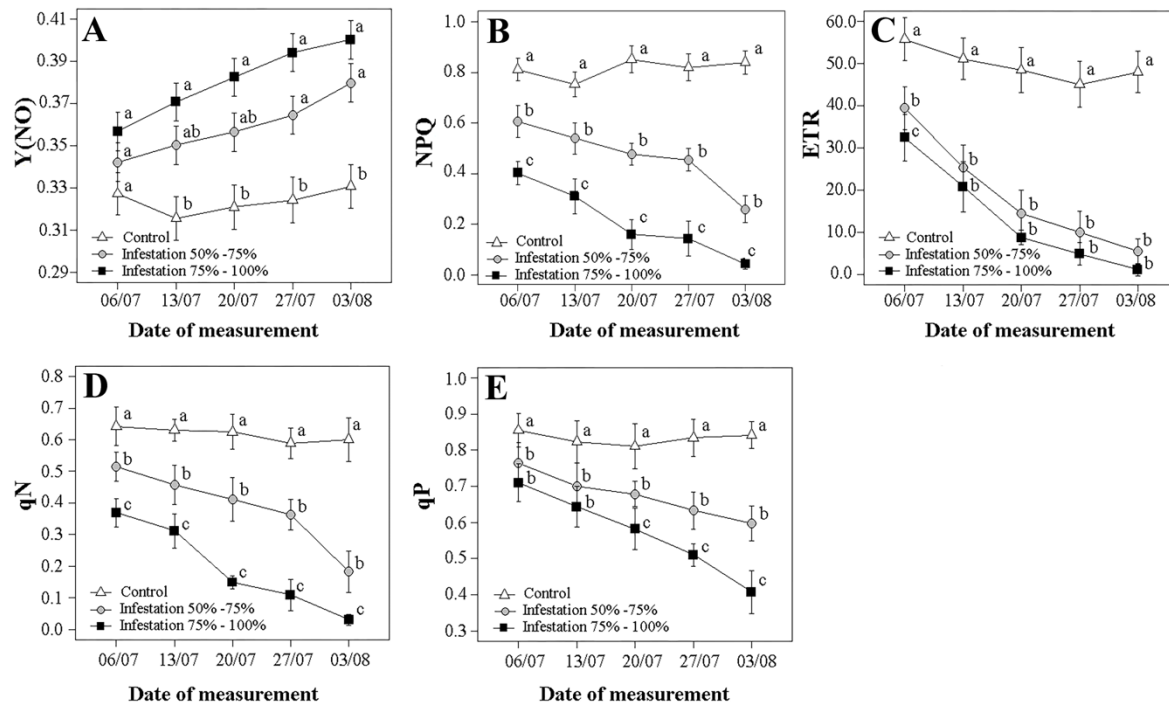


Figure 3. Progress curves of slow fluorescence kinetics parameters measured on leaves of *Q. robur* seedlings at high light conditions ($1389 \mu\text{mol} (\text{photon}) \text{m}^{-2} \text{s}^{-1}$). **A** - Quantum yield of light-induced non-photochemical fluorescence quenching ($Y(\text{NO})$ [relative units]); **B** - Stem-Volmer type non-photochemical fluorescence quenching (NPQ [relative units]); **C** - Relative electron transport rate (ETR [$\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$]); **D** - Coefficient of non-photochemical fluorescence quenching ($q\text{N}$ [relative units]); **E** - Coefficient of photochemical fluorescence quenching ($q\text{P}$ [relative units]). All values are presented as means \pm standard errors ($n = 10$). The different small letters next to error bars indicate significant differences between the values (Tukey's honestly significant difference (HSD) test; $p \leq 0.05$).

3.4. Stomatal traits

Regarding stomatal characteristics, our results showed that infection by *E. alphitoides* caused a significant increase in SD and a simultaneous reduction in W_b (Figure 4a and 4e). In case of SD, the highest values were observed in leaves covered 75% by mycelia at the start of the measurements, as well as at the end of the experiment, when the mycelia reached 100% of leaf coverage. Moreover, the infection occupying 50% of leaf area showed to cause higher values of SD in comparison with the SD of plants in the control group, during the first conducted measurements, as well as at the end, with the infection reaching a 75% leaf coverage (Figure 4a). On the other hand L_A , W_B and L_a were not significantly affected by the mentioned pathogen (Figure 4b, 4c and 4d).

4. Discussion

Results from the present study show the adverse effects of powdery mildew infection on the photosynthesis of pedunculate oak seedlings. The effect of the observed biotic stress on the values of polyphasic fluorescence transient parameters depended on the intensity of infection, except for F_i in the first recording. A positive correlation between the degree of the *E. alphitoides* infection and the severity of damage on the photosynthetic apparatus, affecting the level of net photosynthesis, has been documented by several studies (Hajji et al. 2009; Pap et al. 2014). The mentioned decline of photosynthesis was partially explained by a reduced quantity of irradiance reaching the photosynthetic apparatus due to the shedding effect of the mycelia occupying the surface of the leaves (Misaghi, 1982).

Nevertheless, according to Mitchell et al. (1979) the major factor causing the decline is the reduction of chlorophylls.

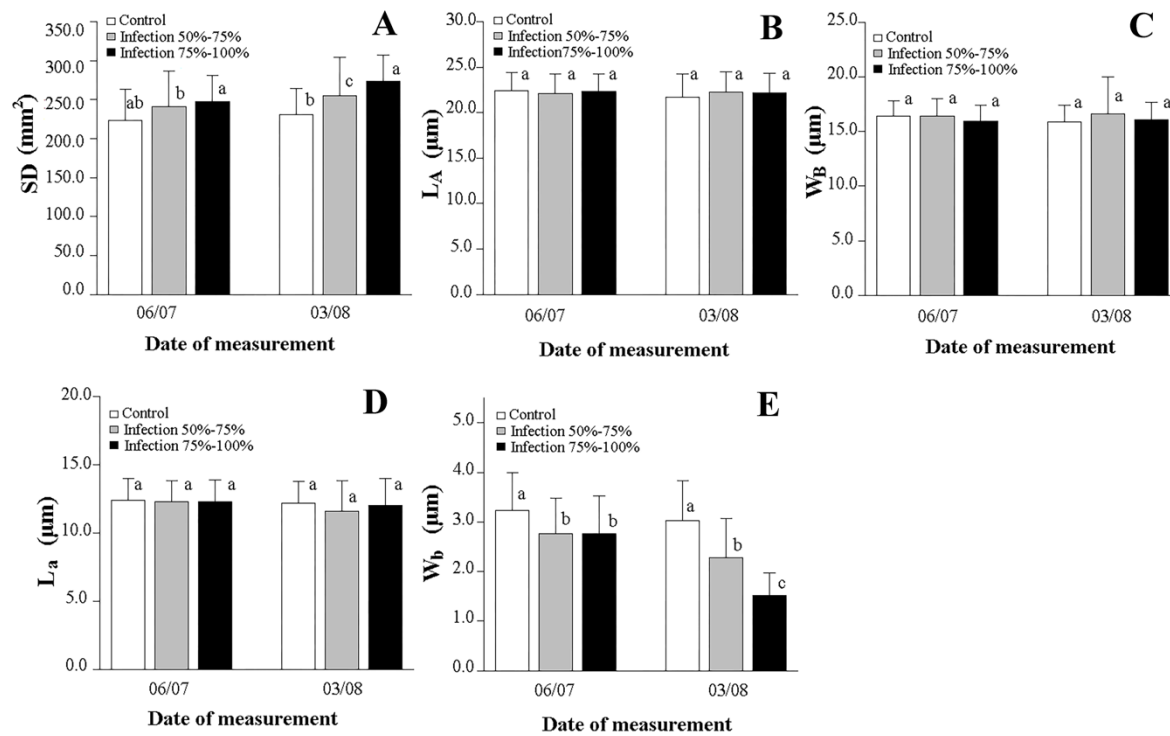


Figure 4. Stomatal traits of leaves of *Q. robur* seedlings. The different small letters next to error bars indicate significant differences between the values (Tukey test; $P \leq 0.05$). **A** - stomatal density per mm² (SD [number per mm²]); **B** - stomata guard cell length (L_A [μm]); **C** - stomata guard cell width (W_B [μm]); **D** - stomatal aperture length (L_a [μm]); **E** - stomatal aperture width (W_b [μm]). All values are presented as means ± standard errors (n = 10). The different small letters next to error bars indicate significant differences between the values (Tukey's honestly significant difference (HSD) test; $p \leq 0.05$).

Only a few studies approached the effects of *E. alphitoides* infection on light-harvesting apparatus by applying fluorescence measurements techniques (Percival and Fraser, 2002; Dąbrowski et al. 2017), even though it has been proven to be a rapid, accurate and non-invasive method (Stirbet, 2014; Kalaji et al. 2018; Song et al. 2018). Likewise, the above-mentioned studies only dealt with a very scarce number of chlorophyll fluorescence parameters, insufficient for getting the entire picture of damage made on PSII and PSI reaction centers.

The results from the present study evidenced a significant reduction of F_i and F_j parameters of chlorophyll *a* fluorescence transient within leaves covered 50% or 75% by *E. alphitoides* mycelia, which decreased gradually in time as the infection progressed. Similarly, a reduction of the above-mentioned parameters was observed by Zhorri et al. (2015) on leaves of *Euphorbia cyparissias* infected by *Uromyces pisi*, as well as by Kaur et al. (2018) on leaves of *Tinospora cordifolia* inoculated with *Phoma putaminum* Speg. A decline of the F_i and F_j parameters was observed not only as the effect of biotic stressors but in abiotic stress conditions as well, reported for salt stress (Oyiga et al. 2016; Zushi and Matsuzoe, 2017), drought (Banks, 2018; Wang et al. 2018), temperature stress (Martinazzo et al. 2012; Chen et al. 2013) and chemical influence (Dąbrowski et al. 2017). According to Kaiser et al. (2014) every change in environmental conditions presses the photosynthetic system to adjust through its physiological state. This adjustment is particularly observable in the shape of the fast polyphasic fluorescence transient (Xia et al. 2004; Mehta et al. 2010), and thus can be successfully applied for the detection and monitoring of the deviation in the behavioural patterns of stressed plants (Cetner et al. 2017). This claim was

evidenced by Percival (2005) as well, stating that the shape of the OJIP curve identified herbicide and heat damage, 24h after the applied treatments.

A decrease in the F_v parameter, evidenced by the present study, is associated with the disorder of energy transformation in PS II, as well as with the partial closing of reaction centers. This results in a fragmented damage of their function during the conversion of light energy into chemical potential (Saakov et al. 2015), indicating less photosynthetic competence per unit of chlorophyll followed by a lower photosynthetic activity of PSII. Under different stresses F_v was reported to diminish, i.e., during freezing stress (Forney et al. 2000), drought stress (Paknejad et al. 2007; Zlatev, 2009), salt stress (Hniličková et al. 2017). Furthermore, its decline is coupled with the reduction of Φ_{Po} , the most frequently used fluorescence parameter for the detection of a plant's physiological state under different unfavorable conditions (Roháček et al. 200; Kalaji and Guo 2008).

In our study, the biotic stress caused by powdery mildew lead to a significant reduction of Φ_{Po} parameter within leaves covered 50% or 75% by mycelia, in the first round of measurements, and it declined further as the infection progressed. The study conducted by Percival and Fraser (2002) demonstrated that the values of Φ_{Po} remained constant until 11%-25% of *Q. robur* leaf surface area was occupied with the mycelia of powdery mildew, while afterward, the values showed a steady decline until the end of the experiment when the leaves became necrotic and stunned. Similarly, Kurjak et al. (2019) observed a much more pronounced decrease of the above-mentioned parameter in previously stressed *Fagus sylvatica* L. trees. In addition, Wang et al. (2014) reported a slight Φ_{Po} decline of 7% in five days after inoculation in rubber trees infected with *Oidium heveae* Steinm., while a significant reduction (16%) was observed after a further five days. According to Guo et al. (2015), the decline of Φ_{Po} is presumably the result of a reduction of the original light energy conversion efficiency, showing the inhibition of a potential active center. As a result of inhibition, the active center suppresses the reaction center of leaves to photosynthesize (Rong-Hua et al. 2006). Nevertheless, this parameter provides only partial information about the state of photosynthetic apparatus, thus should be carefully interpreted and combined with additional parameters indicating its function (Pšidová et al. 2018). In some cases, the F_v/F_o parameter has been proven to be a better alternative to Φ_{Po} , being more susceptible to environmental changes (Maxwell and Johnson, 2000), as supported by our study as well, showing a more rapid decrease in comparison with Φ_{Po} , in both of the treatment groups during our experiment.

In order to get further insight into the functionality of PS II and PSI, as well as to evaluate the current status of plants' performance under stress (Strasser et al. 2004), PI_{ABS} values were obtained and their decline was observed under biotic stress. Results of the study conducted by Percival and Fraser (2002) evidenced that the PI_{ABS} value is a highly sensitive indicator of *E. alphitoides* infection, and therefore of plant vitality in general, manifesting its effects even prior to the visible signs of the infection. The effectiveness of this parameter has been noted by Banks (2018), as well, responding most significantly during drought stress of *Acer* sp. genotypes. The gradual decrease of this parameter has been associated with disturbances at the PSII acceptor side (Brestic and Zivcak, 2013), while its decrease reflects the loss of the density of reaction centers (Dudeja and Chaudhary, 2005).

In terms of slow fluorescence kinetics, biotic stress caused by *E. alphitoides* mildly affected the $Y(NO)$ parameter. An increase in this parameter is associated with a loss of energy along the electron transport chain, as well as with the destruction of the D1 protein in PSII, which overall presents the destruction of PSII (Olmos and Posada, 2013; Li and Zhang, 2016). In accordance with our results, Bürling et al. (2009) demonstrated that $Y(NO)$ was unable to detect differences between control and inoculated wheat plants with concentrations up to 100.0 spores/ml of *Puccinia triticina*. Similarly, Li et al. (2008) noted the absence of significant differences in the mentioned parameter between control, mildly and severely drought-stressed cucumber seedlings.

According to Demmig-Adams et al. (2014), NPQ reflects molecular adaptation which is, indeed, the fastest response of the photosynthetic membrane exposed to excess light. In terms of NPQ, our results suggest that nonphotochemical dissipation was insufficient to avoid photoinhibitory damage, which was indicated by the decrease of the Φ_{Po} parameter, as well. Our findings are in accordance with

the results of Scholes and Rolfe (2009) who reported a decrease of NPQ during necrotrophic phases of *Mycosphaerella graminicola* and wheat interactions. Similarly, Ghosh et al. (2017) evidenced the same trend in rice leaves infected with *Rhizoctonia solani* Kuhn. Unfavorable environmental changes, such as infection by a pathogen, affect the saturation of the electron transport chain and increase the accumulation of protons, which results in increased values of NPQ (Porcar-Castell et al. 2014). Decreased values of NPQ reflect the destruction of photosynthesis systems, which results in an incapability to transport the absorbed light energy to PS I as photochemical quenching (Li et al. 2016).

In a study conducted by Chávez-Arias et al. (2019), a statistically significant reduction of ETR was found in leaves of *Physalis peruviana* L. on the 33rd day after the infection with *Fusarium oxysporum* f. sp. *physalis*. Few additional studies have evidenced the decline of ETR during the infection process with pathogens (Prokopová et al. 2010; Wang et al. 2014; Bermúdez-Cardona et al. 2015), as detected in our experiment as well. Furthermore, a sharp decrease, similar to our findings of the mentioned parameter, was observed in *Vitis vinifera* when the relative volume of water decreased down to approximately 50%-55%, resulting in irreversible damage on the photosynthetic apparatus (Ortoidez, 2016).

According to Chen et al. (2016) apart from Φ_{Po} , qP and qN are the other commonly used parameters of fluorescence that showed to be feasible for detecting damages resulting from subtle, transient stress, as well as from long term and severe stress. Indeed, many studies have reported that severe stress conditions causes a decrease in qN and qP parameters indicating a severe loss of functionality of the PSII (Horton et al., 2008; Murchie and Lawson, 2019). Our results, in accordance with the above-mentioned findings, show significant differences in qN and qP between healthy and infected plants. Additionally, qN distinguished significant differences between the two treatment groups in the first round of measurements, while qP succeeded that in the later stages of infection, namely, during the third round of measurements. The prolonged time needed for the detection of stress levels with qP was also noted by Hazrati et al. (2016), stating that this parameter was insufficient for the detection of differences between 20% and 40% of applied water regimes. At the end of our experiment its values were reduced to 0.59 and 0.40 in leaves infected 75% and 100%, showing that most of the PSII reaction centers were closed and their Q_a was in a reduced state (Zlatev, 2013; Kalaji et al. 2014).

SD is marked as an important eco-physiological parameter affecting gas exchange and photosynthesis, which is highly susceptible to changes in the environment, i.e., drought (Mansouri and Radhouane 2015, Shekari et al. 2016), high irradiance (James et al. 2000; O’Carrigan et al. 2014), pathogen infection (Lake and Wade, 2009; Gomes de Araujo, 2010; Chattopadhyay et al. 2014), etc. Several studies have demonstrated that SD increases with the intensity and duration of stress, as evidenced by our study as well (Djauhari et al. 2008; Djaenuddin and Talanca, 2019). Namely, under stress conditions the pathogen signaling system is triggered in order to increase stomatal numbers, as a protective mechanism to fight against the stressor (Reyna and Yang, 2006; Lake and Wade, 2009). On the other hand, W_b was shown to decrease in the first conducted measurements in leaves infected 50% and 75 %, showing a further decrease in the second treatment group. It has been well documented that even early biotic stress causes the closure of stomata and a reduction in photosynthesis, while severe stress, as in our case, results in the severe disruption of stomatal function and an inhibition of photosynthesis (Zou et al. 2017; Wang et al. 2018). Although Holland and Richardson (2009) noted that apart from SD, L_A is also considered to be sensitive to changes in environmental conditions, our study showed the absence of significant differences between control and two treatment groups. Similar findings were obtained by Stojnić et al. (2015) and Richardson et al. (2001), suggesting that SD is more plastic to environmental influences, which results in its higher ability to adjust to different conditions.

5. Conclusions

The results of the present study point to a disturbance in the photosynthetic apparatus under biotic stress caused by *E. alphitoides*, which progressed in time and depended on the severity of the

infection. Statistically significant negative effects of the studied pathogen on leaf physiology were detected by all of the observed parameters of the chlorophyll *a* fluorescence transient, showing its effectiveness in the detection of severe biotic stress. Furthermore, parameters of slow fluorescence kinetics were considerably influenced as well, with *qN* and *NPQ* parameters showing the fastest responses. Concerning the harmful effects of the studied pathogen on stomatal traits, we detected a significant increase of *SD* followed by a simultaneous reduction of *W_b*, as a protective mechanism of *Q. robur* against the stressor.

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